Low input DNA size selection on the Pippin Prep System using the Agilent 2100 Bioanalyzer with the Agilent High Sensitivity DNA kit

Application Note

Genomics

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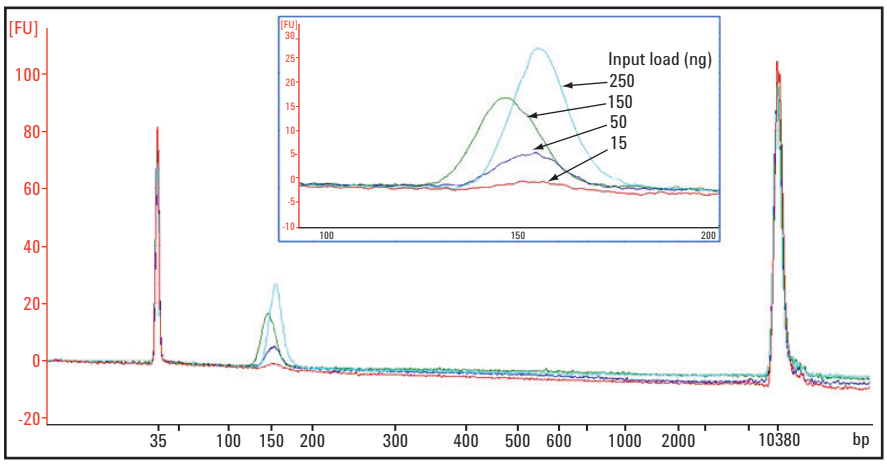

Abstract

With the advent of next-generation sequencing (NGS), library preparation has become a much more streamlined process utilizing smaller amounts of input genomic DNA, thus allowing for more narrow fragmentation profiles. This Application Note demonstrates the usefulness of the Agilent 2100 Bioanalyzer and the Agilent High Sensitivity DNA kit in complementing the Pippin Prep automated size selection workflow from Sage Science, Inc. The 2100 Bioanalyzer is capable of analyzing the size distribution of fragmented input DNA, permitting users to specify the appropriate size selection range on the Pippin Prep system. Together, these technologies enable fine control and increased efficiency with the critical first step of library generation.

Agilent Technologies

Introduction

As the efficiency of NGS sample preparation improves, sequencing labs are using increasingly smaller amounts of genomic DNA for library generation. This presents two challenges. First, there is reduced margin for error in the fragmentation process: lack of desired distribution leads to insufficient amount of DNA in the appropriate size range to generate the intended library. If size constraints on library insert size are not restrictive enough, this problem can be corrected by changing the size selection process so that a more concentrated region of the input distribution is collected. To accomplish this adjustment, the user must know the input sample size distribution before setting up size selection. This requirement leads to the second problem: the pre-fractionation analysis method must be extremely sensitive so that only a small fraction of the input sample is consumed.

This Application Note illustrates a synergy between the Agilent 2100 Bioanalyzer, the Agilent High Sensitivity (HS) DNA kit, and the Pippin Prep system from Sage Science, Inc. that address these problems. As shown below, the 2100 Bioanalyzer with the HS DNA kit is ideal to analyze the fragmented input DNA size distribution, since it requires only single nanogram (ng) of DNA for analysis. Knowing the input size distribution, the user can choose the appropriate size selection range on the Pippin Prep system, thereby increasing user control over the fragmentation and size selection steps of library generation.

Materials and methods

Automated size selection

DNA electrophoresis was performed on the Pippin Prep system (Sage Science, Inc.) which uses pre-cast agarose gel cassettes for the preparation of size-fractionated DNA samples. Each disposable cassette has four sample lanes and one lane for a DNA reference ladder (provided by Sage Science, Inc.). The sample lanes are physically separate, preventing any possibility of cross-contamination. Size ranges to be extracted are entered in the software, and a non-UV optical detection system tracks the DNA migration with accurate timing of the electro-elution cut. All experiments were performed according to the manufacturer's instructions.

Electrophoresis

DNA samples were sized and quantified on the 2100 Bioanalyzer before and after size selection, according to manufacturer's instructions. Standard DNA samples were measured with the Agilent DNA 1000 kit. Samples diluted 50-fold, 100-fold, and 150-fold were analyzed with the High Sensitivity DNA kit.

