

Oakland Police Department Criminalistics Laboratory
DNA Instrumentation and Analysis and Software
Surveillance Impact Report
April 2022

1. Description

The Oakland Police Department (OPD) Criminalistics Laboratory's (Crime Lab) Forensic Biology/DNA unit utilizes specialized DNA collection and analysis instrumentation and software to perform forensic DNA testing. This is a biometric analysis which produces potentially sensitive information.

During the lengthy and complicated process to obtain a DNA profile from evidence or a reference sample, numerous steps may be necessary including, but not limited to: Digestion, Extraction, Quantitation, Normalization/Amplification, Typing, Interpretation, and Database upload.

OPD does not use Forensic DNA Analysis to surveil residents of Oakland; indeed, it is unlawful to analyze samples and upload them to Combined DNA Index System (CODIS) when no articulable nexus to a crime exists.

2. Purpose

At the end of all DNA analysis processes described previously, a determination can be made as to whether a DNA sample collected from a crime scene can be associated with a known individual through a comparison of evidentiary (crime scene) and reference DNA profiles.

3. Location

The DNA instruments and analysis software are housed in the Criminalistics Laboratory and may not be used elsewhere without disclosure to the Laboratory's accreditation agency ANAB [ANAB = American National Standards Institute (ANSI) National Accreditation Board] and revalidation.

4. Impact

The proposed biometric use policy covers how and when information is to be disseminated, as well as prohibitions against disclosures outside those listed. Civil Rights and liberties are adequately protected in that all samples are to be collected pursuant to search warrant, other legal means, or by documented consent. Nothing in the forensic DNA analysis allows for data collection to be discriminatory, viewpoint-based or biased by algorithm; in fact, the results of DNA analysis can, in a scientifically unbiased manner, include or (more importantly to privacy) exclude a person of interest. OPD recognizes that biometric analysis technology and associated data, if used in ways that violate accreditation, legal standards and uses described and referenced herein, would constitute inappropriate use.

5. Mitigations

The OPD Crime Lab mitigates against the impact of unlawful evidence submissions by requiring that all samples subject to DNA analysis are collected pursuant to search warrant, other legal means, or by documented consent.

Inappropriate uses of DNA biometric analysis technology and associated data are mitigated by:

- (1) Limiting access to the instrumentation and records.
 - a. Only staff authorized to work in the Crime Lab have access.
 - b. Sign-in and escort are required of all guests.
 - c. The laboratory is locked during business hours and locked and alarmed after hours.
- (2) Existence of written policies regarding care of data and casefiles.
 - a. Instrument software is in limited access locations and are hosted on secure servers.
 - b. DNA analytical data are kept on secure network drives.
- (3) Existence of written policies precluding wide dissemination of records.
 - a. Legal Discovery for Criminal or Civil trials is honored.
 - b. California Public Records Act (CPRA) requests are subject to limitations as specified in the Biometric Technology Use Policy.

6. Data Types and Sources

The instruments described previously collect data during one step in the process and may be passed along to another. Data generated by each instrument are stored in a proprietary format readable only by the protocol software or may be converted to tables to be used electronically or printed. The Use Policy indicates how raw data and paper casefiles are to be handled and stored.

7. Data Security

Criminalists and Forensic Technicians with duties in the Forensic Biology/DNA unit shall be the only Crime Laboratory personnel authorized to use the DNA collection and analysis software in casework, and only after completing a comprehensive training program and qualifying test, at which time, with the Supervisor's recommendation, the Crime Laboratory Manager issues a written authorization. No one else shall have the authority to grant access to use the DNA instrumentation or software in casework. Criminalists and Forensic Technicians are granted access to one another's cases only for the purpose of complying with discovery, documenting quality checks, verifications or peer review. Interns also are authorized to use the DNA collection and analysis software for special projects, not casework, and only after receiving necessary training and under the supervision of a qualified Criminalist. Data are stored on secure servers hosted in the Laboratory or by the Department.

8. Fiscal Cost

Digestion / Extraction

- Three EZ1 Advanced XL DNA purification instruments and software are in the laboratory; the cost of one new instrument is approximately \$63,000. Currently, two EZ2 DNA purification instruments have had grant funds identified for purchase. The current ongoing annual upkeep of the instrument is approximately \$3,100 per instrument.
- One Versa 1100 liquid handler instrument is in the laboratory; the cost of one replacement instrument is approximately \$85,000. The annual maintenance cost is approximately \$6,800 per instrument.

DNA Quantitation

- Three Qiagility liquid handler instruments are in the laboratory; the cost of one replacement instrument is approximately \$33,100. The annual maintenance cost is approximately \$2,700 per instrument.
- Two QuantStudio 5 Real-Time PCR DNA quantitation instruments are in the laboratory; the cost of two new replacement instruments is \$114,000. The current ongoing annual upkeep of both instruments is approximately \$10,200.

DNA Normalization / Amplification

- One SpeedVac concentrator is in the laboratory; the cost of one replacement instrument is approximately \$4,000. No annual maintenance cost.
- Two ProFlex thermal cyclers are in the laboratory; the cost of one replacement ProFlex instrument is approximately \$14,000. No annual maintenance cost.

DNA Typing

- One 3500 genetic analyzer; the cost of which was \$135,000. The annual maintenance cost is approximately \$6,000.

DNA Interpretation

- STRmix upgrade cost \$66,000; annual maintenance costs run ~\$12,000 annually
- FaSTR cost approximately \$37,000.
- Armed Expert acquisition cost approximately \$15,000

Grants, Proposition 69 funds, and Operations and Maintenance budgets have historically covered these costs.

9. Third Party Dependence

Electronic data are retained indefinitely on secure server or network drives and do not require a third party. Hardcopy data present in paper casefiles are currently stored under laboratory control. In the future, if storage needs for hardcopy files exceed capacity, a Departmentally-approved records retention facility will be used as articulated in the Biometric Use policy.

10. Alternatives

The DNA analysis instruments and software have been validated and meet or exceed both accreditation requirements and industry standards. Alternatives have either been found to be inferior or would require time-exhaustive and expensive validation to replace the current platform with other technology.

11. Track Record

STR-based DNA analysis as a technology has extensive and longstanding documentation as a standard and effective method to analyze DNA. The methods using these technologies in total are employed by many private and government (local, state, federal) forensic and clinical laboratories. There is no known adverse information extant about the technology.

Version 1

February 2007

BioRobot® EZ1 DSP Workstation User Manual



IVD

CE

REF 9001360

HB 1037181

QIAGEN GmbH, D-40724 Hilden

R1



Sample & Assay Technologies

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1 Safety Information

This manual contains information and warnings that must be followed by the user to ensure safe operation of the BioRobot EZ1 DSP workstation and to maintain the instrument in a safe condition.

Possible hazards that could harm the user or result in damage to the instrument are clearly stated at the appropriate places throughout this manual.

The following safety conventions are used throughout this manual.


<p>WARNING</p> 	<p>The term WARNING is used to inform you about situations that could result in personal injury to you or other persons. Details about these circumstances are given in a box like this one.</p>
<p>CAUTION</p> 	<p>The term CAUTION is used to inform you about situations that could result in damage to the instrument or other equipment. Details about these circumstances are given in a box like this one.</p>

Before using the instrument, it is essential to read this manual carefully and to pay particular attention to any advice it contains concerning hazards that may arise from use of the instrument.


The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

Safety Information


1.1 Proper use

<p>WARNING</p> 	<p>Risk of personal injury and material damage [W1]</p> <p>Improper use of the BioRobot EZ1 DSP may cause personal injuries or damage to the instrument. The BioRobot EZ1 DSP must only be operated by qualified personnel who have been appropriately trained.</p> <p>Servicing of the BioRobot EZ1 DSP must only be performed by QIAGEN Instrument Service Specialists.</p>
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Use only QIAGEN® components, otherwise your right to make a claim under the guarantee may be invalidated. Carry out the maintenance regularly in accordance with the operating instructions. QIAGEN charges for repairs that prove to be required due to incorrect maintenance.

<p>CAUTION</p> 	<p>Damage to the instrument [C1]</p> <p>Avoid spilling water or chemicals onto the BioRobot EZ1 DSP. Instrument damage caused by water or chemical spillage will void your warranty.</p>
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In case of an emergency, switch off the BioRobot EZ1 DSP at the power switch on the rear of the instrument and unplug the power cord from the wall power outlet.

<p>CAUTION</p> 	<p>Damage to the instrument [C2]</p> <p>Ensure that the BioRobot EZ1 DSP is switched off before you manually move the mechanical components of the instrument.</p>
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1.2 Electrical safety

To ensure satisfactory and safe operation of the BioRobot EZ1 DSP, it is essential that the line power cord is connected to true electrical earth (ground).

Safety Information


<p>WARNING</p> 	<p>Electrical hazard [W2]</p> <p>Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous. Intentional interruption is prohibited.</p> <p>Lethal voltages inside the instrument</p> <p>When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.</p>
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When working with the BioRobot EZ1 DSP:

- Make sure the line power cord is connected to a line power outlet that has a protective conductor (earth/ground).
- Do not attempt to make any internal adjustments or replacements.
- Do not operate the instrument with any covers or parts removed.
- If water or reagent has spilled inside the instrument, switch off the instrument and disconnect it from the line power supply. Call QIAGEN Technical Services.
- Servicing should be carried out only by QIAGEN Instrument Service Specialists.
- If the instrument becomes electrically unsafe for use, make the instrument inoperative and secure it against unauthorized or unintentional operation. Call QIAGEN Technical Services.

The instrument is likely to be electrically unsafe when:

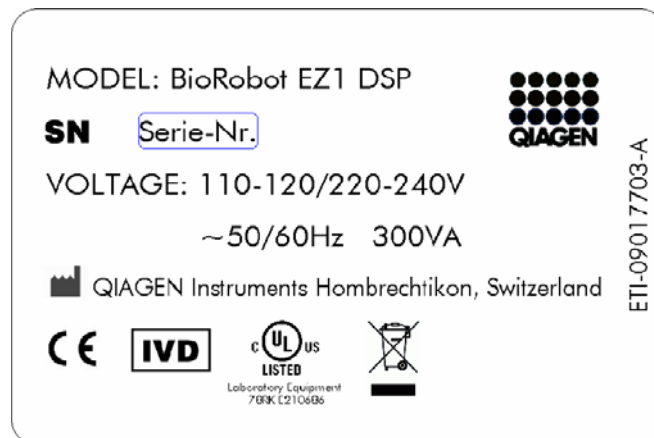
- It shows visible damage
- The line power cord shows signs of damage
- It has been stored under unfavorable conditions for a prolonged period
- It has been subjected to severe transport stresses

<p>WARNING</p> 	<p>Risk of electric shock [W3]</p> <p>Do not touch the BioRobot EZ1 DSP with wet hands.</p>
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Safety Information


Voltage rating label

The following label appears on the back of the BioRobot EZ1 DSP. Ensure that the voltage rating specified on the label matches the voltage available at the installation site.



1.3 Environment

Operating conditions

WARNING 	Explosive atmosphere [W4] The BioRobot EZ1 DSP is not designed for use in an explosive atmosphere.
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The BioRobot EZ1 DSP will operate correctly under the following conditions:

- Indoors
- Ambient temperature of 5–40°C (41–104°F)
- Ambient relative humidity of <80% at <31°C (88°F) (no condensation)

Storage conditions


If you intend to store the instrument for a prolonged period of time, unplug the power cord from the wall power outlet. The instrument can be stored at –25°C to 70°C (–13°F to 158°F).

1.4 Biological safety

Specimens and reagents containing materials from humans should be treated as potentially infectious. Use safe laboratory procedures as outlined in publications such as *Biosafety in Microbiological and Biomedical Laboratories*, HHS (www.cdc.gov/od/ohs/biosfty/biosfty.htm).

Samples

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents and should use, store, and dispose of such samples in accordance with the required safety regulations.

<p>WARNING</p> 	<p>Samples containing infectious agents [W5]</p> <p>Some samples used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations.</p> <p>Always wear safety glasses, two pairs of gloves, and a lab coat.</p> <p>The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe, and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Material Safety Data Sheets (MSDSs) or OSHA, ACGIH, or COSHH documents.</p> <p>Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.</p>
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OSHA: Occupational Safety and Health Administration (United States of America).

ACGIH: American Conference of Government Industrial Hygienists (United States of America).

COSHH: Control of Substances Hazardous to Health (United Kingdom).

Safety Information

1.5 Chemicals

<p>WARNING</p> 	<p>Hazardous chemicals [W6]</p> <p>Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run.</p> <p>Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDSs) or OSHA, ACGIH, or COSHH documents.</p> <p>Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.</p>
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OSHA: Occupational Safety and Health Administration (United States of America).

ACGIH: American Conference of Government Industrial Hygienists (United States of America).

COSHH: Control of Substances Hazardous to Health (United Kingdom).

Toxic fumes

If you work with volatile solvents, toxic substances, etc., you must provide an efficient laboratory ventilation system to remove vapors that may be produced.

1.6 Waste disposal



Used labware, such as reagent cartridges and filter-tips, may contain hazardous chemicals or infectious agents from the purification process. Such wastes must be collected and disposed of properly in accordance with the local safety regulations.

For disposal of waste electrical and electronic equipment (WEEE), see page A-4.

Safety Information


1.7 Mechanical hazards

The worktable of the BioRobot EZ1 DSP moves during operation of the instrument.

WARNING 	Moving parts [W7] Never open the workstation door while the BioRobot EZ1 DSP is operating.
WARNING 	Risk of overheating [W8] To ensure proper ventilation, maintain a minimum clearance of 15 cm and 30 cm at the rear and sides of the BioRobot EZ1 DSP, respectively. Slits and openings which ensure the ventilation of the BioRobot EZ1 DSP must not be covered.







1.8 Heat hazard

The BioRobot EZ1 DSP worktable contains a heating system.


WARNING 	Hot surface [W9] The heating system can reach high temperatures. Avoid touching it when it is hot.
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Safety Information



1.9 Symbols on the BioRobot EZ1 DSP

Symbol	Location	Description
	Heating system	Heat hazard — the temperature of the heating system can reach up to 95°C
	Near the tip rack	Biohazard — the tip rack may be contaminated with biohazardous material and must be handled with gloves
	Type plate on the back of the instrument	CE mark for Europe
	Type plate on the back of the instrument	UL listing mark for Canada and the USA
	Type plate on the back of the instrument	WEEE mark for Europe
	Type plate on the back of the instrument	In vitro diagnostic medical device

Safety Information

Symbol	Location	Description
SN	Type plate on the back of the instrument	Serial number
	Type plate on the back of the instrument	Legal manufacturer

1.10 Additional symbols in this user manual

Symbol	Location	Description
	Front cover	Catalog number
	Front cover	Handbook

Safety Information

Symbol on the Heating System



Symbol near the Tip Rack



2 Introduction

Thank you for choosing the QIAGEN BioRobot EZ1 DSP workstation. We are confident it will become an integral part of your laboratory.

Before using the instrument, it is essential to read this manual carefully and to pay particular attention to any advice it contains concerning hazards that may arise from use of this instrument.

2.1 About this user manual

This manual provides information about the BioRobot EZ1 DSP. It guides you systematically through the following sections:

1. Safety Information
 2. Introduction
 3. BioRobot EZ1 DSP — General Description
 4. Installation Procedures
 5. General Operation
 6. Preventive Maintenance
 7. Troubleshooting
 8. Glossary
- Appendices

The Appendices contain the following:

- Contact information for technical assistance
- Technical data
- Warranty terms

2.2 General information

2.2.1 Technical assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of

Introduction

QIAGEN products. If you have any questions regarding the BioRobot EZ1 DSP or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

2.2.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this publication. Please contact QIAGEN Technical Services.

2.2.3 Version management

This document is the *BioRobot EZ1 DSP Workstation User Manual* version 1, revision R1.

2.3 Intended use of the BioRobot EZ1 DSP

The BioRobot EZ1 DSP workstation is designed to perform automated purification of nucleic acids for in vitro diagnostic purposes. The system is intended for use by professional users, such as technicians and physicians trained in molecular biological techniques and the operation of the BioRobot EZ1 DSP. Any diagnostic results that are generated using the sample preparation procedure in conjunction with any downstream diagnostic assay must be interpreted in conjunction with other clinical or laboratory findings.

2.3.1 Requirements for BioRobot EZ1 DSP users

This table covers the general level of competence and training necessary for transportation, installation, use, maintenance, and servicing of the BioRobot EZ1 DSP.

Type of task	Personnel	Training and experience
Transportation	No special requirements	No special requirements
Installation	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Routine use (running protocols)	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Preventive maintenance	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Servicing and annual preventive maintenance	QIAGEN Instrument Service Specialists only	

Introduction

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3 General Description

The BioRobot EZ1 DSP workstation performs fully automated nucleic acid purification from up to 6 samples using magnetic particles. The automated steps include:

- Lysis of samples
- Binding of nucleic acids to magnetic particles
- Washing and elution of nucleic acids

The user inserts an EZ1 Card containing protocol(s) into the BioRobot EZ1 DSP. After starting worktable setup using the control panel, the user loads samples, reagent cartridges, filter-tips in tip holders, and elution tubes onto the BioRobot EZ1 DSP worktable. The user then closes the workstation door and starts the protocol. The protocol provides all the necessary instructions for the BioRobot EZ1 DSP to carry out automated nucleic acid purification.

The aspiration and dispensing of samples and reagents and the separation of magnetic particles are performed by the 6-channel pipettor head. The temperature of samples is regulated by a heating system.

General Description

3.1 External features of the BioRobot EZ1 DSP

Front View of the BioRobot EZ1 DSP Workstation



- | | | | |
|---|------------------|---|-------------|
| 1 | Workstation door | 4 | EZ1 Card |
| 2 | Control panel | 5 | Status LEDs |
| 3 | EZ1 Card slot | | |

3.1.1 Workstation door

The workstation door can be manually opened by the user in order to gain access to the worktable. A protocol cannot be started until the workstation door is closed.

3.1.2 Control panel

The control panel is the user interface that allows the user to operate the BioRobot EZ1 DSP. It consists of a keypad and a liquid-crystal display (LCD).

General Description

Keypad

The following keys of the control panel are available to the user.

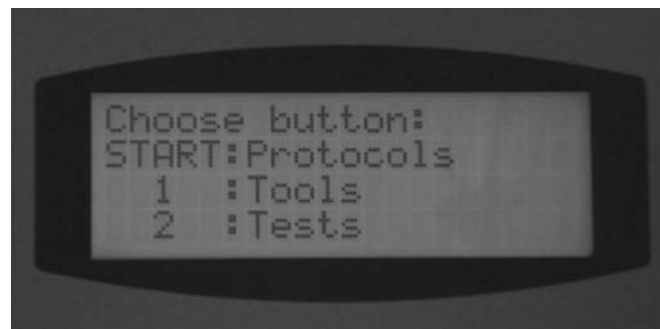
Key	Description
0–9	Selects a menu.
START	Initiates an action or starts a protocol.
STOP	Interrupts a protocol run.
ESC	Displays the previous menu or text.

The other keys of the control panel are for service personnel only.

Liquid-crystal display

The LCD consists of 4 lines. There are 20 spaces per line.

Liquid-Crystal Display



3.1.3 EZ1 Card slot

The EZ1 Card slot accepts an EZ1 Card. Each EZ1 Card contains one or more protocols that allow the BioRobot EZ1 DSP to perform nucleic acid isolation.

Note: The workstation should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted! Otherwise essential instrument data

General Description

could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the workstation is switched on. Do not remove the EZ1 Card while the machine is switched on. Also, take care not to expose the EZ1 Card to electric shock, water, or dirt.

EZ1 Card Completely Inserted into EZ1 Card Slot



3.1.4 Status LEDs

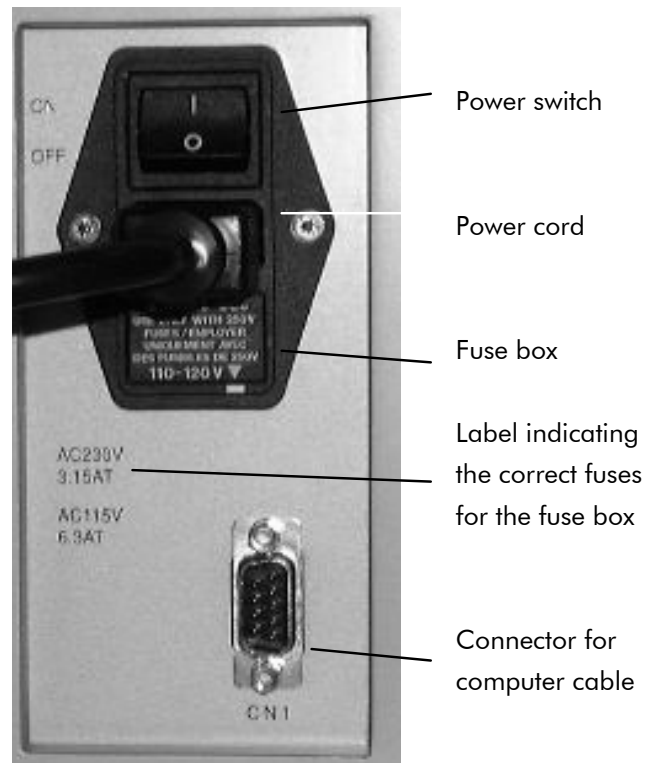
There are 2 light-emitting diodes (LEDs) at the front of the BioRobot EZ1 DSP:

- Green LED — indicates that the instrument is receiving power
- Red LED — indicates that an error has occurred

General Description

3.1.5 Connector panel

The connector panel is located at the rear of the BioRobot EZ1 DSP.

Connector Panel

It contains the power switch, the socket for the power cord, the fuse box, and a connector for a computer cable (for service use only).

3.2 Internal features of the BioRobot EZ1 DSP

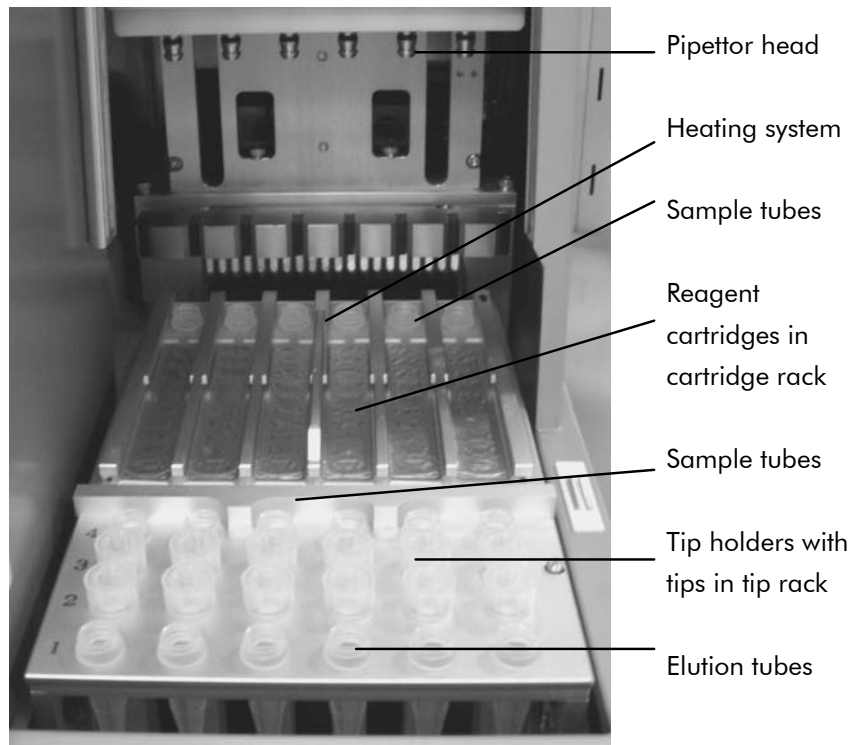
The interior of the BioRobot EZ1 DSP contains the worktable and the pipettor head.

General Description

3.2.1 Worktable

The worktable contains various racks for holding the labware, samples, and reagent cartridges that are required for the protocol run. The worktable also contains a heating system for controlling the temperature of samples.

The BioRobot EZ1 DSP Worktable

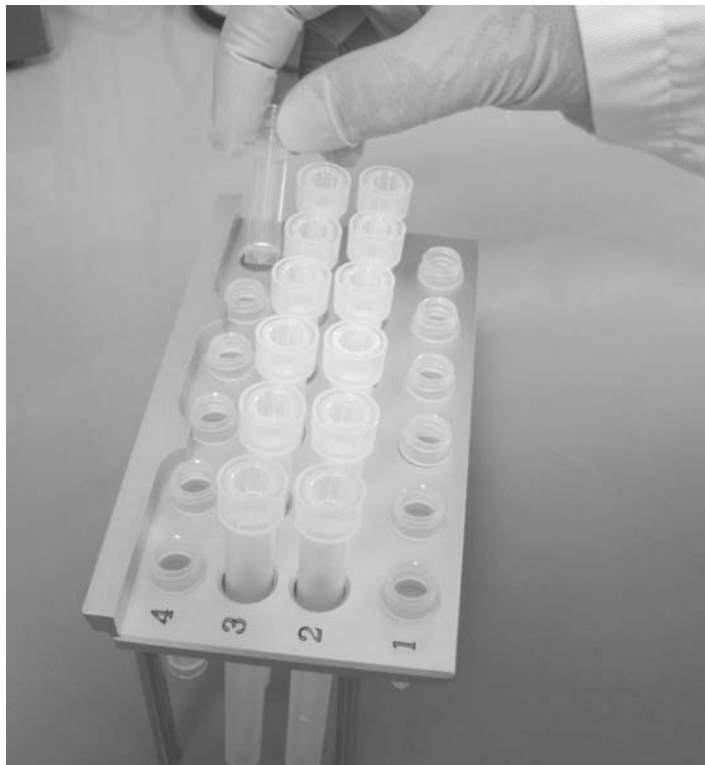


Tip rack

This rack is located in the front of the worktable. The front row holds up to 1.5 ml elution tubes. The recommended elution tubes are screw-capped, made of polypropylene, and supplied by Sarstedt (cat. no. 72.692).

The next 2 rows hold up to 12 tip holders containing filter-tips. (Note: Some protocols require only one row of filter-tips.) The remaining row holds up to 6 sample tubes. Sample tubes are 2 ml in volume, screw-capped, made of polypropylene, and available from Sarstedt (cat. no. 72.693).

General Description

Tip Rack

Both tip holders and filter-tips are made of polypropylene and available from QIAGEN. The filter-tips have a capacity of 50–1000 μ l.

Tip Holders and Filter-tips

General Description

Cartridge rack

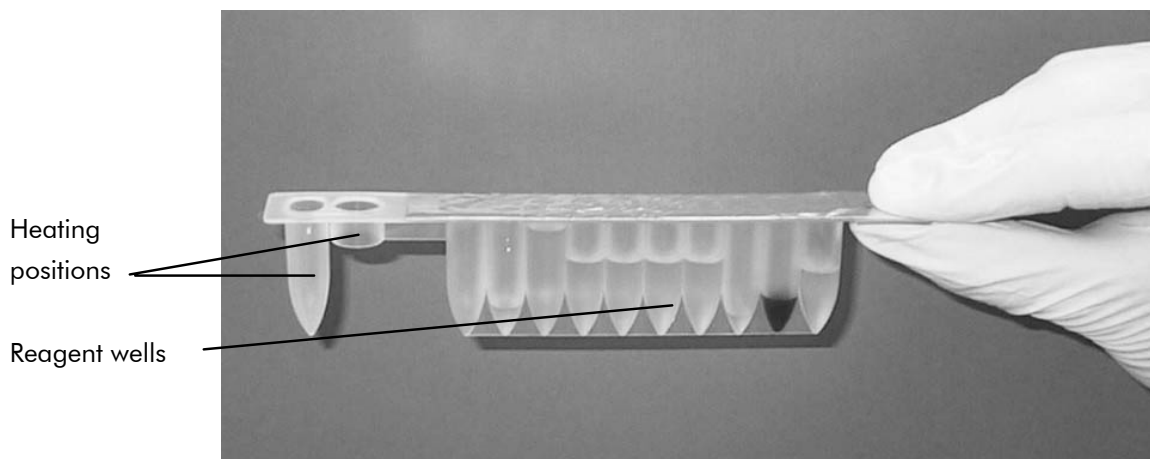
This rack is located behind the tip rack and holds up to 6 reagent cartridges.

Cartridge Rack



Reagent cartridges are made of polypropylene, contain prefilled reagents, and are available from QIAGEN.

Reagent Cartridges



A reagent cartridge consists of 10 reagent wells and 2 heating positions. One heating position is a well, the other is a slot that can accept a tube.

General Description

Heating system

The heating system is located under the far end of the cartridge rack. It holds the heating positions of the reagent cartridges.

Tray

The tray is located under the racks and collects any drops of liquid that may fall.

Tray**3.2.2 Pipettor head**

The pipettor head is mounted above the worktable and moves in the Z direction (i.e., up and down) in order to reach the samples and reagents on the worktable. The worktable itself moves in the Y direction (i.e., front to back) in order to present different samples and reagents under the pipettor head.

The pipettor head contains 6 high-precision syringe pumps that are connected to tip adapters that can be attached to filter-tips. The syringe pumps operate simultaneously to allow aspiration or dispensing of small volumes of liquid (50–1000 μ l) via the filter-tips.

The pipettor head also contains a magnet whose distance from the attached filter-tips can be varied. This feature allows the capture of magnetic particles that are present in the liquid aspirated into the filter-tips.

Located behind the tip adapters is the piercing unit, a row of 6 metal spikes for puncturing the foil that covers the reagent cartridges.

General Description

During operation of the BioRobot EZ1 DSP, the piercing unit first exposes the reagents in the reagent cartridges. The pipettor head then picks up filter-tips from the tip rack, and performs aspiration and dispensing operations at different locations on the worktable before ejecting the tips back into the tip rack.

4 Installation Procedures

The BioRobot EZ1 DSP is a plug-and-play instrument, making unpacking and installation easy. A member of your group who is familiar with laboratory equipment should oversee the installation.

4.1 Requirements

Site

The BioRobot EZ1 DSP must be located out of direct sunlight, away from heat sources, and away from sources of vibration and electrical interference. Refer to Appendix A for the operating conditions (temperature and humidity).

Use a level workbench that is large and strong enough to accommodate the BioRobot EZ1 DSP. Refer to Appendix A for the weight and dimensions of the BioRobot EZ1 DSP.

The BioRobot EZ1 DSP must be placed near to a properly grounded (earthed) AC power outlet. The power line to the instrument should be voltage regulated and surge protected.

4.2 AC power connection

Power requirements

The BioRobot EZ1 DSP operates at:

- 200–240 V AC \pm 10%, 50/60 Hz, 300 VA
- 100–120 V AC \pm 10%, 50/60 Hz, 300 VA

Make sure the voltage rating of the BioRobot EZ1 DSP is compatible with the AC voltage available at the installation site.

Grounding requirements

To protect operating personnel, the BioRobot EZ1 DSP must be correctly grounded (earthed). The workstation is equipped with a 3-conductor AC power cord that, when connected to an appropriate AC power outlet, grounds (earths) the

Installation Procedures

workstation. To preserve this protection feature, do not operate the workstation from an AC power outlet that has no ground (earth) connection.

Installation of AC power cord

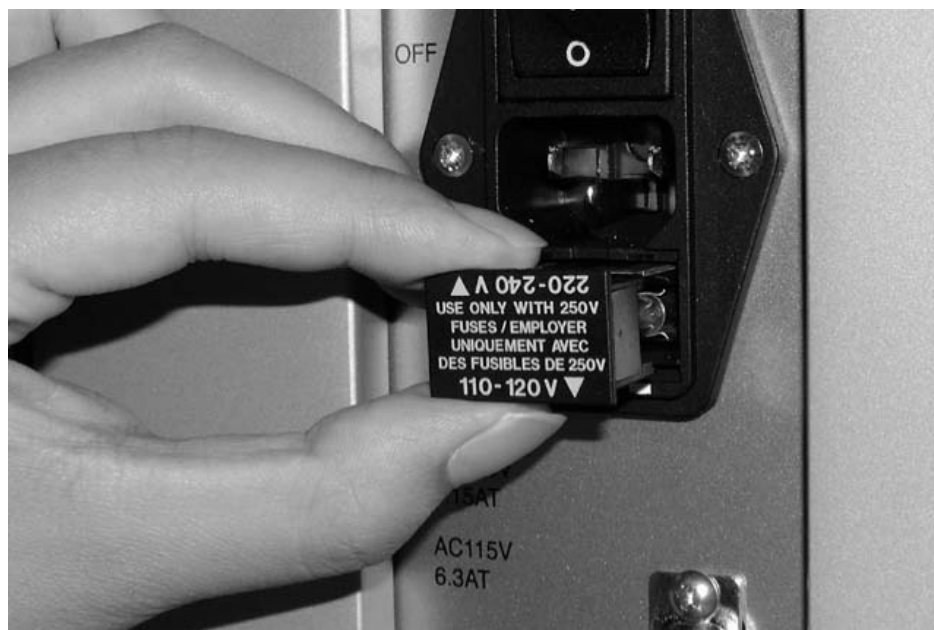
Connect one end of the AC power cord to the socket located on the rear of the BioRobot EZ1 DSP, and the other end to the AC power outlet.

The fuse box of the BioRobot EZ1 DSP is located under the socket for the power cord and contains 2 fuses which are labeled as follows:


- **110–120 V** — a 6.3 A (250 V) fuse compatible with 110–120 V power supplies
- **220–240 V** — a 3.15 A (250 V) fuse compatible with 220–240 V power supplies

Before connecting the instrument to the power supply, you may need to rotate the fuse box in order to select the correct fuse. A fuse is correctly selected when its label is readable and closer to the bottom of the instrument.

Selecting the Correct Fuse for a 110–120 V Power Supply



Installation Procedures

WARNING 	Electrical hazard [W12] Never install a fuse different from that specified in the user manual.
---	---

4.3 Hardware installation

The following items are delivered:

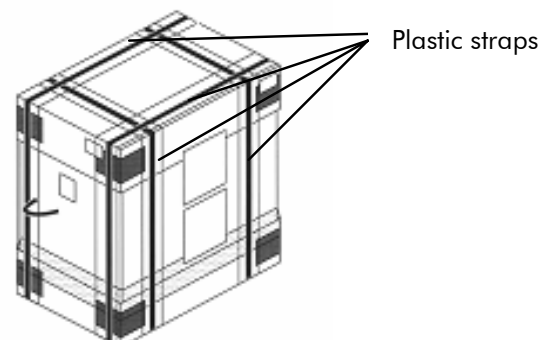
- BioRobot EZ1 DSP workstation
- Power cord (8 pieces)
- BioRobot EZ1 DSP accessories
- *BioRobot EZ1 DSP Workstation User Manual*
- 6GC teaching data sheet

The BioRobot EZ1 DSP accessories include:

- Cartridge rack
- Tip rack
- Tray
- O-ring (pack of 6) and silicon grease
- Fuses (1 each: 6.3 A and 3.15 A)

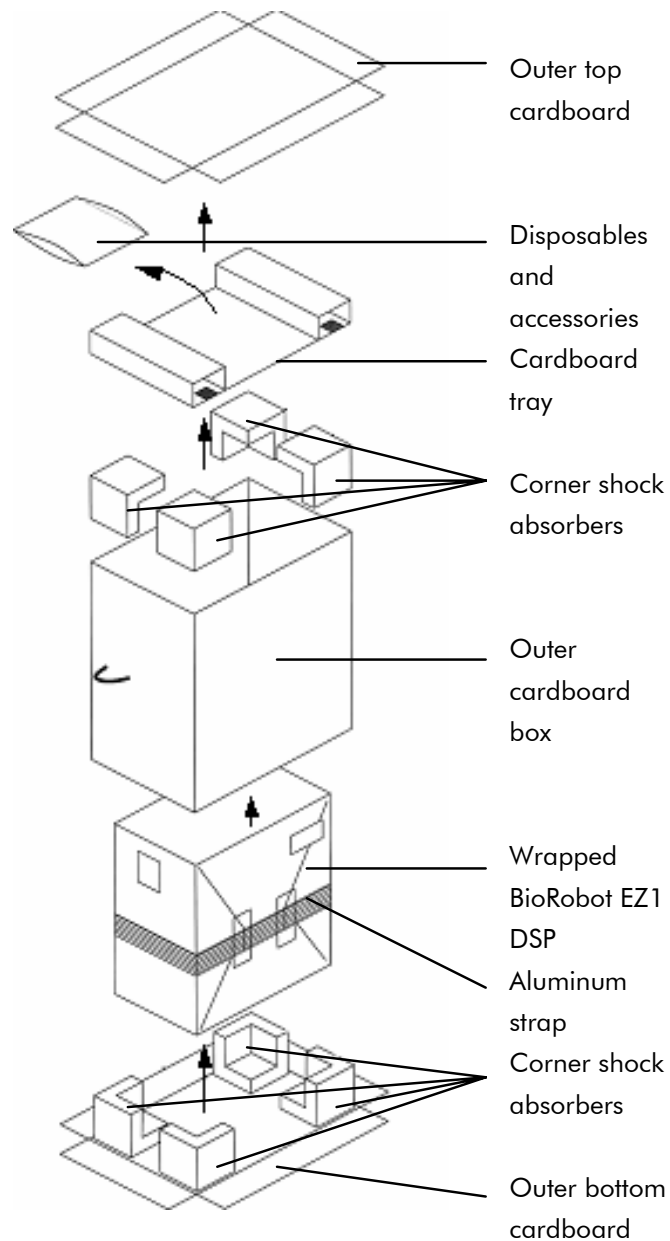
Unpacking the BioRobot EZ1 DSP

1. Cut and remove the plastic straps.



Installation Procedures

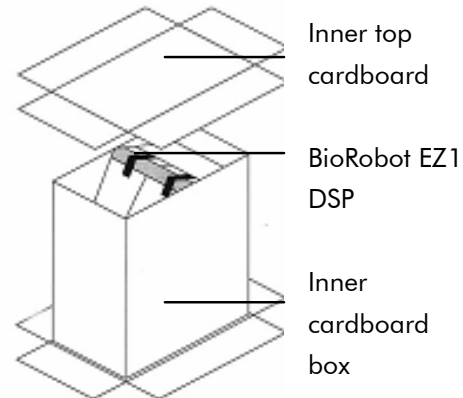
2. Remove the outer top cardboard.



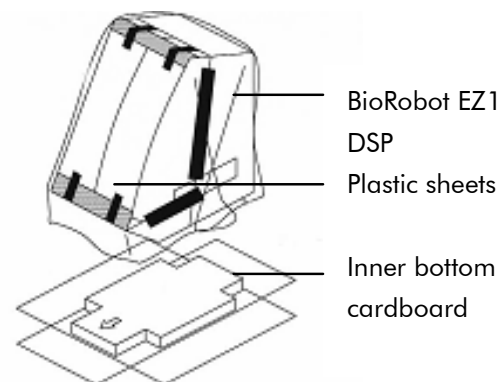
3. Take out the packages with the disposables and accessories.
4. Remove the cardboard tray.
5. Pull out the 4 corner shock absorbers at the top, and remove the outer cardboard box by pulling it up.
6. Take out the wrapped BioRobot EZ1 DSP, and remove the aluminum strap.

Installation Procedures

7. Remove the inner top cardboard, and remove the BioRobot EZ1 DSP from the inner cardboard box.



8. Remove all plastic sheets.



Note: Be careful not to damage the surface of the BioRobot EZ1 DSP after removing the plastic sheets.

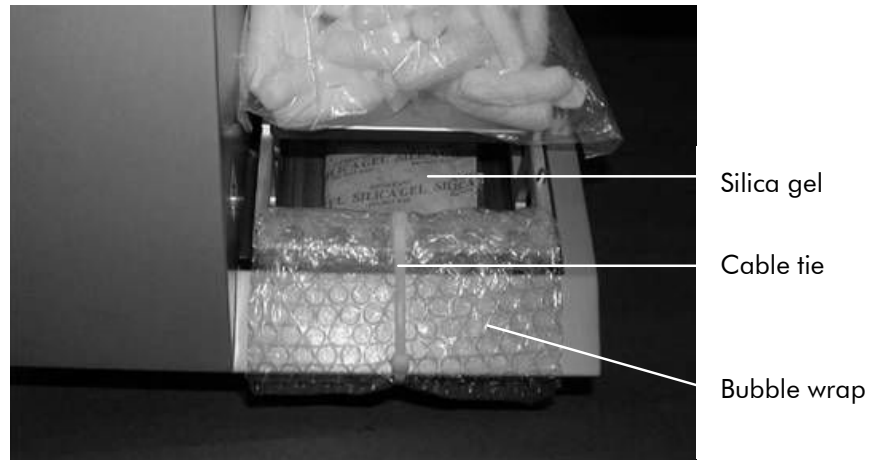
Note: It is recommended to save the original packaging material for later transportation of the BioRobot EZ1 DSP.

Removing the protectors for the Y- and Z-axes

During transportation, protectors prevent the moveable parts of the BioRobot EZ1 DSP from moving along the Y- and Z-axes. Before using the instrument, these protectors must be removed.

Installation Procedures

1. Cut and remove the cable tie.



2. Remove the bubble wrap and the silica gel.
3. Push the pipettor head upward.



4. Remove the bag of foam packaging material.



Installation Procedures

Note: It is recommended to save the original packaging material for later transportation of the BioRobot EZ1 DSP.

Installing the BioRobot EZ1 DSP

1. Adjust the fuse box if necessary (Section 4.2, page 4-1).
2. Connect the BioRobot EZ1 DSP to the power cord.

Installation Procedures

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5 General Operation

This section describes how to operate the BioRobot EZ1 DSP.

Before proceeding, it is recommended that you familiarize yourself with the features of the BioRobot EZ1 DSP by referring to Section 3.

5.1 Overview

The steps for operating the BioRobot EZ1 DSP are presented below. Further details are provided later in this section.

1. Insert the EZ1 Card completely into the EZ1 Card slot.
2. Switch on the BioRobot EZ1 DSP.
3. Press "START" to start worktable setup, and press any key to proceed through the messages displayed by the LCD.
4. Open the workstation door.
5. Set up the worktable according to the messages displayed by the LCD:
 - Load the reagent cartridges into the cartridge rack; then load the rack onto the worktable
 - Load elution tubes into the tip rack
 - Load filter-tips and sample tubes into the tip rack; then load the rack onto the worktable
6. Close the workstation door.
7. Press "START" to start the protocol.
8. At the end of the protocol run:
 - Press "STOP" twice if you do not want to run another protocol
 - Press "ESC" if you want to run another protocol
9. Remove the purified nucleic acid samples.
10. Clean the BioRobot EZ1 DSP.
11. Run another protocol or switch off the BioRobot EZ1 DSP.

General Operation

5.2 Inserting and removing the EZ1 Card

CAUTION**Damage to the instrument**

[C3]

Ensure that the BioRobot EZ1 DSP is switched off before you insert or remove the EZ1 Card. Otherwise, the card and/or the instrument may be damaged.

5.2.1 Inserting the EZ1 Card

1. Lift up the cover of the EZ1 Card slot.



2. Insert the EZ1 Card into the EZ1 Card slot.



Orientate the card so that the picture faces to the left and the triangle symbol is at the bottom.

General Operation

Ensure that the card is completely inserted into the slot.



It is extremely important that the EZ1 Card is completely inserted to avoid memory error and loss of essential instrument data.

3. Close the cover of the EZ1 Card slot.
4. Switch on the BioRobot EZ1 DSP (Section 5.3.1, page 5-4).

Note: Do not remove the EZ1 Card while the machine is switched on.

5.2.2 Removing the EZ1 Card

1. Switch off the BioRobot EZ1 DSP (Section 5.3.2, page 5-5).
2. Lift up the cover of the EZ1 Card slot.

General Operation

3. Press the button at the bottom of the EZ1 Card slot.

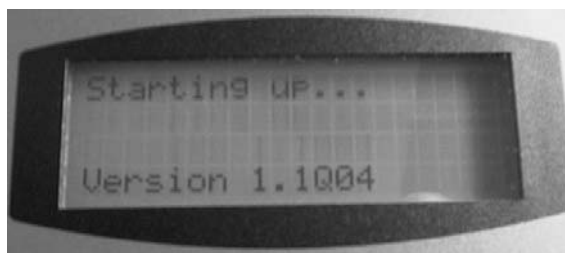


The EZ1 Card is ejected.

5.3 Switching the BioRobot EZ1 DSP on and off

5.3.1 Switching on the BioRobot EZ1 DSP

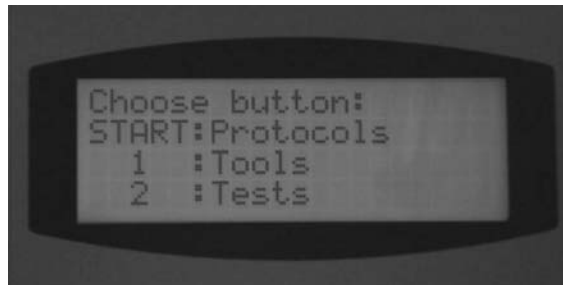
1. Insert the EZ1 Card (Section 5.2.1, page 5-2).
2. Switch on the BioRobot EZ1 DSP at the rear power switch.
3. The LCD displays the following text for a few seconds.



This text indicates that the BioRobot EZ1 DSP is initializing. All motors move to their home positions.

General Operation

4. After initialization, the main menu appears.




You can now operate the BioRobot EZ1 DSP.

5.3.2 Switching off the BioRobot EZ1 DSP

1. If you had run a protocol, refer to “End of the protocol run” on page 5-7.
2. Switch off the BioRobot EZ1 DSP at the rear power switch.

5.4 Opening and closing the workstation door

<p>WARNING</p> 	<p>Moving parts [W7]</p> <p>Never open the workstation door while the BioRobot EZ1 DSP is operating.</p>
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General Operation

5.4.1 Opening the workstation door

1. Slide your fingers under the door and push it upward.



2. Rest the door on the door lock that is located near the top of the BioRobot EZ1 DSP.

5.4.2 Closing the workstation door

1. Press the door down to disengage the door lock.
2. Gradually lower the door until it rests on the worktable. Take care that your fingers do not get caught between the door and worktable.

5.5 Starting and stopping a protocol run

WARNING



Moving parts

[W7]

Never open the workstation door while the BioRobot EZ1 DSP is operating.

5.5.1 Starting a protocol run

After you have inserted the EZ1 Card and switched on the BioRobot EZ1 DSP, start a protocol as follows:

1. Press "START" on the control panel.

General Operation

2. Start worktable setup by pressing the appropriate number key (e.g., "1" or "2").
For details, refer to the handbook supplied with the EZ1 DSP Kit you are using.
3. Follow the instructions displayed by the LCD.
4. Open the workstation door and set up the worktable according to the messages displayed by the LCD (Section 5.6, page 5-8).
5. Close the workstation door.
The protocol run cannot start until the door is closed.
6. Press "START" to start the protocol run.

End of the protocol run

After a protocol run ends, the LCD displays "Finished":

1. Press "STOP" twice if you are not running another protocol. The LCD displays the main menu and the motors return to their home positions.
Alternatively, press "ESC" if you are running another protocol. The LCD displays "Protocols", from which you can start the next worktable setup.
2. Check that the BioRobot EZ1 DSP is no longer operating. Then open the workstation door and remove the elution tubes, which contain the purified nucleic acid samples.
3. Clean the workstation (Section 6, page 6-1).
4. Run another protocol or switch off the BioRobot EZ1 DSP.

5.5.2 Stopping a protocol run

When a protocol is running, you have the option of either pausing or canceling it:

General Operation

1. Press "STOP". The protocol run pauses and the LCD displays the following text.



2. To continue the protocol run, press "START".
The protocol run continues from where it had stopped.
3. To cancel the protocol run, press "STOP". The LCD displays the main menu.

Then follow these steps:

- Ensure that the workstation door is closed
- Press "1" to display "Manual" in the LCD



- Press "2" to return the tips to the tip rack and to return the motors to their home positions
- When "Manual" reappears in the LCD, press "ESC" to redisplay the main menu
- Another protocol can now be run

5.6 Setting up the worktable

Note: For specific details about setting up the worktable, refer to the onscreen instructions, which are also included in the handbook supplied with the EZ1 DSP Kit you are using.

5.6.1 Removing and replacing the tray

The tray is located under the worktable and can be removed for cleaning:

1. Switch off the BioRobot EZ1 DSP.
2. Push the worktable toward the back of the BioRobot EZ1 DSP.
3. Remove the tray using its handle.



Reverse this procedure to replace the tray. To avoid instrument damage, ensure that the tray is positioned correctly.

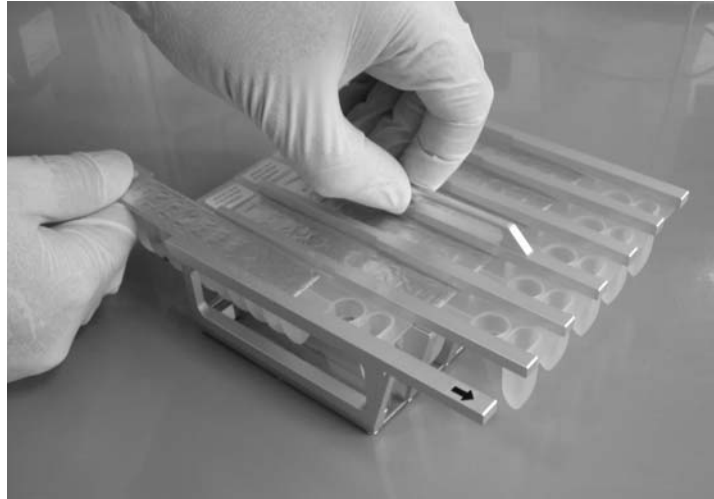
5.6.2 Loading the reagent cartridges

Note: Do not remove the foil from the reagent cartridges.

1. Invert the reagent cartridges several times to mix the magnetic particles.
2. Tap the reagent cartridges until the reagents are deposited at the bottom of the wells.
3. Remove the cartridge rack from the worktable.

General Operation

- Slide the reagent cartridges into the cartridge rack in the direction of the arrow, as shown below, until you feel resistance.



Press down the cartridges until they click into place.

- Return the cartridge rack to the worktable.



Ensure that the 2 heating positions of each cartridge fit into the heating system.

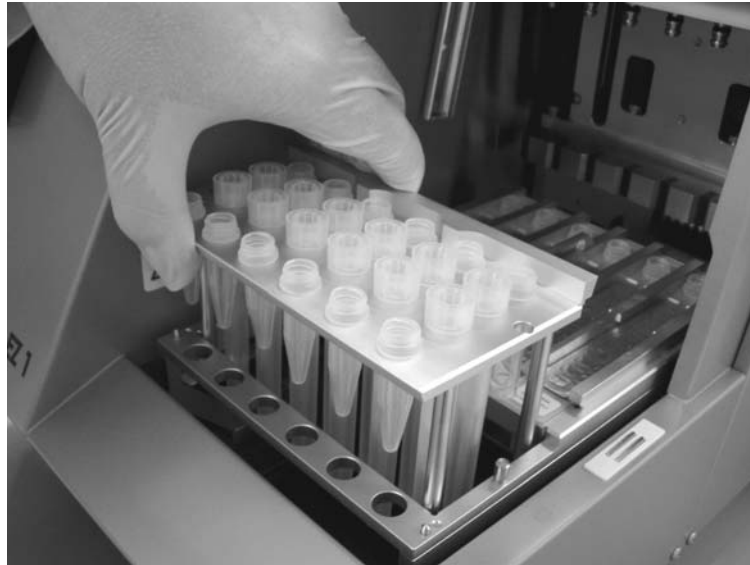
5.6.3 Loading the elution tubes, filter-tips, and sample tubes

1. Remove the tip rack from the worktable.
2. Load the elution tubes into row 1 of the tip rack.
Label the elution tubes before you load them into the rack. Ensure that the caps of the tubes are removed before you start the protocol.
3. Place the filter-tips into the tip holders.
Ensure that the tips are positioned correctly in the holders.
4. Load the tip holders into rows 2 and 3 of the tip rack.
Note: Some protocols require only one row of filter-tips. Please follow the messages displayed by the LCD.
5. Load the sample tubes into row 4 of the tip rack.
Ensure that the caps of the tubes are removed before you start the protocol.



General Operation

6. Return the tip rack to the worktable.




Ensure that the tips, tip holders, and tip rack are correctly positioned on the worktable.

6 Preventive Maintenance

The following preventive maintenance procedures must be carried out to ensure reliable operation of the BioRobot EZ1 DSP:

- **Regular preventive maintenance** — after each protocol run
- **Daily preventive maintenance** — after the last protocol run of the day
- **Weekly preventive maintenance** — every week
- **Annual preventive maintenance** — every year; removal and replacement of parts are carried out by QIAGEN Instrument Service Specialists only

WARNING 	Risk of electric shock [W10] Do not open any panels on the BioRobot EZ1 DSP. Risk of personal injury and material damage Only perform maintenance which is specifically described in this manual.
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Servicing



Each BioRobot EZ1 DSP workstation is supplied with a one-year warranty that includes all repairs due to mechanical breakdown. Worldwide, the maximum time for response to a breakdown is 5 days. Application development, software upgrades, worktable accessories, disposable items, and replacement of spare parts such as syringes, tubing, and pipet tips are not included in the warranty.

QIAGEN offers comprehensive Service Support Agreements, including IQ/OQ, Warranty Extensions, Full Cover Support Agreements, and Preventive Maintenance Agreements. Service Support Agreements ensure high performance from your workstation. In addition, service histories are fully documented and all parts are certified and guaranteed.

Contact your local QIAGEN Instrument Service representative, or your local distributor for more information about flexible Service Support Agreements from QIAGEN.

Preventive Maintenance

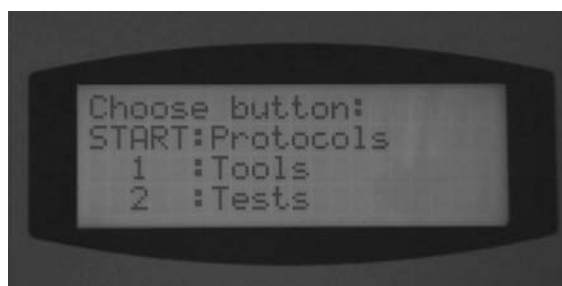
6.1 Regular maintenance procedure

<p>WARNING</p> 	<p>Risk of personal injury and material damage [W1]</p> <p>Improper use of the BioRobot EZ1 DSP may cause personal injuries or damage to the instrument. The BioRobot EZ1 DSP must only be operated by qualified personnel who have been appropriately trained.</p> <p>Servicing of the BioRobot EZ1 DSP must only be performed by QIAGEN Instrument Service Specialists.</p>
<p>WARNING</p> 	<p>Hazardous chemicals and infectious agents [W11]</p> <p>The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly. Refer to your local safety regulations for proper disposal procedures.</p>

If working with potentially infectious materials, such as human blood, serum, or plasma, the BioRobot EZ1 DSP system should be decontaminated after use (Section 6.4, page 6-6).

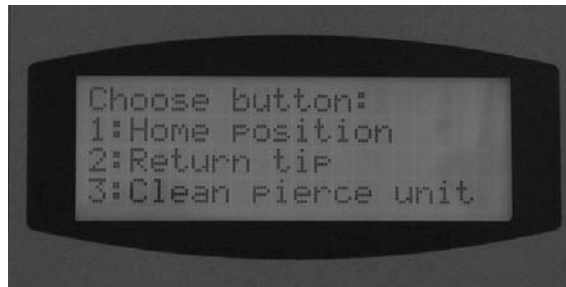
After running a protocol, clean the piercing unit of the pipettor head:

1. Remove used disposable labware and unwanted samples and reagents. Discard them according to your local safety regulations.
2. In the main menu, press "1" to display "Tools".



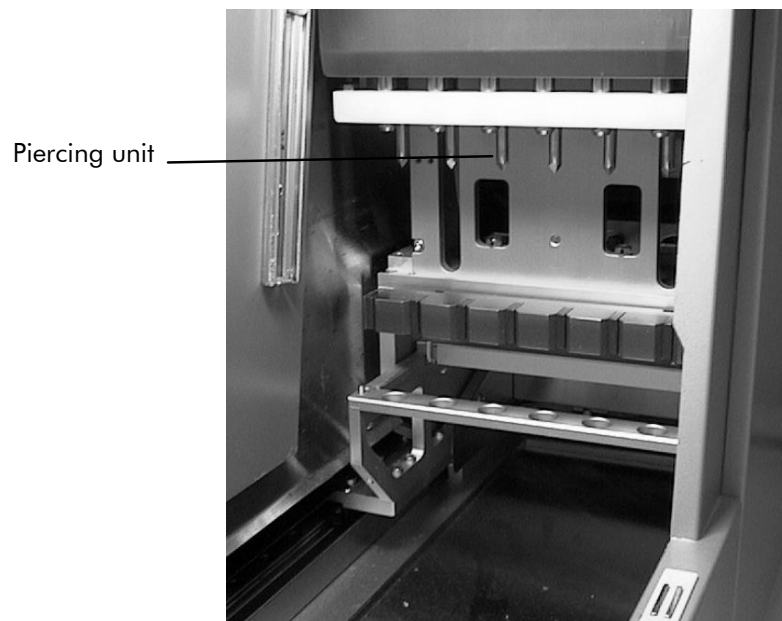
Preventive Maintenance

3. Press "3" to select "Clean pierce unit".

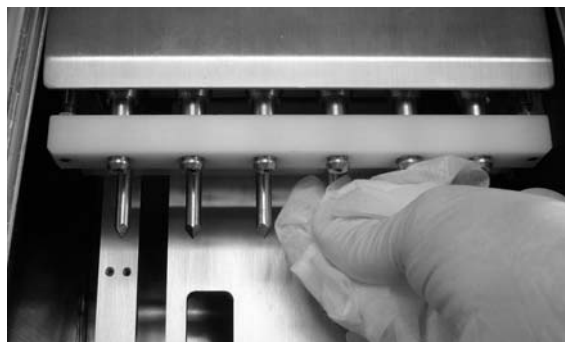


The piercing unit moves downward.

4. Wipe the piercing unit using a soft tissue moistened with 70% ethanol. The piercing unit is sharp. Double gloves are recommended.




5. Wipe the piercing unit using a soft tissue moistened with distilled water.



Preventive Maintenance

6. Press "ESC" to return the piercing unit to its original position.
 7. Clean the tray and racks with 70% ethanol and then with distilled water.
 8. Clean the BioRobot EZ1 DSP worktable with 70% ethanol and then with distilled water.
 9. Wipe the other surfaces of the worktable with a diluted neutral soap solution and then with distilled water.
- You can now run another protocol or switch off the BioRobot EZ1 DSP.

6.2 Daily maintenance procedure

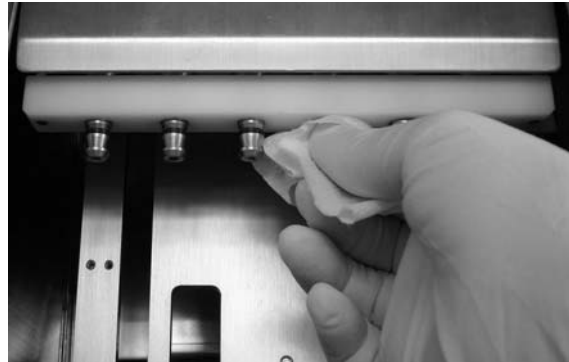
<p>WARNING</p> 	<p>Hazardous chemicals and infectious agents [W11]</p> <p>The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly. Refer to your local safety regulations for proper disposal procedures.</p>
--	--

After running the last protocol of the day, perform the daily maintenance procedure:

1. Clean the piercing unit (Section 6.1, page 6-2).
2. Remove used disposable labware and unwanted samples and reagents, and discard them according to your local safety regulations.
3. Check that the tray is clean. If necessary, clean it with 70% ethanol, and then with distilled water.
4. Clean the worktable and its racks with 70% ethanol, and then with distilled water.
5. Wipe the other surfaces of the workstation with diluted neutral soap solution, and then with water.

Preventive Maintenance

6. Wipe the O-rings of the tip adapters with tissue.

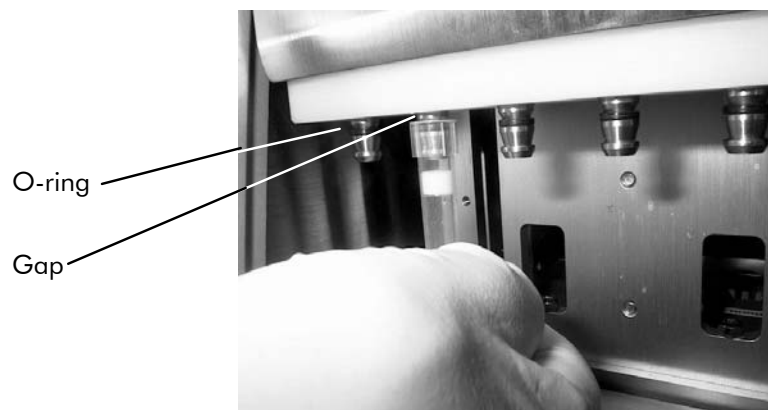


6.3 Weekly maintenance procedure

Perform the daily maintenance procedure before you perform the weekly maintenance procedure.

To maintain good contact between tip adapters and filter-tips and to prevent liquid leaking from the tips, grease the O-rings of the tip adapters every week:

1. Apply a small amount of silicon grease to the end of a filter-tip.
2. Apply the silicon grease to the surface of the O-rings.
3. Place the tip onto the pipettor head, and rotate the tip on the pipettor head to distribute the silicon grease evenly.




Note: The filter-tips should sit flush against the upper white plastic bar if the O-rings are properly greased. There should not be a gap. Excess or insufficient grease can affect the performance of the BioRobot EZ1 DSP.

Preventive Maintenance

6.4 Reagents for decontamination

The following disinfectants and detergents are compatible with surfaces and components of the BioRobot EZ1 DSP system. Use according to manufacturers' instructions for effective disinfection.

WARNING 	Toxic fumes [W13] Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers can produce toxic fumes.
---	---

- **Mikrozyd® Liquid** (Schülke & Mayr GmbH; www.schuelke-mayr.com)* — ethanol-based disinfectant for cleaning surfaces, such as the worktable (consists of 25 g ethanol and 35 g 1-propanol per 100 g Mikrozyd Liquid)
- **Lysetol® AF or Gigasept® Instru AF** (Schülke & Mayr GmbH)* — quaternary ammonium solution for submerging worktable items, such as holders (consists of 14 g cocosporylene-diamine-guanidine diacetate, 35 g phenoxypropanols, and 2.5 g benzalkonium chloride per 100 g, with anticorrosion components, fragrance, and 15–30% nonionic surfactants)

Note: If you want to use disinfectants different from those recommended, ensure that their compositions are similar to those described above. A suitable alternative to Mikrozyd Liquid is Incidin Liquid (EcoLab; www.ecolab.com). A suitable alternative to Lysetol AF or Gigasept Instru AF is DECON-QUAT® 100 (Veltek Associates, Inc.; www.sterile.com).*

Note: Do not spray cleaning or disinfectant liquids onto surfaces of the BioRobot EZ1 DSP. Spray bottles should be used only for items that have been removed from the workstation.

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

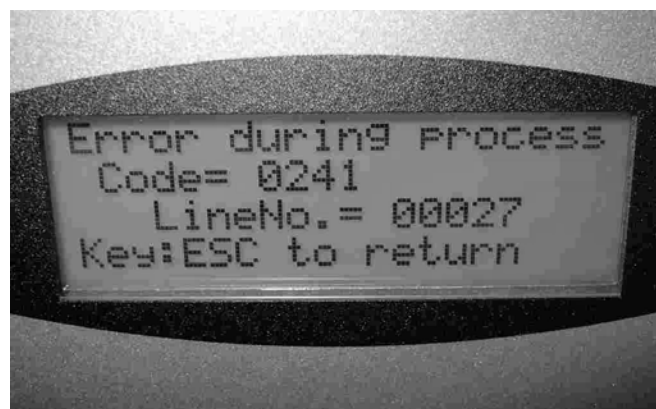
7 Troubleshooting

7.1 Detecting an error

If a protocol run is interrupted due to an error:

- The red LED flashes
- An alarm sounds
- The LCD displays an error message

LCD Displaying an Error Message



The second line of the error message gives the error code (see list below). The third line indicates the line number of the protocol at which the error occurred.

Record the error code and the line number, and contact QIAGEN Technical Services. Then reset the BioRobot EZ1 DSP:

1. Press "ESC" to display the main menu in the LCD.
2. Ensure that the workstation door is closed.
3. Press "1" to display "Manual" in the LCD.
4. Press "2" to return the tips to the tip rack and to return the motors to their home positions.
5. When "Manual" reappears in the LCD, press "ESC" to redisplay the main menu.

Another protocol can now be run.

Note: It is not possible to continue a protocol run that has been interrupted due to an error.

Troubleshooting

7.2 Error codes

Error code	Description
------------	-------------

10	Failed to return to origins, protocol cannot run
11	Limit error, protocol cannot run
12	Failed to return to Z axis, protocol in run
13	Failed to return to P axis, protocol in run
14	Failed to return to M axis, protocol in run
15	Failed to return to Y axis, protocol in run
16	Z axis limit error, protocol in run
19	Y axis end limit, protocol in run
20	Z axis time-out, protocol in run
21	P axis time-out, protocol in run
22	M axis time-out, protocol in run
23	Y axis time-out, protocol in run
24	Open door in motion
25	Abnormal input from bottom sensor in motion
26	Failed to initialize heating block
27	Failed to initialize motion control board
29	Memory error

8 Glossary

Term	Description
Cartridge rack	A metal rack that accommodates reagent cartridges on the worktable.
Connector panel	The panel on the rear of the BioRobot EZ1 DSP. It contains the power switch, the socket for the power cord, the fuse box, and a connector for a computer cable.
Control panel	The user interface that allows the user to operate the BioRobot EZ1 DSP. The control panel consists of an LCD and a keypad.
Elution tube	A polypropylene, screw-capped 1.5 ml tube for collecting purified nucleic acids.
Error code	A 1 or 2 digit number that indicates a particular error of the BioRobot EZ1 DSP.
EZ1 Card	A card that contains one or more protocols for the BioRobot EZ1 DSP and is inserted into the instrument.
EZ1 Card slot	A slot at the front of the BioRobot EZ1 DSP that accepts an EZ1 Card.
Filter-tip	An item of labware that is picked up by a tip adapter during operation of the BioRobot EZ1 DSP. Liquid is aspirated into and dispensed from a filter-tip. A filter-tip is also the location where separation of magnetic particles occurs.
Heating system	A component of the BioRobot EZ1 DSP that accommodates the heating positions of the reagent cartridges and heats samples.

Glossary

Term	Description
O-ring	A ring that is fitted at the bottom of a tip adapter. It is required for good contact between the tip adapter and a filter-tip.
Pipettor head	The component of the BioRobot EZ1 DSP that aspirates and dispenses liquid and separates magnetic particles. The pipettor head moves up and down above the worktable and contains 6 syringe pumps, each of which is connected to a tip adapter.
Protocol	A set of instructions for the BioRobot EZ1 DSP that allows the instrument to automate a nucleic acid purification procedure. Protocols are run using the control panel.
Reagent cartridge	An item of labware that contains 10 wells and 2 heating positions. One heating position is a well, the other is a slot that can accept a tube. A reagent cartridge is prefilled with reagents.
Sample tube	A polypropylene, screw-capped 2 ml tube for holding a sample containing nucleic acids to be purified.
Tip adapter	One of 6 metal probes installed on the pipettor head. During operation of the BioRobot EZ1 DSP, the tip adapters pick up filter-tips from the worktable.
Tip holder	A polypropylene tube that holds a single filter-tip. Tip holders are loaded onto the tip rack.
Tip rack	A metal rack that accommodates tip holders containing filter-tips on the worktable. The tip rack also accommodates sample tubes and elution tubes.
Tray	A metal tray is located under the worktable. It collects any drops of liquid that may fall.

Glossary

Term	Description
Workstation door	The main door at the front of the BioRobot EZ1 DSP. When open, it provides complete access to the worktable.
Worktable	The surface of the BioRobot EZ1 DSP that contains racks and is where samples, reagent cartridges, and disposable labware are loaded. The worktable moves backwards and forwards to present different samples and reagents under the pipettor head.

Glossary

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Appendix A

Technical data

Environmental conditions

Power 100–120/200–240 V AC \pm 10%, 50/60 Hz, 300 VA

Fuses 6.3 A (250 V) (for 110–120 V AC)
3.15 A (250 V) (for 220–240 V AC)

WARNING 	Electrical hazard [W12] Never install a fuse different from that specified in the user manual.
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Operating temperature 5–40°C (41–104°F)

Humidity Maximum 80% relative humidity for temperatures up to 31°C (88°F), decreasing linearly to 50% humidity at 40°C (104°F) (no condensation)

Altitude Up to 2000 m (6500 ft.)

Place of operation For indoor use only

Storage temperature –25°C to 70°C (–13°F to 158°F)

Pollution level 2

Overvoltage category II

Appendix A

Mechanical data and hardware features

Dimensions Width: 0.32 m (13 in.)
 Height: 0.54 m (21 in.) with door closed
 0.73 m (29 in.) with door open
 Depth: 0.50 m (20 in.)

Mass 25.8 kg (57 lb.)

Instrument features

- Automated nucleic acid isolation using magnetic particles
- Desktop instrument
- Protocols stored on EZ1 Cards
- Aspirates and dispenses 6 samples or reagents simultaneously using a 6-channel pipettor head
- Separates magnetic particles using patented technology
- Processes up to 6 samples in one run
- Controlled through LCD user interface
- Temperature control through a heating system

Pipettor head	<p>Contains 6 high-precision syringe pumps, each containing a tip adapter which attaches to filter-tips. Each tip aspirates and dispenses 50–1000 μl liquid.</p> <p>Pipetting accuracy is as follows:</p> <ul style="list-style-type: none">■ 50–100 μl $\pm 5\%$■ 100–1000 μl: $\pm 2\%$ <p>Syringe pumps are air-filled.</p> <p>Liquids containing salts, alcohol, solvents, and/or magnetic particles can be aspirated and dispensed.</p> <p>Air gaps can be aspirated to prevent aspirated liquid from dripping.</p> <p>The pipettor head contains a magnet that allows separation of magnetic particles from the aspirated liquid.</p> <p>Filter-tips are picked up from the tip rack and ejected back into the tip rack.</p> <p>The pipettor head moves in the Z direction above the worktable.</p>
Heating system	<p>Accommodates the heating positions of reagent cartridges and has a temperature range of between ambient temperature and 95°C (203°F).</p> <p>Heating block accuracy at 60°C is $\pm 2^\circ\text{C}$.</p>
Filter-tips	<p>Attach to the tip adapters of the pipettor head to allow liquid aspiration and dispensing. Capacity of 50–1000 μl.</p> <p>The BioRobot EZ1 DSP accommodates up to 12 tip holders, each containing a filter-tip, in the tip rack on the worktable.</p>

Appendix A

Labware

Reagents are loaded onto the worktable using the reagent cartridges. These cartridges are already prefilled with reagents by QIAGEN.

Several reagent cartridges are accommodated together on the worktable by a cartridge rack.

Samples are loaded onto the worktable using 2 ml sample tubes.

Steps which require heating occur on the heating system, which accommodates the heating positions of the reagent cartridges.

Purified nucleic acids are collected in 1.5 ml elution tubes.

Waste Electrical and Electronic Equipment (WEEE)

This section provides information about disposal of waste electrical and electronic equipment by users in the European Union.

The European Directive 2002/96/EC on WEEE requires proper disposal of electrical and electronic equipment when it reaches its end of life. The crossed-out wheeled bin symbol (see below) indicates that this product must **not** be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local legislation. The separate collection and recycling of waste electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.



QIAGEN accepts its responsibility in accordance with the specific WEEE recycling requirements and, where a replacement product is being supplied by QIAGEN, provides free recycling of its WEEE-marked electronic equipment in Europe. If a replacement product is not being purchased from QIAGEN, recycling can be provided upon request at additional cost. To recycle electronic equipment, contact your local QIAGEN sales office for the required return form. Once the form is submitted, you will be contacted by QIAGEN either to request follow-up information for scheduling collection of the electronic waste or to provide you with an individual quote.

Appendix A

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Appendix B

Warranty statement

Thank you for your purchase of QIAGEN instrumentation. Your instrument has been carefully tested to ensure optimum operating efficiency and reproducibility of results. QIAGEN warrants that all new instrumentation manufactured by QIAGEN will correspond to the product specifications and be free from defects in workmanship and materials for a period of twelve (12) months from the original date of shipment. Repair or replacement of defective parts will be provided to the purchaser during this time period provided the QIAGEN instrumentation is operated under conditions of normal and proper use, but not for damage caused by the customer. If any part or subassembly proves to be defective, it will be repaired or replaced at QIAGEN's sole option, subsequent to inspection at the factory, or in the field by an authorized factory representative, provided that such defect manifested under normal and proper use. The shipper will pay all transport fees.

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THE FOREGOING WARRANTY IS QIAGEN'S SOLE AND EXCLUSIVE WARRANTY, AND REPAIR OR REPLACEMENT OF DEFECTIVE PARTS IS THE SOLE AND EXCLUSIVE REMEDY. THERE ARE NO OTHER WARRANTIES OR GUARANTEES, EXPRESS OR IMPLIED. THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE EXPRESSLY EXCLUDED, TO THE FULLEST EXTENT PERMITTED BY LAW. (NOTE: SOME STATES DO NOT PERMIT DISCLAIMERS OF IMPLIED WARRANTIES SO THIS LIMITATION MAY NOT APPLY TO YOU). WITH THE EXCEPTION OF THE ABOVE-REFERENCED REPAIR OR REPLACEMENT REMEDY, QIAGEN SHALL HAVE NO OBLIGATION OR LIABILITY OF ANY NATURE WHATSOEVER WITH RESPECT TO THE QIAGEN INSTRUMENTATION, WHETHER ARISING IN CONTRACT, TORT, STRICT LIABILITY, OR OTHERWISE, INCLUDING BUT NOT LIMITED TO, LIABILITY FOR INDIRECT, CONSEQUENTIAL, INCIDENTAL AND/OR SPECIAL, PUNITIVE, MULTIPLE AND/OR EXEMPLARY DAMAGES AND/OR OTHER LOSSES (INCLUDING LOSS OF USE, LOST REVENUES, LOST PROFITS AND DAMAGE TO REPUTATION), EVEN IF SUCH DAMAGES WERE FORESEEN OR FORSEEABLE, OR WERE BROUGHT TO QIAGEN'S ATTENTION. IN NO EVENT SHALL QIAGEN'S LIABILITY TO YOU EXCEED THE PURCHASE PRICE OF THE PRODUCT.

Appendix B

Liability clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications.

All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

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Japan ■ Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

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USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)



EZ1[®] DNA Investigator Handbook

For automated purification of DNA from
forensic and biosecurity samples using
EZ1 instruments



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Kit Contents

EZ1 DNA Investigator Kit	(48)
Catalog no.	952034
Number of preps	48
Reagent Cartridge, DNA Investigator*	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Sample Tubes (2 ml)	50
Elution Tubes (1.5 ml)	50
Buffer G2	1 x 11 ml
Proteinase K	2 x 250 µl
Carrier RNA†	1 x 310 µg
Q-Card‡	1
Handbook	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 8 for safety information.

† Use of carrier RNA is optional. See “Description of protocols”, page 12 and Appendix A for more information.

‡ The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ1 Advanced or EZ1 Advanced XL instrument.

Additional filter-tips and tip holders are available separately. Additional Buffer G2 and QIAGEN Proteinase K, required for some protocols, are available separately. See page 57 for ordering information.

Storage

The EZ1 DNA Investigator Kit is shipped at ambient temperature. All buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges. When stored properly, the reagent cartridges are stable until the expiration date on the Q-Card. Lyophilized carrier RNA is stable until the expiration date on the Q-Card when stored at room temperature.

The ready-to-use proteinase K solution is stable for up to one year after delivery when stored at room temperature.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1 DNA Investigator Kits is tested against predetermined specifications to ensure consistent product quality. Functional QC testing ensures that the EZ1 DNA Investigator Kit meets the high standards required by forensic scientists.

Product Use Limitations

The EZ1 DNA Investigator Kit is intended for molecular biology applications. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the EZ1 DNA Investigator Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.



CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffers in the reagent cartridges contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If liquid containing potentially infectious agents is spilt on the EZ1 Advanced XL, EZ1 Advanced, or BioRobot® EZ1, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

The following risk and safety phrases apply to components of the EZ1 DNA Investigator Kit.

Reagent cartridge

Contains ethanol, guanidine hydrochloride, and guanidine thiocyanate: highly flammable, harmful, and irritant. Risk and safety phrases: * R11-20/21/22-32-36/38, S13-26-36/37/39-46

QIAGEN proteinase K

Contains proteinase K: sensitizer, irritant. Risk and safety phrases: * R36/37/38-42/43, S23-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R11: Highly flammable; R20/21/22: Harmful by inhalation, in contact with skin, and if swallowed; R32: Contact with acids liberates very toxic gas; R36/38: Irritating to eyes and skin; R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact. S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breathe spray; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37: Wear suitable protective clothing and gloves; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

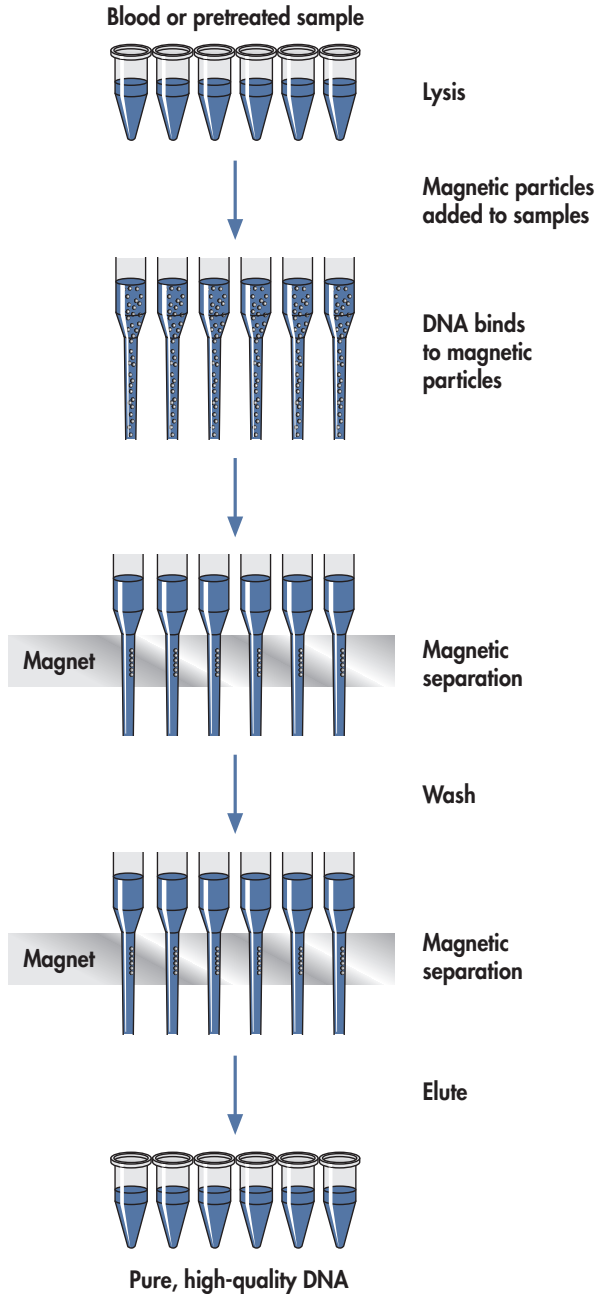
EZ1 instruments and the EZ1 DNA Investigator Kit reproducibly automate purification of genomic DNA from 1–6 samples (EZ1 Advanced and BioRobot EZ1) or 1–14 samples (EZ1 Advanced XL) encountered in forensic, human-identity, and biosecurity applications. Purification is efficient and purified DNA performs well in downstream analyses, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications such as STR analysis or other enzymatic reactions. EZ1 instruments perform all steps of the sample preparation procedure, and the user can choose sample input volumes of 200 µl or 500 µl, allowing purification from varying amounts of starting material. Up to 6 samples (BioRobot EZ1, EZ1 Advanced) or up to 14 samples (EZ1 Advanced XL) are processed in a single run.

Principle and procedure

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see flowchart, page 10). DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in the user's choice of either water or TE buffer. The user can choose elution volumes of 40 µl (EZ1 Advanced XL only), 50 µl, 100 µl, or 200 µl.

EZ1 DNA Investigator Procedure



Description of protocols

This handbook contains two types of protocols.

- Pretreatment protocols detail the preliminary steps, such as proteinase K digestion, prior to processing on the EZ1 instrument.
- DNA purification protocols describe setting up the EZ1 instrument and starting a fully automated run.

Pretreatment protocols

Since the type of samples that can be processed using the EZ1 DNA Investigator Kit can vary greatly, there is also a variety of different pretreatments, optimized for specific sample types. For sample types not specifically included in this handbook, the Protocol: Pretreatment for Other Forensic Samples, page 42, provides a generic protocol that can serve as a starting point for optimizing pretreatment for other sample types.

DNA purification protocols

There are 3 DNA purification protocols, which can be used in conjunction with the pretreatment protocols. Within each protocol, the user can specify elution in water or TE buffer, with elution volumes of 40 µl (EZ1 Advanced XL only), 50 µl, 100 µl, or 200 µl. The standard **Protocol: DNA Purification (Trace Protocol)**, page 44, can be used with all sample types.

In the **Protocol: DNA Purification (“Tip Dance” Protocol)**, page 46, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials, such as swabs, fabrics, blood discs, or cigarette butts, directly in the sample tube. There is generally no need for prior centrifugation to remove solid materials that could clog the tip. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or the sample material is precious.

The **Protocol: DNA Purification (Large-Volume Protocol)**, page 49, enables fully automated processing of starting volumes up to 500 µl. This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. The ability to process larger sample volumes — with the same elution volume as the standard trace protocol — enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Attachment B

The protocol for **purification of low amounts of DNA** in Appendix A, describes the optional use of carrier RNA in the purification procedure. Carrier RNA enhances binding of DNA to the silica surface of the magnetic particles, especially if the sample contains low amounts of DNA (<100 ng). Recently published data suggest that addition of carrier RNA enables more efficient isolation of low amounts of DNA from forensic samples and may, for some sample types, provide improved DNA yields. Addition of carrier RNA to sample lysates did not interfere with downstream STR analyses. This protocol has not been thoroughly tested and optimized by QIAGEN.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

All protocols

- Thermomixer, heating block, or water bath
- Vortexer
- Pipets and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipet tips with aerosol barriers)
- Distilled water

For BioRobot EZ1 users

- BioRobot EZ1 instrument (cat. no. 9000705) and disposables
- EZ1 DNA Investigator Card (cat. no. 9016387)

For EZ1 Advanced users

- EZ1 Advanced instrument (cat. no. 9001410)
- EZ1 Advanced DNA Investigator Card (cat. no. 9018302)

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 Advanced XL DNA Investigator Card (cat. no. 9018699)

For EZ1 Advanced and EZ1 Advanced XL users

For documentation purposes, one of the following is required:

- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced and EZ1 Advanced XL instruments), PC (can be connected with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments), and monitor (cat. no. for PC and monitor 9016643)
- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced and EZ1 Advanced XL instruments) and your own PC and monitor (connection with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments not recommended)
- Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

For purification of DNA from dried blood

- Filter paper (e.g., QIAcard® FTA® Spots, see “Ordering Information”, page 57)
- Manual paper punch, 3 mm (e.g., Harris UNI-CORE 3.00 mm Punch Kit (4), cat. no. 159331, or equivalent punch with cutting mat)

Attachment B

For purification of DNA from forensic surface and contact swabs

- Plastic swabs with cotton or Dacron® tips (Puritan® applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, www.hwppuritan.com, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, www.daigger.com, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.*

For purification of DNA from chewing gum

- Forceps

For purification of DNA from human tissues

- 1.5 ml screw-capped tubes

For purification of DNA from epithelial cells mixed with sperm cells

- Buffer G2, cat. no. 1014636
- 1 M dithiothreitol (DTT)
- Microcentrifuge
- Forceps

For purification of DNA from hair

- QIAGEN Proteinase K, cat. no. 19131 or 19133
- DTT solution (1 M dithiothreitol, 10 mM sodium acetate, pH 5.2)

For purification of DNA from bones or teeth

- QIAGEN Proteinase K, cat. no. 19131 or 19133
- 0.5 M EDTA, pH 8.3
- Liquid nitrogen
- 2 ml microcentrifuge tubes
- Microcentrifuge
- TissueLyser II, cat. no. 85300, with the Grinding Jar Set, S. Steel, cat. no. 69985, or an equivalent bead mill

For purification of DNA from soil

- InhibitEX® tablets (contact QIAGEN Technical Services, see back cover)
- Microcentrifuge

For DNA purification, large-volume protocol

- Buffer MTL (contact QIAGEN Technical Services, see back cover)

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Starting material

The amount of starting material for use in EZ1 DNA Investigator procedures can vary greatly, depending on the amount of DNA in the sample. Specific guidance for starting amounts is given in the individual protocols. EZ1 instruments can process 200 µl pretreated samples using the trace protocol (page 44) or the “tip dance” protocol (page 46) for DNA purification. With the large-volume protocol (page 49), up to 500 µl pretreated samples can be processed.

