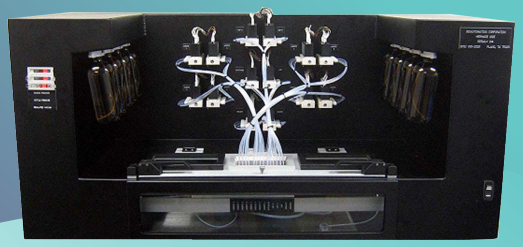
Original instructions



# MerMade 192E user's manual

**GEN/0645/MW/0225**

**For Research Use Only. Not for use in diagnostic procedures.**


BIOSEARCH™ TECHNOLOGIES

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# General safety

**⚠WARNING**

Read and understand equipment operator's manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

**⚠WARNING**

Always follow your facility's PPE program when operating this instrument or performing maintenance.

## Safety first

Accidents can be prevented by recognising the causes or hazards before an accident occurs and doing something about them.

## Safety symbols

Ensure all instrument operators are aware of dangers indicated by safety decals applied to instrument, and be certain they follow all safety decal instructions. Contact company for safety decal replacement.

**⚠ DANGER**

DANGER indicates a hazardous situation which, if not avoided, will result in death or serious injury.

**⚠WARNING**

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury.

**⚠ CAUTION**

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

***NOTICE***

NOTICE is used to address practices not related to physical injury.

Biosearch Technologies cannot anticipate every possible circumstance which involves potential hazard. Warnings and notifications in manual are not all inclusive.

Please obey following warning labels that are posted in potentially dangerous areas on instrument.

Indicates an electrical hazard. Turn off power and completely disconnect power supply to equipment before entering this area.

Indicates pinch point. When equipment is powered up, never put hand in these areas, a mechanical component could move unexpectedly and cause injury.

Indicates area where caution is required to prevent personal injury.

Indicates surface is hot and there is a burn hazard.

## Waste Electrical and Electronic Equipment (WEEE)

EU Waste Electrical and Electronic Equipment (WEEE) Directive is to minimise volume of electrical and electronic waste disposal and to encourage re-use and recycling at the end of life. Products bearing this label should not be disposed of in a landfill or with municipal household waste in EU to prevent potential negative consequences to the environment and human health.

Biosearch Technologies offers a free of charge return and collection service for the disposal of these products. For a copy of Biosearch Technologies's Selective Treatment of Waste Electrical and Electronic Equipment and a list of hazardous materials outlined under Articles 14 and 15 and Annex VII of the EU WEEE Directive 2012/19/EU please contact Biosearch Technologies.

## Owner responsibilities

**Notice**

Biosearch Technologies shall have no liability for loss of profit, loss of business or revenue, loss of data or business, loss of anticipated savings, depletion of goodwill, any third party claims, or any indirect or consequential loss or damage, which arises out of or in connection with any contract.

- Basic safety rules serve as a guide for proper operation of Biosearch Technologies equipment. All personnel who work with this instrument should learn this information.
- User must follow all procedures and precautions. Users should establish appropriate procedures for continued safe operation of instrument. Biosearch Technologies is not responsible for any deviations from instructions in this manual.
- Equipment is designed for generally accepted safety standards. Users are responsible for following the operating, maintenance, and servicing procedures outlined in this manual to ensure safe operation of this equipment.
- Do not allow persons to operate instrument until they have read user's manual and are completely familiar with all safety precautions.
- Always wear safety glasses/goggles and any other required safety equipment as required by your company's Personal Protective Equipment (PPE) policy.
- Do not allow persons under the influence of alcohol, medications, or other drugs that can impair judgment or cause drowsiness to operate or maintain instrument.
- Instrument should not be used to handle materials other than those which were specified as part of its design. It is operator's responsibility to be aware of instrument capacities.
- Ensure operator's area is clear of any distracting objects. Keep work areas clean and free of debris to avoid slipping or falling.
- Operators are responsible to know the location and function of all emergency stop and safety switches.
- Periodically check all guards, safety switches, emergency stop buttons and instrument structure. Replace or repair anything that could cause a potential hazard.
- If any safety devices are not functioning properly, do not use instrument. Remove it from service until it has been properly repaired. Contact Biosearch Technologies.
- Do not replace components or parts with other than factory-recommended parts. To do so could lead to injury or possible death. It may also decrease the effectiveness of the unit.
- When doing maintenance work on structural parts or repairing any moving parts: Disconnect and lockout and tagout all power sources. Know Occupational Safety and Health Standard (OSHA) requirements.
- Do not perform maintenance while instrument is running unless noted otherwise in a procedure within this manual.
- Modifying equipment using unapproved factory recommended service parts or consumables may result in death, injury, voided warranty, and/or decrease equipment effectiveness.
- Always use proper lifting techniques while operating, loading, maintaining, or troubleshooting equipment.
- Be aware of overhead objects while working in or around instrument to prevent head bumps or injury from falling objects.
- Be aware of cords/trailing cables while working around the instrument to prevent tripping.
- Always follow OSHA 1910 and also National Health and Safety Requirements.
- Operate and maintain this instrument in a safe manner and in accordance with all applicable local, state, and federal codes, regulations and/or laws; and in compliance with on-product labeling and this user's manual instructions.

- These are general safety considerations. Additional precautions may be necessary to operate your instrument in a safe manner. Be certain you are operating your equipment in accordance with all safety codes, OSHA rules and regulations, insurance requirements; and local, state, and federal laws.
- It is user's responsibility to ensure that a compatible electromagnetic environment for equipment can be maintained in order that device will perform as intended.
- Electromagnetic environment should be evaluated prior to operation of instrument.
- Do not use device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with proper operation.

Biosearch Technologies does not cover any defects or damage resulting from any of following:

- Neglect, carelessness, or misuse of instrument including without limitation any use which is not in accordance with documentation or contract, or improper or inadequate handling, storage and maintenance of instrument.
- Manufacture of instrument in accordance with custom specifications provided by customer.
- Any products of third parties purchased through Biosearch Technologies (such as third party computers and laptops that may be governed by third party manufacturer's own terms).
- Modification, servicing or repair of an instrument other than by Biosearch Technologies or a party authorised by Biosearch Technologies.
- Installation of any software or hardware, or use of instrument in combination with software or products that Biosearch Technologies did not supply or authorise.
- Any external sources, including without limitation any electrical surges, incorrect voltages, incorrect water supply or any damage caused by computer viruses or hackers.
- Transportation or relocation of an instrument by any party not authorised by Biosearch Technologies.
- Any events, circumstances or causes beyond Biosearch Technologies reasonable control, including without limitation any acts of God, governmental action, war or national emergency, acts of terrorism, riot, civil commotion, fire, explosion, flood, tornado, earthquake, hurricane, and lightning.

## Operating area

- Only operator(s) and other authorised personnel should work within operating area during operation.
- Do not keep tools or other equipment within operating area.
- Always use instrument in a sufficiently lit area.

### Lockout/Tagout

**⚠WARNING**

Failure to follow correct lockout and tagout procedures could result in death or serious injury.

Lockout and tagout procedures have three main purposes. First to prevent unexpected or accidental start-up of instrument, secondly, to notify other users when an instrument is unsafe to operate, and finally to prevent injury to personnel from energy that may be stored in devices installed on instrument.

To lockout and tagout, disconnect instrument from main power source. Disconnect air and release any stored pressure. Place one or more tags on instrument controls or access doors to inform other users that maintenance is being performed or that instrument is unsafe to operate.

According to 29 CFR part 1910 of OSHA (Occupational Safety and Health Administrations) regulations, employer must establish a lockout and tagout system of procedures, training, and periodic inspection before any employee operates, or services an instrument. All employees are responsible for seeing that instrument is locked out and tagged out to facilities policy.

Instrument must be locked out and tagged out under following circumstances:

- Any time repairs or maintenance is being performed on instrument.
- When cleaning or lubricating instrument.
- When cleaning blocked or jammed mechanisms.

If several users are working instrument, each person must apply their own tag and ensure all work is complete prior to instrument being powered on.

## Installation

Only trained and authorised personnel should install electric and pneumatic power sources. Installations must comply with all applicable codes and standards, including those established by OSHA or equivalent.

## Chemical spills

Chemical spills should be cleaned up immediately using recommendations listed in appropriate Safety Data Sheet.

### Chemical safety

- Follow all Safety Data Sheet (SDS) recommendations.

Follow facility's safety requirements when working with samples.

# MerMade safety

**⚠WARNING**

Read and understand operator's manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

Always follow your facility's PPE program when operating or performing maintenance on this instrument.

## Safety

All operators should be well versed in good laboratory practices, be trained in safe operation and use of instrument and be familiar with all SDS's for chemicals involved in oligonucleotide synthesis. The information and instructions contained in this user manual are accurate and reliable to the best of our knowledge.

Standard ANSI Z35.4 convention is used throughout manual.

Instrument must be operated in manner specified by Biosearch Technologies.

It is important that instrument is installed and operated in compliance with health and safety requirements. User is responsible to ensure all requirements are identified and followed. Failure to do so may result in injury or damage to instrument. Read and understand user guide before operating instrument.

Instrument incorporates a motion system and stores and delivers hazardous chemicals. Possible injury may result from motion system, electrical shock, and from inappropriate handling of chemicals.

## Reagent Delivery System

Instrument uses solenoid valves to deliver reagents, under argon/nitrogen pressure, to each of columns on machine in a specific order for synthesis to occur. Once delivered, reagents are removed from columns through a vacuum system.

## Motion system

**⚠WARNING**

Do not attempt to disable slide door sensor. Do not use Injection Head door or Reaction Chamber window to access reaction chamber while table is in motion. This could result in serious injury.

Injection head access door is fitted with a sensor to disable slide movement if door is opened.

Do not attempt to disable this sensor and never use Injection Head door or Reaction Chamber window to access Reaction Chamber while table is in motion.

Should an accident or collision occur, operator can disable slide by turning off power switch on side of instrument. This will close all valves, stop all motion and release motor so that slide may be moved by hand. User should familiarise themselves with power switch location in case of emergency.

Under no circumstances will equipment supplier be liable for any incidental, consequential or any special damages of any kind whatsoever, including but not limited to lost profits arising from or in any way connected with use of equipment or this user manual.

Communication between motion system and computer is over a serial cable connected between computer and instrument. COM port used by software is set in configuration files for instrument. On most instruments software is set to use COM1. If communication cannot be established please contact Biosearch Technologies Field Service for assistance.

## Electrical system

**⚠WARNING**

Remove power supply from instrument before opening back cabinet. Failure to do so may result death or serious injury.

Power supply and electronics are in a control box which can be accessed from back of instrument. Unplug instrument from main power supply before opening control cabinet unless instructed to do so by Biosearch Technologies Field Service. If opening cabinet with power on, ensure proper grounding and pay careful attention to warning labels inside cabinet. See component identification section for more information

## Chemical safety

All of chemicals used by MerMade are hazardous. Each reagent is accompanied by a warning on bottle or canister label. Read these warnings carefully and follow instructions for handling and storage. Refer to SDS from manufacturer and follow any instructions regarding preparation, storage, handling and disposal of chemicals. It is user's responsibility to determine suitability of any chemicals used on MerMade and to develop a safe procedure for use.

**⚠WARNING**

Chemicals are stored under pressure in bottles when in use on MerMade. Bottles are not designed for use at high pressure.

## Pressurised solvent bottles

**⚠WARNING**

Use minimum level A3 cut resistant gloves underneath appropriate PPE gloves when installing and removing bottles unless additional grip is needed.

**⚠WARNING**

LGC Biosearch Technologies is aware of the increased inherent risk of bottle breaking from repeated heating and cooling associated with cleaning process. Biosearch Technologies recommends that bottles are not reused or cleaned via a heating/cooling process.

Bottles are not designed for use at higher pressures and may explode if argon/nitorgen bottle pressure exceeds 30psi (2bar). There is a safety relief valve on instrument that is set to automatically relieve pressure in excess of 25psi (1.7bar). If pressure rises beyond recommended safety limit and relief valve does not engage immediately turn off gas flow at regulator on gas cylinder and loosen a reagent/amidite bottle cap to vent pressure on bottles.

## Cleaning and decontaminating chemical spills

***Notice***

Biosearch Technologies recommends users to follow their company's safety procedures on cleaning, decontaminating and disposal of hazardous chemicals. If needed, consult Biosearch Technologies for assistance in creating safety procedures.

In event of a chemical spill either on, inside, or on outside of instrument, Biosearch Technologies requires that if such an event occurs, user must immediately discontinue use of instrument and address chemical spill.

## Risk reduction: Solvent flammability

All solvents used on instrument are extremely flammable. Biosearch Technologies recommends that all users follow safe laboratory practice procedures when handling solvents on instrument. This includes keeping bottles tightly closed, stored in an appropriate flammable cabinet when not in use, and that all spills are immediately addressed according to facility policy. Any and all possible sources of static electricity or ignition should be avoided when instrument is in use.

Additional safety notes:

- Do not operate instrument unless you have been trained to do so.
- Do not operate instrument until you read and understand operating instructions. Thoroughly familiarise yourself with instrument and its controls.
- Always wear safety goggles and any other required safety equipment as required by your company's Personal Protective Equipment (PPE) policy.
- Never remove warnings displayed on instrument. Replace any worn or damaged labels. Contact Biosearch Technologies for replacement labels.
- Do not operate this instrument in an atmosphere containing explosive gases.
- Only electrical cords supplied by Biosearch Technologies are approved for use with this instrument.
- Instrument doors must be securely closed while instrument is in operation.
- If it is necessary to utilise an electrical extension cord to support this instrument, it is required that cord be grounded and rated to correct amperage.
- Never operate an instrument with safety guards removed.
- Electrical covers on instrument should only be removed by trained personnel.
- Disconnect main power supply before removing any covers.
- Do not substitute fuse or circuit breaker ratings.
- Connect instrument to suitable power supply in accordance with local electrical safety regulations.
- Instrument must be grounded during operation.
- Connect instrument to a suitable electrical supply according to local regulations.
- Do not break external connectors or connections while system is on.

- Do not bypass safety switches on instrument.
- Lock out-tag out all energy sources before servicing instrument.
- Check that all weights are supported before dismantling or adjusting any part of instrument.
- After performing adjustments or part replacement ensure that all parts are moving freely and will not cause damage to instrument.
- Do not modify instrument in any way. Unauthorised modifications can cause serious damage and void warranty.
- Disconnect air supply and electrical supply prior to removing safety guards.
- Certain components become hot during correct operation of instrument. Components are marked and care should be taken to avoid personal injury.
- Instrument should only be used in a ventilated area.
- Instrument should not be immersed in solvents.
- Do not use Acetone or abrasive cleaners.
- Biosearch Technologies accepts no responsibility for misuse of instrument.

### General maintenance safety

- Biosearch Technologies is responsible for instrument repairs. Always contact Biosearch Technologies before performing any repairs or maintenance on instrument.
- Do not operate faulty or damaged equipment. Always perform proper service and maintenance procedures.
- Do not service an instrument without thorough qualifications. Ensure familiarity with necessary service tasks.

### Cleaning safety

- Always wear safety goggles and any other required safety equipment as required by your company's Personal Protective Equipment (PPE) policy.
- Clean bottle threading and bottle caps before attaching new bottle.

### Heat safety

- Do not touch heated surfaces.

### Chemical safety

- Follow all Safety Data Sheet (SDS) recommendations.
- Do not touch, ingest, or inhale samples.

### Electrical safety

- Instrument operates on a ~110V/250VAC single phase supply. Electronics are located in lower back cabinets. Unless specifically instructed by a Biosearch Technologies representative you should unplug instrument from wall supply before opening control cabinet. Failure to do so exposes a possibility of an electrical shock. If it is necessary to open box with power ensure you are properly grounded and pay careful attention to warning labels inside box.

## Recognising safety precautions

**Notice**

If any safety stickers are damaged or missing, contact Biosearch Technologies for replacements. All warning symbols must be accordance with IEC 417.

### Warning safety precautions

Figure 1

Warning - Read Owners Manual Before Operation *(Figure 1)*.

### Caution safety precautions

Figure 2

Warning - Exclamation Point *(Figure 2)*.

Alerts user to presence of important operating and servicing instructions.

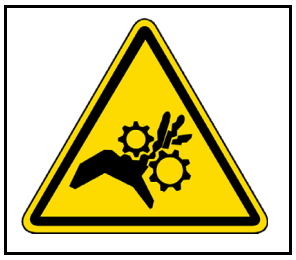


Figure 3

Warning - Pinch Point *(Figure 3)*.

Found on movable components where there is a chance of a body part getting caught in instrument.

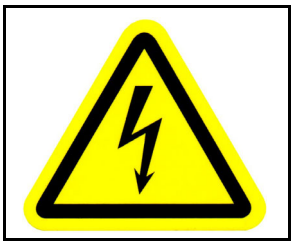


Figure 4

Warning - Electrical Shock Risk *(Figure 4)*.

Alerts user to presence of dangerous voltage and risk of electric shock.

Figure 5

Warning - Poison/Toxic Material *(Figure 5)*.

Indicates presence of substances that may cause harm if they enter body. Possible routes of exposure are through inhalation, skin contact, and ingestion. Hazards depend on toxic material, route of exposure, and concentration of material. Please refer to SDS for hazards associated with each chemical used on synthesizer.

Figure 6

Warning - Corrosive Material *(Figure 6)*.

Indicates corrosive substances that can eat away skin if there is direct contact. Such materials should always be stored at proper humidity and temperature conditions in proper cabinets. All employees who handle corrosive substances should be properly trained and wear gloves, protective clothing, and face protection.

## Stopping instrument

**⚠CAUTION**

Push Power Button (1) *(Figure 7)* to stop instrument in an emergency.

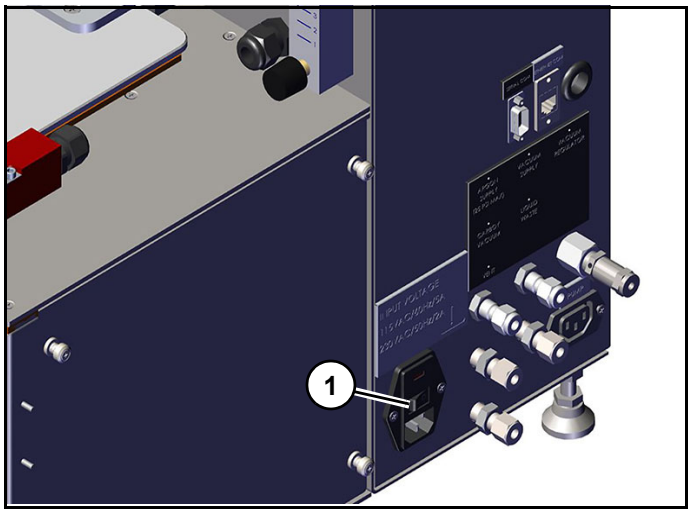



Figure 7

In case of emergency, operator can disable instrument by turning off power button (1) *(Figure 7)* to cut power to synthesizer. This will close all valves, stop all motion and release motor so that slide may be moved by hand.

Note: *(Figure 7)* shows power button on side of instrument. Some instrument models may have power button on back of instrument,

# MerMade 192E component identification

**⚠WARNING**

Read and understand equipment operators manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

## Component identification

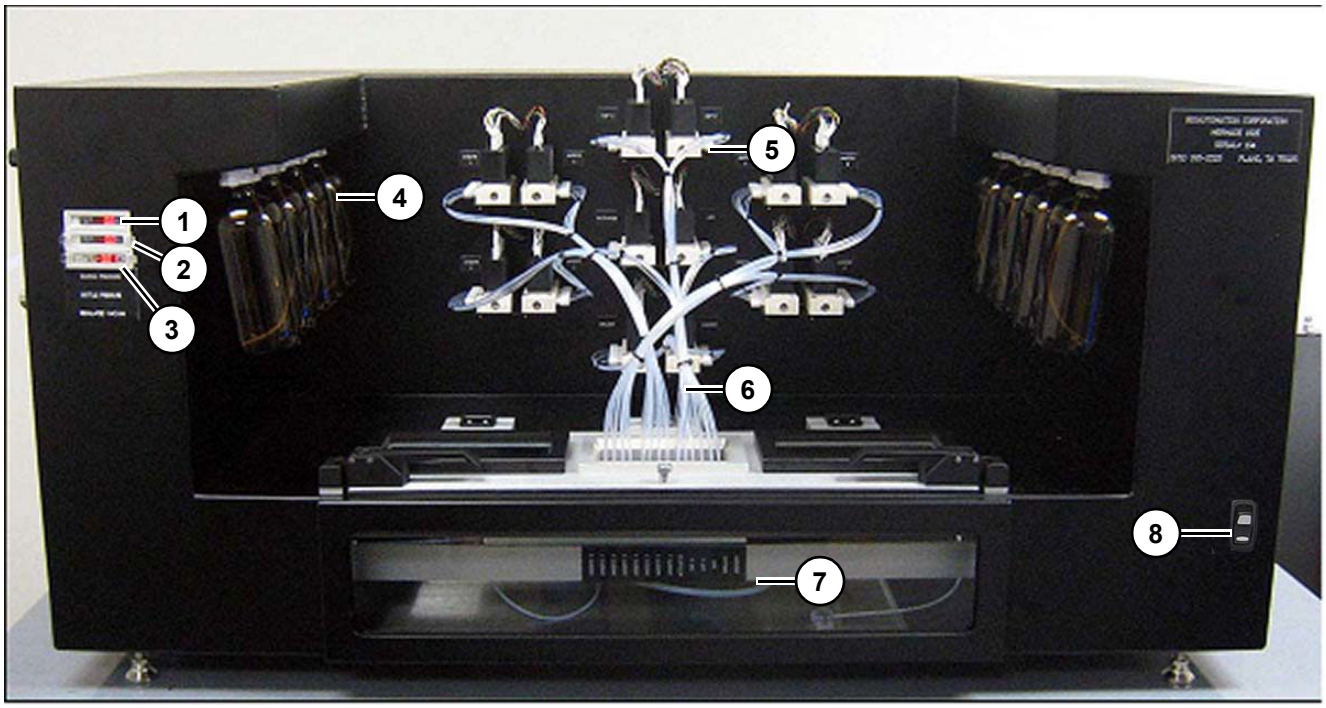



Figure 1

| REF# | DESCRIPTION | REF# | DESCRIPTION |
|---|---|---|---|
| 1 | Source Pressure | 5 | Dispense Valves |
| 2 | Bottle Pressure | 6 | Injections Lines |
| 3 | Vacuum Pressure | 7 | Drain Lines |
| 4 | Amidite Bottles | 8 | Light Switch |

## Component identification left side panel

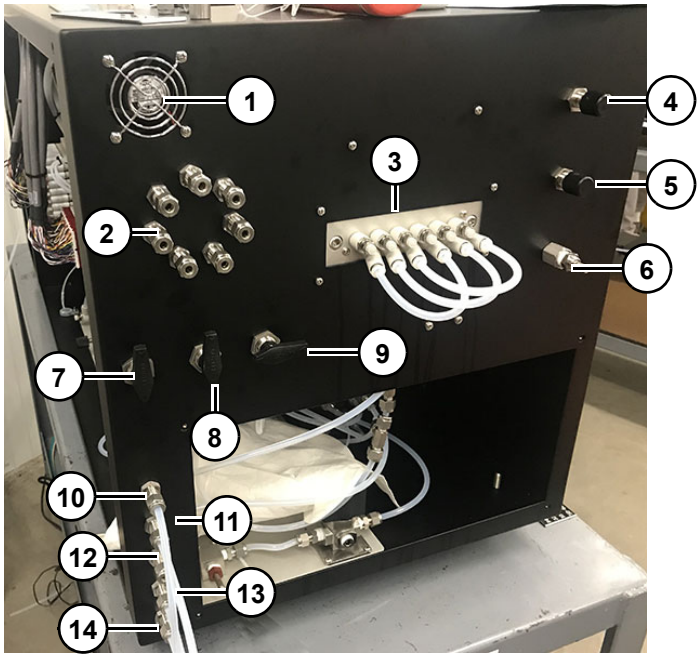



Figure 2

| REF# | DESCRIPTION | REF# | DESCRIPTION |
|---|---|---|---|
| 1 | Fan | 8 | Amidite Pressure Valve |
| 2 | Liquid Bulkhead | 9 | Reagent Pressure Valve |
| 3 | Gas Bulkhead | 10 | Argon In |
| 4 | Amidite Pressure Regulator | 11 | Vacuum In |
| 5 | Reagent Pressure Regulator | 12 | Vent |
| 6 | Vacuum Regulator | 13 | Carboy Vacuum |
| 7 | Source Pressure Valve | 14 | Waste Out |

## Component identification right side panels

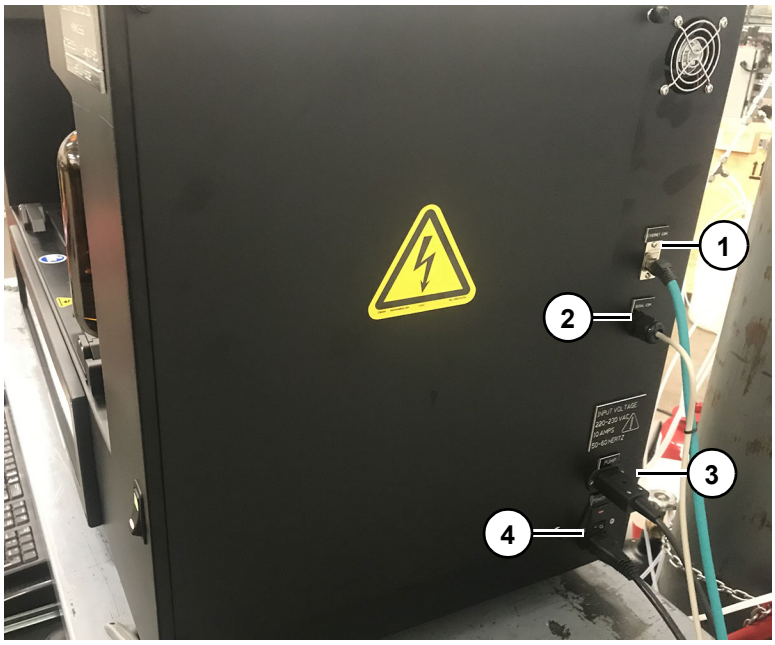



Figure 3

| REF# | DESCRIPTION | REF# | DESCRIPTION |
|---|---|---|---|
| 1 | Ethernet Connection | 3 | Pump Power |
| 2 | Serial Connection | 4 | Power Switch |

# MerMade 192E decal identification

**⚠WARNING**

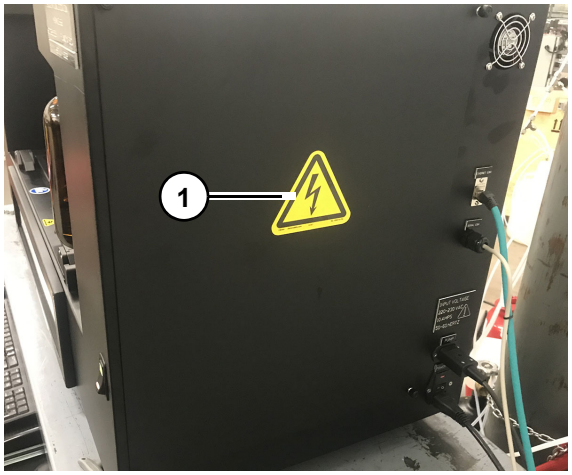
Read and understand equipment operators manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

## Decal identification top deck


Figure 1

| REF# | DESCRIPTION | REF# | DESCRIPTION |
|---|---|---|---|
| 1 | Biological Hazard<br>-Top of instrument (Qty-1) | 4 | Read Owner's Manual<br>-Top of instrument deck (Qty-1) |
| 2 | Corrosive Substance<br>-Top of instrument (Qty-1) | 5 | Pinch Point/Hand Crush Decal<br>Top instrument deck (Qty-1) |
| 3 | Toxic Material<br>-Top of instrument deck (Qty-1) | 6 | Instrument Identification (Qty-1) |

## Decal identification back panel and right side panel



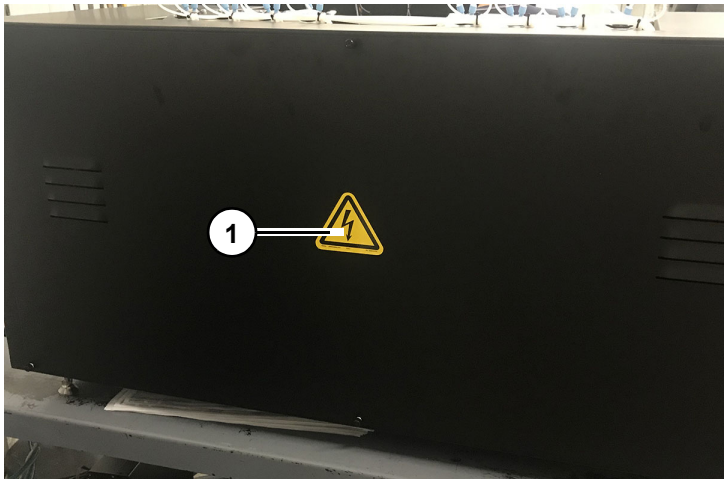




Figure 2

| REF# | DESCRIPTION | REF# | DESCRIPTION |
|---|---|---|---|
| 1 | High Voltage Decal<br>-Back Panel (Qty-1)<br>-Right Panel (Qty-1) | | |

# MerMade 192E description

**⚠WARNING**

Read and understand equipment operators manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

## Instrument overview

***Notice***

Biosearch Technologies accepts no responsibility for misuse of instrument.

Mermade 192E Oligonucleotide Synthesizer is designed to synthesise up to 192 columns, two 96 well plates of oligonucleotides in a single run using standard or modified phosphoramidite chemistry. Run may be paused to allow addition or removal of columns while synthesizer is in operation. Generating custom run protocols allows synthesis of oligos using standard or modified chemistry making instrument adaptable to a wide range of applications. Synthesis scales may be varied from 25 nmole to over 5 micromole for each plate.

Typical applications for oligos are for use in dye terminator sequencing reactions, gene building, polymerase chain reactions (PCR), hybridization, RT-PCR, anti-sense studies, siRNA, aptamers, and dual labeled probes. Oligos up to 150 bases in length have been made with a coupling efficiency in excess of 99%.

Software wizard guides operator through a series of checks and tests which ensure proper operation and prompts user for intervention when required. Once a synthesis has been started, all subsequent operation is handled by software and no further intervention is required for successful completion of synthesis. Software also provides a status window that reports on all aspects of synthesis.

During setup process, columns are placed in appropriate column chucks located in Synthesis Chamber. When synthesis is started chamber is flooded with argon to create a moisture free environment which is required for synthesis chemistry to work most efficiently. When synthesis begins, slide will move to align each row of columns under appropriate injection pins and dispense reagent by firing valve(s). This is repeated for each reagent specified by protocol.

### Hardware

Computer provides user with an interface to synthesizer. Communication between computer and hardware is via a motion controller and a digital Input/output card. Computer receives and processes synthesis information and translates it into motion and injection commands.

There are three main hardware components in instrument, Synthesis Chamber, Injection Head and Motion system. Columns are loaded into column plates which are then mounted on to a single axis slide which moves to align different reagent injection pins with each active column in appropriate order. A vacuum is applied to each plate in such a way that it may be independently drained. During course of synthesis argon is continually introduced into Synthesis Chamber and allowed to flow through a small vent at top of chamber. This ensures that any residual vapors are removed after each injection cycle to maintain an optimal synthesis environment.

### Large scale reagent delivery system

Scales as large as 5 umol per column have been validated on 192E and there is a special configuration for this instrument. Configuration uses 0.030" tubing from valve to injection head, which allows for faster delivery of larger volumes.

Either configuration will allow a customer to perform synthesis at scales of 50 nmol to 5 umol. However, there is a trade-off between speed and accuracy. Standard configuration provides more accuracy, but has longer delivery times at higher volumes for larger scales, and large scale configuration provides much fast delivery times but some accuracy is lost if used for smaller scales.

Note: There are special column holders, slide positioning changes, and specific columns which are all required to run 5 umol scale.

Please contact Bioserach Technologies for more information about larger scale synthesis on 192E.

## Software and machine operation

A PC running Poseidon synthesis software provides user interface for controlling synthesizer. Program controls all aspects of instrument during synthesis process as well as routines to simplify startup and shutdown procedures. A setup wizard is also provided to guide user through startup process.

Software uses script files to specify synthesis process. Script files contain all information needed to control way instrument synthesises oligos. Script files can be created and modified using supplied script file editor.

In addition, two log files are created for each plate used in a synthesis:

**Plate Log.** File records information about all events pertaining to the synthesis for that column. This includes data about oligo sequence and name, synthesis protocol and all injection and reaction time information generated during a synthesis.

**Plate Log Summary.** File is a short run summary that gives important information about synthesizer set up. These files may be referred to at a later date to aid analysis or protocol development.

Software also facilitates routine maintenance of instrument via service screen. These features control low level service such as motion and valve settings and can be password protected.

***Notice***

Windows and Anti virus software may update automatically and interrupt synthesis run. Perform system updates on a regular basis to avoid loss.

