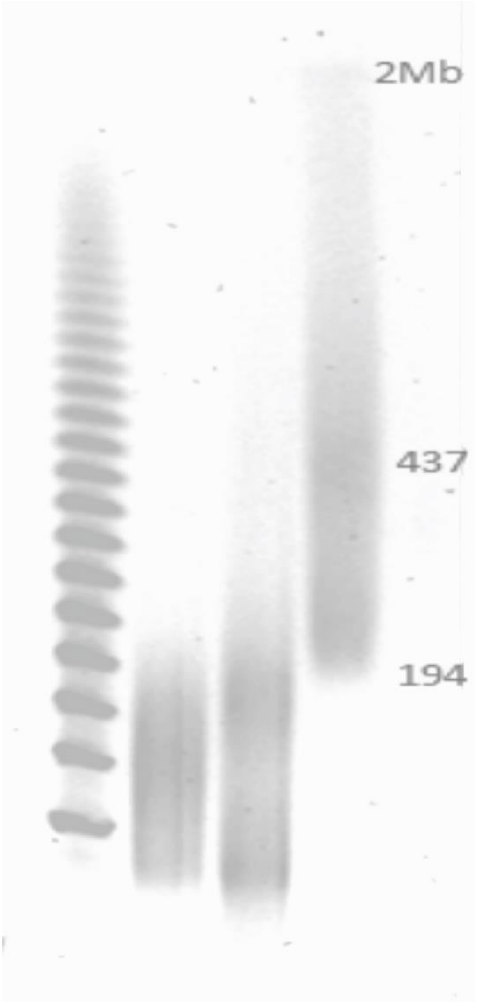
sage science

SageHLS Cassette Kit
Workflow Guide

High Molecular Weight DNA Extraction

PN# HEX-0004 or HEX-0012



Important! Read carefully before starting!

A. <u>One week before running the SageHLS</u>:
The following materials must be obtained or prepared by users prior to using this method. Custom reagents can require a week or more to receive, check with the supplier.

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

B. <u>One day before</u> running the SageHLS:
Schedule availability of cells <u>one day before</u> running the SageHLS:

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

- Recommended cell load per lane for HLS cassettes contains **8-10 µg of genomic DNA per lane**, (~1.2-1.5 million human diploid cells) in a maximum loading volume of **70 µl**.

A. <u>The following items are supplied Cassette Kits (HEX-1004 and HEX-1012).</u> When the kit is received, open immediately to inspect the contents. Contact Sage Science if any items are missing or damaged

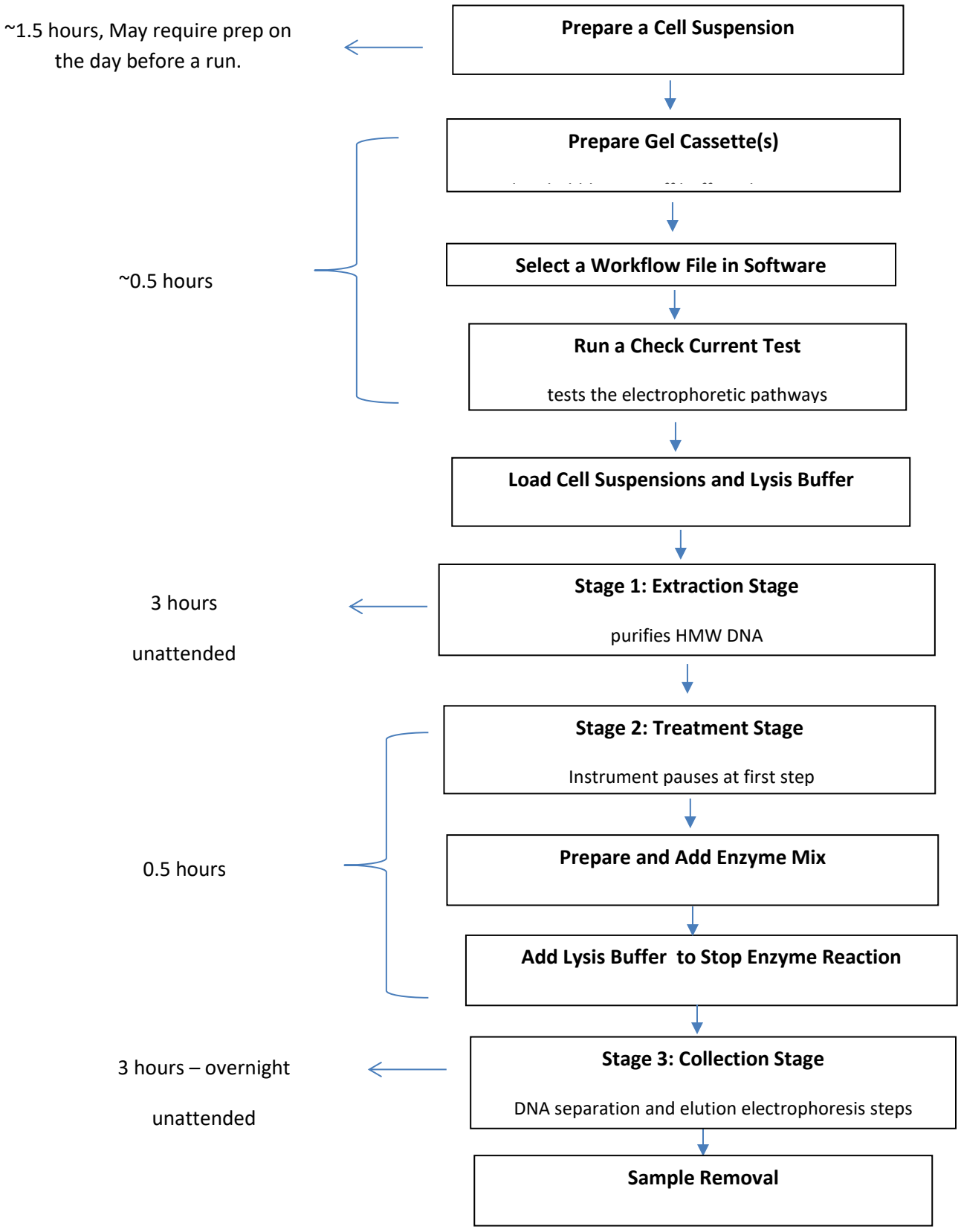
<u>Note the storage conditions and store accordingly.</u>

#	Item	Label	Storage Temp.
4 / 12 ea.	Agarose gel cassettes		RT
20 / 60 ea.	Adhesive Tape Strips		N/A
1 ea.	HLS Lysis Reagent 3% SDS, EDTA, 10 / 30 ml	**A1**	RT
1 ea.	HLS Enzyme Buffer, 15 / 40 ml	**C**	**4ºC**
1 ea.	Running Buffer, 40 / 115 ml	**E**	RT

