Faster DNA size-selection on the Pippin Prep

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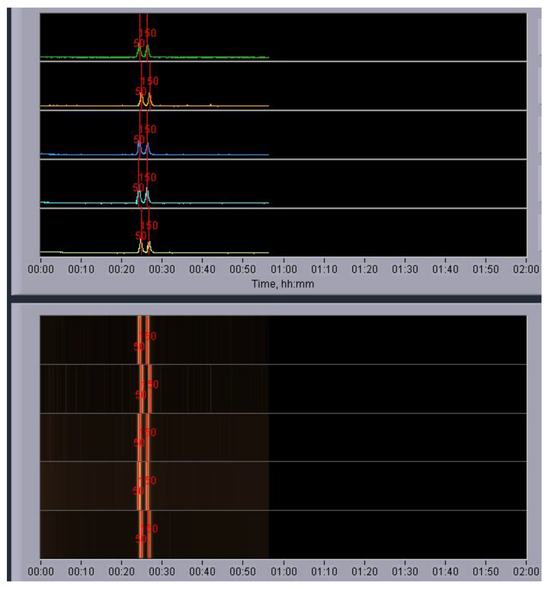
Abstract

Sage Science has introduced a new 1.5% dye-free (DF) cassette that runs at a higher voltage than our standard 2% ethidium cassettes (recommended in the original PGM™ protocols). The new 1.5% DF cassettes run approximately 3X faster than standard 2% ethidium cassettes: size-selection at 500 bp takes 35 minutes on the 1.5% DF cassette, whereas the same selection takes ~1.5 hours on the 2% cassette. To ensure good accuracy and reproducibility on the new cassette, we have developed fluorescently-labeled internal size standards which are mixed with samples prior to loading. This eliminates the effects of lane-to-lane variation, which is more significant at the higher voltage. The internal standards are designed to be much smaller than the lower limit of the selection range, which minimizes the risk of marker contamination in the library sample. The internal standard strategy also allows all five cassette lanes to be used for samples, since there is no need for a dedicated marker lane.

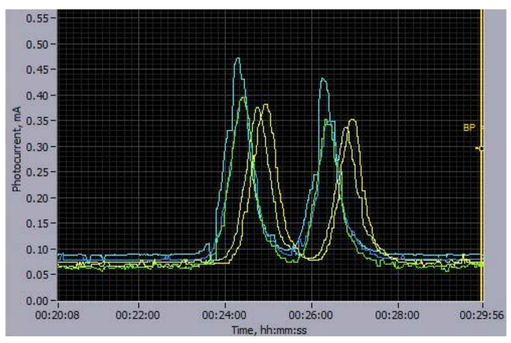
The Pippin Prep System

The Pippin Prep system is an automated preparative electrophoresis system especially designed for NGS library applications. It includes a disposable five-channel pre-cast agarose gel cassette, and a computerized instrument that combines a power supply for electrophoresis with a fluorescence-based DNA detection unit. The cassette lanes are physically isolated from each other to prevent sample cross-contamination. Each lane has a tapered separation channel that branches into two channels with positive electrodes at their termini. Through independent control of these positive electrodes, DNA exiting the separation channel can be directed left or right at the branch point. DNA for library formation is collected in a membrane-delimited elution module in one of the two branches. Fractionated DNA products are recovered in liquid buffer, and no gel extraction is required. Timing of DNA collection is determined by the onboard computer, which uses optical data from fluorescently labeled DNA markers to determine the mobility of DNA through the cassette.

