sage science

HLS Cassette Kit
Workflow Guide

HLS2 and SageHLS:

Ultra HMW DNA Size Selection

PN# HSS-0004 or HSS-0012

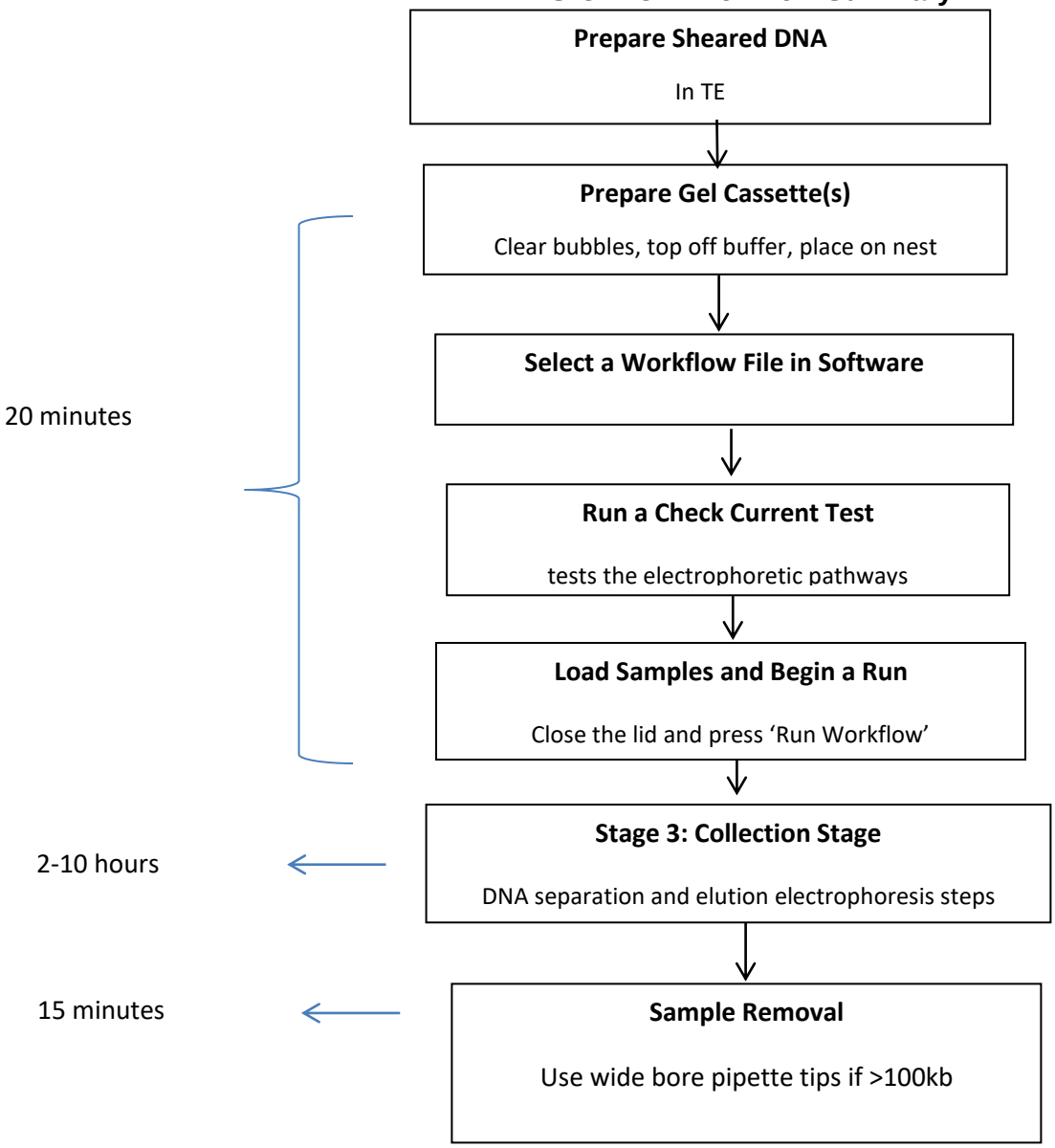
Note: This workflow guide is to be used with either the HLS or HLS2 instrument platforms. In this document both instruments will be referred to as “HLS”.

The following items are supplied Cassette Kits (HIT-0004 and HIT-0012). 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.	Running Buffer, 40 / 115 ml	E	RT
1 ea.	Loading Solution, 1000 / 1000 ul		RT

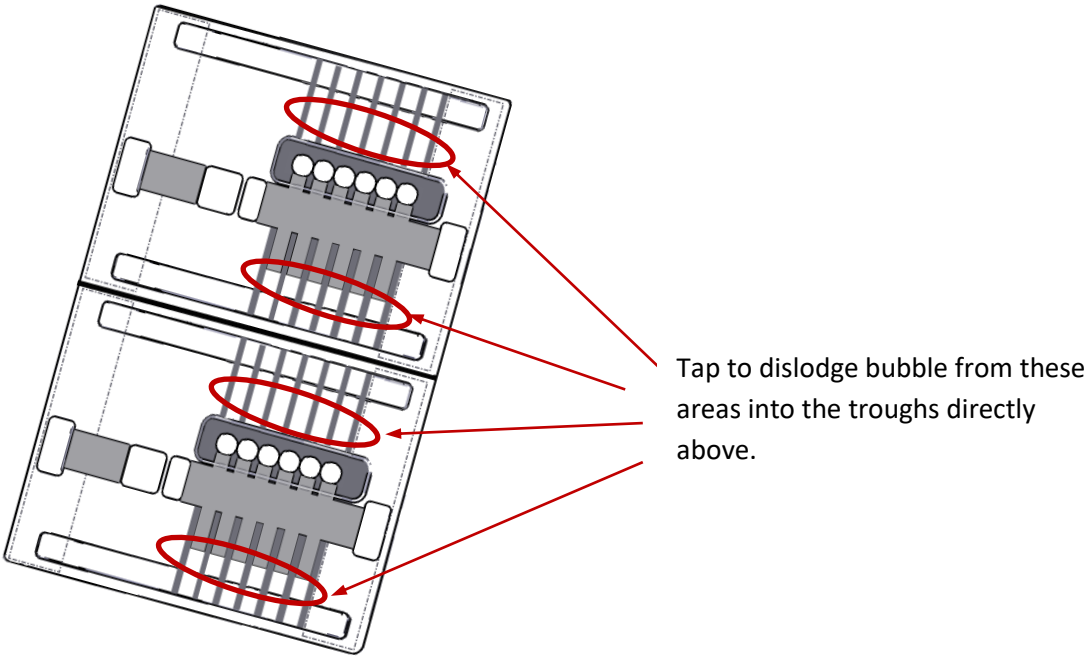
HLS-CATCH Workflow Summary

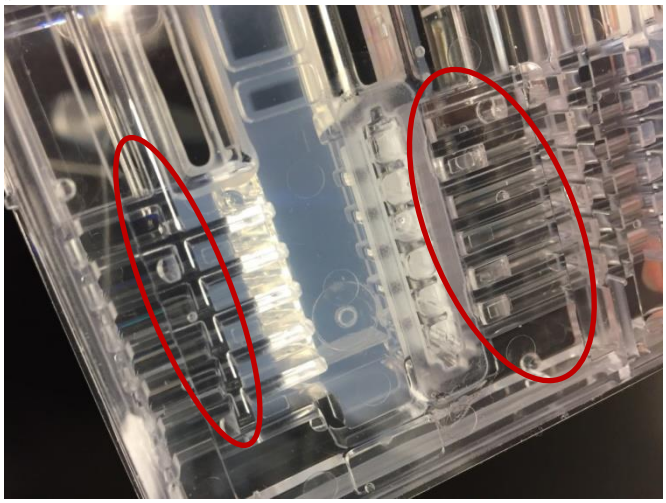


A. Prepare the Gel Cassette(s)

1. Remove the gel cassette from the foil bag.

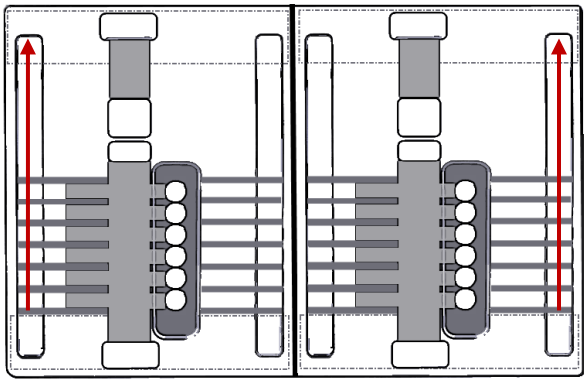
2. **Before removing tape!** – Hold the cassette with top surface almost vertical and the elution modules above the gel channel. Tap the cassette to dislodge any bubbles that are trapped behind the elution wells, or in the elution channels. Repeat if necessary. Allow the bubbles to collect in the electrode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



