Original instructions

MerMade 6 & 12 user's manual

GEN/0627/MW/0225

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

BIOSEARCH™ TECHNOLOGIES

Documentation disclaimer

LGC Biosearch Technologies™ desires to ensure the accuracy and confidentiality of its documentation, including without limitations to print or electronic versions of assembly and electrical drawings, electrical programmes, operator manuals, service manuals, job aids, and bill of materials. Unauthorised use of any kind, including without limitation to modifications, reformatting, or transfer of information other than the first user (the buyer) is not permitted without the express written consent of Biosearch Technologies. Any unauthorised use may void the instrument's warranty. All drawings and designs are and shall remain the property of Biosearch Technologies. No rights or obligations other than those expressly recited herein are to be implied from this agreement and no license hereby granted.

www.biosearchtech.com

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.

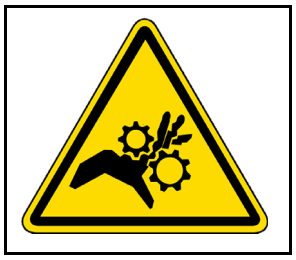

Figure 3

Warning - Pinch Point *(Figure 3)*.

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

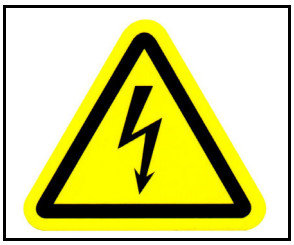

Figure 4

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.

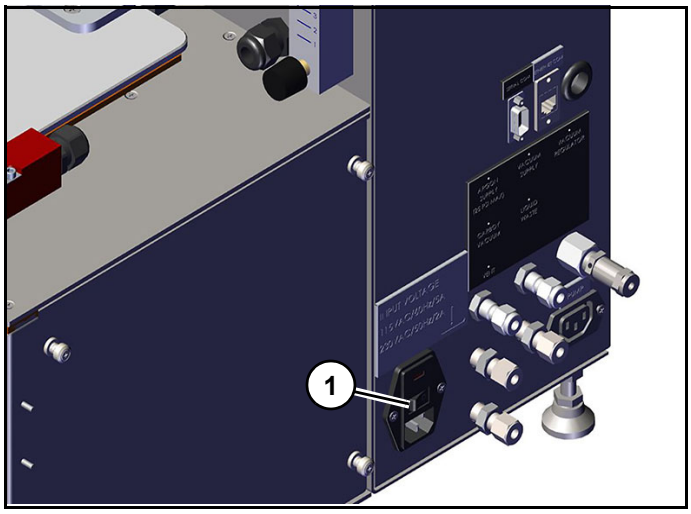

Figure 7

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 6 & 12 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

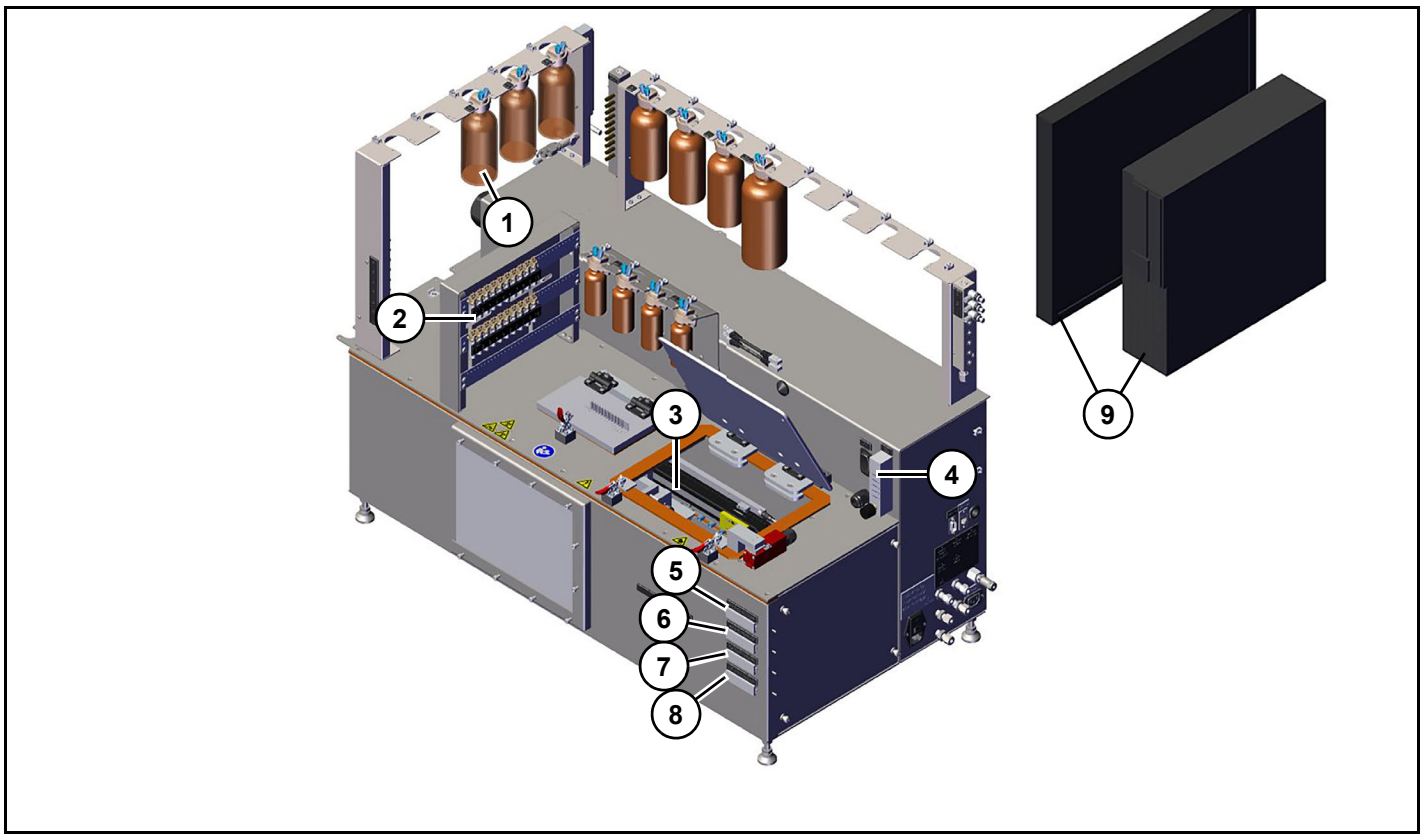

Figure 1

REF#	DESCRIPTION	REF#	DESCRIPTION
1	250 (ml). 30 (ml), 450 (ml), 15 (ml) bottles	7	Reagent Pressure
2	Dispense Valves	8	Column Vacuum
3	Column Rack	9	PC and Monitor
4	Flow Meter	NS	Drain Lines
5	Source Pressure	NS	Injection Lines
6	Amidite Pressure		

Component identification side panel

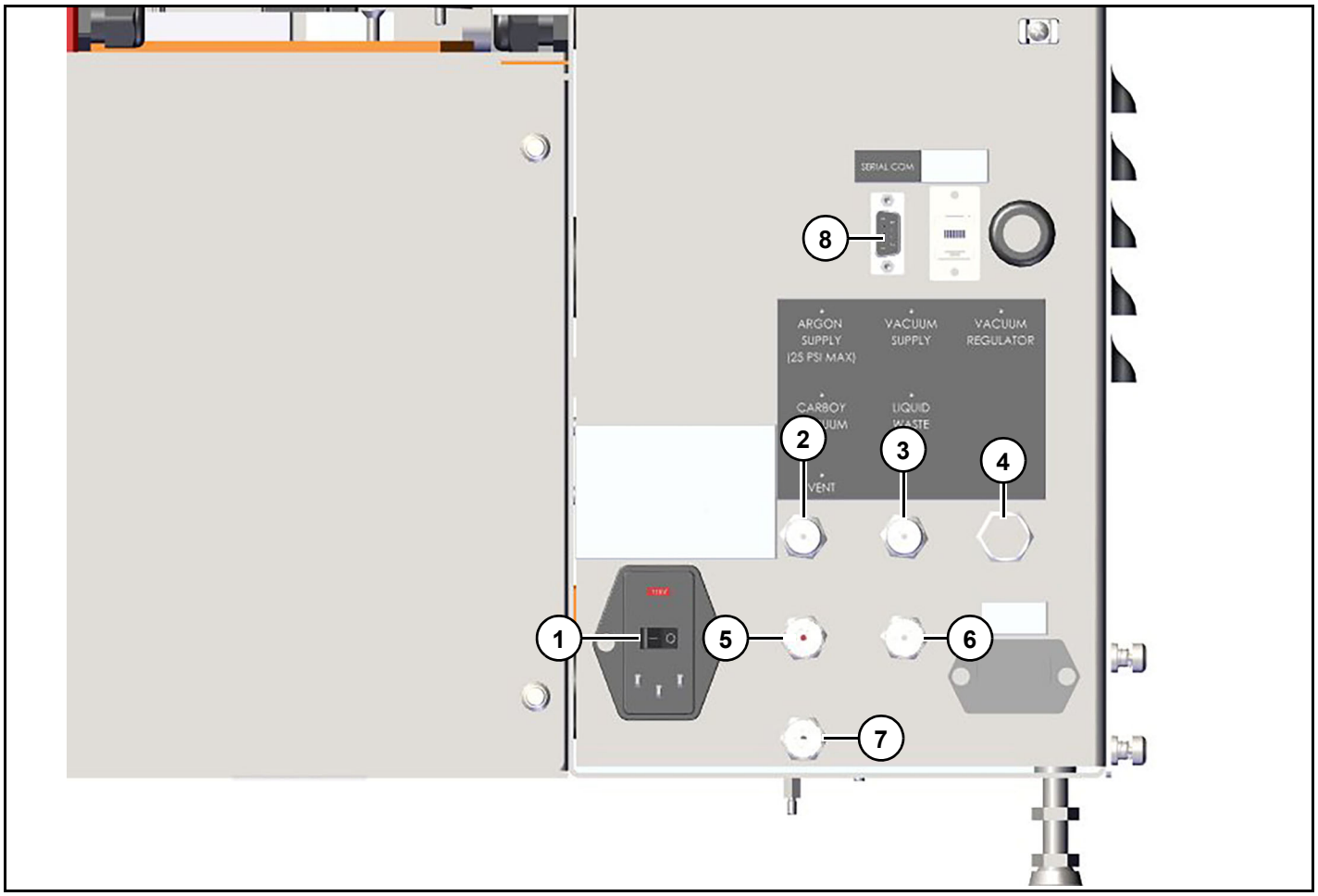

Figure 2

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Power Switch	5	Carboy Vacuum Connection
2	Argon/Nitrogen Supply Connection	6	Liquid Waste Connection
3	Vacuum Supply Connection	7	Vent Connection
4	Vacuum Breaker	8	Serial Port

MerMade 6 & 12 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

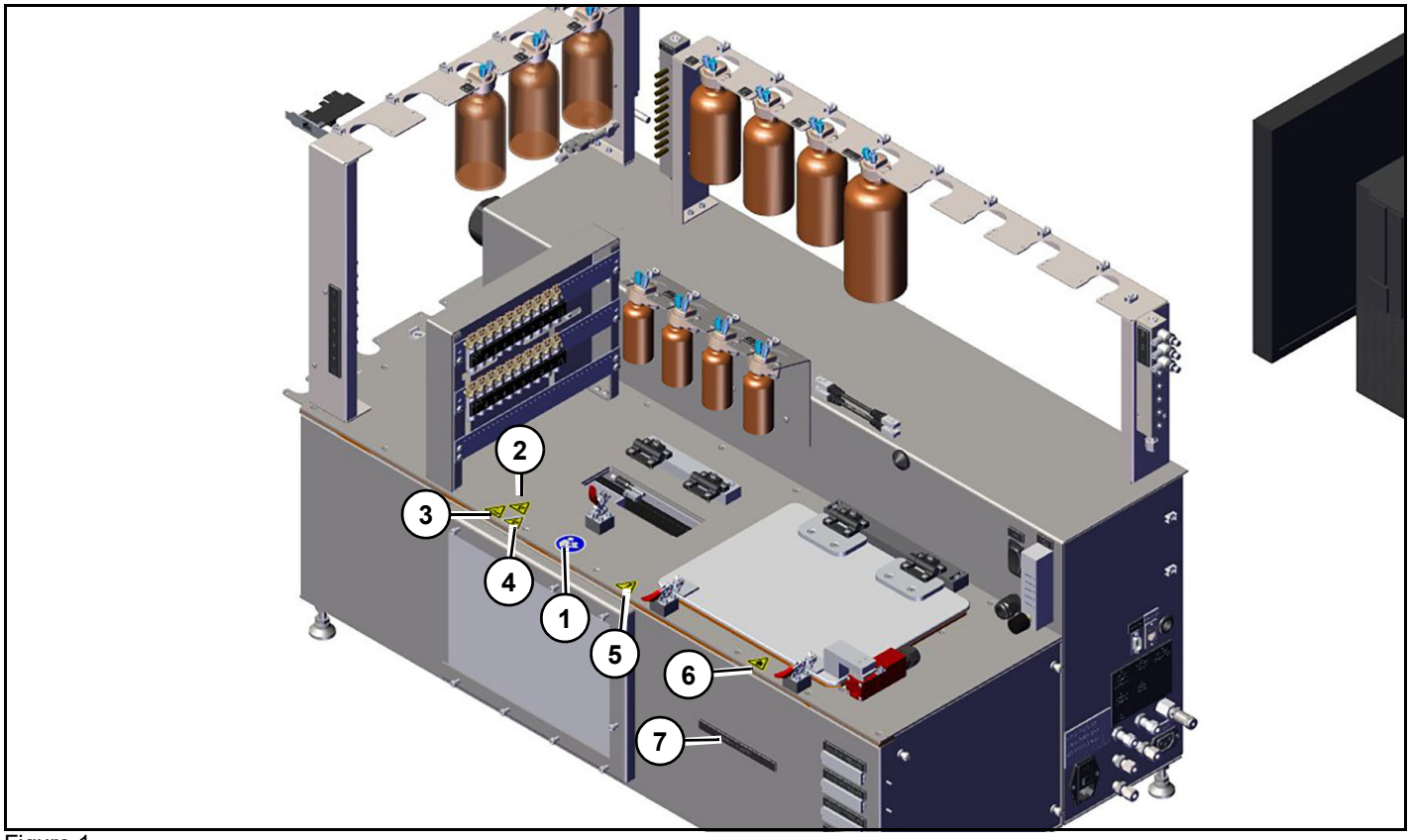

Figure 1

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Biological Hazard -Top of instrument deck (Qty-1)	5	Warning Exclamation Point -Top of instrument deck (Qty-1)
2	Read Owner's Manual -Top of instrument deck (Qty-1)	6	Pinch Point/Hand Crush Decal Top of instrument deck (Qty-1)
3	Toxic Material -Top of instrument deck (Qty-1)	7	Instrument Identification Front Panel (Qty-1)
4	Corrosive Substance -Top of instrument deck (Qty-1)		

Decal identification back panel

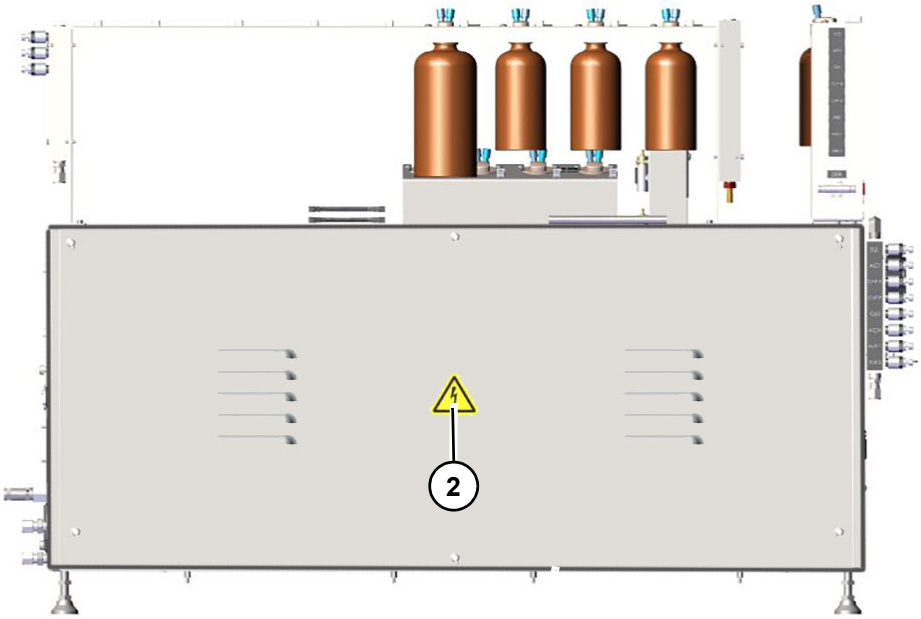

Figure 2

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

MerMade 6 & 12 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 12 is a single axis (x only) bench top syntheziser and needs to be located so there is adequate space to access front and sides to allow unrestricted flow through gas and vacuum lines. There must be sufficient vertical clearance to allow for a ventilation system to vent any hazardous fumes that may be present when lid is open or reagent bottles are being changed.

Safety requirements

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

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

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

Space requirements:

Width

- 90 cm (42")

Height

- 72 cm (28")

Depth

- 62 cm (24")

Weight

- 45 kg (100 lbs)

Clearance requirements:

Left side

- 31 cm (12")

Right side

- 31 cm (12")

Back

- 31 cm (12")

Front

- Access required at all times

Ventilation requirements:

⚠WARNING

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

For safe operation one of following must be installed.

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

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

There are four sources of fumes:

Vent line.

Vent line is located on right side of instrument. This is necessary because Synthesis Chamber is sealed and a 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 Vacuum Chucks. User is exposed to any fumes that may have accumulated inside chamber. Particularly a concern when a run has just completed since fumes will still be concentrated inside syntheziser. 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 syntheziser 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.

Environmental conditions

Temperature

- 5° C to 25° C(41 °F to 77 °F)
- Out if direct sunlight

Relative Humidity

- 40-60% humidity at 25 °C - 35 °C

Pollution Degree: 2

Altitude RangeTested

- to 8200 ft (2500 m) above sea level

Installation Category: 2

Mains Supply Fluctuations

- 115VAC/230VAC

Electrical requirements

Table specifies electrical operating requirements for instruments in various locations:

Location	Voltage	Amps	Frequency
USA/Canada	115 VAC	4A	60Hz
EC	230 VAC	2A	50Hz
Japan	115 VAC	4A	60Hz

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

Fuses:

- Main disconnect: 2 x 10A
- Power Supply 10A
- Terminal blocks: 1 x 2A & 1 x 3A

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 during operation. 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:

- Regulating range: 0-30 psi
- Temperature Range: 40 °F - 150 °F (4 °C - 66 °C)

Regulators rated outside these specifications may damage regulators on instrument.

