deepTools Documentation

Release 2.0.1

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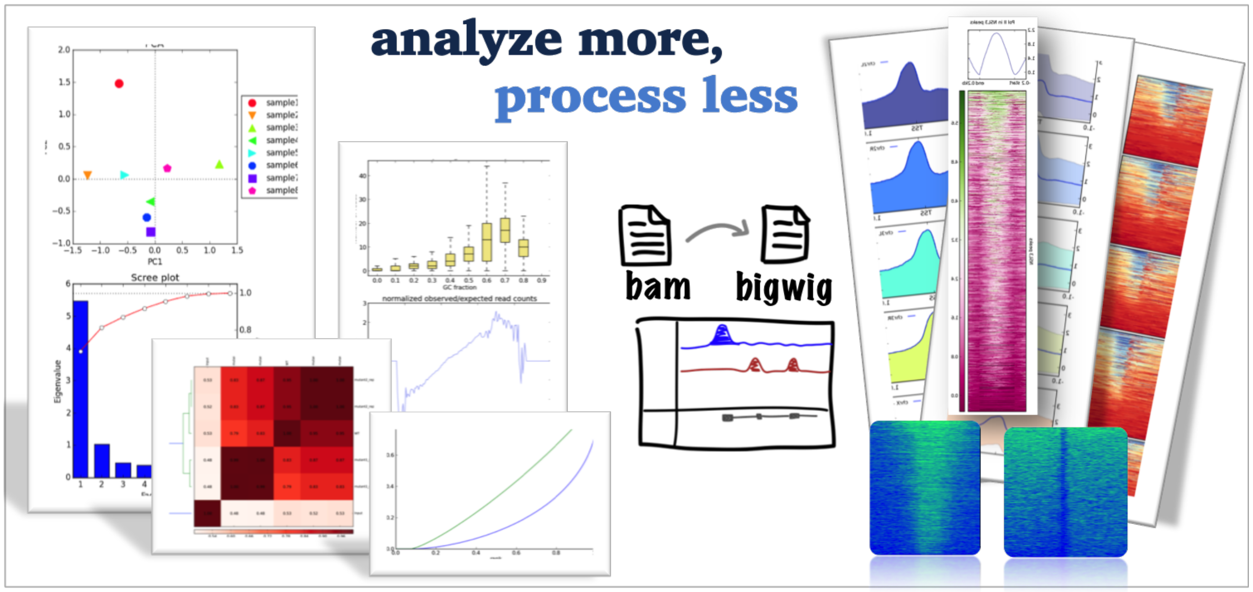
January 25, 2016

Contents



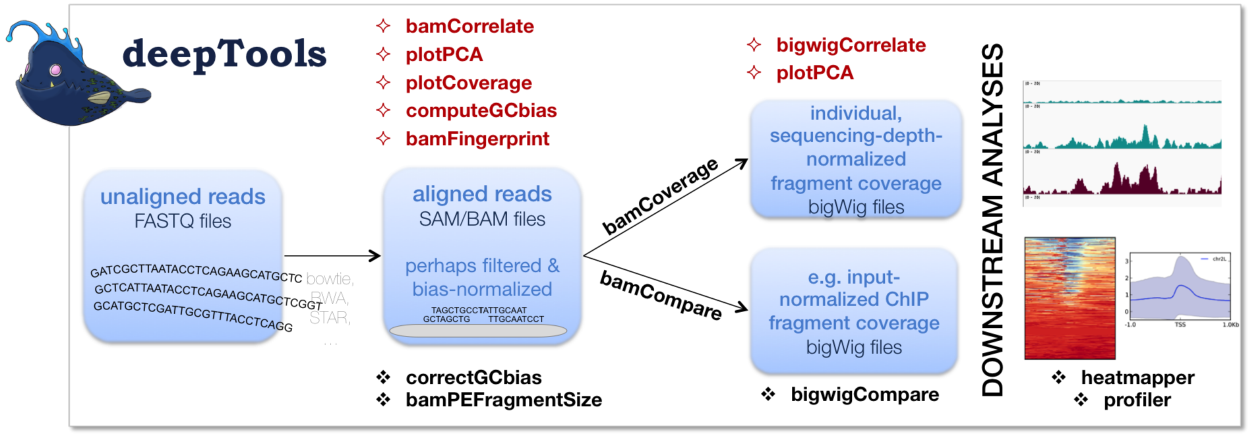
QUALITY CHECKS – FORMAT CONVERSION & NORMALIZATION – PLOTTING

deepTools is a **suite of python tools** particularly developed for the efficient analysis of high-throughput sequencing data, such as ChIP-seq, RNA-seq or MNase-seq.

There are 3 ways for using deepTools:

- **Galaxy usage** – our public deepTools Galaxy server let's you use the deepTools within the familiar Galaxy framework without the need to master the command line
- **command line usage** – simply download and install the tools
- **API** – make use of your favorite deepTools modules in your own python programs

The flow chart below depicts the different tool modules that are currently available (deepTools modules are written in bold red and black font).



If the file names in the figure mean nothing to you, please make sure to check our Glossary of NGS terms.

CHAPTER 1

Contents:

1.1 Installation

Remember – deepTools are available for **command line usage** as well as for **integration into Galaxy servers**!

1.1.1 Requirements

- Python 2.7
- numpy, scipy, bx-python, and pyBigWig
- pysam >= 0.8

The fastet way to obtain **Python 2.7 together with numpy and scipy** is via the Anaconda Scientific Python Distribution. Just download the version that's suitable for your operating system and follow the directions for its installation. All of the requirements for deepTools can be installed in Anaconda with:

> $ conda install -c bioconda deeptools

1.1.2 Command line installation using `pip`

Install deepTools using the following command:

```
$ pip install deeptools
```

All python requirements are automatically installed.

1.1.3 Command line installation without `pip`

1. Download source code

```
$ git clone https://github.com/fidelram/deepTools.git
```

or if you want a particular release, choose one from https://github.com/fidelram/deepTools/releases:

```
$ wget https://github.com/fidelram/deepTools/archive/1.5.12.tar.gz
$ tar -xzvf
```

2. The config file will tell you what deepTools expects to be installed properly:

```
$ cat deepTools/deeptools/config/deeptools.cfg

[external_tools]
sort: sort

[general]
# if set to max/2 (no quotes around)
# half the available processors will
# be used
default_proc_number: max/2
test_root: ../deeptools/test/

# temporary dir:
# deepTools bamCoverage, bamCompare and correctGCbias
# write files to a temporary dir before merging them
# and creating a final file. This can be speed up
# by writting to /dev/shm but for this a large
# physical memory of the server is required. If
# this is the case in your system, uncomment
# the following line. Otherwise, setting the
# variable to 'default', deepTools will use the
# temporary file configured in the system.
# Any other path that wants to be used for temporary
# files can by given as well (ie, /tmp)
#tmp_dir: /dev/shm
tmp_dir: default
```

3. install the source code (if you don't have root permission, you can set a specific folder using the `--prefix` option)

```
$ python setup.py install --prefix /Users/frd2007/Tools/deepTools
```

1.1.4 Galaxy installation

deepTools can be easily integrated into a local Galaxy. All wrappers and dependencies are available in the Galaxy Tool Shed.

Installation via Galaxy API (recommended)

First generate an API Key for your admin user and run the the installation script:

```
$ python ./scripts/api/install_tool_shed_repositories.py \
        --api YOUR_API_KEY -l http://localhost:8080 \
        --url http://toolshed.g2.bx.psu.edu/ \
        -o bgruening -r <revision> --name deeptools \
        --tool-deps --repository-deps --panel-section-name deepTools
```

The `-r` argument specifies the version of deepTools. You can get the latest revsion number from the test tool shed or with the following command:

```
$ hg identify http://toolshed.g2.bx.psu.edu/view/bgruening/deeptools
```

You can watch the installation status under: Top Panel –> Admin –> Manage installed tool shed repositories

Installation via web browser

- go to the admin page
- select *Search and browse tool sheds*
- Galaxy tool shed –> Sequence Analysis –> deeptools
- install deeptools

remember: for support, questions, or feature requests contact: deeptools@googlegroups.com

1.2 The tools

Note: With the release of deepTools 2.0, we renamed a couple of tools:

- **heatmapper** to `tools/plotHeatmap`
- **profiler** to `tools/plotProfile`
- **bamCorrelate** to `tools/multiBamSummary`
- **bigwigCorrelate** to `tools/multiBigwigSummary`
- **bamFingerprint** to `tools/plotFingerprint`.

For more, see Changes in deepTools2.0.

tool	type	input files	main output file(s)	application
`tools/multiBamSummary`	data integration	2 or more BAM	interval-based table of values	perform cross-sample analyses of read counts –> plotCorrelation, plotPCA
`tools/multiBigwigSummary`	data integration	2 or more bigWig	interval-based table of values	perform cross-sample analyses of genome-wide scores –> plotCorrelation, plotPCA
`tools/plotCorrelation`	visualization	bam/multiBigwigSummary output	clustered heatmap	visualize the Pearson/Spearman correlation
`tools/plotPCA`	visualization	bam/multiBigwigSummary output	2 PCA plots	visualize the principal component analysis
`tools/plotFingerprint`	QC	2 BAM	1 diagnostic plot	assess enrichment strength of a ChIP sample
`tools/computeGCBias`	QC	1 BAM	2 diagnostic plots	calculate the exp. and obs. GC distribution of reads
`tools/correctGCBias`	QC	1 BAM, output from computeGCbias	1 GC-corrected BAM	obtain a BAM file with reads distributed according to the genome's GC content
`tools/bamCoverage`	normalization	BAM	bedGraph or bigWig	obtain the normalized read coverage of a single BAM file
`tools/bamCompare`	normalization	2 BAM	bedGraph or bigWig	normalize 2 files to each other (e.g. log2ratio, difference)
`tools/computeMatrix`	data integration	1 or more bigWig, 1 or more BED	zipped file for plotHeatmap or plotProfile	compute the values needed for heatmaps and summary plots
`tools/plotHeatmap`	visualization	computeMatrix output	heatmap of read coverages	visualize the read coverages for genomic regions
`tools/plotProfile`	visualization	computeMatrix output	summary plot ("meta-profile")	visualize the average read coverages over a group of genomic regions
`tools/plotCoverage`	visualization	1 or more bam	2 diagnostic plots	visualize the average read coverages over sampled genomic positions
`tools/bamPEFragmentSize`	information	1 BAM	text with paired-end fragment length	obtain the average fragment length from paired ends

1.2.1 General principles

A typical deepTools command could look like this:

```
$ bamCoverage --bam myAlignedReads.bam \
--outFileName myCoverageFile.bigWig \
--outFileFormat bigwig \
--fragmentLength 200 \
--ignoreDuplicates \
--scaleFactor 0.5
```

You can always see all available command-line options via –help:

```
$ bamCoverage --help
```

- Output format of plots should be indicated by the file ending, e.g. `MyPlot.pdf` will return a pdf file, `MyPlot.png` a png-file

- All tools that produce plots can also output the underlying data - this can be useful in cases where you don't like the deepTools visualization, as you can then use the data matrices produced by deepTools with your favorite plotting tool, such as R
- The vast majority of command line options are also available in Galaxy (in a few cases with minor changes to their naming).

Parameters to decrease the run time

- **`numberOfProcessors` - Number of processors to be used**

  For example, setting `--numberOfProcessors 10` will split up the workload internally into 10 chunks, which will be processed in parallel.
- **`region` - Process only a single genomic region.** This is particularly useful when you're still trying to figure out the best parameter setting. You can focus on a certain genomic region by setting, e.g., `--region chr2` or `--region chr2:100000-200000`

These parameters are optional and available throughout almost all deepTools.

Filtering BAMs while processing

Several deepTools modules allow for efficient processing of BAM files, e.g. `bamCoverage` and `bamCompare`. We offer several ways to filter those BAM files on the fly so that you don't need to pre-process them using other tools such as samtools

- **`ignoreDuplicates`** Reads with the same orientation and start position will be considered only once. If reads are paired, the mate is also evaluated
- **`minMappingQuality`** Only reads with a mapping quality score of at least this are considered
- **`samFlagInclude`** Include reads based on the SAM flag, e.g. `--samFlagInclude 64` gets reads that are first in a pair. For translating SAM flags into English, go to: https://broadinstitute.github.io/picard/explain-flags.html
- **`samFlagExclude`** Exclude reads based on the SAM flags - see previous explanation.

These parameters are optional and available throughout deepTools.

Warning: If you know that your files will be strongly affected by the filtering of duplicates or reads of low quality then consider removing those reads *before* using `bamCoverage` or `bamCompare`, as the filtering by deepTools is done *after* the scaling factors are calculated!

1.2.2 Tools for BAM and bigWig file processing

`tools/multiBamSummary`

`tools/multiBigwigSummary`

`tools/correctGCBias`

`tools/bamCoverage`

`tools/bamCompare`

`tools/bigwigCompare`

`tools/computeMatrix`

1.2.3 Tools for QC

`tools/plotCorrelation`

`tools/plotPCA`

`tools/plotFingerprint`

`tools/bamPEFragmentSize`

`tools/computeGCBias`

`tools/plotCoverage`

1.2.4 Heatmaps and summary plots

`tools/plotHeatmap`

`tools/plotProfile`

1.3 Example usage

1.3.1 Step-by-step protocols

How can I do...?

This section should give you a quick overview of how to do many common tasks. We're using screenshots from Galaxy here, so if you're using the command-line version then you can easily follow the given examples by typing the program name and the help option (e.g. /deepTools/bin/bamCoverage –help), which will show you all the parameters and options (most of them named very similarly to those in Galaxy).

For each "recipe" here, you will find the screenshot of the tool and the input parameters on the left hand side (we marked non-default, *user-specified entries*) and screenshots of the output on the right hand side. Do let us know if you spot things that are missing, should be explained better, or are simply confusing!

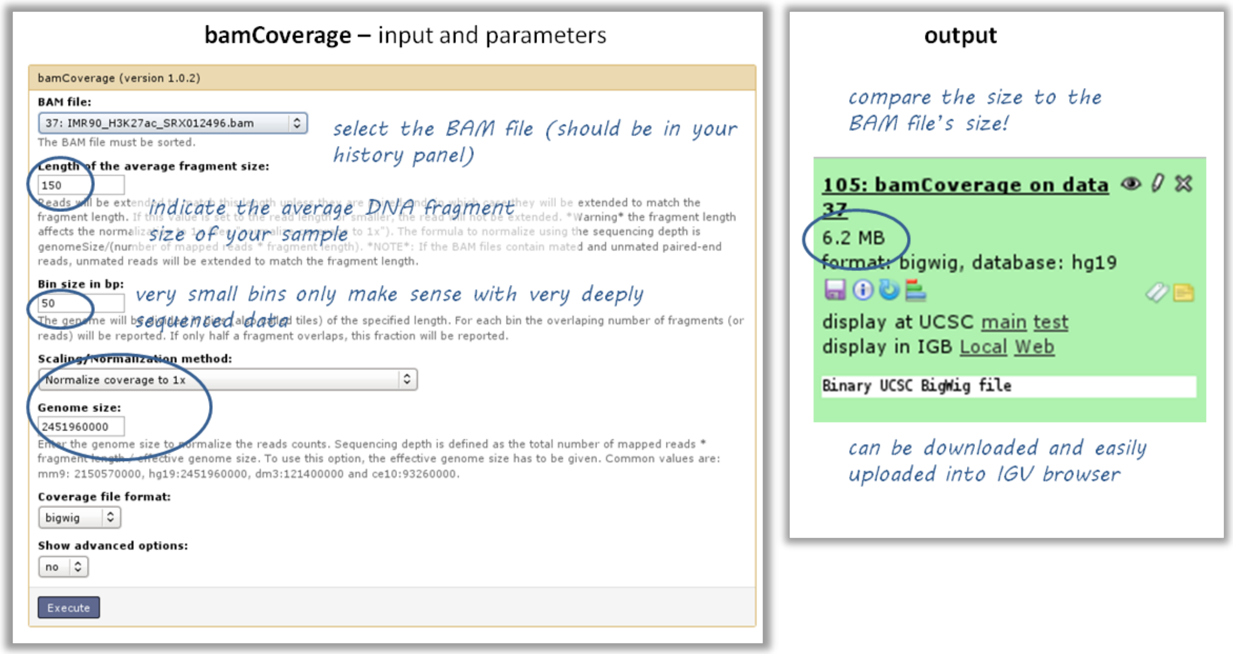
There are many more ways in which you can use deepTools Galaxy than those described here, so be creative once you're comfortable with using them. For detailed explanations of what the tools do, follow the links.

> All recipes assume that you have uploaded your files into a Galaxy instance with a deepTools installation, e.g., deepTools Galaxy
>
> If you would like to try out the protocols with **sample data**, go to deepTools Galaxy –> "Shared Data" –> "Data Libraries" –> "deepTools Test Files". Simply select BED/BAM/bigWig files and click, "to History". You can also download the test datasets by clicking "Download" at the top.

I have downloaded/received a BAM file - how do I generate a file I can look at in a genome browser?

- tool: `tools/bamCoverage`
- input: your BAM file

Note: BAM files can also be viewed in genome browsers, however, they're large and tend to freeze the applications. Generating bigWig files of read coverages will help you a lot in this regard. In addition, if you have more than one sample you'd like to look at, it is helpful to normalize all of them to 1x sequencing depth.



bamCoverage – input and parameters

bamCoverage (version 1.0.2)

BAM file:
37: IMR90_H3K27ac_SRX012496.bam
The BAM file must be sorted.

select the BAM file (should be in your history panel)

Length of the average fragment size:
150
Reads will be extended to match this length unless they are paired-end, in which case they will be extended to match the fragment length. If this value is set to the read length or smaller, the read will not be extended. *Warning* the fragment length affects the normalization to 1x (see "normalize coverage to 1x"). The formula to normalize using the sequencing depth is genomeSize/(number of mapped reads * fragment length). *NOTE*: If the BAM files contain mated and unmated paired-end reads, unmated reads will be extended to match the fragment length.

indicate the average DNA fragment size of your sample

Bin size in bp:
50
The genome will be divided in bins (also called tiles) of the specified length. For each bin the overlaping number of fragments (or reads) will be reported. If only half a fragment overlaps, this fraction will be reported.

very small bins only make sense with very deeply sequenced data

Scaling/Normalization method:
Normalize coverage to 1x

Genome size:
2451960000
Enter the genome size to normalize the reads counts. Sequencing depth is defined as the total number of mapped reads * fragment length / effective genome size. To use this option, the effective genome size has to be given. Common values are: mm9: 2150570000, hg19:2451960000, dm3:121400000 and ce10:93260000.

Coverage file format:
bigwig

Show advanced options:
no

output

compare the size to the BAM file's size!

105: bamCoverage on data 37
6.2 MB
format: bigwig, database: hg19
display at UCSC main test
display in IGB Local Web
Binary UCSC BigWig file

can be downloaded and easily uploaded into IGV browser

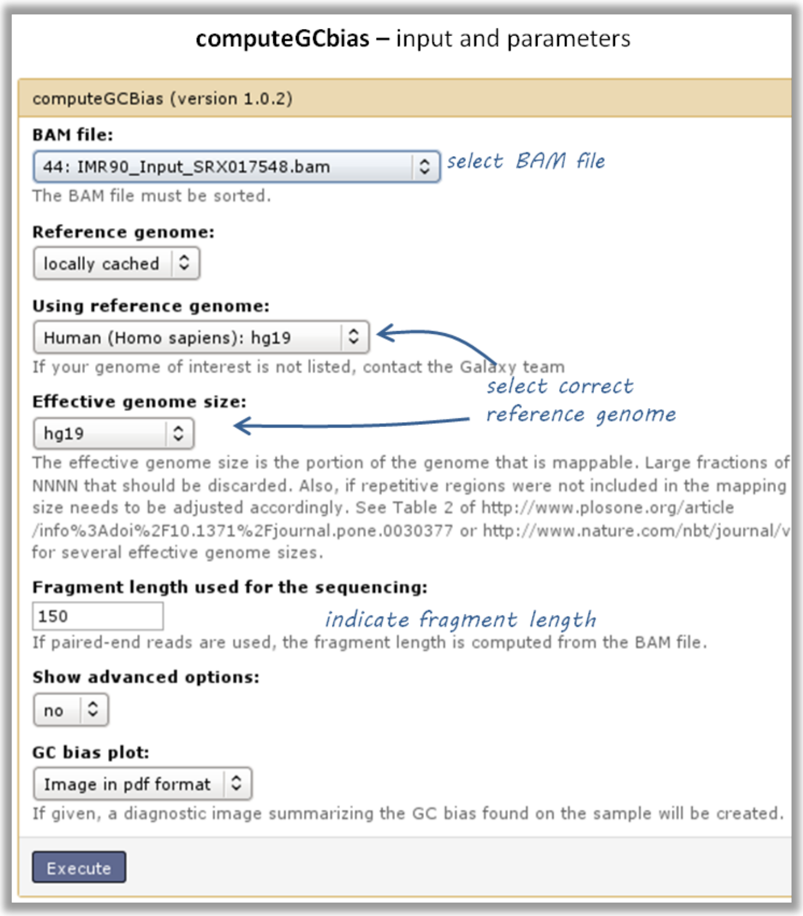
How can I assess the reproducibility of my sequencing replicates?

- tool: `tools/multiBamSummary`
- **input: BAM files**
  - you can compare as many samples as you want, though the more you use the longer the computation will take
- output: heatmap of correlations - the closer two samples are to each other, the more similar their read coverages will be

content/../images/GalHow_multiBamSummary.png

How do I know whether my sample is GC biased? And if it is, how do I correct for it?

- you need a BAM file of your sample
- use the tool `tools/computeGCBias` on that BAM file (default settings, just make sure your reference genome and genome size are matching)



computeGCbias – input and parameters

computeGCBias (version 1.0.2)

BAM file:
44: IMR90_Input_SRX017548.bam
The BAM file must be sorted.

Reference genome:
locally cached

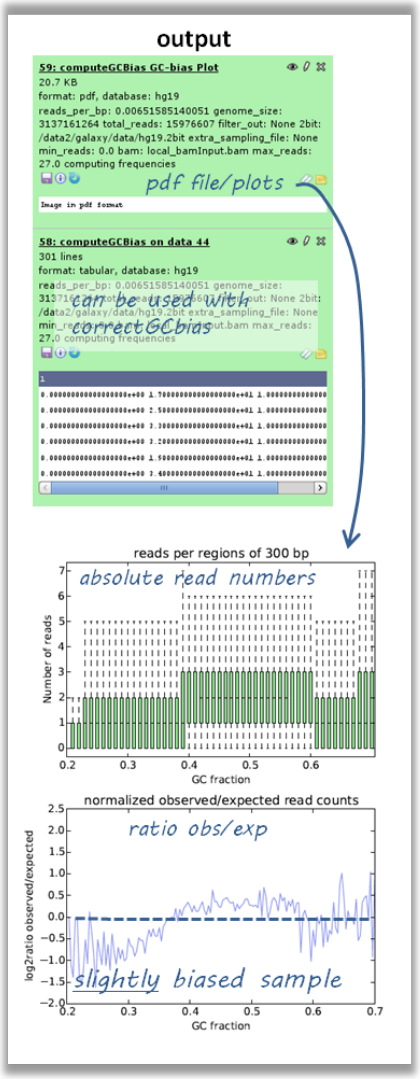
Using reference genome:
Human (Homo sapiens): hg19
If your genome of interest is not listed, contact the Galaxy team

Effective genome size:
hg19
The effective genome size is the portion of the genome that is mappable. Large fractions of
NNNN that should be discarded. Also, if repetitive regions were not included in the mapping
size needs to be adjusted accordingly. See Table 2 of http://www.plosone.org/article
/info%3Adoi%2F10.1371%2Fjournal.pone.0030377 or http://www.nature.com/nbt/journal/v
for several effective genome sizes.

Fragment length used for the sequencing:
150
If paired-end reads are used, the fragment length is computed from the BAM file.

Show advanced options:
no

GC bias plot:
Image in pdf format
If given, a diagnostic image summarizing the GC bias found on the sample will be created.



output

59: computeGCBias GC-bias Plot
20.7 KB
format: pdf, database: hg19
reads_per_bp: 0.00651585140051 genome_size: 3137161264 total_reads: 15976607 filter_out: None 2bit: /data2/galaxy/data/hg19.2bit extra_sampling_file: None min_reads: 0.0 bam: local_bamInput.bam max_reads: 27.0 computing frequencies

Image in pdf format

58: computeGCBias on data 44
301 lines
format: tabular, database: hg19
reads_per_bp: 0.00651585140051 genome_size: 3137161264 total_reads: 15976607 filter_out: None 2bit: /data2/galaxy/data/hg19.2bit extra_sampling_file: None min_reads: 0.0 bam: local_bamInput.bam max_reads: 27.0 computing frequencies

```
1
0.00000000000000000000e+00 1.70000000000000000000e+01 1.00000000000000
0.00000000000000000000e+00 2.50000000000000000000e+01 1.00000000000000
0.00000000000000000000e+00 3.30000000000000000000e+01 1.00000000000000
0.00000000000000000000e+00 3.20000000000000000000e+01 1.00000000000000
0.00000000000000000000e+00 1.50000000000000000000e+01 1.00000000000000
0.00000000000000000000e+00 3.40000000000000000000e+01 1.00000000000000
```

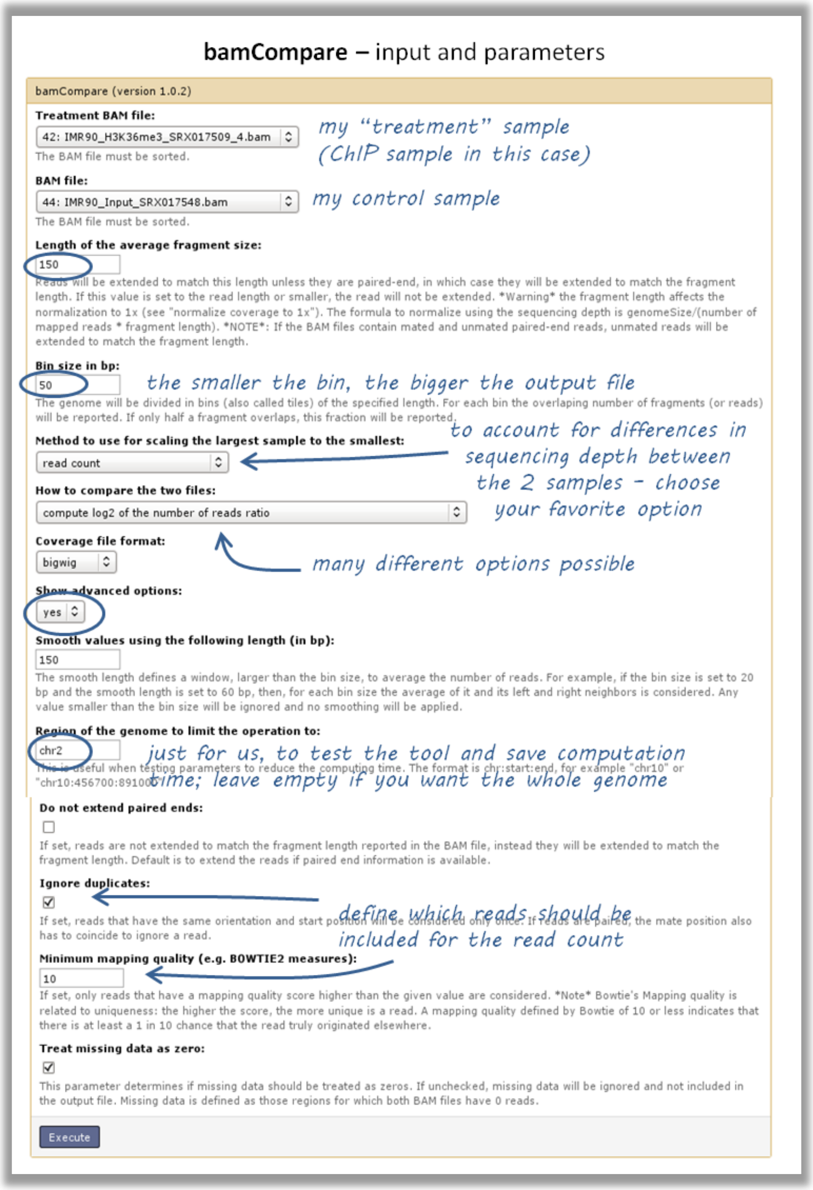
pdf file/plots
can be used with correctGCbias
reads per regions of 300 bp
absolute read numbers
Number of reads
GC fraction
normalized observed/expected read counts
ratio obs/exp
log2ratio observed/expected
slightly biased sample
GC fraction

- have a look at the image that is produced and compare it to the examples here
- if your sample shows an almost linear increase in exp/obs coverage (on the log scale of the lower plot), then you should consider correcting the GC bias - *if* you think that the biological interpretation of this data would otherwise be compromised (e.g. by comparing it to another sample that does not have an inherent GC bias)
  - the GC bias can be corrected with the tool `tools/correctGCBias` using the second output of the computeGCbias tool that you had to run anyway
  - CAUTION!! correctGCbias will add reads to otherwise depleted regions (typically GC-poor regions), that means that you should **not** remove duplicates in any downstream analyses based on the GC-corrected BAM file (we therefore recommend removing duplicates before doing the correction so that only those duplicate reads are kept that were produced by the GC correction procedure)

`content/../images/GalHow_correctGCbias.png`

How do I get an input-normalized ChIP-seq coverage file?

- input: you need two BAM files, one for the input and one for the ChIP-seq experiment
- tool: `tools/bamCompare` with ChIP = treatment, input = control sample



bamCompare – input and parameters

bamCompare (version 1.0.2)

Treatment BAM file:
42: IMR90_H3K36me3_SRX017509_4.bam
The BAM file must be sorted.

my "treatment" sample (ChIP sample in this case)

BAM file:
44: IMR90_Input_SRX017548.bam
The BAM file must be sorted.

my control sample

Length of the average fragment size:
150
Reads will be extended to match this length unless they are paired-end, in which case they will be extended to match the fragment length. If this value is set to the read length or smaller, the read will not be extended. *Warning* the fragment length affects the normalization to 1x (see "normalize coverage to 1x"). The formula to normalize using the sequencing depth is genomeSize/(number of mapped reads * fragment length). *NOTE*: If the BAM files contain mated and unmated paired-end reads, unmated reads will be extended to match the fragment length.

Bin size in bp:
50

the smaller the bin, the bigger the output file

The genome will be divided in bins (also called tiles) of the specified length. For each bin the overlaping number of fragments (or reads) will be reported. If only half a fragment overlaps, this fraction will be reported.