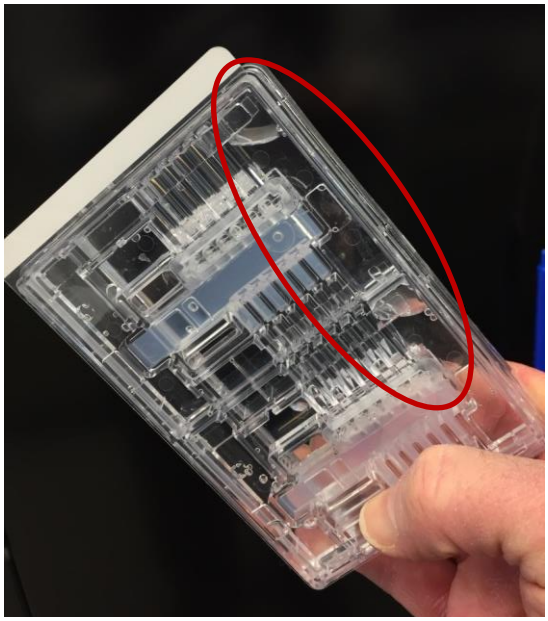


Bubbles in the elution paths can interfere with collection

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



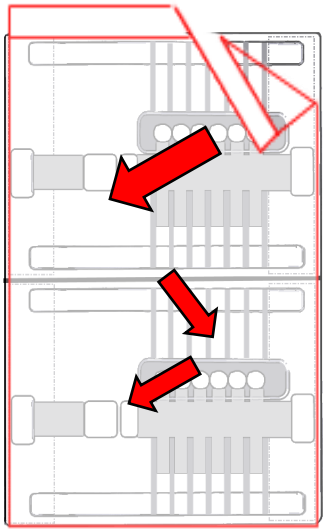
Move any bubbles to the upper buffer area.



Aggregate and collect air bubbles in the upper buffer chambers

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

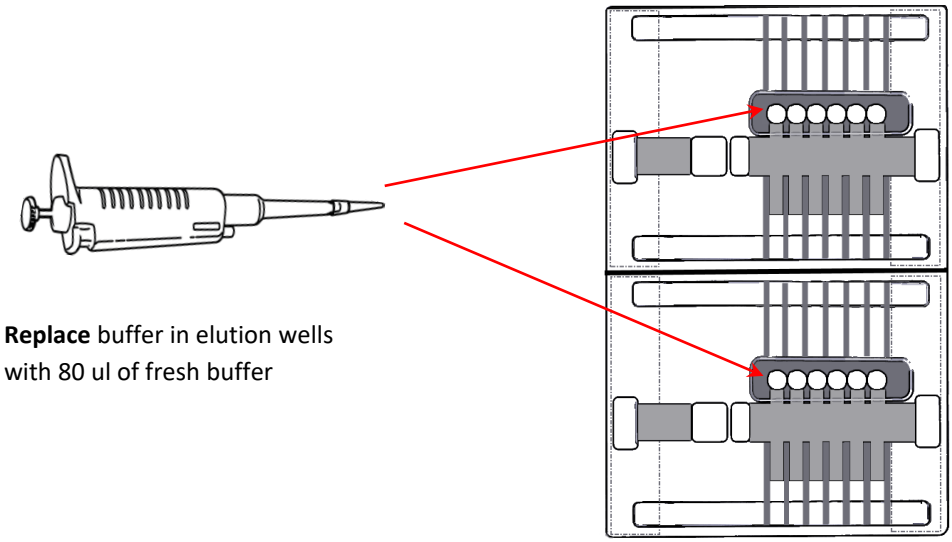
5. While holding the cassette firmly in place on the nest, grab the tape tab, and pull the tape off at an angle, slowly and firmly. Alternate the pulling angle if the tape resists peeling.



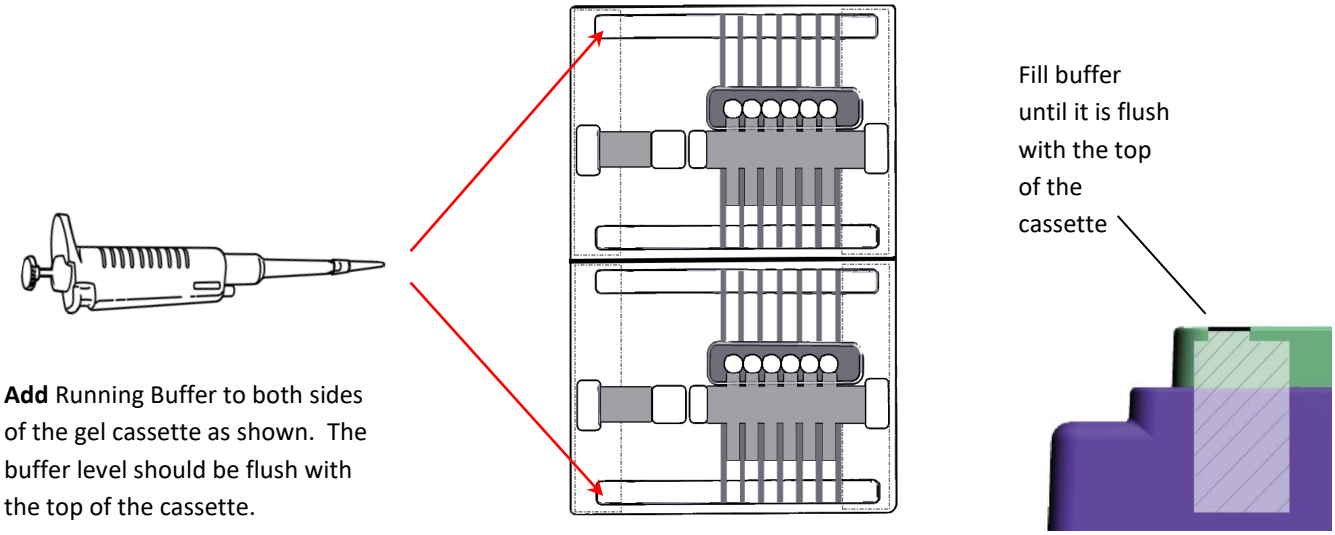
Peel back tape at an angle. Alternate angles if the tape resists peeling.

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

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



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 HLS.



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

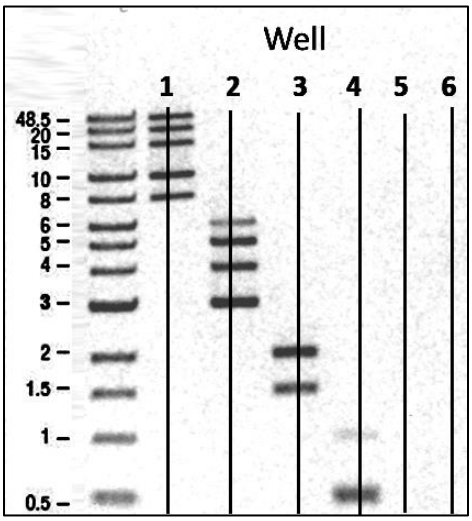
B. Guidelines for Selecting a Size Selection Workflow

Size Selection Workflow files reside in a folder labelled “Size-Selection_Workflows”. A workflow file must be loaded into the HLS instrument software before proceeding to the next step (current test of the cassette). The following pages provide guidelines for selecting a size selection workflow that best suits experimental requirements.

