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

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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 method for chromatin studies, given the accuracy and coverage scale 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.

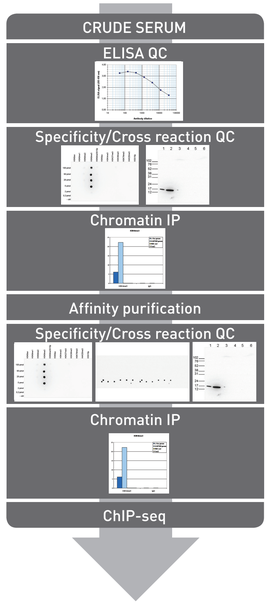
The Premium antibodies passed the most stringent QC tests (including bioinformatics and comparison with Encode reference data sets from the Broad Institute). Our partners consider these the best possible, highest performance antibodies available and they will likely become the next international standards. 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 any epigenome project: H3K4me3, H3K9me3, H3K27me3, H3K27ac, H3K4me1 and H3K36me3. The presence of these marks is indicative of active promoters (H3K4me3, H3K27ac), active enhancers (H3K4me1, H3K27ac), actively transcribed genes (H3K36me3) or heterochromatin regions (H3K9me3, H3K27me3). Diagenode has followed these guidelines to generate specific antibodies recognizing these histone modifications.

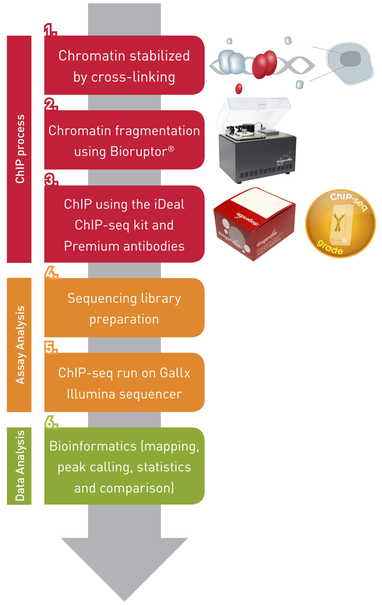
Methods

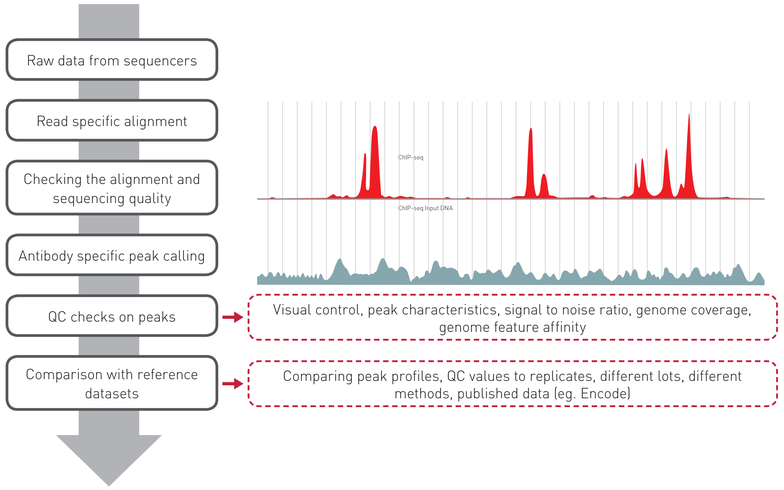
Diagenode's antibody quality control and ChIP-seq workflows:

QC from crude sera to purified ChIP-seq grade antibodies



Diagenode ChIP-seq workflow





Results

ChIP-seq experiments were performed on different blood cell types (Figure 1: K562 - Diagenode; Figure 2: neutrophils - Diagenode's external partner). Typically, cells are grown under standard conditions, harversted by trypsinization and fixed with formaldehyde for 8 min. at room temperature. Crosslinked cells are then sonicated using the Bioruptor® 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 iDeal 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 for visualization in the UCSC browser and checked using our bioinformatics QC workflow.

