

## **ChIP-seq Service Guidelines** **ChIP-seq 解析 サービスガイドライン**

In order to optimize results, we adhere to stringent guidelines for sample preparation (cell collection and fixation). 結果を最大化するため、細胞採取と固定に関するガイドライン(別紙「ChIP-seq 解析サンプル調製・送付ガイドライン」)を厳守してください。

Standard quantities per IP:

1回の免疫沈降実験に必要な標準細胞数

- ChIP on **transcription factors**: 4 million cells
- ChIP on **histones**: 1 million cells
- ChIP on **low cell amount** (histone only): 10,000-500,000 cells

These quantities are adaptable depending on sample availability. The total number of cells required will depend on the experimental design of the project.

必要な細胞数はサンプルの状況や実験目的によって異なります。

### **ChIP-seq workflow**

#### **ChIP-seq 解析内容**

##### **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® Pico.
- 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 or True MicroChIP kit.

#### **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 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 and DNA quantification.

or

#### **By sequencing**

If control primer pairs or public data for primer design are unavailable:

- Quantification and library preparation with the MicroPlex Kit
- Illumina sequencing and peak calling

**2. End-to-end ChIP-seq service**

Three biological replicates are recommended for samples of interest.

**2.1. Chromatin shearing**

- Utilizes optimal shearing conditions (with the Bioruptor® Pico) based on previous validation experiment (see above) or based on experience with known cell types/tissues.
- DNA analysis to assess the shearing profile.

**2.2. ChIP**

- Utilizes optimal antibody amount defined during validation experiment.
- Control IP for H3K4me3 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) and DNA quantification.

**2.3. Library preparation**

Use of the MicroPlex Kit (starting material down to 50 pg of DNA).

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

**2.4. Sequencing**

Illumina technology

Single-Read 50 bp (other sequencing parameters on request).

Marks	Mark category	Row read numbers (millions) for human, mouse and rat
Input	Input	30 / 40 / 50
Non-histone protein	TF	30
H3K4me1	narrow	30
H3K4me2	narrow	30
H3K4me3	narrow	30
H3K9me1	ubiquitous	50
H3K9me2	ubiquitous	50
H3K9me3	ubiquitous	50
H3K27me1	broad	40
H3K27me2	broad	40
H3K27me3	broad	40
H2A.z	broad	40
H3K36me3	broad	40
H3K79me2	broad	40
H3K79me3	broad	40

H3K9ac	narrow	30
H3K27ac	narrow	30
H4K16ac	narrow	30
H4K20me1	broad	40
H3PanAc	ubiquitous	50

## 2.5. Bioinformatic analysis

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

### Provided files:

#### 納品データ

- 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

### 追加解析オプション

- **Differential binding analysis:** Identification and annotation (human, mouse, rat, drosophila) of differential binding between samples based on previously identified ChIPseq 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

### その他の注意事項

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.

サンプル調製・送付については必ず弊社のガイドライン(別紙「ChIP-seq 解析 サンプル調製・送付ガイドライン」及び「ChIP-seq 解析 Cell Fixation Protocol」各種)に従ってください。ご提供いただいたサンプルの品質が基準に満たない場合、再度新しいサンプルをご提供いただき追加の品質確認を行います。再提供および品質確認にかかる追加料金はお客様負担となります。弊社施設へのサンプル提供の遅れは、納期に影響する可能性があります。

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.

解析結果データは弊社 WEB サイトよりダウンロード可能です。解析終了後、Diagenode SA (ベルギー) よりログイン及びダウンロード方法についてご連絡いたしますので、1 ヶ月以内にダウンロードをお願いいたします。また、予備期間として 3 か月間はサーバー上に保存されていますが、それ以降は削除されますので、長期間の保存を希望される場合は早めにご連絡ください。必要であれば解析結果について Diagenode SA(ベルギー) の担当者と 1 時間通話することもできます(アポイントメント後、英語でのご説明となります)。

Original samples are stored at Diagenode during 12 months after project completion, but will be discarded once this time is exceeded. Return shipment of samples is available upon request.

お客様からお預かりしたサンプルは弊社施設にて解析終了から 12 か月間保管した後、順次廃棄いたします。お客様のご要望に応じて、サンプルのご返却も可能です(追加費用がかかります)。

Any additional service which is beyond the current project scope will be charged to the customer. ご依頼いただいた内容の範囲外となる追加サービスの料金はお客様負担となります。