Working with EZ1 instruments

The main features of the EZ1 instruments include:

- Purification of high-quality nucleic acids from 1–6 or 1–14 samples per run
- Small footprint to save laboratory space
- Preprogrammed EZ1 Cards containing ready-to-use protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup of EZ1 instruments
- Complete automation of nucleic acid purification, from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps

Additional features of the EZ1 Advanced and EZ1 Advanced XL include:

- Bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV lamp to help eliminate sample carryover from run-to-run and to allow pathogen decontamination on the worktable surfaces

Note: UV decontamination helps to reduce possible pathogen contamination of the EZ1 Advanced and EZ1 Advanced XL worktable surfaces. The efficiency of inactivation has to be determined for each specific organism and depends, for example, on layer thickness and sample type. QIAGEN cannot guarantee complete eradication of specific pathogens.

EZ1 Cards, EZ1 Advanced Cards, and EZ1 Advanced XL Cards

Protocols for nucleic acid purification are stored on preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Advanced XL Card into the EZ1 Advanced XL, an EZ1 Advanced Card into the EZ1 Advanced, or an EZ1 Card into the BioRobot EZ1, and the instrument is then ready to run a protocol (Figure 1). The availability of various protocols increases the flexibility of EZ1 instruments.



Figure 1. Ease of protocol setup using EZ1 Cards. Inserting an EZ1 Card, containing a protocol, into an EZ1 instrument. The instrument should only be switched on after an EZ1 Card is inserted. EZ1 Cards should not be exchanged while the instrument is switched on.

The EZ1 DNA Investigator Kit requires use of the EZ1 Advanced XL DNA Investigator Card with the EZ1 Advanced XL, or use of the EZ1 Advanced DNA Investigator Card with the EZ1 Advanced, or use of the EZ1 DNA Investigator Card with the BioRobot EZ1. These EZ1 Cards contain protocols for purification of DNA from forensic and human-identity samples.

EZ1 instruments should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted (Figure 2), otherwise essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.

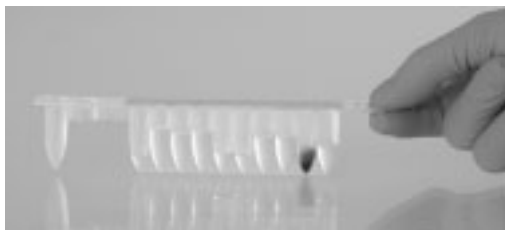


Figure 2. Complete insertion of EZ1 Card. The EZ1 Card must be completely inserted before the EZ1 instrument is switched on.

Reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 3). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Since each well contains only the required amount of reagent, generation of waste due to leftover reagent at the end of the purification procedure is avoided.

A



B



Figure 3. Ease of setup using reagent cartridges. **A** A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. **B** Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

Worktable

The worktable of EZ1 instruments is where the user loads samples and the components of the EZ1 DNA Investigator Kit (Figure 4).

Details on worktable setup are provided in the protocols in this handbook and are also displayed in the vacuum fluorescent display (VFD) of the EZ1 Advanced and EZ1 Advanced XL or the liquid-crystal display (LCD) of the BioRobot EZ1 control panel when the user starts worktable setup.

The display also shows protocol status during the automated purification procedure.

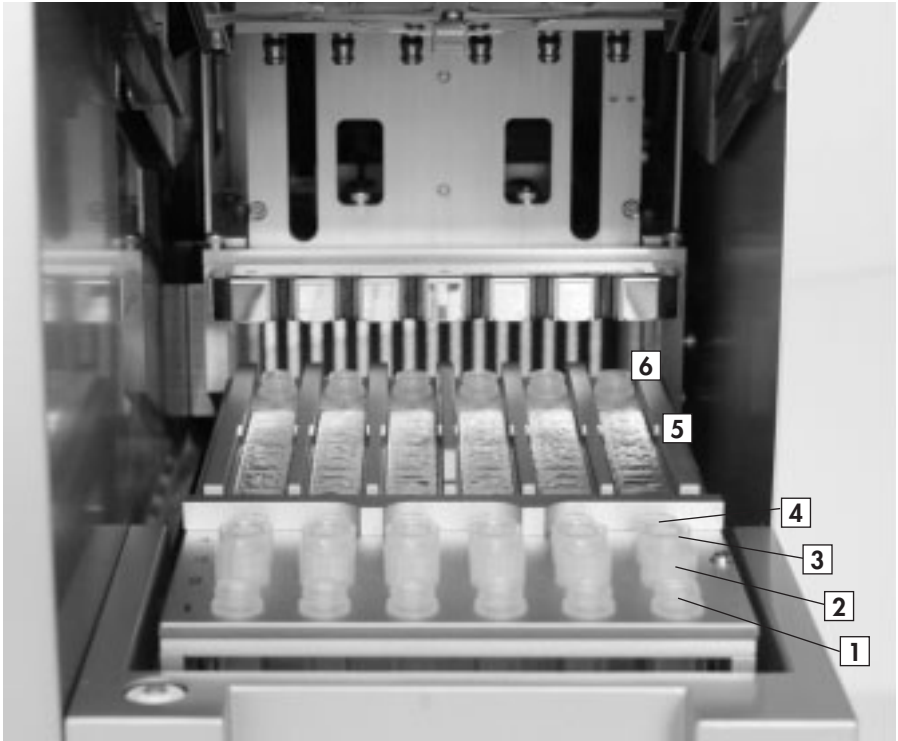


Figure 4. Typical EZ1 worktable.

1. First row: Elution tubes (1.5 ml) are loaded here.
2. Second row: Tip holders containing filter-tips are loaded here.
3. Third row: Tip holders containing filter-tips are loaded here. (In some protocols, this row is empty or loaded with 2 ml Sarstedt tubes.)
4. Fourth row: Sample tubes (2 ml) are loaded here.
5. Reagent cartridges are loaded into the cartridge rack.
6. Heating block with 2 ml tubes in the reagent cartridges for lysis.

Data tracking with the EZ1 Advanced and EZ1 Advanced XL

The EZ1 Advanced and EZ1 Advanced XL enable complete tracking of a variety of data for increased process control and reliability. The EZ1 Kit lot number and expiration date are entered at the start of the protocol using the Q-Card bar code. A user ID and the Q-Card bar code can be entered manually via the keypad or by scanning bar codes using the handheld bar code reader. Sample and assay information can also be optionally entered at the start of the protocol. At the end of the protocol run, a report file is automatically generated. The EZ1 Advanced and EZ1 Advanced XL can store up to 10 result files, and the data can be transferred to a PC or directly printed on a printer (for ordering information, see "Equipment and Reagents to Be Supplied by User" on page 13).

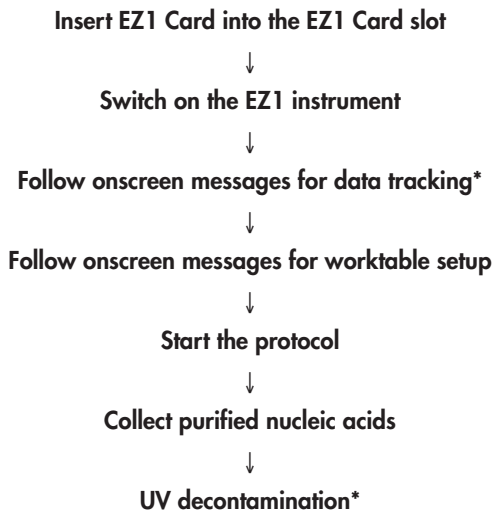
Attachment B

To receive report files on a PC, the EZ1 Advanced Communicator software needs to be installed. The software receives the report file and stores it in a folder that you define. After the PC has received the report file, you can use and process the file with a LIMS (Laboratory Information Management System) or other programs. An example of a report file is shown in Appendix B (page 55). In report files, the 6 pipetting channels of the EZ1 Advanced are named, from left to right, channels A to F or the 14 pipetting channels of the EZ1 Advanced XL are named, from left to right, channels 1–14.

When scanning a user ID or Q-Card bar code with the bar code reader, a beep confirms data input. After the information is displayed for 2 seconds, it is automatically stored, and the next display message is shown. When scanning sample ID, assay kit ID, or notes, a beep confirms data input, the information is displayed, and a message prompts you to enter the next item of information. After scanning sample ID, assay kit ID, and notes, press “ENT” once to confirm that the information entered is correct. If, for example, a wrong bar code was scanned for one of the samples, press “ESC” and then rescan all sample bar codes according to the onscreen instructions. For user ID and notes, you can enter the numbers using the keypad, or you can easily generate your own bar codes to encode these numbers.

For details about data tracking and using EZ1 Advanced Communicator software, see the *EZ1 Advanced User Manual* or the *EZ1 Advanced XL User Manual*.

Workflow of EZ1 operation



* EZ1 Advanced and EZ1 Advanced XL only.

Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, and the protocol used for DNA purification. Table 1 shows typical yields for some common reference sample types.

Table 1. DNA yields from common reference sample types using EZ1 DNA Investigator procedures

Sample type	Sample amount	Protocol	DNA yield
Blood*	10–200 µl	Trace or Tip dance	150 ng–2 µg
Dried blood	4 x 3 mm discs	Tip dance	0.2–0.5 µg
Buccal cells	1 swab	Tip dance	100 ng–2 µg

* Whole blood with $3\text{--}7 \times 10^6$ white blood cells/ml; elution volume 200 µl.

Precipitate in reagent cartridge

The buffer in well 1 of the reagent cartridge (the well that is nearest to the front of the EZ1 instrument when the reagent cartridge is loaded) may form a precipitate upon storage. If necessary, redissolve by mild agitation at 37°C and then place at room temperature (15–25°C).

Equilibrating reagent cartridges

If reagent cartridges have been stored at 2–8°C, they must be equilibrated to operating temperature before use. Place the reagent cartridge into a shaker–incubator and incubate at 30–40°C with mild agitation for at least 2 hours before use. If precipitates are visible at the bottom of the wells, redissolve by incubating at 30–40°C with mild agitation for a further 2 hours. Do not use the reagent cartridges if the precipitates do not redissolve.

Lysis with proteinase K

The EZ1 DNA Investigator Kit contains proteinase K, which is the enzyme of choice for lysis buffers used in EZ1 DNA Investigator protocols. Proteinase K is a recombinant protein expressed in *Pichia pastoris* and is particularly suitable for short digestion times. It possesses a high specific activity and remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures. The activity of the proteinase K solution is 600 mAU/ml solution (or 40 mAU/mg protein). This activity provides optimal results in EZ1 DNA Investigator protocols.

Additional QIAGEN Proteinase K is required for purification of DNA from hair, bones, or teeth (see page 57 for ordering information).

Quantification of DNA

Depending on the sample type, the yields of DNA obtained in the purification procedure may be below 1 µg and therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with carrier RNA may contain much more carrier RNA than target nucleic acids. We recommend using quantitative amplification methods to determine yields.

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings.

To eliminate carried-over magnetic particles, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube.

Protocol: Pretreatment for Whole Blood

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from fresh or frozen blood.

Starting material

This protocol is designed for processing up to 200 μ l of human whole blood.

Storage of blood samples

Whole blood samples treated with EDTA, ACD, or heparin* can be used, and may be either fresh or frozen. Frozen samples should be thawed at room temperature (15–25°C) with mild agitation before beginning the procedure. Yield and quality of the purified DNA depend on storage conditions of the blood. Fresher blood samples may yield better results.

- For short-term storage (up to 10 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.
- For long-term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store the tubes at –70°C.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Proteinase K is not required in this protocol.

Procedure

1. **Thaw and equilibrate up to 6 whole blood samples at room temperature (15–25°C).**
2. **Transfer 200 μ l of each sample into EZ1 sample tubes (2 ml).**
For samples <200 μ l, bring the volume up to 200 μ l with Buffer G2.
3. **Continue with Protocol: DNA Purification (Trace Protocol), page 44.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Protocol: Pretreatment for Dried Blood

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from dried blood. The protocol describes sample collection and the preliminary lysis of dried blood samples using proteinase K.

Starting material

Drying blood on filter paper is an effective form of storage and samples prepared in this manner are cheaper and safer to transport. A disc (3 mm diameter) punched out from filter paper stained with dried blood contains white blood cells from approximately 5 μ l whole blood; we recommend using 4 punched-out discs as starting material.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 15.

Things to do before starting

- As filter paper tends to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 4. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 6.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 7.

Procedure

- 1. Collect 70 μ l of each blood sample onto a ring marked on filter paper. Allow the blood to air-dry.**
Either untreated blood or blood containing an anticoagulant (EDTA, ACD, or heparin)* can be used.
- 2. For each dried blood sample, use the manual paper punch to cut out four 3 mm diameter discs.**
- 3. Transfer each set of 4 discs to a 2 ml sample tube.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Attachment B

4. **Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.**

Note: Prepare diluted Buffer G2 as described in “Things to do before starting”.

5. **Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
6. **Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

7. **Recommended: Incubate at 95°C for 5 min.**

Incubating the sample at 95°C may increase the yield of DNA.

8. **If necessary, flick the tube to remove drops from inside the lid.**
9. **Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Saliva

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from saliva samples. The protocol describes the preliminary lysis of saliva samples using proteinase K.

Starting material

The amount of saliva should not exceed 50 μ l. For larger volumes, if the sample is very dilute, see Protocol: DNA Purification (Large-Volume Protocol), page 49.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

1. Place up to 50 μ l saliva in a 2 ml sample tube.
2. Add 140–190 μ l Buffer G2 to the sample to bring the total volume up to 190 μ l.
3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.
4. Incubate at 56°C for 15 min.
Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. If necessary, flick the tube to remove drops from inside the lid.
6. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Forensic Surface and Contact Swabs

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic surface and contact swabs. The protocol describes the preliminary lysis of forensic surface and contact swabs using proteinase K.

Starting material

Swabs may be processed on the same day as collection or stored for future processing. While storage at -20°C is recommended, DNA of suitable quality for single-copy gene amplification has been documented from swabs stored at room temperature for 24 months.

Important points before starting

- This protocol has been tested using the following swab types: plastic swabs with cotton or Dacron tips. (Puritan applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, www.hwppuritan.com, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, www.daigger.com, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.
- Before beginning the procedure, read "Important Notes", page 15.

Things to do before starting

- Allow the swab or brush to air-dry for at least 2 h after sample collection.
- As swabs tend to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

Procedure

1. **Carefully cut or break off the end part of the swab or brush into a 2 ml sample tube, using an appropriate tool (e.g., scissors).**

2. **Add 290 μ l of diluted Buffer G2 to the sample.**

Note: Prepare diluted Buffer G2 as described in “Things to do before starting”.

3. **Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**

If processing brush samples, centrifuge the tube briefly (at 10,000 x g for 30 s) to force the brush to the bottom of the tube.

4. **Incubate at 56°C for 15 min.**

Vortex the tube 1–2 times during the incubation, or place in a thermomixer.

5. **Recommended: Incubate at 95°C for 5 min.**

Incubating the sample at 95°C may increase the yield of DNA.

6. **If necessary, flick the tube to remove drops from inside the lid.**

7. **Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.**

Using the “tip dance” protocol, there is generally no need to remove the swab or brush from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove the swab or brush from the tube. Using forceps, press the swab or brush against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Nail Scrapings

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic nail-scraping samples. The protocol describes the preliminary lysis of nail-scraping samples using proteinase K.

Starting material

The amount of biological sample material should not exceed 40 mg.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Place the nail-scraping sample in a 2 ml sample tube.**
- 2. Add 190 µl Buffer G2 to the sample.**
- 3. Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.**
- 4. Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

- 5. If necessary, flick the tube to remove drops from inside the lid.**
- 6. Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Chewing Gum

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic chewing-gum samples. The protocol describes the preliminary lysis of chewing-gum samples using proteinase K.

Starting material

Use of up to 40 mg of chewing gum cut into small pieces is recommended.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

1. Place the chewing-gum sample in a 2 ml sample tube.
2. Add 190 µl Buffer G2 to the sample.
3. Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.
4. Incubate at 56°C for 15 min.
Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. If necessary, flick the tube to remove drops from inside the lid.
6. Remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Cigarette Butts

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic cigarette-butt samples. The protocol describes the preliminary lysis of saliva and epithelial cells on paper from cigarette butts using proteinase K.

Starting material

Use of approximately 1 cm² paper from the end of the cigarette or filter is recommended.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As cigarette butts tend to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for n+1 samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

Procedure

1. **Place the cigarette-butt sample in a 2 ml sample tube.**
2. **Add 190 µl diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 µl.**

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

3. **Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C for 15 min.**
Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. **Recommended: Incubate at 95°C for 5 min.**
Incubating the sample at 95°C may increase the yield of DNA.

Attachment B

- 6. If necessary, flick the tube to remove drops from inside the lid.**
- 7. Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Postage Stamps

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from postage stamps. The protocol describes the preliminary lysis of postage-stamp samples using proteinase K.

Starting material

Use of a 0.5–2.5 cm² piece of postage stamp is recommended.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As postage stamps tend to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for n+1 samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

Procedure

1. **Place the piece of postage stamp in a 2 ml sample tube.**
2. **Add 190 µl diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 µl.**

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

3. **Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

5. **Recommended: Incubate at 95°C for 5 min.**

Incubating the sample at 95°C may increase the yield of DNA.

Attachment B

- 6. If necessary, flick the tube to remove drops from inside the lid.**
- 7. Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Stains on Fabric

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from stains on fabric (e.g., blood- or saliva-stained fabrics or leather). The protocol describes the preliminary lysis of stains on fabric using proteinase K. Some samples may require larger volumes for lysis; see Protocol: DNA Purification (Large-Volume Protocol), page 49.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As fabrics tend to be very absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

Procedure

1. Place the fabric sample in a 2 ml sample tube.
2. Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.
4. Incubate at 56°C for 15 min.

Vortex the tube once or twice during the incubation, or place in a thermomixer.

5. Recommended: Incubate at 95°C for 5 min.

Incubating the sample at 95°C may increase the yield of DNA.

6. If necessary, flick the tube to remove drops from inside the lid.

7. Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Human Tissues

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from human tissues. The protocol describes the preliminary lysis of tissues using proteinase K.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Transfer the tissue sample into a 1.5 ml screw-capped tube (not supplied).**
- 2. Add 190 µl Buffer G2.**
Ensure that tissue pieces are fully submerged in Buffer G2.
- 3. Add 10 µl proteinase K solution and mix by tapping the tube gently.**
- 4. Incubate at 56°C until the tissue is completely lysed. Vortex 2–3 times per hour during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.**

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 3 h. Lysis overnight is possible and does not influence the preparation.

- 5. Homogenize the sample by pipetting up and down several times. Transfer the supernatant to a new 2 ml sample tube.**
- 6. Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Remove large pieces of insoluble material and centrifuge at 300 x g for 1 min. The sample volume should be approximately 200 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Epithelial Cells Mixed with Sperm Cells

This protocol is designed for purification of total (genomic and mitochondrial) DNA from epithelial cells mixed with sperm cells. The protocol describes the preliminary lysis of samples using proteinase K and dithiothreitol (DTT).

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- As some sample types (e.g., fabrics) tend to be very absorbent, it may be necessary to add a greater volume of digestion buffer to the sample in step 2.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in steps 4 and 12.

Procedure

1. **Place the forensic sample in a 1.5 ml or 2 ml sample tube.**
2. **Add 190 µl Buffer G2 to the sample.**
3. **Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C for 15 min.**
Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. **Centrifuge the tube briefly to remove drops from inside the lid.**
6. **Remove any solid material from the tube.**
Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.
The sample volume should be approximately 200 µl.
7. **Centrifuge the tube at 15,000 x g for 5 min. Carefully transfer the supernatant to a new tube without disturbing the sperm cell pellet.**
DNA from epithelial cells can be purified from the tube containing the supernatant following Protocol: DNA Purification (Trace Protocol), page 44, or, if the epithelial-cell fraction is very dilute, Protocol: DNA Purification (Large-Volume Protocol), page 49.

Note: The cell pellet may not be visible.

Attachment B

8. Wash the sperm cell pellet by resuspending the pellet in 500 μ l Buffer G2. Centrifuge the tube at 15,000 \times *g* for 5 min and discard the supernatant.
9. Repeat step 8 two or three times.
10. Add 180 μ l Buffer G2 to the pellet and resuspend the pellet.
11. Add 10 μ l proteinase K and 10 μ l 1 M DTT, and mix thoroughly by vortexing for 10 s.
12. Incubate at 56°C overnight at 850 rpm in a shaker–incubator or thermomixer.
13. Centrifuge the tube briefly to remove drops from inside the lid. DNA from sperm cells can now be purified from this tube.
14. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

The two tubes in which the epithelial and sperm cells have been separated are now ready for DNA purification.

Protocol: Pretreatment for Hair

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from the root ends of plucked hair samples. The protocol describes the preliminary lysis of hair samples using proteinase K and dithiothreitol (DTT).

Starting material

We recommend using 0.5–1 cm from the root ends of plucked hair samples.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in steps 4 and 6.

Procedure

1. **Place the hair sample in a 2 ml sample tube.**
2. **Add 180 µl Buffer G2 to the sample.**
3. **Add 10 µl proteinase K and 10 µl DTT solution, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C for at least 6 h.**
Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. **Add another 10 µl proteinase K and 10 µl DTT solution, and mix thoroughly by vortexing for 10 s.**
6. **Incubate at 56°C for at least 2 h or until the hair samples are completely dissolved.**
7. **If necessary, flick the tube to remove drops from inside the lid.**
8. **Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: Pretreatment for Bones or Teeth

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from bones or teeth. The protocol describes the preliminary grinding, decalcification using EDTA, and lysis of bone or teeth samples using proteinase K.

Starting material

The amount of biological sample material should not exceed 200 mg.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Take time to familiarize yourself with the TissueLyser before starting this protocol. See the *TissueLyser Handbook*.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 37°C for the decalcification in step 3.

Procedure

- 1. Remove and discard the bone or teeth surfaces. Grind the remaining bone or tooth root to a fine powder using the TissueLyser system or an equivalent bead mill.**

When using the TissueLyser, transfer the bone sample and the ball into the grinding jar. Pour liquid nitrogen into the grinding jar over the ball and bone fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the TissueLyser. Grind the bone at 30 Hz for 1 min or until the bone is pulverized (grinding times depend on type, condition, and size of bone).

- 2. Place 150–200 mg of powdered bone into a 2 ml microcentrifuge tube.**
- 3. Add 600–700 μ l 0.5 M EDTA (pH 8.3), and incubate at 37°C for 24–48 h.**
After incubation, set the temperature to 56°C for the next incubation step.
- 4. Add 20 μ l QIAGEN Proteinase K, and incubate at 56°C for 3 h.**
- 5. Centrifuge at 6000 rpm for 4 min. Transfer 200 μ l of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Trace Protocol) or transfer 500 μ l of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Large-Volume Protocol).**
- 6. Continue with Protocol: DNA Purification (Trace Protocol), page 44, or Protocol: DNA Purification (Large-Volume Protocol), page 49.**

Protocol: Pretreatment for Soil

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from soil. The protocol describes the preliminary lysis of soil samples and adsorption of inhibitors using InhibitEX tablets (contact QIAGEN Technical Services, see back cover).

Starting material

Up to 0.5 g of soil can be used, depending on the type of soil. With flocculent soil samples, less starting material should be used.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Proteinase K is not required in this protocol.
- This protocol requires InhibitEX tablets (contact QIAGEN Technical Services, see back cover).

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 95°C for use in step 2.

Procedure

1. Place the soil sample in a 2 ml sample tube.
2. Add 900 μ l distilled water. Resuspend the soil by vortexing, and incubate at 95°C for 10 min.
3. Centrifuge the tube at 4000 \times g for 10 min. Transfer the supernatant to another 2 ml sample tube and add 190 μ l Buffer G2. Mix by vortexing.
4. Add 1 InhibitEX tablet and incubate at room temperature (15–25°C) for 1 min.
5. Mix by vortexing and centrifuge at 10,000 \times g for 2 min. Transfer 200 μ l of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Trace Protocol) or transfer 500 μ l of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Large-Volume Protocol).
6. Continue with Protocol: DNA Purification (Trace Protocol), page 44, or Protocol: DNA Purification (Large-Volume Protocol), page 49.

Protocol: Pretreatment for Other Forensic Samples

This protocol is designed as a generic protocol for isolation of total (genomic and mitochondrial) DNA from various forensic samples. The protocol describes the preliminary lysis of samples using proteinase K.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As some sample types (e.g., bloodstained fabrics) tend to be very absorbent, it may be necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 can be diluted with distilled water before use. If necessary, dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

1. **Place the forensic sample in a 2 ml sample tube.**
2. **Depending on the type of sample, follow either step 2a (for non-absorbent samples) or step 2b (for absorbent samples).**
 - 2a. **Non-absorbent samples:**
Add 190 μ l Buffer G2 to the sample.
 - 2b. **Absorbent samples:**
Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.
Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.
3. **Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**

Attachment B

4. Incubate at 56°C for 15 min.

Vortex the tube once or twice during the incubation, or place in a thermomixer.

5. If necessary, flick the tube to remove drops from inside the lid.

6. Continue with Protocol: DNA Purification (“Tip Dance” Protocol), page 46.

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: DNA Purification (Trace Protocol)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (pages 22–43). The protocol describes the simple procedure for setting up the EZ1 instrument and starting a run.

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read “Important Notes” (page 15).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 8 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● (red) if using the BioRobot EZ1.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See “Equilibrating reagent cartridges”, page 20.
- Remove any solid material from the sample tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Insert ▲ the EZ1 Advanced DNA Investigator Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Investigator Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Investigator Card completely into the EZ1 Card slot of the BioRobot EZ1.
2. Switch on the EZ1 instrument.
3. Press “START” to start protocol setup. ▲ Follow the onscreen instructions for data tracking.
4. Press “1” (for Trace protocol).
5. Choose the elution buffer and volume: press “1” to elute in water or “2” to elute in TE buffer. Then press “1”, “2”, or “3”, (or “4”, EZ1 Advanced XL only) to select the elution volume.
6. Press any key to proceed through the text shown on the display and start worktable setup.

The text summarizes the following steps which describe loading of the worktable. Wear gloves when loading the required items on the worktable.

7. **Open the instrument door.**
8. **Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.**
9. **Load the reagent cartridges into the cartridge rack.**

Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
10. **Load opened elution tubes into the first row of the tip rack.**
11. **Load tip holders containing filter-tips into the second row of the tip rack.**
12. **Load opened sample tubes containing digested samples into the back row of the tip rack.**

Pretreat the samples following the individual protocols in this handbook.

Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.

13. **Close the instrument door.**
14. **Press "START" to start the purification procedure.**

The automated purification procedure takes 15–20 min.
15. **When the protocol ends, the display shows "Protocol finished". ▲ Press "ENT" to generate the report file.**

The EZ1 Advanced and the EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

16. **Open the instrument door.**
17. **Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.***

If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.

18. **▲ Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
19. **To run another protocol, press "ESC", prepare samples as described in the relevant protocol, and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**
20. **Clean the EZ1 instrument.**

Follow the maintenance instructions in the user manual supplied with your EZ1 instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 8 for safety information.

Protocol: DNA Purification (“Tip Dance” Protocol)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (pages 22–43). This protocol describes the simple procedure for setting up the EZ1 instrument and starting a run.

In the “tip dance” protocol, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials, such as swabs, fabrics, blood discs, or cigarette butts, directly in the sample tube. There is generally no need for prior centrifugation to remove solid materials that could clog the tip. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or the sample material is precious. (Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.)

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read “Important Notes” (page 15).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 8 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● (red) if using the BioRobot EZ1.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See “Equilibrating reagent cartridges”, page 20.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Insert ▲ the EZ1 Advanced DNA Investigator Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Investigator Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Investigator Card completely into the EZ1 Card slot of the BioRobot EZ1.

Attachment B

2. Switch on the EZ1 instrument.
3. Press "START" to start protocol setup. ▲ Follow the onscreen instructions for data tracking.
4. Press "2" (for Trace TD protocol).
5. Choose the elution buffer and volume: press "1" to elute in water or "2" to elute in TE. Then press "1", "2", or "3", (or "4", EZ1 Advanced XL only) to select the elution volume.
6. Press any key to proceed through the text shown on the display and start worktable setup.

The text summarizes the following steps which describe loading of the worktable. Wear gloves when loading the required items on the worktable.

7. Open the instrument door.
8. Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.
9. Load the reagent cartridges into the cartridge rack.
Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
10. Load opened elution tubes into the first row of the tip rack.
11. Load tip holders containing filter-tips into the second row of the tip rack.
12. Load opened sample tubes containing digested samples into the back row of the tip rack.

Pretreat the samples following the individual protocols in this handbook.

Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.

13. Close the instrument door.
14. Press "START" to start the purification procedure.
The automated purification procedure takes 15–20 min.
15. When the protocol ends, the display shows "Protocol finished". ▲ Press "ENT" to generate the report file.

The EZ1 Advanced and the EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

16. Open the instrument door.

Attachment B

17. Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.*

If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.

18. ▲ **Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
19. To run another protocol, press “ESC”, prepare samples as described in the relevant protocol, and follow the procedure from step 4 onward. Otherwise, press “STOP” twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.
20. Clean the EZ1 instrument.

Follow the maintenance instructions in the user manual supplied with your EZ1 instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 8 for safety information.

Protocol: DNA Purification (Large-Volume Protocol)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (pages 22–43). This protocol describes the simple procedure for setting up the EZ1 instrument and starting a run.

Starting material

Using this protocol, up to 500 µl of pretreated sample can be processed. This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. For these samples, increase the amount of Buffer G2 as required. The amount of proteinase K generally does not need to be increased.

The ability to process larger sample volumes — with the same elution volume as the standard trace protocol — enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read “Important Notes” (page 15).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 8 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- This protocol requires extra Buffer MTL (contact QIAGEN Technical Services, see back cover).
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● (red) if using the BioRobot EZ1.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See “Equilibrating reagent cartridges”, page 20.
- Remove any solid material from the sample tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Insert ▲ the EZ1 Advanced DNA Investigator Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Investigator Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Investigator Card completely into the EZ1 Card slot of the BioRobot EZ1.
2. Switch on the EZ1 instrument.
3. Press "START" to start protocol setup. ▲ Follow the onscreen instructions for data tracking.
4. Press "3" (for Large-Volume protocol).
5. Choose the elution buffer and volume: press "1" to elute in water or "2" to elute in TE buffer. Then press "1", "2", or "3", (or "4", EZ1 Advanced XL only) to select the elution volume.
6. Press any key to proceed through the text shown on the display and start worktable setup.

The text summarizes the following steps which describe loading of the worktable. Wear gloves when loading the required items on the worktable.

7. **Open the instrument door.**
8. **Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.**
9. **Load the reagent cartridges into the cartridge rack.**
Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
10. **Load opened elution tubes into the first row of the tip rack.**
11. **Load tip holders containing filter-tips into the second row of the tip rack.**
12. **Add 400 µl Buffer MTL to each sample tube containing digested samples. Load opened sample tubes containing Buffer MTL and digested samples into the back row of the tip rack.**

Pretreat the samples following the individual protocols in this handbook.

Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.

13. **Close the instrument door.**
14. **Press "START" to start the purification procedure.**

The automated purification procedure takes 15–20 min.

Attachment B

15. When the protocol ends, the display shows "Protocol finished". ▲ Press "ENT" to generate the report file.

The EZ1 Advanced and the EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

16. Open the instrument door.

17. Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.*

If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.

18. ▲ Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.
19. To run another protocol, press "ESC", prepare samples as described in the relevant protocol, and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

20. Clean the EZ1 instrument.

Follow the maintenance instructions in the user manual supplied with your EZ1 instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 8 for safety information.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com)

Comments and suggestions

General handling

- | | | |
|----|-------------------------------------|---|
| a) | Error message in instrument display | Refer to the user manual supplied with your EZ1 instrument. |
| b) | Report file not printed | Check whether the printer is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port.

Check whether the serial port is set for use with a printer. |
| c) | Report file not sent to the PC | Check whether the PC is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port.

Check whether the serial port is set for use with a PC. |
| d) | Wrong Q-Card ID entered | If the wrong ID was entered instead of the Q-Card ID, the EZ1 Advanced/EZ1 Advanced XL will not accept the ID and will prompt for the Q-Card ID until the correct ID is entered. Press "STOP" twice to go to the main menu. |

Low DNA yield

- | | | |
|----|---|--|
| a) | Magnetic particles not completely resuspended | Ensure that you invert the reagent cartridges several times to resuspend the magnetic particles. |
| b) | Insufficient reagent aspirated | After inverting the reagent cartridges to resuspend the magnetic particles, ensure that you tap the cartridges to deposit the reagents at the bottom of the wells. |

- c) Purified DNA stored in water Elute in TE buffer instead of water. Elution in TE buffer gives comparable performance and provides increased stability for long-term storage of small amounts of purified DNA.
- d) Varying pipetting volumes To ensure pipetting accuracy, it is important that buffer volumes in the reagent cartridges are correct and that the filter tips fit optimally to the tip adapter. Ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date. Perform regular maintenance as described in the instrument user manual. Check the fit of the filter tips regularly as described in the user manual.

DNA does not perform well in downstream applications

- a) Insufficient DNA used in downstream applications If possible, repeat the downstream application using more eluate.
- b) Excess DNA used in downstream application Excess DNA can inhibit some enzymatic reactions. Dilute the eluate or use less in the downstream application. Quantify the purified DNA by measurement of the absorbance using an appropriate method.

Appendix A: Purification of Low Amounts of DNA

This protocol is designed for purification of isolation of total (genomic and mitochondrial) DNA from forensic samples that contain <100 ng DNA. The protocol describes the addition of carrier RNA to sample lysates. For full details, refer to Kishore, R., Hardy, W.R., Anderson, V.J., Sanchez, N.A., and Buoncristiani, M.P.H. (2006) Optimization of DNA extraction from low-yield and degraded samples using the BioRobot EZ1 and BioRobot M48. *J. Forensic Sci.* Vol. **51**, No. 5, 1055.

The procedure has not been thoroughly tested and optimized by QIAGEN.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Add 310 µl nuclease-free water or TE buffer to the tube containing carrier RNA (310 µg) to obtain a solution of 1 µg/µl.
- Dissolve the carrier RNA thoroughly, divide it into single-use aliquots, and store at -70°C.

Procedure

1. **Pretreat samples according to the appropriate pretreatment protocol given on pages 22–43 of this handbook.**
2. **Add 1 µl of thawed carrier RNA solution (1 µg) to each lysate. It is not necessary to incubate the carrier RNA and sample lysate.**
3. **Continue immediately with Protocol: DNA Purification (Trace Protocol), Protocol: DNA Purification (“Tip Dance” Protocol), or Protocol: DNA Purification (Large-Volume Protocol) on pages 44, 46, or 49 of this handbook.**

Appendix B: Example of an EZ1 Advanced Report File

This appendix shows a typical report file generated on the EZ1 Advanced. The values for each parameter will differ from the report file generated on your EZ1 Advanced. Please note that "User ID" is allowed a maximum of 9 characters, and that "Assay kit ID" and "Note" are allowed a maximum of 14 characters.

The EZ1 Advanced XL generates a similar report file containing instrument and protocol information relevant to the EZ1 Advanced XL and information for channels 1–14.

REPORT - FILE EZ1 Advanced:

Serial No. EZ1 Advanced: _____ 0301F0172
User ID: _____ 4121
Firmware version: _____ V 1.0.0
Installation date of instr.: _____ Jan 05, 2008
Weekly maintenance done on: _____ Apr 15, 2008
Yearly maintenance done on: _____ Mar 10, 2008
Date of last UV-run: _____ Apr 20, 2008
Start of last UV-run: _____ 16:06
End of last UV-run: _____ 16:26
Status UV-run: _____ o.k.

Protocol name: _____ DNA Investigator
_____ Trace

Date of run: _____ Apr 21, 2008
Start of run: _____ 12:57
End of run: _____ 13:31
Status run: _____ o.k.
Error Code: _____
Sample input Vol [ul]: _____ 200
Elution volume [ul]: _____ 100

Channel A:
Sample ID: _____ 123456789
Reagen Kit number: _____ 9801301
Reagen Lot number: _____ 23456789
Reagent Expiry date: _____ 1208
Assay kit ID: _____ 848373922
Note: _____ 2000

Attachment B

Channel B:

Sample ID: _____ 234567890
Reagen Kit number: _____ 9801301
Reagen Lot number: _____ 23456789
Reagent Expiry date: _____ 1208
Assay kit ID: _____ 836266738
Note: _____

Channel C:

Sample ID: _____ 345678901
Reagen Kit number: _____ 9801301
Reagen Lot number: _____ 23456789
Reagent Expiry date: _____ 1208
Assay kit ID: _____ 883727832
Notes: _____ 1000

Channel D:

Sample ID: _____ 456789012
Reagen Kit number: _____ 9801301
Reagen Lot number: _____ 23456789
Reagent Expiry date: _____ 1208
Assay kit ID: _____ 763684837
Note: _____

Channel E:

Sample ID: _____ 567890123
Reagen Kit number: _____ 9801301
Reagen Lot number: _____ 23456789
Reagent Expiry date: _____ 1208
Assay kit ID: _____ 4387728002
Note: _____

Channel F:

Sample ID: _____ 678901234
Reagen Kit number: _____ 9801301
Reagen Lot number: _____ 23456789
Reagent Expiry date: _____ 1208
Assay kit ID: _____ 509389403
Note: _____ 50

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers and Reagents; includes Certificate of Analysis	952034
EZ1 Advanced XL	Robotic instrument for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, 1-year warranty on parts and labor*	9001492
EZ1 Advanced	Robotic instrument for automated purification of nucleic acids using EZ1 Kits, 1-year warranty on parts and labor*	9001410
EZ1 Advanced XL DNA Investigator Card	Preprogrammed card for EZ1 Advanced XL DNA Investigator protocols on the EZ1 Advanced XL	9018699
EZ1 Advanced DNA Investigator Card	Preprogrammed card for EZ1 Advanced DNA Investigator protocols	9018302
EZ1 DNA Investigator Card	Preprogrammed card for BioRobot EZ1 DNA Investigator protocols	9016387
Accessories		
Filter-Tips and Holders, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1 Kits	994900
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
Buffer G2 (260 ml)	Lysis buffer for EZ1 DNA procedures	1014636
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
TissueLyser II	Universal laboratory mixer-mill disruptor	85300

* Warranty PLUS 2 (cat. no. 9237720) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and repair parts.

Ordering Information

Product	Contents	Cat. no.
Grinding Jar Set, S. Steel (2 x 10 ml)	2 Grinding Jars (10 ml), 2 Stainless Steel Grinding Balls (20 mm)	69985
PC and TFT Monitor, 17"	PC capable of connection with up to 4 EZ1 Advanced or EZ1 Advanced XL instruments; Monitor for use with PC	9016643
Printer	Printer for connection with EZ1 Advanced or EZ1 Advanced XL instrument	9018464
Printer Accessory Package	Accessories for printer connected to EZ1 Advanced or EZ1 Advanced XL instrument	9018465
QIAcard FTA One Spot (100)	For collection and storage of 100 samples: 100 QIAcard FTA One Spots	159201
QIAcard FTA Two Spots (100)	For collection and storage of 100 x 2 samples: 100 QIAcard FTA Two Spots	159203
QIAcard FTA Four Spots (100)	For collection and storage of 100 x 4 samples: 100 QIAcard FTA Four Spots	159205
QIAcard FTA Indicator Four Spots (25)	For collection and storage of 25 x 4 samples: 25 QIAcard FTA Indicator Four Spots	159214
QIAcard FTA Purification Reagent (500 ml)	For use with QIAcard FTA Spots: 500 ml QIAcard FTA Purification Reagent	159300
Related products		
EZ1 DNA Blood 200 µl Kit (48)	48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes	951034
EZ1 DNA Blood 350 µl Kit (48)	48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes	951054

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Tissue Kit (48)	48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes, Buffer G2, Proteinase K	953034
EZ1 Virus Mini Kit v2.0 (48)	For 48 virus nucleic acid preps: Reagent Cartridges (Virus Mini v2.0), Disposable Tips, Disposable Tip-Holders, Sample Tubes, Elution Tubes, Buffers	955134

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Visit www.qiagen.com/goto/EZ1Advanced to find out more about other EZ1 Kits!

Notes

Notes

Notes

Attachment B

Trademarks: QIAGEN®, QIAcard®, BioRobot®, EZ1®, InhibitEX® (QIAGEN Group); 903® (S&S Biosciences, Inc.); Dacron® (E. I. du Pont de Nemours and Company); FTA® (Whatman PLC); Puritan® (Hardwood Products Company).

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Austria = Orders 0800/28-10-10 = Fax 0800/28-10-19 = Technical 0800/28-10-11

Belgium = Orders 0800-79612 = Fax 0800-79611 = Technical 0800-79556

Brazil = Orders 0800-557779 = Fax 55-11-5079-4001 = Technical 0800-557779

Canada = Orders 800-572-9613 = Fax 800-713-5951 = Technical 800-DNA-PREP (800-362-7737)

China = Orders 0086-21-3865-3865 = Fax 0086-21-3865-3965 = Technical 800-988-0325, 800-988-0327

Denmark = Orders 80-885945 = Fax 80-885944 = Technical 80-885942

Finland = Orders 0800-914416 = Fax 0800-914415 = Technical 0800-914413

France = Orders 01-60-920-926 = Fax 01-60-920-925 = Technical 01-60-920-930 = Offers 01-60-920-928

Germany = Orders 02103-29-12000 = Fax 02103-29-22000 = Technical 02103-29-12400

Hong Kong = Orders 800 933 965 = Fax 800 930 439 = Technical 800 930 425

Ireland = Orders 1800-555-049 = Fax 1800-555-048 = Technical 1800-555-061

Italy = Orders 02-33430-420 = Fax 02-33430-426 = Technical 800-787980

Japan = Telephone 03-6890-7300 = Fax 03-5547-0818 = Technical 03-6890-7300

Korea (South) = Orders 1544 7145 = Fax 1544 7146 = Technical 1544 7145

Luxembourg = Orders 8002-2076 = Fax 8002-2073 = Technical 8002-2067

Mexico = Orders 01-800-7742-639 = Fax 01-800-1122-330 = Technical 01-800-7742-639

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Sweden = Orders 020-790282 = Fax 020-790582 = Technical 020-798328

Switzerland = Orders 055-254-22-11 = Fax 055-254-22-13 = Technical 055-254-22-12

UK = Orders 01293-422-911 = Fax 01293-422-922 = Technical 01293-422-999

USA = Orders 800-426-8157 = Fax 800-718-2056 = Technical 800-DNA-PREP (800-362-7737)





PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

12.0 Certification of a New or Reconditioned VERSA 1100 Liquid Handler

12.1 Purpose:

Acceptance of a new or reconditioned VERSA 1100 Liquid Handler before implementation in the laboratory relies upon (1) the proper installation (2) proper internal calibration checks (3) protocol set-up, and (4) a certification exercise.

Documentation of these exercises will be retained in the appropriate VERSA use log next to the instrument in the lab. The certification protocol will ensure that a new or reconditioned VERSA is calibrated so that it functions reliably in achieving specified liquid handling results when operated according to the user manual and laboratory defined protocols.

Thereafter, internal calibration checks of consumable calibration settings will be performed according to the manufacturer's instructions on an as needed basis or during the annual maintenance service call by Aurora Biomed. Records of these tests will be kept in the appropriate VERSA use log next to the robots in the lab.

12.2 References:

VERSA 100 Operation Manual January

1. HARDWARE INSTALLATION AND SETUP

The VERSA 1100 is approximately 920W x 615 L x 999H (mm) including bio-safety hood. It is recommended that the user provide enough space on all sides of the instrument, especially at the back where the Priming, Waste Bottles, and Reagent Box will be situated. The workbench should be stable, vibration free, and capable of handling up to 90kg.

There are a total of five tubing connections to be made on the VERSA 1100.

- **A out (Vacuum)** – This hole is open to air.
- **V in (Vacuum) Blue** – This tubing provides vacuum and is connected to the Waste Bottle. Make sure that this tubing DOES NOT touch the waste solution.
- **W (Waste) Blue** – This tubing connects to the Waste Bottle. Waste liquid that comes from the priming block of the single channel and 4-channel pumps is deposited here.
- **PB (Pressure Box)** – This tubing is used for providing pressure to the reagent bottles. This tubing goes to the 3-way reagent bottle valve.
- **P (Pressure) Red** – This tubing is used to provide air pressure and is connected to the Priming Bottle. This tubing should not touch the water in the bottle.
- **L (Priming Solution) Red Cap** – This tubing supplies the priming solution for both the 4-channel and single channel syringe pumps and is connected to the Priming Bottle. Fill this bottle with distilled water.



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The Priming, Waste, and Reagent bottles are provided with 3-hole bottle caps. Two holes are used either for the solution, pressure, or vacuum. The third hole is provided with either a stopper or a plug. Please observe that a bottle cap with the plug on the outside is used for the Waste Bottle and the bottom cap with the plug inside is used for the Priming and Reagent bottles. It is very important that the correct cap is used for its designated bottle.

The connection to the computer is via USB cable. Please plug the cable into the USB port at the back panel near the power outlet and to the personal computer socket labeled as USB. The power cable should then be plugged into the power source. The power switch is located above the power cable connection.

12.3 Software

Open the software by double-clicking the VERSAware 1100 icon. The username is “admin”. No password is required. The Shell Status will appear first, followed by the VERSAware.

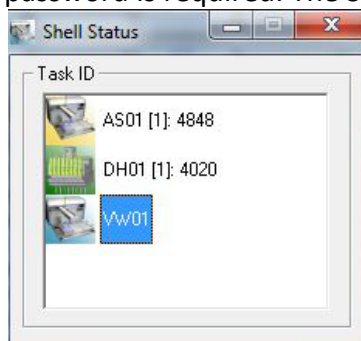




Figure 1: Shell Status window.


There are three interfaces to the software: VERSAware, Aurora Station, and PWM Pump.

- **VERSAware**  – VERSAware is the main interface that the user will use to set up and run protocols
- **Aurora Station**  – Aurora station is a support interface that usually runs alongside VERSAware. Aurora Station controls all robotic movement and all manual manipulations of the robot. Position and plate calibration checks, along with control of the heat block, shaker, gripper, syringe pump, and tip changing can be accessed here. There are two ways to access the Aurora Station:
 1. Open the “Shell Status” Window which runs alongside VERSAware. Double-click on “AS01 [1]: 1172” and Aurora Station will open from the background.
 2. In VERSAware, go to “Tools”, then click on “Aurora Station”. A checkmark will appear next to the interfaces if it is already opened.



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

DO NOT CLOSE THE AURORA STATION. It is required for VERSAware to run. Simply minimize the Aurora station window when it is not used.

- **PWM Pump**  – The PWM Pump is a support interface that controls all of the liquid pumps, including the single channel syringe, 4-channel syringe, and reagent drop. All of the settings regarding the liquid pumps can be accessed here. Volume calibration checks can also be performed here. There are two ways to access the PMP Pump:
 1. Open the “Shell Status” Window which runs alongside VERSAware. Double-click on “DH01 [1]: 5616” and the PWM Pump will open from the background.
 2. In VERSAware, go to “Tools”, then click on “PWM Pump”. A checkmark will appear next to the interfaces if it is already opened.

12.4 Aurora Station

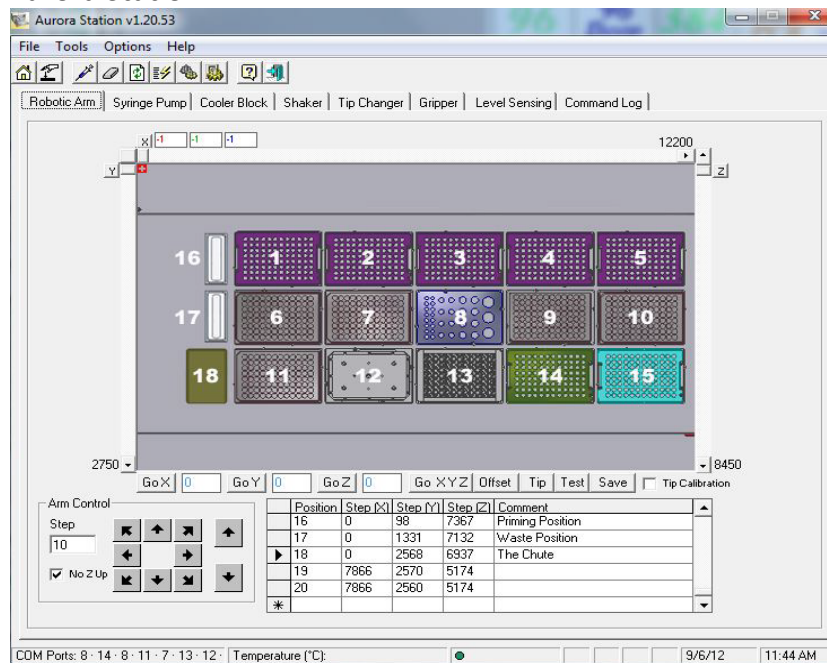


Figure 2: Aurora Station/Robotic Arm Tab

12.4.1 Robotic Arm Tab

The Robotic Arm Tab is the control center for all manual manipulation of the robotic arm. It is crucial that there are no obstructions in the path of the robotic arm prior to movement of the robotic arm.

To initialize all axes of the robotic arm, click on the unlabeled button between the X and Y axes. The robotic arm should travel towards the upper left corner of the workstation. The robotic arm movement can be controlled using two methods:



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

1. **Scroll bars** – The scroll bars labeled X (on the top), Y (on the left), and Z (on the right) can be used to control the X, Y, Z axes independently. The current robotic arm coordinates will be displayed in text boxes in the upper left corner. Each scroll bar shows the maximum travel range for that particular axis. The scroll bars are used for bigger movement of the robotic arm.
2. **Step/Arrow Buttons** – Enter a step value from 1 to 1,000 and use one of the eight arrows on the left side to control the horizontal X and Y axes. The two arrows on the right will control the vertical Z axis. Please be aware of the amount of space between the robotic arm and the surface when selecting the step value. The step/arrow buttons are used for finer movement of the robotic arm.

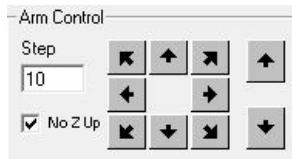


Figure 3: Step/arrow buttons

There are several functions listed above the table:

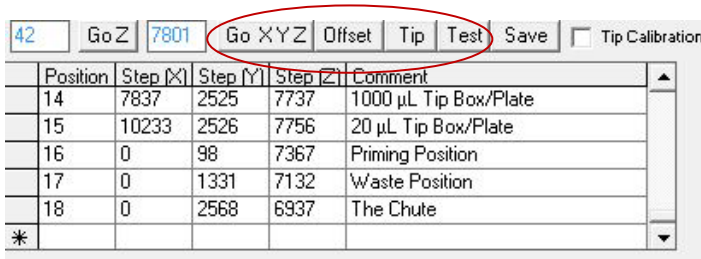


Figure 4

- **Go X, Y, Z**

To test previously saved default teaching positions, double-click on the record selector of the desired position. The X, Y, Z coordinates will be displayed in the text boxes “Go X, Go Y, and Go Z”. Click on “Go X, Y, Z” to have the arm moved to the last calibrated position.

Note: Only use “Go X, Y, Z” if **no tip is engaged**. The robotic arm will move to the position directly above the surface of the teaching plate. Make sure that a teaching plate and not the plate type is in place to avoid crashing the robotic arm.

- **Offset**

The offset calculator will automatically calculate the X, Y, and Z offsets of two different records in the table. Double-click one position record, then click on a second position record, then click “Offset”. The difference in X, Y, and Z positions between the first and second record will be displayed.



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

- **Tip**

To engage a tip, double-click on the record selector of the position from which the tip will be engaged. Positions 2 & 3 hold 50 μ L tips; positions 4 & 5 hold 1000 μ L tips. Click on “Tip” to have the arm move to the tip position and engage a tip.

- **Test**

To test previously saved default teaching positions with **tips engaged**, first engage tips by following the instructions above on “Tip”. Double-click on the record selector of the desired position. The X, Y, Z coordinates will be displayed in the text boxes “Go X, Go Y, and Go Z”. Click on “Test” to have the robotic arm moved to the last calibrated position. The tip offset difference is incorporated in the Z coordinate.

Note: Make sure that a teaching plate and not the plate type is in place to avoid crashing the robotic arm.

Position Calibration Check

VERSA 1100 workstation features 15 positions for various lab ware types, tips, blocks, tubes, reservoirs, etc. Positions 16, 17, and 18 are the priming, waste, and chute positions, respectively. All the deck positions are taught with a 96-well plate. The shaker position is taught as is. All positions should be taught to locate the surface of the upper left well (A1) of the 96-well teaching plate. No pipetting tip should be engaged. The X & Y coordinates should be adjusted so the tip barrel is positioned in the center of the well. For calibration check of the Z coordinate, a piece of paper can be placed in between the tip barrel and the surface of the 96-well teaching plate. Fine adjustments are made until the tip barrel just touches the paper. Slight resistance should be felt when inserting or removing the paper. Upon completion of all position calibration check, the position setting for all other plate types which are available in the Labware library will be automatically calibrated with respect to the 96-well teaching plate based on the offsets in the plate type library.

Calibrate a Position

To calibrate or teach a new plate type, double-click on the position you want to teach. No tip should be engaged. Place a 96-well teaching plate on the deck. Set the Z coordinate to 0 in the “Go Z” text box. Click “Go X, Y, Z”. If necessary, adjust the X and Y coordinates. Click on the down arrow to adjust the Z coordinates. Reduce the steps of the arm control to 5 or 10 steps for more precise and safe control of the arm movement. The tip barrel should be directly above the teaching plate so that a piece of paper should be able to pass through between the teaching plate and the barrel tip with slight resistance. Click “Save”.


Note: If a tip was engaged to calibrate a position, make sure that the “**Tip Calibration**” box is checked. This informs the robot to account for the height differences of a tip.



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Testing Plate Types

Aurora Station allows you to test previously or newly taught positions for all plate types. It is a good practice to test positions periodically to ensure that the calibration checks for a particular plate type and position is still accurate.

First, engage a tip following the instruction above on “Tip”. Place the plate type onto the position to be tested. To test the position with that particular plate type, go to “Options → Aurora Station Settings” or click on the “Aurora Station settings” icon . Click the “Advanced” tab. On the bottom left corner of the screen under “Test Position [Plate Type]”, select the position to test (ignore the description of the position). Click “Apply”.

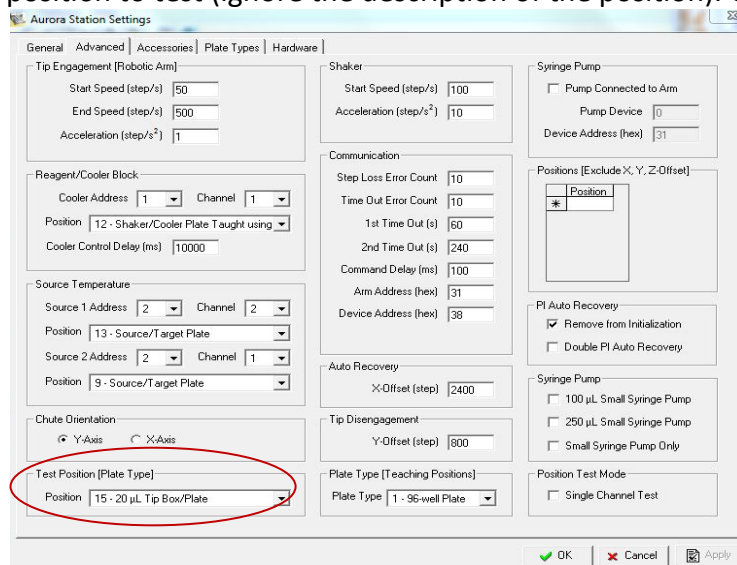


Figure 5: Aurora Station settings/Advanced tab.

Next, click on the “Plate Types” tab. Under the “Symmetrical Plates” section, select the plate type that you want to test in that position by highlighting the selection. Click on the icon with a small red square on the top left corner to test the top left corner of the plate type or click on the icon with a small red square on the bottom right corner to test the bottom right corner of the plate type.

- SlicPrep™ Plate: Plate type #68
- 32-well sample block and reagent cooler block: Plate type #70
(Testing the 32-well sample block includes the height of the samples tubes)



Oakland Police Department Criminalistics Laboratory
Forensic Biology Unit
Quality Control Manual

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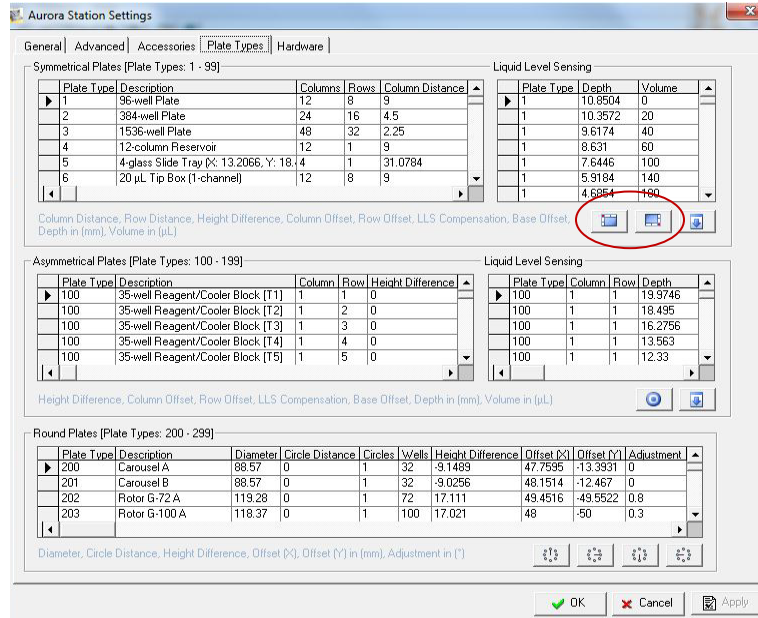


Figure 6: Aurora Station settings/Plate Types tab.

The tip should be in the center of the plate type. If the tip isn't in the center of the plate, then either the position (1) wasn't calibrated correctly or the plate type off-set is not calibrated correctly. Test that particular plate type on a different position (2) by repeating the steps above. If the tip is now centered, then that indicates that the position (1) was calibrated incorrectly. Re-calibrate the position (1) by following the steps on how to "Calibrate a Position".

If the tip is still off-centered, then it indicates that the plate type off-set is not calibrated correctly. To adjust the plate type off-set, following the steps below on how to "Adjust Plate Type Off-set".

Adjust Plate Type Offset

Sometimes, the plate type off-sets that are saved in the Labware library can be slightly off. If that plate type is applied to a position, the tip will not be centered above a well but will instead be off-centered, depending on the severity. An off-centered calibration can result in inaccurate liquid pipetting or potential crashing of the robotic arm in severe cases.

To adjust the plate type off-set, bring the robotic arm to the calibrated plate type position by following the steps above on how to "Test Plate Position". Close the "Aurora Station Settings" screen. The X, Y, Z coordinates on the top left corner of the screen (in red, green, and blue numbers) indicates the current position of the robotic arm.

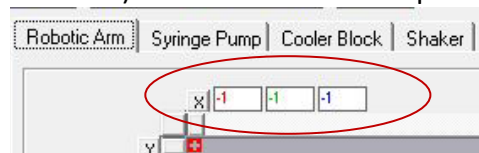


Figure 7: The current X, Y, and Z coordinates.



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

Go to a new row on the record selector table, indicated with an “*”. Type in an arbitrary position number, such as “100”, to distinguish from an actual position. Under the comments column, type “Original”. Select the new position by highlighting the selection, then click “Save”. The current robotic arm coordinates are now saved onto this new position.

	Position	Step [X]	Step [Y]	Step [Z]	Comment
	14	7837	2525	7737	1000 µL Tip Box/Plate
	15	10233	2526	7756	20 µL Tip Box/Plate
	16	0	98	7367	Priming Position
	17	0	1331	7132	Waste Position
	18	0	2568	6937	The Chute
	*				

Figure 8: The position records table.

Adjust the robotic arm using the “Step/Arrow Buttons” until the tip is in the center of the well. Create a new row on the record selector table. Type in an arbitrary position number, such as “101”. Under the comments column, type “Adjusted”. Select the new position by highlighting the selection, then click “Save”. The adjusted robotic arm coordinates are now saved onto this new position.

Double-click on the “Adjusted” position record, then click on the “Original” position record, then click “Offset”. The “Offset Calculator” screen will appear, showing the X, Y, and Z steps of both records and the difference between the two records will appear in the light blue numbers in millimeters (mm). Record the “Offset” value. Click “OK” to close.

Figure 9: Offset calculator


Go to “Options → Aurora Station Settings”. Click on the “Plate Types” tab and select the plate type to be adjusted by highlighting the selection. Scroll to the right until you see the “Height Difference”, “Column Offset”, and “Row Offset” columns. Adjust the offsets by adding/subtracting the value(s) recorded from the “Offset Calculator”. Click “Apply”, then “OK” to close.



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	Coordinate	Positive (+) Value	Negative (-) Value
Column Distance	X	To the right	To the left
Row Distance	Y	Towards user	Away from User
Height Difference	Z	Up	Down

Adjusting the Column and Row Offset Within a Plate Type

Position calibrations align the robot to well A1 of each plate type. Column and row offsets calibration settings for each plate type allow the robot to determine the distance from all other wells with respect to well A1. If it is observed that the calibrations for column 1 is correct but gets increasingly off as it moves to column 12, then the column and row differences may be off for that plate type. To adjust the column and row offset, go to “Options → Aurora Station Settings” or click on the “Aurora Station settings” icon . Click the “Plate Type” tab. The following instructions are an example of adjusting the column and row offset for tip racks.

- Place an empty tip rack in the position to test
- In Aurora Station Settings → Accessories tab, change the tip availability to Next Row: 1, Next Column 12
- Go to Aurora Station and double click on the position, then click “Tip”
- Lower the robotic arm until it is right above the tip rack
- Adjust the positioning until it is centered, keeping track of the number of steps moved (x or y direction)
- Convert from steps to mm by going to Aurora Station settings → Hardware → Robotic Arm → Scale (x or y). This number is mm/step
- Divide the value in mm by 11
- Go to Plate type, column or row distance, and subtract the value from it.

Heater/Shaker Unit Position Calibration Check

The heater/shaker unit should be calibrated with the plate type used (SlicPrep™ Plate)

Reagent Drop Pin Position Calibration Check

To calibrate the reagent drop pin, click “Options → Aurora Station Settings”. Click on the “Advanced” tab. In the bottom left corner under “Test Position [Plate Type]”, select the test position. To the right under “Plate Type [Teaching Position]”, select “1 – 96 well plate” and click “Apply”. Place the 96-well teaching plate in that position. Click on the “Accessories” tab. Under the “Pin Groups” section on the bottom, select the pin number to test by double-clicking. (Only Pin 2 and 3 are used). Click “RD Test”. The robotic arm will go to that position and align the reagent drop pin on the surface of the A1 well of the 96-well teaching plate.



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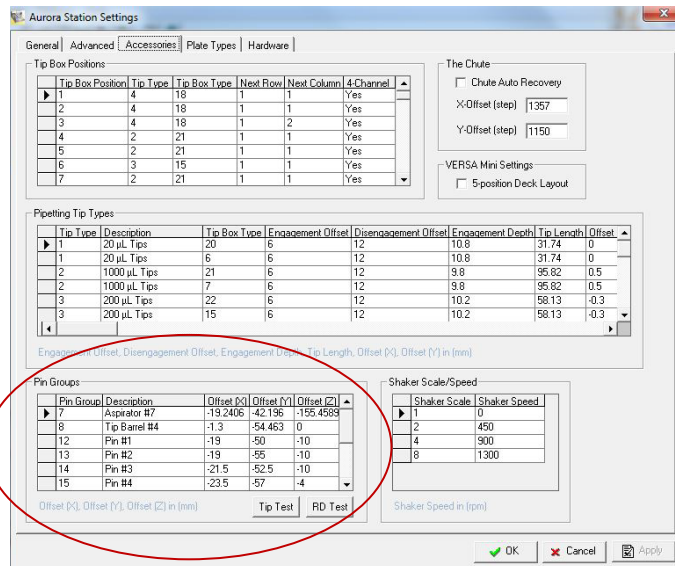


Figure 10: Aurora Station settings/Accessories tab.

To check if the pin is centered on the well, dispense some solution (e.g. 100µL) manually using the PWM Pump. In the VERSAware interface, go to “Tools → PWM Pump”. Click on the “C” icon to go into the control mode. Under the “Valves” section, type “100” in the “Volume” column under the desired valve (Valve 1 for Pin 1, etc.). Watch the pin while it dispenses the liquid. If the pin is not dispensing in the center of the well, change the X and Y Offsets in the “Aurora Station Settings → Accessories tab → Pin Groups” to center the pin.

- Negative value in the X-offset moves the robotic arm/pin to the left side
- Negative value in the Y-offset moves the robotic arm/pin away from the user

When finished, go to the PWM Pump interface and click on the “A” icon to change back to assay mode.

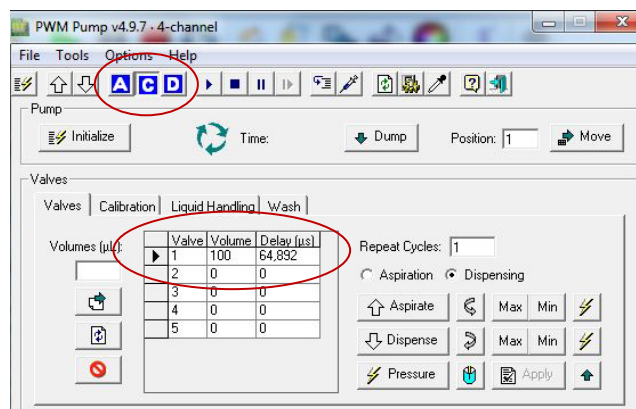


Figure 11: PWM Pump/C Mode.



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In the VERSAware interface, click “Tools → Options”. Click on the “Reagent Drop” tab. Adjust the reagent drop dispensing Z-offset as necessary. This is the height difference between the reagent drop pins and a plate type when dispensing liquid.

- Negative value moves the arm up
- Positive value moves the arm down

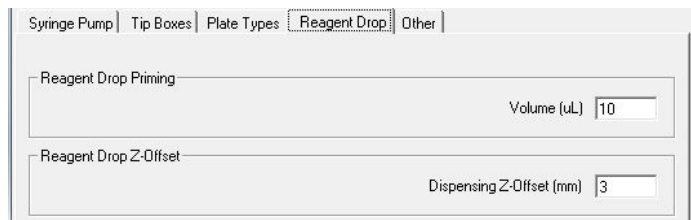


Figure 12: VERSAware/Tools/Options/Reagent Drop Tab

12.4.2 Syringe Pump Tab

The Syringe Pump Tab allows access to manual control of the syringe pump. The syringe pump can be controlled manually to aspirate and dispense liquid and is used for the **syringe pump calibration check**.

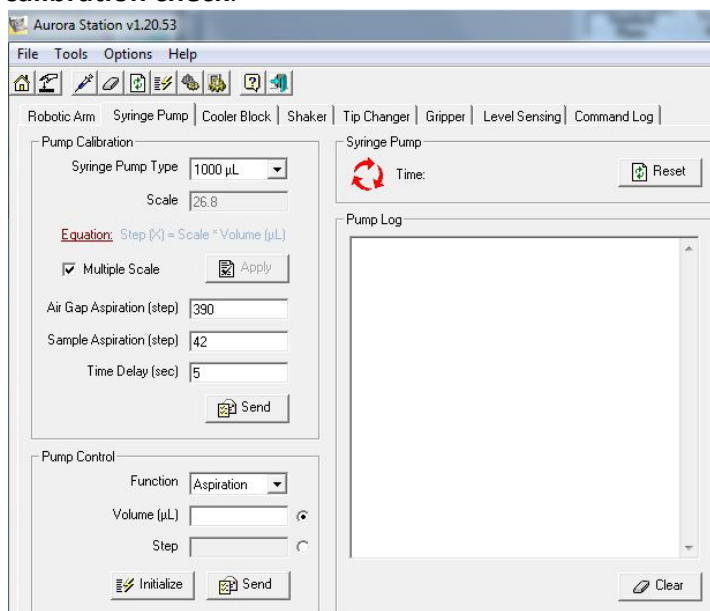


Figure 13: Aurora Station/ Syringe Pump Tab

Syringe Pump Calibration Check

There are two methods to perform the calibration check procedure. The general sequence of aspirating and dispensing liquid for the calibration is AIR GAP ASPIRATION → LIQUID ASPIRATION → DISPENSE LIQUID.

1. **Auto Mode** – The auto mode runs the general sequence listed above automatically, incorporating delays in between each step.



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- Under the “Pump Calibration” section, type in the steps (1 μ L = 10 steps) for the air gap aspiration & sample aspiration and the number of seconds for the delay time.
 - Click “Send”. The instrument will now aspirate X steps of air, wait for Y seconds, aspirate Z steps of sample, wait for Y seconds, then dispense X + Z steps of volume.
 - During the first delay step, place the sample tube containing water underneath the pipette tip, making sure that the tip is submerged in the water.
 - During the second delay step, remove the sample tube and place the empty weigh boat underneath the pipette tip.
2. **Pump Control Mode** – The pump control mode requires manual control of each step in the general sequence listed above.
- Under the “Pump Control” section, select either the “Volume” or “Steps” option.
 - Volume option: Used for testing particular volumes of liquid to determine if the accurate volume of liquid is aspirated/dispensed
 - Steps option: Used for adjusting or creating a new calibration table
 - Select “Aspiration” as the function, then enter the desired amount of steps (X) for air gap aspiration (1 μ L ~ 10 steps).
 - Click “Send”. The instrument will now aspirate the air gap.
 - Now enter the desired amount of steps (Z) for sample aspiration.
 - Place a sample tube containing water under the pipette tip. Click “Send”. The instrument will now aspirate the sample.
 - Select “Dispensing” as the function, then enter the total amount of steps (X + Z) for the air gap aspiration and the sample aspiration.
 - Place an empty weigh boat underneath the pipette tip. Click “Send”. The instrument will now dispense the air gap and the sample.


Prior to performing a syringe pump calibration check, prime the syringe pump to eliminate all air bubbles in the system. It is recommended to perform 8-10 cycles of priming to fill up the tubing with the system fluid and remove most of the air trapped within the system. For instructions on how to perform a syringe pump priming, refer to Section 8.3 – Syringe Pump Priming.

Switch to the *Robotic Arm Tab*, initialize the robotic arm by pressing the button between the X and Y axes, then move the robotic arm towards you by dragging the Y axis scroll bar down. This allows the user to have easier access to the syringe pump during the calibration check. Switch back to the *Syringe Pump Tab*.



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Manually engage a tip onto the first barrel. Use a 50µL tip for the 20-50µL range and a 1000µL tip for the 200-1000µL range. Initialize the pump by clicking the “Initialize” button on the bottom of the screen. Under the “Pump Calibration” section, select 4x1000µL as the syringe pump type. Select “Multiple Scale”. Prepare a sample tube with water and an empty, pre-tared weigh boat. Using either the **Auto Mode** or the **Pump Control Mode**, aspirate and dispense the desired amount of liquid into the empty, pre-tared weigh boat. Weigh the weigh boat. (Note: When using the Pump Control Mode/Volume option, be sure to aspirate an air gap prior to aspirating the liquid. When dispensing, add an extra 50µL to the dispense volume to make sure that all the air and liquid is pushed out.)

In Aurora Station, click on the “Syringe” icon . The calibration table screen should appear. Select the 4x1000µL pump type. Make sure that “Multiple Table” is checked. This allows for multiple calibration tables. Adjust either the existing calibration table values or create a new scale by clicking on an empty row under the “Scale” section. Record the sample aspiration step under the “Step” column and the volume measured in the “Volume” column (1mg = 1µL). Click “Apply”, then “OK”.

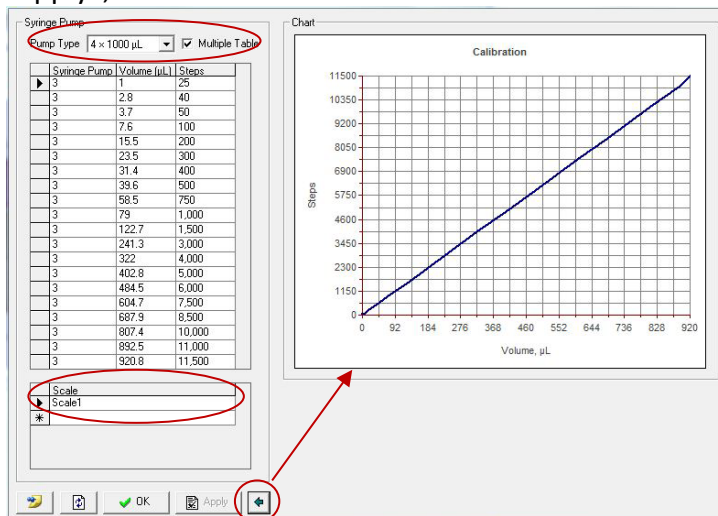


Figure 14: Aurora Station/Syringe Pump Calibration

Measure the volume at different steps by repeating the steps above. Cover the range from 40 – 11,500 steps (~3 – 800µL).

Reagent drop calibration check

Calibration check of the reagent drop is performed using the PWM Pump. Refer to the Section 4.1 – Reagent Drop Calibration Check for instructions on how to perform a reagent drop calibration check.



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12.4.3 Cooler Block Tab

The Cooler Block Tab sets and monitors the temperature of the cooler/heater block in the range of 0 – 90°C with 0.25°C increments. The tab allows the user to check or change COM ports or current temperature. The recommended temperature for the cooler/heater block is 4°C, which is also the default value. Unlock the COM port by click “Unlock” to observe readings of actual and current temperatures.

The equation that the instrument uses to calculate and set the temperature is:

$$\text{Actual Temperature (}^\circ\text{C)} = [\text{Parameter A} \times \text{Set Temperature (}^\circ\text{C)}] + \text{Parameter B}$$

- Parameter A – Usually set at 1
- Parameter B – Adjusts for the difference between the temperature set on the instrument and the actual temperature in the sample. This might be necessary because the transfer of heat from the cooler/heater block may not be 100% efficient.

To change the current temperature, go to the “Temperature” field and input the new temperature. Click “Apply”, then “Send”. The current temperature of the cooler/heater block will be displayed in the “Temperature Monitor” section.

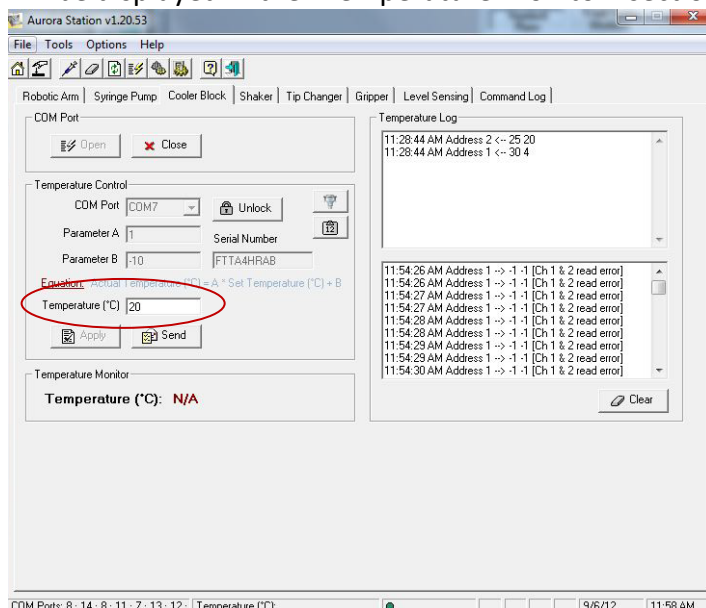


Figure 15: Aurora Station/ Cooler Block Tab

12.4.4 Shaker Tab

The Shaker Tab allows the user to set shaker Com ports and test the speed of the shaker manually. Shaker speed can be tested between 100-2200rpm. Change “Speed” and “Time” settings, then click “Apply” to implement the settings. The new settings can now be tested by clicking the “Shake” button.



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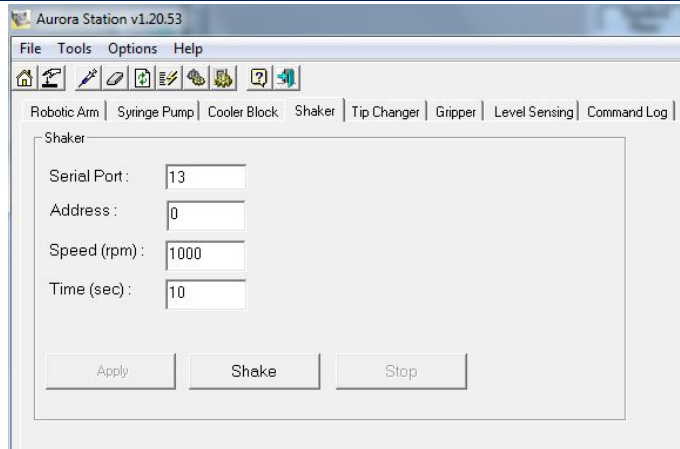


Figure 16: Aurora Station/ Shaker Tab

12.4.5 Tip Changer Tab

The Tip Changer Tab will allow the user to command the instrument to release the tips that are currently engaged. The settings currently set do not need to be changed.

Initialize the instrument by clicking the “Initialize” button. Place a container under the tip barrels to catch the tips. Click “Send” to signal the instrument to release the tips.

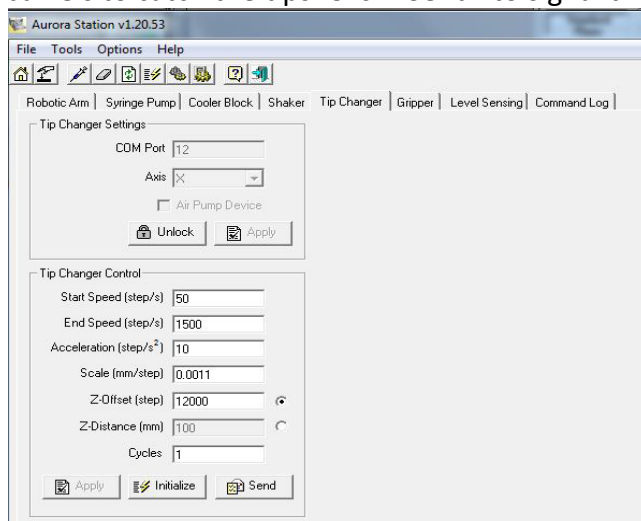


Figure 17: Aurora Station/ Tip Changer Tab

12.4.6 Gripper Tab

The Gripper Tab displays all the settings for the gripper, including the speed settings and gripper control settings.

- **Gripper X-Offset** – Determines where on the plate the gripper will pick up. This setting will be used to center the grip on the plate.
- **Gripper Y-Offset** – Determines the Y-offset for the gripper to pick up the plate. Increasing the number brings the gripper towards the user.



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- **Gripper Z-Offset** – Determines how far down the gripper will go to pick up the plate. Increasing the number makes the gripper go lower.
- **Engagement Off-set** – The height where the gripper claw opens up. The number is the height above the plate surface
- **Gripper Safe Off-set** – The height in which the gripper will lift the plate during transport. The number is the height above the plate surface
- **Gripper Max Range** – The maximum z range the gripper can move up or down
- **Gripper Pad Clearance** – The height that gripping of the plate occurs. 0 is the top surface of the plate.
- **Gripper Plate Type** – Plate type used
- **Source Position** – Deck position where the plate is initially located
- **Target Position** – Deck position where the plate will be transferred to

To test the gripper, place a plate (96-well Slicprep™ Plate) onto deck position 11. Select “11” as the “Source Position”. Select “12” as the “Target Position”. Initialize the robot by clicking the “Initialize” button”. Click “Move” to move the plate from position 11 to 12 using the gripper.

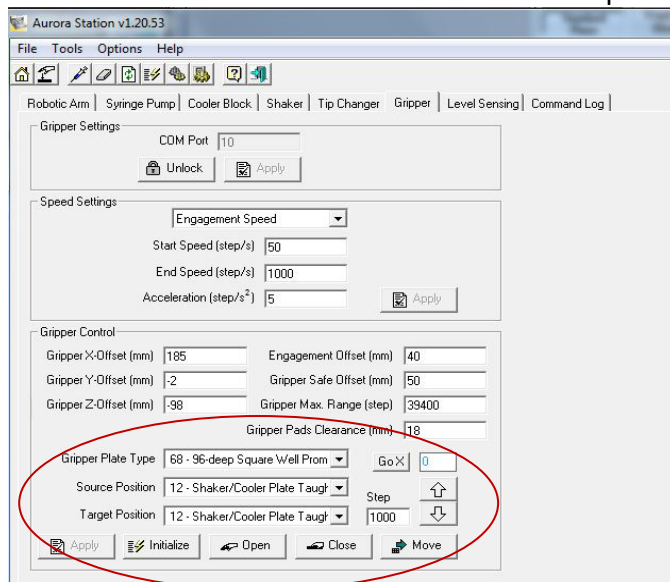


Figure 18: Aurora Station/ Gripper Tab

12.4.7 Level Sensing Tab

The Liquid Sensing Tab displays the liquid level sensing signals for all four channels. The signal from each channel is read every second and is recorded in the “Level Sensing Log” on the right. The signal of each channel is also displayed on the bottom right of the screen next to the date and time. A real-time graph of the log is displayed in the “Level Sensing Monitor”. The signal is typically around 100 when no liquid is detected. Once a tip detects liquid, the signal will increase.



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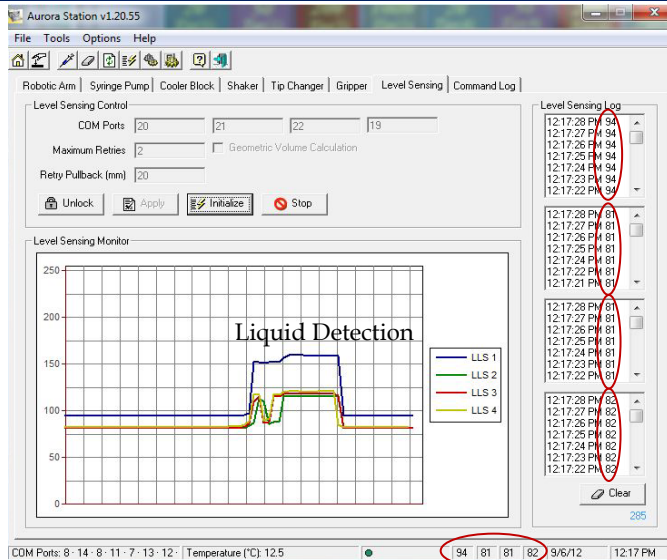


Figure 19: Aurora Station/ Level Sensing Tab

12.4.8 Command Log Tab

The Command Log screen displays the summary of all the executed steps created since the VERSAware was last opened. Click “Clear” to erase logged message once they have been identified and rectified.

12.5 Aurora Station Settings

Click on the “Aurora Station settings” icon  to configure settings of the system.

- **General Settings**

The General Settings Tab can be used to assign deck positions, activate or deactivate various display icons or tabs. These settings are pre-configured and should optimize the performance of the instrument. They are specific to each workstation leaving Aurora’s manufacturing facilities. Contact Aurora Biomed should changes need to be made. Ensure that you click “Apply” to implement any changes in the settings.

