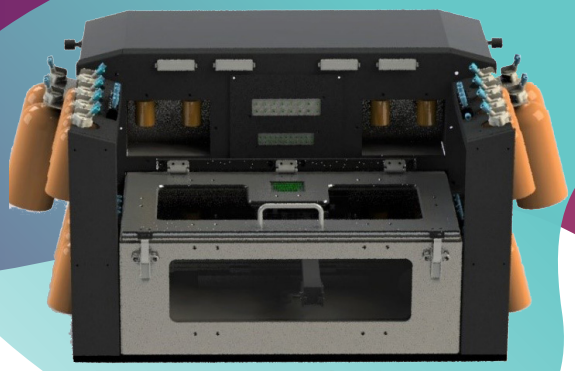
Original instructions



MerMade 48X user's manual

GEN/0636/MW/0225

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

BIOSEARCH™ TECHNOLOGIES

Documentation disclaimer

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General safety

⚠WARNING

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

⚠WARNING

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

Safety first

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

Safety symbols

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

⚠ DANGER

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

⚠WARNING

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

⚠ CAUTION

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

NOTICE

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

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

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

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

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

Indicates area where caution is required to prevent personal injury.

Indicates surface is hot and there is a burn hazard.

Waste Electrical and Electronic Equipment (WEEE)

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

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

Owner responsibilities

Notice

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

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

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

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

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

Operating area

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

Lockout/Tagout

⚠WARNING

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

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

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

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

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

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

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

Installation

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

Chemical spills

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

Chemical safety

- Follow all Safety Data Sheet (SDS) recommendations.

Follow facility's safety requirements when working with samples.

MerMade safety

⚠WARNING

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

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

Safety

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

Standard ANSI Z35.4 convention is used throughout manual.

Instrument must be operated in manner specified by Biosearch Technologies.

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

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

Reagent Delivery System

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

Motion system

⚠WARNING

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

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

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

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

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

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

Electrical system

⚠WARNING

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

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

Chemical safety

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

⚠WARNING

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

Pressurised solvent bottles

⚠WARNING

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

⚠WARNING

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

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

Cleaning and decontaminating chemical spills

Notice

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

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

Risk reduction: Solvent flammability

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

Additional safety notes:

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

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

General maintenance safety

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

Cleaning safety

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

Heat safety

- Do not touch heated surfaces.

Chemical safety

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

Electrical safety

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

Recognising safety precautions

Notice

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

Warning safety precautions

Figure 1

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

Caution safety precautions

Figure 2

Warning - Exclamation Point *(Figure 2)*.

Alerts user to presence of important operating and servicing instructions.

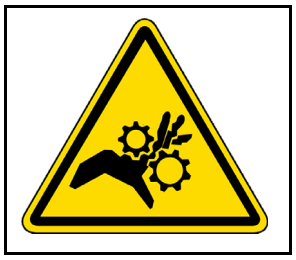

Figure 3

Warning - Pinch Point *(Figure 3)*.

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

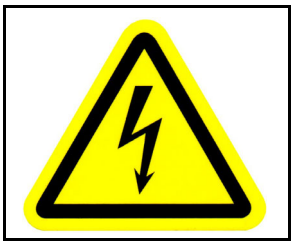

Figure 4

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

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

Figure 5

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

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

Figure 6

Warning - Corrosive Material *(Figure 6)*.

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

Stopping instrument

⚠CAUTION

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

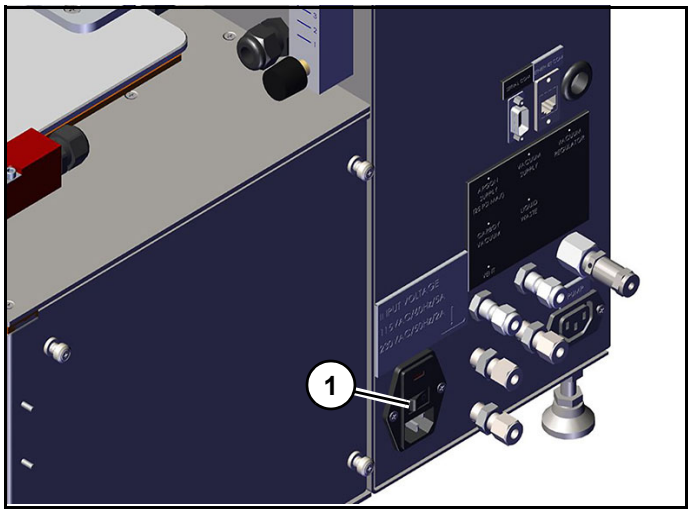

Figure 7

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

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

MerMade 48X 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

Note: Configuration may change with model.

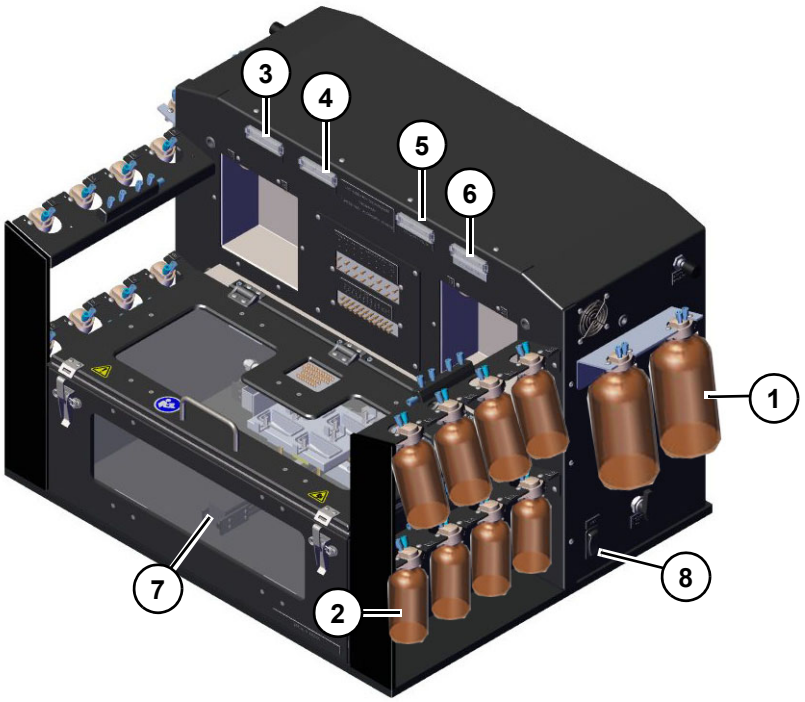

Figure 1

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Reagent Bottles	5	Monomer Pressure
2	Monomer Bottles	6	Reagent Pressure
3	Regulated Vacuum	7	Reaction Chamber
4	Source Pressure	8	Light

Component identification right side and left side

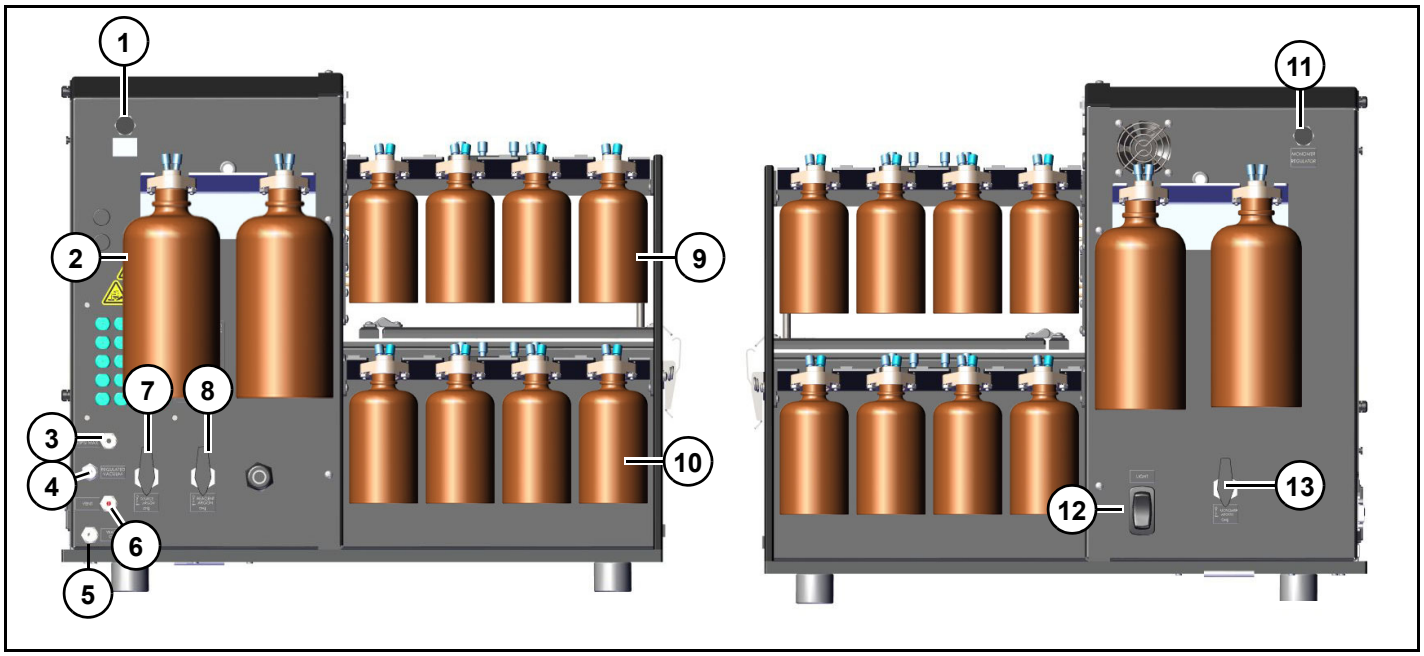

Figure 2

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Reagent Regulator	8	Reagent Pressure On/Off
2	Reagent Bottles	9	Amidite Bottles
3	Argon/Nitrogen Supply Connection	10	Amidite Bottles
4	Regulated Vacuum Conection	11	Amidite Regulator
5	Waste Out Connection	12	Light Switch
6	Vent Connection	13	Amidite Pressure On/Off
7	Source Pressure On/Off		

Component identification back

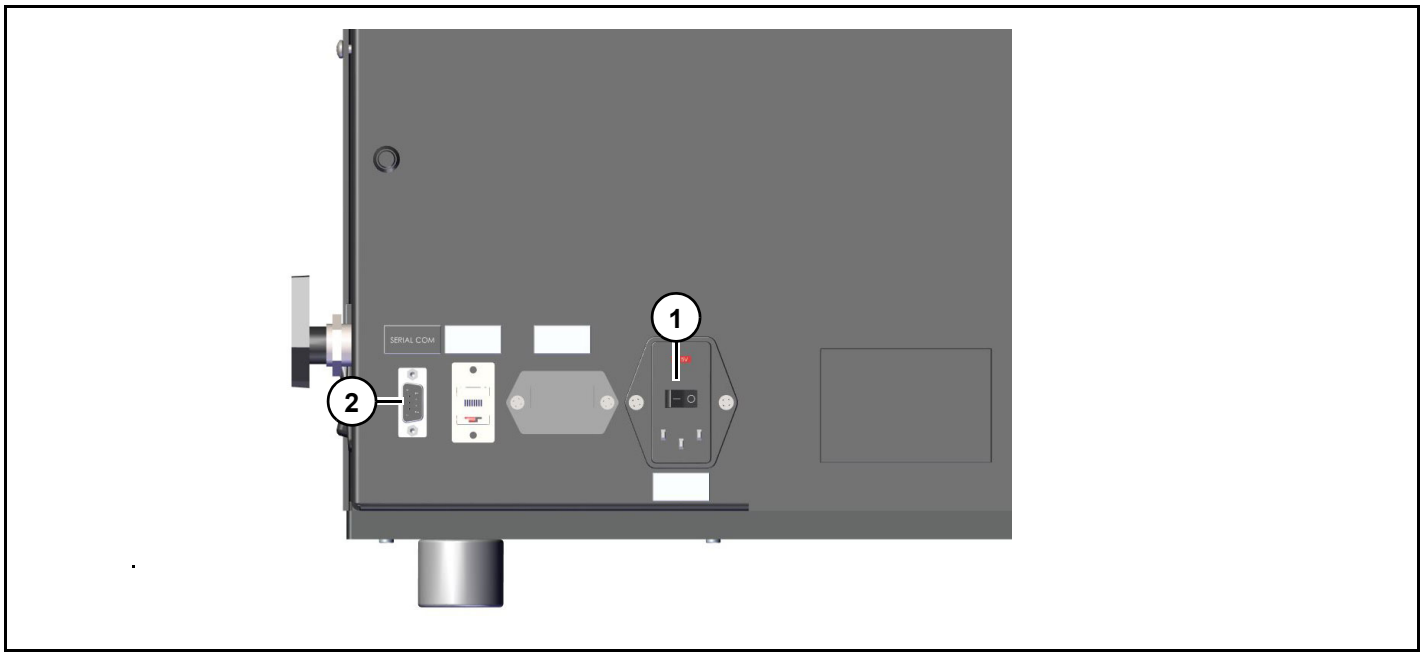

Figure 3

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Power Button	8	Serial Port

MerMade 48X decal identification

⚠WARNING

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

Decal identification main instrument

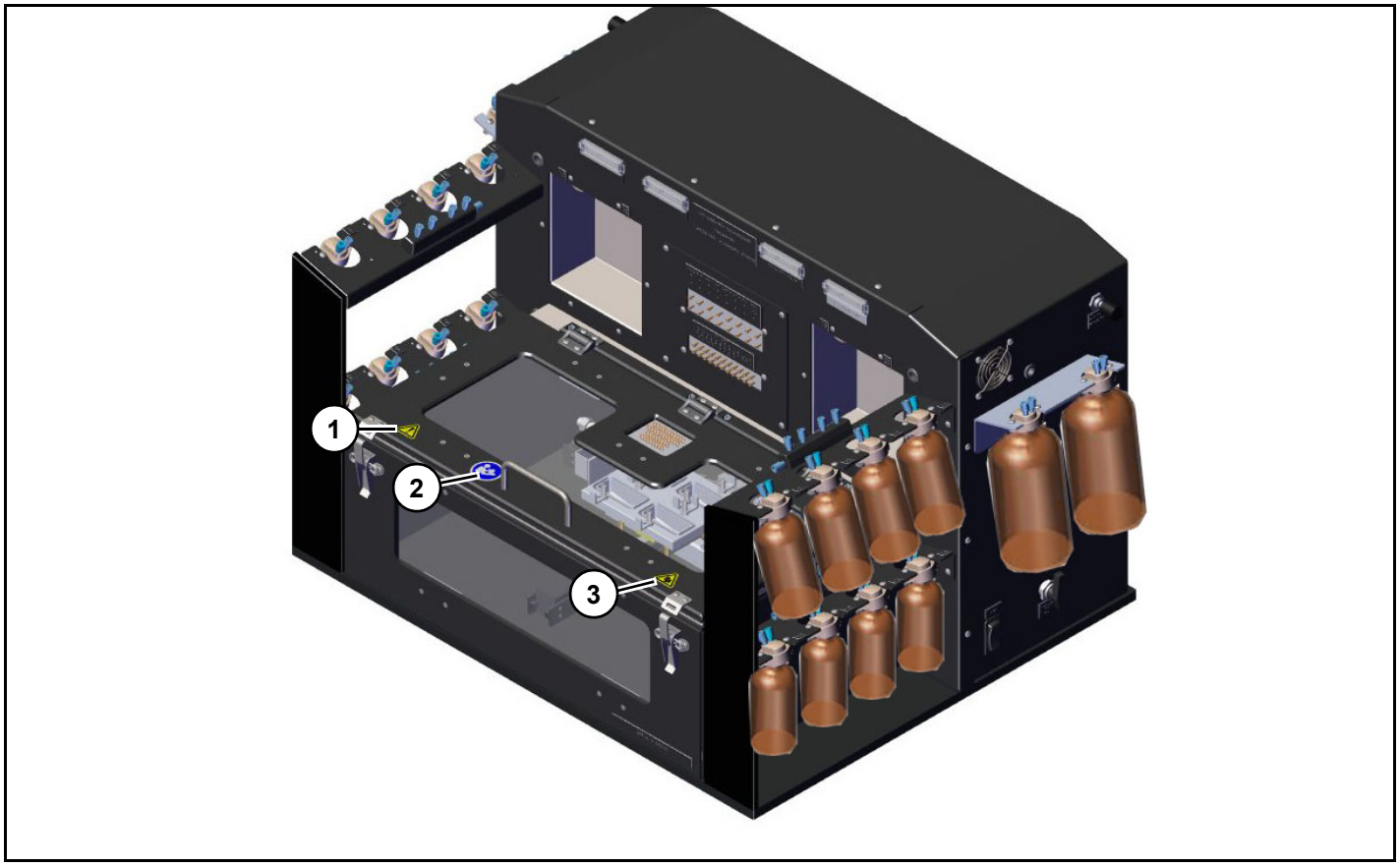

Figure 1

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Warning Exclamation Point -Top of instrument deck (Qty-1)	3	Pinch Point -Top and both sides of Plate Shuttle (Qty-1)
2	Read Owner's Manual -Guard Door (Qty-1)		Pinch Point -Top and both sides of Plate Shuttle (Qty-1)

Decal identification left side

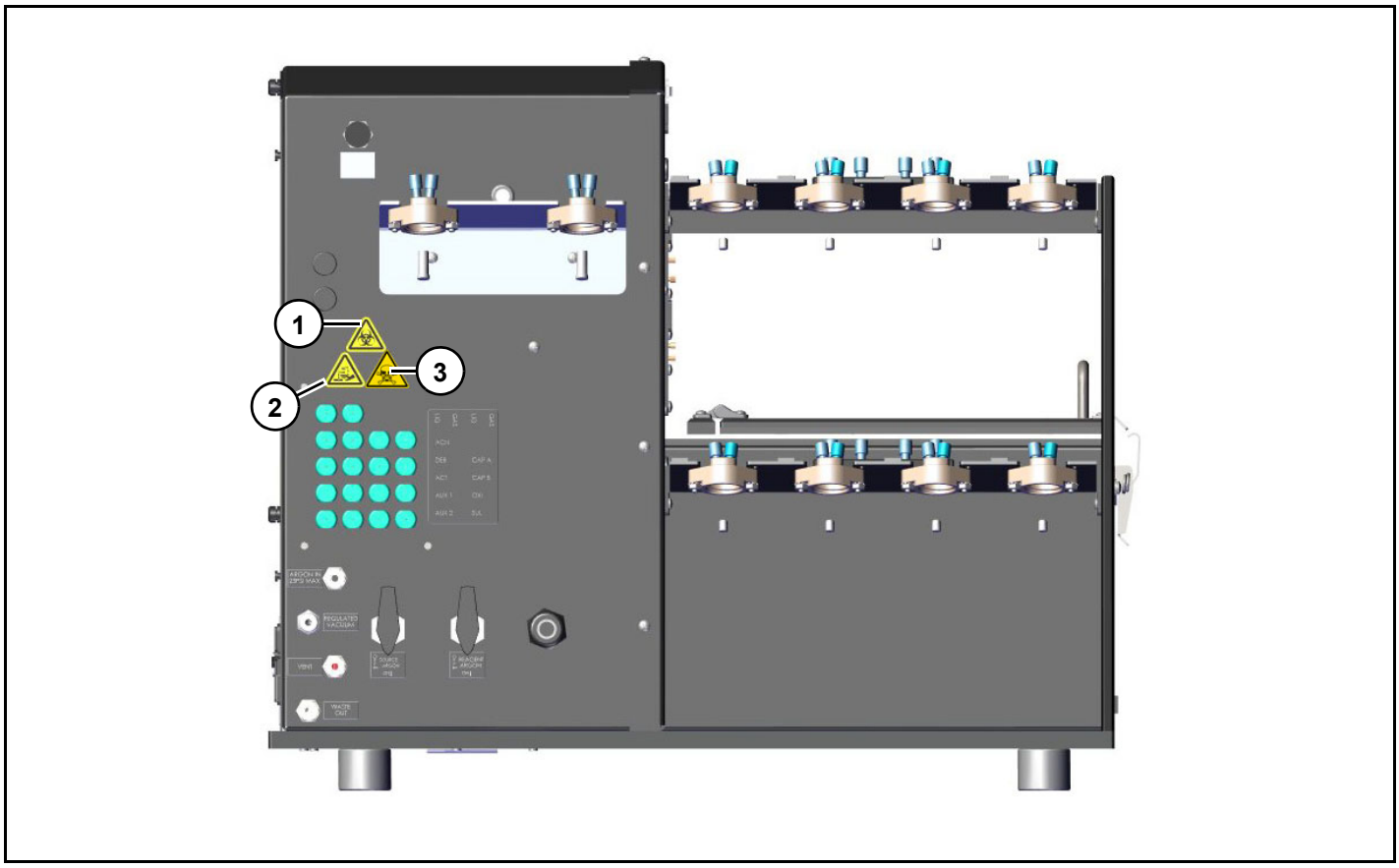

Figure 2

REF#	DESCRIPTION	REF#	DESCRIPTION
1	Biological Hazard -Left side instrument (Qty-1)	3	Toxic Material -Left side instrument (Qty-1)
2	Corrosive Substance -Left side instrument (Qty-1)		

MerMade 48X installation/site preparation

⚠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

All operators should be well versed in good laboratory practices and trained in safe operation and use of instrument. Information and instructions contained in user manual are accurate and reliable to best of our knowledge.

Overview

MerMade Oligonucleotide Synthesizer is designed to synthesise up to 48 columns, four 12 well plates of oligonucleotides in a single run using standard or modified phosphoramidite chemistry. Run may be paused to allow addition or removal of plates while synthesizer is in operation. Generating custom run protocols allows synthesis of oligos using standard or modified chemistry making MerMade adaptable to a wide range of applications. Synthesis scales may be varied from 50 nmole to over 5 micromole for each well.

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

Poseidon software wizard guides operator through a series of checks and tests which ensure proper operation of instrument and prompts user when required. Once a synthesis has been started, all subsequent operation is handled by software and reported on status window.

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

Installation

Mermade 48X is a dual axis (X&Y)bench top synthesizer, and needs to be installed with adequate space to access all four sides. There must be sufficient vertical clearance to allow for a ventilation system to vent any hazardous fumes.

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

- 79 cm (31")

Height

- 51 cm (20")

Depth

- 62 cm (24")

Weight

- 34 kg (75 lbs)

Clearance requirements:

- Left, right and back 4-12".
- Front, access required at all times.
- Ability to remove power cord at all times from back of instrument.

Environmental conditions

- Temperature: 5 °C to 25 °C / 41 °F to 77 °F
- Out of direct sunlight
- Relative humidity: 40-60% at 25-35 °C
- Pollution degree: 2
- Altitude range: Tested to 820 ft (2500 m) above sea level.
- Installation category: 2

Electrical requirements

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

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

Fuses:

- Main disconnect: 2 x 10A
- Power Supply: 8A
- Terminal blocks: 1 x 3A & 1 x 1A

Startup equipment

In order to perform a synthesis on instrument, 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 left 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.

Gas pressure supply

A high purity argon or nitrogen for bottle supply to maximise life of chemicals and to ensure best quality product (A/N 5.0 / ultra high purity grade maximum).

Collection tubes

Collection vial are needed for post processinf run when synthesis is complete. I screw cap vial is recemmended since it will be under pressure and a snap cap can be forceded open.

Sample dryer

Oligo will need to be dried down after deprotection stage is complete so it can be re-suspended in appropriate media. Please consult Biosearch Technologies. for help in choosing and appropriate unit for application.

