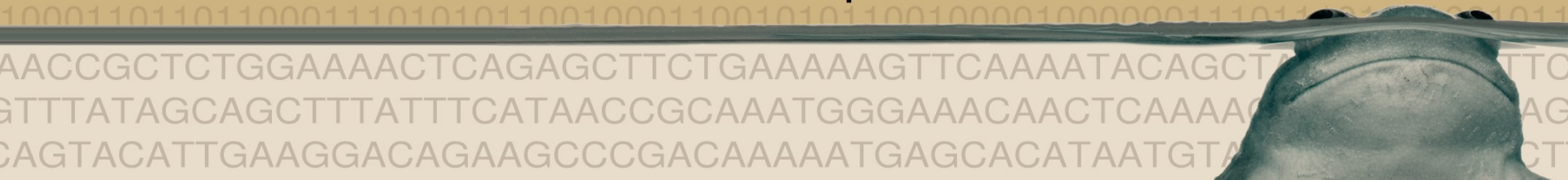
Xenopus functional genomics: Computational analysis of ChIP-seq data

Simon J. van Heeringen

14th International Xenopus conference



Support and more info

http://simonvh.github.com/xenopus2012

- Contains all presentations
- Links to databases, tools and materials

Mail me: s.vanheeringen@ncmls.ru.nl

Our website:
http://www.ncmls.nl/gertjanveenstra

Beyond browsing

- Previous workshop showed a visual approach
  - Good to look at your favorite gene
  - Generate hypothesis
- Usually, a more detailed analysis is necessary
  - Visual inspection can be deceiving
  - Need to know general, genome-wide patterns

Disclaimer

- There are commercial solutions
  - Genomatix
  - CLC bio
  - Avadis
  - And other...

- However, check if they support *Xenopus!*

Disclaimer

- There are commercial solutions
- If you're going to do a lot of analysis, it's worth learning command-line approaches
  - Even if you don't have your own Linux server / computer (“cloud”, Amazon EC2)

Disclaimer

- There are commercial solutions
- If you're going to do a lot of analysis, it's worth learning command-line approaches

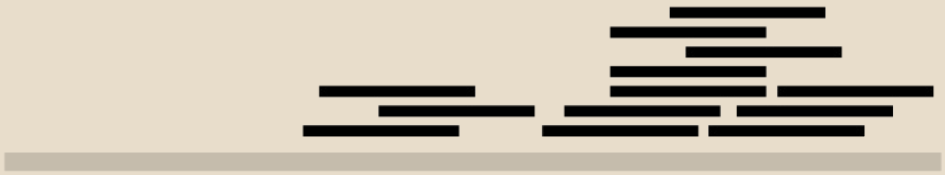
- This is an introduction to basic analysis using freely available (web) resources
  - Which can get you quite far

ChIP-seq workflow

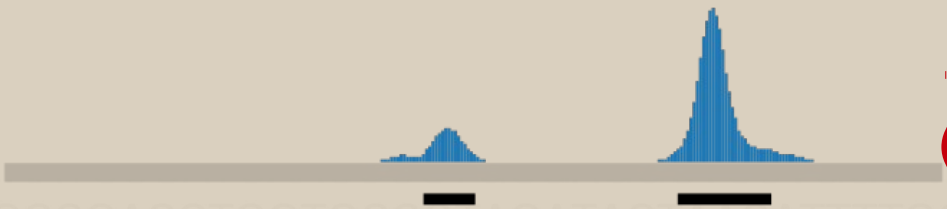
Data type

TAACGTGAACCCCTCTATCTTCCTTCACAGATTG
TAGTTTCTCACTTCAAGTTATCCAGCAACCTTGGA
TTTGAACATCATGTTCCTGCATGTTTTGGTGCTTG
GACTGCTAATATCCTTATCATTTACAAAAGGGTAC
GCTTTTTACATTCGGACCACTTAATAAATGACTAG
TCCTTATCCTATGCTCTTATACCCCATATTACTGC
CAGAACAGGAATGAGGGGTCTCTAAATGGCTGATA
CTGCTAAATGTCAATAACTATAATAGCTATGATTT
TGTGGTATTTTATCAAATACATGTTTAAACAAATG
TCCCTATCTTTAAAATCCAGTGCACTAAAGAATTG

raw reads



aligned data



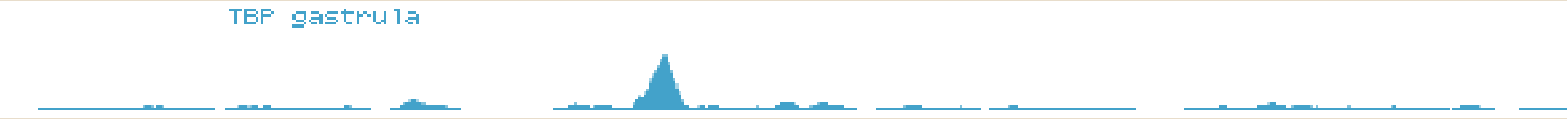
visualization, peak calling

Peak calling

- Sounds easy, doesn't it?

Peak calling

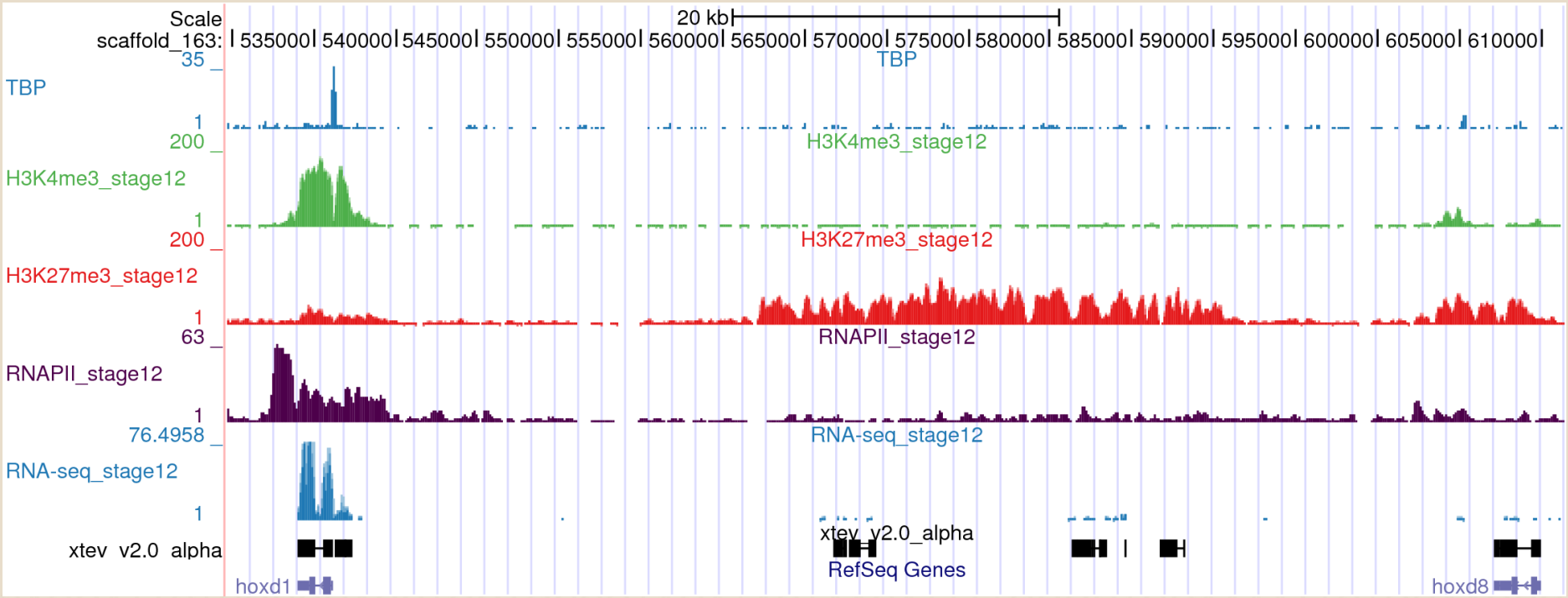
- Sounds easy, doesn't it?