HEX kit Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	12-2	Added Revision Change Log
D	E	6/18/18	12-1	Updated NEB fragmentase part number
D	E	6/18/18	12-7	Revise Workflow File Names
E	F	8/17/18	12-6	Replaced buffer chamber image to show even fill line.
E	F	8/17/18	12-12	Corrected dilution and math for E.coli fragmentase dilution.
F	J	12/12/19	12-1, 12-9	Removed Lysis Reagents: Sarkosyl and 1% SDS
F	J	12/12/19	all	Separate Guide for HLS Operations Manual, Create stand alone document with page numbering changes

HMW DNA Extraction Workflow Summary

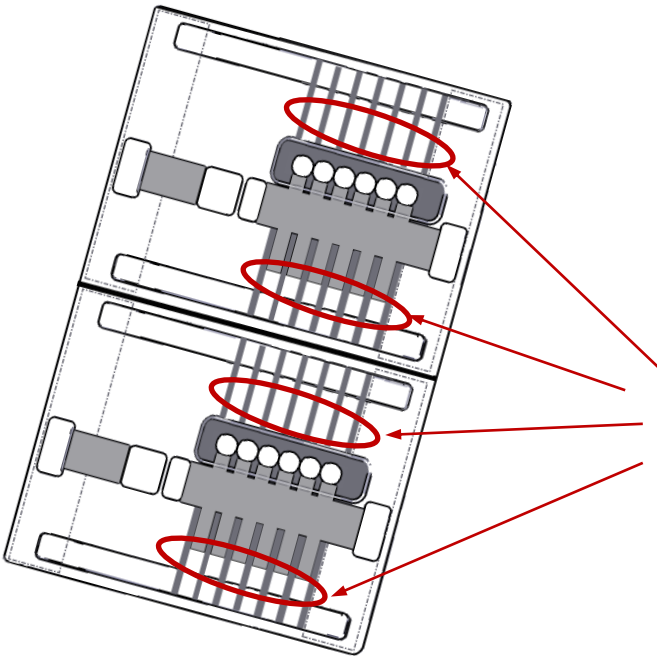


A. Prepare cells using Cell Suspension Guide (Appendix A)

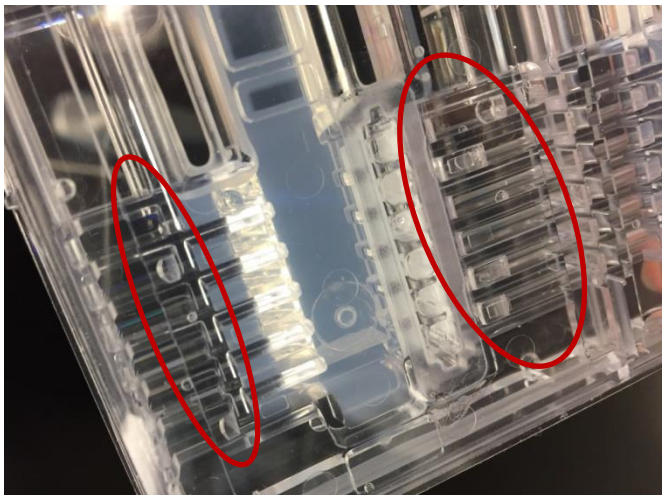
B. Prepare the Gel Cassette(s)

1. Remove the gel cassette from the foil bag.

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

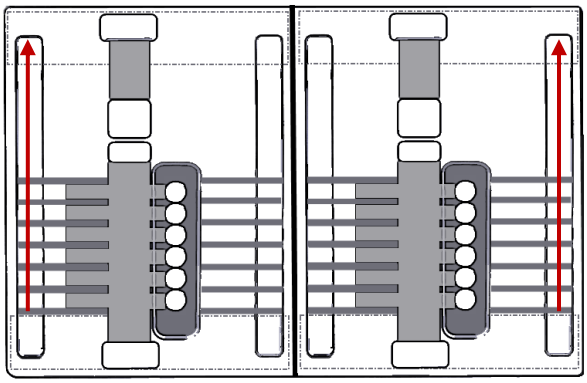


Tap to dislodge bubble from these areas into the troughs directly above.

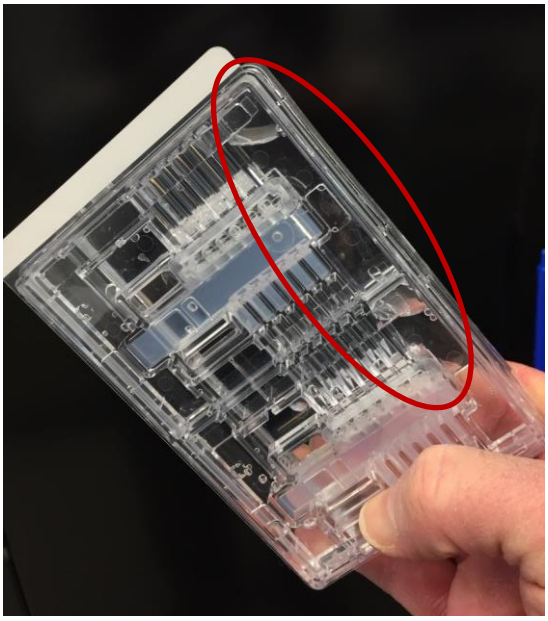


Bubbles in the elution paths can interfere with collection

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



Move any bubbles to the upper buffer area.

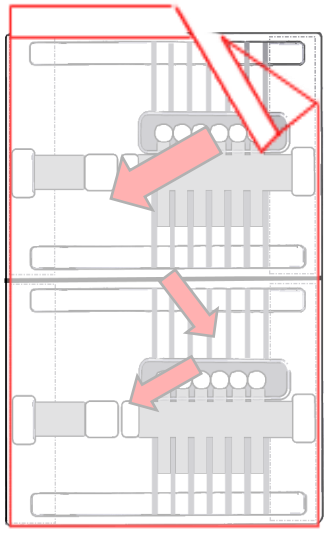


Aggregate and collect air bubbles

in the upper buffer chambers

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

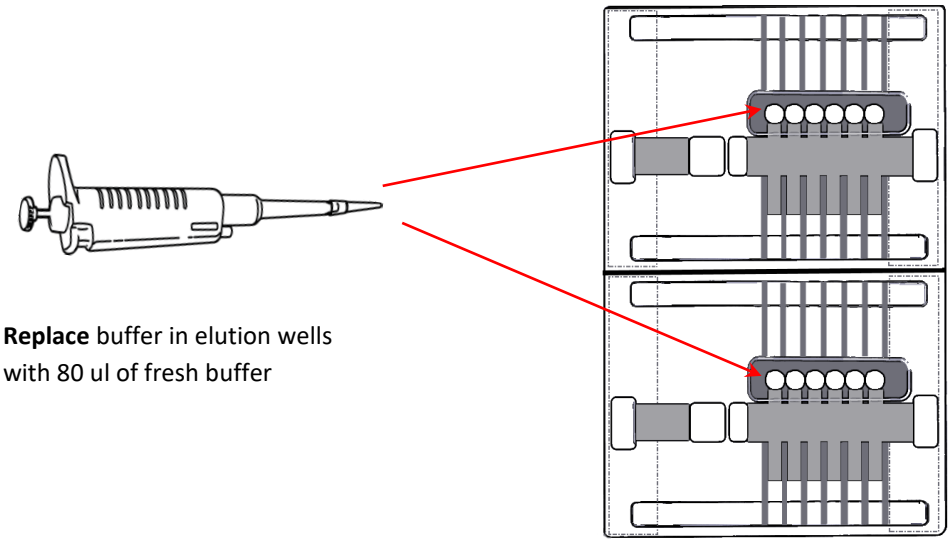
5. While holding the cassette firmly in place on the nest, grab the tape tab, and pull the tape off at an angle, slowly and firmly. Alternate the pulling angle if the tape resists peeling.