Higher Voltage, Internal Standards for Faster runtimes



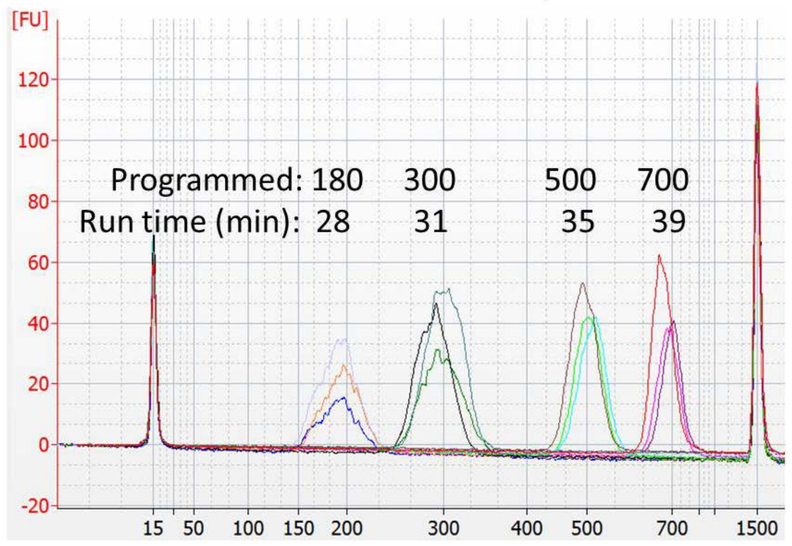
Run-time view of home screen showing normal appearance of internal standards.



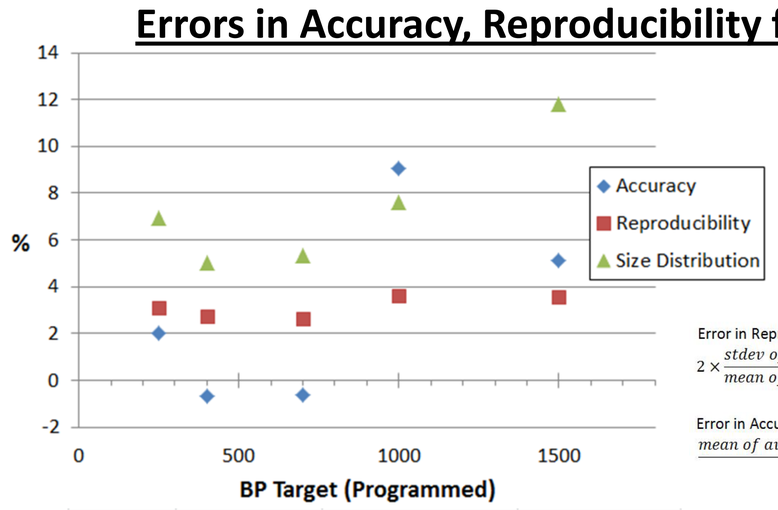
Log review of internal standards in 5 lanes of a single cassette, showing lane to lane variation.

We find that increasing voltage from the original standard value of 100V to 150V decreases runtime by about 3-fold for a 500 bp size selection (from ~1.5 hrs to ~0.5 hrs). However, increasing the voltage substantially increases lane to lane variation. This variation dramatically decreased accuracy and reproducibility when using an external standard lane to time collections. To address this problem, we have developed internal standard reagents and software to correct for the lane to lane variation. The markers are random sequence TAMRA-labeled 50 and 150 bp oligo duplexes. These markers run well ahead of the earliest collectable fraction (~250bp) thereby minimizing the risk of library contamination with the marker DNA. (The sequences of the markers are available on our website.)

1.5% dye-free marker K cassette – 3 cassettes, 4 size cuts in each cassette



Errors in Accuracy, Reproducibility for Tight Cuts



BP Target	Error Accuracy	Error Reproducibility	Size Distribution
250	2.00	3.09	6.93
400	-0.69	2.74	5.05
700	-0.61	2.65	5.33
1000	9.05	3.64	7.63
1500	5.13	3.56	11.80

