APPLICATION NOTE

Epigenetic landscape of proximal tubular epithilial cells using CHiPseq

Epigenetic landscape of proximal tubular epithelial cells using ChIPseq

by Robert C. Akkers[1], Gerben Duns[2] and Klaas Kok[2]
[1] BIOKÉ, Schuttersveld 2, 2316 ZA Leiden, The Netherlands
[2] University Medical Center Groningen, Department of Genetics, 9713 GZ Groningen, The Netherlands

Background

Gene regulation plays an important role in maintaining cell identity. Aberrant control of gene expression can lead to a variety of diseases including cancer. Gene expression is regulated at different levels by epigenetic phenomena, like DNA methylation and histone modifications. The most well studied histone modifications in gene regulation are histone H3 lysine 4 trimethylation (H3K4me3) and trimethylation of lysine 27 at histone H3 (H3K27me3), markers of transcriptionally active and inactive chromatin, respectively. Transcriptional read-out is frequently studied by micro-array and RNA sequencing or alternatively by profiling RNA polymerase II and the trimethylation status of lysine 36 of histone H3 (H3K36me3). The epigenetic status of genes can be assayed by chromatin immunoprecipitation (ChIP). ChIP is a powerful technique to study DNA-protein interactions. To explore the genome-wide binding of transcription factors and the epigenetic landscape ChIP can be combined with sequencing (ChIPseq). This technique enables exploration of the epigenetic state in a global fashion. To date the ChIPseq workflow was not reported as a streamlined process from a single company. Here we report on the ChIPseq workflow from chromatin harvesting to sequencing data analysis using products supplied by BIOKÉ.

Results

To generate epigenetic profiles chromatin immunoprecipitation experiments were performed using the SimpleChIP® enzymatic chromatin IP kit with magnetic beads (#9003S) from Cell Signaling Technology. The SimpleChIP kit is an all-inclusive reagent set that uses an enzymatic digestion of chromatin with micrococcal nuclease sustaining the chromatin integrity which results in high ChIP efficiencies and high ChIP sensitivity. Chromatin was harvested from proximal tubular epithelial cells (PTEC) and assayed using different antibodies to determine the epigenetic environment of target genes. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an example of a transcriptionally active gene as is seen by the enrichment of H3K4me3 at the 5' end and H3K36me3-enrichment in the gene body by quantitative PCR (Figure 1). Myogenic differentiation 1 (MyoD) is a transcriptionally inactive gene as determined by high H3K27me3-enrichment (Figure 1). These ChIP-qPCR results reveal a functional ChIP in PTEC.

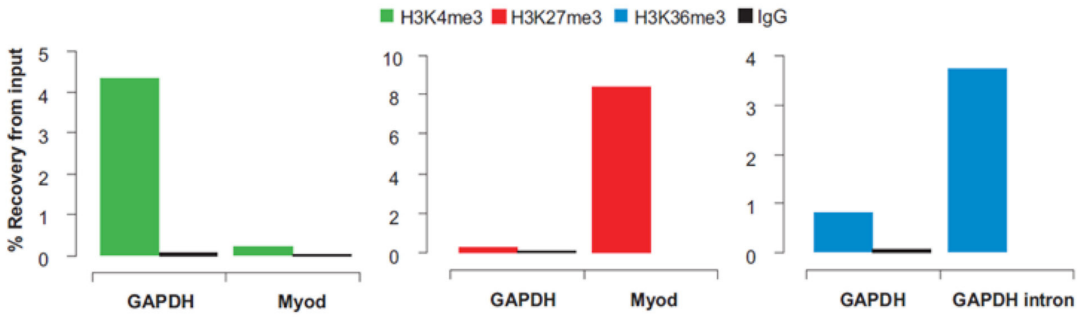

Figure 1: ChIP assessment of individual targets by quantitative PCR.
Percentage recovery of H3K4me3 (green, left panel) H3K27me3 (red, middle panel), H3K36me3 (blue, right panel) and an IgG control (black) for the indicated genes.

