diagenode
Innovating Epigenetic Solutions

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ChIP-seq Service Guidelines

In order to optimize results, we adhere to stringent guidelines for sample preparation (cell collection and fixation). Please ask your Diagenode contact for your project specific guidelines.

Standard quantities per IP:

- ChIP on **transcription factors**: >4 million cells
- ChIP on **histones**: >1 million cells
- ChIP on **low cell amount** (histone only): >20,000-500,000 cells

Your Service specialist will help you to determine the cell amount per sample needed for your project.

ChIP-seq workflow

1. Sample/Ab validation service

Use of a representative sample (each cell/tissue type).

1.1. Chromatin shearing validation

- Test of two shearing times in duplicate using the Bioruptor®.
- DNA analysis to assess the shearing profile.

1.2. ChIP validation

Test ChIP (for each target) with 2 antibody quantities using iDeal ChIP-seq Kits (standard amounts) or True MicroChIP kit (low input).

By qPCR

If available, primers against control regions (one positive and one negative) should be provided by the customer. Otherwise Diagenode recommends primer design based on data from public databases or publications provided by the customer.

- Control IP for H3K4me3/PolII and IgG are included in parallel.
- Validation of qPCR primers on a serial dilution of gDNA.
- Measure of the percentage of input by qPCR, calculation of the ratio of specific enrichment.

or

By sequencing

- Quantification and library preparation with the MicroPlex Kit
- Illumina sequencing, Paired-reads 2x50 bp.
- Quality check, alignment to reference genome, identification of enriched regions (peak calling)

2. End-to-end ChIP-seq service

Two biological replicates are required for samples of interest.

2.1. Chromatin shearing

- Utilizes optimal shearing conditions (with the Bioruptor®) based on previous validation experiment (see above).
- DNA analysis to assess the shearing profile.

2.2. ChIP

- Utilizes optimal antibody amount defined during validation experiment or based on previous experience.
- Control IP for H3K4me3/PolII and IgG are included in parallel.
- Evaluation of the ChIP efficiency in qPCR using primers for the control regions (provided by customer or designed based on validation sequencing results).

2.3. Library preparation

Use of the MicroPlex Kit (starting material down to 500 pg of DNA).

- Control ChIP DNA is included in parallel.
- Libraries are quantified and analysed to assess their size.

2.4. Sequencing

Illumina technology, Paired-reads 2x50 bp.
Other sequencing parameter are available on request.

Marks	Mark category	Raw read numbers (millions) for human, mouse and rat
Input	Input	30/50 (as respective IP)
Non-histone protein	TF	30
Histone	Narrow (H3K4me3,…)	30
Histone	Broad (H3K27me3,…) / Ubiquitous (H3PanAc,..)	50

2.5. Bioinformatic analysis

Quality check, alignment to reference genome, identification of enriched regions (peak calling).

Provided files:

- Report with sequencing statistics
- Raw data in FASTQ format
- FastQC reports
- Alignment files in BAM format
- Peak files in BED format

2.6. Additional bioinformatics analysis on request

- **Differential binding analysis:** Identification and annotation (human, mouse, rat, drosophila) of differential binding between samples based on previously identified ChIP-seq peaks.
  Provided files:
  - Report with summary of differential binding analysis and plots,
  - Files containing differentially bound sites or unique peaks and breakdown of those in annotated regions: introns, exons, promoters, 1-to-5 kb upstream-TSS and intergenic regions for human, mouse, rat and drosophila.

- **Annotation in genomic regions:** Annotation of ChIP-seq peaks with genomic regions: introns, exons, promoters, 1-to-5 kb upstream-TSS and intergenic regions for human, mouse, rat and drosophila.

- **Gene ontology terms analysis:** Enrichment analysis on gene sets. Gene Ontology terms that are overrepresented in bound regions or differentially bound regions may indicate the underlying biological processes involved.

- **Pathway analysis:** Identify biochemical pathways in which genes associated with bound regions or differentially bound regions may be overrepresented.

- **Visualization of specific genomic regions:** Visualization of results (i.e. sequencing data, peaks) at specific genomic regions (e.g. genes, promoters) in publication-ready images (human, mouse, rat).

2.7. Additional information

For sample preparation and sample shipment it is mandatory to follow Diagenode's guidelines. If customer samples do not meet Diagenode's quality requirements, any additional QC of new samples will be charged to the customer. Any delay in sample shipment to Diagenode's facilities might result in delaying customer's project.

Generated files will be available for download during 1 month and stored for an additional period of 3 months on Diagenode's servers. Additional long-term storage of data is available upon request. This offer includes a one-hour call to walk you through the results if needed.

Original samples are stored at Diagenode during 4 months after project completion, but will be discarded once this time is exceeded. Return shipment of samples is available upon request.

Any additional service which is beyond the current project scope will be charged to the customer.