sageHLS™

High Molecular Weight DNA Library System

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SageHLS Operations Manual
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C	D	12/12/17	Appendices, Sections 6.16 – 6.24	Workflow guides added as appendices. Software Log Review and software updating added.
D	E	2/8/18	Section 13 HIT kits	Several workflow changes see Section 13 change log
E	F	6/18/18	Section 13 HIT kits	Several workflow changes see Section 13 change log
E	F	6/18/18	Section 12 HEX kits	Updated NEB Fragmentase ordering part number. Revised Workflow File names.
F	G	8/17/18	Section 12 and 13	Update cassette buffer filling instructions
F	G	8/17/18	Section 12	Corrected Dilution and math are for E.coli fragmentase dilution.
G	H	10/24/18	Section 13	Remove -20°C storage recommendation for cas9 gRNA assemblies (not verified)

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EQUIPMENT RATING AND SAFETY

1.1 Safety Icons Used in this Manual

In this manual, the following icons will be used to provide the user with information pertinent to the use of the SageHLS.

Caution! Warns the user that injury or instrument damage may occur if the contents of the warning are not properly followed.

High Voltage! Warns of the risk of electrical shock if the contents of the warning are not properly followed.

Hot Surface! Warns of a hot surface that may cause injury or irritation if touched.

Important! Provide important information about the proper use of the system that may influence the quality of the result.

Information. Provides additional information regarding the function of the system or applications for which is used.

1.2 SageHLS Equipment Ratings

- Input supply voltage: 100-240V
- Frequency range: 50-60Hz
- Current Rating: 2.8 A

1.3 SageHLS Instrument Safe Use Guidelines

The SageHLS system is designed to operate under the following environmental conditions:

- Pollution Degree 2
- Installation category 2
- Altitude 2000m
- Indoor use
- Ambient temperature 17-25°C
- Humidity 10-80%, non-condensing

Caution! The SageHLS was designed to be operated on a flat surface. Do not operate on a tilted surface or tilt during operation.

1.4 Consumables Safe Use Guidelines

There are two types of consumables that are used on the SageHLS instrument:

1. **Agarose gel cassette.** This is an item that is manufactured by Sage Science. It consists of a styrene cassette into which an agarose gel is cast. The gel is immersed in liquid electrophoresis buffer, and all ports and openings are sealed with adhesive tape. The buffer is formulated with Tris-TAPS and is not hazardous.

2. **Reagent Kits**. Reagent kits consist of reagent formulations that are manufactured by Sage Science or third-party suppliers that are provided as liquids in tubes or bottles. These include lysis reagents, buffers, enzymes (dilute, in buffer), dyes, and in some cases short DNA or RNA that have been manufactured (not originating from plants or animals). All components are non-hazardous.

A partial list of reagent concentrations is provided on the next page.

Important! Reagent kits do not contain hazardous, known mutagenic, or known carcinogenic substances. Some chemicals may be skin irritants at higher concentrations than supplied in our kits (e.g. Sodium Dodecyl Sulfate).

Important! Users should refer to the Material Safety Data Sheets (MSDS) for comprehensive outline of the consumables safety classifications. These are posted at www.SageScience.com/product-support/sagehls-support/

Important! Users should use the SageHLS and related consumables in accordance to safe laboratory guidelines including the use of gloves, safety glasses, and lab coats.

Important! SageHLS consumables do not contain any gases or volatile compounds that can adversely affect health.

Unpacking and Installation

2.1 Unpacking the SageHLS

The SageHLS instrumentation is shipped in two boxes: one will contain the SageHLS and Accessories and the second box will contain the computer monitor in the manufacturer's original packaging. With the boxes in the upright position, open and confirm that the following items are enclosed:

Monitor

- LCD computer monitor
- Video cable
- Power cord

SageHLS

- SageHLS Instrument
- Accessory box
  - Computer keyboard, USB
  - Computer mouse, USB
  - Rinse cassettes (for maintenance of electrodes)
  - Power supply
  - Power cord

Caution! Do not substitute the power cord. A replacement cord with an incorrect rating can damage the instrument or power supply.

2.2 Installing the SageHLS

1. Open the LCD monitor box, and assemble it according to the manufacturer instructions

2. Using a box cutter, open the top seal on the SageHLS box, and open.

3. Remove the accessory box located just inside, atop the instrument. Remove the keyboard, mouse, and instrument power supply and cords from their packaging.

4. Firmly grip both sides of the **bottom** of the instrument and lift it along with the foam packaging inserts. The SageHLS weighs approximately **20 lbs**. Place the unit onto the bench top.

Important! Do not lift the instrument by the lid or lid retainer. This can damage the mechanism or impede the function of the unit.

Important! After removing the instrument, open the lid and remove the foam insert and discard. The instrument can get damaged if operated with the insert in place.

5. Connect the LCD monitor (VGA port) to the SageHLS (VGA port, back panel) using supplied video cable. Connect monitor to the power outlet using the power supply supplied with monitor (**Figure 2.2**).

6. Insert USB connector from the computer **keyboard** into any USB port located on the back panel of the SageHLS (**Figure 2.2**).

7. Insert USB connector from the computer **mouse** into any USB port located on the back panel of the SageHLS (**Figure 2.2**).

8. Connect SageHLS instrument to the power outlet using the SageHLS power supply and power cable. The power input connector is in the lower left hand corner on the back panel of SageHLS, below the power switch (**Figure 2.2**).

9. Press power switch located on the rear of the instrument, and wait for software to launch (approximately 30 seconds)

10. When powered on, a **blue light** on the front panel of the Instrument will be on (**Figure 2.3**).

    The SageHLS is ready for use. The software should automatically launch – allow 30 seconds.

Figures 2.2 and 2.3 show the rear and front panels of the SageHLS.

Figure 2.2. Rear Panel of the SageHLS

Figure 2.3. Front Panel of the SageHLS

2.3 Unpacking and Storage of Gel Cassettes

Store boxes in the upright position and confirm the following contents are present.

- 4 or 12 foil-sealed gel cassettes – store at room temperature.
- 1 bag with reagent bottles
- 1 package of adhesive tape for sealing elution wells (in the reagent bag)

2.4 Unpacking Reagent Kits

SageHLS reagent kits can contain reagents with varying storage requirements. These will be noted on the with the documentation included with the kits and on the labels on the reagent bottles.

Important! Storing reagents at the correct temperatures is required, refer to the documentation provided with reagent kits. Improper storage can affect system performance or reagent shelf-life. Contact support@sagescience for the replacement of reagents if necessary.

Introduction

Thank you for purchasing the SageHLS from Sage Science. We urge you to read this manual to familiarize yourself with the system's capabilities and precautions.

Caution! Use the SageHLS only as indicated in this operations manual. Injury or instrument damage can occur with improper usage.

The SageHLS is a platform on which users may purify intact DNA from cell suspensions and immobilize the DNA in the sample well as it enters the agarose gel surface. Once immobilized, the DNA can be enzymatically treated. The immobilized DNA is subsequently cleaved, electrophoretically size-selected, and collected in buffer in six wells.

3.1 System Overview

There are four components to the SageHLS system:

- **Instrument –** The instrument is comprised of two heated gel cassette nests, an electrophoresis power supply, two electrode arrays embedded in the lid, and a single-board computer. The system computer is accessed by an external LCD monitor, mouse, and keyboard.
- **Software** – System software allows the user to program run parameters to operate the system (e.g. heat, electrophoresis voltage, and time), operate the system (e.g. Start, Stop, Pause), and monitor operations (e.g. time remaining, electrophoresis current). The software also collects log files and allows users to review operation data from previous runs.
- **Gel Cassettes** – Pre-cast, disposable agarose cassettes are manufactured by Sage Science. Gel cassettes have the capacity to process 2 samples per run.
- **Reagent Kits** -- Reagent kits are formulated by Sage Science and contain the necessary components to complete the processing of samples. This may include lysis reagents, conditioning buffers, enzyme mixes, electrophoresis buffers, or oligo adapters.

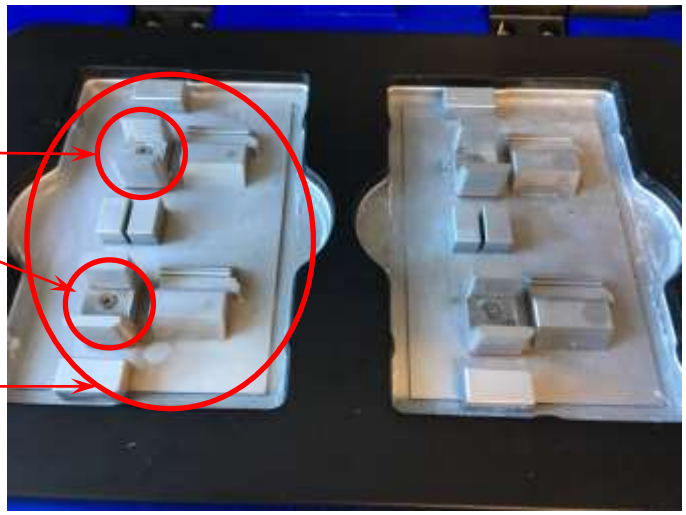
The SageHLS instrument
(the monitor and keyboard are not shown)

3.2 The Heated Nest

The SageHLS is equipped with a heated aluminum nest to control the reagent/enzyme reaction temperature conditions, and electrophoresis temperature. The nest accommodates the agarose gel cassette within which purification, enzymatic reactions, and electrophoresis occurs. The instrument is equipped with two nests, and each nest has two zones of temperature control:

Reaction Zones
(Peltier heating/cooling)
15°C-50°C

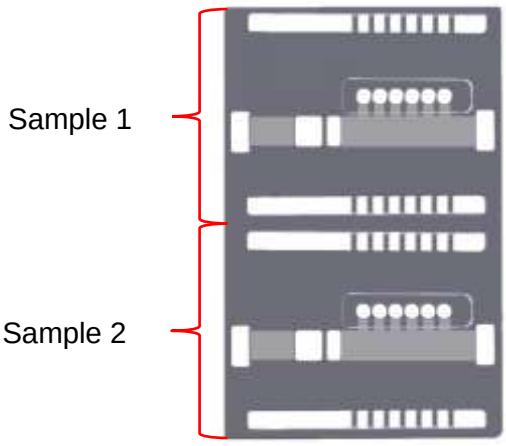
Electrophoresis Zone
(heating)
Ambient - 40°C



Hot Surface! Use caution when using the SageHLS heated nest.

3.3 How the System Works

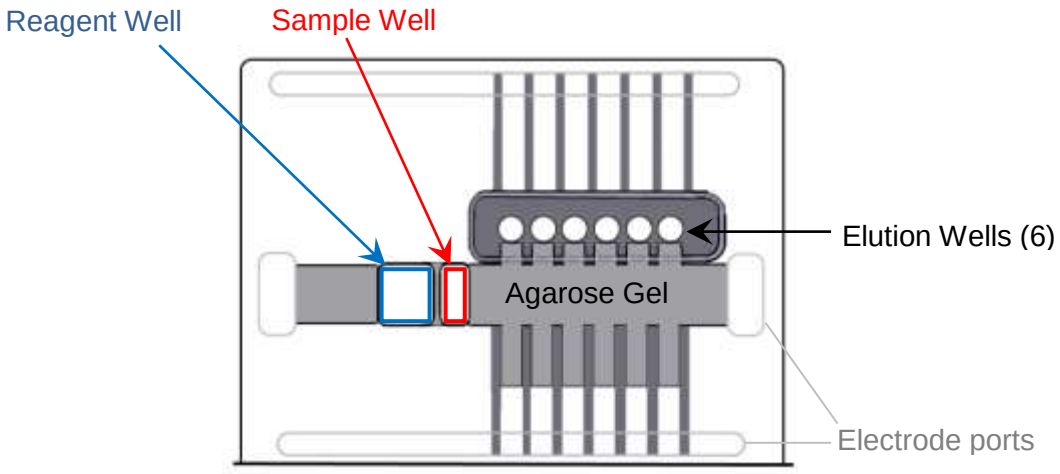
The SageHLS system uses pre-cast and disposable agarose gel cassettes. Each cassette has a 2-sample capacity.



Gel Cassette
Schematic (top)

A schematic of a single sample lane is shown below. It consists of an agarose column, with two wells into which reagents, buffers, or cell suspensions are added.

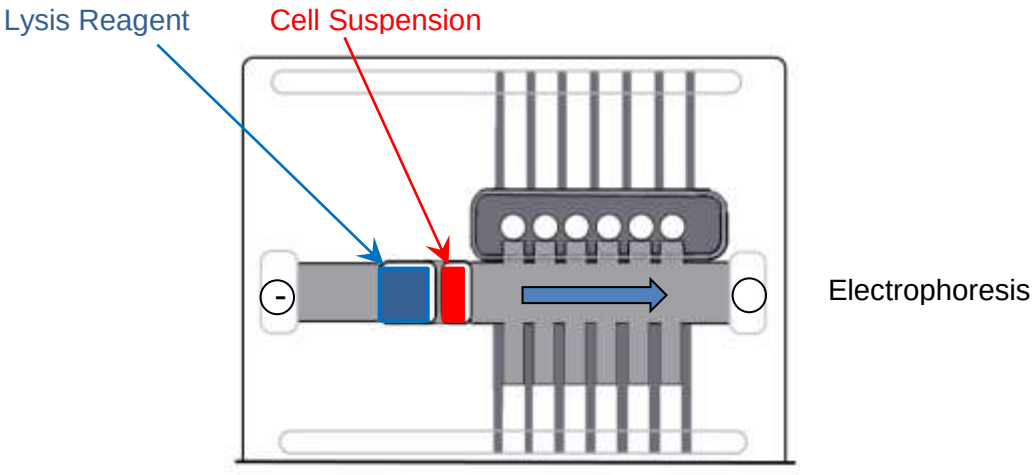
Electrophoretic current can be applied in two (perpendicular) directions. DNA is collected in six membrane-bound elution wells.



Single Sample Schematic

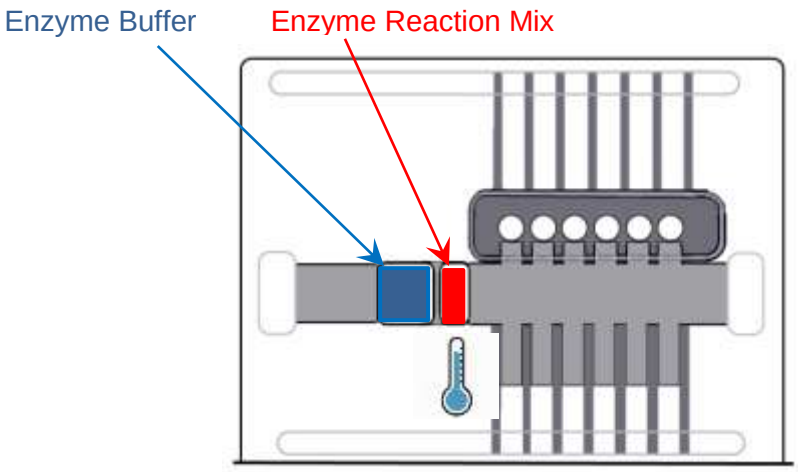
Cell suspensions are processed in three workflow stages:

STAGE 1. EXTRACTION



Cells are lysed. Electrophoresis is used to remove solubilized proteins and small nucleic acids. HMW DNA remains immobilized in the wall of the agarose sample well.

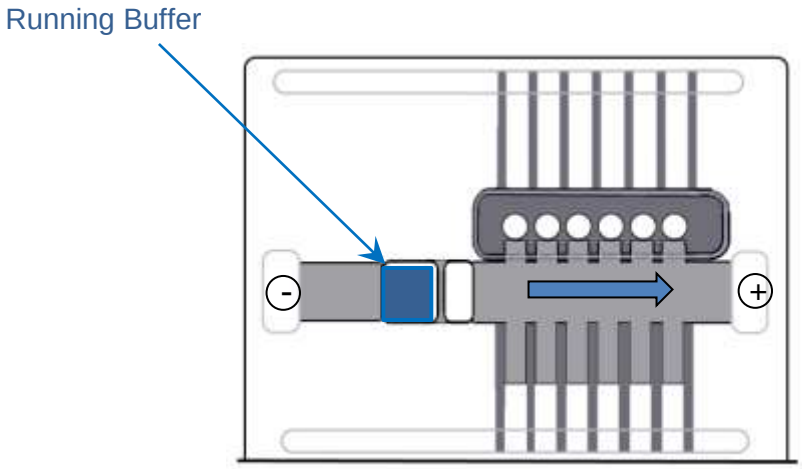
STAGE 2. TREATMENT



Temperature control and time

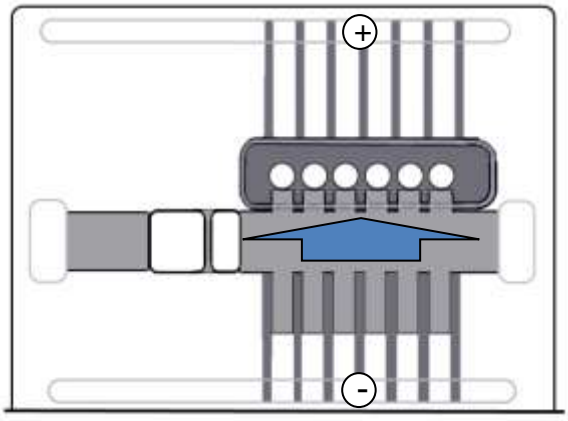
DNA is subject to enzymatic treatment that cleaves the DNA into electrophoretically mobile fragment sizes. Cleavage may be random or targeted.

STAGE 3. COLLECTION



a. Electrophoresis (separation)

Electrophoresis separates DNA by size within the gel column.



b. Electrophoresis (elution)

Lateral electrophoresis elutes the DNA into 6 size-binned wells with buffer, and DNA is removed with wide-bore pipet tips.

3.4 Workflows

Cell Suspensions
Kits provided by Sage Science should be used to prepare cells, spheroplasts, or nuclei prior to DNA purification in the Sage HLS. *Cell suspension workflows may require steps one day before running the SageHLS.*

SageHLS
The processing of samples on the SageHLS requires user interactions with the instrument (opening and closing the lid), software (pausing and resuming), and reagent agarose gel cassette (reagent transfers with a pipette). *SageHLS workflows may require up to one day and an overnight run to complete.*

These interactions are guided by a **Workflow File**. A workflow file is comprised of three **Stages**, each of which consist of multiple **Steps**. Samples are processed by linking stages into a workflow.

Sage Science provides several pre-programmed workflow files for supported application. User can modify the pre-programmed workflows and save the customized workflows under new names.

Overview of sageHLS Workflow Stages

Stage 1: Extraction Stage – (cell suspension kit)
Extracts DNA from cell suspensions and removes residual byproducts.

Step types:
sample temperature, time
electrophoresis voltage, time
pause/resume for reagent transfer

Stage 2: Treatment Stage – (gel cassette kit)
Enzymatic treatment of DNA with cleavase, targeted endonuclease, or other enzymatic processes

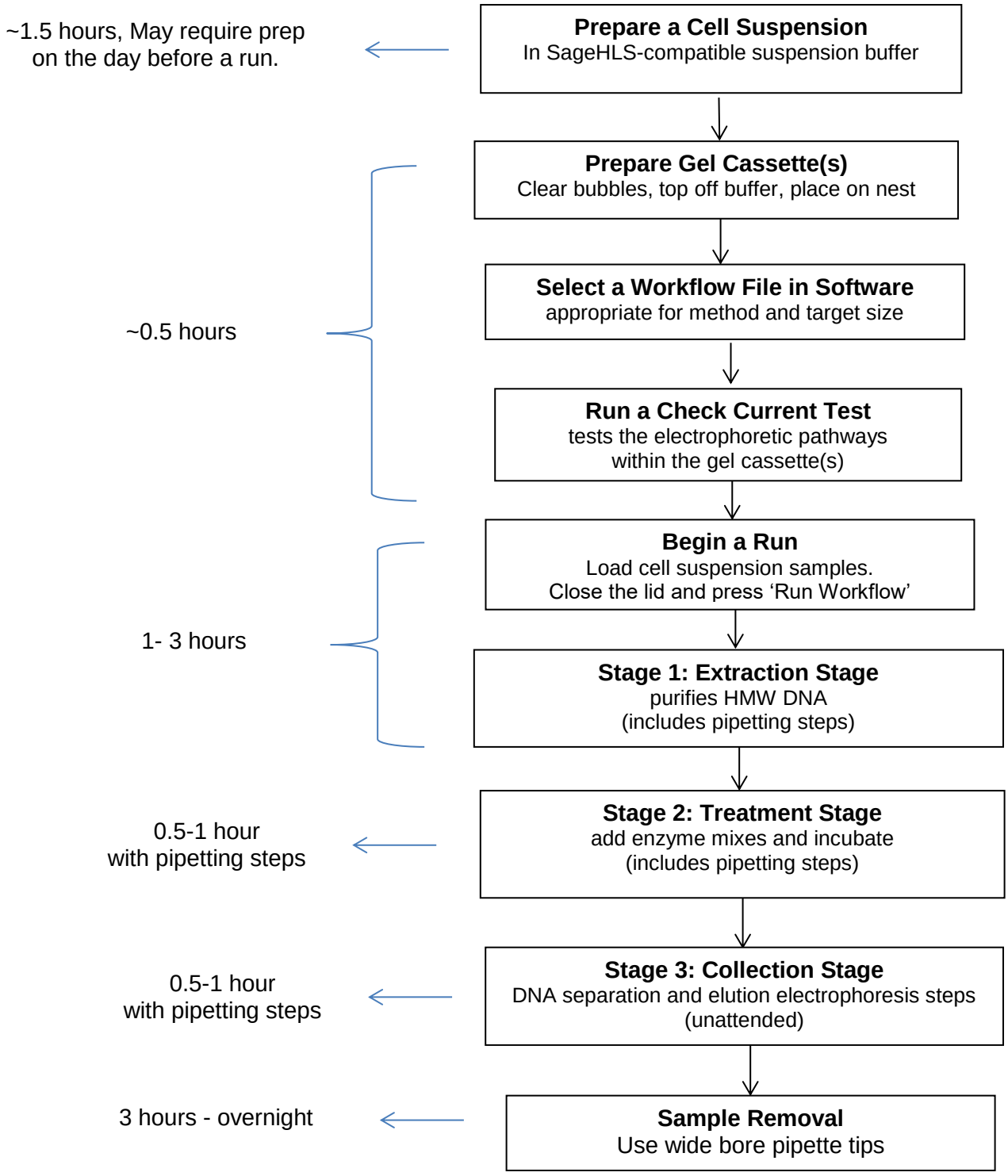
Step types:
sample temperature, time
electrophoresis voltage, time
pause/resume for reagent transfer

Stage 3: Collection Stage - (DNA size selection options)
Size Separation and collection (DNA size selection). These differ by target collection size requirements.

Step types:
separation voltage, pulsed-field, time
separation voltage, DC, time
elution voltage, DC, time
elution reverse voltage, DC, time

3.5 Workflow Summary

This workflow summary should be used as a guideline. Refer to specific Workflow Guides (Appendix A and B) for detailed workflow information.



Maintenance and Cleaning

4.1 Electrode Rinse: Weekly or After 5 Runs

Rinsing the SageHLS Electrodes is an important maintenance function. It is recommended that they be rinsed after every 5 runs, or at the weekly (whichever occurs first)

1. Place the blank SageHLS cassette bodies (provided by Sage Science) onto the Nests.
2. Completely fill the cassettes with deionized water.
3. Close the instrument Lid.
4. In the Main Tab, press the “Clear Run Data” button on the Commend Menu Bar.
5. In the cassette and protocol fields, enter the pre-programmed “Rinse Cassette” and “Rinse Protocol” options from the drop-down menus:
6. Press “Run” on the Command Menu. The protocol will take about 6 minutes to run.
7. Open the lid. Remove the rinse cassettes and pour out the wash into a drain.
8. Save the rinse cassettes for later use.

4.2 Cleaning the Nest: As Needed

Salts may accumulate on the nest from spilt buffer. The aluminum blocks should be cleaned, as needed, using deionized laboratory water. 70% ethanol may also be used, if users are concerned about biological cross contamination.

4.3 Warranty and Service

The SageHLS does not require field preventative maintenance other than what is described above. The system is subject to depot repair at the Sage Science facility. The warranty period is one year, and covers all expenses including parts, labor and shipping. The system is subject to full replacement at Sage Science’s discretion.

Getting Started

A complete description of the SageHLS software is provided in the next section. Workflow guides for preparing HLS-compatible cell suspensions are provided in Appendix A of this document. Detailed workflow guides that include workflow-specific software screens, gel cassette usage, and reagent preparations are provided in Appendix B of this document:

- **Appendix A - Cell Preparation Workflow Guides**
- **Appendix B - SageHLS Workflow Guides**

These guides are individually provided with the appropriate consumable kits and posted on the Sage Science support site. These should be used at the bench top for step-by-step instructions for SageHLS standard workflows.

Before starting, users should be aware of the following and prepare accordingly:

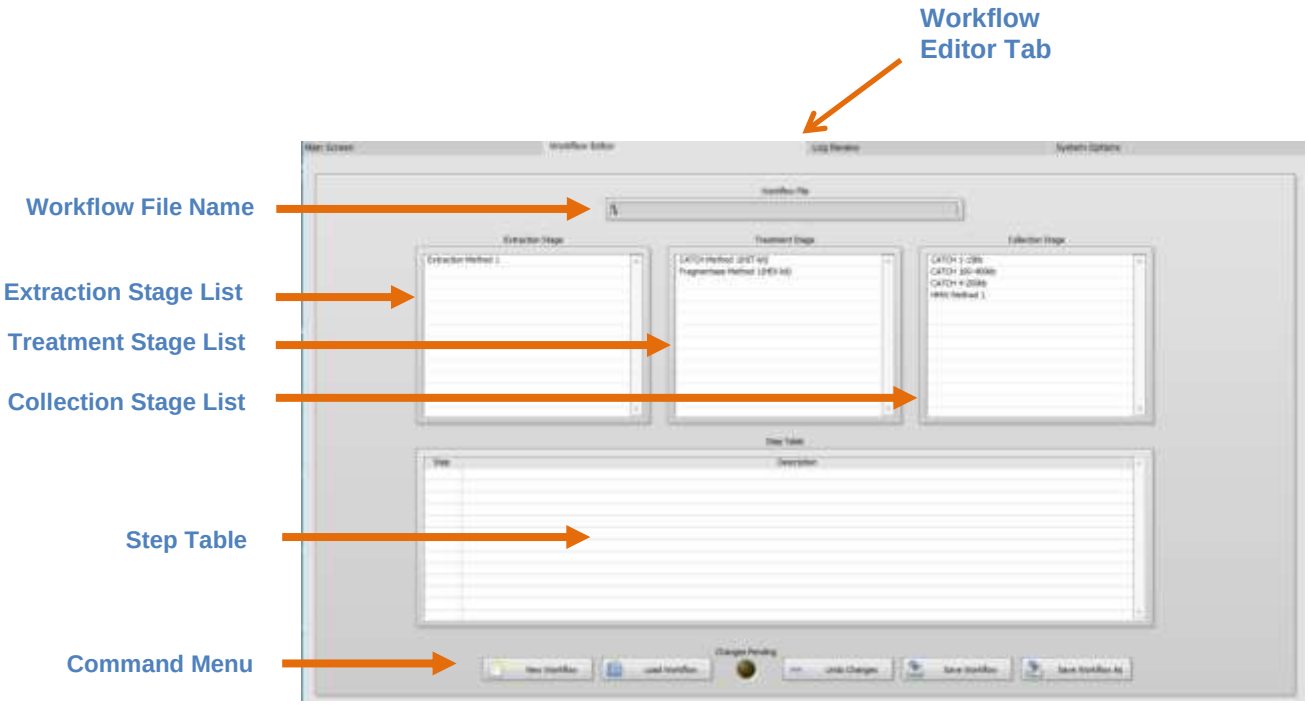
- **Some kits may require the purchase of reagents from 3rd party suppliers**
- **Some cell suspensions may require preparation steps one or more days before a SageHLS run**
- **Some SageHLS runs require an overnight electrophoresis step**

Important! New workflows are under development, and existing workflows may be subject to improvements or modifications. Make sure to check the Revision and Release Date of this document with the Manual or Guides posted on the SageHLS support page on the Sage Science website (http://www.sagescience.com/product-support/sagehls-support/).

SageHLS software

6.1 Workflow Editor Tab

In the SageHLS software, navigate to the **Workflow Editor Tab**. It is the second tab from the left:



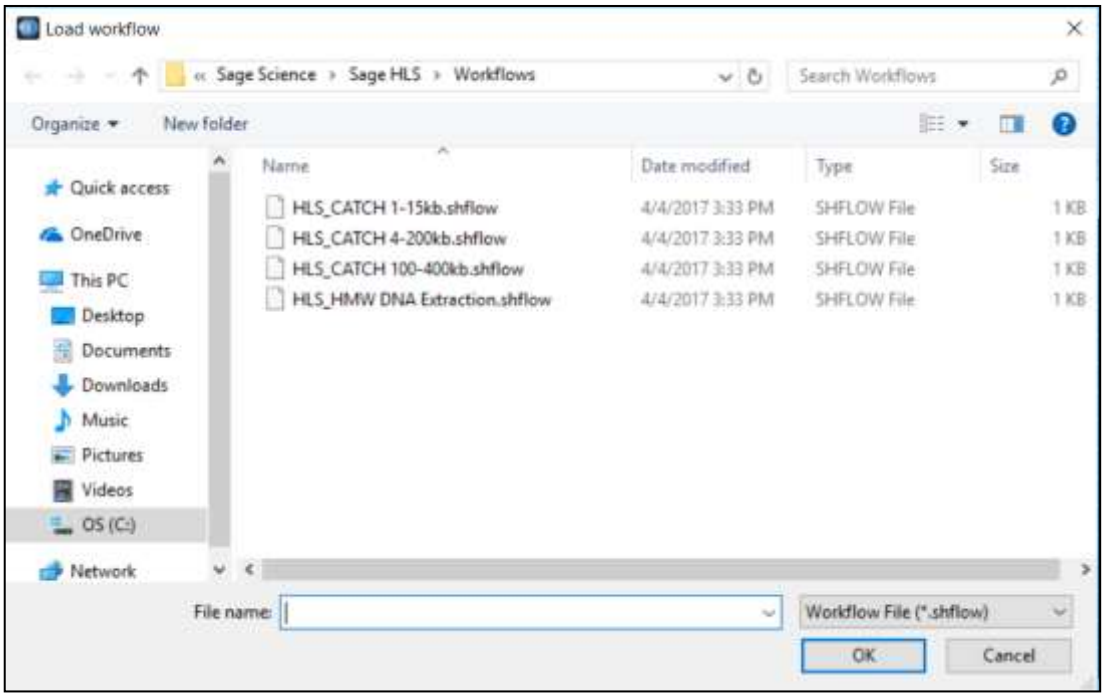
The Stage List fields are populated with pre-set stage protocols.

6.2 Workflow File

A **Workflow File** links the three process **Stages**. The SageHLS instrument follows the Workflow file to run every **Step** of a process, in succession. The Workflow File uses a .shflow file extension.

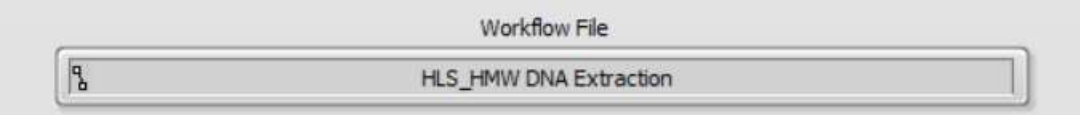
Workflow Files are accessed by pressing the “Load Workflow” button in the Command Menu:

A file list will pop-up from the SageHLS/Workflows folder.

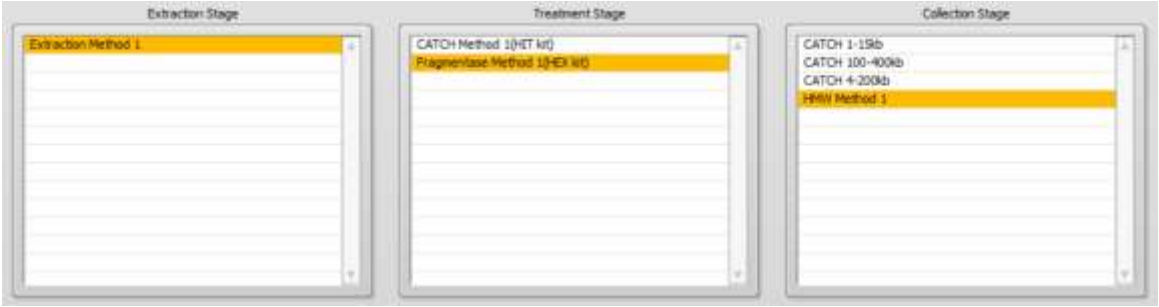


When a Workflow File is selected;

a) the file name will appear in the Workflow File field,



b) the three Stage Protocols that comprise the workflow will become highlighted with a yellow background



c) the Steps that comprise each highlighted Stage will appear in the Step Table. The step numbers include a prefix that corresponds to the Stage number. Each stage's step list ends with the step sub-number (i.e. 1-001 for Stage 1).

Extraction Stage (1)

Treatment Stage (2)

Collection Stage (3)

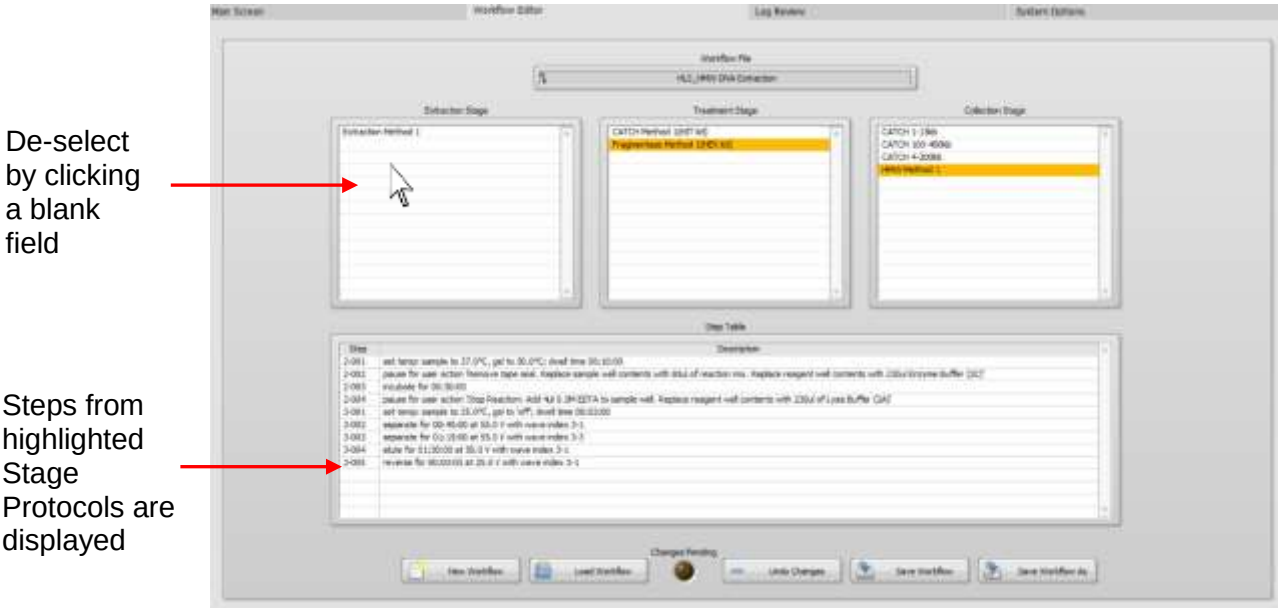
Step Table

Step	Description
1-001	set temp: sample to 25.0°C, gel to 30.0°C; dwell time 00:02:00
1-002	pause for user action 'Replace sample well contents with 70ul of prepared sample. Replace reagent well contents with 230ul of
1-003	separate for 01:00:00 at 55.0 V with wave index 1-1
2-001	set temp: sample to 37.0°C, gel to 30.0°C; dwell time 00:10:00
2-002	pause for user action 'Remove tape seal. Replace sample well contents with 80ul of reaction mix. Replace reagent well content
2-003	incubate for 00:30:00
2-004	pause for user action 'Stop Reaction: Add 4ul 0.5M EDTA to sample well. Replace reagent well contents with 230ul of Lysis Buff
3-001	set temp: sample to 25.0°C, gel to 'off'; dwell time 00:02:00
3-002	separate for 00:45:00 at 55.0 V with wave index 3-1
3-003	separate for 01:15:00 at 55.0 V with wave index 3-3
3-004	elute for 01:30:00 at 50.0 V with wave index 3-1
3-005	reverse for 00:00:05 at 25.0 V with wave index 3-1

Important! Stage Protocols may be edited or created in the Stage Editor. This is accessed in the factory setting using a super-user password. See Section 6.9.

