sageHLS
High Molecular Weight DNA Library System

Operations Manual
Revision C
11_28_17
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EQUIPMENT RATING AND SAFETY

1.1 Safety Icons Used in this Manual

In this manual, the following icons will be used to provide the user with information pertinent to the use of the SageHLS.

- **Caution!** Warns the user that injury or instrument damage may occur if the contents of the warning are not properly followed.

- **High Voltage!** Warns of the risk of electrical shock if the contents of the warning are not properly followed.

- **Hot Surface!** Warns of a hot surface that may cause injury or irritation if touched.

- **Important!** Provide important information about the proper use of the system that may influence the quality of the result.

- **Information.** Provides additional information regarding the function of the system or applications for which is used.

1.2 SageHLS Equipment Ratings

- Input supply voltage: 100-240V
- Frequency range: 50-60Hz
- Current Rating: 2.8 A
1.3 SageHLS Instrument Safe Use Guidelines

The SageHLS system is designed to operate under the following environmental conditions:

- Pollution Degree 2
- Installation category 2
- Altitude 2000m
- Indoor use
- Ambient temperature 17-25°C
- Humidity 10-80%, non-condensing

Caution! The SageHLS was designed to be operated on a flat surface. Do not operate on a tilted surface or tilt during operation.

1.4 Consumables Safe Use Guidelines

There are two types of consumables that are used on the SageHLS instrument:

1. **Agarose gel cassette.** This is an item that is manufactured by Sage Science. It consists of a styrene cassette into which an agarose gel is cast. The gel is immersed in liquid electrophoresis buffer, and all ports and openings are sealed with adhesive tape. The buffer formulated with Tris-TAPS and is not hazardous.

2. **Reagent Kits.** Reagent kits consist of reagent formulations that are manufactured by Sage Science or third party suppliers that are provided as liquids in tubes or bottles. These include lysis reagents, buffers, enzymes (dilute, in buffer), dyes, and in some cases short DNA or RNA that have been manufactured (not originating from plants or animals). All components are non-hazardous.

A partial list of reagent concentrations is provided on the next page.

Important! Reagent kits do not contain hazardous, known mutagenic, or known carcinogenic substances. Some chemicals may be skin irritants at higher concentrations than supplied in our kits (e.g. Sodium Dodecyl Sulfate).

Important! Users should refer to the Material Safety Data Sheets (MSDS) for comprehensive outline of the consumables safety classifications. These are posted at www.SageScience.com/product-support/sagehls-support/

Important! Users should use the SageHLS and related consumables in accordance to safe laboratory guidelines including the use of gloves, safety glasses, and lab coats.

Important! SageHLS consumables do not contain any gases or volatile compounds that can adversely affect health.
Unpacking and Installation

2.1 Unpacking the SageHLS

The SageHLS instrumentation is shipped in two boxes: one will contain the SageHLS and Accessories and the second box will contain the computer monitor in the manufacturer’s original packaging. With the boxes in the upright position, open and confirm that the following items are enclosed:

**Monitor**
- LCD computer monitor
- Video cable
- Power cord

**SageHLS**
- SageHLS Instrument
- Accessory box
  - Computer keyboard, USB
  - Computer mouse, USB
  - Rinse cassettes (for maintenance of electrodes)
  - Power supply
  - Power cord

**Caution!** Do not substitute the power cord. A replacement cord with an incorrect rating can damage the instrument or power supply.
2.2 Installing the SageHLS

1. Open the LCD monitor box, and assemble it according to the manufacturer instructions.

2. Using a box cutter, open the top seal on the SageHLS box, and open.

3. Remove the accessory box located just inside, atop the instrument. Remove the keyboard, mouse, and instrument power supply and cords from their packaging.

4. Firmly grip both sides of the bottom of the instrument and lift it along with the foam packaging inserts. The SageHLS weighs approximately 20 lbs. Place the unit onto the bench top.

   **Important!** Do not lift the instrument by the lid or lid retainer. This can damage the mechanism or impede the function of the unit.

   **Important!** After removing the instrument, open the lid and remove the foam insert and discard. The instrument can get damaged if operated with the insert in place.

5. Connect the LCD monitor (VGA port) to the SageHLS (VGA port, back panel) using supplied video cable. Connect monitor to the power outlet using the power supply supplied with monitor (Figure 2.2).

6. Insert USB connector from the computer keyboard into any USB port located on the back panel of the SageHLS (Figure 2.2).

7. Insert USB connector from the computer mouse into any USB port located on the back panel of the SageHLS (Figure 2.2).

8. Connect SageHLS instrument to the power outlet using the SageHLS power supply and power cable. The power input connector is in the lower left hand corner on the back panel of SageHLS, below the power switch (Figure 2.2).

9. Press power switch located on the rear of the instrument, and wait for software to launch (approximately 30 seconds).

10. When powered on, a blue light on the front panel of the Instrument will be on (Figure 2.3).

   *The SageHLS is ready for use. The software should automatically launch – allow 30 seconds.*
Figures 2.2 and 2.3 show the rear and front panels of the SageHLS.

Figure 2.2. Rear Panel of the SageHLS

Figure 2.3. Front Panel of the SageHLS

When in “Run” mode, a green light is also indicated on the front panel.

After the power button is pressed, the front panel LED will light, and the software will launch.
2.3 Unpacking and Storage of Gel Cassettes

Store boxes are in the upright position and confirm that following contents are present.

- 4 or 12 foil-sealed gel cassettes – store at room temperature.
- 1 package of adhesive tape for sealing elution wells
- 1 package of reagents

2.4 Unpacking Reagent Kits

SageHLS reagent kits can contain reagents with varying storage requirements. These will be noted on the reagent kit packaging and with the documentation included with the kits.

**Important!** Storing reagents at the correct temperatures is required, refer to the documentation provided with reagent kits. Improper storage can affect system performance or reagent shelf-life. Contact support@sagescience for the replacement of reagents if necessary.
Introduction

Thank you for purchasing the SageHLS from Sage Science. We urge you to read this manual to familiarize yourself with the system’s capabilities and precautions.

Caution! Use the SageHLS only as indicated in this operations manual. Injury or instrument damage can occur with improper usage.

The SageHLS is a platform on which users may purify intact DNA from cell suspensions, and immobilize the DNA in the sample well as it enters the agarose gel surface. Once immobilized, the DNA can be enzymatically treated. The immobilized DNA is subsequently cleaved, electrophoretically size-selected, and collected in buffer in six wells.

3.1 System Overview

There are four components to the SageHLS system:

- **Instrument** – The instrument is comprised of two heated gel cassette nests, an electrophoresis power supply, two electrode arrays embedded in the lid, and a single-board computer. The system computer is accessed by an external LCD monitor, mouse, and keyboard.

- **Software** – System software allows the user to program run parameters to operate the system (eg. heat, electrophoresis voltage, and time), operate the system (eg. Start, Stop, Pause), and monitor operations (eg. time remaining, electrophoresis current). The software also collects log files, and allows users to review operation data from previous runs.

