sage science

HLS Cassette Kit
Workflow Guide

HLS-CATCH*:
Targeted Collection of High Molecular Weight Genomic Regions

PN# HIT-0004 or HIT-0012

***For use with either the SageHLS or HLS2 instrument platforms**

Important!
Read carefully before at least one week before running HLS-CATCH!

A. Cell Suspension vs Cell Nuclei Preparation

If possible, using cell nuclei with HLS-CATCH is recommended for highest reaction efficiency (higher enrichment and lower off-target background). 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#
SpyFi Cas9 Nuclease *or* Alt-R S.p.HiFi Cas9 Nuclease V3 *or* EnGen® Spy Cas9 HF1 ***(Recommended)****	Aldevron *or* Integrated DNA Technologies (IDT) *or* New England Biolabs (NEB)	**1081060** (100 µg,~ 7 samples) **1081061** (500 µg,~ 37 samples) **9214-0.25MG (**250µg,~ 18 samples) (Aldevron and IDT enzymes sold at 10µg/µl = 61µM) **M0667M** (500 pmol, 823µg,~5 samples) **M0667T** (2,000 pmol, 328µg,~23 samples) (NEB enzymes sold at 3.3µg/µl = 20µM)
Guide RNAs (crRNAs and tracrRNA)[1, 2]	Integrated DNA Technologies(IDT)	custom
Qubit™ Fluorometer/HS DNA Assay kit[1]	Thermo Fisher Scientific	**Q32851** (100 assays)
**TaqMan™ Target Detection Reagents[1]	Thermo Fisher Scientific	custom
TaqMan™ RNase P Detection Reagents Kit[1]	Thermo Fisher Scientific	**4316831** (100 reactions)
(optional) SYBR Green reagent and primers[1]	preferred vendor	custom primers

*** High Fidelity enzymes from Aldevron, IDT and NEB provide higher enrichment and less off-target collection.**

**** TaqMan or SYBR chemistry can be used for PCR. TaqMan is preferred and highly recommended.**

[1]For instructions on purchasing guideRNAs and primers for demonstrating HLS-CATCH for BRCA1 (human) and/or brca1 (mouse) refer to the following guide:

Sourcing Guide: HLS-CATCH BRCA1 Enrichment in Human and Mouse

[2]For recommendations and instructions on guide RNA design and selection refer to the following guide:

Cas9 Guide RNA Design Tutorial for Sage HLS-CATCH

These guides may be downloaded from http://www.sagescience.com/product-support/HLS-support/

C. One day before running HLS-CATCH:

Schedule availability of cells one day before running the HLS (if using cell suspensions):

- 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. Higher input loads will result in lower recovery and less target enrichment of CATCH targets.

D. Morning of HLS-CATCH Experiment:

- 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/).

- Assembly of Cas9 requires about 30 minutes of hand-on effort. Ideally, Cas9 assembly should be performed the day of CATCH procedure, preferably during the 3hr extraction stage.

E. The following items are supplied Cassette Kits (HIT-0004 and HIT-0012).

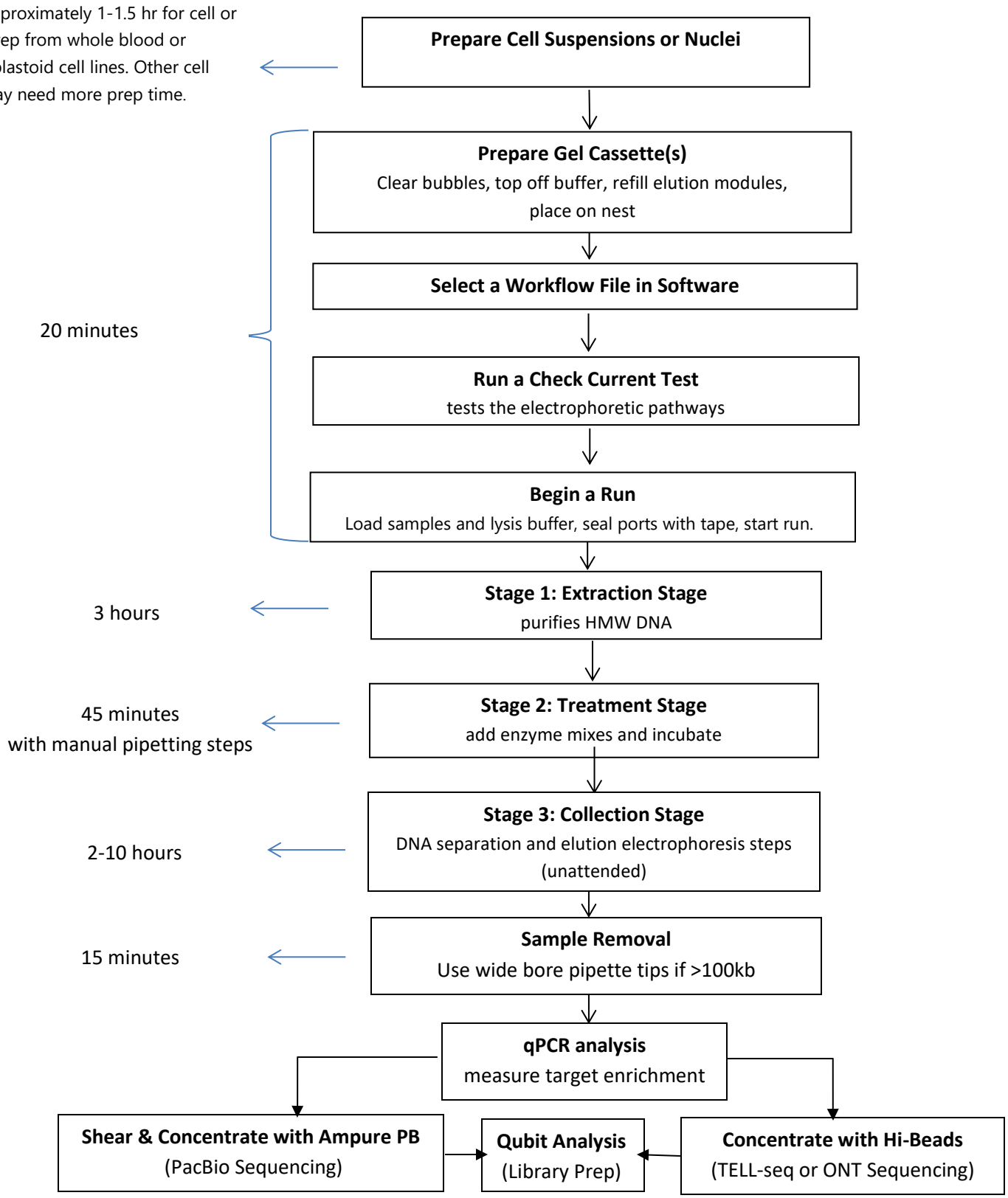
When the kit is received, open immediately to inspect the contents. Contact Sage Science if any items are missing or damaged

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.	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
1 ea.	4X Enzyme Buffer (for Cas9-Guide RNA Mix), 1 ml	F	**-20ºC**
2 ea.	Hydroxypropyl-beta-cyclodextrin 0.33%, 1 ml / 6ml	J	**-20ºC**
1 ea.	Hi-Bead HMW DNA Concentration Reagents		**4ºC**

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

HLS-CATCH Workflow Summary



A. GuideRNA and PCR Primer Design Recommendations

Users may wish to train on HLS-CATCH workflow with known guides and primers. BRCA1 purification from human or mouse can be demonstrated with the validated guide and primer sequences provided in the document (linked below).* These instructions may also be useful when acquiring custom guides and primers.

- **Sourcing guide for Guide-RNAs and qPCR Primers: HLS-CATCH BRCA1 Enrichment in Human or Mouse**

For designing new guide-RNA design for specific targets, using the **UCSC Genome Browser** is recommended. A tutorial for design guides can also be found on the HLS support site:

- **Cas9 guide-RNA Design Tutorial for HLS-CATCH**

*These may be found the HLS support site (http://www.sagescience.com/product-support/HLS-support).

B. Prepare cells or nuclei using Cell Suspension Guide

Prepare cells using HLS cell prep kit instructions (kits/protocols for mammalian WBCs, tissue culture cells, and bacterial spheroplasts are available). Recommended input will contain cells or nuclei containing a maximum of 5 µg of genomic DNA. 5µg is the genomic DNA content of about 1.5 million human diploid cells. Higher cell loads will result in lower recovery of genomic CATCH targets.

B. Prepare the Cas9 Enzyme Assembly

For best results, the Cas9 RNP reagents should be assembled the day of the CATCH procedure, ideally during the 3hr HLS extraction period. The mix should be stored on ice prior to use. Prep time is ~15-30 minutes.

Summary

- The two-part guide RNAs are annealed by heating to 95°C and cooling to room temperature.
- The annealed guide RNAs are mixed with the Cas9 enzyme and incubated briefly at 37°C.
- Cas9 mixture volume should be 80µl.

1. Anneal the two-part IDT ALT-R™ guide RNAs (crRNA and tracrRNA)

a. Dissolve each of the crRNAs and tracrRNAs to be used at **100uM** concentration in IDT Duplexing Buffer. The correct volume for resuspension in microliters will be 10 times the number of nanomoles of RNA in the tube supplied by IDT.

b. Use the table below to prepare the Guide RNA Annealing Mix to a volume of 11µl for the preparation of one sample. Users should scale accordingly if the same guides will be used for multiple sample treatments.

To a 200 µl PCR tube, add:

order of addition	reagent	vol. µl	stock [ ] µM	Final [ ] Annealed gRNA Mix µM
1	**IDT Duplexing Buffer**	7.7		
2	**Pooled crRNAs***	2	100	18.2
briefly mix and spin				
4	**tracrRNA**	1.3	100	11.8
Briefly mix and spin				
	Total	**11**		

***For instance, if two crRNAs are to be used, add 2 ul of each crRNA solution. If four crRNAs are to be used, add 1 ul of each crRNA solution. For more crRNAs, mix equal volumes of each crRNA (using some convenient volume), and then add 4 ul of the resulting crRNA mixture to the PCR tube. (**The goal is to saturate the tracrRNA with a 1.5-fold molar excess of total crRNA**.)

c. Heat the mixture at 95ºC for 5 minutes on a thermal cycler with a heated lid.

d. Remove the mixture from heat and allow to cool on the bench-top for 3-5 minutes.

e. Vortex briefly and centrifuge to collect any condensation.

f. Store on ice or at 4C until assembly with Cas9.

g. Stored up to 4 days at 4 ºC.

NOTE: Annealed gRNAs can be prepared in larger batches and stored frozen at -20C indefinitely without loss of activity.

2. Assemble Annealed Guide RNAs and Cas9 mixture

a. Thaw 4X Enzyme buffer at room temperature.

NOTE: Do not heat 4X Enzyme buffer above 37°C or components will form a precipitate.

b. Cas9 is not frozen, and should be kept in freezer until ready to be mixed.

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

To a 200 µl PCR tube, add in order:

order of addition	reagent	vol. µl	stock [ ] µM	final [ ] in Enzyme Mix µM
1	4X Enzyme Buffer (F)	5	4	1
2	Annealed Guide RNA Mix	11	11.8 (tracrRNA)	6.5 (tracrRNA)
mix and spin				
3	Molecular biology-grade water	2.65		
4	Cas9* nuclease, wt	1.35	61	4
	Total**	**20**		

* If using NEB s.pyogenes (20uM), use 4 ul with no molecular biology-grade water

**20 ul will be diluted to 80 ul before loading in a single sample well. Users should scale accordingly.

d. After addition of Cas9, mix thoroughly by gentle pipetting and briefly spin to collect all liquid at bottom of tube.

e. Incubate at 37°C for 5 minutes in a thermal cycler with a heated lid to assemble enzyme and gRNAs.

f. Proceed to next step (Dilution with HLS Enzyme Buffer).

3. Dilute to loading concentration with HLS Enzyme Buffer

a. Dilute 20ul assembled Cas9 RNP from Step 2, with 60 ul of 1X HLS Enzyme buffer (C).