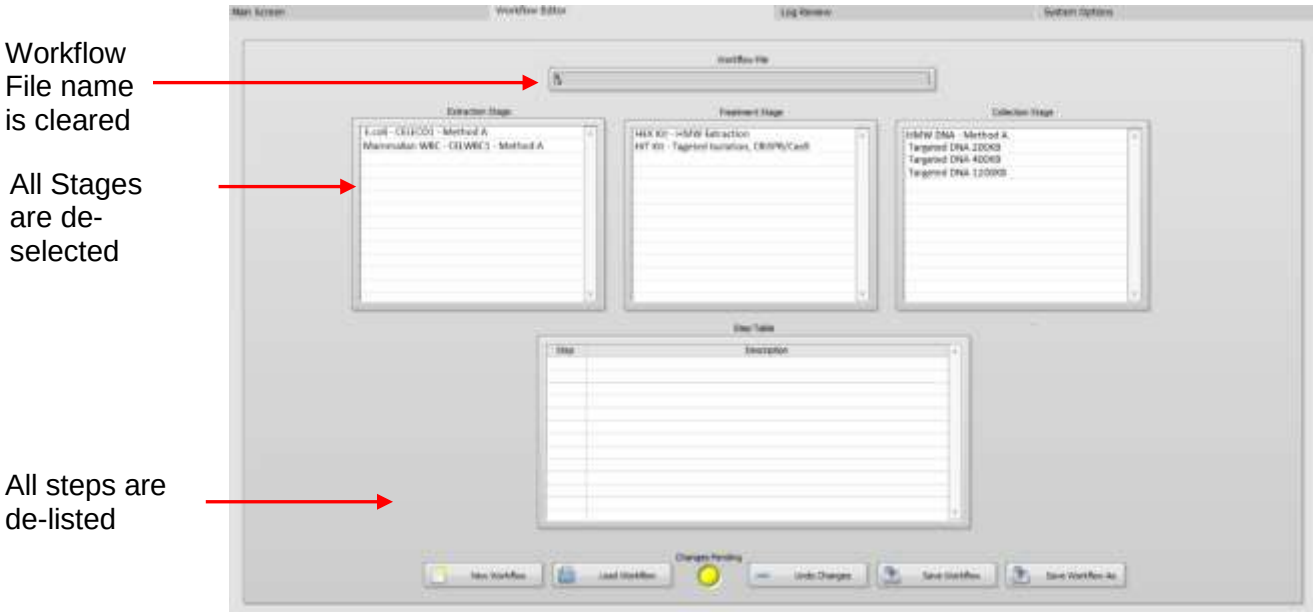
The steps of any Stage Protocol can be viewed by highlighting the Stage Protocol name within a list.

A Stage Protocol can be de-selected by clicking on a blank field within the list. This will remove all of the Stage's steps from the Step Table.



The Workflow Editor screen can be reset by pressing the New Workflow button.

This will clear the Workflow File name and deselect all highlighted Stages:



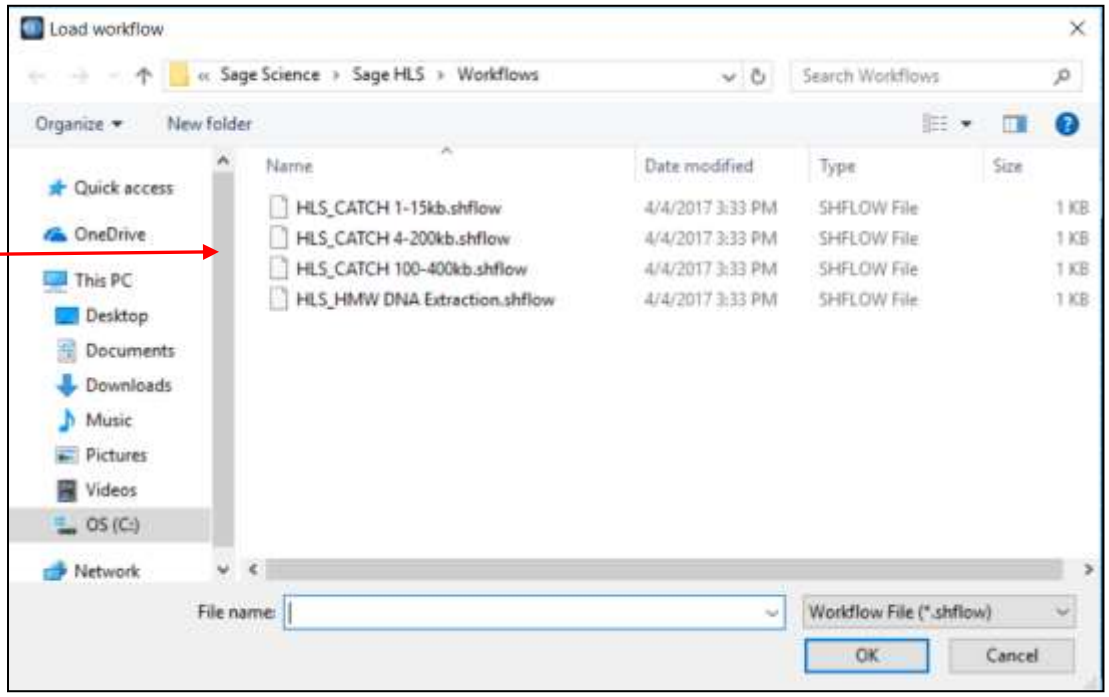
6.3 Saving a Workflow File

If a Workflow File has been modified by replacing or eliminating a step, it can be renamed and resaved using the “Save As” button.

The workflow file list will pop-up. Users may save new workflows or existing workflows with new file names.

Pre-set Workflows (with an HLS prefix), cannot be changed and re-saved. They may be saved with a new file name or modified and saved with a new file name.

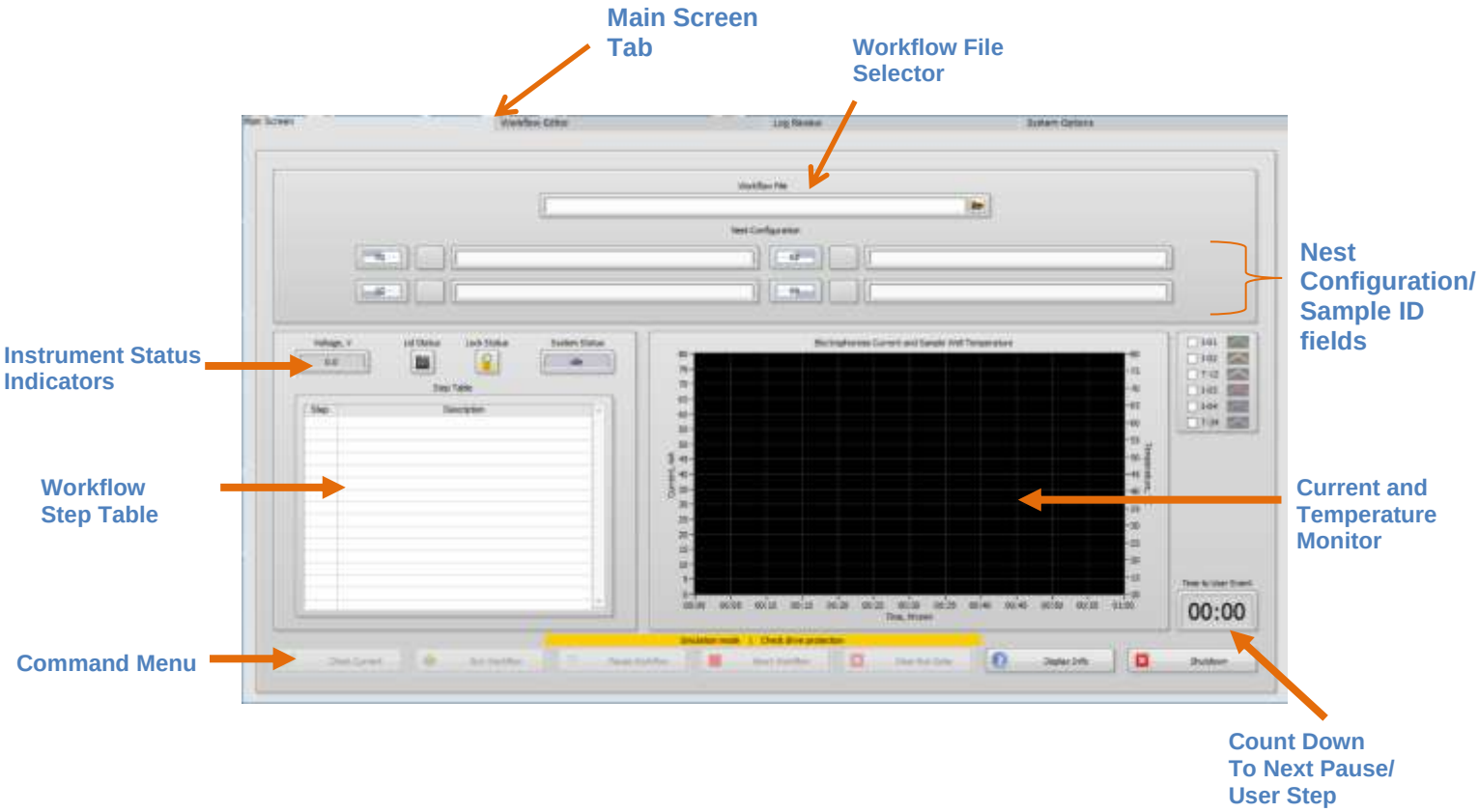
Pre-set Workflow files (HLS prefix) cannot be modified



6.4 Main Screen Tab

The Main Screen tab is the screen with which users interface with the SageHLS instrument. This includes initiating a run, monitoring a run, and pausing/resuming to conduct a manual pipetting step.

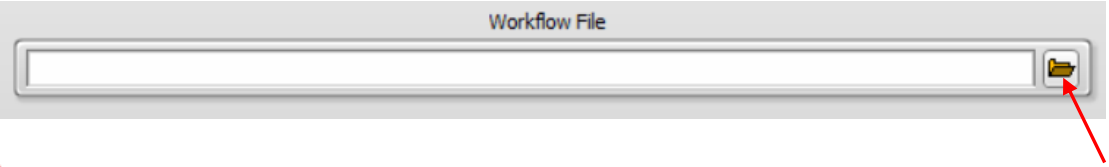
The default software screen on the SageHLS has a tabbed format. The Main Menu is the first tab.



6.5 Loading a Workflow File

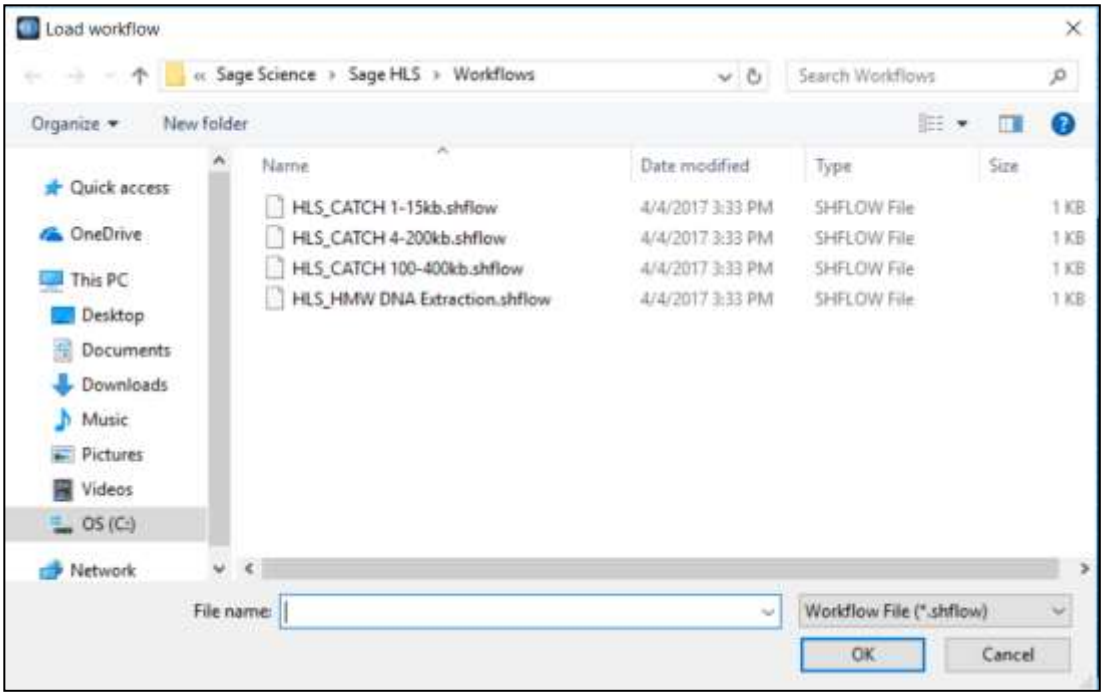
1. If the System status in on Idle (there was a previous run), select “Clear Run” data to clear all fields and bring the system to an Idle System State:

2. Click the folder icon next to the Workflow File Field.

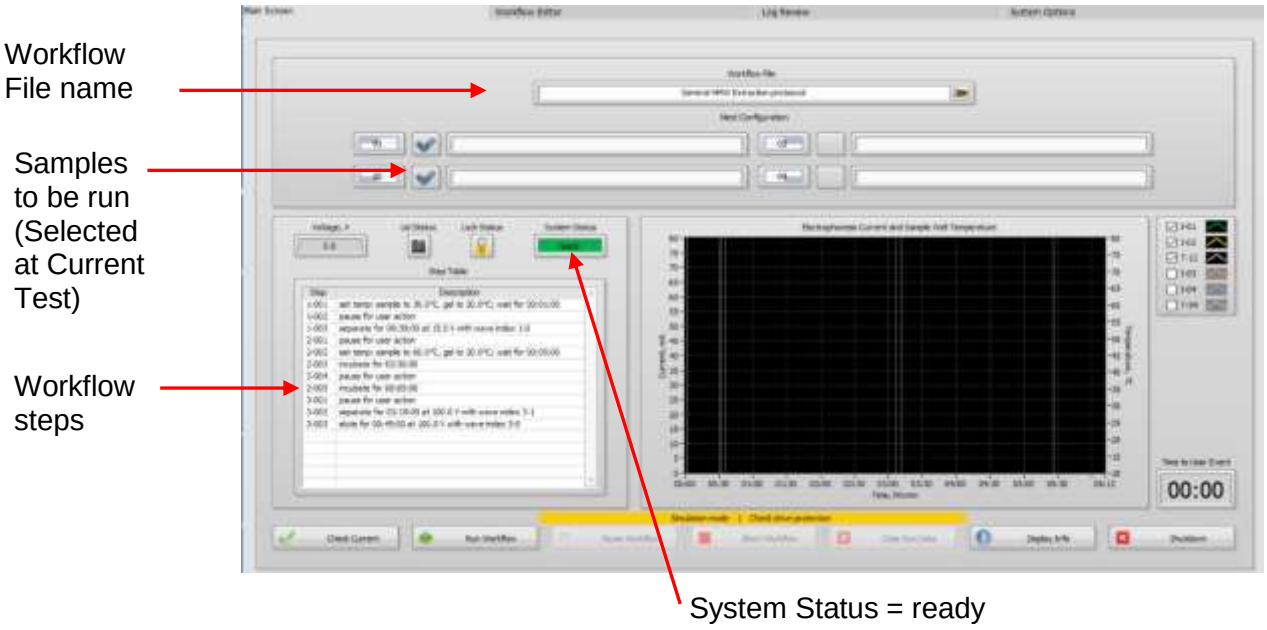


Important! The System Status must display “Idle”, or the Workflow File directory will not open.

3. A Workflow File directory will open. Select a Workflow File and select “OK”.

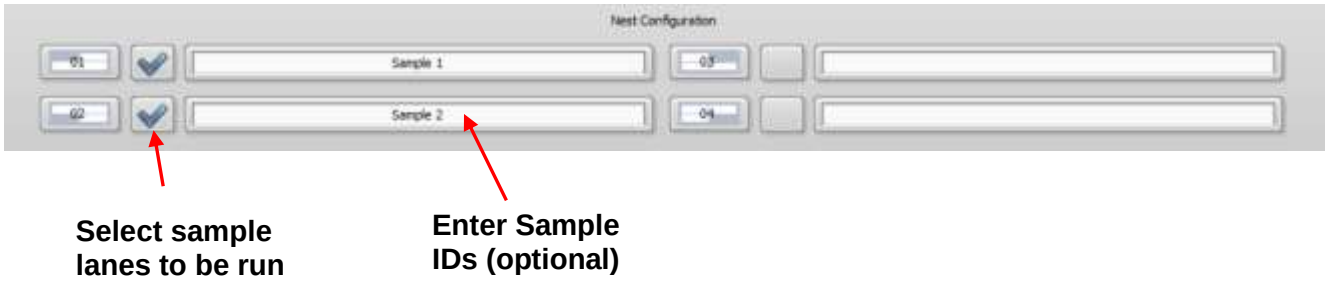


4. The Workflow File name will populate the Workflow File field, the Workflow steps will populate the Step Table, and the System Status will indicate “ready”



5. Select the samples to be run by clicking the check box next to the sample position.

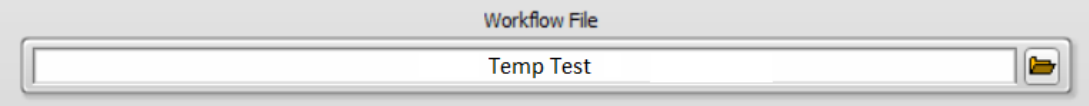
6. Enter Sample ID information into the Nest Configuration fields (optional). Sample IDs will be saved in the log file.



6.6 Temperature Test Workflow (Optional)

A preset workflow, named “Temp Test”, is provided for users who wish to verify that the sample well temperature control is working properly. This test can be used for QC or IQ/OQ purposes. Note that the Temp Test workflow (run after the Current Test in the next section) requires approx. 10 minutes. After it is complete, a new workflow file must be loaded, and the Current Test re-run.

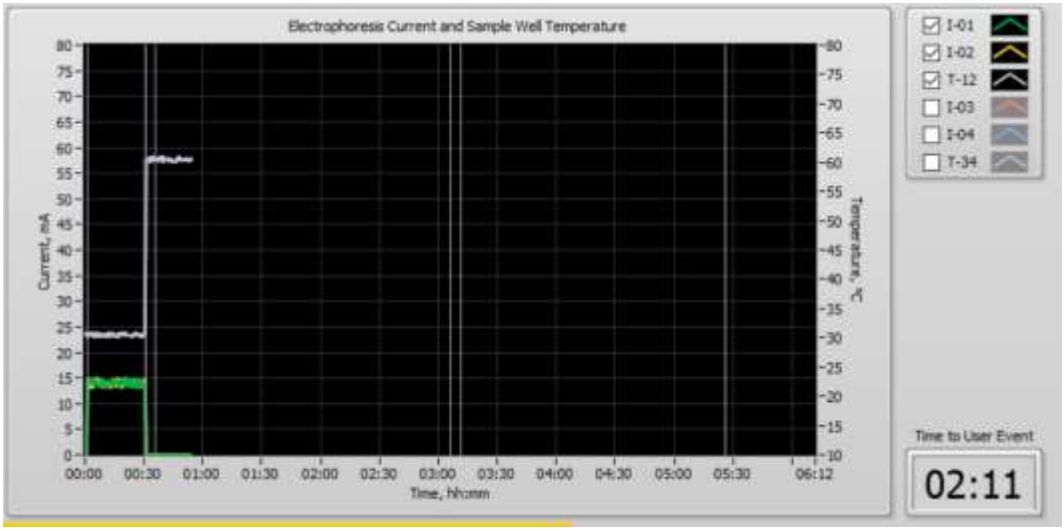
1. In the Workflow File field load the “Temp Test” workflow file.



2. Run the Current Test (next section)

3. Run the “Temp Test” workflow (section 6.8).

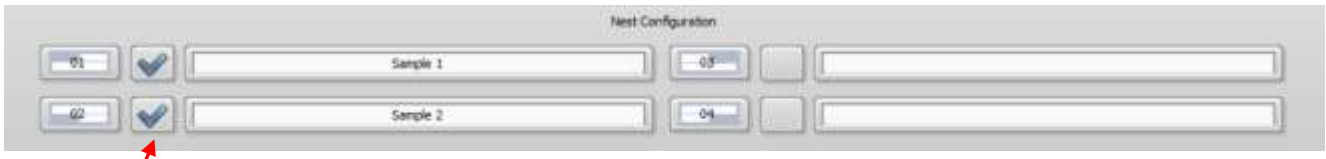
4. At the end of the run, verify that the temperature profile in the Current and Temperature Monitor conforms to the Temp Test step values:



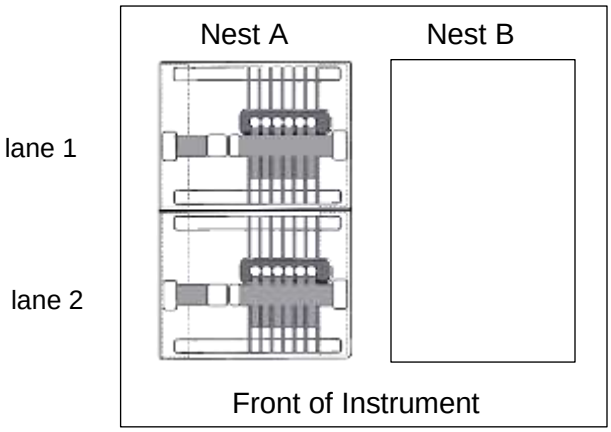
6.7 Running the Electrophoresis Current Test

Prior to loading a sample and running a cassette, the current test should be run to ensure that the electrophoretic properties of the cassette, based on measuring the electrical currents between electrodes, are within expected limits.

Before starting, make sure cassettes have been prepared (outlined in the Workflow Guides) and that they are placed on the nests that correspond to the sample locations that have been checked:



Current Test will run on lanes 1 and 2 on Nest A.



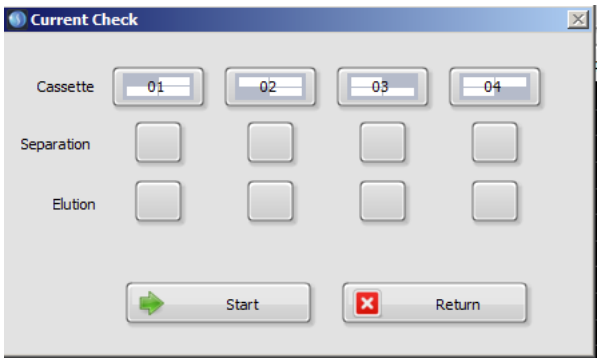
Important! Make sure that all cassette preparation steps in Section 5 have been completed.

The Current Test Procedure:

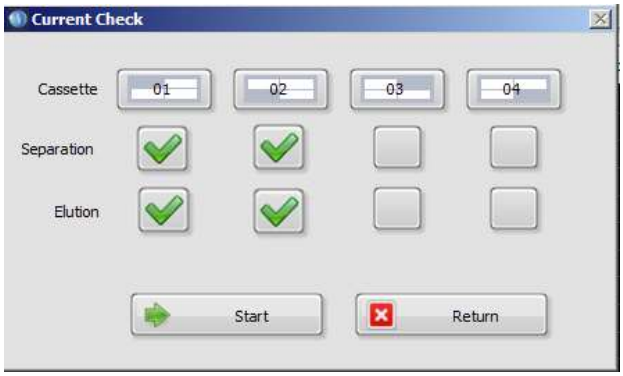
1. Close the SageHLS lid.
2. Once the lid is closed, press the “Check Current” button on the command button menu.

Press “Check Current”

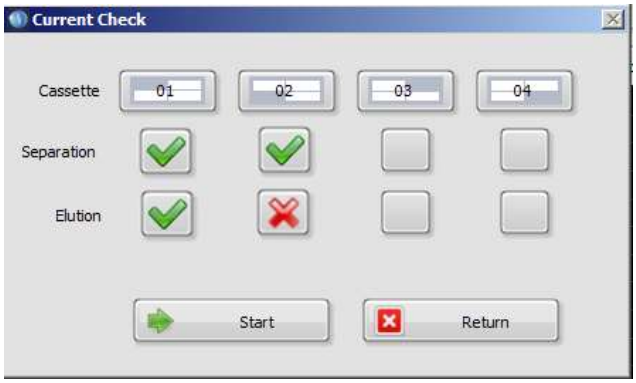
3. A pop-up window will appear:



4. Press “Start”. The lid lock mechanism will engage, and the nest motor will activate. As the test progresses, a check mark will appear in the box corresponding to the electrophoretic path if the measured current falls within the expected limits.



5. If the test fails, the nest will automatically lower, and the lid will disengage until the issue is rectified.



Important! If a current check fails, check the buffer levels in the affected lane, and replace the buffer as needed. Contact Sage Science support if the current test continues to fail.

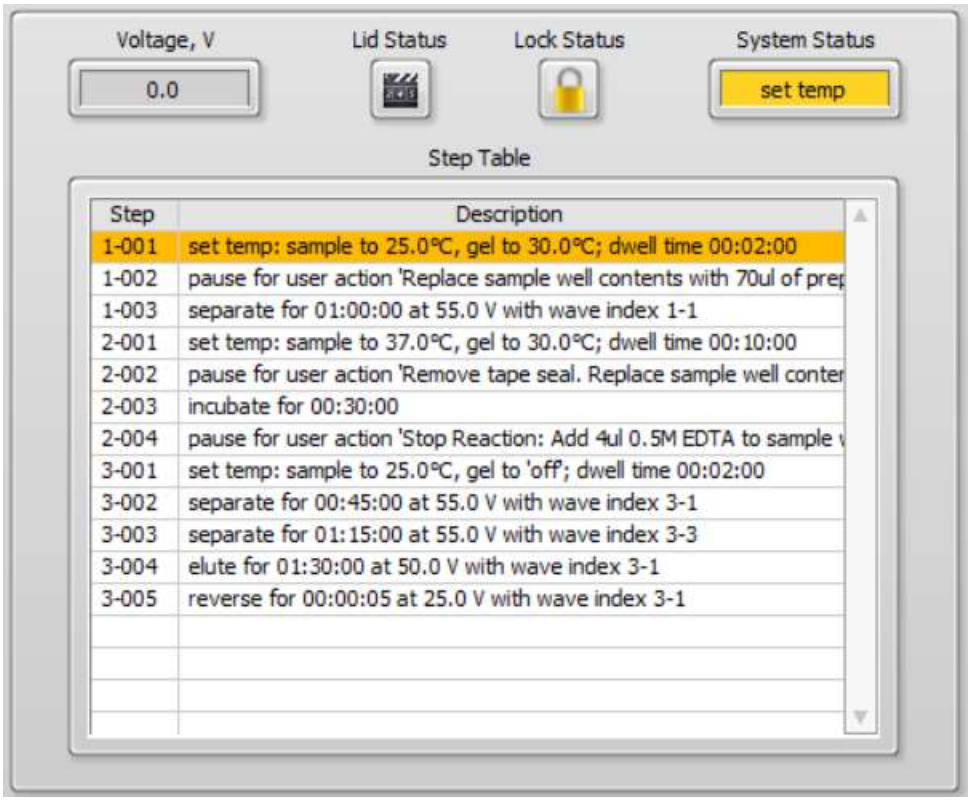
6. Press “Return” to return to the Main screen.

6.8 Running a Workflow

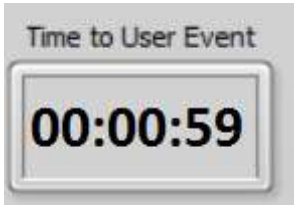
Press “Run Workflow”.

The active step in the Workflow Step Table will be highlighted in yellow.

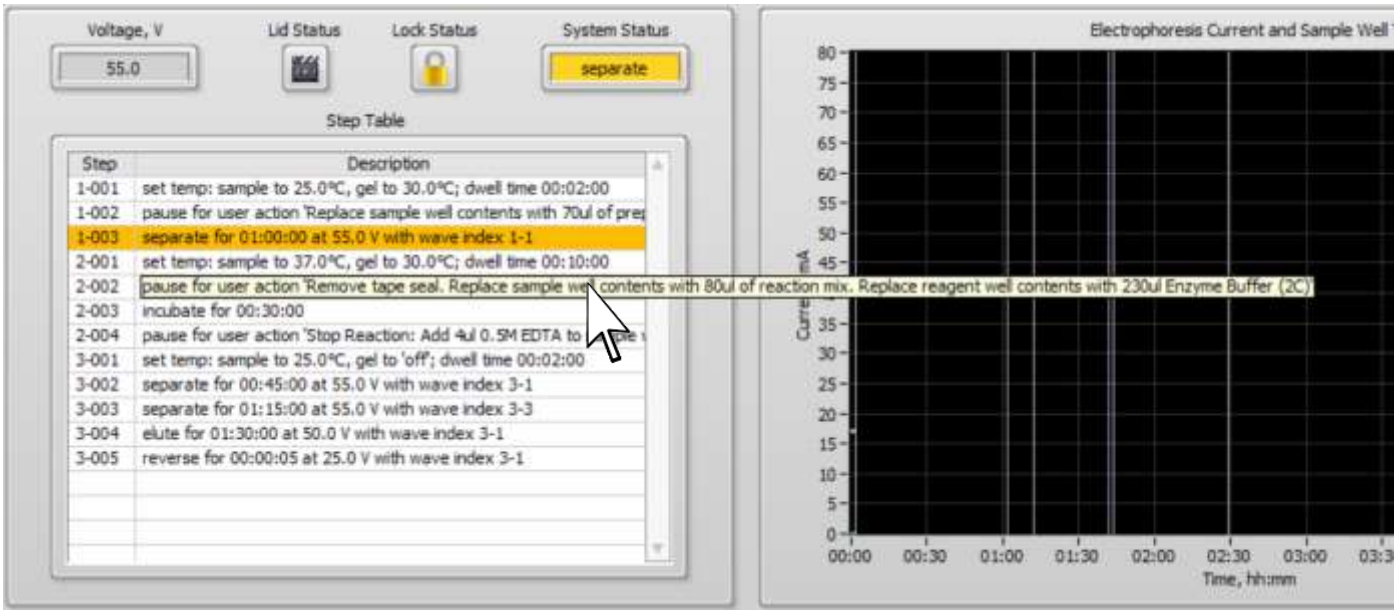
The System Status field will indicate the type of step being run.



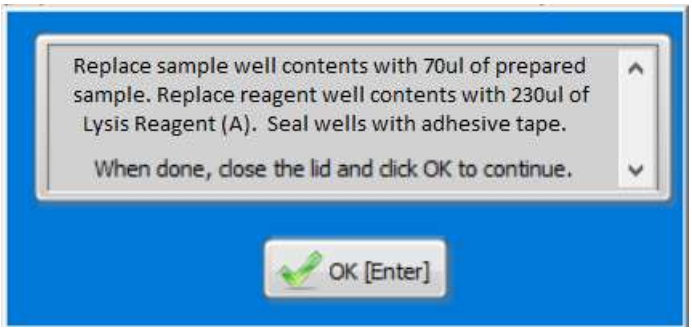
The “Time to User Event” timer will start a countdown until the next Pause step, which will require the lid to be opened, and reagent transfer steps with a pipette.



If a pause step contains instructions that exceed the Step Table display, hovering the mouse pointer over a step will display the complete instruction.



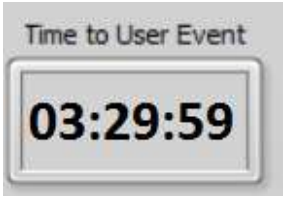
When the instrument reaches a Pause step, a pop-up window will appear with instructions for a pipetting or manual procedure. When the manual procedure is complete, and the lid has been closed, users should press “OK” to continue the Workflow.



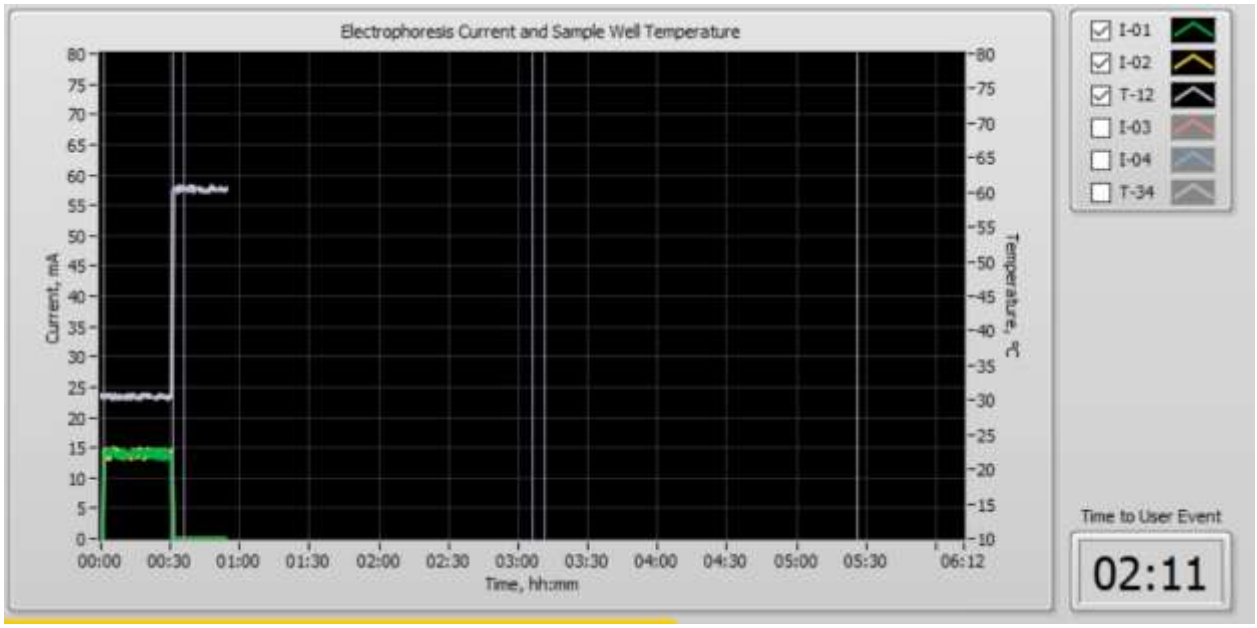
If the instrument is inadvertently resumed, press the “Pause Workflow” button in the Command Menu to re-pause the instrument and continue with the manual user action.

Pause Workflow

After the last Pause step in the Workflow, the “Time to User Event” will begin to countdown to the end of the run, typically a few hours.