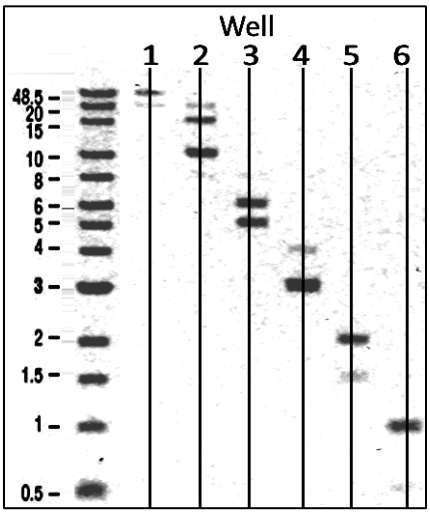
Direct Current Protocols (2-50 kb)

When using continuous field electrophoresis (DC voltage), electrophoretic resolution of DNA fragments from 500bp to approximately 20-30kb can be well-resolved under certain voltage and run time combinations. In general, lower voltages (<50V) and longer run times will increase the resolution (separation of DNA of different size). For these small fragments, we have tested size resolution by running DNA marker ladders at different separation times at 55V DC, eluting the separated fragments and analyzing the contents of each elution module on an analytical agarose gel.

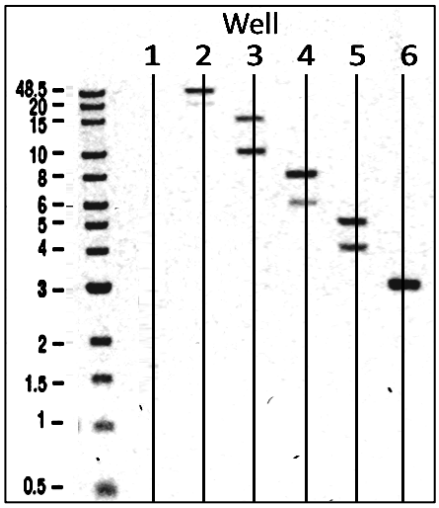
DNAs larger than about 20-30kb tend to migrate at the same rate, termed the limiting low mobility (for those specific electrophoresis conditions). This phenomenon is familiar to most molecular biologists, and results in a high molecular weight compression band near the top of the gel lane when running high molecular weight genomic DNA that has been digested with a rare-cutting enzyme, or gently sheared.



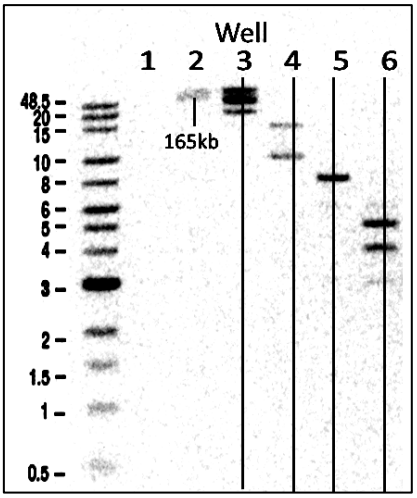
Workflow: Size-select 55V DC 30m



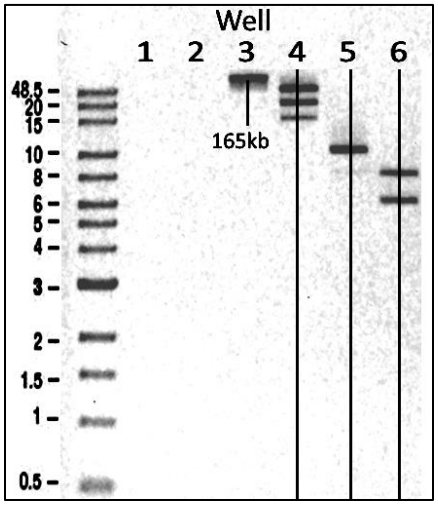
Workflow: Size-select 55V DC 45m



Workflow: Size-select 55V DC 60m



Workflow: Size-select 55V DC 75m



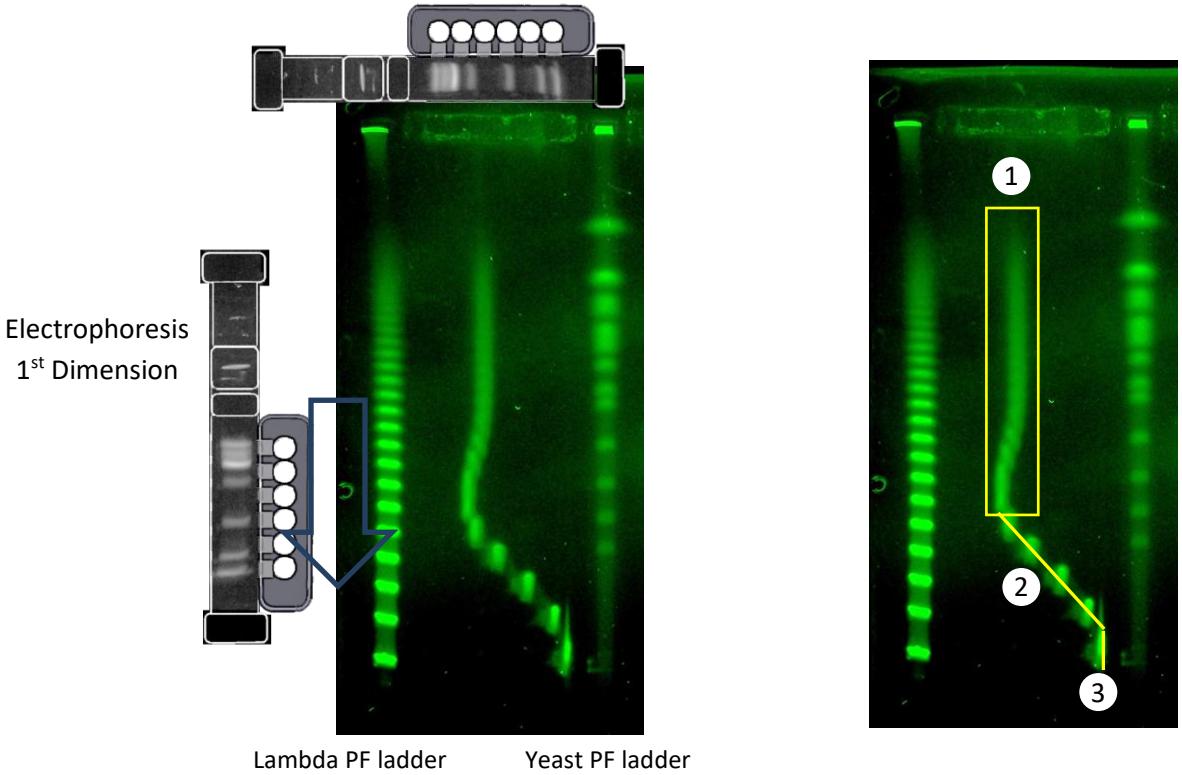
Workflow: Size-select 55V DC 90m

Summary of Direct Current Workflows

Workflow name	Elution time	Region of good resolution	Onset of HMW compression	Voltage	Waveform
Size-select 55V DC 30m	90m	0.5-5kb	6-8kb	55V	Continuous field
Size-select 55V DC 45m	90m	1-10kb	~20kb	55V	Continuous field
Size-select 55V DC 60m	90m	3-15kb	~20kb	55V	Continuous field
Size-select 55V DC 75m	90m	6-20kb	~40kb	55V	Continuous field
Size-select 55V DC 90m	90m	8-20kb	~50kb	55V	Continuous field

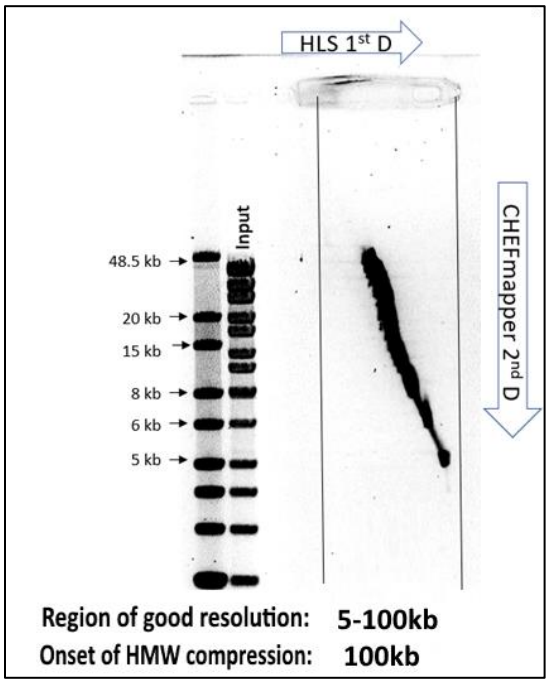
Pulsed-Field Protocols (5 kb – 2 MB)

Electrophoretic resolution of large DNA fragments can be improved through the use of field-inversion pulsed field (PF) electrophoresis. However, this style of electrophoresis can result in unexpected elution profiles. For instance, for a given pulsed field program, there will usually be a range with good electrophoretic resolution, bracketed by high and low molecular weight compression regions where there is little or no resolution, as shown below. To develop useful PF conditions, we have developed a two-dimensional analytical electrophoresis procedure in which a sample of phage lambda DNA concatemers is electrophoresed in an HLS lane without elution. The cassette is cut open and the HLS gel lane is removed and cast into the sample well of a high-resolution Bio-Rad CHEFmapper gel. The CHEFmapper gel is run under conditions where linear separations of DNA up to 2Mb in size can be accomplished. This procedure allows us to unambiguously determine the range of linear DNA resolution along with the positions of HMW and LMW compression.

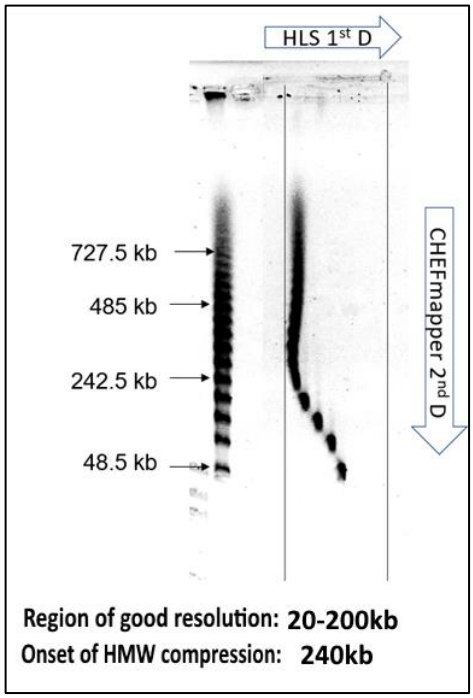


1. HMW compression (with mobility inversion)
2. Size Range with good resolution
3. LMW compression

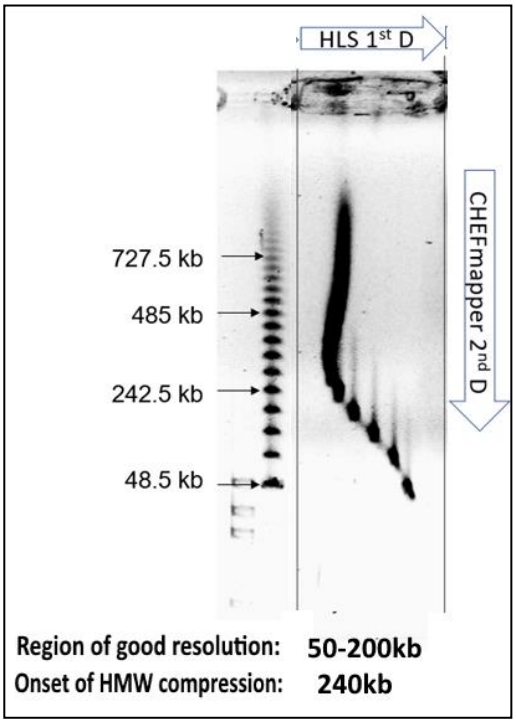
Use the following guidelines to determine the best collection stage for a given application.



