Enabling solutions for fast and cost-effective ChIP-sequencing using Diagenode iDeal ChIP-seq kit and antibodies with the Ion Torrent PGM™ sequencer

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Introduction

ChIP-seq has become the gold standard for whole-genome mapping of protein-DNA interactions. The generalized adoption of this technology is currently limited by 4 main technical hurdles.

First, the reproducibility and biological relevance of DNA-associated protein landscapes depend on the specificity and performance of the antibodies in the context for which they are used. Second, the ChIP-seq method requires optimized protocols ensuring high recovery and increased signal-to-noise ratio. Third, as an effort to reduce the cost per sample and improve reproducibility, the ChIP-seq method should be compatible with automation. Finally, the economical and widespread use of ChIP-seq requires access to a fast and high value/quality next-generation sequencing platform.

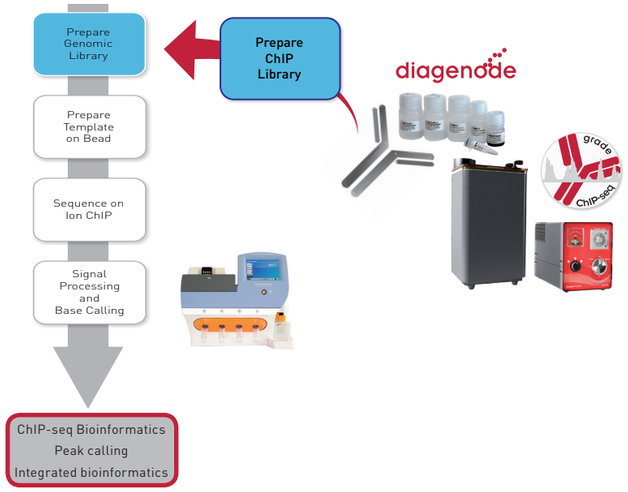
In this poster, we demonstrate the successful use of the Diagenode iDeal ChIP-seq kit (Cat. No. AB-001-0024) for ChIP-seq with the Ion Torrent Personal Genome Machine™.

Methods A real breakthrough in Epigenetics

Ion Torrent has partnered with Diagenode's world-class upstream sonication and epigenetic technologies.

The Bioruptor® sonicator is recommended for mechanical DNA shearing for Ion Torrent PGM™ library preparation. Diagenode has created and validated the iDeal ChIP-seq kit for Ion Torrent PGM™ sequencing. The iDeal ChIP-seq kit is available for purchase from the Ion Torrent Store.

Diagenode and Ion Torrent share the same goal : to provide every sequencing lab a simple, efficient and cost-effective sequencing workflow for ChIP-seq.



Results

1. Efficient and easy chromatin shearing using the Bioruptor® and Shearing buffer iS1 from the iDeal ChIP-seq kit

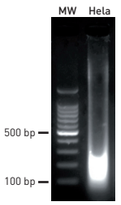


Figure 1.

Chromatin from 1 million of Hela cells was sheared using the Bioruptor® combined with the Bioruptor® Water cooler (Cat No. BioAcc-cool) during 3 rounds of 10 cycles of 30 seconds "ON" / 30 seconds "OFF" at HIGH power setting (position H). Diagenode 1.5 ml TPX tubes (Cat No. M-50001) were used for chromatin shearing.

Samples were gently vortexed before and after performing each sonication round (rounds of 10 cycles), followed by a short centrifugation at 4°C to recover the sample volume at the bottom of the tube. The sheared chromatin was then decross-linked as described in the kit manual and analyzed by agarose gel electrophoresis.

2. Validation of ChIP: reliable results using Diagenode's ChIP-seq grade H3K4me3 antibody, isotype control and sets of validated primers

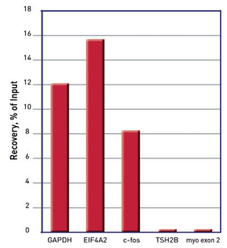


Figure 2.

Specific enrichment on positive loci (GAPDH, EIF4A2, c-fos promoter regions) comparing to no enrichment on negative loci (TSH2B promoter region and Myoglobin exon 2) was detected by qPCR.

Samples were prepared using the Diagenode iDeal ChIP-seq kit. Diagenode ChIP-seq grade antibody against H3K4me3 and the corresponding isotype control IgG were used for immunoprecipitation. qPCR amplification was performed with sets of validated primers.

3. Superior performance of the Diagenode iDeal ChIp-seq kit with the IPure DNA purification module and reliable sequencing with the Ion Torrent PGM™ sequencer

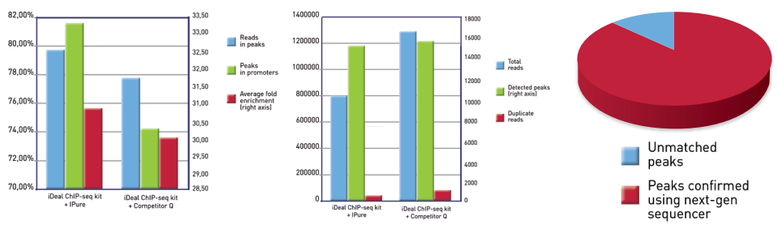


Figure 3.