- **Advanced settings**

In the Advance Settings tab, changes regarding Tip Engagement (speed), Cooling Block, Shaker (speed), Communication, Auto Recovery, Tip Disengagement, Syringe Pump Priming, Air Gap Adjustments, and etc. can be performed. However, it is strongly recommended not to change the pre-set parameters. In most cases, the users only need to use the “Test Position” function on the bottom left corner to perform testing and offset adjustments. Remember to click “Apply” to implement any changes made.



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- Accessories Settings**

The Tip Box positions, Chute, Pipetting Tip Types, Pin Groups, and Shaker configurations are tabulated in the Accessories Settings. Depending on the configuration of the system and the modules in the workstation, some of these options may not apply. These settings come pre-configured or your specific applications. Check with Aurora Biomed for application specific development.

Tip Availability can be change/adjusted in the “Tip Box Positions” section. Under the correct Tip Box Position, change the value for the “Next Row” and “Next Column”. Since the instrument uses a 4-channel pipettor head, the only values used for the “Next Row” field should be 1 or 2. The values available for the “Next Column” field ranges from 1-13; 13 indicates that the tip box is empty.

The screenshot shows the 'Accessories' tab in the software. The 'Tip Box Positions' table is highlighted with a red box. The table has columns: Tip Box Position, Tip Type, Tip Box Type, Next Row, Next Column, and Channel. The 'Pipetting Tip Types' table is also visible below it.

Tip Box Position	Tip Type	Tip Box Type	Next Row	Next Column	Channel
1	4	18	1	1	Yes
2	4	18	1	1	Yes
3	4	18	1	2	Yes
4	2	21	1	1	Yes
5	2	21	1	1	Yes
6	3	15	1	1	Yes
7	2	21	1	1	Yes

Tip Type	Description	Tip Box Type	Engagement Offset	Disengagement Offset	Engagement Depth	Tip Length	Offset
1	20 µL Tips	20	6	12	10.8	31.74	0
2	1000 µL Tips	21	6	12	9.8	95.82	0.5
3	200 µL Tips	22	6	12	10.2	58.13	-0.3
4	200 µL Tips	15	6	12	10.2	58.13	-0.3

- Plate Type Settings**

All plate types configuration are tabulated in the Plate Type settings tab. Depending on the configuration of the system and the modules in the workstation, some of these options may not apply. Specific settings, including plate offsets, are recorded for each plate type. The column, row, and height offsets account for the differences between the 96-well teaching plate and the specific plate type. If the pipette tip is not centered after calibrating the teaching position, adjustments to the plate offsets can be made here.

- Hardware Settings**

In the Hardware Settings tab, the devices attached to the workstation along with the associated COM ports are configured here. Speed and acceleration steps of the robotic arm



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and pump can also be configured. Maximum moving range of the Robotic Arm has already been set and configured. Click “Apply” to implement any changes made.

12.6 PWM Pump


The PWM Pump is a support interface that controls all of the liquid pumps, including the single channel syringe, 4-channel syringe, and reagent drop. All of the settings regarding the liquid pumps can be accessed here. Volume calibration checks can also be performed here. There are two ways to access the PMP Pump:

1. Open the “Shell Status” Window which runs alongside VERSAware. Double-click on “DH01 [1]: 4292” and the PWM Pump will open from the background.
2. In VERSAware, go to “Tools”, then click on “PWM Pump”. A checkmark will appear next to the interfaces if it is already opened.

There are three modes in the PWM Pump:

1. **A (Assay Mode)** – In order to run a protocol, the instrument must be in assay mode
2. **C (Control Mode)** – This mode allows the user to gain access to manual control of the pump
3. **D (Demo Mode)**

12.7 Reagent Drop Calibration Check

Prior to performing a reagent drop calibration check, prime the reagent drop system by clicking the “Pump” icon  on the VERSAware interface. Type “1000” into the Reagent Drop Priming Volume. Click on the “Reagent” button on the bottom.

On the PWM Pump interface, initialize the pump by clicking the “Initialize” button on the top left corner. To perform volume calibration check, click on the “C” icon to switch into control mode. When the pump is operational, the pressure can be observed by clicking the “Chart” button on the bottom right corner. A graphical pressure curve in real-time will appear, showing the user the current pressure maintained. The pressure should be maintained at about 10 psi.



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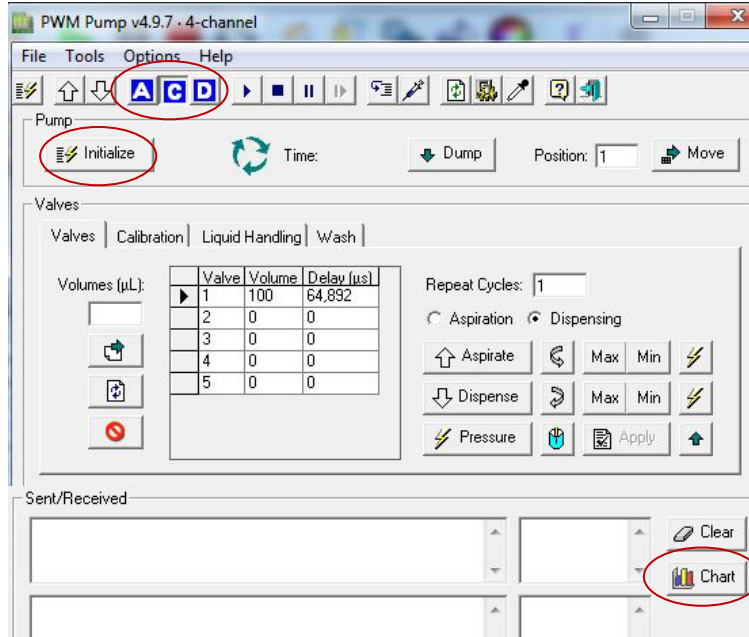




Figure 20: PWM Pump interface

Click on the “Syringe” icon  on the top. This puts the instrument in calibration mode. When the calibration mode is selected, the values that the user inputs for volume is actually in terms of delay time, which is the length of time the valve is opened for in micro-seconds (μ s).

Click on the “Pump” icon  to open the pump calibration table. This is location where all of the calibration scales for the reagent drop is maintained. New calibration data will be recorded here. The volumes for the reagent drop calibration check can be adjusted in the “Dispensing” table. A new scale can be created instead by clicking on the empty row in the “Scales” table. Enter the date and the liquid used to perform the volume calibration check. Under the “Aspiration” table, enter the numbers 1 – 5 in the “Valves” column. Enter “0” in the “Volumes” column. Enter “1” in the “Cycles” column. Enter “0” in the “Pulse” column. Enter “0” in the “Delay” column. Click “Apply”, then “OK”



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Aspiration:

Valve	Volume (µL)	Cycles	Pulse (µs)	Delay (µs)
1	0	1	0	0
2	0	1	0	0
3	0	1	0	0
4	0	1	0	0
5	0	1	0	0

Dispensing:

Valve	Volume (µL)	Cycles	Pulse (µs)	Delay (µs)
1	0	1	0	0
1	1.8	1	20,000	20,000
1	3.1	1	30,000	30,000
1	4.2	1	40,000	40,000
1	5.9	1	50,000	50,000
1	7.2	1	60,000	60,000
1	15.8	2	60,000	120,000
1	23.9	3	60,000	180,000
1	40.7	5	60,000	300,000
1	83.8	10	60,000	600,000
1	340	40	60,000	2,400,000
1	509.6	60	60,000	3,600,000
1	849.5	100	60,000	6,000,000
1	1019.2	120	60,000	7,200,000
2	0	1	0	0
2	1.6	1	20,000	20,000
2	3.2	1	30,000	30,000
2	4.2	1	40,000	40,000
2	5.8	1	50,000	50,000
2	6.7	1	60,000	60,000
2	22.5	3	60,000	180,000
2	38	5	60,000	300,000
2	69.9	10	60,000	600,000
2	283.2	40	60,000	2,400,000

Scales:

Scale
Scale - 8/2/2012 Water @ 10 psi
Scale - 12/13/2011
Scale1

Figure 21: Pump Calibration table

In the PWM Pump main screen, select the “Valves” *Tab* in the “Valves” section. Select the pin valve to calibrate (*Only valves 1 – 3 are available on this instrument*). Type “50,000” in the “Volume” column. Make sure that the other valves have “0”. Remember, in the calibration mode, this is actually the delay time in µs that the valve will be opened for. Type “1” for the number of “Repeat Cycles” on the right. Make sure that “Dispensing” is selected. Place an empty, pre-tared weigh boat underneath the reagent drop valve, then click “Dispense”. Weigh the volume and record in the pump calibration table.

Valves

Valves | Calibration | Liquid Handling | Wash


Volumes (µL):

Valve	Volume	Delay (µs)
1	100	64,892
2	0	0
3	0	0
4	0	0
5	0	0

Repeat Cycles: 1

Aspiration Dispensing

Figure 22: PWM Pump/Valves Tab

Click on the “Pump” icon  to go into the Pump Calibration screen. In the “Dispensing” table, type the pin valve number that is being calibrated in the “Valve” column. Type 0, 1, 0 in the “Volume, Cycles, Pulse” columns. In the next row, type “1” in the “Cycles” column and “100,000” in the “Pulse” column. The “Delay” will automatically be calculated using the equation: Cycles x Pulse = Delay. Record the volume that was measured in the weigh boat (1mg = 1µL).



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Measure the volume at multiple cycles by changing the number of “Repeat Cycles” in the main screen. Cover the range from 100,000 – 6,000,000µs (~10 – 750µL). There is no need to do every cycle to reach 6,000,000. Measure an average of about 10 – 15 cycles. After calibration check of one pin valve is completed, repeat for the 2nd and 3rd valve. Enter 0, 1, 0 in the “Volume, Cycles, Pulse” columns for Valves 4 and 5. Click “Apply”, then “OK”. Switch back to the Assay Mode by clicking on the “A” icon when finished with the volume calibration checks.

12.8 Test Volume Accuracy

The PWM Pump can be used to manually dispense certain volumes of liquid. This function allows the user to periodically test to see if the pump is delivering the accurate volume of liquid.

In the PWM Pump main screen, click the “C” icon to go into Control Mode. Make sure the instrument is not in calibration mode – the “Syringe” icon should not be selected. Type “1” for the number of “Repeat Cycles” on the right. Type the volume of liquid to dispense in the “Volume” column next to the valve that is to be tested. *(Note: do not put the type the volume of liquid to dispense in the “Volume” box on the left).* Place an empty, pre-tared weigh boat below the reagent drop pins and click “Dispense”. Weigh the weigh boat and determine if the instrument is accurately dispensing the volume of liquid. If it is not, a volume calibration check might need to be performed. Refer to **Figure 21**.

12.9 Versaware

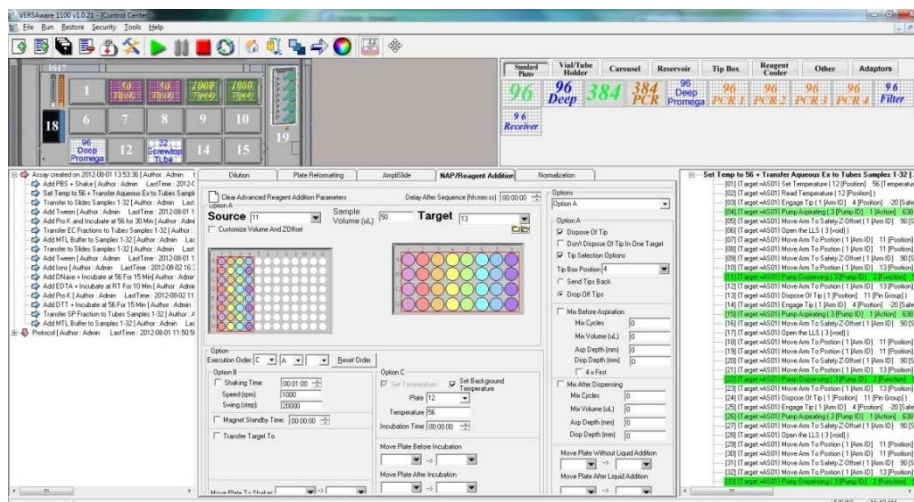


Figure 23: VERSAware interface















12.9.1 Icon Tool Bar

There are a total of 17 user icons available for one-click operations.

- New assay** – Click to add a new assay. Enter new assay’s name and click “OK”. The user can then add any number of sequences to the assay.






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- **New Sequence**  – Click to add a new sequence to an assay. Sequence created can be saved to any existing assay by selecting/highlighting the assay present on the “Assay/Sequence Window” on the left side of the main interface screen.
- **Save Current Sequence**  – Click to save any changes made to any existing sequence. It is important to update any changes made to an existing sequence by clicking the “Save” icon, otherwise the changes will not take effect.
- **Delete**  – Click to delete a selected assay or sequence. Select an assay/sequence by highlighting it, then click the “Delete” icon.
- **Restore**  – Click to auto backup created assays/sequences or restore after deleting assays/sequences. Selected assay/sequence can be restored by highlighting it, then clicking the “Restore” icon.
- **Options**  – Click to enter the VERSAware options. User can make changes to various settings related to the workstation. For further detailed instructions, see the section “Menu Drop-Downs → Options”.
- **Start**  – Click to start a selected assay/sequence.
- **Pause**  – Click to pause an assay/sequence. User can pause at any stage of the sequence. Unclick to resume the run. Sequence will continue from where it left off. The pause function can be used when changes need to be made or to refill tip boxes.
- **Stop**  – Click to stop an assay/sequence. Sequence will start again from the very beginning if user chooses to run the sequence again.
- **Run Continuously**  – Click to run an assay/sequence continuously. Assay/sequence will run continuously until the “Stop” icon is clicked.
- **Home**  – Click to send the robotic arm to the home position and to initialize all devices.
- **Pump**  – Click to prime the syringe pump system.
- **Reload Tip Box**  – Click to reload tip boxes. Used to reload tips when prompted with an audible buzz after the robotic arm has picked up its last tip from any box.
- **Transfer Tip**  – Click for single channel operation. Arm proceeds to transfer tips from the 8-channel tip box of specified position to the targeted single-channel tip box position for single channel use.
- **Color Selector**  – Click to select colors for samples. Color coding the samples will allow the user to follow where a particular sample has been transferred to during sample transferring steps or slide preparation steps.



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- Single/Four Channel**   – Click to switch between single and 4-channel operation. The icon will change from a single channel to multi-channel to reflect the specific operation that is selected.
- Restore Interface**  – Click to refresh the VERSAware main interface.

12.9.2 Drag-Drop Plate Library

Aurora Biomed has accounted for a wide array of plate types found in the market. These have been configured for use on the VERSA 1100 platform to allow users to aspirate from and dispense into. Offsets have also been preset during the manufacturing and testing process. Thus all plate types are ready to use without further user calibration check.

To use a particular plate, move the mouse cursor to that plate type's icon located in the "Plate Library" section on the top right side of the main screen. Click and hold onto that icon while dragging it over to the "Working Deck Configuration" section on the top left side of the main screen. Release to drop the icon onto any of the number position (1 – 15) on the deck.

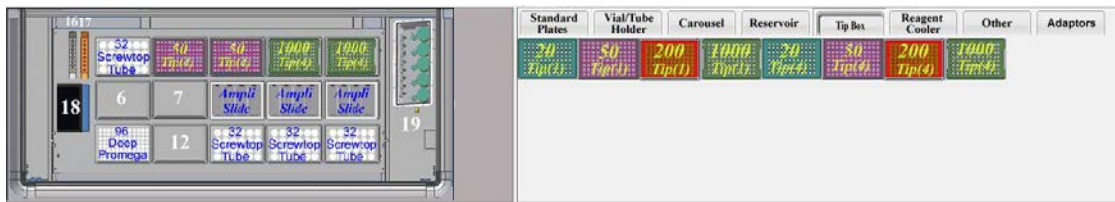


Figure 24: Position decks and plate type library

12.9.3 Menu Drop-Downs

File

The "File" drop-down menu can be used to add an assay/sequence, update a sequence, or delete an assay/sequence. Users can also export or import assays/sequences to/from different file formats. Click on "Export" to save a file or picture (screen shot) to a text (.txt) or picture (.bmp) file, respectively. Users can also print the present screen picture or sequence.

Run

The "Run" drop-down menu can be used to start an assay/sequence, to immediately start a newly created sequence on-screen, and to pause or stop any assay/sequence that is currently running.

Tools

The "Tools" drop-down menu can be used to access the various options that can be used to edit various system settings. The Aurora Station and Aurora PWM Pump can be accessed in this drop-down menu.



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Options

The Options window allows the user to edit or change various system/instrument/plate type settings according to their requirements. When the “Options” window is accessed while a sequence is opened, the changes made in the “Options” will only apply for that particular sequence and not for the entire system/instrument. It is important to click on the “Apply” button and click the “Save” icon for the changes to take effect.

- **Syringe Pump Setting**

The option to prime the syringe pump before running each sequence to remove air bubbles from the tubing system is available. To do so, check the “Prime syringe pump before running each sequence” option on the very top.

Users can also set air gap volumes, mixing air gap volumes, extra aspiration/dispensing volumes for either the single 1000µL pump or the 4-channel 1000µL pump. Delays after aspiration/dispensing can also be added in the sequence. This is useful in cases where the solution is viscous. Pump aspiration/dispensing speeds for solutions and air gap aspiration speed can be set.

- Max Volume – The maximum amount of volume that the syringe pump will aspirated.
- Air Gap Volume – Volume of air that the tip will aspirate prior to aspirating the sample
- Extra Aspiration Volume – Extra volume of liquid that will be aspirated for multiple dispense options
- Extra Dispensing Volume – Extra volume of liquid that will be dispensed
- Priming Cycles – Number of cycles to prime the syringe pump. 8-10 cycles is generally sufficient
- Back Aspiration Volume – The volume of liquid the syringe pump uses to prime for each cycle.
- Mixing Air Gap Volume – The volume of air that the tip will aspirate between each cycle during a mixing step.

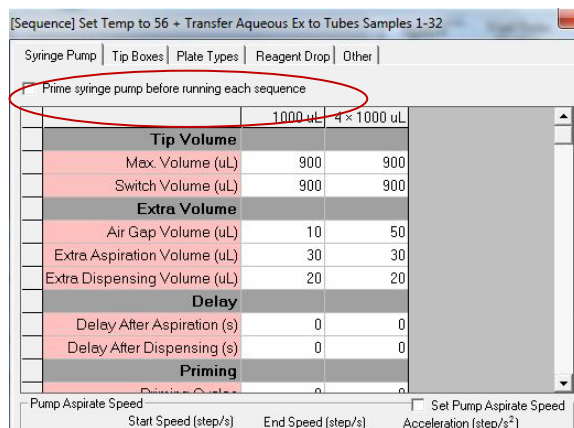


Figure 25: VERSAware/Tools/Options/Syringe Pump Tab



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- Tip Boxes Setting

The Tip Box tab includes the safe Z-offset for safe arm movement above the tip boxes to avoid hitting it. The values set here are set as default data. No change is necessary. The maximum volume which can be aspirated/dispensed for each tip type can also be set here.

- Plate Type Settings

The Plate Type tab allows the user to change Z-offsets for any particular plate type. This tab also displays the plate types available for this system that have been pre-specified by the manufacturer. Any changes made here will only affect the current sequence and not the whole system.

The “Aspiration and Dispensing Z-Offset” specifies the height at which the pipette tip will aspirate or dispense. An offset of 0mm is equivalent to the surface of the plate. If the tip dispenses liquid **too far from the bottom** of the well of a plate, **increase** the Z-Offset for dispense by increments of 1 or 2mm first, click “Apply, then click the “Save” icon. Run the sequence again to test if the tip is going far down enough. The same applies for aspiration.

The “Movement Z-offset” specifies the height in which the robotic arm will move above the plate type. **Decreasing the Z-offset** will make the arm move at a position **closer to the plate**. This will save some time during multiple dispensing.

Likewise, the Reagent/Cooler Block Z-offsets can be adjusted here. These can be changed for each sequence depending upon the volumes in the tubes.

Plate Type	Aspiration Z-Offset (mm)	Dispensing Z-Offset (mm)	Movement Z-Offset (mm)	Volume (uL)
96-Well Plate	10	10	15	350
384-Well Plate	7	5	15	120
12-Column Reservoir	11	10	15	40000
AmpliSlide	-1	-0.1	8	100
96-Deep Well Plate	39	35	15	1000
96-Well PCR Plate	16	12	20	300
384-Well PCR Plate	12	10	25	120
12-Deep Column Reservoir	23	20	15	40000
8-Deep Column Reservoir	23	20	15	40000

Figure 26: VERSAware/Tools/Options/Plate Types Tab

- Reagent Drop Settings

The priming volume for the reagent drop can be changed here, along with its dispensing Z-offset. The dispensing Z-offset is the height above the plate type in which the reagent drop



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will dispense liquid. **Increasing** the “Dispensing Z-offset” will make the reagent drop dispense **further from the surface** of the well.

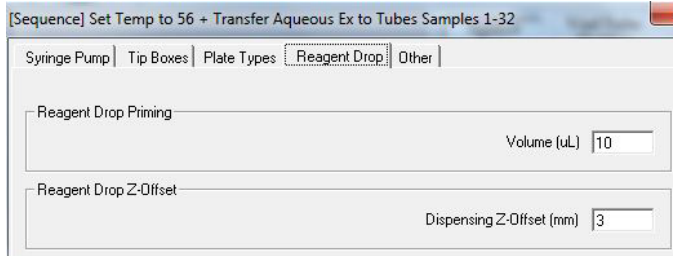


Figure 27: VERSAware/Tools/Options/Reagent Drop Tab

- Other Settings

The options in this tab allow the user to set different Z-offsets for mixing, air gap, and arm movement from one position to another. These settings, if used properly, reduce the total time of an assay. Arm speed for dispensing and handling waste can be set here. Liquid Level Sensing (LLS) can be activated here for each sequence. Arm initialization options for before and after any sequence can be adjusted here.

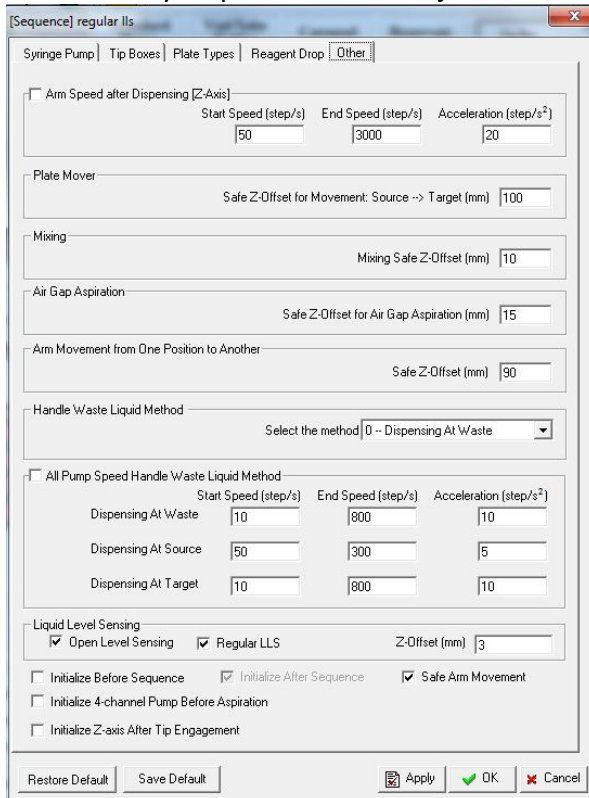


Figure 28: VERSAware/Tools/Options/Others Tab



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Configuration

The Configurations window specifies basic settings/accessories for the instrument. The instrument type is set to "VERSA 1100". The "Shaker" position is set to "12". The "Reagent/Cooler Block" position is set to "12". No Magnetic Stand position is specified as it does not exist in this instrument platform. The "Plate Mover" is set to "Gripper". The "Reagent Drop Pins" is set to "2-5".

Help


The "Help" drop-down menu provides details of the software version type for the VERSAware 1K. For further assistance regarding this software, please contact Aurora's service center.

12.9.4 Application Tabs

Plate Reformatting

This application tab allows user to transfer from one plate type to another. For example, samples from a 96-well plate can be reformatted/transferred to a 32-sample tube block (4x8). The ability to transfer to/from a 6x8 sample tube block exists but will be in a single-channel mode. Other options include dilution with buffer from the reservoir/reagent cooler block, mixing of the diluted target sample, and delays before and after the application run. Advance options include plate replication.

To perform a plate reformatting from a 96-well plate to a 32-sample tube block, follow the steps below:

1. Drag and drop the plate/tube block type from the "Plate Library" on the top right side to the "Deck Configuration" on the top left side to their corresponding positions. Also drag the necessary 4-channel pipette tips into the tip positions. (*The 4-channel tip boxes are indicated with a 4 in parentheses*).
2. Select the position of the source from the "Source" drop-down menu. (In this case, it will be the 96-well plate).
3. Select the position of the target from the "Target" drop-down menu. (In this case, it will be the 32-sample tube block).
4. Make sure that the 4-channel pump is selected in the "Single/Four Channel" icon . In the source section, select the wells to be transferred. Then in the target section, select the wells for the samples to be transferred into.
5. In the target section under the "Sample Volume" field, input the volume of the sample to be transferred.
6. Other options are available which can be included in the sequence (mixing, delays, tip disposal after every dispense, plate replication)



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- Remember to save your created sequence by clicking on the “New Sequence” icon. If this is an addition to an existing assay, highlight the assay name of the left screen and then proceed to click the “New Sequence” icon. You would be given the option to enter the new sequence name.

If a new assay has not yet been created, then proceed to click the “New Assay” icon, name the assay, then click “OK”. Then, click on the “New Sequence” icon and rename the sequence, then click “OK”.

If dilution is required, drag and drop a diluent source (reservoir or cooling block) onto the deck interface, and select it under the “Reservoir Position” field. A “Final Volume” field will appear under the target section which you will then input the final amount for each well.

The plate replication option allows the user to reformat from one source plate to 4 similar target plate types.

AmpliSlide

This application tab allows the user to set up a sequence in which reagents are transferred onto a microscope slide. One slide adaptor can hold up to 4 microscope slides.

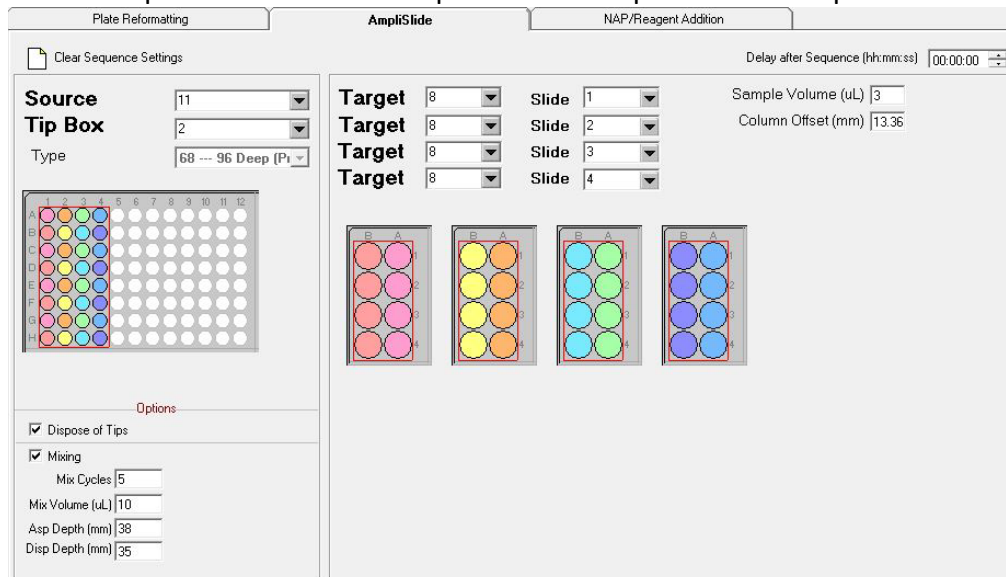



Figure 29: VERSAware/AmpliSlide Tab

To create an AmpliSlide sequence, follow the steps below:

- Drag and drop the AmpliSlide adaptors and any other plate/tube block type from the “Plate Library” on the top right side to the “Deck Configuration” on the top left side to their corresponding positions. Also drag the necessary 4-channel pipette tips into the tip positions. (*The 4-channel tip boxes are indicated with a 4 in parentheses*).



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2. Select the position of the source from the “Source” drop-down menu. (In this case, it will be the 96-well plate).
3. Select the position of the target from the “Target” drop-down menu. (In this case, it will be the AmpliSlide). The target position and the slide position will need to be selected for each of the four slides on the AmpliSlide adaptor.
4. Make sure that the 4-channel pump is selected in the “Single/Four Channel” icon . In the source section, select the wells from which the samples will be transferred. Then in the target section, select the wells for the samples to be transferred into.
5. In the target section under the “Target Volume” field, input the volume of the sample to be transferred.
6. Other options are available which can be included in the sequence (mixing, delays, tip disposal after every dispense)
7. Remember to save your created sequence by clicking on the “New Sequence” icon. If this is an addition to an existing assay, highlight the assay name of the left screen and then proceed to click the “New Sequence” icon. You would be given the option to enter the new sequence name.

If a new assay has not yet been created, then proceed to click the “New Assay” icon, name the assay, then click “OK”. Then, click on the “New Sequence” icon and rename the sequence, then click “OK”.

The mixing parameters to resuspend the sperm pellet can be changed.

- Mixing Cycles – The number of mixing cycles
- Mix Volume – The volume aspirated and dispensed during the mixing cycle
- Asp Depth – The depth the pipette tip will go down to do aspirate the sample to mix
- Disp Depth – The depth the pipette tip will go down to dispense the mixed sample

(Note: the aspiration and dispense depth in the mixing is separate from the aspiration and dispense depth in the options menu, which specifies the depth to aspirate and dispense the sample for transferring from the plate to slides)

NAP/Reagent Addition

This application tab allows the user to set up the workstation for multi-dispensing reagents from reservoir/96-well plates to target plates. This application tab also allows the user incorporate the shaker and the cooler/heater block. The order of execution can be specified under the “Options” section. Options A, B, or C can be selected in the drop-down menus. To change the order, click the “Reset Order” button.



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- **Option A – Liquid transfer**
- **Option B – Shaker**
Select the shaking time, speed, and swing. A plate can be moved from its original position to the shaker and back by selecting the positions on the bottom of the Option B section.
- **Option C – Cooler/Heater Block**
This option allows for incubation steps. The temperature and incubation time can be set. Make sure that position 12 is selected for the “Plate” field, as this is where the cooler/heater block is located. A plate can be moved from its original position to the cooler/heater block and back by selecting the positions on the bottom of the Option C section.

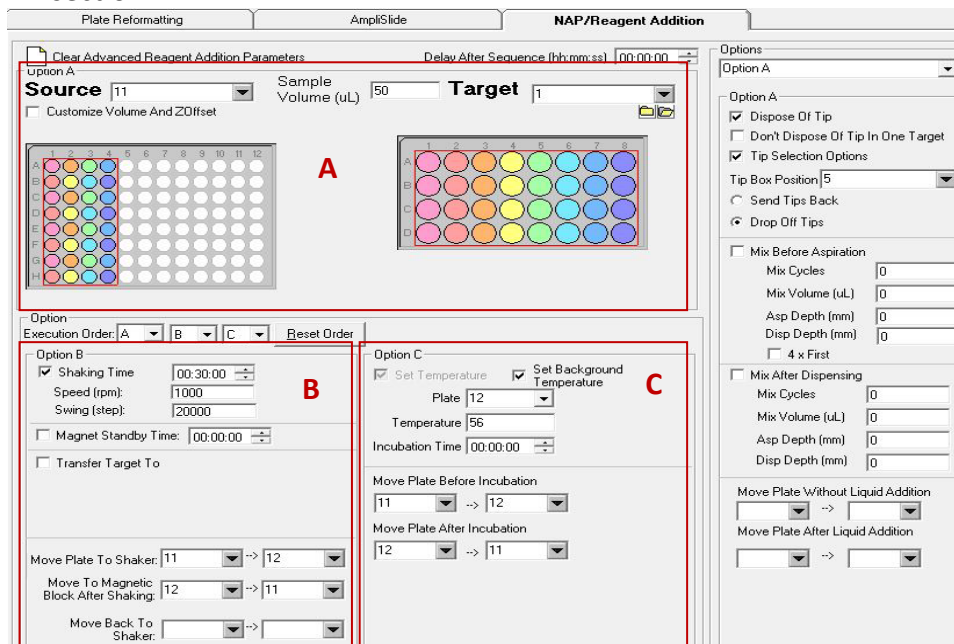



Figure 30: VERSAware/NAP Reagent Addition

To create a NAP/Reagent Addition sequence, follow the steps below:

1. Drag and drop the plate/tube block type from the “Plate Library” on the top right side to the “Deck Configuration” on the top left side to their corresponding positions. Also drag the necessary 4-channel pipette tips into the tip positions. *(The 4-channel tip boxes are indicated with a 4 in parentheses).*
2. Select the position of the source from the “Source” drop-down menu. To use the Reagent Drop, select position 19.
3. Select the position of the target from the “Target” drop-down menu.
4. Make sure that the 4-channel pump is selected in the “Single/Four Channel” icon . In the source section, select the wells from which the samples will be transferred. Then in the target section, select the wells for the samples to be transferred into.



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- In the “Sample Volume” field, input the volume of the sample to be transferred.
- Other options are available which can be included in the sequence (mixing, delays, tip disposal after every dispense)
- Remember to save your created sequence by clicking on the “New Sequence” icon. If this is an addition to an existing assay, highlight the assay name of the left screen and then proceed to click the “New Sequence” icon. You would be given the option to enter the new sequence name.

12.9.5 Assay/Sequence Screen

The Assay/Sequence screen is located on the left side of the main interface screen. It contains all of the available assays (indicated by a red arrow). Click on the assay to display all of the sequences within the assay (indicated by a blue arrow). To start running an assay, highlight the assay/sequence in this screen, then click the “Start” icon. The assays/sequences can be exported by highlighting it, then clicking “File → Export → Export Assay/Sequence”. Assays can also be imported by clicking “File → Import”.

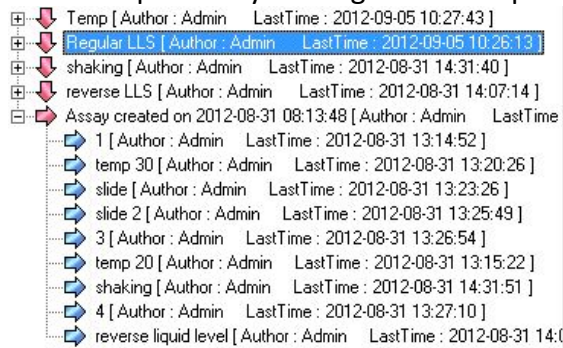


Figure 31: Assay/Sequence screen

12.9.6 Sequence Steps Screen

The Sequence Steps screen is located on the right side of the main interface screen. When an assay/sequence is selected, it will display every single step or action that the instrument will take during that particular assay/sequence. Make sure that the assay/sequence is displayed on the Sequence Steps Screen before starting a run. A particular sequence can be resumed by right click on the sequence, then clicking “Resume the step”.

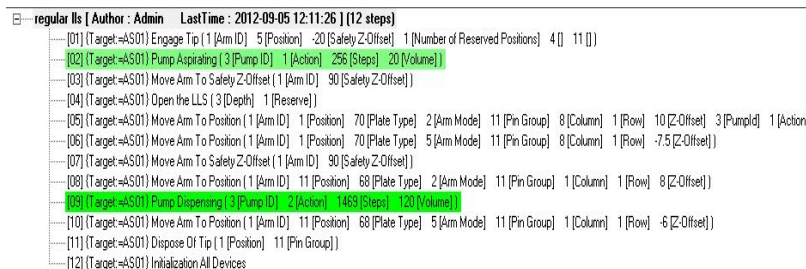


Figure 32: Sequence steps screen



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4. The “Level Sensing Log” on the right side will show the signal levels following the time at various refreshments rates
5. Place your finger slightly below the syringe/tip barrel (without a tip)
6. Note the jump of the signal from ~100 to ~200
7. Insert a black, conductive tip into the barrel
8. Place a tube with ~5-7mL distilled water underneath the tip until the tip is slightly submerged
9. Note the signal level changing from ~100 to ~200

If the above are observed when performing steps 1 – 9, then the liquid sensor is working as it should. Proceed to calibrate or check COM ports if you observe no change in the signal level.

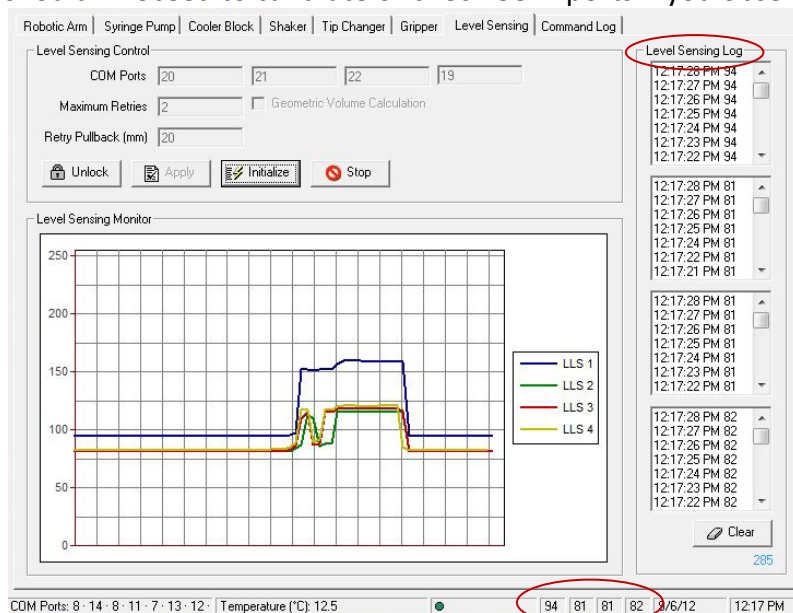



Figure 34: Aurora Station/Level Sensing Tab

LLS Calibration Check for a Current Plate Type

1. Open the Aurora Station interface and click on the “Robotic Arm tab”
2. Select an empty row in the bottom of the table, type in a random position number (100) and a comment (LLS Check – Surface Position). Leave all the other fields empty and highlight the new row entry.

Position	Step (X)	Step (Y)	Step (Z)	Comment
14	7837	2525	7737	1000 µL Tip Box/Plate
15	10233	2526	7756	20 µL Tip Box/Plate
16	0	98	7367	Priming Position
17	0	1331	7132	Waste Position
18	0	2568	6937	The Chute
*				

Figure 35: Robotic Arm Tab/Position record table

3. Click on the “Settings” icon  in the top menu, then the “Advanced” tab.



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4. On the bottom left corner under “Test Position”, select the position to test. To the right under “Plate Type”, select the plate type to test.

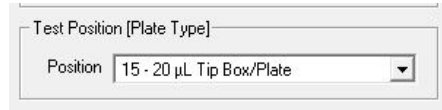


Figure 36: Aurora Station Settings/Advanced Tab

5. Click on the “Plate Types” tab.
6. Under the “Symmetrical Plates” section, select the same plate type by highlighting the field.
7. In the “Liquid Level Sensing” table to the right, also select the corresponding plate type (refer to the plate type number). There will be several entries of the same plate type with different depth & volume. Select the entry with the lowest volume (not including 0).

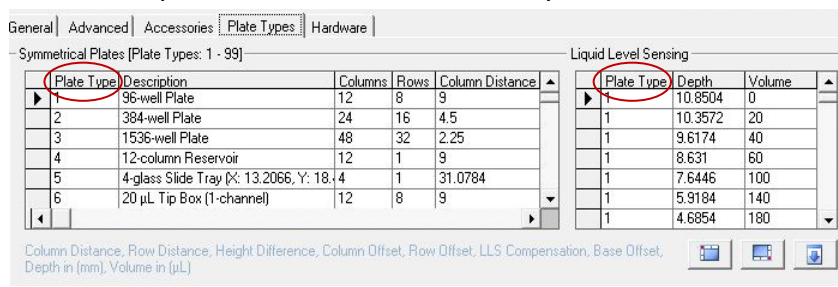


Figure 37: Aurora Station Settings/Plate Type Tab


8. Using a manual pipette, dispense an equal amount of that volume into the top left well (A1) of the testing plate. For example, if 10µL was selected in the table, then there should be 10µL of liquid in the well.
9. Manually insert a black, conductive tip into the first barrel.
10. Click the “Test” icon . The robot will move to well A1 position just above the surface of the plate.
11. Click on the “Robotic Arm” tab.
12. Ensure that the coordinates of the current robotic arm position is present in the “Go X, Go Y, Go Z” fields above the table.



Figure 38: Aurora Station/Robotic Arm Tab

13. Select the new entry that was created (Position 100) by highlighting it, then click the “Save” button. The current coordinates should now be saved onto the entry. This is the plate surface coordinates.
14. In the “Arm Control” section on the bottom left, enter the “10” in the “Steps” field and check “No Z Up”.



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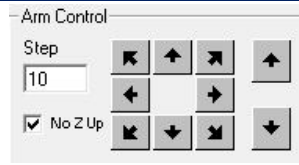


Figure 39: Aurora Station/Robotic Arm Tab

15. Note the liquid level sensing signal on the bottom right corner of the interface next to the date and time. After making a visual record of this value, attempt to move the tip down with the step arrow controls. Move down until the tip barely touches the surface of the 10 μ L liquid. You will notice the signal increasing as the tip moves down.
 - The difference in signal detection from the plate surface to the surface of the liquid (10 μ L) is the “Detection Range” in the “Plate Types” *tab*, “Liquid Level Sensing” section.
 - Detection Range: The lowest point that the robot will move down to detect liquid level. During liquid level sensing, the difference in signal must be greater than the detection range for the instrument to register that there is sufficient liquid. If the difference in signal is less than the detection range, an insufficient liquid message will pop up.
 - Base Offset: A safety mechanism to prevent the instrument from hitting the bottom of the tube/plate. If the instrument reaches the base offset depth, an insufficient liquid message will pop up.
 - This should be similar to what is detected currently. For example, if the signal was 142 at the plate surface and was 154 at the liquid surface, then the difference is 12 \pm 1 for the detection range.
 - The value in the “Detection Range” field for the plate type should be equal to or slightly greater than the “Depth” field for Volume 0.



Figure 40: Bottom of Aurora Station screen

16. To check the depth offset at the 10 μ L surface, click on an empty row in the “Robotic Arm” *tab*. Make sure that the tip is touching the surface of the liquid.
17. The new coordinates should be present in the “Go X, Go Y, Go Z” fields above the table.
18. Click on the “Offset” button to view the “Offset Calculator”.
19. Under the “Height Difference” section in the “Offset” field, the value is the height difference from the plate surface to the liquid level surface. This value should correspond to or be similar to the “Depth” value of the 10 μ L volume in the “Aurora Station Settings \rightarrow Plate Types *tab*”. If it isn’t, change or edit to the updated value.



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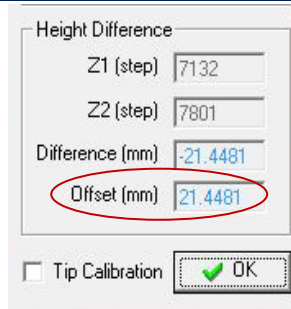


Figure 41: Aurora Station/Robotic Arm Tab/Offset Calculator

Plate Type	Depth	Volume
1	21.4481	10
1	10.3572	20
1	9.6174	40
1	8.631	60
1	7.6446	100
1	5.9184	140
1	4.6854	180

Figure 42: Aurora Station Settings/Plate Types Tab

20. Next, check the minimum level sensing at “0” depth.
21. In the “Robotic Arm” *tab*, move the tip further down with the step arrow controls until the tip surface barely touches the bottom of the well. The signal value should be similar to the 10µL liquid surface value.
22. Repeat steps 16 – 19 for the 0µL volume and depth.
23. Perform the same steps as above to check for the 20µL volume and depth. Remember to manually pipette an additional 10µL of liquid to the same well of the plate.

Check the 30µL volume using the same steps as above and so on. If the checks seem within range of the depths listed in the “Plate Types” *tab*, “Liquid Level Sensing” section, discontinue the calibration check procedures for the rest of the volumes. A rule of thumb would be to perform checks for the volumes used most frequently regarding a specific plate type. Remember to have the right amount of liquid in the well when testing for that volume.

12.10.3 Adjusting the Depth to Match Remaining Volume

For the Reverse LLS method, if the actual remaining volume is less than the targeted remaining volume, the following steps should be taken to correct the difference.

1. Check the teaching position for the source plate (Refer to Section 3.1.1.1 – Calibrate a Position)
2. Check the plate type offsets (Refer to Section 3.1.1.3 – Adjust Plate Type Offset)
3. If the teaching position and plate type offsets are correct and the actual remaining volume is still less than the targeted remaining volume, the depth in the “Aurora Station Settings Liquid Level Sensing” can be adjusted.
 - In the Aurora Station interface, click on the “Settings” *icon*, then the “Plate Types” *tab*.
 - On the right under the “Liquid Level Sensing” section, find the plate type of the source plate.
 - Adjust the depth of the corresponding volume. The value entered in for “Depth” is how far below the surface of the plate that the pipette will go down to to leave the



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corresponding volume of liquid. To leave more volume, decrease the value for “Depth” and vice versa. If the targeted volume in the sequence does not correspond to any volume in the table, the depth will be calculated via a linear relation to the volume.

- When finished, click on another cell to switch records, click “Apply”, then “OK”.

Plate Type	Depth	Volume
1	10.8504	0
1	10.3572	20
1	9.6174	40
1	8.631	60
1	7.6446	100
1	5.9184	140
1	4.6854	180

Figure 43: Aurora Station Settings/Plate Types Tab

12.11 Setting Up A Run

The VERSAware interface allows the option to set up runs with various options and tasks. Only the sequences that will be used for the automated differential digestion protocol will be described. To set up an assay/sequence, go to the VERSAware interface and click on one of the “Application Tabs”. After setting up a sequence by following the steps below, click the “Add Sequence” button. If this is the very first sequence in an assay, the software will prompt for you to name the assay, then the sequence. To add more sequences to the same assay, make sure the assay is selected prior to clicking the “Add Sequence” button. If any changes/updates are made after adding the sequence, make sure to click the “Save Current Sequence” icon  to save those changes.

The following are instructions and parameters on how to set-up the various sequences used:

- Shake**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option B	
Shaking Time (min)	30
Shaking Speed	1,000
Shaking Swing	20,000
Move Plate to Shaker	11 → 12
Delay After Sequence	9 hr
Execution Order	B



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- **Transfer Aqueous Extract to Tubes (1-32) & Set Background Temp to 56°C**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	11
Target	13
Remaining Vol. (µL)	50
Dispose of Tip	Yes
Tip Selection Option	5
Drop Off Tips	Yes
Execution Order A	

- Select wells A1 – H4 on the source plate and wells A1 – D8 on the target plate

OPTIONS TAB						
Syringe Pump (4 x 1000µL)		Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (µL)	250	96 Deep (Promega)	36	8	Open Level Sensing	Yes
		32 Screwtop Tube Holder	10	8	Regular LLS	No

- **Transfer Aqueous Extract to Tubes (33-64)**
 - Same as Transfer Aqueous to Tubes (1-32), except select position #14 as target.
 - Select wells A5 – H8 on the source plate and wells A1 – D8 on the target plate
- **Transfer Aqueous Extract to Tubes (65-96)**
 - Same as Transfer Aqueous to Tubes (1-32), except select position #15 as target and add Options B and C.
 - Select wells A9 – H12 on the source plate and wells A1 – D8 on the target plate

APPLICATION TAB – NAP/REAGENT ADDITION						
Option A		Option B			Option C	
Source	11	Shaking Time (min)	2		Set Temperature (°C)	Yes
Target	13	Shaking Speed	1,000		Set Background Temperature (°C)	Yes
Remaining Vol. (µL)	50	Shaking Swing	20,000		Plate	12
Dispose of Tip	Yes	Move Plate to Shaker	11 → 12		Temperature	56
Tip Selection Option	5	Move to Magnetic Block After Shaking	12 → 11			
Drop Off Tips	Yes					
Execution Order A, B, C						



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

- Transfer to Slides (1-32)**

APPLICATION TAB – AMPLISLIDE			
Source	11		Mixing Yes
Tip Box	2		Mix Cycles 3
Target	8		Mix Volume(μL) 20
Slides	1 – 4		Asp Depth (mm) 38
Sample Volume (μL)	5		Disp Depth (mm) 30
Dispose of Tips	Yes		

- Select wells A1 – H4 on the source plate and wells A1 – B4 for all four slides.

OPTIONS TAB							
Syringe Pump			Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (μL)	20		96 Deep (Promega)	38	8	Open Level Sensing	No
Mix Air Gap (μL)	15		AmpliSlide	-5	-5	Regular LLS	No

- Transfer to Slides (33-64)**


- Same as Transfer to Slides (1-32), except select position #9 as target
- Select wells A5 – H8 on the source plate and wells A1 – B4 for all four slides

- Transfer to Slides (65-96)**

- Same as Transfer to Slides (1-32), except select position #10 as target
- Select wells A9 – H12 on the source plate and wells A1 – B4 for all four slides
- Add Delay After Sequence: 9 hr

- Add Tween**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	19
Target	12
Sample Volume (μL)	510
Execution Order	A

- Make sure single channel mode  is selected
- Select valve #2 on the source section and select samples wells A1 – H12 on target plate



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

- **Add Pro K & Incubate at 56°C for 30 Min**

APPLICATION TAB – NAP/REAGENT ADDITION			
Option A		Option B	
Source	1	Shaking Time (min)	30
Target	11	Shaking Speed	1,000
Sample Volume (µL)	10	Shaking Swing	20,000
Dispose of Tip	Yes	Move Plate to Shaker	11 → 12
Tip Selection Option	2	Move to Magnetic Block After Shaking	12 → 11
Drop Off Tips	Yes		
Delay After Sequence	9 hr		
Execution Order A, B			

- Select wells A8 – D8 on the source plate and wells A1 – H12 on the target plat

OPTIONS TAB						
Syringe Pump (4 x 1000µL)		Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (µL)	30	96 Deep (Promega)	36	28	Open Level Sensing	No
		32 Screwtop Tube Holder	43	8	Regular LLS	No

- **Transfer EC Fractions to Tubes (1-32)**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	11
Target	13
Remaining Vol. (µL)	50
Dispose of Tip	Yes
Tip Selection Option	5
Drop Off Tips	Yes
Execution Order A	



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS


- Select wells A1 – H4 on the source plate and wells A1 – D8 on the target plate

OPTIONS TAB						
Syringe Pump		Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (μL)	250	96 Deep (Promega)	36	8	Open Level Sensing	Yes
		32 Screwtop Tube Holder	10	8	Regular LLS	No

- **Transfer EC Fractions to Tubes (33-64)**
 - Same as Transfer EC Fractions to Tubes (1-32), except select position #14 as target
 - Select wells A5 – H8 on the source plate and wells A1 – D8 on the target plate
- **Transfer EC Fractions to Tubes (65-96)**
 - Same as Transfer EC Fractions to Tubes (1-32), except select position #15 as target
 - Select wells A9 – H12 on the source plate and wells A1 – D8 on the target plate

- **Add MTL Buffer (1-32)**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	19
Target	13
Sample Volume (μL)	400
Execution Order A	

- Make sure single channel mode  is selected
- Select valve #3 on the source section and select samples wells A1 – D8 on target plate
- **Add MTL Buffer (33-64)**
 - Same as Add MTL Buffer (1-32), except select position #14 for target



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

- **Add MTL Buffer (65-96)**

- Same as Add MTL Buffer (1-32), except select position #15 for target and add Option B.

APPLICATION TAB – NAP/REAGENT ADDITION			
Option A		Option B	
Source	19	Shaking Time (min)	2
Target	13	Shaking Speed	1,000
Sample Volume (µL)	400	Shaking Swing	20,000
		Move Plate to Shaker	11 → 12
		Move to Magnetic Block After Shaking	12 → 11
Execution Order A, B			

- **Transfer to Slides (1-32)**

APPLICATION TAB – AMPLISLIDE			
Source	11	Mixing	Yes
Tip Box	2	Mix Cycles	3
Target	8	Mix Volume(µL)	20
Slides	1 – 4	Asp Depth (mm)	38
Sample Volume (µL)	5	Disp Depth (mm)	30
Dispose of Tips	Yes		

- Select wells A1 – H4 on the source plate and wells A1 – B4 for all four slides.

OPTIONS TAB						
Syringe Pump		Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (µL)	20	96 Deep (Promega)	38	8	Open Level Sensing	No
Mix Air Gap	15	AmpliSlide	-5	-5	Regular LLS	No

- **Transfer to Slides (33-64)**

- Same as Transfer to Slides (1-32), except select position #9 as target
 - Select wells A5 – H8 on the source plate and wells A1 – B4 for all four slides

- **Transfer to Slides (65-96)**


- Same as Transfer to Slides (1-32), except select position #10 as target
 - Select wells A9 – H12 on the source plate and wells A1 – B4 for all four slides
 - Add Delay After Sequence: 9 hr



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

- **Add Tween**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	19
Target	12
Sample Volume (µL)	290
Execution Order	A

- Make sure single channel mode  is selected
- Select valve #2 on the source section and select samples wells A1 – H12 on target plate

- **Add Mg/Ca Salts**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	1
Target	11
Sample Volume (µL)	25
Dispose of Tip	Yes
Tip Selection Option	2
Drop Off Tips	Yes
Execution Order A	

- Select wells A7 – D7 on the source plate and wells A1 – H12 on the target plate
- Select the option “Prime syringe pump before running sequence”.

OPTIONS TAB								
Syringe Pump (4 x 1000µL)			Plate Types	Aspiration Offset	Dispensing Offset		Other	
Air Gap (µL)	20		96 Deep (Promega)	36	38		Open Level Sensing	No
Priming Cycles	8		32 Screwtop Tube Holder	43	8		Regular LLS	No
Back Aspiration (µL)	100							



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- Add DNase & Incubate at 56°C for 15 min

APPLICATION TAB – NAP/REAGENT ADDITION			
Option A		Option C	
Source	1	Set Temperature	Yes
Target	11	Set Background Temperature	No
Sample Volume (µL)	15	Plate	12
Dispose of Tip	Yes	Temperature (°C)	56
Tip Selection Option	2	Incubation Time (min)	15
Drop Off Tips	Yes	Move Plate Before Incubation	11 → 12
Mix After Dispensing	Yes	Move Plate After Incubation	12 → 11
Mix Cycle	1		
Mix Volume (µL)	30		
Asp Depth (mm)	36		
Disp Depth (mm)	15		
Execution Order: A, C			

- Select wells A6 – D6 on the source plate and wells A1 – H12 on the target plate

OPTIONS TAB								
Syringe Pump (4 x 1000µL)			Plate Types	Aspiration Offset	Dispensing Offset		Other	
Air Gap (µL)	30		96 Deep (Promega)	36	38		Open Level Sensing	No
Mixing Air Gap (µL)	15		32 Screwtop Tube Holder	43	8		Regular LLS	No



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

- **Add EDTA & delay for 10 min**

APPLICATION TAB – NAP/REAGENT ADDITION			
Option A			
Source	1	Mix After Dispensing	Yes
Target	11	Mix Cycle	2
Sample Volume (µL)	20	Mix Volume (µL)	30
Dispose of Tip	Yes	Asp Depth (mm)	36
Tip Selection Option	2	Disp Depth (mm)	15
Drop Off Tips	Yes		
Delay After Sequence	10 min		
Execution Order: A			

- Select wells A5 – D5 on the source plate and wells A1 – H12 on the target plate

OPTIONS TAB							
Syringe Pump (4 x 1000µL)		Plate Types	Aspiration Offset	Dispensing Offset		Other	
Air Gap (µL)	20	96 Deep (Promega)	36	38		Open Level Sensing	No
Mixing Air Gap (µL)	15	32 Screwtop Tube Holder	43	8		Regular LLS	No

- **Add Pro K**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	1
Target	11
Sample Volume (µL)	10
Dispose of Tip	Yes
Tip Selection Option	2
Drop Off Tips	Yes
Execution Order: A	

- Select wells A8 – D8 on the source plate and wells A1 – H12 on the target plate



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OPTIONS TAB						
Syringe Pump (4 x 1000µL)		Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (µL)	30	96 Deep (Promega)	36	38	Open Level Sensing	No
		32 Screwtop Tube Holder	43	8	Regular LLS	No

- **Add DTT & Incubate at 56°C for 15 min**

APPLICATION TAB – NAP/REAGENT ADDITION			
Option A		Option C	
Source	1	Set Temperature	Yes
Target	11	Set Background Temperature	No
Sample Volume (µL)	20	Plate	12
Dispose of Tip	Yes	Temperature (°C)	56
Tip Selection Option	2	Incubation Time (min)	15
Drop Off Tips	Yes	Move Plate Before Incubation	11 → 12
		Move Plate After Incubation	12 → 11
Execution Order: A, C			

- Select wells A4 – D4 on the source plate and wells A1 – H12 on the target plate

OPTIONS TAB						
Syringe Pump (4 x 1000µL)		Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (µL)	20	96 Deep (Promega)	36	38	Open Level Sensing	No
		32 Screwtop Tube Holder	43	8	Regular LLS	No



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

- **Transfer SP Fraction to Tubes (1-32)**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	11
Target	13
Sample Vol. (µL)	700
Dispose of Tip	Yes
Tip Selection Option	5
Drop Off Tips	Yes
Execution Order A	

- Select wells A1 – H4 on the source plate and wells A1 – D8 on the target plate

OPTIONS TAB						
Syringe Pump		Plate Types	Aspiration Offset	Dispensing Offset	Other	
Air Gap (µL)	200	96 Deep (Promega)	38	35	Open Level Sensing	No
Delay After Aspiration	4	32 Screwtop Tube Holder	10	8	Regular LLS	No

- **Transfer SP Fractions to Tubes (33-64)**

- Same as Transfer SP Fractions to Tubes (1-32), except select position #14 as target
- Select wells A5 – H8 on the source plate and wells A1 – D8 on the target plate

- **Transfer SP Fractions to Tubes (65-96)**


- Same as Transfer SP Fractions to Tubes (1-32), except select position #15 as target
- Select wells A9 – H12 on the source plate and wells A1 – D8 on the target plate



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- **Add MTL Buffer (1-32)**

APPLICATION TAB – NAP/REAGENT ADDITION	
Option A	
Source	19
Target	13
Sample Volume (μL)	400
Execution Order	A

- Make sure single channel mode  is selected
- Select valve #3 on the source section and select samples wells A1 – D8 on target plate

- **Add MTL Buffer (33-64)**

- Same as Add MTL Buffer (1-32), except select position #14 for target

- **Add MTL Buffer (65-96)**

- Same as Add MTL Buffer (1-32), except select position #15 for target
- Set background temperature to 15°C.
- Set Execution order to be “A, C”.


12.12 Running A Protocol

- **Start-up**

Start the VERSAware software by double-clicking on the VERSAware 1100 icon on the desktop. Turn on the robot by turning clockwise the red button on the button right corner of the robot.



- **Initializing**


Each component of the instrument (syringe pump, robotic arm, shaker, etc.) must be initialized before it can function. The “Home”  will initialize all devices, drop off any tips that are engaged, and return the robotic arm to its safe position. It is a good practice to “home” the robot once it is turned on.

- **Syringe Pump Priming**

The syringe pump must be primed before each run. This will remove any air leaks or air bubbles in the tubing system, which may cause inaccurate pipetting volumes. To do so, click



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on the “Syringe Pump”  , then the “4x1000µL” *tab*. Check the “Priming” option, and type in “8” and “100” for the “Priming Cycles” and “Back Aspiration Volume”, respectively. Click the “Prime” button to start.

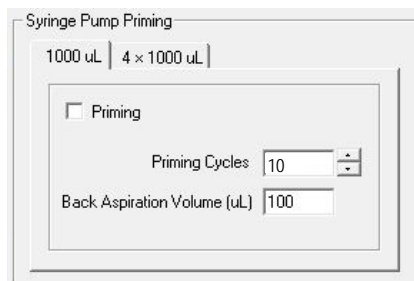



Figure 44: Syringe Pump Priming

- **Reagent Drop Priming**

At the end of a run, the reagent drop tubings will be filled with water to prevent crystallization within the tubings. Prior to starting a run, these tubings must be filled with the appropriate buffers for the protocol. To distinguish between water and buffers, the water in the tubings will first be flushed out with air, then filled with buffer.

Place a container under position 6 to catch the liquid. Remove the two red bottle caps from the deionized water bottles and place them in empty bottles. Click on the “Syringe Pump”  . In the “Reagent Drop Priming” section, type in “2000” for the “Priming Volume”. Click on the “Reagent” button to start priming. This will remove any water in the tubes and fill it with air. After the run is finished, place each of the caps in their corresponding reagent bottle (Bottle 2: Tween 80 ; Bottle 3: MTL Buffer). In the “Reagent Drop Priming” section, type in “4000” for the “Priming Volume”. Click on the “Reagent” button to start. The first 1000 – 2000µL will be just air while the system is beginning to fill itself with the reagents. When liquid is dispensed from the reagent drop valves, this indicates that the tubes are now filled with the reagent and is ready for use.

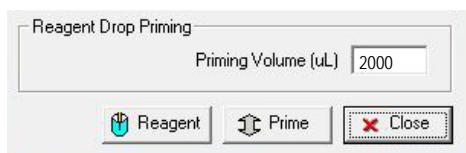


Figure 45: Reagent Drop Priming

- **Starting an assay/sequence**

In the VERSAware interface on the left side of the screen, click on the assay/sequence to run. To run an entire assay, click on the assay. To run a specific sequence, click on the sequence. Make sure that the assay/sequence is displayed in the “Sequence Steps Screen” on the very right.



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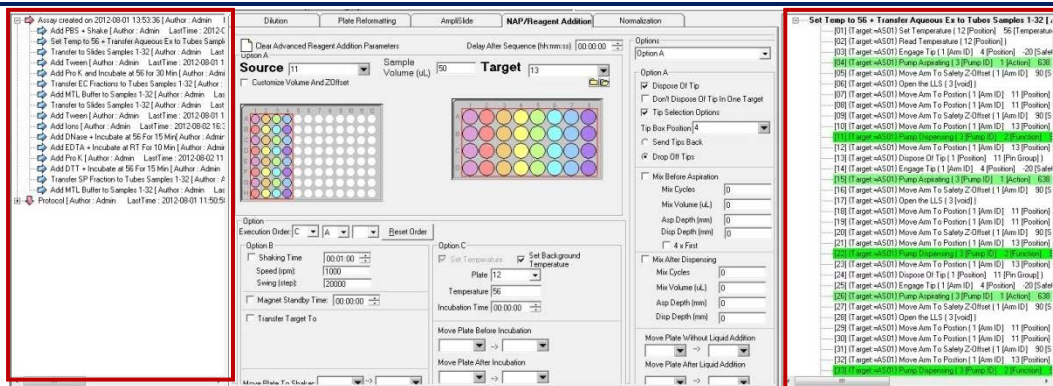



Figure 46: VERSAware – Assay/sequence screen & sequence steps screen

- **Resuming from the middle of an assay/sequence**


An assay/sequence can be resumed from any step. Prior to resuming a step, “home”  the robot first to ensure that the robotic arm is in a safe position. In the “Sequence Steps Screen”, right-click on the step to resume from, and click “Resume the step”. If this is a pipetting step, resume from its previous “Engage Tip” step.

- **Emergency stop button**

The red button on the bottom right corner of the instrument can be pushed anytime to completely stop the instrument. This should be used when it is observed that the robotic arm might crash into something. After pushing the emergency stop button, the robot will be turned off. The software must also then be turned off, restarted, then the robot turned on again so that the software can be synced with the robot.



- **End of run priming**

Prior to turning off the instrument at the end of the run, prime the reagent drop valves with water. The reagents that are used, especially the MTL Buffer, have salts in the solution that might crystallize and plug up the tubings. To prevent this, the reagents in the reagent drop valves must be flushed out after every run and filled with water instead. To do so, remove the caps from the reagent bottles and place them into the deionized water bottles. Click on the “Syringe Pump”  icon. In the “Reagent Drop Priming” section, type “4000” for the “Priming Volume”. Click the “Reagent” button to start.

12.13 UV/HEPA Bio-Containment Hood

The bio-containment hood is designed to provide sterile air quality for the assay sequence workspace by utilizing a high efficiency particulate air filter (HEPA). By performing filtration of the air within the workspace, the hood prevents contamination of the agents used during any



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given sequence. In addition, cross-contamination by harmful particulate or microbes in the air surrounding the workspace is reduced with the built in UV lamp.

- **UV Switch** – Turns the UV light source on and off. Used to de-contaminate the deck.
- **Fan Switch** – Turns on and off the containment hood’s fan to draw external air inward. With the fan running, air is filtered as it enters the hood.
- **Light Switch** – Turns on and off the containment hood’s fluorescent light, which allows for a brighter workspace environment.
- **UV Timer Control** – Along with the UV switch being turned on, the UV Timer Control can be used to set the duration of the UV light source. It can be set for different time intervals (e.g. 5, 15, and 30 minutes)

12.14 Maintenance

This section will discuss how to provide maintenance for tubing, how to handle system fluid and waste bottles, and how to replace syringes.

12.14.1 General Guidelines

It is important to follow the general guidelines and instructions describe in this manual in order to maintain the proper function of the VERSA Workstation.

Use only clean vessels. Do not leave vessels exposed to dust and dirt overnight. It is recommended to use sterile pipetting tips with filter barrier. Run additional priming sequence after the PCR application sequence is finished to avoid air bubble from forming. Use only distilled water or deionized water as the system fluid. It is recommended to use degassed system fluid to avoid excess air bubbles in the system.

12.14.2 Tubing

All tubes should be tightly connected to adaptors and should not be twisted or bent. All tubing is made of durable Teflon which cannot be easily ruptured or bent. When aspirating or dispensing viscous liquids, please prime the instrument more frequently to remove build-up in the tubes and lines.

12.14.3 System Fluid

System fluid and waste liquid bottles are pressurized. Therefore the caps should be tightly sealed to avoid accidental pressure/vacuum release. All tubing should be inserted into the bottles as described in the “Hardware Installation and Set-up” section of this manual.

Only distilled or deionized water should be used as the system fluid. It is highly recommended to use degassed system fluid to avoid excess air bubbles in the system.



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It is very important that the waste liquid bottle does not get full. Otherwise, the waste liquid may fill the vacuum tube and cause damage to the vacuum pump.

12.14.4 Internal Calibration Checks

Position and volume calibration checks should be performed semi-annually.

Instructions on how to perform position calibration checks are found in Section 3.1.1 – Position Calibration Check. Each deck position, except position #5 and 6, should be tested/calibrated to determine if slight offsets may have occurred over the time. Accurate position calibrations will provide increased pipetting accuracy.

Instructions on how to perform volume calibration checks on the syringe pump and reagent drop are found in Section 3.2.1 – Syringe Pump Calibration Check and Section 4.1 – Reagent Drop Calibration Check. Instructions on how to perform volume calibration checks for liquid level sensing are found in Section 6.2.2 – LLS Calibration check for a current plate type. The volumes that are frequently used in the protocols should be tested by instructing the robot to manually dispense liquid. If the actual volume dispensed is inaccurate, additional steps should be taken to created/adjust the calibration table. However, if the volume dispensed is accurate, no further calibration is needed.

12.14.5 Cleaning

The instrument deck surface should be routinely cleaned with water and ethanol to remove any contaminants. The plate adaptor blocks should be cleaned at least quarterly with soap water and ethanol.

12.15 Trouble Shooting

This section will discuss the following potential problems users might encounter with the VERSA 1100 workstation.

VERSA 1100 Troubleshooting	
Problem 1	Power is off
Problem 2	No communication with the VERS
Problem 3	Aurora Arm does not respond to operator's command
Problem 4	Aurora Arm does not move to correct positions
Problem 5	Aurora Arm stops during application sequence run
Problem 6	Syringe pumps do not respond to operator's command
Problem 7	Syringe pumps do not aspirate or dispense any reagent or buffer
Problem 8	Syringe pumps do not aspirate or dispense accurate volume
Problem 9	VERSA producing unusual noise
Problem 10	Pipetting tips are damaged or bent
Problem 11	Excessive air bubbles are introduced into the system tubing



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Problem 12	Pressure and vacuum pumps are not working
Problem 13	Syringes are leaking or broken
Problem 14	While running the sequence, a hanging drop is formed outside of the tip
Problem 15	The pipetting tip does not aspirate the master mix in the reagent block
Problem 16	Pipetting tips do not aspirate or dispense accurate volume
Problem 17	Pipetting tips do not dispense small volumes (1-5 μ L)
Problem 18	VERSA stops working suddenly or operation hangs midway

The VERSAware software provides advance error messaging and a detailed log for both Aurora Arm and Syringe Pumps. Most common problems and solutions are discussed below.

1. Problem – Power is off

Solution – Please check if power cable is properly connected to the VERSA and AC outlet. Make sure that the power switch is on.

2. Problem – No communication with the VERSA

Solution – Please ensure that USB cable is tightly connected to the VERSA and computer USB port. Please check correct COMP port configurations in MS Windows XP/2000.

Start → Settings → Control Panel → System → Hardware → Device Manager → Ports
Device status of COM ports should read: “This device is working properly”. All COM port numbers not exceed 16.

3. Problem – Aurora Arm does not respond to operator’s command

Solution – Please ensure that the arm is initialized in Aurora Station software. Make sure that the COM port settings are correct in the Aurora Station software. If the problem still remains, try to shut down and restart the VERSA and initialize the robotic arm once again.

4. Problem – Aurora Arm does not move to correct positions

Solution – Please refer to Section 3.1.1 – Position Calibration Check to adjust or repeat the teaching procedure for all positions. If the problem still remains, try to initialize the robotic arm again.

5. Problem – Aurora Arm stops during application sequence run

Solution – Please refer to the Section 3.9 – Aurora Station Settings to decrease robotic arm speed and acceleration settings for every axis (X, Y, and Z). It is recommended to gradually reduce the end speed by 500 or 1000 steps/sec. Acceleration should be reduced to 1 or 3 steps/sec². The user can start the sequence from the step it last stopped at by left clicking the step and clicking “Resume this step”.



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6. Problem – Syringe pumps do not respond to operator’s command
Solution – Please ensure that both syringe pumps are initialized. Make sure that the COM port settings are correct. If the problem still remains, try to shut down and restart the VERSA 1K software and initialize the syringe pump one more time.

7. Problem – Syringe pumps do not aspirate or dispense any reagent or buffer
Solution – Please visually confirm that the plunger of the syringe pump is moving down for the aspiration function or moving up for the dispensing function. If the plunger is moving, consult Section 1 – Hardware Installation and Set-up to check for proper syringe pump tubing connections. Ensure there is no air bubbles observed in the syringe or tubes.

8. Problem – Syringe pumps do not aspirate or dispense accurate volume
Solution – Check that the priming bottle is tightly sealed. Usually if the bottle is over-turned, no liquid should be leaking from the bottle cap. If it is, the seal is open and the user should open and re-align the rubber seal along with the screw cap before closing again. Please also refer to Section 3.2.1 – Syringe Pump Calibration Check section of this manual and perform new calibration check procedures if necessary.

9. Problem – VERSA producing unusual noise
Solution – This is normal. During the priming sequence, pressure and vacuum pumps start working and produce unusual noises. The purpose of the pressure pump is to remove air bubbles from the system’s syringes and tubing. The vacuum pump is used to remove waste liquid from the priming station.

10. Problem – Pipetting tips are damaged or bent
Solution – In case of accidental tip damage, press the “Home” icon in the VERSAware software to initialize the robotic and disengage the damaged tip.

11. Problem – Excessive air bubbles are introduced into the system tubing
Solution – Prime the syringe pumps to remove air bubbles.

12. Problem – Pressure and vacuum pumps are not working
Solution – Please contact Aurora Biomed to obtain new pressure and vacuum pumps and instructions on how to install them or request technical assistance.

13. Problem – Syringes are leaking or broken
Solution – Please contact Aurora Biomed to obtain new pressure and vacuum pumps and instructions on how to install them or request technical assistance.



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14. Problem – While running the sequence, a hanging drop is formed outside of the tip
Solution – The engaged pipetting tip is probably loose. Check the position calibration of that tip box. Also, check to see if the tip box is placed in the correct orientation by verifying that the white dot on the tip box is on the upper left corner. Putting in the tip box in an incorrect orientation can cause the position calibration to be slightly off, causing an imperfect engagement of the tip barrel and the tip. After engagement, tip should be tightly fitted to the barrel.
15. Problem – The pipetting tip does not aspirate the master mix in the reagent block
Solution – The pipetting tip probably touches the bottom of the 7 mol vial. Please refer to Section 5.3.3.1 – Options/Plate Type Settings to decrease the Master Mix Block Depth (mm). Or, the pipetting tip might be above the master mix volume level. Increase the Master Mix Block Depth (mm) in this case. Alternatively, click “Pause” when the tip goes to aspirate the solution and subsequently check/fix the Z-offset (calculate with Z-Offset calculator).
16. Problem – Pipetting tips do not aspirate or dispense accurate volume
Solution – Check if there are air bubbles in the tubing. If so, prime the syringe pumps to remove the air bubbles from the syringes and system tubing. Another reason might be that the engaged pipetting tip is loose. Please teach the related tip box positions approximately 20-30 steps lower (Z-axis) as described in Section 3.1.1 – Position Calibration Check. The syringes installed within the syringe pump might be loose as well. Contact Aurora Biomed for assistance.
17. Problem – Pipetting tips do not dispense small volumes (1-5 μ L)
Solution – The pipetting tip should slightly touch the bottom of the micro plate. Please check the Z-offset in the VERSAware option for that particular plate type and increase/decrease the plate’s Z-offset (mm) for any application tab.
18. Problem – VERSA stops working suddenly or operation hangs midway
Solution – Hibernation, standby, or sleep mode is enabled on system software. To change these settings, refer to the “Power Savings Settings” section of the physical manual. Try closing and re-opening the software again. If the problem persists, restart the PC and re-open the software.



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12.16 Certification Exercise

Before the acceptance of a new or reconditioned VERSA liquid handler, the following certification exercise will be carried out. This will consist of setting up and performing the selective degradative differential digestion on multiple known samples as described in the laboratory's Chemical Batch quality control (i.e. known blood, NIST standard, differential digestion fractions, associated reagent blanks). The reagent kit(s) utilized should be previously QC'ed and certified for use.

Materials:

- VERSA Liquid Handler
- Previously QC'ed and certified for use: PBS, Tween Buffer (TwB), MTL Buffer, Pro K, DTT, DNase I, Mg/Ca Salts and EDTA.
- Laboratory QC samples and controls.

Method(s):

1. Set up the VERSA and perform the selective degradative differential digestion according to laboratory protocol.
2. Extract, quantify, and type the samples according to the standard laboratory protocol.
3. Examine the DNA quantitation and STR typing results obtained.

12.17 Conclusion of Certification Process

If the new or reconditioned VERSA 1100 passes installation, internal calibration checks, protocol setup and a certification exercise, it will be placed into service in the DNA laboratory. If noticeable differences are found, repeat the procedure. If there are still significant differences, contact Aurora Biomed for resolution. Do not use the new VERSA until this process has yielded acceptable results.

12.18 Documentation

Place all data in the appropriate VERSA Use Log under the "Certification" section.



PART 2. GENERAL APPROACH TO QUALITY CONTROL OF EQUIPMENT USED IN DNA ANALYSIS

13.0 Maintenance Procedures for Aurora Biomed VERSA 1100

13.1 Scope

To describe the internal calibration checks and maintenance procedures for the VERSA 1100 liquid handler.

13.2 References

- VERSA 1100 Operation Manual

13.3 Test equipment and material

- Alcohol solution
- Neutral soap solution
- Deionized water
- Kimwipes
- 50µL and 1000µL pipette tips
- AmpliSlide adaptors, 32 Sample blocks
- 96-well teaching plate
- SlicPrep™ Plate

13.4 Summary of the tests and frequencies

Test	Frequency
Clean worktable, plate adaptors, and instrument surfaces. NO BLEACH	Weekly Clean-up/annual
UV Decontamination	Weekly Clean-up/ annual
Empty/fill system fluid bottles	As needed
Annual Preventative Maintenance Visit	Annual Maintenance – Conducted by Service Engineer
Position Calibration Check	Annual Maintenance – Conducted by Service Engineer
Volume Calibration Check	Annual Maintenance – Conducted by Service Engineer

13.5 Procedure

There are two cleaning procedures: Cleaning the VERSA and UV Decontamination.

There are two internal calibration check tests: position and volume. The position calibration check test needs to be conducted first using a 96-well teaching plate. The appropriate adaptor blocks, plates, and tubes are then calibrated. The volume calibration check includes the syringe pump, reagent drop, and liquid level sensing.



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If the manufacturer or design of consumable parts (96-well plates, reagent tubes, sample tubes) is changed, run the position calibration check test prior to use in casework.

13.6 Empty/Fill System Fluid Bottles

It is very important that the waste liquid bottle does not get full. Otherwise, the waste liquid may fill the vacuum tube and cause damage to the vacuum pump. Periodically empty the waste bottle to prevent the liquid volume from becoming too high.

The priming liquid bottle is used to prime the syringe pumps. The priming liquid should not reach below the tubing at any time. Otherwise, air will be pumped through the syringe pumps. Periodically fill the priming liquid bottle with sterile deionized water.

13.7 Clean

The instrument deck surface should be wiped down with water and ethanol to remove any contaminants. The plate adaptor blocks should be cleaned weekly with soap water and ethanol.

13.8 UV Decontamination

UV decontamination is performed by turning on the UV light source. The UV time control can be used to set the duration of the UV light for different time intervals. The bio-containment hood of the instrument is made of plexiglass and protects the user against UV radiation.

13.9 Position Calibration Check

Each deck position, except position #6 and 7, should be tested/calibrated to determine if slight offsets may have occurred over the time. Accurate position calibration checks will provide increased pipetting accuracy.

All the deck positions are taught with a 96-well plate. The shaker position and the reagent cooler block positions are taught as is. All positions should be taught to locate the surface of the upper left well (A1) of the 96-well teaching plate. The X & Y coordinates should be adjusted so the tip is positioned in the center of the well. For calibration check of the Z coordinate, a piece of paper can be placed in between the tip barrel and the surface of the 96-well teaching plate. Fine adjustments are made until the tip barrel just touches the paper. Slight resistance should be felt when inserting or removing the paper. Upon completion of all position calibration check, the position setting for all other plate types which are available in the Labware library will be automatically calibrated with respect to the 96-well teaching plate.

For semi-annual check calibration check, first test the various adaptors, plate types, and tubes in their corresponding deck positions to determine if the current calibration check is still accurate. Follow the instructions on "TESTING PLATE TYPES". Check to see if the pipette tip that is engaged is right above the surface of the plate/tube. A piece of paper should be able to slide snugly between the tip and the plate. Also check to see if the pipette tip is centered in the well or tube. If the current calibration check is still accurate, no further calibration check is needed for that particular




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position. If the current calibration check is off, then either the position (1) wasn't calibrated correctly or the plate type off-set is not calibrated correctly. Test that particular plate type on a different position (2). If the tip is now centered, then that indicates that the position (1) was calibrated incorrectly. Re-calibrate the position (1) by following the steps on how to "CALIBRATE A POSITION".

If the tip is still off-centered, then it indicates that the plate type off-set is not calibrated correctly. To adjust the plate type off-set, following the steps below on how to "ADJUST PLATE TYPE OFF-SET".

All position calibrations are performed in the Aurora Station interface. There are two ways to access the Aurora Station:

1. Open the "Shell Status" Window which runs alongside VERSAware. Double-click on "AS01 [1]: 1172" and Aurora Station will open from the background.
2. In VERSAware, go to "Tools", then click on "Aurora Station". A checkmark will appear next to the interfaces if it is already opened.

*Initialize the instrument by clicking the "Home" 

Calibrate a Position

To calibrate or teach a new plate type, go to the "Robotic Arm Tab" and double-click on the position you want to teach in the record selector. No tip should be engaged. Place a 96-well teaching plate on the deck. Set the Z coordinate to 0 in the "Go Z" text box. Click "Go X, Y, Z". If necessary, adjust the X and Y coordinates. Click on the down arrow to adjust the Z coordinates. Reduce the steps of the arm control to 5 or 10 steps for more precise and safe control of the arm movement. The tip barrel should be directly above the teaching plate so that a piece of paper should be able to pass through between the teaching plate and the barrel tip with slight resistance. Click "Save".

Note: If a tip was engaged to calibrate a position, make sure that the "Tip Calibration" box is checked. This informs the robot to account for the height differences of a tip.

Tip


To engage a tip, go to the "Robotic Arm Tab" and double-click on the record selector of the position from which the tip will be engaged. Positions 2 & 3 hold 50µL tips; positions 4 & 5 hold 1000µL tips. Click on "Tip" to have the arm move to the tip position and engage a tip.

Testing Plate Types

Aurora Station allows you to test previously or newly taught positions for all plate types. It is a good practice to test positions periodically to ensure that the calibration checks for a particular plate type and position is still accurate.



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First, engage a tip following the instruction above on “Tip”. Place the plate type (tube/slide/plate) onto the position to be tested. For the SlicPrep™ plate, only use the base plate; do not include the insert. To test the position with that particular plate type, go to “Options → Aurora Station Settings” or click on the “Aurora Station settings” icon . Click the “Advanced” tab. On the bottom left corner of the screen under “Test Position [Plate Type]”, select the position to test (ignore the description of the position). Click “Apply”.

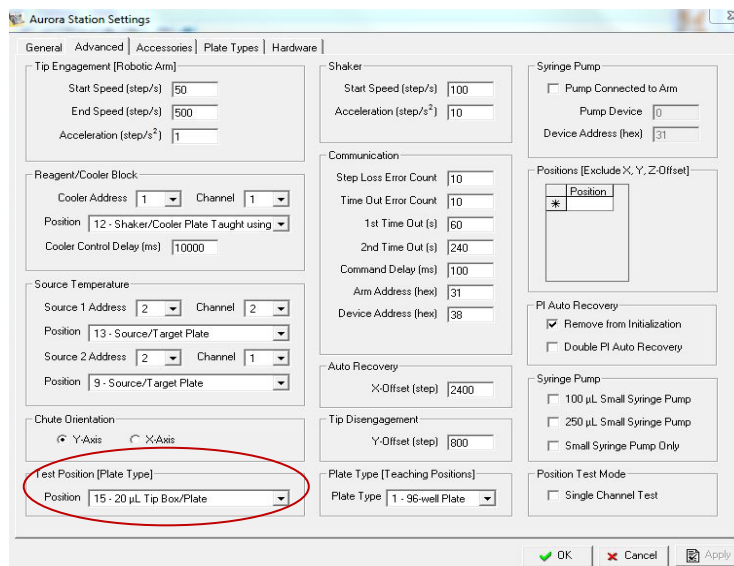


Figure 1: Aurora Station settings/Advanced tab.

Next, click on the “Plate Types” tab. Under the “Symmetrical Plates” section, select the plate type that you want to test in that position by highlighting the selection. Click on the icon with a small red square on the top left corner to test the top left corner of the plate type or click on the icon with a small red square on the bottom right corner to test the bottom right corner of the plate type.

- Slides: Plate type #5
- 50µL Tips: Plate type #18
- 1000µL Tips: Plate type #21
- SlicPrep™ Plate: Plate type #68
- 32-well sample block and reagent cooler block: Plate type #70
(Testing the 32-well sample block includes the height of the samples tubes)



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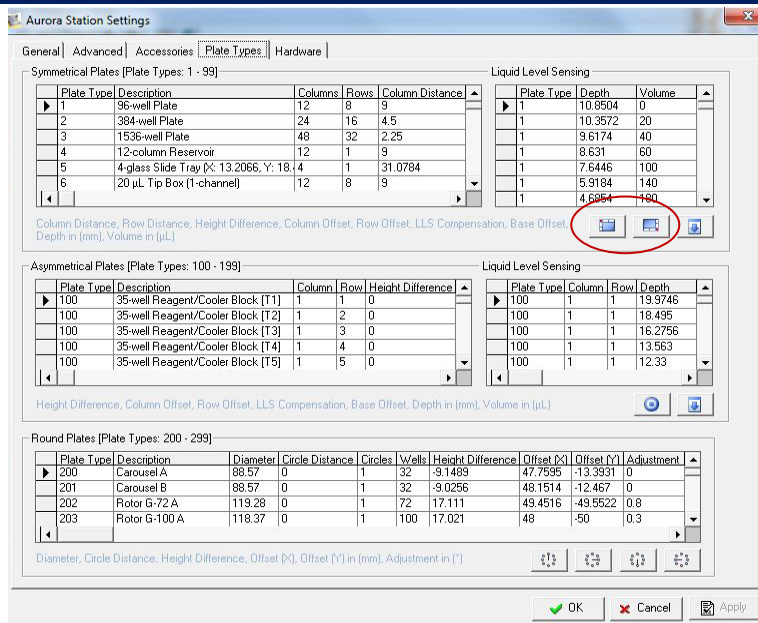


Figure 2: Aurora Station settings/Plate Types tab.