Results and discussion

Analysis of input DNA profile

For the analyses shown, we used a restriction digest of *E. coli* genomic DNA to simulate a sheared input sample. Figure 1 shows that this digest has a broad size range, approximately 20-1000 bp, when analyzed with the DNA 1000 kit on the 2100 Bioanalyzer. The loadings are 100 ng (red) and 50 ng (blue). The most abundant size fraction, around 100 bp, shows an intensity of about 40 fluorescent units in the 50 ng loading.

When the same sample is run on the HS DNA chip, a roughly equivalent signal is achieved with 1/50th of the input (compare 50 ng loading in Figure 1 with 1.2 ng loading in Figure 2). Furthermore, a reasonable picture of the input size distribution can be obtained on the HS DNA chip with even less sample (3-fold less, see 0.375 ng load in Figure 2). These data clearly demonstrate the value of the HS DNA kit chip for characterization of low concentration genomic samples. For samples in the 10's of nanograms, a good analytical profile can be obtained from 5% or less of the

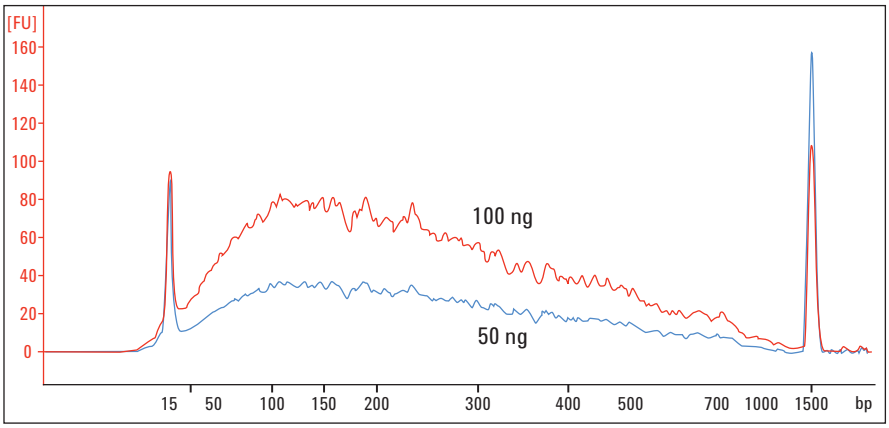

Figure 1
Restriction digest of *E. coli* genomic DNA characterized with the DNA 1000 kit on the Agilent 2100 Bioanalyzer.

input sample. Having a good profile of the input DNA allows the investigator to tailor fractionation settings for the Pippin Prep system, and maximize chances for successful library construction.

Analysis of Pippin Prep fractionation products

The HS DNA kit is also beneficial for post-fractionation analyses of Pippin Prep products. To illustrate this, we ran the *E. coli* genomic digest at four different input loads (total DNA load per lane, 15 ng, 50 ng, 150 ng, and 250 ng) in a 2% Pippin Prep cassette, and collected a tight size fraction centered on 150 bp from each lane. A screen shot of the Pippin Prep home screen at the end of the run is shown in Figure 3.

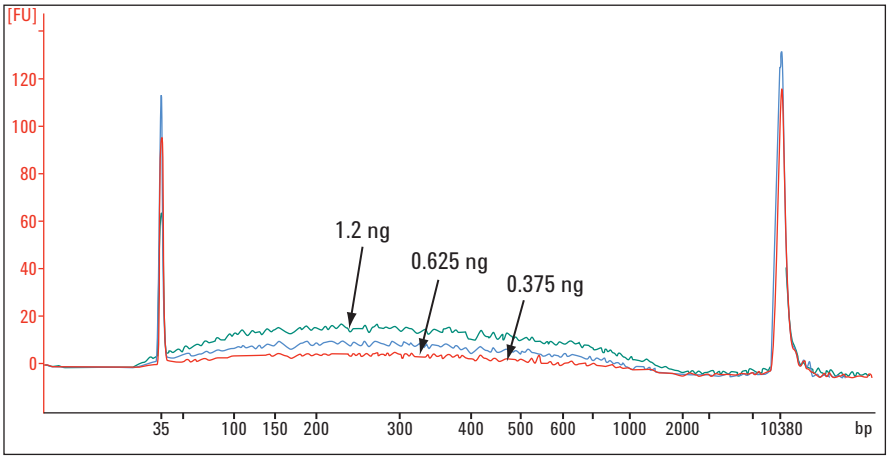

Figure 2
Pre-fractionation analysis of low concentration genomic DNA samples on the HS DNA kit. Total input load for the indicated channel are shown.