Throughout the workflow the “Electrophoresis Current and Sample Well Temperature” monitor will graphically display (in real time) the electrophoresis current for each sample, and the combined sample well temperature for each nest:



Electrophoresis current (mA) Cassette 1

Electrophoresis current (mA) Cassette 2

Sample Well Temperatures Nest 1 (Cassettes 1 and 2)

6.9 Other Run Commands

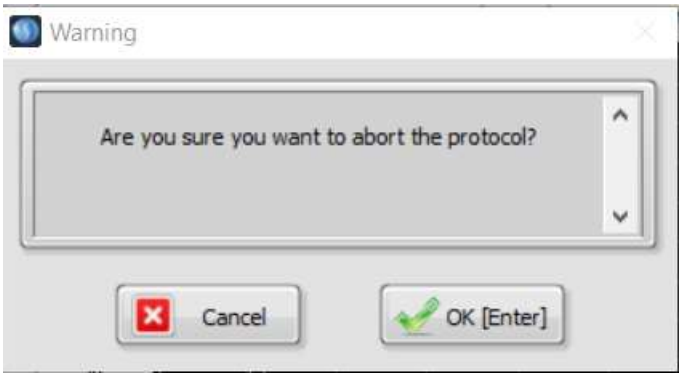
A workflow may be paused (and then resumed), or stopped at any time during a Workflow:

Pause Workflow

Abort Workflow

If a Workflow is manually paused, the following window will appear. Press “OK” to continue the Workflow.

If a Workflow is stopped, the following window will appear. Press “OK” to abort the protocol, or “Cancel” to return to the Workflow.



6.10 Running a Single Lane on a Cassette

A single lane can be run on a cassette allowing for the second lane to be used at a later date:

1. Make sure only the lane to be used is checked in the Nest Configuration in the Main Screen.

2. Save the original adhesive tape that the cassette was packaged with.

3. The unused lane can be left unsealed during the entire run
4. .

4. At the end of the run, re-seal the cassette with the original adhesive tape. Make sure to apply pressure (with a finger or lab marker) around all wells and chambers. With a marker, indicate which lane is unused.

5. Store the cassette at room temperature until needed.

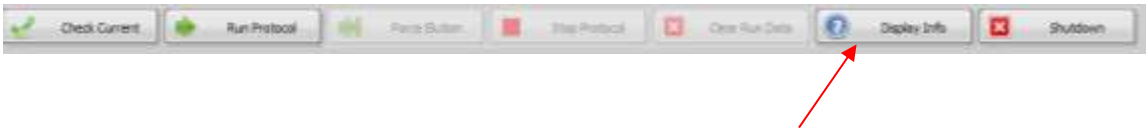
6. The unused lane can be stored until the original expiration date of the cassette.

6.11 Stage Editor Tab

Workflow stages can be created or edited in the Stage Editor.

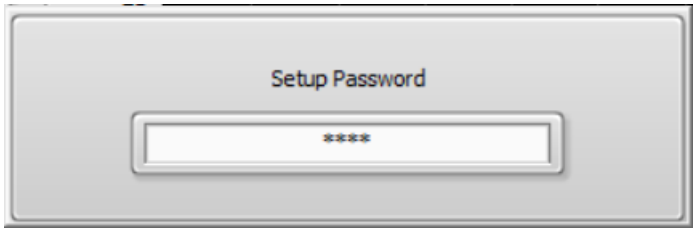
The Stage Editor screen is hidden under one level of user control, and can be accessed by entering a password:

1. In the Main Screen, press the “Display Info” button in the Command Menu.

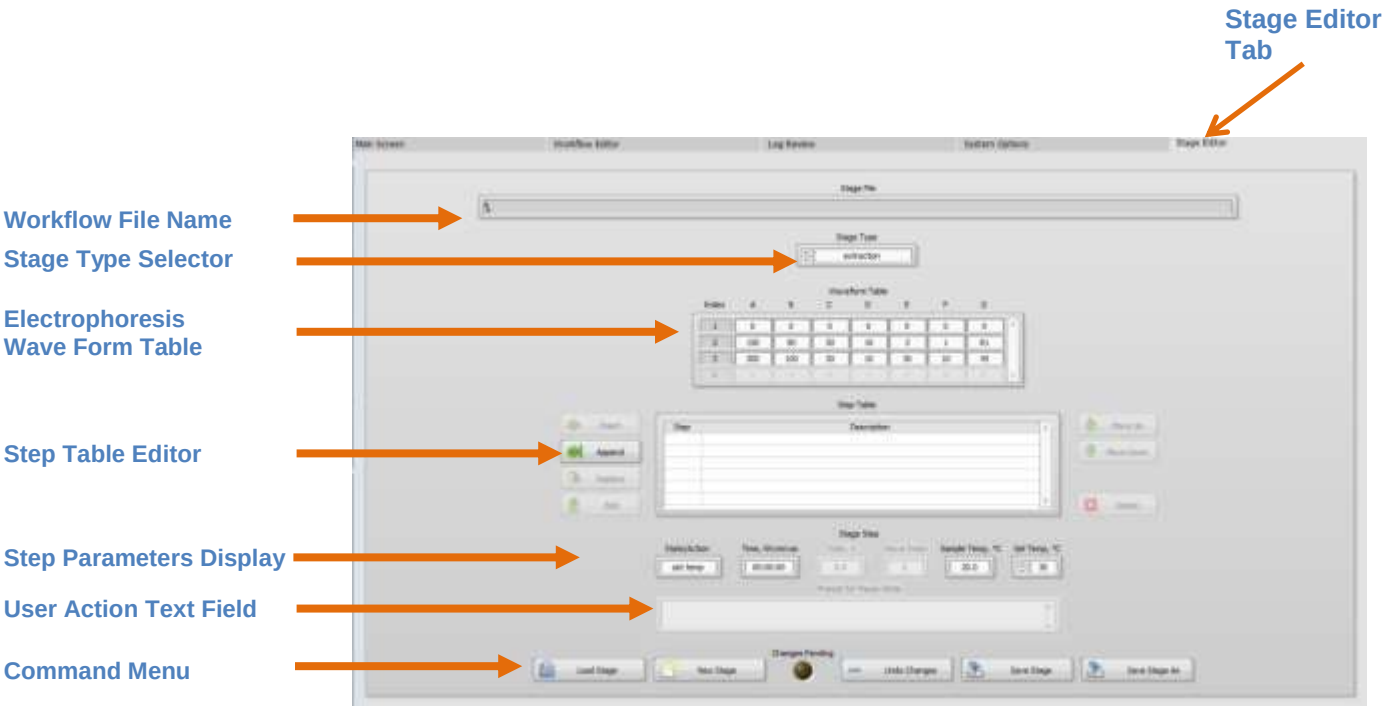


2. The “Display Info” window will appear. Press the “Advanced Tabs” button.

3. A Setup Password window will appear. Enter the password **“pips”:**



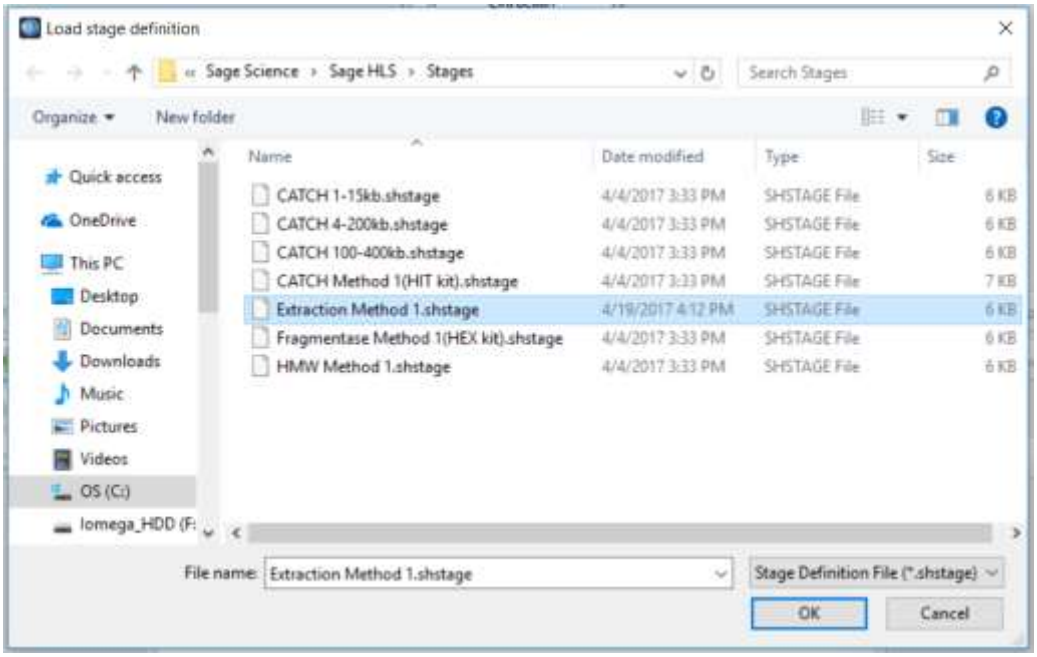
The Stage Editor will appear as a fifth tab on the right side of the screen.



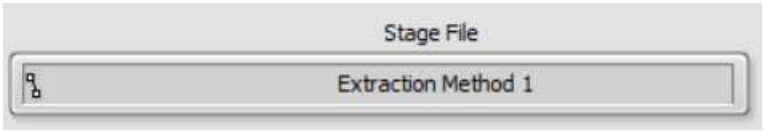
6.12 Opening a Stage File

1. In the Command Menu, press “Load Stage”:

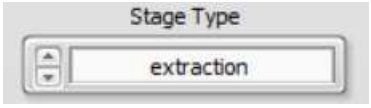
2. A file folder window will appear listing the pre-set protocols in the “Preset Stages” folder. All stage files have a .shstage extension. Select a Stage file to load and press “OK”



3. When a Stage file is loaded, the following fields will populate:

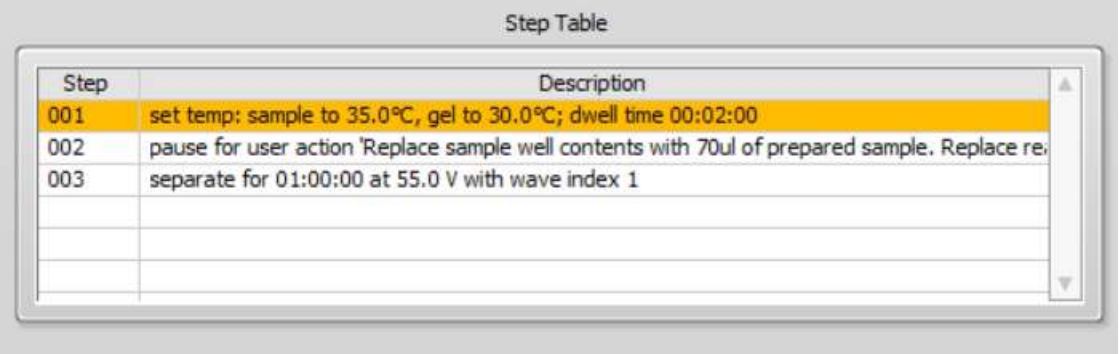


Stage File: The name of the loaded file



Stage Type: The type of stage assigned the loaded file (extraction, treatment, or collection)

Waveform Table: Parameters for electrophoresis waveforms. Index 1 indicates direct current. Others are pulsed-field. This includes preset waveforms and any new waveforms that may have been programmed for the loaded protocol.



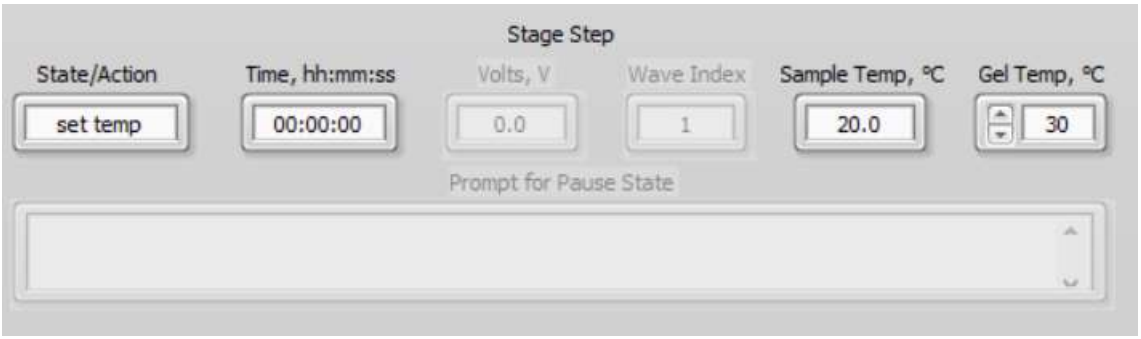
Step Table: The Step assigned to the loaded Stage file.

6.13 Changing the Stage Type

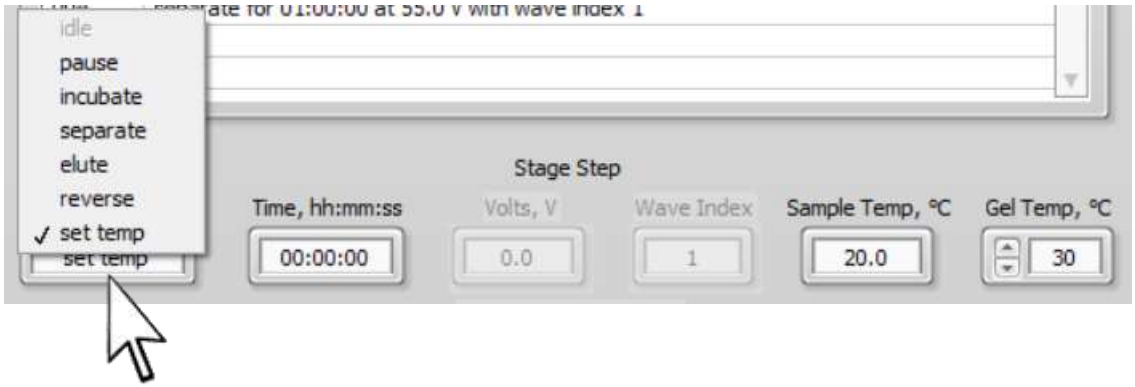
Click the arrows in the Stage type field to toggle between the three stages.

6.14 Editing a Stage Step

A new Step can be programmed in the Stage Step fields:

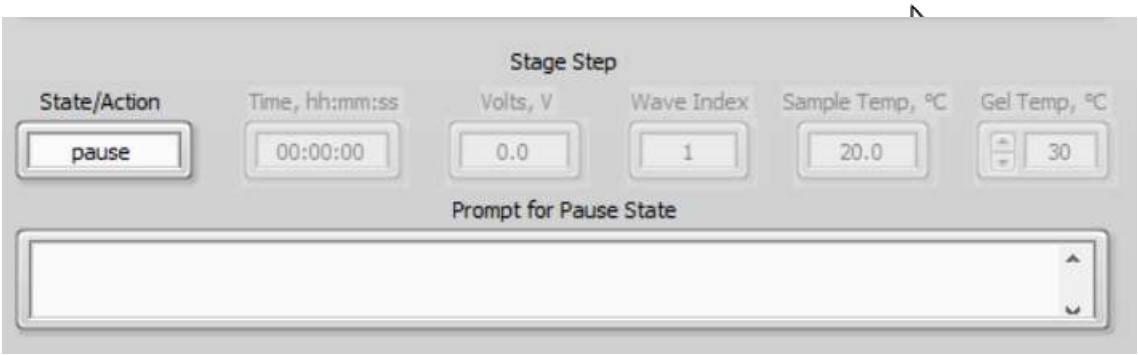


There are several State/Actions that can be programmed. These are selected from a drop-down menu which appears when the “State/Action” field is clicked:

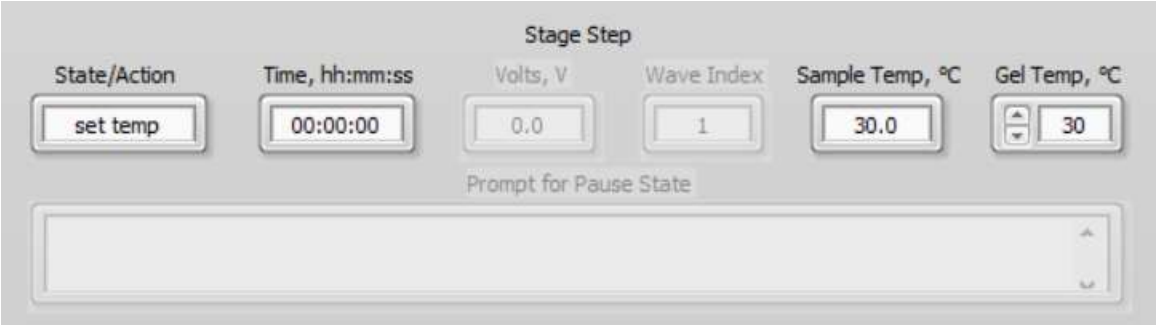


The programmable State/Action Steps are:

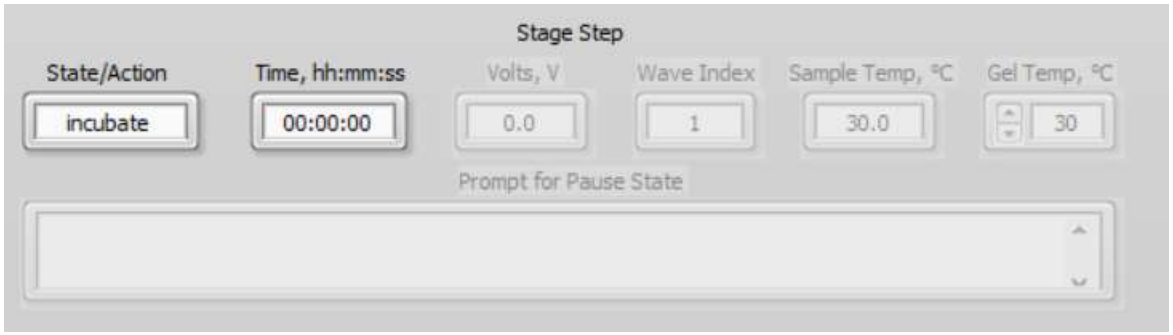
Pause: Pauses a run, and a pop-up window appears. User can enter instructions as text that will appear in the pop-up. Text is entered in the “Prompt for Pause State” field. No other parameters are entered.



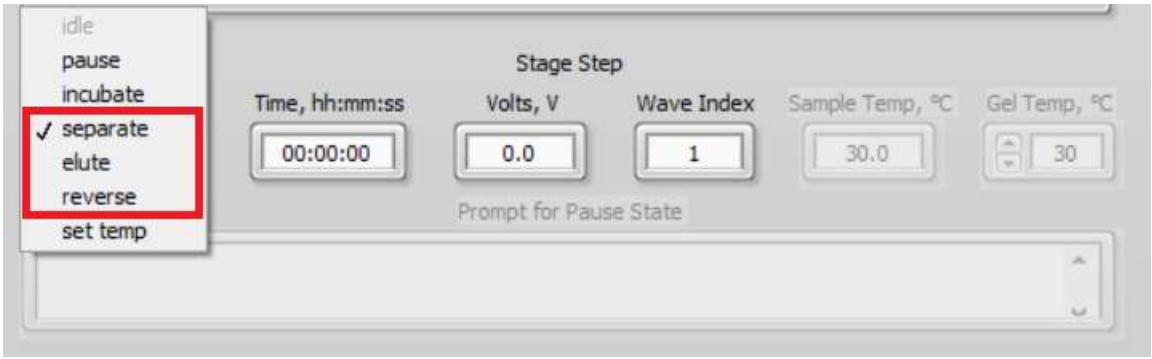
Set Temp: Sets the temperature of the sample well and gel column. The sample well can be set between 15-50ºC. The gel column can be set to 30ºC, or OFF. In a workflow, the temperatures will remain at the set points, until another "set temp" step is reached, or at the end of a run, when the heating function turns off.



Incubate: The instrument remains at an active state for a period of time, with no electrophoresis. This defines the amount of time that a temperature is applied to a sample. The amount of time is only parameter entered.



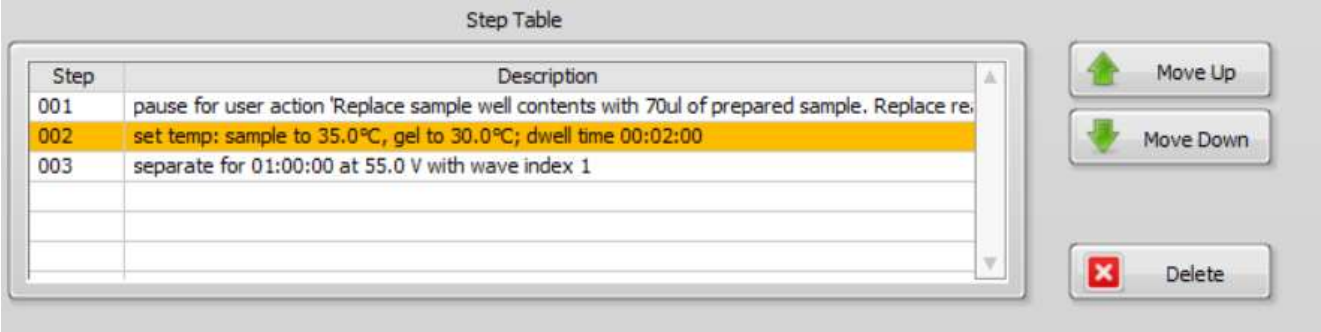
Separate, Elute, and Reverse: Electrophoresis parameters. "Separate" is electrophoresis along the separation column. "Elution" is electrophoresis along the elution pathway (perpendicular to the separation pathway, into the elution wells). "Reverse" is the reverse direction of the Elution pathway - typically for a short reverse to release DNA from the elution well membrane. Time of electrophoresis, electrophoresis voltage, and Wave Index (a waveform from the Waveform Table) are entered.



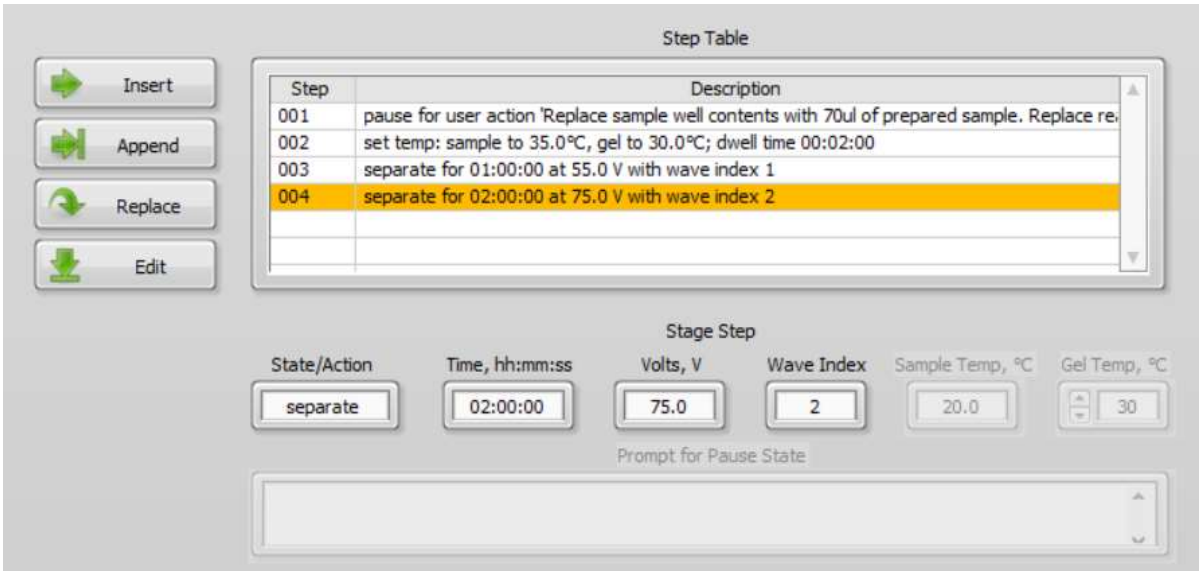
6.15 Editing the Step Table

A step can be highlighted by clicking with within the step. Users can move the highlighted steps up and down the list by pressing the “Move Up” and “Move Down” buttons.

A highlighted step can be deleted by selecting the “Delete” button.



A step can be added to the Step Table in several ways using the buttons on the left of the table:



Insert: Inserts the programmed step in the table **before** the highlighted step.

Append: Appends the programmed step to the **end** of the list.

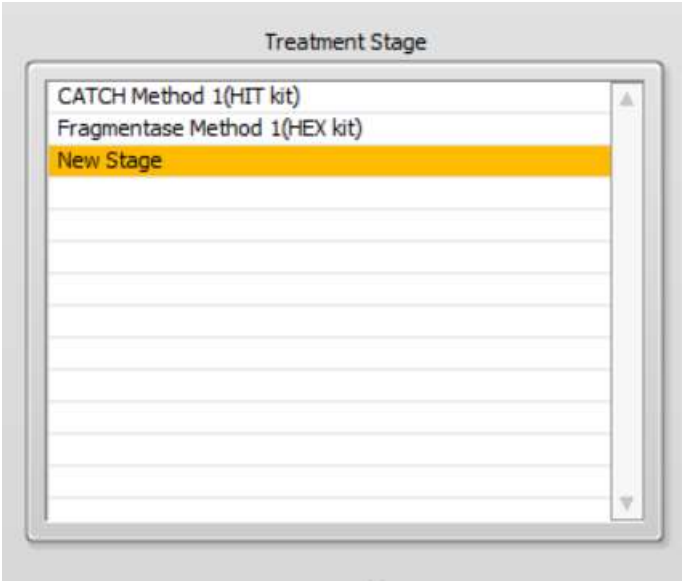
Replace: Replaces the parameters in the **highlighted** step.

Edit: Enters the parameters in the highlighted step into Stage Step fields.

6.16 Saving a Stage File

Press “Save Stage As” to save a Stage File. Preset protocols cannot be altered and saved with the same file name.

After saving the new stage will appear in the Workflow Editor Screen.



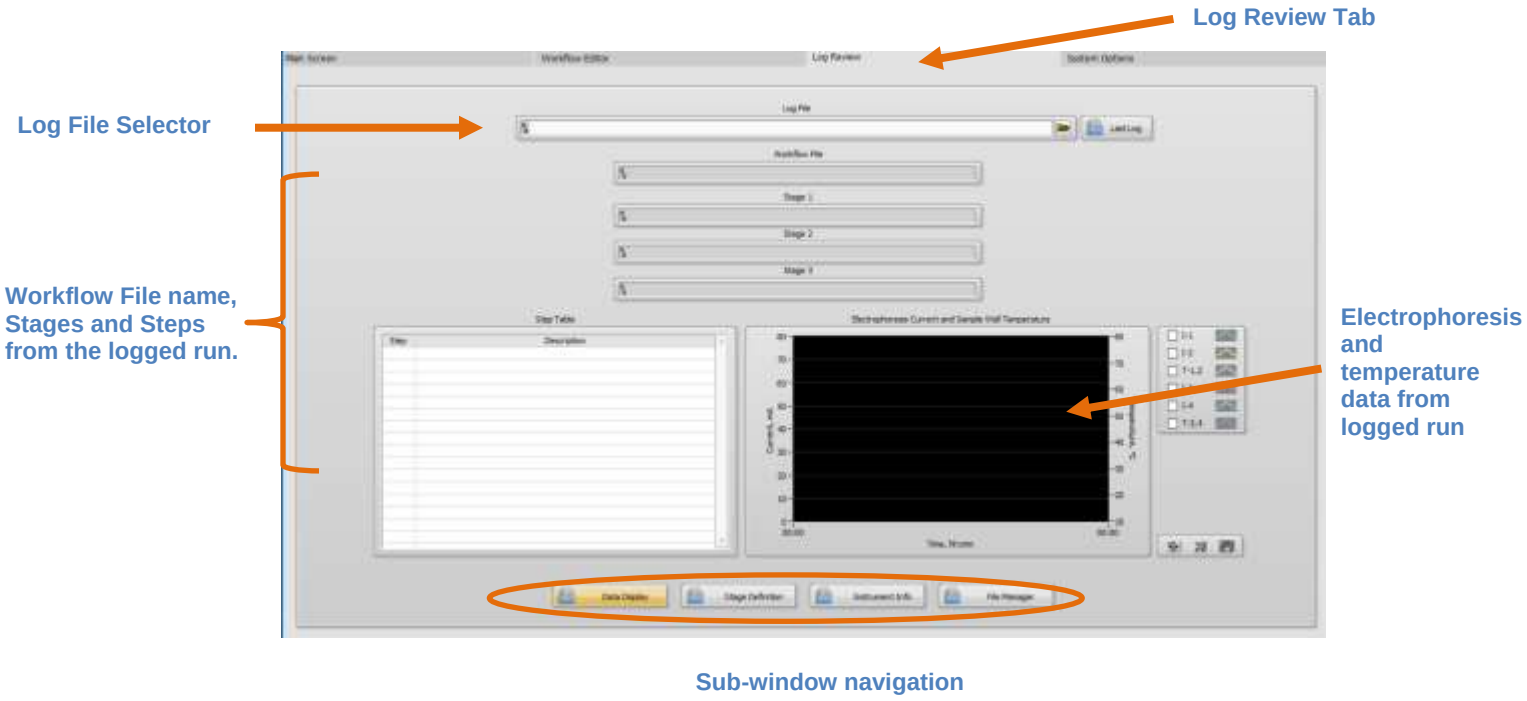
6.17 Log Files

At the end of every workflow, the SageHLS will save a log file (.txt) and an image file (.png) of the Main Screen at the end of the run. Additionally, a log file (.txt) is saved after every Check Current Test. The Table below summarizes the SageHLS log files.

File Type	Folder	Contents	Use
Workflow Log (.txt)	\Logs	Date/time, electrophoretic current data (mA), Temperature data, Sample ID, workflow stages and steps	View in Log Review screen; support for troubleshooting
End of Run screenshot (.png)	\Logs	Image of Main Screen at the end of the run	support for troubleshooting
Current Test Log	\QC	Date/time, electrophoretic current data (mA) from Current Test	support for troubleshooting

6.18 Log Review Tab

In the SageHLS software, navigate to the **Log Review**. It is the third tab from the left.

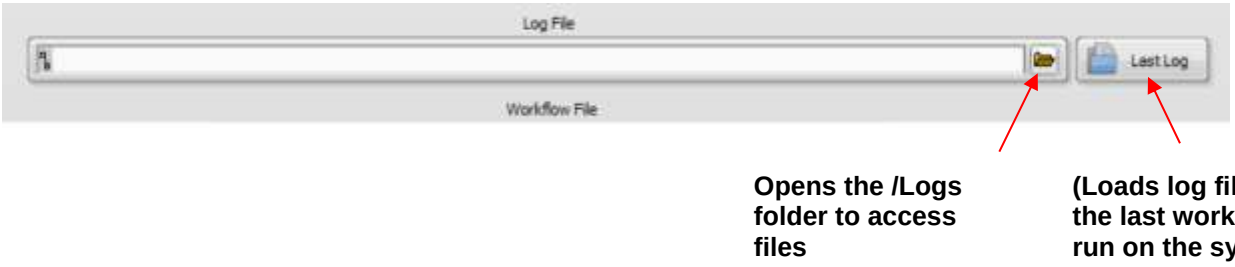


The Log Review has four sub-windows:

- **Data Display (Default Window):** Shows Workflow information and collected data from a log file.
- **Stage Definition:** Shows parameter detail for each Stage in the workflow included from a log file
- **Instrument Info:** Display Instrument information (Name, serial number and software version) included in the log file.
- **File Manager:** A allows copying, deletion and transfer (to and from a USB storage device) of SageHLS files.

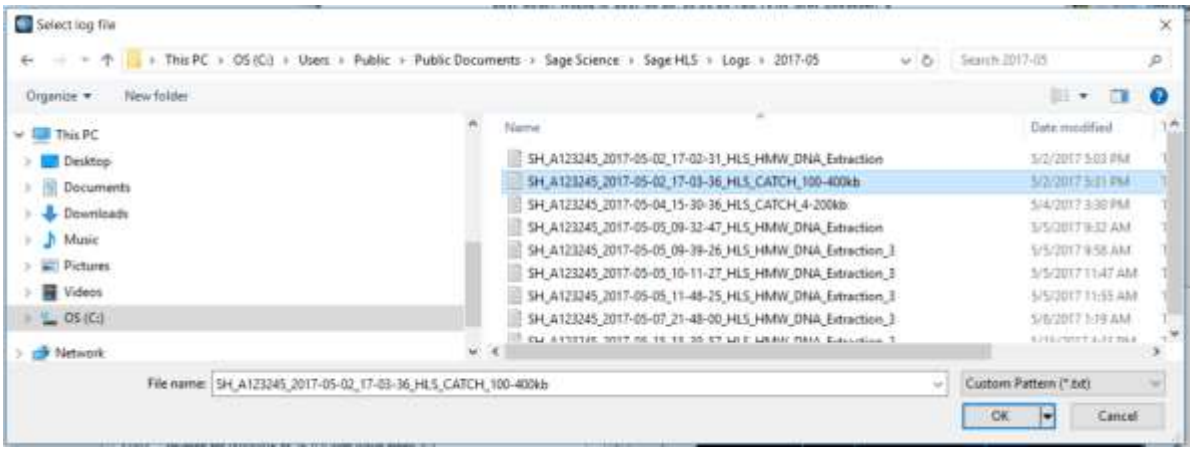
6.19 Log Data Display

From the Data Display sub-window, click the folder icon on the Log File field:

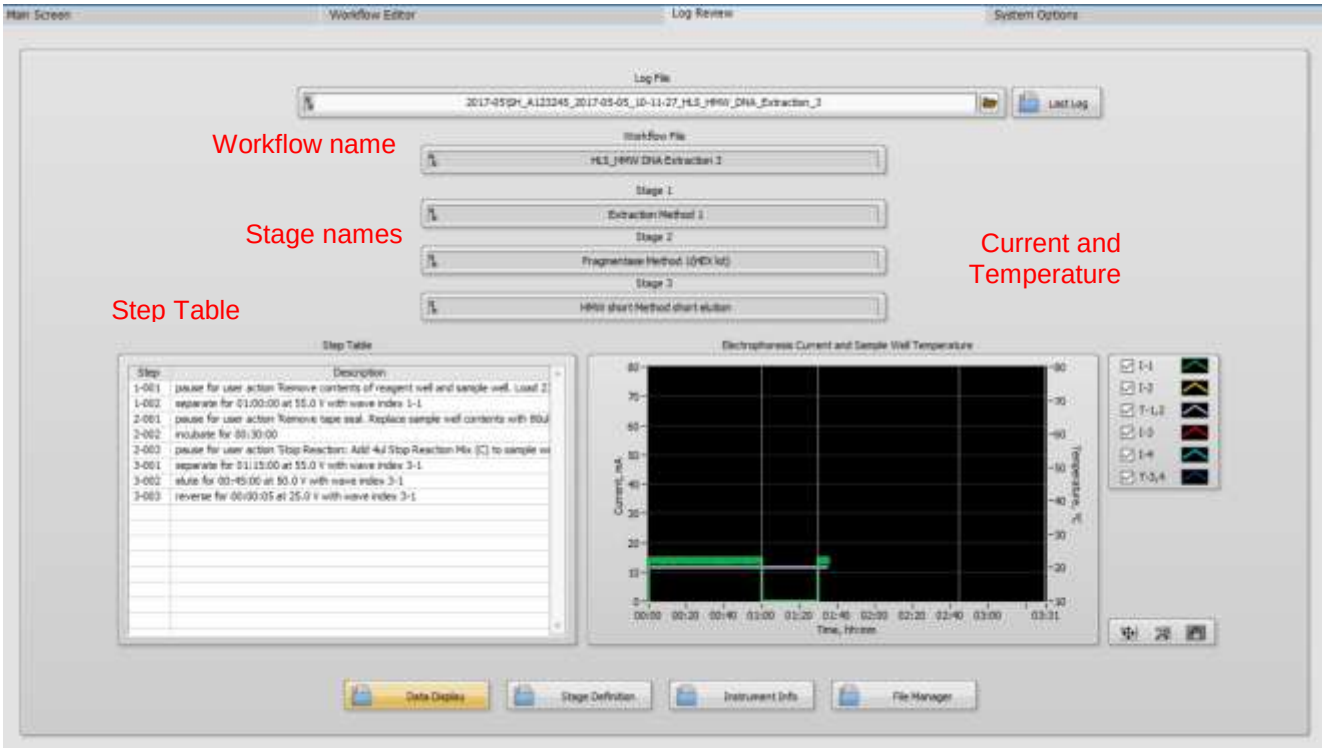


A window will appear listing the log files in the /Logs folder. Older log files are stored in sub-folders that are automatically created and labelled by year/month (yyyy-mm).

