sageHLS™
HMW Library System

Operations Manual:

sage science

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Table of Contents

1 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^{o}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 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.

1.5 Reagents Used

This following is a list of materials that may be included in SageHLS reagent kits, and the relative concentrations at which they may be found. This list is for reference only, actual formulations will vary.

Reagent	Concentration
Tris (hydroxymethyl)aminomethane (Tris)	<5%
3-(Tris(hydroxymethyl)methylamino)propane-1-sulphonic acid (TAPS)	<5%
Ethylenediaminetetraacetic acid disodium dehydrate (EDTA)	<1%
Phenol red	10ng/ul
Ficoll	80 ug/ul
Hydroxy Propyl betaCyclodextrin	32 ug/ul
Bovine Serum Albumin (BSA)	50ng/ul
Glycerol	2%
Sodium Dodecyl Sulfate (SDS)	3%
dsDNA Fragmentase*	

* Proprietary, New England Biolabs.

2 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.

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 on the next page 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 and the Cassette nest

2.3 Unpacking and Storage of Gel Cassettes

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

- 4 or 12 foil-sealed gel cassettes – store at room temperature.
- 1 package of adhesive tape for sealing elution wells
- 1 package of reagents

2.4 Unpacking Reagent Kits

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

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.

3 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 (eg. heat, electrophoresis voltage, and time), operate the system (eg. Start, Stop, Pause), and monitor operations (eg. 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.

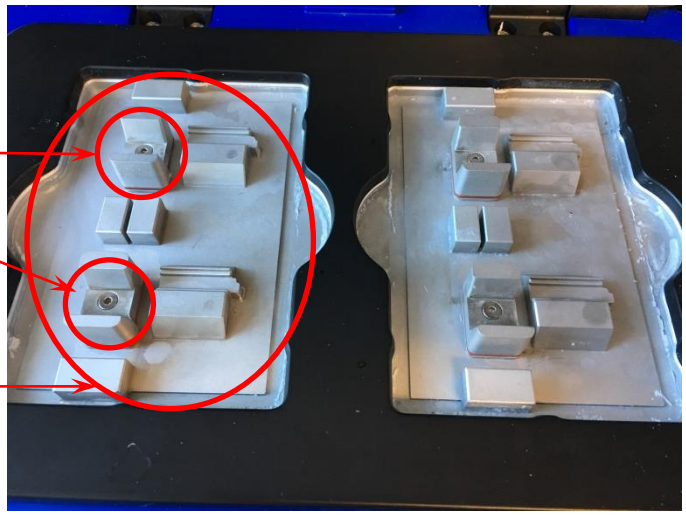
The SageHLS instrument
(the monitor and keyboard are not shown)

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-65°C

Electrophoresis Zone
(heating)
Ambient - 40°C



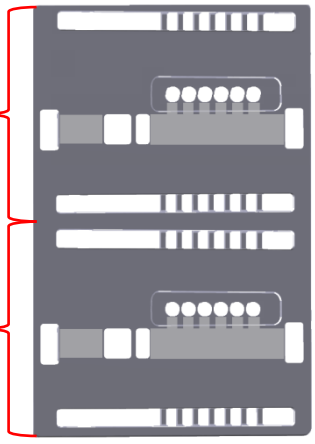
Hot Surface! Use caution when using the SageHLS heated nest.

3.3 How the System Works

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

Sample 1

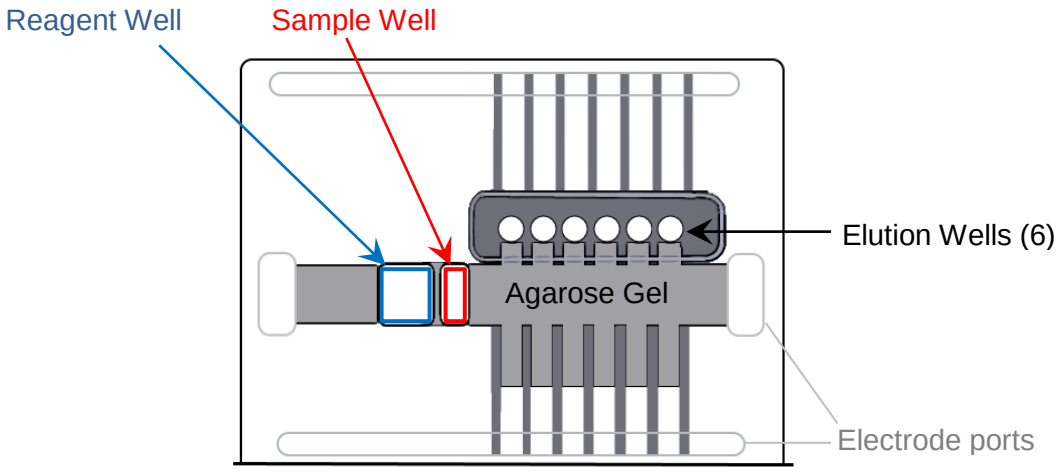
Sample 2



Gel Cassette
Schematic (top)

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

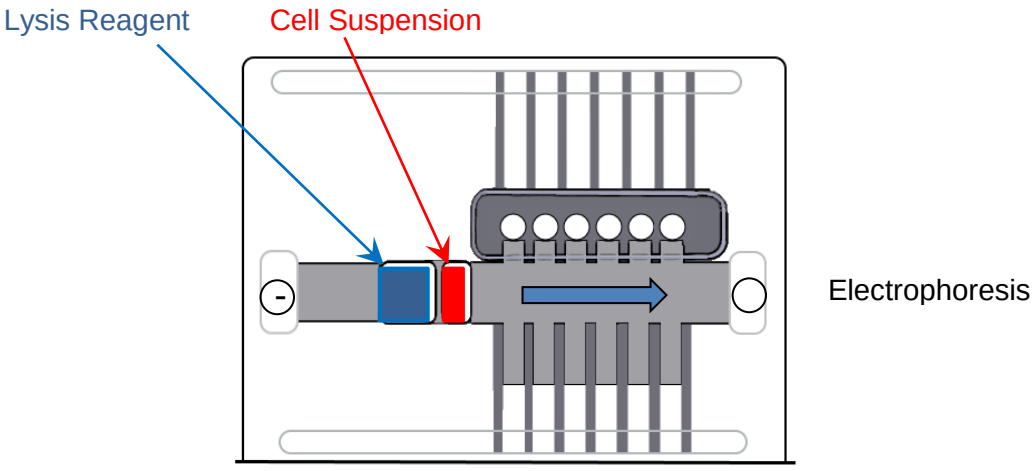
Electrophoretic current can be applied in two (perpendicular) directions. DNA is collected in six membrane-bound elution wells.



Single Sample Schematic

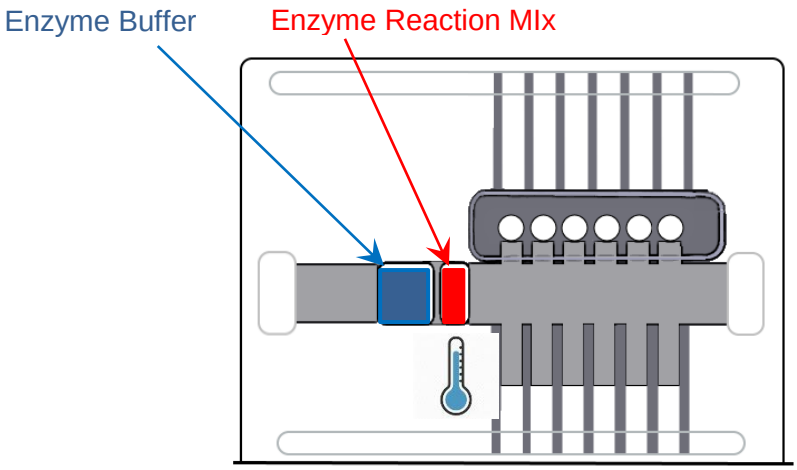
Cell suspensions are processed in three workflow stages:

STAGE 1. EXTRACTION



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

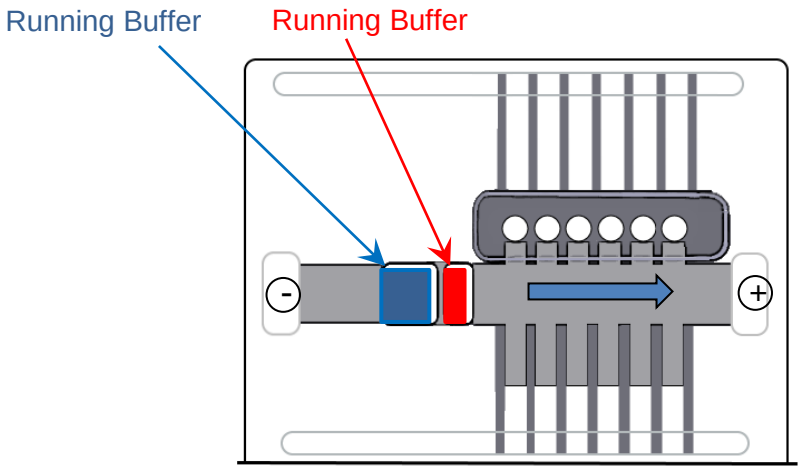
STAGE 2. TREATMENT



Temperature control and time

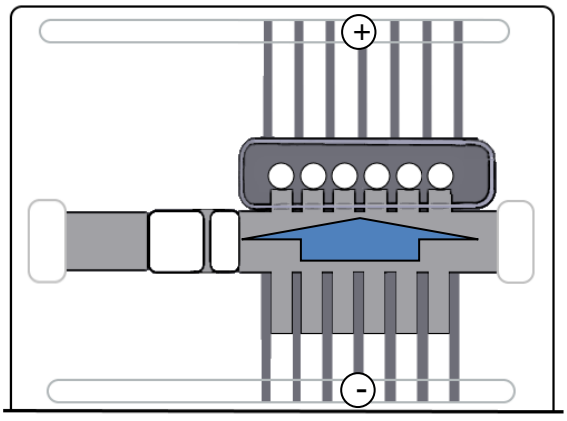
DNA is subject to enzymatic treatment that cleaves the DNA into electrophoretically mobile fragment sizes. Cleavage may be random or targeted.

STAGE 3. COLLECTION



a. Electrophoresis (separation)

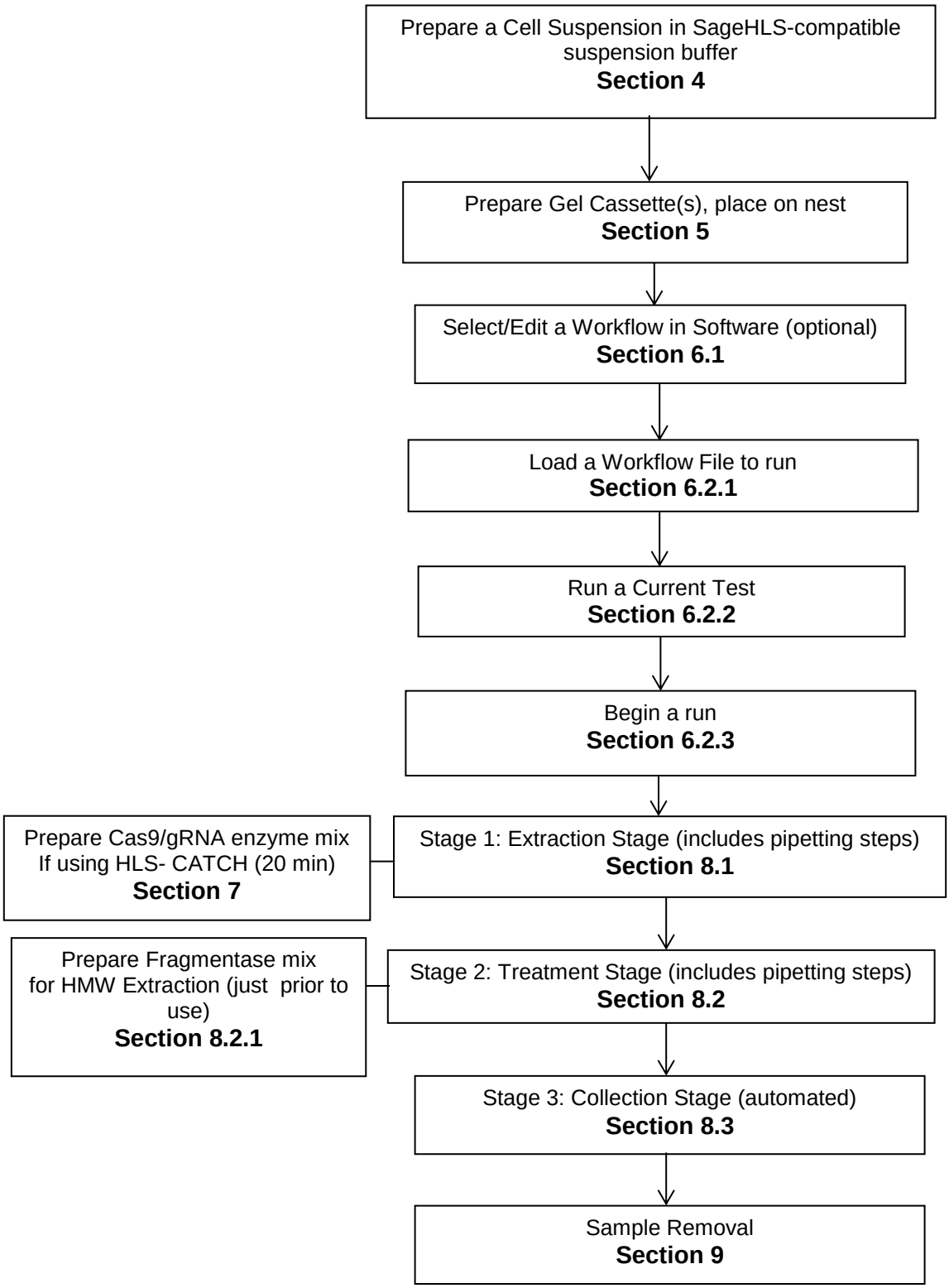
Electrophoresis separates DNA by size within the gel column.



b. Electrophoresis (elution)

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

3.4 Typical workflow



4 Making Cell Suspensions

Important! The SageHLS purifies DNA from cells from varying origins. Different types of cells will require specific suspension protocols. User should refer to instructions provided with the specific reagent kit for the correct guidelines.

4.1 Cell Suspension Procedure

1. Follow the reagent kit instructions for preparing cells. Make sure the cells to be used are in suspension, and not lysed. If lysis hasd occurred, the solution will become viscous.

2. Adjust all concentrations with **HLS Suspension Buffer (or spheroplast buffer if perparing bacterial samples)** to achieve an effective genomic concentration of approximately 10μg/70μl.

3. Hold cells on ice until needed.

Dilution buffer contains sucrose, phenol red dye, EDTA, and electrophoresis buffer (Tris-TAPS).

Important! HLS Suspension buffer does not contain hazardous, known mutagenic, or known carcinogenic substances. Refer to the Material Safety Data Sheets (MSDS) for this kit for a comprehensive outline of the safety classifications (www.SageScience.com/product-support/sagehls-support/). Users should follow safe laboratory practices. Contact Sage Science support about missing or expired reagent

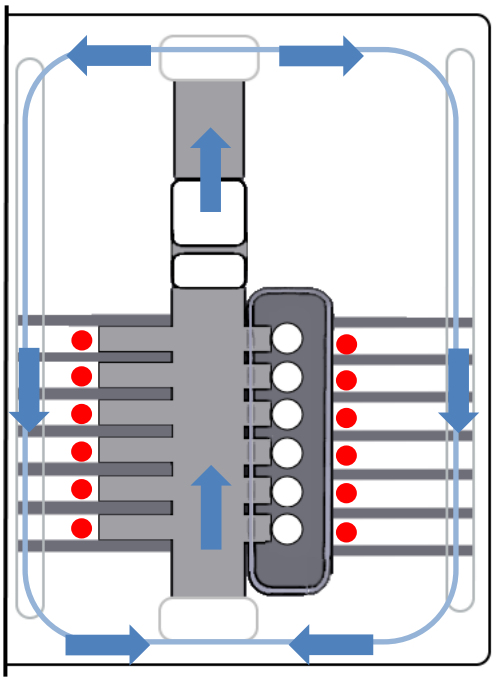
5 Preparing the SageHLS Gel Cassette

5.1 Recommended Laboratory Equipment

- P100 single-channel pipette
- Running Buffer (included with the cassette package)
- Elution well sealing tape ((included with the cassette package)

5.2 On the Bench Top

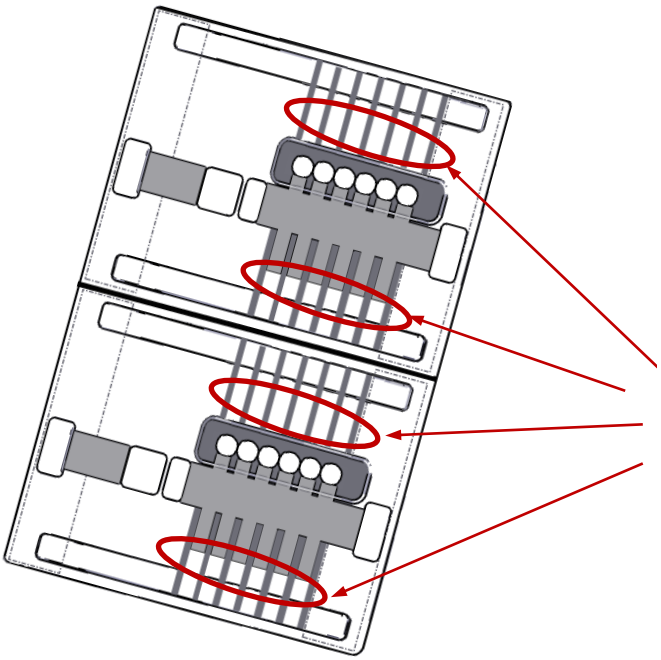
When agarose gel cassettes are manufactured, some air is left within the cassettes to provide effective adhesive sealing during shipment. Before use, the following procedures must be undertaken to clear the headspace, and ensure sufficient buffer capacity.