Figure 1

Un-interruptible power supply (UPS)

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

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. 1g for phosphoramidites, 1L 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)

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

When first starting, smaller size bottle should be used because of smaller sample runs. Amidites are only viable for one week after dissolution and ancillary reagents (Caps, Activator, ACN, Oxi and Deblock) are stable for up to 3 months under argon/ nitrogen. Once protocols are established larger reagent bottle can be used.

Recommended DNA Amidite Dilution is 1 gram in 20 mLs of ACN or ~0.05M concentration.

Biosearch Technologies can assist with chemical packages tailored to synthesis needs and chemistry.

Reagent preparation

Phosphoramidites will need to be prepared in an inert environment away from moisture. If good techniques are used it not necessary to prepare reagents in an argon filled chamber. Reagent preparation training is available from Biosearch Technologies during instrument installation.

Acetonitrile

Acetonitrile is available in a range of sizes (and quality). To ensure optimal quality in final product, use a acetonitrile with a water content with less than 10pp.

A 4L bottle, which is sufficient for a typical four plate run, when changed allows moisture to enter system. Preferable method is to use a drum as a source, which ensures purity of acetonitrile is maintained over time. It also minimises startup time since bottles do not need to be changed before each run.

Larger Stainless Steel drums and a fitting kit are available through Biosearch Technologies.

Final connection between bottle/drum and instrument will be made upon installation. Please inform Biosearch Technologies with supply information so necessary plumbing connections can be made in advance.

Ventilation requirements

Fumes generated during synthesis are hazardous, and adequate ventilation is necessary to ensure a safe operating environment. One of following is required:

- 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:
Located on left hand side of instrument and necessary due to continuous flow of argon/nitrogen into the Inner Chamber which causes a gradual buildup of pressure as run proceeds. Vent line ensures pressure can be reduced by directing excess gas away to a suitably vented place such as a fume hood.

Starting & ending a run:
When a run is started or completed, it is necessary to open lid to Inner Chamber to load or remove Synthesis Plates. It is critical to have adequate ventilation to clear fumes before operator is exposed.

Waste system:
Although waste system is a closed system it is necessary to replace waste container before each run. When replacing waste container adequate ventilation is required.

Vacuum source:
Fumes from vacuum pump must also be vented. Vent port on pump must be attached to a line running to a suitably vented place such as a fume hood.

Columns

Mermade synthesizes oligos in column format. Synthesizing in this format has the following advantages:

- Ease of use
- Higher Yield
- Better Quality
- Longer Length

If using standard columns with first base derivatized offer additional benefit of being color coded to reduce chance of loading wrong column during setup process. During run set up user is presented with a color coded column map and preparing run simply requires putting appropriate colored column in each location.

Amidite, column and reagent life

Reagents on instrument

DNA Amidites

One week under argon and with a Molecular Trap. May be able to two weeks, however there may be lower yields, due to amidite degradation. Avoid pouring old amidites into a bottle of fresh amidites.

RNA Amidites

Three days under argon and with a Molecular Trap. May be able to stretch to seven days; however, there may be lower yields due to amidite degradation. Avoid pouring old amidites into a bottle of fresh amidites.

Amidites are re-suspended in ACN. ACN needs to have a water content of less than 10 ppm. Water/ moisture will kill synthesis reaction.

Activator

Three months under argon and with a Molecular Trap. Avoid pouring old Activator into a bottle of fresh Activator.

Activator is re-suspended in ACN. ACN needs to have a water content of less 10ppm. Water/Moisture will kill synthesis reaction.

Acetonitrile (ACN)

Three months under argon and with a Molecular Trap.

Deblock, Cap A and Cap B and Oxidizer

Three months on instrument under Argon

Reagents off instrument

Shelf Life of Sealed, Unopened Reagents in Bottles:

- Activator: One year
- Deblock, Cap A and Cap B: One year
- Oxidiser: One year
- ACN: One year

Shelf Life of Amidites & Columns:

- Six months at +4 °C
- Two years kept at -20 °C – probably longer.

Note: Shipping amidites in dry ice is not necessary. Excessive heat (+100 °F), during shipment of dC(Ac) synthesis column, could cause protecting group to "fall off", resulting in a lower synthesis yield.

Chemical safety

SDS

Most chemicals used on instrument are hazardous to varying degrees. Safety Data Sheets provide a detailed summary regarding safe handling and storage of each chemical used including:

- 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

Notice

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

Waste disposal requirements will vary by location, follow local and federal regulations regarding storage and disposal of waste.

Always wear safety goggles and gloves and ensure that area is well ventilated when removing waste. Spill clean-up kits should be located near waste removal area and operators should know location of nearest first aid and eye wash stations.

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

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

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 column plates which are then mounted on to a dual axis slide which moves to align different reagent injection pins with each active column in appropriate order. A vacuum is applied to each plate in such a way that plates may be independently drained. During course of synthesis, argon/nitrogen is continually introduced into synthesis chamber and allowed to flow out through a small vent into chamber. This ensures that any residual vapors are removed after each injection cycle to maintain an optimal synthesis environment by removing excess water.

Software and machine operation

A PC running MerMade synthesis software provides the user interface for controlling synthesizer. Software controls all aspects of instrument during synthesis, 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 how instrument synthesises oligos. Script files can be created and modified using supplied Script File Editor.

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

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

Report Log: File is a short run summary which gives important information about synthesizer set up. Files may be referred to at a later date to aid analysis or protocol development.

Software also facilitates routine maintenance of instrument via a series of Service Screens. These features, control low level services such as Motion and Valve Settings, and can be password protected.

Notice

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

Air supply

⚠WARNING

Do not exceed maximum air pressure. Exceeding maximum air pressure may cause damage to instrument.

Electrical power supply

⚠WARNING

Connect instrument to a suitable electrical power supply in a safe and reliable manner in accordance with local electrical safety regulations. Instrument is intended to be hard wired to an electrical supply or connected using a suitable industrial electrical connector.

Instrument must be grounded.

Instrument installation

1. Position instrument in area of use.
2. Plug instrument into main electrical supply.
3. Connect instrument to argon.
4. Connect computer to via Ethernet port.
5. Turn on instrument and computer.

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)*.

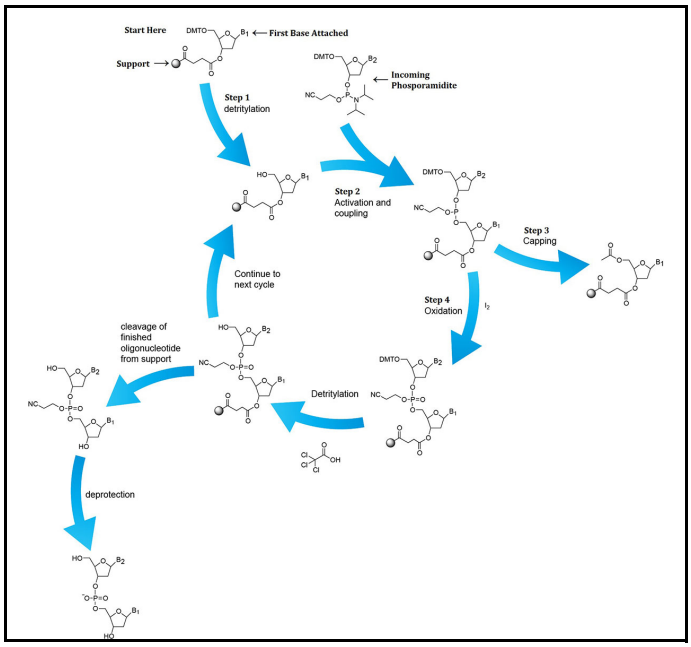

Figure 1

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)*.

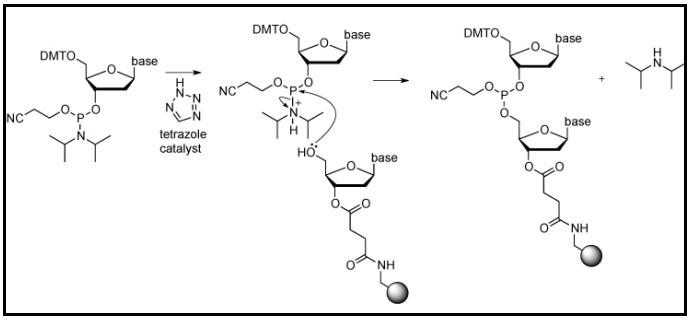

Figure 2

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)*.

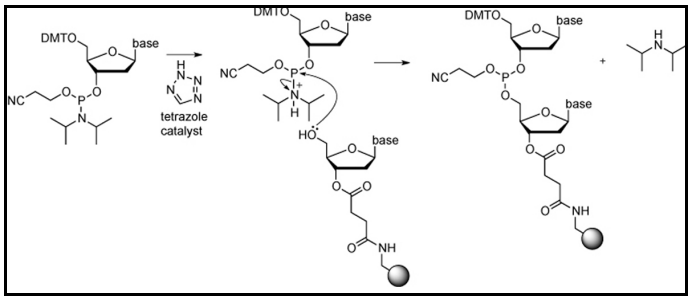

Figure 3

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)*.

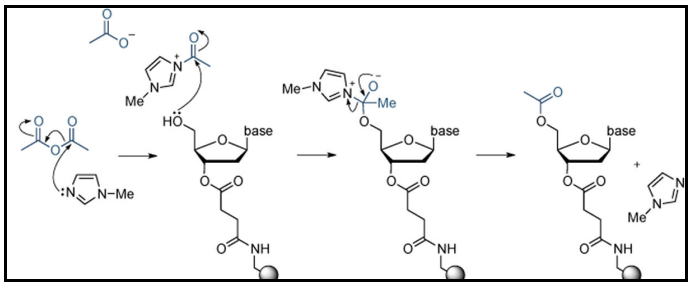

Figure 4

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)*.

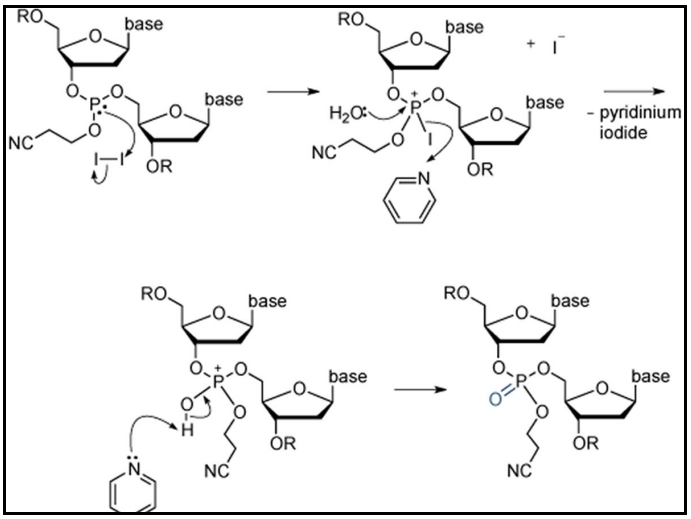

Figure 5

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 48X operation

⚠WARNING

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

Software overview

Notice

Biosearch Technologies accepts no responsibility for misuse of instrument.

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

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

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

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

Example of Plate Log *(Figure 1)*.

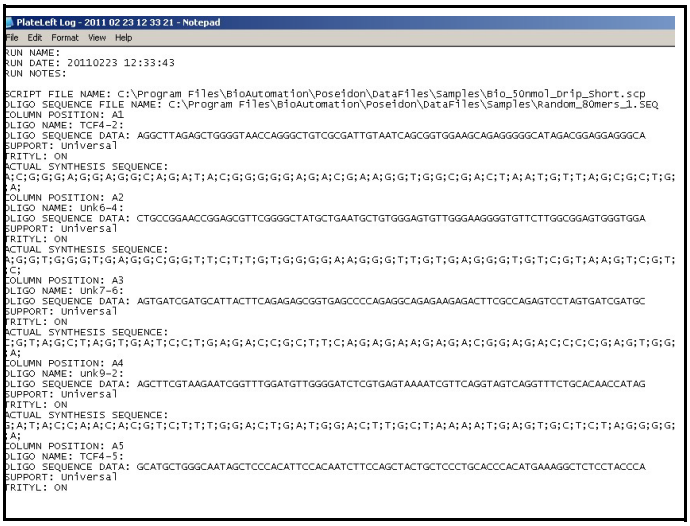

Figure 1

Synthesis process

Synthesis is typically carried out from 3'->5' end of oligonucleotide. Reactions take place in columns using a controlled pore glass (CPG) or polystyrene substrate (PS) contained between two frits. Operator 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 operator through setup process to start synthesis. Usually, before synthesis begins, chamber is flooded with argon/nitrogen to create a moisture free environment. When argon/nitrogen purge initialisation step is completed, software moves XY table to align each column well under appropriate injection lines 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 operator in script file. Reagents are then removed from column(s) by applying vacuum to underside of synthesis plate.

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

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

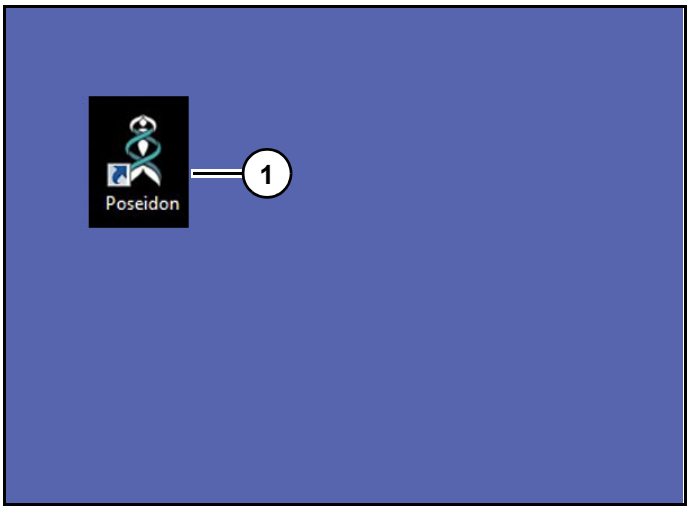

Figure 2

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

Initialisation screen

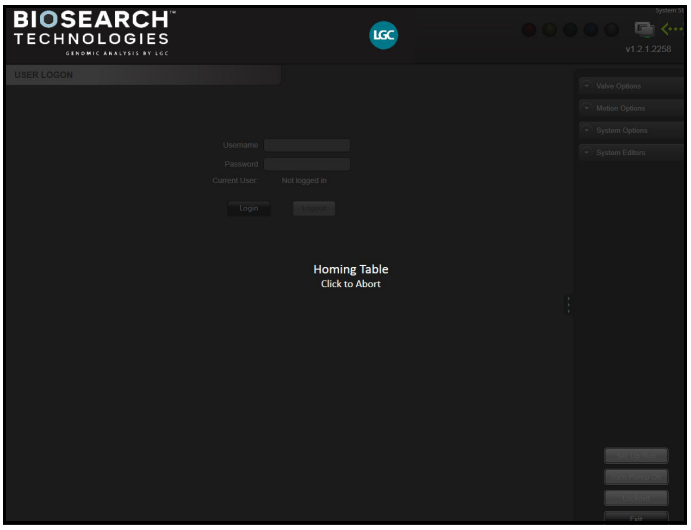

Figure 3

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

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

Log in screen

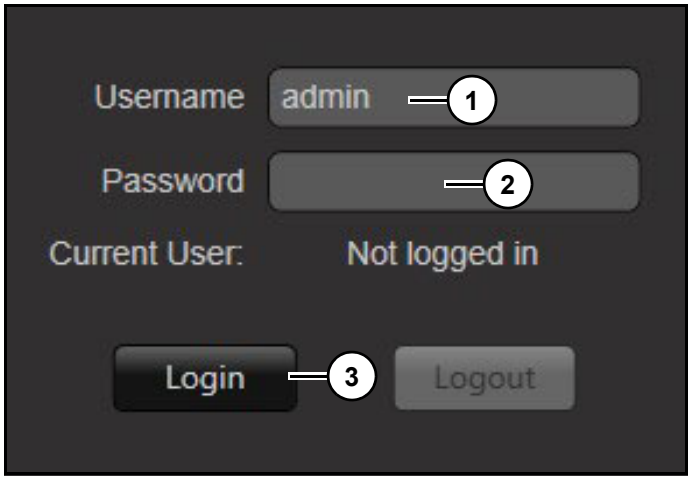

Figure 4

Refer to *(Figure 4)*.

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

Main screen

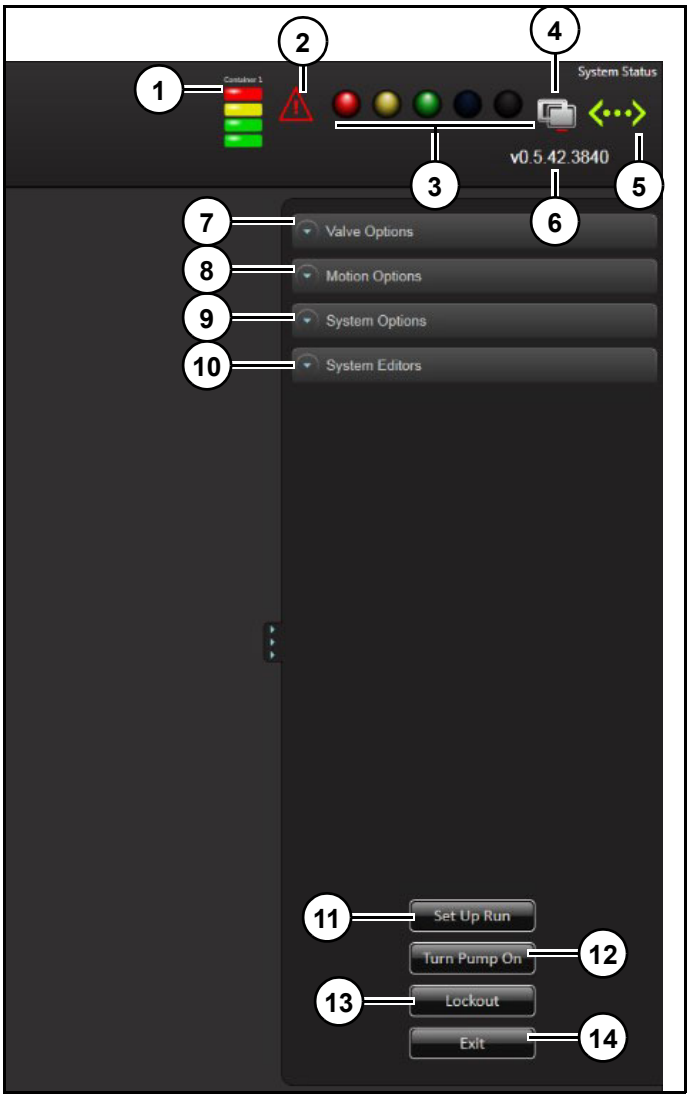

Figure 5

Refer to *(Figure 5)*.

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

Error indicator (2): Displays instrument error.

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

Remote connection (4): Indicates remote connection.

Communication status (5): Displays communication status.

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

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

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

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

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

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

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

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

Exit (14): Exits software.

Valve Options screen

Accesses controls involving liquid, vacuum and argon/nitrogen purge valves.

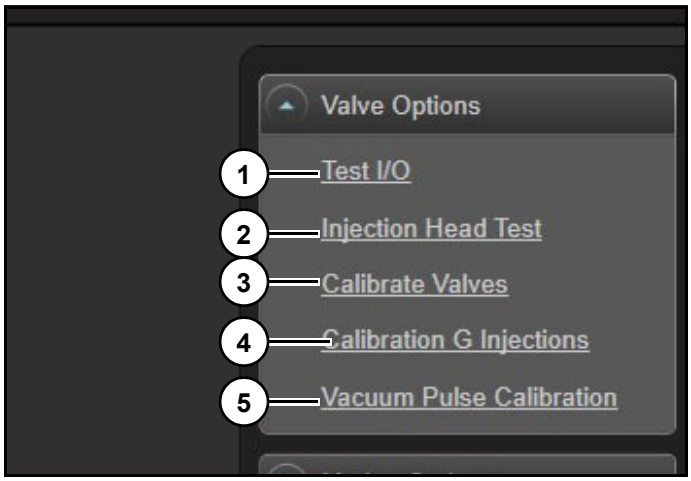

Figure 6

Refer to *(Figure 6)*.

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

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

Calibrate Valves (3): Opens valve calibration screen.

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

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

Test I/O screen

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

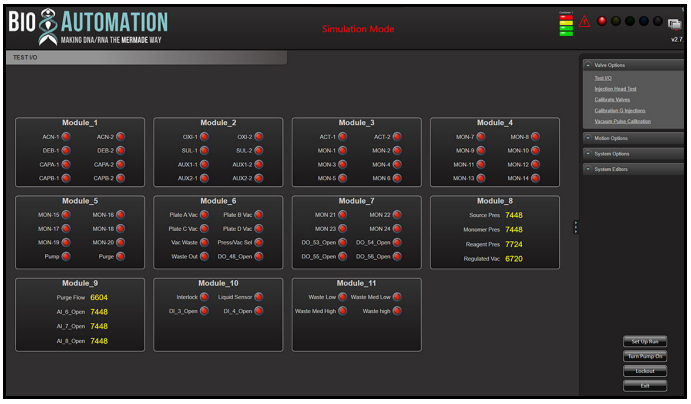

Figure 7

Refer to *(Figure 7)*.

To test a valve:

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

Note: Value will stay open until button is clicked again. In case of emergency operator can turn off main power on back if instrument to shut all valves off.

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

Injection Head Test screen

Allows user to test and prime lines.

⚠WARNING

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

Always wear safety goggles and gloves when using this screen.

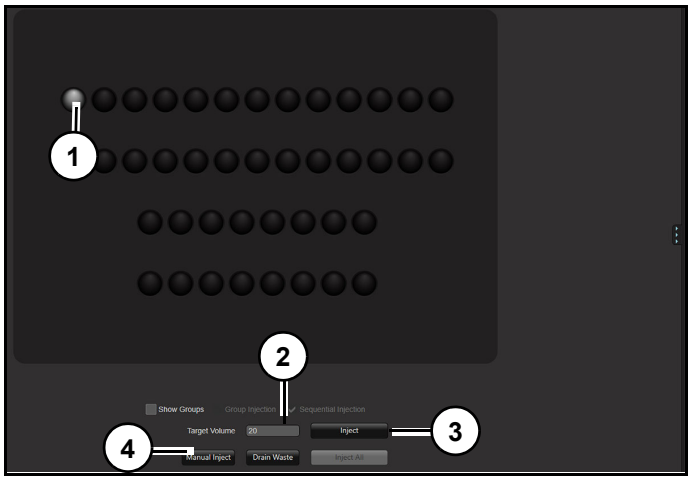

Figure 8

Refer to *(Figure 8)*.

To fire a single valve:

1. Select one valve in displayed valve array (1).
2. Enter a volume (2).
3. Click "Inject" (3)Note: Note: Pressing and holding manual inject button (4) will open selected valve until button is released. This is often used for priming lines.

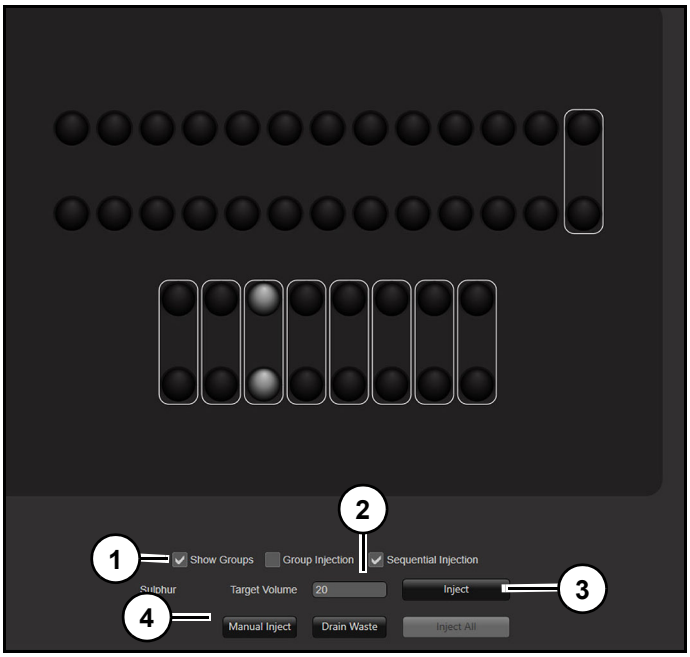

Figure 9

Refer to *(Figure 9)*.