To learn more about the global epigenetic state of the PTEC, ChIP-enriched DNA was processed for sequencing using the sample preparation NEBNext® ChIPseq Sample Prep Master Mix Set 1 from New England Biolabs (#E6240). The NEBNext® kit is a convenient set of reagents that facilitates the sample preparation procedure for most sequencing platforms (Figure 2). In short, the ends of ChIP-enriched DNA were repaired and accommodated with an A-tail. Adaptors were ligated and DNA was amplified by PCR. For each reaction DNA can be purified using the clean-up NucleoSpin® gel and PCR clean-up columns from MACHEREY-NAGEL (#740609). Subsequently sample-prepped DNA was sequenced on an HiSeq2000® sequencer[1].

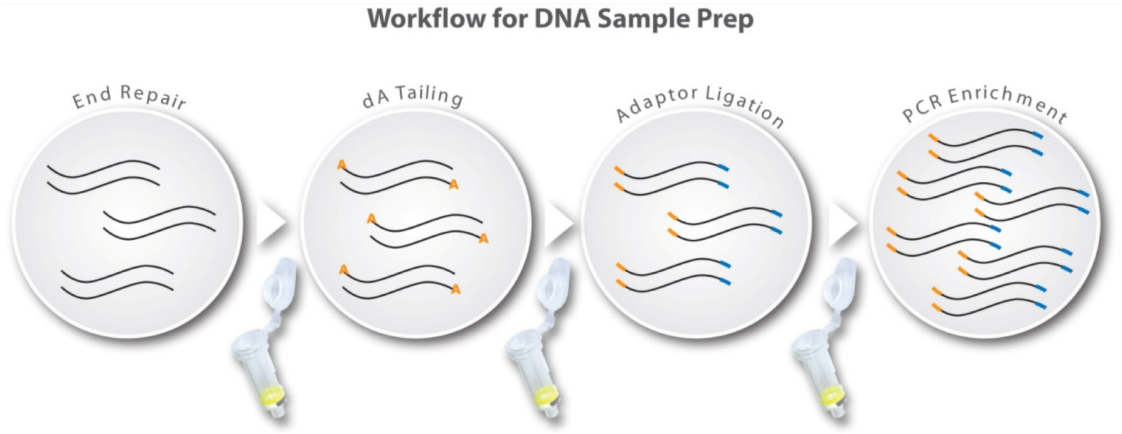

Figure 2: NEBNext® ChIPseq library prep set for Illumina
The NEBNext® sets include reagents for the enzymatic steps of the sample preparation for DNA sequencing on the ILLUMINA®, SOLiD®, 454® and Ion PGM® platforms.

Analysis of next generation sequencing (NGS) data is highly complex and requires extensive bioinformatic skills. NextGENe, from SoftGenetics, is a biologist friendly solution for analyzing NGS data. NextGENe is used for a wide variety of applications including amongst others SNP/InDel discovery (Rossetti et al., 2012), RNAseq analysis and trisomy analysis (van den Oever et al., 2012). The NextGENe project wizard guides researchers through the analysis set-up, analyses the data and allows editing and viewing in the NextGENe Viewer. In short, raw NGS data is converted to FASTA format which is used for the alignment against a custom made reference or a whole genome reference index.

After processing, the projects are automatically opened in the NextGENe Viewer for viewing and editing the results. The ChIPseq profiles reveal that H3K4me3 is enriched at the 5' end of genes (Figure 3A). H3K4me3-enrichment coincides with the presence of H3K36me3 at the gene body. Broad domains of H3K27me3 are seen for inactive loci that are lacking H3K36me3 signals. NextGENe includes a peak identification tool for identifying enriched regions and marks these regions in dark red in the Viewer.

The peak identification results are stored in the peak ID report that includes the position and the peak sequence for downstream analysis (Figure 3B). To determine the extent of H3K36me3 enrichment, the expression report counts the sequence reads per gene and are calculated as a normalized RPKM value (Figure 3C). These results show that ChIPseq approaches are easily performed and analyzed using the BIOKÉ/CST products to obtain the epigenetic status of genes in a genome-wide approach.