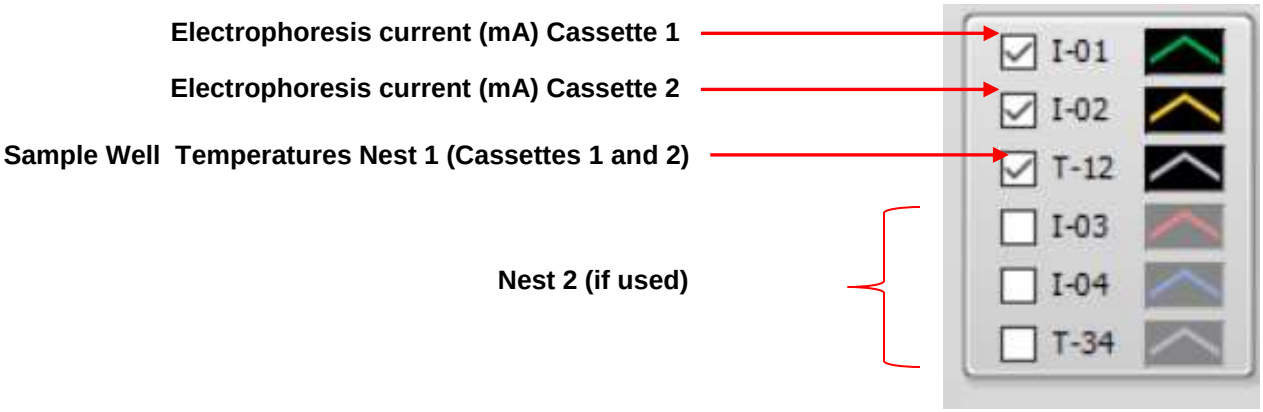
Log files are automatically named with instrument name_serial number_date_ time (at end of run)_workflow name.



When a log file is selected the data will populate the designated fields. The Data Display sub-window will display the Stage, Steps, Electrophoresis current and Sample well Temperature data.

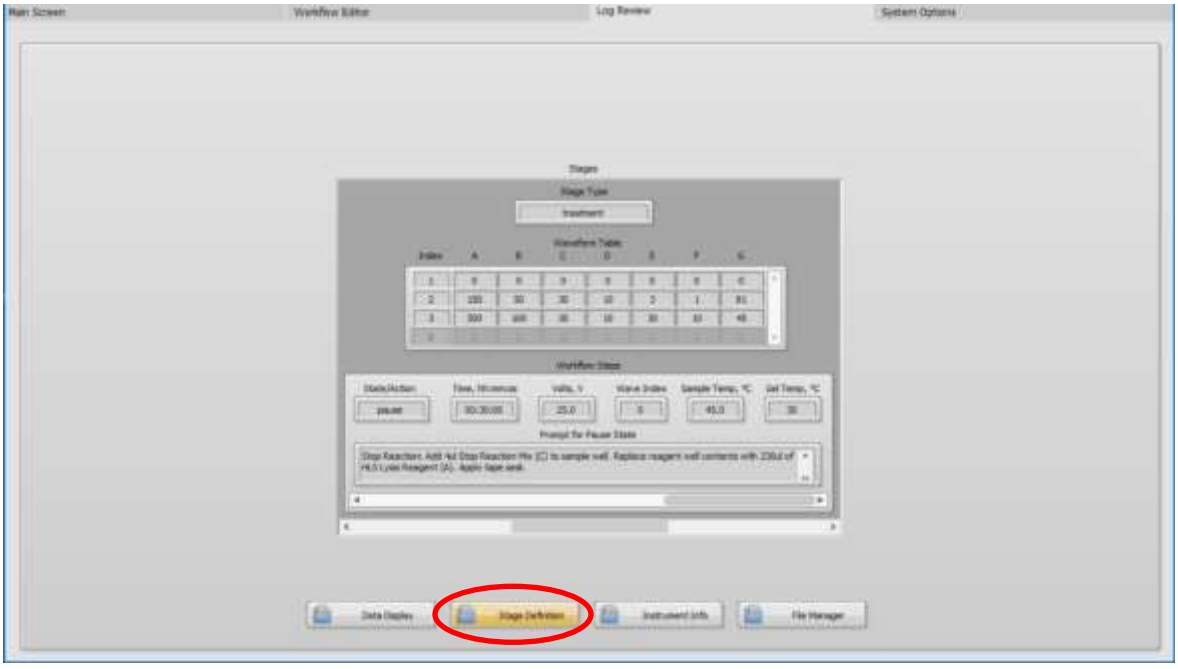


The current and temperature are displayed as follows. The current (mA) is on the left y axis, and the temperature is on the right y-axis. Time (during the run) is on the x-axis.



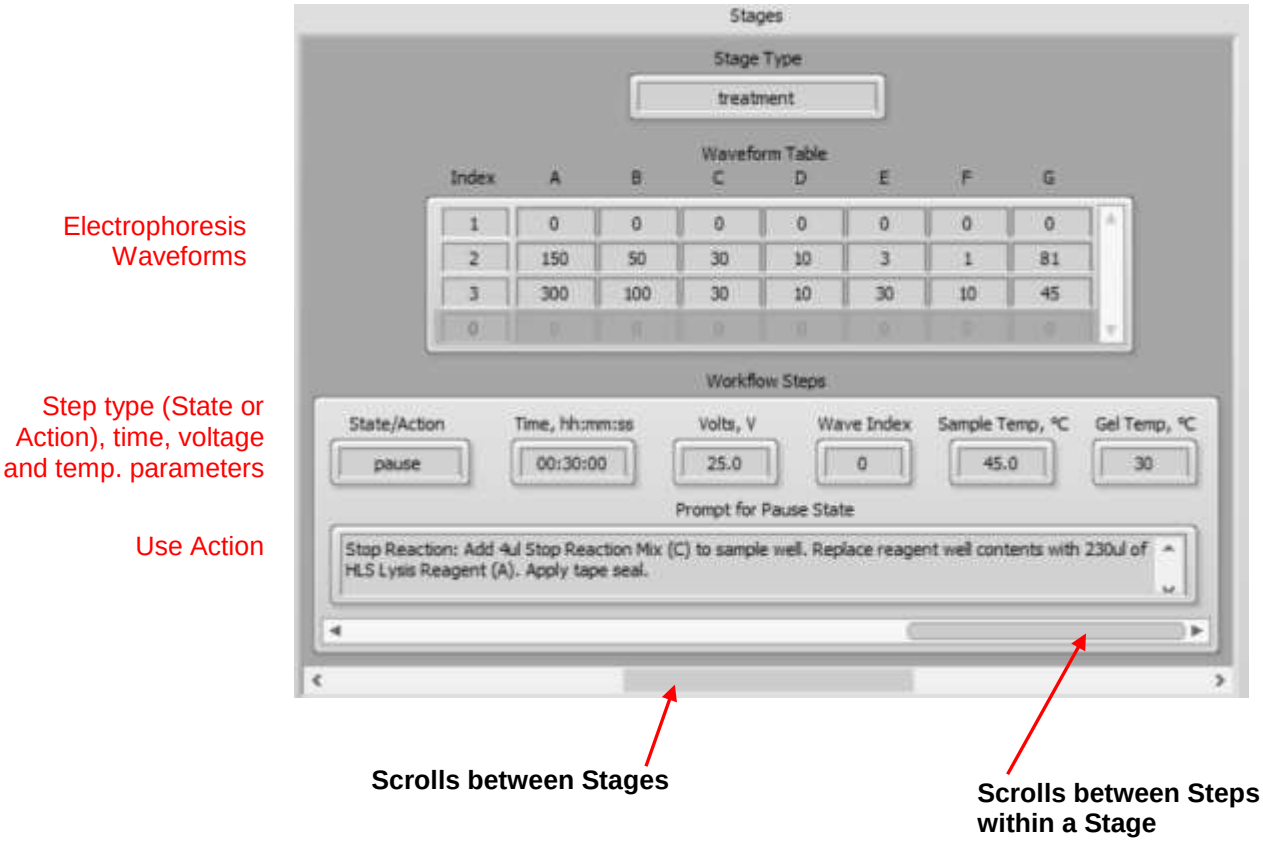
6.20 Stage Definition Sub-Window

The Stage Definition sub-window displays the parameter details for each Step in each Stage for the Workflow that was associated with the loaded log file.



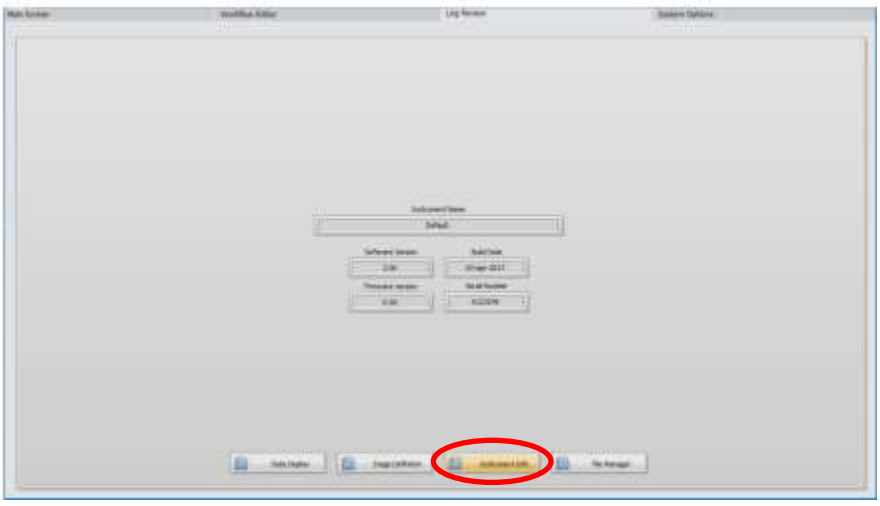
Stage Definition

The Stage Definition Window includes 2 slide bars. The lower slide bar scrolls between Stages. The upper slide bar scrolls between the Steps within the Stage.



6.21 Instrument Info Sub-Window

The Instrument sub-window displays instrument information that was associated with the loaded log file.

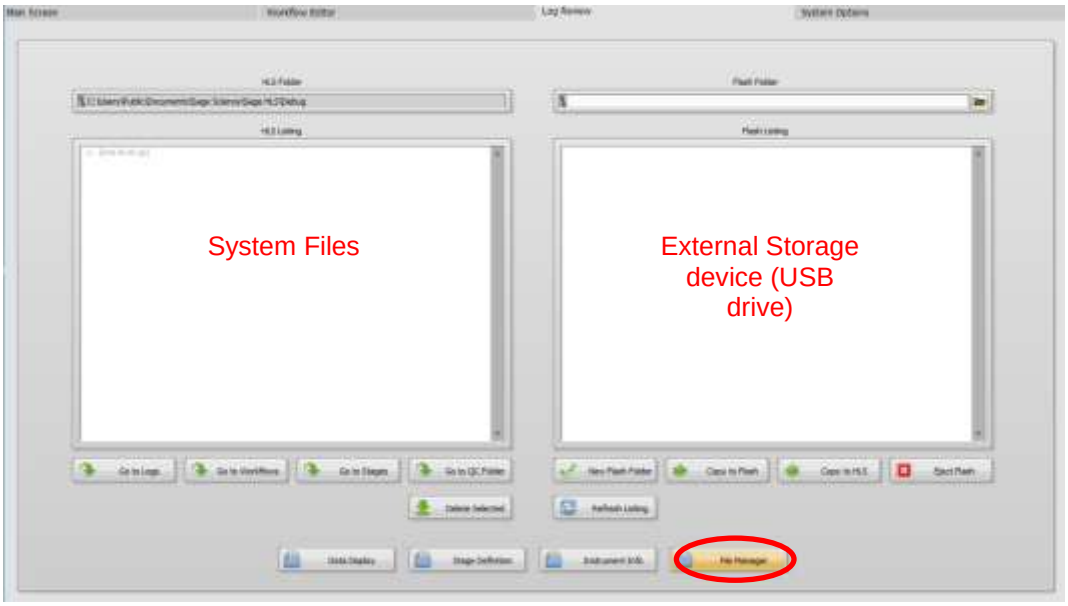


Instrument Info

The Instrument Info displays information about the instrument and software revision of the system at the time the log file was collected.

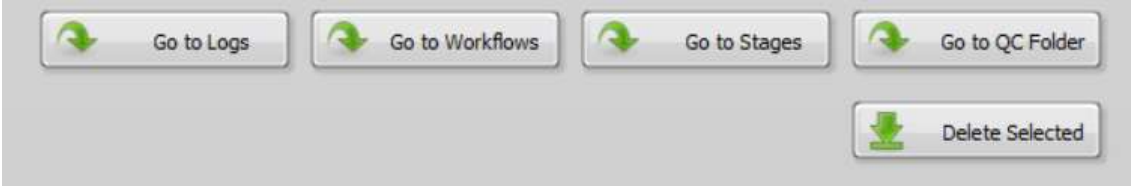
6.22 File Manager Sub-Window

The File Manager sub-window provides an interface for users to manage files on the SageHLS system, including Log files, Workflow files, and Stage files. The File manager allows the transfer of file between SageHLS system and an external USB drive.



File Manager

Several buttons are associated with managing files on the SageHLS instrument, or between the instrument and an external USB drive.



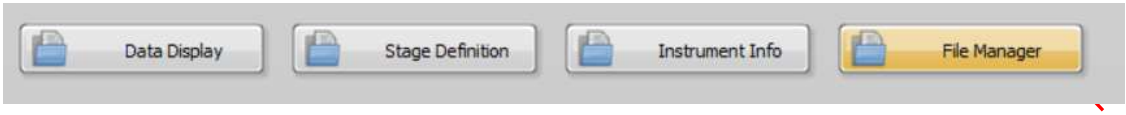
- **Go to Logs**: goes to the log file directory
- **Go to Workflows**: goes to the workflow file directory
- **Go to Stages**: goes to the stage file directory
- **Go to QC folder**: goes to the Current Test log file directory
- **Delete Selected**: deletes files that are highlighted

- **New Flash Folder**: creates a new folder on a connected USB device
- **Copy to Flash**: copies highlighted files from the SageHLS directory to a USB device
- **Copy to HLS**: copies highlighted files from a USB device to the SageHLS
- **Eject Flash**: allows safe removal of USB device
- **Refresh Listing**: deletes files that are highlighted

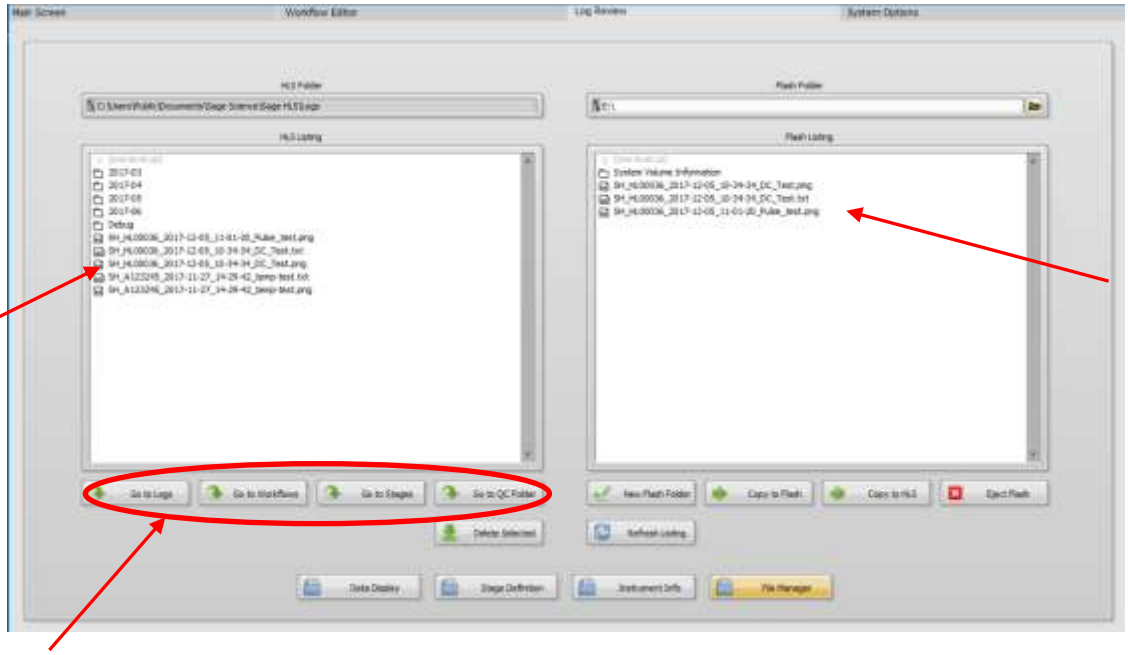
6.23 Copying Files To and From a USB Device

1. Insert a USB storage device into the USB port on the front panel of the SageHLS.
2. In the software, click on the Log Review Tab.

3. At the bottom of the Log Review Screen, press the “File Manager” button.



4. Files located on the SageHLS system will appear in the directory window on the lefts side of the screen, and the files located on the USB device will appear on the right side of the screen.



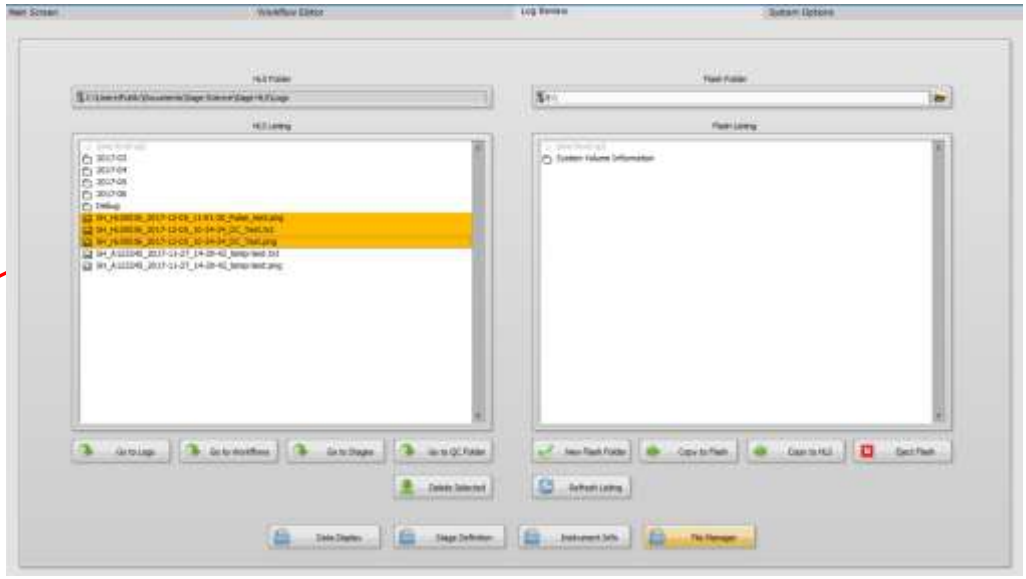
Files stored on the SageHLS

Files stored on the USB device

Buttons navigate to file folders containing types of SageHLS generated files.

5. Files can be highlighted by clicking the file names. Multiple files can be highlighted by holding the Shift button and clicking.

Click to highlight. Shift-click to highlight multiple files.

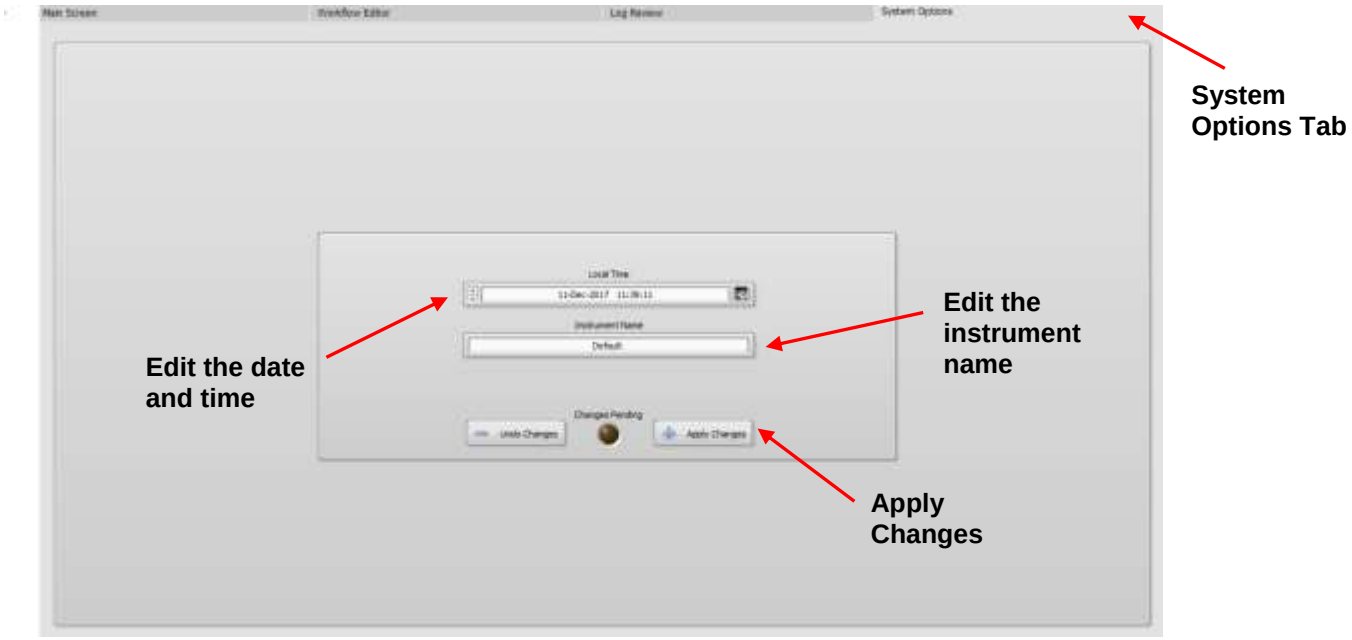


6. Press “Copy to Flash” (or “Copy to HLS”, if moving files from a USB Device to the SageHLS).

7. Press “Eject Flash” to safely unmount the USB device. The USB device can be removed from the USB port on the front panel.

6.24 System Options Tab

The System Options Tab is fourth Tab on the right. The time and date can be adjusted from this screen. The SageHLS may also be given an Instrument name ("Default" is the default name). These may be changed or edited. The "Changes Pending" light will flash if an edit is made. Press "Apply Changes" to accept the edits.



6.25 Updating SageHLS Software and Workflows

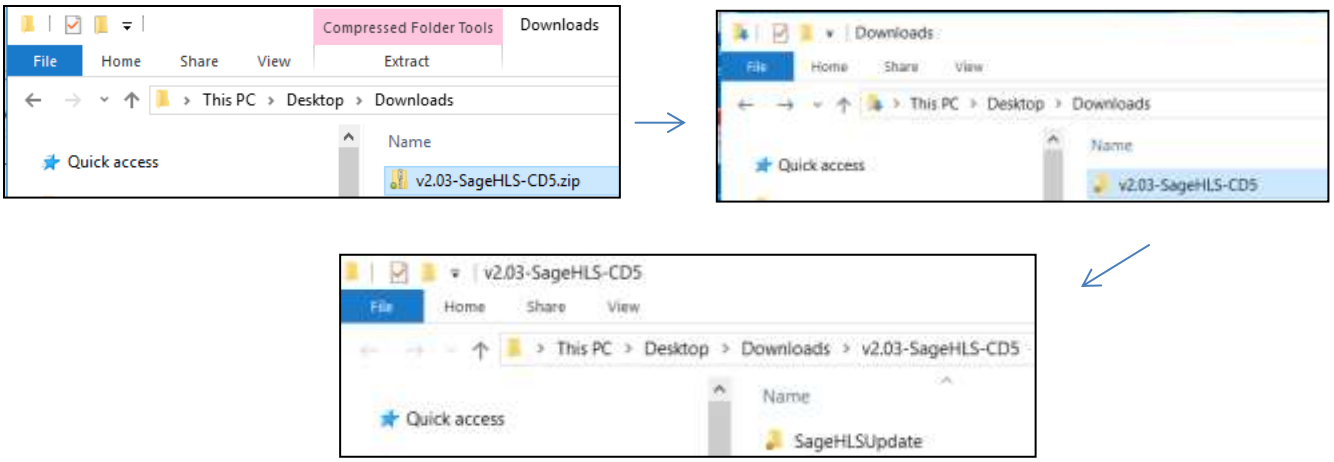
Extracting the file to a USB flash drive

SageHLS upgrade software is available for download at www.sagescience.com/support/. The files are provided in a zip file, and the contents must be extracted to the root directory of a USB flash drive:

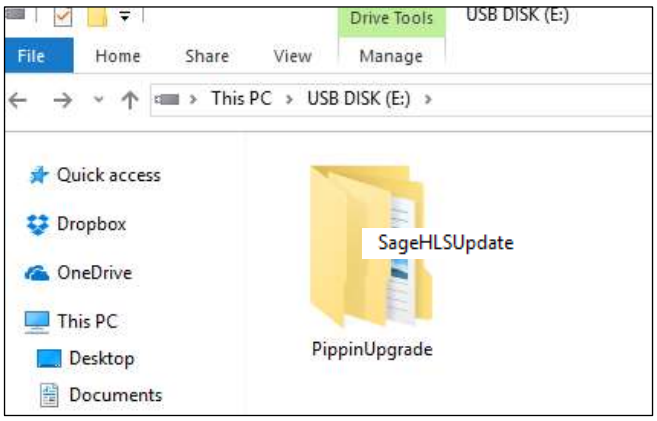
1. Download the Software Upgrade package by pressing the appropriate link on www.sagescience.com/product-support/sagehls-support/ (in the "Software Downloads" section).

2. Browsers may prompt users to save the file. The zip file will likely save to a "Downloads" folder on your computer. The file will be named *[version number]-[instrument]-CD[cassette definition version].zip* (e.g. v2.03-SageHLS-CD5.zip).

3. Open the zip file or unzip its contents onto your computer. It should contain a single folder with the same name as the zip file.



4. Within this folder is a subfolder called “SageHLSUpdate”. Transfer this “SageHLSUpdate” folder into the root directory of a USB drive.

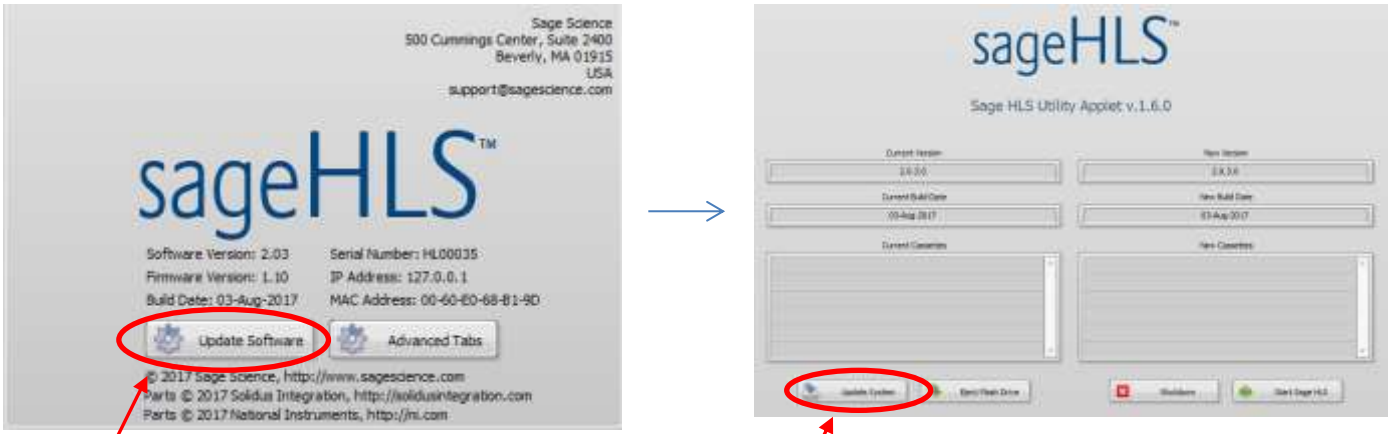


Upgrading SageHLS Instrument Software

1. Insert the USB drive with the upgrade folder into the USB port on the front panel of the SageHLS instrument.

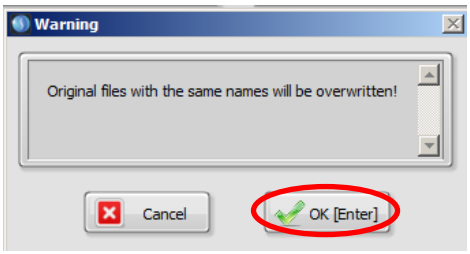
2. From the Main Tab, Press “Display Info”. This will open the information window.

3. From the information window, press “Update Software”. This will open the Software Upgrade window (SageHLS Utility Applet). Press the “Update System” button.

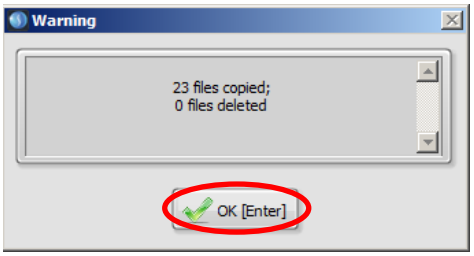


Update Software **Update System**

4. A warning window will appear. Press “OK”:



5. After the files have copied, a second window will appear. Press “OK”.



6. Press “Eject Flash Drive”, then remove the USB drive.

7. Press “Start SageHLS” to return to the main screen. The upgrade will be installed and the instrument ready to use.

8. To check that the software instrument has been updated, press “Display Info” again. In the information window, the software version number is listed on the left side:

Appendix A: Cell Suspension Workflow Guides

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Cell Suspension
Workflow Guide

E. Coli Spheroplasts
PN# CEL-ECO1

Reagents Supplied by Sage Science			Storage Temp.
1 ea.	Wash Buffer, 25 ml	**E1**	4ºC
1 ea.	Spheroplast Buffer, 40 ml	**E2**	4ºC
1 ea.	Qubit Lysis Buffer, 25 ml	**E3**	RT

Materials Supplied or Prepared by User	Supplier	Cat#
Ready-Lyse™ Lysozyme Solution, 4 X 10	Epicentre®	**R1804M**
Molecular Biology Grade BSA, 20 mg/ml	NEB®	**B9000S**
Qubit™ Fluorometer and HS DNA Assay kit	Thermofisher	**Q32851**
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)	N/A	
Rich broth media, LB broth or Trypticase Soy Broth	N/A	

Important!

- **A day prior to HLS procedure**, start an overnight culture of the strain to be analyzed in rich broth media (e.g., LB or Trypticase Soy Broth).
- Maximum recommended cell load per lane for HLS cassettes contains **2.5-10 μg of genomic DNA**.
- Irrespective of input cell load, final input sample volume should be fixed at **70 μl per lane**.

CELECO1 Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	8-2	Added Revision Change Log

Cell / Spheroplast preparation

1. A day prior to HLS procedure, start an overnight culture of the strain to be analyzed in rich broth media (e.g., LB or Trypticase Soy Broth).

2. The next day, start two 3ml rich broth cultures from saturated overnight culture by 1/50 dilution (60 ul of overnight diluted into 3ml rich broth).

3. Grow the two 3ml cultures at 37°C, with shaking for 2 hours. (OD 600 should be around 1 for wild type E. coli strains like MG1655).

4. Pool the two log phase 3ml cultures and mix. Dispense four 1.0 ml aliquots of log phase cell culture into four 1.5ml micro tubes. Immediately chill all four tubes on ice for a few minutes.

5. Pellet cells at room temperature 14,000Xg (max speed in Eppendorf microfuge) for 1 min. Discard all supernatant.

6. Wash each of the 4 pellets by complete resuspension (with vigorous vortexing) in 1ml of Sage Wash Buffer (**E1**). Re-pellet cells as in step 5. Use a P1000 to carefully remove all supernatants. Keep tubes on ice when not handling them.

7. Resuspend each of the 4 pellets in 200 ul Sage Wash Buffer (**E1**) by pipetting and vigorous vortexing.

8. Pool all four 200 ul aliquots of resuspended cells into one 1.5 tube, pellet again, and resuspend entire cell population in 200 ul of Sage Wash Buffer (**E1**).

9. Prepare fresh Sage Spheroplast buffer+BSA by mixing 1 mL of Sage Spheroplast Buffer (**E2**) with 5uL of NEB nuclease-free BSA (20 mg/ml).

10. Dilute Ready-Lyse lysozyme (Epicentre Technologies, Cat #R1804M): 2.5 ul Ready-Lyse into 100 ul of Sage Spheroplast Buffer+BSA. Mix by gentle pipetting several times. (Lysozyme is prone to shear denaturation.)

11. Add 1.5 ul of diluted lysozyme in Spheroplast Buffer +BSA to the 200 ul of E. coli cells. Mix by gentle pipetting several times.

12. Allow lysozyme digestion to proceed on lab bench at room temperature for 30 minutes, then hold digest on ice.

13. Measure DNA content of spheroplast preparation using Qubit lysis method (Next Page).

14. Adjust spheroplast concentration to desired DNA content using Sage Wash Buffer (**E1**). Input volume should be 70ul irrespective of cell load. Maximum cell load should be limited to 10 ug genomic DNA per HLS lane

Quantification of DNA in a cell suspension using the Qubit HS assay

1. Gently mix the cells/spheroplasts by swirling, gentle vortexing, or pipetting. Transfer 10uL aliquots in triplicate to 1.5 ml microcentrifuge tubes.

2. To each microfuge tube add 190uL of Sage Qubit lysis buffer (**E3**) and mix by pipetting up and down several times. The mixing should be rapid but should not generate bubbles or foaming - the goal is to disperse the cells quickly before they lyse and form a condensed clot of DNA.

3. Vortex the tubes as vigorously as possible for 30 – 60 seconds.

4. Add 600uL of TE (10 mM TrisHCl, 1mM EDTA, pH 7.5 to 8.0) to each tube, and vortex at maximum speed for 30-60 seconds.

5. If necessary, centrifuge (maximum speed) for 2 to 5 seconds to reduce any foam.

6. Dispense 5 ul of diluted lysed samples from step 5 into Qubit assay tubes.

7. Perform Qubit HS assay on the 5 ul sample from step 6. Use 195 of Qubit HS reagent for each lysed sample, and pipette Qubit assay mixture up and down several times to ensure homogeneity.

8. Read the Qubit assay tube concentration. Depending on the Qubit model, the Qubit tube concentration can be expressed as ng/ml or ng/ul (check!). Assuming the readout is ng/ml, DNA content of the original cell/spheroplast suspension can be calculated as:

[Qubit tube conc, ng/ml] X (800/10) X (200/5) = [DNA conc original cell suspension, ng/ml]

The first term is the dilution factor involved in making the diluted lysate (steps 2 and 4, above), and second term is the dilution factor for the Qubit HS assay (steps 6 and 7).

