

HLS2

HMW Library System

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HLS2 and related products or for Research use only.



Indicates disposal instruction.

DO NOT throw this unit into a municipal trash bin when this unit has reached the end of its lifetime. To ensure utmost protection of the global environment and minimize pollution, please recycle this unit.

HLS2 Operations Manual Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
A	B	9/15/23	3-1, 3-2	Remove references to cassette nests being heated

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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 HLS2.



Warning! 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 HLS2 Equipment Ratings

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

1.3 HLS2 Instrument Safe Use Guidelines

The HLS2 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



Warning! The HLS2 was designed to be operated on a flat surface. Do not operate on a tilted surface or tilt during operation.

Standard laboratory precautions should be taken when handling SageHLS and HLS2 Gel cassettes and operating the SageHLS or HLS2:

- Wear a lab coat, safety glasses, and gloves.
- Use in proximity of an eye wash station and/or running water.

Disposal



DO NOT throw this unit into a municipal trash bin when this unit has reached the end of its lifetime. To ensure utmost protection of the global environment and minimize pollution, please recycle this unit.

1.4 Consumables Safe Use Guidelines

There are two types of consumables that are used on the HLS2 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/HLS2-support/



Important! Users should use the HLS2 and related consumables in accordance to safe laboratory guidelines including the use of gloves, safety glasses, and lab coats.



Important! HLS2 consumables do not contain any gases or volatile compounds that can adversely affect health.

Unpacking and Installation

2.1 Unpacking the HLS2

The HLS2 instrumentation is shipped in two boxes: one will contain the HLS2 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

HLS2

- HLS2 Instrument
- Accessory box
 - Computer keyboard, USB
 - Computer mouse, USB
 - Rinse cassettes (for maintenance of electrodes)
 - Power supply
 - Power cord



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

2.2 Installing the HLS2

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 HLS2 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 HLS2 weighs approximately **20 lbs**. Place the unit onto the bench top.



Warning! Do not lift the instrument by the lid or lid retainer. This can damage the mechanism or impede the function of the unit. Pressure should never be applied to the retainer, including sliding the instrument along a bench surface.



Lift from the bottom.

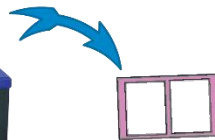


Do not lift by the retainer or lid.

5. Remove the foam insert which is on the **inside of the lid**.



Warning! 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.



Discard
foam insert.

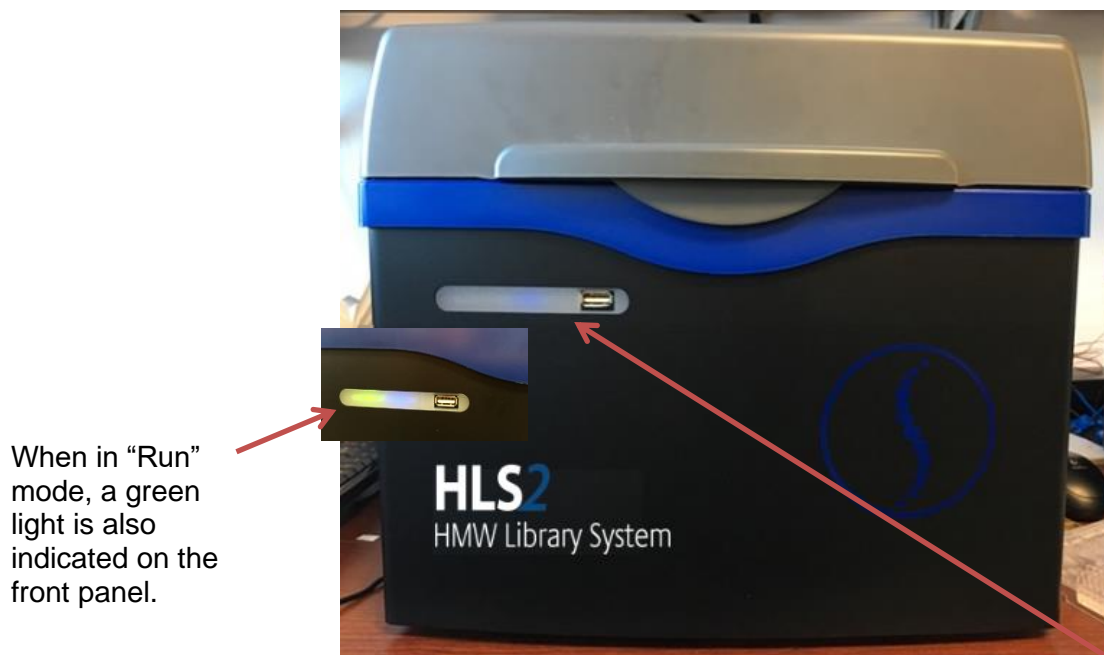


Figure 2.3. Front Panel of the HLS2

After the power button is pressed, the front panel LED will light, and the software will launch.

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

HLS2 reagent kits can contain reagents with varying storage requirements. These will be noted on the bag label, on the provided documentation included with the kits, and on the labels on the reagent bottles.



Warning! 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 HLS2 from Sage Science. We urge you to read this manual to familiarize yourself with the system's capabilities and precautions.



Warning! Use the HLS2 only as indicated in this operations manual. Injury or instrument damage can occur with improper usage.

The HLS2 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 HLS2 system:

- **Instrument** – The instrument is comprised of two aluminum 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.

Nests for agarose gel cassettes (two samples per cassette).



Electrodes for electrophoresis are embedded in the lid.

The HLS2 instrument
(the monitor and keyboard are not shown)

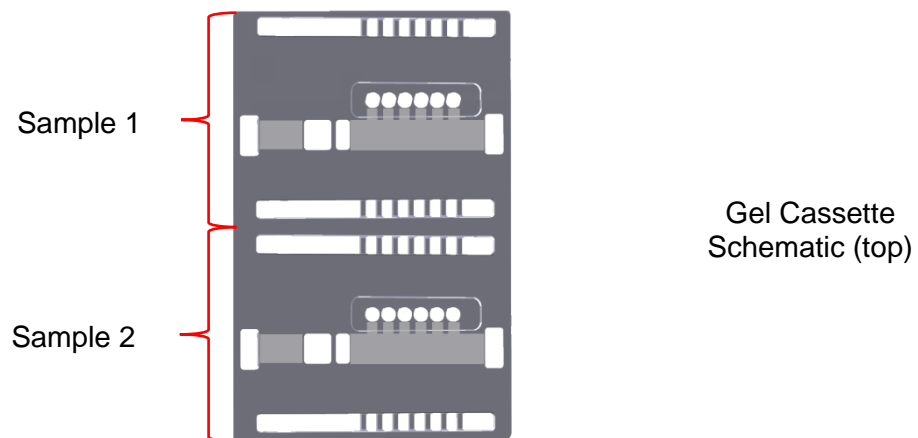
3.2 The Cassette Nest

The HLS2 is equipped with two aluminum nests to hold the agarose gel cassettes.

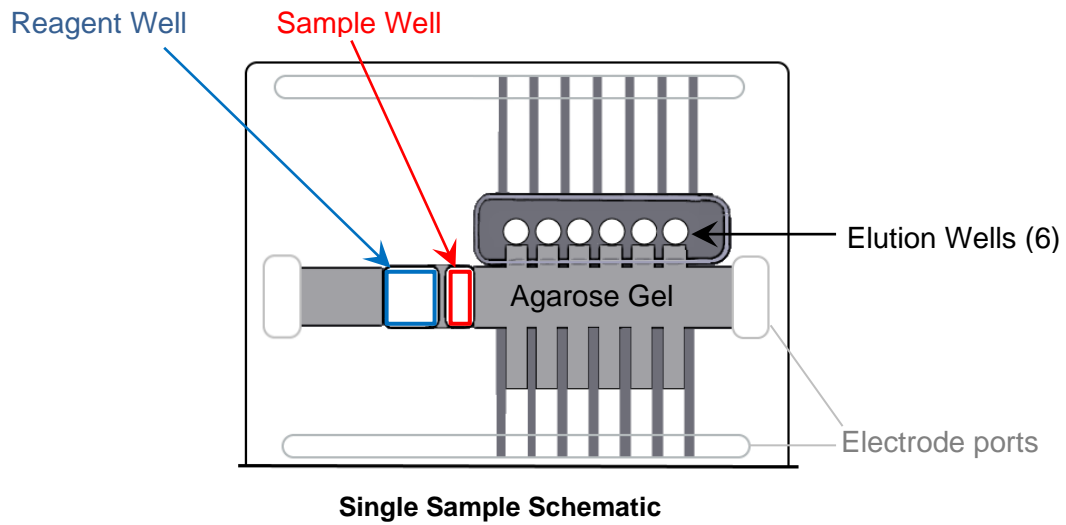


3.3 How the System Works

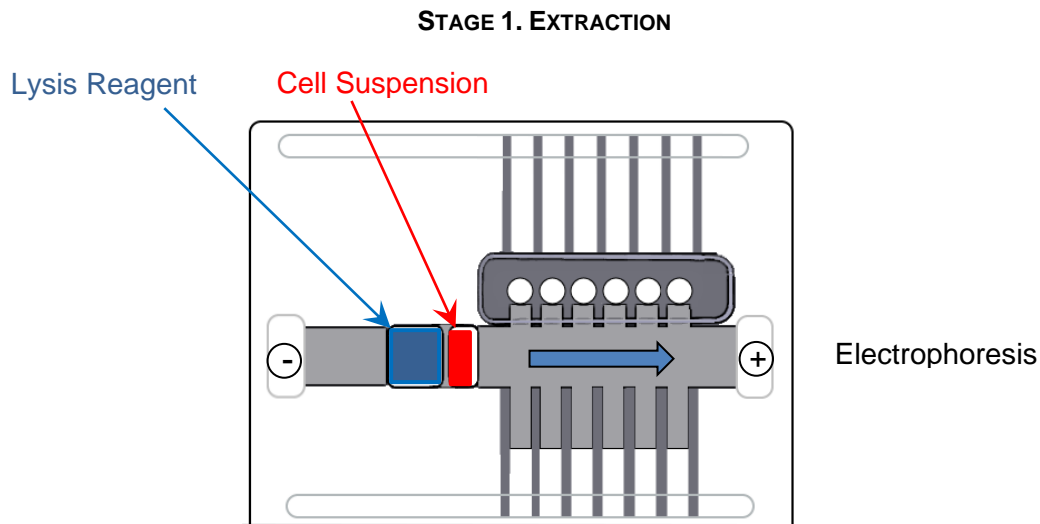
The HLS2 system uses pre-cast and disposable agarose gel cassettes. Each cassette has a 2-sample capacity.



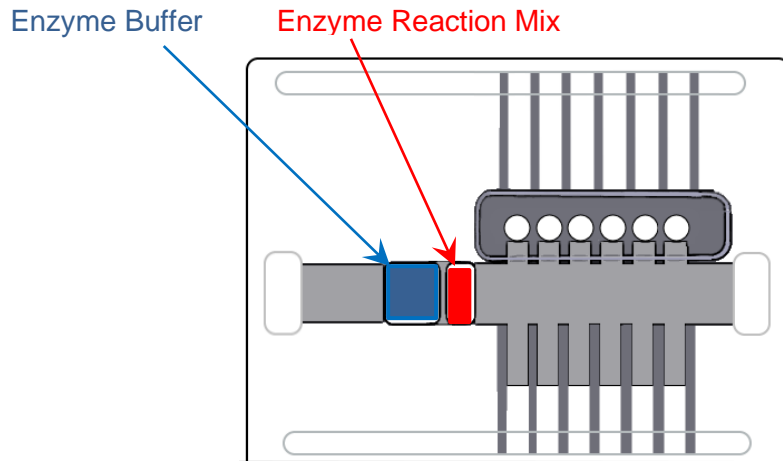
A schematic of a single sample lane is shown below. It consists of an agarose column, with two wells into which reagents, buffers, or cell suspensions are added. Electrophoretic current can be applied in two (perpendicular) directions. DNA is collected in six membrane-bound elution wells.



Cell suspensions are processed in three workflow stages:



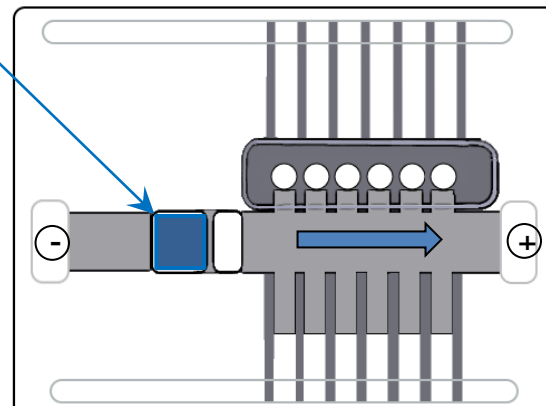
Cells are lysed. Electrophoresis is used to remove solubilized proteins and small nucleic acids. HMW DNA remains immobilized in the wall of the agarose sample well.

STAGE 2. TREATMENT

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

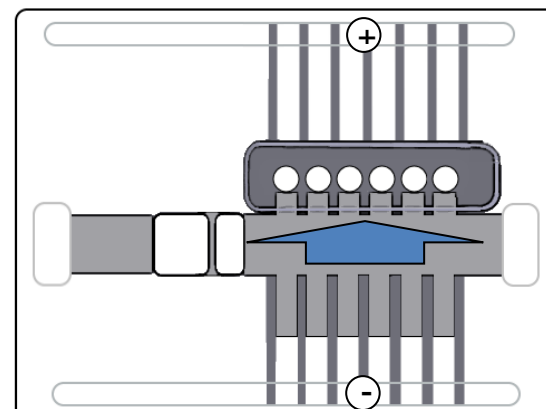
STAGE 3. COLLECTION

Running (or Lysis) Buffer



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 HLS2.*

HLS2

The processing of samples on the HLS2 requires user interactions with the instrument (opening and closing the lid), software (pausing and resuming), and reagent addition and removal from the cassette using a manual pipetter. *HLS2 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 HLS2 Workflow Stages

Stage 1: Extraction Stage – (cell suspension kit)

Extracts DNA from cell suspensions and removes residual byproducts.

Step types:

- Electrophoresis voltage, time.
- Pause for operator action to add or remove sample and/or reagents.