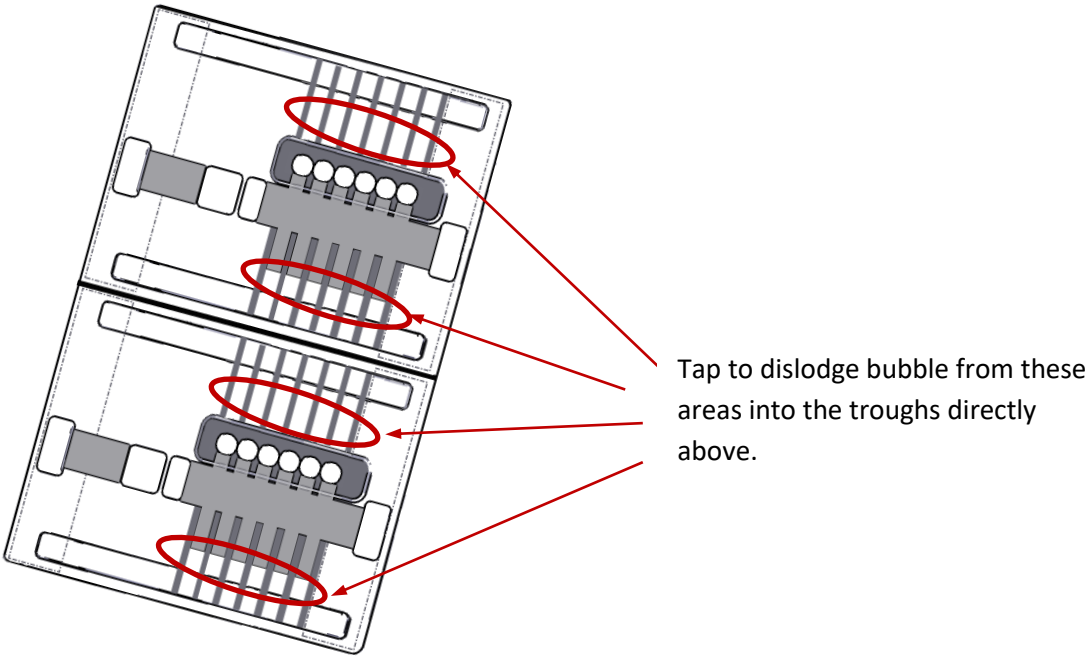
b. Mix thoroughly by gentle pipetting of the entire mixture (80 ul). (Final concentration: 2 µM Cas9 assembled with 3.25 µM pool of annealed two-part gRNAs)

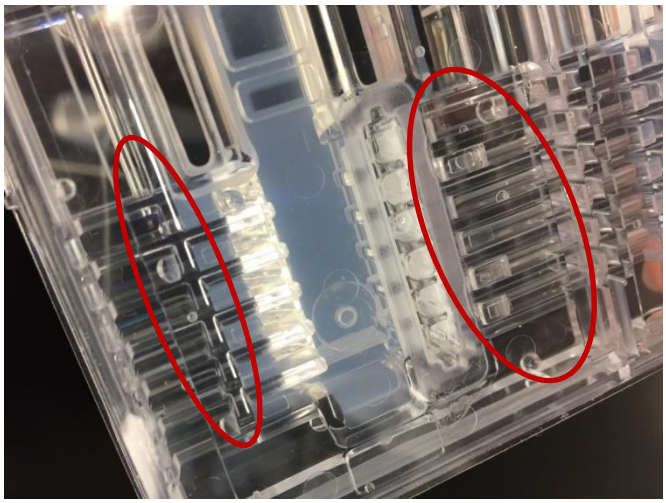
At this point the Cas9 mixture is ready to load in the HLS sample well. The mixture can be stored on ice, or at 4C, for several hours.

C. Prepare the Gel Cassette(s)

1. Remove the gel cassette from the foil bag.

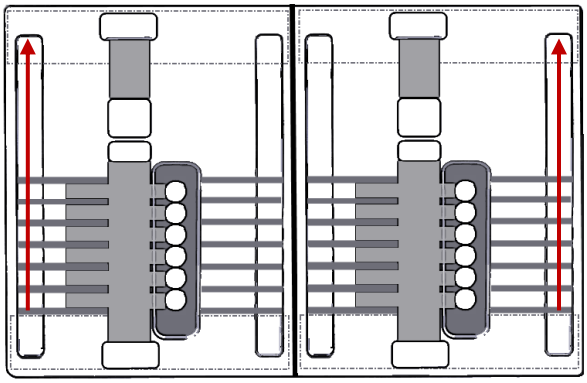
2. **Before removing tape!** – Hold the cassette with top surface almost vertical and the elution modules above the gel channel. Tap the cassette to dislodge any bubbles that are trapped behind the elution wells, or in the elution channels. Repeat if necessary. Allow the bubbles to collect in the electrode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



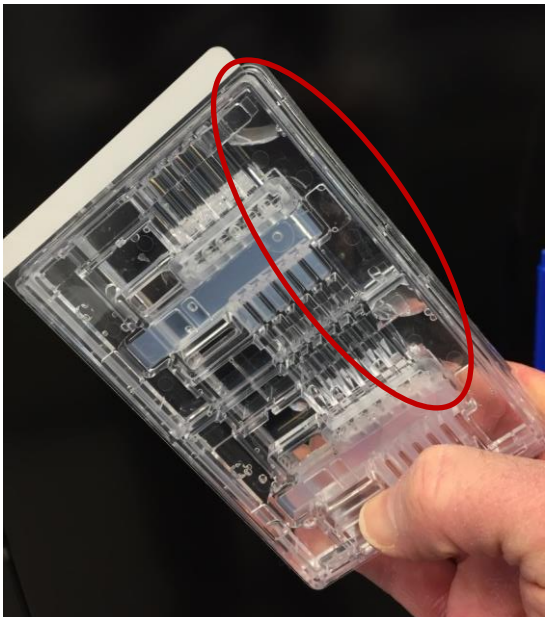


Bubbles in the elution paths can interfere with collection

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



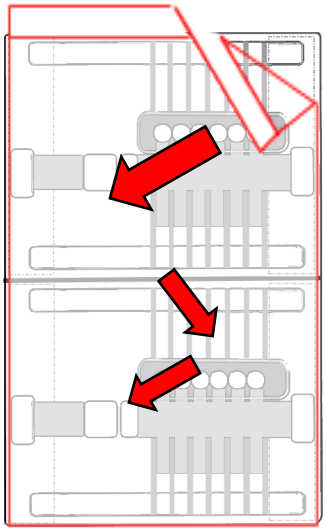
Move any bubbles to the upper buffer area.



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.

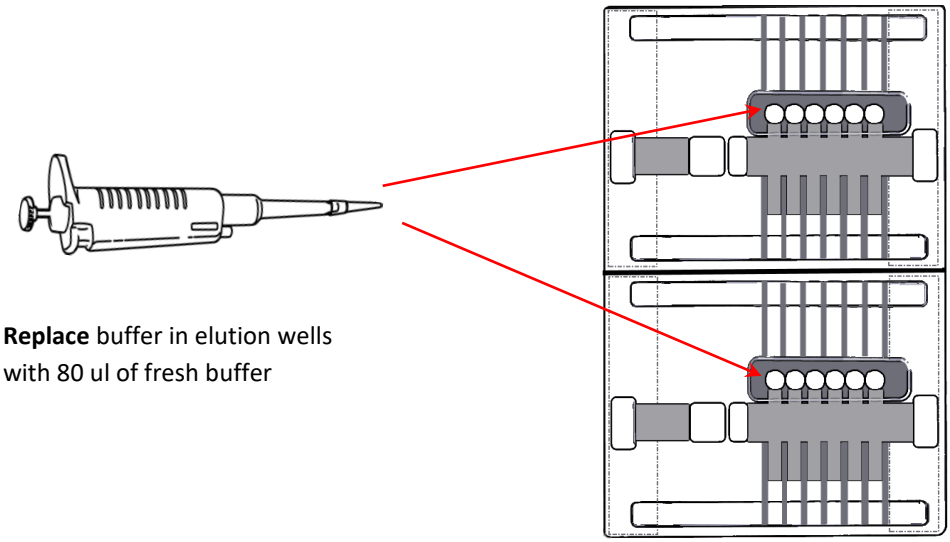
5. While holding the cassette firmly in place on the nest, grab the tape tab, and pull the tape off at an angle, slowly and firmly. Alternate the pulling angle if the tape resists peeling.



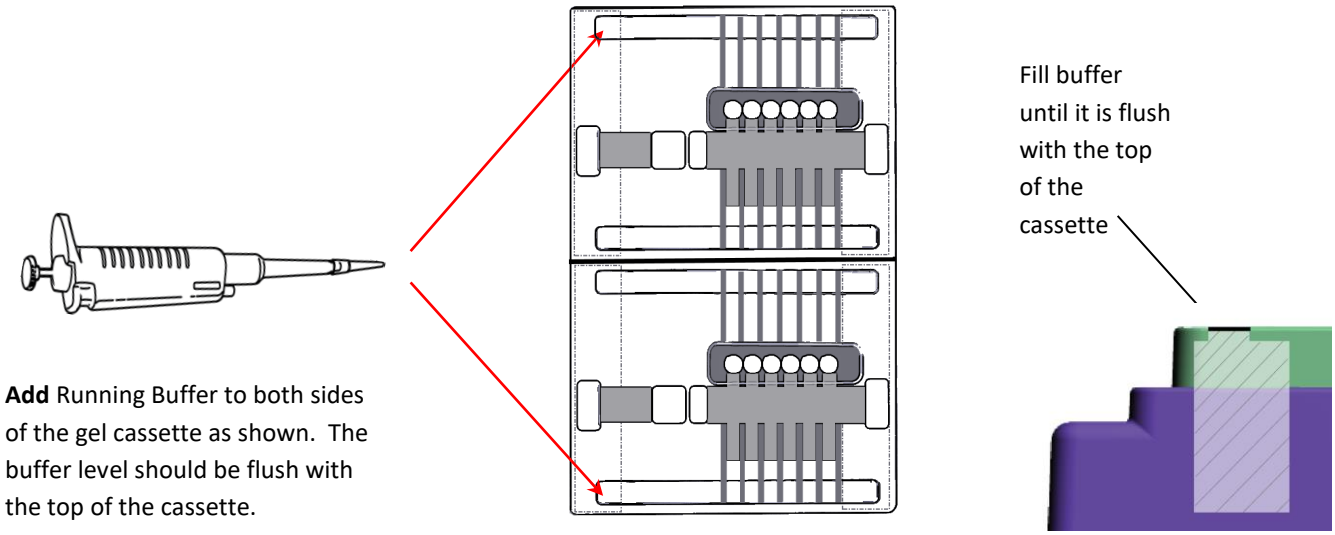
Peel back tape at an angle. Alternate angles if the tape resists peeling.

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.



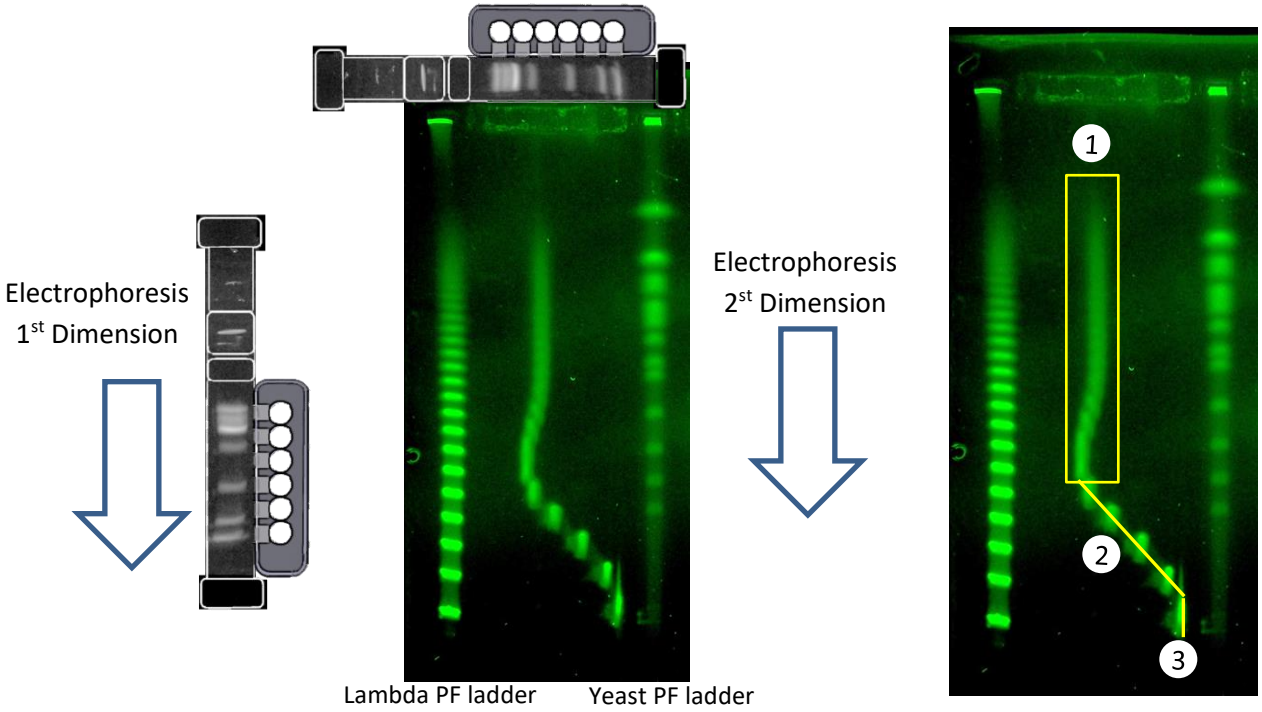
Important! Fill until the buffer level visually reaches the bottom side of the cassette cover

D. Guidelines for Selecting an HLS-CATCH Workflow

HLS-CATCH Workflow files reside in a folder labelled "CATCH_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.

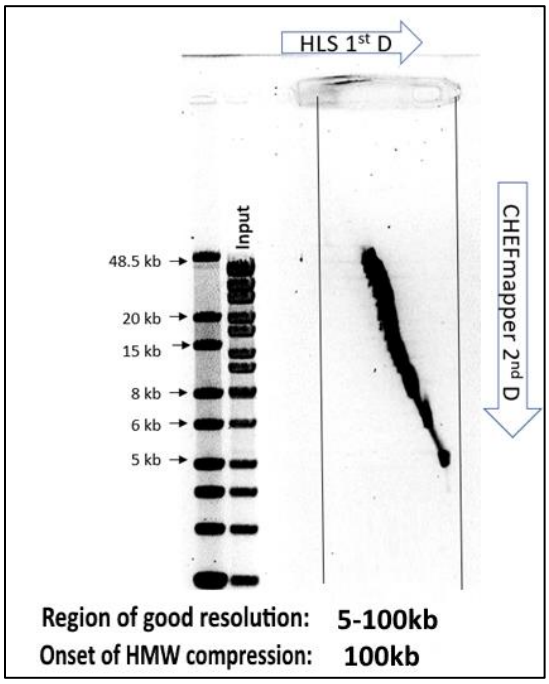
Selecting DNA Target Collection Sizes Ranges: Pulsed-Field Protocols (5 kb – 2 MB)

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.