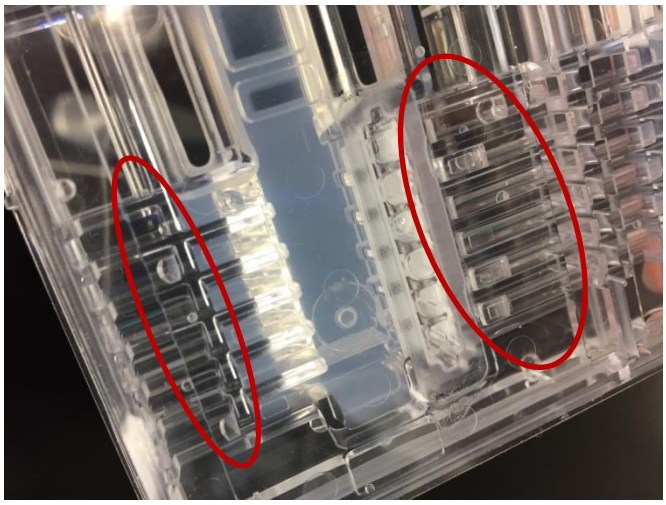
SageHLS Gel Cassette Buffer movement during Electrophoresis
Upper and lower buffer compartments are connected by side channels. Air trapped in elution channels (red dots) needed to be cleared before opening cassette.

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 directy above.

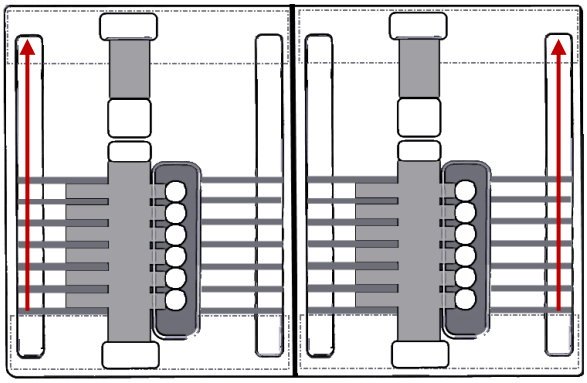


Tap to dislodge bubble from these areas into the troughs directly above.

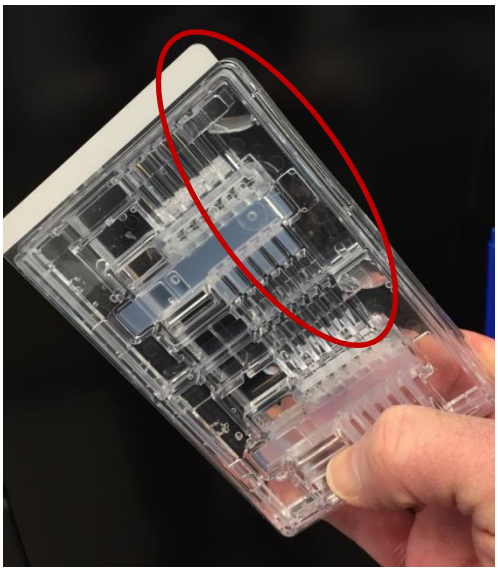


Bubbles in the elution paths

3 Slowly rotate the cassette 180 degree in the clock-wise direction. Allow the bubbles to collect in the upper buffer area. Repeat the tapping procedure. Gently tap if necessary.



Move any bubbles to the upper buffer area.



Aggregate and collect air bubbles in the upper buffer chambers

5.3 On the Instrument Nest

1. With cassette held at a slight angle to keep bubbles loacated 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.

Important! The cassette must be placed on the nest gently to avoid scratching the thin walls of the sample well area against the edges of sample well heater, which protrudes up ½ inch from the floor of the nest.

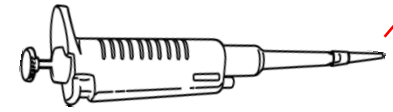
2. While holding the cassette firmly in place on the nest, grab the tape tab, and pull the tape off slowly and firmly, changing the direction of force back and forth sideways.

3. Remove all buffer from all 12 (6 per side) 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.

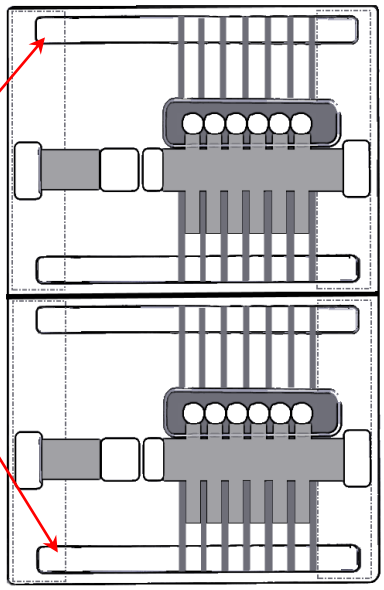
4. Taking care not to introduce additional bubbles into the elution modules, add 85 µl of buffer to all 6 wells

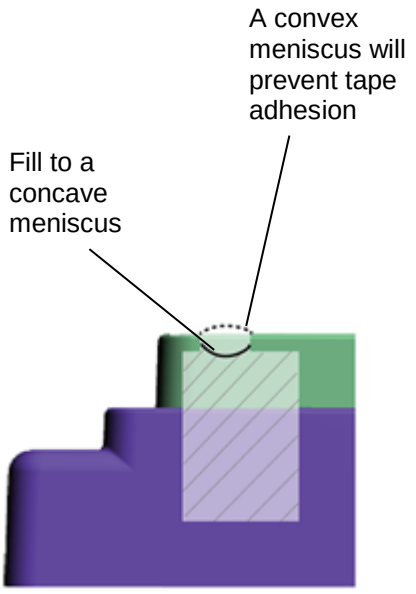
   Add **Running Buffer** to the upper buffer chamber on each side of the gel cassette until the level is flush with the top of the cassette. An adequate buffer level is important for achieving best results from the SageHLS.

Important! A properly filled reagent well will have a **concave meniscus**. If overfilled, the excess Lysis reagent will prevent adhesion of sealing tape in the next step.



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





cassette side view

6 Using the SageHLS

6.0 Overview

The processing of samples on the SageHLS requires combined interactions with the instrument (opening and closing the lid), software (pausing and resuming), and reagent agarose gel cassette (reagent transfers with a pipette).

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 offers several pre-programmed workflow files for supported application. User can modify the pre-programmed workflows and save the customized workflows under new names. Users can also program completely new workflows from scratch using advanced programming features (**see Section10**)

General Overview of sageHLS Workflows

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

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

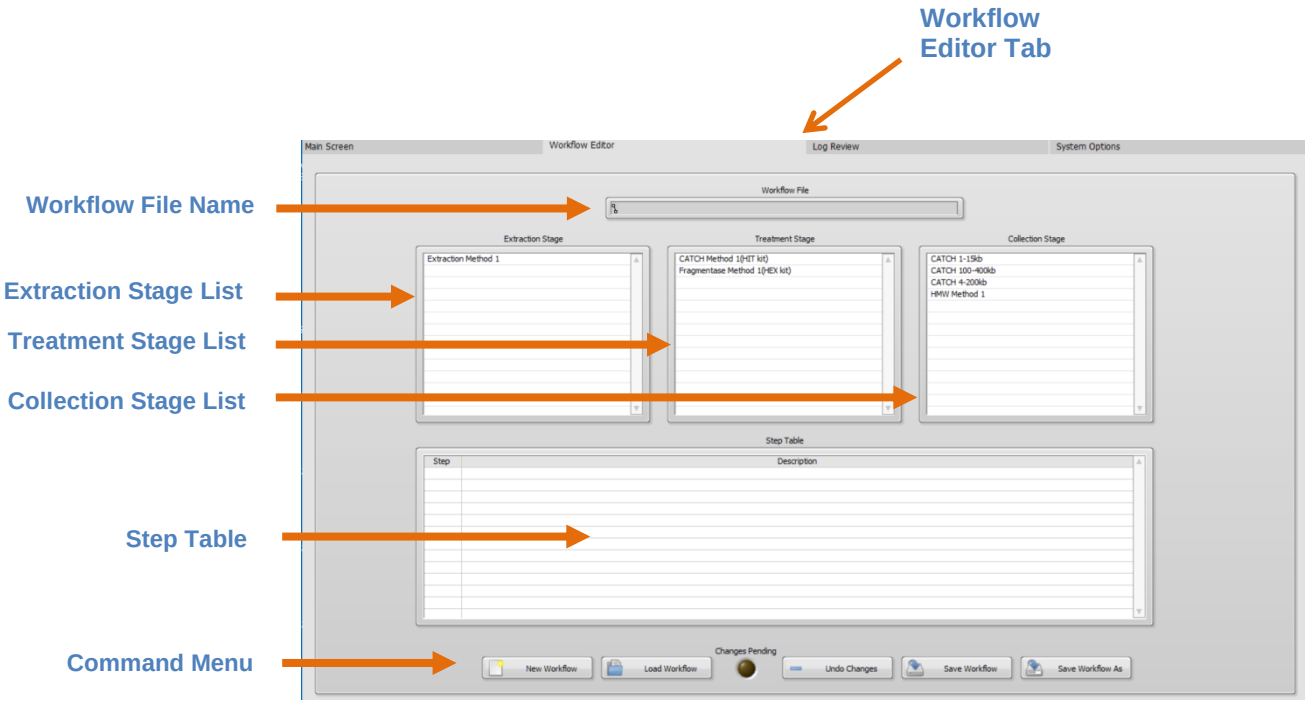
Stage 3: Collection Stage - (DNA size selection options)
Size Separation and collection (DNA size selection). These differ by target collection size requirements.

Step types:

separation voltage, pulsed-field, time
separation voltage, DC, time
elution voltage, DC, time
elution reverse voltage, DC, time

6.1 Creating a Workflow

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

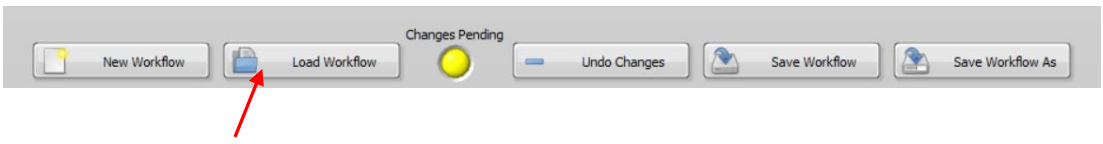


The Stage List fields are populated with pre-set stage protocols.

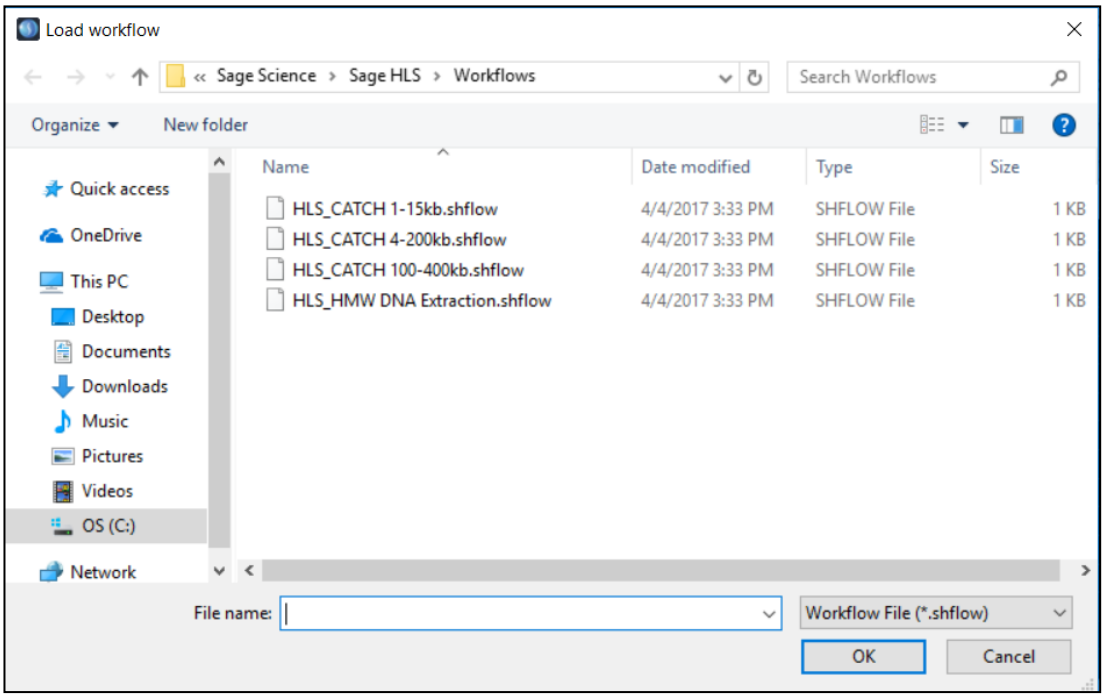
6.1.1 Workflow File

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 .shsflow file extension.

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

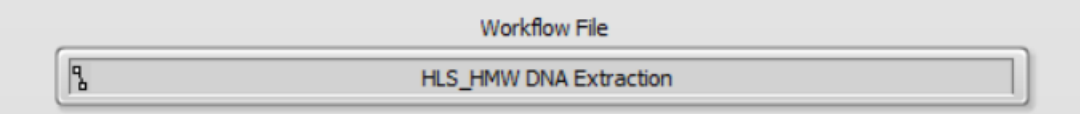


A file list will pop-up from the SageHLS/Workflows folder:

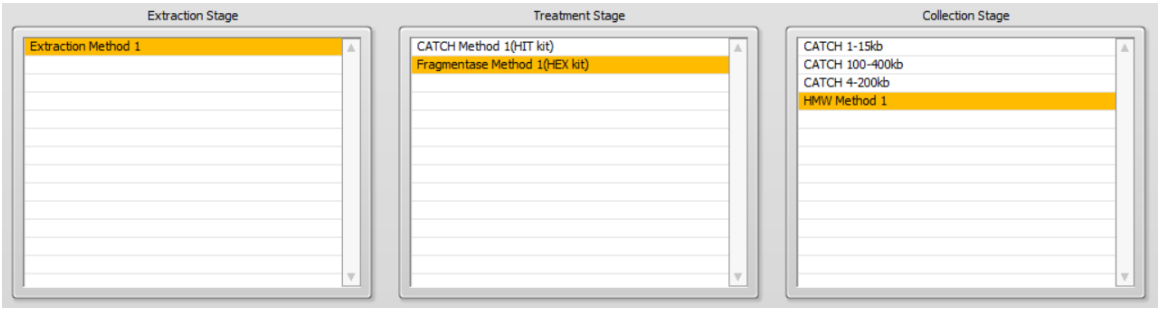


When a Workflow File is selected;

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 begins with the step sub-number (i.e. 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

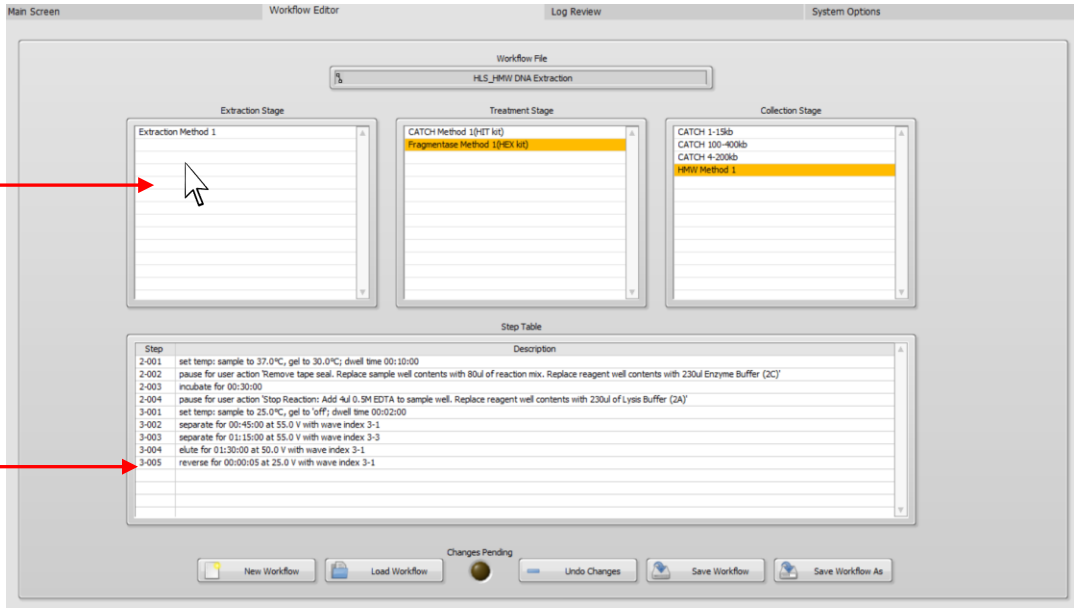
Important! Stage Protocols may be edited or created in the Stage Editor. This is accessed in the factory setting using a super-user password. See Section 10.

