Manual

sbeadex Lightning DNA purification protocol for the Hamilton STAR

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BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

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Contents

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1. Introduction

sbeadex™ Lightning chemistry offers the speed and simplicity of crude extractions combined with the high yields and purity of classical nucleic acid purification technologies including the original sbeadex workflow. sbeadex Lightning uses superparamagnetic particles and a novel one-step binding mechanism to bind and purify nucleic acids. Combined with a single water washing step, this unique process removes impurities and potential inhibitors of enzymatic reactions very effectively, preparing nucleic acids for use in downstream applications including PCR and RT-PCR, sequencing, NGS and restriction analysis. The very simple purification workflow involves only 3 steps and typically requires just 5 minutes from lysate to nucleic acid eluate while delivering high yields of pure nucleic acid.

Alongside time saving benefits, sbeadex Lightning has further benefits that make it very cost-effective, sustainable and eco-friendly. The chemistry utilises biodegradable detergents and, by avoiding chaotropes and chaotropic salts, contains fewer hazardous chemicals than conventional purification kits. In addition, it offers reduced volumes of both plastic and liquid waste.

sbeadex Lightning chemistry has been designed for automation. Here we provide an overview of how to use the Hamilton STAR™ Lightning protocol along with example protocols for purification of nucleic acids from common plant and livestock tissue types. The protocol can be user adjusted using the protocol optimisation panel to meet requirements for specific sample types and methods. A list of required equipment and reagents to perform this protocol is provided in section 3.

2. Protocol setup overview and general notes

Storage: store reagents as directed in table 1.

Safety: Ensure that waste collection and removal is in compliance with your local law. Ensure that the correct PPE is worn while handling and disposing of reagents.

Protocol: The user should be competent to operate a Hamilton STAR instrument and perform tasks including defining and setting up labware and containers and performing liquid class and volume delivery setup with the instrument. Please seek advice from Hamilton before running the script on your machine as height settings and correct plasticware are critical to success of the protocol; failure to adjust them correctly can cause damage to the instrument. The sbeadex_Lightning_Hamilton_Template.pkg file contains the instrument files used for this protocol.

A user will need to:

- Verify deck positions and plate definitions: heights and plateware deck positions will change between instruments and should be verified and adjusted if required.
- Verify liquid calibration curve using reagents such as lysis buffers, water, and elution buffer. Different lysis buffers and sample types can drastically change the liquid properties. Optimisation and verification of liquid classes should be the first point of an optimisation when setting up a new protocol.

3. Materials

3.1 Materials and Hamilton deck equipment supplied by the user.

Materials or equipment needed	Vendor	Required
Hamilton STAR base system	Hamilton Part Number: 807101	Yes
Hamilton CO-RE II 1000 µL tips × 3	Hamilton Part Number: 235979	Yes
Hamilton Modular Arm × 1	Hamilton Part Number: 173051	Yes
Hamilton WASTE MPH L FRONT × 1	Hamilton Part Number: 188125	Yes
Hamilton CO-RE GRIPPER 1000 µL on Waste Block × 1	Hamilton Part Number: 184089	Yes
Hamilton MPH 96 TADM, 1000 µl, CO-RE II, STAR × 1	Hamilton Part Number: 10120001	Yes
Hamilton Heater Shaker, flat bottom adapter (APE) × 2	Hamilton Part Number: 188319	Yes
Hamilton MFX Gravity Waste Module	Hamilton Part Number: 10102492	Yes
Alpaqua Magnum FLX A000400	Hamilton Part Number: 10103443	Yes
Hamilton MULTIFLEX CARRIER BASE × 1	Hamilton Part Number: 188039	Yes
Hamilton MultiFlex Deepwell position (XXL) × 1	Hamilton Part Number: 188293	Yes
Hamilton Custom Milled Shaking Block Adapter for AB-0932	Hamilton Part Number: 10120083	Yes
ThermoFisher – 2.2 mL 96 square well block × 2	AB-0932	Yes
Hamilton Customer Milled Shaking Block Adapter for AB-0765	Hamilton Part Number: 10120083	Yes
ThermoFisher – 800 µL 96 well round deep well block × 1	AB-0765	Yes
Axygen – DNAse and RNase Free Reservoir	Axygen: RES-SW96-HP-SI	Yes
Axygen – DNAse and RNAse Free 96 well PCR Microplate	Axygen: PCR-96-FS-C	Yes
Centrifuge – SBS plate swing out rotor capable of 2700 × g	Customer supplied	

Table 1. Hamilton STAR required modules for supplied sbeadex Lightning protocol. For liquid blocks, a shaking plate adapter will need to be used.

