Antibodies you can trust: Unparalleled ChIP-Seq results with the most rigorously validated antibodies

Jan Hendrickx, Géraldine Goens, Catherine D'Andrea, Mustafa Tammoh, Kana Sumikawa, Céline Sabatell, Miklos Laczik, Sharon Squazzo*, Hélène Pendeville, Dominique Poncelet

Diagenode sa, CHU, Tour GIGA B34, 3ème étage 1 Avenue de l’Hôpital, 4000 Liège, Sart-Tilman, Belgium | *Diagenode inc., 400 Morris Avenue, Suite 101, Denville, NJ 07834, USA

Introduction

Epigenetic research tools have evolved over time from endpoint PCR to qPCR to the analyses of large sets of genome-wide sequencing data. ChIP sequencing (ChIP-seq) has now become the gold standard methodology for chromatin studies, given the accuracy and coverage of the approach over other methods. Successful ChIP-seq, however, requires a higher level of experimental accuracy and consistency in all steps of ChIP than ever before. Particularly crucial is the quality of ChIP antibodies.

In view of such requirements of highly qualified antibodies, Diagenode has established the most rigorous QC procedure for its new Premium Class of ChIP-seq grade antibodies. The Diagenode’s Premium antibodies have reached the highest level of validation from the extensive work realized in numerous collaborations and the IHEC community of epigenetic experts. All are validated for ChIP-seq and exhibit superior performance for virtually any epigenetic application.

The Premium antibodies passed the most stringent QC tests (including bioinformatics and comparison with Encode reference data sets from the Broad Institute). Our partners consider these the best possible, highest performance antibodies available and they will likely become the next international standards. Our R&D work is in accordance with the overall objectives of the IHEC.

A guideline given by the IHEC consortium suggests to map the following 6 histone modifications within the genome feature affinity

- H3K4me1, H3K27ac, actively transcribed genes (H3K36me3), or heterochromatin regions (H3K9me3, H3K27me3). Diagenode has followed these guidelines to generate specific antibodies recognizing these histone modifications.

Methods

Diagenode’s antibody quality control and ChIP-seq workflows:

Diagenode’s ChIP-seq workflow:

- CRUDE SERUM
- ELISA QC
- Affinity purification
- Chromatin IP
- Specificity/Cross reaction QC
- ChIP using the Diagenode ChIP-seq kit and Premium antibodies
- Chromatin stabilization by cross-linking
- Chromatin fragmentation using sonicator
- ChIP-seq run on GAIIx (Illumina sequencer)
- Sequencing library preparation
- Immunoprecipitation
- Chromatin IP
- ChIP-seq workflow

Results

ChIP-seq experiments were performed on different blood cell types (Figure 1: HS62 – Diagenode, Figure 2: neutrophils – Diagenode’s external partner). Typically, cells are grown under standard conditions, fixed with formaldehyde for 8 min. at room temperature. Crosslinked cells are then lysed and the chromatin is tested on an agarose gel and Bioanalyzer for a proper fragment distribution check. Immunoprecipitation was performed on 100,000 cells using the Diagenode ChIP-seq kit and ChIP-seq Premium antibodies, crosslinks were reversed and purified DNA was tested for specific enrichments at pre-defined genomic regions using qPCR. If necessary, the IP’d DNA from different ChIPs is pooled and sequenced using standard or multiplexed library preparation, cluster generation and sequencing on an Illumina GAIIx. The sequenced tags were mapped to hg18, processed by cross-linking and comparison. If peaks were generated by cross-linking and comparison.

Conclusions

As part of our philosophy to apply the highest quality standards to our antibodies and in an effort to continuously improve our QC procedure, we have introduced the validation of the Diagenode Premium antibodies using highly standardized ChIP-seq workflows. The Premium antibodies passed the most stringent QC tests (including general data analysis e.g. reads in peak) and comparisons with the Encode reference data sets from the Broad Institute. Our partners consider these as the best possible, highest performance antibodies available and they will likely become the next international standards.

Diagenode’s Premium Antibodies

For more information, please contact: for Europe, info@diagenode.com | for the US and Canada, contact info.na@diagenode.com