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

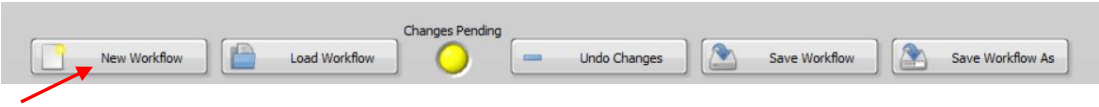
A Stage Protocol can be de-selected by clicking on a blank field within the list. This will remove all of those Stage's steps from the Step Table.

De-select by clicking a blank field

Steps from highlighted Stage Protocols are displayed



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

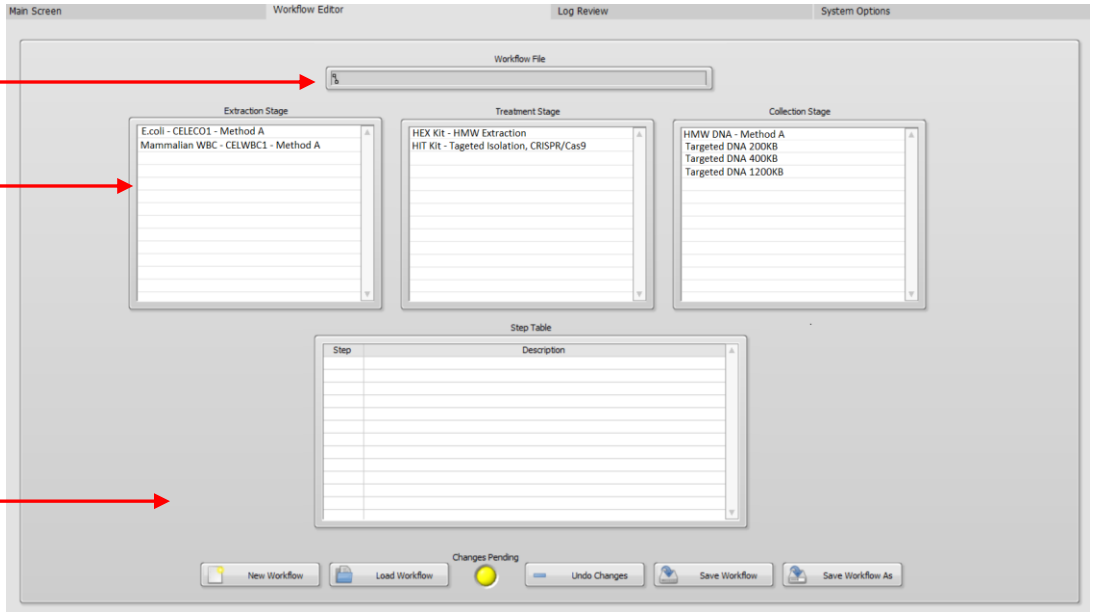


This will clear the Workflow File name and deselect all highlighted Stages:

Workflow File name is cleared

All Stages are de-selected

All steps are de-listed



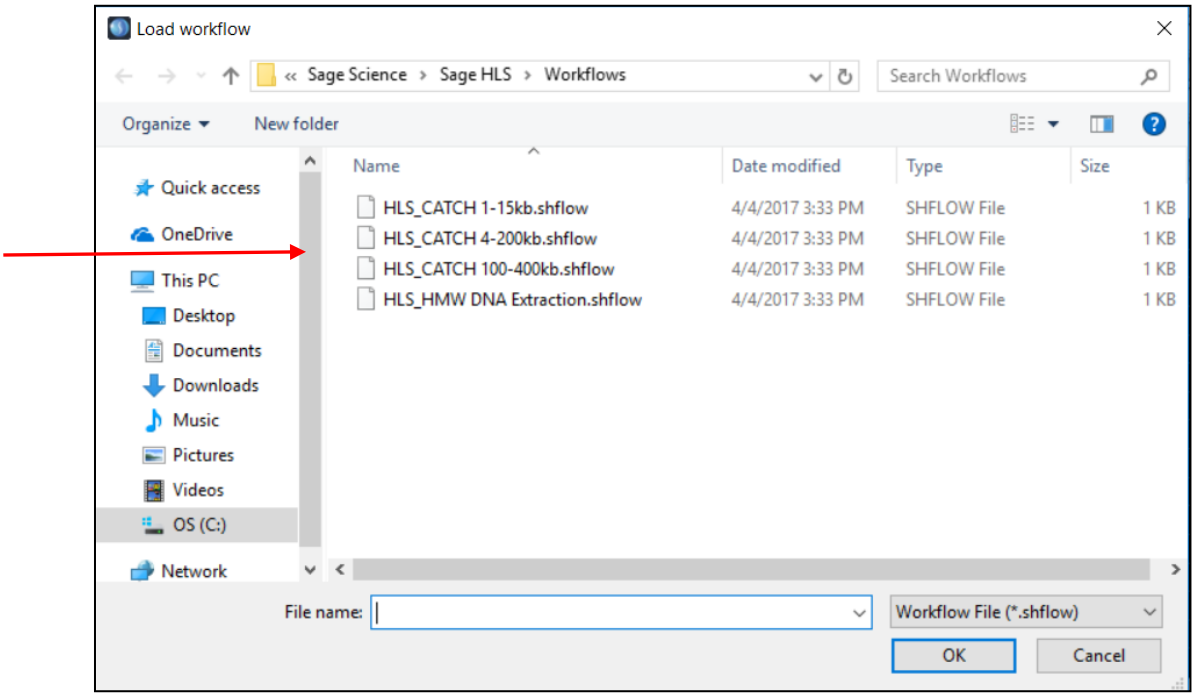
6.1.2 Saving a Workflow File

If a Workflow File has been modified by replacing or eliminating a step, if 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.

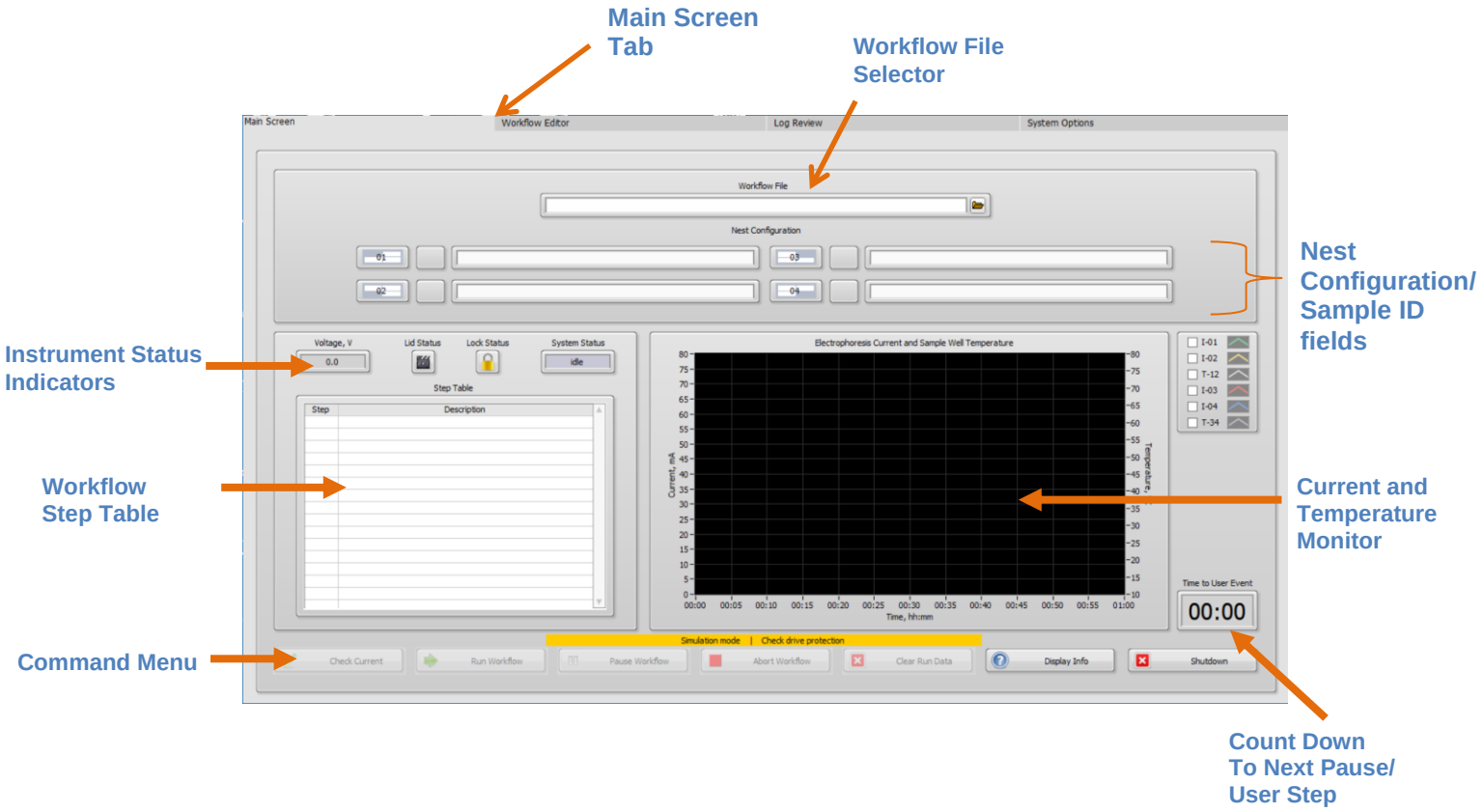
Pre-set Workflow files (HLS prefix) cannot be modified



6.2 Running the SageHLS - Main Screen

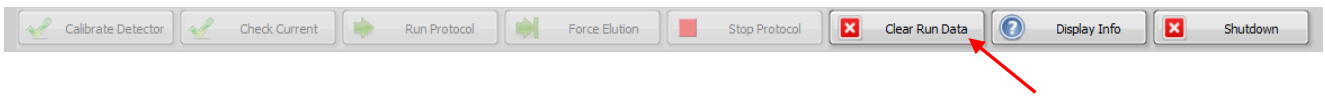
The Main Screen tab is the screen with which users interface with the SageHLS instrument. This includes initiating a run, monitoring a run, and pausing/resuming to conduct a manual pipetting step.

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

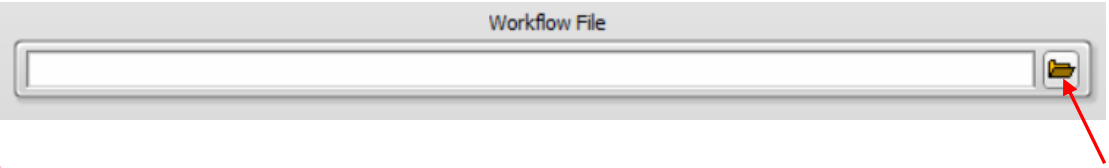


6.2.1 Load a Workflow File

1. If a 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:

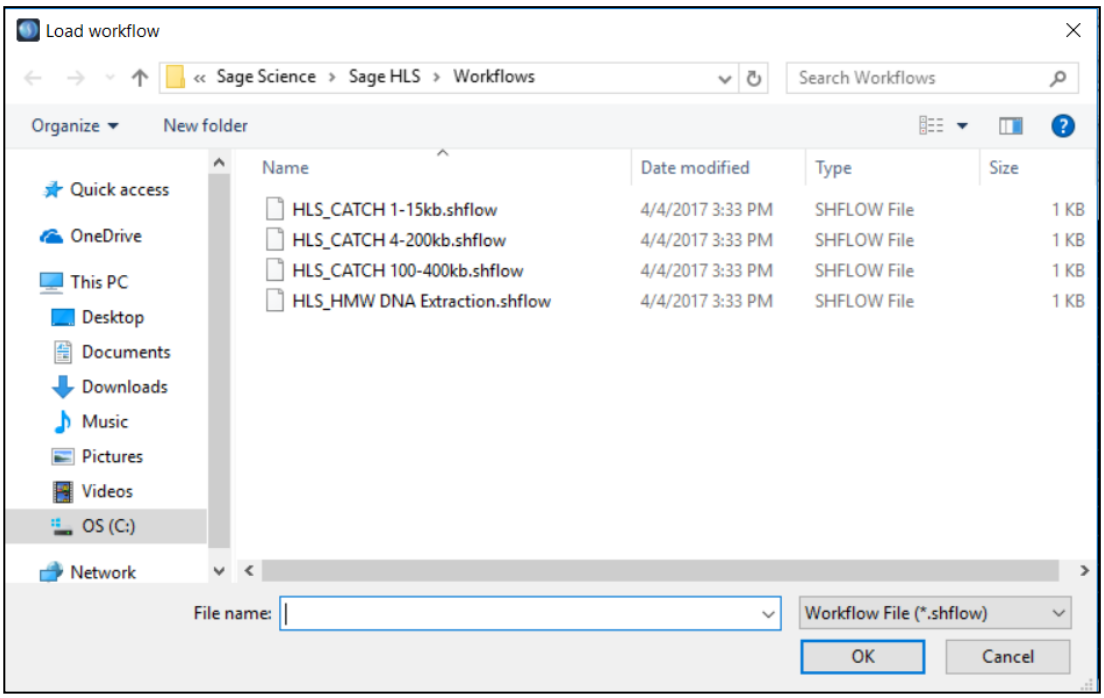


2. Click the folder icon next to the Workflow File Field.

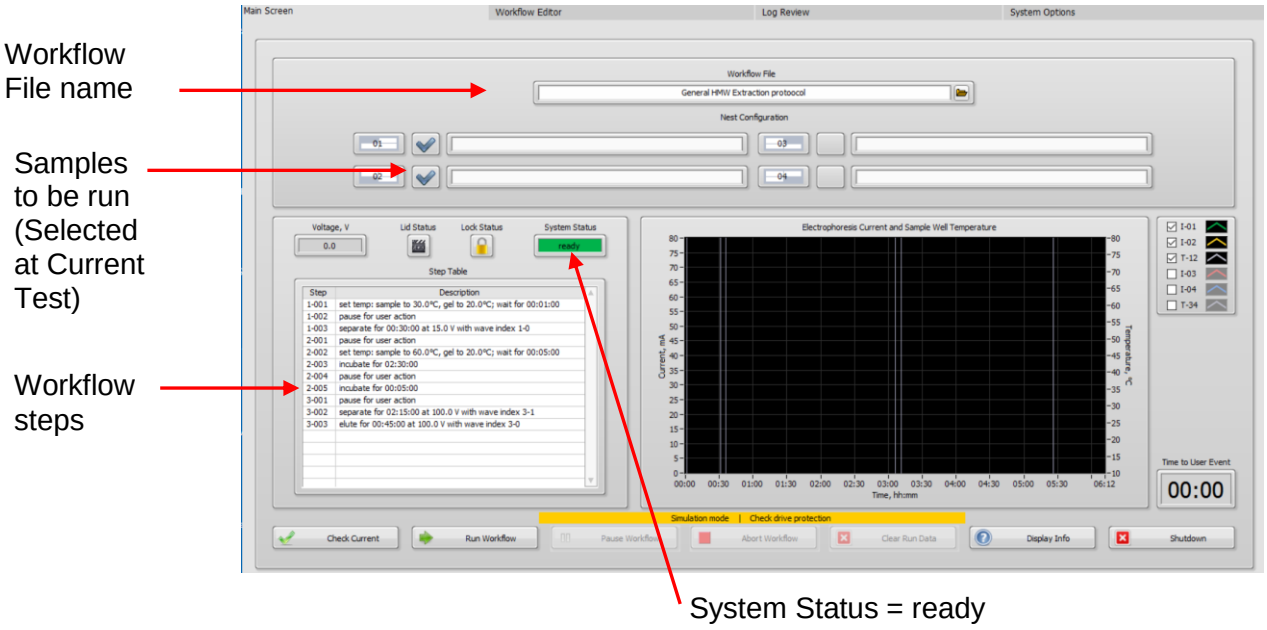


Important! The System Status must display “Idle”, or the Workflow File directory will not open.

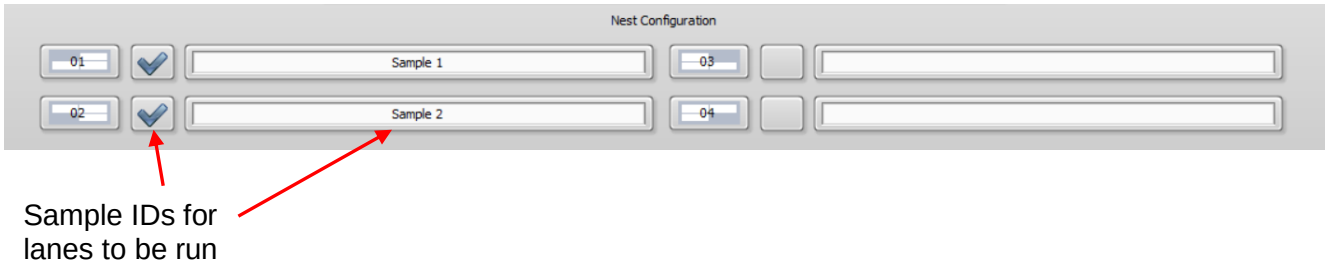
3. A Workflow File directory will open. Select a Workflow File and select “OK”.