- **Gel Cassettes** – Pre-cast, disposable agarose cassettes are manufactured by Sage Science. Gel cassettes have the capacity to process 2 samples per run.

- **Reagent Kits** – Reagent kits are formulated by Sage Science, and contain the necessary components to complete the processing of samples. This may include lysis reagents, conditioning buffers, enzyme mixes, electrophoresis buffers, or oligo adapters.
3.2 The Heated Nest

The SageHLS is equipped with a heated aluminum nest to control the reagent/enzyme reaction temperature conditions, and electrophoresis temperature. The nest accommodates the agarose gel cassette within which purification, enzymatic reactions, and electrophoresis occurs. The instrument is equipped with two nests, and each nest has two zones of temperature control:

- **Reaction Zones (Peltier heating/cooling)**
  - 15°C-50°C
- **Electrophoresis Zone (heating)**
  - Ambient - 40°C

![Image of heated nest](image)

**Hot Surface!** Use caution when using the SageHLS heated nest.

3.3 How the System Works

The SageHLS system uses pre-cast and disposable agarose gel cassettes. Each cassette has a 2 sample capacity.

![Image of gel cassette schematic](image)

A schematic of a single sample lane is shown below. It consists of a agarose column, with two wells into which reagents, buffers, or cell suspensions are added.
Electrophoretic current can be applied in two (perpendicular) directions. DNA is collected in six membrane-bound elution wells.

Cell suspensions are processed in three workflow stages:

**STAGE 1. EXTRACTION**

Cells are lysed. Electrophoresis is used to remove solubilized proteins and small nucleic acids. HMW DNA remains immobilized in the wall of the agarose sample well.
**STAGE 2. TREATMENT**

DNA is subject to enzymatic treatment that cleaves the DNA into electrophoretically mobile fragment sizes. Cleavage may be random or targeted.

**STAGE 3. COLLECTION**

Electrophoresis separates DNA by size within the gel column.

- a. Electrophoresis (separation)
- b. Electrophoresis (elution)

Lateral electrophoresis elutes the DNA into 6 size-binned wells with buffer, and DNA is removed with wide-bore pipet tips.
3.4 Workflows

Cell Suspensions
Kits provided by Sage Science should be used to prepare cells, spheroplasts, or nuclei prior to DNA purification in the Sage HLS. 

*Cell suspension workflows may require steps one day before running the SageHLS.*

SageHLS
The processing of samples on the SageHLS requires user interactions with the instrument (opening and closing the lid), software (pausing and resuming), and reagent agarose gel cassette (reagent transfers with a pipette). 

*SageHLS workflows may require up to one day and an overnight run to complete.*

These interactions are guided by a Workflow File. A workflow file is comprised of three Stages, each of which consist of multiple Steps. Samples are processed by linking stages into a workflow.

Sage Science provides several pre-programmed workflow files for supported application. User can modify the pre-programmed workflows and save the customized workflows under new names.

Overview of sageHLS Workflow Stages

**Stage 1: Extraction Stage – (cell suspension kit)**

*Extracts DNA from cell suspensions and removes residual byproducts.*

**Step types:**
- sample temperature, time
- electrophoresis voltage, time
- pause/resume for reagent transfer

**Stage 2: Treatment Stage – (gel cassette kit)**

*Enzymatic treatment of DNA with cleavase, targeted endonuclease, or other enzymatic processes*

**Step types:**
- sample temperature, time
- electrophoresis voltage, time
- pause/resume for reagent transfer

**Stage 3: Collection Stage - (DNA size selection options)**

*Size Separation and collection (DNA size selection). These differ by target collection size requirements.*

**Step types:**
- separation voltage, pulsed-field, time
- separation voltage, DC, time
- elution voltage, DC, time
- elution reverse voltage, DC, time
3.5 Workflow Summary

This workflow summary should be used a guideline. Refer to specific Workflow Guides (Appendix A and B) for detailed workflow information.

- **Prepare a Cell Suspension**
  In SageHLS-compatible suspension buffer

- **Prepare Gel Cassette(s)**
  Clear bubbles, top off buffer, place on nest

- **Select a Workflow File in Software**
  Appropriate for method and target size

- **Run a Check Current Test**
  Tests the electrophoretic pathways within the gel cassette(s)

- **Begin a Run**
  Close the lid and press ‘Run Workflow’

- **Stage 1: Extraction Stage**
  Purifies HMW DNA (includes pipetting steps)

- **Stage 2: Treatment Stage**
  Add enzyme mixes and incubate (includes pipetting steps)

- **Stage 3: Collection Stage**
  DNA separation and elution electrophoresis steps (unattended)

- **Sample Removal**
  Use wide bore pipette tips

~1.5 hours, May require prep on the day before a run.

~0.5 hours

1-3 hours

0.5-1 hour with pipetting steps

0.5-1 hour with pipetting steps

3 hours - overnight
Maintenance and Cleaning

4.1 Electrode Rinse: Weekly or After 5 Runs

Rinsing the SageHLS Electrodes is an important maintenance function. It is recommended that they be rinsed after every 5 runs, or at the weekly (whichever occurs first)

1. Place the blank SageHLS cassette bodies (provided by Sage Science) onto the Nests.
2. Completely fill the cassettes with deionized water.
3. Close the instrument Lid.
4. In the Main Tab, press the “Clear Run Data” button on the Command Menu Bar.
5. In the cassette and protocol fields, enter the pre-programmed “Rinse Cassette” and “Rinse Protocol” options from the drop-down menus:
6. Press “Run” on the Command Menu. The protocol will take about 6 minutes to run.
7. Open the lid. Remove the rinse cassettes, and pour out the wash into a drain.

4.2 Cleaning the Nest: As Needed

Salts may accumulate on the nest from spilt buffer. The aluminum blocks should be cleaned, as needed, using deionized laboratory water. 70% ethanol may also be used, if users are concerned about biological cross contamination.

4.3 Warranty and Service

The SageHLS does not require field preventative maintenance other than what is described above. The system is subject to depot repair at the Sage Science facility. The warranty period is one year, and covers all expenses including parts, labor and shipping. The system is subject to full replacement at Sage Science’s discretion.
Getting Started

A complete description of the SageHLS software is provided in the next section. Workflow guides for preparing HLS-compatible cell suspensions are provided in Appendix A of this document. Detailed workflow guides that include workflow-specific software screens, gel cassette usage, and reagent preparations are provided in Appendix B of this document:

- Appendix A - Cell Preparation Workflow Guides
- Appendix B - SageHLS Workflow Guides

These guides are individually provided with the appropriate consumable kits, and posted on the Sage Science support site. These should be used at the bench top for step-by-step instructions for SageHLS standard workflows.

Before starting, users should be aware of the following and prepare accordingly:

- Some kits may require the purchase of reagents from 3rd party suppliers
- Some cell suspensions may require preparation steps one or more days before a SageHLS run
- Some SageHLS runs require and overnight electrophoresis step

Important! New workflows are under development, and existing workflows may be subject to improvements or modifications. Make sure to check the Revision and Release Date of this document with the Manual or Guides posted on the SageHLS support page on the sage science website (http://www.sagescience.com/product-support/sagehls-support/).
SageHLS software

In the SageHLS software, navigate to the **Workflow Editor screen**. It is the second tab from the left:

The Stage List fields are populated with pre-set stage protocols.

