Original instructions

MerMade 192X user's manual

GEN/0654/MW/0225

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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.

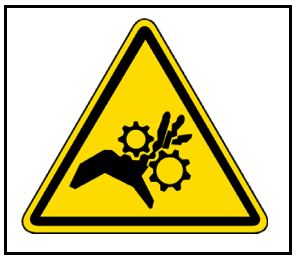

Figure 3

Warning - Pinch Point *(Figure 3)*.

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

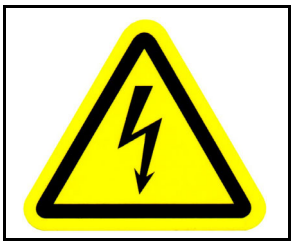

Figure 4

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.

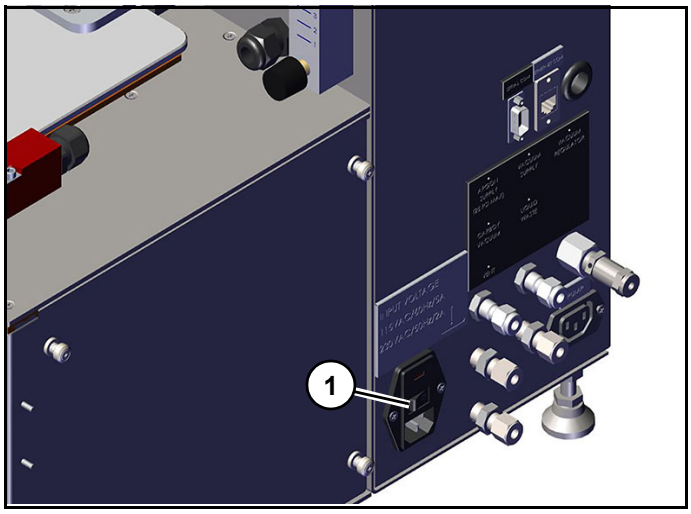

Figure 7

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 192X 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

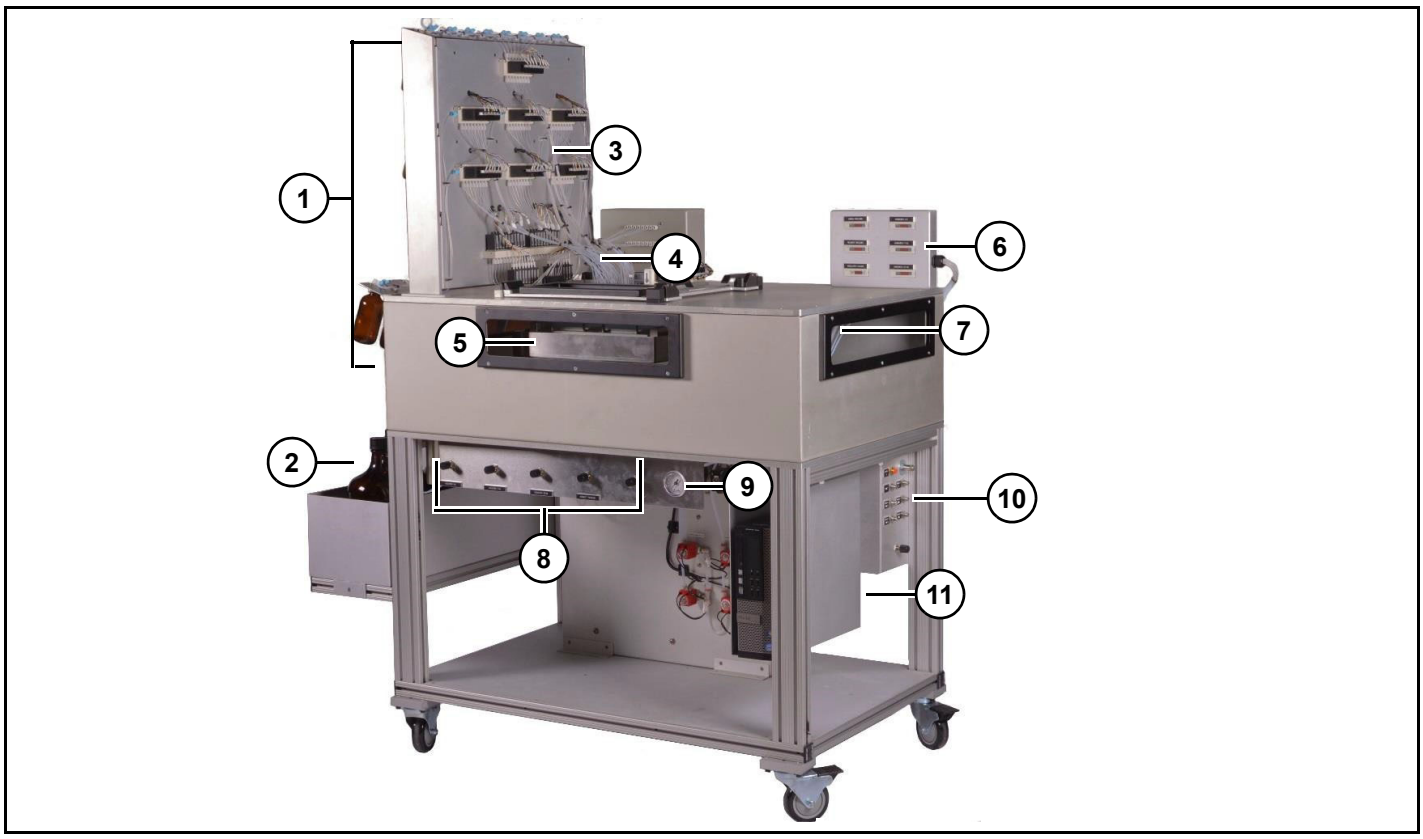

Figure 1

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Monomer Bottles	7	Drain Lines
2	Reagent Bottles	8	Pressure Regulators
3	Dispense Valves	9	Auxiliary Pressure Gauge
4	Injections Lines	10	Connection Panel
5	Plate Carraige	11	Power Switch
6	Gauge Display		

Component identification bottles, pressure regulators and side panel

Note: 192X has multiple configurations, bottle layout may change.

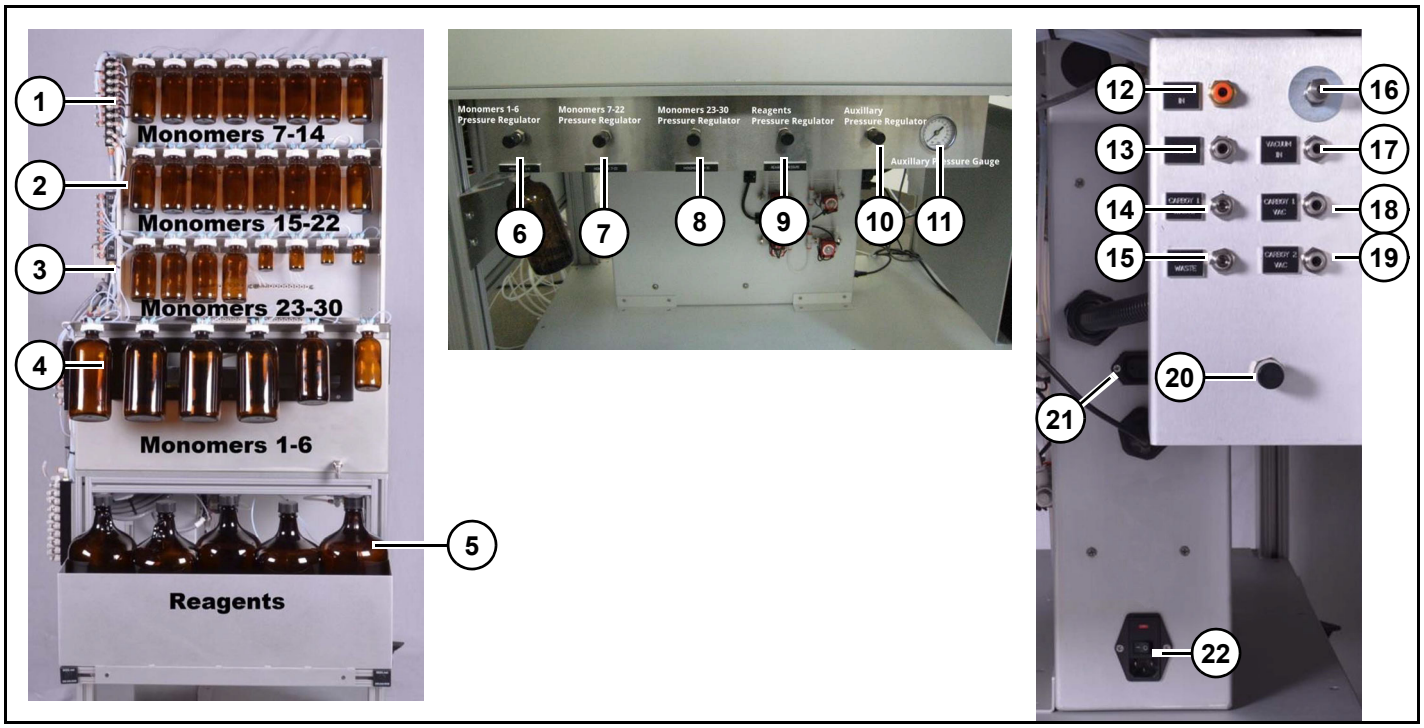

Figure 2

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Monomer Bottles 7-14	12	Argon In
2	Monomer Bottles 15-22	13	Vent
3	Monomer Bottles 23-30	14	Carboy 1 Waste
4	Monomer Bottles 1-6	15	Carboy 2 Waste
5	Reagent Bottles	16	Vacuum Switch
6	Monomer 1-6 Pressure Regulator	17	Vacuum In
7	Monomer 7-22 Pressure Regulator	18	Carboy 1 Vacuum
8	Monomer 23-30 Pressure Regulator	19	Carboy 2 Vacuum
9	Reagent Pressure Regulator	20	Fuse
10	Auxiliary Pressure Regulator	21	Pump Connection
11	Auxiliary Pressure Gauge	22	Power Switch

Component identification back and side panel

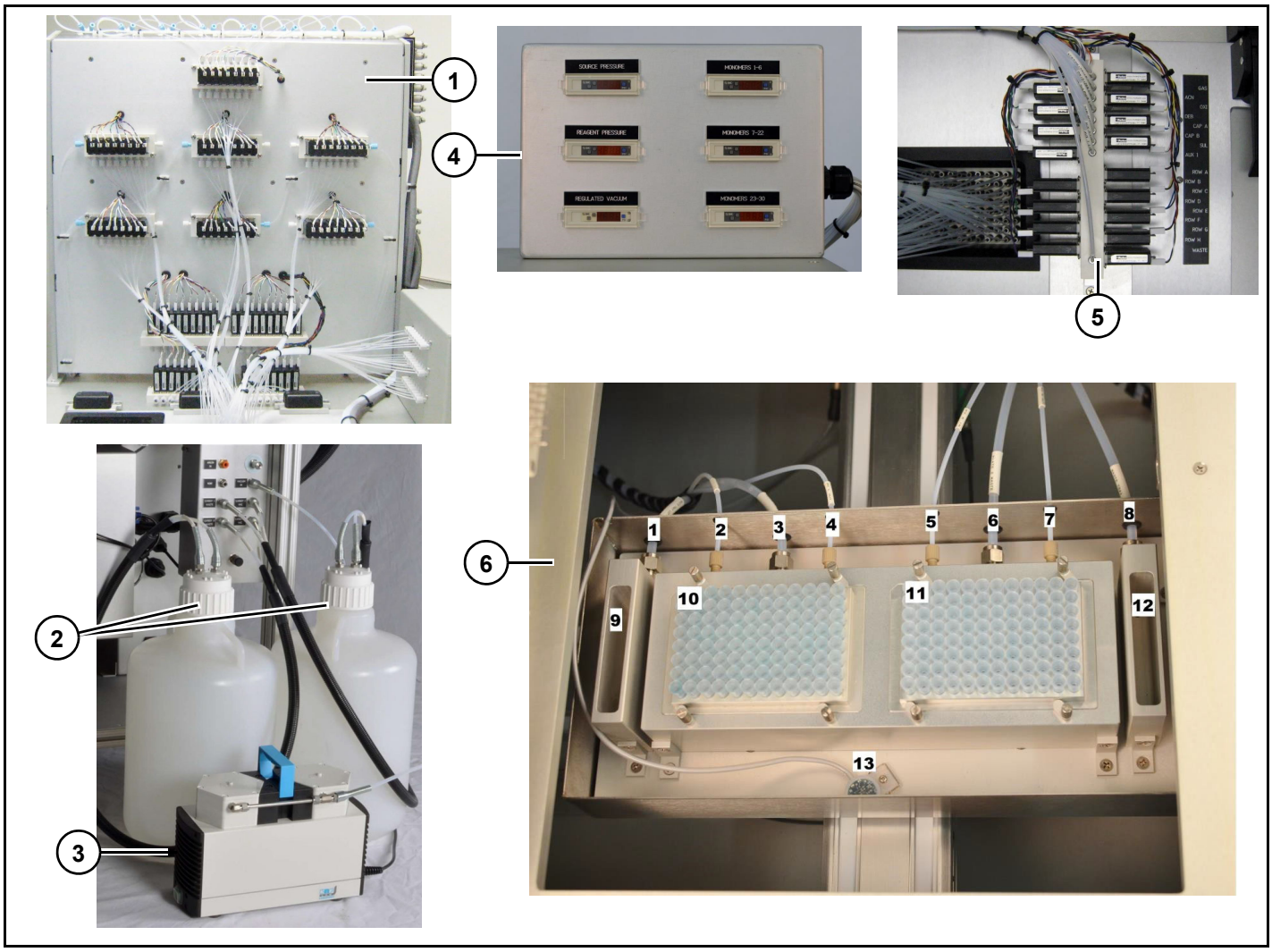

Figure 3

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Valve Panel	4	Gauge Display
2	Waste Carboys	5	Multi Manifold
3	Vacuum Pump		

REF#	DESCRIPTION	REF#	DESCRIPTION
6	Synthesis Chamber 1. Left Waste Tray Out 2. Left Plate Pressure In 3. Left Plate Drain 4. Left Plate Pressure Out 5. Right Plate Pressure In 6. Right Plate Drain 7. Right Plate Pressure Out 8. Right Waste Tray Out 9. Left Waste Tray 10. Left Plate 11. Right Plate 12. Right Waste Tray 13. Liquid Sensor		

Component identification Pneumatic panel

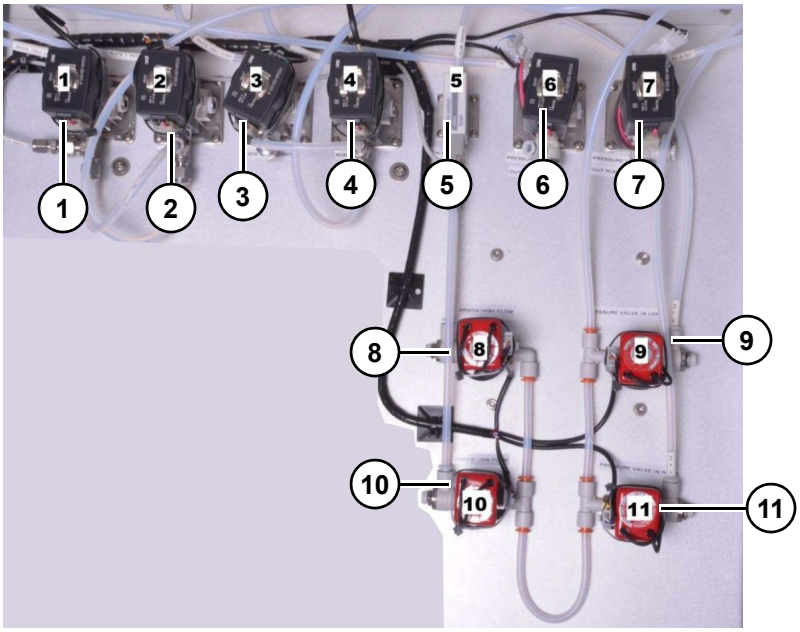

Figure 4

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Multi-Manifold Waste Valve	7	Right Plate Pressure Out
2	Left Plate Waste Valve	8	Argon High Flow
3	Waste Tray Valve	9	Left Plate Pressure In
4	Right Plate Waste Valve	10	Argon Low Flow
5	Argon Purge Flow Sensor	11	Right Plate Pressure In
6	Left Plate Pressure Out		

MerMade 192X 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

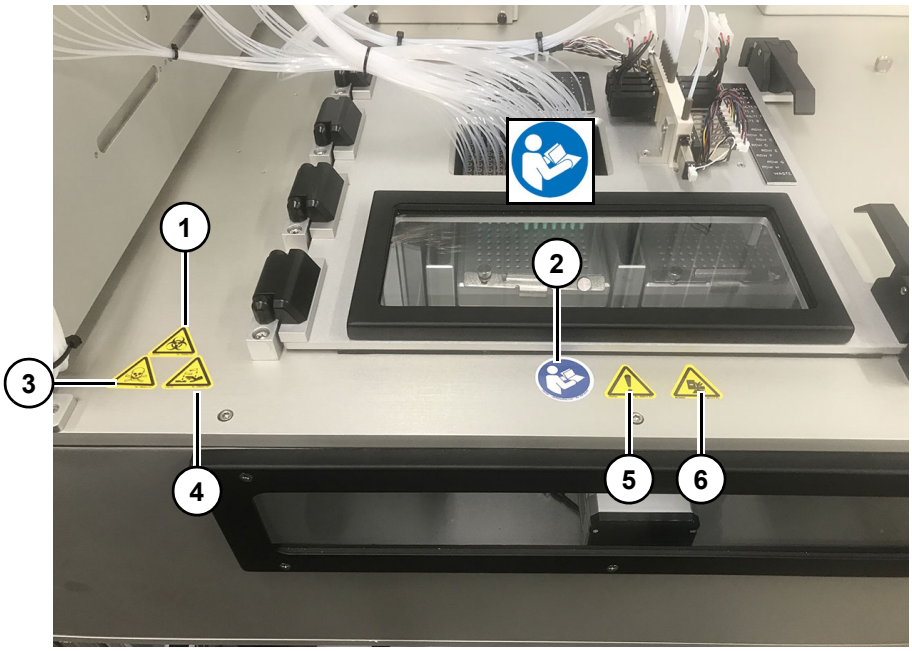

Figure 1

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Biological Hazard -Top of instrument deck (Qty-1)	4	Corrosive Substance -Top of instrument deck (Qty-1)
2	Read Owner's Manual -Top of instrument deck (Qty-1)	5	Warning Exclamation Point -Top of instrument deck (Qty-1)
3	Toxic Material -Top of instrument deck (Qty-1)	6	Warning Pinch Point -Top of instrument deck (Qty-1)

Decal identification back panel

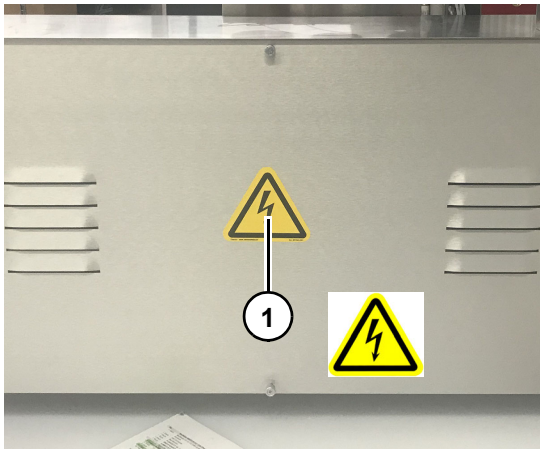

Figure 2

REF#	DESCRIPTION	REF#	DESCRIPTION
1	High Voltage Decal -Back Panel (Qty-1)		

Decal identification front panel

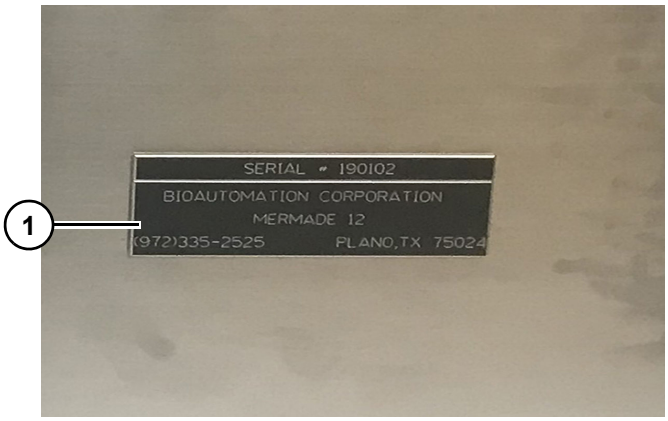

Figure 3

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Instrument Identification (Qty-1)		

MerMade 192X 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 192X is a dual axis (x and y) Oligonucleotide synthesizer designed to synthesise up to 192 columns in a single run. Synthesis takes place in two 96 well plates 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 50nmole 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), hybridisation, 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. Log files are generated for each plate and report all synthesis parameters and events.

During setup process, columns are placed in appropriate plates located in Synthesis Chamber. When synthesis is started chamber is flooded with argon/nitrogen to create a moisture free environment which is required for synthesis chemistry to work most efficiently. When synthesis begins, XY Table will move to align each row of columns under appropriate injection pins and dispense reagent by actuating 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 plates which are then mounted on to a dual 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 drained independently of other plates. During course of synthesis argon/nitrogen 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.

Pneumatic panel located underneath synthesis chamber, contains vacuum waste valves as well as pressure valves to help regulate exact conditions for

successful synthesis. Argon/nitrogen flow valves, allow operator to control flow of inert gas into synthesis chamber during oligo synthesis. Valves allow operator to control flow of argon/nitrogen underneath synthesis plates during synthesis if pressurise option is selected in script file for a certain step. Vacuum valves on pneumatic panel, evacuate liquids from waste trays, left and right plates, and multi-manifold.

Connection panel located on lower right side of the synthesizer, is where gas, vacuum, vent, and waste connections are attached. Vacuum breaker is located in top right hand side of panel. To change the vacuum pressure you will need to remove the vacuum breaker casing, labeled "C". Screwing in or unscrewing the inside stainless steel nut, labeled "A" figure 1-3e, will increase or decrease the overall vacuum pressure of the synthesizer. The outer nut, labeled "B" in figure 1-3e, is used to lock the inner nut in place to keep the vacuum level constant. The regulated vacuum gauge is located on bottom left of the gauge display (figure 1-3h), which sits on top of the synthesizer.

Software and machine operation

A PC running Poseidon synthesis software provides user interface for controlling synthesizer. Program controls all aspects of machine 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 synthesis:

Plate Log. Records information about all events pertaining to synthesis for plate. Includes data about oligo sequence and name, synthesis protocol and all injection and reaction time information generated during a synthesis.

Plate Log Summary. Log is a short run summary that gives 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 192X 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 synthesizer needs to be located so there is adequate space to access front and sides and 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 injection head door 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:

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

Space requirements:

Width

- 137 cm (54")

Height

- 173 cm (68")
  Can change depending on configuration.

Depth

- 76 cm (30")

Weight

- 272 kg (600 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 lower right side of instrument. This is necessary because Synthesis Chamber is sealed and continuous flow of argon/nitrogen 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 synthesis is started or completed, it is necessary to open door to Reaction Chamber in order to load or remove synthesis plates. 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.

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

Pump/external connection.

