

Ion Torrent Process Improvement: PippinPrep™ vs. SizeSelect™ in Ion Library Construction

Lisa Green, Scott Anderson, Jonna Grimsby, Andrew Hollinger, Dana Robbins, Alyssa Rosenthal, Elizabeth Ryan, Austin Tzou, Qing Yu, Niall J Lennon

Genome Sequencing Platform, Broad Institute of MIT & Harvard

Since Ion Torrent's release, the Broad Institute has worked to optimize all parts of the Ion Torrent workflow, including developing and testing a scalable production process. Sustained improvements to Ion's library construction (LC) process aim to be faster, automatable, and less expensive.

Background

E-Gel® SizeSelect™ pre-cast agarose gels allow DNA band excision without the need for manual gel cutting and purification, similar to the PippinPrep (SAGE Science). The recovered DNA requires no additional clean-up necessary before proceeding to the nick translation and amplification steps of Ion library construction. The standard Ion LC protocol uses the Pippin Prep method to size select the library prior to amplification. The SizeSelect™ system is less expensive per sample and has faster run times when compared to PippinPrep.

Design of Experiment

Genomic DH10B E. coli (1.5 µg from Ion LC kit) was sheared to 150bp (Figure 1) and then split into two aliquots. Both aliquots proceeded through standard Ion LC. One sample was size selected using the PippinPrep (Library ID: ION-658), and the other was size selected using SizeSelect™ (Library ID: ION-660). The target size for both libraries was 185bp. No clean-up was performed following elution of the SizeSelected™ aliquot. PCR (15 cycles) followed size selection for both samples.

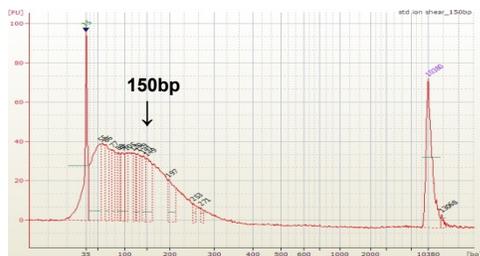


Fig. 1. DNA (1.5 µg) sheared to a target of 150bp.

Analysis and Results

ION-658 and ION-660 libraries were run on an Agilent High Sensitivity DNA chip (Figure 2) to assess final library size and concentration.

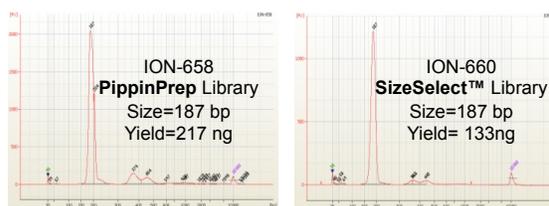


Fig. 2. ION-658 (size selected by PippinPrep), and ION-660, (size selected on SizeSelect gel), were run on an Agilent high sensitivity chip.

ION-658 had a final size of 187bp, and yield of 217 ng. ION-660 had a final size of 187bp, and yield of 133 ng. ION-658 had some higher molecular weight products at 375bp and 450bp. ION-660 had these same products, but at a lower concentration with respect to the library peak.

Recommendations

Although only two libraries were made to evaluate PippinPrep and SizeSelect™ for the size selection step in Ion LC, these preliminary results show comparable final libraries, with respect to size range and size specificity. The yield of the SizeSelect gel was slightly lower, however both methods produced plenty of library molecules for multiple downstream templating reactions.

For groups who are currently evaluating which size selection method to choose, further investigation into the relative cost/time benefits and throughput between these two systems are warranted prior to making a purchase.