

Introducing the Sage ELF: Multiple DNA size fractions from the same sample

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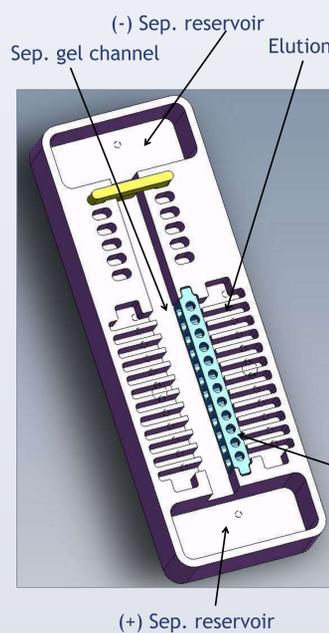


Abstract

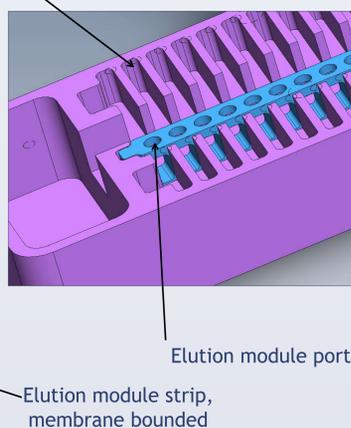
Sage Science has developed a new preparative electrophoresis system for size-fractionating genomic DNA samples. The system utilizes precast agarose cassettes that are processed in two automated steps. In the first step, DNA is electrophoretically separated in an agarose channel. In the second step, DNA is electroeluted sideways out of the separation channel into a linear array of 12 membrane-bounded elution modules that are located alongside the lower two-thirds of the separation channel. The DNA fractionation range can be adjusted through selection of gel concentration and run time. In addition, pulsed field power can be used to resolve high molecular weight fractions for mate-pair libraries. The instrument uses a CCD camera to track movement of standard electrophoresis tracking dyes through the cassette and determine run times. The first ELF instrument will accommodate 2 samples per run with manual cassette loading and unloading. Follow-on instruments will feature automated sample and cassette handling for higher-throughput sample processing.

ELF cassette design

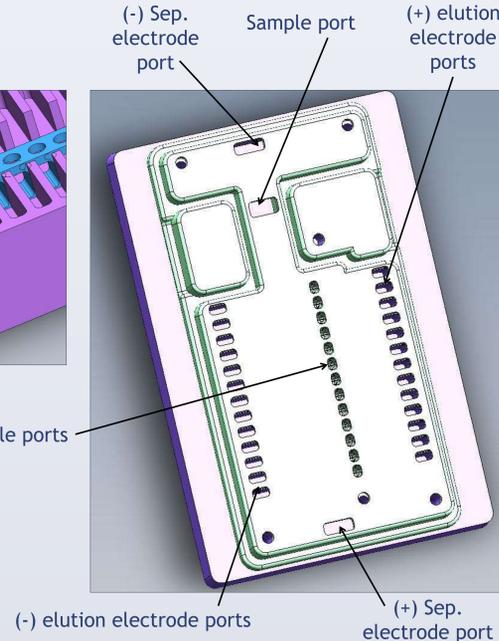
Prototype ELF cassette



Detail of elution channels in prototype.

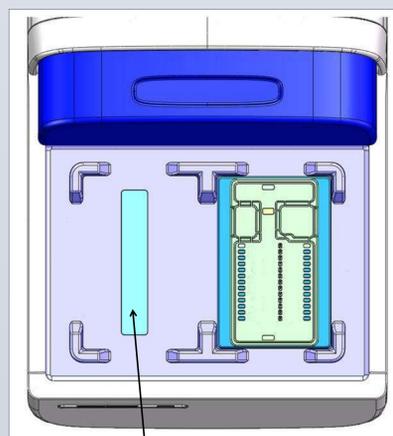
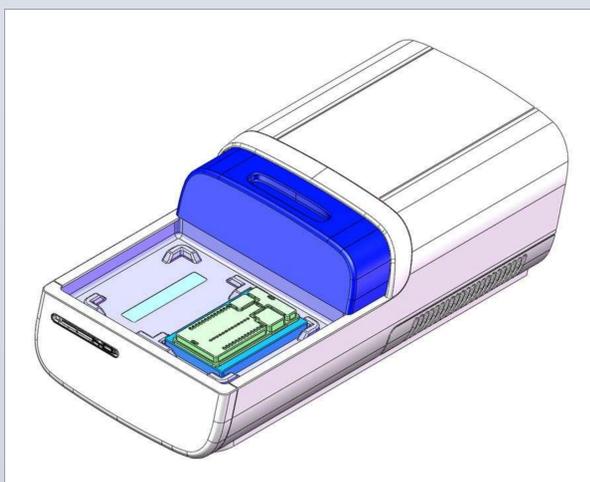


Top view of production ELF cassette.