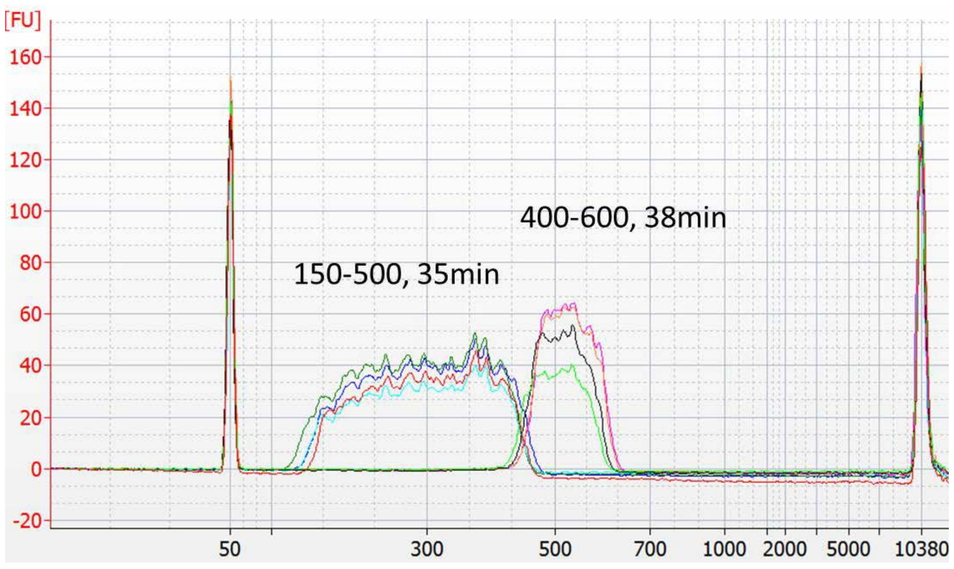
Error in Reproducibility (%) defined as:

$$2 \times \frac{stdev\ of\ ave.cut\ sizes, Agilent\ data}{mean\ of\ ave.cut\ sizes, Agilent\ data} \times 100$$

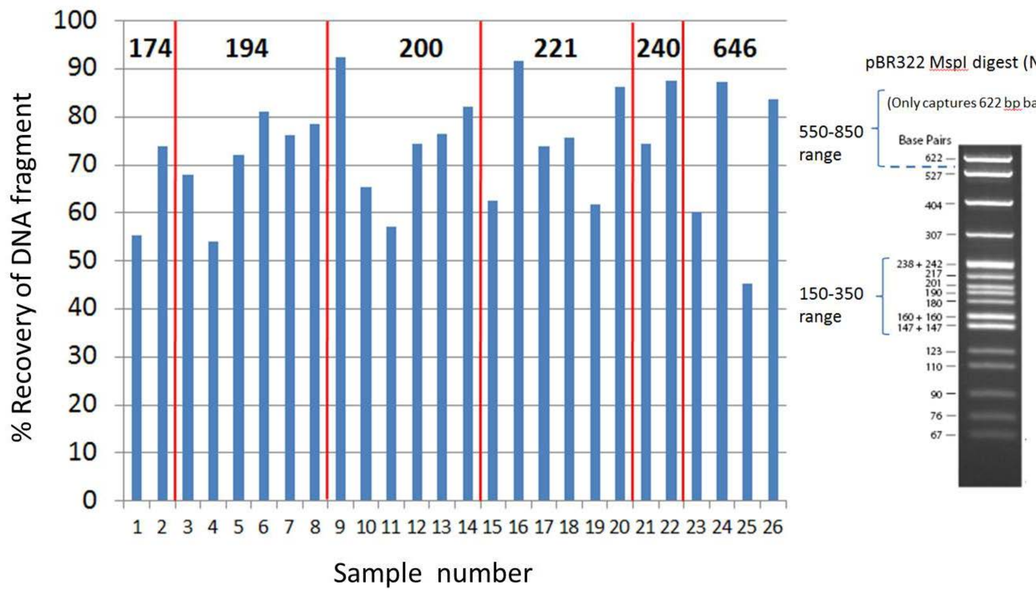
Error in Accuracy (%) defined as:

$$\frac{mean\ of\ ave.cut\ sizes, Agilent\ data - programmed\ bptarget}{programmed\ bptarget} \times 100$$

Broad cuts, 1.5% DF marker K cassette. 4 cassettes, 2 different cuts



Intrinsic recovery of DNA restriction fragments, 1.5% DF marker K cassette



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