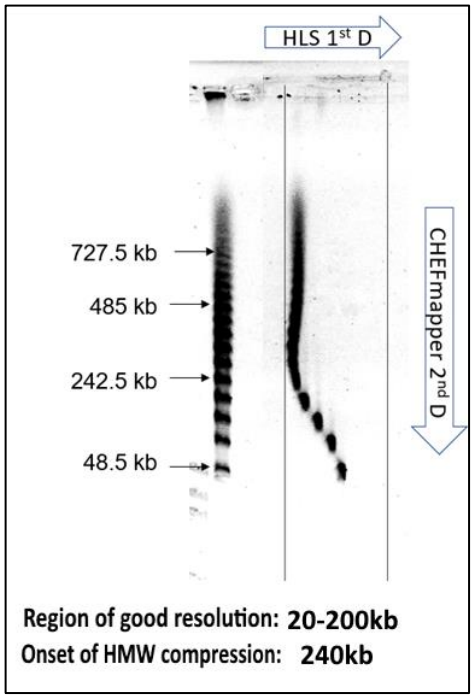


1. HMW compression (with mobility inversion)
2. Size Range with good resolution
3. LMW compression

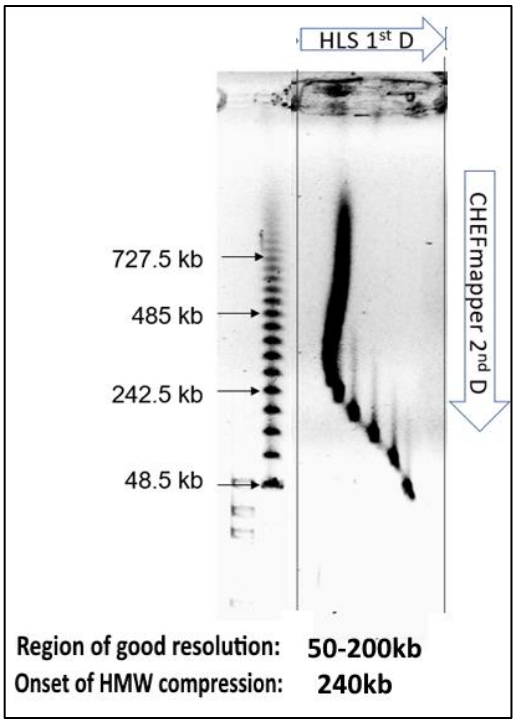
Use the following guidelines to determine the best collection stage for a given application.



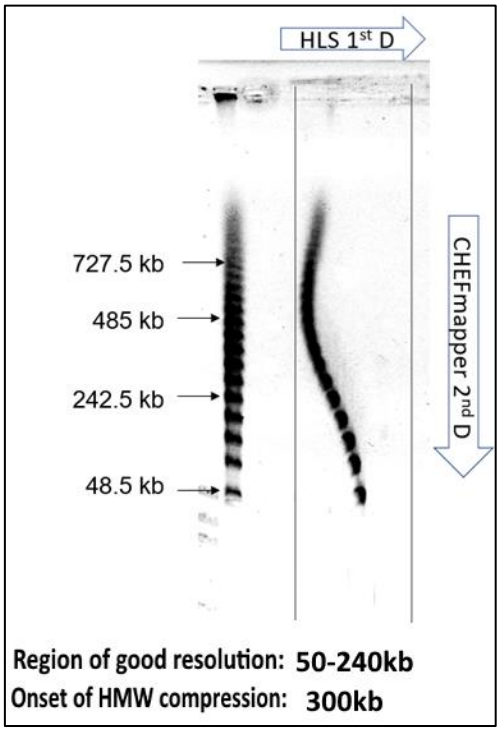
Workflow: CATCH 5-100kb sep2.5h



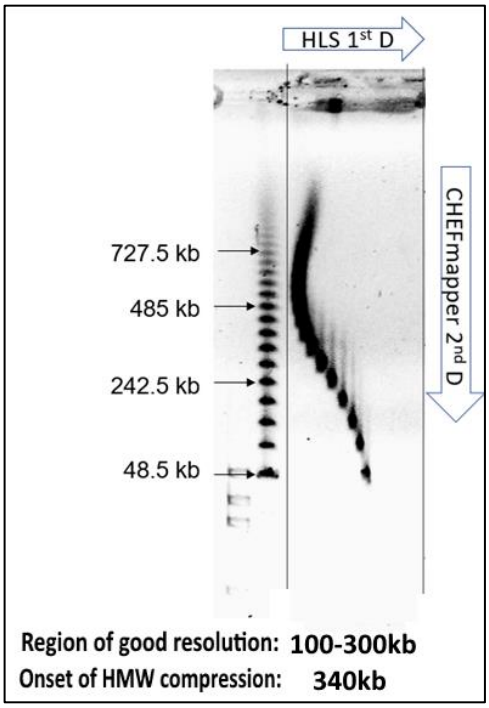
Workflow: CATCH 20-200kb sep3h



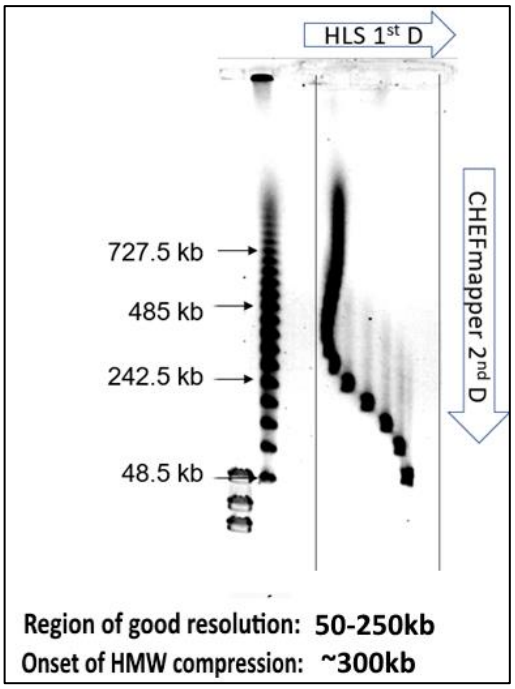
Workflow: CATCH 50-200kb sep4h



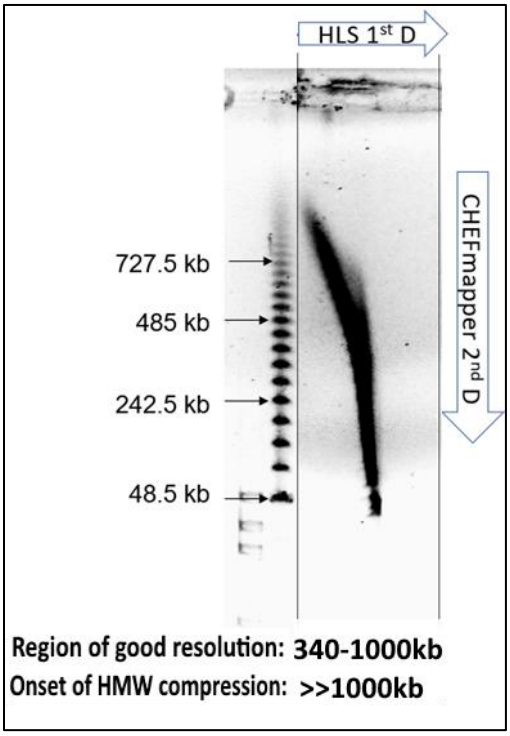
Workflow: CATCH 100-300kb sep3h



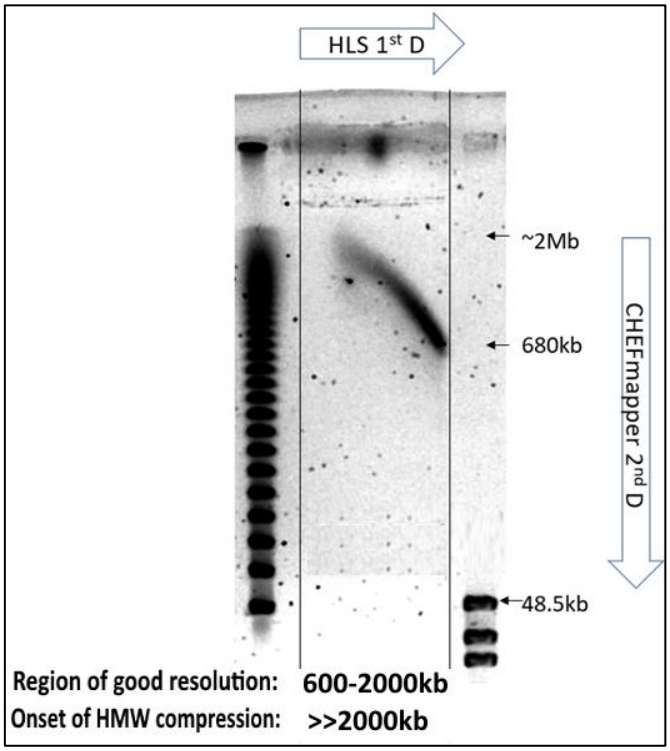
Workflow: CATCH 100-300kb sep4h



Workflow: CATCH 50-250kb sep8h



Workflow: CATCH 340-1000kb sep3h



Workflow: CATCH 600-2000kb sep8h

Summary of Pulsed-Field Workflows

CATCH Workflow File name	Elution time	Region of good resolution	Onset of HMW compression	Voltage	Waveform**
CATCH 5-100kb inj80V 2m sep2.5h	90m	5-100kb	100kb	80V	25 10 25 10 5 2 21
CATCH 20-200kb inj80V 2m sep3h	90m	20-200kb	240kb	55V	150 50 30 10 3 1 81
CATCH 50-200kb inj80V 2m sep4h	90m	50-200kb	240kb	55V	150 50 30 10 3 1 81
CATCH 100-300kb inj80V 2m sep3h	90m	50-240kb	300kb	55V	150 50 60 20 6 2 81
CATCH 100-300kb inj80V 2m sep4h	90m	100-300kb	340kb	55V	150 50 60 20 6 2 81
CATCH 50-250kb inj80V 2m sep8h	90m	50-250kb	~300kb	37V	1000 333 240 80 0 0 101
CATCH 340-1000kb 80V 2m sep3h	90m	340-1000kb	>>1000kb*	55V	3000 1000 2550 850 0 0 24
CATCH 600-2000kb inj80V 2m sep8h	90m	600-2000kb	>>2000kb*	55V	3000 1000 2550 850 0 0 24

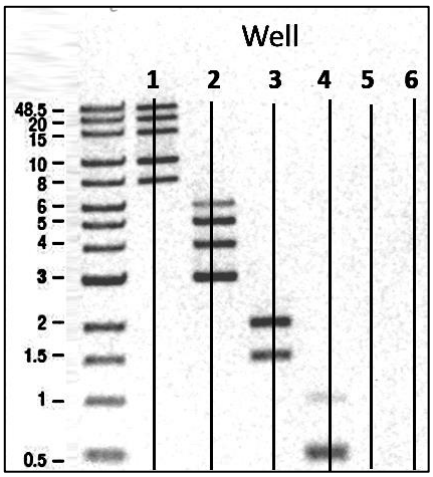
***** These stages are not useful for high-pass size selections because HMW compression is not seen with this stage up to >2Mb.

** 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.

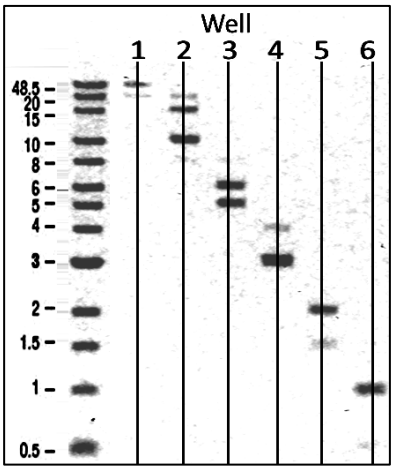
Selecting DNA Target Collection Sizes Ranges: Direct Current Protocols (2-50 kb)

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.

