New automated systems for size-selection in NGS library construction

Chris Boles, Simran Singh, Todd Barbera, Ezra Abrams

Sage Science, Inc., Beverly, MA, USA

sage science

Introduction

Virtually all genomic library construction methods use some kind of size-selection in order to avoid adapter artifacts, and to place boundaries on the library fragment size. The gold standard method for size-selection is preparative manual agarose gel electrophoresis, a procedure that is laborious, irreproducible, and difficult to automate. In response to this need, Sage Science introduced its Pippin Prep automated preparative electrophoresis system in 2010.

Sage is introducing two new preparative electrophoresis systems. The first system, called the ELF (Electrophoretic Lateral Fractionator) is designed to fractionate a single genomic sample into 12 contiguous size fractions. The system uses a two-dimensional process to separate DNA through an agarose column in a first direction, and then move the separated DNA fragments sideways into a linear array of buffer-filled elution modules that are positioned alongside the separation column. Each disposable cassette will process a single sample, and the instrument will process two cassettes per run. The instrument can operate in direct current mode for samples up to mid-single kilobases in size, or in pulsed field mode for samples up to 50 kilobases in size. Fractionation range is controlled by selection of voltage protocol, gel concentration, and run timing. Internal standards can be used to improve run-to-run reproducibility, and the instrument can read fluorescein-labeled markers.
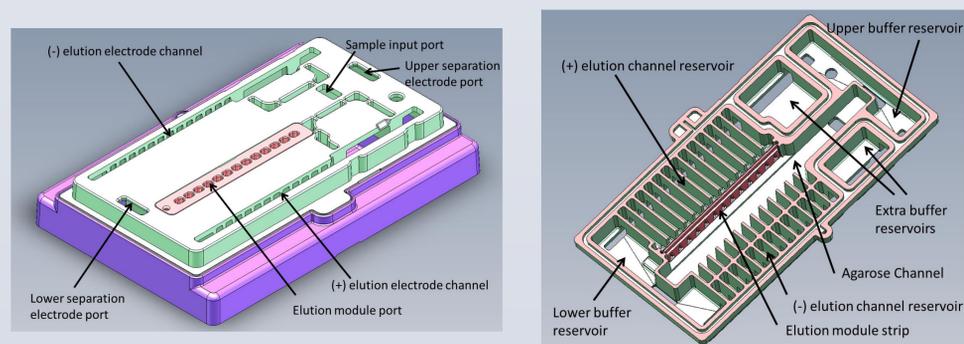
ELF Concept
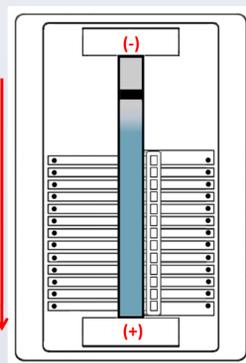
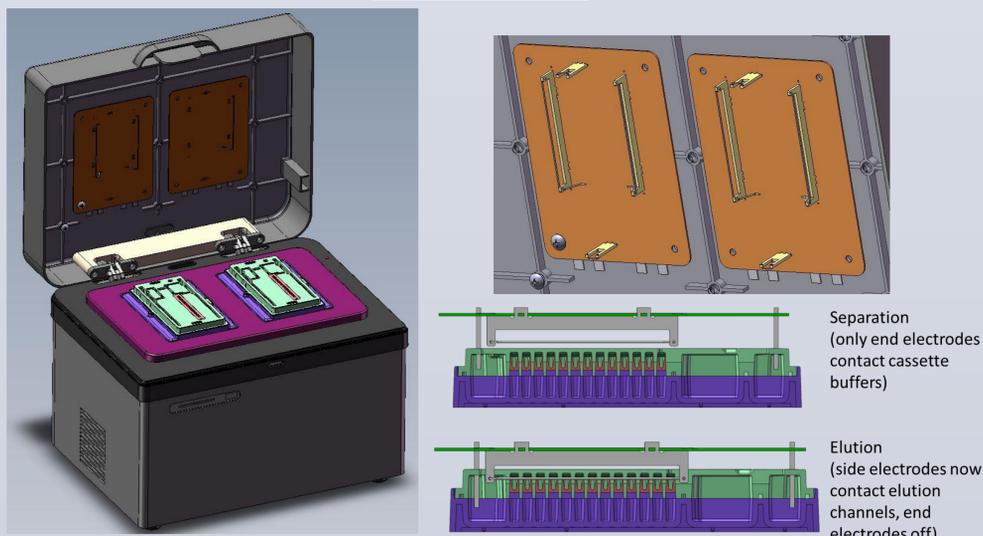Step 1. Separation electrophoresis through agarose channel
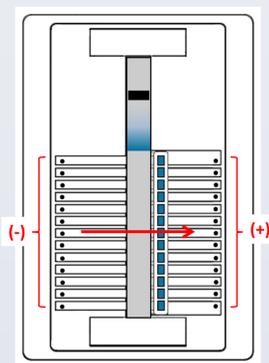
Step 2. Elution electrophoresis into buffer filled elution module strip