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”



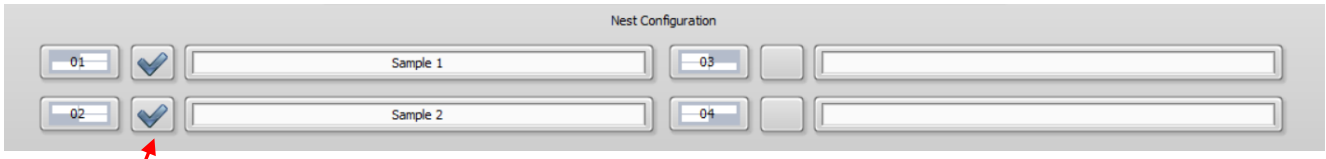
5. Enter Sample ID information into the Nest Configuration fields (optional). Sample IDs will be saved in the log file.



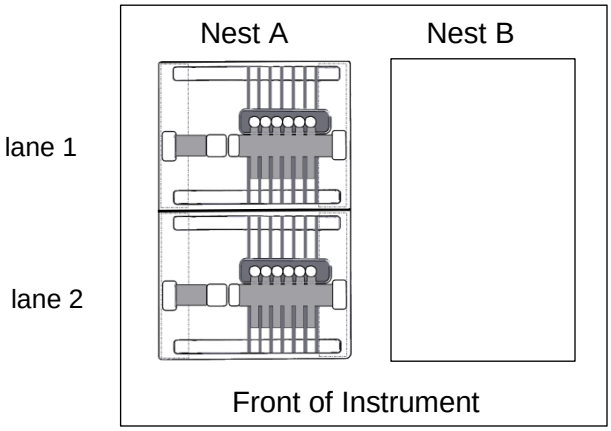
6.2.2 Run the Electrophoresis Current Test

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 (Section 5) 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.

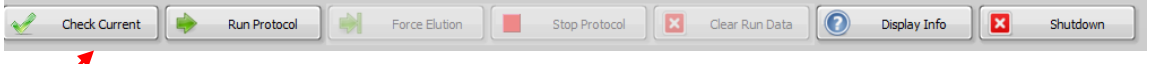


Important! Make sure that all cassette preparation steps in Section 5 have been completed.

The Current Test Procedure:

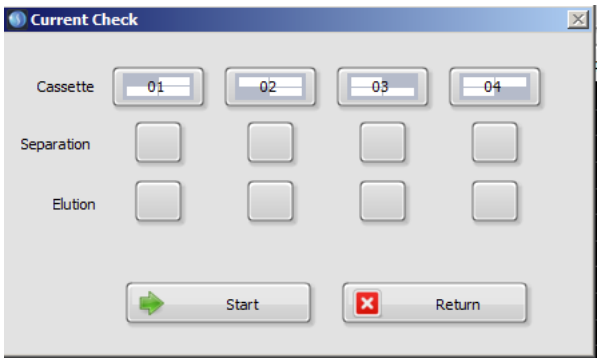
1. Close the SageHLS lid.

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

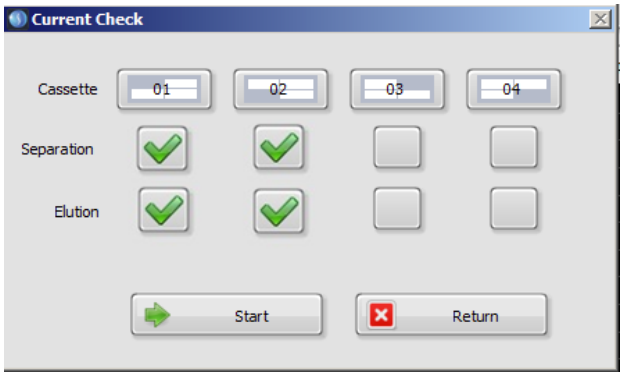


Press “Check Current”

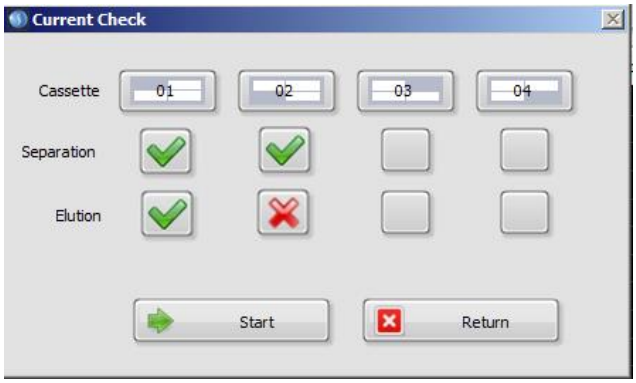
3. A pop-up window will appear:



4. Press “Start”. The lid lock mechanism will engage, and the nest will 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.

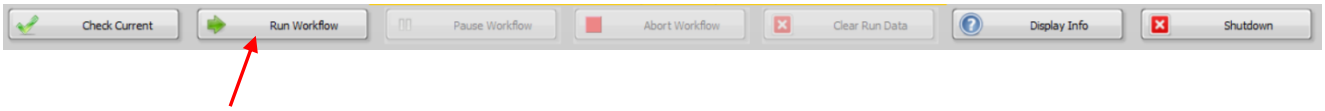


Important! If a current check fails, check the buffer levels in the affected lane, and replace the buffer as needed. Contact Sage Science support if the current test continues to fail.

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

6.2.3 Run the Workflow

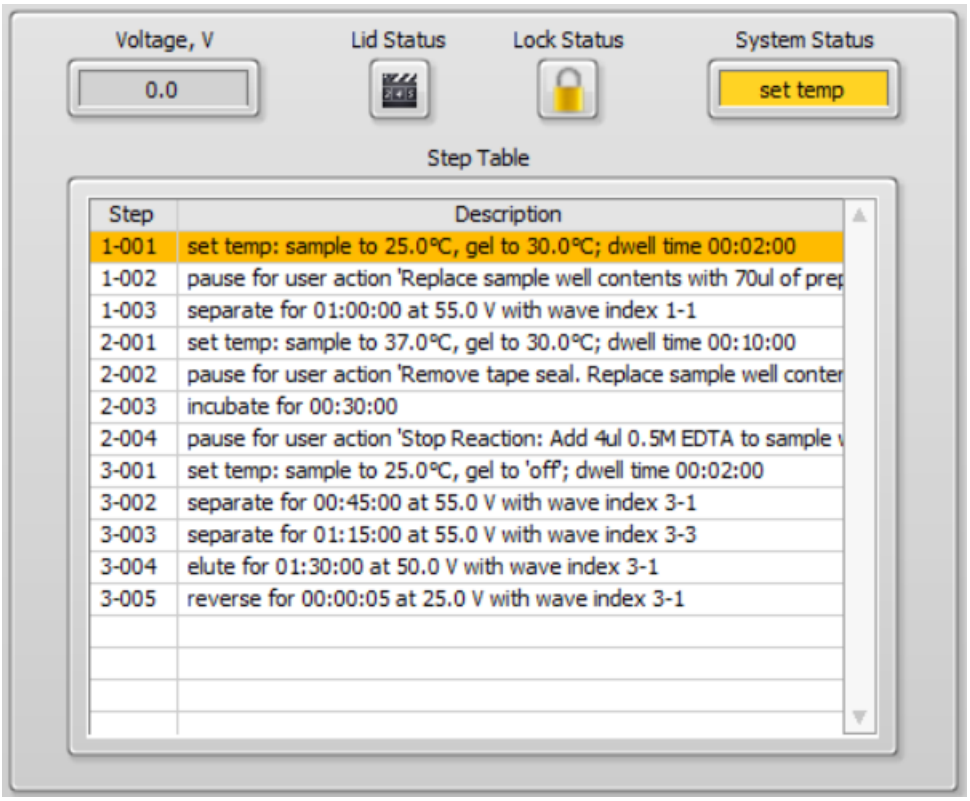
Press “Run Workflow”.



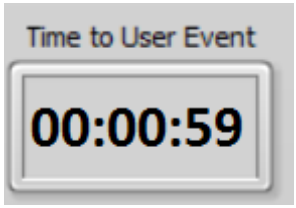
The active step in the Workflow Step Table will be highlighted in yellow.

In some cases, the first step will most likely require a temperature pre-heat step to ensure a constant nest temperature throughout the workflow, and to set the reagent and sample well temperatures to an optimal temperature for the first processing step (typically lysis).

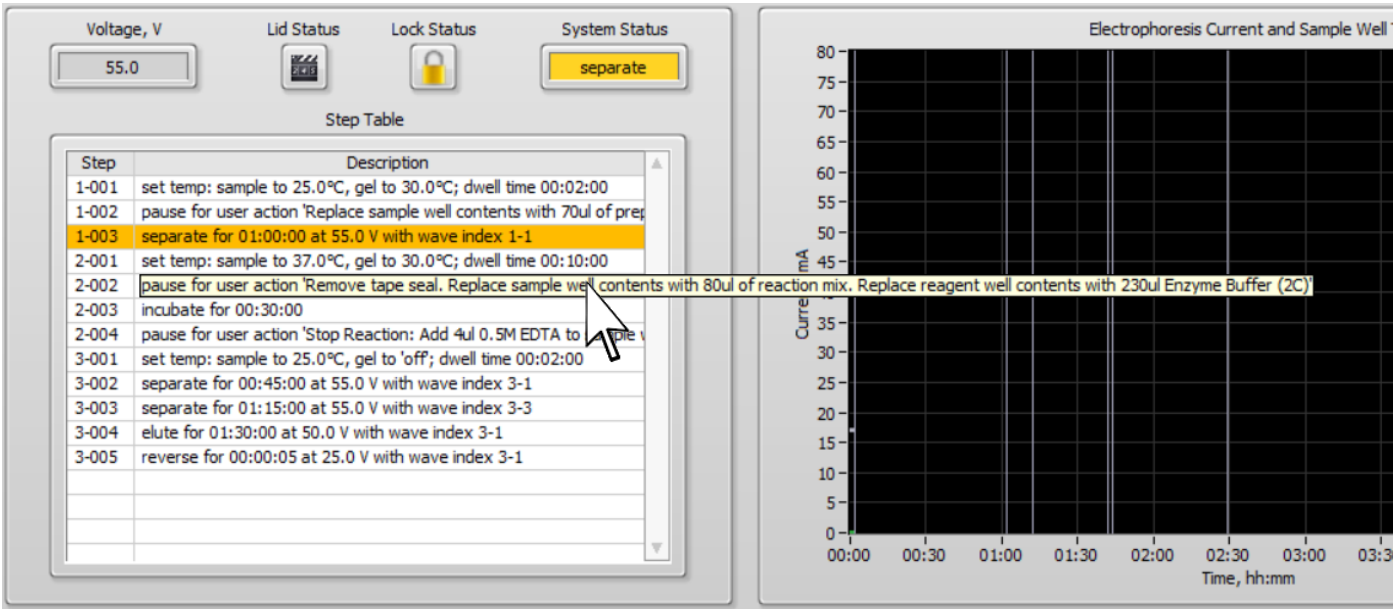
The System Status field will indicate the type of step being run.



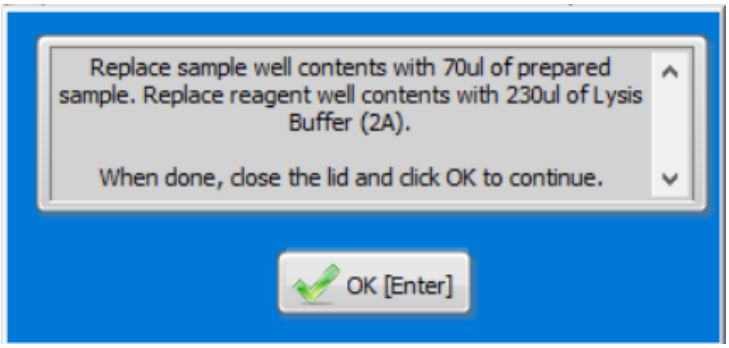
The “Time to User Event” timer will start a countdown until the next Pause step, which will require the lid to be opened, and reagent transfer steps with a pipette.



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

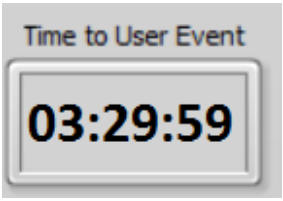


When the instrument reaches a Pause step, a pop-up window will appear with instructions for a pipetting or manual procedure. When the manual procedure is complete, and the lid has been closed, users should press “OK” to continue the Workflow.

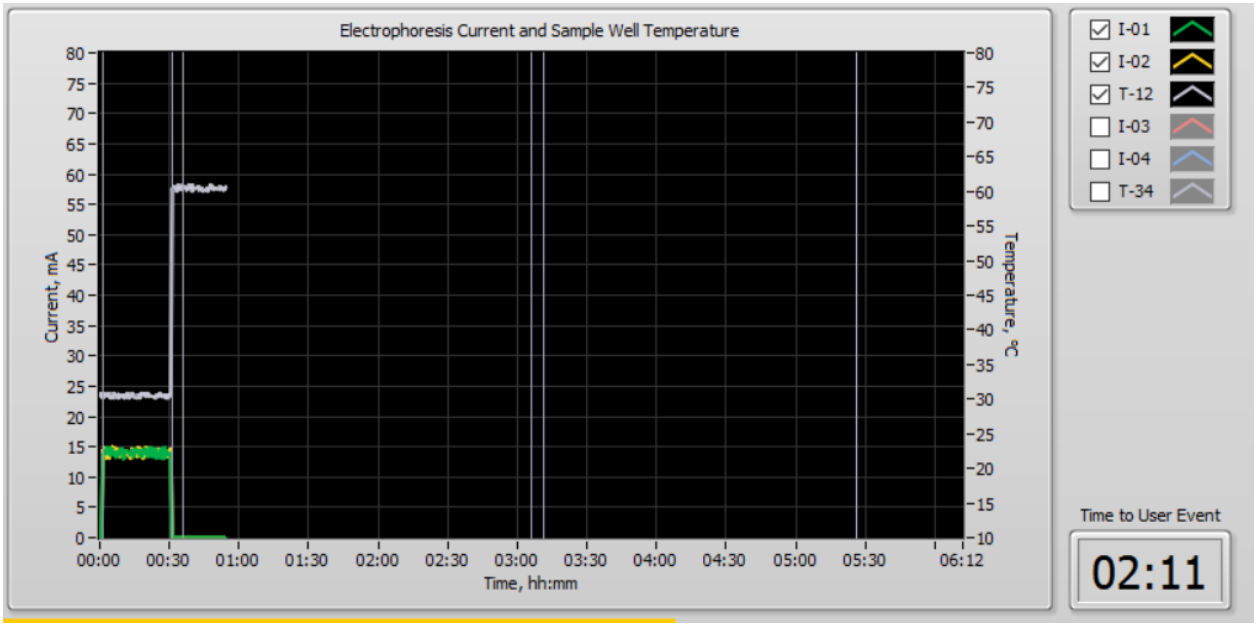


Important! For best practices and important details on the Stage manual steps, review Section 7.

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.

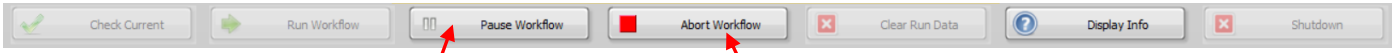


Throughout the workflow the “Electrophoresis Current and Sample Well Temperature” monitor will graphically display (in real time) the electrophoresis current for each sample, and the combined sample well temperature for each nest:



6.3 Other Run Commands

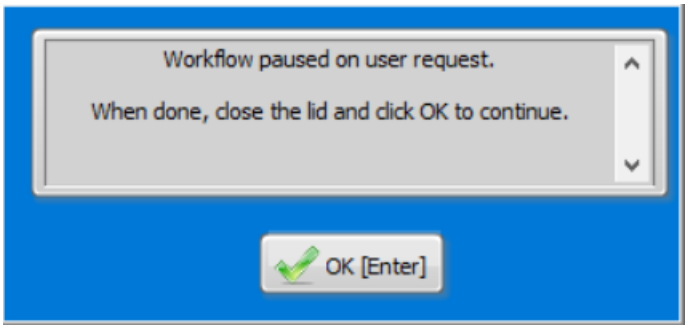
A worklflow may be paused (and then resumed), or stopped at any time during a Workflow:



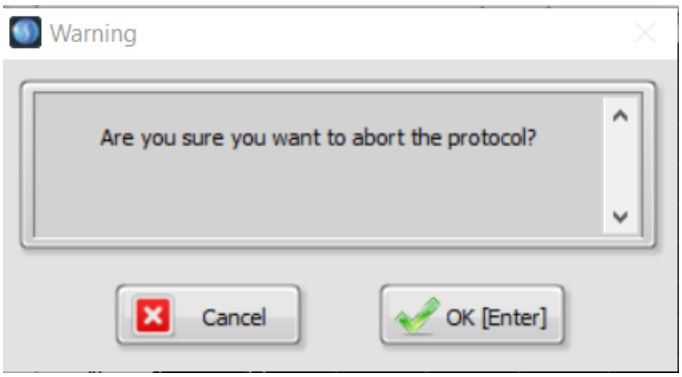
Pause Workflow

Abort Workflow

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



If a Workflow is stopped, the following window will pop-up. Press “OK” to stop the protocol, or “Cancel” to return to the Workflow.