3.2 sbeadex Lightning reagents

Tables 2 and 3 detail the suggested reagents required to perform sbeadex Lightning purification using the Hamilton Star. Sufficient reagents will be required for multiple 96-well blocks. Ultrapure water at p.H. ≤6.5 is required for washing.

	Core Kit A			Core Kit B			
	100 purifications	1,000 purifications	10,000 purifications	100 purifications	1,000 purifications	10,000 purifications	
Component	NAP40-030-01	NAP40-030-02	NAP40-030-03	NAP40-031-01	NAP40-031-02	NAP40-031-03	**Storage conditions**
Binding buffer LP	22 mL	220 mL	2200 mL	-	-	-	Room temperature
Binding buffer LU	-	-	-	22 mL	220 mL	2200 mL	Room temperature
sbeadex particle suspension	2.2 mL	22 mL	220 mL	2.2 mL	22	220 mL	Room temperature
Elution buffer AMP	11 mL	110 mL	1100 mL	11 mL	110	1100 mL	Room temperature

Table 2. Components supplied in the sbeadex Lightning core kits.

Component	Part codes	Volume	Storage conditions
Lysis buffer PN	NAP10-006-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer PVP	NAP10-010-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer UR	NAP10-004-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer BL	NAP10-002-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer H	NAP10-003-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer LI	NAP10-005-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Protease K solution	NAP30-002-01/...-02/...-03/...-04/...- 05/...-06	1-1,000 mL	Room temperature
RNase A solution	NAP30-003-01/...-02/...-03	1-100 mL	4 °C
Debris capture beads	NAP20-005-00/...-01/...-02/...-03/...-04/...- 05	1-1,000 mL	Room temperature
Ultra pure water	Customer supplied	-	Room temperature

Table 3. Lysis buffers and supplements for sbeadex Lightning (purchased separately).

4. Protocol overview

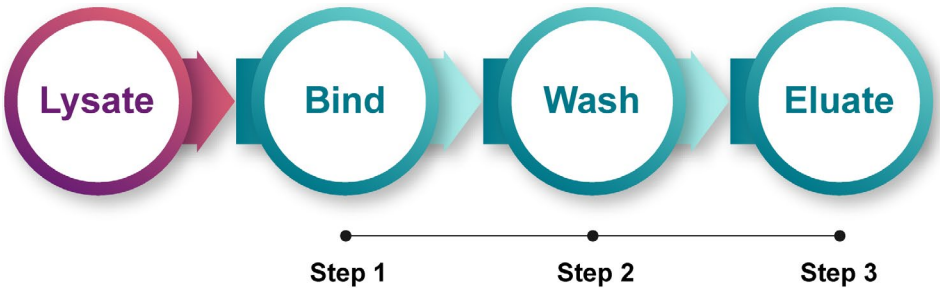


Figure 1. Schematic of the key steps in sbeadex Lightning nucleic acid purification.

Following the off-deck lysis step, the three key protocol steps for performing sbeadex Lightning nucleic acid purification using the Hamilton STAR instrument are followed as shown in figure 1.

Step 1: The lysis plate is then transferred on deck along with all reagents, troughs and load tips. The appropriate binding buffer containing sbeadex particles is added and binds the DNA within 1 minute.

Step 2: Supernatant is removed whilst samples are on a magnet, and nucleic acids are washed using ultra pure water.

Step 3: Water is removed whilst samples are on a magnet, and elution buffer is added to eluate nucleic acids from the beads. Purified nucleic acids are transferred to the final destination plate ready for use.

5. Instrument setup

The images below detail various aspects of the Hamilton STAR instrument set up for performing the sbeadex Lightning nucleic acid purification protocol.

