User´s Manual

Prelude® X

Parallel Synthesis and Rapid Heating

GYROS PROTEIN
Technologies

WARNING ALL REACTION VESSELS MUST BE IN PLACE AT ALL TIMES.

WARNING COLLECTION VIALS MUST BE IN PLACE AT ALL TIMES.

WARNING SAFETY SHIELD DOORS MUST BE CLOSED WHILE A SYNTHESIS OR CLEAVAGE IS RUNNING.

WARNING SYNTHESIS WILL HALT IF WASTE CONTAINER IS FULL.

WARNING DO NOT ATTEMPT TO MOVE THE INSTRUMENT WHILE ANY OF THE SOLVENT OR WASTE CONTAINERS CONTAIN LIQUIDS.

WARNING THIS INSTRUMENT CONTAINS SOLVENTS AND CHEMICALS THAT SHOULD BE HANDLED CAREFULLY. MANY ARE EASILY ABSORBED THROUGH THE SKIN AND CAN CAUSE ADVERSE HEALTH EFFECTS. WEAR SAFETY GLASSES, PROTECTIVE CLOTHING AND RUBBER GLOVES AT ALL TIMES. FOLLOW MSDS HANDLING GUIDELINES PROVIDED WITH THE INDIVIDUAL REAGENTS. RESPIRATORS AND ABSORBENT SHOULD BE AVAILABLE IN THE EVENT OF A SPILL.

WARNING: BURN HAZARD

TOUCHING THIS SURFACE OR SURFACES INSIDE THE RV ENCLOSURE COULD RESULT IN BODILY INJURY.

TO REDUCE RISK OF INJURY,
ALLOW TO COOL BEFORE TOUCHING.

WARNING: PINCHING POINT HAZARD

MOVING PARTS. KEEP HANDS CLEAR FROM SURFACES INSIDE THE RV ENCLOSURE COULD RESULT IN BODILY INJURY.

TO REDUCE RISK OF INJURY,
ALLOW TO STOP BEFORE TOUCHING.

AVERTISSEMENT : TOUS LES RÉCIPIENTS DE REACTION DOIVENT ÊTRE EN PLACE À TOUT MOMENT.

AVERTISSEMENT: LES FLACONS DE COLLECTION DOIVENT ÊTRE EN PLACE À TOUT MOMENT.

AVERTISSEMENT : LE COUVERCLE DE SÉCURITÉ DOIT ÊTRE TOUJOURS FERMÉ PENDANT LA SYNTHÈSE OU LE CLIVAGE.

AVERTISSEMENT: LA SYNTHÈSE S'ARRETERA SI LE CONTENEUR DE DECHETS EST PLEIN.

AVERTISSEMENT: NE PAS TENTER DE DÉPLACER L'INSTRUMENT PENDANT QU'UN DES SOLVANTS OU DES CONTENEURS DE DÉCHETS CONTIENNENT DES LIQUIDES.

ATTENTION : CET INSTRUMENT CONTIENT DES SOLVANTS ET DES PRODUITS CHIMIQUES QUI DOIVENT ETRE MANIPULÉS ATTENTIVEMENT. NOMBREUX SONT FACILEMENT ABSORBÉS PAR LA PEAU ET PEUVENT PROVOQUER DES EFFETS NÉFASTES SUR LA SANTÉ. PORTER DES LUNETTES DE SÉCURITÉ, VÊTEMENTS DE PROTECTION ET DES GANTS DURANT LEUR UTILISATION.
SUIVRE LES DIRECTIVES DE MANIPULATION FOURNIS AVEC LES REACTIFS INDIVIDUELS. DES RESPIRATEURS ET ABSORBANT DOIVENT ÊTRE DISPONIBLE EN CAS DE FUITE.

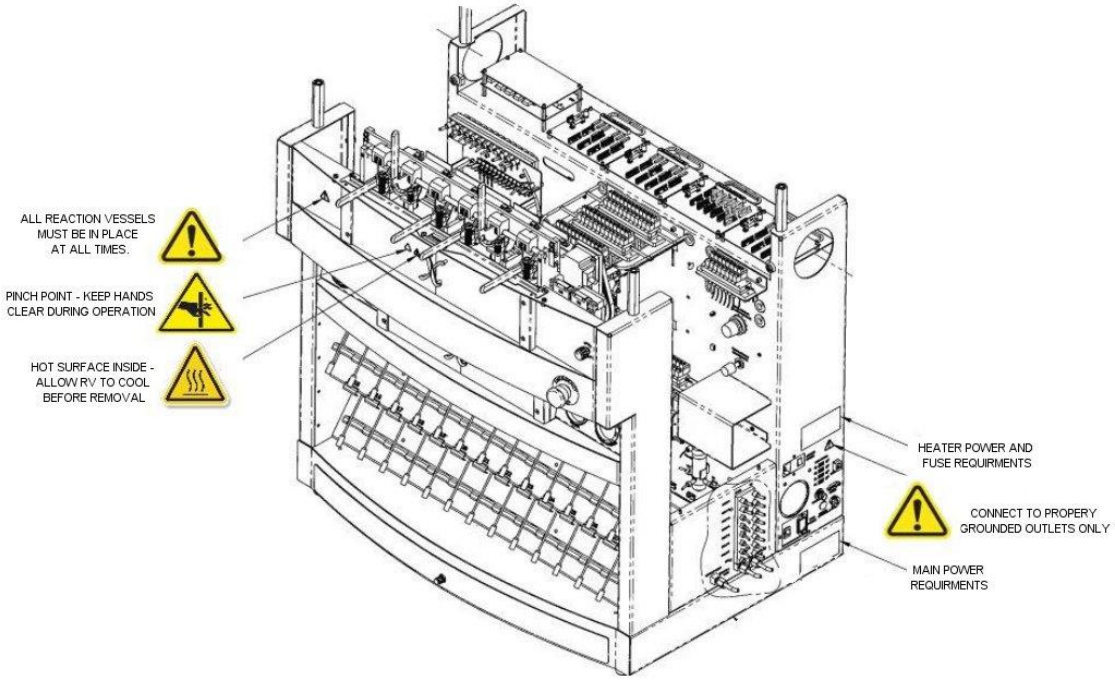
<u>WARNING:</u> BURN HAZARD

TOUCHER CETTE SURFACE OU SURFACES À L'INTÉRIEUR DE L'ENCEINTE DE VACANCES POURRAIENT RESULTANT DE BLESSURES CORPORELLES.

POUR RÉDUIRE LE RISQUE DE BLESSURES, PERMET DE REFRIGER AVANT DE TOCER.

<u>WARNING:</u> PINCHING POINT HAZARD

PIÈCES MOBILES. GARDER LES MAINS À L'ÉCART DES SURFACES À L'INTÉRIEUR DE L'ENCEINTE DU VR POURRAIT CAUSER DES BLESSURES CORPORELLES.


ALL REACTION VESSELS MUST BE IN PLACE AT ALL TIMES.
PINCH POINT - KEEP HANDS CLEAR DURING OPERATION
HOT SURFACE INSIDE - ALLOW RV TO COOL BEFORE REMOVAL
HEATER POWER AND FUSE REQUIRMENTS
CONNECT TO PROPERY GROUNDED OUTLETS ONLY
MAIN POWER REQUIRMENTS

Contents

Introduction

Thank you for purchasing your new ***Prelude*® *X*** peptide synthesizer from Gyros Protein Technologies (formerly Protein Technologies, Inc.). Building upon the strength of the original Prelude platform, the ***Prelude X*** adds heating, oscillation mixing, and UV monitoring to deliver uncompromised speed, yield, reagent savings, and flexibility, creating the most complete peptide synthesis solution available. Heating conditions in each of the 6 reaction vessels can be independently set to enable flexible, customized reactions. Real time UV monitoring with available Single-Shot™ amino acid delivery ensures that reactions are completed efficiently and no excess reagent is wasted. Combined with automatic cleavage and flexible pre-activation options, ***Prelude X*** enables researchers to synthesize routine and difficult peptides with unparalleled speed and efficiency.

I.1 About The Manual

In this manual:

- Chapter 1, **General Information**, describes the instrument layout, basic installation procedures and ***Prelude X*** accessories available for purchase from Gyros Protein Technologies (www.gyrosproteintechnologies.com)
- Chapter 2, **Introduction to Software**, explains the basics of using the software
- Chapter 3, **Basic Synthesis Operations**, describes the basic procedures for setting up a synthesis on ***Prelude X***
- Chapter 4: **Advanced Synthesis Operations & Optional Features,** describes advanced features and options on ***Prelude X***
- Chapter 5, **Cleaning & Maintenance**, explains the cleaning procedures for ***Prelude X*** and its maintenance schedule
- Chapter 6, **Errors & Recovery**, describes common instrument errors and how to recover from them

I.2 About The Company

Gyros Protein Technologies (GPT) resulted from the 2016 merger between Gyros, the leading manufacturer of nanoliter scale immunoassays and Protein Technologies, Inc., leading manufacturer of peptide synthesizers. GPT is built on the belief that our products and services are of the highest possible quality. Products from GPT are supported by a dedicated global field service and technical support team, and we are proud of our reputation for reliability. Founded in 1985 by researchers affiliated with the University of Arizona, GPT

launched its first peptide synthesizer in 1990. Since then, GPT has manufactured and sold the world's finest solid-phase synthesizers. Today, we are growing and innovating to serve the needs of the solid-phase synthesis market. If you have any questions concerning your GPT synthesizer, please feel free to contact us:

+1 520.629.9626 • 800-477-6834
Email: peptides@gyrosproteintech.com
www.gyrosproteintechnologies.com

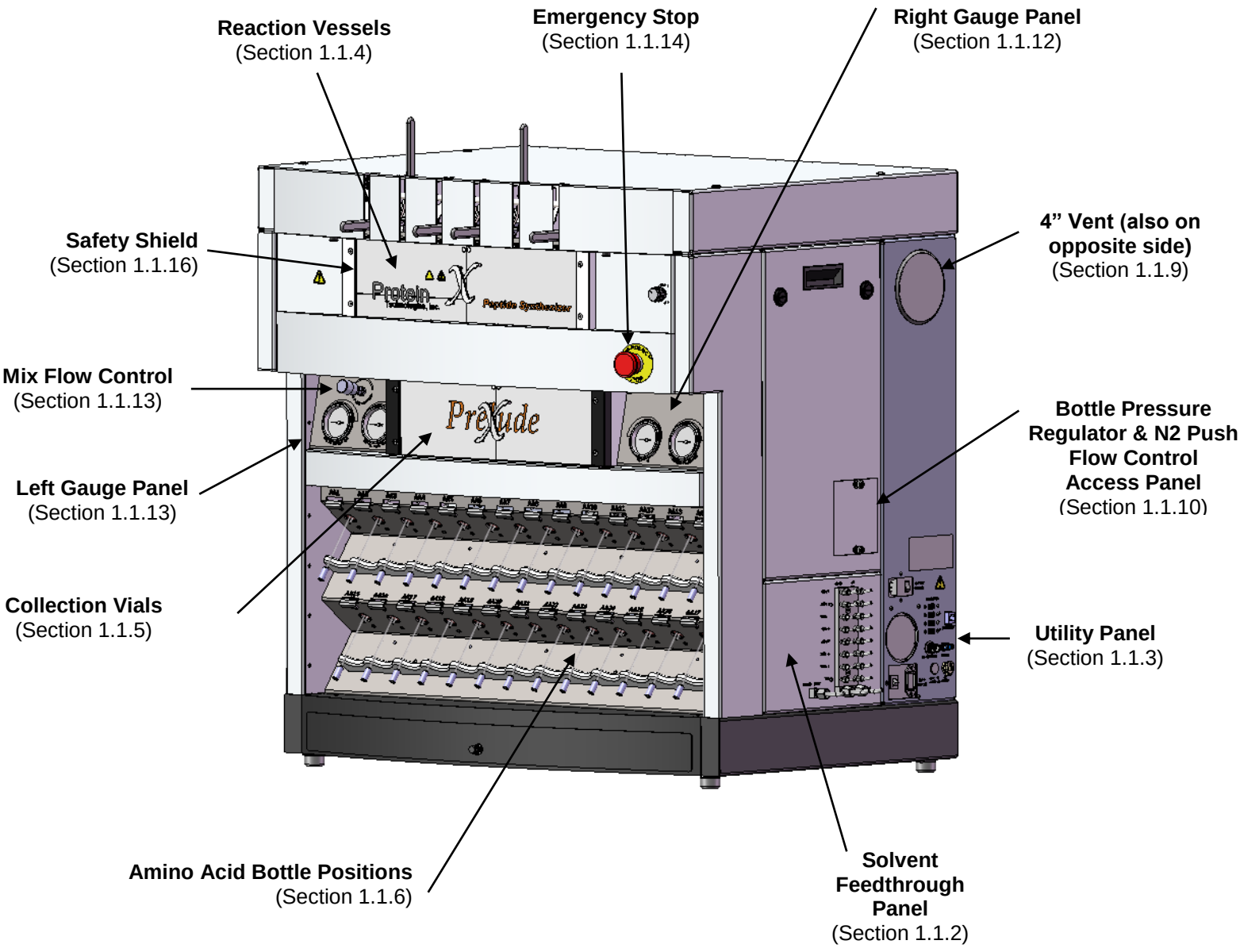
I.3 Common Abbreviations

AA – Amino Acid
Act – Activator or Action
Conc – Concentration
DCM – Methylene Chloride or Dichloromethane
Dep – Deprotection Solution or Deprotected
DIPEA – Diisopropylethylamine
DMA – Dimethylacetamide
DMF – Dimethylformamide
Fmoc – 9-Fluorenylmethyloxycarbonyl
GLP – Good Laboratory Practice
HBTU – 2-(1H-Benzotriazol-1-yl-)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCTU – 1H-Benzotriazolium 1-[bis(dimethylamino)methylene]-5chloro-hexafluorophosphate (1-),3-oxide
In Hg – Inches of Mercury
M – Molarity (moles/liter)
μL – Microliters
mL – Milliliters
MW – Molecular Weight
N2 – Nitrogen
NMM – *N*-Methylmorpholine
NMP – *N*-Methyl-2-Pyrrolidone
NPT – National Pipe Thread
Pip – Piperidine
Pro – Protected
Psi & Psig – pound(s) per square inch gauge
PVC – Polyvinylchloride
Reag – Reagent
Rep – Repetition
Res – Residues
RV – Reaction Vessel
Solv – Solvent
TFA – Trifluoroacetic Acid
THF – Tetrahydrofuran
Vac – Vacuum
Vol – Volume

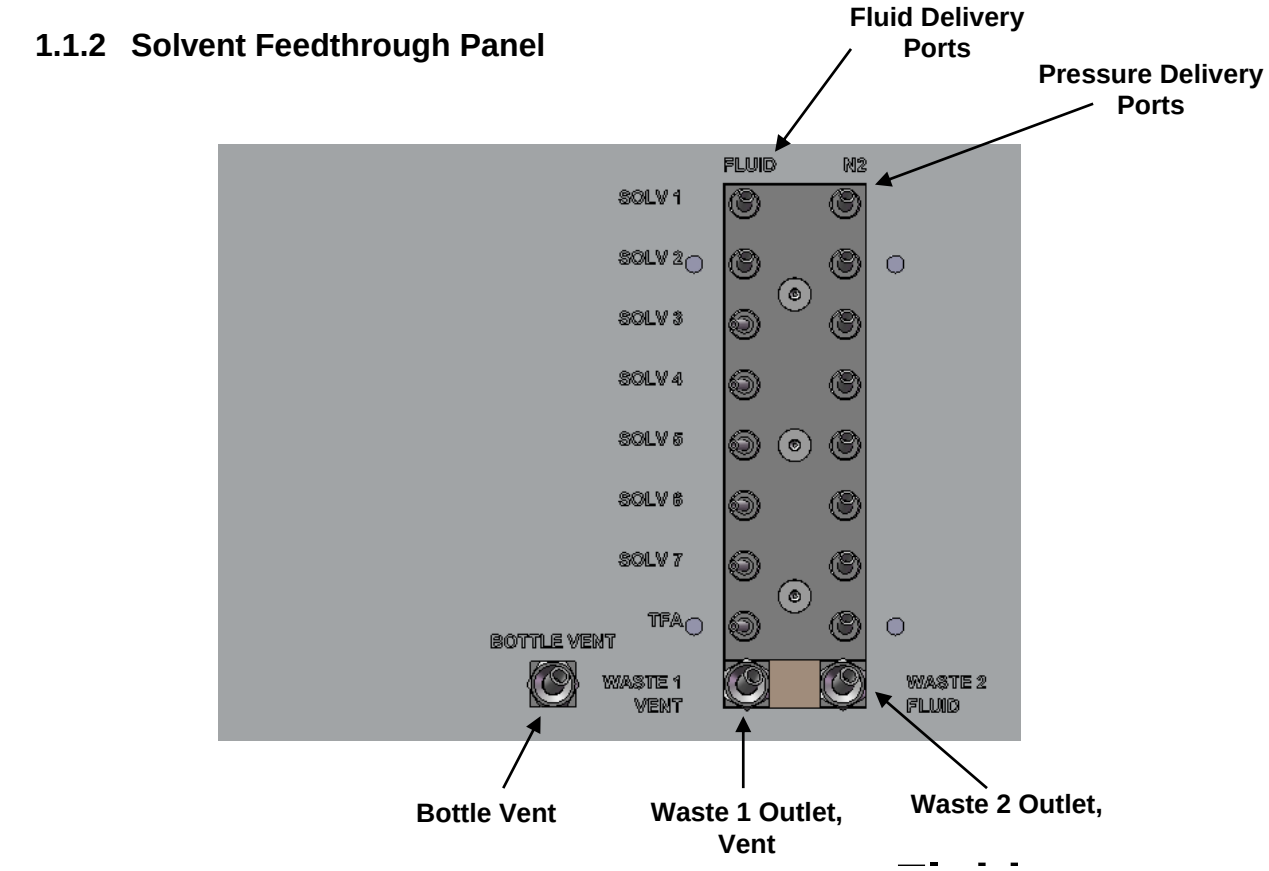
Chapter 1 General Information

1.1 General System Description

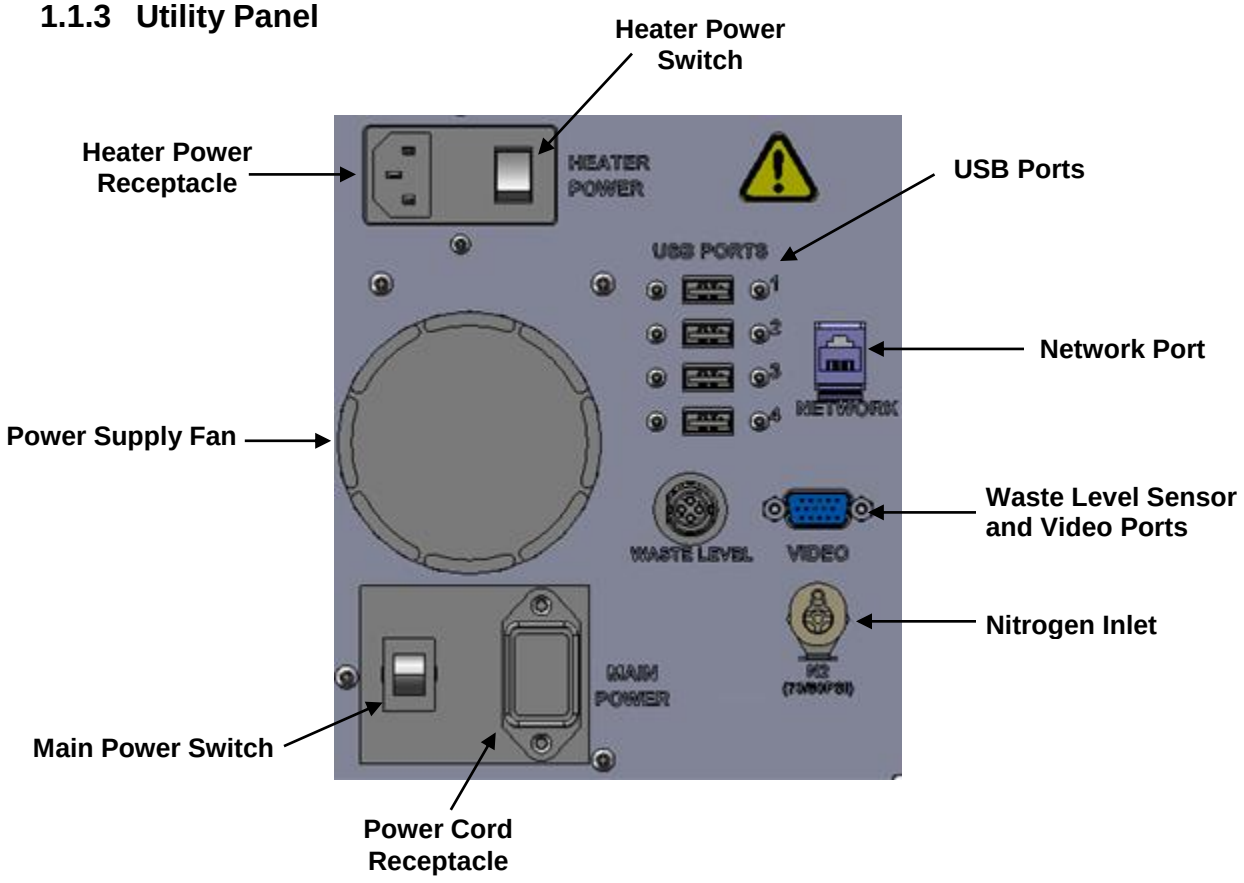
1.1.1 *Prelude*® *X* Front



1.1.2 Solvent Feedthrough Panel



1.1.3 Utility Panel



1.1.4 Reaction Vessel System

Prelude X reaction vessel system is designed around a simple and reliable quick release mechanism. Cam levers allow the operator to remove and install the six reaction vessels quickly and easily. An 'In-Place' detection sensor verifies that all RVs are present prior to executing a synthesis. ***Prelude X*** is also available with an induction heating and shaking option. Induction heating makes it possible to assign unique heating temperatures to each reaction vessel position. GPT strongly recommends using resin that is 74 microns or larger (mesh size 200 or smaller), including standard 100-200 mesh resins. Using resin of a smaller size (larger mesh) will produce poor results and may damage the synthesizer. Using resins that are not recommended may void GPT warranty and service contract coverage.

CAUTION All RV positions that are being heated must use an induction compatible reaction vessel. Failure to use the correct RV will cause the heater to time out and result in a system error. See Section 1.3.1 for a listing of available reaction vessels and accessories.

NOTE All RV positions (including unused positions) must have an RV present for the instrument to function.

1.1.5 Cleavage Collection System

The collection system for ***Prelude X*** accepts 50 mL polypropylene vials. The system is made of materials resistant to the aggressive reagents associated with cleavage solutions. A positive seal ensures cleavage solution vapors are vented. An 'In-Place' detection sensor verifies that a vial is present at all times. These vial positions may be also used as Single-Shot positions as described in Section 3.8.

NOTE All six collection vials must be in place at all times for the instrument to function.

CAUTION The collection tubing is not rinsed automatically since it exits into the collection vials. Therefore, it is important to perform a cleaning process after each collection operation is completed and the collection vials have been replaced. The cleaning operation is denoted as a **Collect Back Flush** under **Cleaning** in the **Operations** menu. See Section 2.5.3.5 for detailed information on the **Collect Back Flush**.

1.1.6 Amino Acid Bottle System

27 amino acid bottle positions are located on the front of ***Prelude X***. Amino acid bottles are available in 10, 120 and 400 mL capacities and can be connected or detached quickly and easily. Pressurizing the bottles with nitrogen accomplishes solution transfer, and this and other operations are controlled using the **Bottle Preparations** screen (Section 2.5.1). Each amino acid bottle has a bottle filter to prevent particulates from entering the fluid system. These filters should be changed on a regular basis depending on the quality and concentration of reagent utilized. See Section 4.2.4, **Bottle Filter Replacement** for instructions.

CAUTION DO NOT install or remove amino acid bottles when they are pressurized.

IMPORTANT All 27 amino acid bottles are vented or pressurized together. For this reason, all amino acid positions must have bottles in place for the pressurization to occur. Empty bottles should be placed in any unused positions.

IMPORTANT The amino acid manifold seals are not affected by DMF or NMP. Contact GPT's Technical Service Department if alternative solvents are desired. Under no circumstances should TFA be used in the amino acid manifold system—destruction of the seals will occur! See Section 4.2.5 **Amino Acid Bottle Seal Replacement** for replacement procedures.

1.1.7 Solvent/Reagent Bottle System

The eight solvent and reagent bottles are located outside the unit in a bottle container and are attached to ***Prelude X*** via the solvent feedthrough panel. These glass bottles are pressurized with nitrogen to accomplish solution transfer. For the safety of the user, safety-coated glass bottles should always be used. The solvent and reagent bottles are controlled using the **Bottle Preparations** screen (Section 2.5.1).

CAUTION Safety-coated glass bottles are supplied by GPT with each instrument and should always be used with this instrument. Using regular glass bottles may result in serious bodily injury.

Bottle positions 1-4 are intended for solvents, and volumes are measured out by timed deliveries. These positions are appropriate for the primary and secondary wash solvents, deprotectant and capping reagent. Bottle positions 5-7 are intended for reagents, and can precisely measure volumes in 150, 500 and 1000 μL aliquots using a metering loop. These positions are appropriate for coupling solutions. Bottle position 8 is specifically intended for the delivery of cleavage solution. Volumes for bottle 8 are measured by timed deliveries using the "cleave

and collect" operation and upon draining from the reaction vessel the contents are transferred to the collection vials. Throughout ***Prelude X*** software program, each bottle position is referred to by an abbreviated title. These assignments may be changed in the **Solvent/Reagent Editor** (Section 2.4.2). The titles, standard abbreviations, bottle volumes, and typical solution composition of each bottle position for Fmoc chemistry are as follows:

SOLV 1 or DMF: Two daisy-chained 4 L safety-coated glass bottles for the primary wash solvent, typically reagent grade DMF. DMA or NMP may also be used. Because this solvent is quite stable, the 4 L containers may be installed and left in place throughout several sets of syntheses. This solvent is utilized in the automated cleaning operations for the valve fluid system. Therefore, SOLV 1 must be in place for normal operation of the instrument. This solvent position is also utilized during the **Bottle Position Flush** (Section 2.5.3.2), **Rinse All Blocks** (Section 2.5.3.5) and **Wash RVs** (Section 2.5.3.7) cleaning operations.

SOLV 2 or DCM: 1 L safety-coated glass bottle for a secondary wash solvent, such as DCM to wash the peptide-resin in preparation for automated cleavage. This bottle position is utilized during the **System Clean** (Section 2.5.3), **Cleave Bottle Solvent Back Flush** (Section 2.5.3.3), and **Collect Back Flush** (Section 2.5.3.4) cleaning operations. DCM may be installed and left in place for several sets of syntheses.

SOLV 3 or Dep: 1 L safety-coated glass bottle for deprotectant to remove the N-terminal Fmoc protecting group. The standard composition is 20% (v/v) piperidine in DMF. This solution is also quite stable and may be installed and used for several sets of syntheses. Other reagents may be loaded for alternate chemistries.

SOLV 4 or Cap: 1 L safety-coated glass bottle for capping solution to permanently block any unreacted amino groups following a coupling reaction or to acetylate the N-terminus of a completed peptide. Typical compositions include 1:1:3 acetic anhydride/pyridine (or DIPEA)/DMF. Other reagents may be loaded for alternate chemistries.

SOLV 5 or Base: 1 L safety-coated glass bottle for base, if separation of base and coupling reagent is desired. The standard base composition is 0.4 M NMM in DMF. Bottle 6 or 7 should then contain a coupling reagent such as 0.1 M HBTU in DMF. Alternatively, base and coupling reagent may be combined into a single activator solution as described below.

SOLV 6 or Act1: 1 L safety-coated glass bottle for activator solution to form the activated Fmoc amino acid for the coupling reaction. The standard composition is 0.1 M HBTU in 0.4 M NMM in DMF. Other reagents may be loaded for alternate chemistries.

NOTE The activator solution must be equimolar with the amino acid solutions to prevent undesirable side reactions. The activator solution should be prepared fresh for each synthesis.

SOLV 7 or Act2: 1 L safety-coated glass bottle for additional reagent.

SOLV 8 or CLEAV: 1 L safety-coated glass bottle of cleavage reagent for cleavage of the peptide from the resin after synthesis is complete. This position is specifically designed to handle the aggressive TFA cleavage solution. It can only be accessed through the **Cleave and Collect** operation. When necessary, other solvents/reagents may be used from this position.

NOTE The cleavage solution should be prepared fresh for each synthesis.

NOTE The **No Prime** feature must be selected for the SOLV 8 bottle in the **Special Bottles** screen (Section 2.5.1.2) when in use. However, the **No Prime** feature should be deselected for the SOLV 8 bottle during **Solvent Calibration** (Section 2.5.1.3).

Each solvent position has a bottle filter to prevent particulates from entering the fluid system. For replacement procedures, see Section 4.2.4. An encapsulated o-ring in the bottle cap insert establishes the bottle seals and is inert to the reagents. Damage to the insert or o-rings will result in nitrogen leakage and potential loss of reagent (volatiles like TFA, DCM). For bottle seal replacement procedures see Section 4.2.6.

Custom bottle configurations and assemblies can be arranged through technical service.

1.1.8 Waste System

The only exit for the closed fluid flow paths of the instrument is through the waste system. Waste exits ***Prelude X*** to the waste container through three ports on the Solvent Feedthrough Panel. The waste container is vented through a fourth tube attached to a fitting on the 4” vent duct. The waste container is a 5 gallon carboy fitted with a waste level sensor to prevent overfilling. If the waste container is full, all operations in the instrument will stop automatically and all the bottles will vent. No operations will be allowed until the container is emptied and reconnected.

IMPORTANT The waste container being full is a critical error on ***Prelude X***. To prevent overfilling, the instrument will automatically pause all operations and vent all bottles. To resume operations, first empty and reconnect the waste container,

then re-pressurize and prime all the bottles using the **Bottle Preparations** screen (Section 2.5.1). Go to the **RV Status** screen (Section 2.5.2) and press the **Start** button to continue the operations on the paused reaction vessels.

The waste level sensor is wired in a normally closed (NC) configuration so if the switch is disconnected, it is the same as if the container is full. This logic prevents waste from being delivered when the container is not connected. The connectors are resistant to the aggressive waste solutions. Do not attempt to disassemble the switch connector assembly. It is not a field service item and damage may occur.

CAUTION Be sure to backflush bottles before removing the waste level sensor connector. If the sensor connector is removed from the waste container while any bottles are primed, the bottles will vent, and fluid may remain in the lines.

1.1.9 Ventilation System

Prelude X has two 4-inch vent holes—one on each side of the instrument. It comes equipped with an adjustable angle adaptor for one hole, and a vent cover for the other. The adaptor has a tube fitting for attaching the waste container vent line. ***Prelude X*** should be connected to lab ventilation with a 4-inch (10 cm) duct supplied by the user. The ducting should be made of a chemically resistant material (PVC or urethane, but no rubber). A minimum flow of 50 cubic feet/min (CFM) must be maintained at the instrument.

1.1.10 Nitrogen System

The nitrogen inlet is located on the utility panel (Section 1.1.3). A minimum of 70 psi (70-80 psi / 482-551 kPa) must be supplied for the instrument to operate. The lack of nitrogen is a critical error, and the instrument will pause all operations, vent all bottles, and display an error message. No operations will be allowed until the supply is restored.

The high pressure nitrogen is diverted into three regulators:

1. **Valve Pressure** – Used to seal the valve membranes. Should be set to 30-40 psi. User should not adjust. Displayed on Left Gauge Panel (See Section 1.1.13).

2. **Nitrogen Pressure** – Used for mixing and delivering fluid. Should be set to 5 psi. User should not adjust. Displayed on Right Gauge Panel (See Section 1.1.12).

3. **Bottle Pressure** – Used to pressurize the bottles. Should be set to 9 psi. Regulator is accessible through the side access panel. Displayed on Left Gauge Panel (See Section 1.1.13).

CAUTION Timed delivery volumes from solvent bottles 1-4 and 8 are dependent on the **Bottle Pressure** setting. Any adjustments made to this regulator will require reverification of **Solvent Calibration** (Section 2.5.1).

The intensity of the mixing and fluid deliveries are controlled using the following two flow controls:

1. **Mix Flow Control** – Controls the nitrogen flow during a mix. Located on Left Gauge Panel (See Section 1.1.13). Counter clockwise to increase, clockwise to decrease.

2. **Nitrogen Push Flow Control** – Controls the nitrogen flow during fluid delivery. Control can be accessed through the side access panel.

CAUTION The **Nitrogen Push Flow Control** is factory set. Adjusting the **Nitrogen Push Flow Control** will affect the way the instrument delivers fluid to the reaction vessels. If it is adjusted too low, it may cause the instrument to fail in its delivery and error out. If you feel an adjustment is needed, please contact a GPT customer service representative for proper instructions prior to adjusting this control.

IMPORTANT Adjusting the mixing or delivery flows too high can cause resin to be pushed to the top of the reaction vessels and possible reagent loss. This can lead to incomplete reactions.

1.1.11 Vacuum System

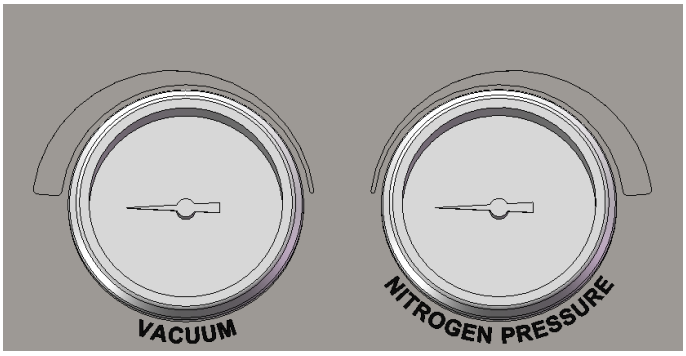
Vacuum is supplied by a vacuum pump located inside the instrument. The vacuum is displayed on the vacuum gauge on the front of the instrument. The normal operating range is 10-22 in Hg. When the vacuum drops to 10 in Hg, the vacuum pump will turn on. The vacuum is diverted directly to the valve blocks and is used to lift the valve membranes to allow fluid flow from different locations. The lack of vacuum is a critical error, and the instrument will pause all operations, vent all bottles, and display an error message. This occurs when the vacuum pump fails to bring the vacuum >10 in Hg. No operations are allowed until the vacuum is restored.

1.1.12 Right Gauge Panel

The right gauge panel contains:

1. **Vacuum** - Displays the vacuum (in mm Hg). See Section 1.1.11.

2. **Nitrogen Pressure** - Displays the nitrogen pressure (in psi). See Section 1.1.10.



1.1.13 Left Gauge Panel

The left gauge panel contains:

1. **Valve Pressure** – Displays the valve pressure (in psi). The valve pressure should be at 30-40 psi.

2. **Bottle Pressure** – Displays the solvent bottle pressure (in psi). The bottle pressure should be at 9 psi.

3. **N2 Mix Flow Control** - Flow knob controls the nitrogen flow during a mix. Counter clockwise to increase, clockwise to decrease.

1.1.14 Emergency Stop Button

Press down to stop ***Prelude X*** in the event of an emergency. All actions will cease and all bottles will be vented. Simply twist clockwise to release. This button should only be used in the event of an emergency and is not a viable way

to pause an operation during a synthesis. Pressing the Emergency Stop Button can result in a loss of reagents and/or the current synthesis.

1.1.15 Computer System

Prelude X has an internal computer that operates the instrument and user software. A monitor, keyboard and mouse are supplied with the instrument. They are connected to the instrument's internal computer via the utility panel. Extra USB ports allow data to be transferred from the computer via a user-supplied memory stick.

1.1.16 Safety Shield

Safety doors are installed for the protection of the user. The doors in front of the reaction vessels MUST be CLOSED when ***Prelude X*** is running. Before opening the doors, all of the reaction vessels must be in a non-operational state (i.e. paused or completed) and drained of all fluids. Opening the doors while a synthesis is running will result in an error and the synthesis will be paused.

IMPORTANT Minimum safety equipment to be used at all times are: NIOSH/MSHA-approved respirator, face shield, chemically resistant gloves, and other protective clothing.

1.1.17 Service and instrument modifications

The service, operation, and/or repair of the instrument by persons other than GPT designated technicians may result in the voiding of any existing warranty coverage. Damages to the instrument from service rendered or use of non-GPT parts without the prior written or oral approval of GPT is the responsibility of the customer and may be just cause for voiding any existing warranty coverage.

1.1.18 Unauthorized software

No additional software or programs should be installed onto the GPT-supplied computer unless otherwise instructed by GPT. GPT cannot guarantee that installation of unauthorized programs will not impact instrument performance.

1.2 Instrument Setup

1.2.1 Laboratory Requirements

Ensure a flat sturdy surface capable of safely supporting 136Kg (300 lbs) and to allow for easy access for loading reagents and viewing and operating the computer. The surface should be near a primary power outlet, a fume hood and a nitrogen source. The power outlet needs to be clear at all times. If main power needs to be disconnected, unplug BOTH cords at the power outlet. Only use the power cords supplied with the instrument. Ambient temperature should be 10-37.8°C(50-100ºF) with relative humidity below 90%. Do not place the instrument where it can be exposed to extreme temperatures, e.g. near heating or cooling ducts, near open windows or in direct sunlight. The elevation should be between sea level and 2000 meters.

In order to install and run ***Prelude X***, 100-240 VAC 50/60Hz (Main Power: 1.0A (Internally Fused), Heater Power:10.0A (Fuse Value = 10.0A Ceramic 250V Slo-Blo), / Main Power: 0.5A (Internally Fused), Heater Power: 5.0A (Fuse Value = 5.0A Ceramic 250V Slo-Blo)) a laboratory must be able to supply the following:

1. **Ventilation System**
   Prelude X has a 4-inch vent equipped with an adjustable angle adaptor.
   Prelude X should be connected to a lab ventilation system with a 4-inch (10 cm) duct supplied by the user. The ducting should be made of a chemically resistant material (PVC or urethane, but no rubber). A minimum flow of 50 cubic feet/min (CFM) must be maintained at the instrument.

2. **Nitrogen Supply**
   A relatively pure (>99.9%) and dry source of pressurized nitrogen is recommended. The system uses nitrogen for solution transfers and agitation/mixing. Alternative gases can be utilized if desired, e.g., Argon. The user must supply all necessary regulators and nitrogen tanks. One male and one female 1/4" NPT fittings are provided with the unit to connect it to the tank.

IMPORTANT Securely fasten cylinders with safety straps to prevent them from falling, and do not move a cylinder or undo safety straps unless the safety cap is in place.

3. **Secondary container for waste (recommended)**

The supplied 5 gallon waste carboy should be placed in a secondary container that is resistant to the harsh chemicals in the waste. The capacity of the secondary container should be enough to contain a 5 gallon spill.