- 115V/3.2A/60Hz
- 230V/1.6A/50Hz

Operating conditions

Temperature

- 5 °C to 25 °C(41 °F to 77 °F)

Relative Humidity

- 40-60% humidity at 25 to 35 °C

Pollution Degree

- 2

Altitude Range

- FAT at 1400' (427m)
- Components rated up to 8200 ft (2500 m)

Installation Category

- 2

Mains Supply Fluctuations

- 230VAC/115VAC

Shipping/storage conditions

Temperature

- -30 °C to 40 °C(-22 °F to 104 °F)

Electrical requirements

Table specifies electrical operating requirements for instruments in various locations:

Location	Voltage	Amps	Frequency
USA/ Canada/ Japan	115 VAC	8A	60 Hz
EC	230 VAC	5A	50 Hz

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

Fuses:

- ABC-5A I.R. 10K
- ABC-2A I.R. 10K
- MDA-3A I.R. 10K
- MDA-1A I.R. 10K

- MDA-5A I.R. 10K

Pressure requirments

Pressure Requirements	
Instrument Inlet Pressure	<25 psi (1.7 bar)
Regulating range	0-30 psi (0-2 bar)
Temperature Range	40 °F - 150 °F (4 °C – 66 °C)
Tubing	PTFE - 1/4“ ID x 1/8” OD
Amidites/ monomer	5-8 psi

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

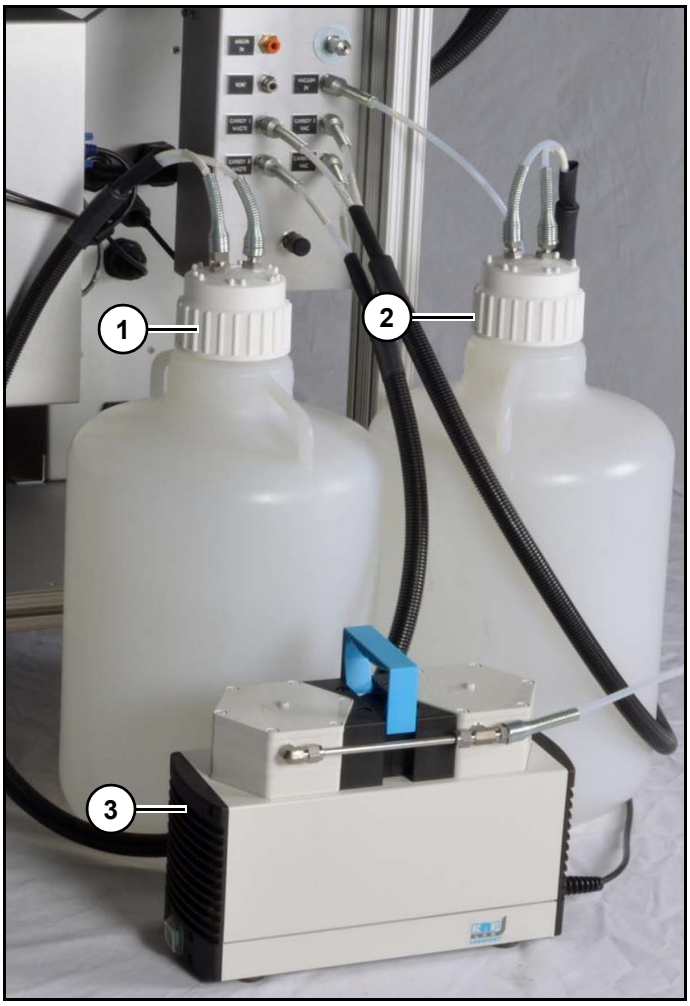

Figure 1

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.

Note: Waste system may look different depending on waste option.

Startup equipment

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

Gas regulators.

Instrument requires an argon/nitrogen source to pressurise bottles and to purge Synthesis Chamber before starting synthesizer. Argon/nitrogen inlet port, a ¼” Male NPT Swage Lok compression fitting, is

located on right hand side of instrument. A regulator with following rating will need to be supplied:

- Inlet pressure:150 psi (10 bar)
- Regulating range: 0-30 psi
- Maximum Outlet Pressure: 15 - 150 psi (1 - 10 bar)
- Temperature Range: 40 °F - 150 °F (4 °C - 66 °C)

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.(A/N 5.0 / Ultra high purity grade minimum).

Collection plates and plate sealers.

Collection vials are needed for post processing when synthesis is complete. A vial with screw cap is recommend since it will be under pressure and a snap cap may be forced open.

Sample dryer.

Once deprotection stage is complete, oligo 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 synthesis can continue when power is restored. MerMade will pause all plates 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

Note: Size depends on required run time.

Synthesis chemicals

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

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% Methylimidazole/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 (~0.05M)

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

Reagent preparation

Phosphoramidites are susceptible to degradation when exposed to moisture and amidite dilution 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, acetonitrile with a water content of no more than 10 ppm for amidites should be used.

Acetonitrile in 4L 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.

Using standard columns with first base derivatized also offer 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 50nmole to 5µmole sizes and can be ordered directly from Biosearch Technologies.

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.

Always use vacuum-rated polyethylene waste containers, do not use containers larger than 20L as this can cause draining problems if vacuum source is too weak.

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.

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

Overview of Synthetic Oligonucleotide Synthesis on a solid-phase support. Most common types of support used in synthesising 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)*.

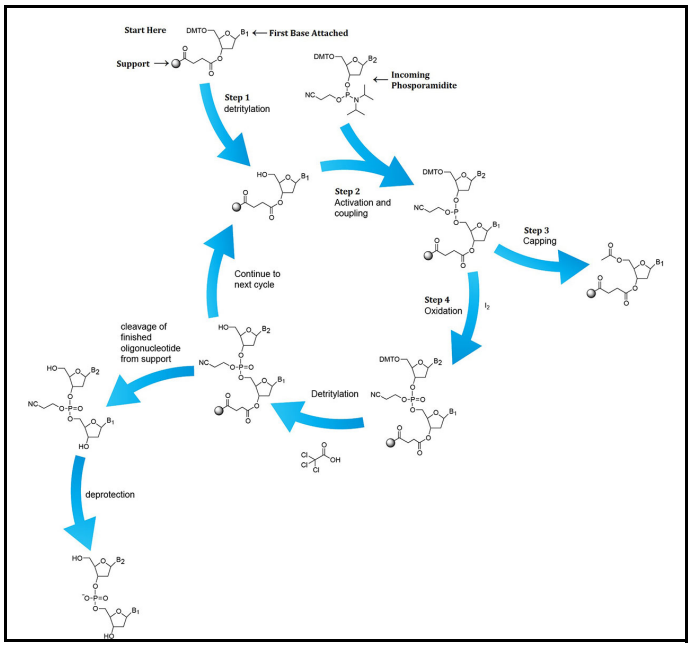

Figure 1

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)*.

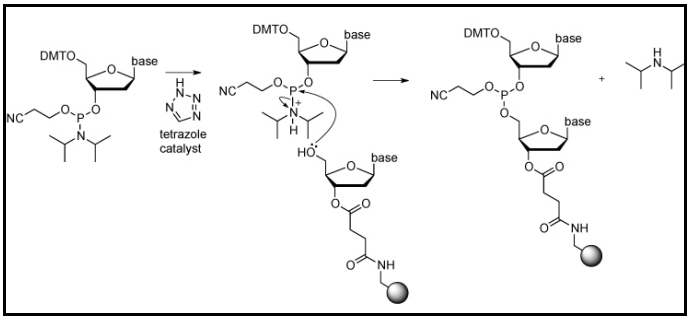

Figure 2

Activation and coupling

Once DMT group is removed, 5'-hydroxy on existing oligonucleotide is now open to react 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 phosphoramidites. Refer to *(Figure 3)*.

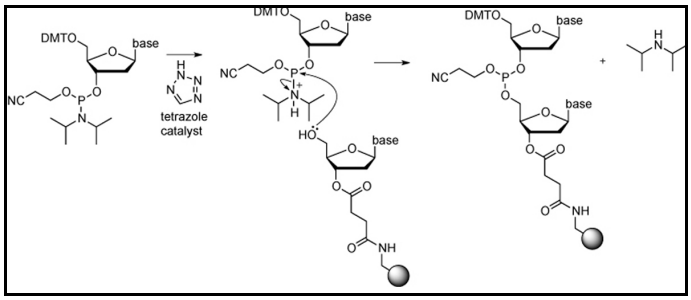

Figure 3

Capping

After coupling has been 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)*.

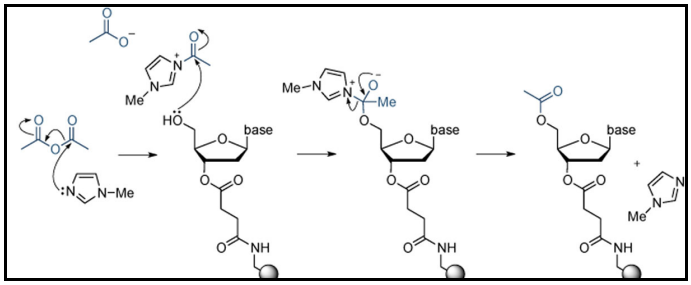

Figure 4

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)*.

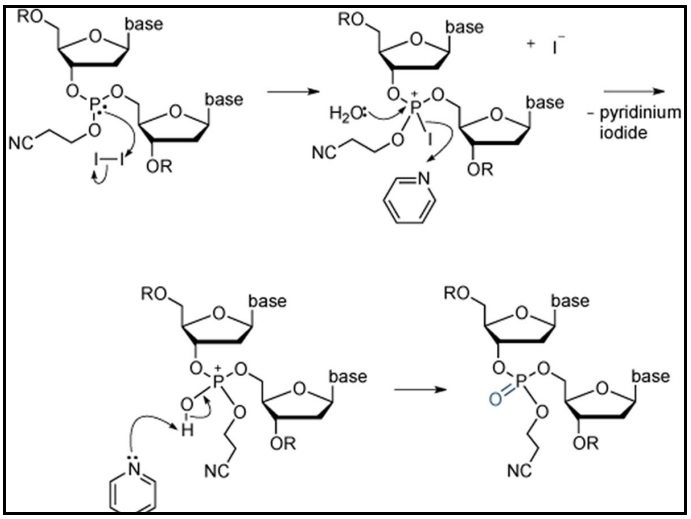

Figure 5

Oligonucleotide Synthesis reagents and solid supports

Biosearch Technologies recommends use of its phosphoramidites, modified oligonucleotide reagents, such as spacers, LNA, 2'O Methyl and 2'Floro modifiers, fluorophores and quenchers, modifications relating to cell delivery and update for therapeutic manufacture and nucleosides.

As manufactures of these products, we ensure that our reagents work with optimal efficiency on all our instruments and should you have any quires, you may contact our support team by telephone on

+44 (0) 1698 849911 or

email us at techsupport@lgcgroup.com

A range of Cap A and Cap B ancillary reagents are available from Biosearch Technologies depending on specific phosphoramidite.
Refer to Oligonucleotide Synthesis Reagents Catalogue for full details.

MerMade 192x 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 synthesiser. 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 all injection and reaction times. *(Figure 1)* Logs are located at C:\Users\Public\Documents\BioAutomation\Poseidon\Logs
- Plate Log Summary: Log saves important information about synthesiser 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)*.

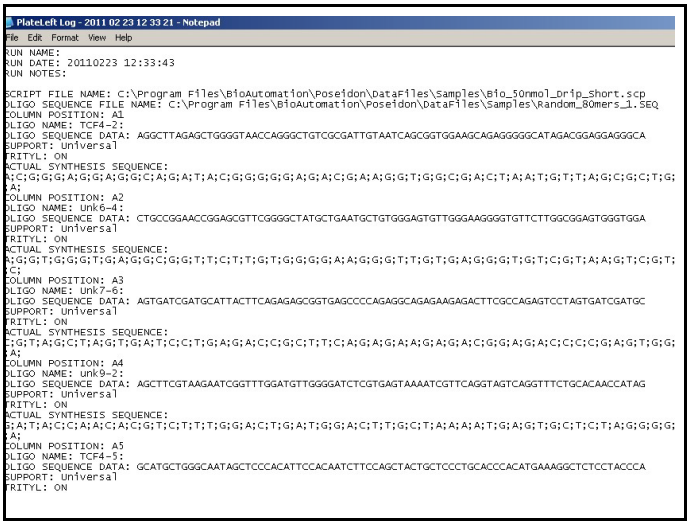

Figure 1

Synthesis process

Synthesis is typically carried out from 3'->5' end of oligonucleotide. Reactions take place in columns using a controlled pore glass (CPG) or polystyrene substrate (PS) contained 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/nitrogen to create a moisture free environment. When argon/nitrogen purge initialisation step is completed, software moves XY table 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, reagent quality, 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 synthesis plate.

Once plate is drained, underside of column(s) is equalised to same 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 Oxidization 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 and heated (if required) to fully deprotect the oligo. 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.

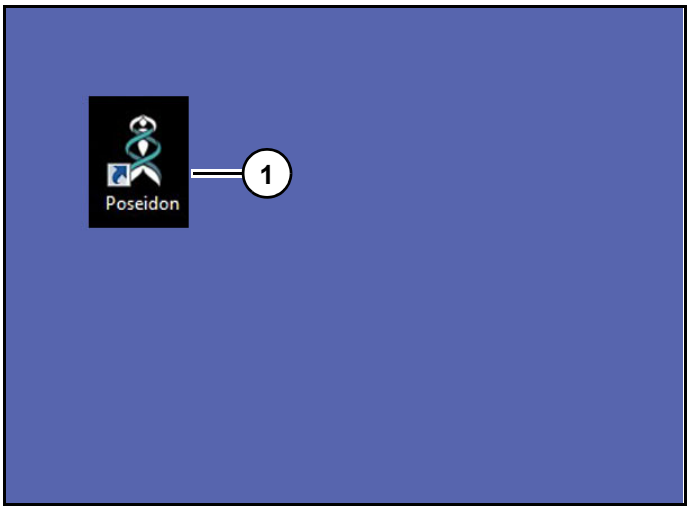

Figure 2

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

Initialization screen

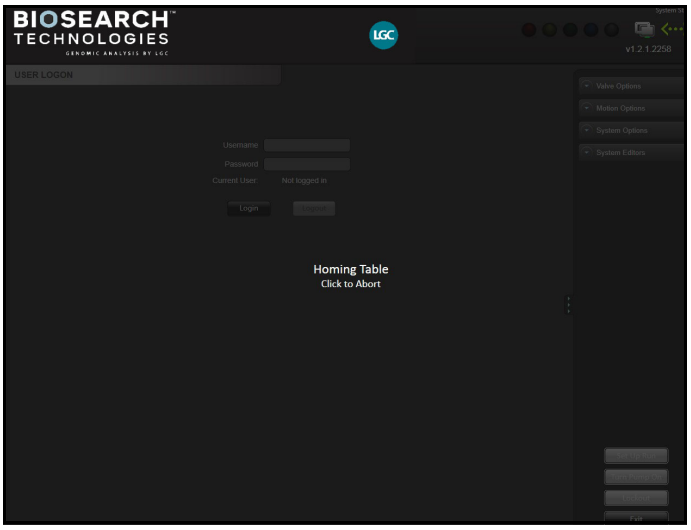

Figure 3

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

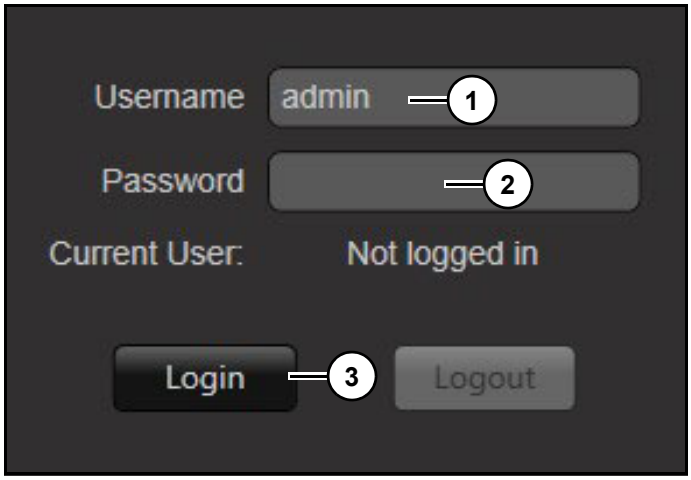

Figure 4

Refer to *(Figure 4)*.

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

Main screen

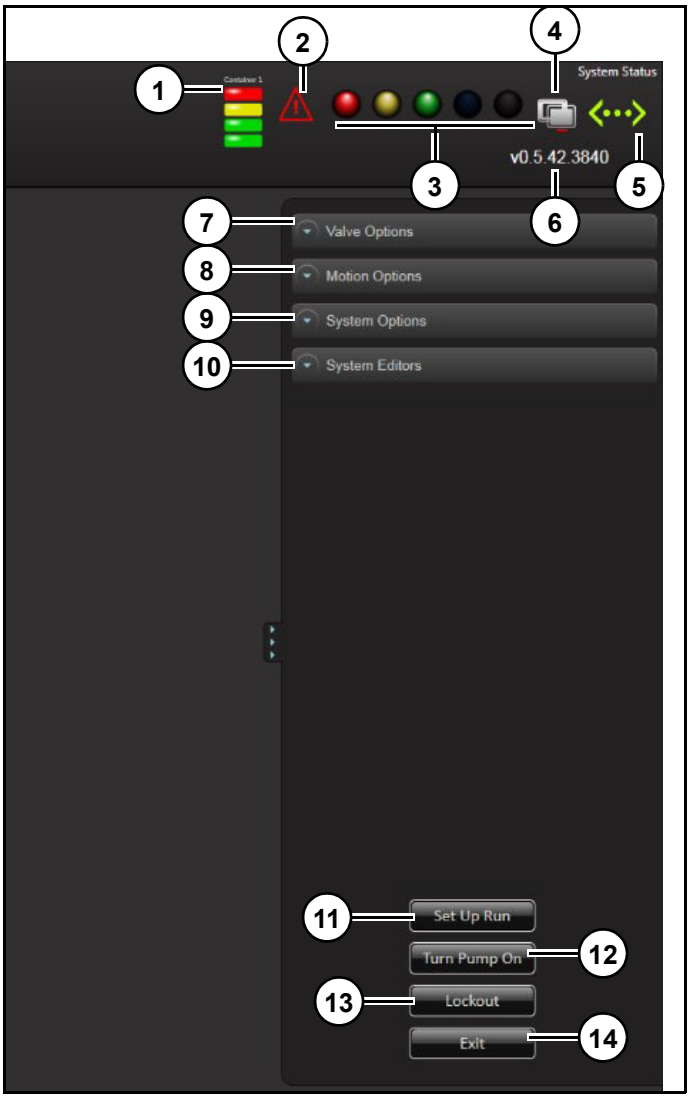

Figure 5

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 synthesiser is ready to perform a synthesis.

Turn Pump On (12): Instrument is equipped with ability to control a vacuum 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/nitrogen purge valves.

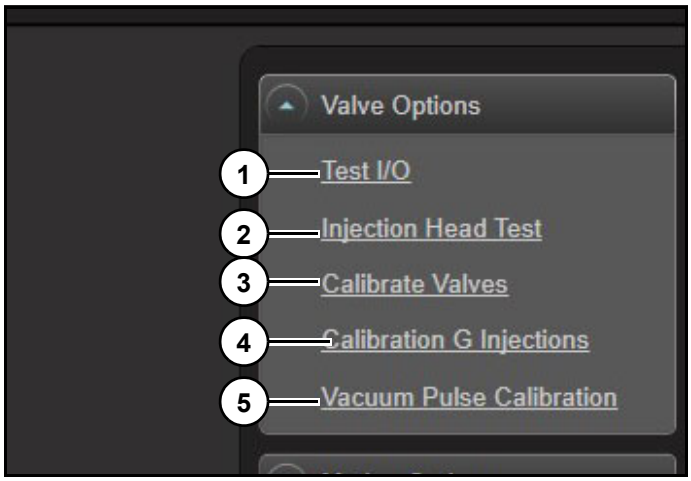

Figure 6

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 valve group 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/nitrogen valves.

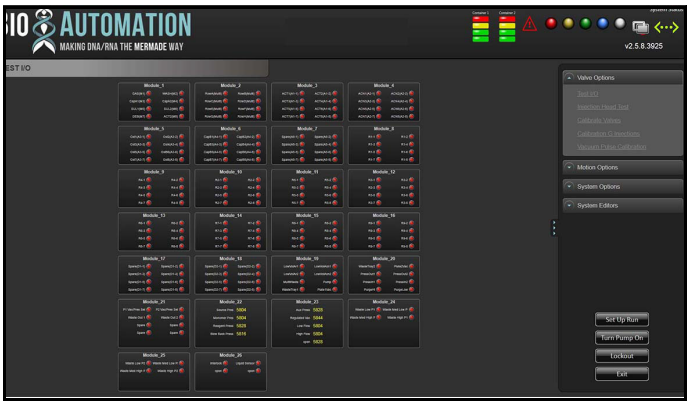

Figure 7

Refer to *(Figure 7)*.

To test a valve:

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

Note: Valve will stay open until button is clicked again. In case of emergency user can turn main off power on lower 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.

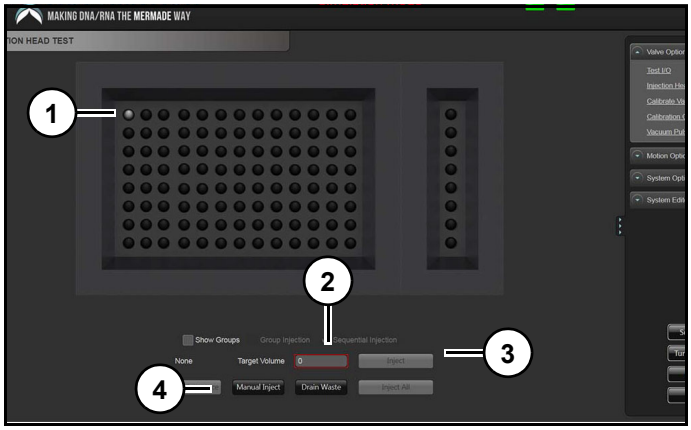

Figure 8

Refer to *(Figure 8)*.

To test 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 selected valves until button is released. This is often used for priming lines.

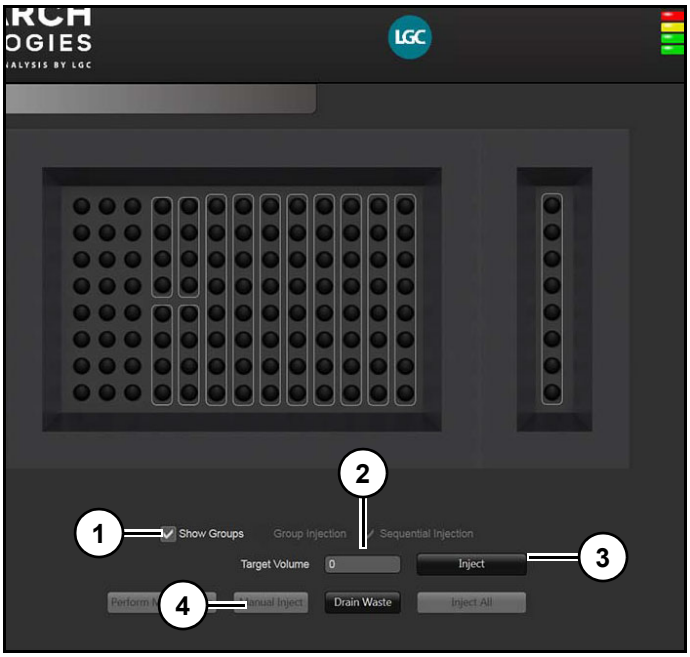

Figure 9

Refer to *(Figure 9)*.

