

Application of the Bioruptor® Pico for best shearing of yeast genomic DNA for NGS

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Introduction

DNA shearing is important step for next-generation sequencing library preparation. Important is reproducibility and efficiency of shearing. *S. cerevisiae* is yeast and have smaller amount of DNA per cells. They have a cell wall so the conditions which we use for the genomic DNA from yeast are different than for mammalian cells.

Shearing performance

We extracted the genomic DNA from $3.6 \cdot 10^9$ cells of *S. cerevisiae*. The genomic DNA was extracted with QIAGEN Genomic-tip 100/G, resuspended in Tris EDTA, pH 8.0 buffer. Samples was diluted with Tris EDTA, pH 8.0 buffer till 50 ng/μl. We sheared 100 μl of the genomic DNA in 0.65 ml Bioruptor® Microtubes. The DNA was sheared using the following Bioruptor® settings: 30 seconds ON/30 seconds OFF, taking 1 μl of samples after 10, 13, 15, 17, 20, 23, 25, 27, 30, 35, 40 cycles, followed by a short centrifugation after each round of 10 cycles. To the samples we added 9 μl of H₂O and purified with DNA Clean & Concentrator™-5 Zymo Research kit and eluted in 10 μl of elution buffer. We analyzed size distribution on an Agilent Bioanalyzer 2100 with Agilent High Sensitivity DNA Kit. An example result is shown here:

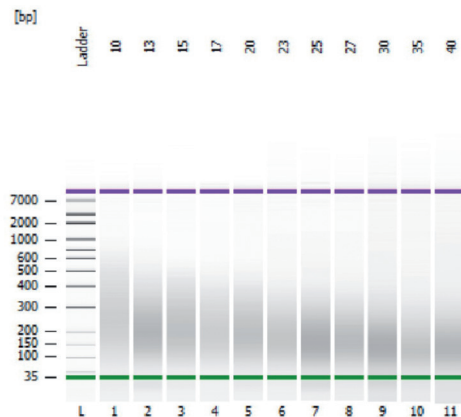


Figure 1: The resulting lengths of the sheared DNA with different sonication duration.

Reproducibility

For our purpose we chose 30 cycles condition which give us fragments with average size of 150 bp. To test reproducibility of the Bioruptor® Pico, we took 11 samples of genomic DNA from different biological replicates. We observed a very high reproducibility with the following Bioruptor® settings: 30 cycles (Sonication parameters 30 seconds ON/30seconds OFF, followed by a short centrifugation after each round of 10 cycles. All samples have an average size of 150 bp (Figure 2).

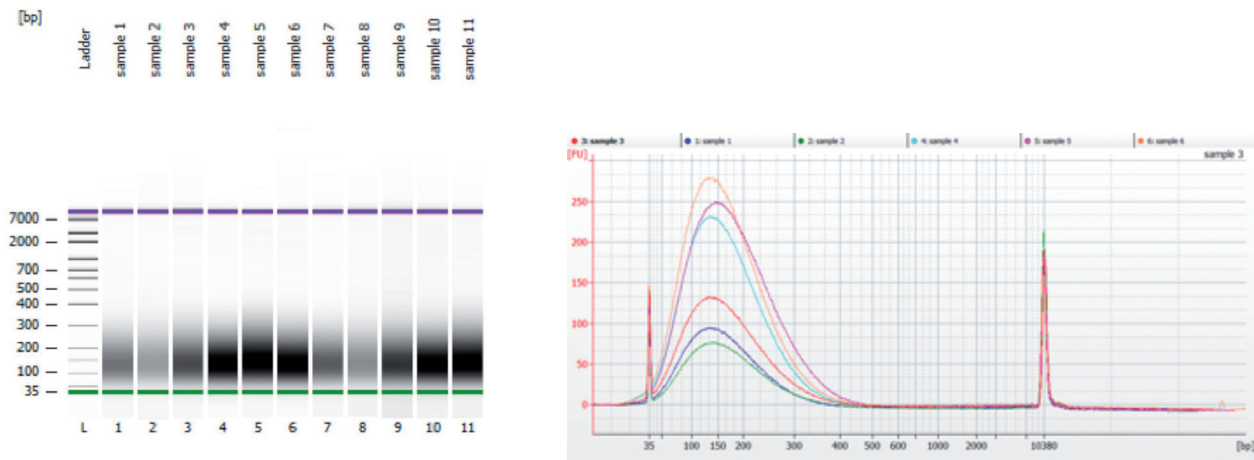


Figure 2: The resulting lengths of the sheared DNA from different biological replicate.

Library preparation

With the help of IMB Genomic Core Facility we used these samples for library preparation with Accel-NGS 1S DNA Library Kit from Swift Bioscience for Next-Generation Sequencing. For the library preparation we used 10 ng of sheared DNA per sample. Since the size of fragment were rather small we used relaxing the SPRI ratios: Post-Extension SPRI: 1.8X; Post-Ligation SPRI: 1.6X; Post-PCR SPRI: perform TWO 1.6X cleanups. With this condition we created library with size of 300 bp (Figure 3).

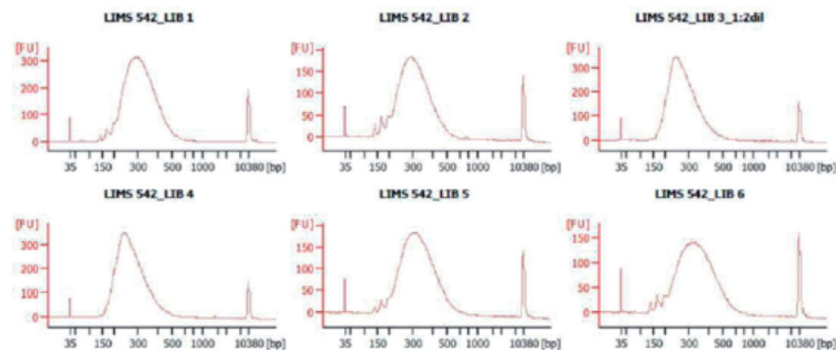


Figure 3: The resulting lengths of the library for Next-Generation Sequencing from different biological replicate.

Conclusion

Bioruptor® Pico provide a simple, reproducible and straight forward method for DNA shearing to obtain small fragment library for Next-Generation Sequencing.