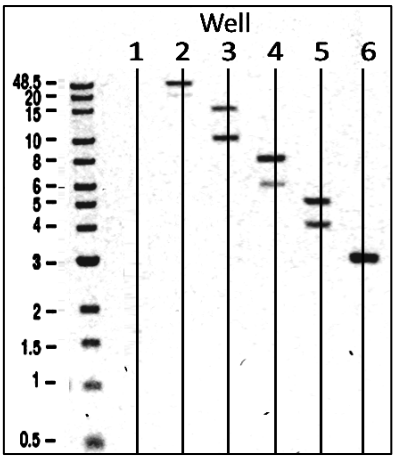
DNAs larger than about 20-30kb tend to migrate at the same rate, termed the limiting low mobility (for those specific electrophoresis conditions). This phenomenon is familiar to most molecular biologists, and results in a high molecular weight compression band near the top of the gel lane when running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



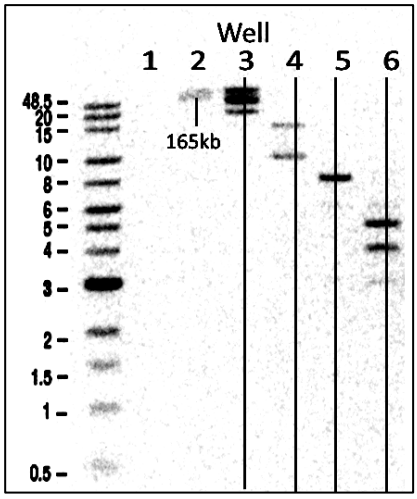
Workflow: CATCH 55V DC 30m



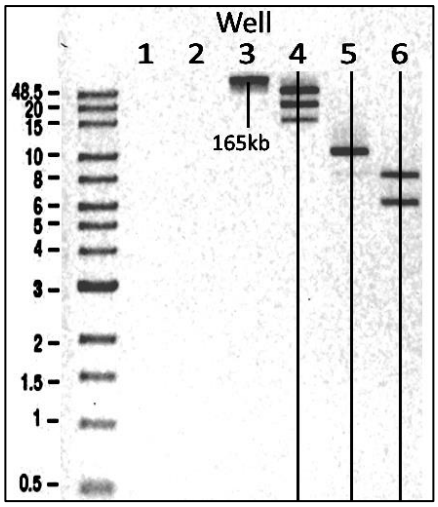
Workflow: CATCH 55V DC 45m



Workflow: CATCH 55V DC 60m



Workflow: CATCH 55V DC 75m

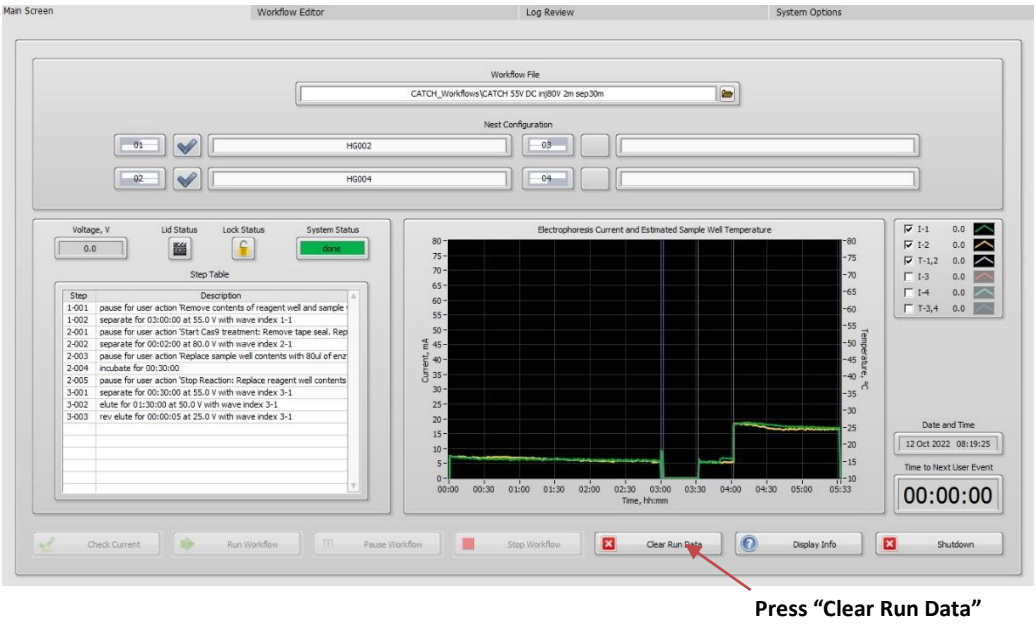


Workflow: CATCH 55V DC 90m

Summary of Direct Current Workflows

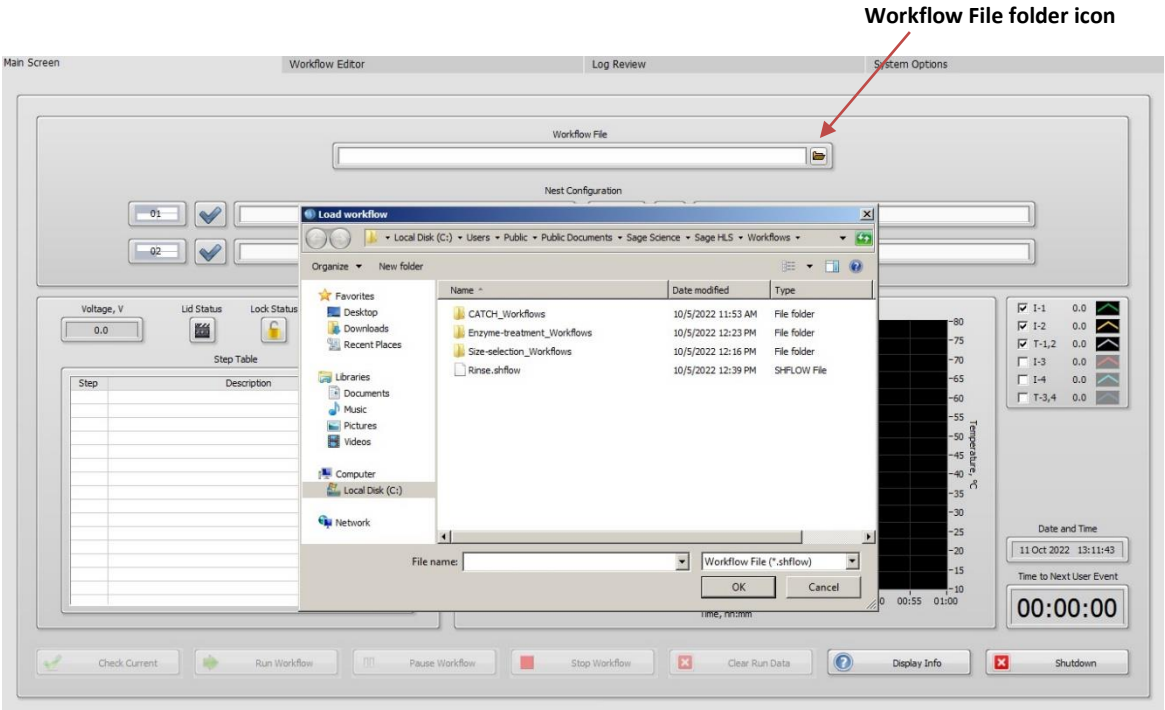
CATCH workflow file name	Elution time	Region of good resolution	Onset of HMW compression	Voltage	Waveform
CATCH inj80V 55V DC 30m	90m	0.5-5kb	6-8kb	55V	Continuous field
CATCH inj80V 55V DC 45m	90m	1-10kb	~20kb	55V	Continuous field
CATCH inj80V 55V DC 60m	90m	3-15kb	~20kb	55V	Continuous field
CATCH inj80V 55V DC 75m	90m	6-20kb	~40kb	55V	Continuous field
CATCH inj80V 55V DC 90m	90m	8-20kb	~50kb	55V	Continuous field

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.

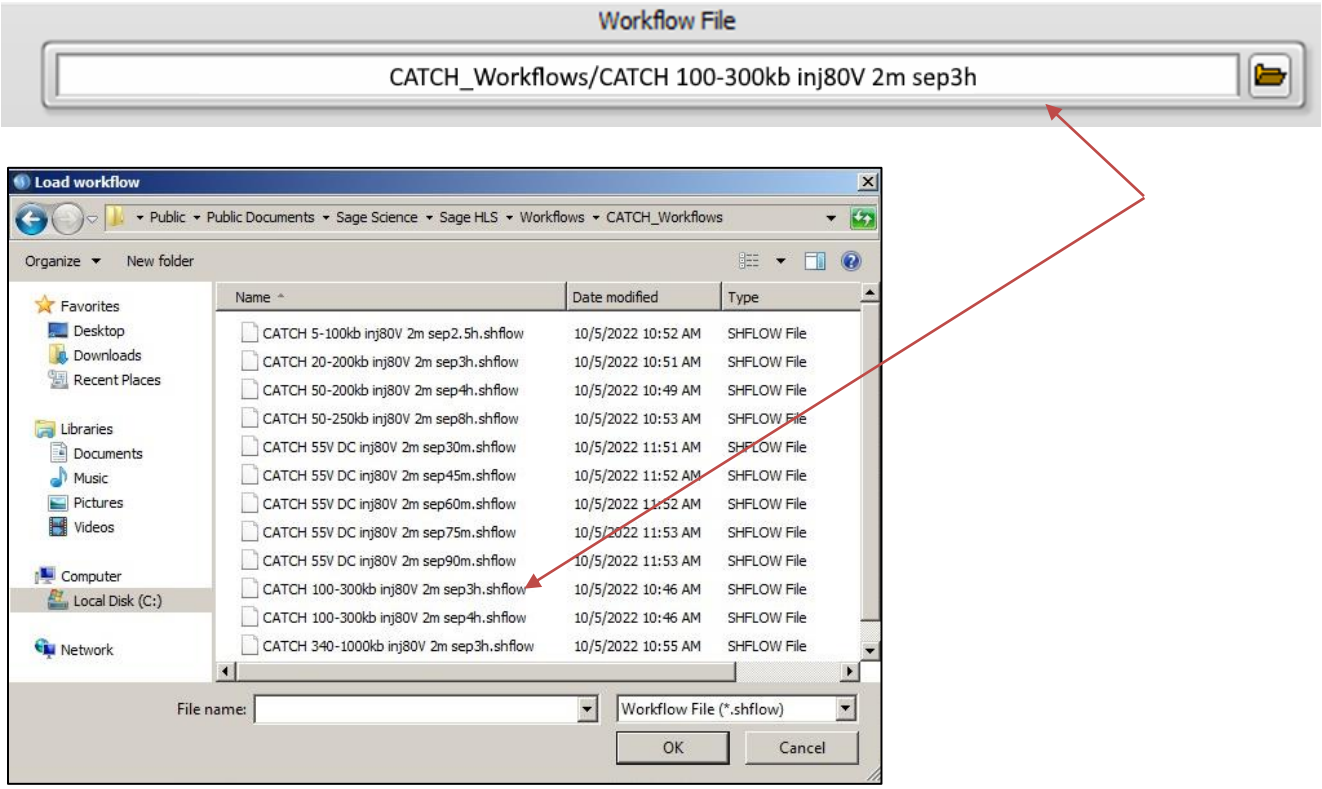


2. Select the Workflow File folder icon.

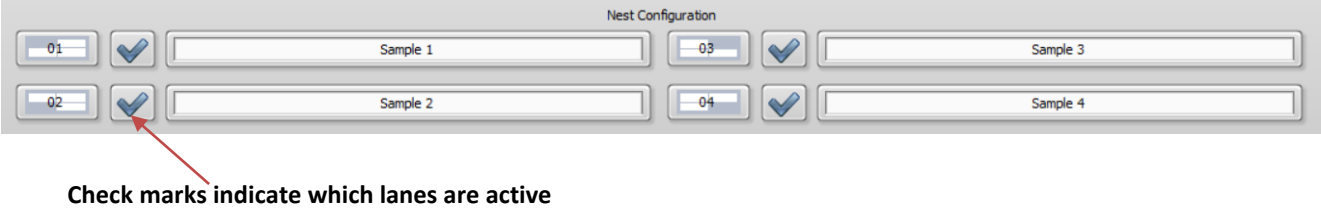
A workflow file directory window will open, select the folder labelled ‘CATCH Workflows”



3. Select the appropriate Workflow File from the folder. The file name will appear in the Workflow File field.

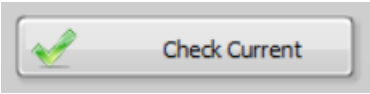


4. Choose the lanes to be used by clicking the boxes next to the lane numbers and enter sample IDs into the adjacent fields (sample IDs are optional or can be entered later).

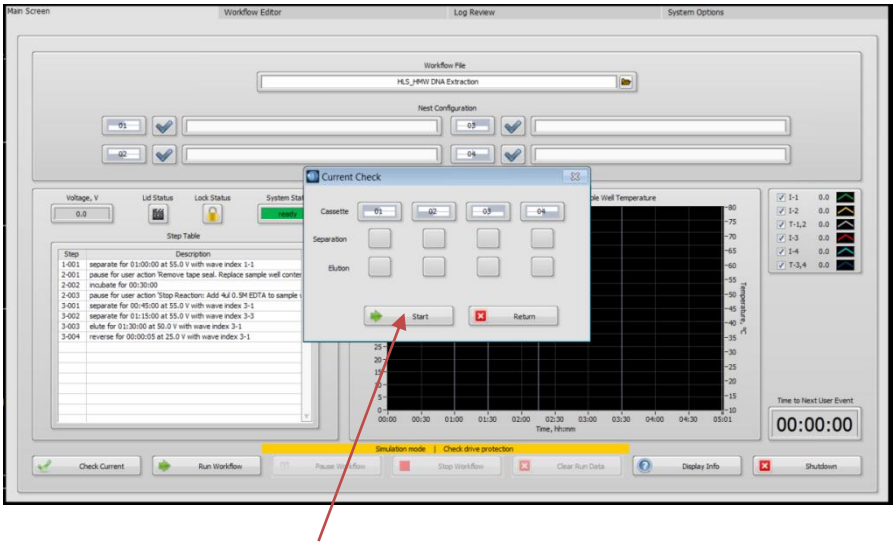


E. Run the Check Current Test

1. Press the “Check Current” button.

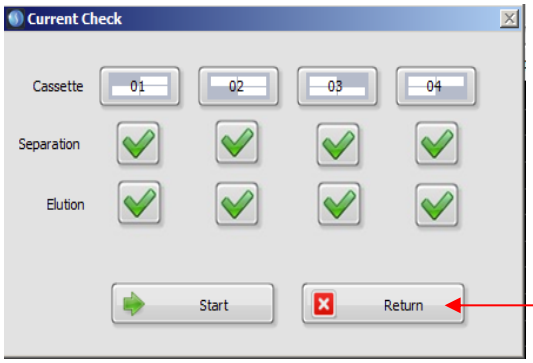


2. A pop-up window will appear. Press “Start” to begin the Check Current routine.



Press “Start”