The tip should be in the center of the plate type. If the tip isn't in the center of the plate, then either the position (1) wasn't calibrated correctly or the plate type off-set is not calibrated correctly. Test that particular plate type on a different position (2) by repeating the steps above. If the tip is now centered, then that indicates that the position (1) was calibrated incorrectly. Re-calibrate the position (1) by following the steps on how to "Calibrate a Position".

If the tip is still off-centered, then it indicates that the plate type off-set is not calibrated correctly. To adjust the plate type off-set, following the steps below on how to "Adjust Plate Type Off-set".

Adjusting Plate Type Offset

Sometimes, the plate type off-sets that are saved in the Labware library can be slightly off. If that plate type is applied to a position, the tip will not be centered above a well but will instead be off-centered, depending on the severity. An off-centered calibration check can result in inaccurate liquid pipetting or potential crashing of the robotic arm in severe cases.

To adjust the plate type off-set, bring the robotic arm to the calibrated plate type position by following the steps above on how to "Test Plate Position". Close the "Aurora Station Settings" screen. The X, Y, Z coordinates on the top left corner of the screen (in red, green, and blue numbers) indicates the current position of the robotic arm.

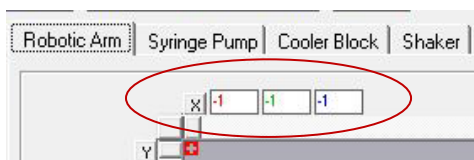


Figure 3: The current X, Y, and Z coordinates.



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Go to a new row on the record selector table, indicated with an “*”. Type in an arbitrary position number, such as “100”, to distinguish from an actual position. Under the comments column, type “Original”. Select the new position by highlighting the selection, then click “Save”. The current robotic arm coordinates are now saved onto this new position.

	Position	Step [X]	Step [Y]	Step [Z]	Comment
	14	7837	2525	7737	1000 µL Tip Box/Plate
	15	10233	2526	7756	20 µL Tip Box/Plate
	16	0	98	7367	Priming Position
	17	0	1331	7132	Waste Position
	18	0	2568	6937	The Chute
	*				

Figure 4: The position records table.

Adjust the robotic arm using the “Step/Arrow Buttons” until the tip is in the center of the well. Create a new row on the record selector table. Type in an arbitrary position number, such as “101”. Under the comments column, type “Adjusted”. Select the new position by highlighting the selection, then click “Save”. The adjusted robotic arm coordinates are now saved onto this new position.

Double-click on the “Adjusted” position record, then click on the “Original” position record, then click “Offset”. The “Offset Calculator” screen will appear, showing the X, Y, and Z steps of both records and the difference between the two records will appear in the light blue numbers in millimeters (mm). Record the “Offset” value. Click “OK” to close.

Column Distance	
X1 (step)	0
X2 (step)	662
Total Columns	2
Distance (mm)	37.6843
Offset (mm)	37.6843

Row Distance	
Y1 (step)	1331
Y2 (step)	42
Total Rows	2
Distance (mm)	99.253
Offset (mm)	-99.253

Height Difference	
Z1 (step)	7132
Z2 (step)	7801
Difference (mm)	-21.4481
Offset (mm)	21.4481

Tip Calibration

Figure 5: Offset calculator

Go to “Options → Aurora Station Settings”. Click on the “Plate Types” tab and select the plate type to be adjusted by highlighting the selection. Scroll to the right until you see the “Height Difference”, “Column Offset”, and “Row Offset” columns. Adjust the offsets by adding/subtracting the absolute value(s) recorded from the “Offset Calculator”. Click “Apply”, then “OK” to close.



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	Coordinate	Positive (+) Value	Negative (-) Value
Column Distance	X	To the right	To the left
Row Distance	Y	Towards user	Away from User
Height Difference	Z	Up	Down

Example: Height difference offset: 21.4481. The current position is too low and you want to bring the z-axis up. According to the table, to bring the z-axis up, the height difference should become more positive so you would add 21.4481 to the original value.

Heater/Shaker Unit Position Calibration Check

The heater/shaker unit should be calibrated with the plate type used (SlicPrep™ Plate)

Reagent Drop Pin Position Calibration Check

To calibrate the reagent drop pin, click “Options → Aurora Station Settings”. Click on the “Advanced” tab. In the bottom left corner under “Test Position [Plate Type]”, select the test position. To the right under “Plate Type [Teaching Position]”, select “1 – 96 well plate” and click “Apply”. Place the 96-well teaching plate in that position. Click on the “Accessories” tab. Under the “Pin Groups” section on the bottom, select the pin number to test by double-clicking. (Only Pin 2 and 3 are used). Click “RD Test”. The robotic arm will go to that position and align the reagent drop pin on the surface of the A1 well of the 96-well teaching plate.

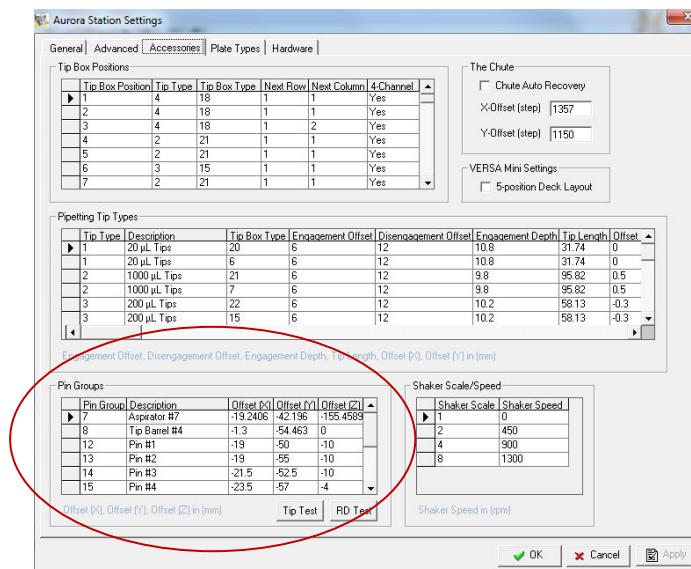


Figure 6: Aurora Station settings/Accessories tab.

To check if the pin is centered on the well, dispense some solution (e.g. 100µL) manually using the PWM Pump. In the VERSAware interface, go to “Tools → PWM Pump”. Click on the “C” icon to go into the control mode. Under the “Valves” section, type “100” in the “Volume” column under the desired valve (Valve 1 for Pin 1, etc.). Watch the pin while it dispenses the liquid. If the pin is not



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dispensing in the center of the well, change the X and Y Offsets in the “Aurora Station Settings → Accessories tab → Pin Groups” to center the pin.

- Negative value in the X-offset moves the robotic arm/pin to the left side
- Negative value in the Y-offset moves the robotic arm/pin away from the user

When finished, go to the PWM Pump interface and click on the “A” icon to change back to assay mode.

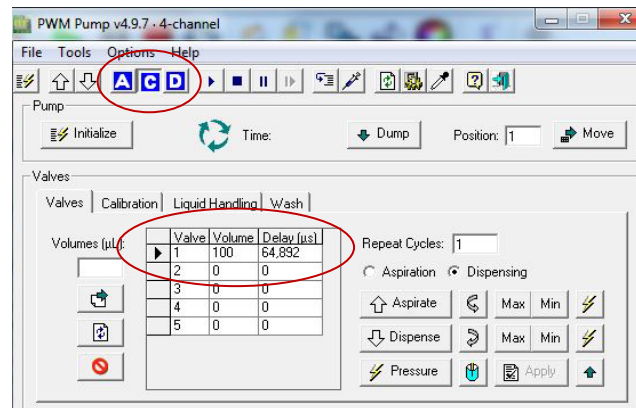


Figure 7: PWM Pump/C Mode.

13.10 Volume Calibration Check

There are three volume calibration checks that are performed: Syringe Pump, Reagent Drop, and Liquid Level Sensing. The volumes that are frequently used in the protocols should be tested by instructing the robot to manually dispense liquid. If the actual volume dispensed is inaccurate, additional steps should be taken to create/adjust the calibration table. However, if the volume dispensed is accurate, no further calibration check is needed. The acceptable range of the actual dispensed liquid volume is within 10% of the targeted volume.

All volume calibrations are performed in either the Aurora Station or PWM Pump interface.

There are two ways to access the Aurora Station:

1. Open the “Shell Status” Window which runs alongside VERSAware. Double-click on either “AS01 [1]: 316” or “DH01 [1]: 5616” to open either the Aurora Station or PWM Pump from the background.
2. In VERSAware, go to “Tools”, then click on either “Aurora Station” or “PWM Pump”. A checkmark will appear next to the interfaces if it is already opened.

Syringe Pump Calibration Check

There are two methods to perform the calibration check procedure. The general sequence of aspirating and dispensing liquid for the calibration check is AIR GAP ASPIRATION → LIQUID ASPIRATION → DISPENSE LIQUID.



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1. **Auto Mode** – The auto mode runs the general sequence listed above automatically, incorporating delays in between each step.
 - Under the “Pump Calibration” section, type in the steps (1µL = 10 steps) for the air gap aspiration & sample aspiration and the number of seconds for the delay time.
 - Click “Send”. The instrument will now aspirate X steps of air, wait for Y seconds, aspirate Z steps of sample, wait for Y seconds, then dispense X + Z steps of volume.
 - During the first delay step, place the sample tube containing water underneath the pipette tip, making sure that the tip is submerged in the water.
 - During the second delay step, remove the sample tube and place the empty weigh boat underneath the pipette tip.

***The Auto Mode should be used for creating new calibration data or adjusting existing data**

2. **Pump Control Mode** – The pump control mode requires manual control of each step in the general sequence listed above.
 - Under the “Pump Control” section, select either the “Volume” or “Steps” option.
 - Volume option: Used for testing particular volumes of liquid to determine if the accurate volume of liquid is aspirated/dispensed
 - Steps option: Used for adjusting or creating a new calibration table
 - Select “Aspiration” as the function, then enter the desired amount of steps for air gap aspiration (1µL ~ 10 steps).
 - Click “Send”. The instrument will now aspirate the air gap.
 - Now enter the desired amount of steps for sample aspiration.
 - Place a sample tube containing water under the pipette tip. Click “Send”. The instrument will now aspirate the sample.
 - Select “Dispensing” as the function, then enter the total amount of steps for the air gap aspiration and the sample aspiration.
 - Place an empty weigh boat underneath the pipette tip. Click “Send”. The instrument will now dispense the air gap and the sample.

*** The Pump Control Mode should be used during normal quarterly checks where the goal is to check if the instrument is dispensing the correct volume of liquid.**

Prior to performing a syringe pump calibration check, prime the syringe pump to eliminate all air bubbles in the system. It is recommended to perform 8-10 cycles of priming to fill up the tubing with the system fluid and remove most of the air trapped within the system. For instructions on how to perform a syringe pump priming, refer to section 8: Syringe Pump Priming.



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Switch to the *Robotic Arm Tab*, initialize the robotic arm by pressing the button between the X and Y axes, then move the robotic arm towards you by dragging the Y axis down. This allows the user to have easier access to the syringe pump during the calibration check. Switch back to the *Syringe Pump Tab*.

Checking Volume Dispense

Manually engage a tip onto the first barrel. Use a 50 μ L tip for the 20-50 μ L range and a 1000 μ L tip for the 200-1000 μ L range. Initialize the pump by clicking the “Initialize” button on the bottom of the screen. Under the “Pump Calibration” section, select 4x1000 μ L as the syringe pump type. Select “Multiple Scale”. Prepare a sample tube with water and an empty, pre-tared weigh boat. Use the **Pump Control Mode**, select the “volume” option, and aspirate and dispense the desired amount of liquid into the empty, pre-tared weigh boat. Weigh the weigh boat. (Note: When using the Pump Control Mode/Volume option, be sure to aspirate an air gap prior to aspirating the liquid. When dispensing, add an extra 50 μ L to the dispense volume to make sure that all the air and liquid is pushed out.) Multiple the value by 1000 to get the volume (1mg = 1 μ L). Record this value.

Measure the volume 5, 10, 15, 20, 25, and 550 μ L by repeating the steps above. If the actual volume is significantly different from the targeted volume adjusted the record in the calibration table. However, if all of the actual volumes are significantly off from the targeted volume, create a new calibration table and measure the volume from 3 – 1000 μ L (~40 – 11,500 steps). Follow instructions below for adjusting or creating a new calibration table.

Adjusting/Creating a New Calibration Table

In Aurora Station, click on the “Syringe” icon . The calibration table screen should appear. Select the 4x1000 μ L pump type. Make sure that “Multiple Table” is checked. This allows for multiple calibration tables. Use the **Auto Mode** or the **Pump Control Mode (step option)**. Aspirate and dispense the desired amount of liquid into the empty, pre-tared weigh boat. Weigh the weigh boat. Adjust either the existing calibration table values or create a new scale by clicking on an empty row under the “Scale” section. Record the sample aspiration step under the “Step” column and the volume measured in the “Volume” column (1mg = 1 μ L). Click “Apply”, then “OK”.

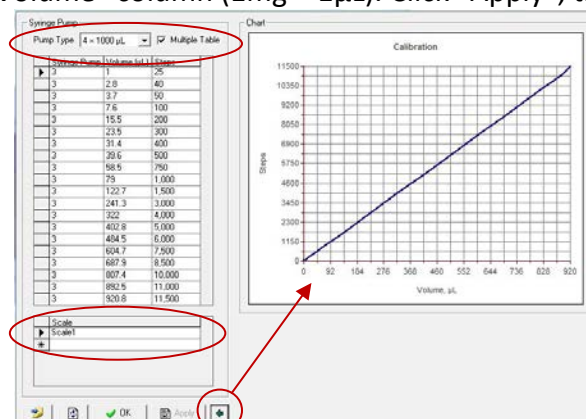



Figure 9: Aurora Station/Syringe Pump Calibration



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Reagent Drop Calibration Check

Prior to performing a reagent drop calibration check, prime the reagent drop system by clicking the “Pump” icon  on the VERSAware interface. Type “1000” into the Reagent Drop Priming Volume. Click on the “Reagent” button on the bottom.

On the PWM Pump interface, initialize the pump by clicking the “Initialize” button on the top left corner. To perform volume calibration check, click on the “C” icon to switch into control mode. When the pump is operational, the pressure can be observed by clicking the “Chart” button on the bottom right corner. A graphical pressure curve in real-time will appear, showing the user the current pressure maintained. The pressure should be maintained at about 10 psi.

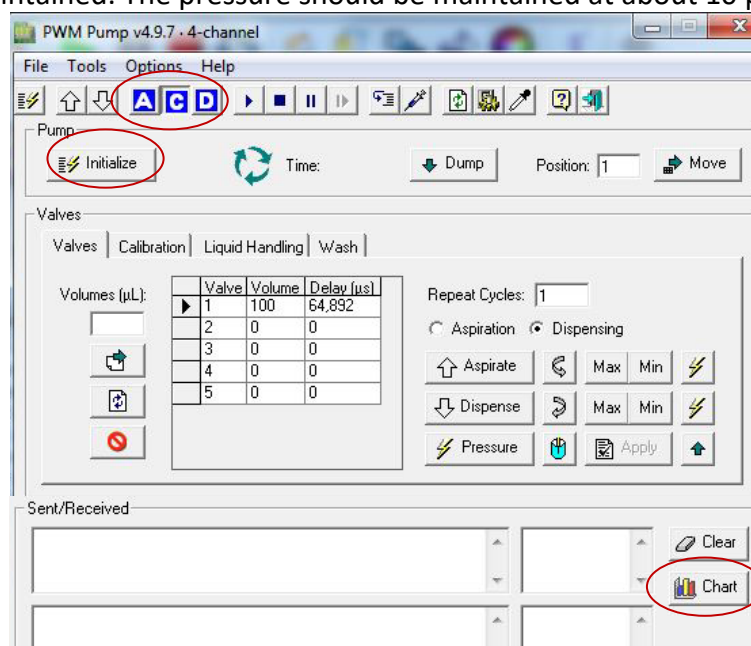




Figure 10: PWM Pump interface

Make sure the instrument is not in calibration mode – the “Syringe” icon  should not be selected. Type “1” for the number of “Repeat Cycles” on the right. Type the volume of liquid to dispense in the “Volume” column next to the valve that is to be tested (290, 400, 510, and 650µL), then click on a different field for the software to refresh and update. (Note: do not put the type the volume of liquid to dispense in the “Volume” box on the left). Place an empty, pre-tared weigh boat below the reagent drop pins and click “Dispense”. Weigh the weigh boat and determine if the instrument is accurately dispensing the volume of liquid. If the volume dispensed is accurate, no further calibration check is needed. If it is not, a volume calibration check might need to be performed by following the instructions below.

Click on the “Syringe” icon  on the top. This puts the instrument in calibration mode. When the calibration mode is selected, the values that the user inputs for volume is actually in terms of delay time, which is the length of time the valve is opened for in micro-seconds (µs).



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Click on the “Pump” icon  to open the pump calibration table. This is location where all of the calibration scales for the reagent drop is maintained. New calibration data will be recorded here. The volumes for the reagent drop calibration check can be adjusted in the “Dispensing” table. A new scale can be created instead by clicking on the empty row in the “Scales” table. Enter the date and the liquid used to perform the volume calibration check. Under the “Aspiration” table, enter the numbers 1 – 5 in the “Valves” column. Enter “0” in the “Volumes” column. Enter “1” in the “Cycles” column. Enter “0” in the “Pulse” column. Enter “0” in the “Delay” column. Click “Apply”, then “OK”


Figure 1: Pump Calibration table

In the PWM Pump main screen, select the “Valves” Tab in the “Valves” section. Select the pin valve to calibrate (*Only valves 1 – 3 are available on this instrument*). Type “50,000” in the “Volume” column. Make sure that the other valves have “0”. Remember, in the calibration mode, this is actually the delay time in μs that the valve will be opened for. Type “1” for the number of “Repeat Cycles” on the right. Make sure that “Dispensing” is selected. Place an empty, pre-tared weigh boat underneath the reagent drop valve, then click “Dispense”. Weigh the volume and record in the pump calibration table.

Figure 2: PWM Pump/Valves Tab



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Click on the “Pump” icon  to go into the Pump Calibration screen. In the “Dispensing” table, type the pin valve number that is being calibrated in the “Valve” column. Type 0, 1, 0 in the “Volume, Cycles, Pulse” columns. In the next row, type “1” in the “Cycles” column and “100,000” in the “Pulse” column. The “Delay” will automatically be calculated using the equation: Cycles x Pulse = Delay. Record the volume that was measured in the weigh boat (1mg = 1µL).

Measure the volume at multiple cycles by changing the number of “Repeat Cycles” in the main screen. Cover the range from 100,000 – 6,000,000µs (~10 – 750µL). There is no need to do every cycle to reach 6,000,000. Measure an average of about 10 – 15 cycles. After calibration check of one pin valve is completed, repeat for the 2nd and 3rd valve. Enter 0, 1, 0 in the “Volume, Cycles, Pulse” columns for Valves 4 and 5. Click “Apply”, then “OK”. Switch back to the Assay Mode by clicking on the “A” icon when finished with the volume calibration checks.

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2. About This Manual

Important: Prior to the initial operation of the instrument, please read these operating instructions carefully to make sure you prevent possible damage to the instrument.

Instructions for the VERSA 1100 workstation include the following general areas:

- ✓ Installing the VERSA1100 System
- ✓ Configuration the VERSA hardware for specific application requirements
- ✓ Creating or modifying assays
- ✓ Running assays
- ✓ Performing preventive maintenance

3. Disclaimer

Application of this system for analytical purposes, utilizing third party, or in-house methods, remains the professional responsibility of the end user. The system is not intended to diagnose, mitigate, prescribe, treat, cure, or prevent any disease or illness or symptoms when used in any analytical procedure.

Aurora Biomed Inc., Aurora Instruments Ltd. or any of their employees, shall not be held liable in any way for problems encountered by the use of this product or procedures performed on this product outside of its intended purpose.

4. Warranty

The warranty, where applicable, only covers defects arising from normal usage of the instrument and does not cover malfunction or failures resulting from misuse, abuse, neglect, alteration, modification, erratic AC power, natural disasters or repairs by someone other than an authorized Service Representative. The Warranty includes unlimited telephone and e-mail access to our Service Engineers between our standard hours of operation. Please refer to your purchase agreement for details of your warranty coverage.

5. Safety Precautions

Use this product only in the manner described in this manual. When used in a manner other than specified, user safety may be jeopardized.

Arm Safety

Attention: Always keep body parts, hair, jewelry and clothing away from the robotic arm while it is operating. Failure to do so may result in injury.

Deck units

Attention: Some deck unit modules can be set at high temperatures. When operating the instrument be aware of these units to prevent accidental burns.

Some deck unit modules are replaceable. Strictly follow the instructions in this manual when replacing them.

Some deck unit modules include a motor. Never try to physically stop them or use an object to interfere with their action. Press the emergency stop bottom in the event of any unexpected situations.

Station Safety

Caution: Risk of electric shock. To reduce the risk of electric shock, do not remove the cover on the back of the instrument. There are no user-serviceable parts inside.

Never force any component to fit if it does not do so easily.

Attention: Be sure that the workstation and all its deck components are assembled and in their proper positions before beginning operation. Failure to do so may result in injury and/or damage to the equipment.

Pump Safety

Caution: The Pump is equipped with both positive-pressure and a vacuum that is controlled by the system's software. Please keep the caps of the buffer bottles tightened in order to avoid pressure/vacuum release.

Solvents & Reagent Drop

The VERSA 1100 Reagent Drop valve is equipped to handle most organic solvents. However special care should be taken with some solvents. Please consult Aurora representative regarding solvent use if you are unsure. Aurora is not responsible for damage incurred to the valves / tubing if the Reagent Drop is used with strong solvents. Contact Aurora for more information prior to use.

Attention: Reagent box is under pressure; take an extra care while opening this box. Always release pressure from the pressure release valve before opening.

6. System Overview

The VERSA1100 is an automated liquid handler which can be used to fully automate the following applications upon requested:

Serial Dilution

Plate Reformatting

Nucleic Acid Preparation (NAP)

AmpliSlide

The VERSA 1100 (*Figure 6-1 Front View*) is a multichannel system with an arm equipped with 8 barrels, a plate gripper to move the plate from one position to another, and up to 5 reagent drop pins for bulk dispensing of reagents. There are 15 deck positions which have been customized for the specific needs of the user(*Figure 6-2 VERSA 1100 Deck Layout*).



Figure 6-1 Front View

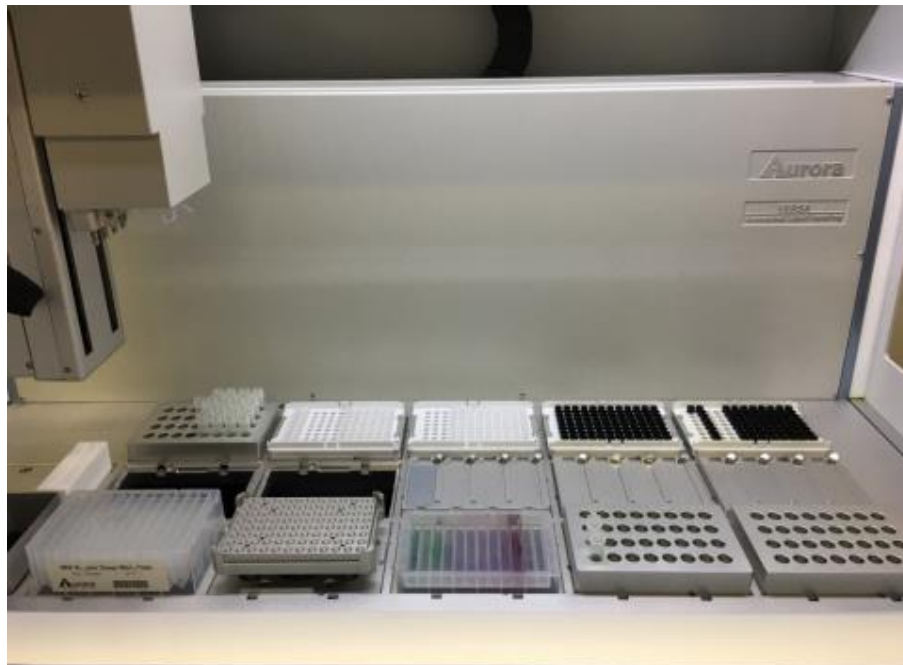


Figure 6-2 VERSA 1100 Deck Layout

7. Hardware Installation

7.1. Workbench Unpacking & Preparing for Installation

The VERSA 1100 is approximately 920W x 615L x 999H (mm) including the bio-safety hood. However, we recommend that the user also provide additional space on all sides of the instrument (especially at the back where the Waste Bottles and Reagent Box will be situated). The workbench should be stable, vibration free and capable of handling up to 100 kg.

The VERSA 1100 instrument will arrive securely packaged in a wooden crate specially sized for this instrument. Open the wooden crate, and remove the instrument carefully and place on a sturdy work bench. After removing the cellophane wrapping (*Figure 7-1 VERSA 1100 with cellophane wrapping*), carefully remove the packing material from all sides of the arm (as well as the track that the arm moves along). After clearing the instrument of all packing material, move the arm manually to the far left corner. Connect all the tubings to waste bottles & pressure box as shown below.



Figure 7-1 VERSA 1100 with cellophane wrapping

7.2. Tubing and Bottles

There are two tubing connections to be made on the VERSA 1100. See the picture below (*Figure 7-2 Reagent drop tubing Connections*) to familiarize yourself with all the tubing connections for pressure, vacuum and waste (Please note that depending on your application your unit may not have all the below features and connections).

General Waste: This bottle collects the waste coming from the protocols run on this system. This is directly connected from the waste position # 17 to the waste bottle. There is no vacuum or pressure required for this tubing.

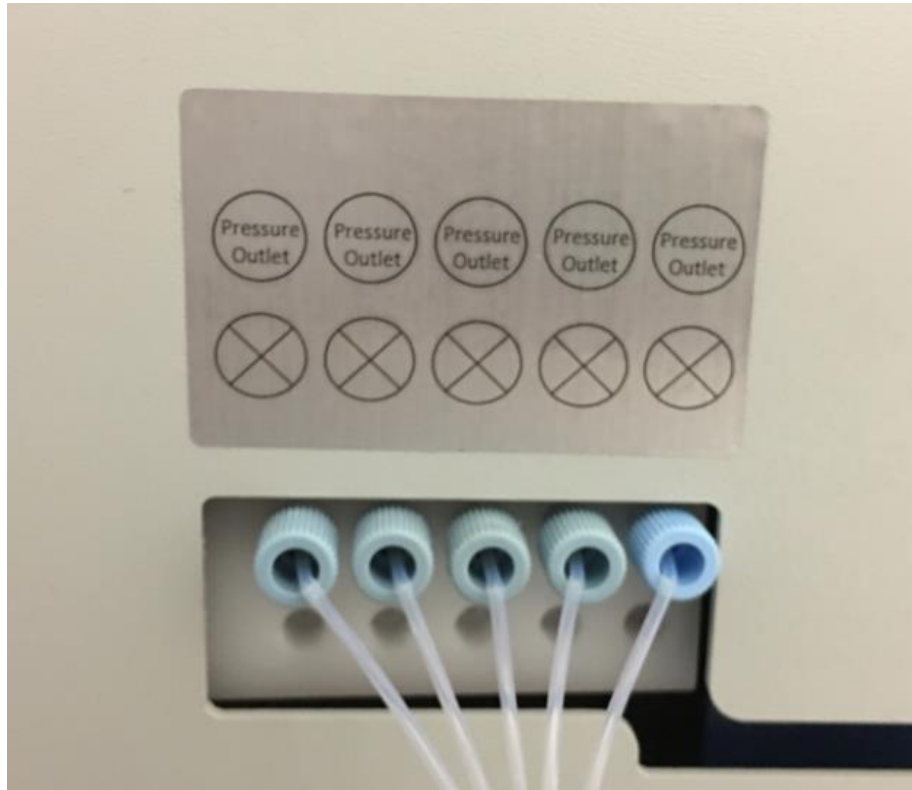


Figure 7-2 Reagent drop tubing Connections

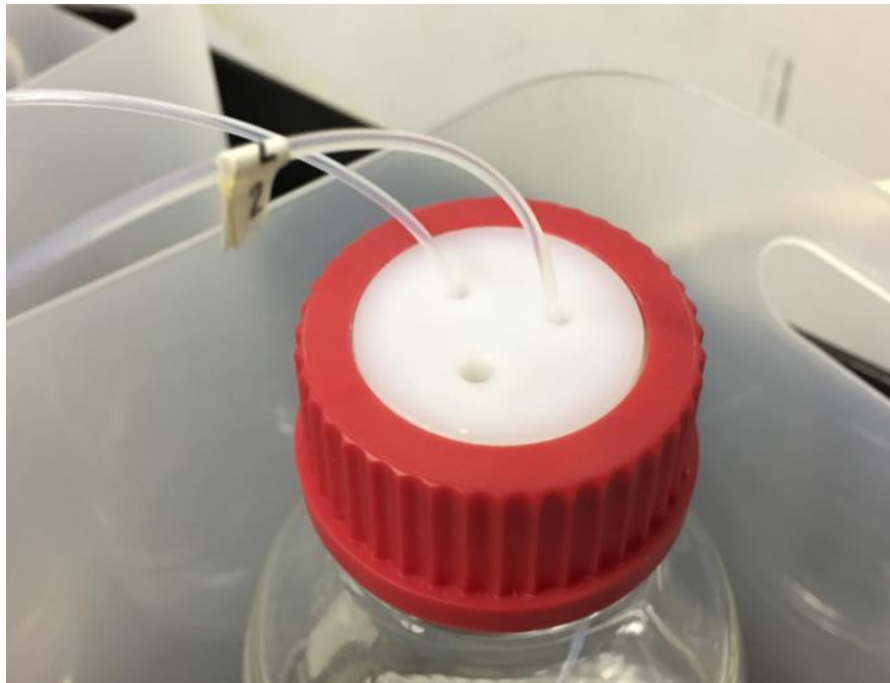


Figure 7-3 Reagent Bottles for reagent drop pins

7.3. External Equipment Connections

The VERSA 1100 has two external devices to be connected to the main unit; the UV/HEPA cover(*Figure 7-4 UV/HEPA power cable connection*) and the vacuum pump for the 96 channel aspirator.



Figure 7-4 UV/HEPA power cable connection

7.4. Power & USB Connection

The connection to the computer is via a USB cable(*Figure 7-5 USB Cable Connections*). Please plug the cable into the USB port at the back panel near the power outlet and on the personal computer. The power cable should then be plugged into the power source. The power switch is located above the power cable connection (*Figure 7-6 VERSA and Vacuum pump Power Connections*). Connect the vacuum pump power connection too if the unit is equipped with 96 channel aspirator or vacuum modules for DNA/RNA extraction protocols.

Switch on the instrument. There is an emergency switch in front of the unit which can be used to turn the instrument off quickly in an emergency(*Figure 7-7 Emergency Switch*). Switch Ensure this emergency switch is not tripped when turning on the system.



Figure 7-5 USB Cable Connections

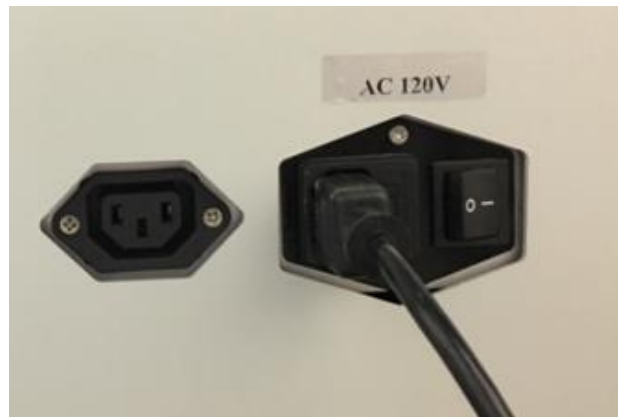


Figure 7-6 VERSA and Vacuum pump Power Connections



Figure 7-7 Emergency Switch

Note: In case of any emergency, if the instrument is turned off from this emergency switch, remember to pull the switch back out when ready to restart the instrument.

8. Software Installation

The software will come pre-installed on the computer that is shipped with your instrument, therefore once switching on the computer look for the software shortcut icon titled "VERSAware" on the desktop.

Double click on the icon to open the software. There is no password set to open the software (just click ENTER), a password can be set at a later time if desired. Once the software opens proceed to next section.

If however you are installing the software on a new computer from the CD, please follow the instructions as below.

8.1. VERSAware Installation

1. Log on to Windows with the Administrator Account or any account (local or domain) that belongs to the Administrator group to install and run the VERSAware 1100A software.
2. Disable User Account Control: In "Control Panel → User Accounts → Change User Account Control settings → set Never Notify".

Restart the computer.

3. Following restart, go to "Control Panel → Power Options → Change plan settings". Select the current power plan.

"Put the computer to sleep → Never".

Go to "Control Panel → Power Options → Change plan settings → Change advanced power settings".

"Hard disk → Turn off hard disk after → Never".

"Sleep → Sleep after → Never".

"Allow hybrid sleep → Off".

"Hibernate after → Never".

"Allow wake timers → Disable".

"Power buttons and lid → Power button action → Shut down".

4. If this or any previous version the VERSAware software is installed on the computer, please rename the previous VERSAware 1100A software folder if available:

C:\Program Files\Aurora Biomed\VERSAware 1100A

to C:\Program Files\Aurora Biomed\VERSAware 1100A_old

("C:\Program Files (x86)\Aurora Biomed\VERSAware 1100A" in case of Windows Vista (64-bit), Windows 7, Windows 8)

5. Copy the entire contents of the installation CD to any local hard drive folder before installation. Install the software by running "Setup.exe" from local hard drive folder (*Figure 8-1VERSAWare 1100A Installation Folder Selection-click Setup.exe*).

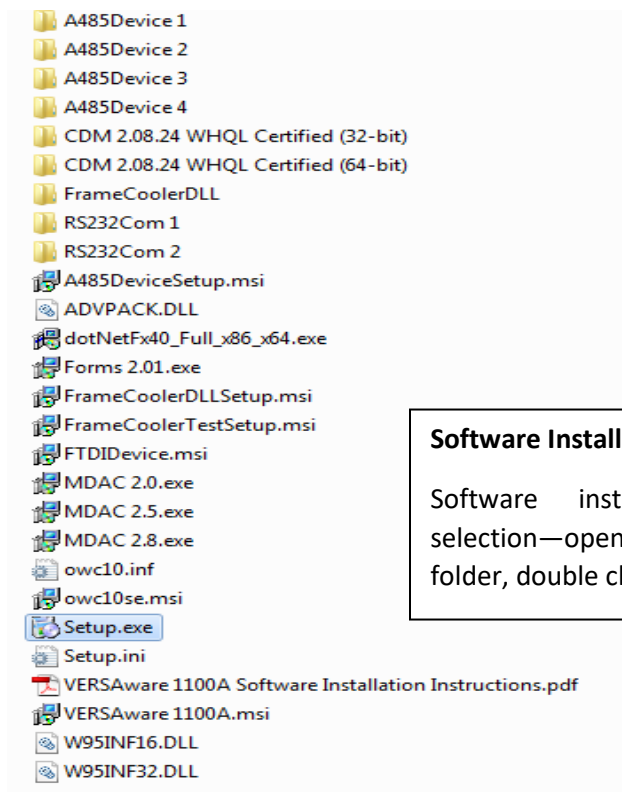
Follow the installation process. Press "Finish" and/or "Close" buttons each time after RS232Com and A485Device component installation windows request user input.

Do not press the "Cancel" button during the installation process.

Choose "Repair" options for libraries and components.

Wait until Microsoft .NET Framework 4 is fully installed.

Total software installation time should be between 1 - 5 min. depending on the computer speed.(*Figure 8-3 VERSAWare 1100A Software installed successfully window*)



Software Installation:
Software installation folder selection—open release package folder, double click "Setup.exe"

Figure 8-1VERSAWare 1100A Installation Folder Selection-click Setup.exe

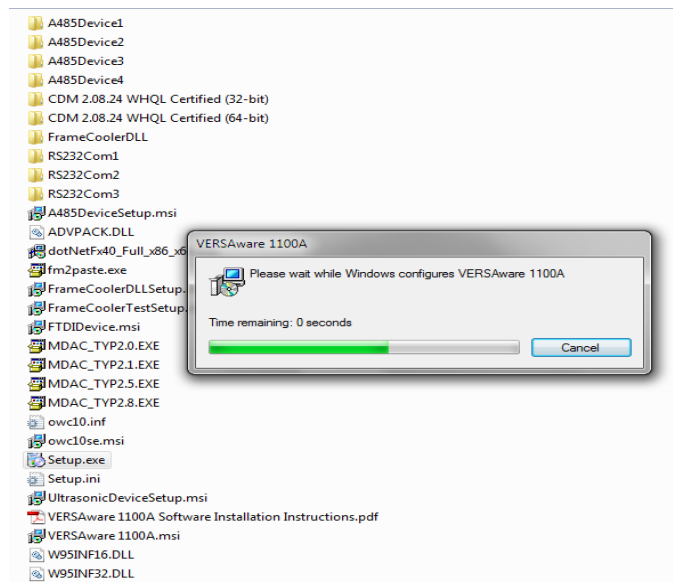


Figure 8-2 VERSAware 1100A Software installation window

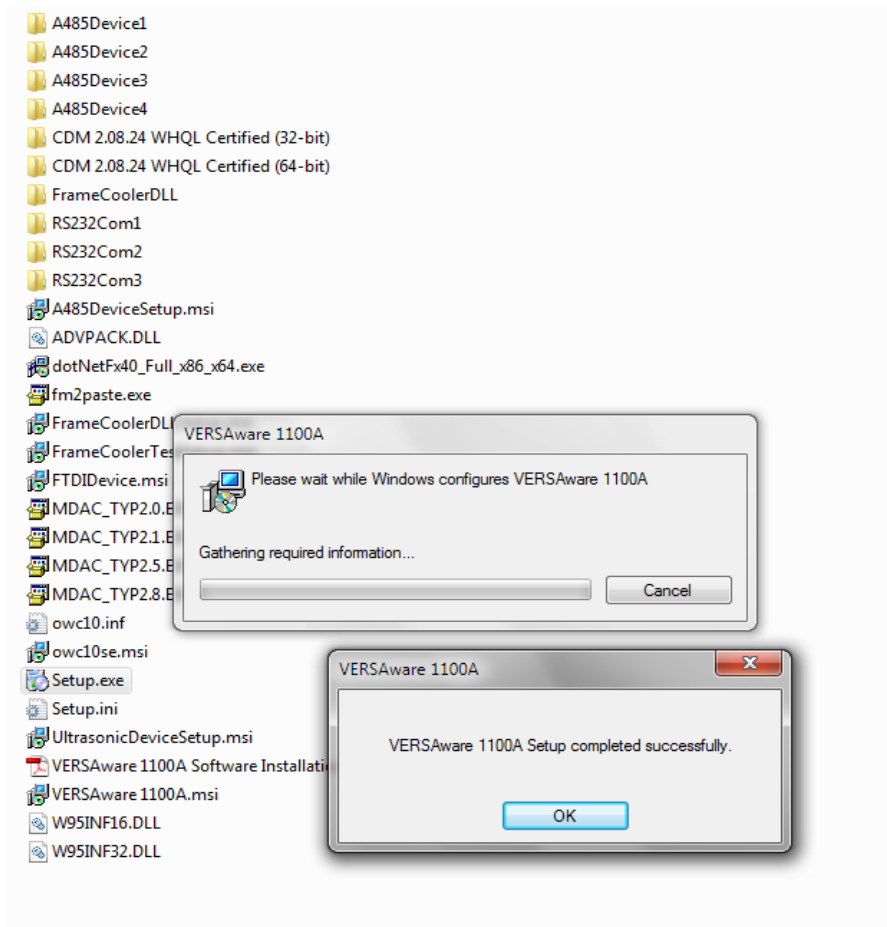


Figure 8-3 VERSAware 1100A Software installed successfully window

After the software is installed successfully, software shortcut icon will appear on the monitor screen (Figure 8-4 VERSAware1100 software shortcut icon on the screen).

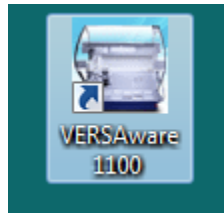


Figure 8-4 VERSAware1100 software shortcut icon on the screen

Software folder will be created at C drive-program files(x86) Aurora Biomed as shown below:

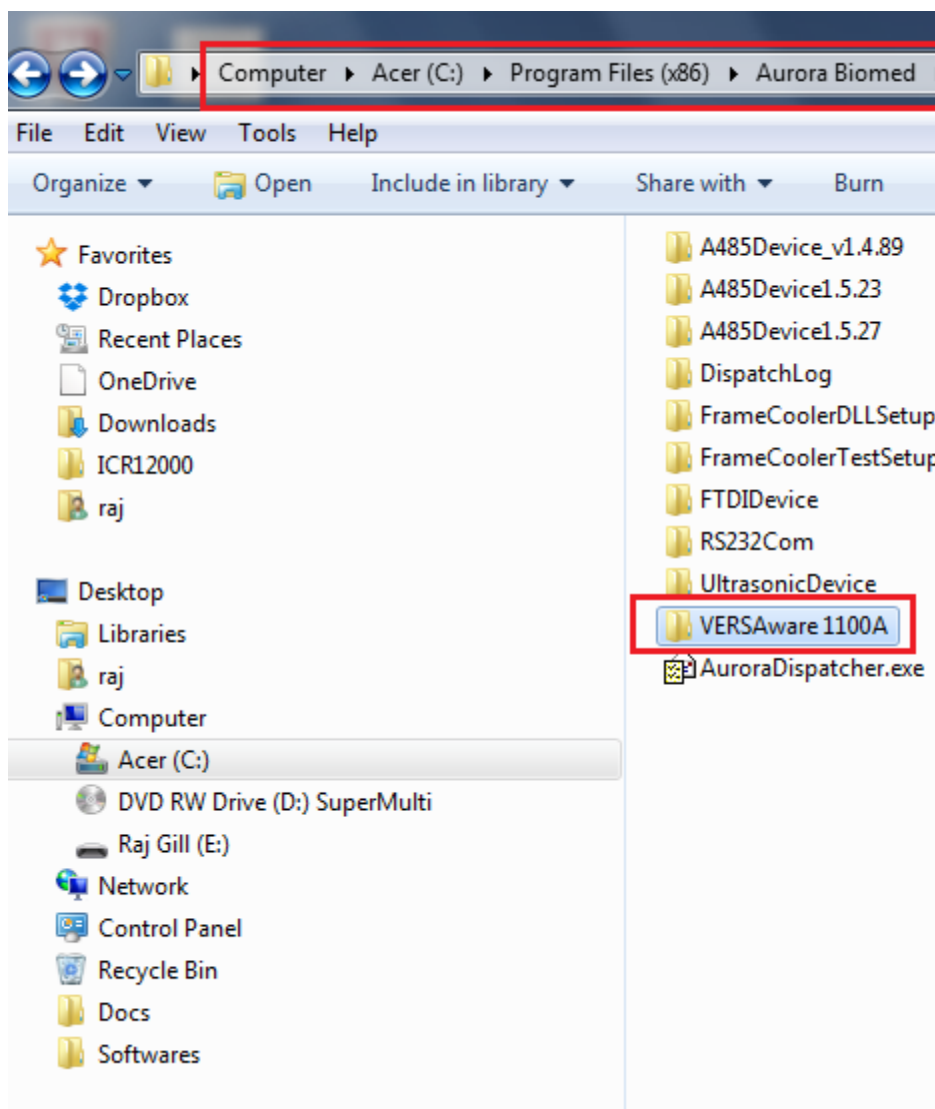


Figure 8-5 VERSAware1100 software installed folder

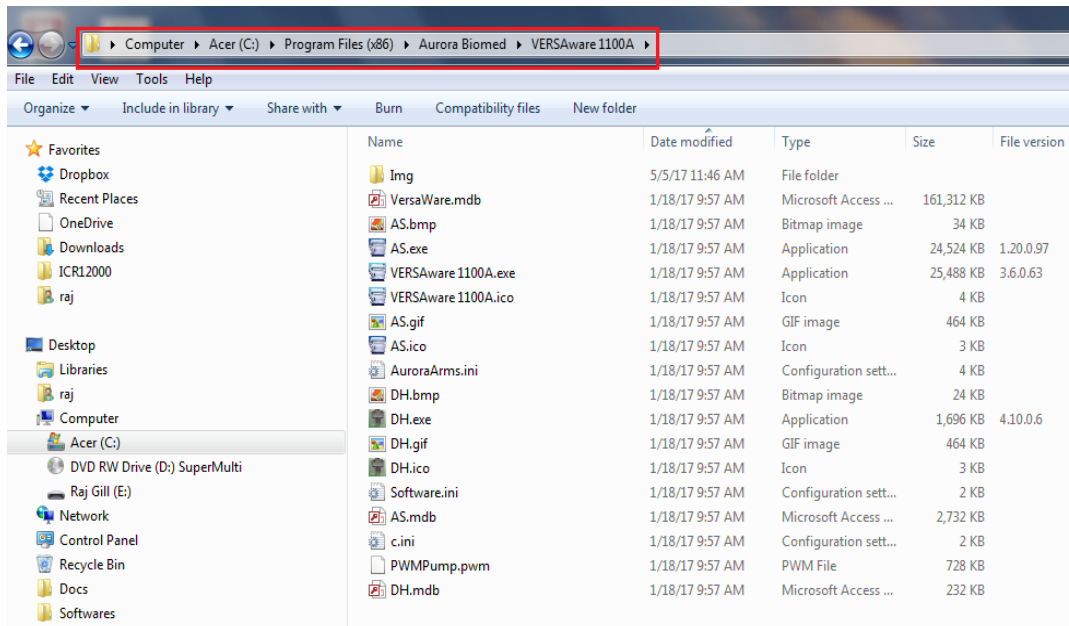


Figure 8-6 VERSAware1100 –Open VERSAware1100 software to see all files

Restart the computer after the VERSAware 1100A software installation.

7. Connect the instrument using the USB cable, and turn the instrument power on.

If the computer is connected to the Internet, confirm that COM ports are automatically installed one by one in "Control Panel → Device Manager → Ports (COM & LPT)".

If no Internet connection is available, install the FTDI USB-to-RS232 driver for each "Control Panel → Device Manager → Other devices → USB Serial Port → right-mouse click → Properties → Driver → Update Driver..." from one of the folders located on the installation CD:

"CDM 2.08.24 WHQL Certified (32-bit)" in case of Windows XP, Windows Vista (32-bit)

"CDM 2.08.24 WHQL Certified (64-bit)" in case of Windows Vista (64-bit), Windows 7, Windows

8.2. COM Ports – Changing or Reassigning

Usually, if the above mentioned steps are followed, COM ports should be assigned correctly and automatically. Make sure all comports are working first under Control Panel. If necessary, COM ports can be reassigned correctly under the associated directories as follows:

For Windows XP (Start --> Control Panel --> System --> Hardware --> Device Manager)

For Windows Vista (Start --> Control Panel --> Device Manager):

Change or adjust COM port numbers (if necessary) in Control Panel as displayed in table below (Figure 8-7 COM Ports Allocation) :

Device	Description	COM Port	Serial Number	Range (X)	Range (Y)	Range (Z)	Start Speed (X)	End Speed (X)
0	Syringe Pump (1000 µL)	4	FTT7Q0V4A	20000			10	1000
1	Robotic Arm	3	C	12100	2700	7800	50	1500
3	Syringe Pump (8 × 1000 µL)	4	FTTA4HRAC	20000			10	1000
4	Gripper Arm	12	FTT7Q0V4C	46000			100	2000
*								

Figure 8-7 COM Ports Allocation

8.3. Changing COM Ports in Control Panel for Windows Vista

The below section will discuss how to change COM port numbers in the Control Panel for Windows Vista. COM port numbers should be adjusted in such way that they match COM ports configured in Aurora Station (Figure 8-8 Aurora Station Settings) and Device Manager DH (if the instrument features the Reagent Drop).

Please check COM port configuration in Aurora Station .

Aurora Station → Aurora Station Settings → Hardware

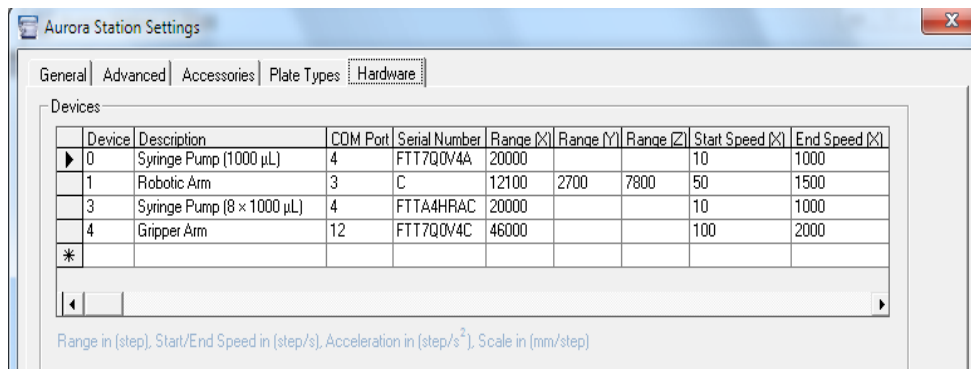


Figure 8-8 Aurora Station Settings

COM port numbers should be adjusted in Control Panel (Figure 8-9 Windows Vista Control Panel) of Windows Vista starting with the COM port for the robotic arm :

Start → Control Panel → Device Manager

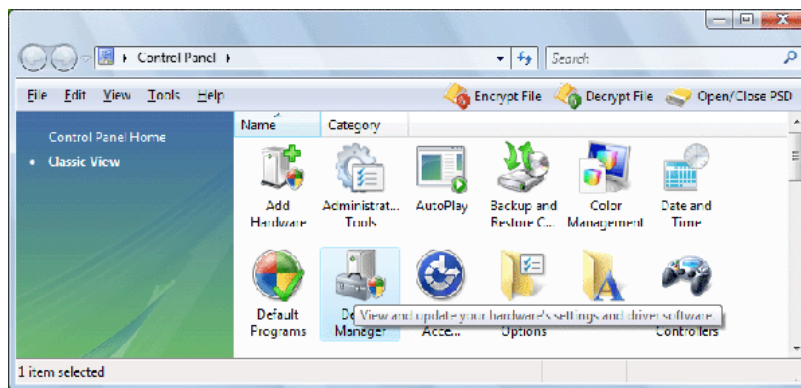


Figure 8-9 Windows Vista Control Panel

Display available COM ports in Device Manager of Windows Vista as shown in below Figure(Figure 8-10 Windows Vista Device Manager).

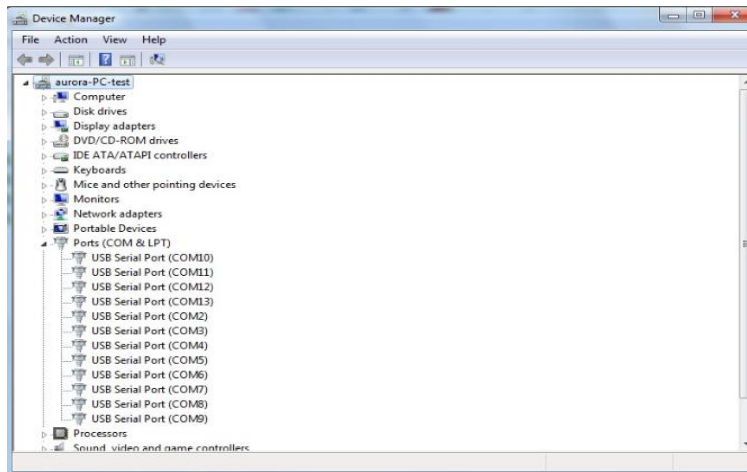


Figure 8-10 Windows Vista Device Manager

This example will illustrate how to change one particular COM port 3 to COM port 11 (as an example) and this process can be applied to all other COM ports.

COM port numbers in Device Manager should match the COM ports in Aurora Station and Device Manager DH (if the system features the Reagent Drop).

Standard COM port configuration in Device Manager might have 4, 8 or 12 individual COM ports (Prolific USB-to-Serial Bridge).

Any existent COM port should only be changed to the new COM port number which is not available in Device Manager to avoid communication errors. All COM port numbers that are featured in Device Manager should not exceed 16. This is one of the basic requirements for the software configuration. Use left double-click with your mouse to display COM port properties as shown in below Figure(Figure 8-11 COM Port Properties).

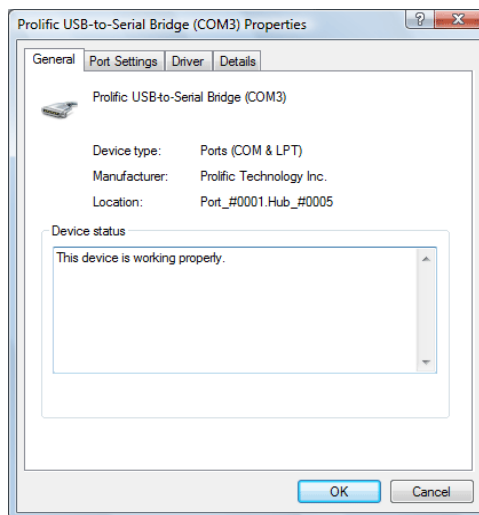


Figure 8-11 COM Port Properties

Please switch to 'Port Settings' tab as displayed in the below Figure(Figure 8-12 COM Port Settings).

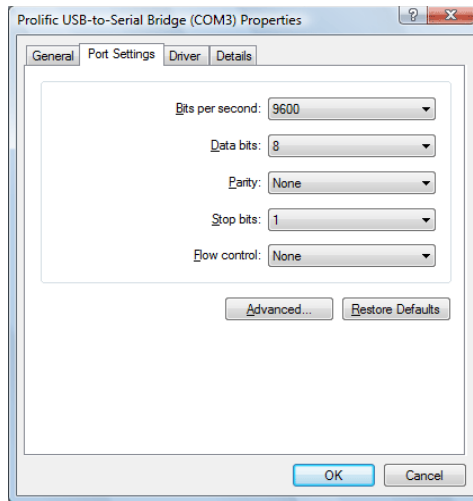


Figure 8-12 COM Port Settings

Click 'Advanced' button as shown in the below Figure(Figure 8-13 Advanced COM Port Settings).

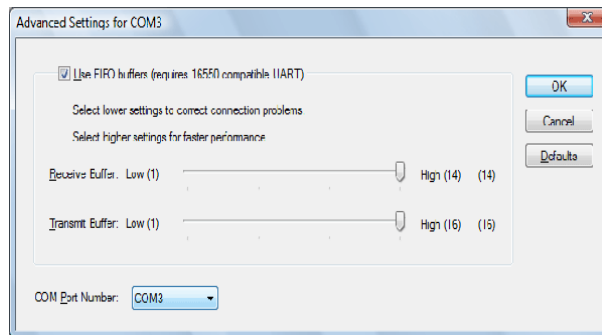


Figure 8-13 Advanced COM Port Settings

Change COM port number to 11 (just for this example) as displayed in below Figure(Figure 8-14 COM Port Number) and press 'OK' button.

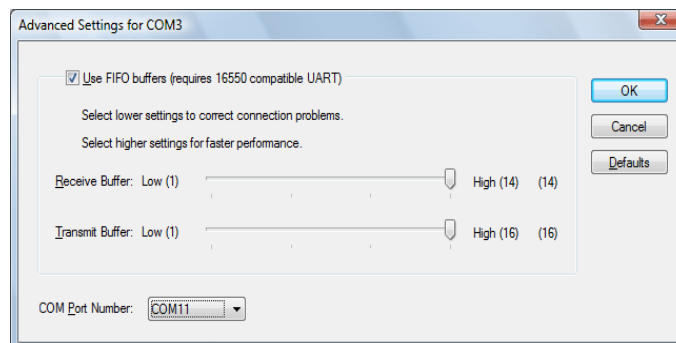


Figure 8-14 COM Port Number

The new COM port number will appear in Windows Vista Aurora Station shown in below Figure (Figure 8-15 Windows Vista Device Manager). Please perform the same procedure for all COM ports that need to be adjusted.

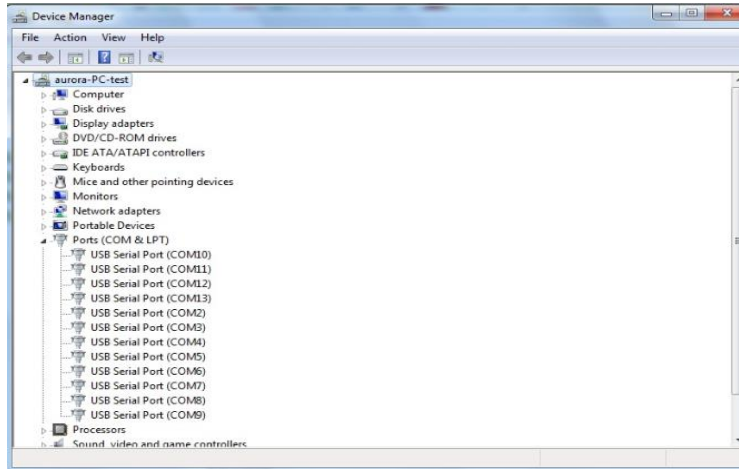


Figure 8-15 Windows Vista Device Manager

8.4. Power Options and User Accounts

To reduce the probability of robotic interruption during operation, it is strongly advised that you disable any screen savers, hibernation or standby features.

Power Saving Settings (Windows XP):

These settings should be incorporated for VERSAWare to run without interruptions and be compatible to Windows XP

Go to:

Control Panel --> Power Options --> Power Schemes:

Set the following options for the:

1. Settings for power scheme:

Turn off monitor: Never

Turn off hard disks: Never

System standby: Never

Control Panel --> Power Options --> Hibernate:

2. Disable hibernation/ Uncheck hibernation

Power Saving Settings (Windows Vista):

These settings should be changed for VERSAWare to run without interruptions and be compatible to Windows Vista

Go to:

Control Panel --> User Accounts --> Turn User Account on or off

Use User Account Control to help protect your computer (Not checked)

Control Panel --> Power Options --> Change plan settings --> Put the computer to sleep: Never

Control Panel --> Power Options --> Change plan settings --> Change advanced power settings

Set the following options for the:

1. Hard disk

Turn off hard disk after

On battery: Never

Plugged in: Never

2. Sleep

Sleep after

On battery: Never

Plugged in: Never

Allow hybrid sleep

On battery: Off

Plugged in: Off

Hibernate after

On battery: Never

Plugged in: Never

3. Power buttons and lid

Lid close action

On battery: Do nothing

Plugged in: Do nothing

Power button action

On battery: Shut down

Plugged in: Shut down

Start menu power button

On battery: Shut down

Plugged in: Shut down

4. Battery

Critical battery action

On battery: Shut down

Plugged in: Do nothing

Low battery action

On battery: Do nothing

Plugged in: Do nothing

8.5. Software Upgrading (Optional)

Aurora advises the following when performing an upgrade or software change. Clients or users will be informed through email or by phone of the upgrade being made available and be given instructions of how to download the upgraded version from Aurora's FTP site or by mail.

To install or upgrade to a new version, please follow the instructions below:

- 1) Backup all necessary sequences or assays by opening the current version. In the main interface screen, highlight the first assay you want backed up by clicking on the "file" drop down menu and clicking the 'export file' option. If you do not wish to keep the assays or sequences created, skip this step and proceed to step 3).
- 2) A new window will appear to confirm the location of the exported file. Click save and note the location of the backed-up assay file. Renaming can be performed once you import into the new software version.
- 3) Proceed then to rename the folder containing the current software in your PC. This is performed if you still want to keep the old version. If you do not wish to keep it, just delete without renaming. If you do not perform this step, the new version will attempt to save over the old and file types may conflict. Remember the location of the saved assay files if you want to still import them into the new software later.
- 4) Next, ensure that the new version is compatible to your workstation. Download the new software or install the received CD version into your PC. We strongly advise that you install the software into the same PC that came with the workstation.
- 5) After the installation procedure is completed, you may then want to import the files back into the new version by first adding an assay. Then by highlighting the created assay, right clicking and selecting 'import assay'.
A new window will appear to prompt you to select the location of the .txt assay file previously saved. After locating the file, complete the import of data. Your old assays along with the sequences attached should now be imported.
- 6) You can now rename the assays. Sequence names should have remained from the previous version and shouldn't need renaming.

9. VERSA1100 Set up - Aurora Station

This chapter will discuss how to set up and teach positions using Aurora Station and VERSAware, and how to set up, calibrate and prime the tubes with wash buffer and/or reagent.

NOTE: Aurora Station usually runs alongside VERSAware. Do not attempt to close Aurora Station but instead minimize the interface. In case of closure, follow the steps below:



Figure 9-1 Shell Status

Shell Status

- 1) Open shell status window (*Figure 9-1 Shell Status*) (which runs alongside VERSAware), double click onto AS01 (1): 1564 and Aurora Station will reopen. Do not attempt to close shell status window whenever VERSAware is running.
- 2) Alternatively, under VERSAware tools, <click> onto the Aurora Station option to reopen the interface. To close, click again.

9.1. Robotic Arm Tab

This section will discuss how to set up and teach the robotic arm positions. Please start VERSAware 1100 software by selecting:

Start → All Programs → Aurora Biomed → VERSAware1100

Switch to Aurora Station application by selecting the following menu option:

Tools → Aurora Station

The Aurora Station software should appear on the screen. Switch to the 'Robotic Arm' tab Press the unlabeled button (*Figure 9-2 Initialize Button*) between X and Y-axes to initialize all axes of the robotic arm. The robotic arm should travel towards the upper left corner of the workstation.



Caution Please ensure there are no obstructions in the path of the robotic arm!!!

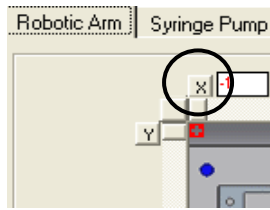


Figure 9-2 Initialize Button

Switch to the Robotic Arm tab (Figure 9-3 Aurora Station Robotic Arm Control). The Figure below explains how to control Aurora Arm. Use the scroll bars to control X, Y, Z-axes separately. The current arm coordinates will be displayed in text boxes in the upper left corner.

Every scroll bar shows the maximum range of this axis.

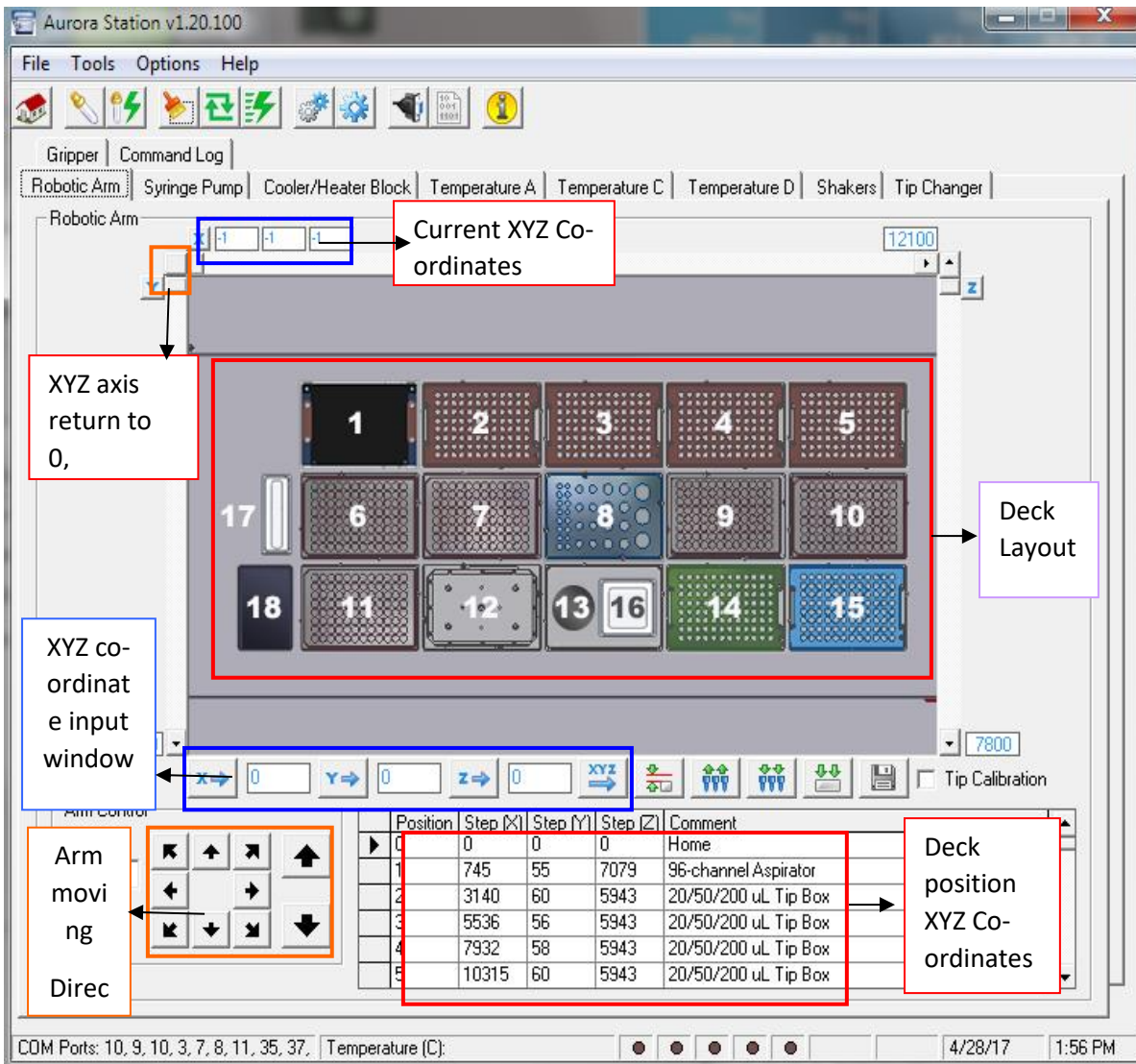







Figure 9-3 Aurora Station Robotic Arm Control

Aurora Station position teaching icons:

Click XYZ -     -- Arm will go to the loaded XYZ co-ordinates.

Click this icon  to pick tips from any tip box position on the deck to teach positions

After picking tips, Click this icon  to test the any position on the deck

After teaching position, click on this icon  Tip Calibration to save the position (check on Tip calibration)

This icon is used  to calculate the XYZ off sets

Please use the scroll bars to control X, Y, Z-axes independently. The current robotic arm coordinates will be displayed in text boxes in the upper left corner. Each scroll bar shows the maximum travel range for this axis. X, Y, and Z buttons will initialize every individual axis. There are maximum ranges for X, Y, and Z coordinate – for e.g. for the Z axis, the maximum range is 60000 steps.

Another option to control the Aurora Arm would be to use the step/arrow control (*Figure 9-4 Aurora Station Step/Arrow Control*). Enter a step value from 1 to 1,000 and use one of the eight arrows on the left side to control horizontal X and Y-axes. Two arrows on the extreme right side will control vertical Z-axis.

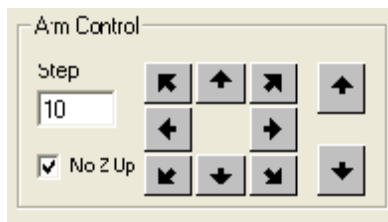


Figure 9-4 Aurora Station Step/Arrow Control

VERSA 1100 workstation features 15 positions for various lab ware types, tips, blocks, tubes, reservoirs etc. Positions 16 & 17 are the waste & chute positions respectively. All the positions are taught with a 96 well plate (*Figure 9-5 Plate Teaching Positions*) except reagent cooler block position which is taught as is. Once taught, the robotic arm will be automatically calibrated for liquid handling of all other plate types which are stored in the VERSAWare plate library.

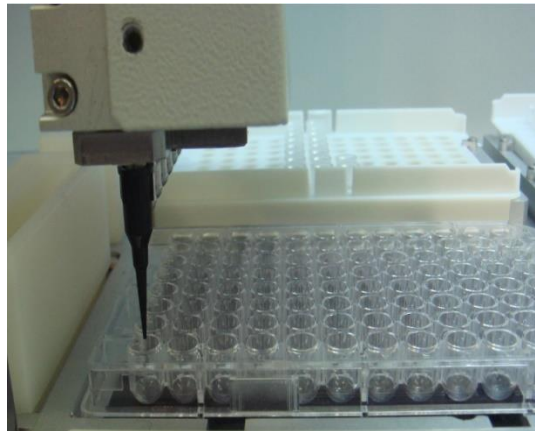


Figure 9-5 Plate Teaching Positions

In order to test previously saved default teaching positions; please double-click the record selector of the desired position as displayed in below Figure (Figure 9-6 Aurora Station Teaching Positions). The X, Y, Z-coordinates will be displayed in text boxes GoX, GoY, and GoZ. Next, <Click> on “Go XYZ” to have the arm move to the default position set by the manufacturer to confirm correct teach position has been set.

To recalibrate or teach a new plate type, click on the position you want to teach, place a 96-well plate on that position on the deck, set the step (Z) coordinate to “0” and press “Go XYZ”. If the XY positions are right, click the down arrow until desired Z position is reached. Please note to reduce the steps of the arm control to 5 or 10 for more precise and safe control of arm movement. Once syringe barrel (w/o tip) is close to the top left corner first well of the 96 well plate, correct position is taught or determined by passing through a paper in between the 96-well plate and the barrel tip barely touching it.

Similarly check for all other positions. Adjust the other positions if necessary and save the new coordinates.

GoX | 0 | GoY | 0 | GoZ | 0 | Go XYZ | Offset | Tip | Test | Save | Tip Calibration

Arm Control

Step: 10

No Z Up

Position	Step (X)	Step (Y)	Step (Z)	Comment
16	1000	1000	3000	Priming Position
17	30	1370	6000	Waste Position
18	0	2550	5000	The Chute
100	8794	2652	7184	
*				

Figure 9-6 Aurora Station Teaching Positions



Caution Inaccurate robotic arm positions may cause damage to liquid handling head and tip barrels!!

9.2. Teaching Positions

The VERSA 1100 Workstation uses a standard 96-well plate for teaching all positions except the Reagent Cooler Block. Only the reagent block, chute and waste positions are taught as shown below. All other positions should be taught on the surface of the upper left well of the standard 96-well micro-plate as shown in the figures below.

Initialize the robotic arm by pressing the button between X and Y-axes (Robotic Arm tab). The top left well A1 (column 1, row 1) should be taught on the surface of the plate. Move the arm towards you and manually (with your hands) engage a 20 μ L tip to barrel # 1(outer one).

This tip can be clear or black liquid level sensing tip if available. Place a small sheet of paper (~4 x 4 mm) on top of the 96-well plate. Move the arm down to slightly touch this sheet of paper with the tip as shown in *Figure 9-6 Aurora Station Teaching Positions*. Remove the sheet of paper and align the arm in the center of the well (X and Y-axes) without changing Z-axis.

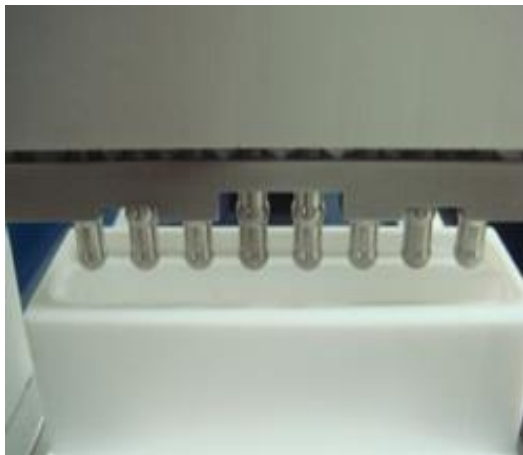


Figure 9-7 Aurora Station Chute Position Teaching



Figure 9-8 Aurora Station Waste Position Teaching

Open the position to be taught by double clicking from AS e.g. position # 6 (*Figure 9-9 Aurora Station- Position Teaching for # 6 deck position*). Make the Z co-ordinate 0 and then click 'Test'. The arm will execute the command and go to position # 6 but very high as Z is set zero.

The 20 μ L tip should be positioned exactly on the surface level of the extreme left row of the standard 96-well micro-plate and positioned at the centre of each well (X-axis & Y-axis alignment).

For verification of z-axis positioning, a piece of paper can be placed in between the tip and the well of the 96-well micro-plate (*Figure 9-10 96-well Micro-plate Teaching Position*). Fine adjustment is done by commanding the z-axis robotic arm in descending movement till the

barrel tips just touching the surface of the paper (*Figure 9-11 96-well Micro-plate Teaching Position*).

Slight resistance is felt when inserting or removing the paper. After setting all the co-ordinates to make the tip in the center of the wells, save this position by clicking 'Tip Calibration' as shown in *Figure 9-12 Aurora Station- Saving Position*.

Teach all other position same way.

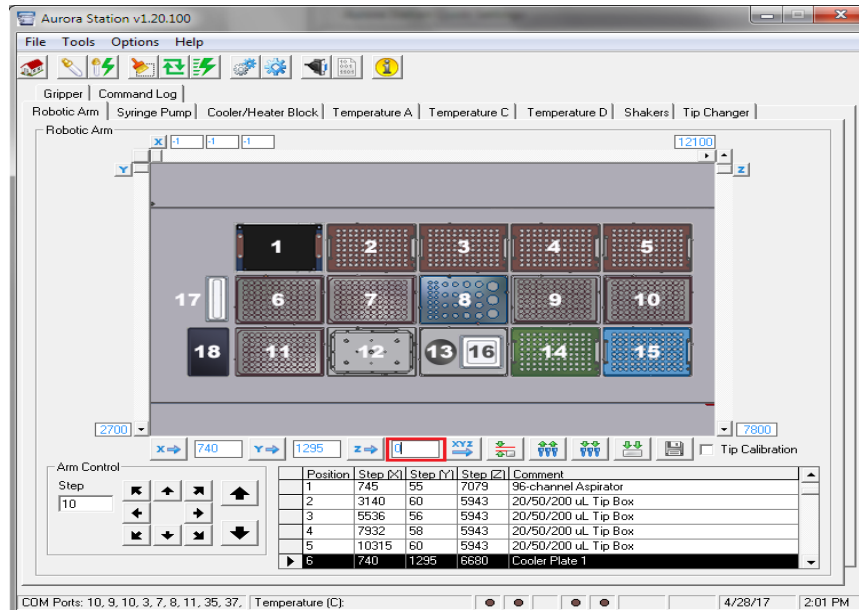


Figure 9-9 Aurora Station- Position Teaching for # 6 deck position

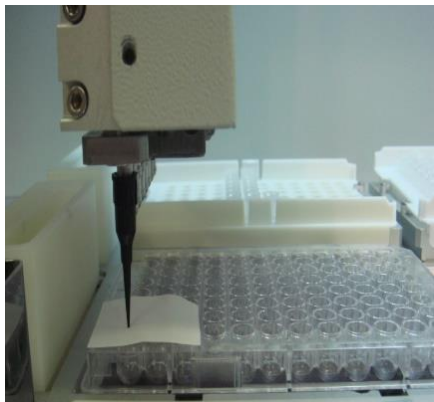


Figure 9-10 96-well Micro-plate Teaching Position

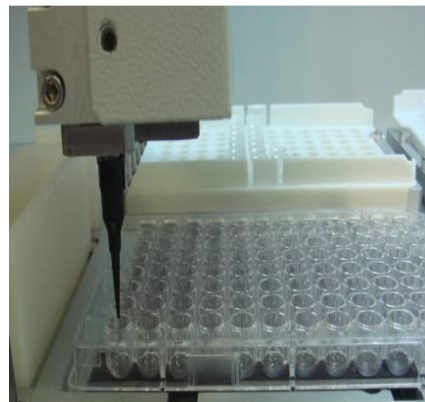


Figure 9-11 96-well Micro-plate Teaching Position

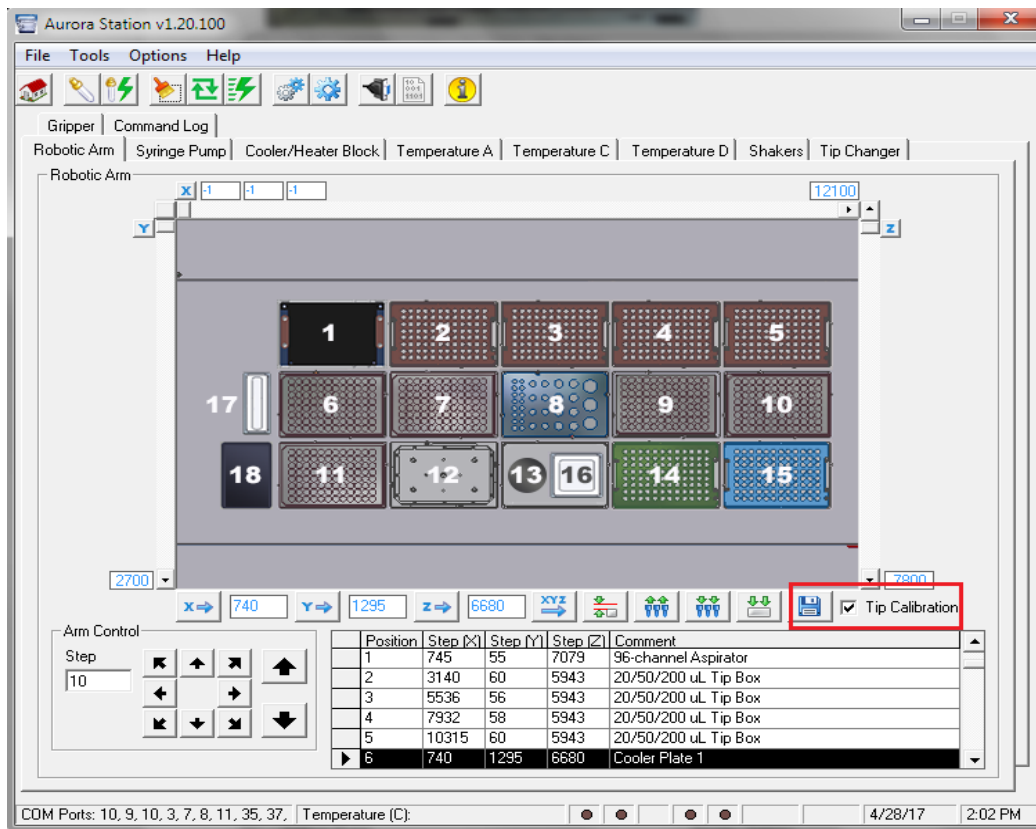


Figure 9-12 Aurora Station- Saving Position

In the VERSAware software interface, drag the 20ul tip box on to the deck (e.g. position # 2), put one 20ul tip in this box (A1 well), click that position from AS & click tip. Arm will go to position # 2 to pick the tips or tip.

Double click the cooler block position, make Z co-ordinate zero, and click 'Test'. Arm will go to T1 column of the block, adjust X Y Z co-ordinates to position the tip on the surface and the centre of the tube as shown in *Figure 9-13 Aurora Station- Picking 20ul tip from position #2*.

The z-axis height position can be checked with a piece of paper in such a way that it can pass under the tip with slight resistance. After adjusting it in the center of the T1 tube, save the position by clicking 'Tip Calibration'.

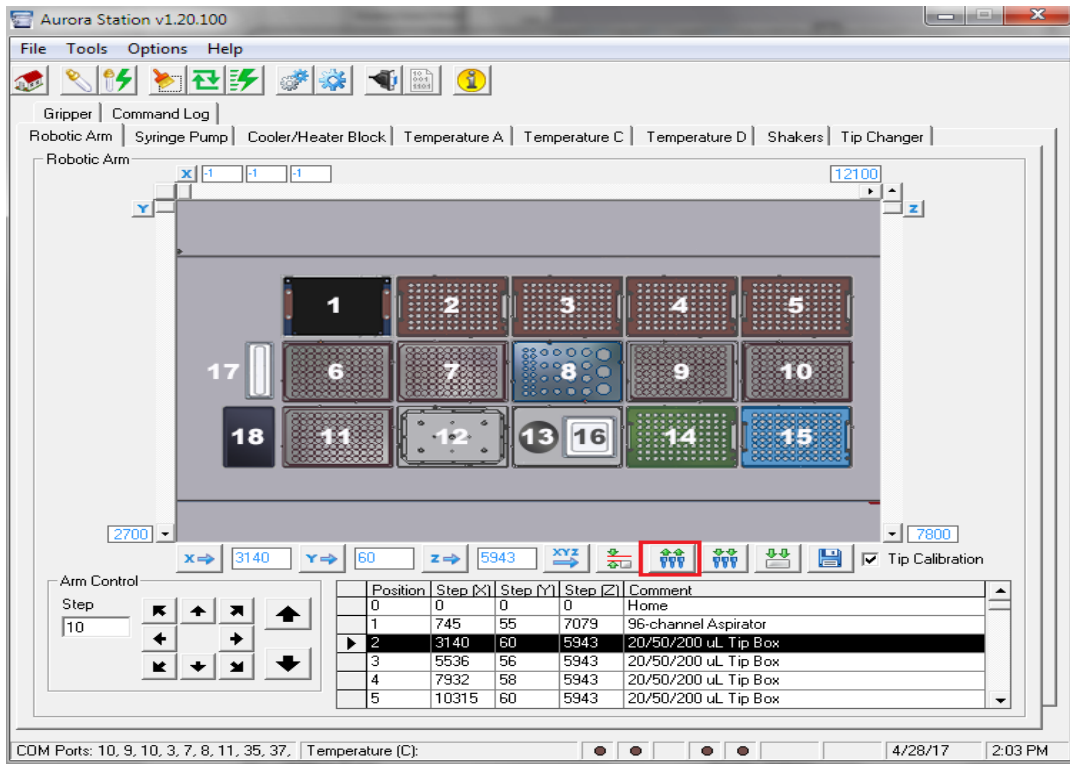


Figure 9-13 Aurora Station- Picking 20ul tip from position #2

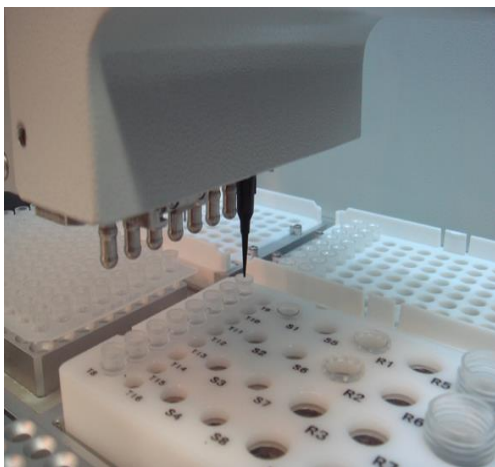


Figure 9-14 Reagent Cooler Block Teaching Position

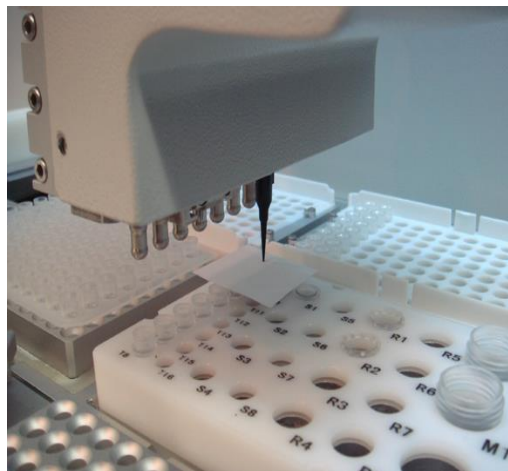


Figure 9-15 Reagent Cooler Block Teaching Position

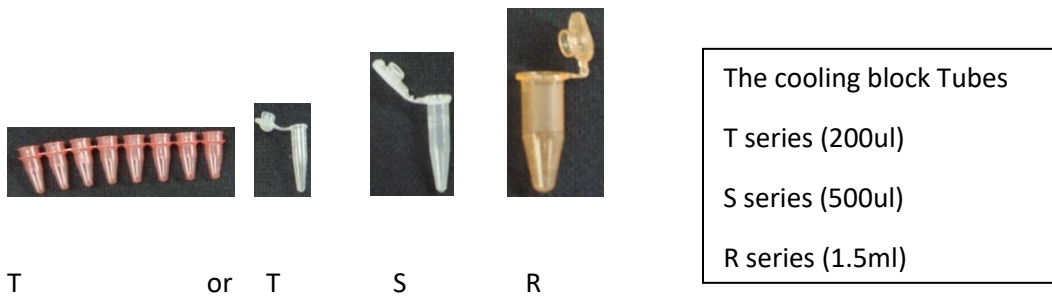


Figure 9-16 Reagent Cooler Block Tubes

Once you are done teaching the positions for the 8-Channel Head, the system is ready to run the application tabs.