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

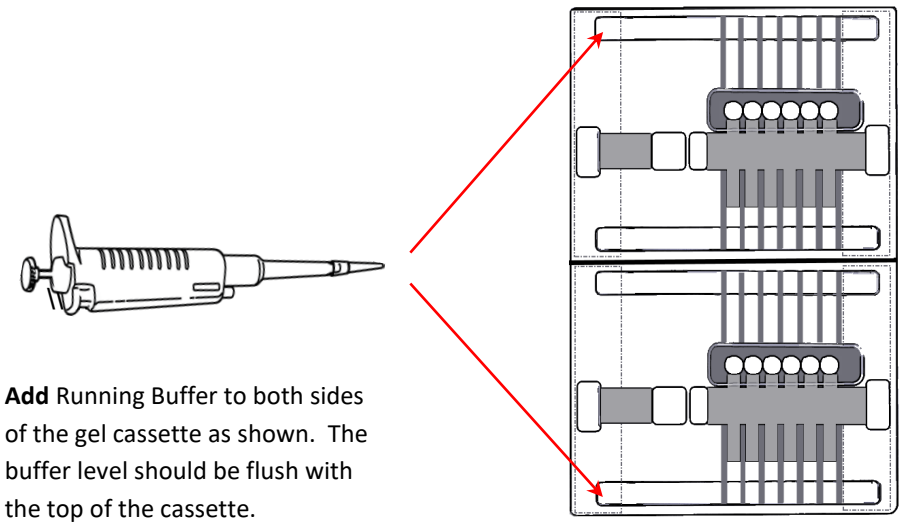
6. Remove all buffer from all elution wells (set a pipette to 100 µl to completely empty the wells). Keep pipette tips vertical in the well to avoid damage to the membranes.

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



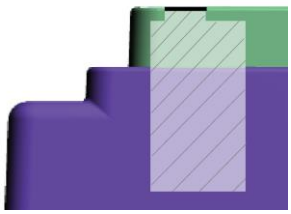
Replace buffer in elution wells with 80 ul of fresh buffer

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



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

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



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

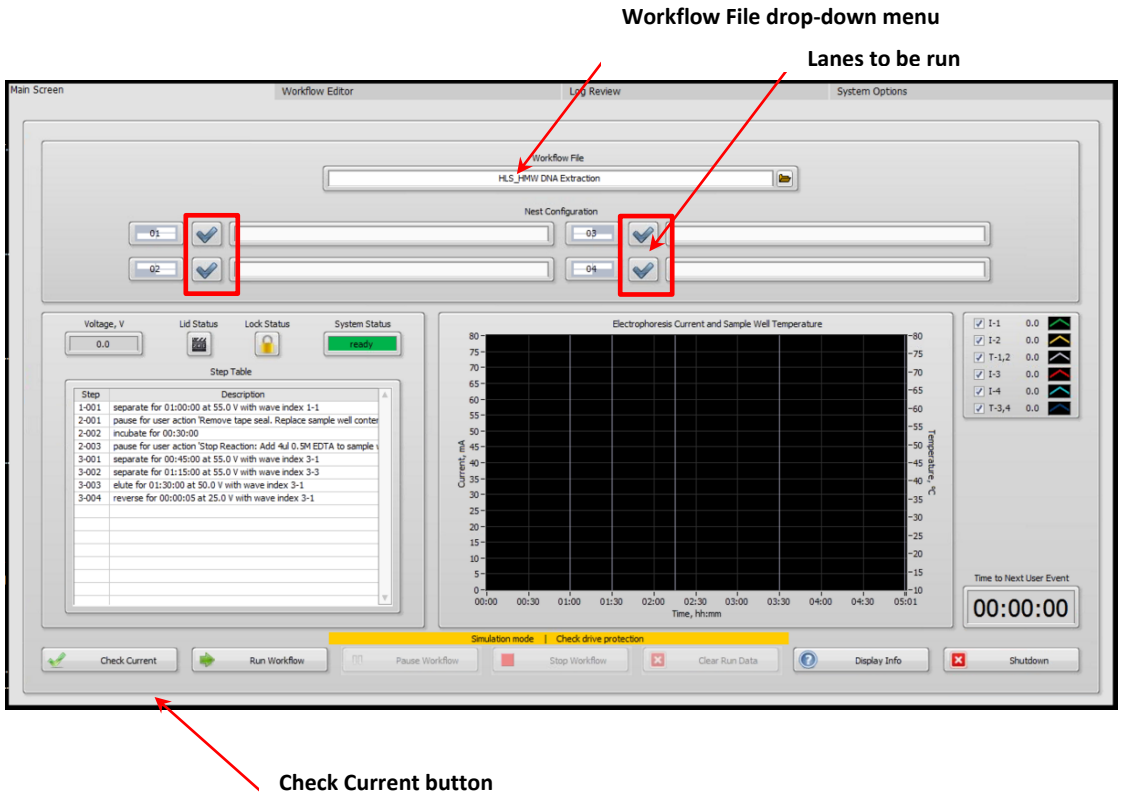
C. Load the Workflow File

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

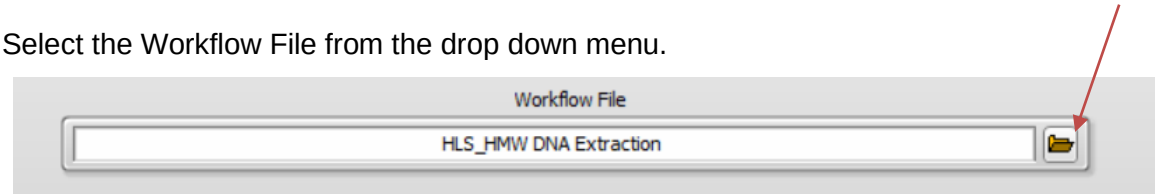
Core Workflow* File Name	Description	Run Time
HMW DNA Extraction	Maximum separation, ultra-HMW fragments in well 2	5:00
HMW High-Pass 50kb elu45	Compression band, 50kb and above, in wells 2 and 3, lower yield	3:30
HMW High-Pass 50kb	Compression band, 50kb and above, in wells 2 and 3	4:20
HMW High-Pass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