Stage 2: Treatment Stage – (gel cassette kit)

Enzymatic treatment of extracted DNA (in sample well wall) with cleavase, targeted endonuclease, or other enzymatic processes

Step types:

- Electrophoresis voltage, time.
- Pause for operator action to add or remove sample and/or reagents.
- Incubation time.

Stage 3: Collection Stage - (DNA size selection options)

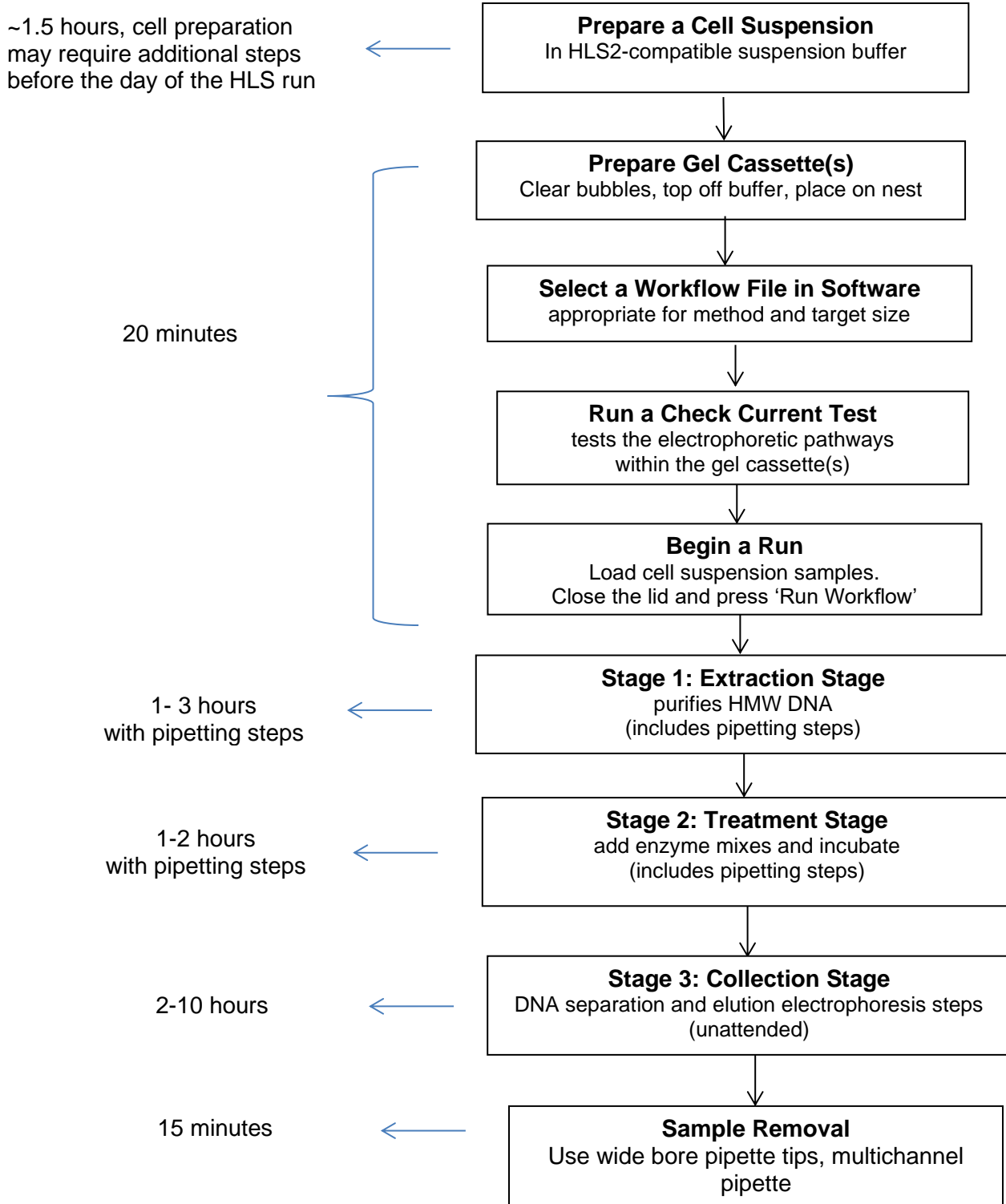
Size Separation and collection (DNA size selection). These differ by target collection size requirements.

Step types:

- Separation electrophoresis
- Elution electrophoresis
- Reverse Elution electrophoresis

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 HLS2 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 HLS2 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 Command 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.

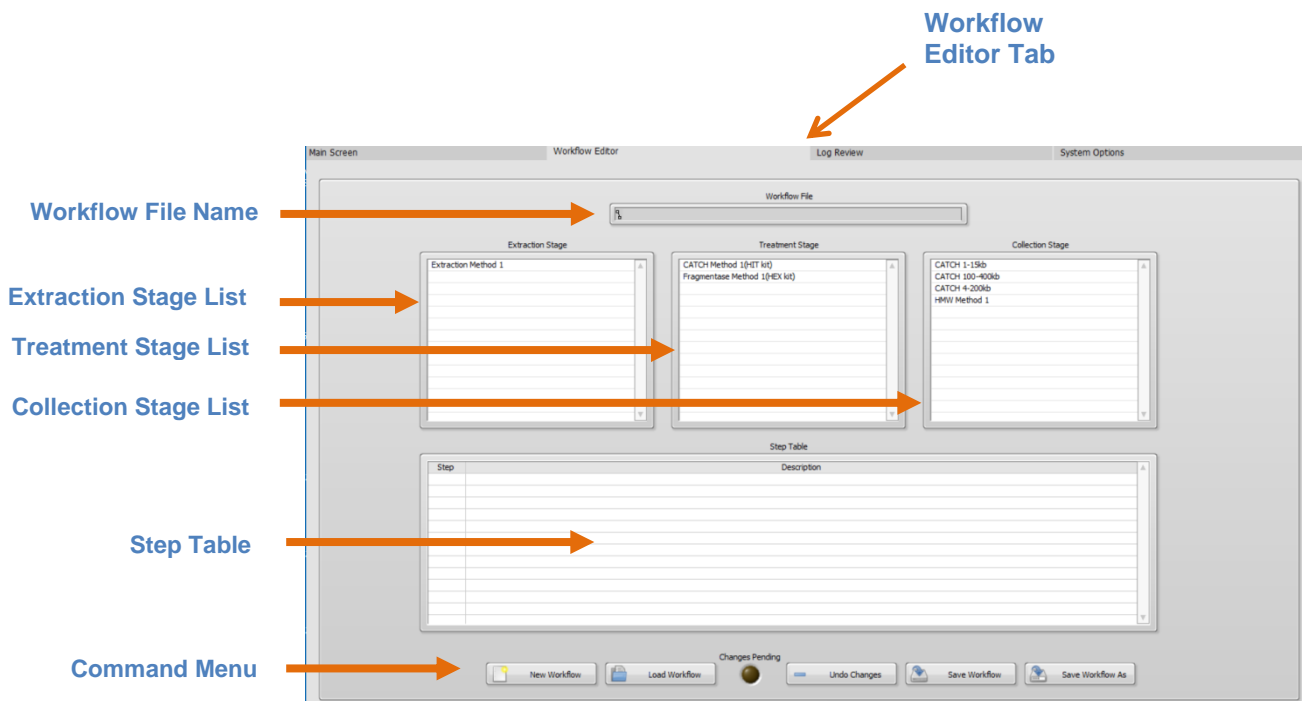
4.3 Warranty and Service

The HLS2 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.

HLS2 software

5.1 Workflow Editor Tab

In the HLS2 software, navigate to the **Workflow Editor Tab**. It is the second tab from the left:

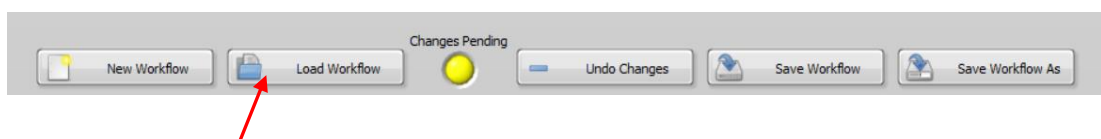


The Stage List fields are populated with pre-set stage protocols.

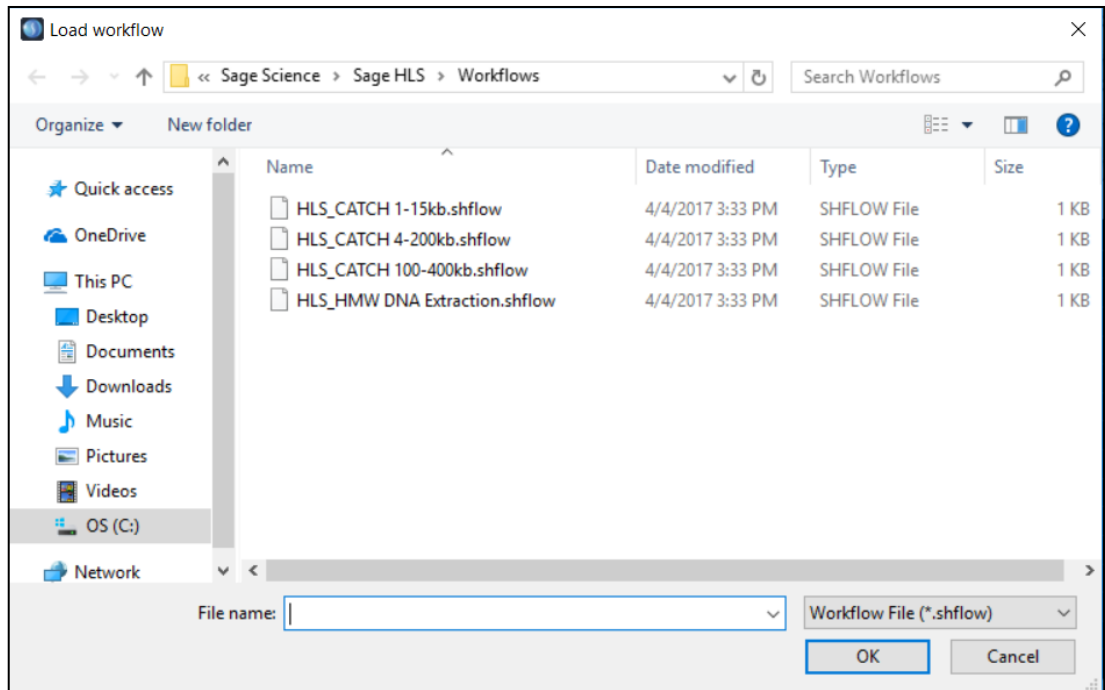
5.2 Workflow File

A **Workflow File** links the three process **Stages**. The HLS2 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:

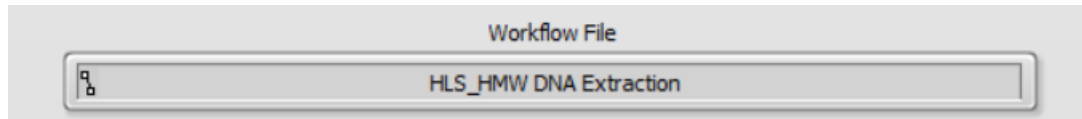


A file list will pop-up from the HLS2/Workflows folder.

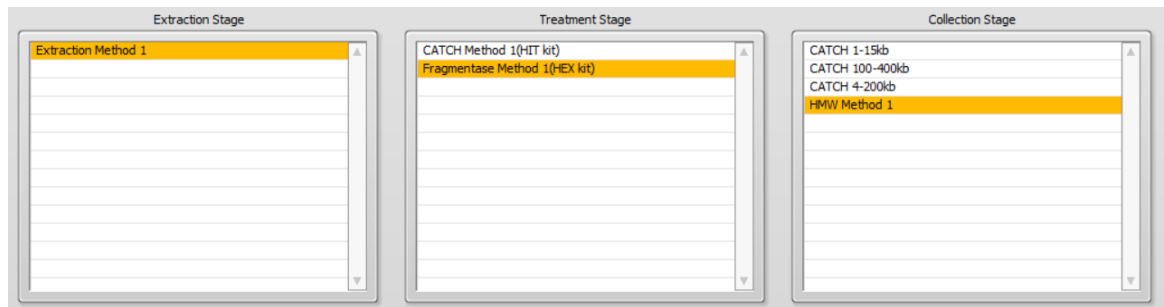


When a Workflow File is selected;

- 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	pause for user action 'Replace sample well contents with 70ul of prepared sample. Replace reagent well contents with 230ul of
1-002	separate for 01:00:00 at 55.0 V with wave index 1-1
1-003	pause for user action 'Remove tape seal. Replace sample well contents with 80ul of reaction mix. Replace reagent well content
2-001	incubate for 00:30:00
2-002	pause for user action 'Stop Reaction: Add 4ul 0.5M EDTA to sample well. Replace reagent well contents with 230ul of Lysis Buff
2-003	separate for 00:45:00 at 55.0 V with wave index 3-1
2-004	separate for 01:15:00 at 55.0 V with wave index 3-3
3-001	elute for 01:30:00 at 50.0 V with wave index 3-1
3-002	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.

De-select
by clicking
a blank
field

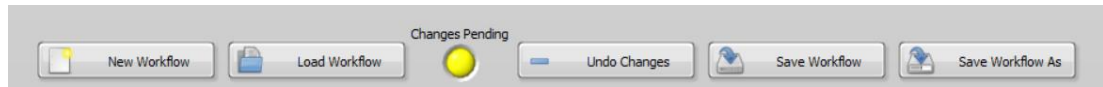
Steps from
highlighted
Stage
Protocols are
displayed

The screenshot shows the Workflow Editor interface with the following components:

- Workflow File:** HLS_JMW DNA Extraction
- Extraction Stage:** A list with one item, "Extraction Method 1", which is currently unselected. A mouse cursor is positioned over a blank field in this list.
- Treatment Stage:** A list with two items: "CATCH Method 1(HIT kit)" and "Fragmentase Method 1(FEX kit)". The latter is highlighted in yellow.
- Collection Stage:** A list with three items: "CATCH 1-194b", "CATCH 100-400b", and "CATCH 4-200b". The latter is highlighted in yellow.
- Step Table:** A table with two columns: "Step" and "Description". It contains five rows of steps, all of which are displayed, corresponding to the highlighted protocols in the Treatment and Collection stages.
- Buttons:** "New Workflow", "Load Workflow", "Changes Pending", "Undo Changes", "Save Workflow", and "Save Workflow As".

