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ChIP-exo: High Resolution Identification of Protein-DNA Binding Events and Quality Control

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Abstract

Recently, ChIP-exo has been developed to investigate protein-DNA interaction in higher resolution compared to popularly used ChIP-Seq. Although ChIP-exo has drawn much attention and is considered as powerful assay, currently, no systematic studies have yet been conducted to determine optimal strategies for experimental design and analysis of ChIP-exo. In order to address these questions, we evaluated diverse aspects of ChIP-exo and found the following characteristics of ChIP-exo data. First, Background of ChIP-exo data is quite different from that of ChIP-Seq data. However, sequence biases inherently present in ChIP-Seq data still exist in ChIP-exo data. Second, in ChIP-exo data, reads are located around binding sites much more tightly and hence, it has potential for high resolution identification of protein-DNA interaction sites, hence the space to allocate the reads is greatly reduced. Third, although often assumed in the ChIP-exo data analysis methods, the peak pair assumption does not hold well in real ChIP-exo data. Fourth, spatial resolution of ChIP-exo is comparable to that of PET ChIP-Seq and both of them are significantly better than resolution of SET ChIP-Seq. Finally, for given fixed sequencing depth, ChIP-exo provides higher sensitivity, specificity, and spatial resolution than PET ChIP-Seq.

In this article, we provide a quality control pipeline which visually assesses ChIP-exo biases and calculates a signal-to-noise measure. Also, we updated dPeak [1], which makes a striking balance in sensitivity, specificity, and spatial resolution for ChIP-exo data analysis.

Keywords: ChIP-exo; QC

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1 Background

ChIP-exo (Chromatin Immunoprecipitation followed by exonuclease digestion and next generation sequencing) Rhee and Pugh ([2]) is the state-of-the-art experiment developed to attain single base-pair resolution of protein binding site identification and it is considered as a powerful alternative to popularly used ChIP-Seq (Chromatin Immunoprecipitation coupled with next generation sequencing) assay. ChIP-exo experiments first capture millions of DNA fragments (150 - 250 bp in length) that the protein under study interacts with using random fragmentation of DNA and a protein-specific antibody. Then, exonuclease is introduced to trim 5' end of each DNA fragment to a fixed distance from the bound protein. As a result, boundaries around the protein of interest constructed with 5' ends of fragments are located much closer to bound protein compared to ChIP-Seq. This is the step unique to ChIP-exo that could potentially provide significantly higher spatial resolution compared to ChIP-Seq. Finally, high throughput sequencing of a small region (25 to 100 bp) at 5' end of each fragment generates millions of reads or tags.

While the number of produced ChIP-exo data keeps increasing, characteristics of ChIP-exo data and optimal strategies for experimental design and analysis of ChIP-exo data are not fully investigated yet, including issues of sequence biases inherent to ChIP-exo data, choice of optimal statistical methods, and determination of optimal sequencing depth. However, currently, the number of available ChIP-exo data is still limited and their sequencing depths are still insufficient for such investigation. To address this limitation we gathered ChIP exo data from diverse organisms: CTCF factor in human [2]; ER factor in human and FoxA1 factor in mouse from [?]; and generated σ^{70} factor in *Escherichia coli* (E. Coli) under aerobic (+O₂) condition, and treated by rifampicin by 0 and 20 minutes.

2 Results and discussion

2.1 Characteristics of ChIP exo data

2.2 ChIP exo Quality Control pipeline

Methods

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References

1. Chung, D., Park, D., Myers, K., Grass, J., Kiley, P., Landick, R., Keleş, S.: dpeak, high resolution identification of transcription factor binding sites from pet and set chip-seq data. *PIOS, Computational Biology* (2013)
2. Rhee, H.S., Pugh, F.: Comprehensive genome-wide protein-dna interactions detected at single-nucleotide resolution. *Cell* (2011)
3. Mendenhall, E., Bernstein, B.: Dna-protein interactions in high definition. *Genome Biology* (2012)