To test a row of reagents:

1. Check "Show Groups" (1). Grouped valves will be marked by 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.

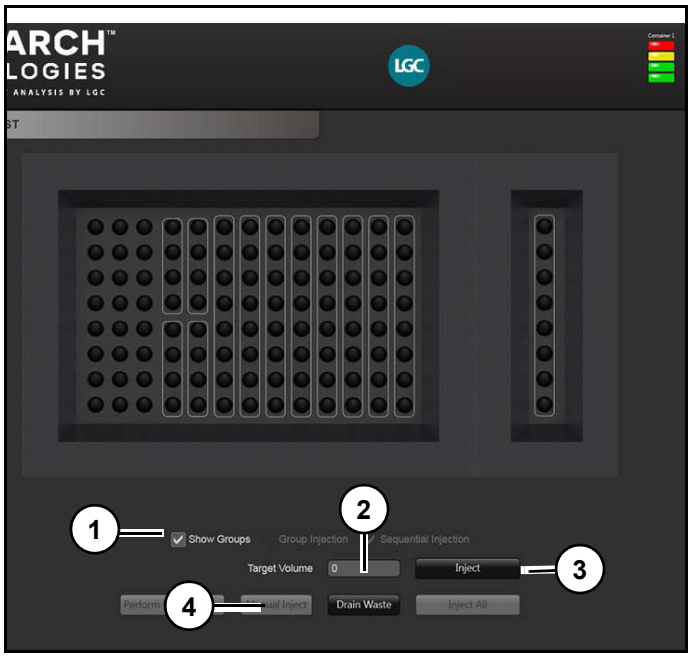

Figure 10

Refer to *(Figure 10)*.

To test valves in group individually:

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).

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

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 when injected as part of a group. Amidite/reagent pressure must be kept stable to ensure calibration accuracy.

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

Liquid dispensing valves must 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. Liquid valve delivery will be more accurate if more points are calibrated between minimum and maximum point. It is highly recommended to have calibration points similar to injection volumes that will be used during synthesis.

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 curve 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.

Lowest calibrated point must be equal or less than lowest volume use. Highest calibration point must be equal or greater than highest volume being delivered in all script files being used. This includes priming.

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 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 to determine if time that valve remains open is correct. If delivered volume is not enough, valve open time can be increased (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 must be calibrated manually by using a collection tube and a pipette, or with a scale, if calibration by weight selection is preferred.

Switching calibration methods

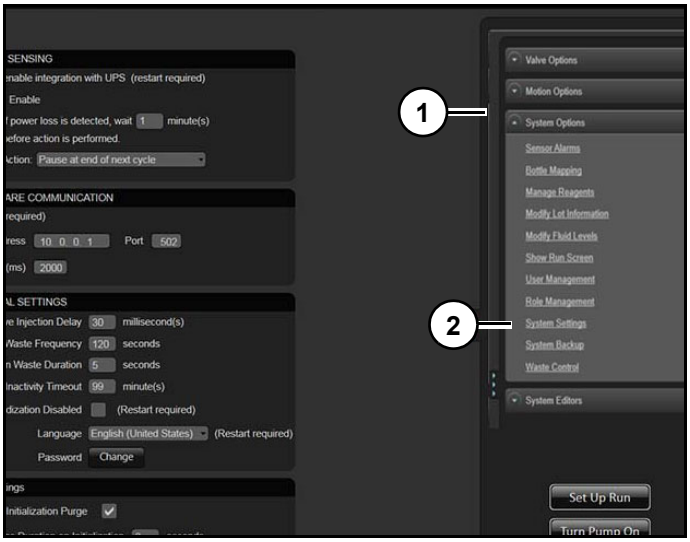

Figure 11

Refer to *(Figure 11)*.

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

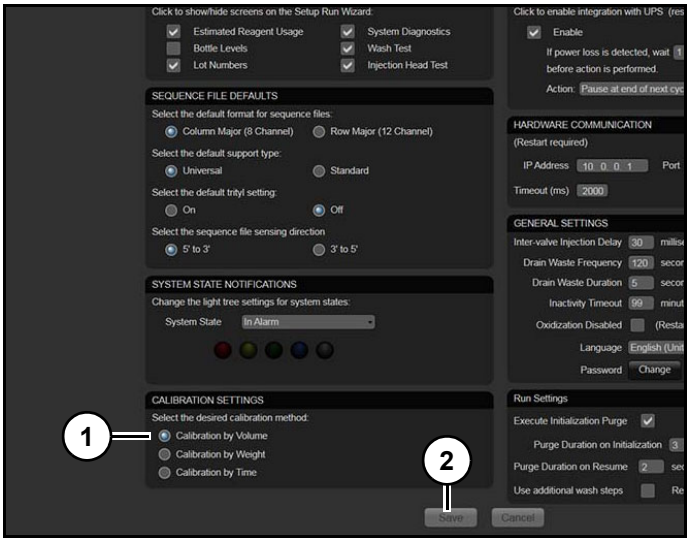

Figure 12

Refer to *(Figure 12)*.

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 must add more points, delete points, etc. as necessary. Calibration is an important aspect of instrument setup. It is crucial to be consistent.

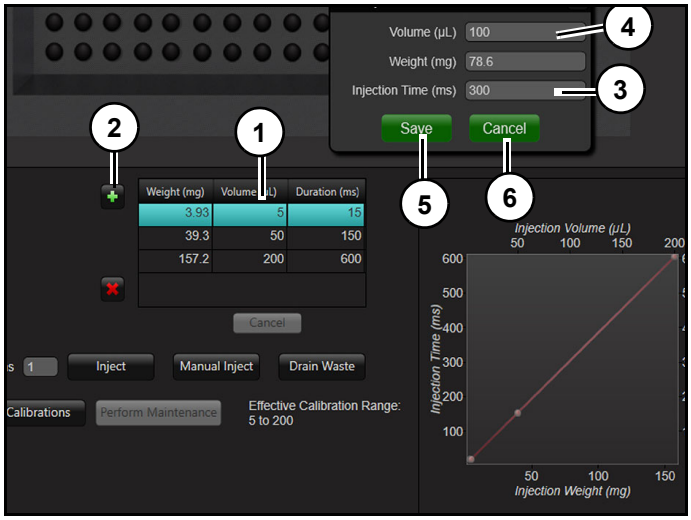

Figure 13

In *(Figure 13)*, Acetonitile Valve has volume points for 5 µL, 50 µL, and 200 µL (1).

To add calibration point:

1. Click "+" (2) to add a calibration point.
2. Enter estimated time (ms) in "Injection Time" (3).
3. Enter estimated delivery volume (µL) in "Injection Volume (µL)" (4).

Note: 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 14)*.

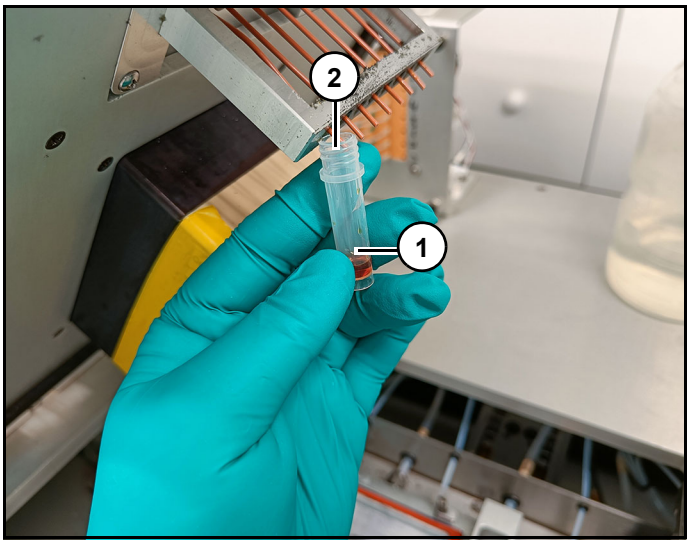

Figure 14

5. Place 2ml tube (1) under correct injection pin (2) with lid open. *(Figure 14)*

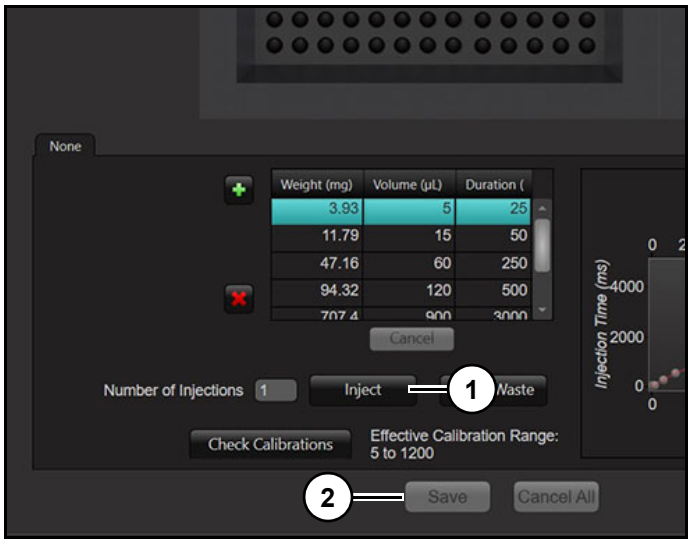

Figure 15

6. Click "Inject" (1) *(Figure 15)*. Instrument will open valve for 25 ms, injecting liquid into collection vile.

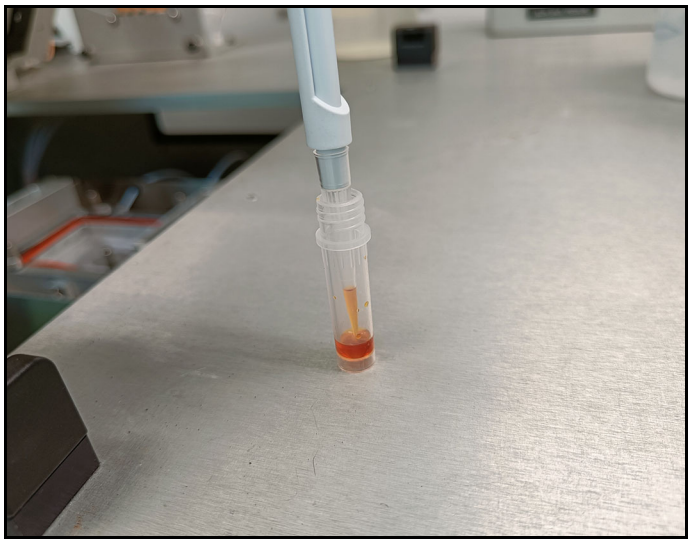

Figure 16

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 16)*

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)".

9. Click "Save" (2) *(Figure 15)*. To calibrate another point, highlight appropriate line with a mouse click.

Updating calibration point

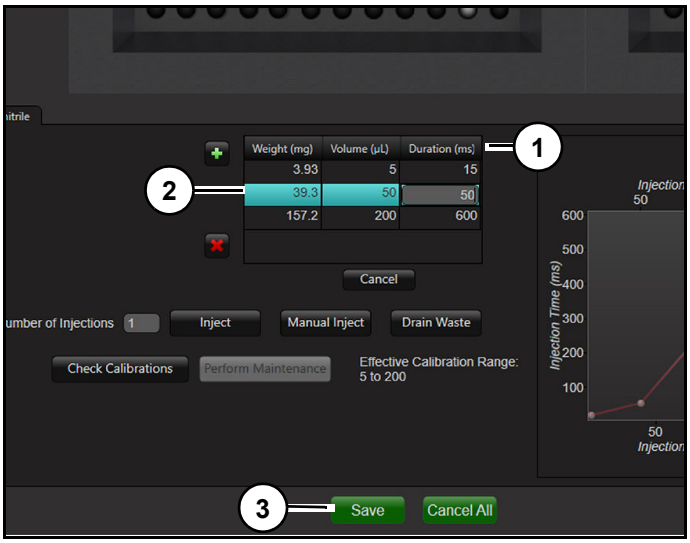

Figure 17

Refer to *(Figure 17)*.

1. Select calibration point to change. Either Weight/ Volume(µL)/Duration(ms). (1)
2. Change desire field in tab (2).
3. Click "Save" (3).

Deleting calibration point

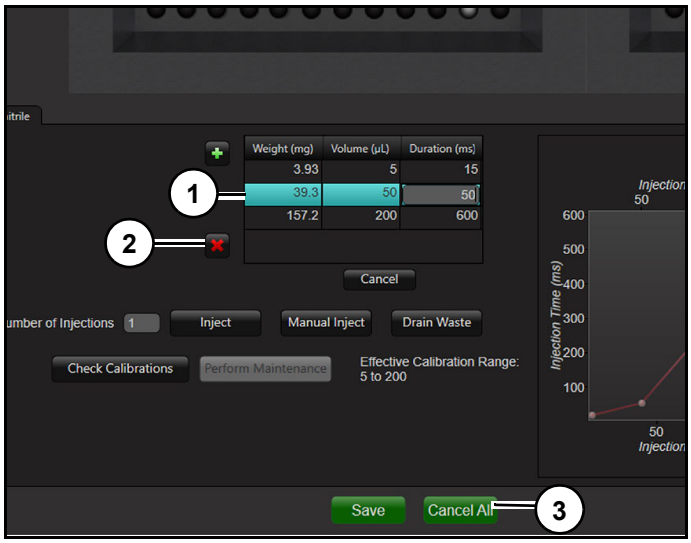

Figure 18

Refer to *(Figure 18)*.

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

Note: If "X" (1) is accidentally pressed, hit "Cancel All" (3) undo delete. All changes since your last save will be lost.

Checking calibrations

Allows user to check calibration curve for each valve.

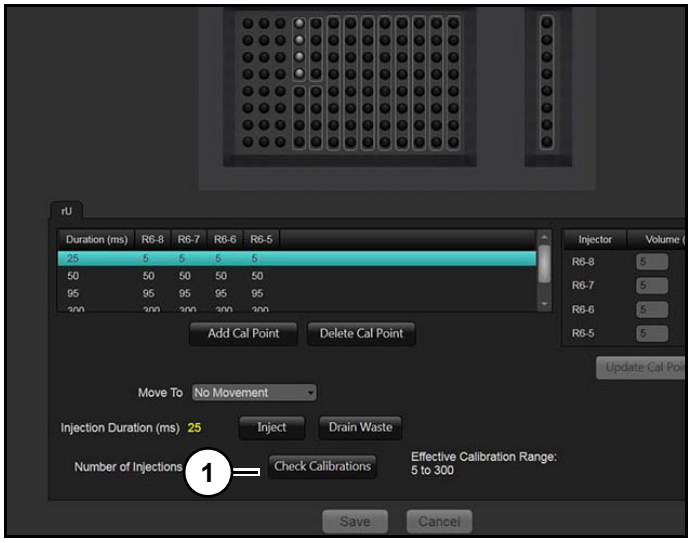

Figure 19

1. Click "Check Calibration" (1) *(Figure 19)*.

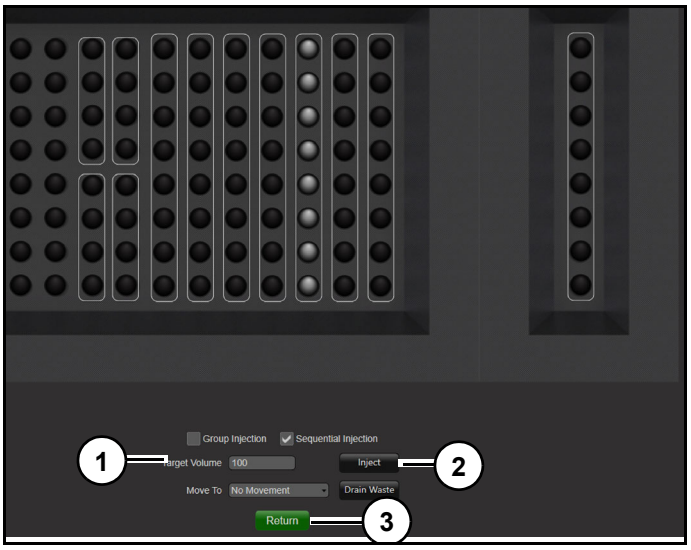

Figure 20

Refer to *(Figure 20)*.

For example, Oxidizer 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 Eppendorf tubes.
5. Check volume with a pipette. Software will calculate necessary valve open times, 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

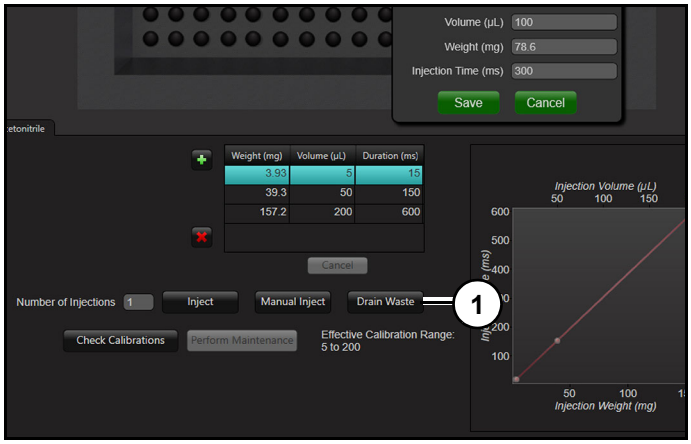

Figure 21

Click "Drain Waste" (1) *(Figure 21)* to drain waste from waste tray.

Clone calibration

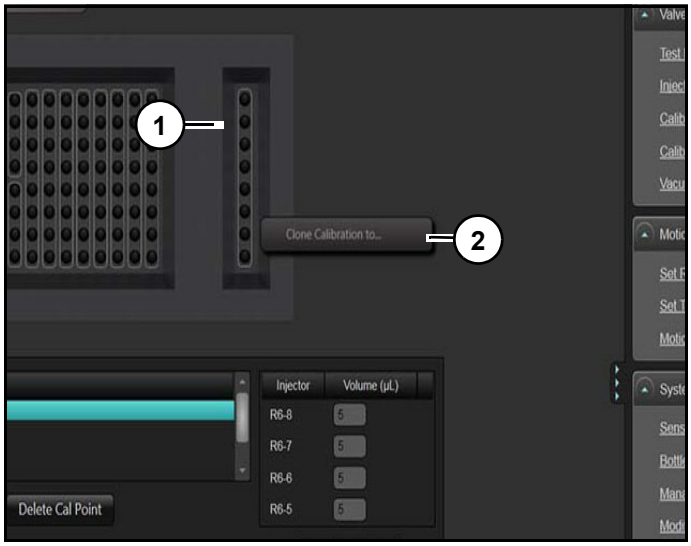

Figure 22

In multi-manifold array graphic (1), right mouse clicking on a single valve will bring up a dialog box with "Clone Calibration to" (2).

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

Calibrate G injections

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.

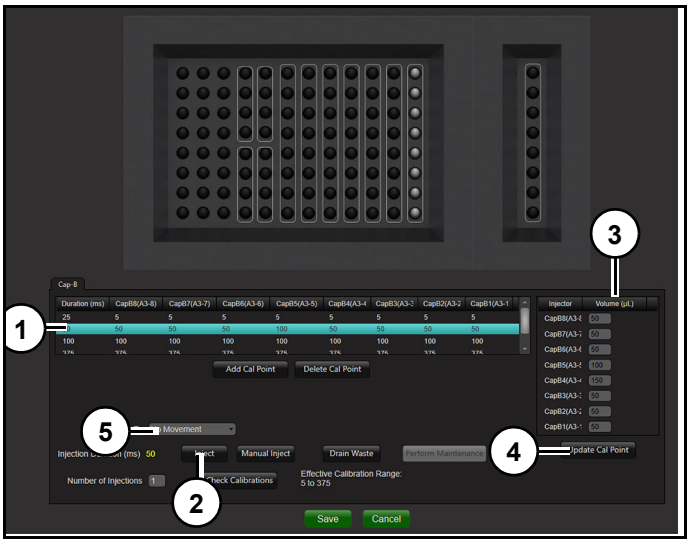

Figure 23

Refer to *(Figure 23)*.

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.

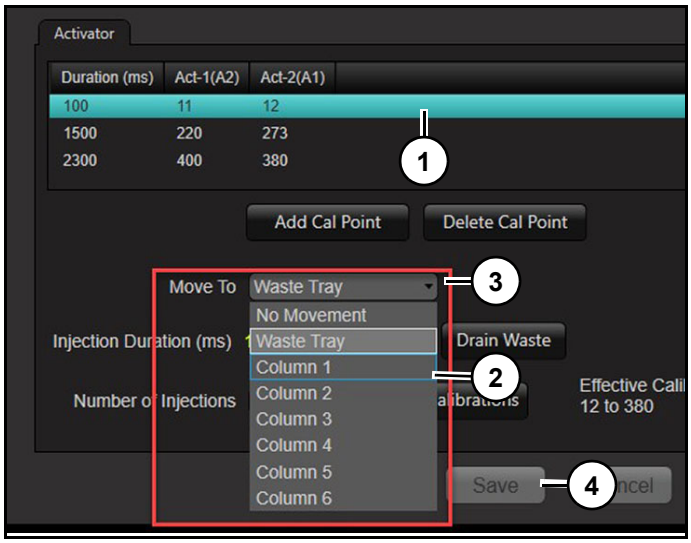

Figure 24

Refer to *(Figure 24)*.

For example:

1. User selects activator 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 pull 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. Vacuum pulses will determine reaction time for reagents and is important to performance of instrument.

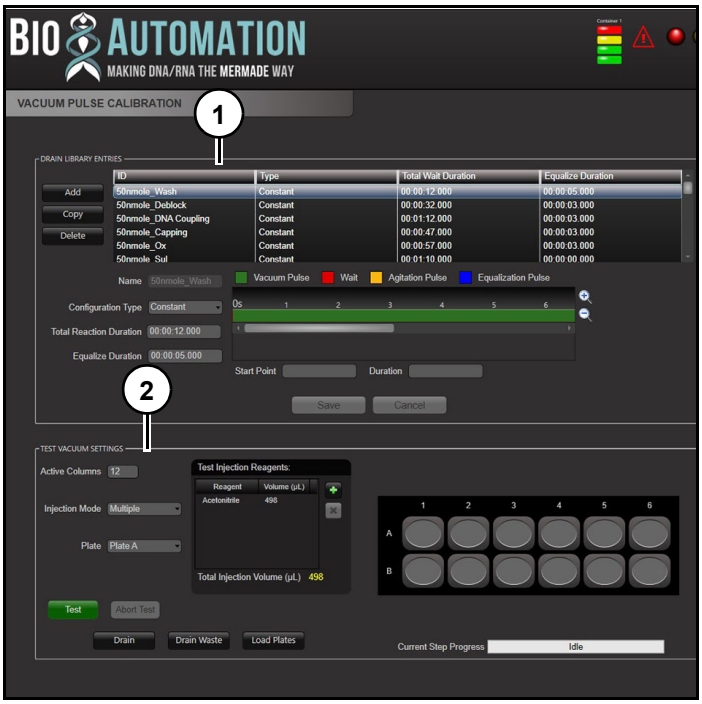

Figure 25

Refer to *(Figure 25)*.

Drain Library Entries (1): Displays saved drain types. These may or may not be calibrated. 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. All pre-configured vacuum library entries should be reviewed prior to use.

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

Types of vacuum pulses

Different pulse calibrations allow instrument to completely drain a full plate of synthesis columns regardless of number of active columns (1-96) on a plate. As columns drop out of synthesis cycle, due to difference in length, adjustments to a vacuum pulse may be necessary because empty columns may no longer be getting liquid injections. The empty column allows vacuum to easily dissipate and therefore makes it harder to drain remaining columns that did get injections.

There are two types of vacuum pulses on instrument.

Pulse types:

Constant Vacuum Pulse.

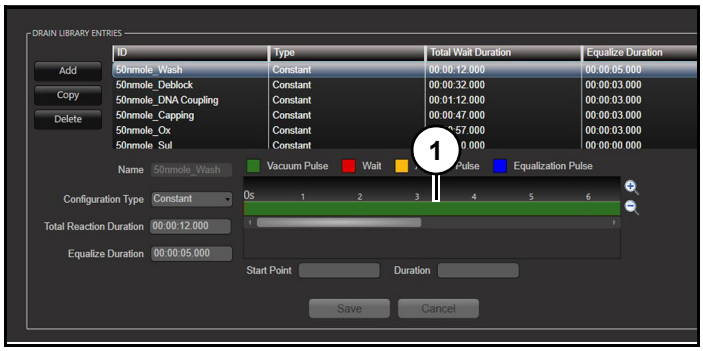

Figure 26

A constant vacuum pulse (1) *(Figure 26)* is a pulse that applies same length of vacuum to synthesis plate 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.