Vacuum Positions: These positions (Filter & Receiver) are taught on the surface of the filter plate on top of both positions (Filter & Receiver positions) as shown in Figure 9-17 Vacuum Wash & Sample Collection Positions.



Figure 9-17 Vacuum Wash & Sample Collection Positions

9.3. Testing New and Default Plate Types

Aurora Station allows you to test previously or newly taught positions for all lab-ware types. It is a good practice to test positions periodically to ensure that the arm calibrated for a particular plate type and position is still intact.

To test any position on the deck, enter Aurora Station settings and <click> on the Advanced Options tab as depicted below (*Figure 9-18 Aurora Station Settings Main Interface – Advanced Settings Tab*). Next, on the bottom left corner of the interface screen, select the position to test. After which you'd want to select the plate type or some other listed lab-ware to test on that position. <Click> onto the Plate Type settings tab and select for a listed plate type by highlighting the selection.

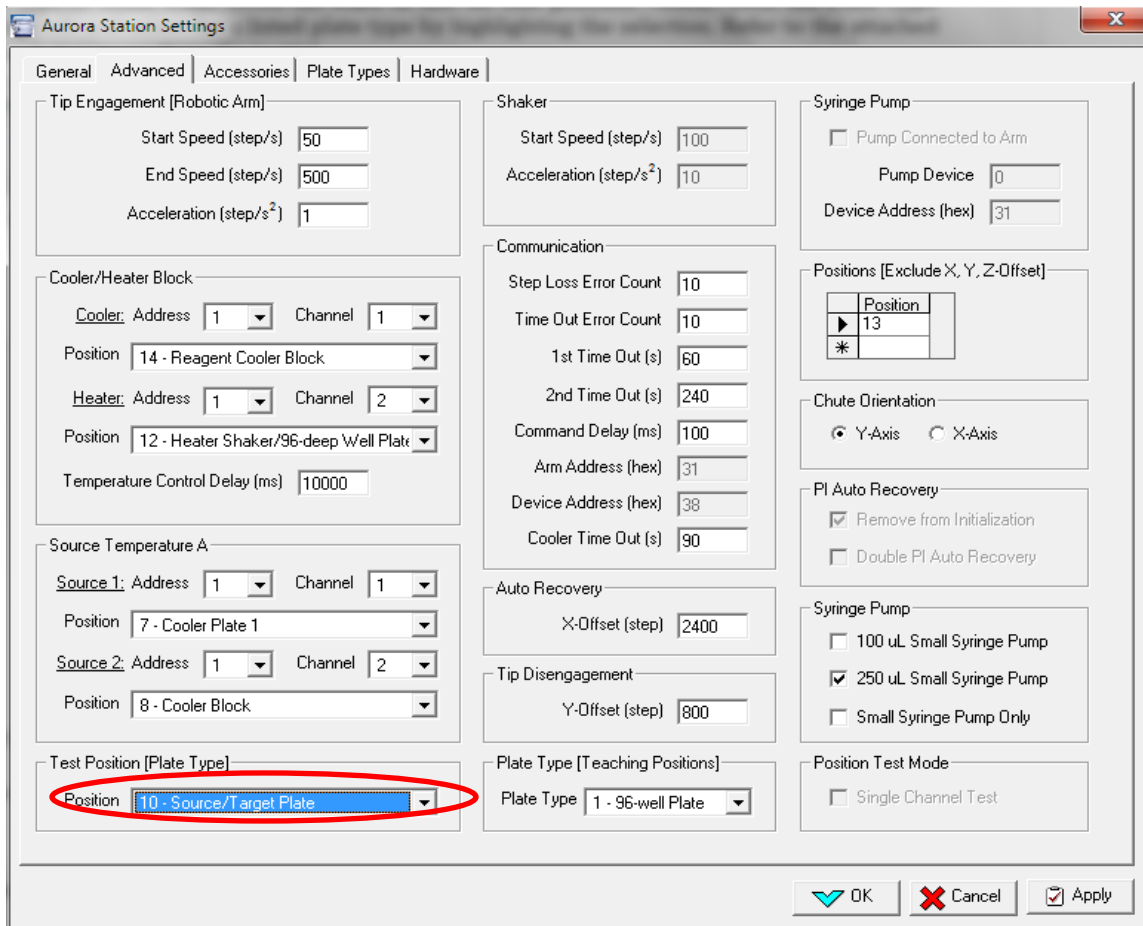


Figure 9-18 Aurora Station Settings Main Interface – Advanced Settings Tab

To pick up a 200µL Tip for teaching position verification, ensure you have selected Single-Channel operation mode, and then go to the Aurora Station main screen to select position (for this example we will use position 15).

To let the software know that you have a full tip-box you must “reload the tip boxes” by dragging and dropping the correct size of tip box in the software to the location where it exists on the deck (Figure 9-19 Aurora Station –Reload Tip Box). In the VERSA1100 main interface menu, drag 200 Tip (1) representing single channel 200µL Tip Box to position 15. Or under Reload Tip Boxes mode, drag 200Tip (1) to position 15.

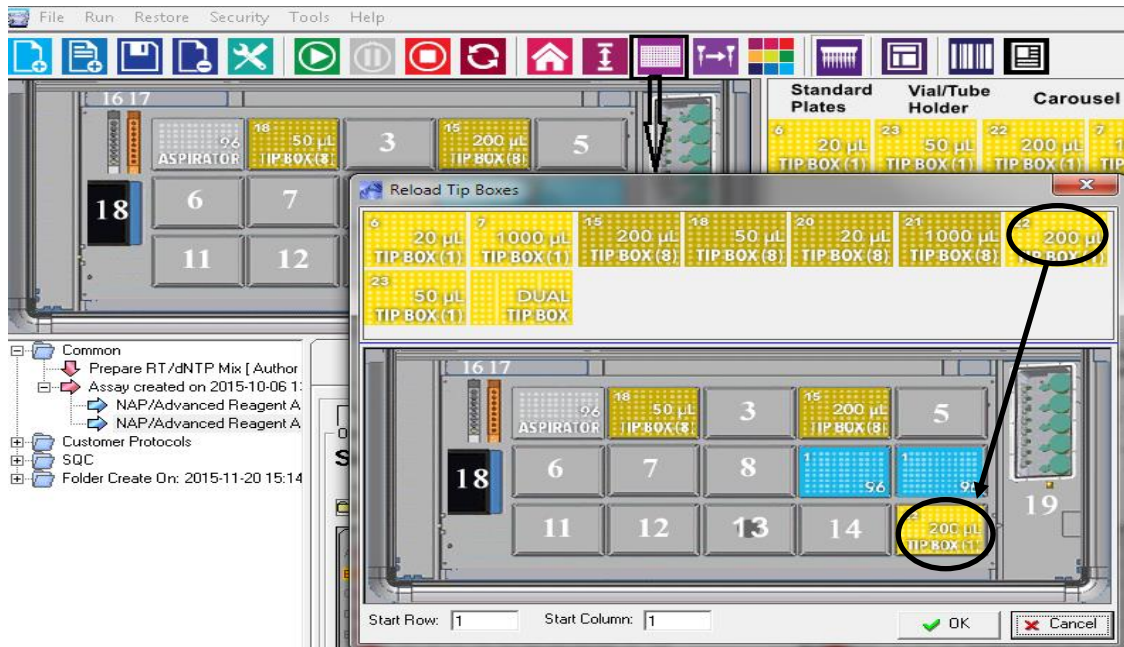


Figure 9-19 Aurora Station –Reload Tip Box

To ask the software to pick up a single tip, in single channel mode simply click Tip. The robotic arm will execute the command to pick up the extreme top left corner tip (Figure 9-20 Aurora Station –Pick Tip). Black tips have been used in this example (Figure 9-21 Aurora Station –Picking Tip) for better photo illustration, however clear tips can also be used to check positions.

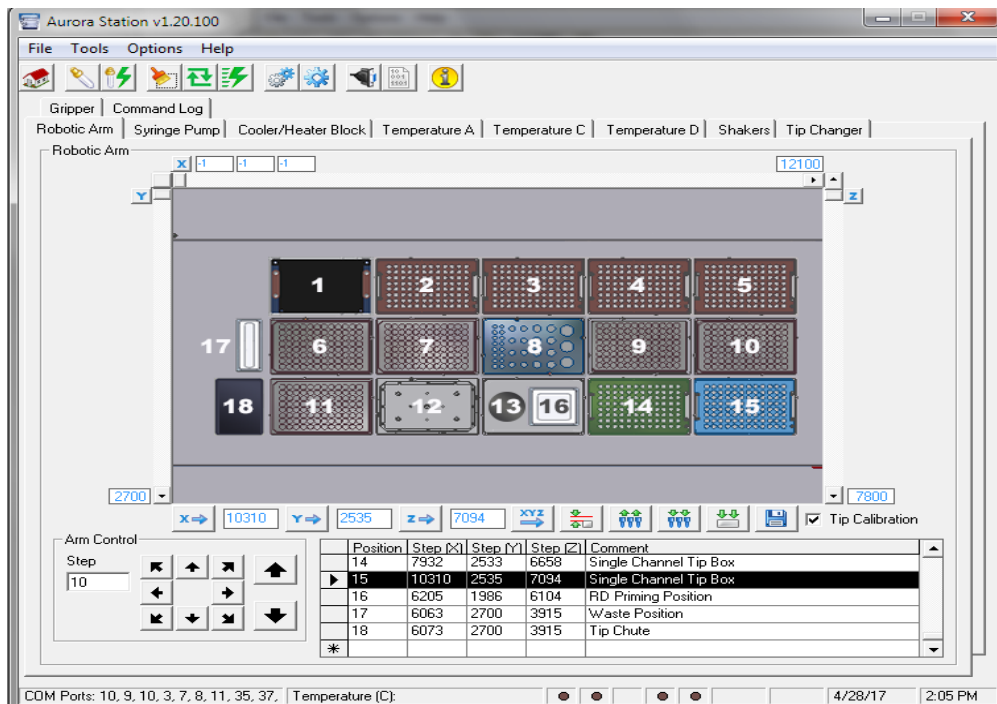


Figure 9-20 Aurora Station –Pick Tip

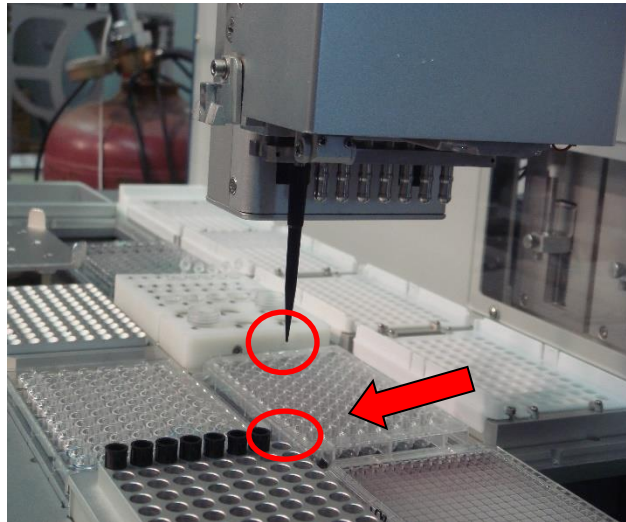


Figure 9-21 Aurora Station –Picking Tip

In Aurora Station Settings, all the parameters for various plates are tabulated as shown below (Figure 9-22 Aurora Station Settings Main Interface – Plate Types Settings Tab) and these settings are used for verification and fine adjustment of position.

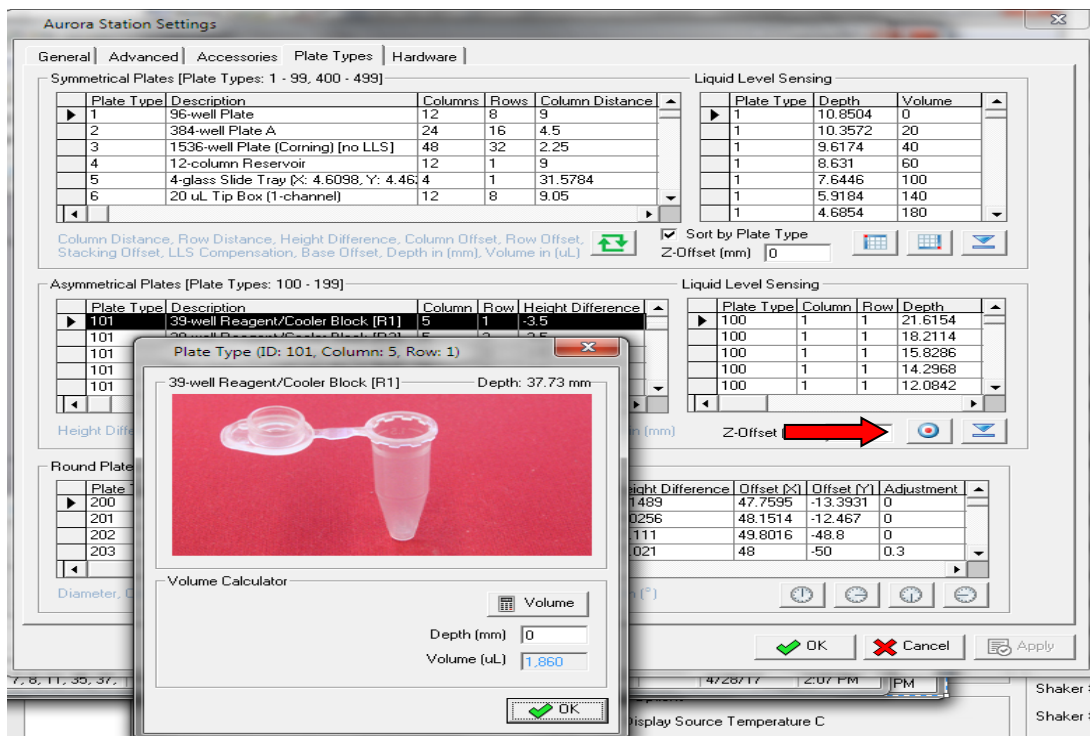


Figure 9-22 Aurora Station Settings Main Interface – Plate Types Settings Tab

To select a plate type: (in this example a 39-well Reagent/Cooler Block is used) double click to highlight the desired position (highlighted in black) as shown in the above screen shot (Figure 9-22 Aurora Station Settings Main Interface – Plate Types Settings Tab). Press the centre

position icon (test button red arrow) & the arm will go to T1 position of the reagent cooler block. Check if the tip is in the center of T1 position tube, (shown below, *Figure 9-23 39 Well Reagent cooler Block-Testing T1 Position*)

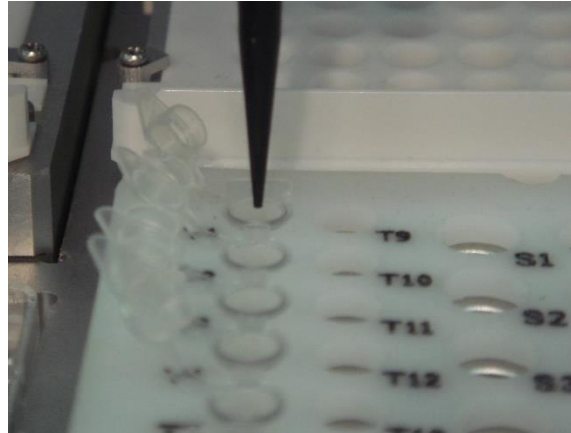


Figure 9-23 39 Well Reagent cooler Block-Testing T1 Position

Likewise, you can perform other location verification such as W position.

Scroll down the table to select Plate type 101: 39-well Reagent/Cooler Block (W).

Press the centre position icon to verify its position.

If you find that the tip is away from the centre position, it may have a significant impact on application (like tip hitting the wall of the vial), it can be re-align the tip back to the centre position by adjusting the Column (X-axis) Offset and Row (Y-axis) Offset accordingly.

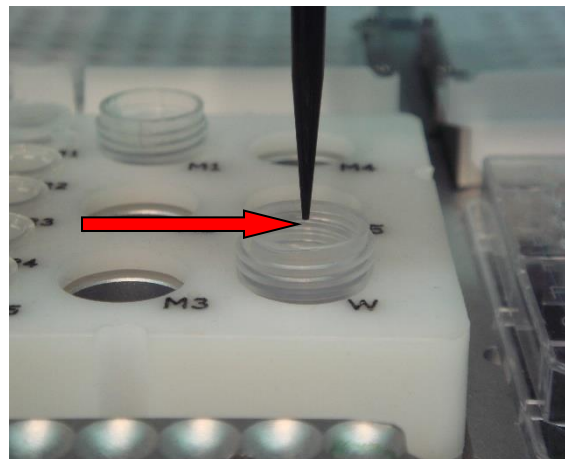


Figure 9-24 39 Well Reagent cooler Block-Testing W Position

In the above photo illustration (*Figure 9-24 39 Well Reagent cooler Block-Testing W Position*), the tip is slightly offset to the left away from the centre position. By adding a small positive value increment say +0.1 to the existing column offset value, followed by clicking the centre position tab (*Figure 9-25 Test position*), the robotic arm will move towards the centre in the X-axis direction. Once finalized, the Column offset value can be adopted for the holes M4 and M5 which are located in the same column.

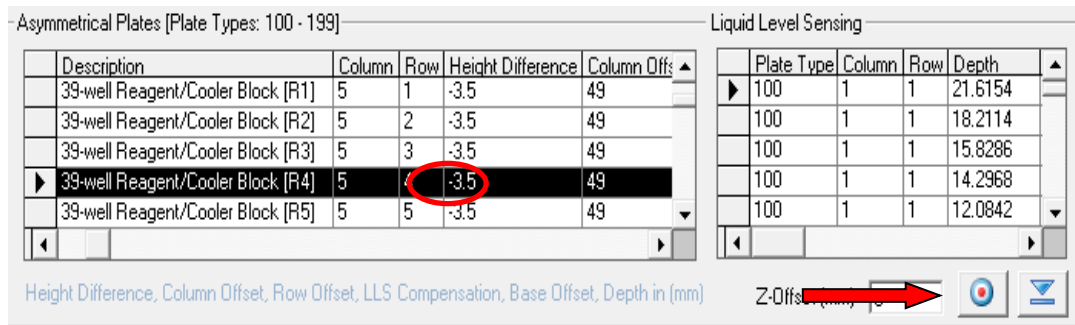


Figure 9-25 Test position

In general, the same methodology is adopted to verify and fine-tune all the other plate types and accessories in terms of height offset, column offset and row offset.

9.4. Reagent Drop Pin Position Teaching

This section will give the details to teach/check reagent drop pin position for 96 well plate.

Put the 96 position teaching plate on any position from 11-15 on the deck.

Open Aurora station from Tools>Open Settings>Advanced, and select the test position (the location where you put the plate) e.g. position 12 in figure.

Open accessories tab from Aurora Station settings (Figure 9-26 Reagent Drop pin position testing-test position selection), check the pin group, highlight the XYZ coordinates (double click) & click RD Test.

The arm will go to position 15 & align the reagent drop pin on the surface of the A1 well of the 96 well plate. To check if the pin is in the center of the well, try dispensing some solution e.g. 100ul manually using PWM pump in C mode (Figure 9-27 Reagent Drop pin position testing-Accessories) & watch the pin while its dispensing, if the pin is not dispensing in the center of the well, otherwise change the X (more negative value of X offset moves the pin/arm to left side of the well relative to the standard position of the A1 well of the plate—increase/decrease it to align in the center of the well) & Y (more negative value of Y offsets moves the pin/arm/ backward) change it to align the pin in the center of the well.

Likewise, you can perform other pins position testing/verification (Figure 9-28 PWM pump-Dispense 100ul volume-Pin # 1).

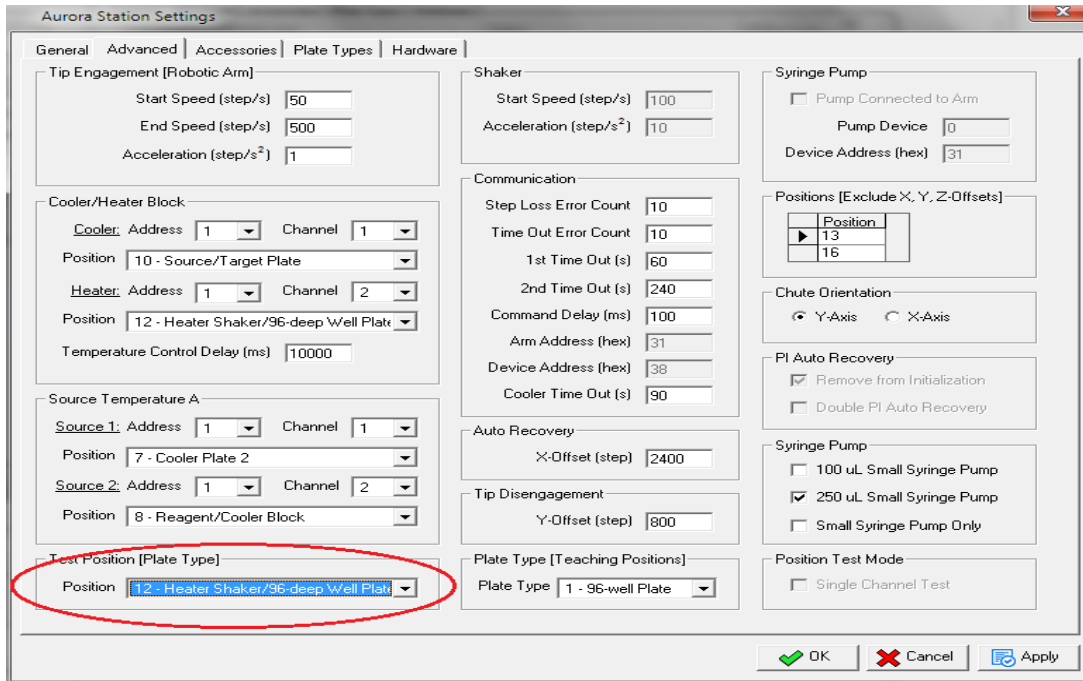


Figure 9-26 Reagent Drop pin position testing-test position selection

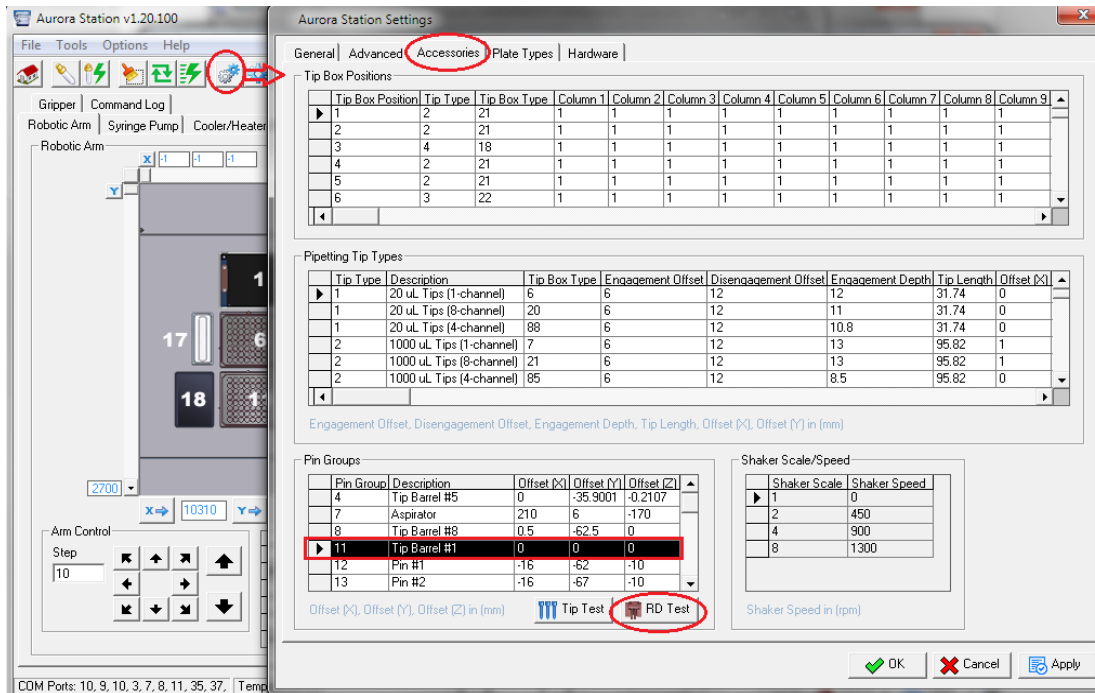


Figure 9-27 Reagent Drop pin position testing-Accessories

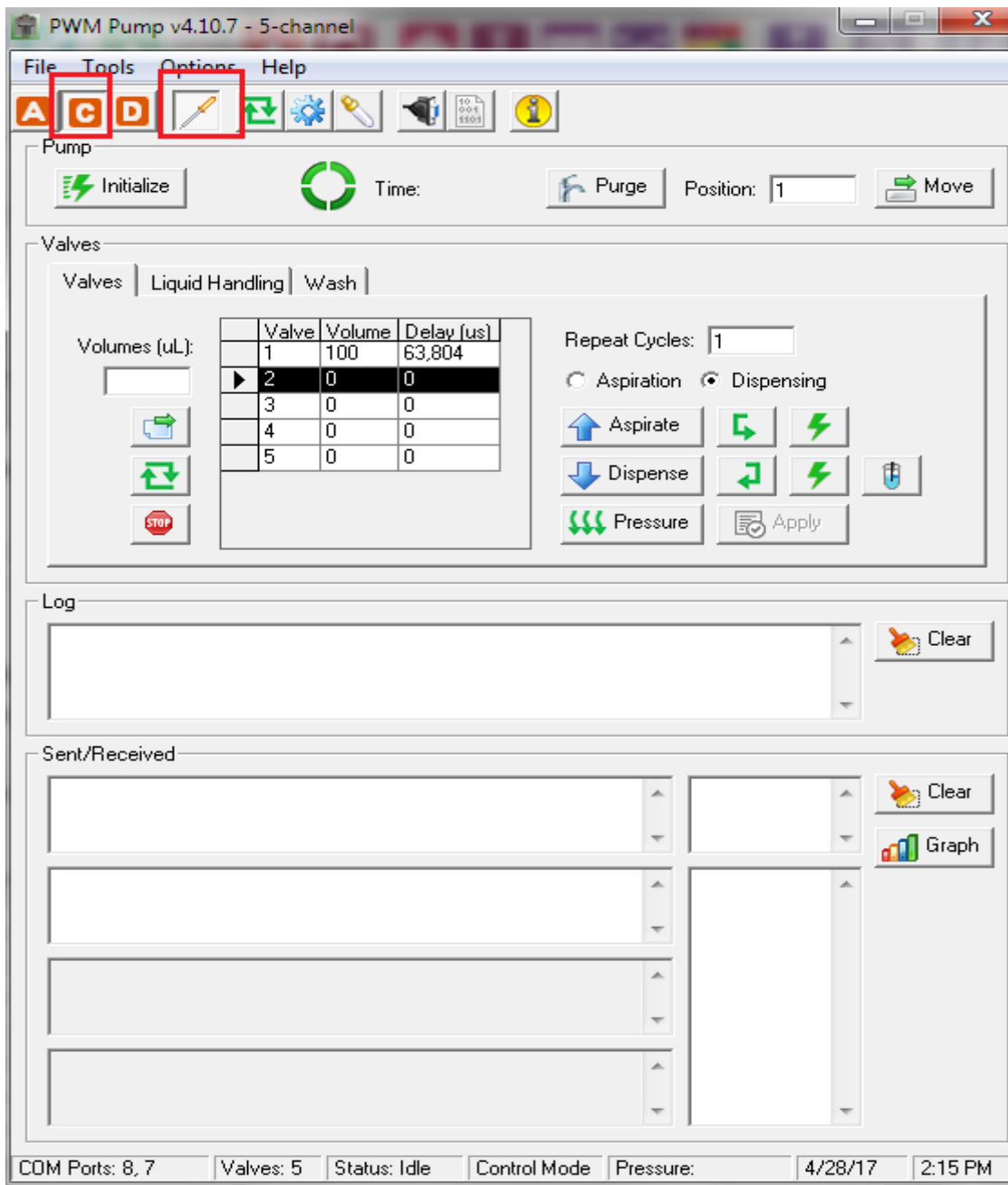


Figure 9-28 PWM pump-Dispense 100ul volume-Pin # 1

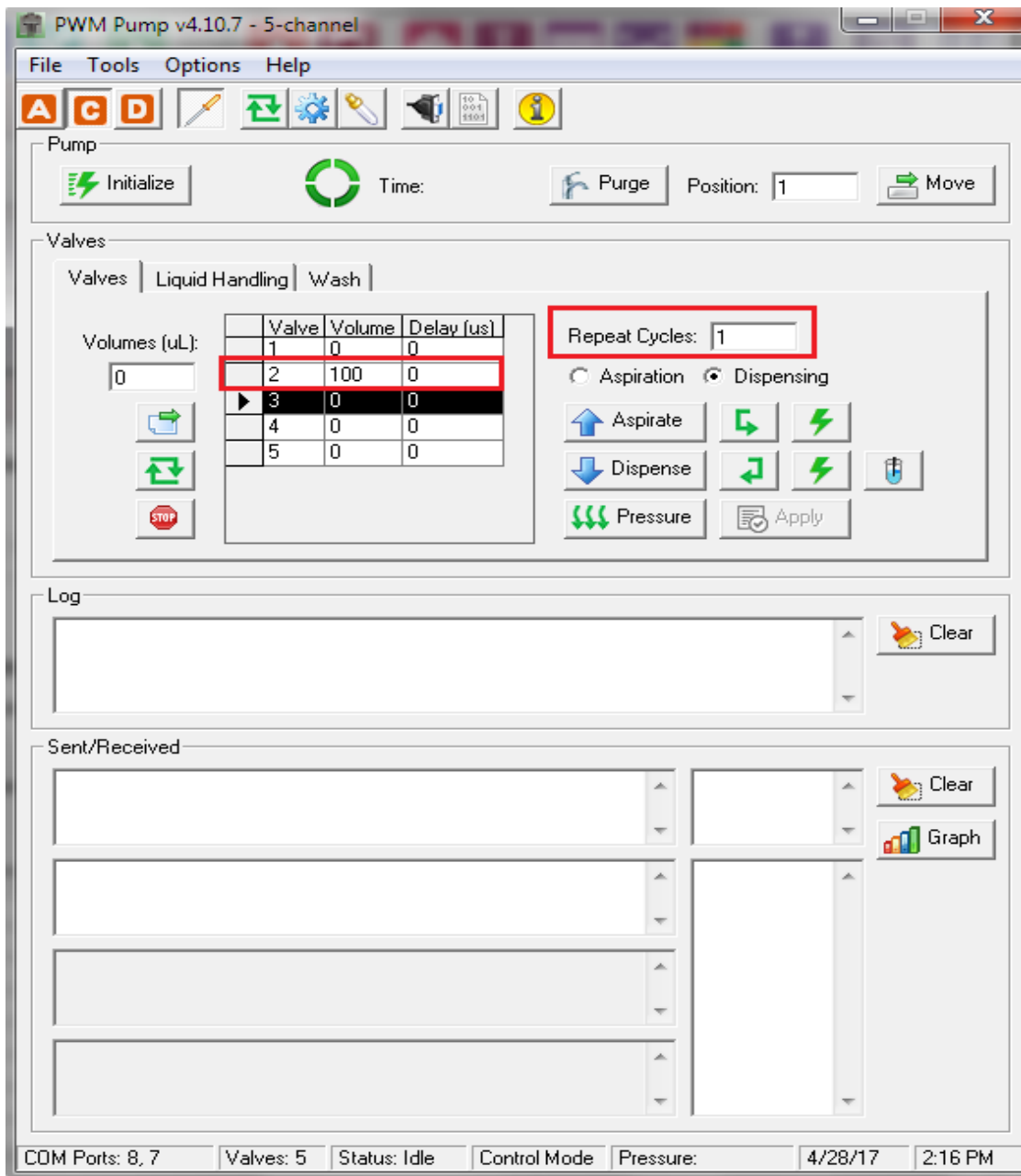


Figure 9-29 PWM pump-Dispense 100ul volume-Pin # 2

After all the pin positions are tested and you have verified that these are correct, set the reagent drop Z offset before creating sequences in VERSAware.

Open OPTIONS from Tools>Reagent Drop and change the Z off set setting (more negative value move the arm up & positive value move the arm down). You can also change the priming volume for reagent drop (*Figure 9-30 Reagent Drop-Z-offset*)

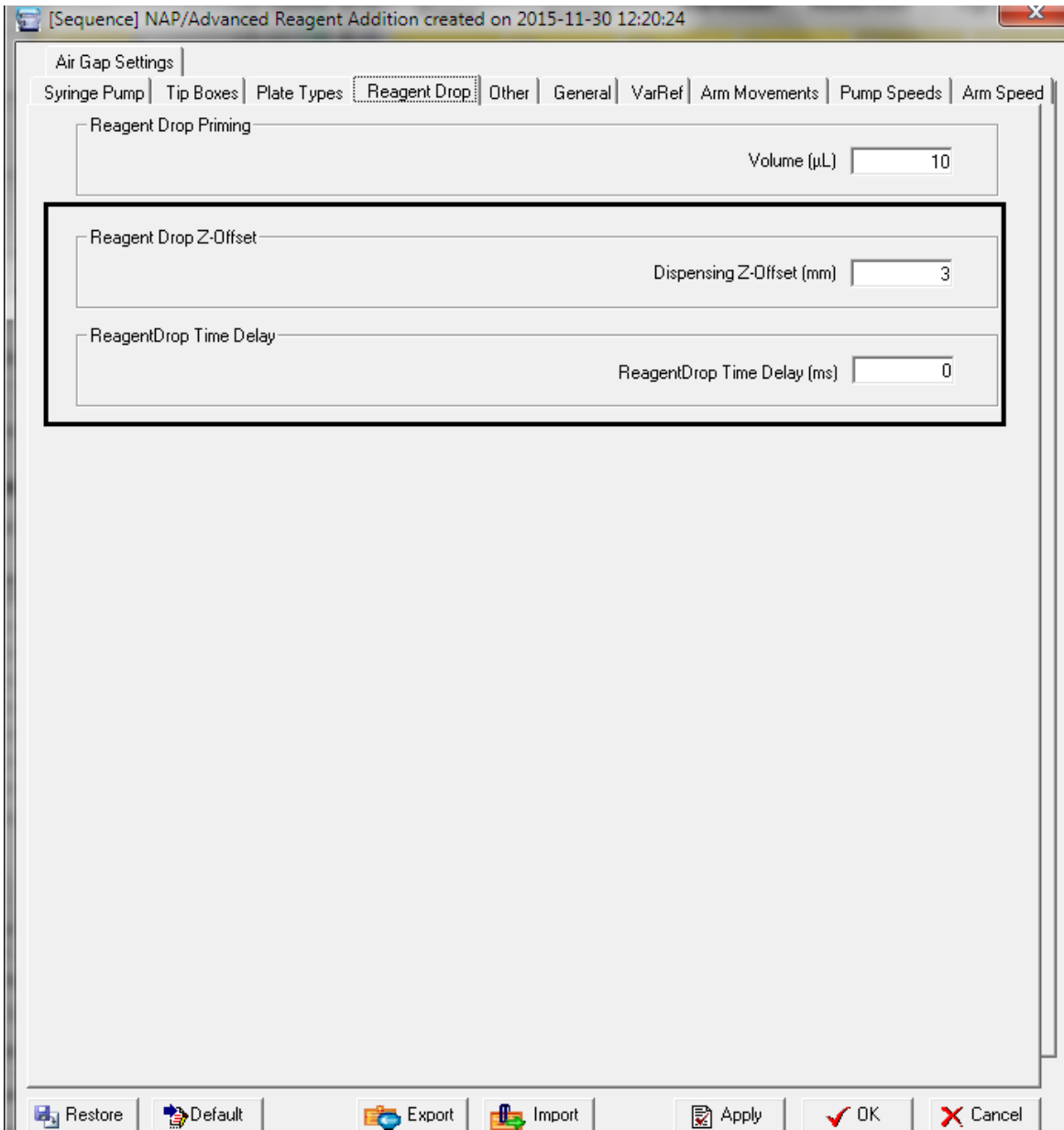


Figure 9-30 Reagent Drop-Z-offset

9.5. Reagent Drop Calibration (PWM PUMP)

Note: Calibration of the PWM pump is recommended in case a different reagent is being used which may affect the pressure setting or use multiple table calibration mode

PWM Pump interface is used for calibration. To open the interface window, follow the steps below:



Shell Status

- 1) Open shell status window (which runs alongside VERSAware), double click onto DH01 (1):584 and PWM Pump interface will open. Do not attempt to close shell status window whenever VERSAware is running.
- 2) Alternatively, under VERSAware tools, click onto the Aurora PWM Pump option to reopen the interface. To close, click again.

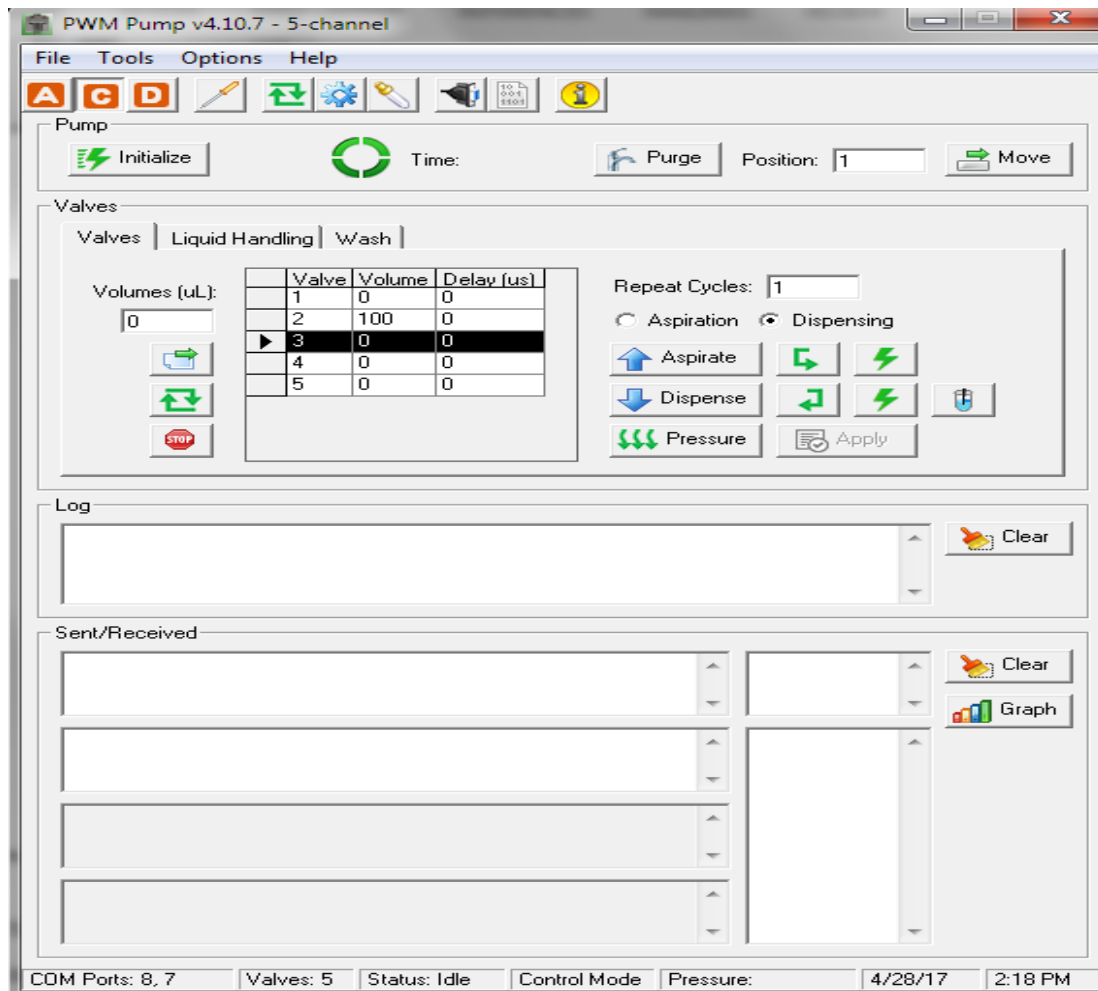



Figure 9-31 PWM Pump Calibration Mode

To change pressure in PWM Pump, click onto the “C” button  to switch to pump control mode (*Figure 9-31 PWM Pump Calibration Mode*).

When pump is operational, pressure can be observed by clicking the chart tab (bottom right of interface).

A graphical pressure curve in real-time appears which shows the user the current pressure maintained.

If changes are needed, open PWM settings from settings icon as shown below (*Figure 9-32 PWM Pump-Settings*). Change the pressure setting (if the pressure setting is lower than before, please release pressure manually by pulling the pressure tubing from priming bottle) and click OK, apply and close this window.

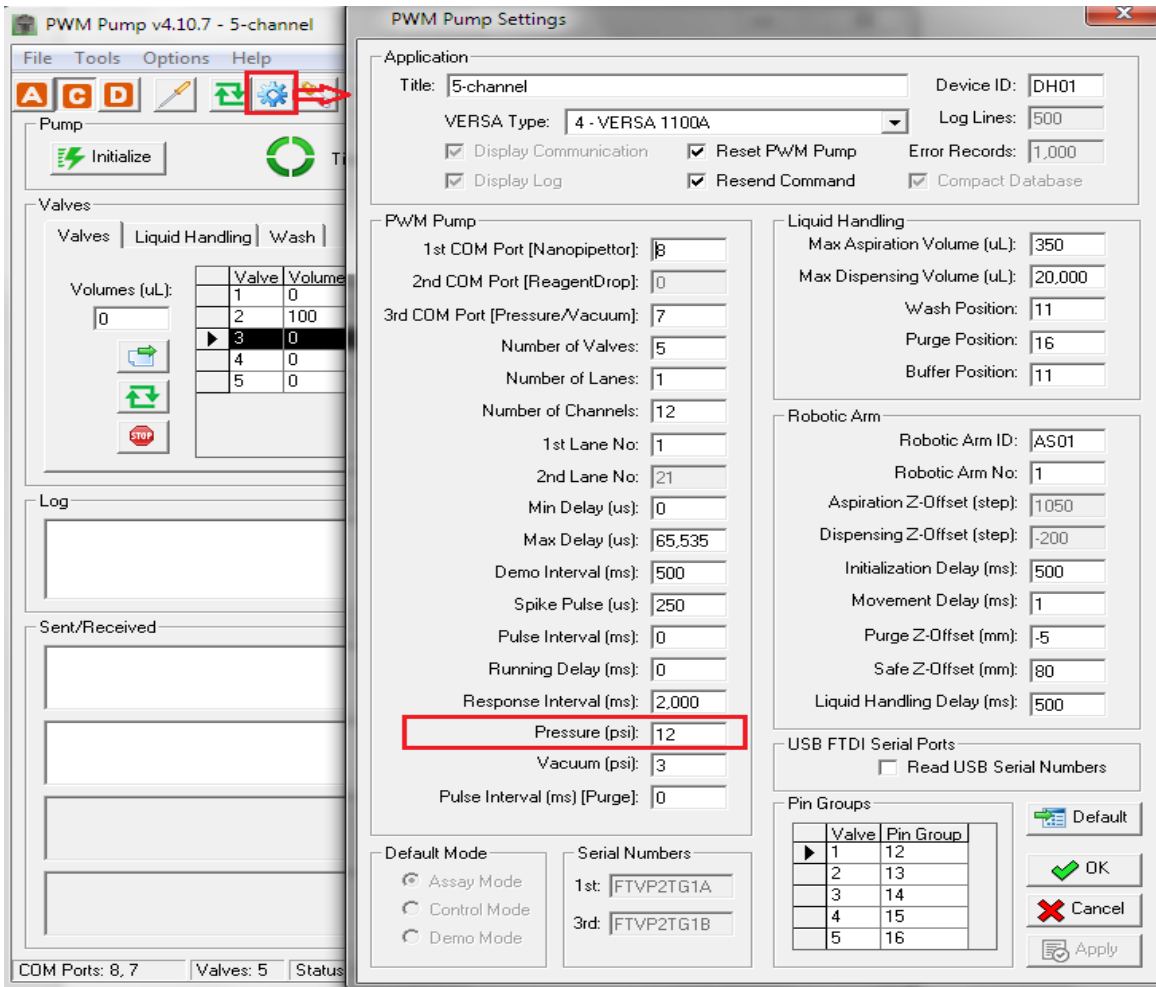




Figure 9-32 PWM Pump-Settings

To set the new pressure setting, go to main interface and click the initialize button , then click the “pressure” button  to change settings as shown below (Figure 9-33 PWM Pump- New Pressure settings).

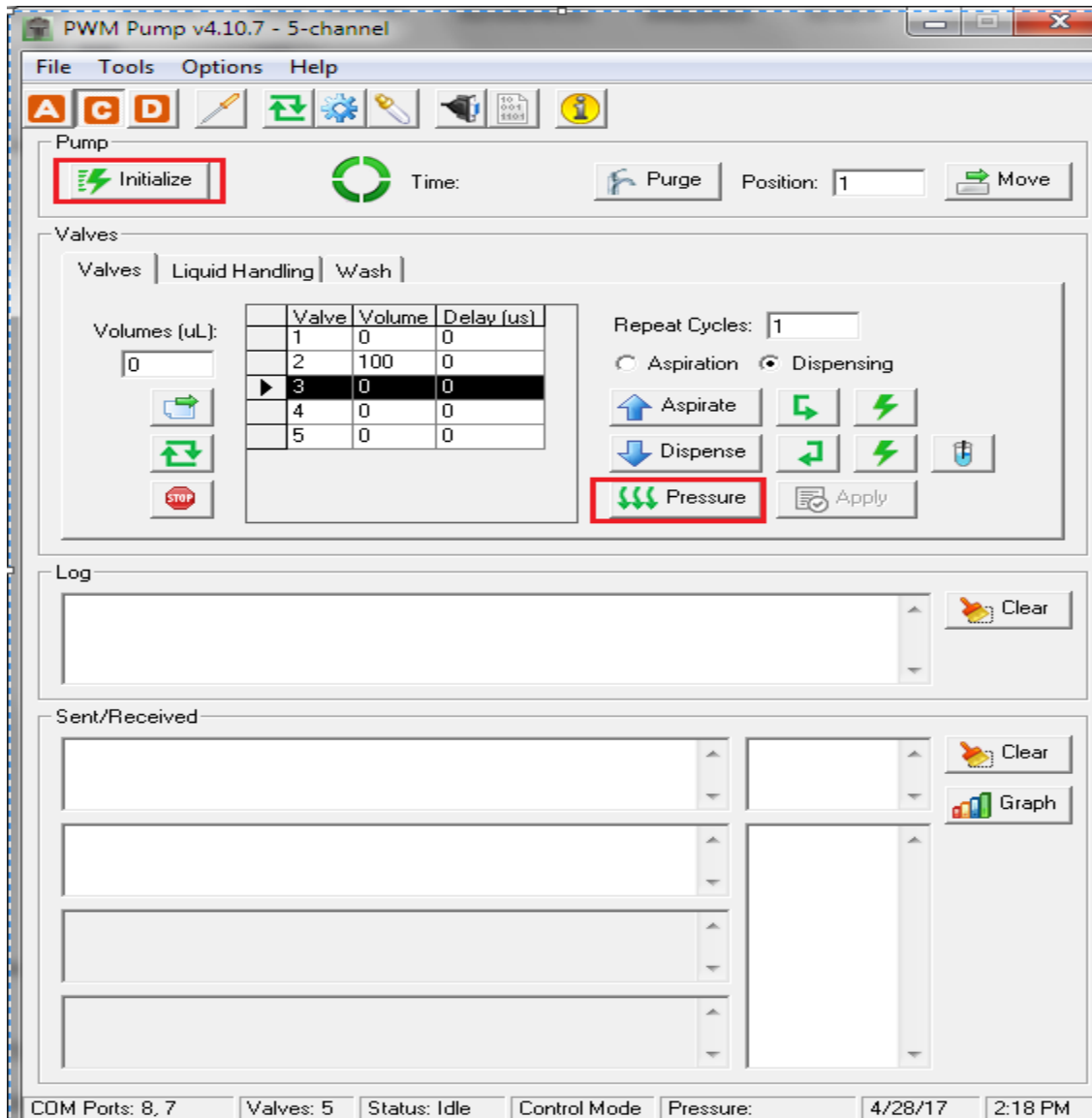


Figure 9-33 PWM Pump- New Pressure settings

- Open valves mode using the “valves” button
- Initialize the pump using the initialize button
- Click the syringe button icon which switches the pump into pulse mode of calibration in micro-seconds (us).
- Open calibration data table by clicking the dropper icon . See the table data for pin # 1. You can choose to start the calibration from a higher or lower value

e.g. Add pulse value 50000, repeat cycles 10 as shown in *Figure 9-35 Add pulse & repeat cycle*, switch to pin # 2 (add 0 value for all other 4 pins) apply, dispense (new reagent or with new pressure setting) in a pre-tared weighing boat to check weight on a balance or in a tube to check the volume with a pipettor. Add this new weight/volume value in the calibration table & switch records to add the new volume/weight as shown in *Figure 9-36 Add new volume/weight value*. This way, check all the pulse values & repeat cycles which are there in the table like up to 30000 & keep adding the new weight values in the table. This is just repeating the table pulse time & cycles with new reagent or pressure setting as with pressure & different reagent weights/volumes change.

- In case, user want to add new data point e.g. 50000 pulse & 9 cycles. Check the weight by adding new # of cycles & add the new data point as shown in *Figure 9-37 Adding new data point in the calibration table*
- Similarly, calibrate other pins & keep adding weight values in the calibration table for pin # 2, 3, 4 and 5.

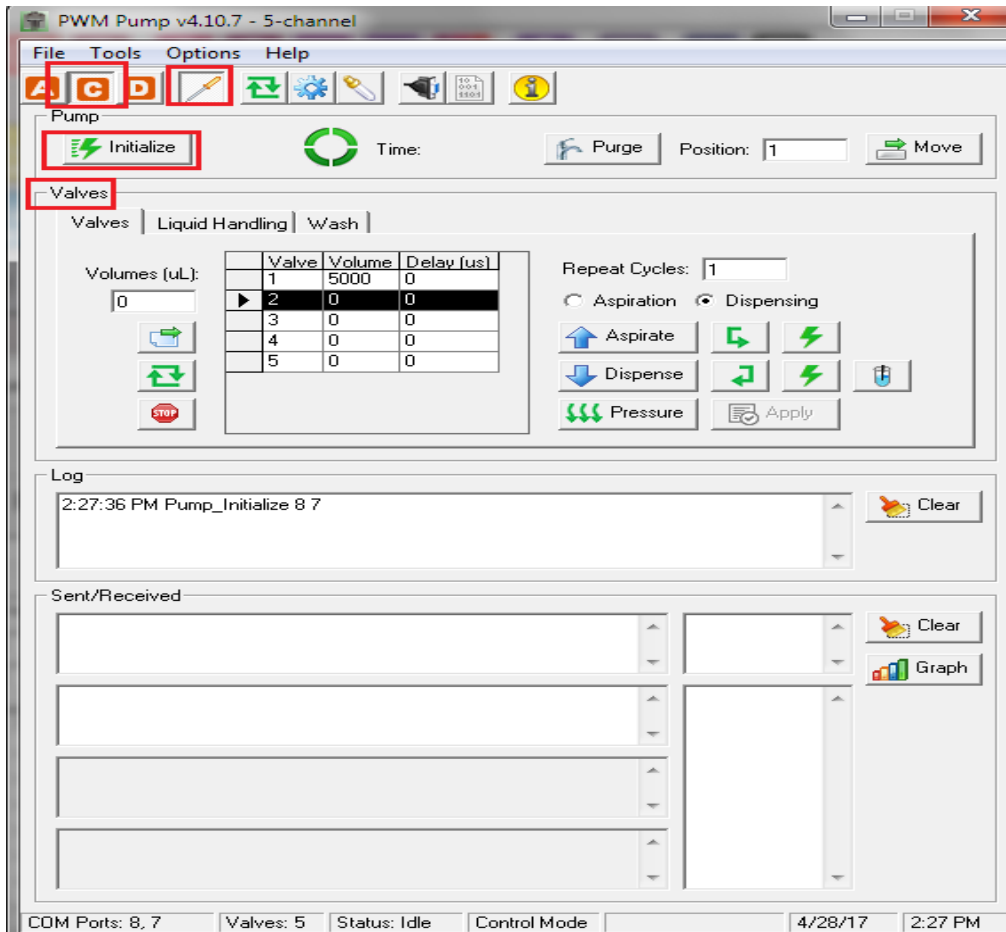


Figure 9-34 PWM Pump in Pulse mode of calibration

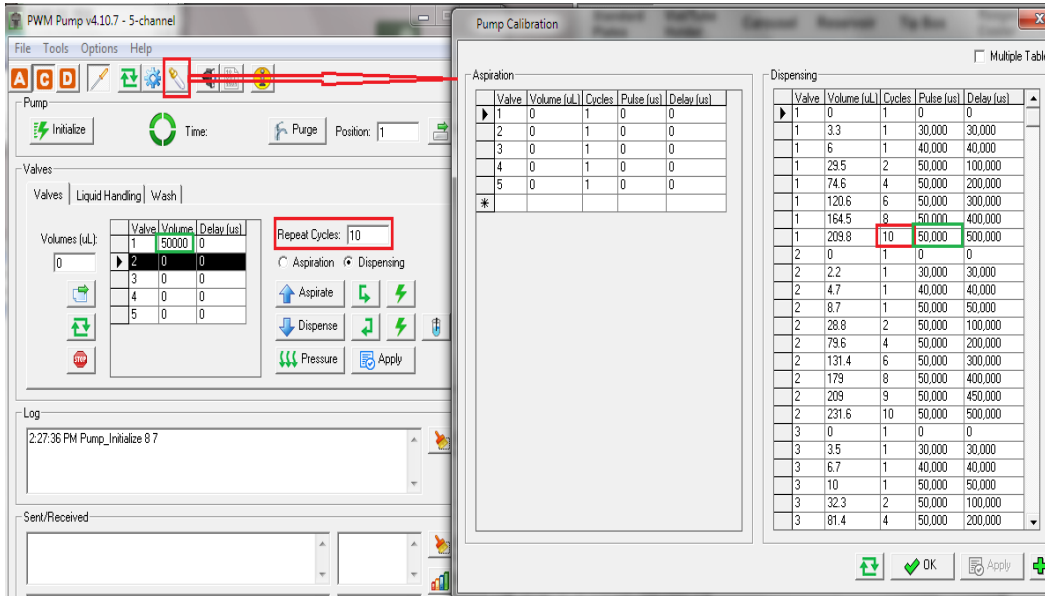


Figure 9-35 Add pulse & repeat cycle

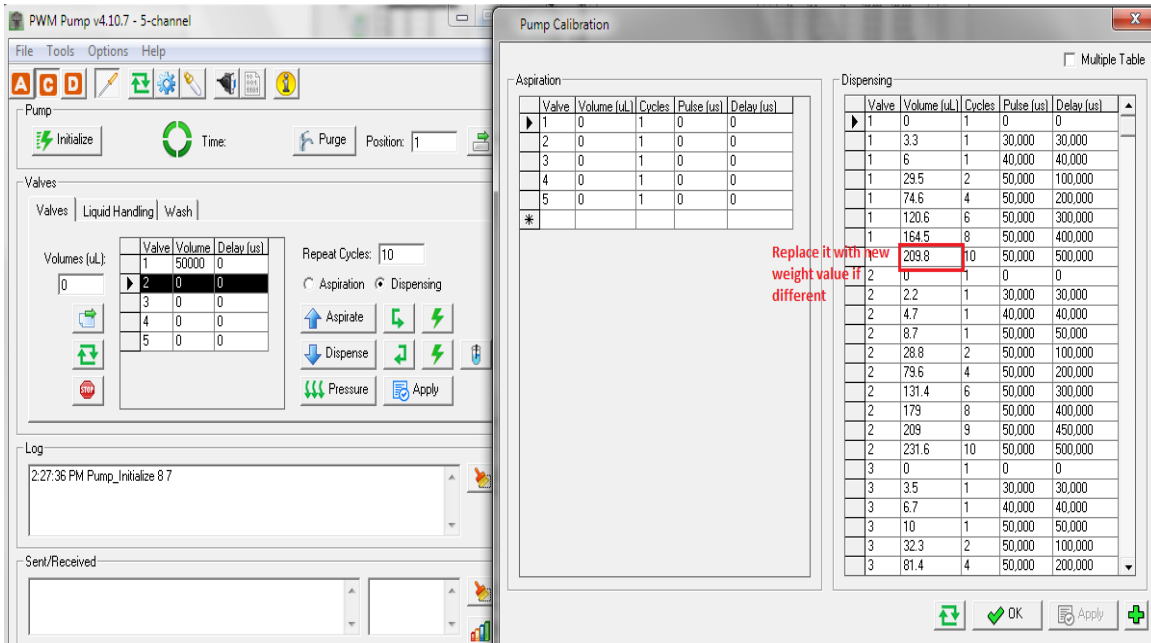


Figure 9-36 Add new volume/weight value

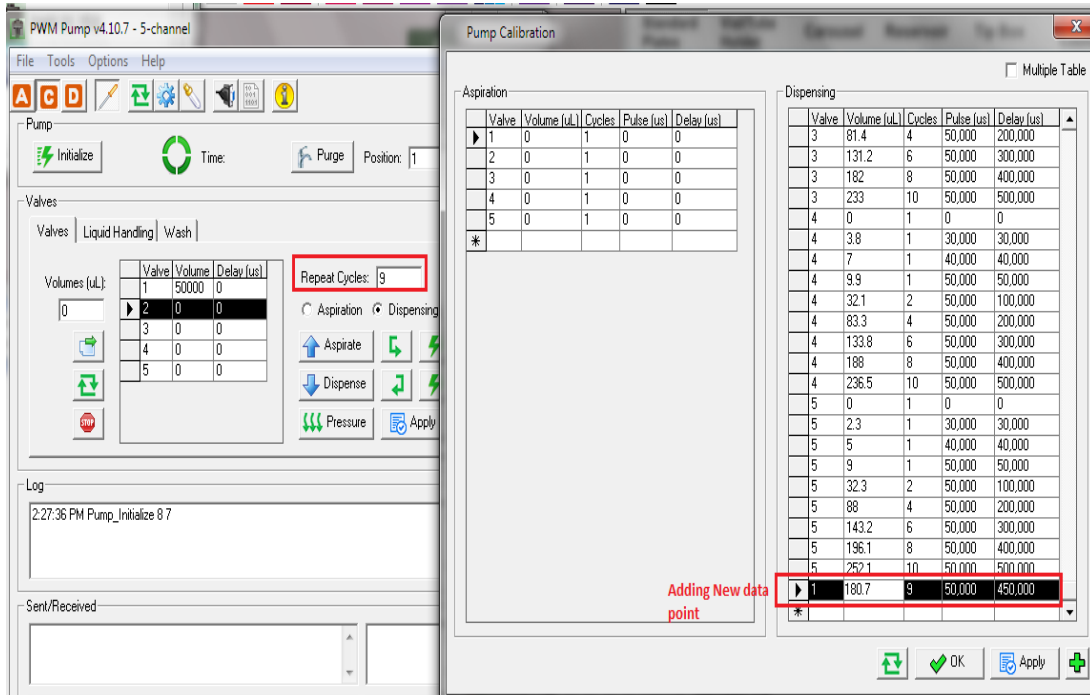


Figure 9-37 Adding new data point in the calibration table

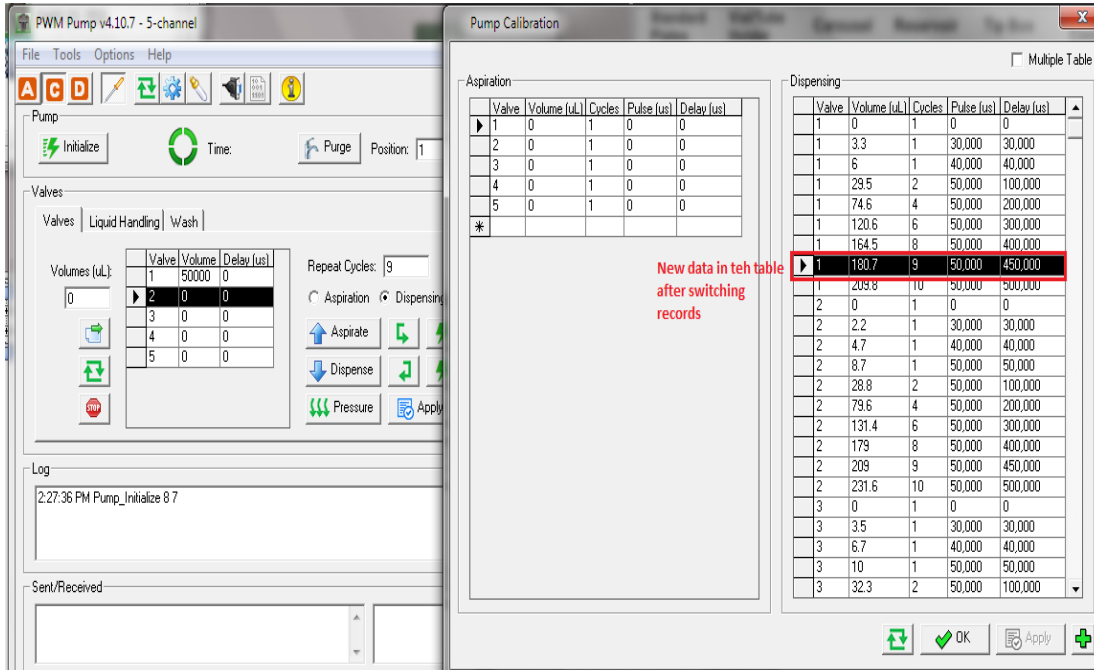


Figure 9-38 New data point in the calibration table for channel # 1

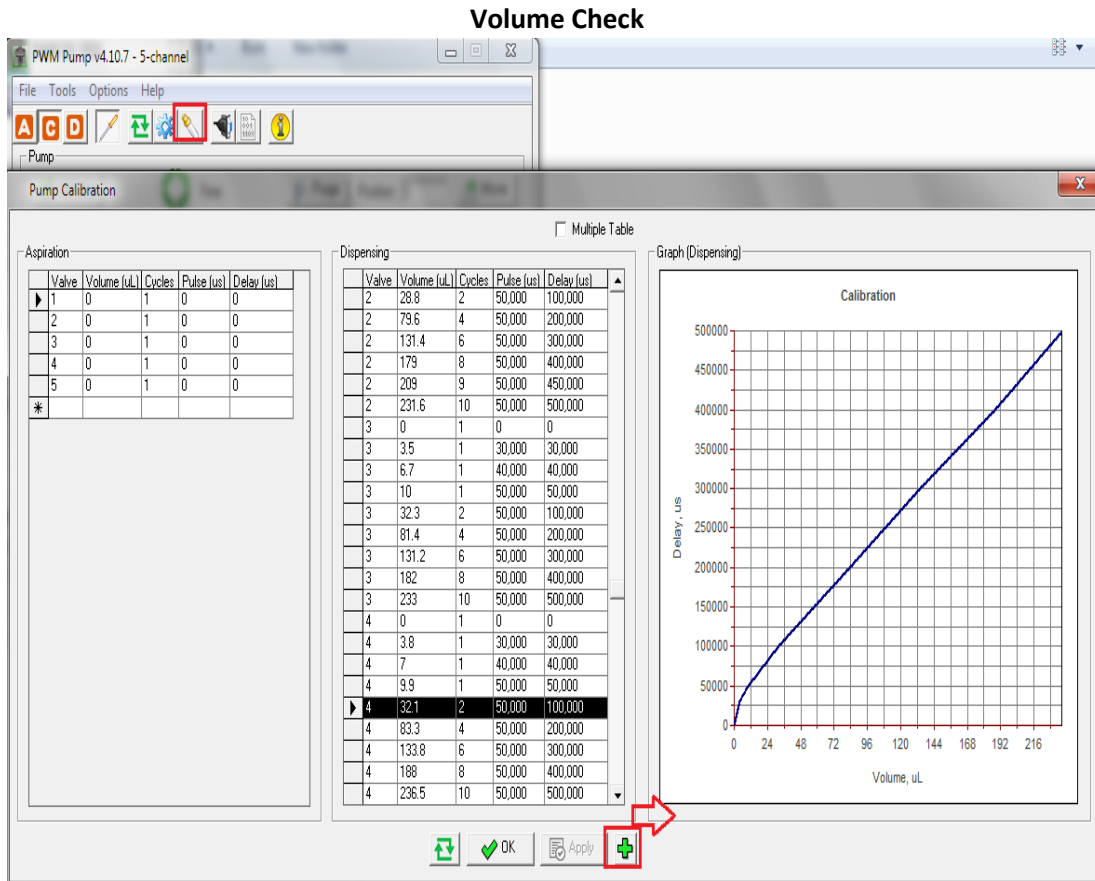




Figure 9-39 Checking data in a graph

- After the calibration is finished, you can check different volumes for accuracy.

Switch to volume mode by clicking the syringe icon  again and it will appear unclicked . Then you can add 100µL volume for valve 1 (Pin 1) & repeat 1 cycle. You can then switch to valve 2 as shown in figure 42 & then dispense in a pre-tared weighing boat & check the volume on a balance or with pipettor. Similarly, other volumes within the range of the calibration table/data can be checked. (Figure 9-40 Testing volumes)

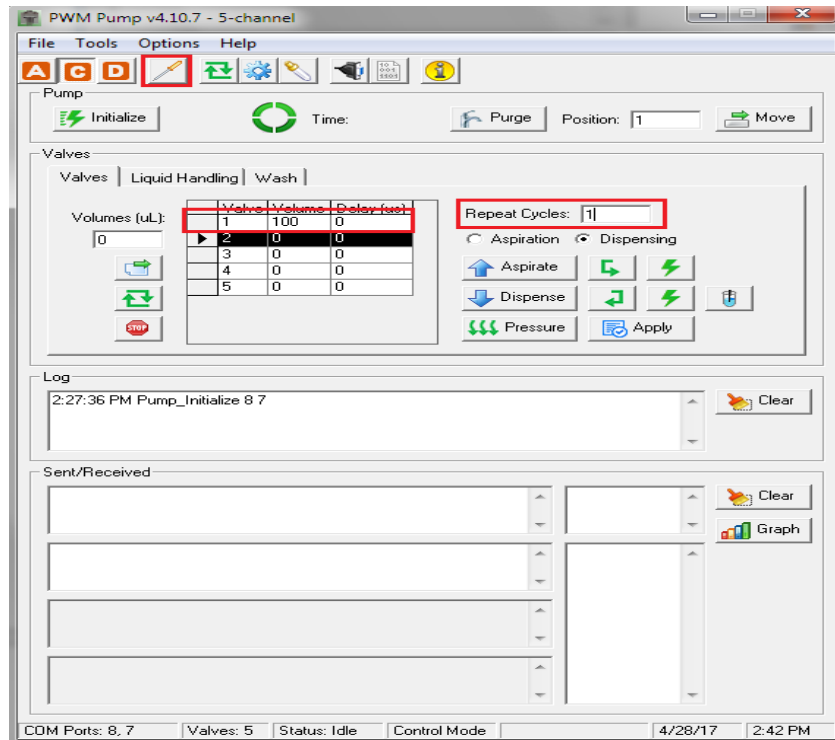


Figure 9-40 Testing volumes

User can also calibrate reagent drop pins for different reagents/solvents (ethanol, DMSO etc.) by using multiple table option, whenever, there is switch to different reagent—use that particular calibration table as shown in *Figure 9-41 Multiple table option* below.

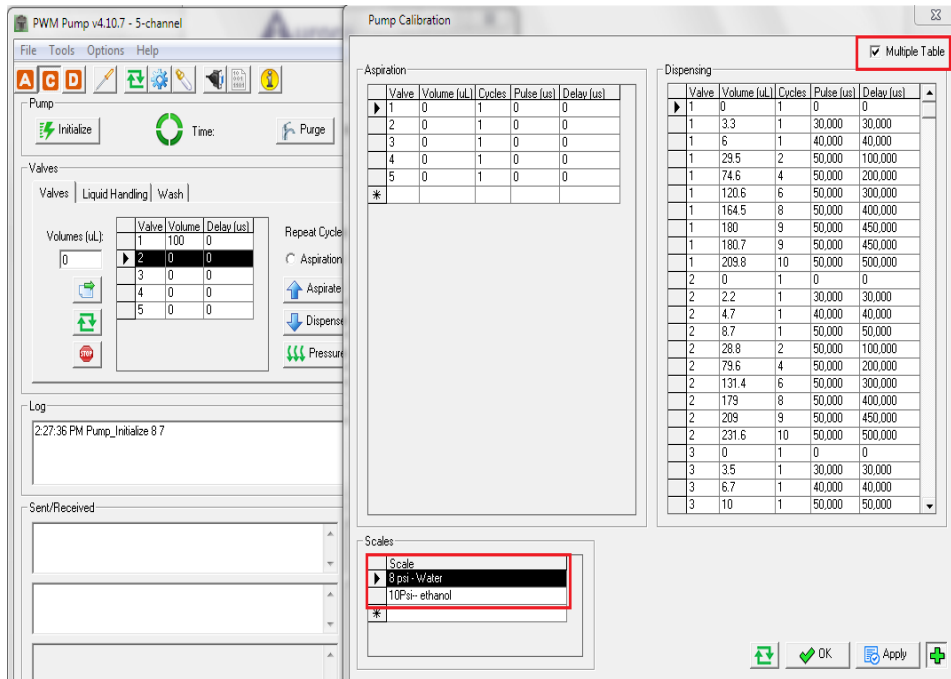


Figure 9-41 Multiple table option

9.6. Single Channel Barrel Position Teaching

This system has the option of single channel operation as well. The first barrel from outside of the deck functions as a single channel head. The position for this barrel is aligned like the reagent drop pins.

Select the test position, open settings>accessories and then double click Tip barrel #8. This will highlight the XYZ co-ordinates, and then click 'Tip Test'.

The first barrel from outside will go to that position. If the barrel is not in the center of the well, adjust X & Y settings. This testing can be done by picking 20ul tip too using single channel test mode (Figure 9-42 Single Channel Barrel Position teaching).

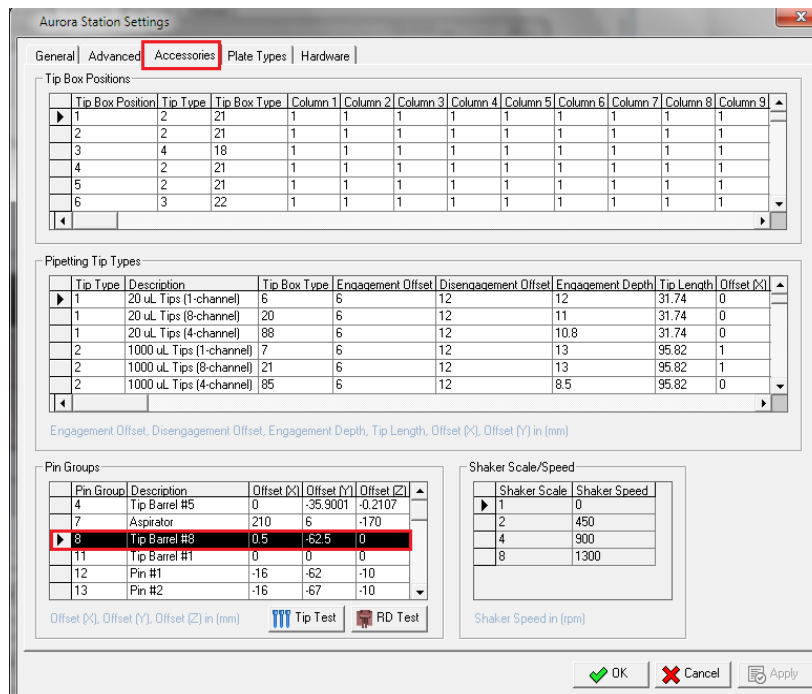


Figure 9-42 Single Channel Barrel Position teaching

9.7. Syringe Pump Tab

Syringe Pump Tab is used to calibrate the pump/piston and to check the volume accuracy manually. (Figure 9-43 Syringe Pump Control)

Press the Initialize button and the plunger /piston of the selected pump should move upward to its initialized position. Typical Syringe Pump Type includes 8-channel x 250µl or 1000µl.

Select the step option to perform aspiration of 10,000 steps and press the Send button. The piston should move downward. There are two options to check volume or perform calibration. Pump control is completely manual and other one is little bit automated.

NOTE: For units with air displacement option—both single channel and 8 channel have same calibration data as all 8 pistons move together with one plunger. Therefore, same data is used in both scales. Water displacement units have different calibration data as the single channel syringes (25µl & 1000µl) are separate from the 8 channel syringes.

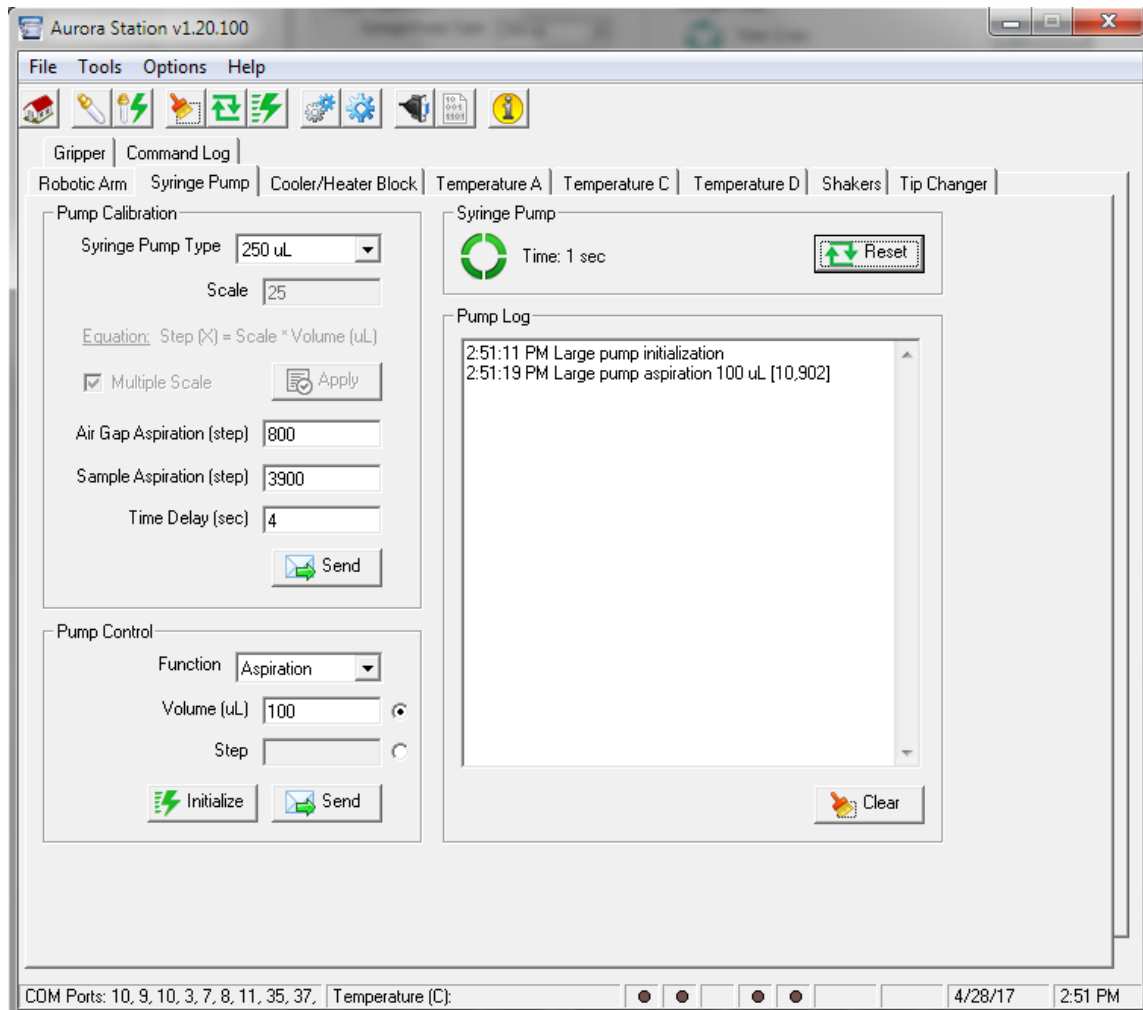


Figure 9-43 Syringe Pump Control

NOTE: If there is no response from the syringe pump, refer to the Syringe Pump Settings section of this manual to adjust the COM port.

9.8. Calibration of Syringe Pumps (8-channel x 1000µL)

Syringe Pump Scale Calibration

This chapter will discuss the procedure of syringe pump calibration which applies to both syringe pumps: 1000µL (single channel) and 8x1000µL (8 channel) pumps. The “Multiple Scale” option should be selected, and the single scale option selected. (*Figure 9-44 Syringe Pump Calibration*)

Open Aurora Station (Tools → Aurora Station). Initialize the robotic arm by pressing the button between X and Y-axes ('Robotic Arm' tab) and move the arm towards you.

Manually engage a tip onto the first outer barrel. (Use a 25µL tip for calibrations up to 20µL volume, a 50µL tip for calibrations in the 20-50µL range, a 200µL tip for calibrations in the 50-200µL range and a 1000µL tip for calibrations in the 200-1000 µL range.

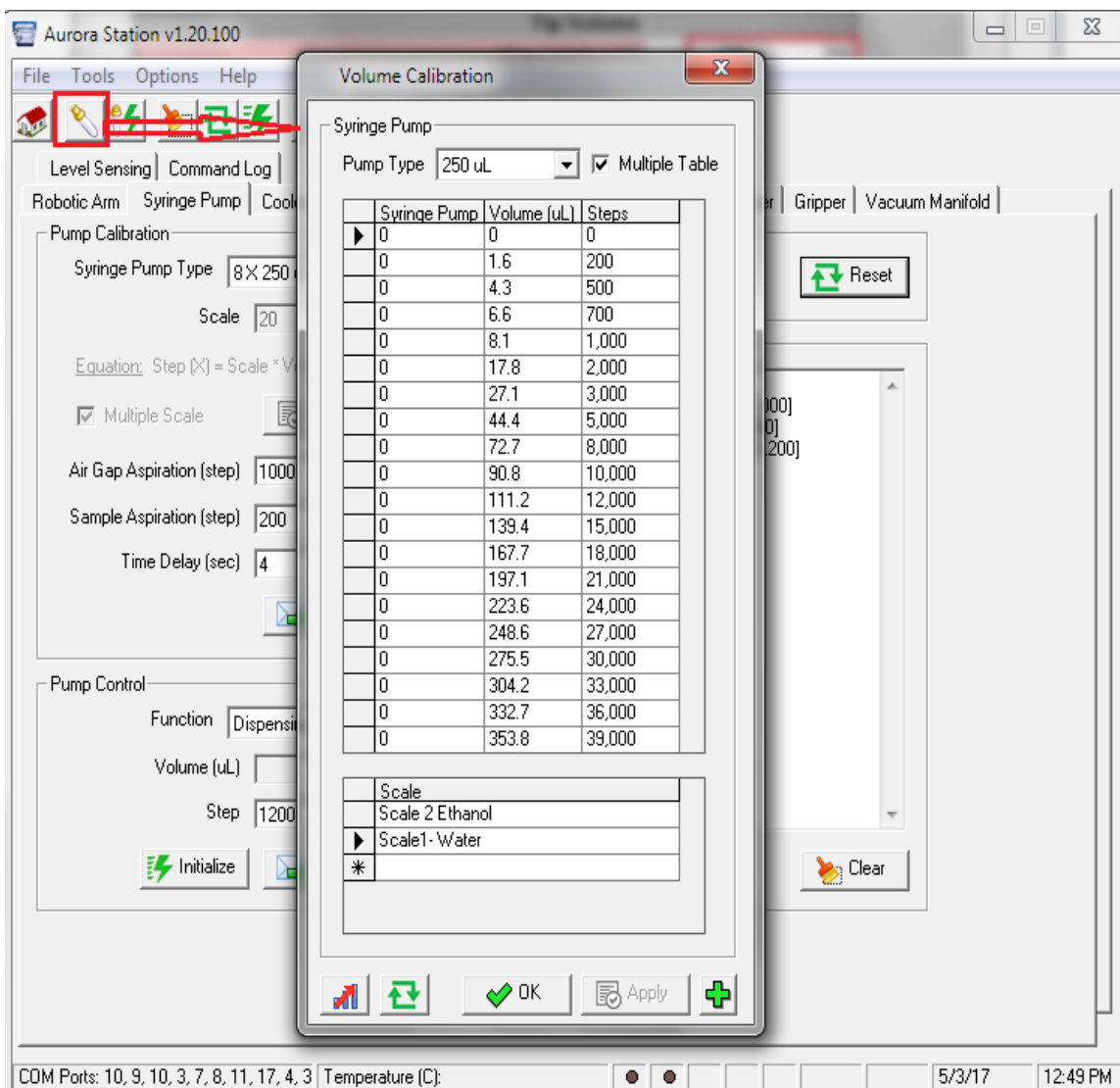


Figure 9-44 Syringe Pump Calibration

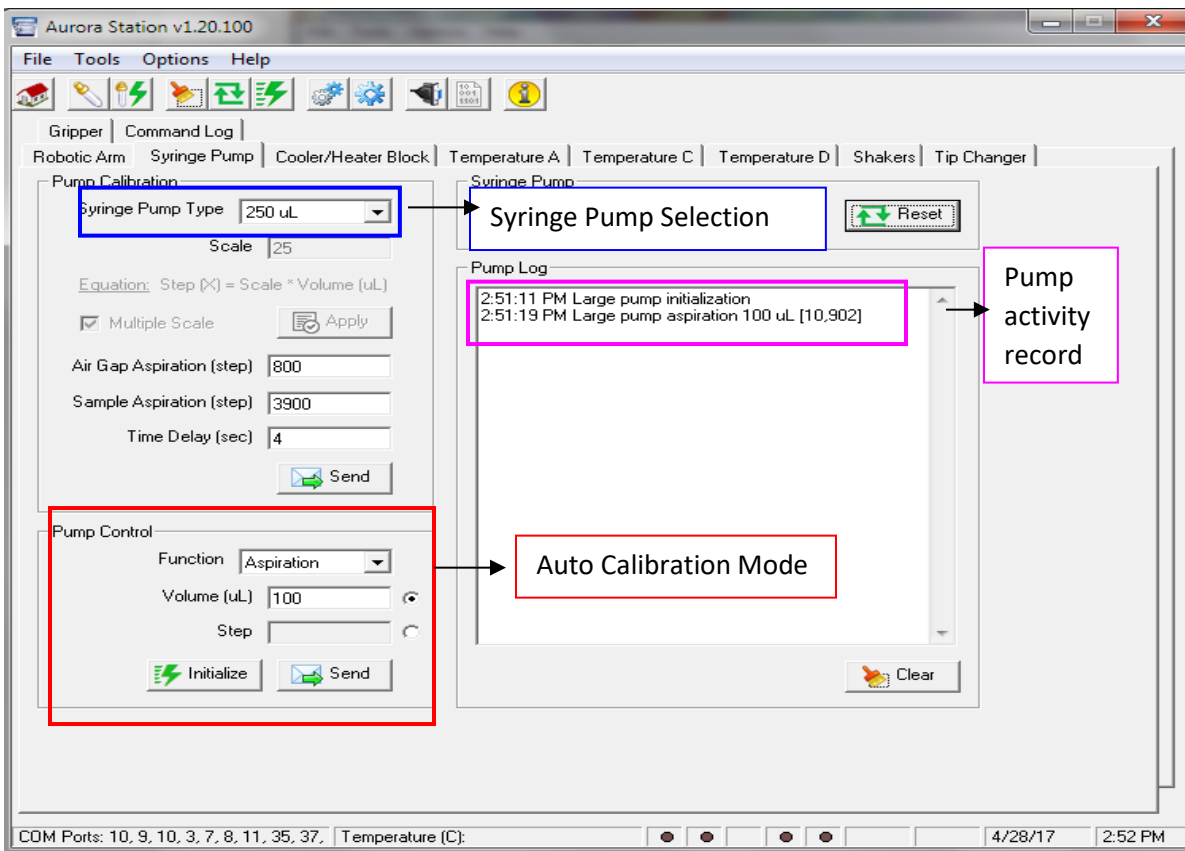


Figure 9-45 Syringe Pump

Switch to 'Syringe Pump' tab (*Figure 9-45 Syringe Pump*) from the Aurora Station main menu. Select the desired syringe pump and click 'Initialize' button.