9. Average the three replicates to estimate the DNA content of the original suspension.

10. If replicates vary by >15%, the cells in the original suspension may be lysed or clumped.

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Cell Suspension Workflow Guide

Mammalian White Blood Cells from Whole Blood PN# CEL-MWB1

Reagents Supplied by Sage Science			Storage Temp.
1 ea.	10X RBC Lysis Buffer, 275 ml	**M1**	4ºC
1 ea.	Suspension Buffer, 30 ml	**M2**	4ºC
1 ea.	Qubit Lysis Buffer, 25 ml	**M3**	RT
12 ea.	Cell strainers		

Materials Supplied or Prepared by User	Supplier	Cat#
Qubit™ Fluorometer and HS DNA Assay kit	Thermofisher	**Q32851**
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)	N/A	

Important!

- Whole blood should be collected with Acid Citrate Dextrose (ACD) or sodium EDTA anticoagulants, and stored at 4°C.
- Blood should be used within 5 days of collection.
- Maximum recommended cell load per lane for HLS cassettes contains **10 ug of genomic DNA**.
- Irrespective of input cell load, final input sample volume should be fixed at **70 ul per lane**.

CELMWB1 Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	9-2	Added Revision Change Log

Isolation of WBC: all steps at 4°C

(Dilute the provided 10X RBC Lysis Buffer (**M1**) in distilled H_20 to make 1X RBC Lysis Buffer. You will need 80ml of 1X RBC Lysis Buffer per cassette. Chill on ice before use.)

1. Mix the blood to ensure that it is a homogeneous solution. Add 12 mL whole blood to 37mL of cold 1X RBC lysis buffer.

2. Incubate for 5 minutes at 4°C. Halfway through this incubation gently invert tube 3 times to mix. The initial opaque, dark red solution will clear and become lighter in color.

3. Centrifuge at 2,400 x g for 4 minutes, decant and discard the supernatant.

4. Add 20mL 1X RBC lysis buffer.

5. Resuspend the cell pellet gently by pulsing 3 times for 2 to 5 seconds each, on a vortex set to 1800rpm. There should be no visible clumps left after vortexing.

6. Centrifuge at 2,200 x g for 2 minutes. Decant and discard the supernatant.

7. Repeat steps 4-6. The pellet should be almost completely clear of red or pink color.

8. After decanting the supernatant, let the tube sit for 1 minute, then aspirate the remaining buffer using a P1000 pipettor with 1ml tip.

Resuspension of WBCs in HLS Suspension Buffer

1. Add 1mL of Sage HLS Suspension Buffer (**M2**) to the cells and resuspend them by slow, gentle pipetting with a P1000 pipettor. Note that the HLS Suspension Buffer solution is slightly more viscous than RBC lysis buffer due to a higher concentration of sucrose.

2. Examine the solution carefully for clumps, this is best done by drawing the solution into a 1mL pipet tip and holding the tip up to the light.

3. If clumps are visible, filter the suspension through a 40-micron cell strainer (place the strainer in a new tube, and pour or pipet the cells into the strainer. Some tapping may be needed to start the liquid flow). Remove and discard the strainer.

4. Centrifuge for 10 to 20 seconds at 200 RPM to collect all the liquid at the bottom of the tube; the cells should not settle in this step.

5. Quantify the cells using a cell counter or a hemocytometer. Alternatively, determine the DNA content per unit volume of the suspension using the Qubit lysis procedure (Next Page). (The expected concentration of gDNA in the resuspended cell prep is 200 – 300 ng/ul.)

Quantification of DNA in a cell suspension using the Qubit HS assay

1. Gently mix the cells/spheroplasts by swirling, gentle vortexing, or pipetting. Transfer 10uL aliquots in triplicate to 1.5 ml microcentrifuge tubes.

2. To each microfuge tube add 190uL of Sage Qubit lysis buffer (**M3**) and mix by pipetting up and down several times. The mixing should be rapid but should not generate bubbles or foaming - the goal is to disperse the cells quickly before they lyse and form a condensed clot of DNA.

3. Vortex the tubes as vigorously as possible for 30 – 60 seconds.

4. Add 600uL of TE (10 mM TrisHCl, 1mM EDTA, pH 7.5 to 8.0) to each tube, and vortex at maximum speed for 30-60 seconds.

5. If necessary, centrifuge (maximum speed) for 2 to 5 seconds to reduce any foam.

6. Dispense 5 ul of diluted lysed samples from step 5 into Qubit assay tubes.

7. Perform Qubit HS assay on the 5 ul sample from step 6. Use 195 of Qubit HS reagent for each lysed sample, and pipette Qubit assay mixture up and down several times to ensure homogeneity.

8. Read the Qubit assay tube concentration. Depending on the Qubit model, the Qubit tube concentration can be expressed as ng/ml or ng/ul (check!). Assuming the readout is ng/ml, DNA content of the original cell/spheroplast suspension can be calculated as:

   [Qubit tube conc, ng/ml] X (800/10) X (200/5) = [DNA conc original cell suspension, ng/ml]

The first term is the dilution factor involved in making the diluted lysate (steps 2 and 4, above), and second term is the dilution factor for the Qubit HS assay (steps 6 and 7).

9. Average the three replicates to estimate the DNA content of the original suspension.

10. If replicates vary by >15%, the cells in the original suspension may be lysed or clumped.

Cell Suspension
Workflow Guide

Mammalian Tissue Culture Cells
PN# CEL-MWB1

Reagents Supplied by Sage Science			Storage Temp.
1 ea.	10X RBC Lysis Buffer, 275 ml (not used)	M1	4ºC
1 ea.	Suspension Buffer, 30 ml	**M2**	4ºC
1 ea.	Qubit Lysis Buffer, 25 ml	**M3**	RT
12 ea.	Cell strainers		

Materials Supplied or Prepared by User	Supplier	Cat#
Phosphate-buffered saline (PBS)	N/A	
Qubit™ Fluorometer and HS DNA Assay kit	Thermofisher	**Q32851**
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)	N/A	

Important!

- Maximum recommended cell load per lane for HLS cassettes contains **10 ug of genomic DNA**, (approximately 1.5e06 human diploid cells).
- Irrespective of input cell load, final input sample volume should be fixed at **70 ul per lane**.

CELMWB1 Workflow Guide
Mammalian Tissue Culture
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
D	E	12/12/17	10-2	Added Revision Change Log

Quantification of DNA in a cell suspension using the Qubit HS assay

1. Obtain cells from culture flask and wash at least 3 times by centrifugation and resuspension in phosphate-buffered saline (PBS) to remove media (centrifugation at 100-200Xg for 5-10 minutes, depending on cell type).

2. After third wash, resuspend cells in a small volume of PBS, and quantify cell concentration with cell counter or hemocytometer. Alternatively, determine the DNA content per unit volume of the suspension using the Qubit lysis procedure (next page).

3. Repellet the cells and resuspend them in Suspension Buffer (**M2)** by gentle pipetting with a 1000ul pipette. The volume of HLS Suspension Buffer used should give a cell suspension containing less than or equal to 10 ug of genomic DNA per 70 ul (the loading volume for the HLS cassette). Note that the HLS Suspension Buffer solution is viscous.

   Note: While cell samples containing less than 10 ug of genomic DNA can be used, the loading volume should remain fixed at 70 ul for best DNA extraction results.

Quantification of DNA in a cell suspension using the Qubit HS assay

1. Gently mix the cells/spheroplasts by swirling, gentle vortexing, or pipetting. Transfer 10uL aliquots in triplicate to 1.5 ml microcentrifuge tubes.

2. To each microfuge tube add 190uL of Sage Qubit lysis buffer (**M3**) and mix by pipetting up and down several times. The mixing should be rapid but should not generate bubbles or foaming - the goal is to disperse the cells quickly before they lyse and form a condensed clot of DNA.

3. Vortex the tubes as vigorously as possible for 30 – 60 seconds.

4. Add 600uL of TE (10 mM TrisHCl, 1mM EDTA, pH 7.5 to 8.0) to each tube, and vortex at maximum speed for 30-60 seconds.

5. If necessary, centrifuge (maximum speed) for 2 to 5 seconds to reduce any foam.

6. Dispense 5 ul of diluted lysed samples from step 5 into Qubit assay tubes.

7. Perform Qubit HS assay on the 5 ul sample from step 6. Use 195 of Qubit HS reagent for each lysed sample, and pipette Qubit assay mixture up and down several times to ensure homogeneity.

8. Read the Qubit assay tube concentration. Depending on the Qubit model, the Qubit tube concentration can be expressed as ng/ml or ng/ul (check!). Assuming the readout is ng/ml, DNA content of the original cell/spheroplast suspension can be calculated as:

   [Qubit tube conc, ng/ml] X (800/10) X (200/5) = [DNA conc original cell suspension, ng/ml]

   The first term is the dilution factor involved in making the diluted lysate (steps 2 and 4, above), and second term is the dilution factor for the Qubit HS assay (steps 6 and 7).

9. Average the three replicates to estimate the DNA content of the original suspension.

10. If replicates vary by >15%, the cells in the original suspension may be lysed or clumped.

Appendix B: HLS Cassette Kit Workflow Guides

sage science

SageHLS Cassette Kit
Workflow Guide

High Molecular Weight DNA Extraction
PN# HEX-0004 or HEX-0012

Reagents Supplied by Sage Science			Storage Temp.
4 / 12 ea.	Agarose gel cassettes		RT
20 / 60 ea.	Adhesive Tape Strips		N/A
1 ea.	HLS Lysis Reagent 3% SDS, 10 / 30 ml	A	RT
1 ea.	Enzyme Buffer, 15 / 40 ml	C	4°C
1 ea.	Running Buffer, 40 / 115 ml	E	RT
1 ea.	HLS Lysis Reagent 3% Sarkosyl, 10 / 30 ml	G	RT
1 ea.	HLS Lysis Reagent 1% SDS, 10 / 30 ml	H	RT

Materials Supplied or Prepared by User	Supplier	Cat#
NEBNext® dsDNA Fragmentase®	New England Biolabs	**M0348S**

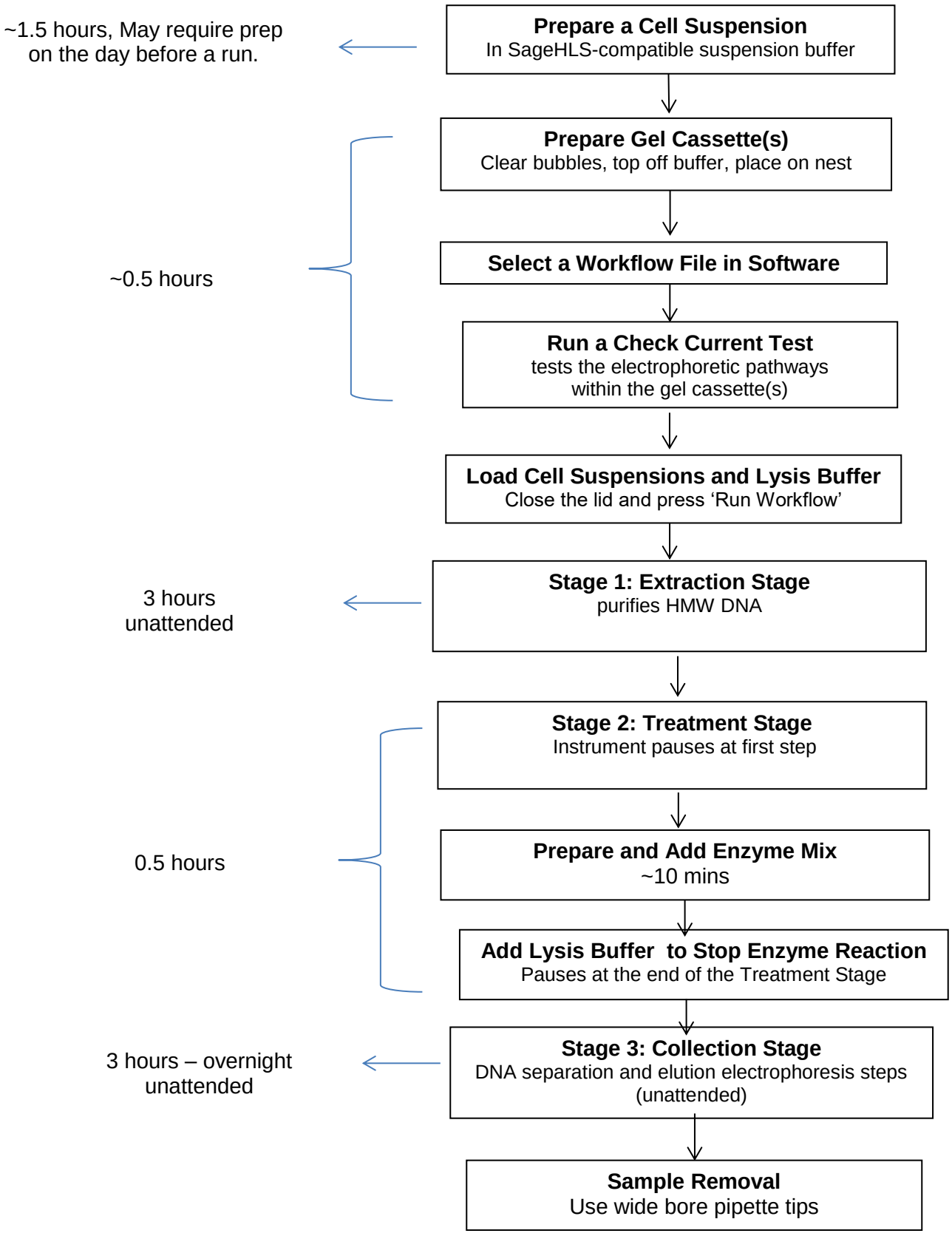
Important!

- Prior to day of extraction, schedule availability of cells. Check that cell preparation reagents are ready.
- Maximum recommended cell load per lane for HLS cassettes contains **2.5-10 ug of genomic DNA**, (approximately 1.5e06 human diploid cells).
- Irrespective of input cell load, final input sample volume should be fixed at **70 ul per lane**.

HEX kit Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	12-2	Added Revision Change Log
D	E	6/18/18	12-1	Updated NEB fragmentase part number
D	E	6/18/18	12-7	Revise Workflow File Names
E	F	8/17/18	12-6	Replaced buffer chamber image to show even fill line.
E	F	8/17/18	12-12	Corrected dilution and math for E.coli fragmentase dilution.

HMW DNA Extraction Workflow Summary

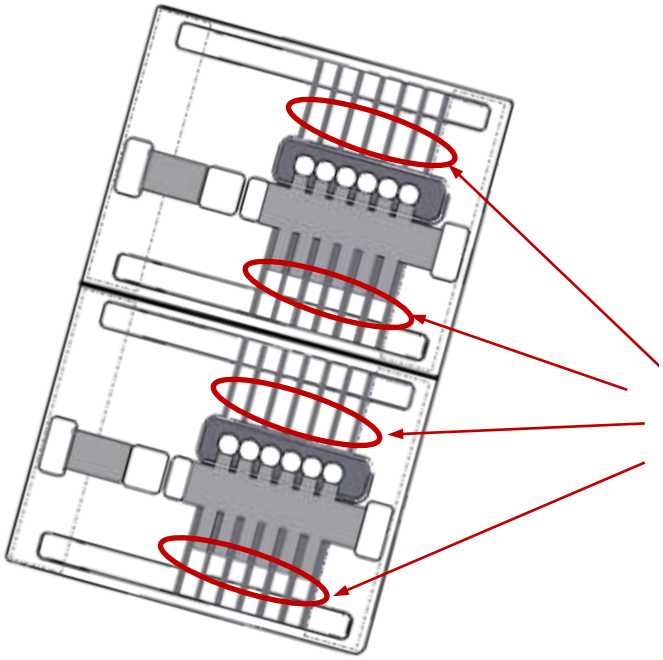


A. Prepare cells using Cell Suspension Guide (Appendix A)

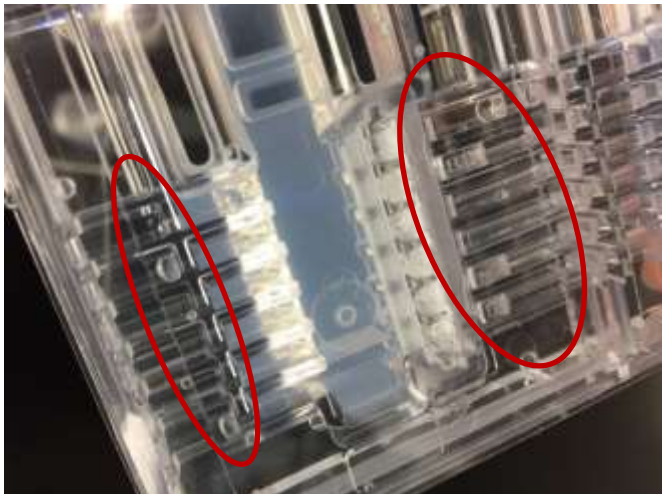
B. Prepare the Gel Cassette(s)

1. Remove the gel cassette from the foil bag.

2. **Before removing tape!** – Hold the cassette with top surface almost vertical and the elution modules above the gel channel. Tap the cassette to dislodge any bubbles that are trapped behind the elution wells, or in the elution channels. Repeat if necessary. Allow the bubbles to collect in the electrode channel directly above.

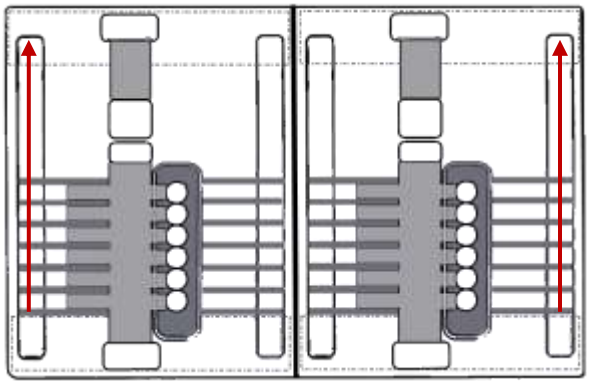


Tap to dislodge bubble from these areas into the troughs directly above.

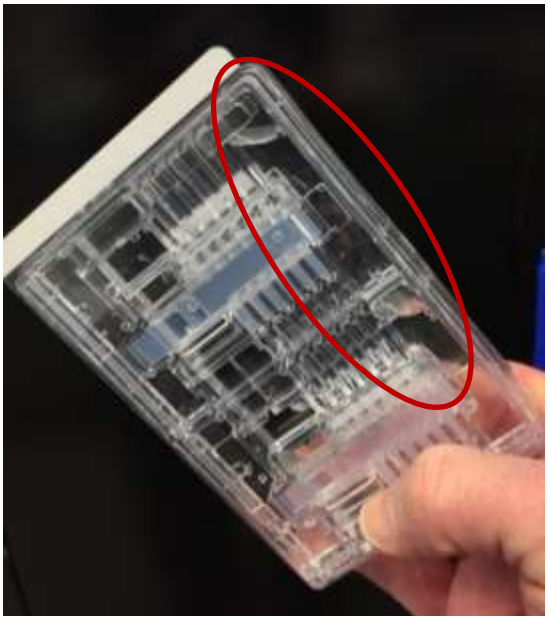


Bubbles in the elution paths can interfere with collection

3 Slowly rotate the cassette to allow the bubbles to collect in the upper buffer area. Gently tap if necessary.



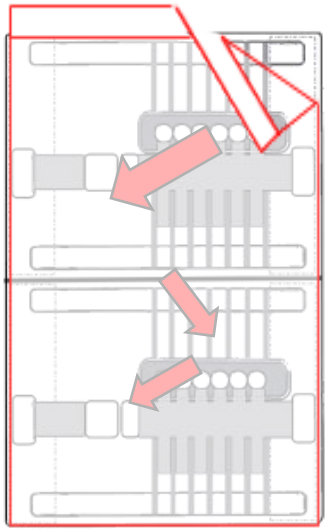
Move any bubbles to the upper buffer area.



Aggregate and collect air bubbles in the upper buffer chambers

4. With cassette held at a slight angle to keep bubbles located in the upper buffer chamber, gently place the cassette onto the nest. The upper buffer chamber should be on the left side of the nest.

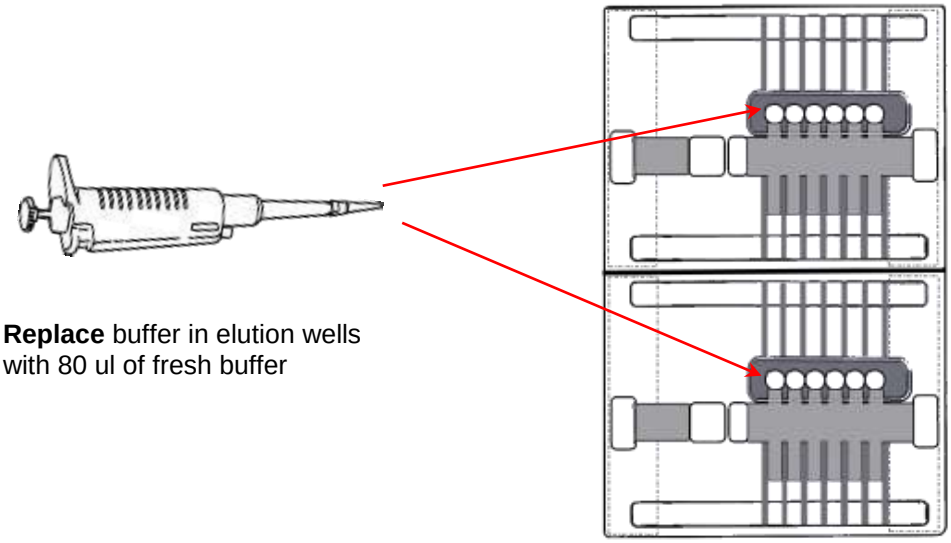
5. While holding the cassette firmly in place on the nest, grab the tape tab, and pull the tape off at an angle, slowly and firmly. Alternate the pulling angle if the tape resists peeling.



Peel back tape at an angle. Alternate angles if the tape resists peeling.

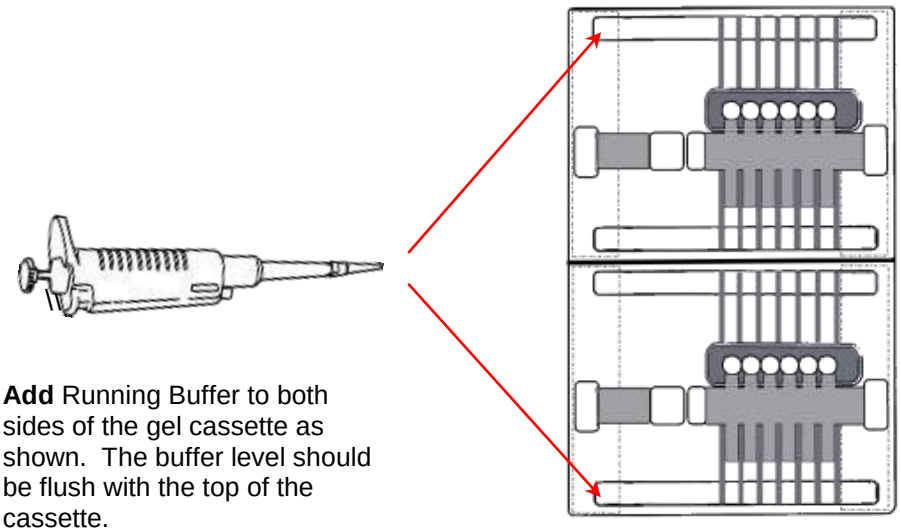
6. Remove all buffer from all elution wells (set a pipette to 100 µl to completely empty the wells). Keep pipette tips vertical in the well to avoid damage to the membranes.

7. Taking care not to introduce additional bubbles into the elution modules, add 80 µl of buffer to all elution wells.



Replace buffer in elution wells with 80 ul of fresh buffer

8. Add **Running Buffer** to the upper buffer chamber on each side of the gel cassette until the level is flush with the top of the cassette. An adequate buffer level is important for achieving best results from the SageHLS.



Add Running Buffer to both sides of the gel cassette as shown. The buffer level should be flush with the top of the cassette.

Fill buffer until it is flush with the top of the cassette

Important! Fill until the buffer level visually reaches the bottom side of the cassette cover.

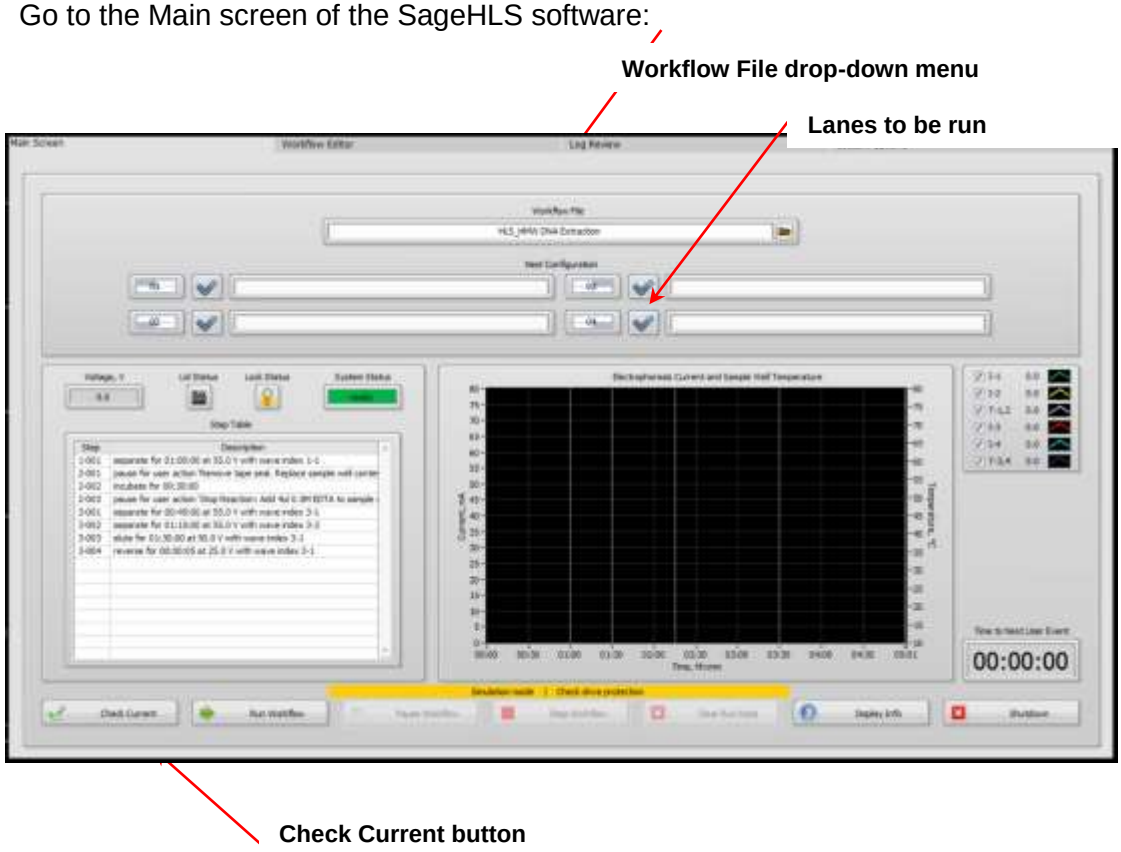
C. Load the Workflow File

1. Use the following Table as a guide to select the most appropriate Workflow File:

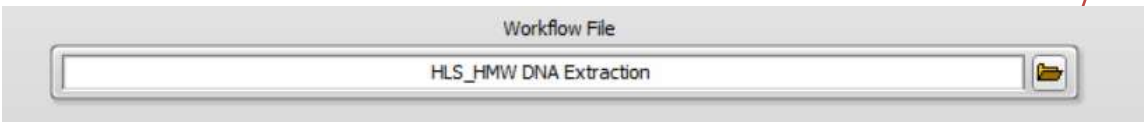
Core Workflow* File Name	Description	Run Time
HMW DNA Extraction	Maximum separation, ultra-HMW fragments in well 2	5:00
HMW High-Pass 50kb elu45	Compression band, 50kb and above, in wells 2 and 3, lower yield	3:30
HMW High-Pass 50kb	Compression band, 50kb and above, in wells 2 and 3	4:20
HMW High-Pass 300kb	3 hour extraction, fragments >300 in well 2	13:00
Non-Core Workflow File Name	**Description**	**Run Time**
HMW High-Pass 250kb	3 hour extraction, fragments >250 in well 2	13:00
HMW High-Pass 350kb	3 hour extraction, fragments >350 in well 2	13:00
HMW High-Pass 500kb	3 hour extraction, fragments >500 in well 2	13:00

*Core work flows have been extensively tested with biological samples. Non-core workflows have only been tested with model DNA samples.

2. Go to the Main screen of the SageHLS software:



3. Select the Workflow File from the drop down menu.



4. Choose the lanes to be used by clicking the boxes next to the lane numbers and enter sample IDs into the adjacent fields (sample IDs are optional or can be entered later).

Check marks indicate which lanes are active

D. Run the Check Current Test

1. Press the “Check Current” button.

2. A pop-up window will appear. Press “Start” to begin the Check Current routine.

Press “Start”

3. The routine will first test the separation electrodes, then test the elution electrodes, and complete within a few minutes. After a successful test, all boxes will fill with green check marks. Press “Return” to continue

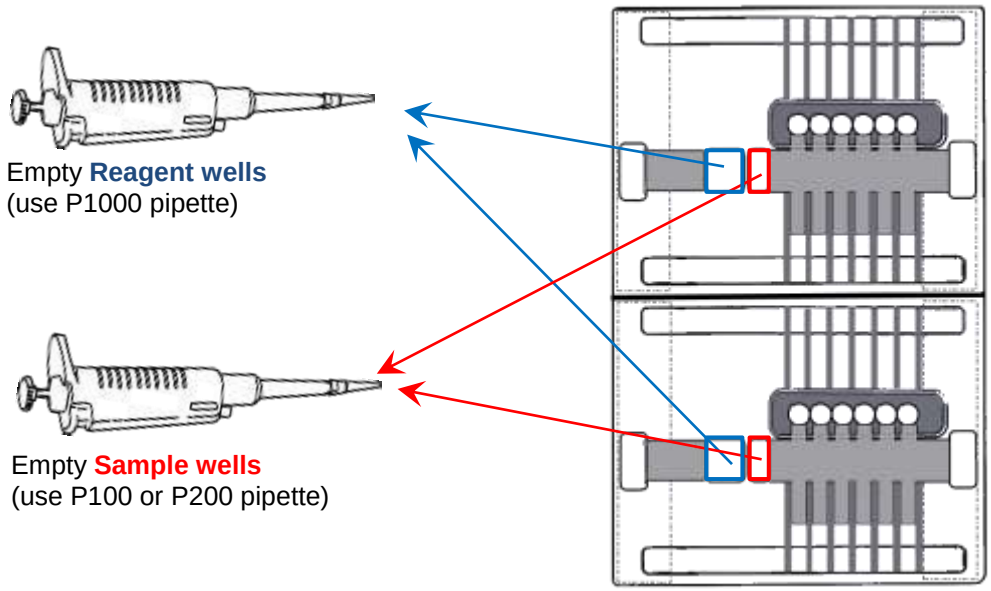
Press “Return”

E. Stage 1: Extraction

1. Use the following Table as a guide to select the most appropriate Lysis Reagent. The extraction step will take 3 hours:

Lysis Reagent Name	Description
HLS Lysis Reagent 3% SDS (**A**)	Maximum DNA recovery, residual SDS (0.01-0.03%) in eluant
HLS Lysis Reagent 1% SDS (**H**)	Less DNA recovery (25-30%), less residual SDS (0.001 – 0.009%)
HLS Lysis Reagent 3% Sarkosyl (**G**)	Less DNA recovery (25-30%), for samples with potassium (alternative to SDS, which co-precipitates in the presence of potassium)

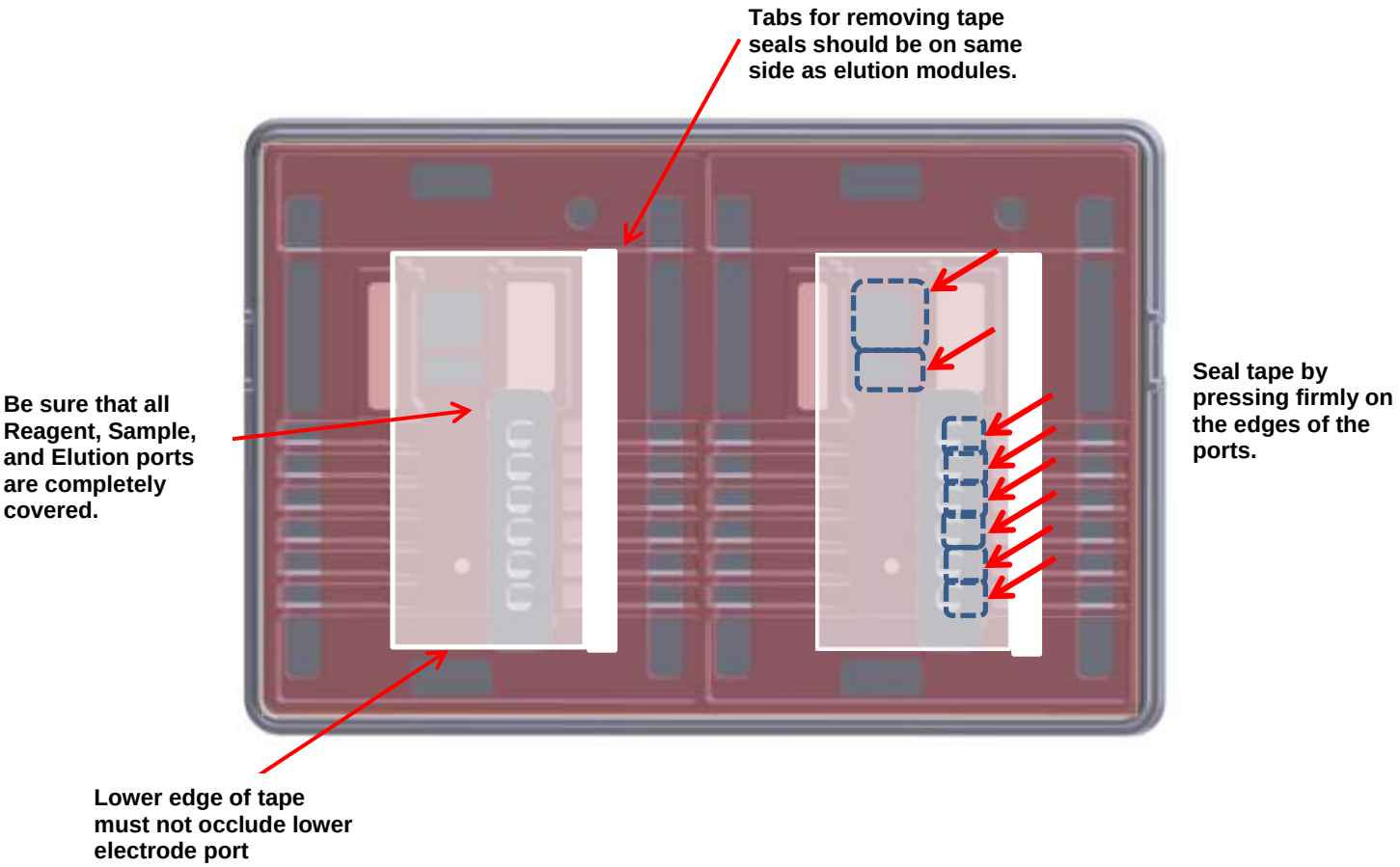
2. Empty all sample and reagent wells. Use caution not to pierce agarose at the bottom of the wells.