# MerMade 192E installation

**⚠WARNING**

Read and understand equipment operators manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

## Installation

MerMade 192E is a single axis (x only) bench top synthesizer and needs to be located so there is adequate space to access front and sides to allow unrestricted flow through gas and vacuum lines. There must be sufficient vertical clearance to allow for a ventilation system to vent any hazardous fumes that may be present when lid is open or reagent bottles are being changed.

### Safety requirements

Instrument must always be operated in manner specified by Biosearch Technologies. All operators should be versed in good laboratory practices and trained in safe operation and use of instrument.

Following equipment should be available or readily accessible at all times:

- Gloves
- Lab Coat
- Eye Protection
- Eyewash Station
- Fire Extinguisher (Halon)
- First Aid Equipment
- Spill Cleanup Kit

### Space requirements:

Width

- 122 cm (48")

Height

- 61 cm (24")

Depth

- 51 cm (20")

Weight

- 34 kg (75 lbs)

### Clearance requirements:

Left side

- 31 cm (12")

Right side

- 31 cm (12")

Back

- 31 cm (12")

Front

- Access required at all times

### Ventilation requirements:

**⚠WARNING**

Fumes generated in Reaction Chamber are hazardous and adequate ventilation is necessary to ensure a safe operating environment.

For safe operation one of following must be installed.

- Fume Hood with an average airflow of 300-500 scfm.

- A dedicated duct for exhaust of chemical vapors with a draw at least equal to 100 scfm.

There are four sources of fumes:

**Vent line.**

Vent line is located on right side of instrument. This is necessary because Synthesis Chamber is sealed and continuous flow of argon into chamber causes a gradual build up of pressure as run proceeds. Vent Line ensures pressure can be reduced by directing excess gas away in a controlled manner. Vent line must be suitably vented, such as a fume hood.

**Starting/ending a run.**

When a run is started or completed, it is necessary to open door to Reaction Chamber in order to load or remove Vacuum Chucks. User is exposed to any fumes that may have accumulated inside chambers. Particularly a concern when a run has just completed since fumes will still be concentrated inside synthesizer. It is important to have adequate ventilation to clear fumes before they are inhaled by operator.

**Waste system.**

Although waste system is a closed system from time to time it is necessary to replace waste container when full. This should be performed in vicinity of synthesizer and where there should be adequate ventilation.

**Vacuum source.**

Vented fumes from vacuum pump must be controlled in same manner as Vent Line from Reaction Chamber. Vent port on pump must be attached to a line which runs to a suitably vented place such as a fume hood.

## Environmental conditions

Temperature

- 5 °C to 35 °C (41 °F to 95 °F)

Relative Humidity

- 40-60% relative humidity at 25 °C - 35 °C

Pollution Degree

- 2

Altitude RangeTested

- to 8200 ft (2500 m) above sea level

Installation Category

- 2

Mains Supply Fluctuations

- 110 VAC/230 VAC

## Electrical requirements

Table specifies electrical operating requirements for instruments in various locations:

| Location | Voltage | Amps | Frequency |
|---|---|---|---|
| USA/ Canada | 110 VAC | 4 A | 60 Hz |
| EC | 230 VAC | 2 A | 50 Hz |
| Japan | 110 VAC | 4 A | 60 Hz |

MerMade requires a dedicated 1.5 kVA power line and associated ground connection.

Fuses:

- Power Supply 3 A

## Waste system

Waste system consists of two 20 liter waste carboys and a dual headed vacuum pump.

| Vacuum pump requirements | |
|---|---|
| No. of Heads | 2 |
| Inlet Rating | 30 inHg |
| Valve Rating | 30 inHg |
| Permissible temperature | 5-40 °C |
| Flow Requirements | 17 Liters/minute (48Torr/28inHg) |
| Port Connection | ¼" Stainless Steel Swagelok |
| Tubing | PTFE – ¼" OD – 1/8" ID |

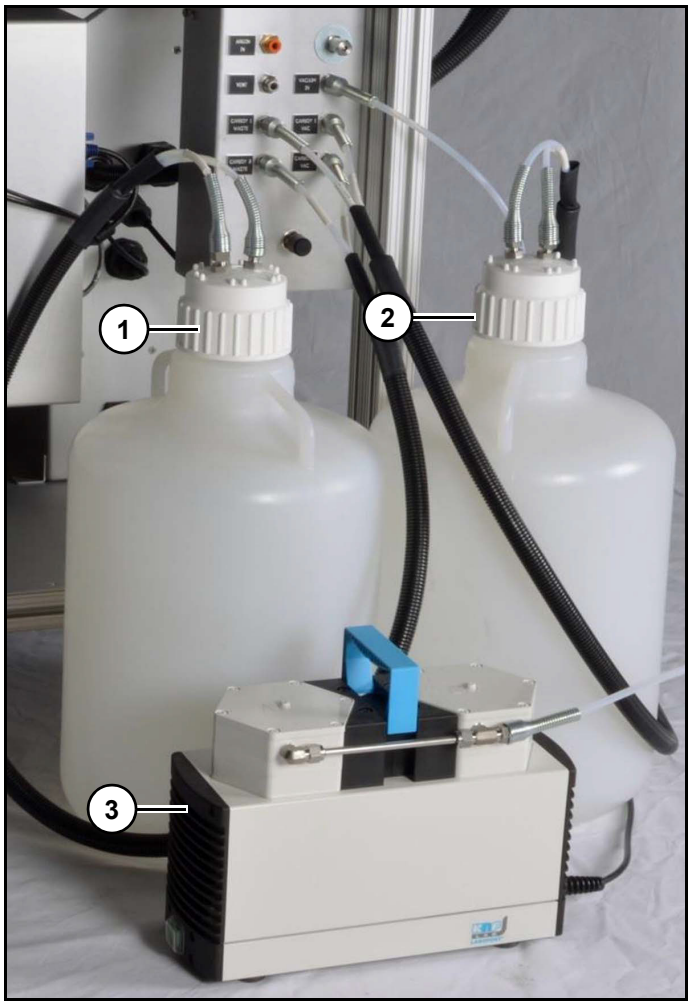



Figure 1

Refer to *(Figure 1)*.

The carboy on left (1) collects waste from plate 1 and waste trays. Carboy on right (2) collects waste from plate 2. Vacuum pump (3) must always be in ON position for software to properly control it.

## Startup equipment

In order to perform a synthesis run on MerMade, following supplies will be needed:

**Gas Regulators.**

Instrument requires an argon source to pressurise bottles and to purge Synthesis Chamber before starting synthesizer. Argon inlet port, a ¼" Swage Lok compression fitting, is located on right hand side of the Synthesis Chamber. A regulator with following rating will need to be supplied:

- Inlet pressure:150 psi (10 bar)
- Regulating range: 0-50 psi (0-1 bar)
- Maximum Outlet Pressure: 15 - 150 psi (1 - 10bar)
- Temperature Range: 40 °F - 150 °F (4 °C - 66 °C)
- Amidites/monomer pressure 6-8 psi

Regulators rated outside these specifications may damage regulators on instrument.

**Gas Supply.**

Use high purity argon or Nitrogen for bottle supply to maximise life of chemicals and to ensure best quality product.

**Collection Plates.**

Collection plates are needed for post processing run when synthesis is complete.

Example: PAX2000150 2.0 Ml 96 Well ritter plate.

**Sample Dryer.**

Once Deprotection stage is complete, product will need to dried down so it can be re-suspended in appropriate media. Please consult Biosearch Technologies for help in choosing an appropriate unit for application.

**Uninterruptible Power Supply (UPS).**

A SMART UPS from APC is strongly recommended so instrument can perform an intelligent shutdown in event of power failure and run can continue when power is restored. MerMade will pause all columns prior to Deblocking step which will allow for resumption of synthesis with minimum impact on quality.

Biosearch Technologies recommends:

UPS: APC Smart-UPS 1000XL
120V Model Part # SUA1000XL
www.apc.com

## Synthesis chemicals

Biosearch Technologies will assist you in selecting an appropriate set of chemicals based on your synthesis needs at start-up.

When first starting MerMade it is recommended to buy smaller size bottles (e.g. 0.5 g or 1 g for phosphoramidites, 250 mL for reagents) since instrument will be starting a with smaller numbers of samples. Once protocols have been established and usage has been increased switch to larger sizes of reagents.

Recommended Chemicals for Synthesis:

| Reagent | Formulation |
|---|---|
| Acetonitrile | <10 ppm |
| Deblock | 3% DCA in DCM |
| Cap A | THF/Lut/$Ac_2O$ (8:1:1) |
| Cap B | 16% MeIm/THF |
| Activator | 0.25 M ETT in ACN |
| Oxidizer | 0.02 M $I_2$ in THF/Pyridine/$H_2O$ 70/20/10 (w/v/v) |
| Amidite | 1 g in 20 ml ACN (~0.05 M) |

Other chemical formulations will work but may need adjustments to Standard Biosearch Technologies protocols to get optimal results.

## Reagent preparation

Phosphoramidites are susceptible to moisture and needs to be done in an inert environment. If good techniques are developed when diluting chemicals it is not necessary to perform this task in an argon filled chamber. Please contact Biosearch Technologies if help is needed preparing reagents.

**Acetonitrile.**

Acetonitrile is available in a range of sizes and quality. To ensure optimal quality in final product, an acetonitrile with a water content of not more than 30 ppm for amidites should be used. Biosearch Technologies recommends less than 10 ppm.

Acetonitrile in 4 L bottles of acetonitrile should be used. If smaller bottles are used, they will need to changed more often, which allows a greater chance of moisture to enter system. If purchasing large quantities of acetonitrile, contact your local safety officer or authorities for more information on safety and storage regulations.

## Columns

MerMade synthesises oligos in column format. Synthesising in this format has following advantages:

- Ease of use.
- Higher Yield.
- Better Quality.
- Ability to synthesise longer oligos.

Columns with first base derivatised also has additional benefit of being color coded to reduce chance of loading wrong column during setup process. During run set up the user is presented with a color coded column map and preparing run simply requires putting appropriate colored column in each location.

Columns are available in 50 nmole to 5 µmole sizes and can be ordered directly from Biosearch Technologies.

100 columns (25 of each base) will be provided to perform some test runs.

Columns can also be packed with custom CPG. Contact Biosearch Technologies for more information.

## Chemical safety

**⚠WARNING**

Chemicals used on instrument are hazardous to varying degrees. Be aware of these hazards and review Safety Data Sheets for safe handling and storage of each chemical.

## SDS

Safety date sheets provide information regarding:

- Safety considerations.
- Physical properties.
- Health warnings.
- First aid procedures.
- Disposal procedures.
- Spill cleanup procedures.

SDS may vary between manufacturers and may be periodically updated. Ensure current SDS is provided by supplier.

## Waste disposal

**⚠WARNING**

Follow all local and national regulations for waste storage and disposal.

When working with waste system always wear safety goggles and gloves and ensure that area is well ventilated. Always have a spill clean up kit within easy reach and be aware of location of nearest first aid and eye wash stations.

Use proper waste containers for the waste classification.

Use stainless steel Swagelok fittings in waste disposal system. Waste chemicals will corrode brass fittings.

## Setting up MerMade

**⚠CAUTION**

Installing instrument should only be done by a Biosearch Technologies Service Technician.

## Shut down

### Period of inactivity (more than a few days)

1. Remove reagents from instrument and replace with bottles of acetonitrile.
2. Flush any residual chemicals from lines and replaced with acetonitrile. Particularly important for activator lines, most activators reagents will crystallise over time and permanently damage valves.
3. On a daily basis flush all reagent lines for a few seconds to remove any moisture that reagents may have accumulated. Do this from Injection Head Service Screen.

# Solid-phase Oligonucleotide Synthesis

## Introduction

**⚠WARNING**

Read and understand equipment operator's manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

Always follow your facility's PPE program when operating or performing maintenance on this instrument.

### Solid-phase Oligonucleotide Synthesis

Most common types of support used in Synthetic Oligonucleotide Synthesis single stranded DNA are Controlled Pore Glass (CPG) and Polystyrene (PS). Supports with first nucleotide already attached are referred to as (Standard) and supports without a nucleotide attached are known as (Universal). Phosphoramidite synthesis proceeds in 3' to 5' direction with one nucleotide added per cycle. Refer to *(Figure 1)*.

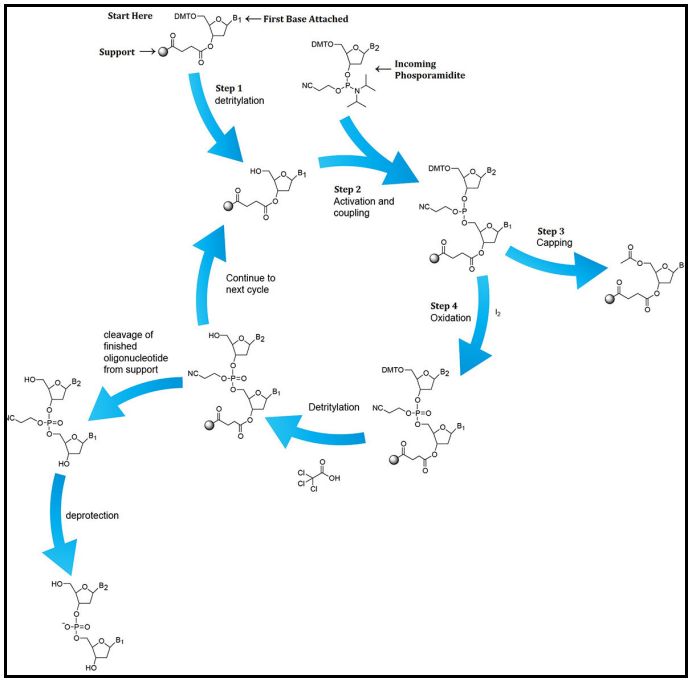



Figure 1

### Detritylation

Also known as Deblock step, is process of removing Dimethyloxytrityl (DMT) group from 5′ end of existing nucleotide using an acid. Most common acids used are 3% trichloroacetic acid (TCA) or 3% dichloroacetic acid (DCA), in an inert solvent such as dichloromethane or toluene. Molar exposure required to reach saturation is similar for both DCA and TCA but DCA is preferred over TCA because stronger acidity of TCA makes depurination of oligo a greater concern. When DMT group is successfully removed an orange solution can be observed. Solid support is then washed with acetonitrile (ACN) to remove any acid left behind. Refer to *(Figure 2)*.

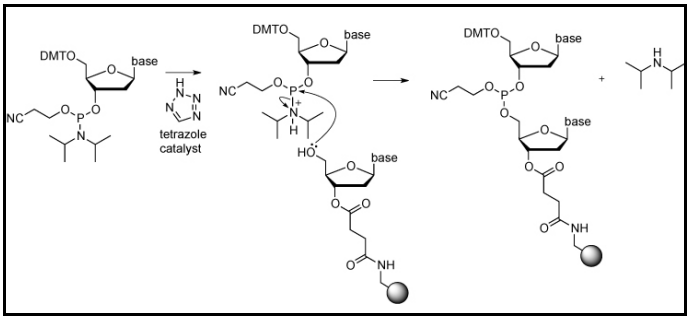



Figure 2

### Activation and Coupling

Once DMT group is removed, 5'-hydroxy on existing oligonucleotide is now open to bond with next incoming base. An acidic catalyst, also known as an Activator, is combined with new incoming phosphoramidite in a solution to remove 3' protecting group on phosphoramidite. 5'-hydroxy group then reacts with incoming nucleoside phosphoramidite to form a weak phosphite triester linkage, known as Coupling. Some common acidic catalysts used are 2-ethylthiotetrazole (ETT) and 4, 5-dicyanoimidazole (DCI). Solid support is then washed with acetonitrile (ACN) to remove any acidic catalysts and uncoupled incoming phosphoramidites. Refer to *(Figure 3)*.

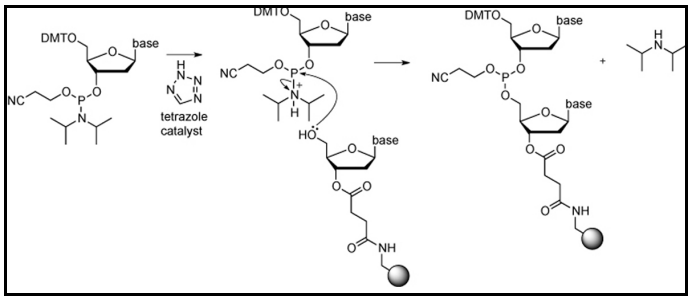



Figure 3

## Capping

After coupling has completed, a small percentage, (<0.1 to 1.0%), of 5'-hydroxy groups on existing oligonucleotide may not have reacted. These sites need to be permanently blocked off to prevent any further chain elongation which will lead to sequences with deleted bases, also known as (n-1) shortmers. Solid support is washed with a mixture of acetic anhydride in solution (Cap A) and 1-methylimidazole in solution (Cap B) to "Cap off" these active bonding sites.Solid support is then washed with acetonitrile (ACN) to remove any excess capping solution. Refer to *(Figure 4)*.

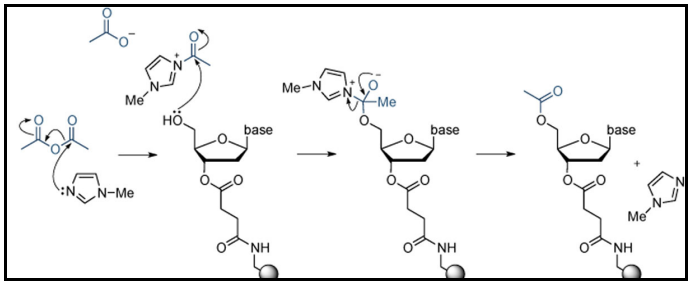



Figure 4

## Oxidation

Newly formed phosphite triester linkage is weak and will need to be reinforced to further elongate single stranded DNA. A mixture of water, iodine, and a weak base (pyridine, lutidine, or collidine), known as Oxidiser, oxidises phosphite triester linkage creating a strong phosphate diester internucleosidic linkage. Solid support is then washed with acetonitrile (ACN) to remove any excess water left behind in oxidation solution. Refer to *(Figure 5)*

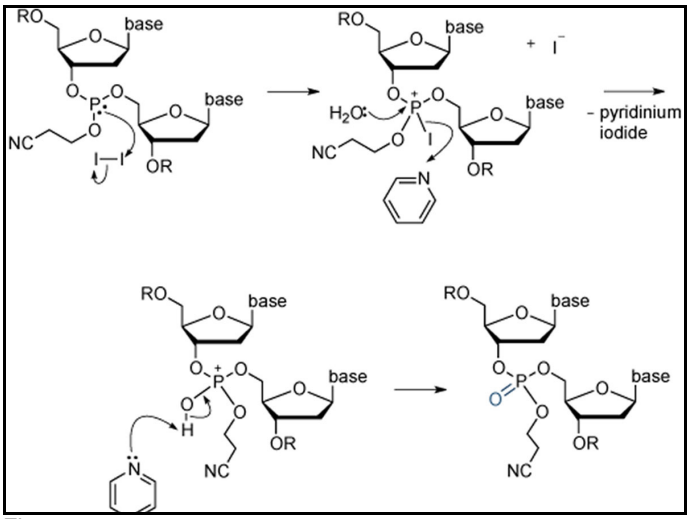



Figure 5

# MerMade 192E operation

**⚠WARNING**

Read and understand equipment operator's manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

## Software overview

***Notice***

Biosearch Technologies accepts no responsibility for misuse of instrument.

A PC running Poseidon synthesis software provides a user interface for controlling synthesizer. Software controls all aspects of operation. A setup wizard is also provided to guide user through startup process.

Software uses script files to specify synthesis process. Script files can be created and modified using supplied Script File Editor.

Two log files are created for each column used in a synthesis.

- Plate Log: File records information about all events pertaining to synthesis for specific plate including data about oligo sequence, name, synthesis protocol and all injection and reaction time. *(Figure 1)* Logs are located at C:\Users\Public\Documents\BioAutomation\Poseidon\Logs
- Plate Log Summary: File saves important information about synthesizer set up. Files can be referenced to aid analysis or protocol development. File does not contain all injection and drain step information and provides necessary information to duplicate a run.

**Example of Plate Log *(Figure 1)*.**

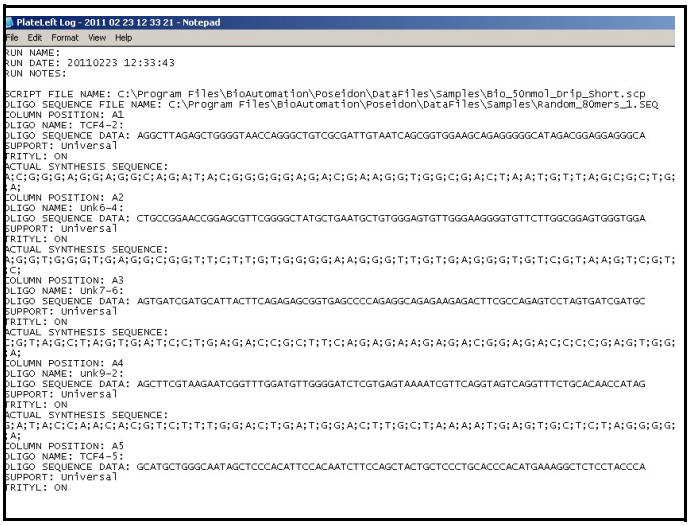



Figure 1

Software also facilitates routine maintenance of instrument through service screens which control low level services such as motion and valve settings.

Note: Service screens can be password protected to prevent unauthorised access.

## Synthesis process

Synthesis is typically carried out from 3'->5' end of oligonucleotide. Reactions take place in columns and are initialised using a controlled pore glass (CPG) or polystyrene substrate (PS) sandwiched between two frits. User specifies support, bases and software makes necessary adjustments during synthesis. A hole in bottom of each column allows removal of reagents by vacuum when reaction is complete.

Software guides user through setup process to start synthesis. Usually, before synthesis begins, chamber is flooded with argon to create a moisture free environment. When argon purge initialisation step is completed, software moves slide to align each column well under appropriate injection line and actuates corresponding reagent valve or valves. When reagent injections are finished, software pauses for chemical reactions to complete inside columns.

Synthesis quality and yield depend on synthesis scale wait times, volumes, and vacuum pulse settings specified by user in script file. Reagents are then removed from column(s) by applying vacuum to underside of reaction block.

Once column is drained, underside of column(s) is returned to pressure of synthesis chamber to prevent premature drainage of reagents during next injection cycle. After each stage, columns can be washed with acetonitrile several times to make sure all unused reagents are removed prior to next stage of synthesis. Number of washes required between each reagent addition will vary depending on desired quality and yield of the synthesis.

Example, in a synthesis generating a higher quality product for use in applications such as gene building, a higher number of washes is recommended after Deblock and Oxidisation steps since these reagents contain acid or water which will affect synthesis reaction and ultimately quality of final product. By modifying script files, user can easily create custom protocols to meet specific requirements.

Post-synthesis processing stage is a three-step process. First, oligonucleotide product is cleaved from solid support by application of a suitable reagent. Reagent will vary according to chemistry but typically ammonium hydroxide is used. Second, product is drawn through column, via vacuum, into a receiving vessel which is then sealed. Final stage allows sample to cool (if it was heated), evaporating cleaving reagent and then re-suspending product in an appropriate buffer.

## Instrument start-up

Poseidon software provides a user interface to control all aspects of instrument including synthesis setup and service routines.

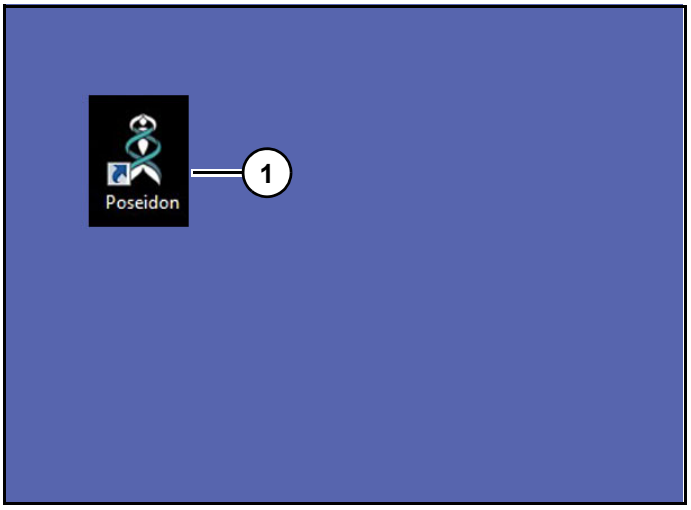



Figure 2

1. Double click "Poseidon program icon" (1) *(Figure 2)* on desktop.

### Initialization screen

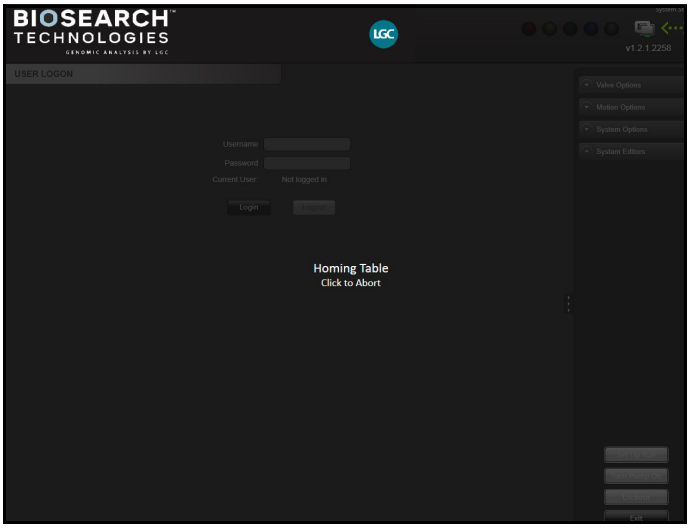



Figure 3

Initialization screen *(Figure 3)* is shown after startup and instrument will home motion system.

Note: If homing procedure fails there may be a problem with communication to instrument or an issue with motion hardware. Please contact Biosearch Technologies for support.

## Log in screen

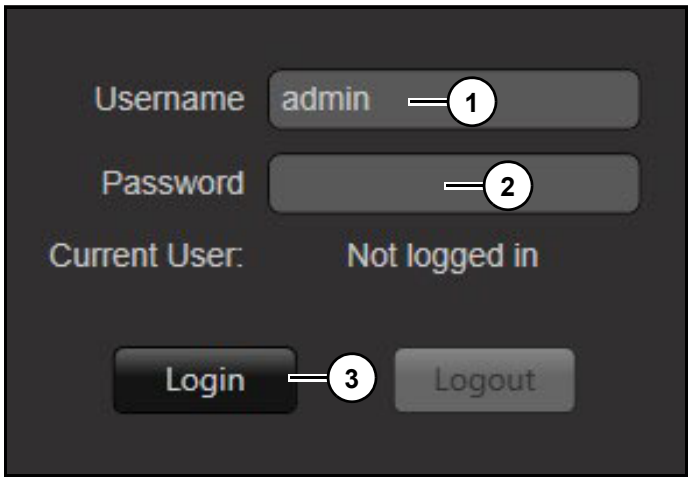



Figure 4

Refer to *(Figure 4)*.

1. Enter admin in "Username" (1). Leave "Password" (2) blank.
2. Click "Login" (3).

## Main screen

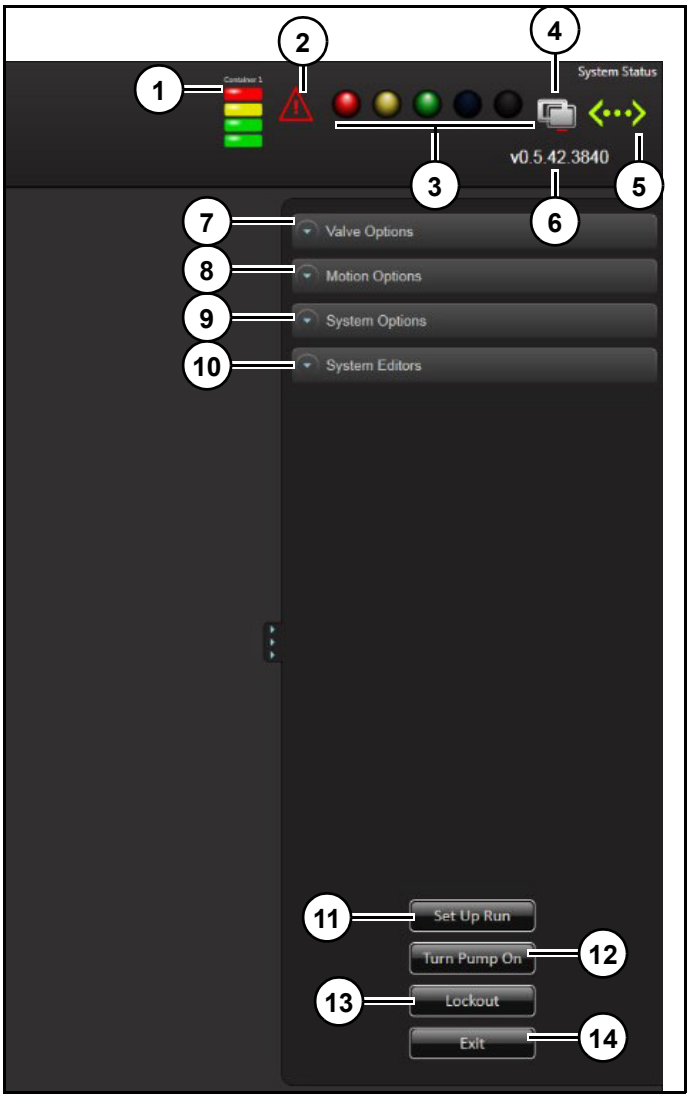



Figure 5

Refer to *(Figure 5)*.

**Waste level indicator (1):** Displays waste level (instrument specific).

**Error indicator (2):** Displays instrument error.

**Light tree (3):** Displays instrument status.
Note: Light tree is customisable.

**Remote connection (4):** Indicates remote connection.

**Communication status (5):** Displays communication status.

**Software version (6):** Displays instrument's software version.

**Valve Options (7):** Opens valve options display screen.

**Motion Options (8):** Opens motion options display screen.

**System Options (9):** Opens system options display screen.

**System Editors (10):** Opens system editors display screen.

**Set Up Run (11):** Opens setup wizard. Allows user to use various steps to ensure synthesizer is ready to perform a synthesis.

**Turn Pump On (12):** Instrument is equipped with ability to control pump during and after synthesis. When starting a run, pump will automatically turn on and then turn off once synthesis is complete. In screens where pump is needed it may be necessary to use this button to turn pump on. Pump will need to be turned on for vacuum calibrations.

**Lockout (13):** Logs out current user and returns to login screen.

**Exit (14):** Exits software.

## Valve Options screen

Accesses controls involving liquid, vacuum and argon purge valves.

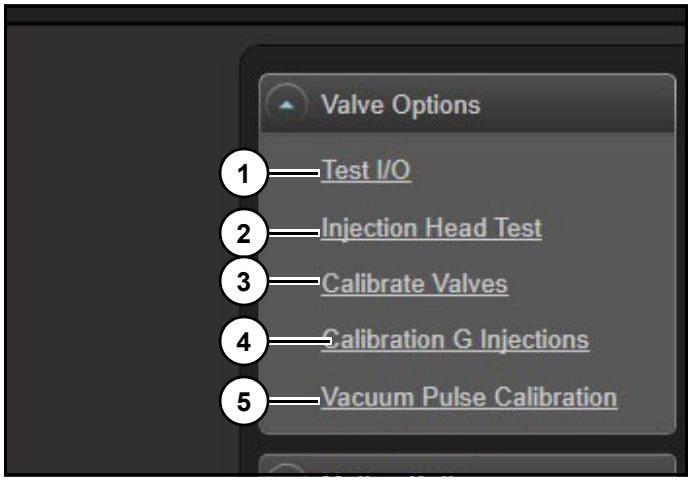



Figure 6

Refer to *(Figure 6)*.

**Test I/O (1):** Opens valve input and outputs screen.

**Injection Head Test (2):** Opens head valve test screen.

**Calibrate Valves (3):** Opens valve calibration screen.

**Calibration G Injections (4):** Opens injections calibration screen.

**Vacuum Pulse Calibration (5):** Open vacuum calibration screen.

### Test I/O screen

Allows user to operate all liquid valves in system as well as vacuum and argon valves.

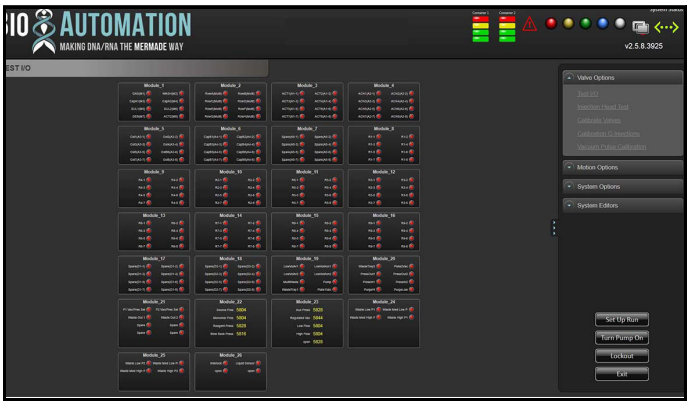



Figure 7

Refer to *(Figure 7)*.