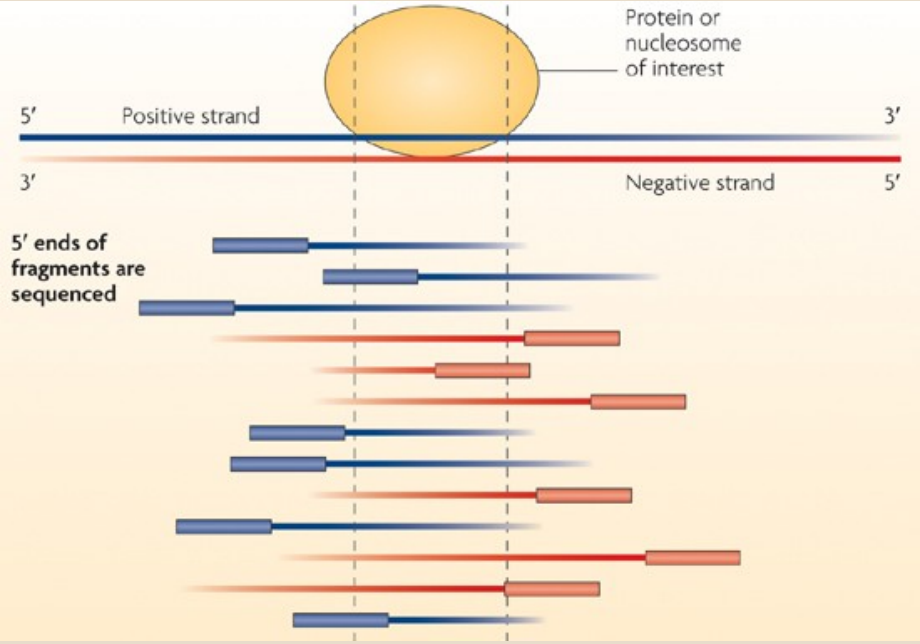
Peak!

Peak calling

- One peak-caller to call them all?

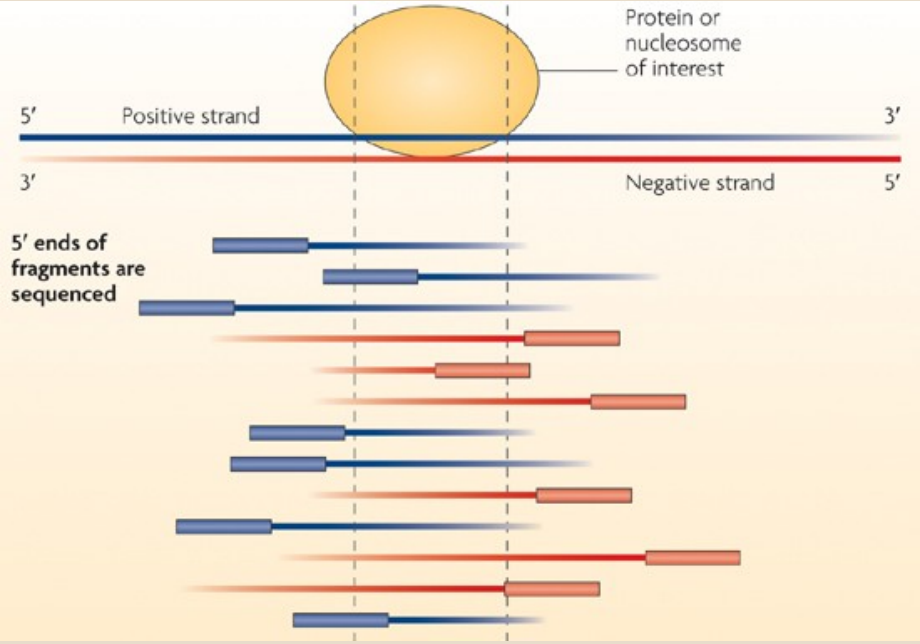


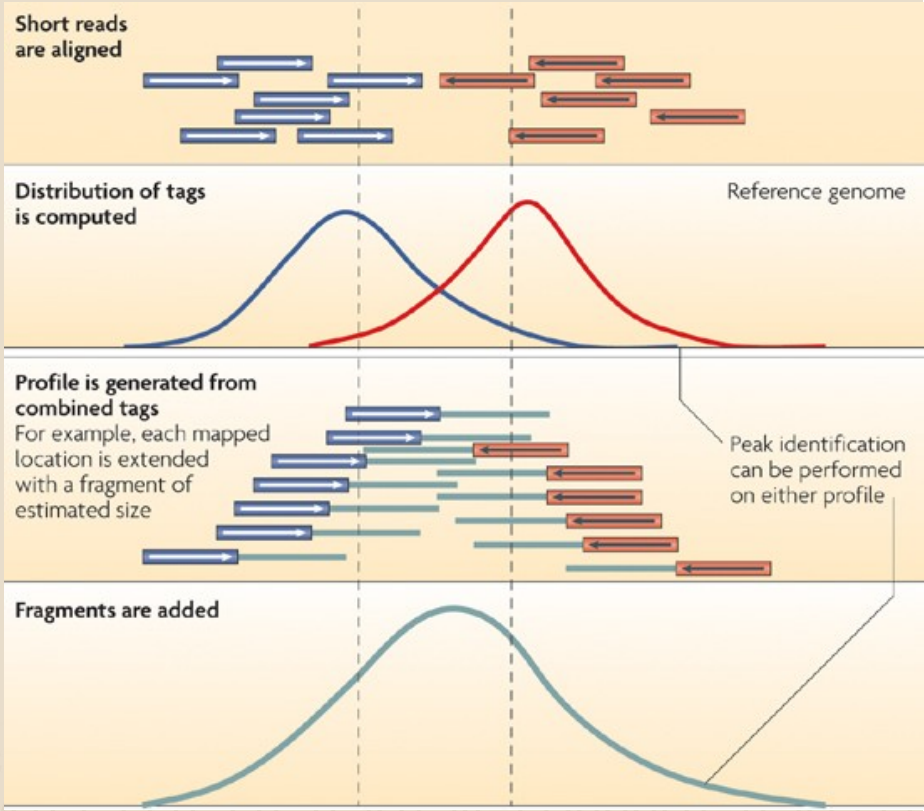
Strand information



Adapted from: Park, Nature, 2009

Strand information





Adapted from: Park, Nature, 2009

Which one to use?

- Many options, I've lost count...
- Many don't support *Xenopus* out-of-the-box
  - “What's 'scaffolds', Precious?”
- My personal (likely biased) advice
  - MACS (widely used)
    - With or without control
  - PeakRanger (modENCODE)
    - With control
- YMMV

Galaxy

- https://main.g2.bx.psu.edu/
- Web-based analysis
- Easy-to-use
- Lots of tutorials
- First choice if:
  - you don't have your own analysis server
  - you are unfamiliar with Linux, command-line based tools

Opera | X. tropicalis scaffol... | Galaxy | GEO Accession vie...