Method to use for scaling the largest sample to the smallest:
read count

to account for differences in sequencing depth between the 2 samples - choose your favorite option

How to compare the two files:
compute log2 of the number of reads ratio

Coverage file format:
bigwig

many different options possible

Show advanced options:
yes

Smooth values using the following length (in bp):
150
The smooth length defines a window, larger than the bin size, to average the number of reads. For example, if the bin size is set to 20 bp and the smooth length is set to 60 bp, then, for each bin size the average of it and its left and right neighbors is considered. Any value smaller than the bin size will be ignored and no smoothing will be applied.

Region of the genome to limit the operation to:
chr2
This is useful when testing parameters to reduce the computing time. The format is chr:start:end, for example "chr10" or "chr10:456700:891000".

just for us, to test the tool and save computation time; leave empty if you want the whole genome

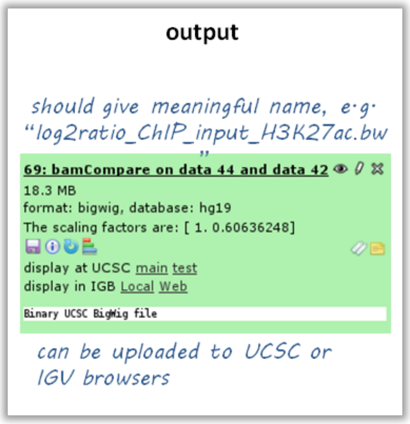
Do not extend paired ends:
☐
If set, reads are not extended to match the fragment length reported in the BAM file, instead they will be extended to match the fragment length. Default is to extend the reads if paired end information is available.

Ignore duplicates:
☑
If set, reads that have the same orientation and start position will be considered only once. If reads are paired, the mate position also has to coincide to ignore a read.

define which reads should be included for the read count

Minimum mapping quality (e.g. BOWTIE2 measures):
10
If set, only reads that have a mapping quality score higher than the given value are considered. *Note* Bowtie's Mapping quality is related to uniqueness: the higher the score, the more unique is a read. A mapping quality defined by Bowtie of 10 or less indicates that there is at least a 1 in 10 chance that the read truly originated elsewhere.

Treat missing data as zero:
☑
This parameter determines if missing data should be treated as zeros. If unchecked, missing data will be ignored and not included in the output file. Missing data is defined as those regions for which both BAM files have 0 reads.



output

should give meaningful name, e.g. "log2ratio_ChIP_input_H3K27ac.bw"

69: bamCompare on data 44 and data 42
18.3 MB
format: bigwig, database: hg19
The scaling factors are: [1. 0.60636248]
display at UCSC main test
display in IGB Local Web

Binary UCSC BigWig file

can be uploaded to UCSC or IGV browsers

How can I compare the ChIP strength for different ChIP experiments?

- tool: `tools/plotFingerprint`
- input: as many BAM files as you'd like to compare. Make sure you get all the labels right!

content/../images/GalHow_plotFingerprint.png

How do I get a (clustered) heatmap of sequencing-depth-normalized read coverages around the transcription start site of all genes?

- tools: `tools/computeMatrix`, then `tools/plotHeatmap`
- **inputs:**
  - 1 bigWig file of normalized read coverages (e.g. the result of bamCoverage or bamCompare)
  - 1 BED or INTERVAL file of genes, e.g. obtained through Galaxy via "Get Data" –> "UCSC main table browser" –> group: "Genes and Gene Predictions" –> (e.g.) "RefSeqGenes" –> send to Galaxy (see screenshots below)

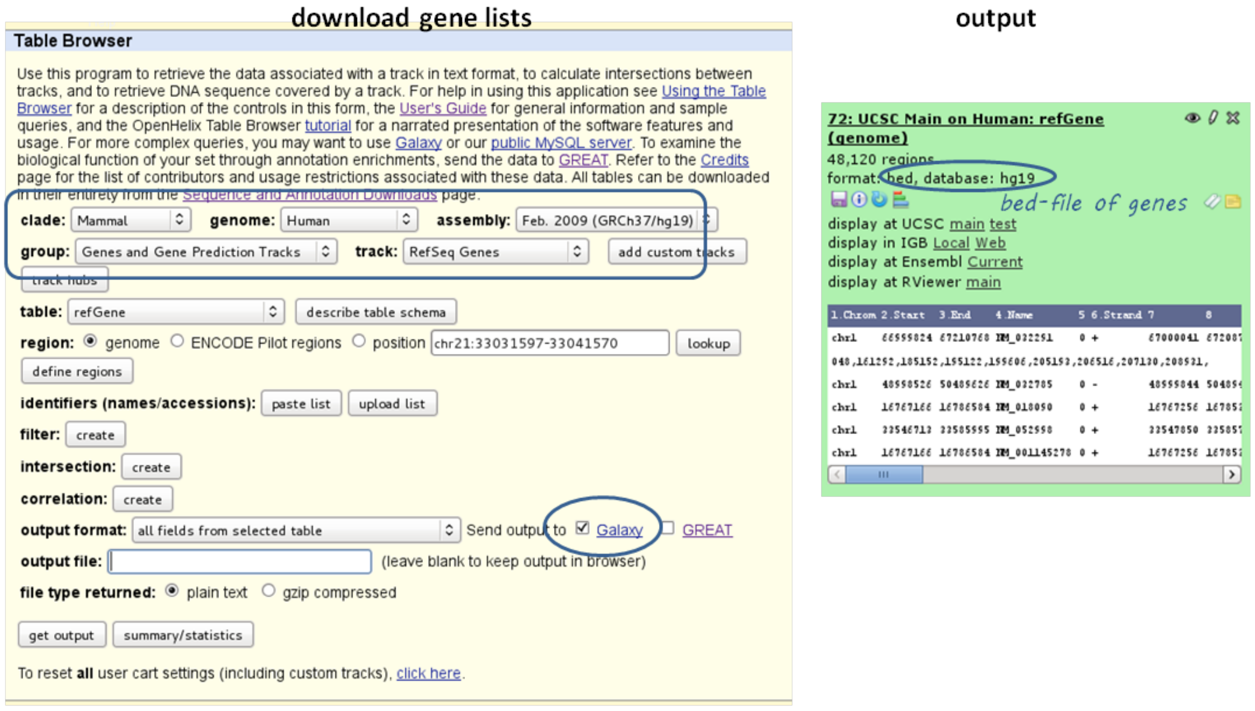


Table Browser

Use this program to retrieve the data associated with a track in text format, to calculate intersections between tracks, and to retrieve DNA sequence covered by a track. For help in using this application see Using the Table Browser for a description of the controls in this form, the User's Guide for general information and sample queries, and the OpenHelix Table Browser tutorial for a narrated presentation of the software features and usage. For more complex queries, you may want to use Galaxy or our public MySQL server. To examine the biological function of your set through annotation enrichments, send the data to GREAT. Refer to the Credits page for the list of contributors and usage restrictions associated with these data. All tables can be downloaded in their entirety from the Sequence and Annotation Downloads page.

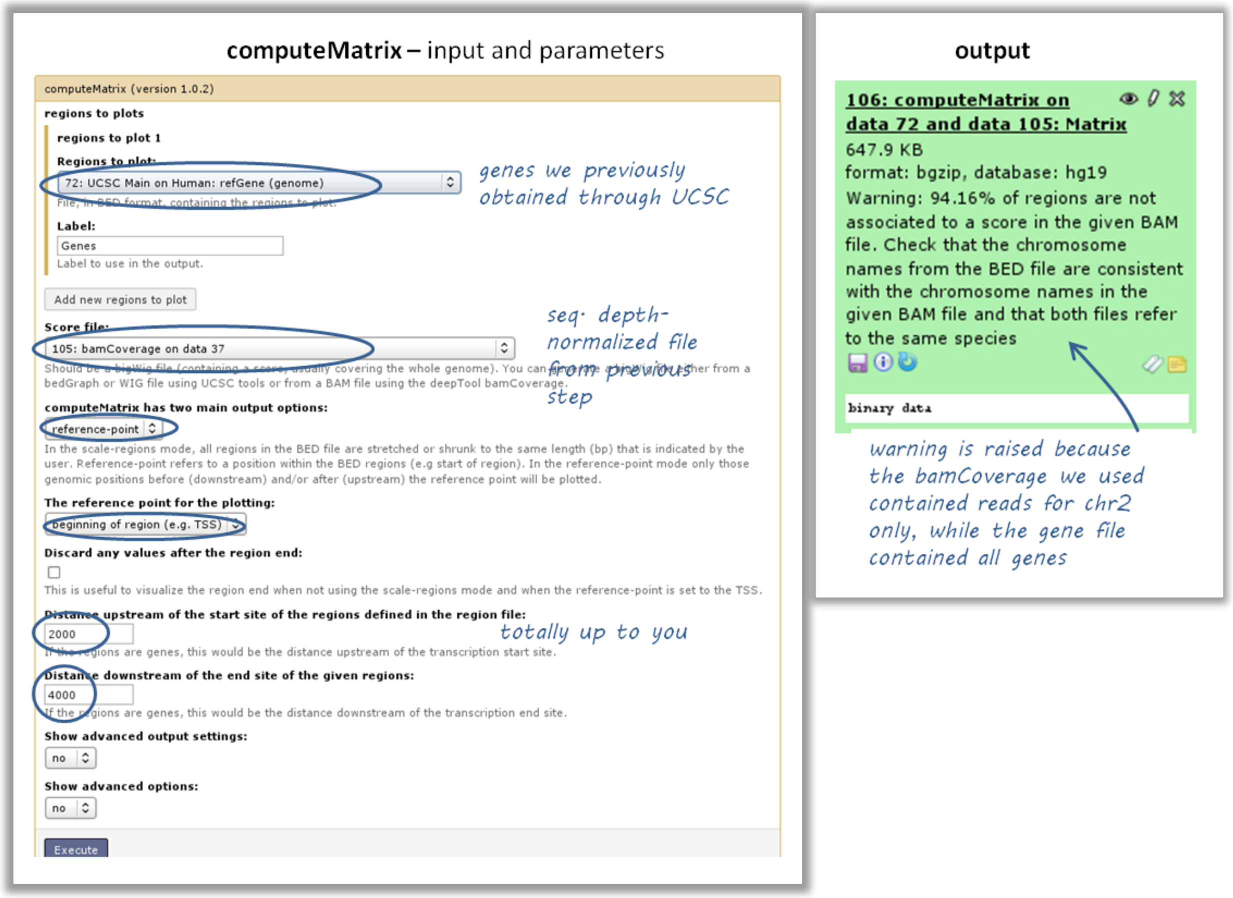
clade: Mammal **genome:** Human **assembly:** Feb. 2009 (GRCh37/hg19)
group: Genes and Gene Prediction Tracks **track:** RefSeq Genes
table: refGene
region: (●) genome ( ) ENCODE Pilot regions ( ) position chr21:33031597-33041570
identifiers (names/accessions):
filter:
intersection:
correlation:
output format: all fields from selected table Send output to ☑ Galaxy ☐ GREAT
output file: (leave blank to keep output in browser)
file type returned: (●) plain text ( ) gzip compressed

To reset **all** user cart settings (including custom tracks), click here.

72: UCSC Main on Human: refGene (genome)
48,120 regions
format: bed, database: hg19
display at UCSC main test
display in IGB Local Web
display at Ensembl Current
display at RViewer main

```
1.Chrom 2.Start   3.End    4.Name       5 6.Strand 7        8
chr1    66999824  67210768 NM_032291    0 +        67000041 67208
048,161292,185152,195122,199606,205193,206516,207130,208931,
chr1    48998526  50489626 NM_032785    0 -        48999844 50489
chr1    16767166  16786584 NM_018090    0 +        16767256 16785
chr1    33546713  33585995 NM_052998    0 +        33547850 33585
chr1    16767166  16786584 NM_001145278 0 +        16767256 16785
```

- use `tools/computeMatrix` with the bigWig file and the BED file
- indicate "reference-point" (and whatever other option you would like to tune, see screenshot below)


computeMatrix – input and parameters
output
genes we previously obtained through UCSC
seq. depth-normalized file from previous step
totally up to you
warning is raised because the bamCoverage we used contained reads for chr2 only, while the gene file contained all genes
Add new regions to plot
Execute

computeMatrix (version 1.0.2)

regions to plots

regions to plot 1

Regions to plot: 72: UCSC Main on Human: refGene (genome)

File, in BED format, containing the regions to plot.

Label: Genes

Label to use in the output.

Score file: 105: bamCoverage on data 37

Should be a bigWig file (containing a score, usually covering the whole genome). You can generate a bigWig file either from a bedGraph or WIG file using UCSC tools or from a BAM file using the deepTool bamCoverage.

computeMatrix has two main output options: reference-point

In the scale-regions mode, all regions in the BED file are stretched or shrunk to the same length (bp) that is indicated by the user. Reference-point refers to a position within the BED regions (e.g start of region). In the reference-point mode only those genomic positions before (downstream) and/or after (upstream) the reference point will be plotted.

The reference point for the plotting: beginning of region (e.g. TSS)

Discard any values after the region end: ☐

This is useful to visualize the region end when not using the scale-regions mode and when the reference-point is set to the TSS.

Distance upstream of the start site of the regions defined in the region file: 2000

If the regions are genes, this would be the distance upstream of the transcription start site.

Distance downstream of the end site of the given regions: 4000

If the regions are genes, this would be the distance downstream of the transcription end site.

Show advanced output settings: no

Show advanced options: no

106: computeMatrix on data 72 and data 105: Matrix

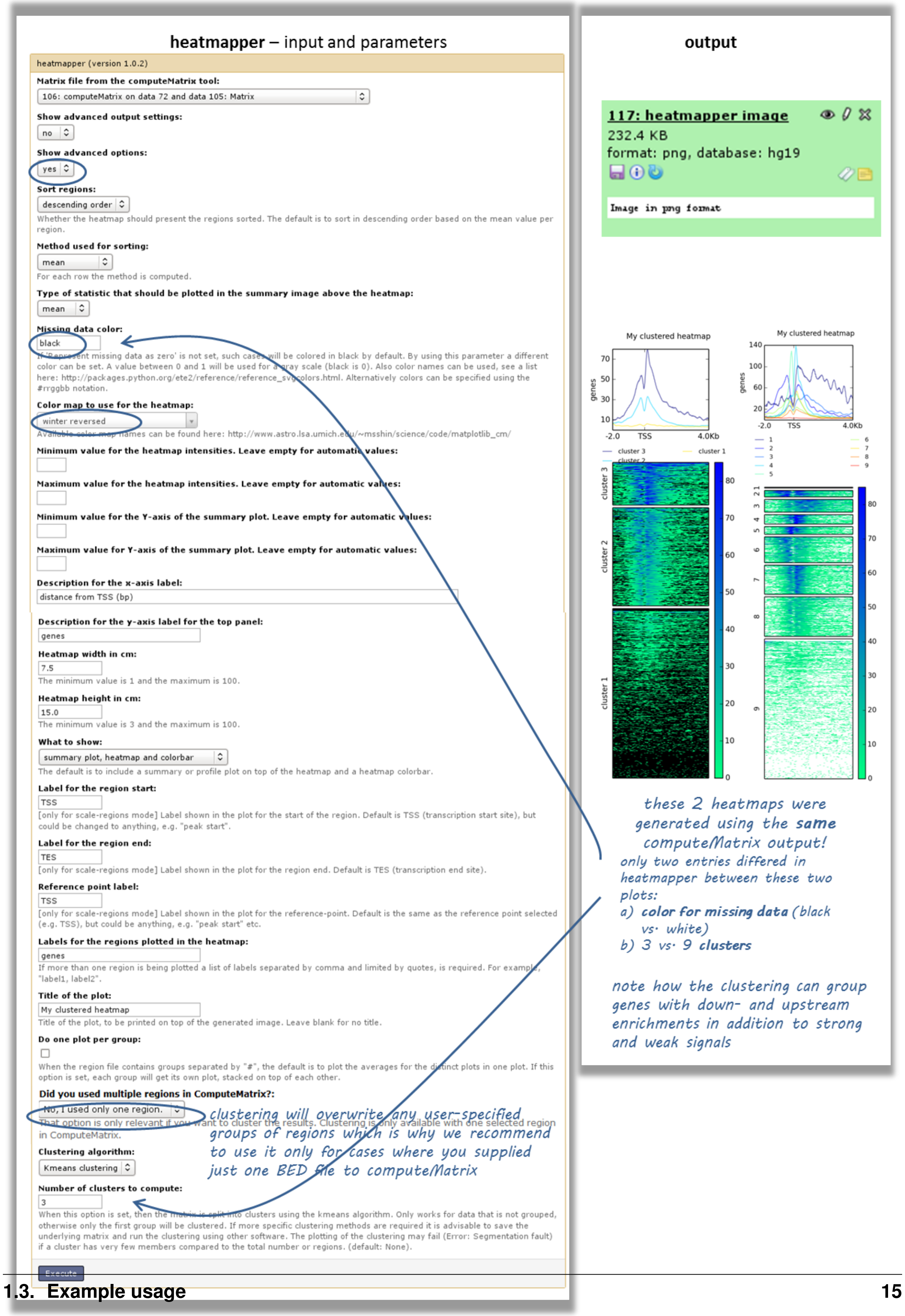
647.9 KB

format: bgzip, database: hg19

Warning: 94.16% of regions are not associated to a score in the given BAM file. Check that the chromosome names from the BED file are consistent with the chromosome names in the given BAM file and that both files refer to the same species

binary data

- **use the output from computeMatrix with `tools/plotHeatmap`**
  - if you would like to cluster the signals, choose "k-means clustering" (last option of "advanced options") with a reasonable number of clusters (usually between 2 to 7)



heatmapper – input and parameters

heatmapper (version 1.0.2)

Matrix file from the computeMatrix tool:
106: computeMatrix on data 72 and data 105: Matrix

Show advanced output settings:
no

Show advanced options:
yes

Sort regions:
descending order
Whether the heatmap should present the regions sorted. The default is to sort in descending order based on the mean value per region.

Method used for sorting:
mean
For each row the method is computed.

Type of statistic that should be plotted in the summary image above the heatmap:
mean

Missing data color:
black
If 'Represent missing data as zero' is not set, such cases will be colored in black by default. By using this parameter a different color can be set. A value between 0 and 1 will be used for a gray scale (black is 0). Also color names can be used, see a list here: http://packages.python.org/ete2/reference/reference_svgcolors.html. Alternatively colors can be specified using the #rrggbb notation.

Color map to use for the heatmap:
winter reversed
Available color map names can be found here: http://www.astro.lsa.umich.edu/~msshin/science/code/matplotlib_cm/

Minimum value for the heatmap intensities. Leave empty for automatic values:

Maximum value for the heatmap intensities. Leave empty for automatic values:

Minimum value for the Y-axis of the summary plot. Leave empty for automatic values:

Maximum value for Y-axis of the summary plot. Leave empty for automatic values:

Description for the x-axis label:
distance from TSS (bp)

Description for the y-axis label for the top panel:
genes

Heatmap width in cm:
7.5
The minimum value is 1 and the maximum is 100.

Heatmap height in cm:
15.0
The minimum value is 3 and the maximum is 100.

What to show:
summary plot, heatmap and colorbar
The default is to include a summary or profile plot on top of the heatmap and a heatmap colorbar.

Label for the region start:
TSS
[only for scale-regions mode] Label shown in the plot for the start of the region. Default is TSS (transcription start site), but could be changed to anything, e.g. "peak start".

Label for the region end:
TES
[only for scale-regions mode] Label shown in the plot for the region end. Default is TES (transcription end site).

Reference point label:
TSS
[only for scale-regions mode] Label shown in the plot for the reference-point. Default is the same as the reference point selected (e.g. TSS), but could be anything, e.g. "peak start" etc.

Labels for the regions plotted in the heatmap:
genes
If more than one region is being plotted a list of labels separated by comma and limited by quotes, is required. For example, "label1, label2".

Title of the plot:
My clustered heatmap
Title of the plot, to be printed on top of the generated image. Leave blank for no title.

Do one plot per group:
☐
When the region file contains groups separated by "#", the default is to plot the averages for the distinct plots in one plot. If this option is set, each group will get its own plot, stacked on top of each other.

Did you used multiple regions in ComputeMatrix?:
No, I used only one region.
That option is only relevant if you want to cluster the results. Clustering is only available with one selected region in ComputeMatrix.

clustering will overwrite any user-specified groups of regions which is why we recommend to use it only for cases where you supplied just one BED file to computeMatrix

Clustering algorithm:
Kmeans clustering

Number of clusters to compute:
3
When this option is set, then the matrix is split into clusters using the kmeans algorithm. Only works for data that is not grouped, otherwise only the first group will be clustered. If more specific clustering methods are required it is advisable to save the underlying matrix and run the clustering using other software. The plotting of the clustering may fail (Error: Segmentation fault) if a cluster has very few members compared to the total number or regions. (default: None).

Execute

output

117: heatmapper image
232.4 KB
format: png, database: hg19

Image in png format

*these 2 heatmaps were generated using the **same** computeMatrix output!*
only two entries differed in heatmapper between these two plots:
*a) **color for missing data** (black vs. white)*
*b) 3 vs. 9 **clusters***

note how the clustering can group genes with down- and upstream enrichments in addition to strong and weak signals

How can I compare the average signal for X- and autosomal genes for 2 or more different sequencing experiments?

Make sure you're familiar with computeMatrix and profiler before using this protocol.

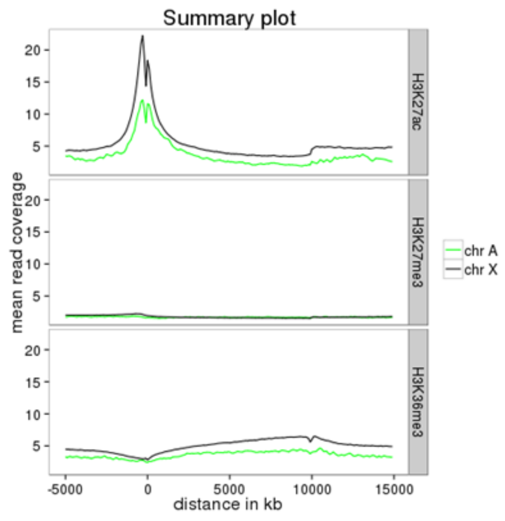
- **tools:**
  - Filter data on any column using simple expressions
  - computeMatrix
  - profiler
  - (plotting the summary plots for multiple samples)
- **inputs:**
  - several bigWig files (one for each sequencing experiment you would like to compare)
  - two BED files, one with X-chromosomal and one with autosomal genes

How to obtain a BED file for X chromosomal and autosomal genes each

1. download a full list of genes via "Get Data" –> "UCSC main table browser" –> group:"Genes and Gene Predictions" –> tracks: (e.g.) "RefSeqGenes" –> send to Galaxy
2. filter the list twice using the tool **"Filter data on any column using simple expressions"**
   - first use the expression: c1=="chrX" to filter the list of all genes –> this will generate a list of X-linked genes
   - then re-run the filtering, now with c1!="chrX", which will generate a list of genes that do not belong to chromosome X (!= indicates "not matching")

Compute the average values for X and autosomal genes

- use `tools/computeMatrix` for all of the signal files (bigWig format) at once
  - supply both filtered BED files (click on "Add new regions to plot" once) and label them
  - indicate the corresponding signal files
- now use `tools/plotProfile` on the resulting file
  - important: display the "advanced output options" and select "save the data underlying the average profile" –> this will generate a table in addition to the summary plot images



1.3.2 Gallery of deepTools plots

Note: If you have a nice deepTools plot that you'd like to share, we'll be happy to add it to our Gallery! Just send us an email: deeptools@googlegroups.com

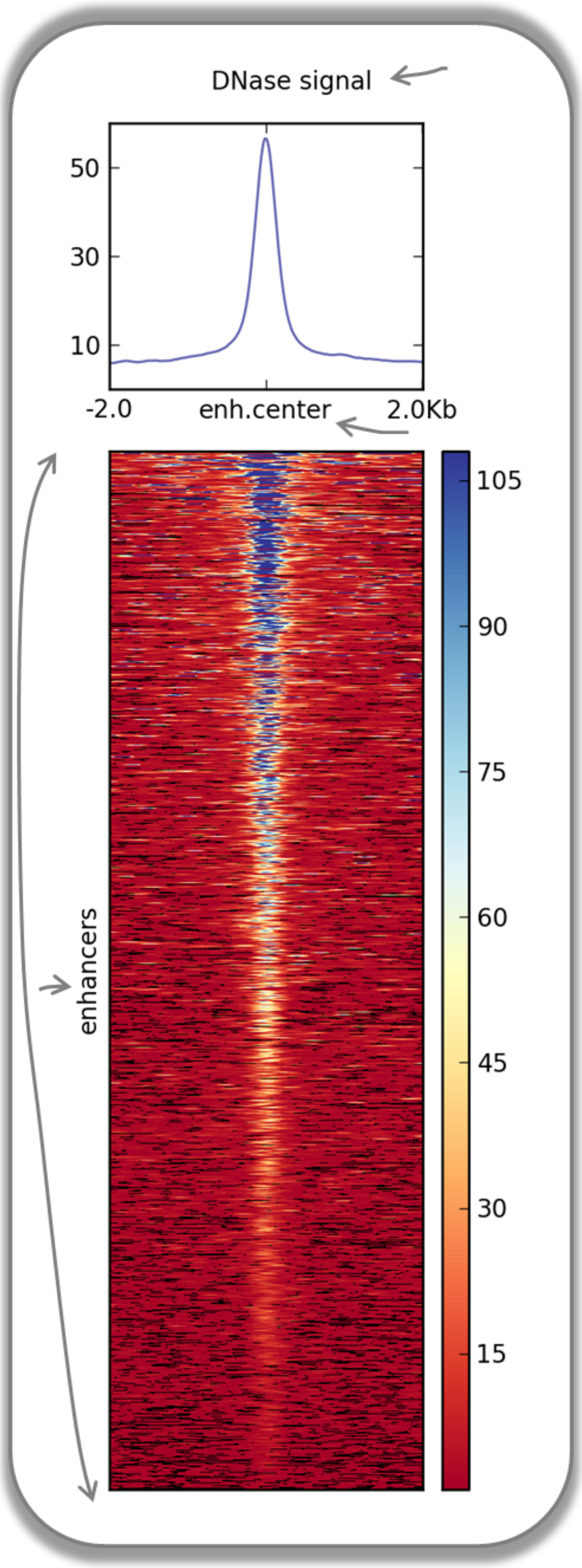
Published example plots

- *DNase accessibility at enhancers in murine ES cells*
- *TATA box enrichments around the TSS of mouse genes*
- *Visualizing the GC content for mouse and fly genes*
- *CpG methylation around murine transcription start sites in two different cell types*
- *Histone marks for genes of the mosquito* Anopheles gambiae
- *Signals of repressive chromatin marks, their enzymes and repeat element conservation scores*
- *Normalized ChIP-seq signals and peak regions*

We're trying to collect a wide variety of plots generated using deepTools. For the plots that we created ourselves, we try to point out the options that were used to create each image, so perhaps these can serve as inspiration for you.

DNase accessibility at enhancers in murine ES cells

The following image demonstrates that enhancer regions are typically small stretches of highly accessible chromatin (more information on enhancers can be found, for example, here). In the heatmap, yellow and blue tiles indicate a large numbers of reads that were sequenced (indicative of open chromatin) and black spots indicate missing data points. An appropriate labeling of the y-axis was neglected.


DNase signal
50
30
10
-2.0
enh.center
2.0Kb
105
90
75
60
45
30
15
enhancers

Fast Facts:

- *computeMatrix* mode: reference-point
- *regions file*: BED file with typical enhancer regions from Whyte et al., 2013 (download here)
- *signal file*: bigWig file with DNase signal from UCSC
- *heatmap cosmetics*: labels, titles, heatmap height

Command:

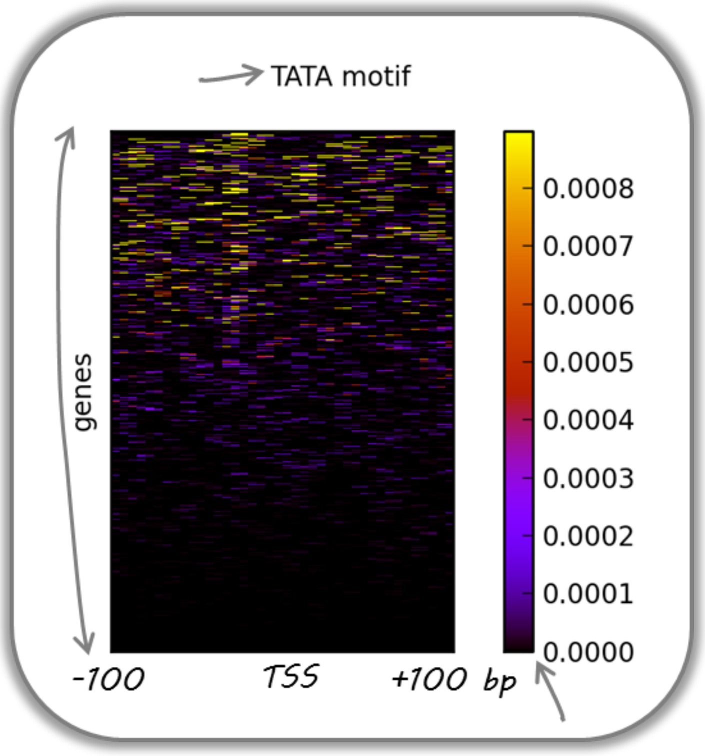
```
$ deepTools-1.5.7/bin/computeMatrix reference-point \
 -S DNase_mouse.bigwig \
 -R Whyte_TypicalEnhancers_ESC.bed \
 --referencePoint center \
 -a 2000 -b 2000 \ ## regions before and after the enhancer centers
 -out matrix_Enhancers_DNase_ESC.tab.gz

$ deepTools-1.5.7/bin/heatmapper \
 -m matrix_Enhancers_DNase_ESC.tab.gz\
 -out hm_DNase_ESC.png \
 --heatmapHeight 15  \
 --refPointLabel enh.center \
 --regionsLabel enhancers \
 --plotTitle 'DNase signal' \
```

TATA box enrichments around the TSS of mouse genes

Using the TRAP suite, we produced a bigWig file that contained TRAP scores for the well-known TATA box motif along the mouse genome. The TRAP score is a measure for the strength of a protein-DNA interaction at a given DNA sequence; the higher the score, the closer the motif is to the consensus motif sequence. The following heatmap demonstrates that:

- TATA-like motifs occur quite frequently
- there is an obvious clustering of TATA motifs slightly upstream of the TSS of many mouse genes
- there are many genes that do not contain TATA-like motifs at their promoter

Note that the heatmap shows *all* mouse RefSeq genes, so ca. 15,000 genes!