4. **Memory Stick (optional)**
   Files may be transferred from ***Prelude X*** computer to an external computer using the USB port and a memory stick.

1.2.2 Instrument Installation Procedure

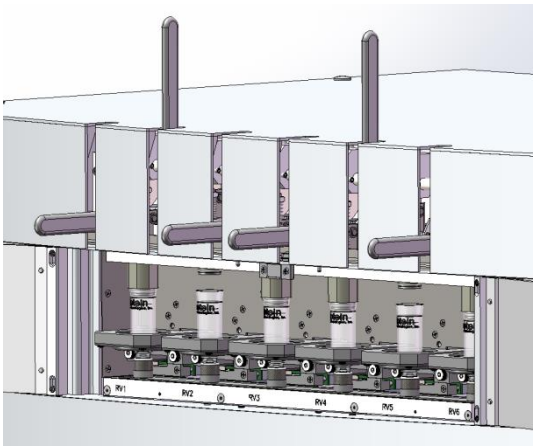
IMPORTANT Installation of ***Prelude X*** should be performed by trained personnel only. Improper installation may result in damage to the instrument or operators. Please contact GPT if ***Prelude*** X needs to be moved after installation.

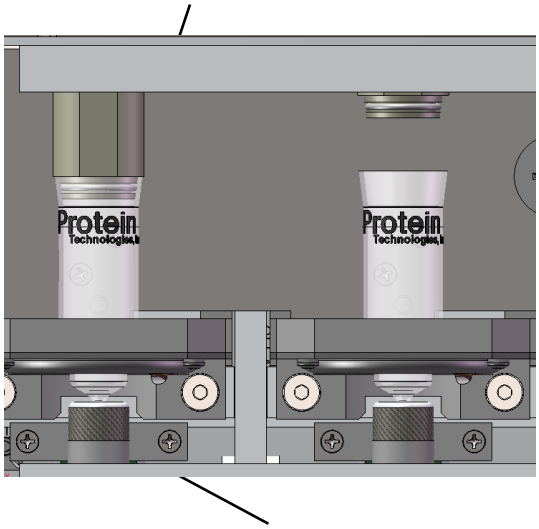
- Uncrate main unit, safely (bend at the knees) lift off pallet (use caution >300 lbs).
- Remove all materials from crate. Check off list:
  - (1) Prelude X user manual
  - (2) 4L safety-coated glass bottles
  - (7) 1L safety-coated glass bottles
  - (1) 5 gal. waste container
  - (12) 10 ml reaction vessel assemblies
  - (12) Package of collection tubes
  - (27) Package of amino acid bottles
  - (1) Waste duct assembly
  - (1) Nitrogen tubing assembly
  - (1) Flow meter assembly
  - (1) Bottle tray
  - (1) Bottle cap and tubing assembly
  - (1) Waste tubing assembly
  - (1) Waste cable assembly
  - (1) Power cord
  - (1) Monitor
  - (1) USB Keyboard
  - (1) USB Mouse
  - (1) Mouse pad
  - (1) Plastic fitting, 1/4" FPT x 1/4" tube
  - (1) Peptide Predictor software
  - (1) AA replacement filters (100 pack)
  - (1) Solvent bottle filter (100 pack)

- Remove top panel and side panel where vent will be installed. Install adjustable vent adaptor and bend flaps out to fasten. Place vent cap in other side panel vent hole.
- Attach waste level sensor cable connector (4 pin) to utility panel (Section 1.1.3).
- Place waste tank in user-provided secondary container and dress cleanly. Attach the other end of the connector to the waste level sensor on the waste container by lining up the red dots, then pushing down.
- Attach the three shorter 1/4" waste lines from waste tank to waste fittings on solvent feedthrough panel (Section 1.1.2).
- Attach the long 1/4" vent line from waste tank to vent duct adaptor fitting.
- Install RV's and collection tubes into positions (Sections 1.2.3 & 1.2.4).
- Install Solvent 1 through Solvent 8 bottles (Section 1.2.6) and loctite fittings to solvent feedthrough panel connectors
- Install 27 empty amino acid bottles (Section 1.2.6).
- Unpack and setup monitor/keyboard/mouse and connect to utility panel (Section 1.1.3).
- Attach power cord to power cord receptacle (Section 1.1.3) and plug in.
- Attach nitrogen supply lines.
- Turn on main power switch and the three circuit breakers.
- Turn on computer monitor.
- Verify all systems alarms are OK. (4 icons on lower right corner of main screen are green)
- Perform Nitrogen Leak Check (see Section 4.2.3).
- Back flush Solvent 1 to all 27 amino acid lines and verify liquid delivery to each amino acid bottle using the **Bottle Preparations** screen (Section 2.5.1.1).
- Use the **Manual Operations** screen (Section 2.5.2.5) to deliver 1000 µL of Solvent 1 to all RV's. Verify liquid delivery and drain into waste (check for leaks on all waste valve fittings).
- Pressurize amino acid bottles using the **Bottle Preparations** screen (Section 2.5.1.1) and check for leaks.

1.2.3 RV & O-Ring Installation

To install or remove reaction vessel:





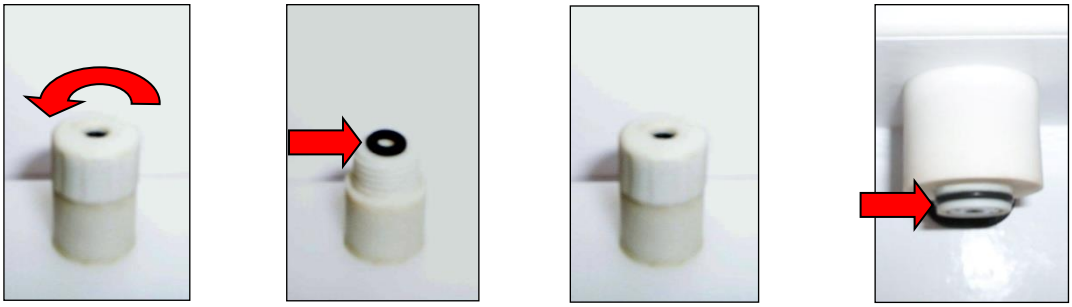
1. When installing an RV, gently insert the RV into the bottom seat. Be sure to line the RV up with the upper seat. Hold the RV with one hand while carefully lowering the cam lever to the horizontal position to lock the RV in place.

2. To remove an RV, apply slight downward pressure to the RV with one hand while lifting the cam lever with the other until the lever locks into the vertical position. Pull the RV gently up and out of the lower seat. A slight twisting motion may help release the RV from the bottom seat.

CAUTION Make sure the RV bottom is pressed through the bottom o-ring in the lower RV seat, or the RV may leak. Be cautious when lowering the RV cam lever. It can snap closed and break the RV if it is not guided down.

To install reaction vessel o-rings:

1 **2** **3** **4**



Bottom o-rings:

1. Unscrew the cap from the lower RV seat on the instrument.
2. Place a reaction vessel bottom o-ring in the center of the lower RV seat.
3. Screw the cap on over the o-ring until tight.

Top o-rings:

1. Slide a reaction vessel top o-ring into the groove on the upper RV seat.
2. Test for leaks by installing an empty reaction vessel (below) and performing a **DMF Top Wash** using the **Manual Operations** screen (Section 2.5.2.5).

1.2.4 Collect Vial Installation

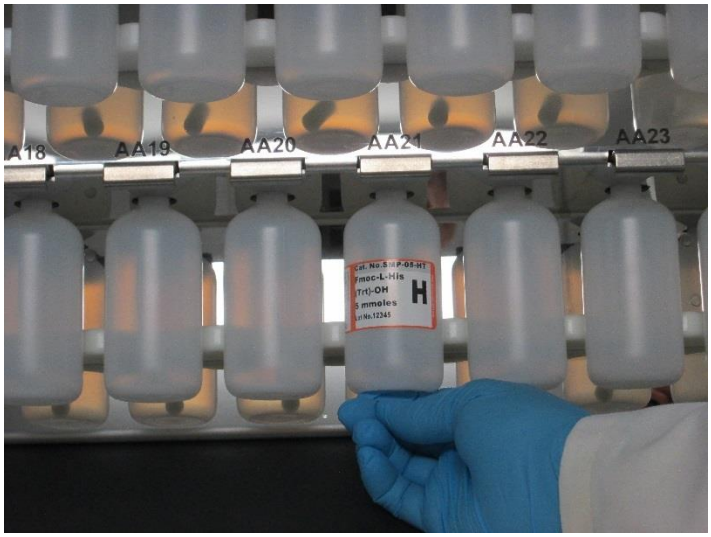
The collection vials install into a threaded port on the machine. Install by turning the vial clockwise, by hand, until the top of the vial is seated firmly against the seal inside the threaded port. To remove, turn the vial counterclockwise.

IMPORTANT It is recommended that collection vials be ordered from Gyros Protein Technologies, or, if ordered from another source, that only “VWR” branded tubes are used. Other similar products may not connect properly.

IMPORTANT It is not recommended to have cold ether in the collection vial when the cleavage solution is collected. The vial may overfill during the collection of the product causing both loss of the product and potential damage to the instrument from the TFA solution. Rather, collect cleavage solution, remove collection vial from instrument, then precipitate peptide with cold ether (< 0°C).

1.2.5 Amino Acid Bottle Installation

To install an amino acid bottle, first make sure the bottle position is vented and if necessary, back flushed with nitrogen (See **Bottle Preparations** screen, Section 2.5.1.1) then:

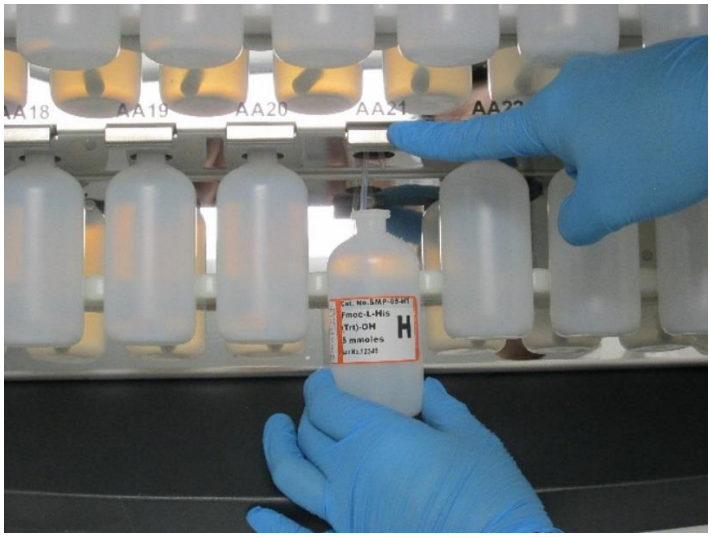


1. Make sure the metal slide is pushed all the way in. Insert the bottle filter and tube into the bottle, and push the amino acid bottle upward.

2. The metal slide is spring-loaded and will pop out when the bottle is in place.

NOTE Check that the bottle filter is resting against the lower rear of the bottle. This will ensure that all of the reagent in the bottle will be used.

To release the bottle, make sure the bottle position is vented. Hold the amino acid bottle with one hand while pushing in the metal slide with the other. Carefully slide the bottle off the tubing and filter.



CAUTION Failure to hold the bottle while releasing will cause the bottle to fall and spill, which may result in personal injury, loss of reagent and/or damage to the instrument.

1.2.6 Solvent/Reagent Bottle Installation

To install a solvent/reagent bottle:

1. Make sure the solvent/reagent bottle position is vented (See **Bottle Preparations** screen, Section 2.5.1.1).

2. Verify the o-ring is properly installed on the cap insert and that the insert is in the cap. Also, verify that the fluid line has a bottle filter with frit attached.

3. Place the bottle in the bottle container. Insert the line so that it is straight and at the bottom of the bottle (the tubing can be 'molded' by gentle bending—Do not 'kink' or the tubing integrity will be compromised).

4. Attach the cap and tighten to a firm hand tight.

To remove the bottle, make sure the bottle position is vented and unscrew the cap while carefully guiding the tubing and filter out of the bottle.

1.2.7 Waste Container Installation

To install the waste container:

1. Place the waste container into a secondary containment vessel (if available).

2. Align both red dots to properly insert the waste level cable connector into the waste level sensor located at the top of the waste container. Plug the other end of the waste level cable connector into the waste level sensor port located on the utility panel.

3. Connect the longer 1/4" vent line to the exhaust vent adaptor fitting and insert the other end into the waste tank cap.

4. Screw the cap onto the waste tank.

To empty a full container:

1. Carefully disconnect the waste level sensor connector by grasping the knurled area of the fitting firmly and pulling directly up. Unscrew the cap.

2. Empty the waste container and place it back into the secondary containment vessel.

3. Screw the cap back on. Do not force.

1.3 Accessories

1.3.1 Reaction Vessels & O-Rings

10 mL, Induction Compatible
Cat#: PPX-FGRV10-1, 1 ea.
Cat#: PPX-FGRV10-1, Pkg. of 6

25 mL, Induction Compatible
Cat#: PPX-FGRV25-1, 1 ea.
Cat#: PPX-FGRV25-1, Pkg. of 6

40 mL, Induction Compatible
Cat#: PPX-FGRV40-1, 1 ea.
Cat#: PPX-FGRV40-1, Pkg. of 6

10 mL, Glass
Cat#: TPS-GRV10-1, 1 ea.
Cat#: TPS-GRV10-10, Pkg. of 10

40 mL, Glass
Cat#: TPS-GRV40-1, 1 ea.
Cat#: TPS-GRV40-10, Pkg. of 10

10 mL, Disposable
Cat#: PPS-R10-030, Pkg. of 30
Cat#: PPS-R10-090, Pkg. of 90
Cat#: PPS-R10-180, Pkg. of 180

45 mL, Disposable
Cat#: PPS-R45-030, Pkg. of 30
Cat#: PPS-R45-090, Pkg. of 90
Cat#: PPS-R45-180, Pkg. of 180

Reaction Vessel O-Rings:

- Bottom, Premium:
  Cat#: PPS-ORING-BK-06, Pkg. of 6
- Top, Premium:
  Cat#: PPS-ORING-TK-06, Pkg. of 6

1.3.2 Amino Acid Bottles

10 mL Single-Shot™
Cat#: AAR-SSI, 1 ea
Cat#: AAR-SSX, Pkg. of 10

120 mL
Cat#: SMP-VX-20, Pkg. of 20
Cat#: SMP-VX-100, Pkg. of 100

400 mL
Cat#: AAR-400-I, 1 ea.
Cat#: AAR-400-X, Pkg. of 10

1.3.3 Collection Vials

50 mL
Cat#: CLV-050-030, Pkg. of 30
Cat#: CLV-050-090, Pkg. of 90
Cat#: CLV-050-180, Pkg. of 180

1.3.4 Amino Acids & Reagents for Peptide Synthesis

GPT supplies high quality, pre-tested N-Fmoc-protected amino acids preweighed in 5 mmol, 10 mmol and 20 mmol quantities in synthesizer-ready bottles (see Appendix A.1 for listings), as well as bulk N-Fmoc-protected amino acids pre-weighed in 5 g, 25 g 100 g, and 1 kg quantities (See Appendix A.2 for listings). We recommend using our amino acids for all of your synthesis needs.

GPT also supplies reagents for peptide synthesis on ***Prelude X*** (See Appendix A.3 for listings).

1.3.5 Replacement Parts & Additional Accessories

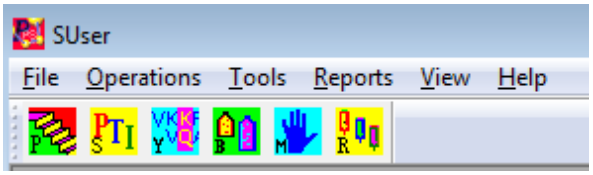
GPT supplies a full line of replacement parts for ***Prelude X***, as well as various accessories, including solvent/reagent bottles and waste containers. A partial listing of replacement parts and accessories is located in Appendix C. For additional parts and accessory information, please contact our customer support desk at 800.477.6834 or peptides@gyrosproteintech.com.

Chapter 2 Introduction to Software

This Chapter covers the components of each screen in the software. ***Prelude X*** software was designed to mimic the Windows® format to ease customer learning, however, there are some differences. The main difference is the **File Manager** screen, which is explained in Section 2.3.

2.1 Main Menu

The **Main Menu** is located at the top of the main SUser window and contains the following menus:



2.2 Shortcut buttons

Six shortcut buttons are located below the main menu. They open the following screens:

 – Program File Manager

 – Sequence File Manager

 – Synthesis File Manager

 – Bottle Preparations Screen

 – Manual Operations Screen

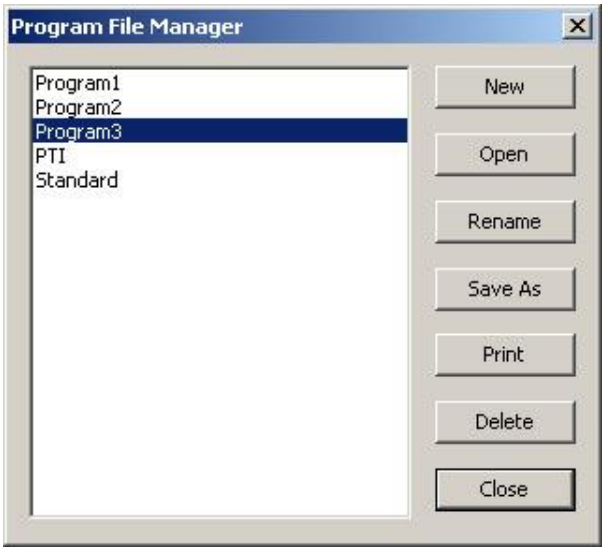
 – Reaction Vessel Operations Screen

2.3 File Manager

The **File Manager** is the main interface screen for manipulating the following file types:

1. Amino Acid
2. Solvent/Reagent
3. Program
4. Sequence
5. Synthesis

As an example, the **Program File Manager** is shown below.



The **File Manager** lists all files of that type in the box to the left of the screen with function buttons to the right. To select a file, click on its name to highlight it. When a file is highlighted, all buttons to the right activate. The function of each button is:

1. **New** - creates a new file.

    a. Click on the **New** button. This will open a new window.

b. Enter a name for the new file (or accept the default name) and click **OK** to create a new file or click **Cancel** to return to the **File Manager** screen without creating a new file.

c. After clicking **OK**, the **File Editor** for that file type will open with the new file ready for editing.

2. **Open** - opens an existing file.

   a. Click on the desired file name to highlight it. Once a file is highlighted, the **Open** button becomes active.

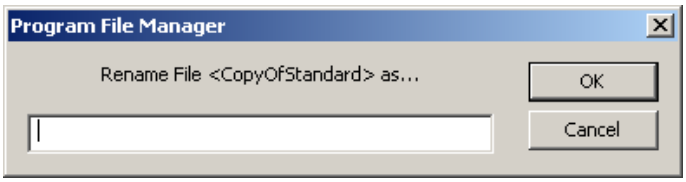
   b. Click on the **Open** button.

   c. The **File Editor** for that file type will open with the file ready for viewing or editing.

3. **Rename** - assigns a new name to an existing file.

   a. Click on the desired file name to highlight it. Once a file is highlighted, the **Rename** button becomes active.

   b. Click on the **Rename** button. This will open a new window.



   c. Enter the new name and click **OK** to rename the file, or click **Cancel** to return to the **File Manager** without renaming the file.

4. **Save As** - creates a copy of an existing file.

   a. Click on the desired file name to highlight it. Once a file is highlighted, the **Save As** button becomes active.

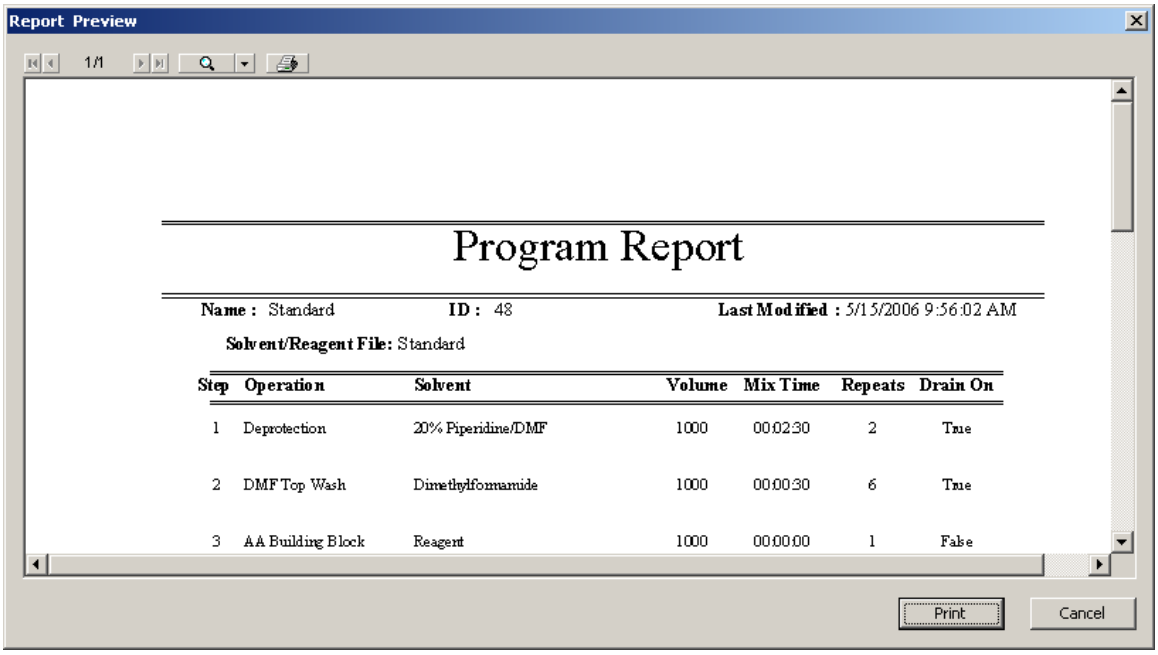
   b. Click on the **Save As** button. This will open a new window with the name “CopyOfX,” where “X” is the name of the original file, displayed as the default name for the copy.

c. You may choose to keep this name or enter a different name. Click **OK** to create a copy of the file, or click **Cancel** to return to the **File Manager** screen without copying the file.

5. **Print** - prints a file.

   a. Click on the desired file name to highlight it. Once a file is highlighted, the **Print** button becomes active.

   b. Click on the **Print** button. This will open the **Report Preview** window with a preview of how the document will look when it is printed. Use the magnifying glass at the top of the screen to view the document at different magnifications and the left and right arrows to navigate between pages.



   c. To print the file, click the **Print** button or click on the printer icon at the top of the screen. Alternatively, click on **Cancel** to return to the **File Manager** screen without printing the file.

   d. Click on the **X** in the upper right corner of the window or click **Cancel** to close the **Report Preview** window.

6. **Delete** - deletes an existing file.

   a. Click on the desired file name to highlight it. Once a file is highlighted, the **Delete** button becomes active.

b. Click on the **Delete** button. This will open a new window with the message “You are about to delete 1 file. Are you sure?”

c. Click **OK** to delete the file or click **Cancel** to return to the **File Manager** screen without deleting the file.

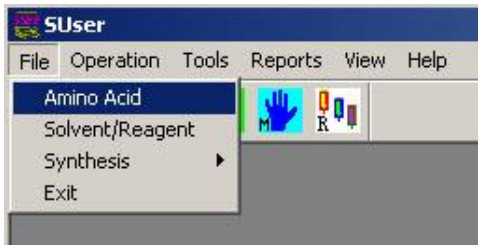
7. **Close** - closes the **File Manager** screen. Click the **Close** button or the **X** in the top right corner of the screen to exit the **File Manager**.

2.4 File Menu

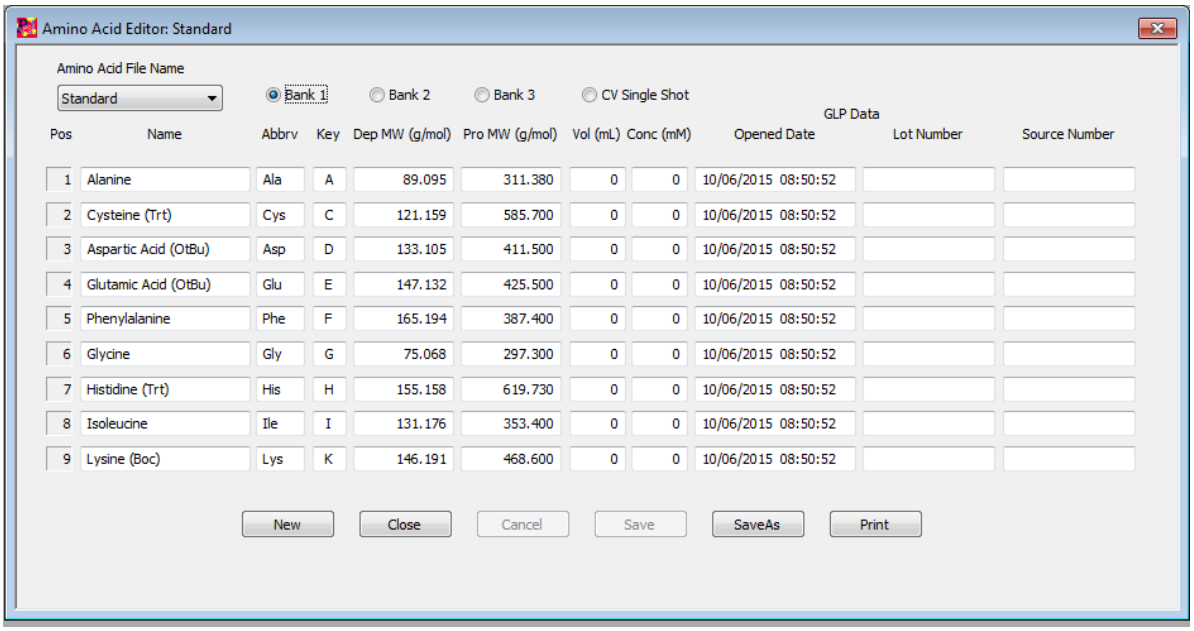
2.4.1 Amino Acid Editor

The **Amino Acid Editor** allows the user to describe the contents of each amino acid bottle and create a good laboratory practice record of the volumes and concentrations of amino acids consumed, as well as the date opened, the lot number and the source number.

To open the **Amino Acid Editor**, click on the **File** menu and select **Amino Acid.**



The **Amino Acid File Manager** will open as a new screen. To create a new amino acid file, click the **New** button, enter a name for the new file, and click **OK**. Alternatively, select an existing amino acid file from the list and click the **Open** button. The amino acid file will open in the **Amino Acid Editor**.



The **Amino Acid File Name** box displays the name of the currently open amino acid file. To open a different file, select a different file from the pull-down menu.

Bank 1, Bank 2, and Bank 3 selections determine which amino acid positions are displayed. Bank 1 displays positions 1-8, Bank 2 displays positions 9-17, Bank 3 displays positions 19-27 and CV Single Shot displays positions 28-33.

The columns are labeled as follows:

1. **Pos** – Amino acid bottle position (1-33)
2. **Name** – Name of amino acid (or other chemical)
3. **Abbrv** – Three-letter abbreviation for amino acid (or other chemical)
4. **Key** – One-letter abbreviation
5. **Dep MW (g/mol)** – Molecular Weight without protecting groups
6. **Pro MW (g/mol)** – Molecular Weight with protecting groups
7. **GLP Data**
    a. **Vol (mL)** – Volume used for synthesis (in mL).
    b. **Conc (mM)** – Concentration of amino acid solution used for synthesis (mM).
    c. **Opened Date** – Date (and time) solution was opened.

d. **Lot Number** – Lot number.

e. **Source Number** – Source information (catalog number, company, etc.).

By default, a new file contains the names and molecular weight values of 20 standard amino acids. Double-click in a cell to modify its value.

The function of each button is:

1. **New** – The **New** button creates a new file. The **Amino Acid File Manager** will open. Enter a new name or use the default and click **OK**, or click the **Cancel** button to return to the **Amino Acid Editor** without creating a new file.

2. **Close** – The **Close** button is not active until the file is saved. After saving the file, click the **Close** button to exit the **Amino Acid Editor** screen.

3. **Cancel** – The **Cancel** button removes any changes made since the last **Save**. A screen will appear reading, "Abandon All Changes?" The **Yes** button will permanently remove the changes; the **No** button will leave the changes.

4. **Save** – Once a change is made to the file the **Save** button becomes active. Click **Save** to save the changes. The **Save** and **Cancel** buttons will deactivate once the file is saved.

5. **Save As** – To copy the file on the screen, click the **Save As** button. The **Amino Acid File Manager** will appear with a default name for the copy. Change the file name or accept the default and click **OK**. The **Cancel** button will close the screen and return to the **Amino Acid Editor**.

6. **Print** – To print the open amino acid file click the **Print** button. It will go automatically to the printer.

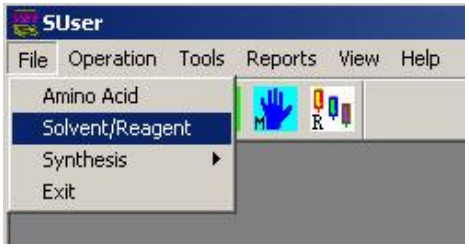
NOTE To view the report before printing, click the **Close** button and preview the file using the **Print** button in the **Amino Acid File Manager** (Section 2.3).

NOTE *Prelude X* software comes with a standard amino acid file that can be copied, opened or viewed by selecting the file "Standard" in the **Amino Acid File Manager** (See Section 2.3). The "Standard" file contains the names and molecular weight values of 20 standard amino acids.

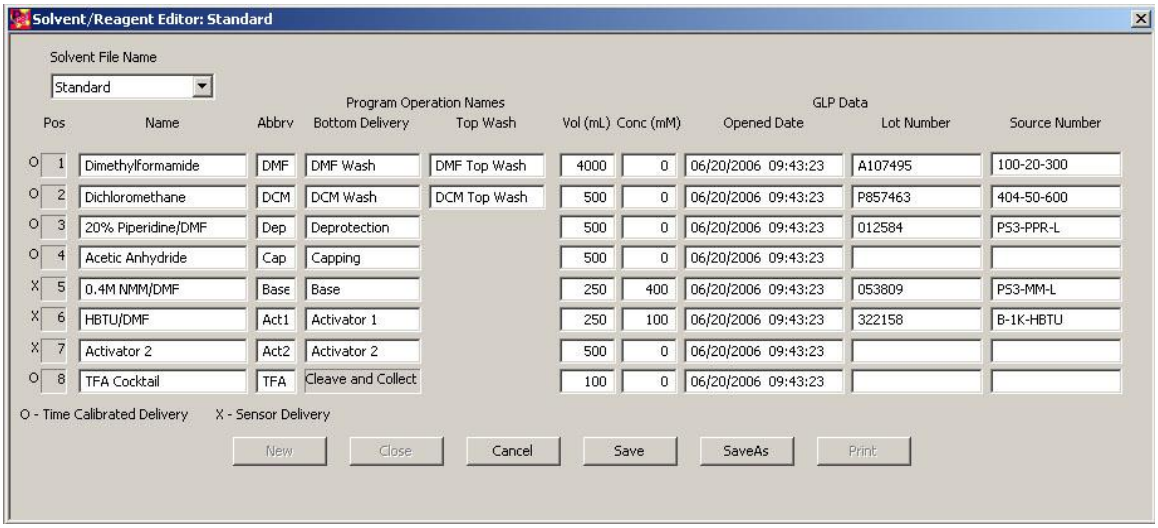
2.4.2 Solvent/Reagent Editor

The **Solvent/Reagent Editor** allows the user to assign abbreviations and operation names to each solvent/reagent bottle and create a good laboratory practice record of the volumes and concentrations of solvents or reagents consumed, as well as the date opened, the lot number and the source number.

To open the **Solvent/Reagent Editor**, click on the **File** menu and select **Solvent/Reagent** similar to the Amino Acid file.



The **Solvent/Reagent File Manager** will open as a new screen. To create a new solvent/reagent file, click the **New** button, enter a name for the new file, and click **OK**. Alternatively, select an existing solvent/reagent file from the list and click the **Open** button. The solvent/reagent file will open in the **Solvent/Reagent Editor**.



The **Solvent File Name** box displays the name of the currently open solvent/reagent file. To open a different file, select a different file from the pull-down menu.

To the left of each row is an “O” or an “X.” “O” indicates volumes for that bottle position are measured by timed deliveries, while “X” indicates volumes for that bottle position are measured by sensor-controlled deliveries using a metered loop.

The columns are:

1. **Pos** – Solvent/reagent bottle position (1-8)

2. **Name** – Name of solvent or reagent

3. **Abbrv** – Abbreviation for solvent or reagent

4. **Program Operation Names** – Names used to describe program operations. These operation names are displayed in programs, on the synthesis log, and in the **Manual Operations** screen.

    a. **Bottom Delivery** – Solvent or reagent is delivered through the bottom of the RV

    b. **Top Wash** – Solvent is delivered through the top of the RV

NOTE Only Solvents 1 & 2 can be delivered through the top of the RV.

5. **GLP Data**

    a. **Vol (mL)** – Volume used for synthesis (in mL).

    b. **Conc (mM)** – Concentration of reagent solution used for synthesis (mM).

    c. **Opened Date** – Date and time solution was opened.

    d. **Lot Number** – Lot number.

    e. **Source Number** – Source information (catalog number, company, etc.).

By default, a new file contains the standard solvents and reagents. Double-click in a cell to modify the value.

The buttons are:

1. **New** – The **New** button creates a new file. The **Solvent/Reagent File Manager** will open. Enter a new name or use the default and click **OK**, or click the **Cancel** button to return to the **Solvent/Reagent Editor** without creating a new file.

2. **Close** – The **Close** button is not active until the file is saved. After saving the file, click the **Close** button to exit the **Solvent/Reagent Editor** screen.

3. **Cancel** – The **Cancel** button removes any changes made since the last **Save**. A screen will appear reading, “Abandon All Changes?” The **Yes** button will permanently remove the changes; the **No** button will leave the changes.

4. **Save** – Once a change is made to the file, the **Save** button becomes active. Click **Save** to save the changes. The **Save** and **Cancel** buttons will deactivate once the file is saved.

5. **Save As** – To copy the file on the screen, click the **Save As** button. The **Solvent/Reagent File Manager** will appear with a default name for the copy. Change the file name or accept the default and click **OK**. The **Cancel** button will close the screen and return to the **Solvent/Reagent Editor**.

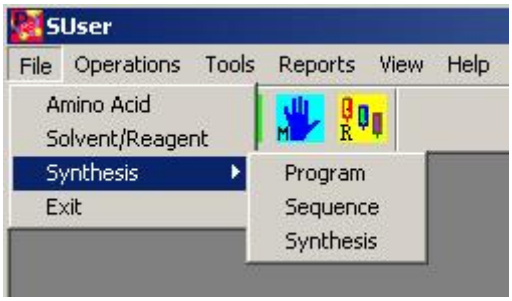
6. **Print** – To print the open solvent/reagent file click the **Print** button. It will go automatically to the printer.

NOTE To view the report before printing, click the **Close** button and preview the file using the **Print** button in the **Solvent/Reagent File Manager** (Section 2.3).

NOTE *Prelude X* software comes with a standard solvent/reagent file that can be copied, opened or viewed by selecting the file “Standard” using the **Solvent/Reagent File Manager** (See Section 2.3). The “Standard” file contains standard solvents and reagents for Fmoc chemistry.

2.4.3 Synthesis Editor

To open the **Synthesis Editor** related screens, click on the **File** menu and select **Synthesis.**



Then select from one of the following three screens:

1. Program
2. Sequence
3. Synthesis

The function of each of these screens will be reviewed in each subsection below.

2.4.3.1 Program Editor

Three programs are recommended to run a synthesis on ***Prelude X:***

1. **Swelling Program** (Synthesis program with extended initial wash times to swell the resin during the first step)

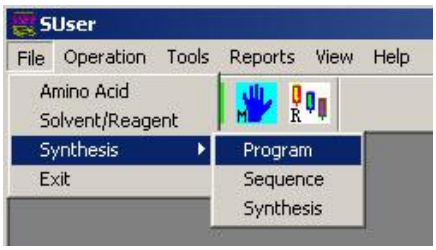
2. **Synthesis Program**

3. **End Program** (Same as Synthesis program with extended washings with DMF and DCM and a drying step at the end)

If automated cleavage is desired, a **Cleavage Program** is also necessary.

NOTE ***Prelude X*** software has standard swelling, synthesis, and cleavage program files called “Standardsw,” “Standard,” and “StandardCleave,” respectively, that can be opened and viewed by selecting a file and opening it using the **Program File Manager** (See Section 2.3). These files are the standard programs installed by GPT, and are used for the synthesis of GPT’s test peptides.

To open the **Program Editor**, click on the **File** menu, select **Synthesis**, and select **Program** or click on the shortcut button.



Or

The **Program File Manager** will open as a new screen. To create a new program file, click the **New** button, enter a name for the new file, and click **OK**. Alternatively, select an existing program file from the list and click the **Open** button. The program file will open in the **Synthesis Editor** under the **Program** tab.

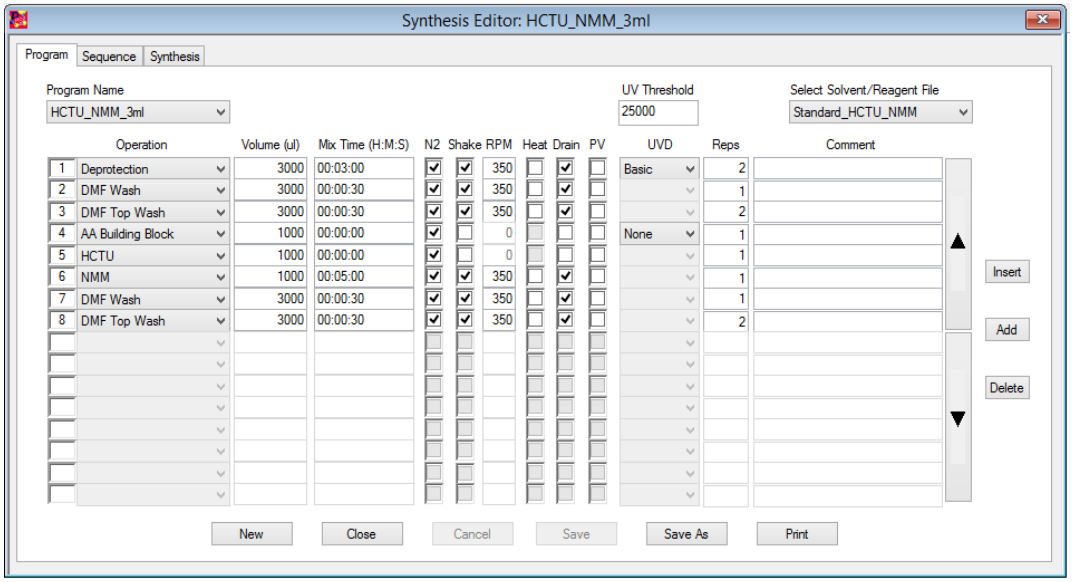


Figure 1. Example synthesis program.