Secure main.g2.bx.psu.edu galaxy genome

Galaxy

Analyze Data | Workflow | Shared Data | Visualization | Cloud | Help | User | Using 0%

Tools

search tools

- Get Data
- Send Data
- ENCODE Tools
- Lift-Over
- Text Manipulation
- Convert Formats
- FASTA manipulation
- Filter and Sort
- Join, Subtract and Group
- Extract Features
- Fetch Sequences
- Fetch Alignments
- Get Genomic Scores
- Operate on Genomic Intervals
- Statistics
- Graph/Display Data
- Regional Variation
- Multiple regression
- Multivariate Analysis
- Evolution
- Motif Tools
- Multiple Alignments
- Metagenomic analyses
- Phenotype Association
- Genome Diversity
- EMBOSS

NGS TOOLBOX BETA

- NGS: QC and manipulation
- NGS: Mapping
- NGS: SAM Tools
- NGS: GATK Tools (beta)
- NGS: Indel Analysis
- NGS: Peak Calling
- NGS: RNA Analysis

Live Quickies

Galaxy is an open, web-based platform for data intensive biomedical research. Whether on this free public server or your own instance, you can perform, reproduce, and share complete analyses. The Galaxy team is a part of BX at Penn State, and the Biology and Mathematics and Computer Science departments at Emory University. The Galaxy Project is supported in part by NSF, NHGRI, The Huck Institutes of the Life Sciences, The Institute for CyberScience at Penn State, and Emory University.

Galaxy build: **$Rev 7614:e6444e7a1685$**

galaxyproject

galaxyproject Advanced NGS Course: RNA-seq, @ Amsterdam Medical Centre (AMC) 15 - 17 October. Galaxy & R practicals. bit.ly/O8TLHc #usegalaxy
2 days ago · reply · retweet · favorite

caseybergman .@rvosa shows us how to use phylotastic tools inside galaxy (youtu.be/kMME658xOu4) #phylogenetics #bioinformatics #usegalaxy
4 days ago · reply · retweet · favorite

caseybergman .@rvosa shows us how to add a phylotastic service to galaxy (youtu.be/d-fDngweW-M) #phylogenetics #bioinformatics #usegalaxy
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more ...

This is a free, public, internet accessible resource. Data transfer and data storage are not encrypted. If there are restrictions on the way your research data can be stored and used, please consult your local institutional review board or the project PI before uploading it to any public site, including this Galaxy server. If you have protected data, large data storage requirements, or short deadlines you are encouraged to setup your own local Galaxy instance or run Galaxy on the cloud.

History

0 bytes

Your history is empty. Click 'Get Data' on the left pane to start

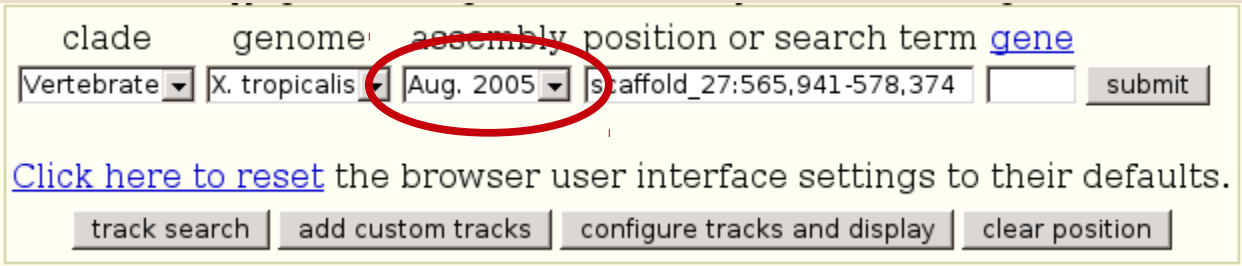
Example

- Peak calling using Galaxy

- TBP ChIP-seq (van Heeringen et al., 2011)
- Download from GEO
- Peak calling with MACS
- Upload peaks to Genome Browser for inspection

Try it out yourself!

- Resources are linked on the tutorial page
- Notice: I'm using **JGI 4.1 / xenTro2** data



Scope: Self Format: HTML Amount: Quick GEO accession: GSM537039 GO

Sample GSM537039

Query DataSets for GSM537039

Status	Public on Dec 30, 2010
Title	TBP_ChIPSeq
Sample type	SRA
Source name	whole embryo
Organism	Xenopus (Silurana) tropicalis
Characteristics	development stage: 12 antibody: SL33 against TBP
Growth protocol	Xenopus tropicalis embryos were obtained from a natural mating procedure after human chorion gonadotropin injection, dejellied in 3% cysteine and collected at Nieuwkoop-Faber stage 12
Extracted molecule	genomic DNA
Extraction protocol	Chromatin (400 embryos) was fixed in 1% formaldehyde, sheared using a Branson sonifier, immunoprecipitated using 2 µl of antibody (SL33 against TBP, Ruppert et al. 1996,PMID: 9064287), washed, decrosslinked, DNA was purified and sequenced using a Illumina Genome Analyzer according to the manufacturer.
Library strategy	ChIP-Seq
Library source	genomic
Library selection	ChIP
Instrument model	Illumina Genome Analyzer
Description	Chromatin IP against TBP
Data processing	Reads were mapped to the X.tropicalis genome (version JGI4.1) with ELAND, reads in identical positions were removed and peaks were called using MACS with p-value 1e-7 The included bed-files contain xenTro2/JGI4.1 coordinates. These tab-delimited files contain up to 6 columns according to the BED specification. 1) chromosome 2) start 3) end 4) score (see description below) 5) not used 6) strand The file TBP.bed contains the coordinates of the filtered unique 35-basepair reads, mapped strand-specific to the xenTro2 genome. The TBP_peaks.bed file contain the peaks as determined by MACS using a

The included bed-files contain xenTro2/JGI4.1 coordinates. These tab-delimited files contain up to 6 columns according to the BED specification.
1) chromosome
2) start
3) end
4) score (see description below)
5) not used
6) strand

The file TBP.bed contains the coordinates of the filtered unique 35-basepair reads, mapped strand-specific to the xenTro2 genome.
The TBP_peaks.bed file contain the peaks as determined by MACS using a p-value threshold of 1e-7. The score column contains the number of reads in the peak as determined by MACS.

Submission date	Apr 22, 2010
Last update date	Dec 30, 2010
Contact name	Gert Jan Veenstra
E-mail(s)	g.veenstra@ncmls.ru.nl
Phone	+31 24 3610541
Fax	+31-24-3610523
URL	http://www.ncmls.nl/gertjanveenstra/home.htm
Organization name	Radboud University
Department	Molecular Biology
Street address	Geert Grooteplein 28
City	Nijmegen
ZIP/Postal code	6525 GA
Country	Netherlands

Platform ID GPL9405
Series (1) GSE21482 Nucleotide composition-linked divergence of vertebrate core promoter architecture

Relations
SRA SRX019583

Supplementary file	Size	Download	File type/resource
SRX/SRX019/SRX019583		(ftp)	SRA Experiment
GSM537039_TBP.bed.gz	68.9 Mb	(ftp)(http)	BED
GSM537039_TBP_peak.bed.gz	60.7 Kb	(ftp)(http)	BED

Raw data provided as supplementary file
Processed data provided as supplementary file

Opera | X. laevis - UCSC G... | Galaxy | GEO Accession vie...

Web | main.g2.bx.psu.edu | Search with Google

Galaxy

Analyze Data | Workflow | Shared Data | Visualization | Cloud | Help | User | Using 0%

Tools

search tools

Get Data

- Upload File from your computer
- UCSC Main table browser
- UCSC Archaea table browser
- BX table browser
- EBI SRA ENA SRA
- BioMart Central server
- GrameneMart Central server
- Flymine server
- modENCODE fly server
- modENCODE modMine server
- Ratmine server
- YeastMine server
- modENCODE worm server
- WormBase server
- EuPathDB server
- EncodeDB at NHGRI
- EpiGRAPH server

Send Data

ENCODE Tools

Lift-Over

Text Manipulation

Convert Formats

FASTA manipulation

Filter and Sort

Join, Subtract and Group

Extract Features

Fetch Sequences

Fetch Alignments

Get Genomic Scores

Upload File (version 1.1.3)

File Format:

Auto-detect

Which format? See help below

File:

"/home/simon/tmp/GSM | Choose...

