Automated preparative gel electrophoresis using the Pippin Prep system

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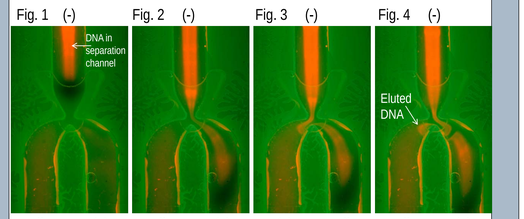
Introduction

Size fractionation of randomly sheared DNA is a laborious process that often forms a bottleneck in next-generation sequencing applications. Sage Science has developed a novel preparative electrophoresis system to automate this process. The system includes a disposable, five-channel pre-cast agarose cassette, and a computerized instrument which combines a power supply for electrophoresis with a fluorescence-based DNA detection unit. During operation, system software uses the optical system to detect DNA corresponding to a user-programmed size range, and controls the application of voltage to the cassette so that selected DNA fractions are electroeluted into a buffer-filled sample recovery compartment. At the end of a run, fractionated samples can be recovered from the cassette using standard micropipettes or other liquid handling devices.

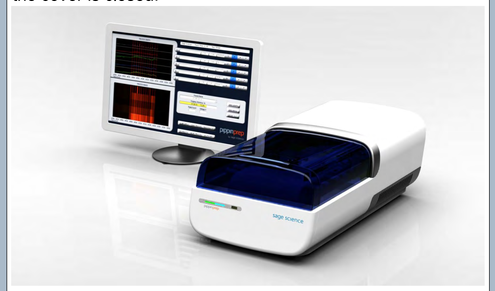
Principle of the System

Each lane of the cassette has a gel-filled separation channel extending from the sample well down to a branch point (see cassette schematics at right). At the branch point, DNA can be directed into the left or right lower channels by switching on or off (+) electrodes located at the termini of the lower channels. Figures 1-4 (below) show time lapse photography of a genomic restriction digest being fractionated in a prototype cassette. The Pippin Prep cassette uses this principle to electroelute user-selected fractions into a buffer-filled elution chamber. DNA is retained in the elution chamber by an ultrafiltration membrane.

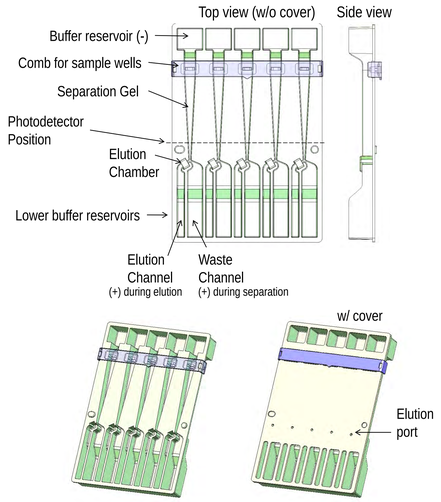


Instrument

The Pippin Prep instrument houses an electrophoresis power supply, a real-time fluorescence detection unit (for DNA-bound ethidium), and a single-board PC. The blue cover slides back for insertion of the cassette. The electrode assembly is integrated into the cover, and is lowered into the cassette as the cover is closed.

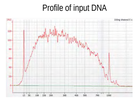


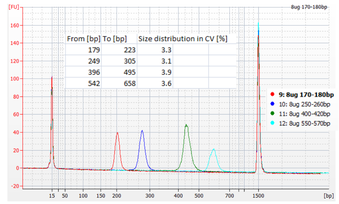
Precast agarose gel cassette



Performance: Size resolution, sheared genomic DNA

Using 2% agarose Pippin Prep cassettes, CV's of 3-4% are routinely obtained (edge-to-edge distribution, 6-8% of average length).

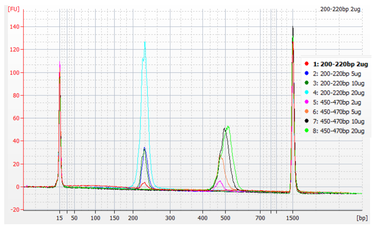


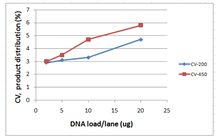


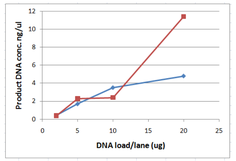
Genomic E. coli DNA was restricted to give fragments from 50 – 1500 bp. Four 8ug aliquots were fractionated in a Pippin Prep to produce tight distributions ranging from approx. 180, 250, 400, and 550 bp.