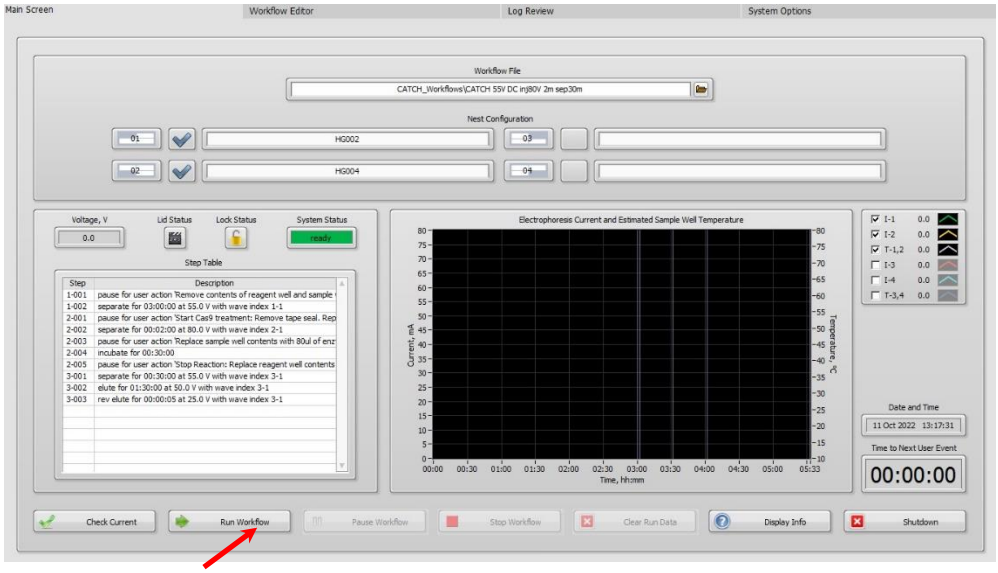
3. The routine will first test the separation electrodes, then test the elution electrodes, and complete within a few minutes. After a successful test, all boxes will fill with green check marks. Press “Return” to continue



Press “Return”

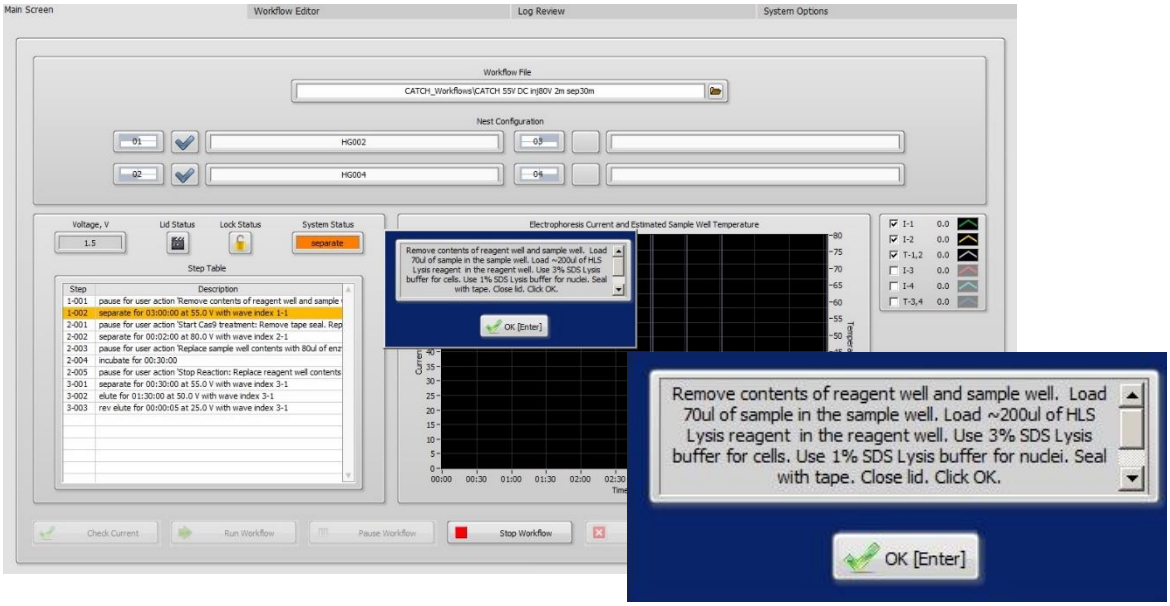
F. Stage 1: Extraction

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



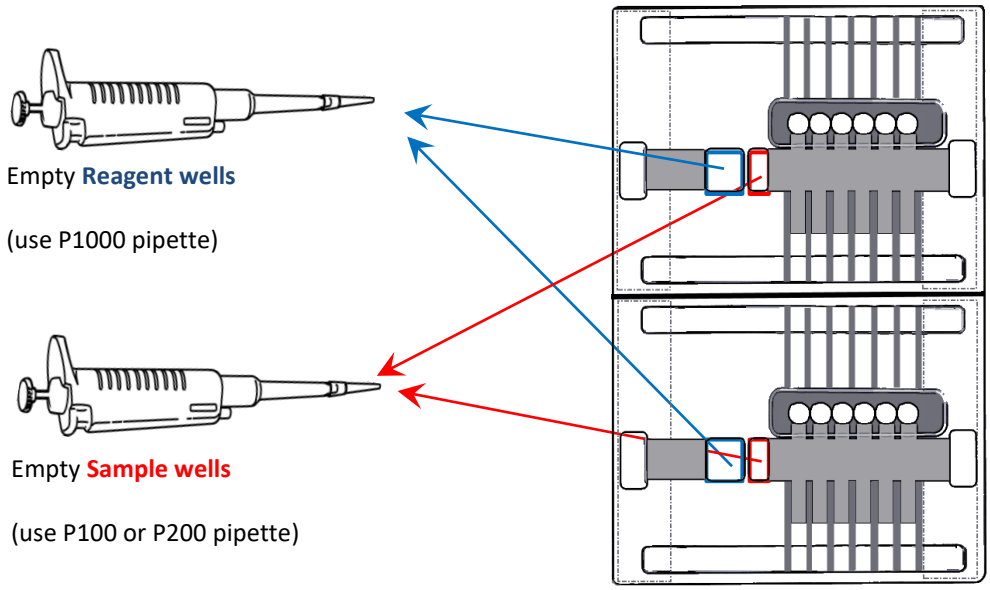
Press “Run Workflow”

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.

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

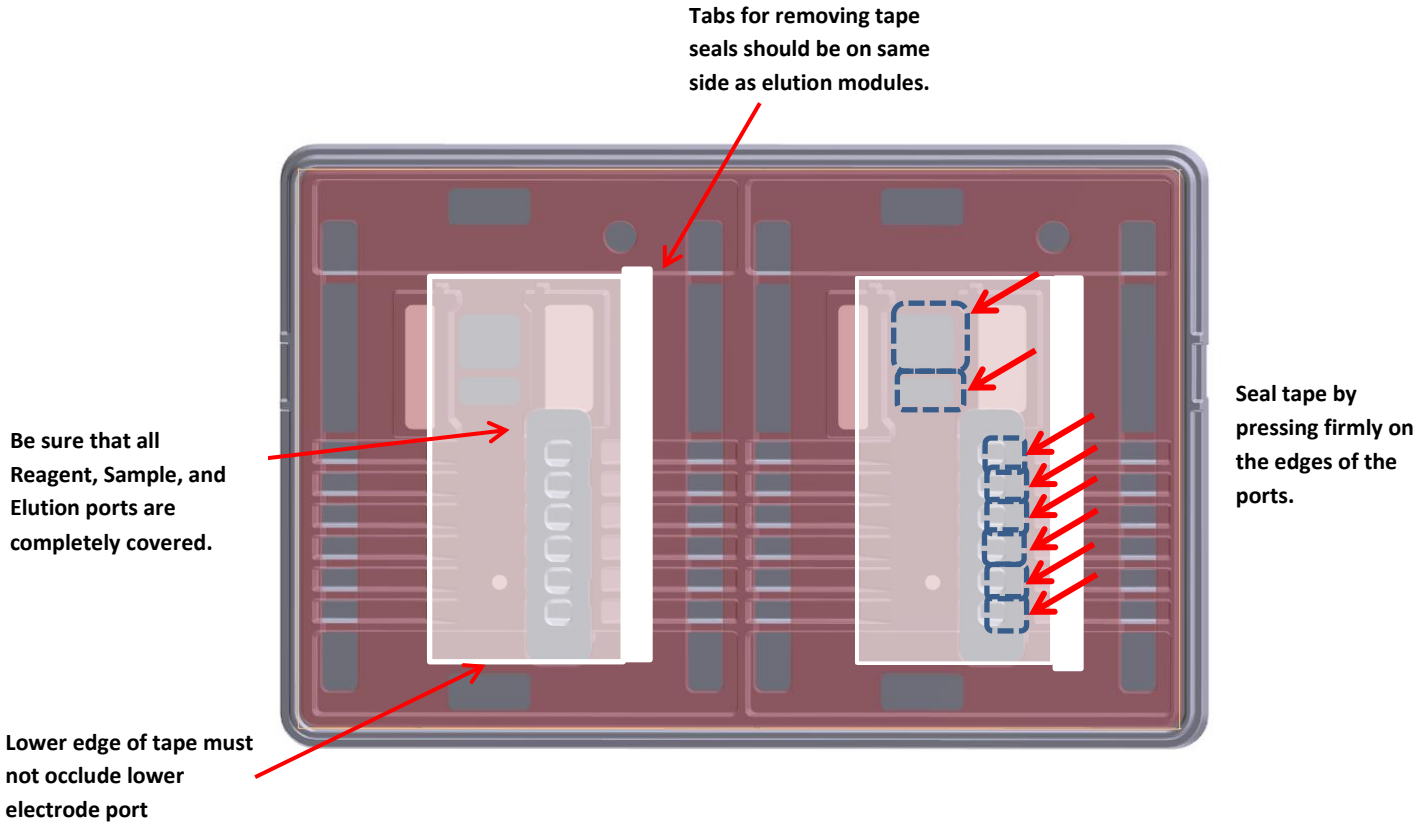


4. Load samples (cells or nuclei) in all lanes. Use a **70ul sample loading volume**. (Sample wells will not be completely full.)

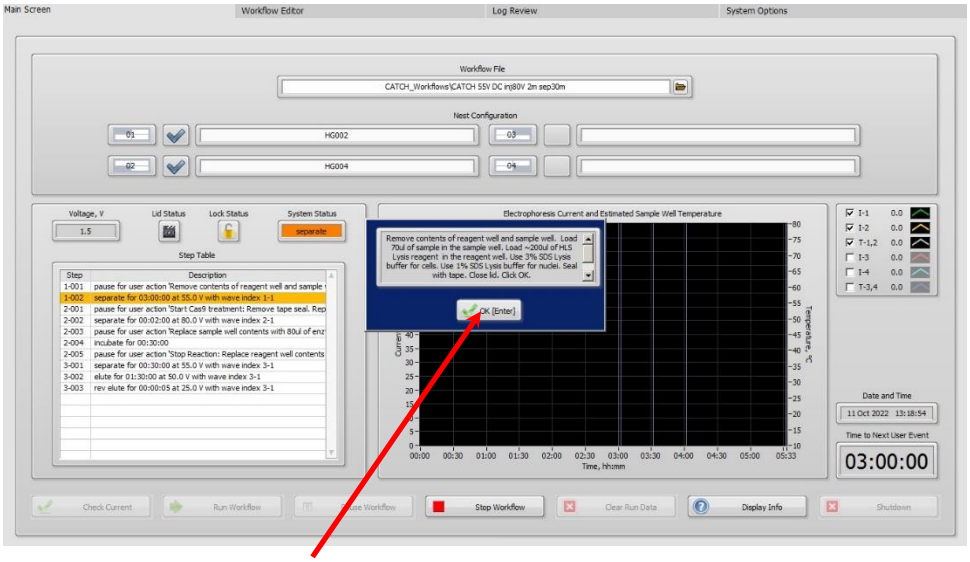
5. **Add 200 µl of HLS Lysis Buffer** to the reagent Wells with. For extractions with nuclei use HLS Lysis Reagent 1% SDS (**H**). For extractions with cells, use HLS Lysis Reagent 3% SDS (**A1**).