One-time initialization is required for each syringe pump for the entire calibration procedure.

Manually place a tip onto the barrel. Check the calibration table for pre-calibrated data.

Calibration can be done in steps (0) or volume mode (0).

Calibration Using Steps

There are two ways to perform calibration procedure.

Auto Mode: In this mode, the air-gap is adjusted in steps, the volume is adjusted in steps and the time delay after air-gap/before dispensing the volume is accounted for automatically.

Follow the below steps:

- 1 According to the calibration pump scale, add the desired air-gap (e.g. for 8x250 μ L pump, add 1000steps (10 μ L volume) in the air-gap field, and 200 steps (1.6 μ L) in the sample aspiration field and a four second time delay).
- 2 By clicking send, the instrument will draw in an air gap (1000 steps/10 μ L), wait for four seconds, aspirate 200 steps worth of volume (at this time put your vial with liquid under the tip), wait for four seconds again, dispense 1200 steps/ volume (1000 steps for the air-gap and 200 steps for the aspiration volume) into a pre-tared weigh boat. Weigh this weigh boat and confirm the volume is correct.

If the volume is not correct, replace the old value (1.6 μ L) to the new weight value in the calibration table (*Figure 9-46 Syringe Pump Calibration—Auto mode*).

Using the same process check whole range of steps (500, 700,3000) and keep changing the volume value in the calibration table if different from the previous value.

Pump Control Mode: In this mode, both the air-gap and the volume is sent separately, and then the volume is dispensed.

Follow the steps as below:

- 1 Under “pump control”, select ‘steps’. This will make steps the active field for data entry. Select “aspiration” as the function and enter 1000 steps (10 μ L) which will serve as the air gap, then click the send button. You will see 1000 steps in the command log. (*Figure 9-47 Syringe Pump Calibration-Manual mode*)
- 2 Enter 200 steps in the “Pump Control” section (1.6 μ L volume), and before clicking send insert a sample source under the pipette tip (*Figure 9-48 Syringe Pump Calibration-Aspirate volume*). You will see the solution aspirated into the tip.
- 3 Next tare a weigh boat on a scale. In the software, click “dispensing” and enter the 1200 steps (1000 air-gap +200 aspiration volume steps = 1200steps) and click send and collect the volume in the pre- tared weigh boat (*Figure 9-49 Syringe Pump*).Weigh the collected sample and enter the weight (if different than the previous value) in the volume section of the calibration data table.
- 4 Repeat this procedure for whole range of this pump (100, 200...) and keep adding the weight/volume in the calibration table if different from pre-calibrated volumes.
- 5 After calibration, check the calibration data, and click the green arrow to show the graph (*Figure 9-50 Syringe Pump Calibration-Graph & Scale*). Also check the scale for maximum volume by clicking the icon below the calibration table which will open the ‘Maximum Volume’ window. This window shows the maximum volume of the pump that can be aspirated/ dispensed (*Figure 9-50 Syringe Pump Calibration-Graph & Scale*). Add these maximum volume values in OPTIONS for all the three pumps (*Figure 9-51 OPTIONS-Add Maximum volume*).

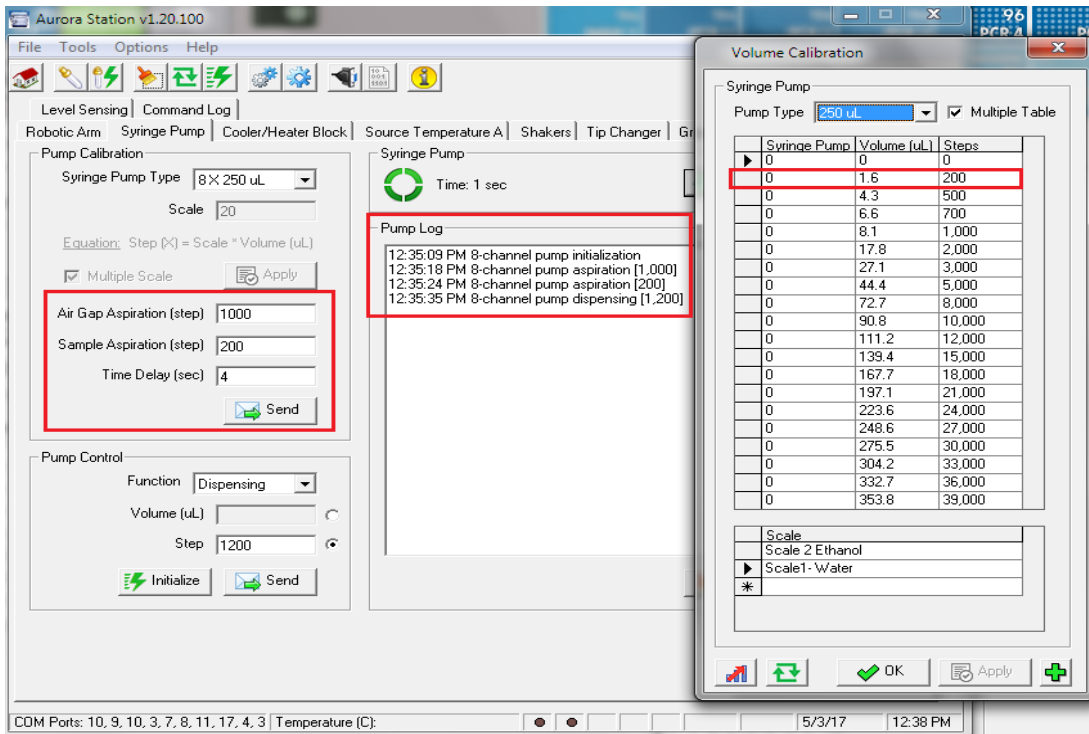


Figure 9-46 Syringe Pump Calibration—Auto mode

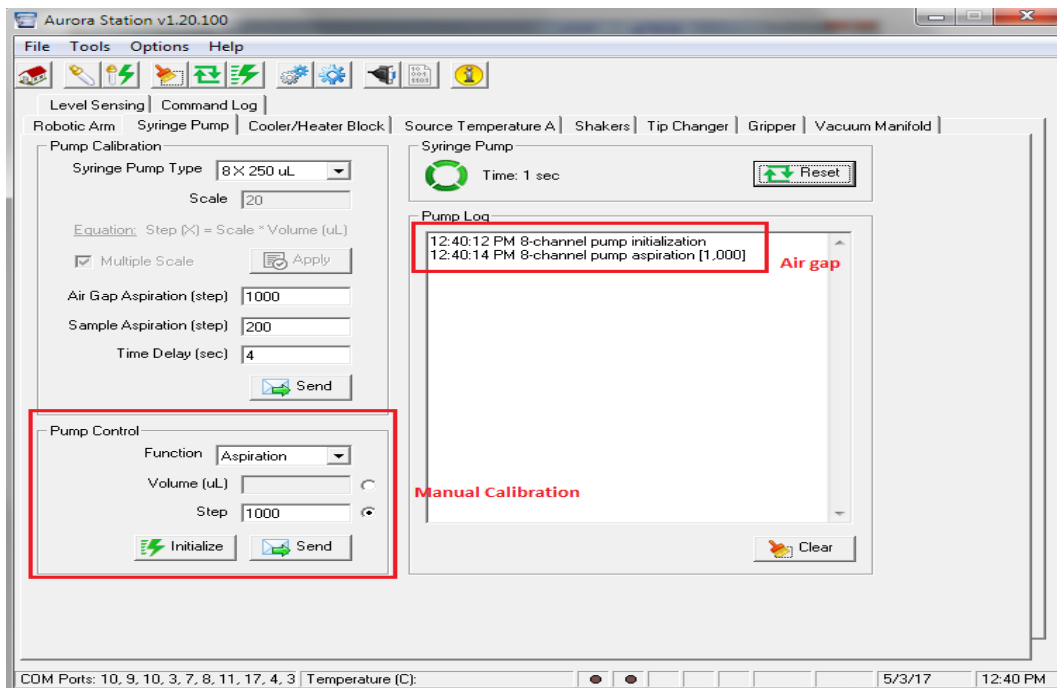


Figure 9-47 Syringe Pump Calibration-Manual mode

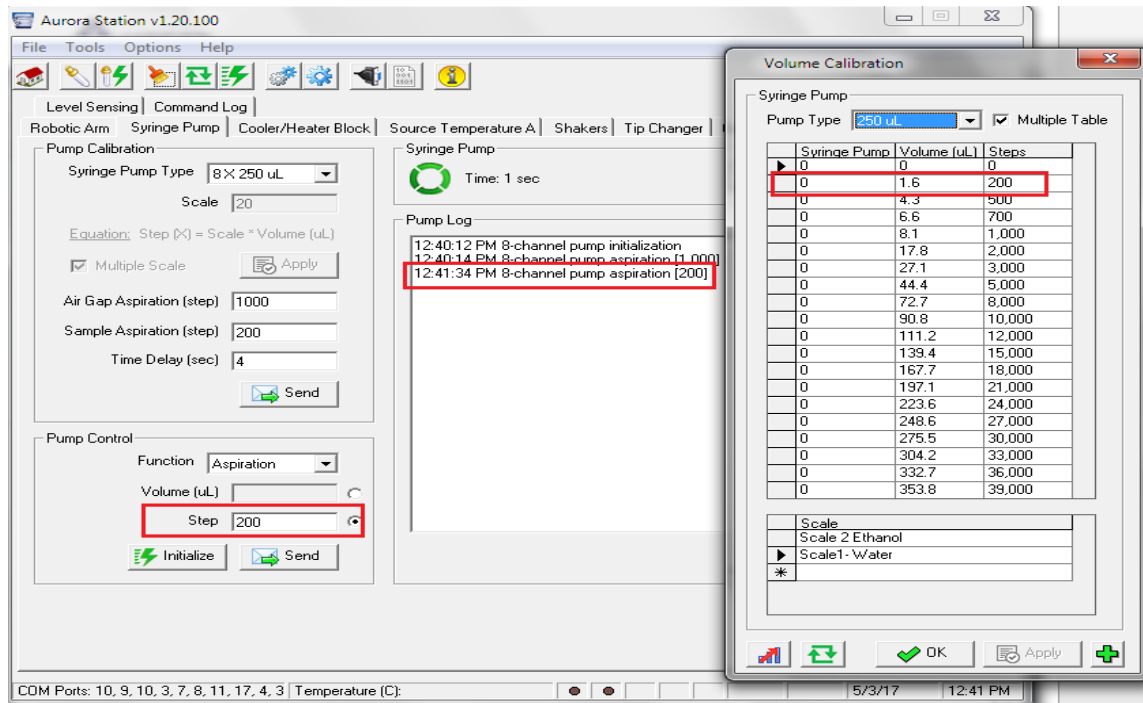


Figure 9-48 Syringe Pump Calibration-Aspirate volume

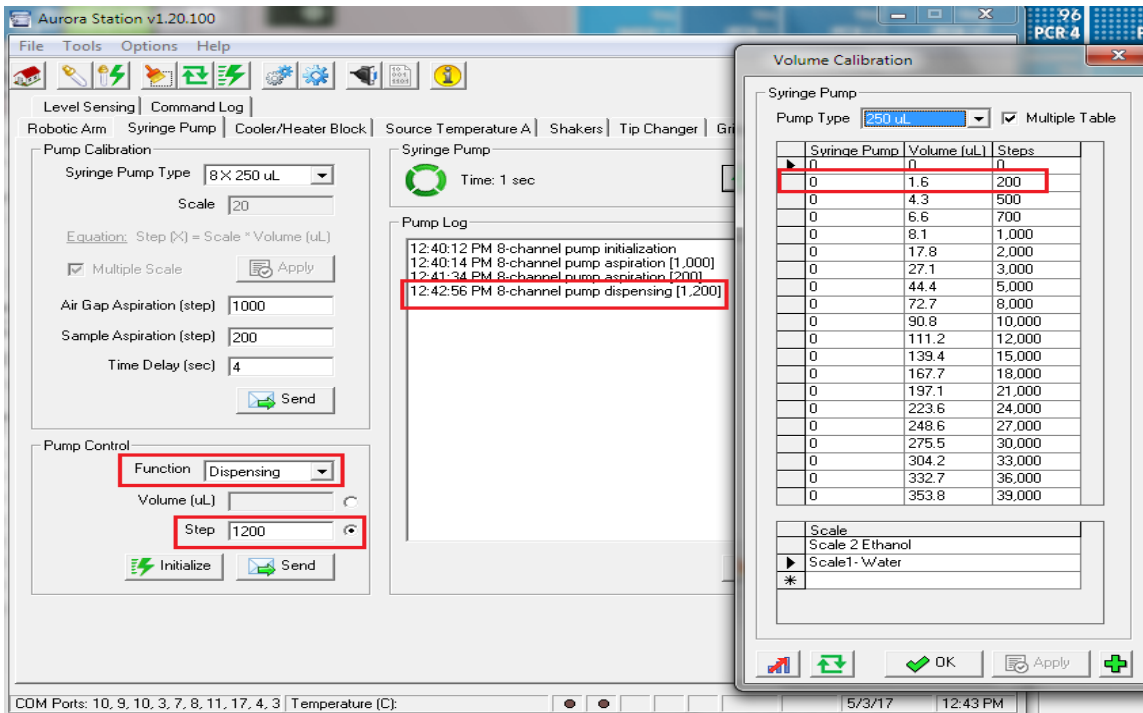


Figure 9-49 Syringe Pump

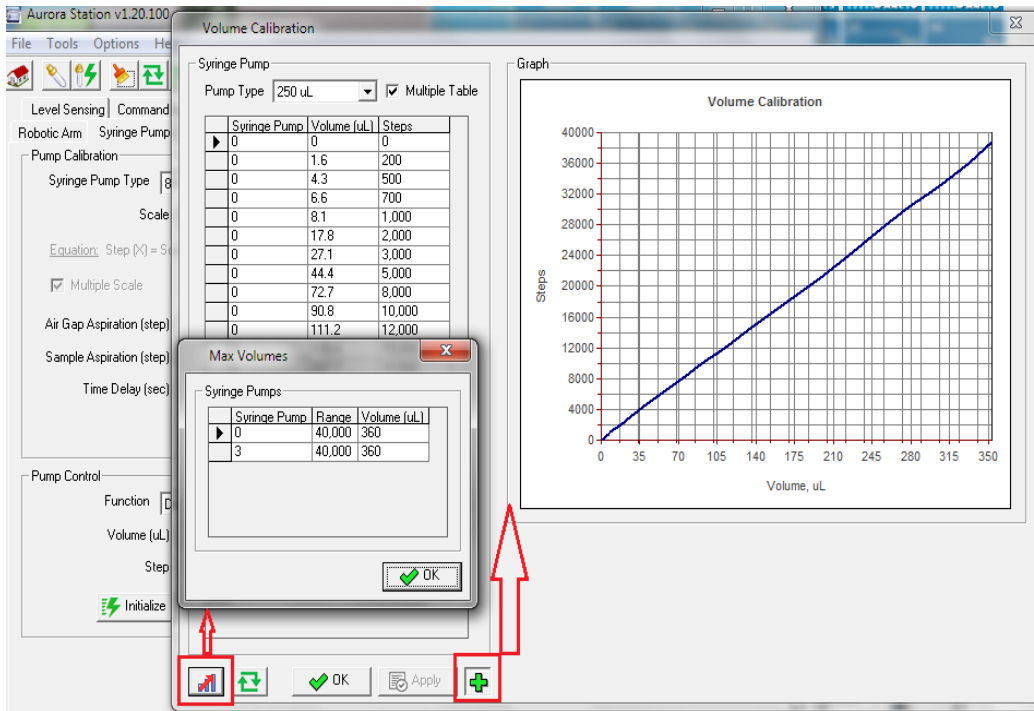


Figure 9-50 Syringe Pump Calibration-Graph & Scale

Options X

Syringe Pump | Tip Boxes | Plate Types | Reagent Drop | Other

Prime syringe pump before running each sequence

	1000 uL	4 × 1000 uL
Tip Volume		
Max. Volume (uL)	900	900
Switch Volume (uL)	900	900
Extra Volume		
Air Gap Volume (uL)	30	30
Extra Aspiration Volume (uL)	0	0
Extra Dispensing Volume (uL)	0	0
Delay		
Delay After Aspiration (s)	0	0
Delay After Dispensing (s)	0	0
Priming		
Priming Cycles	0	0

Pump Aspirate Speed Set Pump Aspirate Speed

	Start Speed (step/s)	End Speed (step/s)	Acceleration (step/s ²)
1000 uL Pump	10	600	5
4 ?1000 uL Pump	10	600	5

Pump Dispense Speed Set Pump Dispense Speed

	Start Speed (step/s)	End Speed (step/s)	Acceleration (step/s ²)
1000 uL Pump	30	800	10
4 ?1000 uL Pump	20	1000	10

Pump Air Aspirate Speed Set Pump Air Aspirate Speed

	Start Speed (step/s)	End Speed (step/s)	Acceleration (step/s ²)
1000 uL Pump	10	600	5
4 ?1000 uL Pump	10	600	5

Restore Default | Save Default | Apply | OK | Cancel

Figure 9-51 OPTIONS-Add Maximum volume

7 If you would like to add a new data point, note the weight of the volume and the number of steps from the 'Pump Log' as shown in *Figure 9-52 Calibration Table New Data Point Entry*. Then add the weight/volume and steps at the end of the calibration table, and click "Apply".

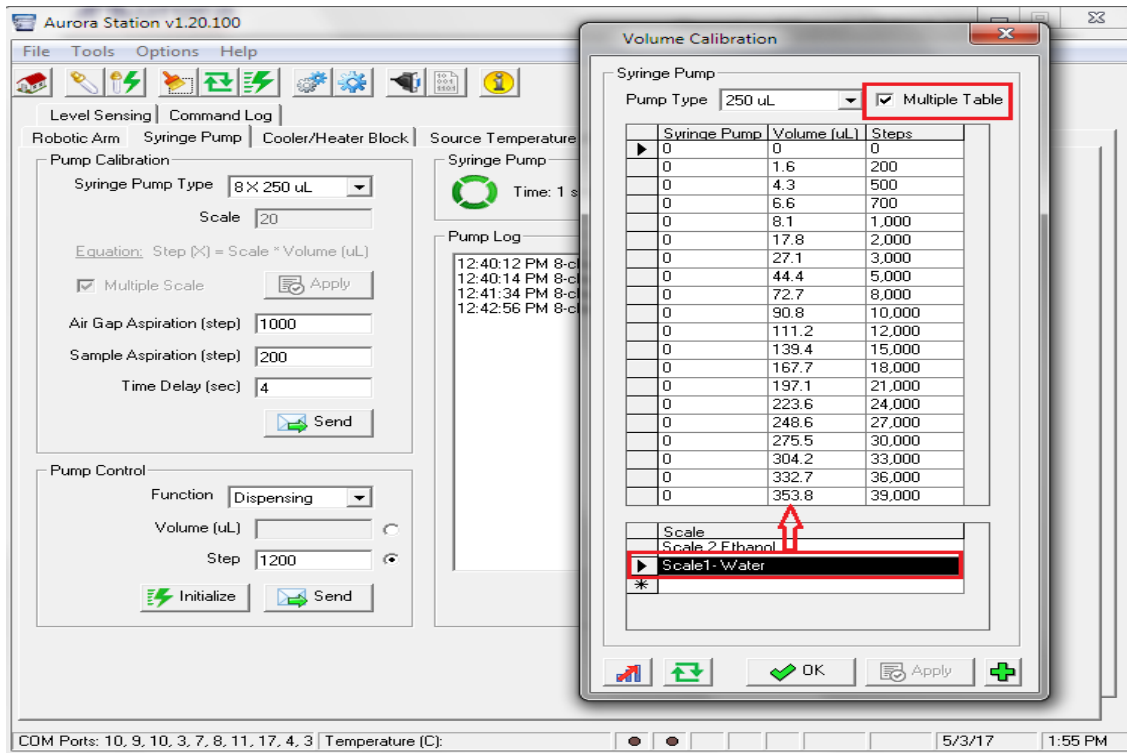


Figure 9-53 Multiple Table- Scale-1

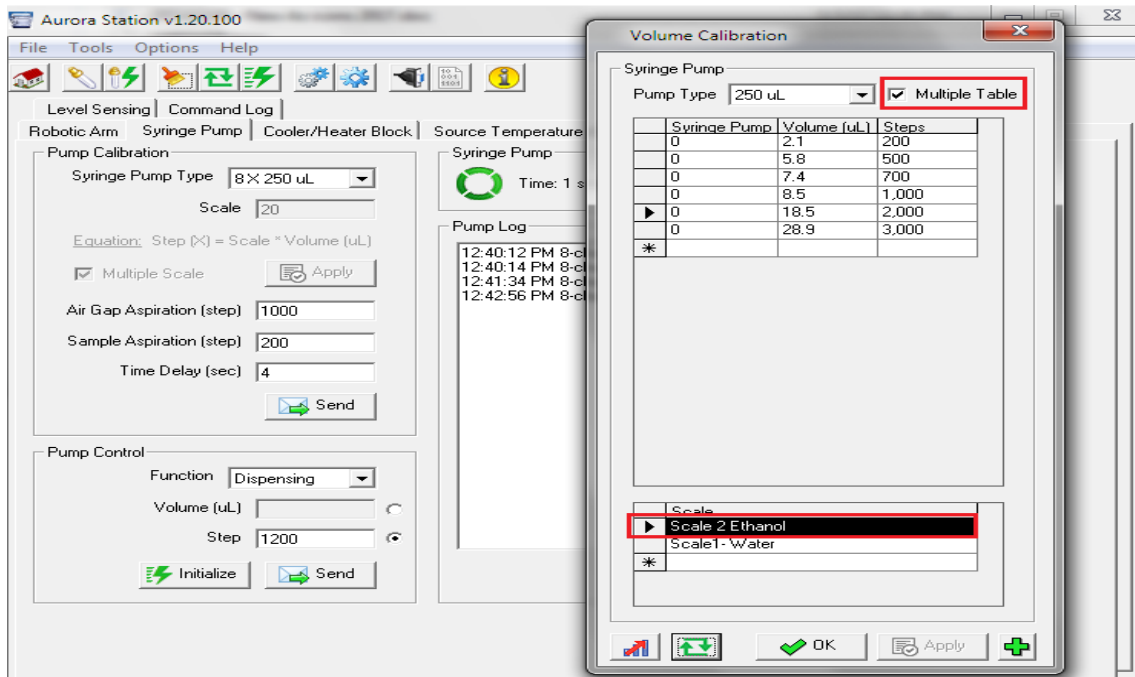


Figure 9-54 Multiple Table scale-2

Testing Small volumes using Multiple Cycles:

This method of testing is recommended for small volumes (1, 2 & 5µL) which cannot be weighed properly on the balance with one dispense/cycle only.

Follow the steps below:

1. Open Aurora Station, and open the Syringe pump tab. Select the pump that you wish to check the volume of.
2. Go to 'manual calibration' with volume selected as shown in figure.
3. Initialize the pump.
4. Manually engage one 50µL tip onto the desired barrel by hand.
5. Select an aspiration option
6. Add a 20µL air-gap in the volume column and hit send (there will be volume & steps displayed in the log table on the left. (*Figure 9-55 Syringe Pump –Aspirate 20µL Air-gap*))

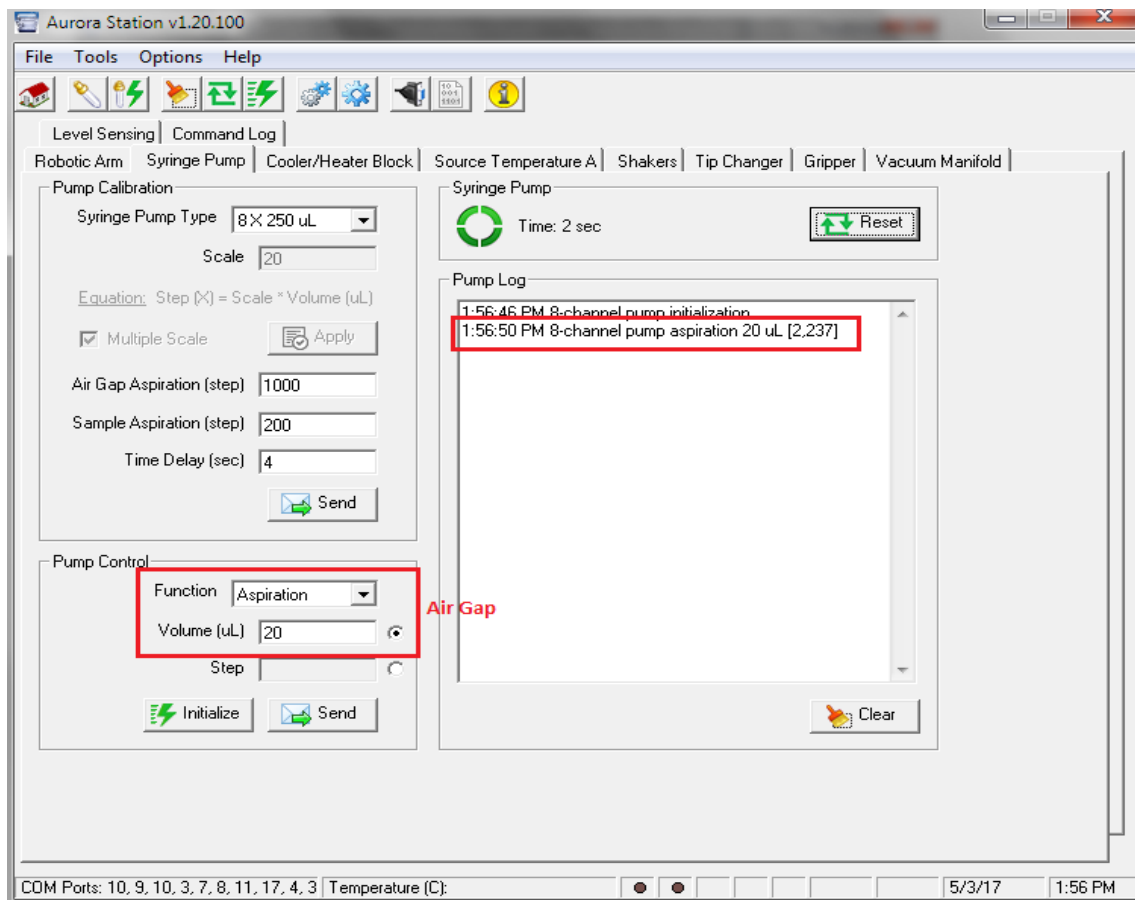


Figure 9-55 Syringe Pump –Aspirate 20µL Air-gap

7. Enter a 50µL volume in the pump control, and click send (Again place a tube of distilled water below the barrel to aspirate the 50 µL volume). (Figure 9-56 Syringe Pump –Aspirate 50µL volume)

8. Change to dispense mode

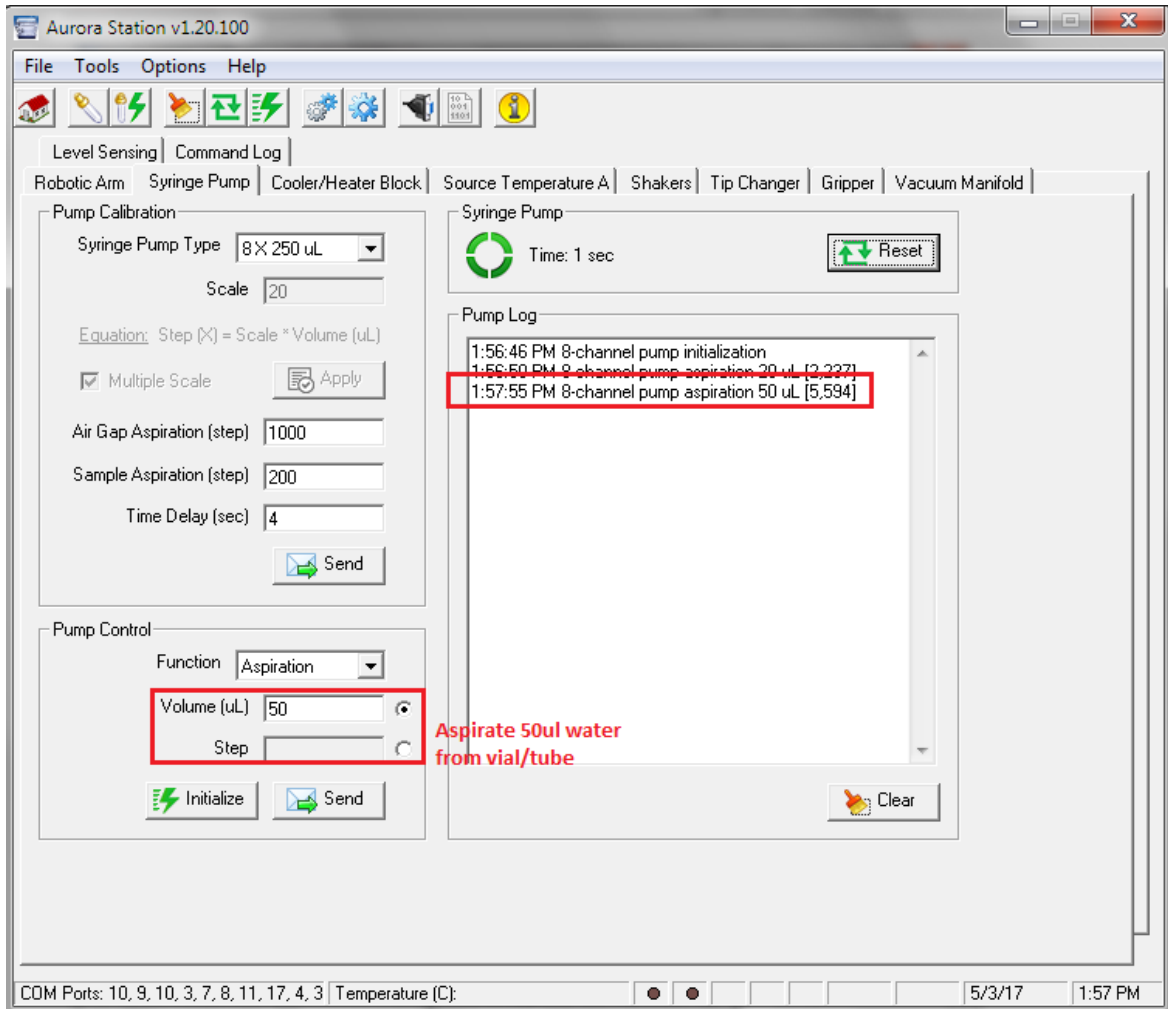


Figure 9-56 Syringe Pump –Aspirate 50µL volume

9. Add 10µL & click send & dispense back to the same tube. This dispense back volume is to compensate any backlash of the system (Figure 9-57 Syringe Pump –Dispense back to vial 10µL)

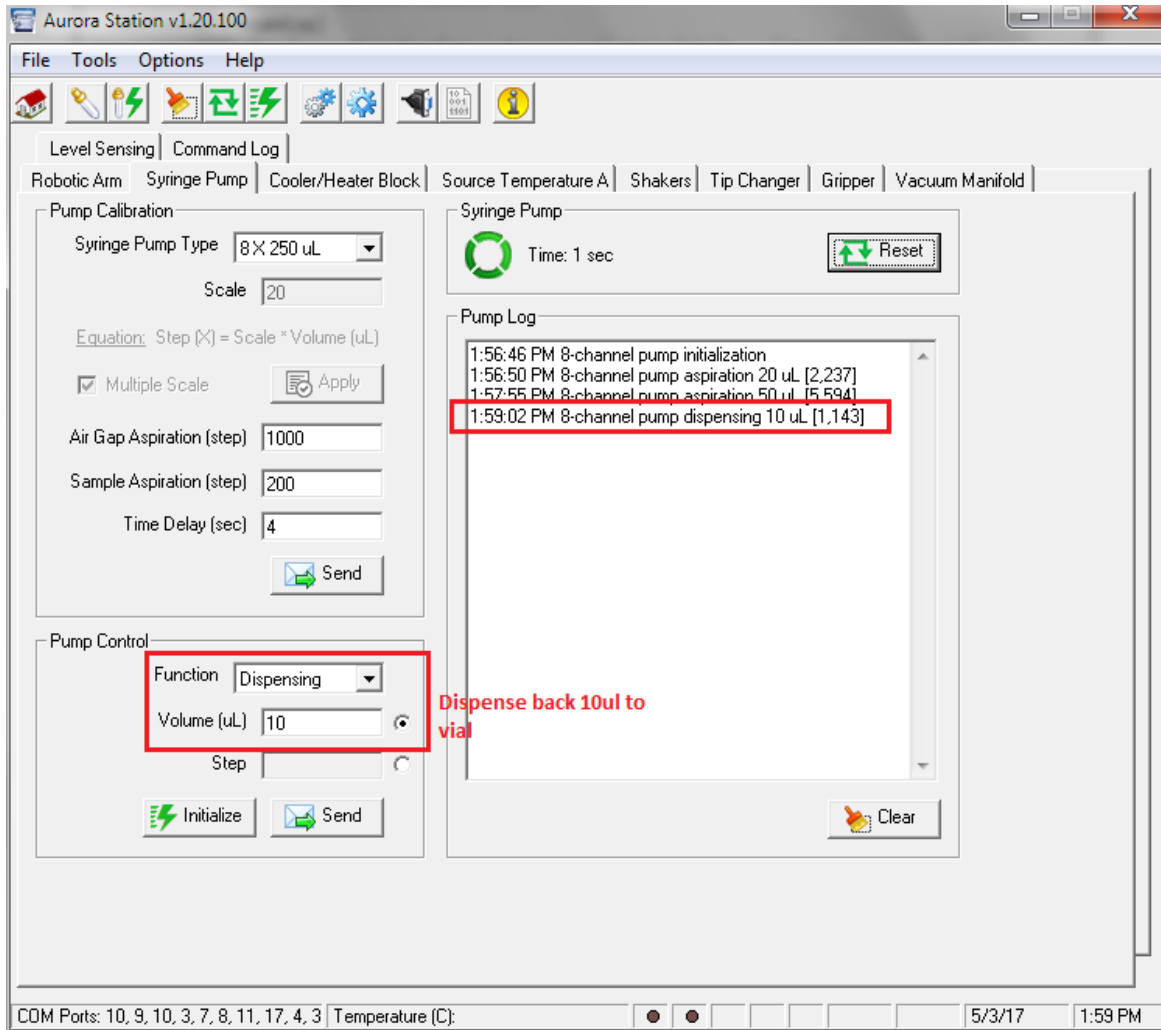


Figure 9-57 Syringe Pump –Dispense back to vial 10 μ L

10. Enter 1 μ L volume in the pump control, then aspirate and dispense it into a pre-tared weigh boat (making sure the tip touches the boat while dispensing). Repeat this 1 μ L aspiration and dispensing cycle in the same boat five times & then weigh the boat—it should be around 5 μ L. (Figure 9-58 Syringe Pump –Dispense 1ul (five times) to weighing boat) Divide this weight by 5 & get the volume of 1 μ L. Repeat it 10 times to calculate the CV. Repeat steps 5-9 again to finish 10 replicates. If the accuracy is not good, calibrate the system for 1 μ L again.

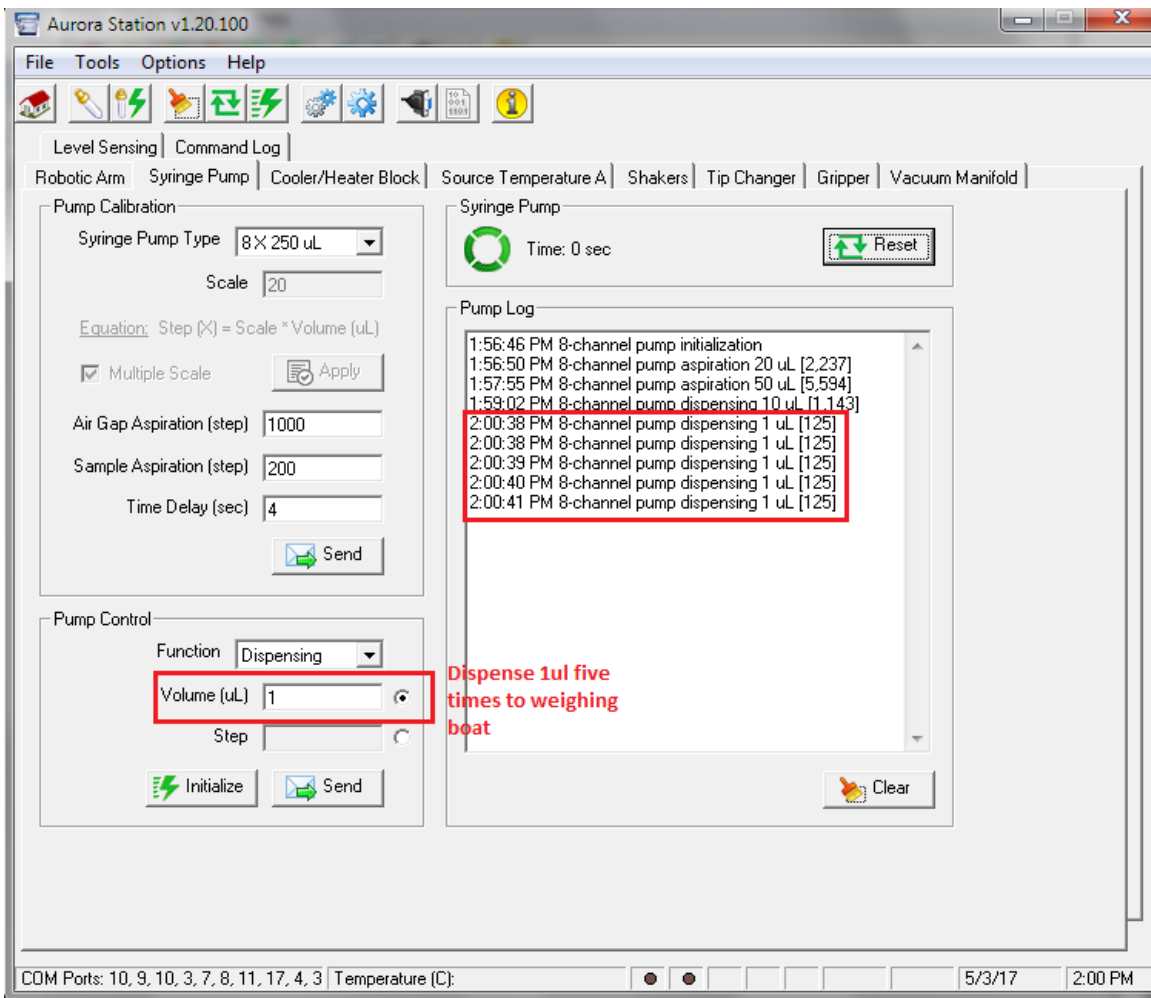


Figure 9-58 Syringe Pump –Dispense 1ul (five times) to weighing boat

9.9. Reagent Cooler Block Tab

This tab sets and monitors the temperature of the reagent/cooler block in the range of 2 - 90°C with 0.25°C increments.

The tab allows the user to check or change COM ports or present temperature. The recommended temperature for the reagent/cooler block is 4 °C which is also the default value.

Unlock the COM port as displayed in the figure below (*Figure 9-59 Cooler Block Control*) to observe readings of actual and pre-set temperatures. If there is no response, proceed to change the COM port. Test the new COM port by clicking the “open” tab.

To change present temperature, go to the temperature field and input the temperature required and click the “apply” tab to save the new settings.

After implementing the change, allow up to 30 minutes for the change to take effect before loading reagents and enzymes onto the block. The temperature log provides a record of any changes made. Click the “clear” button to erase the log records.

If the temperature reading from an external thermometer does not match the display temperature, parameter B can be adjusted to match the true temperature. Wait and check after it stabilizes at the required temperature (*Figure 9-59 Cooler Block Control*).

NOTE: If there is no response from the cooler block, refer to the Temperature Control section of this manual to adjust the COM port. Use only installed COM ports for cooler block configuration (refer to software installation chapter of this manual for more details).

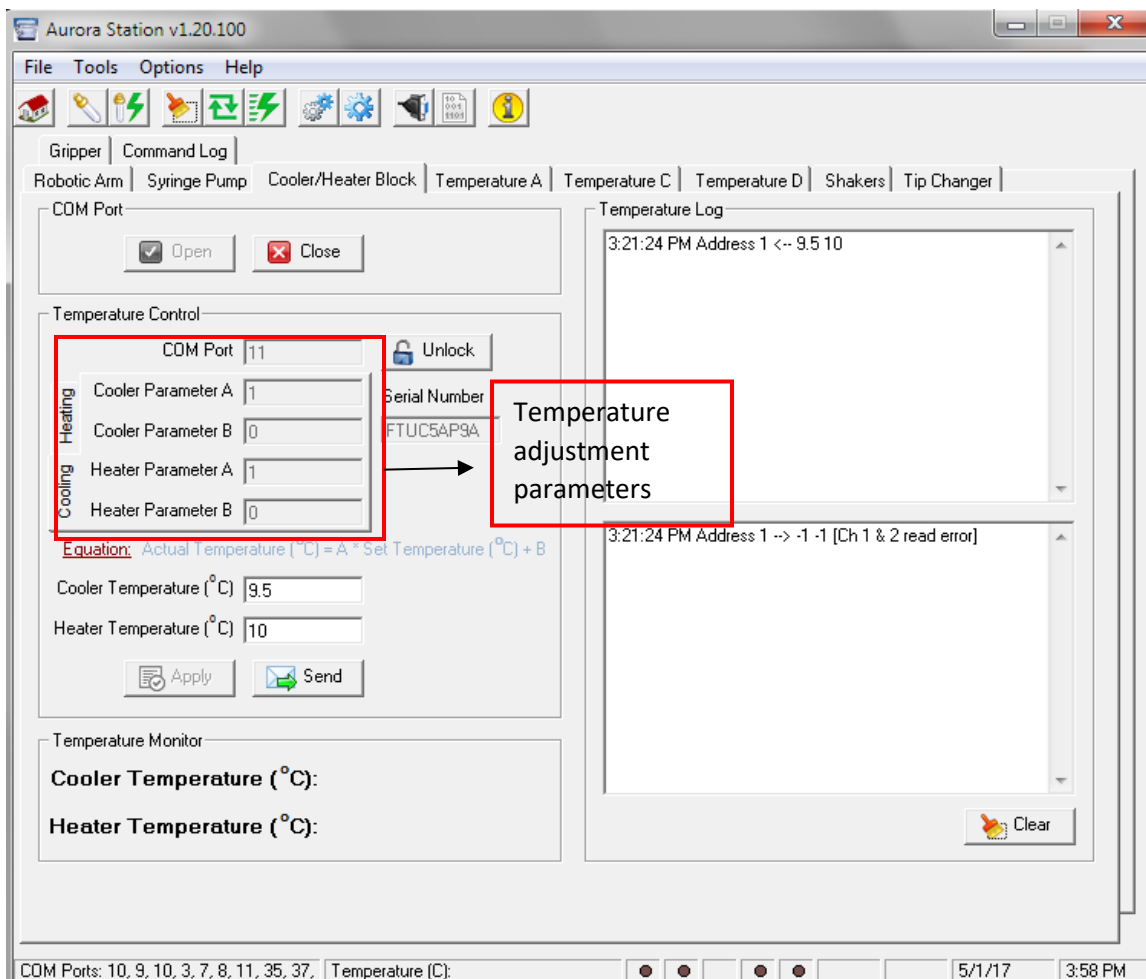


Figure 9-59 Cooler Block Control

9.10. Source Temperature Tab

This tab allows the user to change/ set the COM ports, as well as the temperature for other cooling/ heating adaptors that have been fitted onto the deck.

The temperature can be changed or set here for up to two different temperature adaptors (e.g. plate coolers/ heaters, shaker with heater etc.). Once the new set temperature takes effect, it can be monitored under the “temperature monitor” part of the window (bottom of screen) for each adaptor (*Figure 9-60 Source Temperature Settings*).

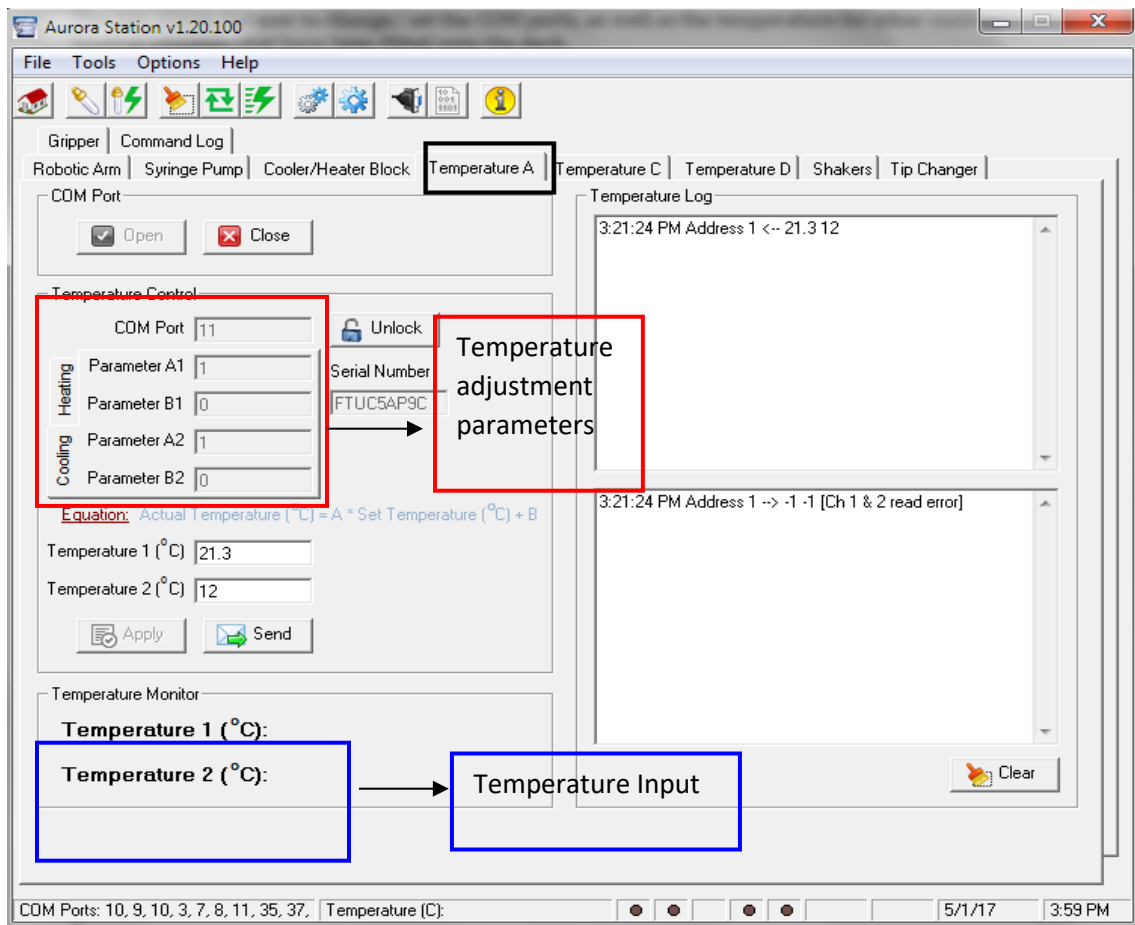


Figure 9-60 Source Temperature Settings

9.11. Shaker Tab

This tab allows the operator to set shaker COM ports (already specified) and test the speed of the shaker.

Shaker speeds can be set between 200-2500 rpm. To change the shaker control settings, click “apply” to implement the settings. You can test the new settings by clicking on the “send” tab (*Figure 9-61 Shaker Settings*).

In the application tabs, the user can use any speed between 100 – 2500 rpm depending upon the protocol requirements and the plate being used on the shaker.

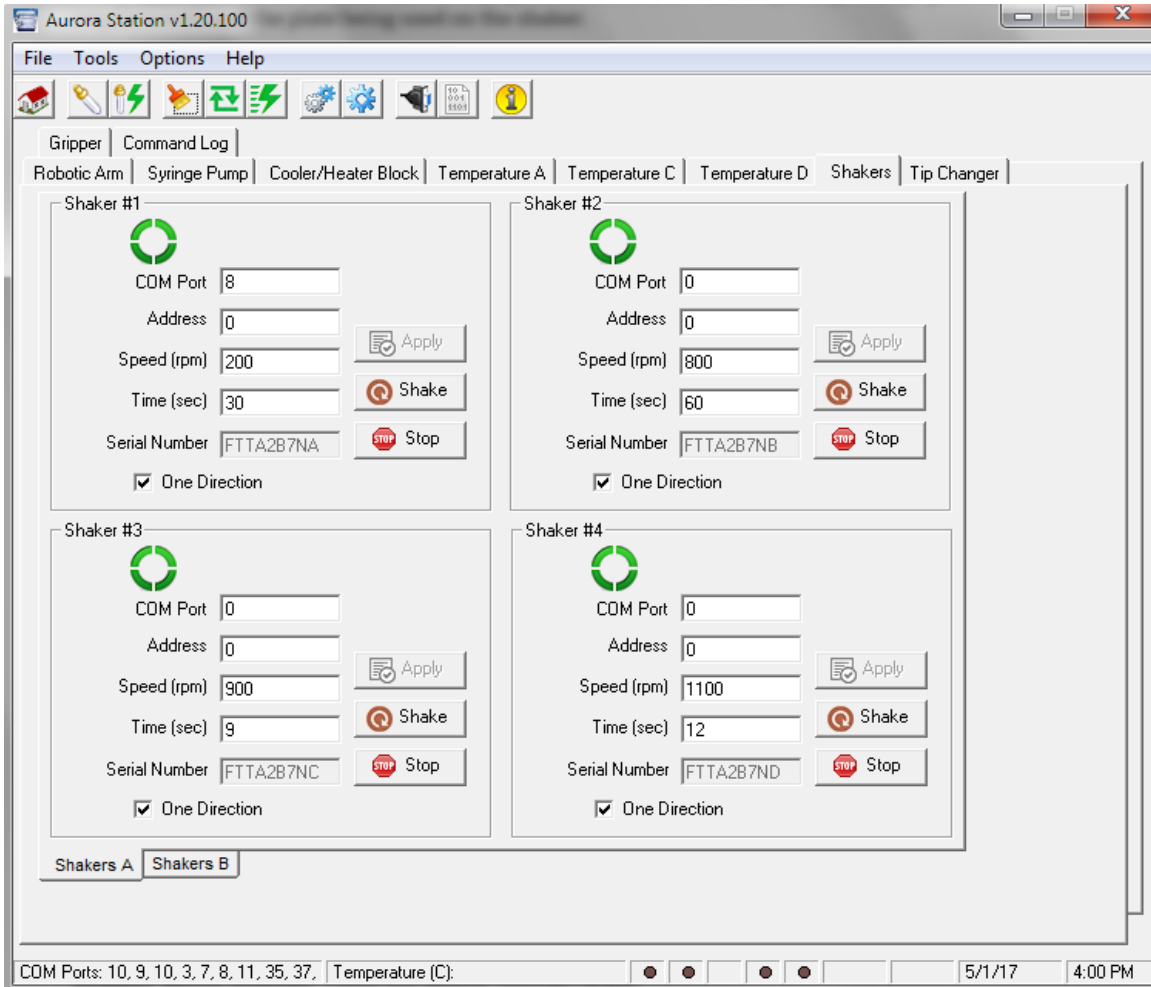


Figure 9-61 Shaker Settings

9.12. Gripper & Aspirator Tab

Gripper: This tab shows all the settings for gripper. To set the COM port for the gripper the user can test/set the gripper position using the gripper engagement offset, XYZ offsets, speed, and positions

Gripper Speed Settings: These are default settings (Figure 9-62 Gripper Settings)

Gripper XYZ Settings: These are also default settings, but can be adjusted depending on the plate being moved. The gripper X-Offset value, if increased, will change the gripper position on the right side of the plate and if decreased will change the gripper position on the left side.

Gripper Y-Offset value if increased will move the gripper outwards and vice versa.

Gripper Z-Offset is the value which allows the gripper to grab the plate at a specific Z distance (Figure 9-63 Gripper Holding Deep Well Plate).

Gripper Offset Settings: Engagement Offset is used to set the gripper Z setting to open the gripper when moving the plate from one position another. Other settings like max range, pad clearance etc. are also set here. Plate count is the number of plates to be stacked at one position.

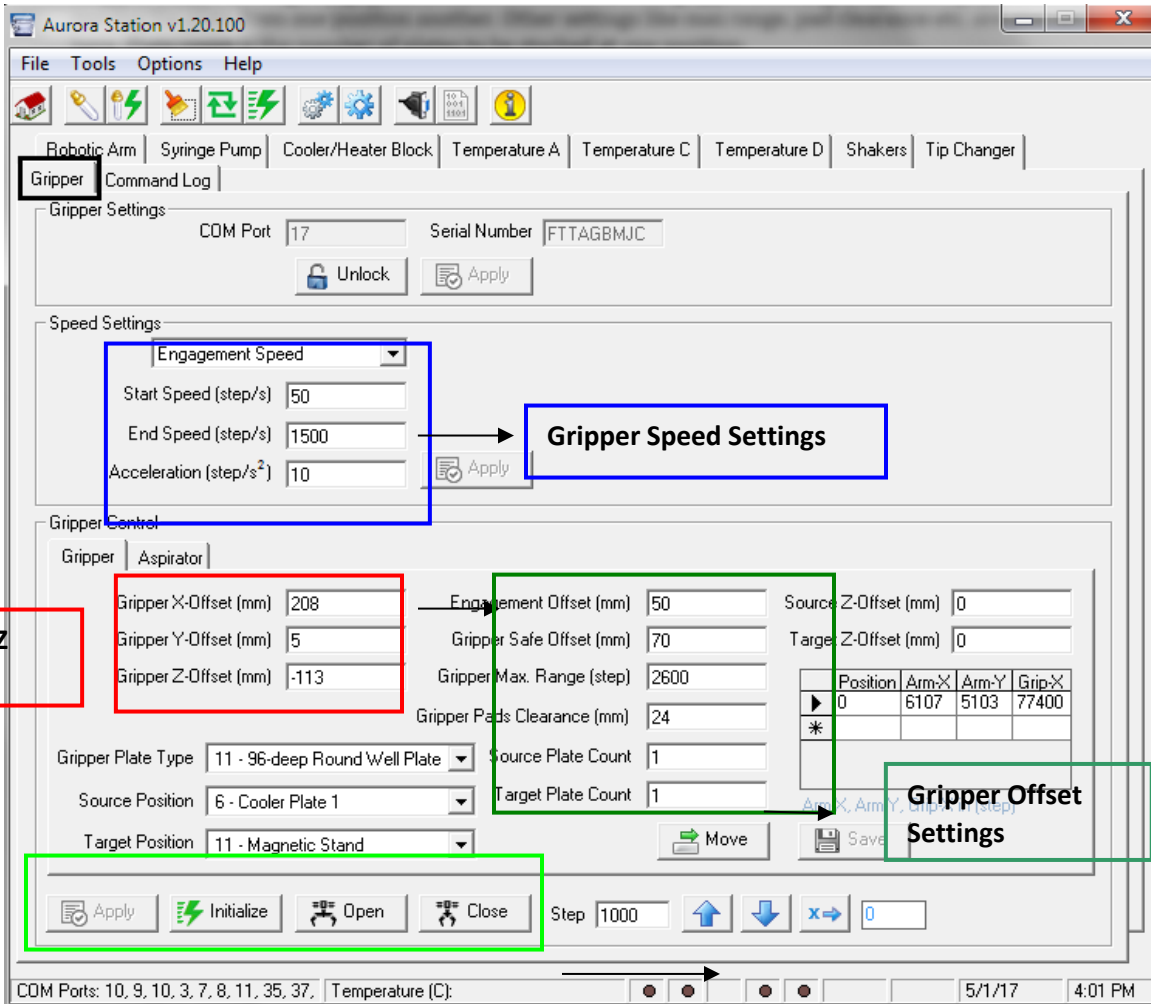


Figure 9-62 Gripper Settings

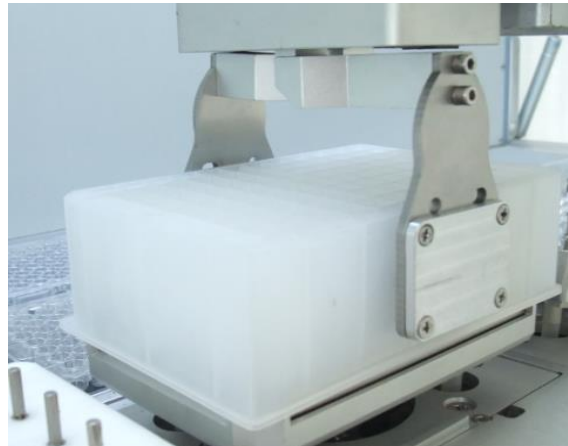


Figure 9-63 Gripper Holding Deep Well Plate

9.13. Vacuum Tab

This tab allows user to test vacuum manually. After the COM port is set, the vacuum can be tested for valves 1 & 2 (*Figure 9-64 Vacuum Settings*). This tab is also used for RD Level Sensing.

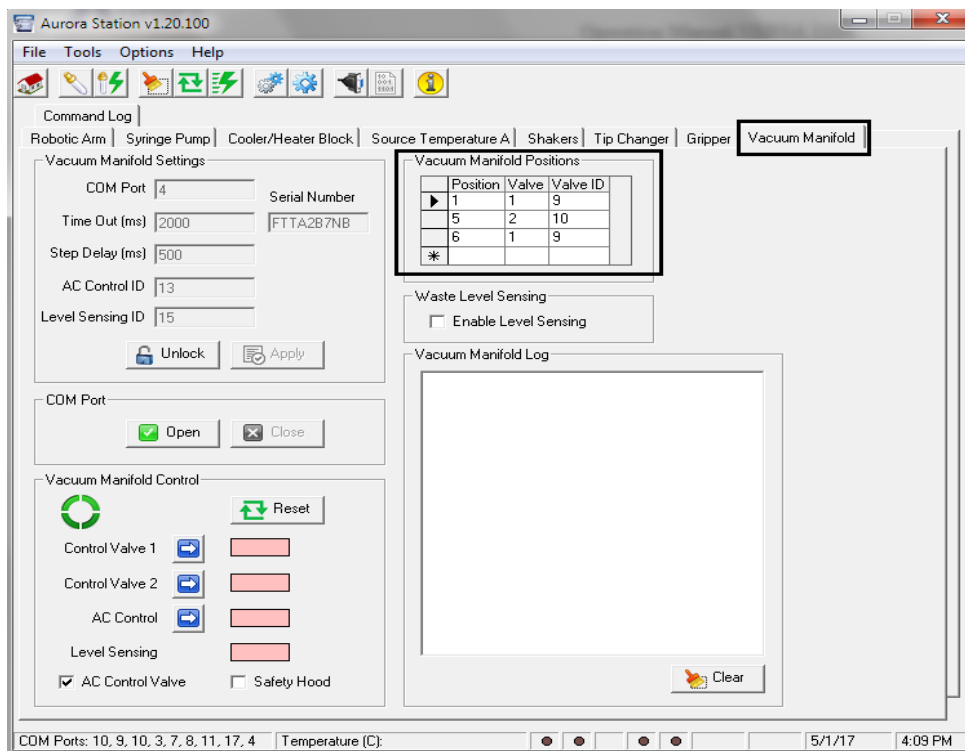


Figure 9-64 Vacuum Settings

9.14. Command Log Tab

This screen displays the summary of all the executed steps created since the VERSAware was last opened. Click on the “clear” tab to erase logged message once they have been identified and rectified (Figure 9-65 Command Log Settings).

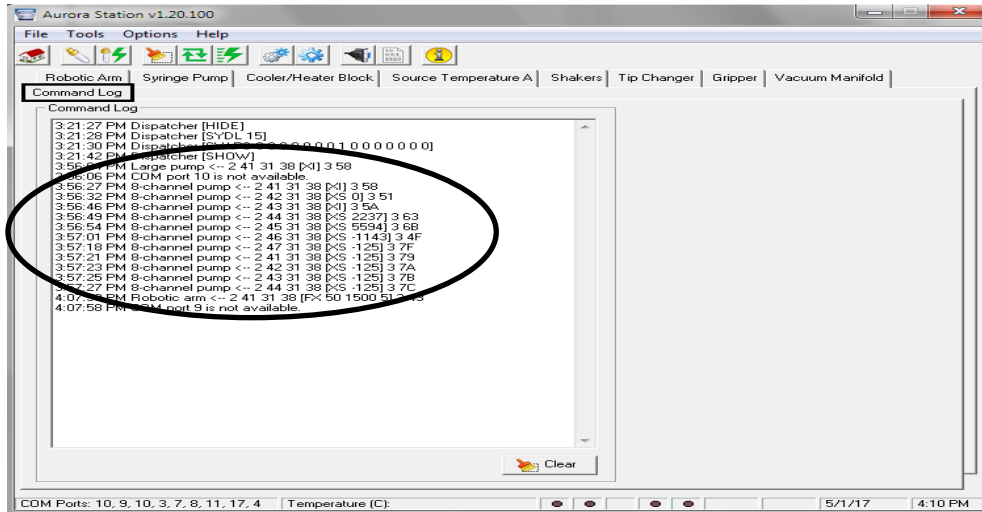


Figure 9-65 Command Log Settings

NOTE: Consult the manufacturer on rectification of errors if unsure or if errors persist.

9.15. Liquid level Sensing (Optional-Single Channel - LLS Hardware Required)

This feature of liquid level sensing on the unit provides user the ease of aspirating the liquid after sensing the level in the tube/plate and then aspirating the liquid from that level with accuracy.

In the figure below (Figure 9-66 Liquid Level Sensing Settings), LLS comport can be set, LLS can be tested manually to make test it before running any assays.

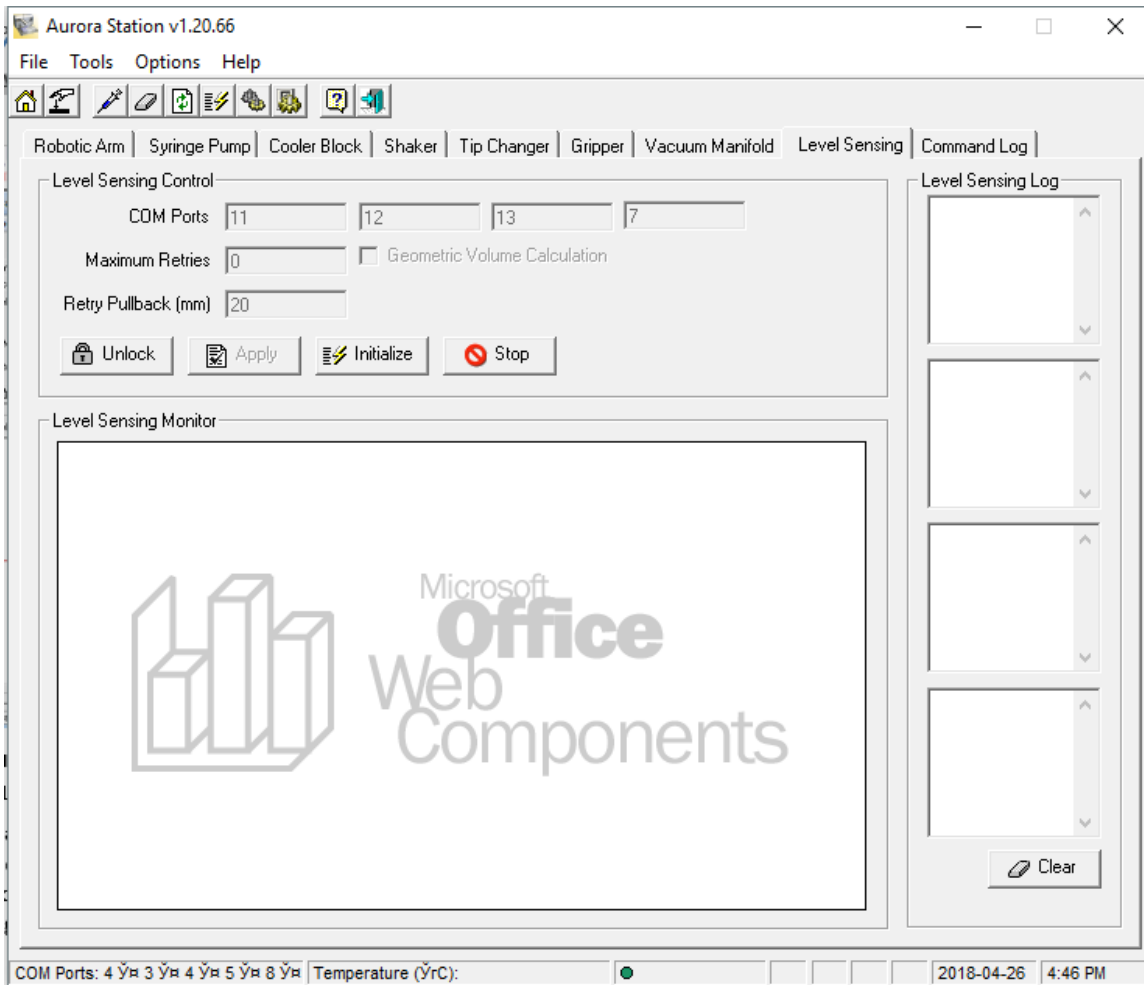


Figure 9-66 Liquid Level Sensing Settings

Liquid Level Sensing (LLS) Calibration

This chapter deals with both manual checking and calibration of the liquid level sensor equipped. You are advised to manually check the sensor’s operation before or after calibration. It is good practice to manually check the sensor’s operation periodically to ensure it is in good working condition.

Manual checking LLS operational capability

Steps:

1. Move the arm to an empty position anywhere on the deck
2. Open Aurora Station and click on the level sensing tab.
3. Click “initialize”.
4. A dialog will pop up saying, “initializing in progress.”
5. The log will show the signal levels at various rates

6. Place your finger slightly below the syringe/tip barrel (no tip engaged)
7. Note the jump of the signal from 100 to approximately 200 (*Figure 9-67 Base Signal*).
8. Next, insert a black conductive 20 μ L tip into the barrel.
9. Insert the tip into a 5 to 7mL vial with distilled water.
10. Note the signal level changing from 100 to 200 (*Figure 9-68 Signal Level Change – Barrel with Tip Immersed in Liquid*).
11. If the above are observed when performing steps 1 to 10, then the liquid sensor is working as it should. Proceed to calibrate or check COM ports if you observe no change in the signal level.

The below figures provide examples of what to expect when level sensing is operational around the barrel and in a liquid.

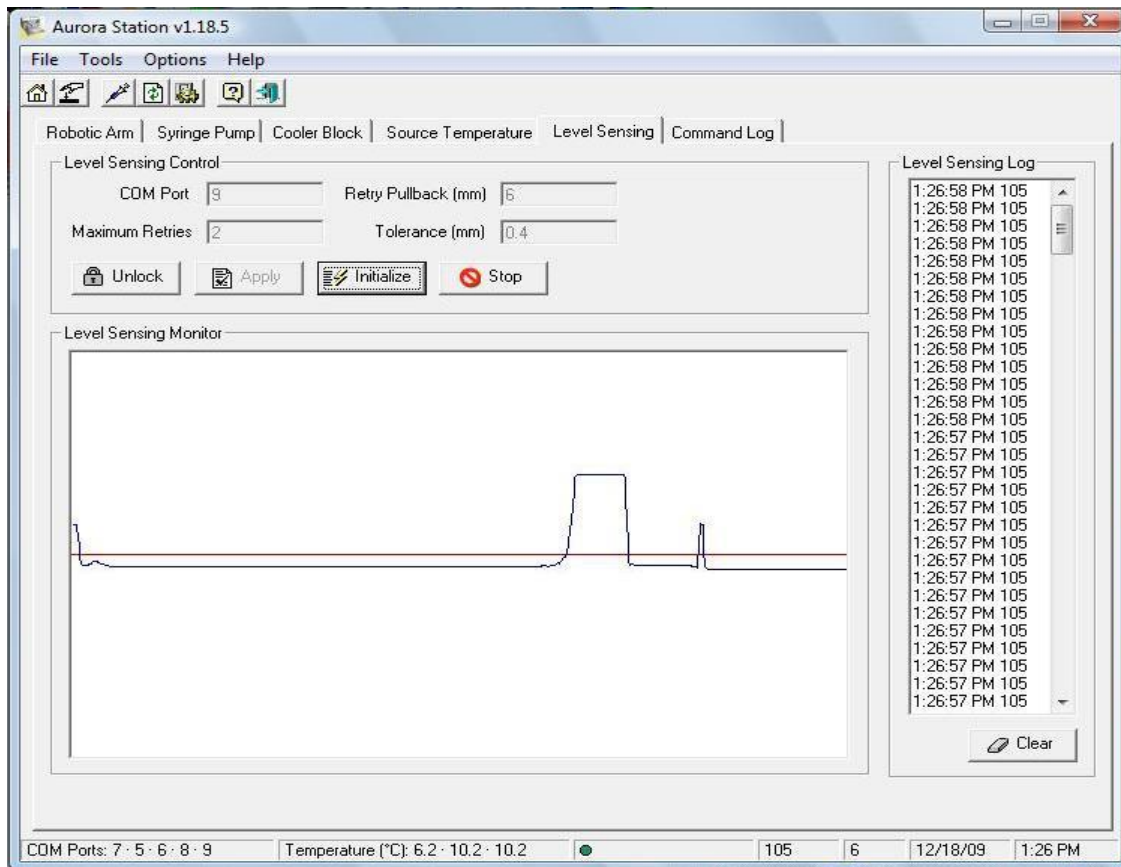


Figure 9-67 Base Signal

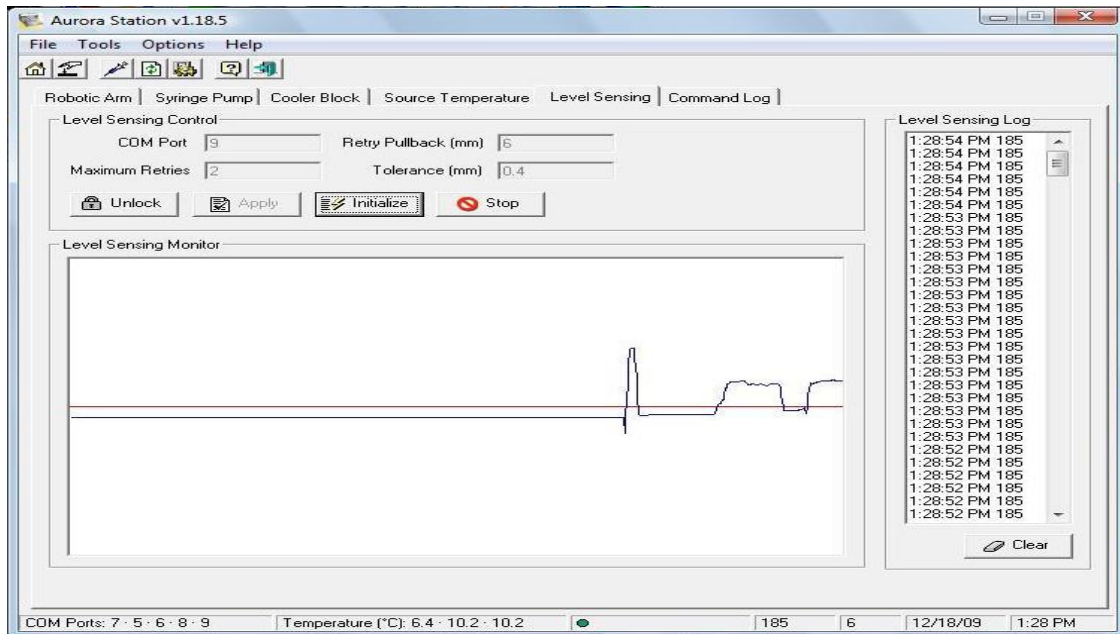


Figure 9-68 Signal Level Change – Barrel with Tip Immersed in Liquid

LLS calibration check for a current plate type

Steps:

1. Open Aurora Station and select a new blank or empty row for use in the coordinates table. Under the position field, type in a position number and a comment (this can be any number or name that don't coincide with the default teach positions). Leave all other fields empty and highlight the new row entry (Figure 9-69 New Row Entry for Coordinates).



Figure 9-69 New Row Entry for Coordinates

2. Again from Aurora Station, select advanced settings, select single channel

Mode to test LLS as shown below in Figure(Figure 9-70 Setting the Tip Box Position, Plate Position & single channel mode for Calibration).

NOTE: You'd have to ensure that the test position for both the tip box and plate type are set by clicking onto the "advanced" tab in Aurora Station Settings. To change or set, click on the arrow to open options and select the position of choice. Remember that if you select e.g. position 14 for 20/50/200 µL tip box that you have a tip box placed in position 14 on the deck available. You'd also want to drag the appropriate tip box icon over onto the working deck interface in VERSAware to position 14. After which, you'd want to "reload" tip boxes. The same goes for the plate type you'd want to test. If test position for a plate is selected as 1, load appropriate plate type for the test should be in that position and icon dragged over to that position on the working deck interface in VERSAware.

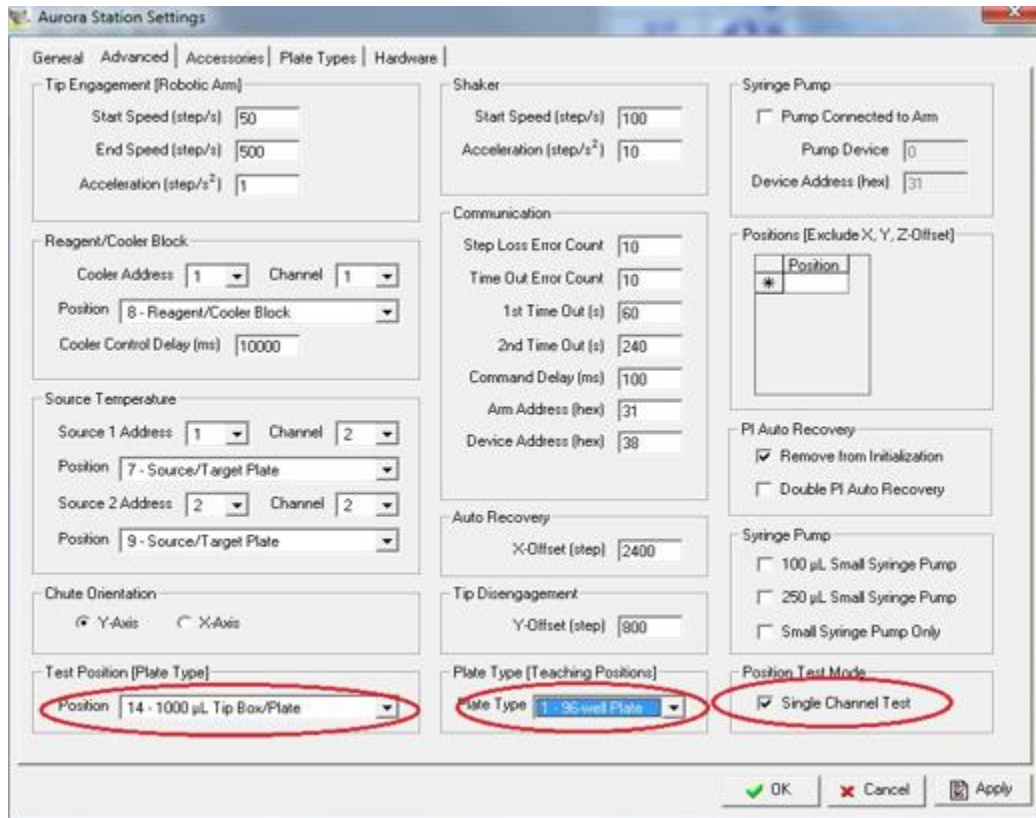


Figure 9-70 Setting the Tip Box Position, Plate Position & single channel mode for Calibration

- Click on the plate type icon in Aurora Station. Focus on the topmost table to test LLS parameters. To achieve this, click on the particular plate type field for test (Figure 9-71 Testing LLS Parameters under Aurora Station Settings Plate Types Tab).

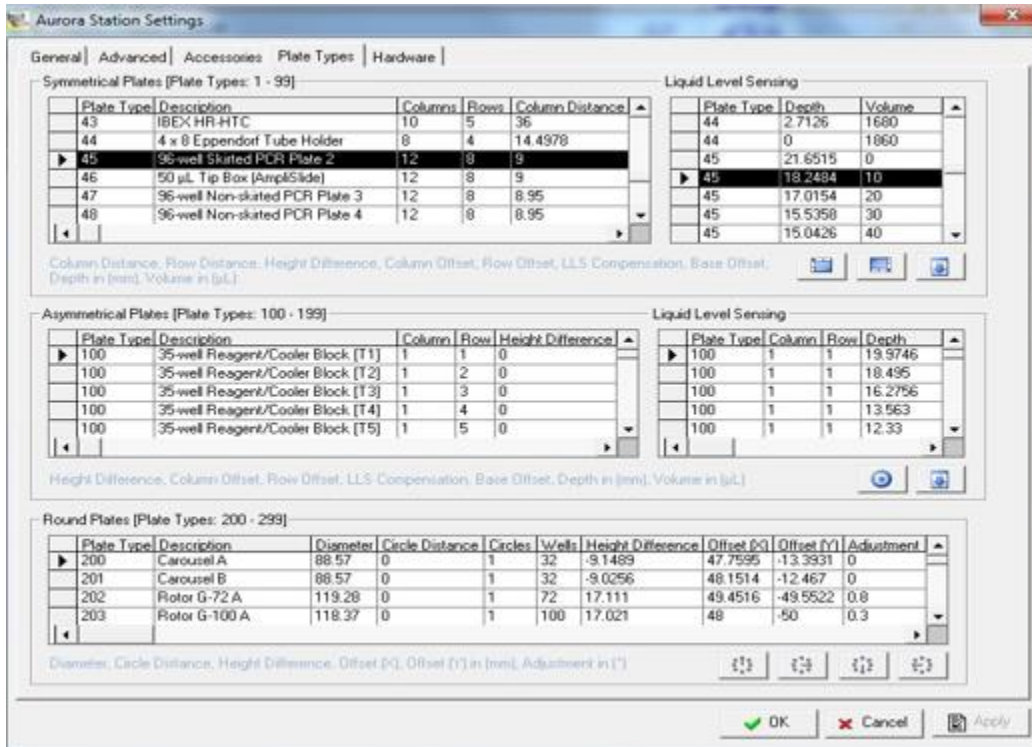


Figure 9-71 Testing LLS Parameters under Aurora Station Settings Plate Types Tab

- Return to the “plate types” tab again to start the LLS calibration. Then select the plate type field that you want to test by highlighting that field with your mouse. Usually the detection range field for that plate type would have been set by default, but if a new plate is entered or reset for the detection range, this value would have to be changed. To illustrate this, we first explain how to test default positions:

Step 1: Click on plate type number to test. (Figure 9-72 Testing Default Positions under Aurora Station Settings Plate Types Tab)

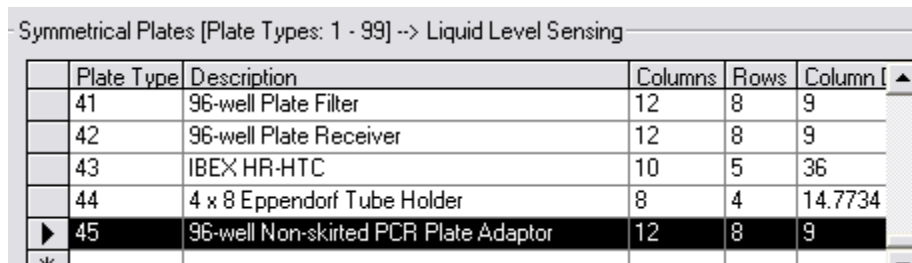


Figure 9-72 Testing Default Positions under Aurora Station Settings Plate Types Tab

Step 2: In the rightmost table, scroll up to the corresponding plate type no. and note the depth and volume. You'd want to test from the lowest volume all the way to the highest volume. But essentially sampling/ calibrating anywhere from 12 to 16 volumes would be sufficient. (Figure 9-73 Testing LLS Volume and Depth)

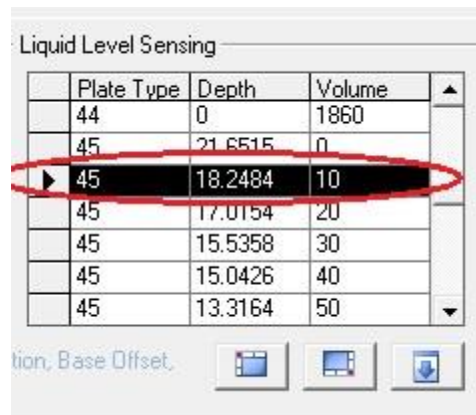


Plate Type	Depth	Volume
44	0	1860
45	21.6515	0
45	18.2484	10
45	17.0154	20
45	15.5358	30
45	15.0426	40
45	13.3164	50

Figure 9-73 Testing LLS Volume and Depth

Step 3: Then with a manual pipette, dispense the same volume into the first well of the plate to test. If 10µL is selected, then there should be 10µL of liquid in that well. (Figure 9-74 LLS Calibration Check with selected Plate Type)

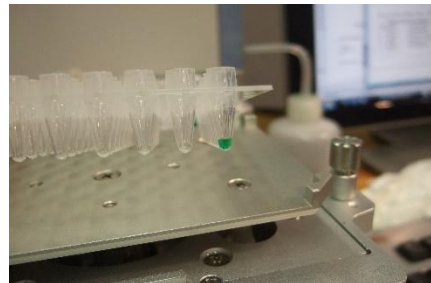


Figure 9-74 LLS Calibration Check with selected Plate Type

Step 4: Next click “First icon” and the robotic arm will move to pick up a tip from the specified tip position (first tip) as specified under the “advanced” tab. If you click on the second icon, the arm will pick up a tip from last well (96) of the tip box. Once a tip is automatically

engaged by the barrel, you can click “Downward arrow icon to test LLS” as shown in Figure.(Figure 9-75 Testing Depth and Volume Parameters under Aurora Station Settings Plate Types Tab)

Liquid Level Sensing

	Plate Type	Depth	Volume	
	44	0	1860	▲
	45	21.6515	0	
▶	45	18.2484	10	
	45	17.0154	20	
	45	15.5358	30	
	45	15.0426	40	
	45	13.3164	50	▼

tion, Base Offset

Figure 9-75 Testing Depth and Volume Parameters under Aurora Station Settings Plate Types Tab

You will notice that the tip moves to a position just above the first well. By default and at all times, the tip will always move to the surface position. (Figure 9-76 Surface Position Coordinates Achieved)



Figure 9-76 Surface Position Coordinates Achieved

Step 5: Next you may return to Aurora Station robotic arm interface and ensure that the surface coordinates are already present in the GoX, GoY, and GoZ fields before clicking the “save” tab to save this surface position into the new line created. Highlight the field coordinates, then click the “save” button. (Figure 9-77 Saving Surface Coordinates into Aurora Station Robotic Arm Interface)

2800 52000

Go X 8108 Go Y 2450 Go Z 50111 Go XYZ Offset Tip Test Save Tip Calibration

Arm Control

Step 10

No Z Up

Position	Step [X]	Step [Y]	Step [Z]	Comment
15	10495	2550	51011	20 µL Tip Box/Plate
16	273	75	47414	Priming Position
17	287	1310	46000	Waste Position
18	87	2509	45984	The Chute
▶ 111	8108	2450	50111	Rename "Temp LLS Check"

Figure 9-77 Saving Surface Coordinates into Aurora Station Robotic Arm Interface

Step 6: After saving the surface position coordinates, start working from this position for LLS calibration of individual volumes. In the arm control portion of the interface (as above), enter “10” as a value for the step and check off “no Z up”.