Figure A: The iDeal ChIP-seq kit with the IPure DNA purifcation module provides lower noise (more reads in the peaks, less in the background) and higher specificity (more peaks found in the promoter regions for the promoter specific H3K4me3 antibody – less false positives). The lower noise level and the subsequent higher read accumulation tendency is demonstrated by a higher average fold enrichment ratio as well for the iDeal ChIP-seq kit with the IPure DNA purification module.

Figure B: Using the IPure DNA purification module with the iDeal ChIP-seq, kit we detected approximately the same number of peaks as with the column-based purification kit from Competitor Q from roughly 40% less reads, using the same H3K4me3 antibody. This is due to the more specific captured reads and the lower noise level maintained by the IPure unique technology. The filtered duplicate read ratio was also lower, 4.0% compared to the Competitor's 5.6%.

Figure C: Using another Massive Parallel Sequencing system and the iDeal ChIP-seq kit with same antibody confirmed most of the peaks, further proving the high ratio of true positive hits.

4. ChIP-seq data acquired by PGM maintain high specificity.

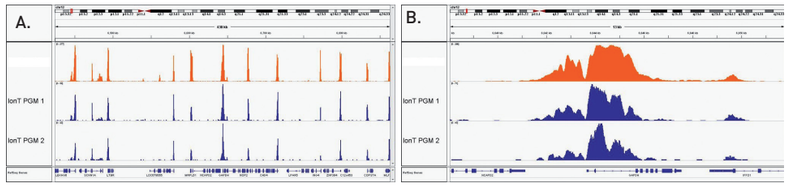


Figure 4.

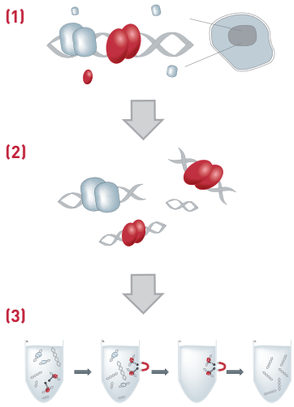
The high consistency of the iDeal ChIP-seq kit is proven by multiple sequencings on Ion Torrent PGM™ and another Massive Parallel Sequencer (MPS-I).

Figure A shows a several hundred bp stretch along 12th chromosome, where a high similarity of read distribution can be observed, despite the radically different characteristics of the sequencers.

Figure B is a close capture focusing on the well-known GAPDH gene (see dotted rectangle). The detailed view reveals that even the peak structure is similar.

The iDeal ChIP-seq kit is designed specifically for ChIP-seq

To provide the most robust and versatile ChIP-seq, we optimized the key steps of the ChIP workflow:



1. Cell fixation and DNA-protein cross-linking.
2. Cell lysis and chromatin shearing using the Bioruptor®.
3. Binding of the antibody to beads and chromatin.
4. Magnetic immunoprecipitation.
5. Washes of immune complexes.
6. DNA purification and recovery of ChIP'd DNA.

Optimized steps	Benefits
Chromatin buffers and shearing conditions (1, 2)	High fragmentation efficiency, easy to set up, reproducible results
IP buffer (3)	Optimal conditions for antibody binding
Affinity enrichment using ChIP-seq grade Ab (3, 4)	Improved specificity, reliable results
Washes and washing buffers (5)	Higher signal-noise ratio, less background in sequencing
DNA purification using the IPure kit (6)	Improved consistency and maximum sample recovery

Related Products

Description	Cat. No.	Description	Cat. No.
Bioruptor® Standard	4465622	H3K9ac polyclonal antibody	pAb-004-050
iDeal ChIP-seq kit x24 for PGM	AB-PGM-0024	H3K9ac polyclonal antibody	pAb-177-050
IPure kit x100	AL-100-0100	H3K9/14ac polyclonal antibody	pAb-005-044
H3K4me3 polyclonal antibody	pAb-003-050	H3K27ac polyclonal antibody	pAb-174-050
H3K4me3 monoclonal antibody	MAb-152-050	H4K20me3 polyclonal antibody	pAb-057-050
H3K4me2 polyclonal antibody	pAb-035-050	Pol II monoclonal antibody	AC-055-100
H3K4me1 polyclonal antibody	pAb-037-050	ER monoclonal antibody	AC-066-100
H3K9me3 polyclonal antibody	pAb-056-050	TBP monoclonal antibody	MAb-002-100
H3K27me3 polyclonal antibody	pAb-069-050	RARA polyclonal antibody	CS-155-100
H3K36me3 polyclonal antibody	pAb-058-050	Human c-fos promoter primer air	pp-1004-050/500
H3K36me3 polyclonal antibody	CS-058-100	Human TSH2B primer pair	pp-1041-050/500
H3K79me3 polyclonal antibody	pAb-068-050	Human myoglobin exon 2 primer pair	pp-1006-050/500

Conclusions

- The Diagenode iDeal ChIP-seq kit is tailor-made for ChIP-seq.
- Performance of the iDeal ChIP-seq kit is optimal with Diagenode's ChIP-seq grade antibodies and IPure Magnetic DNA Purification module.
- Proven efficiency and robustness of the iDeal ChIP-seq kit with Ion Torrent PGM™ sequencing platform.
- Extremely high reproducibility and high peak overlap with PGM using the iDeal ChIP-seq kit starting from as low as 10ng ChIP'd DNA.
- Compatible with automation using the Auto iDeal ChIP-seq kit using the SX-8G IP-Star® and SX-8G IP-Star® Compact Automated Systems (data not shown).

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