Note – Inlet pressure is not depending on system. Customer's tank or house system needs to be regulated down to the range needed.

Gas supply.

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

Collection tubes.

Collection vials are needed for post processing run 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, product will need to dried down so it can be re-suspended in appropriate media. Please consult Biosearch Technologies for help in choosing an appropriate unit for application.

Uninterruptible Power Supply (UPS).

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

Reagent delivery system

Instrument uses solenoid valves to deliver reagents, under argon 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. Standard protocols on both systems operate under following conditions:

- Source Pressure Setting = 20 PSI
- Reagent Pressure Setting = 6 PSI
- Amidite Pressure Setting = 6-8 PSI
- Vacuum Regulator Setting = -5 inHg

Synthesis chemicals

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

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

Recommended Chemicals for Synthesis:

Reagent	Formulation
Acetonitrile	<10ppm
Deblock	3% DCA in DCM
Cap A	THF/Lut/Ac_2O (8:1:1)
Cap B	16% Methylimidazole/THF
Activator	0.25M ETT in ACN
Oxidiser	0.02M I_2 in THF/Pyridine/H_2O 70/20/10 (w/v/v)
Amidite	1g in 20ml 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/ nitrogen filled chamber. Please contact Biosearch Technologies if help is needed preparing reagents

Acetonitrile.

Acetonitrile is available in a range of sizes and quality. To ensure optimal quality in final product, an acetonitrile with a water content of not less than 70ppm for amidites should be used. Biosearch Technologies recommends less than 10ppm.

Acetonitrile in 4L bottles 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.

If using standard columns with first base derivatised offer additional benefit of being color coded to reduce chance of loading the 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 100μmole 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 vacum-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.

Shut down

Period of inactivity (more than a few days)

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

MerMade 6 & 12 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 12 Oligonucleotide Syntheziser is designed to synthesise up to 12 columns of oligonucleotides in a single run using standard or modified phosphoramidite chemistry. MerMade 6, is limited to six column positions.

Each column may be assigned a different protocol to permit synthesis of oligos of different quality and yield within same run. Run may be paused to allow addition or removal of columns while syntheziser is running. 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 200micromole for each column.

Typical applications for oligos include 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. During synthesis, user can also monitor removal of trityl when instrument is equipped with optional monitors. Log files are generated for each column and report all synthesis parameters and events.

During setup process, columns are placed in appropriate column chucks 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, slide will move to align each injection pin over appropriate column and dispense reagent by actuating valve(s). This is repeated for each reagent in turn.

Hardware

Computer provides user with an interface to syntheziser. Communication between computer and hardware is established 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 individual column chucks which are then mounted onto a single axis slide which moves to align different reagent injection pins with each active column in appropriate order. A vacuum is applied to each column in such a way that it may be drained independently of other columns. 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.

Software and instrument operation

A PC running Poseidon synthesis software provides user interface for controlling syntheziser. 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, three log files are created for each block used in synthesis:

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

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

Trityl Log. Files are collections of all raw data from trityl monitors that are collected during Deblock steps in a synthesis. A folder is created for every column in a run and a file is created with trityl data for each cycle. This data is interpreted in software under the "Show Trityl Data" screen.

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.

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)*.

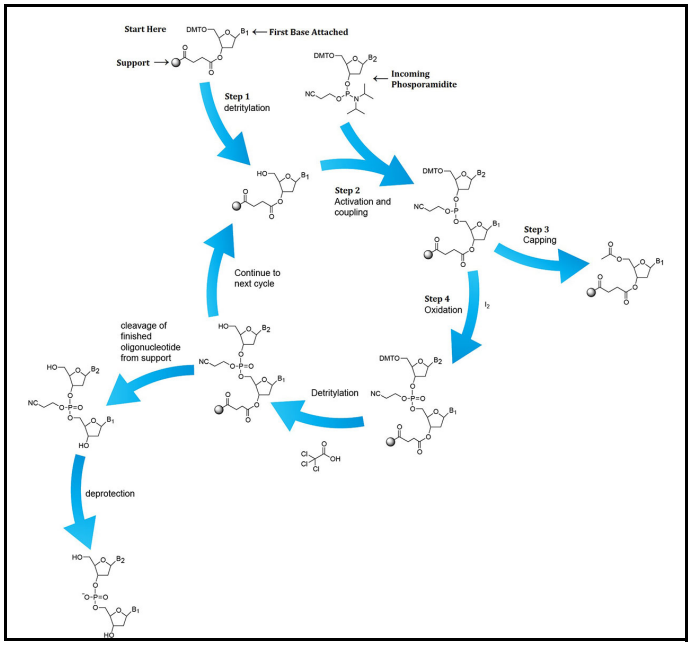

Figure 1

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)*.

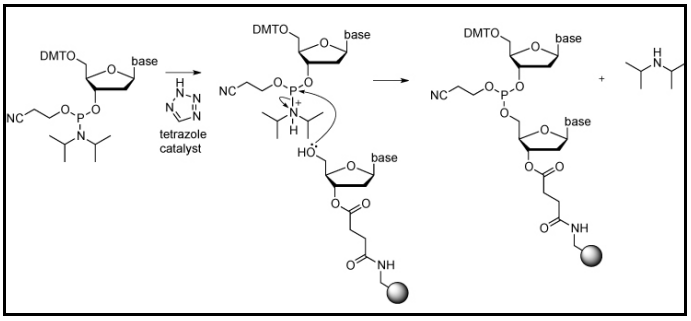

Figure 2

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)*.

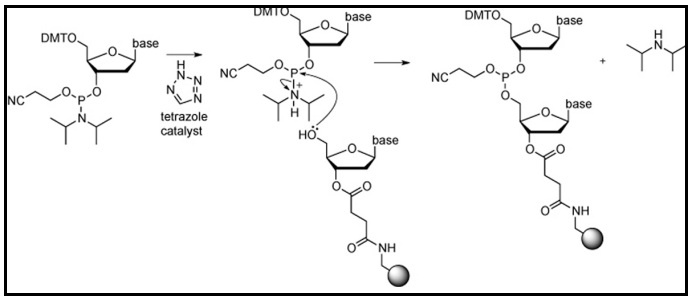

Figure 3

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)*.

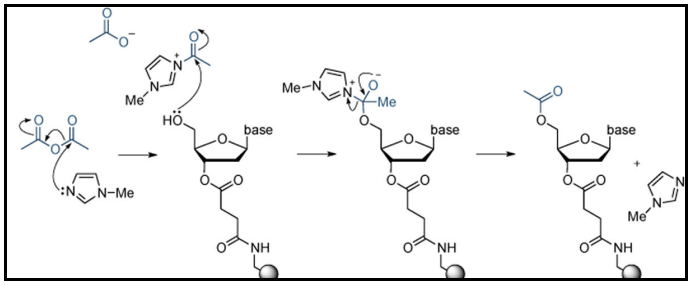

Figure 4

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)*.

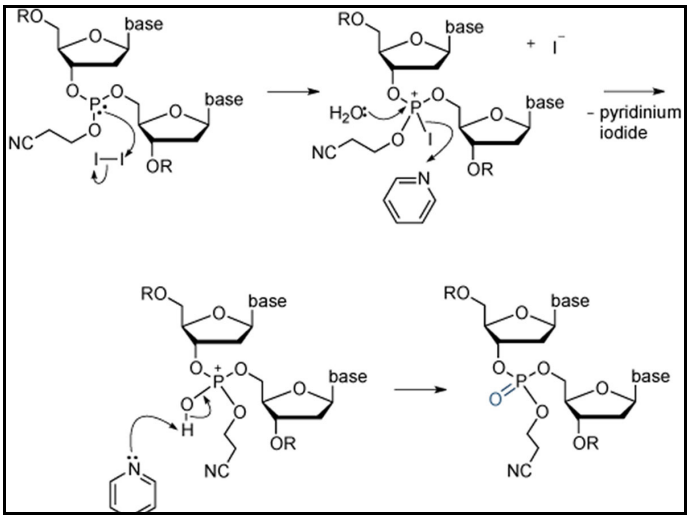

Figure 5

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 6 & 12 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 start-up 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.

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

Example of Block Log *(Figure 1)*.

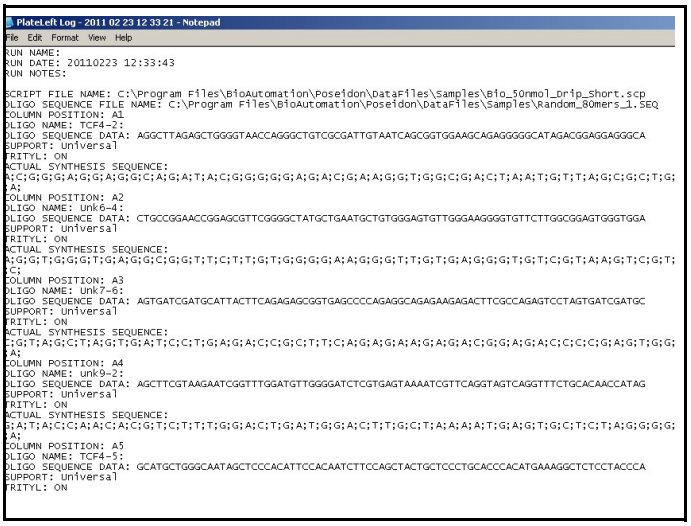

Figure 1

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

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

Synthesis process

Synthesis is typically carried out from 3'->5' end of oligonucleotide. Reactions take place in columns and are initialised using a controlled pore glass (CPG) or polystyrene substrate (PS) 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/nitogen to create a moisture free environment. When argon/nitrogen purge initialisation step is completed, software moves slide to align each column well under appropriate injection line and actuates corresponding reagent valve or valves. When reagent injections are finished, software pauses for chemical reactions to complete inside columns.

Synthesis quality and yield depend on synthesis scale, 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 reaction block.

Once column is drained, underside of column(s) is equlised to pressure of synthesis chamber to prevent premature drainage of reagents during next injection cycle. After each stage, columns can be washed with acetonitrile several times to make sure all unused reagents are removed prior to next stage of synthesis. Number of washes required between each reagent addition will vary depending on desired quality and yield of the synthesis.
Example, in a synthesis generating a higher quality product for use in applications such as gene building, a higher number of washes is recommended after Deblock and Oxidisation steps since these reagents contain acid or water which will affect synthesis reaction and ultimately quality of final product. By modifying script files, user can easily create custom protocols to meet specific requirements.

Post-synthesis processing stage is a three-step process. First, oligonucleotide product is cleaved from solid support by application of a suitable reagent. Reagent will vary according to chemistry but typically ammonium hydroxide is used. Second, product is drawn through column, via vacuum, into a receiving vessel which is then sealed and then 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.

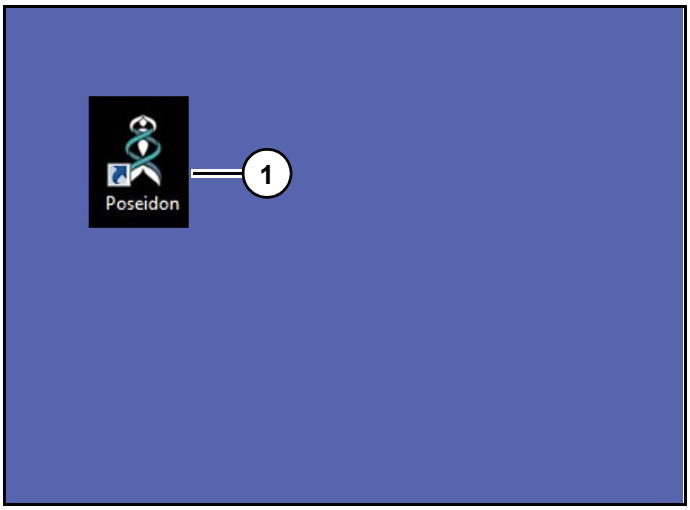

Figure 2

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

Initialisation screen

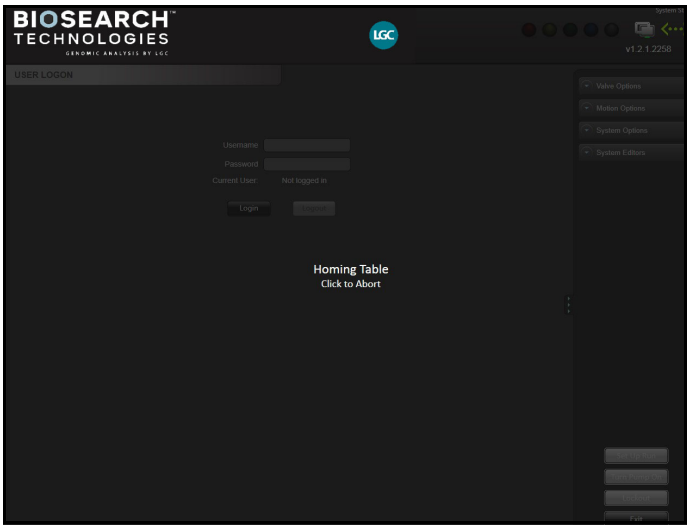

Figure 3

Initialisation screen *(Figure 3)* is shown after start-up 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

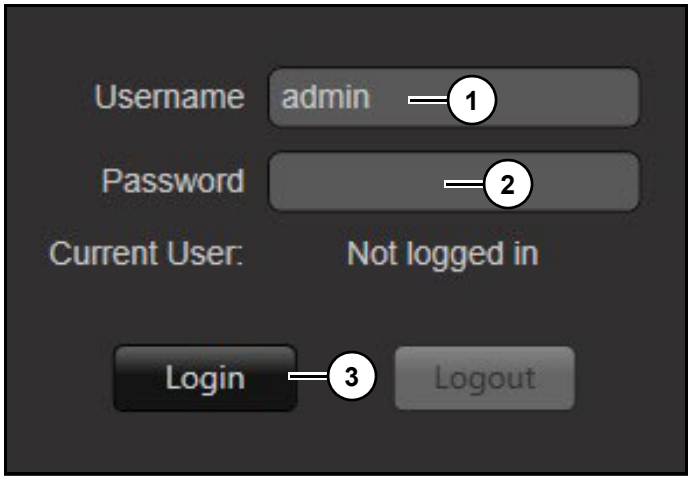

Figure 4

Refer to *(Figure 4)*.

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

Main screen

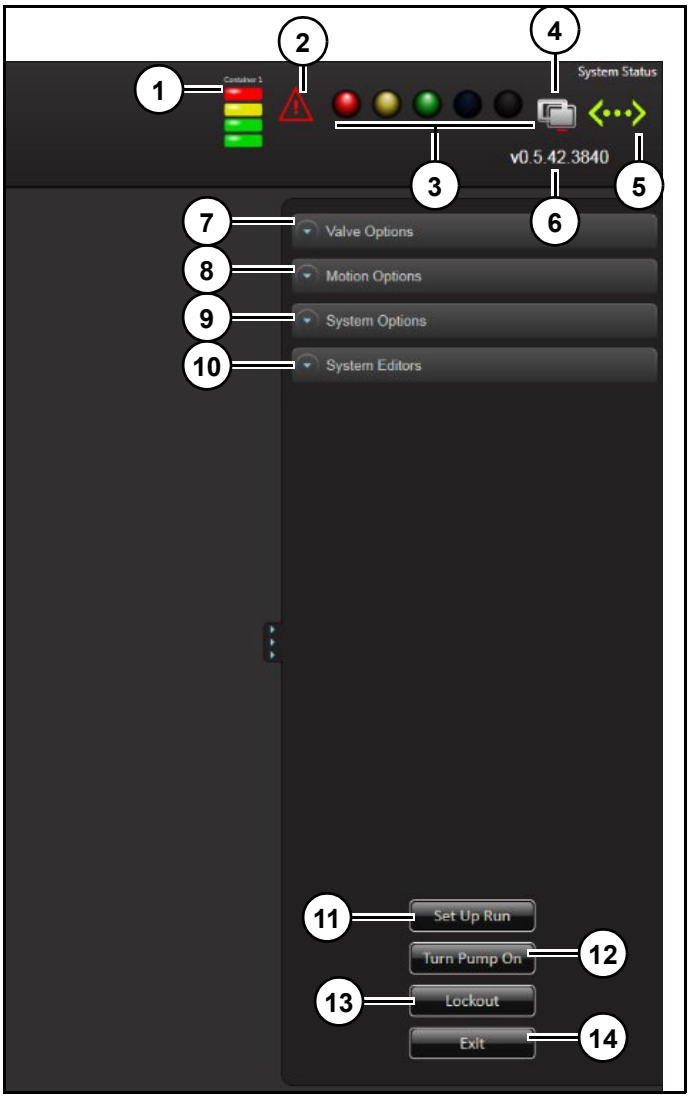

Figure 5

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.

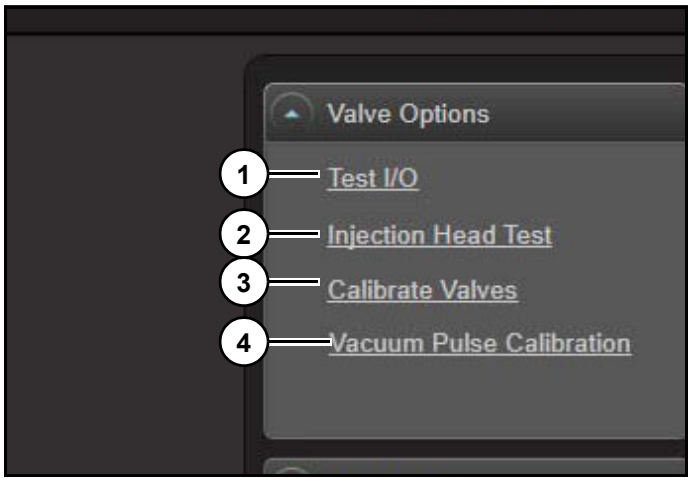

Figure 6

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.

Vacuum Pulse Calibration (4): 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.
2. Click button again to close valve.

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

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

Injection Head Test screen

Allows user to test and prime lines.

⚠WARNING

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

Always wear safety goggles and gloves when using this screen.

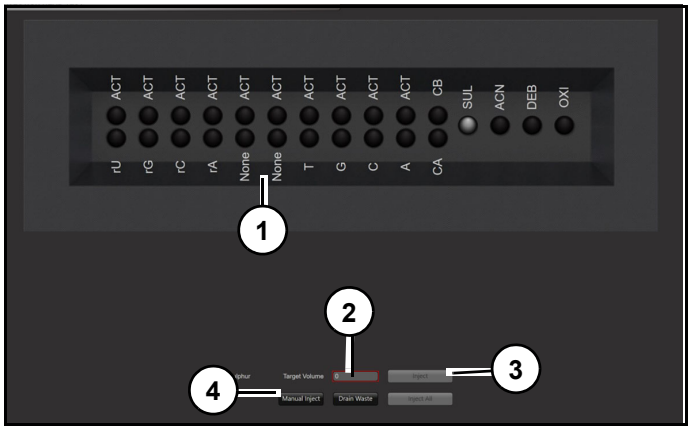

Figure 8

Refer to *(Figure 8)*.