ELF instrument

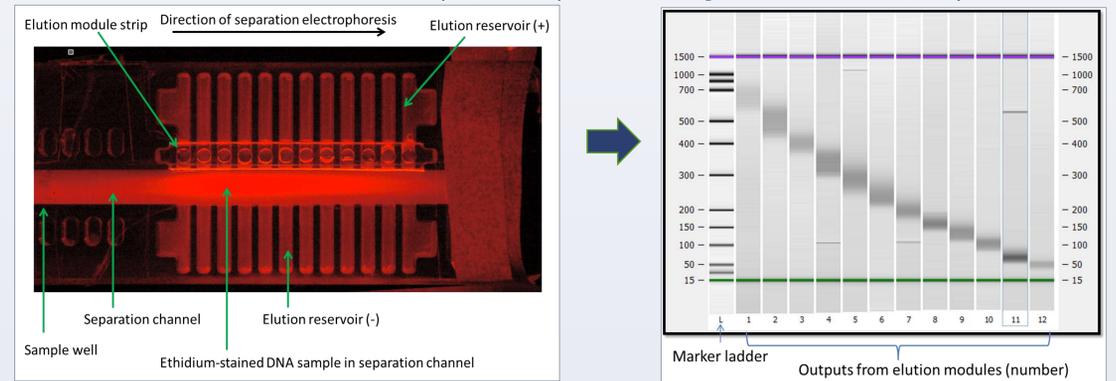
The ELF instrument is based on the Sage BluePippin, and can separate DNA using DC or pulsed field (field inversion) power modes. The instrument can run two independently programmable cassettes simultaneously. The electrode arrays are housed in the sliding lid. Runtimes vary between 1.5 hours (for DNA up to 1000 bp) and 6.5 hour (pulsed field protocols for DNA up to 50kb). The progress of the run is monitored by following the movement of colored tracking dyes using a CCD camera, which is located under the separation channel. Separation and elution steps are automated, under control of an on-board computer.



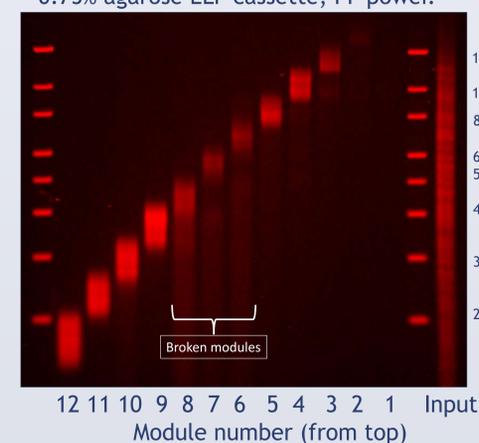
Examples of fractionations obtained with ELF prototype cassette

E. coli genomic DNA was digested with combinations of restriction enzymes to provide input samples with broad size distributions. Aliquots of 5 μ g were loaded in the ELF prototype and fractionated. Output samples were analyzed by Agilent Bioanalyzer (for DNA <2000 bp), or by analytical pulsed field agarose gel electrophoresis (Pippin Pulse power supply, ethidium bromide post staining). Yields have been estimated from experiments using plasmid and phage restriction ladders. For fragments <1000, yields are 50-70%, For fragments >1kb, up to 20kb, recovery averages 40%.

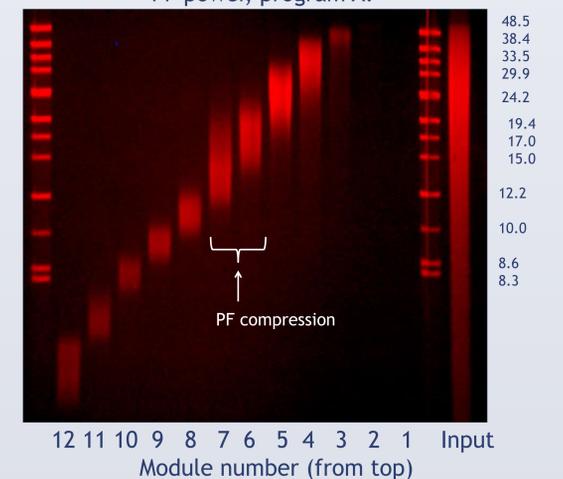
Fractionation of restricted sample, <700 bp in size, 2% agarose ELF cassette, DC power



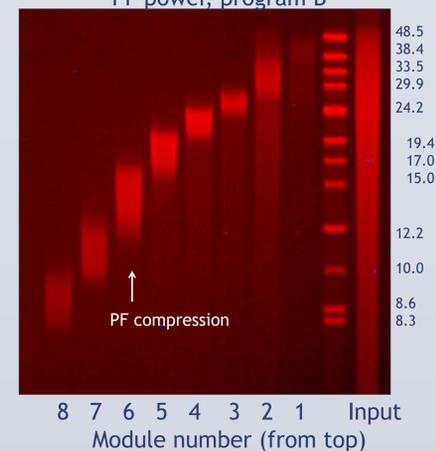
Fractionation of restricted sample, 1-15kb 0.75% agarose ELF cassette, PF power.



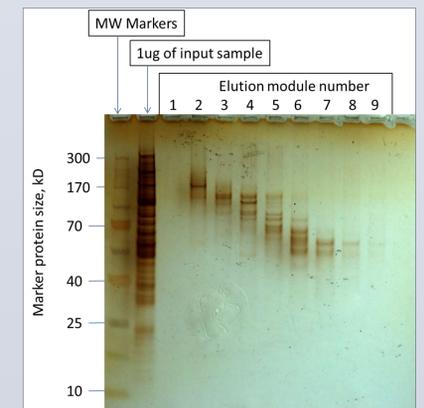
Fractionation of a 4-50kb sample, 0.75% cassette, PF power, program A.



Fractionation of 4-50kb sample, 0.75% cassette, PF power, program B



The ELF system can also be used for protein size fractionation. A 3% SDS-agarose ELF cassette was used to fractionate a mouse brain extract. Fractions were analyzed on SDS PAGE gels with silver staining.



Conclusions

The ELF system is well suited for applications where multiple size fractions of the same sample are needed, such as mate-pair library construction, and studies of marker phasing (haplotyping). Since run conditions can be configured to collect most or all of the original sample, the ELF system is also valuable for size fractionation of precious samples of unknown quality, such as DNA from clinical samples.

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