To test a valve:

1. Click button corresponding to valve.
2. Click button again to close valve.

Note: Value will stay open until button is clicked again. In case of emergency user can turn off power on right side of control box to shut all valves off.

Note: Valve test box is used mainly for diagnostic purposes and to drain bottles.

## Injection Head Test screen

Allows user to test and prime lines.

**⚠WARNING**

Do not put hand in synthesis chamber when using Injection Head Test screen. Instrument moves XY table to align injection head over waste tray.

Always wear safety goggles and gloves when using this screen.

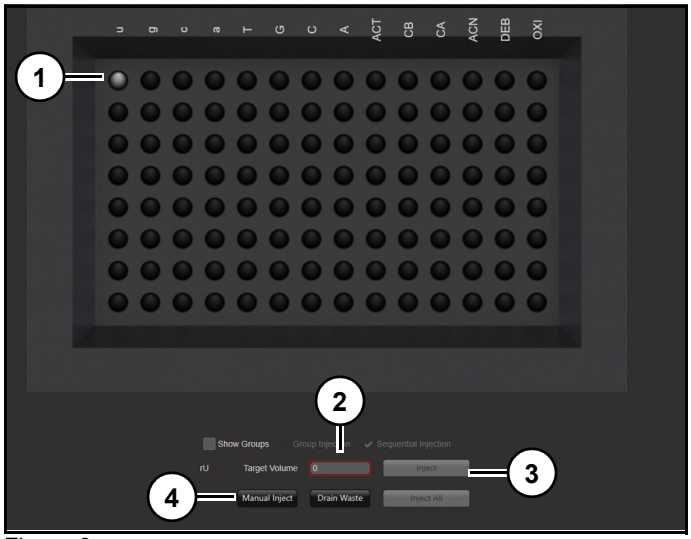



Figure 8

Refer to *(Figure 8)*.

To fire a single valve:

1. Select one valve in displayed valve array (1).
2. Enter a volume (2).
3. Click "Inject" (3)

Note: Pressing and holding manual inject button (4) will open all valves in selected row until button is released. This is often used for priming lines.

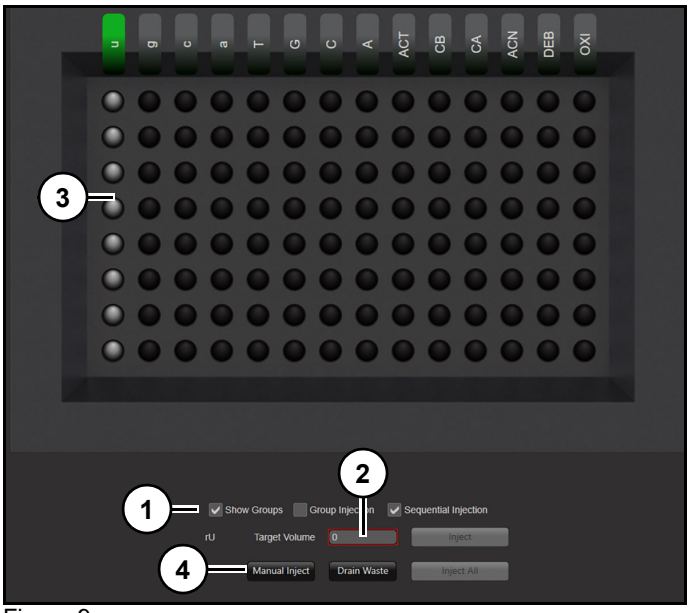



Figure 9

Refer to *(Figure 9)*.

To fire a group of valves:

1. Check "Show Groups" (1).
2. Enter a volume (2).
3. Select group of valves (3).
4. Click and hold "Manual Inject" (4) to inject all eight lines of a reagent.

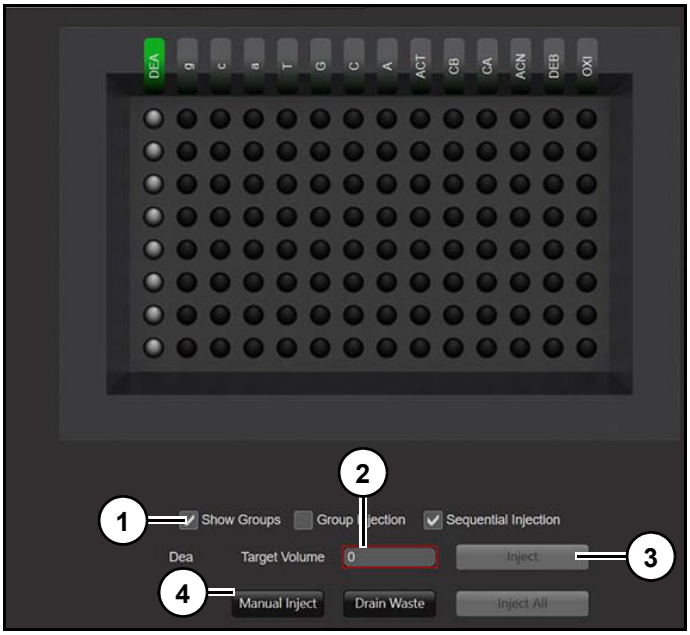



Figure 10

Refer to *(Figure 10)*.

To test a row of reagents:

1. Check "Show Groups" (1). Valve will be groups with a white line.
2. Enter a value into target volume box (2).
3. Click "Inject" (3).

This will inject selected volume, for all valves in array according to calibration table, into waste tray. To prime lines and confirm valves as a group are working, press and hold manual inject button (4).

Note: Pressing and holding manual inject button (4) will open all valves in selected row until button is released. This is often used for priming lines.

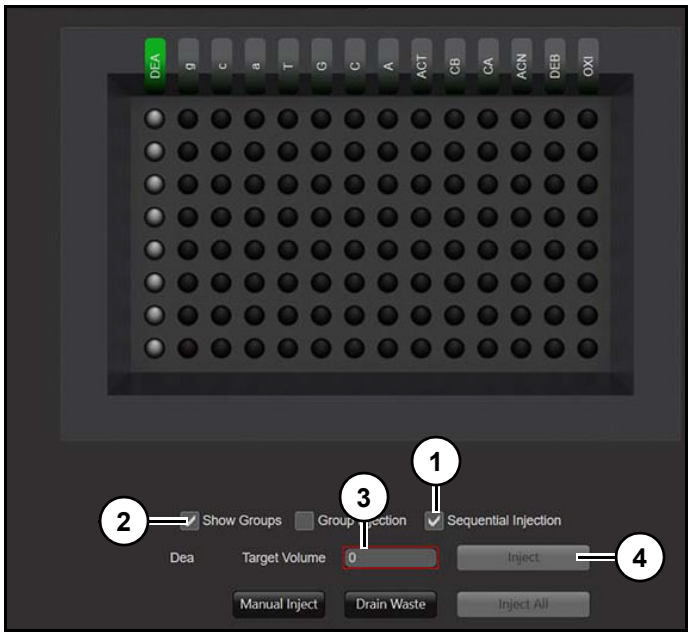



Figure 11

Refer to *(Figure 11)*.

To test valves in group individually:

1. Check "Sequential Injection" (1).
2. Check "Show Groups" (2). Valve will be groups with a white line.
3. Enter a value into target volume box (3).
4. Click "Inject" (4).

Valves will be fired in group, one at a time, in order of back to front. Valves that are grouped together will be fired as a group, sequentially in a group. Click on "Show Groups" (2) to display valves that are grouped together.

Note: Target volume must be within calibrated volume range. If box is outlined in red valve cannot be fired.

## Calibrate Valves screen

Allows user to check reagent valves to ensure correct volume is being delivered. Valve calibration is used to determine number of milliseconds that valve must be open to get correct volume delivered as defined by user in script file.

Each valve can be calibrated individually when injected by itself and not injected as part of a group. Amidite/reagent pressure must be kept stable to insure calibration accuracy.

Note: If using single injection setting in script file it is not necessary to calibrate for group injections.

Liquid dispensing valves can each be calibrated for a minimum volume and a maximum volume, a minimum time and a maximum time, or a minimum weight and a maximum weight. Software will then determine all (volumes/times/weights) between minimum and maximum points. Each valve must be calibrated for a minimum and a maximum point and values outside these points cannot be injected. Valve liquid delivery will be more accurate if more points are calibrated between these points.

For example, Aux 2 valve is calibrated for 5 µL, 50 µL, and 200 µL. If a volume of 75 µL is specified in a script file, then software will calculate volume based on line information created from the 2 closest calibration points for Aux 2 valve.

Note: An uncalibrated/computer calculated point will be more accurate if it is located closer to a calibrated point.

Note: Valves can be calibrated three different ways; by time, by volume, and by weight. Time option is selected by default.

Minimum calibrated point must be equal or less than lowest volume and maximum calibration point must be equal or greater than highest volume being delivered in all script files being used.

**Calibration by volume:**
Volume is entered and valve will open for a set number of milliseconds (ms). User can only change injection volume amount (µL) based on volume measured by user, typically using a pipette. Software will inject liquid at a set injection time (ms). This is most preferred method and is default setting.

**Calibration by weight:**
User will collect liquid injected at a given time (ms) and record injection weight (mg). Uses measured weight to calculate volume based on density. This option will only be accurate if correct density (g/cm³) of reagent is entered in manage reagents screen.

**Calibration by time:**
Valve will remain open for set time (ms) to reach desired volume. User must measure dispensed volume in order to determine if time that valve remains open is correct. If delivered volume is not enough, valve open time can be opened for more time (ms). Customers typically measure dispensed volume with a calibrated pipette.

## Calibrating valves

**⚠WARNING**

Always wear safety goggles and nitrile gloves and be careful not to open head so far that lines are pointing directly at your face. Head should only be open far enough to permit collection of reagent from valve being calibrated.

Valves have to be calibrated manually by using an Eppendorf tube and a pipetman, or with a scale if by weight selection is preferred.

## Switching calibration methods

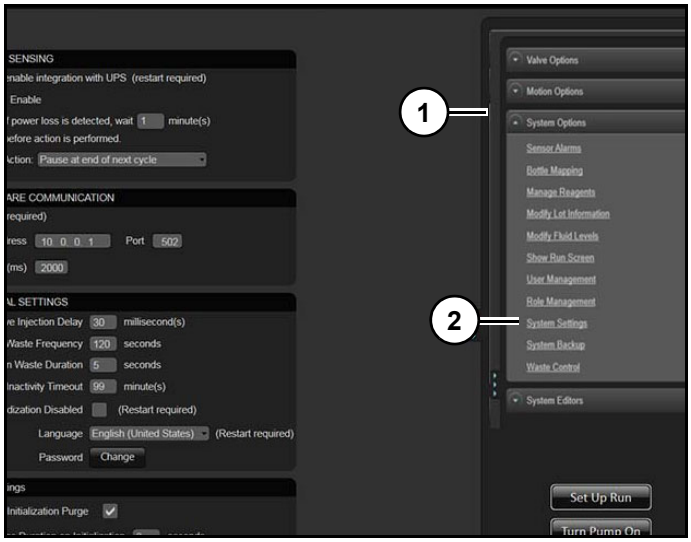



Figure 12

Refer to *(Figure 12)*.

1. Click "System Options" (1).
2. Click "System Settings" (2).

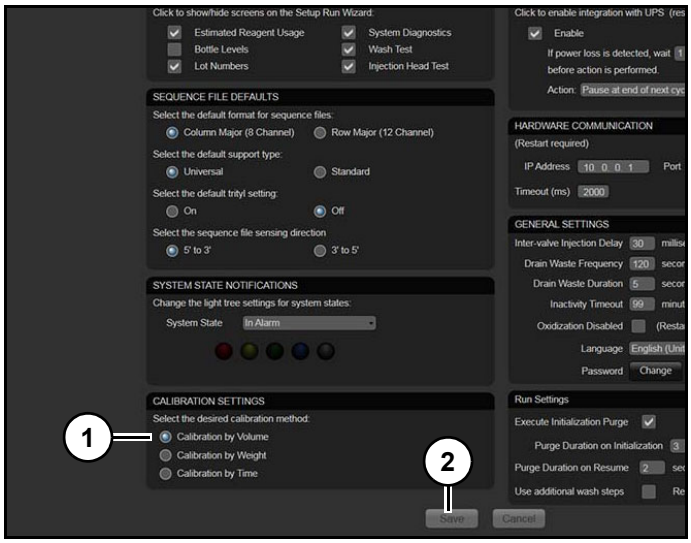



Figure 13

Refer to *(Figure 13)*.

3. Select desired calibration method (1).
4. Select "Save" (2).

Note: Changes in calibration method may result in changes to calculated values as calibration points. Please verify calibration values after changing method.

## Adding calibration points

**⚠WARNING**

Calibrate with caution as exposure to chemicals and fumes is possible. Please wear appropriate PPE. Refer chemical to SDS for appropriate handling.

Allows user to add a calibration point to current set of points for a single valve. Software comes pre-loaded with reference points for all valves. However, user should add more points, delete points, etc. as necessary. Calibration is an important aspect of instrument setup. It is crucial to be consistent.

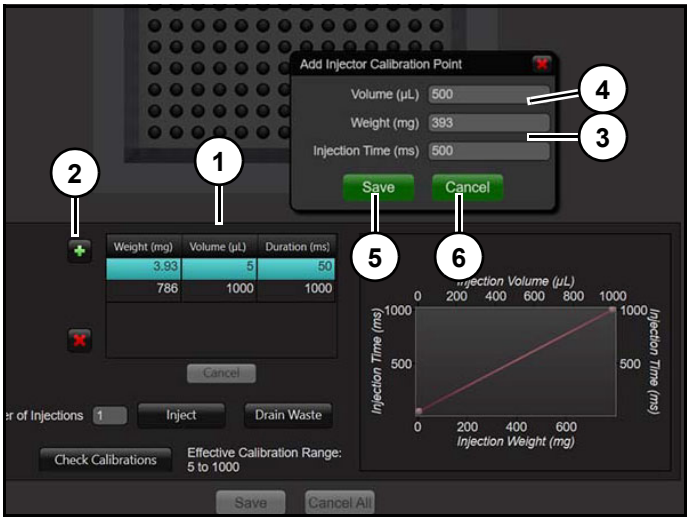



Figure 14

Refer to *(Figure 14)*.

In *(Figure 14)*, Aux 2 Valve has volume points for 5 µL, 50 µL, and 200 µL (1).

To add calibration point:

1. Click "Add Cal Point" (2).
2. Enter estimated time (ms) in "Injection Time" (3).

Note: Valve should be open to inject at least 400mL.

3. Enter estimated delivery volume (µL) in "Injection Volume (µL)" (4).

Note: Valve should be open to inject at least 400 µL. Delivery volume will be adjusted to be accurate in calibration procedure.

4. Click "Save" (5). New data point will appear in valve screen.

Note: Click "Cancel" (6) to delete changes.

**⚠WARNING**

Always wear safety goggles and nitrile gloves and be careful not to open head so far that lines are pointing directly at your face. Head should only be open far enough to permit collection of reagent from valve being calibrated.

Once desired point is added it is necessary to adjust calibration entry so correct volume is injected. User will need to collect dispensed liquid, measure it, and then enter value into "Injection Volume (µL)" (1).

Typically, this is done by collecting liquid into a 1-2ml conical bottom Eppendorf tube and measuring dispensed volume using a pipette.

When performing valve calibration it is easier to unlock and raise Injection Head so that injection lines are more accessible *(Figure 15)*.

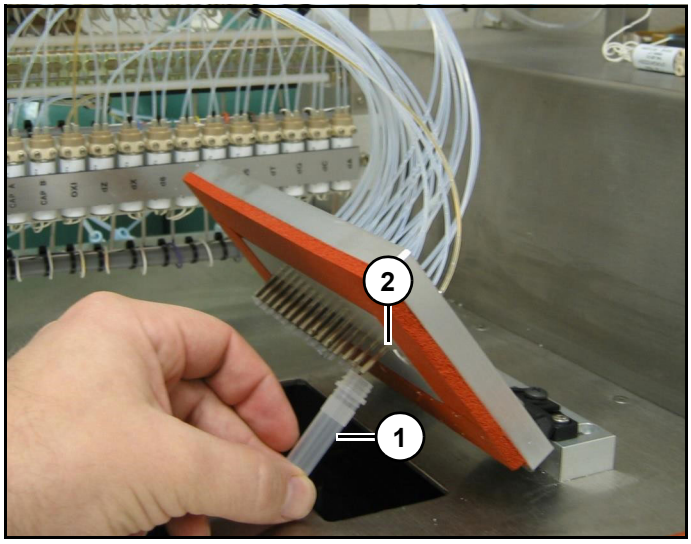



Figure 15

5. Place 2 ml tube (1) under correct injection pin (2) with lid open. *(Figure 15)*

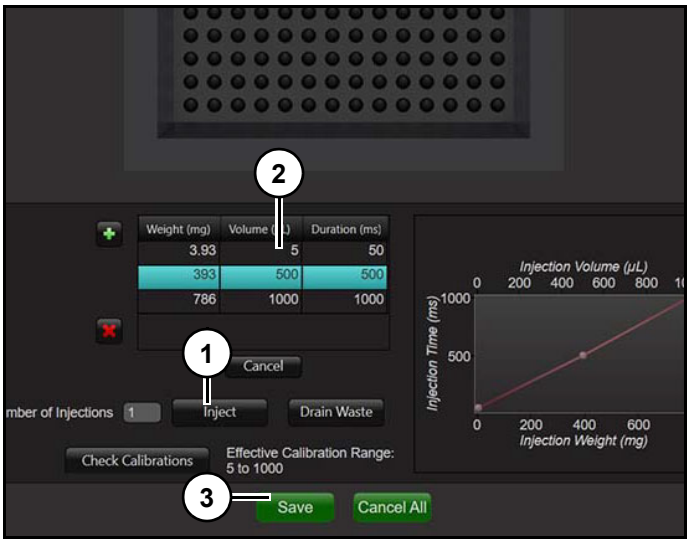



Figure 16

6. Click "Inject" (1) *(Figure 16)*. Instrument will open valve for 1200 ms, injecting liquid into collection vile.

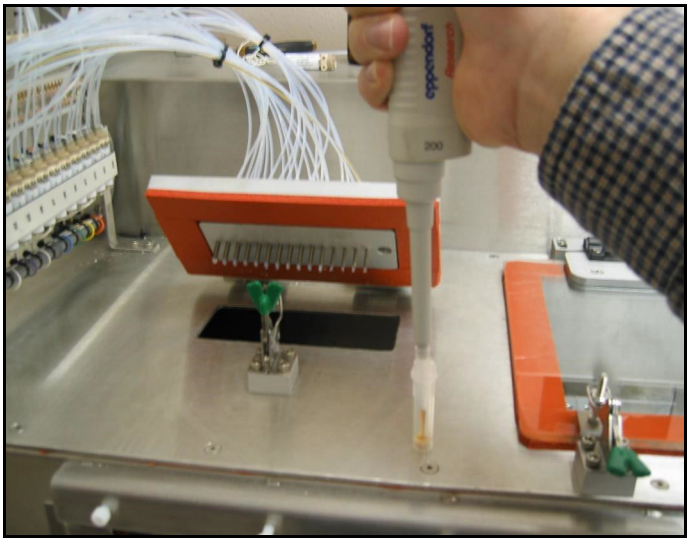


Figure 17

7. Draw liquid into pipette and adjust until volume can be determined. It may be necessary to draw liquid in and out a few times to determine volume accurately. *(Figure 17)*

Note: Some liquids are more difficult to measure due to viscosity and volatility. Deblock is most difficult. Repeat procedure until a confident measurement is obtained.

8. Enter measured volume into "Injection Volume (µL)" (2) *(Figure 16)*.

9. Click "Save" (3) *(Figure 16)*. To calibrate another point, highlight appropriate line with a mouse click.

## Updating calibration point

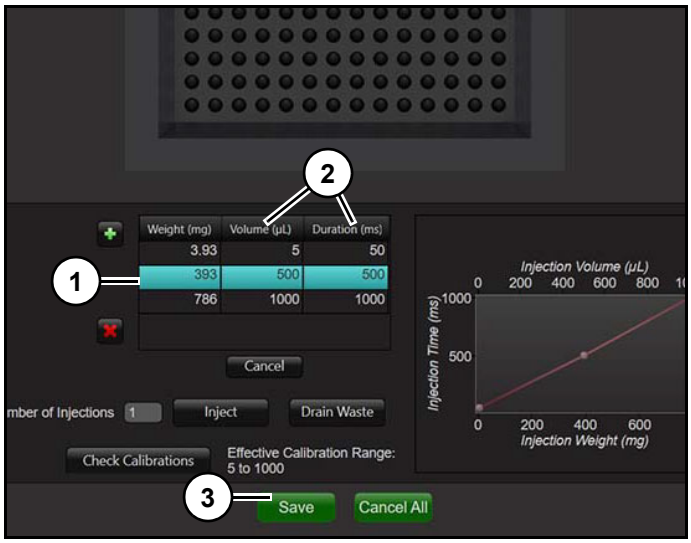



Figure 18

Refer to *(Figure 18)*.

1. Select line to change (1).
2. Select box to change (2) "Volume (µL)" or "Duration".
3. Edit box.
4. Click "Save" (3).

Note: If "Update Cal Point" (1) is not pressed, but "Save (3) button is, calibration point will be updated as well.

## Deleting calibration point

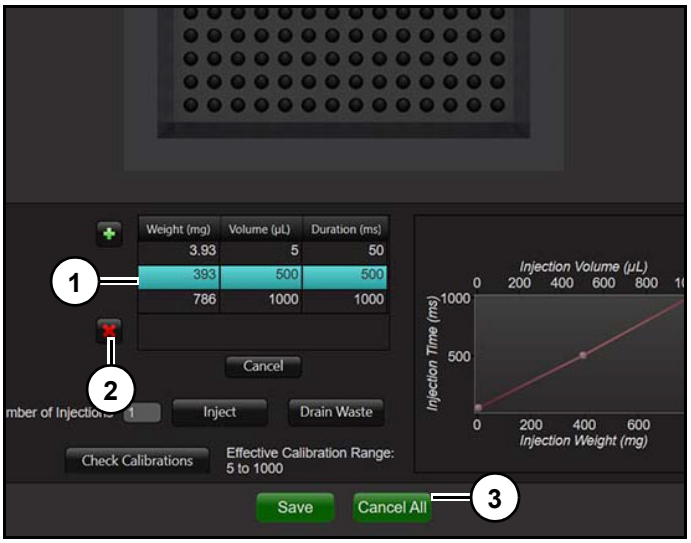



Figure 19

Refer to *(Figure 19)*.

1. Select calibration point to delete (1).
2. Click "X" (2).

Note: If "X" (2) is accidentally pressed, hit "Cancel" (3) undo delete. All changes since your last save will be lost.

## Checking calibrations

Allows user to check calibration curve for each valve.

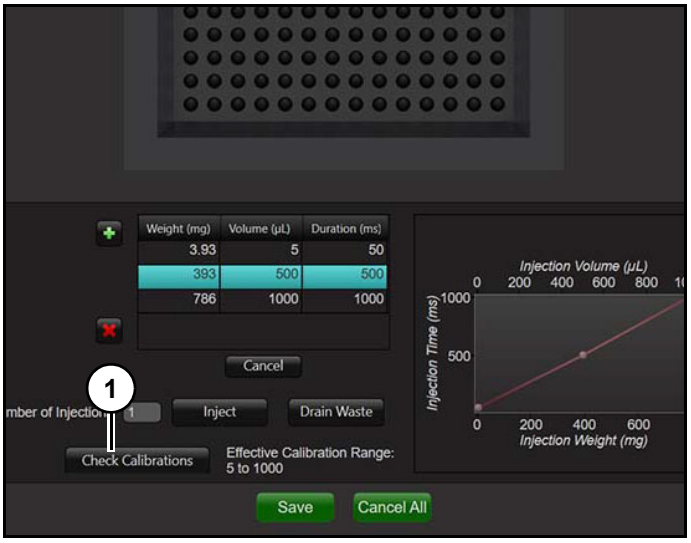



Figure 20

1. Click "Check Calibration" (1) *(Figure 20)*.

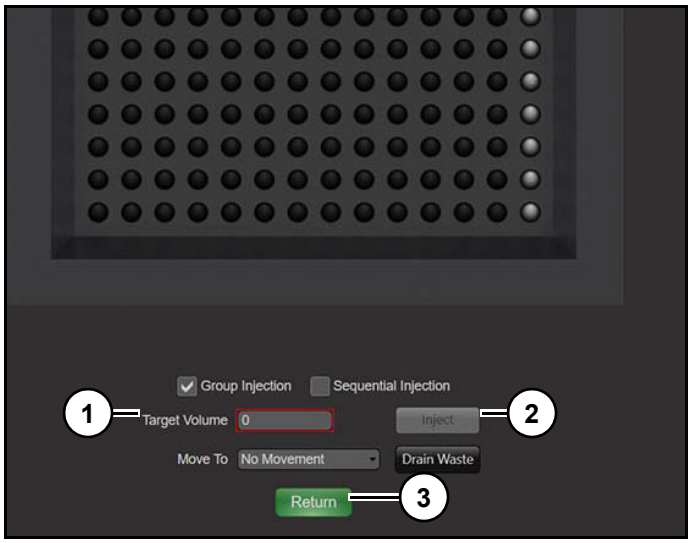



Figure 21

Refer to *(Figure 21)*.

For example, Oxidiser Valve is calculated for 1 for 10 µL and 250 µL and an entered target volume of 100 µL.

2. Enter 100 µL in "Target Volume" (1).
3. Click "Inject" (2).
4. Collect reagent in an Eppendorf tube.
5. Check volume with a pipette. Software will calculate necessary valve open time, based on volume to be delivered and slope between two calibration points.
6. Click "Return" (3) to return to check calibration screen.

## Drain waste

During calibration user can dispense any collected reagent into waste tray for disposal. Drain waste button will allow user to drain the waste tray when it becomes full.

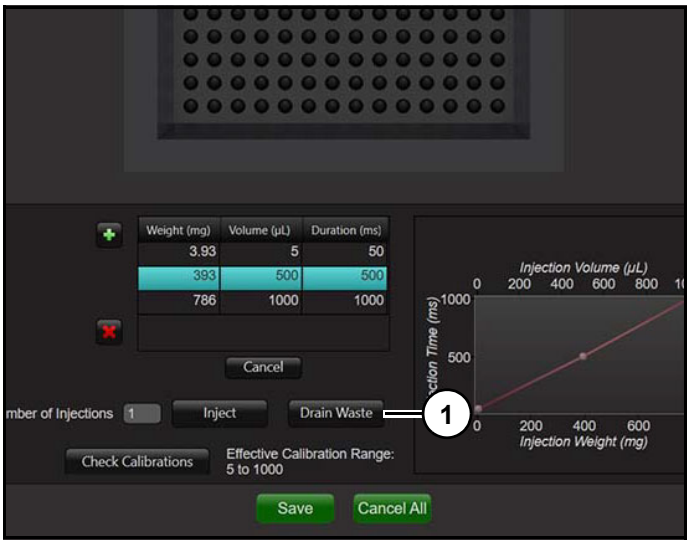



Figure 22

Click "Drain Waste" (1) *(Figure 22)* to drain waste from waste tray.

## Assign to.. Assign delta to

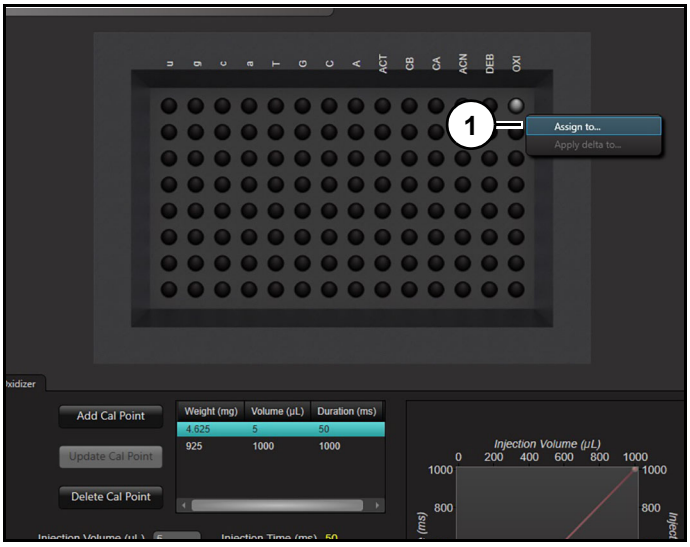



Figure 23

Refer to *(Figure 23)*.

In valve array graphic, right mouse clicking on a single valve will bring up a dialog box with "Assign to... Assign delta to" (1).

Assign to allows user to copy a calibration point from one reagent to another on multi-manifold.

If calibration changes 30ms for any/all calibrations on a single point, Assign delta to will apply changes to chosen/all valves in selected group.

## Group injection calibration

Calibration of valves in an array is known as G or Group Calibration. Valves are calibrated on a curve, default points are standard in software and user can/ should add, remove, update, and delete points as necessary. Calibrations are utilised when script file calls for a multiple injection for a group of valves requiring same volume. If any numbers of valves less than group size are required to be injected, then single injections will be used.

## Calibrating a group injection

1. Place a collection plate/tube under all valves in group.

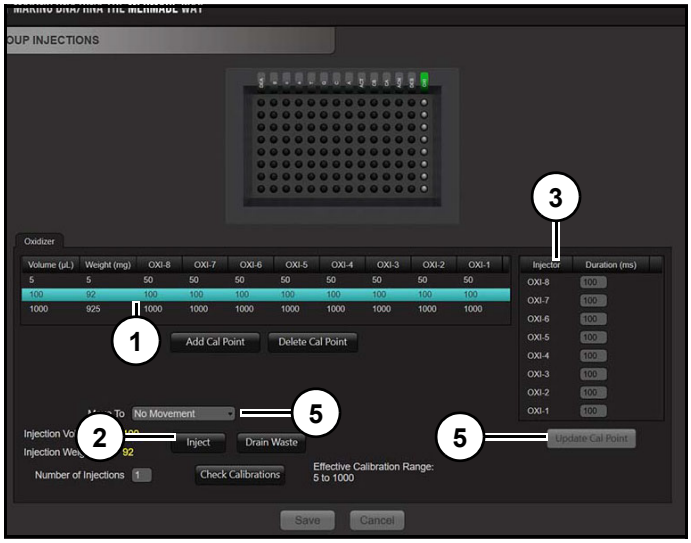



Figure 24

Refer to *(Figure 24)*.

2. Select a point to calibrate (1).
3. Click "Inject" (2).
4. Volumes in each tube will be measured and recorded in Volume column (3).
5. Record all volumes.
6. Click "Update Cal Point" (4) to commit changes.

Note: Failure to click "Update Cal Point" (4) will not save changes.

"Move to" (5) selects a column in Plate 1 plate to inject into.

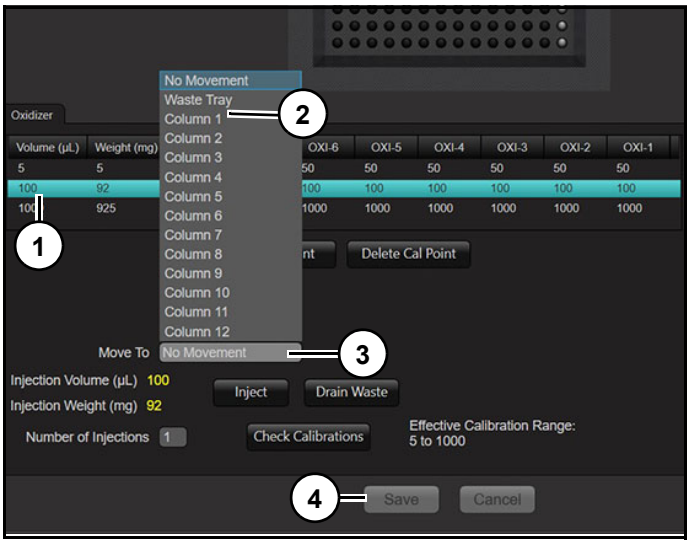



Figure 25

Refer to *(Figure 25)*.

For example:

1. User selects oxidizer tab at top of screen
2. Selects "100 µL calibration point" (1).
3. Selects "Column 1" (2) in "Move to" drop down (3).
4. Click "Inject" (Not shown).

Instrument will inject 100 µL into column 1 of Plate 1. This can be useful if a collection tube is placed in column positions. Most user's prefer to open door and simply hold collection tubes under the injection head to collect injections.

Process is repeated for each calibration point for each reagent. When completed, click "Save" (4) to save changes.
Note: Click "Save" (4) frequently to avoid loss of work.

## Vacuum Pulse Calibration screen

After instrument dispenses a reagent, software will use vacuum pulses to move reagents through columns at a calibrated rate. Instrument has a steady vacuum level which is set by adjusting vacuum breaker. Typical vacuum levels are between 9 inHg and 3 inHg. To flow reagents delivered to column through support bed after injection software applies short vacuum pulses which pull reagents through column at a calibrated rate. Vacuum pulses will determine reaction time for reagents and is important to performance of instrument.