The **Program Name** box lists the name of the currently open program file. To open a different file, select a different file from the pull-down menu.

The **Select Solvent/Reagent File** box lists the name of the solvent/reagent file whose values determine what operations are available under the **Operation** column pull-down menu (see below). To use a different solvent/reagent file, select a different file from the pull-down menu. Make sure to select the **Solvent/Reagent File** before writing the program, since changing the file after operations are set the program will be deleted.

In addition to the operations defined by the selected **Solvent/Reagent File**, the following operations may be selected:

1. **Cleave and Collect** – Delivers Solvent 8, mixes every 2 minutes for the specified time, and drains to collection vial. Rinses for 30 seconds with specified volume of solvent 8 for specified number of reps.

2. **Cleave Mix** – Performs a mix every 2 minutes for the specified time.

3. **Collect** – Drains RV contents to collection vial. Rinses with specified volume of solvent 8 for specified mix time. Repeats rinse for specified number of reps.

4. **Drain/Dry** – Drains RV contents and flushes RV with nitrogen for the specified mix time.

5. **E-Mail Notification** – Sends an email describing the instrument status to the address in the **Settings** screen (See Section 3.7).

6. **Mix** – Mixes RV contents for the specified time.

7. **PV to RV** - Transfers contents from PV positions 2, 4, and 6 to corresponding RVs 1, 3, and 5.

8. **Waste Collect** – Drains RV contents to collection vial.

The columns are:

1. **Operation** – Use the pull-down menu to select an operation for each step.

2. **Volume (uL)** – Enter a volume in microliters. ***Prelude X*** delivers in 150, 500 and/or 1000 μL increments. Entries will be rounded up to the nearest value possible with a minimum volume of 150 μL and a maximum volume of 10,000 μL for the 10 mL RV. The maximum volume increases to 12,500 μL for the 25 mL RV and 20,000 μL for the 40 mL RV. The size of the RV must be specified in the **Settings** screen (Section 2.6.3) for these volumes to take effect.

3. **Mix Time (H:M:S)** – Enter the mix time in Hours:Minutes:Seconds. The maximum mix time is 99:59:59.

4. **N2** - Click to check or uncheck the box. When checked, the mix will occur with N2 bubbling.

5. **Shake** - Click to check or uncheck the box. When checked, during the mix, the RVs will shake.

6. **RPM** - Enter a value for RPM.

7. **Heat** - Click to check or uncheck the box. When checked, the RV will be heated when the mix occurs at the specified temperature in the sequence.

8. **Drain** – Click to check or uncheck the box. When checked, the reaction vessel will be drained at the end of the program step. When unchecked, the reaction vessel will not be drained at the end of the program step.

NOTE It is important to leave **Drain** unchecked when delivering amino acid so the amino acid remains in the reaction vessel for the activator delivery.

CAUTION When **Drain** is unchecked, multiple deliveries may be made to the same RV without draining. Be careful not to exceed the RV's maximum capacity as this may force resin into the showerhead causing clogs or contamination.

9. **RV/PV** - Click to check or uncheck the box. When checked the specified operation will be done on the pre-activation vessel (PV), for example a DMF wash will be delivered to the PV and not the RV. When unchecked the step will occur on the RV.

NOTE If adding reagents to PV (like amino acid and coupling reagents for pre-activation), it is recommended that PV wash steps be included in the program to avoid cross-contamination during following cycle.

10. **UVD** - Use the pull-down menu to select an UV mode during a Deprotection operation. The options are: None, Basic, Xtend, XtendRep. To apply an extended time of the feedback of a UV deprotection, in an AA Building Block, Base, Activator 1 or Activator 2 operation select Use Fb.

11. **Reps** – Enter the number of times to repeat the step. The maximum number of repetitions is 9.

12. **Comment** – Record comments for the step.

The buttons are:

1. **Insert** – To insert a step, move the cursor to a step and click the **Insert** button. A new step will be inserted above that step.

2. **Add** – To add a step to the bottom of the program click the **Add** button.

3. **Delete** – To delete a step, move the cursor to that step and click the **Delete** button.

4. **New** – The **New** button creates a new file. The **Program File Manager** will open. Enter a new name or use the default and click **OK**, or click the **Cancel** button to return to the **Program Editor** without creating a new file.

5. **Close** – The **Close** button is not active until the program is saved. After saving the program, click the **Close** button to exit the **Program Editor** screen.

6. **Cancel** – The **Cancel** button removes any changes made since the last **Save**. A screen will appear reading, "Abandon All Changes?" The **Yes**

button will permanently remove the changes, while the **No** button will leave the changes.

7. **Save** – Once a change is made to the program, the **Save** button becomes active. Click **Save** to save the changes. The **Save** and **Cancel** buttons will deactivate once the program is saved.

8. **Save As** – To copy the file on the screen, click the **Save As** button. The **Program File Manager** will appear with a default name for the copy. Change the file name or accept the default and click **OK**. The **Cancel** button will close the screen and return to the **Program Editor**.

9. **Print** – To print the open program file click the **Print** button. It will go automatically to the printer.

NOTE To view the program before printing, click the **Close** button and preview the file using the **Print** button in the **Program File Manager** (Section 2.3).

Pre-loaded programs are available to be used as initial guidance. The Standard programs included are:

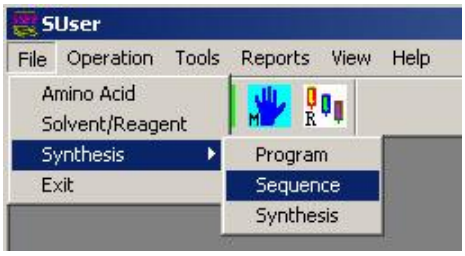
1. **Stand_3ml** – Consists of a simple coupling cycle composed of 2 x 3 min deprotection, 3 x DMF washes, 10 min coupling (1 mL AA, 1 mL activator, 1 mL base), and 3 x DMF washes with a total RV volume of 3 mL.
2. **Stand_3ml_cap** – Same as Stand_3ml with an added 5 min capping step followed by 5 x DMF washes.
3. **Stand_3ml_dblcouple** – Same as Stand_3ml with an added coupling step (1 mL AA, 1 mL activator, 1 mL base) with 1 x DMF wash between coupling steps.
4. **Stand_3ml_heat** – Same as Stand_3ml with heat selected on the deprotection and coupling steps.
5. **Stand_3ml_PV** – Same as Stand_3ml with the coupling reagents sent to the PV, mix for 3 min, followed by a PVtoRV transfer followed by DMF washes to the PV.
6. **Stand_3ml_short** – Same as Stand_3ml with shorter reaction times: 2 x 1 min for deprotection and 3 min coupling.
7. **StandardCleave** – Cleave program including the final Fmoc-deprotection step, followed by DMF and DCM washes and resin drying. Followed by TFA cleavage, collect and final resin washes.
8. **Stand_Cleave_Nodep** – Is a cleave program that starts with the TFA delivery followed by resin washing and drying.
9. **Standard** – Consists of a simple coupling step with 2 x 30 s deprotection and a 3 min coupling reaction (1 mL AA and 1 mL activator).
10. **Longpep_snglcouple_UV** – Similar to the standard programs with deprotection UV monitoring turned on. Consists of 3 x 5 min deprotections and 1 x 30 min coupling reactions (1 mL AA, 1 mL activator, 1 mL base)

11. **Longpep_dblcouple_UV** – Same as Longpep_snglcouple_UV with repeated coupling steps.
12. **Longpep_trpcouple_UV** – Same as Longpep_snglcouple_UV with reapeted coupling steps twice.
13. **AA_only** – Only delivers AA.
14. **Finish** – Consists of DCM washes and resin drying.

A "**_swll**" and "**_end**" programs have also been included where an extra DMF wash is added at the beginning and final DCM washes and drying steps are added at the end, respectively.

2.4.3.2 Sequence Editor

To open the **Sequence Editor**, click on the **File** menu, select **Synthesis**, and select **Sequence** or select the shortcut button below the main menu.



Or

The **Sequence File Manager** will open as a new screen. To create a new sequence file, click the **New** button, enter a name for the new file, and click **OK**. Alternatively, select an existing sequence file from the list and click the **Open** button. The sequence file will open in the **Synthesis Editor** under the **Sequence** tab.

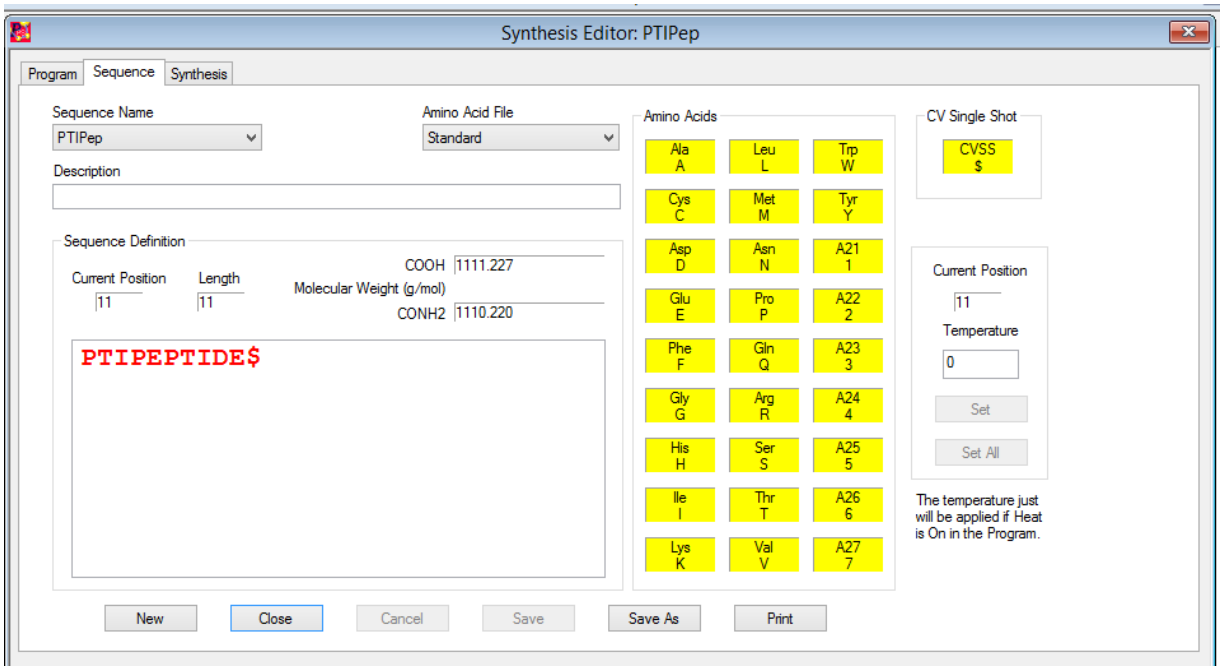


Figure 2. Example of sequence editor set up.

The **Sequence Name** box displays the name of the currently open sequence file. To open a different file, select a different file from the pull-down menu.

The **Select Amino Acid File** box displays the name of the amino acid file whose values will determine what amino acids (or other chemicals) are available for inclusion in the sequence. To use a different **amino acid** file, select a different file from the pull-down menu.

The **Amino Acids** box contains buttons for each of the 27 bottle positions on the instrument. The buttons are labeled with the abbreviations and key codes of the currently open amino acid file. Enter a sequence by clicking the buttons with the mouse or using the keyboard and entering the single letter code from N to C-terminal.

The **CV Single Shot** box contains a button to add the key of the CV single shot amino acid. Each CV Single Shot position is tied to a specific RV. For example, CV1 can only deliver to RV1.

The **Temperature box** to the right of the screen allows a temperature to be set for each position in the sequence. Each position can have a unique temperature or a single temperature can be used across all positions. Entering a "0" in the Temperature box will leave the position unheated.

Enter comments in the **Description** box.

The **Sequence Definition** box contains the following boxes:

1. **Current Position** – lists the current position of the cursor within the sequence

2. **Length** – lists the total number of characters in the sequence

3. **Molecular Weight COOH** – displays the molecular weight of the deprotected peptide with an acid C-terminus

4. **Molecular Weight CONH2** – displays the molecular weight of the deprotected peptide with an amino C-terminus

NOTE The peptide molecular weights are calculated from the amino acid molecular weight values entered in the amino acid file.

5. **Sequence Box** – large white box where the sequence is entered. Click in the box to place the cursor. Enter a sequence by clicking on the buttons to the right of the screen or by using the keyboard.

NOTE Entered characters must match those available in the current amino acid file.

The remaining buttons are as follows:

1. **New** – The **New** button creates a new file. The **Sequence File Manager** will open. Enter a new name or use the default and click **OK**, or click the **Cancel** button to return to the **Sequence Editor** without creating a new file.

2. **Close** – The **Close** button is not active until the sequence is saved. After saving the sequence, click the **Close** button to exit the **Sequence Editor** screen.

3. **Cancel** – The **Cancel** button removes any changes made since the last **Save**. A screen will appear reading, “Abandon All Changes?” The **Yes** button will permanently remove the changes; the **No** button will leave the changes.

4. **Save** – Once a change is made to the sequence, the **Save** button becomes active. Click **Save** to save the changes. The **Save** and **Cancel** buttons will deactivate once the sequence is saved.

5. **Save As** – To copy the file on the screen, click the **Save As** button. The **Sequence File Manager** will appear with a default name for the copy.

Change the file name or accept the default and click **OK**. The **Cancel** button will close the screen and return to the **Sequence Editor**.

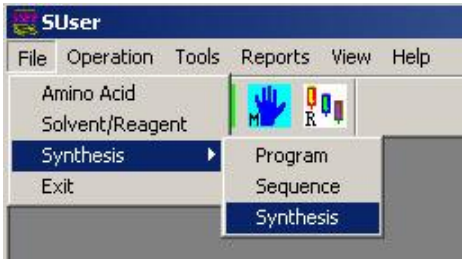
6. **Print** – To print the open sequence file click the **Print** button. It will go automatically to the printer.

NOTE To view the sequence before printing, click the **Close** button and preview the file using the **Print** button in the **Sequence File Manager** (Section 2.3).

2.4.3.3 Synthesis Editor

The **Synthesis Editor** is used to create synthesis files. Synthesis files are used to assign sequences to reaction vessels, choose an acid or amino C-terminus for each peptide, and assign programs to each cycle of the synthesis. A program assigned to a given cycle is run on all active reaction vessels in parallel unless **Dynamic Sequence Programming** is used (Section 4.2).

To open the **Synthesis Editor**, click on the **File** menu, select **Synthesis**, and then select **Synthesis** or select the shortcut button.



Or

The **Synthesis File Manager** will open as a new screen. To create a new synthesis file, click the **New** button, enter a name for the new file, and click **OK**. Alternatively, select an existing synthesis file from the list and click the **Open** button. The synthesis file will open in the **Synthesis Editor** under the **Synthesis** tab.

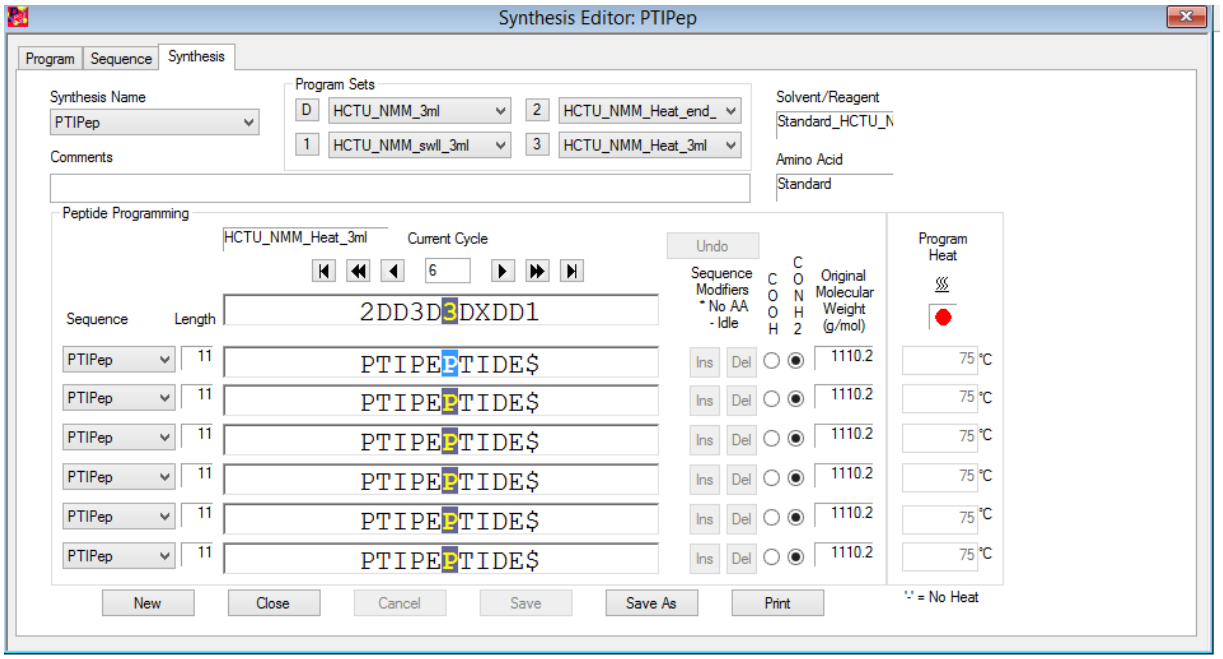


Figure 3. Example of synthesis editor set up.

The **Synthesis Name** box lists the name of the currently open synthesis file. To open a different file, select a different file from the pull-down menu.

Enter synthesis comments in the **Comments** box.

The **Program Sets** section defines programs that can be assigned to different cycles of the synthesis. Use the pull-down menus to assign programs to each button: **D**, **1**, **2** and **3**. **D** stands for the default program, which is initially assigned to every cycle in the synthesis. If more than four programs are needed, additional programs may be assigned using buttons **1**, **2**, and **3**. See **Assign Sequence** (#8 in **Peptide Programming** section below) for more information about programming assignments.

The **Solvent/Reagent** box displays the solvent/reagent file assigned to the synthesis. This file is determined based on the first sequence entered.

The **Amino Acid** box lists the current amino acid file assigned to the synthesis. This file is determined based on the first sequence entered.

The **Peptide Programming** section has the following functionalities:

1. **Sequence** – Assign a sequence to each RV using the pull-down menus in this column. The first row corresponds to RV1, the second row corresponds to RV2, and so on down to RV6.

NOTE The amino acid and solvent/reagent files assigned to the first sequence chosen will define those files for the synthesis. Only sequences with amino acid and solvent/reagent files matching those of the first sequence chosen will be allowed in the synthesis. To run a sequence with a different amino acid or solvent/reagent file, a different synthesis file must be created.

2. **Length** – The length of the peptide is displayed in the **Length** column.

3. **Peptide Sequence** – The sequence is displayed to the right of the **Length** column, and is right justified with the N-terminus to the left, and the C-terminus to the right. The residues involved in the current cycle are highlighted.

4. **Dynamic Sequence Programming** – The function of the **Ins**, **Del**, and **Undo** buttons next to the text “Sequence Modifiers *No AA – Idle” are part of this optional advanced feature described in detail in Section 3.6.

5. **COOH/CONH2** – Click under COOH or CONH2 to choose an acid or amino C-terminus, respectively, for the peptide. When COOH is chosen, the sequence will shift one character to the right, and the first character will not be included in the synthesis.

6. **Molecular Weight** – The molecular weight of the peptide with the assigned COOH or CONH2 C-terminus is displayed. The molecular weight is calculated using the deprotected molecular weight values in the amino acid file assigned to the sequence.

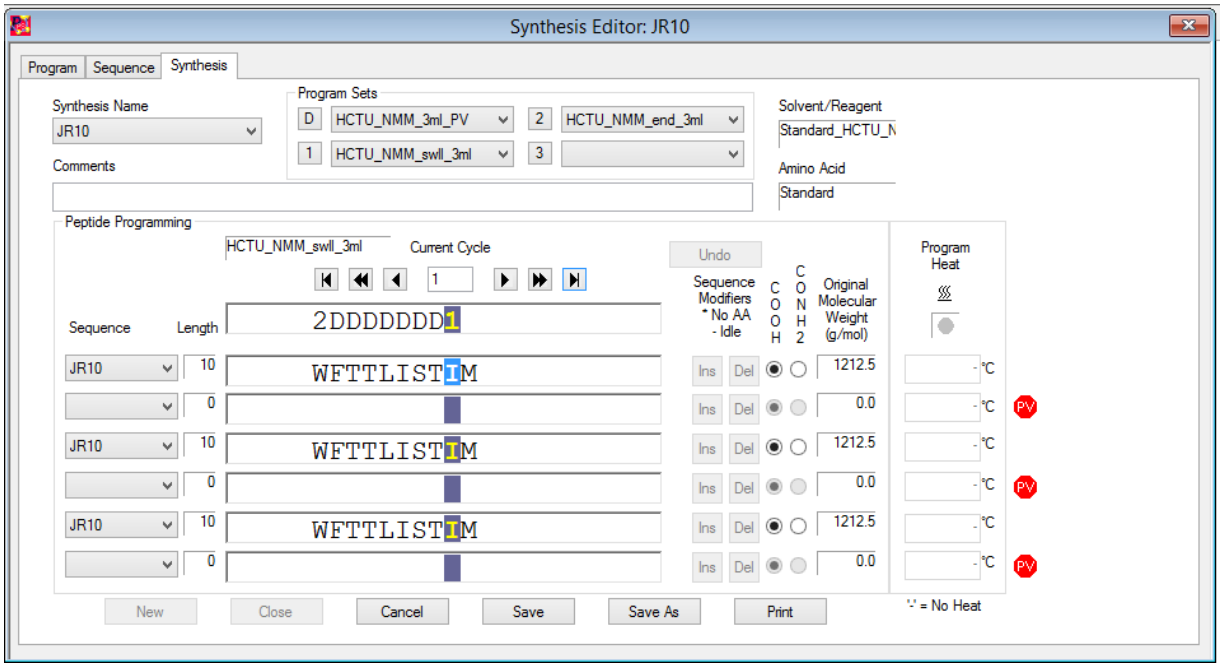
7. **Current Cycle** – The box to the left of “Current Cycle” displays the name of the program assigned to the current cycle. The box below “Current Cycle” displays the current cycle position.

8. **Assign Sequence** – The box above the peptide sequence column lists the programs assigned to a given cycle of the synthesis. Use the arrow keys on the keyboard or the arrow buttons on the screen to move to different amino acids in the sequence. Then click on a program button (**D**, **1**, **2**, or **3**) in the **Program Sets** section to assign a program to the cycle. If more than four programs are needed, additional programs may be assigned using the numbered buttons. To assign an additional program, select a new program using the pull-down menu next to one of the numbered buttons. Programs previously assigned with that number will be represented by an X. This process may be repeated to assign additional programs. Only the latest program selection will be represented by the number. All others will be represented with an X.

The **Program Heat** box shows a red dot icon if the program has the Heat option enabled, and a gray icon if it is not. The boxes below the icon show the

temperature assigned to each Reaction Vessel. These are informative only and cannot be edited from this screen.

If a program that has operations done on a PV the synthesis screen will identify RVs 2, 4, and 6 as the PVs with a red PV icon on the right of the **Program Heat** box.



The buttons are:

1. **New** – The **New** button creates a new file. The **Synthesis File Manager** will open. Enter a new name or use the default and click **OK**, or click the **Cancel** button to return to the **Synthesis Editor** without creating a new file.

2. **Close** – The **Close** button is not active until the synthesis is saved. After saving the synthesis, click the **Close** button to exit the **Synthesis Editor** screen.

3. **Cancel** – The **Cancel** button removes any changes made since the last **Save**. A screen will appear reading, “Abandon All Changes?” The **Yes** button will permanently remove the changes; the **No** button will leave the changes.

4. **Save** – Once a change is made to the synthesis, the **Save** button will become active. Click **Save** to save the changes. The **Save** and **Cancel** buttons deactivate once the synthesis is saved.

5. **Save As** – To copy the file on the screen, click the **Save As** button. The **Synthesis File Manager** will appear with a default name for the copy. Change the file name or accept the default and click **OK**. The **Cancel** button will close the screen and return to the **Synthesis Editor**.

6. **Print** – To print the open synthesis file click the **Print** button. It will go automatically to the printer.

The **Exit** function closes ***Prelude X*** SUser software. To exit the user software, click on the **File** menu and select **Exit**.

NOTE To view the synthesis before printing, click the **Close** button and print the file using the Print button in the **Synthesis File Manager** (Section 2.3).

Example syntheses have been included in the software to serve as templates for future syntheses including:

1. Double coupling
2. Heated
3. Heat scans
4. Pre-activation
5. Short cycle times
6. Capping
7. Single Shot deliveries
8. Long peptide with UV monitoring and double and triple coupling steps

2.5 Operations Menu

2.5.1 Bottle Preparations

The **Bottle Preparations** screen automatically opens when ***Prelude X*** software opens. To open the **Bottle Preparations** screen, click on the shortcut button:

or click on the **Operations** menu and select **Bottle Preparations.**

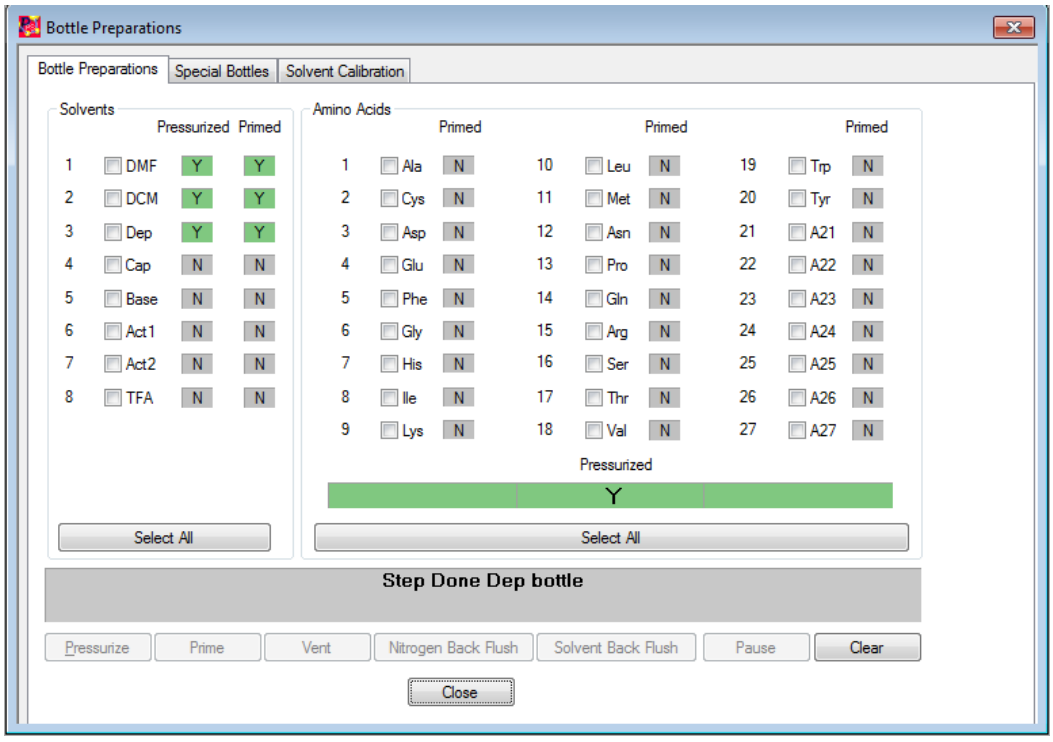
Then select from one of the following three screens:

1. Bottle Preparations
2. Special Bottles
3. Solvent Calibration

The function of each of these screens will be reviewed in each subsection below.

2.5.1.1 Bottle Preparations

The **Bottle Preparations** screen is located under the **Bottle Preparations** tab in the **Bottle Preparations** window. It allows the user to pressurize, prime, vent, and back flush the 8 solvent/reagent bottles and the 27 amino acid bottles. Each solvent/reagent bottle has a separate pressure valve, while amino acid bottles 1-9, 10-18 and 19-27 share common pressure manifolds. Each solvent and amino acid bottle is primed separately so it is not necessary to keep solutions in unused bottles during a synthesis. Position 8 is the only position available for the **Cleave and Collect** operation, while positions 1 and 2 are used specifically for the primary and secondary wash solvents that are also used in certain cleaning operations.



The **Solvents** section lists the 8 solvent/reagent positions, while the **Amino Acids** section lists the 27 amino acid positions. Select a position by clicking in

the box to the right of a position number. This will place a check mark in the box. Click again to uncheck the position.

NOTE The **Bottle Preparations** screen is set to display the abbreviations from the amino acid and solvent/reagent files entitled "**Standard**." To change the labels next to each bottle position, change the abbreviation for that position in the amino acid or solvent/reagent file entitled "**Standard**." To set the **Bottle Preparations** screen to display abbreviations from a different amino acid or solvent/reagent file, the default file name must be changed in the **Settings** screen (See Section 2.6.3).

The **Pressurized** columns indicate the pressurization status. "**Y**" indicates the position is pressurized, and "**N**" indicates the position is vented. Amino acids 1-27 are pressurized together.

The **Primed** columns indicate the prime status. "**Y**" indicates the position is primed, while "**N**" indicates the position is not primed.

The **Status Bar** near the bottom of the screen displays the status of the current operation.

The buttons are:

1. **Select All/Clear All** – The **Select All/Clear All** buttons simplify the bottle preparations process. Click on **Select All** to select all bottle positions in the **Solvents** or **Amino Acids** sections. When one or more positions are selected, the **Select All** button will be replaced by the **Clear All** button. Click on **Clear All** to deselect all bottle positions in the **Solvents** or **Amino Acids** sections.

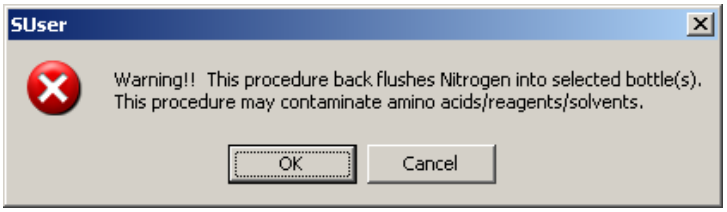
2. **Pressurize** – Click on the **Pressurize** button to pressurize all selected bottles. When pressurization is complete, the **Pressurized** column will display a "**Y**" next to the selected bottles. Allow a minute for the bottle(s) to equilibrate.

NOTE Because amino acid bottle positions 1-9, 10-18 and 19-27 share a common pressure manifold, when one bottle in the column is selected and pressurized, all bottles sharing the same valve will be pressurized.

3. **Prime** – Click on the **Prime** button to prime all selected bottles. When priming is complete, the **Primed** column will display a "**Y**" next to the selected bottles.

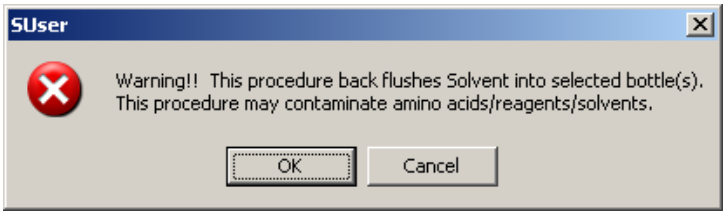
4. **Vent** – Click on the **Vent** button to vent all selected bottles. When complete, the **Pressurized** column will display an "**N**" next to the vented positions. Allow a minute for the pressure to return to 1 atm (14.7 psi) before opening the bottle.

5. **Nitrogen Back Flush** – Click the **Nitrogen Back Flush** button to back flush selected bottles with nitrogen gas. A SUser warning window will open.



   Click **OK** to proceed, or click **Cancel** to return to the **Bottle Preparations** screen without back flushing. If the bottle is not already vented, the bottle will vent prior to back flushing.

6. **Solvent Back Flush** – Click the **Solvent Back Flush** button to back flush selected bottles with Solvent 1, DMF. A SUser warning window will open.



   Click **OK** to proceed, or click **Cancel** to return to the **Bottle Preparations** screen without back flushing. If the bottle is not vented, the bottle will vent prior to back flush with Solvent 1.

7. **Pause/Resume** – Click the **Pause** button to pause an active process. When the action is paused, the **Pause** button will be replaced by the **Resume** button. Click the **Resume** button to continue the paused process.

CAUTION Fluid may not immediately stop when the process is paused.

8. **Cancel** – Click the **Cancel** button to cancel an active process.

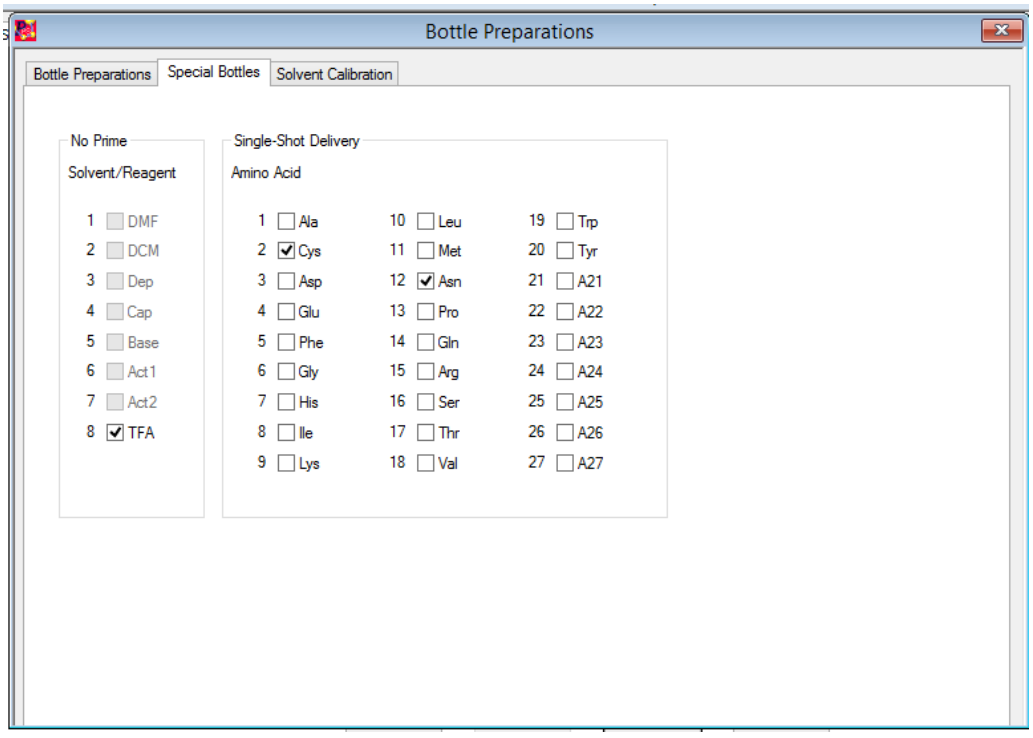
CAUTION Fluid may not immediately stop when the process is cancelled. Cancelling an action may result in solution left in the lines and valve blocks, which could lead to cross contamination. Always prime either Solvent 1 or Solvent 2 following a cancel to clear the valve blocks and lines.

9. **Close** – Click the **Close** button or click the **X** in the upper right corner to close the **Bottle Preparations** screen.

2.5.1.2 Special Bottles

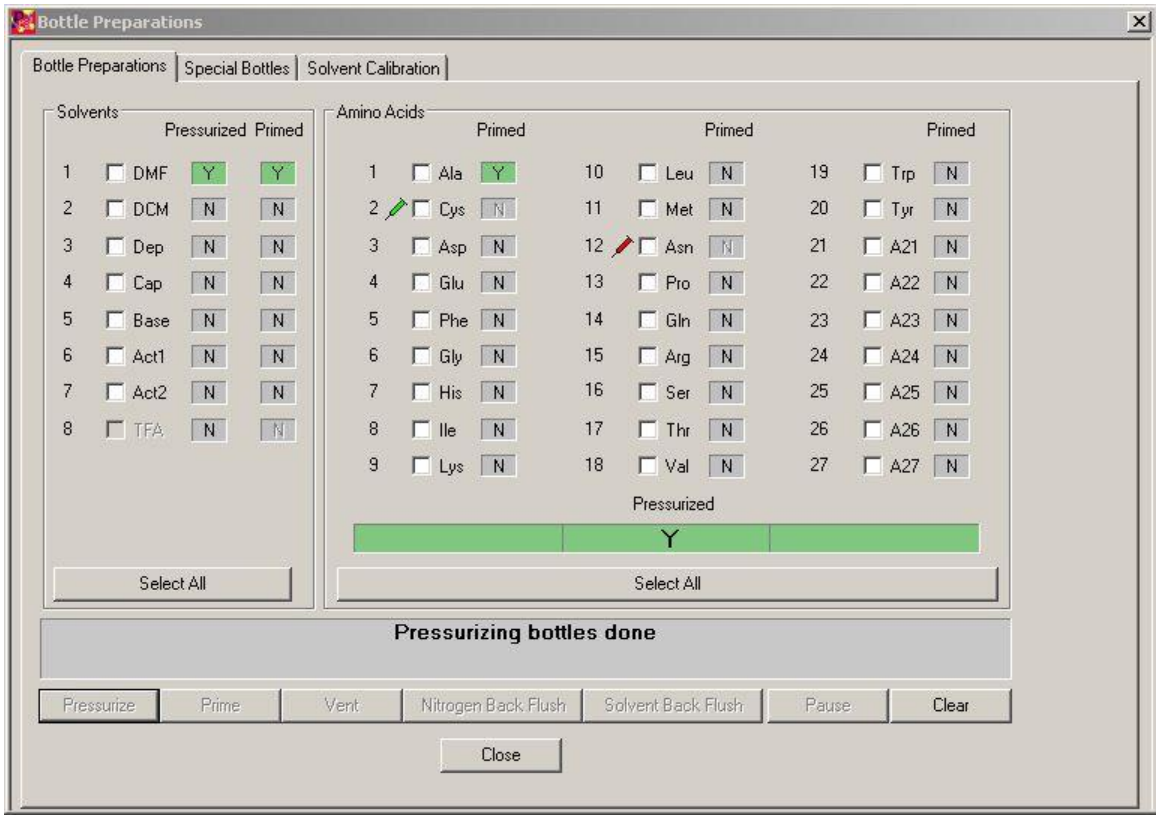
Special Bottles allows the user to prevent solvent/reagent bottles from being manipulated in the **Bottle Preparations** screen and set individual amino acid bottles to perform a **Single-Shot** delivery. To open the **Special Bottles** screen, click on the **Operations** menu and select **Special Bottles**.

This will open the **Bottle Preparations** screen with the **Special Bottles** tab active.



