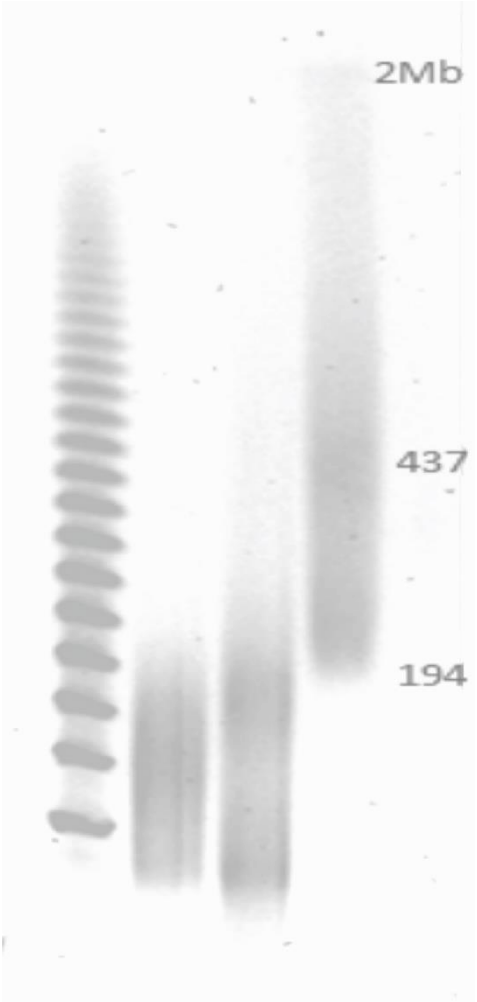
sage science

HLS Cassette Kit
Workflow Guide

High Molecular Weight DNA Extraction

PN# HEX-0004 or HEX-0012



Important! Read carefully before starting!

Note: This workflow guide is to be used with either the HLS or HLS2 instrument platforms. In this document both instruments will be referred to as "HLS".

A. Cell Suspension vs Cell Nuclei Preparation

Whole cell suspensions or cell nuclei may be used for HMW extractions. Nuclei from lymphoblastoid cells are very stable and can be frozen before use. If using whole cells, check that viability is >85%

B. One week before running the HLS:

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**
-or- (for partial restriction) Msp1 and Mse1	New England Biolabs	**R106S and R0525S**

C. One day before running the HLS:

Schedule availability of cells one day before running the HLS:

- Recommended input load of cells or nuclei should contain 5 µg of genomic DNA in a volume of 70ul. 5µg of human genomic DNA is equivalent to about 1.5 m diploid cells.

D. Morning of the HMW Extraction:

- Check that cell/nuclei preparation reagents are ready (PN# CEL-MWB1). Prepare cells or nuclei according to the Cell Suspension and Preparation of Nuclei from Mammalian WBC from Whole Blood guide (https://sagescience.com/product-support/sagehls-support/).

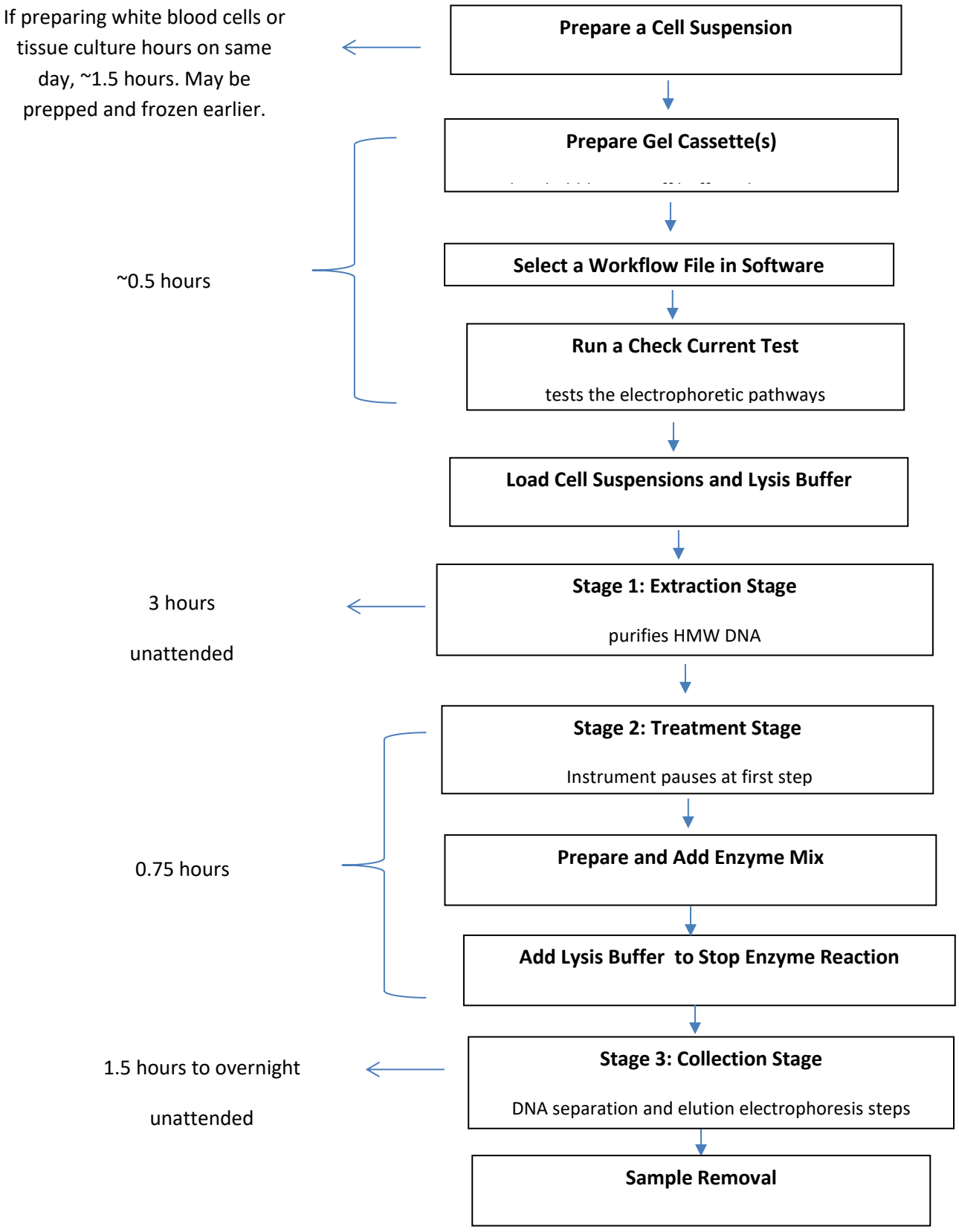
A. **The following items are supplied Cassette Kits (HEX-1004 and HEX-1012).** When the kit is received, open immediately to inspect the contents. Contact Sage Science if any items are missing or damaged

Note the storage conditions and store accordingly.

#	Item	Label	Storage Temp.
4 / 12 ea.	Agarose gel cassettes		RT
20 / 60 ea.	Adhesive Tape Strips		N/A
1 ea.	HLS Lysis Reagent 1% SDS, EDTA, 10 / 30 ml*	H	RT
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