To test a row of reagents:

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

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

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

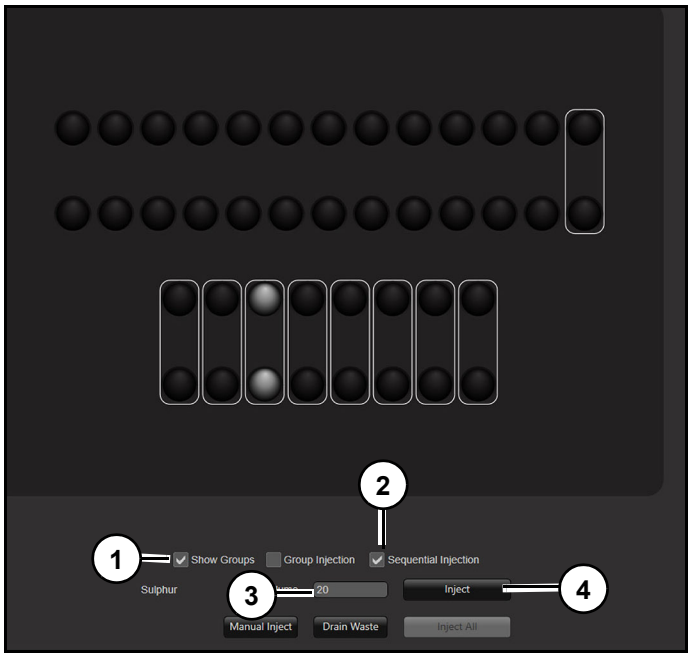

Figure 10

Refer to *(Figure 10)*.

To test valves in group individually:

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

If "Sequential Injection" (2) is checked, valves will be fired in group, both at same time.

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

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.

Each valve can be calibrated individually when injected by itself and when injected as part of a group. Amidite/reagent pressure must be keep stable to insure calibration accuracy.

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

Liquid dispensing valves 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.

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 a curve information created from the 2 closest calibration points for Aux 2 valve.

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

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

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

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

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

Calibration by time:
Valve will remain open for set time (ms) to reach desired volume. User must measure dispensed volume to determine if time that valve remains open is correct. If delivered volume is not enough, valve open time can be 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 must be calibrated manually by using an Eppendorf tube and a pipette, or with a scale if by weight selection is preferred.

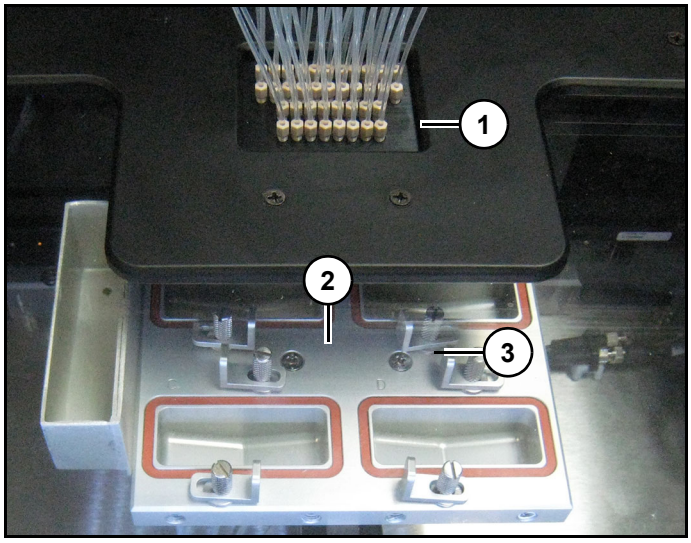

Figure 11

When performing valve calibration it is easier to open and raise Injection Head (1) *(Figure 11)* so that injection lines are more accessible.

1. Collect injection with a small Eppendorf tube.

Measure actual delivered volume with a pipette or measure weight by scale and then adjust Weight (mg) / Volume (µl) / Duration (ms) of valve until correct volume is delivered.

Switching calibration methods

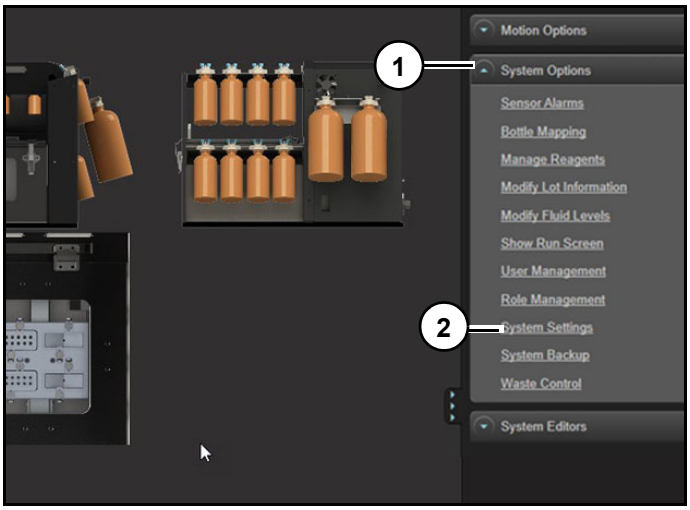

Figure 12

Refer to *(Figure 12)*.

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

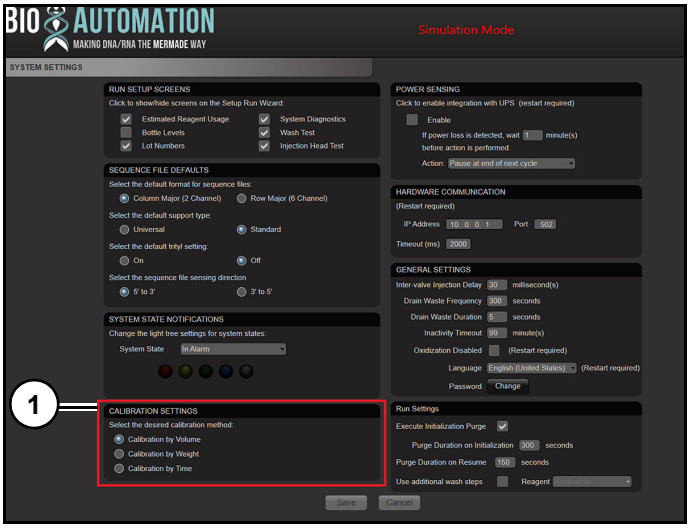

Figure 13

3. Select desired calibration method (1) *(Figure 13)*.

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

Adding calibration points

⚠WARNING

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

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

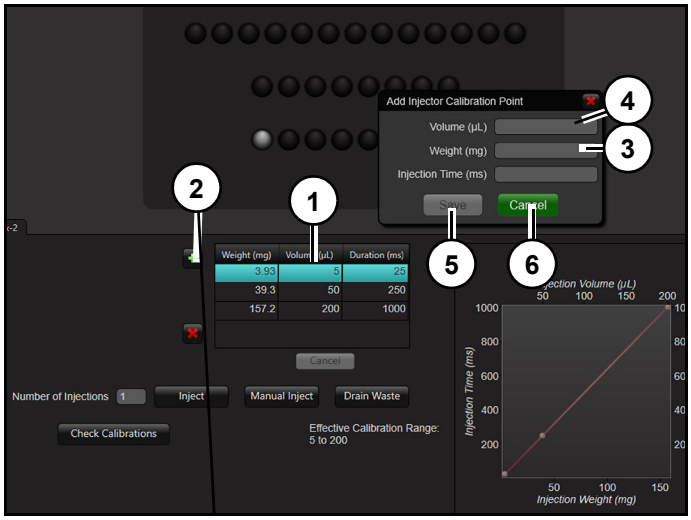

Figure 14

Refer to *(Figure 14)*.

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

To add calibration point:

1. Click "Add Cal Point" (2).
2. Enter estimated time (ms) in "Injection Time" (ms) (3).
3. Enter estimated delivery volume (µL) in "Injection Volume (µL)" (4).
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. Then change desired colume in calibration table (1).

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

5. Place 2ml tube under correct injection pin with lid open.

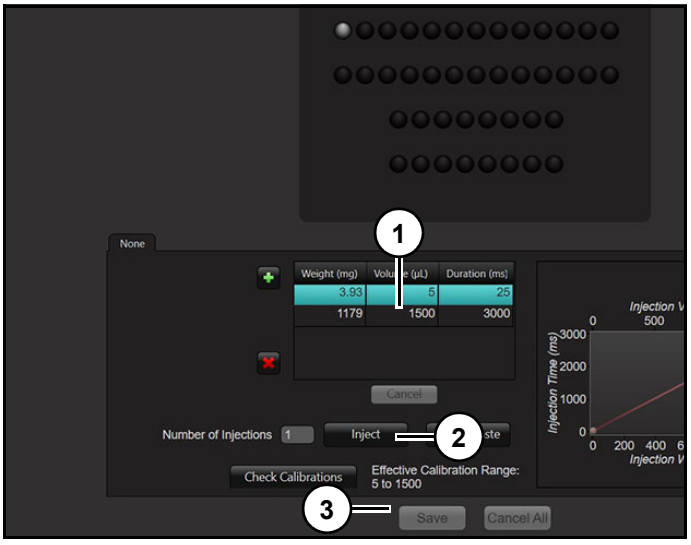

Figure 15

6. Click "Inject" (2) *(Figure 15)*. Instrument will open valve for 3000ms, injecting liquid into collection vile.
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. *(6.)*

Note: Some liquids are more difficult to measure due to viscosity and volatility. Deblock and Oxidizer is

most difficult. Repeat procedure until a confident measurement is obtained.

8. Click "Save" (3) *(Figure 15)*. To calibrate another point, highlight appropriate line with a mouse click.

Updating calibration point

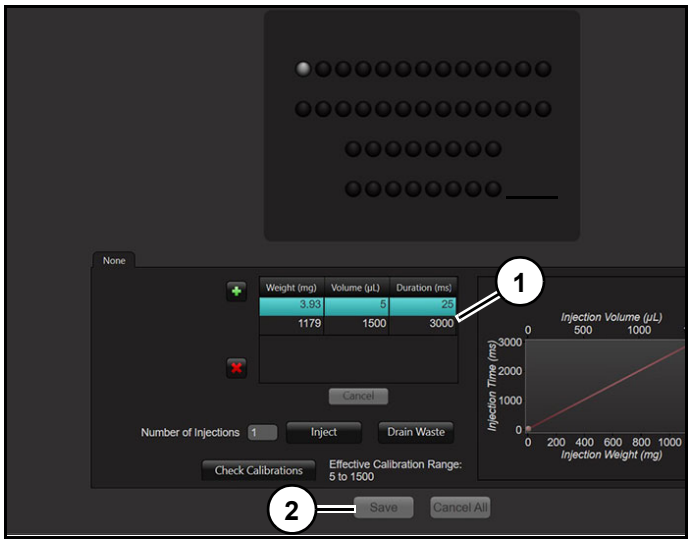

Figure 16

Refer to *(Figure 16)*.

1. Select calibration point (1) to change.
2. Change desired settings.
3. Click "Save" (2).

Deleting calibration point

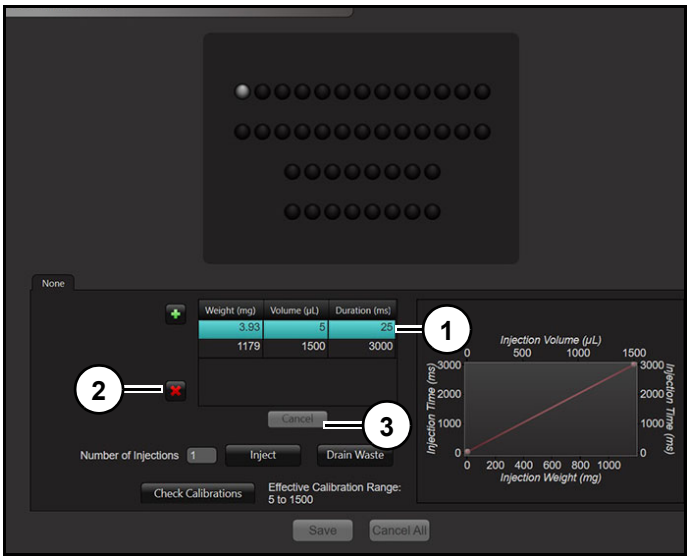

Figure 17

Refer to *(Figure 17)*.

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

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

Checking calibrations

Allows user to check calibration curve for each valve.

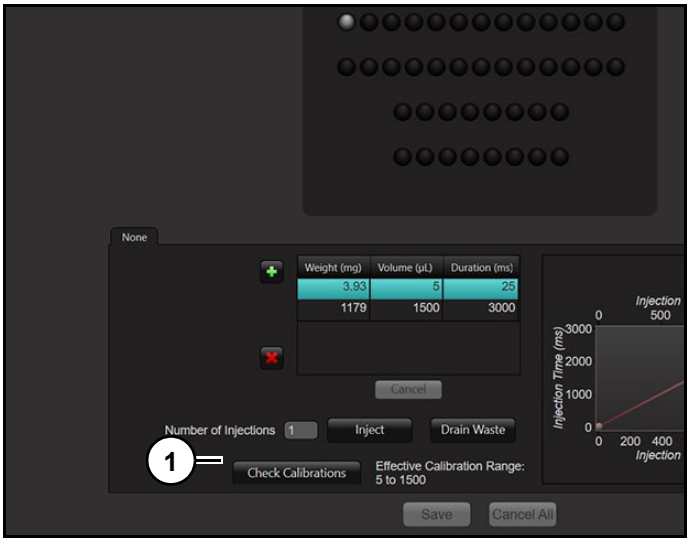

Figure 18

1. Click "Check Calibration" (1) *(Figure 18)*.

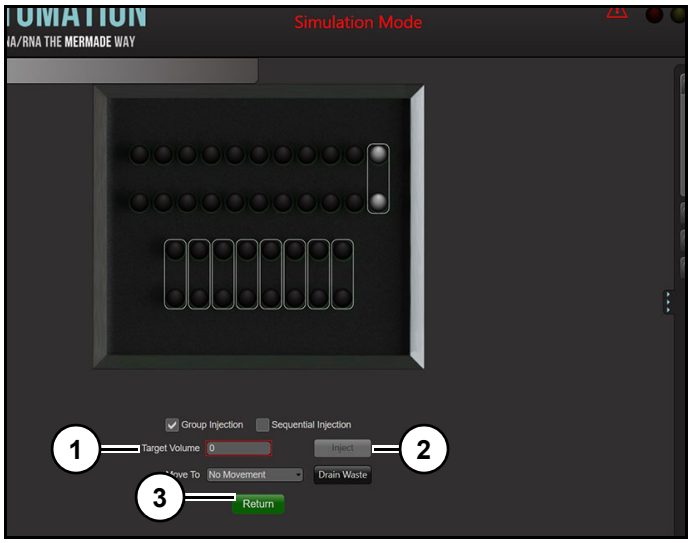

Figure 19

Refer to *(Figure 19)*.

For example, Oxidizer Valve is calculated for 1µL for 10 µL and 250 µL. Clibration curve is tested with a target volume of 100 µL.

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

Drain waste

Allows user to drain waste tray from instrument into waste container.

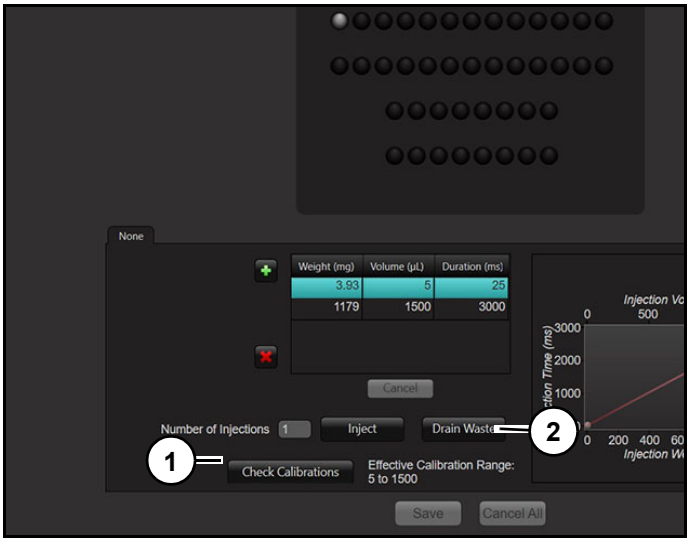

Figure 20

Refer to *(Figure 20)*.

1. Click "Check Calibration" (1).
2. Click "Drain Waste" (2).

Calibrate G injections

Calibration of valves in an array (group) is known as G or Group Calibration. Valves are calibrated on a curve like single valve calibrations. Default points are standard in software and user must add, remove, update, and delete points as necessary. G Calibrations are utilised when script file calls for a "multiple" injection of a group of valves requiring same volume. If both valves aren't required, or the volume injected are not the same for both valves, then single injections will be used.

Calibrating a group injection

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

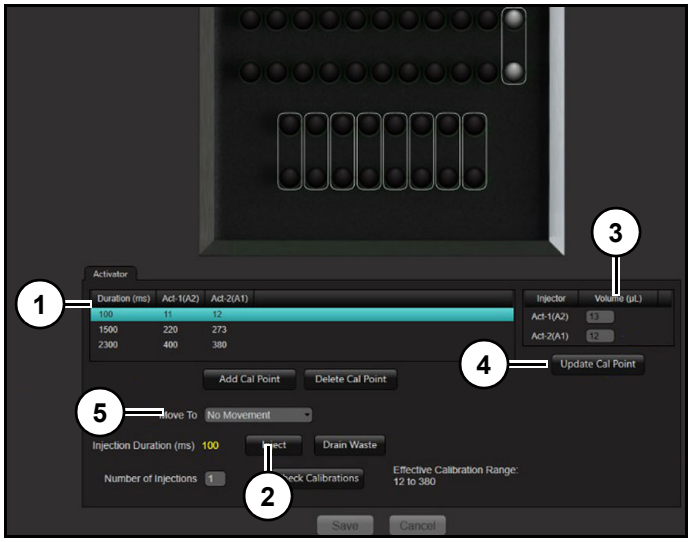

Figure 21

Refer to *(Figure 21)*.

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

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

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

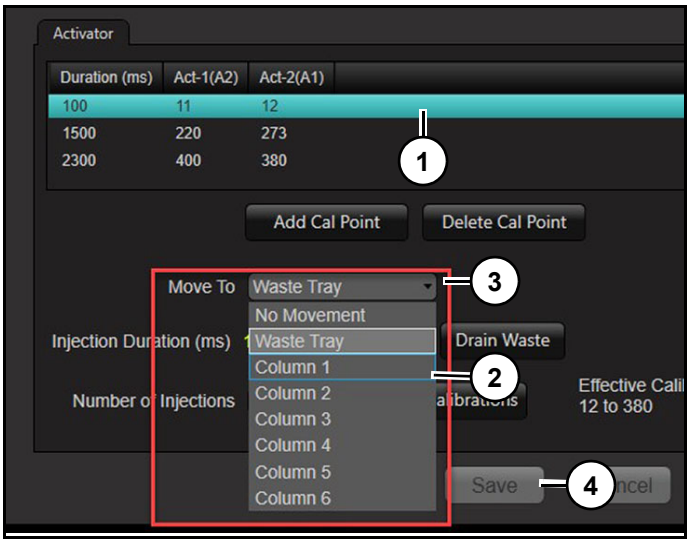

Figure 22

Refer to *(Figure 22)*.

For example:

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

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

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

Vacuum Pulse Calibration screen

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

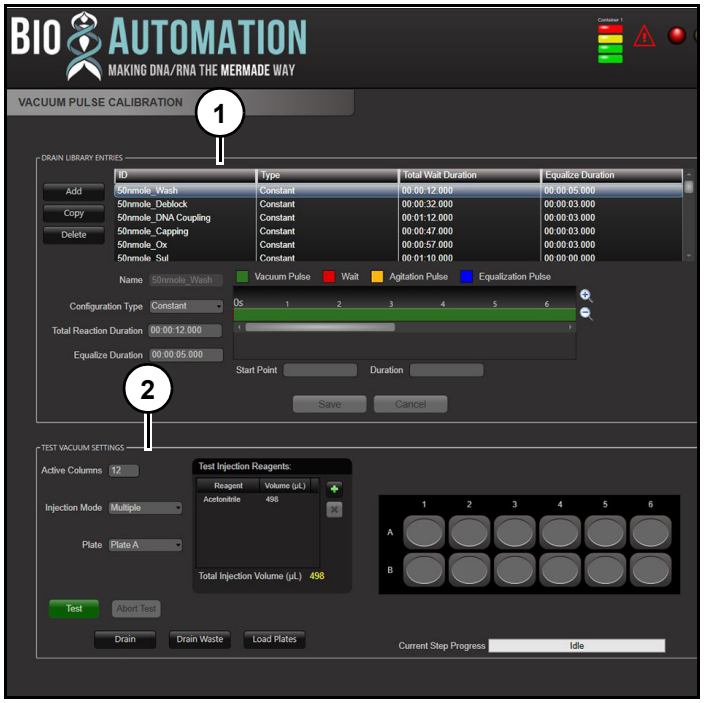

Figure 23

Refer to *(Figure 23)*.

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

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

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

Types of Vacuum Pulses

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

Pulse types:

Constant Vacuum Pulse.

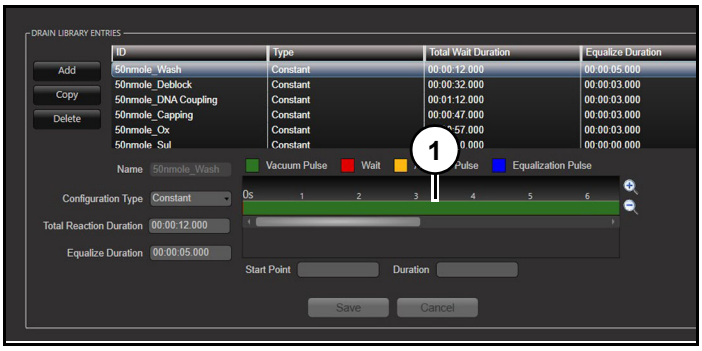

Figure 24

A constant vacuum pulse (1) *(Figure 24)* is a pulse that applies same length of vacuum to column chuck regardless of number of active columns. This is default vacuum setting when a new pulse is added to drain library and used most often. There is no compensation for dropped columns and is possible to find a good working range where dropouts do not negatively affect other columns.

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

Fixed Increment.

Figure 25

Refer to *(Figure 25)*.

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

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

Vacuum Pulse Structure screen

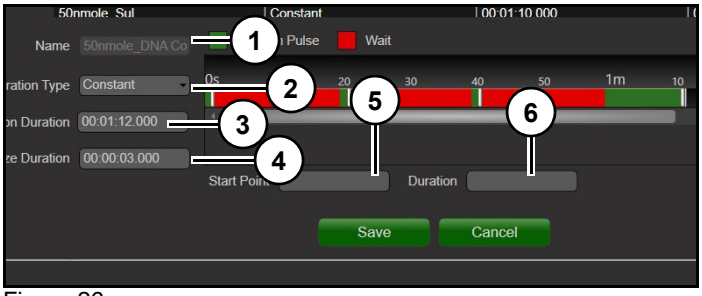

Figure 26

Refer to *(Figure 26)*.