### 6.1 Workflow File

A **Workflow File** links the three process **Stages**. The SageHLS instrument follows the Workflow file to run every **Step** of a process, in succession. The Workflow File uses a `.shflow` file extension.

Workflow Files are accessed by pressing the “Load Workflow” button in the Command Menu:
A file list will pop-up from the SageHLS/Workflows folder.
When a Workflow File is selected:

a) the file name will appear in the Workflow File field,

b) the three Stage Protocols that comprise the workflow will become highlighted with a yellow background

c) the Steps that comprise each highlighted Stage will appear in the Step Table. The step numbers include a prefix that corresponds to the Stage number. Each stage’s step list ends with the step sub-number (i.e. 1-001 for Stage 1).

Important! Stage Protocols may be edited or created in the Stage Editor. This is accessed in the factory setting using a super-user password. See Section 10.
The steps of any Stage Protocol can be viewed by highlighting the Stage Protocol name within a list.

A Stage Protocol can be de-selected by clicking on a blank field within the list. This will remove all of those Stage’s steps from the Step Table.
The Workflow Editor screen can be reset by pressing the New Workflow button.

This will clear the Workflow File name and deselect all highlighted Stages:

6.2 Saving a Workflow File

If a Workflow File has been modified by replacing or eliminating a step, it can be renamed and resaved using the "Save As" button.

The workflow file list will pop-up. Users may save new workflows or existing workflows with new file names.

Pre-set Workflows (with an HLS prefix), cannot be changed and re-saved. They may be saved with a new file name or modified and saved with a new file name.
Pre-set Workflow files (HLS prefix) cannot be modified.
6.3 Running the SageHLS - Main Screen

The Main Screen tab is the screen with which users interface with the SageHLS instrument. This includes initiating a run, monitoring a run, and pausing/resuming to conduct a manual pipetting step.

The default software screen on the SageHLS has a tabbed format. The Main Menu is the first tab.

6.4 Loading a Workflow File

1. If the System status is on Idle (there was a previous run), select “Clear Run” data to clear all fields and bring the system to an Idle System State:
2. Click the folder icon next to the Workflow File Field.

![Workflow File](image)

**Important!** The System Status must display “Idle”, or the Workflow File directory will not open.

3. A Workflow File directory will open. Select a Workflow File and select “OK”.

![Workflow File Directory](image)
4. The Workflow File name will populate the Workflow File field, the Workflow steps will populate the Step Table, and the System Status will indicate “ready”.

5. Enter Sample ID information into the Nest Configuration fields (optional). Sample IDs will be saved in the log file.

6.5 Running the Electrophoresis Current Test

Prior to loading a sample and running a cassette, the current test should be run to ensure that the electrophoretic properties of the cassette, based on measuring the electrical currents between electrodes, are within expected limits.
Before starting, make sure cassettes have been prepared (Section 5) and that they are placed on the nests that correspond to the sample locations that have been checked:

- Make sure that all cassette preparation steps in Section 5 have been completed.

**The Current Test Procedure:**

1. Close the SageHLS lid.

2. Once the lid is closed, press the “Check Current” button on the command button menu.

Press “Check Current”
3. A pop-up window will appear:

4. Press “Start”. The lid lock mechanism will engage, and the nest will motor will activate. As the test progresses, a check mark will appear in the box corresponding to the electrophoretic path if the measured current falls within the expected limits.

5. If the test fails, the nest will automatically lower, and the lid will disengage until the issue is rectified.

6. Press “Return” to return to the Main screen.

⚠️ Important! If a current check fails, check the buffer levels in the affected lane, and replace the buffer as needed. Contact Sage Science support if the current test continues to fail.
6.6 Running a Workflow

Press “Run Workflow”.

The active step in the Workflow Step Table will be highlighted in yellow.

The System Status field will indicate the type of step being run.
The “Time to User Event” timer will start a countdown until the next Pause step, which will require the lid to be opened, and reagent transfer steps with a pipette.

![Time to User Event](image)

If a pause step contains instructions that exceed the Step Table display, hovering the mouse pointer over a step display the complete instruction.
When the instrument reaches a Pause step, a pop-up window will appear with instructions for a pipetting or manual procedure. When the manual procedure is complete, and the lid has been closed, users should press “OK” to continue the Workflow.

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.

After the last Pause step in the Workflow, the “Time to User Event” will begin to countdown to the end of the run, typically a few hours.
Throughout the workflow the “Electrophoresis Current and Sample Well Temperature” monitor will graphically display (in real time) the electrophoresis current for each sample, and the combined sample well temperature for each nest:

![Electrophoresis Current and Sample Well Temperature Monitor](image)

### 6.7 Other Run Commands

A workflow may be paused (and then resumed), or stopped at any time during a Workflow:
If a Workflow is manually paused, the following window will pop-up. Press “OK” to continue the Workflow.

If a Workflow is stopped, the following window will pop-up. Press “OK” to abort the protocol, or “Cancel” to return to the Workflow.
6.8 Running a Single Lane on a Cassette

A single lane can be run on a cassette and used a later date:

1. Make sure only the lane to be used is checked in the Nest Configuration in the Main Screen.

2. Save the original adhesive tape that the cassette was packaged with.

3. The unused lane can be left unsealed during the entire run on the active lane.

4. At the end of the run, re-seal the cassette with the original adhesive tape. Make sure to apply pressure (with a finger or lab marker) around all wells and chambers. With a marker, indicate which lane is unused.

5. Store the cassette at room temperature until needed.
6.9 Accessing the Stage Editor Screen

Workflow stages can be created or edited in the Stage Editor screen.

The Stage Editor screen is hidden under one level of user control, and can be accessed by entering a password:

1. In the Main Screen, press the “Display Info” button in the Command Menu.

2. The “Display Info” window will pop up. Press the “Advanced Tabs” button.

3. A Setup Password window will appear. Enter the password “pips”:
The Stage Editor will appear as a fifth tab on the right side of the screen.

6.10 Opening an Stage File

1. In the Command Menu, press “Load Stage”:
2. A file folder window will pop-up listing the pre-set protocols in the “Preset Stages” folder. All stage files have a.shstage extension. Select a Stage file to load and press “OK”.

![File folder window with stage files]

3. When a Stage file is loaded, the following fields will become populated:

- **Stage File**: The name of the loaded file
- **Stage Type**: The type of stage assigned the loaded file (extraction, treatment, or collection)
Waveform Table: Parameters for electrophoresis waveforms. Index 1 indicates direct current. Others are pulsed-field. This includes preset waveforms and any new waveforms that may have been programmed for the loaded protocol.

Step Table: The Step assigned to the loaded Stage file.

