Galaxy: Analyze, Visualize, Communicate

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http://UseGalaxy.org

Overview

- What is Galaxy?
- Galaxy for Experimental Biologists
- Galaxy for Bioinformaticians

Galaxy, a web-based genome analysis platform

- An open-source **framework** for integrating various computational tools and databases into a cohesive workspace
- A web-based **service** we provide, integrating many popular tools and resources for comparative genomics
- A completely **self-contained application** for building your own **Galaxy** style sites

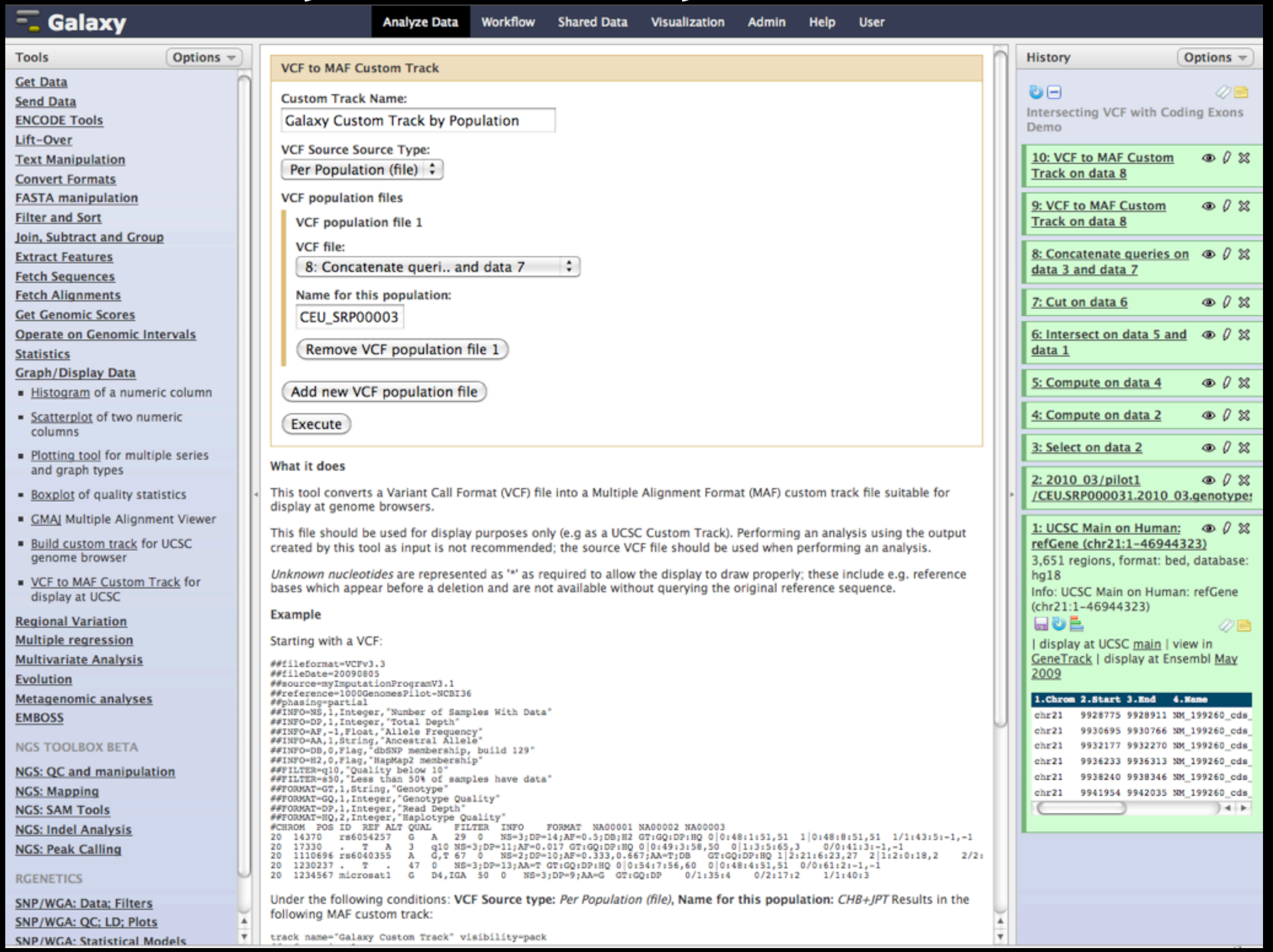
Overview

- What is Galaxy?
- Galaxy for Experimental Biologists
- Galaxy for Bioinformaticians