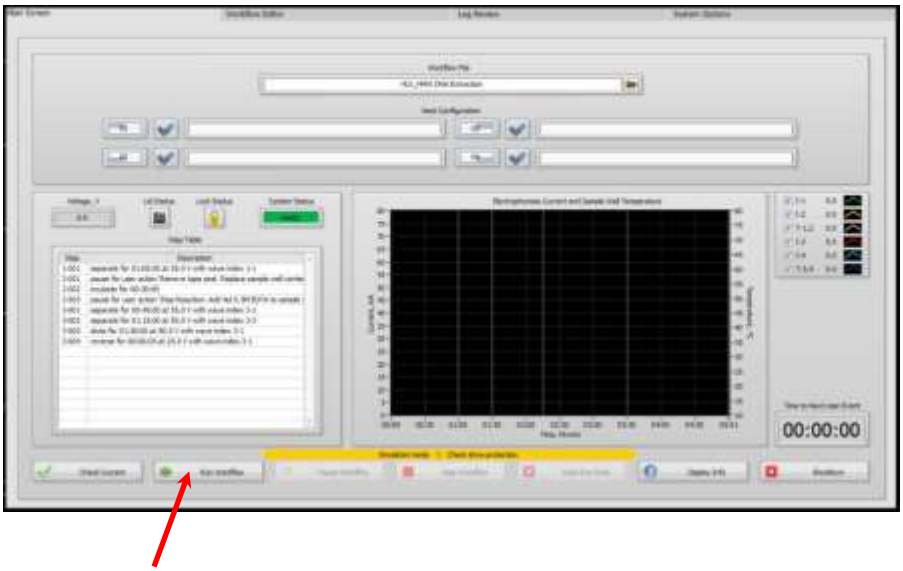
3. Load samples in all lanes. Always use 70ul sample loading volume. (Sample wells will not be completely full.)

4. Fill Reagent Wells with HLS Lysis Buffer. Fill, but do not overfill! Leave a concave meniscus to prevent contact with sealing tape in next step. Approximate volume needed will be 220-230 ul.

5. Seal reagent, sample, and elution ports with supplied tape without occluding the electrode ports. Press tape firmly around edges of the ports.

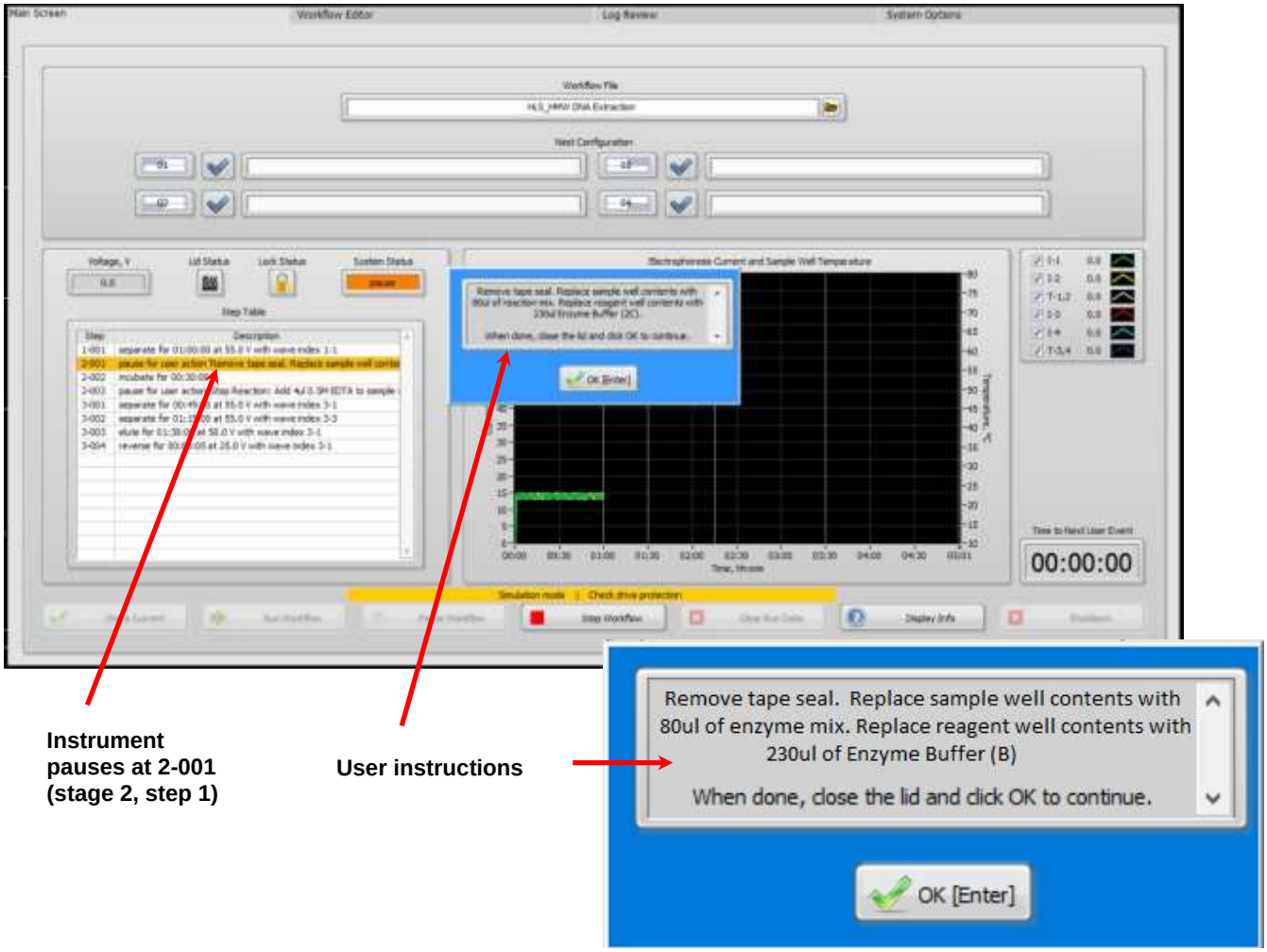


6. Close the lid and press “Run Workflow”. The Extraction step will take 3 hours of unattended operation.



F. Stage 2: Treatment Stage

1. At the end of the Extraction Stage/Step, the SageHLS will pause on the first step of the Treatment Stage and a pop-up window will appear with user instructions.



Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user action.

2. Prepare the Enzyme Reaction Mix:

   a. Remove NEB Fragmentase from the freezer, briefly vortex (1s) to mix

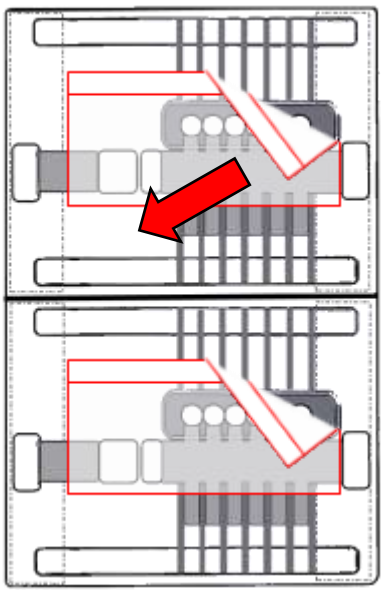
   b. Dilute the NEB Fragmentase (NF) with Enzyme Buffer (**C**) as follows:

Cell Type	Dil.Factor	Fragmentase	Procedure
E.coli	1:600	0.01µl / reaction	i. add 2µl of NF to 28µl of Enzyme Buffer **C**, vortex to mix ii.add 20µl of dilution to 780µl of Enzyme Buffer **C**, vortex to mix
White Blood Cells	1:400	0.02µl / reaction	i. add 2µl of NF to 798µl of EB, vortex to mix

Important! Fragmentase Enzyme Mix should be used within minutes of preparation. It can be prepared at the end of the extraction step and kept on ice. Preparing the mix within 15 minutes of use is recommended.

3. Open the lid and carefully remove the adhesive tape(s). To remove the tapes, grab the tab in right upper corner and peel diagonally with a slow smooth motion.

grab the tab in right upper corner and peel diagonally with a slow smooth motion



Important! Pulling the tape in a diagonal fashion prevents liquid transfer between adjacent elution ports and transfer between the sample/reagent ports and the elution ports.

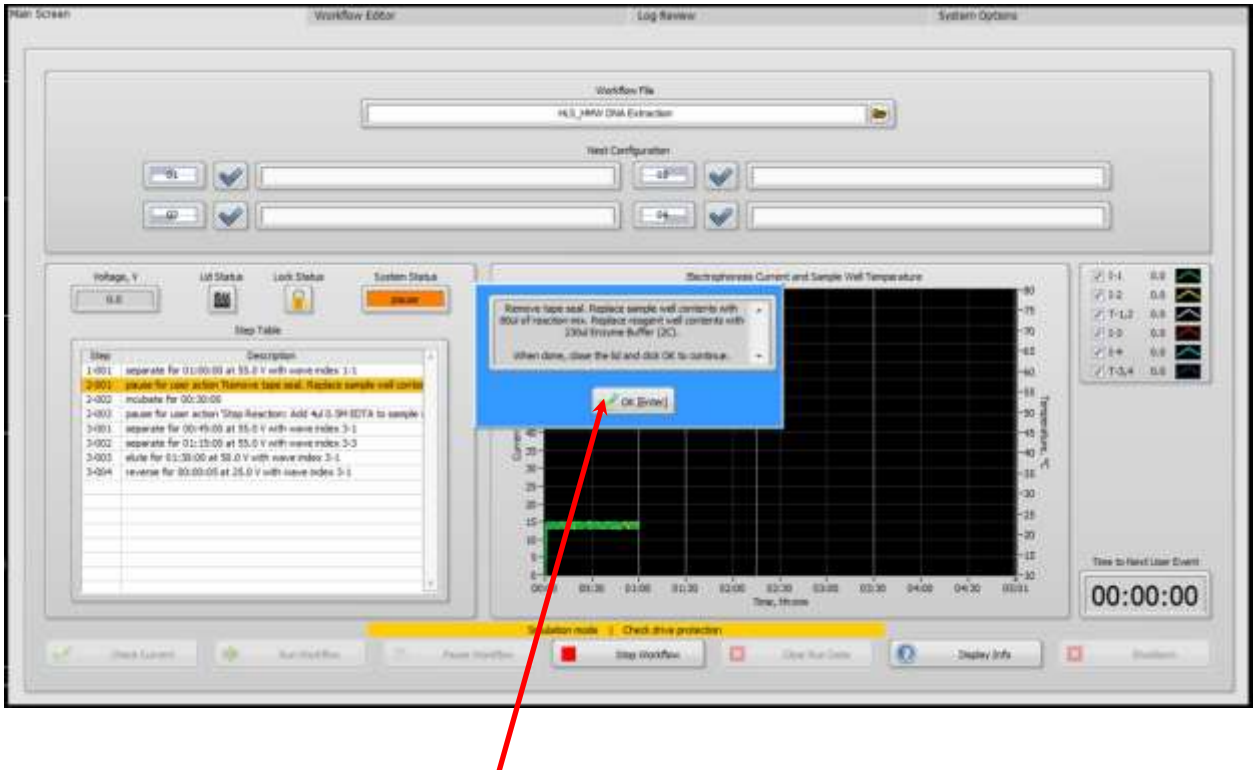
4. Remove <u>all</u> contents from the Reagent wells and Sample wells in the cassettes to be run. The well volumes are 270 µl and 85 µl, respectively.

5. Add 70 µl of the Fragmentase Enzyme Mix to the Sample well.

6. Add 230 µl Enzyme Buffer (**C**) to the Reagent well.

7. Close the lid (**do <u>not</u> re-seal the wells with tape**).

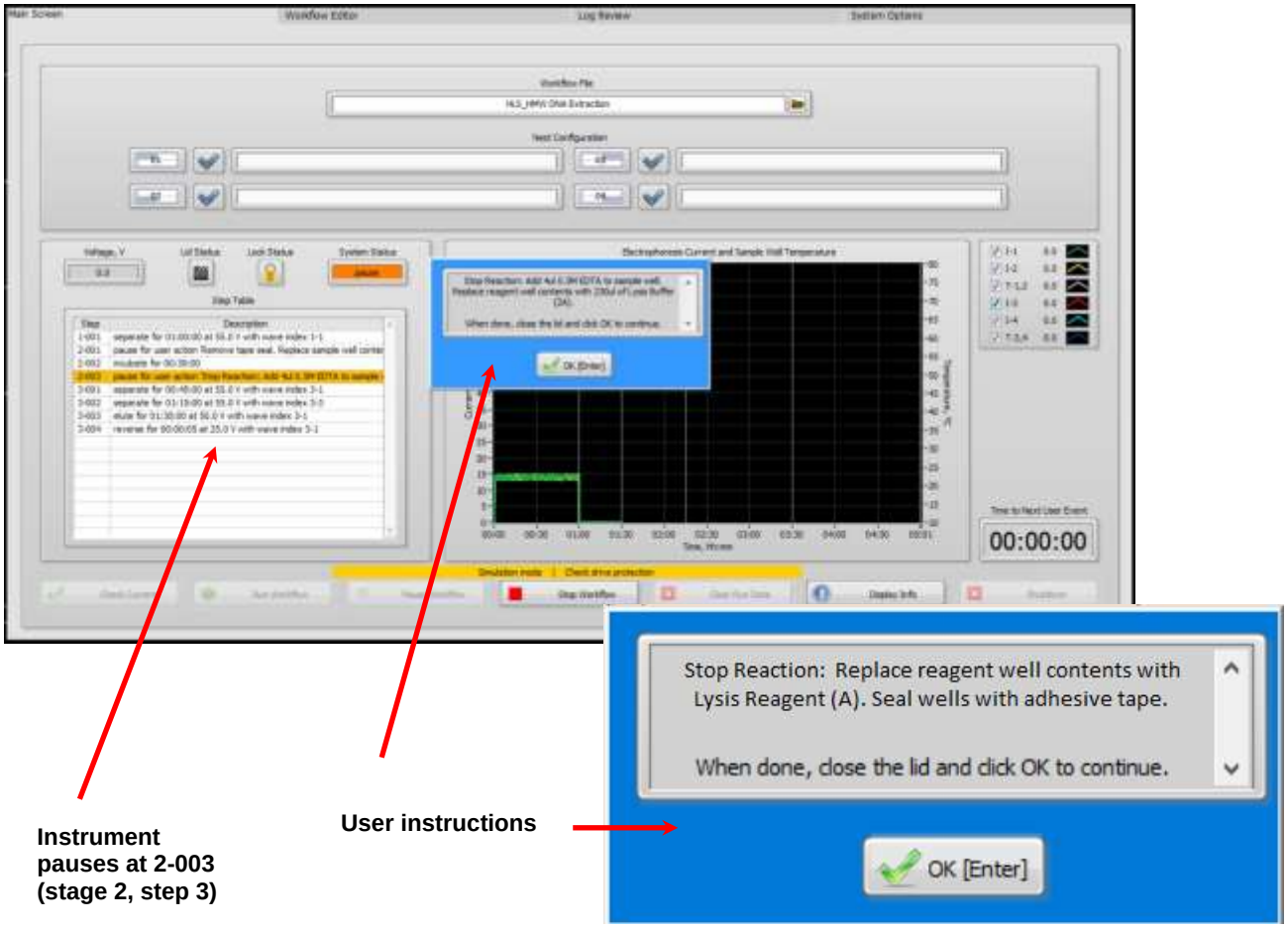
8. Press “OK” in the pop-up window to resume the workflow.



Press “OK” to resume

9. The enzymatic treatment will take **30 minutes**.

10, At the end of **30 minutes** the SageHLS will pause, and a pop-up window with user instructions will appear.

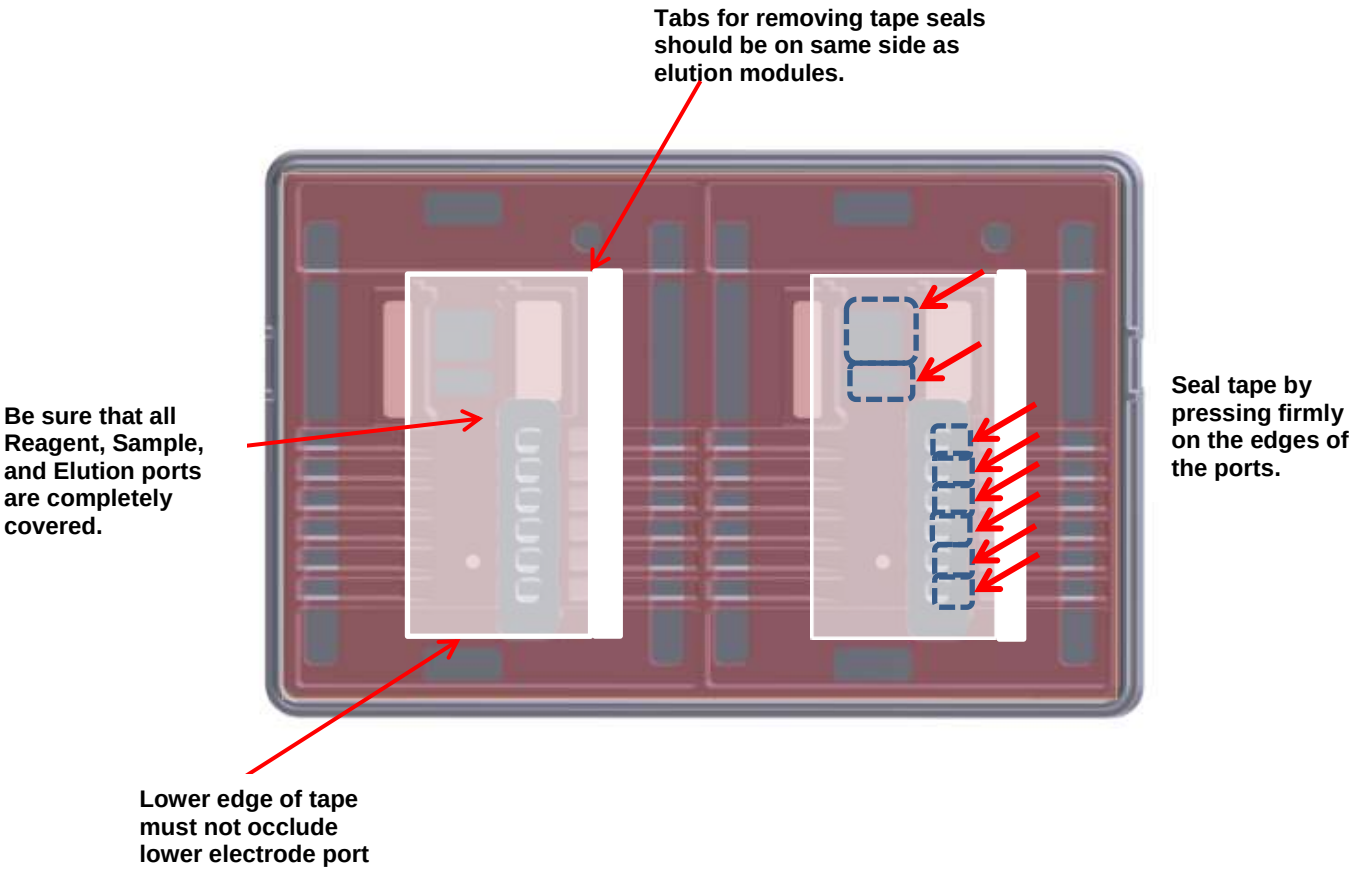


Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user action.

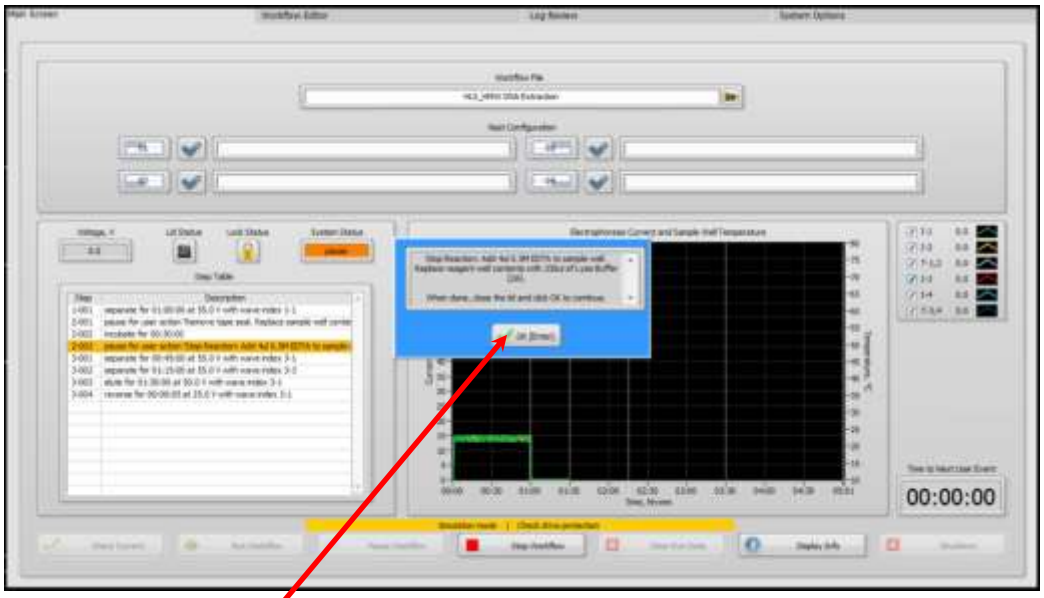
11. Open the lid and remove the contents of the Reagent well.

12. Replace the Reagent well contents with Lysis Reagent (**A**, **G**, or **H**), ~230 ul.

13. Close the lid and **re-seal the cassette wells with tape**.

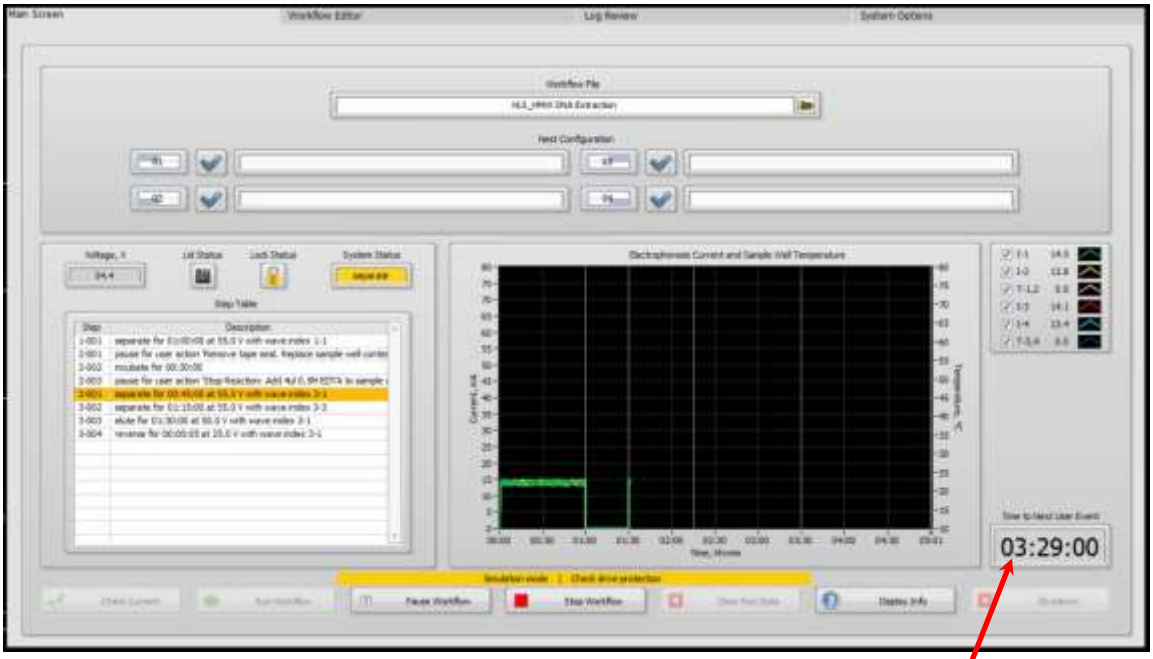


15. Close the lid and press “OK” in the pop-up window to resume the workflow.



G. Stage 3: Collection Stage

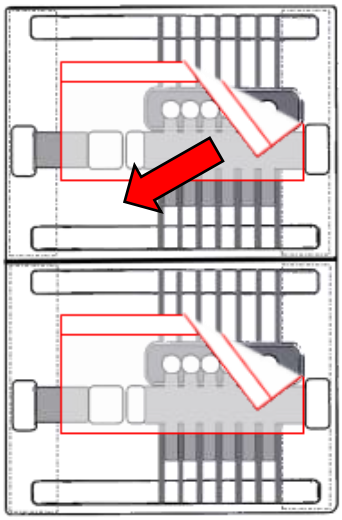
1. The Collection Stage will require several hours of unattended operations. Users should note the time remaining, after which the samples can be collected.



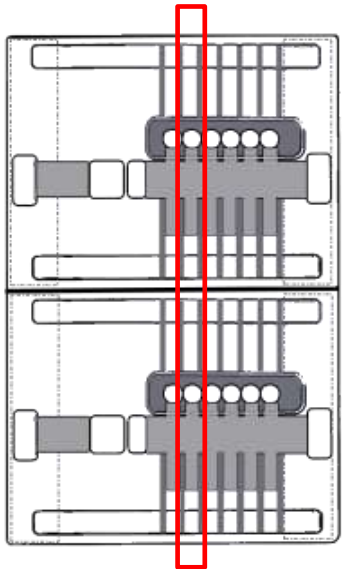
Time remaining to the end of the run

2. After the run is complete, open the lid and remove the sealing tape from the cassette(s).

grab the tab in right upper corner and peel diagonally with a slow smooth motion



3. SageHLS workflows are designed to collect the target range (indicated by target size in HLS-CATCH or highest molecular weight DNA in HMW workflows) in well number 2. This does not guarantee that all targets will be in that well, and users may have multiple size targets. It is highly recommended that the adjacent wells,1 and 3, are sampled for target DNA at the least.



Workflows are designed to collect most targets in well #2

4. Using a wide-bore pipette tip, remove the contents of the elution modules.

Important! Pipette as slowly as possible to avoid shearing the HMW DNA. Use of an electronic pipettor at low speed settings may be helpful. There should be 70-80 ul of liquid in each module.

5. Extremely HMW DNA will be very inhomogeneously distributed in the elution product. To quantify, we recommend Qubit assays using at least three 1 ul aliquots from different locations within the tube. Average the three readings. A high average value with a high CV is diagnostic of very HMW DNA.

For Qubit assays, using at least three 1 ul aliquots from different locations within the tube

sage science

SageHLS Cassette Kit Workflow Guide

HLS-CATCH

PN# HIT-0004 or HIT-0012

Reagents Supplied by Sage Science			Storage Temp.
4 / 12 ea.	Agarose gel cassettes		RT
20 / 60 ea.	Adhesive Tape Strips		N/A
1 ea.	HLS Lysis Reagent 3% SDS, 10 / 30 ml	A	RT
1 ea.	HLS Enzyme Buffer, 15 / 40 ml	C	4°C
1 ea.	Running Buffer, 40 / 115 ml	E	RT
1 ea.	4X Enzyme Buffer (for Cas9-Guide RNA Mix), 1 ml	F	-20°C
1 ea.	HLS Lysis Reagent 3% Sarkosyl, 10 / 30 ml	G	RT
1 ea.	HLS Lysis Reagent 1% SDS, 10 / 30 ml	H	RT

Materials Supplied or Prepared by User	Supplier	Cat#
S.pyogenes Cas9 enzyme, wild type:	New England Biolabs (NEB)	**M0386T** (400 pmol) **M0386M** (2,000 pmol)
Guide RNAs (crRNAs and tracrRNA)	Integrated DNA Technologies(IDT)	custom
Qubit™ Fluorometer/HS DNA Assay kit	Thermo Fisher Scientific	**Q32851** (100 assays)
TaqMan™ RNase P Detection Reagents Kit	Thermo Fisher Scientific	**4316831** (100 reactions)
TaqMan™ Target Detection Reagents	Thermo Fisher Scientific	custom

Important!

- Prior to day of extraction, schedule availability of cells. Check that cell preparation reagents are ready. Prepare cells using Sage HLS cell prep kit instructions.

- Recommended cell load per lane for HLS cassettes contains **2.5 µg of genomic DNA per lane**, (for example ~375,000 human diploid cells) in a maximum loading volume of **70 µl**. Higher cell loads will result in lower recovery of genomic CATCH targets.

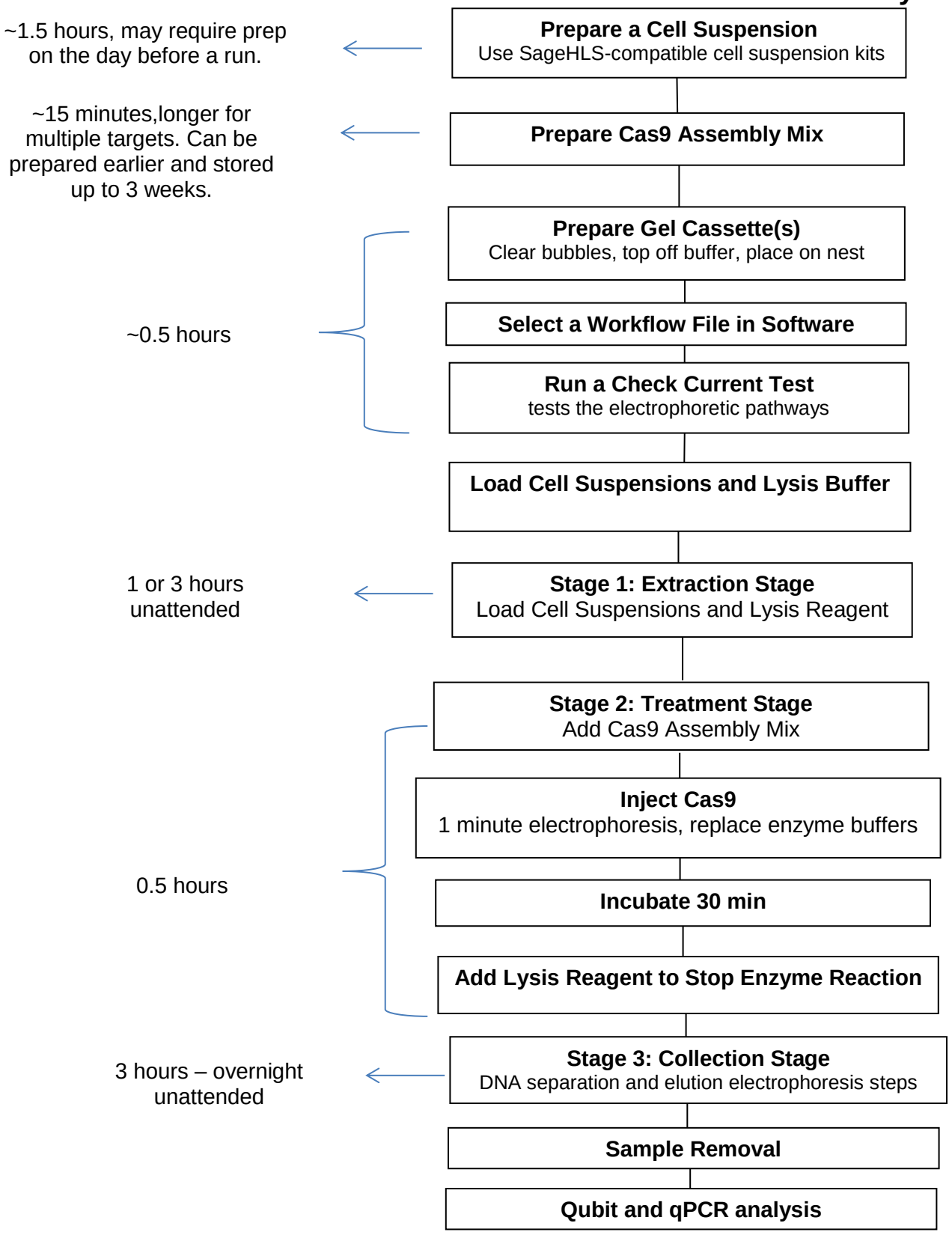
- Assembly of Cas9 require about 30 minutes of effort and should be prepared before starting a SageHLS run. It may also be prepared up to four days in advance and stored at 4°C. Users should take Cas9 reagent preparation time into consideration when planning HLS procedures.

- In addition to the design of Cas9 guide RNAs, users should design and order primer sets and reagents for qPCR quantitation of enriched targets.

HIT kit Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	13-2	Added Revision Change Log
D	E	2/8/18	13-3	Extraction stage in workflow chart changed from 3hr to 1-3 hr
D	E	2/8/18	13-12	Added an optional step at the end of DNA extraction for removal of SDS in the buffer chamber around the anode
D	E	2/8/18	13-15 13-16	Added step to replace Enzyme buffer in the Reagent well in addition to the well after Cas9 injection
E	F	6/18/18	13-1	Change DNA equivalency load recommendation from 10ug to 2.5 ug
E	F	6/18/18	13-3,4,5	Revised Cas9 guideRNA assembly procedure
E	F	6/18/18	13-9,10	New workflow file naming conventions and descriptions
E	F	6/18/18	13-18	New step to (remove running buffer) to reduce SDS in eluants
E	F	6/18/18	13-23	New step to replenish running buffer after the treatment stage
E	F	6/18/18	13-9,27	Modified extraction times – 1 hr for 10X platforms, 3 hr for PacBio and Oxford platforms
E	F	6/18/18	13-27,28	Added description of Qubit and qPCR analyses
F	G	8/17/18	13-6	Replaced buffer chamber image to show even fill line.
G	H	10/24/18	13-4 13-5	Removed Cas9 gRNA assembly -20C storage recommendations. Not verified at that temperature.

HLS-CATCH Workflow Summary



A. Prepare cells using Cell Suspension Guide

Prepare cells using SageHLS cell prep kit instructions (kits/protocols for mammalian WBCs, tissue culture cells, and bacterial spheroplasts are available). Recommended cell load will contain ~2.5 ug genomic DNA per lane (for example, ~375,000 human diploid cells per lane) in a maximum loading volume of 70 ul. Higher cell loads will result in lower recovery of genomic CATCH targets.

B. Prepare the Cas9 Enzyme Assembly (can be done in advance)

The Cas9 assembly can be prepared ahead of time and stored up to 4 days at 4 °C without loss of activity. The mix can be stored on ice (thawed first, if necessary) prior to use.

Summary

- The two-part guide RNAs are annealed by heating to 95C and cooling to room temperature.
- The annealed guide RNAs are mixed with the Cas9 enzyme and incubated briefly at 37C.
- The final assembled enzyme is diluted to the correct volume (80 ul) for loading into the HLS sample well.

1. Anneal the two-part IDT ALT-R™ guide RNAs (crRNA and tracrRNA)

a. Dissolve each of the crRNAs and tracrRNAs to be used at **100uM** concentration in IDT Duplexing Buffer. The correct volume for resuspension in microliters will be 10 times the number of nanomoles of RNA in the tube supplied by IDT.

b. Use the table below to prepare the Guide RNA Annealing Mix to a volume of 22 ul for the preparation of one sample. Users should scale accordingly if the same guides will be used for multiple sample treatments.