Step	Description
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 80µl of reaction mix. Replace reagent well contents with 230µl Enzyme Buffer (2C)'
2-003	incubate for 00:30:00
2-004	pause for user action 'Stop Reaction: Add 4µl 0.2M EDTA to sample well. Replace reagent well contents with 230µl Lysis Buffer (2A)'
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 3-1
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

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

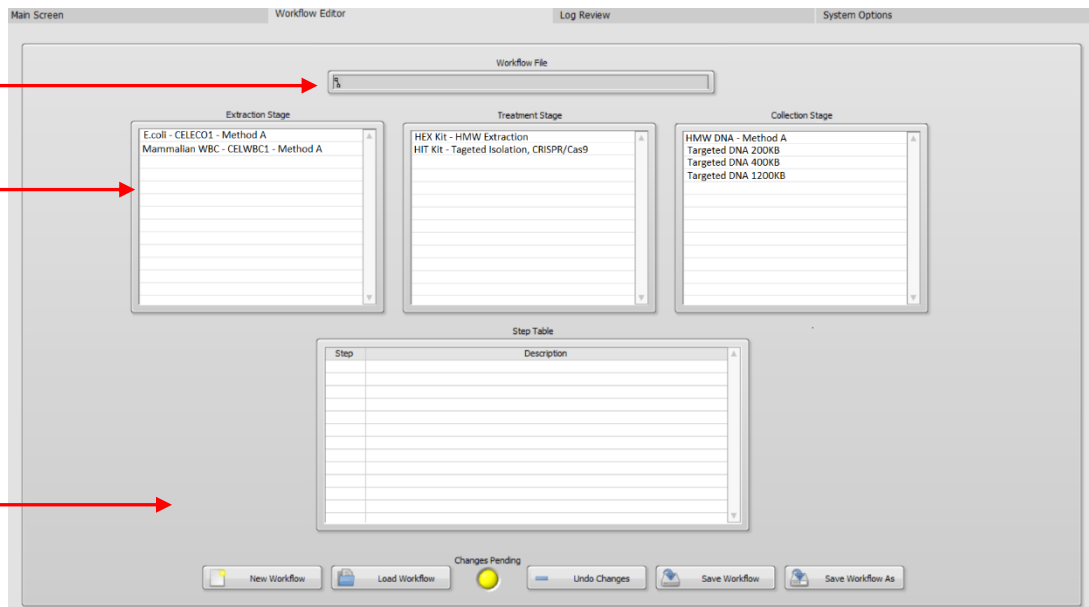


This will clear the Workflow File name and deselect all highlighted Stages:

Workflow File name is cleared

All Stages are de-selected

All steps are de-listed



5.3 Saving a Workflow File

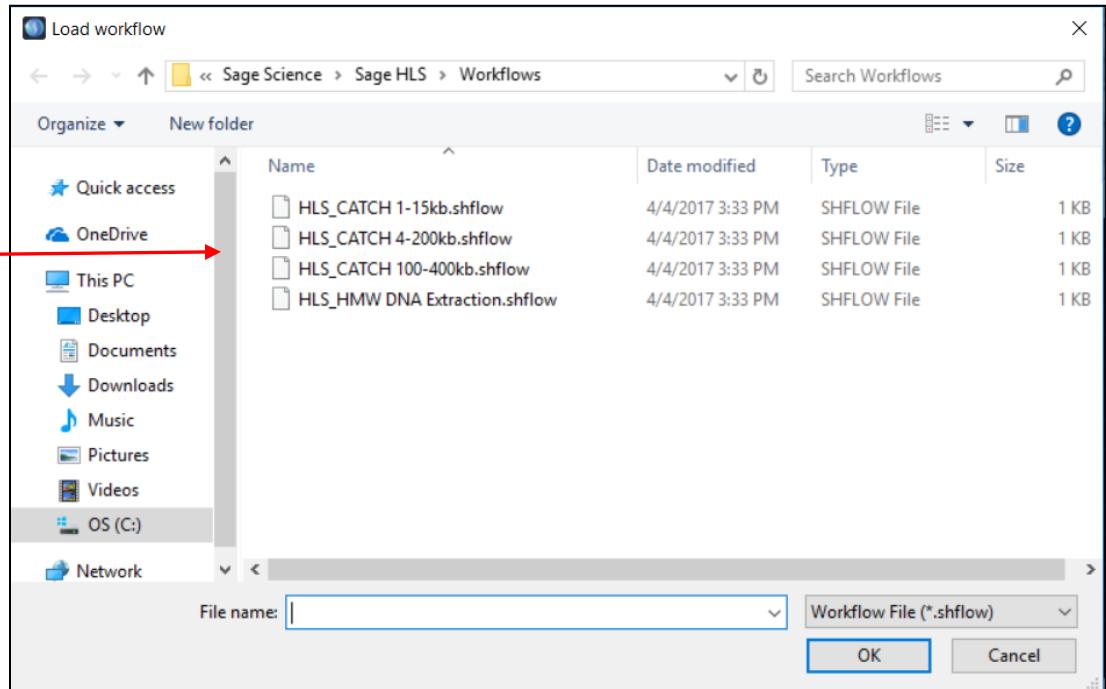
If a Workflow File has been modified by replacing or eliminating a step, it can be renamed and resaved using the “Save As” button.



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.

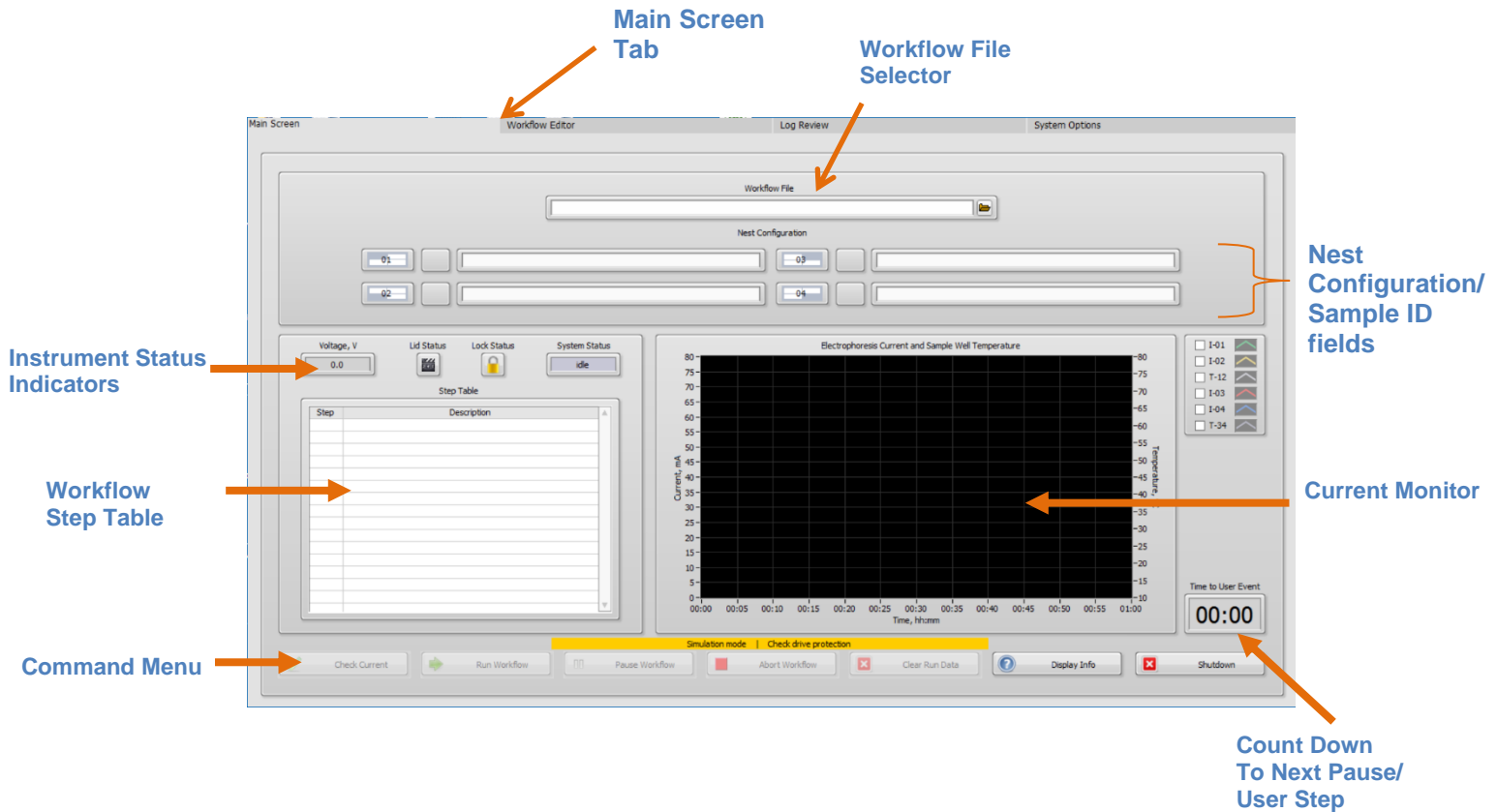
Pre-set
Workflow
files (HLS
prefix)
cannot be
modified



5.4 Main Screen Tab

The Main Screen tab is the screen with which users interface with the HLS2 instrument. This includes initiating a run, monitoring a run, and pausing/resuming to conduct a manual pipetting step.

The default software screen on the HLS2 has a tabbed format. The Main Menu is the first tab.

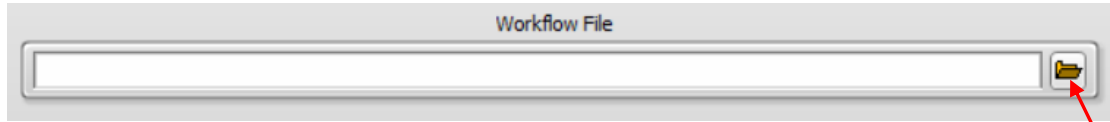


5.5 Loading a Workflow File

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

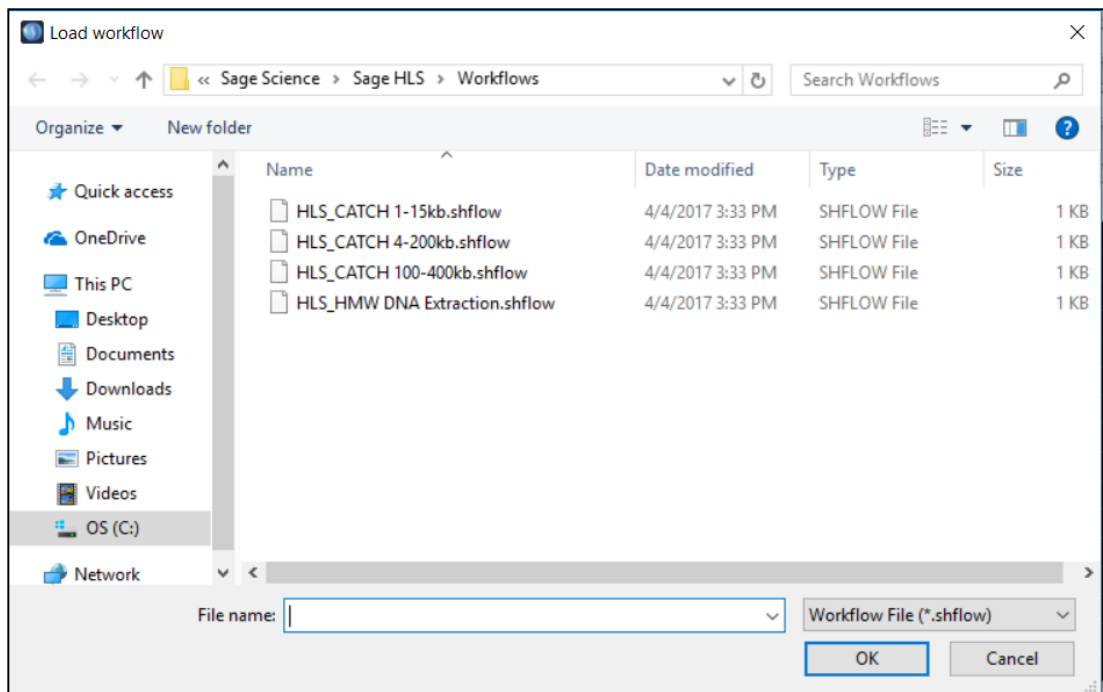


2. Click the folder icon next to the Workflow File Field.



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”

The screenshot shows the Workflow Editor interface with the following components and annotations:

- Workflow File name:** A red arrow points to the 'Workflow File' field, which contains 'General HMW Extraction protocol'.
- Samples to be run (Selected at Current Test):** A red arrow points to the 'Nest Configuration' section, where sample lanes 01 and 02 are selected with checkmarks.
- Workflow steps:** A red arrow points to the 'Step Table' which lists 10 steps with descriptions such as 'set temp: sample to 30.0°C, gel to 20.0°C; wait for 00:01:00'.
- System Status = ready:** A red arrow points to the 'System Status' indicator, which is a green button labeled 'ready'.

Other visible elements include a graph titled 'Electrophoresis Current and Sample Well Temperature' and a 'Time to User Event' display showing '00:00'.

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.

The close-up shows the 'Nest Configuration' section with the following details:

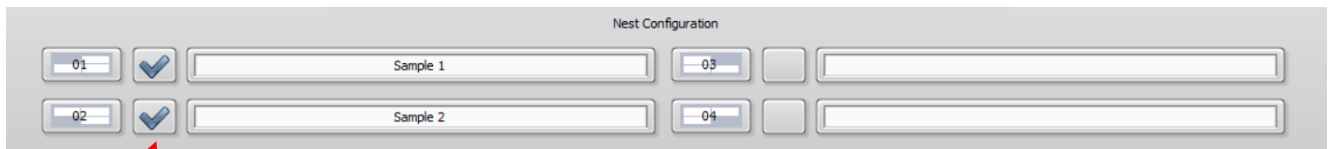
- Select sample lanes to be run:** A red arrow points to the checkmark next to sample lane '02'.
- Enter Sample IDs (optional):** A red arrow points to the text input field for 'Sample 2'.

Other visible elements include sample lanes '01' and '03', and a 'Sample 1' label.