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:

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

Please create or log in to a Galaxy account to view files uploaded via FTP.

This Galaxy server allows you to upload files via FTP. To upload some files, log in to the FTP server at **main.g2.bx.psu.edu** using your Galaxy credentials (email address and password).

Convert spaces to tabs:

☐ Yes

Use this option if you are entering intervals by hand.

Genome:

·opicalis Aug. 2005 (JGI 4.1/xenTro2) (xenTro2)

Execute

Auto-detect

The system will attempt to detect Axt, Fasta, Fastqsolexa, Gff, Gff3, Html, Lav, Maf, Tabular, Wiggle, Bed and Interval (Bed with headers) formats. If your file is not detected properly as one of the known formats, it most likely means that it has some format problems (e.g., different number of columns on different rows). You can still coerce the system to set your data to the format you think it should be. You can also upload compressed files, which will automatically be decompressed.

Ab1

A binary sequence file in 'ab1' format with a '.ab1' file extension. You must manually select this 'File Format' when uploading the file.

History

0 bytes

Your history is empty. Click 'Get Data' on the left pane to start

Opera | X. laevis - UCSC G... | Galaxy | GEO Accession vie...

Web | main.g2.bx.psu.edu | Elements: 65/65 | Search with Google

Galaxy

Analyze Data | Workflow | Shared Data | Visualization | Cloud | Help | User | Using 0%

Tools

- Statistics
- Graph/Display Data
- Regional Variation
- Multiple regression
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- Genome Diversity
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NGS TOOLBOX BETA

- NGS: QC and manipulation
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- NGS: Indel Analysis
- NGS: Peak Calling
  - MACS Model-based Analysis of ChIP-Seq
  - SICER Statistical approach for the Identification of ChIP-Enriched Regions
  - GeneTrack indexer on a BED file
  - Peak predictor on GeneTrack index
- NGS: RNA Analysis
- NGS: Picard (beta)
- BEDTools
- snpEff

RGENETICS

- SNP/WGA: Data; Filters
- SNP/WGA: QC; LD; Plots
- SNP/WGA: Statistical Models

MACS (version 1.0.1)

Experiment Name:
MACS in Galaxy

Paired End Sequencing:
Single End

ChIP-Seq Tag File:
2: TBP_scaffold_1.bed

ChIP-Seq Control File:
Selection is Optional

Effective genome size:
1500000000
default: 2.7e+9

Tag size:
35

Band width:
300

Pvalue cutoff for peak detection:
1e-05
default: 1e-5

Select the regions with MFOLD high-confidence enrichment ratio against background to build model:
32

Parse xls files into into distinct interval files:
☐

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:
☐
up to 9X more time consuming

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:

History

0 bytes

2: TBP_scaffold_1.bed

Opera | Manage Cust... | Galaxy | GEO Accessio... | X. tropicalis s... | Veenstra Lab... | Postvak IN - ... | simonvh/xen... | Xenopus2012

Web main.g2.bx.psu.edu

Search with Google

Galaxy

Analyze Data | Workflow | Shared Data | Visualization | Cloud | Help | User | Using 0%

Tools

Statistics
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snpEff

RGENETICS

SNP/WGA: Data; Filters
SNP/WGA: QC; LD; Plots
SNP/WGA: Statistical Models

The following job has been successfully added to the queue:

3: MACS on data 2 (peaks: bed)

4: MACS on data 2 (html report)

You can check the status of queued jobs and view the resulting data by refreshing the **History** pane. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History

2.0 MB

4: MACS on data 2 (html report)

3: MACS on data 2 (peaks: bed)

52 regions, 1 comments
format: bed, database: xenTro2

display at UCSC main
view in GeneTrack
display in IGB Local Web
display at Ensembl Current

1. Chrom	2. Start	3. End	4. Name	5
track name="MACS peaks for MACS_in_Galaxy"				
scaffold_1	135	425	MACS_peak_1	57.64
scaffold_1	90495	92200	MACS_peak_2	99.93
scaffold_1	46425	46467	MACS_peak_3	64.97
scaffold_1	49413	49572	MACS_peak_4	109.53
scaffold_1	55904	56086	MACS_peak_5	243.24

2: TBP_scaffold_1.bed

Opera | X. tropicalis scaffol... | Galaxy

Web genomes.nimr.mrc.ac.uk/cgi-bin/hgTracks

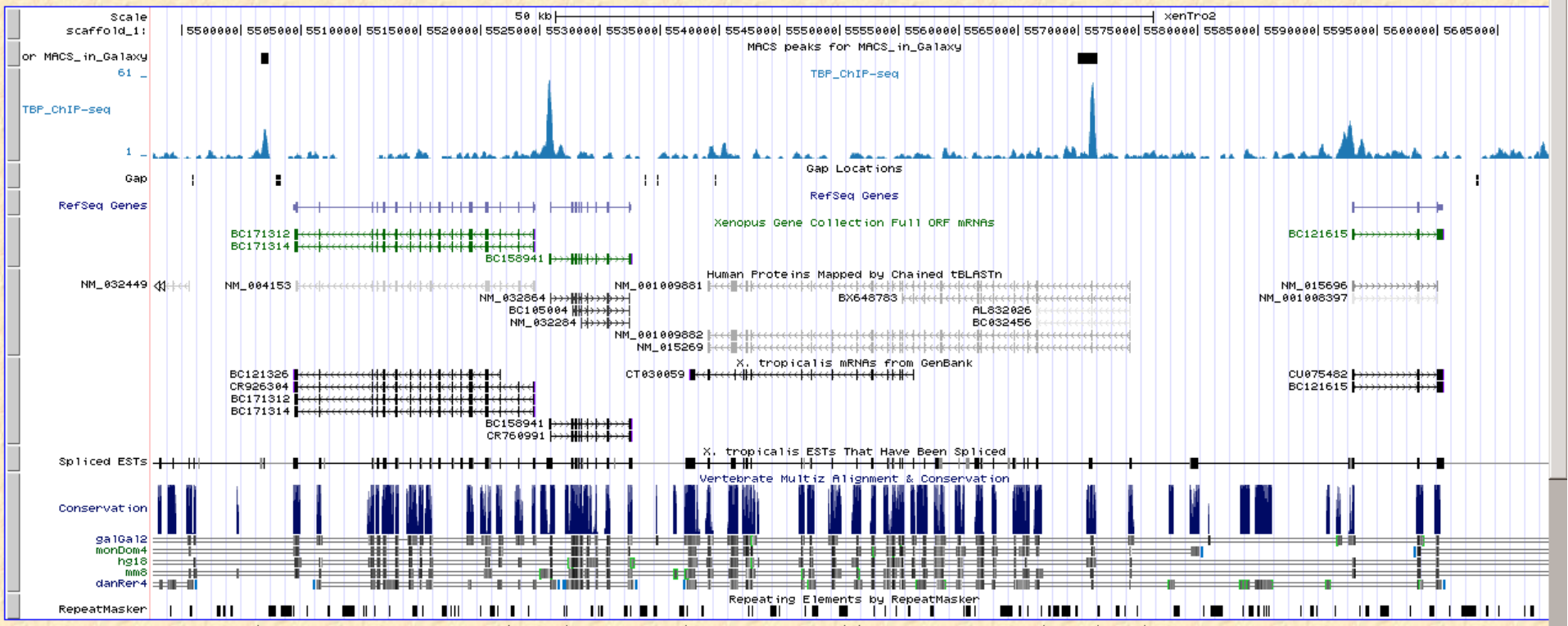
Search with Google

Home | Genomes | Blat | Tables | DNA | Convert | PDF/PS | Session | Help

UCSC Genome Browser on X. tropicalis Aug. 2005 Assembly (xenTro2)