*Use 1% SDS for nuclei preps and 3% SDS for cell suspensions

HMW DNA Extraction Workflow Summary

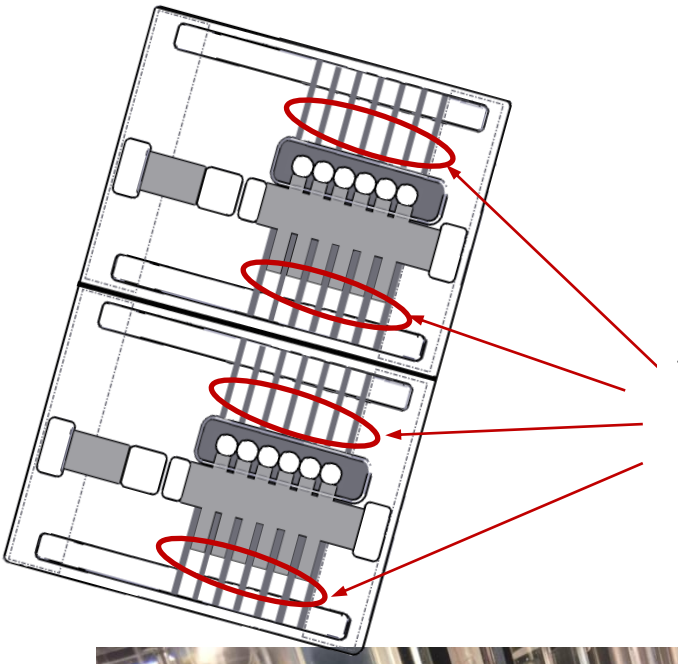


A. Prepare cells using Cell Suspension Guide

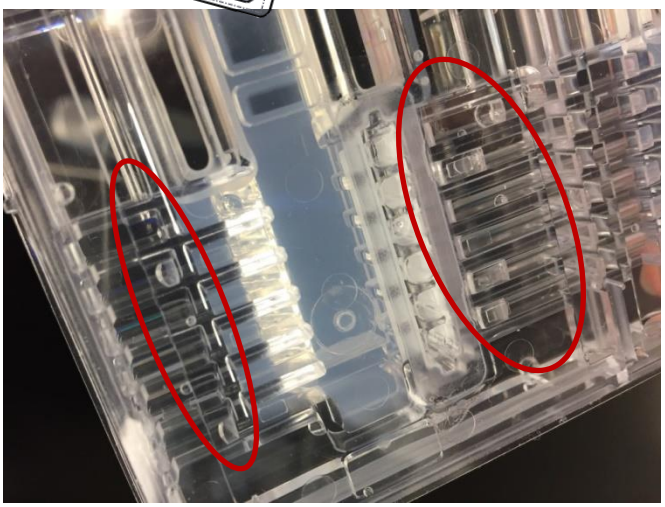
B. Prepare the Gel Cassette(s)

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.



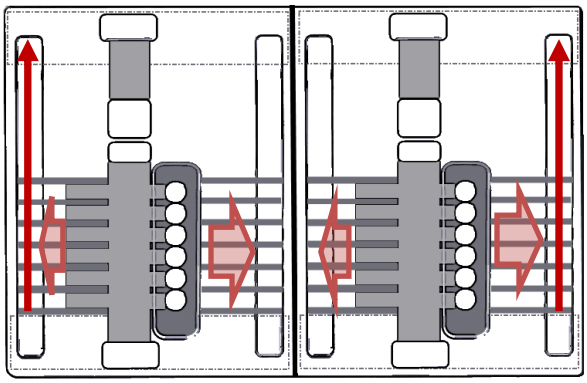
Tap to dislodge bubble from these areas into the troughs directly above.



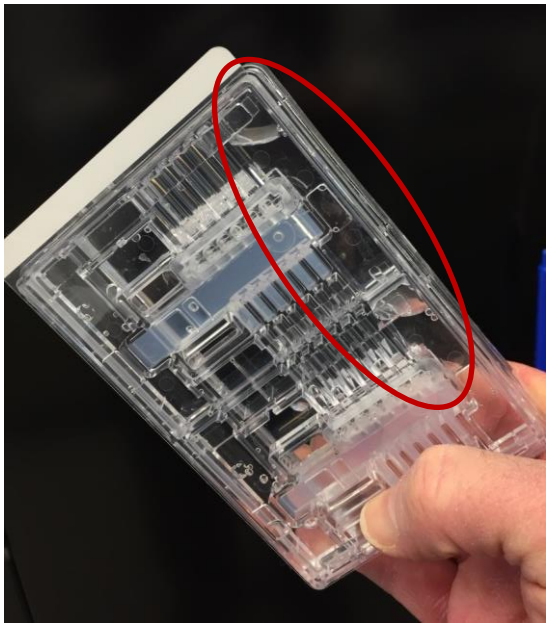
Bubbles in the elution paths can interfere with collection

3. Slowly rotate the cassette to allow the bubbles to collect in the upper buffer area. Tap the cassette to dislodge bubbles.

4. Repeat tapping to dislodge any bubbles in the elution pathways and aggregate the bubbles into the corners of the cassette in the upper buffer chambers.



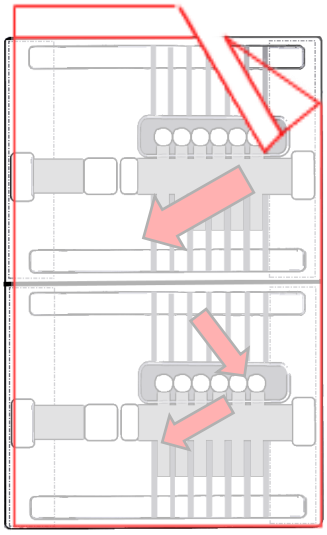
Move any bubbles to the upper buffer area.



Aggregate and collect air bubbles in the upper buffer chambers

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

6. 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.

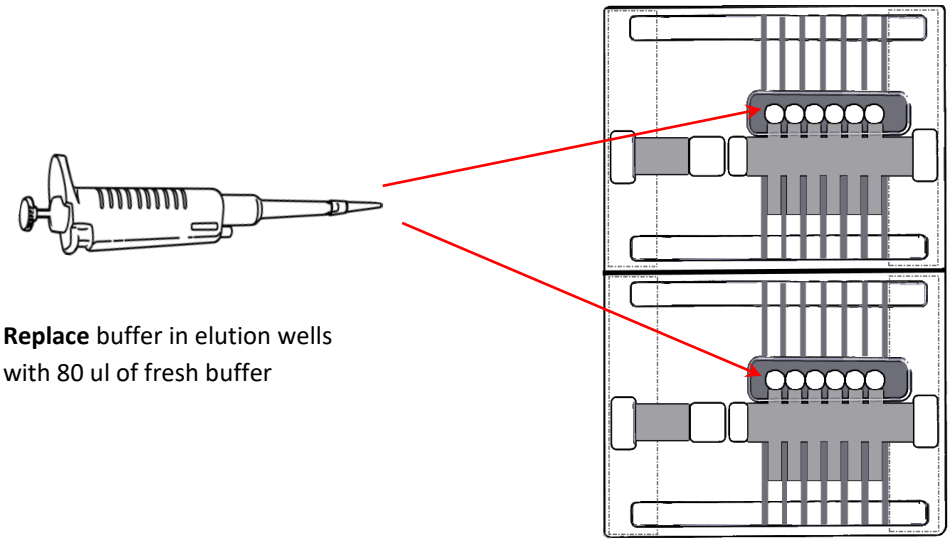


Peel back tape at an angle.

Alternate angles if the tape resists peeling.

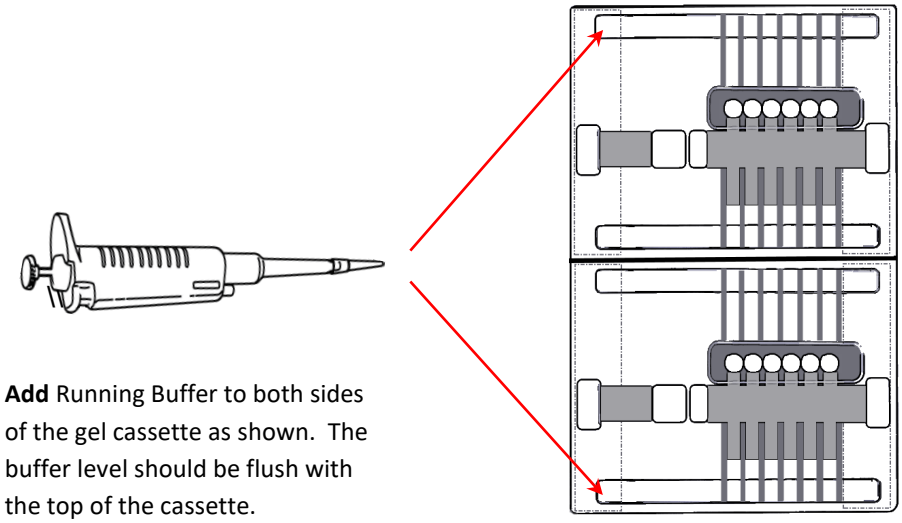
5. Remove all buffer from all elution wells (set a pipette to 100 µl to completely empty the wells). Keep pipette tips vertical in the well to avoid damage to the membranes.

