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 electroeject 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 to the left to allow for insertion of the cassette. The electrode assembly is integrated into the cover, and is lowered into the cassette as the cover is closed.

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).

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.

Performance: Loading capacity

For 20ug input, suggesting that useful capacity of the cassette is between 10 and 20 ug at that fragment size. 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: Reproducibility

Shown at right are fractionations using Rug 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 recovery values are within a few bp of each other.

Performance: Reproducibility

Typical run times range from just under an hour to just over 1 1/2 hours. Sage will introduce cassettes with different acrylamide concentrations for other DNA fragment sizes.

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