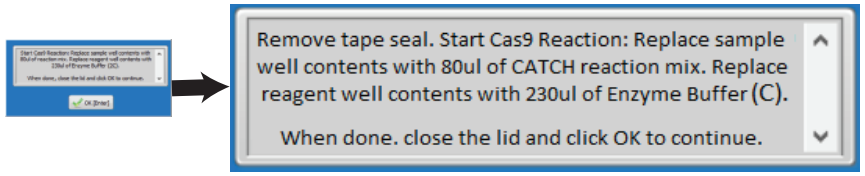
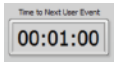
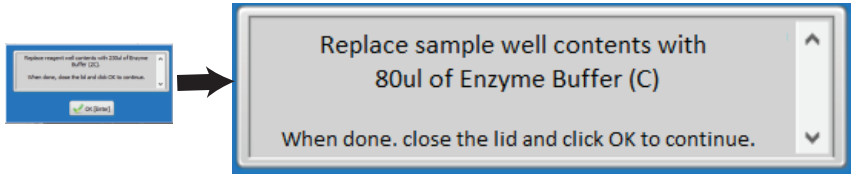


2. Open the lid and remove the adhesive seals from the cassette(s)
4. Replace the contents of the sample wells with **CATCH Reaction Mix, 80µl**
4. Replace the contents of the reagent wells with **Enzyme Buffer** (**C**), **230µl**
5. Close the lid (do not re-seal the wells).
6. In the pop-up window, press "OK" to resume the workflow
7. The User Event timer will countdown 1 minute.

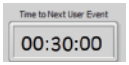


8. At the end of the count down, the instrument will pause and a pop-up window will appear:

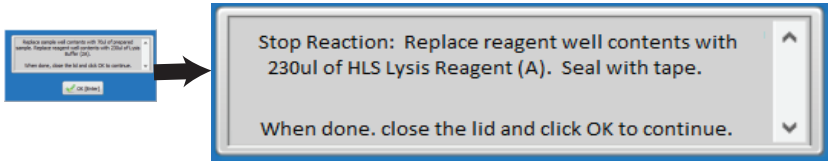


8. Open the lid
9. Replace the contents of the sample wells with **Enzyme Buffer** (**C**), **80µl**
10. Close the lid (do not re-seal the wells).
11. In the pop-up window, press "OK" to resume the workflow
12. The User Event timer will countdown 30 minutes.

H. Stage 3: Collection



1. At the end of the count down, the instrument will pause and a pop-up window will appear:



2. Open the lid
3. Replace the contents of the reagent wells with Lysis Reagent (**A, H or G**), **230µl**
4. Seal the wells with new adhesive tapes and close the lid
5. In the pop-up window, press "OK" to resume the workflow
6. The User Event timer will countdown base on the target range (2-6hrs)
7. At the end of the count down, the run will end, and samples may be removed.

⚠ **Caution!** Slowly pipette eluants with wide-bore tips to prevent shearing of DNA fragments

sageHLS™
HMW Library System
Quick Guide
HLS-CATCH™ Kit

Kit Product#: HIT-0004 and HIT-0012

Materials supplied by Sage Science:

4 /12 ea. Agarose Gel Cassettes
20 /60 ea. Adhesive Tape Strips
1 ea. HLS Lysis Reagent 10/30 ml (use one of the following):

HLS Lysis Reagent 3% SDS (**A**)
HLS Lysis Reagent 1% SDS (**H**)
HLS Lysis Reagent 3% Sarkosyl (**G**)

1 ea. Enzyme Buffer, 15/40 ml; **C**
1 ea. Running Buffer, 40/115 ml **E**
1 ea. 4X Enzyme Buffer for CATCH Mix 250µl/1ml **F**

Materials supplied or prepared by user:

S.pyogenes Cas9 enzyme, wild Type:

New England Biolabs Cat#		
M0386T	400 pmol	20 µM
M0386M	2,000 pmol	20 µM

GuideRNAs*:

Integrated DNA Technologies (IDT) - custom order
Provided in two halves; crRNA and tracRNA. crRNAs must be designed to flank the target sequence, the tracRNA is identical for all gRNAs.

*Refer to the Guidelines for Preparing HLS-CATCH Enzyme Mix for instructions on preparation.