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

position/search scaffold_1:5,492,633-5,609,93 gene jump clear size 117,300 bp. configure



move start < 2.0 >

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.

move end < 2.0 >

track search | default tracks | default order | hide all | manage custom tracks | 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

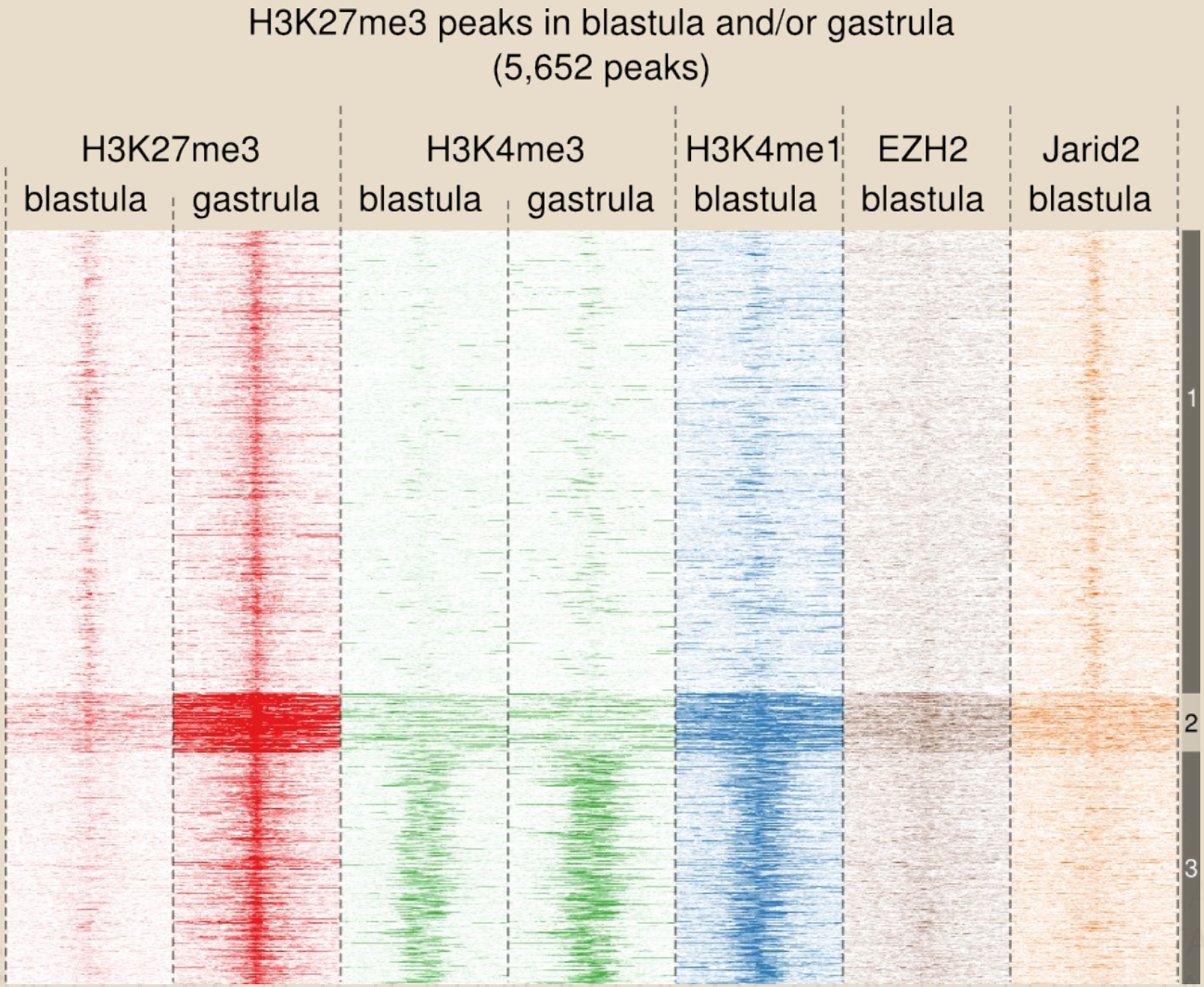
MACS peaks for MACS_in_Galaxy — dense

TBP_ChIP-seq — full

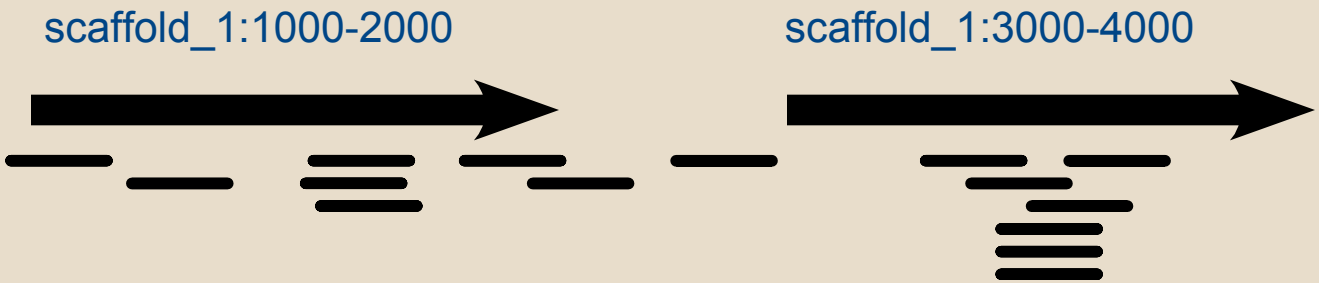
Mapping and Sequencing Tracks

refresh

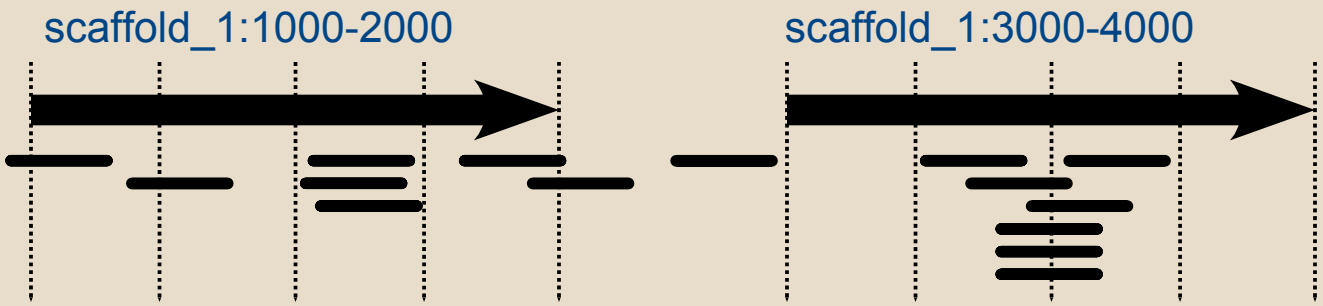
Heatmaps


H3K27me3 peaks in blastula and/or gastrula
(5,652 peaks)
H3K27me3
blastula
gastrula
H3K4me3
blastula
gastrula
H3K4me1
blastula
EZH2
blastula
Jarid2
blastula
1
2
3