To fire a single valve:

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

Note: 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.

Calibrate Values 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.

To ensure calibration accuracy, amidite/ reagent pressure must be kept stable. When calibrating, each valve must be calibrated individually. Valves can be calibrated three different ways: by time, by volume and by weight. Time option is selected by default.

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 these points.

Liquid valve delivery will be more accurate if more points are calibrated between these points. 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 line information created from the 2 closest calibration points for Aux 2 valve.

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

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

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

Calibrating valves

⚠WARNING

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

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

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

1. Collect injection with a small Eppendorf tube.
2. Measure actual delivered volume with a pipette or measure weight by scale and then adjust Injection Time of valve until correct volume is delivered.

Switching calibration methods

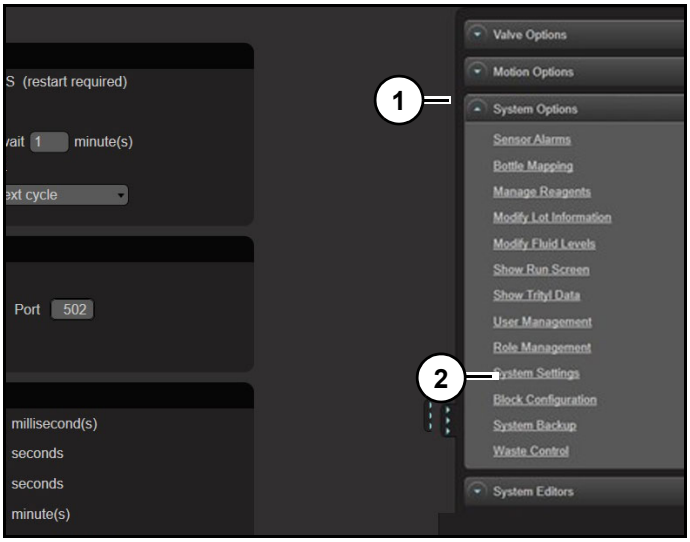

Figure 9

Refer to *(Figure 9)*.

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

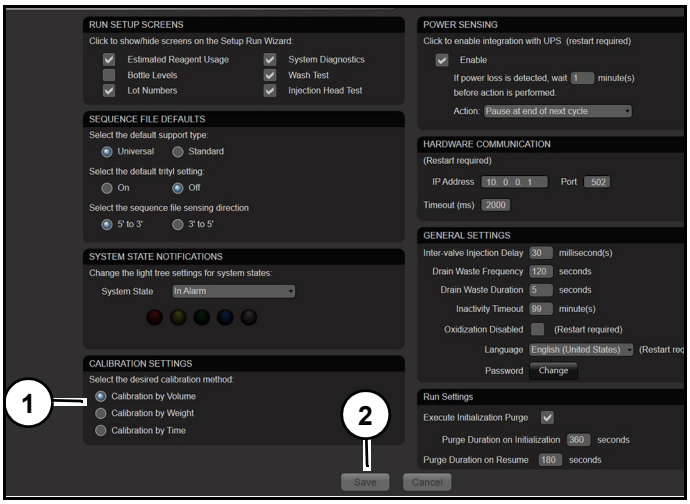

Figure 10

Refer to *(Figure 10)*.

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

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

Adding calibration points

⚠WARNING

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

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

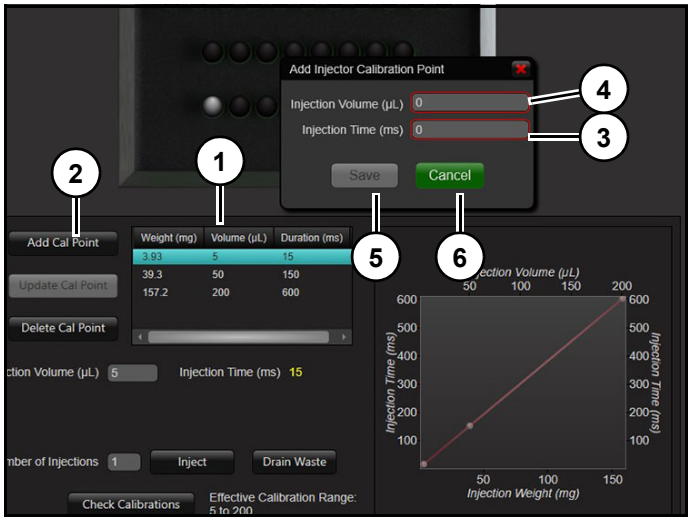

Figure 11

Refer to *(Figure 11)*.

In *(Figure 11)*, Aux 2 Valve has volume points for 5 µL, 50 µL, and 200 µL (1).

1. Click "Add Cal Point" (2).
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 12)*.

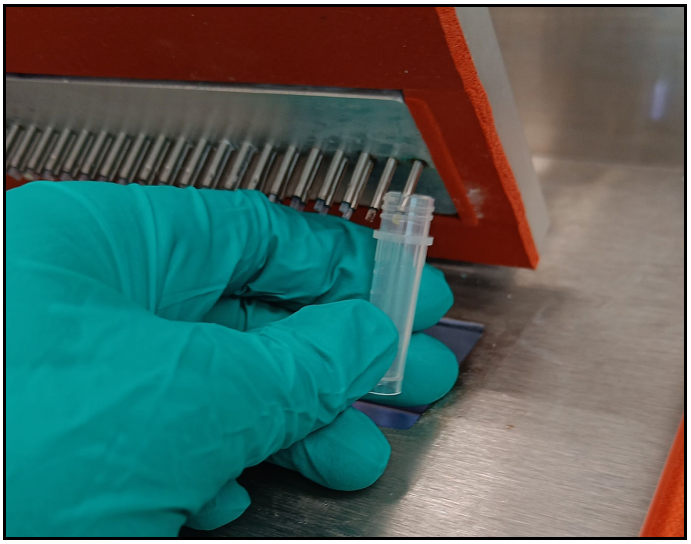

Figure 12

5. Place 2ml tube (1) under correct injection pin (2) with lid open. *(Figure 12)*

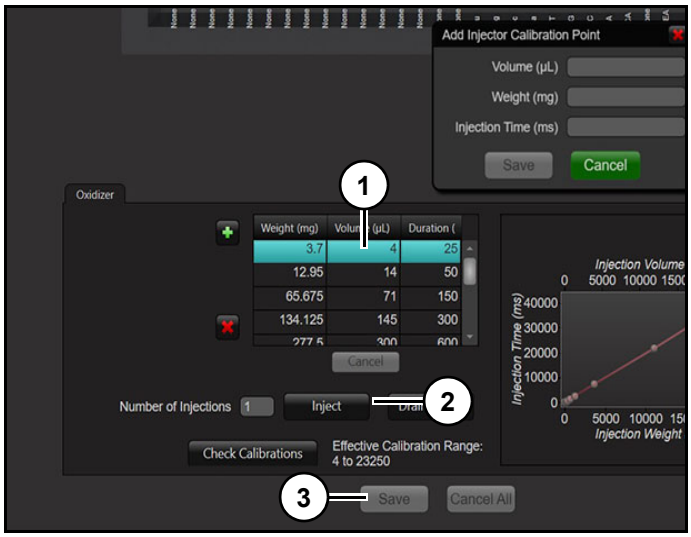

Figure 13

6. Click "Inject" (2) *(Figure 13)*. Instrument will open valve for 1200ms, injecting liquid into collection vile.

Figure 14

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 14)*

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)" (1) *(Figure 13)*.

9. Click "Save" (3) *(Figure 13)*. To calibrate another point, highlight appropriate line with a mouse click.

Updating calibration point

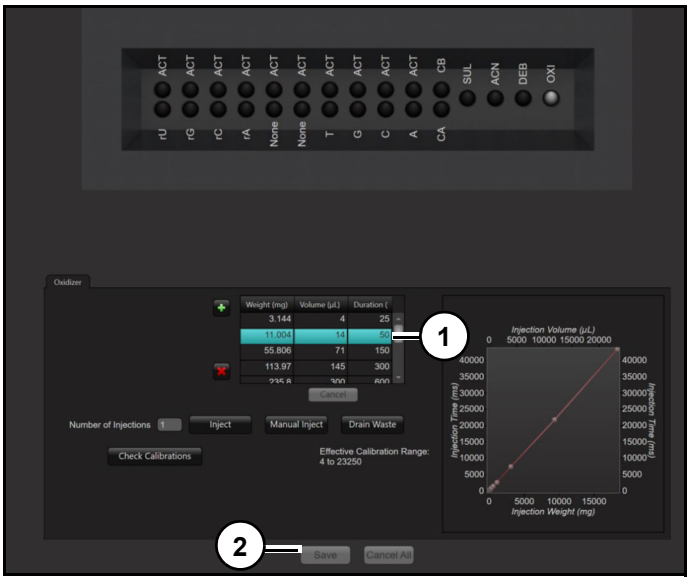

Figure 15

Refer to *(Figure 15)*.

1. Select line (1) to calibrate.

2. Update time/volume/weight higher or lower as needed to get correct volume.

3. Click "Save" (2).

Note: + is add point, X is delete point update is save.

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

Deleting calibration point

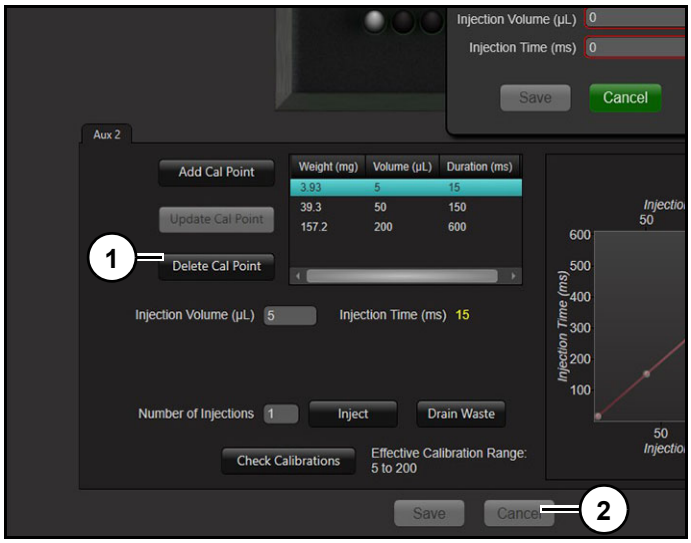

Figure 16

Refer to *(Figure 16)*.

1. Select calibration point to delete.
2. Click "Delete Cal Point" (1).

Note: If "Delete Cal Point" (1) is accidentally pressed, hit "Cancel" (2) undo delete. All changes since your last save will be lost.

Checking calibrations

Allows user to check calibration curve for each valve.

Figure 17

1. Click "Check Calibration" (1) *(Figure 17)*.

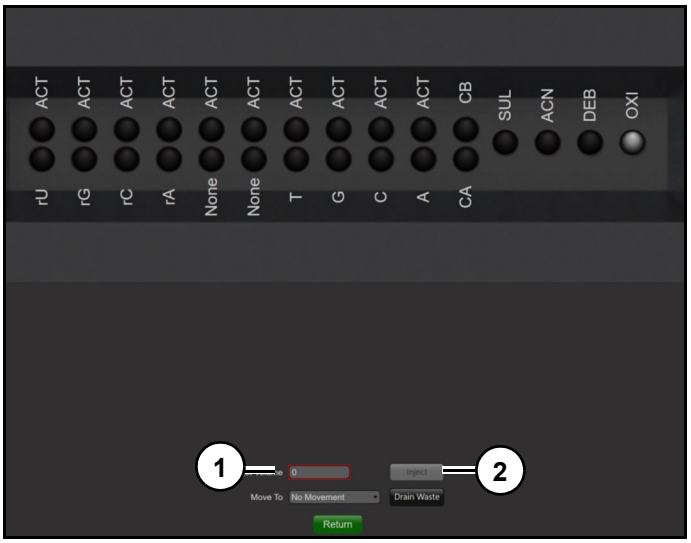

Figure 18

Refer to *(Figure 18)*.

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

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

Drain waste

Allows user to drain waste reagent from waste tray into waste tank.

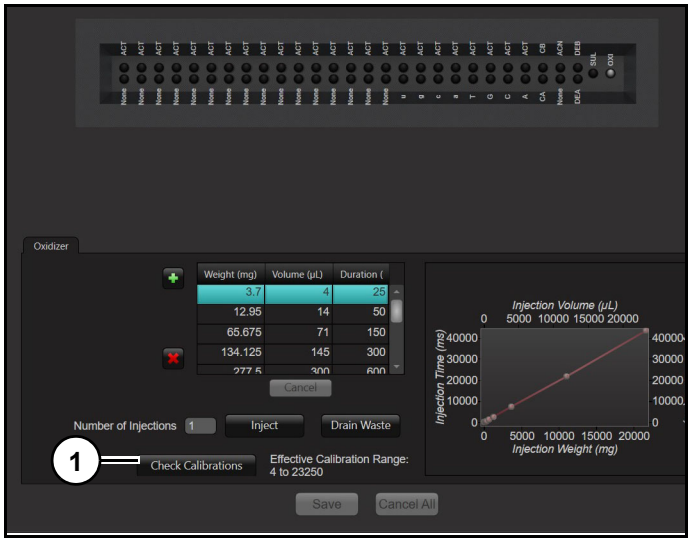

Figure 19

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

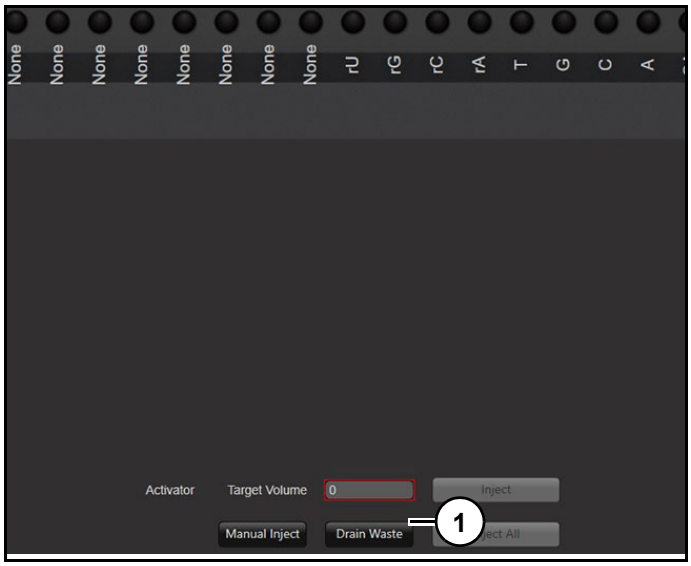

Figure 20

2. Click "Drain Waste" (1) *(Figure 20)*.

Vacuum Pulse Calibration screen

After instrument dispenses a reagent, software will use short 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.

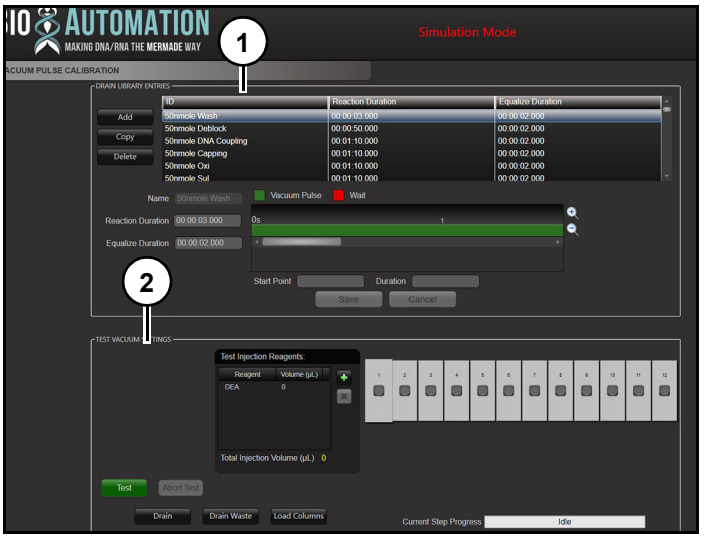

Figure 21

Refer to *(Figure 21)*.

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 existing drain ID's in drain library will need to be re-calibrated for new vacuum pressures.

Types of vacuum pulses

There is one type of vacuum pulse on instrument along with wait functionality.

Constant vacuum pulse.

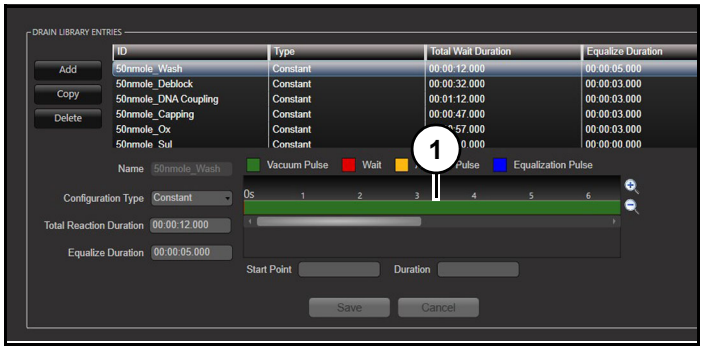

Figure 22

A constant vacuum pulse (1) *(Figure 22)* is a pulse that applies same length of vacuum to column chuck.

Vacuum Pulse Structure screen

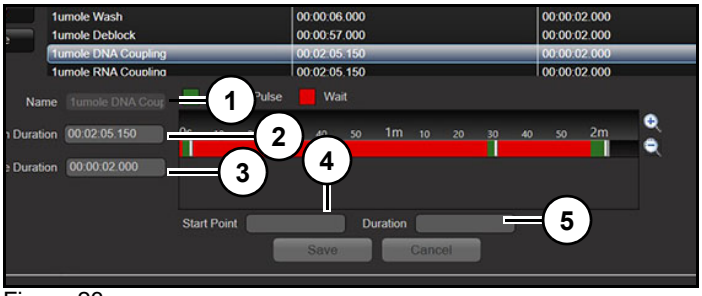

Figure 23

Refer to *(Figure 23)*.

Name (1): ID of specific drain.

Reaction Duration (2): Total time of complete vacuum step encompassing all wait times, vacuum pulses and final drain time.

Equalize Duration (3): Time after pulses are finished, needed for column to recover from residual vacuum so that system is ready for next vacuum pulse/drain.

Start Point (4): Starting time of currently selected vacuum pulse.

Duration (5): Total length of currently selected vacuum.

Red (waits), and green bars (pulses) are visually represented of selected drains. Any lengthy vacuum pulse placed at end of vacuum pulse box is called a final drain. This is to remove all reagents from column.

For instance, some modified amidites require a 12 minute reaction time which would be reflected by Reaction Duration (2). 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

Add Vacuum Pulse Here: Adds a vacuum pulse at selected location with a right mouse click. Will pull freah 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 drain 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 dependent on vacuum level set by vacuum breaker/regulator and synthesis scale. These levels will need to be adjusted if vacuum level changes.