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



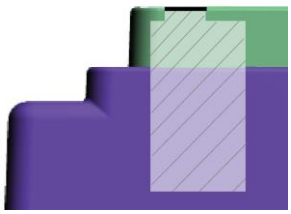
Replace buffer in elution wells with 80 ul of fresh buffer

7. 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 HLS.



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

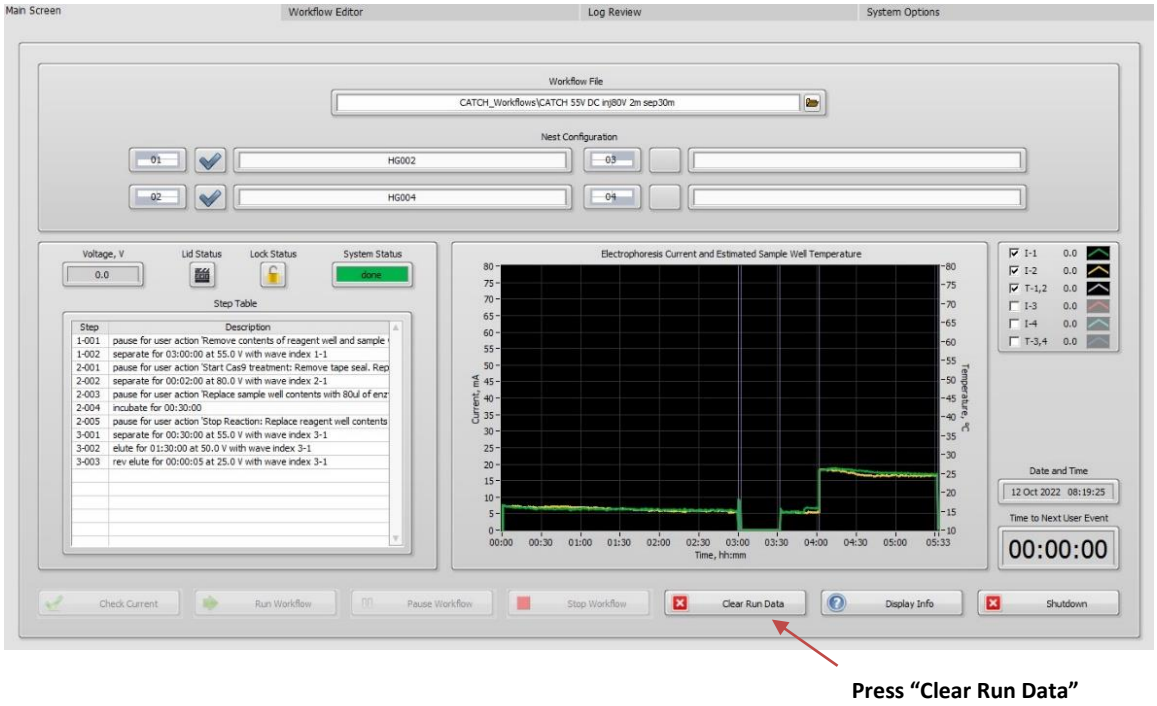
Fill buffer until it is flush with the top of the cassette



Important! Fill until the buffer level until it is flush with the top of the cassette cover.

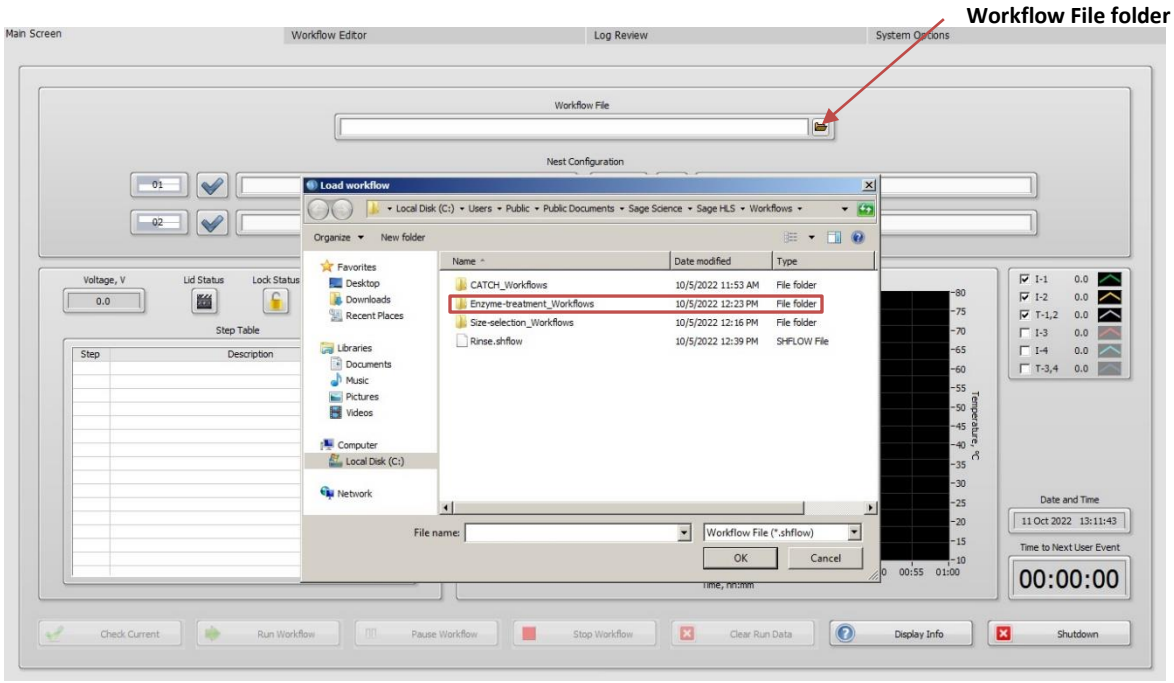
C. Load the Workflow File

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



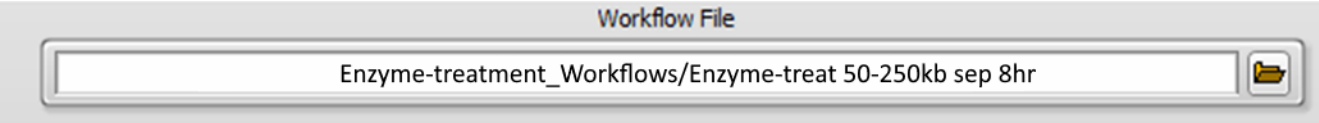
2. Select the Workflow File folder icon.

   A workflow file directory window will open, select the folder labelled ‘Enzyme Treatment Workflows”



3. Select the file named “Enzyme-treat 50-250kb sep 8hr”. It will appear in the Workflow File window.

   This protocol uses a collection waveform that is designed to collect DNA fragment above 250kb as a compression.

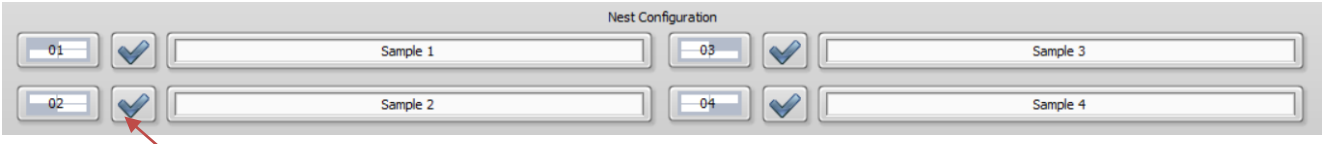


Note: Workflow names include characterization of the DNA collection profiles. In the example above, “50-250kb” indicates the range within which DNA fragments will be well-resolved electrophoretically. Fragments above 250 kb, and those below 50 kb, will move as a compression through the gel column during the Collection Stage. “sep 8hr” indicates that the Collection Stage will require approximately 8 hours of run time. Users may replace the Collection Stage parameters in the Workflow Editor if they choose to experiment with the collection conditions. Collection Stage options are shown and described in the Table below.