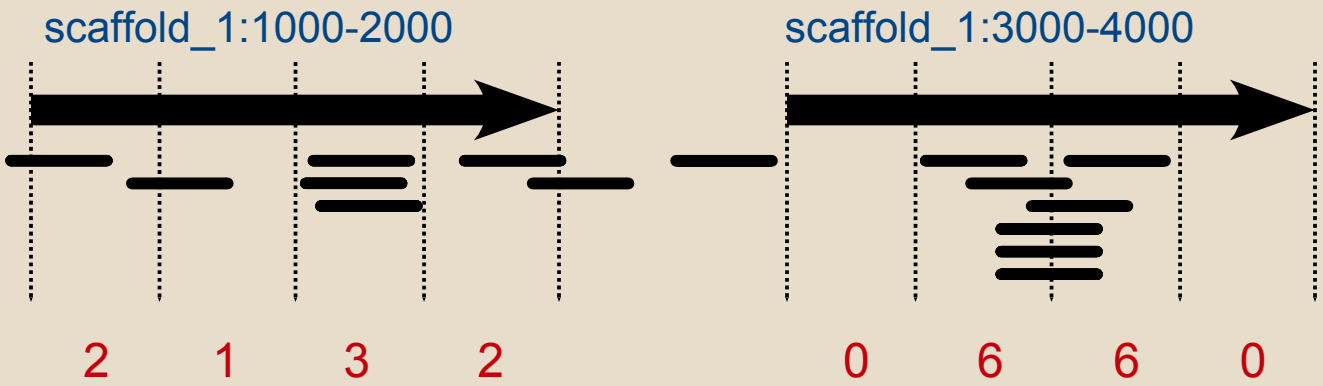
Heatmaps



Heatmaps



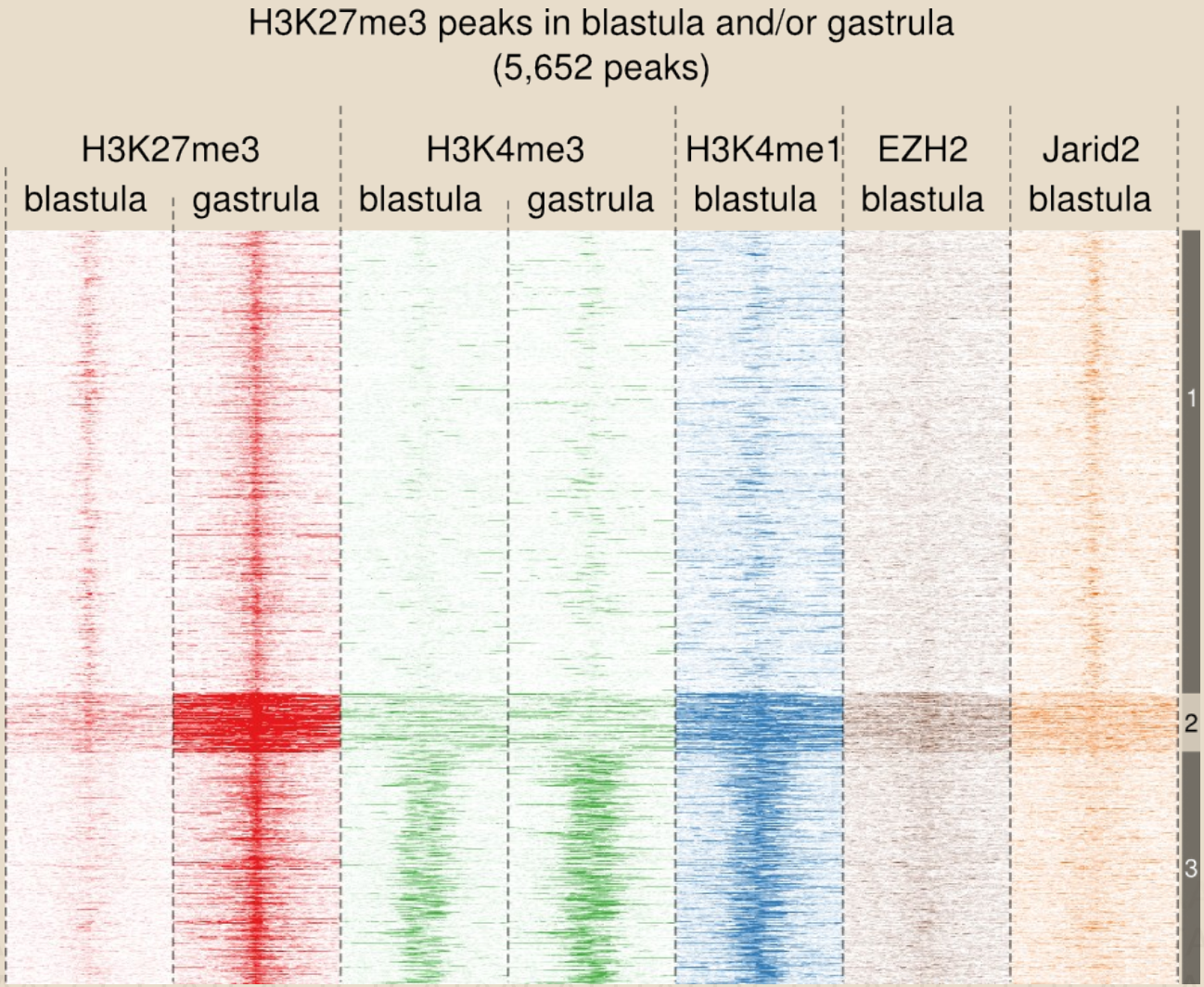
Heatmaps



Heatmap data:

scaffold_1	1000	2000	2	1	3	2
scaffold_1	3000	4000	0	6	6	0

Heatmaps


H3K27me3 peaks in blastula and/or gastrula
(5,652 peaks)
H3K27me3
blastula
gastrula
H3K4me3
blastula
gastrula
H3K4me1
blastula
EZH2
blastula
Jarid2
blastula
1
2
3

Heatmaps (from easy to “difficult”)

- seqMINER http://bips.u-strasbg.fr/seqminer/tiki-index.php
  - Graphical Interface
  - Easy to use
- fluff http://github.com/simonvh/fluff
  - Command-line
  - Colorful plots
- R
  - Powerful language for statistical computing
  - Many plotting functions
  - Heatmaps using gplots library

seqMINER

- Java program
  - Runs on Windows, Mac OS X, Linux
  - Easy to use
  - Easy to play around with settings
  - Runs on modest hardware
    - However: more tracks = more memory needed
  - Export is somewhat iffy
    - it gets confused by scaffolds

Simple example

- Goal: heatmap of H3K4me3 and H3K27me3 around TSS of genes

- Download BED data from GEO (GSE14025)
- Create the TSS of Xtev genes using UCSC Table Browser

Xtev genes

- Based on Xenbase, JGI, Ensembl, Refseq genes and EST data (Akkers, 2009)
- Available at http://www.ncmls.nl/gertjanveenstra

- Upload track as custom track in UCSC Genome Browser
- Then, use the Table Browser

Web genomes.nimr.mrc.ac.uk/cgi-bin/hgTables Search with Google

Home Genomes Genome Browser Blat Tables Session FAQ Help

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: Vertebrate **genome:** X. tropicalis **assembly:** Aug. 2005

group: Custom Tracks **track:** Xtev_v1.0 manage custom tracks

table: ct_Xtev10_8432 describe table schema

region: genome position scaffold_163:520000-6900 lookup define regions

identifiers (names/accessions): paste list upload list

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

get output summary/statistics

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

Using the Table Browser

This section provides brief line-by-line descriptions of the Table Browser controls. For more information on using this program, see the Table Browser User's Guide.