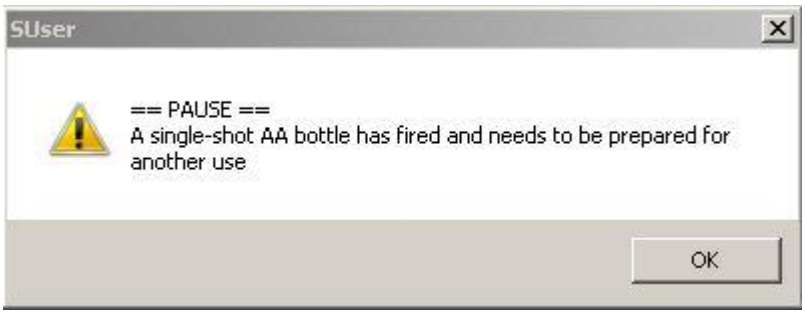
Bottle 8 has a special feature called **No Prime**; it will prevent the user from manipulating the bottle using the **Bottle Preparations** screen by making the position inactive. This feature keeps the TFA bottle vented and unprimed except

during a delivery for safety reasons and to minimize the release of fumes. This feature must be selected for the TFA bottle during use, since the **Cleave Collect** operation includes pressurizing and priming the bottle prior to the delivery, and venting and back flushing the TFA bottle following a delivery. This feature must be deselected for the TFA bottle during **Solvent Calibration** (Section 2.5.1.3).



The amino acid bottles have a special feature called a **Single-Shot™** delivery (**One-Shot Delivery** section), which will deliver all of the amino acid in a bottle to a specific reaction vessel. To activate this feature, check the box next to an amino acid. Selected amino acid bottles will display a picture of a green syringe next to their position in the **Bottle Preparations** screen (see figure above) after pressurization, indicating that it is ready for use. When the **Sequence** reaches the amino acid in the **Synthesis**, ***Prelude X*** will deliver all of the amino acid solution in the selected bottle to the specified reaction vessel. Once the position has been used, the syringe will turn red, to notify that the position is not ready.

NOTE If a bottle is set to perform more than one **Single-Shot** delivery from the same bottle position during the same synthesis, the system will pause at the start of the cycle following delivery requiring a reset of the **Single-Shot** position (See message below). To reset vent, refill, and repressurize the bottle before resuming. Otherwise, the instrument will error when it reaches the second delivery.



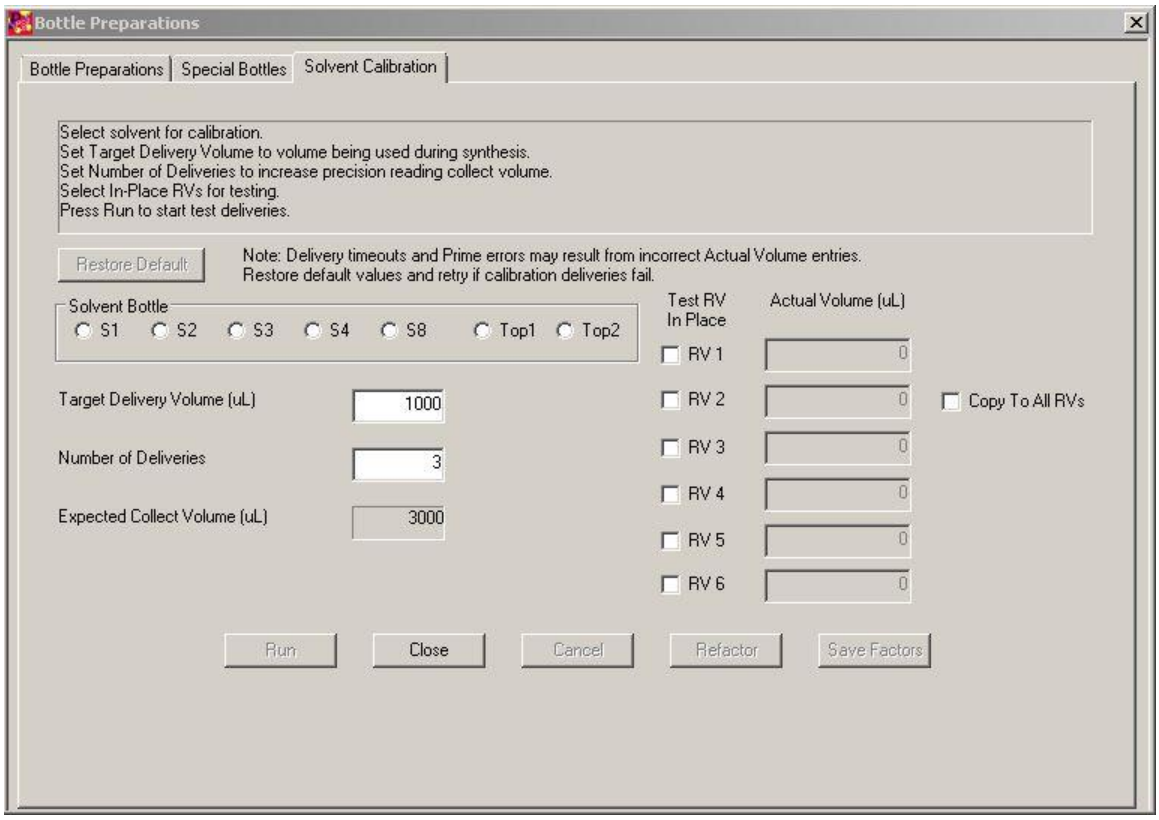
2.5.1.3 Solvent Calibration

The **Solvent Calibration** screen is located under the **Solvent Calibration** tab in the **Bottle Preparations** window. It allows the user to calibrate the volume deliveries of the timed solvent/reagent bottles (1-4, and 8).

NOTE Calibration accuracy is dependent on the **Bottle Pressure** (Section 1.1.10). If the pressure reading on the **Bottle Pressure** gauge changes by more than ¼ psi in either direction following a calibration, the calibration should be checked.

To open the **Solvent Calibration** screen, click on the **Operations** menu, select **Bottle Preparations**, and select **Solvent Calibration**.

This will open the **Bottle Preparations** screen with the **Solvent Calibration** tab active.



The **Solvent Calibration** screen has the following buttons:

1. **Run** – Starts running a calibration.

2. **Close** – Closes the window.

3. **Cancel** – Cancels a running calibration.

4. **Refactor** – Calculates new calibration factors based on **Expected Collect Volume (uL)** and **Actual Volume (uL)** values.

5. **Save Factors** – Saves new calibration factors. Click the **Save Factors** button when the **Actual Volume (uL)** matches the **Expected Collect Volume (uL)**.

6. **Restore Default** – Restores default calibration factors to selected solvent

The **Status Box**, located at the top of the screen, displays instructions for how to perform a calibration. While a calibration is running, it displays the current actions of the instrument.

The **Solvent Bottle** section is where you select a solvent bottle for calibration. To select a bottle, click in the circle next to the appropriate label (S1 – S8 stand for Solvent 1 – Solvent 8 bottom deliveries, while Top1 & Top2 stand for the Solvent 1 and Solvent 2 top wash deliveries, respectively). Only one solvent bottle may be selected at a time.

The **Target Delivery Volume (uL)** box is where you enter the calibration delivery volume. For best results, this volume should be the same as the volume (in microliters) that will be delivered by the selected bottle during a synthesis.

The **Number of Deliveries** box is where you enter the number of times the volume will be delivered to an RV(s) during the calibration. The higher this value, the more accurate the calibration.

The software calculates the theoretical volume that should be in the collection vial at the end of the calibration and displays it in the **Expected Collect Volume (uL)** box.

The **Test RV In Place** column allows you to select the RV positions to be calibrated. Check the box to the left of the desired RV(s) to select an RV. Clicking the box a second time will deselect the RV.

The **Copy To All RVs** selection runs the calibration in RV 2 only, then copies the resulting calibration factors to all RVs.

NOTE If instrument is equipped with UV monitoring, RV 1's timed delivery calibration has to be done separately to account for the extra tubing due to the UV.

CAUTION Using the **Copy To All RVs** feature significantly shortens the calibration time, but may result in a delivery volume variation of up to 10%, which is sufficient for most solvents. The greatest accuracy is obtained when all 6 RVs are selected during a calibration. This is recommended for reagent deliveries that require greater accuracy.

After running a calibration, the **Actual Volume (uL)** column becomes active. Measure the actual volumes delivered to the collection vial (s) and enter them in the **Actual Volume (uL)** column.

CAUTION Make sure RVs and collection vials are installed on the instrument for the selected RV positions before running a calibration.

To calibrate a bottle:

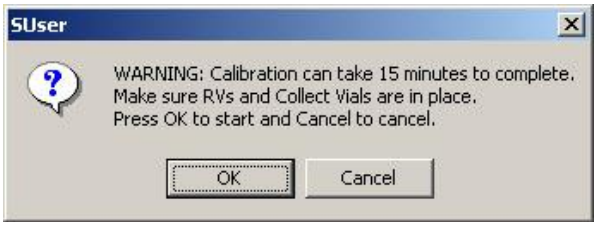
1. Select a solvent bottle in the **Solvent Bottle** section by clicking in the appropriate circle.

2. Enter the volume (in microliters) that will be delivered to an RV from the selected bottle during a synthesis in the **Target Delivery Volume (uL)** box.

3. Enter the number of times the volume will be delivered to the selected RV(s) in the **Number of Deliveries** box.

4. Select the RV positions to be tested by clicking in the box to the left of the desired RV(s) in the **Test RV In Place** column. Click in the box a second time to deselect an RV. Alternatively, select the **Copy To All RV's** feature.

5. Click the **Run** button. A SUser window will open with the message "WARNING: Calibration can take 15 minutes to complete. Make sure RVs and Collect Vials are in place. Press OK to start and Cancel to cancel."



   Click **OK** to continue or **Cancel** to return to the **Solvent Calibration** screen without starting the calibration.

6. After the calibration is complete, measure the volumes in the collection vials(s) using a calibrated collection vial or a graduated cylinder, and enter the values (in microliters) in the **Actual Volume (uL)** column next to the appropriate RV.

7. Click the **Refactor** button to calculate new calibration factors.

8. Repeat steps 1-7 until the **Actual Volume (uL)** and **Expected Collect Volume (uL)** values match. Then, click the **Save Factors** button.

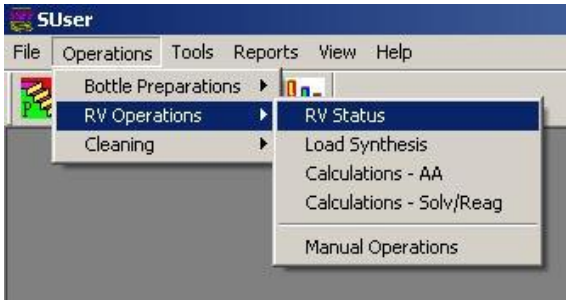
9. Click **Close** to exit the **Solvent Calibration** screen.

CAUTION After calibration is complete, the fluid system may be contaminated. Perform a **Rinse All Blocks** followed by a **Clear All Blocks** to clean the instrument (Sections 2.5.3.6 & 2.5.3.7).

2.5.2 RV Operations

The **Reaction Vessel Operations** screen automatically opens when *Prelude X* software is opened. To open the **Reaction Vessel Operations** screen, click on the shortcut button:

or click on the **Operations** menu, select **RV Operations**, and select a sub-section.



This will open the **Reaction Vessel Operations** screen with the sub-section tab active.

The **Reaction Vessel Operations** screen has 5 sub-sections:

1. RV Status
2. Load Synthesis
3. Calculations – AA
4. Calculations – Solv/Reag
5. Manual Operations

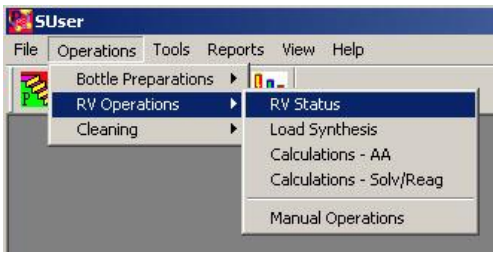
The function of each of these sub-sections will be reviewed in each subsection below.

2.5.2.1 RV Status

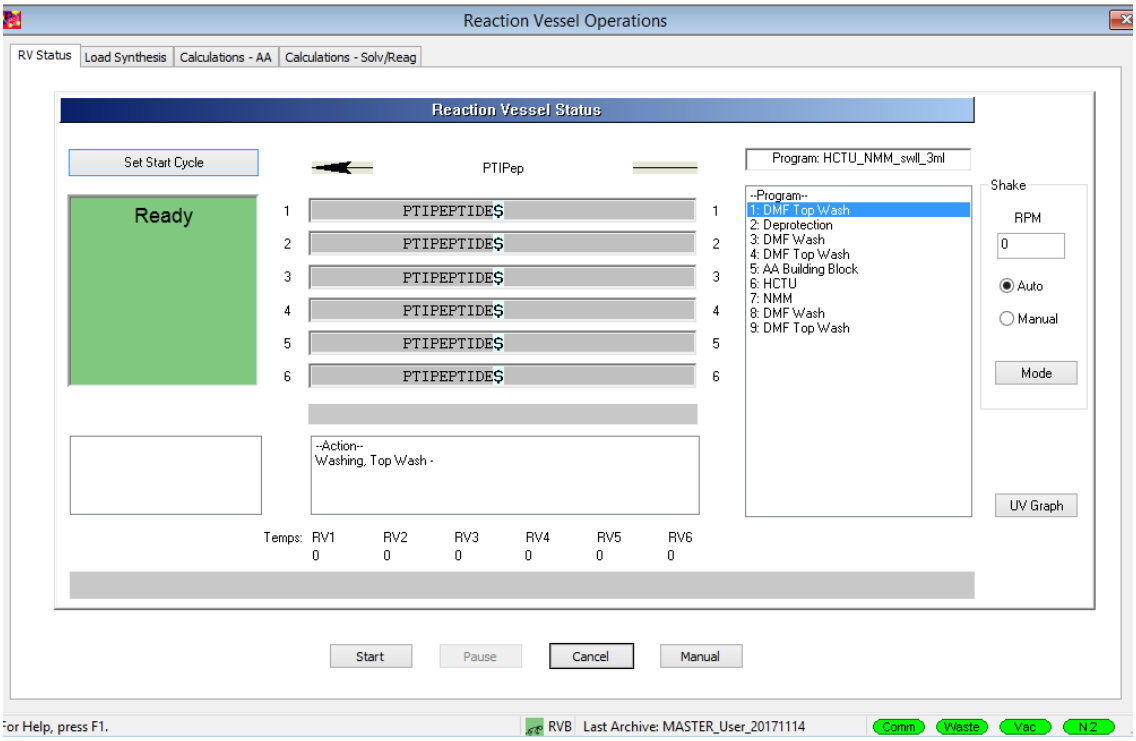
The **RV status** screen is located under the **RV Status** tab in the **Reaction Vessel Operations** window. Once a synthesis has been loaded, this screen allows the user to set a new start cycle, start a synthesis, and monitor a running synthesis.

To open the **RV status** screen, click on the shortcut button:

or click on the **Operations** menu, select **RV Operations,** and then select **RV Status**.



The **Reaction Vessel Operations** screen will open with the **RV Status** tab active.



On the left side of the **RV Status** screen is the **Set Start Cycle** button, the **Colored Status Box**, and the **Error Reporting Box**.

The **Colored Status Screen** displays various messages on a colored background depending on the status of the synthesis as follows:

1. **Synthesis is Loaded** – “Ready” is displayed on a dark green background

2. **Synthesis is Running** – Cycle, step, program operation, repetition and current action are displayed on a light green background

3. **Synthesis is Paused** – Cycle, step, program operation, repetition and "Pause" are displayed on a yellow background

4. **Synthesis is in Error** – Cycle, step, program operation, repetition and action are displayed on a flashing red and yellow background

IMPORTANT It is important to manually drain all reaction vessels prior to resuming after an error. When a synthesis is resumed following an error, it will start at the beginning of the step, not where it left off as in a regular pause. Thus, if reaction vessels 1-3 were filled prior to the error, it will start filling at reaction vessel 1, and reaction vessels 1-3 will have been filled twice.

5. **Synthesis is Complete** – "Done" is displayed on a white background

When there is an error, the details of the error are reported in the **Error Reporting Box** located in the lower left corner.

In the center of the screen, the synthesis name is displayed at the top over an arrow that indicates the direction of the synthesis. Below the synthesis name, peptide sequences are displayed in numbered rows corresponding to the 6 reaction vessels. The white box at the lower center of the screen displays the status of the current operation.

On the right side of the screen, the name of the currently running program is displayed in the upper box, while the steps of the program are displayed in the lower box.

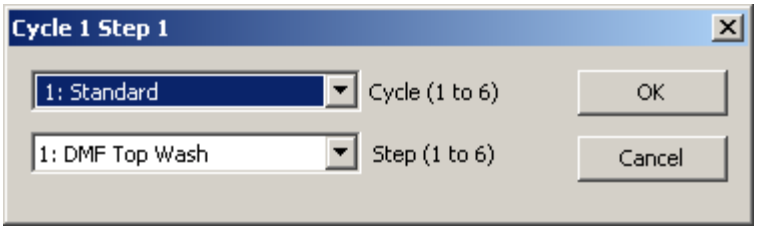
The **Shake** box allows the user to change between Shake modes: Auto will keep the set RPM and Manual will set the RPM with the knob on the instrument. When a synthesis is running the RPM box shows the current RPM.

The current temperature of each RV will be shown in the bottom labels.

The UV Graph dialog will be shown clicking in the UV Graph button.

The buttons are as follows:

1. **Set Start Cycle** – By default, the synthesis will start at cycle 1, step 1. To change the starting cycle and step of the synthesis, click on the **Set Start Cycle** button. This will open a new window:



Use the upper pull-down menu to select a starting cycle. Use the lower pull-down menu to select a starting step. Click the **OK** button to accept the changes, or click the **Cancel** button to return to the **RV Status** screen without changing the start settings.

After clicking **OK**, a SUser screen will appear with the message, "This will set the synthesis to Cycle < # >, Step< # >. Are you sure?" Click the **Yes** button to continue or **No** to cancel.

2. **Start** – Click the **Start** button to start the synthesis.

3. **Pause** – Click the **Pause** button to pause the synthesis. The synthesis will stop after the current step is complete to allow the manifold blocks to be washed. This eliminates the problem of residual fluid in the lines contaminating the next fluid delivery. Click the **Start** button to resume the synthesis.

4. **Cancel/E-Stop** – Click the **Cancel** button during a pause or before the synthesis is started to delete the synthesis from the **RV Status** screen. While a synthesis is running, the **Cancel** button is replaced with an **E-Stop** button. The **E-Stop** button ends the synthesis immediately and cancels the synthesis.

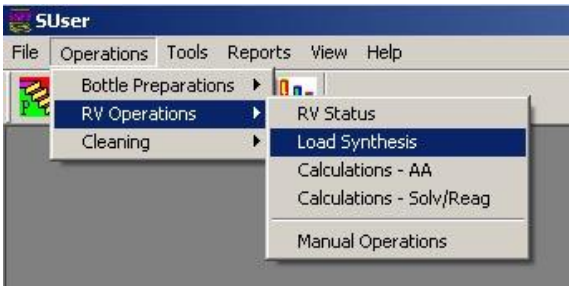
CAUTION There may be fluid left in the reaction vessels and/or lines after using the **E-Stop**. Use the **Manual** button to open the **Manual Operations** screen and run a Drain/Dry and a DMF wash step if necessary to clean the synthesizer after an emergency stop and avoid contaminating the next fluid delivery.

NOTE The synthesis must be reloaded to resume after an **E-Stop**. Reload the synthesis using the **Load Synthesis** screen, then select the starting cycle and step using the **Set Start Cycle** button on the **RV Status** screen. The synthesis will start at the beginning of the step.

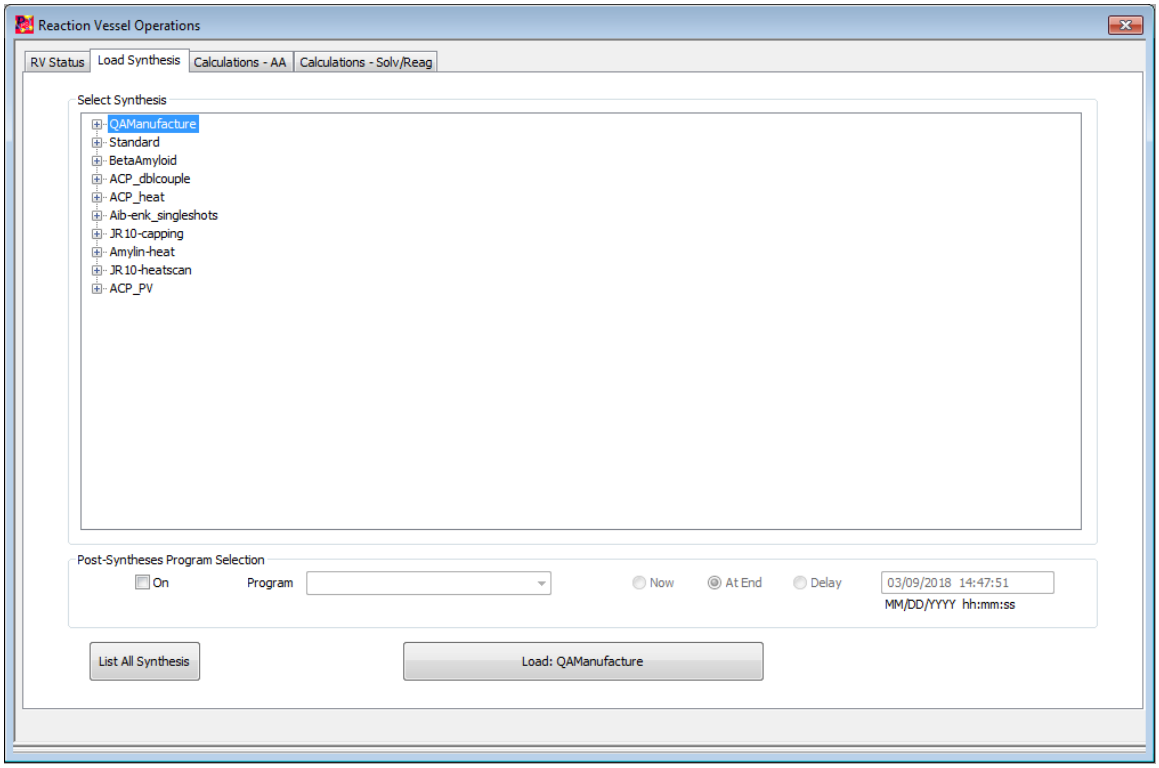
5. **Manual** – The manual button will open the **Manual Operations** screen (See Section 2.5.2.5).

2.5.2.2 Load Synthesis

The **Load Synthesis** screen is located under the **Load Synthesis** tab in the **Reaction Vessel Operations** window. It allows the user to view the details of a synthesis file, load a synthesis file onto the **RV Status** screen, and assign a cleavage program to a synthesis. To open the **Load Synthesis** screen, click on **RV Operations** under the **Operations** menu and select **Load Synthesis**.

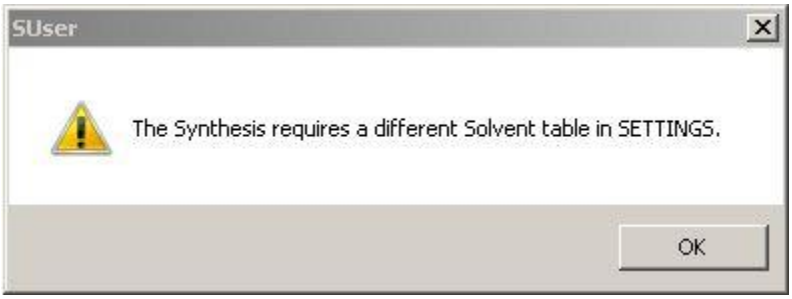


The **Reaction Vessel Operations** window opens with the **Load Synthesis** tab active.



The **Select Synthesis** section displays all synthesis files saved on the instrument. Only the ten most recent synthesis will show up in the screen, click “List All Syntheses” to show every synthesis stored on the instrument. If a selected synthesis does not match the Amino Acid File or the Solvent/Reagent File selected on the instrument’s settings a message will appear to notify of the

mismatch (see below). Click on [+] to view more details. Click on [–] to hide details.



The **Post-Synthesis Program Selection** section assigns a post-synthesis, including final washes and resin drying for example, or a cleavage program to a synthesis.

After selecting a synthesis, turn on the Post-Synthesis option by clicking in the **On** box. When the box is checked, the functions in the **Post-Synthesis Program Selection** section become active. Select a program using the pull-down menu in the **Program** box. The **Program** box menu is populated with programs that do not include an “AA delivery” operation. Set the program start time by selecting one of three options:

1. **Now** – To start the selected program immediately, click the circle next to **Now**. If this is selected, the loaded synthesis will be ignored and only the cleavage program will be performed.

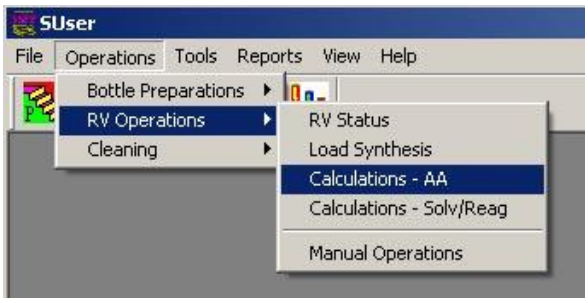
2. **At End** – To start the selected program at the end of the synthesis click the circle next to **At End**.

3. **Delay** – To delay the start of the selected program, click the circle next to **Delay**, then enter a date and time to start the cleavage program. There is no need to delete or backspace in the date box. Type over the date and time to change the numbers (MM:DD:YYYY hh:mm:ss).

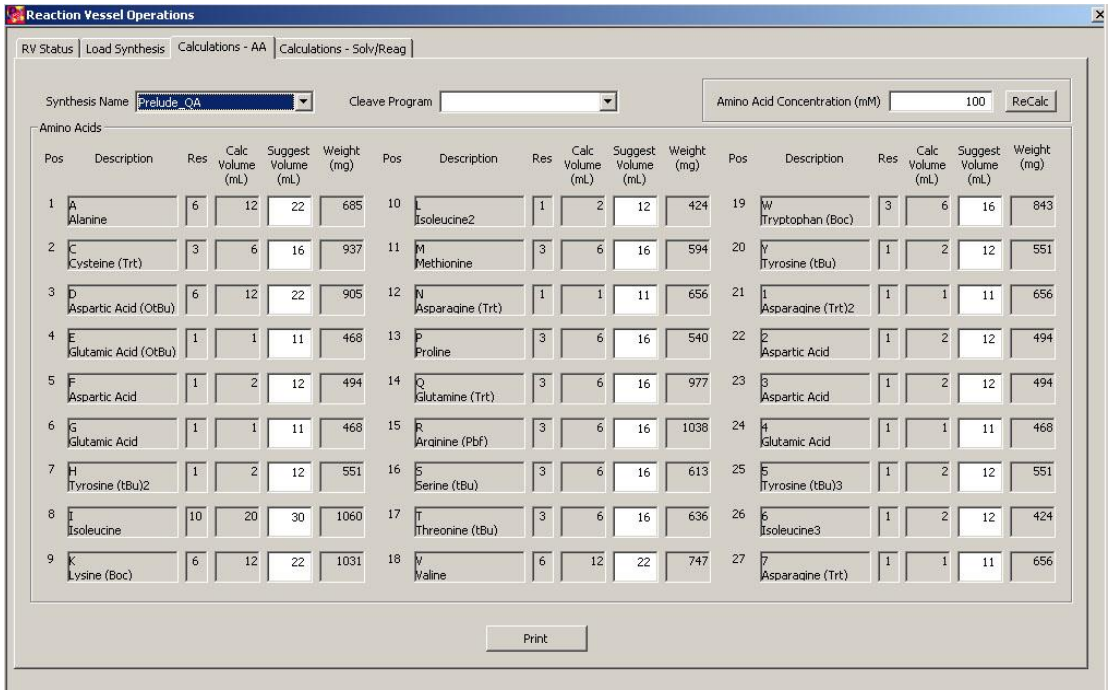
The **Load** button loads the selected synthesis (and cleavage) onto the **RV Status** screen, and opens the **Calculations – AA** screen.

2.5.2.3 Calculations – AA

The **Calculations – AA** screen is located under the **Calculations – AA** tab in the **Reaction Vessel Operations** window. It calculates solution volumes and the amount of dry amino acid necessary for a given concentration. To open the **Calculations – AA** screen click on the **Operations** menu, select **RV Operations** and then select **Calculations – AA**:



or load a synthesis in the **Load Synthesis** screen and the **Calculations – AA** screen will open automatically.



Pos	Description	Res	Calc Volume (mL)	Suggest Volume (mL)	Weight (mg)	Pos	Description	Res	Calc Volume (mL)	Suggest Volume (mL)	Weight (mg)	Pos	Description	Res	Calc Volume (mL)	Suggest Volume (mL)	Weight (mg)
1	A Alanine	6	12	22	685	10	L Isoleucine2	1	2	12	424	19	W Tryptophan (Boc)	3	6	16	843
2	C Cysteine (Trt)	3	6	16	937	11	M Methionine	3	6	16	594	20	Y Tyrosine (tBu)	1	2	12	551
3	D Aspartic Acid (OtBu)	6	12	22	905	12	N Asparagine (Trt)	1	1	11	656	21	1 Asparagine (Trt)2	1	1	11	656
4	E Glutamic Acid (OtBu)	1	1	11	468	13	P Proline	3	6	16	540	22	2 Aspartic Acid	1	2	12	494
5	F Aspartic Acid	1	2	12	494	14	Q Glutamine (Trt)	3	6	16	977	23	3 Aspartic Acid	1	2	12	494
6	G Glutamic Acid	1	1	11	468	15	R Arginine (Pbf)	3	6	16	1038	24	4 Glutamic Acid	1	1	11	468
7	H Tyrosine (tBu)2	1	2	12	551	16	S Serine (tBu)	3	6	16	613	25	5 Tyrosine (tBu)3	1	2	12	551
8	I Isoleucine	10	20	30	1060	17	T Threonine (tBu)	3	6	16	636	26	6 Isoleucine3	1	2	12	424
9	K Lysine (Boc)	6	12	22	1031	18	V Valine	6	12	22	747	27	7 Asparagine (Trt)	1	1	11	656

The **Synthesis Name** box displays the current synthesis. Use the pull-down menu to select a synthesis.

The **Cleave Program** box displays the current cleavage program. Use the pull-down menu to select a cleavage program, or leave the box blank. When a cleavage program is displayed, calculated values will correspond to both the selected synthesis and the selected cleavage program.

The **Amino Acid Concentration (mM)** box allows the user to input a solution concentration in mM. When the **ReCalc** button is pressed, amino acid weights will be calculated based on this value and the **Suggest Volume (mL)** value explained below.

The **Amino Acids** section displays a table of the 27 amino acid bottle positions. The columns are labelled as follows:

1. **Pos** – Amino acid bottle position

2. **Description** – Displays the 1 letter abbreviation and full name of the amino acid or other chemical based on the amino acid file associated with the selected synthesis

3. **Res** – Displays the number of times the amino acid solution will be delivered during the synthesis

4. **Calc Volume (mL)** – Displays the calculated minimum volume (in mL) necessary for the synthesis.

CAUTION It is not recommended to use less than the minimum volumes. Reagent bottles may run out of fluid during the synthesis.

5. **Suggest Volume (mL)** – Displays the suggested volume (in mL) to place in the bottle at the start of the synthesis. The user may change the value in this box for recalculation of amino acid to be measured.

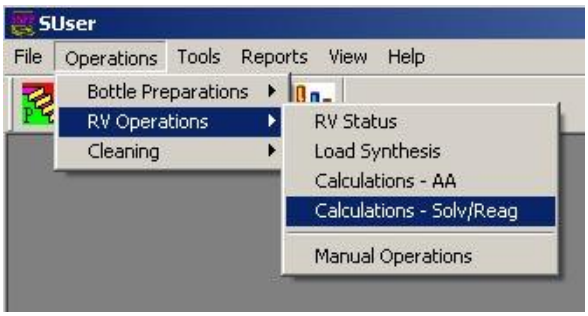
6. **Weight (mg)** – Displays the amount of dry amino acid (in mg) needed for the amino acid solution. The software calculates this value based on the **Amino Acid Concentration (mM)** and the **Suggest Volume (mL)** values.

The **Print** button prints the calculated values from the **Calculations – AA** and **Calculations – Solv/Reag** screens.

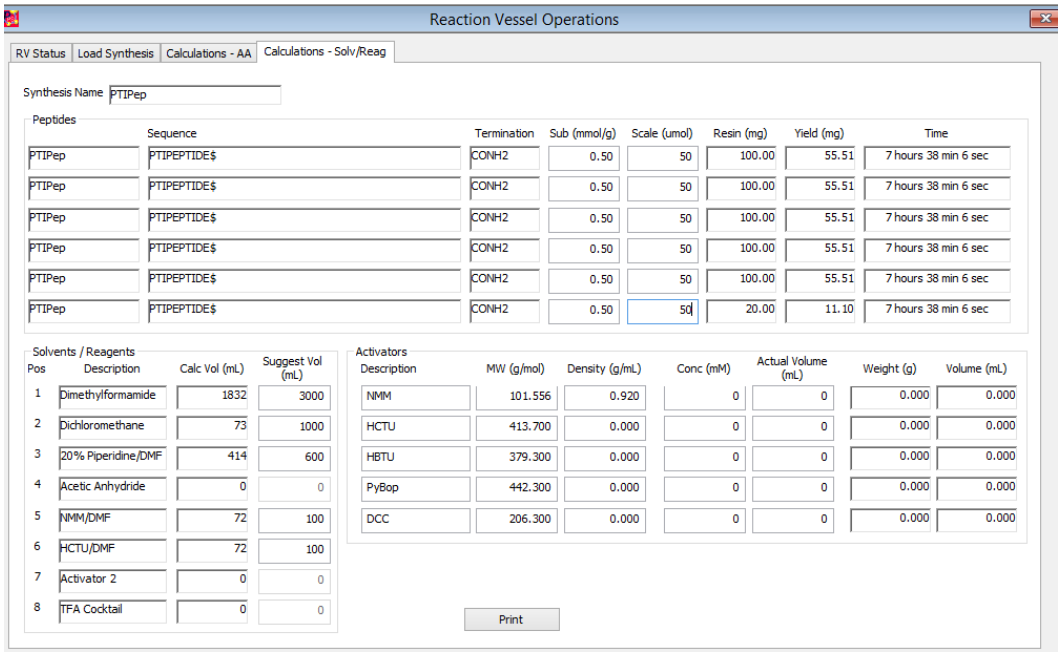
NOTE Use the **Print** button only after modifying both the **Calculations – AA** and the **Calculations – Solv/Reag** screens to avoid having to print twice.

2.5.2.4 Calculations – Solv/Reag

The **Calculations – Solv/Reag** screen is located under the **Calculations – Solv/Reag** tab in the **Reaction Vessel Operations** window. It aids the user in preparing chemicals for a synthesis by calculating resin weights, solvent/reagent minimum volumes and the amount of activator necessary for the coupling solutions in a given synthesis. To open the **Calculations – Solv/Reag** screen click on the **Operations** menu, select **RV Operations** and then select **Calculations – Solv/Reag**:



The **Reaction Vessel Operations** screen will open with the **Calculations – Solv/Reag** tab active.



The **Synthesis Name** box displays the current synthesis. Use the pull-down menu in the **Calculations – AA** screen (Section 2.5.2.3) to select a different synthesis.

The **Peptides** section calculates the resin amount needed for each reaction vessel. It displays a table with columns labelled as follows:

1. **Sequence File** – Displays the name of the sequence file in each RV. Row 1 corresponds to RV1, row 2 to RV2 and so on down to RV6.

2. **Sequence** – Displays the amino acid sequence of the peptide

3. **Termination** – Displays the termination group on the C-terminus (COOH or CONH2)

4. **Sub (mmol/g)** – Displays the substitution on the resin (in mmol/g). The user may change the value in this column.

5. **Scale (umol)** – Displays the synthesis scale (in μmol). The user may change the value in this column.

6. **Resin (mg)** – Displays the amount of resin (in mg) to weigh out into each RV. ***Prelude X*** software calculates this amount based on the values in the **Sub (mmol/g)** and **Scale (umol)** columns set by the user.

7. **Yield (mg)** – Displays the theoretical amount of peptide (in mg) expected from the synthesis. ***Prelude X*** software calculates this amount based on the value in the **Scale (umol)** column and the peptide's molecular weight.

8. **Time** – Displays the estimated time for the synthesis (in hours, min, sec).

The **Solvents/Reagents** section calculates the minimum volume of solvent or reagent needed for each of the 8 solvent/reagent bottle positions. The columns are labelled as follows:

1. **Pos** – Displays the bottle position number

2. **Description** – Displays the full name of the solvent or reagent based on the solvent/reagent file associated with the selected synthesis

3. **Calc Vol (mL)** – Displays the calculated minimum volume (in mL) necessary for the selected synthesis.

4. **Suggest Vol (mL)** – Displays the suggested volume (in mL) to use for the synthesis. The user may change the value in this column.

The **Activators** section calculates the volume and/or weight of various activators needed for the activator solutions. All values can be edited by the user and used as a calculator.

The columns are:

1. **Description** – Displays the name of the activator as entered in the solvent/reagent file associated with the selected synthesis.

2. **MW (g/mol)** – Displays the molecular weight (in g/mol) of the activator as entered in the solvent/reagent file associated with the selected synthesis.

3. **Density (g/mL)** – Displays the density (in g/mL) of the activator as entered in the solvent/reagent file associated with the selected synthesis.

4. **Conc (mM)** – Displays the concentration of the activator solution (in mM). The user may change the value in this column.

5. **Actual Volume (mL)** – Displays the total volume of activator solution. The user may change the value in this column.

6. **Weight (mg)** – Displays the weight (in mg) of activator needed. The software calculates this value based on the entry in the **MW (g/mol)** column.

7. **Volume (mL)** – Displays the volume (in mL) of activator needed. The software calculates this value based on the entries in the **MW (g/mol)** and **Density (g/mL)** columns. If there is no entry in the **Density (g/mL)** column, the value will be displayed as 0.000.

The **Print** button prints the calculated values from the **Calculations – AA** and **Calculations – Solv/Reag** screens.