Name (1): ID of specific drain.

Configuration (2): Type of drain pulse.

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

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

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

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

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

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

Adding/removing a vacuum pulse

There are four options:

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

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

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

Delete: Deletes current yellow or green selected pulse box.

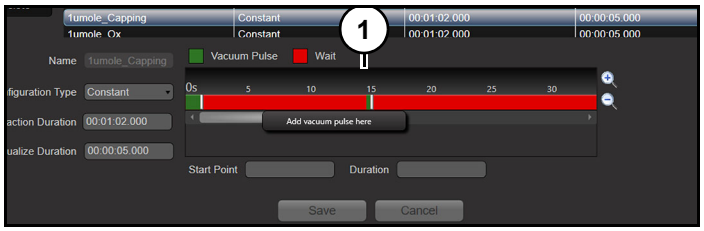

Figure 27

Refer to *(Figure 27)*.

1. Right click on one of boxes (1).

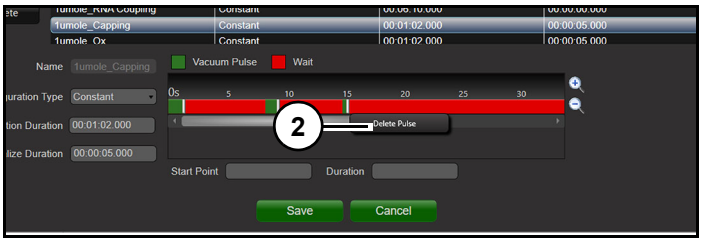

Figure 28

2. Select a pulse option (2) *(Figure 28)* from menu.

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

Editing Pulse Segments

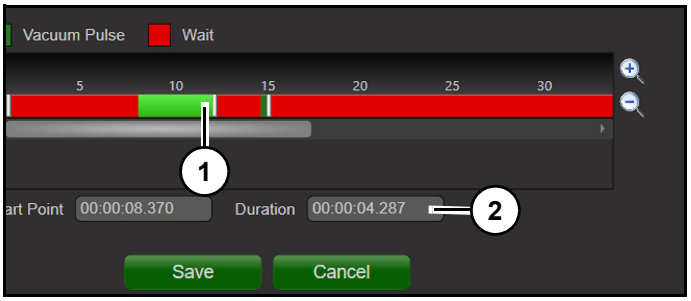

Figure 29

Refer to *(Figure 29)*.

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

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

Test vacuum settings

After calibration, vacuum pulses can be double checked using test vacuum settings box *(Figure 30)*.

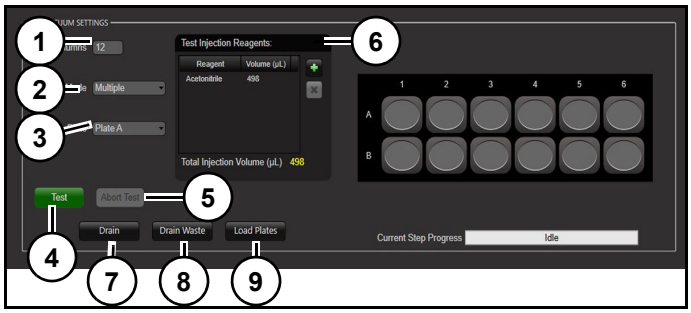

Figure 30

Refer to *(Figure 30)*.

Active Columns (1): Allows user to change the number of columns that will have reagent dispensed into them during to vacuum pulse test; useful for checking fixed increment vacuum pulse options and <12 column plate calibrations. Injections will start in row one.

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

Plate (3): Choses plate to be tested.

Test (4): Tests selected vacuum pulse setting for calibration verification. Includes injection of a specified reagent at a certain volume followed by selected vacuum pulse step. Allows user to test and adjust vacuum pulse settings so that reagents flow through column without premature draining or under draining. Once settings are accepted users can make observations during a run in order to fine tune settings.

Abort Test (5): Aborts test during execution.

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

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

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

Drain Waste (8): Drains waste tray.

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

Testing vacuum pulses

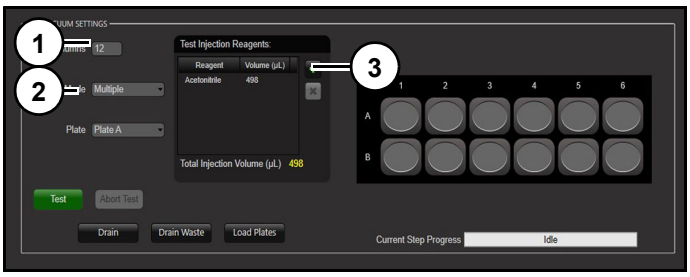

Figure 31

Refer to *(Figure 31)*.

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

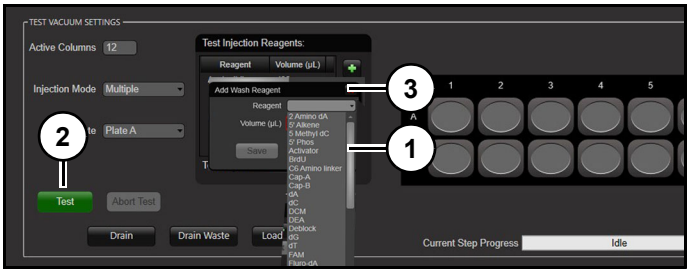

Figure 32

Refer to *(Figure 32)*.

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

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

5. Click "Test" (2).

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

Motion Options

Allows user access to motion options (1) *(Figure 33)*,. Option available are: Set Reference Positions, Set Table Parameters, and Motion.

Figure 33

Set Reference Positions

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

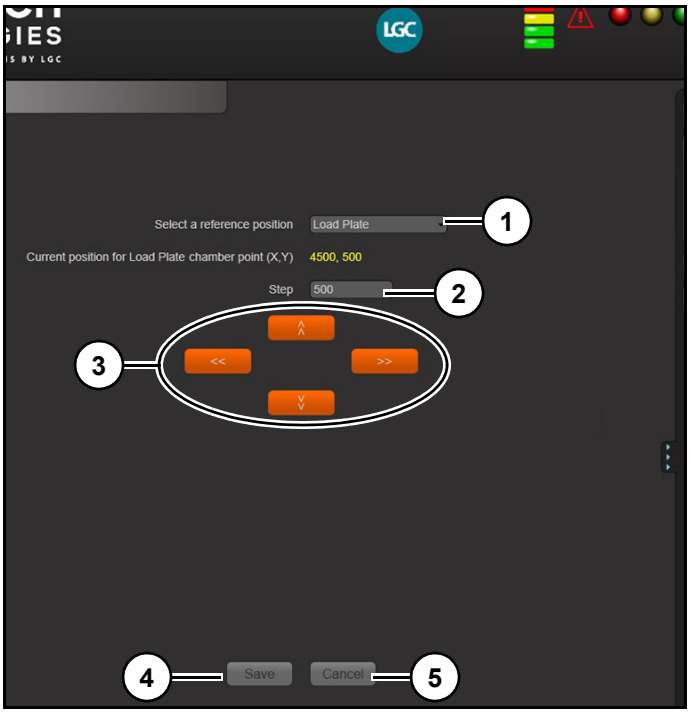

Figure 34

Changing reference positions

User tells instrument where to start by aligning injection head. Alignment can be changed in Injection Head Align screen *(Figure 34)*.

To adjust injection head for plate A:

1. Select plate A from "Select a reference position" (1).
2. Enter a value in "Step" (2), for example 500.
3. Press "←, →, ↑, ↓" (3) to move plate left, right, up, or down in relation to user. Farthest left monomers on injection head and column A1 and B1 on plate A should be centered, so any injected reagents will go into the columns without causing spills.
4. Click "Save" (4) to save position.

Note: Click "Cancel" to undo changes.

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

Set Table Parameters

Notice

Failure to adhere to instructions can cause damage to motion system.

Determines speed at which slides will move during various operations. Values are set at factory and should not be changed unless instructed so by Biosearch Technologies. *(Figure 35)*

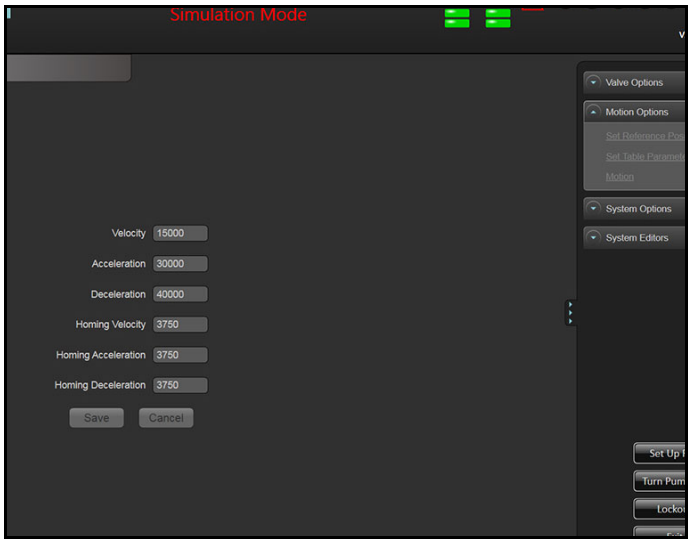

Figure 35

Motion

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

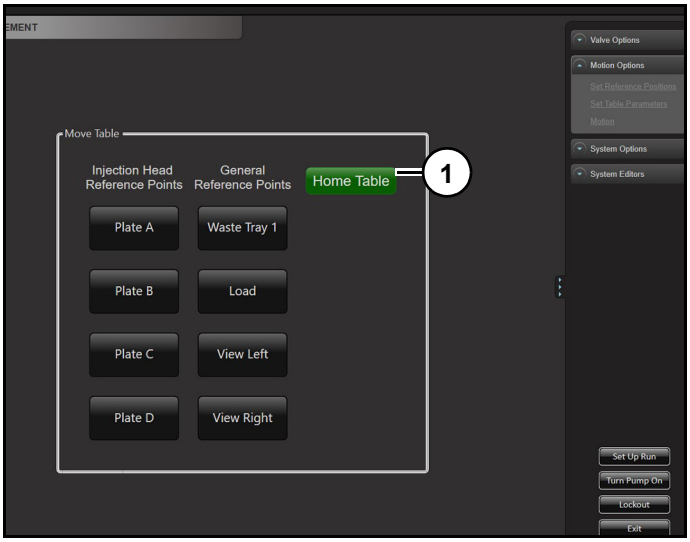

Figure 36

Refer to *(Figure 36)*.

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

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

System Options

Allows user access controls involving system related options *(Figure 37)*:

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

Figure 37

Sensor Alarms

Contains settings relating to sensor information coming from instrument

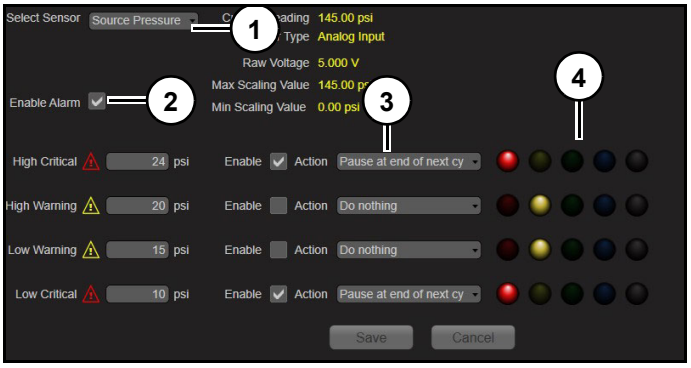

Figure 38

Refer to *(Figure 38)*.

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

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

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

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

- Do Nothing: No action is taken upon conditin trigger. Light tree will still be changed, and sensor events recorded in log files. Useful for troubleshooting.
- Pause After Current Step: Pauses synthesis after current step. Allows current injection and drain steps to complete then instrument will be paused.
- Pause After Next Wash: Pauses after next Wash Step. Will finish current injection and will continue until it encounters a wash step in script file. After that wash step is executed including injection and drain instrument will pause.

- Pause at End of Cycle: Pauses at end of cycle for current base (safety pause). Will continue until end of current base addition and then pause, typically after the last wash step and just before a deblock step. Safest place to pause instrument from a chemistry perspective.
- Pause Immediately: Pauses as soon as sensor is triggered. Least desirable and least stable point to pause. Typically, only sensors set to pause immediately is interlock sensor and liquid sensor.

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

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

High Critical: Highest alarm.

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

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

Low Critical: Lowest alarm.

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

Interlock Sensor

⚠WARNING

Do not insert hand into a running instrument, doing so could result in serious injury. Ensure it 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.

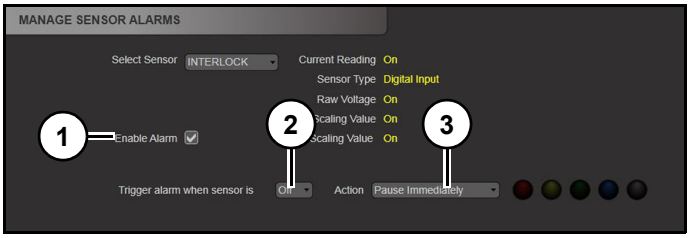

Figure 39

Refer to *(Figure 39)*.

Interlock sensor factory settings:

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

Liquid Sensor

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

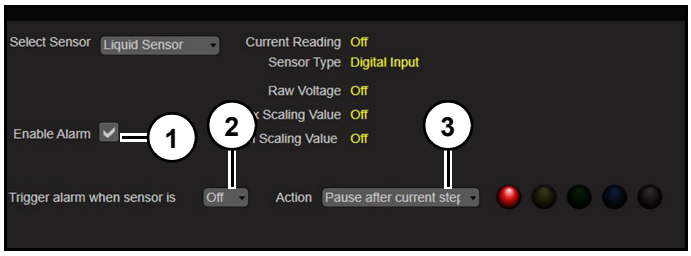

Figure 40

Refer to *(Figure 40)*.

Liquid sensor factory settings:

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

Source Pressure Sensor

Notice

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

Notice

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

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

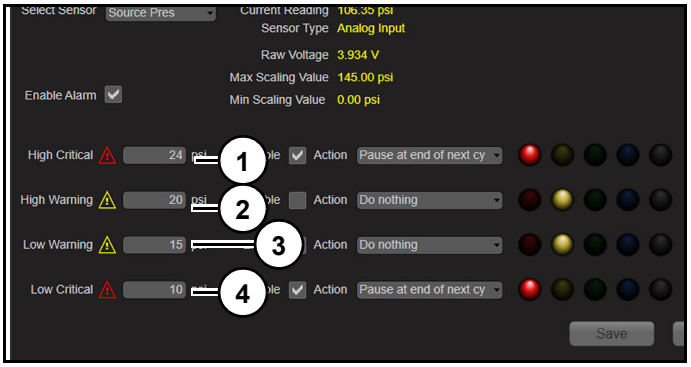

Figure 41

Refer to *(Figure 41)*.

Pressure sensor factory settings:

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

Source pressure is adjusted using source pressure regulator. Actual source pressure can fluctuate slightly without need to recalibrate valves, as 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 12 psi.

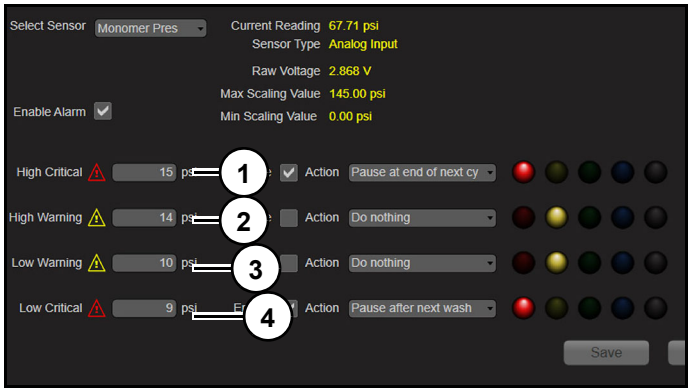

Figure 42

Refer to *(Figure 42)*.

Monomer sensor factory settings:

High Critical (1): 15 psi
High Warning (2): 14 psi
Low Warning (3): 10 psi
Low Critical (4): 9 psi

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

Note: Monomer bottle pressures should not be set higher than 15 psi and lower than 10 psi to eliminate splashing during reagent dispensing.

Note: Activator and amidite valves are held closed by pressure. If bottle pressure drops below 10 psi, valves can start leaking from the injection nozzles.

Reagent Pressure

Notice

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

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

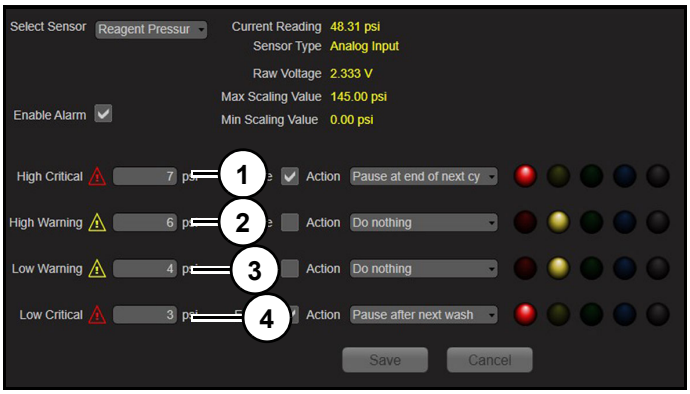

Figure 43

Refer to *(Figure 43)*.

Reagent sensor factory settings:

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

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

Regulated Vac

Monitors vacuum system during synthesis.

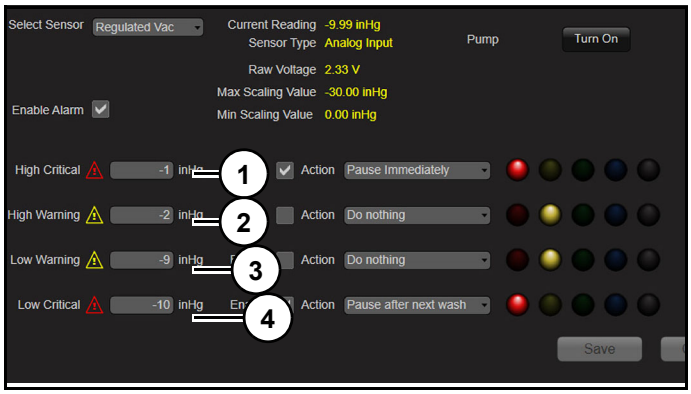

Figure 44

Refer to *(Figure 44)*.

Vacuum flow factory settings:

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

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

Purge Flow

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

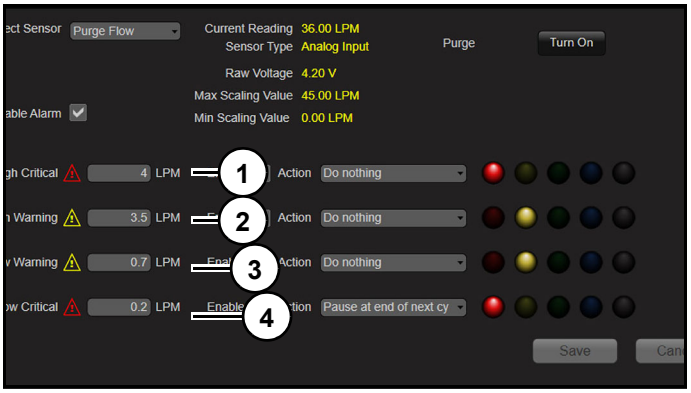

Figure 45

Refer to *(Figure 45)*.

Purge flow factory settings:

High Critical (1): 4 LPM
High Warning (2): 3 LPM
Low Warning (3): .7 LPM
Low Critical (4): 0.2 LPM

Bottle Mapping

Screen 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.

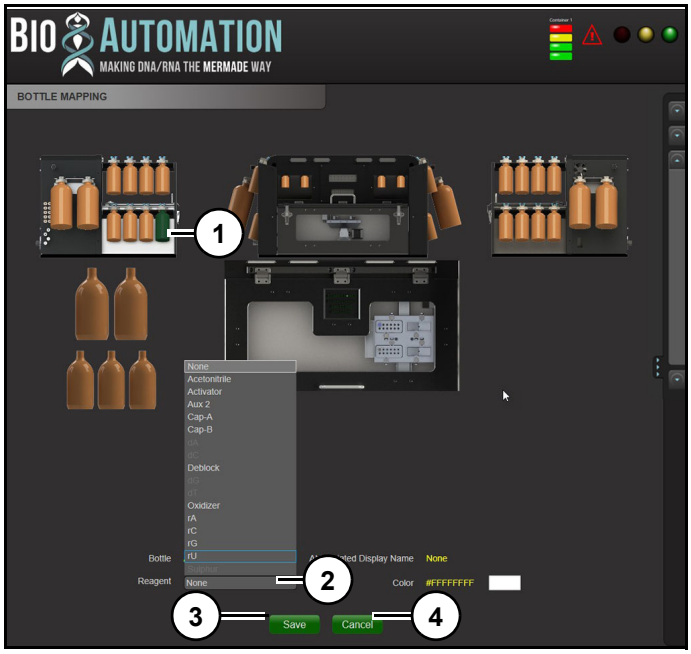

Figure 46

Refer to *(Figure 46)*. Can change due to instrument configuration.

Each bottle is mapped on instrument to injection head in a default pattern. If user would like to change a reagent or monomer to a differnt one that reagent will need to be physically changed in bottle on synthesizer and in software. When a bottle is selected, an 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.

Note: Same reagent/monomer cannot be mapped to multiple bottle positions at the same time. To move a reagent to a different position, select the original position and set the reagent to "None". Now the reagent is ready to be mapped to another position.

To change default reagent configuration:

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

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

Reagents can be added where desired and some rules apply:

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

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.

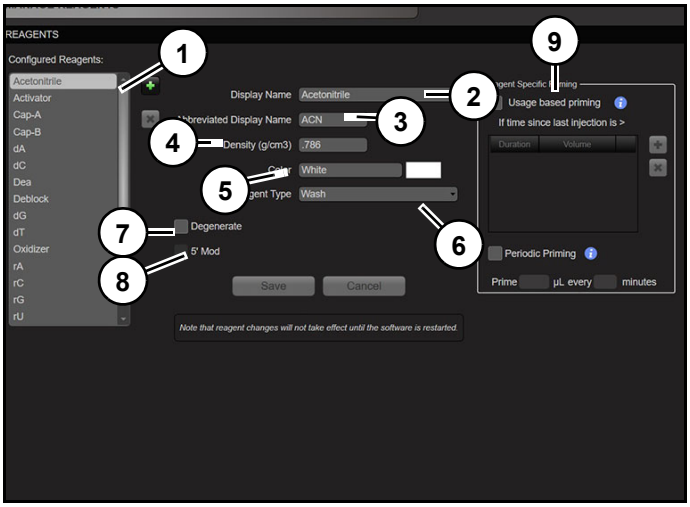

Figure 47

Refer to *(Figure 47)*.

Click on reagent (1) to view properties.

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

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

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

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. Used to narrow choices when in script editor and other screens. This serves as a contingency to prevent human error while building scripts.
Example: When adding a reagent to a Deblock step in script only reagents programmed to be of deblock type will be displayed.

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

5′ Mod (8):

Allows user to designate a monomer as a 5" modification. Monomer can still be used internally and will behave as any other monomer if it is not the last base added to oligo.
If 5' mod is detected as the last base addition of that specific monomer the coupling of the 5' mod will be postponed until all oligos in the synthesis plate are ready for the addition . Saves in reagent from priming waste, and reduces run time for slow coupling modifications.

Reagent Specific Priming (9): Priming parameters for selected reagent. Can be set individually for each 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.

