DNA input Load dependence of fractionated DNA size on the 0.75% cassette

Introduction

This technical note describes how accuracy of fractionated DNA size is dependent on the amount of DNA loaded per lane on the 0.75% Pippin Prep cassette.

Analysis

To illustrate the effect of varying the amount of DNA loaded in a lane, we did some initial testing at three different size cuts, 3kb, 5kb and 8kb with four different DNA inputs: 1ug, 3ug, 6ug and 10ug. A stock of 1 ug/ul of E coli genomic DNA from Sigma (Type VIII), digested with Dra I, was used as the input sample for this experiment. The distribution of the input DNA is shown in Figure 1.

All samples were prepared in 30ul of TE. 10ul of loading solution was added to each, and this 40ul mix was loaded on to each lane of the Pippin Prep. Three cassettes were run: one each for 3kb, 5kb and 8kb cuts. All cuts were performed using the 'Target' setting in the Pippin Prep protocol in order to achieve the narrowest allowable fraction. Elution wells were sealed using tape as described in Tech Note 4, allowing all elution volumes to be consistent at 40ul.

Analysis of collected fractions was done by loading 1ul from a total of 40ul of each eluted sample, on a DNA 12000 chip on the Agilent 2100 Bioanalyzer.

Conclusion

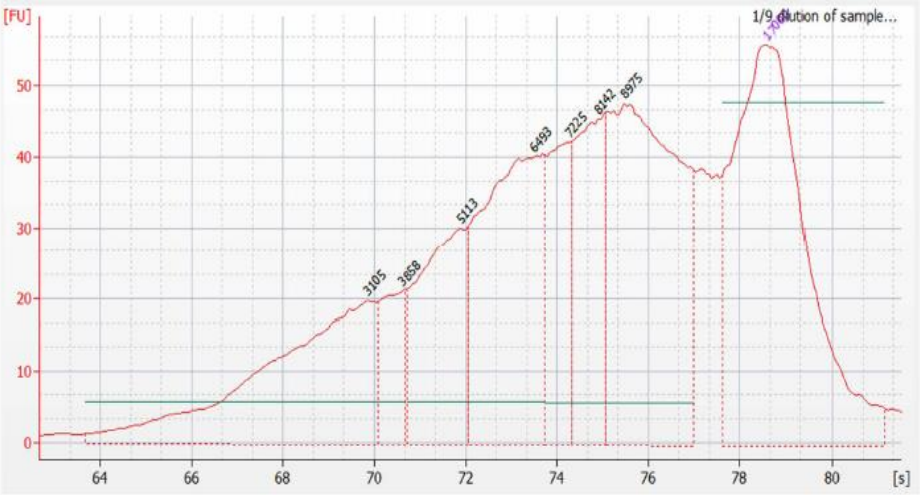
The 0.75% cassette is very sensitive to input load. The Pippin Prep protocols are most accurate at an input load of 5ug. At lower or higher input loads, the accuracy decreases. Agilent electropherograms from the 12000 chip are shown in Figure 2-4. Results are summarized in Table 1.

As a work-around for this issue users should refer to Figure 6. This gives some suggestions on how to adjust your bp target or range settings. Find your desired elution size on the y axis of the graph, read the value on the x axis where the desired size intersects with the input load you will be using. Adjust the bp settings in the protocol to the x axis value determined from the graph to achieve the desired fraction.

For example: If a 5kb fraction is desired from a 3ug input DNA load, set a Target cut for 4000bp in the protocol.

This data can be used for semi-quantitative suggestions. In addition, we suspect that the shape of the input size distribution may affect accuracy of selection. We are still gathering data and will update you as we do more studies towards more accurate predictions.

Input DNA profile:
Ecoli genomic DNA from Sigma (Type VIII), digested with Dra I (NEB).
Analysis on Agilent 12k chip.



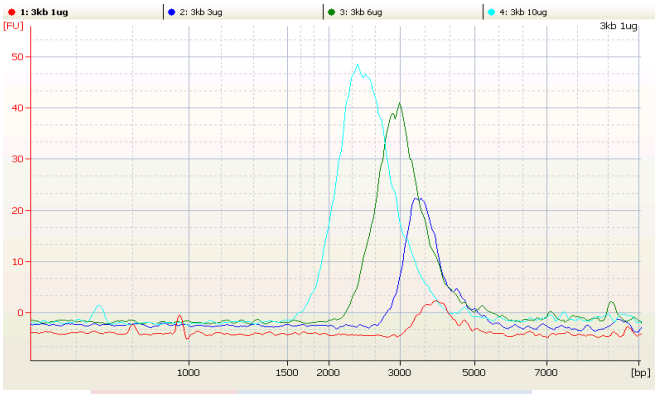


Figure 2

Cassette A	ug load	Programmed Target (bp)	From [bp]	To [bp]	Average Size [bp]	Size distribution in CV [%]
sample 1	1	3000	3,005	5,267	4,043	11.9
sample 2	3	3000	2,674	5,462	3,720	15
sample 3	6	3000	2,108	4,981	3,156	17.3
sample 4	10	3000	1,546	4,347	2,579	18.1