Delete: Deletes current green selected pulse box.

Figure 24

Refer to *(Figure 24)*.

1. Right click on one of boxes (1).
2. Select a "Add vacuum pulse here" (2).

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

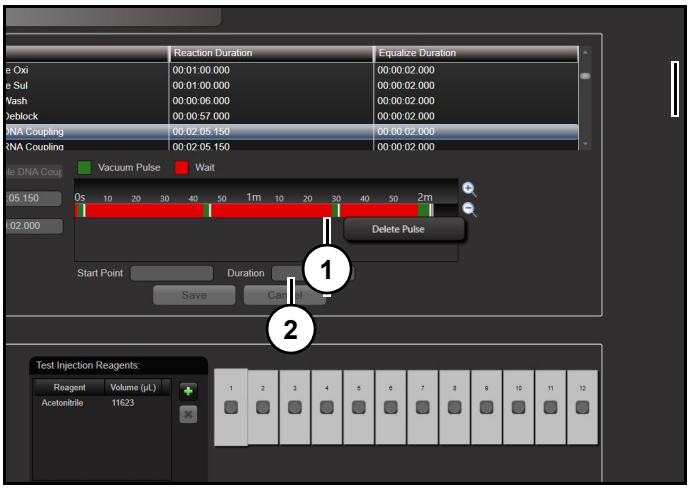

Figure 25

Refer to *(Figure 25)*.

1. Grab right edge of green 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 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 26)*.

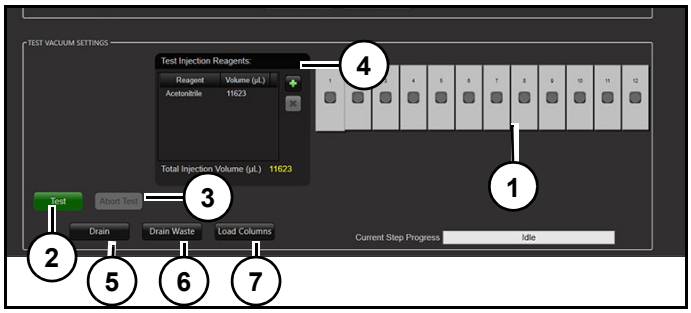

Figure 26

Refer to *(Figure 26)*.

Block (1): Choses column to be tested.

Test (2): Tests selected vacuum pulse setting for calibration verification. Includes injection of a specified reagent at a certain volume followed by selected vacuum pulse step. Allows user to test and adjust vacuum pulse settings to calibrate vacuum pulses so that reagents flow through column during reaction time and does not over or underdrain. Once vacuum pulses are calibrated, screen can be used to make observations during a run in order to fine tune settings.

Abort Test (3): Aborts test during execution.

Test Injection Reagents (4): Allows user to add or remove type and/or volume of reagent(s) to be dispensed into columns.

Drain (5): Drains liquid from selected block before or after test.

Drain Waste (6): Drains waste tray.

Load Columns (7): Moves table forward so columns can be added or removed.

Testing vacuum pulses

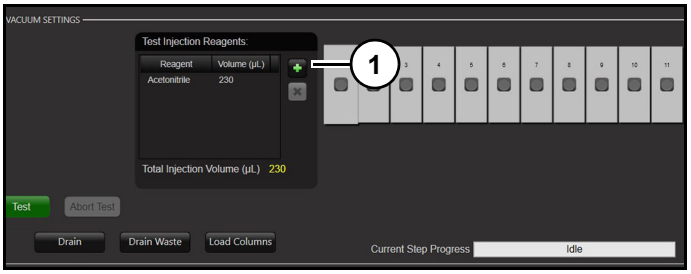

Figure 27

Refer to *(Figure 27)*.

1. Click "+" (1).

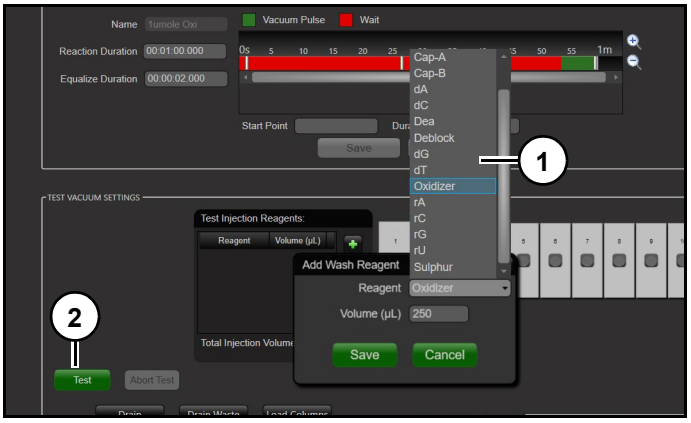

Figure 28

Refer to *(Figure 28)*.

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

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

3. Click "Test" (2).

Motion Options

Allows user access to motion options (1) *(Figure 29)* including, Set Reference Point, Set Table Parameters, and Motion options.

Figure 29

Select Reference Positions

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

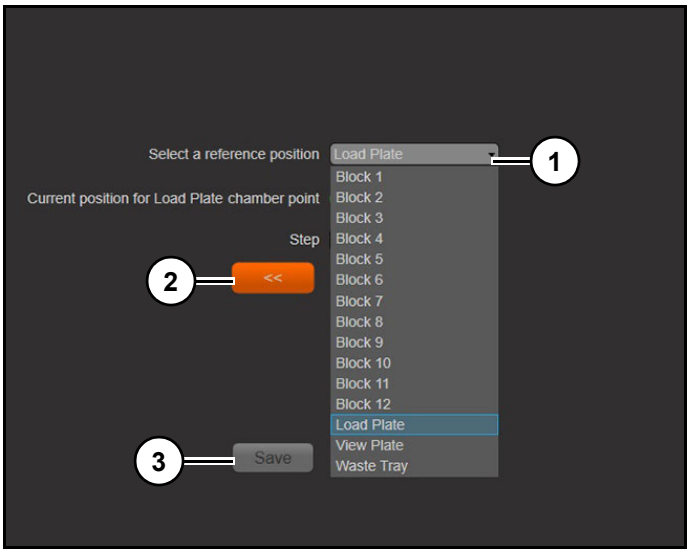

Figure 30

User tells instrument where to start by aligning injection head. Alignment can be changes in Injection Head Align screen *(Figure 30)*.

To adjust injection head:

1. Select a position from "Select a reference position" (1).
2. Use "<<" or ">>" (2) to adjust the position.
3. Click "Save" (4) to save position.

Table should be moved in X directions in order to align column center under OXI on far right injection pin so injections enter columns without leakage.

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

Set Table Parameters

Determines speed at which slide will move during its' various operations. Values are set at factory and should not be changed unless instructed so by Biosearch Technologies. *(Figure 31)*

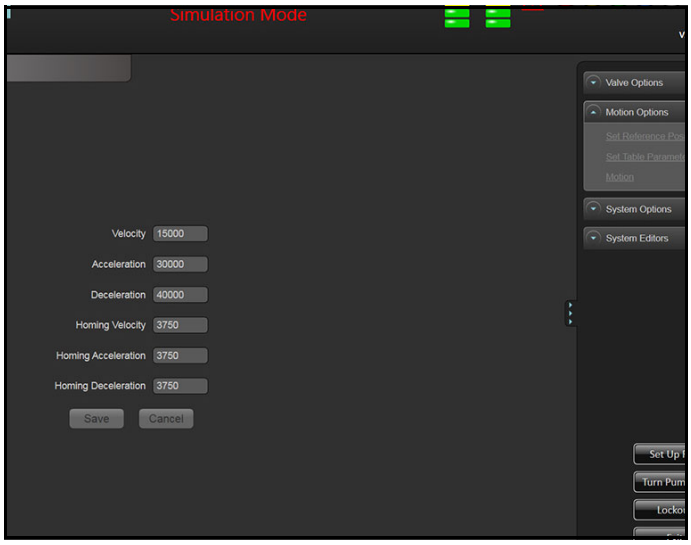

Figure 31

Motion

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

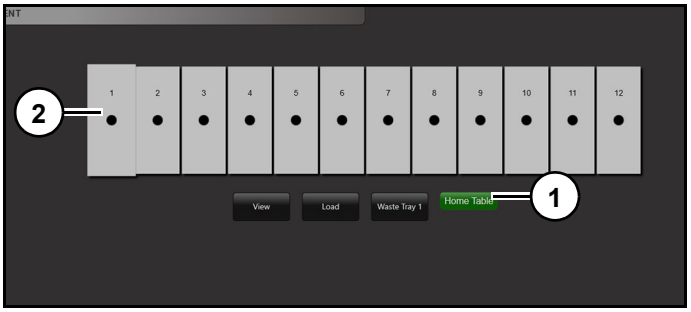

Figure 32

Refer to *(Figure 32)*.

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

Reference Points (2): Displays reference points. This will move selected column chuck to left-most injection position.

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

System Options

Allows user access controls involving system related options *(Figure 33)*:

- Sensor Alarms
- Bottle Mapping
- Manage Reagents
- Modify Lot Information
- Modify Fluid Levels
- Show Run Screen
- Show Trityl Data
- User Management
- Role Management
- System Settings
- Block Configuration
- System Backup
- Waste Control

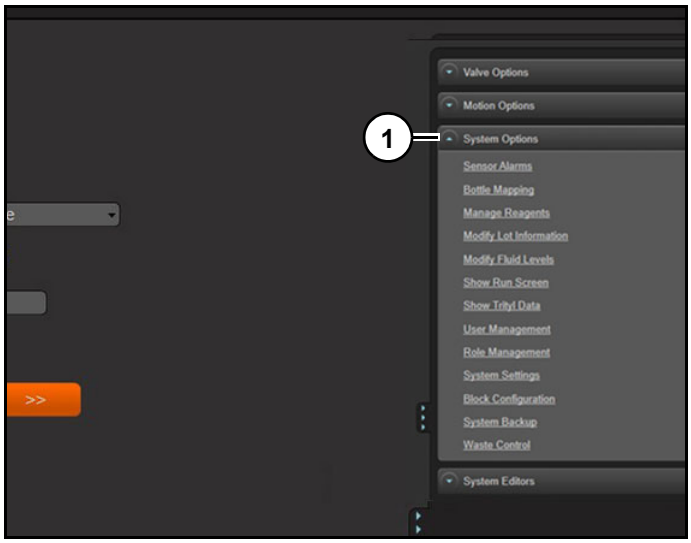

Figure 33

Sensor Alarms

Contains information relating to sensor information coming from instrument

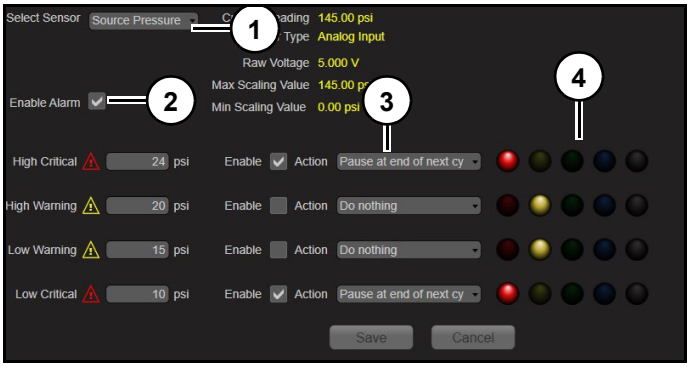

Figure 34

Refer to *(Figure 34)*.

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. Not recommended to change these from factory defaults as it can result in alarms not being detected. Please contact Biosearch Technologies before changing setting.

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

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

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

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

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

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

High Critical: Highest alarm.

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

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

Low Critical: Lowest alarm.

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

Interlock sensor

⚠WARNING

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

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

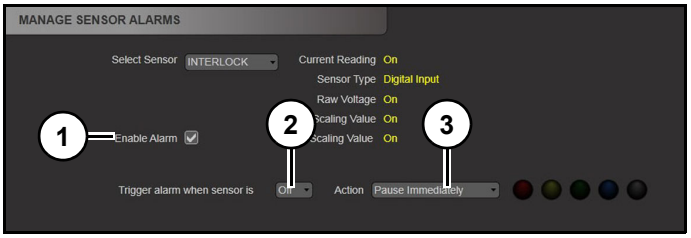

Figure 35

Refer to *(Figure 35)*.

Interlock sensor factory settings:

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

Liquid Sensor

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

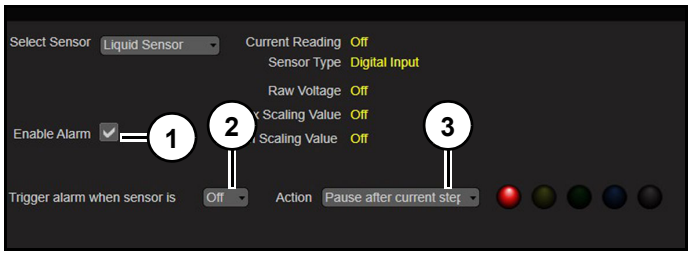

Figure 36

Refer to *(Figure 36)*.

Liquid sensor factory settings:

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

Source Pressure

Notice

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

Notice

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

Analog sensor that monitors source pressure feeding Monomer and Reagent regulators. Sensor measures pressure after client-side pressure regulator. Gas is supplied to instrument at no more than 23 psi via a customer supplied gas line to gas inlet of instrument. Gas is then used to feed Monomer and Reagent gas regulators where pressure is reduced further and is distributed to bottles. If source gas pressure exceeds 25 psi then relief valve will begin to leak.

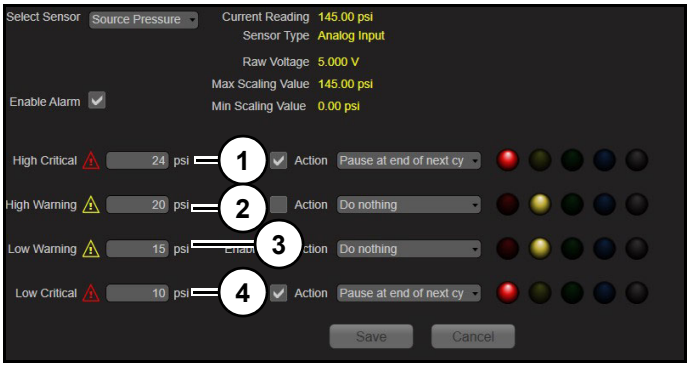

Figure 37

Refer to *(Figure 37)*.

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 client supplied gas regulator. Actual source pressure can fluctuate slightly without need to recalibrate valves, as pressure does not dip below operating pressures of monomer and reagent regulators.

Monomer Pressure

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

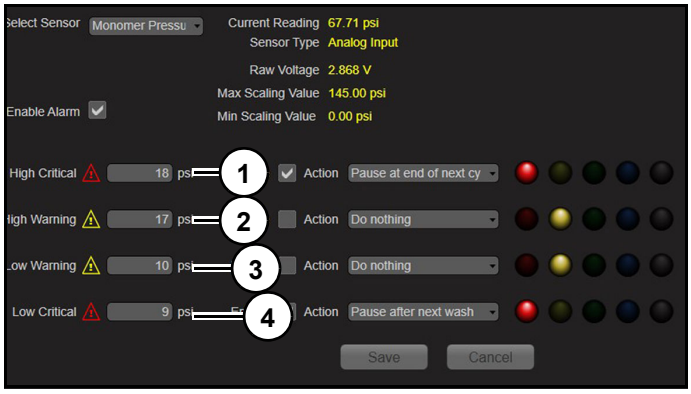

Figure 38

Refer to *(Figure 38)*.

Monomer sensor factory settings:

High Critical (1): 10 psi
High Warning (2): 8 psi
Low Warning (3): 4 psi
Low Critical (4): 3 psi

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

Reagent Pressure

Notice

Reagent bottle pressures can be set lower than 5 psi, but not higher than 6 psi because splashing may occur during reagent dispensing. Glass bottles greater than 10 L are not recommended as they can break easily while under pressure. Glass bottles should be inside of secondary containment and/or be plastic coated.

Monitors actual pressure of ancillary reagent bottles. Sensor typically includes all deblocks, oxidisers, capping reagents, and wash reagents. Confirm tubing on actual instrument if there is any question whether chemicals can be supplied by same regulator due to compatibility concerns. Recommended reagent pressure setting is 6 psi.

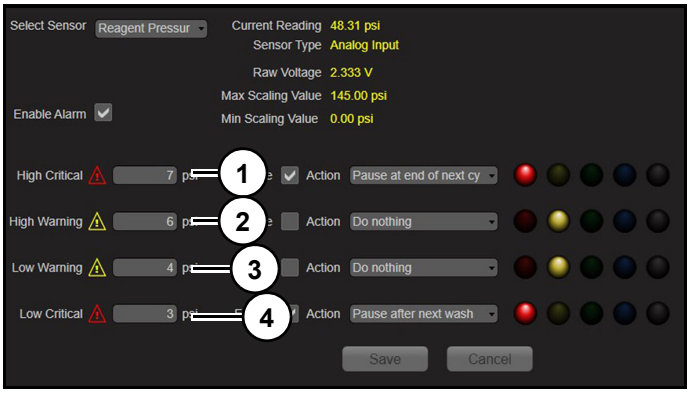

Figure 39

Refer to *(Figure 39)*.

Reagent sensor factory settings:

High Critical (1): 9 psi
High Warning (2): 8 psi
Low Warning (3): 3 psi
Low Critical (4): 2 psi

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

Regulated Vac

Monitors vacuum system during synthesis.

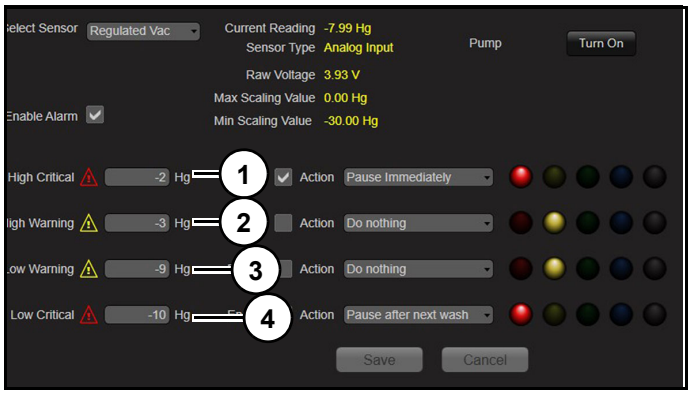

Figure 40

Refer to *(Figure 40)*.

Vacuum flow factory settings:

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

Vacuum level can be changed by adjusting vacuum breaker. 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 Hg. Very long drains, utilised often in a script file, can also cause vacuum level to drop during a run and should be avoided.