Collection Stage	Region of good resolution	Onset of HMW compression	Voltage	Waveform*
5-100kb sep2.5h	5-100kb	100kb	80V	25 10 25 10 5 2 21
20-200kb sep3h	20-200kb	240kb	55V	150 50 30 10 3 1 81
50-200kb sep4h	50-200kb	240kb	55V	150 50 30 10 3 1 81
100-300kb sep3h	50-240kb	300kb	55V	150 50 60 20 6 2 81
100-300kb sep4h	100-300kb	340kb	55V	150 50 60 20 6 2 81
50-250kb sep8h	50-250kb	~300kb	37V	1000 333 240 80 0 0 101
340-1000kb sep3h	340-1000kb	>>1000kb	55V	3000 1000 2550 850 0 0 24
600-2000kb sep8h	600-2000kb	>>2000kb	55V	3000 1000 2550 850 0 0 24

* Waveform definitions (all in milliseconds): initial F time, initial R time, initial F increment, initial R increment, incr. to F incr., incr. to R incr., number of F/R cycles until return to initial conditions.

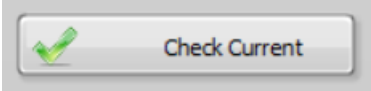
4. Choose the lanes to be used by clicking the boxes next to the lane numbers and enter sample IDs into the adjacent fields (sample IDs are optional or can be entered later).



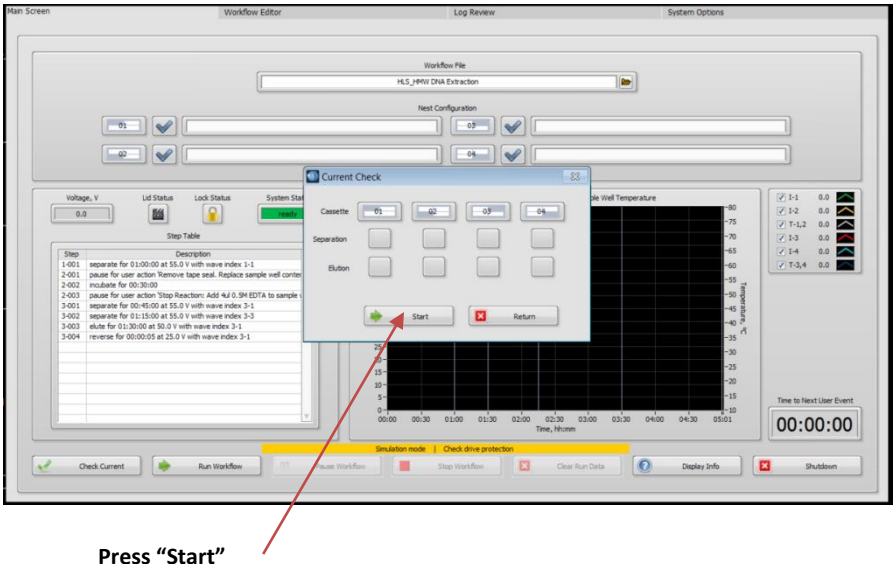
Check marks indicate which lanes are active

D. Run the Check Current Test

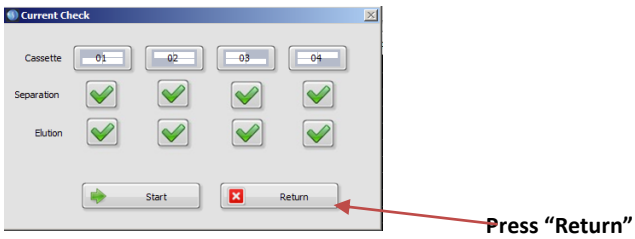
1. Press the “Check Current” button.



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

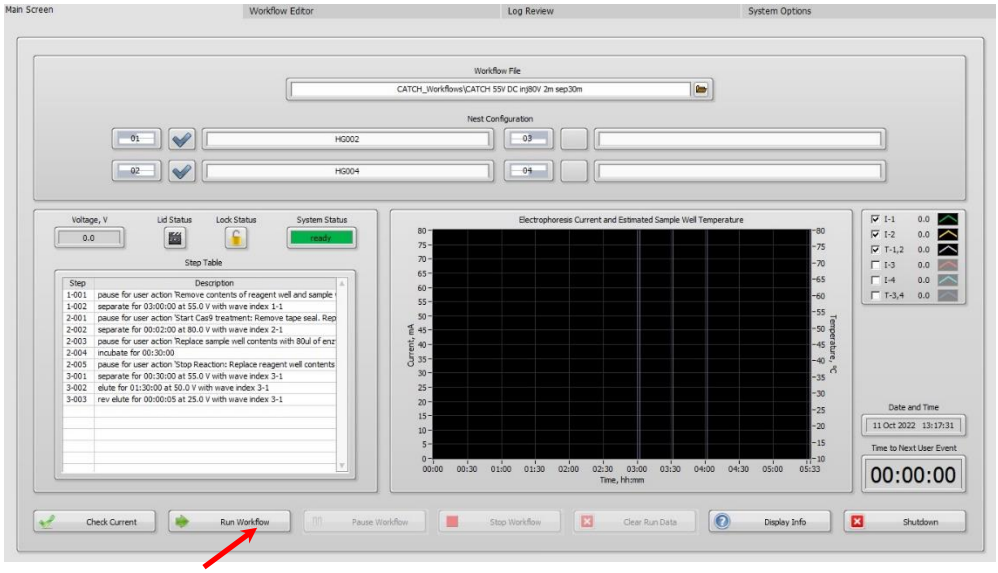


3. The routine will first test the separation electrodes, then test the elution electrodes, and complete within a few minutes. After a successful test, all boxes will fill with green check marks. Press “Return” to continue