6.11 Changing the Stage Type

Click the arrows in the Stage type field to toggle between the three stages.
6.12 Editing a Stage Step

A new Step can be programmed in the Stage Step fields:

There are several State/Actions that can be programmed. These are selected from a drop-down menu which appears when the “State/Action” field is clicked with a mouse:

The programmable State/Action Steps are:

**Pause:** Pauses a run, and a pop-up window appears. User can enter instructions as text that will appear in the pop-up. Text is entered in the “Prompt for Pause State” field. No other parameters are entered.
**Set Temp:** Sets the temperature of the sample well and gel column. The sample well can be set between 15-50°C. The gel column can be set to 30°C, or OFF. In a workflow, the temperatures will remain at the set points, until another “set temp” step is reached, or at the end of a run, when the heating function turns off.

**Incubate:** The instrument remains at an active state for a period of time, with no electrophoresis. This defines the amount of time that a temperature is applied to a sample. The amount of time is only parameter entered.

**Separate, Elute, and Reverse:** Electrophoresis parameters. “Separate” is electrophoresis along the separation column. “Elution” is electrophoresis along the elution pathway (perpendicular to the separation pathway, into the elution wells). “Reverse” is the reverse direction of the Elution pathway - typically for a short reverse to release DNA from the elution well membrane. Time of electrophoresis, electrophoresis voltage, and Wave Index (a waveform from the Waveform Table) are entered.
6.13 Editing the Step Table

A step can be highlighted by clicking with a mouse pointer. Users can move the highlighted steps up and down the list by pressing the “Move Up” and “Move Down” buttons.

A highlighted step can be deleted by pressing the “Delete” button.

To edit the Step Table, program a State/Action in the Stage Step fields (previous section). The step can be added to the Step Table in several ways using the buttons on the left of the table:
**Insert:** Inserts the programmed step in the table **before** the highlighted step.

**Append:** Appends the programmed step to the **end** of the list.

**Replace:** Replaces the parameters in the **highlighted** step.

**Edit:** Enters the parameters in the highlighted step into Stage Step fields.

### 6.14 Saving a Stage File

Press “Save As” to save a Stage File. Preset protocols cannot be altered and saved with the same file name.

After saving the new stage will appear in the Workflow Editor Screen.
Appendix A: Cell Suspension Workflow Guides
E. Coli Spheroplasts
PN# CEL-ECO1

Reagents Supplied by Sage Science

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ea. Wash Buffer, 25 ml</td>
<td>E1 4°C</td>
</tr>
<tr>
<td>1 ea. Spheroplast Buffer, 40 ml</td>
<td>E2 4°C</td>
</tr>
<tr>
<td>1 ea. Qubit Lysis Buffer, 25 ml</td>
<td>E3 RT</td>
</tr>
</tbody>
</table>

Materials Supplied or Prepared by User

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
<th>Cat#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-Lyse™ Lysozyme Solution, 4 X 10</td>
<td>Epicentre®</td>
<td>R1804M</td>
</tr>
<tr>
<td>Molecular Biology Grade BSA, 20 mg/ml</td>
<td>NEB®</td>
<td>B9000S</td>
</tr>
<tr>
<td>Qubit™ Fluorometer and HS DNA Assay kit</td>
<td>Thermofisher</td>
<td>Q32851</td>
</tr>
<tr>
<td>TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Rich broth media, LB broth or Trypticase Soy Broth</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Important!

- **A day prior to HLS procedure**, start an overnight culture of the strain to be analyzed in rich broth media (e.g., LB or Trypticase Soy Broth).

- Maximum recommended cell load per lane for HLS cassettes contains **10 ug of genomic DNA**.

- Irrespective of input cell load, final input sample volume should be fixed at **70 ul per lane**.
Cell / Spheroplast preparation

1. A day prior to HLS procedure, start an overnight culture of the strain to be analyzed in rich broth media (e.g., LB or Trypticase Soy Broth).

2. The next day, start two 3ml rich broth cultures from saturated overnight culture by 1/50 dilution (60 ul of overnight diluted into 3ml rich broth).

3. Grow the two 3ml cultures at 37°C, with shaking for 2 hours. (OD 600 should be around 1 for wild type E. coli strains like MG1655).

4. Pool the two log phase 3ml cultures and mix. Dispense four 1.0 ml aliquots of log phase cell culture into four 1.5ml micro tubes. Immediately chill all four tubes on ice for a few minutes.

5. Pellet cells at room temperature 14,000Xg (max speed in Eppendorf microfuge) for 1 min. Discard all supernatant.

6. Wash each of the 4 pellets by complete resuspension (with vigorous vortexing) in 1ml of Sage Wash Buffer (E1). Re-pellet cells as in step 5. Use a P1000 to carefully remove all supernatants. Keep tubes on ice when not handling them.

7. Resuspend each of the 4 pellets in 200 ul Sage Wash Buffer (E1) by pipetting and vigorous vortexing.

8. Pool all four 200 ul aliquots of resuspended cells into one 1.5 tube, pellet again, and resuspend entire cell population in 200 ul of Sage Wash Buffer (E1).

9. Prepare fresh Sage Spheroplast buffer+BSA by mixing 1 mL of Sage Spheroplast Buffer (E2) with 5uL of NEB nuclease-free BSA (20 mg/ml).

10. Dilute Ready-Lyse lysozyme (Epicentre Technologies, Cat #R1804M): 2.5 ul Ready-Lyse into 100 ul of Sage Spheroplast Buffer+BSA. Mix by gentle pipetting several times. (Lysozyme is prone to shear denaturation.)

11. Add 1.5 ul of diluted lysozyme in Spheroplast Buffer +BSA to the 200 ul of E. coli cells. Mix by gentle pipetting several times.

12. Allow lysozyme digestion to proceed on lab bench at room temperature for 30 minutes, then hold digest on ice.


14. Adjust spheroplast concentration to desired DNA content using Sage Wash Buffer buffer (E1). Input volume should be 70ul irrespective of cell load. Maximum cell load should be limited to 10 ug genomic DNA per HLS lane.
Quantification of DNA in a cell suspension using the Qubit HS assay

1. Gently mix the cells/spheroplasts by swirling, gentle vortexing, or pipetting. Transfer 10uL aliquots in triplicate to 1.5 ml microcentrifuge tubes.

2. To each microfuge tube add 190uL of Sage Qubit lysis buffer (E3) and mix by pipetting up and down several times. The mixing should be rapid, but should not generate bubbles or foaming - the goal is to disperse the cells quickly before they lyse and form a condensed clot of DNA.

3. Vortex the tubes as vigorously as possible for 30 – 60 seconds.

4. Add 600uL of TE (10 mM TrisHCl, 1mM EDTA, pH 7.5 to 8.0) to each tube, and vortex at maximum speed for 30-60 seconds.

5. If necessary, centrifuge (maximum speed) for 2 to 5 seconds to reduce any foam.

6. Dispense 5 ul of diluted lysed samples from step 5 into Qubit assay tubes.

7. Perform Qubit HS assay on the 5 ul sample from step 6. Use 195 of Qubit HS reagent for each lysed sample, and pipette Qubit assay mixture up and down several times to ensure homogeneity.