Purge Flow

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

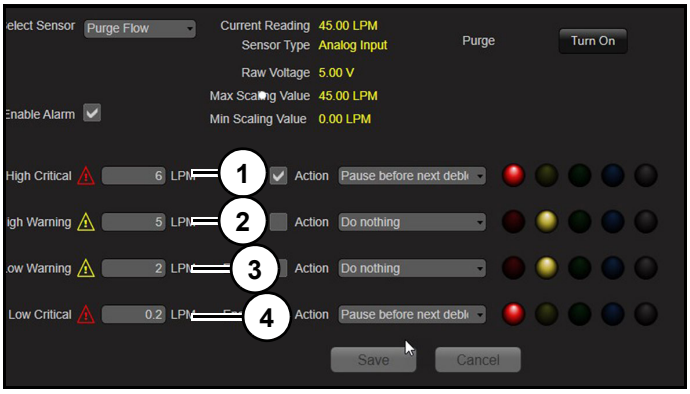

Figure 41

Refer to *(Figure 41)*.

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

UI displays an image of instrument and associated bottles and their positions. Clicking on a bottle will display name of reagent mapped to that bottle and actual position on injection head.

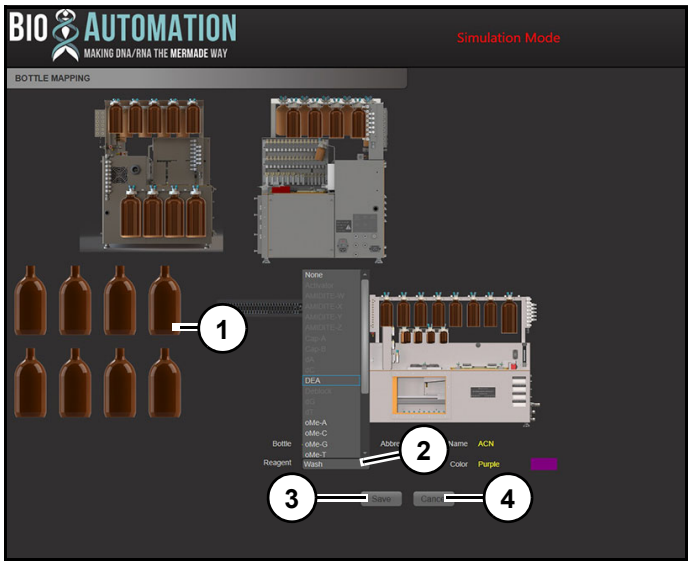

Figure 42

Refer to *(Figure 42)*.

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 synthesiser and new position of reagent changed in software using Bottle Mapping screen. When a bottle is selected, injection head preview will show user which injection line, or lines, on injection head will dispense reagent. Hovering over injection head image with cursor will enlarge display.

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:

- Cap A and Cap B should be mapped on same injection column. If not, there will be excess movements and synthesis will slow down.
- Reagents can be mapped to multiple bottles positions at same time.

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.

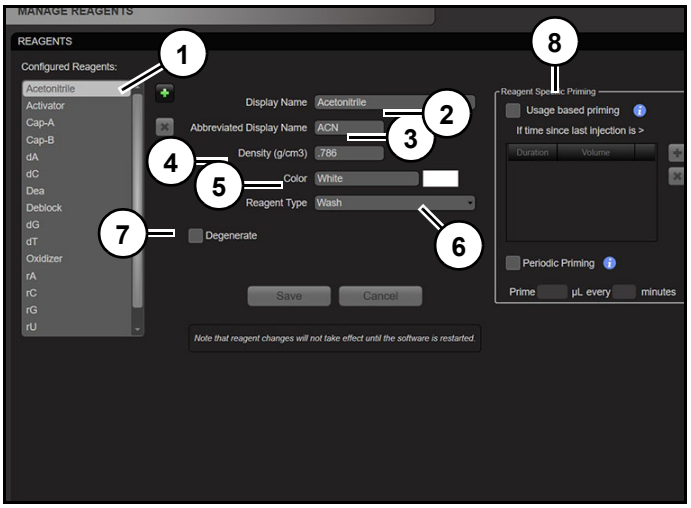

Figure 43

Refer to *(Figure 43)*.

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

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

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

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

Color (5): Color of reagent shown in column well display during a synthesis. HEX code of a color can

be used or standard Name for HEX color can be entered. Example: Red = #FF0000, either can be used.

Reagent Type (6): Type of reagent in bottle. Used to narrow choices when in script editor and other screens. It also acts as a contingency, so deblock cannot be used as an activator.
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.

Reagent Specific Priming (8): 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

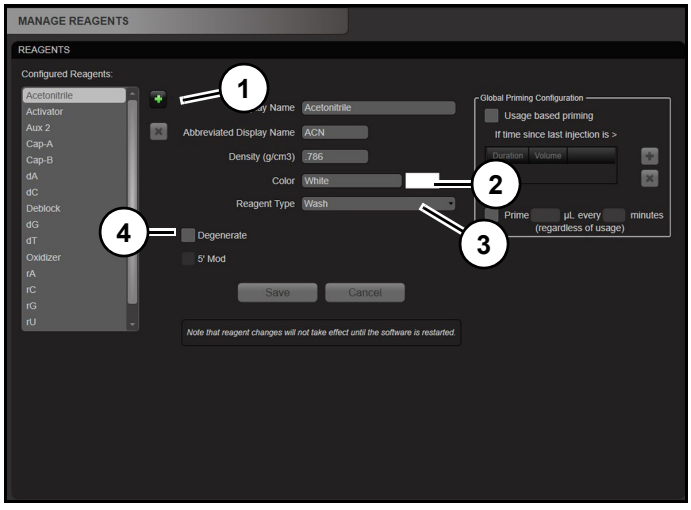

Figure 44

1. Click "+" (1) *(Figure 44)*.

Figure 45

Refer to *(Figure 45)*.

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 44)*

Adding Degenerate Base

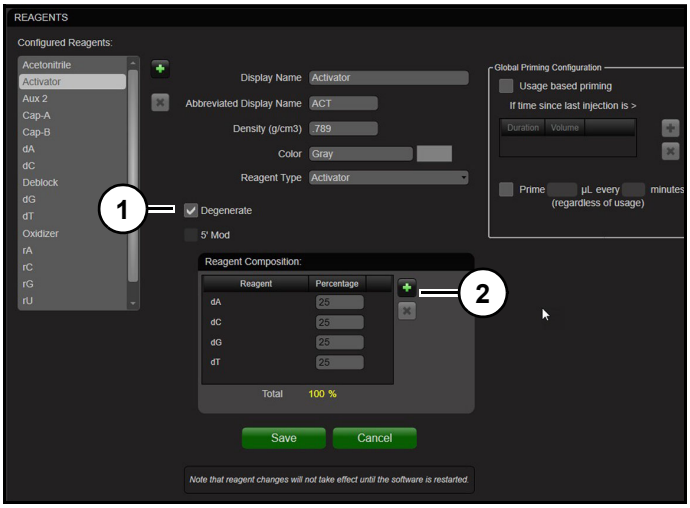

Figure 46

Refer to *(Figure 46)*.

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 must 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.

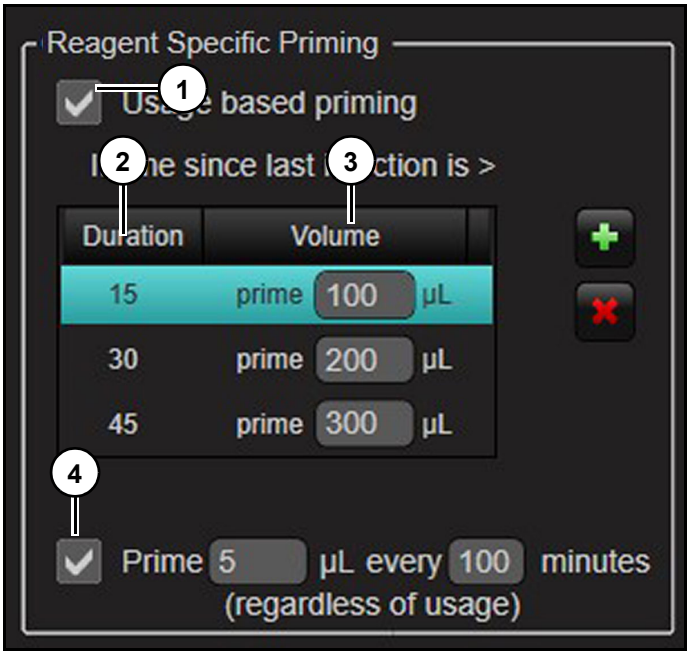

Figure 47

Refer to *(Figure 47)*.
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 next time 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 45min. This allows user to prime more if reagent has had more time to crystallise due to lack of use. Value must be under max value of liquid calibration table.

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

If lower box (4) is checked, then reagent line will be primed every 5 minutes with 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.

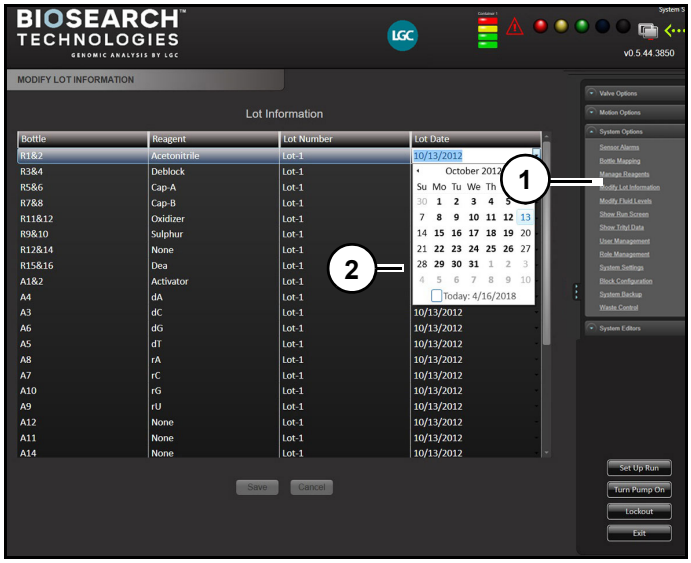

Figure 48

Refer to *(Figure 48)*.

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.

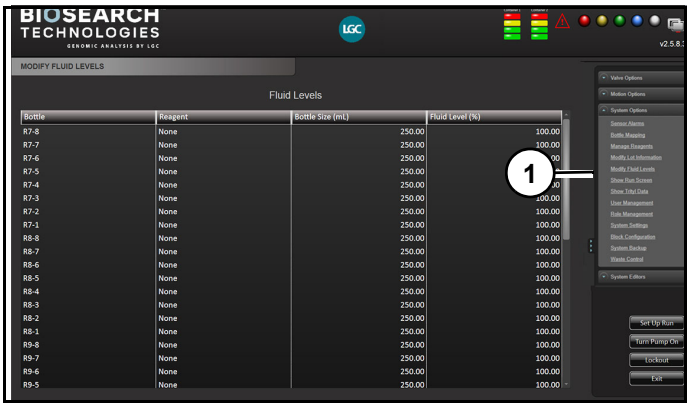

Figure 49

Modify Fluid Levels (1) *(Figure 49)* 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 to diagnostics and testing when instrument is paused.

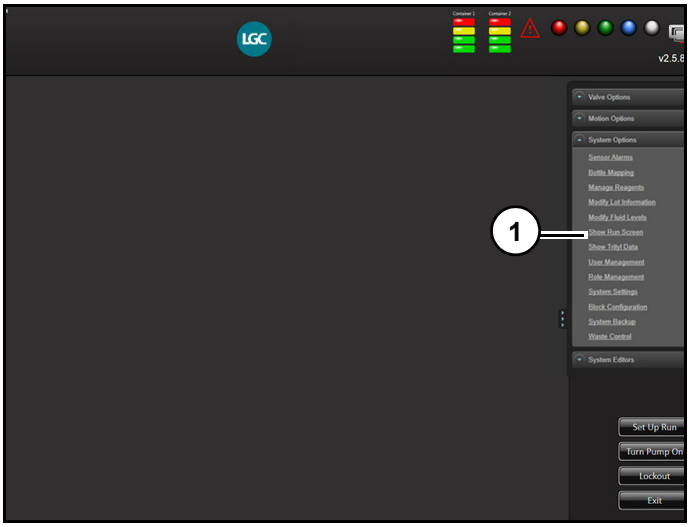

Figure 50

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.

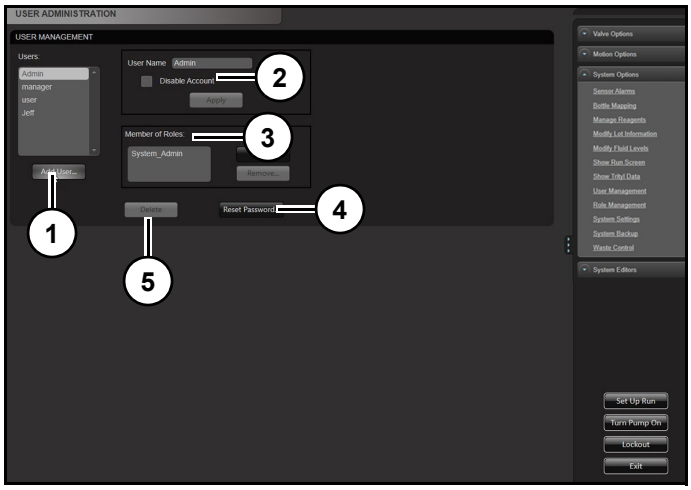

Figure 51

Refer to *(Figure 51)*.

Add User (1): Adds additional user.

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

Role 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

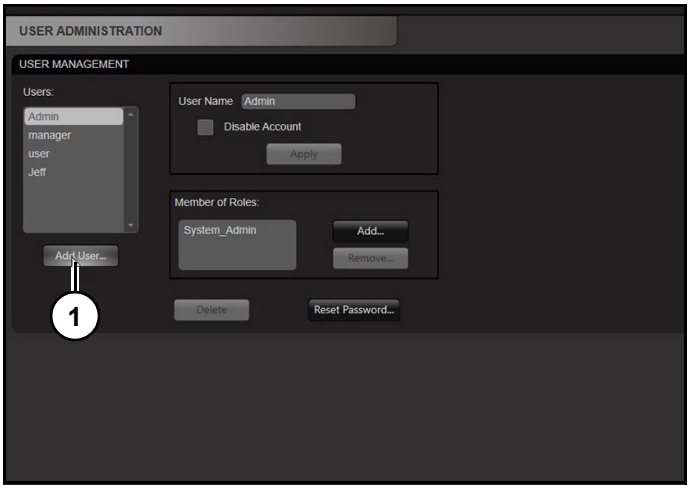

Figure 52

1. Click "Add User" (1) *(Figure 52)*.

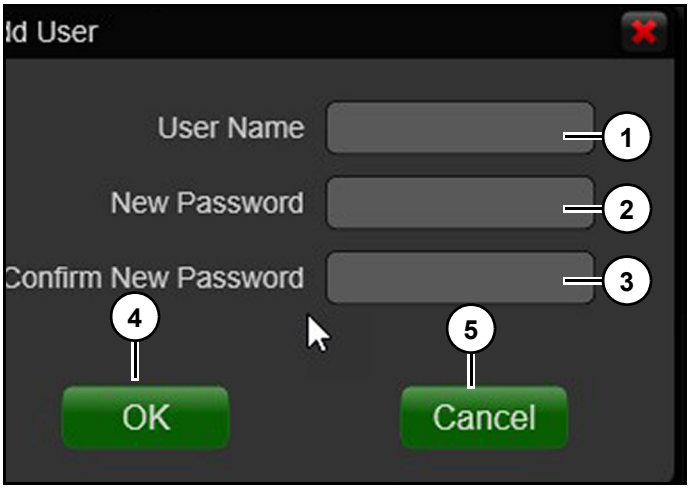

Figure 53

Refer to *(Figure 53)*.

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.

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

Role Management

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

Figure 54

Refer to *(Figure 54)*.

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

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

Delete (3): Deletes selected user role.

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

Permissions (5): Selects permission for selected role.

Permissions:

- Reset Password: Allows role to reset password for account
- Enable/Disable Sensor Alarms: Allows role to change sensor alarm settings.
- Override Support Type: Allows CPG type (universal vs standard) to be overridden after loading a sequence file which has support type designated. Allows a user to change support type form universal to standard and vice versa during the run start up process.
- Override DMT On/Off: Allows final DMT state (On vs Off) to be overridden after loading a sequence file which contains designated trityl information. Allows user to change trityl setting for any oligo form on to off and vice versa during run start up process.
- Modify Setup Screens: Allows change to setup screen defaults.
- Modify Security: Allows modification of security settings in user account management
- Reposition During Synthesis: Allows user to reposition a synthesis while active.
- Cancel Purge: Allows user to ignore initial chamber purge.
- Toggle Sequence Sensing Direction: Changes direction in which sequence file is read from loaded file; 5' -> 3' or 3' -> 5'.
- Modify Reagents: Allows user to make changes to reagents.
- Bypass Failed Sensor Checks: Allows user to continue to 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

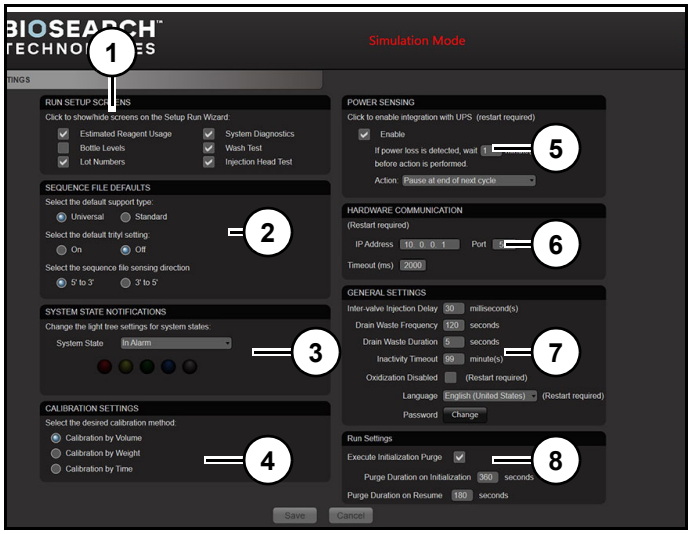

Figure 55

Refer to *(Figure 55)*.

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):

- 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 Finalisation 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 (5to3) it will be between G and C and in other (3to5) this bond will end up between C and A.

'*' indicates that sulfurisation 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 pause position to be set. Safest place to pause is before next deblock. 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 synthesiser is running instrument will open waste tray valves at this frequency to ensure that reagents, which are primed into it 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 Initialisation: 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 columns are added and removed and when 'Resume With Purge' option is used.

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.

Block Configuration

Allows user to adjust block configuration when switching from smaller blocks to larger blocks.
Note: Large scale sythesis block (8ml-35ml) take up two small scale block positions. Only one vacuum line is needed and other freeline is placed in park position.