Fast Facts:

- *computeMatrix mode*: reference-point
- *regions file*: BED file with all mouse genes (from UCSC table browser)
- *signal file*: bigWig file of TATA psem scores
- *heatmap cosmetics*: color scheme, labels, titles, heatmap height, only showing heatmap + colorbar

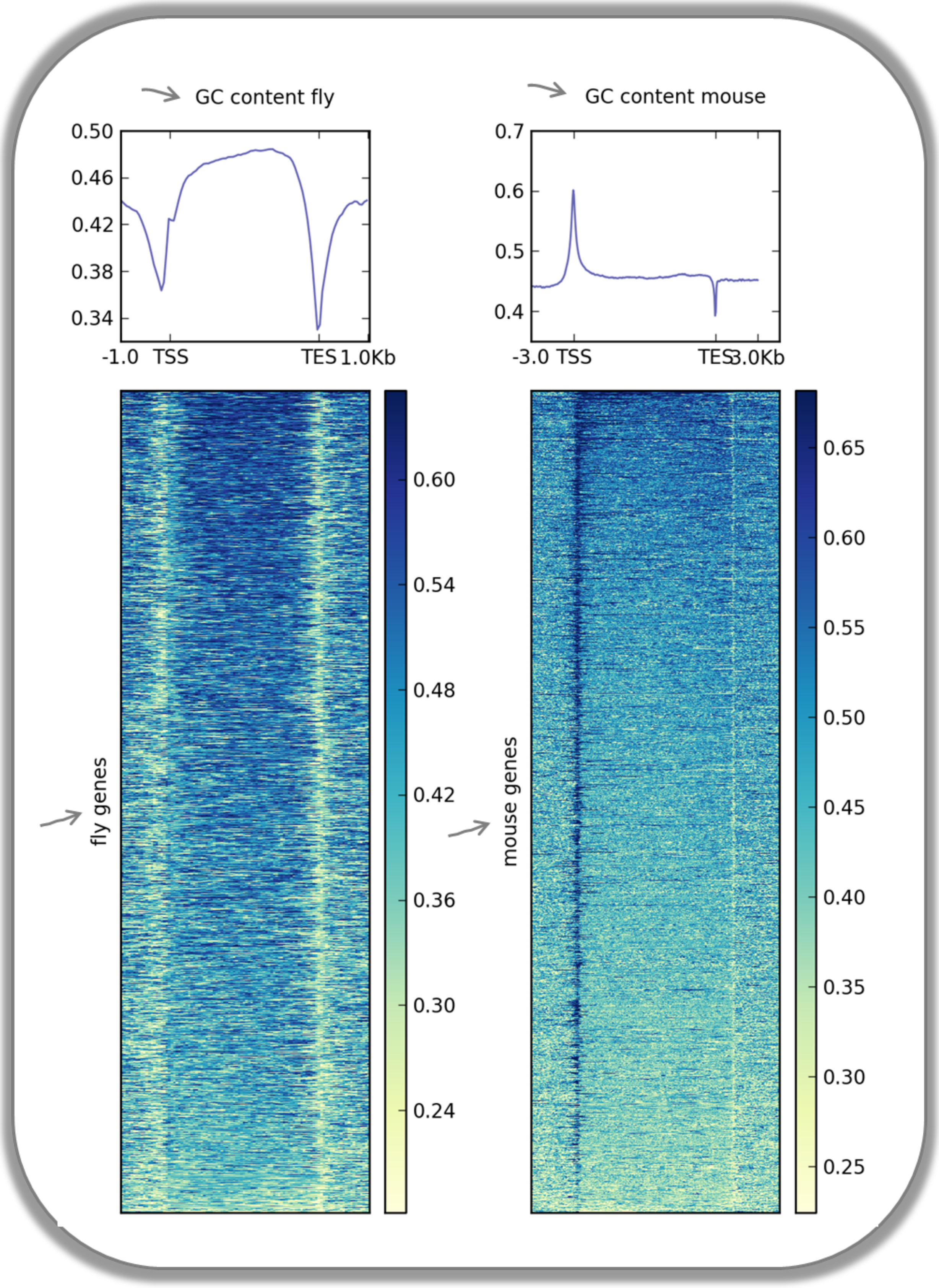
Command:

```
$ deepTools-1.5.7/bin/computeMatrix reference-point \
 -S TATA_01_pssm.bw \
 -R RefSeq_genes.bed \
 --referencePoint TSS \
 -a 100 -b 100 \
 --binSize 5 \

$ deepTools-1.5.7/bin/heatmapper \
 -m matrix_Genes_TATA.tab.gz  \
 -out hm_allGenes_TATA.png \
 --colorMap hot_r \
 --missingDataColor .4 \
 --heatmapHeight 7 \
 --plotTitle 'TATA motif' \
 --whatToShow 'heatmap and colorbar' \
 --sortRegions ascend
```

Visualizing the GC content for mouse and fly genes

It is well known that different species have different genome GC contents. Here, we used two bigWig files where the GC content was calculated for 50 base windows along the genome of mice and flies and the resulting scores visualized for gene regions.

The images nicely illustrate the completely opposite GC distributions in flies and mice: while the gene starts of mammalian genomes are enriched for Gs and Cs, fly promoters show depletion of GC content.


GC content fly
0.50
0.46
0.42
0.38
0.34
-1.0
TSS
TES
1.0Kb
0.60
0.54
0.48
0.42
0.36
0.30
0.24
fly genes
GC content mouse
0.7
0.6
0.5
0.4
-3.0
TSS
TES
3.0Kb
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
mouse genes

Fast Facts	
computeMatrix mode	scale-regions
regions files	BED files with mouse and fly genes (from UCSC table browser)
signal file	bigwig files with GC content
heatmap cosmetics	color scheme, labels, titles, color for missing data was set to white, heatmap height

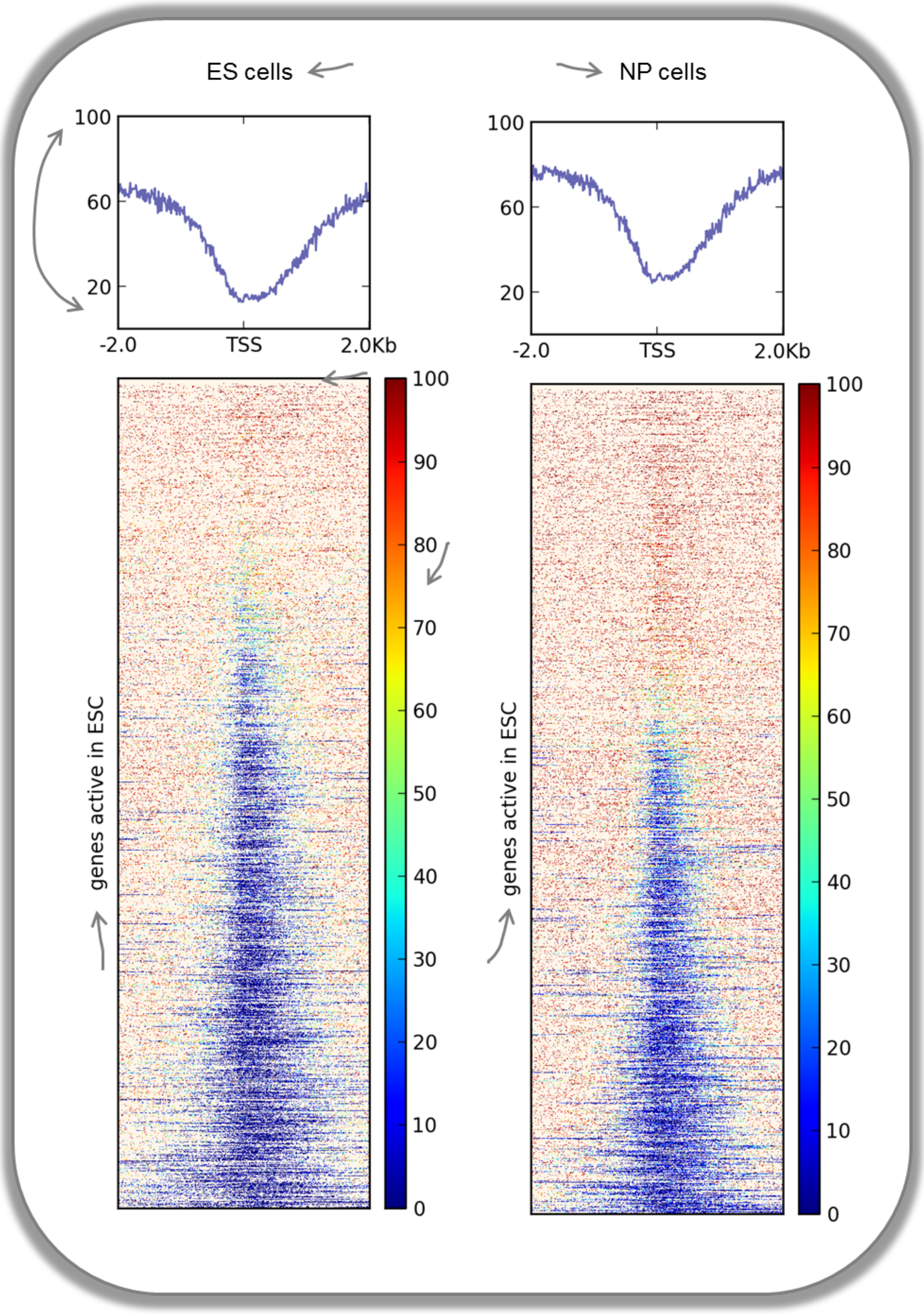
Fly and mouse genes were scaled to different sizes due to the different median sizes of the two species' genes (genes of *D.melanogaster* contain many fewer introns and are considerably shorter than mammalian genes). Thus, computeMatrix had to be run with slightly different parameters while the heatmapper commands were virtually identical (except for the labels).

```
$ deepTools-1.5.7/bin/computeMatrix scale-regions \
 -S GCcontent_Mm9_50_5.bw \
 -R RefSeq_genes_uniqNM.bed \
 -bs 50
 -m 10000 -b 3000 -a 3000 \
 -out matrix_GCcont_Mm9_scaledGenes.tab.gz \
 --skipZeros \
 --missingDataAsZero

$ deepTools-1.5.7/bin/computeMatrix scale-regions \
 -S GCcontent_Dm3_50_5.bw \
 -R Dm530.genes.bed \
 -bs 50
 -m 3000 -b 1000 -a 1000 \
 -out matrix_GCcont_Dm3_scaledGenes.tab.gz \
 --skipZeros --missingDataAsZero

$ deepTools-1.5.7/bin/heatmapper \
 -m matrix_GCcont_Dm3_scaledGenes.tab.gz \
 -out hm_GCcont_Dm3_scaledGenes.png \
 --colorMap YlGnBu \
 --regionsLabel 'fly genes' \
 --heatmapHeight 15 \
 --plotTitle 'GC content fly' &

$ deepTools-1.5.7/bin/heatmapper \
 -m matrix_GCcont_Mm9_scaledGenes.tab.gz \
 -out hm_GCcont_Mm9_scaledGenes.png \
 --colorMap YlGnBu \
 --regionsLabel 'mouse genes' \
 --heatmapHeight 15 \
 --plotTitle 'GC content mouse' &
```

CpG methylation around murine transcription start sites in two different cell types

In addition to the methylation of histone tails, the cytosines can also be methylated (for more information on CpG methylation, read here). In mammalian genomes, most CpGs are methylated unless they are in gene promoters that need to be kept unmethylated to allow full transcriptional activity. In the following heatmaps, we used genes expressed primarily in ES cells and checked the percentages of methylated cytosines around their transcription start sites. The blue signal indicates that very few methylated cytosines are found. When you compare the CpG methylation signal between ES cells and neuronal progenitor (NP) cells, you can see that the majority of genes remain unmethylated, but the general amount of CpG methylation around the TSS increases, as indicated by the stronger red signal and the slight elevation of the CpG methylation signal in the summary plot. This supports the notion that genes stored in the BED file indeed tend to be more expressed in ES than in NP cells.

This image was taken from Chelmicki & Dündar et al. (2014), eLife.


ES cells
NP cells
100
60
20
-2.0
TSS
2.0Kb
100
90
80
70
60
50
40
30
20
10
0
genes active in ESC
genes active in ESC

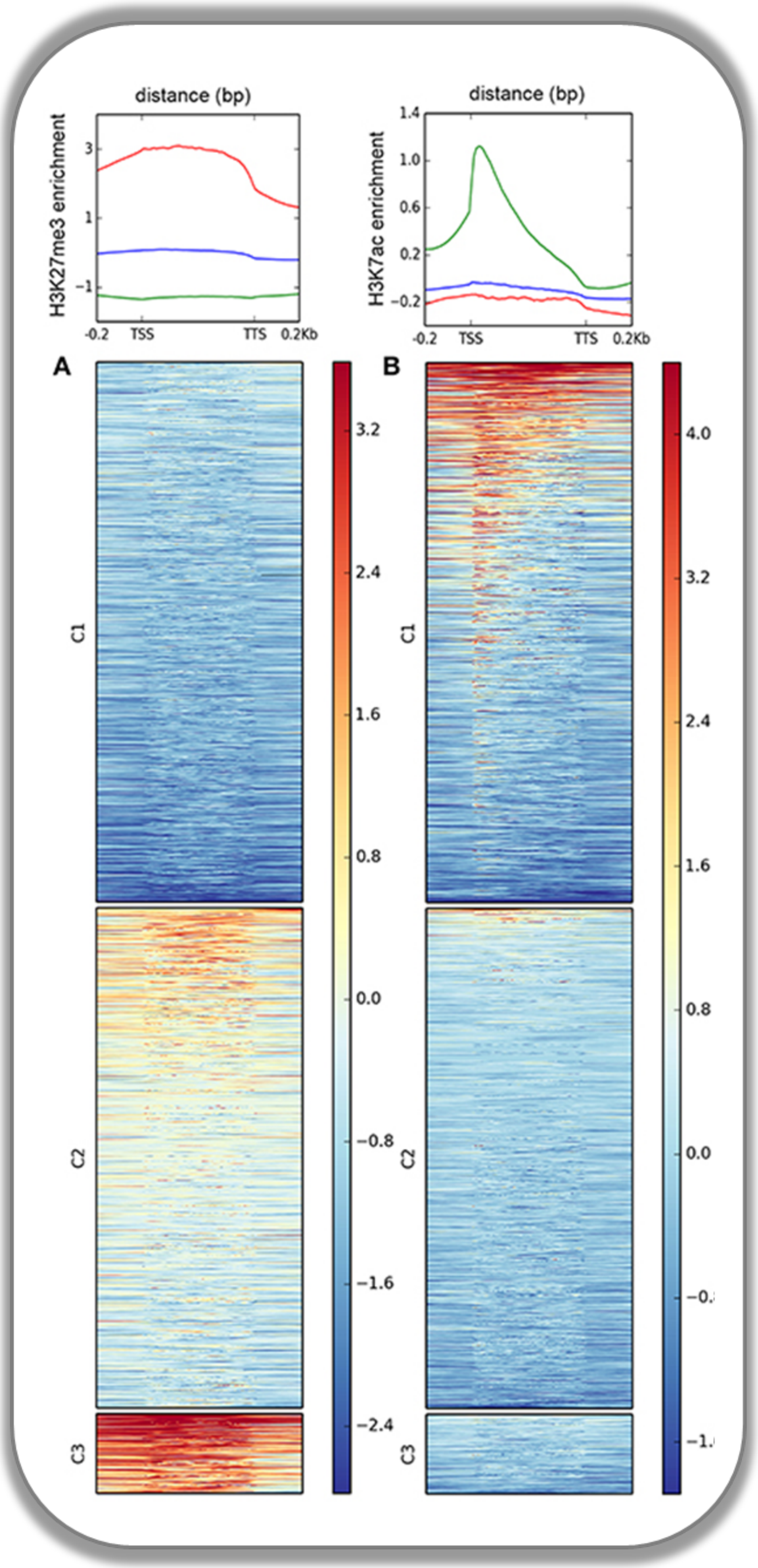
Fast Facts	
computeMatrix mode	reference-point
regions files	*BED* file mouse genes expressed in ES cells
signal file	*bigWig* files with fraction of methylated cytosins (from Stadler et al., 2011)
heatmap cosmetics	color scheme, labels, titles, color for missing data was set to customized color, y-axis of profiles were changed, heatmap height

The commands for the bigWig files from the ES and NP cells were the same:

```
$ deepTools-1.5.7/bin/computeMatrix reference-point \
 -S GSE30202_ES_CpGmeth.bw \
 -R activeGenes_ESConly.bed \
 --referencePoint TSS \
 -a 2000 -b 2000 \
 -out matrix_Genes_ES_CpGmeth.tab.gz

$ deepTools-1.5.7/bin/heatmapper \
 -m matrix_Genes_ES_CpGmeth.tab.gz \
 -out hm_activeESCGenes_CpG_ES_indSort.png \
 --colorMap jet \
 --missingDataColor "#FFF6EB" \
 --heatmapHeight 15 \
 --yMin 0 --yMax 100 \
 --plotTitle 'ES cells' \
 --regionsLabel 'genes active in ESC'
```

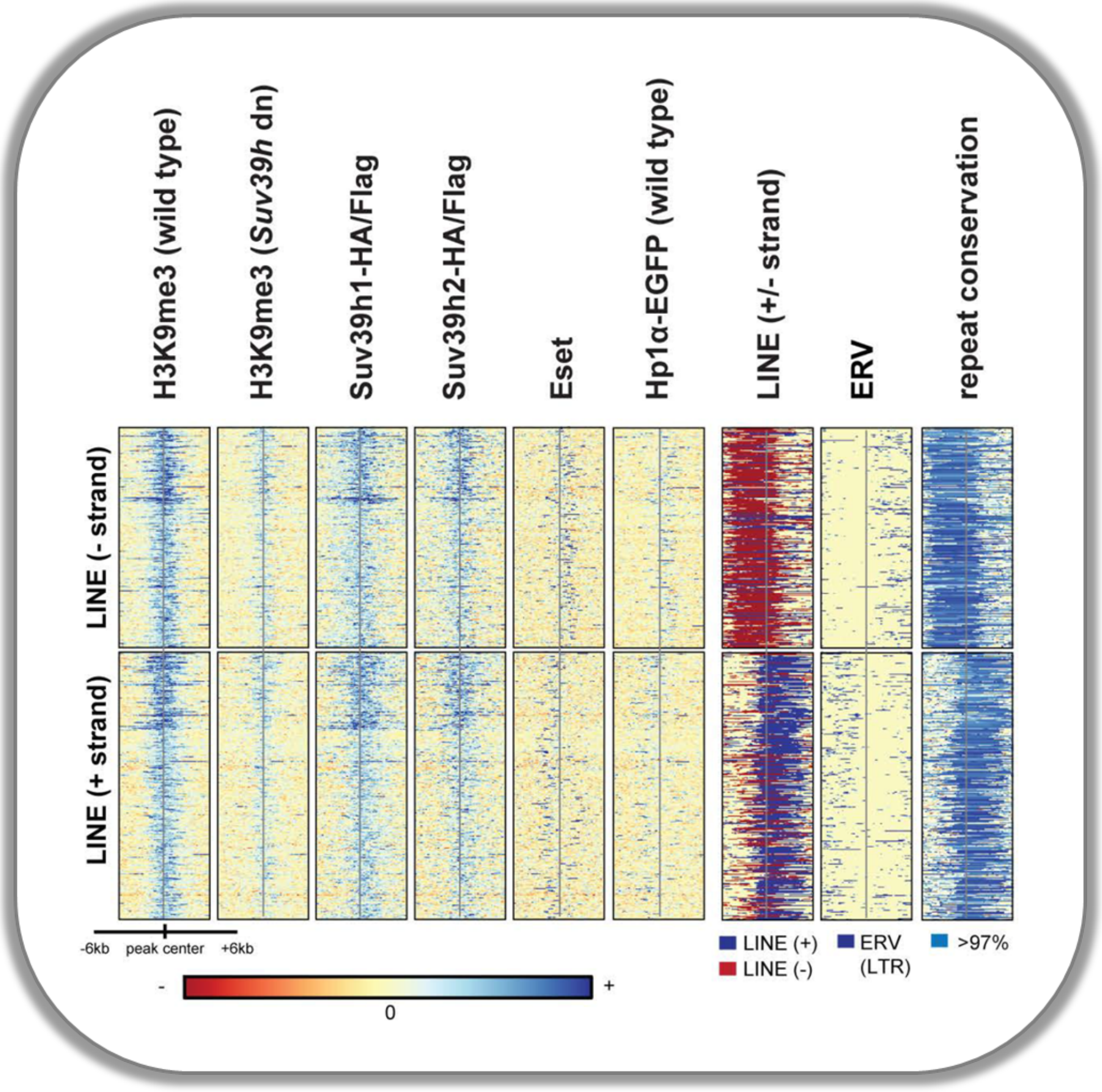
Histone marks for genes of the mosquito *Anopheles gambiae*

This figure was taken from Gómez-Díaz et al. (2014): Insights into the epigenomic landscape of the human malaria vector *Anopheles gambiae*. From Genet Aug15;5:277. It shows the distribution of H3K27Me3 (left) and H3K27Ac (right) over gene features in *A. gambiae* midguts. The enrichment or depletion is shown relative to chromatin input. The regions in the map comprise gene bodies flanked by a segment of 200 bases at the 5 end of TSSs and TTSs. Average profile across gene regions ±200 bases for each histone modification are shown on top.


distance (bp)
H3K27me3 enrichment
3
1
−1
-0.2
TSS
TTS
0.2Kb
distance (bp)
H3K7ac enrichment
1.4
1.0
0.6
0.2
−0.2
-0.2
TSS
TTS
0.2Kb
A
B
C1
C2
C3
3.2
2.4
1.6
0.8
0.0
−0.8
−1.6
−2.4
4.0
3.2
2.4
1.6
0.8
0.0
C1
C2
C3

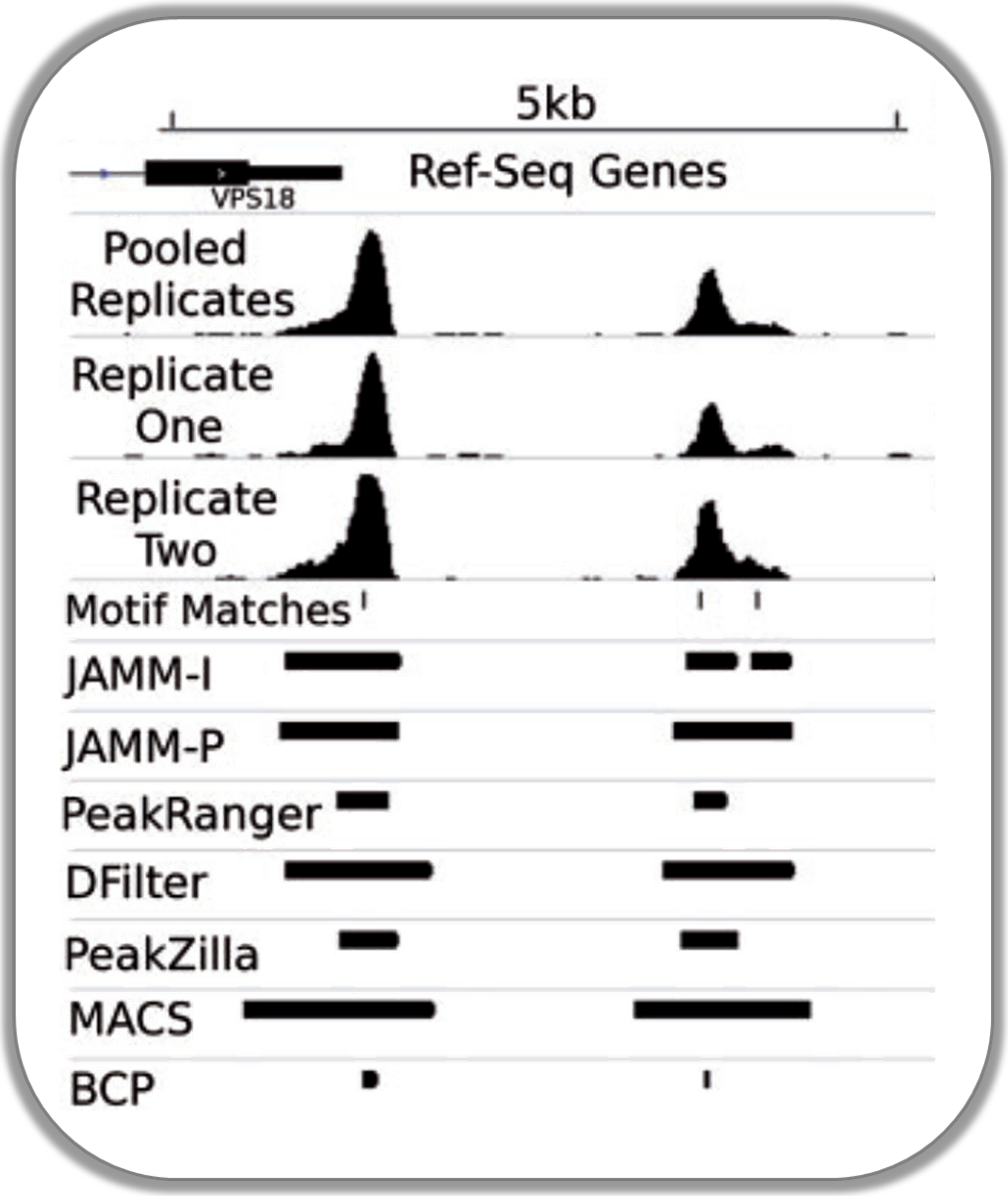
Signals of repressive chromatin marks, their enzymes and repeat element conservation scores

This image is from Bulut-Karsliogu and De La Rosa-Velázquez et al. (2014), Mol Cell. The heatmaps depict various signal types for unscaled peak regions of proteins and histone marks associated with repressed chromatin. The peaks were separated into those containing long interspersed elements (LINEs) on the forward and reverse strand. The signals include normalized ChIP-seq signals for H3K9Me3, Suv39h1, Suv39h2, Eset, and HP1alpha-EGFP, followed by LINE and ERV content and repeat conservation scores.



Normalized ChIP-seq signals and peak regions

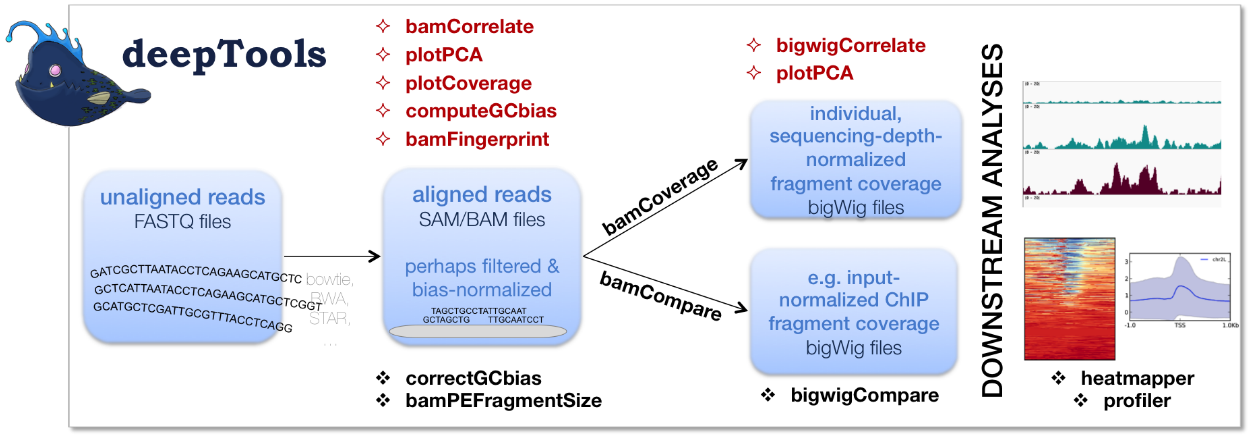
This image was published by Ibrahim et al., 2014 (NAR). They used deepTools to generate extended reads per kilobase per million reads at 10 base resolution and visualized the resulting coverage files in IGV.



1.3.3 How we use deepTools for ChIP-seq analyses

deepTools started off as a package for ChIP-seq analysis, which is why you'll find many ChIP-seq examples in our documentation. Here are slides that we used for teaching at the University of Freiburg, with more details on the deepTools usage and aims in regard to ChIP-seq. To get a feeling fo what deepTools can do, we'd like to give you a brief glimpse into how we typically use deepTools for ChIP-seq analyses.

Note: While some tools, such as `plotFingerprint`, specifically address ChIP-seq-issues, the majority of tools is widely applicable to deep-sequencing data, including RNA-seq.



As shown in the flow chart above, our work usually begins with one or more *FASTQ* file(s) of deeply-sequenced samples. After preliminary quality control using FASTQC, we align the reads to the reference genome, e.g., using bowtie2. The standard output of bowtie2 (and other mapping tools) is in the form of sorted and indexed BAM files that provide the common input and starting point for all subsequent deepTools analyses. We then use deepTools to assess the quality of the aligned reads:

1. **Correlation between BAM files** (`multiBamSummary` and `plotCorrelation`). Together these two modules perform a very basic test to see whether the sequenced and aligned reads meet your expectations. We use this check to assess reproducibility - either between replicates and/or between different experiments that might have used the same antibody or the same cell type, etc. For instance, replicates should correlate better than differently treated samples.

2. **Correlation between bigWig files** (`multiBigwigSummary` and `plotCorrelation`). Sometimes we want to compare our alignments with genome-wide data stored as "tracks" in public repositories or other more general scores that are not necessarily based on read-coverage. To this end, we provide an efficient module to handle bigWig files and compare them and their correlation for several samples. In addition we provide a tool (`plotPCA`) to perform a Principle Component Analysis of the same underlying data.

3. **GC-bias check** (`computeGCbias`). Many sequencing protocols require several rounds of PCR-based DNA amplification, which often introduces notable bias, due to many DNA polymerases preferentially amplifying GC-rich templates. Depending on the sample (preparation), the GC-bias can vary significantly and we routinely check its extent. In case we need to compare files with different GC biases, we use the *correctGCbias* module to match the GC bias. See the paper by Benjamini and Speed for many insights into this problem.

4. **Assessing the ChIP strength**. We do this quality control step to get a feeling for the signal-to-noise ratio in samples from ChIP-seq experiments. It is based on the insights published by Diaz et al..