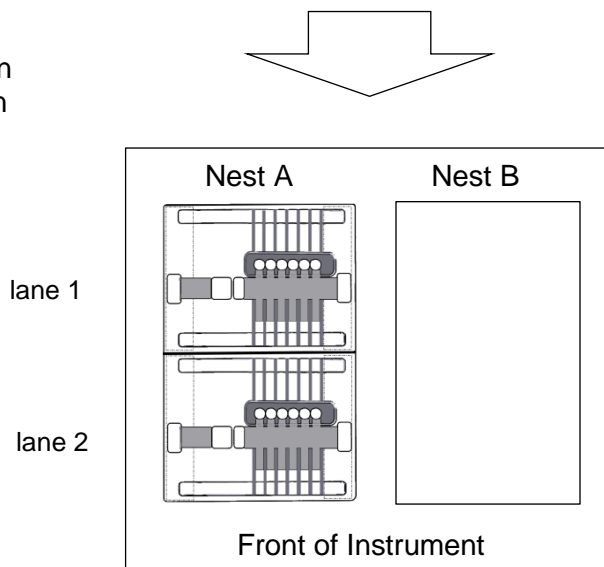
5.6 Running the Electrophoresis Current Test

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:

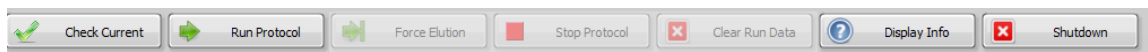


Current Test will run on lanes 1 and 2 on Nest A.



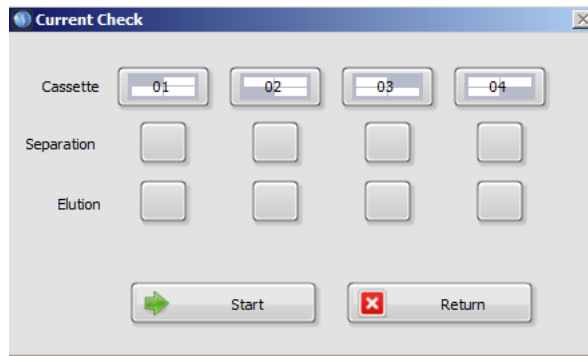
The Current Test Procedure:

1. Close the HLS2 lid.
2. Once the lid is closed, press the “Check Current” button on the command button menu.

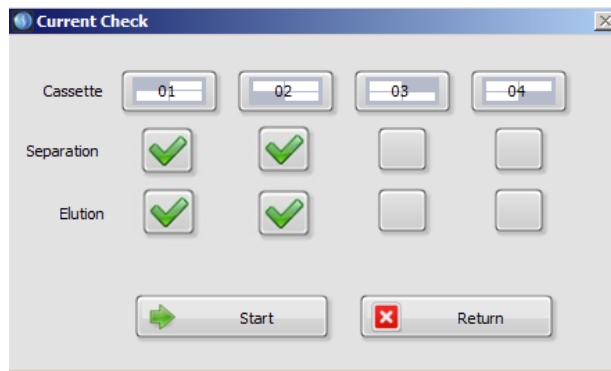


Press
“Check Current”

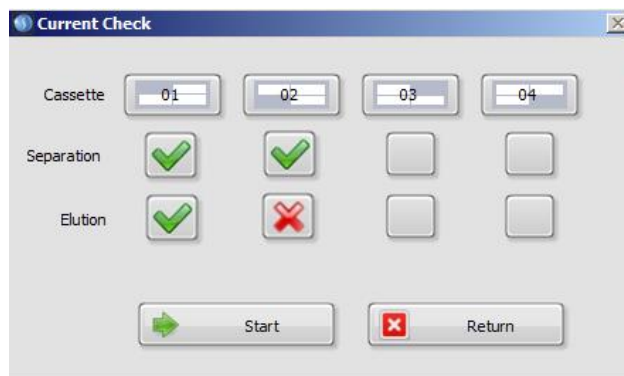
3. A pop-up window will appear:



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.

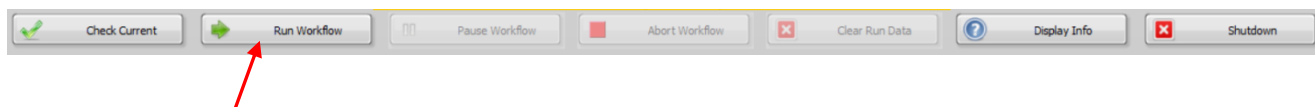


Important! If a current check fails, check the buffer levels in the affected lane, and replace the buffer as needed. Contact Sage Science support if the current test continues to fail.

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

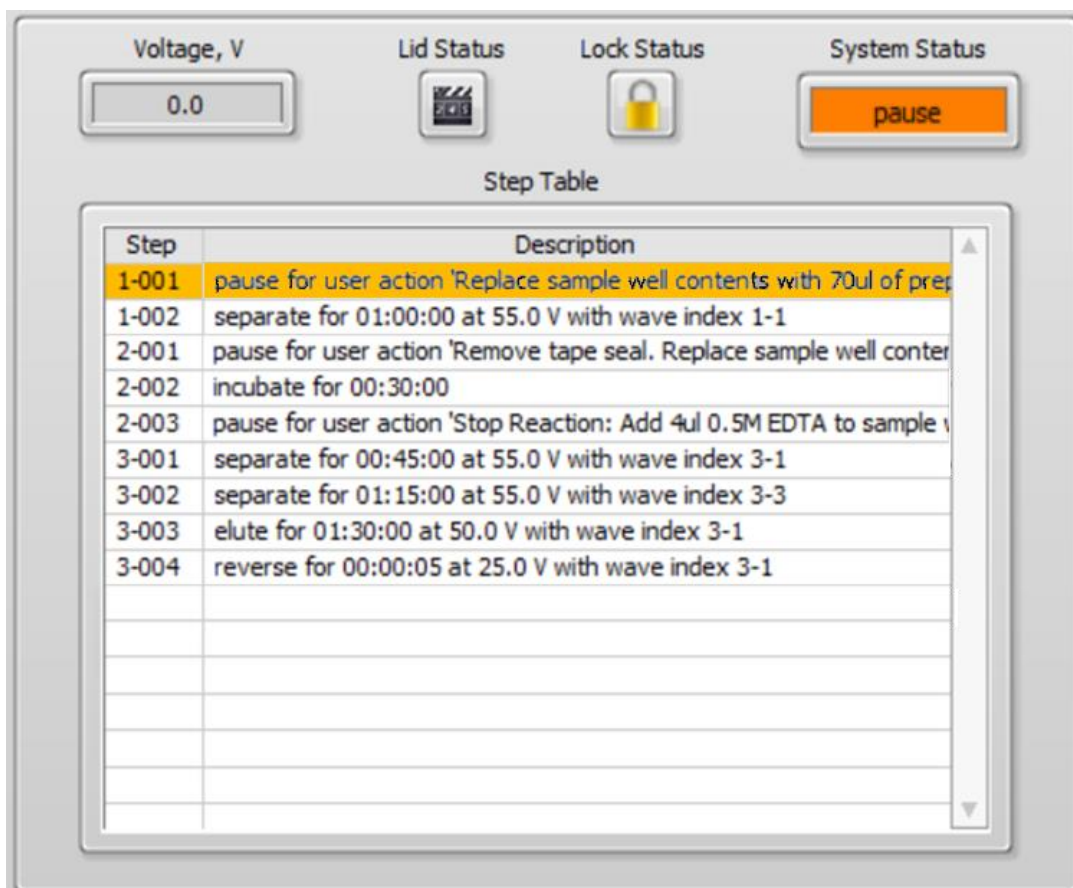
5.7 Running a Workflow

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.

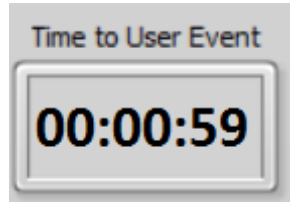


The screenshot shows a control panel with the following elements:

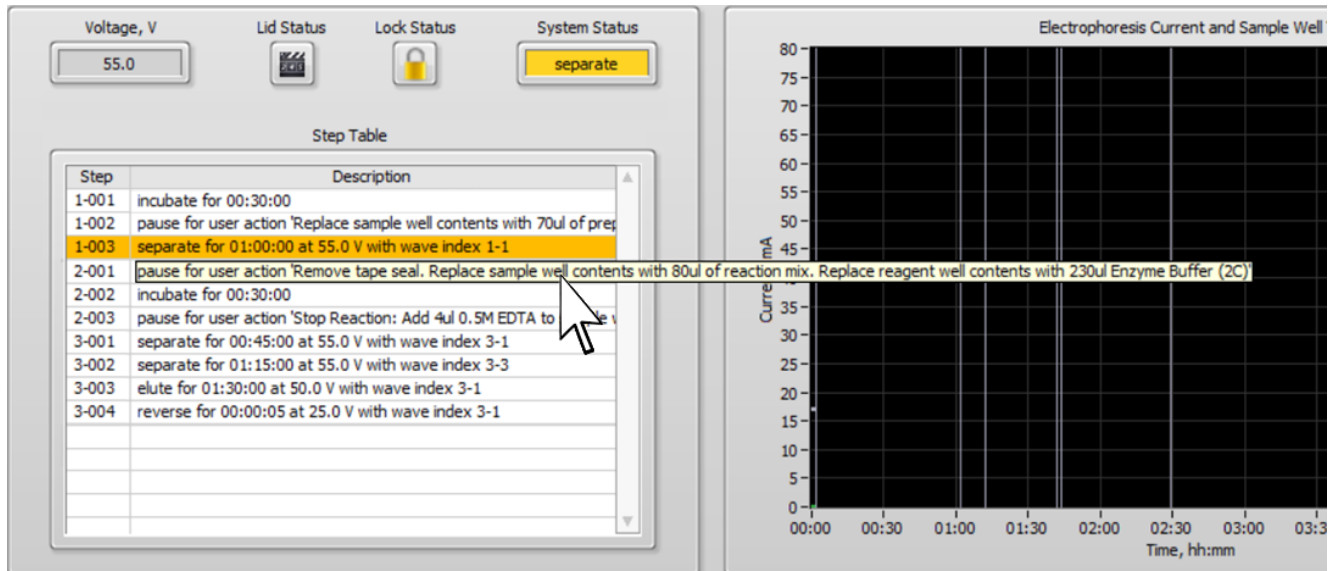
- Voltage, V:** 0.0
- Lid Status:** Closed (film strip icon)
- Lock Status:** Locked (yellow padlock icon)
- System Status:** pause (orange button)
- Step Table:** A table with two columns: Step and Description. The first row is highlighted in yellow.

Step	Description
1-001	pause for user action 'Replace sample well contents with 70ul of prep
1-002	separate for 01:00:00 at 55.0 V with wave index 1-1
2-001	pause for user action 'Remove tape seal. Replace sample well conter
2-002	incubate for 00:30:00
2-003	pause for user action 'Stop Reaction: Add 4ul 0.5M EDTA to sample v
3-001	separate for 00:45:00 at 55.0 V with wave index 3-1
3-002	separate for 01:15:00 at 55.0 V with wave index 3-3
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

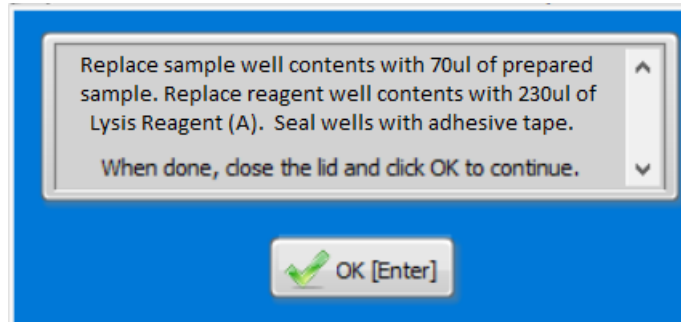
The “Time to User Event” timer will start a countdown until the next Pause step, which will require the lid to be opened, and reagent transfer steps with a pipette.



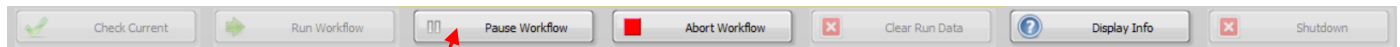
If a pause step contains instructions that exceed the Step Table display, hovering the mouse pointer over a step will display the complete instruction.



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.

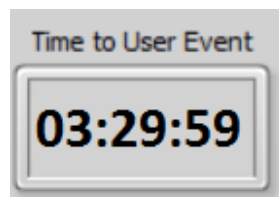


If the instrument is inadvertently resumed, press the “Pause Workflow” button in the Command Menu to re-pause the instrument and continue with the manual user action.

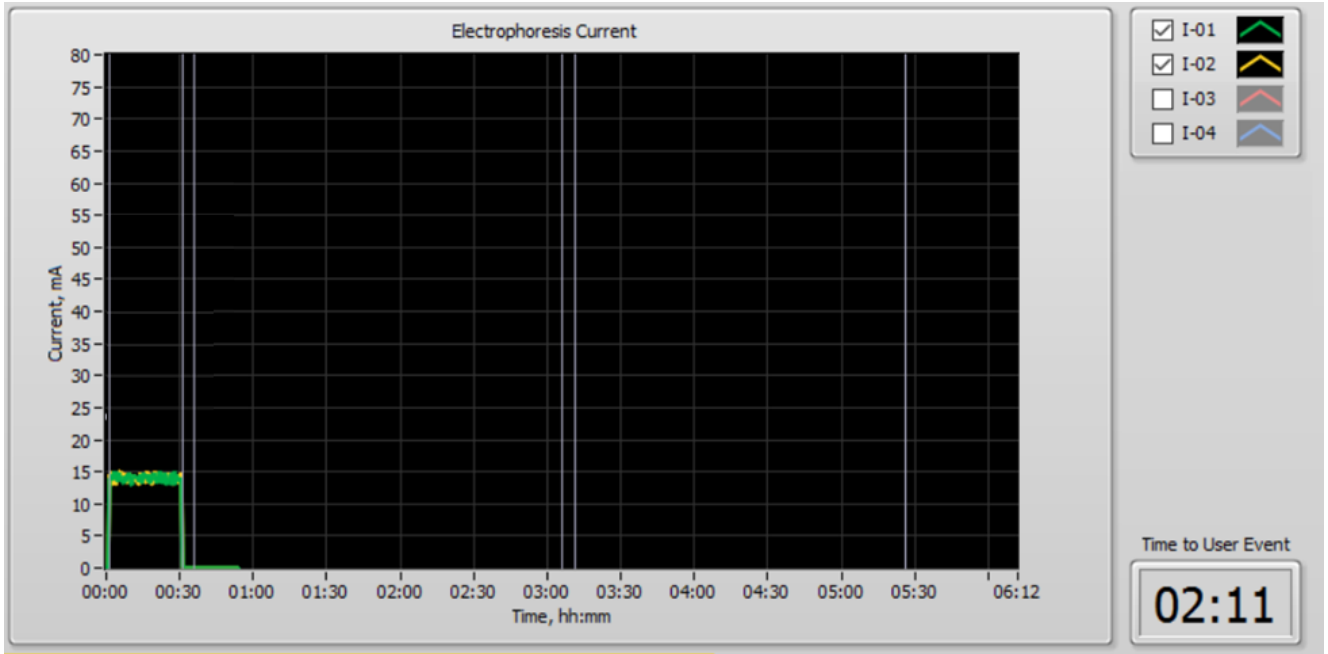


Pause Workflow

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.