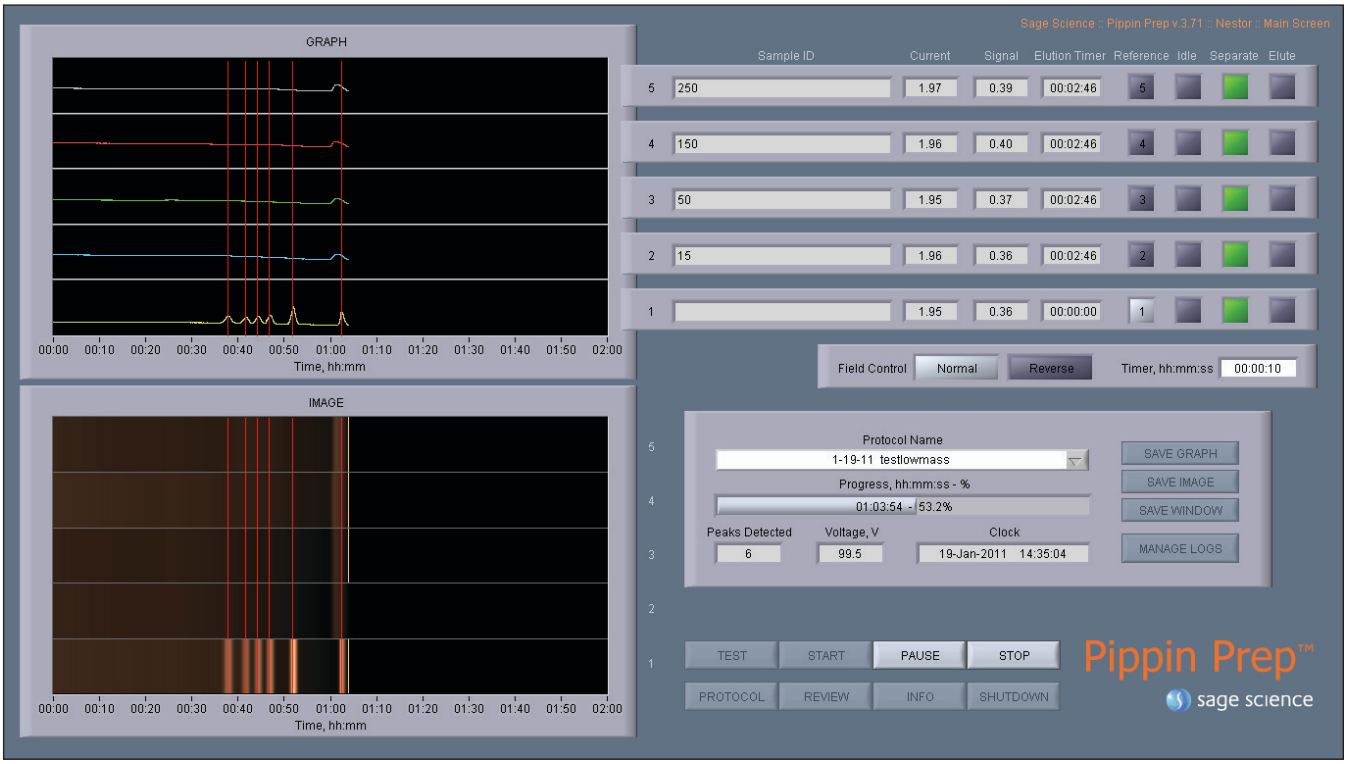

Figure 3
Home screen from the end of a Pippin Prep fractionation run using restricted *E. coli* genomic DNA as input. Reference markers are in lane 1 (bands at 20, 50, 75, 100, 150, 250bp). Genomic DNA input loads were 15, 50, 150, and 250 ng in lanes 2-5, respectively. No optical signal is observed from the genomic DNA samples due to low load. The small bump in signal that coincides with the 250 bp marker is caused by the increase in background when ethidium bromide from the elution channel moves up past the detector during elution.