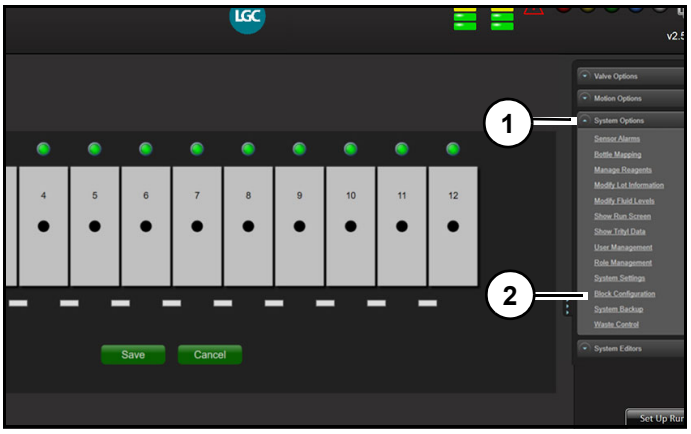

Figure 56

Refer to *(Figure 58)*.

1. Click "System Options" (1).
2. Click "Block Configuration" (2).

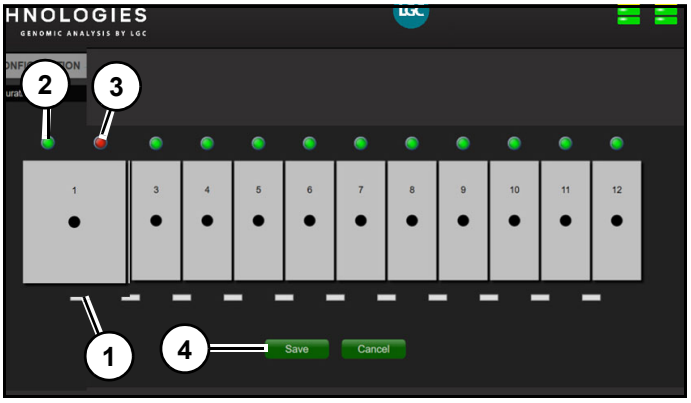

Figure 57

Refer to *(Figure 58)*.

3. Click rectangle (1) under block to switch between large scale column chuckor two small scale column chucks.

Note: Green circle (2) denotes drain line being used, red circle (3) denotes drain line not being used.

4. Click "Save" (4) to save changes.

System backup instructions

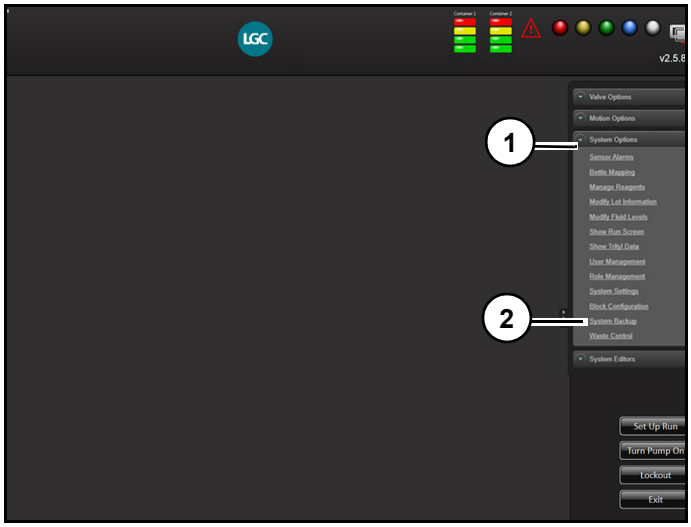

Figure 58

Refer to *(Figure 58)*.

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.

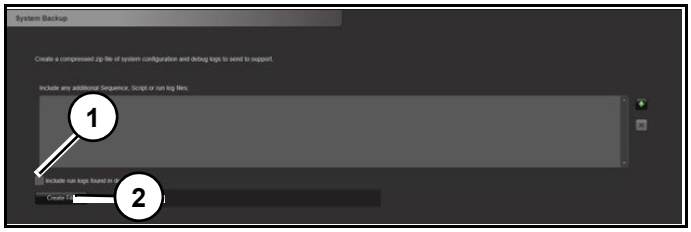

Figure 59

Refer to *(Figure 59)*.

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.

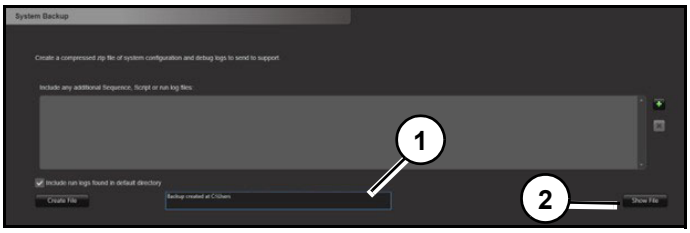

Figure 60

Refer to *(Figure 60)*.

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

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

Export Backup—Additional Files Selected.

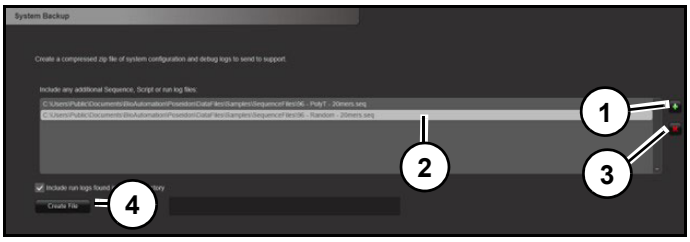

Figure 61

Refer to *(Figure 61)*.

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.

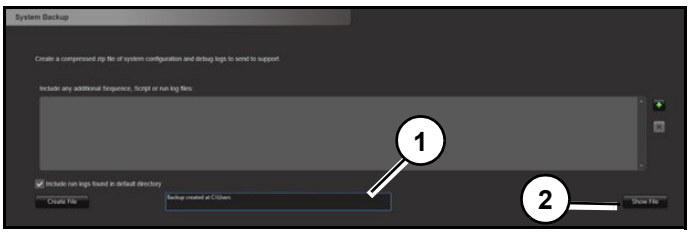

Figure 62

Refer to *(Figure 62)*.

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.

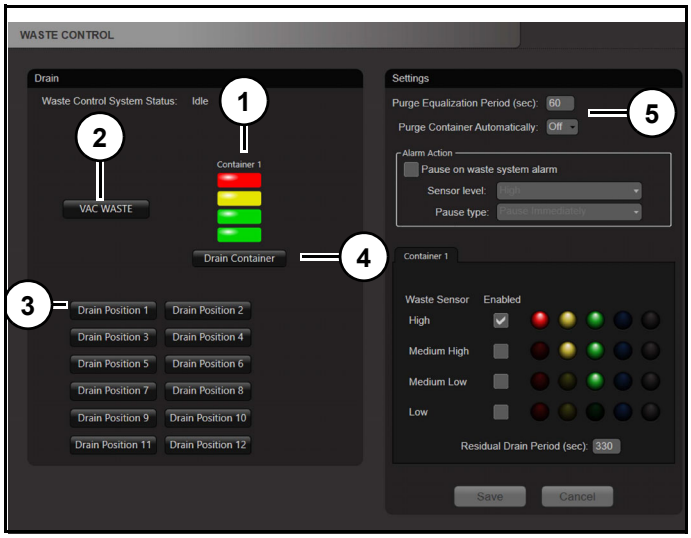

Figure 63

Refer to *(Figure 63)*.

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

Vac Waste (2): Will drain waste tray.

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

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

If using "Purge Container Automatically" (5), set instrument will 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 waste tank 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 64)* allows user to modify and create script files.

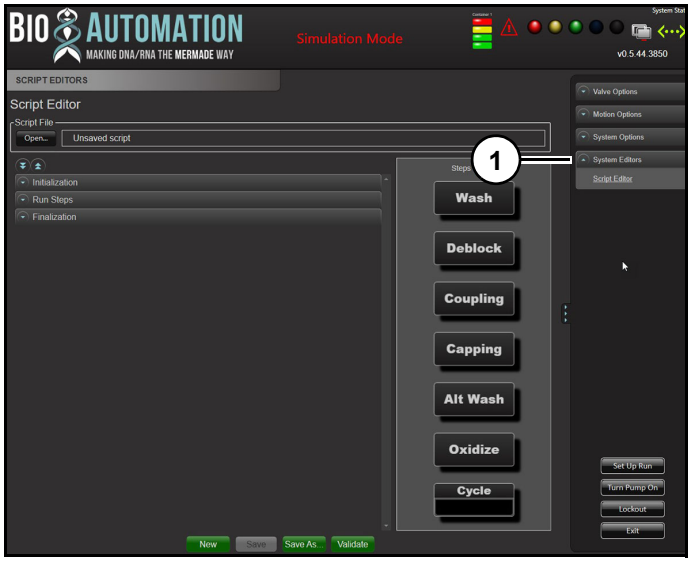

Figure 64

Using script files to set synthesis parameters

During process of setting up a run, user will be asked to specify a script file for scale of synthesis to perform on each column position 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 synthesiser will execute when synthesising oligos. Users can assign a different script to each column position.

Opening a script file

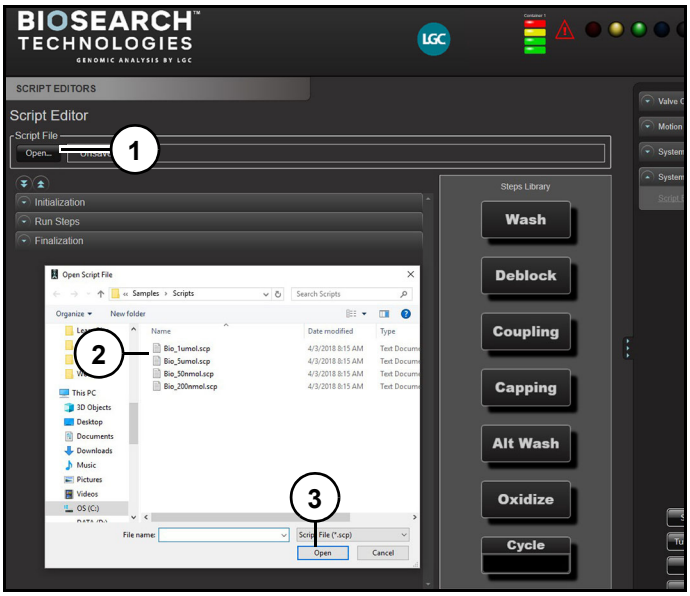

Figure 65

Refer to *(Figure 65)*.

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 66

Refer to *(Figure 66)*.

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/ Sulfurisation). Steps will repeat however 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.

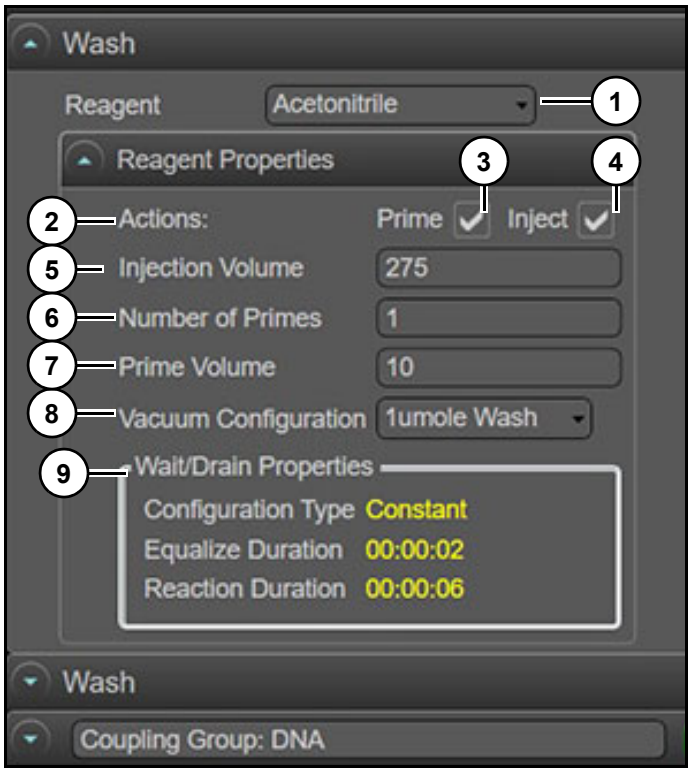

Figure 67

Refer to *(Figure 67)*.

Reagent (1): 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 (2): 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" (3). "Inject" (4) can be deselected to execute primes or drains without injecting reagent.

Injection Volume (5): Actual volume that will be delivered during step-in microliters. Liquid calibrations are critical to actual delivered volumes.

Number of Primes (6): 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 (7): 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 (8): 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 (9): Displays drain configuration type 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 support for next step in cycle. Since there is no reaction time necessary these steps are usually programmed to drain to completion as soon as delivery is finished. Acetonitrile is most common wash solvent used.

Deblock

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

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

Coupling

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

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

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

Capping

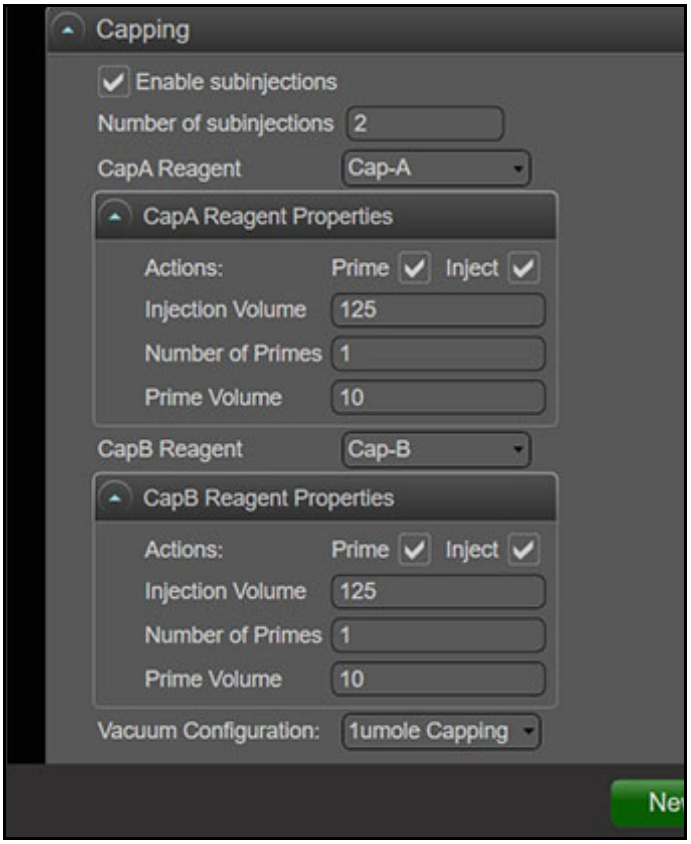

Figure 68

Refer to *(Figure 68)*.

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

Oxidizer & Sulfur

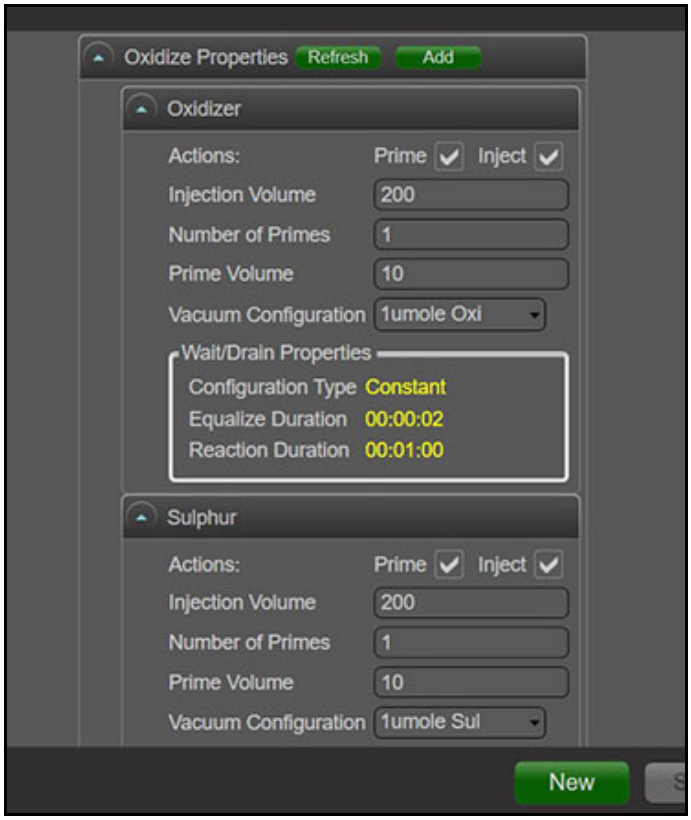

Figure 69

Refer to *(Figure 69)*.

Oxidation steps are used to put newly added backbone linkage into a stable state. This can be done with either oxygen or sulfur. When oxygen is used, result will be a phosphodiester back bone. When sulfur is used a phosphothiolated oligo will be generated. Each place on an oligo's backbone can be programmed to receive either oxygen or sulfur. Which of reagents that will be used is designated by either a ';' delimiter for oxygen or a '*' for sulfur in sequence file. Additional oxidisers can be added to and delimitators can be changed.
Contact Biosearch Technologies for support if more than two oxidation reagents are required. There are many types of oxidisers and thiolation reagents. Best choice for any given application will depend on factors including monomers being added and any modifiers that are being used.

Alt Wash

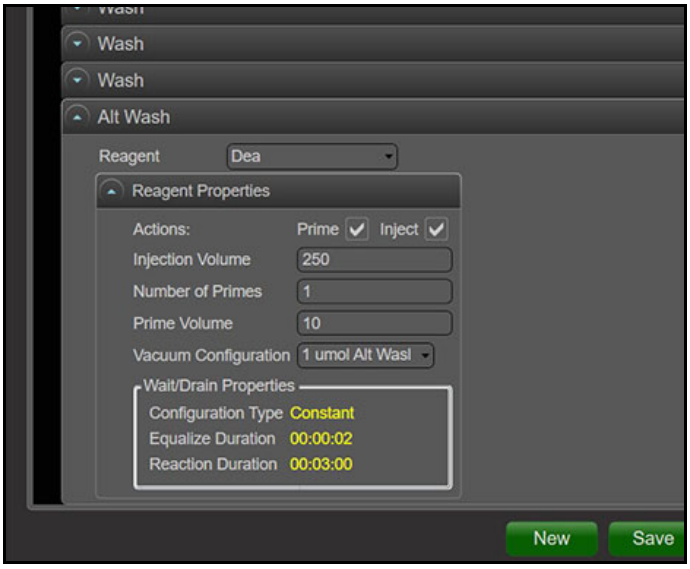

Figure 70

Refer to *(Figure 70)*.

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

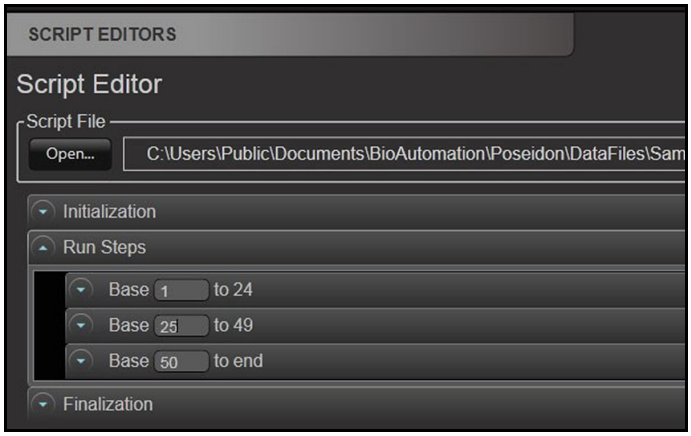

Figure 71

Refer to *(Figure 71)*.

