Research Article
COPAR: A ChIP-Seq Optimal Peak Analyzer

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Sequencing data quality and peak alignment efficiency of ChIP-seq profiles are directly related to the reliability and reproducibility of NGS experiments. Till now, there is no tool specifically designed for optimal peak alignment estimation and quality-related genomic feature extraction for ChIP-seq profiles. We developed open-sourced COPAR, a user-friendly package, to statistically investigate, quantify, and visualize the optimal peak alignment and inherent genomic features using ChIP-seq data from NGS experiments. It provides a versatile perspective for biologists to perform quality-check for high-throughput experiments and optimize their experiment design. The package COPAR can process mapped ChIP-seq read file in BED format and output statistically sound results for multiple high-throughput experiments. Together with three public ChIP-seq data sets verified with the developed package, we have deposited COPAR on GitHub under a GNU GPL license.

1. Introduction

Next-generation sequencing (NGS) integrated with ChIP technology provides a genome-wide perspective for biomedical research and clinical diagnosis applications [1–3]. Data quality and peak alignment of ChIP-seq profiles are directly related to the reliability and reproducibility of analysis results. For example, ChIP-seq data characterize alteration evidence for transcription factor (TF) binding activities in response to chemical or environmental stimuli, but if the ChIP-seq alignment is poorly selected, any follow-up analysis may lead to inaccurate TF binding results and inevitable loss of biological meanings [4, 5].

The mostly investigated items in ChIP-seq peak calling procedures are peak number, false discovery rate (FDR), corresponding bin-size, and other statistical thresholds selected in each analysis. Without exception, such arguments form impenetrable barriers for biologists and bioinformaticians to choose a suitable pair condition for analyzing experimental results.

And to our knowledge, few literatures or application notes focus on such topics; thus herein we propose a flexible package based on feature extraction and signal processing algorithms for solving such an argument-selection optimization problem in optimal peak alignment.

In summary, the package COPAR can quantitatively measure NGS/ChIP-seq experiment quality through global peak alignment comparison and extract genomic features based on spectrum method for in-depth analysis of ChIP-seq profiles.

2. Materials and Methods

2.1. Optimal Peak Alignment Estimation. For determining optimal ChIP-seq alignment, we need to analyze peak numbers under specific argument constraints. Thus we acquire optimal peak numbers by constraining specific arguments, which can be formalized as a class of optimal track analysis, illustrated as

\[
\arg\max_{i} P_i, \quad i \in N
\]

s.t. \( f_i \leq \chi \),

\( b_i = \beta \),

\( \rho_i \leq \delta \),

(1)
Optimal estimation for NGS peak alignment

(ii) Genomic feature extraction & comparison

(iii) Genome-wide analysis for multiple samples

**Figure 1:** Flowchart for optimal peak alignment estimation and genomic feature analysis with COPAR. The package can perform optimal peak estimation based on global alignment of ChIP-seq data; then it can utilize the frequency spectrum approach for genomic feature extraction and carries out statistical comparison for multiple ChIP-seq samples.

where $P_i$ denotes a set of optimal peak numbers under corresponding argument constraints, $f_i$ stands for argument FDR, $b_i$ stands for bin-size, $p_i$ denotes $p$ value threshold, and $\chi$, $\beta$, and $\delta$ represent the presupposed argument values, respectively.

2.2. Spectrum-Based Genomic Feature Extraction. For a finite random variable sequence, its power spectrum is normally estimated from its autocorrelation sequence by use of discrete-time Fourier transform (DTFT), denoted as \[ P(\omega) = \frac{1}{2\pi} \sum_{n=-\infty}^{\infty} C_{xx}(n) e^{-j\omega n}, \] (2)

where $C_{xx}$ denotes autocorrelation sequence of a discrete signal $x_n$, defined as

\[ C_{xx}(i, j) = \frac{E[(X_i - \mu)(X_j - \mu)]}{\sigma_i \sigma_j}, \] (3)

where $\mu$ and $\sigma$ stand for mean and variance, respectively.

In our study, for consideration of the ChIP-seq data characteristics, we use 128 sampling points to calculate discrete Fourier transform, with the related sampling frequency 1 KHz.

3. Results

The COPAR package was developed and open-sourced for academic biologists, and it uses built-in functions for determining optimal peak alignment candidate and extracting genomic features from ChIP-seq dataset.

The package is designed to handle BED-formatted ChIP-seq data as input [9], and it can process single ChIP-seq for optimal peak alignment and feature extraction analysis, together with the capability to perform genome-wide statistical comparison for multiple ChIP-seq samples. The analysis flowchart for the package is given in Figure 1.

It can automatically determine the optimal peak alignment with statistically meaningful FDR through fast global alignment comparison; the global comparison is subject to two statistical arguments, namely, bin-size and $p$ value threshold.

The functionalities of our developed package are largely complementary to and extend current tools used for ChIP-seq data analysis. The optimal peak alignment estimation is shown in Figures 2(a) and 2(b); and the spectrum-based feature extraction is given in Figures 2(c) and 2(d). Figures 2(a) and 2(b) utilize heatmap to represent peak number and corresponding FDR candidate subject to each argument pair, bin-size (vertical axis), and $p$ value threshold (horizontal axis), respectively; Figure 2(c) denotes the spectrum distribution of the global peak alignment candidate sequence, normalized with its frequency range [0, 500] Hz and magnitude within $[-40, -3]$ dB; Figure 2(d) denotes the randomized case.

4. Conclusions

Based on global peak alignment, COPAR optimizes the argument selection in ChIP-seq analysis; meanwhile, COPAR utilizes the signal spectrum processing method to further extract genomic features and statistically compare multiple ChIP-seq samples for NGS high-throughput experiments.

In summary, our developed package COPAR can process mapped read file in BED format and output statistically sound
results for diverse high-throughput sequencing experiments; we further verified the package with three GEO ChIP-seq datasets as study cases, and we included the analysis results into the package manual. The developed package COPAR is currently available under a GNU GPL license from https://github.com/gladex/COPAR.

Abbreviations
NGS: Next-generation sequencing
ChIP-seq: Chromatin immunoprecipitation-sequencing
FDR: False discovery rate
TF: Transcription factor
DTFT: Discrete-time Fourier transform.

Competing Interests
The authors declare that they have no competing interests.

Authors’ Contributions
Binhua Tang and Victor X. Jin conceived the method; Binhua Tang and Xihan Wang wrote and compiled the package; Binhua Tang, Xihan Wang, and Victor X. Jin drafted and proof-checked the manuscript.

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