Pippin Prep™ DNA Size Selection System

A Rapid Proteinase K Cleanup Method for Illumina Adapter Ligation Reactions Prior to Pippin Prep Fractionation

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Introduction

DNA in next generation sequencing library preparation is usually size-selected post adapter ligation. Until now this has been done on standard horizontal submarine agarose gels which has proved to be tedious, sometimes inaccurate and time consuming. The Pippin Prep automates this process and allows for preparative size selection in cartridges preloaded with an agarose matrix.

Resolving Illumina ligation reactions directly has been observed to be problematical due to the affect the DNA bound ligase has on migration rates, and this can lead to extraction of the incorrect DNA size. The ability to load samples directly onto the Pippin Prep without a clean-up step would save time and potentially improve yield.

Here we show a simple method of proteinase K treatment which allows post-ligation material to be loaded directly onto the Pippin Prep system.

Methods

Approximately 3 µg of 200 bp sheared genomic DNA was ligated with 4 µl of 100 µM (approx. 10 µg) Illumina paired-end adapter. The reaction mixture was divided into three aliquots, which were treated as follows:

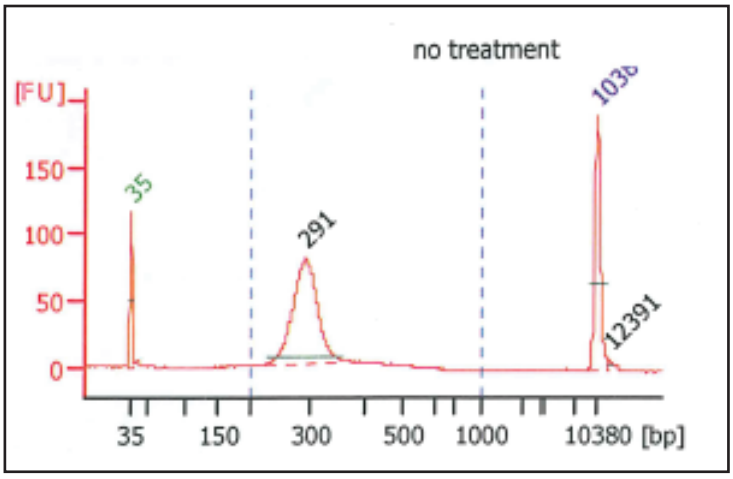
1. Cleaned on QIAquick PCR Purification column (Qiagen).
2. Treated with 1µl Proteinase K (Roche recombinant PCR grade) for 10 minutes at 37ºC.
3. No treatment.

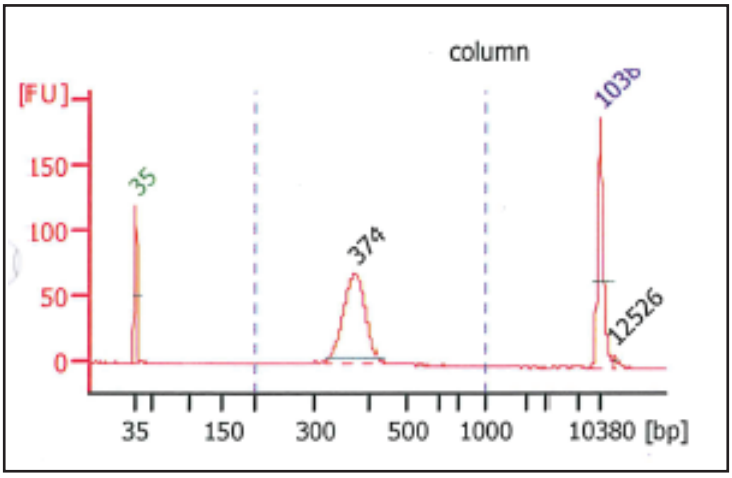
The samples were then loaded onto a 2% Pippin Prep cassette using settings to collect a tight distribution centered on 335bp (range: 308 to 362 bp). **Signal monitoring was turned off in the sample lanes due to the large amount of unligated adapter.** (High concentrations of unligated adapter create intense optical signals that can adversely affect reference marker recognition, see Tech Note 01.)

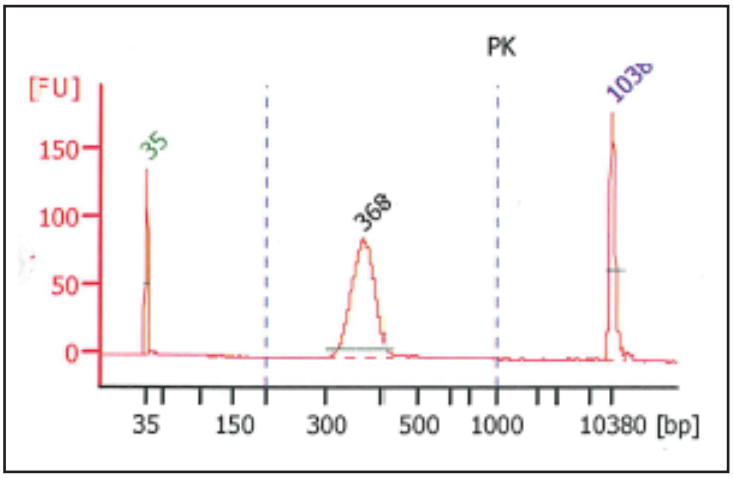
Fractionated samples were recovered and purified on a Qiagen column in order to remove ethidium bromide and reduce the sample volume.

Results and Discussion

Agilent Bioanalyzer traces of the recovered fractionated DNA are shown below. The 'no treatment' trace shows a peak at a lower than expected size suggesting retardation of the DNA byT4 DNA ligase and subsequent fractionation at the wrong size. Both the 'column-cleaned' and 'Proteinase K' traces show similar peak sizes at 374 and 368 bp respectively. This is approximately what we would expect when running adapter-ligated DNA on the Pippin Prep system. Illumina paired-end adapters are 'Y' shaped structures and we routinely observe these partially single-stranded fragments to run 30-40 bp faster on the Pippin Prep than their fully double stranded counterparts. Moreover, post-ligation material fractionated on the Pippin Prep often produces a broader peak, or indeed double or triple peaks, when run on an Agilent Bioanalyzer. We believe this is because post-ligation DNA that is fractionated on the Pippin Prep is composed of both fully double-stranded and partially single-stranded fragments and these have different electrophoretic mobilities on the Agilent Bioanalyzer, and are thus partially resolved in that instrument.







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