Once we're satisfied with the basic quality checks, we normally **convert** the large *BAM* files into a leaner data format, typically *bigWig*. bigWig files have several advantages over BAM files, mainly stemming from their significantly decreased size:

- useful for data sharing and storage
- intuitive visualization in Genome Browsers (e.g. IGV)
- more efficient downstream analyses are possible

The deepTools modules `bamCompare` and `bamCoverage` not only allow for simple conversion of BAM to bigWig (or bedGraph for that matter), but also for normalization, such that different samples can be compared despite differences in their sequencing depth, GC biases and so on.

Finally, once all the files have passed our visual inspections, the fun of downstream analysis with `computeMatrix`, `heatmapper` and `profiler` can begin!

1.4 Changes in deepTools2.0

1.4.1 Major changes

Note: The major changes encompass features for **increased efficiency**, **new sequencing data types**, and **additional plots**, particularly for QC.

Moreover, deepTools modules can now be used by other python programs. The *deepTools API example* is now part of the documentation.

Accommodating additional data types

- correlation and comparisons can now be calculated for **bigWig files** (in addition to BAM files) using `multiBigwigSummary` and `bigwigCompare`
- **RNA-seq:** split-reads are now natively supported
- **MNase-seq:** using the new option `--MNase` in `bamCoverage`, one can now compute read coverage only taking the 2 central base pairs of each mapped fragment into account.

Structural updates

- All modules have comprehensive and automatic tests that evaluate proper functioning after any modification of the code.
- Virtualization for stability: we now provide a `docker` image and enable the easy deployment of deepTools via the Galaxy `toolshed`.
- Our documentation is now version-aware thanks to readthedocs and `sphinx`.
- The API is public and documented.

Renamed tools

- **heatmapper** to `tools/plotHeatmap`
- **profiler** to `tools/plotProfile`
- **bamCorrelate** to `tools/multiBamSummary`
- **bigwigCorrelate** to `tools/multiBigwigSummary`
- **bamFingerprint** to `tools/plotFingerprint`

Increased efficiency

- We dramatically improved the **speed** of bigwig related tools (`tools/multiBigwigSummary` and `computeMatrix`) by using the new pyBigWig module.
- It is now possible to generate one composite heatmap and/or meta-gene image based on **multiple bigwig files** in one go (see `tools/computeMatrix`, `tools/plotHeatmap`, and `tools/plotProfile` for examples)
- `computeMatrix` now also accepts multiple input BED files. Each is treated as a group within a sample and is plotted independently.
- We added **additional filtering options for handling BAM files**, decreasing the need for prior filtering using tools other than deepTools: The `--samFlagInclude` and `--samFlagExclude` parameters can, for example, be used to only include (or exclude) forward reads in an analysis.
- We separated the generation of read count tables from the calculation of pairwise correlations that was previously handled by `bamCorrelate`. Now, read counts are calculated first using `multiBamSummary` or `multiBigWigCoverage` and the resulting output file can be used for calculating and plotting pairwise correlations using `plotCorrelation` or for doing a principal component analysis using `plotPCA`.

New features and tools

- Correlation analyses are no longer limited to BAM files – bigwig files are possible, too! (see `tools/multiBigwigSummary`)
- Correlation coefficients can now be computed even if the data contains NaNs.
- **Added new quality control tools:**
  - use `tools/plotCoverage` to plot the coverage over base pairs
  - use `tools/plotPCA` for principal component analysis
  - `tools/bamPEFragmentSize` can be used to calculate the average fragment size for paired-end read data
- Added the possibility for **hierarchical clustering**, besides *k*-means to `plotProfile` and `plotHeatmap`

1.4.2 Minor changes

Changed parameters names and settings

- `computeMatrix` can now read files with DOS newline characters.
- `--missingDataAsZero` was renamed to `--skipNonCoveredRegions` for clarity in `bamCoverage` and `bamCompare`.

- Read extension was made optional and we removed the need to specify a default fragment length for most of the tools: `--fragmentLength` was thus replaced by the new optional parameter `--extendReads`.
- Added option `--skipChromosomes` to `multiBigwigSummary`, which can be used to, for example, skip all 'random' chromosomes.
- Added the option for adding titles to QC plots.

Bug fixes

- Resolved an error introduced by `numpy version 1.10` in `computeMatrix`.
- Improved plotting features for `plotProfile` when using as plot type: 'overlapped_lines' and 'heatmap'
- Fixed problem with BED intervals in `multiBigwigSummary` and `multiBamSummary` that returned wrongly labeled raw counts.
- `multiBigwigSummary` now also considers chromosomes as identical when the names between samples differ by 'chr' prefix, e.g. chr1 vs. 1.
- Fixed problem with wrongly labeled proper read pairs in a BAM file. We now have additional checks to determine if a read pair is a proper pair: the reads must face each other and are not allowed to be farther apart than 4x the mean fragment length.
- For `bamCoverage` and `bamCompare`, the behavior of `scaleFactor` was updated such that now, if given in combination with the normalization options (`--normalizeTo1x` or `--normalizeUsingRPKM`), the given scaling factor will be multiplied with the factor computed by the respective normalization method.

1.5 Using deepTools within Galaxy

Galaxy is a tremendously useful platform developed by the Galaxy Team at Penn State and the Emory University. This platform is meant to offer access to a large variety of bioinformatics tools that can be used without computer programming experiences. That means, that the basic features of Galaxy will apply to every tool, i.e. every tool provided within a Galaxy framework will look very similar and will follow the concepts of Galaxy.

Our publicly available deepTools Galaxy instance can be found here: deeptools.ie-freiburg.mpg.de. This server also contains some additional tools that will enable users to analyse and visualize data from high-throughput sequencing experiments, starting from aligned reads.

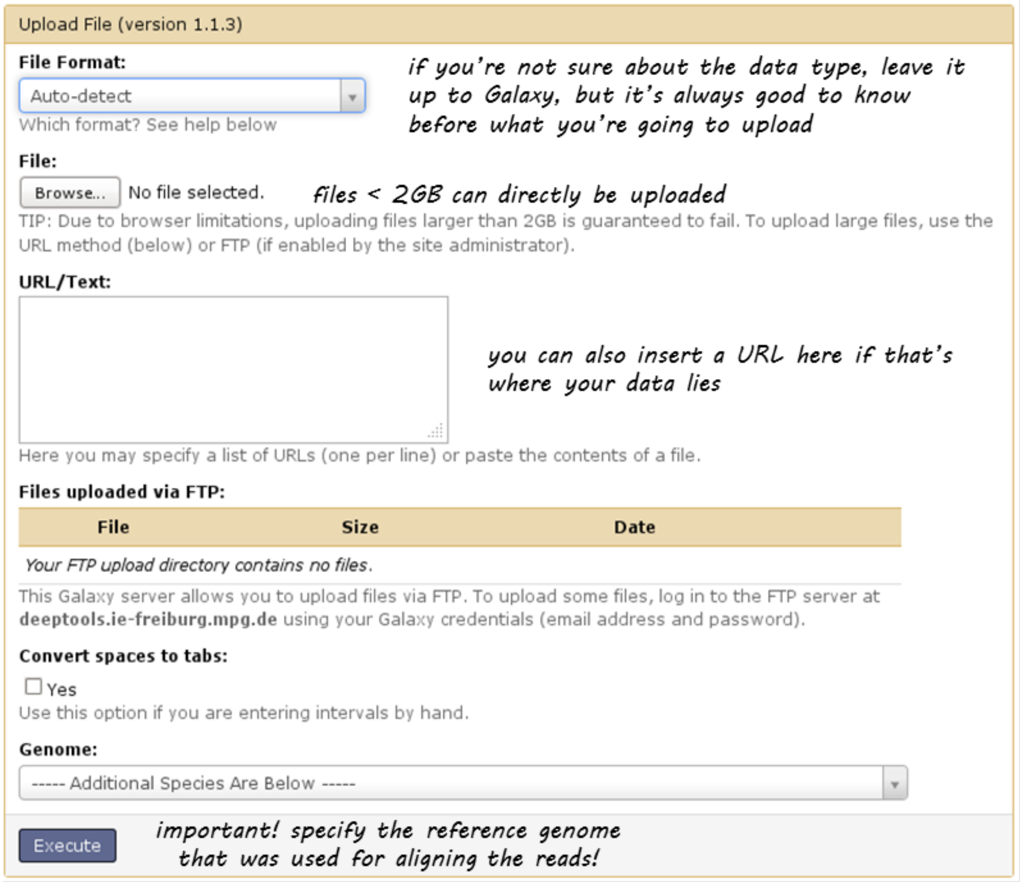
Table of content

1.5.1 Data import into Galaxy

There are three main ways to populate your Galaxy history with data files plus an additional one for sharing data within Galaxy.

Upload files from your computer

The data upload of files **smaller than 2 GB** that lie on your computer is fairly straight-forward: click on the category "Get data" and choose the tool "Upload file". Then select the file via the "Browse" button.


Upload File (version 1.1.3)

File Format:

Auto-detect

Which format? See help below

if you're not sure about the data type, leave it up to Galaxy, but it's always good to know before what you're going to upload

File:

Browse... No file selected.

files < 2GB can directly be uploaded

TIP: Due to browser limitations, uploading files larger than 2GB is guaranteed to fail. To upload large files, use the URL method (below) or FTP (if enabled by the site administrator).

URL/Text:

you can also insert a URL here if that's where your data lies

Here you may specify a list of URLs (one per line) or paste the contents of a file.

Files uploaded via FTP:

File	Size	Date
Your FTP upload directory contains no files.		

This Galaxy server allows you to upload files via FTP. To upload some files, log in to the FTP server at **deeptools.ie-freiburg.mpg.de** using your Galaxy credentials (email address and password).

Convert spaces to tabs:

☐ Yes

Use this option if you are entering intervals by hand.

Genome:

----- Additional Species Are Below -----

Execute

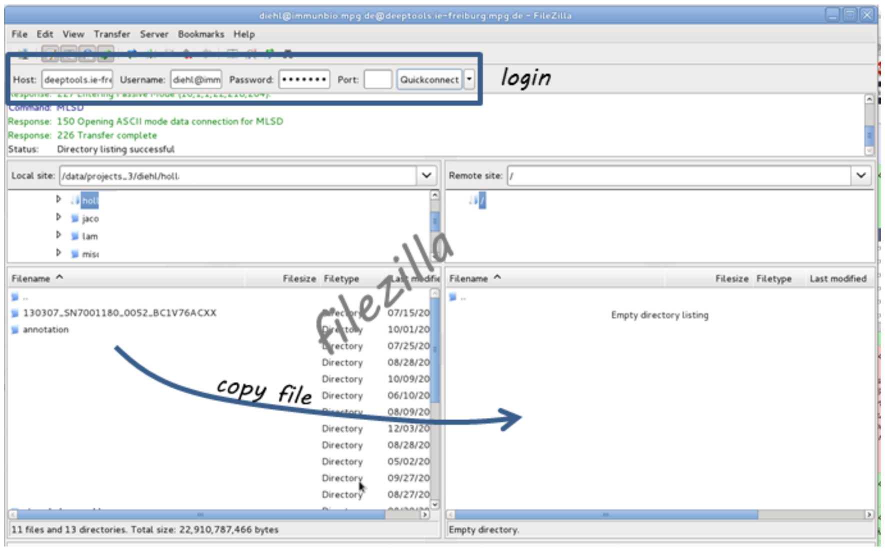
important! specify the reference genome that was used for aligning the reads!

For files **greater than 2GB**, there's the option to upload via an FTP server. If your data is available via an URL that links to an FTP server, you can simply paste the URL in the empty text box.

If you do not have access to an FTP server, you can directly upload to our Galaxy's FTP.

1. Register with deeptools.ie-freiburg.mpg.de (via "User" "register"; registration requires an email address and is free of charge)
2. You will also need an FTP client, e.g. filezilla.
3. Then login to the **FTP client** using your **deepTools Galaxy user name and password** (host: deeptools.ie-freiburg.mpg.de). Down below you see a screenshot of what that looks like with filezilla.
4. Copy the file you wish to upload to the remote site (in filezilla, you can simply drag the file to the window on the right hand side)

5. Go back to deepTools Galaxy.
6. Click on the tool "Upload file" ("Files uploaded via FTP") - here, the files you just copied over via filezilla should appear. Select the files you want and hit "execute". They will be moved from the FTP server to your history (i.e. they will be deleted from the FTP once the upload was successful).


diehl@immunbio.mpg.de@deeptools.ie-freiburg.mpg.de - FileZilla
File Edit View Transfer Server Bookmarks Help
login
filezilla
copy file
Quickconnect

Host: deeptools.ie-fre Username: diehl@imm Password: ••••••• Port:

```
Command:  MLSD
Response: 150 Opening ASCII mode data connection for MLSD
Response: 226 Transfer complete
Status:   Directory listing successful
```

Local site: /data/projects_3/diehl/holl.

Remote site: /

Filename	Filesize	Filetype	Last modified
..			
130307_SN7001180_0052_BC1V76ACXX		Directory	07/15/20
annotation		Directory	10/01/20
		Directory	07/25/20
		Directory	08/28/20
		Directory	10/09/20
		Directory	06/10/20
		Directory	08/09/20
		Directory	12/03/20
		Directory	08/28/20
		Directory	05/02/20
		Directory	09/27/20
		Directory	08/27/20

11 files and 13 directories. Total size: 22,910,787,466 bytes

Empty directory listing

Empty directory.

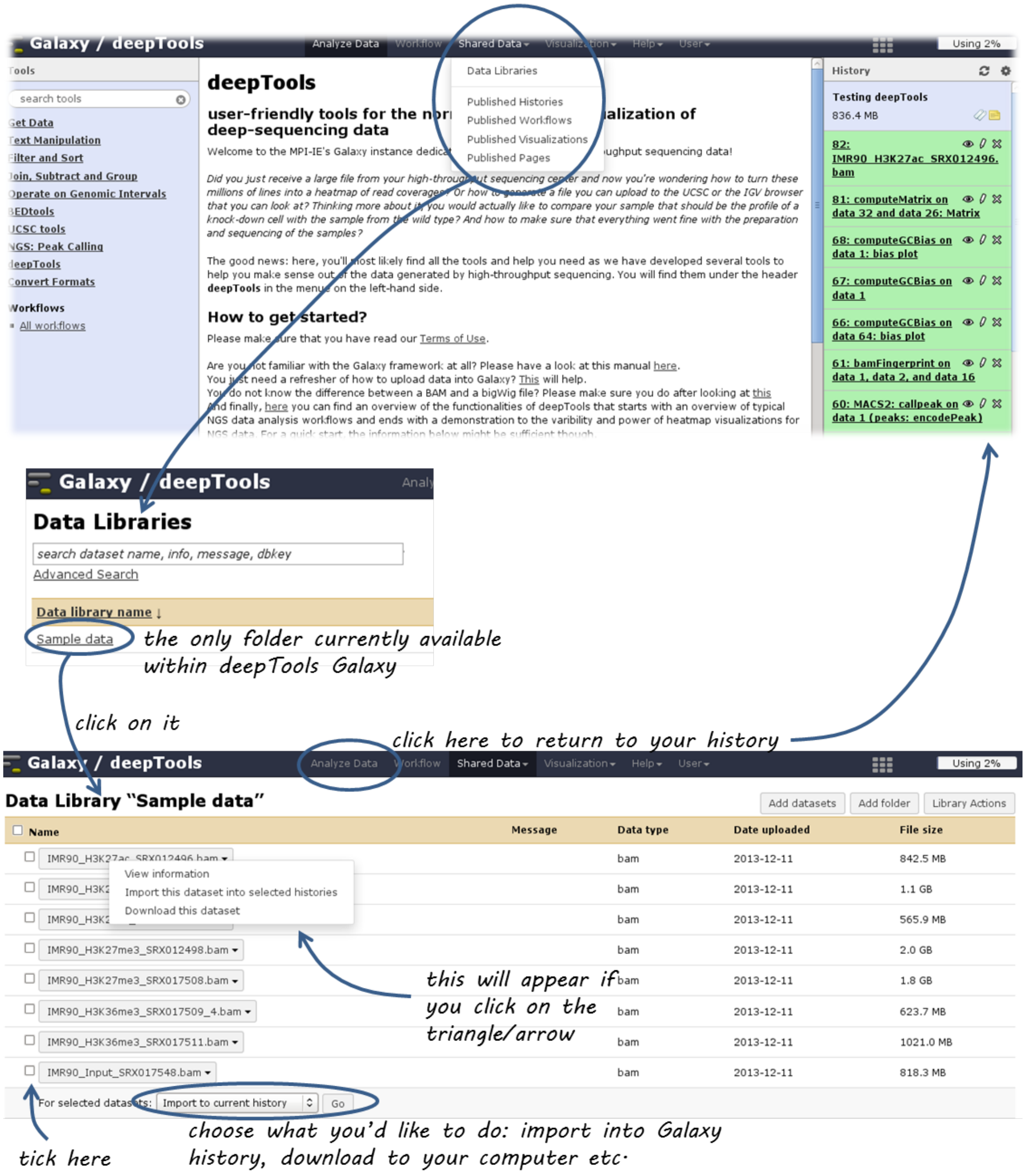
Import data sets from the Galaxy data library

If you would like to play around with sample data, you can import files that we have saved within the general data storage of the deepTools Galaxy server. Everyone can import them into his or her own history, they will not contribute to the user's disk quota.

You can reach the data library via "Shared Data" in the top menu, then select "Data Libraries".

Within the Data Library you will find a folder called "Sample Data" that contains data that we downloaded from the Roadmap project and UCSC More precisely, we donwloaded the [FASTQ][] files of various ChIP-seq samples and the corresponding input and mapped the reads to the human reference genome (version hg19) to obtain the [BAM][] files you see. In addition, you will find bigWig files created using `bamCoverage` and some annotation files in BED format as well as RNA-seq data.

Note: To keep the file size smallish, all files contain data for chromosome 19 and chromosome X only!


Galaxy / deepTools
Analyze Data
Workflow
Shared Data
Visualization
Help
User
Using 2%
Data Libraries
Published Histories
Published Workflows
Published Visualizations
Published Pages
Tools
search tools
Get Data
Text Manipulation
Filter and Sort
Join, Subtract and Group
Operate on Genomic Intervals
BEDtools
UCSC tools
NGS: Peak Calling
deepTools
Convert Formats
Workflows
All workflows
History
Testing deepTools
836.4 MB
82: IMR90_H3K27ac_SRX012496.bam
81: computeMatrix on data 32 and data 26: Matrix
68: computeGCBias on data 1: bias plot
67: computeGCBias on data 1
66: computeGCBias on data 64: bias plot
61: bamFingerprint on data 1, data 2, and data 16
60: MACS2: callpeak on data 1 (peaks: encodePeak)
Data Libraries
search dataset name, info, message, dbkey
Advanced Search
Data library name ↓
Sample data
the only folder currently available within deepTools Galaxy
click on it
click here to return to your history
Data Library "Sample data"
Add datasets
Add folder
Library Actions
View information
Import this dataset into selected histories
Download this dataset
this will appear if you click on the triangle/arrow
For selected datasets:
Import to current history
Go
tick here
choose what you'd like to do: import into Galaxy history, download to your computer etc.

deepTools

user-friendly tools for the nor alization of deep-sequencing data

Welcome to the MPI-IE's Galaxy instance dedica ughput sequencing data!

Did you just receive a large file from your high-throughput sequencing center and now you're wondering how to turn these millions of lines into a heatmap of read coverages? Or how to generate a file you can upload to the UCSC or the IGV browser that you can look at? Thinking more about it, you would actually like to compare your sample that should be the profile of a knock-down cell with the sample from the wild type? And how to make sure that everything went fine with the preparation and sequencing of the samples?

The good news: here, you'll most likely find all the tools and help you need as we have developed several tools to help you make sense out of the data generated by high-throughput sequencing. You will find them under the header **deepTools** in the menue on the left-hand side.

How to get started?

Please make sure that you have read our Terms of Use.

Are you not familiar with the Galaxy framework at all? Please have a look at this manual here.
You just need a refresher of how to upload data into Galaxy? This will help.
You do not know the difference between a BAM and a bigWig file? Please make sure you do after looking at this
And finally, here you can find an overview of the functionalities of deepTools that starts with an overview of typical NGS data analysis workflows and ends with a demonstration to the varibility and power of heatmap visualizations for NGS data. For a quick start, the information below might be sufficient though.

Name	Message	Data type	Date uploaded	File size
IMR90_H3K27ac_SRX012496.bam		bam	2013-12-11	842.5 MB
IMR90_H3K2		bam	2013-12-11	1.1 GB
IMR90_H3K2		bam	2013-12-11	565.9 MB
IMR90_H3K27me3_SRX012498.bam		bam	2013-12-11	2.0 GB
IMR90_H3K27me3_SRX017508.bam		bam	2013-12-11	1.8 GB
IMR90_H3K36me3_SRX017509_4.bam		bam	2013-12-11	623.7 MB
IMR90_H3K36me3_SRX017511.bam		bam	2013-12-11	1021.0 MB
IMR90_Input_SRX017548.bam		bam	2013-12-11	818.3 MB

Download annotation files from public data bases

In many cases you will want to query your sequencing data results for known genome annotation, such as genes, exons, transcription start sites etc. These information can be obtained via the two main sources of genome annotation, UCSC and BioMart.

> **Warning:** UCSC and BioMart cater to different ways of genome annotation, i.e. genes defined in UCSC might not correspond to the same regions in a gene file downloaded from BioMart. (For a brief overview over the issues of genome annotation, you can check out Wikipedia, if you always wanted to know much more about those issues, this might be a good start.)

You can access the data stored at UCSC or BioMart conveniently through our Galaxy instance which will import the resulting files into your history. Just go to **"Get data"** "UCSC" or "BioMart".

The majority of annotation files will probably be in [BED][] format, however, you can also find other data sets. UCSC, for example, offers a wide range of data that you can browse via the "group" and "track" menus (for example, you could download the GC content of the genome as a signal file from UCSC via the "group" menu ("Mapping and Sequencing Tracks").

> **Warning:** The download through this interface is limited to 100,000 lines per file which might not be sufficient for some mammalian data sets.

Here's a screenshot from downloading a BED-file of all RefSeq genes defined for the human genome (version hg19):

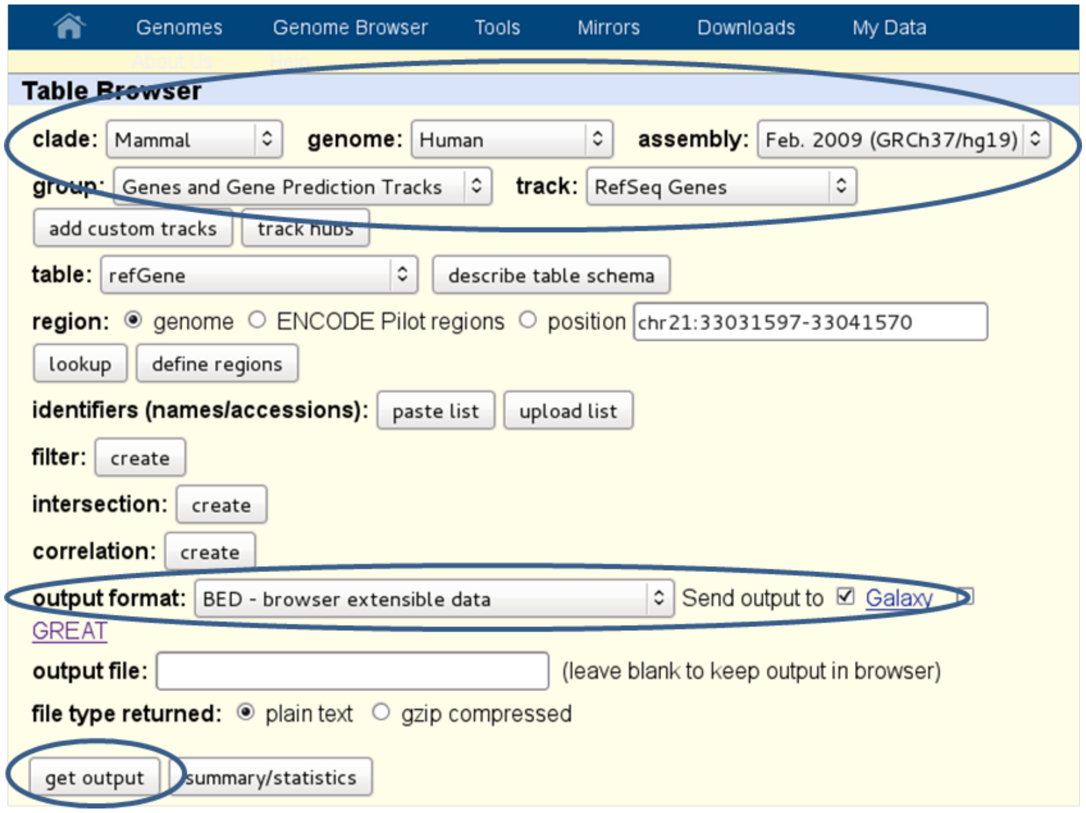


Table Browser

clade: Mammal genome: Human assembly: Feb. 2009 (GRCh37/hg19)

group: Genes and Gene Prediction Tracks track: RefSeq Genes

table: refGene

region: [✓ genome] ○ ENCODE Pilot regions ○ position chr21:33031597-33041570

identifiers (names/accessions):

filter: create

intersection: create

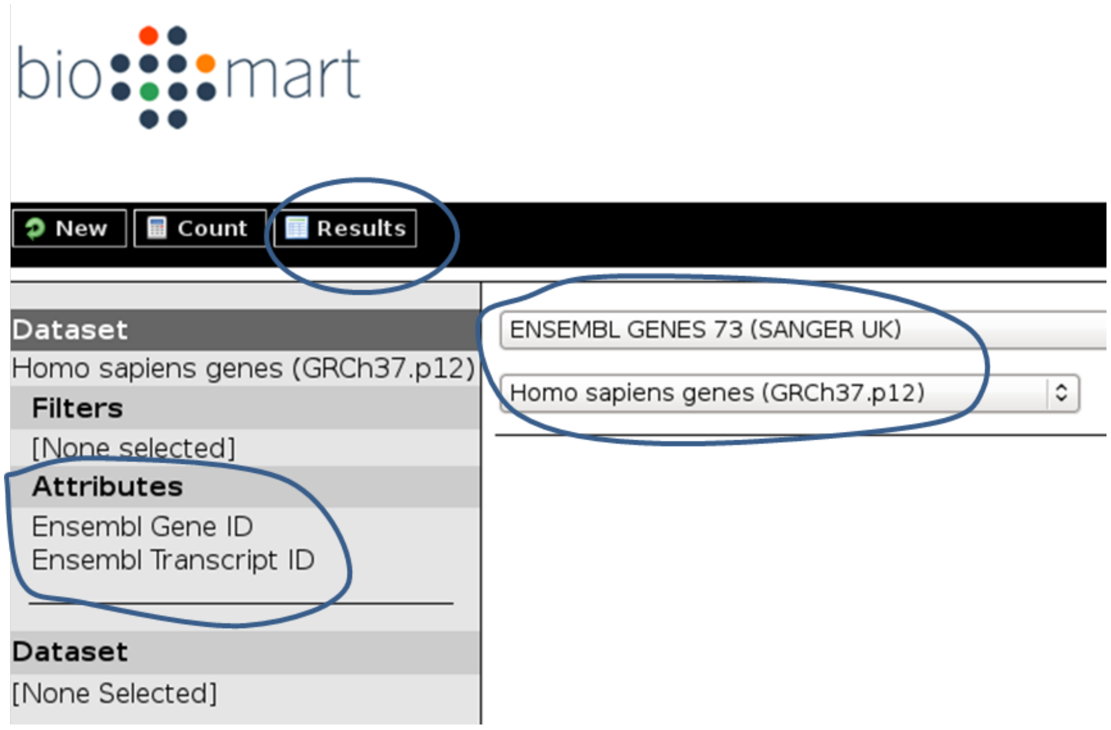
correlation: create

output format: BED - browser extensible data Send output to ☑ Galaxy ☐ GREAT

output file: (leave blank to keep output in browser)

file type returned: [✓ plain text] ○ gzip compressed

And here's how you would do it for the BioMart approach:



Tip: Per default, **BioMart will not output a BED file** like UCSC does. It is therefore important that you make sure you get all the information you need (most likely: chromosome, gene start, gene end, ID, strand) via the "Attributes" section. You can click on the "Results" button at any time to check the format of the table that will be sent to Galaxy (Note that the strand information will be decoded as 1 for "forward" or "plus" strand and -1 for "reverse" or "minus" strand).

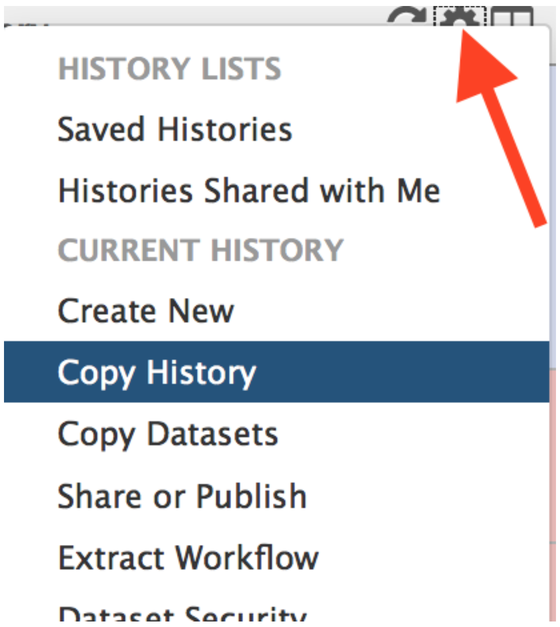
Warning: Be aware, that BED files from UCSC will have chromosomes labelled with "chr" while ENSEMBL usually returns just the number – this might lead to incompatibilities, i.e. when working with annotations from UCSC and ENSEMBL, you need to make sure to use the same naming!

Copy data sets between histories

If you have registered with deepTools Galaxy you can have more than one history.

In order to minimize the disk space you're occupying we strongly suggest to **copy** data sets between histories when you're using the same data set in different histories.

Note: Copying data sets is only possible for registered users.



Copying can easily be done via the History panel's `option` button "Copy dataset". In the main frame, you should now be able to select the history you would like to copy from on the left hand side and the target history on the right hand side.

More help

Hint: If you encounter a failing data set (marked in red), please **send a bug report** via the Galaxy bug report button and we will get in touch if you indicate your email address.

http://wiki.galaxyproject.org/Learn	Help for Galaxy usage in general
deepTools Galaxy FAQs	Frequently encountered issues with our specific Galaxy instance
deeptools@googlegroups.com	For issues not addressed in the FAQs

1.5.2 Which tools can I find in the deepTools Galaxy?

As mentioned before, each Galaxy installation can be tuned to the individual interests. Our goal is to provide a Galaxy that enables you to **quality check, process and normalize and subsequently visualize your data obtained by high-throughput DNA sequencing**.

