CGAT: computational genomics analysis toolkit

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ABSTRACT

Summary: Computational genomics seeks to draw biological inferences from genomic datasets, often by integrating and contextualizing next-generation sequencing data. CGAT provides an extensive suite of tools designed to assist in the analysis of genome scale data from a range of standard file formats. The toolkit enables filtering, comparison, conversion, summarization and annotation of genomic intervals, gene sets and sequences. The tools can both be run from the Unix command line and installed into visual workflow builders, such as Galaxy.

Availability: The toolkit is freely available from http://github.com/CGATOxford/cgat

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1 INTRODUCTION

A central task in computational genomics is to extract biologically meaningful summaries and annotations from short read sequences to facilitate both visualization and statistical analysis. Commonly, this process starts by mapping next-generation sequencing (NGS) reads and quantifying their distribution in genomic features such as transcripts with expression level and transcription factor binding sites with peak scores. This initial contextualization phase is well supported by specialized tools such as Tophat/Cufflinks (Trapnell et al., 2012) or MACS (Feng et al., 2012). In a second phase, datasets are typically integrated to allow interpretation, asking, for example, how many transcription factor binding sites are associated with various classification schemes for transcript data or interval data. RNA-seq-derived transcripts can be marked as instances, fragments or alternative versions of transcripts in a reference gene set. Chromatin immunoprecipitation-sequencing (ChIP-Seq) intervals can be marked as intronic, intergenic or within the UTR, upstream or downstream regions of transcript models. Finally, the toolkit provides tools to summarize genomic datasets, reporting the number of intervals or transcripts per chromosome, size distributions of features and more.

2 OVERVIEW

The computational genomic analysis toolkit comprises >50 tools, each with documentation and examples. Tools are tagged to facilitate discovery. Tags associate tools with broad themes (genomic intervals, gene sets, sequences), standard genomic file formats (BED, GTF, BAM, FASTA.Q) and the type of computation performed by the tool, such as statistical summary, format conversion, annotation, comparison or filtering.

As an illustrative example, a gene set can be annotated with the tool gtf2table. In fact, gtf2table provides >25 different methods to annotate transcript models. Annotation is dependent on auxiliary data: given a genome sequence, transcripts can be annotated by composition (e.g. %GC); given a reference gene set, transcripts can be marked as fragments or extensions, enabling the user to ascertain the completeness of transcript models built for RNaseq data. Given a BAM file with NGS read data, gtf2table can compute coverage in sense/antisense direction over transcript models; another example, bam2geneprofile computes and plots metagene-profiles from mapped NGS read data in BAM format (Fig. 1a). Different metagene models (with/without UTRs/introns, etc.) and various normalization options are available. Finally, the tool bam2peakshape computes read densities in specified genomic intervals to generate matrix data suitable for visualization in heatmaps (Fig. 1b). The toolkit also contains standard sequence analysis utilities such as fasta2table, which annotates sequences with CpG frequencies, codon frequencies and amino acid composition.

To assist the interpretation of NGS data, the toolkit implements various classification schemes for transcript data or interval data. RNA-seq-derived transcripts can be marked as instances, fragments, extensions or alternative versions of transcripts in a reference gene set. Chromatin immunoprecipitation-sequencing (ChIP-Seq) intervals can be marked as intronic, intergenic or within the UTR, upstream or downstream regions of transcript models. Finally, the toolkit provides tools to summarize genomic datasets, reporting the number of intervals or transcripts per chromosome, size distributions of features and more.

3 USAGE

We introduce the usage of the computational genomics analysis toolkit with a brief example. The fully worked example can be found online. Given a set of transcription factor binding intervals from a ChIP-seq experiment in BED format (nfkb.bed), we wish to determine how many binding intervals lie within exons, introns or intergenic sequence using a reference gene set from
GENCODE (Harrow et al., 2012), in GTF format (hg19.gtf). We then want to plot the density of binding relative to transcript
features are normalized from multiple possible alternative
tions.gff'
filter
 retained the longest
approximate promoter regions (5000–10000 bp upstream and 10000–50000 bp downstream)'s annotation genome
tracks go beyond simple intertranscript intersection, as gene struc-
tures are normalized from multiple possible alternative
transcripts to a single transcript that is chosen by the user de-
pending on what is most relevant for the analysis.

The generated annotations are then used to classify the tran-
scription factor binding sites using bed2table (4). The profile of
ChIP-seq binding over genes can be calculated and plotted using
bam2geneprofile (Fig. 4a). Chromatin state at ChIP-seq peaks can be investigated by integrating H3K4me1 and H3K4me3
data for a relevant tissue (ENCODE Project Consortium, 2012) using bam2peakshape and plotted in R (R Core Team, 2012) (Fig. 1b). Statistical significance can be assessed using tools such as GAT (Heger et al., 2013). More usage examples, including testing for functional enrichment, assessment of CpG
content in long non-coding RNA promoters and clustering meta-
genomic contigs on tetranucleotide frequency, can be found online.

4 IMPLEMENTATION

We aim to write legible and maintainable code that can serve as
an entry point into computational methods for biologists. The
toolkit is implemented in the Python language (van Rossum, 1995). Some performance-critical sections have been imple-
mented in Cython (Behnel et al., 2011). The toolkit can be
installed from common Python package repositories. Dependencies will be installed automatically, although some tools require external software to be installed. All tools are freely available under the BSD 3-clause licence. The toolkit is under constant development, and community involvement in the project is welcome. Regression tests ensure that core functionality is maintained as scripts are extended. All tools are built using a common coding style and follow a naming scheme centred on common genomic file formats. The tools have a consistent command line interface enabling them to be combined into work flows using Unix pipes and integrated into automated pipelines allowing automated and parallel execution. They use a consistent logging mechanism to facilitate issue tracking. Furthermore, the use of common genomic formats means that tools can be easily combined with other popular genomic software such as BEDtools (Quinlan and Hall, 2010), University of California, Santa Cruz tools (Kuhn et al., 2013) or biopieces, http://www.

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