Note: Often referred to as "Global Priming"

Adding Reagents

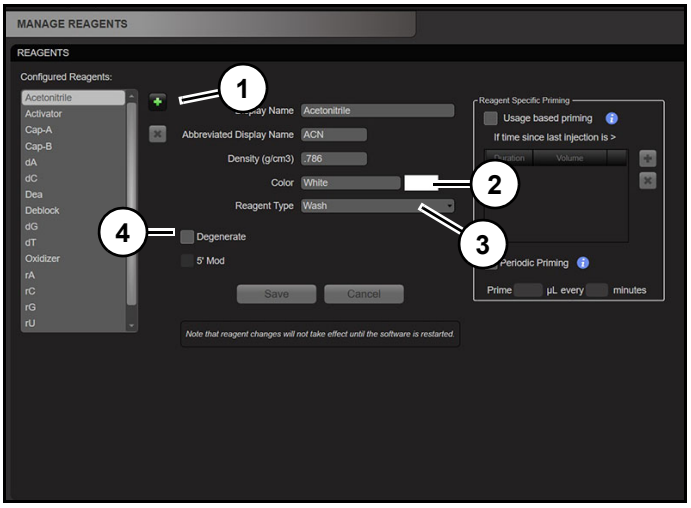

Figure 48

1. Click "+" (1) *(Figure 48)*.

Figure 49

Refer to *(Figure 49)*.

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 48)*

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

Adding Degenerate Base

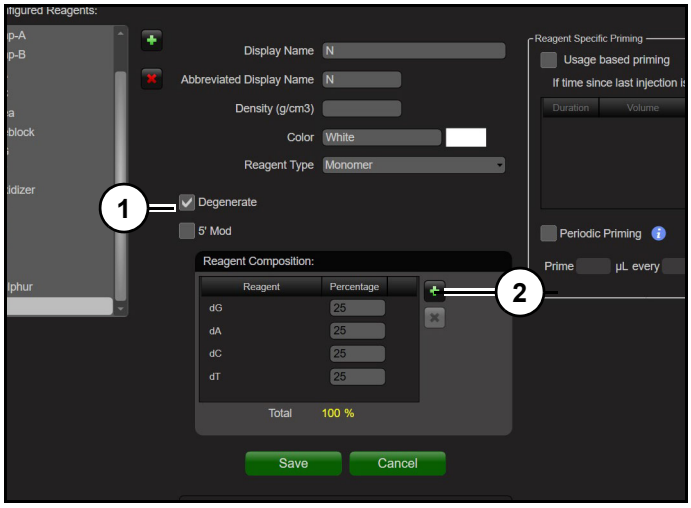

Figure 50

Refer to *(Figure 50)*.

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%. Reagent volume of selected reagents must be within calibrated range.
Example: In a coupling step if 100µl 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.

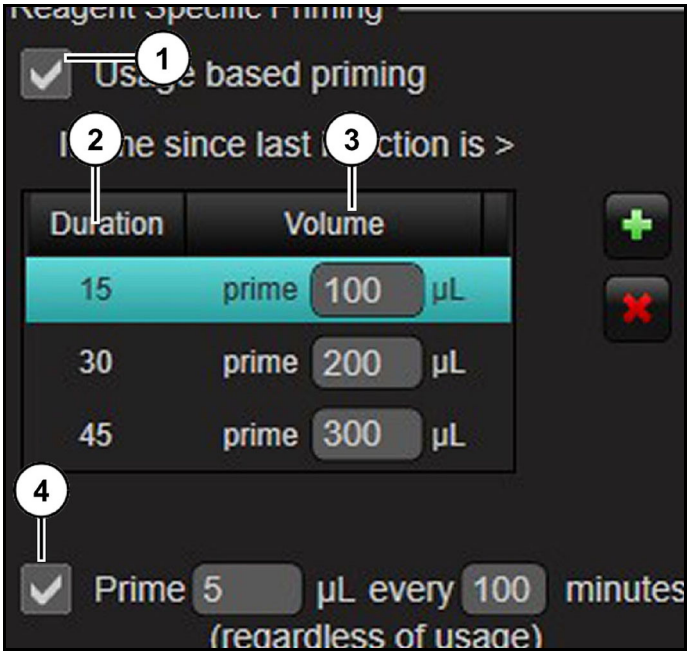

Figure 51

Refer to *(Figure 51)*.
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 in the last 15 minutes, reagent valve will be open, and line will be primed for 100 µL the next time a prime is called for in script file.

User can enter multiple time and volume combinations.
Example: User can designate a reagent to prime 100 µL if it has been more than 15 minuted since last use. 200 µL if it has been more than 30 minutes and 300 µL if it has been more than 45 minutes. This allows user to increase priming based on time, if reagent has had more time to crystallise due to lack of use or volatility. Prining value must be within min/ max calibrated value in calibration table.

Note: 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. Subsequent primes in that step will inject as a set in script file. These primes are recorded in log file as soft primes.

If lower box Periodic Priming (4) is checked, then reagent line will be primed every 5 minutes for 100 µL regardless if that reagent has been used during that time frame or not.

Note: Interval and volume can be customized.

It will periodically prime reagent if it is used at any point during current synthesis, regardless of when it is used. Primes will take place during first priming cycle of any reagent after periodic priming interval for specific reagent has been reached. 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 reagent specific priming parameters (Global priming) 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.

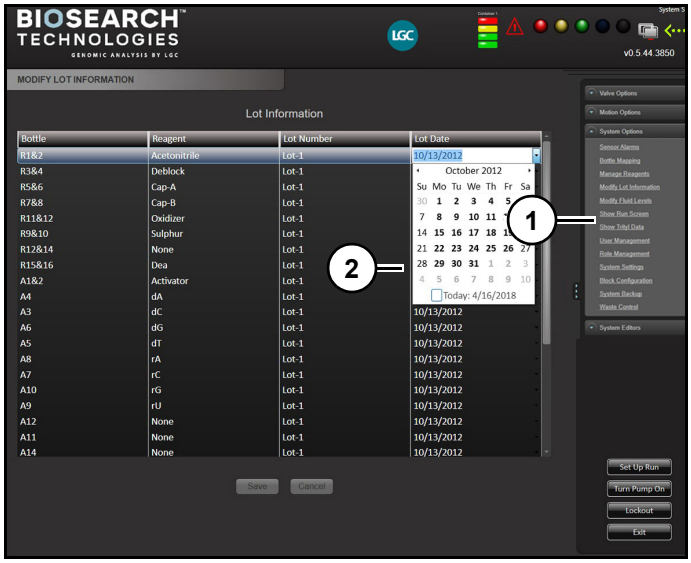

Figure 52

Refer to *(Figure 52)*.

Modify Lot Information (1) is used to track reagent batch information on instrument. It records reagent, mapped position, lot number, and when reagent was added to instrument to each run log generated after a synthesis is completed. By clicking date section a pop-up calendar (2). When entering a new lot number, date will automatically be updated. .

Modify Fluid Levels screen

Allows user to track fluid levels.

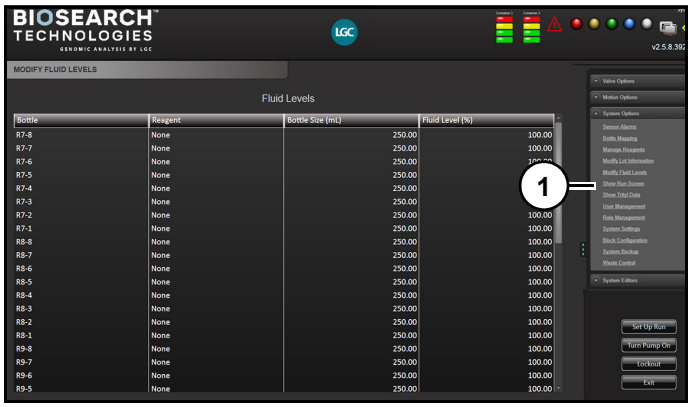

Figure 53

Modify Fluid Levels (1) *(Figure 53)* is used to track fluid levels of bottle positions. Bottle size can be entered, and after each run, user can manually subtract amount used in previous run. Helps keep track of chemical consumption and ensure user checks reagent levels to prevent failed runs due to insufficient chemicals.

Note: Fuild level screen is only as accurate as calibrations. It is strongly recommended to visually verify fluid level.

Show Run Screen

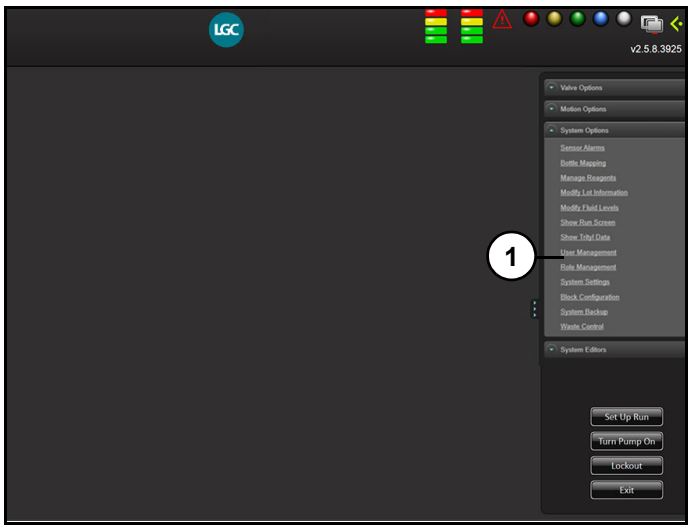

Figure 54

Show Run Sceen (1) *(Figure 55)* allows user to return to "run screen" after limited access for diagnostics and testing when instrument is paused.

User Management screen

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

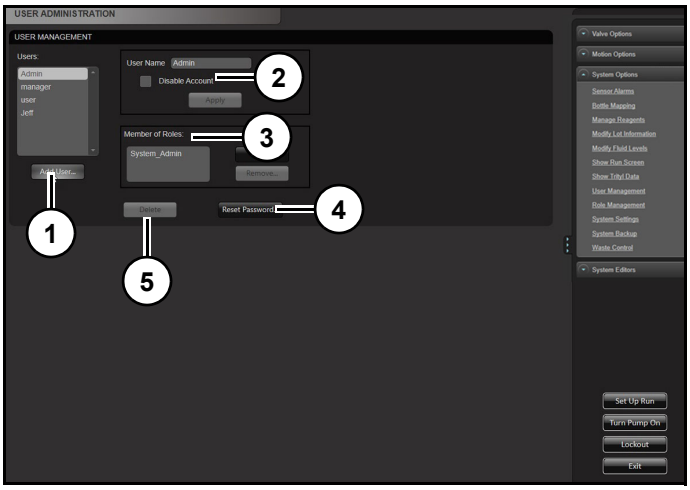

Figure 55

Refer to *(Figure 55)*.

Add User (1): Adds additional user.

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

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

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

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

Adding User

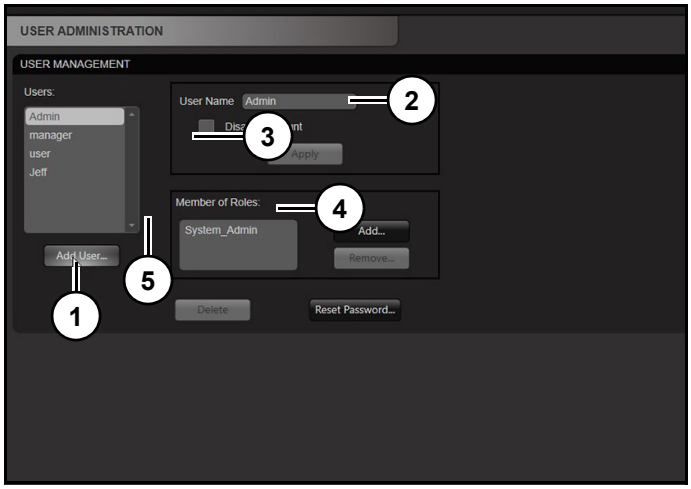

Figure 56

1. Click "Add User" (1) *(Figure 56)*.

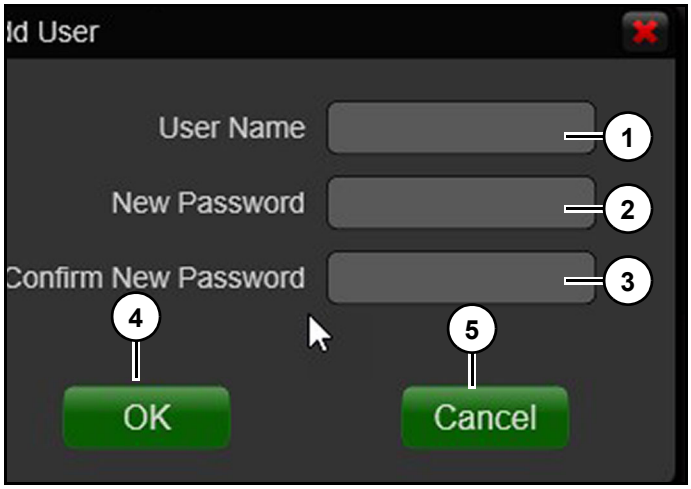

Figure 57

Refer to *(Figure 57)*.

2. Enter "User Name" (1).
3. Enter "New Password" (2).
4. Enter password in "Confirm New Password" (3).
5. Click "Save" (4).

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

6. Assigned a role to new user.

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

Role Management

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

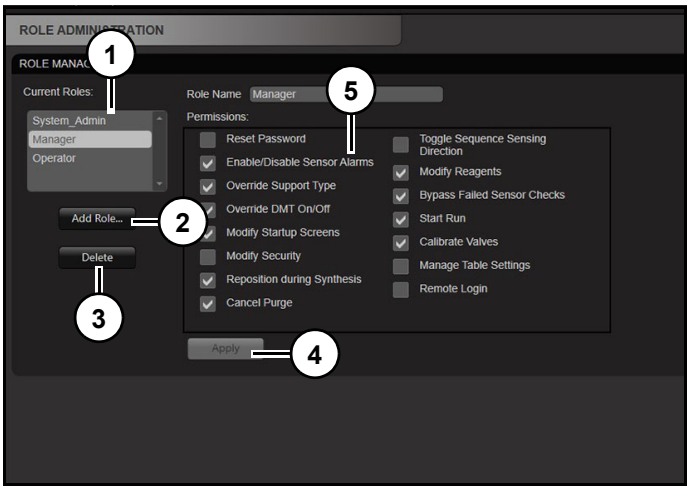

Figure 58

Refer to *(Figure 58)*.

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 from on vs off and vice versa during run start up process.

- Modify Setup Screens: Allows change to setup screen defaults.
- Modify Security: Allows modification of security settings in user account management
- Reposition During Synthesis: Allows user to reposition a synthesis while active.
- Cancel Purge: Allows user to ignore initial chamber purge.
- Toggle Sequence Sensing Direction: Changes direction in which sequence file is read from loaded file; 5' -> 3' or 3' -> 5'.
- Modify Reagents: Allows user to make changes to reagents.
- Bypass Failed Sensor Checks: Allows user to continue 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

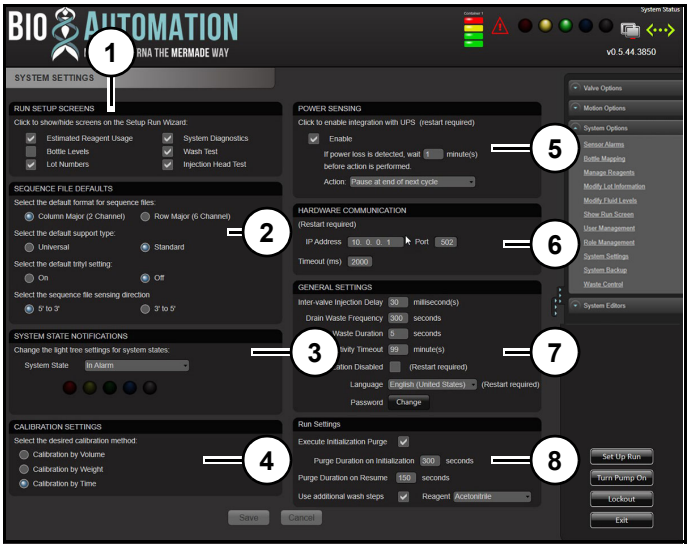

Figure 59

Refer to *(Figure 59)*.

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

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

Sequence File Defaults (2): Determines how sequence file entered during run start-up process will be interpreted by software. Sequence file is a 12 line file of sequence information. First oligo/line is always well-plate position A1. But should the second line be A2 or B1? Following defaults may be configured:

- Row Major: Sequences will be loaded across plate from A1 to A6. A1 would be first sequence in list and A2 being second.
- Column Major: Sequence loaded down plate from A1 to B1. Example: A1 being first sequence in list and B1 being second.
  Note: This is the default setting, and strongly recommend as it keeps synthesis speed high.
- Default Support Type: Select either Universal or Standard support type.

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

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

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

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

  Trityl Off: Software will remove final DMT group from oligo.
  Note: Calibrated Deblock step(s) must be included in Finalization step of cycle 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 sulfurized positions. Example: In one setting (5' to 3') the PS bond will be between G and C and in the other setting (3' to 5') this bond will end up between C and A. See below:

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

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

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

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

- In Alarm: One or more sensor is in alarm state.
- Machine Paused: Instrument is paused for any reason.
- Machine Pausing: A pause has been set but instrument has not yet paused.
- Machine Running: Instrument is currently active.
- Not in Alarm: No alarms are currently active.
- Offline: Instrument is not currently connected to controlling computer.
- Online: Instrument is currently connected to controlling computer.
- Synthesis Completed: All plates are finished but have not yet been removed.
- Synthesis Running: Instrument is current synthesising oligos.
- Synthesis Run Setup: Instrument is currently in set-up process.

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

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

Power Sensing (5): A separate UPS may be added to instrument. Software can be enabled to detect a power failure and a time in minutes may be entered before machine pauses if power has not been restored. . Also allows a sequence position to be set. Safest place to pause is after wash steps of oxidiser step. Software is only tested with APC brand of UPS although it may work with other brands.

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

Notice

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

General Settings (7):

- Drain Waste Frequency: How often waste tray is drained (time in seconds). While synthesizer is

running instrument will open waste tray valve at this frequency to ensure that reagents, which are primed into tray a during run, 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. Currently only English is supported.
- 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 purge on/off. At beginning of a run, argon/nitrogen is used to fill synthesis chamber with inert gas to remove humidity and reduce risk of fire by maintaining a low oxygen atmosphere inside chamber.
- "Purge Duration on Initialization" Refers to time (sec) of initialisation purge executed during synthesis startup.
- "Purge Duration on Resume" Refers to time (sec) for an argon/nitrogen purge to occur after a pause. Usually used when plates are added and removed and when 'Resume With Purge' option is used.
- Use Additional Wash Steps: When running oligos of different lengths, if turned on this function will fill completed/empty columns with a similar volume of ACN as active columns are receiving current reagent.
  Example: Active columns receive 150 µL of Deblock, completed columns receive 150 µL of ACN so there are no changes in vacuum conditions across plate due to completed columns being empty.

System Backup

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

Without additional files selected the zipped folder will contain:

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

With additional files selected, the zipped folder will contain:

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

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

System backup instructions

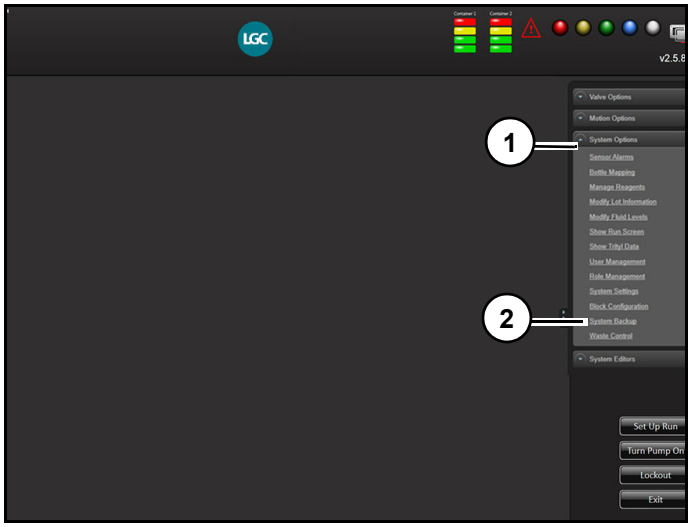

Figure 60

Refer to *(Figure 60)*.

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.

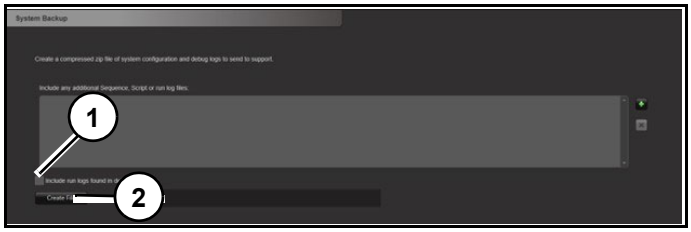

Figure 61

Refer to *(Figure 61)*.

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.

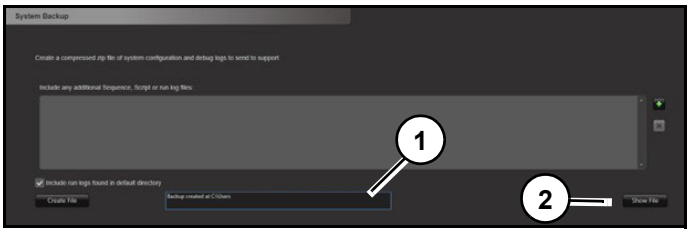

Figure 62

Refer to *(Figure 62)*.

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

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

Export Backup—Additional Files Selected.

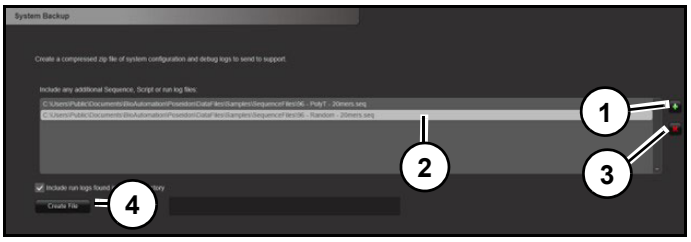

Figure 63

Refer to *(Figure 63)*.

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.

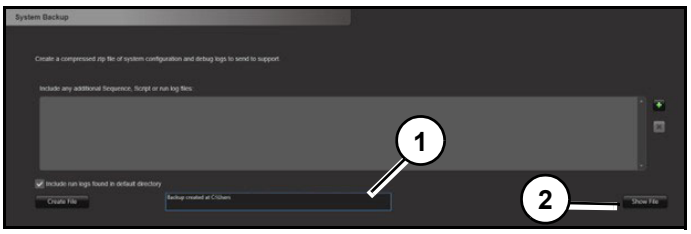

Figure 64

Refer to *(Figure 64)*.

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 waste level sensors and to set parameters to empty waste containers.

Note: Waste control section is only present on instruments with stainless steel automatic waste upgrades.

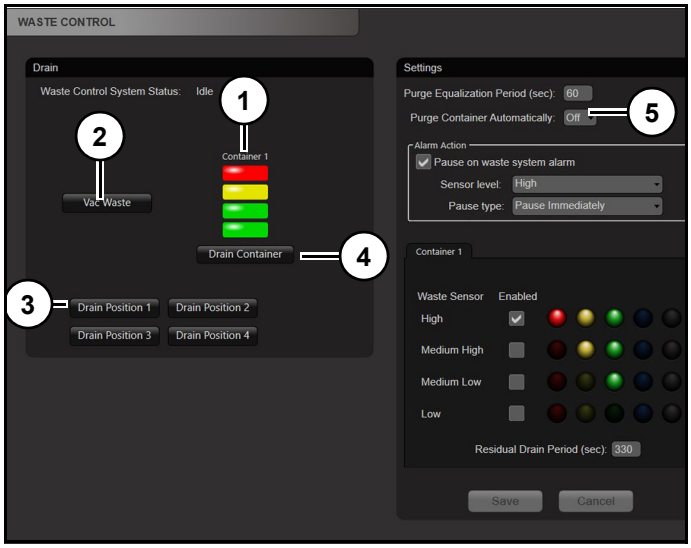

Figure 65

Refer to *(Figure 65)*.