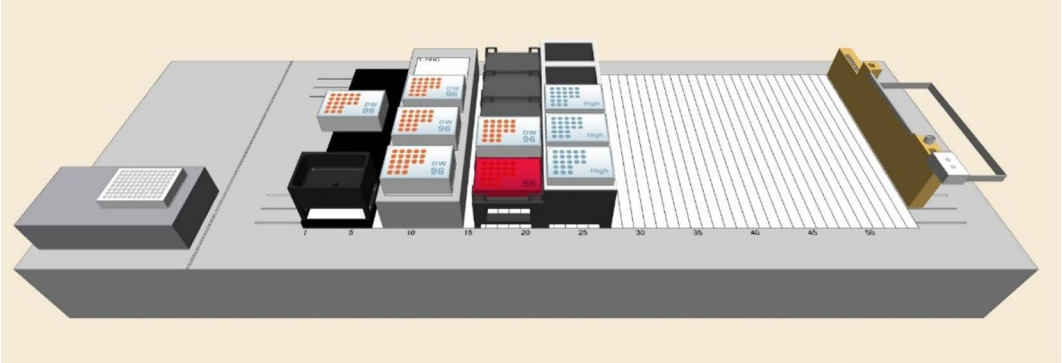


Figure 2. Hamilton STAR deck layout.

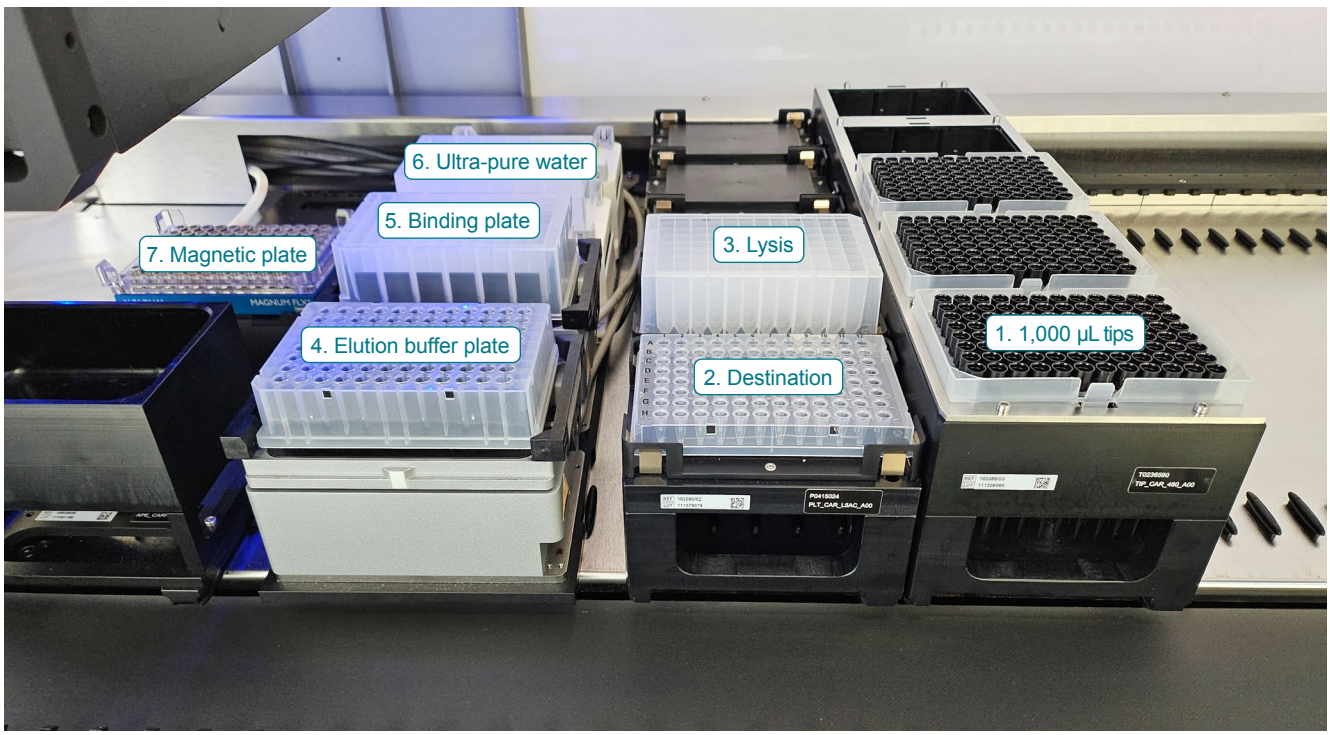


Figure 3. Hamilton STAR deck layout view occupied with plasticware and ready to run the sbeadex Lightning protocol. See tables 1 and 2 for equipment and reagents required to complete the protocol.

5.1 The Protocol Optimisation Menu (POM)

Figure 4 shows the POM for the sbeadex Lightning protocol on the Hamilton STAR.