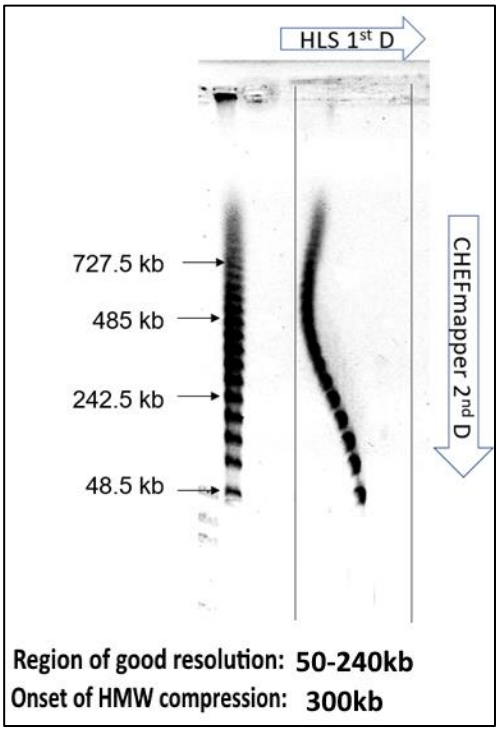
Workflow: Size-select 5-100kb sep2.5h



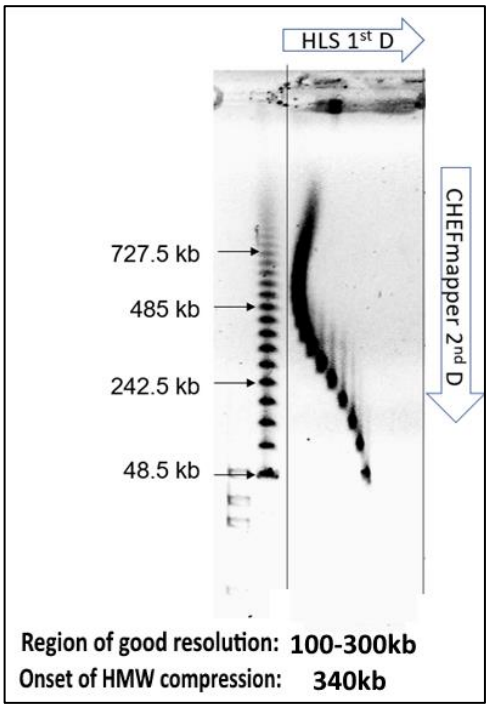
Workflow: Size-select 20-200kb sep3h



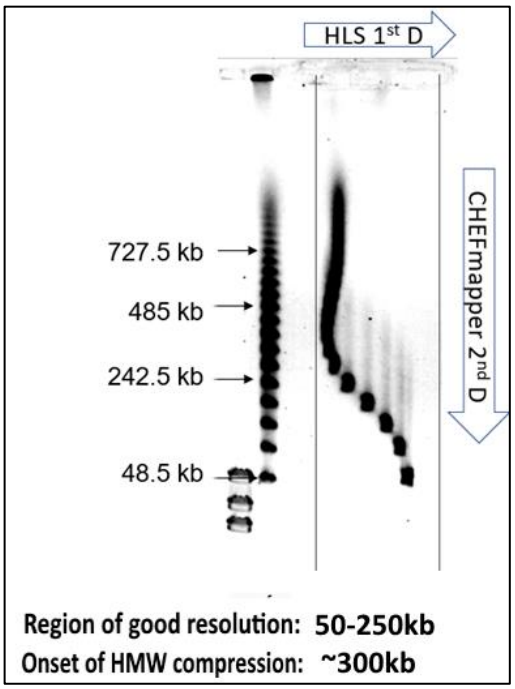
Workflow: Size-select 50-200kb sep4h



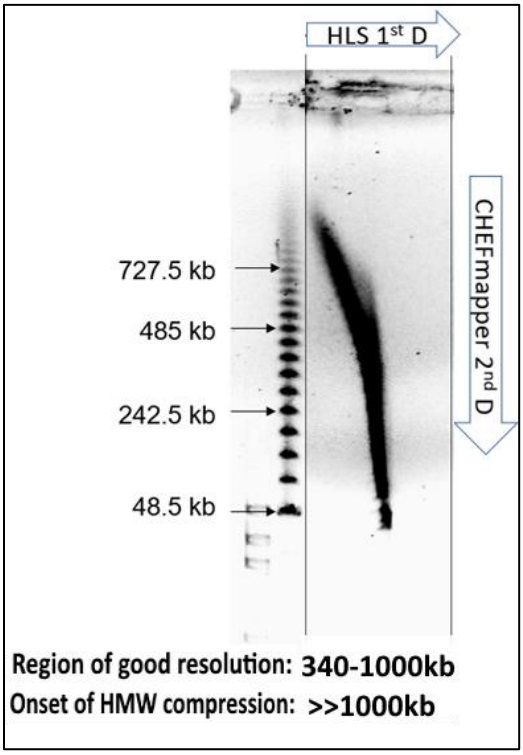
Workflow: Size-select 100-300kb sep3h



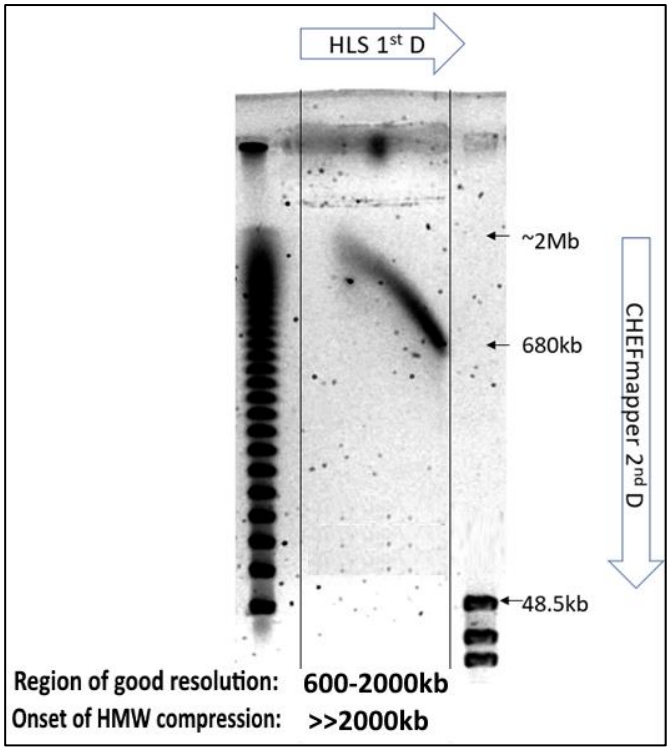
Workflow: Size-select 100-300kb sep4h



Workflow: Size-select 50-250kb sep8h



Workflow: Size-select 340-1000kb sep3h



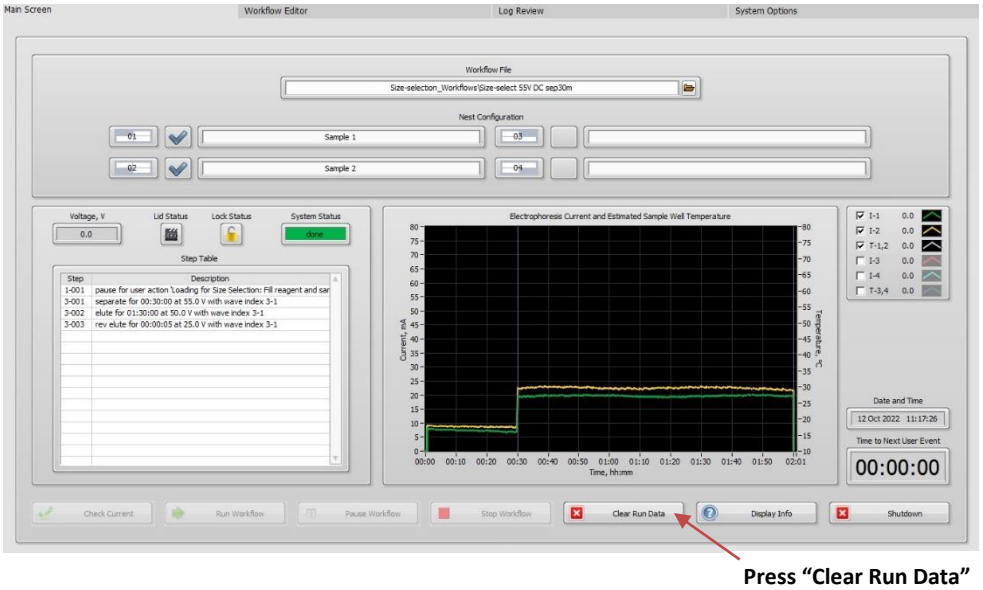
Workflow: Size-select 600-2000kb sep8h

Summary of Pulsed-Field Workflows

Workflow name	Elution time	Region of good resolution	Onset of HMW compression	Voltage	Waveform**
Size-select 5-100kb sep2.5h	90m	5-100kb	100kb	80V	25 10 25 10 5 2 21
Size-select 20-200kb sep3h	90m	20-200kb	240kb	55V	150 50 30 10 3 1 81
Size-select 50-200kb sep4h	90m	50-200kb	240kb	55V	150 50 30 10 3 1 81
Size-select 100-300kb sep3h	90m	50-240kb	300kb	55V	150 50 60 20 6 2 81
Size-select 100-300kb sep4h	90m	100-300kb	340kb	55V	150 50 60 20 6 2 81
Size-select 50-250kb sep8h	90m	50-250kb	~300kb	37V	1000 333 240 80 0 0 101
Size-select 340-1000kb sep3h	90m	340-1000kb	>>1000kb*	55V	3000 1000 2550 850 0 0 24
Size-select 600-2000kb sep8h	90m	600-2000kb	>>2000kb*	55V	3000 1000 2550 850 0 0 24

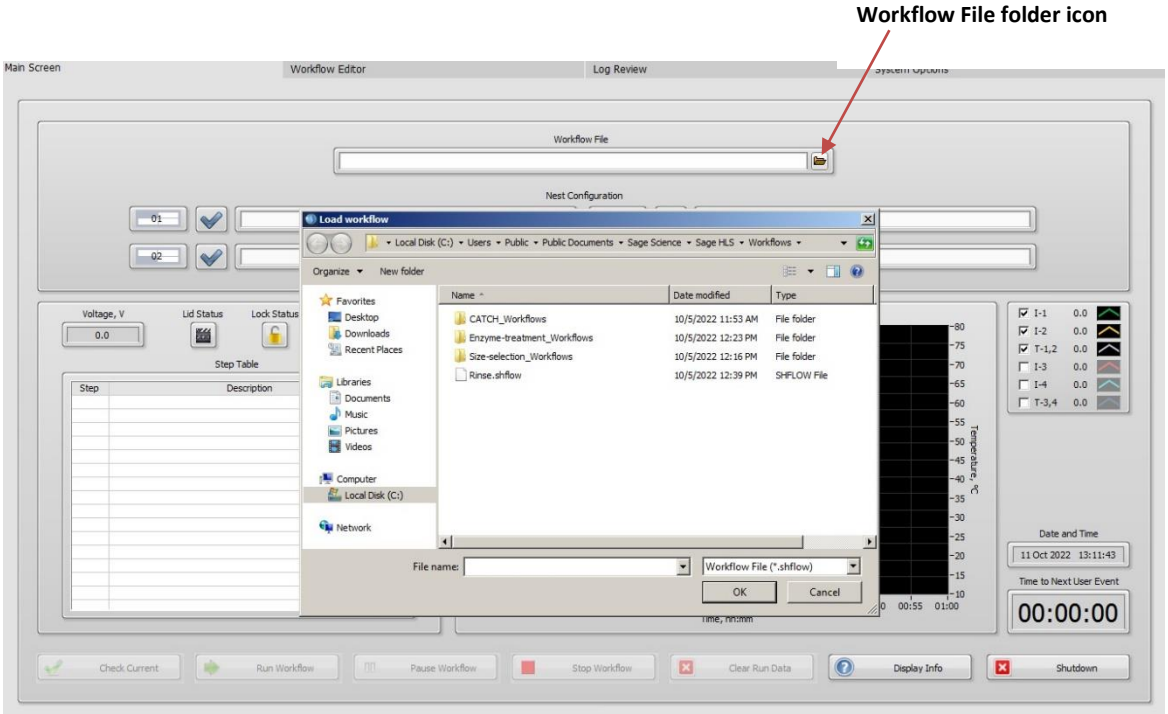
C. Load the Workflow File

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.

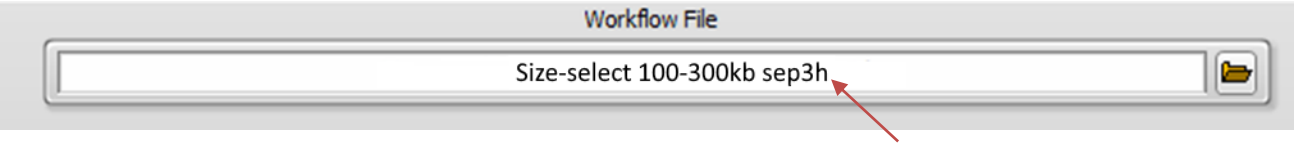


2. Select the Workflow File folder icon.

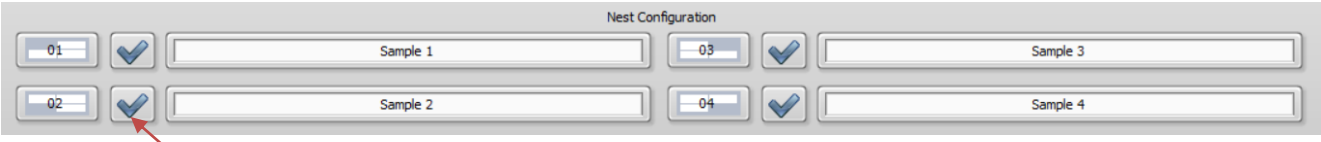
   A workflow file directory window will open, select the “Size-Selection_Workfow”. From the list of workflow, select the workflow file that was selected based on the guidelines provided on the previous pages.