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

Vac Waste (2): Will drain waste tray until turned off.

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

Drain Container (4): Will execute drain container procedure. Instrument will switch from a vacuum inside waste container to a positive pressure, driving the waste out of "Waste out" valve into a client supplied receptacle.

If "Purge Container Automatically" (5) is set to "On", instrument will pause when high sensor is triggered. Instrument will then purge/empty waste container into designated receptacle. Once purging is completed, instrument will resume synthesis. This allows waste to be removed without operator intervention while instrument is running.

Notice

Check that instrument is connected to an adequate waste management system.

Whether system is told to drain waste by pressing "Drain Container" or is triggered automatically by High-level sensor during a run, execution is as follows:

After being triggered, instrument pauses based on 'Pause Type' selected, and then actuates a 3-way (Vac-Pressure Select) valve that shuts off vacuum supplied from pump to waste container and simultaneously opens a path-way for gas to pressurise waste container to 6 psi (15 psi max). Waste 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 "waste low" sensor turns off.

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

After "Residual Drain Period" expires a 3-way valve and a 2-way valve will be actuated. This will shut "waste out" valve and reconnect waste 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 66)* allows user to modify and create script files.

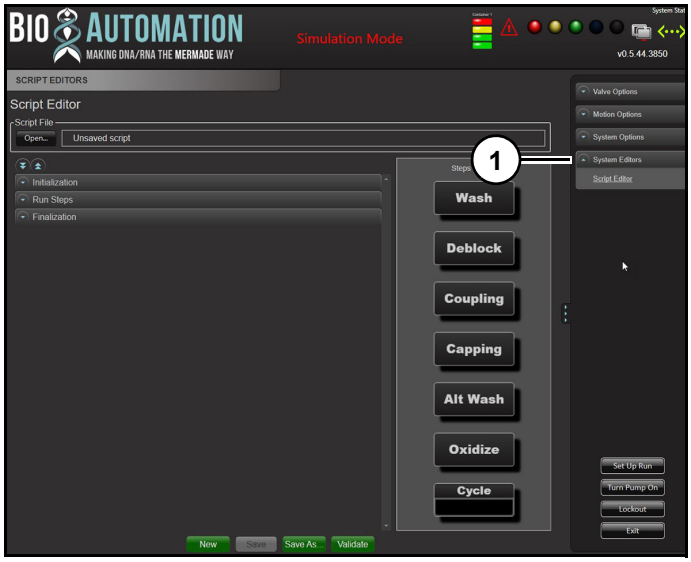

Figure 66

Using script files to set synthesis parameters

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

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

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

Opening a script file

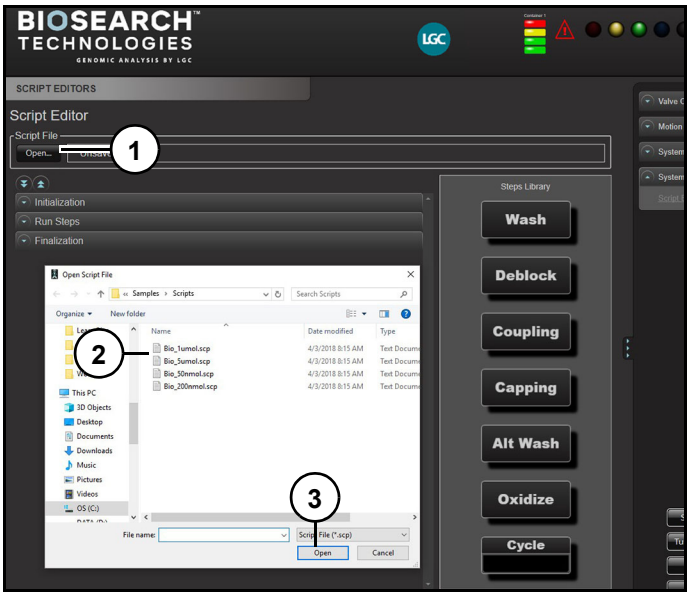

Figure 67

Refer to *(Figure 67)*.

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 68

Refer to *(Figure 68)*.

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

Script file structure

A script file is composed of three main parts:

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

Run Steps (4): Actual synthesis cycle (Deblock, Wash, Coupling, Capping, and Oxidation/ Sulfurization). Steps will repeat 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.

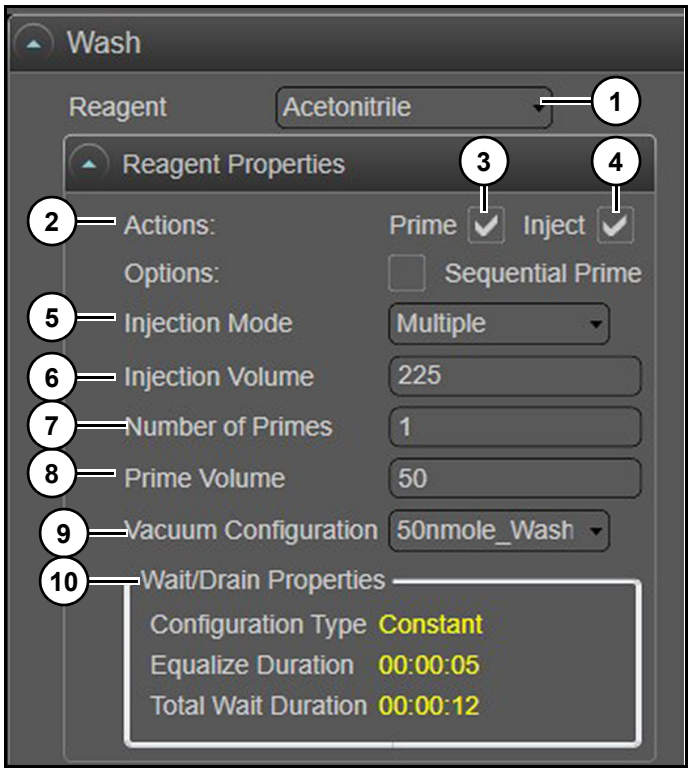

Figure 69

Refer to *(Figure 69)*.

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 Mode (5): There are three types of injection modes.

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

Injection Volume (6): Actual volume that will be delivered in microliters. Calibrations are critical to actual delivered volumes.

Number of Primes (7): 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 (8): 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 (9): 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 (10): Displays drain configuration type (constant or fixed increment), and equalise and total wait durations (in M:S:MS) of chosen vacuum pulse library entry. Gives user reference as to characteristic of drain without the need to open Vacuum Pulse Calibration screen.

Wash

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

Deblock

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

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

Coupling

Coupling is most important reaction. Many things affect coupling efficiency such as moisture, monomer to activator ratio, reagent quality, and drain characteristics.

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

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

Capping

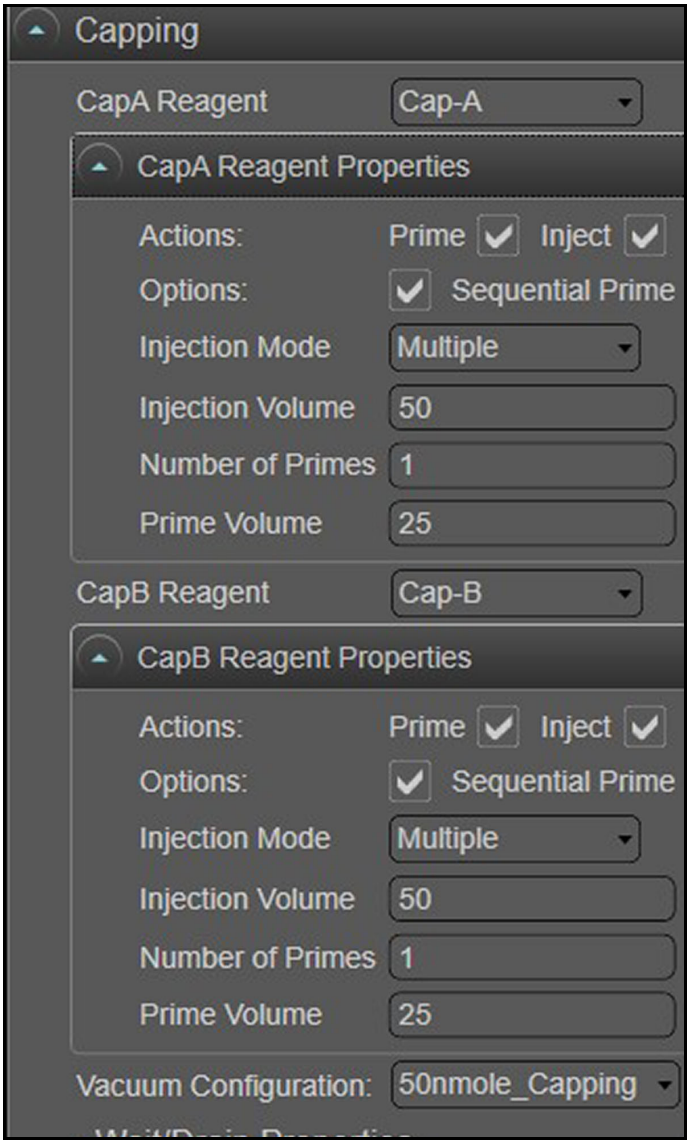

Figure 70

Refer to *(Figure 70)*.

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

Oxidation and Sulfurization

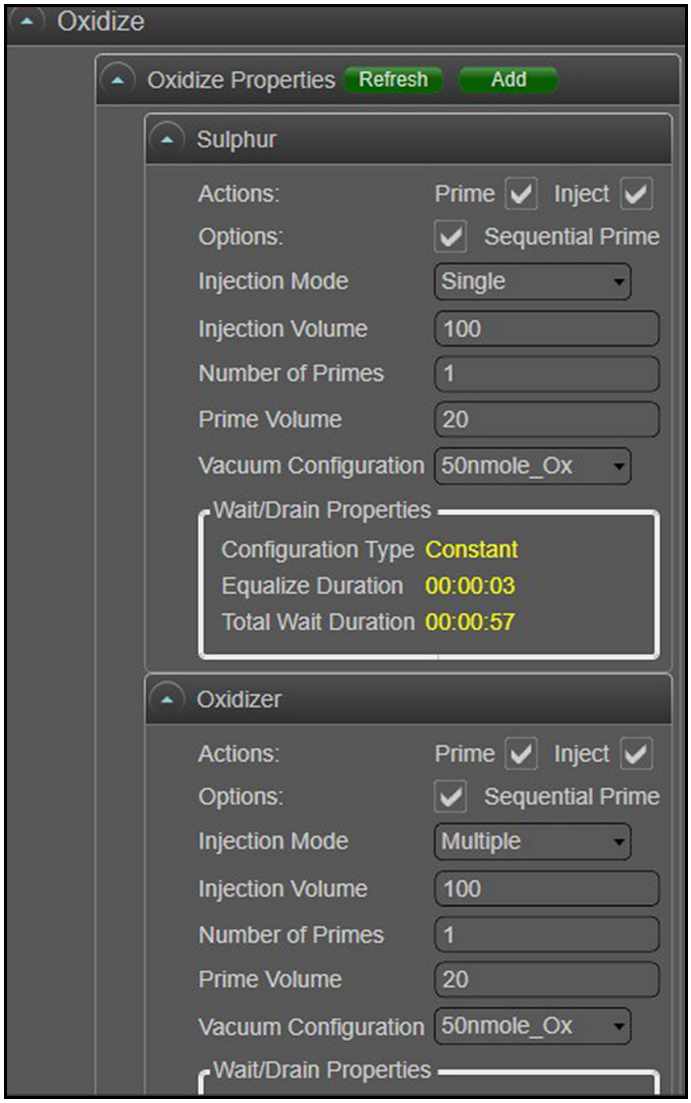

Figure 71

Refer to *(Figure 71)*.

Oxidation steps are used to put newly added backbone linkage into a stable state. This can be done with either oxygen, sulfur or a customized backbone. When oxygen is used, result will be a phosphodiester back bone. When sulfur is used a phosphothiolated oligo will be generated. Each place on an oligo's backbone can be programmed to receive either oxygen, sulfur or a tertiary option. To designate which reagent is used, its designated symbol will be utilized in the sequence file: a ';' delimiter for oxygen or a '*' for sulfur. Additional oxidisers can be added, and delimitators can be changed.

Contact Biosearch Technologies Field Service 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

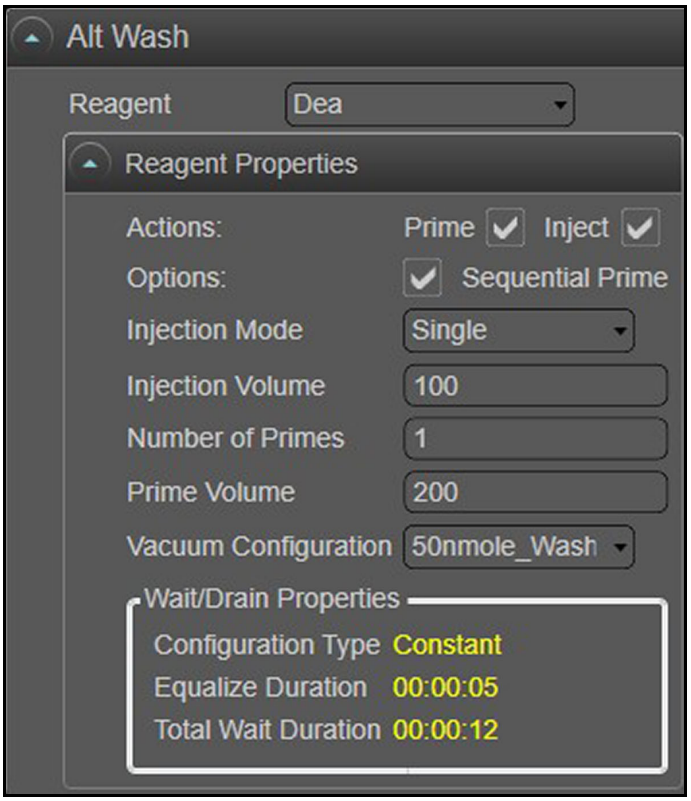

Figure 72

Refer to *(Figure 72)*.

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

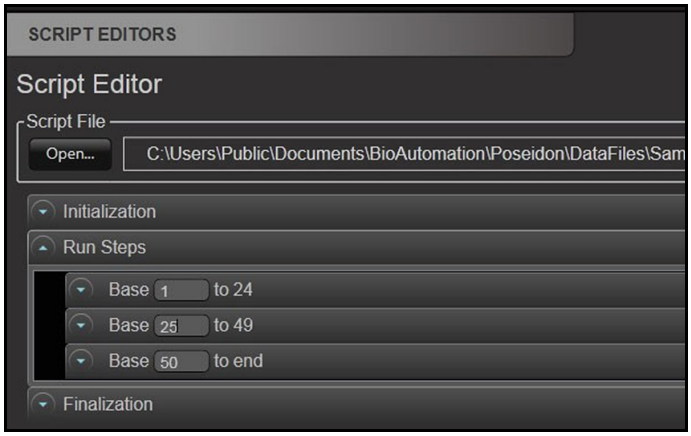

Figure 73

Refer to *(Figure 73)*.

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 73)*.

Making Changes to a Script File

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

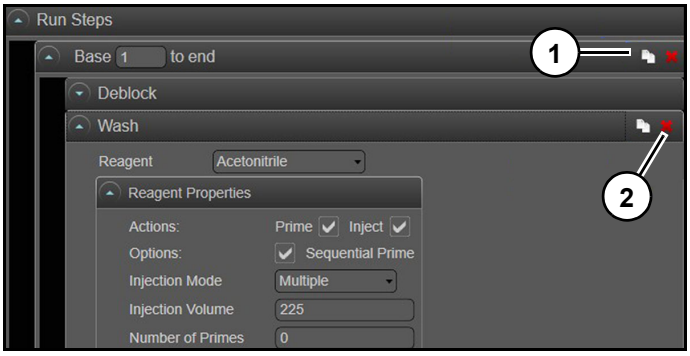

Figure 74

Refer to *(Figure 74)*.

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

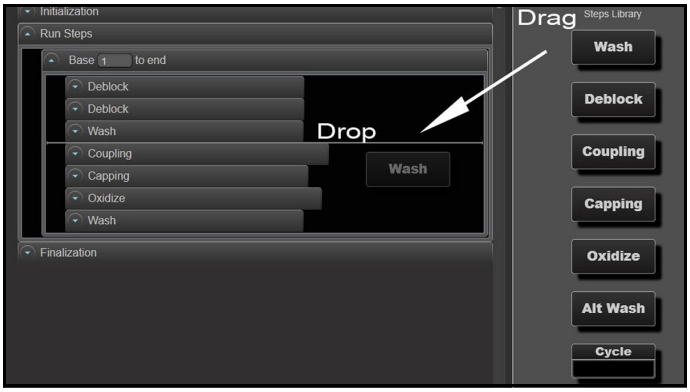

Figure 75

Refer to *(Figure 75)*.

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

There are default script files that come standard on instrument.

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

Starting a run

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

Steps for necessary to start a run:

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

Setup screen

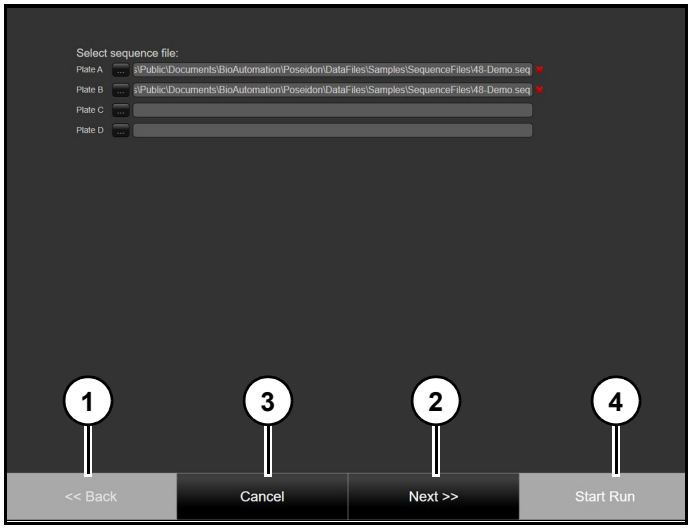

Figure 76

Refer to *(Figure 76)*.

Back (1): Returns to a previous step.

Next (2): Advances to next step.

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

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

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

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

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

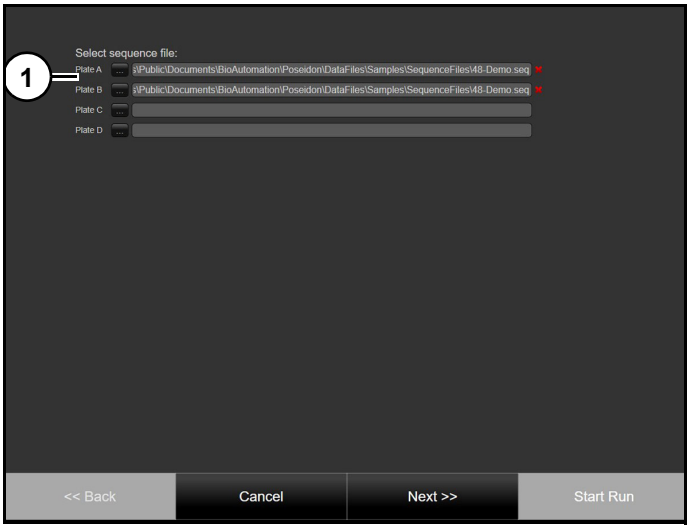

Figure 77

Refer to *(Figure 77)*.

1. Select "Plate" (1).

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

Load Sequence Files.

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

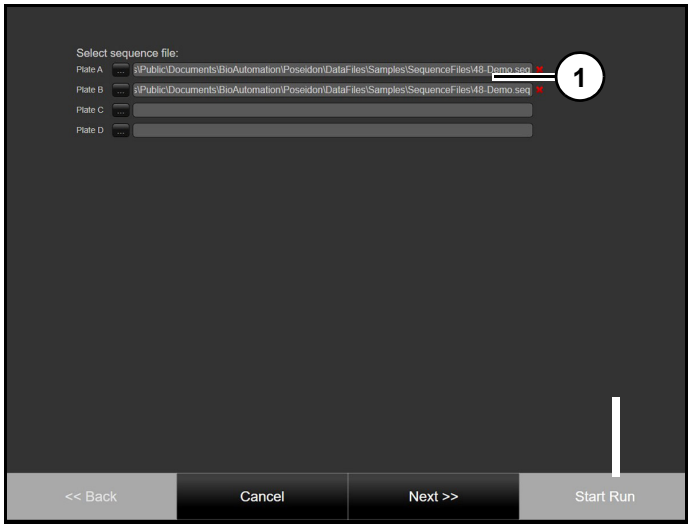

Figure 78

Refer to *(Figure 78)*.

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

- Sequence entry for each well is located on a separate line. Any sequence information beyond line 12 will produce too many Oligos error.
- Each line must contain an oligo name and an oligo sequence it may also contain information regarding type of CPG being used (Universal or Standard) and desired state of final DMT group (On or Off).
- Oligo name must be delimited from oligo sequence by a comma when using text files.
- Oligo name may contain any combination of characters and numbers including punctuation and spaces if they are part of an abbreviated display name for a monomer on instrument.
- Oligo sequence may contain combinations of upper and lower case characters as well as spaces.

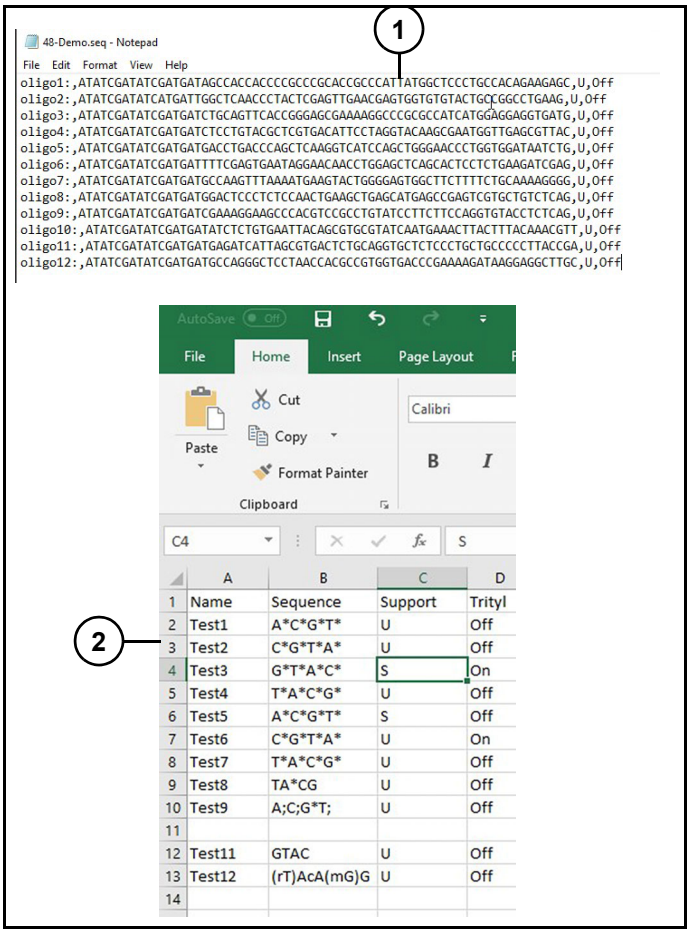

Figure 79

Refer to *(Figure 79)*.

