

# An introduction to ChIP-seq analysis with Galaxy

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# An introduction to ChIP-seq analysis with Galaxy

## 1.0 Preface

In this brief tutorial we will learn how to use the excellent tool [Galaxy](http://galaxyproject.org/) (<http://galaxyproject.org/>) to analyse data from a chromatin immunoprecipitation followed by sequencing (ChIP-seq) experiment. It is part of a series of introductory tutorials that can be found at <http://sschmeier.github.io/bioinf-workshop/>.

A PDF-version of this tutorial can be downloaded [here](#) or at [http://sschmeier.github.io/bioinf-workshop/galaxy-intro/doc/AnIntroductionToChIPseqAnalysisWithGalaxy\\_sschatmeier.pdf](http://sschmeier.github.io/bioinf-workshop/galaxy-intro/doc/AnIntroductionToChIPseqAnalysisWithGalaxy_sschatmeier.pdf)

An accompanying lecture for this tutorial is available at figshare (<http://dx.doi.org/10.6084/m9.figshare.1545468>).

## 1.1 Overview

In this brief tutorial we will learn how to use the excellent tool [Galaxy](#) to analyse ChIP-seq data. If you are new to [Galaxy](#), you might want to start with the [Galaxy introductory tutorial](http://sschmeier.github.io/bioinf-workshop/#galaxy-intro/) (<http://sschmeier.github.io/bioinf-workshop/#galaxy-intro/>).

## 1.2 The task at hand

The overall purpose in this tutorial is to:

- Understand better the [Galaxy](#) system ([1.3-1.4](#)).
- Understand how to get your data of interest into the system ([1.5](#)).
- Understand how to quality control your sequencing data ([1.7](#)).
- Understand how to map sequence reads to a reference genome ([1.8](#)).
- Understand how to call ChIP-peaks based on the mapped reads ([1.9](#)).
- Understand how to gather additional information about your data ([2.0](#)).

In order to develop an understanding of the points above, you will run through the workflow to analyse ChIP-seq data (see *Figure 1*):

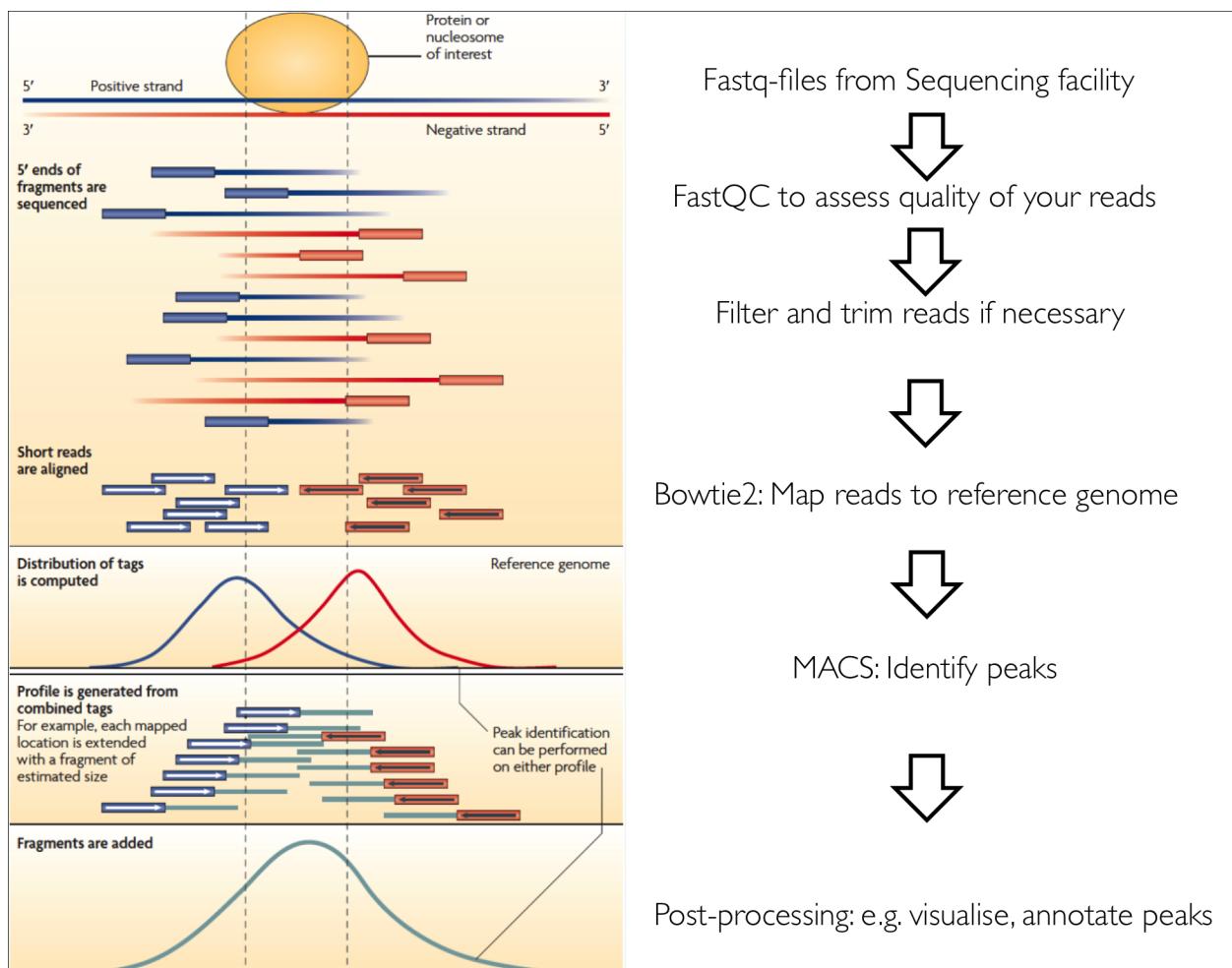


Figure 1: ChIP-workflow (adjusted from Park2009).

The individual tasks are:

1. Load the dataset.
2. Quality assess the reads.
3. Map the reads to the genome using Bowtie2.
4. Call peaks using MACS.
5. Run Enrichr with genes and GREAT with the peak regions to find enriched annotations.
6. Visualise the peaks in UCSC browser.
7. Prepare peak data and use MEME to find TFBS motifs.

### 1.3 Log into Galaxy

First, go to <https://usegalaxy.org/> and log into your Galaxy account (see Figure 2 and Figure 3).

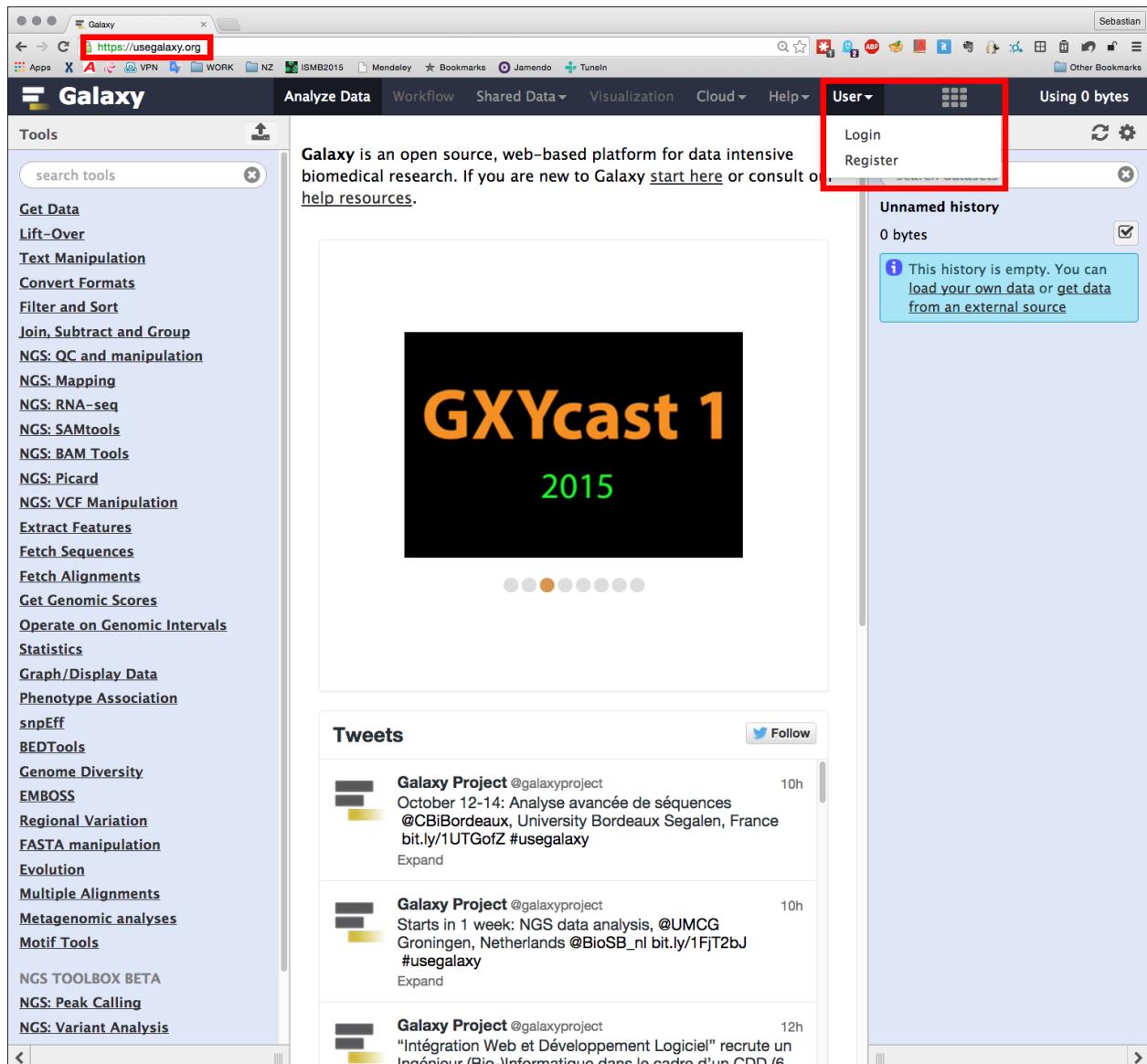


Figure 2: Log into your Galaxy account.