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



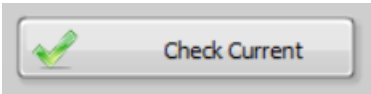
4. Choose the lanes to be used by clicking the boxes next to the lane numbers and enter sample IDs into the adjacent fields (sample IDs are optional or can be entered later).



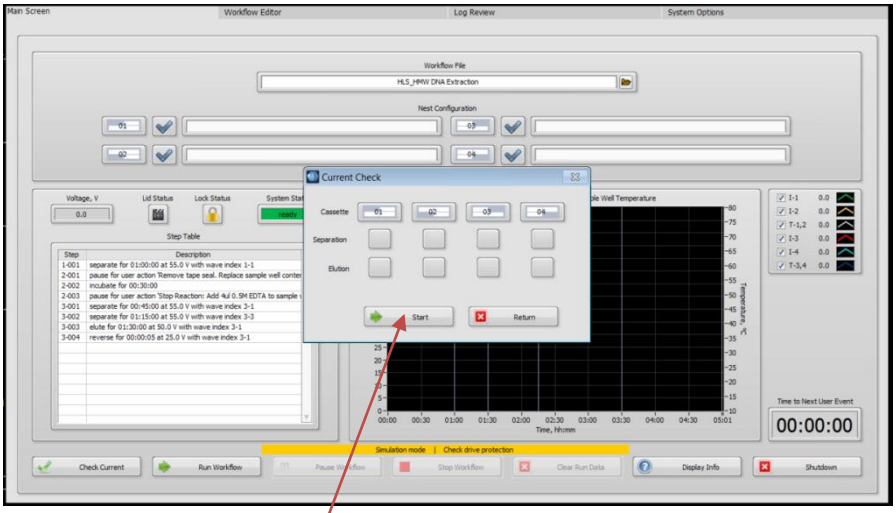
Check marks indicate which lanes are active

D. Run the Check Current Test

1. Press the “Check Current” button.

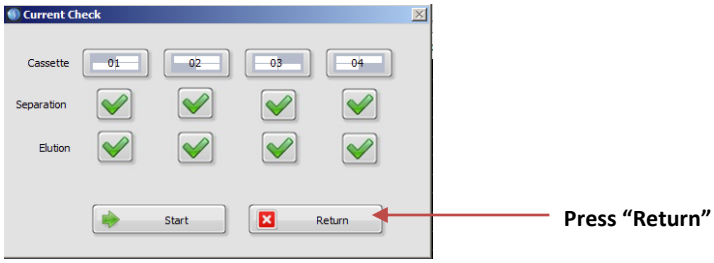


2. A pop-up window will appear. Press “Start” to begin the Check Current routine.



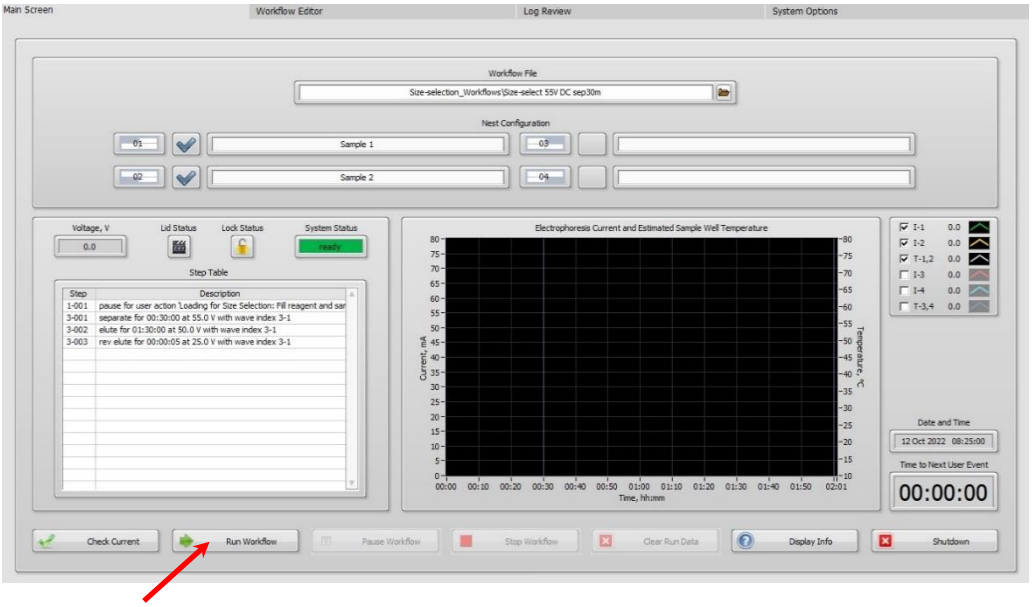
Press “Start”

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

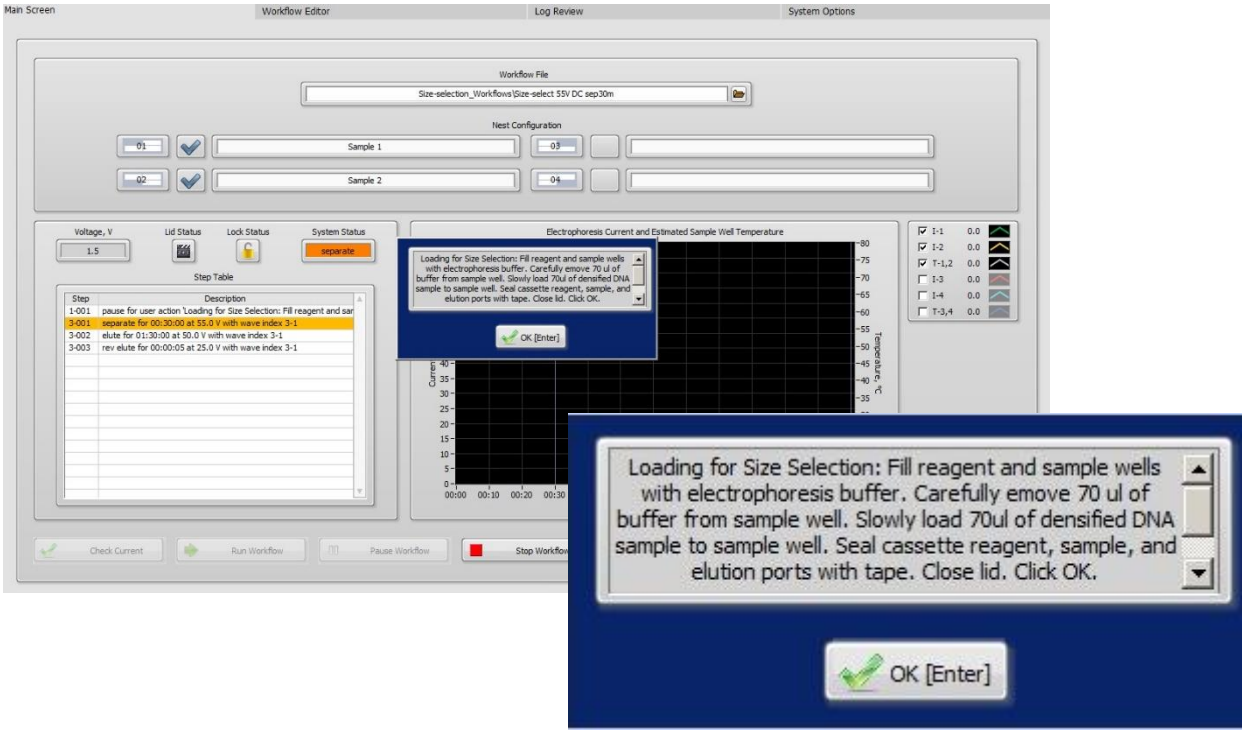


E. Start the Workflow

1. Press “Run Workflow”.



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

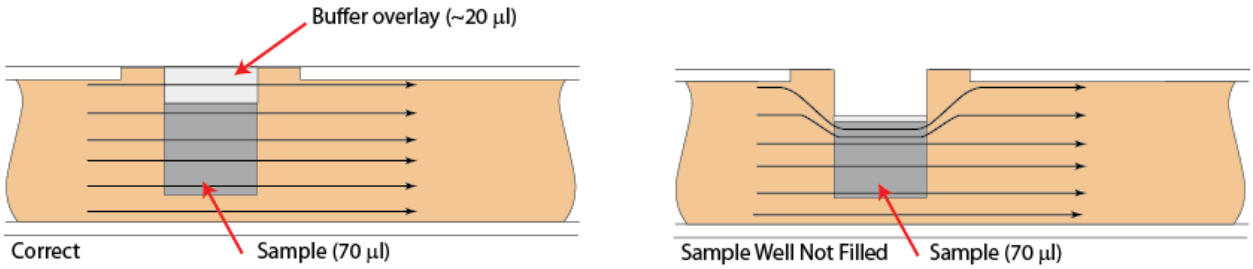


Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the “OK” button is pressed. If the instrument is inadvertently resumed, press the “Pause Workflow” button in the Command Menu to re-pause the instrument and continue with the manual user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