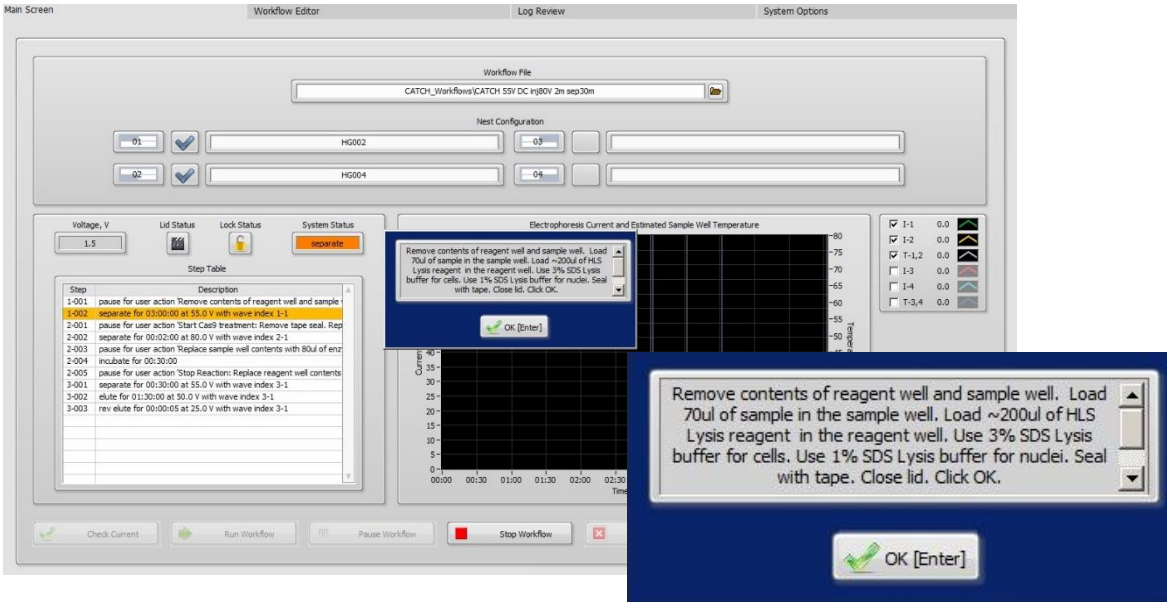
E. Stage 1: Extraction

1. When the current test is complete, press “Run Workflow”



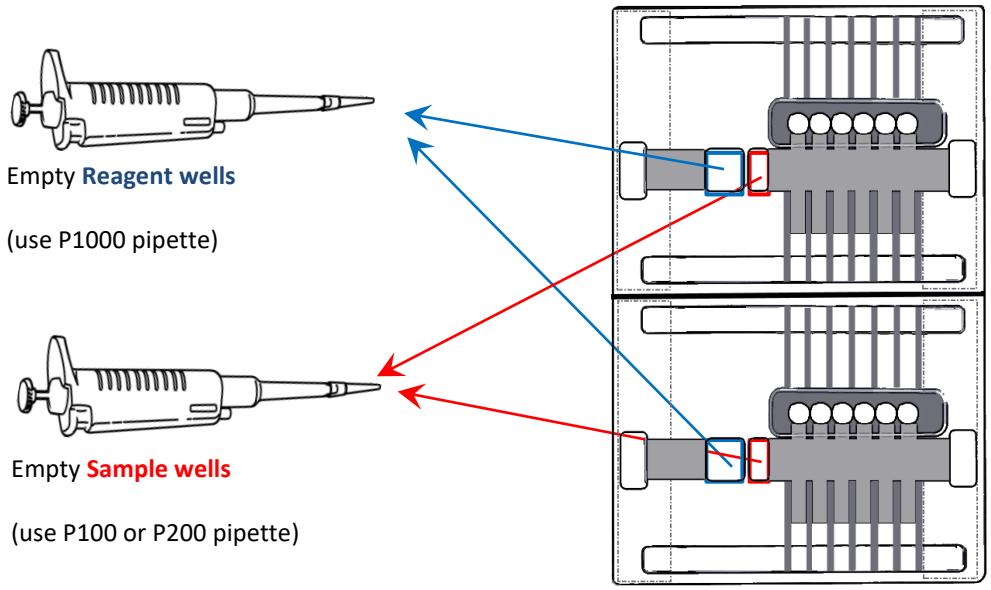
Press “Run Workflow”

1. A window will pop-up describing the next instructions to be undertaken:



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.

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

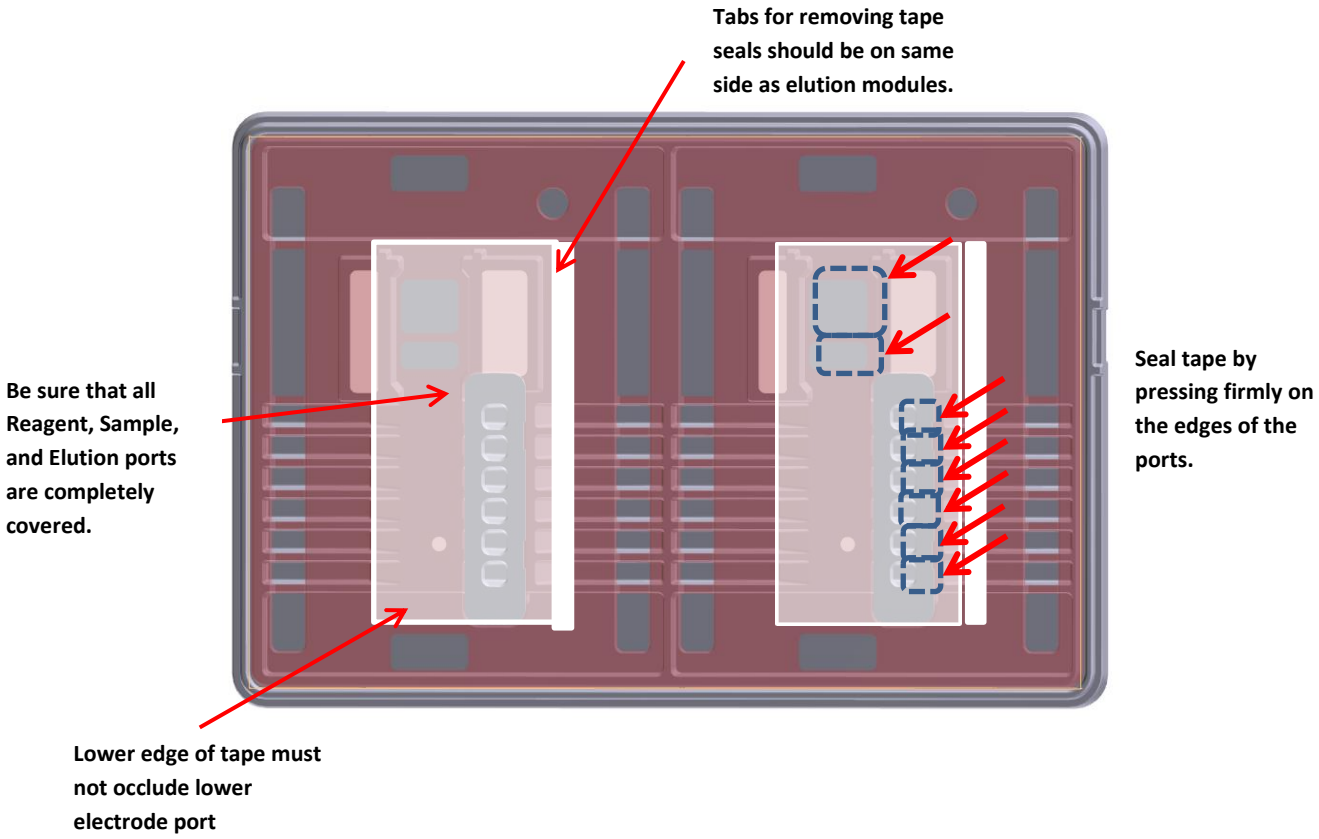


4. Load samples (cells or nuclei) in all lanes. Use a **70ul sample loading volume**. (Sample wells will not be completely full.)

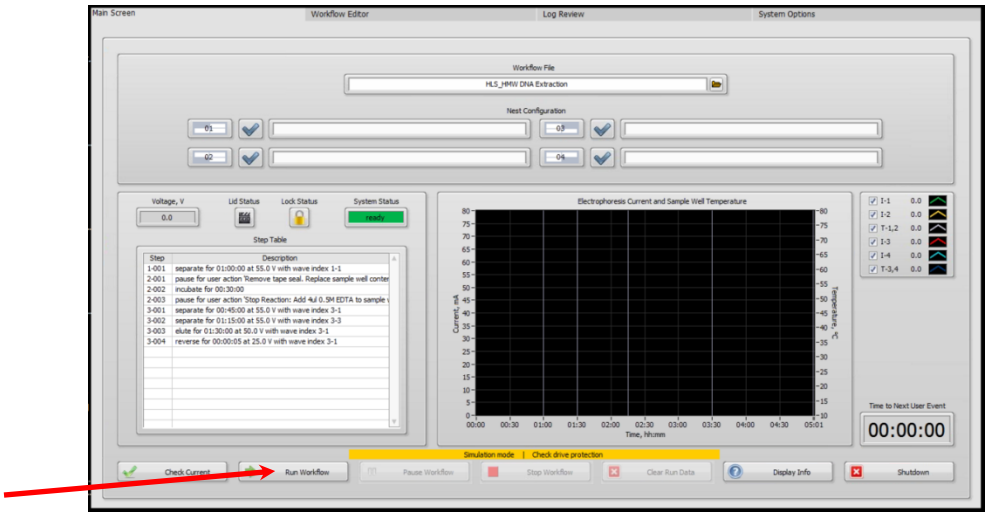
5. **Add 200 µl of HLS Lysis Buffer** to the reagent Wells with. For extractions with nuclei use HLS Lysis Reagent 1% SDS (**H**). For extractions with cells, use HLS Lysis Reagent 3% SDS (**A1**).

Important! Do not overfill ! Leave a concave meniscus to prevent contact with sealing tape in next

4. Seal reagent, sample, and elution ports with supplied tape without occluding the electrode ports. Press tape firmly around edges of the ports. Use an object with a smooth round tip if possible (the pointed end of a 15 ml conical tube is useful for this task).