The “Display Info” button can also be accessed during a Workflow. Pressing this button will not affect the Workflow progress.

The following window will pop-up, Press “Advanced Tabs” to enter a password to access advanced tabs. This will allow users to access the Stage Editor tab, which could be useful for troubleshooting under certain circumstances. See **Section 11, Stage Editor** for more information.

To quit the “Display Info” screen, click the SageHLS logo.

7 Preparing the HLS-CATCH Enzyme Mix

Important! The HLS-CATCH enzyme mix requires 30 minutes and should prepared prior to starting the Workflow, or during the Extraction Stage (1 hour, 10 min, Section 8.1). The Fragmentase reaction is very brief and can be prepared just prior to use in the Treatment Stage.

Guide RNAs should be purchased from Integrated DNA Technologies (IDT, ALT-R CRISPR-Cas9 gRNAs). These are provided in two halves; crRNA and tracRNA, and must be annealed before they will assemble with the S.pyogenes Cas9 enzyme. The crRNAs are designed to flank the target sequence, and the tracRNA is identical for all gRNAs.

Cas9 Enzyme should be purchased from New England BioLabs

7.1 Anneal the tracRNA and crRNAs

1. Add equimolar concentrations of tracRNA and crRNAs. Stocks are dissolved in IDT Duplex Buffer (30mM HEPES, pH7.5, 100 mM Potassium Acetate):

	vol. µl	stock [ ] µM	final [ ] in anneal mixture µM
tracRNA	20	100	50
crRNA1	10	100	25
crRNA2	10	100	25
TOTAL	40		

2. Heat the mix at 95^{o}C for 5 minutes (in a heating block or thermal cycler)

3. Remove the mix from heat, and allow to cool on the bench-top

Note: In some cases it might be useful to use gRNAs which cut at closely spaced sites on either side of the target sequence. In this instance, anneal with equimolar amounts of each crRNA, with the total moles of crRNA equal to the total moles of tracRNA. IFdesigning a system with two cut sites on either side of the target, anneal with all four crRNAs at 12.5µM and tracRNA at 50 µM.

7.2 Assemble the Cas9-gRNA complete reaction mixture

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

order of addtion	vol. µl	stock [ ] µM	final [ ] in enzyme mixture µM	
1	16	4	1	**Nuclease free H2O**
2	20	10	25	**4X Enzyme Buffer (provided by Sage Science)**
3	40	20	1	**Annealed gRNA mix (from Section 7.1, above)**
4	4			**NEB Cas9 nuclease, wt**
TOTAL	80			

order of addtion	vol. µl	stock [ ] µM	final [ ] in enzyme mixture µM	
1	16	4	1	**Nuclease free H2O**
2	20	10	25	**4X Enzyme Buffer (provided by Sage Science)**
3	40	20	1	**Annealed gRNA mix (from Section 7.1, above)**
4	4			**NEB Cas9 nuclease, wt**
TOTAL	80			

2. Mix by pipetting up and down.

3. Incubate at 37^{o}C for 10 minutes in a thermal cycler or heat block.

4. The Enzyme Mix can be placed on ice for up to 3 hours prior to use.

8 Workflow Stages: Manual Steps

Important! The HLS-CATCH enzyme mix requires 30 minutes and should prepared prior to starting the Workflow, or during the Extraction Stage (1 hour, 10 min). The Fragmentase reaction is very brief and can be prepared just prior to use in the Treatment Stage.

8.1 Extraction Stage

DNA extraction from cells requires lysis of cell suspensions. In the sageHLS system, electrophoresis is used to apply the lysis reagents to the cells without mixing or viscous shear. Electrophoresis also separates non-DNA contaminants and lysis reagents from the extracted DNA. The extracted DNA remains trapped in the wall of the sample well, due its large size (>>2mb).

The Extraction Stage requires approximately 1 hour of instrument run time. If users are using the HLS-CATCH targeted isolation kits, enzyme reaction component can be prepared during this time, particularly if multiple regions are being targeted. The reaction mix can be kept on ice. For HMW DNA extractions, a simple 1:400 dilution with Fragmentase is required, and it is recommended that this be prepared right before using.

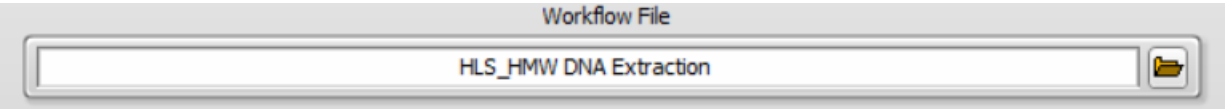
Important! The SageHLS can process 1 or 2 gel cassettes per run (1 to 4 samples). However the same protocol must be used, and the same sample types must be used, for a single run.

Important! Reagents in this section do not contain hazardous, known mutagenic, or known carcinogenic substances. Refer to the Material Safety Data Sheets (MSDS) for this kit for a comprehensive outline of the safety classifications (www.SageScience.com/product-support/sagehls-support/). Users should follow safe laboratory practices. Contact Sage Science support about missing or expired reagents.

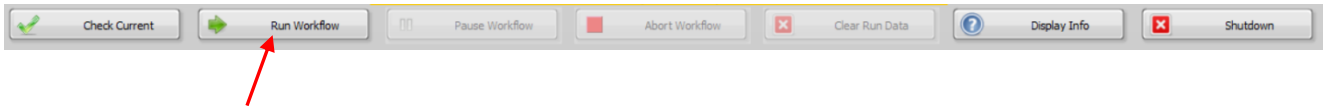
1. Cell suspensions should be prepared should be prepared at a concentration equivalent to a genomic DNA concentration of 10μg/70μl in HLS Suspension Buffer

2. The gel cassette(s) should be on the nest(s) and have been prepared and current-tested (see Sections 5 and 6)

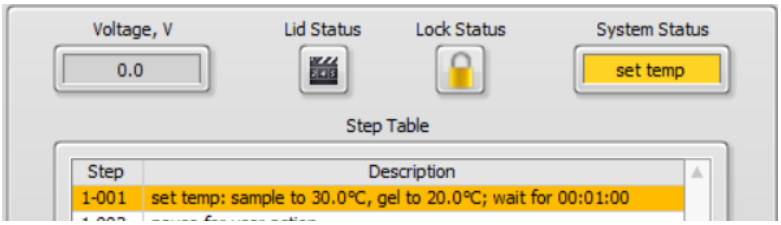
3. In the Main Screen of SageHLS software, load the appropriate Workflow file. The the cell suspension Kit documentation for guidance, or use the Workflow Editor screen to create a new Workflow file.



4. Close the lid and press “Run Workflow”:

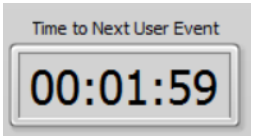


5. The first step in the Workflow will likely heat the sample wells and nest(s) to a set temperature:

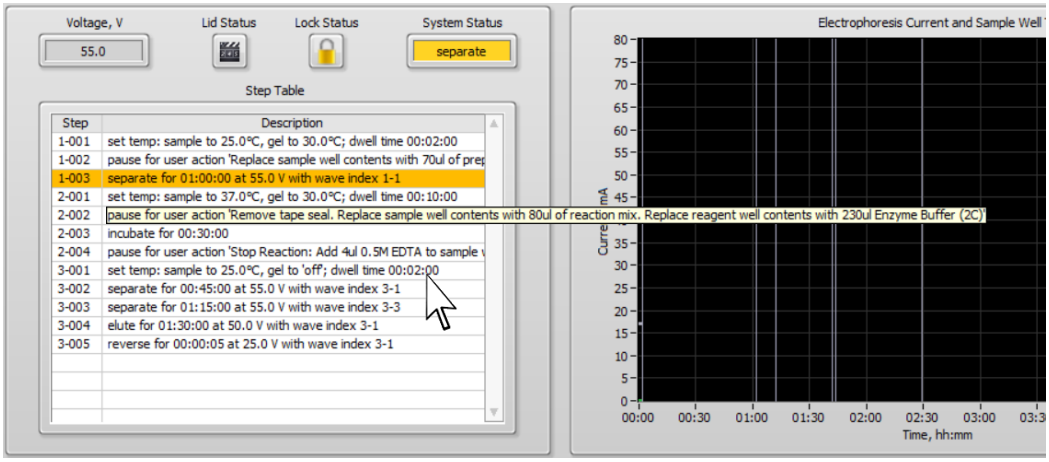


Hot Surface. From this point until the end of the workflow the aluminum blocks within the nests have active heat control. The operating temperatures are not likely to cause injury, but users should be aware and avoid incidental contact with exposed aluminum blocks.

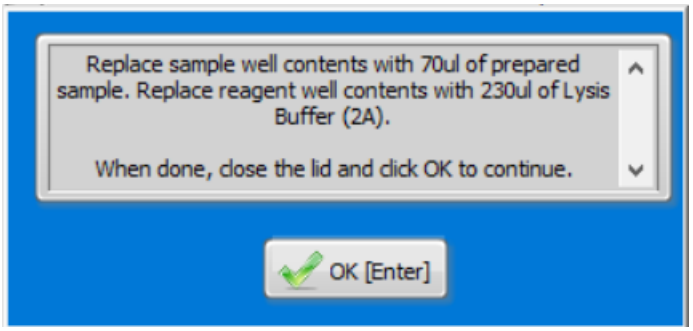
6. The “Time to User Event” countdown timer will indicate the time until the pause required for the first pipetting step.



7. Hovering the mouse pointer over the step field to display the entire instruction for for users to review the upcoming user action step:

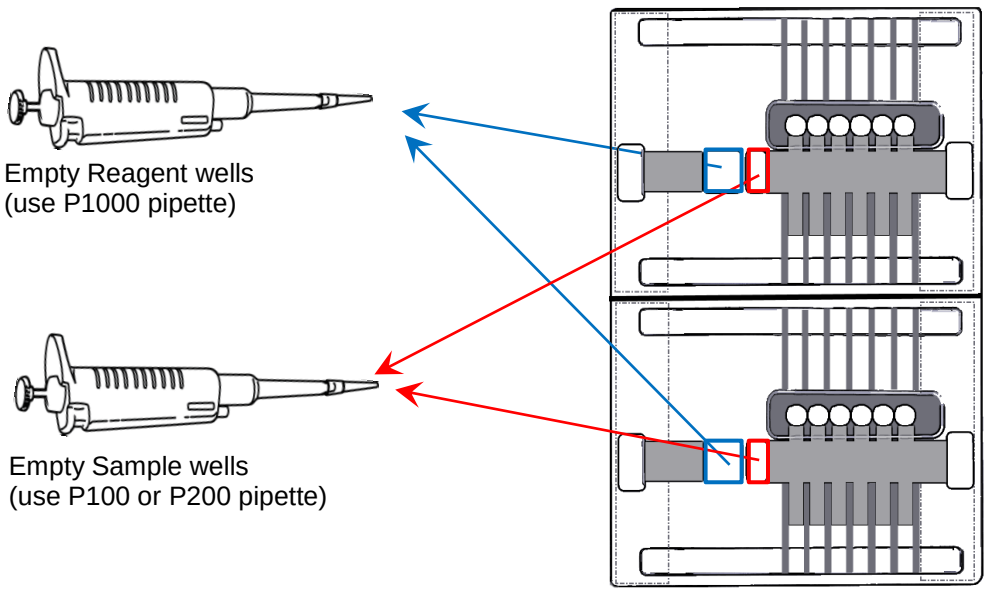


8. When user action is required, the instrument will pause and a window will pop-up. The window will display the user instruction step that was programmed into the workflow.



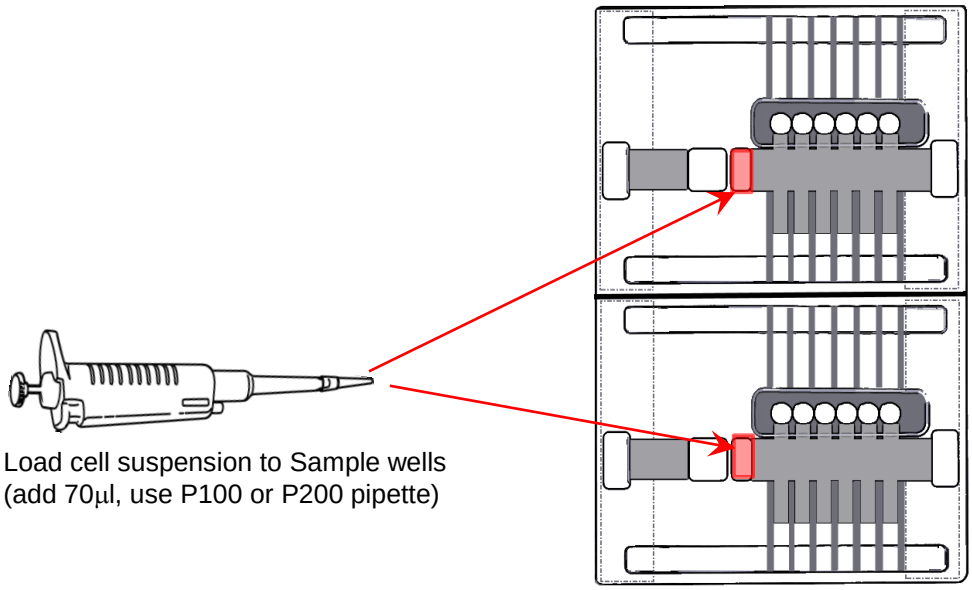
Important! The instrument will remained paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user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

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



10. Load **70µl of the cell suspension** (previously prepared, see Section 4) into each Sample Well.

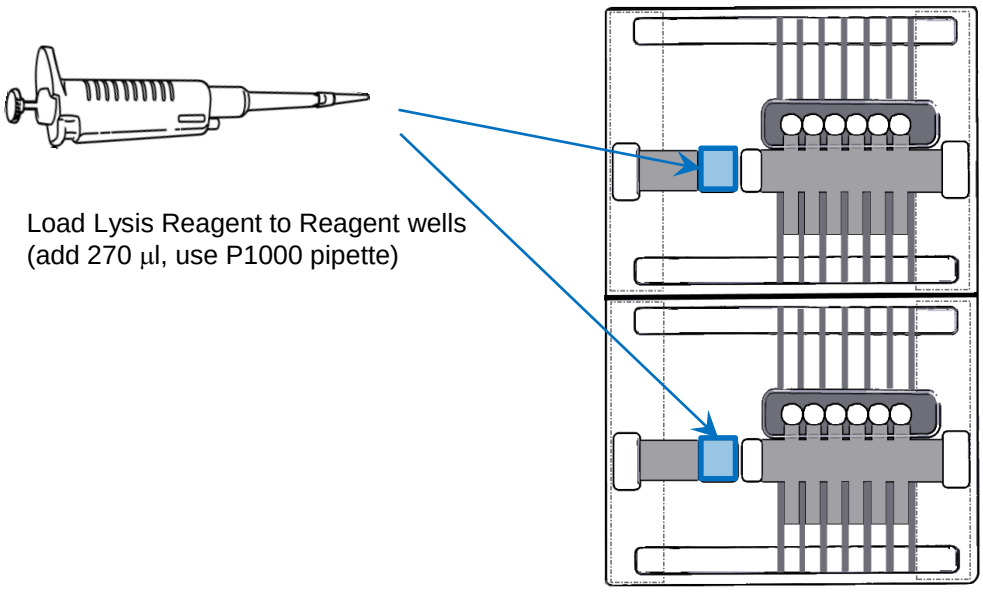
Important! The loading volume should remain fixed at 70 ul regardless of the cell number used.



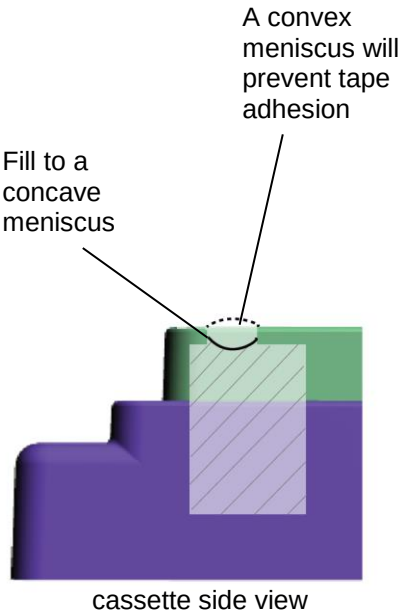
Load cell suspension to Sample wells
(add 70µl, use P100 or P200 pipette)

11. Load approximately **230µl of Lysis Reagent** into each Reagent well. Lysis Reagent is supplied with the reagent kit.

Important! A properly filled reagent well will have a **concave meniscus**. If overfilled, the excess Lysis reagent will prevent adhesion of sealing tape in the next step.