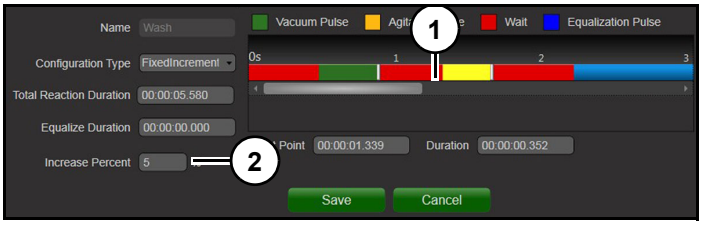

Figure 27

Refer to *(Figure 27)*.

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 250ms pulse, a one-second wait, a 900ms 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 5% would increase each vacuum setting by 5% per column. So, a 10-second drain for 12 columns would be a 10.5-second drain for 11 columns, 11 seconds for 10 columns, and so on.

Vacuum Pulse Structure screen

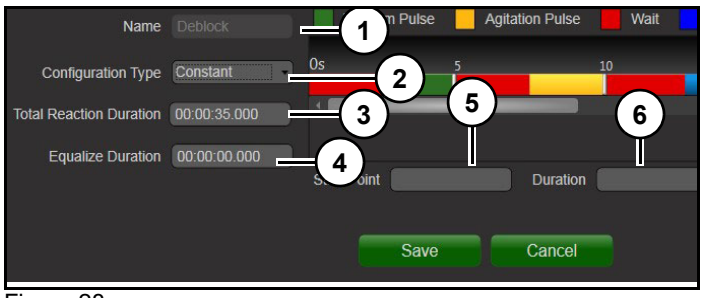

Figure 28

Refer to *(Figure 28)*.

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, equalize 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 agitation, or equalisation pulse.

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

Red (waits), yellow (agitation pulses), blue (equalisation pulses), and green bars (vacuum 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 final 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 reflected by Reaction Duration (3). It is vital to observe the calibrations to ensure there is no premature drainage, which would have a negative impact on coupling.

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 pull reagent through column to maximise solvent usage and reaction efficiency. 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 usually achieve 2-3 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/nitrogen into bottom of columns by pressurising bottom of synthesis plate. An argon/nitrogen valve (Press IN) connected under plate position opens. Allows for argon/nitrogen 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 1.3ml and when no top frit over support bed would be used.

Add Equalization Pulse Here: Adds an equalize 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 blue, green or yellow selected pulse box.

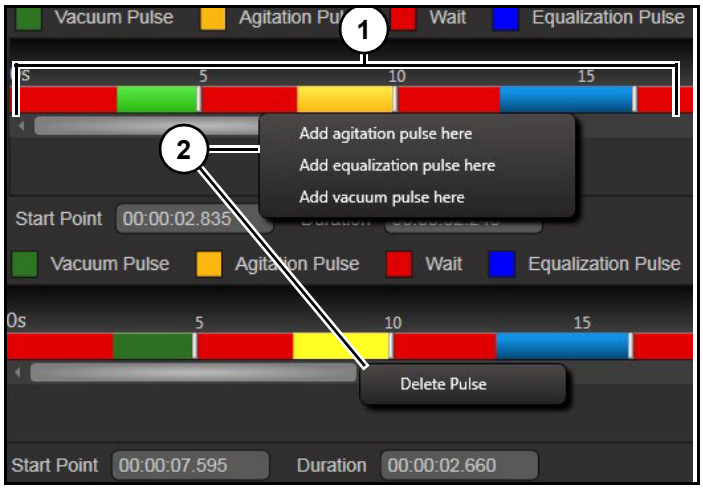

Figure 29

Refer to *(Figure 29)*.

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

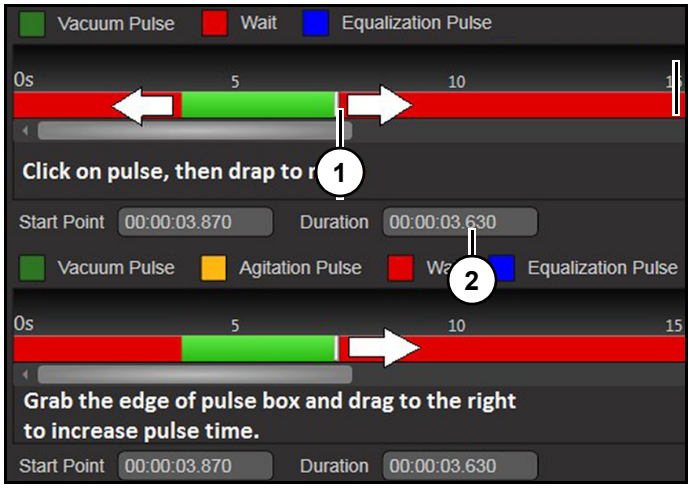

Figure 30

Refer to *(Figure 30)*.

1. Grab right edge of blue, 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 31)*.

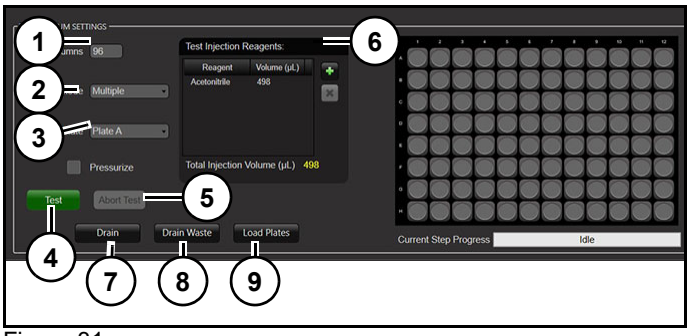

Figure 31

Refer to *(Figure 31)*.

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 fixed increment vacuum pulse options, and <96 well plates. 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 press IN valve 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 so that reagents flow through column without premature draining or under draining. Once settings are accepted users can 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 during calibration.

Note: If multiple reagents are added to table, all reagents on table will be injected from top to bottom.

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

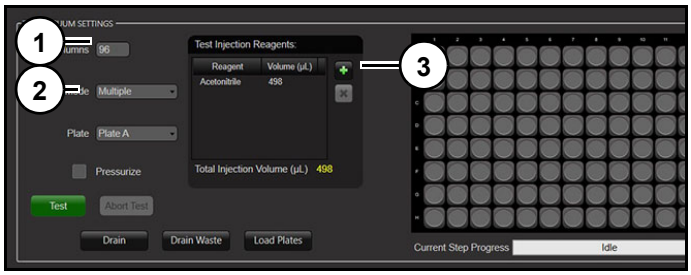

Figure 32

Refer to *(Figure 32)*.

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

Figure 33

Refer to *(Figure 33)*.

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 34)*. Options available are: Set Reference Positions, Set Table Parameters, and Motion.

Figure 34

Set Reference Positions

Instrument arrives from factory with injection head already aligned to synthesis plates. Well to well distance of columns in synthesis plates 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.

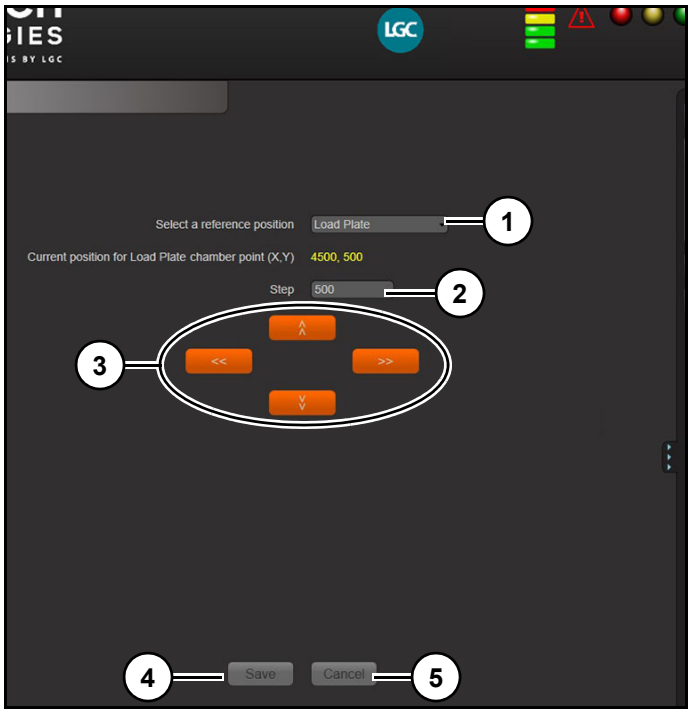

Figure 35

Changing reference positions:

User tells instrument where to start by aligning injection head. Alignment can be changed in Injection Head Align screen *(Figure 35)*.

To adjust injection head for "Load Plate:

1. Select "Load Plate" from "Select a reference position" (1).
2. Enter a value in "Step" (2), for example 500.
3. Press "←, →, ↑, ↓" (3) to move plate left, right, up, or down in relation to user. Farthest left row of monomers on injection head and row 1 on synthesis plate should be centered, so any injected reagents will go into the columns without causing spills.
4. Click "Save" (4) to save position.

Note: Click "Cancel" to undo changes.

Alignment process is then repeated for both base plate positions and both waste trays. 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 slides will move during various operations. Values are set at factory and should not be changed unless instructed so by Biosearch Technologies. Failure to adhere to instructions can cause damage to motion system. *(Figure 36)*

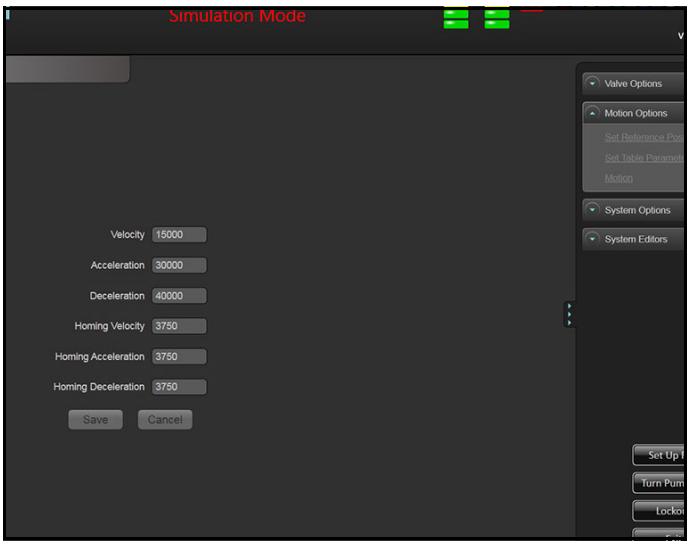

Figure 36

Motion

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

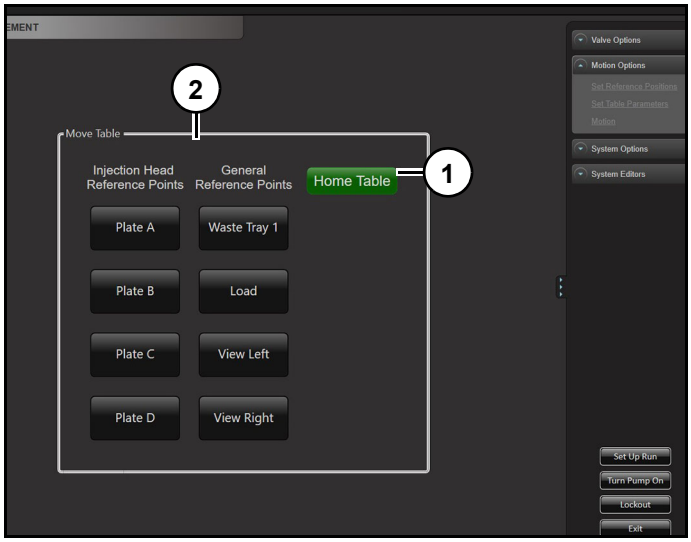

Figure 37

Refer to *(Figure 37)*.

Home Table (1): Homes XY table. Software will re-zero slides 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): Allows movement to reference positions.

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

System Options

Allows user to access controls involving system related options *(Figure 38)*:

- Sensor Alarms
- Bottle Mapping
- Manage Reagents
- Modify Lot Information
- Modify Fluid Levels
- Show Run Screen
- User Management
- Role Management
- System Settings
- System Backup
- Waste Control (Only shown if autowaste is installed)

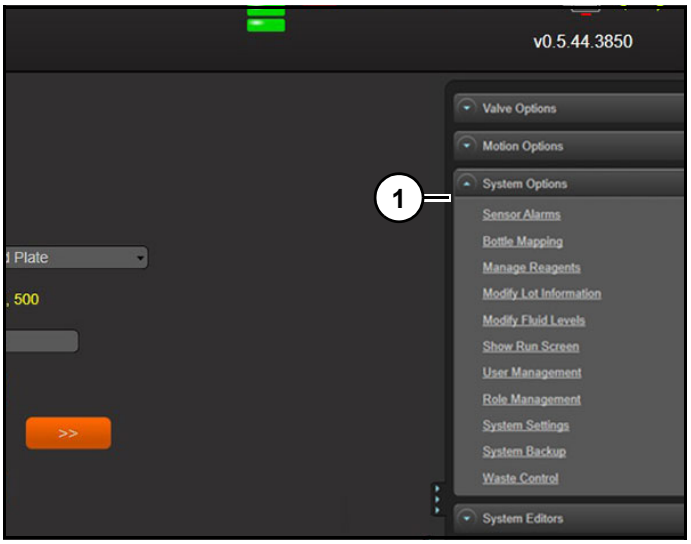

Figure 38

Sensor Alarms

Contains settings relating to sensor information coming from instrument

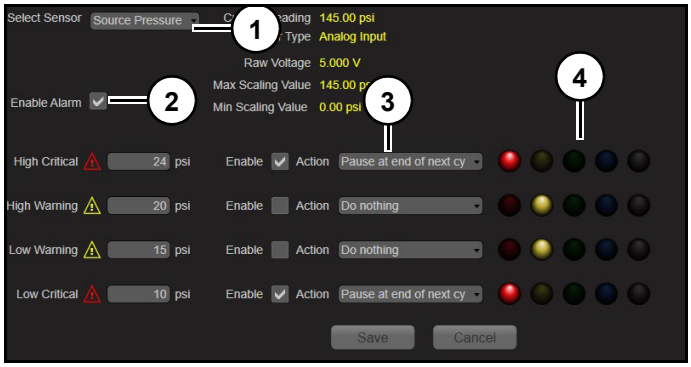

Figure 39

Refer to *(Figure 39)*.

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

Enable Alarm (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. It is 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 action is taken upon condition trigger. Light tree will still be changed, and sensor events recorded in log files. Useful for troubleshooting.
- 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. After that 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. Example: 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 instrument is paused and not in motion.

Monitors door switch, located on top right of chamber door. 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. Caution is adviced when moving slides by hand, as excessive force can damage components.

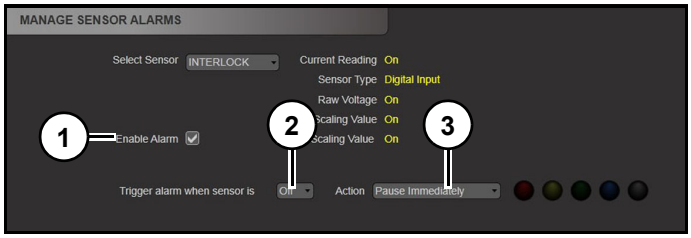

Figure 40

Refer to *(Figure 40)*.

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 plate carraige 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 returning valves to a closed state to prevent further spills. Power to valves will be cut regardless of whether sensor alarm is enabled in software 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.

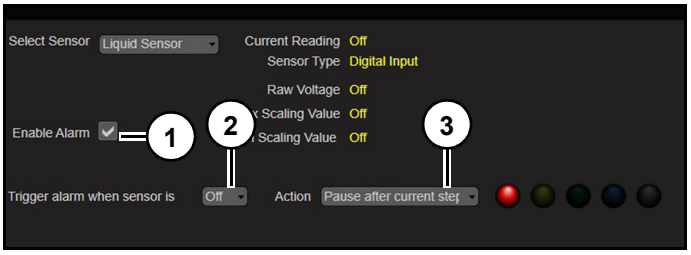

Figure 41

Refer to *(Figure 41)*.

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.

Recommended source pressure is 20psi.

Analog sensor that monitors source pressure feeding Monomer, Reagent, Auxiliary, and Blowback regulators. Sensor measures pressure after source pressure regulator. Gas is supplied to instrument at no more than 25 psi via a gas line to a customer supplied gas regulator. Gas enters source regulator where it is regulated down to recommended source pressure setting. Gas is then used to feed Monomer, Reagent, Auxiliary and Blowback regulators where pressure is dropped further and is distributed to bottles. If source pressure exceeds 25 psi then relief valve will begin to leak.

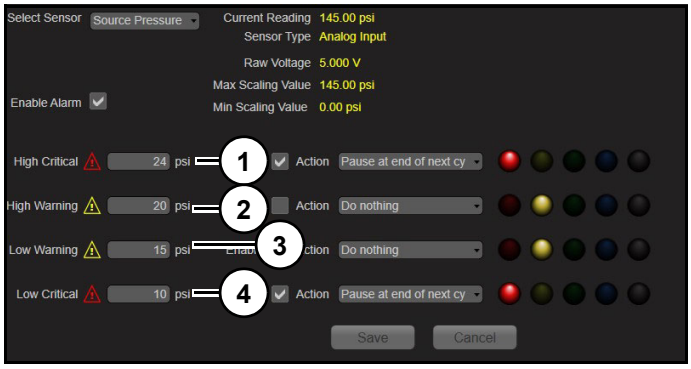

Figure 42

Refer to *(Figure 42)*.

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 bottles. Recommended setting is 6 psi.

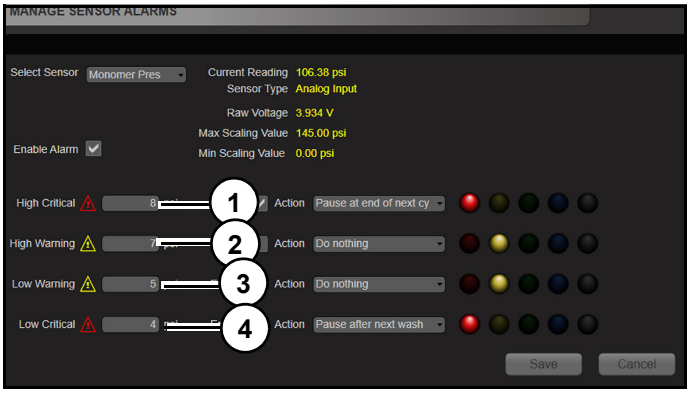

Figure 43

Refer to *(Figure 43)*.

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 not 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 7 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 and auxiliary reagent bottles. Typically consists of all reagents not used in coupling steps. Sensor includes all deblocks, oxidizers, capping reagents, and wash reagents. Confirm tubing plumbing 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.

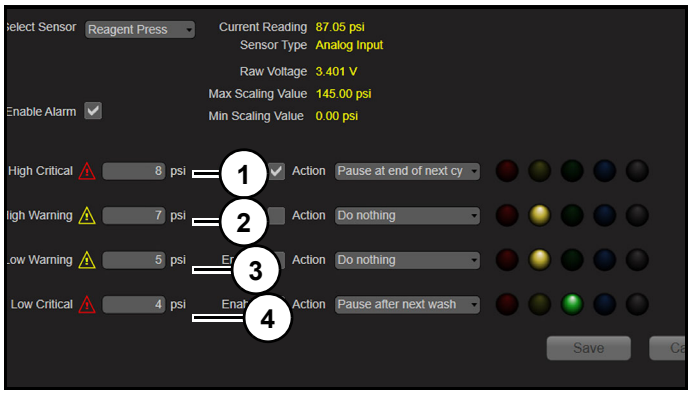

Figure 44

Refer to *(Figure 44)*.

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.

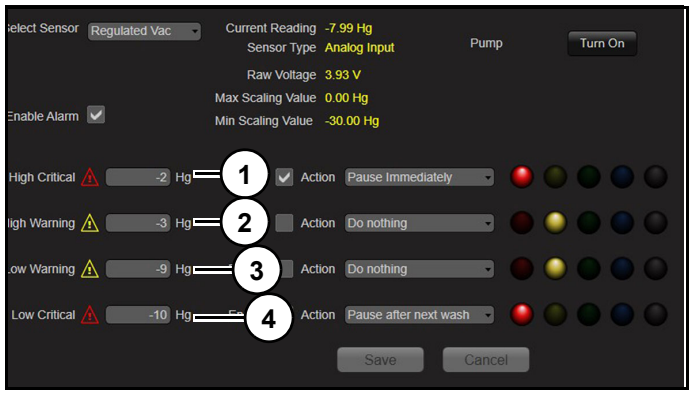

Figure 45

Refer to *(Figure 45)*.

Vacuum flow factory settings:

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

Vacuum level can be changed by adjusting vacuum breaker. 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 a should not be more than +/- 1 inHg. Very long drains in rapid succession can cause vacuum level to drop during a run and should be avoided.

Purge Flow

Analog sensor monitors argon/nitrogen purge valve responsible for delivering inert gas to chamber before and during synthesis. Chamber purge reduces humidity in chamber as well as acting as a fire prevention measure.

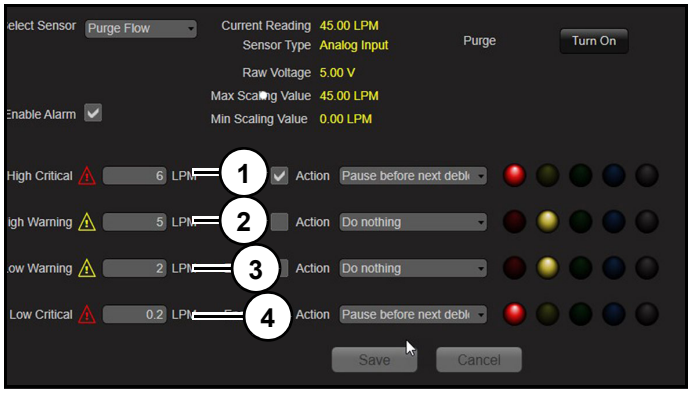

Figure 46

Refer to *(Figure 46)*.

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 192X has a group default configuration for following bottle positions:

Multi-Manifold.

- Bottle 1 (Argon)
- Bottle 2
- Bottle 3
- Bottle 4
- Bottle 5
- Bottle 6
- Bottle 7

Main Injection Head.

- Bottle 8
- Bottle 9
- Bottle 10
- Bottle 11
- Bottle 12
- Bottle 13
- Bottle 14
- Bottle 15
- Bottle 16

Group injection bottles should be reserved for user's default reagents (Oxidizer, Cap A, Cap B, De-block, Acetonitrile, Activator, Sulfur, Aux Wash 1, Aux Wash 2) and most used monomers (dA, dC, dG, dT, etc.). These positions can either be injected into columns as a group to save time, or one at a time sequentially in an array from back to front.

192X has a single line default configuration for following bottle positions:

Single Injection Positions on the Main Injection Head.

- Bottle 17
- Bottle 18
- Bottle 19

- Bottle 20
- Bottle 21
- Bottle 22
- Bottle 23
- Bottle 24
- Bottle 25
- Bottle 26
- Bottle 27
- Bottle 28
- Bottle 26
- Bottle 30
- Bottle 31
- Bottle 32
- Bottle 33
- Bottle 34
- Bottle 35
- Bottle 36
- Bottle 37
- Bottle 38
- Bottle 39
- Bottle 40

Single line bottle injection positions are all located on main injection head and should bereserved for any other monomers, dyes, or specialty modifiers the user will be using sparingly. These positions can only be injected one at a time into columns.