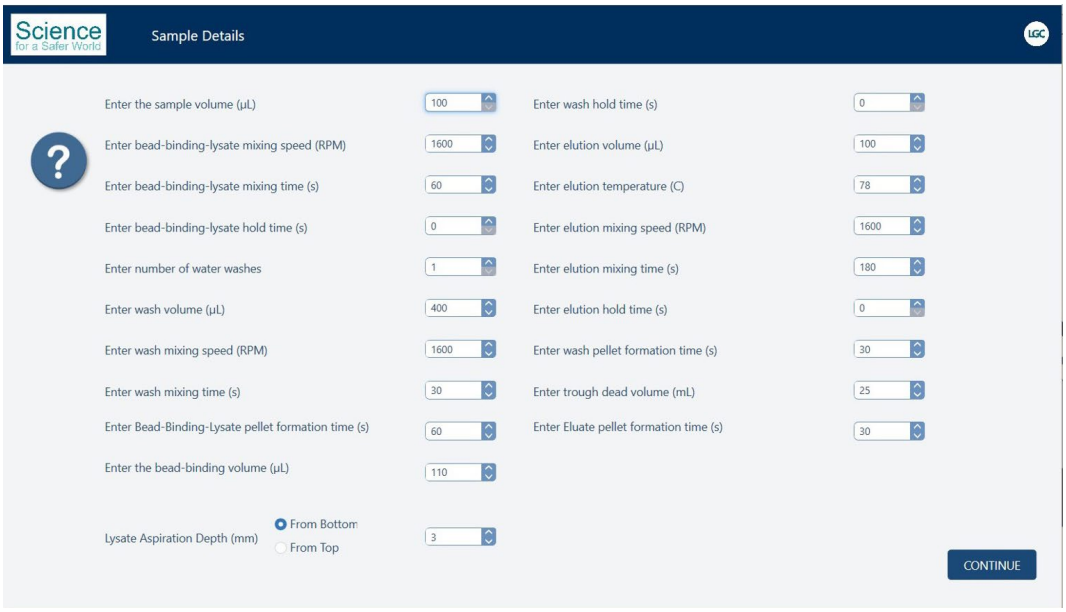


Figure 4. Overview of the Protocol Optimisation Menu (POM) for the sbeadex Lightning protocol. The POM allows for optimisation to be carried out without modification of the main script. Parameters provided are for a standard protocol but can be adjusted as required to suit your samples.

See section 8 for details on how to save a modified script once you are happy with any adjustments.

Details of POM parameters:

- Mixing time and mixing speeds: this refers to how long (seconds) mixing is required and at what speed mixing should take place (rpm). Mixing speed is capped at 1600 rpm; this is suitable for a maximum volume of 420 µL and can be lowered as required.
- Lysate, binding, washing, elution volumes: this allows control of delivery volumes (µL) for each stage of the protocol. When adjusting volumes, it is recommended not to exceed 420 µL or to reduce mixing speed.
- Pellet formation and hold time: this refers to the duration (seconds) allowed for the beads to form a pellet on the magnet prior to removal of supernatant and the duration (seconds) to allow beads to settle after mixing.
- Number of water washes: this refers to the total number of water washes performed post-binding. The recommended number is one, but this can be increased which will result in the water wash step being repeated the specified number of times using the same tips.

6. Before you start

Before commencing the sbeadex Lightning protocol on the Hamilton STAR, please read and follow the guidelines below.

6.1 General guidelines

- Always wear gloves when handling sbeadex Lightning reagents.
- Ensure reagent troughs are filled as specified by the script's load instructions and place on deck directly prior to beginning a run. Do not leave sbeadex Lightning reagents on deck and uncovered for longer than 1 hour.
- Ensure all robotic lab equipment is functional and in the same positions, as indicated in the sbeadex_Lightning_Hamilton_Template.lay file, prior to starting the protocol.
- Ensure that you have taught and verified all the deck positions and are sure they match your instrument. It is recommended to teach labware positions to the bottom of each container. Any liquid handling steps that require position changes can be adjusted within the liquid handling step using the method editor.
- Ensure that you have verified the liquid volume delivery for each liquid type used in the protocol. This is especially important between different sample types and lysis conditions. Each sample-specific protocol may require adjustment to ensure correct delivery.
- Ensure that waste capture for tips and for liquid wastes are in place and functional prior to starting.