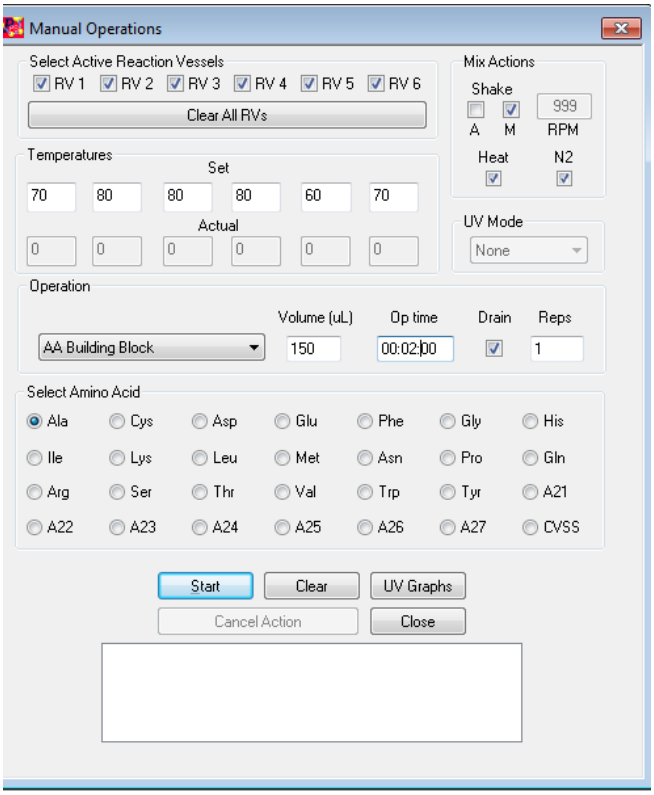
NOTE Use the **Print** button only after modifying both the **Calculations – AA** and the **Calculations – Solv/Reag** screens to avoid having to print twice.

2.5.2.5 Manual Operations

The **Manual Operations** screen allows the user to perform individual operations on the instrument outside of a synthesis. To open the **Manual Operations** screen, click on the shortcut button:

or click on the **Operations** menu, select **RV Operations**, and then select **Manual Operations**.

Alternatively, open the **Manual Operations** screen by clicking the **Manual** button on the **RV Status** screen (See Section 2.5.2.1).



Select one or more RVs for an operation in the **Select Active Reaction Vessels** section. Click on the **Select All RVs/Clear All RVs** button to select or deselect all RVs, respectively. The **Clear All RVs** button replaces the **Select All RVs** button when one or more RVs are selected.

Select a mix mode in **Mix Actions**. Checking the "A" box under **Shake** and entering a value in the **RPM** box sets a specific shake RPM. Checking the **Heat** box allows the user to assign temperatures to each RV, with RV1 starting at the left. Checking the **N2** box activates N2 bubbling during the mix.

Select a mix mode in Mix Actions, checking the box of Shake and introducing a RPM for automatic shake, checking Heat and setting the temperatures at the left side, corresponding to each RV, and checking the N2 box for N2 bubbles.

The actual temperatures are shown in the **Actual** boxes, and the actual RPM is also shown in the **RPM** box when a manual operation is running.

If a Deprotection (or Mix) step is selected the **UV Mode** box becomes active. Select a Basic or Xtend UV deprotection.

Use the pull-down menu in the **Operation** section to select an operation. Input the delivery volume in microliters in the **Volume (uL)** box. Volumes will be rounded up to volumes that can be delivered in increments of 150, 500 or 1000 μL up to a maximum volume of 5,000 μL for 10 mL RV's, 13,000 μL for 25 mL

RV's, and 20,000 μL for 40 mL RV's. The RV size should be selected in the **Settings** screen (Section 2.6.3). Input the mix time in Hours: Minutes: Seconds in the **Mix time** box. Use the **Drain** box to select whether the selected RVs will be drained at the end of the operation. If the **Drain** box is checked, the selected RVs will drain at the end of the operation. If the **Drain** box is unchecked, the RVs will not drain at the end of the operation. Input the number of times the operation will be repeated (up to a maximum of 9) in the **Reps** box.

CAUTION When **Drain** is unchecked, multiple deliveries may be made to the same RV without draining. Be careful not exceed the RV's maximum capacity as this may force resin into the showerhead causing clogs or contamination.

When the operation "AA Building Block" is selected, the **Select Amino Acid** section becomes active. Click in the circle next to the desired amino acid position to select an amino acid for delivery. If a CV Single Shot is desired, then click in circle next to CVSS.

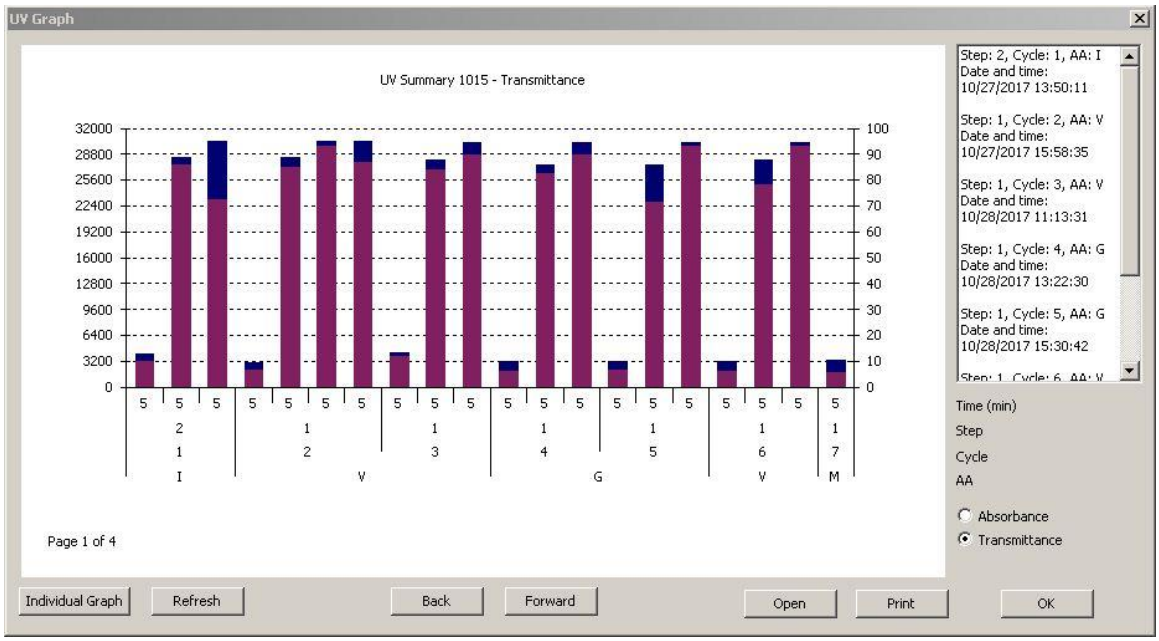
The buttons are as follows:

1. **Start** – Starts the manual operation.
2. **Close** – Closes the **Manual Operations** screen.
3. **Clear** – Resets the **Manual Operations** screen to default values.
4. **Cancel Action** – Cancels the running operation.
5. **UV Graphs** - Shows the UV Graph dialog.

CAUTION If an operation is cancelled during a delivery or drain, residual fluid in the lines may contaminate the next operation. Perform a **Drain** operation to remove fluid from the lines prior to running a new operation.

The **Status Box** at the bottom of the screen displays the actions of the running operation, as well as errors

The UV Graph screen shows the user the UV Summary and Individual graphs of a synthesis. To open it, click on the **UV Graphs** button in the Manual Operation and RV Status screens.



The buttons are as follows:

1. **Individual Graph/Summary Graph** - Allows the user to change between graphs of a general UV synthesis or an individual repetition of deprotection.

2. **Refresh** - Redraw the graph.

3. **Back** - Move back through pages of the same graph, or between data files.

4. **Forward** - Move forward through pages of the same graph, or between data files.

5. **Open** - Shows an Open dialog where can be selected the UV file to see the graph.

6. **Print** - Prints an image of the current graph, and also the text information is at the right side.

7. **OK** - Closes the dialog.

2.5.3 Cleaning

The cleaning operations are:

1. System Clean
2. Bottle Position Flush
3. Cleave Bottle Solvent Back Flush
4. Cleave Bottle Nitrogen Back Flush
5. Collect Back Flush
6. Rinse All Blocks
7. Clear All Blocks
8. Wash RVs

Each operation is described in the sections below.

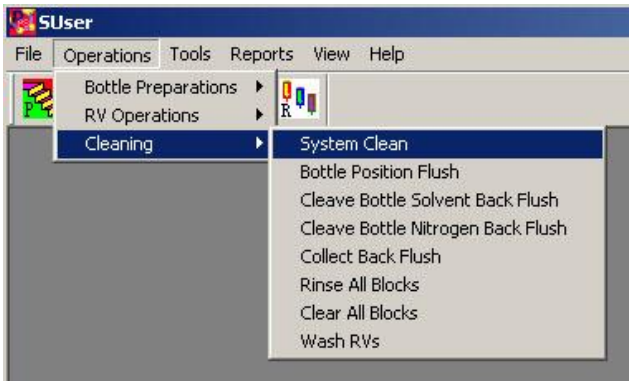
2.5.3.1 System Clean

System Clean flushes the entire fluid system with Solvent 2. This operation should be executed every two weeks in order to prevent precipitate from building up. A system clean performs the **Bottle Position Flush**, **Cleave Bottle Solvent Back Flush**, **Cleave Bottle Nitrogen Back Flush**, **Collect Back Flush**, **Rinse All Blocks**, **Clear All Blocks**, and **Wash RVs** operations.

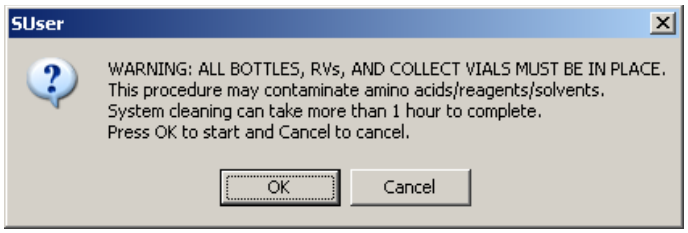
1. To perform a **System Clean**, place empty amino acid and solvent/reagent bottles in all positions. Place empty RVs and collection vials in position.

2. Place 1 L of methanol or Premium Wash solvent in the Solvent 2 bottle. Please contact GPT to order Premium Wash Solvent.

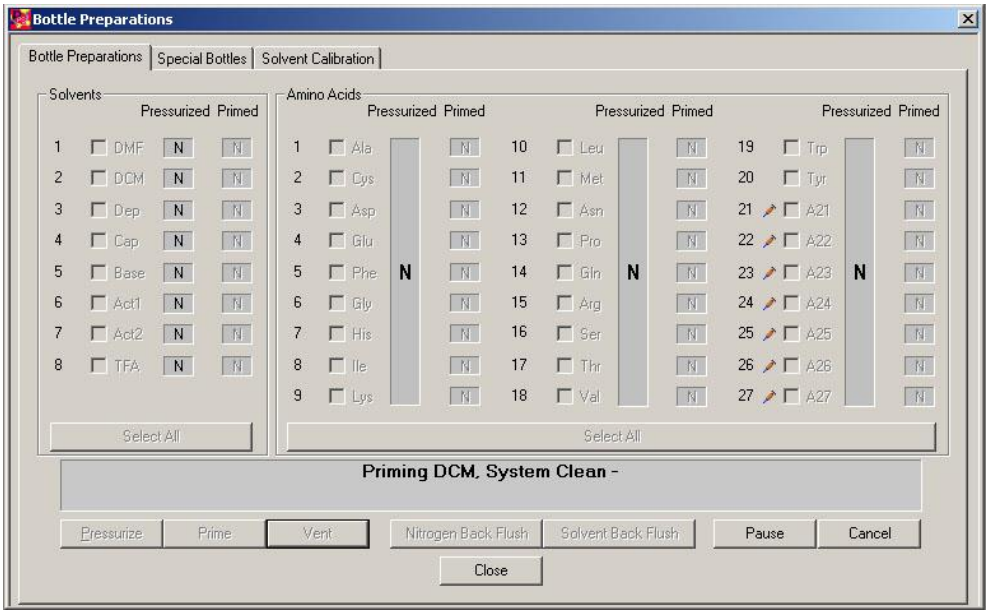
3. Click on the **Operations** menu, select **Cleaning**, and then select **System Clean**.



4. The following warning window will open. Verify all bottles, RVs and collection vials are in place, then click **OK** to start the procedure or **Cancel** to exit the cleaning procedure.



5. During the **System Clean**, the **Bottle Preparations** screen will open.



The status of the operation will be displayed in the status bar. Click the **Pause** button to pause the operation. Click **Resume** to resume a paused operation. Click the **Cancel** button to cancel the operation. Click the **Close** button to close the **Bottle Preparations** screen.

6. After the cleaning operation is complete, remove the bottles and collection vials, dispose of the rinse solution, and replace the bottles and collection vials with clean ones for the next synthesis.

7. If Premium Wash Solvent is used for the System Clean, it is recommended to perform a second System Clean with DMF or NMP in the Solvent 2 bottle in order to remove the Premium Wash Solvent from the lines. Any remaining Premium Wash Solvent in the system can adversely affect chemistry.

NOTE The **System Clean** takes over 1 hour to complete.

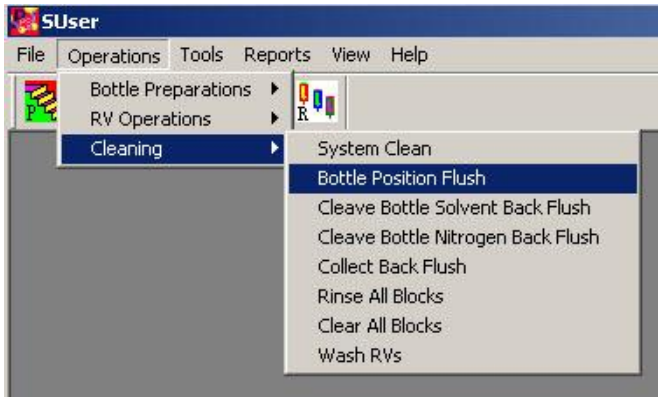
CAUTION Remove all chemicals and resins from the ***Prelude X***® before the **System Clean** because they will be contaminated with Solvent 2.

2.5.3.2 Bottle Position Flush

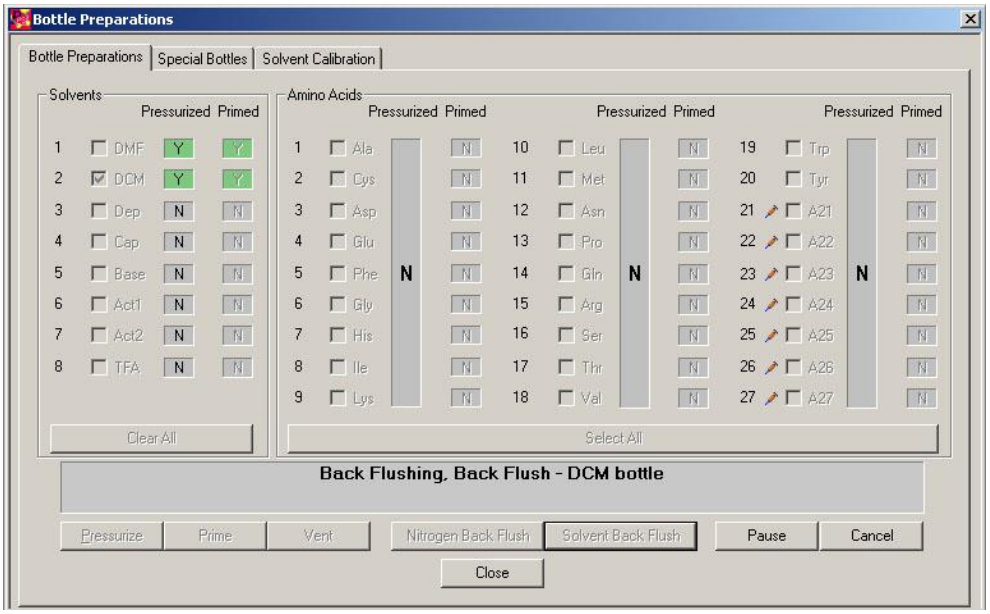
Bottle Position Flush flushes selected amino acid and/or solvent/reagent bottle lines with Solvent 1, DMF, or nitrogen. **Bottle Position Flush** should be performed when changing reagents or to clear a clog caused by amino acid precipitate in the line. **Bottle Position Flush** is performed as part of a **System Clean**, but it can also be performed alone as follows:

1. Replace amino acid and/or solvent/reagent bottles to be flushed with empty bottles.

2. Click on the **Operations** menu, select **Cleaning**, and then select **Bottle Position Flush**.



3. This will open the **Bottle Preparations** screen.



4. If a **Solvent Back Flush** will be performed, Solvent 1, DMF, must be pressurized and primed first. A **Nitrogen Back Flush** operation does not require Solvent 1, DMF, to be pressurized and primed. To pressurize and prime Solvent 1, DMF, check the box next to Solvent 1, DMF, by clicking in it. Click the **Pressurize** button. When complete, select Solvent 1 again. Click the **Prime** button.

5. Check the box(es) next to the bottle(s) that will be flushed.

6. To back flush bottle(s) with Solvent 1, DMF, click on the **Solvent Back Flush** button. To back flush bottle(s) with nitrogen, click on the **Nitrogen Back Flush** button.

NOTE When changing reagents, it is suggested to perform a **Nitrogen Back Flush** to flush reagent back into the bottle. Replace the bottle with an empty bottle, and perform **Solvent Back Flush** to flush residual reagent from the line. Wipe excess fluid off the bottle tubing and load the new reagent bottle. When trying to loosen a clog, remove bottle filter and use a **Solvent Back Flush**.

NOTE Different flushing solvents may be used by placing them in the Solvent 1 bottle.

CAUTION Under no circumstances should TFA be used in the amino acid manifold system—destruction of the bottle seals will occur! See Section 4.2.5 **Amino Acid Bottle Seal Replacement** for replacement procedures.

7. After the cleaning operation is complete, empty the flushed bottles of any rinse fluid and replace with clean bottles for the next synthesis.

2.5.3.3 Cleave Bottle Solvent Back Flush

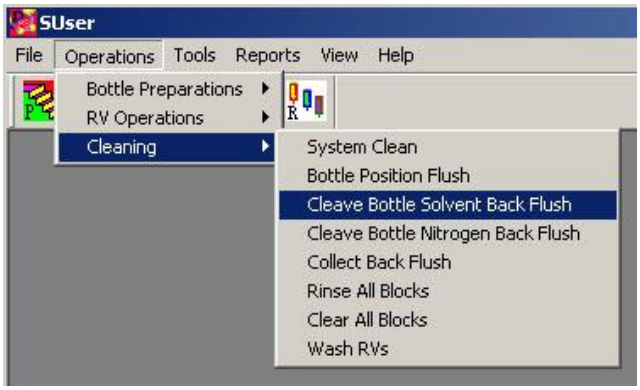
The **Cleave Bottle Solvent Back Flush** cleaning procedure flushes solvent bottle 8 with Solvent 2, DCM, to remove TFA solution from the line. **Cleave Bottle Solvent Back Flush** is performed as part of a **System Clean**, but it can also be performed alone.

NOTE When changing cleavage solutions, it is suggested to perform a **Cleave Bottle Nitrogen Back Flush** (Section 2.5.3) to flush reagent back into the bottle. Replace the bottle with an empty bottle, and perform a **Cleave Bottle Solvent Back Flush** to clear residual reagent from the line. Wipe excess fluid off the bottle tubing and load the new reagent bottle.

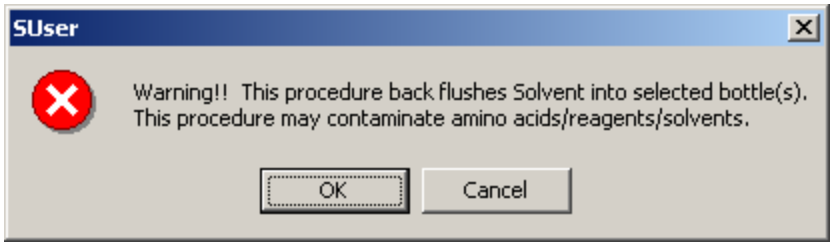
To perform a **Cleave Bottle Solvent Back Flush**:

1. Pressurize and prime Solvent 2, DCM, using the **Bottle Preparations** screen (See Section 2.5.1).

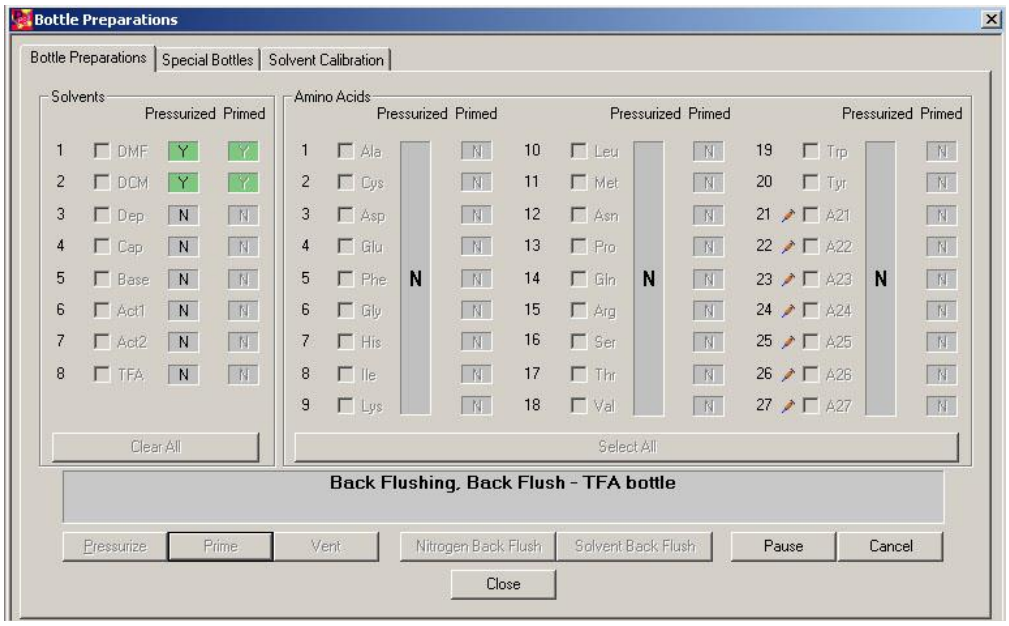
2. Click on the **Operations** menu, select **Cleaning**, and then select **Cleave Bottle Solvent Back Flush**.



3. The following warning window will open. Click **OK** to continue or **Cancel** to cancel the **Cleave Bottle Solvent Back Flush** operation.



4. During the **Cleave Bottle Solvent Back Flush**, the **Bottle Preparations** screen will open.



The status of the operation will be displayed in the status bar. Click the **Pause** button to pause the operation. Click **Resume** to resume a paused operation. Click the **Cancel** button to cancel the operation. Click the **Close** button to close the **Bottle Preparations** screen.

5. After the cleaning operation is complete, empty solvent bottle 8 of any rinse fluid and replace with a clean solvent bottle for the next cleavage.

2.5.3.4 Collect Back Flush

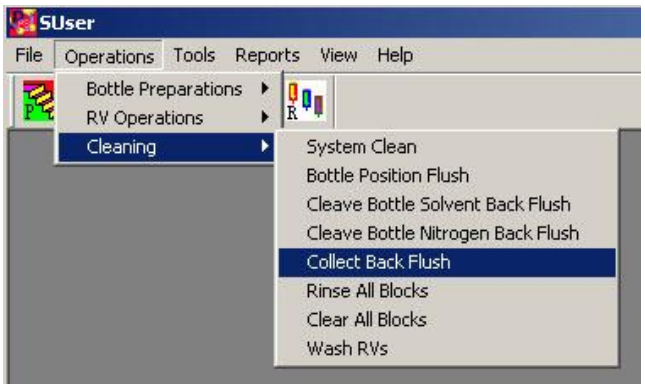
The **Collect Back Flush** cleaning procedure flushes Solvent 2, DCM, through the cleave system and collection lines into the collection vials to remove TFA solution and any residual peptide from the system. **Collect Back Flush** should be performed after every collection to prevent contamination of the next synthesis product. **Collect Back Flush** is performed as part of a **System Clean**, but it can also be performed alone.

To perform a **Collect Back Flush**:

1. Install empty collection vials to receive the rinse solvent.

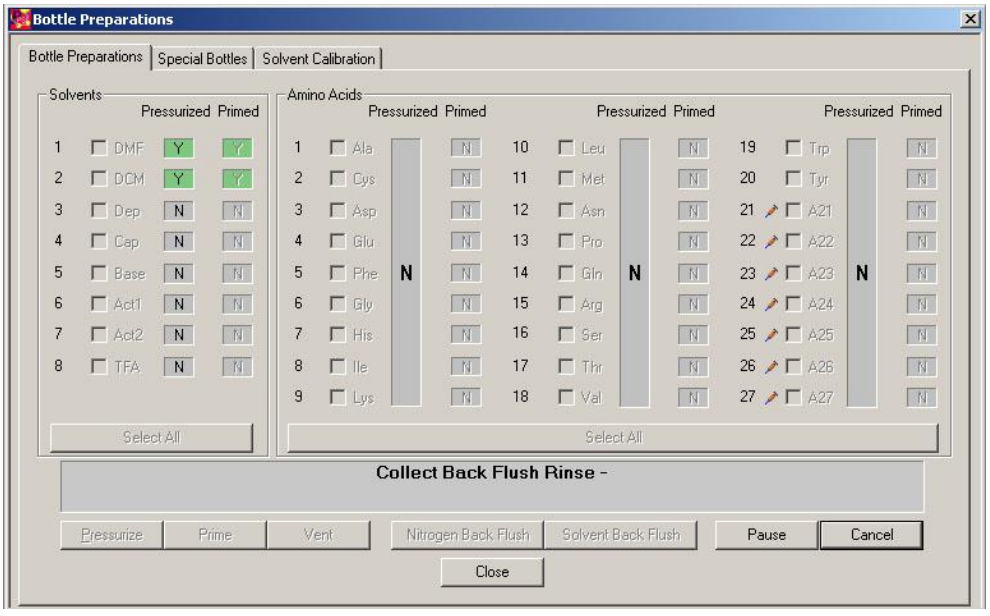
2. Pressurize and prime Solvent 2, DCM, using the **Bottle Preparations** screen (See Section 2.5.1.1).

3. Click on the **Operations** menu, select **Cleaning**, and then select **Collect Back Flush**.



4. The following warning window will open. Verify the empty collection vials are in place and click **OK**. Click the **Cancel** button to cancel the **Collect Back Flush** operation.

5. During the **Collect Back Flush**, the **Bottle Preparations** screen will open.



The status of the operation will be displayed in the status bar. Click the **Pause** button to pause the operation. Click **Resume** to resume a paused operation. Click the **Cancel** button to cancel the operation. Click the **Close** button to close the **Bottle Preparations** screen.

CAUTION If the procedure is cancelled there may be fluid left in the instrument lines or blocks. Repeat the **Collect Back Flush** procedure to clear the lines.

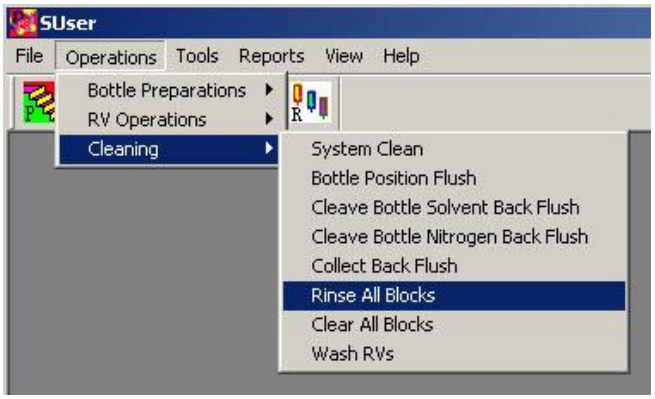
6. After the cleaning operation is complete, remove the collection vials and discard the rinse solution. Place clean, empty collection vials on the instrument for the next collection.

2.5.3.5 Rinse All Blocks

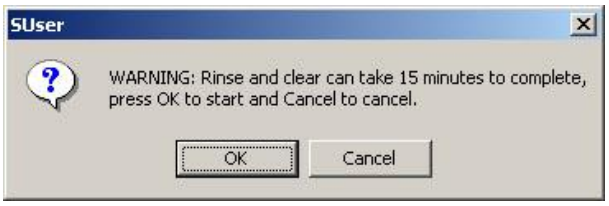
Rinse All Blocks rinses each block with Solvent 1, DMF, then flushes each block with nitrogen to remove residual fluid. It does so without venting the bottles to prevent contamination of the amino acids, solvents and reagents. **Rinse All Blocks** is performed using Solvent 2 as part of a **System Clean**, but it can also be performed alone using Solvent 1, DMF.

1. To perform a **Rinse All Blocks** operation, pressurize and prime Solvent 1 using the **Bottle Preparations** screen (See Section 2.5.1.1).

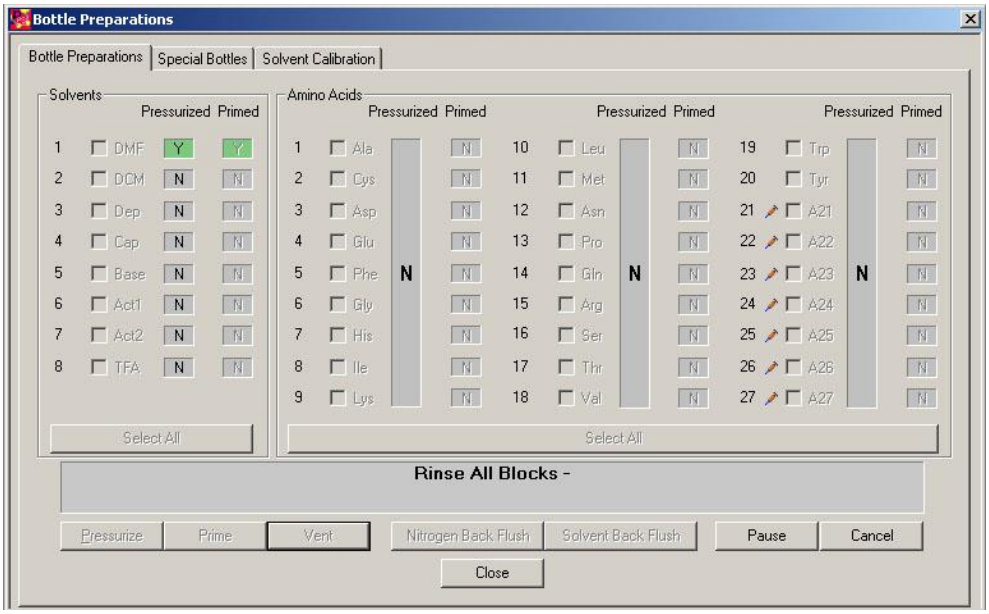
2. Click on the **Operations** menu, select **Cleaning**, and then select **Rinse All Blocks**.



3. The following warning window will open. Click **OK** to proceed, or click the **Cancel** button to cancel the **Rinse All Blocks** operation.



4. During the **Rinse All Blocks** operation, the **Bottle Preparations** screen will open.



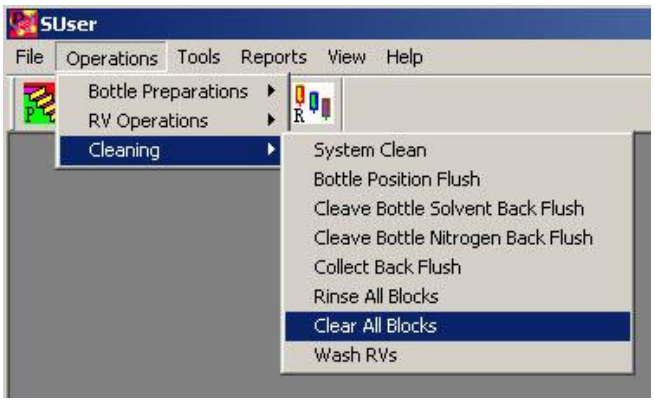
The status of the operation will be displayed in the status bar. Click the **Pause** button to pause the operation. Click **Resume** to resume a paused operation. Click the **Cancel** button to cancel the operation. Click the **Close** button to close the **Bottle Preparations** screen.

CAUTION If the procedure is cancelled there may be fluid left in the lines or blocks. Perform a **Clear All Blocks** operation (See Section 2.5.3.7) to remove any residual fluid.

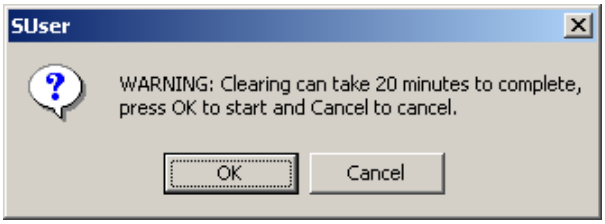
2.5.3.6 Clear All Blocks

The **Clear All Blocks** function flushes each block with nitrogen gas to remove residual fluid without venting the bottles and contaminating the amino acids, solvents and reagents. **Clear All Blocks** is performed as part of a **System Clean**, but it can also be performed alone as follows:

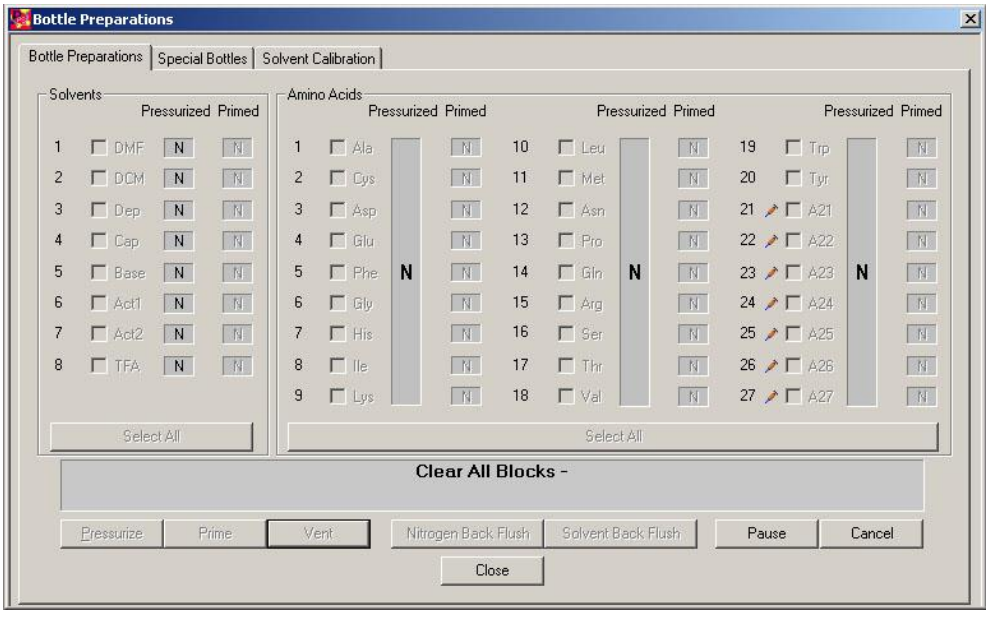
1. To perform a **Clear All Blocks** operation, click on the **Operations** menu, select **Cleaning**, and select **Clear All Blocks**.



2. The following warning window will open. Click **OK** to proceed, or click the **Cancel** button to cancel the **Clear All Blocks** operation.



3. During the **Clear All Blocks** operation, the **Bottle Preparations** screen (See Section 2.5.1.1) will open.



The status of the operation will be displayed in the status bar. Click the **Pause** button to pause the operation. Click **Resume** to resume a paused operation. Click the **Cancel** button to cancel the operation. Click the **Close** button to close the **Bottle Preparations** screen.

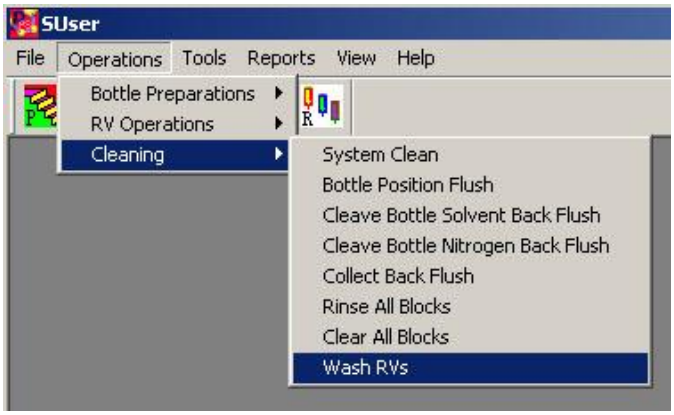
CAUTION If the procedure is cancelled there may be fluid left in the lines or blocks. Repeat a **Clear All Blocks** to remove any residual fluid.

2.5.3.7 Wash RVs

Wash RVs rinses the reaction vessels and lines by delivering Solvent 1, DMF, from the top of all 6 reaction vessels through the vessels to waste. After draining the solvent, nitrogen is delivered from the top to dry the RVs. **Wash RVs** should be performed every time a reaction vessel is used to remove residual reagent from the RVs, delivery lines and block. **Wash RVs** is performed as part of a **System Clean**, but it can also be performed alone using Solvent 1, DMF.

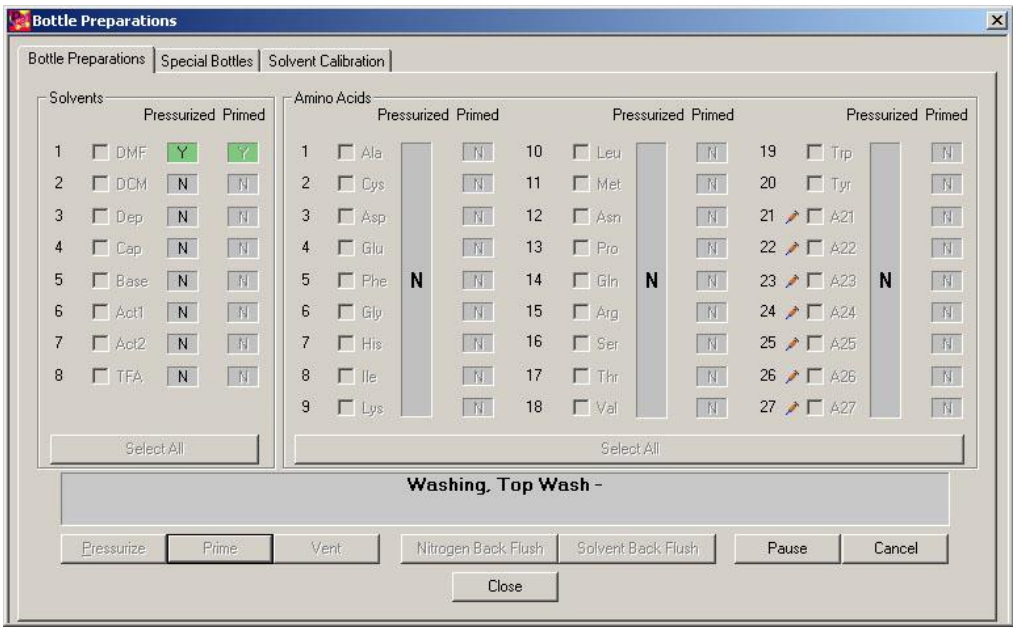
1. To perform a **Wash RVs**, pressurize and prime Solvent 1, DMF, using the **Bottle Preparations** screen (See Section 2.5.1.1).