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

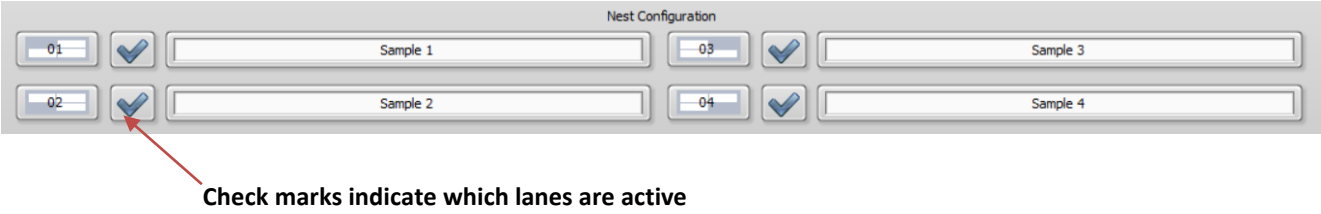
2. Go to the Main screen of the SageHLS software:



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

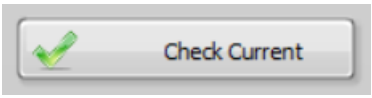


4. Choose the lanes to be used by clicking the boxes next to the lane numbers and enter sample IDs into the adjacent fields (sample IDs are optional or can be entered later).

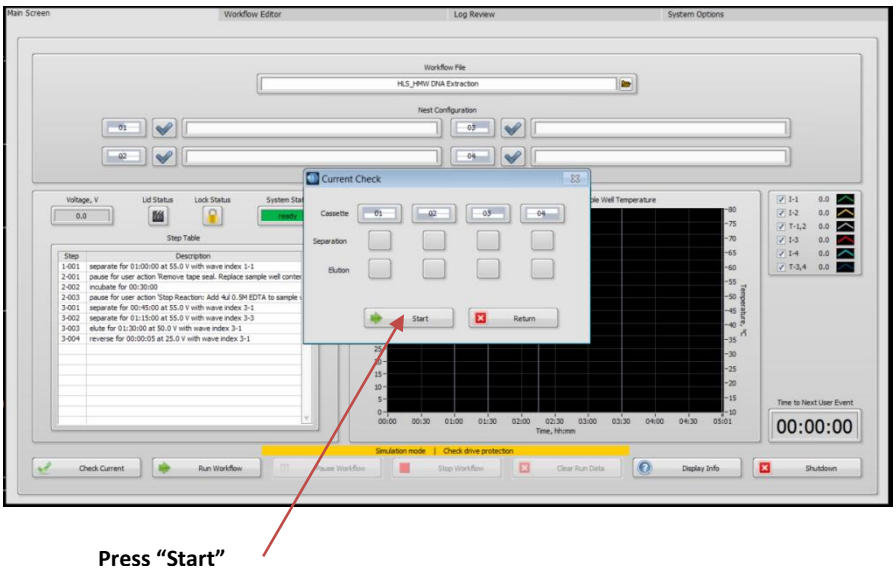


D. Run the Check Current Test

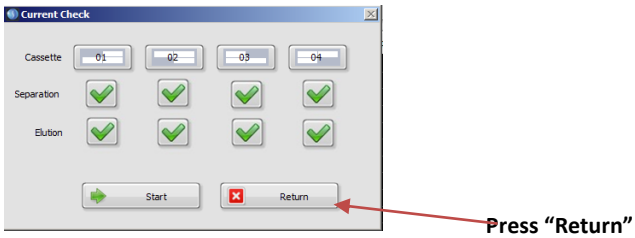
1. Press the “Check Current” button.



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

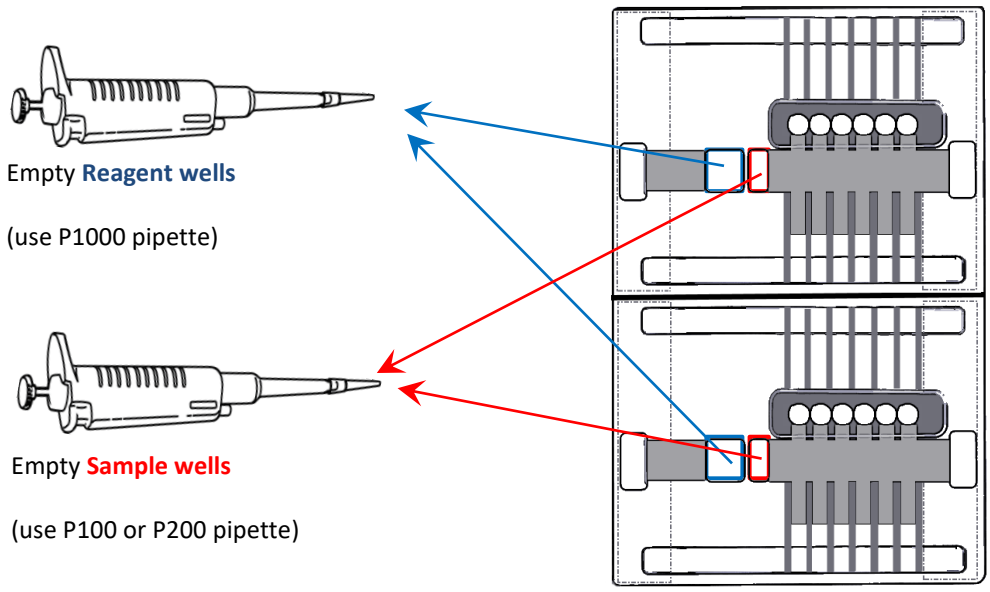


3. The routine will first test the separation electrodes, then test the elution electrodes, and complete within a few minutes. After a successful test, all boxes will fill with green check marks. Press “Return” to continue