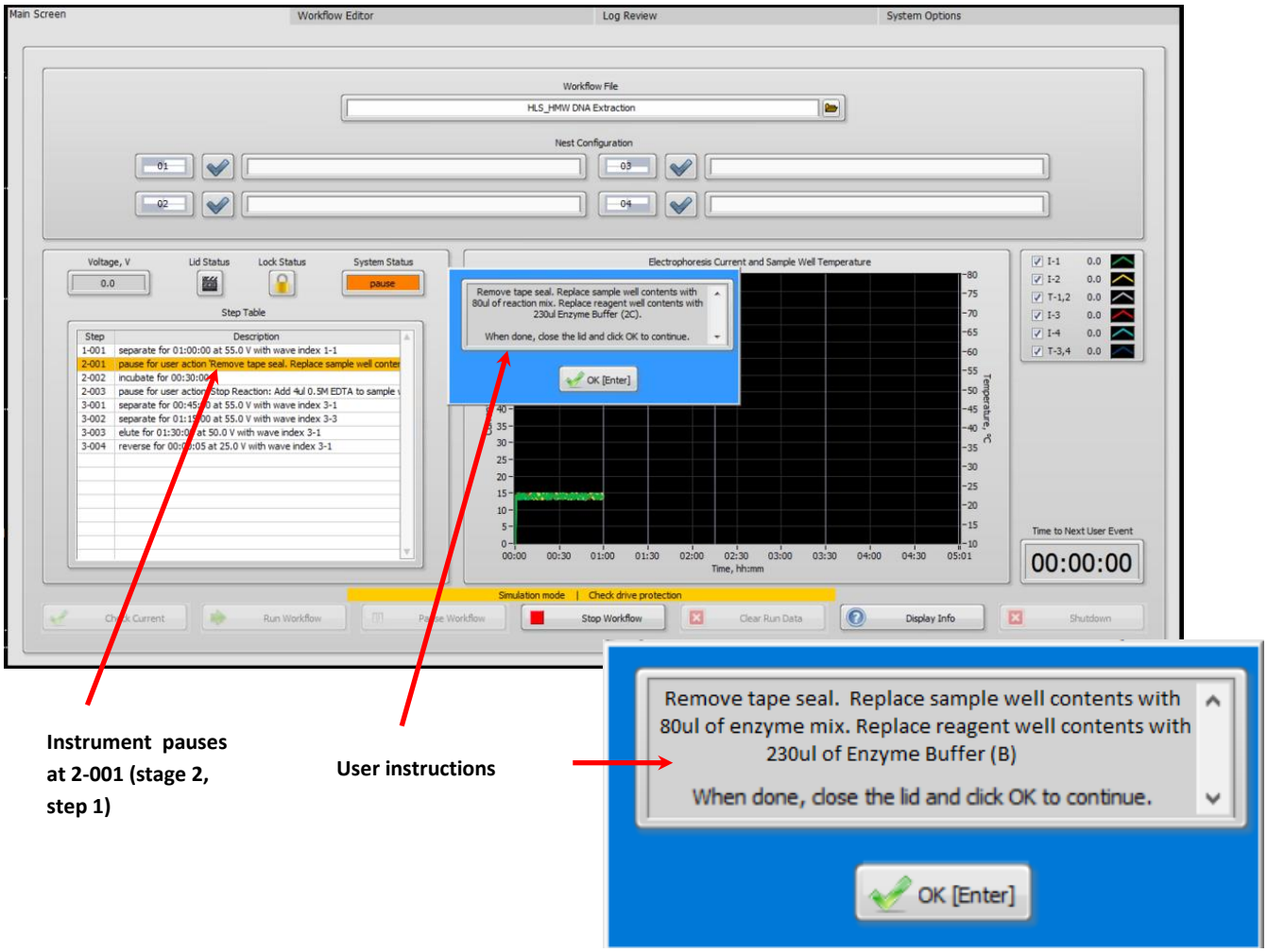


5. Close the lid and press “Run Workflow”. The Extraction step will take 3 hours of unattended operation.



F. Stage 2: Treatment Stage

1. At the end of the Extraction Stage/Step, the HLS 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 or B below):

A: For Fragmentase:

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

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

Dil.Factor	Fragmentase	Procedure
1:400	0.2µl / 80µl reaction	i. add 2µl of NF to 800µl of Enzyme Buffer (**C**) ii. mix gently by pipetting

.

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.

B: For Partial Restriction (Msp1 and Mse1 Restriction Enzymes)

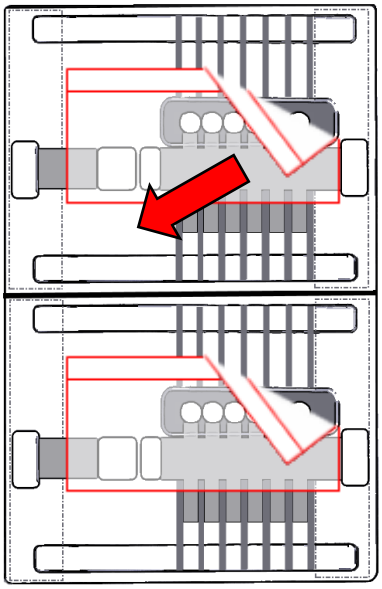
a. Remove stock enzymes from the freezer and keep on ice.

b. Dilute the Restriction Enzymes with Enzyme Buffer (**C**) as follows:

Dil.Factor	Fragmentase	Procedure
1:400*	0.50 µl / 80µl reaction	i. add 1.1µl of Msp1 and 1.1µl Mse1 to 350µl of Enzyme Buffer (**C**) ii. mix gently by pipetting, place on ice

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.

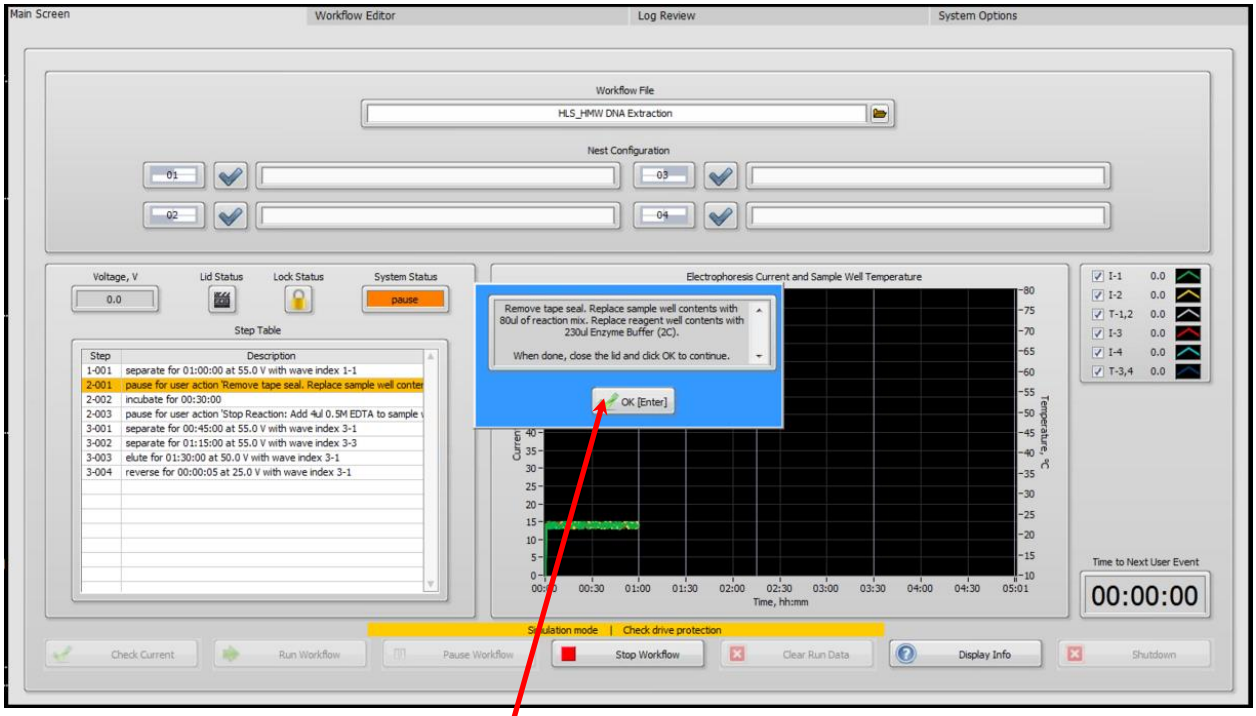
4. Remove <u>all</u> contents from the Reagent wells and Sample wells in the cassettes to be run. The well volumes are 270 µl and 85 µl, respectively. Take care not to damage the agarose walls or floor fo the wells with the pipette tip.

5. Fill the Reagent Well with Enzyme Buffer (**C**) (approx. 220 µl).

6. Add 80 µl of the Fragmentase Enzyme Mix or Partial Restriction Mix to the sample well.

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

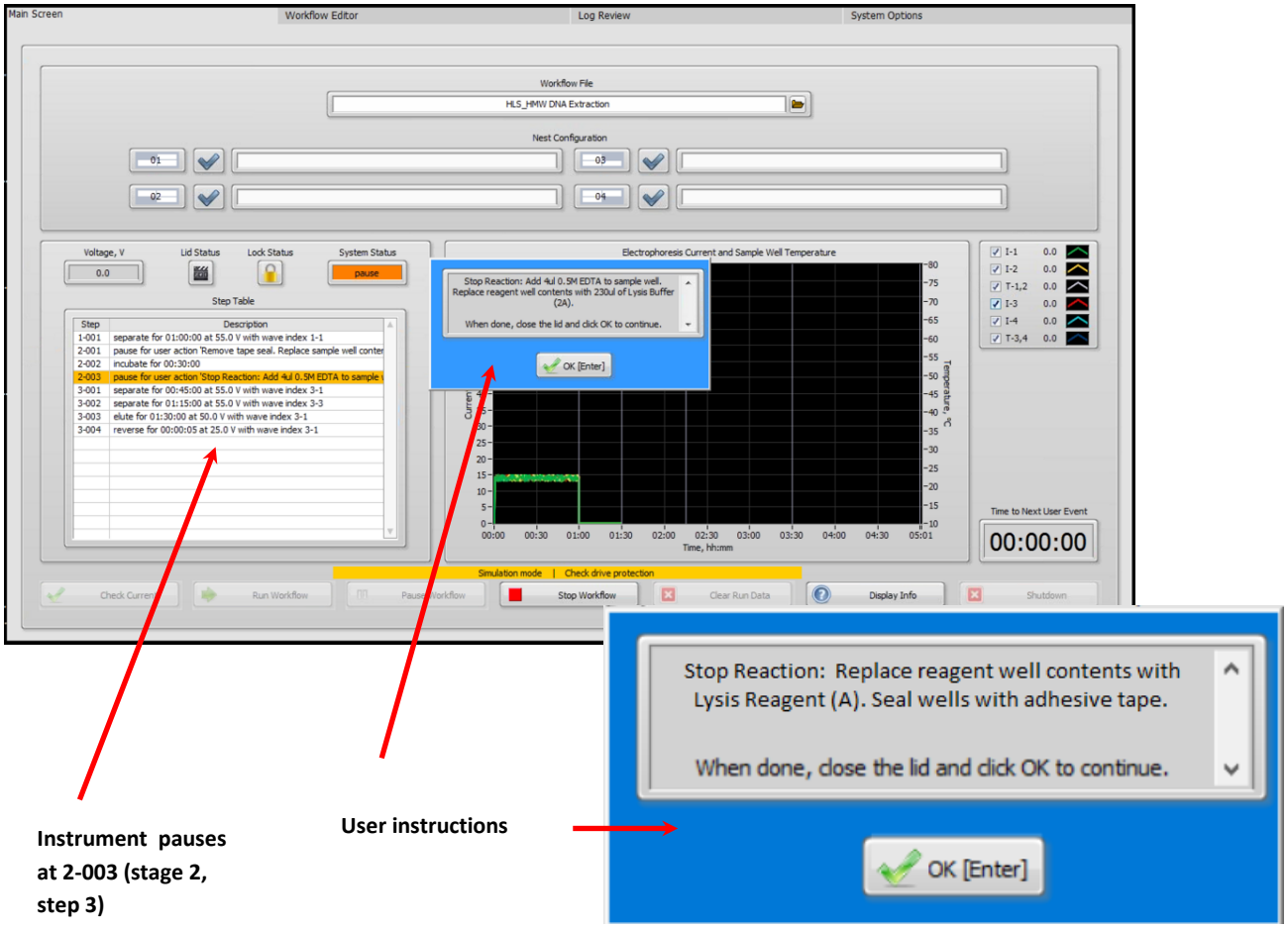
8. Press “OK” in the pop-up window to resume the workflow.



Press “OK” to resume

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

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

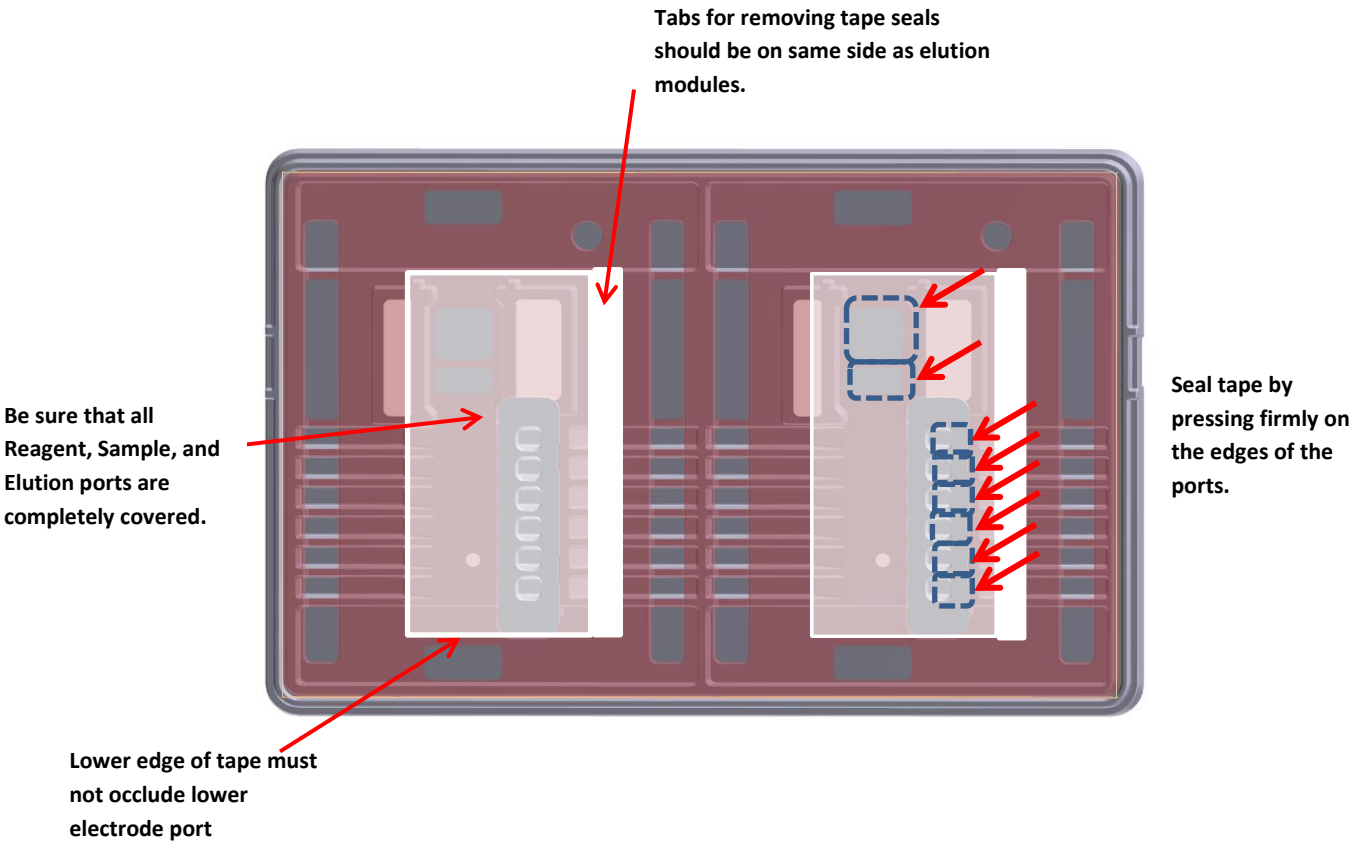


Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user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