8. Read the Qubit assay tube concentration. Depending on the Qubit model, the Qubit tube concentration can be expressed as ng/ml or ng/ul (check!). Assuming the readout is ng/ml, DNA content of the original cell/spheroplast suspension can be calculated as:

\[ \text{[Qubit tube conc, ng/ml]} \times (800/10) \times (200/5) = \text{[DNA conc original cell suspension, ng/ml]} \]

The first term is the dilution factor involved in making the diluted lysate (step 5), and second term is the dilution factor for the Qubit HS assay.

9. Average the three replicates to estimate the DNA content of the original suspension.

10. If replicates vary by >15%, the cells in the original suspension may be lysed or clumped.
Mammalian White Blood Cells from Whole Blood
PN# CEL-MWB1

Reagents Supplied by Sage Science

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Description</th>
<th>Supplier</th>
<th>Cat#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ea.</td>
<td>10X RBC Lysis Buffer, 275 ml</td>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>1 ea.</td>
<td>Suspension Buffer, 30 ml</td>
<td></td>
<td>M2</td>
</tr>
<tr>
<td>1 ea.</td>
<td>Qubit Lysis Buffer, 25 ml</td>
<td></td>
<td>M3</td>
</tr>
<tr>
<td>12 ea.</td>
<td>Cell strainers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Materials Supplied or Prepared by User

<table>
<thead>
<tr>
<th>Description</th>
<th>Supplier</th>
<th>Cat#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qubit™ Fluorometer and HS DNA Assay kit</td>
<td>Thermofisher</td>
<td>Q32851</td>
</tr>
<tr>
<td>TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)</td>
<td></td>
<td>N/A</td>
</tr>
</tbody>
</table>

Important!

- Whole blood should be collected with Acid Citrate Dextrose (ACD) or sodium EDTA anticoagulants, and stored at 4°C.
- Blood should be used within 5 days of collection.
- Maximum recommended cell load per lane for HLS cassettes contains **10 ug of genomic DNA**.
- Irrespective of input cell load, final input sample volume should be fixed at **70 ul per lane**.
Isolation of WBC: all steps at 4°C

(Dilute the provided 10X RBC Lysis Buffer (M1) in distilled H₂O to make 1X RBC Lysis Buffer. You will need 80ml of 1X RBC Lysis Buffer per cassette. Chill on ice before use.)

1. Mix the blood to ensure that it is a homogeneous solution. Add 12 mL whole blood to 37mL of cold 1X RBC lysis buffer.

2. Incubate for 5 minutes at 4°C. Halfway through this incubation gently invert tube 3 times to mix. The initial opaque dark red solution will clear and become lighter in color.

3. Centrifuge at 2,400 x g for 4 minutes, decant and discard the supernatant.

4. Add 20mL 1X RBC lysis buffer.

5. Resuspend the cell pellet gently by pulsing 3 times for 2 to 5 seconds each, on a vortex set to 1800rpm. There should be no visible clumps left after vortexing.

6. Centrifuge at 2,200 x g for 2 minutes, decant and discard the supernatant.

7. Repeat steps 4-6. The pellet should be almost completely clear of red or pink color.

8. After decanting the supernatant, let the tube sit for 1 minute, then aspirate the remaining buffer using a P1000 pipettor with 1ml tip.

Resuspension of WBCs in HLS Suspension Buffer buffer

1. Add 1mL of Sage HLS Suspension Buffer (M2) to the cells and resuspend them by slow gentle pipetting with a P1000 pipettor. Note that the HLS Suspension Buffer solution is slightly more viscous than RBC lysis buffer due to a higher concentration of sucrose.

2. Examine the solution carefully for clumps, this is best done by drawing the solution into a 1mL pipet tip, and holding the tip up to the light.

3. If clumps are visible, filter the suspension through a 40 micron cell strainer (place the strainer in a new tube, and pour or pipet the cells into the strainer. Some tapping may be needed to start the liquid flow). Remove and discard the strainer.

4. Centrifuge for 10 to 20 seconds at 200 RPM to collect all the liquid at the bottom of the tube; the cells should not settle in this step.

5. Quantify the cells using a cell counter or a hemocytometer. Alternatively, determine the DNA content per unit volume of the suspension using the Qubit lysis procedure in (Next Page). (The expected concentration of gDNA in the resuspended cell prep is 200 – 300 ng/ul.)
Quantification of DNA in a cell suspension using the Qubit HS assay

1. Gently mix the cells/spheroplasts by swirling, gentle vortexing, or pipetting. Transfer 10uL aliquots in triplicate to 1.5 ml microcentrifuge tubes.

2. To each microfuge tube add 190uL of Sage Qubit lysis buffer (M3) and mix by pipetting up and down several times. The mixing should be rapid, but should not generate bubbles or foaming - the goal is to disperse the cells quickly before they lyse and form a condensed clot of DNA.

3. Vortex the tubes as vigorously as possible for 30 – 60 seconds.

4. Add 600uL of TE (10 mM TrisHCl, 1mM EDTA, pH 7.5 to 8.0) to each tube, and vortex at maximum speed for 30-60 seconds.

5. If necessary, centrifuge (maximum speed) for 2 to 5 seconds to reduce any foam.

6. Dispense 5 ul of diluted lysed samples from step 5 into Qubit assay tubes.

7. Perform Qubit HS assay on the 5 ul sample from step 6. Use 195 of Qubit HS reagent for each lysed sample, and pipette Qubit assay mixture up and down several times to ensure homogeneity.

8. Read the Qubit assay tube concentration. Depending on the Qubit model, the Qubit tube concentration can be expressed as ng/ml or ng/ul (check!). Assuming the readout is ng/ml, DNA content of the original cell/spheroplast suspension can be calculated as:

\[
[\text{Qubit tube conc, ng/ml}] \times (800/10) \times (200/5) = [\text{DNA conc original cell suspension, ng/ml}]
\]

The first term is the dilution factor involved in making the diluted lysate (step 5), and second term is the dilution factor for the Qubit HS assay.

9. Average the three replicates to estimate the DNA content of the original suspension.

10. If replicates vary by >15%, the cells in the original suspension may be lysed or clumped.
Cell Suspension Workflow Guide

Mammalian Tissue Culture Cells
PN# CEL-MWB1

Reagents Supplied by Sage Science

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Description</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ea.</td>
<td>10X RBC Lysis Buffer, 275 ml</td>
<td>M1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4°C</td>
</tr>
<tr>
<td>1 ea.</td>
<td>Suspension Buffer, 30 ml</td>
<td>M2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4°C</td>
</tr>
<tr>
<td>1 ea.</td>
<td>Qubit Lysis Buffer, 25 ml</td>
<td>M3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT</td>
</tr>
<tr>
<td>12 ea.</td>
<td>Cell strainers</td>
<td></td>
</tr>
</tbody>
</table>

Materials Supplied or Prepared by User

<table>
<thead>
<tr>
<th>Description</th>
<th>Supplier</th>
<th>Cat#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qubit™ Fluorometer and HS DNA Assay kit</td>
<td>ThermoFisher</td>
<td>Q32851</td>
</tr>
<tr>
<td>TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Important!