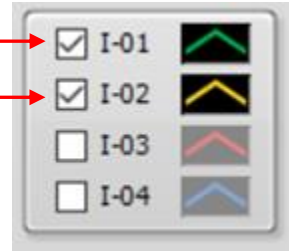


Throughout the workflow the “Electrophoresis Current” monitor will graphically display (in real time) the electrophoresis current for each sample.



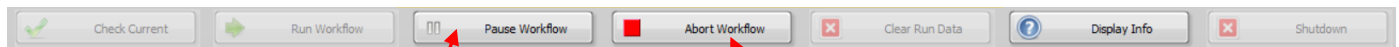
Electrophoresis current (mA) Cassette 1

Electrophoresis current (mA) Cassette 2



5.8 Other Run Commands

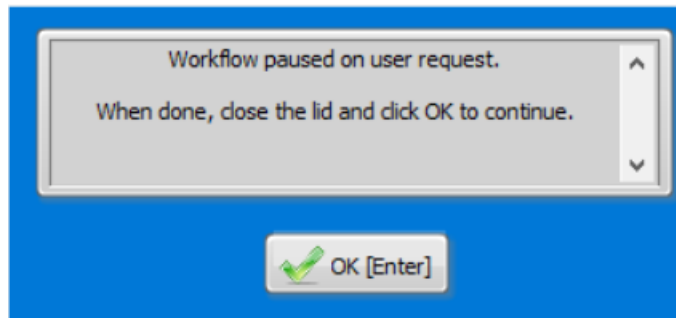
A workflow may be paused (and then resumed), or stopped at any time during a Workflow:



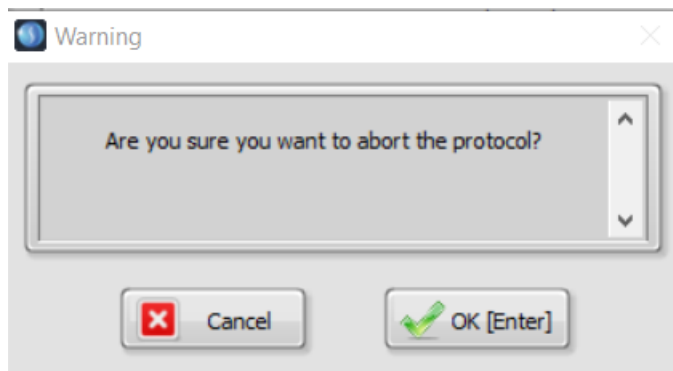
Pause Workflow

Abort Workflow

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



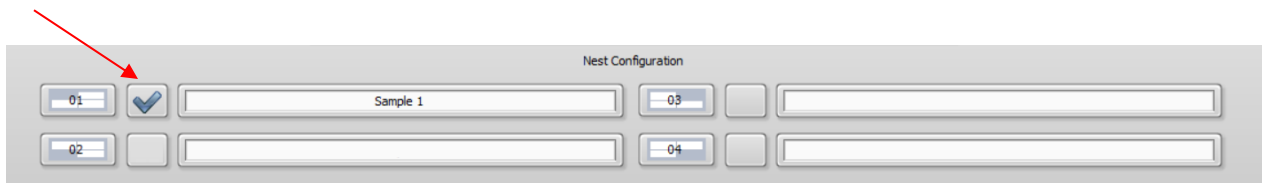
If a Workflow is stopped, the following window will appear. Press “OK” to abort the protocol, or “Cancel” to return to the Workflow.



5.9 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.



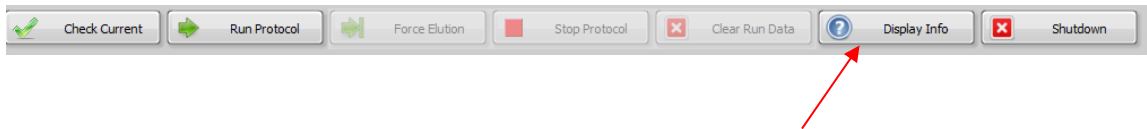
2. Save the original adhesive tape that the cassette was packaged with.
3. The unused lane can be left unsealed during the entire run
4. At the end of the run, re-seal the cassette with the original adhesive tape. Make sure to apply pressure (with a finger or lab marker) around all wells and chambers. 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.

5.10 Stage Editor Tab

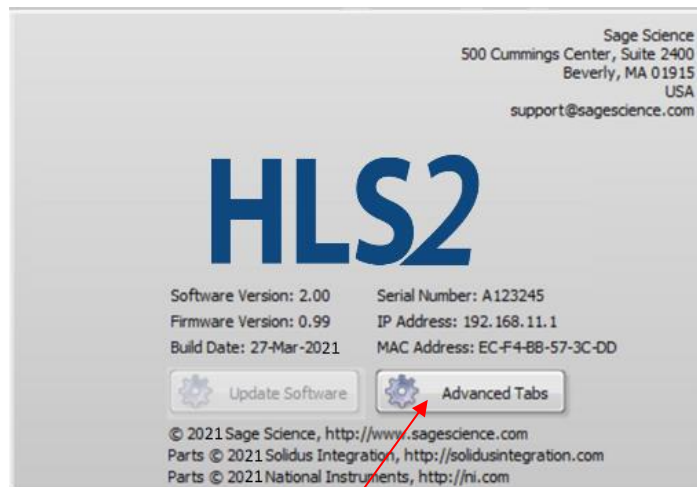
Workflow stages can be created or edited in the Stage Editor.

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

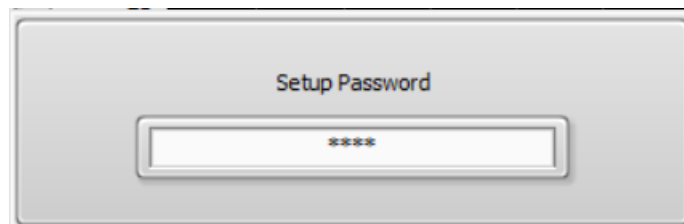
1. In the Main Screen, press the “Display Info” button in the Command Menu.



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.

Stage Editor Tab

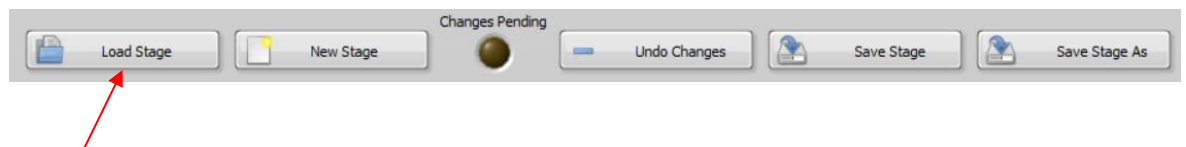
The screenshot shows the Stage Editor interface with the following components and callouts:

- Workflow File Name**: A text input field at the top.
- Stage Type Selector**: A dropdown menu showing "extraction".
- Electrophoresis Wave Form Table**: A table with columns Index, A, B, C, D, E, F, G.

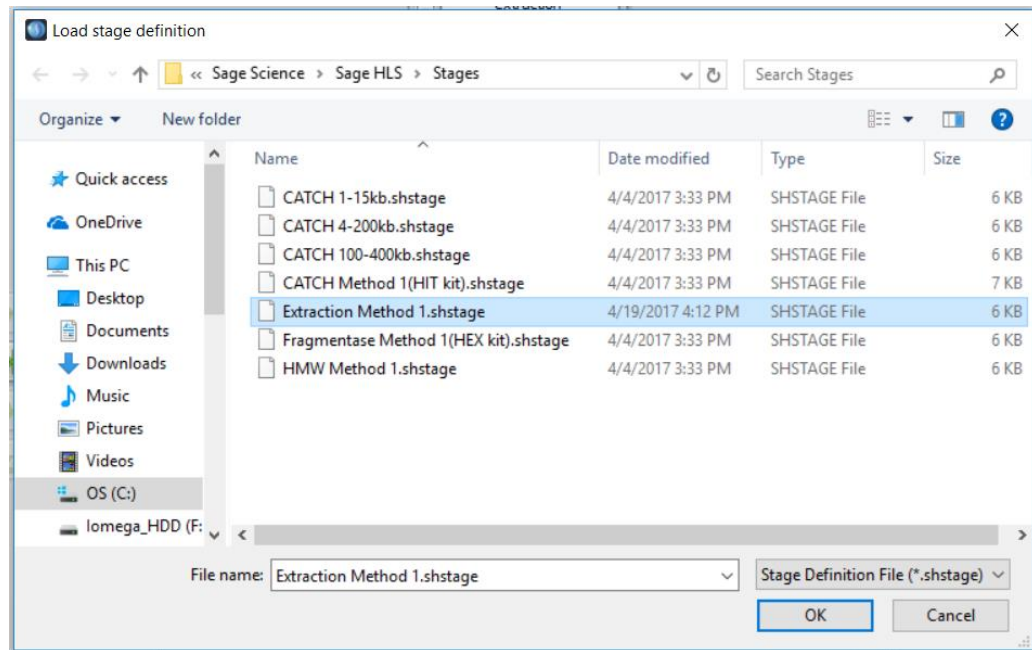
Index	A	B	C	D	E	F	G
1	0	0	0	0	0	0	0
2	150	50	30	10	3	1	81
3	300	100	30	10	30	10	45
- Step Table Editor**: A table with columns Step and Description, and buttons for Insert, Append, Replace, Edit, Move Up, Move Down, and Delete.
- Step Parameters Display**: A section for Stage Step parameters including State/Action (set temp), Time (00:00:00), Volts (V) (0.0), Wave Index (0), Sample Temp (°C) (20.0), and Gel Temp (°C) (30).
- User Action Text Field**: A text input field for "Prompt for Pause State".
- Command Menu**: A row of buttons including Load Stage, New Stage, Changes Pending indicator, Undo Changes, Save Stage, and Save Stage As.

5.11 Opening a Stage File

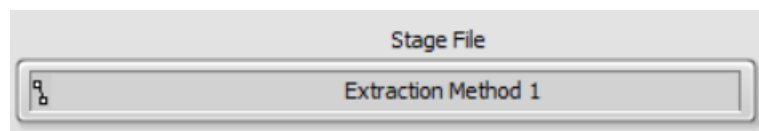
1. In the Command Menu, press "Load Stage":



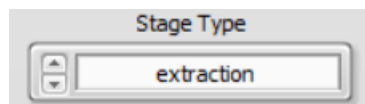
2. A file folder window will appear listing the pre-set protocols in the “Preset Stages” folder. All stage files have a .shstage extension. Select a Stage file to load and press “OK”



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)

Index	A	B	C	D	E	F	G
1	0	0	0	0	0	0	0
2	150	50	30	10	3	1	81
3	300	100	30	10	30	10	45
0	0	0	0	0	0	0	0

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	Description
001	pause for user action 'Replace sample well contents with 70ul of prepared sample. Replace re.
002	separate for 01:00:00 at 55.0 V with wave index 1

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

5.12 Changing the Stage Type

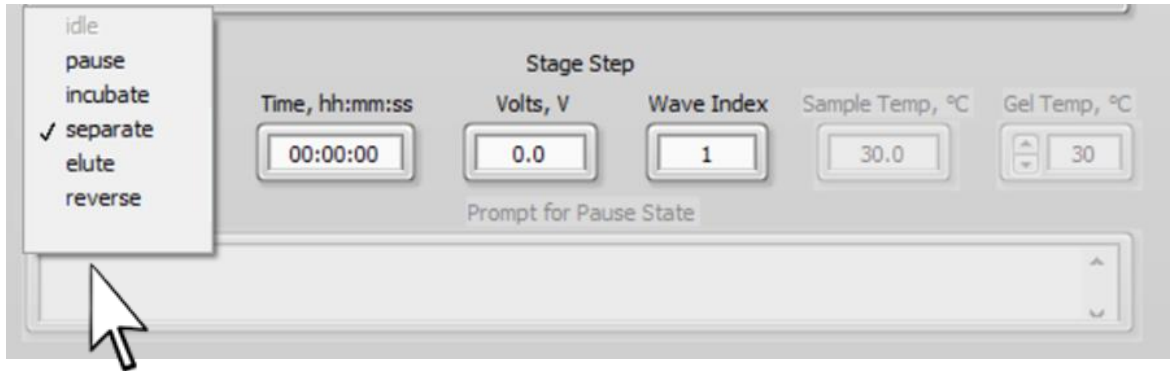
Click the arrows in the Stage type field to toggle between the three stages.



5.13 Editing a Stage Step

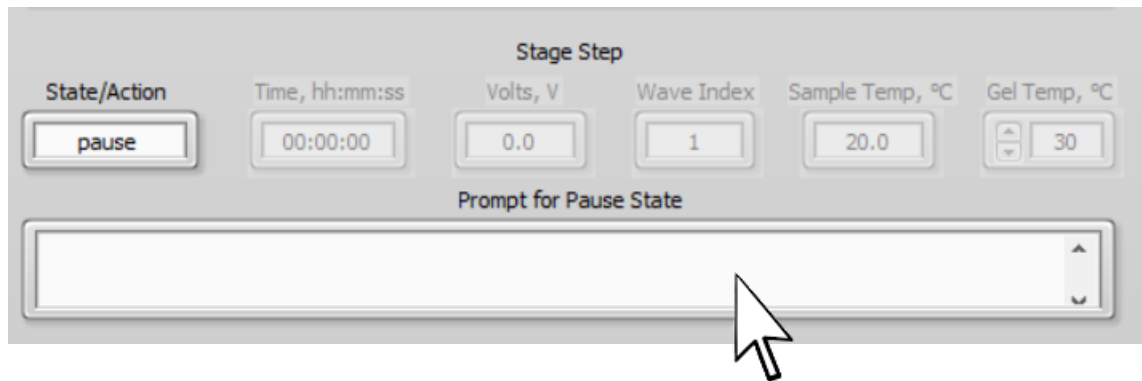
A new Step can be programmed in the Stage Step fields:

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.