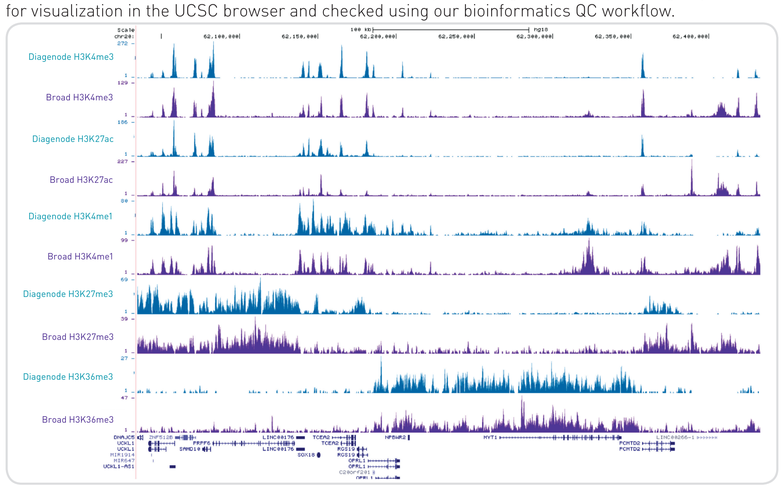


Figure 1

H3K4me3, H3K27ac, H3K4me1, H3K36me3, H3K27me3 ChIP-seq profiles in K562 cells (Blue profile: Diagenode's data; Violet profile: Broad data).

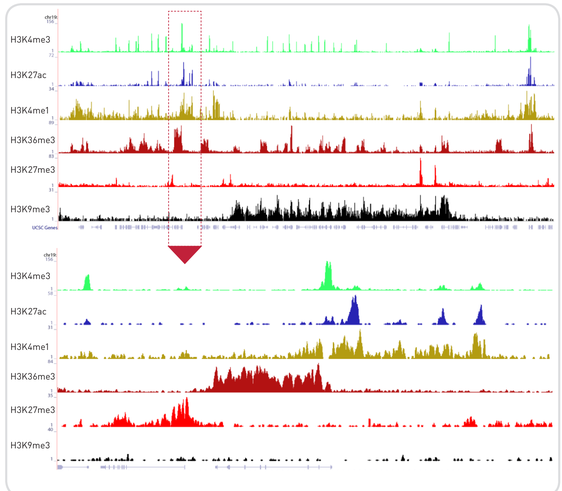


Figure 2

H3K4me3, H3K27ac, H3K4me1, H3K36me3, H3K27me3 and H3K9me3 ChIP-seq profiles in neutrophils (bottom panel – chromosome 19 views for 2 zoom-in levels

(Data kindly provided by the Blueprint consortium, Joost Martens, Willem Ouwehand & Henk Stunnenberg's teams (Radboud University Nijmegen, The Netherlands; University of Cambridge, UK)

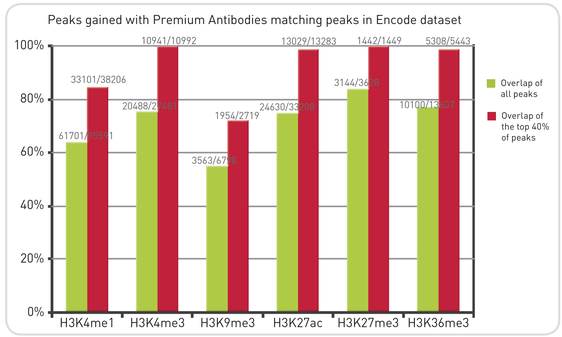


Figure 3

We used reference datasets from the Encode project to confirm the peaks detected with our Premium antibodies, direct matches were required (no distance between peaks), and we achieved exceptional overlap rates: the best 40% of our peaks (likely containing no false peaks) often achieve nearly 100% overlap ratio.

These overlapping figures are normally characteristic of replicate datasets.

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 stantardized ChIP-seq workflows.

The Premium antibodies passed the most stringent QC tests (including general data analysis (eg. reads in peaks) 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 ★★★★★

Products	Cat No.
H3K4me3 polyclonal antibody - Premium	pAb-003-050
H3K36me3 polyclonal antibody - Premium	pAb-192-050
H3K9me3 polyclonal antibody - Premium	pAb-193-050

Products	Cat No.
H3K4me1 polyclonal antibody - Premium	pAb-194-050
H3K27me3 polyclonal antibody - Premium	pAb-195-050
H3K27ac polyclonal antibody - Premium	pAb-196-050

Products	Cat No.
H3K9/14ac polyclonal antibody - Premium	C15410200
H2A.Zac polyclonal antibody - Premium	C15410202
H4K20me3 polyclonal antibody - Premium	C15410207

PO-Prem_Abs-A3-V1_21_01_13