To a 200 µl PCR tube, add:

order of addition	reagent	vol. µl	stock [ ] µM	Final [ ] Annealed gRNA Mix µM
1	**IDT Duplexing Buffer**	15.4		
2	**Pooled crRNAs***	4	100	18.2
mix and spin				
4	**tracrRNA**	2.6	100	11.8
mix and spin				
	Total	**22**		

***For instance, if two crRNAs are to be used, add 2 ul of each crRNA solution. If four crRNAs are to be used, add 1 ul of each crRNA solution. For more crRNAs, mix equal volumes of each crRNA (using some convenient volume), and then add 4 ul of the resulting crRNA mixture to the PCR tube. **The goal is to saturate the tracrRNA with a 1.5-fold molar excess of total crRNA**.)

d. Remove the mix from heat and allow to cool on the bench-top for 3-5 minutes.

e. Centrifuge for 30-60 seconds to collect any condensation.

f. Stored up to 4 days at 4 ºC.

2. Assemble Annealed Guide RNAs and Cas9 mixture

a. Make sure the frozen reagents have been thawed: 4X Enzyme Buffer (**F**), NEB Cas9 nuclease

b. Using the following order of addition, assemble the gRNA-Cas9 reaction mixture:

To a 200 µl PCR tube, add:

order of addition	reagent	vol. µl	stock [ ] µM	final [ ] in Enzyme Mix µM
1	4X Enzyme Buffer (**F**)	10	4	1
2	Annealed Guide RNA Mix	22	11.8 (tracrRNA)	6.5 (tracrRNA)
mix and spin				
3	NEB Cas9 nuclease, wt	8	20	4
	Total*	**40**		

***40 ul will be diluted to 80 ul before loading in a single sample well. Users should scale accordingly.

c. Mix by pipetting up and down.

d. Incubate at 37ºC for 10 minutes in a thermal cycler with a heated lid to assemble enzyme and gRNAs.

e. Proceed to next step (Dilution with HLS Enzyme Buffer) or place on ice until ready.

3. Dilute to loading concentration with HLS Enzyme Buffer

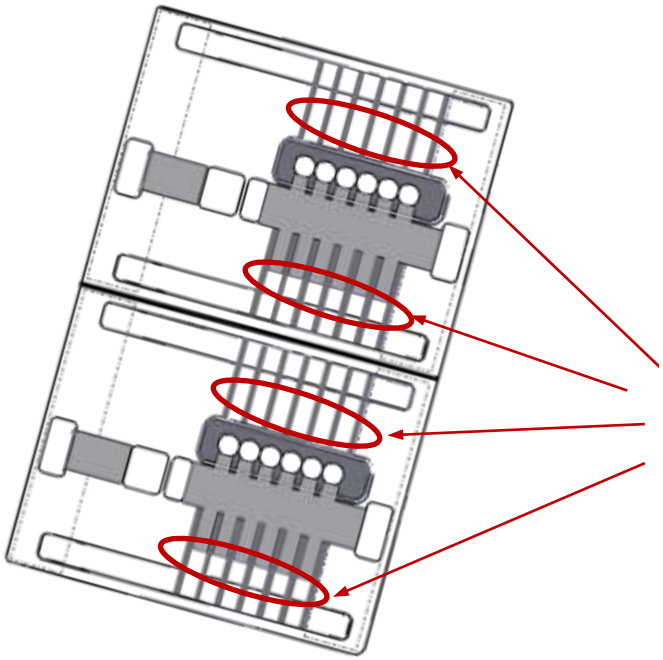
Dilute the assembled Annealed Guides/Cas9 mixture from Step 2 (40ul), with 40 ul of HLS Enzyme Buffer (**C**). Mix thoroughly by gentle pipetting of the entire mixture (80 ul). (Final conc: 2 uM Cas9 assembled with 3.25 uM pool of annealed two-part gRNAs)

At this point the Cas9 mixture is ready to load in the HLS sample well. The mixture can be stored on ice, or at 4C, for several hours.

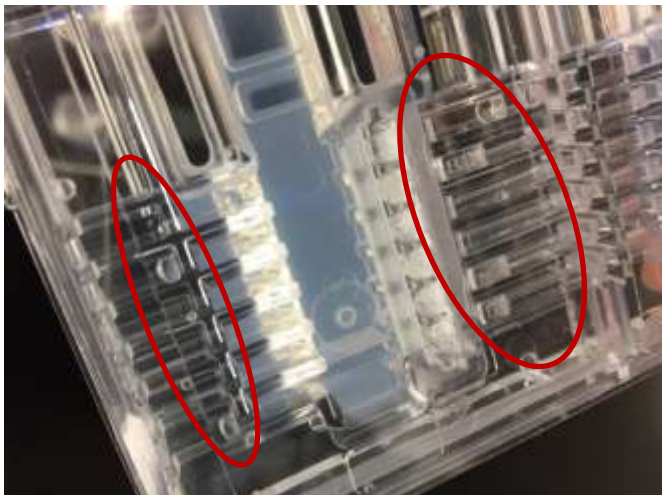
C. Prepare the Gel Cassette(s)

1. Remove the gel cassette from the foil bag.

2. **Before removing tape!** – Hold the cassette with top surface almost vertical and the elution modules above the gel channel. Tap the cassette to dislodge any bubbles that are trapped behind the elution wells, or in the elution channels. Repeat if necessary. Allow the bubbles to collect in the electrode channel directly above.

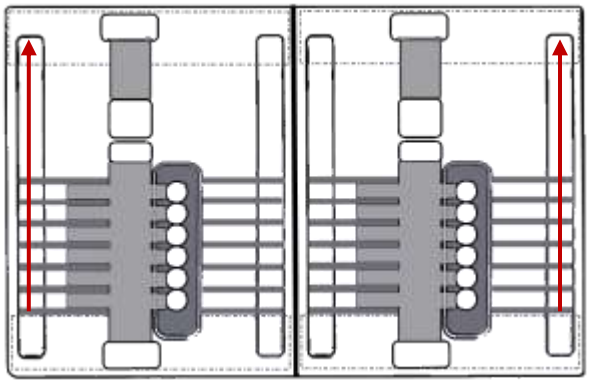


Tap to dislodge bubble from these areas into the troughs directly above.

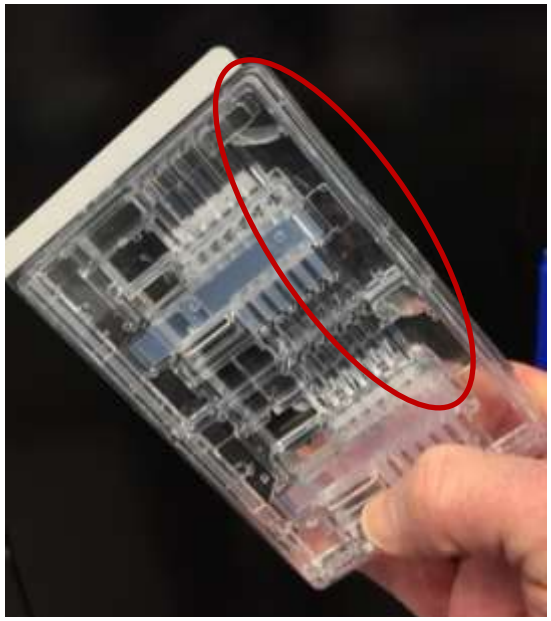


Bubbles in the elution paths can interfere with collection

3. Slowly rotate the cassette to allow the bubbles to collect in the upper buffer area. Gently tap if necessary.



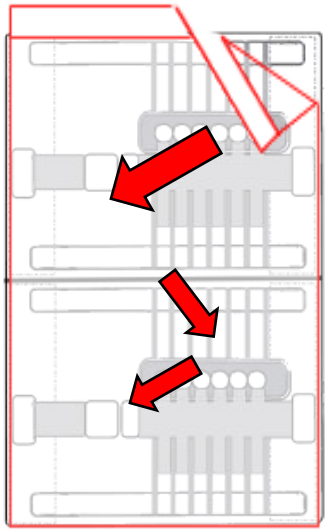
Move any bubbles to the upper buffer area.



Aggregate and collect air bubbles in the upper buffer chambers

4. With cassette held at a slight angle to keep bubbles located in the upper buffer chamber, gently place the cassette onto the nest. The upper buffer chamber should be on the left side of the nest.

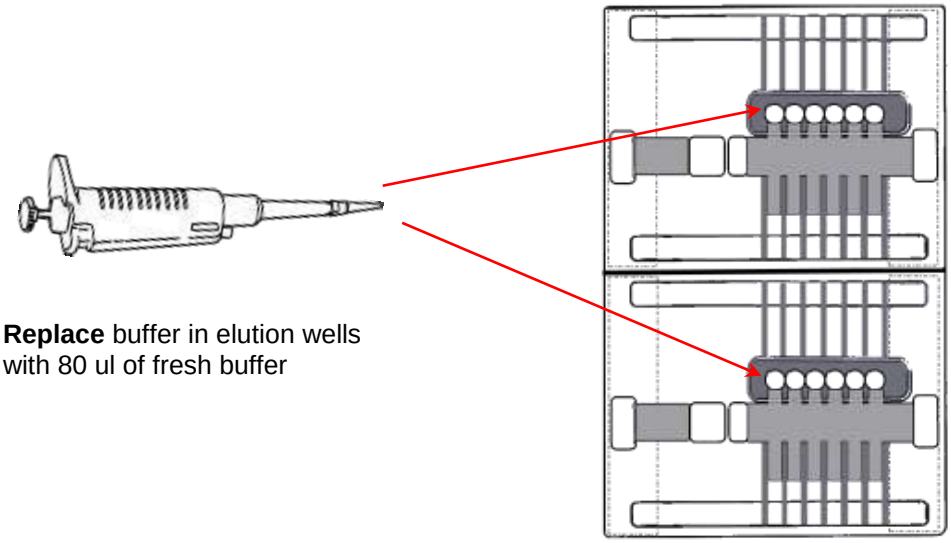
5. While holding the cassette firmly in place on the nest, grab the tape tab, and pull the tape off at an angle, slowly and firmly. Alternate the pulling angle if the tape resists peeling.



Peel back tape at an angle. Alternate angles if the tape resists peeling.

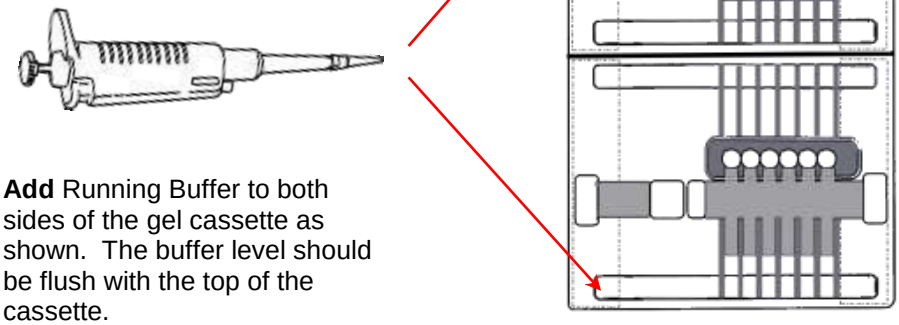
6. Remove all buffer from all elution wells (set a pipette to 100 µl to completely empty the wells). Keep pipette tips vertical in the well to avoid damage to the membranes.

7. Taking care not to introduce additional bubbles into the elution modules, add 80 µl of Running Buffer (**E**) to all elution wells.



Replace buffer in elution wells with 80 ul of fresh buffer

8. Add **Running Buffer** to the upper buffer chamber on each side of the gel cassette until the level is flush with the top of the cassette. An adequate buffer level is important for achieving best results from the SageHLS.



Add Running Buffer to both sides of the gel cassette as shown. The buffer level should be flush with the top of the cassette.

Fill buffer until it is flush with the top of the cassette

Important! Fill until the buffer level visually reaches the bottom side of the cassette cover

D. Load the Workflow File

Workflow File Guidelines:

There are two types of workflow files that are based on level of method validation at Sage Science:

Core Workflows – Tested extensively on biological samples
Non-core Workflows – Tested with model DNA sample input only

File Naming Conventions
For convenience, Sage-provided workflow file names follow the following convention (if practical):

[Application type]_[DNA Size Target]_[DNA extraction time]_[DNA separation time].shflow

Example: **CATCH 100-300kb extr 1h sep 3h.shlfow**

Selecting DNA Target Sizes
Core workflows have been tested for targets of 100, 200, and 400kb ("100-300kb" files) and smaller targets up to 30kb ("55V" files). Although all elution wells should be interrogated for enriched product (see pages 13-27 and 13-28), workflows are designed to collect targets in well 3 and 4. Longer separation times will collect larger targets in the same wells:

≥ 100kb: use "100-300kb"
≤ 30kb: use "55V"

Selecting Extraction Times
Variable amounts of non-specifically cleaved DNA (50kb to 200kb) can be liberated from non-viable cells during the extraction step. With a one hour extraction, some of these fragments will co-elute with CATCH targets. These fragments benefit 10X Genomics linked-read library construction by providing a reference:

10X Genomics linked reads: use 1 hr extraction
Pac-Bio or Oxford nanopore: use 3 hr extraction

Selecting Separation Times
For a given target range, a longer separation time will collect larger DNA targets in the same elution wells. The following targets have been extensively tested using following core workflows:

100kb: 3 hour separation (CATCH 100-300kb extr 3h sep 3h.shlfow)
200kb: 3 hour separation (CATCH 100-300kb extr 3h sep 3h.shlfow)
400kb: 3 hour separation (CATCH 100-300kb extr 3h sep 4h.shlfow)
16kb: 45 min separation (CATCH DC55V extr 3h sep 45m.shlfow)
30kb: 75 min separation (CATCH DC55V extr 3h sep 75m.shlfow)

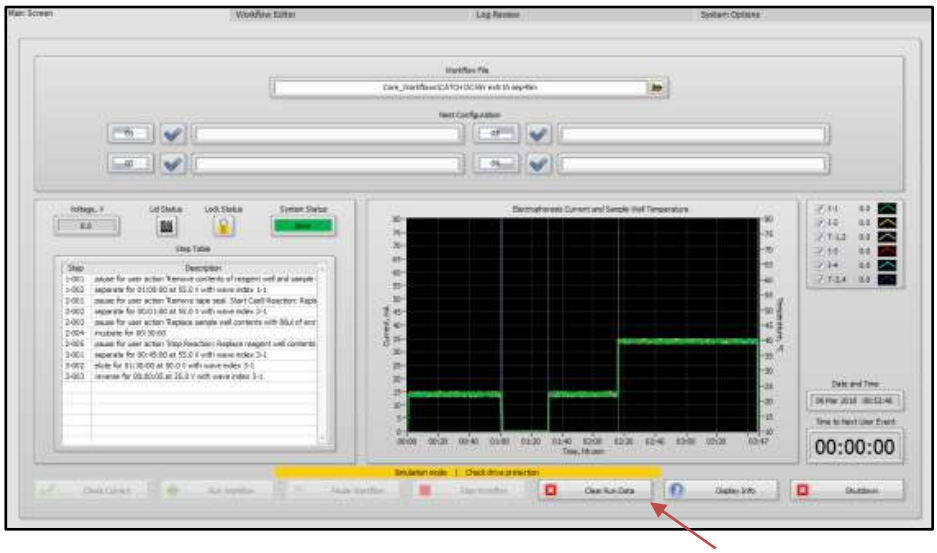
Workflow File Summary

Workflow Files	
Core Workflows	**~ Run Time (hr)**
CATCH 100-300kb extr 1h sep 3h.shlfow	6
CATCH 100-300kb extr 1h sep 4h.shlfow	7
CATCH 100-300kb extr 3h sep 3h.shlfow	8
CATCH 100-300kb extr 3h sep 4h.shlfow	9
CATCH DC55V extr 1h sep 45m.shlfow	4
CATCH DC55V extr 1h sep 75m.shlfow	4.5
CATCH DC55V extr 3h sep 45m.shlfow	6
CATCH DC55V extr 3h sep 75m.shlfow	6.5
Non-Core Workflows	**~ Run Time (hr)**
CATCH 100-400kb extr 1h sep 3h.shlfow	6
CATCH 100-400kb extr 1h sep 4h.shlfow	7
CATCH 100-400kb extr 3h sep 3h.shlfow	8
CATCH 100-400kb extr 3h sep 4h.shlfow	9
CATCH 145kb extr 1h.shlfow	11
CATCH 145kb extr 3h.shlfow	13
CATCH 200kb extr 1h.shlfow	11
CATCH 200kb extr 3h.shlfow	13
CATCH 400kb extr 1h.shlfow	11
CATCH 400kb extr 3h.shlfow	13

About non-core workflows

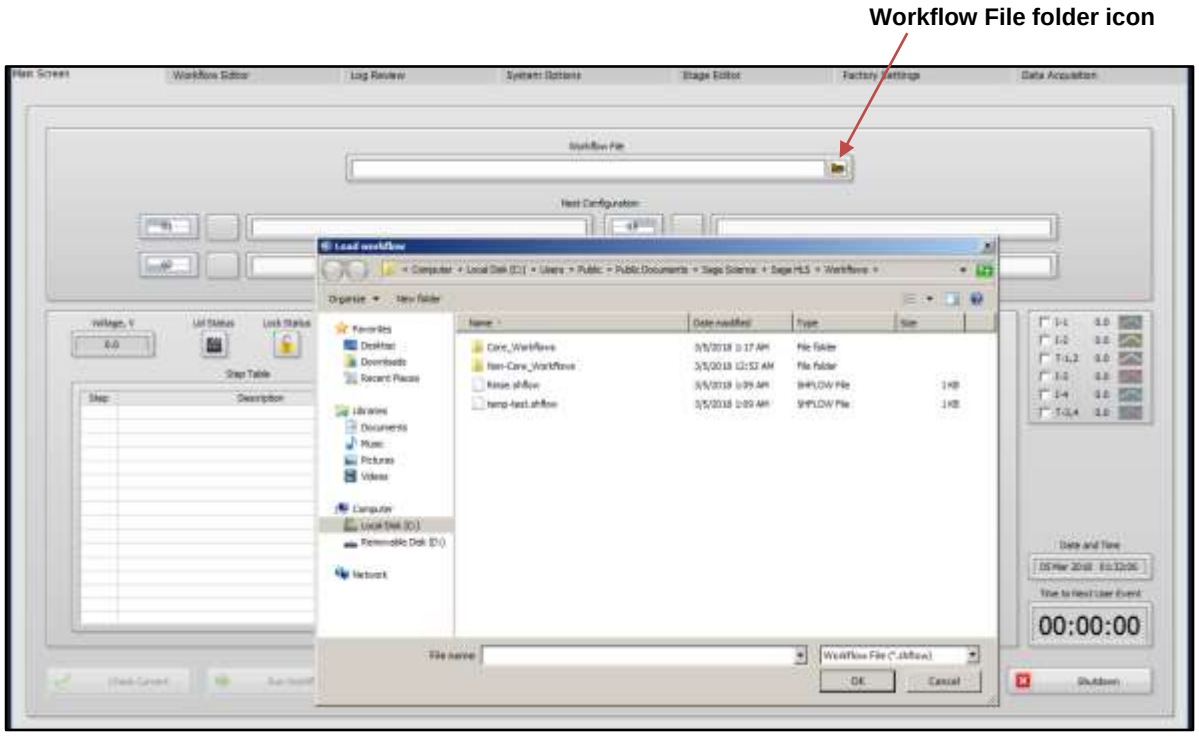
Non-core workflows have been designed for special purposes. For instance, the CATCH 100-400k workflows are designed to have a broader collection range than the core 100-300kb workflows, whereas the “CATCH <xxx>kb” workflows are designed to have expanded resolution in a narrow window centered on <xxx>kb. In general, Sage has tested the non-core workflows with model samples but doesn’t yet have extensive test data from biological samples.

1. Go to the Main screen of the SageHLS software. If there is data from a previous run, the "Clear Data" button must be pressed to clear all fields.

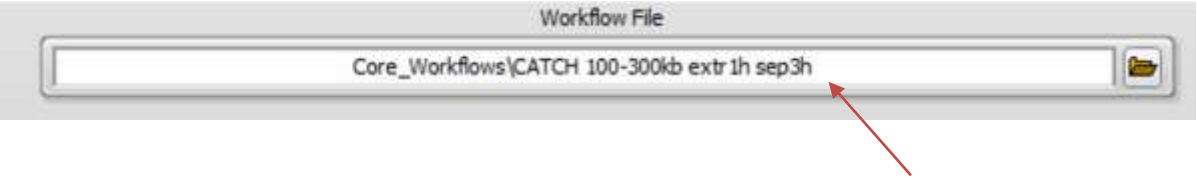


2. Select the Workflow File folder icon.

   A workflow file directory window will open, select the folder containing either core or non-core workflows.



3. Select the Workflow File from the folder. The file name will appear in the Workflow File field.



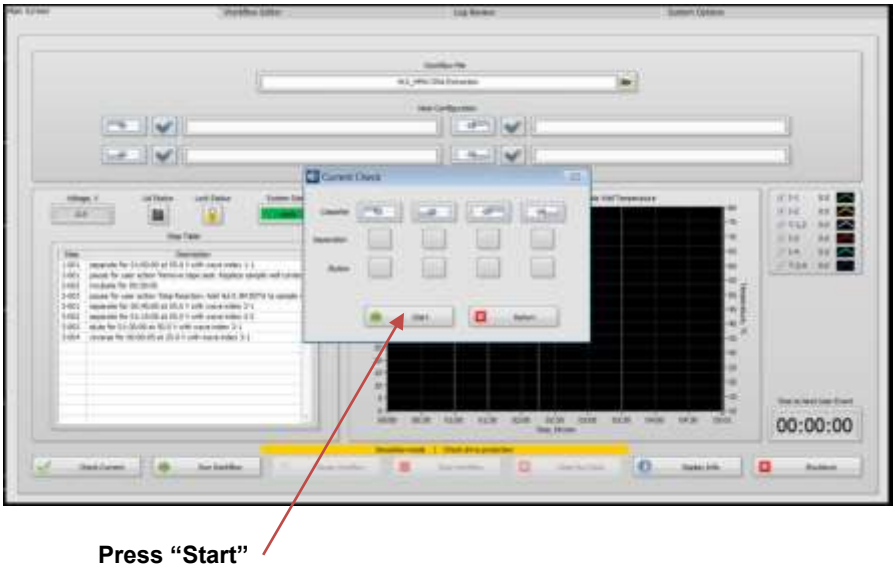
4. Choose the lanes to be used by clicking the boxes next to the lane numbers and enter sample IDs into the adjacent fields (sample IDs are optional or can be entered later).

Check marks indicate which lanes are active

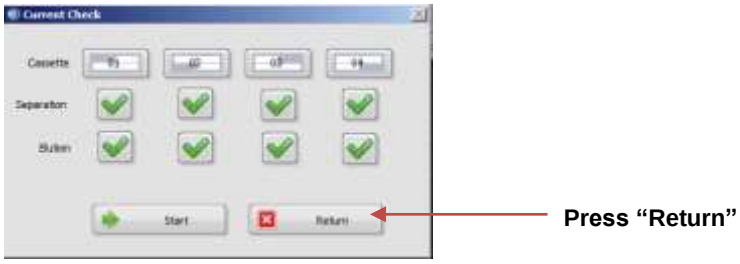
E. Run the Check Current Test

1. Press the “Check Current” button.

2. A pop-up window will appear. Press “Start” to begin the Check Current routine.



3. The routine will first test the separation electrodes, then test the elution electrodes, and complete within a few minutes. After a successful test, all boxes will fill with green check marks. Press “Return” to continue

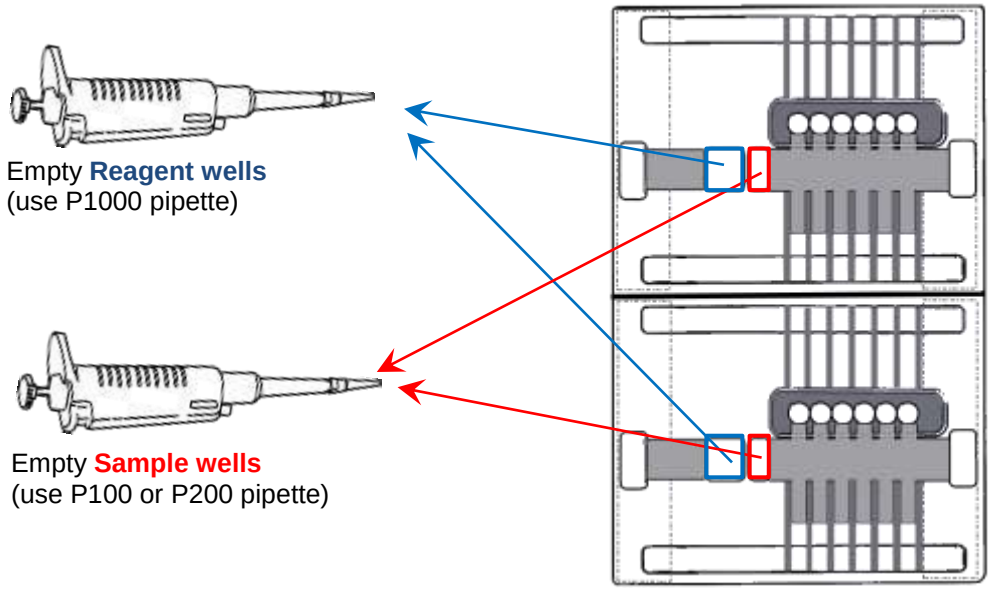


F. Stage 1: Extraction

1. Use the following Table as a guide to select the most appropriate Lysis Reagent.:

Lysis Reagent Name	Description
HLS Lysis Reagent 3% SDS (**A**)	Maximum DNA recovery, residual SDS (0.01-0.03%) in eluant
HLS Lysis Reagent 1% SDS (**H**)	Less DNA recovery (25-30%), less residual SDS (0.001 – 0.009%)
HLS Lysis Reagent 3% Sarkosyl (**G**)	Less DNA recovery (25-30%), for samples with potassium (alternative to SDS, which co-precipitates in the presence of potassium)

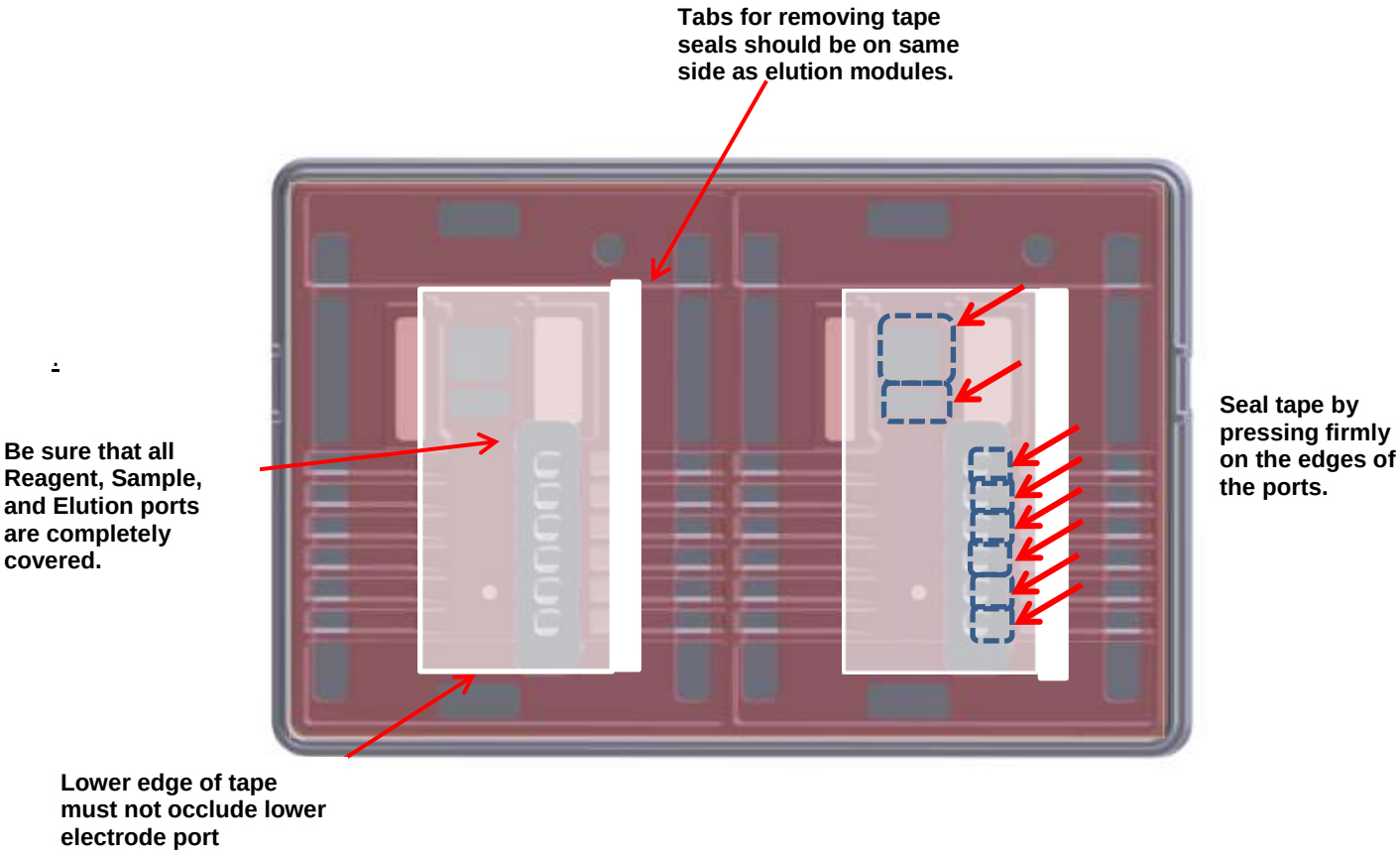
2. Empty all sample and reagent wells. Use caution not to pierce agarose at the bottom of the wells.



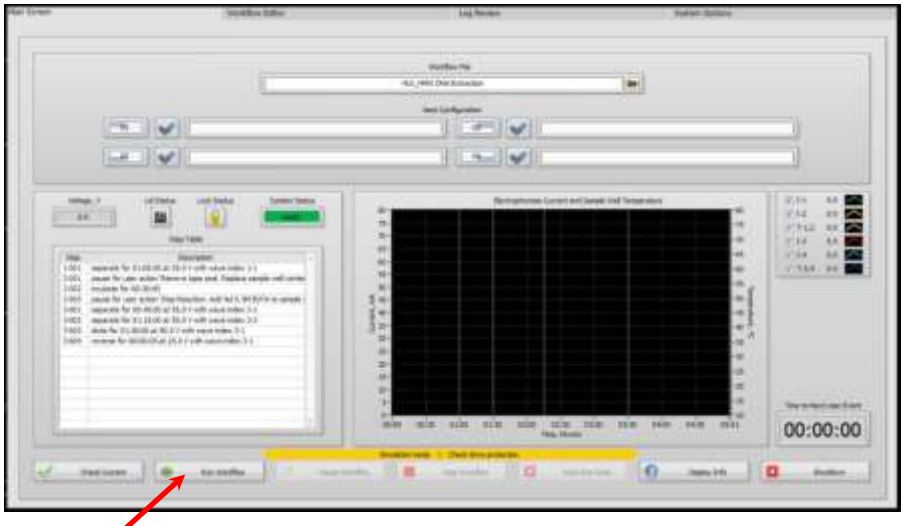
3. Load samples in all lanes. Use a **70ul sample loading volume**. (Sample wells will not be completely full.)

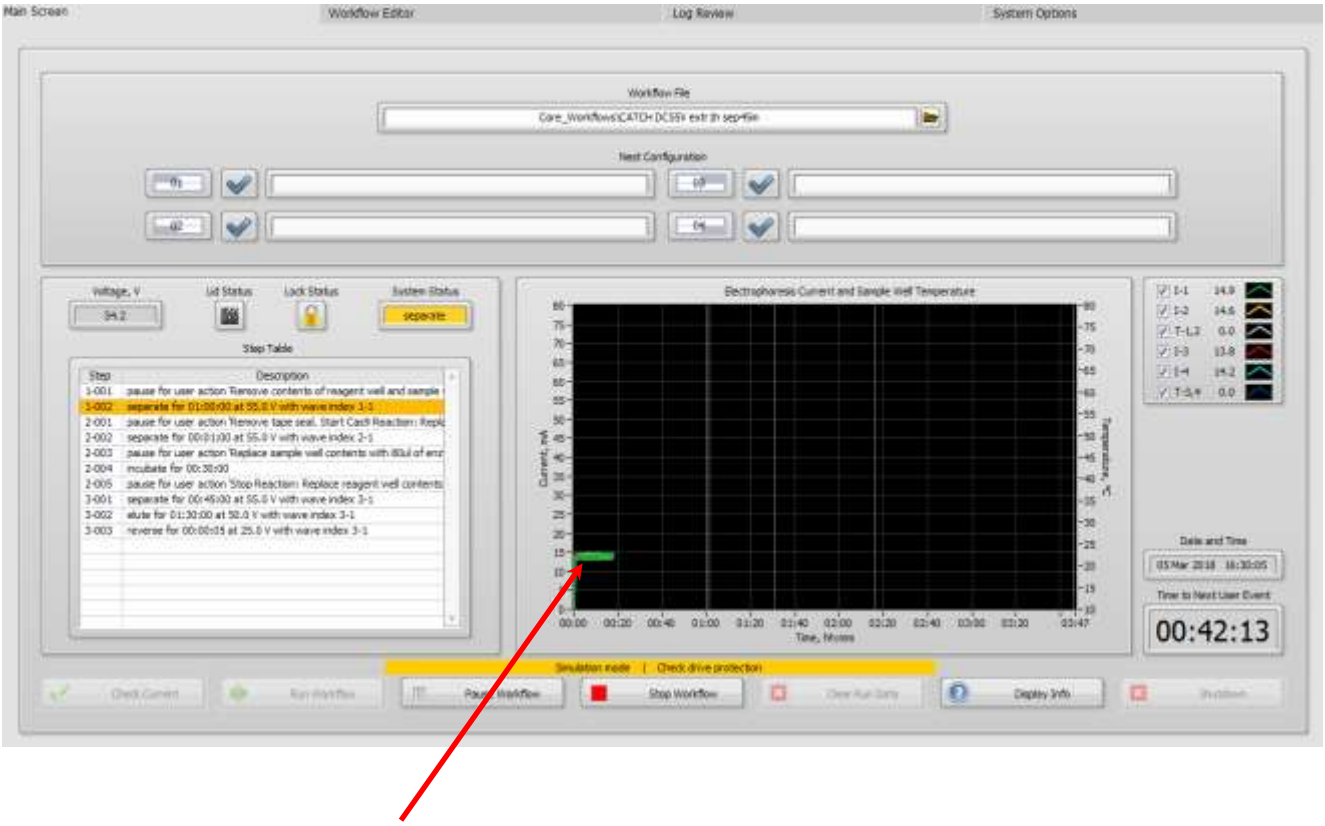
4. **Add 220 ul of HLS Lysis Buffer** (**A** or **G**) to the reagent Wells with. Do not overfill! Leave a concave meniscus to prevent contact with sealing tape in next step.

5. Seal reagent, sample, and elution ports with supplied tape without occluding the electrode ports. Press tape firmly around edges of the ports.



6. Close the lid and press “Run Workflow”. The extraction step will require the amount of time indicated in the workflow file name, typical 1 or 3 hours.