Tip: If you do not know the difference between a BAM and a BED file, that's fine. You can read up on them in our Glossary of NGS terms.

Tip: For more specific help, check our Galaxy-related FAQ and the Step-by-step protocols.

We provide the following kinds of tools:

deepTools

The most important category is called **"deepTools"** that contains all the main tools we have developed.

Tools for BAM and bigWig file processing

`multiBamSummary`	get read counts for the binned genome or user-specified regions
`multiBigwigSummary`	calculate score summaries for the binned genome or user-specified regions
`correctGCBias`	obtain a BAM file with reads distributed according to the genome's GC content
`bamCoverage`	obtain the normalized read coverage of a single BAM file
`bamCompare`	normalize 2 BAM files to each other (e.g. log2ratio, difference)
`bigwigCompare`	normalize the scores of two bigWig files to each other (e.g., ratios)
`computeMatrix`	compute the values needed for heatmaps and summary plots

Tools for QC of NGS data

`plotCorrelation`	calculate and visualize the pairwise Spearman or Pearson correlation of read counts (or other scores)
`plotPCA`	perform PCA and visualize the results
`plotFingerprint`	assess the ChIP enrichment strength
`bamPEFragmentSize`	obtain the average fragment length for paired-end samples
`computeGCBias`	assess the GC bias by calculating the expected and observed GC distribution of aligned reads
`plotCoverage`	obtain the normalized read coverage of a single BAM file

Heatmaps and summary plots

`plotHeatmap`	visualize read counts or other scores in heatmaps with one row per genomic region
`plotProfile`	visualize read counts or other scores using average profiles (e.g., meta-gene profiles)

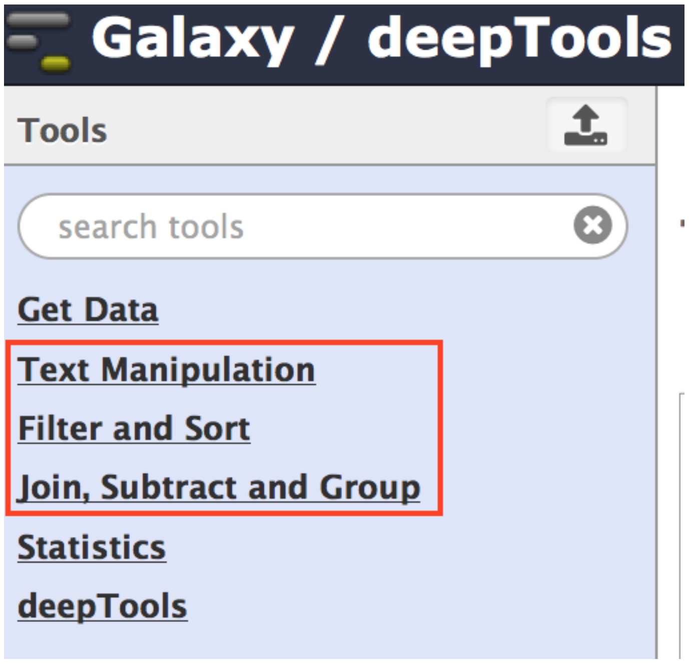
For each tool, you can find example usages and tips within Galaxy once you select the tool.

In addition, you may want to check our pages about Example usage, particularly Step-by-step protocols.

Working with text files and tables

In addition to deepTools that were specifically developed for the handling of NGS data, we have incorporated several standard Galaxy tools that enable you to manipulate tab-separated files such as gene lists, peak lists, data matrices etc.

There are 3 main categories;



Text manipulation

Unlike Excel, where you can easily interact with your text and tables via the mouse, data manipulations within Galaxy are strictly based on commands.

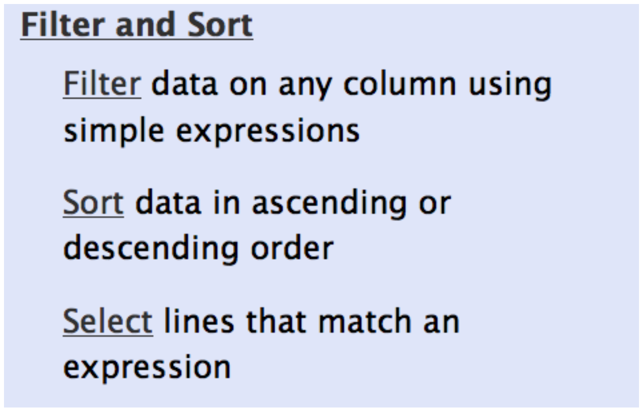
If you feel like you would like to do something to certain *columns* of a data set, go through the tools of this category!

Example actions are: * adding columns * extracting columns * pasting two files side by side * selecting random lines * etc.

A very useful tool of this category is called `Trim`: if you need to **remove some characters from a column**, this tool's for you! (for example, sometimes you need to adjust the chromosome naming between two files from different source - using `Trim`, you can remove the "chr" in front of the chromosome name)

Filter and Sort

In addition to the common sorting and filtering, there's the very useful tool to `select lines that match an expression`. For example, using the expression `c1=='chrM'` will select all rows from a BED file with regions located on the mitochondrial chromosome.


Filter and Sort

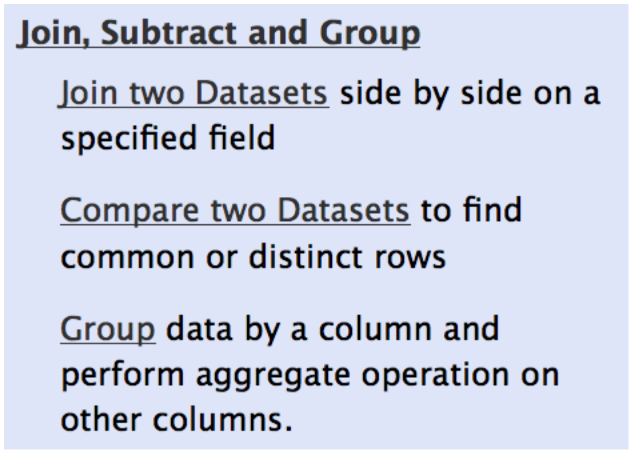
Filter data on any column using simple expressions

Sort data in ascending or descending order

Select lines that match an expression

Join, Subtract, Group

The tools of this category are very useful if you have several data sets that you would like to work with, e.g. by comparing them.


Join, Subtract and Group

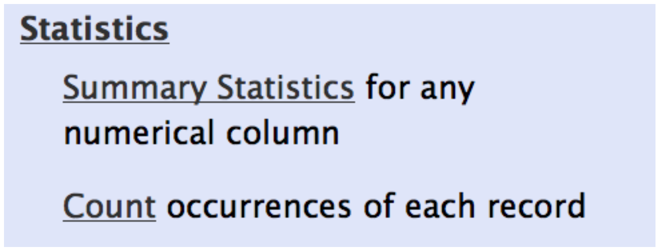
Join two Datasets side by side on a specified field

Compare two Datasets to find common or distinct rows

Group data by a column and perform aggregate operation on other columns.

Basic arithmetics for tables

We offer some very basic mathematical operations on values stored with tables. The `Summary Statistics` can be used to calculate the sum, mean, standard deviation and percentiles for a set of numbers, e.g. for values stored in a specific column.


Statistics

Summary Statistics for any numerical column

Count occurrences of each record

More help

Hint: If you encounter a failing data set (marked in red), please **send a bug report** via the Galaxy bug report button and we will get in touch if you indicate your email address.

http://wiki.galaxyproject.org/Learn	Help for Galaxy usage in general
deepTools Galaxy FAQs	Frequently encountered issues with our specific Galaxy instance
deeptools@googlegroups.com	For issues not addressed in the FAQs

1.5.3 Basic features of Galaxy

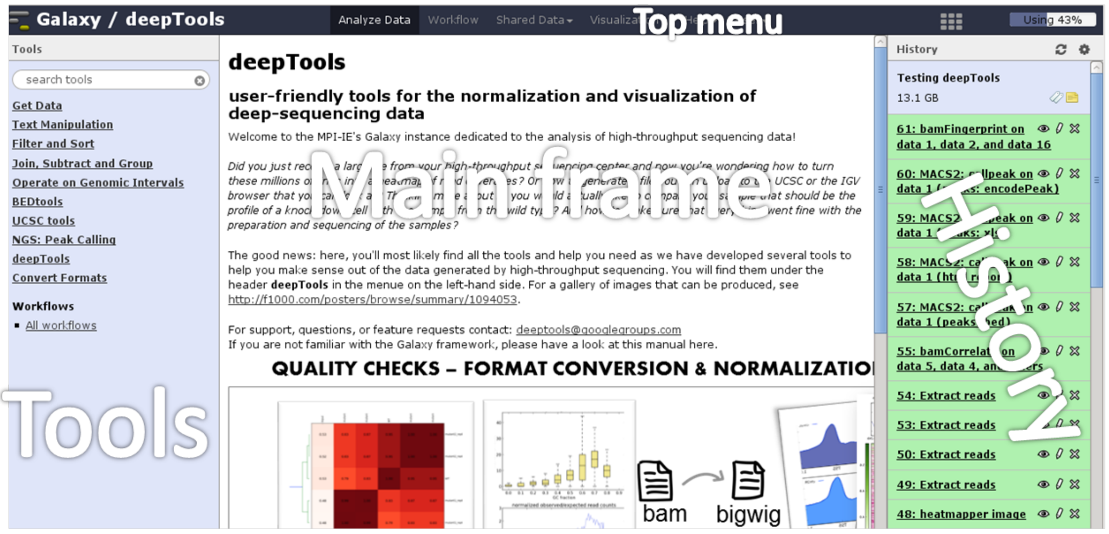
Galaxy is a web-based platform for data intensive, bioinformatics-dependent research and it is being developed by Penn State and John Hopkins University. The original Galaxy can be found here.

Since it is impossible to meet all bioinformatics needs – that can range from evolutionary analysis to data from mass spectrometry to high-throughput DNA sequencing (and way beyond) – with one single web server, many institutes have installed their own versions of the Galaxy platform tuned to their specific needs.

Our deepTools Galaxy is such a specialized server dedicated to the analysis of high-throughput DNA sequencing data. The overall makeup of this web server, however, is the same as for any other Galaxy installation, so if you've used Galaxy before, you will learn to use deepTools in no time!

The start site

Here is a screenshot of what the start site will approximately look like:



deepTools

user-friendly tools for the normalization and visualization of deep-sequencing data

Welcome to the MPI-IE's Galaxy instance dedicated to the analysis of high-throughput sequencing data!

Did you just rec[illegible] a larg[illegible] from your high-throughput s[illegible]uencing center and now you're wondering how to turn these millions o[illegible] UCSC or the IGV browser that yo[illegible] you w[illegible] hat should be the profile of a knoc[illegible] wild typ[illegible] went fine with the preparation and sequencing of the samples?

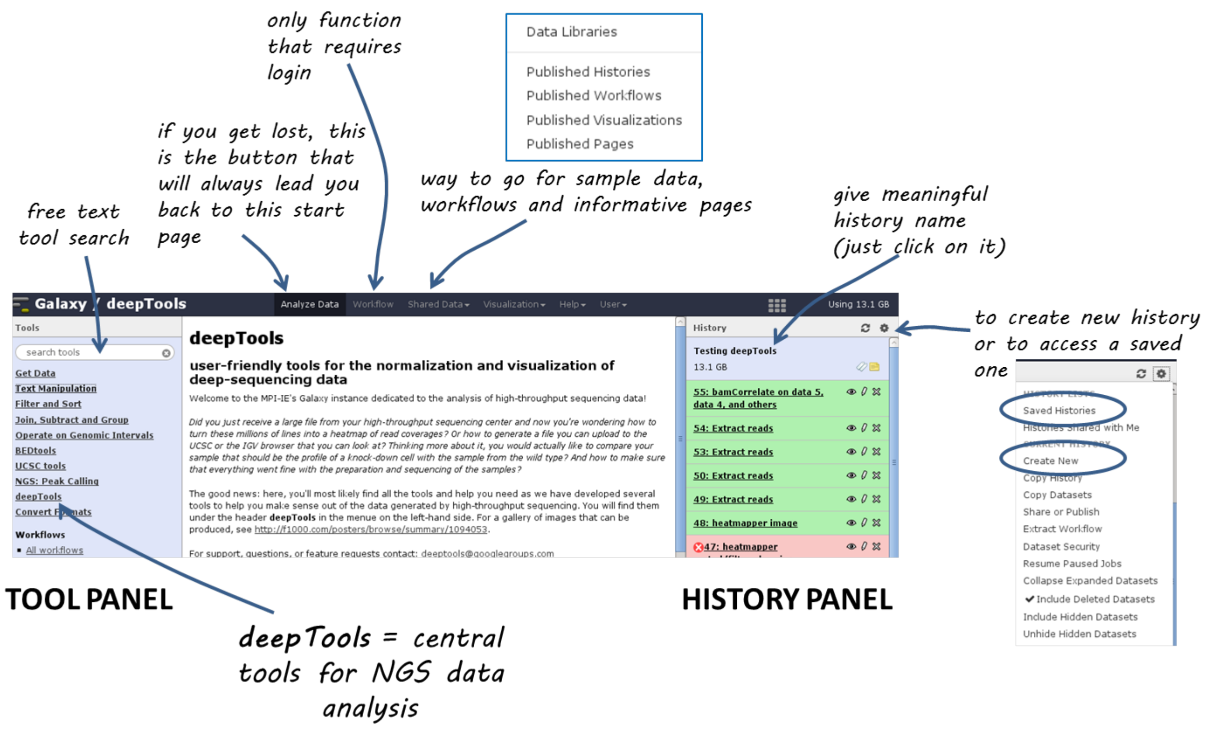
The good news: here, you'll most likely find all the tools and help you need as we have developed several tools to help you make sense out of the data generated by high-throughput sequencing. You will find them under the header **deepTools** in the menue on the left-hand side. For a gallery of images that can be produced, see http://f1000.com/posters/browse/summary/1094053.

For support, questions, or feature requests contact: deeptools@googlegroups.com
If you are not familiar with the Galaxy framework, please have a look at this manual here.

The start site contains 4 main features:

Top menu	Your gateway away from the actual analysis part into other sections of Galaxy, e.g. **workflows** and **shared data**.
Tool panel	*What can be done?* Find all tools installed in this Galaxy instance
Main frame	*What am I doing?* This is your main **working space** where input will be required from you once you've selected a tool.
History panel	*What did I do?* The history is like a **log book**: everything you ever did is recorded here.

For those visual learners, here's an annotated screenshot:



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Did you just receive a large file from your high-throughput sequencing center and now you're wondering how to turn these millions of lines into a heatmap of read coverages? Or how to generate a file you can upload to the UCSC or the IGV browser that you can look at? Thinking more about it, you would actually like to compare your sample that should be the profile of a knock-down cell with the sample from the wild type? And how to make sure that everything went fine with the preparation and sequencing of the samples?

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For support, questions, or feature requests contact: deeptools@googlegroups.com

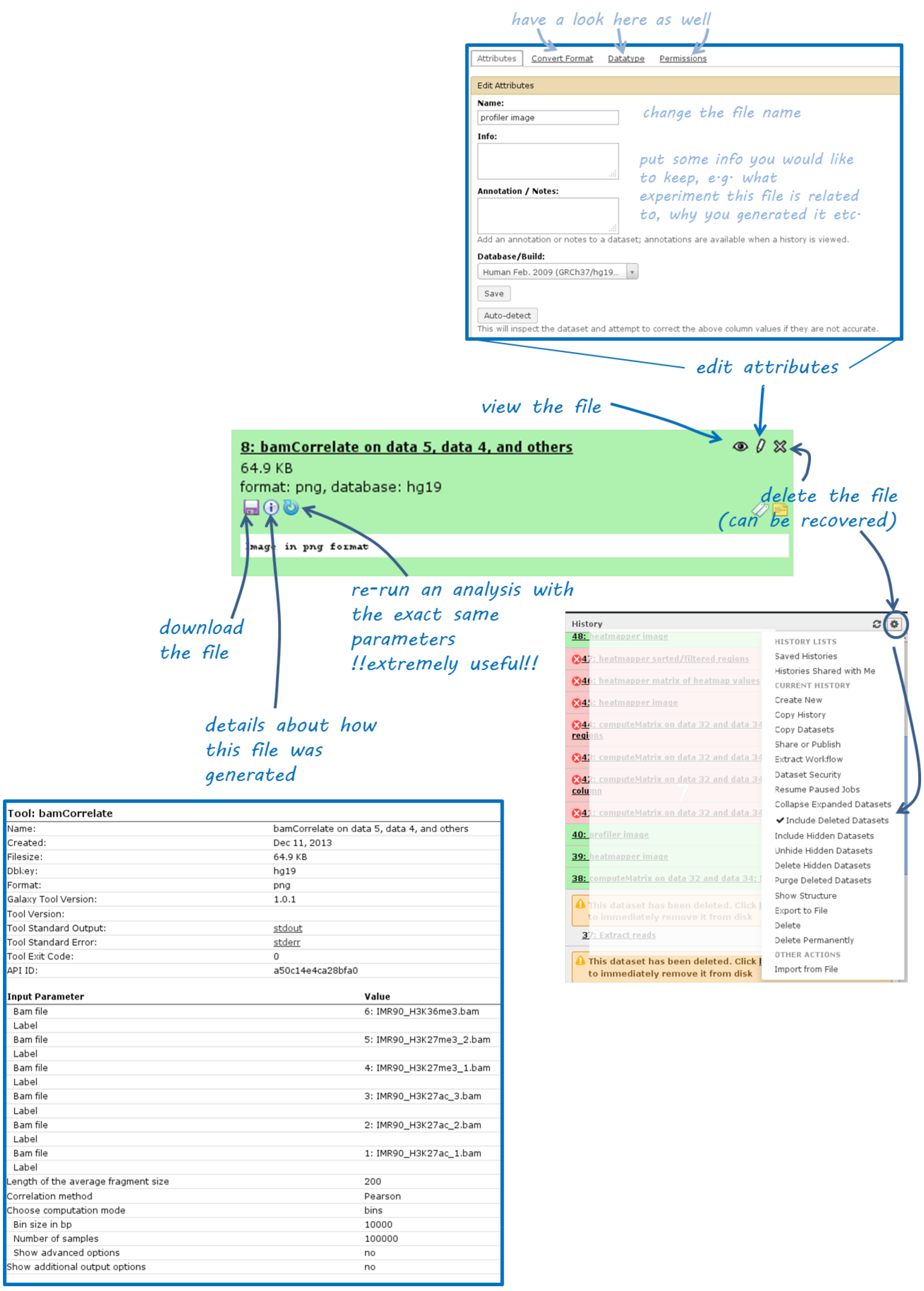
Details

In the default state of the tool panel you see the **tool categories**, e.g. “Get Data”. If you click on them, you will see the **individual tools** belonging to each category, e.g. “Upload File from your computer”, “UCSC Main table browser” and “Biomart central server” in case you clicked on “Get Data”. To use a tool such as “Upload File from your computer”, just click on it.

The **tool *search* panel** is extremely useful as it allows you to enter a key word (e.g. “bam”) that will lead to all the tools mentioning the key word in the tool name.

Once you’ve uploaded any kind of data, you will find the history on the right hand side filling up with green tiles. Each tile corresponds to one data set that you either uploaded or created. The data sets can be images, raw sequencing files, text files, tables - virtually anything. The content of a data set *cannot* be modified - every time you want to change something *within* a data file (e.g. you would like to sort the values or add a line or cut a column), you will have to use a Galaxy tool that will lead to a *new* data set being produced. This behaviour is often confusing for Galaxy novices (as histories tend to accumulate data sets very quickly), but it is necessary to enforce the strict policy of documenting *every modification* to a given data set. Eventhough your history might be full of data sets with strange names, you will always be able to track back the source and evolution of each file. Also, every data set can be downloaded to your computer individually. Alternatively, you can *download* an entire history or *share* the history with another user.

Have a look at the following screenshot to get a feeling for how many information Galaxy keeps for you (which makes it very feasible to reproduce any analysis):


have a look here as well
Attributes
Convert Format
Datatype
Permissions
change the file name
put some info you would like to keep, e.g. what experiment this file is related to, why you generated it etc.
edit attributes
view the file
delete the file (can be recovered)
download the file
re-run an analysis with the exact same parameters !!extremely useful!!
details about how this file was generated
History
HISTORY LISTS
Saved Histories
Histories Shared with Me
CURRENT HISTORY
Create New
Copy History
Copy Datasets
Share or Publish
Extract Workflow
Dataset Security
Resume Paused Jobs
Collapse Expanded Datasets
✓ Include Deleted Datasets
Include Hidden Datasets
Unhide Hidden Datasets
Delete Hidden Datasets
Purge Deleted Datasets
Show Structure
Export to File
Delete
Delete Permanently
OTHER ACTIONS
Import from File
48: heatmapper image
47: heatmapper sorted/filtered regions
46: heatmapper matrix of heatmap values
45: heatmapper image
44: computeMatrix on data 32 and data 34 regions
43: computeMatrix on data 32 and data 34
42: computeMatrix on data 32 and data 34 column
41: computeMatrix on data 32 and data 34
40: profiler image
39: heatmapper image
38: computeMatrix on data 32 and data 34
This dataset has been deleted. Click to immediately remove it from disk
37: Extract reads
This dataset has been deleted. Click to immediately remove it from disk

Edit Attributes

Name: profiler image

Info:

Annotation / Notes:

Add an annotation or notes to a dataset; annotations are available when a history is viewed.

Database/Build: Human Feb. 2009 (GRCh37/hg19...

Save

Auto-detect

This will inspect the dataset and attempt to correct the above column values if they are not accurate.

8: bamCorrelate on data 5, data 4, and others

64.9 KB

format: png, database: hg19

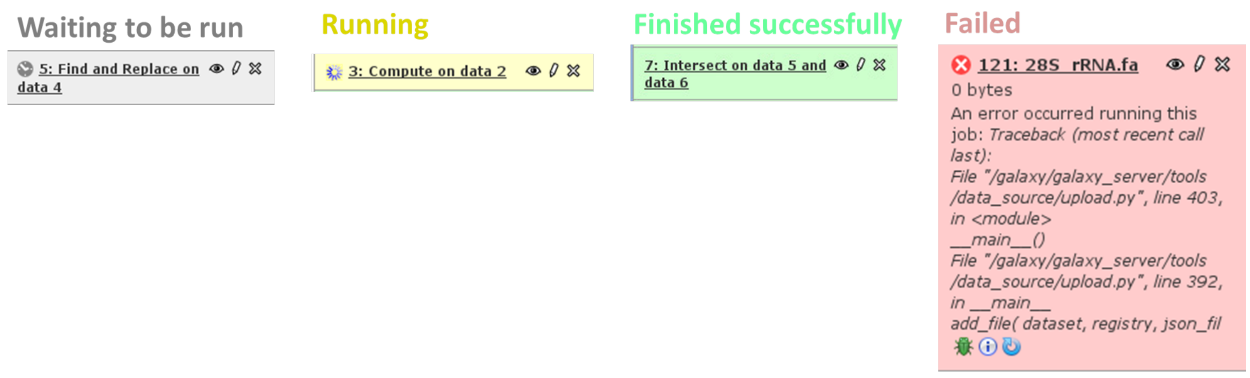
Image in png format

Tool: bamCorrelate

Name:	bamCorrelate on data 5, data 4, and others
Created:	Dec 11, 2013
Filesize:	64.9 KB
Dbkey:	hg19
Format:	png
Galaxy Tool Version:	1.0.1
Tool Version:	
Tool Standard Output:	stdout
Tool Standard Error:	stderr
Tool Exit Code:	0
API ID:	a50c14e4ca28bfa0

Input Parameter	Value
Bam file	6: IMR90_H3K36me3.bam
Label	
Bam file	5: IMR90_H3K27me3_2.bam
Label	
Bam file	4: IMR90_H3K27me3_1.bam
Label	
Bam file	3: IMR90_H3K27ac_3.bam
Label	
Bam file	2: IMR90_H3K27ac_2.bam
Label	
Bam file	1: IMR90_H3K27ac_1.bam
Label	
Length of the average fragment size	200
Correlation method	Pearson
Choose computation mode	bins
Bin size in bp	10000
Number of samples	100000
Show advanced options	no
Show additional output options	no

Each data set can have 4 different states that are intuitively color-coded:



0 bytes
An error occurred running this job: *Traceback (most recent call last):*
File "/galaxy/galaxy_server/tools/data_source/upload.py", line 403, in <module>
__main__()
File "/galaxy/galaxy_server/tools/data_source/upload.py", line 392, in __main__
add_file(dataset, registry, json_fil

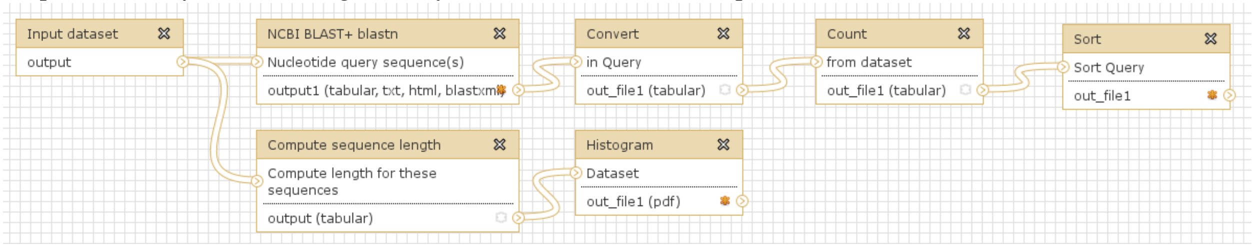
Handling failed files

If you encounter a failed file after you've run a tool, please do the following steps (**in this order**):

1. click on the center button on the lower left corner of the failed data set `(i)`: did you chose the **correct data files**?
2. if you're sure that you chose the correct files, hit the `re-run button` (blue arrow in the lower left corner) - check again whether your files had the **correct file format**. If you suspect that the format might be incorrectly assigned (e.g. a file that should be a BED file is labelled as a tabular file), click the `edit button` (the pencil) of the input data file - there you can change the corresponding attributes
3. if you've checked your input data and the error is persisting, click on the `green bug` (lower left corner of the failed data set) and send the **bug report** to us. You do not need to indicate a valid email-address unless you would like us to get in touch with you once the issue is solved.

Workflows

Workflows are Galaxy's equivalent of protocols. This is a very useful feature as it allows users to *share their protocols and bioinformatic analyses* in a very easy and transparent way. This is the graphical representation of a Galaxy workflow that can easily be modified via drag'n'drop within the workflows manual (you must be registered with deepTools Galaxy to be able to generate your own workflows or edit published ones).



More help

Hint: If you encounter a failing data set (marked in red), please **send a bug report** via the Galaxy bug report button and we will get in touch if you indicate your email address.

http://wiki.galaxyproject.org/Learn	Help for Galaxy usage in general
deepTools Galaxy FAQs	Frequently encountered issues with our specific Galaxy instance
deeptools@googlegroups.com	For issues not addressed in the FAQs

1.6 General FAQ

Below are issues we have frequently encountered. Feel free to contribute your questions via deeptools@googlegroups.com We also have a Galaxy-related FAQ.

1.6.1 How does deepTools handle data from paired-end sequencing?

Generally, all the modules working with [BAM] files (`multiBamSummary`, `bamCoverage`, `bamCompare`, `plotFingerprint`, `computeGCBias`) recognize paired-end sequencing data. You can by-pass the typical fragment handling on mate paires using the option `--doNotExtendPairedEnds` ("advanced options" in Galaxy).

1.6.2 How can I test a tool with little computation time?

When you're playing around with the tools to see what kinds of results they will produce, you can limit the operation to one chromosome or a specific region to save time. In Galaxy, you will find this under "advanced output options" &rarr; "Region of the genome to limit the operation to"; the command line option is called "–region" (CHR:START:END).

The following tools currently have this option:

- `tools/multiBamSummary`
- `tools/plotFingerprint`
- `tools/computeGCBias`, `tools/correctGCBias`
- `tools/bamCoverage`, `tools/bamCompare`

It works as follows: first, the *entire* genome represented in the *BAM* file will be regarded and sampled, *then* all the regions or sampled bins that do not overlap the region indicated by the user will be discarded.

Beware that you can limit the operation to only *one* chromosome (or *one* specific locus on a chromosome). If you would like to limit the operation to more than one region, see the next question.

1.6.3 Can I specify more than one chromosome in the `--regions` option?

Several programs allow specifying a specific regions. For these, the input must be in the format of `chr:start:end`, for example "chr10" or "chr10:456700:891000". For these programs, it is not possible to indicate more than one region, e.g. chr10, chr11 - **this will not work**!

Here are some ideas for workarounds if you none-the-less need to do this:

- **general workaround**: since all the tools that have the `--region` option work on BAM files, you could *filter your reads* prior to running the program, e.g. using `intersectBed` with `--abam` or `samtools view`. Then use the resulting (smaller) BAM file with the deepTools program of your choice.

```
samtools view -b -L regionsOfInterest.bed Reads.bam > ReadsOverlappingWithRegionsOfInterest.bam
```

or

```
intersectBed -abam Reads.bam -b regionsOfInterest.bed > ReadsOverlappingWithRegionsOfInterest.bam
```

However, `computeGCBias` and `multiBamSummary` do offer in-build solutions:

- **`multiBamSummary`**

  multiBamSummary has two modes, `bins` and `BED`. If you make use of the BED mode (as opposed to `bin`, wherein consecutive bins of equal size are used for the coverage calculation), you can supply a BED file of regions that you would like to limit the operation to. This will do the same thing as in the general workaround mentioned above.

- `computeGCBias`: You can make use of the `--filterOut` option of `tools/computeGCBias`. You will first need to create a BED file that contains all the regions you are **not** interested in. Then supply this file of RegionsOf__Non__Interest.bed to computeGCBias.

1.6.4 When should I exclude regions from computeGCBias?

In general, we recommend only correcting for GC bias (using `tools/computeGCBias` followed by `tools/correctGCBias`) if the majority of the genome (the region between 30-60%) is GC-biased *and* you want to compare this sample with another sample that is not GC-biased.

Sometimes, a certain GC bias is expected, for example for ChIP samples of H3K4Me3 in mammalian samples where GC-rich promoters are expected to be enriched. To not confound the GC bias caused by the library preparation with the inherent, expected GC-bias, we incorporated the possibility to supply a file of regions to `computeGCBias` that will be excluded from the GC bias calculation. This file should typically contain those regions that one expects to be significantly enriched. This allows `computeGCBias` to focus on background regions.

1.6.5 When should I use bamCoverage or bamCompare?

Both tools produce bigWig files, i.e. they translate the read-centered information from a *BAM* file into scores for genomic regions of a fixed size. The only difference is the *number of BAM files* that the tools use as input: while bamCoverage will only take one BAM file and produce a coverage file that is mostly normalized for sequencing depth, `bamCompare` will take *two BAM* files that can be compared with each other using several mathematical operations. bamCompare will always normalize for sequencing depth like bamCoverage, but then it will perform additional calculations depending on what the user chose, for example:

- **`bamCompare:`**
  - ChIP vs. *input* → obtain a bigWig file of log2ratios(ChIP/input)
  - treatment vs. control → obtain a bigWig file of differences (Treatment - control)
  - Replicate 1 and Replicate 2 → obtain a bigWig file where the values from two BAM files are summed up

1.6.6 How does computeMatrix handle overlapping genome regions?

If the *bed* file supplied to `tools/computeMatrix` contains regions that overlap, computeMatrix will report those regions and issue warnings, but they will just be taken as is. If you would like to prevent this, then clean the BED file before using computeMatrix. There are several methods for modifying your BED file. Let's say your file looks like this:

```
$ cat testBed.bed
chr1        10      20      region1
chr1        7       15      region2
chr1        18      29      region3
chr1        35      40      region4
chr1        10      20      region1Duplicate
```

- if you just want to eliminate *identical* entries (here: region1 and region1Duplicate), use sort and uniq in the shell (note that the label of the identical regions is different - as uniq can only ignore fields at the beginning of a file, use rev to revert the sorted file, then uniq with ignoring the first field (which is now the name column) and then revert back:

```
    $ sort -k1,1 -k2,2n testBed.bed | rev | uniq -f1 | rev
    chr1        10      20      region1
    chr1        7       15      region2
    chr1        18      29      region3
    chr1        35      40      region4
```

- if you would like to *merge all overlapping regions* into one big one, use the BEDtool mergeBed
  - again, the BED file must be sorted first
  - -n and -nms tell mergeBed to output the number of overlapping regions and the names of them
  - in the resulting file, regions 1, 2 and 3 are merged

```
        $ sort -k1,1 -k2,2n testBed.bed | mergeBed -i stdin -n -nms
        chr1      7       29      region2;region1;region1Duplicate;region3        4
        chr1      35      40      region4 1
```

- if you would like to *keep only regions that do not overlap* with any other region in the same *BED* file, use the same mergeBed routine but subsequently filter out those regions where several regions were merged
  - the awk command will check the last field of each line ($NF) and will print the original line ($0) only if the last field contained a number smaller than 2

```
        $ sort -k1,1 -k2,2n testBed.bed | mergeBed -i stdin -n -nms | awk '$NF < 2 {print $0}'
        chr1      35      40      region4 1
```

1.6.7 Why does the maximum value in the heatmap not equal the maximum value in the matrix?

Additional processing, such as outlier removal, is done on the matrix prior to plotting the heatmap. We've found this beneficial in most cases. You can override this by manually setting *–zMax* and/or *–zMin* appropriately.

1.6.8 The heatmap I generated looks very "coarse", I would like a much more fine-grained image.

- decrease the *bin size* when generating the matrix using `computeMatrix`

- go to "advanced options" –> "Length, in base pairs, of the non-overlapping *bin* for averaging the score over the regions length" –> define a smaller value, e.g. 50 or 25 bp

- make sure, however, that you used a sufficiently small *bin* size when calculating the bigWig file, though (if generated with deepTools, you can check the option "bin size")

1.6.9 How can I change the automatic labels of the clusters in a k-means clustered heatmap?

Each cluster will get its own box, exactly the same way as different groups of regions. Therefore, you can use the same option to define the labels of the final heatmap: In Galaxy: Heatmapper –> "Advanced output options" –> "Labels for the regions plotted in the heatmap".

If you indicated 3 clusters for k-means clustering, enter here: C1, C2, C3 –> instead of the full default label ("cluster 1"), the heatmap will be labeled with the abbreviations.

In the command line, use the `--regionsLabel` option to define your customized names.

1.6.10 How can I manually specify several groups of regions (instead of clustering)?

Simply specify multiple BED files (e.g., genes.bed, exons.bed and introns.bed). This works both in Galaxy and on the command line.

1.6.11 What do I have to pay attention to when working with a draft version of a genome?

If your genome isn't included in our standard dataset then you'll need the following:

1. **Effective genome size** - this is mostly needed for `bamCoverage` and `bamCompare`, see *below* for details
2. **Reference genome sequence in 2bit format** - this is needed for `computeGCBias`, see *2bit* for details

1.6.12 How do I calculate the effective genome size for an organism that's not in your list?

At the moment we do not provide a tool for this purpose, so you'll have to find a solution outside of deepTools for the time being.

The "real" effective genome size is the part of the genome that is *uniquely mappable*. This means that the value will depend on the genome properties (how many repetitive elements, quality of the assembly etc.) and the length of the sequenced reads as 100 million 36-bp-reads might cover less than 100 million 100-bp-reads.

We currently have these options for you:

1. Use an *external tool*
2. Use *faCount* (only if you let reads be aligned non-uniquely, too!)
3. Use *bamCoverage*
4. Use *genomeCoverageBed*

1. Use an external tool There is a tool that promises to calculate the mappability for any genome given the read length (k-mer length): GEM-Mappability Calculator . According to this reply here, you can calculate the effective genome size after running this program by counting the numbers of ”!” which stands for uniquely mappable regions. **2. Use faCount** If you are using bowtie2, which reports *multimappers* (i.e., *non-uniquely* mapped reads) as a default setting, you can use **faCount from UCSC tools** to report the total number of bases as well as the number of bases that are missing from the genome assembly indicated by 'N'. The effective genome size would then be the total number of base pairs minus the total number of 'N'. Here's an example output of faCount on *D. melanogaster* genome version dm3:

```
$ UCSCtools/faCount dm3.fa
#seq                len             A       C       G        T       N       cpg
chr2L               23011544        6699731 4811687 4815192  6684734 200     926264
chr2LHet    368872          90881   58504   57899    90588   71000   10958
chr2R               21146708        6007371 4576037 4574750  5988450 100     917644
chr2RHet    3288761          828553  537840   529242  826306  566820  99227
chr3L               24543557        7113242 5153576  5141498 7135141 100     995078
chr3LHet    2555491          725986  473888   479000  737434 139183  89647
chr3R               27905053        7979156 5995211  5980227 7950459 0       1186894
chr3RHet    2517507          678829  447155   446597  691725  253201  84175
chr4                1351857         430227  238155   242039  441336  100     43274
chrU                10049037        2511952 1672330  1672987 2510979 1680789 335241
chrUextra   29004656        7732998 5109465  5084891 7614402 3462900 986216
chrX                22422827        6409325 4742952  4748415 6432035 90100   959534
chrXHet             204112          61961   40017    41813   60321   0       754
chrYHet             347038          74566   45769    47582   74889   104232  8441
chrM                19517           8152    2003     1479    7883    0       132
total               168736537       47352930 33904589 33863611 47246682 6368725 6650479
```

In this example: Total no. bp = 168,736,537 Total no. 'N' = 6,368,725

NOTE: this method only works if multimappers are randomly assigned to their possible locations (in such cases the effective genome size is simply the number of non-N bases). **3. Use bamCoverage** If you have a sample where you expect the genome to be covered completely, e.g. from genome sequencing, a very trivial solution is to use bamCoverage with a bin size of 1 bp and the –outFileFormat option set to 'bedgraph'. You can then count the number of non-Zero bins (bases) which will indicate the mappable genome size for this specific sample. **4. Use genomeCoverageBed** The BEDtool genomeCoverageBed can be used to calculate the number of bases in the genome for which 0 reads can be found overlapping. As described on the BEDtools website (go to genomeCov description), you need:

- a file with the chromosome sizes of your sample's organism
- a position-sorted BAM file

```
bedtools genomecov -ibam sortedBAMfile.bam -g genome.size
```

1.6.13 Where can I download the 2bit genome files required for `computeGCBias`?

The 2bit files of most genomes can be found here. Search for the .2bit ending. Otherwise, **fasta files can be converted to 2bit** using the UCSC program faToTwoBit (available for different platforms from UCSC here).

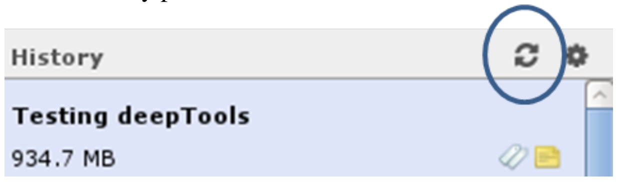
1.7 Galaxy-related FAQ

1.7.1 I've reached my quota - what can I do to save some space?

1. make sure that all the data sets you deleted are **permanently** eliminated from our disks: go to the history option button and select "Purge deleted data sets", then hit the "refresh" button on top of your history panel
2. download all data sets for which you've completed the analysis, then remove the data sets (click on the "x" and then **make sure they're purged** (see above))

1.7.2 Copying from one history to another doesn't work for me - the data set simply doesn't show up in the target history!

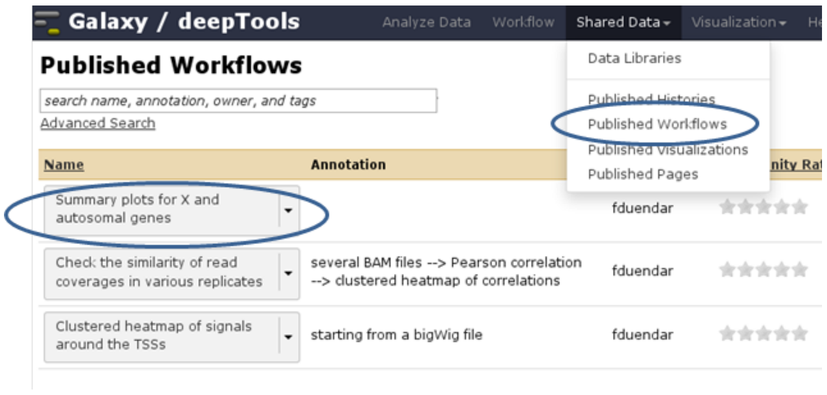
Once you've copied a data set from one history to another, check two things: * do you see the destination history in your history panel, i.e. does the title of the current history panel match the name of the destination history you selected in the main frame? * hit the refresh button



1.7.3 How can I use a published workflow?

You **must register** if you want to use the workflows within deepTools Galaxy. ("User" –> "Register" - all you have to supply is an email address)

You can find workflows that are public or specifically shared with you by another user via "Shared Data" –> "Published Workflows". Click on the triangle next to the workflow you're interested in and select "import".

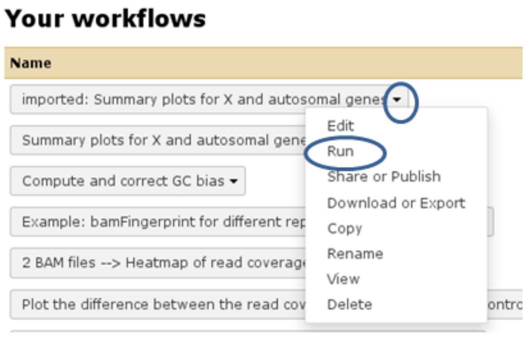


Published Workflows

search name, annotation, owner, and tags
Advanced Search

Name	Annotation	Owner
Summary plots for X and autosomal genes		fduendar
Check the similarity of read coverages in various replicates	several BAM files --> Pearson correlation --> clustered heatmap of correlations	fduendar
Clustered heatmap of signals around the TSSs	starting from a bigWig file	fduendar

A green box should appear, there you select "start using this workflow", which should lead you to your own workflow menu (that you can always access via the top menu "Workflow"). Here, you should now see a workflow labeled "imported: ....". If you want to use the workflow right away, click on the triangle and select "Run". The workflow should now be available within the Galaxy



Your workflows

Name
imported: Summary plots for X and autosomal genes
Summary plots for X and autosomal gene
Compute and correct GC bias
Example: bamFingerprint for different rep
2 BAM files --> Heatmap of read coverag
Plot the difference between the read cov

main data frame and should be waiting for your input.

1.7.4 I would like to use one of your workflows - not in the deepTools Galaxy, but in the local Galaxy instance provided by my institute. Is that possible?

Yes, it is possible. The only requirement is that your local Galaxy has a recent installation of deepTools.

Go to the workflows, click on the ones you're interested in and go to "Download". This will save the workflows into .ga files on your computer. Now go to your local Galaxy installation and login. Go to the workflow menu and select "import workflow" (top right hand corner of the page). Click on "Browse" and select the saved workflow. If you have the same tool versions installed in your local Galaxy, these workflows should work right away.

1.7.5 How can I have a look at the continuous read coverages from bigWig files? Which genome browser do you recommend?

There are 2 popular genome browsers for visualizing continuous data: UCSC and IGV.

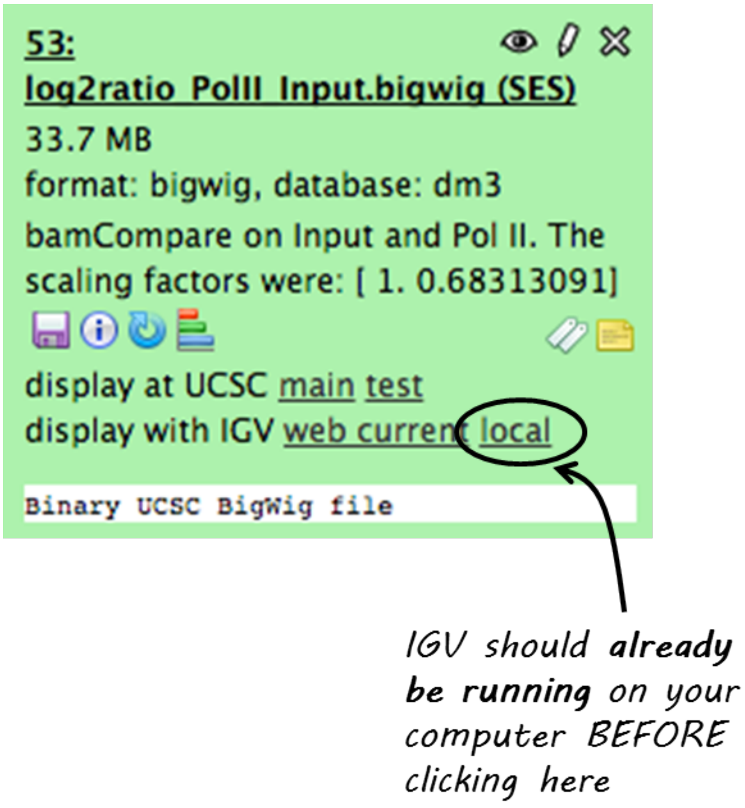
IGV (recommended)

We recommend downloading IGV, which is free for academic use. IGV itself needs an up-to-date Java installation and a considerable amount of RAM. It's usage is rather intuitive and the display can be easily customized. In addition, you can download genome-wide annotation data that can be displayed together with your own data.

To display data in IGV, do the following:

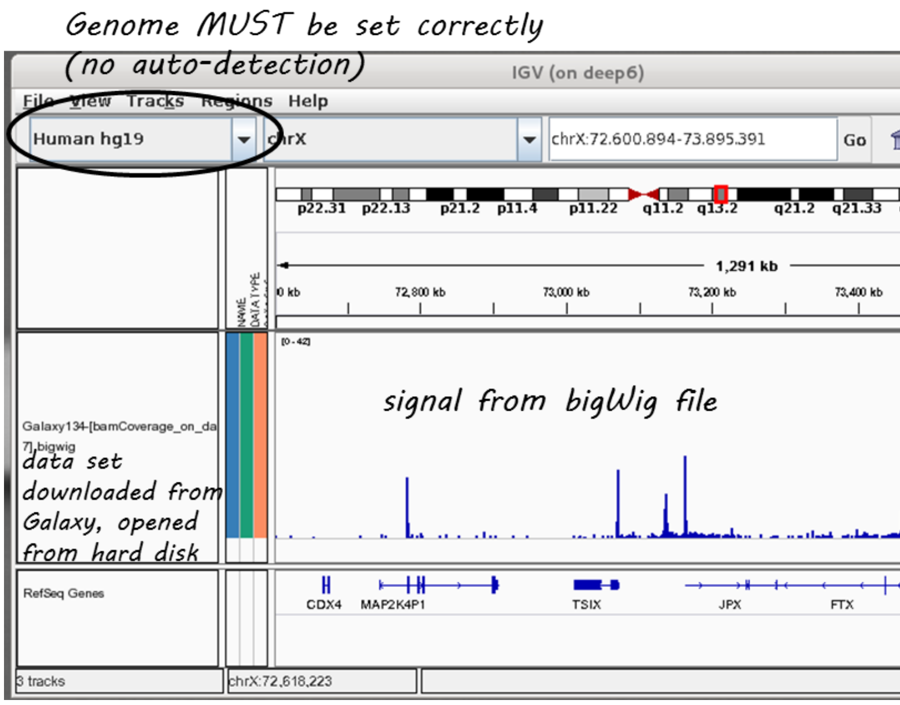
1. Go to http://www.broadinstitute.org/igv/, register and download IGV
2. Unpack the IGV archive and change to the extracted IGV folder

3. Use the igv.bat (Windows), igv.sh (Linux) or igv.command (OSX) to start IGV (for more information please read the included readme.txt file or the IGV documentation)
4. Choose the genome version of the file(s) you would like to visualize (e.g. dm3) THIS IS THE MOST IMPORTANT STEP! IGV will not detect the genome version automatically, i.e. if you select mm9 but your file is based on human data, it will still be displayed without an error message (but with the wrong positions, obviously!)
5. Go to your deepTools Galaxy server (http://deeptools.ie-freiburg.mpg.de/) and navigate to your data set of choice
6. Click on your data set so that you see its details like in the screenshot below (**Keep in mind that not all datasets can be visualized in IGV or UCSC.** We recommend to use *bigWig* or *bed* files for visualization.)


53:
log2ratio PolII Input.bigwig (SES)
33.7 MB
format: bigwig, database: dm3
bamCompare on Input and Pol II. The scaling factors were: [1. 0.68313091]
display at UCSC main test
display with IGV web current local
Binary UCSC BigWig file

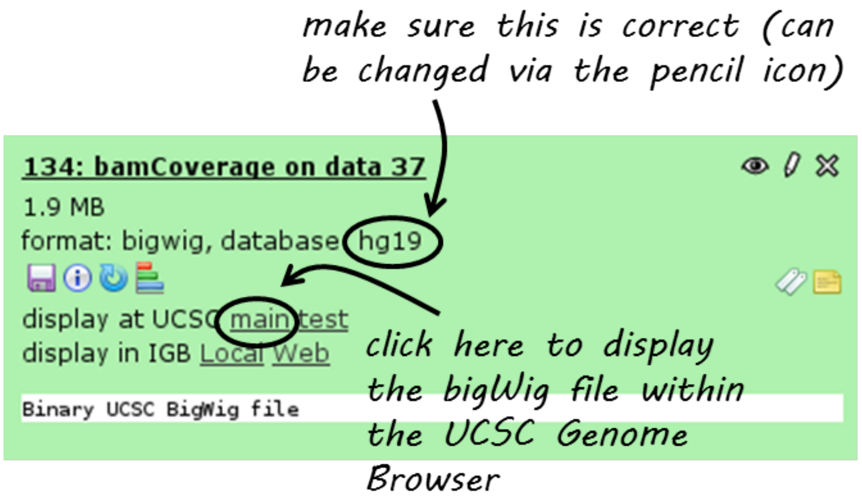
IGV should already be running on your computer BEFORE clicking here

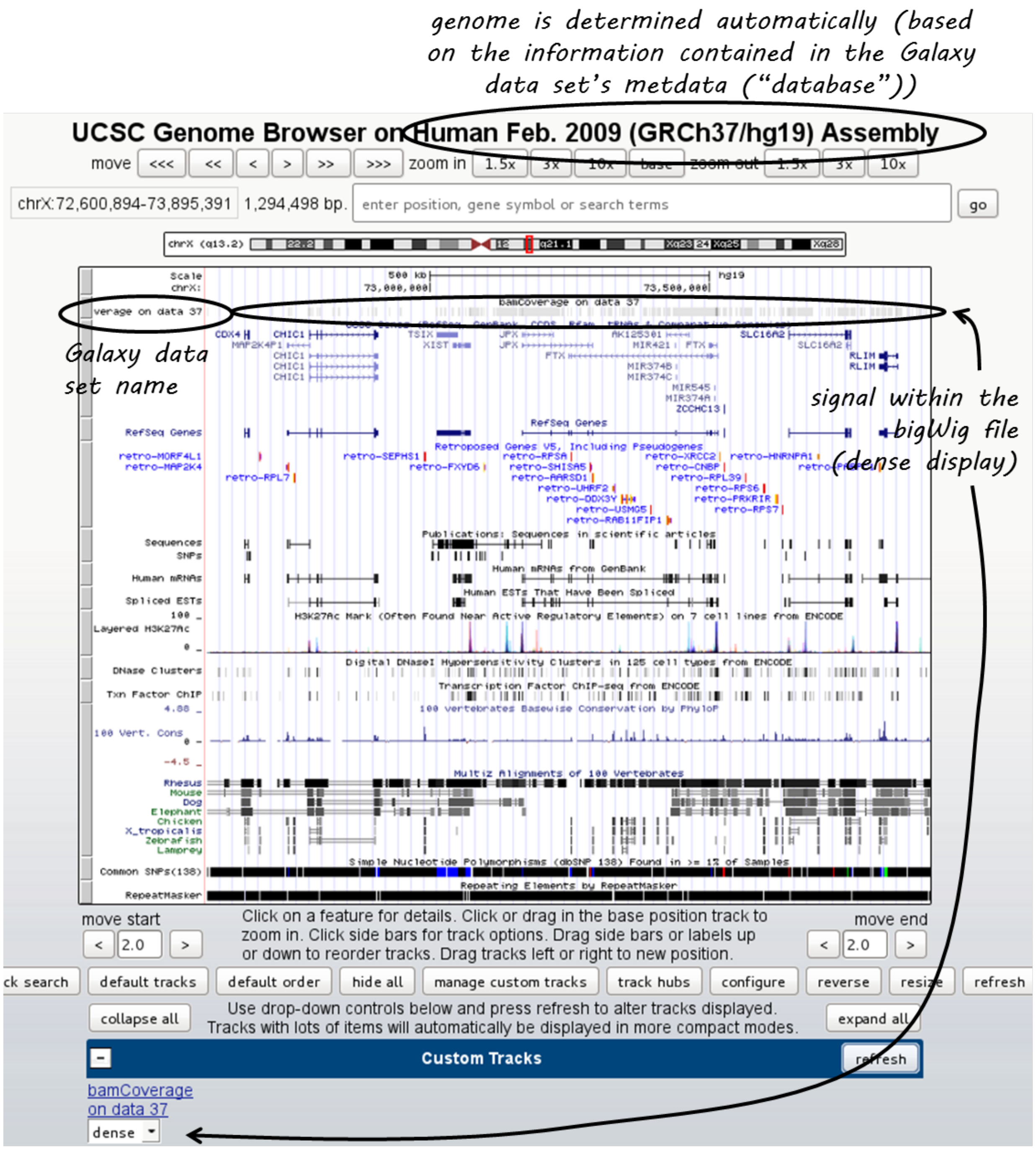
Now click on "display with IGV local" to visualize your data set in IGV that should already be running on your computer. *"display with IGV Web current" can be used if you do not have an installed IGV. It will start an IGV web start version. We do not recommend that option.*

Here's a screenshot of a typical bigWig file display: 
Genome MUST be set correctly (no auto-detection)
IGV (on deep6)
File View Tracks Regions Help
Human hg19
chrX
chrX:72.600.894-73.895.391
Go
p22.31 p22.13 p21.2 p11.4 p11.22 q11.2 q13.2 q21.2 q21.33
1,291 kb
72,800 kb
73,000 kb
73,200 kb
73,400 kb
[0 - 42]
signal from bigWig file
Galaxy134-[bamCoverage_on_da 7].bigwig
data set downloaded from Galaxy, opened from hard disk
RefSeq Genes
CDX4
MAP2K4P1
TSIX
JPX
FTX
3 tracks
chrX:72,618,223
For more information, check out the IGV documentation.

UCSC

There is a direct link from within deepTools Galaxy to stream a data set to UCSC. You can find it in the data set tiles:


make sure this is correct (can be changed via the pencil icon)
134: bamCoverage on data 37
1.9 MB
format: bigwig, database hg19
display at UCSC main test
display in IGB Local Web
Binary UCSC BigWig file
click here to display the bigWig file within the UCSC Genome Browser
"display at UCSC", like here:
Click on "main" and the UCSC browser should open within a new window, displaying the data set that you chose. The default setting for bigWig files is the "dense" display that looks like a heatmap.


genome is determined automatically (based on the information contained in the Galaxy data set's metdata ("database"))
UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x
chrX:72,600,894-73,895,391 1,294,498 bp.
enter position, gene symbol or search terms
go
verage on data 37
bamCoverage on data 37
Galaxy data set name
signal within the bigWig file (dense display)
RefSeq Genes
Sequences
SNPs
Human mRNAs
Spliced ESTs
Layered H3K27Ac
DNase Clusters
Txn Factor ChIP
100 Vert. Cons
Common SNPs(138)
RepeatMasker
move start
move end
Click on a feature for details. Click or drag in the base position track to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position.
ck search
default tracks
default order
hide all
manage custom tracks
track hubs
configure
reverse
resize
refresh
collapse all
Use drop-down controls below and press refresh to alter tracks displayed. Tracks with lots of items will automatically be displayed in more compact modes.
expand all
Custom Tracks
refresh
bamCoverage on data 37
dense

If you would like to display the continuous profile in a "valley-mountain" fashion like the one shown in the IGV screenshot, go to the drop-down menu underneath your custom track and choose "full".

UCSC has large amounts of public data that you can display which you can find by scrolling down the page, beyond your custom track entry. For more information on how to use the UCSC Genome Browser, go here.

Known issues with UCSC

- **chromosome naming**: UCSC expects chromosome names to be indicated in the format "chr"Number, e.g. chr1. If you mapped your reads to a non-UCSC-standard genome, chances are that chromosomes are labeled just with their number. bigWig files generated from these BAM files will not be recognized by UCSC, i.e. you will see the data set name, but no signal.

- **no upload of bigWig files from your hard drive**: to minimize the computational strains, UCSC relies on streaming bigWig files (i.e. there's no need to load the entire file at once, the browser will always just load the data for the specific region a user is looking at).

1.7.6 What's the best way to integrate the deepTools results with other downstream analyses (outside of Galaxy)?

- you can save all the data tables underlying every image produced by deepTools, i.e. if you would like to plot the average profiles in a different way, you could download the corresponding data (after ticking the profiler option at "advanced output options") and import them into R, Excel, GraphPadPrism etc.

1.7.7 How can I determine basic parameters of a BAM file, such as the number of reads, read length, duplication rate and average DNA fragment length?

Even though MACS is meant to do peak calling for you, it also outputs additional useful information, such as that listed above. Simply run MACS on the BAM file in question and check the .xls file from the MACS output. It will list:

- tag length = read length
- duplication rate
- number of tags = number of reads
- d = distance = average DNA fragment size

1.8 Glossary of NGS terms

Like most specialized fields, next-generation sequencing has inspired many an acronyms. We are trying to keep track of those *Abbreviations* that we heavily use. Do make us aware if something is unclear: deeptools@googlegroups.com

We have also assembled a short glossary of common *NGS and generic terminology* and *File Formats* of next-generation sequencing data.

1.8.1 Abbreviations

Reference genomes are usually referred to by their abbreviations, such as:

- hg19 = human genome, version 19
- mm9 = *Mus musculus* genome, version 9
- dm3 = *Drosophila melanogaster*, version 3
- ce10 = *Caenorhabditis elegans*, version 10

For a more comprehensive list of available reference genomes and their abbreviations, see the UCSC data base.

Acronym	full phrase	Synonyms/Explanation
<ANYTHING>-seq	sequencing	indicates that an experiment was completed by DNA sequencing using NGS
ChIP-seq	chromatin immunoprecipitation sequencing	NGS technique for detecting transcription factor binding sites and histone modifications (see entry *Input* for more information)
DNase	deoxyribonuclease I	DNase I digestion is used to determine active (“open”) chromatin regions
HTS	high-throughput sequencing	next-generation sequencing, massive parallel short read sequencing, deep sequencing
MNase	micrococcal nuclease	MNase digestion is used to determine sites with nucleosomes
NGS	next-generation sequencing	high-throughput (DNA) sequencing, massive parallel short read sequencing, deep sequencing
RPGC	reads per genomic content	normalize reads to 1x sequencing depth, sequencing depth is defined as: (mapped reads x fragment length) / effective genome size
RPKM	reads per kilobase per million reads	normalize read numbers: RPKM (per bin) = reads per bin / (mapped reads (in millions) x bin length (kb))

For a review of popular *-seq applications, see Zentner and Henikoff.

1.8.2 NGS and generic terminology

The following are terms that may be new to some:

bin

- synonyms: window, region
- A ‘bin’ is a subset of a larger grouping. Many calculations calculation are performed by first dividing the genome into small regions (bins), on which the calculations are actually performed.

Input

- Control experiment typically done for ChIP-seq experiments
- While ChIP-seq relies on antibodies to enrich for DNA fragments bound to a certain protein, the input sample should be processed exactly the same way, excluding the antibody. This allows one to account for biases introduced by sample handling and the general chromatin structure of the cells

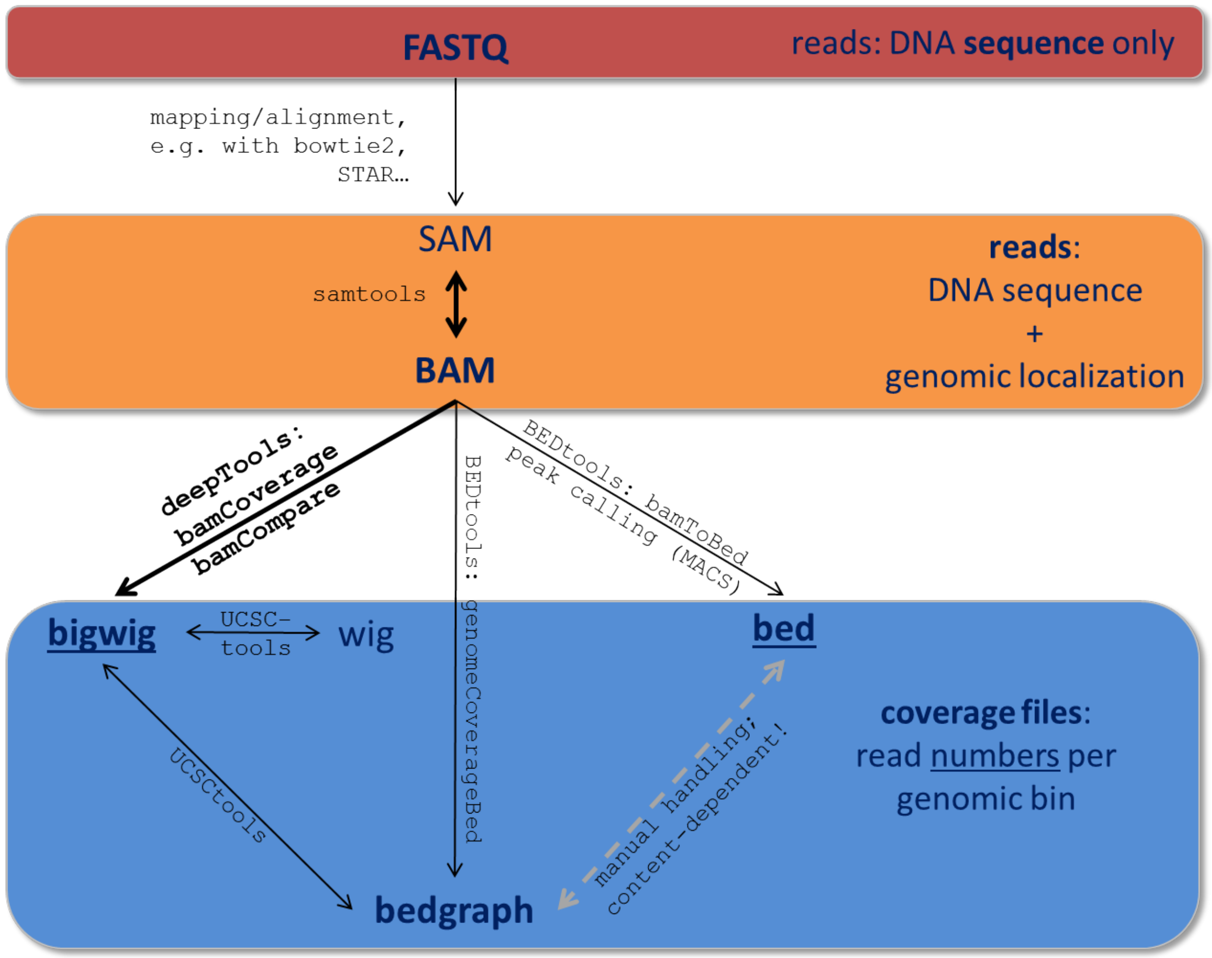
read

- synonym: tag
- This term refers to the piece of DNA that is sequenced (“read”) by the sequencers. We try to differentiate between “read” and “DNA fragment” as the fragments that are put into the sequencer tend to be in the range of 200-1000 bases, of which only the first 50 to 300 bases are typically sequenced. Most of the deepTools will not only take these reads into account, but also extend them to match the original DNA fragment size. (The original size will either be given by you or, if you used paired-end sequencing, be calculated from the distance between the two read mates).

1.8.3 File Formats

Data obtained from next-generation sequencing data must be processed several times. Most of the processing steps are aimed at extracting only that information needed for a specific down-stream analysis, with redundant entries often discarded. Therefore, **specific data formats are often associated with different steps of a data processing pipeline**.

Here, we just want to give very brief key descriptions of the file, for elaborate information we will link to external websites. Be aware, that the file name sorting here is alphabetical, not according to their usage within an analysis pipeline that is depicted here:


FASTQ
reads: DNA sequence only
mapping/alignment, e.g. with bowtie2, STAR...
SAM
samtools
BAM
reads: DNA sequence + genomic localization
deepTools: bamCoverage bamCompare
BEDtools: genomeCoverageBed
BEDtools: bamToBed
peak calling (MACS)
bigwig
UCSC-tools
wig
bed
UCSCtools
manual handling; content-dependent!
coverage files: read numbers per genomic bin
bedgraph

Follow the links for more information on the different tool collections mentioned in the figure:

samtools | UCSCtools | BEDtools |

2bit

- compressed, binary version of genome sequences that are often stored in *FASTA*
- most genomes in 2bit format can be found at UCSC
- *FASTA* files can be converted to 2bit using the UCSC programm *faToTwoBit*, which is available for different platforms at UCSC
- more information can be found here

BAM

- typical file extension: .bam
- *binary* file format (complement to *SAM*)
- contains information about sequenced reads (typically) *after alignment* to a reference genome

- **each line = 1 mapped read, with information about:**
  - its mapping quality (how likelihood that the reported alignment is correct)
  - its sequencing quality (the probability that each base is correct)
  - its sequence
  - its location in the genome
  - etc.
- highly recommended format for storing data
- to make a BAM file human-readable, one can, for example, use the program *samtools view*
- for more information, see below for the definition of *SAM* files

bed

- typical file extension: .bed
- text file
- used for genomic intervals, e.g. genes, peak regions etc.
- the format can be found at UCSC
- for deepTools, the first 3 columns are important: chromosome, start position of the region, end position of the genome
- do not confuse it with the *bedGraph* format (although they are related)
- example lines from a BED file of mouse genes (note that the start position is 0-based, the end-position 1-based, following UCSC conventions for BED files):

```
    chr1    3204562 3661579 NM_001011874 Xkr4   -
    chr1    4481008 4486494 NM_011441    Sox17  -
    chr1    4763278 4775807 NM_001177658 Mrpl15 -
    chr1    4797973 4836816 NM_008866    Lypla1 +
```

bedGraph

- typical file extension: .bg, .bedgraph
- text file
- similar to BED file (not the same!), it can *only* contain 4 columns and the 4th column *must* be a score
- again, read the UCSC description for more details
- 4 example lines from a bedGraph file (like BED files following the UCSC convention, the start position is 0-based, the end-position 1-based in bedGraph files):

```
chr1 10 20 1.5
chr1 20 30 1.7
chr1 30 40 2.0
chr1 40 50 1.8
```

bigWig

- typical file extension: .bw, .bigwig
- *binary* version of a *bedGraph* or *wiggle* file
- contains coordinates for an interval and an associated score
- the score can be anything, e.g. an average read coverage
- UCSC description for more details

FASTA

- typical file extension: .fasta
- text file, often gzipped (.fasta.gz)
- very simple format for **DNA/RNA** or **protein** sequences, this can be anything from small pieces of DNA or proteins to an entire genome (most likely, you will get the genome sequence of your organism of interest in fasta format)
- see the *2bit* file format entry for a compressed alternative
- example from wikipedia showing exactly one sequence:

```
>gi|5524211|gb|AAD44166.1| cytochrome b [Elephas maximus maximus]
 LCLYTHIGRNIYYGSYLYSETWNTGIMLLLITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLV
 EWIWGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLG
 LLILILLLLLLALLSPDMLGDPDNHMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSIVIL
 GLMPFLHTSKHRSMMLRPLSQALFWTLTMDLLTLTWIGSQPVEYPYTIIGQMASILYFSIILAFLPIAGX
 IENY
```

FASTQ

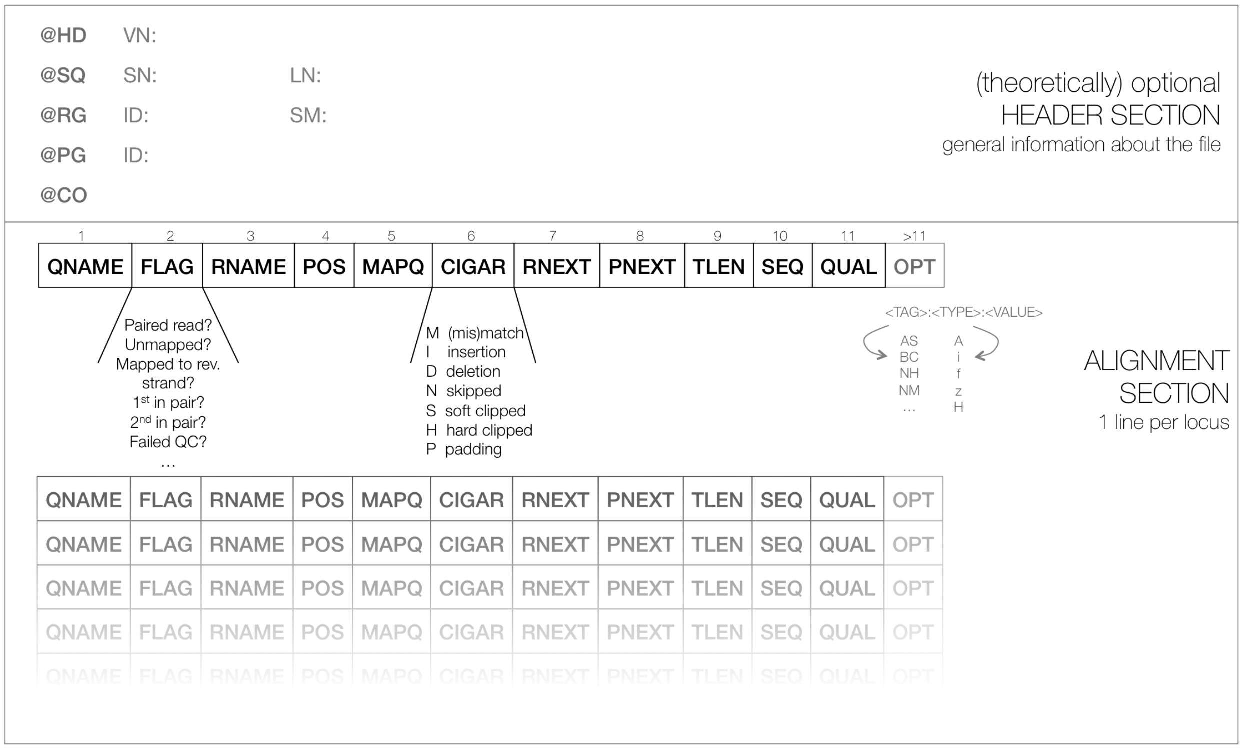
- typical file extension: .fastq, fq
- text file, often gzipped (–> .fastq.gz)
- **contains raw read information – 4 lines per read:**
  - read ID
  - base calls
  - additional information or empty line
  - sequencing quality measures - 1 per base call
- note that there is no information about where in the genome the read originated from
- example from the wikipedia page, which contains further information:

```
      @read001
      GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT        # read sequence
      +
      !''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65        # ASCII-encoded quality scor
```

- if you need to find out what type of ASCII-encoding your .fastq file contains, you can simply run FastQC – its summery file will tell you

SAM

- typical file extension: .sam
- usually the result of an alignment of sequenced reads to a reference genome
- contains a short header section (entries are marked by @ signs) and an alignment section where each line corresponds to a single read (thus, there can be millions of these lines)



- **header section**:
  - tab-delimited lines, beginning with @, followed by tag:value pairs
  - *tag* = two-letter string that defines the content and the format of *value*
- **alignment section**:
  - each line contains information about its mapping quality, its sequence, its location in the genome etc.

```
r001 163 chr1 7 30 8M2I4M1D3M = 37 39 TTAGATAAAGGATACTG *
r002 0 chr1 9 30 3S6M1P1I4M * 0 0 AAAAGATAAGGATA *
```

  - the **flag in the second field** contains the answer to several yes/no assessments that are encoded in a single number
  - for more details on the flag, see this thorough explanation or this more technical explanation
  - the **CIGAR string in the 6th field** represents the types of operations that were needed in order to align the read to the specific genome location:
    - insertion
    - deletion (small deletions denoted with *D*, bigger deletions, e.g., for spliced reads, denoted with *N*)
    - clipping (deletion at the ends of a read)

Warning: Although the SAM/BAM format is rather meticulously defined and documented, whether an alignment program will produce a SAM/BAM file that adheres to these principles is completely up to the programmer. The mapping score, CIGAR string, and particularly, **all optional flags** (fields >11) are often **very differently defined depending on the program**. If you plan on filtering your data based on any of these criteria, make sure you know exactly how these entries were calculated and set!

1.9 deepTools API

deepTools consists of several command line and Galaxy wrappers for summarizing the information of Next Generation Sequencing data that can be mapped to a reference genome. Through the API, the engine powering the deepTools commands can be used for other purposes as well.

Our deepTools API example explains step-by-step how to make use of some deepTools modules to achieve analyses outside the scope of the deepTools suite such as counting reads for certain genome regions and computing the FRiP score.

1.9.1 deepTools API example

The following is a short overview of the most useful methods and classes from deepTools. Complete information can be found in the following links: genindex and modindex

Finding read coverage over a region

With deepTools, the read coverage over multiple genomic regions and multiple files can be computed quite quickly using multiple processors. First, we start with a simple example that is later expanded upon to demonstrate the use of multipe processors. In this example we compute the coverage of reads over a small region for bins of 50bp. For this we need the *`deeptools.countReadsPerBin`* class.

```
import deeptools.countReadsPerBin
```

We also need a BAM file containing the aligned reads. The BAM file must be indexed to allow quick access to reads falling into the regions of interest.

```
bam_file = "file.bam"
```

Now, the `countReadsPerBin` object can be initialized. The first argument to the constructor is a list of BAM files, which in this case is just one file. We are going to use a `binLength` of 50 bases, with subsequent bins adjacent (i.e., the `stepSize` between bins is also 50 bases). Overlapping bin coverages can be used by setting a `stepSize` smaller than `binLength`.

```
cr = countReadsPerBin.CountReadsPerBin([bam_file], binLength=50, stepSize=50)
```

Now, we can compute the coverage over a region in chromosome 2 from position 0 to 1000.

```
cr.count_reads_in_region('chr2L', 0, 1000)
```

```
array([[ 2.],
       [ 3.],
       [ 1.],
       [ 2.],
       [ 3.],
       [ 2.],
       [ 4.],
       [ 3.],
       [ 2.],
       [ 3.],
       [ 4.],
       [ 6.],
       [ 4.],
       [ 2.],
       [ 2.],
       [ 1.]])
```

The result is a numpy array with one row per bin and one column per bam file. Since only one BAM file was used, there is only one column.

Filtering reads

If reads should be filtered, the relevant options simply need to be passed to the constructor. In the following code, the reads are filtered such that only those with a mapping quality of at least 20 and not aligned to the reverse strand are kept (samFlag_exclude=16, where 16 is the value for reverse reads, see the [SAM Flag Calculator](http://broadinstitute.github.io/picard/explain-flags.html) for more info). Furthermore, duplicated reads are ignored.

```
cr = countReadsPerBin.CountReadsPerBin([bam_file], binLength=50, stepSize=50,
                                        minMappingQuality=20,
                                        samFlag_exclude=16,
                                        ignoreDuplicates=True
                                        )
cr.count_reads_in_region('chr2L', 1000000, 1001000)
```

```
array([[ 1.],
       [ 1.],
       [ 0.],
       [ 0.],
       [ 0.],
       [ 0.],
       [ 2.],
       [ 3.],
       [ 1.],
       [ 0.],
       [ 1.],
       [ 2.],
       [ 0.],
       [ 0.],
       [ 1.],
       [ 2.],
       [ 1.],
       [ 0.],
       [ 0.],
       [ 0.]])
```

Sampling the genome

Instead of adjacent bins, as in the previous cases, a genome can simply be sampled. This is useful to estimate some values, like depth of sequencing, without having to look at the complete genome. In the following example, 10,000 positions of size 1 base are going to be queried from three bam files to compute the average depth of sequencing. For this, we set the *numberOfSamples* parameter in the object constructor. The *skipZeros* parameter is added to exclude regions lacking reads in all BAM files. The *run()* method is used instead of *count_reads_in_region*.

```
cr = countReadsPerBin.CountReadsPerBin([bam_file1, bam_file2, bam_file3],
                                        binLength=1, numberOfSamples=10000,
                                        numberOfProcessors=10,
                                        skipZeros=True)
sequencing_depth = cr.run()
print sequencing_depth.mean(axis=0)
```

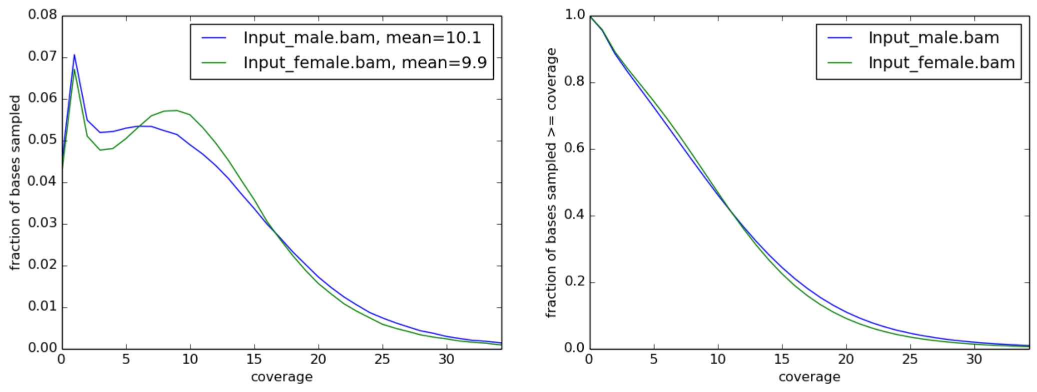
```
[  1.98923924   2.43743744  22.90102603]
```

The *run()* method splits the computation over 10 processors and collates the results. When the parameter *numberOfSamples* is used, the regions selected for the computation of the coverage are not random. Instead, the genome is split into 'number-of-samples' equal parts and the start of each part is queried for its coverage. You can also compute coverage over selected regions by inputting a BED file.

Now it is possible to make some diagnostic plots from the results:

```
fig, axs = plt.subplots(1, 2, figsize=(15,5))
# plot coverage
for col in res.T:
    axs[0].plot(np.bincount(col.astype(int)).astype(float)/total_sites)
    csum = np.bincount(col.astype(int))[::-1].cumsum()
    axs[1].plot(csum.astype(float)[::-1] / csum.max())
axs[0].set_xlabel('coverage')
axs[0].set_ylabel('fraction of bases sampled')
# plot cumulative coverage

axs[1].set_xlabel('coverage')
axs[1].set_ylabel('fraction of bases sampled >= coverage')
```



Computing the FRiP score

The FRiP score is defined as the fraction of reads that fall into a peak and is often used as a measure of ChIP-seq quality. For this example, we need a BED file containing the peak regions. Such files are usually computed using a peak caller. Also, two bam files are going to be used, corresponding to two biological replicates.

```
bed_file = open("peaks.bed", 'r')
cr = countReadsPerBin.CountReadsPerBin([bam_file1, bam_file2],
                                        bedFile=bed_file,
                                        numberOfProcessors=10)
reads_at_peaks = cr.run()
print reads_at_peaks
```

```
```

```
array([[ 322.,  248.],
       [ 231.,  182.],
       [ 112.,  422.],
       ...,
       [ 120.,   76.],
       [ 235.,  341.],
       [ 246.,  265.]])
```

The result is a numpy array with a row for each peak region and a column for each BAM file.

```
reads_at_peaks.shape
```

```
(6295, 2)
```

Now, the total number of reads per peaks per bam file is computed:

```
total = reads_at_peaks.sum(axis=0)
```

Next, we need to find the total number of mapped reads in each of the bam files. For this we use the pysam module.

```
import pysam
bam1 = pysam.AlignmentFile(bam_file1)
bam2 = pysam.AlignmentFile(bam_file2)
```

Now, *bam1.mapped* and *bam2.mapped* contain the total number of mapped reads in each of the bam files, respectively.

Finally, we can compute the FRiP score:

```
frip1 = float(total[0]) / bam1.mapped
frip2 = float(total[1]) / bam2.mapped
print frip1, frip2
```

```
0.170030741997, 0.216740390353
```

Using mapReduce to sample paired-end fragment lengths

deepTools internally uses a map-reduce strategy, in which a computation is split into smaller parts that are sent to different processors. The output from the different processors is subsequently collated. The following example is based on the code available for *bamPEFragmentSize.py*

Here, we retrieve the reads from a BAM file and collect the fragment length. Reads are retrieved using pysam, and the *read* object returned contains the *template_length* attribute, which is the number of bases from the leftmost to the rightmost mapped base in the read pair.

First, we will create a function that can collect fragment lengths over a genomic position from a BAM file. As we will later call this function using mapReduce, the function accepts only one argument, namely a tuple with the parameters: chromosome name, start position, end position, and BAM file name.

```
import pysam
import numpy as np
def get_fragment_length(args):
    chrom, start, end, bam_file_name = args
    bam = pysam.Aligmementfile(bam_file_name)
    f_lens_list = []
    for fetch_start in range(start, end, 1e6):
        # simply get the reads over a region of 10000 bases
        fetch_end = min(end, start + 10000)
        f_lens_list.append(np.array([abs(read.template_length)
                              for read in bam.fetch(chrom, fetch_start, fetch_end)
                              if read.is_proper_pair and read.is_read1]))

    # concatenate all results
    return np.concatenate(fragment_lengths)
```

Now, we can use *mapReduce* to call this function and compute fragment lengths over the whole genome. mapReduce needs to know the chromosome sizes, which can be easily retrieved from the BAM file. Furthermore, it needs to know the size of the region(s) sent to each processor. For this example, a region of 10 million bases is sent to each processor using the *genomeChunkLength* parameter. In other words, each processor executes the same *get_fragment_length* function to collect data over different 10 million base regions. The arguments to mapReduce are the list of arguments sent to the function, besides the first obligatory three (chrom start, end). In this case only one extra argument is passed to the function, the BAM file name. The next two positional arguments are the name of the function to call (*get_fragment_length*) and the chromosome sizes.

```
import deeptools.mapReduce
bam = pysam.Aligmentfile(bamFile)
chroms_sizes = zip(bam.references, bam.lengths)

result = mapReduce.mapReduce((bam_file_name, ),
                              get_fragment_length
                              chrom_sizes,
                              genomeChunkLength=10000000,
                              numberOfProcessors=20,
                              verbose=True)

fragment_lengths =  np.concatenate(result)

print "mean fragment length {}".format(fragment_lengths.mean()"
print "median fragment length {}".format(np.median(fragment_lengths)"
```

```
0.170030741997, 0.216740390353
```

Indices and tables

- genindex
- modindex
- search

1.9.2 deeptools package modules

deeptools.SES_scaleFactor module

class `deeptools.SES_scaleFactor.`**`Tester`**
    Bases: `object`

`deeptools.SES_scaleFactor.`**`estimateScaleFactor`**(*bamFilesList*, *binLength*, *numberOfSamples*, *normalizationLength*, *avg_method='median'*, *numberOfProcessors=1*, *verbose=False*, *chrsToSkip=[]*)
    Subdivides the genome into chunks to be analyzed in parallel using several processors. The code handles the

creation of workers that compute fragment counts (coverage) for different regions and then collect and integrates the results.

Parameters **bamFilesList** : list

list of bam files to normalize

binLength : int

the window size in bp, where reads are going to be counted.

numberOfSamples : int

number of sites to sample from the genome. For more info see the documentation of the CountReadsPerBin class

normalizationLength : int

length, in bp, to normalize the data. For a value of 1, on average 1 read per base pair is found

avg_method : str

defines how the different values are to be summarized. The options are 'mean' and 'median'

chrsToSkip : list

name of the chromosomes to be excluded from the scale estimation. Usually the chrX is included.

Returns dict

Dictionary with the following keys:: 'size_factors' 'size_factors_based_on_mapped_reads' 'size_factors_SES' 'size_factors_based_on_mean' 'size_factors_based_on_median' 'mean' 'meanSES' 'median' 'reads_per_bin' 'std' 'sites_sampled'

Examples

```
>>> test = Tester()
>>> bin_length = 50
>>> num_samples = 4
>>> _dict = estimateScaleFactor([test.bamFile1, test.bamFile2], bin_length, num_samples,  1)
>>> _dict['size_factors']
array([ 1. ,  0.5])
>>> _dict['size_factors_based_on_mean']
array([ 1. ,  0.5])
```

deeptools.bamHandler module

deeptools.bamHandler.**openBam**(*bamFile*)

deeptools.correctReadCounts module

deeptools.correctReadCounts.**computeCorrectedReadcounts**(*tileCoverage*, *args*)

This function is called by the writeBedGraph workers for every tile in the genome that is considered

It computes a pvalue based on an expected lambda comming from the correction of treatment when the input is considered.

`deeptools.correctReadCounts.`**`computeLambda`**(*tileCoverage*, *args*)

This function is called by the writeBedGraph workers for every tile in the genome that is considered

`deeptools.correctReadCounts.`**`computePvalue`**(*tileCoverage*, *args*)

This function is called by the writeBedGraph workers for every tile in the genome that is considered

It computes a pvalue based on an expected lambda comming from the correction of treatment when the input is considered.

`deeptools.correctReadCounts.`**`controlLambda`**(*tileCoverage*, *args*)

`deeptools.correctReadCounts.`**`correctReadCounts`**(*bamFilesList*, *binLength*, *numberOfSamples*, *defaultFragmentLength*, *outFileName*, *outFileFormat*, *outFileNameCorr=None*, *region=None*, *extendPairedEnds=True*, *numberOfProcessors=1*, *Nsigmas=2*, *maxSignalRatio=10*, *verbose=False*)

deeptools.correlation module

class `deeptools.correlation.`**`Correlation`**(*matrix_file*, *corr_method=None*, *labels=None*, *remove_outliers=False*, *skip_zeros=False*, *log1p=False*)

class to work with matrices having sample data to compute correlations, plot them and make scatter plots

`compute_correlation`()

computes spearman or pearson correlation for the samples in the matrix

The matrix should contain the values of each sample per column that's why the transpose is used.

```
>>> matrix = np.array([[1, 2, 3, np.nan],
...                     [1, 2, 3, 4],
...                     [6, 4, 3, 1]]).T
>>> np.savez_compressed("/tmp/test_matrix.npz", matrix=matrix, labels=['a', 'b', 'c'])
```

```
>>> c = Correlation("/tmp/test_matrix.npz", corr_method='pearson')
```

the results should be as in R

```
>>> c.compute_correlation().filled(np.nan)
array([[ 1.        ,  1.        , -0.98198051],
       [ 1.        ,  1.        , -0.98198051],
       [-0.98198051, -0.98198051,  1.        ]])
>>> c.corr_method = 'spearman'
>>> c.corr_matrix = None
>>> c.compute_correlation()
array([[ 1.,  1., -1.],
       [ 1.,  1., -1.],
       [-1., -1.,  1.]])
```

static **`get_outlier_indices`**(*data*, *max_deviation=200*)

The method is based on the median absolute deviation. See Boris Iglewicz and David Hoaglin (1993), "Volume 16: How to Detect and Handle Outliers", The ASQC Basic References in Quality Control: Statistical Techniques, Edward F. Mykytka, Ph.D., Editor.

returns the list, without the outliers

The max_deviation=200 is like selecting a z-score larger than 200, just that it is based on the median and the median absolute deviation instead of the mean and the standard deviation.

load_matrix(*matrix_file*)
loads a matrix file saved using the numpy savez method. Two keys are expected: 'matrix' and 'labels'. The matrix should contain one sample per row

plot_correlation(*plot_fiilename*, *plot_title=''*, *vmax=None*, *vmin=None*, *colormap='jet'*, *image_format=None*, *plot_numbers=False*)
plots a correlation using a symmetric heatmap

plot_pca(*plot_filename*, *plot_title=''*, *image_format=None*, *log1p=False*)
Plot the PCA of a matrix

plot_scatter(*plot_filename*, *plot_title=''*, *image_format=None*, *log1p=False*)
Plot the scatter plots of a matrix in which each row is a sample

remove_outliers(*verbose=True*)
get the outliers *per column* using the median absolute deviation method

Returns the filtered matrix

remove_rows_of_zeros()

save_corr_matrix(*file_handle*)
saves the correlation matrix

deeptools.correlation_heatmap module

deeptools.correlation_heatmap.**plot_correlation**(*corr_matrix*, *labels*, *plotFileName*, *vmax=None*, *vmin=None*, *colormap='jet'*, *image_format=None*, *plot_numbers=False*, *plot_title=''*)

deeptools.countReadsPerBin module

class deeptools.countReadsPerBin.**CountReadsPerBin**(*bamFilesList*, *binLength=50*, *numberOfSamples=None*, *numberOfProcessors=1*, *verbose=False*, *region=None*, *bedFile=None*, *extendReads=False*, *minMappingQuality=None*, *ignoreDuplicates=False*, *chrsToSkip=[]*, *stepSize=None*, *center_read=False*, *samFlag_include=None*, *samFlag_exclude=None*, *zerosToNans=False*, *smoothLength=0*, *out_file_for_raw_data=None*)
Bases: `object`

Collects coverage over multiple bam files using multiprocessing

This function collects read counts (coverage) from several bam files and returns an numpy array with the results. This class uses multiprocessing to compute the coverage.

Parameters **bamFilesList** : list

List containing the names of indexed bam files. E.g. ['file1.bam', 'file2.bam']

binLength : int

Length of the window/bin. This value is overruled by `bedFile` if present.

numberOfSamples : int

Total number of samples. The genome is divided into `numberOfSamples`, each with a window/bin length equal to `binLength`. This value is overruled by `stepSize` in case such value is present and by `bedFile` in which case the number of samples and bins are defined in the bed file

numberOfProcessors : int

Number of processors to use. Default is 4

verbose : bool

Output messages. Default: False

region : str

Region to limit the computation in the form chrom:start:end.

bedFile : file_handle

File handle of a bed file containing the regions for wich to compute the coverage. This option overrules `binLength`, `numberOfSamples` and `stepSize`.

extendReads : bool, int

Whether coverage should be computed for the extended read length (i.e. the region covered by the two mates or the regions expected to be covered by single-reads). If the value is 'int', then then this is interpreted as the fragment length to extend reads that are not paired. For Illumina reads, usual values are around 300. This value can be determined using the peak caller MACS2 or can be approximated by the fragment lengths computed when preparing the library for sequencing. If the value is of the variable is true and not value is given, the fragment size is sampled from the library but only if the library is paired-end. Default: False

minMappingQuality : int

Reads of a mapping quality less than the give value are not considered. Default: None

ignoreDuplicates : bool

Whether read duplicates (same start, end position. If paired-end, same start-end for mates) are to be excluded. Default: false

chrToSkip: list

List with names of chromosomes that do not want to be included in the coverage computation. This is useful to remove unwanted chromosomes (e.g. 'random' or 'Het').

stepSize : int

the positions for which the coverage is computed are defined as follows: `range(start, end, stepSize)`. Thus, a stepSize of 1, will compute the coverage at each base pair. If the stepSize is equal to the binLength then the coverage is computed for consecutive bins. If seepSize is smaller than the binLength, then teh bins will overlap.

center_read : bool

Determines if reads should be centered with respect to the fragment length.

samFlag_include : int

Extracts only those reads having the SAM flag. For example, to get only reads that are the first mates a samFlag of 64 could be used. Similarly, the samFlag_include can be used to select only reads mapping on the reverse strand or to get only properly paired reads.

samFlag_exclude : int

Removes reads that match the SAM flag. For example to get all reads that map to the forward strand a samFlag_exlude 16 should be used. Which translates into exclude all reads that map to the reverse strand.

zerosToNans : bool

If true, zero values encountered are transformed to Nans. Default false.

out_file_for_raw_data : str

File name to save the raw counts computed

Returns numpy array

Each row correspond to each bin/bed region and each column correspond to each of the bamFiles.

