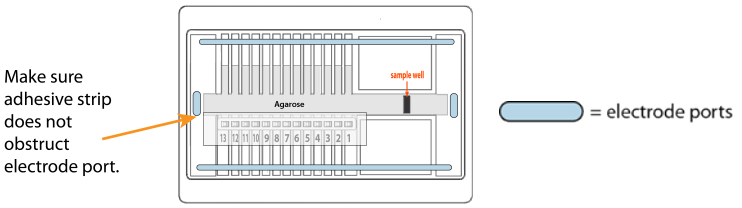
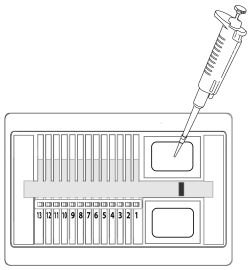
8. From left-to-right, seal the elution modules with the adhesive tape provided. Firmly rub the tape with a smooth round plastic object (like a lab marker pen) to firmly seal the elution elution wells.



9. Carefully move the cassette to the SageELF nest.

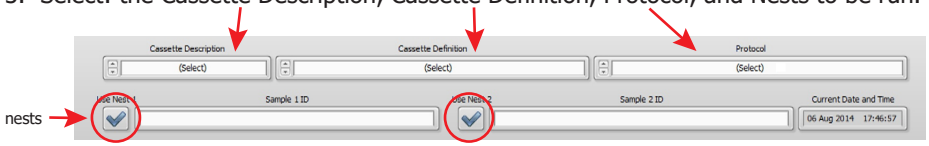
10. Top up the liquid level in the upper buffer area. Add buffer until it is completely full (>2ml). This is a critical step, refer the operation manual if unfamiliar with the proper level.

11. Remove 1.0 ml of buffer from the upper buffer area to set the correct volume.

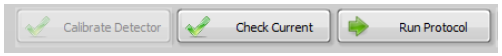


E. Run the Electorphoresis Current Test

1. Close the lid.
2. In the Main Tab, press "Clear Run Data".
3. Select: the Cassette Description, Cassette Definition, Protocol, and Nests to be run.

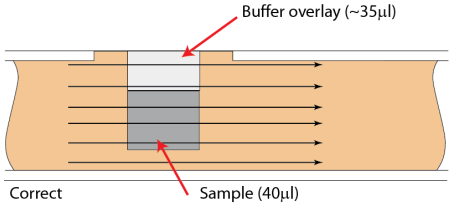


4. Press "Check Current". When the test is complete, open the lid.



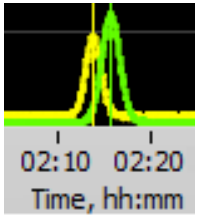
F. Load Sample(s)

1. Make sure the sample well is full. If not, top it off with buffer. Remove 40µl of buffer from the sample well, and load 40µl of sample into that well. Make sure the sample well is full after loading.



G. Run

1. Close the lid.
2. Press "Run Protocol".
3. During a run, a marker peak should be detected for each cassette that is run. The marker runs ahead of the fractionation range.



Marker 75

SageELF™ Quick Guide

0.75% Agarose Gel Cassette

for DNA fractionation between 1kb -18kb

Product No.:	**ELD7510**
Cassette Definition:	**0.75% 1kb - 18kb V2**
Cassette and Marker:	**0.75% Agarose / Marker 75**

Research Use Only

Recommended Sample Load Guidelines

Ionic strength: The ionic strength of the sample should be lower than the ionic strength of the buffer (80mM monovalent ions).

Protein in the sample: For best results, samples should be de-proteinized prior to loading.

Maximum Load: 5 µg Minimum Load: 100 ng

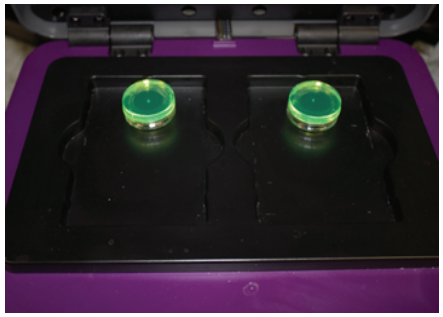
Note: This guide describes the workflow for using an internal marker. Refer to the SageELF operations manual for running timed fractionation.

A. Prepare DNA samples

1. Bring DNA sample up to 30µl with TE.
2. Bring Loading Solution/Marker 75 mix to room temperature.
3. For each sample, combine 30µl of DNA sample with 10µl of loading solution/marker mix.
4. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

B. Calibrate the Optics with the Calibration Fixtures

1. Place calibration fixture(s) onto the optical nest(s) as shown.
2. Close the instrument lid.
3. Go to the "Main" tab in the software.
4. Clear the protocol field by pressing the "Clear Run Data" button (if necessary).
5. Press the "Calibrate Detector" button.



6. In the Detector Calibration pop-up window, select the nest(s) to be calibrated.

7. Press "Start".

8. When calibration is complete, press "Return".

C. Program a Protocol

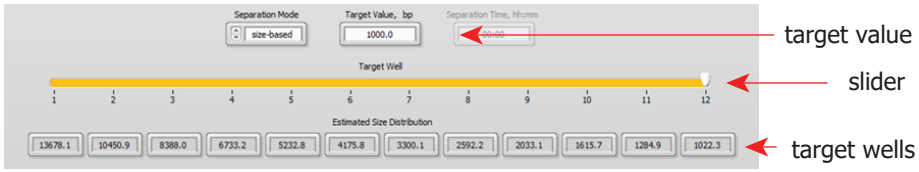
1. Go to the "Protocol Editor" tab in the software.

2. Select "New Protocol" in the menu bar.

3. Select **0.75% 1kb-18kb V2** from the "Casssette Definition" drop-down menu.

4. Using "size-based" mode, move the slider to a target elution well number.

5. Enter a value in to the "Target Value" window. This defines the range of fragments that will be collected in the target elution well. The collection range for the remaining wells will be calculated in the software.



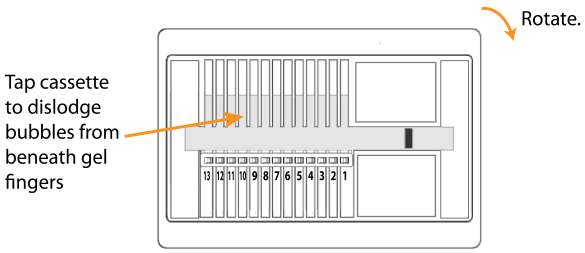
6. The chart below can be used as a guideline to estimate fractionation values.

Est. Run Time (hr)	1	2	3	4	5	6	7	8	9	10	11	12
1.94	8896	5857	4382	3009	2222	1580	1115	665	410	137	79	-
2.22	10558	7558	5566	4098	3041	2251	1650	1319	821	493	297	99
2.5	12221	9258	7044	5460	4156	3184	2415	2072	1434	1016	675	428
2.78	13883	10958	8815	7098	5567	4380	3411	2922	2247	1706	1214	880
3.05	15546	12659	10880	9009	7274	5838	4636	3870	3262	2561	1913	1455
3.33	17208	14359	13239	11196	9277	7559	6093	4915	4477	3583	2773	2154
3.61	18870	16059	15891	13656	11575	9543	7779	6058	5893	4771	3794	2975

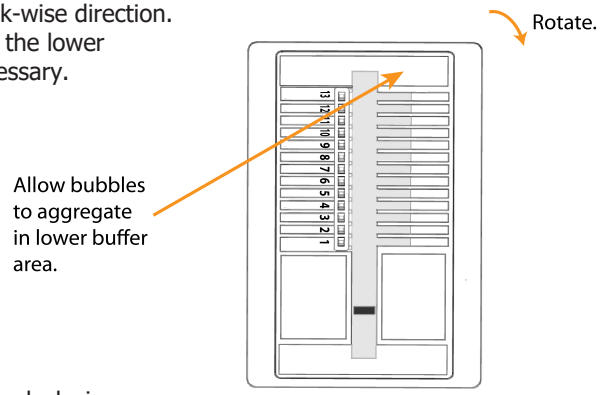
7. Press "Save As" and name the fractionation protocol.

D. Prepare the Cassette(s)

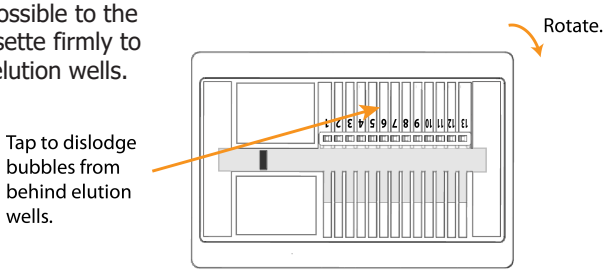
1. **While still taped closed!** Hold the cassette sideways with the elution port side down. Tap to remove air bubbles from beneath the gel fingers.



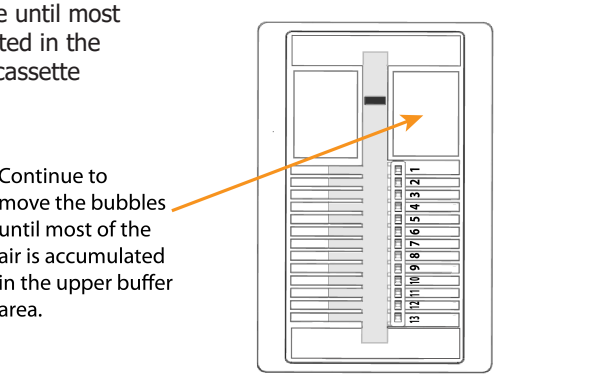
2. Rotate the cassette in the clock-wise direction. Allow the bubbles to collect in the lower buffer area. Gently tap if necessary.



3. Continue to rotate the cassette clockwise, moving as many bubbles as possible to the side buffer area. Tap the cassette firmly to dislodge any bubbles behind elution wells.



4. Continue to rotate the cassette until most of the bubbles have accumulated in the upper buffer area. Place the cassette onto a benchtop.



5. **On a flat bench top:** Peel off the adhesive tape. Grab the tape tab, hold the cassette firmly down, and pull the tape with a steady motion.

6. Remove all buffer from all 13 elution wells (set pipette to 40 µl to completely empty wells). Keep pipette tips vertical in the well to avoid damage to the membranes.

7. Add 30 µl of buffer to all 13 elution wells.