ChIP-Seq FAQ

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Contents

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1. Is there a limitation on species for ChIP-Seq? What kinds of samples are accepted for ChIP -Seq? What kinds of antibodies for ChIP–Seq are available?

    - ChIP -Seq is available for all species that have a reference genome.
    - Currently the samples we accept are as follows: 1) cell lines; 2) liver or brain tissue of animal or human being, and 3) ChIP-ed DNA prepared by the client for the samples that have a reference genome.
    - Currently BGI uses the following antibody for immunoprecipitation: H3K4Me3, H3K4Me2 H3K9Me2, H3K9Me3, H3K27me3, H3K27Me2.

2. Why are there two types of cell delivery requirements in ChIP Seq?

    BGI developed two ChIP platforms: X-ChIP and N-ChIP. X-ChIP is suitable for most of DNA-protein interaction studies, whereas N-ChIP targets research on histone modifications, but not the weak binding of DNA-protein interactions. As there are different pre-processing requirements for cells in ChIP experiment, the requirement of sample delivery are different. We recommend that you choose one of the methods depending on your research goals.

3. How many cells are required for ChIP-Seq?

    The experimental conditions and the yield of ChIP DNA of different samples will not be the same. For most cell types, each ChIP reaction uses 5*10e7 cells. This ensures that the amounts of DNA are enough to build libraries for subsequent sequencing.
    Note: Extra samples of the same origin are required for QC testing. The amount needed is equivalent to one-tenth of a ChIP reaction.

4. For ChIP experiments, how are client samples tested?

    After receiving and pre-treating the samples, we extract DNA and evaluate the quantity of DNA. This allows us to understand the initial conditions (e.g. if digestion is involved in N-ChIP we explore the relevant conditions). Next, the DNA quantity is used indirectly to determine whether the sample sizes can meet the conditions. However, this approach may not always be sufficient to determine that the samples meet the criteria. Therefore, the sample information sheet must indicate the actual number of cells (can be approximate, but clients should ensure that the sample size is large enough).

5. If the ChIP experiment is conducted by the customer, what are the requirements for the samples?

    The quality and fragment size of ChIP DNA exert significant influence on the result. To ensure the accuracy of data generated, delivery of DNA samples after immunoprecipitation is rigidly controlled.

Sample quantity should have DNA concentration ≥10 ng/ul and total DNA should be ≥10 ng. (The minimum sample amount is 5ng.) *Sample purity* should have OD260/280 = 1.8~2.2'

If the DNA amount from single ChIP is insufficient, we recommend conducting the immunoprecipitation 2 to 3 times and combining the DNA obtained. Also, please provide the image of electrophoresis gels showing the DNA fragments. The main band should be located between 100 and 500bp, and the main peak should be at 250bp.

6. Is it necessary to do PCR amplification during the library construction, and will PCR amplification influence the final results?

   Based on the new generation sequencing technology, PCR is necessary for ChIp-Seq sample preparation, but only a few cycles are needed (fewer than 15 cycles), which minimizes the effect of amplification bias. Also, based on the compared results of reads after sequencing, the influence of duplication can be removed during the bioinformatics analysis. If the samples have undergone PCR amplification after immunoprecipitation and the sample undergoes a second round of amplification during library construction, the quality of the results will be adversely impacted.

7. What are the risks for ChIP-Seq, if the ChIPed DNA is less than 10ng?

   - The risk of library construction is higher.
   - A low mapping rate will result.
   - Because of low mapping rate, sufficient data may not be generated, which can have a significant impact on the accuracy of subsequent bioinformatic analysis.

8. What are the data requirements for ChIP-Seq?

   We offer three types of data: 10M, 20M, and 50M reads (50SE).

   Theoretically, we rely on the proportion of the target protein in the whole-genome binding sites to determine the amount of data. Given our previous experience and results of saturation analyses, we recommend that the number of reads should be at least 20M for a sample when performing histone modifications. For non-histone proteins, we recommend that the number of reads be at least 10M.

9. Why is it that sometimes the target region cannot be found from the result of analysis?

   Some of the ChIP samples may meet the criteria of Q-PCR proving the existence of the target region. However, with sequencing analysis the lack of data generated may not cover the regions of interest. In addition, ChIP experiments may affect the analysis.