- **clade:** Specifies which clade the organism is in.
- **genome:** Specifies which organism data to use.
- **assembly:** Specifies which version of the organism's genome sequence to use.
- **group:** Selects the type of tracks to be displayed in the *track* list. The options correspond to the track groupings shown in the Genome Browser. Select 'All Tracks' for an alphabetical list of all available tracks in all groups. Select 'All Tables' to see all tables including those not associated with a track.
- **database:** (with "All Tables" group option) Determines which database should be used for options in table menu.
- **track:** Selects the annotation track data to work with. This list displays all tracks belonging to the group specified in the group list.

Web genomes.nimr.mrc.ac.uk/cgi-bin/hgTables Search with Google

Home Genomes Genome Browser Blat Tables Session FAQ Help

Output ct_Xtevv10_8432 as BED

☐ **Include custom track header:**

name= xtev_tss

description= TSS of Xtev models

visibility= pack

url=

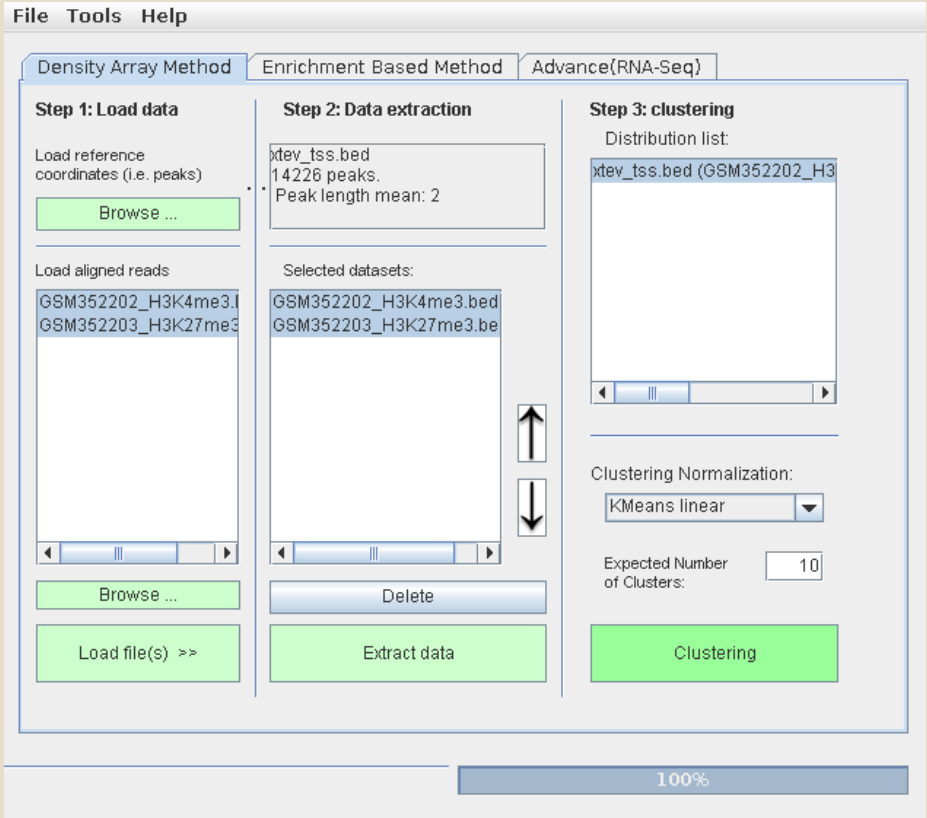
Create one BED record per:

- ○ Whole Gene
- ◉ Upstream by 1 bases
- ○ Exons plus 0 bases at each end
- ○ Introns plus 0 bases at each end
- ○ 5' UTR Exons
- ○ Coding Exons
- ○ 3' UTR Exons
- ○ Downstream by 200 bases

Note: if a feature is close to the beginning or end of a chromosome and upstream/downstream bases are added, they may be truncated in order to avoid extending past the edge of the chromosome.