F. Loading Samples

1. Sample Loading Guidelines

Proper sample loading is critical for best performance of HLS cassettes. For maximum reproducibility and accuracy, the sample should travel through the central section of the gel column, and should be bounded on all four sides by uniformly conductive media – either gel or electrophoresis buffer. The goal of the loading procedure is to produce this geometry in the sample loading well, as illustrated below. Properly prepared samples will be 70 µl in total volume. Loading solution used during sample prep contains concentrated Ficoll as a densifying agent, and therefore the samples will sink and form a high density layer beneath the electrophoresis buffer when pipetted slowly into the sample wells. If there is insufficient conductive buffer over the sample, the electrophoretic forces lines will curve upward as the sample exits the well (see the figure below, right), and the sample will be drawn to the top of the cassette where it can travel out of the gel into the gap between the gel column and the plastic top of the channel. Sample moving in this gap will travel at a different rate than the sample inside the gel column, and will lead to elution of undesired size fractions in the eluted material. In such cases, the contaminating sample will usually (but not always) be higher in molecular weight than the anticipated fractions.



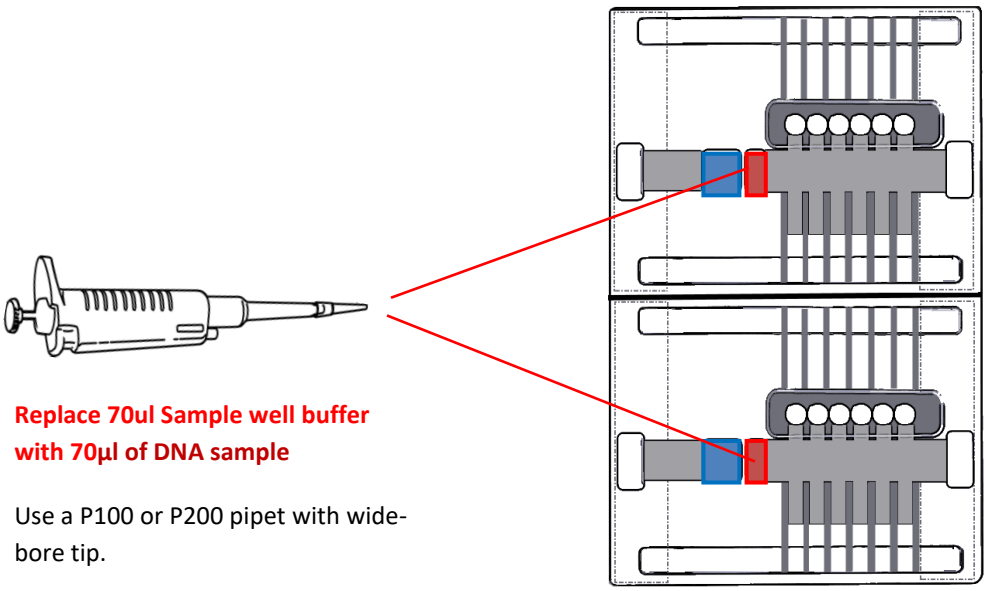
1. Bring a DNA samples up to **56µl** in TE. The maximum DNA input amount should not exceed **7µg**

Important! The ionic strength of the sample should be lower than the ionic strength of the running buffer (80 mM monovalent ions). For best results, samples should be de-proteinized prior to loading if possible.

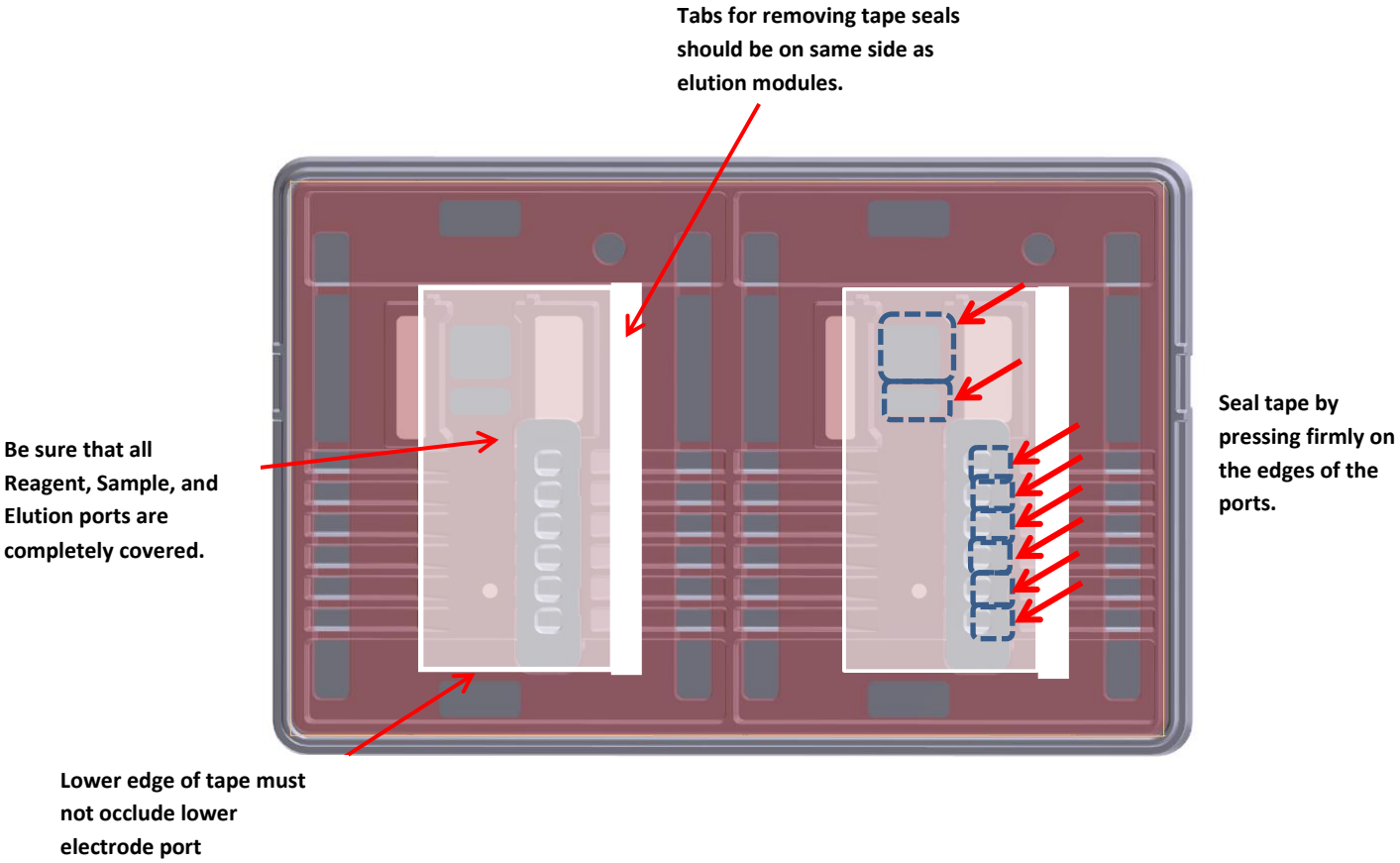
2. Combine each DNA sample with **14µl of Loading Solution.** Using a wide-bore pipette tip, mix thoroughly by pipetting up and down.

