Ion Torrent Applications Development:

Evaluation of ChIP-seq on Ion Torrent

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Background

We want to assess the utility of the Ion Torrent technology for ChIP-seq applications. There are two main issues that may cause problems when attempting to sequence ChIP-seq samples on Ion Torrent:
1) ChIP DNA fragments are usually 100-500 bp and peak at 300 bp. DNA fragments that are over 200bp are currently (at the time of this expt) suboptimal to amplify on Ion Spheres.
2) ChIP DNA is usually very low yield (0.5ng to 5ng), which is a magnitude lower than the recommended starting material for the standard Ion Torrent library construction (1ug). The low amount of starting material precludes shearing or other size selection methods to push more of the molecules into the correct starting size range.

Design of Pilot Experiment

We performed Ion library construction on 3 ChIP samples, H3K4me3 (2.7ng/ul), polII (0.2ng/ul) and IgG (0.07ng/ul) from mouse dendritic cells stimulated with LPS (0.1ug/ml) for 2 hours. Unligated ChIP DNA samples went through Ion LC without shearing. Post adapter ligation, samples were size selected at 200bp using the Pippin Prep (Sage Science). Nick Translation and amplification was performed (9 cycles). The 200bp libraries were templated according to standard protocols and enriched ISPs were sequenced on Ion 316 chips.

Analysis and Results

We compared histone mark (H3K4me3) and RNA polymerase II ChIP-seq enrichment peaks between Ion Torrent and previous data from a Massively Parallel Sequencer (MPS) and found that ChIP-seq enrichment signals from 2 million Ion Torrent reads (a single run of an Ion 316 chip) is comparable to 10-18 million MPS reads (Figure 1).

When comparing Ion Torrent H3K4me3 ChIP-seq enrichment scores from 2 millions reads with MPS H3K4me3 ChIP-seq enrichment scores from 18 million reads over a 200bp window size, we found that the two ChIP-seq datasets are highly correlated with a Pearson correlation coefficient of 0.92 (Figure 2).

Figure 1. Comparing histone mark and RNA polymerase II ChIP-seq enrichment peaks between Ion Torrent and Massively Parallel Sequencer (MPS).

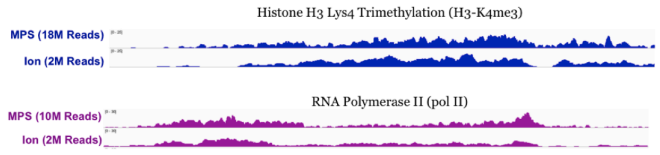
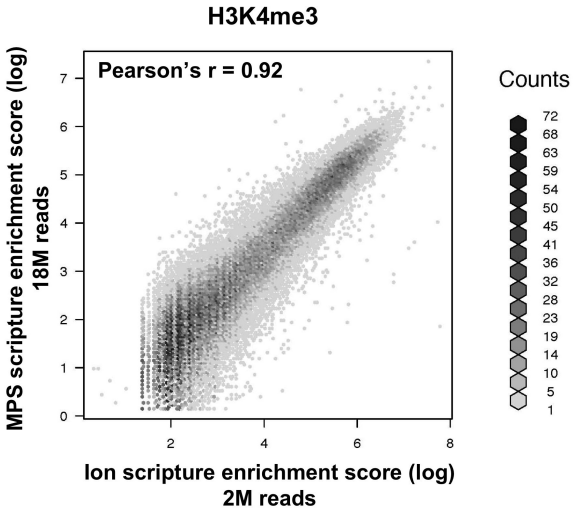


Figure 2. Ion Torrent H3K4me3 ChIP-seq enrichment scores are highly correlated with MPS ChIP-seq



Conclusions

The results shown here indicate that the Ion Torrent sequencing technology is fully capable of producing ChIP-seq data. It should be noted that these data are preliminary and follow-up studies, with the same biological samples on both Ion Torrent and a MPS, with technical repeats, and titration of ChIP DNA inputs, are underway.