- Maximum recommended cell load per lane for HLS cassettes contains 10 ug of genomic DNA, (approximately 1.5e06 human diploid cells).
- Irrespective of input cell load, final input sample volume should be fixed at 70 ul per lane.
Isolation of WBC: all steps at 4°C

1. (Dilute the provided 10X RBC Lysis Buffer (M1) in distilled H₂O to make 1X RBC Lysis Buffer. You will need 80ml of 1X RBC Lysis Buffer per cassette. Chill on ice before use.)

2. Mix the blood to ensure that it is a homogeneous solution. Add 12 mL whole blood to 37mL of cold 1X RBC lysis buffer.

3. Incubate for 5 minutes at 4°C. Halfway through this incubation gently invert tube 3 times to mix. The initial opaque dark red solution will clear and become lighter in color.

4. Centrifuge at 2,400 x g for 4 minutes, decant and discard the supernatant

5. Add 20mL 1X RBC lysis buffer.

6. Resuspend the cell pellet gently by pulsing 3 times for 2 to 5 seconds each, on a vortex set to 1800rpm. There should be no visible clumps left after vortexing.

7. Centrifuge at 2,200 x g for 2 minutes, decant and discard the supernatant

8. Repeat steps 4-6. The pellet should be almost completely clear of red or pink color.

9. After decanting the supernatant, let the tube sit for 1 minute, then aspirate the remaining buffer using a P1000 pipettor with 1ml tip.

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2. Examine the solution carefully for clumps, this is best done by drawing the solution into a 1mL pipet tip, and holding the tip up to the light.

3. If clumps are visible, filter the suspension through a 40 micron cell strainer (place the strainer in a new tube, and pour or pipet the cells into the strainer. Some tapping may be needed to start the liquid flow). Remove and discard the strainer.

4. Centrifuge for 10 to 20 seconds at 200 RPM to collect all the liquid at the bottom of the tube; the cells should not settle in this step.

5. Quantify the cells using a cell counter or a hemocytometer. Alternatively, determine the DNA content per unit volume of the suspension using the Qubit lysis procedure in (next page). (The expected concentration of gDNA in the resuspended cell prep is 200 – 300 ng/ul.)
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2. To each microfuge tube add 190uL of Sage Qubit lysis buffer (M3) and mix by pipetting up and down several times. The mixing should be rapid, but should not generate bubbles or foaming - the goal is to disperse the cells quickly before they lyse and form a condensed clot of DNA.

3. Vortex the tubes as vigorously as possible for 30 – 60 seconds.

4. Add 600uL of TE (10 mM TrisHCl, 1mM EDTA, pH 7.5 to 8.0) to each tube, and vortex at maximum speed for 30-60 seconds.

5. If necessary, centrifuge (maximum speed) for 2 to 5 seconds to reduce any foam.

6. Dispense 5 ul of diluted lysed samples from step 5 into Qubit assay tubes.

7. Perform Qubit HS assay on the 5 ul sample from step 6. Use 195 of Qubit HS reagent for each lysed sample, and pipette Qubit assay mixture up and down several times to ensure homogeneity.

8. Read the Qubit assay tube concentration. Depending on the Qubit model, the Qubit tube concentration can be expressed as ng/ml or ng/ul (check!). Assuming the readout is ng/ml, DNA content of the original cell/spheroplast suspension can be calculated as:

\[
\text{[Qubit tube conc, ng/ml]} \times \frac{800}{10} \times \frac{200}{5} = \text{[DNA conc original cell suspension, ng/ml]}
\]

The first term is the dilution factor involved in making the diluted lysate (step 5), and second term is the dilution factor for the Qubit HS assay.

9. Average the three replicates to estimate the DNA content of the original suspension.

10. If replicates vary by >15%, the cells in the original suspension may be lysed or clumped.
Appendix B: HLS Cassette Kit Workflow Guides
High Molecular Weight DNA Extraction
PN# HEX-0004 or HEX-0012

Reagents Supplied by Sage Science

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Description</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 / 12 ea.</td>
<td>Agarose gel cassettes</td>
<td>RT</td>
</tr>
<tr>
<td>20 / 60 ea.</td>
<td>Adhesive Tape Strips</td>
<td>N/A</td>
</tr>
<tr>
<td>1 ea.</td>
<td>HLS Lysis Reagent 3% SDS, 10 / 30 ml</td>
<td>A, RT</td>
</tr>
<tr>
<td>1 ea.</td>
<td>Enzyme Buffer, 15 / 40 ml</td>
<td>C, 4°C</td>
</tr>
<tr>
<td>1 ea.</td>
<td>Running Buffer, 40 / 115 ml</td>
<td>E, RT</td>
</tr>
<tr>
<td>1 ea.</td>
<td>HLS Lysis Reagent 3% Sarkosyl, 10 / 30 ml</td>
<td>G, RT</td>
</tr>
<tr>
<td>1 ea.</td>
<td>HLS Lysis Reagent 1% SDS, 10 / 30 ml</td>
<td>H, RT</td>
</tr>
</tbody>
</table>

Materials Supplied or Prepared by User

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Cat#</th>
</tr>
</thead>
<tbody>
<tr>
<td>New England Biolabs</td>
<td>Q32851</td>
</tr>
</tbody>
</table>

Important!

- Prior to day of extraction, schedule availability of cells. Check that cell preparation reagents are ready.
- Maximum recommended cell load per lane for HLS cassettes contains 10 ug of genomic DNA, (approximately 1.5e06 human diploid cells).
- Irrespective of input cell load, final input sample volume should be fixed at 70 ul per lane.
A. Prepare cells using Cell Suspension Guide (Appendix A)

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

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.
6. Remove all buffer from all (6 per side) 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 buffer to all 6 wells (6 per side).

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 SageHLS.