Figure 47

Refer to *(Figure 47)*.

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 be located right of all amidites. If not, there will be excess movements and synthesis will slow down.
- Cap A must be located 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.
Note: Any changes made in "Manage Reagents" screen will not take effect until software is rebooted.

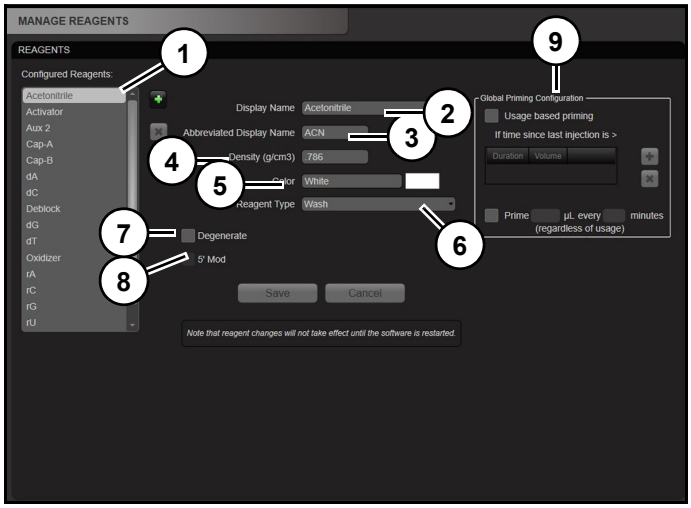

Figure 48

Refer to *(Figure 48)*.

Click on reagent (1) 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 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

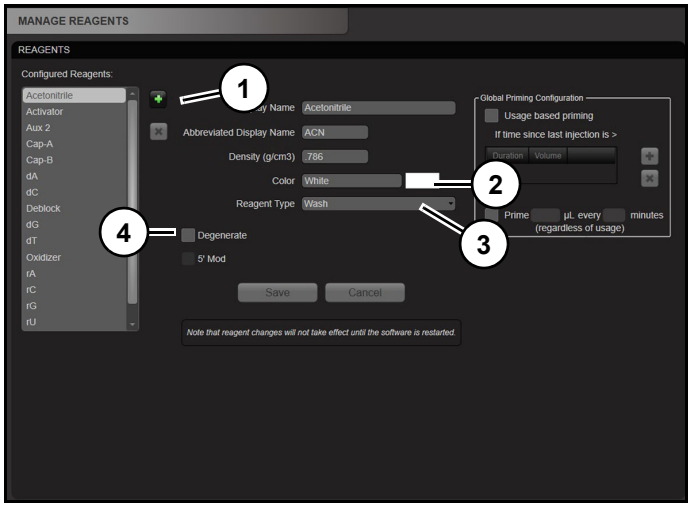

Figure 49

1. Click "+" (1) *(Figure 49)*.

Figure 50

Refer to *(Figure 50)*.

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 49)*

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

Adding Degenerate Base

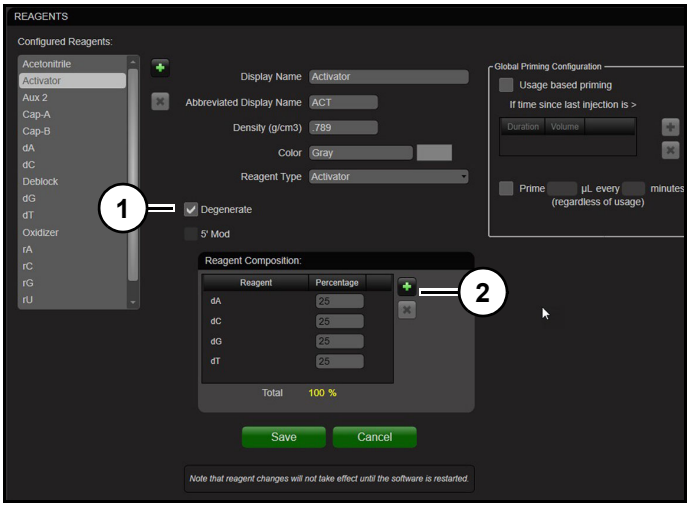

Figure 51

Refer to *(Figure 51)*.

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.

Note: Be sure liquid calibrations are accurate in lower ranges to accommodate for decrease in volume injected.

Reagent Specific Priming

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

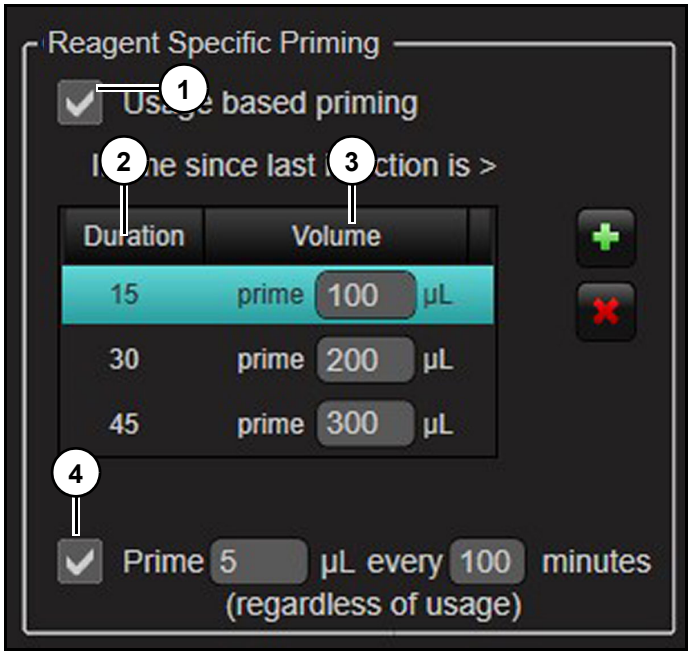

Figure 52

Refer to *(Figure 52)*.
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 within min/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.

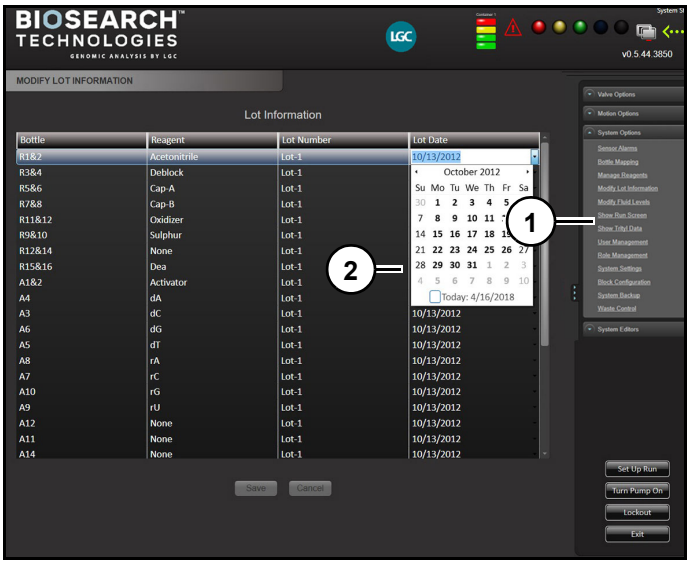

Figure 53

Refer to *(Figure 53)*.

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.

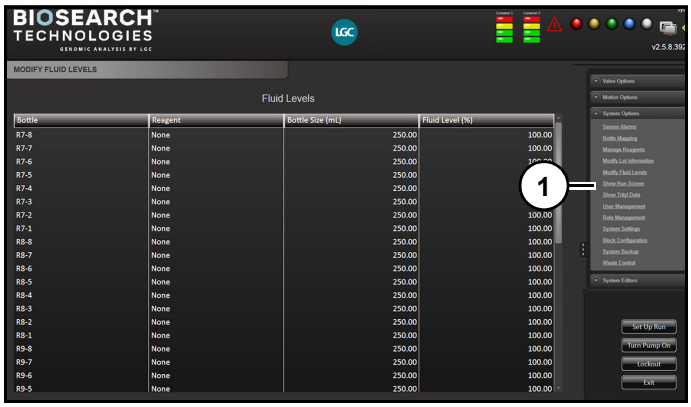

Figure 54

Modify Fluid Levels (1) *(Figure 54)* 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 to return to "run screen" after limited access diagnostics and testing when instrument is paused.

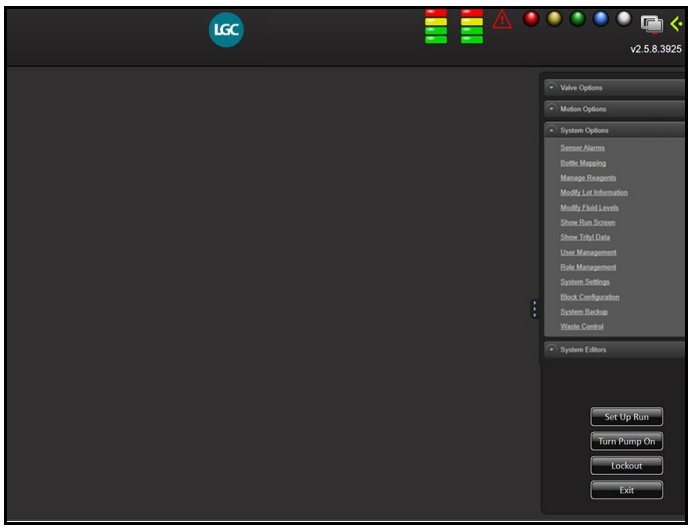

Figure 55

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.

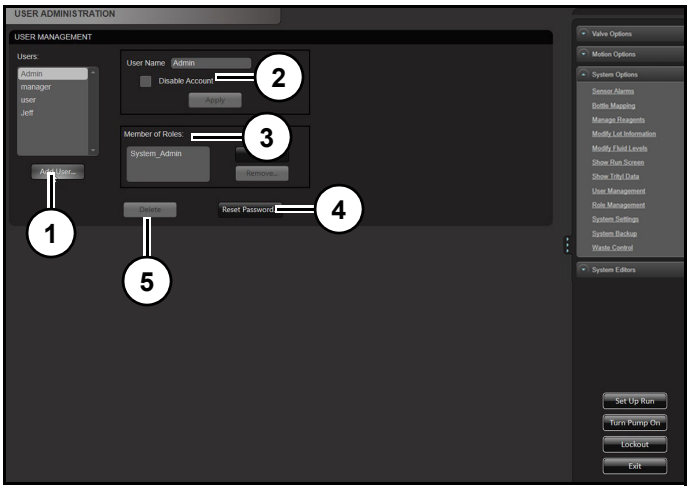

Figure 56

Refer to *(Figure 56)*.

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

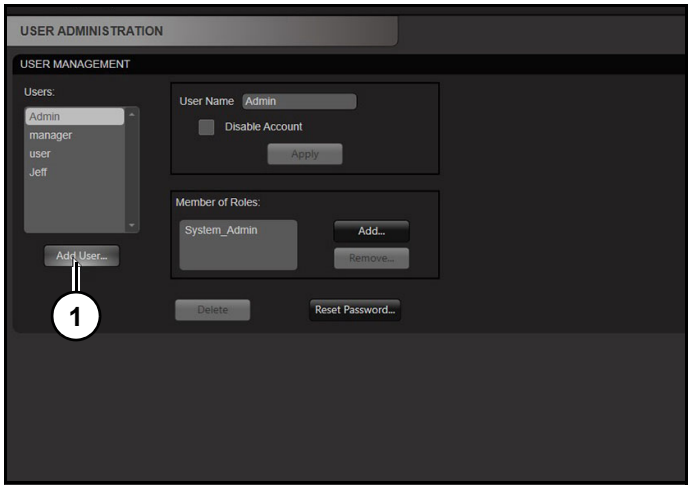

Figure 57

1. Click "Add User" (1) *(Figure 57)*.

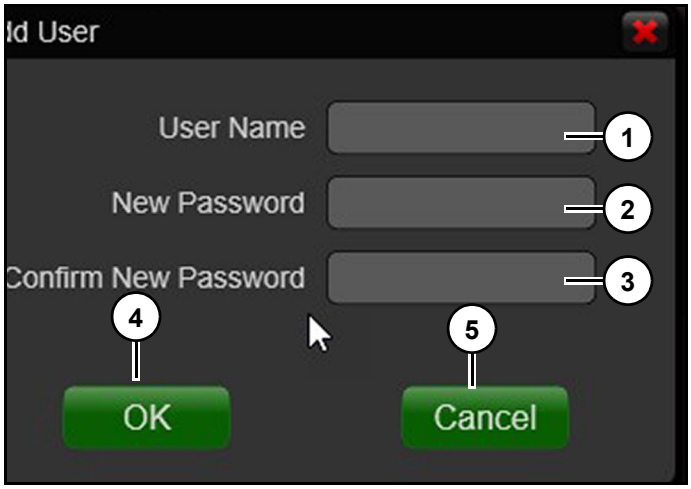

Figure 58

Refer to *(Figure 58)*.

2. Enter "User Name" (1).
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.

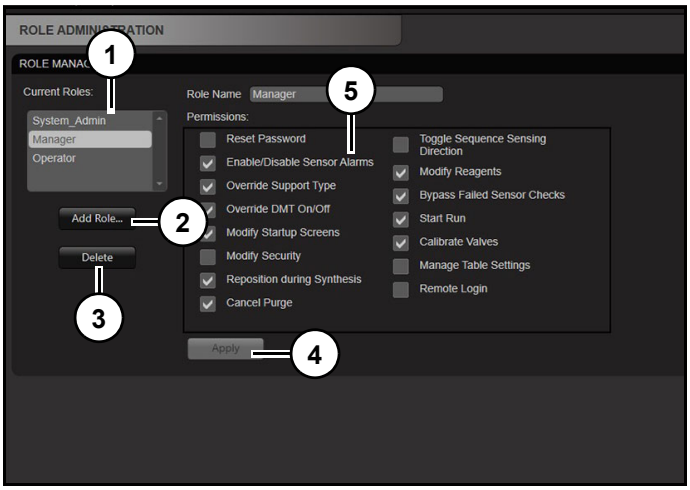

Figure 59

Refer to *(Figure 59)*.

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 vs 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

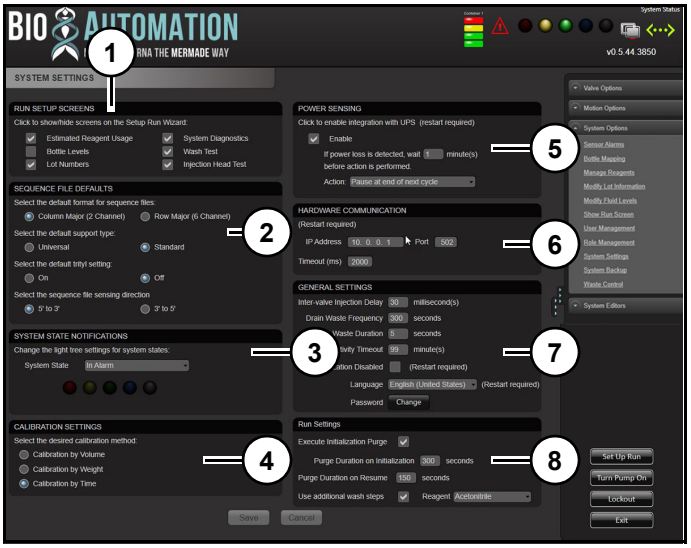

Figure 60

Refer to *(Figure 60)*.

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 line 2.
- 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 (3 to 5) 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;

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 oxidizer 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.
- Password: Allows changes to opening screen software password if permissions allow.

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

- Execute Initiation Purge: Turns initialisation argon/nitrogen purge on/off. At beginning of a run, argon/nitrogen 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/nitrogen 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.

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

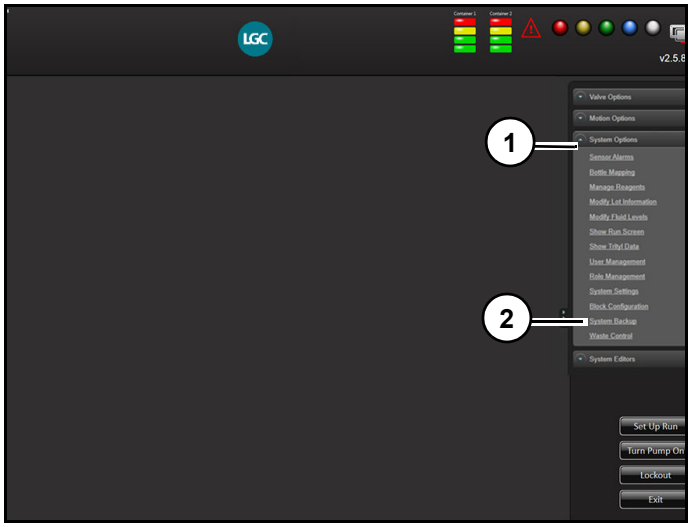

Figure 61

Refer to *(Figure 61)*.

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.

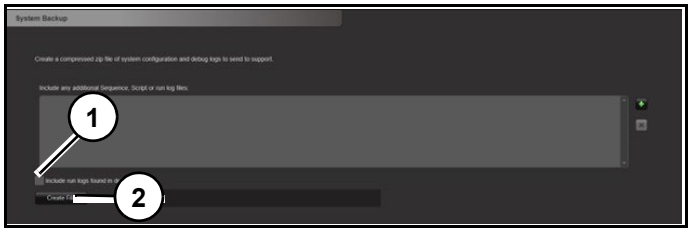

Figure 62

Refer to *(Figure 62)*.

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.

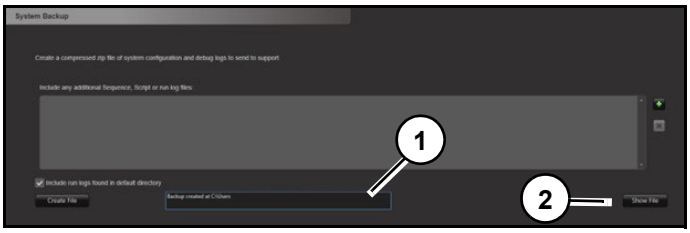

Figure 63

Refer to *(Figure 63)*.

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

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

Export Backup—Additional Files Selected.

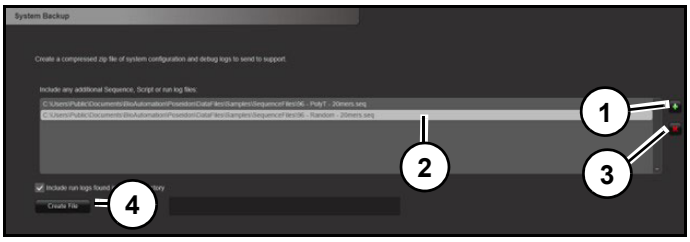

Figure 64

Refer to *(Figure 64)*.

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.

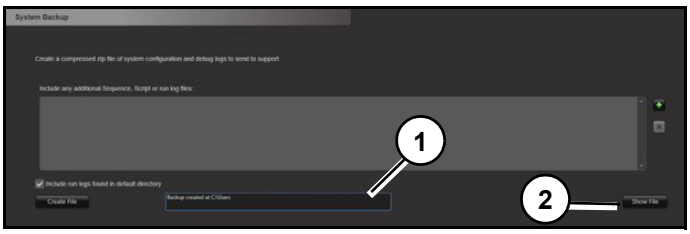

Figure 65

Refer to *(Figure 65)*.

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.

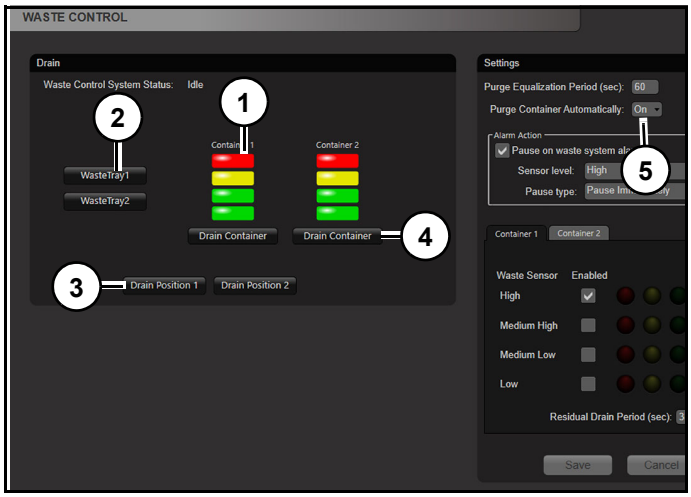

Figure 66

Refer to *(Figure 66)*.

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.

Waste Tray 1/2 (2): Selects waste tray to drain.

Drain Position 1 (3): Will drain plate position 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 67)* allows user to modify and create script files.

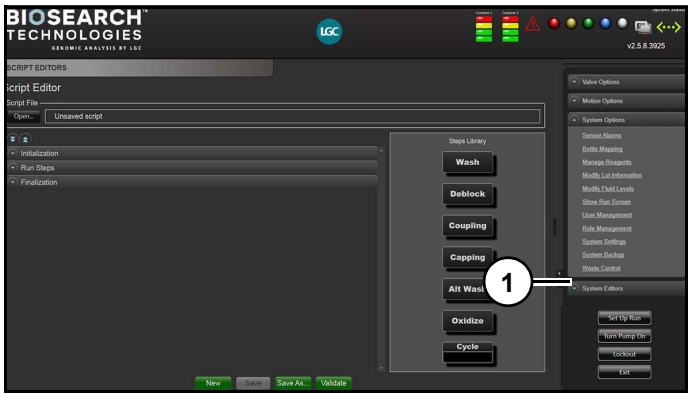

Figure 67

Using script files to set synthesis parameters

During process of setting up a run, users 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.

Note: Script files should always be reviewed before first use.

Opening a script file

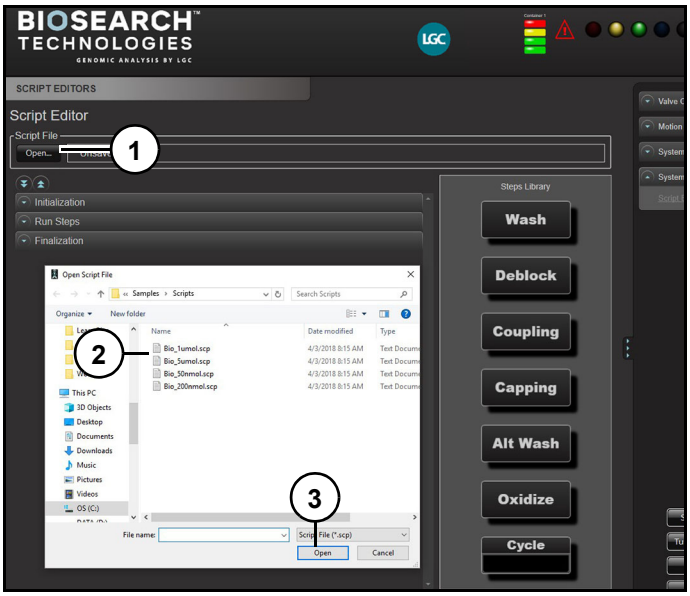

Figure 68

Refer to *(Figure 68)*.

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 69

Refer to *(Figure 69)*.

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.

