sage science

Cell Suspension
Workflow Guide

E. Coli Spheroplasts
PN# CEL-ECO1

Reagents Supplied by Sage Science			Storage Temp.
1 ea.	Wash Buffer, 25 ml	E1	4°C
1 ea.	Spheroplast Buffer, 40 ml	E2	4°C
1 ea.	Qubit Lysis Buffer, 25 ml	E3	RT

Materials Supplied or Prepared by User	Supplier	Cat#
Ready-Lyse™ Lysozyme Solution, 4 X 10	Epicentre®	**R1804M**
Molecular Biology Grade BSA, 20 mg/ml	NEB®	**B9000S**
Qubit™ Fluorometer and HS DNA Assay kit	Thermofisher	**Q32851**
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 to 8.0)	N/A	
Rich broth media, LB broth or Trypticase Soy Broth	N/A	

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**.

CELECO1 Workflow Guide
Revision Change Log

Last Rev	New Rev	Date	Page#	Notes
C1	D	12/12/17	8-2	Added Revision Change Log

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.

13. Measure DNA content of spheroplast preparation using Qubit lysis method (Next Page).

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:

[Qubit tube conc, ng/ml] X (800/10) X (200/5) = [DNA conc original cell suspension, ng/ml]

The first term is the dilution factor involved in making the diluted lysate (steps 2 and 4, above), and second term is the dilution factor for the Qubit HS assay (steps 6 and 7).

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.