E. Stage 1: Extraction

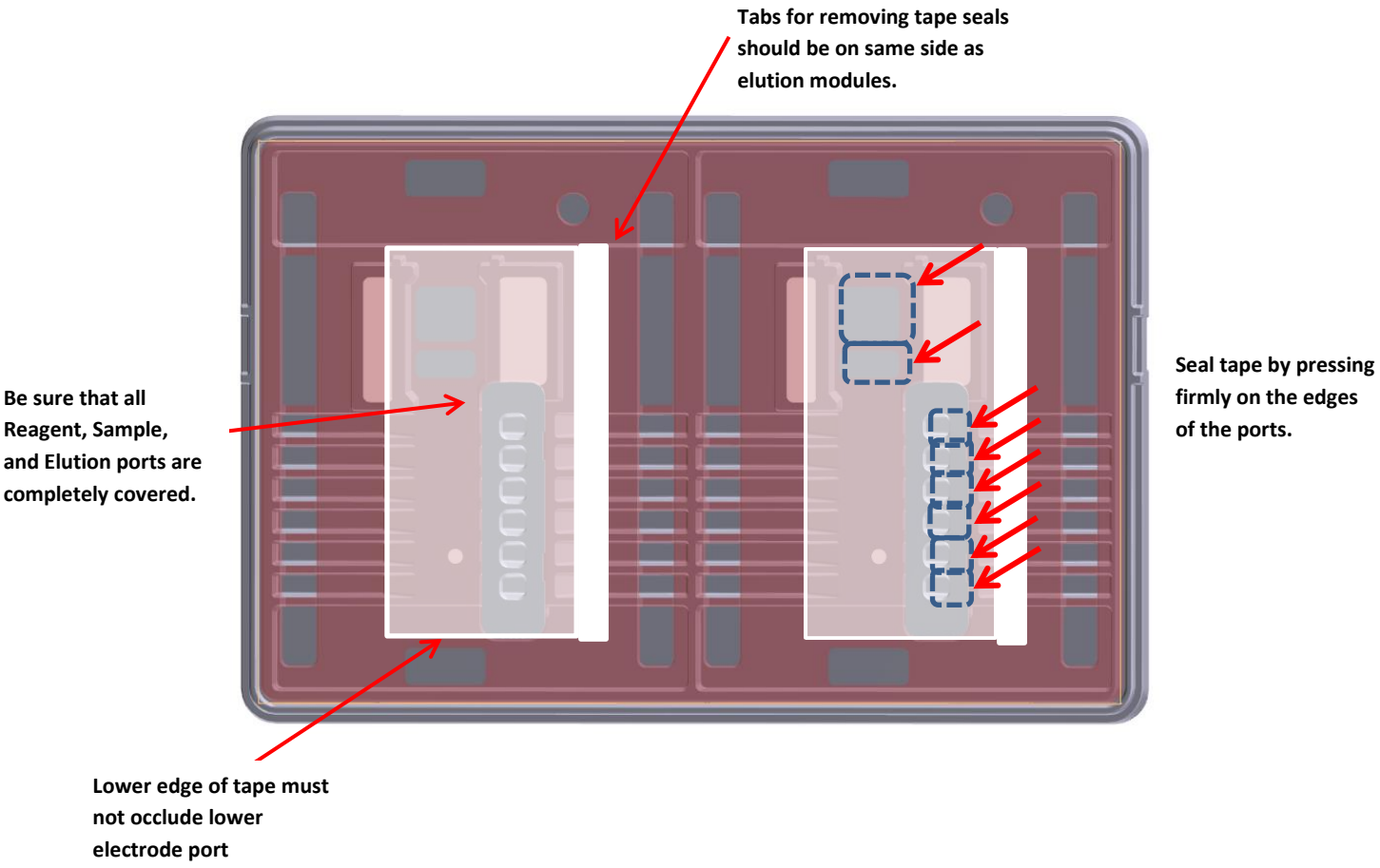
1. Empty all sample and reagent wells. Use caution not to pierce agarose at the bottom of the wells.



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

3. Fill Reagent Wells with HLS Lysis Reagent (**A1**). Fill, but do not overfill! Leave a concave meniscus to prevent contact with sealing tape in next step. Approximate volume needed will be 220-230 ul.

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



press “Run Workflow”. The Extraction step will take 3 hours of unattended

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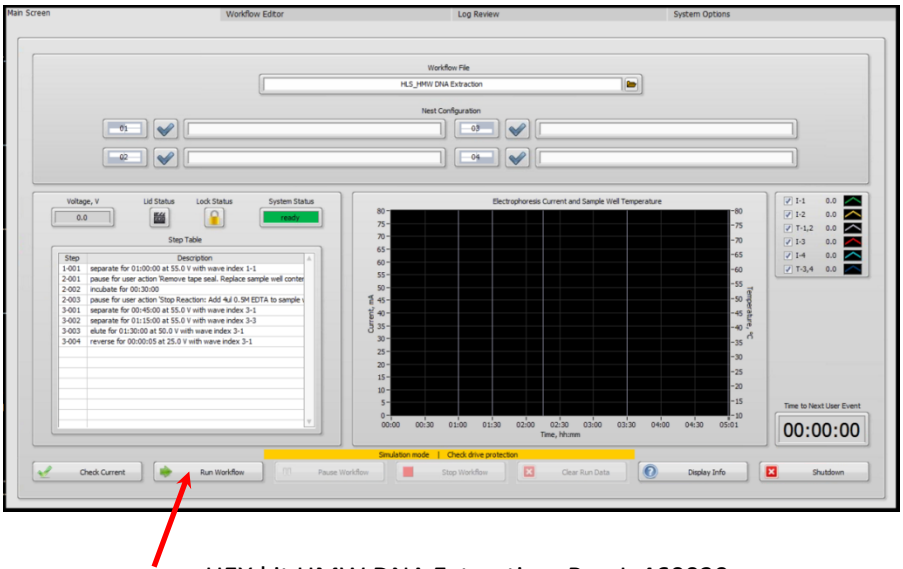
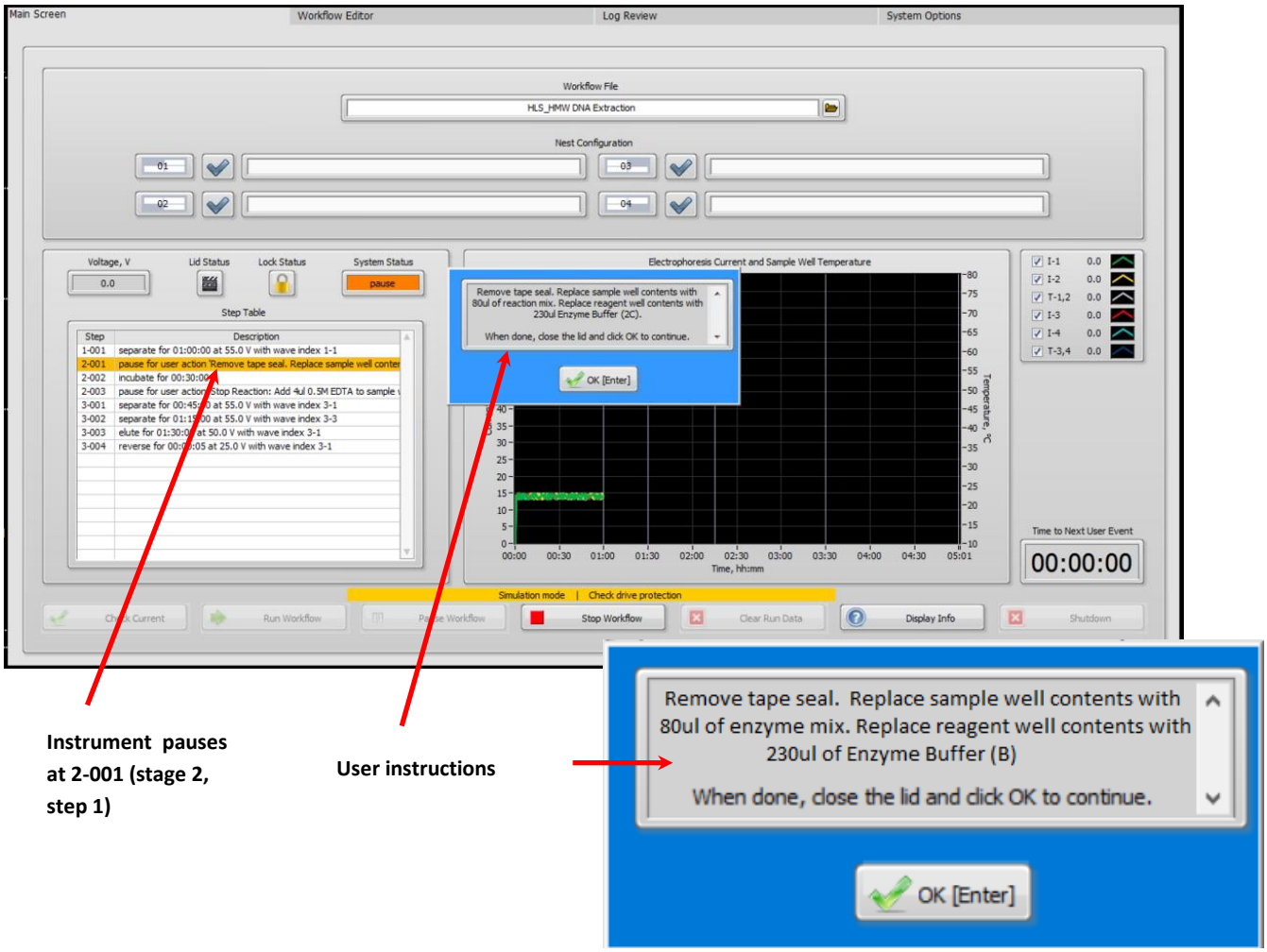