Performance: Loading capacity

The Pippin Prep system has the same loading capacity as standard preparative agarose gels. There is little change in product fraction CV's at genomic DNA loads of up to 20ug. Similarly, DNA recovery linearly increases with input loads up to 20ug for fragments around 200 bp. There is a slight deviation from linearity of yield for the 500 bp fragments at 20ug input, suggesting that useful capacity of the cassette is between 10 and 20 ug at that fragment size.



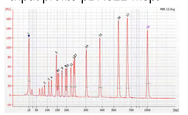




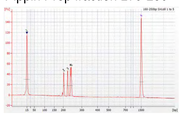
Performance: Efficiency of recovery

Recovery was evaluated by fractionating plasmid restriction digests. Efficiency of recovery in 2% Pippin Prep cassettes ranged from 50-70% for fragments 200-400 bp in length.

Input profile pBR322-MspI



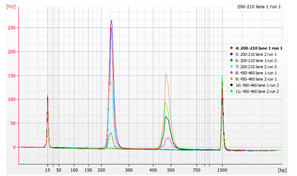
Pippin Prep fraction 170-250



Fragment size (bp)	Total plasmid input, cassette lane(ng)	Fragment input, cassette lane (ng)	Recovered fragment, Bioanalyzer (ng/ul)	Dilution factor of Bioanalyzer sample	Total volume of eluted product (ul)	Total fragment recovered (ng)	Percent recovery
201	2000	92	0.67	5	15	50	55%
217	2000	100	1.81	2	15	54	54%
238	2000	110	0.8	5	15	60	55%
242	2000	110	1.07	5	15	80	73%
307	2000	140	0.94	5	15	71	50%
404	2000	186	0.89	10	15	134	72%

Performance: Reproducibility

Shown at right are fractionations using 8ug E.coli genomic DNA per lane. Each fractionation was carried out in duplicate in each of two Pippin Prep cassettes. In all four fractionations of each type (230 or 480 bp), the average values are within a few bp of each other.



Cassette	Lane	Programmed start	Average Size (bp)	CV (%)	Conc. (ng/µl)
1	1	200	236	3.7	17.48
1	2	200	239	3.5	17.27
2	1	200	235	3.1	2.58
2	2	200	236	3.4	8.29
1	1	450	486	4.1	1.52
1	2	450	482	4.4	5.53
2	1	450	482	4.2	3.72
2	2	450	481	4.4	5.16

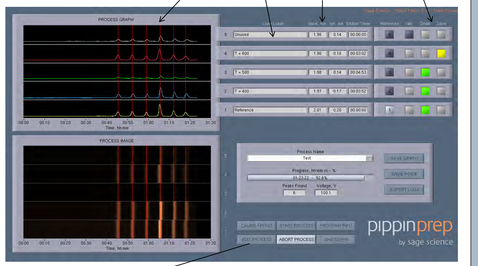
Run times

Typical run times range from just under an hour to just over 1 ½ hours. Sage will introduce cassettes with different agarose concentrations for other DNA fragment sizes.

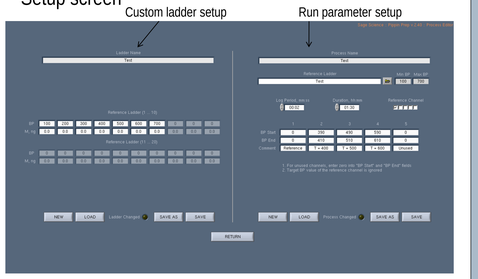
start bp	start coll. (min)	duration elution (min)	total run time (min)
170	55	2	57
200	59	2	61
250	62	2	64
400	79	4	83
450	84	5	89
550	96	6	102

System Software

Run-time screen



Setup screen



Pippin Prep Cassette Kits

Kits will contain:

- 10 five-lane Pippin Prep cassettes
- 2 tubes DNA marker ladder
- 2 tubes Ficoll loading solution
- 1 bottle 1X electrophoresis buffer

Initially, Pippin prep cassettes will be available in 1% and 2% agarose concentrations, covering size ranges from ~150 bp to ~5 kb. Later, Sage will offer 0.5% and 3% agarose, as well as acrylamide cassettes.

Contact information

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