The ELF system is useful for constructing multiple libraries with different insert sizes from the same sample (mate-pair libraries for analysis of structural variation, RNA-seq libraries). The system is also useful for situations when the user wants to recover and save unused portions of the sample for future analyses.
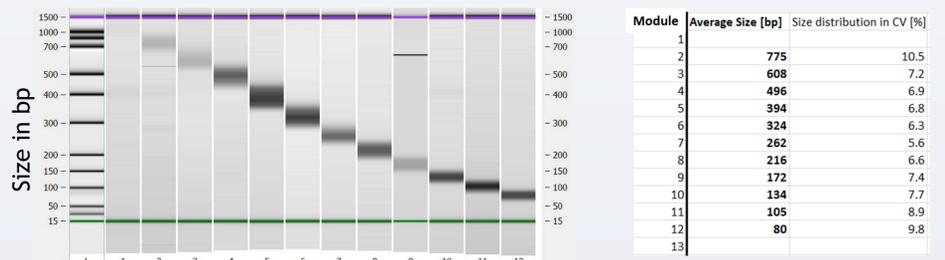
ELF Cassette

ELF Instrument

Separation (only end electrodes contact cassette buffers)

Elution (side electrodes now contact elution channels, end electrodes off)

The ELF instrument will run 2 cassettes (1 sample/cassette). The electrodes are contained in the instrument lid, and are exposed when the lid is closed. The instrument has an on-board computer, and is supplied with a monitor and keyboard. Dimensions: 9.9"(high), 13.3"(wide), 11.5"(deep).
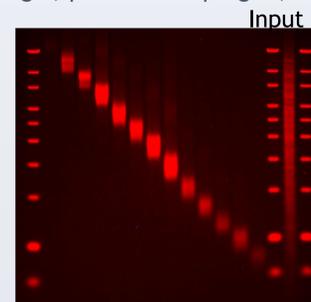
Examples of ELF performance

2% agarose gel, continuous field, sample of Ecoli genomic DNA digested with 4base cutters

Elution module number, 1 (top) to 12 (bottom)

Module	Average Size [bp]	Size distribution in CV [%]
1		
2	775	10.5
3	608	7.2
4	496	6.9
5	394	6.8
6	324	6.3
7	262	5.6
8	216	6.6
9	172	7.4
10	134	7.7
11	105	8.9
12	80	9.8
13		

0.75% agarose gel, pulsed field prog. 1, restricted Ecoli gDNA, 2.5 hours

Input

elution position	avg (bp)	bp start	bp end	CV%
1				
2	11962	10018	15309	11.06
3	9446	7750	11550	10.06
4	7260	5880	9661	13.02
5	5439	4302	7228	13.45
6	4351	3337	5592	12.96
7	3382	2633	4314	12.43
8	2476	2025	3337	13.25
9	1952	1598	2374	9.94
10	1548	1314	1873	9.03
11	1282	1121	1502	7.43
12	1107	964	1274	7.00
13	953	862	1028	4.35