> ! **Important!** Fill until the buffer level visually reaches the bottom side of the cassette cover.
C. Load the Workflow File

1. Use the following Table as a guide to select the most appropriate Workflow File:

<table>
<thead>
<tr>
<th>Workflow File Name</th>
<th>Description</th>
<th>Run Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMW High-Pass 242 kb vs 2</td>
<td>3 hour extraction, fragments &gt;242 in well 2</td>
<td>13:00</td>
</tr>
<tr>
<td>HMW High-Pass 291 kb vs 2</td>
<td>3 hour extraction, fragments &gt;291 in well 2</td>
<td>13:00</td>
</tr>
<tr>
<td>HMW High-Pass 340 kb vs 2</td>
<td>3 hour extraction, fragments &gt;340 in well 2</td>
<td>13:00</td>
</tr>
<tr>
<td>HMW High-Pass 400 kb vs 2</td>
<td>3 hour extraction, fragments &gt;400 in well 2</td>
<td>13:00</td>
</tr>
<tr>
<td>HLS HMW DNA Extraction</td>
<td>Maximum separation, ultra-HMW fragments in well 2</td>
<td>5:00</td>
</tr>
<tr>
<td>HLS HMW DNA Extraction 2</td>
<td>Compression band, 50kb and above, in wells 2 and 3</td>
<td>4:20</td>
</tr>
<tr>
<td>HLS HMW DNA Extraction 3</td>
<td>Compression band, 50kb and above, in wells 2 and 3, lower yield</td>
<td>3:30</td>
</tr>
</tbody>
</table>

2. Go to the Main screen of the SageHLS software:

   ![Workflow File drop-down menu](image)

   ![Lanes to be run](image)

   ![Check Current button](image)

3. Select the Workflow File from the drop down menu.
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](image)

D. Run the Check Current Test

1. Press the “Check Current” button.

   ![Check Current](image)

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

   ![Press “Start”](image)

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

   ![Press “Return”](image)
E. Stage 1: Extraction

1. Use the following Table as a guide to select the most appropriate Lysis Reagent. The extraction step will take 3 hours:

<table>
<thead>
<tr>
<th>Lysis Reagent Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLS Lysis Reagent 3% SDS (A)</td>
<td>Maximum DNA recovery, residual SDS (0.01-0.03%) in eluant</td>
</tr>
<tr>
<td>HLS Lysis Reagent 1% SDS (H)</td>
<td>Less DNA recovery (25-30%), less residual SDS (0.001 – 0.009%)</td>
</tr>
<tr>
<td>HLS Lysis Reagent 3% Sarkosyl (G)</td>
<td>Less DNA recovery (25-30%), for samples with potassium (SDS will co-precipitate in the presence of potassium)</td>
</tr>
</tbody>
</table>

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

3. Load samples in all lanes. Always use 70ul sample loading volume. (Sample wells will not be completely full.)

4. Fill Reagent Wells with HLS Lysis Buffer. Fill, but do not overfill! Leave a concave meniscus to prevent contact with sealing tape in next step. Approximate volume needed will be 220-230 ul.
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 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 SageHLS 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.
2. Prepare the Enzyme Reaction Mix:

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

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

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Dil.Factor</th>
<th>Fragmentase</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>1:800</td>
<td>0.01μl / reaction</td>
<td>i. add 2μl of NF to 398μl of Enzyme Buffer C, vortex to mix</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii. add 20μl of diluted to 780μl of Enzyme Buffer C, vortex to mix</td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>1:400</td>
<td>0.02μl / reaction</td>
<td>i. add 2μl of NF to 798μl of EB, vortex to mix</td>
</tr>
</tbody>
</table>

**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 withing 15 minutes of use is recommended.

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.

**Important!** Pulling in a the tape in diagonal fashion prevents liquid transfer between adjacent elution ports and transfer between the sample/reagent ports and the elution ports.
4. Remove all contents from the Reagent wells and Sample wells the cassettes to be run. The well volumes are 270 μl and 85 μl, respectively.

5. Add 70 μl of the Fragmentase Enzyme Mix to the Sample well.

6. Add 230 μl Enzyme Buffer (C) to the Reagent well.

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

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

9. The enzymatic treatment will take 30 minutes.
10. At the end of **30 minutes** a the SageHLS will pause, and a pop-up window with user instructions will appear.

**Important!** The instrument will remained 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. Replace the Reagent well contents with Lysis Reagent (A, G, or H), ~230 ul.

13. Close the lid and **re-seal the cassette wells with tape**.
15. Close the lid and press “OK” in the pop-up window to resume the workflow.

Be sure that all Reagent, Sample, and Elution ports are completely covered.

Tabs for removing tape seals should be on same side as elution modules.

Seal tape by pressing firmly on the edges of the ports.

Lower edge of tape must not occlude lower electrode port.

Press “OK” to resume.
F. 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.

2. After the run is complete, open the lid and remove the sealing tape from the cassette(s).
3. SageHLS workflows are designed to collect the target range (indicated by target size in HLS-CATCH or highest molecular weight DNA in HMW workflows) in well number 2. This does not guarantee that all targets will be in that well, and users may have multiple size targets. It is highly recommended that the adjacent wells, 1 and 3, are sampled for target DNA at the least.

4. 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.
SageHLS Cassette Kit Workflow Guide

HLS-CATCH
PN# HIT-0004 or HIT-0012

Reagents Supplied by Sage Science

<table>
<thead>
<tr>
<th>Reagent Details</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 / 12 ea. Agarose gel cassettes</td>
<td>RT</td>
</tr>
<tr>
<td>20 / 60 ea. Adhesive Tape Strips</td>
<td>N/A</td>
</tr>
<tr>
<td>1 ea. HLS Lysis Reagent 3% SDS, 10 / 30 ml</td>
<td>A, RT</td>
</tr>
<tr>
<td>1 ea. HLS Enzyme Buffer, 15 / 40 ml</td>
<td>C, 4°C</td>
</tr>
<tr>
<td>1 ea. Running Buffer, 40 / 115 ml</td>
<td>E, RT</td>
</tr>
<tr>
<td>1 ea. 4X Enzyme Buffer (for Cas9-Guide RNA Mix), 250µl / 1 ml</td>
<td>F, -20°C</td>
</tr>
<tr>
<td>1 ea. HLS Lysis Reagent 3% Sarkosyl, 10 / 30 ml</td>
<td>G, RT</td>
</tr>
<tr>
<td>1 ea. HLS Lysis Reagent 1% SDS, 10 / 30 ml</td>
<td>H, RT</td>
</tr>
</tbody>
</table>

Materials Supplied or Prepared by User

<table>
<thead>
<tr>
<th>Materials Type</th>
<th>Supplier</th>
<th>Cat#</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pyogenes Cas9 enzyme, wildType:</td>
<td>New Enland Biolabs (NEB)</td>
<td>M0386T (400 pmol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M0386M (2,000 pmol)</td>
</tr>
<tr>
<td>Guide RNAs (crRNAs and tracRNA)</td>
<td>Integrated DNA Technologies (IDT)</td>
<td>custom</td>
</tr>
</tbody>
</table>

Important!

- Prior to day of extraction, schedule availability of cells. Check that cell preparation reagents are ready.

- Assembly of Cas9 require about 30 minutes of effort, and should be prepared before starting a SageHLS run. It may also be prepared up to four days in advance and stored at 4°C. Users should take Cas9 reagent preparation time into consideration when planning HLS procedures.

- Maximum recommended cell load per lane for HLS cassettes contains 10 ug of genomic DNA, (approximately 1.5e06 human diploid cells).