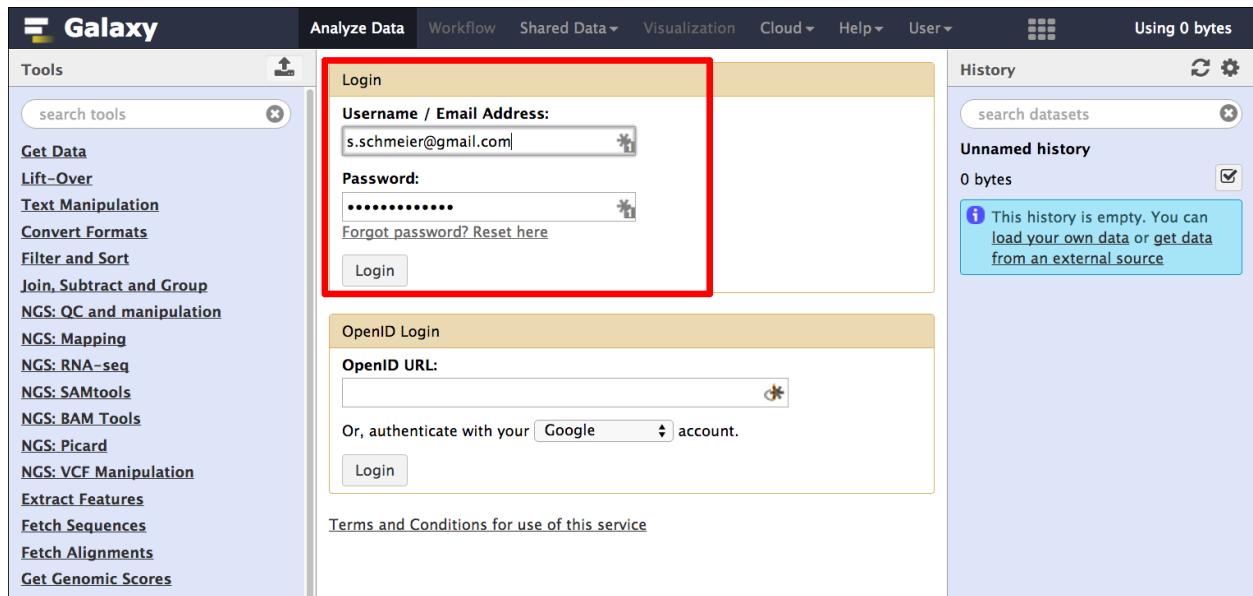


Figure 3: Log into your Galaxy account with your credentials.

## 1.4 Create a new history

Create a new history (see *Figure 4*) and rename it to something useful (see *Figure 5*).

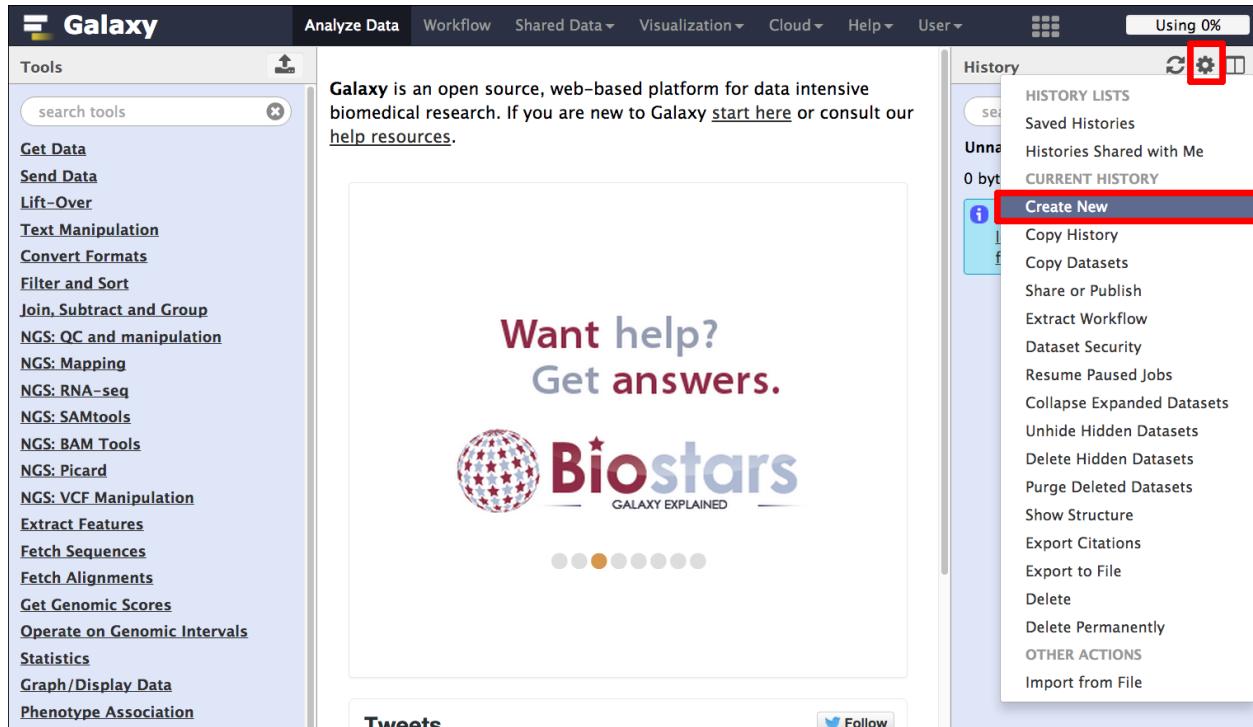


Figure 4: Log into your Galaxy account.

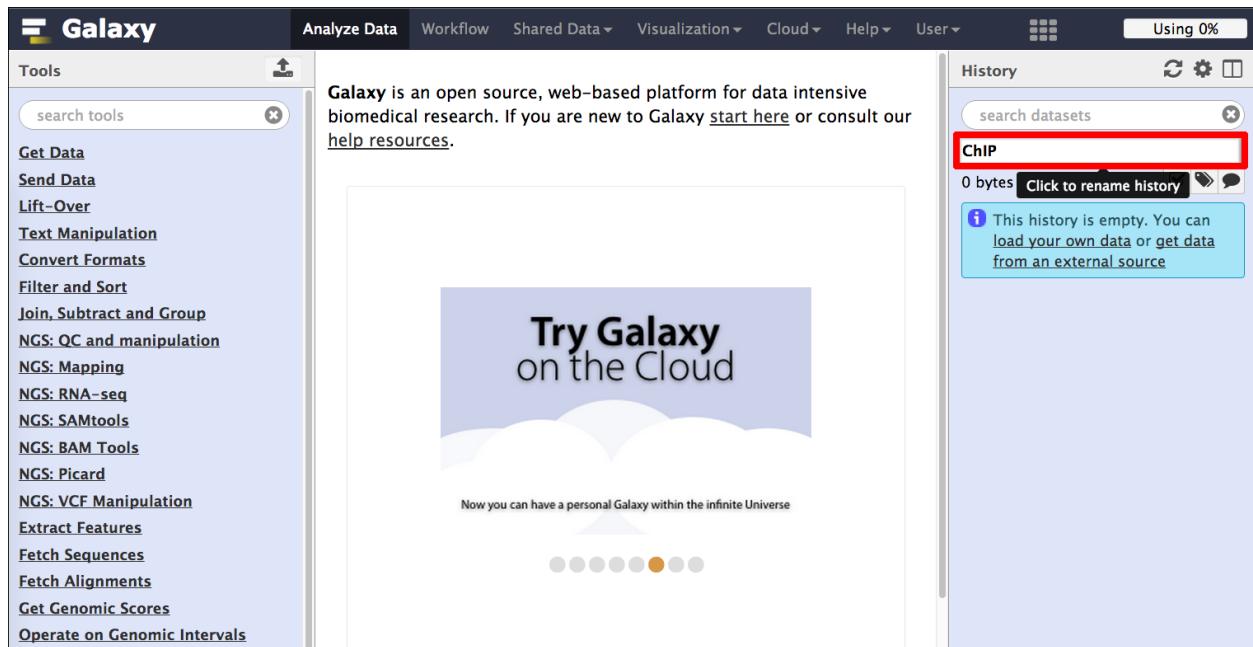


Figure 5: Log into your Galaxy account with your credentials.

## 1.5 Loading the data

We are going to use some *Shared Data* from the Galaxy Demonstration dataset.

1. Click on the *Shared Data* tab (see *Figure 6*).
2. Search for the *Demonstration Datasets*. (see *Figure 7*)

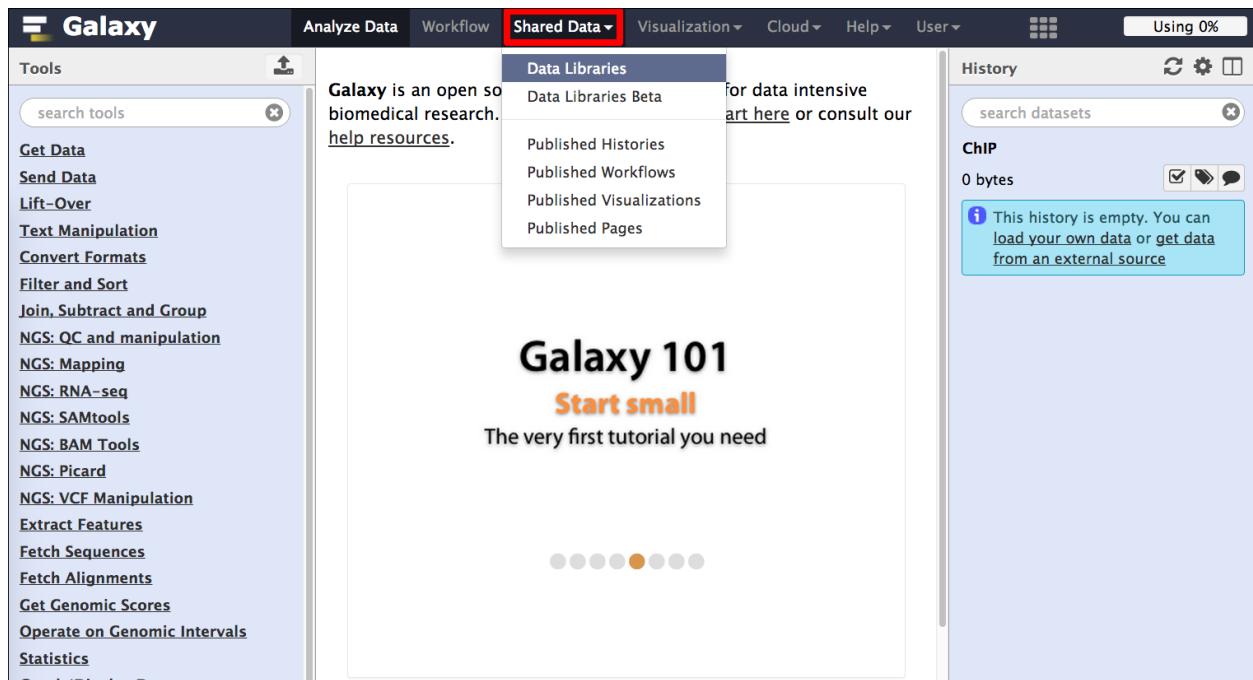


Figure 6: Load shared data tab.

The screenshot shows the Galaxy interface with the 'Data Libraries' tab selected. A search bar at the top has 'search dataset name, info, message, dbkey' and a magnifying glass icon. Below it is a link to 'Advanced Search'. The main area displays a table of datasets:

Data library name	Data library description
<a href="#">1000 Genomes</a>	Data from the 1000 Genomes Project FTP site
<a href="#">AC-exome</a>	
<a href="#">Bushman</a>	Data for two papers about the Khoisan and other populations.
<a href="#">Charts Example Data</a>	
<a href="#">ChIP-Seq Mouse Example</a>	Data used in examples that demonstrate analysis of ChIP-Seq data
<a href="#">Chobi</a>	
<a href="#">CloudMap</a>	Contains userguide, reference files, and configuration files for the Cloudmap WGS analysis pipeline
<a href="#">Codon Usage Frequencies</a>	
<a href="#">Coleman</a>	IonPGM
<a href="#">DataImport-00107dec-86f4-44f6-af87-cb20d69c2fe9@createprivatelibRARYexample.com</a>	
<a href="#">DataImport-ff2b1cbd-ded0-41b8-afdb-741f1534ceb8@createprivatelibRARYexample.com</a>	
<a href="#">Demonstration Datasets</a>	Demonstration datasets collected from various Galaxy tutorials
<a href="#">Denisovan sequences</a>	Files from 'A high-coverage genome sequence from an archaic Denisovan Individual' Meyer et al. Science 2012 and basic processed data.
<a href="#">Erythroid Epigenetic Landscape</a>	Dynamics of the epigenetic landscape during erythroid differentiation after GATA1 restoration

Figure 7: Look for the Demonstration Datasets.

Load the following 4 files: G1E CTCF, G1E\_ER4 CTCF, G1E ER4 input, G1E input (see Figure 8).

The screenshot shows the 'Data Library "Demonstration Datasets"' page. It lists datasets under 'Human RNA-seq: CHB ENCODE Exercise' and 'Mouse ChIP-seq: G1E CTCF binding'. A red box highlights the 'Import to current history' button at the bottom.

Name	Message	Data type	Date uploaded	File size
Human RNA-seq: CHB ENCODE Exercise	Data on h1-hESC and CD20 produced by ENCODE and used by the CHB Sequencing Workshops			
Mouse ChIP-seq: G1E CTCF binding	Sample datasets from Hardison lab for ChIP-seq analysis			
G1E CTCF (chr19)		fastqsanger	Sun Jan 11 18:38:58 2015 (UTC)	29.2 MB
G1E_ER4 CTCF (chr19)		fastqsanger	Sun Jan 11 18:38:57 2015 (UTC)	29.2 MB
G1E_ER4 input (chr19)	None	fastqsanger	Sun Jan 11 18:38:58 2015 (UTC)	17.1 MB
G1E input (chr19)		fastqsanger	Sun Jan 11 18:38:59 2015 (UTC)	28.5 MB

Figure 8: Load the datasets.

Once the files are loaded we can switch back to the analysis window by clicking *Analyze Data* tab (see Figure 9). We should find four datasets in the history panel (see Figure 10).

The screenshot shows the Galaxy Data Library interface. At the top, there is a red box around the "Analyze Data" tab. Below it, a green message bar says "4 datasets imported into 1 history: ChIP". The main area displays a table of datasets:

Name	Message	Data type	Date uploaded	File size
Human RNA-seq: CHB ENCODE Exercise	Data on h1-hESC and CD20 produced by ENCODE and used by the CHB Sequencing Workshops			
Mouse ChIP-seq: G1E CTCF binding	Sample datasets from Hardison lab for ChIP-seq analysis			
G1E CTCF (chr19)		fastqsanger	Sun Jan 11 18:38:58 2015 (UTC)	29.2 MB
G1E_ER4 CTCF (chr19)		fastqsanger	Sun Jan 11 18:38:57 2015 (UTC)	29.2 MB
G1E_ER4 input (chr19)	None	fastqsanger	Sun Jan 11 18:38:58 2015 (UTC)	17.1 MB
G1E input (chr19)		fastqsanger	Sun Jan 11 18:38:59 2015 (UTC)	28.5 MB

At the bottom, there is a button for "Import to current history".

Figure 9: Load the datasets.

The screenshot shows the Galaxy interface with the history panel open. On the left, there is a sidebar with "Get Data" options like "Upload File" and various table browsers. The central area has a "Try Galaxy on the Cloud" banner. The history panel on the right shows four datasets under the "ChIP" category:

- 4: G1E input (chr19)
- 3: G1E\_ER4 input (chr19)
- 2: G1E\_ER4 CTCF (chr19)
- 1: G1E CTCF (chr19)

Each dataset entry includes a preview icon, edit icon, and delete icon.

Figure 10: Loaded data in history panel

Alternatively, you can download the data [chipdata.zip](#) or (~40MB) from <http://sschmeier.github.io/bioinf-workshop/galaxy-chipseq/data/chipdata.zip>, unzip it and upload the files to the Galaxy history.

Hint! Should you need to refresh how to upload data to Galaxy, have a look at the [Galaxy introductory tutorial](#) (<http://sschmeier.github.io/bioinf-workshop/#!galaxy-intro/>)).

## 1.6 Investigate the data

The four files that we have now in our history are: G1E CTCF, G1E\_ER4 CTCF, G1E ER4 input, G1E input. A closer look reveals that they are in fastq-sanger format (see [Figure 11](#) and [Figure 12](#)).

The screenshot shows the Galaxy web interface. On the left, there's a 'Tools' sidebar with various options like 'Upload File from your computer', 'UCSC Main table browser', etc. In the center, a warning message says: 'This dataset is large and only the first megabyte is shown below. Show all | Save'. Below this is a large block of sequence data starting with '@HWUSI-EAS610:2:1:4:959#0/1'. On the right, the 'History' panel shows four datasets: '4: G1E input (chr19)', '2: G1E\_ER4 CTCF (chr19)', '3: G1E\_ER4 input (chr19)', and '1: G1E CTCF (chr19)'. The 'G1E input' dataset is highlighted with a red border around its preview area. The 'format: fastqsanger' and 'database: mm9' labels are also highlighted with red boxes.

Figure 11: Information about the data.

## FastQ-format

Sequence id: @EAS139:136:FC706VJ:2:2104:15343:197393 1:Y:18:ATCACG

Sequence: GATTGGGGTCAAGCAGTATCGATCAAATAGTAAATCCATTGTTC

Phred quality of the corresponding nucleotide (ASCII code): +'\*(((((\*\*\*+))%%++)(%%%)).1\*\*\*-+\*'')\*\*55CCF>>>

**Phred quality**

- One ASCII character per nucleotide.
- Encodes for a quality  $Q = -10 \log_{10}(P)$ , where P is the error probability

The Relationship Between Quality Score and Base Call Accuracy		
Quality Score	Probability of Incorrect Base Call	Inferred Base Call Accuracy
10 (Q10)	1 in 10	90%
20 (Q20)	1 in 100	99%
30 (Q30)	1 in 1000	99.9%

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Figure 12: The fastq-format.

What we are looking at is data from the [G1E mouse cell-line from Gata1-null mouse embryonic stem cells](#). We are looking at two conditions, normal G1E cell-lines and G1E-ER4 cell-lines, where the effect of Gata1 deletion is restored. Under both conditions Ctcf has been ChIP'ed and sequenced. The "input" samples denote samples where the DNA was fragmented but before the immunoprecipitation against Ctcf and can thus be used as controls. We

are also only looking at a subset of the full dataset, only chr19. Thus, we can compare the CTCF occupancy between G1E and G1E-ER4 cell-lines.

Note!

**TODO:**

1. Find out what **Ctcf** is.
2. Find out why studying **Gata1** in mouse embryonic stem cells is of interest?

Hint! You can use [NCBI gene](#) or [wikigenes](#) or even [wikipedia](#) to find out about **Ctcf** and **Gata1**.

## 1.7 Quality assessment

Now we need to assess the quality of the reads in each sample and filter and quality trim the reads if necessary.

### 1.7.1 FastQC

First, we run FastQC on each sample to get a feel for the overall quality of the data (see *Figure 13*).

Figure 13: FastQC.

Have a look at the HTML result page. Depending on what the results are you might want to do some filtering and quality trimming.

Note!

**TODO:** Run FastQC on all four files and investigate the quality. Note for each sample the nucleotide number where the quality markedly drops.

### 1.7.2 Read filtering

Here, we want to get rid of all reads that are of low quality. This strongly depends on your definition for “low quality”. In the figure below the default values are used (see *Figure 14*). The *Quality cut-off* value is 20 and 90% of all nucleotides of the read need to be equal or above this cut-off value to be accepted.

*Figure 14: Filtering reads of bad quality.*

Furthermore, I edited the dataset name (1) to keep track the kind of data (see *Figure 15*). The original name I copied into the notes field (2), however it is not strictly necessary as the information from which dataset this one was derived is still available when clicking the info button (see *Figure 16*). Finally, I renamed the dataset to something useful (3, see *Figure 15*).

*Figure 15: Rename the dataset to keep track.*

The screenshot shows the Galaxy web interface. On the left, a sidebar lists various NGS tools: NGS: Mapping, NGS: RNA-seq, NGS: SAMtools, NGS: BAM Tools, NGS: Picard, and NGS: VCF Manipulation. The main panel is titled 'Tool: Filter by quality' and shows details for a dataset named 'G1E input filtered'. The dataset was created on Tue Sep 22 01:23:37 2015 (UTC), has a file size of 21.9 MB, and is in mm9 format. It was run using fastqsanger. The Galaxy Tool ID is toolshed.g2.bx.psu.edu/repos/devteam/fastq\_quality\_filter/cshl\_fastq\_quality\_filter/1.0.0, and the Galaxy Tool Version is 1.0.0. The Tool Standard Output is stdout and the Tool Standard Error is stderr. The Tool Exit Code is 0. The API ID is bbd44e69cb8906b505edda71e814f4ab, and the History ID is 62fc6065d03a7359. The UUID is 6d8cd290-e71f-4f23-979e-b7de3bc731e4. Below this, there is a table for 'Input Parameter' with rows for 'Library to filter' (Value: 4: G1E input (chr19), Note for rerun: 4: G1E input (chr19)), 'Quality cut-off value' (Value: 20, Note for rerun: 20), and 'Percent of bases in sequence that must have quality equal to / higher than cut-off value' (Value: 90, Note for rerun: 90). An 'Inheritance Chain' section shows 'G1E input filtered' as the final step. To the right, a 'History' panel shows a list of datasets, with 'Copy of 'ChIP'' (active items only) highlighted. A sequence viewer shows a portion of a DNA sequence with a red box around the 'Info' button.

Figure 16: Detailed information about a dataset can be gathered by clicking the info button.

Note!

**TODO:** Run the filtering on all four files and note how many reads got excluded for each sample (see the next section on how to speed this process up by re-running analyses).

### Re-running an analysis

Click on the re-run button of the analysis (1) you would like to re-run (see *Figure 17*). The parameter window pops up with all the original parameters used. Now you can select a different dataset (2) and run the original analysis with the same parameters (see *Figure 17*).

Figure 17: Re-run button to re-run the same analysis.

### 1.7.3 Quality trimming

Finally, we can use a quality trimmer to get rid of bad starts and ends of reads (see *Figure 19*). To do so, select the *FASTQ Quality Trimmer* (1). Choose the **filtered** dataset from the step before (2). In *Figure 19* I use a simple window size of 1 (3) and a quality score of 20 (4) to just trim off the ends on both sides.

The screenshot shows the Galaxy web interface with the following details:

- Tools Panel:** On the left, a list of tools including 'FASTQ Quality Trimmer by sliding window' (highlighted with a red box and labeled 1).
- Tool Configuration:**
  - FASTQ File:** Set to '9: G1E input filtered' (highlighted with a red box and labeled 2).
  - Window size:** Set to '1' (highlighted with a red box and labeled 3).
  - Quality Score:** Set to '20' (highlighted with a red box and labeled 4).
  - Aggregate action for window:** Set to 'min score'.
  - Trim until aggregate score is:** Set to '>='.
- History Panel:** Shows several datasets:
  - ChIP (6 shown, 3 deleted)
  - 127.1 MB
  - 9: G1E input filtered
  - 5: FastQC on data 4: Web page
  - 4: G1E input (chr19)
  - 3: G1E\_ER4 input (chr19)
  - 2: G1E\_ER4 CTCF (chr19)
  - 1: G1E CTCF (chr19)

Figure 19: Filtering reads of bad quality.

Note!

**TODO:** Run the quality trimmer on all *filtered* datasets and rename the sets to something meaningful.

## 1.8 Mapping reads

By now we should have 4 sets of filtered and trimmed reads with a meaningful name (see Figure 19). These form the basis for the subsequent analyses. Now we are going to map the reads to the reference genome.

The screenshot shows the Galaxy web interface. On the left, the 'Tools' panel is open, displaying a list of bioinformatics tools categorized under 'NGS: Mapping'. In the center, a 'Galaxy 101' tutorial is displayed with the heading 'Start small' and the subtext 'The very first tutorial you need'. At the bottom of this section, there is a horizontal navigation bar with several dots, one of which is orange. On the right, the 'History' panel is shown, listing 12 datasets under the 'ChIP' category. The datasets are: 16: G1E\_ER4 input trimmed, 15: G1E\_ER4 CTCF trimmed, 14: G1E CTCF trimmed, 13: G1E CTCF filtered, 12: G1E\_ER4 CTCF filtered, 11: G1E\_ER4 input filtered, 10: G1E input trimmed, 9: G1E input filtered, 4: G1E input (chr19), 3: G1E\_ER4 input (chr19), 2: G1E\_ER4 CTCF (chr19), and 1: G1E CTCF (chr19). The datasets from 10 to 16 are highlighted with a red border.

Dataset ID	Dataset Name
16	G1E_ER4 input trimmed
15	G1E_ER4 CTCF trimmed
14	G1E CTCF trimmed
13	G1E CTCF filtered
12	G1E_ER4 CTCF filtered
11	G1E_ER4 input filtered
10	G1E input trimmed
9	G1E input filtered
4	G1E input (chr19)
3	G1E_ER4 input (chr19)
2	G1E_ER4 CTCF (chr19)
1	G1E CTCF (chr19)

Figure 19: The datasets for mapping.

### 1.8.1 Bowtie2

We can now map the trimmed data to the reference genome using Bowtie2. Select Bowtie2 in the tools panel under section *NGS: Mapping* (1, see *Figure 19*). We select the *trimmed* dataset we want to map (2) and select an appropriate reference genome (3).

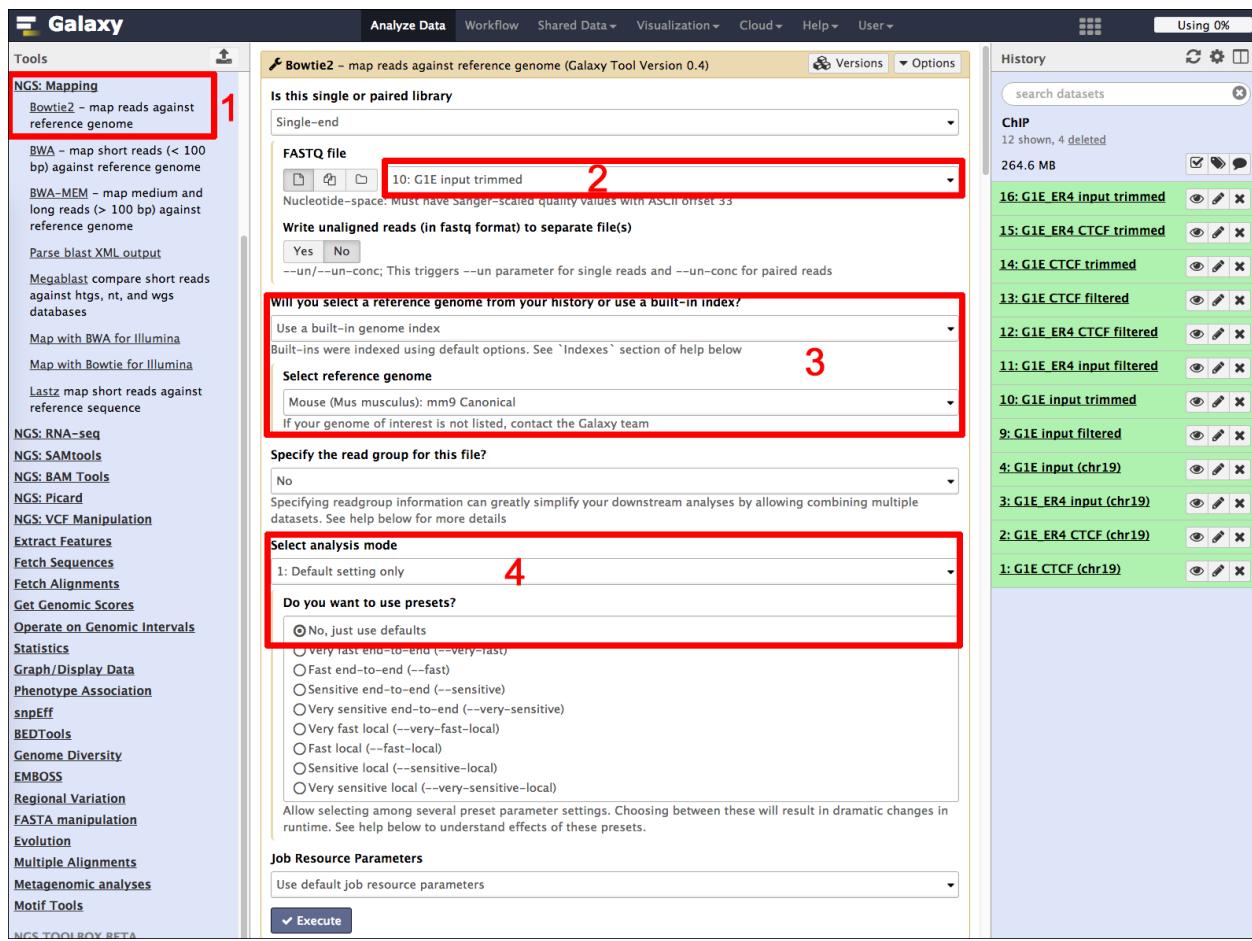


Figure 19: Bowtie2.

Using an inbuilt index choose the same genome built as your data is (here we are looking at mouse mm9 data). Choose the canonical index, **mm9 Canonical**.

From the Galaxy Bowtie tool description:

#### A Note on Built-in Reference Genomes

The default variant for all genomes is “Full”, defined as all primary chromosomes (or scaffolds/contigs) including mitochondrial plus associated unmapped, plasmid, and other segments. When only one version of a genome is available in this tool, it represents the default “Full” variant. Some genomes will have more than one variant available. The “Canonical Male” or sometimes simply “Canonical” variant contains the primary chromosomes for a genome. For example a human “Canonical” variant contains chr1-chr22, chrX, chrY, and chrM. The “Canonical Female” variant contains the primary chromosomes excluding chrY.

Finally, we just use the default parameters of Bowtie2 (4) and execute the analysis (see *Figure 19*).

We can not look at the resulting data in detail, as the output is in a format called *bam* which is a binary version of the Sequence Alignment/Map (SAM) format (see <http://genome.ucsc.edu/goldenpath/help/bam.html> and <http://samtools.sourceforge.net/> for an explanation). However, by clicking the dataset-name, we get more detailed information about the mapping (see *Figure 20*)

**22: G1E4 input bowtie2**

10.2 MB  
format: **bam**, database: **mm9**

```
[samopen] SAM header is present: 22 sequences.  
212756 reads; of these:  
212756 (100.00%) were unpaired; of these:  
1975 (0.93%) aligned 0 times  
204916 (96.32%) aligned exactly 1 time  
5865 (2.76%) aligned >1 times  
99.07% overall alignment
```

[display at UCSC main](#)  
[display at Ensembl Current](#)  
[display with IGV local Mouse mm9](#)  
[display in IGB View](#)

**Binary bam alignments file**

Figure 20: Bowtie2 mapping information.

Note!

**TODO:** Run Bowtie2 on each of the four trimmed datasets. Note for each sample the number of reads that could be aligned exactly once to the genome and the overall alignment percentage.

## 1.9 Peak calling

### 1.9.1 MACS

Select the MACS tool in the **NGS Peak Calling** section:

1. Once you have the tool open (see *Figure 21*), give it a useful name.
2. We are dealing with single-end reads, so select this option.
3. We give it the Bowtie mapped file of the CTCF-ChIP'ed experiment and the “input” of the same cell-line as a control-file.
4. We need to adjust the genome size to that of mm9 Canonical which is 1.87e+9.
5. We also change the tag-size to 36.
6. Finally, we adjust the peak detection method to the “new” one.

The screenshot shows the Galaxy web interface with the MACS Model-based Analysis of ChIP-Seq tool selected. The interface is organized into several panels:

- Left Sidebar (Tools):** Contains a search bar and a list of tools categorized under NGS Toolbox BETA, including NGS: Peak Calling, MACS Model-based Analysis of ChIP-Seq, SICER Statistical approach for the Identification of ChIP-Enriched Regions, NGS: Variant Analysis, NGS: GATK Tools (beta), NGS: Picard (beta), RNA Structure Prediction, and Workflows.
- Central Panel (MACS Tool):**
  - Experiment Name:** MACS G1E CTCF (1)
  - Paired End Sequencing:** Single End (2)
  - Chip-Seq Tag File:** 18: G1E CTCF bowtie2 (3)
  - Effective genome size:** 187000000 (4)
  - Tag size:** 36 (5)
  - Band width:** 300
  - Pvalue cutoff for peak detection:** 0.00001
  - Select the regions with MFOLD high-confidence enrichment ratio against background to build model:** 32
  - Parse xls files into distinct interval files:** Yes
  - Save shifted raw tag count at every bp into a wiggle file:** Do not create wig file (faster)
  - Use fixed background lambda as local lambda for every peak region:** Yes (checkbox)
  - 3 levels of regions around the peak region to calculate the maximum lambda as local lambda:** 1000,5000,10000
  - Build Model:** Build the shifting model
  - Diagnosis report:** Do not produce report (faster)
  - Perform the new peak detection method (futurefdr):** Yes (checkbox) (6)
- Right Panel (History):** Shows a list of datasets related to the ChIP experiment, including G1E input bowtie2, G1E\_ER4 input bowtie2, G1E\_ER4 CTCF bowtie2, G1E CTCF bowtie2, G1E\_ER4 input trimmed, G1E\_ER4 CTCF trimmed, G1E CTCF trimmed, G1E CTCF filtered, G1E\_ER4 CTCF filtered, G1E\_ER4 input filtered, G1E input trimmed, G1E input filtered, G1E input (chr19), G1E\_ER4 input (chr19), G1E\_ER4 CTCF (chr19), and G1E CTCF (chr19).

Figure 21: MACS peak calling.

The results of the MACS run are two datasets (see Figure 22). One bed-file that contains the enriched regions and a html-file that provides more information about the MACS run, e.g. we can have a look at the estimated peak model (see Figure 23) or get more information about the peaks in the created xls-file (see Figure 24).

Additional output created by MACS (MACS\_G1E\_CTCF)

**Additional Files:**

- MACS\_G1E\_CTCF\_model.pdf
- MACS\_G1E\_CTCF\_model.r
- MACS\_G1E\_CTCF\_model.r.log
- MACS\_G1E\_CTCF\_negative\_peaks.xls
- MACS\_G1E\_CTCF\_peaks.xls

**Messages from MACS:**

```

INFO  @ Mon, 21 Sep 2015 23:36:23:
# ARGUMENTS LIST:
# name = MACS_G1E_CTCF
# format = BAM
# ChIP-seq file = /galaxy-repl/main/files/012/526/dataset_12526748.dat
# control file = /galaxy-repl/main/files/012/526/dataset_12526910.dat
# effective genome size = 1.87e+09
# tag size = 36
# band width = 300
# model fold = 32
# pvalue cutoff = 1.00e-05
# Ranges for calculating regional lambda are : peak_region,1000,5000,10000
INFO  @ Mon, 21 Sep 2015 23:36:23: #1 read tag files...
INFO  @ Mon, 21 Sep 2015 23:36:23: #1 read treatment tags...
WARNING @ Mon, 21 Sep 2015 23:36:26: NO records for chromosome chr13, minus stra
WARNING @ Mon, 21 Sep 2015 23:36:26: NO records for chromosome chr10, minus stra
WARNING @ Mon, 21 Sep 2015 23:36:26: NO records for chromosome chr18, minus stra
INFO  @ Mon, 21 Sep 2015 23:36:26: #1.2 read input tags...
WARNING @ Mon, 21 Sep 2015 23:36:28: NO records for chromosome chr16, minus stra
WARNING @ Mon, 21 Sep 2015 23:36:28: NO records for chromosome chr15, plus stra
INFO  @ Mon, 21 Sep 2015 23:36:28: #1 Background Redundant rate: 0.01
INFO  @ Mon, 21 Sep 2015 23:36:28: #1 finished!
INFO  @ Mon, 21 Sep 2015 23:36:28: #2 Build Peak Model...
INFO  @ Mon, 21 Sep 2015 23:36:34: #2 number of paired peaks: 9733
INFO  @ Mon, 21 Sep 2015 23:36:34: #2 finished!

```

Figure 22: MACS peak calling results.

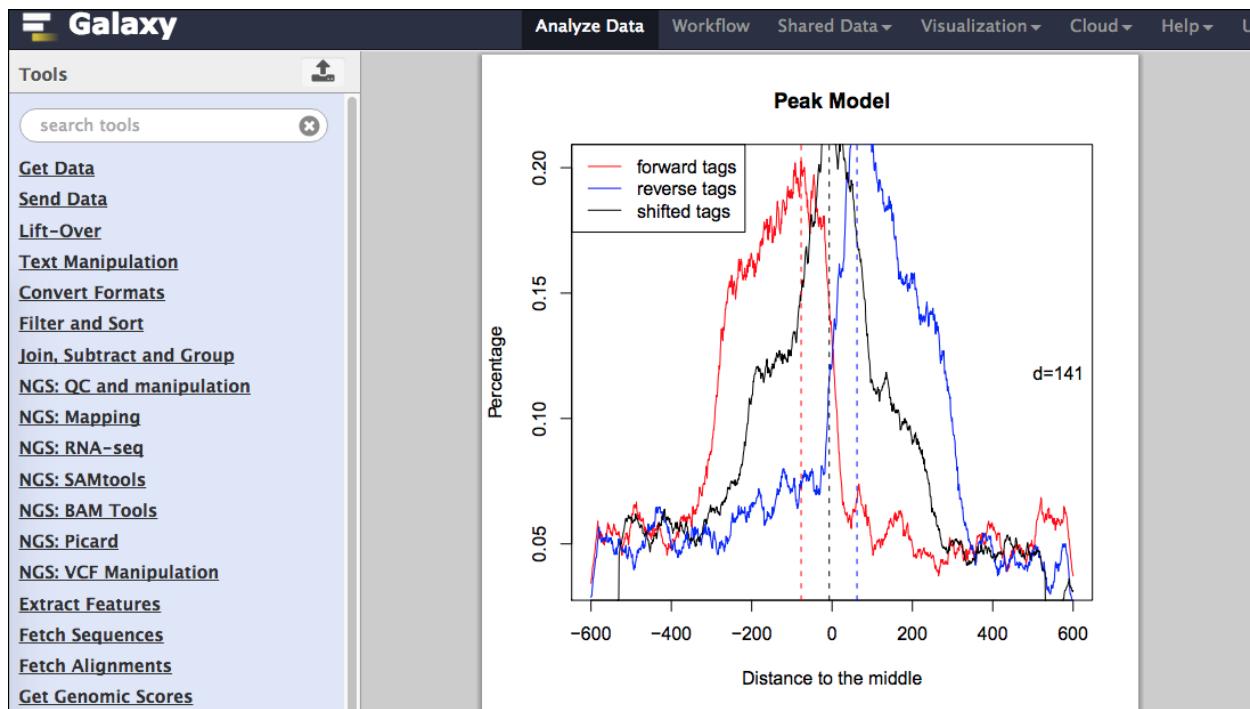


Figure 23: MACS peak model.

	A	B	C	D	E	F	G	H	I
1	# This file is generated by MACS								
2	# ARGUMENTS LIST:								
3	# name = MACS_G1E_CTCF								
4	# format = BAM								
5	# ChIP-seq file = /galaxy-repl/main/files/012/526/dataset_12526748.dat								
6	# control file = /galaxy-repl/main/files/012/526/dataset_12526910.dat								
7	# effective genome size = 1.87e+09								
8	# tag size = 36								
9	# band width = 300								
10	# model fold = 32								
11	# pvalue cutoff = 1.00e-05								
12	# Ranges for calculating regional lambda are : peak_region,1000,5000,10000								
13	# unique tags in treatment: 213711								
14	# total tags in treatment: 214878								
15	# unique tags in control: 210164								
16	# total tags in control: 210781								
17	# d = 141								
18	chr	start	end	length	summit	tags	-10*LOG10(pvalue)	fold_enrichment	FDR(%)
19	chr19	3204403	3204776	374	266	12	92.61	17.73	0
20	chr19	3291824	3292396	573	337	35	132	10.3	0
21	chr19	3450652	3452121	1470	765	44	64.64	10.69	0.34
22	chr19	3587687	3588189	503	257	29	103.41	12.95	0
23	chr19	3623514	3624226	713	381	29	71.88	11.44	0
24	chr19	3723759	3725525	1767	1270	40	53.23	12.07	1.32
25	chr19	3946485	3947973	1489	438	33	51.4	14.51	2.27
26	chr19	3980149	3981146	998	393	49	156.53	12.41	0
27	chr19	4012707	4013391	685	242	28	95.26	11.32	0
28	chr19	4047685	4048485	801	365	22	56.6	6.3	0.84
29	chr19	4098871	4099526	656	340	30	120	16.92	0

Figure 24: MACS peak details.

Note!

**TODO:**

1. Do the MACS peak calling for both cell-lines. Look at both peak models and note the distance and differences between the models.
2. Rename the peak-files to something meaningful and while you are doing it change the **score**-column to **5**.
3. What do you expect in terms of called peaks if you would run G1E-CTCF without a control (the “input”-file)?
4. RUN G1E-CTCF without the input control. Note the differences.

## 2.0 Post-processing

Now that we established the peaks, we can do several different analyses to gain information about the genes they regulate or differences in peak abundance as well as functional association.

### 2.1 Overlap peaks with promoter regions

#### 2.1.1 Get genes

Let's upload some genes and extract promoter information for them. Please download the following file ([mm9\\_chr19\\_NCBigenes.bed](#) or from [http://sschmeier.github.io/bioinf-workshop/galaxy-chipseq/data/mm9\\_chr19\\_NCBigenes.bed](http://sschmeier.github.io/bioinf-workshop/galaxy-chipseq/data/mm9_chr19_NCBigenes.bed)) and upload to your Galaxy history (see Figure 25). the file contains 1428 gene regions in bed-format.

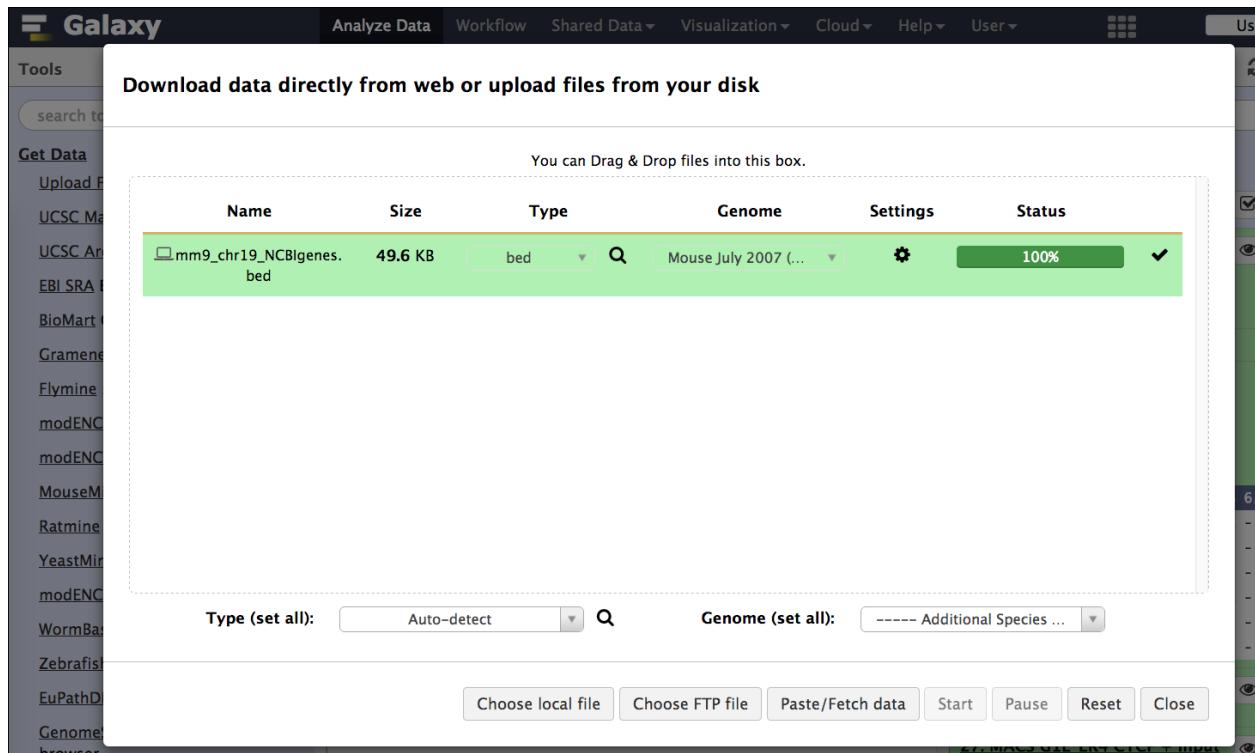


Figure 25: Upload the gene bed-file.

1	2	3	4	5	6
chr19	3065710	3197714	AK077035	1	-
chr19	3153210	3197714	AK006563	1	-
chr19	3153798	3197714	AK007025	1	-
chr19	3259075	3283010	Ighmbp2	1	-
chr19	3264810	3283010	Ighmbp2	1	-
chr19	3272720	3283010	Ighmbp2	1	-
chr19	3283046	3291197	Mrlp21	1	+
chr19	3283046	3292837	Mrlp21	1	+
chr19	3323300	3385733	Cpt1a	1	+
chr19	3388868	3398168	Mtl5	1	+
chr19	3388868	3407785	Mtl5	1	+
chr19	3389400	3407785	Mtl5	1	+
chr19	3409916	3414457	Gal	1	-
chr19	3454927	3575749	Ppp6r3	1	-
chr19	3477775	3575749	Ppp6r3	1	-
chr19	3483527	3494038	mKIAA1558	1	-
chr19	3483527	3575749	Ppp6r3	1	-
chr19	3510945	3575749	mKIAA1558	1	-
chr19	3584824	3615879	Lrp5	1	-
chr19	3584824	3686564	Lrp5	1	-
chr19	3621680	3686564	Lrp5	1	-
chr19	3689686	3708168	AK144662	1	-

Figure 26: The file is in bed-format.

### 2.1.2 Get promoter

Get the promoter regions by using **Operate on Genomic Intervals => Get flanks**. Choose the upstream regions and 10,000 bases (see *Figure 27*). Rename the promoter-set to something meaningful.

The screenshot shows the Galaxy web interface. On the left, the 'Tools' sidebar is open, showing various genomic analysis tools. The main panel displays the 'Get flanks' tool, which is used to return flanking region/s for every gene. The configuration includes:

- Select data:** Input dataset is '51: mm9\_chr19\_NCBigenes.bed'.
- Region:** Set to 'Around Start'.
- Location of the flanking region/s:** Set to 'Upstream'.
- Offset:** Set to '0'.
- Length of the flanking region(s):** Set to '10000'.

The 'Execute' button is highlighted. Below the tool panel, a note states: "This tool finds the upstream and/or downstream flanking region(s) of all the selected regions in the input file." The right panel shows the 'History' section with the results of the 'Get flanks' tool, labeled '51: mm9\_chr19\_NCBigenes.bed'. It lists 1,428 regions in bed format for mm9. Below this, another entry '28: MACS G1E\_ER4 CTCF + input (html)' is shown.

Figure 27: Get upstream flanking regions of the TSS of genes.

### 2.1.3 Join

Now we are going to join (overlap) the peaks with the promoter regions by choosing the tool: **Operate on Genomic Intervals => Join** (see Figure 28). Again rename the resulting dataset to something useful.

The screenshot shows the Galaxy web interface. The 'Tools' sidebar is open, showing various genomic analysis tools. The main panel displays the 'Join' tool, which is used to join the intervals of two datasets side-by-side. The configuration includes:

- Join:** First dataset is '52: Gene promoters chr19'.
- with:** Second dataset is '25: MACS G1E CTCF + input (peaks)'.
- with min overlap:** Set to '1' (bp).
- Return:** Set to 'Only records that are joined (INNER JOIN)'.

The 'Execute' button is highlighted. A tip message states: "TIP: If your dataset does not appear in the pulldown menu, it means that it is not in interval format. Use "edit attributes" to set chromosome, start, end, and strand columns." Below the tool panel, sections for 'Screencasts!' and 'Syntax' are visible. The right panel shows the 'History' section with the results of the 'Join' tool, labeled '52: Gene promoters chr19'. It lists 1,428 regions in interval format for mm9. Below this, other entries like '51: mm9\_chr19\_NCBigenes.bed' and '28: MACS G1E\_ER4 CTCF + input (html)' are shown.

Figure 28: Overlap promoter and peaks with the join tool.

Note!

**TODO:** Join the peak file for G1E CTCF and G1E\_ER4 CTCF with the gene promoter regions. Note the numbers and differences in promoter numbers that overlap Ctcf peaks for both peak-files.

## 2.2 Enrichment analysis (genes) with Enrichr

Now lets take the genes with Ctcf in their promoter regions and do some functional annotation. To do this, we need the unique genes from the overlap of peaks and promoters from the step before. We will be using the tool: **Join, Subtract and Group => Group** to do this. **Group** aggregates data in a certain column. We will use it to aggregate column 4, the gene symbol column (see *Figure 29*). Copy the resulting genes symbol (see *Figure 30*).

Chrom	Start	End	Name	Strand
chr19	3197714	3207714	AK077035	1 -
chr19	3197714	3207714	AK006563	1 -
chr19	3197714	3207714	AK007025	1 -
chr19	3283010	3293010	Ighmbp2	1 -
chr19	3283010	3293010	Ighmbp2	1 -
chr19	3283010	3293010	Ighmbp2	1 -

Figure 29: Aggregate the gene symbol column.

The screenshot shows the Galaxy web interface. On the left, the 'Tools' panel is open, displaying a list of available tools categorized under 'Text Manipulation' and 'NGS: QC and manipulation'. In the main workspace, a history dataset titled '55: Group on data 53' is shown. This dataset contains 156 lines of gene symbols, grouped by column 4 (c4). The grouped data is displayed in a table format. Below the table, the dataset is described as 'format: tabular, database: mm9'. A secondary dataset, '53: Overlap promoter + G1E CT', is also visible in the history.

Figure 30: The aggregated gene symbols.

Now, go to the online tool [Enrichr](http://amp.pharm.mssm.edu/Enrichr/) (<http://amp.pharm.mssm.edu/Enrichr/>). Enrichr provides a way to analyse mammalian gene lists to find enriched annotation terms to get a better understanding of the functions of the gene list under investigation. Go to **Analyze** tab and paste your gene list into the field (see *Figure 31*). Click on the arrow.

 [Login | Register](#)  
**Analyze** What's New? Libraries Find A Gene About Help

## Input data

Choose an input file to upload. Either in BED format or a list of genes. For a quantitative set, add a comma and the level of membership of that gene. The membership level is a number between 0.0 and 1.0 to represent a weight for each gene, where the weight of 0.0 will completely discard the gene from the enrichment analysis and the weight of 1.0 is the maximum. Try an example [BED file](#).

No file chosen

Or paste in a list of gene symbols optionally followed by a comma and levels of membership. Try a [regular example](#) or an [example of a quantitative set](#).

Ift12  
Ighmbp2  
Kazald1  
Kcnip2  
Kcnk4  
Lbx1  
Lcor  
Lrp5  
Lrrn4cl  
Map4k2  
Mark2

156 gene(s) entered 

Enter a brief description for the list in case you want to share it. (Optional)

Contribute

Please acknowledge Enrichr in your publications by citing the following reference:  
 Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, Ma'ayan A. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*. 2013;128(14).

Figure 31: The Enrichr tool.

On the result pages (see Figure 32) you will find several different categories (e.g. *Transcription*, *Pathways*, etc.) of with different databases where term-gene association information was extracted. Figure 32 for example shows the enriched pathways from the [Reactome](http://www.reactome.org/) (<http://www.reactome.org/>) database.

 **Enrichr**

Login | Register

Transcription Pathways Ontologies Disease/Drugs Cell Types Misc Legacy Crowd

Description No description available (156 genes)  

**KEGG 2015**

**WikiPathways 2015**

**Reactome 2015** Bar Graph Table Grid Network 

Hover each row to see the overlapping genes.

10 entries per page Search:

Index	Name	P-value	Z-score	Combined Score
1	Signaling by Wnt	0.03101	-2.24	1.74
2	Organelle biogenesis and maintenance	0.004934	-2.20	1.71
3	misspliced LRP5 mutants have enhanced beta-catenin-dependent signaling	0.02128	-2.19	1.70
4	RNF mutants show enhanced WNT signaling and proliferation	0.02128	-2.16	1.68
5	XAV939 inhibits tankyrase, stabilizing AXIN	0.02128	-2.16	1.68
6	TCF dependent signaling in response to WNT	0.02128	-2.16	1.68
7	Signaling by WNT in cancer	0.03189	-2.13	1.65
8	Polymerase switching on the C-strand of the telomere*	0.007373	-2.07	1.61
9	Telomere C-strand (Lagging Strand) Synthesis*	0.01758	-2.07	1.61
10	Lagging Strand Synthesis*	0.01499	-2.03	1.57

Showing 1 to 10 of 415 entries | [Export entries to table](#)  Previous  Next

Terms marked with an \* have an overlap of less than 5

Figure 32: The Enrichr results show enriched term associations to the input gene list.

Note!

TODO:

1. Find and note the top 5 enriched Gene Ontology process terms for both the G1E and G1E\_ER4 genes that have Ctcf in their promoters.
2. Now that you have unique gene lists for G1E and G1E\_ER4, how many genes are in common, e.g. which genes in both cases have Ctcf in their promoter region?

Hint! For point 2. you can use the **Join, Subtract and Group => Compare two Datasets** tool.

## 2.3 Enrichment analysis (peaks) with GREAT

Here we are going to use another tool called **GREAT** (<http://bejerano.stanford.edu/great/public/html/>). Great as opposed to **Enrichr** excepts bed-regions directly, thus we do not need to get the genes that overlap our peak regions. Take the results from MACS, cut out the first 4 columns with **Text Manipulation => Cut** (as GREAT does not except floats as scores and will produce errors), copy the regions and paste them into the **GREAT** interface.

**GREAT** Overview News Use GREAT Demo Video How to Cite Help Forum

GREAT version 3.0.0 current (02/15/2015 to now)

## GREAT predicts functions of cis-regulatory regions.

Many coding genes are well annotated with their biological functions. Non-coding regions typically lack such annotation. GREAT assigns biological meaning to a set of non-coding genomic regions by analyzing the annotations of the nearby genes. Thus, it is particularly useful in studying cis functions of sets of non-coding genomic regions. Cis-regulatory regions can be identified via both experimental methods (e.g. ChIP-seq) and by computational methods (e.g. comparative genomics). For more see our [Nature Biotech Paper](#).

### News

- Feb 15, 2015: GREAT version 3.0 switches to Ensembl genes, adds the mouse mm10 assembly, and adds new ontologies.
- Apr 3, 2012: GREAT version 2.0 adds new annotations to human and mouse ontologies and visualization tools for data exploration.
- Feb 18, 2012: The [GREAT forums](#) are released, allowing increased user-to-user interaction

[More news items...](#)

**Species Assembly**

- Human: GRCh37 ([UCSC hg19, Feb/2009](#))
- Mouse: NCBI build 37 ([UCSC mm9, Jul/2007](#))
- Mouse: NCBI build 38 ([UCSC mm10, Dec/2011](#))
- Zebrafish: Wellcome Trust Zv9 ([danRer7, Jul/2010](#)) Zebrafish CNE set

[Can I use a different species or assembly?](#)

**Test regions**

- BED file: [Choose File](#) Galaxy25...bed]] .bed
- BED data:
 

chr19	60968112	60968799	MACS_peak_401
chr19	61160078	61161098	MACS_peak_402
chr19	61173687	61174023	MACS_peak_403
chr19	61185950	61186399	MACS_peak_404
chr19	61275219	61276078	MACS_peak_405

[What should my test regions file contain?](#)  
[How can I create a test set from a UCSC Genome Browser annotation track?](#)

**Background regions**

- Whole genome
- BED file: [Choose File](#) No file chosen
- BED data:

[When should I use a background set?](#)  
[What should my background regions file contain?](#)

**Association rule settings**

Show settings »

**Submit** **Reset**

Figure 33: The GREAT website.

**GREAT** Overview News Use GREAT Demo Video How to Cite Help Forum Bejerano Lab, Stanford University

GREAT version 3.0.0 current (02/15/2015 to now)

**Job Description**

**Region-Gene Association Graphs**

**Global Controls** Global Export Which data is exported by each option?

**GO Molecular Function (4 terms)** Global controls

Table controls: Export Shown top rows in this table: 20 Set Term annotation count: Min: 1 Max: Inf Set Visualize this table: [select one]

Term Name	Binom Rank	Binom Raw P-Value ▲	Binom FDR Q-Val	Binom Fold Enrichment	Binom Observed Region Hits	Binom Region Set Coverage	Hyper Rank	Hyper FDR Q-Val	Hyper Fold Enrichment	Hyper Observed Gene Hits	Hyper Total Genes	Hyper Gene Set Coverage
oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water	1	2.6326e-16	9.1693e-13	78.0167	10	2.47%	1	8.8353e-6	37.2489	6	9	1.58%
iron ion binding	4	2.2764e-12	1.9822e-9	5.2559	28	6.91%	3	3.7623e-3	3.9732	16	225	4.22%
stearoyl-CoA 9-desaturase activity	6	1.7961e-11	1.0426e-8	69.6114	7	1.73%	2	2.5647e-3	37.2489	4	6	1.06%
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	17	6.7018e-8	1.3731e-5	3.9978	22	5.43%	4	2.1452e-2	3.7607	14	208	3.69%

The test set of 405 genomic regions picked 379 (2%) of all 21,176 genes. GO Molecular Function has 3,483 terms covering 15,735 (74%) of all 21,176 genes, and 181,165 term - gene associations. 3,483 ontology terms (100%) were tested using an annotation count range of [1, Inf].

**GO Biological Process (1 term)** Global controls

Table controls: Export Shown top rows in this table: 20 Set Term annotation count: Min: 1 Max: Inf Set Visualize this table: [select one]

Term Name	Binom Rank	Binom Raw P-Value ▲	Binom FDR Q-Val	Binom Fold Enrichment	Binom Observed Region Hits	Binom Region Set Coverage	Hyper Rank	Hyper FDR Q-Val	Hyper Fold Enrichment	Hyper Observed Gene Hits	Hyper Total Genes	Hyper Gene Set Coverage
Wnt receptor signaling pathway	2	1.4738e-14	7.4104e-11	4.1678	42	10.37%	3	4.7691e-2	3.7249	15	225	3.96%

Figure 34: GREAT result page.

Note!

**TODO:** Run **GREAT** for both MACS result-files and note the top 5 **GO Biological processes**. Are they different to the ones from **Enrichr**?

## 2.4 Visualisation

Let us now create a visualisation track of the promoters that overlap G1E CTCF peaks and G1E\_ER4 CTCF peaks. Use **Graph/Display Data => Build custom track** (see Figure 33). Also add the two MACS peak bed-files. Look at the track at UCSC (see Figure 36 and Figure 37).

The screenshot shows the Galaxy web interface with the 'Build custom track for UCSC genome browser' tool selected. The tool is configured in three steps:

- Step 1: Track**
  - Dataset:** 56: Overlap promoter + G1E\_ER4 CTCF
  - name:** Gene promoter overlapping G1E\_ER4 CTCF
  - description:** User Supplied Track (from Galaxy)
  - Color:** Green
  - Visibility:** Dense
- Step 2: Track**
  - Dataset:** 53: Overlap promoter + G1E CTCF
  - name:** Gene promoter overlapping G1E CTCF
  - description:** User Supplied Track (from Galaxy)
  - Color:** Black
  - Visibility:** Dense
- Step 3: Track**
  - Dataset:** 25: MACS G1E CTCF + input (peaks)
  - name:** (empty)

The 'Graph/Display Data' section on the left sidebar is highlighted with a red box, and the specific tool being used is also highlighted.

Figure 35: Building a custom UCSC track.

Galaxy

Analyze Data Workflow Shared Data Visualization Cloud Help User

History

search datasets

ChIP  
28 shown, 33 deleted  
324.2 MB

60: Build custom track on data 27, data 25, and others  
1,641 lines, 5 comments  
format: customtrack, database: mm9  
Generated a custom track containing 4 subtracks.

display at UCSC main

1	2	3	4
chr19	3197714	3207714	0
chr19	3197714	3207714	1
chr19	3197714	3207714	2
chr19	3283010	3293010	3
chr19	3283010	3293010	4
chr19	3283010	3293010	5
chr19	3313300	3323300	6
chr19	3575749	3585749	7
chr19	3575749	3585749	8
chr19	3575749	3585749	9
chr19	3575749	3585749	10
chr19	3615879	3625879	11
chr19	3615879	3625879	12
chr19	3698332	3708332	13
chr19	3698410	3708410	14
chr19	3976570	3986570	15
chr19	3976660	3986660	16
chr19	3990579	4000579	17
chr19	3990743	4000743	18
chr19	3993384	4003384	19
chr19	4012725	4022725	20
chr19	4037912	4047912	21
chr19	4042221	4052221	22

Figure 36: Visualising a Galaxy dataset/track.

Genomes Genome Browser Tools Mirrors Downloads My Data View H

JCSC Genome Browser on Mouse July 2007 (NCBI37/mm9) Assembly

move <<< << < > >> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x

chr19:3,152,709-3,252,718 100,010 bp. enter position, gene symbol or search terms go hg38

replaces hg19 as default human assembly

chr19 (qA) 19qA 19qB 19qC1 19qC2 19qC3 19qD1 19qD2 qD3

Scale chr19: 50 kb mm9

ping G1E\_ER4 CTCF User Supplied Track (from Galaxy)

User Track User Supplied Track

lapping G1E CTCF User Supplied Track (from Galaxy)

for MACS\_G1E\_CTCF MACS peaks for MACS\_G1E\_CTCF

MACS\_G1E\_ER4\_CTCF MACS peaks for MACS\_G1E\_ER4\_CTCF

ENCCFF447V0V ChIP-seq of MEL cell line - ENCSR705HGT(Target - USF1)

STS Markers drag select or click to zoom in on Genetic and Radiation Hybrid Maps

AK0877035 UCSC Genes (RefSeq, GenBank, tRNAs & Comparative Genomics)

AK006563

HK007025

Figure 37: Custom UCSC track at the UCSC genome browser website.

## 2.5 Motif finding

Here we want to establish enriched sequence motifs in the peak regions to hypothesise on the actual binding site of Ctcf. We are going to use **MEME-ChIP** (<http://meme.ebi.edu.au/meme/tools/meme-chip>) for this. However, **MEME-ChIP** expects fasta-sequence data as an input, not bed-files. So, we need to extract for our peak bed-files the actual sequence. Another restriction is, that **MEME-ChIP** expects regions of similar size, this is also not a given in the MACS results. The workflow for this analysis looks like this:

1. Find the center of each MACS peak region.
2. Get the flanking region  $\pm 250$  bases (as the recommended region size for MEME-ChIP is 500bp).
3. Extract the fasta-sequence for the regions.
4. Download the fasta-file and upload to [MEME-ChIP](#).
5. Run [MEME-ChIP](#).

### 2.5.1 Find the peak center

We have the start and stop position in our bed-files, thus we can calculate the center point with **start + ((stop-start)/2)**. The tool we need is: **Text Manipulation => Compute** (see *Figure 38*). Make sure you round the results.

*Figure 38: Calculate center peak position.*

Now we cut out the first column (chromosome) and last column (center) with **Text Manipulation => Cut** (see *Figure 39*).

*Figure 39: Cut columns.*

We add another column to the result that will represent the stop-position with **Text Manipulation => Compute**. Make this **c2 + 1** (see *Figure* ).

The screenshot shows the Galaxy web interface with the 'Tools' sidebar open. Under 'Text Manipulation', the 'Compute an expression on every row' tool is selected. The configuration panel shows the expression 'c2+1' entered in the 'Add expression' field. The 'as a new column to' dropdown is set to '63: Cut on data 62'. The 'Round result?' dropdown is set to 'YES'. A large blue 'Execute' button is at the bottom.

Figure 40: Compute the stop-position.

Now, the resulting data is in *tabular* format (see Figure 41) and we need to change it first to *bed-format* (see Figure 42).

1	2	3
chr19	3204589	3204590
chr19	3292109	3292110
chr19	3451386	3451387
chr19	3587937	3587938
chr19	3623869	3623870
chr19	3724641	3724642
chr19	3947228	3947229
chr19	3980647	3980648
chr19	4013048	4013049
chr19	4048084	4048085
chr19	4099198	4099199
chr19	4206891	4206892
chr19	4228453	4228454
chr19	4309935	4309936
chr19	4338322	4338323
chr19	4442144	4442145
chr19	4455946	4455947
chr19	4755852	4755853
chr19	4847634	4847635
chr19	4908202	4908203
chr19	4961967	4961968

History panel details:

- Job ID: 64: Compute on data 6 3
- 30 shown, 34 deleted
- 324.2 MB
- 405 regions
- format: tabular, database: mm9
- Creating column 3 with expression c2+1
- kept 100.00% of 405 lines.

Figure 41: Center peaks.

Change data type

New Type:

bed

This will change the datatype of the existing dataset but *not* modify its contents. Use this if Galaxy has incorrectly guessed the type of your dataset.

Save

History

ChIP  
30 shown, 34 deleted  
324.2 MB

**64: Compute on data 6** 3  
405 regions  
format: tabular, database: mm9  
Creating column 3 with expression c2+1  
kept 100.00% of 405 lines.

1	2	3
chr19	3204589	3204590
chr19	3292109	3292110
chr19	3451386	3451387
chr19	3587937	3587938
chr19	3623869	3623870
chr19	3724641	3724642

Figure 42: Change the data-format to bed.

### 2.5.2 Get flanking regions

Use **Operate on Genomic Intervals => Get flanks**. Extend **both** sides of the start position by **500** bases (see Figure 43).

Get flanks returns flanking region/s for every gene (Galaxy Tool) Version 1.0.0

Select data

64: Compute on data 63

Region

Around Start

Location of the flanking region/s

Both

Offset

0

Use positive values to offset co-ordinates in the direction of transcription and negative values to offset in the opposite direction.

Length of the flanking region(s)

500

Use non-negative value for length

Execute

History

ChIP  
30 shown, 34 deleted  
324.2 MB

**64: Compute on data 6** 3  
405 lines  
format: bed, database: mm9  
Creating column 3 with expression c2+1  
kept 100.00% of 405 lines.

1.Chrom	2.Start	3.End
chr19	3204589	3204590
chr19	3292109	3292110
chr19	3451386	3451387
chr19	3587937	3587938
chr19	3623869	3623870
chr19	3724641	3724642

Figure 43: Get flanking regions.

### 2.5.3 Extract fasta-sequence

Use **Fetch Sequences => Extract Genomic DNA** to extract for the regions the genomic DNA (see *Figure 44*).

The screenshot shows the Galaxy web interface. On the left, the 'Tools' sidebar is open, showing various bioinformatics tools under categories like 'Get Data', 'Send Data', 'Text Manipulation', and 'Fetch Sequences'. The main workspace displays the configuration for the 'Extract Genomic DNA' tool. The tool's parameters are set as follows:

- Fetch sequences for intervals in:** 65: Get flanks on data 64
- Interpret features when possible:** No
- Source for Genomic Data:** Locally cached
- Output data type:** FASTA

Below the tool configuration, there are three warning messages:

- This tool requires interval or gff (special tabular formatted data). If your data is not TAB delimited, first use *Text Manipulation->Convert*.
- Make sure that the genome build is specified for the dataset from which you are extracting sequences (click the pencil icon in the history item if it is not specified).
- All of the following will cause a line from the input dataset to be skipped

The 'History' panel on the right shows the output dataset 65: Get flanks on data 64, which contains 810 regions in mm9 format. The list of genomic coordinates is as follows:

```

1.Chrom 2.Start 3.End
chr19 3204339 3204589
chr19 3204589 3204839
chr19 3291859 3292109
chr19 3292109 3292359
chr19 3451136 3451386
chr19 3451386 3451636

```

Figure 44: Extract DNA for regions.

The screenshot shows the Galaxy web interface. The 'Tools' sidebar is open, showing various bioinformatics tools. The main workspace displays the output of the 'Extract Genomic DNA' tool, which is presented in FASTA format. The sequence begins with:

```

>mm9_chr19_3204089_3204589_+ 3204589
TTTAAATACCAAGGGAGTAAAAATCTCACTATTCCACATCTGTTATATA
AAACTGTACAAAGAAGGGCTTAGCACAAGCTTGGGTACACAGCTTCCTT
AACACCTGTTCTGCTGATACACACCCATTCAGCTTCCAG
TAAAGATCTCTAGAACACCAGGTCTCTACCCAGACTAGTTTATCATACCTT
ACACGATAAAAGGCACAGCAGCTGAGTTCAAACGAAGGGACAGANTTCACA
TGCCAGGTAAACACAGGGTGTAAAATAAAAGGGAAACTCTGTTAAAGG
AGAGATAAAGATTAGACACCCCTGAAACTGAGAAGGCATAGCCCAGGGAG
AACAGATGTGAGGGTTTCAATGCTGAATACTAGGGATAAARACTTTCT
TCCCTTAAGATTGCCACCTGGCCTGGAGAGTAAGTTGCCCCAGACAGAG
TTCTCTAGACAGTTCTGTTCTGGGGAAACGGTTTGGCTCAGATTTGA
>mm9_chr19_3204589_3205089_+ 3205089
GGTGCACAGGGTCTTACAACAACTTGAACTTGAATTCTACAGTCTGTAGT
CTGCTAGTGTGCTGCTGAAACAGGGCTCAAGGGGCCACCTCTGGAG
GATGCTCCCTGCATCCCTACATCTCTCTATCCCTCTGAATGCTCTAGTT
TCTTATCTGTTGCTGATAAAGAAACTAGTTCCCTTTCTGTTCTGCTCAGCTG
CCTCATCTCCCAACCCCTCAGAACACATTATTCCTGCTGATTCTTTAA
TGTCTAGTGTCTCTCTCTCAActccgcctccctccacaggggctctgt
gatcccccgaggctccatcatagtagttccaaataatctccatccatc
catacatctccacagtcccttctgttgtCTCAGGTAGCAGACCTAGAT
AGTGGGTGTCCTGGGGTCAACATCTGTCACCATATCTCTGTTCTGGG
TAATTCCACACTGTCCTCTGCCATCATCACCATGAAAGAAAGAAC
>mm9_chr19_3291609_3292109_+ 3292109
CTTCCCTGATCCATGCTCTATGACAGGGGTCAAGTACAGGGTCAG
TGCTCCAGCAGCCCCCAGGGGTGGGGGAGCACAGTGACAATAGGGCA
GAGGGTGAGGCAAGGTTCTGATCTAGGAACGTTTGGGGCTCAAGGGC
TATGACCCCTGTGAGTCAGGCCACCTTGGGGGAAGACCAACAGGAAGGTC
ACACAGGCTCAAGGATCAAGCCAGAAGCATGGAATCAGAGAGGAAGCAC

```

The 'History' panel on the right shows the output dataset 72: Extract Genomic DNA on data 71, which contains 810 sequences in mm9 format.

Figure 45: Region in fasta-format.

### 2.5.4 Run MEME-ChIP

Go to [MEME-ChIP](http://meme.ebi.edu.au/meme/tools/meme-chip) (<http://meme.ebi.edu.au/meme/tools/meme-chip>) and copy the fasta-sequences into the field and run the application (see *Figure 45*). This may result in enriched sequence motifs that were found in the uploaded sequences (see *Figure 46*).

**MEME-ChIP**  
Motif Analysis of Large Nucleotide Datasets

Version 4.10.1

**MEME Suite 4.10.1**

- ▶ Motif Discovery
- ▶ Motif Enrichment
- ▶ Motif Scanning
- ▶ Motif Comparison
- ▶ Manual
- ▶ Guides & Tutorials
- ▶ Sample Outputs
- ▶ File Format Reference
- ▶ Databases
- ▶ Download & Install
- ▶ Help
- ▶ Alternate Servers
- ▶ Authors & Citing
- ▶ Recent Jobs

← Previous version  
4.10.0

**Data Submission Form**

Perform motif discovery, motif enrichment analysis and clustering on large nucleotide datasets.

**Input the sequences**  
Enter the (equal-length) nucleotide sequences to be analyzed. [?](#)  
 Type in sequences [DNA](#) [Protein](#) [X](#) [?](#)

**Input the motifs**  
Select, upload or enter a set of known motifs. [?](#)  
 Vertebrates (In vivo and in silico) [?](#)

**Input job details**  
(Optional) Enter your email address. [?](#)  
  
 (Optional) Enter a job description. [?](#)

**Universal options**

**MEME options**

**DREME options**

**CentriMo options**

Note: if the combined form inputs exceed 80MB the job will be rejected.

Start Search  Clear Input

Figure 45: MEME-ChIP interface.



Figure 46: MEME-ChIP results.

Note!

**TODO:** Note the enriched motif for the G1E CTCF and G1E\_ER4 peak regions. Are there any differences?

## 2.6 References

Hawkins RD, Hon GC & Ren B. Next-generation genomics: an integrative approach. *Nature Reviews Genetics*. 2010; 11, 476-486

Park PJ. ChIP-seq: advantages and challenges of a maturing technology. *Nature Reviews Genetics*. 2009; 10, 669-680

## 2.7 Web links

Galaxy: <https://usegalaxy.org>

Enrichr: <http://amp.pharm.mssm.edu/Enrichr/>

GREAT: <http://bejerano.stanford.edu/great/public/html/>

Gene Ontology: <http://amigo.geneontology.org/>

MEME-ChIP: <http://meme.ebi.edu.au/meme/tools/meme-chip>

This tutorial: <http://sschmeier.github.io/bioinf-workshop/galaxy-chipseq/>