2. Click on the **Operations** menu, select **Cleaning**, and then select **Wash RVs**.



3. The following warning window will open. Verify all 6 reaction vessels are in place and click **OK**, or click the **Cancel** button to cancel the **Wash RVs** operation.

4. During the **Wash RVs** operation, the **Bottle Preparations** screen will open.



The status of the operation will be displayed in the status bar. Click the **Pause** button to pause the operation. Click **Resume** to resume a paused operation. Click the **Cancel** button to cancel the operation. Click the **Close** button to close the **Bottle Preparations** screen.

CAUTION If the procedure is cancelled there may be fluid left in the RVs, lines or blocks. Repeat the operation, or perform a **Drain** operation using the **Manual Operations** screen (See Section 2.5.2.5) to remove any residual fluid.

5. After the cleaning operation is complete, remove the reaction vessels and replace with clean, resin-filled reaction vessels for the next synthesis.

2.6 Tools Menu

2.6.1 Database

Synthesis data and other information are saved in a database. The two functions available to help maintain this database are described in the following sections.

2.6.1.1 Rebuild Errors Table

The **Rebuild Errors Table** function is available for technician use only. It is used to reconfigure the database as needed following a software upgrade. If you encounter an error message pertaining to this function, please contact GPT customer service at 1-800-477-6834.

2.6.1.2 Archive All Data

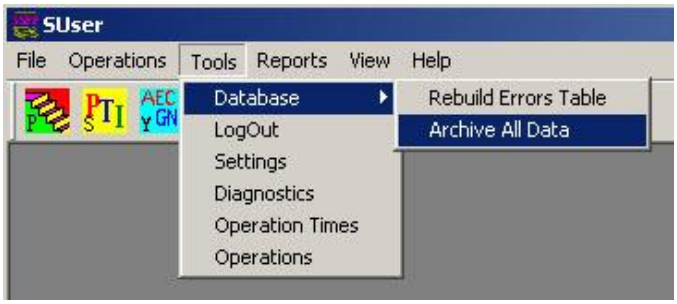
The **Archive All Data** function archives all synthesis data and compacts the database. It is located in the **Tools** menu under **Database**. The selection becomes active when a supervisor logs in (Section 2.6.2). This function makes a back-up copy of all data from syntheses run since the last **Archive All Data** was performed. The **Archive All Data** command archives all the synthesis log data contained in the current database file into an archive file named after the database file and the archive date: Name_YYYYMMDD.mdb. It is recommended to archive data every month depending on the number of syntheses run. This makes the system more responsive. In addition, data should be archived prior to deleting old program, sequence and synthesis files as part of regular file maintenance.

NOTE Halt all other instrument operations before running an **Archive All Data**.

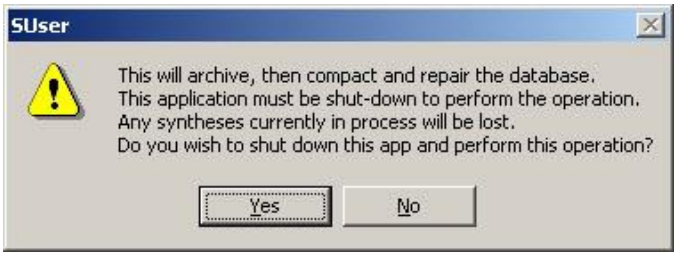
To **Archive All Data**:

1. Click on the **Tools** menu and select **LogIn**. Enter the supervisor password and click **OK**.

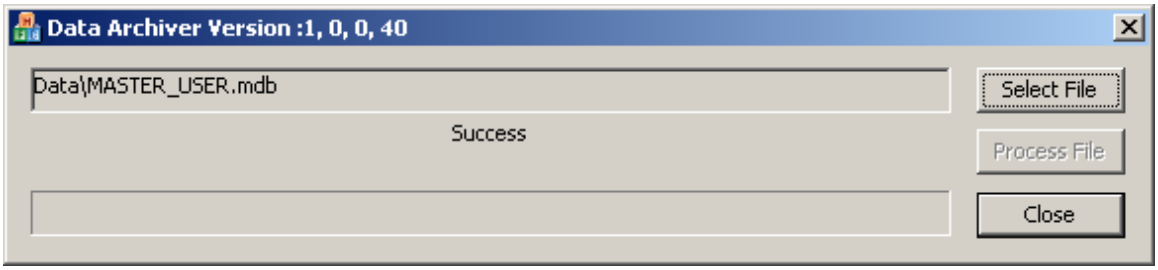
2. Click on the **Tools** menu, select **Database**, and select **Archive All Data**.



3. The following warning window will open. Click **Yes** to continue, or click **No** to cancel the operation.

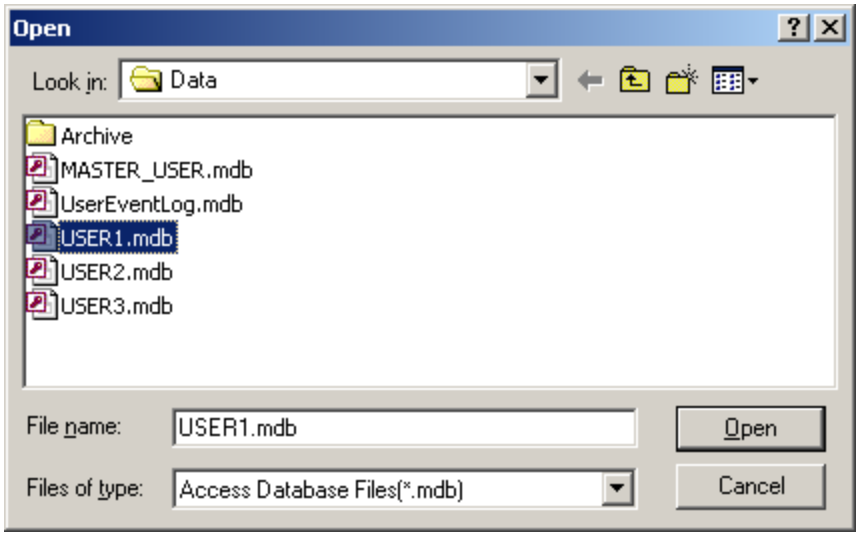


4. The **SUser** software will exit and the **Data Archiver** window will open.



5. When the archiving process is complete, the word “Success” will be displayed.

6. To archive a database file other than the current database file specified under **Settings** (Section 2.6.3), click the **Select File** button. This will open a new window.



7. Select a user database file from the **Data** folder, and click **Open**. This will return the user to the **Data Archiver** window where the **Process File** button will have become active.

8. Click the **Process File** button to archive the file.

9. Click the **Close** button or click on the **X** in the upper right corner to close the window.

10. Double-click on the SUser icon to restart the **SUser** software.

Archived data is located in the C:\Program Files\PTI\Prelude X\Data\Archive folder, and is located in a single file named according to the database file name and archive date (Name_YYYYMMDD.mdb).

NOTE It is also recommended to copy the current .mdb database file to an external location as a backup. Save to a memory stick inserted in one of the USB ports, then transfer to another computer where the file may be saved to the hard drive or other external drive.

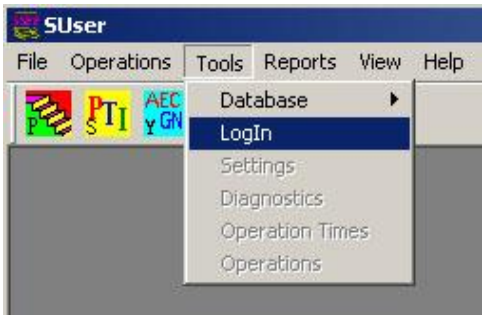
To print a job report from an archived file, see **Jobs** (Section 2.7.1).

2.6.2 LogIn/LogOut

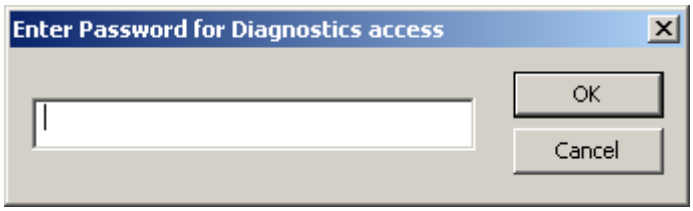
The **LogIn/LogOut** feature prevents unauthorized users from accessing certain functions under the **Tools** menu. The **Archive All Data** (Section 2.6.1.2) function and the **Settings** (Section 2.6.3) screen are only accessible with a supervisor password, while the remaining selections under the **Tools** menu, and **Comm Log** under the **Reports** menu may only be accessed by a GPT technician or factory password. For general use, access to the **Tools** menu is not required to run the instrument.

To **LogIn**:

1. Click on the **Tools** menu and select **LogIn**.



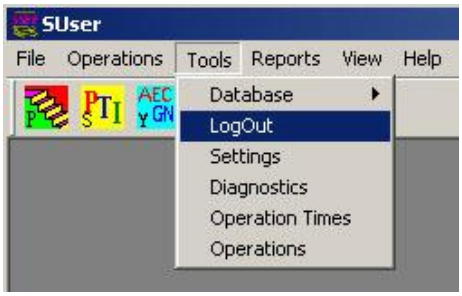
2. The following window will open.



3. Enter the appropriate password and click **OK**. Click **Cancel** to cancel the login.

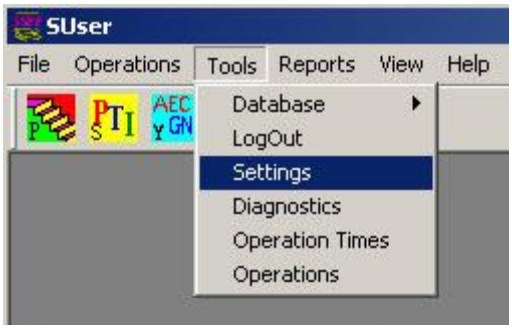
To **LogOut**:

Click on the **Tools** menu and select **LogOut**.

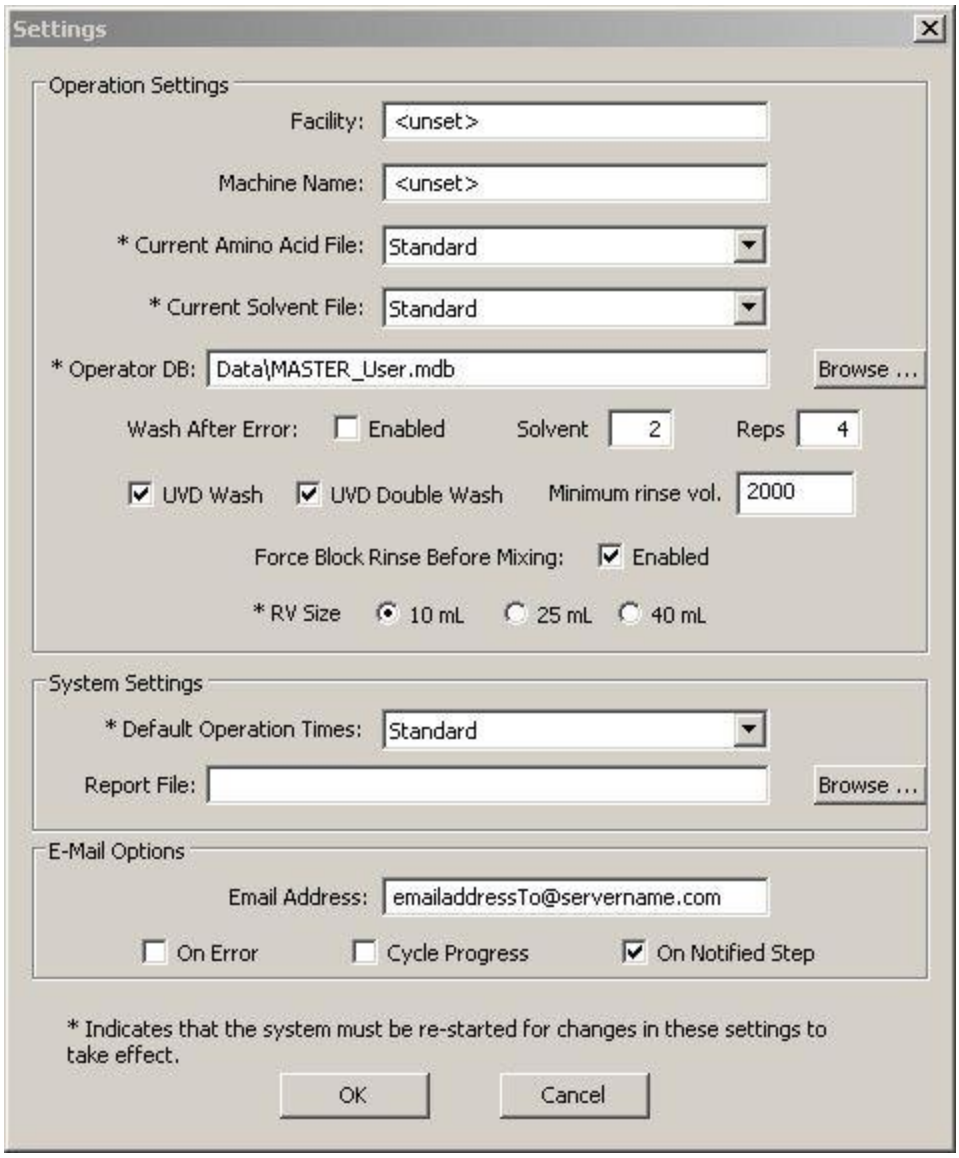


2.6.3 Settings

The **Settings** window allows a logged-in customer or technician (Section 2.6.2) to set the system defaults. Click on the **Tools** menu and select **Settings**.



This will open the **Settings** window.



The **Operation Settings** are as follows:

1. **Facility** – Enter name of Facility

2. **Machine Name** – Enter name for ***Prelude® X*** computer

3. **Current Amino Acid File** – Values from this file are displayed in the **Bottle Preparations** screen in the **Name** column. The default setting is **Standard**. Select a different amino acid file using the pull down menu. ***Prelude X*** software must be restarted for changes to this setting to take effect.

4. **Current Solvent File** – Values from this file are displayed in the **Bottle Preparations** screen in the **Name** column. The default setting is **Standard**. Select a different solvent/reagent file using the pull down menu. ***Prelude X*** must be restarted for changes to this setting to take effect.

5. **Operator DB** – This database file will store all system data, bottle settings, synthesis definitions and the synthesis log file data of any syntheses run on the instrument while it is selected. Individual operators may have their own database files for their own syntheses. The default setting is **MASTER_USER.mdb**. ***Prelude X*** software must be restarted for changes to this setting to take effect. To select a different file, click the **Browse…** button. To create a new database file, make a copy of the **MASTER_USER.mdb** file (preferably after an archive) and rename it using Windows® Explorer®.

6. **Wash After Error** – When enabled, ***Prelude X*** will wash all six RVs with the entered solvent for the entered repetitions if an error occurs. Click in the **Enabled** box to enable this safety feature. A check mark indicates the feature is enabled. Enter a wash solvent in the **Solvent** box (Solvents 1-2), and the number of repetitions in the **Reps** box (1-9).

7. **UVD Wash/UVD Double Wash** – When using an “XtendRep” program for deprotection, the instrument will use a single wash with Solvent 1 (generally DMF) between repetitions when “UVD Wash” is selected, and two washes between repetitions when “UVD Double Wash” is selected. if the user is using a deprotection volume (from the program) smaller than the minimum volume indicated, then it would use the “settings” minimum volume for the DMF rinses in between repetitions. If the deprotection volume is greater, then it will use the program volumes instead of the “settings” minimum volume.

8. **Force Block Rinse Before Mixing** – When enabled, ***Prelude X*** will perform a block rinse before mixing rather than after draining. This is useful when long mix times are used to prevent reagents from sitting in the valve system for extended periods of time prior to being rinsed out.

9. **RV Size (10 mL, 25 mL or 40 mL)** – Adjusts calibration curve, Wash After Error wash volume, and maximum delivery volume based on RV size. The maximum single delivery volume is 5,000 µL for the 10 mL RV,13,000 µL for the 25 mL RV and 20,000 µL for the 40 mL RV. After changing this setting, the software needs to be restarted for the change to take effect.

The **System Settings** are as follows:

System Settings are not accessible under supervisor logins and should not be changed by the user. Improper changes can cause the instrument to malfunction. Only GPT factory engineers should modify **System Settings.**

1. **Default Operation Times** – The contents of this file control the times associated with operations on the instrument. The default setting is **Standard**. Prelude *X* software must be restarted for changes to this setting to take effect.

2. **Report File** – This is the file whose contents are displayed when the **Reporter** window is opened. The default setting is **MASTER_USER.mdb**. To select a different file, click the **Browse…** button.

The function of the **E-Mail Options** section is described in detail in Section 3.7.

To save changes, click the **OK** button, and restart ***Prelude X*** software by clicking on the **X** in the upper right corner of the main screen, then double-clicking on ***Prelude X*** user software icon on the desktop.

To cancel changes, click the **Cancel** button.

2.6.4 Diagnostics

The **Diagnostics** screen is available for technician and factory use only. It is used to troubleshoot and repair the instrument by allowing the technician to control individual valves.

2.6.5 Operation Times

The **Operation Times** screen is available for factory use only. It is used to edit the control times for individual software operations.

2.6.6 Operations

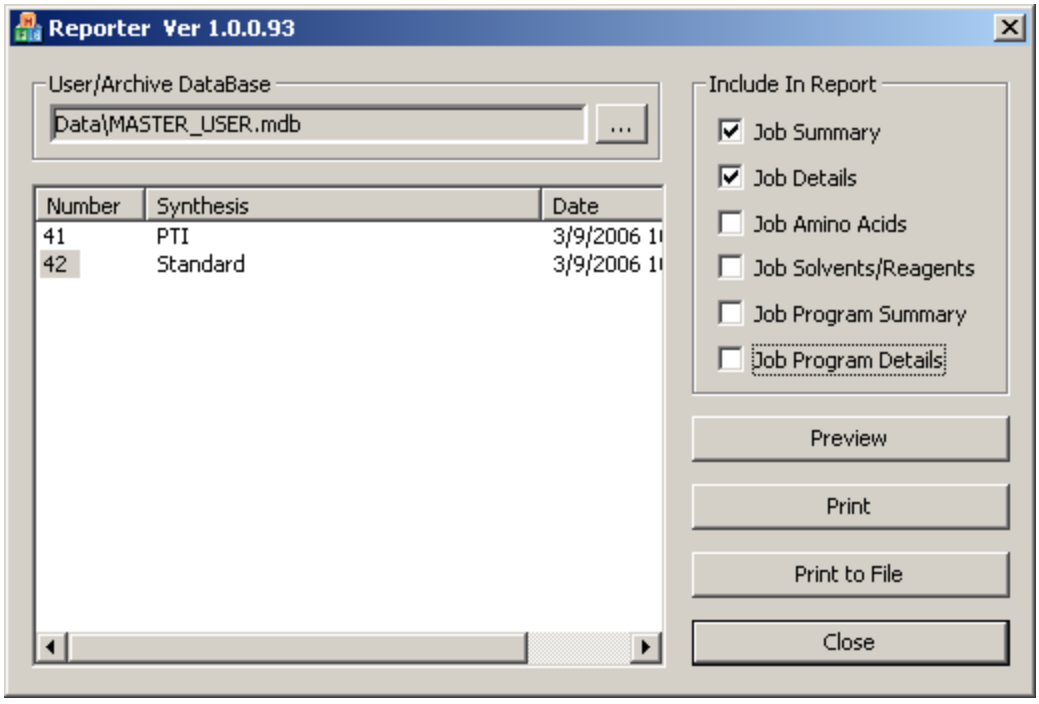
The **Operations** screen is available for factory use only. It is used to edit the software operations available for use on the instrument.

2.7 Reports Menu

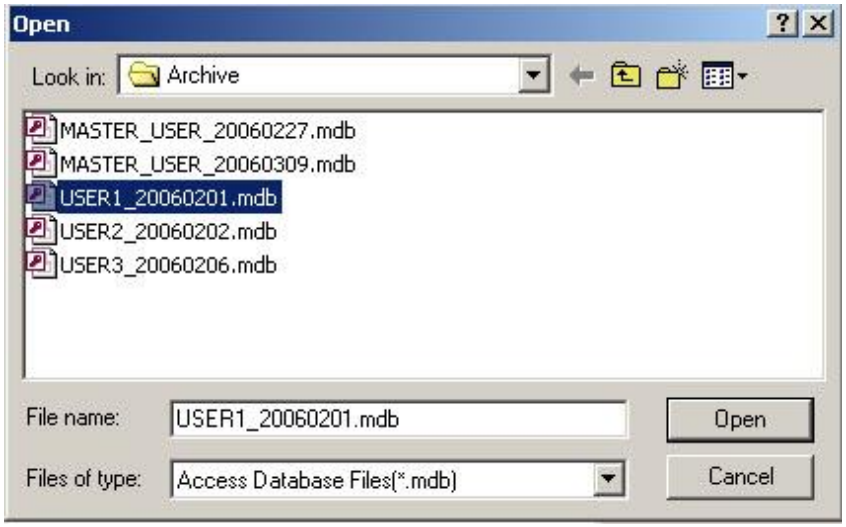
2.7.1 Jobs

When a synthesis is run, all information related to that synthesis is stored in a job file. Job files are assigned a job number, and are stored in the default database file defined under **Settings** (Section 2.6.3). Jobs may be previewed on screen or sent to a printer. To access and print a job file, click on the **Reports** menu and select **Jobs**.

The **Reporter** window will open.



The **User/Archive DataBase** section displays the current database file, while the job files from the database are listed in the table. To select a job file from a different database, or an archived database file, click on the **"…"** button. This will open the **Open** window.



Go to C:\PTI\Prelude\Data\Archive and select an archive file. Click **Open**.

The columns of the job table are labelled as follows:

1. **Number** – Job number
2. **Synthesis** – Name of the Synthesis file
3. **Date** – Date and time synthesis was run

To select a job file, click on and highlight the job number listed in the **Number** column.

In the **Include In Report** section, select the information that will be displayed in the job report. Select from the following:

1. **Job Summary** – Displays the facility, machine, job number, date and synthesis name as well as a table summarizing the following:
    a. **RV** – RV number
    b. **Name** – Name of sequence file
    c. **Sequence** – Amino acid sequence of peptide
    d. **MW (g/mol)** – Molecular weight (in g/mol) of peptide
    e. **CONH2** – Terminal group on C-terminus of peptide. **Yes** for CONH2, **No** for COOH.

2. **Job Details** – Displays the cycle, job number, program, and amino acid added to each RV in the cycle as well as a table summarizing every software operation executed for each cycle. The columns are:

    a. **Step** – Program step

    b. **Act** – Action

    c. **Pos** – Position of RV, bottle, or other code depending on the operation

    d. **Rep** – Repetition

    e. **Operation** – Operation

    f. **Description** – Detailed description of operation

    g. **OT** – Operation Type

        i. **M** – Manual operation

        ii. **A** – Automated operation

        iii. **R** – Restart

        iv. **D** – Done

    h. **Date/Time** – Date and time of operation

3. **Job Amino Acids**– Displays the job number and a table of the information entered in the **Amino Acid Editor** (Section 2.4.1). The columns are:

    a. **Position** – Amino acid bottle position.

    b. **Amino Acid** – Full name of amino acid.

    c. **Source Number** – Source information for amino acid.

    d. **Opened** – Date and time bottle was opened.

    e. **Lot Number** – Lot number of amino acid.

    f. **Concentration (mM)** – Concentration (in mM) of amino acid solution.

    g. **Volume (mL)** – Volume (in mL) of amino acid solution.

4. **Job Solvents/Reagents**– Displays the job number and a table of the information entered in the **Solvent/Reagent Editor** (Section 2.4.2). The columns are:

    a. **Position** – Solvent/reagent bottle position.

    b. **Solvent** – Full name of solvent/reagent.

    c. **Source Number** - Source information for solvent/reagent.

    d. **Opened** – Date and time bottle was opened.

    e. **Lot Number** – Lot number of solvent/reagent.

    f. **Concentration (mM)** – Concentration (in mM) of reagent solution.

    g. **Volume (mL)** – Volume (in mL) of solvent/reagent solution.

5. **Job Program Summary** – Displays the job number, name of the synthesis, ID (synthesis file number), and the date and time the synthesis file was last modified. A table lists the programs run at each cycle. The columns are:

    a. **Starting Cycle** – The first cycle the program was run at.

    b. **Program Run** – The name of the program run at that cycle and subsequent cycles until the next **Starting Cycle** listing.

6. **Job Program Details** – Displays the job number, name of the synthesis, ID (synthesis file number), and the date and time the synthesis file was last modified. A table lists the individual steps of all programs run during the synthesis. The columns are:

    a. **Step** – Program step.

    b. **Operation** – Operation executed during the program step.

    c. **Solvent** – Name of the solvent/reagent/amino acid delivered during the operation.

    d. **Mix Time** – Nitrogen mixing time (in HH:MM:SS).

    e. **Repeats** – Number of times the step was repeated.

f. **Drain On** – Displays a checked box if the RVs were drained following the step, and an unchecked box if they were not.

The buttons are:

1. **Preview** – Clicking the **Preview** button will open the job reports selected in the **Include In Report** section in a **Report Preview** window. Use the magnifying glass at the top of the **Report Preview** window to view the document at different magnifications and the left and right arrows to navigate between pages. Click on the printer icon at the top of the window to print the reports from the **Report Preview** window, and click the **Close** button or click on the **X** in the upper right corner to close the window.

2. **Print** – The **Print** button will automatically send the reports to the printer.

3. **Print to File** – Click the **Print to File** button to save the job report. A **Save As** window will open and allow you to save the report as a text file or other file type.

4. **Close** – Click the **Close** button to exit the **Reporter** window.

2.8 View Menu

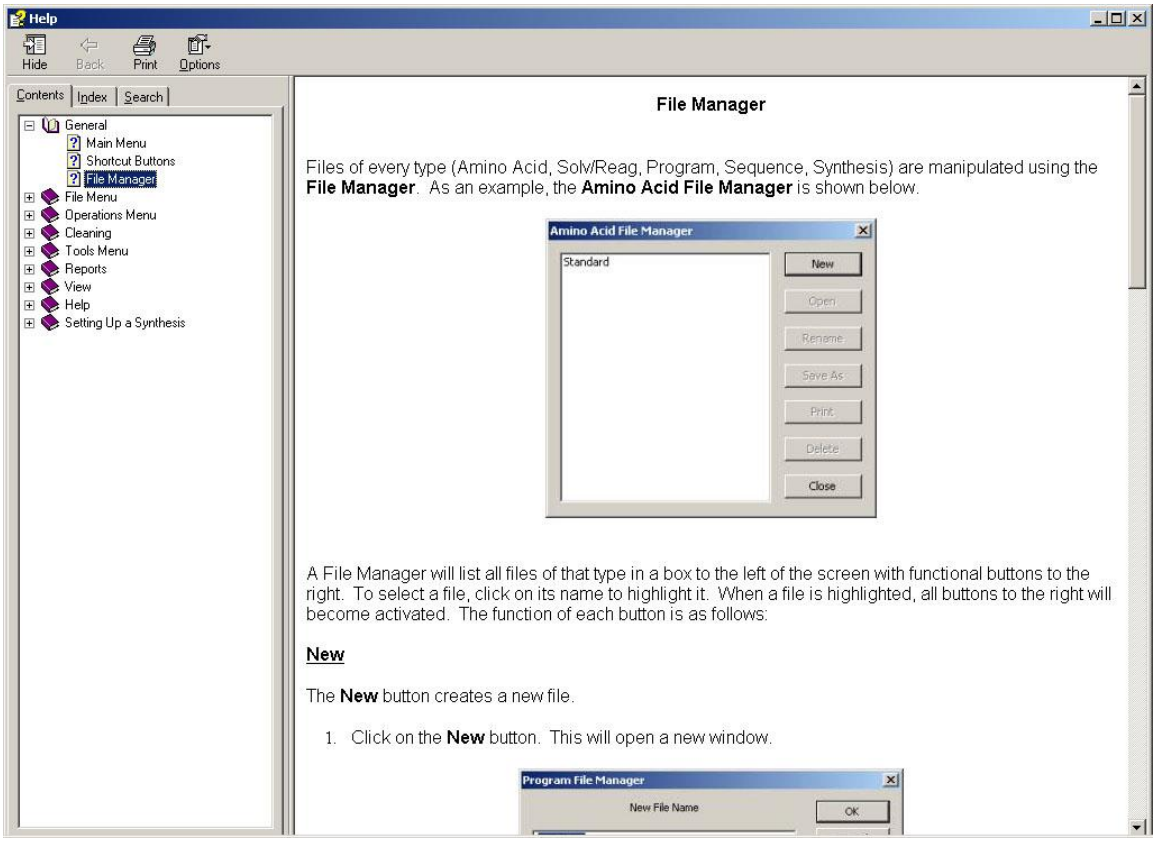
The **View** menu displays the names of all open windows. The active top window is indicated by a checkmark next to its name. Click on the **View** menu and select an open window to go to it quickly.

2.9 Help Menu

2.9.1 Help Topics

To open the **Help Topics** window, click on the **Help** menu and select **Help Topics**.

On the left side of the **Help Topics** window are the **Contents**, **Index**, and **Search** tabs.



The **Contents** tab organizes the help files into directories. A purple book icon indicates directories while a sheet of paper with a question mark icon indicates individual help files. Click on a file name or icon in the Contents list to display the corresponding help file in the window to the right.

Type in a keyword in the box at the top of the **Index** tab to find it and highlight it in the alphabetical list below. Click the **Display** button at the bottom of the **Index** tab to choose from the list of help files containing the keyword. Click the **Display** button in the new window to display the help file in the window to the right.

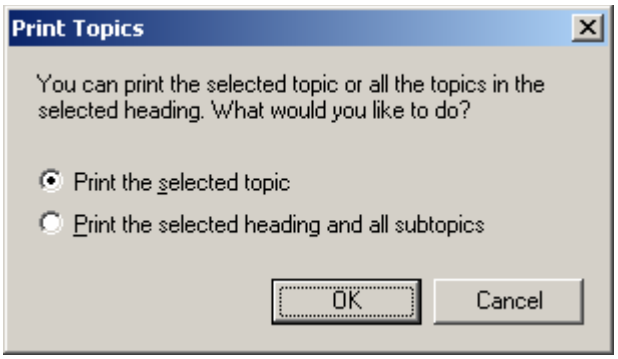
Type in a keyword in the box at the top of the **Search** tab and click the **List Topics** button to display a list of the help files containing the keyword. Click the **Display** button at the bottom of the **Search** tab to display the help file in the window to the right.

The icons are as follows:

1. **Hide/Show** – Click the **Hide** icon to hide the **Contents**/**Index**/**Search** tabs. Click the **Show** icon to show the tabs.

2. **Back** – Click the **Back** button to return to the previously viewed help file.

3. **Print** – Click the **Print** button to print the currently open help file. If the file has been selected from the **Contents** tab, the following window will open:



   This window gives the user the option to print the individual help file or all the help files under the selected heading. Select an option, and then click **OK** to print the file from the **Print** screen. Click **Cancel** to return to the **Help Topics** window without printing.

4. **Options** – Click the **Options** icon to select from the following:

   a. **Hide/Show Tabs** – Hides/Shows the **Contents**/**Index**/**Search** tabs.

   b. **Back** – Returns to the previously viewed help file.

   c. **Forward** – If the **Back** button was pressed, returns user to the previously viewed help file.

   d. **Home** – Returns the user to the **Help Topics Home** page.

   e. **Stop** – Stops the process of opening the help file.

   f. **Refresh** – Restarts the process of opening the help file.

   g. **Internet Options…** – Allows the user to set the Internet options.

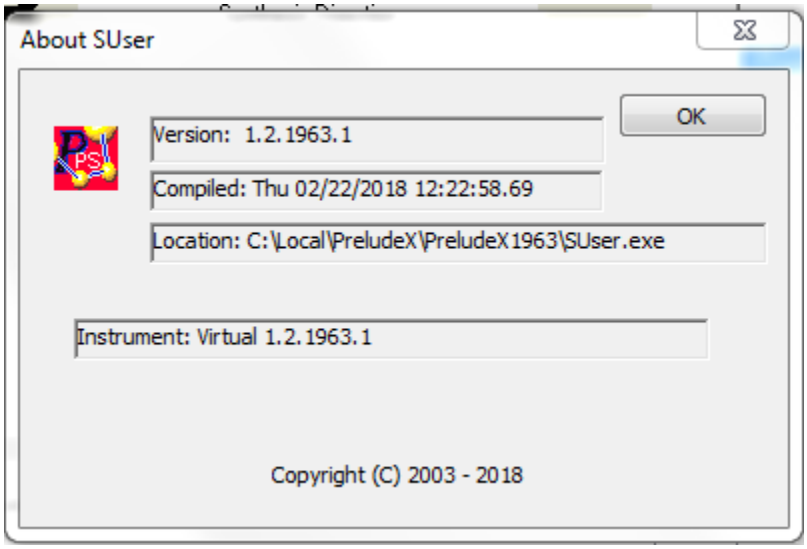
h. **Print…** – Prints the currently open help file.

i. **Search Highlight On/Off** – Highlights the search term in the Help file(s).

2.9.2 About SUser

To open the **About SUser** information box, click on the **Help** menu and select **About SUser…**.

This will open the **About SUser** information box.



Version numbers for ***Prelude X*** User and Instrument software will be displayed.

Chapter 3 Basic Synthesis Operations

3.1 Synthesis Checklist

Steps for running a synthesis without cleavage (**NC**) or with cleavage (**C**) are shown in the table below. See Section 3.5 for more information about automated cleavage.

√	NC	C	**Startup & Instrument Check**
	●	●	Turn on the ***Prelude X***® Peptide Synthesizer (Section 3.2)
	●	●	Check nitrogen supply and gauges (Sections 3.2 & 1.1.10)
	●	●	Check vacuum gauge (Sections 3.2 & 1.1.11)
	●	●	Check waste level (Sections 3.2, 1.1.8 & 1.2.7)
	●	●	Change RV upper and lower o-rings, if necessary (every 2 weeks) (Section 1.2.3)
	●	●	Change bottle filters, if necessary (i.e. change of reagent) (Sections 3.2 & 5.2.4)
	●	●	Select RV size in **Settings** screen (Section 2.6.3)
	●	●	Calibrate solvent delivery volumes, if necessary (Sections 3.2 & 2.5.1.3)
√	**NC**	**C**	**Software Setup**
	●	●	Create amino acid file (Section 2.4.1)
	●	●	Create solvent/reagent file (Section 2.4.2)
	●	●	Create swelling and synthesis program file(s) (Section 2.4.3.1)
		●	Create cleavage program file(s) (Section 2.4.3.1)
	●	●	Create sequence file(s) (Section 2.4.3.2)
	●	●	Create synthesis file (Section 2.4.3.3)
	●	●	Set default amino acid and solvent/reagent files in **Settings** screen (Section 2.6.3)
	●		Load synthesis (Section 2.5.2.2)
		●	Load synthesis & cleavage program (Section 2.5.2.2)
	●	●	Calculate amino acid/solvent/reagent/resin amounts needed (Sections 2.5.2.3 & 2.5.2.4)
√	**NC**	**C**	**Instrument Setup**
	●	●	Prepare amino acids/solvents/reagents and load bottles on instrument (Sections 1.2.5-1.2.6)
	●	●	Add resins to RVs and install on instrument (Section 1.2.3)
		●	Install collection vials on instrument (Section 1.2.4)
		●	Select **No Prime** for Solv 8 bottle (Section 2.5.1.2)
	●	●	Pressurize and prime all bottles needed for the synthesis (Section 2.5.1.1)
√	**NC**	**C**	**Run Synthesis**
	●	●	Click on Start in **RV Status** screen to run synthesis (Section 2.5.2.1)
	●	●	Adjust nitrogen mix flow control, if necessary (Section 1.1.10 & 1.1.13)
√	**NC**	**C**	**Post-Synthesis Procedures**
	●		Cleave peptides from resin (Section 3.3)
		●	Remove collection vials and work up peptides (Section 3.3)
	●	●	Perform **Wash RVs** (Sections 3.3 & 2.5.3.8)
	●	●	Perform **Bottle Position Flush** on used bottles (Sections 3.3 & 2.5.3.2)
		●	Perform **Collect Back Flush** (Section 2.5.3.5)
		●	Perform **Cleave Bottle Solvent Back Flush** (Section 2.5.3.3)
	●	●	Perform **System Clean** if necessary (every 2 weeks) (Section 2.5.3.1)
	●	●	Empty the waste container (Section 3.3 & 1.2.7)

3.2 Startup & Instrument Check

To startup ***Prelude X*** Peptide Synthesizer, turn on the On/Off switch located on the utility panel on the side of the instrument and also the On/Off switch for the heaters (if installed). Turn on the monitor and printer. The SUser software will startup automatically. Before starting a synthesis, perform the following checks:

1. Check the nitrogen supply & gauges. Make sure there is enough nitrogen in the tank for the synthesis and that the tank is on. Check the nitrogen status in the lower right corner of the screen. **N2** should be displayed on a green background. If **N2** is displayed on a red background, nitrogen is not getting to the instrument. Check the tank and lines. Check the pressure gauges. The Valve Pressure Gauge should read 25-35 psi and the setting should not be adjusted. The Nitrogen Pressure Gauge should read 5 psi and the setting should not be adjusted. The Bottle Pressure Gauge should read 9 psi. See Section 1.1.10 for more information.

2. Check the vacuum gauge. The vacuum gauge is located on the front of the instrument and should read 17-22 in Hg. Also check the vacuum status in the lower right corner of the screen. **Vac** should be displayed on a green background. If **Vac** is displayed on a red background, there is a problem with the vacuum system. If this occurs, call your local GPT Service Technician at 1-800-477-6834.

3. Check the waste level. Check the waste status in the lower right corner of the screen. **Waste** should be displayed on a green background. If **Waste** is displayed on a red background, the waste is full, or the waste level sensor is disconnected. Empty the waste if necessary, and make sure the waste level sensor is connected properly. See Sections 1.1.8 and 1.2.7 for more information.

4. Change bottle filters, if necessary. Bottle filters should be changed in the event of a clogged filter or change of reagent. See Section 4.2.4 for instructions.

5. Select RV size (10 mL, 25 mL or 40 mL) in the **Settings** screen (Section 2.6.3).

   Caution – When using the 10 mL reaction vessel, it is possible for resin to be pushed to the top of the RV if the swelled resin bed reaches to where the RV sides are parallel. Bottom deliveries can then push the resin bed to the top of the RV if the resin sticks together as a plug. The formation of a resin plug is resin and sequence dependent. Use of a larger RV is recommended to ensure that this will not occur.