At this point, there should be 10µL of liquid in the well and the barrel with tip should be just above the surface of the well.

Note the signal at this position. It should be around 142 more or less. You can note the liquid level sensing signal value on the bottom right corner of the interface next to the date and time (*Figure 9-78 Level Sensing Signal check under Aurora Station Robotic Arm Interface*).

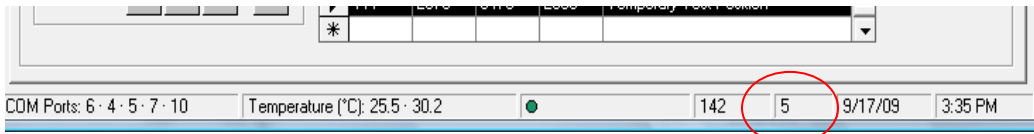


Figure 9-78 Level Sensing Signal check under Aurora Station Robotic Arm Interface

Step 7: After making a record of this value, attempt to move the tip down with the step arrow controls. Move down and until the surface of the tip barely touches the surface of the 10µL liquid. You’ll notice the signal increasing as the tip moves down.

Usually, the detection range that is set in the ‘plate types’ tab accounts for this and you can confirm the difference in signal detection from the plate surface to the surface of the liquid (10µL) to be similar or close to what is set in the detection range field already set in the plate types tab for that particular plate type. (*Figure 9-79 Check signal difference under Aurora Station Plate Types Tab Detection Range Field*)

If for e.g. the signal is 154 and the surface signal is 142, then the difference is 12 +/- 1 for the detection range (limit). To check the depth offset at the surface of 10uL, first single click on the new field in Aurora Station after the visual inspection of tip touching the 10µL surface is achieved. The new coordinates would automatically be present in the GoX, GoY, and GoZ fields. You’d then click on the “offset” tab to view the offset field value (in mm). This value should correspond or be similar to the depth value for volume 10 in the rightmost table of the “Plate Types” tab. If it isn’t, change or edit that value by copying and pasting the new offset into the depth field in the “Plate Types” tab rightmost table. (*Figure 9-81 Check Depth Offset (Calculator) at 10 µL with Depth Value under Aurora Station Plate Types Tab* *Figure 9-79 Check signal difference under Aurora Station Plate Types Tab Detection Range Field*)

Plate Types | Devices |

--> Liquid Level Sensing

Column Offset	Row Offset	Detection Range	Arr	Plate Type
-0.7981	-0.2826	7	100	44
-0.7981	-0.2826	7	100	44
-108.3966	98.5249			44
-2.1357	-2.877	30	100	45
0.4839	-0.348	12	30	45
				45
				45

at Difference, Column Offset, Row Offset, Depth in (mm), Volume in (µL)

Figure 9-79 Check signal difference under Aurora Station Plate Types Tab Detection Range Field

Offset Calculator

Column Distance

X1 (step) 2579

X2 (step) 2579

Total Columns 2

Distance (mm) 0

Offset (mm) 0

Row Distance

Y1 (step) 5470

Y2 (step) 5470

Total Rows 2

Distance (mm) 0

Offset (mm) 0

Height Difference

Z1 (step) 2509

Z2 (step) 3249

Difference (mm) -18.2484

Offset (mm) 18.2484

OK

Figure 9-80 Check Depth Offset (Calculator) at 10 µL with Depth Value under Aurora Station Plate Types Tab

Liquid Level Sensing

Plate Type	Depth	Volume
44	0	1860
45	21.6515	0
45	18.2484	10
45	17.0154	20
45	15.5358	30
45	15.0426	40
45	13.3164	50

sation, Base Offset.

Figure 9-81 Check Depth Offset (Calculator) at 10 µL with Depth Value under Aurora Station Plate Types Tab

Step 8: Next check minimum level sensing at “0” depth approx. 1mm from the very bottom of the well. Move the tip down further with the step arrow controls

until the tip surface barely touches the bottom of the well. At this point, the signal value should be similar to the surface signal value for 10µL (as a guide).

- Step 9: Once that is achieved, highlight the whole field teach position in Aurora Station after the visual inspection of tip touching approx. 1mm from the bottom of the well is achieved. The new coordinates would then automatically be present in the GoX, GoY, and GoZ fields. Then click on the offset tab to view the offset field value (in mm). This value should correspond or be similar to the depth value for volume “0” in the rightmost table of the plate types tab. If it isn’t, edit that value by copying and pasting the new offset (fr.offset tab calculator) into the depth field in the “Plate Types” tab rightmost table.

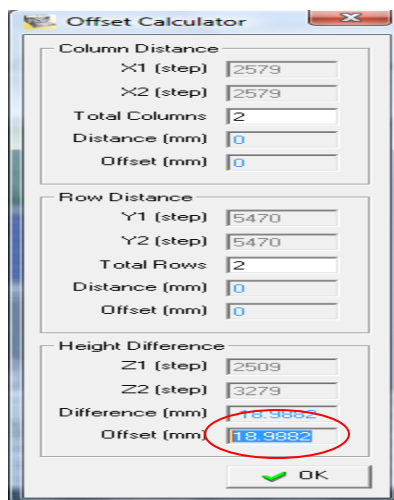


Figure 9-82 Check Depth Offset (Calculator) at 0uL with Depth Value under Aurora Station Plate Types Tab

Liquid Level Sensing

Plate Type	Depth	Volume
44	0	1860
45	21.6515	0
45	18.2484	10
45	17.0154	20
45	15.5358	30
45	15.0426	40
45	13.3164	50

Figure 9-83 Check Depth Offset (Calculator) at 0uL with Depth Value under Aurora Station Plate Types Tab

- Step 10: Perform the same steps to check for 20µL. Remember to manually pipette an additional 10µL to the same well of the plate first before moving the tip down again for the surface level depth check for 20µL.
- Step 11: You would subsequently check for 30µL using the above steps above and so on and so forth. If the checks seem within range of the depths listed in the “Plate

Types” tab rightmost table, discontinue the calibration procedures for the rest of the volumes. A rule of thumb would be to perform checks for the volumes used most frequently for any specific plate type for your work or research. Remember to have the right amount of liquid in the well when testing for that volume.

LLS calibration setup for new volumes - current or new plate type

NOTE: If a new plate type is required that cannot be found in the list of plate types in Aurora Station plate types settings tab, please consult an Aurora representative to aid in entering that plate type for you. But calibration has to be achieved once that is performed by the user. Please follow the below steps for calibration:

Steps:

1. Open Aurora Station and select a new blank or empty row for use in the coordinates table. Under the position field, type in a position number and a comment (this can be any number or name that don't coincide with the default teach positions). Leave all other fields empty and highlight the new row (*Figure 9-84 New Row Entry for Coordinates Table*)

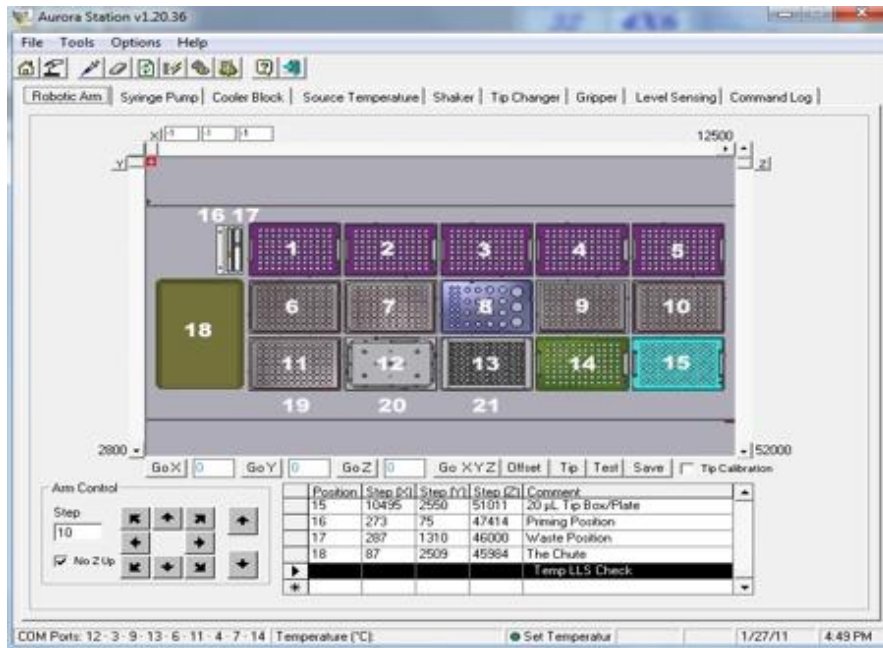


Figure 9-84 New Row Entry for Coordinates Table

2. Next click on the settings icon in Aurora Station. Focus on the topmost table to test LLS parameters. To achieve this, click on the new plate type field created for test (*Figure 9-85 Creating New Plate Type Field under Aurora Station Settings Plate Types Tab*).

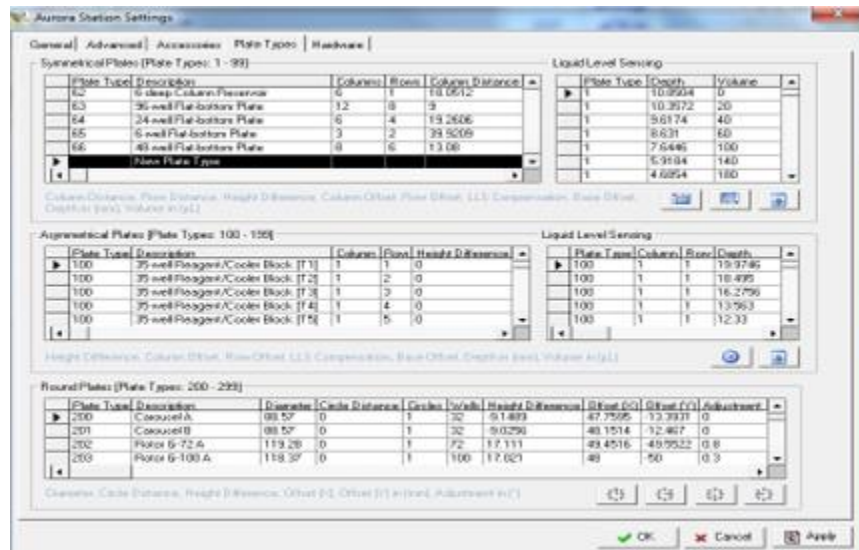


Figure 9-85 Creating New Plate Type Field under Aurora Station Settings Plate Types Tab

NOTE: Columns and rows, offsets and arm parameters shown in the picture above have been left empty but after consultation with your Aurora Representative, it should be entered into the fields. Focus for the LLS calibration for the new plate type should be placed on the detection range field instead. To achieve the detection range field, refer to the following steps below:

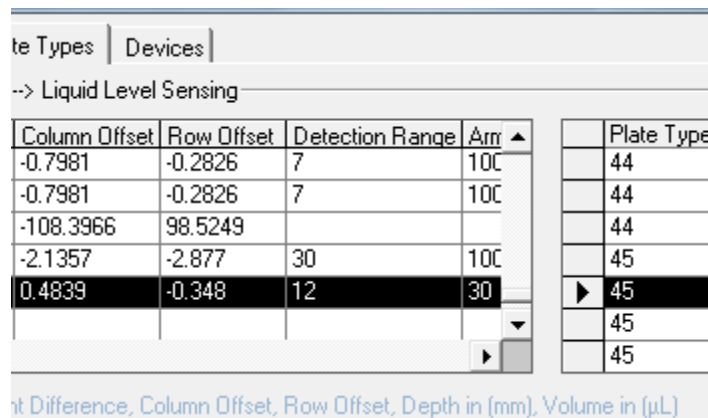


Figure 9-86 Detection Range Field under Aurora Station Settings Plate Types Tab

3. First ensure that the test position for both the tip box and plate type are set by clicking onto the “advanced” tab in Aurora Station Settings. To change or set it, click on the arrow to open options and select the position of choice. Remember that if you select for position 1 (as an example) for 20/50/200 μL tip box that you must place a tip box in position 1 on the deck available. You will need to “reload” tip boxes. The same goes for the plate type you want to test. If the test position for your plate is selected as 5, the appropriate plate type for test should be in that position (Figure 9-87 Setting the Tip Box Position and Plate Position for Calibration).

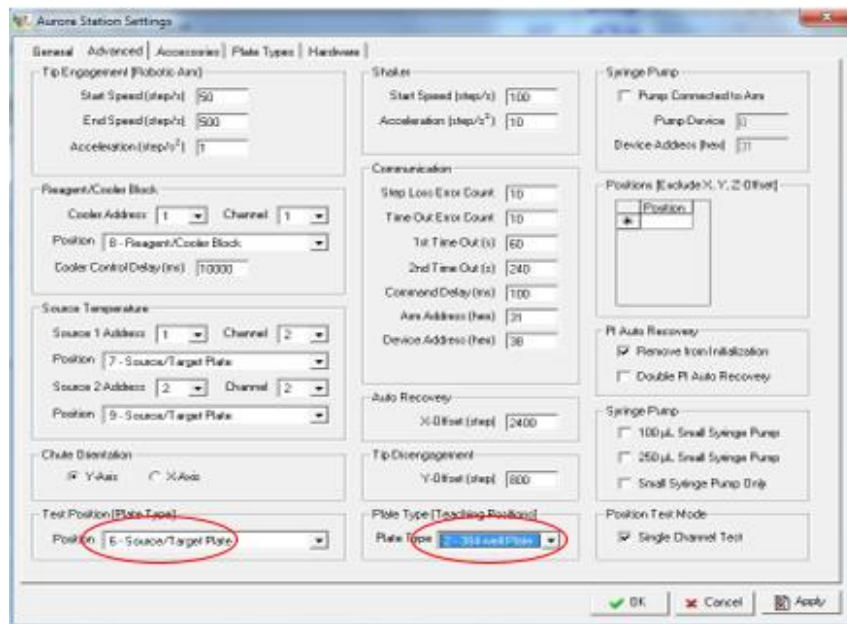


Figure 9-87 Setting the Tip Box Position and Plate Position for Calibration

- Next, ensure that new fields are entered for calibration of LLS for the new plate type, inputting several volumes starting from 0 in increments of 10 μL . Depth will be calculated afterwards. If a new plate type is entered e.g. 46, then enter 46 in the new fields under plate type and for each entry, enter a corresponding volume value in increments of 10 μL .

Liquid Level Sensing

Plate Type	Depth	Volume
45	0	340
47	0	0
47	0	10
47	0	20
47	0	30
47	0	40
47	0	50

Figure 9-88 Entering New Plate Type Numbers with Corresponding Volumes prior to LLS Calibration Test

- Start calibrating with 0 μL , then 10 μL , then 20 μL and so on. Return to the 'plate types' tab (leftmost table) to start the LLS calibration. Highlight the plate type row that you want to test by clicking on that row. The detection range field for that plate type would have to be set if a new plate is entered. To illustrate this, we first explain how to test default surface position:

Step 1: Click on the plate type number to test (note that the all offsets at this point would have been entered by your Aurora representative for a new plate type).

All you need to do is test the default surface position and set the detection range.

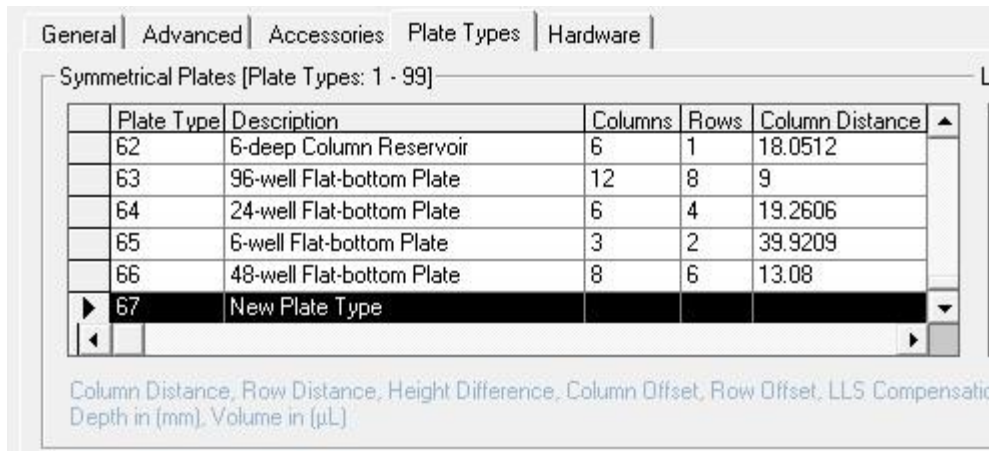


Figure 9-89 Highlight Row of New Plate Type

Step 2: In the rightmost table, scroll up to the corresponding plate type no. and note the depth and volume (in this case 10 μ L). Then test for the unknown depth value along with the detection range. Also test from the lowest volume all the way to the highest volume (or top surface of the well).

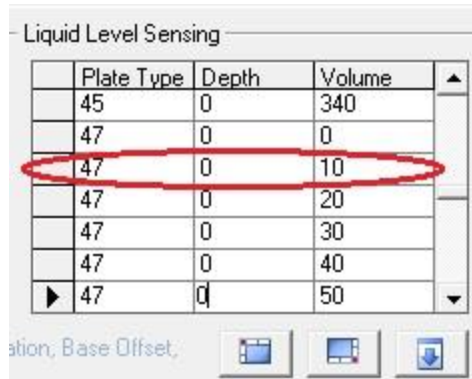


Figure 9-90 Start the Calibration by Highlighting the Row with 10 μ L Volume under Aurora Station Plate Types Tab

Step 3: With a manual pipette, dispense an equal amount of that volume into the first well of the plate to test. If 10 μ L is selected, then there should be 10 μ L of liquid in that well.

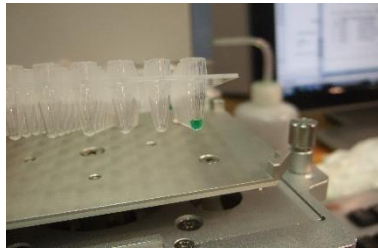


Figure 9-91 LLS Calibration Check with selected New Plate Type

Step 4: Next click “Tip” and the robotic arm will move to pick up a tip from the specified tip position. Once a tip is automatically engaged by the barrel, you can click “test”.

Liquid Level Sensing

	Plate Type	Depth	Volume	
	44	2.7126	1680	▲
	44	0	1860	
	45	21.6515	0	
▶	45	18.2484	10	
	45	17.0154	20	
	45	15.5358	30	
	45	15.0426	40	▼




ation, Base Offset.   

Figure 9-92 Tip Engagement and Surface Test

Notice that after the barrel with tip moves to position itself just above the first well. By default and at all times, the tip would always move to the surface position.



Figure 9-93 Surface Position Achieved

Step 5: Next return to the Aurora Station robotic arm interface and ensure that the surface coordinates are already present in the GoX, GoY, and GoZ fields before clicking the “save” tab to save this surface position into the new row that was originally created. Then click the “save” button.

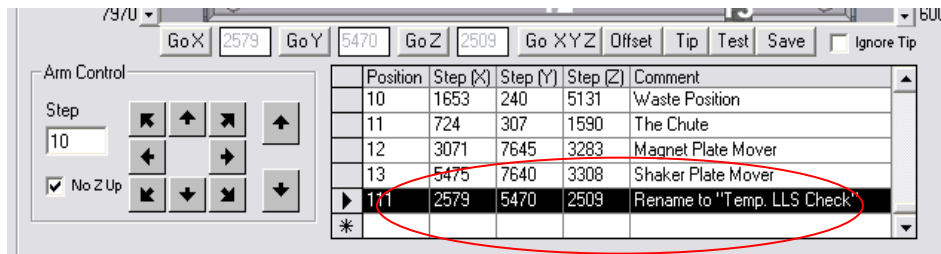


Figure 9-94 Saving Surface Coordinates into Aurora Station Robotic Arm Interface

Step 6: After saving the surface position coordinates, start working from this position for LLS calibration of individual volumes. In the arm control portion of the interface (as in figure above), enter “10” as a value for the step and check off “no Z up”. At this point, there should be 10 μ L of liquid in the well and the barrel with tip should be just above the surface of the well.

Note the signal at this position. Let’s use 142 as an example to illustrate for now. You can note the liquid level sensing signal value on the bottom right corner of the interface next to the date and time.

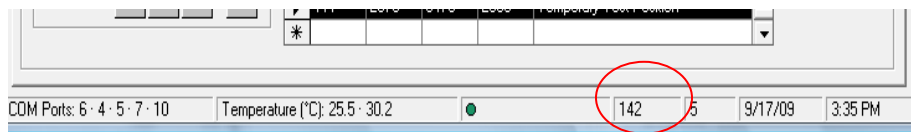


Figure 9-95 Level Sensing Signal check under Aurora Station Robotic Arm Interface

Step 7: After making a record of this value, attempt to move the tip down using the step arrow controls. Move the tip down until the surface of the tip barely touches the surface of the 10 μ L liquid. You will notice the signal increasing as its tip moves down. Note the signal value.

To illustrate this, we’ll use 155 as the signal value when the tip touches the surface of the 10 μ L liquid. If for e.g. the signal is 155 and the surface signal is 142, then the difference is 12 +/- 1 for the detection range (limit). Click on the “plate types” tab and enter the detection range as 12 or 13 in the detection range field.

Click “apply” and then “ok” to save the new setting. Then to check the depth offset at the surface of 10uL, first single clicking on the newly entered field in Aurora Station after the visual inspection of the tip touching the 10uL surface is achieved (Figure 9-98 Check (Offset Calculator) and Enter New Depth Offset for 10uL Volume). The new coordinates will automatically be present in the GoX, GoY, and GoZ fields. Then click on the “offset” tab to view the offset field value (in mm). This value should be entered in the depth field for volume 10 in the rightmost table of the “Plate Types” tab. To perform this, copy the new offset

(using the offset tab calculator) and paste them into the depth field in the “Plate Types” tab rightmost table.

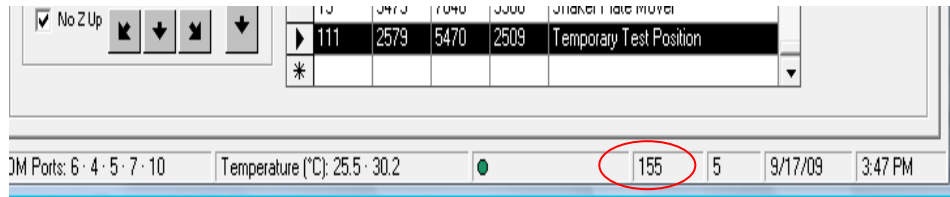


Figure 9-96 Signal at 10uL Surface Noted under Aurora Station Plate Types Tab

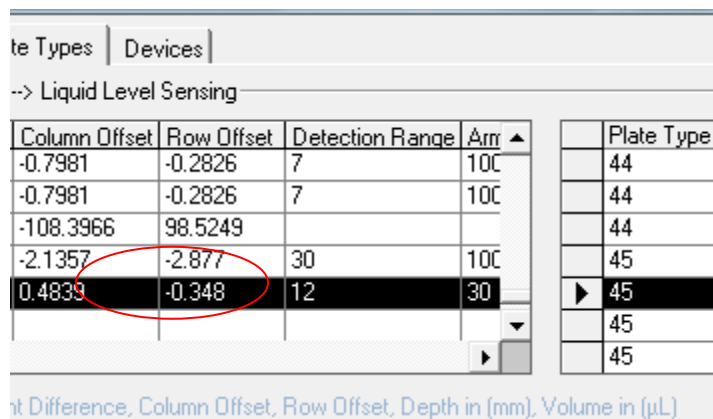


Figure 9-97 Enter Signal Difference under Aurora Station Plate Types Tab Detection Range Field

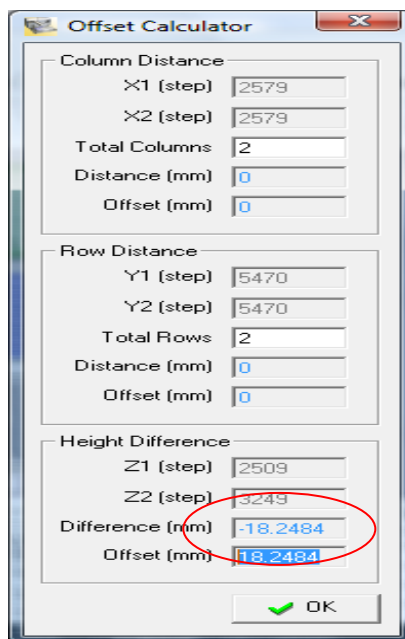


Figure 9-98 Check (Offset Calculator) and Enter New Depth Offset for 10uL Volume

Liquid Level Sensing

	Plate Type	Depth	Volume
	47	0	0
▶	47	18.2484	10
	47	0	20
	47	0	30
	47	0	40
	47	0	50
	47	0	60

tion, Base Offset.




Figure 9-99 Check (Offset Calculator) and Enter New Depth Offset for 10uL Volume

- Step 8: Next check the minimum level sensing at “0” depth for approx. 1mm from the very bottom of the well. Move the tip down further with the step arrow controls till the tip surface barely touches the bottom of the well. At this point, the signal value should be similar to the surface signal value for 10 μ L (as a guide).
- Step 9: Once that is achieved, highlight the whole field teach position in Aurora Station after the visual inspection of tip touching approx. 1mm from the bottom of the well is achieved. The new coordinates would then automatically be present in the GoX, GoY, and GoZ fields. Next, click on the offset tab to view the offset field value (in mm). This value should be entered in the depth field for volume 0 in the rightmost table of the “Plate Types” tab. To perform this, copy the new offset (fr. offset tab calculator) and paste into the depth field in the “Plate Types” tab rightmost table.

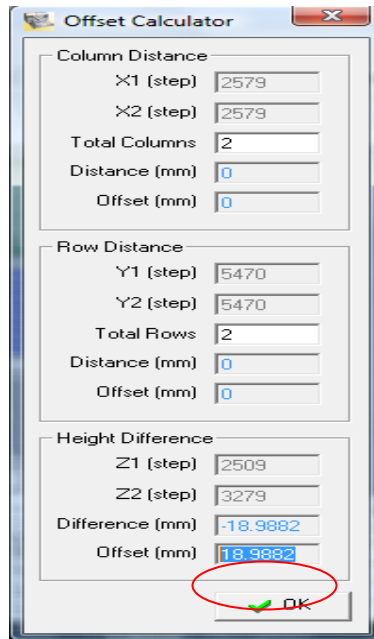


Figure 9-100 Check (Offset Calculator) and Enter New Depth Offset for 0uL Volume

Liquid Level Sensing

Plate Type	Depth	Volume
45	0	340
47	18.9882	10
47	18.2484	20
47	0	30
47	0	40
47	0	50
47	0	60

ation, Base Offset.

Figure 9-101 Check (Offset Calculator) and Enter New Depth Offset for 0uL Volume

- Step 10: Perform the same steps above to check and input depth value for 20uL. Remember to manually pipette an additional 10uL to the same well of the plate first before moving the tip down again for the surface level depth check for 20uL.
- Step 11: Subsequently check and input the depth value for 30uL using the above steps. Continue adding in 10uL increments and perform all the necessary steps for offsets at each until the top of the well is reached. A good rule of thumb would be to perform checks for the volumes used most frequently regarding a specific plate type for your work or research. Remember to have the right amount of liquid in the well when testing for that volume.


Below is the table showing dead volumes for various plate types/vials/tubes/reservoirs etc.

Plate ID	Plate Description	Dead Volume (μL)
1	96-well Plate	34
2	384-well Plate	26
4	12-column Reservoir	910
8	35-vial Holder	141
10	35-tube Holder	57
11	96-deep Round Well Plate (H: 43.57 mm)	44
12	96-well Skirted PCR Plate 1	14
13	384-well PCR Plate	3
14	12-deep Column Reservoir	995
17	4-deep Column Reservoir	1,665
19	48-tube Holder	57
27	48-tube 1.5 mL Holder (Eppendorf)	22
34	48-vial Holder	141
37	192-PCR Tube Block	13
44	4 x 8 Eppendorf 1.5 mL Tube Holder	22
45	96-well Skirted PCR Plate 2	12
47	96-well Non-skirted PCR Plate 3	13
48	96-well Non-skirted PCR Plate 4	12
52	96-deep Square Well Plate (H: 43.91 mm)	43
53	Roche Magpure-32	27
54	96-tube Holder (Micronic)	26
55	48-screwtop 1.25 mL Tube Holder (Sigma)	44
56	96-deep Round Well Plate (H: 31.36 mm)	43
57	96-deep V-bottom Round Well Plate (H: 35.91 mm)	21
58	96-deep Round Well Eppendorf Plate (H: 43.95 mm)	34
59	96-well Skirted PCR Plate 5	13
60	96-well Skirted PCR Plate 6	14
61	96-well Non-skirted PCR Plate 7	14

Plate ID	Plate Description	Dead Volume (µL)
62	6-deep Column Reservoir	1,101
63	96-well Flat-bottom Plate	73
Plate ID	Description	Dead Volume (µL)
101	39-well Reagent/Cooler Block [T1 - T16]	10
101	39-well Reagent/Cooler Block [S1 - S12]	19
101	39-well Reagent/Cooler Block [R1 - R5]	22
101	39-well Reagent/Cooler Block [M1 - M5, W]	150

Figure 9-102 Dead volume for various plate types

9.16. Aurora Station Settings

Click the Aurora Station Settings icon  to configure settings of the system. The figure (*Figure 9-103 Aurora Station General Settings Tab*) below displays the window for the Aurora Station Settings. Browse through the tabs for additional settings, and click “Apply” to implement the setting.

General Settings

The general settings tab can be used to assign deck positions like priming, waste and the tip chute.

NOTE: *These settings are pre-configured and should optimize the performance of your instrument. Contact Aurora Biomed should you need to make some changes. Ensure that you click “Apply” to implement any changes in the settings.*

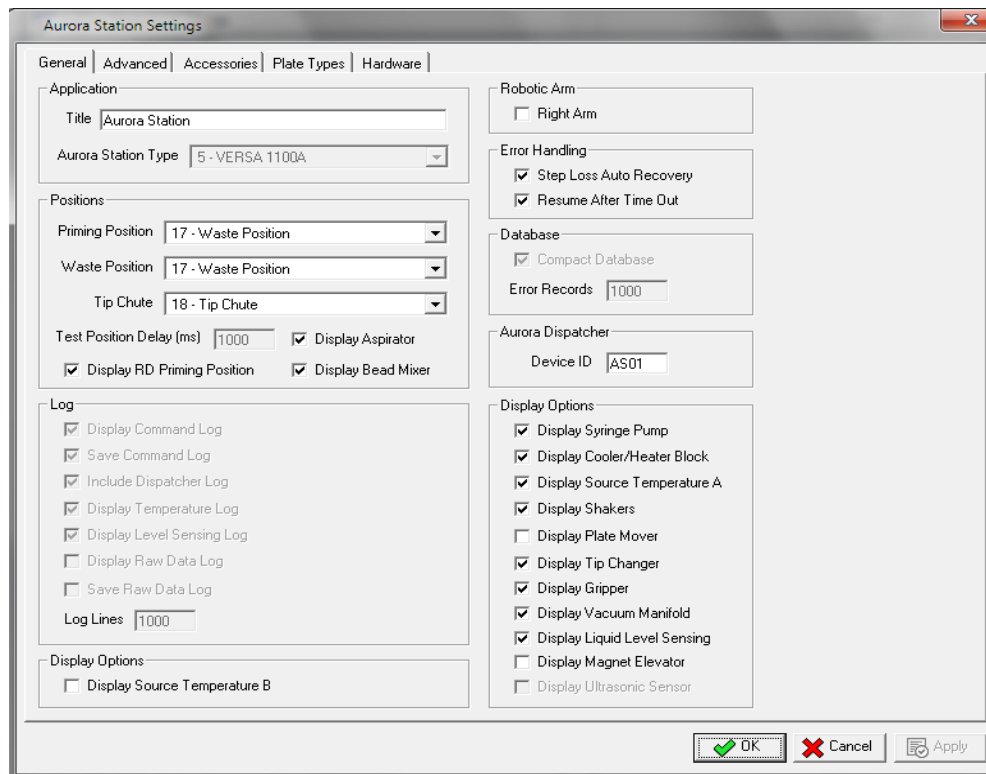


Figure 9-103 Aurora Station General Settings Tab

Advanced Settings

In this tab (*Figure 9-104 Aurora Station Advanced Settings Tab*), changes regarding Tip Engagement (speed), Cooling Block, Shaker (speed), Communication, Auto Recovery, Tip Disengagement, Air Gap adjustment etc. can be performed. However, it is strongly recommended not to change the pre-setting parameters. In most cases, end users only need to use the “Test Position” button to perform testing and offset adjustment for other plate types from plate type tab. Here user will set test position on the deck where any new other plate type is placed to check its position from plate types. Remember to click “Apply” to implement any changes made.

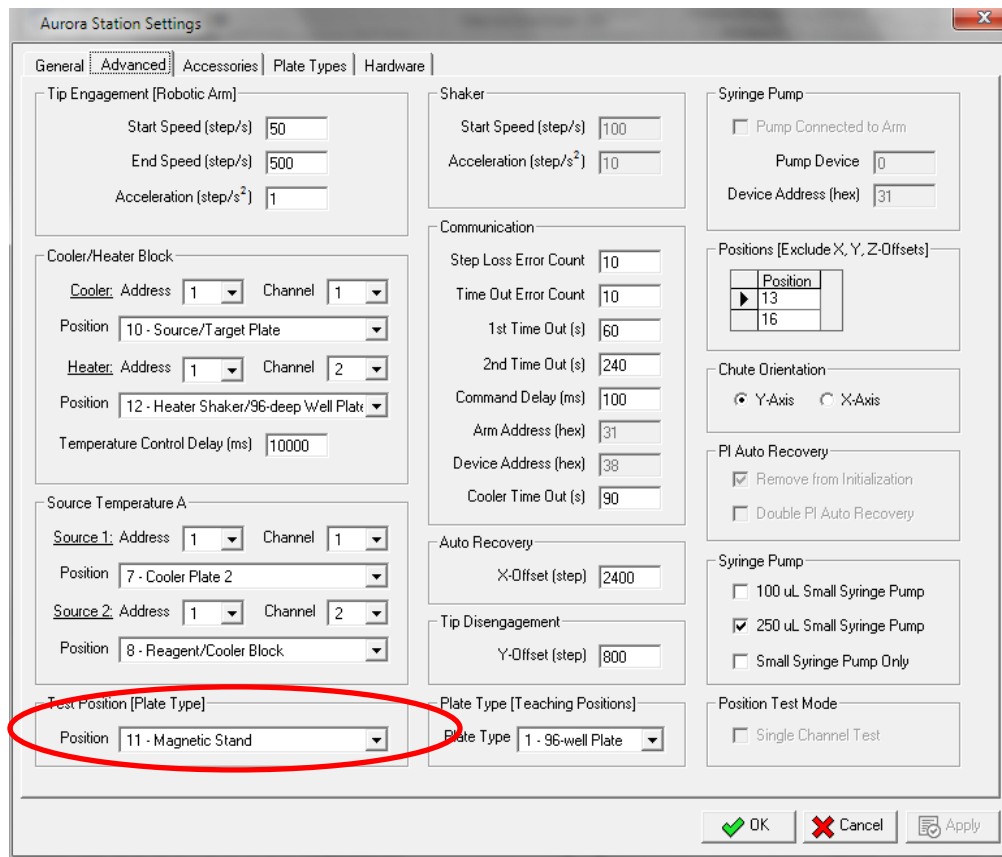


Figure 9-104 Aurora Station Advanced Settings Tab

NOTE: Increased robotic arm speed and acceleration may result in step loss or arm failure.

Accessory Settings

The Tip Box positions, record the used tips and remaining tips is in the “Tip Box Positions” box as shown below. In ‘Pipetting Tip Types’ table, tip parameters are recorded such as engagement offset, depth (how deep barrel goes into the tip to engage it, tip length, and the X/Y offsets for different tip types.

Pin Groups and Shaker configurations are tabulated as shown in below (Figure 9-105 Aurora Station Accessories Settings Tab). Depending on the configuration of the system and the modules in the workstation, some of these options may not apply. These settings come pre-configured for your specific applications. Check with Aurora Biomed for application specific development.

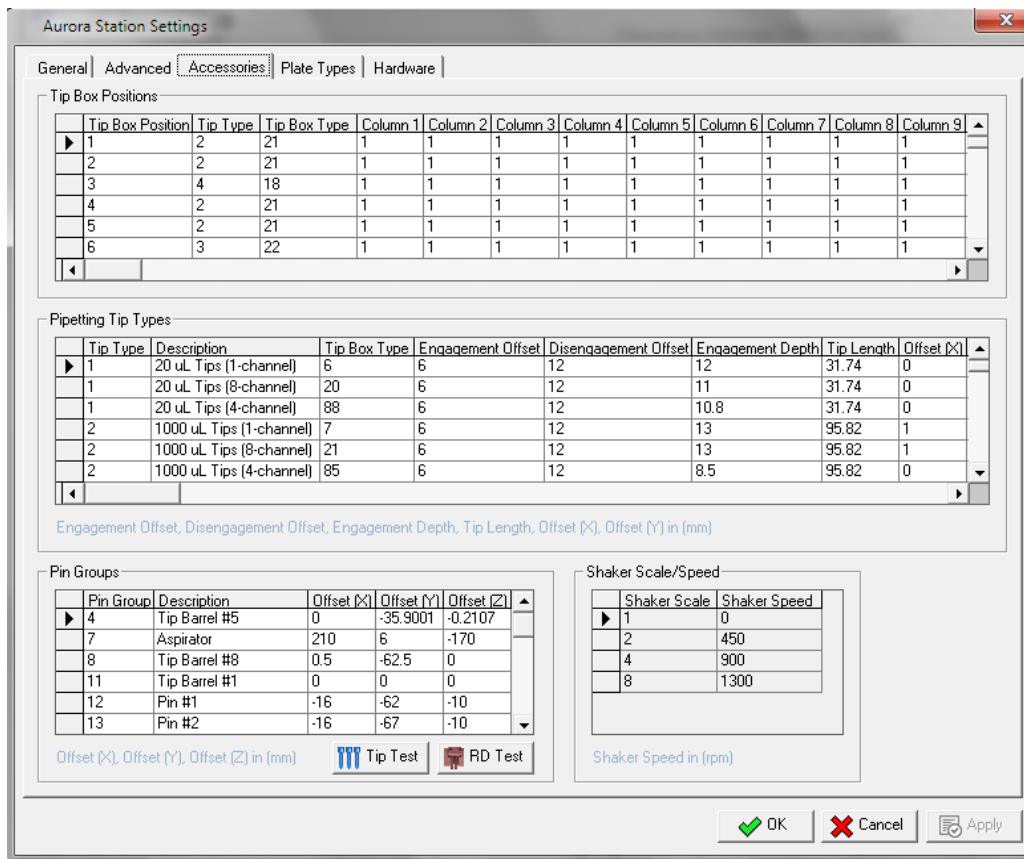


Figure 9-105 Aurora Station Accessories Settings Tab

Plate Types Settings

All Plate Type configurations are tabulated in the ‘Plate Types Settings’ Tab as shown above. Depending on the configuration of the system and the modules in the workstation, some of these options may not apply.

When entering a new plate, enter all relevant information in the symmetrical plates section. Base the offset of the new plate on the 96 deep-well plate. Accurately enter the offset difference (as compared with the 96-well plate) allows the workstation to adjust accordingly regardless of the differing depth of the plates on the deck.

Start with measuring the height difference between your new plate against a standard 96-well plate and enter the difference in mm into the field. Next calculate for X and Y offsets by placing the new plate onto the deck; and using Device Manager AS to move the arm. Figure out the offset distance with the step arrow control or offset calculator of the syringe barrel to the top surface of the new plate.

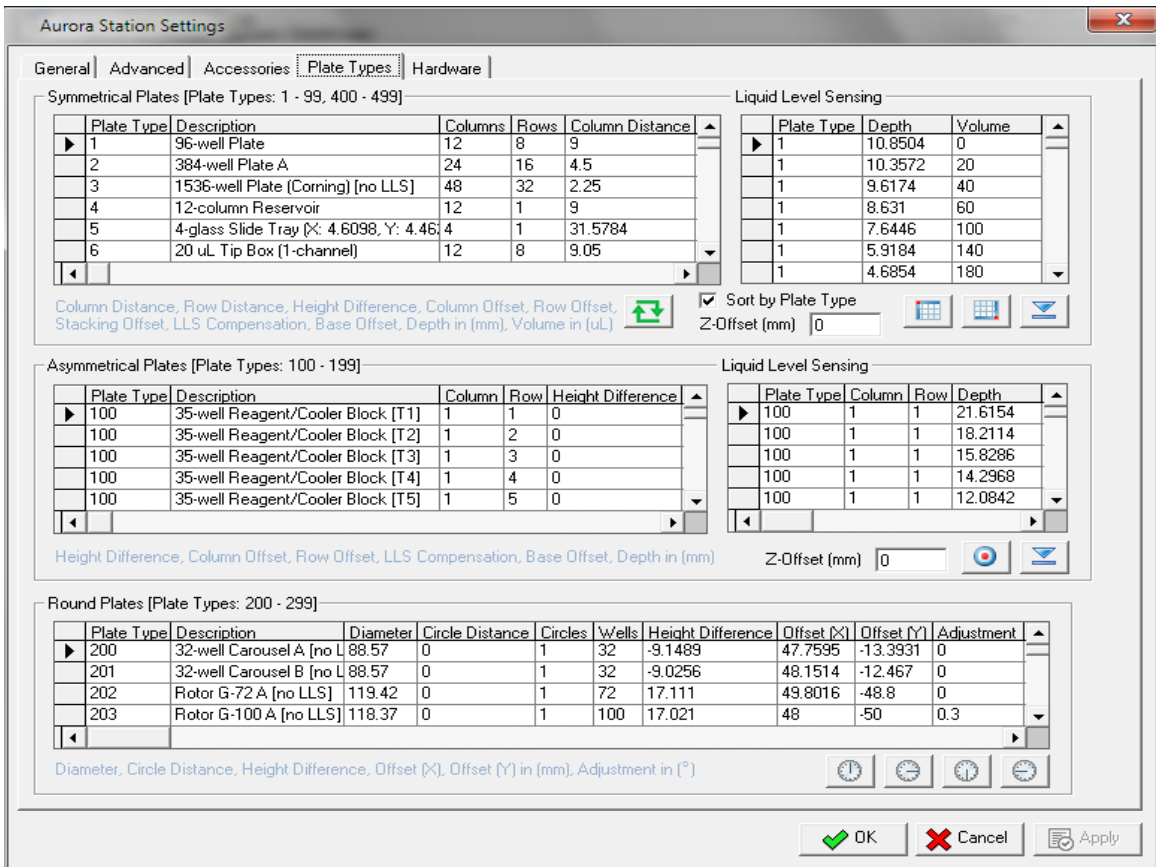


Figure 9-106 Aurora Station Plate Types Settings Tab

NOTE: The settings in your software come pre-configured for your specific applications. Check with Aurora Biomed for application specific development

Hardware Settings

In this window (Figure 9-107 Aurora Station Hardware Settings Tab), the devices attached to the workstation along with the associated COM ports are configured. Speed and acceleration steps of the robotic arm and pump can also be configured. Maximum moving range of the Robotic Arm has already been set and configured. All adaptors used for different PCR plates, magnetic blocks etc. are configured here under Plate/Tip Box Adaptors. Click “Apply” to implement any changes made in this window.

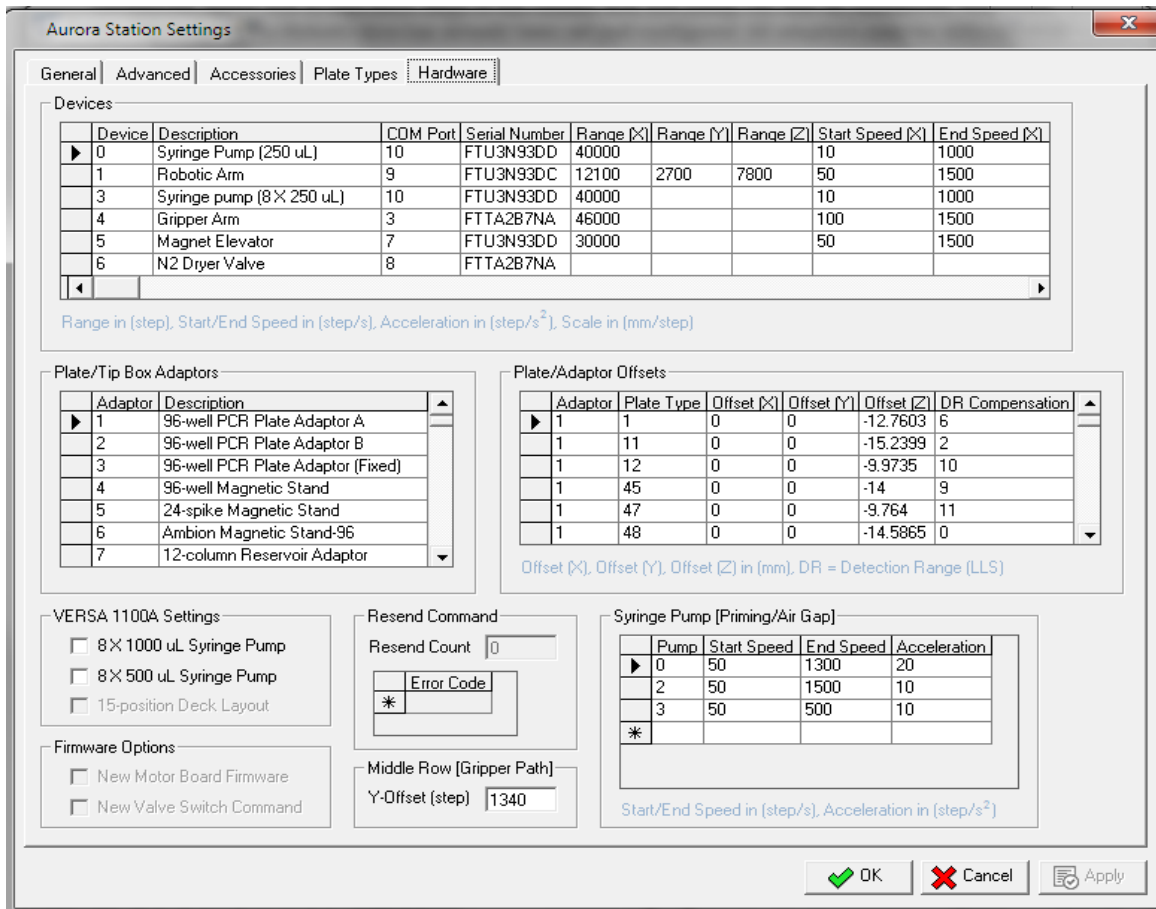


Figure 9-107 Aurora Station Hardware Settings Tab

10.VERSA 1100 Main Interface

1. Menu drop-downs
2. Short cuts
3. Deck configuration
4. Assay/Sequence folder window
5. Application Tabs
6. Sequence Steps window
7. Single channel or 8-channel mode change icon
8. Lab ware Library
9. Tip Transfer for single channel boxes

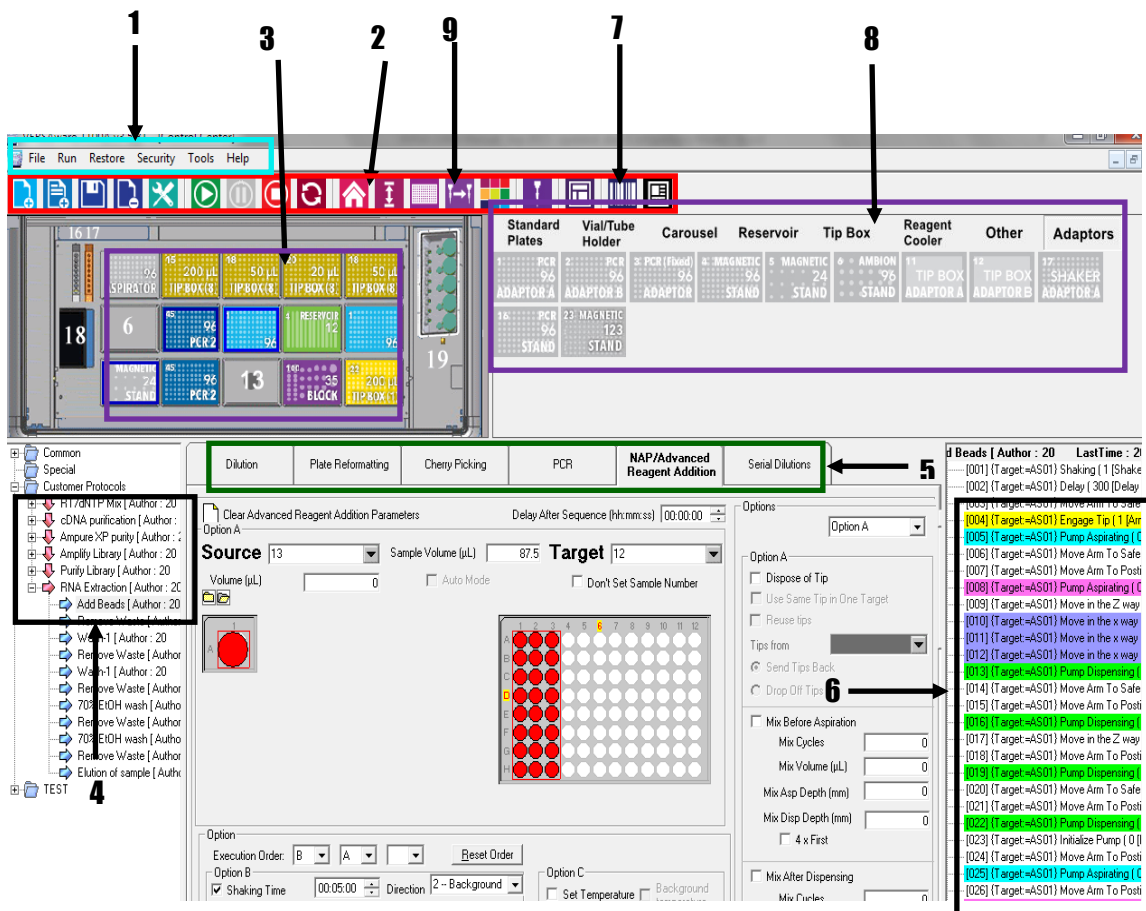


Figure 10-1 VERSAWare Main Interface

Menu Drop-downs – consists of the File, Run, Tools and Help functions.

Icon Shortcuts – provides direct functions like adding, deleting and saving assay/sequence; starting, pausing and stopping assay/sequence runs; and reloading tips

Deck configuration – configuring the deck for use with 96, 384 well plates and reservoir

Saved Assay/Sequence – Lists all the assay/sequence created which can be accessed and used anytime when required

Application Tabs – consists of the applications Dilution, Plate Reformatting, Pooling Application, Cherry Picking, PCR and Advanced Reagent Addition

Sequence Steps – provides the series of commands when running an assay sequence file

Tips Channels Number – Selects Single-Channel or 8-Channel Head for use in the application Tabs.

Lab-ware Library – Lists of Plate types, Tip Boxes, Adaptors and Vail/tube Holders.

Tip Transfer – Helps user to transfer tips with robotic arm from 8-channel tip box to single channel tip box.

10.1. VERSAware Drag-Drop Plate Library

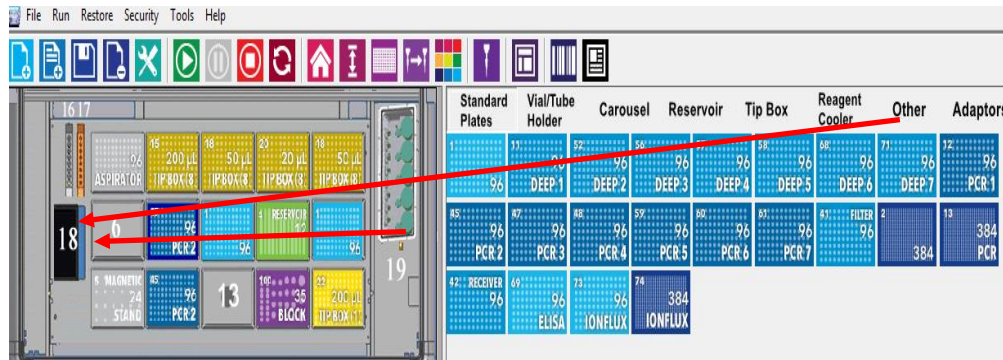


Figure 10-2 VERSAware Deck Configuration and Plate Library

Aurora has accounted for a wide array of plate types found in the market. These have been configured for use on the VERSA platform to allow users to aspirate from and dispense into. Offsets have also been pre-set during the manufacturing and testing process, thus they are ready to use without any further user intervention needed.

To use a particular plate, move the mouse cursor to that plate’s icon located in the plate library on the right side of the main interface screen, click and drag it to the working deck on the left side of the plate library. Release the left mouse button to drop the icon onto any numbered position (1 to 15) on the deck.

Special plates for e.g. the 96 PCR 1, 96 PCR 2, 96 PCR 3 and 96 PCR 4 need to be placed on top of adaptors to be utilized. To view images of these plates before use, right click the plate in the plate library and a pop-up image of the plate will be displayed (*Figure 10-3 VERSAware Plate Library – 96 PCR 2*).

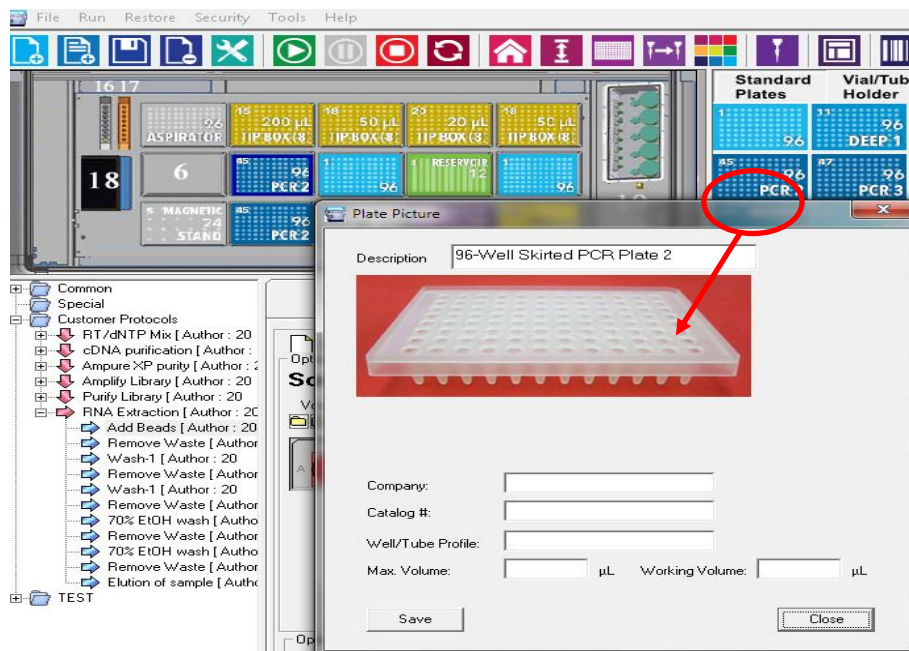


Figure 10-3 VERSAware Plate Library – 96 PCR 2

The above figure depicts the preview picture of 96 PCR 2 plate. We advise using 96 PCR 2, 3 and 4 with adaptors A or B. Please note that the 96 PCR 1 plate can be used directly placed onto the deck without using adaptor. For the use of a plate with an adaptor follow the instructions below:

First select adaptor plate A or B to be used.

Drag and drop one of them (eg. Adaptor B) onto the deck configuration.

Next, select the plate to be uses (e.g. 96 PCR 3) and drag-drop this icon onto adaptor which you previously dropped onto the deck.

When that happens, the 96 PCR 3 will be present for use on the deck and you'd see a colored shadow around the plate. This indicates a plate and adaptor combo as shown in the figure below (*Figure 10-5 VERSAware-Create New Folder*).

VERSAware software has folders, Two folder named as "Common" and "Special" are pre-specified in the software (*Figure 10-4 VERSAWare folders*).

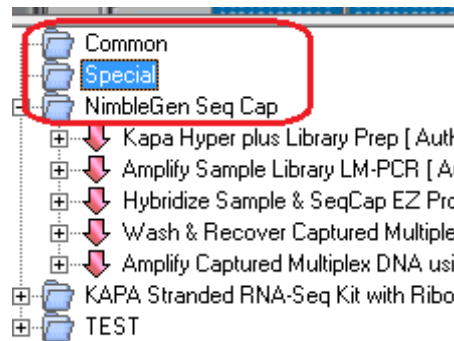


Figure 10-4 VERSAWare folders

VERSAware-pre-specified Folders-common and Special

User can create new folders by right clicking as shown below (*Figure 10-5 VERSAware-Create New Folder*). There are other options too like add message at the end of the sequence (this option must be clicked on from OPTIONS-Other tab), delete, copy, cut/paste any assay or sequence. Sample number can also be in an assay. Assays/sequences can be renamed, imported and saved.

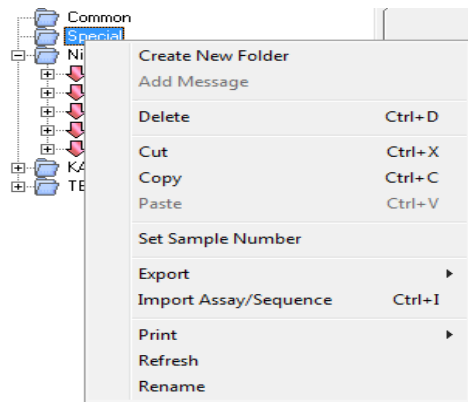



Figure 10-5 VERSAware-Create New Folder

Assays are created under the folder from this  of the tool bar as shown below:

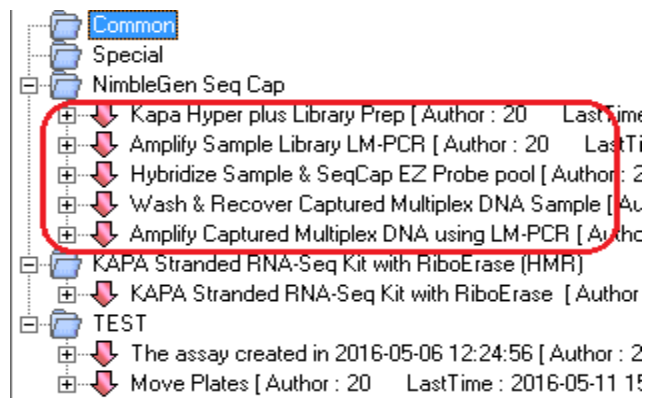



Figure 10-6 VERSAware-Create New Assay

Sequences are created from this  icon of the tool bar and saved under Assays to create all the liquid handling steps as shown below:

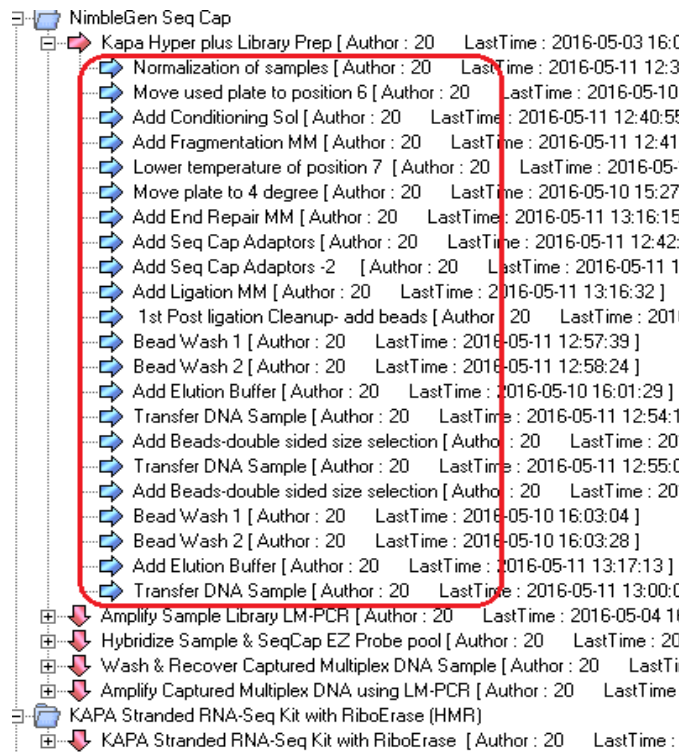




Figure 10-7 VERSAware- Create Sequences/write protocols


There are a total of 17 user icons available for “one-click” operation(s) which will be further explained below:




Figure 10-8 VERSAware Icon Tool Bar


<Click> on the New Assay icon  to add a new assay. Enter new assay’s name and click “ok”. The user can add any number of sequences


After creating a sequence under any of the application tabs, click on the add Sequence icon  to add it as a new sequence. Sequences created can be saved to any existing assay by selecting/ highlighting the assay on the main interface screen.


User can also make changes to any existing sequence and save over it by clicking on the save icon . Click to open any sequence you want to change, make the changes and update the sequence using the save sequence icon. It is important to update the new changes by clicking on this icon, otherwise the changes will not take effect. This icon is also used to update or apply


new changes made to any sequence – be it a Z-offset setting change or any changes made in the options tab.



Click on this icon  to delete a selected assay/ sequence. Selected assay/ sequence is deleted by first highlighting it and then clicking on this icon.


Click on this icon  to enter VERSAware options (ref. VERSAware Options Section). User can make changes to various settings related to the workstation.


Click on this icon  to start/ run any selected assay/ sequence.

Click on this icon  to pause an assay/ sequence. User can pause at any stage of the sequence and unclick to continue the run. Sequence will continue from the where it left off/ paused on. Used when certain changes need to be made or to refill tip boxes.

Click on this icon  to stop an assay/ sequence from running. Sequence will start again from the very beginning if user chooses to run this sequence again.

Click on this icon  to run an assay/ sequence continuously. Assay/ sequence will run continuously until the  icon is “clicked”.

Click on this icon  to send the robotic arm back to the home position

Click on this icon  to reload tip boxes. Used to reload tips when prompted with an audible buzz after robotic arm has picked up its last tip from any box.

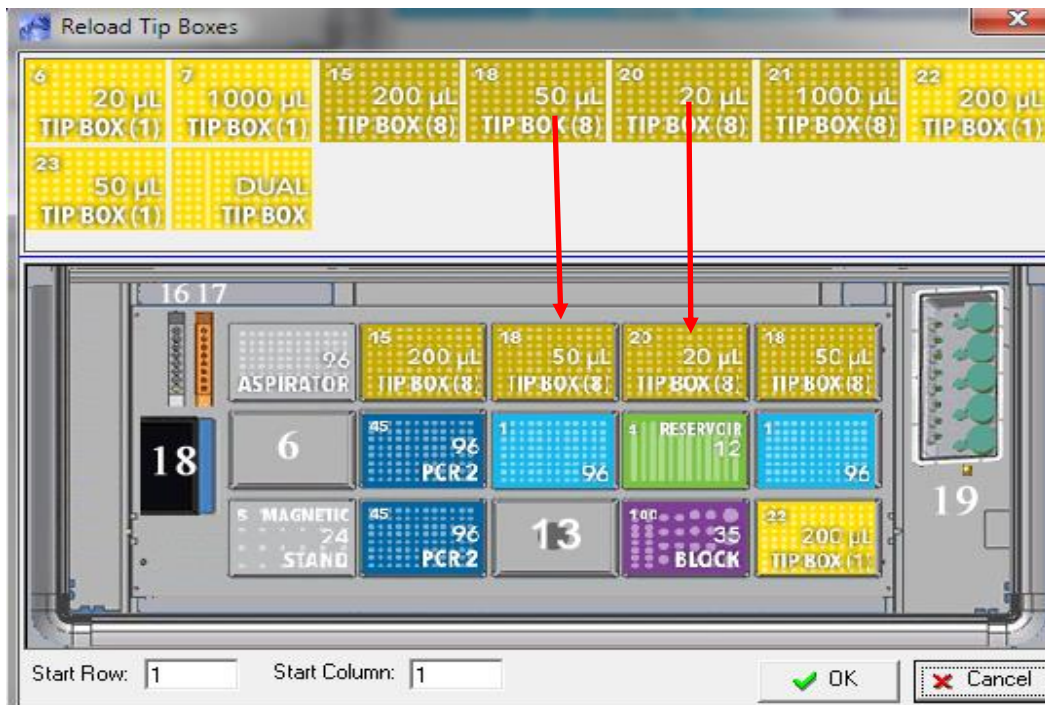



Figure 10-9 VERSAware Reload Tip Boxes Icon Dialog Box

NOTE: Tip boxes have to be dragged and dropped from the library onto the deck to make them available for use.

Click on this icon  to transfer tips from an 8-channel tip box to a single channel tip box operation. The arm proceeds to transfer tips from the 8-channel tip box of specified position (# 2 in the figure below) to the targeted single-channel tip box position (Position 15 in the Figure below) for single channel use (source and target must be specified – 20, 50, 200, 1000 µL Tip (1) and Tip (8). 1 for single channel and 8 for eight channel. Drag source tip box from position 2 to the “Source Tip Box” and Target single channel tip box to “Target Tip Box” as shown by the arrows in the figure below:

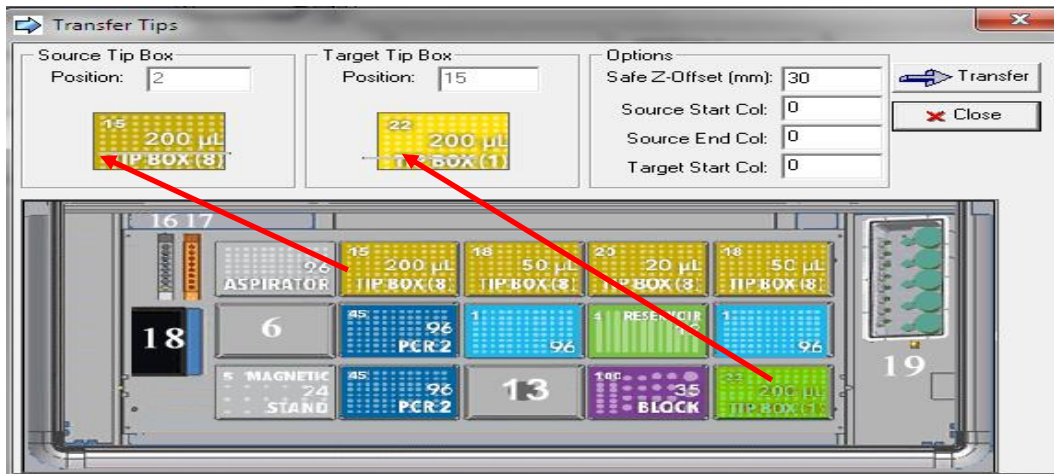



Figure 10-10 VERSAware Tip Transfer for single channel box Dialog Box

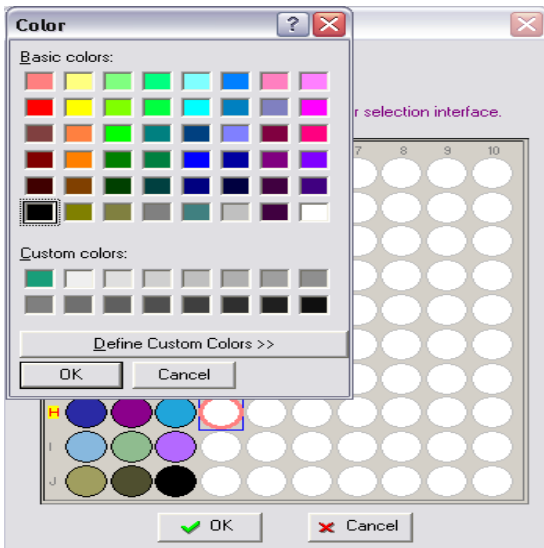
Click on this icon  for the color selector for replicates and Ampligrd application, this icon allows the user to choose or custom choose more colors for use with each well of the slide. A dialog box will open which has several instructions and options for selection. This is to be used when additional colors are needed to code more wells. By default the color selector allows for 30+ colors to be used and displayed.

NOTE: User defined color selector only applies to the Ampligrd application tab. For replicates using PCR and Normalization tab, only 32 colors are allowed by default (color selector does not apply here).





A dialog box will appear that will prompt the user to first select an empty (white) well with the mouse. <Click> to use the palette (ref. below).


Figure 10-11 VERSAware Color Selector Dialog Box



Select from 48 basic colors or define your own by clicking onto the “define custom colors” tab. Create user defined color(s) by adjusting the color range and hue. Click on “add to custom colors” tab, and next click “ok” to insert a new colored well. This will be displayed on the color selector dialog box as shown above. To apply click ok.

Figure 10-12 VERSAware Color Selector Palette

Click on the single-channel  icon to switch to the 8-channel  or single channel mode of the robotic head

Click on the  icon to refresh the VERSAware main interface screen.

10.2. VERSAware 1100 Drop-down Menu

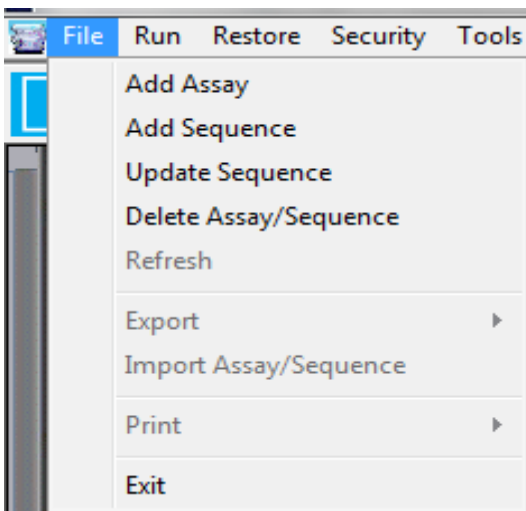


Figure 10-13 VERSAware File Drop-down Menu

File Drop-down Menu

Alternatively, the “file” drop down menu could be used to add an assay, a sequence, to update changes to a sequence or delete an assay or sequence. Users can also export or import assays and sequences to/from different file formats. Click on export to save a file or picture (screen shot) to a text (.txt) or picture (.bmp) file respectively. Users can also choose to print the present screen

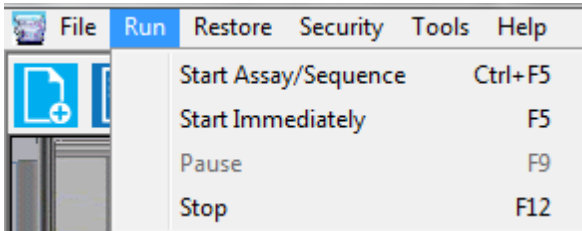


Figure 10-14 VERSAware Run Drop-down Menu

Run Drop-down Menu

Alternatively, the “run” drop down menu could be used to start an assay or sequence, to immediately start a newly created sequence on-screen, and pause or stop any assay/

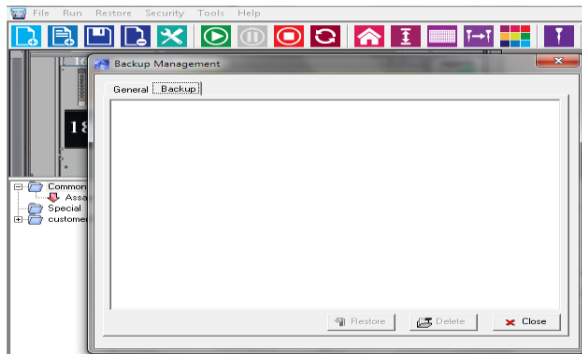


Figure 10-15 VERSAware Restore Drop-down Menu

Restore Drop-down Menu

This icon can be used to restore the deleted sequences which

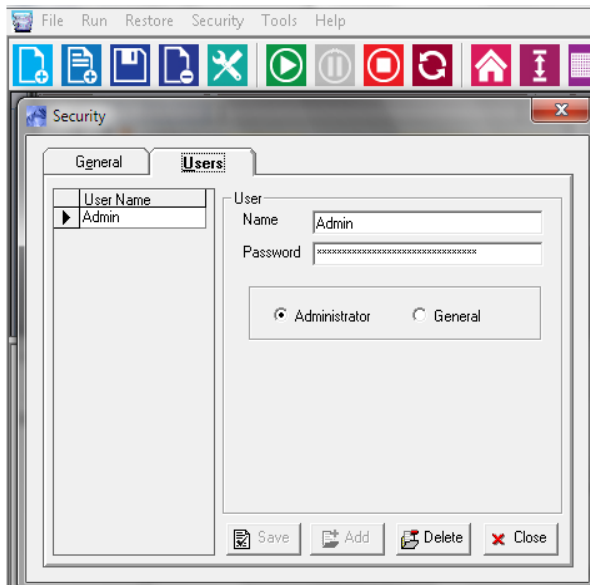


Figure 10-16 VERSAware Security Drop-down Menu

Security Drop-down Menu

Alternatively, the “Security” drop down menu could be used to set the user ID and password for security

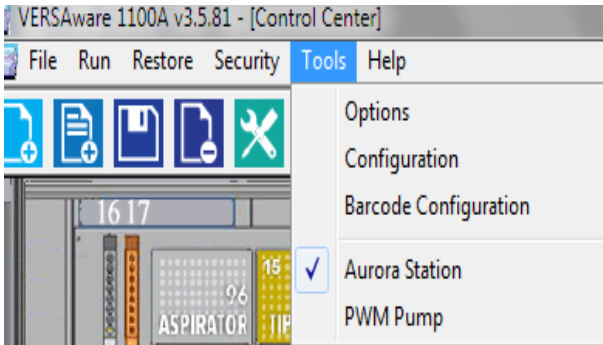


Figure 10-17 VERSAware Tools Drop-down Menu

Tools Drop-down Menu

This menu consists of various options that can be used to edit various system settings and open other sub-menu or interfaces like Device Manager AS, Clicking configuration will bring up another window.

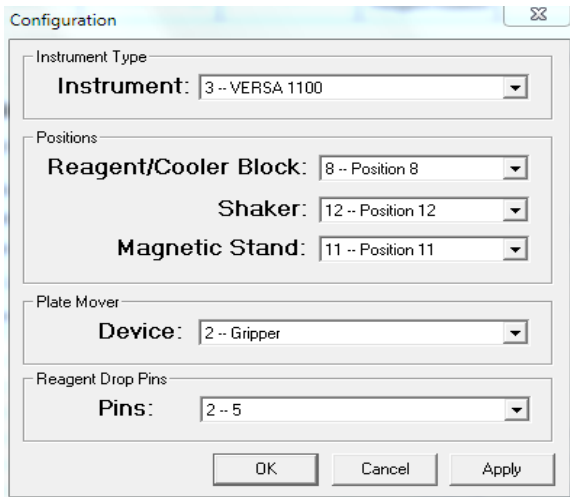


Figure 10-18 VERSAware Tools Drop-down Menu-Configuration

Configuration Window

Instrument type: Set to VERSA1100 NAP by default.

Positions: Used to set or change the reagent block, shaker and magnetic stand position on the deck. At present the default is position 8, 12 and 11 respectively.

Other devices like the plate gripper has already been set and is the default position for that device.

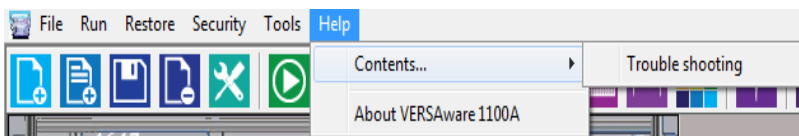



Figure 10-19 VERSAware Help Drop-down Menu

Help Drop-down Menu

This menu provides VERSA1100 trouble shooting details and software version type for VERSA Appl.1100

10.3. VERSAware 1100 Options



The VERSAware options can be accessed by clicking on the  icon or by alternatively clicking on the drop down menu tools>options.

Here, users can edit or change various system/ instrument/ plate type settings according to their requirements. If clicking on the options for any sequence. The new changes will only apply for that sequence and not for the whole system or instrument throughout.

NOTE: Changes made here in the “options” apply only to individual sequences if a particular sequence is highlighted prior to “clicking” on the options icon. It is pertinent that you apply and update the changes by clicking on the “apply” button and then proceed to “click” on the “update” icon for changes to take effect.

Syringe Pump Settings

Depending upon the volume range of the user, some units are configured with 1000ul or 500ul 8 channel syringes. Users can also set air gap volumes, mixing air gap volumes, extra aspiration/dispensing volumes for all plate types for pumps (*Figure 10-20 VERSAware Syringe Pump Settings*). This extra aspiration/dispensing back volume settings help to cover the backlash of the pumps and make first and last well volumes correct. These should be set so that at the end there is little bit (1-3ul) volume left in the tip which can be dispensed at the source or at the waste position. Delays after aspiration/dispensing can also be added in the sequence. This is useful in case the solutions are viscous. Syringe aspiration/dispensing speeds for solutions and for an air gap can be set for all the syringe types depending upon the application.

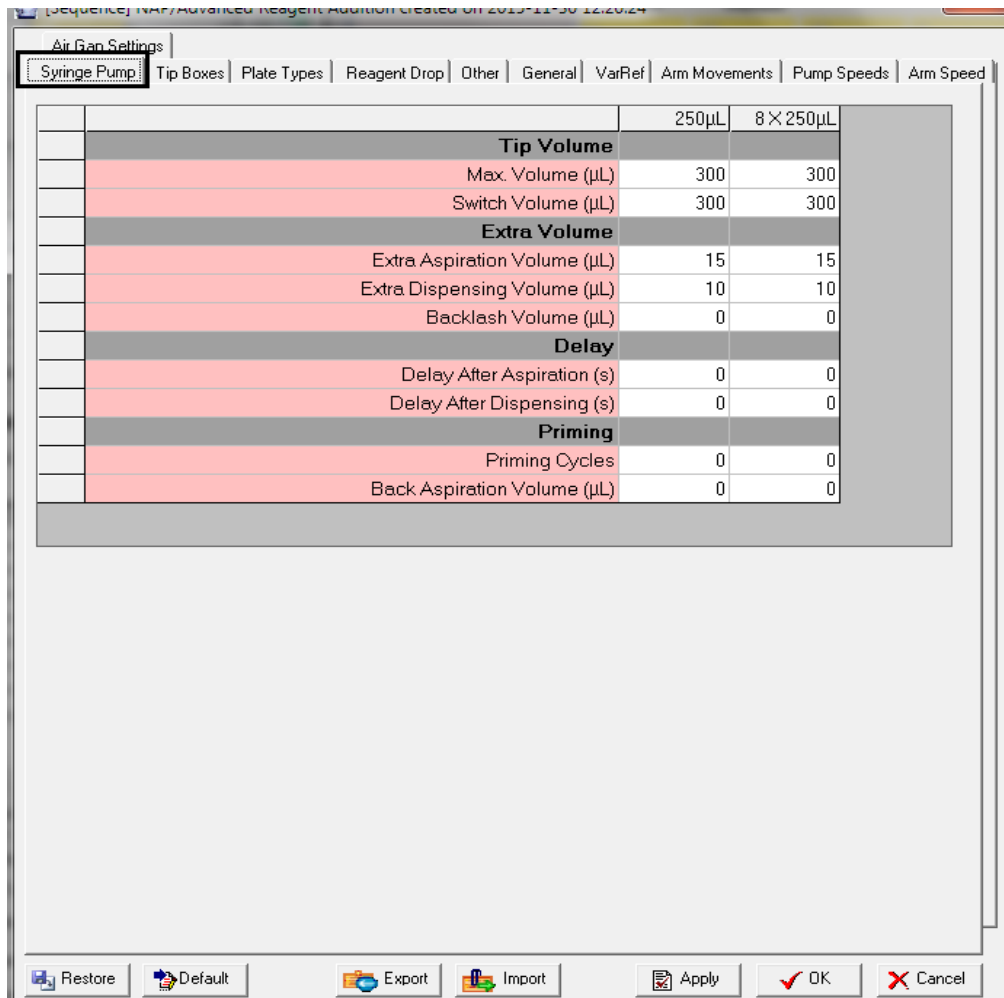


Figure 10-20 VERSAware Syringe Pump Settings

NOTE: The values set here are by default and are specific for each user’s application. No further changes are necessary unless specified by the manufacturer.

Tip Boxes Settings

The instrument comes pre-programmed with safe Z-setting Offsets for safe arm movement above the tip boxes to avoid hitting it. The values here are set as default data. No change is necessary. Maximum volume which can be aspirated/dispensed for each tip type can also be set here. There is provision to add different types of tips in single channel tip box as shown below. First six columns are loaded with 200ul tips and 7-12 columns are loaded with 50ul tips. Depending on the volume used in the sequence, tips will be taken from the single channel tip box (Figure 10-21 Tip Boxes Settings).

As an example, if a tip dispenses liquid too far from the bottom of the well of a 96-well plate, increase the dispensing Z-Offset by increments of 1 or 2 mm first, apply and then update the sequence; and run again to see if tip goes further into the well to dispense.

The same applies for aspiration. For increased accuracy, measure the height from the tip's end to the bottom of the well measured in mm. Make provisions for the required Z-Offset increase in mm.

Likewise, Reagent Cooler Block (columns 1-7) Z-off settings are also found here. These can be changed for each sequence depending upon the volumes in these tubes. The Arm movement Z-offset (mm) helps to move the arm at a specified Z movement within that plate type.

For example, if movement Z-offset is set at 5mm instead of normal setting of 15mm, then arm will move at lower position with respect to the plate. It will save some time during multiple dispensing.

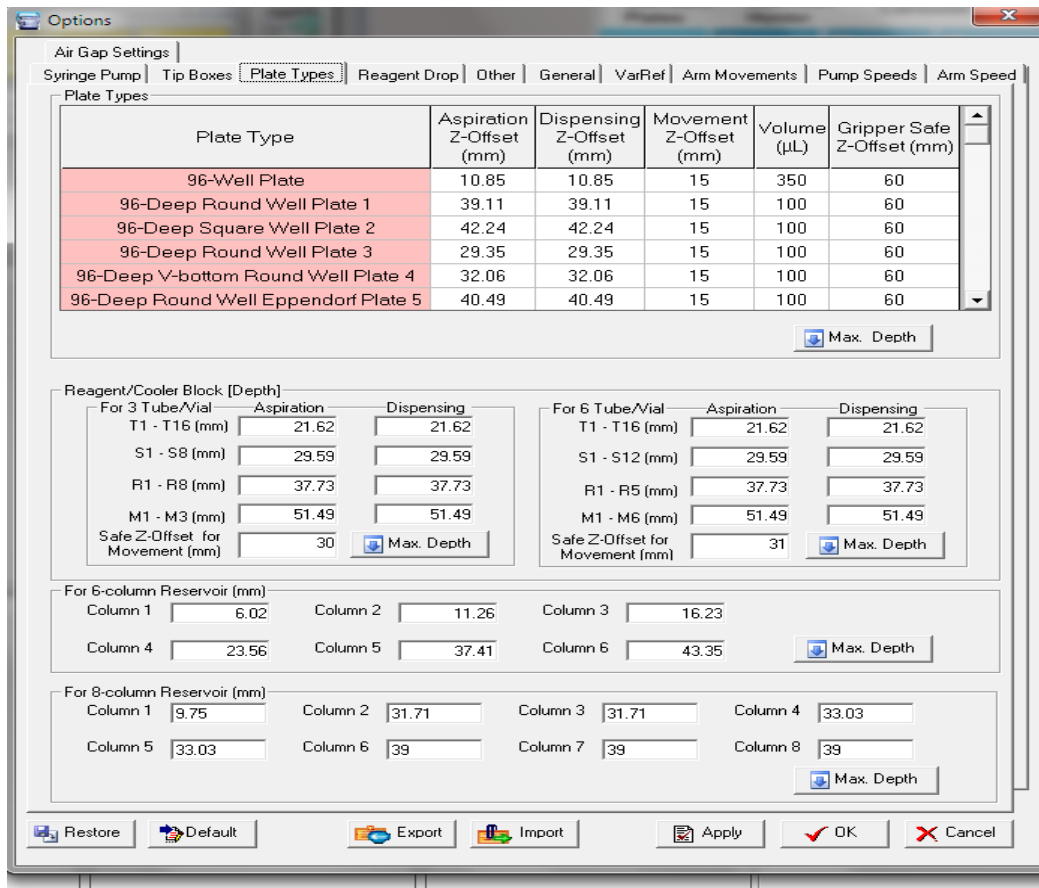


Figure 10-22 VERSAWARE Plate Types Settings

NOTE: The values set here are by default and are specific for each user's application. No further changes are necessary unless specified by the manufacturer or if you experience an insufficient dispense or aspiration height Z-Offset.

