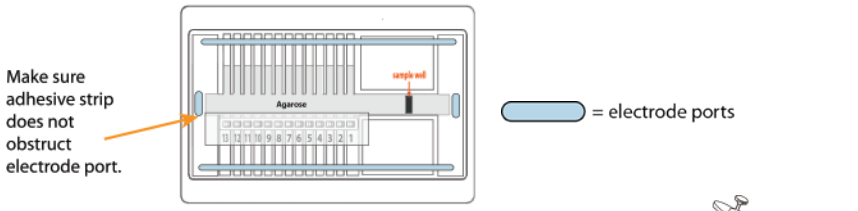
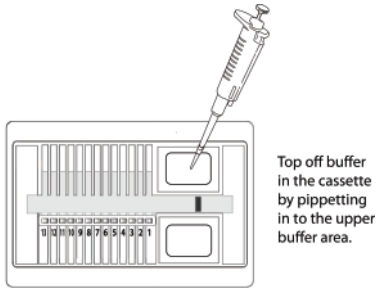
8. From left-to-right, seal the elution modules with the adhesive tape provided. Firmly rub the tape with a smooth round plastic object (like a lab marker pen) to firmly seal the elution elution wells.



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. This is a critical step, refer the operation manual if unfamiliar with the proper level.



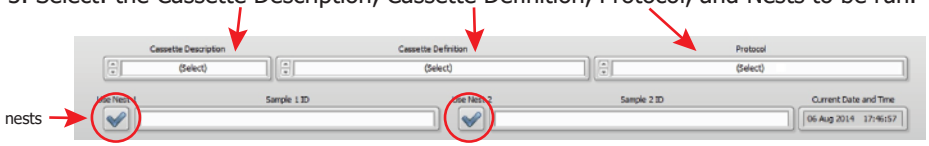
11. Remove 1.0 ml of buffer from the upper buffer area to set the correct volume.

F. Run the Electorphoresis Current Test

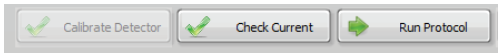
1. Close the lid.

2. In the Main Tab, press "Clear Run Data"

3. Select: the Cassette Description, Cassette Definition, Protocol, and Nests to be run.

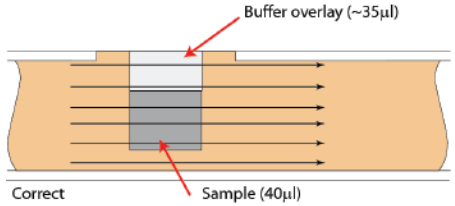


4. Press "Check Current". When the test iscomplete, open the lid.



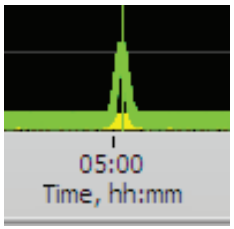
F. Load Sample(s)

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

SageELF™ Quick Guide

0.75% Agarose Gel Cassette

for DNA fractionation between 10kb - 40kb

Product No.:	**ELD4010**
Cassette Description:	**0.75% 10 kb - 40kb**
Cassette Definition:	**0.75% Agarose Marker 40**
Software Version:	**1.03**

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

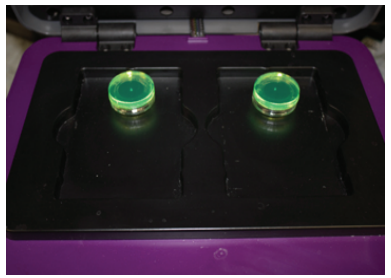
Note: this guide describes workflow using an internal marker. Refer to the SageELF operations manual for running timed fractionation.