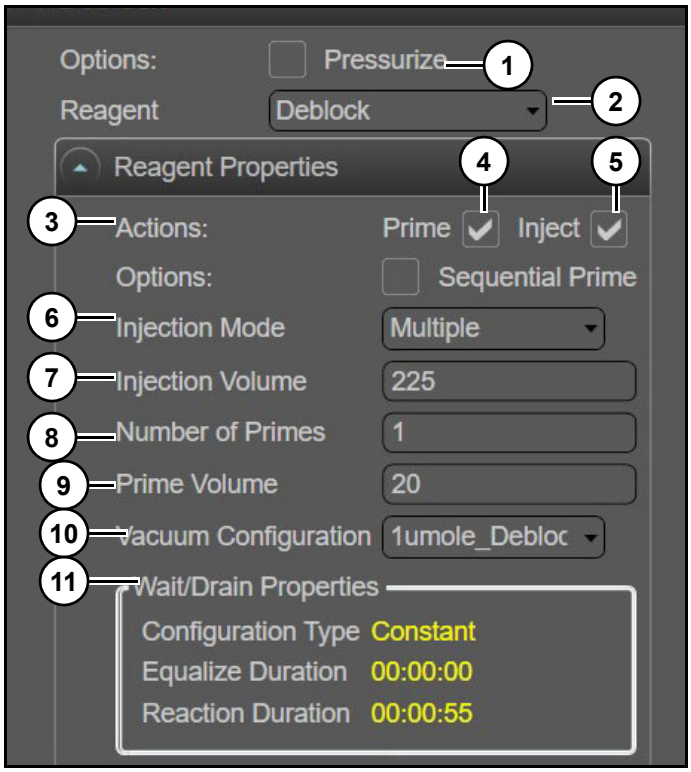

Figure 70

Refer to *(Figure 70)*.

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 (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. Contact LGC representatives to have fast mode enabled.
- 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 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 reaction 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 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 prepare solid support for next step in cycle. Since there is no reaction time necessary for 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 used. Most common deblock reagents are 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. 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 coupling and RNA could have two coupling steps.

Different monomers/amidites can have different reaction times. If all columns within synthesis plate have same vacuum configuration, coupling will be performed on all columns in a single step. If different reaction times, or vacuum profiles are required during same step, software will split step into individual steps for each differing vacuum library entry. If "Use additional wash steps" is enabled in "System Settings" screen, software will add selected reagent to all wells not being coupled to keep vacuum calibrations accurate. It is recommended to run in simulation after creating a new script to confirm proper step execution during synthesis.

Capping

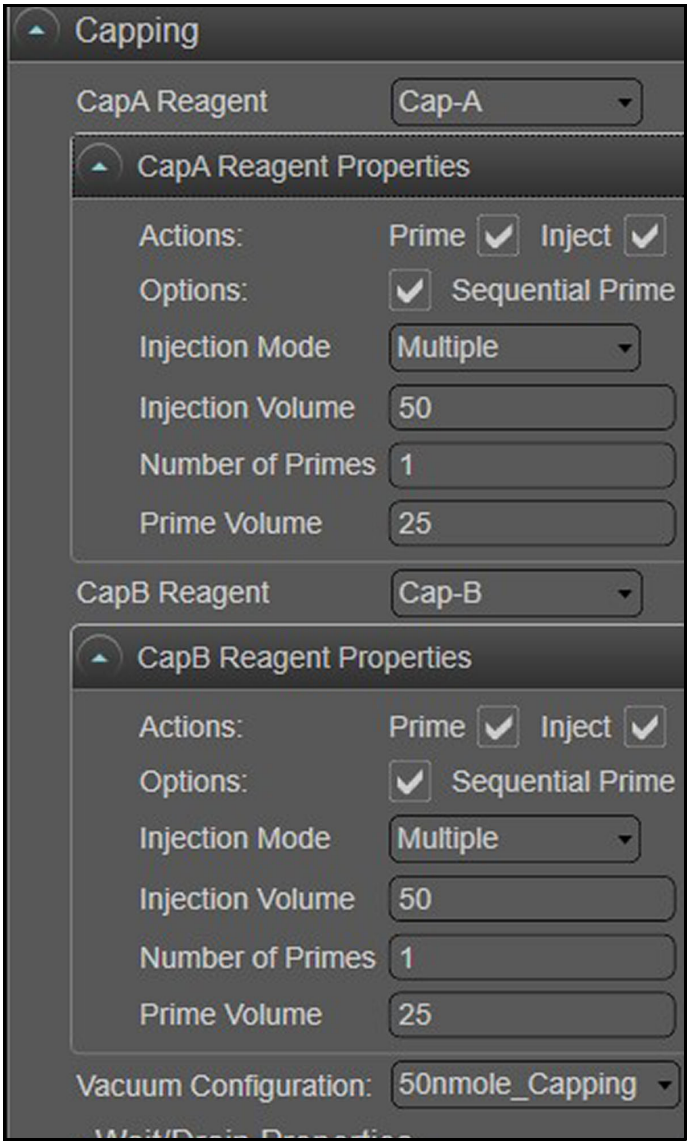

Figure 71

Refer to *(Figure 71)*.

Capping prevents molecules which did not get coupled during coupling step from reacting in future coupling steps. A capping failure during synthesis will lead to poor quality, deletions, and high N- impurities. Cap A and Cap B share one vacuum library entry as reagents are injected into same well and therefore must be drained together. There are several capping formulations available and optimal composition for end application should be determined.

Oxidation and Sulfurization

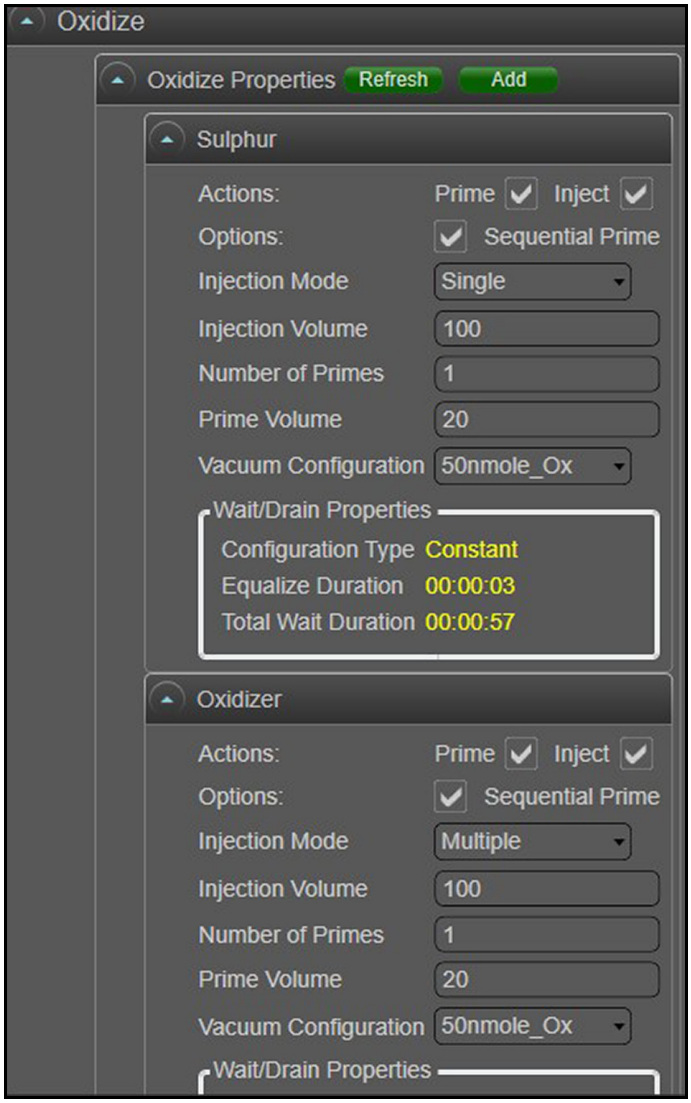

Figure 72

Refer to *(Figure 72)*.

Oxidation steps are used to put newly added backbone linkage into a stable state. This can be done with either oxygen, sulfur or a customized backbone. 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, sulfur or a tertiary option. To designate which reagent is used, its designated symbol will be utilized in sequence file: a ';' delimiter for oxygen or a '*' for sulfur. Additional oxidisers can be added, 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

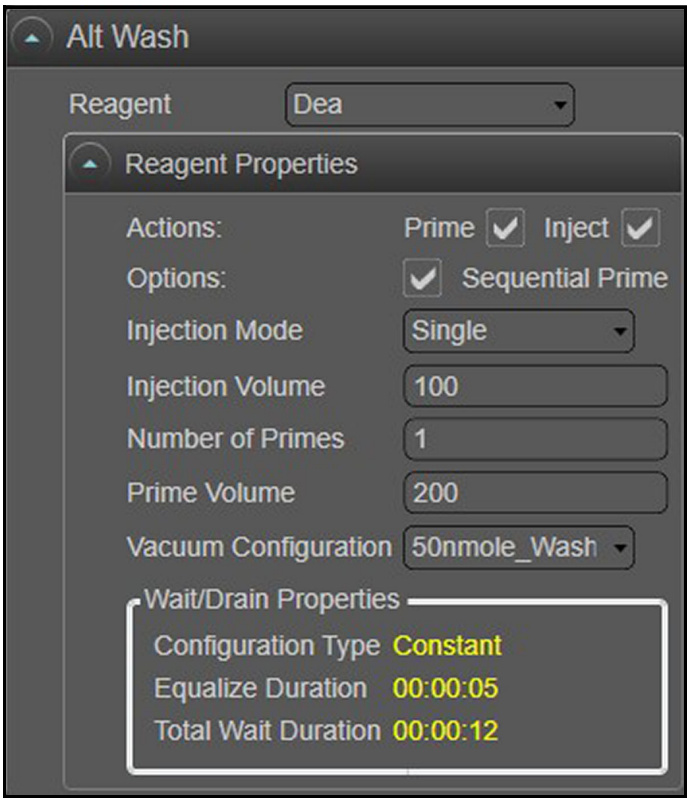

Figure 73

Refer to *(Figure 73)*.

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

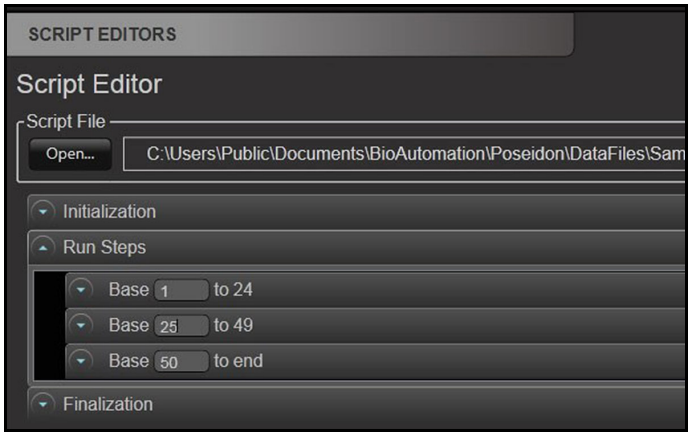

Figure 74

Refer to *(Figure 74)*.

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 74)*.

Making changes to a script file

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

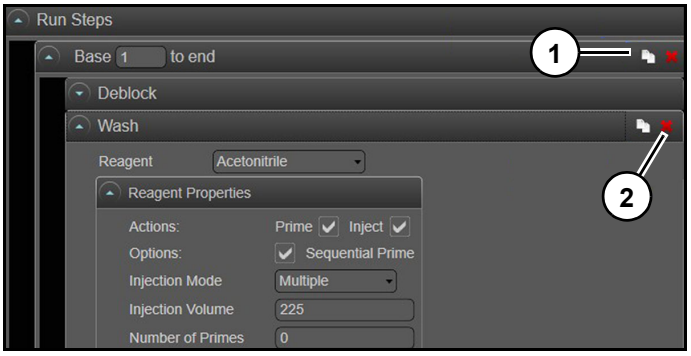

Figure 75

Refer to *(Figure 75)*.

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

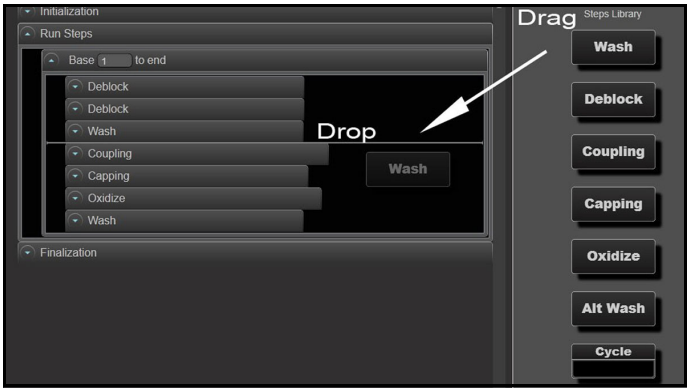

Figure 76

Refer to *(Figure 76)*.

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

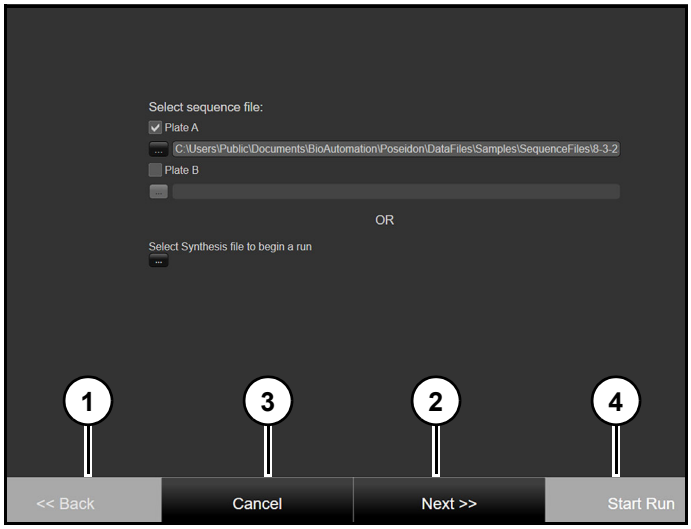

Figure 77

Refer to *(Figure 77)*.

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.

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

Start Run (4): Starts synthesis when all minimum required information has been added. Bypasses several crucial safety checks.

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.

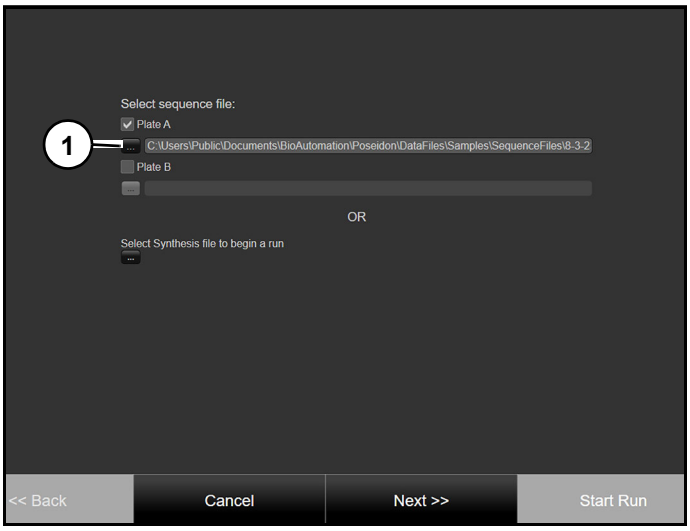

Figure 78

Refer to *(Figure 78)*.

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.

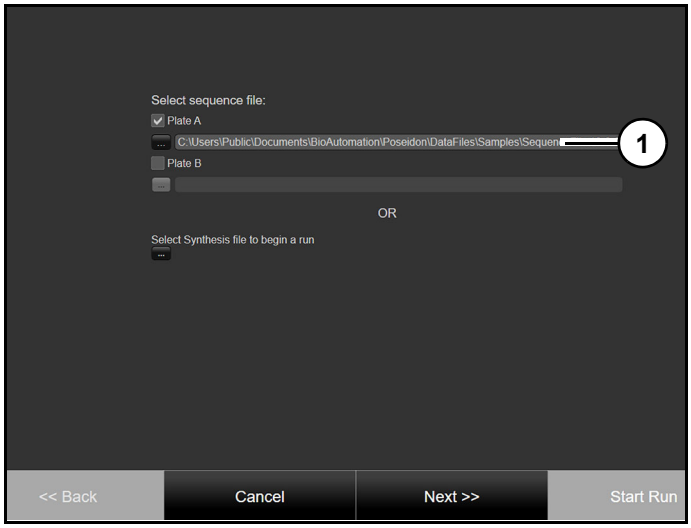

Figure 79

Refer to *(Figure 79)*.

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 produce a "Too Many Oligos" error..
- Each line must contain an oligo name and an oligo sequence. It may also 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.

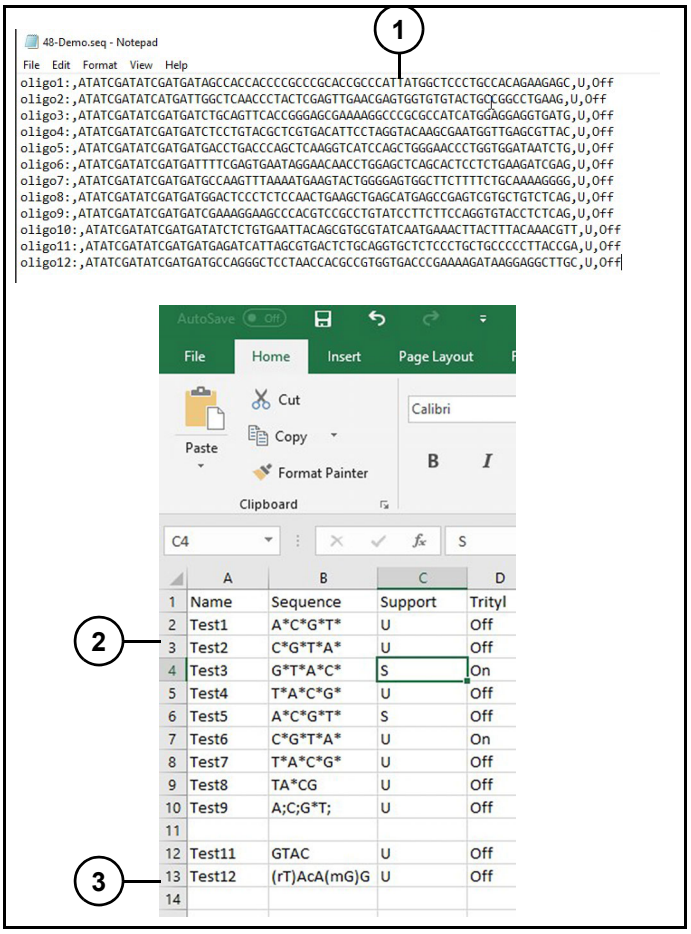

Figure 80

Refer to *(Figure 80)*.

- File must be a text file saved in '.seq' format (1) (for sequence file) or in '.xlsx' Excel format (2). Notepad is used to create a text file saved as a '.seq' file. Excel files can be loaded natively and will ignore all fields except first four columns and first 97 rows.

User can select to have sequence file read so that order of synthesis is A1, B1, A2, B2, etc. (Column Major) or A1, A2, A3…A6, B1, B2, B3…B6 (Row Major). 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 from manage reagents screen is case sensitive and will be used to call a base from sequence file. 'C' is not equivalent to 'c' and 'UsA' is not equivalent to 'uSa'. Abbreviated display names with more than one

character in length must be bracketed by parentheses as shown on line 13 (3) *(Figure 80)*.

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. If alternate oxidisers are being used, it needs to be indicated in sequence file. An "*" must entered 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.

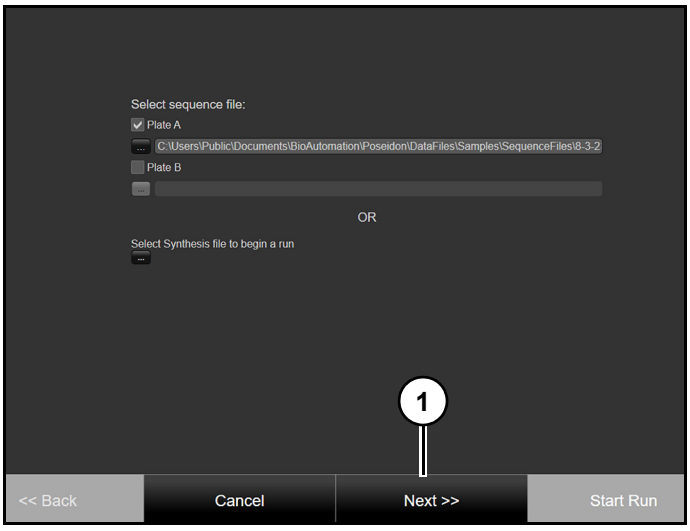

Figure 81

3. Click "Next" (1) *(Figure 81)*.

When sequence file is loaded a validation is performed and any issues that will prevent sequence file from being run 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.

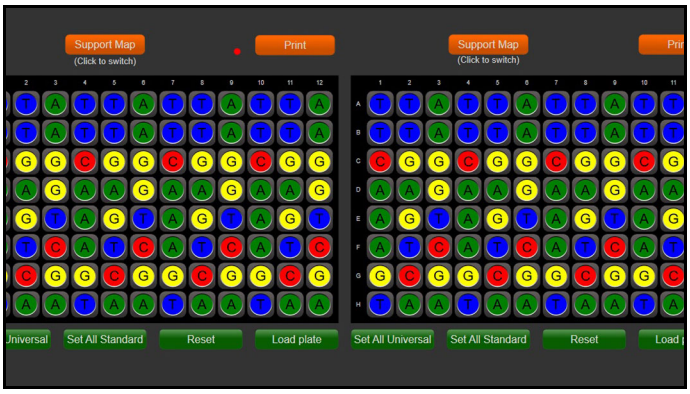

Figure 82

Refer to *(Figure 82)*.

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 be 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.

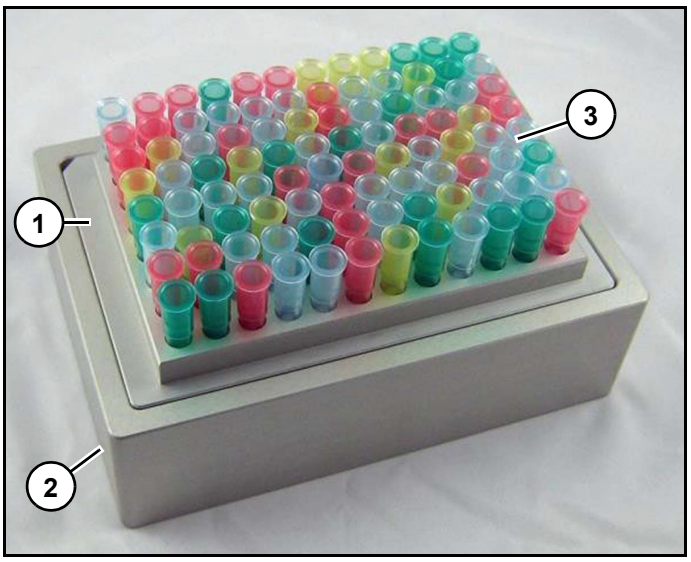

Figure 83

Refer to *(Figure 83)*.

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

Figure 84

Refer to *(Figure 84)*.

3. Using supplied rubber mallet to tap columns into column chuck.

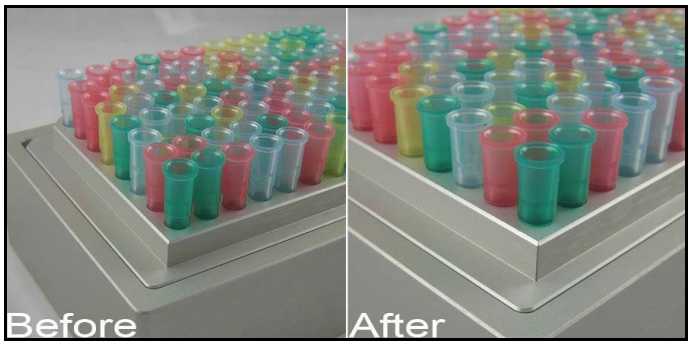

Figure 85

Refer to *(Figure 85)*.

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.