Reagent Drop Settings

Priming volume for the 5 channel reagent drop module can be changed along with its dispensing Z-offset. Increase or decrease if the dispensing height is too low or too high from the surface of the well. Also, add delay time between wells if required for large volumes (*Figure 10-23 VERSAware Reagent Drop Z-offset settings*).

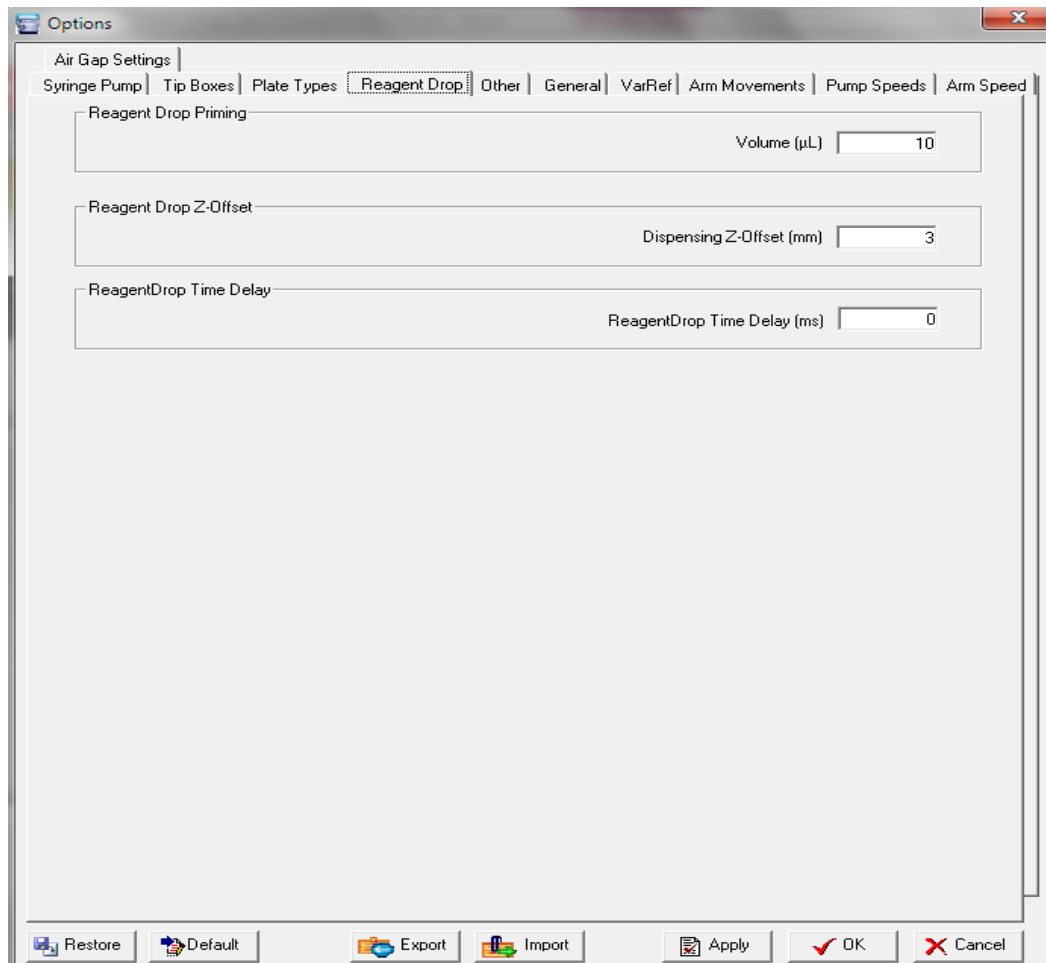


Figure 10-23 VERSAware Reagent Drop Z-offset settings

Other Settings

In other, these options allow the user to set different Z-offsets for mixing, air gap, arm movement from one position to another (*Figure 10-24 VERSAware Other Settings*). These settings if used properly reduce the total timing of an assay.

Also arm speed for dispensing and speeds for handling waste can be set here. In case, the instrument is equipped with Liquid Level Sensing (LLS), it can be activated from here for each sequence. After and before any sequence, arm initializing options are also here. The user can add message after any sequence

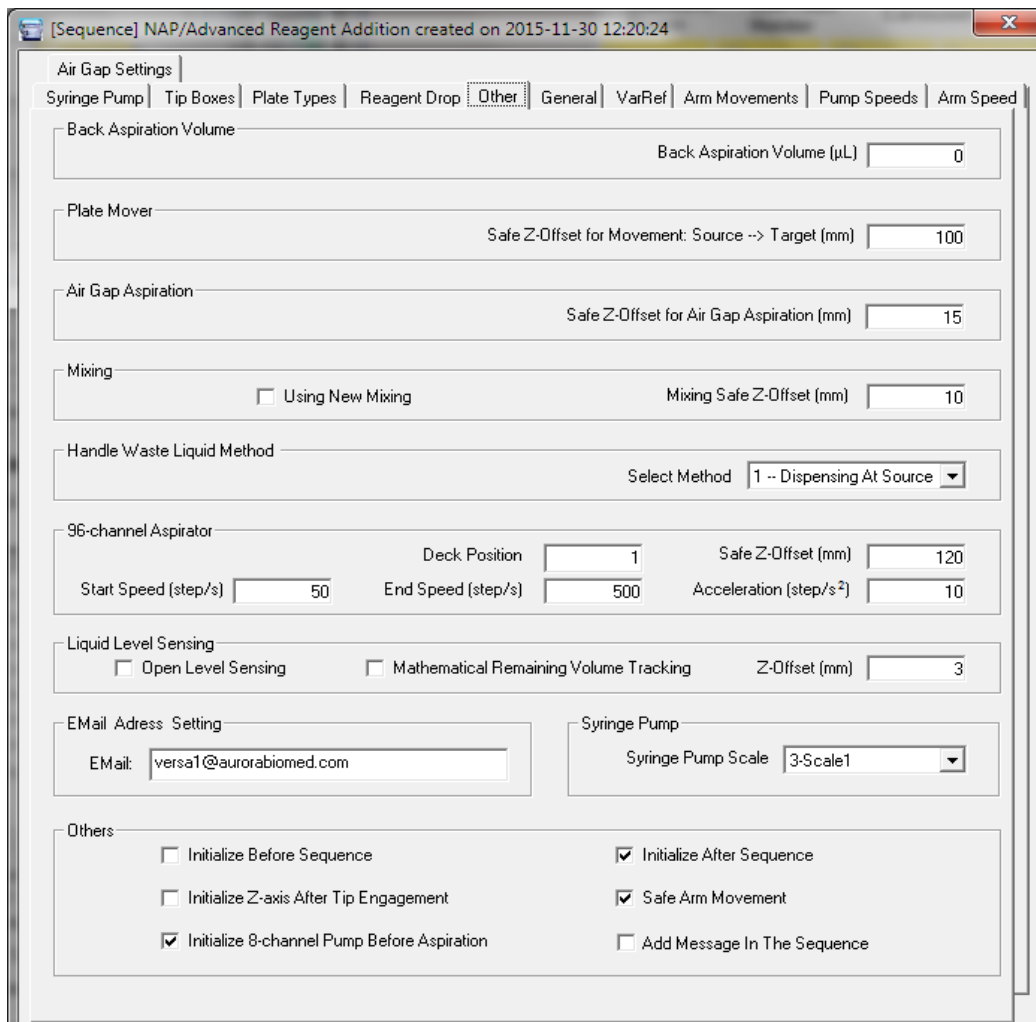


Figure 10-24 VERSAware Other Settings

General Settings

The general settings tab (*Figure 10-25 General Settings*) allows user to hide or to show the application tabs so as not to clutter the VERSAware main interface screen. After the activation of any tab, it will appear on the main interface of the software and after deactivation, it will disappear from the interface. Depending upon the applications, the user can choose show those tabs on the interface.

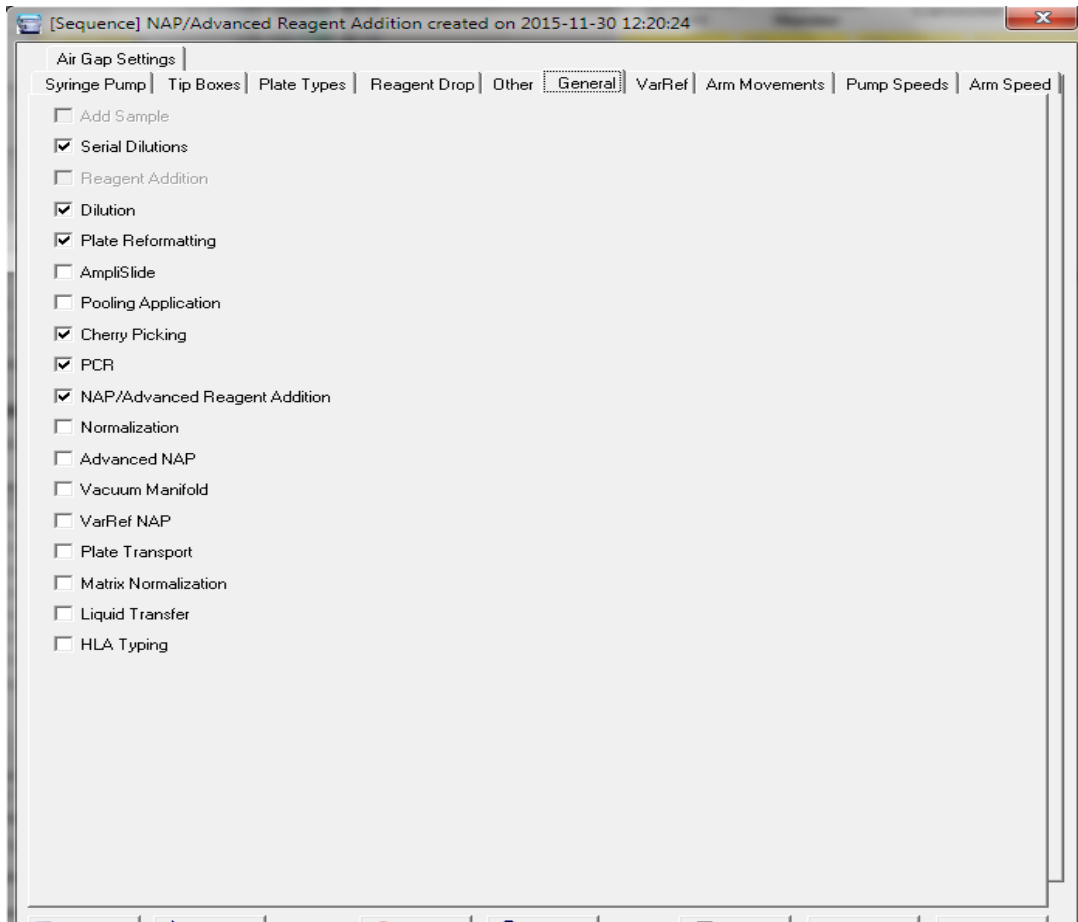


Figure 10-25 General Settings

Arm Movements

Arm movements setting tab allows user to set X/Z way movement after aspiration and after dispensing. These can be set for all the tube types of the reagent cooler block as shown below (*Figure 10-25 General Settings*). These settings help to remove any liquid outside of the tip after aspiration. The arm will move up after aspiration to the specified Z distance like 24mm as shown below in the figure (*Figure 10-26 Arm Movements*) and then move 2mm to right side to touch the side of the well and again move 4mm to left side to touch the left side of the well, move 2mm to the center and move out to dispense the reagent in the target plate.

Likewise X/Y offsets can also be set before aspiration, after aspiration and before dispensing and after dispensing reagent in the wells. These X/Y off sets help to aspirate/dispense reagents at different X/Y positions in a well.

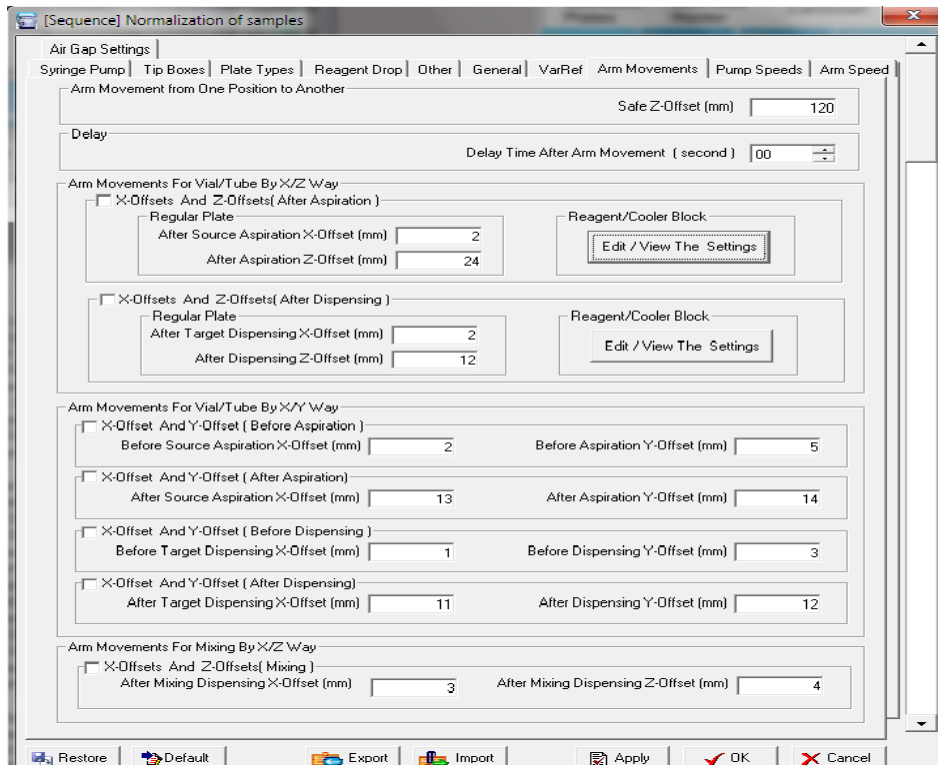


Figure 10-26 Arm Movements

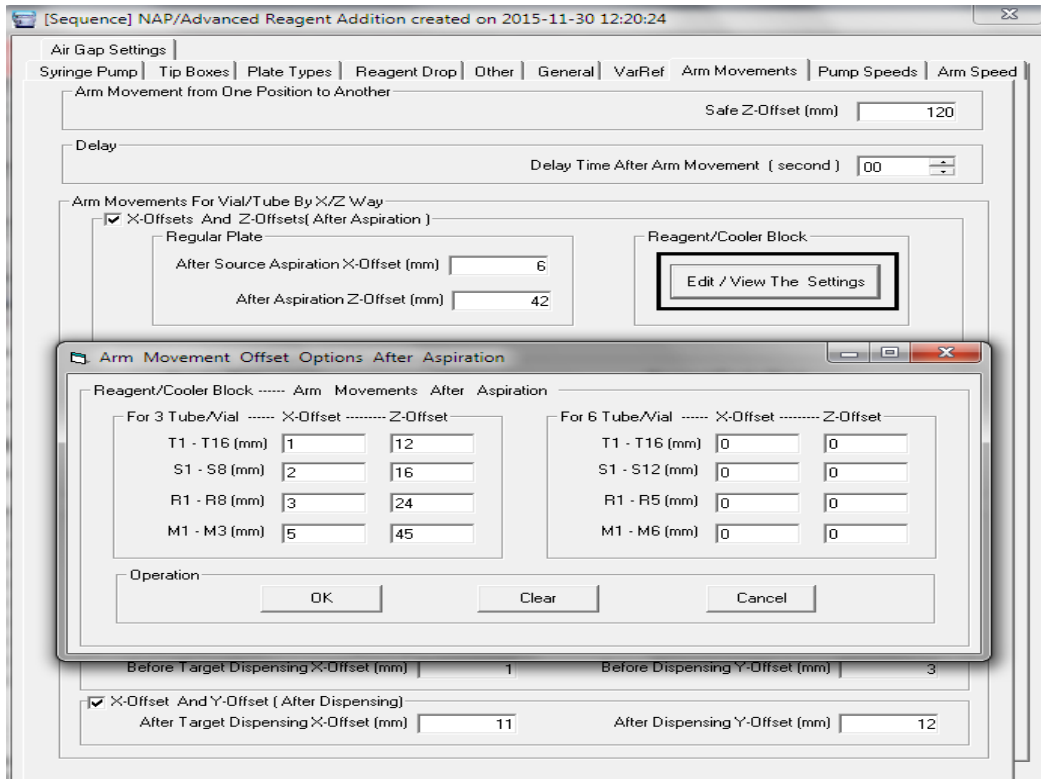


Figure 10-27 Arm Movements-Reagent cooler Block

Pump Speeds

Pump Speed settings tab allows the user to control pump speeds for aspiration and dispensing (Figure 10-28 Pump Speeds). Even the pump speed to take in the air gap can be controlled by the user. Higher/lower speeds can be set to dispense waste at the waste position.

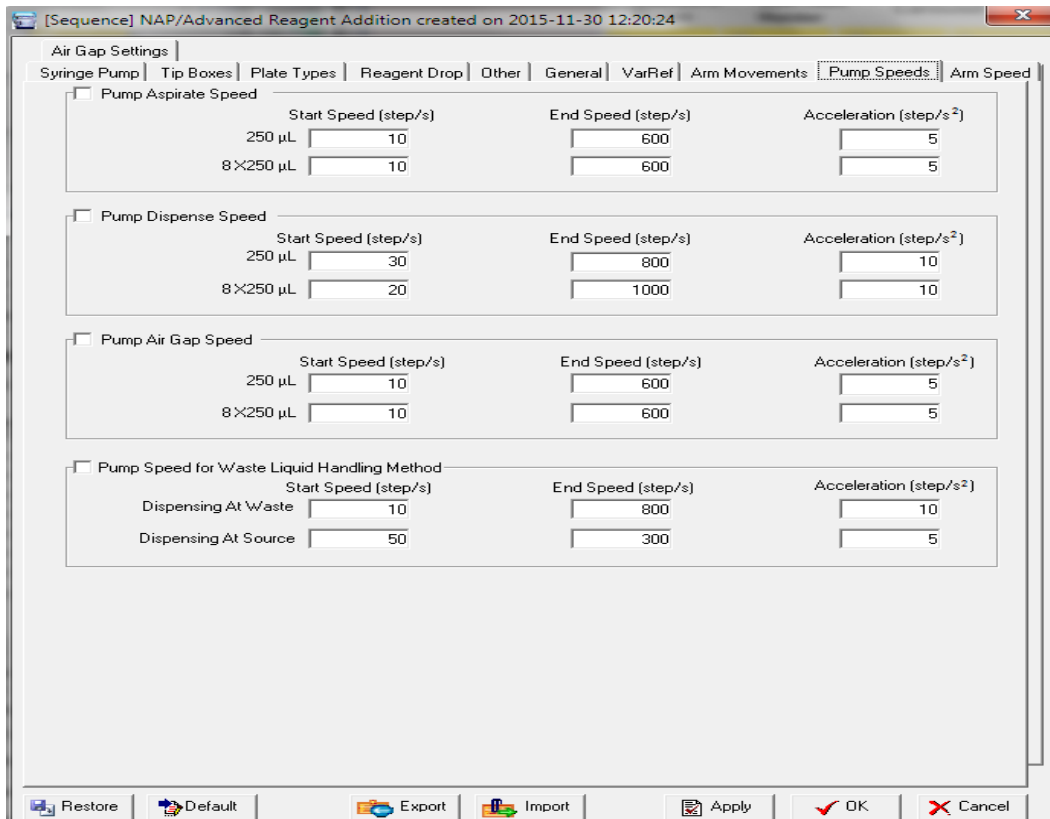


Figure 10-28 Pump Speeds

Arm Speeds

Arm Speed setting tab allows user to control arm speeds after aspiration and dispensing in Z way (Figure 10-29 Arm Speeds).

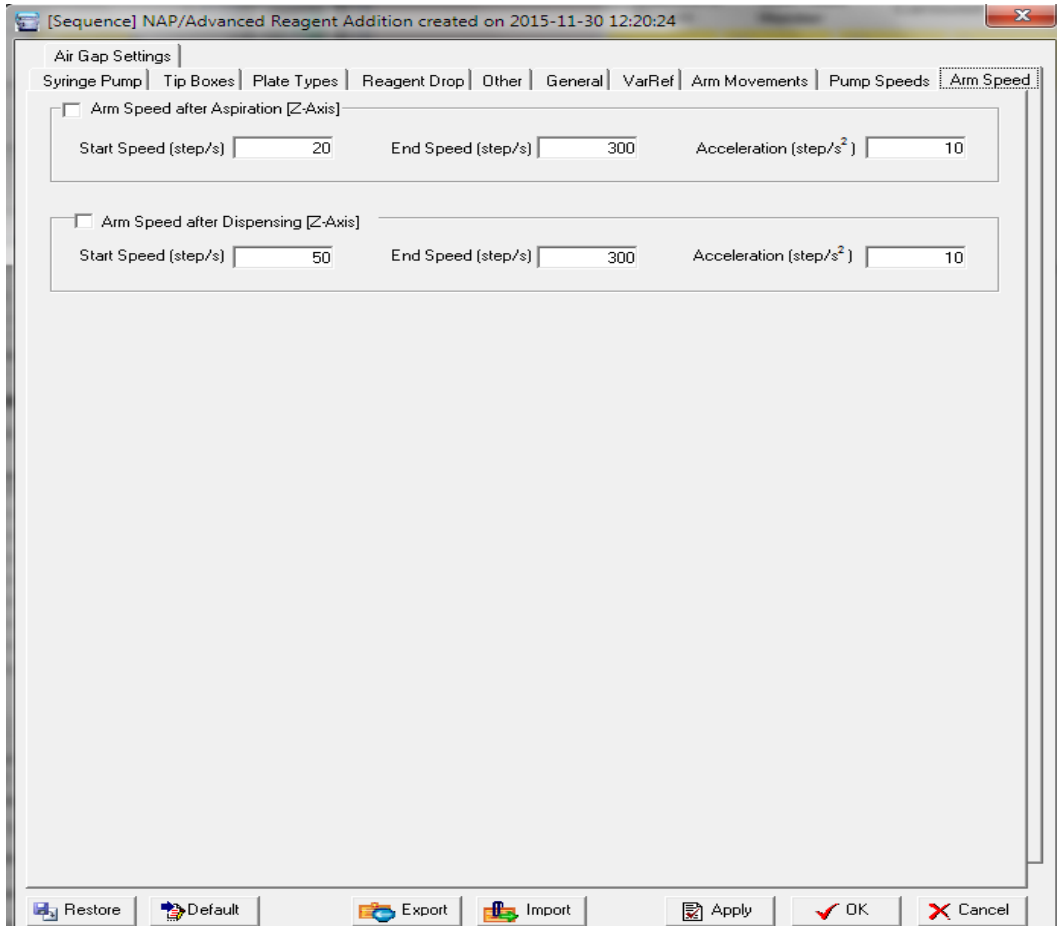


Figure 10-29 Arm Speeds

Air Gap Settings

Air Gap setting tab allows user to set different air gap settings for all the four types of tips available in lab-ware (*Figure 10-30 Air Gap Settings*). There are before aspiration and after dispensing too. Separate air gap settings are there for mixing options too so that user can avoid any bubble formation during mixing—air gap can be reduced for each tip type.

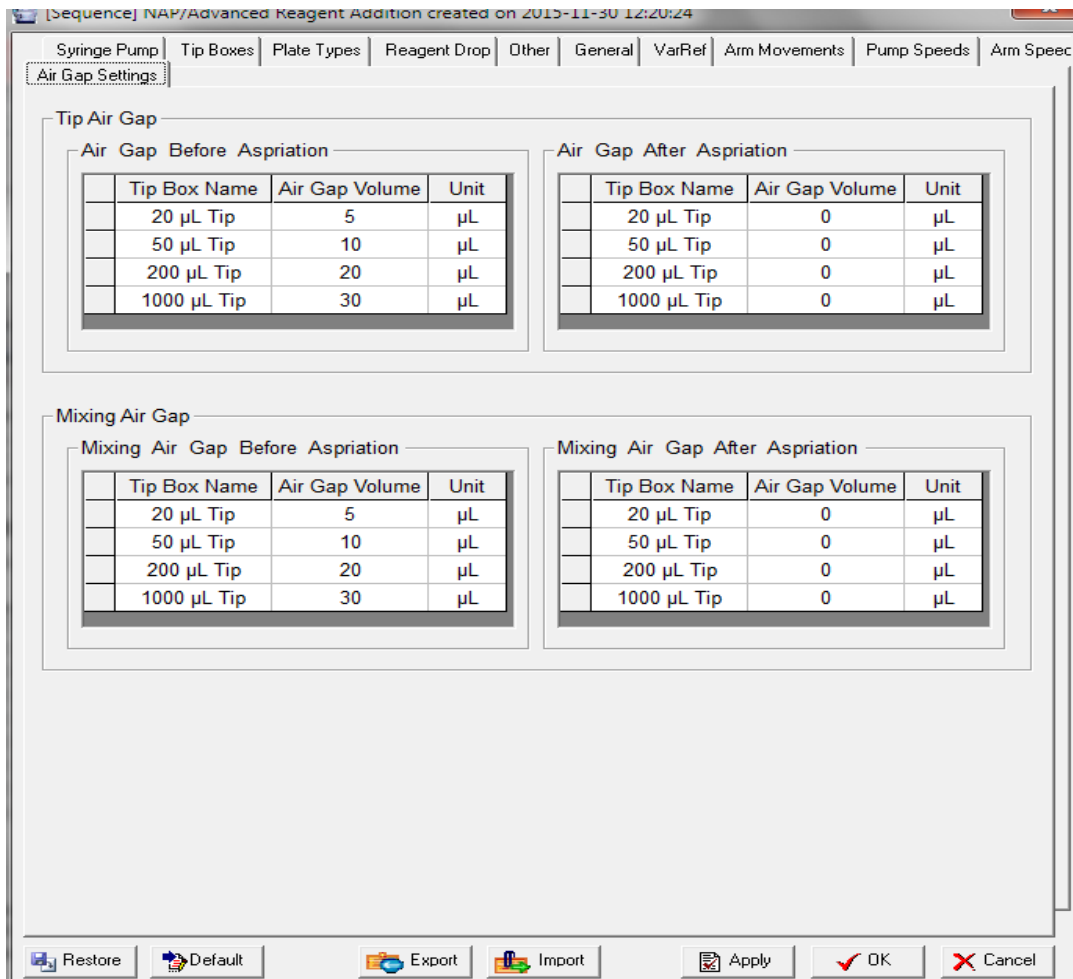


Figure 10-30 Air Gap Settings

11.VERSA Application Tabs

This section will provide information on how to configure and operate VERSAware NAP/PCR, how to design, validate, store and run Serial Dilution, NAP/Reagent Addition, Dilution, Plate Reformatting, Cherry Picking, PCR application and Normalization sequence(s).

11.1. Startup

The VERSAware software provides a user-friendly graphic interface with an easy drag-and-drop technique to create, validate, save, combine and run application sequences defined by parameters such as Serial Dilution, Dilution, Plate Reformatting, Pooling Amplislide, Cherry Picking, PCR [Polymerase Chain Reaction] and Normalization application sequences.

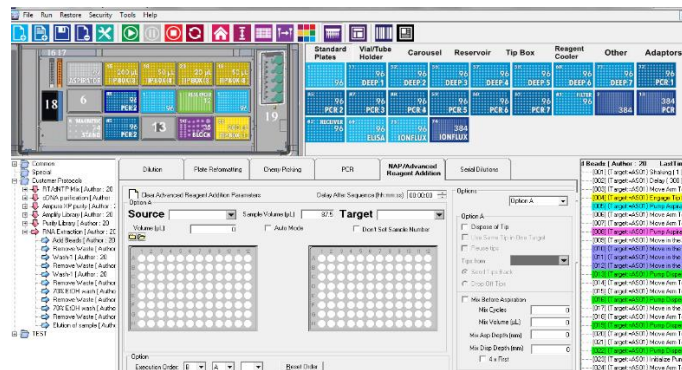


Figure 11-1 VERSAware Workspace Main Interface

VERSAware1100 allow users to configure the deck using different labware types, define, store, combine and run assays or sequences for various applications. Device Manager AS provides robotic arm control, teaching positions, syringe pump control and calibration; and temperature control. Aurora Station on the other hand stores all communication parameters, robotic arm and syringe pump speed, acceleration and travel limit settings along with tip engagement speed and acceleration and tip disengagement offset.

11.2. Basics

The 10 µL or 20 µL pipette tips are generally used with the small 25 µL syringe pump, and 50, 200, 1,000 µL pipette tips are typically used with the large 1,000 µL syringe pump and 200 µL tip for 8-channel x 250 µL pump.

Please contact your Aurora sales representative for pricing and catalogues on consumables. Refer to the Teaching Positions or the VERSAware1100 Main Interface section of this manual for more information on tip box location(s).



Figure 11-2 8-Channel - 200 µL Pipette Tip Box

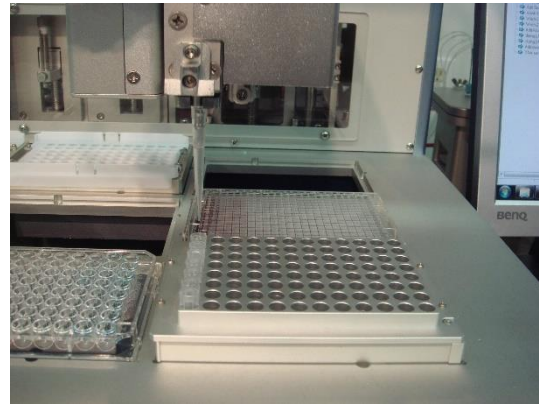


Figure 11-3 Single Channel - 200 µL Pipette Tip Box

The drag-and-drop technique is used to configure the deck with necessary vessels for any particular assay or sequence. All 15 positions can be used for deck configuration. Depending on the configuration of the deck and customer's requirements, the below mentioned guidelines are recommended deck layout configuration for VERSA1100.

Position 1 – 4X8 Tube Adaptor

Positions 2 to 3 – 8 channel -20/50/200µL tip boxes

Positions 4 to 5 – 8 channel -1000µL Conductive tip boxes

Positions 6 & 7 & 13 – Empty

Positions 8 to 10 - AmpliSlide

Position 11 – Deep well plate

Position 12 – Heater/Shaker

Position 14 & 15 – 4X8 Tube Adaptor

NOTE: Drag and drop the required plate or tip type from the library onto the deck interface for use

11.3. Folder Assays and Sequences

Assays and sequences are stored in the database of VERSAware1100 software. Multiple sequences can be stored under each created assay and the storage capacity is unlimited. Any stored assay or a sequence can be selected to run at any time by clicking on it. To rename as assay or sequence, click on any assay or sequence title.

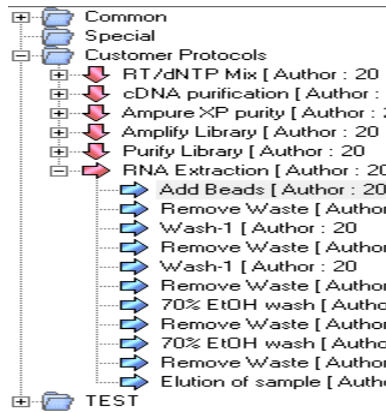


Figure 11-4 Example: Assay and Sequences

An option to cut/copy and paste sequences is also available (Figure 11-5 VERSAWare- Cut, Copy and Paste Sequence). Select the sequence you want to cut/copy and paste and right click to choose the option. The original sequence will be pasted after the selected sequence. There is also an export (to file) and import (from file) function for sequences; along with a print function for individual sequences or steps.

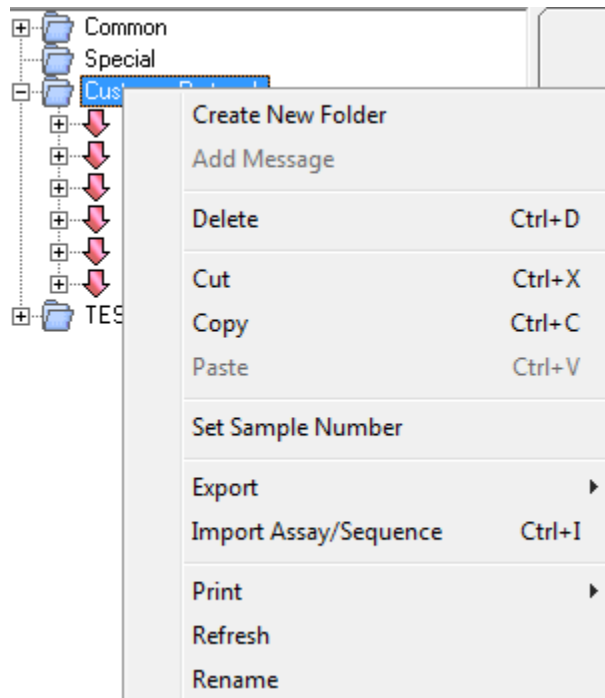


Figure 11-5 VERSAWare- Cut, Copy and Paste Sequence


Follow the steps below for creating and running sequences or assays:

First click on the appropriate application tab.

Then drag and drop required lab-ware or plate types on the main deck interface.

Ensure the actual plates and tips are in place on the physical deck for use.

Design your protocol – highlight wells and input options.

If mistakes are made, erase all parameters by clicking the clear sequence  setting icon.

Recreate and click the Add New Assay icon to create a new blank assay and change the assay name if required.

11.4. Add New Assay Icon

Add a sequence to this new assay by pressing the Add New Sequence button and change the sequence name if needed.

The sequence will be saved in the database if there are no errors.



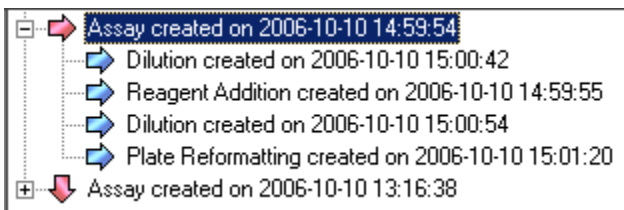
Add New Sequence Icon

Press the Start Assay/Sequence button to run this sequence.



Start Assay/Sequence Icon

If you want to run the entire assay that consists of multiple sequences, select the assay and all attached sequences will run one after another.



There is another option available to run an application without saving the sequence. To do this, click on the Reagent Addition tab and press the Start Assay/Sequence button on the VERSAware V1100 toolbar.

While running an assay, before each sequence, teach and initialize the robotic arm and prime both syringe pumps to prevent possible robotic arm step loss error (ref. Aurora Station section of the manual).

Click the Stop button to terminate the sequence. However, a single command being performed at that moment, whether it is a robotic arm movement or liquid handling operation, will continue running until it is finished.

Click the Pause button to pause or resume the sequence run.

Click the Stop button to stop the sequence run. You have the option of resuming from a particular step of a stopped sequence by left clicking on that step.

Click the Run Continuously button to loop the entire assay if an assay is running or the sequence if only a sequence was started.



Continuous Sequence Run

11.5. Serial Dilution Application Tab

This tab allows the user to perform serial dilutions (log10 & log2 fold). User can do a 1/10 or 1/2 dilution of the samples can be done with single-channel and 8-channel application.

The first dilution can be any fold. For example, if the original concentration of the compound is 1mM, the starting or first well can be any concentration either less than 1mM or the same. The user must select the first well from where the serial dilution starts and specify the concentration in that first well.

After which, the user can choose the type of dilution required (log10, log2, Log5) and specify the source position of the diluent which can be from a reservoir or reagent cooling block as shown in *Figure 11-6 VERSAware Serial Dilution Tab*.

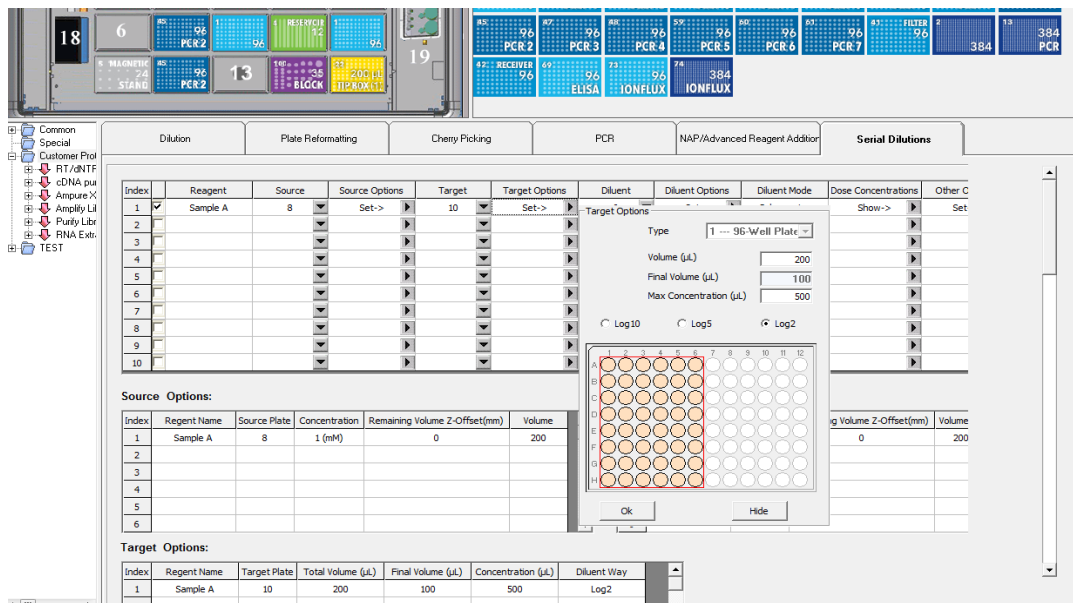


Figure 11-6 VERSAware Serial Dilution Tab

To perform a serial dilution user are to follow the steps below:

Step 1 – Drag and drop the plate or tube types to the correct positions on the deck layout interface segment (as shown in screen shot above).

If utilizing diluent from a source (reservoir or cooling block), drag and drop the diluent type onto the deck. With single channel mode the user can use the reagent cooler block for diluents; and the reservoir can be used for 8-channel mode.

Step 2 – Choose the selection mode for dilution – by row or by column only in single-channel mode.

Step 3 – Next proceed to select the position of the source. In this case, the 96-well plate is the source (as shown in screen shot above) located at position “2” on the deck. Highlight source wells.


Step 4 – Select the target position. This is the position where you would want the source to go. In this case, the 96-well plate is the source (as shown in screen shot above) located at position “3” on the deck. Highlight target wells


NOTE: Targets and sources need not be of the same type. Serial dilution can be performed from tubes to plate, plate to plate etc.


Step 5 – If using diluent from a source for e.g. reservoir or reagent cooling block, under diluent source (as in screen shot above), click onto the “reservoir position” field and select the position of the diluent source.


Step 6 – Proceed then to input a value for the concentration of the source sample. Here we have used 1uM as the sample concentration (as shown in the screen shot above).

Step 7 – Proceed to do the same input values for the original volume and final volume (μL) for the target, along with the maximum concentration allowed. The software automatically calculates for the dilution volume needed.

Step 8 – Other options are also available which can be included in the sequence, for e.g. – Operators can choose to check off “mixing” and “delays” or even “tip disposal after every dispense”. To erase all parameters, click the clear sequence setting  icon.

Step 9 – Lastly, remember to save your created sequence by clicking on the  icon. If this is an addition to an existing assay, highlight the assay name on the left of the screen and then proceed to click on the “add new sequence” icon. You would be given the option to enter the new sequence name. You can also rename after. If a new assay has not yet been created, then

proceed to first click on the  icon, rename the assay, click “OK”. After which, click on the “add new sequence” icon, rename the sequence and click “OK”.

Step 10 – If making changes to an already loaded or existing sequence – load by highlighting and <click> on the sequence you want to edit or change, make the changes, and the <click> the  icon to save or update the new changes. New changes will then be applied to the sequence and system.



Step 11 - To run this sequence, <click> on the icon.

11.6. Plate Reformating Application Tab

This application allows users to format from e.g. a 96-well plate into a 384-well plate or one 96-well plate to another type.

The ability to reformat from e.g. 6x8 tubes to a 96-well plate also exists but in single-channel mode only. Other options include dilution with buffer from the reservoir/ reagent cooling block, mixing of the diluted target sample, and delays before and after the application run. Advanced options include plate replication and reformatting from two source positions. Please follow the steps as shown below to reformat from 96 well plate to 384 well plate:

Step 1 - Configure your deck

Step 2 - Select your Source Plates from where you will aspirate liquid and assign the volume

Step 3 - Select the column on source plate (each source plate column will be the same)

Step 4 - Select your Target 384 well Plate (column will be alternate in 384 well plate)

Step 5- Add volume to be transferred to 394 well plate, select options to change tip or not to change tip

Step 6 - Assign an air gap volume (choose a higher volume for higher source volumes)

Step 7- Save the sequence under an assay

Step 6 - Click the Start Button

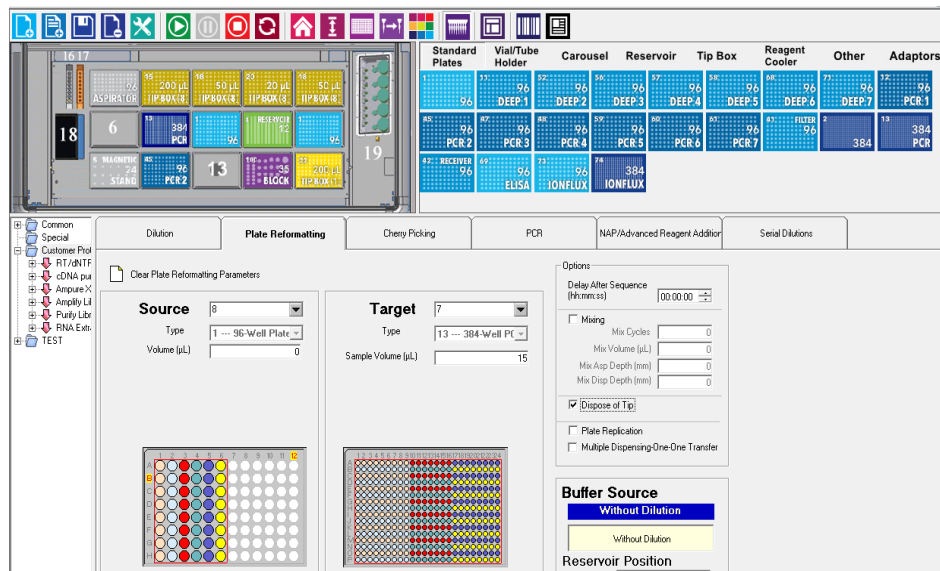


Figure 11-7 VERSAware Plate Reformating Tab

If dilution is needed after reformatting is performed, drag and drop a diluent source (reservoir or cooling block) onto the deck interface, and select under “reservoir position” field, the position of the diluent source. A “final volume” field will appear under the target section which you will then input the final amount for each well.

Source & Target Micro-plates	
96-well	384-well
2 Columns	1 Column
4 Columns	2 Columns
6 Columns	3 Columns
8 Columns	4 Columns
10 Columns	5 Columns
12 Columns	6 Columns

Columns for 96 and 384 Source and Target Plates

Only one sequence is sufficient to reformat a single 96-well micro-plate with 12 columns. However, four 96-well plate sequences are needed to reformat an entire 384-well micro-plate with 24 columns.

Options which include mixing and delays after sequences can be selected if needed. The “dispose of tip” option, when enabled, changes the tip after each dispense. Multiple dispensing using same tip can also be opted when transferring small volumes which cannot be done using the multiple dispensing option.

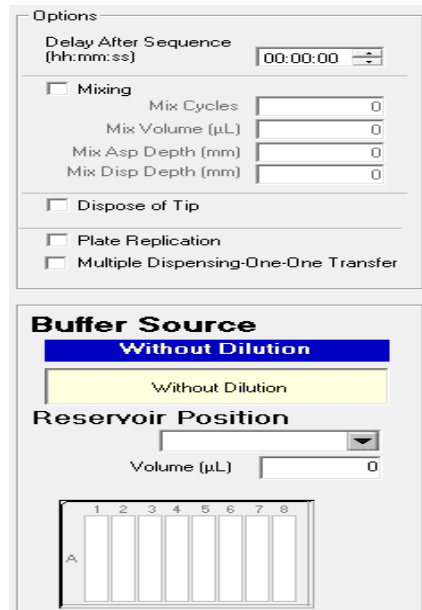


Figure 11-8 VERSAware Plate Reformatting Tab Options

The plate replication option allows one to reformat from one source plate to 5 similar type of target plates as shown below in *Figure 11-9 VERSAware Plate Replication Option*.

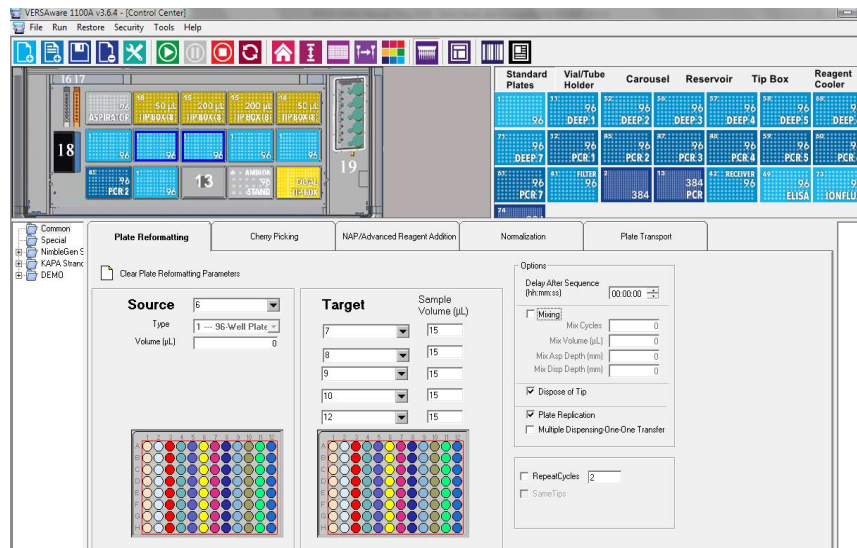


Figure 11-9 VERSAware Plate Replication Option

NOTE: Source and target plates have to be of similar types to perform plate replication

11.7. NAP/Advanced Reagent Addition Application Tab

This tab allows user to set up the workstation for multi-dispensing reagents from reservoir/96 well plates to target plates. Configure the deck by dragging plates/reservoir to source & target positions. Select source position and target position, add volume to be transferred and select option to execute the commands.

Option “A” is for liquid handling options. Reagents can be aspirated from reservoirs, plates, bead mixer etc. and also from reagent drop to target plates. All the parameters required to perform accurate liquid handling can be used to dispose of tips for every well, reuse tips, mix before aspiration, mix after dispensing, distribute liquid in one to one way mode during multiple dispensing using same tip during bead distribution. Plate can be moved with and without liquid handling as shown below in figures *Figure 11-10 Advanced Reagent Addition Tab—Option A parameters* and *Figure 11-11 Advanced Reagent Addition Tab—Option “A” execution order*.

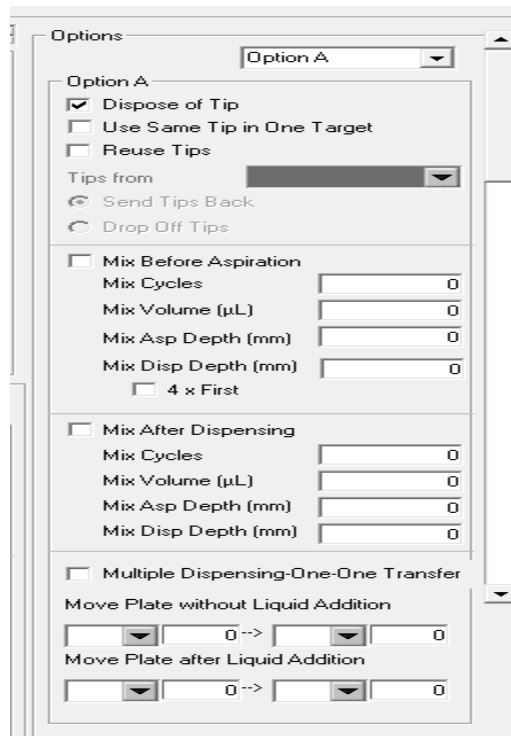


Figure 11-10 Advanced Reagent Addition Tab—Option A parameters

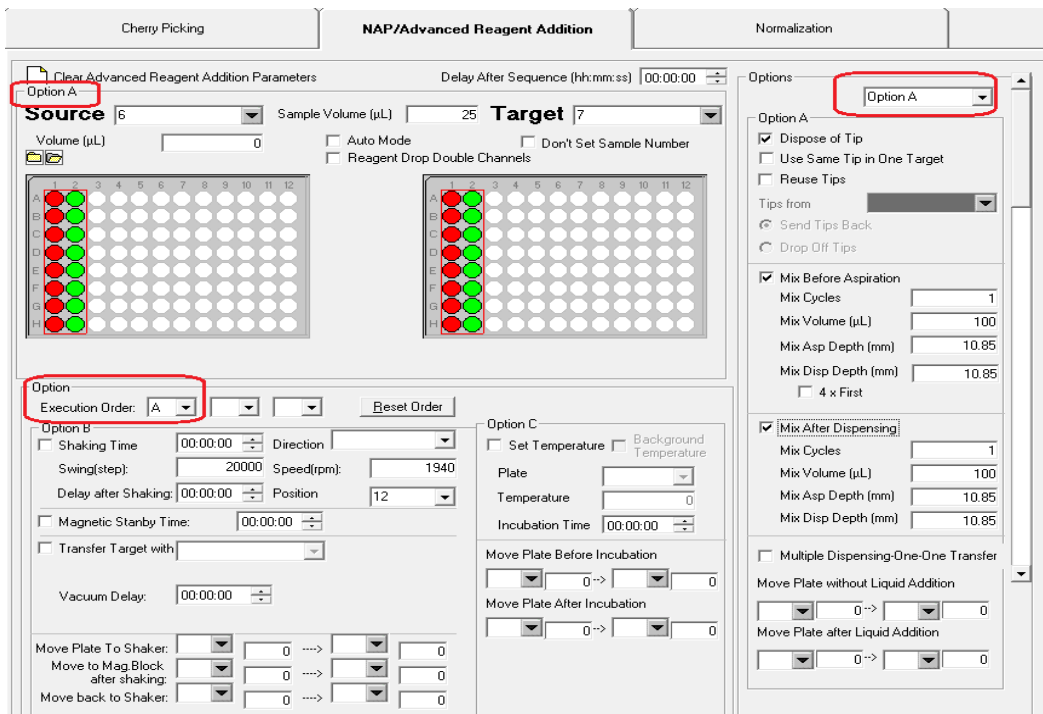


Figure 11-11 Advanced Reagent Addition Tab—Option “A” execution order

Option “B” for shaking , set shaking direction (both directions, one direction, background mode) , speed (rpm), time, plate movement from shaker to magnetic block and back, delay time on magnetic block,

aspirator selection to remove waste using aspirator and vacuum time for aspirator as shown below in figure *Figure 11-12 Advanced Reagent Addition Tab—Option B parameters* and *Figure 11-13 Advanced Reagent Addition Tab—Option “B” execution order*.

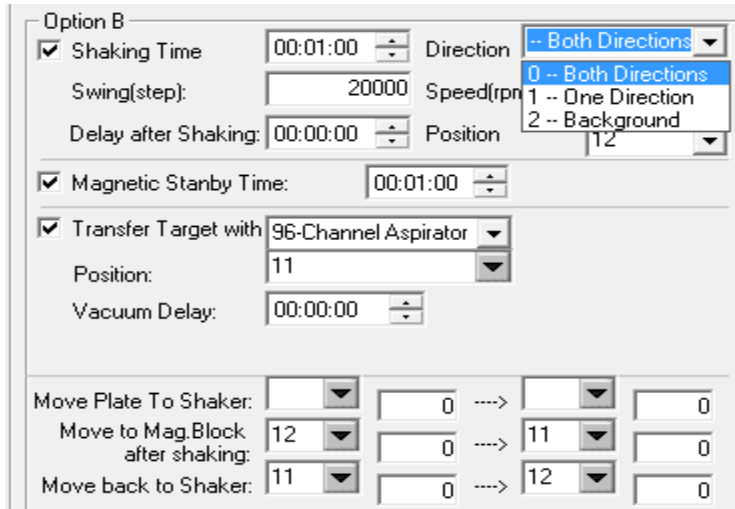


Figure 11-12 Advanced Reagent Addition Tab—Option B parameters

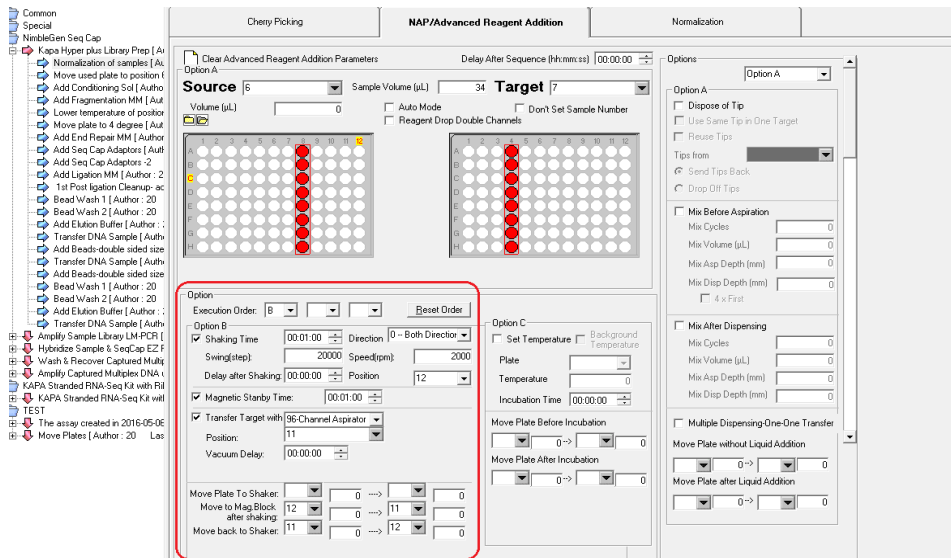


Figure 11-13 Advanced Reagent Addition Tab—Option “B” execution order

Option “C” for incubations of the plate at different temperatures. Select the position to set the plate cooler/heater temperature, set temperature, set the duration of incubation. Temperature can be set in background mode too—this is the option which works in background mode to set the required temperature without interfering the protocol run e.g. if the temperature is required to be raised in the protocol but needed after 40 minutes/10th sequence, a sequence can be created earlier to set temperature in background mode so that by the time it reaches to that sequence, temperature will be almost set (can be read in this sequence). This saves time to raise the temperature during that sequence. Select all the parameters as shown below in *Figure 11-14 Advanced Reagent Addition Tab—Option “C” execution order*.

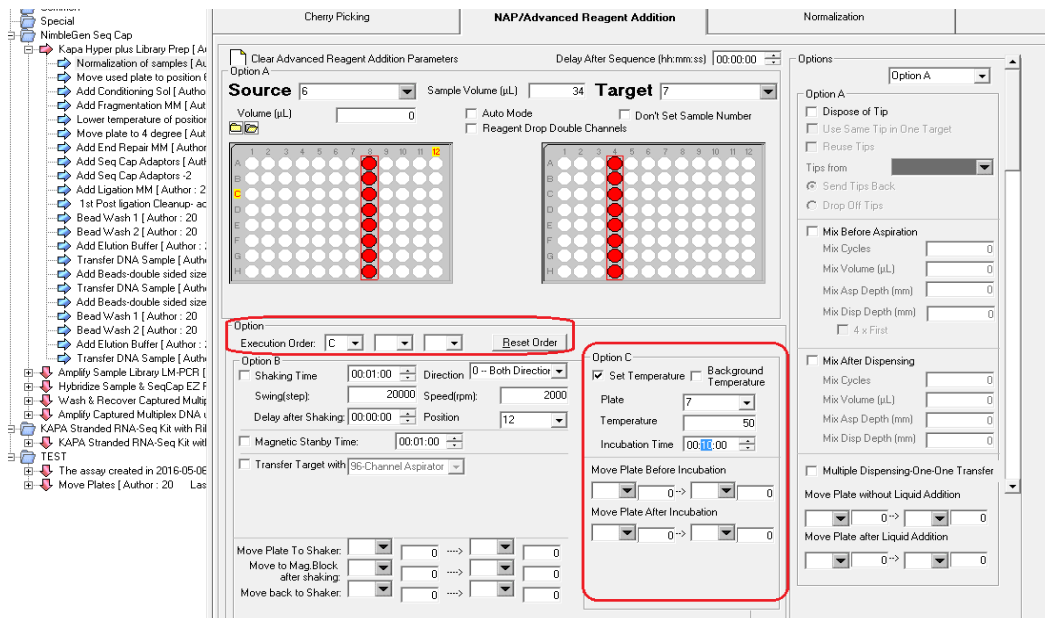


Figure 11-14 Advanced Reagent Addition Tab—Option “C” execution order

Options ABC : Using all the three options—liquid handling in option A, move plate to shaker after liquid handling, shake plate in option B, move plate before incubation in Option C. Select the execution order A, B, C as shown below in *Figure 11-15 Advanced Reagent Addition Tab—Using all three options ABC*. It can be any execution order—B,C,A etc. depending upon protocol requirements like shake, incubate and liquid handling etc.

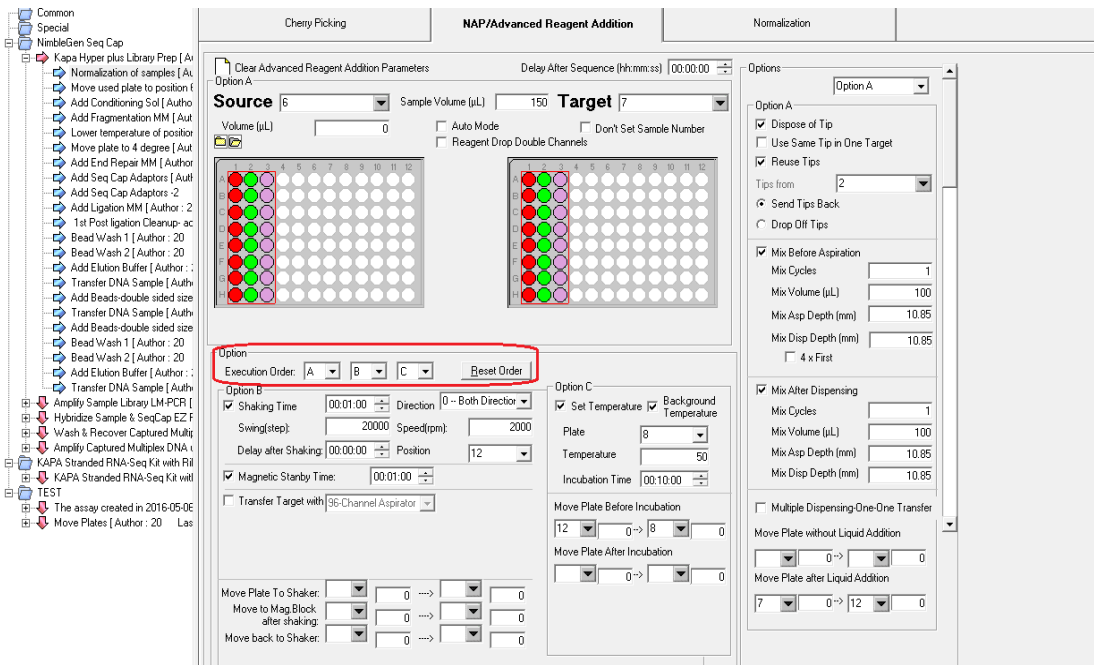


Figure 11-15 Advanced Reagent Addition Tab—Using all three options ABC

Follow the steps below for a typical Nucleic Acid Prep protocol using magnetic beads.

1. Add sample (e.g. buccal swab) into a deep well 96 well plate on the shaker position # 12.
Save this sequence.
2. Add lysis buffer from reservoir to the sample, shake it for 3-5 min depending on the kit requirements. Save the sequence using options A and B
3. Add proteinase K from reservoir & magnetic beads (from bead mixer in single channel mode) to the sample plate, shake again, after shaking move the plate to magnetic block, delay for 1-2min, discard the waste to waste position by changing tips or use aspirator, move the plate back to shaker position
4. Add wash buffer 1 using reagent drop, shake again, after shaking move the plate to magnetic block, delay for 1-2min, discard the waste to waste position by changing tips, move the plate back to shaker position.
5. Repeat step 4 with wash buffer 2 & 3.
6. Add elution buffer, shake again, after shaking move the plate to magnetic block, delay for 1-2min, collect the sample to another plate by changing tips for each sample. Discard the bead plate.

11.8. Slide Tab

This tab allows user to set up sample transfer from source plate/tubes to 2X4 slides. Configure the deck by dragging source plate, slide to source & target positions. Select source position & target position, then assign sample volume. Save this sequence and run it, make multiple test runs to adjust the offsets, please see figures below as samples:

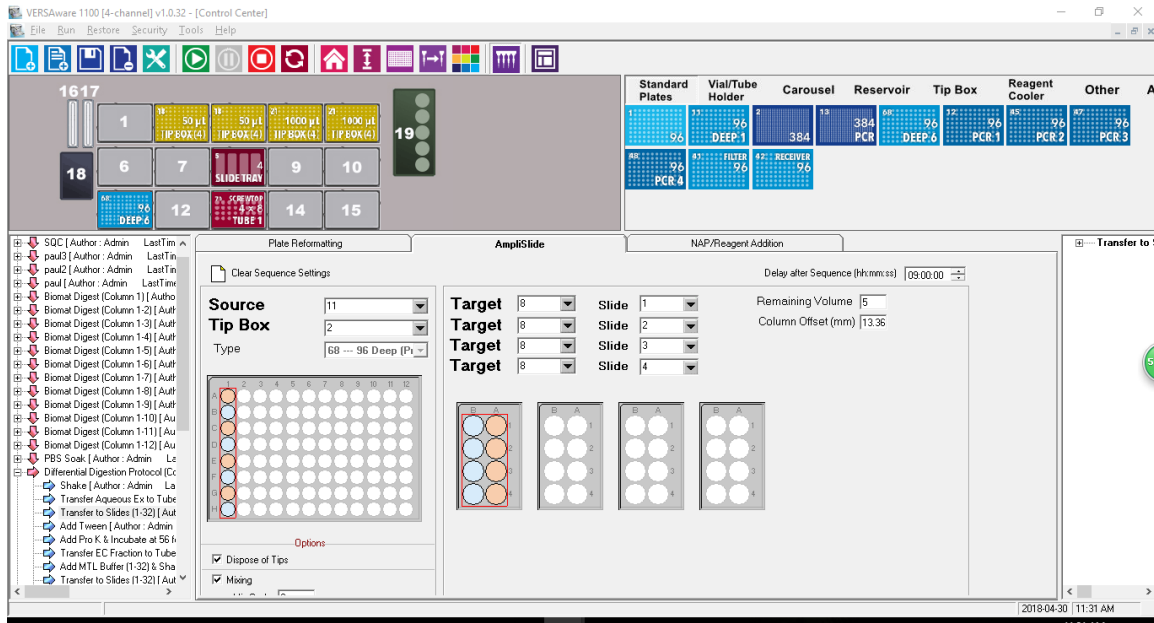


Figure 11-16 Slide Tab--Sequence settings-Protocol 1

Follow the steps below for a typical Slide Application.

- 1 Configure the deck according to protocol by dragging all required plate types, tip boxes etc.. Select sample source, target as well as slides, select sample and transfer target, input volume.
- 2 Select tip box location with black conductive tip, make sure LLS option in option menu is activated.
- 3 Once the sequence has been saved/updated, do multiple test runs to verify the aspiration offsets and dispense offsets, you can modify these offsets inside option menu.
- 4 You can also copy existing Slide sequence, modify and save for new applications.

12. UV/HEPA Hood

The bio-containment hood (*Figure 12-1 UV/HEPA Bio-Containment Hood*) is designed to provide sterile air quality for the assay sequence workspace by utilizing a high efficiency particulate air filter (HEPA). By performing filtration of the air within the workspace, the hood prevents contamination of the agents used during any given sequence. In addition, cross-contamination by harmful particulates or microbes in the air surrounding the workspace is reduced with the built in UV lamp.



Figure 12-1 UV/HEPA Bio-Containment Hood



Figure 12-2 UV/HEPA Bio-Containment Hood—Control Panel

1. Fan Switch – Turns on and off the containment hood’s fan to draw external air inward.

With the fan running, air is filtered as it enters the hood.

2. Light Switch – Turns on and off the containment hood’s fluorescent light, which allows for a brighter workspace environment.

3. UV Switch – Turns on and off the ultraviolet light source, which is used to De-contaminate the workspace.

4. UV Timer Control – Along with the UV switch being turned on, the UV Timer Control is used to set the duration of the UV light source.

13. Workstation Maintenance

13.1. General Guidelines

It is important to follow the general guidelines and instructions described in this manual in order to maintain the proper function of the VERSA 1100 NAP/PCR Workstation:

Use only clean vessels such as micro plates, reservoirs, micro tubes, vials, beakers, flasks, tip boxes and reagent/cooler block

It is recommended to use sterile pipetting tips with filter barrier

Only use 20µL, 50µL and 200µL pipetting tips with the VERSA 1100NAP/PCR

Do not leave vessels exposed to dust and dirt overnight

13.2. Tubing

All tubes should be tightly connected to adapters and should not be twisted or bent. All tubing is made of durable Teflon which cannot be easily ruptured or bent. If aspirating or dispensing viscous liquids, adjust the aspiration/dispensing speeds in the software.

13.3. Waste Bottles

All tubing should be inserted into the bottles as described in Tubing and Bottles section of this manual.

It is very important that the waste liquid bottle does not over fill. Otherwise the waste liquid may fill the vacuum tube and cause damage to the vacuum pump.

S/No	Description of Maintenance Program	Frequency	Good	No Good	Actions Taken / Remarks
A.	Daily Maintenance Program				
1.	Inspect & clean any visible Reagent / Chemical Spillage on the instrument with Isopropyl Alcohol.	Begin / End day			
2.	Do not leave test samples in tube plate stacker placed on Shaker or target plates on the VERSA 1100 when terminating / aborting the protocol operation.	1x (End day)			
3.	Remove & discard all the used tips in the collection tray / bags/ box.	1x (End day)			
4.	VERSA 1100 Module All tubes are correctly inserted into the System Fluid and waste bottles	1x			

	<p>Power Cord & USB cable/COM-Port connection</p> <p>Verify critical teaching positions</p> <p>Visual Inspection for any abnormality in function, misalignment / visible signs of damages:</p> <p>Robotic Arm & Gripper</p> <p>8-channel Syringe Pump Unit</p> <p>5-channel Reagent Drop Unit</p> <p>Shakers</p> <p>Clean the Reagent Drop pins from outside with distilled water at the end of daily operation to prevent any salts depositing to pins</p>				
7.	<p>Gripper</p> <p>Power Cord & USB/COM-Port cable connection</p> <p>Verify gripper position</p>	1x			
B.	Weekly Maintenance Program				
1.	<p>Inspect the Wash Fluid – adequate level required for the operation and visual check of contaminants and potential growth in the bottle. Recommendation – replace the Wash fluid every week.</p>	1x per week			
2.	<p>General cleaning of the exterior instruments –particulates & stain.</p>	1x per week			
3.	<p>Removal of any trapped parts on the deck and below the deck.</p>	1x per week			
4.	<p>Perform routine check on individual hardware modules Visual Inspection for any abnormality in function, misalignment / visible signs of damages</p> <p>VERSA 1100</p> <p>Multi-dispensing RD</p>	1x per week			
C.	Monthly / Yearly Maintenance Program				

1.	Discard the leftover Wash fluid, clean the bottle thoroughly and re-fill with the System fluid/ Wash fluid.	1x per month			
2.	Clean the System Fluid/ Priming Waste / Waste / Reagent Bottles.	1x per month			
3.	Visual inspect and replace any twisted or serious bent tubes (if any).	1x per 6 months			
4.	Perform routine check on individual hardware modules - Visual Inspection for any abnormality in function, misalignment / visible signs of damages, worn out parts. VERSA 1100 Multi-dispensing RD Inspect and add lubricants: WD40/ grease at various critical mechanical moving parts.	1x per month 1x per 6 months			
5.	Perform volume check & calibration VERSA1100: 8-channel Syringe Pump Unit VERSA1100: 5-channel Reagent Drop Unit	1x per 4 months			

14.Troubleshooting

This section will discuss the following potential problems users might encounter with the VERSA1100 Workstation.

VERSA1100 Troubleshooting	
Problem 1	Power is off
Problem 2	No communication with the VERSA
Problem 3	Aurora Arm does not respond to operator's command
Problem 4	Aurora Arm does not move to correct position
Problem 5	Aurora Arm stops during application sequence run
Problem 6	Syringe pumps do not respond to operator's command
Problem 7	Syringe pumps do not aspirate or dispense accurate volume
Problem8	Pipetting tips are damaged or bent

Problem 9	Pressure and vacuum pumps are not working
Problem 10	While running the sequence, a hanging drop is formed outside of the tip
Problem 11	The pipetting tip does not aspirate the master mix in the reagent block
Problem 12	Pipetting tips do not dispense small volumes (1 - 5 μ L)
Problem 13	VERSA stops working suddenly or operation hangs midway
Problem 14	During Multiple dispensing of any reagent, first well and last well volumes are not correct

Trouble Shooting Problems

The VERSAware V1100 software provides advanced error messaging and a detailed log for both Aurora Arm and Syringe Pumps. Most common problems and solutions are discussed below.

Problem: Power is off

Solution: Please check if power cable is properly connected to the VERSA and AC outlet as discussed in the Wire and Cable Connections section. Make sure that the power switch is on. For additional information please refer to the Specifications section of this manual for the correct voltage settings.

Problem: No communication with the VERSA

Solution: Please ensure that USB cable is tightly connected to the VERSA and computer USB port as described in the Wire and Cable Connections section. Please check correct COM port configuration in MS Windows XP/2000.

Start → Settings → Control Panel → System → Hardware → Device Manager → Ports

Device status of COM ports should read: This device is working properly. All COM port numbers should not exceed 16. Please refer to the USB Driver Installation section of this manual for more information how to change the COM port number.

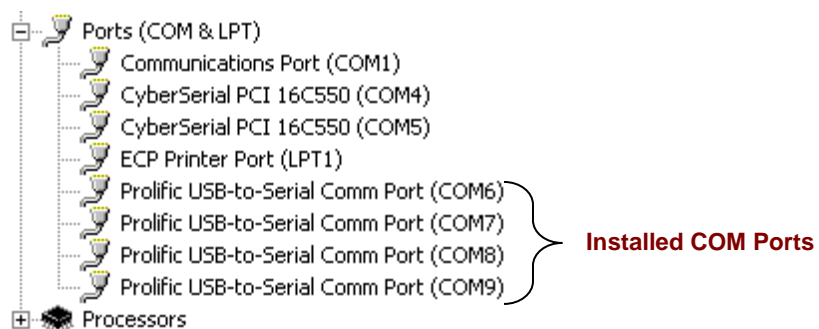


Figure 14-1 Correct COM Port Configuration in MS Windows XP/2000

If COM ports are not visible in Windows XP/2000 Device Manager, install the USB driver for the USB/Serial COM Port Converter contained within the VERSA as described in the USB Driver Installation chapter of this manual.

Problem: Aurora Arm does not respond to operator's command

Solution: Please ensure that the arm is initialized in Device Manager AS software. Make sure that the COM port settings are correct in the Device Manager AS software as explained in the Device Manager AS Settings chapter. If the problem still remains, try to shut down and restart the VERSA and initialize the robotic arm once again.

Problem: Aurora Arm does not move to correct position

Solution: Please refer to the Teaching Positions section to adjust or repeat the teaching procedure for all positions. If the problem still remains, try to initialize the robotic arm again.

Problem: Aurora Arm stops during application sequence run

Solution: Please refer to the Device Manager AS Settings section to decrease robotic arm speed and acceleration settings for every axis (X, Y, and Z). It is recommended to gradually reduce end speed by 500 or 1,000steps/sec. Acceleration should be reduced to 1 or 3 steps/sec². Can start the sequence from the step it last stopped at by left clicking to resume.

Problem: Syringe pumps do not respond to operator's command

Solution: Please ensure that both syringe pumps are initialized in the Device Manager AS software. Make sure that the COM port settings are correct in the Device Manager AS software as explained in the Syringe Pump Settings and Calibration chapter. If the problem still remains, try to shut down and restart the VERSA IK software and initialize the syringe pumps one more time

Problem: Syringe pumps do not aspirate or dispense accurate volume

Solution: Please refer to the Syringe Pump Settings and Calibration section of this manual and perform new calibration procedures if necessary.

Problem: Pipetting tips are damaged or bent

Solution: In case of accidental tip damage, press the Home Aurora Arm button in the VERSAware 1100 software to initialize the robotic arm from home icon to disengage the damaged tip.



Figure 14-2 *VERSAware Toolbar Arm Initialization and Tip Disengagement Icon from Home icon*

Problem: Pressure and vacuum pumps are not working

Solution: Please contact Aurora Biomed Inc. to obtain new pressure and vacuum pumps, instructions on how to install them or request technical assistance.

Problem: While running the sequence, a hanging drop is formed outside of the tip

Solution: The engaged pupating tip is probably loose. Check the position of that tip box. After engagement, tip should be tightly fitted to the barrel.

Problem: The Pipetting tip does not aspirate the master mix in the reagent block

Solution: The pupating tip probably touches the bottom of the 7 mol vial. Please refer to the VERSAware 1100 Settings section of this manual to decrease the Master Mix Block Depth (mm). Or the pupating tip might be above the master mix volume level. Increase the Master Mix Block Depth (mm) in this case. Alternatively, click Pause when the tip goes to aspirate solution and subsequently check/fix the Z-Offset (calculate with Z-Offset calculator).

Problem: Pipetting tips do not dispense small volumes (1 - 5 μ L)

Solution: The pipetting tip should slightly touch the bottom of the micro plate. Please check the Z-offset in VERSAware Option for that particular plate type, to increase / decrease the plate's Z-Offset (mm) for any application tab.

Problem: VERSA1100 stops working suddenly or operation hangs midway

Solution: Hibernation, standby or sleep mode is enabled on system software. To change these settings, please refer to section 2 (power saving settings) of manual for more details. Try closing and reopening the software again. If problem persists, restart the PC and reopen the software.

14 During Multiple dispensing of any reagent-first and last well volumes are not correct

During multiple dispensing of any reagent--to ensure the volumes in first well and last well are correct, following settings need to be optimized.

Extra Aspiration/ Dispense back volume and backlash settings e.g. extra aspiration volume is set 10 μ L, dispense back volume is 8 μ L and backlash is set at 0--according to these settings, at the end after dispensing in all wells--theoretically, there should be 2 μ L (10-8) left in the tip (which can be dispensed back at source) but due to backlash of the pump--it can be more or less--if more--decrease the difference

in Asp/Disp.---10/9/0 and if still more then set backlash too-10/9/2 depending upon how much left in the tip and if less then increase the difference--10/7/0, 10/6/0

First volume correct--dispense back volume settings- 10/8/0 or 12/10/0

Last well Volume correct--difference in the Asp/Disp.--10/7/0 (If left less), 10/9/0 (if left OK), 10/9/2 (if left more). Please check *Figure 14-3 Extra aspiration/dispense back volume settings*.

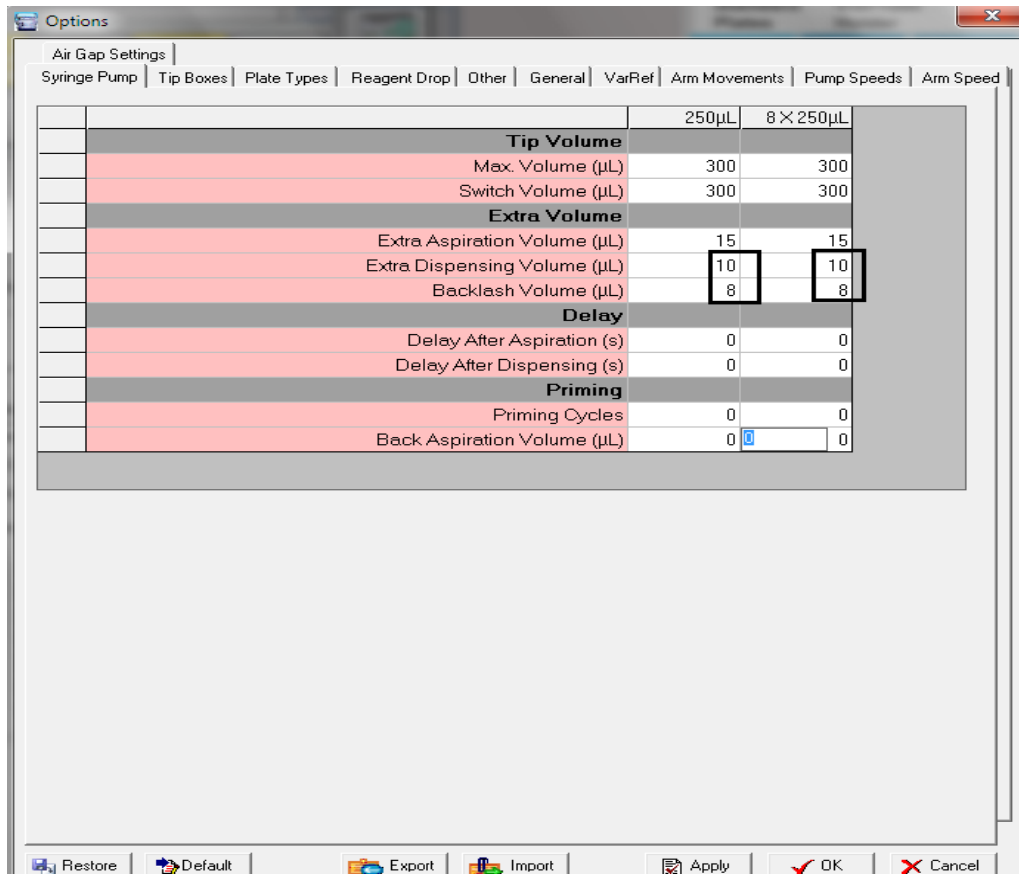


Figure 14-3 Extra aspiration/dispense back volume settings

To add these settings in the sequence—open that particular sequence--open OPTIONS--Syringe PUMP--change the settings for extra Aspiration/Dispense back and if required backlash too and then update the sequence. These setting will come in the steps--can be confirmed by checking steps as shown in the *Figure 14-4 Extra aspiration/dispense back volume showing in the steps.*

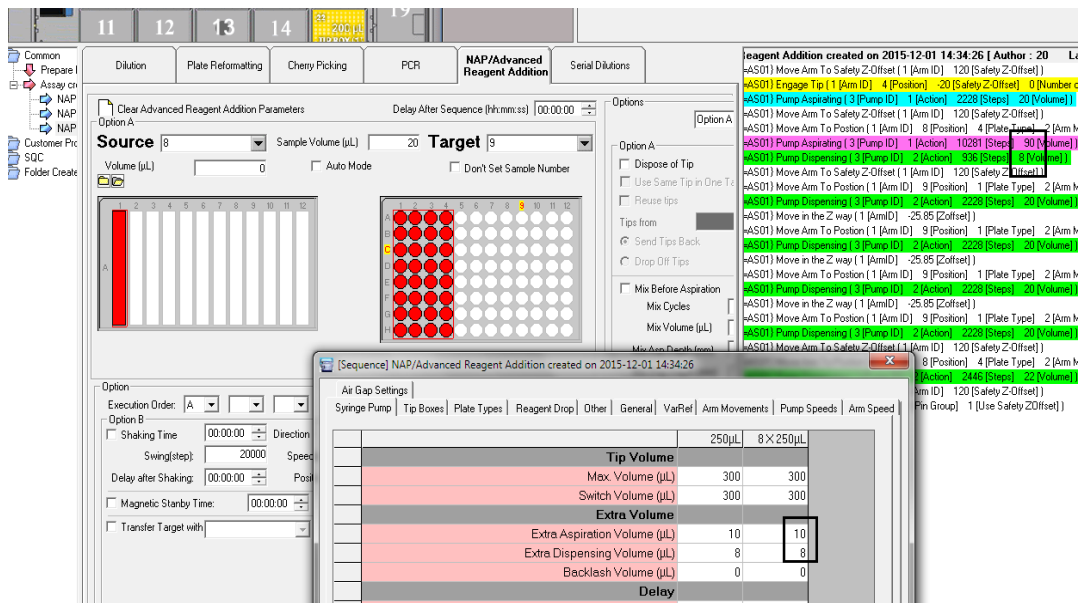


Figure 14-4 Extra aspiration/dispense back volume showing in the steps

Note: If using filter tips—set the Maximum volume in OPTIONS—Tip Box as—20µL as 10 µL, 50 µL —45 µL, 200µL -180µL and 1000 µL--900µL

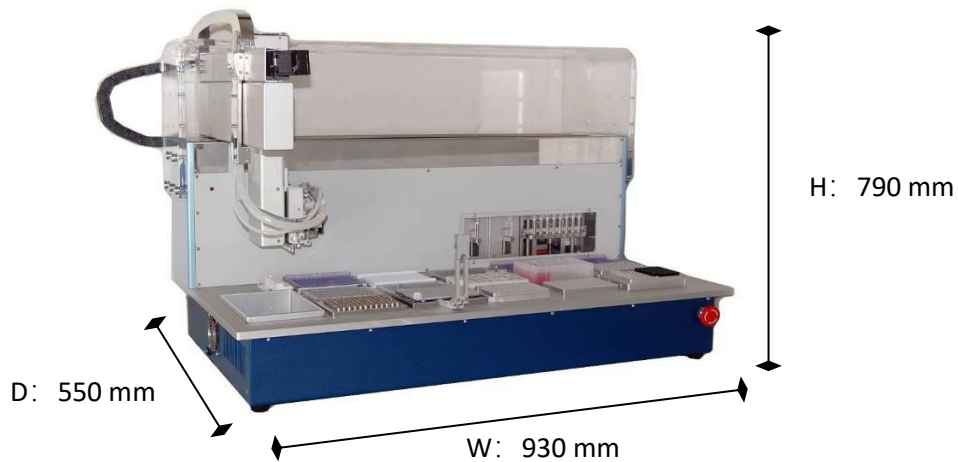
15.Specifications

Weight : 90 kg without hood

Deck Positions : 15 Deck positions

Head : Dual Head Configuration - 8 Channel Tip

Changer & Single Channel



PWM Pump (Reagent Drop)

Pressure : 15 psi
Max. Dispensing Volume : 10ml

250µL Syringe

Asp/Dispensing Volume Range : 1 - 250 µL

1,000 µL Syringe

Asp/Dispensing Volume Range : 2- 1000 µL

Voltage : 100 ~ 120 Volts

Current : 5 A, Fuse: 10 A

Frequency : 50 – 60 Hz

16. System Requirements

This section will provide minimum system requirements to successfully operate the VERSA1100 Workstation.

One dedicated IBM compatible personal computer with Pentium 4 processor, 1.5 GHz or higher
--

1GB RAM (64-bit) or 2GB RAM (32-bit)

100 GB of hard drive space

US version of Microsoft Windows 2000 Professional (Service Pack 4 or greater), Microsoft Windows XP Professional (Service Pack 1 or greater), Microsoft Windows Vista or Window 7
SVGA display (1024 x 768 true color)
PS2 mouse
At least 1 USB 2.0 port
1 CD-ROM drive

System and Computer Requirements

17. Product Service and Customer Support

Aurora Biomed Inc. offers a full range of services to ensure your success. From our original factory warranty through a comprehensive line of customer support plans, Aurora Biomed Inc. offers Field Service Engineers and in-house Specialists who are dedicated to supporting your hardware, software and application development needs.

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