F. Stage 2: Treatment Stage

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



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

1. Prepare the Enzyme Reaction Mix:

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

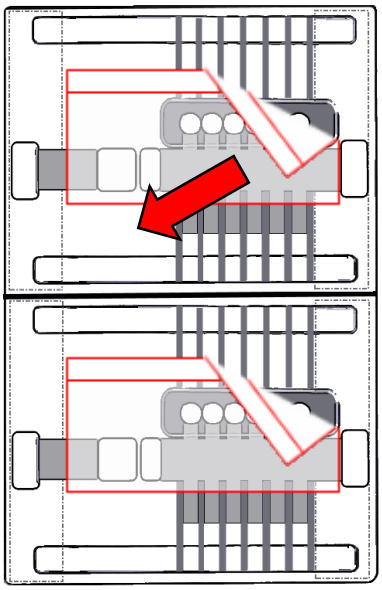
   b. Dilute the NEB Fragmentase (NF) with Enzyme Buffer (**C**) as follows:

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

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

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

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



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

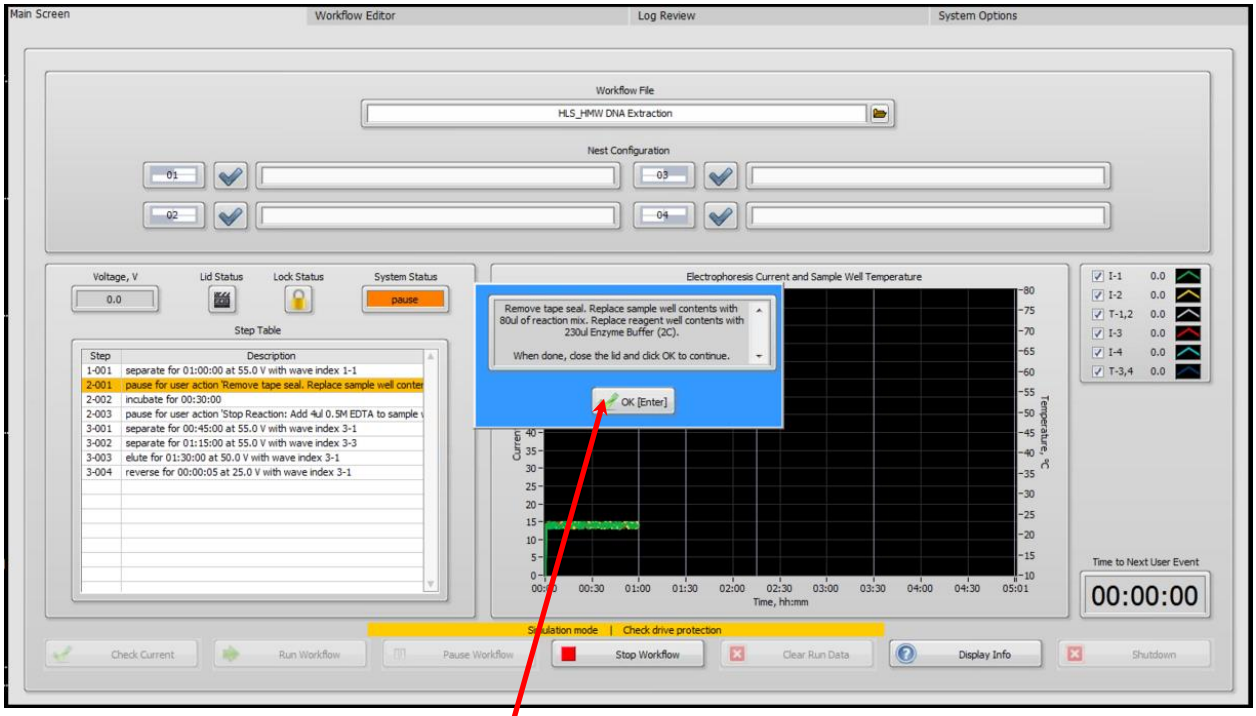
4. Remove <u>all</u> contents from the Reagent wells and Sample wells in the cassettes to be run. The well volumes are 270 µl and 85 µl, respectively.