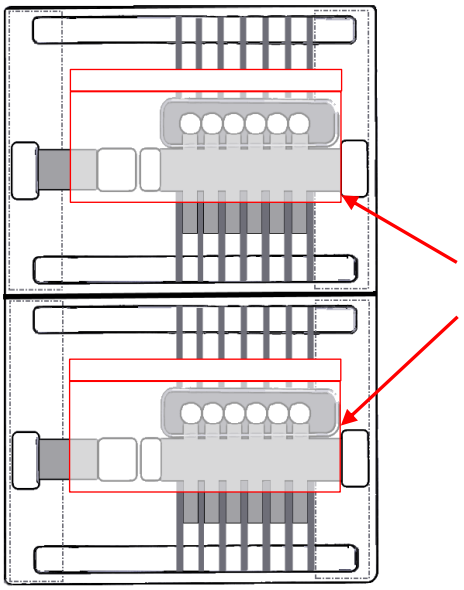
Load Lysis Reagent to Reagent wells
(add 270 µl, use P1000 pipette)



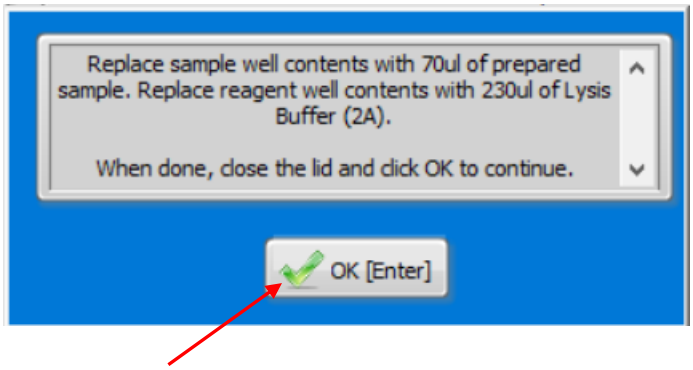
cassette side view

12. Seal the Reagent wells, Sample wells, and Elution wells with the adhesive tape provided. Make sure the tape covers all of the wells and seal tightly.

Ensure adhesive tape tightly seals the wells shown.

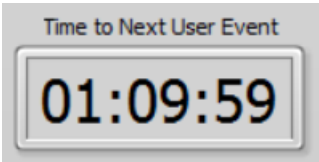


13. Close the Lid and press “OK” in the pop-up window to resume the instrument and workflow.



Important! Users should add the lysis reagent, seal the wells, and resume the software protocol without delay. With a significant delay, the lysis reagent will diffuse into the sample well before electrophoresis begins. This can affect the quality of purification.

11. The counter will reset and begin to count down 1 hour 10 minutes to the next user action pause:.



12. The purification process can approximately 1 hour, 10 minutes.

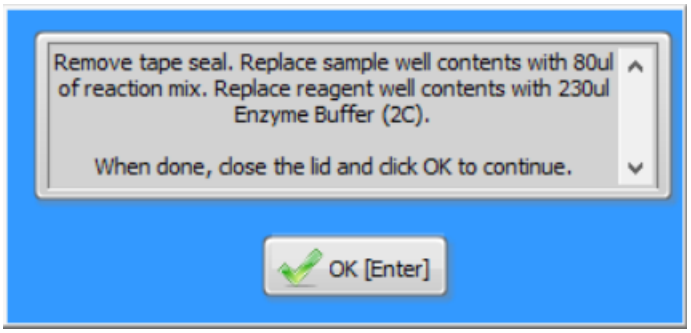
Important! Lysis Reagent does not contain hazardous, known mutagenic, or known carcinogenic substances. Refer to the Material Safety Data Sheets (MSDS) for this kit for a comprehensive outline of the safety classifications (www.SageScience.com/product-support/sagehls-support/). Users should follow safe laboratory practices. Contact Sage Science support about missing or expired reagent

8.2 Treatment Stage

For random cleavage Fragmentase reactions, refer to the next **Section, 7.2.1**. For targeted HLS-CATCH reactions, skip to **Section 7.2.2**.

8.2.1 HMW DNA Extraction: NEB® Fragmentase®

1. After the sample well(s) has reached an initial set temperature, the instrument will pause, and a user action window will pop up. The window will display the user instruction step that was programmed into the workflow:



Important! The instrument will remained 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.

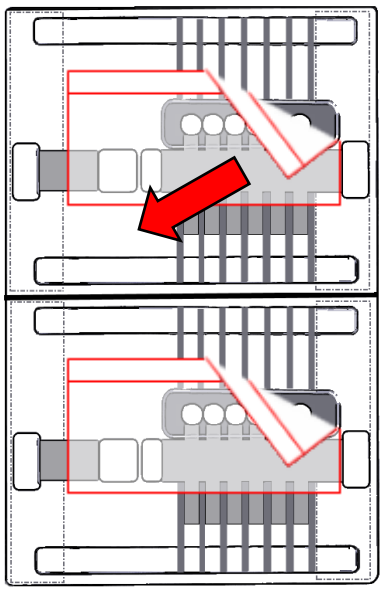
2. Prepare the Enzyme Reaction Mix:

    a. Remove NEB Fragmentase from the freezer, briefy vortex to mix

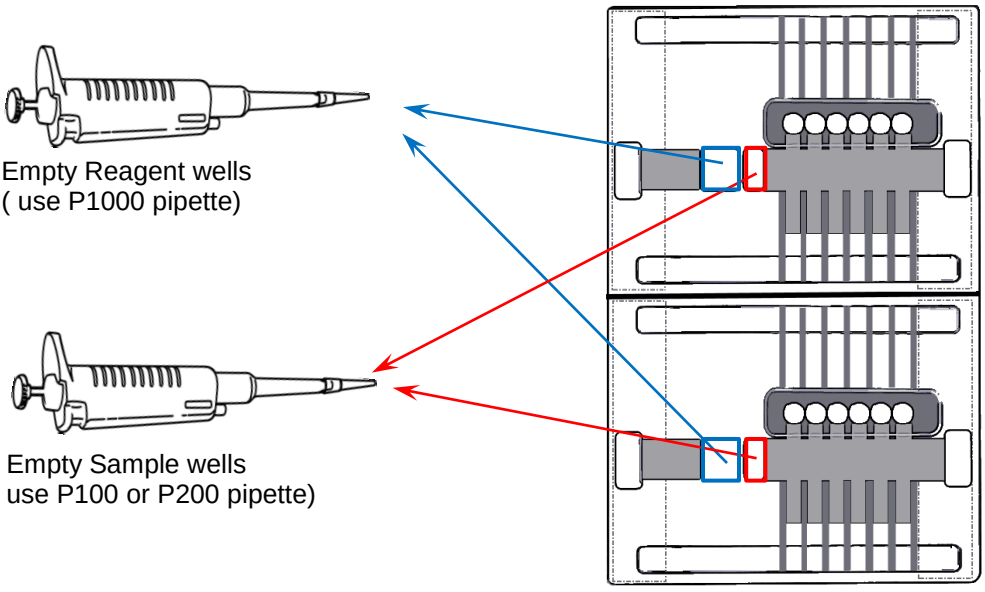
    b. To 800µl of Enzyme Buffer, add 2µl of NEB Fragmentase (1:400 dilution), vortex to mix

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.

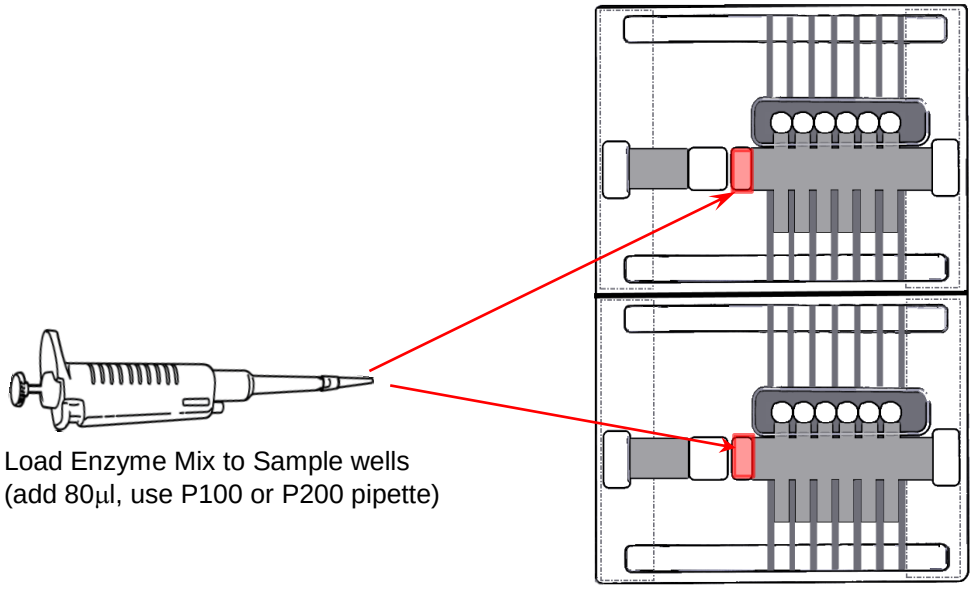
grab the tab in right upper corner and peel diagonally with a slow smooth motion



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

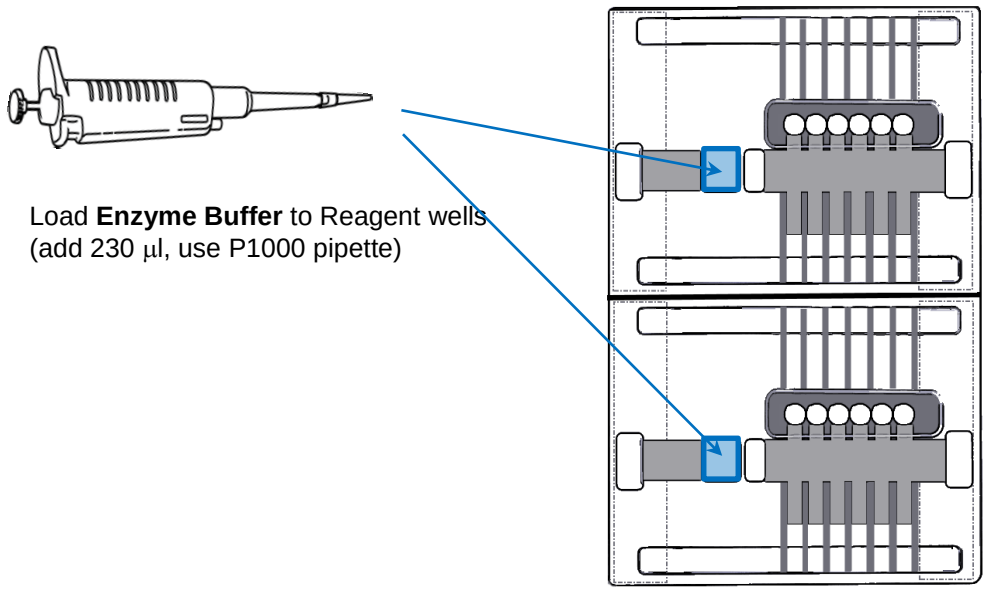


5. Load **<u>80µl</u> of the Enzyme Mix** into each Sample well. Enzyme Mix is prepared with the reagents provided the cassette kit, or with reagents supplied by users and prepared with SageHLS-compatible instructions.



Load Enzyme Mix to Sample wells
(add 80µl, use P100 or P200 pipette)

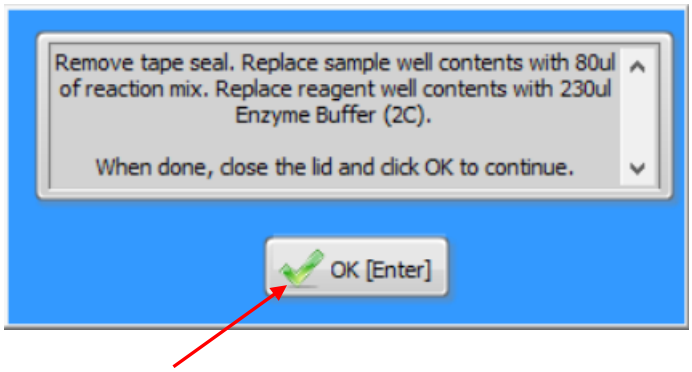
6. Fill the Reagent Wells with **<u>230µl</u> of the Enzyme** Buffer(without enzyme).



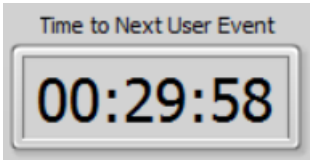
Load **Enzyme Buffer** to Reagent wells
(add 230 µl, use P1000 pipette)

7. Close the Lid and press “OK” in the pop-up window to resume the instrument and

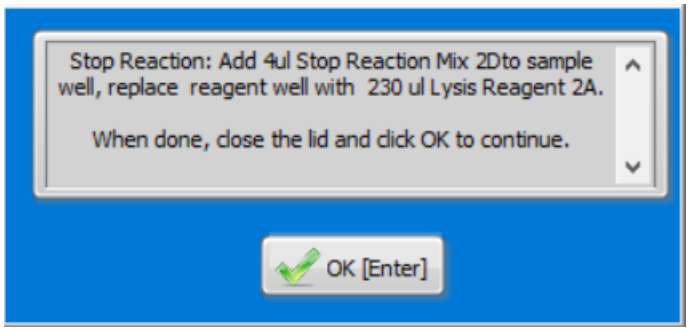
workflow.



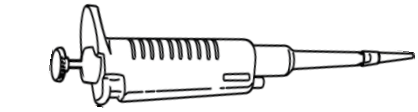
8. The counter will reset and begin to count down 30 minutes to the next user action pause:



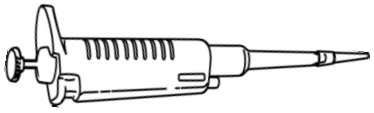
9. At the end of the enzyme reaction incubation, the software will pause for a quenching step



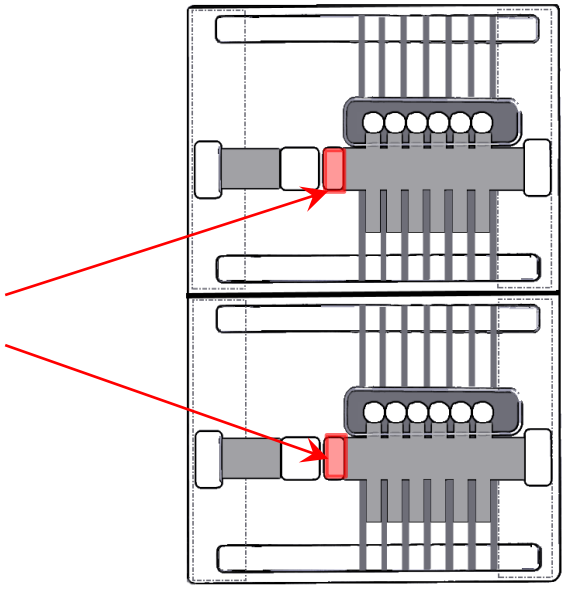
10. Add **4µl Stop Reaction Mix** to the Sample Well, gently pipette up and down 1-2X with a P100 or P200 pipette to mix the reagent into the sample



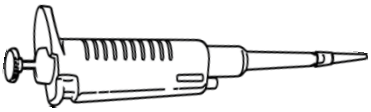
Add **Stop Reaction Mix** wells
(add 4µl, use P10 pipet)



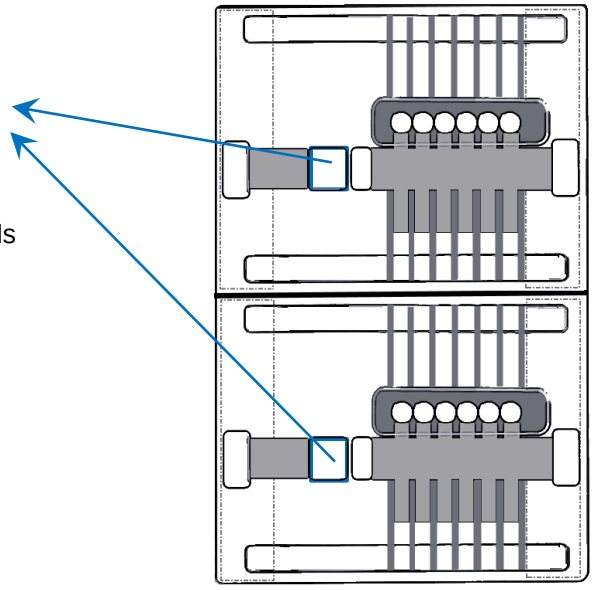
Mix by pipetting up and down
(use P100 or P200 pipet)



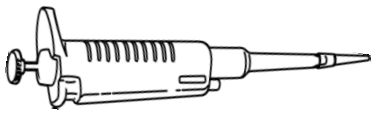
11. Remove the contents of the Reagent Wells (approx. 270µl)



