BIOINFORMATICS

Vol. 00 no. 00 2011
Pages 1–2

Analyzing microbial ChIP-Seq data with Pique

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Received on XXXXX; revised on XXXXX; accepted on XXXXX

Associate Editor: XXXXXXX

ABSTRACT

Motivation:

Most ChIP-Seq peak finders are designed to identify protein-DNA binding events in eukaryotic datasets. To make cost-effective use of current sequencing capacity, the peak finders must be cleverly optimized to work with sparse-coverage data, and must take into account the effect of chromatin structure on the variation in background coverage. While numerous effective peak finders have been developed for eukaryotic data, these algorithmic approaches can be suprisingly error prone in our hands when run on high-coverage bacterial and archaeal ChIP-Seq datasets.

Results:

Fortunately, many of the statistical challenges for peak detection inherent in eukaryotic ChIP-seq data are not present in bacterial and archaeal datasets. This is due in part to higher genome coverage (typically in inverse proportion to genome size) and in part to the absence of non-random coverage variation from to highly structured chromatin. We have developed Pique, a conceptually simple, easy to use ChIP-Seq peak finding application for bacterial and archaeal ChIP-Seq data. The software is cross-platform and Open Source, and based on Open Source dependencies. Output is provided in standard GFF files, and easily imported into the Gaggle Genome Browser for manual curation and data exploration.

Availability:

The software is available under the BSD-3 license at
http://github.com/ryneches/pique.
A tutorial and test data are included with the documentation.

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1 INTRODUCTION

Next generation sequencing coupled with chromatin immunoprecipitation (ChIP-Seq) is revolutionizing our abilty to genomically map protein-DNA associations for a wide variety of proteins. The growing popularity of ChIP-Seq has spurred the development of over thirty peak picking algorithms (for a nearly completely list see [10]). The relative performance represetative peak detection algorithms on eukatyoric data and methods to assess performance have been recently reviewed by several authors [6, 3, 9, 2, 7]. Many of these peak picking methods have employed a number of sophisticated strategies to detect peaks in the typically sparcely covered eukaryotic datasets for which they are designed.

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While ChIP-Seq has been predominantly used to interrogate protein-DNA interactions in eukaryotic systems, there are clear advantages to adopting this technology for studying microbial systems. Microbial genomes typically are much smaller than eukaryotic genomes (the genome of *E. coli* is ≈2000 times smaller than the human genome), and have simpler replicon and chromatin structure. Eukaryotic ChIP-Seq necessarily involves more challenging biochemical and statistical approaches than microbial ChIP-Seq, and so we were surprised to find that software that works well in eukaryotic systems evidently fails when presented with a lesser challenge.

To our knowledge, only one other peak finding package, CSDeconv [5], has been explicitly developed for finding peaks in microbial ChIP-Seq data. This MATLAB package successfully finds peaks in microbial ChIP-Seq data, but its application is limited by its dependency on costly proprietary software, very slow performance, lack of support for manual curation, and high false negative rate. Herein we describe Pique, a conceptually simple, Python-based peak finding package that enables easy and rapid peak finding in bacterial and archaeal ChIP-Seq datasets.

2 APPROACH

ChIP-Seq in bacteria and archaea yields coverage several orders of magnitude larger than in eukaryotic systems. This generates data with continuous signal across the microbial chromosome rather than the sparse coverage typically present in eukaryotic ChIP-Seq data. This feature of microbial ChIP-Seq data permits simpler, faster algorithms to be used. We have based our algorithm on classic noise reduction techniques from signal processing.

Pique is designed for use in systems have genomic complexities such as IS elements, gene dosage polymorphisms and accessory genomes that cause variations in sequence coverage unrelated to ChIP, or in cases where the organism under study is not identical to the reference genome. The resulting enrichment "pedestals" and "holes" can be very problematic for detecting peaks and calculating enrichment levels. Pique allows the user to optionally supply a map of these features as a GFF file, and the software will automatically perform a segmented analysis.

Pique has integrated curation support through the Gaggle Genome Browser. This permits convenient interactive curation of the peak list and analysis of the ChIP-Seq data in the context of other Gaggle-enabled resources. Interactive curation of a microbial ChIP-Seq data set can typically be completed in a few minutes.

Fig. 1. HOW ABOUT A FIGURE OF A PEAK with rectangular envelope and subsequent shape envelope

3 METHODS

Prior to running Pique, Illumina (Solexa) reads should be quality filtered, quality trimmed, and aligned to a reference genome using the user's preferred short-read sequence aligner. Pique requires a BAM file as input [4]. We suggest using all contigs of the reference genome as the mapping target, but the user may prefer to proceed with one contig at a time if desired.

The user may optionally supply a map of coverage features. Pique supports three features; analysis regions, exclusion regions, and normalization regions. By default, Pique treats each contig as a single analysis regions, but the user may designate regions within a contig for separate analysis. This is useful where a gene dosage polymorphism has systematically altered the coverage level in a large region. Exclusion regions are simply masked out of their respective analysis regions, and are useful for removing coverage variation due to repetitive DNA. If the user designates normalization regions within an analysis region, Pique will use them to compensate for total coverage discrepancies between the background and ChIP alignments.

The user launches the primary analysis stage by providing alignment an file for the ChIP data, an alignment file for the control data, and an optional coverage feature map. The primary analysis proceeds thusly :

- The alignment files are digested, and the analysis regions are initialized. If a coverage feature map provided, the analysis regions are separated and the exclusion regions applied.
- The coverage noise threshold is measured in both the ChIP and control alignments by comparing the relative total coverage within the normalization regions. (The user should choose normalization regions that contain neither peaks nor coverage level aberrations.)
- In each analysis region, the "DC" component is removed using linear detrending. This removes effects due to coverage variation features larger than about 100Kb.
- High-k noise in coverage is removed using a Wiener-Kolmogorov filter. The filter delay α is chosen to approximate to the expected footprint size of the targeted protein. The choice of filter implies some specific assumptions about the nature of the coverage noise. The Wiener-Kolmogorov filter was the first and simplest statistical signal filter, first published by Norbert Wiener in 1949, and independently derived in discrete-time form by Andrey Kolmogorov in 1941. The approach assumes the existence of two inputs; a "true" signal, and a noise source. Both are assumed to be stationary stochastic processes combined additively.
- A sliding window average is used to identify regions whose coverage level deviates from the background. Peaks usually contain gaps in coverage on the order of the experimentally selected fragment size; the window width is chosen to correspond to this size. This yields simple rectangular envelopes around putative regions of enrichment.
- To determine if a putative enriched region corresponds to a binding event, we require that the stop coordinate of the forward strand enrichment envelope must fall between the coordinates of the reverse strand enrichment envelope, and that the start coordinate of the reverse strand enrichment envelope fall between the coordinates of the forward strand enrichment envelope. (We call this the overlap criterion.)

4 DISCUSSION

If a putative peak passes all of the tests above, Pique measures the enrichment ratio of the ChIP alignment to the the control alignment,

Fig. 2. GAGGLE GENOME BROWSER

estimates the binding coordinate, and reports to the user, as well as the enrichment normalization factor for that analysis region. Determination of the statistical significance of a peak is highly specific to the experiment, and so Pique does not undertake this calculation.

5 CONCLUSION

We note that Pique should also work well with eukaryotic datasets provided they are gathered with greater coverage than has been previously reported.

ACKNOWLEDGEMENT

Funding: This project was funded by UC Davis startup funds to MTF, NSF graduate fellowship AWARD NUMBER to EGW and DARPA AWARD NUMBER TO RN.

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