Incubate: The instrument remains at an active state for a period of time, with no electrophoresis. The amount of time is only parameter entered.

The screenshot shows the 'Stage Step' control panel. The 'State/Action' dropdown is set to 'incubate'. The 'Time, hh:mm:ss' field is set to '00:00:00'. The 'Volts, V' field is set to '0.0'. The 'Wave Index' field is set to '1'. The 'Sample Temp, °C' field is set to '30.0'. The 'Gel Temp, °C' field is set to '30'. Below the fields is a 'Prompt for Pause State' label and a large empty text area.

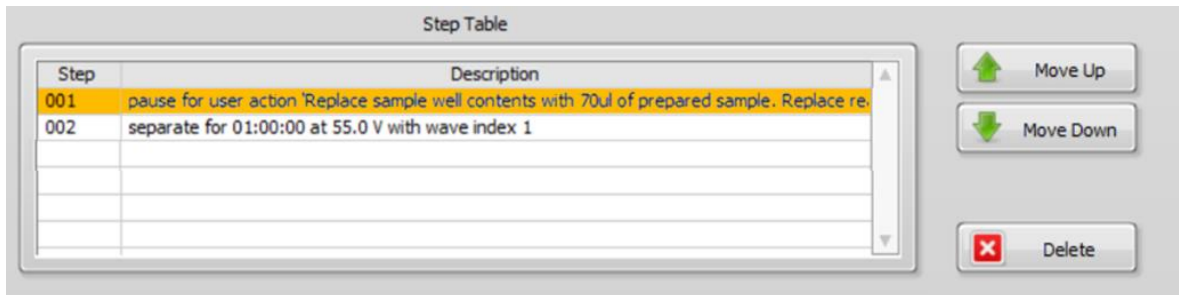
Separate, Elute, and Reverse: Electrophoresis parameters. “Separate” is electrophoresis along the separation column. “Elution” is electrophoresis along the elution pathway (perpendicular to the separation pathway, into the elution wells). “Reverse” is the reverse direction of the Elution pathway - typically 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.

The screenshot shows the 'Stage Step' control panel with a dropdown menu open on the left. The dropdown menu lists the following options: 'idle', 'pause', 'incubate', '✓ separate', 'elute', and 'reverse'. The 'separate' option is selected and highlighted with a red border. The 'Time, hh:mm:ss' field is set to '00:00:00'. The 'Volts, V' field is set to '0.0'. The 'Wave Index' field is set to '1'. The 'Sample Temp, °C' field is set to '30.0'. The 'Gel Temp, °C' field is set to '30'. Below the fields is a 'Prompt for Pause State' label and a large empty text area.

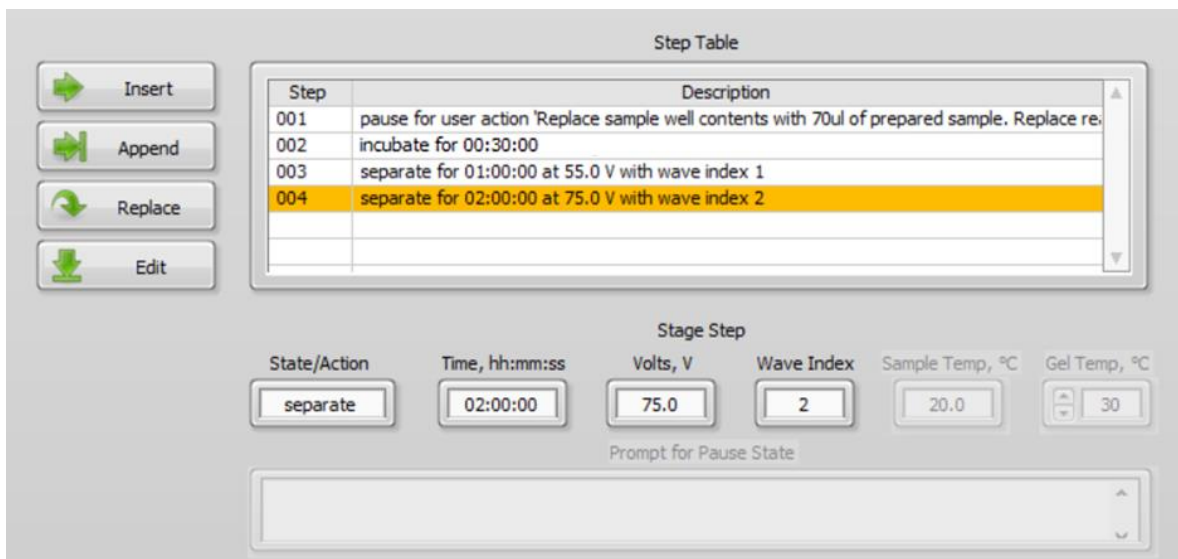
5.14 Editing the Step Table

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.

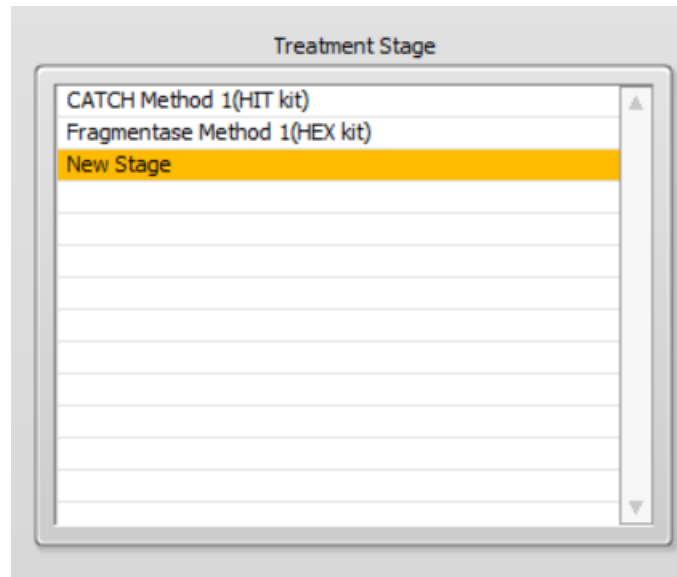
Edit: Enters the parameters in the highlighted step into Stage Step fields.

5.15 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.



5.16 Log Files

At the end of every workflow, the HLS2 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 HLS2 log files.

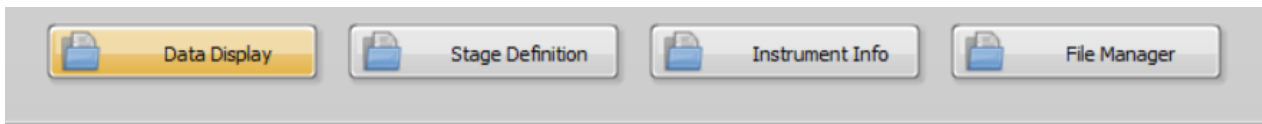
File Type	Folder	Contents	Use
Workflow Log (.txt)	\Logs	Date/time, electrophoretic current data (mA), Sample ID, workflow stages and steps	View in Log Review screen; support for troubleshooting
End of Run screenshot (.png)	\Logs	Image of Main Screen at the end of the run	support for troubleshooting
Current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

5.17 Log Review Tab

In the HLS2 software, navigate to the **Log Review**. It is the third tab from the left.

The screenshot shows the Log Review tab in the HLS2 software. The interface includes a 'Log File' selector at the top, followed by fields for 'Workflow File', 'Stage 1', 'Stage 2', and 'Stage 3'. Below these is a 'Step Table' with columns for 'Step' and 'Description'. To the right is a graph titled 'Electrophoresis Current and Sample Well Temperature' showing 'Current, mA' on the y-axis (0 to 80) and 'Time, h:mm' on the x-axis (00:00 to 00:00). A 'Last Log' button is located in the top right corner. At the bottom, there are four sub-window navigation buttons: 'Data Display', 'Stage Definition', 'Instrument Info', and 'File Manager'. Annotations include: 'Log Review Tab' pointing to the tab header; 'Log File Selector' pointing to the file selection field; 'Workflow File name, Stages and Steps from the logged run.' pointing to the workflow and stage fields; 'Electrophoresis current from logged run' pointing to the graph; and 'Sub-window navigation' pointing to the bottom navigation buttons.

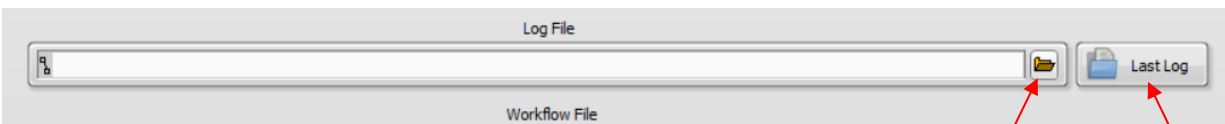
The Log Review has four sub-windows:



- **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 HLS2 files.

5.18 Log Data Display

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

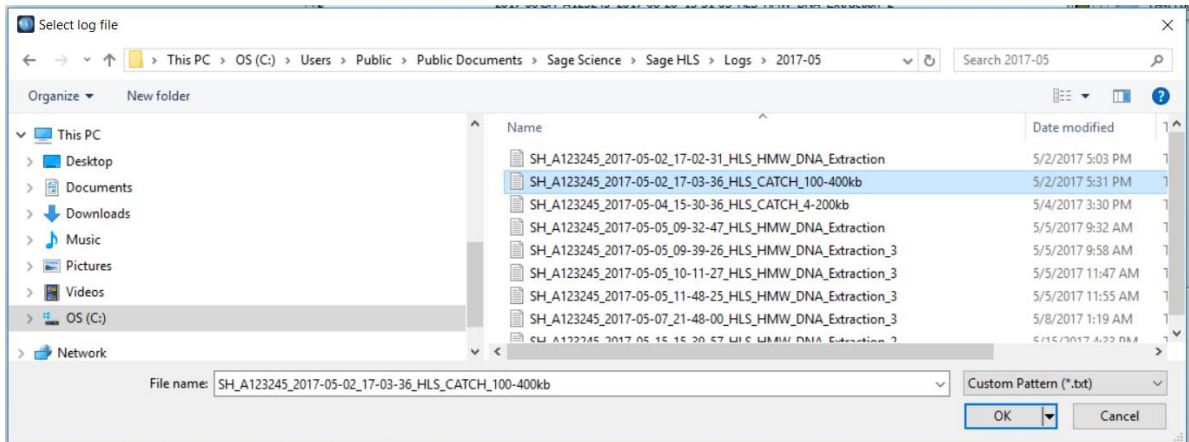


Opens the /Logs folder to access files

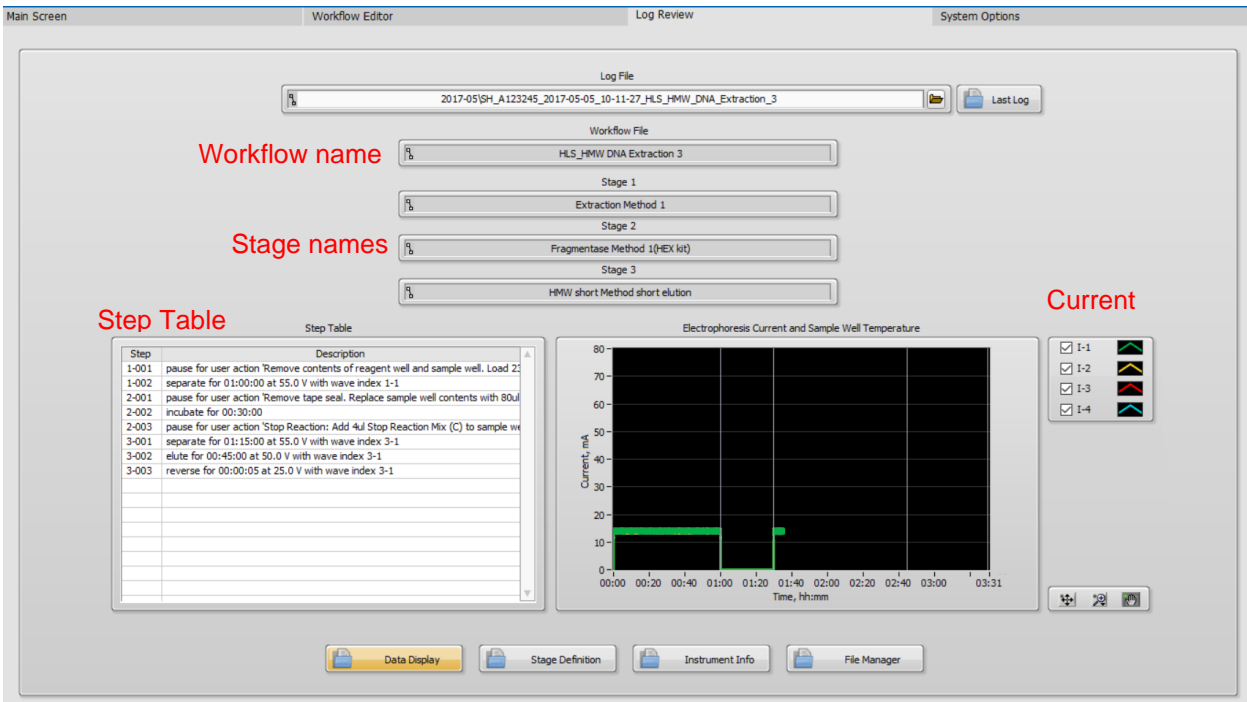
(Loads log file of the last workflow run on the system)

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).

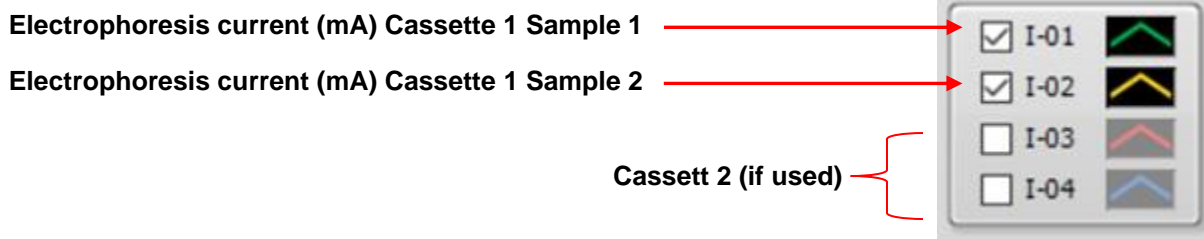
Log files are automatically named with instrument name_serial number_date_time (at end of run)_workflow name.



