Technical Note

Recommendations for DNA Input, Using Adapters, Optimizing PCR, and Loading Sample Libraries

Apollo 324™ System

Overview

This technical note presents recommendations for fragmented DNA input, using adapters, and optimizing the PCR reaction when using the Apollo 324™ System, and loading prepared sample libraries on a sequencer.

DNA Input

- Recommended sample input is 200-1000 ng of DNA, although good results have been seen using amounts as high as 3 µg and as low as 0.5 ng (see Figure 1).
- Protocol size selection ranges are:
  - PrepX™ DNA Library: 350 bp +/- 75 bp (at half-height peak width)
  - PrepX Library BeadX™ High: > 200 bp
  - PrepX-32 DNA Library: 350 bp +/- 75 bp (at half-height peak width)
  - PrepX-454 DNA Library: > 500 bp
- Fragment the DNA by nebulization or Covaris®. It is a useful to verify the fragmented DNA size range with a run on an Agilent® Bioanalyzer. The Apollo 324 System does not support protocols that use DNA sheared with fragmentase.

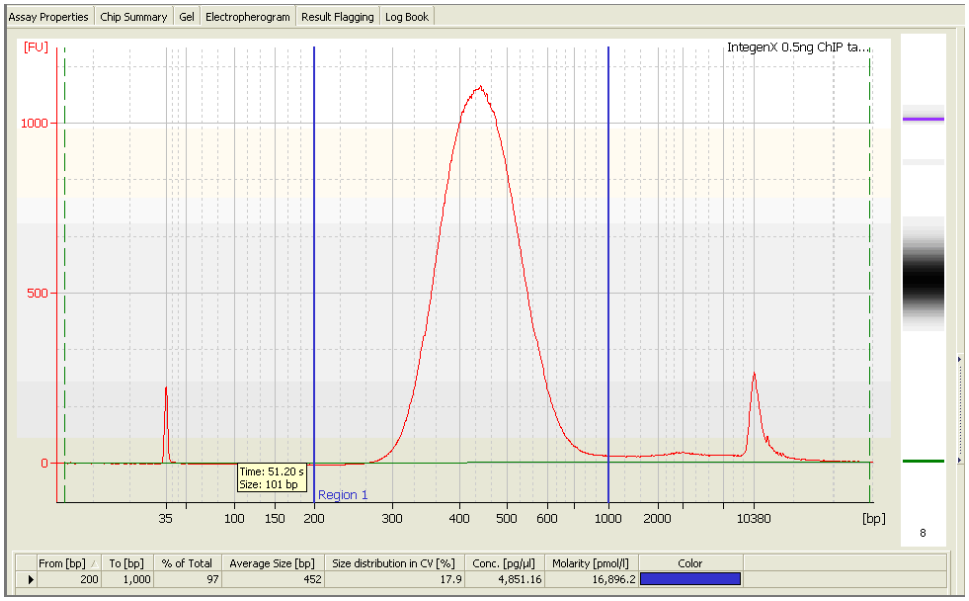

FIGURE 1. 0.5 ng of input library processed with the PrepX DNA Library protocol on the Apollo 324 System. Analysis was performed after 18 cycles of PCR. (See the Application Note, “Automated Library Preparation from 0.5 ng of ChIP DNA,” Document Number D006271.)

Using Adapters

- Illumina® Tru-Seq and Roche RL and MID adapters can be substituted into the Apollo 324 PrepX recipes. Pre-made adapters can also be purchased from Bioo Scientific (http://www.biooscientific.com/). HPLC or PAGE purified custom-made oligos can be ordered from oligo manufacturers such as IDT® and Sigma®. A procedure to make annealed adapters from custom oligos can be found in the *Apollo 324 System User Guide*, Chapter 2, in the section “Preparing Adapter Mix.”
- Recommended adapter concentration is 5 µM. Although good results can be seen with higher concentrations, more adapter dimers can result. It is not recommended to go below a 1.7 µM adapter concentration.
- Quality and age of adapters will impact ligation efficiency and adapter dimer formation. Adapters subject to multiple freeze/thaw cycles might reduce ligation efficiency and increase adapter dimer formation.
- Adapter dimers can always be cleaned up using Ampure® XP beads after PCR. If adapter dimers are still present after clean-up, repeat the Ampure XP bead clean-up.

Optimizing PCR

- Avoid overcycling or overloading the PCR reaction, as product multimers can result (see Figure 2). The recommended amounts for PCR input are:
  - 1 ng of library DNA
  - 10-12 PCR cycles for paired-end adapters
  - 15 PCR cycles for multiplex adapters
- Use primers that complement the adapters used to prepare the library. No amplification will result if primers do not match the adapter sequence.

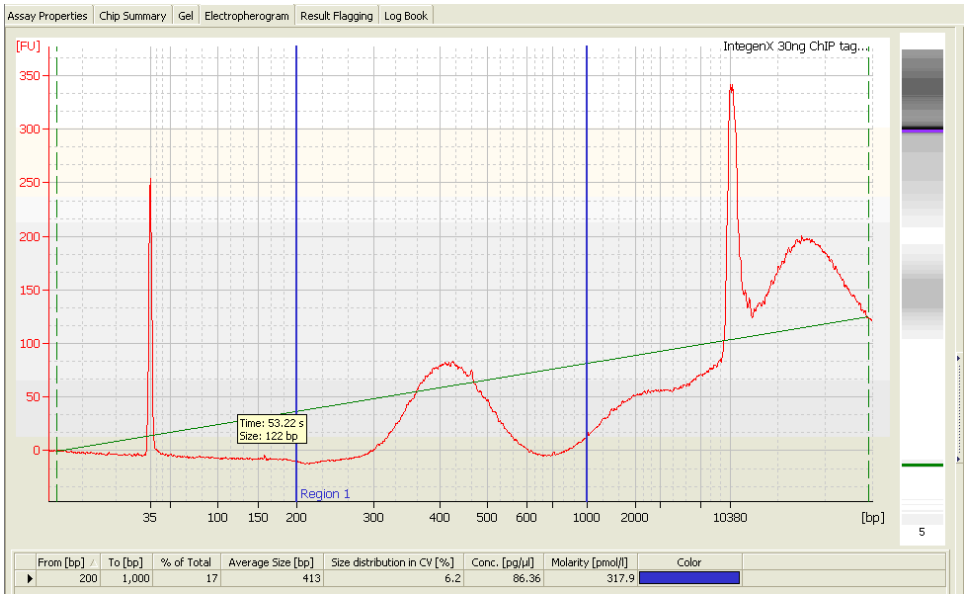

FIGURE 2. Unoptimized PCR conditions can lead to higher molecular weight products, seen here at 2000 bp and above.

Loading DNA Sample Libraries On the Sequencer

- Only DNA with adapters ligated onto both ends of the fragments will provide sequence on a next-generation sequencer. This is typically less than 100% of total DNA recovered after library preparation or PCR.
- Measure the amount of DNA with ligated adapters, using KAPA SYBR® FAST qPCR kits from Kapa Biosystems (http://www.kapabiosystems.com/).

Document Number D006270, 8/2011

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