Help: support@sagescience.com or call 978.922.1832

460030 Rev D

sage science

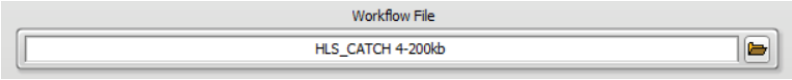
A. Prepare a cell suspension *(refer to the guide provided with the kit)*

B. Prepare the Gel Cassette *(refer to Section 4 in the SageHLS Operations Manual)*

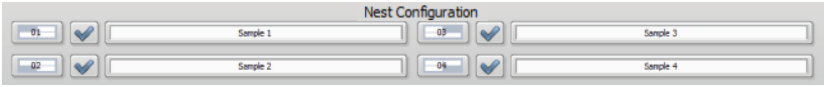
1. Clear air bubbles around the perimeter of the gel columns
2. Clear air bubbles from behind the elution wells
3. Place cassette(s) on instrument nest(s) and remove adhesive tape(s)
4. Replace the contnect of the elution wells with **80µl** of **Running Buffer** (**E**)
5. Add **Running Buffer** (**E**) to upper buffer chambers of each sample column until the buffer level is flush with the cassette cover

C. Prepare for the Run in the SageHLS Software

1. Go to the Main Screen
2. Press the folder icon and select "**HLS_CATCH**" from the file folder pop-up. Select a file with size range suffix that matches the genomic target size:



3. Select the samples lanes to be used by clicking the check boxes, and enter sample IDs in the text fields (optional):



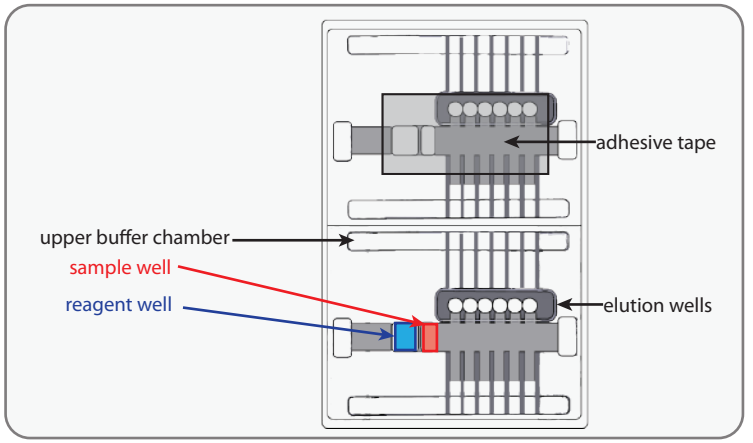
D. Run the Check Current Test

1. Close the lid
2. Press the "Check Current" button in the Command Menu:

3. A pop-up window will appear. Press "Start" in the window.
4. At the completion of a successful Check Current test, press "Return":

E. Begin the Run

1. Press the "Run Workflow" button in the Command Menu:

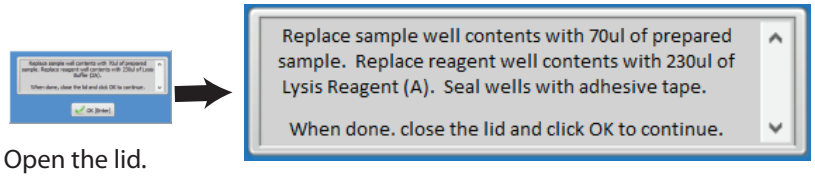


E. Begin the Run

1. Press the "Run Workflow" button in the Command Menu:

F. Stage 1: Extraction

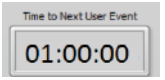
1. The instrument will pause, and a pop up window will appear:



2. Open the lid.
3. Replace the contents of the sample wells with **Cell Suspension, 70µl**
4. Replace the contents of the reagent wells with **Lysis Reagent** (**A, H or G**), **230µl**
5. Seal the wells with adhesive tape
6. Close the lid.
7. In the pop-up window, press "OK" to resume the workflow.

⚠ **Caution!** Without hurrying, users should minimize the length of the pause. It is important to load all sample wells first, and reagent wells second.

8. The User Event timer will countdown ~1 hr.



G. Stage 2: Treatment

1. At the end of the count down, the instrument will pause and a pop-up window will appear: