ChIPdig: a comprehensive user-friendly tool for mining multi-sample ChIP-seq data

Ruben Esse[1*] and Alla Grishok[1]

[1]Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118, USA

*Correspondence: rmesse@bu.edu

Abstract

Background: In recent years, epigenetic research has enjoyed explosive growth as high-throughput sequencing technologies become more accessible and affordable. However, this advancement has not been matched with similar progress in data analysis capabilities from the perspective of experimental biologists not versed in bioinformatic languages. For instance, chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) is at present widely used to identify genomic loci of transcription factor binding and histone modifications. Basic ChIP-seq data analysis, including read mapping and peak calling, can be accomplished through several well-established tools, but more sophisticated analyzes aimed at comparing data derived from different conditions or experimental designs constitute a significant bottleneck. We reason that the implementation of a single comprehensive ChIP-seq analysis pipeline could be beneficial for many experimental (wet lab) researchers who would like to generate genomic data.

Results: Here we present ChIPdig, a stand-alone application with adjustable parameters designed to allow researchers to perform several analyzes, namely read mapping to a reference genome, peak calling, annotation of regions based on reference coordinates (e.g. transcription start and termination sites, exons, introns, 5' UTRs and 3' UTRs), and generation of heatmaps and metaplots for visualizing

coverage. Importantly, ChIPdig accepts multiple ChIP-seq datasets as input, allowing genome-wide differential enrichment analysis in regions of interest to be performed. ChIPdig is written in R and enables access to several existing and highly utilized packages through a simple user interface powered by the Shiny package. Here, we illustrate the utility and user-friendly features of ChIPdig by analyzing H3K36me3 and H3K4me3 ChIP-seq profiles generated by the modENCODE project as an example.

Conclusions: ChIPdig offers a comprehensive and user-friendly pipeline for analysis of multiple sets of ChIP-seq data by both experimental and computational researchers. It is open source and available at https://github.com/rmesse/ChIPdig.

Keywords

ChIP-seq; read mapping; peak calling; genomic region annotation; differential enrichment analysis; heatmaps; metaplots

Background

Interactions between nuclear proteins and DNA are vital for cell and organism function. They control DNA replication and repair, safeguard genome stability, and regulate chromosome segregation and gene expression. Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) is a powerful method for assessing such interactions and has been widely used in recent years to map the location of post-translationally modified histones, transcription factors, chromatin modifiers and other non-histone DNA-associated proteins in a genome-wide manner. This progress has been fostered by the increasing technical feasibility and affordability of this technology, with more documentation and technical support available to researchers, including commercial library preparation kits, aided by the plummeting costs of sequencing and the ease of multiplexing samples. In light of this progress, ChIP-seq data sets are continuously deposited in publicly-accessible databases, such as the National Center for

44 Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO) and the ENCODE consortium portal
45 [1]. Therefore, there is an unprecedented wealth of epigenomic data in the public domain that can be
46 used for integrative and correlative analyses.
47 This important advancement had not been accompanied with similar progress in user-friendly post-
48 sequencing data analysis pipelines, which is still a significant bottleneck often handled by skilled
49 bioinformaticians. A key step in ChIP-seq data analysis is to map reads to a reference genome assembly.
50 Programs such as BWA [2] or Bowtie/Bowtie2 [3,4] are frequently employed for this purpose. Aligned
51 data are then processed to find regions of enrichment along the genome, thereby identifying potential
52 loci of DNA binding by the target protein or of deposition of the histone post-translational modifications
53 of interest. This process is known as peak calling and can be performed by using algorithms such as
54 MACS/MACS2 [5,6] and SICER [7]. SAMtools is another popular software used in next-generation
55 sequencing (NSG) analysis that provides various utilities for manipulating alignments, including sorting,
56 merging, indexing and generating alignments in a per-position format [8].
57 Following the initial and common steps in ChIP-seq data analysis mentioned above, downstream
58 analysis is often more customizable and may present a hurdle, especially in the case of comparing data
59 derived from different conditions or experimental settings. Artifacts arising from bias of DNA
60 fragmentation, variation of immunoprecipitation efficiency, as well as polymerase chain reaction (PCR)
61 amplification and sequencing depth bias, result in ChIP-seq experiments with distinct signal-to-noise
62 ratios and impose great challenges to the computational analysis [9]. Several methods addressing the
63 differential enrichment analysis problem, i.e. the detection of genomic regions with changes in ChIP-seq
64 profiles between two distinct samples or sets of replicate samples, have been proposed [10–12].
65 Generally, these methods rely on the initial detection of candidate peak regions by a conventional peak
66 calling algorithm, and then this peak-defining information is applied to analysis with methods tailored
67 for the differential expression analysis of RNA-seq data such as edgeR [13] or DESeq [14]. Downstream

68 analysis of ChIP-seq data may also involve the annotation of genomic regions based on reference
69 coordinates (e.g. distance from nearest transcription start site) and visualization of normalized coverage
70 by means of heatmaps and metaplots.
71 Importantly, ChIP-seq data analysis typically relies, at least in part, on tools that have been designed to
72 run primarily on Linux/Unix-based systems, while biologists who need to work with NGS may be
73 unfamiliar with such operating systems. In the past few years, several packages designed to handle NGS
74 data have been released for R, a popular platform-independent programming language and software
75 environment for computing and graphics. Here we present ChIPdig, an open-source application that
76 leverages on several R packages to enable comprehensive and modular analysis of multi-sample ChIP-
77 seq data sets through a user-friendly graphical user interface (GUI), allowing any experimental biologist
78 with minimal computational expertise to use it easily.

79 Implementation

80 ChIPdig is developed in R using package Shiny and relies on multiple R/Bioconductor packages, including
81 QuasR and BSgenome [15], for mapping reads to a reference genome assembly [16], BayesPeak for peak
82 calling [17], csaw [12] and edgeR [13] for differential enrichment analysis, ChIPseeker for annotation of
83 genomic regions of interest [18], and EnrichedHeatmap for generating coverage heatmaps [19].
84 Packages used in several analysis modules implemented in ChIPdig are GenomicRanges [20], valr [21],
85 shinyFiles, GenomicFeatures, ggplot2, ggsignif, reshape2 and circlize. A few command lines provided in
86 https://github.com/rmesse/ChIPdig suffice to launch the GUI from within R Studio, allowing integrative
87 and interactive usage of these powerful libraries without requiring programming or statistical
88 experience from the user.
89
90

Results

ChIPdig has the following capabilities: (1) alignment of reads to a reference genome; (2) normalization and comparison of distinct ChIP-seq data sets; (3) annotation of genomic regions; (4) generation of comparative heatmaps and metaplots for visualization of normalized coverage in user-defined specific regions. Each capability corresponds to a specific analysis module which can be loaded separate from other modules and using different input files. To illustrate each module, raw single-end ChIP-seq data for H3K4me3 and H3K36me3 in the model organism *Caenorhabditis elegans* were downloaded from NCBI GEO with series IDs GSE28770 and GSE28776, respectively. These two histone modification marks have different distribution profiles, namely promoter-proximal enrichment with punctuated peaks for H3K4me3, and gene body enrichment with broad peaks for H3K36me3 [22]. Figure 1 shows the GUI displayed upon launching ChIPdig from R Studio. The left pane displays four radio buttons, each corresponding to one of the four analysis modules, as well a clickable box for selection of the folder containing the input files for the analysis.

- **Alignment of ChIP-seq reads**

The aligning module of ChIPdig relies primarily on the QuasR package, which supports the analysis of single-read and paired-end deep sequencing experiments [16]. If desired, read preprocessing can be performed to prepare the input sequence files prior to alignment, e.g. removal of sequence segments corresponding to adapters and low quality reads. Sequence files in FASTQ format for each input DNA and ChIP sample corresponding to the H3K4me3 and H3K36me3 ChIP-seq experiments were aligned to the WS220/ce10 reference genome (available through the BSgenome package [15]) (Fig. 2), producing an average of 10 million mapped reads per sample. A report with several quality metrics for each alignment was automatically generated and is available in Additional file 1.

114 • **ChIP-seq data normalization, peak calling and differential enrichment analysis**

115 A recurrent problem in ChIP-seq data analysis lies at the comparison of multiple coverage profiles
116 generated from different experiments or corresponding to different conditions. This problem is
117 addressed in the second analysis module of ChIPdig. Upon its selection, the user is prompted to provide
118 a tab-delimited text file with the names of each ChIP sample (treatment) mapped reads file in BAM
119 format, the matching input DNA (control) file, a sample ID, the condition or target name, and a color
120 designation for the output peak and coverage files (Fig. 3a). Any of the 657 R built-in colors can be
121 chosen (Additional file 2). A bin size parameter set to 50 bp (base pairs) by default is used in both peak
122 calling and genome-wide library normalization for differential enrichment analysis. If desired, duplicate
123 reads can be removed prior to normalization and sequences can be extended to a median fragment
124 length that can be estimated either computationally or experimentally (e.g. average size of fragments
125 generated by chromatin shearing) (Fig. 3b). The initial mapped read processing outputs a table with
126 library sizes and median fragment sizes, a chromosome size list, and a multidimensional scaling plot
127 representing the similarity of samples in the data set (Fig. 3c). Data are normalized based on library sizes
128 and on the trimmed means of M values (TMM) approach [23], which is implemented in the edgeR
129 package [13].

130 Completion of the initial processing of mapped reads releases options for downstream processing which
131 are posted to the sidebar panel, namely export of files representing sequencing coverage, peak calling
132 and differential enrichment analysis (Fig. 4). Coverage is expressed in log2-transformed counts per
133 million (cpm) for each genomic bin and, for each sample ID indicated by the user, three files with
134 bedGraph format are generated: treatment (ChIP sample), control (input DNA sample) and the
135 treatment-to-control ratio. Each file can be loaded onto a genome browser for visualization (Fig. 5).

136 Peak calling is performed via the BayesPeak package by using a hidden Markov model and Bayesian
137 statistical methodology [17]. The user specifies a posterior probability (PP) threshold and genomic

138 regions with PP above such threshold are identified as peaks. If replicates are indicated, commonly
139 enriched genomic regions representing replicated peaks can be derived, and a track definition line can
140 be added, allowing each file to be loaded onto the UCSC genome browser for visualization. In addition, a
141 consensus peak set representing all candidate enriched regions across the full data set may be obtained.
142 The user may be interested in choosing this option for posterior differential enrichment analysis. Peak
143 calling was performed for the H3K4me3 and H3K36me3 ChIP-seq data sets, yielding 4994 replicated
144 peaks for H3K4me3 (Additional file 3) and 25855 replicated peaks for H3K36me3 (Additional file 4).
145 Differential enrichment analysis resorts to functions implemented in the csaw [12] and edgeR [13]
146 packages. Following library size and TMM normalization, bin-level coverage is computed and, if desired,
147 bins in which coverage for input DNA samples exceeds that of the corresponding ChIP samples are
148 filtered off. If the user is interested in differential enrichment analysis in a specific set of regions, the
149 corresponding file in BED format has to be provided. To illustrate this feature of ChIPdig, differential
150 enrichment analysis was performed for comparing H3K4me3 coverage with that of H3K36me3 using
151 either H3K4me3 replicated peak coordinates (Fig. 6a and Additional file 5) or those corresponding to
152 H3K36me3 peaks (Fig. 6b and Additional file 6), with a false discovery rate (FDR) threshold of 0.1. As
153 expected, at H3K4me3 genomic peak coordinates, H3K4me3 coverage is greater than that of H3K36me3,
154 and the opposite is observed for H3K36me3 peaks.

155 • **Annotation of genomic regions**

156 Annotation of genomic regions of interest is performed via the ChIPseeker package [18]. The user
157 supplies the file with regions in BED format and chooses the reference genome assembly, as well as the
158 distance upstream and downstream of the annotated transcription start site to be considered for
159 assignment of promoter regions (Fig. 7a). Replicated H3K4me3 and H3K36me3 peaks were annotated
160 and, characteristically of these marks [22], most H3K4me3 peaks were assigned to promoters (Fig. 7b

161 and Additional file 7), whereas H3K36me3 peaks lie predominantly at gene bodies (Fig. 7c and Additional
162 file 8).

163 • **Comparative heatmaps and metaplots**

164 The user can supply multiple coverage files in bedGraph format and generate heatmaps and metaplots
165 to visualize coverage in a specific region set in a comparative manner. This module of ChIPdig relies on a
166 custom algorithm which builds a coverage matrix based on bin size and reference coordinates (start, end
167 or both) selected by the user. Such matrix is then plotted in the form of a comparative metaplot and a
168 set of heatmaps. H3K4me3 and H3K36me3 coverage files were loaded onto ChIPdig, along with a BED
169 file with *C. elegans* transcription units in the WS220/ce10 reference assembly. Coverage was computed
170 in 25 bp bins and gene bodies were either expanded or compressed to 500 bp. Windows of 250 bp
171 upstream and downstream of each region were selected (Fig. 8a). Typical of H3K4me3, coverage is
172 higher in the vicinity of the transcription start site, whereas H3K36me3 is enriched at transcription unit
173 bodies.

174 Conclusions

175 ChIPdig is a user-friendly application for handling multiple ChIP-seq data sets and has diverse useful
176 capabilities spanning a comprehensive analysis pipeline, namely: read alignment to a reference genome,
177 ChIP-seq data normalization, peak calling, differential enrichment analysis, annotation of genomic
178 regions, and generation of comparative heatmaps and metaplots for visualization of normalized
179 coverage.

180

181

182

Availability and requirements

- **Project name:** ChIPdig
- **Project home page:** https://github.com/rmesse/ChIPdig
- **Operating system(s):** Platform-independent
- **Programming language:** R, Shiny
- **Other requirements:** None
- **License:** GNU General Public License version 3.0 (GPL-3.0)
- **Any restrictions to use by non-academics:** No

Abbreviations

bp: base pairs; ChIP-seq: chromatin immunoprecipitation followed by next-generation sequencing; cpm: counts per million; FDR: false discovery rate; GEO: Gene Expression Omnibus; GUI: graphical user interface; H3K36me3: histone H3 trimethylated at lysine 36; H3K4me3: histone H3 trimethylated at lysine 4; NCBI: National Center for Biotechnology Information; NGS: next-generation sequencing; PCR: polymerase chain reaction; PP: posterior probability; TMM: trimmed means of M values; TSS: transcription start site; UCSC: University of California Santa Cruz

Acknowledgements

Not applicable.

Funding

This research was supported by the GM107056 R01 grant awarded to AG.

Availability of data and materials

All source code has been made publicly at https://github.com/rmesse/ChIPdig.

Authors' contributions

RE conceived the study and wrote the code. RE and AG wrote the paper. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study does not involve humans, human data or animals.

References

1. Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, et al. The Encyclopedia of DNA elements (ENCODE): data portal update. Nucleic Acids Res. [Internet]. 2017; Available from: http://academic.oup.com/nar/article/doi/10.1093/nar/gkx1081/4595865

2. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform.

220 Bioinformatics. 2009;25:1754–60.

221 3. Langmead B. Aligning short sequencing reads with Bowtie. Curr. Protoc. Bioinforma. 2010;

222 4. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat. Methods. 2012;9:357–9.

223 5. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, et al. Model-based analysis of ChIP-

224 Seq (MACS). Genome Biol. 2008;9.

225 6. Feng J, Liu T, Qin B, Zhang Y, Liu XS. Identifying ChIP-seq enrichment using MACS. Nat. Protoc.

226 2012;7:1728–40.

227 7. Zang C, Schones DE, Zeng C, Cui K, Zhao K, Peng W. A clustering approach for identification of

228 enriched domains from histone modification ChIP-Seq data. Bioinformatics. 2009;25:1952–8.

229 8. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format

230 and SAMtools. Bioinformatics. 2009;25:2078–9.

231 9. Meyer CA, Liu XS. Identifying and mitigating bias in next-generation sequencing methods for

232 chromatin biology. Nat. Rev. Genet. 2014. p. 709–21.

233 10. Liang K, Keles S. Detecting differential binding of transcription factors with ChIP-seq. Bioinformatics

234 [Internet]. 2012;28:121–2. Available from:

235 http://www.ncbi.nlm.nih.gov/pubmed/22057161%5Cnhttp://www.pubmedcentral.nih.gov/articlerende

236 r.fcgi?artid=PMC3244766

237 11. Shao Z, Zhang Y, Yuan G-C, Orkin SH, Waxman DJ. MAnorm: a robust model for quantitative

238 comparison of ChIP-Seq data sets. Genome Biol. [Internet]. 2012;13:R16. Available from:

239 http://genomebiology.biomedcentral.com/articles/10.1186/gb-2012-13-3-r16

240 12. Lun ATL, Smyth GK. Csaw: A Bioconductor package for differential binding analysis of ChIP-seq data

241 using sliding windows. Nucleic Acids Res. 2015;44:1–10.

242 13. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression

243 analysis of digital gene expression data. Bioinformatics [Internet]. 2010;26:139–40. Available from:

244 http://www.ncbi.nlm.nih.gov/pubmed/19910308%255Cnhttp://www.pubmedcentral.nih.gov/articleren
245 der.fcgi?artid=PMC2796818
246 14. Anders S, Huber W. Differential expression of RNA-Seq data at the gene level – the DESeq package.
247 EMBL, Heidelberg, Ger. [Internet]. 2012; Available from:
248 http://gga01.med.wayne.edu/online_help/help_regionminer/DESeq.pdf
249 15. Bonhoure N, Bounova G, Bernasconi D, Praz V, Lammers F, Canella D, et al. An introduction to the
250 Biostrings / BSgenome framework. Nature [Internet]. 2014;11:1–13. Available from:
251 http://www.bioconductor.org/packages/release/bioc/vignettes/csaw/inst/doc/csawUserGuide.pdf%5Cn
252 http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0011471%5Cnhttp://www.ncbi.nlm.ni
253 h.gov/pubmed/18594521$%5C$nhttp://www.pubmedcentral.nih.gov/ar
254 16. Gaidatzis D, Lerch A, Hahne F, Stadler MB. QuasR: Quantification and annotation of short reads in R.
255 Bioinformatics. 2015;31:1130–2.
256 17. Cairns J, Spyrou C, Stark R, Smith ML, Lynch AG, Tavar?? S. BayesPeak-an R package for analysing
257 ChIP-seq data. Bioinformatics. 2011;27:713–4.
258 18. Yu G, Wang LG, He QY. ChIP seeker: An R/Bioconductor package for ChIP peak annotation,
259 comparison and visualization. Bioinformatics. 2015;31:2382–3.
260 19. Gu Z. EnrichedHeatmap: Making Enriched Heatmaps. R package version 1.6.0.
261 https://github.com/jokergoo/EnrichedHeatmap. 2017.
262 20. Lawrence M, Huber W, Pagès H, Aboyoun P, Carlson M, Gentleman R, et al. Software for Computing
263 and Annotating Genomic Ranges. PLoS Comput. Biol. 2013;9.
264 21. A. Riemondy K, M. Sheridan R, Gillen A, Yu Y, G. Bennett C, R. Hesselberth J. valr: Reproducible
265 genome interval analysis in R. F1000Research [Internet]. 2017;6:1025. Available from:
266 https://f1000research.com/articles/6-1025/v1
267 22. Liu T, Rechtsteiner A, Egelhofer TA, Vielle A, Latorre I, Cheung MS, et al. Broad chromosomal

268 domains of histone modification patterns in C. elegans. Genome Res. 2011;21:227–36.

269 23. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-

270 seq data. Genome Biol. 2010;11.

271 Figure legends

272 **Fig. 1**

273 ChIPdig user interface displayed upon launch. The left pane displays four radio buttons for selection of

274 the analysis module and a clickable box for selection of the folder containing the input files. Additional

275 features are sequentially unlocked as the user progresses through the software suite. Outputs

276 generated throughout the analysis and additional instructions are placed in the main panel under a tab

277 corresponding to the analysis module selected by the user.

278 **Fig. 2**

279 Alignment module. The user supplies a text file listing the input files corresponding to unmapped reads

280 in either uncompressed (e.g.: '.fq', '.fastq') or compressed (e.g.: '.gz', '.bz2', '.xz') format. Both single-end

281 and paired-end data are supported. A scroll-down menu displays the reference genome assemblies

282 available for alignment.

283 **Fig. 3**

284 Initial processing of mapped reads. The user supplies a tab-delimited text file listing the input DNA and

285 ChIP sample mapped read files **(a)**. If desired, duplicate read removal and fragment size extension can

286 be performed **(b)**. The application outputs a table with library sizes and median fragment sizes, a

287 chromosome size list, and a multidimensional scaling plot representing the similarity of samples in the

288 data set **(c)**.

289 **Fig. 4**

290 Downstream processing of mapped reads. The user may generate coverage files in bedGraph format,
291 perform peak calling for each sample, and assess differential enrichment for a set of genomic regions of
292 interest.

293 **Fig. 5**

294 H3K4me3 and H3K36me3 ChIP-seq coverage. Values represent log2-transformed cpm for the ChIP
295 sample subtracted by log2-transformed cpm for the input sample. Files in bedGraph format were
296 exported from ChIPdig and loaded onto the UCSC Genome Browser. These profiles exemplify the well-
297 known scenario whereby H3K4me3 accumulates around the transcription start site (TSS) and H3K36me3
298 is enriched at the gene body.

299 **Fig. 6**

300 Differential enrichment analysis results. H3K4me3 ChIP-seq coverage was compared with that of
301 H3K36me3 using either H3K4me3 peak genomic coordinates **(a)** or those of H3K36me3 peaks **(b)** with a
302 false discovery rate (FDR) threshold of 0.1. For each analysis, a mean-different plot representing the
303 library size-adjusted log2-transformed fold change (the difference) against the average log2-
304 transformed coverage (the mean), as well as a box-and-whisker plot showing the global change in
305 normalized coverage between the two conditions, were originated.

306 **Fig. 7**

307 Annotation of genomic regions. The region file in BED format is uploaded and reference genome
308 assembly, as well as distances upstream and downstream of transcription start site, are chosen **(a)**.
309 Replicated H3K4me3 peaks are mostly promoter-proximal **(b)**, whereas H3K36me3 peaks are found
310 predominantly at gene bodies **(c)**.

311 **Fig. 8**

312 Generation of heatmaps and comparative metaplot for visualizing coverage. Coverage files for H3K4me3
313 and H3K36me3 were supplied to ChIPdig, as well as a file with coordinates of *C. elegans* transcription

314 units. The heatmaps and metaplot were generated by considering a 25-bp bin size, and gene bodies
315 were resized to 500 bp. Windows of 250 bp upstream and downstream of each region were selected **(a)**.
316 As observed in both the heatmaps **(b)** and the comparative metaplot **(c)**, coverage for H3K4me3 is
317 overall higher in the vicinity of transcription start sites, while that of H3K36me3 is enriched at
318 transcription unit bodies.

319 Additional file description

320 **Additional file 1**

321 File name and extension: Additional_file_1_alignment_report.pdf

322 Title of data: Read alignment report

323 Description of data: Report generated by the read alignment module of ChIPdig

324 **Additional file 2**

325 File name and extension: Additional_file_2_available_colors.txt

326 Title of data: Available colors

327 Description of data: List of R built-in colors available for the user to choose from

328 **Additional file 3**

329 File name and extension: Additional_file_3_H3K4me3_replicated_peaks.bed

330 Title of data: H3K4me3 replicated peaks

331 Description of data: Peaks found in both H3K4me3 ChIP-seq experimental replicates

332 **Additional file 4**

333 File name and extension: Additional_file_4_H3K36me3_replicated_peaks.bed

334 Title of data: H3K4me4 replicated peaks

335 Description of data: Peaks found in both H3K36me3 ChIP-seq experimental replicates

336 **Additional file 5**

File name and extension: Additional_file_5_H3K4me3_vs_H3K36me3_at_H3K4me3_peaks.csv

Title of data: Differential enrichment analysis of H3K4me3 versus H3K36me3 at H3K4me3 peaks

Description of data: Table summarizing the results of the differential enrichment analysis comparing H3K4me3 coverage with H3K36me3 coverage at replicated H3K4me3 peaks

Additional file 6

File name and extension: Additional_file_5_H3K4me3_vs_H3K36me3_at_H3K36me3_peaks.csv

Title of data: Differential enrichment analysis of H3K4me3 versus H3K36me3 at H3K36me3 peaks

Description of data: Table summarizing the results of the differential enrichment analysis comparing H3K4me3 coverage with H3K36me3 coverage at replicated H3K36me3 peaks

Additional file 7

File name and extension: Additional_file_7_H3K4me3_replicated_peaks_annotation_table.csv

Title of data: Annotation of H3K4me3 peaks

Description of data: Annotation table for H3K4me3 replicated peaks considering 1500 bp upstream and 500 bp downstream of transcription start site for promoter assignment and using *C. elegans* reference assembly ce10

Additional file 8

File name and extension: Additional_file_7_H3K36me3_replicated_peaks_annotation_table.csv

Title of data: Annotation of H3K36me3 peaks

Description of data: Annotation table for H3K36me3 replicated peaks considering 1500 bp upstream and 500 bp downstream of transcription start site for promoter assignment and using *C. elegans* reference assembly ce10

ChIPdig

Select analysis module

- Align reads to the genome
- Normalize and compare ChIP-seq data sets
- Annotate genomic regions
- Visualize coverage in specific regions using background-normalized coverage files

Select input folder and files

Choose folder with files to be analyzed

```
[1] "C:/Users/rmesse/Desktop/ChIPdig-master"
```

Mapping | Initial processing of mapped reads | Peak calling | Differential enrichment analysis | Peak annotation | Heatmaps and metaplots

ChIPdig provides a user-friendly interface for analyzing multi-sample ChIP-seq data using packages implemented in R. First, select the analysis module in the left pane and the input directory containing the files to be analyzed.

ChIPdig

Select analysis module

- (●) Align reads to the genome
- ( ) Normalize and compare ChIP-seq data sets
- ( ) Annotate genomic regions
- ( ) Visualize coverage in specific regions using background-normalized coverage files

Is data single-ended or paired-ended?

- (●) Single-ended
- ( ) Paired-ended

Select input folder and files

Choose folder with files to be analyzed

```
[1] "D:/H3K4me3_H3K36me3"
```

Alignment of reads to reference genome

Alignment

Choose reference genome:

BSgenome.Celegans.UCSC.ce10

Align reads to reference genome

Mapping | Initial processing of mapped reads | Peak calling | Differential enrichment analysis | Peak annotation | Heatmaps and metaplots

Sample list (single-end reads):

FileName	SampleName
H3K4me3_inp_1.fastq	Sample_1
H3K4me3_inp_2.fastq	Sample_2
H3K4me3_IP_1.fastq	Sample_3
H3K4me3_IP_2.fastq	Sample_4
H3K36me3_inp_1.fastq	Sample_5
H3K36me3_inp_2.fastq	Sample_6
H3K36me3_IP_1.fastq	Sample_7
H3K36me3_IP_2.fastq	Sample_8

a

Sample list (single-end reads):

Sample ID	Mapped reads file, treatment	Mapped reads file, control	Condition	Color for exported peak and coverage files
H3K4me3_1	H3K4me3_ChIP_1.bam	H3K4me3_inp_1.bam	H3K4me3	green
H3K4me3_2	H3K4me3_ChIP_2.bam	H3K4me3_inp_2.bam	H3K4me3	green
H3K36me3_1	H3K36me3_ChIP_1.bam	H3K36me3_inp_1.bam	H3K36me3	brown
H3K36me3_2	H3K36me3_ChIP_2.bam	H3K36me3_inp_2.bam	H3K36me3	brown

b

Processing of mapped reads

Bin size (bp):

50

☐ Remove duplicate reads

○ Extend reads to computationally estimated median fragment length

○ Extend reads to experimentally observed median fragment length

Load mapped read files

NOTE: Loading may take a while. Be patient.

c

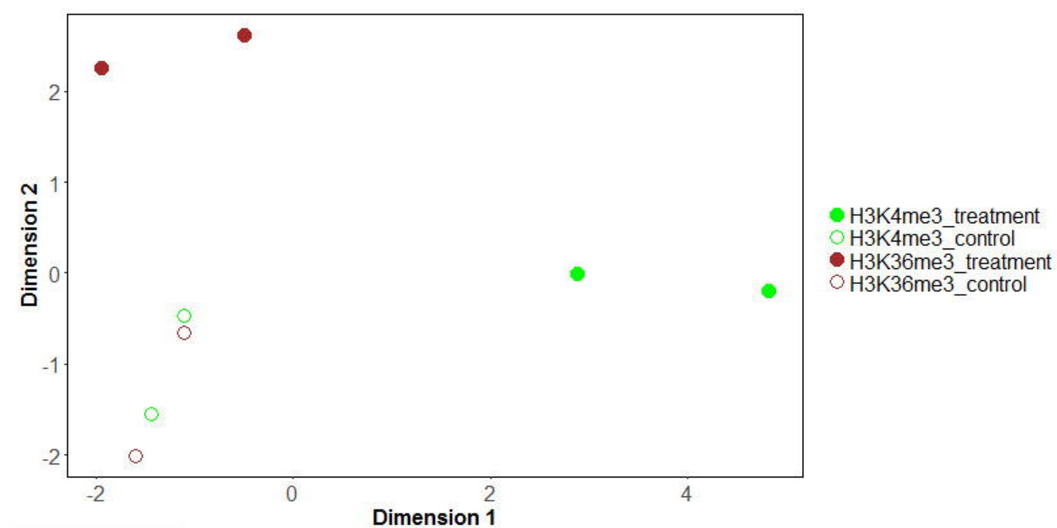
Library sizes and read extension sizes:

Sample ID	Treatment reads extended to:	Control reads extended to:	Library size, treatment:	Library size, control:
H3K4me3_1	149	108	9249098	16478107
H3K4me3_2	136	135	18086821	10484293
H3K36me3_1	172	109	7704106	25318356
H3K36me3_2	138	107	9674205	6574294

Chromosome sizes:

Chromosome	Size
chrI	15072288
chrII	15279344
chrIII	13783518
chrIV	17493610
chrV	20923998
chrX	17718761
chrM	13794

Multidimensional scaling plot (genome-wide):



Download plot

Processing of mapped reads

Coverage files export

Export coverage files

NOTE: Coverage files (treatment, control and normalized) will be created in a subfolder created in the input directory. Be patient.

Peak calling

Posterior probability threshold:

0.5

Call peaks

NOTE: Peak files will be created in a subfolder created in the input directory. Peak calling may take a while. Be patient.

Load peaks called externally

Differential enrichment analysis

False discovery rate threshold (for MA plot):

0.1

Discard bins in which fold enrichment (treatment/control) in all samples is less than (bp):

1

Select regions to limit differential enrichment analysis to (BED format) (please do not include column names):

Browse... No file selected

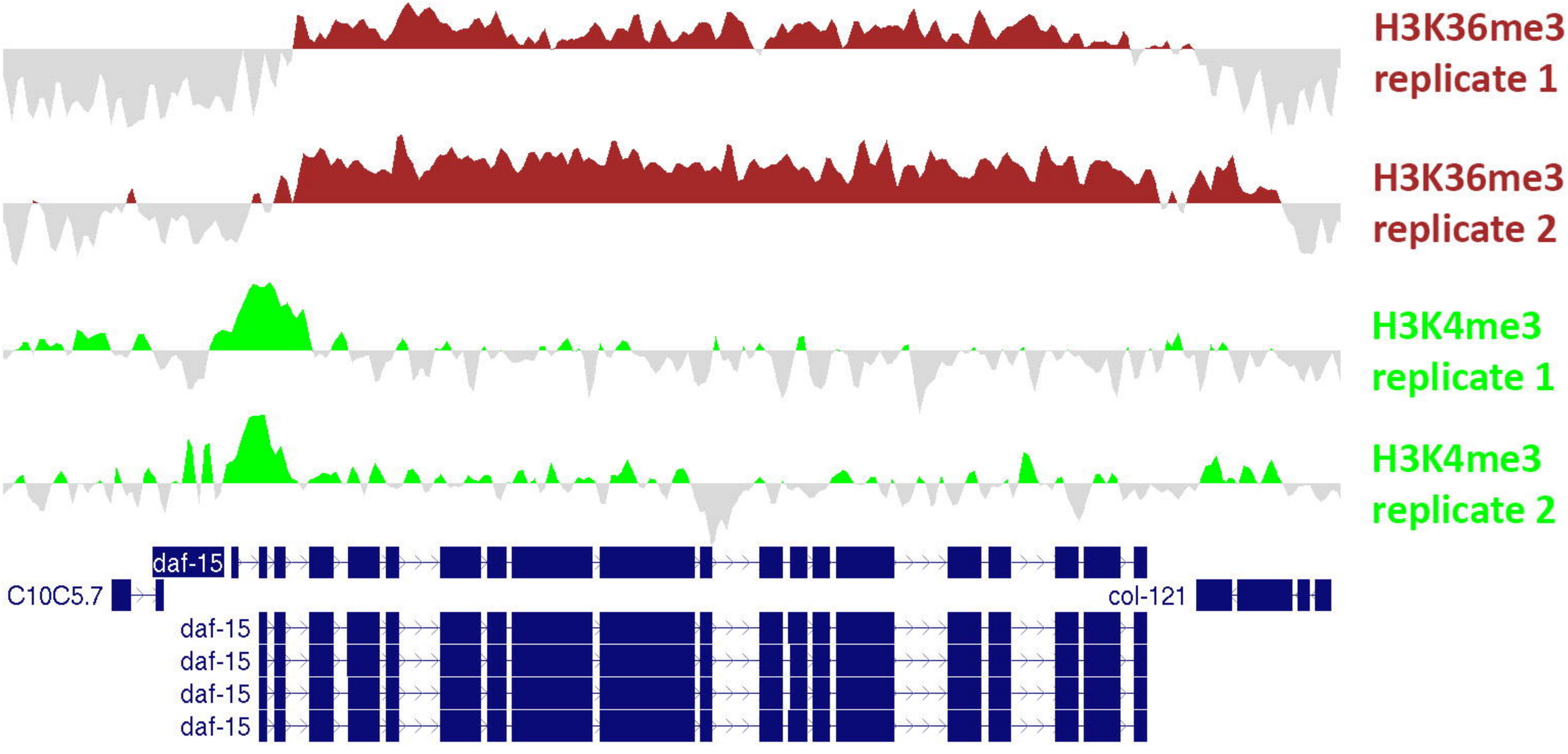
Choose condition to test:

H3K4me3

Choose reference condition:

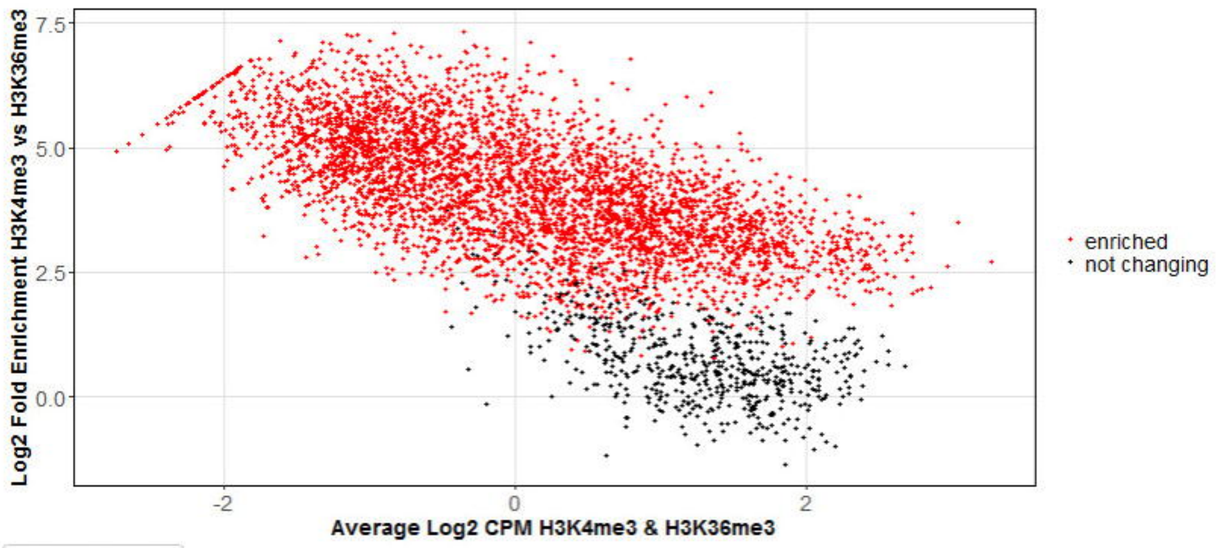
H3K36me3

Perform differential enrichment analysis on chosen contrast.


H3K36me3
replicate 1
H3K36me3
replicate 2
H3K4me3
replicate 1
H3K4me3
replicate 2
daf-15
C10C5.7
col-121
daf-15
daf-15
daf-15
daf-15

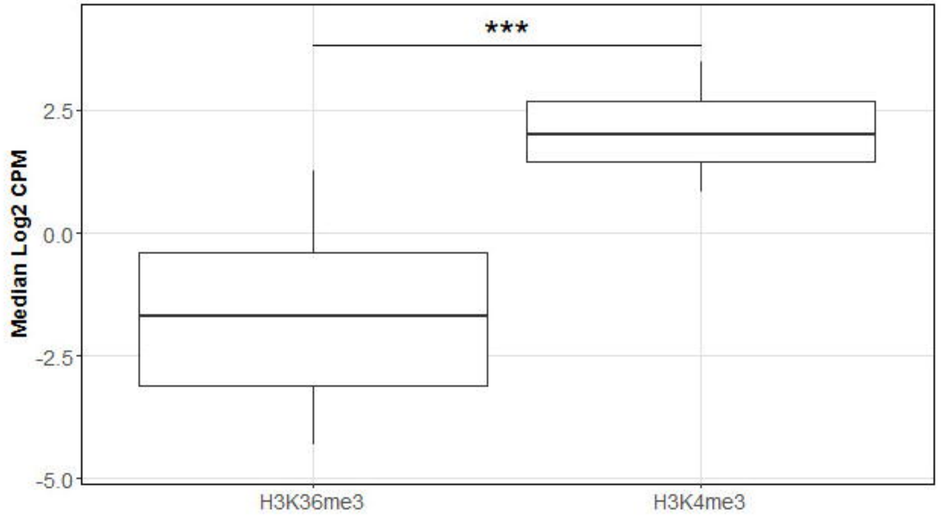
a

Mean-difference plot:



Download plot

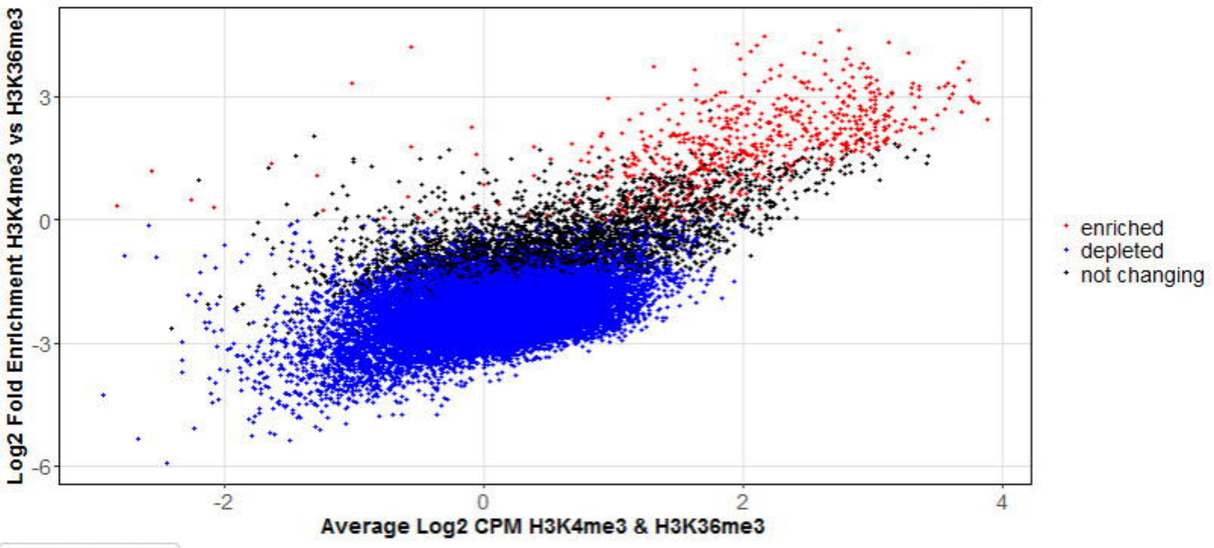
Box-and-whisker plot:



Download plot

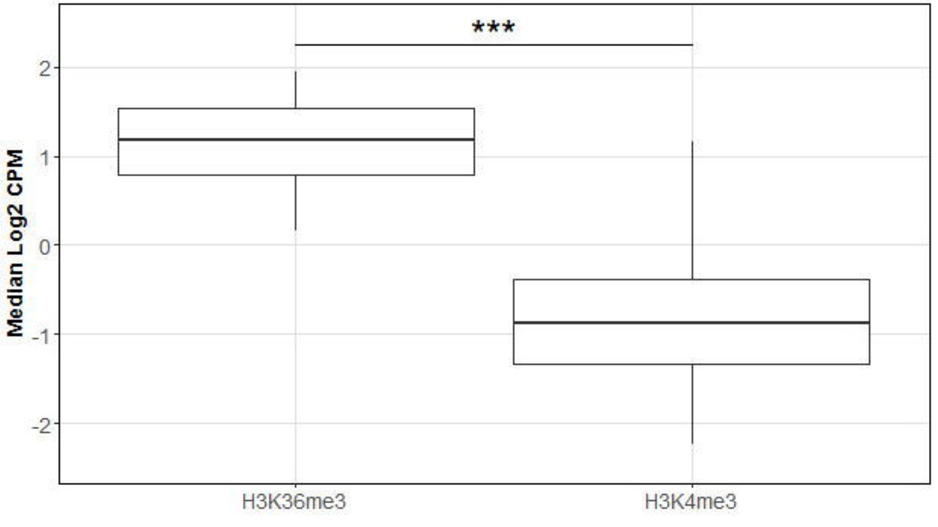
b

Mean-difference plot:

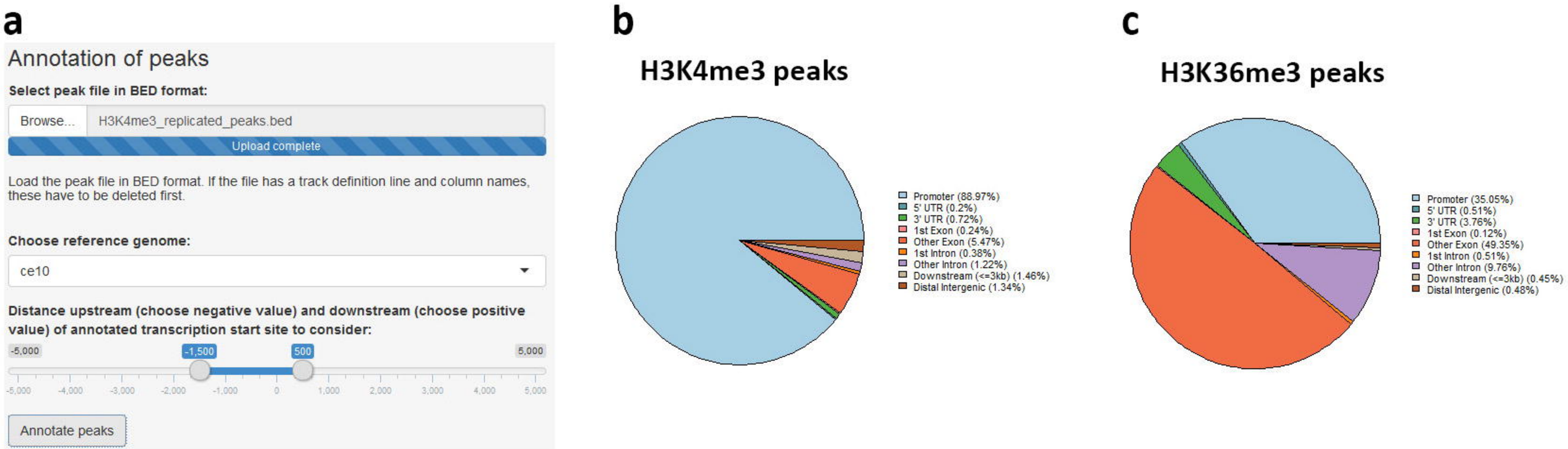


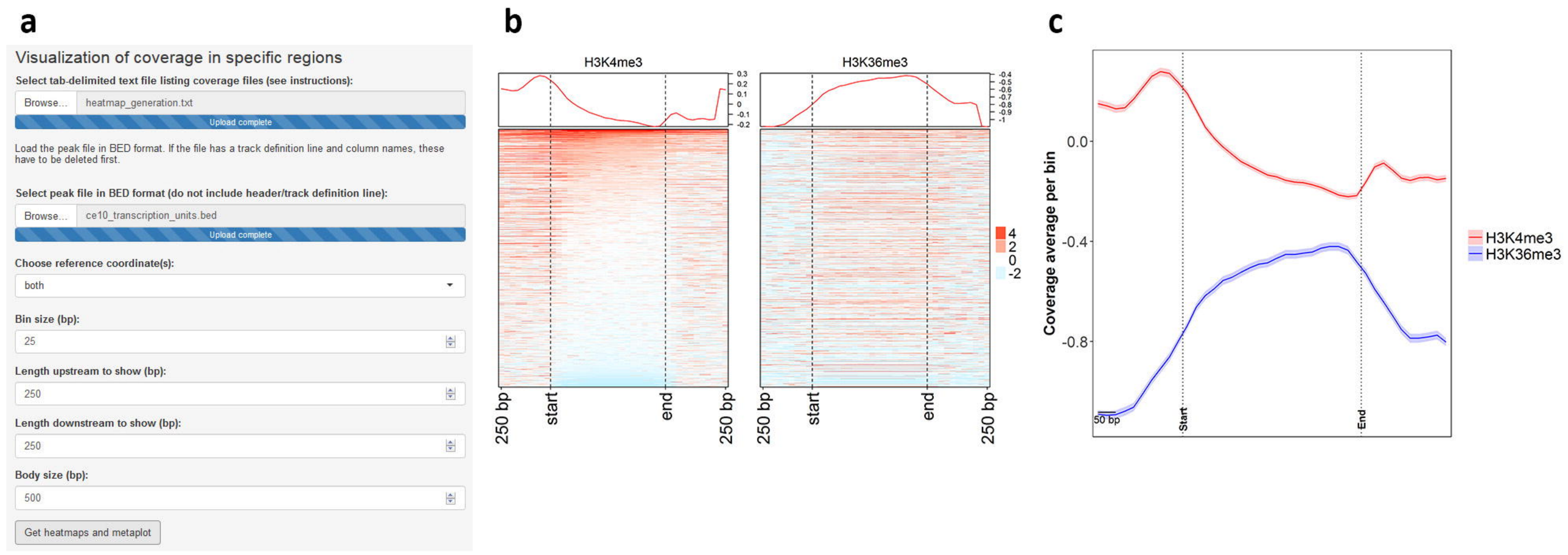
Download plot

Box-and-whisker plot:



Download plot


a
Annotation of peaks
Select peak file in BED format:
Browse...
H3K4me3_replicated_peaks.bed
Upload complete
Load the peak file in BED format. If the file has a track definition line and column names, these have to be deleted first.
Choose reference genome:
ce10
Distance upstream (choose negative value) and downstream (choose positive value) of annotated transcription start site to consider:
-5,000
-1,500
500
5,000
-5,000
-4,000
-3,000
-2,000
-1,000
0
1,000
2,000
3,000
4,000
5,000
Annotate peaks
b
H3K4me3 peaks
Promoter (88.97%)
5' UTR (0.2%)
3' UTR (0.72%)
1st Exon (0.24%)
Other Exon (5.47%)
1st Intron (0.38%)
Other Intron (1.22%)
Downstream (<=3kb) (1.46%)
Distal Intergenic (1.34%)
c
H3K36me3 peaks
Promoter (35.05%)
5' UTR (0.51%)
3' UTR (3.76%)
1st Exon (0.12%)
Other Exon (49.35%)
1st Intron (0.51%)
Other Intron (9.76%)
Downstream (<=3kb) (0.45%)
Distal Intergenic (0.48%)


a
Visualization of coverage in specific regions
Select tab-delimited text file listing coverage files (see instructions):
Browse...
heatmap_generation.txt
Upload complete
Load the peak file in BED format. If the file has a track definition line and column names, these have to be deleted first.
Select peak file in BED format (do not include header/track definition line):
Browse...
ce10_transcription_units.bed
Upload complete
Choose reference coordinate(s):
both
Bin size (bp):
25
Length upstream to show (bp):
250
Length downstream to show (bp):
250
Body size (bp):
500
Get heatmaps and metaplot
b
H3K4me3
H3K36me3
250 bp
start
end
250 bp
4
2
0
-2
c
Coverage average per bin
0.0
-0.4
-0.8
50 bp
Start
End
H3K4me3
H3K36me3