[1] Sequencing can be performed at ServiceXS, the only service provider in the BeNeLux to offer NGS under the ISO/IEC 17025 accreditation scope.

Product Information

Cat. #	Description	Quantity
9003S	SimpleChIP Enzymatic Chromatin IP Kit (Magnetic Beads)	1 Kit (30 immunoprecipitations)
E6240S/L	NEBNext ChIP-Seq Sample Prep Master Mix Set 1	12 / 60 rxns
740609.15 / .50 / .250	NucleoSpin Gel & PCR Clean-up	10 / 50 / 250 preps

Index	Chr	Chromosome Region	Length	Coverage(75%)	Gene Distance	Sequence
3594	3	122399486..122400892	1407	16	PARP14(0)	AGGCCGCCTTCCCGGGGCGCCGCTGGCCA
3595	3	122512075..122513792	1718	10	HSPBAP1(0)	AGCGCATAATCCCTGTTCACAAAAGTCTCA
3596	3	122514297..122514340	44	9	DIRC2(+396)	CAGCTGGGACATCGCGCTGCTCGTGCTGT
3597	3	122786106..122787193	1088	9	PDIA5(+250)	CCCTTCTGCGCTCTCTGCTCCAGCCCTCTG
3598	3	122920396..122921628	1233	10	SEC22A(0)	CGGGGCCAGGTCTTCTCCTTGCTCACCCA
3599	3	123303142..123303238	97	14	PTPLB(+686)	ACTCCCCAGTTTCTTCCCCACCCCAACCCA
3600	3	123304228..123305387	1160	14	LOC100506826(0)	AATCACTGCTTCCATGTTGAATCAAAACAA
3601	3	123602021..123602883	863	12	MYLK(+266)	TGCAGCTCCAGGGCAGGCTCTCAAAGGCT

Chr	Gene	Length	Read Counts	RPKM
3	PARP14; +	50016	672	1554.5182
3	HSPBAP1; -	53805	196	421.4722
3	SEMA5B; -	118534	472	460.7173
3	PDIA5; +	95098	1364	1659.5047
3	SEC22A; +	72210	294	471.0700
3	ADCY5; -	163994	709	500.2116
3	PTPLB; -	90562	397	507.2009
3	MYLK; -	272007	4539	1930.7035

Figure 3: Histone methylation profiles of proximal tubular epithelial cells (PTEC) using NextGENe
(A) ChIPseq profiles of H3K4me3 (upper track), H3K27me3 (middle track) and H3K36me3 (lower track) are visualized with the NextGENe Viewer using the hg19 reference. Blue arrows indicate annotated genes. Green en yellow arrows show coding sequences. Sequence depth is shown in grey. Dark red areas represent ChIP-enriched regions identified by Peak Identification Tool in NextGENe. Transcriptionally active genes parp14, pdia5 and mylk are highlighted in green blocks, whereas transcriptionally inactive genes sema5b and adcy5 are visualized by light red blocks. (B) Peak identification report including position, length, coverage and sequences of each peak. (C) Expression report for ChIPseq read counts and normalized value (RPKM) per gene.

Discussion

BIOKÉ is the first company to have a ready-to-go solution for ChIPseq experiments from chromatin harvesting to data analysis that leads to accurate results. This report describes ChIPseq experiments for epigenetic regulation in PTEC. The results correlate with findings on H3K4me3, H3K27me3 and H3K36me3 from previous studies (Kouzarides, 2007). Better understanding of epigenetic characteristics and transcription networks with techniques like ChIPseq will enhance our understanding on gene regulation and will result in more insights in aberrant gene control in, for example, cancer.

References

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BIOKÉ
www.bioke.com

BIOKÉ
Schuttersveld 2
2316 ZA Leiden
The Netherlands

T. +31 (0)71 568 1000
T. 0800-71640 (BE)
F. +31 (0)71 568 1010
info@bioke.com