A. Prepare DNA samples

1. Bring DNA sample up to 30µl with TE.
2. Bring Loading Solution/Marker 40 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

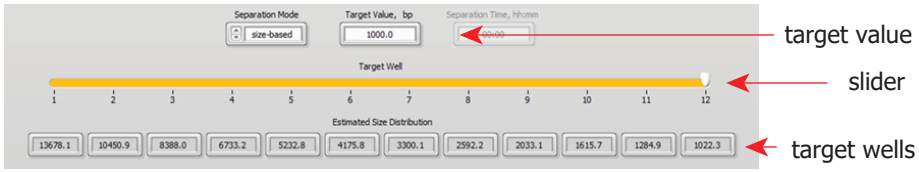
1. Go to the "Protocol Editor" tab in the software.

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

3. Select **0.75% 10 kb-40kb** 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.



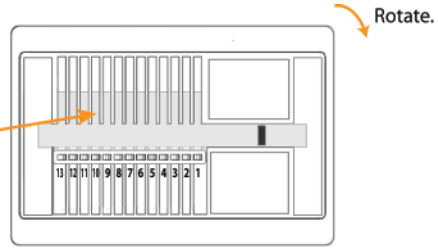
6. The chart below can be used as a guideline to estimate fractionation values. .

Est. Run Time (hr)	1	2	3	4	5	6	7	8	9	10	11	12
6		28000	26500	19400	16862	13569	10872	8860	7646	6588	5000	4500
7		2660	28000	24000	17382	14595	12076	10085	8704	8562	5688	4500
7.5			43538	25474	21963	19076	16125	13544	10819	9452	6465	5200
8			35175	28854	24388	20864	17857	15306	12645	10694	9300	6500
8.5				39014	28658	23963	21338	18509	16278	13856	11530	9809
9				62500	45506	25016	21864	19956	18390	16297	13667	11565
DNA compression occurs at this well												

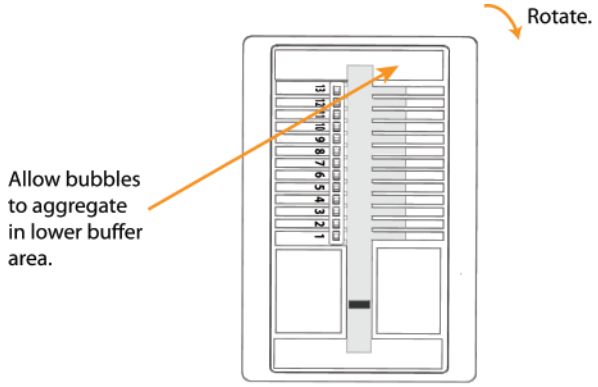
7. Press "Save As" and name the fractionation protocol.

D. Prepare the Cassette(s)

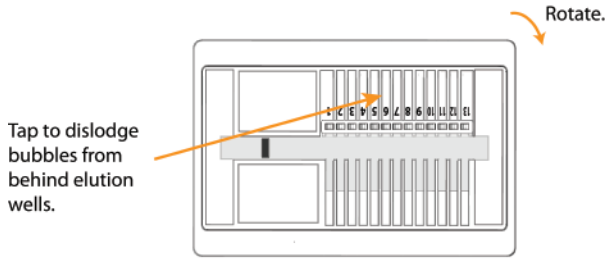
1. **While still taped closed!** Hold the cassette sideways with the elution port side down. Tap to remove air bubbles from beneath the gel fingers.



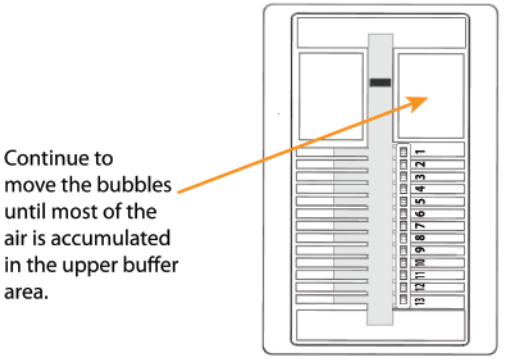
2. Rotate the cassette in the clock-wise direction. Allow the bubbles to collect in the lower buffer area. Gently tap if necessary.



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.