The limiting optical sensitivity of the Pippin Prep instrument is about 5 ng of DNA in a single band, but has been shown to be much lower for genomic samples with a broad size distribution. As a result, even at the highest input load, 250 ng (Fig. 3, lane 5, at top), the genomic DNA is not detected. However, this is not an issue for operation of the Pippin Prep, since all elution timing is based on the reference markers (Fig. 3, lane 1, at bottom).

The size-fractionated products of the Pippin Prep run shown in Figure 3 were analyzed using an Agilent HS DNA chip. In all cases, 1 µL of the elution product (total 40 µL) was loaded directly on the chip, omitting additional purification. Electropherograms from the HS DNA chip are shown in Figure 4. The inset shows a blow-up of the 150 bp region, which contains the product peaks. Table 1 lists the amount of DNA in the peaks of interest, as determined by the Agilent 2100 Expert software.

Figure 4 and Table 1 show that the size-fractionated DNA products from the 50, 150, and 250 ng loads are easily detected on the HS DNA chip. Estimates of the DNA in these product bands are in the 30 to 120 pg range (Table 1). The 150 bp product from the 15 ng input load was barely detected above background, with the product amount estimated in the low single pg range These data demonstrate that the HS DNA kit is an excellent choice for evaluating Pippin Prep product DNA, with sufficient sensitivity to evaluate tight cuts from genomic DNA inputs as low as 50 ng.

It should be noted that the useful sensitivity for characterization of Pippin Prep products on the 2100 Bioanalyzer will depend on the shapes of the input and product distributions. For instance, if Pippin Prep selection in tight mode is carried out on input samples with a narrow distribution around the target size, product characterization will be possible with less input DNA than used for Figures 3 and 4. Similarly, more input DNA would be required if broad bp ranges were collected by the Pippin Prep.

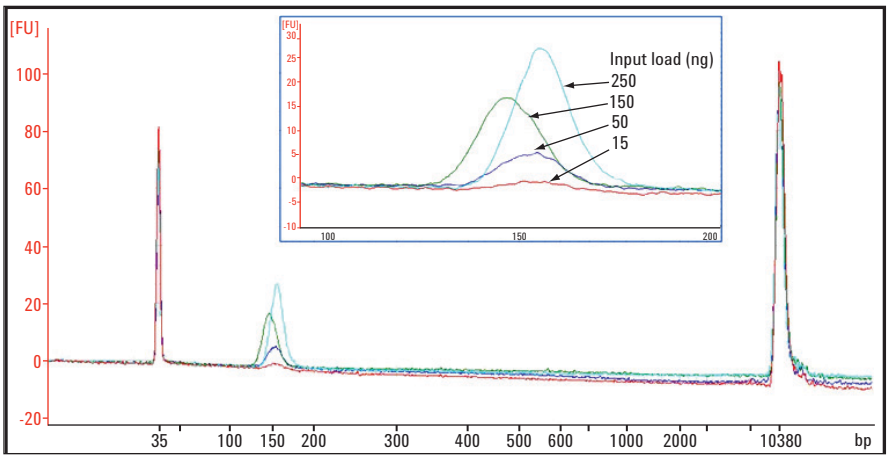

Figure 4
Pippin Prep products from run shown in Figure 3, analyzed on a HS DNA chip. A 2% cassette was used to collect tight distributions centered on 150 bp (Pippin Prep settings: bp target = 150). The inset shows a blow-up of the product peaks region.

Pippin lane	Input [ng]	From [bp]	To [bp]	Corr. area	% of Total	Average size [bp]	Size distribution in CV [%]	Conc. [pg/µL]
2	15	134	173	4.2	32	154	3.9	5.1
3	50	135	174	22.1	72	153	4.7	31.5
4	150	128	170	58.8	86	148	5.0	85.0
5	250	134	180	81.9	90	156	4.5	122.3

Table 1
Agilent 2100 Expert software output for HS DNA chip run shown in Figure 4.

Conclusion

We showed the benefits of using the High Sensitivity DNA kit on the Agilent 2100 Bioanalyzer for pre- and post-fractionation analysis of Pippin Prep samples. The kit provides analytical profiles of very low concentrated genomic samples allowing optimization of the fractionation settings used for the Pippin Prep system. Afterwards, it is used to confirm size ranges, quality, and purity of the Pippin Prep process. In summary, the 2100 Bioanalyzer and Pippin Prep systems work well in concert to increase the efficiency of library generation prior to next generation sequencing.

www.agilent.com/genomics/bioanalyzer

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