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

User can select to have sequence file read so that order of synthesis is A1, B1, A2, B2, ect. (Column Major) or 6 Channel A1, A2, A3…A6, B1, B2, B3…B6 (Row Major). See System Settings for more information on selecting how synthesizer interprets sequence files. If not using a well, set oligo name to 'BLANK' followed by a comma for text files, and skip line in excel files as seen in line 11.

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

Abbreviated display names with more than one character in length must be bracketed by parentheses as shown on line 13 *(Figure 79)* .

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

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

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

Default for software is an assumed semi-colon. If not using delimiters in sequence files, instrument will assume that a standard oxidation chemistry to obtain an unmodified backbone is being used and hence assume sequence has delimiters ";" between each base. If alternate oxidisers are being used, it needs to be indicated in the sequence file. An "*" must entered for all P=S bonds (Sulfurised) in final synthesised oligo.

Example, **A;C;G*T;T;** will only have a P=S bond on the 3rd base 'G'. Sulfurisation reagent will be used in same cycle step as 'G' amidite. All other bonds will be P=O. For instruments with oxidation set as default **A;C;G*T;T;** is same as **ACG*TT.**

Notice

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

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

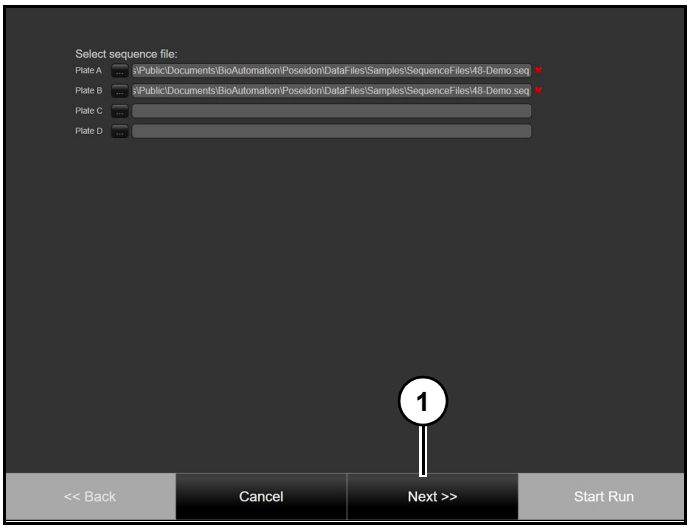

Figure 80

3. Click "Next" (1) *(Figure 80)*.

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

Column Details.
Allows user to select CPG type, final DMT, and start at base position. Also allows access to a GUI to load columns into 12 well column chucks and verify sequence in each column on each plate.

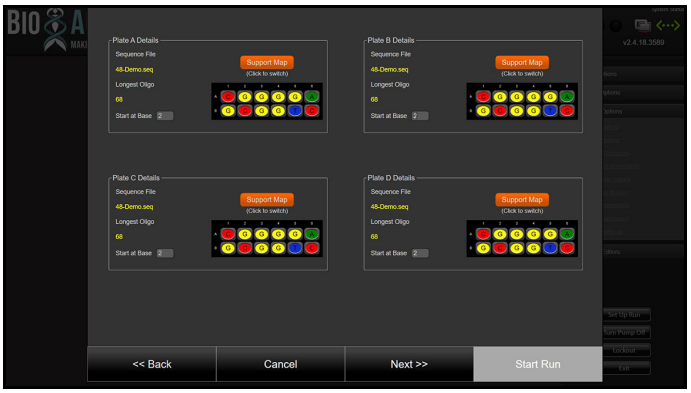

Figure 81

Refer to *(Figure 81)*. Correct Screen Shot?

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

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

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

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

Loading Synthesis Columns.

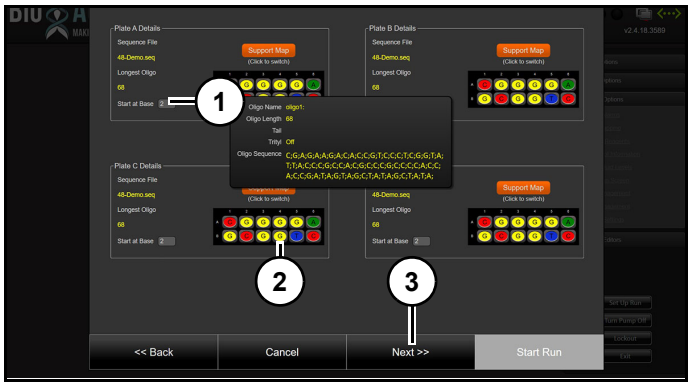

Figure 82

Refer to *(Figure 82)*.

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

5. Push columns into each of 4 column holders, until tip extends through bottom of plate by 1/8". If synthesising less than 12 columns, remaining holes in column holder need to be covered with an aluminum foil or plugs to ensure consistent vacuum.

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

7. Confirm each plate sequence by placing mouse cursor over each well (2) and checking displayed 5' to 3' sequence.

8. Click "Next" (3).

Load Script File.

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

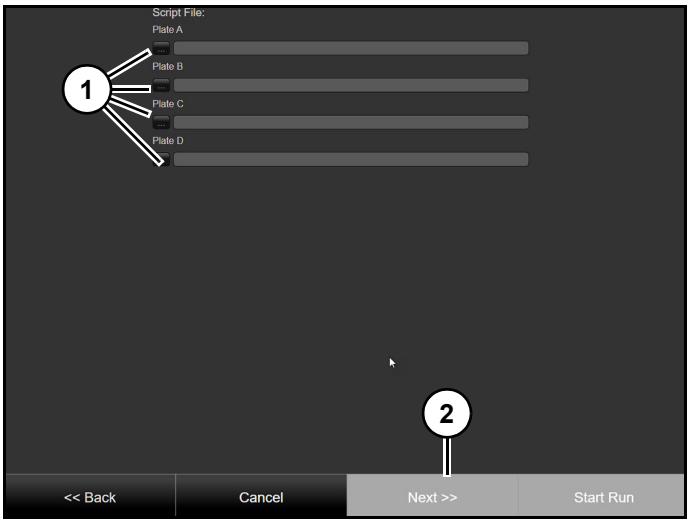

Figure 83

Refer to *(Figure 83)*.

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

10. 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.

11. Click "Next" (2).

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

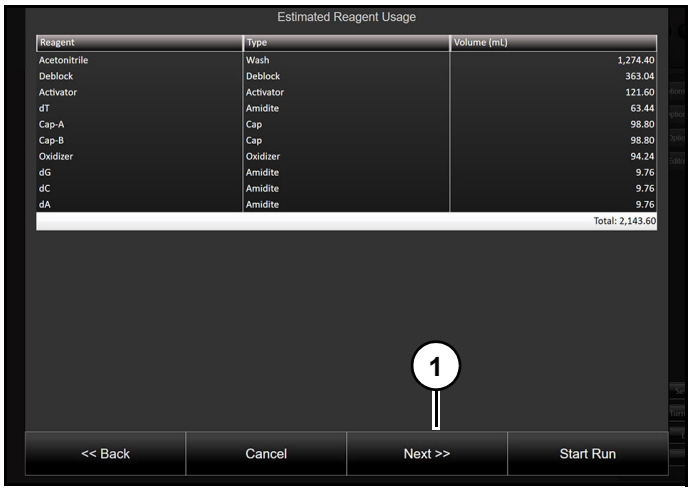

Figure 84

Values shown *(Figure 84)* are calculated from injection volume field in reagent file and are only as accurate as calibration.
Note: synthesizer has no way of determining quantity of reagent in each bottle or how accurate instrument is calibrated.

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

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

Click "Next" (1).

Lot information.

Allows user to enter chemical lot information.

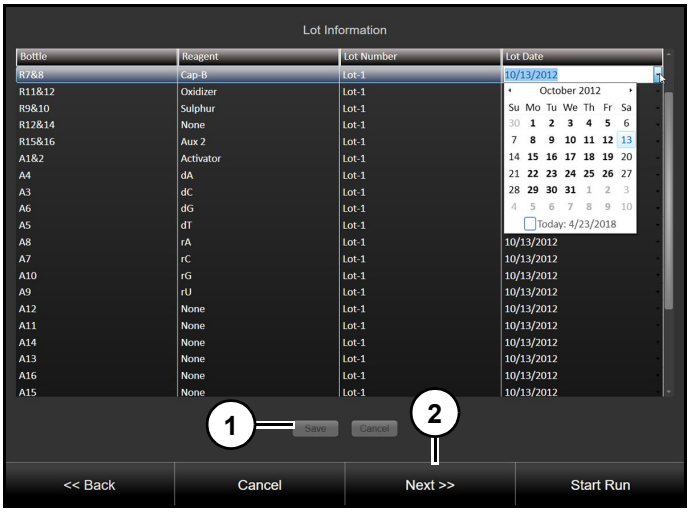

Figure 85

Refer to *(Figure 85)*.

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

13. Click "Save" (1).

14. Click "Next" (2).

Plate Information.

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

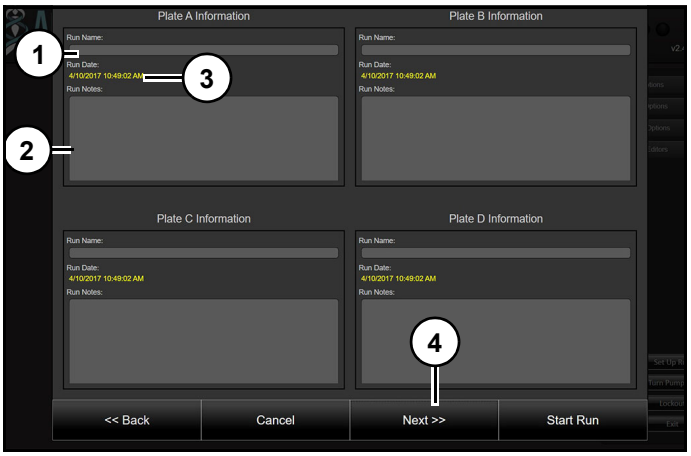

Figure 86

Refer to *(Figure 86)*.

15. Enter log information.

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

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

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

Software automatically generates date and time stamp (3).

Click "Next" (4).

Sensor Test Screen.

Allows user to run a system check on instrument sensors.

Sensors:

Alarm Checks.

Argon Hi Flow Test.

Argon Low Flow Test.

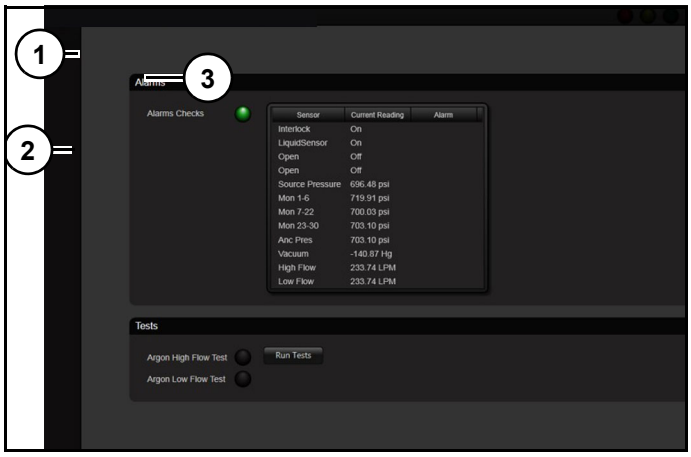

Figure 87

Refer to *(Figure 87)*.

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

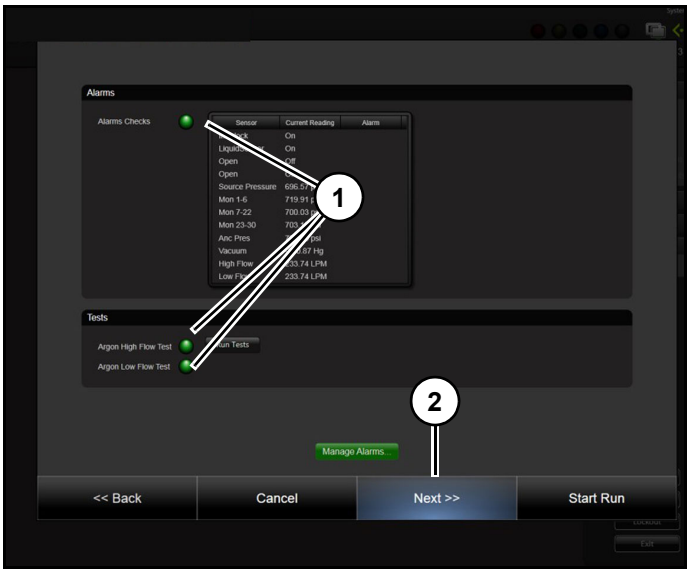

Figure 88

Refer to *(Figure 88)*.

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

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

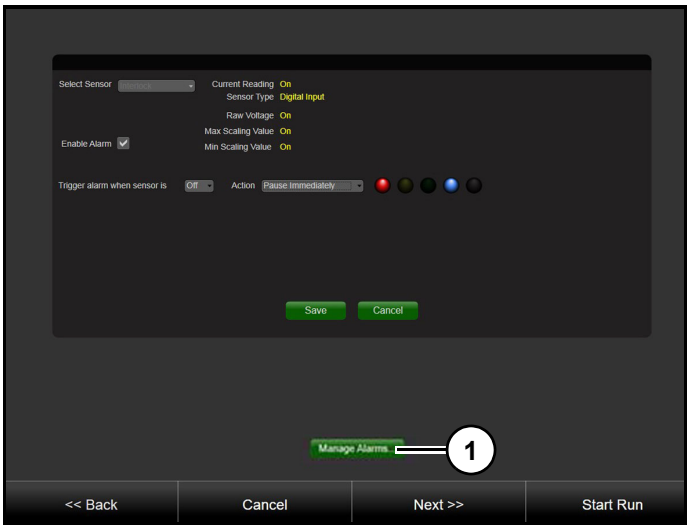

Figure 89

Refer to *(Figure 89)*.

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

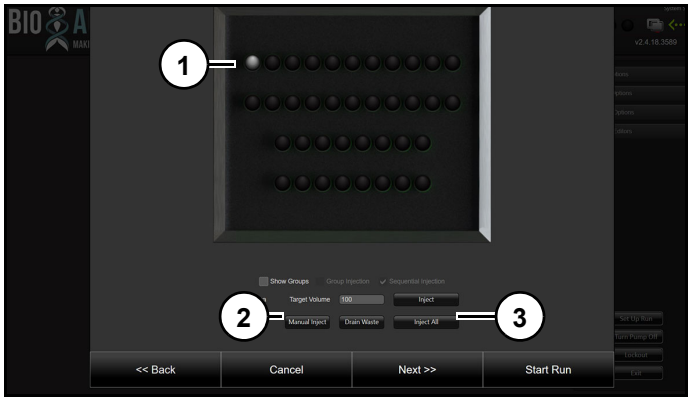

Figure 90

Refer to *(Figure 90)*.

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

ACN Wash Test

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

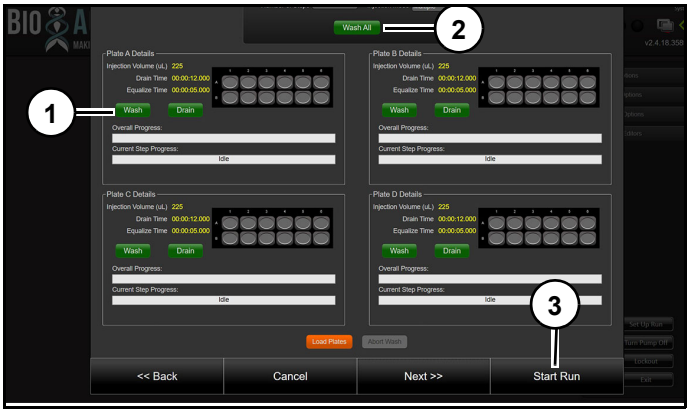

Figure 91

Refer to *(Figure 91)*.

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

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

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

Run Screen

Provides user with control and displays details of ongoing synthesis.

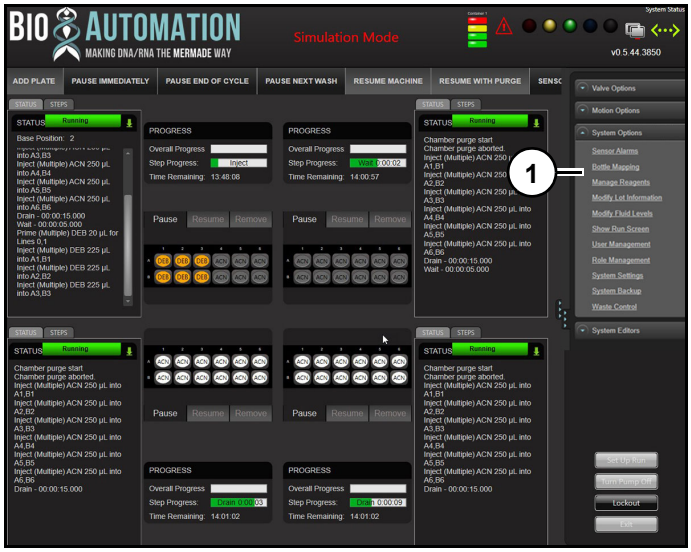

Figure 92

Refer to *(Figure 92)*.

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

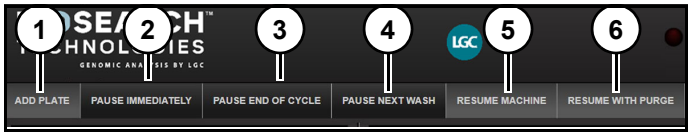

Figure 93

Refer to *(Figure 93)*.

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

Pause Immediately (2): Pauses instrument immediately. Option should rarely be used and considered a last resort as it can cause software to lose track of some events and cause unexpected behavior when restating run.

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

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

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

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

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

Sensors

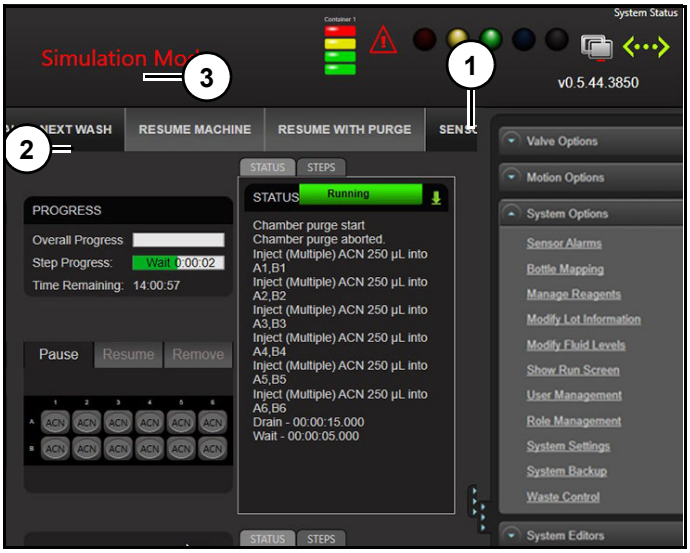

Figure 94

Refer to *(Figure 94)*.

Displays current sensors and their respective values. Sensors cannot be changed, only viewed. To adjust sensors and sensor options (See "Sensor Alarms" on page 43.).

Plate Options Bar Features

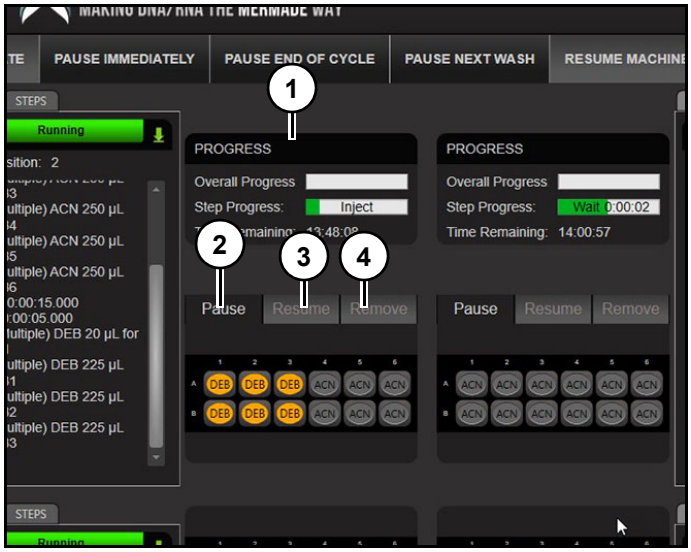

Figure 95

Refer to *(Figure 95)*.

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

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

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

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

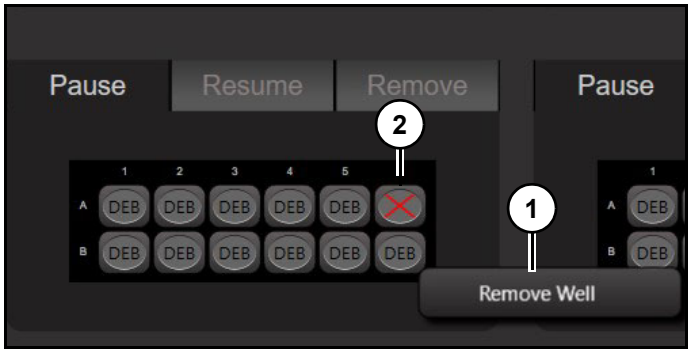

Figure 96

Refer to *(Figure 96)*.

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

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

Steps Control Options

Displays active step in synthesis cycle and provides user with options when right-clicked (1) *(Figure 97)*.

Figure 97

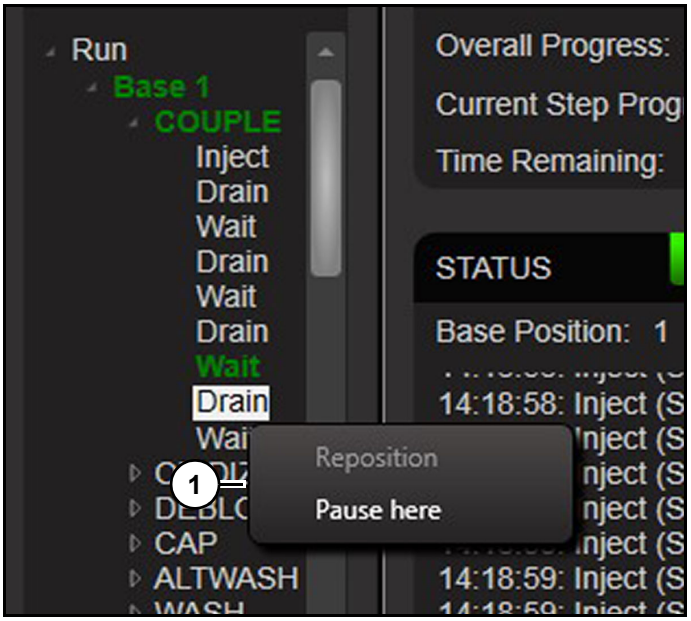

Figure 98

Refer to *(Figure 98)*.