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

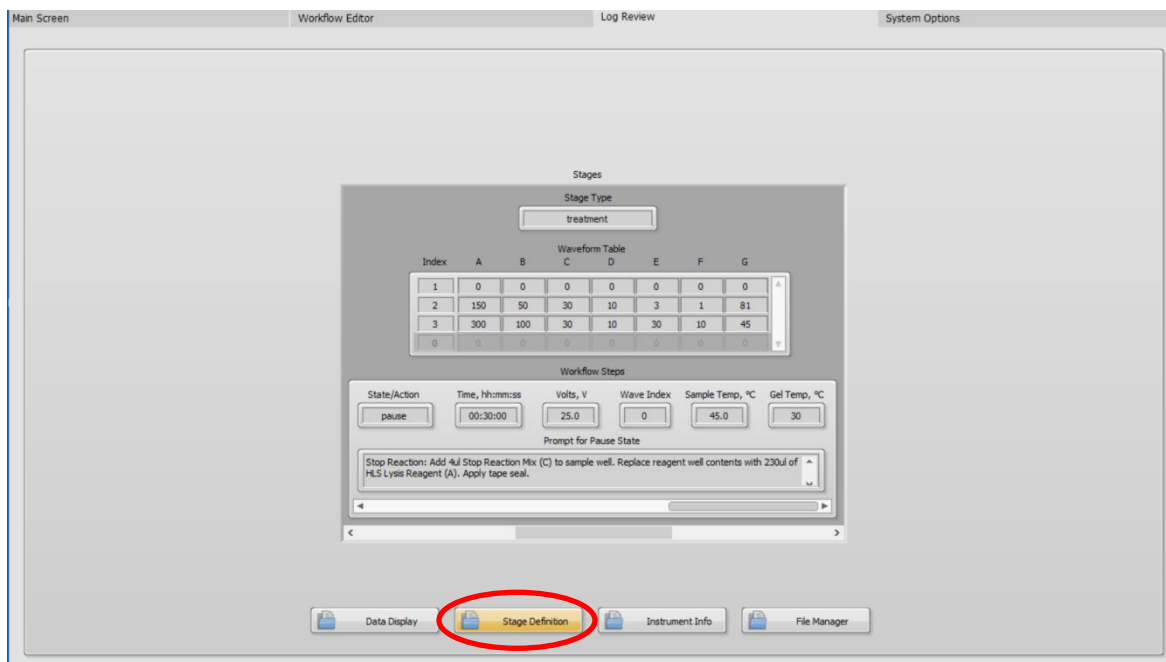


The current is displayed as follows. The current (mA) is on the left y axis, and the time (during the run) is on the x-axis.



5.19 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.

The screenshot shows the Stage Definition Window with the following components:

- Stages:** Stage Type is set to "treatment".
- Waveform Table:** A table with 8 columns (Index, A, B, C, D, E, F, G) and 4 rows (1, 2, 3, 0).

Index	A	B	C	D	E	F	G
1	0	0	0	0	0	0	0
2	150	50	30	10	3	1	81
3	300	100	30	10	30	10	45
0	0	0	0	0	0	0	0
- Workflow Steps:**
 - State/Action: pause
 - Time, hh:mm:ss: 00:30:00
 - Volts, V: 25.0
 - Wave Index: 0
 - Sample Temp, °C: 45.0
 - Gel Temp, °C: 30
- Prompt for Pause State:** Stop Reaction: Add 4ul Stop Reaction Mix (C) to sample well. Replace reagent well contents with 230ul of HLS Lysis Reagent (A). Apply tape seal.

Annotations:

- Electrophoresis Waveforms:** Points to the Waveform Table.
- Step type (State or Action), time, voltage and temp. parameters:** Points to the Workflow Steps parameters.
- Use Action:** Points to the "pause" button.
- Scrolls between Stages:** Points to the lower slide bar at the bottom.
- Scrolls between Steps within a Stage:** Points to the upper slide bar above the prompt.

5.20 Instrument Info Sub-Window

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

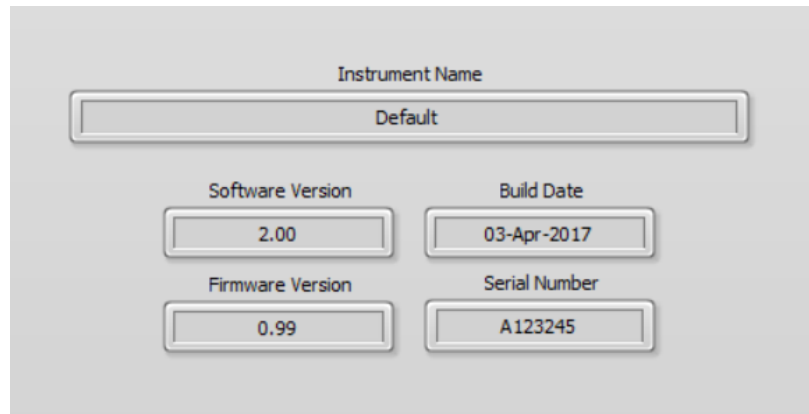
The screenshot shows the Instrument Info Sub-Window with the following information:

- Instrument Name:** Default
- Software Version:** 2.00
- Build Date:** 03-Apr-2017
- Firmware Version:** 0.99
- Serial Number:** A12245

At the bottom, there are four buttons: Data Display, Stage Definition, **Instrument Info** (circled in red), and File Manager.

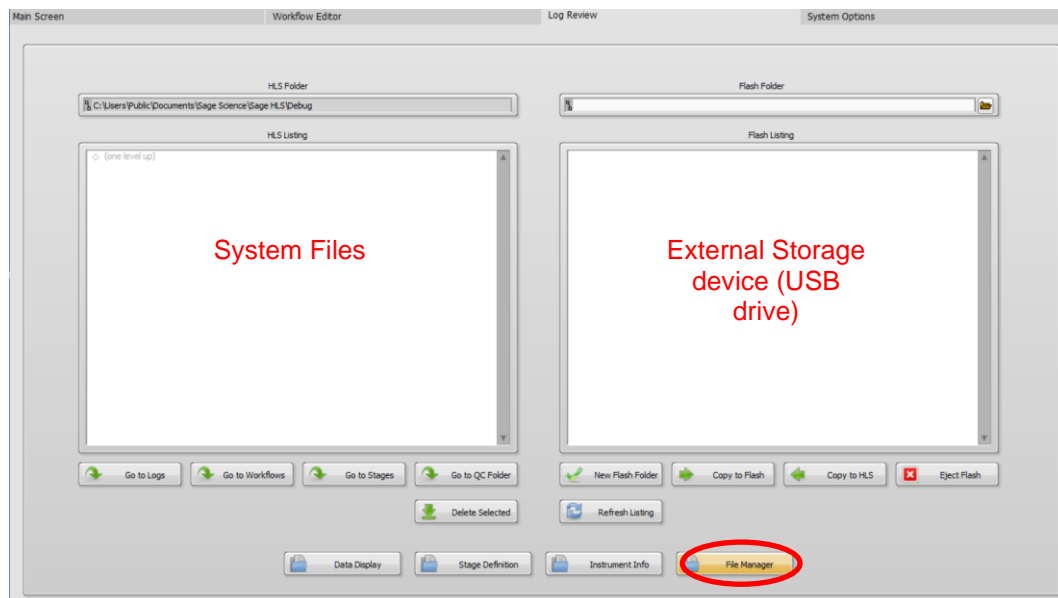
Instrument Info

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



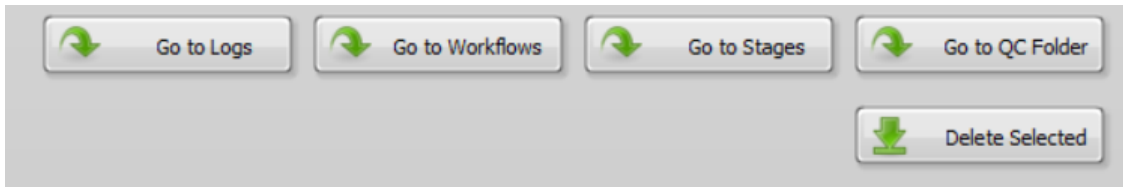
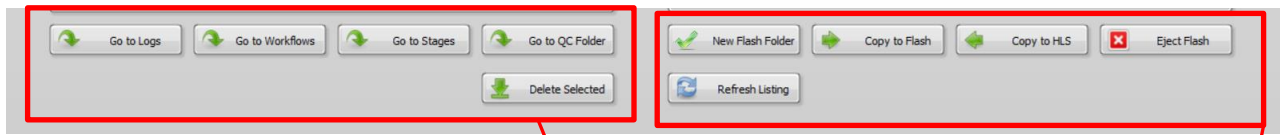
5.21 File Manager Sub-Window

The File Manager sub-window provides an interface for users to manage files on the HLS2 system, including Log files, Workflow files, and Stage files. The File manager allows the transfer of file between HLS2 system and an external USB drive.

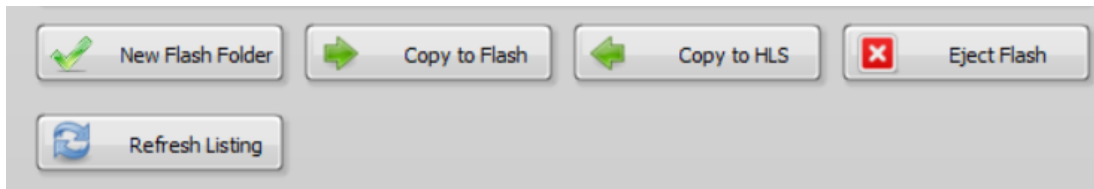


File Manager

Several buttons are associated with managing files on the HLS2 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 HLS2 directory to a USB device
- **Copy to HLS:** copies highlighted files from a USB device to the HLS2
- **Eject Flash:** allows safe removal of USB device
- **Refresh Listing:** deletes files that are highlighted

5.22 Copying Files To and From a USB Device

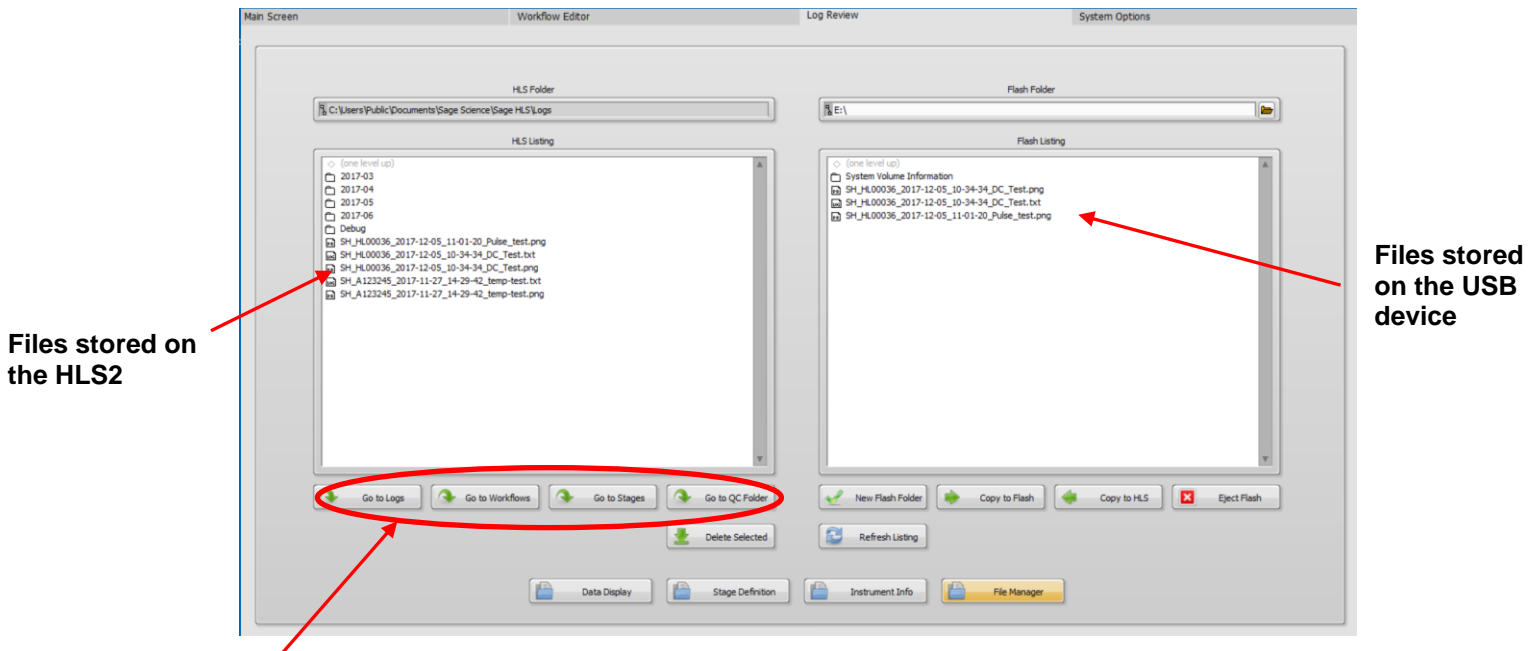
1. Insert a USB storage device into the USB port on the front panel of the HLS2.
2. In the software, click on the Log Review Tab.