Note: Biosearch Technologies supply multiple types of column chucks. Pictures above may differ

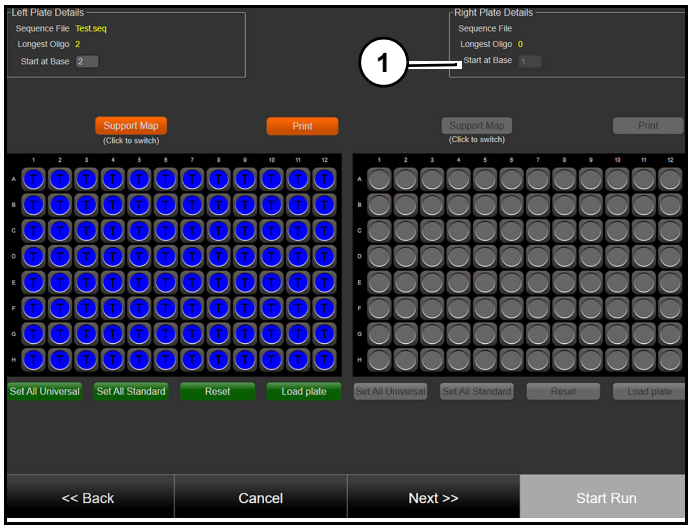

Figure 86

Under plate details box, there is a start at base box (1) *(Figure 86)*. 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.

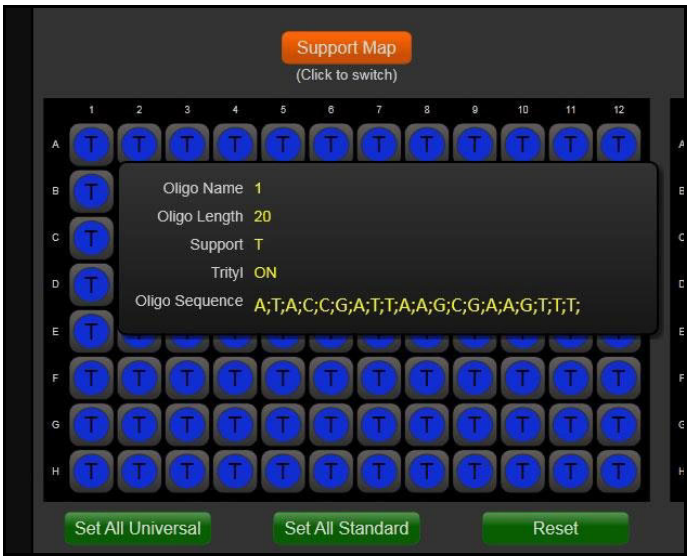

Figure 87

Refer to *(Figure 87)*.

4. 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.

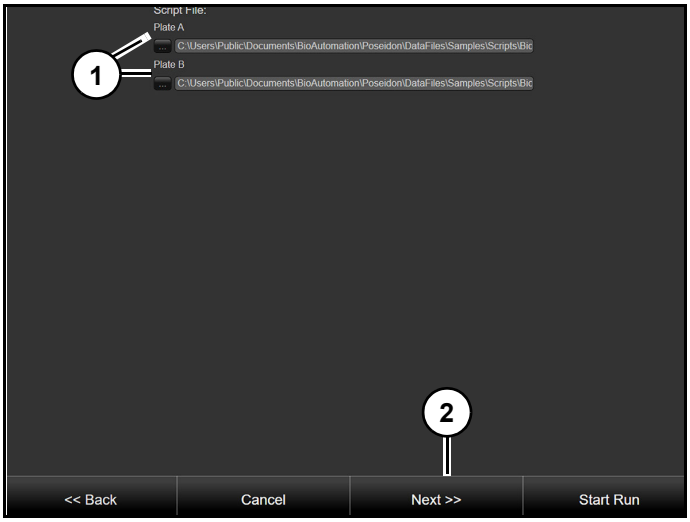

Figure 88

Refer to *(Figure 88)*.

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

6. 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 for each plate.

7. Click "Next" (2).

Estimated Reagent Usage.
Allows user to view an estimate of how much of each reagent will be required to finish a run. It will also display the estimated waste generated.

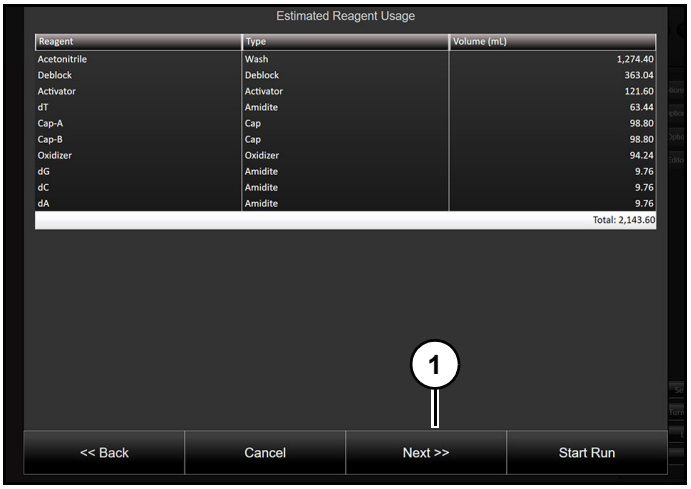

Figure 89

Values shown *(Figure 89)* 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.

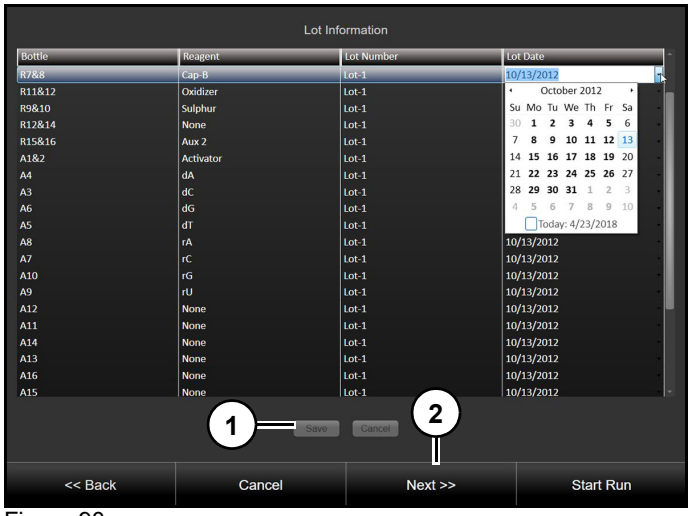

Figure 90

Refer to *(Figure 90)*.

8. Enter lot information for chemicals that are loaded on instrument.
9. Click "Save" (1).
10. Click "Next" (2).

Plate Information.

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

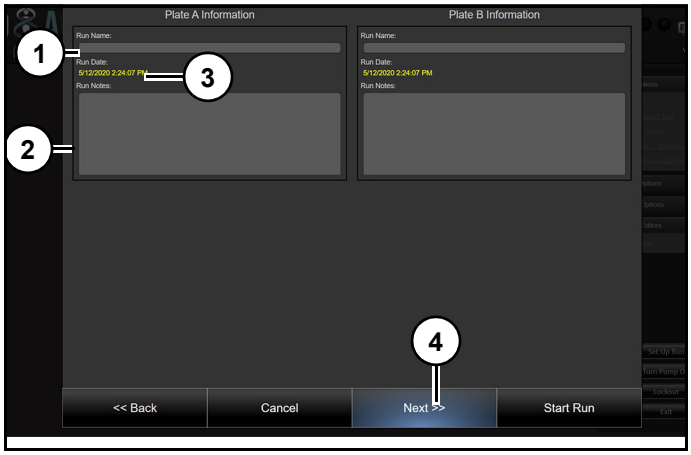

Figure 91

Refer to *(Figure 91)*.

11. 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. Each plate can contain different notes.

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.

Argon Hi Flow Test.

Argon Low Flow Test.

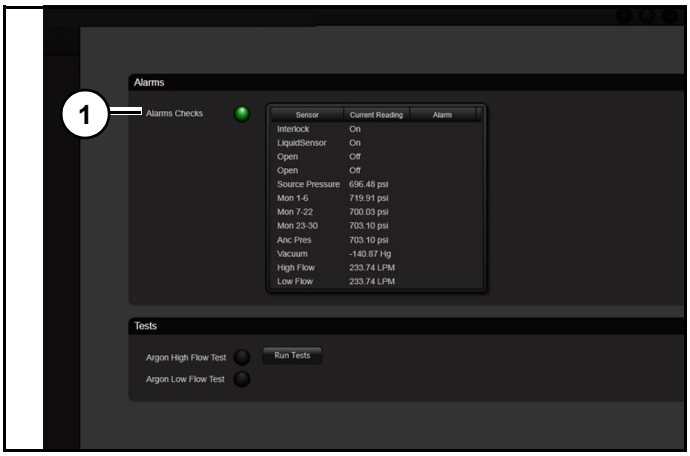

Figure 92

Refer to *(Figure 92)*.

Alarm Checks (1).

- Source Pres
- Monomer Pres
- Reagent Pres
- Blow Back Pres
- Aux Pres
- Regulated Vac

- Purge Flow

Note: Additional alarms can be present depending on instrument configuration.

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

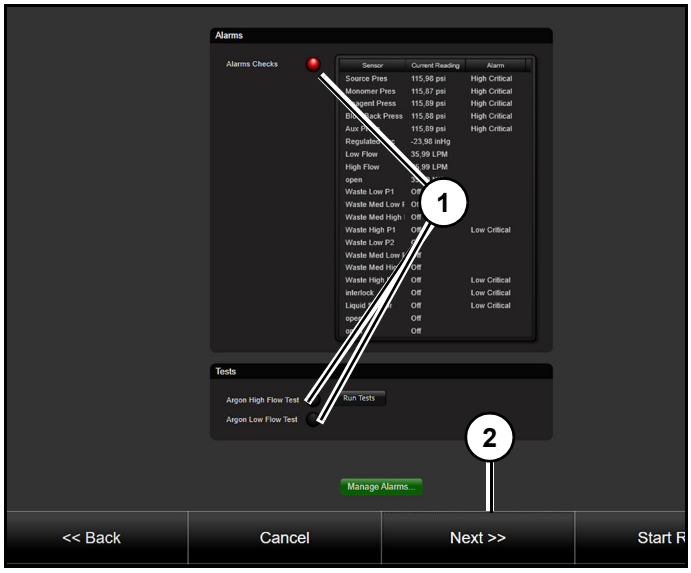

Figure 93

Refer to *(Figure 93)*.

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

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

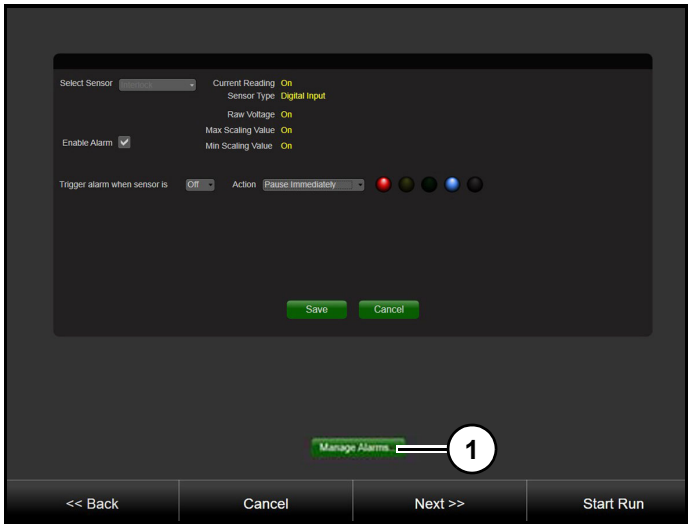

Figure 94

Refer to *(Figure 94)*.

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/nitrogen supply to machine is either very weak or exhausted. Replacing argon/ nitrogen 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

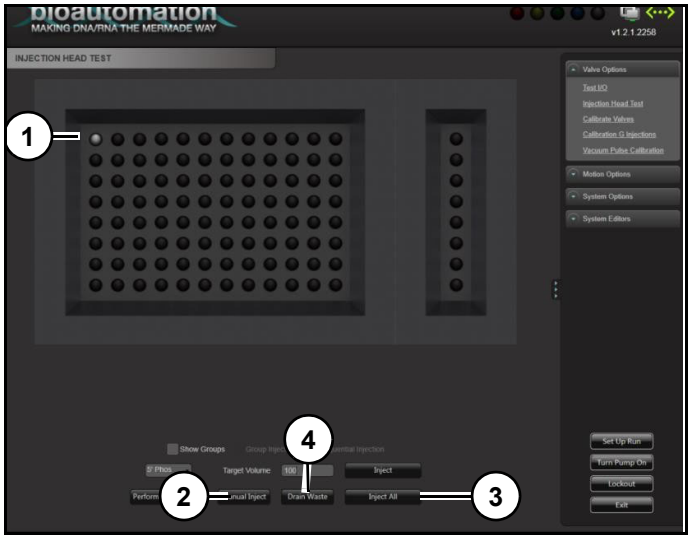

Figure 95

Refer to *(Figure 95)*.

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. Hold down "Drain Waste" (4) to drain waste trays.
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.

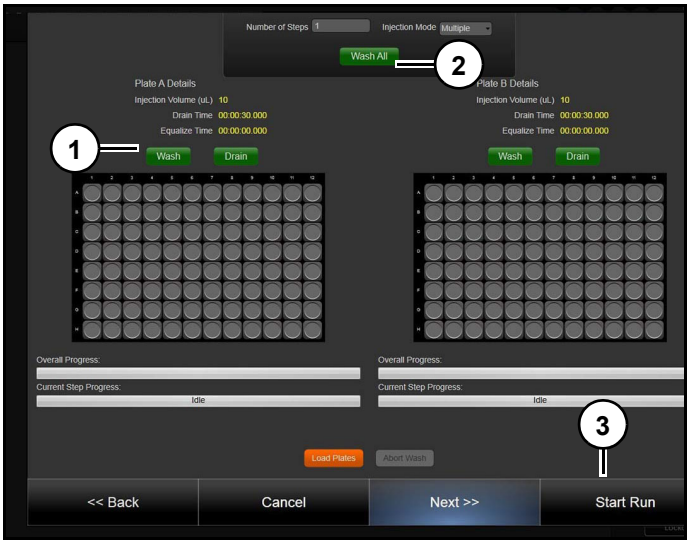

Figure 96

Refer to *(Figure 96)*.

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.

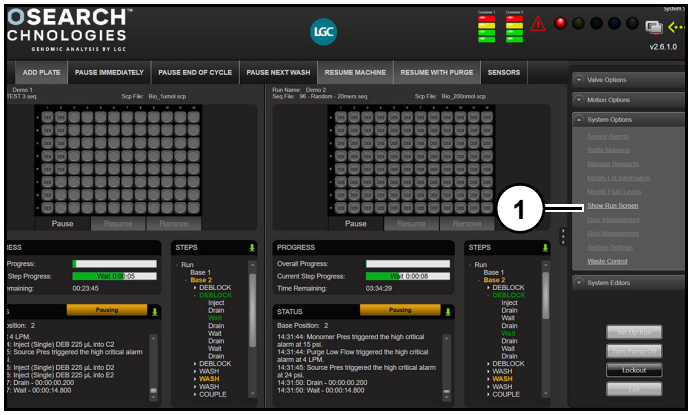

Figure 97

Refer to *(Figure 97)*.

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

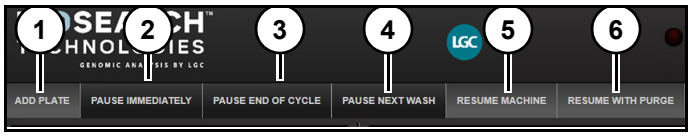

Figure 98

Refer to *(Figure 98)*.

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 startup wizard, which will allow them to add a new plate to synthesizer.

Pause Immediately (2): Pauses instrument immediately. 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/nitrogen chamber purge and then continues from last pause point.

Sensors

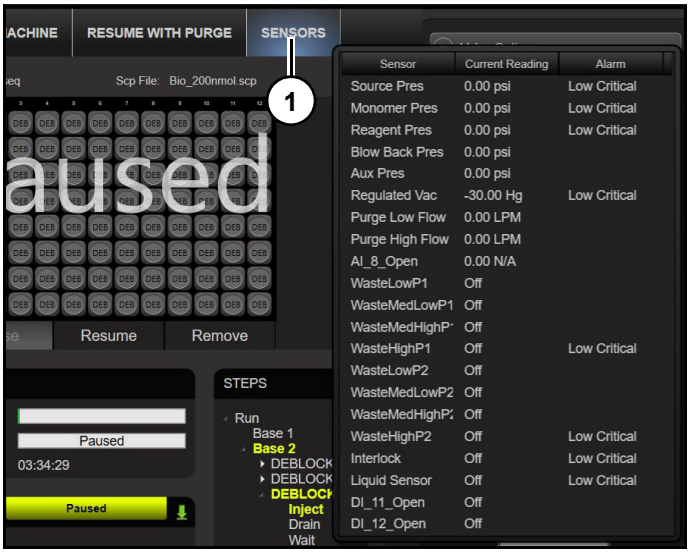

Figure 99

Refer to *(Figure 99)*.

Displays current sensors and their respective values. Sensors cannot be changed, only viewed. To adjust sensors and sensor options (See "Sensor Alarms" on page 47.).

Plate options bar features

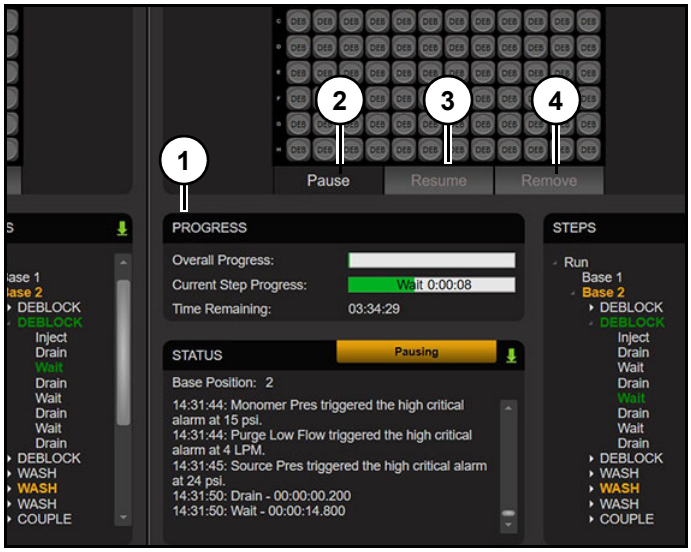

Figure 100

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 a step. Unexpected events can occur when plates are paused immediately.

Resume (3): Will resume a paused plate, if both plates are paused, only selected plate will resume.

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

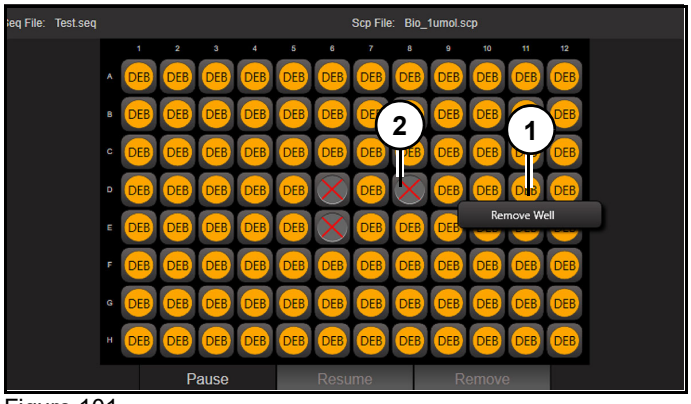

Figure 101

Refer to *(Figure 101)*.

Remove a Well (1): Within plate graphic itself, each synthesis well can be removed by right-clicking and selecting "Remove Well" (1). Synthesis well will become inactive and will have a red X over it (2).Synthesis well will no longer receive any injections form the synthesiser.

Note: This is not recommended. Can affect draining/ vacuum pulses. If columns are terminated, it's best to pause instrument remove column and seal that position with Aluminum tape sealer.

Steps control options

Figure 102

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

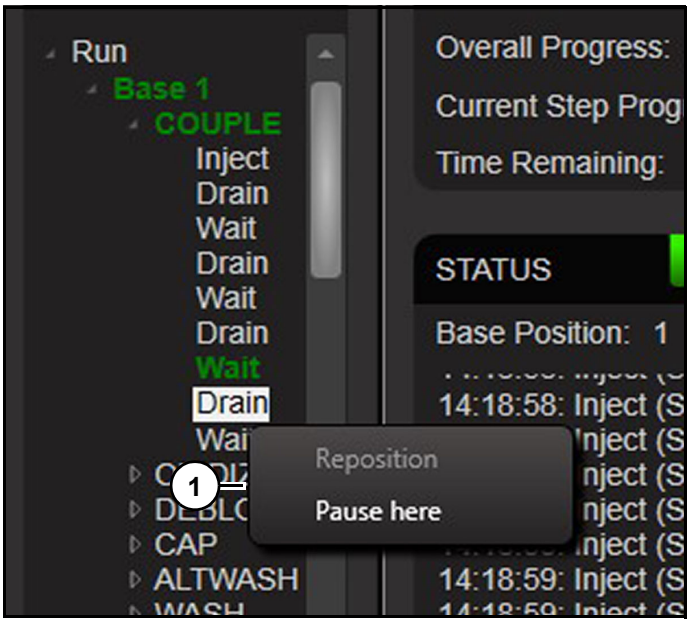

Figure 103

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)*

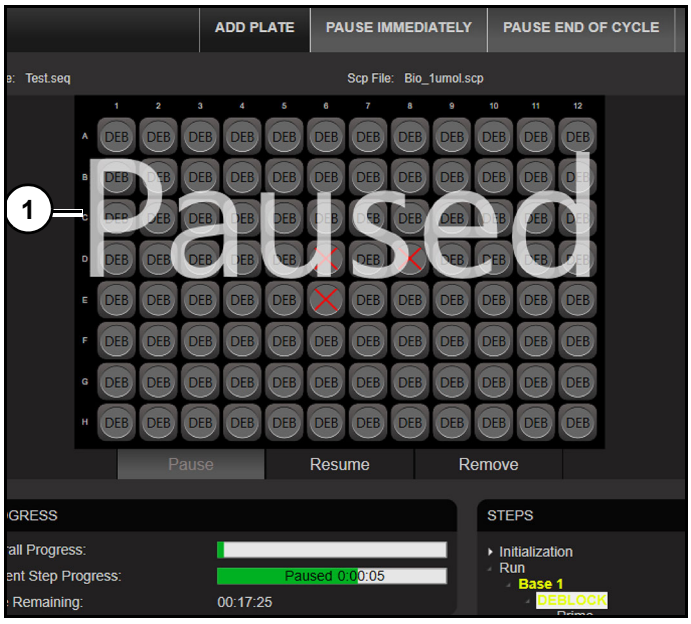

Figure 105

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.

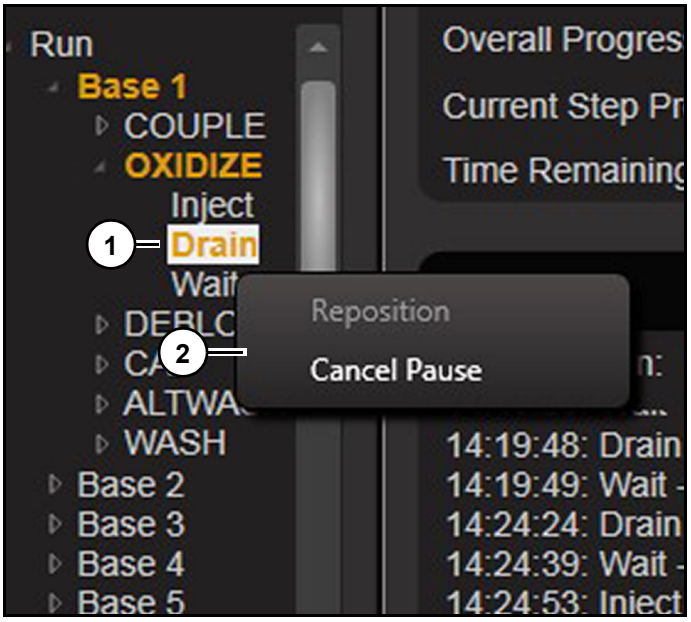

Figure 106

Refer to *(Figure 106)*.