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 99

Once a pause is initiated, location of pause will be highlighted orange (1). *(Figure 99)*

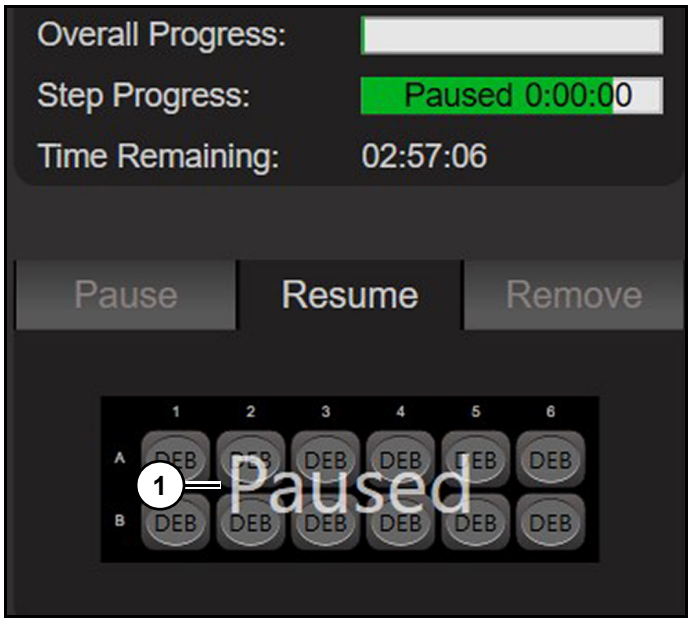

Figure 100

When pause point is reached, instrument will pause, and status will be highlighted yellow and plate graphic will show "Paused" (1). *(Figure 100)*

Canceling a Pause

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

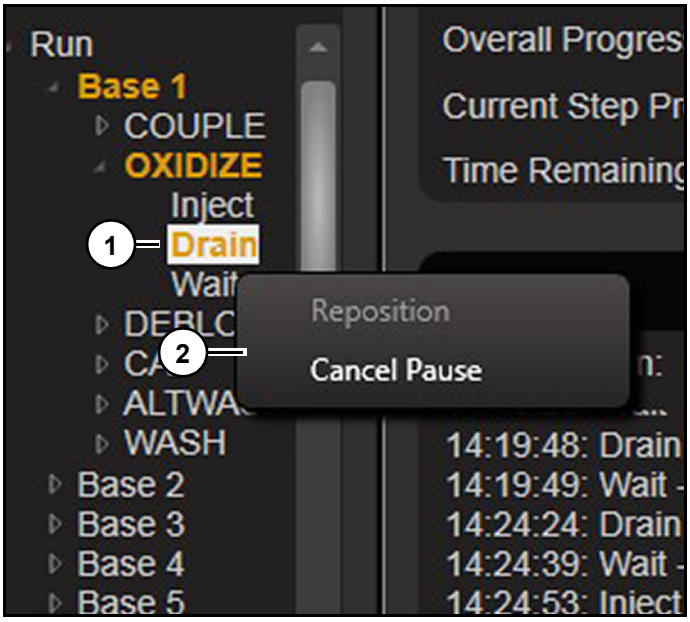

Figure 101

Refer to *(Figure 101)*.

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

Reposition

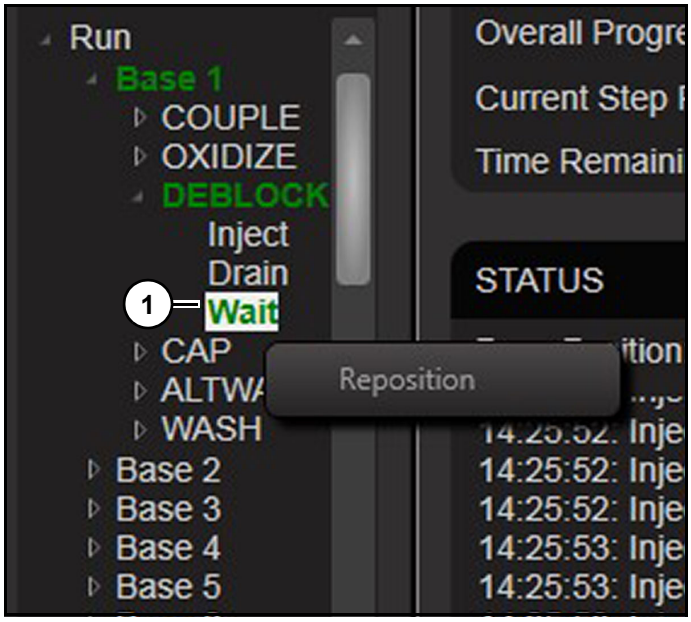

Figure 102

Refer to *(Figure 102)*.

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

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

Post Synthesis

When synthesis is complete screen will show finished.

Removing Synthesis Plate(s)

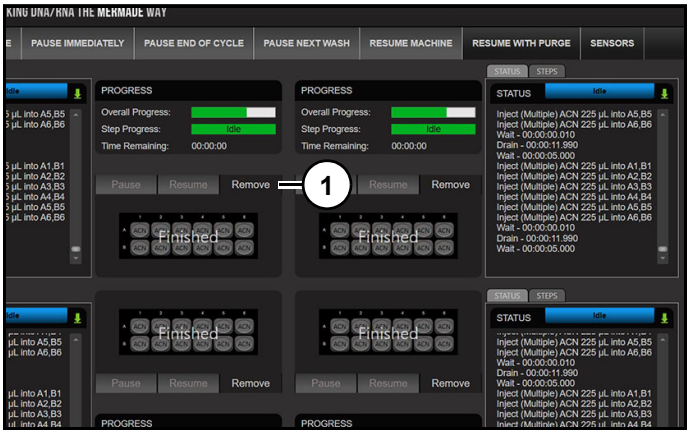

Figure 103

Refer to *(Figure 103)*.

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

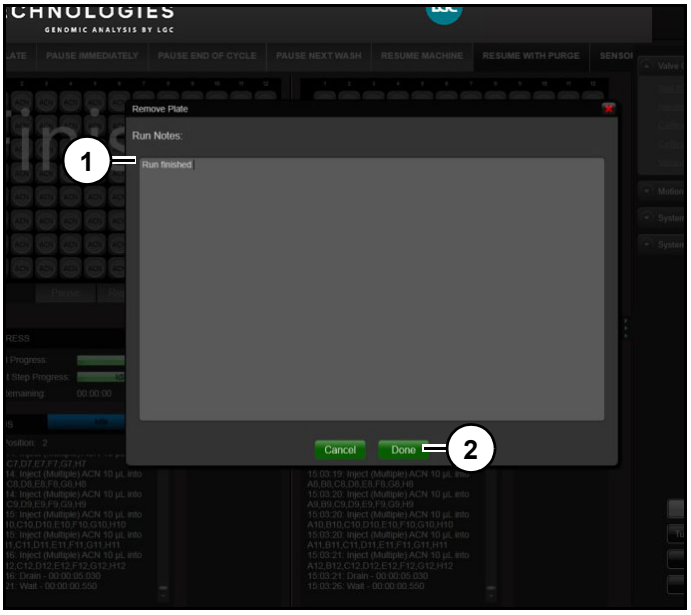

Figure 104

Refer to *(Figure 104)*.

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

Cleavage and Deprotection

Cleavage of Oligonucleotides from columns

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

Cleavage and deprotection chucks are provided in instrument startup kit.

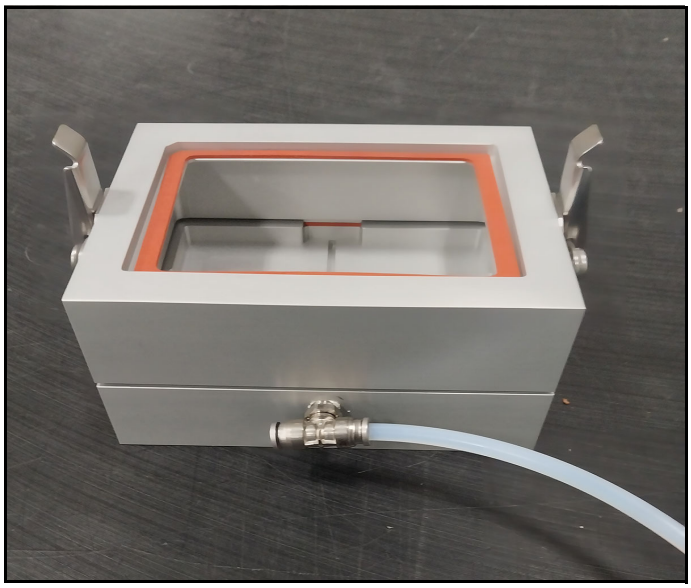

Figure 105

Cleavage Chuck *(Figure 105)*.

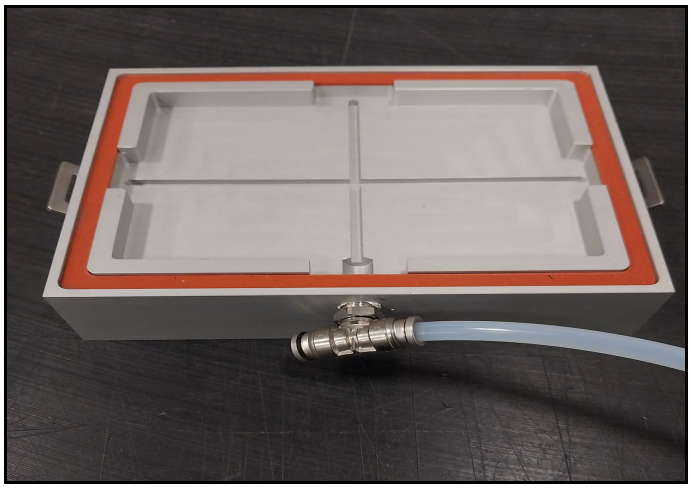

Figure 106

Cleavage Chuck Bottom *(Figure 106)*.

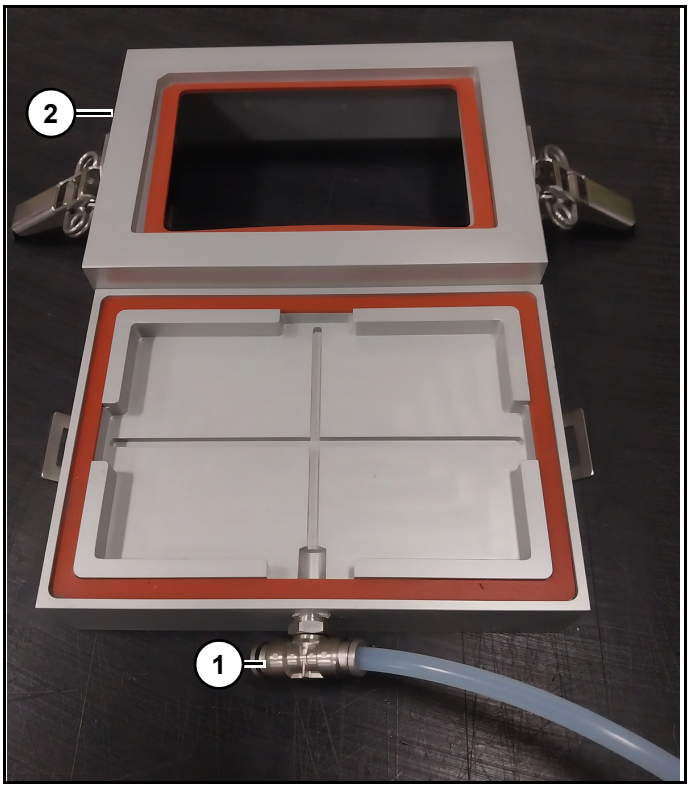

Figure 107

Refer to *(Figure 107)*.

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

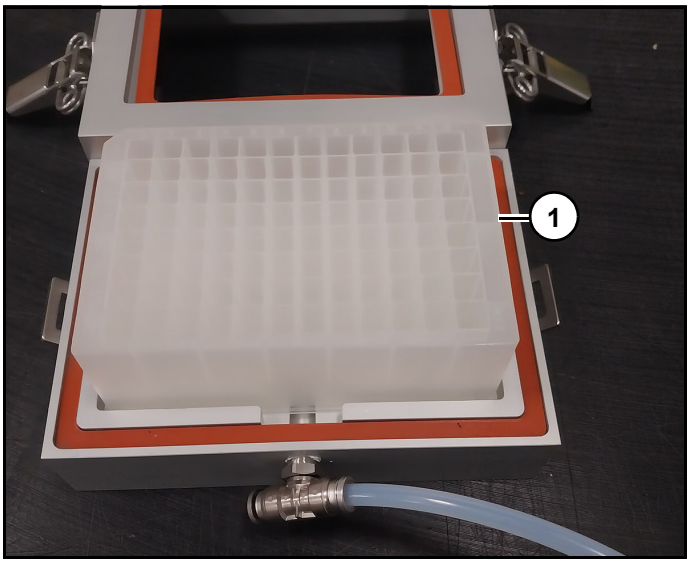

Figure 108

Refer to *(Figure 108)*.

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

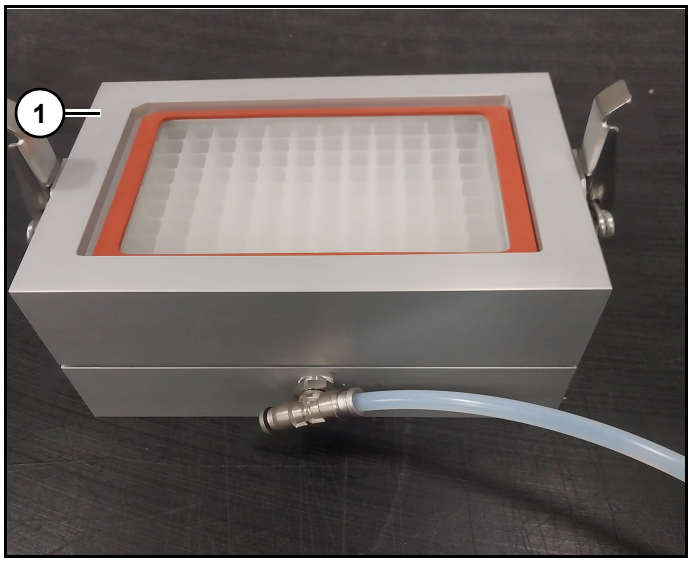

Figure 109

4. Place top portion (1) *(Figure 109)* of cleavage chuck on top of bottom portion of the chuck with 96 well plate.

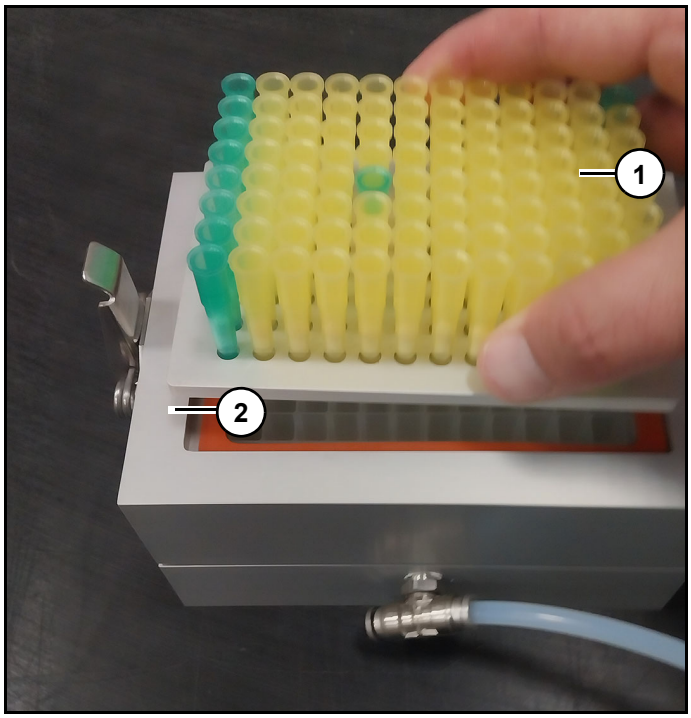

Figure 110

5. Place column chuck (1) into recess of cleavage chuck (2), making sure that column A1 is in upper left hand corner. *(Figure 110)*

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

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

Reference chart for both chemistries:

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

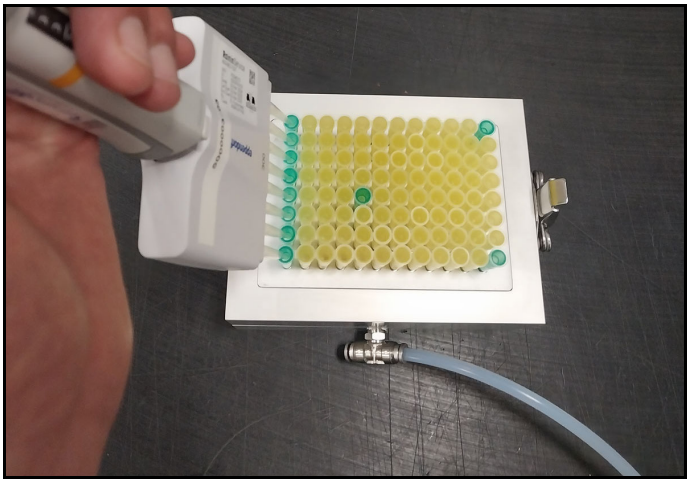

Figure 111

7. Once chemistry is chosen, cleavage cocktail can be applied to columns *(Figure 111)*.
   Note: Apply just enough vacuum to columns to pull cocktail onto CPG bed. Too much vacuum can cause cocktail to pull through columns too quickly increasing chance of incomplete cleavage resulting in lower yields. Repeat with correct wait times according to charts above.

Deprotection of Cleaved Oligonucleotides

1. Remove the 96 well plate from cleavage chuck.

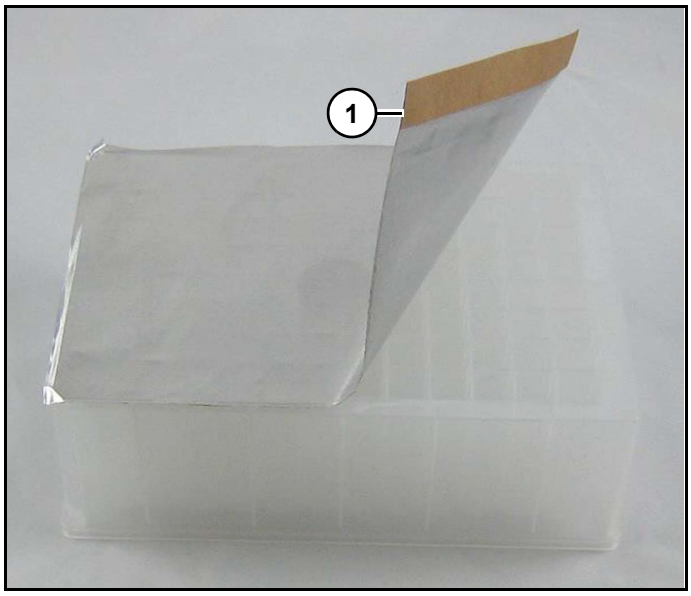

Figure 112

2. Using supplied foil seal (1) *(Figure 112)*, apply seal to top of plate ensuring that all wells are completely covered.

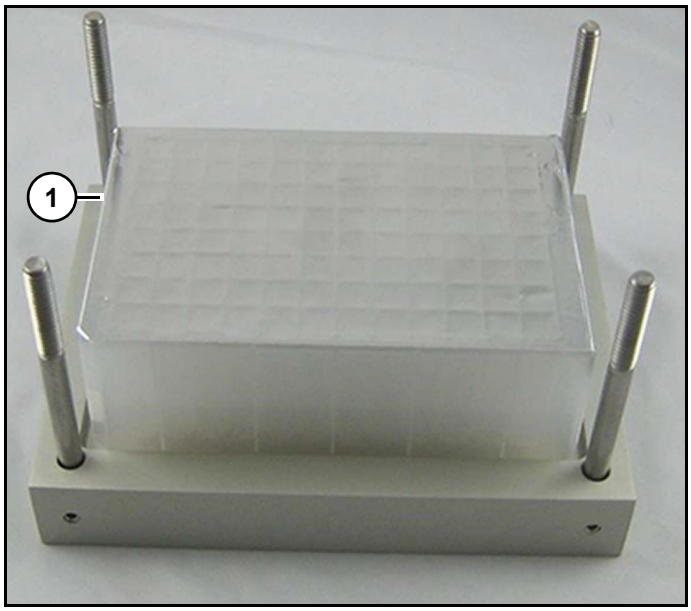

Figure 113

3. Place 96 well plate (1) *(Figure 113)* into bottom of deprotection chuck.

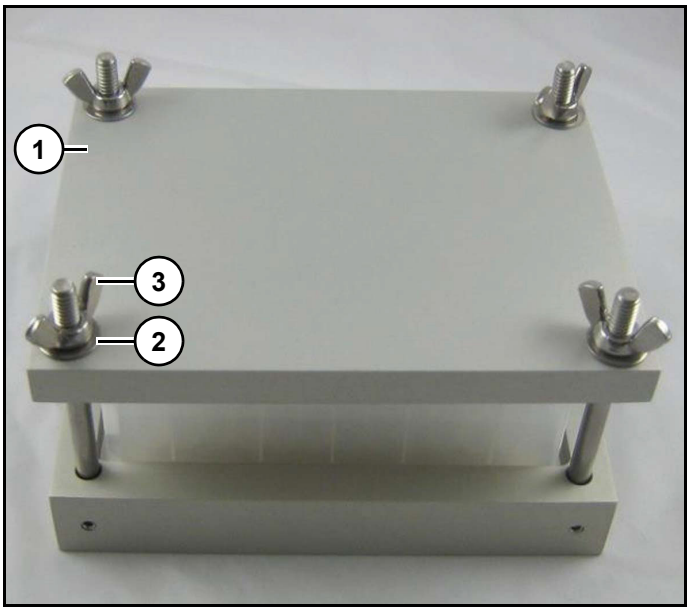

Figure 114

Refer to *(Figure 114)*.

4. Place the silicone spacer mat (not shown) *(Figure 114)* on top of sealed well plate.
5. Place deprotection chuck top (1) on top of entire assembly.
6. Secure top plate with washers (2) and wing nuts (3).
7. Plate is now ready to deprotect according to chart.

Deprotection			
Scale	**Room Temp.**	**65 °C**	**80 °C**
AMA	1 Hour	30 mins.	15 mins
(NH4OH)	Overnight	6 Hours	3 Hours

MerMade 48X 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 spillages, Biosearch Technologies recommends a proprietary cleaning agent and follow manufacturer's instructions.

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

Wipe down plate deck of instrument with a damp cloth.

Maintenance schedule

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

Maintenance and Spare Parts

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

Long-term instrument shutdown procedure

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

Note: 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 plate positions utilizing a plate filled with columns to avoid spills.
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 and that plates are clamped down

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 Lodestar 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:

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

Customer Support Portal

Customer Support Portal will be accessible through following website:

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

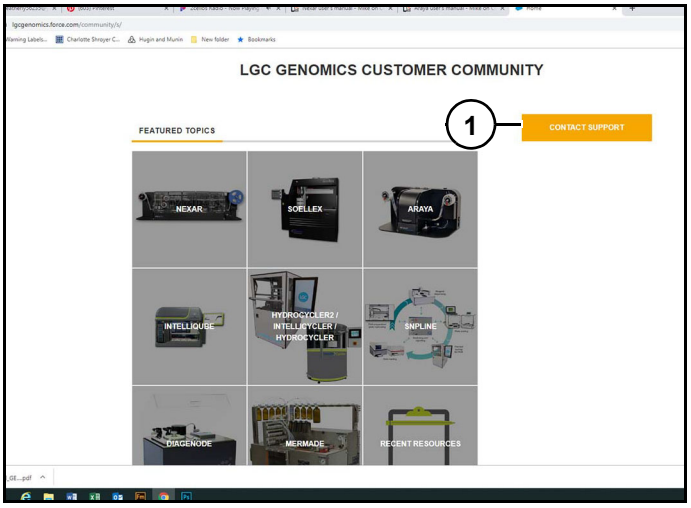

Figure 1

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

Figure 2

Refer to *(Figure 2)*.

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

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