6.2 Hamilton-specific guidelines

1. Import the Hamilton_Lightning.pkg files into Hamilton Run Control software. This will unpackage the method components. Ensure that your system has no duplicate names for plasticwares or liquid classes.
2. Ensure that you use the correct plasticware as indicated in table 1. If using alternatives, avoid plasticware with internal shelves or ridges which can trap reagents and prevent proper mixing and pellet formation.
3. Complete lysate preparation. Lysate volumes can be between 200 and 1600 µL.
4. The elution plate (ABGene 800 µL plate) requires an additional 30 µL of elution buffer as dead volume. A minimum suggested elution volume is 50 µL and the standard elution volume is 100 µL. Ensure that elution buffer is pre-heated to 65 °C.
5. Load three sets of 1000 µL filtered CORE-II 96 well tips to positions as indicated in figure 3.
6. Place a labelled, clean 96-well PCR plate at the destination position.
7. Fill the water trough (Axygen – SW96 – HP - SI) with ultrapure water. The minimum volume required is displayed on the load instruction. From the POM screen, the volume required can be adjusted by decreasing or increasing the dead volume in trough. The standard dead volume is set at 10 mL which requires 50 mL of ultrapure water. Load this trough into the position indicated in figure 3.
8. Binding plates require pre-filling with either Binding Buffer LP or Binding Buffer LU (sample dependent) and the required quantity of beads prior to loading plates onto the instrument. Do not leave beads and binding solution uncovered for longer than 1 hour.

7. Example protocols

7.1 Step-by-step DNA purification method, example 1: maize (corn) seed flour.

7.1.1. Prepare lysates: In each well, place 35 mg (+/-5 mg) of ground maize seed flour. To each sample add 500 µL of Lysis buffer LI, 1 µL of Protease K solution and 2 µL of Debris Capture Beads. Incubate at 65 °C for 30 minutes with constant shaking.

7.1.2. Centrifuge the lysis plate to pellet all materials at 2700 × g for 10 minutes.

7.1.3. Carefully remove the lysis plate from the centrifuge, avoid disturbing the pellet and immediately place in the correct position the Hamilton STAR.

7.1.4. Fill each well of an empty 2.2 mL well plate, binding plate (AB – 0932 2.2 mL) with 200 µL of Binding Buffer LU and 20 µL of sbeadex particles (without EDTA). Load this at position 5 as indicated in figure 3. Load the lysate plates containing the lysis materials at position 3. The instrument will transfer lysate to the binding plate during the protocol.

7.1.5. Load the Hamilton STAR instrument run control software and open the sbeadex_Lightning_ Hamilton_Template.med file.

7.1.6. Start the protocol by clicking on the play button.

7.1.7. Once the script begins, adjust the POM to the following conditions: set lysate aspiration mode to ‘Top’ to avoid sample debris at the bottom of the well. Set the depth to 3 mm below the surface of the liquid, as sensed with cLLD (see figure 5).

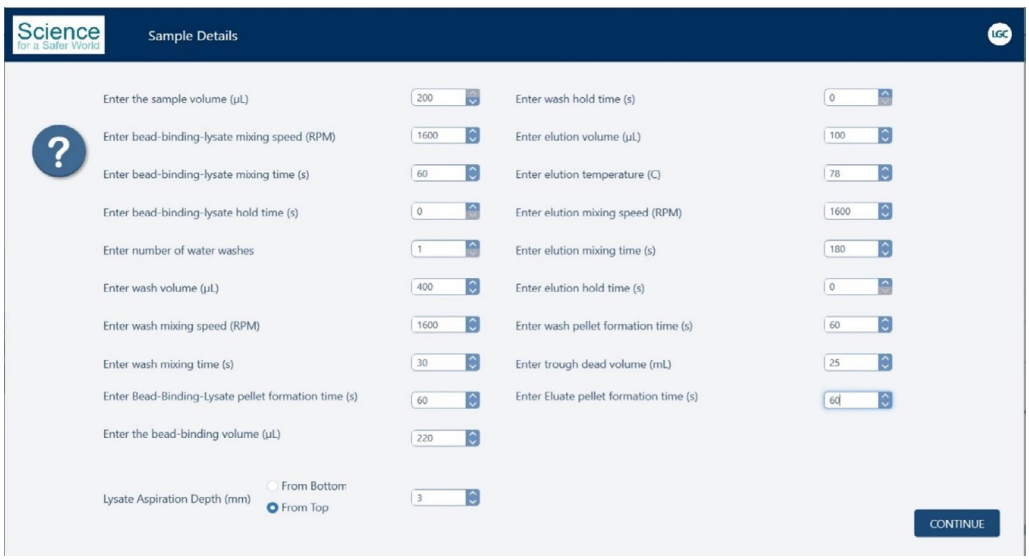


Figure 5. POM settings for purification from maize seed flour.

