Analyzing microbial ChIP-Seq data with Pique

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ABSTRACT

Motivation:
Most ChIP-Seq peak finders are designed to 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 due to highly structured chromatin. In response, we have developed Pique, a conceptually simple, easy to run ChIP-Seq peak finding pipeline for bacterial and archaeal ChIP-Seq data. The software is cross-platform and Open Source, and based on Open Source dependencies. Output is easily imported into the Gaggle Genome Browser for manual curation of peaks and the exploration of the dataset in the context of Gaggle-enabled resources.

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 numerous peak detection algorithms. All but one, (CSDeconv (REF)) have been designed with eukaryotic data sets in mind. To make most cost-effective use of contemporary sequencing abilities, these methods have employed a number of sophisticated strategies to detect peaks in sparsely covered datasets. The problem of finding peaks in such data is apparently so challenging that over thirty different methods have been published since 2007. Eleven representative peak detection algorithms were recently reviewed by Wilbanks and Facciotti (REF).

While ChIP-Seq has been predominantly used to interrogate protein-DNA interactions in eukaryotic systems (REFS) there are clear advantages to adopting this technology for studying microbial systems that are largely associated with the relatively small sizes of microbial genomes (the genome of *E. coli* is ≈2000 times smaller than the human genome). 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 (REF) 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. The output is easily imported into the Gaggle Genome Browser (REF) to enable rapid manual curation and analysis of ChIP-Seq data in the context of other Gaggle-enabled (REF) resources (browsers are that can import GFF data also supported).

Pique is also 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.

2 APPROACH

ChIP-Seq in bacteria and archaea yields coverage several orders of magnitude larger than in eukaryotic systems. This generates data with near-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

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

algorithms to be used. We have based our algorithm on classic noise reduction techniques from signal processing.

Finally, given the importance of manual curation and settings optimization, 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.

3 METHODS

Prior to running Pique, 40-bp 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 [1]. 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 two modes; analysis regions and exclusion 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.

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 adaptive simulated annealing of the cutoff threshold with respect to the peak recovery rate.
- 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.
- 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 yield 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.)

Fig. 2. GAGGLE GENOME BROWSER

4 DISCUSSION

If a putative peak passes all of the tests above, Pique concludes that the peak "looks" like a peak. To make sure that we are not finding horsies by gazing at clouds, we also require that the integral of the coverage in the raw data within the putative peak region exceeds the integral of the coverage in the background by a margin set by the user. (Other tests for statistical significance may also work, be more shiny, et cetera. For example, Monte Carlo simulations of random subsamples of the ChIP track and the background track until a coalescent is found. YES - WHAT/HOW IS THE CURRENT BG SELECTED?

5 CONCLUSION

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. (SOME OF THIS COULD PERHAPS GO ABOVE?) 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. We note that Pique should also work well with eukaryotic datasets provided they are gathered with greater coverage than has been previously reported.

NEED SOME SUMMARY SHOWING THAT THE PIQUE WORKS

NEED SOME RATIONALE FOR SELECTION OF FILTERS

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