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

Reposition

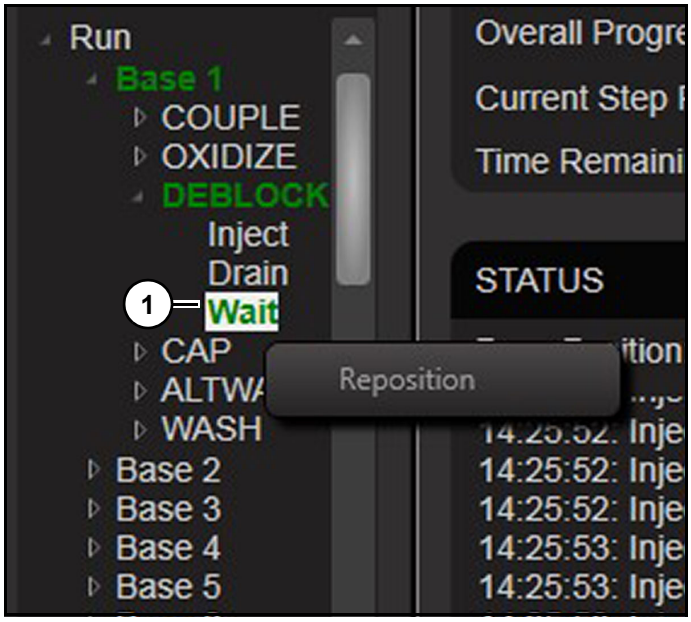

Figure 107

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)

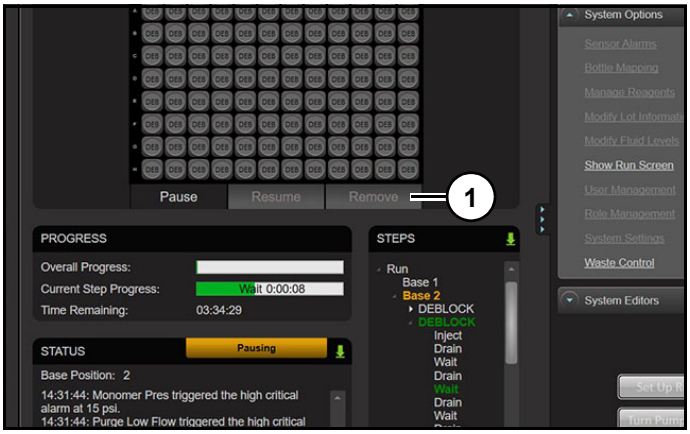

Figure 108

Refer to *(Figure 108)*.

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

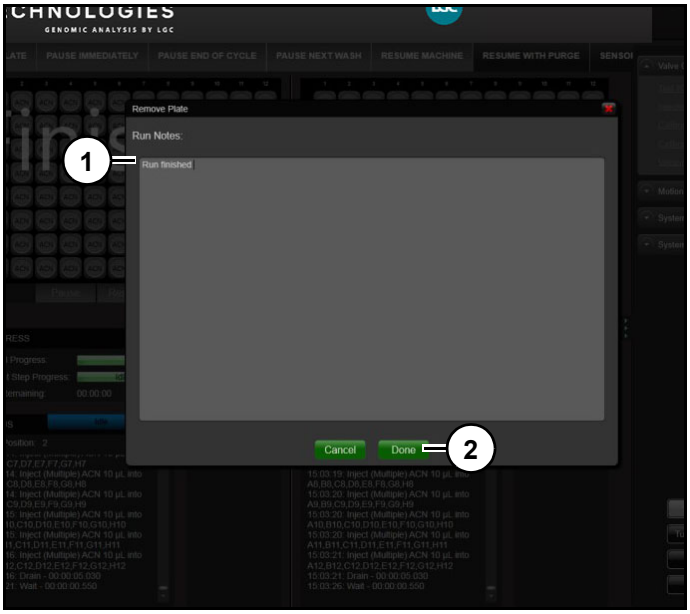

Figure 109

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 192X startup kit.

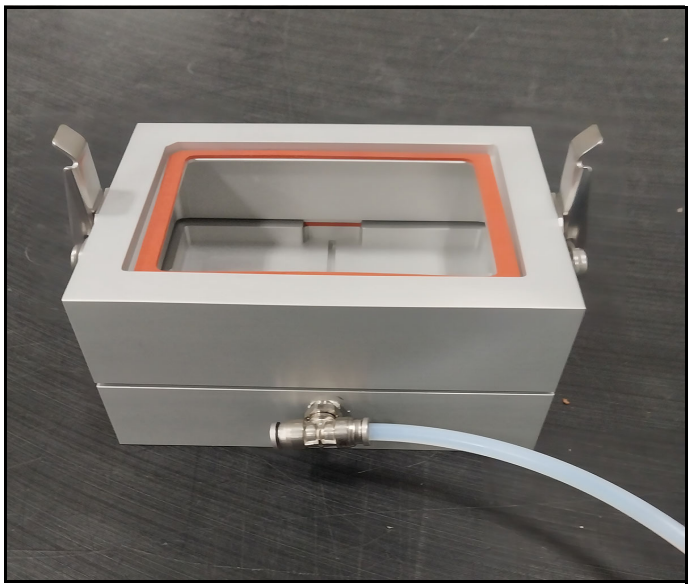

Figure 110

Cleavage Chuck *(Figure 110)*.

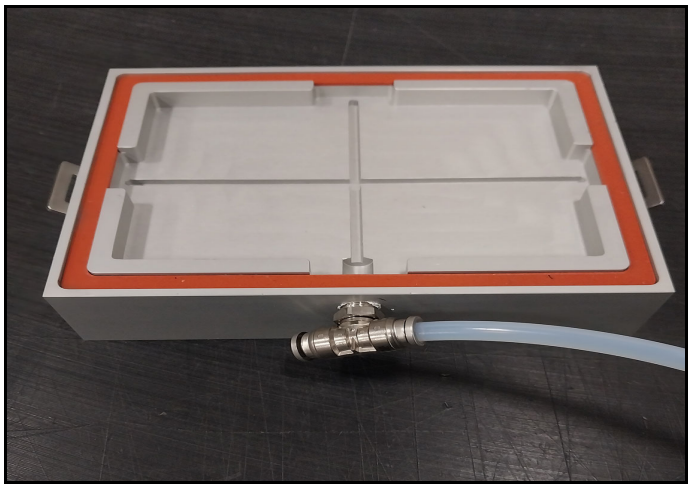

Figure 111

Cleavage Chuck Bottom *(Figure 111)*.

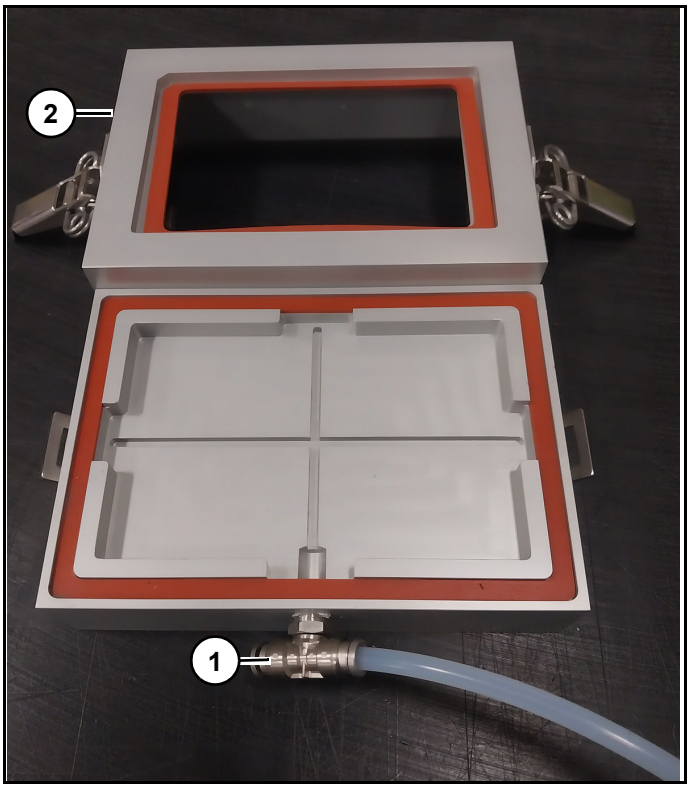

Figure 112

Refer to *(Figure 112)*.

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

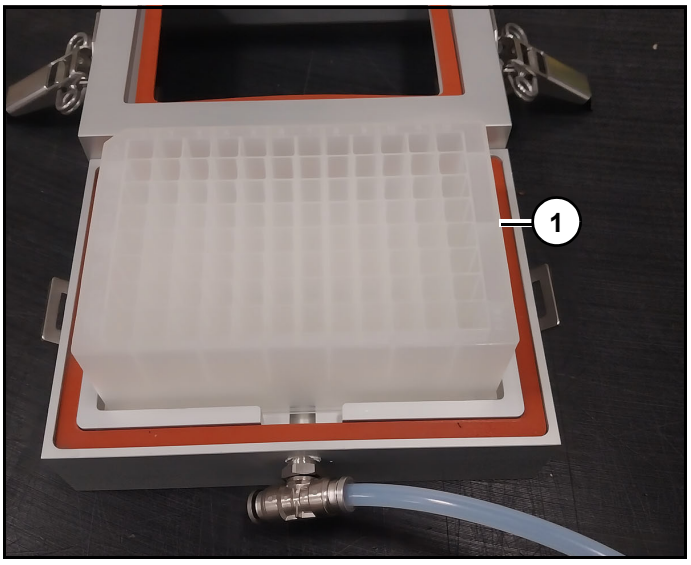

Figure 113

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.

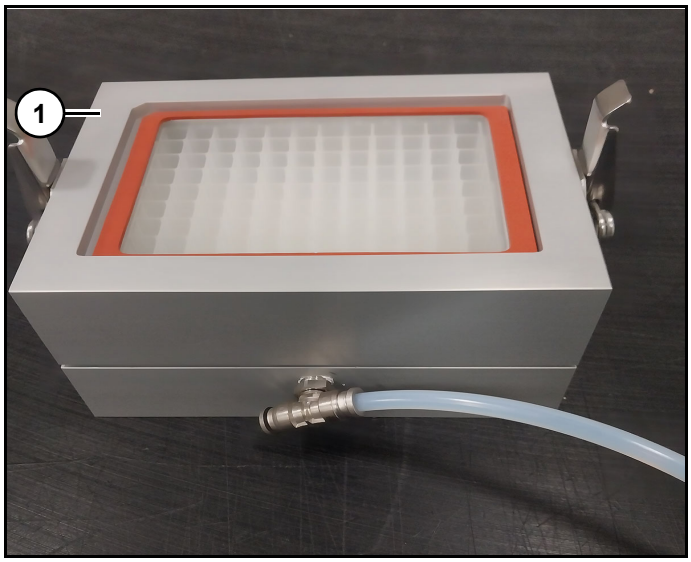

Figure 114

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

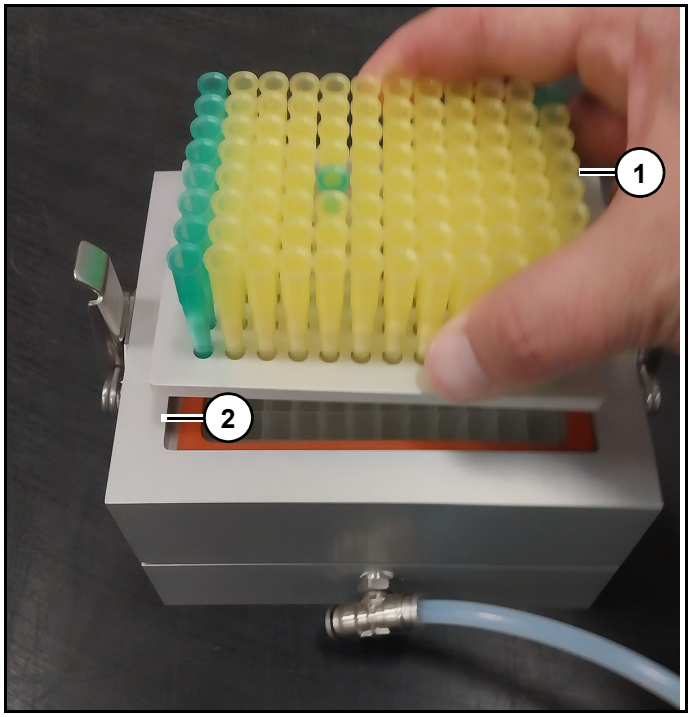

Figure 115

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.
- 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.

Reference chart for both chemistries:

Reagent cleavage				
Scale	**Volume**	**# of Aliquots**	**Time (NH4OH)**	**Time (AMA)**
50 nmol	100 uL	3	15 mins	5 mins
200 nmol	150 uL	3	15 mins	5 mins
1 umol	200 uL	3	15 mins	5 mins

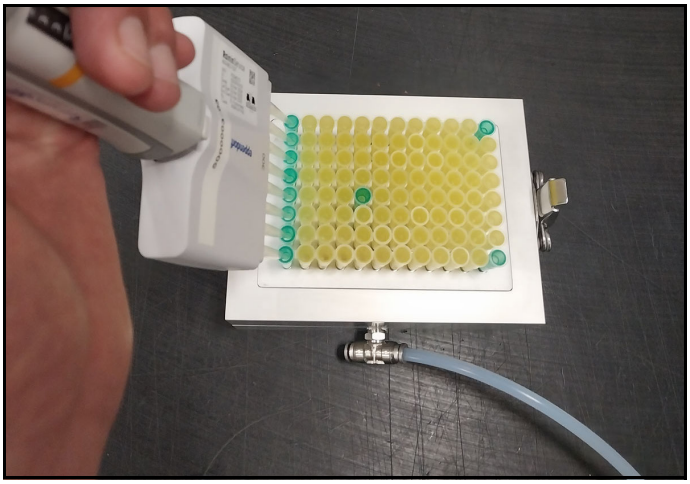

Figure 116

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 incomplete cleavage 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.

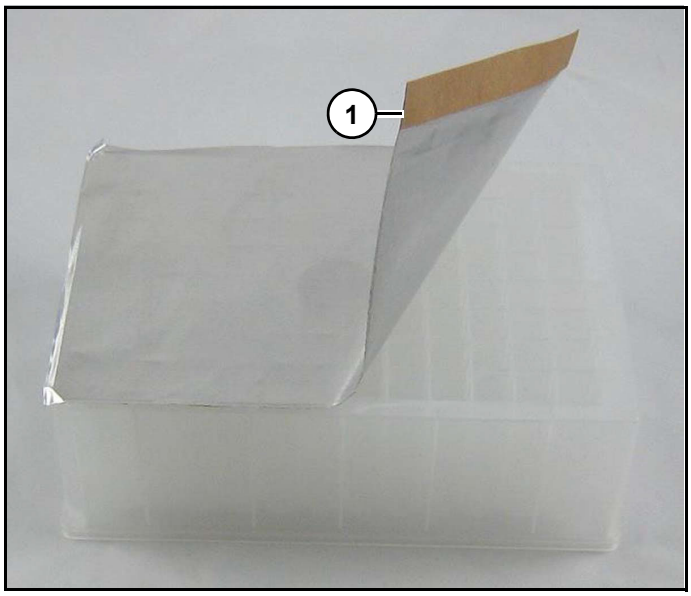

Figure 117

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

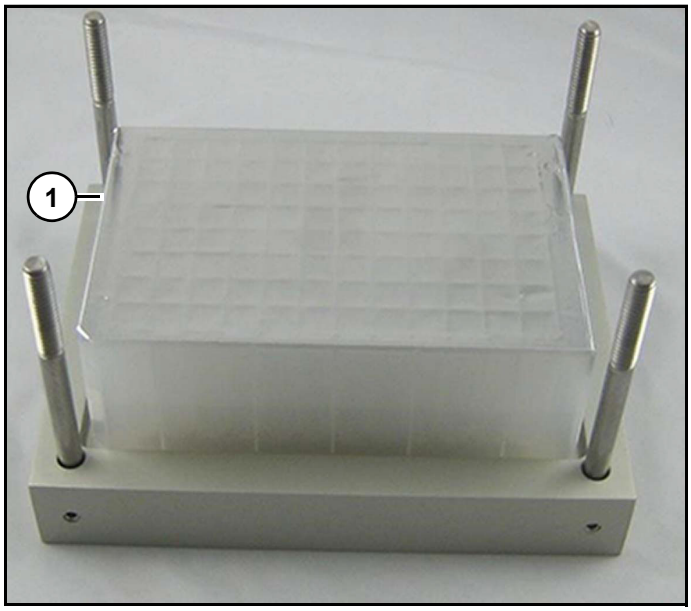

Figure 118

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

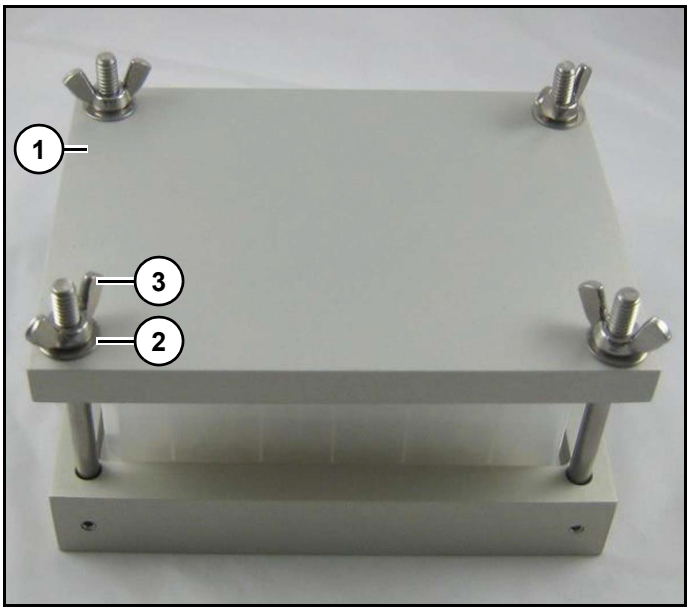

Figure 119

Refer to *(Figure 119)*.

4. Place the silicone spacer mat (not shown) *(Figure 119)* 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

Figure 120

Refer to *(Figure 120)*.

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

MerMade 192X 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, 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)	
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 desire 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.

Long-term instrument shutdown procedure

1. Attach fresh bottles of anhydrous Acetonitrile to each monomer and reagent bottle position on instrument.
2. From Test I/O Screen, flush all injection lines with Acetonitrile by turning on a few injection line locations at a time and allowing liquid to flow out for a few minutes.

Note: Be mindful, if using Test I/O screen, to only turn on a few lines at a time to avoid blowing a fuse.

Note: Verify that vacuum pump is on and waste tray valve is open.

3. Once all lines have been thoroughly flushed with Acetonitrile, inject Acetonitrile into both plate positions utilizing a plate filled with columns to avoid spills.
4. Drain waste tray thoroughly to not leave liquid in drain lines.
   Note: Verify that vacuum pump is turned on and waste tray valve is open.
5. Repeat above step 2-3 times to ensure drain lines are flushed with Acetonitrile.
6. Hold 'Drain' button down in Vacuum pump calibration screen until liquid evacuates drain lines.

Note: Verify that vacuum pump is turned on and that plates are clamped down

7. Place empty bottles in all monomer and reagent bottle locations on instrument.
8. From Test I/O flush all injection lines by turning on a few injection line locations at a time and allowing liquid to flow out for a few minutes. This will evacuate all remaining Acetonitrile from liquid lines.
9. Once all liquid lines and drain lines are completely empty, evacuate any waste remaining in waste container(s).
10. Evacuate all synthesis waste from container into a secondary waste location. Thoroughly rinse container(s) with Acetonitrile.

11. Open container(s) and allow container(s) to air dry in a fume hood or by utilizing a snorkel system.
12. Shut off Argon or Nitrogen to instrument and at gas tank.
13. Gently loosen and remove all monomer and reagent bottles to relieve gas pressure.
14. Disconnect all tubing from gas tank to instrument.
15. Disconnect or coil and zip tie any ventilation tubing that was previously routed to a snorkel system or fume hood.
16. Disconnect reagent bottle caps from instrument if needed for storage or transport.
17. To remove reagent bottle caps, unscrew liquid lines attached to reagent manifolds using an adjustable crescent wrench. Gently press on gray portion of gas line fitting and pull gas line out of fitting.
18. Gently coil reagent bottle cap tubing and secure it with a zip tie while being mindful to not bend of crease tubing. This can be done if removing bottle caps or leaving them attached to the instrument.
19. Empty monomer bottles may be place on instrument depending on storage needs.

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. 2. Check circuit breaker. Activate the circuit breaker if necessary. 3. Check fuses at plug. Replace fuses if necessary. 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. 2. If Dispense Head cannot be moved away from obstacle, disconnect power to instrument. 3. Contact LGC Field Service for additional support.
Instrument has stopped moving.	E-stop pressed.	1. De-press E-stop. 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. 2. Listen for the vacuum valve click on and off. 3. Check for bent drain lines. 4. Check for debris in drain lines. Ex: Crystalized amidite Use a guitar string to unclog the lines. Rinse thoroughly Important: Ensure the clog doesn't move to and get stuck in a cross, manifold, or valve. 5. Change the drain lines Important: Ensure new drain lines are the same length as the old lines to keep drain calibration the same. 6. Replace the vacuum valve 7. Check the manifold for debris or burrs. 8. Trace the problem from vacuum to vacuum. 9. Contact LGC Field Service for additional support.

System	Cause	Solution
Bad synthesis		1. Check all valves – ensuring that all fire as they should. 2. Confirm that instrument has not run dry. 3. Check all gauges – are normal/expected. 4. Check the calibration If outliers are found, check for broken positions. 5. If no leaks are found, replace the valve. 6. Check the vacuum calibration. 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. 2. Check o-rings in all amidite positions and reagents. 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. 4. Contact LGC Field Service for additional support.
Instrument loses alignment		1. Tighten screws on the limit detectors 2. Contact LGC Field Service for additional support.

System	Cause	Solution
Motor error		1. Ensure instrument is connected to the computer. 2. Ensure power is on to the instrument and that power connections are well seated. 3. Check if the liquid sensor is tripped. 4. Ensure that the safety interlock is functioning correctly. 5. Review the Copley logs. 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 3600 Minnesota Street 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/

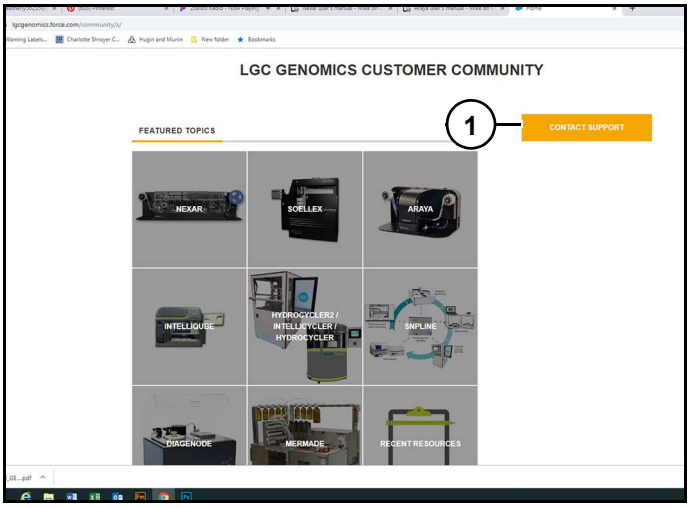

Figure 1

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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