7.1.8. The protocol will ask for confirmation that all consumables have been loaded in the correct positions. Confirm that this is correct once the instrument is loaded correctly.

7.1.9. The protocol will run for approximately 17 minutes, depending on the modifications that are made.

7.1.10. Once the instrument has completed the protocol, the Hamilton Control Software will indicate 'Instrument Idle'; at this stage it is safe to open the main door of the instrument to recover your purified DNA.

7.2 Step-by-step DNA purification method, example 2; trout muscle biopsy.

7.2.1. Carefully transfer 50 mg of trout muscle biopsy to each well of a 2.2 mL (AB-0932) plate. To each sample add 300 µL of Lysis buffer Li and 20 µL of Protease K solution.

7.2.2. Vortex the solution at 1600 rpm for 5 minutes. Following vortexing, centrifuge at 800 × g for 2 minutes to collect all materials into the lysis solution.

7.2.3. Incubate the solution for 16 – 24 hours at 65 °C. This incubation should either have constant shaking at 400 rpm OR samples should be vortexed for 5 minutes every hour for the first 4 hours of incubation time.

7.2.4. Centrifuge the lysate for 10 minutes at 2700 × g prior to loading the lysate plate onto the Hamilton STAR instrument.

7.2.5. Pre-fill a binding plate (AB-0932) with 110 µL of Binding buffer LU and 10 µL of sbeadex beads (no EDTA).

7.2.6. Load the Hamilton Run Control software and open the sbeadex_Lightning_Hamilton_Template.med file.

7.2.7. Once the script begins, adjust the POM as follows; set lysis aspiration height to 3 mm from the top of the liquid height (figure 6). This can be adjusted to avoid a layer of fat that can form with this type of tissue.

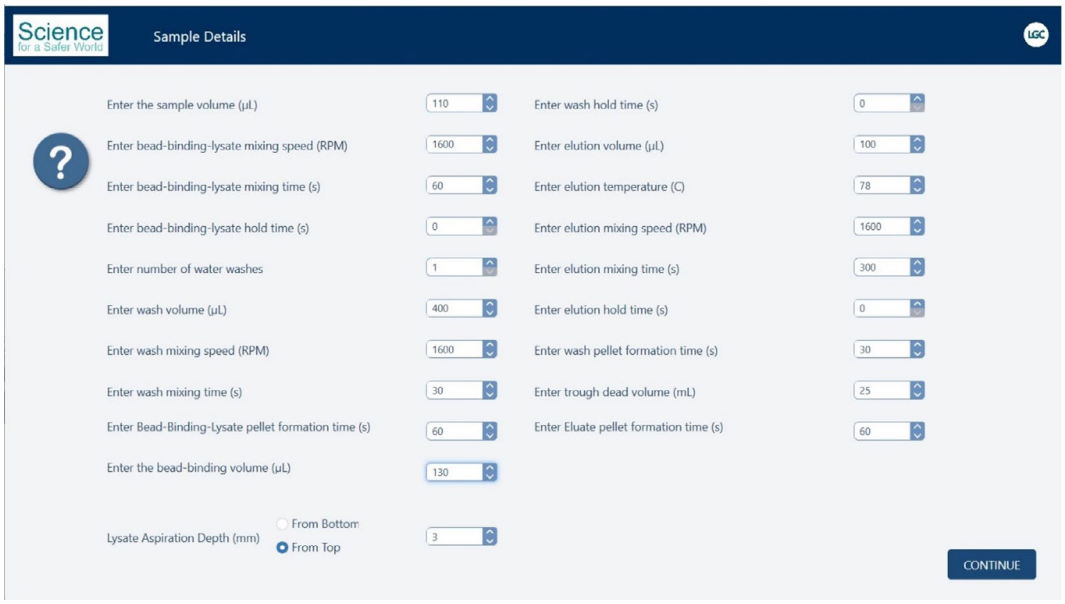


Figure 6. POM settings for purification from trout muscle biopsy.