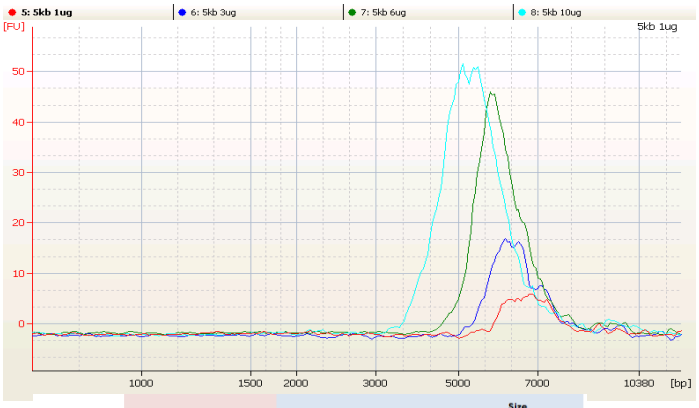


Figure 3

Cassette B	ug load	Programmed Target (bp)	From [bp]	To [bp]	Average Size [bp]	Size distribution in CV [%]
sample 1	1	5000	5,189	8,338	6,775	9.1
sample 2	3	5000	4,993	7,794	6,399	8.9
sample 3	6	5000	4,333	7,766	6,025	10
sample 4	10	5000	3,572	7,424	5,355	13.7

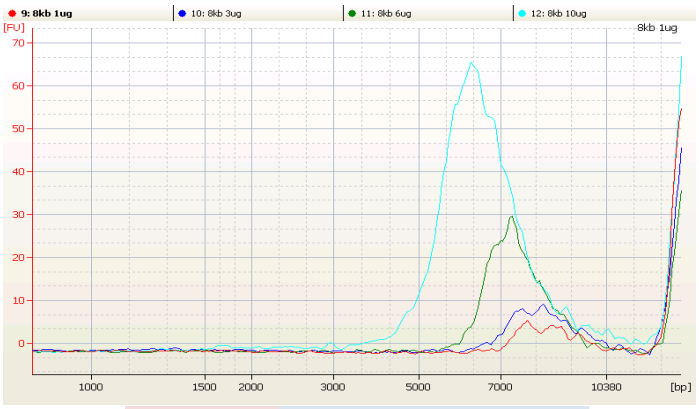


Figure 4

	ug load	Programmed Target (bp)	From [bp]	To [bp]	Average Size [bp]	Size distribution in CV [%]
Cassette C						
sample 1	1	8000	6,924	10,397	8,508	9.7
sample 2	3	8000	6,070	10,867	8,311	12
sample 3	6	8000	5,487	9,799	7,517	11.4
sample 4	10	8000	4,419	9,521	6,560	15.2

Cassette A	ug load	Programmed Target (bp)	From [bp]	To [bp]	Average Size [bp]	Size distribution in CV [%]
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Table 1