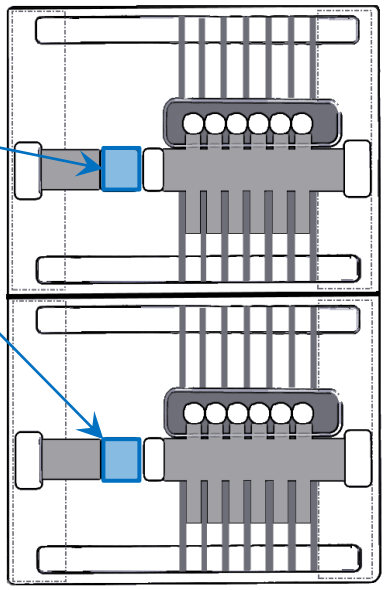
Remove the contents of the Reagent wells
(use P1000 pipette, 300µl)



12. Load **<u>230 µl</u> of the Lysis Reagent** into each Sample well. Lysis Reagent is provided with the reagent kit.

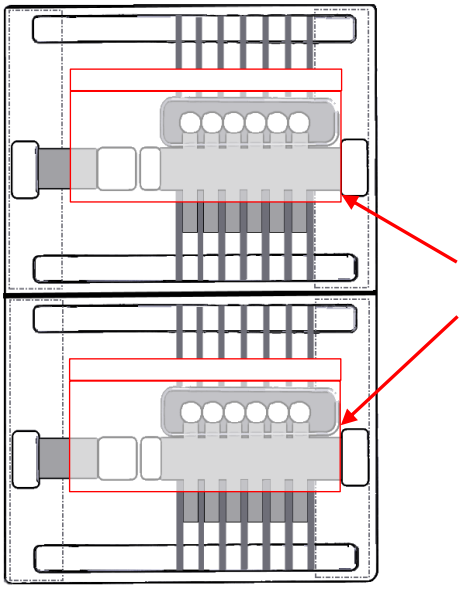


Load **Lysis Reagent** to Reagent wells
Add 270µl, use P1000 pipette)



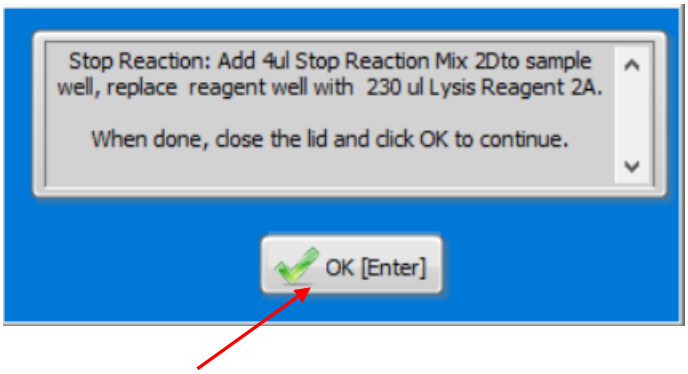
13. Seal the Reagent wells, Sample wells, and Elution wells with the adhesive tape provided. Make sure the tape covers all of the wells and seal tightly.

Ensure adhesive tape tightly seals the wells shown.

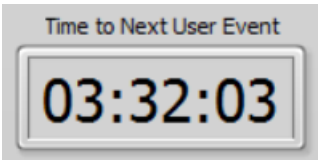


Be sure both electrode ports are not occluded by tape

14. Close the Lid and press “OK” in the pop-up window to resume the instrument and workflow.



15. The remainder of the run, the Collection Stage, is automated and will not require user actions until the end of the run. The Collection Stage will require approximately 3.5 hours



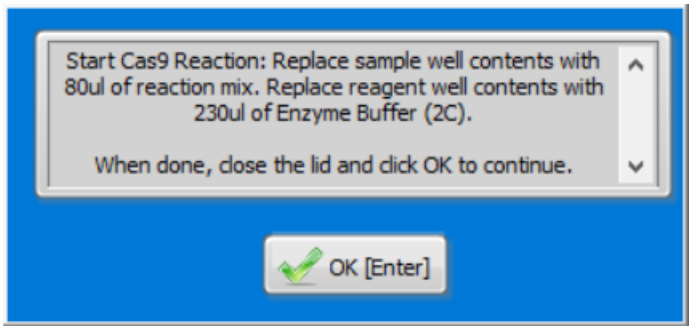
Important! Lysis Reagent and Stop Reagent Mix do not contain hazardous, known mutagenic, or known carcinogenic substances. Refer to the Material Safety Data Sheets (MSDS) for this kit for a comprehensive outline of the safety classifications (www.SageScience.com/product-support/sagehls-support/). Users should follow safe laboratory practices. Contact Sage Science support about missing or expired reagent

Lysis Reagent contains 3% SDS (Sodium Dodecyl Sulfate), glycerol, bromophenol blue dye, and electrophoresis buffer (Tris-TAPS).

8.2.1 Targeted Cas9 Isolation: HLS-CATCH

Important! The HLS-CATCH Enzyme Mix should be prepared ahead of time; either during the Extraction Stage or before beginning the workflow. Preparation requires approximately 30 minutes, and may be kept on ice for several hours. **See Section 7**.

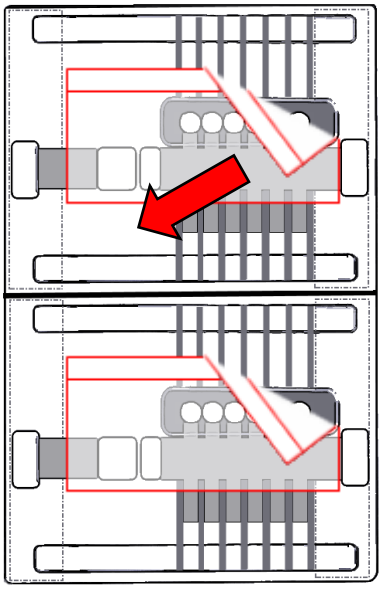
1. After the sample well(s) has reached an initial set temperature, the instrument will pause, and a user action window will pop up. The window will display the user instruction step that was programmed into the workflow:



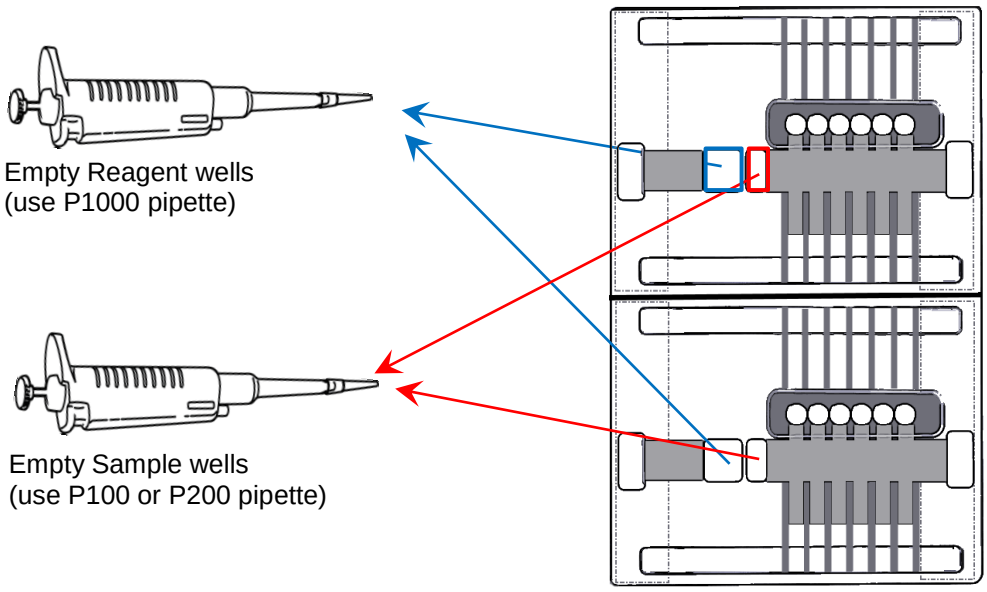
Important! The instrument will remained paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user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

2. 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.

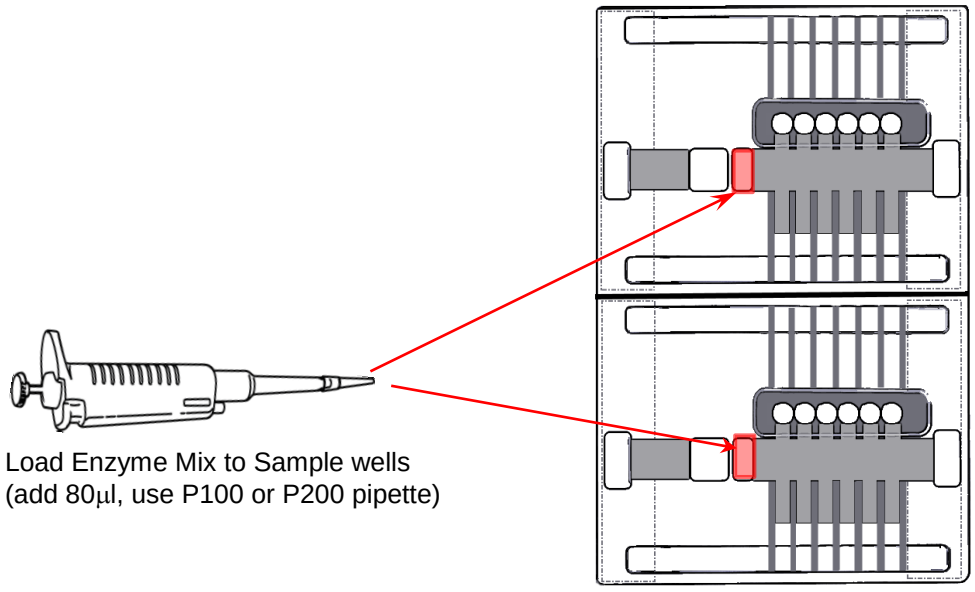
grab the tab in right upper corner and peel diagonally with a slow smooth motion



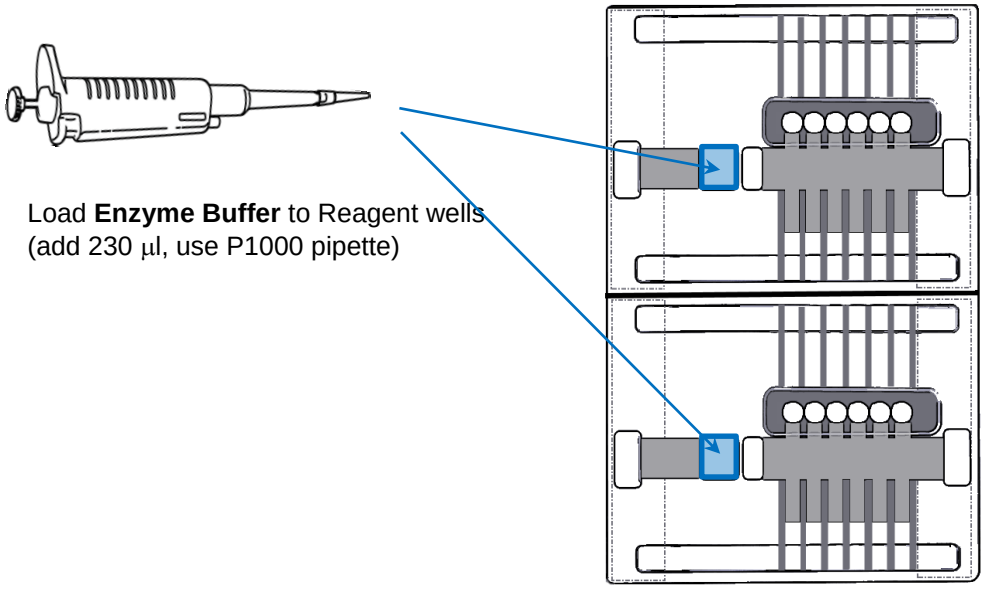
3. Remove <u>all</u> buffer from the Reagent wells and Sample wells the cassettes to be run. The total well volumes are 270 µl and 85 µl, respectively.



4. Load **<u>80µl</u> of the Enzyme Mix** into each Sample well.

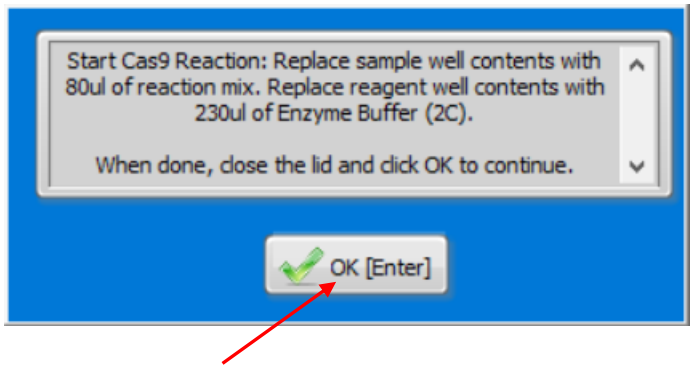


5. Fill the Reagent Wells with **<u>230µl</u> of the Enzyme** Buffer(without enzyme).



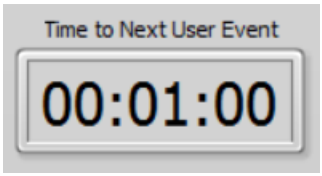
Load **Enzyme Buffer** to Reagent wells
(add 230 µl, use P1000 pipette)

6. Close the Lid and press “OK” in the pop-up window to resume the instrument and workflow.

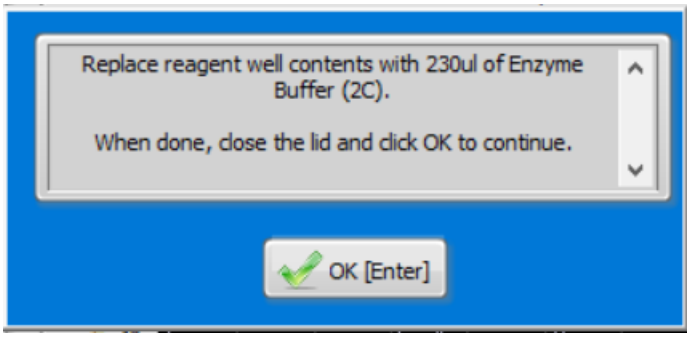


7. The Timer will count-down 1 minute:

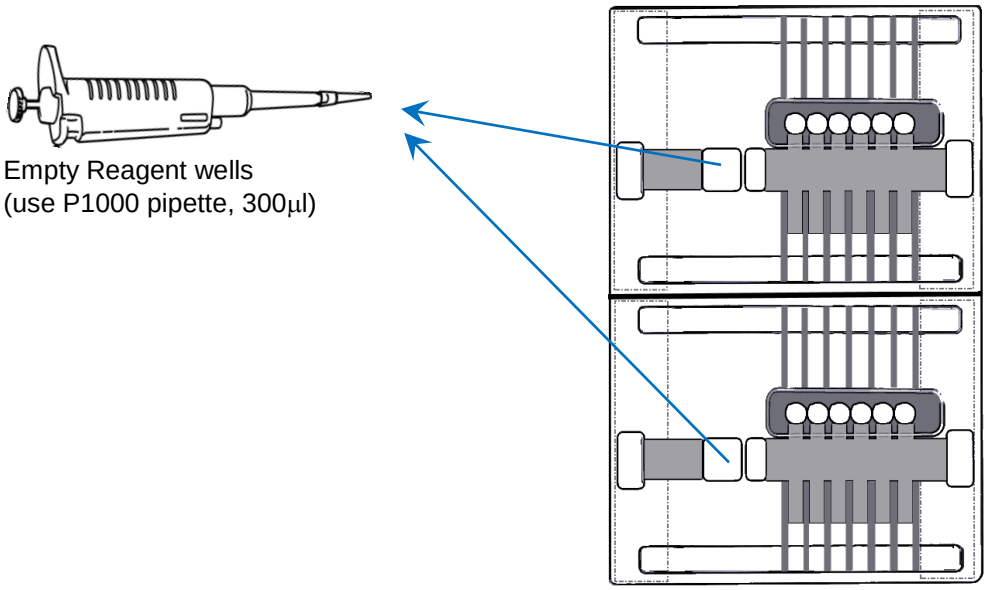
.