7.2.8. The protocol will run for approximately 17 minutes.

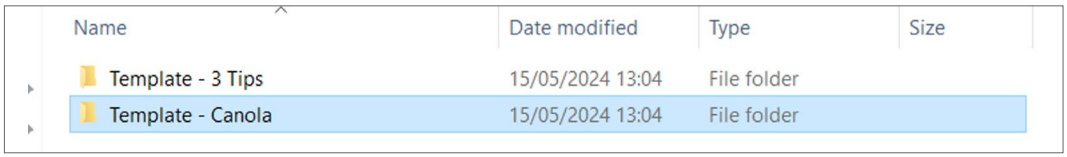
7.2.9. Once the instrument has completed the protocol, the Hamilton Control Software will indicate 'Instrument Idle'; at this stage it is safe to open the main door of the instrument to recover your purified DNA.

8. Adjustment of script to save final conditions.

8.1 Default parameters can be updated for a given protocol and saved. This does not replace correct liquid class and setup of plasticwares and containers; these should be performed and optimised per system before protocol optimisation is performed.

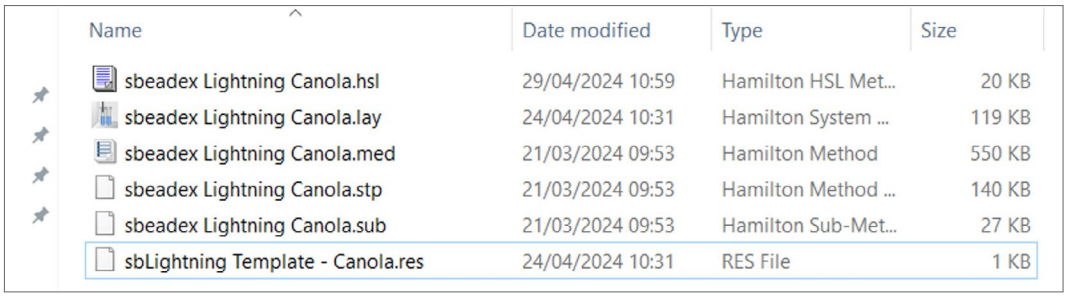
8.2 Copy the contents of the folder named sbeadex_Lightning_Hamilton_Template to a new folder location.

8.3 Rename the folder to a protocol name that is different to the original. You will also need to rename each of the files contained within the folder as per the example below where a copy has been renamed to canola. Once the copy folder is made, each file in turn should be updated and renamed so the .hsl, .lay, .med. .stp, .sub, .res files have a matching file name.



Name	Date modified	Type	Size
Template - 3 Tips	15/05/2024 13:04	File folder	
Template - Canola	15/05/2024 13:04	File folder	

In this screenshot, the 'Template – 3 tips' folder has been copied and the copy renamed to 'Template – Canola'.