Examples

The test data contains reads for 200 bp.

```
>>> test = Tester()
```

The transpose function is used to get a nicer looking output. The first line corresponds to the number of reads per bin in bam file 1

```
>>> c = CountReadsPerBin([test.bamFile1, test.bamFile2], 50, 4)
>>> np.transpose(c.run())
array([[ 0.,  0.,  1.,  1.],
       [ 0.,  1.,  1.,  2.]])
```

count_reads_in_region(*chrom*, *start*, *end*, *bed_regions_list=None*)

Counts the reads in each bam file at each 'stepSize' position within the interval (start, end) for a window or bin of size binLength.

The stepSize controls the distance between bins. For example, a step size of 20 and a bin size of 20 will create bins next to each other. If the step size is smaller than the bin size the bins will overlap.

If a list of bedRegions is given, then the number of reads that overlaps with each region is counted.

Parameters **chrom** : str

Chrom name

start : int

start coordinate

end : int

end coordinate

bed_regions_list: list

List of tuples of the form (chrom, start, end) corresponding to bed regions to be processed. If not bed file was passed to the object constructor then this list is empty.

Returns numpy array

The result is a numpy array that as rows each bin and as columns each bam file.

Examples

Initialize some useful values

```
>>> test = Tester()
>>> c = CountReadsPerBin([test.bamFile1, test.bamFile2], 25, 0, stepSize=50)
```

The transpose is used to get better looking numbers. The first line corresponds to the number of reads per bin in the first bamfile.

```
>>> _array, __ = c.count_reads_in_region(test.chrom, 0, 200)
>>> _array
array([[ 0.,   0.],
       [ 0.,   1.],
       [ 1.,   1.],
       [ 1.,   2.]])
```

getReadLength(*read*)

getSmoothRange(*tileIndex*, *tileSize*, *smoothRange*, *maxPosition*)

Given a tile index position and a tile size (length), return the a new indices over a larger range, called the smoothRange. This region is centered in the tileIndex an spans on both sizes to cover the smoothRange. The smoothRange is trimmed in case it is less than zero or greater than maxPosition

```
---------------|==================|------------------
           tileStart
      |--------------------------------------|
      |    <--      smoothRange     -->      |
      |
tileStart - (smoothRange-tileSize)/2
```

Test for a smooth range that spans 3 tiles.

Examples

```
>>> c = CountReadsPerBin([], 1, 1, 1, 0)
>>> c.getSmoothRange(5, 1, 3, 10)
(4, 7)
```

Test smooth range truncated on start.

```
>>> c.getSmoothRange(0, 10, 30, 200)
(0, 2)
```

Test smooth range truncated on start.

```
>>> c.getSmoothRange(1, 10, 30, 4)
(0, 3)
```

Test smooth range truncated on end.

```
>>> c.getSmoothRange(5, 1, 3, 5)
(4, 5)
```

Test smooth range not multiple of tileSize.

```
>>> c.getSmoothRange(5, 10, 24, 10)
(4, 6)
```

get_coverage_of_region(*bamHandle, chrom, start, end, tileSize, fragmentFromRead_func=None*)

Returns a numpy array that corresponds to the number of reads that overlap with each tile.

```
>>> test = Tester()
>>> import pysam
>>> c = CountReadsPerBin([], stepSize=1, extendReads=300)
```

For this case the reads are length 36. The number of overlapping read fragments is 4 and 5 for the positions tested.

```
>>> c.get_coverage_of_region(pysam.AlignmentFile(test.bamFile_PE), 'chr2',
... 5000833, 5000835, 1)
array([ 4.,  5.])
```

In the following example a paired read is extended to the fragment length which is 100 The first mate starts at 5000000 and the second at 5000064. Each mate is extended to the fragment length *independently* At position 500090-500100 one fragment of length 100 overlap, and after position 5000101 there should be zero reads.

```
>>> c.zerosToNans = True
>>> c.get_coverage_of_region(pysam.AlignmentFile(test.bamFile_PE), 'chr2', 5000090, 5000110,
array([  1.,  nan])
```

In the following case the reads length is 50. Reads are not extended.

```
>>> c.extendReads=False
>>> c.get_coverage_of_region(pysam.AlignmentFile(test.bamFile2), '3R', 148, 154, 2)
array([ 1.,  2.,  2.])
```

get_fragment_from_read(*read*)

Get read start and end position of a read. If given, the reads are extended as follows: If reads are paired end, each read mate is extended to match the fragment length, otherwise, a default fragment length is used. If reads are split (give by the CIGAR string) then the multiple positions of the read are returned. When reads are extended the cigar information is skipped.

Parameters **read** : pysam read object

Returns list of tuples

[(fragment start, fragment end)]

```
>>> test = Tester()
```

```
>>> c = CountReadsPerBin([], 1, 1, 200, extendReads=True)
```

```
>>> c.defaultFragmentLength=100
```

```
>>> c.get_fragment_from_read(test.getRead("paired-forward"))
```

[(5000000, 5000100)]

```
>>> c.get_fragment_from_read(test.getRead("paired-reverse"))
```

[(5000000, 5000100)]

```
>>> c.defaultFragmentLength = 200
>>> c.get_fragment_from_read(test.getRead("single-forward"))
```

[(5001491, 5001691)]

```
>>> c.get_fragment_from_read(test.getRead("single-reverse"))
```

[(5001536, 5001736)]

```
>>> c.defaultFragmentLength = 'read length'
```

```
>>> c.get_fragment_from_read(test.getRead("single-forward"))
```

[(5001491, 5001527)]

```
>>> c.defaultFragmentLength = 'read length'
```

```
>>> c.extendReads = False
```

```
>>> c.get_fragment_from_read(test.getRead("paired-forward"))
```

[(5000000, 5000036)]

Tests for read centering.

```
>>> c = CountReadsPerBin([], 1, 1, 200, extendReads=True, center_read=True)
```

```
>>> c.defaultFragmentLength = 100
```

```
>>> c.get_fragment_from_read(test.getRead("paired-forward"))
```

[(5000032, 5000068)]

```
>>> c.defaultFragmentLength = 200
```

```
>>> c.get_fragment_from_read(test.getRead("single-reverse"))
```

[(5001618, 5001654)]

run()

class deeptools.countReadsPerBin.**Tester**

Bases: object

getRead(*readType*)

prepare arguments for test

deeptools.countReadsPerBin.**countReadsInRegions_wrapper**(*args*)

Passes the arguments to countReadsInRegions_worker. This is a step required given the constrains from the multiprocessing module. The args var, contains as first element the 'self' value from the countReadsPerBin object

deeptools.countReadsPerBin.**remove_row_of_zeros**(*matrix*)

deeptools.getFragmentAndReadSize module

deeptools.getFragmentAndReadSize.**getFragmentLength_worker**(*chrom*, *start*, *end*, *bamFile*)

Queries the reads at the given region for the distance between reads and the read length

Parameters **chrom** : str

chromosome name

start : int

region start

end : int

region end

bamFile : str

BAM file name

Returns np.array

an np.array, where first column is fragment length, the second is for read length

deeptools.getFragmentAndReadSize.**getFragmentLength_wrapper**(*args*)

deeptools.getFragmentAndReadSize.**get_read_and_fragment_length**(*bamFile*, *return_lengths=False*, *numberOfProcessors=None*, *verbose=False*)

Estimates the fragment length and read length through sampling

Parameters **bamFile** : str

BAM file name

return_lengths : bool

numberOfProcessors : int

verbose : bool

Returns **d** : dict

tuple of two dictionaries, one for the fragment length and the other for the read length. The dictionaries summarise the mean, median etc. values

deeptools.getRatio module

deeptools.getRatio.**compute_ratio**(*value1*, *value2*, *args*)

deeptools.getRatio.**getRatio**(*tileCoverage*, *args*)

The mapreduce method calls this function for each tile. The parameters (args) are fixed in the main method.

```
>>> funcArgs= {'valueType': 'ratio', 'scaleFactors': (1,1), 'pseudocount': 1}
>>> getRatio([9, 19], funcArgs)
0.5
>>> getRatio([0, 0], funcArgs)
1.0
>>> getRatio([np.nan, np.nan], funcArgs)
nan
```

```
>>> getRatio([np.nan, 1.0], funcArgs)
nan
>>> funcArgs['valueType'] ='subtract'
>>> getRatio([20, 10], funcArgs)
10
>>> funcArgs['scaleFactors'] = (1, 0.5)
>>> getRatio([10, 20], funcArgs)
0.0
```

The reciprocal ratio is of a and b is: is a/b if a/b > 1 else -1* b/a >>> funcArgs['valueType'] ='reciprocal_ratio' >>> funcArgs['scaleFactors'] = (1, 1) >>> funcArgs['pseudocount'] = 0 >>> getRatio([2, 1], funcArgs) 2.0 >>> getRatio([1, 2], funcArgs) -2.0 >>> getRatio([1, 1], funcArgs) 1.0

deeptools.getScorePerBigWigBin module

class `deeptools.getScorePerBigWigBin.`**`Tester`**

Bases: `object`

`deeptools.getScorePerBigWigBin.`**`countFragmentsInRegions_worker`**(*chrom*, *start*, *end*, *bigWigFiles*, *stepSize*, *binLength*, *save_data*, *bedRegions=None*)

returns the average score in each bigwig file at each 'stepSize' position within the interval start, end for a 'binLength' window. Because the idea is to get counts for window positions at different positions for sampling the bins are equally spaced and *not adjacent*.

If a list of bedRegions is given, then the number of reads that overlaps with each region is counted.

Test dataset with two samples covering 200 bp. >>> test = Tester()

Fragment coverage. >>> np.transpose(countFragmentsInRegions_worker(test.chrom, 0, 200, [test.bwFile1, test.bwFile2], 50, 25, False)[0]) array([[1., 1., 2., 2.],

[1., 1., 1., 3.]])

```
>>> np.transpose(countFragmentsInRegions_worker(test.chrom, 0, 200, [test.bwFile1, test.bwFile2]
array([[ 1.5],
       [ 1.5]])
```

BED regions: >>> bedRegions = [(test.chrom, 45, 55), (test.chrom, 95, 105), (test.chrom, 145, 155)] >>> np.transpose(countFragmentsInRegions_worker(test.chrom, 0, 200,[test.bwFile1, test.bwFile2], 200, 200, False, ... bedRegions=bedRegions)[0]) array([[1. , 1.5, 2.],

[1. , 1. , 2.]])

`deeptools.getScorePerBigWigBin.`**`countReadsInRegions_wrapper`**(*args*)

`deeptools.getScorePerBigWigBin.`**`getChromSizes`**(*bigwigFilesList*)

Get chromosome sizes from bigWig file with pyBigWig

Test dataset with two samples covering 200 bp. >>> test = Tester()

Chromosome name(s) and size(s). >>> getChromSizes([test.bwFile1, test.bwFile2]) ([('3R', 200L)], set([]))

`deeptools.getScorePerBigWigBin.`**`getScorePerBin`**(*bigWigFiles*, *binLength*, *numberOfProcessors=1*, *verbose=False*, *region=None*, *bedFile=None*, *stepSize=None*, *chrsToSkip=[]*, *out_file_for_raw_data=None*)

This function returns a matrix containing scores (median) for the coverage of fragments within a region. Each row corresponds to a sampled region. Likewise, each column corresponds to a bigwig file.

Test dataset with two samples covering 200 bp. >>> test = Tester() >>> np.transpose(getScorePerBin([test.bwFile1, test.bwFile2], 50, 3)) array([[1., 1., 2., 2.],

[1., 1., 1., 3.]])

deeptools.heatmapper module

`deeptools.heatmapper.`**`compute_sub_matrix_wrapper`**(*args*)

class `deeptools.heatmapper.`**`heatmapper`**

Bases: `object`

Class to handle the reading and plotting of matrices.

static **`change_chrom_names`**(*chrom*)

Changes UCSC chromosome names to ensembl chromosome names and vice versa.

`computeMatrix`(*score_file_list*, *regions_file*, *parameters*, *verbose=False*)

Splits into multiple cores the computation of the scores per bin for each region (defined by a hash '#' in the regions (BED/GFF) file.

static **`compute_sub_matrix_worker`**(*score_file_list*, *regions*, *parameters*)

Parameters **score_file_list** : list

List of strings. Contains the list of bam or bigwig files to be used.

regions : list of dictionaries

Each item in the list is a dictionary containing containing the fields: 'chrom', 'start', 'end', 'name' and 'strand.

parameters : dict

Contains values that specify the length of bins, the number of bp after a reference point etc.

Returns numpy matrix

A numpy matrix that contains per each row the values found per each of the regions given

static **`coverage_from_array`**(*valuesArray*, *zones*, *binSize*, *avgType*)

static **`coverage_from_bam`**(*bamfile*, *chrom*, *zones*, *binSize*, *avgType*, *verbose=True*)

currently this method is deactivated because is too slow. It is preferred to create a coverage bigiwig file from the bam file and then run heatmapper.

static **`coverage_from_big_wig`**(*bigwig*, *chrom*, *zones*, *binSize*, *avgType*, *nansAsZeros=False*, *verbose=True*)

uses bigwig file reader from bx-python to query a region define by chrom and zones. The output is an array that contains the bigwig value per base pair. The summary over bins is done in a later step when coverage_from_array is called. This method is more reliable than quering the bins directly from the bigwig, which should be more efficient.

By default, any region, even if no chromosome match is found on the bigwig file, produces a result. In other words no regions are skipped.

zones: array as follows zone0: region before the region start,

zone1: the body of the region (not always present) zone2: the region from the end of the region downstream

each zone is a tuple containing start, end, and number of bins

This is useful if several matrices wants to be merged or if the sorted BED output of one computeMatrix operation needs to be used for other cases

`get_individual_matrices`(*matrix*)
In case multiple matrices are saved one after the other this method splits them appart. Returns a list containing the matrices

`get_num_individual_matrix_cols`()
returns the number of columns that each matrix should have. This is done because the final matrix that is plotted can be composed of smaller matrices that are merged one after the other.

static `get_regions_and_groups`(*regions_file*, *onlyMultiplesOf=1*, *default_group_name='genes'*, *verbose=None*)
Reads a bed file. In case is hash sign '#' is found in the file, this is considered as a delimiter to split the heatmap into groups

Returns a list of regions with a label index appended to each and a list of labels

static `matrix_avg`(*matrix*, *avgType='mean'*)

`matrix_from_dict`(*matrixDict*, *regionsDict*, *parameters*)

static `my_average`(*valuesArray*, *avgType='mean'*)
computes the mean, median, etc but only for those values that are not Nan

`read_matrix_file`(*matrix_file*, *verbose=None*, *default_group_name='label_1'*)

`saveTabulatedValues`(*file_handle*)

`save_BED`(*file_handle*)

`save_matrix`(*file_name*)
saves the data required to reconstruct the matrix the format is: A header containing the parameters used to create the matrix encoded as: @key:value key2:value2 etc... The rest of the file has the same first 5 columns of a BED file: chromosome name, start, end, name, score and strand, all separated by tabs. After the fifth column the matrix values are appended separated by tabs. Groups are separated by adding a line starting with a hash (#) and followed by the group name.

The file is gzipped.

`save_matrix_values`(*file_name*)

deeptools.heatmapper_utilities module

`deeptools.heatmapper_utilities.`**`getProfileTicks`**(*hm*, *referencePointLabel*, *startLabel*, *endLabel*)
returns the position and labelling of the xticks that correspond to the heatmap

`deeptools.heatmapper_utilities.`**`plot_single`**(*ax*, *ma*, *average_type*, *color*, *label*, *plot_type='simple'*)
Adds a line to the plot in the given ax using the specified method

Parameters **ax** : matplotlib axis

matplotlib axis

ma : numpy array

numpy array The data on this matrix is summarized according to the *average_type* argument.

average_type : str

string values are sum mean median min max std

color : str

a valid color: either a html color name, hex (e.g #002233), RGB + alpha tuple or list or RGB tuple or list

label : str

label

plot_type: str

type of plot. Either 'se' for standard error, 'std' for standard deviation, 'overlapped_lines' to plot each line of the matrix, fill to plot the area between the x axis and the value or None, just to plot the average line.

Returns ax

matplotlib axis

Examples

```
>>> import matplotlib.pyplot as plt
>>> fig = plt.figure()
>>> ax = fig.add_subplot(111)
>>> matrix = np.array([[1,2,3],
...                    [4,5,6],
...                    [7,8,9]])
>>> ax = plot_single(ax, matrix -2, 'mean', color=[0.6, 0.8, 0.9], label='fill light blue', plot
>>> ax = plot_single(ax, matrix, 'mean', color='blue', label='red')
>>> ax = plot_single(ax, matrix + 5, 'mean', color='red', label='red', plot_type='std')
>>> ax =plot_single(ax, matrix + 10, 'mean', color='#cccccc', label='gray se', plot_type='se')
>>> ax = plot_single(ax, matrix + 20, 'mean', color=(0.9, 0.5, 0.9), label='violet', plot_type='
>>> ax = plot_single(ax, matrix + 30, 'mean', color=(0.9, 0.5, 0.9, 0.5), label='violet with alp
>>> leg = ax.legend()
>>> plt.savefig("/tmp/test.pdf")
>>> fig = plt.figure()
```

deeptools.mapReduce module

deeptools.mapReduce.**BED_to_interval_tree**(*BED_file*)
Creates an index of intervals, using an interval tree, for each BED entry

Parameters **BED_file** – file handler of a BED file

:return interval tree

deeptools.mapReduce.**getUserRegion**(*chrom_sizes*, *region_string*, *max_chunk_size=1000000.0*)
Verifies if a given region argument, given by the user is valid. The format of the region_string is chrom:start:end:tileSize where start, end and tileSize are optional.

Parameters

- **chrom_sizes** – dictionary of chromosome/scaffold size. Key=chromosome name

- **`region_string`** – a string of the form chr:start:end
- **`max_chunk_size`** – upper limit for the chunk size

Returns tuple chrom_size for the region start, region end, chunk size

#>>> data = getUserRegion({'chr2': 1000}, "chr1:10:10") #Traceback (most recent call last): # ... #NameError: Unknown chromosome: chr1 #Known chromosomes are: ['chr2']

If the region end is biger than the chromosome size, this value is used instead >>> getUserRegion({'chr2': 1000}, "chr2:10:1001") ([('chr2', 1000)], 10, 1000, 990)

Test chunk and regions size reduction to match tile size >>> getUserRegion({'chr2': 200000}, "chr2:10:123344:3") ([('chr2', 123344)], 9, 123345, 123336)

`deeptools.mapReduce.`**`mapReduce`**(*staticArgs*, *func*, *chromSize*, *genomeChunkLength=None*, *region=None*, *bedFile=None*, *numberOfProcessors=4*, *verbose=False*, *self_=None*)

Split the genome into parts that are sent to workers using a defined number of procesors. Results are collected and returned.

For each genomic region the given 'func' is called using the following parameters:

chrom, start, end, staticArgs

The *arg* are static, *pickable* variables that need to be sent to workers.

The genome chunk length corresponds to a fraction of the genome, in bp, that is send to each of the workers for processing.

Depending on the type of process a larger or shorter regions may be preferred

Parameters

- **`chromSize`** – A list of duples containing the chromosome name and its length
- **`region`** – The format is chr:start:end:tileSize (see function getUserRegion)
- **`staticArgs`** – tuple of arguments that are sent to the given 'func'
- **`func`** – function to call. The function is called using the following parameters (chrom, start, end, staticArgs)
- **`bedFile`** – Is a bed file is given, the args to the func to be called are extended to include a list of bed defined regions.
- **`self`** – In case mapreduce should make a call to an object the self variable has to be passed.

deeptools.readBed module

class `deeptools.readBed.`**`BedInterval`**(*field_data_dict*, *line*)

Bases: `object`

simple place holder for the line data of a bed file

class `deeptools.readBed.`**`ReadBed`**(*file_handle*)

Bases: `object`

Reads a bed file. Based on the number of fields it tries to guess the type of bed file used. Current options are bed3, bed6 and bed12

Examples

bed = readBed(open("file.bed", 'r')) for interval in bed: ... print bed['start']

get_no_comment_line()
Skips comment lines starting with '#' "track" or "browser" in the bed files

get_values_from_line(*bed_line*)
Processes each bed line from a bed file and casts the values

guess_file_type(*line_values*)
try to guess type of bed file by counting the fields

next()

Returns bedInterval object

deeptools.utilities module

deeptools.utilities.**copyFileInMemory**(*filePath*, *suffix=''*)
copies a file into the special /dev/shm device which moves the file into memory. This process speeds ups the multiprocessor access to such files

deeptools.utilities.**getCommonChrNames**(*bamFileHandlers*, *verbose=True*)
Compares the names and lengths of a list of bam file handlers. The input is list of pysam file handlers.

The function returns a duple containing the common chromosome names and the common chromome lengths.

Hopefully, only _random and chrM are not common.

deeptools.utilities.**getGC_content**(*dnaString*, *as_fraction=True*)

deeptools.utilities.**getTempFileName**(*suffix=''*)
returns a temporary file name. If the special /dev/shm device is available, the temporary file would be located in that folder. /dv/shm is a folder that resides in memory and which has much faster accession.

deeptools.utilities.**tbitToBamChrName**(*tbitNames*, *bamNames*)
checks if the chromosome names from the two-bit and bam file coincide. In case they do not coincide, a fix is tried. If successful, then a mapping table is returned. tbitNames and bamNames should be lists

deeptools.utilities.**which**(*program*)
method to identify if a program is on the user PATH variable. From: http://stackoverflow.com/questions/377017/test-if-executable-exists-in-python

deeptools.writeBedGraph module

class deeptools.writeBedGraph.**WriteBedGraph**(*bamFilesList*, *binLength=50*, *numberOfSamples=None*, *numberOfProcessors=1*, *verbose=False*, *region=None*, *bedFile=None*, *extendReads=False*, *minMappingQuality=None*, *ignoreDuplicates=False*, *chrsToSkip=[]*, *stepSize=None*, *center_read=False*, *samFlag_include=None*, *samFlag_exclude=None*, *zerosToNans=False*, *smoothLength=0*, *out_file_for_raw_data=None*)
Bases: deeptools.countReadsPerBin.CountReadsPerBin

Reads bam files coverages and writes a bedgraph or bigwig file

Extends the CountReadsPerBin object such that the coverage of bam files is writen to multiple bedgraph files at once.

The bedgraph files are later merge into one and converted into a bigwig file if necessary.

The constructor arguments are the same as for CountReadsPerBin. However, when calling the *run* method, the following parameters have to be passed

Examples

Given the following distribution of reads that cover 200 on a chromosome named '3R':

```
  0                              100                           200
  |------------------------------------------------------------|
A                               ===============
                                               ===============


B                ===============               ===============
                                ===============
                                               ===============
```

```
>>> import tempfile
>>> test_path = os.path.dirname(os.path.abspath(__file__)) + "/test/test_data/"
```

```
>>> outFile = tempfile.NamedTemporaryFile()
>>> bam_file = test_path +  "testA.bam"
```

For the example a simple scaling function is going to be used. This function takes the coverage found at each region and multiplies it to the scaling factor. In this case the scaling factor is 1.5

```
>>> function_to_call = scaleCoverage
>>> funcArgs = {'scaleFactor': 1.5}
```

Restrict process to a region between positions 0 and 200 of chromosome 3R

```
>>> region = '3R:0:200'
```

Set up such that coverage is computed for consecutive bins of length 25 bp >>> bin_length = 25 >>> step_size = 25

```
>>> num_sample_sites = 0 #overruled by step_size
>>> c = WriteBedGraph([bam_file], binLength=bin_length, region=region, stepSize=step_size)
>>> c.run(function_to_call, funcArgs, outFile.name)
>>> open(outFile.name, 'r').readlines()
['3R\t0\t100\t0.00\n', '3R\t100\t200\t1.5\n']
>>> outFile.close()
```

run (*func_to_call*, *func_args*, *out_file_name*, *format='bedgraph'*, *smoothLength=0*)

Given a list of bamfiles, a function and a function arguments, this method writes a bedgraph file (or bigwig) file for a partition of the genome into tiles of given size and a value for each tile that corresponds to the given function and that is related to the coverage underlying the tile.

Parameters **func_to_call** : str

function name to be called to convert the list of coverages computed for each bam file at each position into a single value. An example is a function that takes the ratio between the coverage of two bam files.

func_args : dict

dict of arguments to pass to *func*. E.g. {'scaleFactor':1.0}

out_file_name : str

name of the file to save the resulting data.

smoothLength : int

Distance in bp for smoothing the coverage per tile.

writeBedGraph_worker(*chrom*, *start*, *end*, *func_to_call*, *func_args*, *bed_regions_list=None*)

Writes a bedgraph based on the read coverage found on bamFiles

The given func is called to compute the desired bedgraph value using the funcArgs

Parameters **chrom** : str

Chrom name

start : int

start coordinate

end : int

end coordinate

func_to_call : str

function name to be called to convert the list of coverages computed for each bam file at each position into a single value. An example is a function that takes the ratio between the coverage of two bam files.

func_args : dict

dict of arguments to pass to *func*.

smoothLength : int

Distance in bp for smoothing the coverage per tile.

bed_regions_list: list

List of tuples of the form (chrom, start, end) corresponding to bed regions to be processed. If not bed file was passed to the object constructor then this list is empty.

Returns temporary file with the bedgraph results for the region queried.

Examples

```
>>> test_path = os.path.dirname(os.path.abspath(__file__)) + "/test/test_data/"
>>> bamFile1 = test_path +  "testA.bam"
>>> bin_length = 50
>>> number_of_samples = 0 # overruled by step_size
>>> func_to_call = scaleCoverage
>>> funcArgs = {'scaleFactor': 1.0}
```

```
>>> c = WriteBedGraph([bamFile1], bin_length, number_of_samples, stepSize=50)
>>> tempFile = c.writeBedGraph_worker( '3R', 0, 200, func_to_call, funcArgs)
>>> open(tempFile, 'r').readlines()
['3R\t0\t100\t0.00\n', '3R\t100\t200\t1.0\n']
>>> os.remove(tempFile)
```

`deeptools.writeBedGraph.`**`bedGraphToBigWig`**(*chromSizes*, *bedGraphPath*, *bigWigPath*, *sort=True*)

takes a bedgraph file, orders it and converts it to a bigwig file using pyBigWig.

`deeptools.writeBedGraph.`**`getGenomeChunkLength`**(*bamHandlers*, *tile_size*)

Tries to estimate the length of the genome sent to the workers based on the density of reads per bam file and the number of bam files.

The chunk length should be a multiple of the tileSize

`deeptools.writeBedGraph.`**`ratio`**(*tile_coverage*, *args*)

tileCoverage should be an list of two elements

`deeptools.writeBedGraph.`**`scaleCoverage`**(*tile_coverage*, *args*)

tileCoverage should be an list with only one element

`deeptools.writeBedGraph.`**`writeBedGraph_wrapper`**(*args*)

Passes the arguments to writeBedGraph_worker. This is a step required given the constrains from the multiprocessing module. The args var, contains as first element the 'self' value from the WriteBedGraph object

deeptools.writeBedGraph_bam_and_bw module

`deeptools.writeBedGraph_bam_and_bw.`**`getCoverageFromBigwig`**(*bigwigHandle*, *chrom*, *start*, *end*, *tileSize*, *missingDataAsZero=False*)

`deeptools.writeBedGraph_bam_and_bw.`**`writeBedGraph`**(*bamOrBwFileList*, *outputFileName*, *fragmentLength*, *func*, *funcArgs*, *tileSize=25*, *region=None*, *numberOfProcessors=None*, *format='bedgraph'*, *extendPairedEnds=True*, *missingDataAsZero=False*, *smoothLength=0*, *fixed_step=False*)

Given a list of bamfiles, a function and a function arguments, this method writes a bedgraph file (or bigwig) file for a partition of the genome into tiles of given size and a value for each tile that corresponds to the given function and that is related to the coverage underlying the tile.

`deeptools.writeBedGraph_bam_and_bw.`**`writeBedGraph_worker`**(*chrom*, *start*, *end*, *tileSize*, *defaultFragmentLength*, *bamOrBwFileList*, *func*, *funcArgs*, *extendPairedEnds=True*, *smoothLength=0*, *missingDataAsZero=False*, *fixed_step=False*)

Writes a bedgraph having as base a number of bam files.

The given func is called to compute the desired bedgraph value using the funcArgs

tileSize

`deeptools.writeBedGraph_bam_and_bw.`**`writeBedGraph_wrapper`**(*args*)

Module contents

Complete information can be found in the following links: genindex and modindex

1.10 About

Please cite deepTools as follows: Fidel Ramírez, Friederike Dündar, Sarah Diehl, Björn A. Grüning, and Thomas Manke. deepTools: a flexible platform for exploring deep-sequencing data. Nucl. Acids Res., 2014 doi:10.1093/nar/gku365

This tool suite is developed by the Bioinformatics Facility at the Max Planck Institute for Immunobiology and Epigenetics, Freiburg.

While developing deepTools, we continuously strive to create software that fulfills the following criteria:

- **efficiently extract reads from BAM files** and perform various computations on them
- **turn BAM files of aligned reads into bigWig files** using different normalization strategies
- make use of **multiple processors** (speed!)
- generation of **highly customizable images** (change colours, size, labels, file format, etc.)
- enable **customized down-stream analyses**, meaning that every data set created can be stored by the user
- **modular approach** - compatibility, flexibility, scalability (i.e. we can add more and more modules and make use of established methods)

Tip: For support, questions, or feature requests contact: deeptools@googlegroups.com

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