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

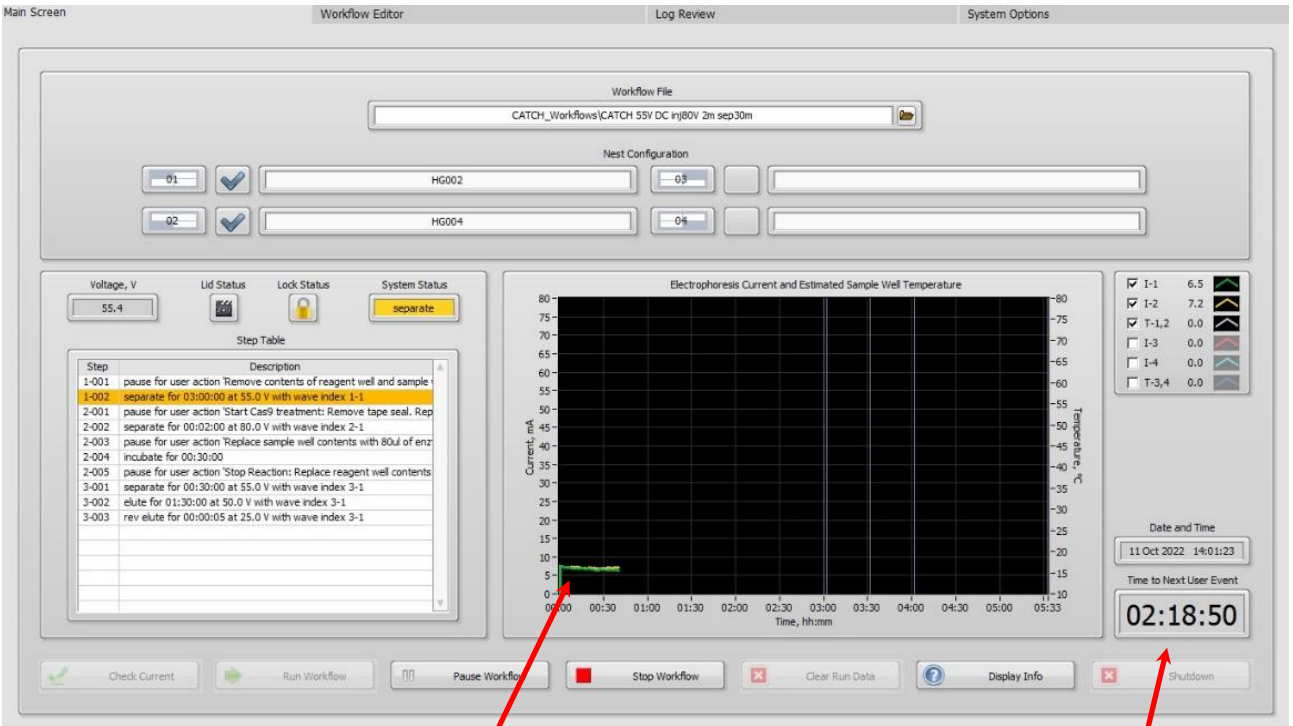
6. Seal reagent, sample, and elution ports with supplied tape without occluding the electrode ports. Press tape firmly around edges of the ports.



7. Close the lid and press “OK”.



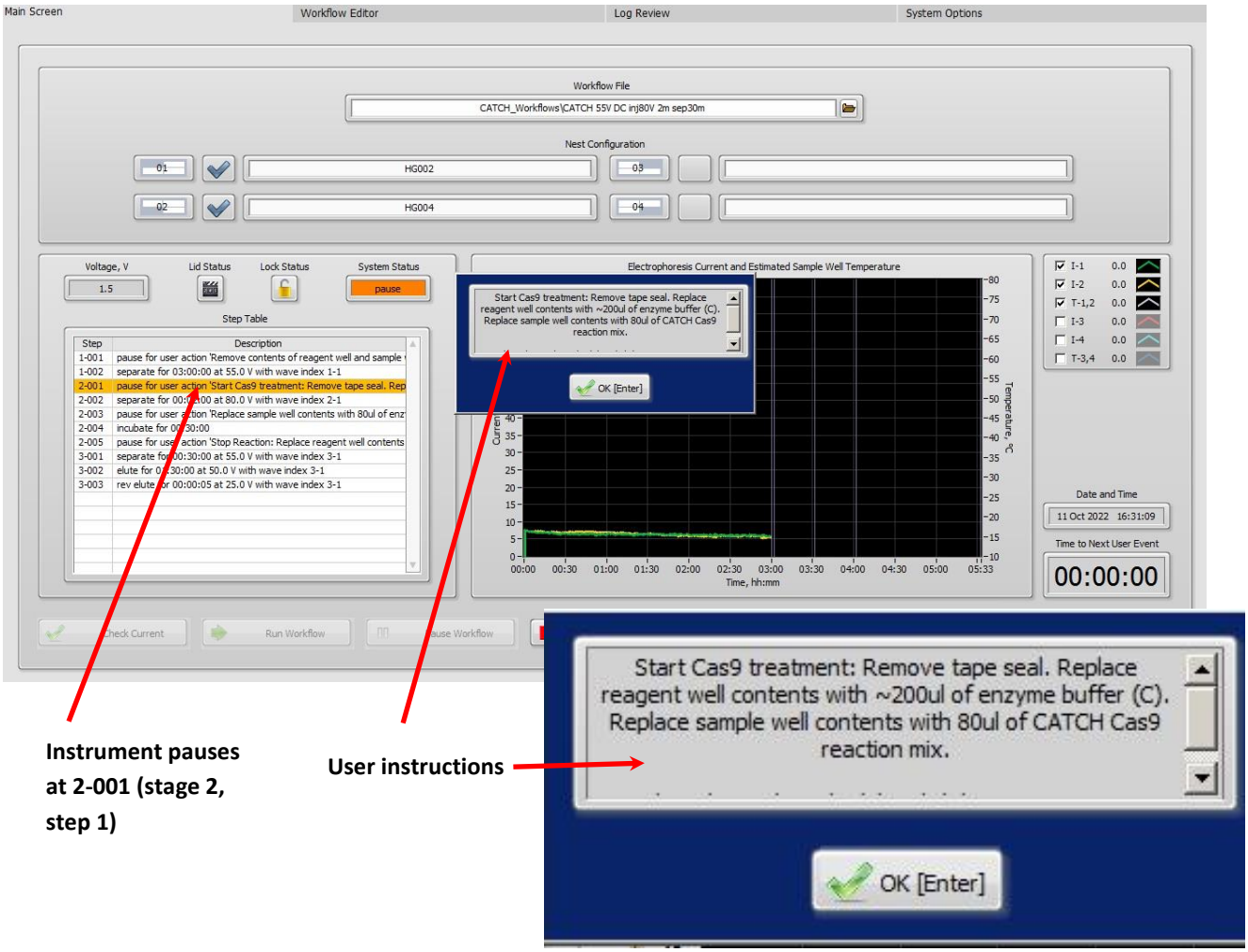
8. The Extraction Step will take 3 hours:



During the run, the Current display should be similar for all lanes, and in the range of 5-10 mA

Time remaining for the extraction stage, until the next user action.

G. Stage 2: Treatment

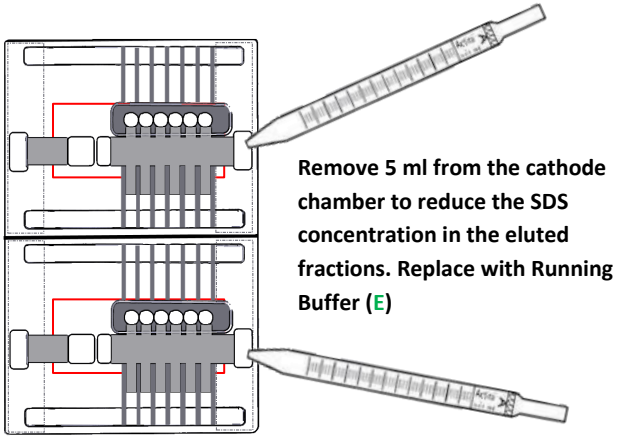


At the end of the Extraction Stage, the HLS will <u>pause</u> on the first step of the Treatment Stage and a pop-up window will appear with user instructions.

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.

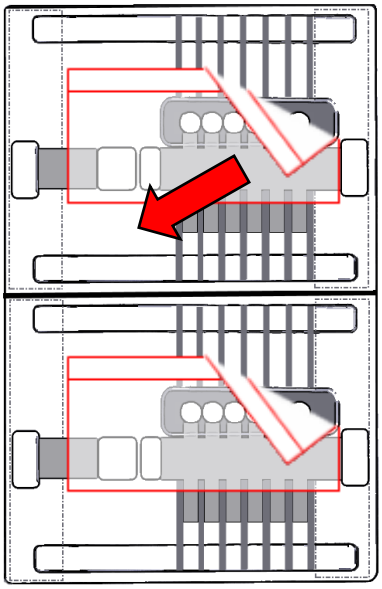
1. When using 3% Lysis Buffer (**A1**): Replace 5 ml of buffer from the anode chamber

   For extractions on cell samples using 3% SDS Lysis buffer, remove 5 ml of SDS-rich electrophoresis buffer from the positive separation electrode port. Add 5 ml of fresh electrophoresis buffer to the negative separation electrode port. This buffer exchange is not necessary for extractions of nuclei using 1% SDS Lysis buffer.
   .



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

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



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

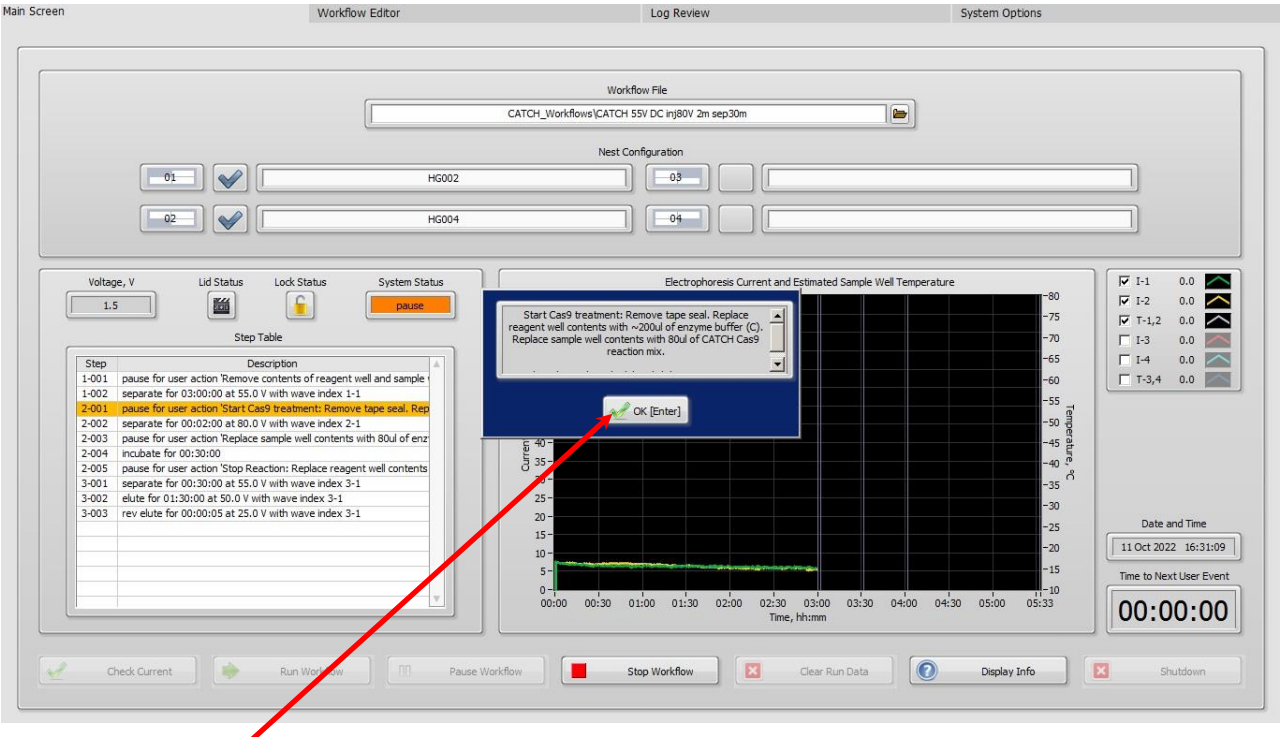
3. Remove the contents from the Reagent wells and Sample wells on the cassette(s). The well volumes are 270 µl and 85 µl, respectively.

4. Fill the Reagent well with HLS Enzyme buffer **(C),** approximately 200µl.

5. Add 80 µl of the assembled Cas9 RNP mixture to the sample well.

6. Close the lid (do not re-seal the wells with tape).

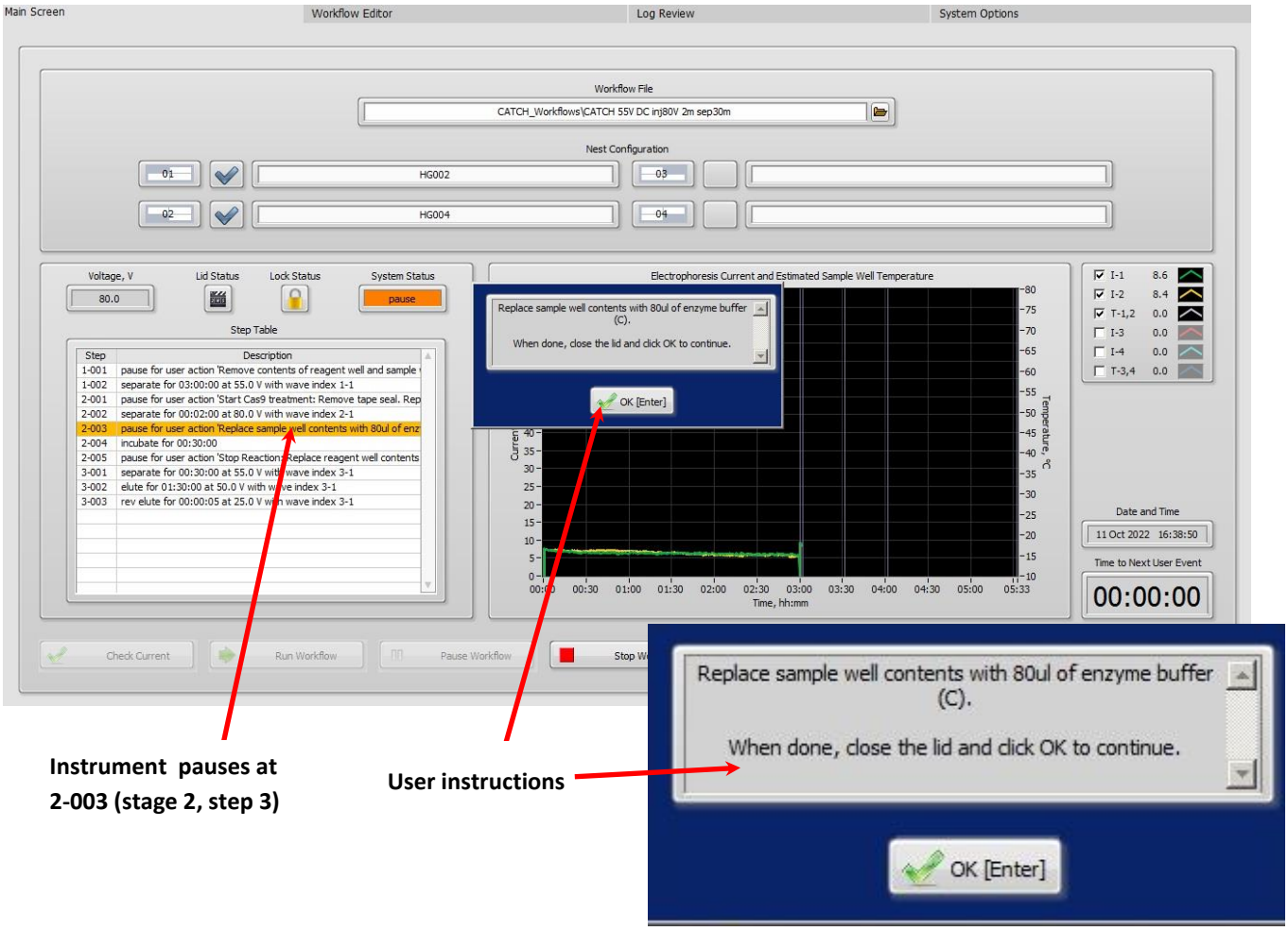
7. Press "OK" in the pop-up window to resume the workflow.