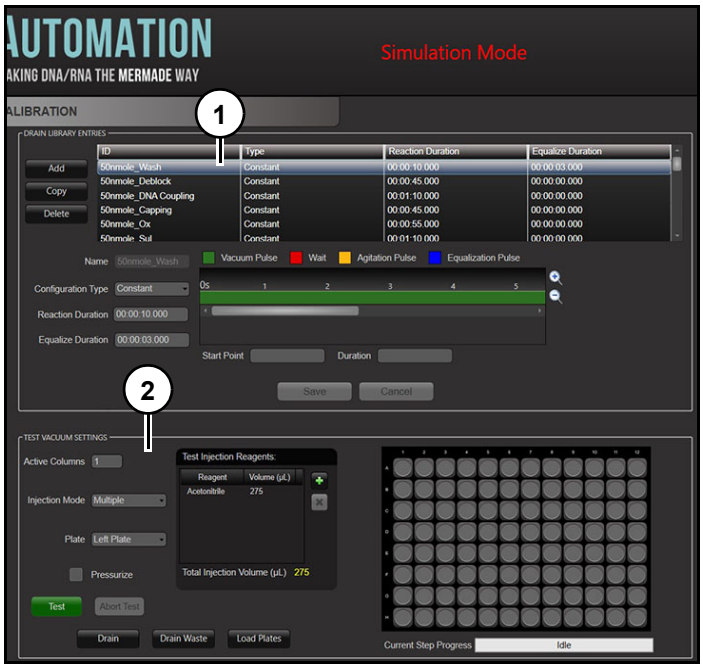



Figure 26

Refer to *(Figure 26)*.

**Drain Library Entries (1):** Displays saved and calibrated drain types. Selecting a drain ID from library will display drain details in "Test Vacuum Settings" (2).

Note: Software comes pre-configured with a working set of vacuum library entries for standard protocol scales.

If user changes vacuum breaker settings, then existing drain ID's in drain library will need to be re-calibrated for new vacuum pressures.

## Types of vacuum pulses

There are three types of vacuum pulses on instrument. Different types allow instrument to completely drain a full plate of synthesis columns regardless of number of active columns (1-12) on a plate. As columns drop out of synthesis cycle, due to length, then adjustments to length of a vacuum pulse may be necessary to drain remaining columns to completion since columns that have dropped out may no longer be getting liquid injections. This allows vacuum to easily dissipate through these empty columns and therefore makes it harder to drain remaining columns that did get injections.

Pulse types:

**Constant Vacuum Pulse.**

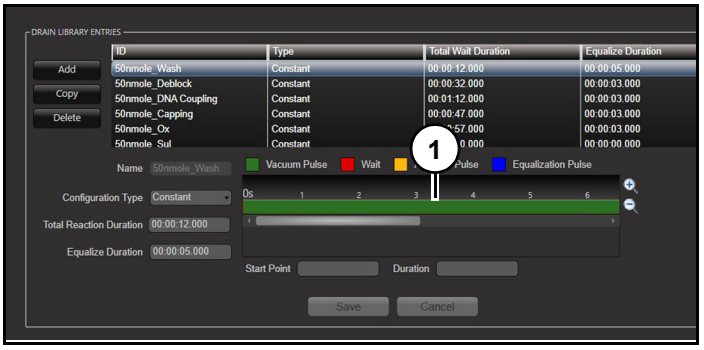



Figure 27

A constant vacuum pulse (1) *(Figure 27)* is a pulse that applies same length of vacuum to column chuck regardless of number of active columns. This is default vacuum setting when a new pulse is added to drain library and used most often. There is no compensation for dropped columns and is possible to find a good working range where dropouts do not negatively affect other columns.

Exceptions occur when oligos of drastically different lengths are combined on same plate. It is recommended that oligos of similar lengths be group whenever possible.This is vacuum pulse of choice when using "Additional Wash Steps" feature.

**Fixed Increment.**

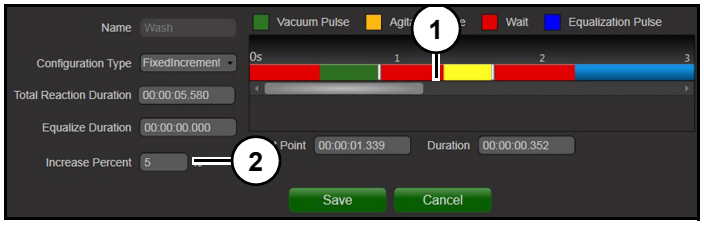



Figure 28

Refer to *(Figure 28)*.

The fixed increment pulse (1) increases vacuum pulse settings at a fixed percentage per column, as columns are completed and become empty.

Example: There is a one second wait followed by a 250 ms pulse, a one-second wait, a 900 ms pulse, etc. "Increase percent" (2) is percentage that vacuum settings will be multiplied by as columns drop out. For example, an increase percent value of 1% would increase each vacuum setting by 1% per column. So, a 10-second drain for 12 columns would be a 10.1-second drain for 11 columns, 10.2 seconds for 10 columns, and so on.

## Vacuum Pulse Structure screen

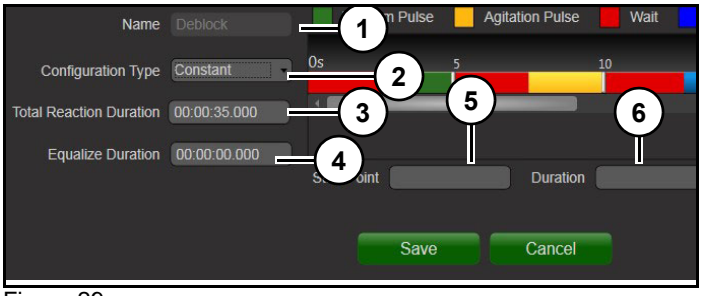



Figure 29

Refer to *(Figure 29)*.

**Name (1):** ID of specific drain.

**Configuration (2):** Type of drain pulse.

**Total Reaction Duration(3):** Total time of complete vacuum step encompassing all wait times, vacuum pulses, agitation pulses, equalise pulses, and final drain time.

**Equalize Duration (4):** Time after pulses are finished, needed for column to recover from loss of vacuum so that system is ready for next vacuum pulse/drain.

**Start Point (5):** Starting time of currently selected vacuum or agitation pulse.

**Duration (6):** Total length of currently selected vacuum or agitation pulse.

Red (waits), yellow (pulses), blue (equalised pulses), and green bars (full pulses) are visually represented of selected drains. Not all instruments have all drain types. Any lengthy vacuum pulse placed at end of vacuum pulse box is considered a drain. Dwell time is specific to reagents being used and type of reaction steps. Dwell time is considered prior to final long drain pulse.

For instance, some modified amidites require a 12 minute reaction time which would be programmed on this screen.

## Adding/removing a vacuum pulse

There are four options:

**Add Vacuum Pulse Here:** Adds a vacuum pulse at selected location with a right mouse click. Will pull unreacted reagent down from on top of reaction bed onto support allowing for chemical reaction to take place. Also allows user to drain column completely after reaction has taken place.

Vacuum pulses are used to move reagent through column to maximise solvent usage. Vacuum pulses must be adjusted so that they do not empty column prematurely and do not leave excess unreacted reagent above support bed at end of reaction time. Typical Pipette tip style columns as sold by Biosearch Technologies will hold up to 300 µL and can achieve three vacuum pulses and are dependant on vacuum level set by vacuum breaker/regulator and synthesis scale. These levels will need to be adjusted if vacuum level changes. It is important for all columns on a plate to have similar flow characteristic. If columns drain slower or faster than others then average synthesis quality will suffer.

**Add Agitation Pulse Here:** Adds an agitation pulse in selected location with a right mouse click. Will push dry argon into bottom of columns by pressurising bottom of synthesis plate. An argon valve (Press IN) connected under plate position opens. Allows for argon to be bubbled up through columns and allows user to extended reaction time by keeping reagent in contact with support for a prolonged period of time. Typically used when using columns greater than 1.3 ml and when no top frit over support bed would be used.

**Add Equalization Pulse Here:** Adds an equalised pulse at selected location with a right mouse click. Will release pressure or vacuum from underside of the columns. A 2-way valve (Press Out) is connected to underside of column plate, which when open, connects to atmosphere. Opening this valve shortens amount of residual drain experienced by system by relieving vacuum remaining after drain valve closes to atmospheric pressure.

**Delete:** Deletes current yellow or green selected pulse box.

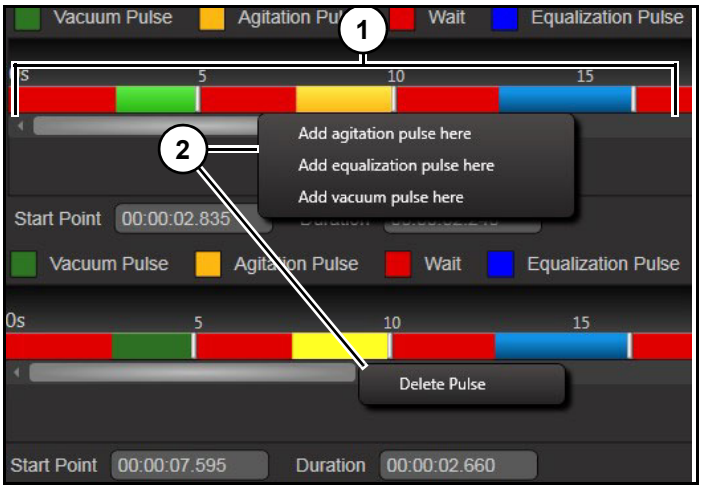



Figure 30

Refer to *(Figure 30)*.

1. Right click on one of boxes (1).
2. Select a pulse option (2) from menu.

Note: Not all pulse types are available on all instruments.

## Editing pulse segments

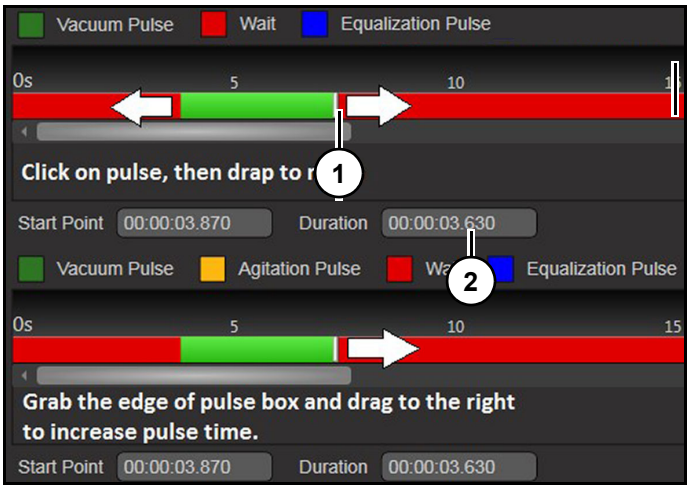



Figure 31

Refer to *(Figure 31)*.

1. Grab right edge of green or yellow pulse box (1).
2. Pull edge to right to make pulse duration longer or to left to make pulse duration shorter.

Note: This can also be accomplished by clicking on green, blue, or yellow pulse segment and editing time in "Duration" (2).

## Test vacuum settings

After calibration, vacuum pulses can be double checked using test vacuum settings box *(Figure 32)*.

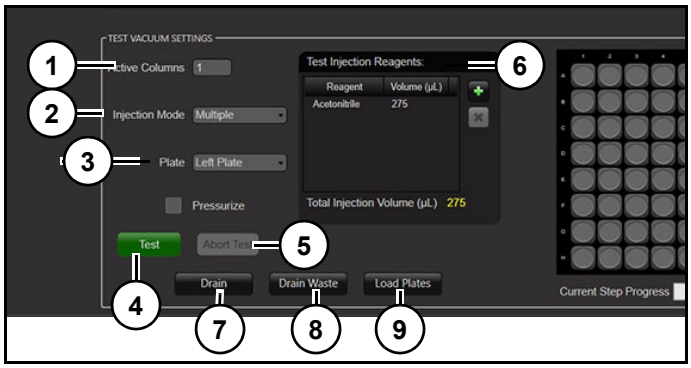



Figure 32

Refer to *(Figure 32)*.

**Active Columns (1):** Allows user to change the number of columns that will have reagent dispensed into them prior to checking the vacuum pulse settings; useful for checking the interpolated and fixed increment vacuum pulse options. Injections will start in row one.

**Injection Mode (2):** Determines injection method, single, multiple, or fast injection.

**Plate (3):** Choses plate to be tested.

**Pressurize (Not shown):** If box is checked, then bottom of plate will be purged with positive pressure before reagent injection to allow for an even liquid level across 96 columns for vacuum pulsing. Will open plate pressure inside and outside of value to allow argon to flow under columns to create back pressure. Prevents columns from draining due to gravity during injection cycle. Optional, not available on all instruments.

**Test (4):**Tests selected vacuum pulse setting for calibration verification. Includes injection of a specified reagent at a certain volume followed by selected vacuum pulse step.Allows user to test and adjust vacuum pulse settings to calibrate vacuum pulses so that reagents flow through column during reaction time and does not over or underdrain. Once settings are calibrated screen can be used to make observations during a run in order to fine tune settings.

**Abort Test (5):** Aborts test during execution.

**Test Injection Reagents (6):** Allows user to add or remove type and/or volume of reagent(s) to be dispensed into columns.

**Drain (7):** Drains liquid from selected plate before or after test.

**Drain Waste (8):** Drains waste tray.

**Load Plates (9):** Moves XY table forward so plates can be added or removed.

## Testing vacuum pulses

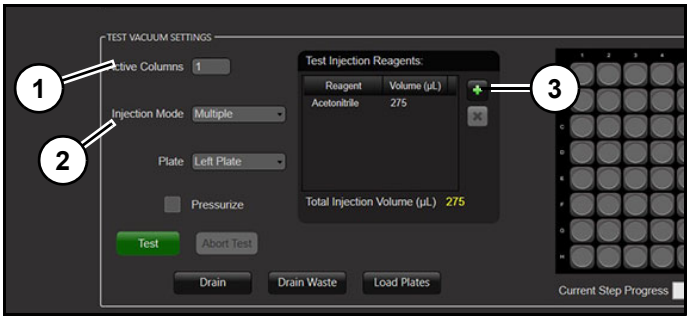



Figure 33

Refer to *(Figure 33)*.

1. Input number of columns in "Active Columns" (1).
2. Select "Injection Mode" (2).
3. Click "+" (3).

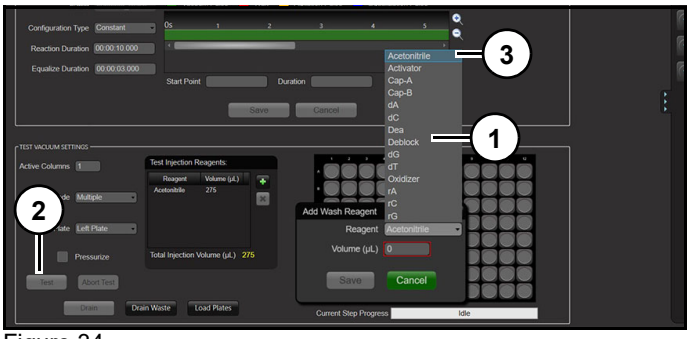



Figure 34

Refer to *(Figure 34)*.

4. Select reagent Type and volume from list (1).

Note: Multiple reagents can be added and synthesizer will inject them in order entered.

5. Click "Test" (2).

Note: A reagent can be removed from queue by clicking Red "X" (3).

## Motion options

Allows user access to motion options (1) *(Figure 35)* including, Set Reference Point, Set Table Parameters, and Motion options.


Figure 35

## Set Reference Positions

Instrument arrives from factory with injection head already aligned to synthesis plates. Well to well distance of columns in column chucks and spacing between each reagent in injection head is a standard value hard-coded in a configuration file and does not need to be changed.

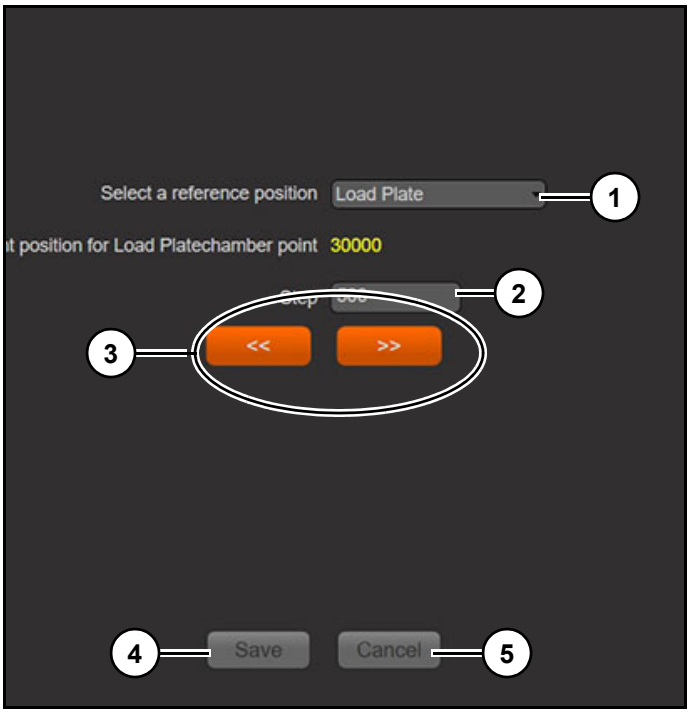



Figure 36

Use

User tells instrument where to start by aligning injection head. Alignment can be changes in Injection Head Align screen *(Figure 36)*.

To adjust injection head for plate A:

1. Select plate A from "Select a reference position" (1).
2. Enter a value in "Step" (2), for example 250.
3. Press "←, →" (3) to move plate left, right, up, or down in relation to user. Farthest left monomer on injection head and column A1 on plate A should be centered.
4. Click "Save" (4) to save position.

Note: Click "Cancel" (5) to undo changes.

Process is then repeated for all base plate positions and waste tray. Load plate position is where plate will be positioned to allow user to remove or add plates to system. View plate left, and view plate right positions are where plate will move after an injection so user can observe vacuum pulses during a run. These can be adjusted independently to suit needs. and each position can be changed without affecting alignment of other positions of slide.

## Set Table Parameters

Determines speed at which slide will move during its' various operations. Values are set at factory and should not be changed unless instructed so by Biosearch Technologies. *(Figure 37)*

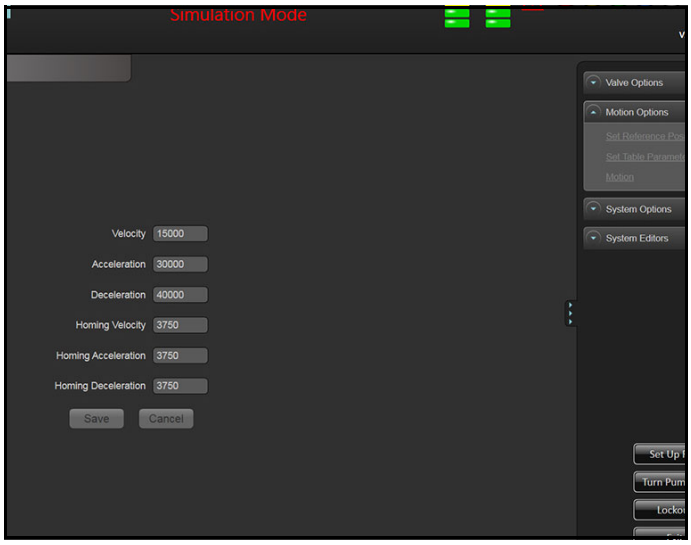



Figure 37

## Motion

Allows user to check motion system and to perform certain motion related functions.

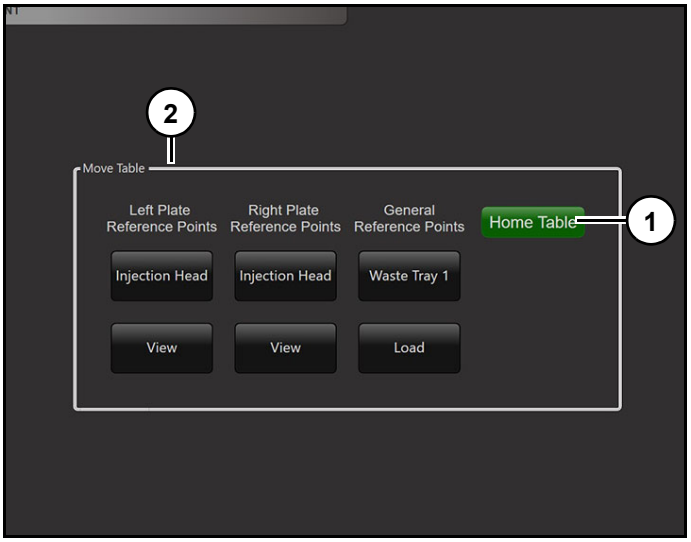



Figure 38

Refer to *(Figure 38)*.

**Home Table (1):** Homes table or synthesis plate chucks. Software will re-zero slide and find leftmost limit switch and front-most limit switch. Homing also happens each time software is initialised. If instrument is not homing properly there will be problems entering a run. Please contact Biosearch Technologies if table is not homing correctly.

**Reference Points (2):** Displays reference points. Reference points will change per instrument configuration.

Note: If alignment is not correct, go to Set References screen to correct alignment.

## System Options

Allows user access controls involving system related options *(Figure 39)*:

- Sensor Alarms
- Bottle Mapping
- Manage Reagents
- Modify Lot Information
- Show Run Screen
- User Management
- Role Management
- System Settings
- System Backup
- Waste Control

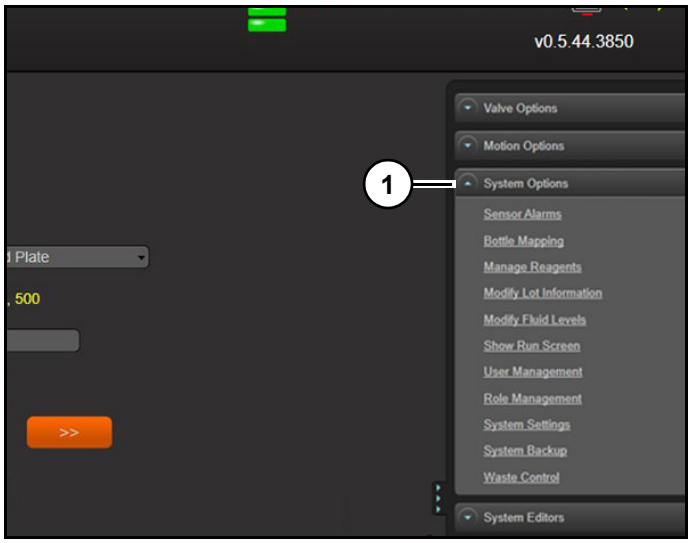



Figure 39

## Sensor Alarms

Contains information relating to sensor information coming from instrument

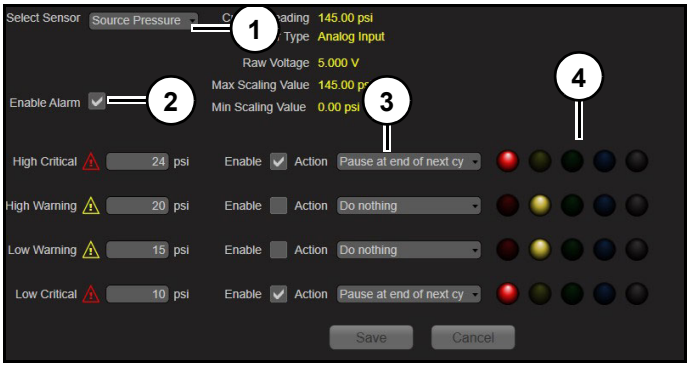



Figure 40

Refer to *(Figure 40)*.

**Select Sensor (1):** Toggles between sensors. Once a sensor is selected, options are displayed based on whether sensor is digital or analog.

**Enable (2):** When checked (on), instrument will respond with action and light tree settings (4) when alarm conditions are met.

**Trigger Alarm When Sensor Is: On or Off (Not shown):** Only visible for digital or On/Off sensors. Refers to state of sensor (on/off) that will create an action. Allows either type of sensor to be used. Not recommended to change these from factory defaults as it can result in alarms not being detected. Please contact Biosearch Technologies before changing setting.

**Action (3):** Allows user to designate a pause should alarm conditions be met.

- Do Nothing: No pause is set. Light tree will still be changed, and sensor events recorded in log files.
- Pause After Current Step: Pauses synthesis after current step. Allows current injection and drain steps to complete then instrument will be paused.
- Pause After Next Wash: Pauses after next Wash Step. Will finish current injection and will continue until it encounters a wash step in script file and after wash step is executed including injection and drain instrument will pause.
- Pause at End of Cycle: Pauses at end of cycle for current base (safety pause). Will continue until

end of current base addition and then pause, typically after the last wash step and just before a deblock step. Safest place to pause instrument from a chemistry perspective.

- Pause Immediately: Pauses as soon as sensor is triggered. Least desirable and least stable point to pause. Typically, only sensors set to pause immediately is interlock sensor and liquid sensor.

**Light Tree (4):** User can modify Light Tree.
Five color choices: Red, Yellow, Green, Blue, and White. A single click on colored circles will illuminate that circle as a solid color during sensor alarm. Double-clicking a color circle will illuminate that circle as a blinking color during alarm.

With analog sensors such as pressure transducers, there are four levels of alarm. Normal operating range of sensor should be between High Warning and Low Warning. Normal operating range of source pressure is between 15 and 20 psi. If instrument transitions up or down outside of range then one of alarms will trigger, and if synthesizer is running, then appropriate action will be executed. If source pressure continues to change further, then eventually High or Low critical alarms will be triggered.

High Critical: Highest alarm.

High Warning: Second most high alarm and this is warning prior to high critical alarm.

Low Warning: Second lowest alarm and is warning prior to low critical alarm.

Low Critical: Lowest alarm.

User can customise sensors to alert themselves as they deem fit.

## Interlock sensor

**⚠WARNING**

Do not insert hand into a running instrument, doing so could result in serious injury. Ensure is paused and not in motion.

Monitors door switch, located on top right of chamber door, and terminates any instrument movement when door is opened to prevent injury or instrument damage. Table can be moved and should go back to correct position after door is closed and instrument un-paused.

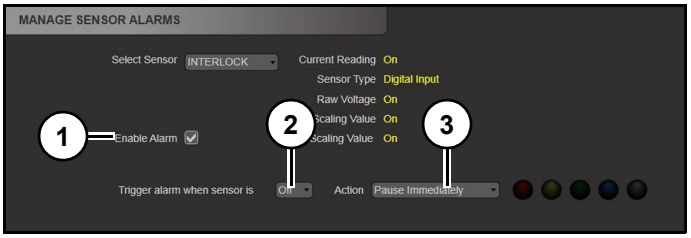



Figure 41

Refer to *(Figure 41)*.

Interlock sensor factory settings:

Enable Alarm (1): On
Trigger Alarm (2): Off
Action (3): Pause immediately.

## Liquid sensor

Liquid sensor is mounted in bottom of synthesis chamber under injection head and monitors any liquid spills from injection head or waste tray. Sensor should be tested periodically to prevent large spills of hazardous chemicals. When triggered, power to all valves will be cut and valves will shut closed if any liquid is detected in synthesizer. Power to valves will be cut regardless of whether sensor is enabled or not. If liquid sensor indicator light is Red, sensor is detecting liquid and will need to be cleaned or fixed before synthesizer will operate.

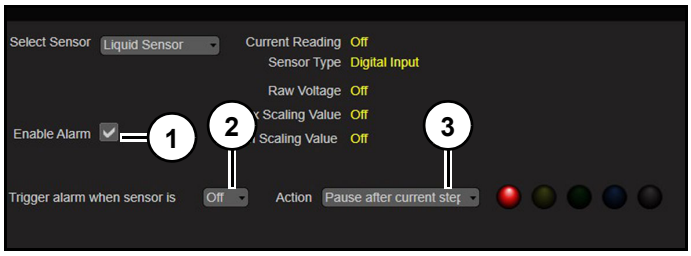



Figure 42

Refer to *(Figure 42)*.

Liquid sensor factory settings:

Enable Alarm (1): On
Trigger Alarm (2): Off
Action (3): Pause immediately.

## Source Pressure sensor

**Notice**

Instrument is equipped with a pressure relief valve that opens at 25 psi to protect bottles from over pressurisation.

**Notice**

Maximum pressure supplied to instrument should not exceed recommendations in site preparation document.

Analog sensor that monitors source pressure feeding Monomer and Reagent regulators. Sensor measures pressure after source pressure regulator. Gas is supplied to instrument at no more than 60 psi via a customer supplied gas line to gas inlet of instrument. Gas enters source regulator where it should be adjusted down to less than 25 psi. Gas is then used to feed Monomer and Reagent gas regulators where pressure is dropped further and is distributed to bottles. If source gas pressure exceeds 25 psi then relief valve will begin to leak.

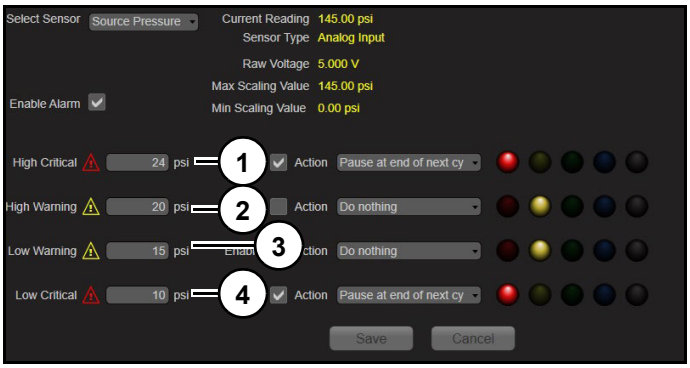



Figure 43

Refer to *(Figure 43)*.

Pressure sensor factory settings:

High Critical (1): 24 psi
High Warning (2): 20 psi
Low Warning (3): 15 psi
Low Critical (4): 10 psi

Source pressure is adjusted using source pressure regulator. Actual source pressure can fluctuate slightly without need to recalibrate valves, as long as pressure does not dip below operating pressures of monomer and reagent regulators.

## Monomer Pressure

Monitors actual pressure of amidite and activator bottles. Recommended setting is 6 psi.

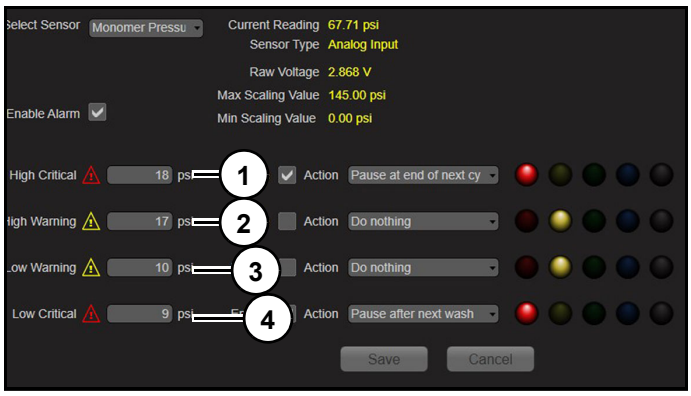



Figure 44

Refer to *(Figure 44)*.

Monomer sensor factory settings:

High Critical (1): 8 psi
High Warning (2): 7 psi
Low Warning (3): 5 psi
Low Critical (4): 4 psi

Pressure can be adjusted using the Monomer regulator. If Monomer pressure level changes for any reason, it is recommended that injection calibrations be checked and updated. Adjustments to pressure level will cause liquid to over or under dispense, depending on whether level goes up or down.

Note: Monomer bottle pressures should be set higher than 7 psi and lower than 3 psi to eliminate splashing during reagent dispensing.

## Reagent Pressure

**Notice**

Reagent bottle pressures can be set lower than 5 psi, but not higher than 10 psi because splashing may occur during reagent dispensing. Glass bottles greater than 10L are not recommended as they can break easily while under pressure. Glass bottles should be inside of secondary containment and/or be plastic coated.

Monitors actual pressure of ancillary reagent bottles. Typically consists of all reagents not used in coupling steps. Sensor typically includes all deblocks, oxidisers, capping reagents, and wash reagents. Confirm tubing on actual instrument if there is any question whether chemicals can be supplied by same regulator due to compatibility concerns. Recommended reagent pressure setting is 6 psi.

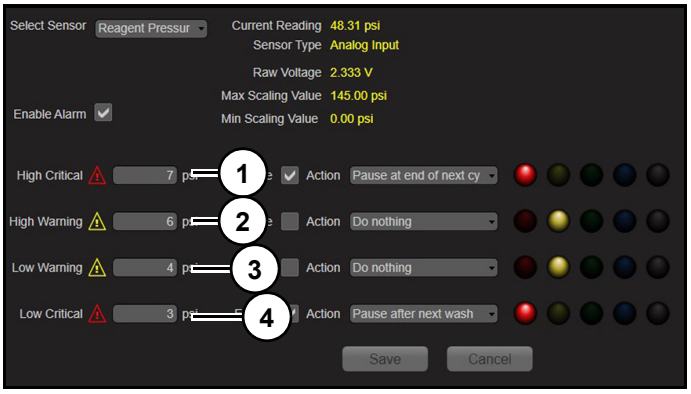



Figure 45

Refer to *(Figure 45)*.

Reagent sensor factory settings:

High Critical (1): 8 psi
High Warning (2): 7 psi
Low Warning (3): 5 psi
Low Critical (4): 4 psi

If Reagent Pressure level changes for any reason, it is recommended that injection calibrations be checked and updated. Adjustments to pressure level will cause liquid to over or under dispense, depending on whether level goes up or down.