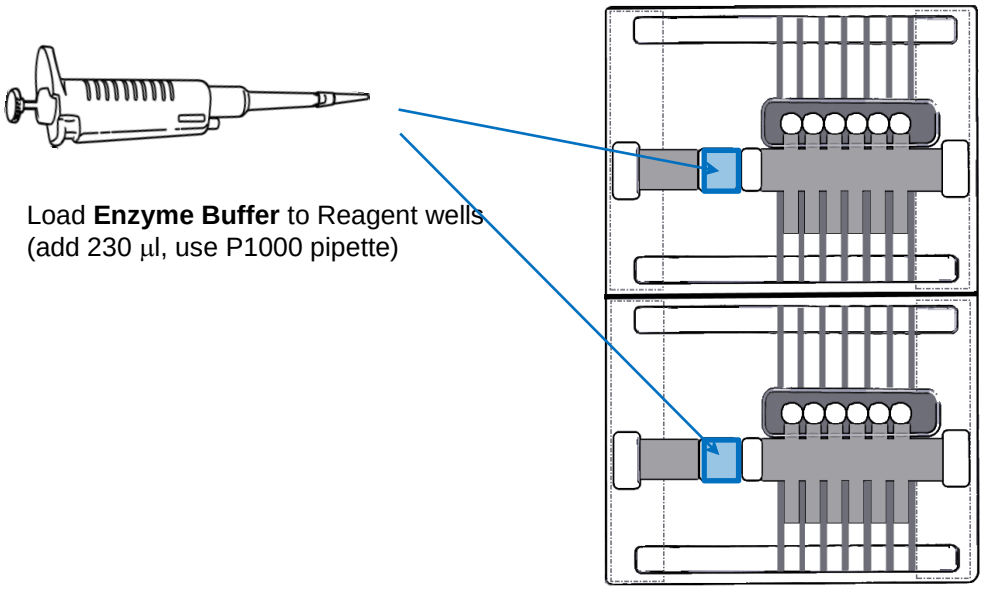
8. The next user action window will pop-up requiring a second replacement of the **Enzyme Buffer** in the Reagent Wells only:



9. Remove the contents of the Reagent Wells (270µl).

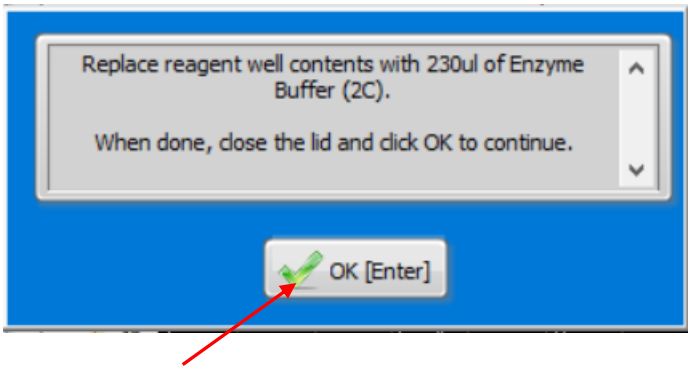


10. Add 230µl of **Enzyme Buffer** to the Reagent Wells.

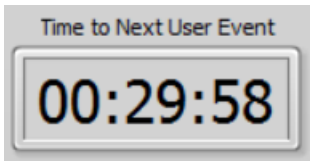


Load **Enzyme Buffer** to Reagent wells
(add 230 µl, use P1000 pipette)

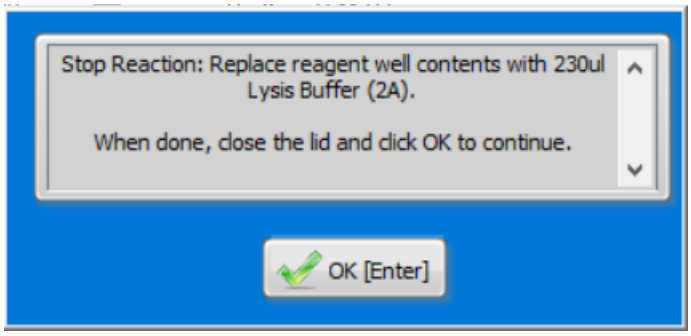
11. Close the Lid and press “OK” in the pop-up window to resume the instrument and workflow.



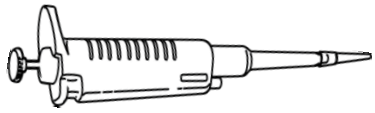
12. The Timer will count down approximately 30 minutes



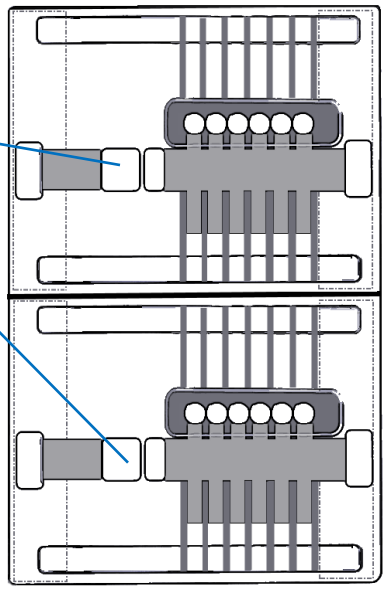
13. The next user action window will pop-up requiring a Stop Reaction: replacement of the Reagent Well contents with **Lysis Buffer**:



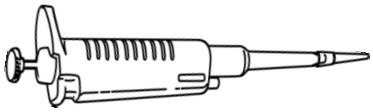
14. Remove the contents of the Reagent Wells (270µl).



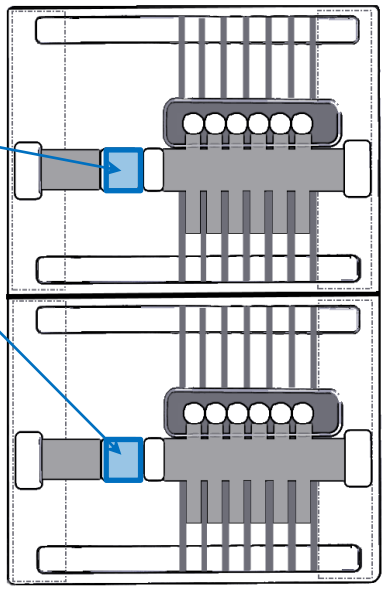
Empty Reagent wells
(use P1000 pipette, 300µl)



15. Add 230µl of **Lysis Buffer** to the Reagent Wells.

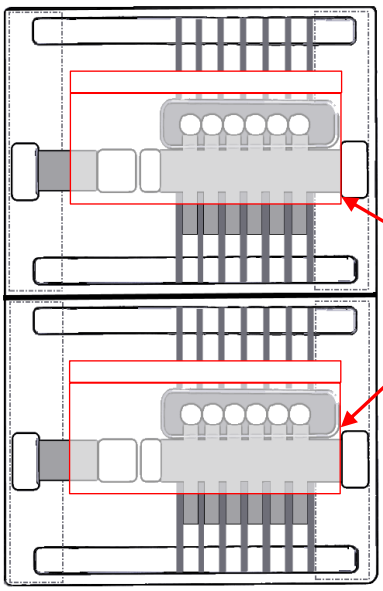


Load **Lysis Buffer** to Reagent wells
(add 230 µl, use P1000 pipette)



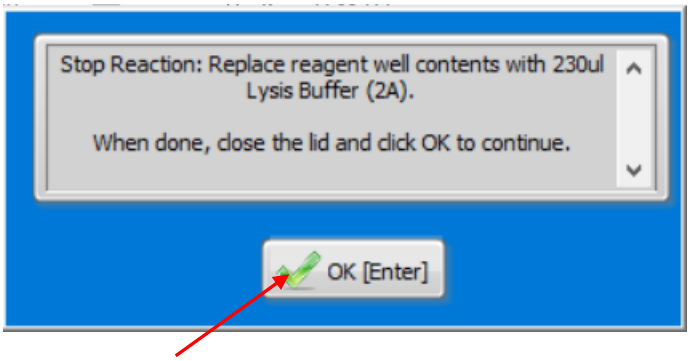
16. Seal the Reagent wells, Sample wells, and Elution wells with the adhesive tape provided. Make sure the tape covers all of the wells and seal tightly.

Ensure adhesive tape tightly seals the wells shown.



Be sure both electrode ports are not occluded by tape

17. Close the Lid and press “OK” in the pop-up window to resume the instrument and workflow.



18. The remainder of the run, the Collection Stage, is automated and will not require user actions until the end of the run. The Collection Stage time is depends on the targeted size range for collection. Run times are between 1.75 hr, up to 5.5 hours

8.3 Collection Stage

There typically no additional user actions during the Collection Stage until the end of the run, after which samples are removed from the SageHLS cassette.

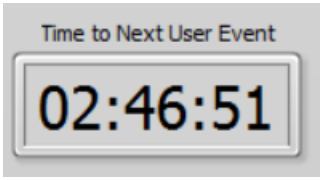
The Step Table will list the several steps that comprise the Collection Stage. This can include a new temperature set point, a separation wave form (pulsed-field, direct current, or a linked combination of wave forms), an elution wave form, and a brief field reversal at the end of the collection:

Collection Stage Steps

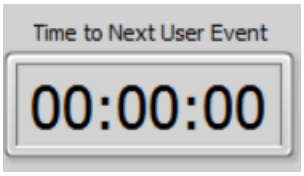
Step Table

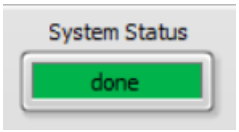
Step	Description
1-001	set temp: sample to 30.0°C, gel to 30.0°C; dwell time 00:02:00
1-002	separate for 01:00:00 at 55.0 V with wave index 1-1
2-001	set temp: sample to 45.0°C, gel to 30.0°C; dwell time 00:01:00
2-002	pause for user action 'Start Fragmentation Reaction: Replace sample
2-003	incubate for 00:30:00
2-004	pause for user action 'Stop Reaction: Add 4ul Stop Reaction Mix 2Dt
3-001	set temp: sample to 28.0°C, gel to 'off'; dwell time 00:02:00
3-002	separate for 01:15:00 at 55.0 V with wave index 3-1
3-003	elute for 01:30:00 at 50.0 V with wave index 3-1
3-004	reverse for 00:00:05 at 25.0 V with wave index 3-1

The counter will reset and begin to count down to the end of the run:



When the timer is reaches zero, the run is complete. The System Status display will indicate “done”:



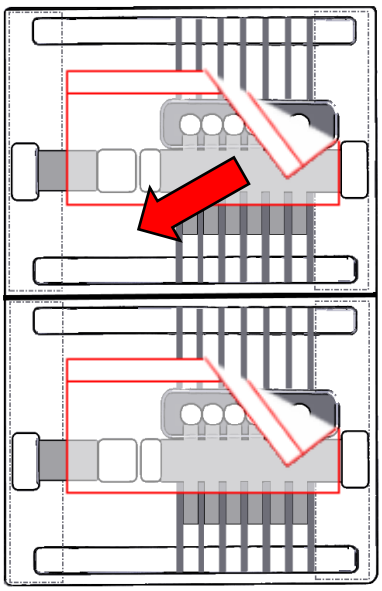


9 Removing Sample Fractions

1. After a run has been completed, open the lid, remove the cassette from the nest(s), and place them on the bench top.

2. 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



3. Using a P200 pipet, and wide bore pipet tips, completely remove the samples from each elution well (total volume is approx. 85µl per well).

10 HMW DNA Extraction: Quick Guide

1. **Prepare a cell suspension**. Follow the instructions provided with the cell suspension kit supplied by Sage Science. A kit provides sufficient reagents for the preparation of 24 SageHLS runs (12 cassettes) with 10μg DNA equivalents per run. If Sage Science does not provide a kit for the a cell type of interest, contact the support team at support@sagescience.com .

2**. Prepare gel cassettes.**

a. Clear bubbles
b. Remove Adhesive Tape
c. Place on Instrument Nest
d. Replace the elution well contents with 80μl of fresh running buffer in each
d. Fill upper buffer chambers with Running buffer, until flush

3. Go to the Main Screen Tab in software

a. Select a Workflow File
b. Check the boxes corresponding to samples to be run in the Nest Configuration
c. Enter Sample ID (optional)

4. Close the Lid

5. In the Main Screen Tab, press "Check Current"

a. When the Current Check screen pops up, press "Start"
b. At the end of the Current Check, press "Return" to the return to the Main Screen

6. At the first pause (Stage 1, Extraction):

a. open the lid
b. replace contents of each Sample Well with 70μl of cell suspension
c. replace contents of each Reagent Well with 230μl of **Lysis Buffer**
d. seal the cassette wells with adhesive tape
e. close the lid

7. Press "OK" in the pause window to continue the run – 1 hr 10 min.

8. At the next pause (Stage 2, Treatment):

a. Remove NEB Fragmentase from the freezer, briefy vortex to mix
b. To 800µl of Enzyme Buffer, add 2µl of NEB Fragmentase (1:400 dilution), vortex to mix
c. open the lid
d. remove the adhesive tape
e. replace contents of each Sample Well with 80µl of **Enzyme Mix**
f. replace contents of each Reagent Well with 230µl of **Enzyme Buffer**
g. close the lid

7. Press "OK" in the pause window to continue the run

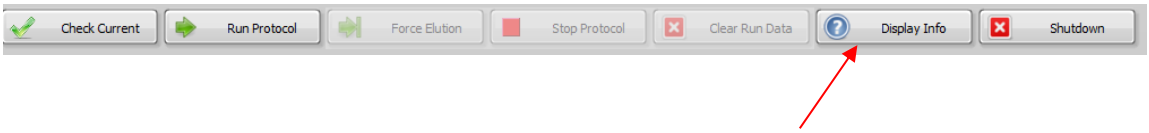
11 Stage Editor: Editing and Creating Stages

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

10.1 Accessing the Stage Editor Screen

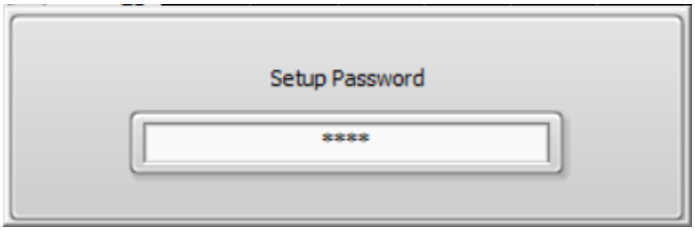
The Stage Editor screen is hidden under one level of user control, and can be accessed by entering a password:

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

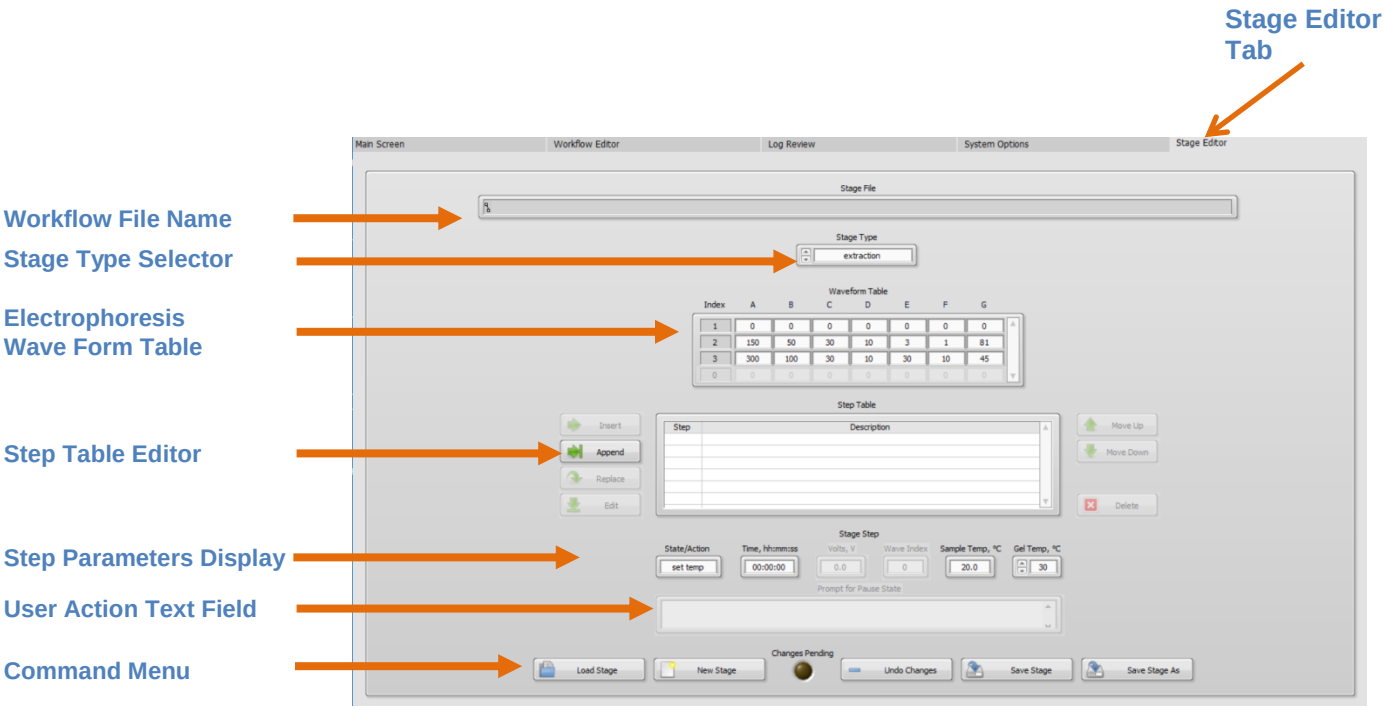


2. The “Display Info” window will pop up. Press the “Advanced Tabs” button.

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

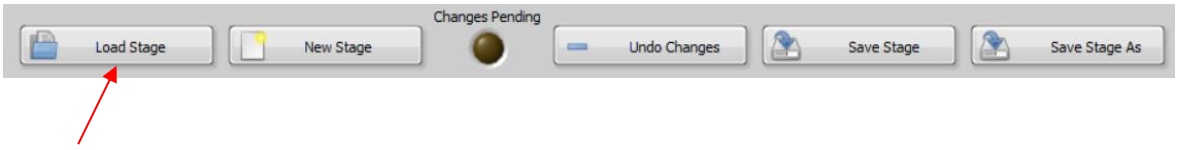


The Stage Editor will appear as a fifth tab on the right side of the screen.



10.2 Editing a Workflow Stage

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



2. A file folder window will pop-up listing the pre-set protocols in the “Presets_Stages” folder. Select a Stage protocol and select

12 Maintenance and Cleaning

11.1 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.

11.2 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.

11.3 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.

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