Press "OK" to resume

10. The instrument will perform a **2 minute electrophoresis step** to inject the Cas9 into the sample wall where the HMW DNA is immobilized.

11. After 2 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.

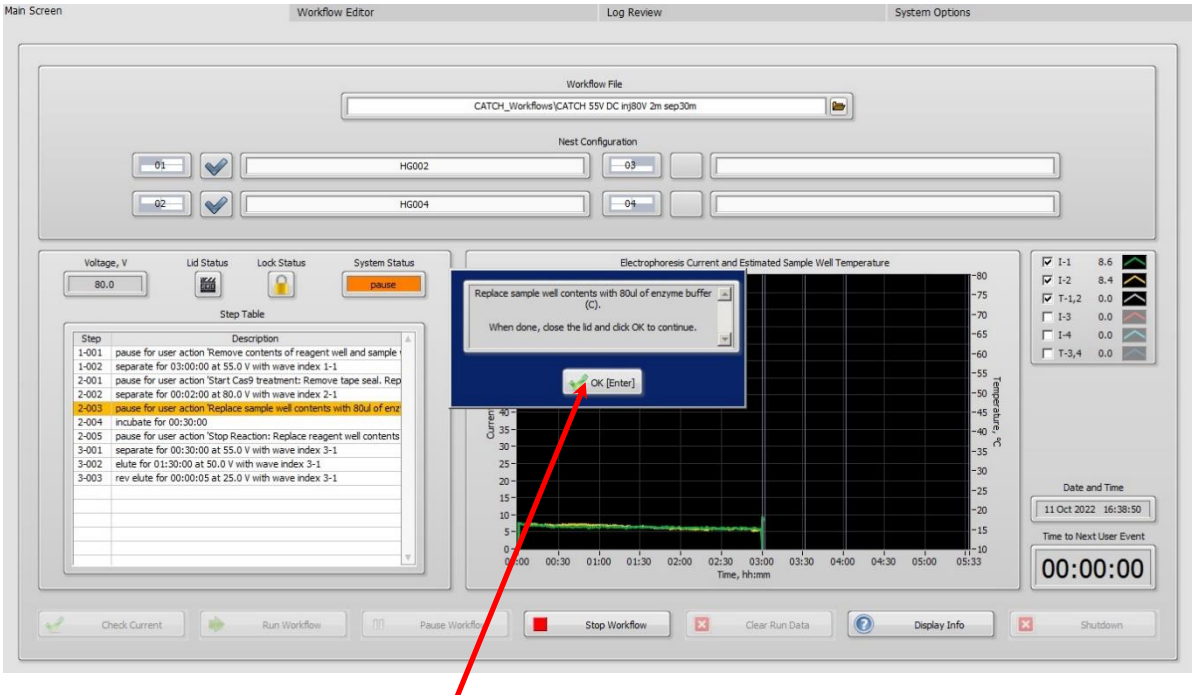
12. Open the lid.

13. Remove the contents from the Sample well(s) on the cassette(s).

14. Add 80 µl of the Enzyme Buffer (C) to the Sample well(s).

15. Close the lid (do not re-seal the wells with tape).

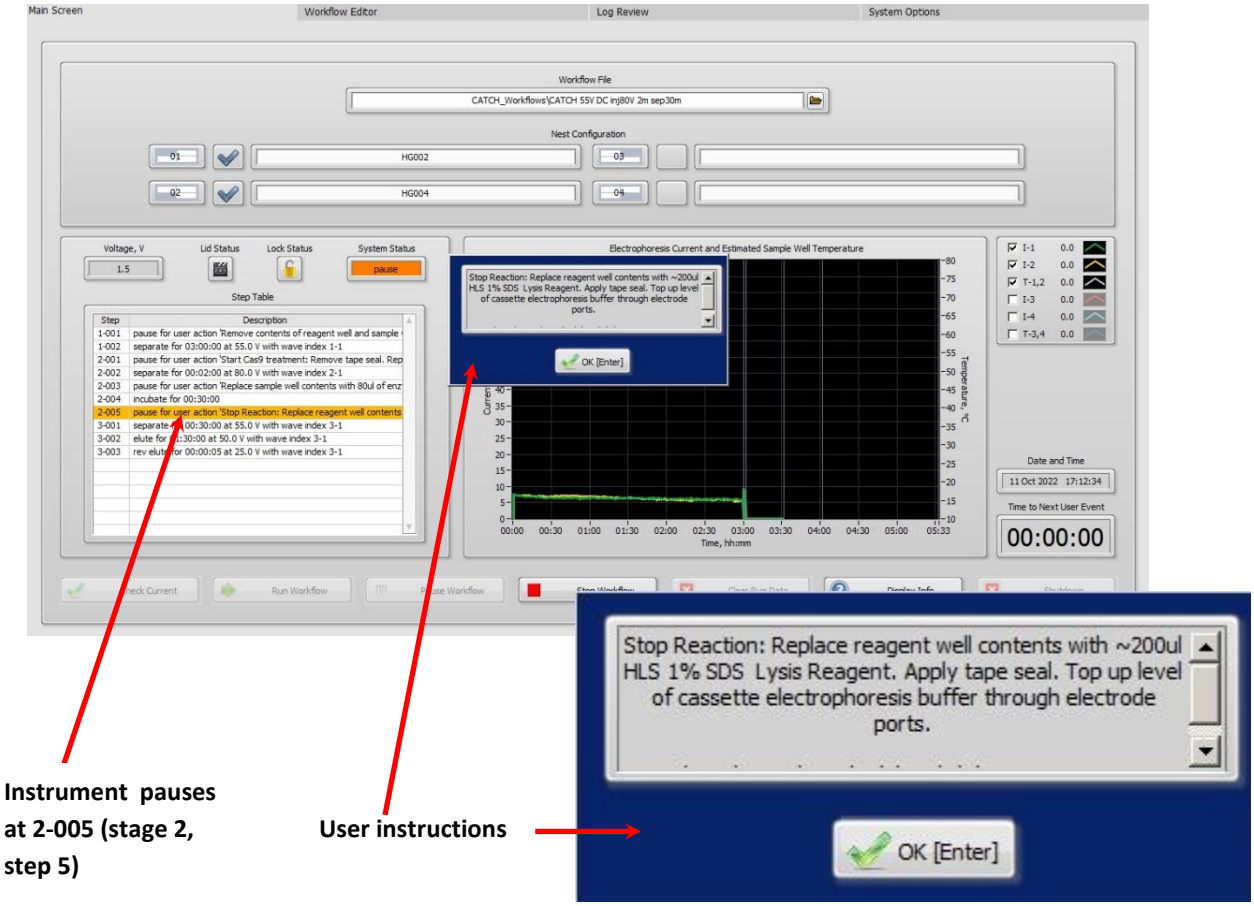
16. Press “OK” in the pop-up window to resume the workflow.



Press “OK” to resume

19. The enzyme treatment step will take **30 minutes**.

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



Instrument pauses at 2-005 (stage 2, step 5)

User instructions

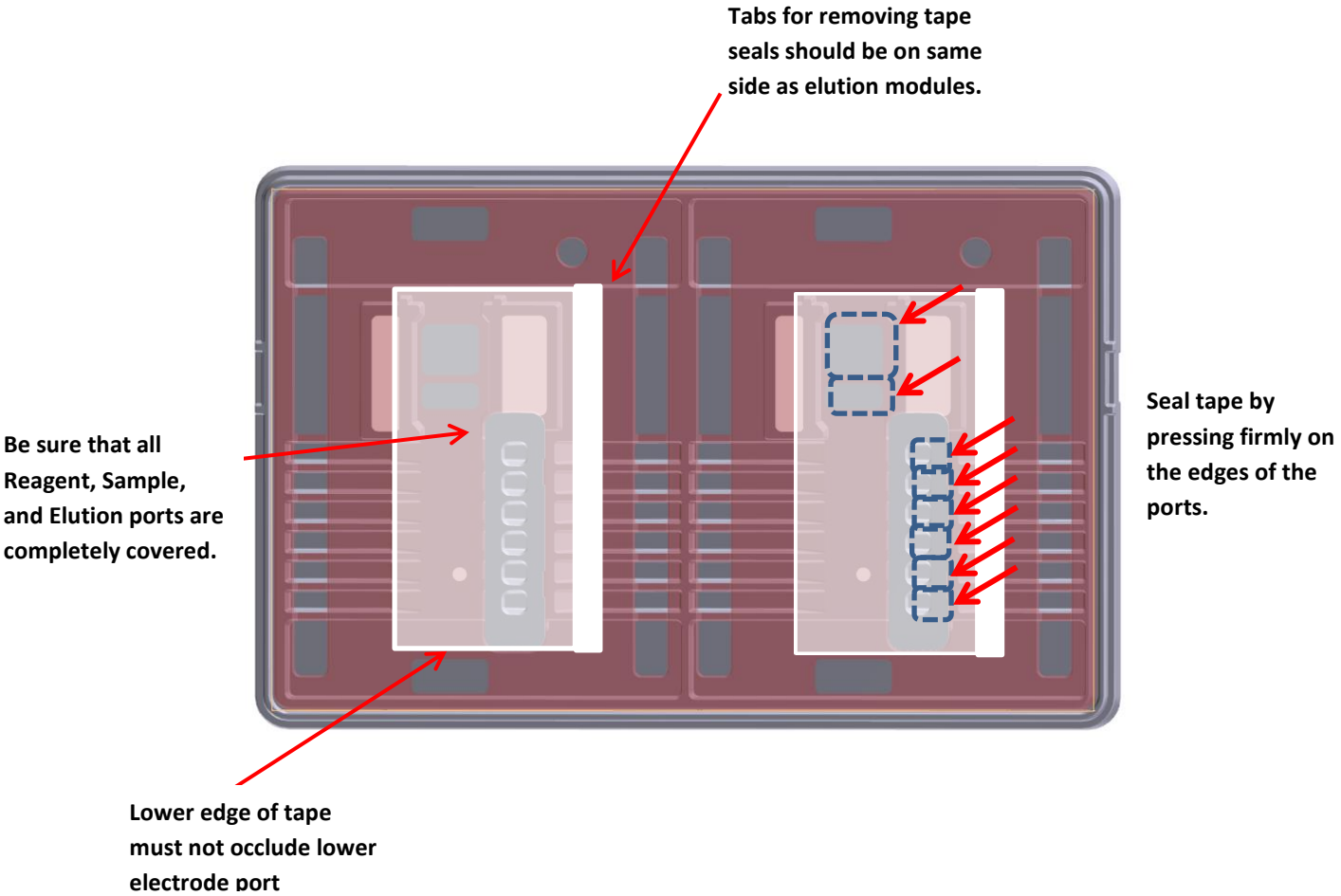
Important! The instrument will remain paused while the user action pop-up window is displayed. It is resumed when the "OK" button is pressed. If the instrument is inadvertently resumed, press the "Pause Workflow" button in the Command Menu to re-pause the instrument and continue with the manual user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

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

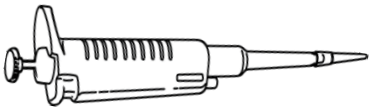
22. Add approximately 200 µl HLS Lysis Reagent 1% SDS (H) to the Reagent well(s).

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

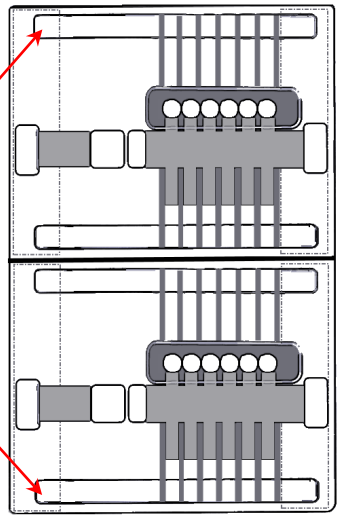
23. Close the lid and **re-seal the cassette wells with tape**.