## Regulated Vac

Monitors vacuum system during synthesis.

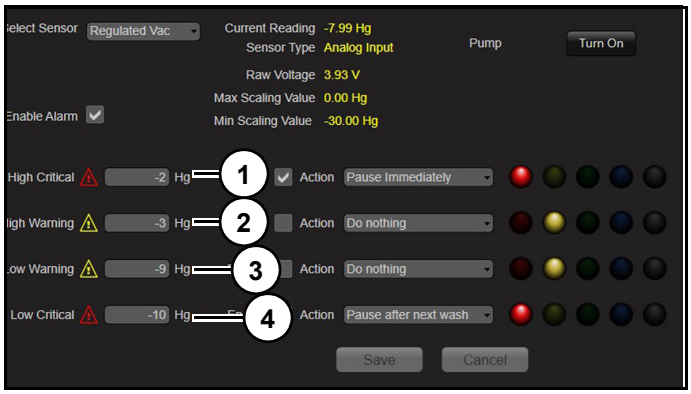



Figure 46

Refer to *(Figure 46)*.

Vacuum flow factory settings:

High Critical (1): -2 Hg
High Warning (2): -3 Hg
Low Warning (3): -9 Hg
Low Critical (4): -10 Hg

Vacuum level can be changed by adjusting vacuum breaker or vacuum regulator. If vacuum level changes for any reason, it is recommended that vacuum settings in drain library be updated and calibrated. Adjustments to vacuum level will cause columns to either drain faster or slower depending on whether level goes up or down. Some fluctuations during a run are expected but it should not be more than 1 Hg. Very long drains, utilised often in a script file, can also cause vacuum level to drop during a run and should be avoided.

## Purge Flow

Analog sensor monitors argon purge valve responsible for delivering gas to chamber before and during synthesis with argon. Chamber purge reduces humidity in chamber as well as acting as a fire prevention measure. During a run it acts to replace gas which is removed via vacuum applied through columns.

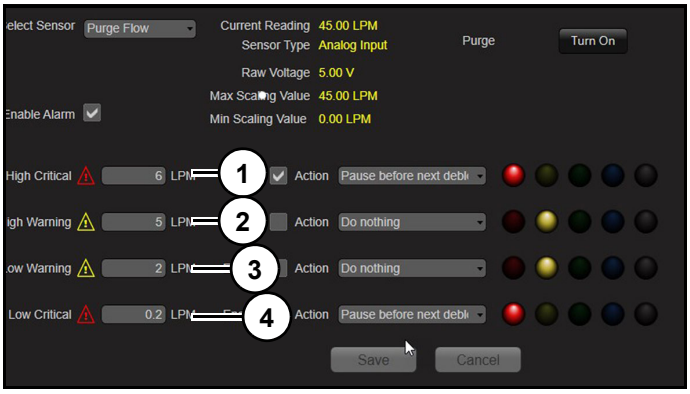



Figure 47

Refer to *(Figure 47)*.

Purge flow factory settings:

High Critical (1): 6 LPM
High Warning (2): 5 LPM
Low Warning (3): 2 LPM
Low Critical (4): 0.2 LPM

## Bottle Mapping

The 192E has a group default configuration for following bottle positions:

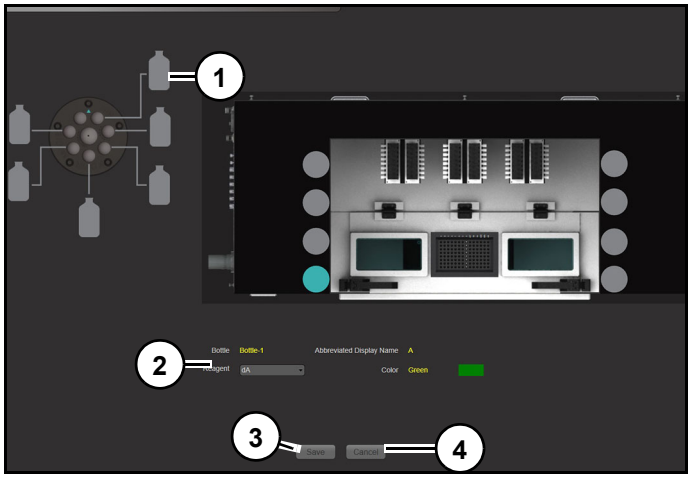



Figure 48

Refer to *(Figure 48)*.

In the bottle mapping screen all of bottle positions is displayed. All of grouped injection reagents are located on row 4. All of single injection reagents are located on rows 1-3. Reagents are located in row 5. To observe which reagent is mapped to which bottle, click on any bottle position. The bottle number, bottle reagent, abbreviated name, and the color matched to that reagent will be displayed. Once the bottle is selected, hover over the layout of the injection head, located on the right of the machine, to see which line(s) will inject that reagent.

Each bottle is mapped on instrument to injection head in a default pattern. If user would like to change a reagent or monomer in a bottle, reagent will need to be change in bottle on synthesizer and new position of reagent changed in software using Bottle Mapping screen.

To change default reagent configuration:

1. Click "Bottle" (1).
2. Click "Reagent" (2) and change reagent in drop-down.
3. Click "Save" (3) to save changes.

Note: Click "Cancel" (4) to cancel changes.

Reagents can be added where desired and some rules apply:

- Activator reagent must not be too far right of all amidites. If not, there will be excess movements and synthesis will slow down.
- Cap A must be to right of Cap B on injection head. If not, there will be excess movements and synthesis will slow down.
- Reagents can only be assigned to one bottle.

Note: Instrument bottle configurations will vary.

## Manage Reagents

Allows user to control properties of each reagent.

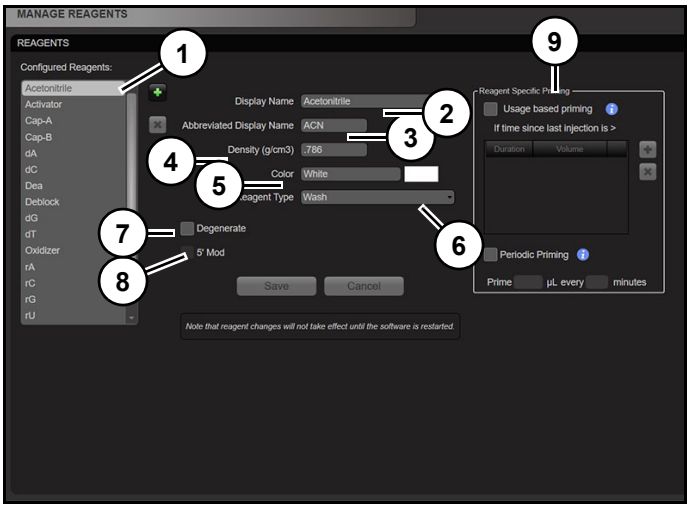



Figure 49

Refer to *(Figure 49)*.

Click on drop-down (1) and click on a preloaded reagent to view properties.

**Display Name (2):** Full name of reagent.
Note: Long names may be cut off in software.

**Abbreviated Display Name (3):** Can be up to 3 characters and are case sensitive. This is name shown in run screen during synthesis and is character set used to call monomers.
Note: If abbreviated display name of a monomer/ amidite is more than one character, it must be bracketed in parentheses in sequence file.
Example: ACGT(rA)ACGT.

**Density (g/cm³) (4):** Density of selected reagent. Used when calibrating by weight and can be left blank. If left blank default density of acetonitrile will be assigned if calibrating by weight.

**Color (5):** Color of reagent shown in column well display during a synthesis. HEX code of a color can be used or standard Name for HEX color can be entered. Example: Red = #FF0000, either can be used.

**Reagent Type (6):** Type of reagent in bottle. Used to narrow choices when in script editor and other screens.
Note: When adding a reagent to a Deblock step for instance only reagents of deblock type will be displayed.

**Degenerate (7):** Allows user to mix multiple reagents in columns. Typically used to mix monomers to generate mix based position such as N (25% A, 25%C, 25%G, 25%T) in an oligo.
Note: Not limited to monomers allowing any reagent on instrument to be mixed. Recommended that users mix degenerate monomers in bottle prior to injection as this will give best distribution of various bases and especially true with degenerates containing more than two constituents.

**5′ Mod (8):** Allows user to designate a monomer as a 5" modification. Monomer can still be used internally and will behave as any other amidite as long as it is not last base added to oligo. If 5' mod is detected in as last base addition of that base will be paused until all oligos are on last base and it will be added at last step. All 5' mods will be added on last step. Saves in reagent from priming waste.

**Global Priming Configuration (9):** Priming parameters for reagent. Can be set per reagent and provides methods to help prevent mis-injection due to crystallisation on low-frequency use monomers or monomers that require a volatile co-solvent such as Dichloromethane.

## Adding reagents

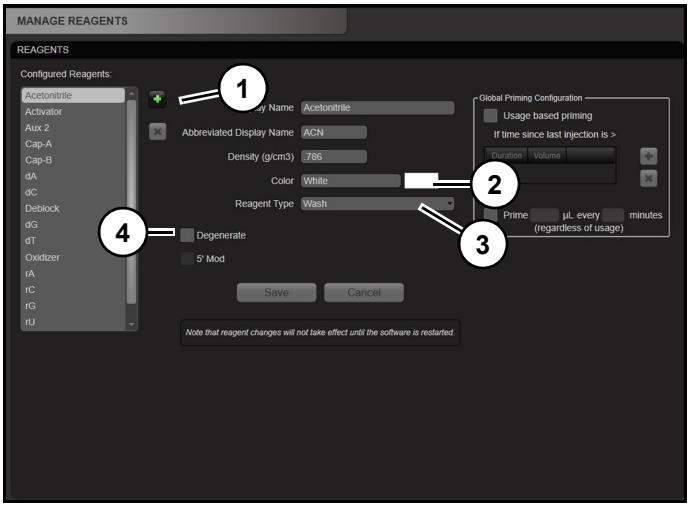



Figure 50

1. Click "+" (1) *(Figure 50)*.


Figure 51

Refer to *(Figure 51)*.

2. Enter "Display Name" (1).
3. Enter "Abbreviated Display Name" (2).
4. Enter "Density" (3).
   Note: Correct density must be entered for calibration weight option to be accurate. Consult reagent supplier or SDS for correct density.
5. Click "OK" (4). Reagent will be added to configured reagents box.
6. Select reagent in reagent box to modify "Color" (2), "Reagent Type" (3) and "Degenerate" (4) reagent. if necessary. *(Figure 50)*

Note: Newly added reagents will not be displayed in bottle mapping screen until software is rebooted.

## Adding degenerate base

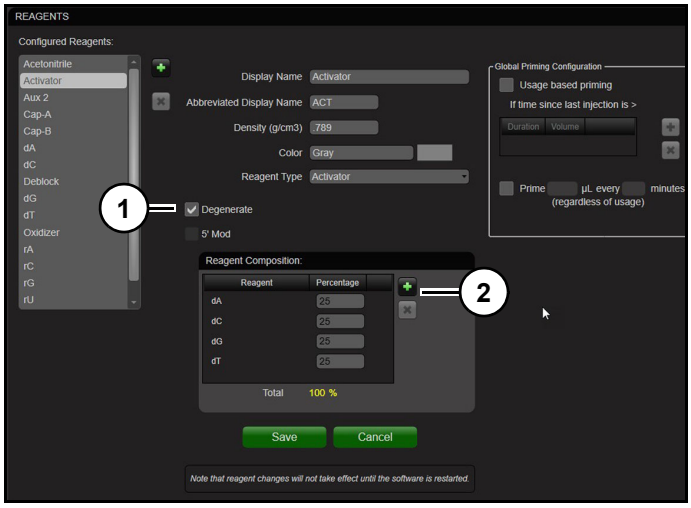



Figure 52

Refer to *(Figure 52)*.

If degenerate box is checked (1), reagent composition will appear. By clicking "+" (2) user can mix multiple reagents together at any percentage listed in configured reagents box. Percentages equal 100%. In order to utilise reagents, volume to be delivered must be within calibrated range.
Example: In a coupling step if 100 µL of N is delivery volume and degenerate is 25% dA then dA must be calibrated for 25 µL in order to be delivered. A warning is presented when starting run and run will not start until two appropriate calibration points are added.

## Reagent Specific Priming

Allows user to periodically prime reagent regardless of current synthesis step.

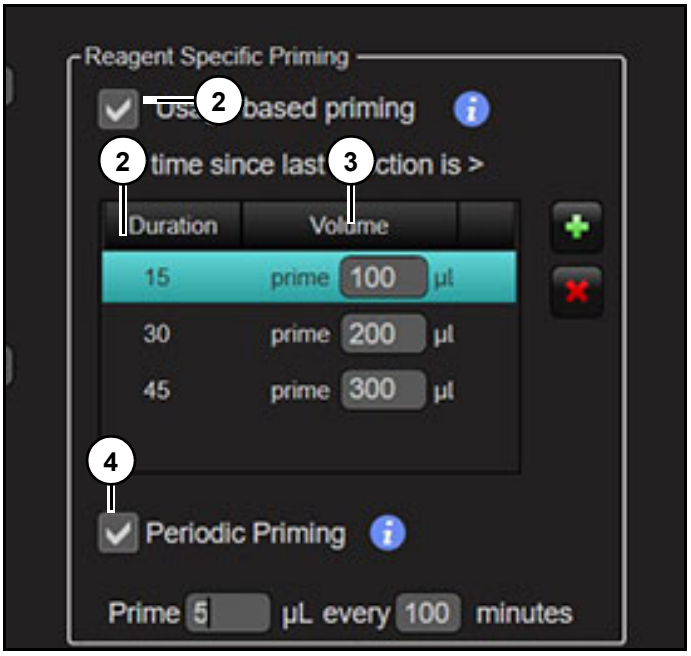



Figure 53

Refer to *(Figure 53)*.
By checking "Usage based priming" (1) and entering a duration (2) and volume (3), software will prime reagent using a volume based on time that has expired since last time reagent was used.
Example: If user enters 15 minutes and 100 µL and reagent has not been injected for greater than 15 minutes, reagent valve will be open, and line will be primed for 100 µL when a prime is called for in script file.

User can enter multiple time and volume combinations. User can designate to prime reagent 100 µL if it has been greater than 15 min since last use but 200 µL if it has been greater than 30 min and 300 µL if it has been greater than 45 min. This allows user to prime more if reagent has had more time to crystallise due to lack of use. Value must be under max value of liquid calibration table.

Primes will occur when a prime for that reagent is called for in script file. If there are no primes called out in script file, no priming will occur. If multiple primes are called for, then only first one will be replaced with priming volume designated. Remaining primes will inject as a set in script file. These primes are recorded in log file as soft primes.

If lower box (4) is checked, then reagent line will be primed every 5 minutes for 100 µL regardless if that reagent has been used during that time frame or not. It will simply periodically prime reagent if it is used during current synthesis, regardless of when it is used. Primes will take place during first priming cycle of any reagent after time has expired. These primes do not require any primes to be set in script file and are recorded in log file as hard primes.

Click "Save" to save new global priming parameters or "Cancel" to return to previous screen without saving changes.

## Modify Lot Information screen

Allows user to track lot numbers and dates when bottles were put on instrument.

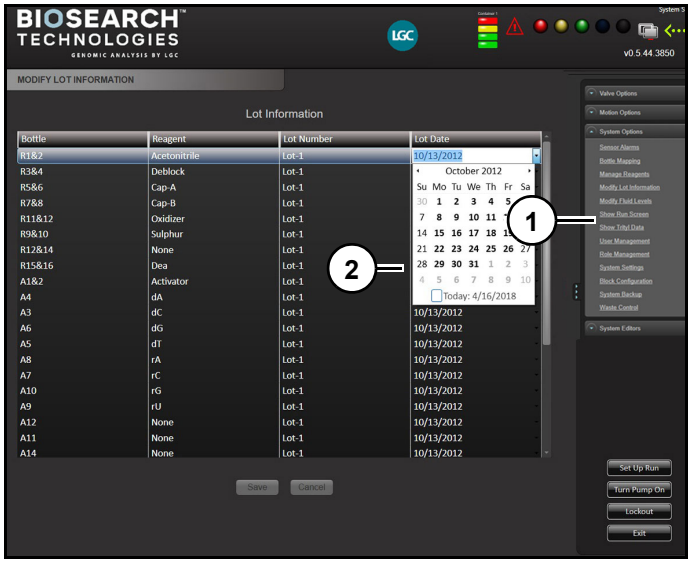



Figure 54

Refer to *(Figure 54)*.

Modify Lot Information (1) is used to track reagent usage on instrument and a pop-up calendar (2) to locate by date. When entering a new lot number, date will automatically be updated. Information is recorded in log files for future reference. Information only needs to change for reagents that have been modified since last run.

## Modify Fluid Levels screen

Allows user to track fluid levels.

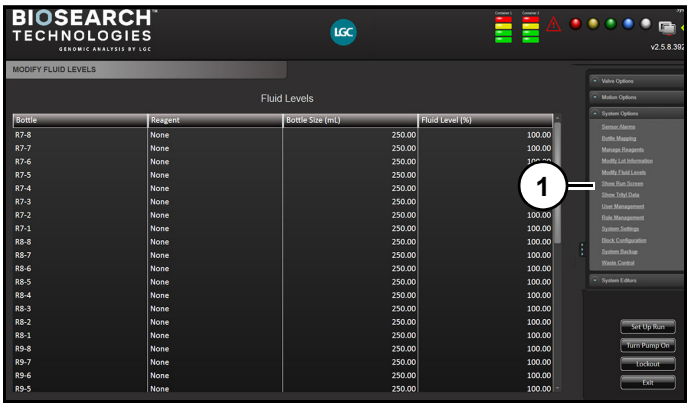



Figure 55

Modify Fluid Levels (1) *(Figure 55)* is used to track fluid levels. Bottle size can be entered, and after each run, user can manually subtract amount used in previous run in terms of percentage loss. Helps keep track of chemical consumption and ensure user checks reagent levels to prevent failed runs due to insufficient chemicals.

## Show Run Screen

Allows user limited access for diagnostics and testing when instrument is paused. Allows user to return to run screen.

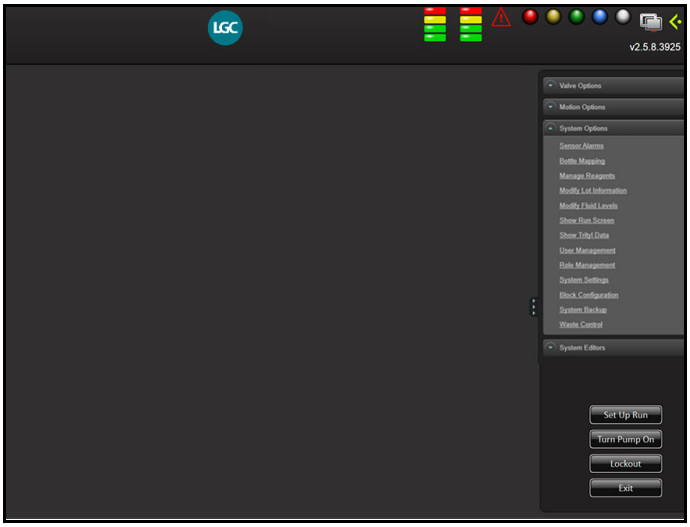



Figure 56

## User Management screen

Allows different levels of user access to software. Username and password can be created and a role may be assigned to user.

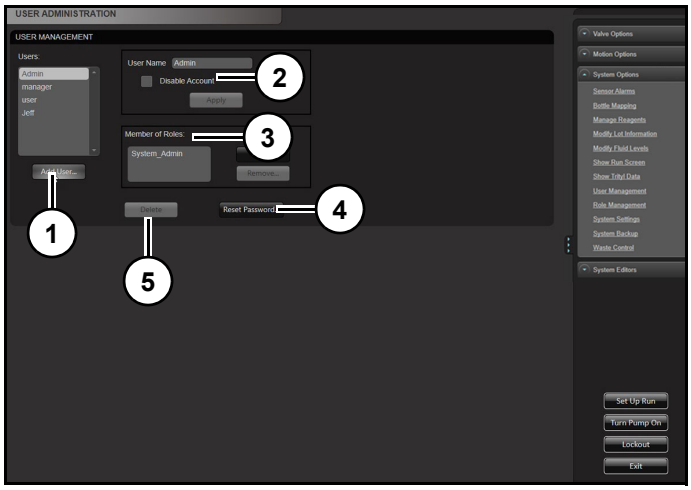



Figure 57

Refer to *(Figure 57)*.

**Add User (1):** Adds additional user.

**Disable Account (2):** Temporarily disables an account**.**

**Row Administration (3):** Assigns a membership role to a user group.

**Reset Password (4):** Resets password selected user account**.**

**Delete (5):** Deletes selected account**.**

## Adding User

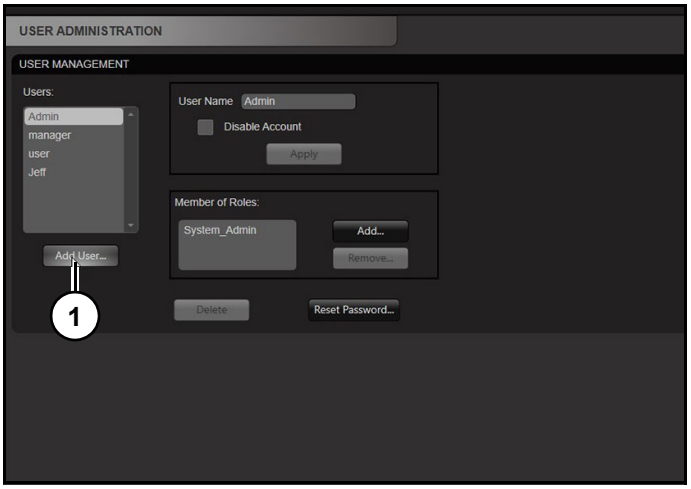



Figure 58

1. Click "Add User" (1) *(Figure 58)*.

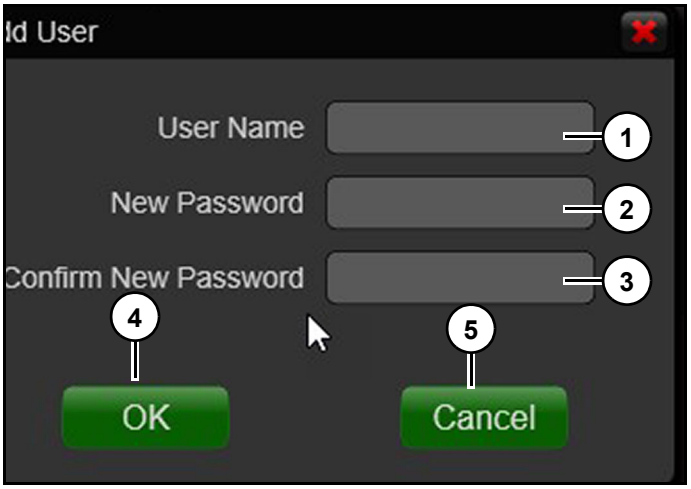



Figure 59

Refer to *(Figure 59)*.

2. Enter "User Name" (2).
3. Enter "New Password" (2).
4. Enter password in "Confirm New Password" (3).
5. Click "Save" (4).

Note: Click "Cancel" (5) to exit without saving changes.

6. Assigned a role to new user.

Note: Biosearch Technologies will not be able to retrieve forgotten passwords.

## Role Management

Creates different levels of user access to software. There can be administrators, managers, users, etc. and prevents excluded user's to make unwanted changes to software.

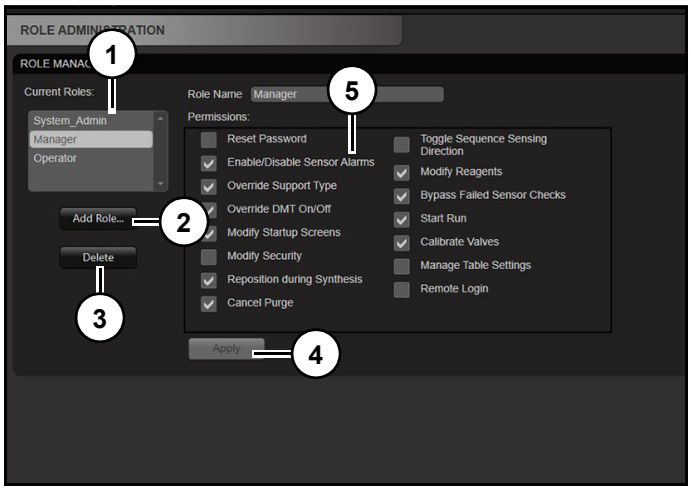



Figure 60

Refer to *(Figure 60)*.

**Current Role (1):** Selects current role to modify.

**Add Role (2):** Adds a new user role. User roles can be renamed. For instance, each user could have their own role.

**Delete (3):** Deletes selected user role.

**Apply (4):** Applies changes made to a user role.

**Permissions (5):** Selects permission for selected role.

Permissions:

- Reset Password: Allows role to reset password for account
- Enable/Disable Sensor Alarms: Allows role to change sensor alarm settings.
- Override Support Type: Allows CPG type (universal vs standard) to be overridden after loading a sequence file which has support type designated. Allows a user to change support type form universal to standard and vice versa during the run start up process.
- Override DMT On/Off: Allows final DMT state (On vs Off) to be overridden after loading a sequence file which contains designated trityl information. Allows user to change trityl setting for any oligo form on to off and vice versa during run start up process.
- Modify Setup Screens: Allows change to setup screen defaults.
- Modify Security: Allows modification of security settings in user account management
- Reposition During Synthesis: Allows user to reposition a synthesis while active.
- Cancel Purge: Allows user to ignore initial chamber purge.
- Toggle Sequence Sensing Direction: Changes direction in which sequence file is read from loaded file; 5' -> 3' or 3' -> 5'.
- Modify Reagents: Allows user to make changes to reagents.
- Bypass Failed Sensor Checks: Allows user to continue past system diagnostic screen even if some sensors are outside their threshold values.
- Start Run: Allows user to start synthesis.
- Calibrate Valves: Allows user access to calibration screens.
- Manage Table Settings: Allows access to movement options screen.
- Remote Login: Allows user to log in with a third-party software utilising API.
  Contact Biosearch Technologies for information. Feature is usually only utilised when instrument is part of an automated process and requires remote control and monitoring.

A role may be created or a current one can be selected, and various permissions can be enabled or disabled according to how Administrator user determines type of access other users may have.

## System Settings

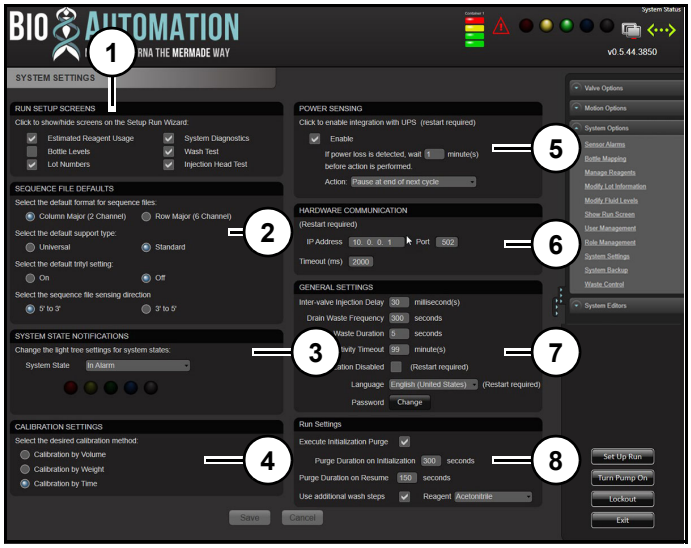



Figure 61

Refer to *(Figure 61)*.

**Run Setup Screens (1):** Following run setup default screens may be enabled or disabled:

Estimated Reagent Usage
Bottle Levels
Lot Numbers
System Diagnostics
Wash Test
Injection Head Test

**Sequence File Defaults (2):** Determines how sequence file entered during run start-up process will be interpreted by software. Sequence file is a 96 line file of sequence information. First oligo/line is always well-plate position A1. But should the second line be A2 or B1? Following defaults may be configured:

- Row Major: Sequences will be loaded across plate from A1 to A12. A1 would be first sequence in list and A2 being second.
- Column Major: Sequence loaded down plate from A1, B1, to F1, etc. A1 being first sequence in list and B1 being second.
- Default Support Type: Select either Universal or Standard support type.

  Universal: Does not have first base attached. Alerts software to add first base in sequence.

  Standard: Has first base attached. Alerts software to skip first base addition and start with second base addition.

- Default Trityl Setting: Tells software to leave or remove final DMT group.

  Trityl On: Software will leave final DMT group on oligo.

  Trityl Off: Software will remove final DMT group from oligo.
  Note: Calibrated Deblock step(s) must be included in Finalization step of script file for Trityl Off option to work properly.

- Sequence Sensing Direction: Chooses how software reads sequence file.

Note: Synthesis will always happen in direction of 3' to 5'. Take note of execution on these positions. In one case (5 to 3) it will be between G and C and in other (3to5) this bond will end up between C and A.

'*' indicates that this reagent will be used after coupling in same cycle step as the base immediately to it's left.
('C' in example).

- 5' to 3' Selected: entered seq= AC*GT actual execution= 3' T;G;C*A;
- 3' to 5' Selected: entered seq =AC*GT actual execution= 3' A;C*G;T;

In example 5' – AC*GT-3' During sequence transposing wild card character (*) gets flipped to 3'-TG*CA-5'. This causes error of sulfurization.

**System State Notifications (3):** Allows user to monitor system remotely and to change light tree illumination based on following machine states:

- In Alarm: One or more sensor is in alarm state.
- Machine Paused: Instrument is paused for any reason.
- Machine Pausing: A pause has been set but instrument has not yet paused.
- Machine Running: Instrument is currently active.
- Not in Alarm: No alarms are currently active.

- Offline: Instrument is not currently connected to controlling computer.
- Online: Instrument is currently connected to controlling computer.
- Synthesis Completed: All plates are finished but have not yet been removed.
- Synthesis Running: Instrument is current synthesising oligos.
- Synthesis Run Setup: Instrument is currently in set-up process.

**Calibration Settings (4):** Allows user to select a preferred calibration method.

Calibration by Volume (µL)
Calibration by Weight (g)
Calibration by Time (s)

**Power Sensing (5):** A separate UPS may be added to instrument. Software can be enabled to detect a power failure and a time in minutes may be entered before machine pauses. Also allows a sequence position to be set. Safest place to pause is after wash steps of oxidiser step. Software is only tested with APC brand of UPS although it may work with other brands.

**Hardware Communication (6):** Allows changes to IP Address, Port and Timeout (in ms) for communication between instrument and software.

***Notice***

Do not change these settings unless instructed by Biosearch Technologies. Sometimes there is a conflict with customers network and it may be necessary to change from our default network address (10.0.0.1 & 10.0.0.2) to an alternative address range.

**General Settings (7):**

- Drain Waste Frequency: How often waste tray is drained (time in seconds). While synthesizer is running instrument will open all waste tray valves at this frequency to ensure that reagents, which are primed into them during runs, do not overflow.
- Drain Waste Duration: How long waste valve is open as determined by drain waste frequency.
- Inactivity Timeout: How long instrument can sit idle before requiring user to log back in; enforces role management aspect of software.
- Oxidation Disable: Turns off oxidation step for special chemistry applications (restart required).
- Language: Different languages may be added and selected. Consult Biosearch Technologies for language packs.
- Password: Allows changes to opening screen software password if permissions allow.
- Inter-valve Injection Delay: Delay to position columns under injection head. Note: It is not recommended to change without approval. Errant changes can lead to injections injecting before movement is complete.

**Run Settings (4):** Allows user to select a preferred run settings.

- Execute Initiation Purge: Turns initialisation argon purge on/off. At beginning of a run, argon is used to fill synthesis chamber to remove humidity and reduce risk of fire by maintaining a low oxygen atmosphere inside chamber.
- Purge Duration on Initialization: Time (sec) for initialisation purge to occur.
- Purge Duration on Resume: Time (sec) for an argon purge to occur after a pause. Usually used when plates are added and removed and when 'Resume With Purge' option is used.
- Use Additional Wash Steps: When running oligos of different lengths, this function will fill completed columns with a similar volume of ACN as active columns are receiving current reagent.
  Example: Active columns receive 150 µL of Deblock, completed columns receive 150 µL of ACN so there are no changes in vacuum conditions across plate due to completed columns being empty.
  Note: Turn this feature off if running oligos that are same length to conserve ACN usage.

## System Backup

Allows user to back up system files or if files are requested by LGC Biosearch Technologies Technical Support. Allows users to export a zipped folder with or without additional files. Export is configurable regarding Log Files Folder. User may wish to only include debug log or include additional run logs. Exported zipped folder will be exported to the computer desktop and named 'Instrument Serial Number_MMDDYYYY_HHMM.zip'.

Without additional files selected the zipped folder will contain:

- Poseidon Configuration Files folder
- Log Files folder
- Windows Event Log folder

With additional files selected, the zipped folder will contain:

- Poseidon Configuration Files folder
- Log Files folder
- Windows Event Log folder
- Additional files the user requests (e.g. Script files, sequence files, specific run log files, etc.)

Note: Export location is not configurable and will always export to the computer's desktop.

## System backup instructions

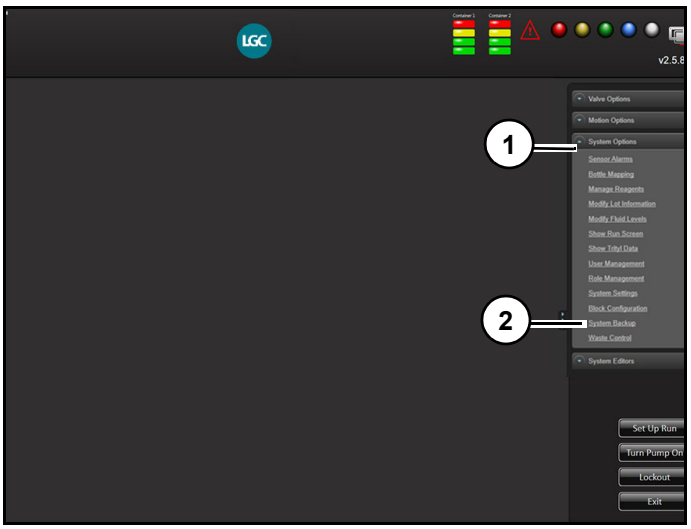



Figure 62

Refer to *(Figure 62)*.

1. Click "System Options" (1).
2. Click "System Backup" (2).

**Export Backup—No Additional Files Selected.**

User can determine if export should contain only the debug log or the debug log and run log files within Log Files folder.

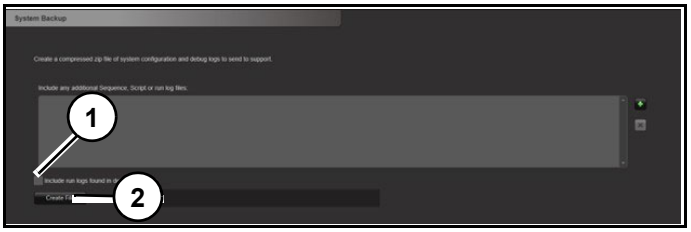



Figure 63

Refer to *(Figure 63)*.

1. To include debug file deselect "Include run logs found in default directory" (1) check box.
   To include debug file and run logs, select "Include run logs found in default directory" (1) check box.
2. Click "Create File" (2) to create export backup.

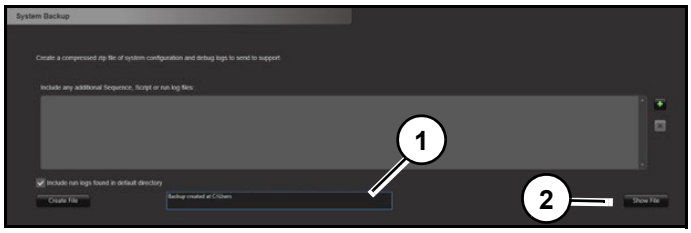



Figure 64

Refer to *(Figure 64)*.

Backup location of zipped folder will appear in black box (1).

3. To view folder, click "Show File" (2).

**Export Backup—Additional Files Selected.**

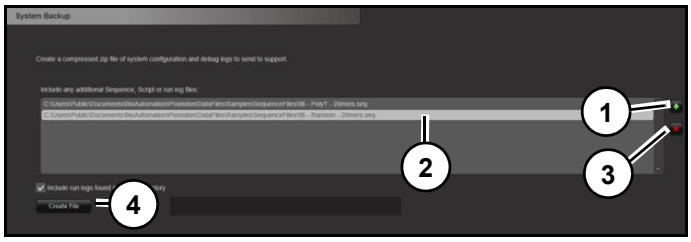



Figure 65

Refer to *(Figure 65)*.

1. Click "green +" (1) to select additional files to add to export. File Explorer will open to selected files.
2. Select desired file and click open.

Note: To delete files from export, select file (2) and click "red X" (3).

3. Click "Create File" (4) to generate backup export.

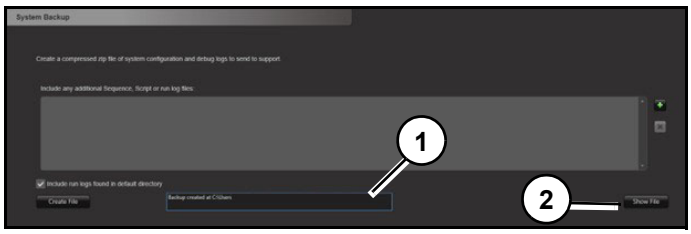



Figure 66

Refer to *(Figure 66)*.

Backup location of zipped folder will appear in black box (1).

4. To view folder, click "Show File" (2).

## Waste Control

Allows user to view current state of liquid level sensors and to set parameters to empty waste containers. Also manually triggers waste removal process.

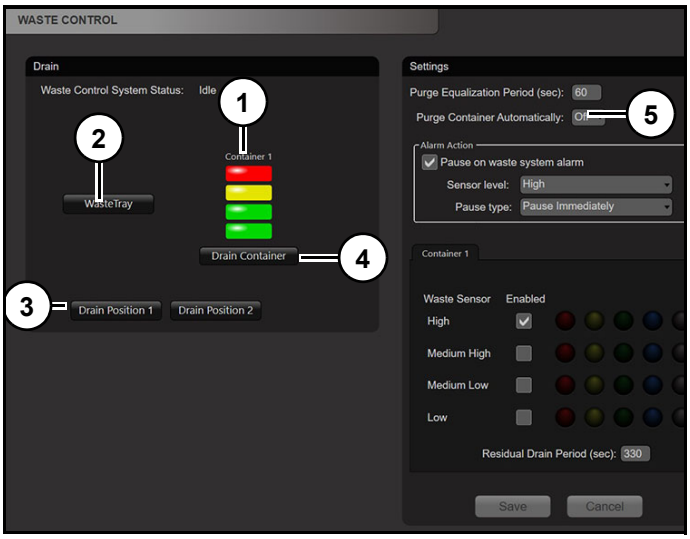



Figure 67

Refer to *(Figure 67)*.

**Level Indicator (1):** Shows state of each of four float switches on level sensor located in waste container. If indicator is lit, then liquid is triggering float switch.

**Vac Waste (2)**: Will drain waste tray.

**Drain Position 1 (3)**: Will drain column chuck associated with plate one.

**Drain Container (4)**: Will execute drain container procedure. Container has different settings on how to pause instrument when waste triggers high-level sensor, what light stack response will be, and parameters used when draining container. Each of four level sensors can have different light tree settings.

If using "Purge Container Automatically" (5), set instrument to pause when high sensor is triggered. When Purge Container Automatically" is checked software will pause instrument while running, as soon as high sensor is triggered, execute waste removal process on all tanks, and then re-initialise synthesis. This allows waste to be removed without operator intervention while instrument is running.

**Notice**

Assure instrument is connected to an adequate waste management system that can accept waste coming from instrument.

Whether system is told to drain waste by pressing "Drain Container" or if it is triggered automatically by High-level sensor during a run, execution is same.

After being triggered, instrument pauses based on 'Pause Type' selected, and then energises a 3-way (Vac-Pressure Select) valve that shuts off vacuum supplied from pump and simultaneously opens a path way for gas to pressurise container to 10 psi (15 psi max). Container will pressurise for a few minutes, then open a 2-way "Waste Out" valve which will allow pressurised gas to push waste out of container.

Waste removal process will continue until low-level indicator turns off.

After low-level indicator turns off, instrument will continue to drain for time indicated in "Residual Drain Period". This allows liquid below low-level sensor to be drained as well.

After "Residual Drain Period" expires, 3-way valve and 2-way valve will be de-energised. This will shut "waste out" valve and reconnect container to supplied vacuum.

"Purge Equalization" period begins and allows vacuum to be re-established in waste container to normal operating state so that instrument can begin synthesis again.

If "Purge Container Automatically" is set to "On" and a run is in progress, then synthesizer will un-pause run and begin synthesising oligos again.

## System Editors

System Editor (1) *(Figure 68)* allows user to modify and create script files.

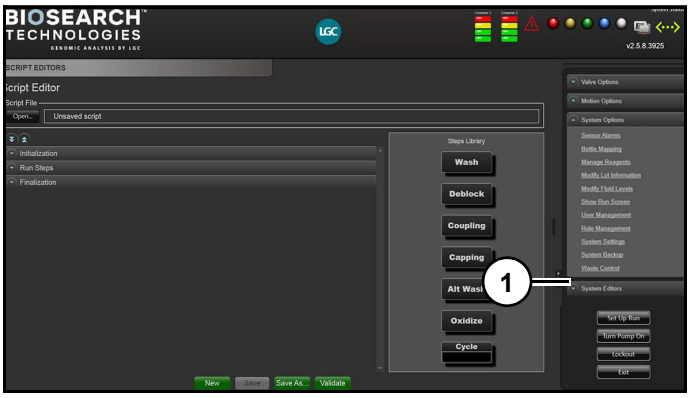



Figure 68

### Using script files to set synthesis parameters

During process of setting up a run, user will be asked to specify a script file for scale of synthesis to perform on each plate used during synthesis process. Script files also specify which parameters are used for each base addition

A series of standard script files have been created by Biosearch Technologies which can be used to synthesise products of different quality and yield. Script files are specific to a scale of synthesis as they contain volume information. Script file is program that synthesizer will execute when synthesising oligos. Users can assign a different script to each plate position.

## Opening a script file

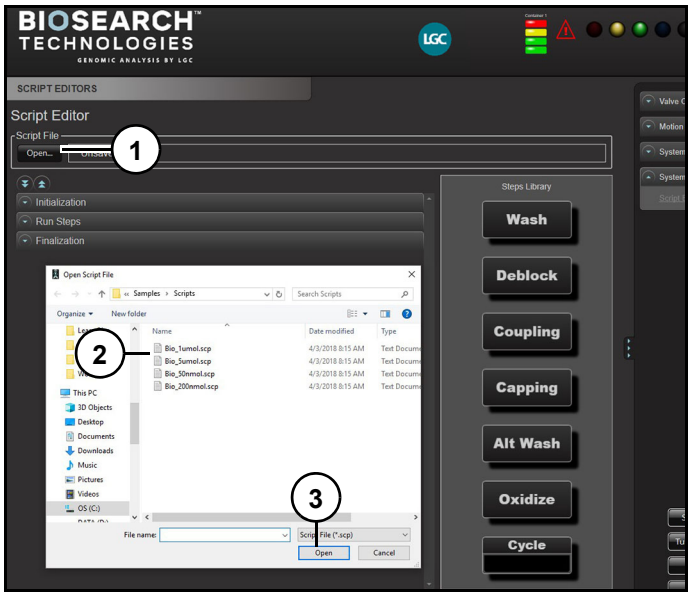



Figure 69

Refer to *(Figure 69)*.

1. Click "Open" (1).
2. Select "Script file" (2).
3. Click "Open" (3).

Script file will be loaded. By default software will return to last location from which a script was successfully loaded.

When opened, script file is validated against instrument. Validation includes calibrations and reagent configurations.

## Editing a script file


Figure 70

Refer to *(Figure 70)*.

Once script file is open, user can add, remove, and modify individual steps as needed. Left window (1) contains loaded script file and right side (2) contains steps library.

### Script file structure

A script file is composed of three main parts:

**Initialization (3):** Steps that take place prior to synthesis such as ACN washes and/or pre-capping**.**

**Run Steps (4):** Actual synthesis cycle (Deblock, Wash, Coupling, Capping, and Oxidation/ Sulfurization). Steps will repeat how ever many times necessary to complete longest oligos.

**Finalization (3):** Consists of post-synthesis steps such as Deblock for trityl off and ACN washes.

## Steps Library

Allows user to select which steps to put into script files. Step available:
Wash
Deblock
Coupling
Capping
Oxidize
Alt Wash
Cycle

Steps are self-explanatory and each applies to standard DNA/RNA chemistry. Software system adds two additional functions Alt Wash and cycle.

## Reagent Properties

Within each step of a cycle, in a script file, there are reagent properties that are unique to that reagent and that specific step.

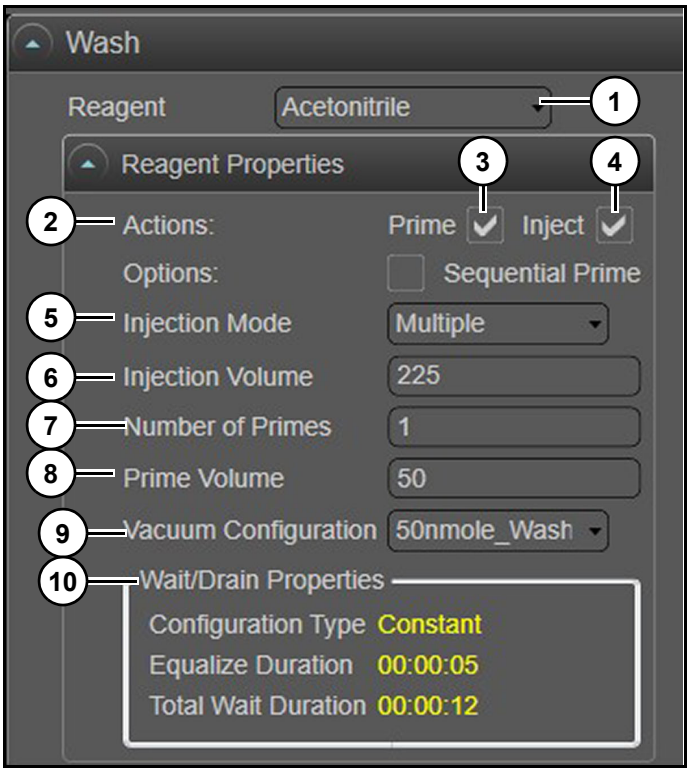



Figure 71

Refer to *(Figure 71)*.

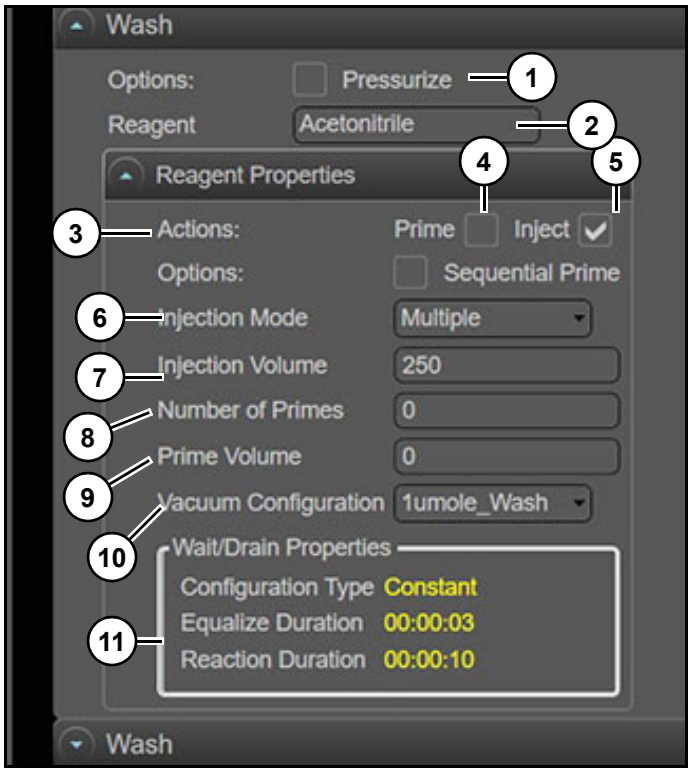



Figure 72

Refer to *(Figure 71)*.

**Pressurize (1):** Tells instrument to open plate pressurisation in and out valves. Opening valves allows argon to flow under plate which causes back pressure which keeps reagents in columns during injection process. When injection is done, valves are closed.

**Reagent Selection (2):** Allows user to select which reagent to use in that step. Drop down will contain more than one reagent if there are multiple reagents in category.
Example: If both ACN and DEA are designated as belonging to wash category in Manage Reagents Screen then drop-down will display both as choices when editing a wash step.

**Actions (3):** Prime and/or inject can be turned on or off. If a script file has multiples of same step in a row it is common not to prime second step since it was just used. Primes can be disabled by unchecking "Prime" (4). "Inject" (5) can be deselected to execute primes or drains without injecting reagent.

**Injection Mode (6):** There are three types of injection modes.

- Fast: Injects reagents as needed in groups; this mode is fast but not accurate and not recommended.
- Multiple: Injects reagents either eight at a time or one at a time, but not in smaller groups, recommended and most accurate.
- Single: Injects reagents one valve/one column at a time, very slow but accurate.

**Injection Volume (7):** Actual volume that will be delivered during step-in microliters. Calibrations are critical to actual delivered volumes.

**Number of Primes (8):** Number of times instrument will prime reagent. Primes will take place immediately before injection.
Note: Valve will only prime if priming box is checked.

**Prime Volume (9):** Prime volume in microliters. Priming is necessary to prevent build up and crystallisation on injection head. Reagents can also evaporate during times of no use. Priming will ensure that reagents is delivered accurately. Amount of priming will depend on reagents being used. Monomers/Amidite and activator typically require more priming due to their tendency to crystallise.

**Vacuum Configuration (10):** User selects a vacuum library entry created in Vacuum Pulse Calibration screen. Selected drain library entry dictates dwell time of reagent and controls liquid flow through column. This property is unique to each step, and each step (even with the same reagent) can have a different vacuum configuration.

**Wait/Drain Properties (11):** Displays drain configuration type (constant, interpolated, or fixed increment), and equalise and total wait durations (in M:S:MS) of chosen vacuum pulse library entry. Gives user reference as to characteristic of drain without the need to open Vacuum Pulse Calibration screen.

## Wash

Wash cycle is used to remove residual reactants and prep support for next step in cycle. Since there is no reaction time necessary these steps are usually programmed to drain to completion as soon as delivery is finished. Acetonitrile is most common wash solvent used.

## Deblock

Has same variables as a wash cycle except when deblock is added to support a reaction time is necessary to remove trityl groups.

Reaction times are usually around 30-90 seconds, depending on oligo length and chemistry. Most common deblock reagents are 2% or 3% DCA or TCA in Dichloromethane. Specific deblock formulations should be chosen based on chemistry being used.
Example: RNA usually uses 3% TCA whereas DNA would typically use 3% DCA. Depurination of the 3' Purines is much more of a concern when making DNA than when making RNA. Compromises will need to be made when making Chimeras.

## Coupling

Coupling is most important reaction when growing oligo is extended and another base is added. Many things affect coupling efficiency such as moisture, monomer to activator ratio, reagent quality, and drain characteristics.

Activator reagent properties will apply to all monomers in coupling step. Activator will be injected first then amidite will follow. User can have multiple coupling steps each with a different set of monomers associated. For instance, DNA could have one step and RNA could have another step.

Amidites can have different reaction times, each reaction time will require a unique vacuum library. Each monomer with a different vacuum library entry within a coupling step will be split into its own coupling step. Where multiple coupling steps and multiple vacuum library entries are used resultant injections will be concatenated and alternated. It is recommended to run in simulation after creating a new script to confirm proper step execution during synthesis.

## Capping

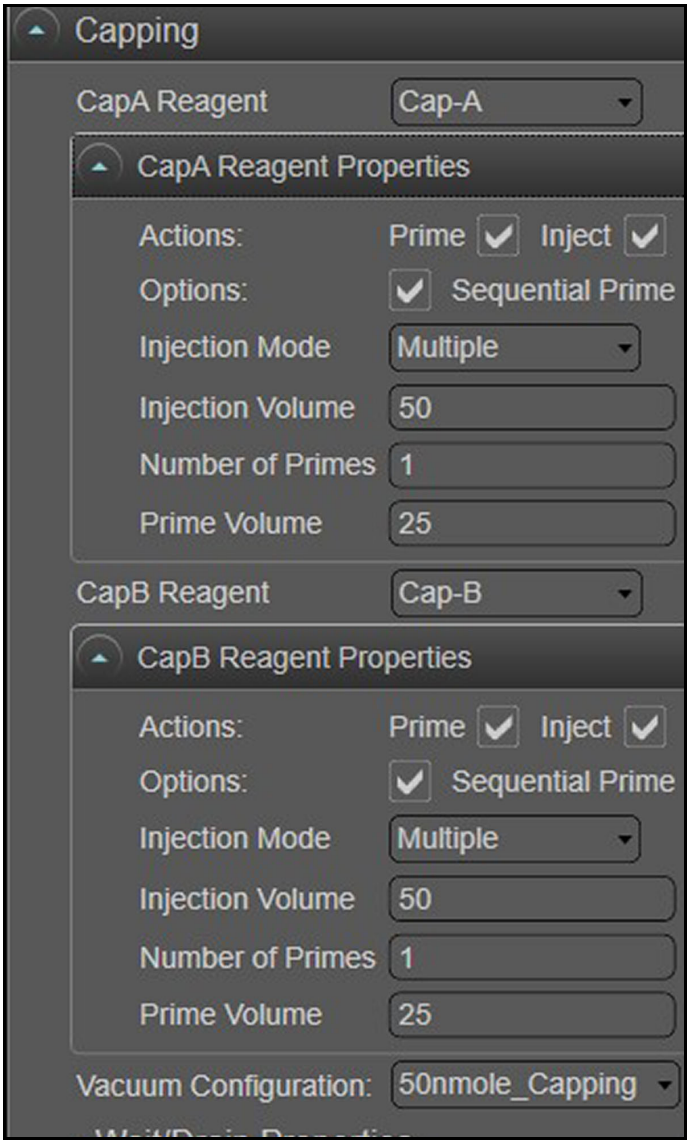



Figure 73

Refer to *(Figure 73)*.

Capping prevents molecules which did not get extended during coupling step to be blocked from further coupling steps. A capping failure during synthesis will lead to poor quality, deletions, and high N- impurities. Two reagents share one vacuum library entry as reagents are injected into same well and therefore must be drained together. There are a few capping formulations available and best for application should be determined.

## Oxidation and Sulfurization

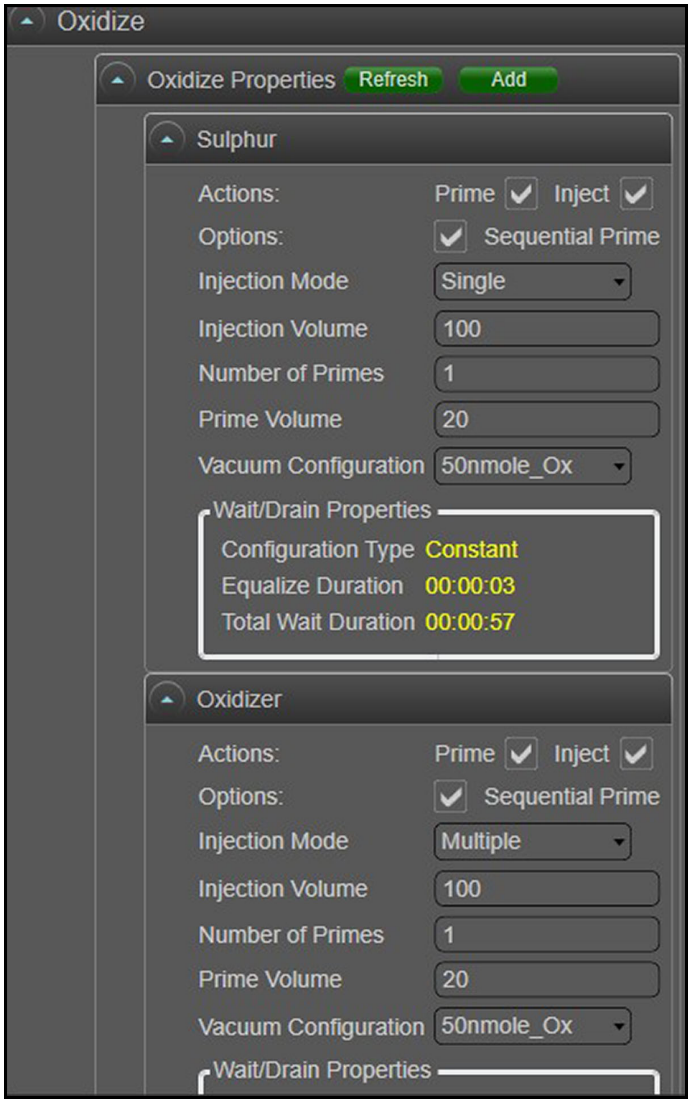



Figure 74

Refer to *(Figure 74)*.

Oxidation steps are used to put newly added backbone linkage into a stable state. This can be done with either oxygen or sulfur. When oxygen is used, result will be a phosphodiester back bone. When sulfur is used a phosphothiolated oligo will be generated. Each place on an oligo's backbone can be programmed to receive either oxygen or sulfur. Which of reagents that will be used is designated by either a ';' delimiter for oxygen or a '*' for sulfur in sequence file. Additional oxidisers can be added to and delimitators can be changed.

Contact Biosearch Technologies for support if more than two oxidation reagents are required. There are many types of oxidisers and thiolation reagents. Best choice for any given application will depend on factors including monomers being added and any modifiers that are being used.

## Alt Wash

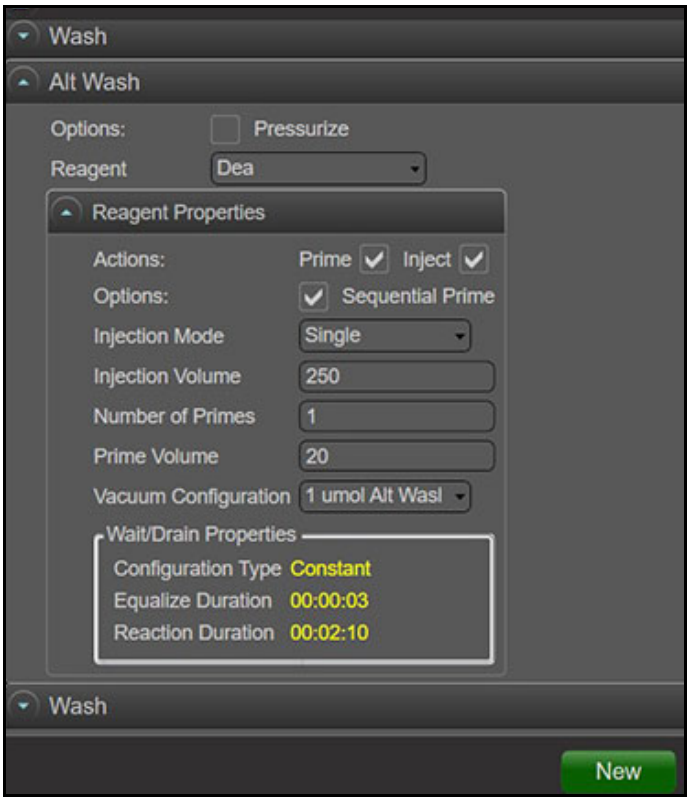



Figure 75

Refer to *(Figure 75)*.

Allows user to use any reagent on instrument as an alternative wash during a cycle. A common use for this cycle is for DEA treatments. Alt wash can also be used to force a prime at a specific point in a synthesis.

## Cycle

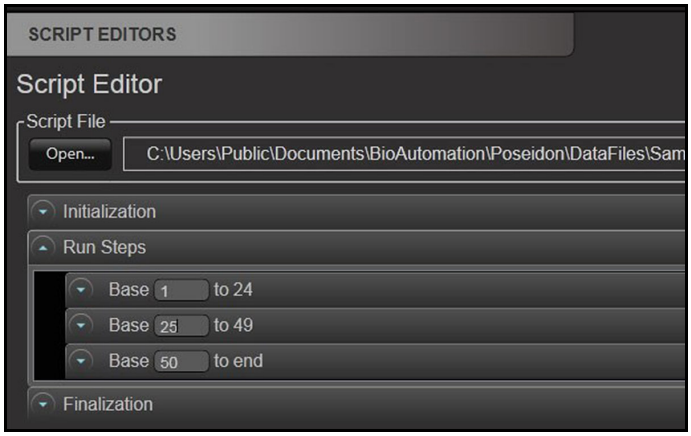



Figure 76

Refer to *(Figure 76)*.

Allows multiple cycles within a given script file. Example: Bases 1-24 can have a specific set of cycle steps, bases 25-49 another set, and bases 50-end a different set. Cycles can be grouped and added as necessary depending on specific chemistry requirements. Cycle can be used to increase number of deblock steps used as oligo grows. Similarly, it can be used to increase number of coupling steps used as oligo gets longer. Another use would be to increase volume of a reagent as oligos becomes longer. Example of a script file with multiple cycle *(Figure 76)*.

## Making changes to a script file

After loading a script file, user can make changes to script file.

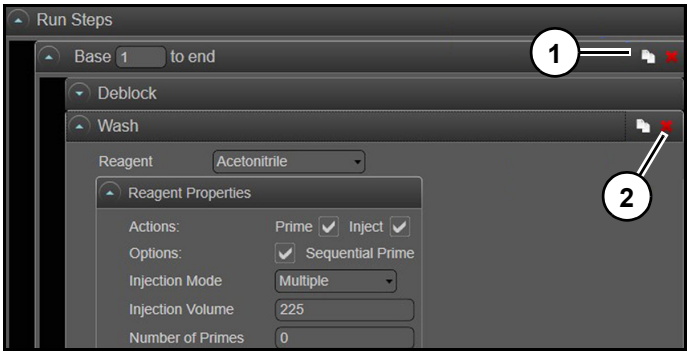



Figure 77

Refer to *(Figure 77)*.

**Copy Icon (1):** Allows individual steps of script file to be copied. Recommended method for adding steps.

**Delete Icon (2):** Deletes steps of script file.

## Drag and Drop

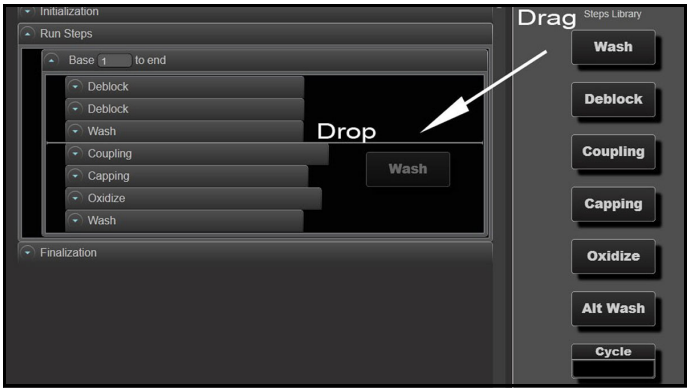



Figure 78

Refer to *(Figure 78)*.

User can drag/drop individual steps within a cycle or from steps library into script file. There are some restrictions on where some steps or cycles can be placed.
Example: Oxidation cannot be placed in finalisation part of the script.

There are default script files that come standard on instrument.

Please contact Biosearch Technologies with questions about changing a script file or help with a custom chemistry application.

## Starting a run

Once instrument has been calibrated for both liquid and vacuum user can start a run.

Steps for necessary to start a run:

- Plate Selection
- Load Sequence Files
- Column Details: CPG Type, Final Deblock, & Start at Base
- Load Script File
- Estimate Reagent Usage
- Plate Information
- Sensor Test Screen
- Injection Head Test
- ACN Wash Test
- Run Screen

### Setup screen

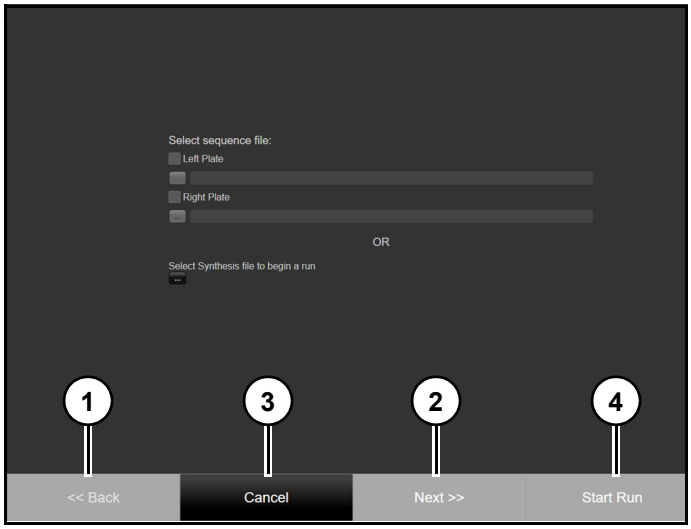



Figure 79

Refer to *(Figure 79)*.

**Back (1):** Returns to a previous step.

**Next (2):** Advances to next step.
Note: If "Next" is disabled, additional input is required before software can proceed. Some of steps, such as reagent usage, can be disabled in system settings screen if not required.

**Cancel (3):** Cancels setup process. Software will prompt user to terminate process.

**Start Run (4):** Activates run set up wizard which will guide user through setup process.

**Plate Selection.**
Allows user to specify which plates and sequence file to use in run.

Note: On some instruments there is an option to select a previously run synthesis file, this includes same sequence(s), plate(s), and same script files(s). Allows user to skip next run setup steps and proceed to run screen.

For a previously run synthesis file to be selected, file must be saved in last run setup screen.

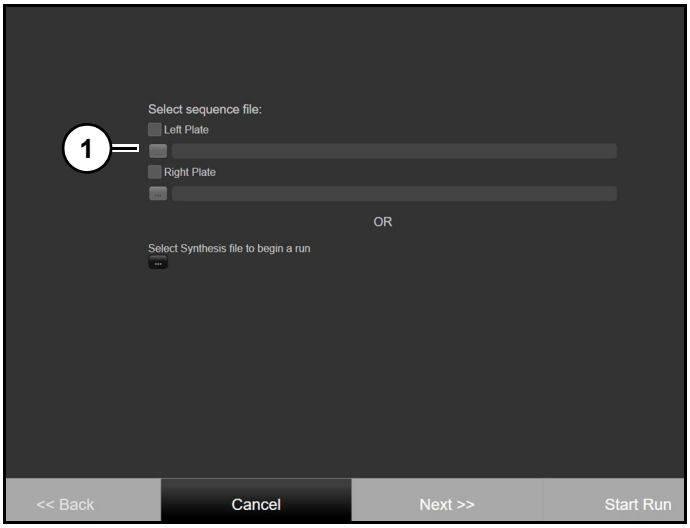



Figure 80

Refer to *(Figure 80)*.

1. Select "Plate" (1).

A plate can be run by itself or both plates at same time. If both plates are selected, instrument will start with left plate and continue to right plate when dispensing reagents.

**Load Sequence Files.**

Sequences can either be written 5' to 3' or 3' to 5', but synthesis will always occur in 3' to 5' direction. Software can be directed on which direction sequences are written on system settings screen.

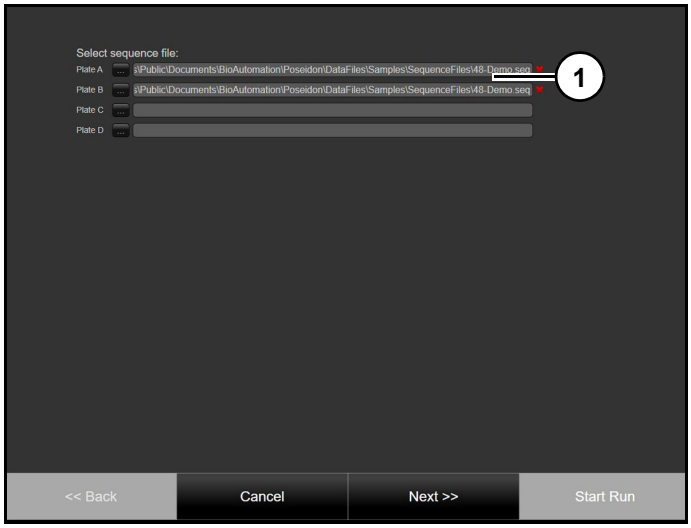



Figure 81

Refer to *(Figure 81)*.

2. Specify file(s) (1) containing sequence of oligos to be synthesise.
   Note: Sequence file can reside in any location and will accept sequence formats with following restrictions:

- Sequence entry for each well is located on a separate line. Any sequence information beyond line 96 will be ignored.
- Each line must contain an oligo name and an oligo sequence and it may contain information regarding type of CPG being used (Universal or Standard) and desired state of final DMT group (On or Off).
- Oligo name must be delimited from oligo sequence by a comma when using text files.
- Oligo name may contain any combination of characters and numbers including punctuation and spaces if they are part of an abbreviated display name for a monomer on instrument.
- Oligo sequence may contain combinations of upper and lower case characters as well as spaces.

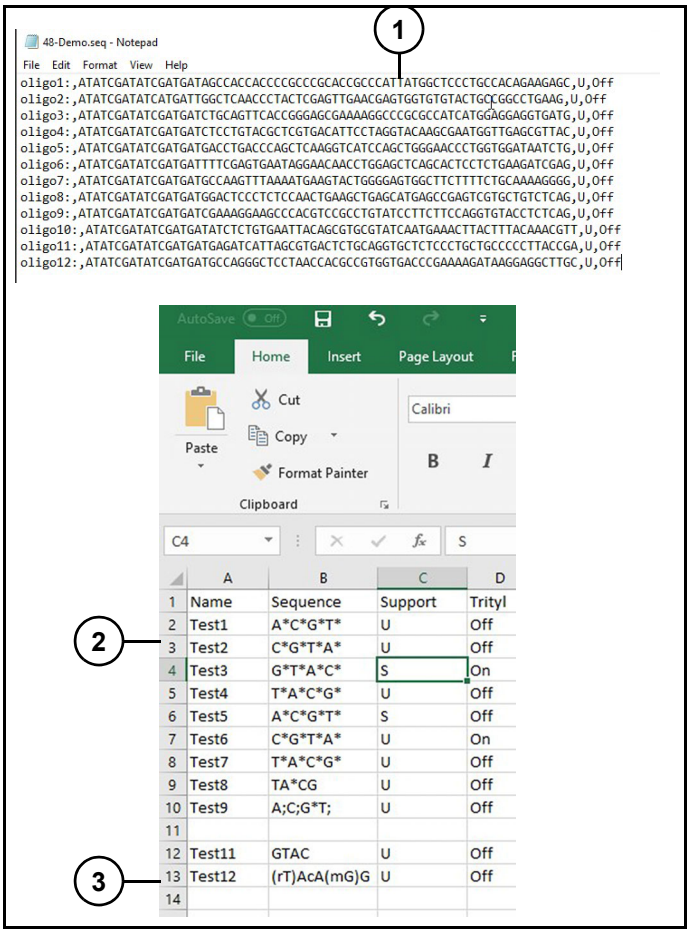



Figure 82

Refer to *(Figure 82)*.

- File must be a text file saved in '.seq' format (1) (for sequence file) or in Excel format (2). Notepad is used to create a text file saved as a '.seq' file. Excel files can be consumed natively and will ignore all fields except first four columns and first 13 rows.

User can select to have sequence file read so that order of synthesis is A1, B1 …A2, B2, …(Column Major or 8 Channel) or A1, A2, A3…A6, B1, B2, B3…B6 (Row Major or 12 Channel). See System Settings for more information on selecting how synthesizer interprets sequence files. If not using a well, set oligo name to 'BLANK' followed by a comma for text files, and skip line in excel files as seen in line 11.

Standard sequences can be specified in upper or lower-case format. Abbreviated Display Name in manage reagents screen is case sensitive and will be used to call a base form sequence file. 'C' is not equivalent to 'c' and 'UsA' is not equivalent to 'uSa'.

Abbreviated display names more than one character in length must be bracketed by parentheses as shown on line 13 (3) *(Figure 82)*.

To differentiate between standard backbone (P=O) and phosphorothioate backbone (P=S) oligos, use following nomenclature:

- Standard Backbone Delimiter: Semi-colon, ";"
- Phosphorthioate Backbone Delimiter: Asterisk, "*"

Note: Delimiters can be customised, *contact Biosearch Technologies for more information on how to change delimiters.*

Default for software is an assumed semi-colon. If not using delimiters in sequence files, instrument will assume that a standard oxidation chemistry to obtain an unmodified backbone is being used and hence assume sequence has delimiters ";" between each base. This needs to be changes to "*" for all P=S bonds (Sulfurised) in final synthesised oligo.
Example, **A;C;G*T;T;** will only have a P=S bond on the 3rd base 'G'. Sulfurisation reagent will be used in same cycle step as 'G' amidite. All other bonds will be P=O. For instruments with oxidation set as default **A;C;G*T;T;** is same as **ACG*TT.**

**Notice**

If uncertain of where P=S bond will be formed, it is recommended a test synthesis be conducted to confirm correct bond order.

Universal vs. Standard support can be designated with a U and S respectively. Trityl information can be designated using either 'On' or 'Off'. If neither is designated then instrument will use defaults set in System Options Screen.

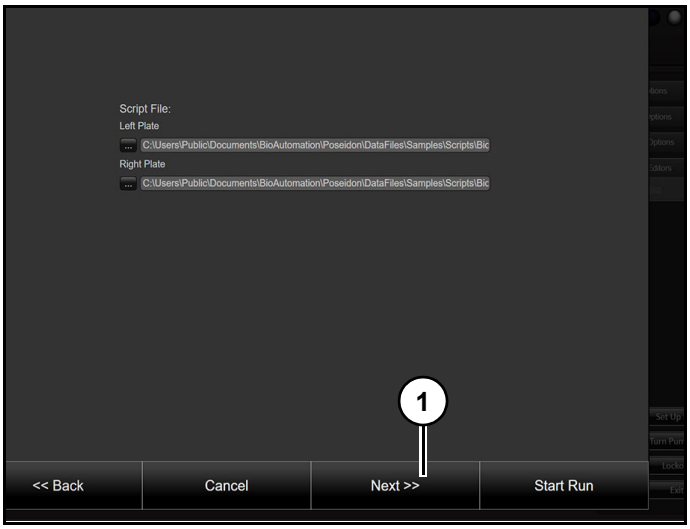



Figure 83

3. Click "Next" (1) *(Figure 83)*.

When sequence file is loaded a validation is run and any issues that will prevent sequence file from being ran and a prompt will be displayed.

**Column Details.**
Allows user to select CPG type, final DMT, and start at base position. Also allows access to a GUI to load columns into 96 well column chucks and verify sequence in each column on each plate.

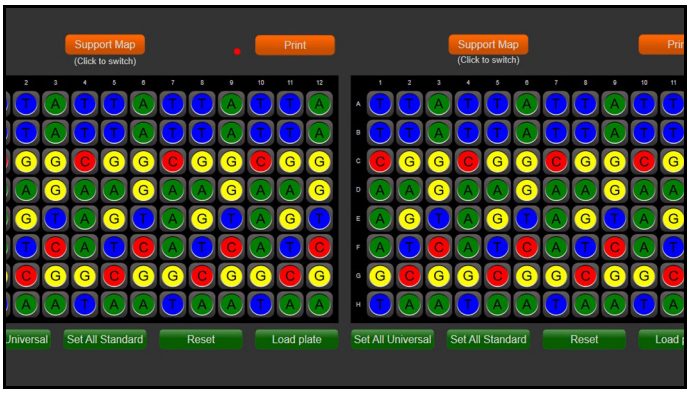



Figure 84

Refer to *(Figure 84)*.

Software default for CPG type is standard, where first base of desired sequence is attached to column. This can be changed in system settings to universal, where first base is not attached to column. In both cases, software reads sequence file and loads first base based on settings stored in system settings. If standard is default support type and a sequence is loaded which specifies universal, then sequence file will take precedence and universal will be displayed.

After loading sequences, to change CPG type click on a well to toggle between standard and universal, or by clicking "Set All Universal" or "Set All Standard". Click "Reset" to undo any changes and return to sequence file default or the system settings default (if there is no selection in the sequence file).

Final DMT selection takes place in same manner as CPG type. This can be specified in system settings, either ON or OFF, or can be specified in sequence file. Once a sequence is loaded, changes can be made to individual columns or entire plate with "Set All On" or "Set All Off buttons". Changes made can be undone by clicking "Reset".

Toggling between CPG type and final DMT selection screens by clicking orange Trityl Map/Support Map button.

## Loading synthesis columns

When CPG screen visible, load synthesis columns into column chucks that will be placed in synthesizer.

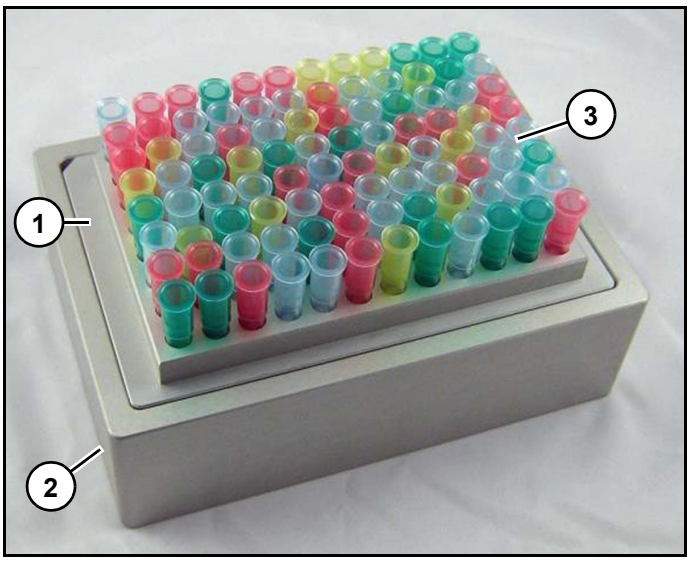



Figure 85

Refer to *(Figure 85)*.

1. Place column chuck (1) into holder chuck (2).
2. Using CPG information screen, load correct columns (3) into appropriate positions.

Figure 86

Refer to *(Figure 86)*.

3. Using supplied rubber mallet (part # 43690239) to tap columns into column chuck.

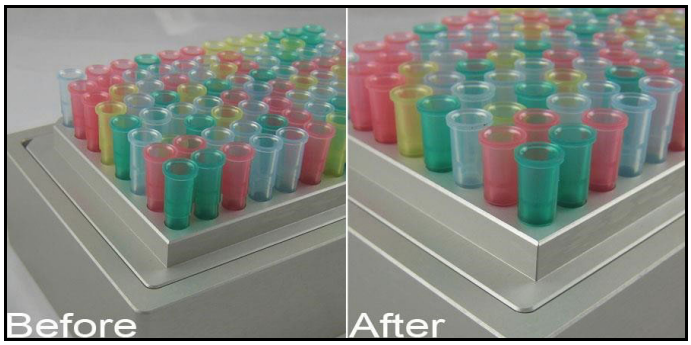



Figure 87

Refer to *(Figure 87)*.

Columns should be tapped so bottom edge of top of column ring is flat against surface of column chuck. If columns are not properly tapped into plate, plate will not drain properly during synthesis.

Figure 88

Refer to *(Figure 88)*.

1. To remove columns, place column chuck into holder chuck upside down.
2. Using rubber mallet. to tap columns out of column chuck and into holder chuck.

Under plate details box, there is a start at base box. For standard CPG, this box will have a value of 2 and for universal a value of 1. If there is a mixture of universal and standard CPG columns in a plate(s), software will start run as if all columns are universal, but not add any reagent or start synthesis in standard CPG columns until Base 2. Only ACN will be injected in standard CPG columns if "use additional wash steps" option is selected in run settings under system settings menu.

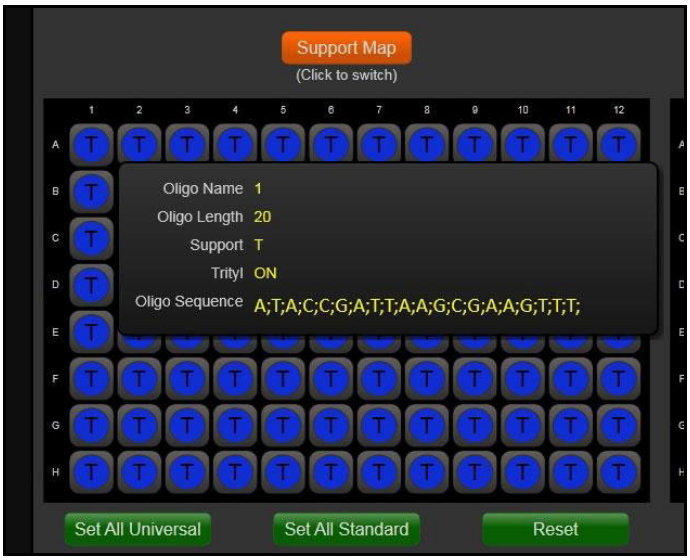



Figure 89

Refer to *(Figure 89)*.

3. Confirm each sequence in a plate by placing mouse cursor over each well and checking displayed 5' to 3' sequence.

**Load Script File.**

Script files are files which control how synthesizer will make oligos.


Figure 90

Refer to *(Figure 90)*.

4. Click "Plate Button" to assign script file to desired plate. This will bring up file explorer.

5. Navigate to correct script file and select.
   Note: Software will always return to last location from which a script file was successfully loaded.

Different script files may be used. However, having two script files in varying liquid dispensing, wait, and drain times will increase synthesis time.

6. Click "Next" (2).

**Estimated Reagent Usage.**

Allows user to view how much of each reagent will be used to determine if there is sufficient reagents to complete run and enough waste capacity.

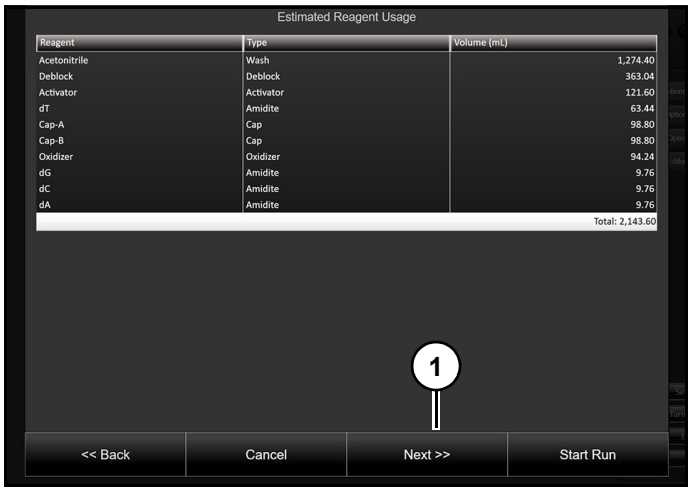



Figure 91

Values shown *(Figure 91)* are calculated from injection volume field in reagent file and are only as accurate as calibration.
Note: Synthesizer has no way of determining quantity of reagent in each bottle or how accurate instrument is calibrated.

If screen fails to show or is shown blank, then there is likely an issue with sequence file. Contact Biosearch Technologies.

Note: Add 20% more reagent to each bottle that software estimates.

Click "Next" (1).

**Lot information.**

Allows user to enter chemical lot information.

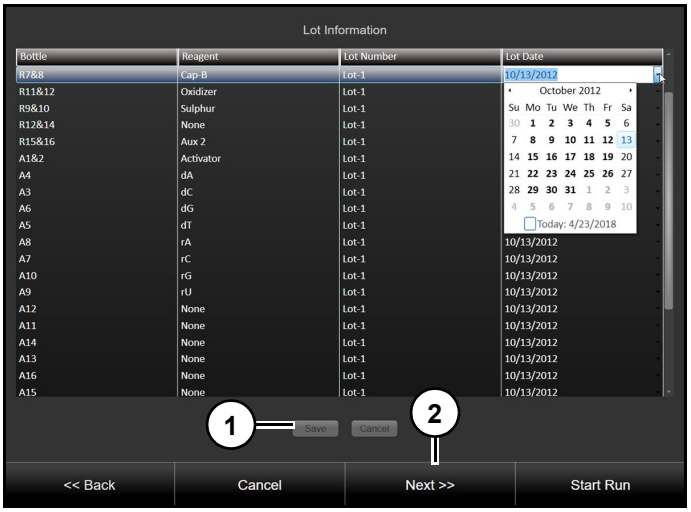



Figure 92

Refer to *(Figure 92)*.

7. Enter lot information for chemicals that are loaded on instrument.

8. Click "Save" (1).

9. Click "Next" (2).

**Plate Information.**

Allows user to enter run information to be recorded at top of log file.

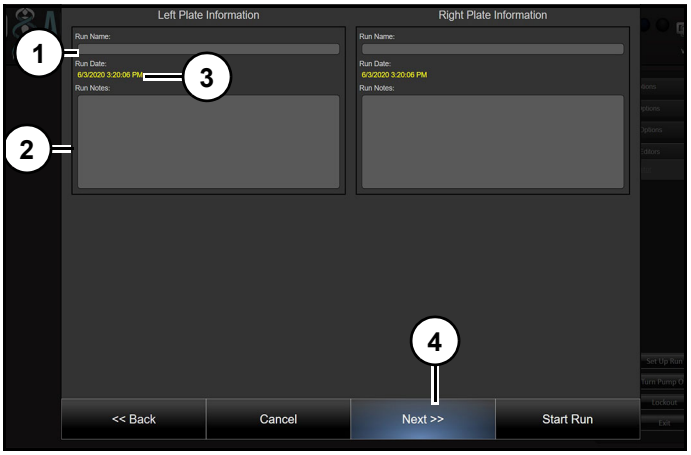



Figure 93

Refer to *(Figure 93)*.

10. Enter log information.

Log files use a date and time stamp to generate a unique name, it is not always obvious which file

corresponds to a run. Run name (1) will also be used when creating log files to allow easy tracking.

Inserting run notes (2) will make it easier to associate a log file with a run and prove helpful when troubleshooting or optimising.

Software automatically generates date and time stamp (3).

Click "Next" (4).

**Sensor Test Screen.**

Allows user to run a system check on instrument sensors.

Sensors:

**Alarm Checks.**

- Interlock
- Liquid Sensor
- Source Pressure
- Monomers Pressure
- Reagent Pressure
- Regulated Vac
- Purge Flow

**Argon Hi Flow Test.**

**Argon Low Flow Test.**

Clicking check button will check sensors for limits and warnings that were established in system options/sensor alarms screen.

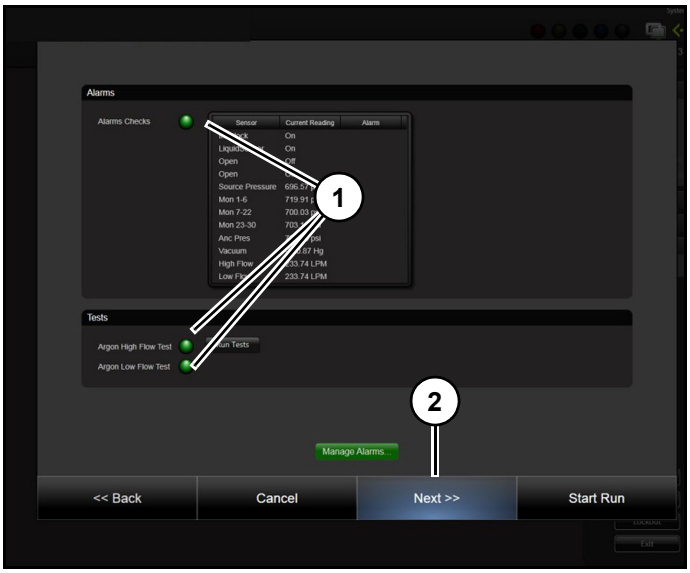



Figure 94

Refer to *(Figure 94)*.

If all three sensor checks have passed, green display lights (1) will light up next to sensor and "Next" (2) will become active.

11. Click "Next" (2), when green lights are display,

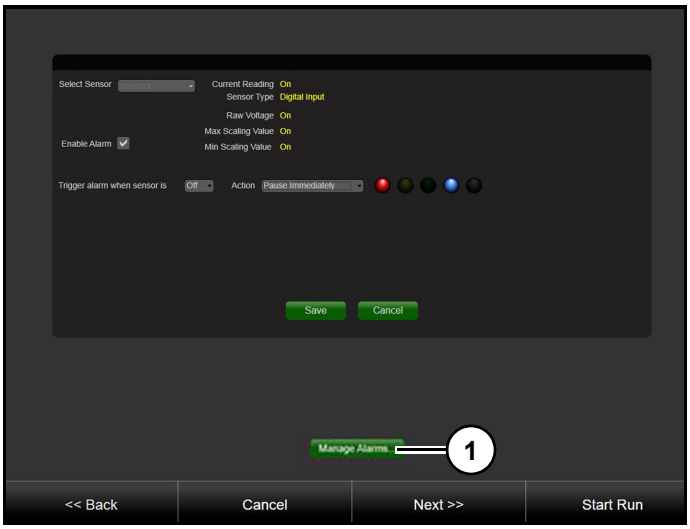



Figure 95

Refer to *(Figure 95)*.

If any sensors fail, then user must determine why they failed. Click "View Diagnostics" (1) to enter sensor alarms screen to check actual feedback values for sensors.

In some cases, sensor alarms may be set too narrow, in terms of trigger values, and so adjusting alarms for a wider tolerance will alleviate failed

sensor checks. In most cases, sensor check screen will fail because argon supply to machine is either very weak or exhausted. Replacing argon cylinder or adjusting cylinder regulator can alleviate potential problems.

Users with appropriate permissions can proceed past this screen after acknowledging errors. If user proceeds past this screen with sensors that are in error states then any sensors that are out of range will be ignored during run until sensor cross a warning threshold.

## Injection Head Test

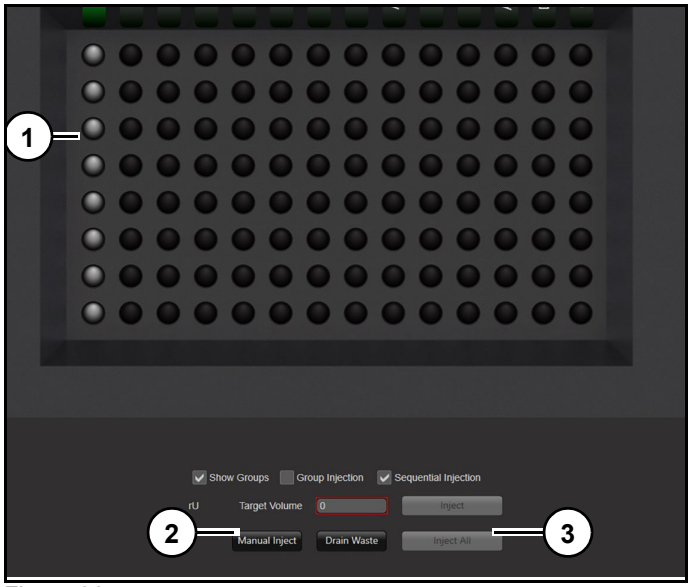



Figure 96

Refer to *(Figure 96)*.

1. Select valve to fire (1).
2. Select "Manual Inject" (2).
3. Confirm liquid is dispensing straight down and in a constant stream (ei,., no air bubbles).
4. Continue until all lines are primed and flowing as expected or click "Inject All" (3) to confirm all lines.
5. Drain waste tray.
6. Ensure all line are free from obstructions and crystal buildup.

## ACN Wash Test

Allows user to check if instrument is dispensing into plate correctly and vacuum is draining plate evenly.

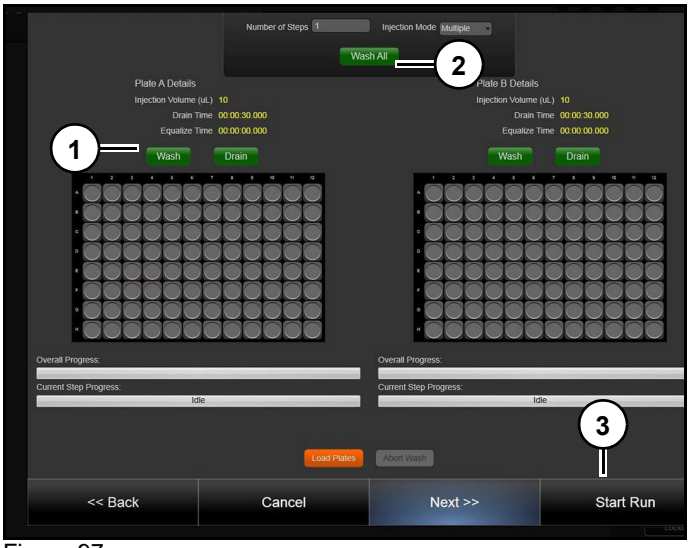



Figure 97

Refer to *(Figure 97)*.

7. Click "Wash" (1) button under each plate, or "Wash All" (2).

Software will dispense ACN into plate, and then drain plate based on parameters outlined in current script file. If columns do not drain at same rate, consider replacing slower draining columns.

8. Ensure plate is completely drained at end of ACN wash test and check that there are no blocked or slow draining columns prior to starting synthesis.
9. Click "Start Run" (3).

## Run Screen

Provides user with control and displays details of ongoing synthesis.

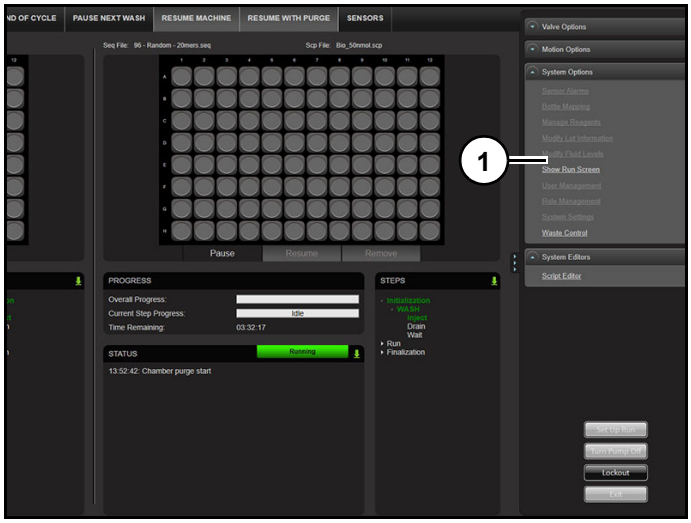



Figure 98

Refer to *(Figure 98)*.

if user selects one of options from navigation menu (1), this screen will no longer be visible. To navigate back, user simply navigates to system options → show run screen.

## Run screen controls

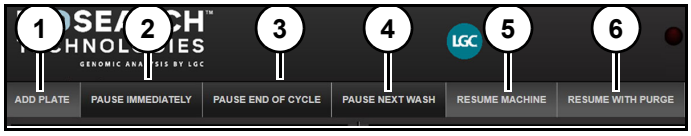



Figure 99

Refer to *(Figure 99)*.

**Add Plate (1):** Allows user to add an additional plate if synthesizer has an idle plate position. Instrument will need to be paused. Once selected operator will be taken through an startup process which will allow them to add a new plate to synthesizer.

**Pause Immediately (2):** Pauses instrument immediately unless synthesis is in a wait step and then will pause as soon as wait step is over. Option should rarely be used and considered a last resort as it can cause software to lose track of some events and cause unexpected behavior when restarting run. Note: Do not pause immediately during an injection step. May cause valve to stay open.

**Pause at End of Cycle (3):** Will pause instrument prior to next Deblock step. Safest way to pause synthesis.

**Pause Next Wash (4):** Will pause after next available ACN wash. If no wash step is called for during synthesis, then no pause will happen.

After pausing, user will have to resume run for synthesis to continue.

**Resume Machine (5):** Resumes from last pause point.

**Resume with Purge (6):** Initiates an argon chamber purge and then continues from last pause point.

## Sensors

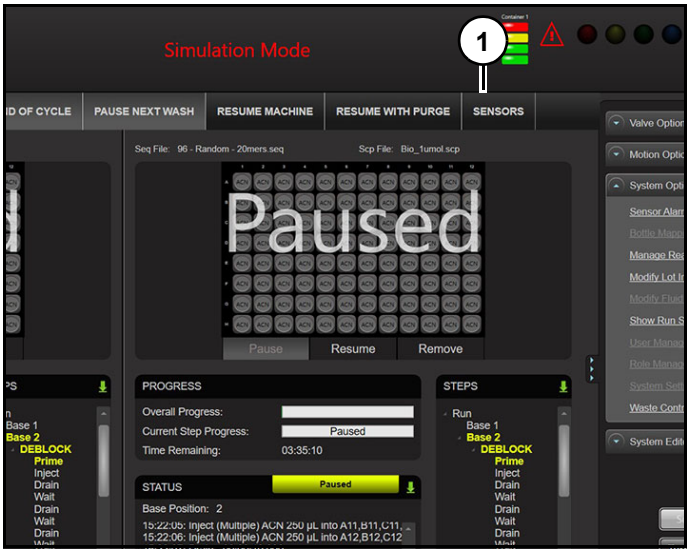



Figure 100

Refer to *(Figure 100)*.

Displays current sensors and their respective values. Sensors cannot be changed, only viewed. To adjust sensors and sensor options (See "Sensor Alarms" on page 44.).

## Plate Options Bar Features

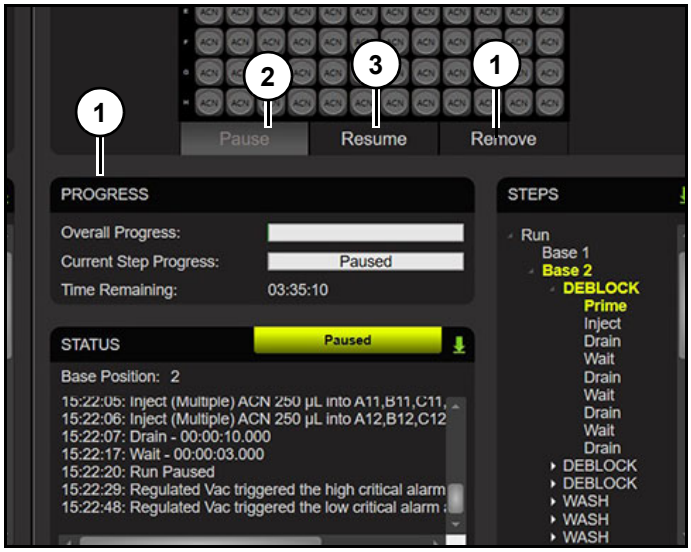



Figure 101

Refer to *(Figure 100)*.

Plate options bar (1) is shown just below graphic of the active plates.

**Pause (2):** Will pause individual plate immediately. Should be used only as a last measure. It is better to program a pause at end of step. Unexpected events can occur when plates are paused immediately.

**Resume (3):** Will resume a paused plate., single phase only.

**Remove (4):** Removes plate from active synthesis queue.

## Steps Control Options

Displays active step in synthesis cycle and provides user with options when right-clicked (1) *(Figure 102)*.


Figure 102

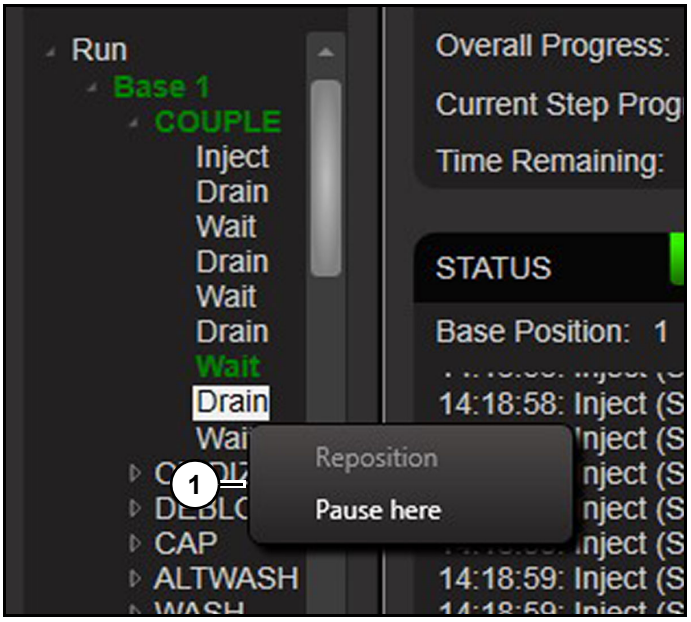



Figure 103

Refer to *(Figure 103)*.

**Pause Here (1):** Will pause instrument at exact location specified by user. Pause can be initiated at a base, cycle step, or cycle sub-step (wait, drain, inject, etc.).


Figure 104

Once a pause is initiated, location of pause will be highlighted orange (1). *(Figure 104)*

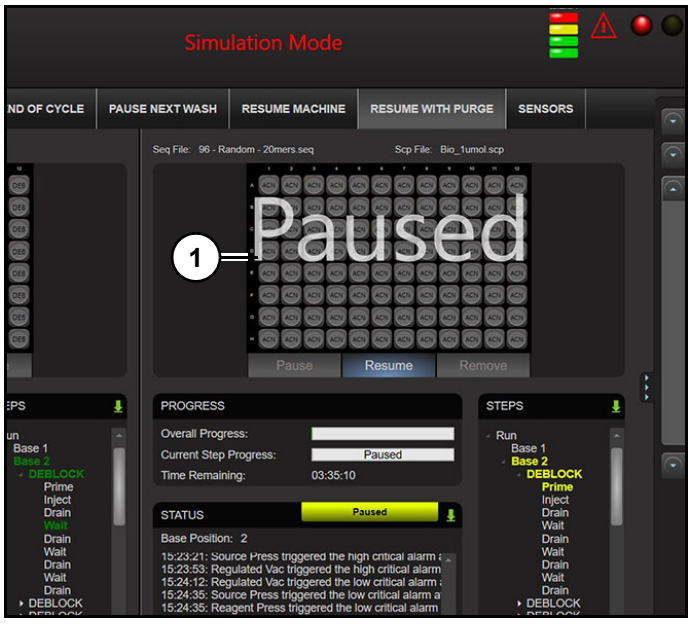



Figure 105

When pause point is reached, instrument will pause, and status will be highlighted yellow and plate graphic will show "Paused" (1). *(Figure 105)*

## Canceling a Pause

A set pause point can be canceled before it is executed.

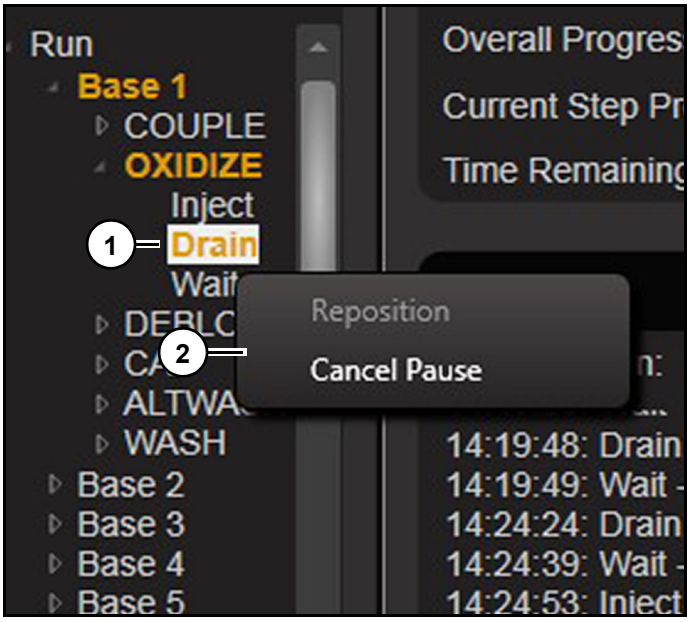



Figure 106

Refer to *(Figure 106)*.

1. Right click pause point (1) highlighter in orange.
2. Click "Cancel Pause" (2).

## Reposition

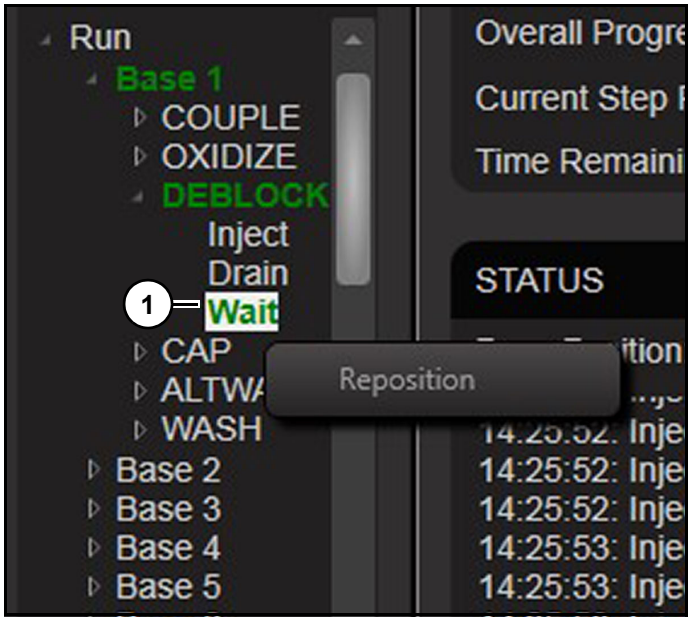



Figure 107

Refer to *(Figure 107)*.

After machine is paused, user can right-click any step or sub-step (1) to reposition synthesis at a different point in cycle. This option is useful for real-time run control and gives step level control of each base in active synthesis.

For example, if user sees that a certain step did not prime properly or drain columns completely then reposition feature can be used to rerun that certain step to ensure proper oligo elongation. Use selectively, to not have unintended consequences.

## Post synthesis

When synthesis is complete screen will show finished.

### Removing synthesis plate(s)

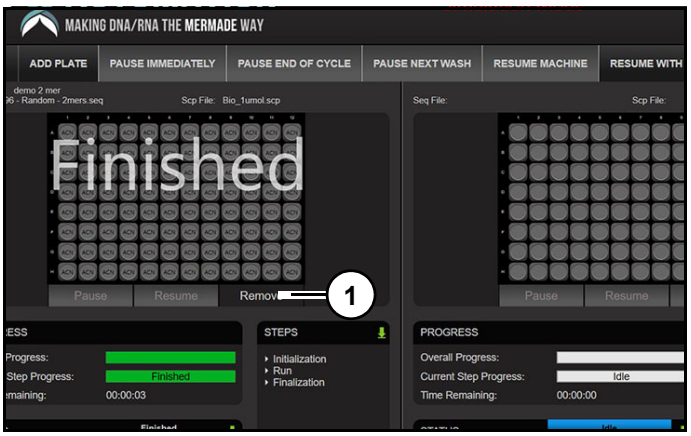



Figure 108

Refer to *(Figure 108)*.

1. Click "Remove" (1) to let software know that plate is no longer active.

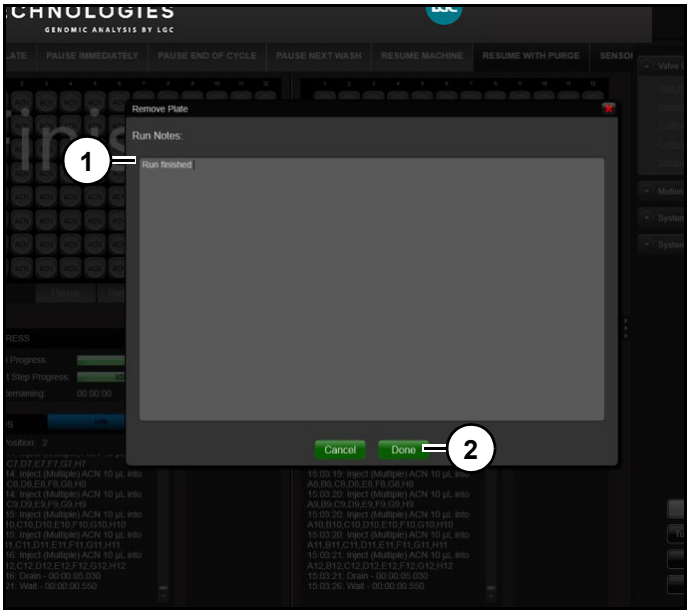



Figure 109

Refer to *(Figure 109)*.

2. Record any synthesis notes in "Run Notes" (1).
3. Click "Done" (2).

# Cleavage and deprotection

## Cleavage of Oligonucleotides from columns

Once synthesis is complete, oligos will need to be cleaved from CPG columns and deprotected before they can be used.

Cleavage and deprotection chucks are provided in the 192E startup kit.

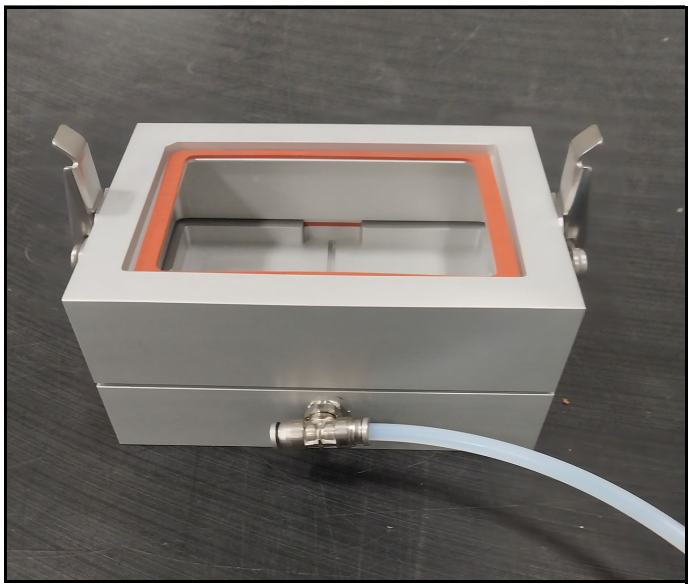


Figure 110

Cleavage Chuck *(Figure 110)*.

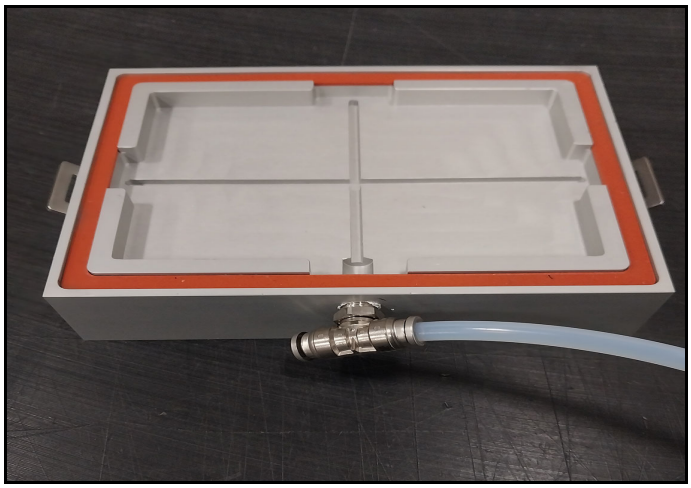


Figure 111

Cleavage Chuck Bottom *(Figure 111)*.

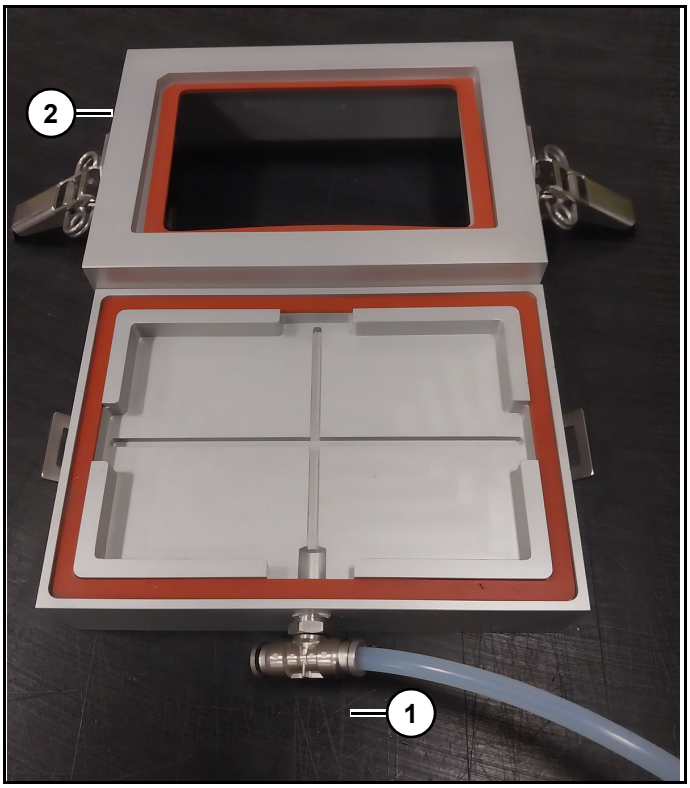



Figure 112

Refer to *(Figure 112)*.

1. Connect cleavage chuck (1) to a vacuum source.
2. Remove top portion (2) of assembly.

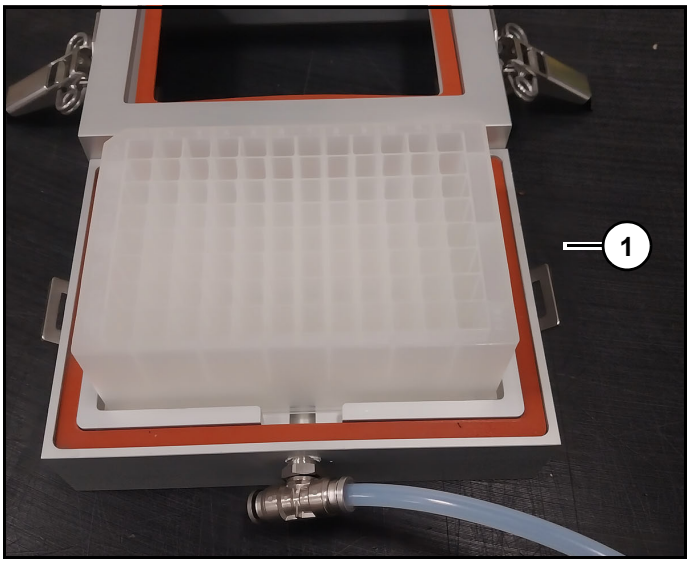



Figure 113

Refer to *(Figure 113)*.

3. Place a clean 96-well plate (1) onto bottom of cleavage chuck. Make sure that A1 of well plate is in upper left hand corner of chuck.

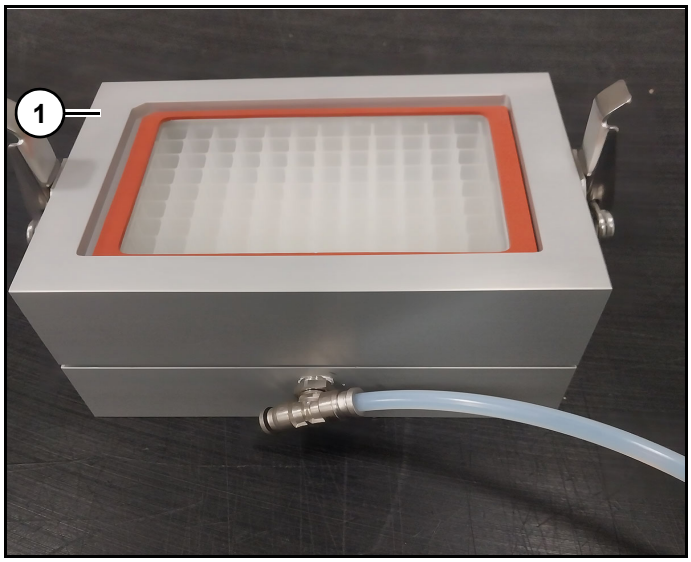



Figure 114

4. Place top portion (1) *(Figure 114)* of cleavage chuck on top of bottom portion of the chuck with 96 well plate.

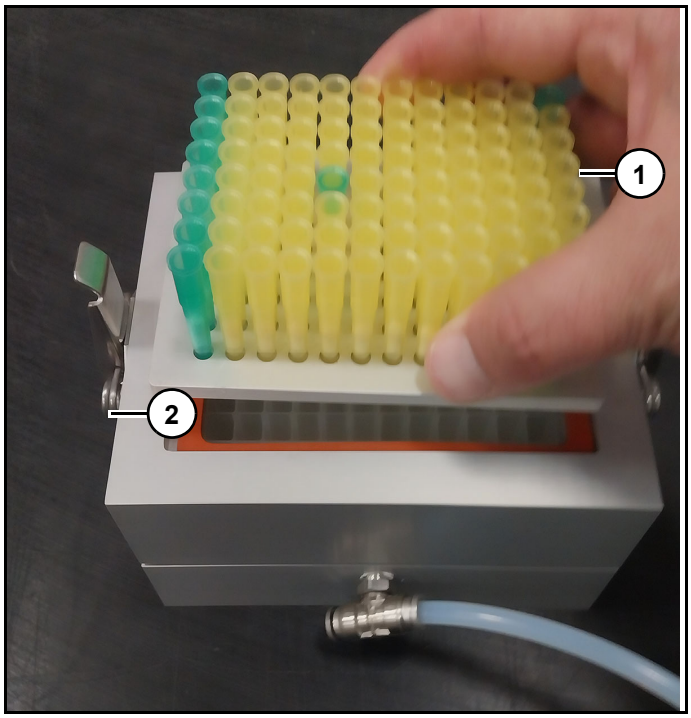



Figure 115

5. Place column chuck (1) into recess of cleavage chuck (2), making sure that column A1 is in upper left hand corner. *(Figure 115)*

6. Add a cleavage solution to columns using an 8 or 12 channel pipette to remove synthetic oligo from solid CPG support.

Note: There are 2 main cleavage cocktails on the market. Pure 28 to 30% Ammonium Hydroxide and second is AMA; a 50:50 solution of Ammonium Hydroxide and Methylamine. AMA is faster, but has 1 specific limitation; you cannot use benzoyl protected dC (bz-dC) with this chemistry and you must use acetyl protected dC (Ac-dC) instead.

Use deprotection condition for base protecting group.

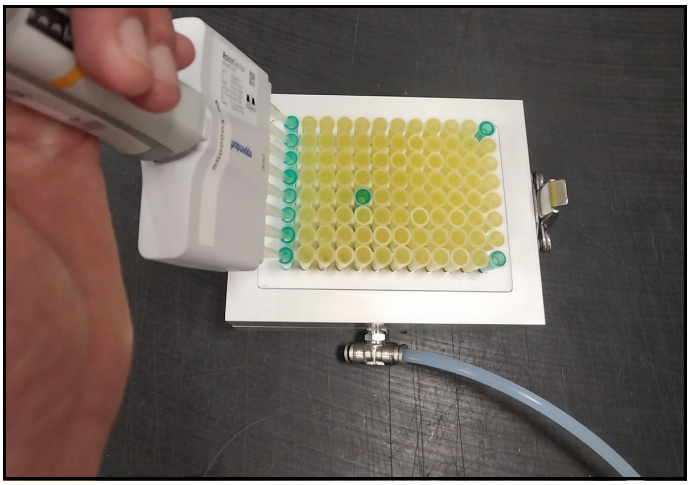


Figure 116

7. Once chemistry is chosen, cleavage cocktail can be applied to columns *(Figure 116)*.
   Note: Apply just enough vacuum to columns to pull cocktail onto CPG bed. Too much vacuum can cause cocktail to pull through columns too quickly increasing chance of improper cleavage and deprotection resulting in lower yields. Repeat with correct wait times according to charts above.

## Deprotection of Cleaved Oligonucleotides

1. Remove the 96 well plate from cleavage chuck.

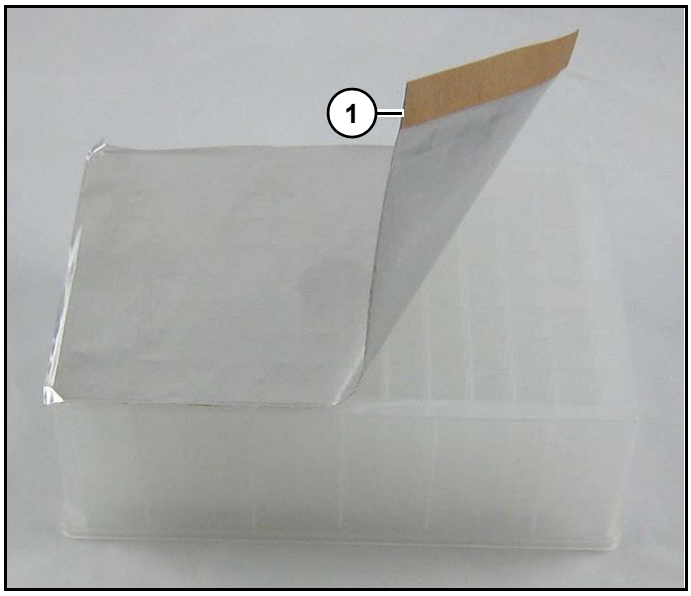



Figure 117

2. Using supplied foil seal (1) *(Figure 117)* (part # 538619), apply seal to top of plate ensuring that all wells are completely covered.

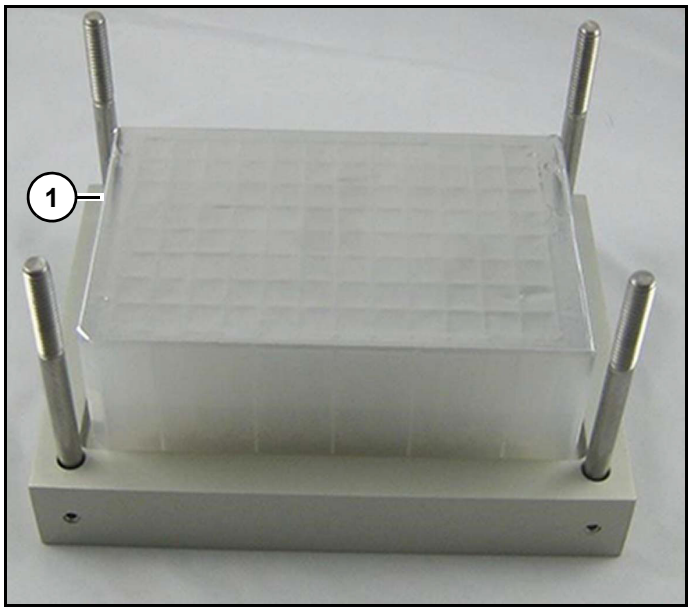



Figure 118

3. Place 96 well plate (1) *(Figure 118)* into bottom of deprotection chuck.

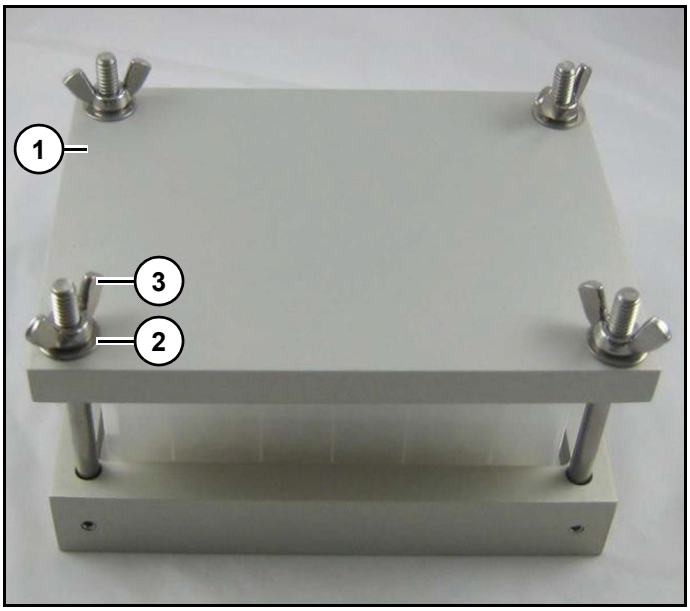



Figure 119

Refer to *(Figure 119)*.

4. Place the silicone spacer mat (not shown) *(Figure 119)* (part # O-SIL-DPG) on top of sealed well plate.
5. Place deprotection chuck top (1) on top of entire assembly.
6. Secure top plate with washers (2) and wing nuts (3).
7. Plate is now ready to deprotect according to chart.

| Deprotection | | | |
|---|---|---|---|
| **Scale** | **Room temp.** | **65 °C** | **80 °C** |
| AMA | 1 hour | 30 mins. | 15 mins |
| **(NH4OH)** | Overnight | 6 hours | 3 hours |

# MerMade 192E maintenance

**⚠WARNING**

Read and understand equipment operators manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

**⚠WARNING**

Shut down main power to instrument before performing any maintenance. Failure to do so could result in serious injury or death.

**⚠WARNING**

Use minimum level A3 cut resistant gloves underneath appropriate PPE gloves when installing and removing bottles unless additional grip is needed.

## Cleaning

**⚠WARNING**

Do not use Acetone or abrasive cleaner on instrument. Do not immerse instrument in solvents.

Before using any cleaning or decontamination method, check with manufacturer that method will not damage instrument.

In case of radioactive spillage's, Biosearch Technologies recommends a proprietary cleaning agent and follow manufacturer's instructions.

Clean instruments cover with a cloth lightly dipped in water, ethanol, methanol or formaldehyde may also be used.

Wipe down plate deck of instrument with a damp cloth.

## Maintenance schedule

| Maintenance checklist | |
|---|---|
| **Weekly (Performed by operator)** | |
| Wipe down surfaces | |
| Wipe down plastic guards with glass cleaner | |
| Check valve station (clean when necessary) | |
| **Quartely (Performed by Biosearch Technologies Field Service Technician)** | |
| Calibrate Instrument (liquid and vacuum). | |
| **Annual (Performed by Biosearch Technologies Field Service Technician)** | |
| Inspect all panels for damage | |
| Check software version and backup. Update software if newer version is available and desired by customer. | |
| Check all terminals | |
| Check all plugs are secure and fitted correctly | |
| Inspect cables | |
| Check on/off switch is functioning | |
| Check input and output sensors | |
| Check outputs are working. | |
| Check fuses | |
| Check pumps, and replace tubing | |
| Inspect fittings for leaks | |

## Maintenance and spare parts

Biosearch Technologies recommends that all service on MerMade instruments be performed by Biosearch Technologies. Whenever instrument has an issue that prevents it from being used safely, Biosearch Technologies recommends removing instrument from service and contacting Biosearch Technologies.

# Troubleshooting

**⚠WARNING**

Read and understand equipment operators manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

***Notice***

Contact LGC Field Service for assistance with troubleshooting and instrument maintenance.

## *Troubleshooting guide*

| System | Cause | Solution |
|---|---|---|
| Instrument will not power up. | No power to the instrument. | 1. Check power to instrument.<br>2. Check circuit breaker. Activate the circuit breaker if necessary.<br>3. Check fuses at plug. Replace fuses if necessary.<br>4. Contact LGC Field Service for additional support. |
| Dispense Head has collided with an obstruction. | Obstruction within instrument cabin. | 1. If possible, manually control Dispense Head through options menu so that it is moved away from obstacle. Remove obstacle.<br>2. If Dispense Head cannot be moved away from obstacle, disconnect power to instrument.<br>3. Contact LGC Field Service for additional support. |
| Instrument has stopped moving. | E-stop pressed. | 1. De-press E-stop.<br>2. Contact LGC Field Service for additional support. |
| Priming does not work. | Low RO water. | 1. Check level of RO water in wash bottle. If bottle is placed on ground, raise bottle higher. |

| System | Cause | Solution |
|---|---|---|
| Instrument will not drain | | 1. Check vacuum gauge for normal display.<br>2. Listen for the vacuum valve click on and off.<br>3. Check for bent drain lines.<br>4. Check for debris in drain lines. Ex: Crystalized amidite Use a guitar string to unclog the lines. Rinse thoroughly<br>Important: Ensure the clog doesn't move to and get stuck in a cross, manifold, or valve.<br>5. Change the drain lines<br>Important:<br>Ensure new drain lines are the same length as the old lines to keep drain calibration the same.<br>6. Replace the vacuum valve<br>7. Check the manifold for debris or burrs.<br>8. Trace the problem from vacuum to vacuum.<br>9. Contact LGC Field Service for additional support. |

| System | Cause | Solution |
|---|---|---|
| Bad synthesis | | 1. Check all valves – ensuring that all fire as they should.<br>2. Confirm that instrument has not run dry.<br>3. Check all gauges – are normal/expected.<br>4. Check the calibration<br>If outliers are found, check for broken positions.<br>5. If no leaks are found, replace the valve.<br>6. Check the vacuum calibration.<br>7. Contact LGC Field Service for additional support. |
| Fails pressure check (source pressure tanks when the argon is disconnected) | | 1. Tighten all amidite positions and check reagent lines – ensuring they are tight.<br>2. Check o-rings in all amidite positions and reagents.<br>3. Over-pressurize the system by a full turn of the regulator. Wait for the system to pressurize and then turn it down. If gauge drops, Check for broken bottle positions.<br>4. Contact LGC Field Service for additional support. |
| Instrument loses alignment | | 1. Tighten screws on the limit detectors<br>2. Contact LGC Field Service for additional support. |

| System | Cause | Solution |
|---|---|---|
| Motor error | | 1. Ensure instrument is connected to the computer.<br>2. Ensure power is on to the instrument and that power connections are well seated.<br>3. Check if the liquid sensor is tripped.<br>4. Ensure that the safety interlock is functioning correctly.<br>5. Review the Copley logs.<br>6. Contact LGC Field Service for additional support. |
| Slide errors | | 1. If drifting is seen, contact LGC Field Service. |

# Customer support

## Customer support

Biosearch Technologies customer support provides unparalleled in-house, field, and remote customer support. Available 24 hours a day and 7 days a week, our experienced technicians provide you with superior knowledge and fast, reliable service. Phones are staffed from 7:00 am to 5:00 pm (CST), Monday through Friday, with after hours and Saturday/Sunday support available through an answering service.

Technicians are cross-trained in mechanical, electrical, and programming competencies. They are equipped with latest portable computers and remote software and are available for preventive maintenance, instrument surveys, instrument modifications, and routine or emergency service work. Biosearch Technologies customer support can be contacted at:

| Biosearch Technologies customer support | |
|---|---|
| Customer support | + 1 866.225.3482 |
| Parts ordering | orders.alex@lgcgroup.com |
| Reagents ordering | orders@berryassoc.com |
| Address: | LGC Biosearch Technologies<br>3600 Minnesota Street<br>Alexandria, MN 56308 |
| Website: | www.biosearchtech.com |

## Customer Support Portal

Customer Support Portal will be accessible through following website:

1. Within an Internet browser, navigate to https://lgcgenomics.force.com/community/s/

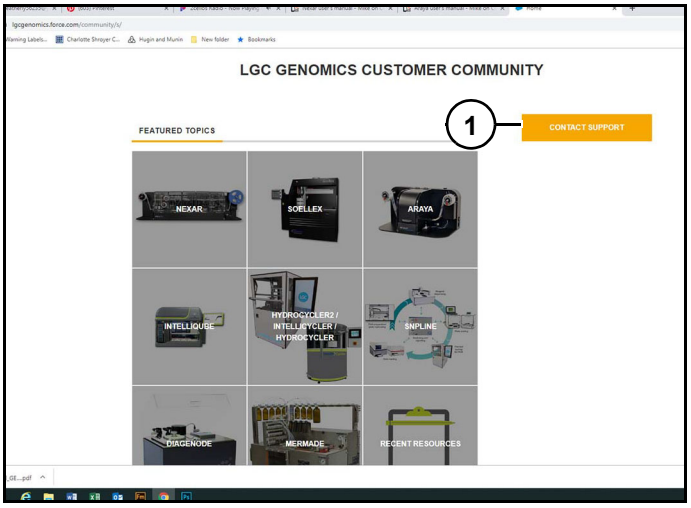



Figure 1

2. Click "Customer Support" (1)*(Figure 1)*.


Figure 2

Refer to *(Figure 2)*.

3. Fill in required fields (1) and attach any related files (2).
4. Check "I'm not a robot" (3).
5. Click "Submit" (4).

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