6. Calibrate solvent delivery volumes, if necessary. If consumption volumes are not matching calculated volumes for solvents 1-4 and 8, it may be necessary to perform a solvent calibration. See Section 2.5.1.3 for instructions.

3.3 Post-Synthesis Procedures

1. Remove collection vials and work up peptides or cleave peptide from resin if on-instrument cleavage was not performed.

2. Perform a **Wash RVs** (Section 2.5.3.7).

3. Perform a **Bottle Position Flush** (Section 2.5.3.2) on all bottles used in the synthesis. First perform a **Nitrogen Back Flush** to flush reagent back into the bottles. Replace used bottles with empty bottles, and perform a **Solvent Back Flush** to flush residual reagent from the lines.

4. If a cleavage was performed, do a **Collect Back Flush** (Section 2.5.3.4) and a **Cleave Bottle Nitrogen Back Flush** (Section 2.5.3.3). Then, replace Solv 8 bottle with an empty bottle and perform a **Cleave Bottle Solvent Back Flush** (Section 2.5.3.3).

5. Discard, store, or reuse used chemicals.

6. Empty the waste container.

7. If the instrument will not be used immediately, shutdown the instrument (Section 3.4).

3.4 Instrument Shutdown

It is not necessary to shutdown ***Prelude X*** following each synthesis. Instrument shutdown is only necessary if the instrument needs to be moved or if the instrument will not be in use for an extended period.

To shutdown ***Prelude X:***

1. Perform a **System Clean** (Section 2.5.3.1) then **Nitrogen Back Flush** all bottles using the **Bottle Preparations** screen (Section 2.5.1.1).
2. Empty all amino acid and solvent/reagent bottles of fluid.
3. Empty the waste container.
4. Close the SUser software.
5. Shutdown the computer by selecting “Shutdown” from the “Start” menu.
6. Turn off the instrument.
7. Disconnect the nitrogen tank.

Advanced Synthesis Operations & Optional Features

In addition to its basic synthesis operations, ***Prelude X*** has the following advanced synthesis operations:

1. Automated Cleavage
2. Dynamic Sequence Programming
3. E-Mail Notification
4. Single-Shot™ Delivery
5. Wash After Error
6. Preactivation
7. UV monitoring
8. Induction heating

Some of these features are optional, but can be purchased by contacting GPT customer service at 1-800-477-6834 or peptides@gyrosproteintech.com. The functions of each feature are reviewed in the subsections below.

3.5 Automated Cleavage

The optional automated cleavage feature performs cleavage and collection on the instrument. To perform an automated cleavage:

1. Select **No Prime** for the Solv 8 bottle using the **Special Bottles** screen (Section 2.5.1.2).

2. Create a cleavage program using the **Program Editor** (Section 2.4.3.1).

3. Set the cleavage to occur after a synthesis or alone using the **Load Synthesis** screen (Section 2.5.2.2).

4. Calculate necessary solvent volumes for cleavage by selecting the cleavage program in the **Calculations – AA** (Section 2.5.2.3) screen and viewing the calculated solvent volumes in the **Calculations – Solv/Reag** (Section 2.5.2.4) screen.

5. Prepare cleavage cocktail in the Solv 8 bottle.

6. Run synthesis and/or cleavage using the **RV Status** screen (Section 2.5.2.1).

7. Following the cleavage, remove collection vials and work up the cleaved peptide.

8. Perform a **Collect Back Flush** (Section 2.5.3.5).

9. Replace Solv 8 bottle with an empty bottle. Perform a **Cleave Bottle Solvent Back Flush** (Section 2.5.3.3).

10. Discard rinse solution.

3.6 Dynamic Sequence Programming

The optional **Dynamic Sequence Programming** feature consists of advanced operations in the **Synthesis Editor** that may be activated by purchasing a license from GPT. To purchase a license, contact GPT customer service at 1-800-477-6834 or peptides@gyrosproteintech.com.

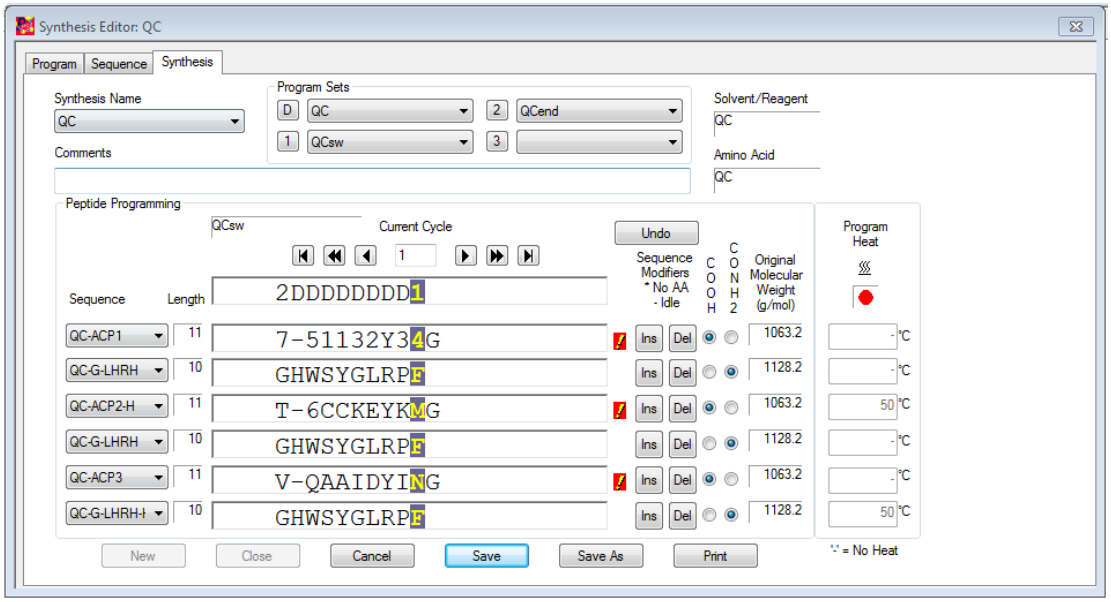
Catalog No.	Description	Quantity
PPS-DYN-SEQ-PGM	Dynamic Sequence Programming License	1

Dynamic Sequence Programming allows the user to:

1. Insert or delete an **Idle** cycle from the sequence. An **Idle** cycle makes the RV inactive during that cycle.

2. Insert or delete a **No AA** cycle from the sequence. A **No AA** cycle performs all program steps in a cycle except for amino acid addition.

If used incorrectly, these operations can easily ruin a synthesis. Therefore, once activated, **Dynamic Sequence Programming** may only be performed when a supervisor is logged in.



The **Dynamic Sequence Programming** buttons are located in the **Synthesis Editor** to the right of the peptide sequences and are as follows:

1. **Ins** – Opens the **Cycle Selection** window which allows the user to choose the type of cycle to insert: **Idle** or **No AA** (See below). Inserts the new cycle to the right of the selected cycle in a sequence.

NOTE Make all cycle inserts prior to assigning programs. When a **No AA** or new amino acid cycle is inserted, all program assignments return to the default program.

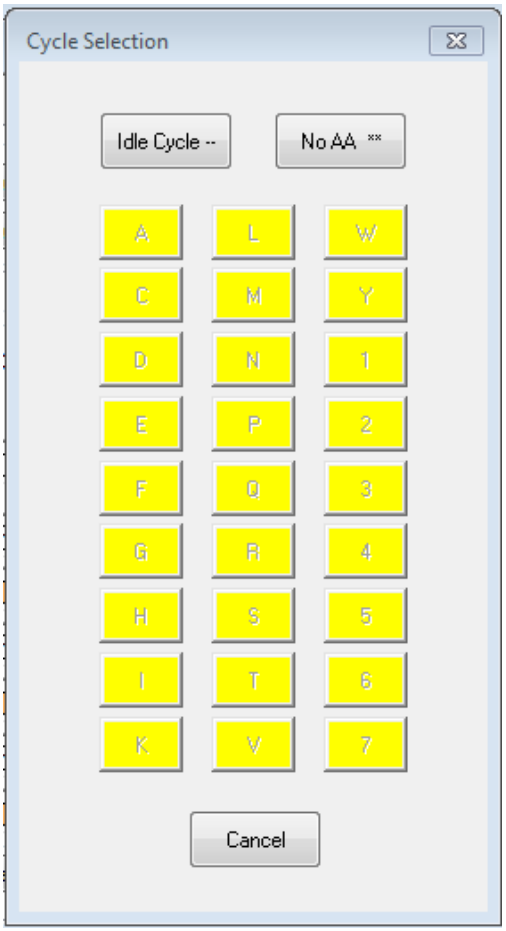
2. **Del** – Deletes the selected cycle in a sequence.

NOTE Make all cycle deletions prior to assigning programs. When an **Idle** or a **No AA** cycle is deleted, all program assignments return to the default program.

CAUTION This feature can delete amino acids in the original sequence file. Use with caution. If an amino acid is deleted by mistake, use the **Undo** button, or reload the original sequence file to start over.

3. **Undo** – Undoes the most recent cycle modification.

The **Cycle Selection** window allows the user to choose the type of cycle that will be inserted when the **Ins** button is clicked.



The buttons are as follows:

1. **Idle Cycle --** – Inserts an **Idle** cycle (represented by a hyphen “-“) to the right of the selected cycle in the sequence.

2. **No AA **** – Inserts a **No AA** cycle (represented by an asterisk “*”) to the right of the selected cycle in the sequence.

3. **Cancel** – Cancels the insert operation

When an advanced operation is assigned to a sequence,  is displayed to the right of the sequence to alert the user.

The following examples demonstrate possible uses of **Dynamic Sequence Programming**.

Example 1:
Run different programs during cycle 1.

Program Assignment:	D 2 1
RV 1 Sequence:	A – A
RV 2 Sequence:	C C –

RV 1 will couple A with program “1” while RV 2 sits idle. When program “1” is complete, RV 2 will couple C with program “2” while RV 1 sits idle. Both RV’s will then couple their next amino acid with the default program, “D.”

Example 2:
Use different activators in cycle 1.

Program Assignment:	D X 3 2 1
RV 1 Sequence:	A * – * A
RV 2 Sequence:	C * * – C

Program 1: Deprotection, DMF wash, amino acid addition (no drain)
Program 2: Activator addition from bottle #5 (no drain)
Program 3: Activator addition from bottle #6 (no drain)
Program X: Coupling mix, DMF wash

RV’s 1 & 2 run program “1”. RV 2 sits idle while RV 1 runs program “2.” When program “2” is complete, RV 1 sits idle while RV 2 runs program “3.” Finally, both RV’s run program “X” to complete the cycle. Both RV’s will then couple their next amino acid with the default program, “D.”

Example 3:
Couple different modifiers at the end of the synthesis.

Program Assignment:	2 1 D D…
RV 1 Sequence:	– 1 A A…
RV 2 Sequence:	2 – A A…

At the end of the sequence (poly-A), RV 1 couples a modifier from amino acid bottle 1 to peptide 1 using program “1” while RV 2 sits idle. When program “1” is complete, RV 1 sits idle, while RV 2 couples a modifier from amino acid bottle 2 to peptide 2 using program “2.”

3.7 E-Mail Notification

The optional **E-Mail Notification** feature allows ***Prelude X*** to send emails to a specified email address under the following conditions:

1. At the beginning of each synthesis cycle

2. At a specific step of a synthesis program

3. When a user error occurs

This feature may be activated by purchasing a license from GPT. To purchase a license, contact GPT customer service at 1-800-477-6834 or peptides@gyrosproteintech.com.

Catalog No.	Description	Quantity
PPS-TXT-EML-RPT	E-Mail Notification License	1

To use:

1. Login as a supervisor (Section 2.6.2).

2. Open the **Settings** screen (Section 2.6.3) and go to the **E-Mail Options** section.

3. Enter an email address in the **Email Address** box.

4. Select one of the following options:

    a. **On Error** – sends email when ***Prelude X*** goes into error.

    b. **Cycle Progress** – sends email at the beginning of each synthesis cycle.

    c. **On Notified Step** – sends email when the operation **E-mail Notification** is performed as part of a synthesis **Program**.

3.8 Single-Shot™ Delivery

The **Single-Shot** delivery feature is an advanced feature that allows the entire contents of an amino acid bottle to be delivered to a specified reaction vessel. A 10 mL amino acid bottle is available for use with this feature.

Catalog No.	Description	Quantity
AAR-SSI AAR-SSX	Bottle, 10 mL Single-Shot™ AA	1 ea. Pkg. of 10

The functions of this feature are described in detail in Section 2.5.1.2, **Special Bottles**.

3.9 Wash After Error

The **Wash After Error** feature can be enabled using the **Settings** screen (Section 2.6.3). When enabled, ***Prelude X*** will wash all six RVs with Solvent 1 or 2 for up to 9 times if an error occurs. This keeps your reaction safe from unwanted side reactions and their resulting impurities. To enable this safety feature, check the **Enabled** box in the **Settings** screen to the right of **Wash After Error**. Enter a wash solvent in the **Solvent** box (Solvent 1 or 2), and the number of repetitions in the **Reps** box (1-9).

3.10 Pre-activation (PV)

The optional **Pre-Activation** feature allows the user to do a pre-activation reaction on a separate RV prior to the coupling reaction. When available the user can select which operations occur in the PV (RVs 2, 4, and 6 when PV is being used) followed by a PV to RV operation for the transfer of the PV contents to the RV and to come in contact with the resin.

NOTE When using the PV feature make sure to include DMF washes on the PV following the PV operation.

3.11 UV Monitoring

The optional **UV Monitoring** feature allows the monitoring the of the Fmoc deprotection by measuring the absorbance at 301 nm of the Fmoc-adduct as described in detail on 1.1.1.1.1.1.Appendix E.

3.12 Induction Heating

The optional **Induction Heating** feature allows for the independent heating of all RVs in the temperature range of 35-90°C as described on 1.1.1.1.1.1.Appendix D.

Chapter 4 Cleaning & Maintenance

4.1 Cleaning & Maintenance Schedule

Every Synthesis	• **Wash RVs** (Section 2.5.3.7) • **Bottle Position Flush** used bottles (Section 2.5.3.2) • **Collect Back Flush** (Section 2.5.3.5) (Only After Cleave) • **Cleave Bottle Solvent Back Flush** (Section 2.5.3.3) (Only After Cleave)
Every Two Weeks	• **System Clean** (Section 2.5.3.1) • Computer Maintenance (Section 4.2.2)
Quarterly or Semi-Annually	• **Solvent Calibration** (Section 2.5.1.3)
Annually	• Amino Acid Bottle Seal Replacement (Section 4.2.5) • Replacement of RV Top Upper valve block membrane (must be performed by GPT service personnel) • Calibration of UV
As Needed	• Nitrogen Leak Check (Section 4.2.3) • Bottle Filter Replacement (Section 4.2.4) • Amino Acid Bottle Seal Replacement (Section 4.2.5) • Solvent Bottle Seal Replacement (Section 4.2.6)

4.2 Operations

4.2.1 Cleaning Operations

The following cleaning operations are available on ***Prelude X*** and are covered in Section 2.5.3:

1. System Clean
2. Bottle Position Flush
3. Cleave Bottle Solvent Back Flush
4. Cleave Bottle Nitrogen Back Flush
5. Collect Back Flush
6. Rinse All Blocks
7. Clear All Blocks
8. Wash RVs

4.2.2 Computer Maintenance

The computer is an important component in the operation of ***Prelude X*** and should be maintained like any other component. Overloading of the GPT directory can occur if the program, sequence and synthesis files are not removed regularly. Files that are no longer required should be removed quarterly. Database files should be archived (Section 2.6.1.2) monthly, and backups of important files (programs, job files) should be performed on a regular basis. When the database has not been archived a message will prompt the user to do so (see messages below). Lost or truncated clusters can occur if power failures or accidental shutdowns occur while the instrument is operating. Perform a Scandisk check or a Disk Cleanup routine to fix disk problems. Use regular Microsoft® Windows® conventions to maintain the computer and operating system.

4.2.3 Nitrogen Leak Check

It is recommended to routinely check the sealing of all the reagent supply bottles.

NOTE For all of the following tests, use only one nitrogen supply. Do not allow the nitrogen tank gauge to fall below 75 psi during the test, or the bottle positions that are pressurized for the test will be automatically vented.

Test A: Regulator & QC Test

1. Remove the nitrogen quick connect, i.e., no nitrogen line connected to unit.

2. Turn off the nitrogen tank valve.

3. Watch nitrogen tank gauge on tank for drop in pressure within 15 minutes, then turn on nitrogen tank valve.

4. If the gauge on the nitrogen tank regulator drops, there is a leak. If this is the case, check the tank regulator and tank fitting for leaks. Also check the tank regulator outlet fitting and gauges.

5. If the gauge does not drop, there is no leak. If there are no leaks, reconnect the nitrogen quick connect.

Test B: Internal Nitrogen System Test

1. Connect a nitrogen flow meter between the nitrogen tank and the nitrogen inlet to the unit.

2. If the flow is greater than 25 cc/min, there is a leak in the internal nitrogen system. Call the GPT Technical Service Department at 1-800-477-6834.

3. If the flow is less than 25 cc/min, proceed to Test C.

Test C: Solvent System Test

1. Connect the nitrogen flow meter and pressurize all solvent bottles in the Bottle Preparations (Section 2.5.1.1) screen.

2. Allow the system to stabilize for 10-15 minutes.

3. Check the nitrogen flow meter to see if there is any flow.

4. If the flow is greater than 25 cc/min, there is a leak in one of the bottles. If the flow is below 25 cc/min, proceed to Test D.

5. To identify the leaky bottle, start at the Solvent #8 position (or #7 if no Solvent #8) and vent the bottle.

6. Check nitrogen flow meter for flow.

7. If the flow is greater than 25 cc/min, move up one bottle and vent Solvent #7 (or #6).

8. Check nitrogen flow meter for flow.

9. If the flow is still greater than 25 cc/min, continue to the next bottle(s) until the flow is below 25 cc/min.

10. When the flow is below 25 cc/min, the last vented bottle has a leak.

11. Check the bottle cap, insert, and o-ring.

12. Check the supply tubing for cracks or leaks.

13. Pressurize the bottle and re-test. If the flow is still above 25 cc/min, call your GPT Technical Service Department representative at 1-800-477-6834. If the flow is below 25 cc/min, proceed to Test D.

Test D: Amino Acid System Test

1. Make sure all 27 amino acid bottles are in place.

2. Pressurize the first amino acid manifold, and let the system stabilize for 5-10 minutes.

3. Check nitrogen flow meter.

4. If the flow is greater than 25 cc/min, vent the system and examine the amino acid bottle seals for solids, cracking, tears or other damage that would interfere with sealing. Pressurize the amino acid manifold again, let stabilize and check the nitrogen flow meter. If the flow is still greater than 25 cc/min, call your local Technical Service Department representative at 1-800-477-6834.

5. If the flow is less than 25 cc/min, proceed to the next manifold. Repeat steps 2-4 for all three amino acid manifolds. If the flow is less than 25 cc/min, the system check is complete.

4.2.4 Bottle Filter Replacement

The bottle filter should be replaced on a regular basis; the frequency depends upon the quality and concentration of the reagents utilized. Always replace filters for reagents that show any precipitation. If a specific reagent cannot be delivered, replacement of the bottle filter should be the first solution.

The bottle filter consists of a filter housing and a frit. The frit is press fit into the housing. On the other side of the housing is a partially threaded entrance for the tube. To thread the filter housing onto a bottle tube, gently twist the housing clockwise while pushing it onto the tube. Be certain to thread the assembly completely onto the tubing or bubbles may be introduced between the top of the housing and the tubing. The filter assemblies are easily removed by gently twisting counterclockwise while pulling down. To remove the filter frit, either press the frit out with a swab, etc., from the top or lift the frit out with a spatula or dental pick. To replace the frit, put the new frit on a clean, flat surface and press the filter housing firmly over the frit.

CAUTION Always wear protective clothing, safety glasses and gloves when working on the filter assemblies.

Replacement Procedure:

1. From the Bottle Preparations screen (Section 2.5.1.1), select the positions that need replacement filters.

2. Press the Nitrogen Back Flush button to blow out the reagent from the lines and filter housing.

3. When the operation is complete, remove bottle(s) and wipe exterior reagent off the filter assembly.

4. Unscrew the filter assembly from the tube and remove filter frit from filter housing.

5. Clean and rinse housing with desired solvent and dry.

6. Install new frit by pressing housing over frit

7. Screw filter assembly back onto tubing.

IMPORTANT When installing the filter assembly onto the tubing, be sure the tube is threaded into the filter housing as far as it will go to prevent nitrogen bubbles from being introduced when the reagent level goes below the top of the filter housing.

NOTE To expedite the replacement procedure, it is best to have extra filter assemblies. The clean filter assembly can be used and the dirty filter can then be cleaned while the instrument is running.

4.2.5 Amino Acid Bottle Seal Replacement

The amino acid bottle seal should be replaced annually or as needed.

1. To remove, use forceps or tweezers to grab the seal and pull it out of the manifold.

2. To replace, remove filter housing, then put new seal over tube and start by feeding one corner into the manifold using a DULL instrument or fingernail to prevent cutting or tearing the surface of the seal.

3. The seal can then be turned and pushed into the manifold in small increments.

4. The metal backing disk 'floats' and can be pressed upward to allow entry of the seal.

5. Reinstall filter housing and frit (see 4.2.5)

4.2.6 Solvent Bottle Seal Replacement

The solvent bottle seal consists of an encapsulated o-ring seated in a bottle cap insert. The o-ring can be damaged if not handled properly and should be replaced if a nitrogen leak is noted. Extra caution should be taken not to damage the insert when replacing the o-ring. To remove the o-ring, simply lift the o-ring off the insert with your fingertip. The protective gloves will assist in preventing damage to the inserts by cushioning against fingernail damage.

IMPORTANT Never use sharp or pointed objects to remove the o-rings from the inserts. Even small nicks may cause a nitrogen leak. Never use a razor blade or knife to cut off the o-rings.

CAUTION Always wear protective clothing, safety glasses and gloves when working on bottle seals.

Chapter 5 Errors & Recovery

IMPORTANT It is important to manually drain all reaction vessels prior to resuming after an error. When a synthesis is resumed following an error, it will start at the beginning of the step, not where it left off as in a regular pause. Thus, if reaction vessels 1-3 were filled prior to the error, it will start filling at reaction vessel 1, and reaction vessels 1-3 will have been filled twice.

5.1 Common Errors

The following table lists common errors, their cause, and possible corrective actions to take. If the error still persists after all suggested actions have been taken, please contact your GPT Technical Service representative.

Error	Cause	Possible Action(s)
FILL ERROR	RV sensor did not sense fluid during a **Fill** operation	• Check for fluid covering the filter in delivery bottle • If many RVs are pausing frequently on the same solvent/reagent, check, clean or replace bottle supply filter at the source. • Check that the bottle pressure gauge is set to 9 psi • Check bottle seal for improper fit/damage/missing parts that may cause a nitrogen leak • Check for nitrogen leaks using external nitrogen flow meter (Section 5.2.3). • Check for plugs or precipitates and perform **Bottle Position Flush** cleaning operation if required (Section 2.5.3.2). • Check waste line for plugs • If only one RV is pausing frequently on numerous solvents, check RV for clogged frits and clean or replace RV if required • Perform **Wash RVs** to clean the RV lines (Section 2.5.3.8). • RV sensor may require service; contact Technical Service Dept.
CLEAR ERROR	The RV fluid sensor senses fluid after the **Clear** operation	• Check/adjust nitrogen pressure gauge to 5 psi (See Section 1.1.10). • Perform **Wash RVs** to clean RV lines (Section 2.5.3.8). • Perform **Rinse All Blocks** to clear waste valve (Section 2.5.3.6). • RV sensor may require service; contact Technical Service Dept.
NOT PRIMED	Sensor does not sense fluid	• Go to **Bottle Preparations** screen and prime bottle (Section 2.5.1.1). • Try FILL ERROR Possible Action(s)
NO PRESSURE	The bottle required by the program is not pressurized	• Check nitrogen supply pressure gauge • Go to **Bottle Preparations** screen to pressurize (Section 2.5.1.1).
TIME OUT	Operation was not performed in the allotted time	• Press **Start** to continue with RV operations.
RV NOT IN PLACE	An RV is removed or not in place when an operation is initiated.	• Replace RV (Section 1.2.3) and press **Start**.
COLLECTION VIAL NOT IN PLACE	A collection vial is removed or not in place when a cleave or collect operation is initiated	• Replace the required collection vial (Section 1.2.4) and press **Start.**
RV DOORS OPEN	RV door sensor senses an open door.	• Check the RV doors are closed • RV door sensor may require service; contact Service Dept.

5.2 Critical Errors/No Operations Allowed

The following table lists critical errors on Prelude*X*, their cause, and possible corrective actions to take. The following errors will cause Prelude*X* to pause all operations immediately and vent all bottles. If the problem persists after the suggested actions are taken, please contact your GPT Technical Service representative at 1-800-477-6834.

Error	Cause	Possible Action(s)
NO NITROGEN	The nitrogen supply switch in the pneumatic inlet assembly senses < 65 psig from the nitrogen supply system.	• Check nitrogen tanks and regulators • Check quick connect fittings for proper fit and/or leaks
NO VACUUM	Vacuum supply switch senses < 10 in Hg vacuum after pump stops running.	• Attach external vacuum gauge. • Check tube fittings on vacuum pump head for leaks and tightness
WASTE FULL	Waste level sensor indicates the tank is full or not connected to the instrument.	• Empty waste tank and reconnect (Section 1.2.7) • Check/reconnect waste tank connector
NOT COMMUNICATING	No communication between the user and instrument software	• Turn *Prelude®X* power off for 30 seconds then back on
E-STOP ENGAGED	The E-stop button is pressed	• Disengage E-stop button
THERMAL CUT-OFF	A thermal cut-off has occurred	• Ensure that Induction Compatible RVs are being used in all heated positions

5.3 Other Errors

The following errors indicate an internal computer problem:

- NO MODULE
- NO COMMAND STRING
- MODULE NOT IN PLACE
- SYSTEM ERROR 1 **
- SYSTEM ERROR 2 **
- SYSTEM ERROR 3 **
- SYSTEM ERROR 4 **

If the error persists, shutdown then restart the instrument. If this does not fix the problem, contact your GPT Technical Service representative at 1-800-477-6834

Appendix A Reagents For Peptide Synthesis

A.1 Prelude X Pre-Packed N-Fmoc-Protected Amino Acids, Preweighed

Catalog No.	Amino Acid	Quantity
SMP-05-A	Fmoc-L-Ala-OH	5 mmol
SMP-10-A		10 mmol
SMP-20-A		20 mmol
SMP-05-RBF	Fmoc-L-Arg(Pbf)-OH	5 mmol
SMP-10-RBF		10 mmol
SMP-20-RBF		20 mmol
SMP-05-NT	Fmoc-L-Asn(Trt)-OH	5 mmol
SMP-10-NT		10 mmol
SMP-20-NT		20 mmol
SMP-05-DB	Fmoc-L-Asp(OtBu)-OH	5 mmol
SMP-10-DB		10 mmol
SMP-20-DB		20 mmol
SMP-05-CT	Fmoc-L-Cys(Trt)-OH	5 mmol
SMP-10-CT		10 mmol
SMP-20-CT		20 mmol
SMP-05-EB	Fmoc-L-Glu(OtBu)-OH	5 mmol
SMP-10-EB		10 mmol
SMP-20-EB		20 mmol
SMP-05-QT	Fmoc-L-Gln(Trt)-OH	5 mmol
SMP-10-QT		10 mmol
SMP-20-QT		20 mmol
SMP-05-G	Fmoc-Gly-OH	5 mmol
SMP-10-G		10 mmol
SMP-20-G		20 mmol
SMP-05-HT	Fmoc-L-His(Trt)-OH	5 mmol
SMP-10-HT		10 mmol
SMP-20-HT		20 mmol
SMP-05-I	Fmoc-L-Ile-OH	5 mmol
SMP-10-I		10 mmol
SMP-20-I		20 mmol
SMP-05-L	Fmoc-L-Leu-OH	5 mmol
SMP-10-L		10 mmol
SMP-20-L		20 mmol
SMP-05-KBC	Fmoc-L-Lys(Boc)-OH	5 mmol
SMP-10-KBC		10 mmol
SMP-20-KBC		20 mmol
SMP-05-M	Fmoc-L-Met-OH	5 mmol
SMP-10-M		10 mmol
SMP-20-M		20 mmol

Catalog No.	Amino Acid	Quantity
SMP-05-F SMP-10-F SMP-20-F	Fmoc-L-Phe-OH	5 mmol 10 mmol 20 mmol
SMP-05-P SMP-10-P SMP-20-P	Fmoc-L-Pro-OH	5 mmol 10 mmol 20 mmol
SMP-05-SB SMP-10-SB SMP-20-SB	Fmoc-L-Ser(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-TB SMP-10-TB SMP-20-TB	Fmoc-L-Thr(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-WBC SMP-10-WBC SMP-20-WBC	Fmoc-L-Trp(Boc)-OH	5 mmol 10 mmol 20 mmol
SMP-05-YB SMP-10-YB SMP-20-YB	Fmoc-L-Tyr(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-V SMP-10-V SMP-20-V	Fmoc-L-Val-OH	5 mmol 10 mmol 20 mmol

A.2 Bulk N-Fmoc-Protected Amino Acids, Preweighed

Catalog No.	Description	Quantity
FLA-5-A FLA-25-A FLA-100-A FLA-1KG-A	Fmoc-L-Ala-OH	5 g 25 g 100 g 1 kg
FLA-5-RBF FLA-25-RBF FLA-100-RBF FLA-1KG-RBF	Fmoc-L-Arg(Pbf)-OH	5 g 25 g 100 g 1 kg
FLA-5-NT FLA-25-NT FLA-100-NT FLA-1KG-NT	Fmoc-L-Asn(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-DB FLA-25-DB FLA-100-DB FLA-1KG-DB	Fmoc-L-Asp(OtBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-CT FLA-25-CT FLA-100-CT FLA-1KG-CT	Fmoc-L-Cys(Trt)-OH	5 g 25 g 100 g 1 kg

Catalog No.	Description	Quantity
FLA-5-EB FLA-25-EB FLA-100-EB FLA-1KG-EB	Fmoc-L-Glu(OtBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-QT FLA-25-QT FLA-100-QT FLA-1KG-QT	Fmoc-L-Gln(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-G FLA-25-G FLA-100-G FLA-1KG-G	Fmoc-Gly-OH	5 g 25 g 100 g 1 kg
FLA-5-HT FLA-25-HT FLA-100-HT FLA-1KG-HT	Fmoc-L-His(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-I FLA-25-I FLA-100-I FLA-1KG-I	Fmoc-L-Ile-OH	5 g 25 g 100 g 1 kg
FLA-5-L FLA-25-L FLA-100-L FLA-1KG-L	Fmoc-L-Leu-OH	5 g 25 g 100 g 1 kg
FLA-5-KBC FLA-25-KBC FLA-100-KBC FLA-1KG-KBC	Fmoc-L-Lys(Boc)-OH	5 g 25 g 100 g 1 kg
FLA-5-M FLA-25-M FLA-100-M FLA-1KG-M	Fmoc-L-Met-OH	5 g 25 g 100 g 1 kg
FLA-5-F FLA-25-F FLA-100-F FLA-1KG-F	Fmoc-L-Phe-OH	5 g 25 g 100 g 1 kg
FLA-5-P FLA-25-P FLA-100-P FLA-1KG-P	Fmoc-L-Pro-OH	5 g 25 g 100 g 1 kg
FLA-5-SB FLA-25-SB FLA-100-SB FLA-1KG-SB	Fmoc-L-Ser(tBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-TB FLA-25-TB FLA-100-TB FLA-1KG-TB	Fmoc-L-Thr(tBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-WBC FLA-25-WBC FLA-100-WBC FLA-1KG-WBC	Fmoc-L-Trp(Boc)-OH	5 g 25 g 100 g 1 kg

Catalog No.	Description	Quantity
FLA-5-YB FLA-25-YB FLA-100-YB FLA-1KG-YB	Fmoc-L-Tyr(tBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-V FLA-25-V FLA-100-V FLA-1KG-V	Fmoc-L-Val-OH	5 g 25 g 100 g 1 kg

A.3 Reagents & Kits

Please see www.gyrosproteintechnologies.com for all of your peptide synthesis reagent needs. Gyros Protein Technologies offers a wide selection of high-quality coupling reagents, resins, and solvents, as well as pseudoprolines and other specialty reagents that can be found at www.gyrosproteintechnologies.com.

Catalog No.	Start-Up Kits	Quantity
PPX-STARTKIT	Fmoc Amino Acid Start-up Kit for the Prelude X. Contains: 30 x 10 mL disposable RVs, 6 x 10 mL coated glass RVs, 6 x 25 mL coated glass RVs, 6 x 40 mL coated glass RVs, 0.9 L of 20% Piperidine/DMF, 0.9 L of 0.4M NMM, 0.1 mmol scale Rink amide resin, 0.1 mmol scale Fmoc-Gly-Wang resin, twenty 5 mmol and twenty 20 mmol prepacked AA bottles (one of each amino acid), 100 g HCTU. Assorted 5 mmol prepacked AA bottles for running test peptides.	1 ea.
PPX-STARTKIT-I	Fmoc Amino Acid Start-up Kit for the Prelude X. Contains: 30 x 10 mL disposable RVs, 6 x 10 mL coated glass RVs, 6 x 25 mL coated glass RVs, 6 x 40 mL coated glass RVs, 0.1 mmol scale Rink amide resin, 0.1 mmol scale Fmoc-Gly-Wang resin, twenty 5 mmol and twenty 20 mmol prepacked AA bottles (one of each amino acid), 100 g HCTU. Assorted 5 mmol prepacked AA bottles for running test peptides.	1 ea.

Catalog No.	Cleavage Kits	Quantity
CLEAVEKIT-U	**GPT Universal Cleavage Kit**. Suitable for cleaving peptides containing all 20 standard amino acids. Contains 95 mL TFA, 2 mL water, 2 mL anisole, 1 mL EDT. Makes 100 mL	1 ea.
CLEAVEKIT-R	**Reagent K Cleavage Kit.** Suitable for cleaving peptides containing all 20 standard amino acids. Contains 82.5 mL TFA, 5 mL thioanisole, 5 mL water, 5 g phenol, and 2.5 mL EDT. Makes 100 mL	1 ea.

Appendix B Reagent Shelf Life & Handling

CAUTION This instrument contains solvents and chemicals that should be handled carefully. Many are easily absorbed through the skin and can cause adverse health effects. Wear safety glasses, protective clothing and rubber gloves at all times. Follow MSDS handling guidelines provided with the individual reagents. Respirators and adsorbent should be available in the event of a spill.

B.1 Reagent Shelf Life

Proper handling and storage of peptide synthesis reagents is important for the successful performance of your instrument. Please review the table on the following pages to be certain that your reagents are properly stored. Be sure to rotate your stock of reagents using a "first in, first out" method so that their shelf-life is not exceeded before use.

Reagent	Temperature	Shelf Life
Amino Acids [Solid, except Fmoc-Trp(Boc)-OH]	20-25°C	Stable
Fmoc-Trp(Boc)-OH (Solid)	-20°C	Stable
Amino Acids [in DMF solution, except Fmoc-Cys(Trt)-OH]	20-25°C	7-14 Days
Fmoc-Cys(Trt)-OH (in DMF solution)	20-25°C	1 Day
N,N-Dimethylformamide	20-25°C	Stable
Methylene Chloride	20-25°C	Stable
20% Piperidine/DMF (v/v)	20-25°C	Stable
Acetic Anhydride	20-25°C	Stable
0.1M HBTU/0.4M 4-Methylmorpholine in DMF	20-25°C	5-7 Days
Trifluoroacetic Acid	20-25°C	Stable
TFA Cocktail	20-25°C	1 Day

B.2 Amino Acid Solubility Testing

Supplies Required:

- Sealable vials or bottles (2-5 mL volume)
- Amino acids
- Solubilizing solvent (i.e. DMF or NMP)*

Protocol:

Determine the concentration of amino acid to be used on ***Prelude X*** instrument. In the **RV Operations** menu of ***Prelude X*** software, open the **Calculations – AA** screen. Calculate the amount of amino acid to dissolve in 2-5 mL of solvent* to create a solution at that concentration. Prepare the solution and mix or sonicate until powder is fully solubilized. Seal vial and place on a shelf with similar temperature and light conditions as would be experienced on the instrument. Examine the vials daily for discoloration or precipitation and record the data. Amino acids should not be left on the instrument longer than the time it takes for discoloration or precipitation to occur.

*__NOTE__ This test should use the same batch of solvent that will be used on the instrument.

B.3 Amino Acid Degradation Testing

Supplies Required:

- Amino acid vials used for solubility study
- HPLC gradient system with 214 nm detection, water/0.1% TFA and acetonitrile/0.1% TFA mobile phases, and a reverse phase column**
- Optional: autosampler

Protocol:

Inject a small amount of each amino acid over a two-week period to determine if there is any degradation of the amino acid. For example, 10 µL of the amino acid solution diluted to 1 mM at the following time periods: initial, 3 days, 7 days, 10 days, and 14 days. This data will provide you with the stability of the amino acid in the primary solvent.

**Gradient is dependent on column. Typically, a 5% to 75% gradient over 20 minutes is used.

Appendix C Accessories

Catalog No.	Accessories	Quantity
PPS-R10-030 PPS-R10-090 PPS-R10-180	Reaction Vessel, 10 mL PP	Pkg of 30 Pkg. of 90 Pkf of 180
PPS-R45-030 PPS-R45-090 PPS-R45-180	Reaction Vessel, 45 mL PP	Pkg. of 30 Pkg. of 90 Pkg. of 180
PPX-FGRV10-6	Reaction Vessel, 10 mL Coated Glass (required for heat)	Pkg. of 6
PPX-FGRV25-6	Reaction Vessel, 25 mL Coated Glass (required for heat)	Pkg. of 6
PPX-FGRV40-6	Reaction Vessel, 40 mL Coated Glass (required for heat)	Pkg. of 6
AAR-SSI AAR-SSX	Bottle, 10 mL Single-Shot AA	1 ea. Pkg. of 10
SMP-VX-20 SMP-VX-100	Bottle, 120 mL AA	Pkg. of 20 Pkg. of 100
AAR-400-I AAR-400-X	Bottle, 400 mL AA	1 ea. Pkg. of 10
CLV-050-030 CLV-050-090 CLV-050-180	Vial, 50 mL Collection	Pkg. of 30 Pkg. of 90 Pkg. of 180

APPENDIX D Induction Heating System

The ***Prelude*®** ***X*** Peptide Synthesizer can be field-upgraded in your facility to a unit with induction heating and shaking (Cat. #: PPX-IHEAT-OPT & PPX-SHAKE-OPT).