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 71)*.

Making changes to a script file

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

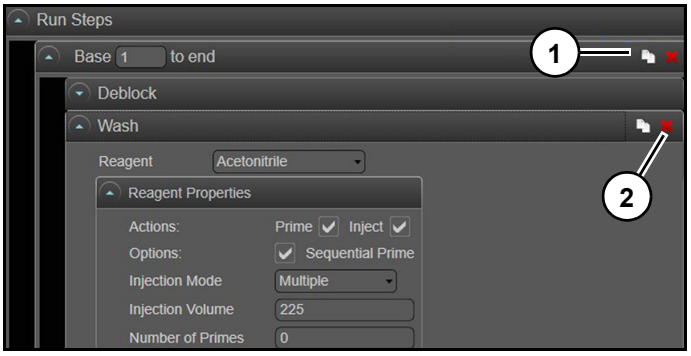

Figure 72

Refer to *(Figure 72)*.

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

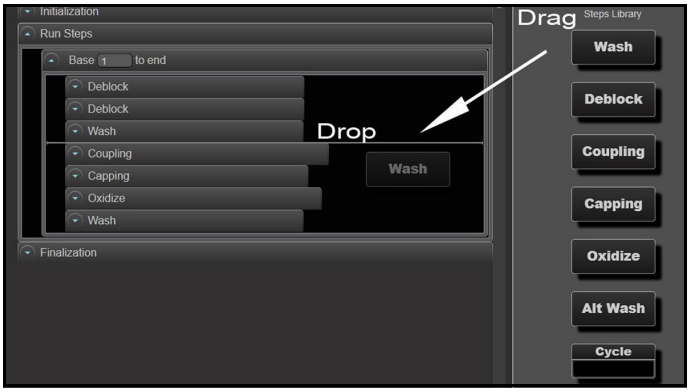

Figure 73

Refer to *(Figure 73)*.

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:

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

Setup screen

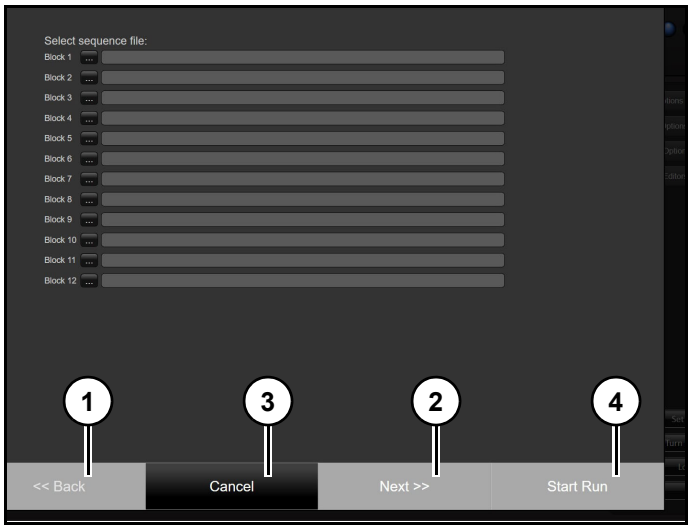

Figure 74

Refer to *(Figure 74)*.

Back (1): Returns to a previous step.

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

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

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

Block Selection.
Allows user to specify which columns 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.

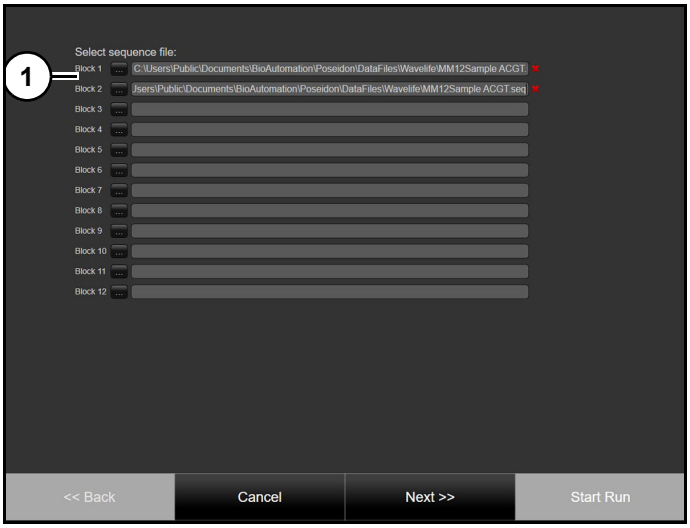

Figure 75

Refer to *(Figure 75)*.

1. Select "Block" (1).

A column can be run by itself, or multiple columns can be run at same time.

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.

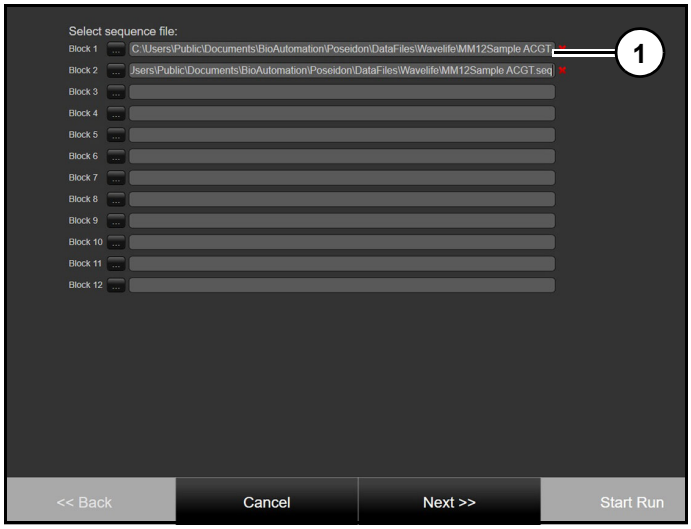

Figure 76

Refer to *(Figure 76)*.

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 file is restricted to 1 sequence per file. If more than 1 sequence is contained in file, it will produce a "Too Many Oligos" error.
- Line must contain an oligo name and an oligo sequence and it may contain information regarding type of CPG being used (Universal or Standard) and desired state of final DMT group (On or Off).
- Oligo name must be delimited from oligo sequence by a comma when using text files.
- Oligo name may contain any combination of characters and numbers including punctuation and spaces.
- Oligo sequence may contain combinations of upper and lower case characters as well as spaces.

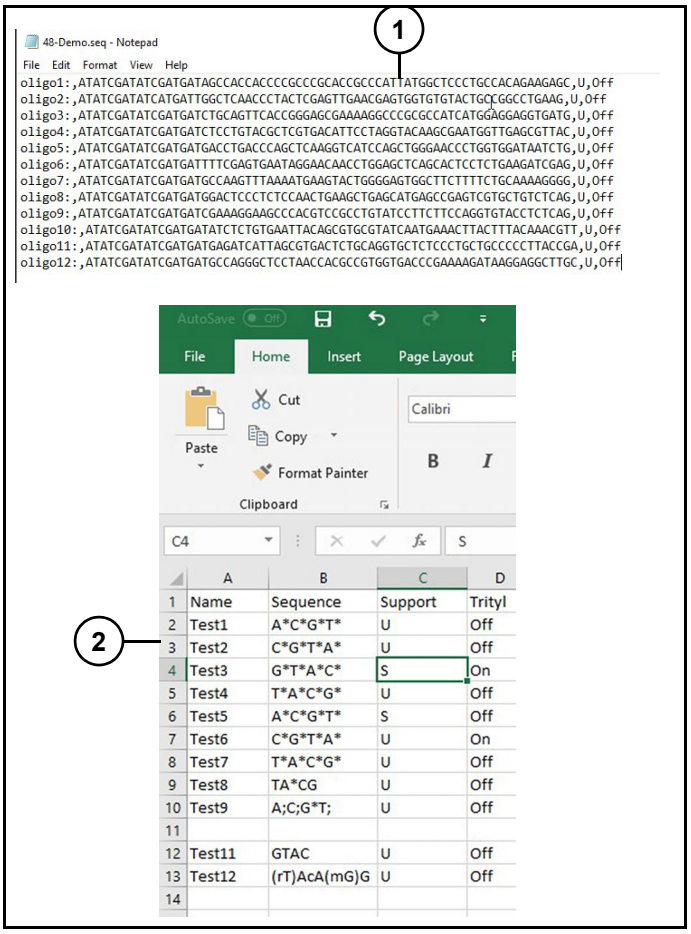

Figure 77

Refer to *(Figure 77)*.

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

Standard sequences can be specified in upper or lower-case format. Abbreviated Display Name in manage reagents screen is case sensitive and will be used to call a base form sequence file. 'C' is not equivalent to 'c' and 'UsA' is not equivalent to 'uSa'. Abbreviated display names more than one character in length must be bracketed by parentheses.

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 P=S bonds are desired in the final synthesized olig a "*" at base positions.
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.

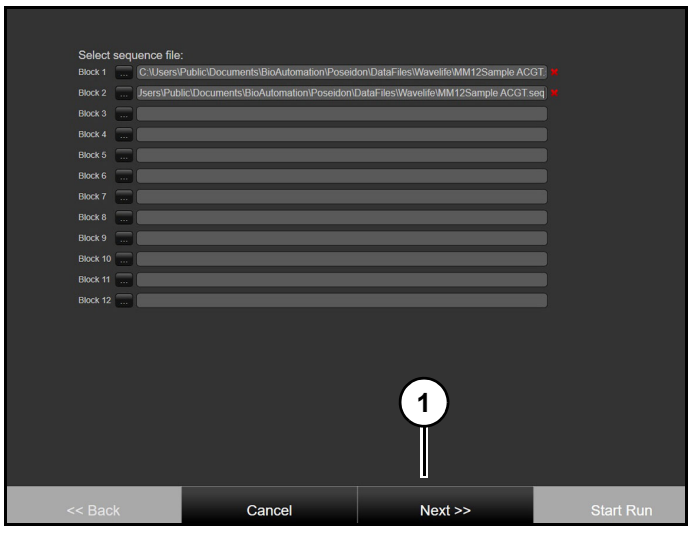

Figure 78

3. Click "Next" (1) *(Figure 78)*.

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

Column Details.

Allows user to select CPG type, final DMT, and start at base position.

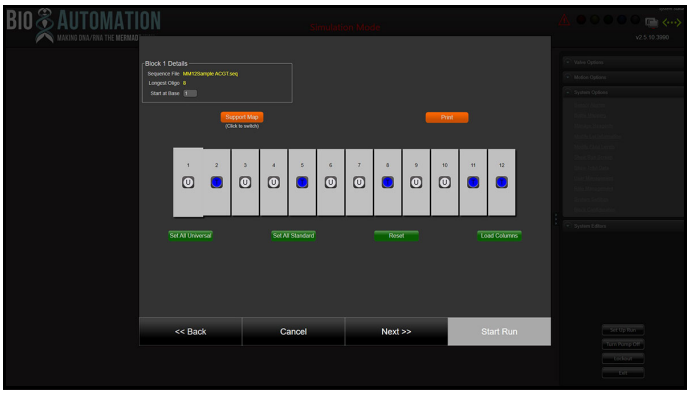

Figure 79

Refer to *(Figure 79)*.

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

After loading sequences, to change CPG type click on a column 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 all positions 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.

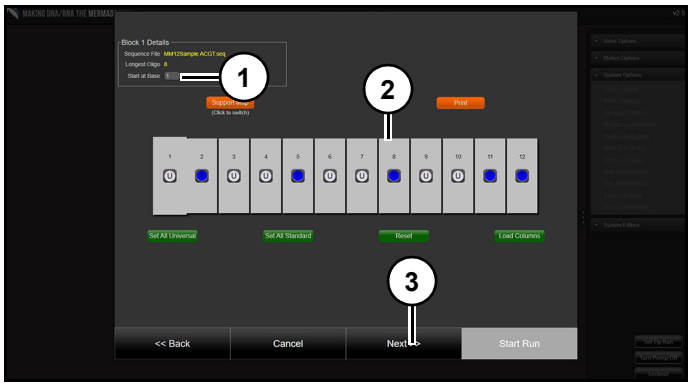

Figure 80

Refer to *(Figure 80)*.

4. When CPG screen is visible, load synthesis columns into column chucks inside synthesiser.
5. For standard CPG, enter 2 in "Start at Base" (1) for universal a value of 1.
6. Confirm each column sequence by placing mouse cursor over each well (2) and checking displayed 5' to 3' sequence.
7. Click "Next" (3).

Load script file.

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

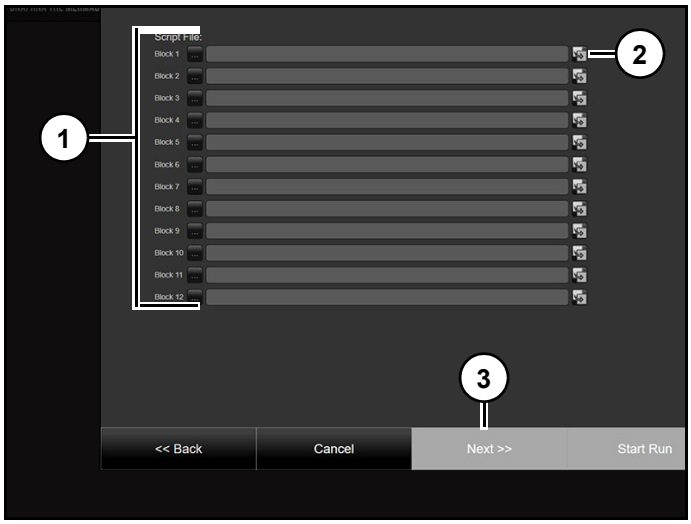

Figure 81

Refer to *(Figure 81)*.

8. Click "Block Button" to assign script file to desired plate. This will bring up file explorer.

Note: Clicking "Copy Icon" (2) will copy script file to all block positions.

9. 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 column position. However, having two script files in varying liquid dispensing, wait, and drain times will increase synthesis time.

10. Click "Next" (3).

Estimated Reagent Usage.

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

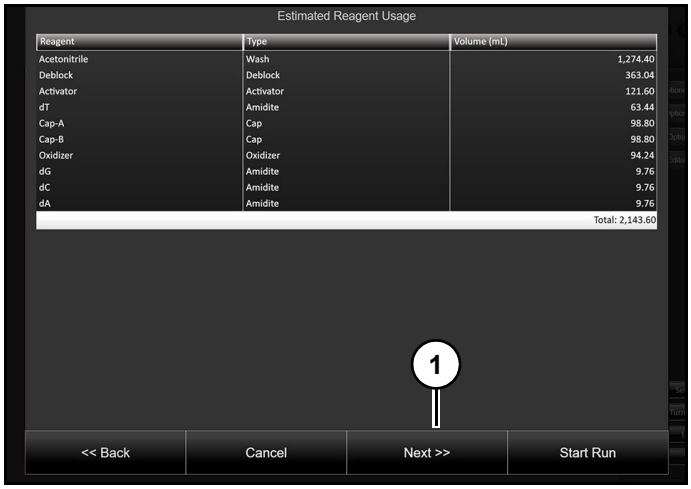

Figure 82

Values shown *(Figure 82)* are calculated from injection volume field in reagent file and are only as accurate as calibration.
Note: Synthesiser 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.

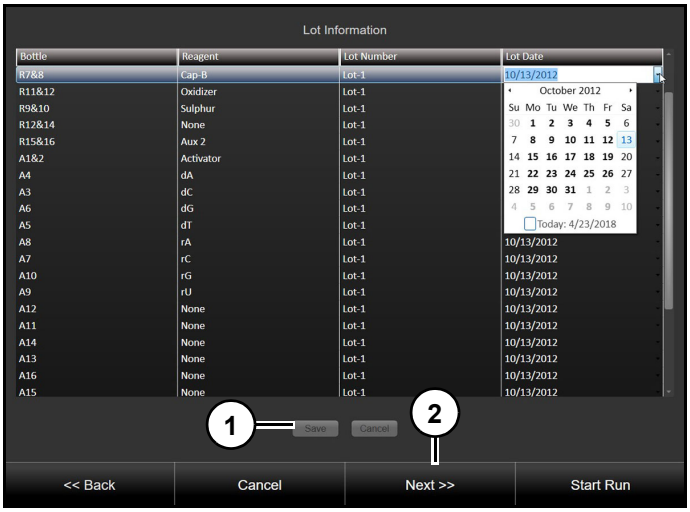

Figure 83

Refer to *(Figure 83)*.

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

12. Click "Save" (1).

13. Click "Next" (2).

Block Information.

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

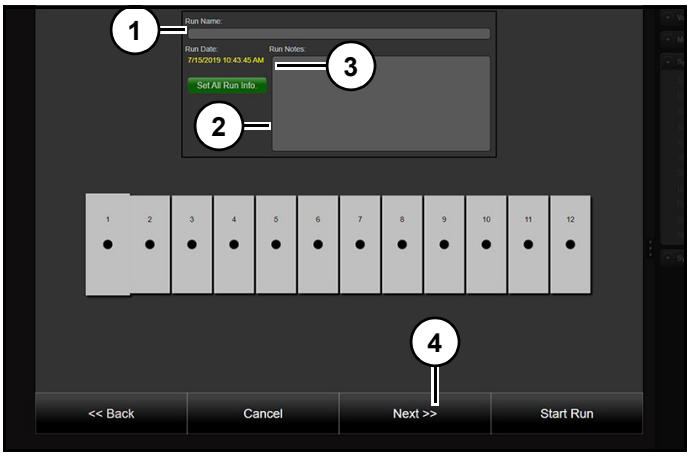

Figure 84

Refer to *(Figure 84)*.

14. 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. To insert a note select individual block.

Software automatically generates date and time stamp (3).

Click "Next" (4).

Sensor Test Screen.

Allows user to run a system check on instrument sensors.

Sensors:

Alarm Checks.

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

Argon Hi Flow Test.

Argon Low Flow Test.

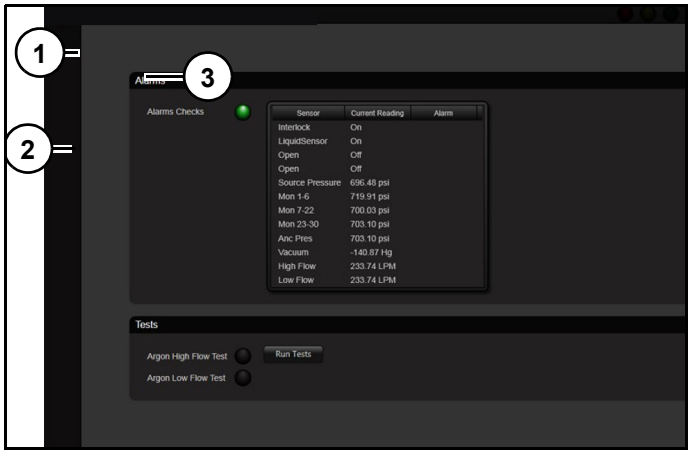

Figure 85

Refer to *(Figure 85)*.