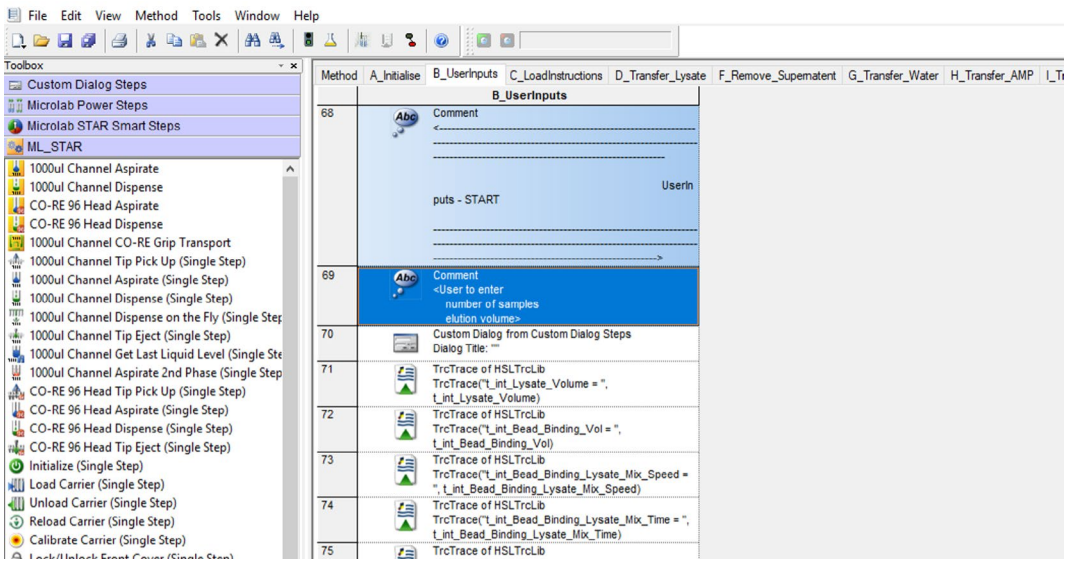


Name	Date modified	Type	Size
sbeadex Lightning Canola.hsl	29/04/2024 10:59	Hamilton HSL Met...	20 KB
sbeadex Lightning Canola.lay	24/04/2024 10:31	Hamilton System ...	119 KB
sbeadex Lightning Canola.med	21/03/2024 09:53	Hamilton Method	550 KB
sbeadex Lightning Canola.stp	21/03/2024 09:53	Hamilton Method ...	140 KB
sbeadex Lightning Canola.sub	21/03/2024 09:53	Hamilton Sub-Met...	27 KB
sbLightning Template - Canola.res	24/04/2024 10:31	RES File	1 KB

In this screenshot, the six files within the 'Template – Canola' folder have been renamed to include 'Canola' instead of '3 tip'.

8.4 Open the renamed ".med" file using the Hamilton Run Control Software. This open the method file and you will be presented with an error. Click on "browse" and select the newly created folder and select the matching "res" file. The protocol will open.

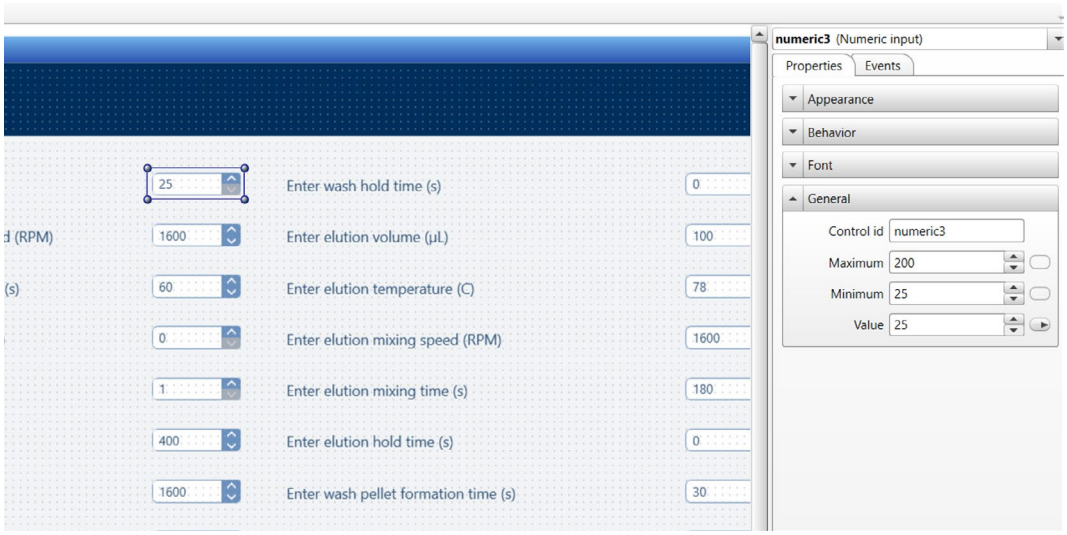
8.5 Select the method editor view by clicking on Tools > Method editor view and then select the tab 'B_User inputs' to make amendments to default parameters.



8.6 From the user inputs tab, select the “User Dialog command” to open the POM view and make adjustments to standard protocol. Doing so means that your optimal setup will be default for this protocol. An example is provided in step 8.7.

8.7 Select one of the dialogue boxes below and double click. Change the ‘value’ by increasing or decreasing within the ranges permitted within the Maximum and Minimum boxes. In the example below a value of 25 µL is changed to 200 µL.

Changing a default value by amending the dialogue box



Updated dialogue box

8.8 Save the new method and close the new method. Re-open and verify all saved changes are correctly in place.

8.9 It is recommended to verify that any changes made function correctly and as expected.

9. Further support

If you require any further support for any of the sbeadex products, please contact our technical support team at techsupport@lgcgroup.com or submit a request for support directly into our case system.

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