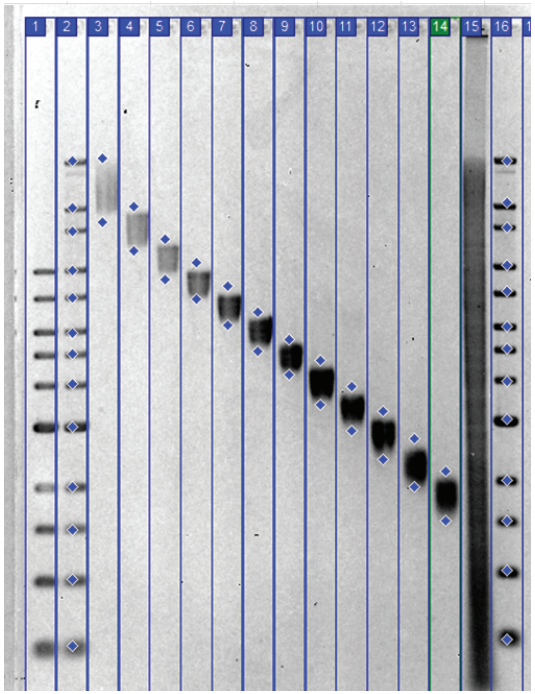
Analysis of Size fractions



Lane	Sample	Start	End	Average Size (kb)	Programmed Target (kb)	Variance
1	1kb ladder					
2	1kb extend					
3	**well 1**	16.4	48.8	32.6	16.2	102%
4	**well 2**	12.2	20.2	16.2	13.3	22%
5	**well 3**	9.1	13.7	11.4	11.7	3%
6	**well 4**	7.8	10.6	9.2	9.8	6%
7	**well 5**	6.3	8.6	7.4	8.0	7%
8	**well 6**	5.0	7.0	6.0	6.5	8%
9	**well 7**	4.2	5.5	4.9	5.2	6%
10	**well 8**	3.4	4.6	4.0	4.3	5%
11	**well 9**	2.8	3.9	3.3	3.7	10%
12	**well 10**	2.3	3.2	2.8	2.9	5%
13	**well 11**	1.9	2.7	2.3	2.2	3%
14	**well 12**	1.5	2.1	1.8	1.7	7%
15	input DNA					
16	1kb extend					

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SageELF™ Control DNA

For Testing and Validation of DNA Fractionation on the SageELF Platform

use with:

0.75% Agarose Gel Cassette

DNA fractionation between 1kb -18kb

Product No.:	**CDE7504**
Cassette Definition:	**0.75% 1kb - 18kb**
Cassette Description:	**75 - 0.75% Agarose**

sage science

About This Product

Every SageELF instrument is validated at Sage Science prior to shipment. As part of this procedure, DNA is fractionated using the agarose gel cassettes that are available to customers. Enzyme digested e.coli that has been prepared to span the specified fractionation range for each cassette is used. The DNA that has been provided with this product is the same sample used for these validations.

Genomic E.coli DNA is digested using the Dra1 restriction enzyme and purified using phenol:chloroform extraction, dialyzed and diluted in TE.

What Is Enclosed

Product number CDE7504 consists of 1 tube containing 180ul of DNA in TE. The DNA concentration is 5ug/30ul, which is the maximum input amount per load. This is sufficient to run at least 4 cassettes, with 60ul of sample remaining. To use this product, users must have the ELD7510 cassette kit, and use the reagents provided with the kit (add 10ul of loading solution/internal standard mix per sample load).

How To Use The Control DNA

1. Carefully follow the cassette preparation instructions outlined in the SageELF operations manual or Quick Guide.

2. Bring the loading solution/marker-mix provided with the ELD7510 cassette kit to room temperature.

3. Combine the 30µl DNA sample with 10µl of loading solution/marker-mix. The total sample volume is 40µl.

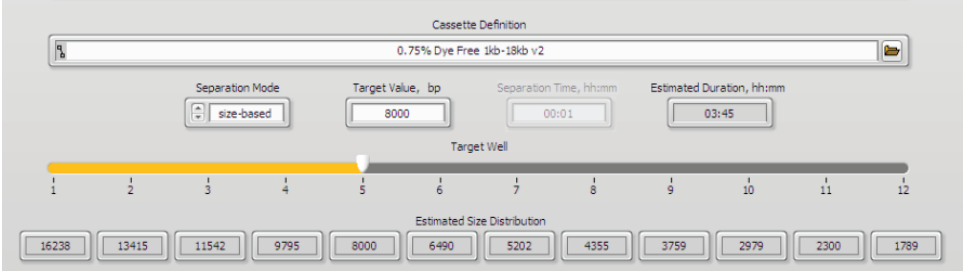
4. Mix the samples thoroughly (vortex mixer). Briefly centrifuge to collect.

5. Load the sample onto the cassette following the instructions outlined in the SageELF operations manual or Quick Guide.

6. Select the "**0.75% 1kb - 18kb**" cassette definition in the software protocol editor.

7. Program a size-based fractionation protocol with an 8000bp collection target in well number 5 (see next page). Initiate the Run.

A Correctly Programmed Protocol



Analysis of Size fractions

Due to the size of the DNA fractions, pulsed-field gel analysis is recommended. For the result below, the Pippin Pulse electrophoresis power supply was used with a 0.75% agarose gel (14 cm, SeqKem Gold from Lonza). The 10-48kb pre-set protocol was used and run for 15 hours.

Fraction sizes were analyzed using TL100™ analysis software from Totallab, Ltd.

The fraction sizes should be within +/- 15% of the target entered except for wells 1 and 2, in which a DNA compression causes a widening of the fragment range withing the wells. Typical results are summarize in the table below, and the gel image is shown on the next page.

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