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

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

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

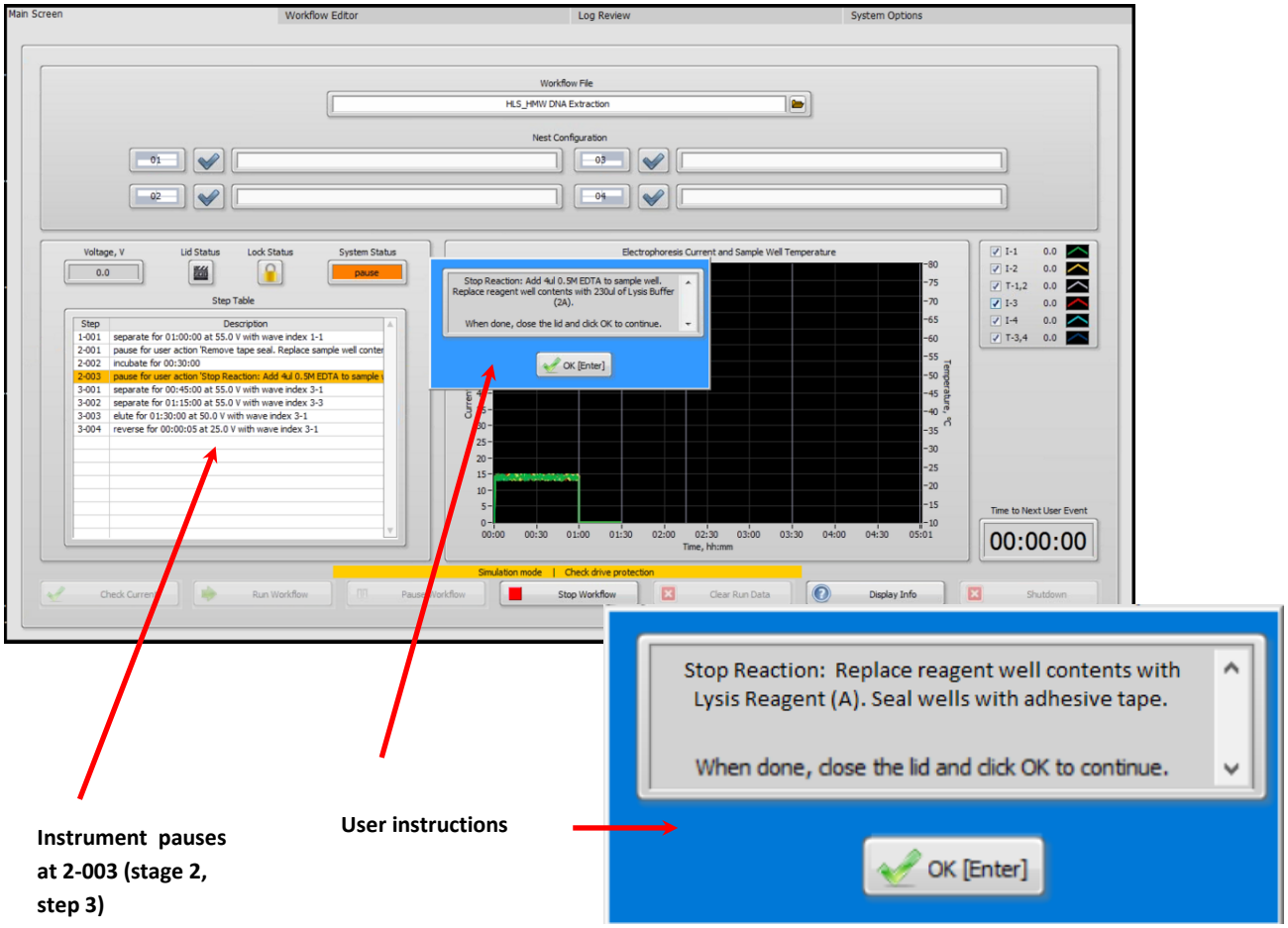
8. Press “OK” in the pop-up window to resume the workflow.



Press “OK” to resume

9. The enzymatic treatme

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

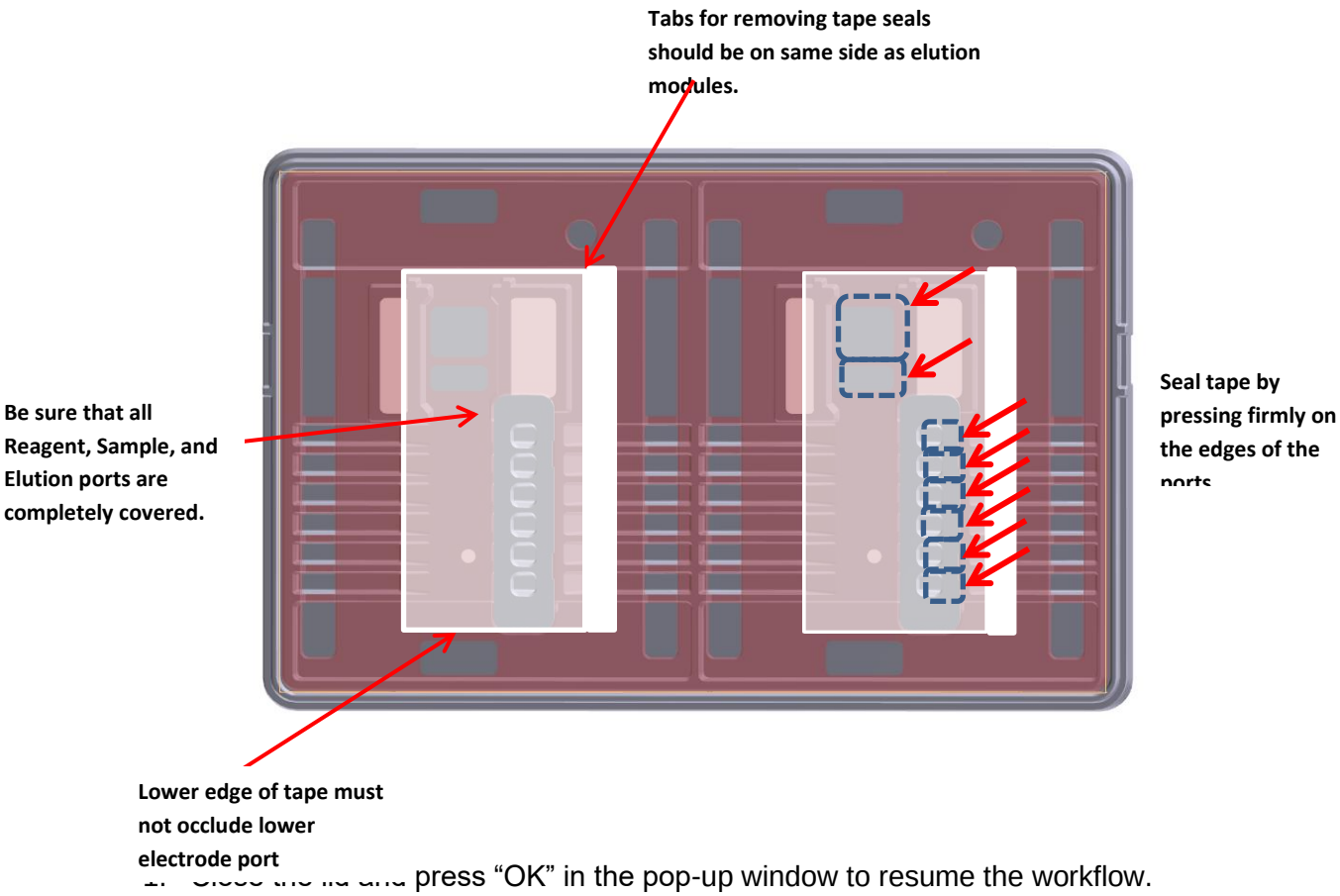


Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user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