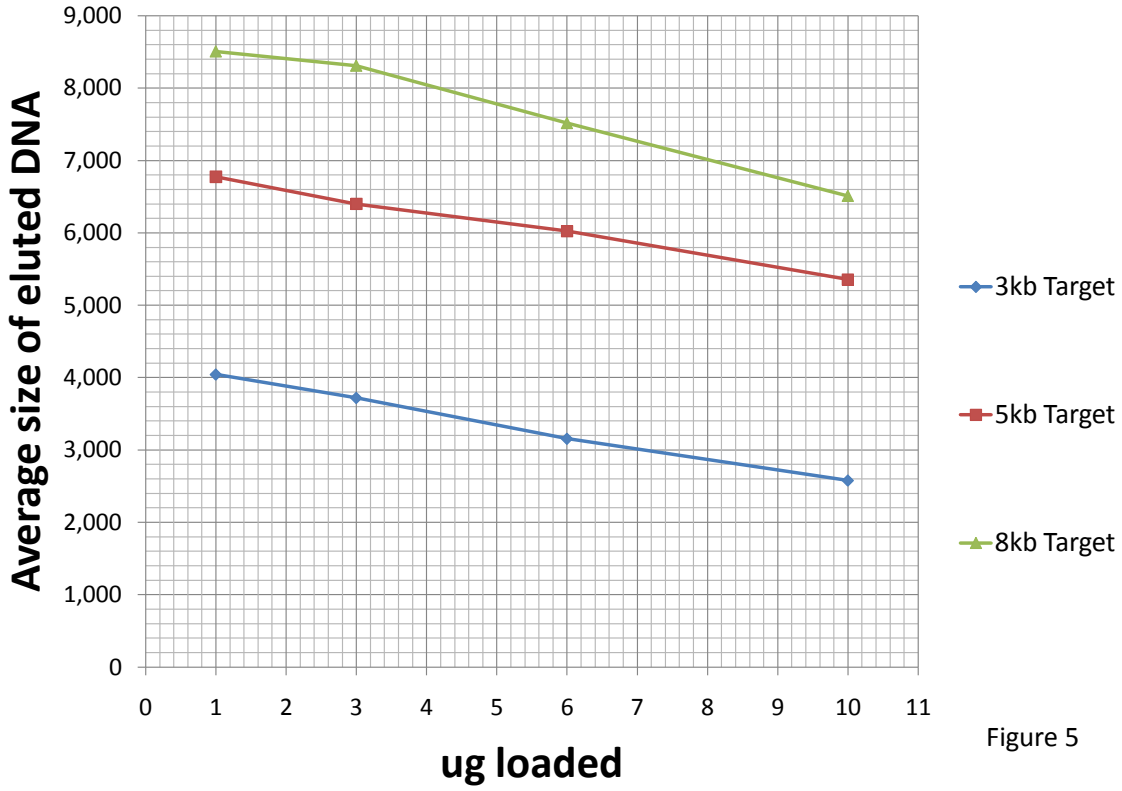

Figure 5

Average size of eluted DNA as a function of input load at Target settings of 3,5 and 8kb

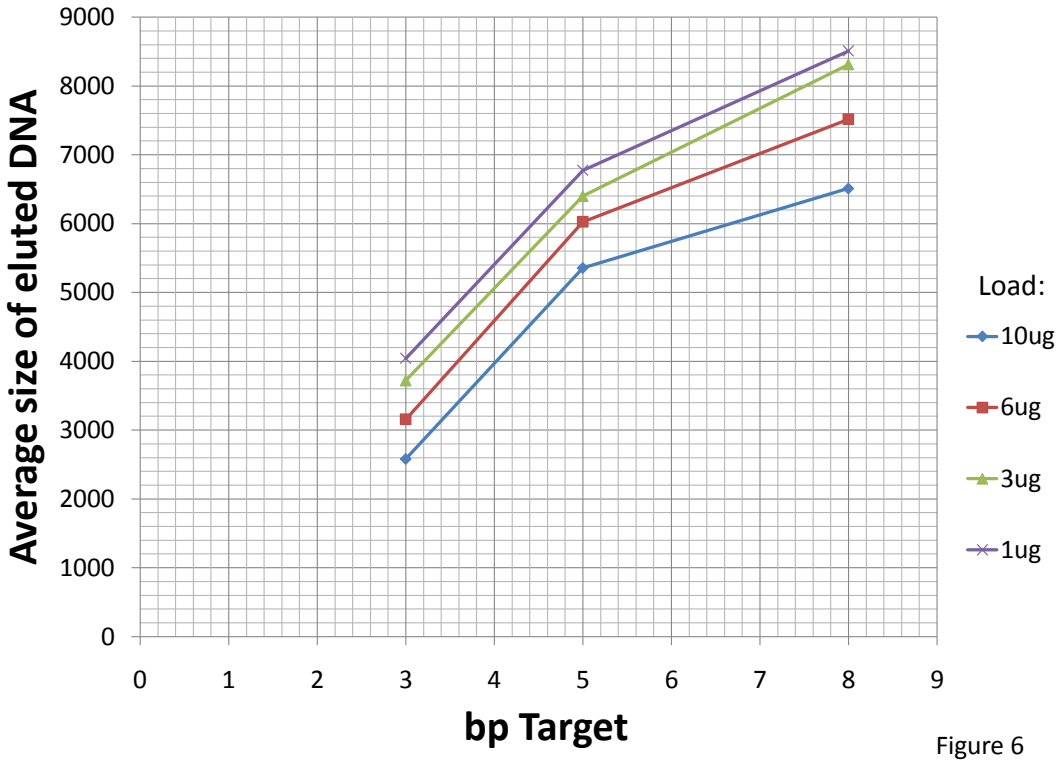

Figure 6

Average size of eluted DNA as a function of bp Target programmed on Pippin Prep