D.1 History of Heat in SPPS

Heat has been used to aid in the syntheses of difficult peptides for the last 30 years. Believed to be first used in 1984 by Janda and colleagues, heating methods range from a simple oil bath, to specially designed heated reaction blocks, to infrared and induction heating today. Below is a brief history of heating methods used in SPPS:

1985 – Tam synthesized TGFα using an oil bath
1986 – Barlos synthesized leucine enkephalin using an oil bath
1991 – Foutch synthesized AT III and other peptides using a recirculating oil bath
1992 – Wang synthesized ACP (65-74) using a domestic microwave
2002 – Erdelyi and Gogoll synthesized TVI and others using a microwave synthesizer
2012 – GPT introduces the first peptide synthesizer to use infrared (IR) heating and synthesizes ACP (65-74), Aib enkephalin, and others on the Tribute®-IR peptide synthesizer
2013 – GPT introduces the Symphony® X Multiplex Peptide Synthesizer available with infrared (IR) heating
2015 – GPT introduces ***Prelude X*** the first peptide synthesizer available with induction heating

D.2 Advantages of the Induction Heating System

The induction heating system from GPT offers extremely fast time to temperature, as well as accurate temperature sensing without overshooting or overcorrecting. Vortex mixing is used to ensure even temperature profiles. With independent control and a compact design, the use of induction heating allows parallel, independent heating of all six reaction vessels.

Instruments from Gyros Protein Technologies are the only rapid heating systems to come with the patented GPT fluidics system giving you maximum up-time, minimum solvent-usage and worry-free operation that lasts for years!

D.3 Induction Heating Parameters

The recommended parameters to use for induction heating on ***Prelude X***. Depending on the RV size and volumes used, it is important that the shaking speed is set to an appropriate value within the RPM range described in the table below. The recommended volume range is the apparent volume with the resin.

RV Size	10 mL	25 mL	40 mL
Volume Range (mL)	3 – 6	6 – 13	13 - 20
Shaker Range (RPM)	350 – 400	300 – 375	350 – 400
Temperature Range (°C)	35 – 90	35 – 90	35 – 90

NOTE Make sure that when using induction heating, the RV has a metal band.

NOTE Please ensure the correct RV size is selected in the settings screen.

D.4 Recommended Use – Tips and Tricks

GPT recommends synthesizing most peptides at room temperature first. The majority of peptides produced worldwide are successfully synthesized at room temperature using conventional methods. When a peptide does not come out well in the first try, it is possible to further optimize the synthesis using more efficient activators, using lower-loaded resins or more hydrophilic solid supports, adding pseudoproline dipeptides or Dmb or Hmb dipeptides, or adding heat (See Sections D.4). In the same way that it does not make sense to use pseudoprolines or more expensive hydrophilic solid supports for every synthesis, it is also not recommended to use heat to synthesize all peptides. In the case of the former, it is a needless expense, in the case of the latter, it is to prevent the acceleration of unwanted side reactions. Heat is best used selectively, ideally just for the specific cycles in which it is needed! Difficult cycles can be identified using the UV monitoring feature on ***Prelude X*** and protocols can be modified accordingly.

Heat works by accelerating reactions. In certain cases, the use of heat promotes various side reactions, and the best results may be obtained by removing the heat altogether. Heating during the coupling of cysteine or histidine can produce unacceptable levels of racemization, and heating during the coupling of arginine

residues may promote gamma-lactam formation. During the synthesis of phosphopeptides, the heater must be turned off during all deprotection reactions once the phosphate group has been incorporated or it will cleave the phosphate group. Care must also be used when synthesizing peptides containing aspartic acid as heat can accelerate aspartimide formation during the deprotection step. Finally, heating during the coupling of amino acids to C-terminal proline residues may accelerate diketopiperazine formation. (See Section D.5)

D.4.1 Starting Protocol

GPT recommends synthesizing all unknown peptide sequences at room temperature, then adding enhancements as needed from there. All reagents listed below are available in our Chemical Catalog. An example starting protocol is:

Resin: Rink amide MBHA resin or preloaded Wang-polystyrene resin (loading ~0.5 mmol/g)

Deprotection: 2 x 5 minutes, 20% piperidine/DMF

Coupling: 2 x 10 minutes, 100 mM AA/100 mM HCTU/200 mM NMM in DMF, 5x excess

Washing: 6 x 30 seconds, DMF

Cleavage: 95:2:2:1 TFA/water/anisole/EDT for 2 hours

Most peptides using the standard 20 amino acids can be made using the above protocol. Additional tools may be used for difficult sequences. GPT recommends using GPT peptide predictor software and/or UV-monitoring to identify difficult cycles. Difficult sequences are caused by aggregation or steric hindrance. Aggregation occurs when hydrophobic side chains clump together causing a single peptide chain to fold in on itself, or neighboring peptide chains to interact with one another, obscuring the reactive group at the end of the growing chains. The presence of highly sterically hindered side chains can also prevent the facile formation of bonds at the end of the growing peptide chain as well. The sections below list different strategies you can use to improve the outcome of difficult cycles.

D.4.2 Low-Loaded Resin

Using a low-loaded resin (< 0.4 mmol/g) can significantly improve purities, presumably by eliminating interchain aggregation.

D.4.3 Coupling reaction

1) **Try increasing the amino acid excess and/or concentration.** Performing the coupling reaction at a higher concentration can significantly improve the coupling efficiency. This can be accomplished simply by increasing the amino acid excess from 5x to 10x, or by decreasing the overall coupling reaction volume (assuming the resin can be sufficiently covered for good mixing).

2) **Try increasing the reaction times, or the number of couplings.** For very sterically hindered amino acids such as Aib, it was found that 4 x 90 minute couplings with HATU were necessary to incorporate them at high efficiency within the sequence (VQ-Aib-Aib-IDYING-OH; 89% final peptide crude purity), although the other amino acids were able to be coupled for just 2 x 1 minute each.

3) **Try dissolving the difficult amino acid in DMSO.** Dissolving hydrophobic, sterically hindered amino acids in DMSO has been found to improve the coupling efficiency in some cases. In the synthesis of ACP (VQAAIDYING-OH), dissolving the final valine in DMSO so its coupling occurred in a 1:1 mixture of DMF/DMSO virtually eliminated the valine deletion peak from the HPLC of the crude peptide.

4) **Try a more efficient activator.** If HCTU was insufficient to do the job, try HATU. In rare cases, using an activator that operates via a different mechanism (i.e. PyBOP or PyClock) can improve results for specific sequences (i.e. C-peptide: H-EAEDLQVGQV ELGGGPGAGS LQPLALEGSL G-OH). GPT offers these high-efficiency coupling reagents and others in our chemical catalog.

5) **Try increasing the temperature.** (See below)

The following four items must be in balance in order to maximize coupling efficiency:

1. Activator Efficiency
2. Reaction Time
3. Temperature
4. Sequence Difficulty

Increasing each of the first three items alone can increase the coupling efficiency. However, increasing all three for an easy sequence may actually allow side reactions to occur, resulting in a lower purity peptide. Therefore, when adding heat, it is important to balance it with the other factors. In general, heat

should be used with lower efficiency activators for short amounts of time (i.e. DIC/HOBt or HBTU for 1-5 minutes at 75°C). If a coupling is extremely difficult (as in the case of Aib or N-methylated amino acids), it may be necessary to use a higher efficiency activator like HATU, and multiple couplings along with elevated temperatures.

D.4.4 Deprotection Reaction

Typically, when a difficult sequence presents itself, it is the coupling reaction that is causing the problem. Occasionally, however, it is possible to improve results by optimizing the deprotection reaction. **Try adding 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to 20% piperidine/DMF.** Prolonged exposure to DBU can promote aspartimide formation in Asp-containing sequences, so in this case reaction times should be decreased to 2 x 30 seconds, or at most 2 x 1 minutes.

D.4.5 Pseudoprolines, Hmb & Dmb Amino Acids and Dipeptides

The addition of a proline can break up aggregation up to 6 residues down the growing peptide chain. Pseudoproline dipeptides, and Hmb and Dmb amino acids and dipeptides are rigid structures that can break up aggregation in a similar way. Strategic placement of such building blocks in a difficult peptide sequence can significantly improve its synthesis, as in the synthesis of h-amylin (1-37): H-KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH_2 (pseudoproline dipeptides underlined). Pseudoproline dipeptides were necessary to obtain the peptide with a usable purity and yield. One limitation is pseudoproline dipeptides must contain a serine or threonine, and can therefore only be used in sequences containing those amino acids. Hmb and Dmb amino acids are less restricted, but are not available for all 20 standard amino acids.

D.5 Side Reactions Accelerated by Heat

1. **Racemization** – Racemization is the partial conversion of a chiral amino acid into its other enantiomeric form. Heat can increase the amount of racemization during the coupling reaction, especially for histidine and cysteine residues. Lowering the temperature to 50°C or below during the coupling of these residues, and the use of coupling methods that do not rely on the presence of base can help to minimize this side reaction.
2. **Aspartimide Formation** – Aspartic acid and the nitrogen of an adjacent residue in a peptide sequence may form an aspartimide in the presence of an acid and/or a base. Once formed, the aspartimide can reopen into various forms. In Fmoc chemistry, the aspartimide can form piperidides when exposed to piperidine in subsequent deprotection steps. Heat can accelerate this side reaction. It is especially prevalent in peptide sequences containing Asp-Gly, Asp-Ala or Asp-Ser. To minimize this side

reaction in aspartimide-prone sequences, HOBt can be added to the piperidine deprotection solution. However, HOBt can interfere with UV-monitoring. If UV-monitoring is being used concurrently with heated protocols, a better solution would be to use an alternative deprotection reagent such as piperazine, or to simply turn the heat off during the deprotection steps after aspartic acid has been incorporated in the sequence. Finally, replacing the amino acid immediately preceding the Asp with the Fmoc-(Hmb)-protected version can also help minimize aspartimide formation.

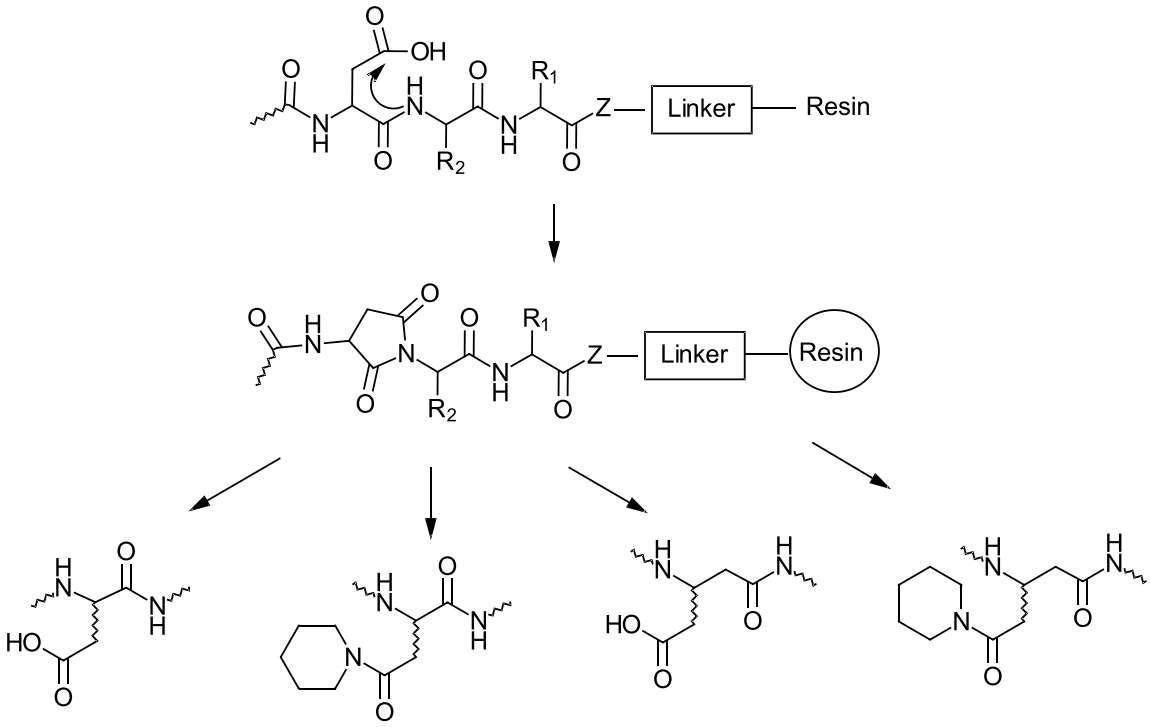


Figure 4. Aspartimide formation products.

3. **Diketopiperazine Formation** – Diketopiperazine formation is an example of cyclative cleavage. In peptide synthesis, peptides containing a C-terminal proline attached to the solid support via a Wang linker can undergo diketopiperazine formation during the addition of the next amino acid. This resulting cyclized product is cleaved from the resin, resulting in lower overall yields for the synthesis. Heat can significantly accelerate this side reaction in peptides containing C-terminal prolines attached to the Wang linker, resulting in very low to negligible yields. It is highly recommended to turn the heat off during the addition of the next amino acid after a C-terminal proline, or to use Pro-2-chlorotrityl resin.

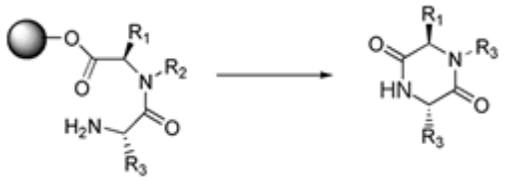


Figure 5. Diketopiperazine formation via cyclative cleavage.

4. **Gamma-Lactam Formation** – Gamma-lactam formation occurs when the activated ester of the incoming Arg amino acid reacts with its own side-chain and forms a ring. This cyclized product is unable to couple to the growing peptide chain, resulting in arginine deletion sequences. Heat accelerates this side reaction, creating higher levels of arginine deletion in sequences prepared with heat. The best way to prevent this side reaction may be to turn the heat off during the arginine coupling step. Double-coupling methods have been reported to aid in the synthesis, however, they include a significant amount of time (25 minutes) at room temperature as part of the first coupling, making it impossible to conclude whether the improved incorporation of Arg was due to the reaction being performed at room temperature before the heat was turned on, or actually double coupling at the higher temperatures.

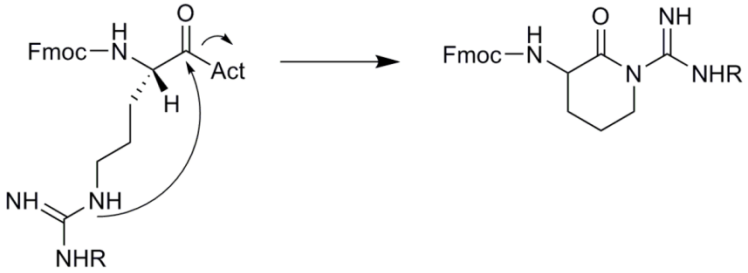


Figure 6. γ-lactam formation.

5. **Phosphate Group Cleavage** – Heat can cleave phosphate groups during the deprotection reaction. Therefore, when synthesizing peptides containing phosphate groups, it is important to perform all deprotection steps (after the phosphate group has been incorporated) with the heat turned off.

APPENDIX E Intellisynth UV Monitoring And UV Extend System

With the UV-monitoring and UV extend control system, it is possible to monitor the extent of the deprotection reaction (Fmoc removal), and use that data to control deprotection times and repeats, and also extend coupling times.

There are 3 main UV-Monitoring operations on the ***Prelude*** X.

1. Basic Monitoring – Qualitatively measures UV absorption of the reaction solution over time to determine the extent of the deprotection reaction, but does not interfere with the synthesis.

2. UV Extend– Measures the UV absorption of the reaction solution to determine the extent of the deprotection reaction and uses that data to control the deprotection reaction times. The deprotection mix time is extended until a specified threshold is met or until the maximum amount of time has elapsed.

3. UV Extend with Repetitions – Measures the extent of the deprotection reaction and uses that data to control the deprotection reaction times and repetitions. First, the deprotection mix time is extended and if reaction is not complete then additional repetitions are done until a specified threshold is met or until the maximum amount of time and repetitions have elapsed.

4. Coupling feedback – when selected in a coupling step, it uses the data from the UV extend to also extend the time for the coupling reaction accordingly.

E.1 How UV Monitoring Works

E.1.1 Chemistry

During the deprotection reaction, piperidine removes the Fmoc group and forms a piperidine-dibenzofulvene adduct with the byproduct (See Figure 7).

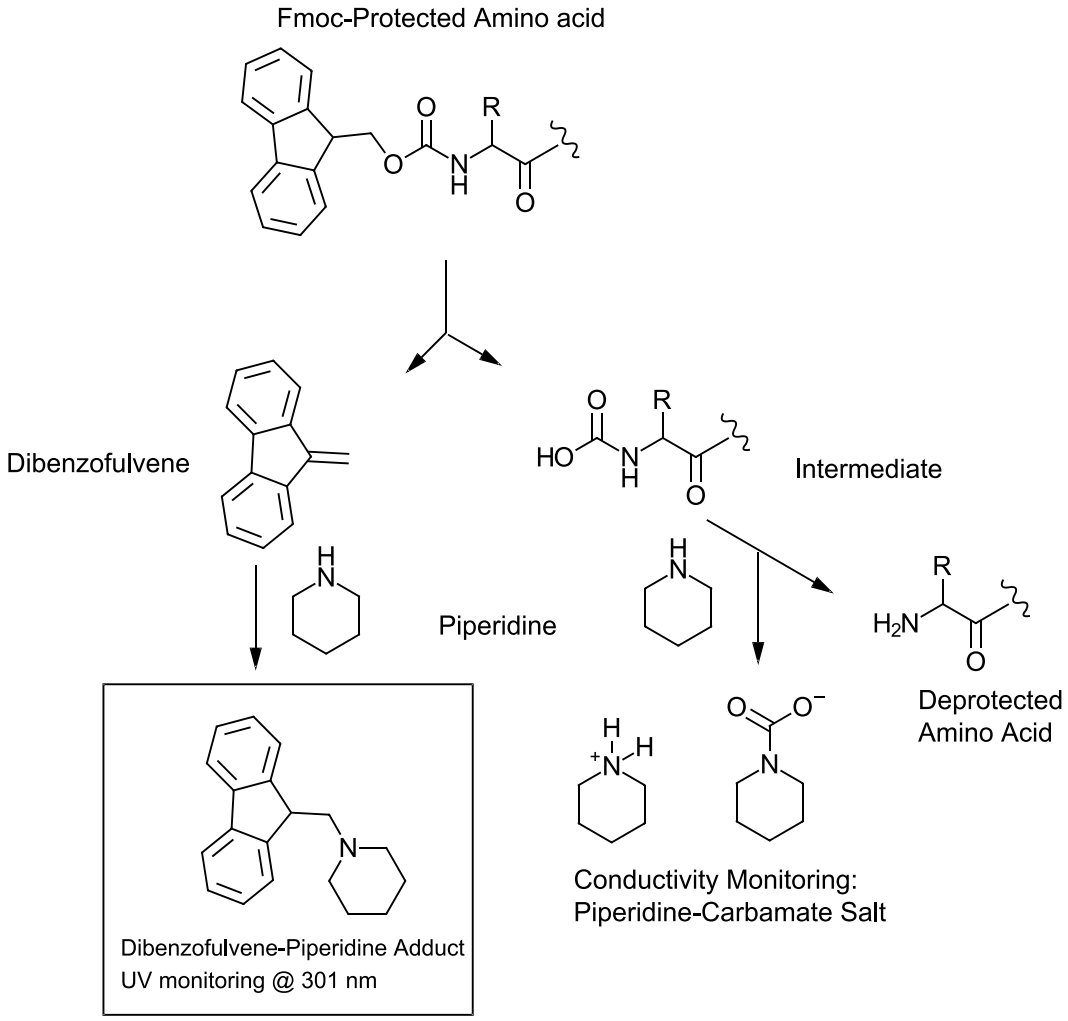
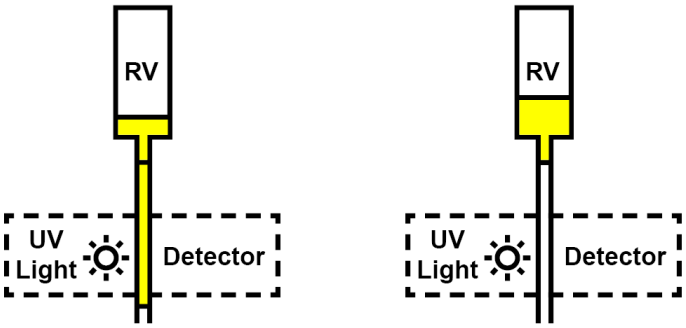


Figure 7. Fmoc- deprotection products. Dibenzofulvene-piperidine adduct is monitored at 301 nm for the extent of deprotection reaction.

The IntelliSynth UV-Monitoring System monitors the absorbance of this adduct at 301 nm during the deprotection reaction.

E.1.2 The IntelliSynth UV Monitoring System



The IntelliSynth UV Monitoring System consists of a light source and detector encased in a 1 3/8” x 1 3/8” x 1 ¾” (3.5 cm x 3.5 cm x 4.5 cm) housing which measures the absorbance/transmittance of the fluid in the tubing directly below the reaction vessel. During a mix, part of the fluid is pushed down into the section of tubing exposed to the light source and detector and a measurement is taken (left diagram). The fluid is then pushed back up into the reaction vessel to resume mixing (right diagram). This process occurs once every 10 seconds during a monitored mix.

E.1.3 Advantages of the IntelliSynth UV Monitoring System

By taking a measurement every 10 seconds during a mix, it is possible to determine when the reaction has stopped progressing. This means unlike other UV monitoring systems on the market, the IntelliSynth system can actually determine the shortest deprotection time required for a step rather than just the number of repeats.

E.2 UV Graph Screen

The **UV Graph** screen displays the UV absorbance/transmittance graphs for individual deprotection reactions as well as overall syntheses. The **UV Graph** screen can be accessed by selecting the **UV Graph** button at the bottom right of the **RV Status** screen or **Manual Operations** screen. UV Graphs generate when UV Monitored steps are included in syntheses which are run in RV Automated Operations.

There are two graph types that can be displayed on the UV Graph screen.

1. UV Summary Graph
2. UV Individual Graph

The buttons at the bottom of the screen are as follows:

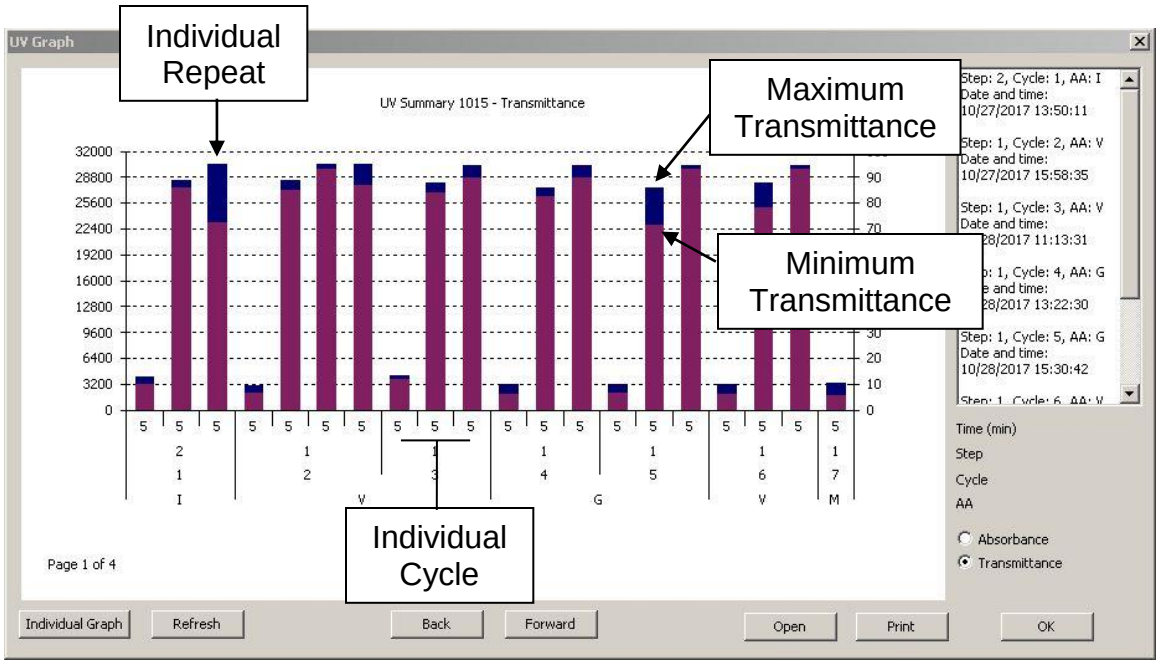
1. Individual Graph / Summary Graph – When viewing a Summary graph, selecting Individual Graph displays UV monitoring data for the Individual Mixes of the latest synthesis. When viewing an Individual graph, selecting Summary Graph displays UV monitoring data for latest synthesis.
2. Refresh -- Refreshes data for current screen.
3. Back – Scrolls the graph to the left when full data set does not fit on screen (Summary Graph) or toggles to previous data screen (Individual Graph)
4. Forward – Scrolls the graph to the right when full data set does not fit on screen (Summary Graph) or toggles to next data screen (Individual Graph)
5. Open – Use to view UV monitoring data from another synthesis.
6. Print – Prints graphs from currently selected synthesis.
7. OK – Use to return to the **RV operations** or **Manual operations** screen.

UV Graph data updates after a UV Monitored step is complete, giving access to view the Summary Graph and Individual Graph data of that step.

E.2.1 Summary Graph

A UV Summary Graph displays a summary of the UV transmittance data for a total synthesis. In a Summary Graph, each peak represents an individual repeat in a UV Monitored step, where the lighter portion represents the minimum transmittance measured during that repeat, and the darker portion represents the maximum transmittance measured during that repeat. Below each cycle are the following labels:

1. Time – Time for UV Monitored step to complete (in minutes)
2. Step – Program step number for monitored step
3. Cycle – Cycle number
4. AA -- One letter code of the AA coupled that cycle

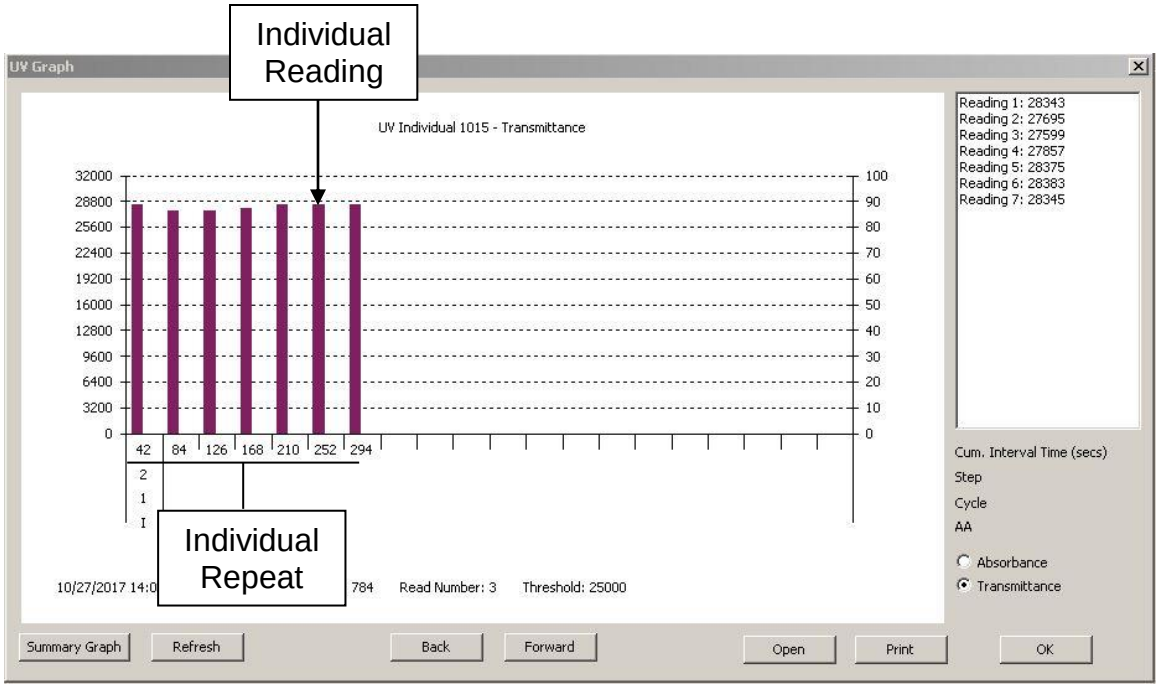


E.2.2 Individual Read Graph

An Individual Read Graph displays UV data for Individual Repeats of UV Monitored steps. A peak is recorded once every 10 seconds when a UV reading is taken.

In an Individual Read graph, each peak represents an individual transmittance reading. Individual readings are shown for each UV Monitored step. Below each cycle are the following labels:

1. Step – Program step number
2. Cycle – Cycle number
3. AA – One letter code of the AA coupled that cycle



E.3 Basic Monitoring Mode

E.3.1 Overview

Basic monitoring takes absorbance/transmittance readings every 10 seconds during the UV Monitored step, but it does not interfere with the synthesis. You can get two types of graphs from this data:

1. UV Summary Graph
2. UV Individual Graph

E.3.2 Writing a Program

To use basic monitoring during a deprotection step, select “Basic” in the UVD drop-down menu for that step.

NOTE The recommended minimum volumes for UV operations are 3000 uL for 10 mL RVs, 4000 uL for 25 mL RVs and 5000 uL for 45 mL RVs.

Example Program

An example program for synthesis using *in situ* couplings and basic UV monitoring is shown below. These programs will produce a UV graph of the deprotection reactions for the synthesis without interfering with the synthesis.

Example UV Monitored Synthesis Program

Step	Operation	Volume	Mix Time	N2	Shake	RPM	Heat	Drain	RV/PV	UVD	Reps
1	Deprotection	3000	0:02:30	X				X	RV	Basic	2
2	DMF Top Wash	3000	0:00:30	X				X	RV		3
3	AA Building Block	1000	0:00:00	X					RV		1
4	Activator 1	1000	0:00:00	X					RV		1
5	Base	1000	0:10:00	X				X	RV		1
6	Top Delivery	3000	0:00:30	X				X	RV		1
7	AA Building Block	1000	0:00:00	X					RV		1
8	Activator 1	1000	0:00:00	X					RV		1
9	Base	1000	0:10:00	X				X	RV		1
10	Top Delivery	3000	0:00:30	X				X	RV		3

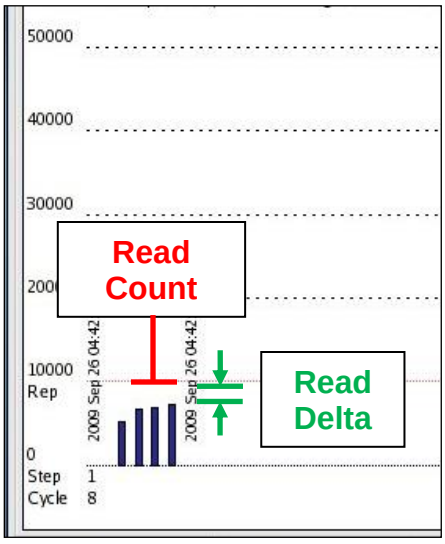
E.4 Deprotection with UV Extend Operations and Extend with Repetitions

E.4.1 Overview

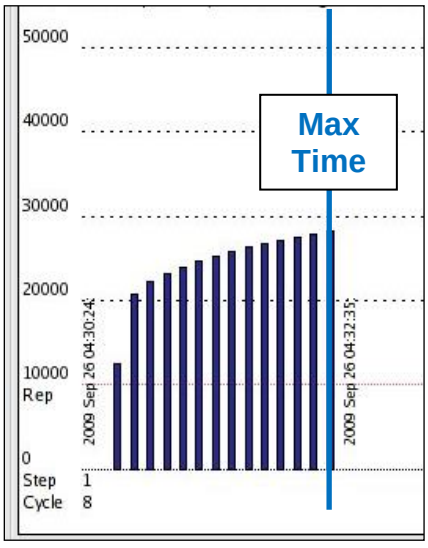
This mode is the same as the Basic Monitoring mode, however the data is used to extend the deprotection time and number of repetitions without affecting any other steps in the cycle. The UV Extend Operations alter the deprotection reaction time in two ways.

1. **XTend** – The time of an Individual Repeat can lengthen depending on the observed UV absorbance/transmittance in an Individual Read Graph. If the absorbance/transmittance changes from one Individual Reading to another above a set threshold another Individual Read will be added to the repeat. If the absorbance/transmittance changes less than the threshold for a set number of reads, the repeat will end. The repeat will end automatically if it has run for the maximum amount time, which is a repeat of the set operation time.

A repeat will end when either condition is met:



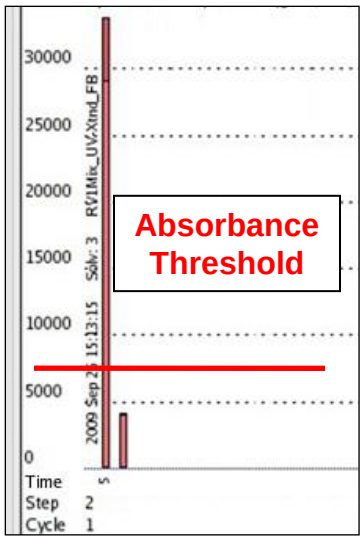
(1) The number of consecutive absorbance readings that fall within the Reaction Read Delta value of each other is equal to the Reaction Read Count.



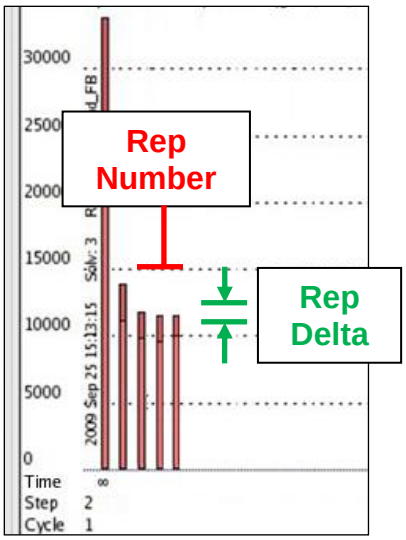
(2) The Maximum Repetition Time is reached.

2. **XTendRep** – The number of repeats can increase depending on the observed UV absorbance/transmittance in a Synthesis Graph. After a minimum number of repeats is completed, the instrument checks if the absorbance/transmittance is above a set absorbance/transmittance threshold. If the absorbance/transmittance is above the threshold, another repeat is added, and if the absorbance/transmittance is below the threshold the operation will end. The operation will end automatically if it has run for a maximum number of repetitions, which can be set by the user.

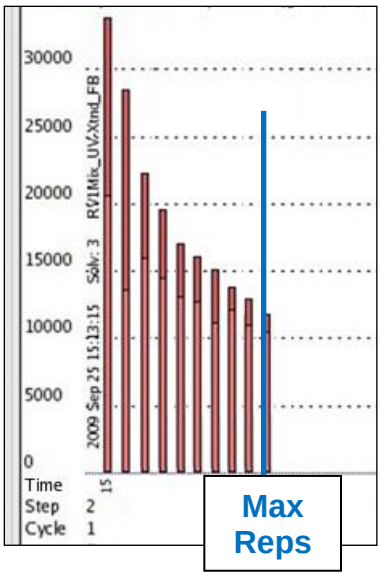
The operation will stop repeating when any condition is met:



(1) The peak height is below the Absorbance Threshold.



(2) The number of consecutive Individual Repeats that fall below the Absorbance Threshold is equal to the Repeat Threshold Count.



(3) The maximum number of repetitions is reached.

E.4.2 Writing a Program

To use UV Extend operations during a deprotection step, select “Xtend” or “XtendRep” in the UVD drop-down menu for that step.

NOTE The recommended minimum volumes for UV operations are 3000uL for 10mL RVs, 4000 uL for 25 mL RVs and 5000uL for 45mL RVs.

WARNING Make sure that extra DMF and deprotection solution has been added since if more repetitions are needed the system will do a DMF wash and add more deprotect solution.

Example Program

An example program using UV Extend Operations is shown below. These programs will produce a UV graph of the deprotection reactions for the synthesis. They will control the deprotection reaction times and repetitions. The coupling reaction is unaffected.

Example UV Deprotect Extend Synthesis Program (*In Situ*)

Step	Operation	Volume	Mix Time	N2	Shake	RPM	Heat	Drain	RV/PV	UVD	Reps
1	Deprotection	3000	0:00:30	X				X	RV	Xtend	1
2	DMF Top Wash	3000	0:00:30	X				X	RV		3
3	AA Building Block	1000	0:00:00	X					RV		1
4	Activator 1	1000	0:00:00	X					RV		1
5	Base	1000	0:10:00	X				X	RV		1
6	Top Delivery	3000	0:00:30	X				X	RV		1
7	AA Building Block	1000	0:00:00	X					RV		1
8	Activator 1	1000	0:00:00	X					RV		1
9	Base	1000	0:10:00	X				X	RV		1
10	Top Delivery	3000	0:00:30	X				X	RV		3

E.5 Deprotection and Coupling with UV Extend Operations

E.5.1 Overview

This mode is the same as the Deprotection with UV Extend Operations, however the UV data from the deprotection step is also used to extend the coupling time in the cycle.

If the deprotection time was extended the original coupling time is multiplied by the Coupling Multiplier (default setting of 2) to obtain the “new” coupling time.

E.5.2 Writing a Program

To use UV Extend and Feedback control operations during a deprotection and coupling step, select “Xtend” in the UVD drop-down menu for the deprotection step and “Use Fb” from the UVD drop-down menu during the coupling step.

NOTE The recommended minimum volumes for UV operations are 3000 uL for 10 mL RVs, 4000 uL for 25 mL RVs and 5000uL for 45 mL RVs.

NOTE If Use Fb is included in a program, in order for its reaction time to calculate correctly, it must be included in a program after an Xtend step.

Example Program

An example program for the Deprotection and Coupling with UV Extend Operations is shown below. These programs will produce a UV graph of the deprotection reactions for the synthesis. They will control the deprotection reaction times and repetitions, and extend the coupling time based on the total deprotection reaction time (does not include wash time between repeats)

Example UV Synthesis Extend Program (*in situ*)

Step	Operation	Volume	Mix Time	N2	Shake	RPM	Heat	Drain	RV/PV	UVD	Reps
1	Deprotection	3000	0:00:30	X				X	RV	Xtend	1
2	DMF Top Wash	3000	0:00:30	X				X	RV		3
3	AA Building Block	1000	0:00:00	X					RV		1
4	Activator 1	1000	0:00:00	X					RV		1

5	Base	1000	0:10:00	X				X	RV	Use Fb	1
6	Top Delivery	3000	0:00:30	X				X	RV		1
7	AA Building Block	1000	0:00:00	X					RV		1
8	Activator 1	1000	0:00:00	X					RV		1
9	Base	1000	0:10:00	X				X	RV	Use Fb	1
10	Top Delivery	3000	0:00:30	X				X	RV		3

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