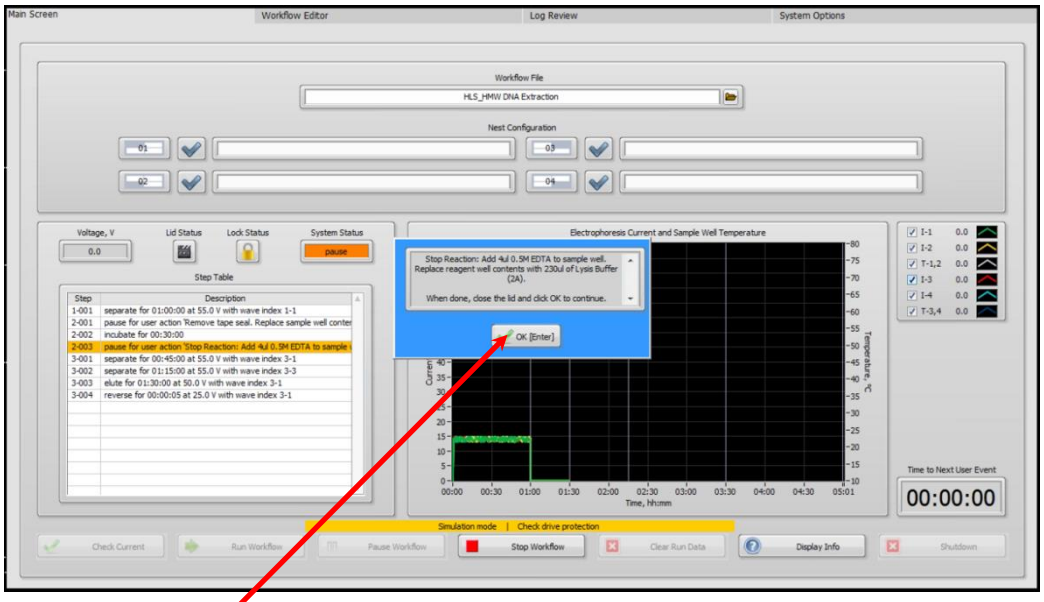
11. Open the lid and remove the contents of the Reagent well.

12. Replace the Reagent well contents with Lysis Reagent (**A1**), ~230 ul.

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

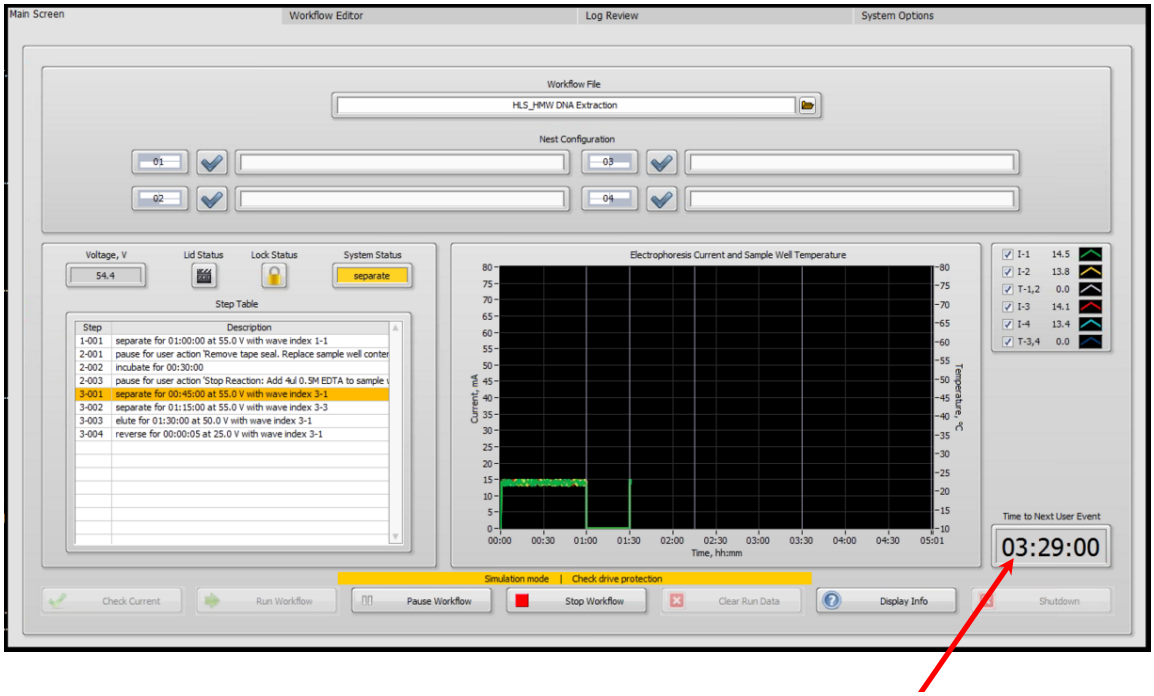


press “OK” in the pop-up window to resume the workflow.



G. Stage 3: Collection Stage

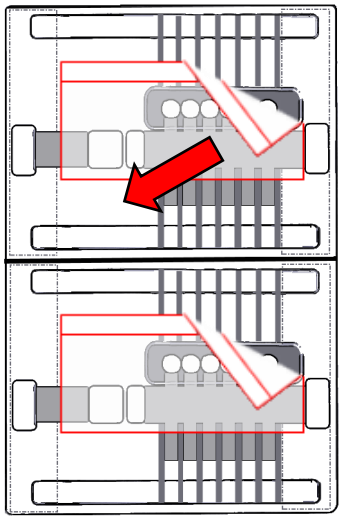
1. The Collection Stage will require several hours of unattended operations. Users should note the time remaining, after which the samples can be collected.



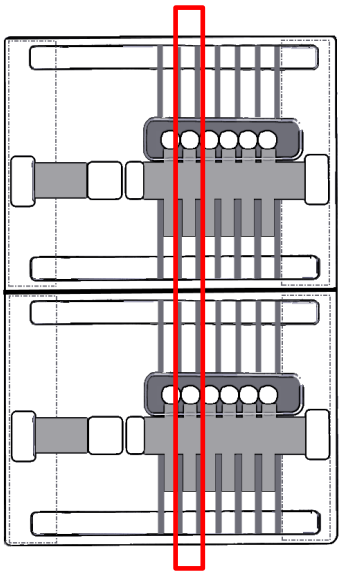
Time remaining to the end of the run

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

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



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

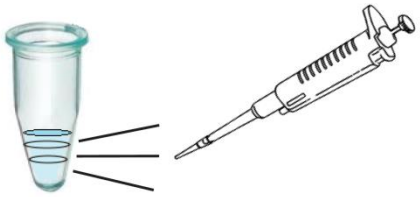


Workflows are designed to collect most targets in well #2

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

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

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



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