get BED cancel

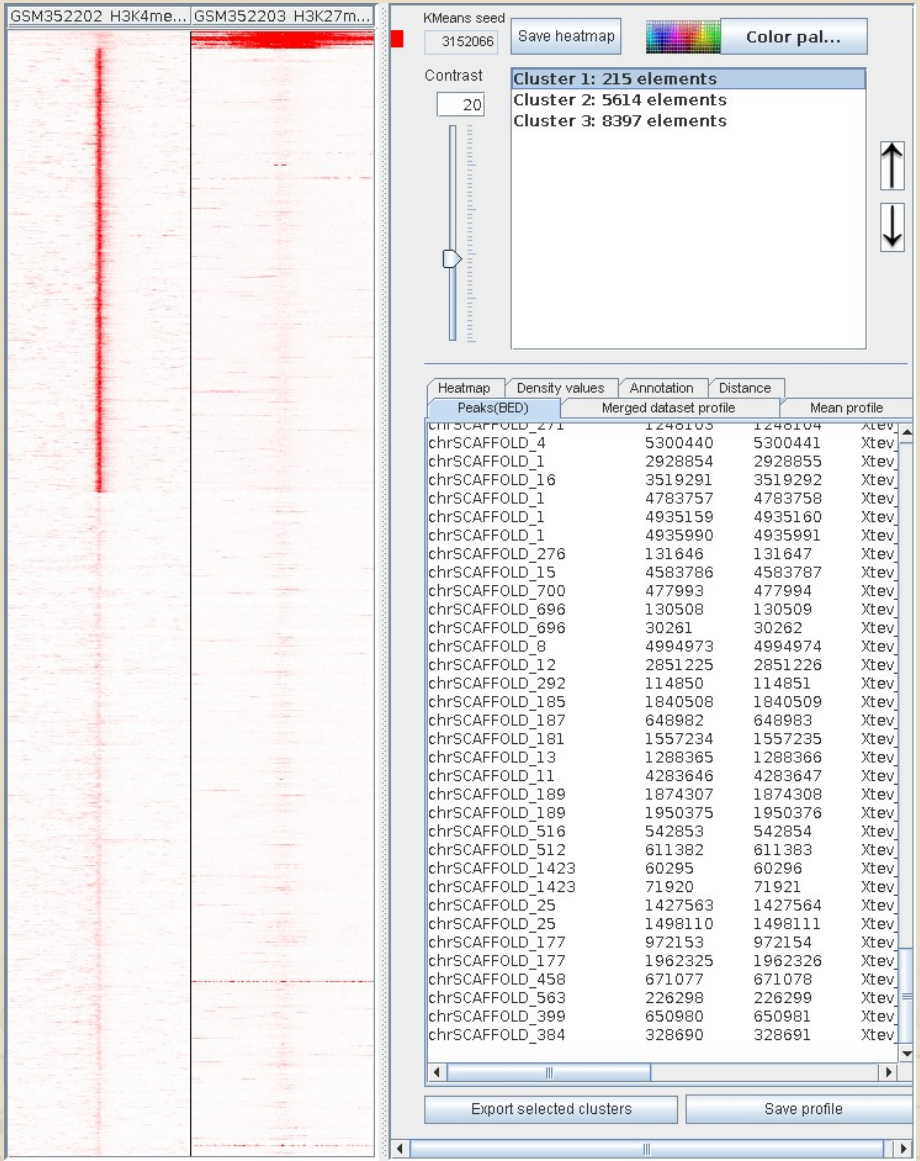
seqMINER



- load reference
- load reads (BED/BAM)
- extract data
- cluster

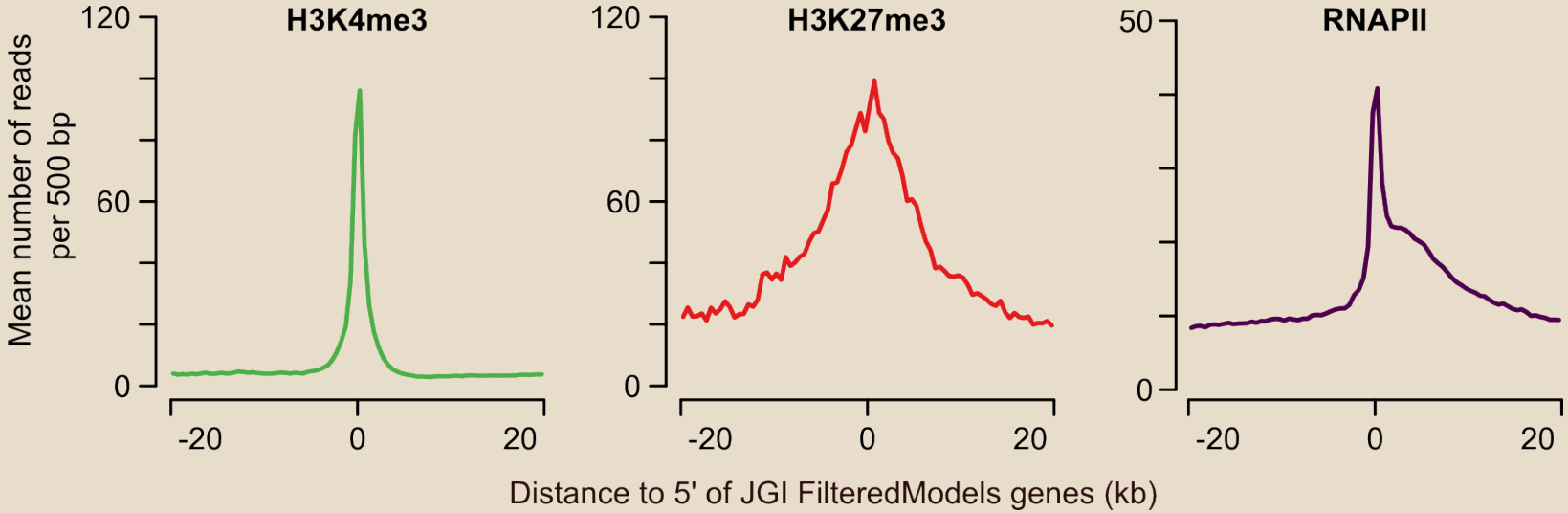
Tip: select window size under Tools

Result



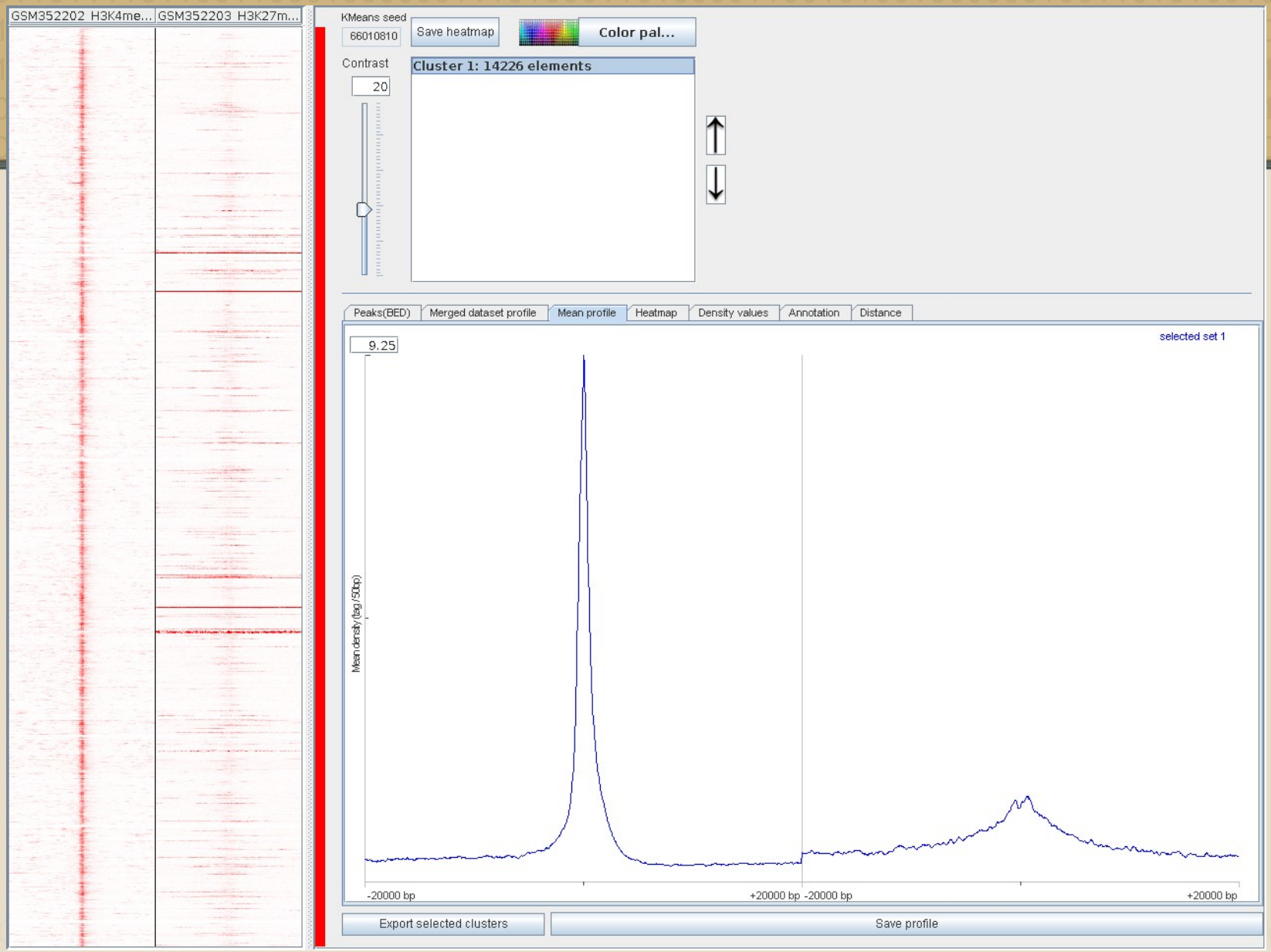
- Play around with number of clusters
- Vary contrast to see how that looks

Profiles


H3K4me3
H3K27me3
RNAPII
Mean number of reads per 500 bp
120
60
0
50
-20
0
20
Distance to 5' of JGI FilteredModels genes (kb)

Creating profiles

- Many ways, as usual
  - R / Bioconductor
  - Python: HTSeq
  - seqMINER
- seqMINER can do it for each cluster
  - To generate a profile of the whole dataset, just use 1 cluster ;-)


GSM352202 H3K4me...
GSM352203 H3K27m...
KMeans seed
66010810
Save heatmap
Color pal...
Contrast
20
Cluster 1: 14226 elements
Peaks(BED)
Merged dataset profile
Mean profile
Heatmap
Density values
Annotation
Distance
9.25
selected set 1
Mean density (tag/50bp)
-20000 bp
+20000 bp
-20000 bp
+20000 bp
Export selected clusters
Save profile

Thank you for your attention

NCMLS
Radboud University, Nijmegen
Robert Akkers
Ozren Bogdanovic
Ulrike Jacobi
Gert Jan Veenstra

MRC National Institute for Medical Research

Mike Gilchrist
Ilya Patrushev

National Institutes of Health

NWO Netherlands Organisation for Scientific Research

Radboud University Nijmegen

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