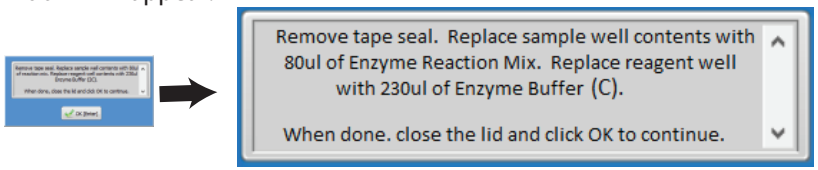
G. Stage 2: Treatment

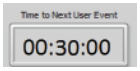
1. At the end of the count down, the instrument will pause and a pop-up window will appear:



2. Prepare the **Enzyme Reaction Mix**:

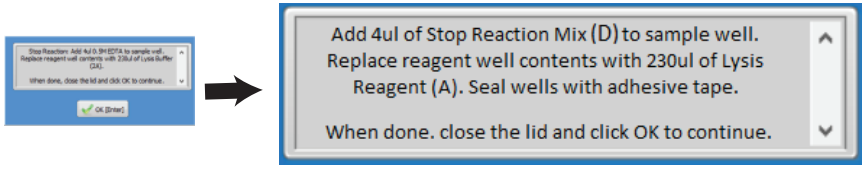
   a. Remove **NEB Fragmentase** from the freezer, briefly vortex to mix
   b. To 800**µl** of **Enzyme Buffer,** add 2**µl** of **NEB Fragmentase** (1:400 dilution), vortex to mix

3. Open the lid
4. Remove the adhesive seals from the cassette(s)
5. Replace the contents of the sample wells with **Fragmentase Reaction Mix, 80µl**
6. Replace the contents of the reagent wells with **Enzyme Buffer** (**C**), **230µl**
7. Close the lid (do not re-seal the wells).
8. In the pop-up window, press "OK" to resume the workflow
9. The User Event timer will countdown ~30 min.



H. Stage 3: Stope Reaction and 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. Remove the adhesive seals from the cassette(s)
4. To the sample wells, add **4µl** of **Stop Reaction Mix** (**D**)
5. With a P100 or 200, pipette up and down 3-4 times to mix
5. Replace the contents of the reagent wells with **Lysis Reagent** (**A, H or G**), **230µl**
6. Seal the wells with new adhesive tapes
7. Close the lid.
8. In the pop-up window, press "OK" to resume the workflow
9. The User Event timer will countdown based on the collection waveform that was selected. At the end of the count down, the run will end, and samples may be removed. This step can take several hours (1 - 4 hours)

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

sageHLS™
HMW Library System
Quick Guide
HMW DNA Extraction Kit

Kit Product #: HEX-0004 and HEX-0012

Materials supplied with Cassette Kits:

4 /12	ea.	Agarose Gel Cassettes	
20 /60	ea.	Adhesive Tape Strips	
1	ea.	HLS Lysis Reagents, 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.	Stop Reaction Mix, 250/250 µl;	**D**
1	ea.	Running Buffer, 40/115 ml	**E**

Materials supplied or prepared by user:

NEBNext® dsDNA Fragmentase® (store at -20°C) **Cat# M0348S**

A. Prepare a cell suspension *(refer to the guide provided with the cell suspension 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 contents of the elution wells with **80µl** of **Running Buffer** (**E**)
5. Add **Running Buffer** (**E**) to upper buffer chambers (see the schematic, next page) of each sample column until the buffer level is flush with the cassette cover

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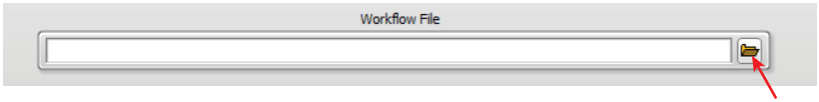
C. Prepare for the Run in the SageHLS Software

1. Go to the Main Screen
2. Press the folder icon and select a workflow from the file folder pop-up:



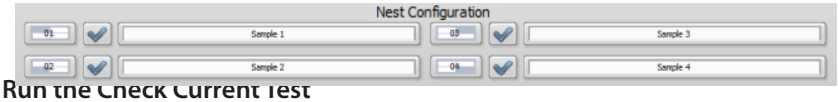
Select from one of the following options:

a. HLS_HMW DNA Extraction 1
Maximum separation, ultra-HMW fragments in well 2: ~5:00 hr total run time

b. HLS_HMW DNA Extraction 2
Compression band, 50kb and above, in wells 2 and 3: ~4:15 hr total run time

b. HLS_HMW DNA Extraction 3
Compression band, 50kb and above, in wells 2 and 3: ~3:30 hr total run time, lower yield

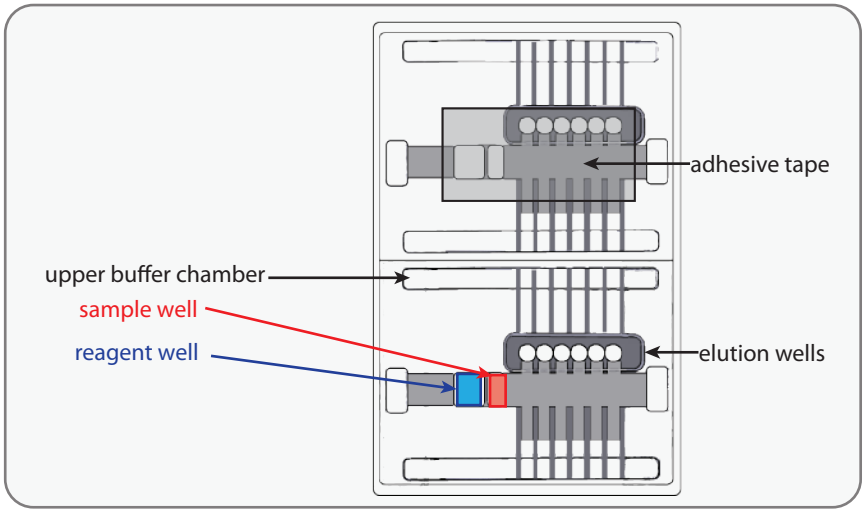
3. Select the sample lanes to be used by clicking the check boxes, and enter sample IDs in the text fields (optional):



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" :

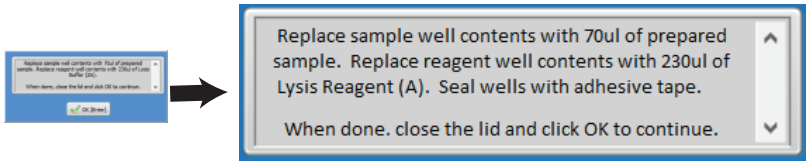


E. Begin the Run

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

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.