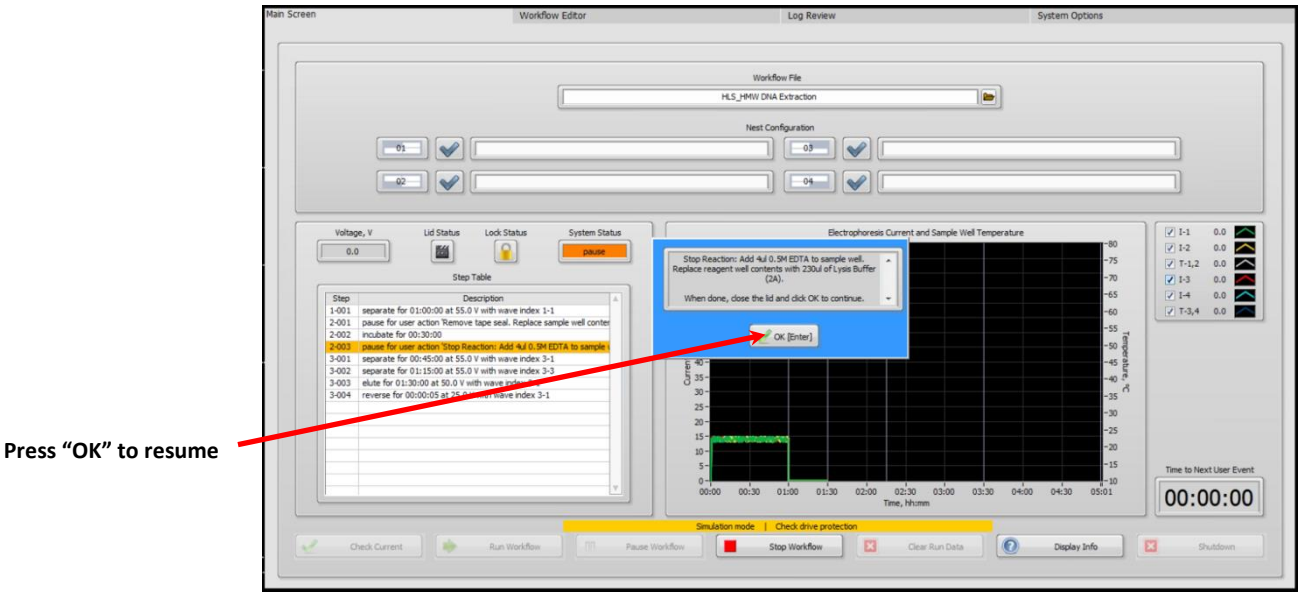
11. Open the lid and remove the contents of the Reagent well.

12. 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 200-210 µl.

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

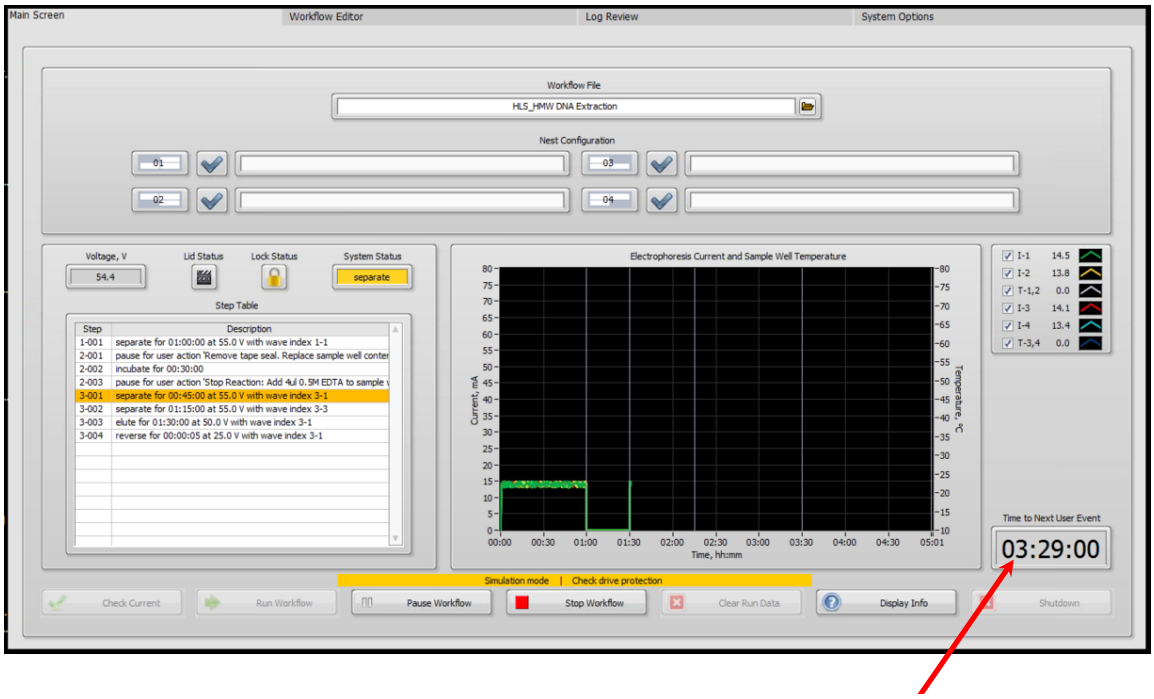


13. Close the lid and press “OK” in the pop-up window to resume the workflow.



G. Stage 3: Collection Stage

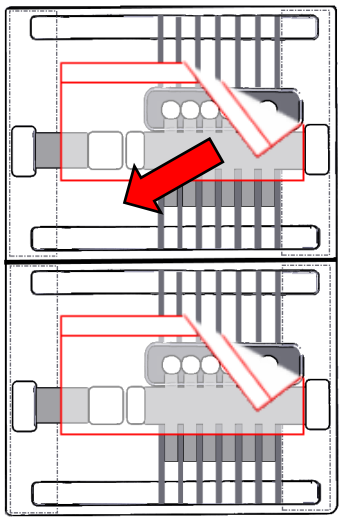
1. The Collection Stage will require several hours of unattended operations. Users should note the time remaining, after which the samples can be collected.



Time remaining to the end of the run

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

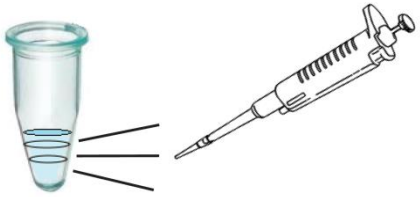
grab the tab in right upper corner and peel diagonally with a slow smooth motion



3. 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

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
J	K	1/27/22	all	SageHLS replaced with “HLS” to indicate both SageHLS and HLS2 instruments
K	L	4/15/22	2	Add restriction enzymes to fragementase as an extraction method
K	L	4/15/22	2	Input load maximum changed from 8-10 ug to 7 ug
K	L	4/15/22	5	Change Treatment stage from 0.5 hrs to 0.75 hrs And Collection stage 3 hrs to 1-5 hours - overnight
K	L	4/15/22	7	Expand description of dislodging and collecting bubbles
K	L	4/15/22	11	The approx.. lysis buffer needed (step 3) is changed from 220-230 to 200 – 210 ul.
K	L	4/15/22	11	Add dilution factor for restriction enzymes (Msp1 and Mse1) for partial restriction fragmentation method.
K	L	4/15/22	15	Step 4 add note to take care to not puncture agarose in wells. Reversed steps 5 and 6.
K	L	4/15/22	16	Step 12 add note to take care to not puncture agarose in wells
K	L	4/15/22	19	Removed Step 3 (note about CATCH products and adjacent wells).
L	M	11/28/22	13	Fixed typo in fragmentation dilution factor (0.02 ul to 0.2ul)
L	M	11/28/22	3,11	Added 1% SDS lysis reagent (**H**) for extracting DNA from Nuclei.
L	M	11/28/22	8,9	Adjust the workflow file convention