- Irrespective of input cell load, final input sample volume should be fixed at 70 ul per lane.
A. Prepare cells using Cell Suspension Guide (Appendix A)

B. Prepare the Guide RNA Annealing Mix

1. Anneal the tracRNA and crRNAs

   a. Users should prepare the Guide RNA Annealing Mix prior to initiating a SagHLS run. The mix can be prepared ahead of time and stored up to 3 weeks at -20°C or 4 days at 4°C without loss of activity. Mix will be combined with Cas9 at the end of the DNA extraction stage of a run, requiring ~15 minutes. The mix can be stored on ice (thawed first, if necessary) prior to use.

   b. Use the Table below to prepare the Guide RNA Annealing Mix to a volume of 20 µl. A 7 µl volume from is required for the treatment of one sample. Users should scale accordingly if the same guides will be used for multiple sample treatments.

   c. In some cases it might be useful to use gRNAs which cut at closely spaced sites on either side of the target sequence, or cut a multiple sites. In these instances, anneal with equimolar amounts of each crRNA, with the total moles of crRNA at slightly higher (1.3X) ratio to total moles of tracRNA. For instance, if designing a system with two cut sites on either side of the target, anneal with all four crRNAs at 10 µM while tracRNA should remain at 30µM.

<table>
<thead>
<tr>
<th>order of addition</th>
<th>reagent</th>
<th>vol. µl</th>
<th>stock [ µM]</th>
<th>Final [ µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IDT Duplexing Buffer</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>crRNA1</td>
<td>4</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>crRNA1</td>
<td>4</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>mix and spin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>tracRNA</td>
<td>6</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>mix and spin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>20</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   *7 µl will be required to treat one sample

d. Heat the mix at 95°C for 20 minutes on a thermal cycler with a heated lid.

e. Remove the mix from heat, and allow to cool on the bench-top for 3-5 minutes.

f. Centrifuge for 30-60 seconds to collect any condensation.

g. Stored up to 3 weeks at -20°C or 4 days at 4°C. Place on ice up to 3 hours before use.

h. The mix will be used after starting a SageHLS run, during the extraction stage.
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 tap if necessary.

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.
6. Remove all buffer from all (6 per side) 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 buffer to all 6 wells (6 per side).

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 SageHLS.

---

**Important!** Fill until the buffer level visually reaches the bottom side of the cassette cover.
C. Load the Workflow File

1. Use the following Table as a guide to select the most appropriate Workflow File:

<table>
<thead>
<tr>
<th>Workflow File Name</th>
<th>Description</th>
<th>Run Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATCH 100-300 kb vs 2</td>
<td>for 100-300kb targets</td>
<td>9</td>
</tr>
<tr>
<td>CATCH 145 kb vs 2</td>
<td>for 100-180kb targets</td>
<td>13</td>
</tr>
<tr>
<td>CATCH 200 kb vs 2</td>
<td>for 145-240kb targets</td>
<td>13</td>
</tr>
<tr>
<td>CATCH 400 kb vs2</td>
<td>for 340-480kb targets</td>
<td>13</td>
</tr>
</tbody>
</table>

2. Go to the Main screen of the SageHLS software:

3. Select the Workflow File from the drop down menu.
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).

![Next Configuration](image)

Check marks indicate which lanes are active

**D. Run the Check Current Test**

2. Press the “Check Current” button.

![Check Current](image)

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

![Start](image)

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

![Return](image)
E. Stage 1: Extraction

1. Use the following Table as a guide to select the most appropriate Lysis Reagent. The extraction step will take 3 hours:

<table>
<thead>
<tr>
<th>Lysis Reagent Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLS Lysis Reagent 3% SDS (A)</td>
<td>Maximum DNA recovery, residual SDS (0.01-0.03%) in eluant</td>
</tr>
<tr>
<td>HLS Lysis Reagent 3% Sarkosyl (G)</td>
<td>Less DNA recovery (25-30%), for samples with potassium (SDS will co-precipitate in the presence of potassium)</td>
</tr>
<tr>
<td>HLS Lysis Reagent 1% SDS (H)</td>
<td>Less DNA recovery (25-30%), less residual SDS (0.001 – 0.009%)</td>
</tr>
</tbody>
</table>

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

3. Load samples in all lanes. Always use 70ul sample loading volume. (Sample wells will not be completely full.)

4. Fill Reagent Wells with HLS Lysis Buffer. Fill, but do not overfill! Leave a concave meniscus to prevent contact with sealing tape in next step. Approximate volume needed will be 220-230 ul.
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 and press “Run Workflow”. The Extraction step will take 3 hours.
Prepare the Cas9-Guide RNA Mix

a. Make sure the frozen reagents have been thawed: 4X Enzyme Buffer (F), NEB Cas9 nuclease, and (if necessary, Guide RNA Mix)

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

<table>
<thead>
<tr>
<th>order of addition</th>
<th>reagent</th>
<th>vol. µl</th>
<th>stock [ ] µM</th>
<th>final [ ] in Enzyme Mix µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nuclease free H20</td>
<td>19</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4X Enzyme Buffer (F)</td>
<td>10</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Annealed Guide RNA Mix</td>
<td>7</td>
<td>30 (tracRNA)</td>
<td>5.25 (tracRNA)</td>
</tr>
<tr>
<td></td>
<td>mix and spin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NEB Cas9 nuclease, wt</td>
<td>4</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

c. Mix by pipetting up and down.

d. Incubate at 37°C for 10 minutes in a thermal cycler with a heated lid.

e. Place on ice until the loading step in the treatment stage.
F. Stage 2: Treatment Stage

1. At the end of the Extraction Stage/Step, the SageHLS 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.
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.

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

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

5. Dilute the Cas9-Guide RNA Mix to 80 µl with HLS Enzyme Buffer (C)

6. Mix thoroughly by pipetting up and down.

5. Add 80 µl of the diluted Cas9-Guide RNA Mix to the Sample well(s).

6. Add 230 µl HLS Enzyme Buffer (C) to the Reagent well(s)

7. Close the lid (do not re-seal the wells with tape).
8. Press “OK” in the pop-up window to resume the workflow.

5. The instrument will perform a **1 minute electrophoresis step** to inject the Cas9 into the sample well where the HMW DNA is immobilized.

10. The SageHLS will pause, and a pop-up window with user instructions will appear.
11. Open the lid.

12. Remove all contents from the **Sample well(s)** on the cassette(s).

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

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

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

16. The enzyme treatment step will take **30 minutes**.
10. At the end of **30 minutes** a the SageHLS will pause, and a pop-up window with user instructions will appear. **Important!** The instrument will remained 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. Replace the Reagent well contents with Lysis Reagent (**A** or **H**), ~230 ul.

13. Close the lid and **re-seal the cassette wells with tape**.
1. Close the lid and press “OK” in the pop-up window to resume the workflow.

Be sure that all Reagent, Sample, and Elution ports are completely covered.

Tabs for removing tape seals should be on same side as elution modules.

Seal tape by pressing firmly on the edges of the ports.

Lower edge of tape must not occlude lower electrode port

Press “OK” to resume
F. 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. SageHLS workflows are designed to collect the target range (indicated by target size in HLS-CATCH or highest molecular weight DNA in HMW workflows) in well number 2. This does not guarantee that all targets will be in that well, and users may have multiple size targets. It is highly recommended that the adjacent wells, 1 and 3, are sampled for target DNA at the least.

4. 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.