3. **Re-check the buffer level in the sample well and reagent wells.** Make sure that reagent and sample wells are completely full to the top with running buffer (**E**). Top off with additional buffer, if necessary. The total volume of the sample well is ~90 □l.

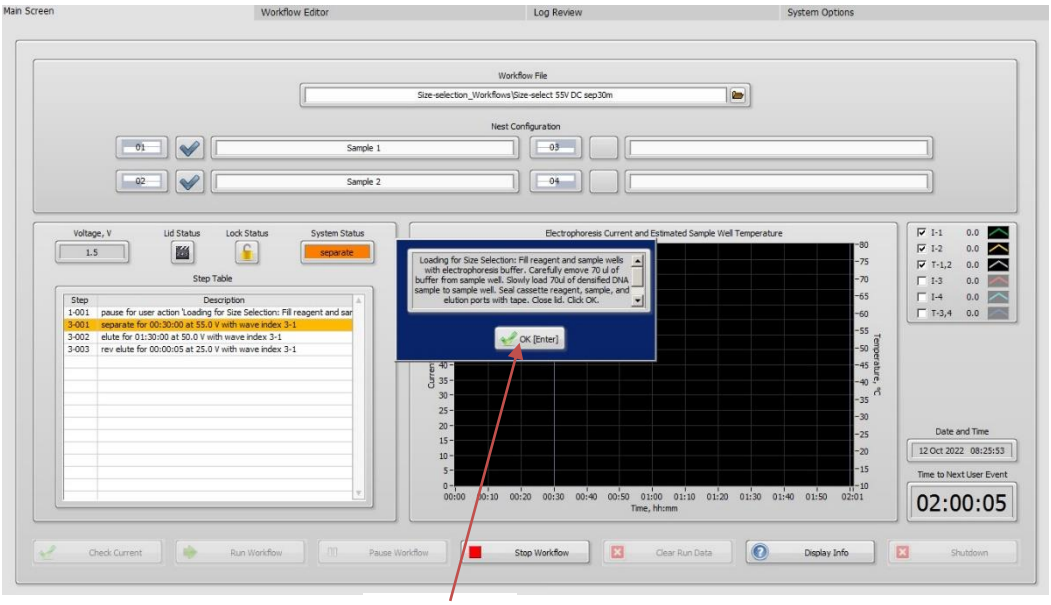
4. **Remove 70µl of buffer from the <u>Sample well</u>, and load 70µl of sample into that well.** Take care not to pierce the agarose with the pipette tip. There is gel on all sides and bottom of the sample well. In addition, there is an agarose "chimney" surrounding the top of the sample well that protrudes up through the cassette cover (see the figure below). When removing buffer, some users find it useful to immerse the pipette tip just below the surface of the buffer and follow the liquid level down with the tip as the buffer is removed. When buffer removal is completed, there will be ~20 µl of buffer left in the well. When adding sample, place tip of pipette just below the surface of the buffer and follow the liquid level up with the tip as the well fills. Don't be concerned if the sample well slightly overfills. The density of the sample will allow it to sink before it can flow out of the well.



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



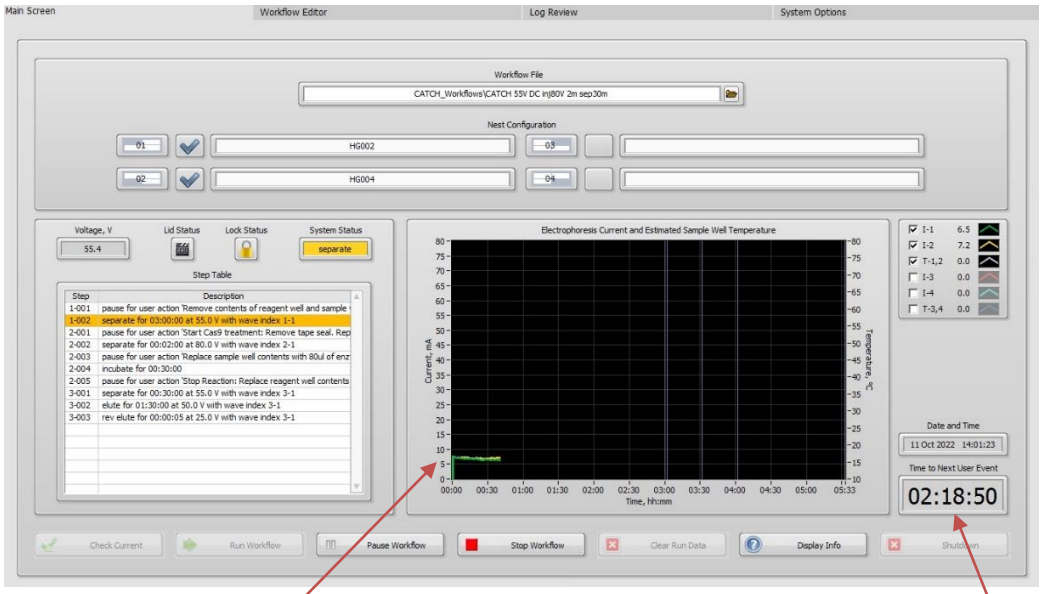
6. Close the Lid. Press “OK” to continue.



Press “OK”.

G. Size Selection and Collection

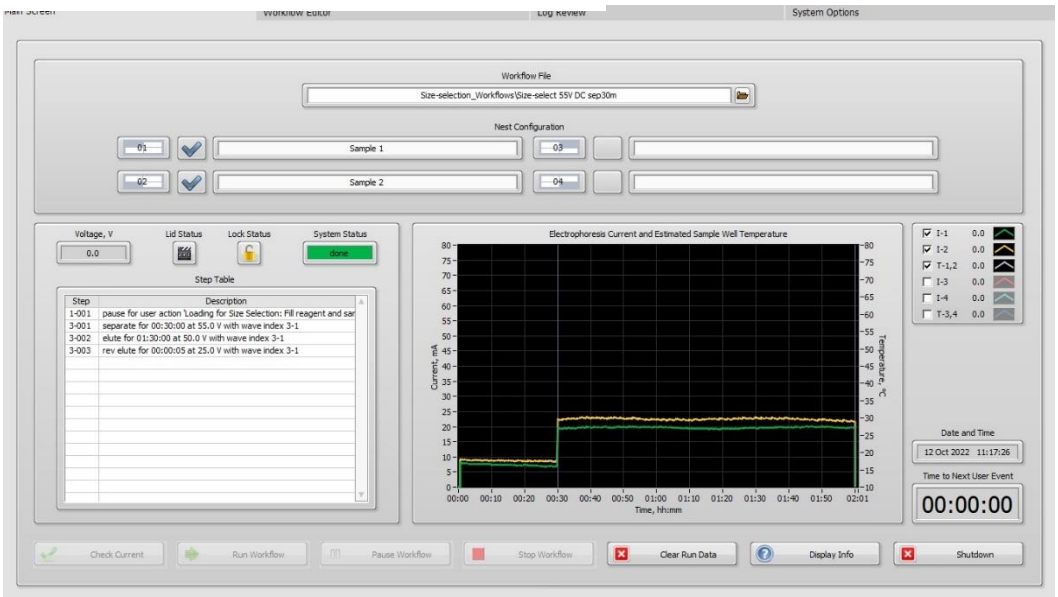
1. The Collection Stage will require several hours of unattended operation. Users should note the time remaining, after which the samples can be collected.



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

Time remaining until the end of the run.

Main Screen at Run Completion:

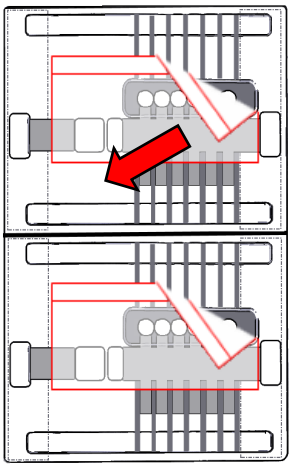


H. Removing Samples

1. After the run is complete, wait at least 45 minutes after run completion before removing contents of elution modules. This allows HMW product to release from the ultrafiltration membranes in the elution modules.

2. 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 (if DNA is >100kb), remove the contents of the elution modules.

Important! Pipette as slowly as possible to avoid shearing the HMW (>100kb DNA). Use of an electronic pipettor at low speed settings may be helpful. There should be 70-80 µl of liquid in each module.

HIT kit Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes