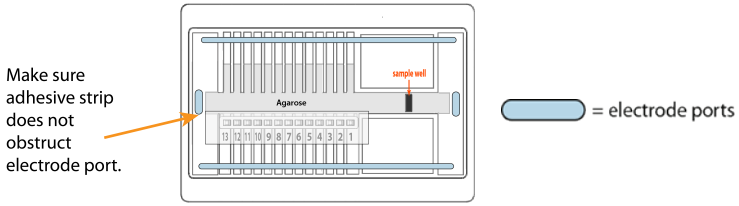
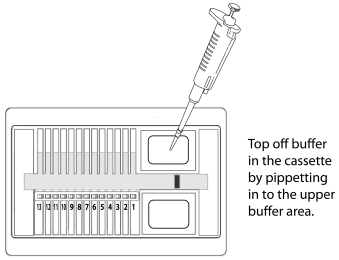
8. From left-to-right, seal the elution modules with the adhesive tape provided. Firmly rub the tape with a smooth round plastic object (like a lab marker pen) to firmly seal the elution elution wells.



9. Carefully move the cassette to the SageELF nest.

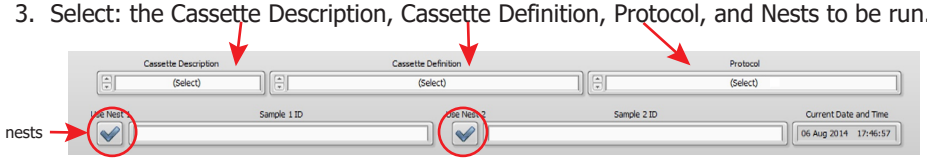
10. Top up the liquid level in the upper buffer area. Add buffer until it is completely full (>2ml). This is a critical step, refer the operation manual if unfamiliar with the proper level.

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

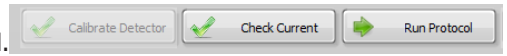


E. Run the Electorphoresis Current Test

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.

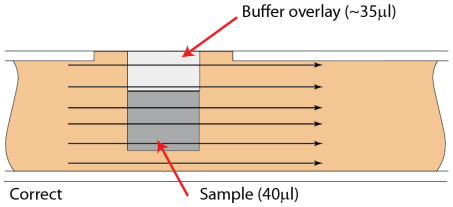


4. Press "Check Current". When the test is complete, open the lid.



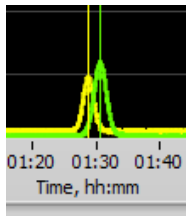
F. Load Sample(s)

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 02

SageELF™ Quick Guide

2% Agarose Gel Cassette

for DNA fractionation between 100bp -2300bp

Product No.:	**ELD2010**
Cassette Definition:	**2% 100bp - 2300bp**
Cassette and Marker:	**2% Agarose / Marker 02**

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

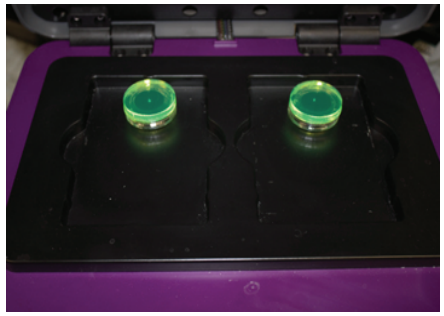
Note: This guide describes the workflow for using an internal marker. Refer to the SageELF operations manual for running timed fractionation.

A. Prepare DNA samples

1. Bring DNA sample up to 30µl with TE.
2. Bring Loading Solution/Marker 02 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

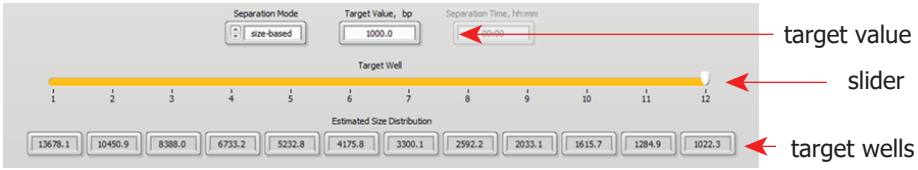
1. Place calibration fixture(s) onto the optical nest(s) as shown.
2. Close the instrument lid.
3. Go to the "Main" tab in the software.
4. Clear the protocol field by pressing the "Clear Run Data" button (if necessary).
5. Press the "Calibrate Detector" button.



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 **2% 100bp - 2300bp** 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.



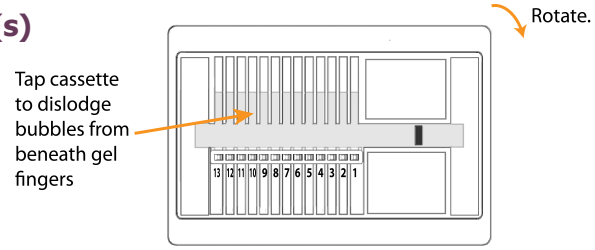
6. The chart below can be used as a guideline to estimate fractionation values.

Est. Run Time (hr)	1	2	3	4	5	6	7	8	9	10	11	12
1.26	1251	797	604	485	436	360	288	226	161	116	64	49
1.32	1349	911	698	563	494	411	334	268	202	153	100	80
1.38	1447	1025	793	640	553	462	380	309	242	190	135	111
1.45	1545	1139	887	718	611	512	426	351	283	227	171	142
1.51	1643	1253	981	795	669	563	472	393	323	264	207	173
1.57	1741	1367	1076	873	728	614	517	434	364	301	242	204
1.64	1839	1481	1170	950	786	665	563	476	404	338	278	235
1.7	1937	1595	1265	1028	845	715	609	518	444	375	313	266
1.76	2035	1709	1359	1105	903	766	655	559	485	412	349	297
1.83	2133	1823	1453	1183	962	817	701	601	525	449	385	328
1.89	2231	1937	1548	1260	1020	868	747	643	566	486	420	359
1.95	2329	2051	1642	1338	1079	918	793	685	606	523	456	390

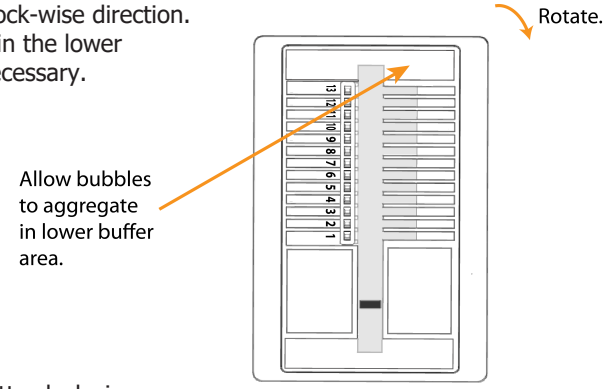
7. Press "Save As" and name the fractionation protocol.

D. Prepare the Cassette(s)

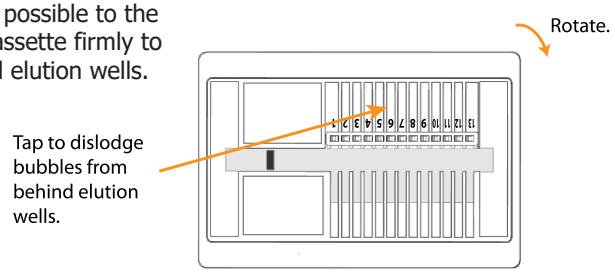
1. **While still taped closed!** Hold the cassette sideways with the elution port side down. Tap to remove air bubbles from beneath the gel fingers.



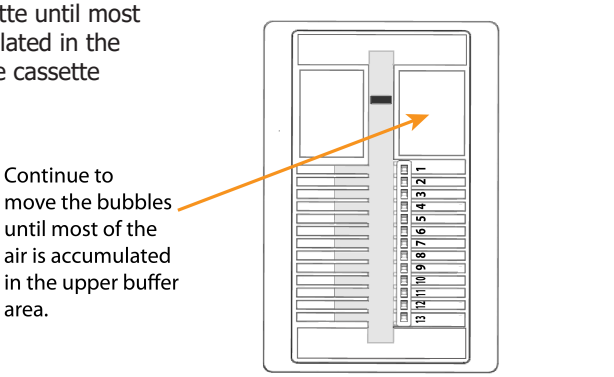
2. Rotate the cassette in the clock-wise direction. Allow the bubbles to collect in the lower buffer area. Gently tap if necessary.



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.