0.75% agarose gel, pulsed field prog. 1, restricted Ecoli gDNA, 5 hours

Input
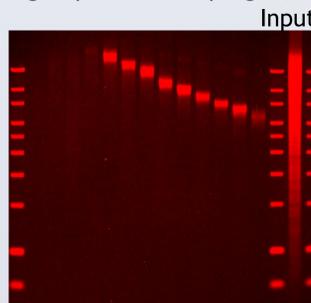
elution module	avg (bp)	bp start	bp end	CV%
1				
2				
3				
4				
5	18753	15398	22366	9.29
6	16179	13567	19093	8.54
7	14452	11842	17146	9.18
8	11847	9919	14182	9.00
9	10662	8908	11917	7.06
10	9367	7899	10770	7.66
11	8360	7161	9223	6.17
12	7520	6152	8657	8.33
13	6659	5542	8024	9.32

075% agarose gel, pulsed field prog. 2, restricted Ecoli genome DNA, 6 hours

Input
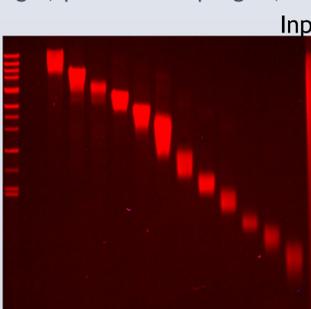
elution module	avg (bp)	bp start	bp end	CV%
1				
2	42379	30450	56790	15.54
3	29587	20487	41304	17.59
4	25661	19939	30041	9.84
5	20891	16890	25104	9.83
6	16821	13324	20978	11.38
7	13512	10948	17830	12.73
8	10922	9856	12326	5.65
9	9908	9400	10547	2.89

10-12 Samples from modules 10-12 are beyond lower markers

Coming in Q'1- 2014: Pippin HT

Sage is also working on a high-throughput version of the Pippin Prep/BluePippin size selection system. The system will feature SBS compliant, 12 channel cassettes. Each channel will accommodate up to 1.5 micrograms of genomic DNA. The instrument will have a similar form factor to the ELF instrument, and will run 2 cassettes, for a total throughput of 24 samples per run. Cassette offerings and size ranges will be similar to the current BluePippin system.

Comparison of Pippin HT and Pippin Prep channel geometry

Top view of Pippin HT instrument nest

Contact
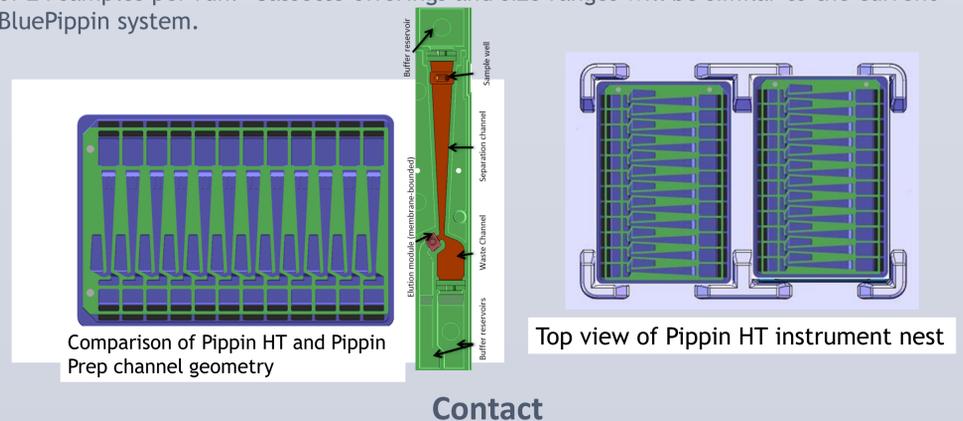
Technical questions: chris.boles@sagescience.com, info@sagescience.com

Sales questions: sales@sagescience.com

Visit our website at www.sagescience.com