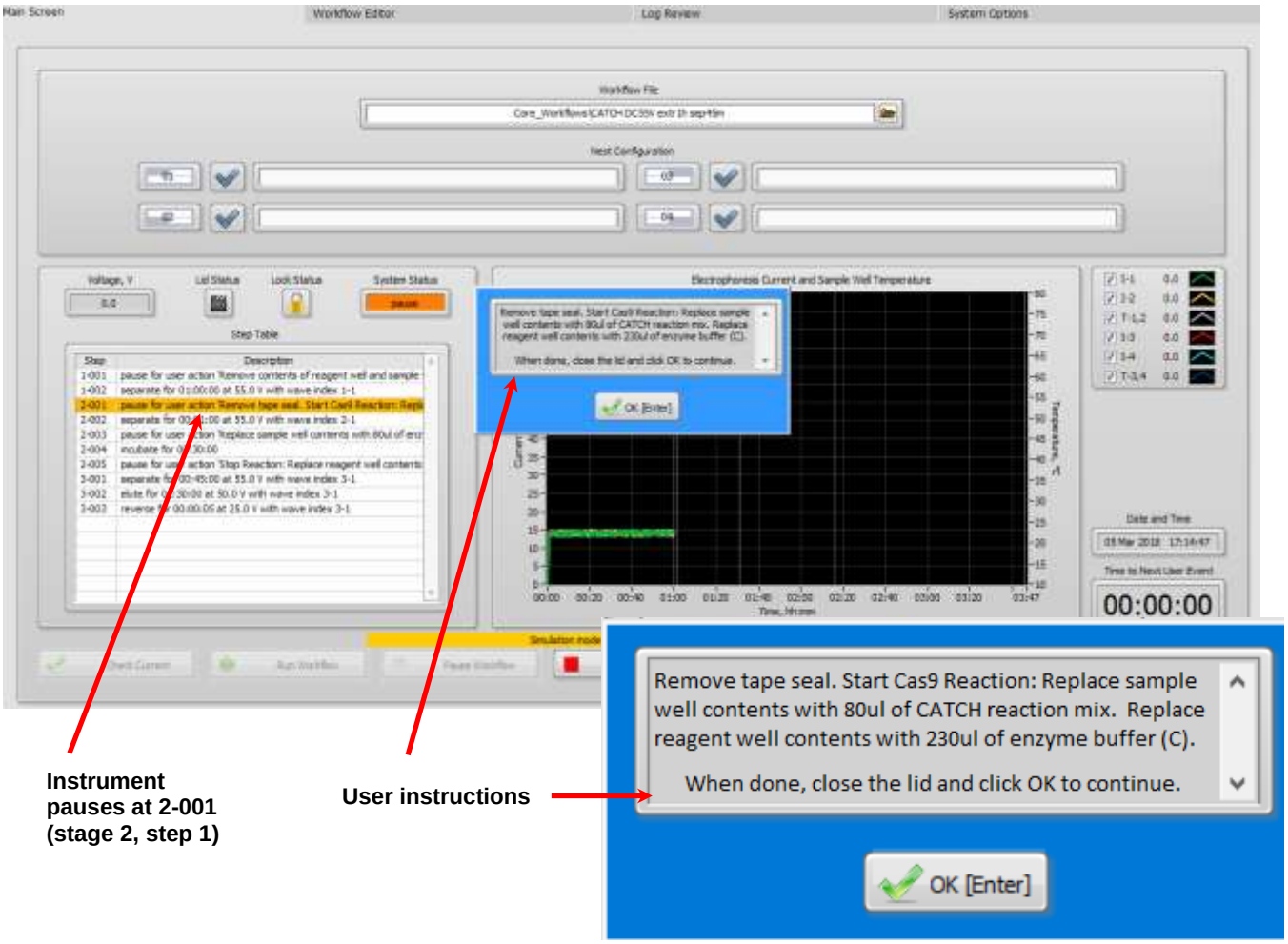




During the run, the Current display should be similar for all lanes, and in the range of 10-15 mA

G. Stage 2: Treatment

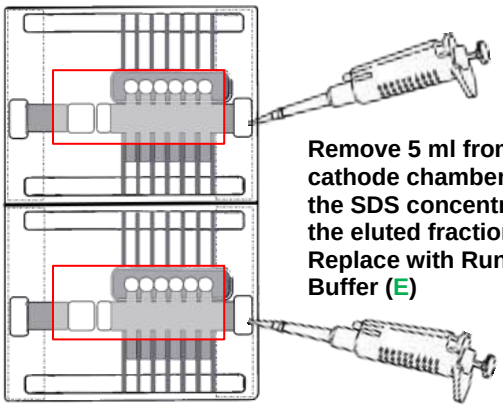
At the end of the Extraction Stage, the SageHLS will pause on the first step of the Treatment Stage and a pop-up window will appear with user instructions.



Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user action.

1. Replace 5 ml of buffer from the anode chamber

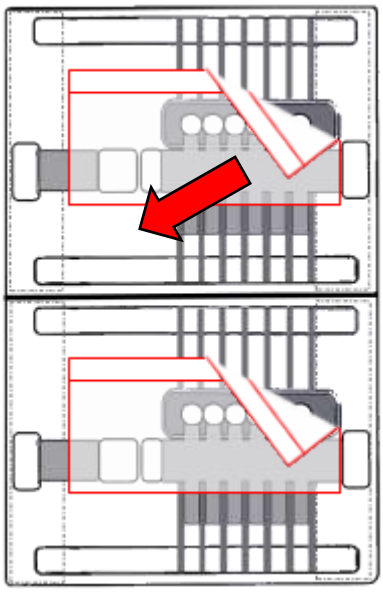
   Detergents (SDS, Sarkosyl) will aggregate near the electrode in the anode chambers of the gel cassette during extraction. By replacing buffer in this area immediately after the extraction stage, the detergent concentration will be reduced in the final eluted samples.



Remove 5 ml from the cathode chamber to reduce the SDS concentration in the eluted fractions. Replace with Running Buffer (E)

2. Open the lid and carefully remove the adhesive tape(s). To remove the tapes, grab the tab in right upper corner and peel diagonally with a slow smooth motion.

grab the tab in right upper corner and peel diagonally with a slow smooth motion



Important! Pulling in the tape in diagonal fashion prevents liquid transfer between adjacent elution ports and transfer between the sample/reagent ports and the elution ports.

3. Remove the contents from the Reagent wells and Sample wells on the cassette(s). The well volumes are 270 µl and 85 µl, respectively.

4. **Dilute 40 ul of the Cas9-Guide RNA Mix with 40 ul HLS Enzyme Buffer** (**C**) (80 ul total)

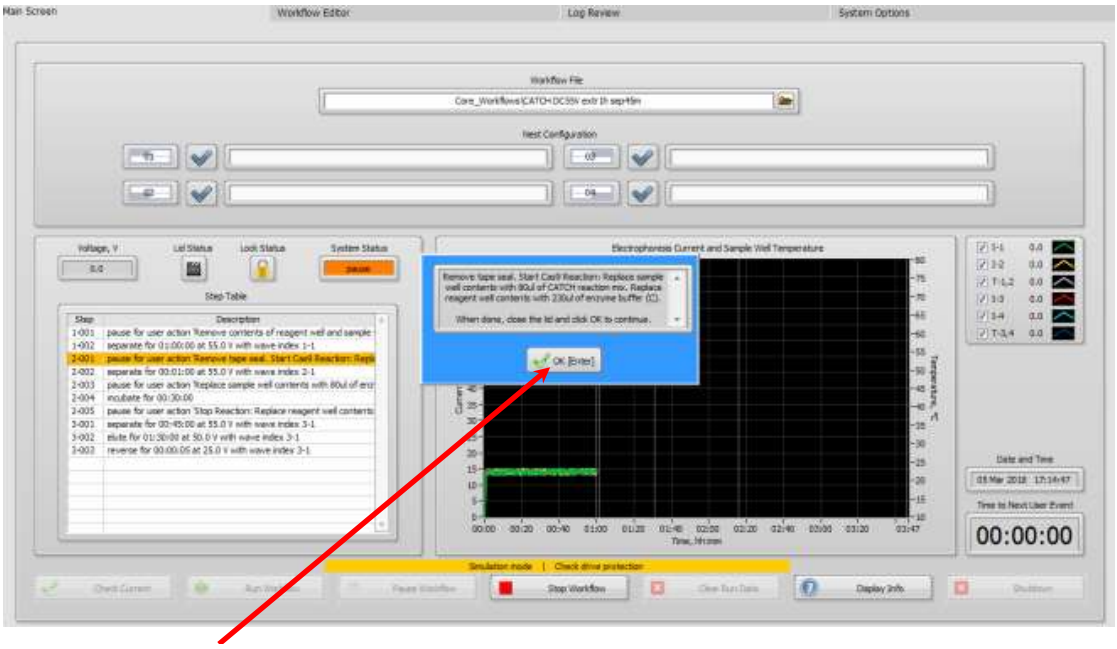
5. Mix thoroughly by pipetting up and down.

6. **Add 80 µl of the diluted Cas9-Guide RNA Mix** to the **Sample well(s)**.

7. **Add 230 µl HLS Enzyme Buffer (C)** to the **Reagent well**(s)

8. Close the lid (do not re-seal the wells with tape).

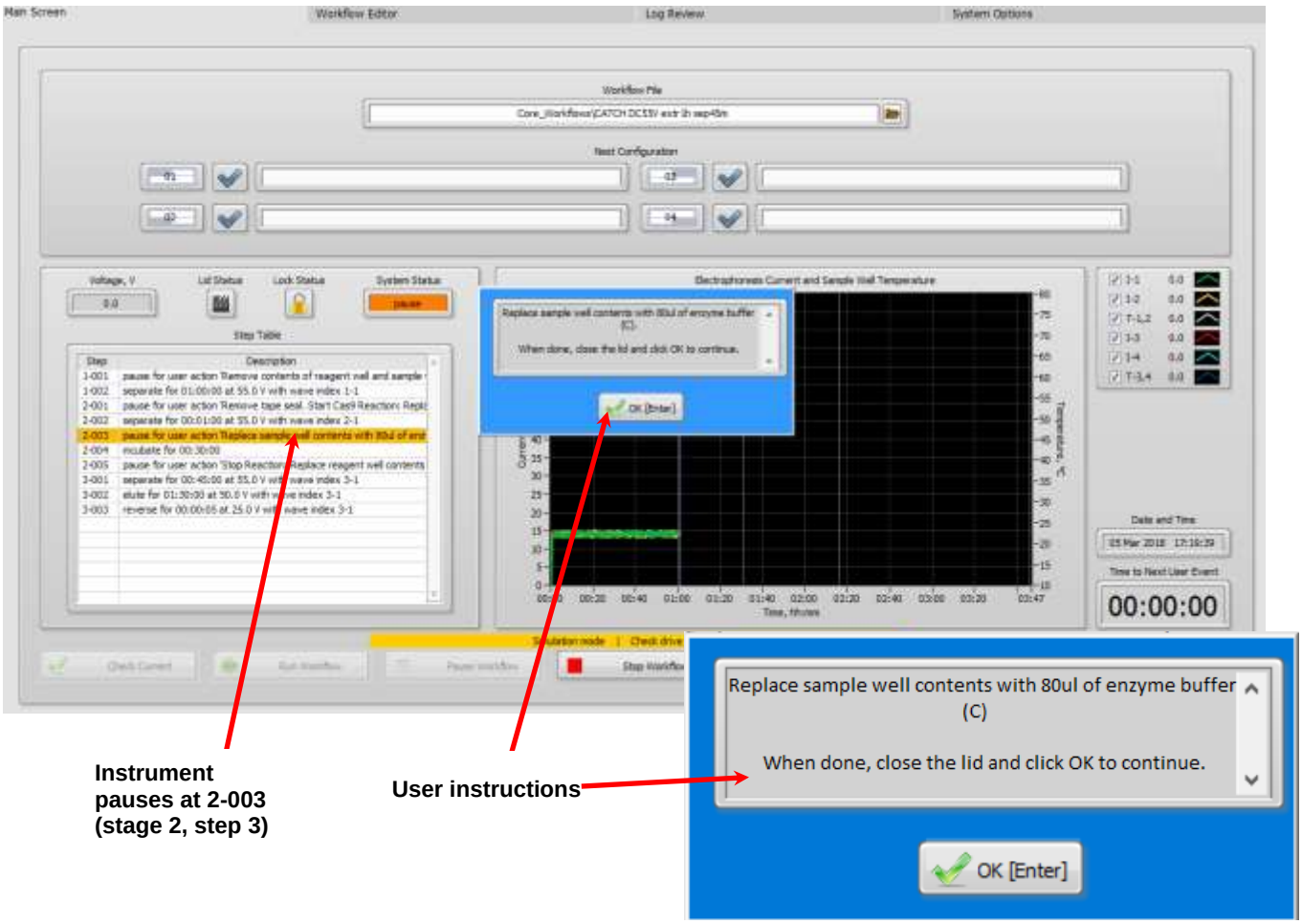
9. Press "OK" in the pop-up window to resume the workflow.



Press "OK" to resume

10. The instrument will perform a **1 minute electrophoresis step** to inject the Cas9 into the sample wall where the HMW DNA is immobilized.

11. The SageHLS will pause, and a pop-up window with user instructions will appear.



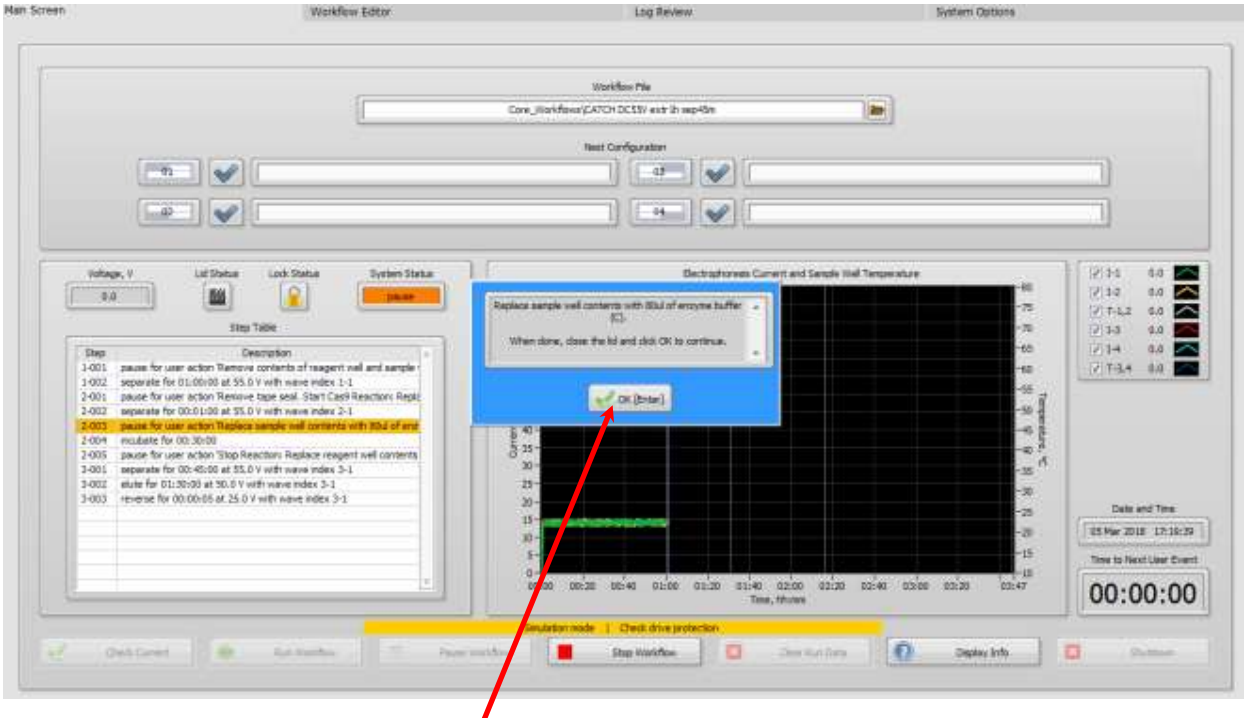
12. Open the lid.

13. Remove the contents from the **Sample well(s)** on the cassette(s).

14. Add **80 µl of the Enzyme Buffer** (**C**) to the **Sample well(s).**

15. Close the lid (do <u>not</u> re-seal the wells with tape).

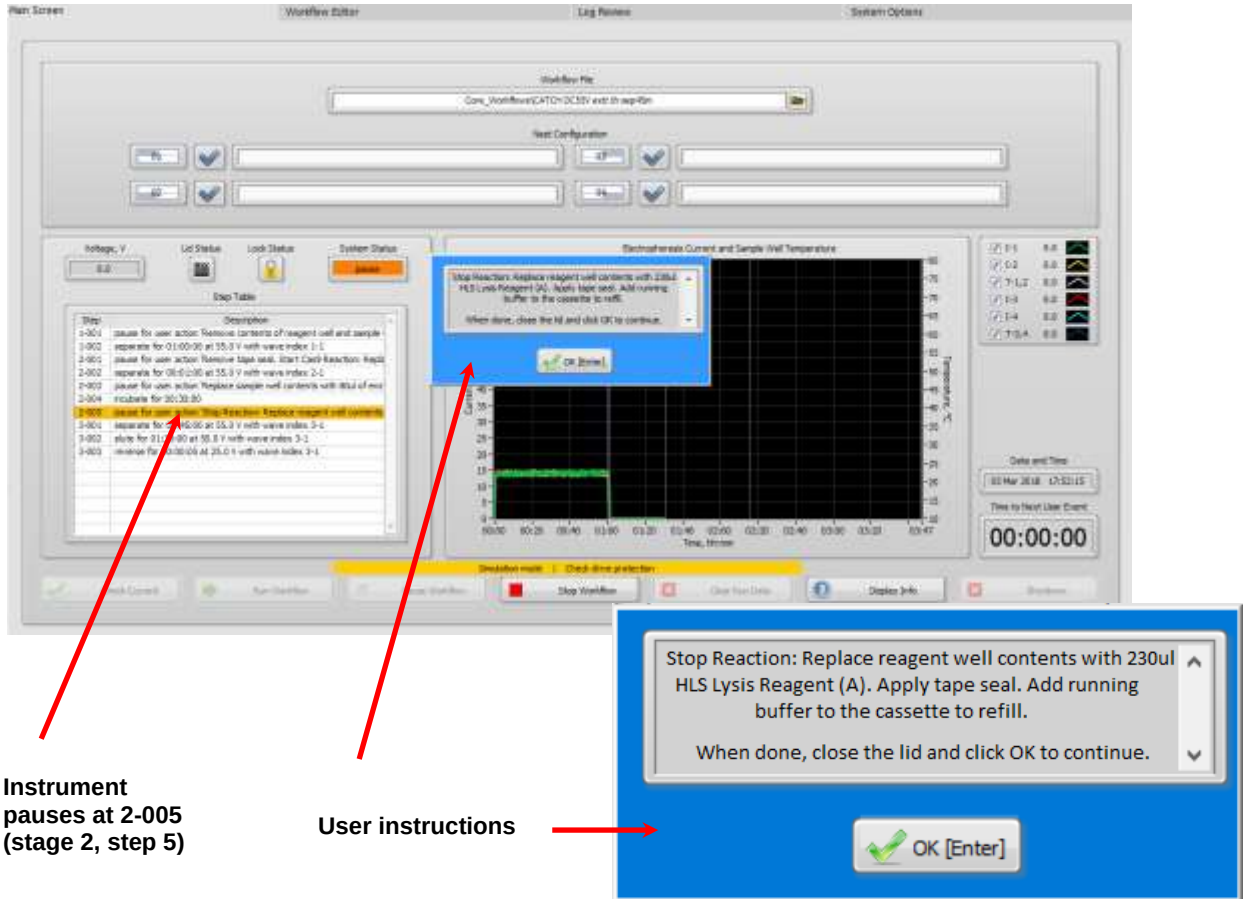
16. Press “OK” in the pop-up window to resume the workflow.



Press “OK” to resume

19. The enzyme treatment step will take **30 minutes**.

20. At the end of **30 minutes** the SageHLS will pause, and a pop-up window with user instructions will appear.

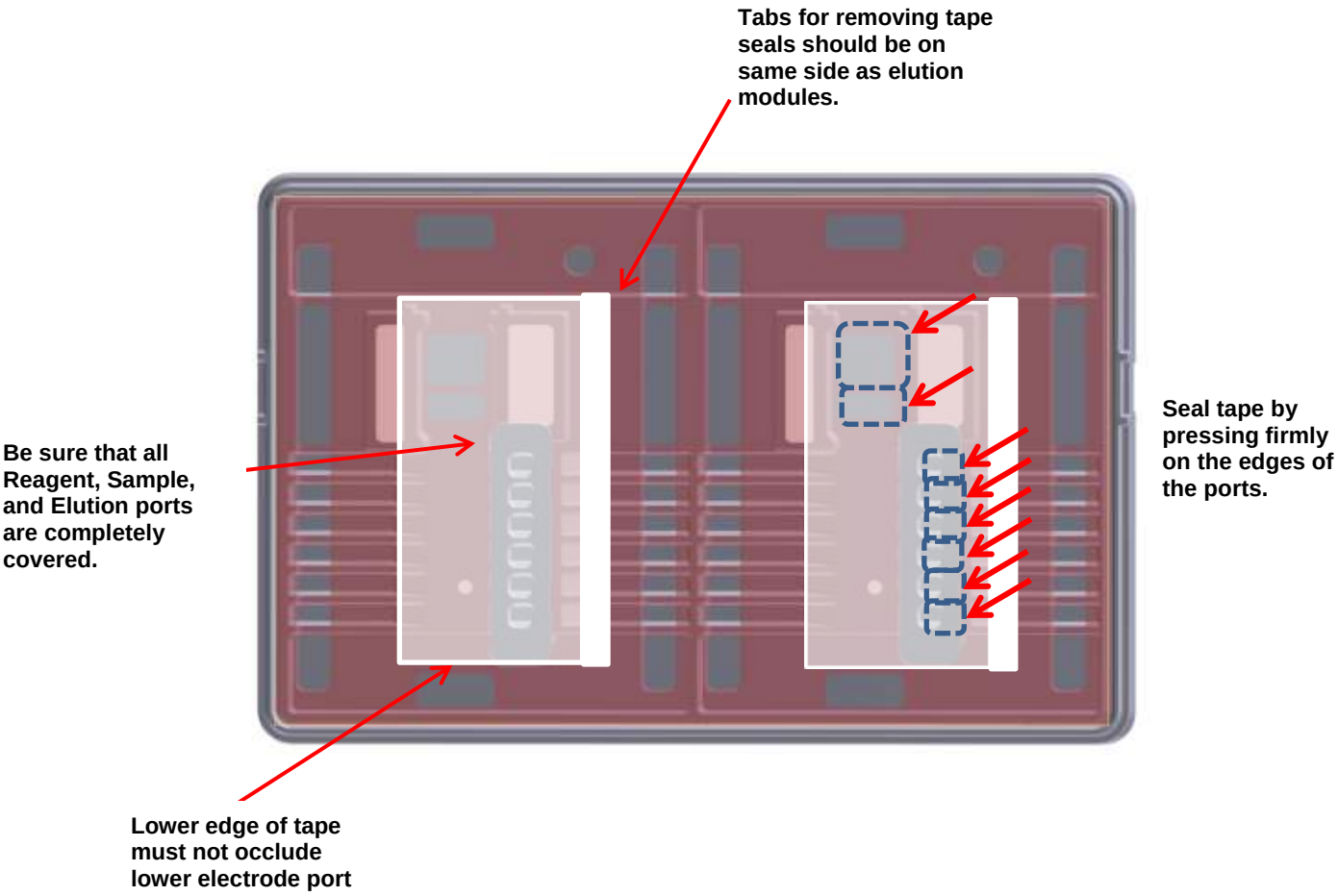


Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user action.

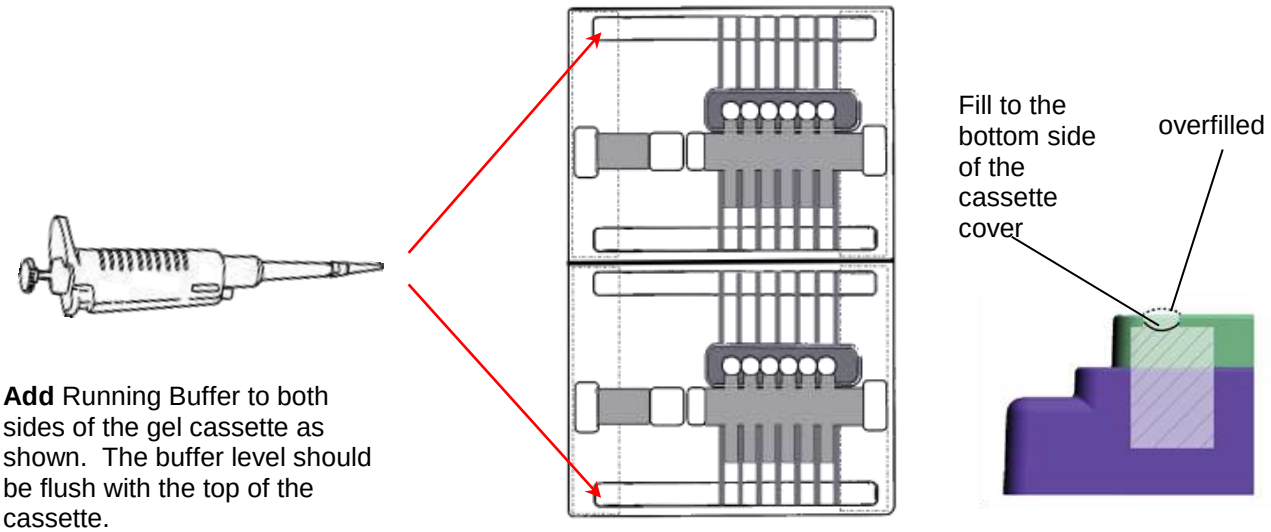
21. Open the lid and remove the contents of the Reagent well.

22. **Add 220 ul Lysis Reagent** (**A, H** or **G**) **to the Reagent well(s).** Do not overfill! Leave a concave meniscus to prevent contact with sealing tape in next step.

23. Close the lid and **re-seal the cassette wells with tape**.

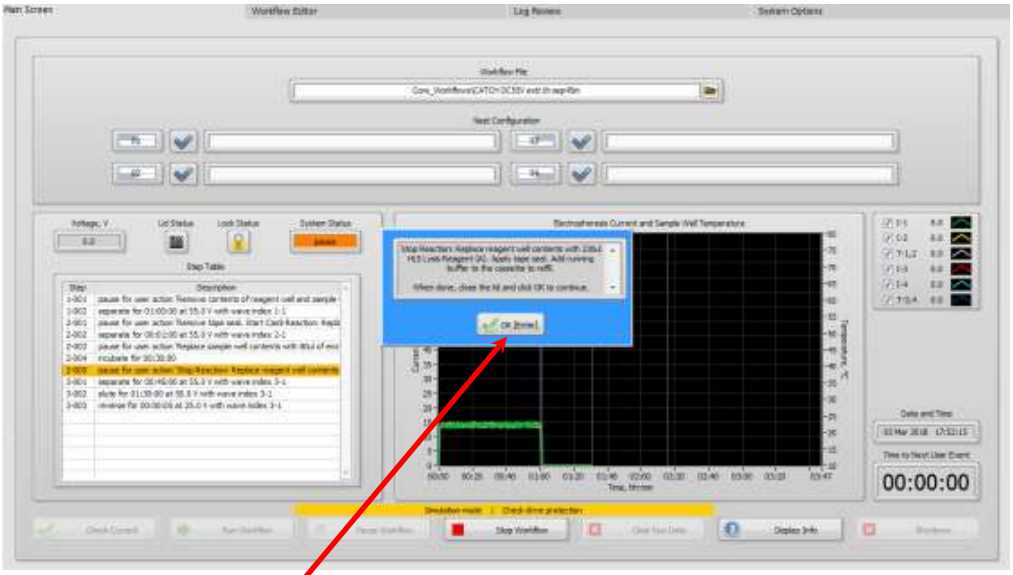


24. Add running buffer (**E**) to the cassette to replace evaporated buffer.



Add Running Buffer to both sides of the gel cassette as shown. The buffer level should be flush with the top of the cassette.

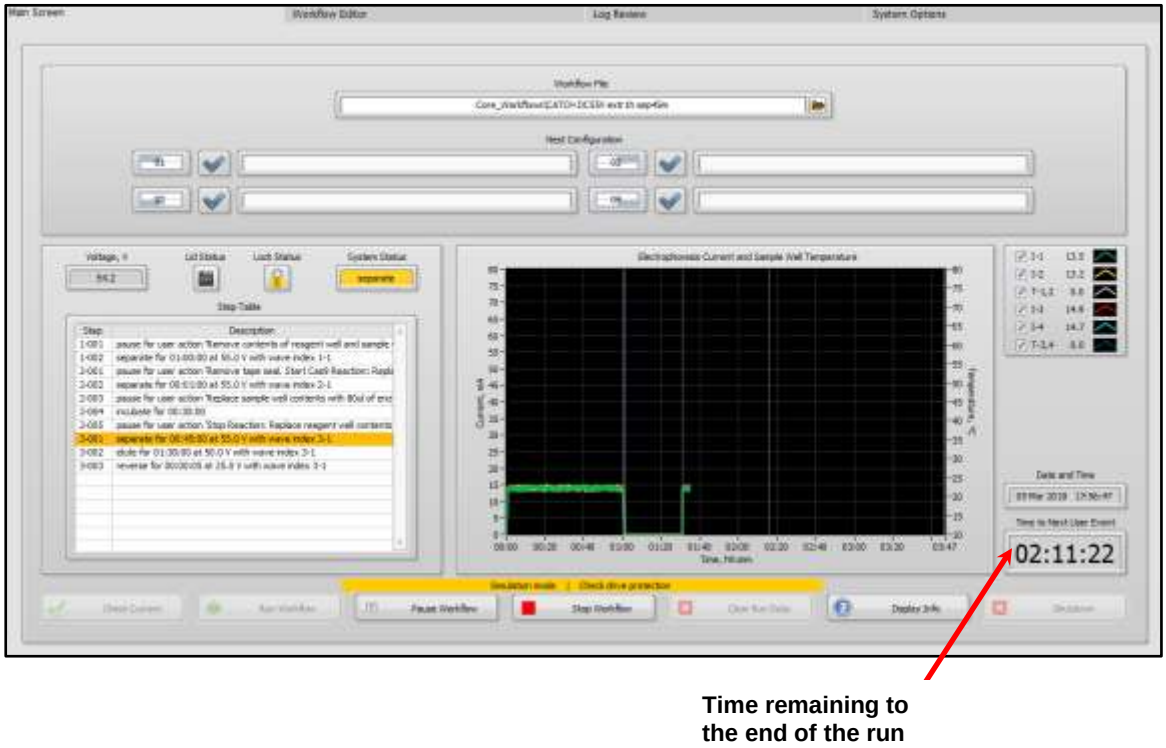
25. Close the lid and press “OK” in the pop-up window to resume the workflow.



Press “OK” to resume

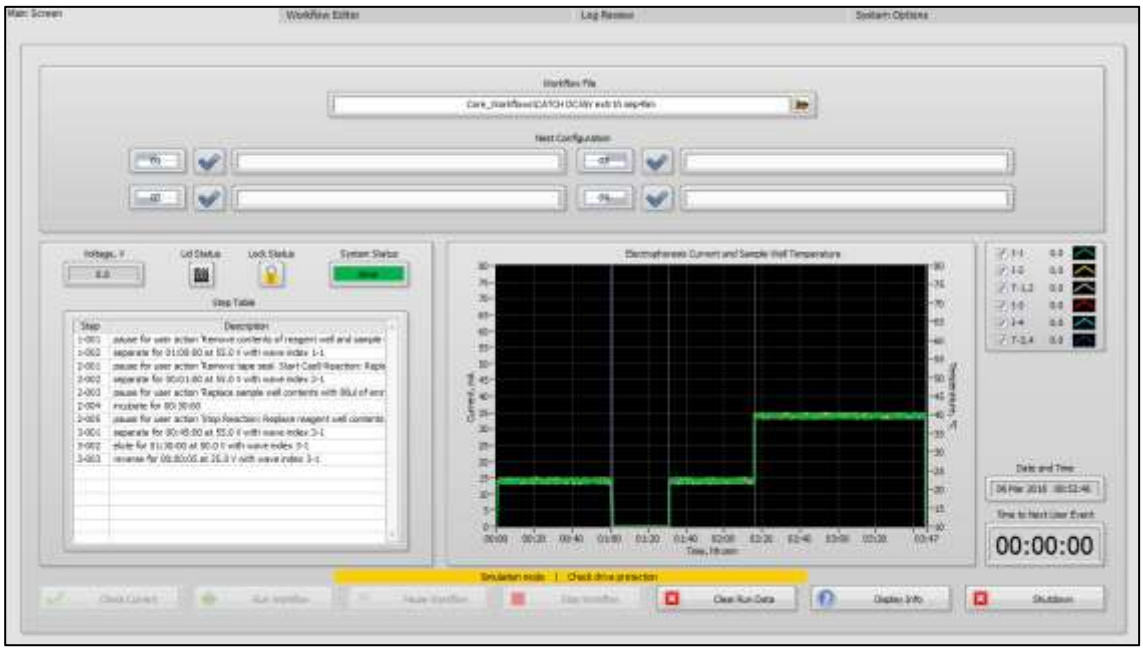
H. Stage 3: Collection Stage

The Collection Stage will require several hours of unattended operation. Users should note the time remaining, after which the samples can be collected.



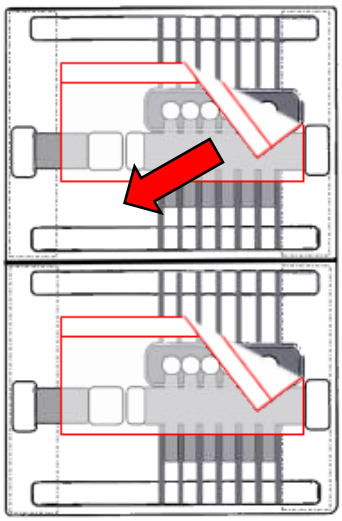
Time remaining to the end of the run

Main Screen at Run Completion:



1. After the run is complete, open the lid and remove the sealing tape from the cassette(s).

grab the tab in right upper corner and peel diagonally with a slow smooth motion



2. Using a wide-bore pipette tip, remove the contents of the elution modules.

Important! Pipette as slowly as possible to avoid shearing the HMW DNA. Use of an electronic pipettor at low speed settings may be helpful. There should be 70-80 ul of liquid in each module.

I. Analysis

To identify the position of CATCH targets in the elution products, following procedures are recommended:

- Perform a Qubit HS assay to determine total DNA content in each elution well. (Use 2-5 ul aliquots of elution product.)
- Perform a qPCR assay for CATCH target and non-target (control) genomic sequences to obtain target copy numbers for each elution well.

1. Qubit Assay

The Qubit assay will give a semi-quantitative evaluation of the quality of the CATCH procedure. Extremely HMW DNA will be very inhomogeneously distributed in the elution product. To quantify, we recommend Qubit assays using at least three 1 ul aliquots from different locations within the tube. Average the three readings. A high average value with a high CV is diagnostic of very HMW DNA.

For Qubit assays, using at least three 1 ul aliquots from different locations within the tube

The expected quantity of DNA detected can depend on the Stage 1 extraction method:

1 hour extraction: ~10ng DNA in each elution well. This is indicative of the presence of non-target DNA, which is beneficial as genomic reference for **10X Genomics linked-read analysis.**

3 hour extraction: ~2ng DNA in each elution well, with up to 20ng in non-target wells (#5 or 6) only under some separation conditions. These more highly purified samples are better suited for **PacBio or Oxford Nanopore sequencing.**

2. qPCR Assay

If possible “custom” Taqman qPCR assays from Thermo Life Technologies are recommended for target detection. SYBR green kits may be used for regions outside of genes.

Based on an input of 300,000-400,000 diploid mammalian cells:

1 hour extraction: For single-plex CATCH target enrichment factors of 15-50x relative to a single copy reference gene (RNaseP RNA gene) is typical.

3 hour extraction: Enrichment relative to a reference gene can be as high as 700-fold.