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

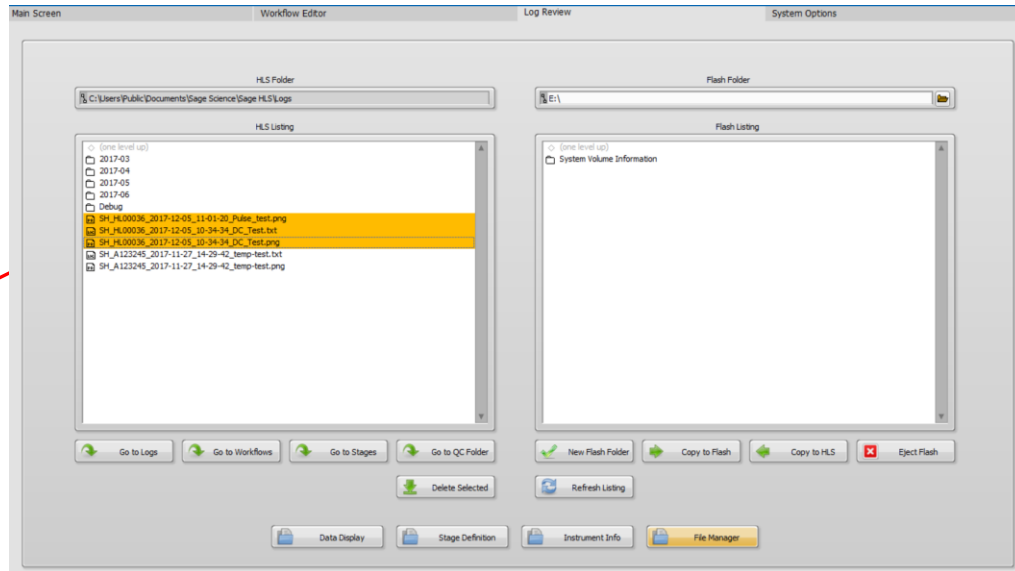


4. Files located on the HLS2 system will appear in the directory window on the left side of the screen, and the files located on the USB device will appear on the right side of the screen.

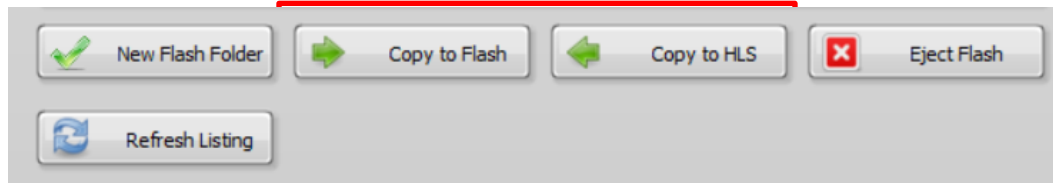


- Files can be highlighted by clicking the file names. Multiple files can be highlighted by holding the Shift button and clicking.

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



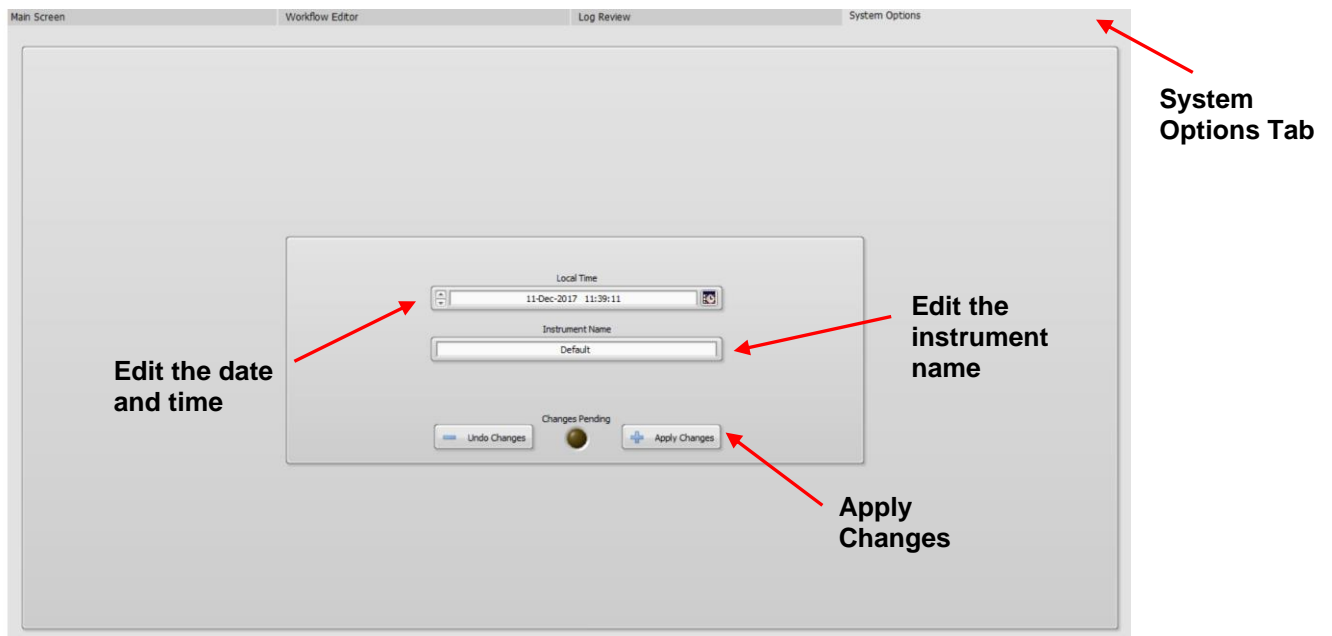
- Press "Copy to Flash" (or "Copy to HLS", if moving files from a USB Device to the HLS2).



- Press "Eject Flash" to safely unmount the USB device. The USB device can be removed from the USB port on the front panel.

5.23 System Options Tab

The System Options Tab is fourth Tab on the right. The time and date can be adjusted from this screen. The HLS2 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.

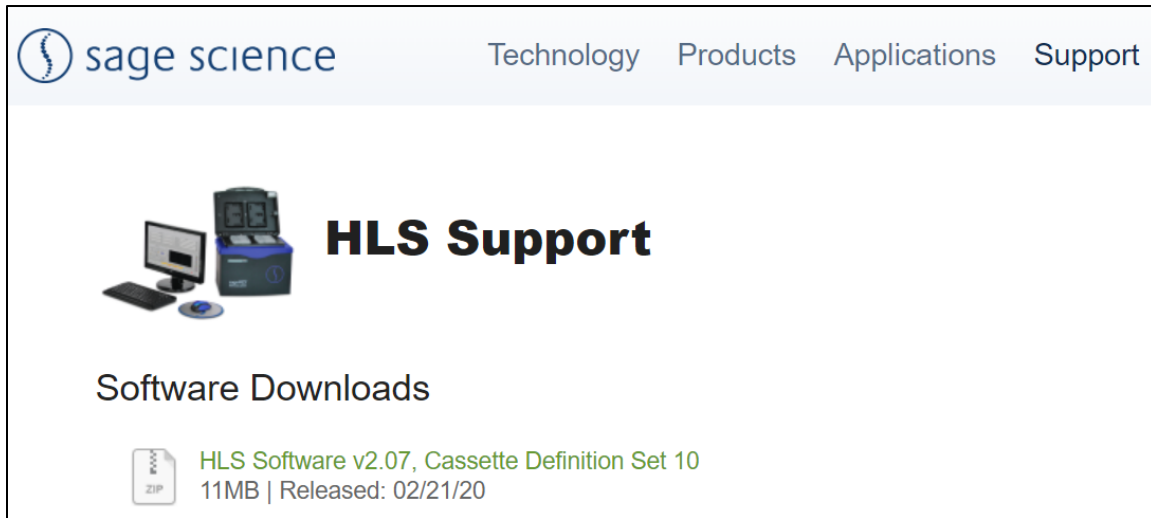


5.24 Updating HLS2 Software and Workflows

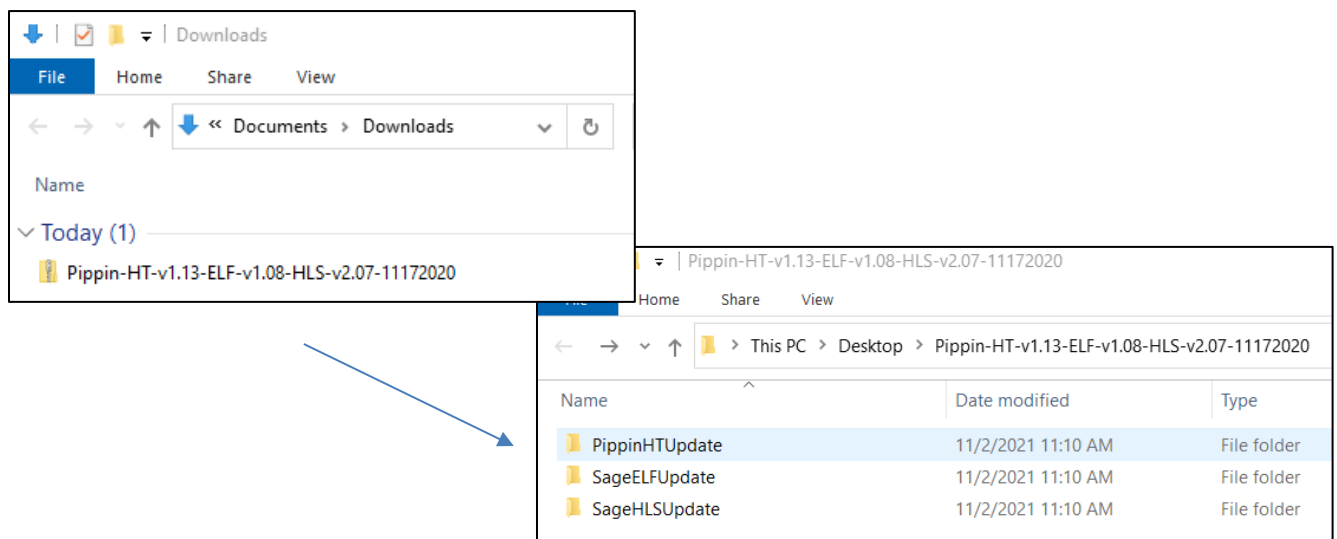
Extracting the file to a USB flash drive

HLS2 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:

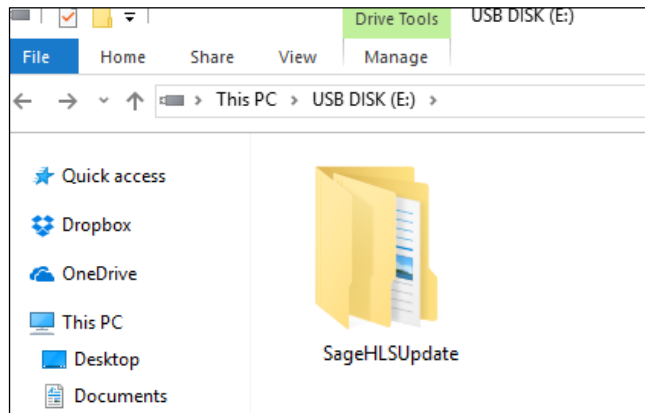
1. Download the Software Upgrade package by pressing the appropriate link on www.sagescience.com/product-support/HLS2-support/ (in the “Software Downloads” section).
2. Browsers may prompt users to save the file. The zip file will likely save to a “Downloads” folder on your computer. The file will include update folders for the PippinHT, SageELF and HLS instruments.



3. Open the zip file or unzip its contents onto your computer. It will contain three folders.

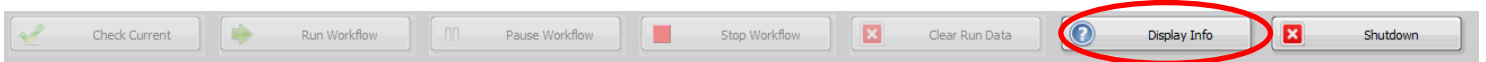


- Transfer this “SageHLSUpdate” folder into the root directory of a USB drive.

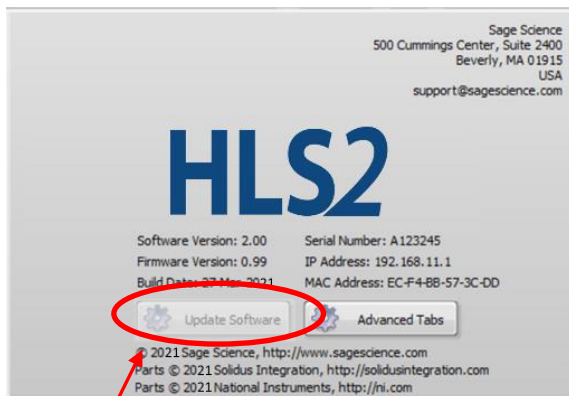


Upgrading HLS2 Instrument Software

- Insert the USB drive with the upgrade folder into the USB port on the front panel of the HLS2 instrument.
- From the Main Tab, Press “Display Info”. This will open the information window.



- From the information window, press “Update Software”. This will open the Software Upgrade window (HLS2 Utility Applet). Press the “Update System” button.

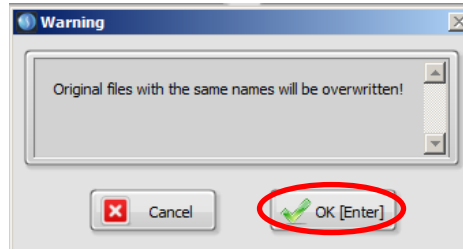


Update Software

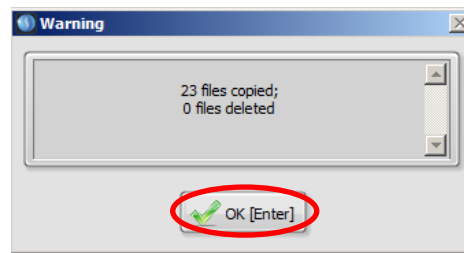


Update System

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 HLS2” 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:

Software Version: 2.03
Firmware Version: 1.10
Build Date: 03-Aug-2017



Getting Started

Workflow guides for preparing HLS-compatible cell suspensions are available separately with purchases of Cell Preparation Kits. They may also be viewed or downloaded at <http://www.sagescience.com/product-support/HLS2-support/>

- **Mammalian White Blood Cell from Whole Blood Preparation**
- **Mammalian Tissue Culture Cell Preparation**
- **E.Coli Sphroplast Perparation**

Application workflow guides are individually provided with the purchase of Gel Cassette Kits and may also be viewed or downloaded from <http://www.sagescience.com/product-support/HLS2-support/>. These include complete step-by-step instructions for HLS2 workflows and associated instructions.

- **HLS-CATCH Targeted Collection of HMW Genomic Regions**
 - **Sourcing guide for for Guide-RNAs and qPCR Primers: HLS-CATCH BRCA1 Enrichment in Human or Mouse**
 - **Cas9 guide-RNA Design Tutorial for HLS-CATCH**
- **High Molecular Weight DNA Extraction**

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 HLS2 run
- Some HLS2 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 HLS2 support page on the Sage Science website (<http://www.sagescience.com/product-support/HLS2-support/>).

