Successful implementation of ChIP-seq Quality Control at Diagenode using automated ChIP protocol on the SX-8G IP-Star® Compact

Jan Hendrickx, Géraldine Goens, Catherine D'andrea, Ignacio Mazon, Geoffrey Berguet, Sharon Squazzo, Céline Sabatel, Miklos Laczik, Dominique Poncelet.

Diagenode sa, CHU, Tour GIGA B34, 3ème étage 1 Avenue de l'Hôpital, 4000 Liège, Sart-Tilman, Belgium

Introduction

Chromatin immunoprecipitation (ChIP) is the most widely used method to study protein-DNA interactions. A successful ChIP, however, is largely depending on the use of well characterised, highly specific ChIP-grade antibodies.

We have established a robust QC procedure to be able to provide researchers with the highest quality ChIP-grade antibodies. The recent development of high throughput sequencing (HTS) technologies has also opened new possibilities for epigenetic research. ChIP followed by HTS (ChIP-seq) enables the extension of the target directed analysis of ChIP results to a genome wide analysis.

In view of the enormous potential and growing interest of this new development and to increase the quality standards of our antibodies, we added this new technique to our QC procedure. Here, we present 19 ChIP-seq grade antibodies that have passed this very strict quality control procedure.

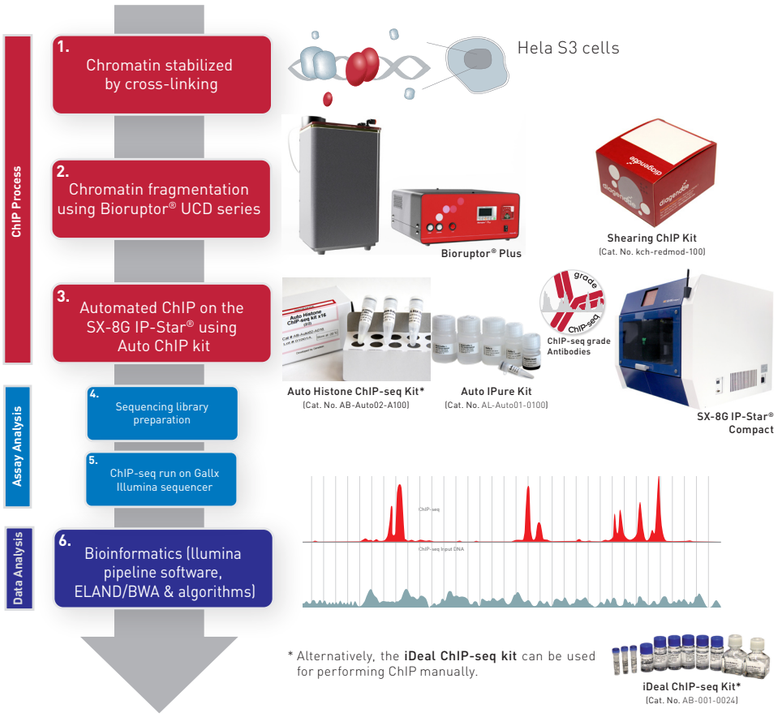
Methods

HeLaS3 were grown under standard conditions, harvested by trypsinisation and fixed for 8 minutes at room temperature with 1% formaldehyde.

After washing of the cells with PBS, chromatin was sheared using the shearing ChIP kit (kch-redmod-100). ChIP was performed on sheared chromatin from 1 million cells with the "Auto Histone ChIP-seq kit protein A" (Cat. No. AB-Auto02-A100) on the SX-8G IP-Star® Automated System using 1 - 2 µg of the respective antibody. IgG (2 µg/IP) was used as a negative IP control. Depending on the antibody, the IP'd DNA from up to 5 different ChIP reactions was pooled and purified.

The IP'd DNA was subsequently analysed on an Illumina Genome Analyzer IIx. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 36 bp tags were aligned to the human genome using the ELAND or BWA algorithm.

Diagenode automated ChIP-seq workflow: overview



Results

We have validated 19 of the Diagenode ChIP-grade antibodies using ChIP-seq (see table 1).

Figure 1 shows the profiles obtained with antibodies against (1) H3K4me3, H3K9ac and H3K9/14ac, histone modifications that are present at active promoters, (2) H3K4me2 which is surrounding promoters of active genes, (3) H3K79me3 present at promoters of active genes and extending into the gene body, and (4) H3K36me3, present at the gene bodies of active genes. The screenshots below show the peak distribution along the complete X-chromosome and in a 75 kb region surrounding the GAPDH gene.

Figure 2, 3 and 4 show the profiles of H3K27me3, H3K36me3 and H3K4me3 in a 500 kb region surrounding the MYT1 gene (figure 2), of H3K9me3 in a 200 kb region surrounding ZNF12 (figure 3), and of PolII in a 75 kb region containing the GAPDH gene (figure 3).