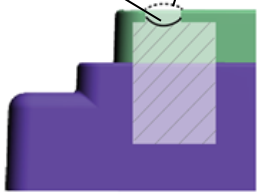


24. Add running buffer (E) to the cassette to replace evaporated buffer.



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





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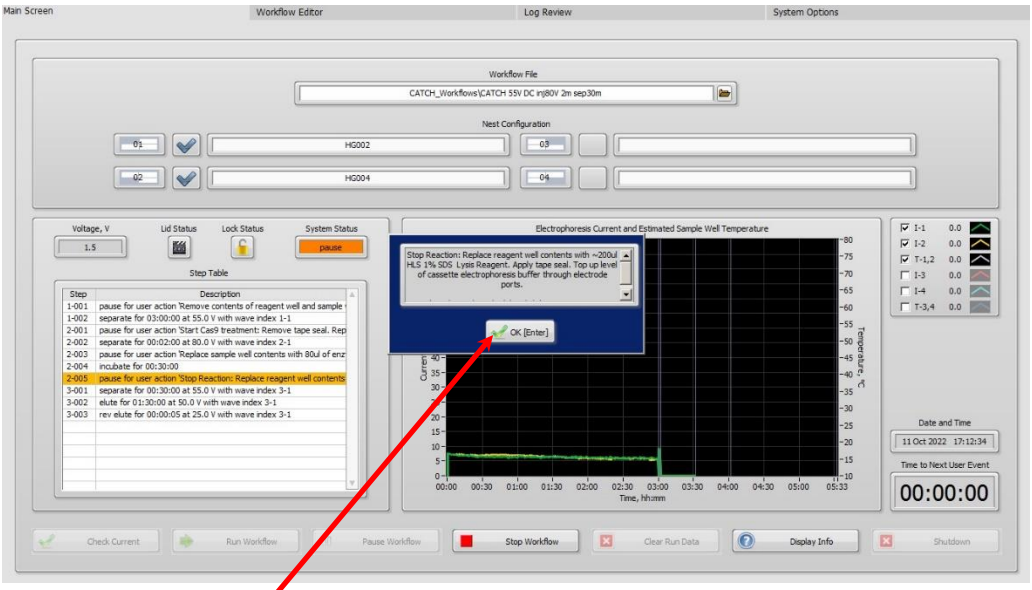
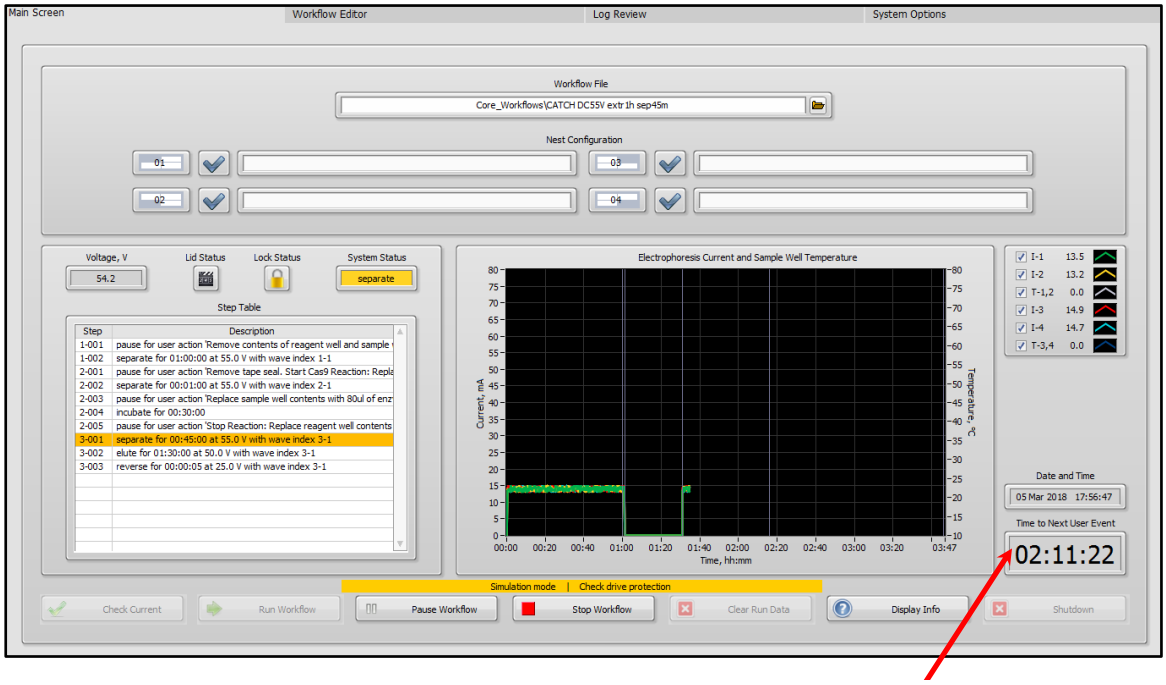


Press "OK" to resume

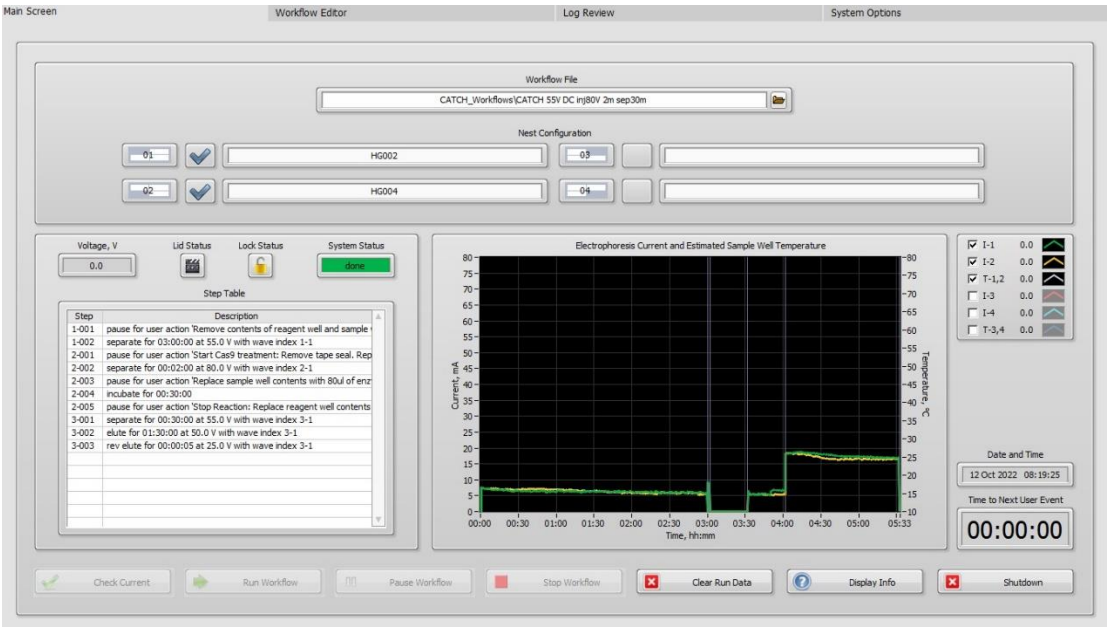
H. Stage 3: Collection Stage

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



Time remaining to the end of the run

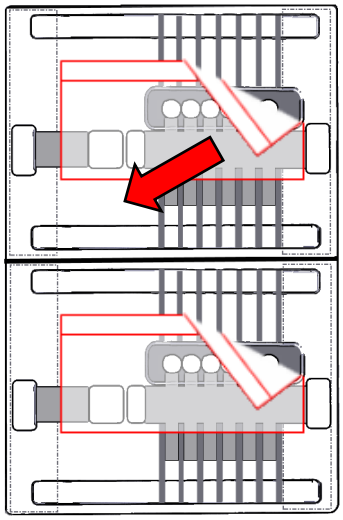
Main Screen at Run Completion:



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

3. 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 CATCH products are >100kb), remove the contents of the elution modules.

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

I. QPCR Analysis

A qPCR (Taqman or SYBR green) analysis of the target(s) should be undertaken for each elution well to verify the enrichment and amount of target DNA. Using a Taqman assay with a reference gene such as RNaseP (Life Technologies) will provide a more accurate result.

Based on an input of 300,000-400,000 diploid mammalian cells, enrichment relative to a reference gene can be as high as 100-700X.

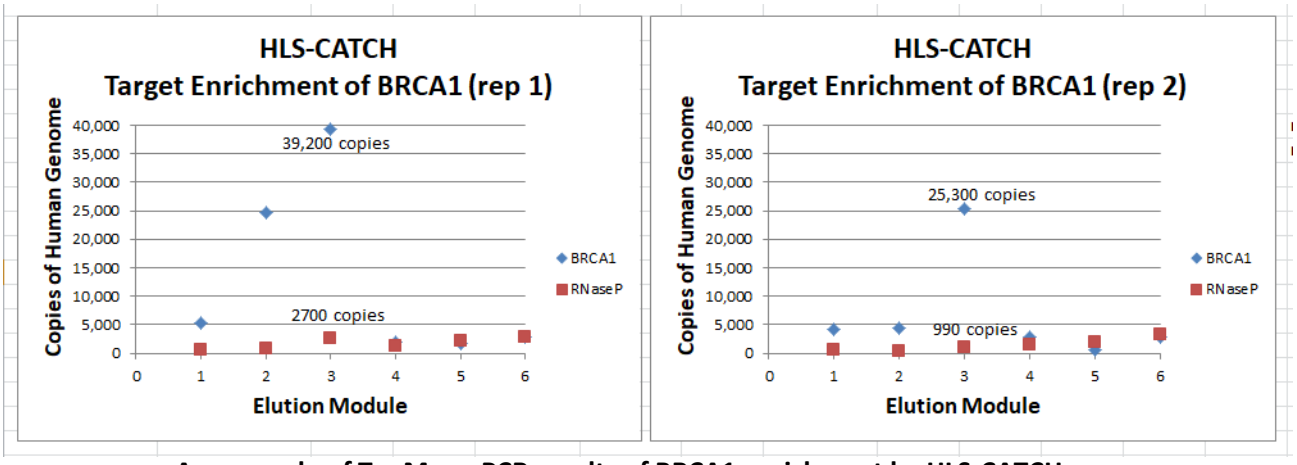
Important! SDS that is present in eluted samples can be inhibitory to qPCR reactions. Hydroxypropyl-beta-cyclodextrin (bCD) eliminates SDS interference. bCD is provided with CATCH kits as an additive for qPCR reactions.

TaqMan Reaction with bCD (J) Additive

Custom primer/probe sets for specific targets can be obtained from Life Technologies. A RNaseP control with a second color is sold as standard product as well. The following reaction set up should be used (includes the addition of bCD):

Suggested qPCR Reaction Set Up

2X master mix	10 ul
Primer/probe reagent	2 ul
0.33% bCD (J)	**6 ul**
DNA sample	2 ul
Total volume	20 ul



An example of TaqMan qPCR results of BRCA1 enrichment by HLS-CATCH

J. Sequencing Preparation

When samples and been have been collected and pooled, it is useful to concentrate the samples. For low molecular weight targets use Ampure beads (or Ampure PB for PacBio libraries). For high molecular weight targets, use Sage Science Hi-Bead reagents are which included in the HLS-CATCH kits. The instructions for using Hi-Beads can be found here:

https://sagescience.com/wp-content/uploads/2022/09/Sage-Hi-Bead-Protocol-460056-Rev-C.pdf

Use 2 µl of the concentrated sample to a run a Qubit HS assay to estimate the DNA amount prior to library construction.

HIT kit Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	13-2	Added Revision Change Log
D	E	2/8/18	13-3	Extraction stage in workflow chart changed from 3hr to 1-3 hr
D	E	2/8/18	13-12	Added an optional step at the end of DNA extraction for removal of SDS in the buffer chamber around the anode
D	E	2/8/18	13-15 13-16	Added step to replace Enzyme buffer in the Reagent well in addition to the well after Cas9 injection
E	F	6/18/18	13-1	Change DNA equivalency load recommendation from 10ug to 2.5 ug
E	F	6/18/18	13-3,4,5	Revised Cas9 guideRNA assembly procedure
E	F	6/18/18	13-9,10	New workflow file naming conventions and descriptions
E	F	6/18/18	13-18	New step to (remove running buffer) to reduce SDS in eluants
E	F	6/18/18	13-23	New step to replenish running buffer after the treatment stage
E	F	6/18/18	13-9,27	Modified extraction times – 1 hr for 10X platforms, 3 hr for PacBio and Oxford platforms
E	F	6/18/18	13-27,28	Added description of Qubit and qPCR analyses
F	G	8/17/18	13-6	Replaced buffer chamber image to show even fill line.
G	H	10/24/18	13-4 13-5	Removed Cas9 gRNA assembly -20C storage recommendations. Not verified at that temperature.
H	I	5/3/19	13-1, 13-21	Add beta cyclodextrin to HIT kits for reducing SDS in qPCR assays
H	I	5/3/19	13-1	Remove 1%SDS and Sarkosyl Lysis reagents from kit and protocol
I	J	12/12/19	all	Separate Guide for HLS Operations Manual, Create stand alone document with page numbering changes
J	K	3/16/2020	7	Add step to gRNA annealing including heating to 95, cooling, and centrifuging
K	L	5/14/2020	3, 6 21	DNA input requirement increased from 5-7 ug Cas9 injection increase from 1 to 2 minutes
L	M	1/27/22	all	SageHLS replaced with "HLS" to indicate both SageHLS and HLS2 instruments
M	P	6/14/23	2	Added NEB HiFi Cas9 Recommendation (EnGen® Spy Cas9 HF1)