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

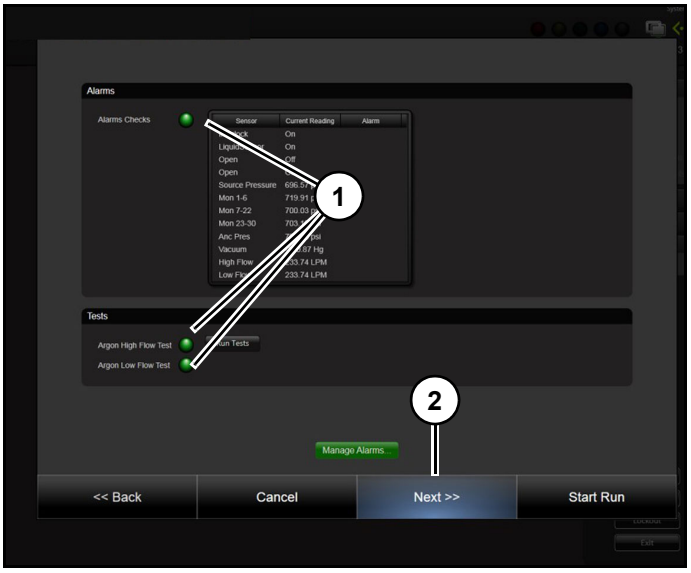

Figure 86

Refer to *(Figure 86)*.

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

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

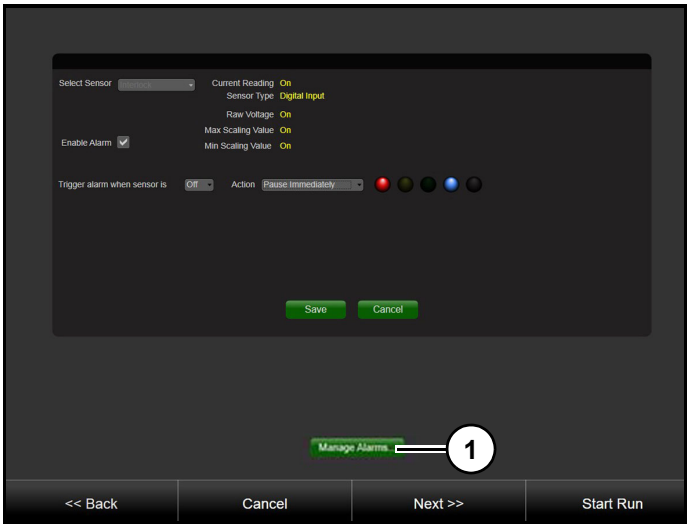

Figure 87

Refer to *(Figure 87)*.

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

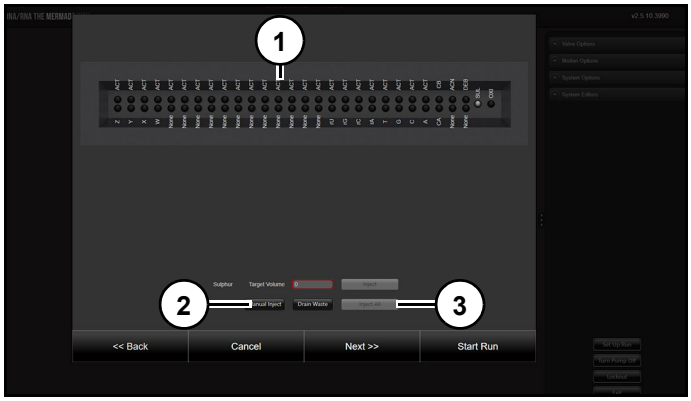

Figure 88

Refer to *(Figure 88)*.

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

Wash Test

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

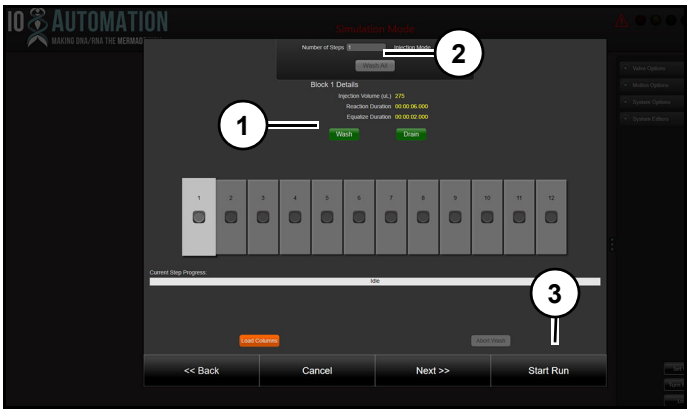

Figure 89

Refer to *(Figure 89)*.

6. Click "Wash" (1) button under each block, or "Wash All" (2).

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

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

Run screen

Provides user with control and displays details of ongoing synthesis.

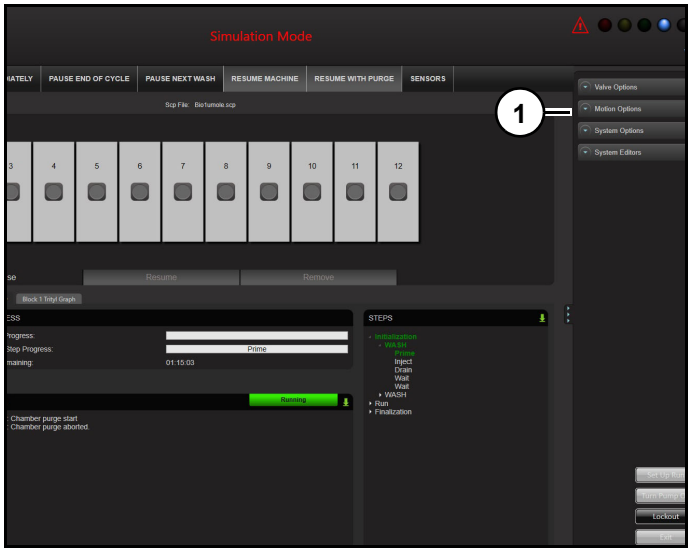

Figure 90

Refer to *(Figure 90)*.

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

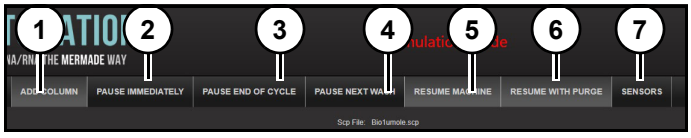

Figure 91

Refer to *(Figure 91)*.

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

Pause Immediately (2): Pauses instrument immediately unless synthesis is in a wait step and then will pause as soon as wait step is over. Option should rarely be used and considered a last resort as it can cause software to lose track of some events and cause unexpected behavior when restating 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 (7): Displays current sensors and their respective values. Sensors cannot be changed, only viewed.

Steps Control Options

Displays active step in synthesis cycle and provides user with options when right-clicked (1) *(Figure 92)*.

Figure 92

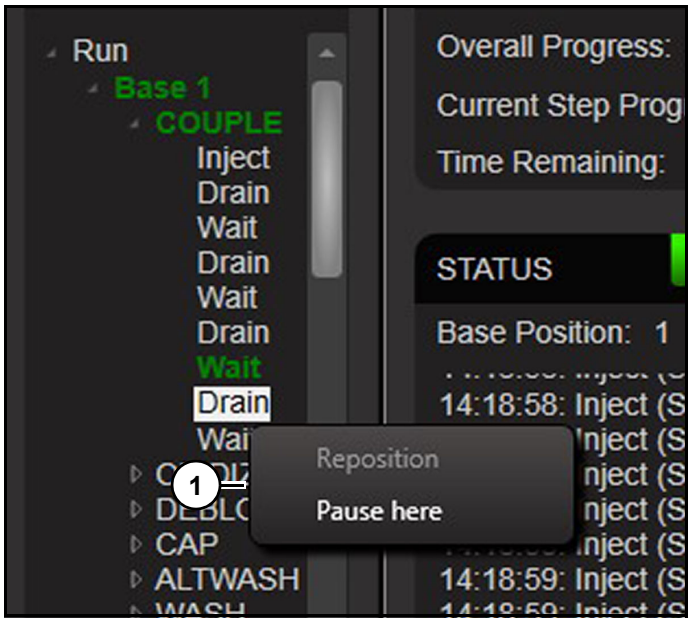

Figure 93

Refer to *(Figure 93)*.

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 94

Once a pause is initiated, location of pause will be highlighted orange (1). *(Figure 94)*

Canceling a pause

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

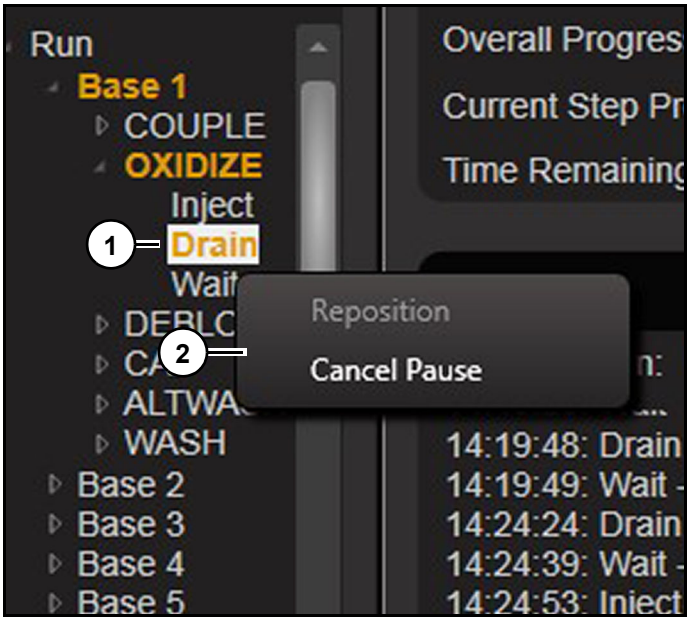

Figure 95

Refer to *(Figure 95)*.

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

Reposition

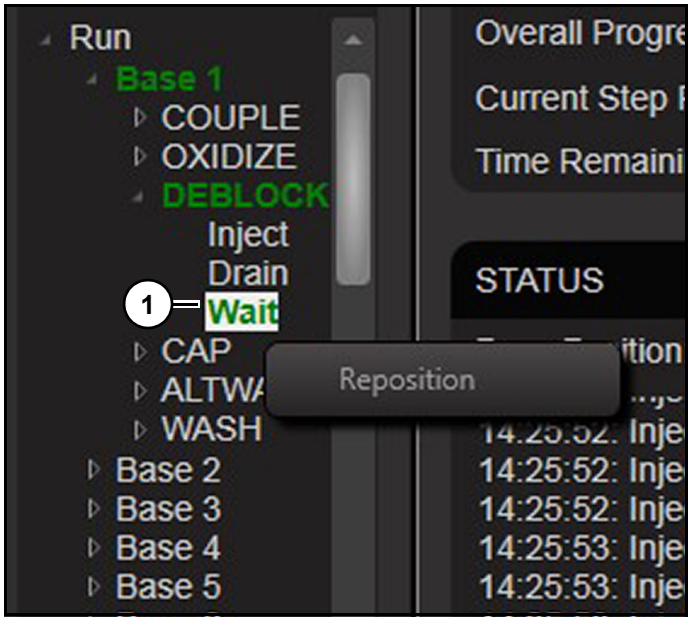

Figure 96

Refer to *(Figure 96)*.

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 column(s)

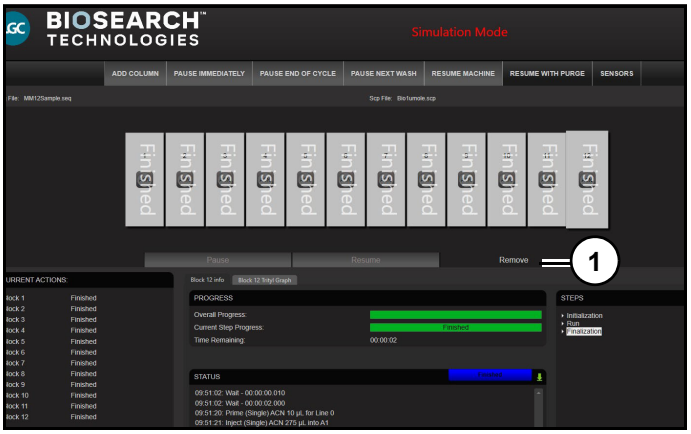

Figure 97

Refer to *(Figure 97)*.

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

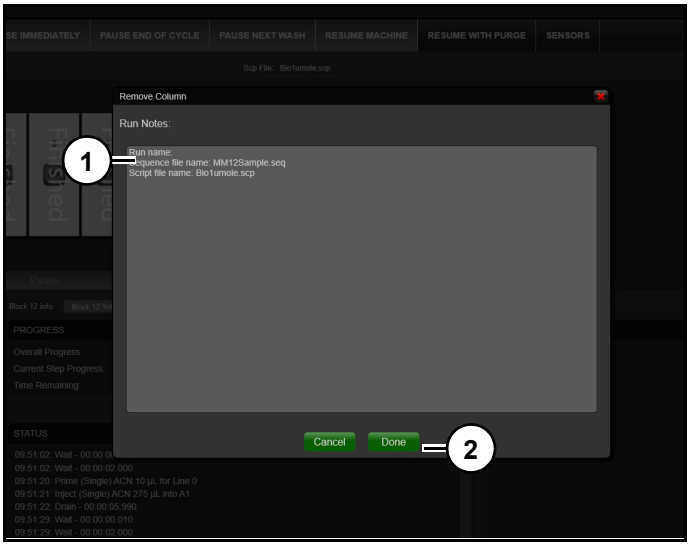

Figure 98

Refer to *(Figure 98)*.

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

MerMade 6 & 12 maintenance

⚠WARNING

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

⚠WARNING

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

⚠WARNING

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

Cleaning

⚠WARNING

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

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

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

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

Wipe down plate deck of instrument with a damp cloth.

Maintenance schedule

Maintenance checklist	
Weekly (Performed by operator)	
Wipe down surfaces	
Wipe down plastic guards with glass cleaner	
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 home switches	
Check pumps, and replace tubing	
Inspect fittings for leaks	
Check load positions	

Vacuum breaker

Figure 1

Vacuum breaker (1) *(Figure 1)* is located on right hand side of instrument.

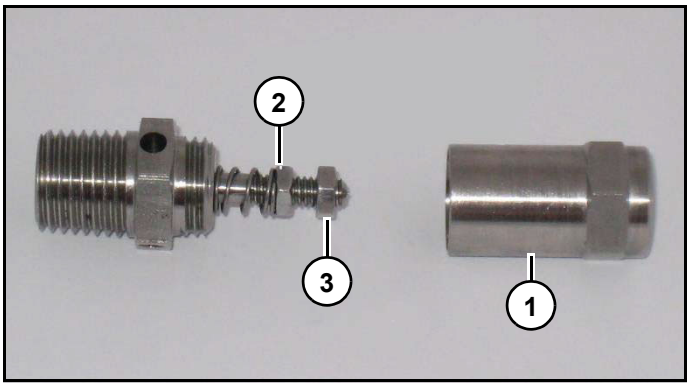

Figure 2

Refer to *(Figure 2)*.

1. Remove vacuum breaker casing (1).
2. Screwing in or unscrewing inside stainless steel nut (2) will increase or decrease vacuum pressure.
3. Using outer nut (3) lock inner nut in place to keep vacuum level constant.

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: mindful, if using Test I/O screen, to only turn on a few lines at a time to avoid a blown 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 waste tray and all block positions.
4. Inject Acetonitrile into waste tray by utilizing any injection line connected to Acetonitrile in Test I/O Screen.
5. 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.
6. Inject Acetonitrile into columns and drain plate locations utilizing the Vacuum Pulse Calibration screen.
7. Repeat above step 2-3 times to ensure drain lines are flushed with Acetonitrile.
8. Hold 'Drain' button down in Vacuum pump calibration screen until liquid evacuates drain lines.

Note: Verify that vacuum pump is turned on.

9. Place empty bottles in all monomer and reagent bottle locations on instrument.
   Note: Do not shut off Argon or Nitrogen pressure to instrument.
10. 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.
11. Return to Vacuum Pulse Calibration screen in Poseidon software. With vacuum pump on, click "Drain" button for several seconds for all plate positions until there is no longer visible liquid in waste container tubing. Repeat using "Drain Waste" button to evacuate all remaining liquid in waste tray drain line.
12. Once all liquid lines and drain lines are completely empty, evacuate any waste remaining in waste container(s).
13. Evacuate all synthesis waste from container into a secondary waste location via 'Waste Control' screen. Thoroughly rinse container(s) with Acetonitrile.
14. This may be done manually or by injecting Acetonitrile into waste tray on instrument and draining waste tray several times.
15. Evacuate all remaining Acetonitrile in waste container(s) once adequately rinsed.
16. Open container(s) and allow container(s) to air dry in a fume hood or by utilizing a snorkel system.
17. Shut off Argon or Nitrogen to instrument and at gas tank.
18. Gently loosen and remove all monomer and reagent bottles to relieve gas pressure.
19. Disconnect all tubing from gas tank to instrument.
20. Disconnect or coil and zip tie any ventilation tubing that was previously routed to a snorkel system or fume hood.
21. Disconnect reagent bottle caps from instrument if needed for storage or transport.
22. 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.
23. 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.

24. 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/

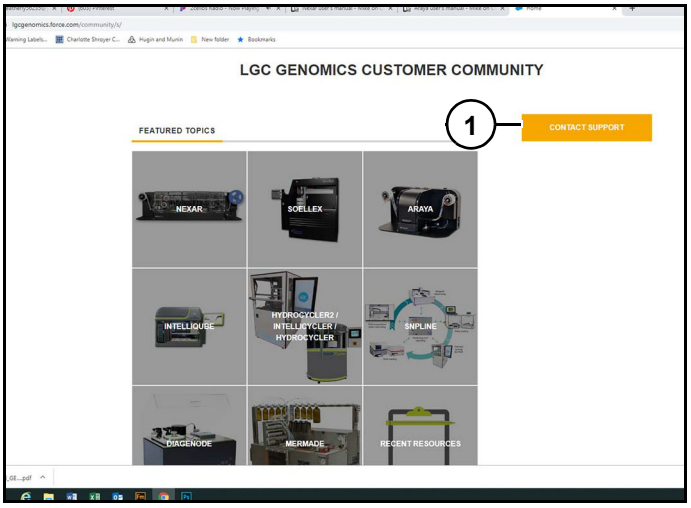

Figure 1

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).

@LGCBiosearch | biosearchtech.com

BIOSEARCH™ TECHNOLOGIES