Figure 1

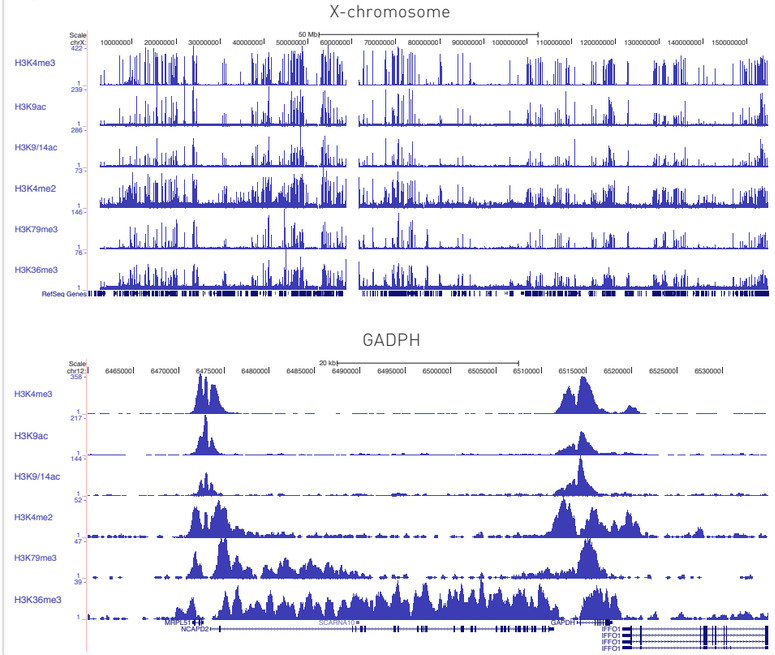


Figure 2

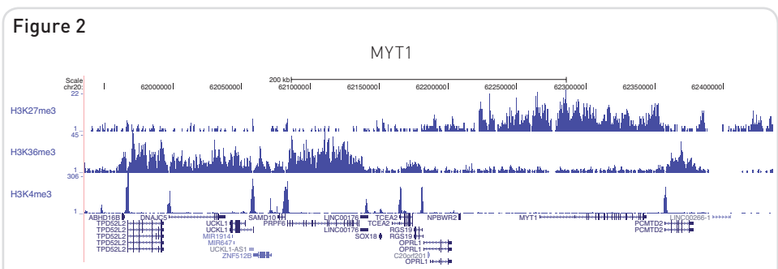


Figure 3

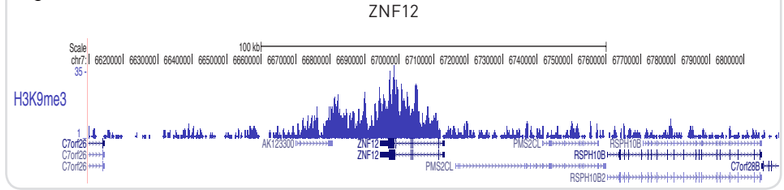
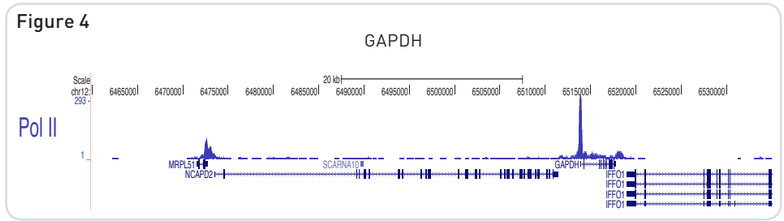


Figure 4



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 antibodies in ChIP-seq. Currently, a total of 19 ChIP-seq grade antibodies, available at Diagenode, have passed this very strict quality control procedure.

Description	Cat No.
H3K4me3 polyclonal antibody	pAb-003-050
H3K4me3 monoclonal antibody	MAb-152-050
H3K4me2 polyclonal antibody	pAb-035-050
H3K4me1 polyclonal antibody	pAb-037-050
H3K9me3 polyclonal antibody	pAb-056-050
H3K27me3 polyclonal antibody	pAb-069-050
H3K36me3 polyclonal antibody	pAb-058-050
H3K36me3 polyclonal antibody	CS-058-100
H3K79me3 polyclonal antibody	pAb-068-050
H3K9ac polyclonal antibody	pAb-004-050
H3K9ac polyclonal antibody	pAb-177-050
H3K9/14ac polyclonal antibody	pAb-005-044
H3K27ac polyclonal antibody	pAb-174-050
H4K20me3 polyclonal antibody	pAb-057-050
Pol II monoclonal antibody	AC-055-100

Description	Cat No.
ER monoclonal antibody	AC-066-100
TBP monoclonal antibody	MAb-002-100
RARA polyclonal antibody	CS-155-100
GR monoclonal antibody	MAb-010-050
Human c-fos promoter primer air	pp-1004-050/500
Human TSH2B primer pair	pp-1041-050/500
Human myoglobin exon 2 primer pair	pp-1006-050/500
SX-8G IP-Star® Automated System	UH-001-0001
SX-8G IP-Star® Compact Automated System	UH-002-0001
Bioruptor® Plus	UCD-300-TO
Shearing ChIP kit	kch-redmod-100
Shearing ChIP kit	kch-redmod-100
IPure kit	AL-100-0100
Auto IPure kit	AL-Auto01-0100
Auto Histone ChIP-seq kit	AB-Auto02-A100
iDeal ChIP-seq kit x24	AB-001-0024