Galaxy: the one-stop shop for Genome Analysis

- Analyze
  - Retrieve data directly from popular data resources or upload your own
  - Interactively manipulate genomic data with a comprehensive and expanding "best-practices" toolset
- Visualize
  - Send data results to external Genome Browsers
  - Build reusable AJAX-based custom Genome Browsers (Trackster)
- Communicate (Publish and Share)
  - Results and step-by-step analysis record (Data Libraries and Histories)
  - Customizable pipelines (Workflows)
  - Complete protocols (Pages)

Galaxy's Analysis Interface



Visualize

- Send data results to external Genome Browsers
- Build reusable and sharable custom Genome Browsers (Trackster)

External Genome Browsers

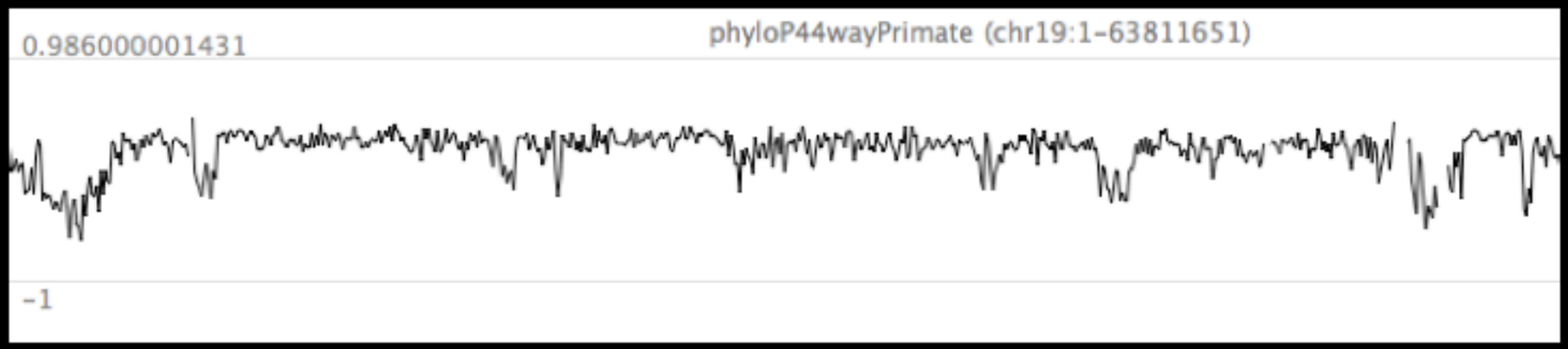
- UCSC
- Ensembl
- GBrowse

- Adding more is easy!
  - https://bitbucket.org/galaxy/galaxy-central/wiki/ExternalDisplayApplications/Tutorial

Trackster

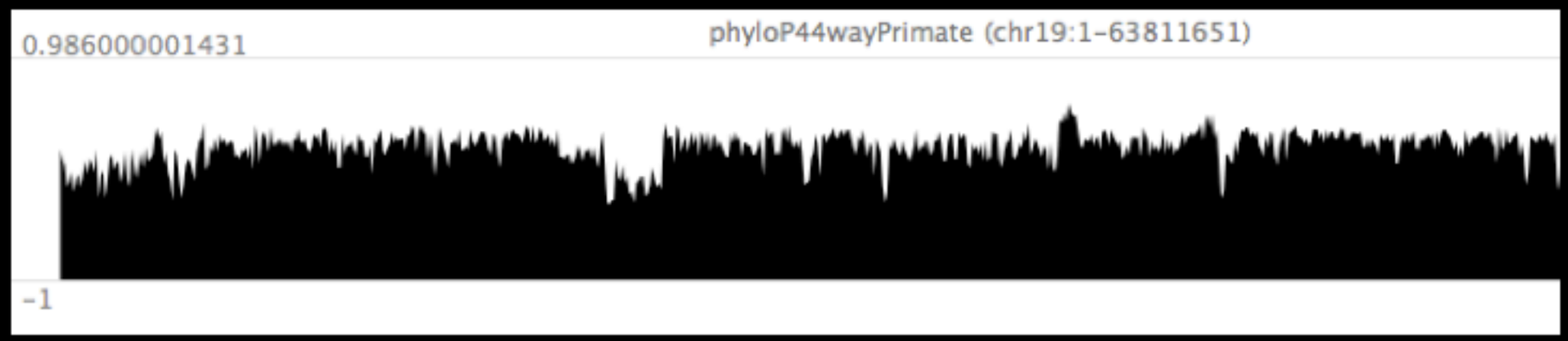
- Track/data viewer in web browser
  - HTML5 Canvas, jQuery
  - Renders in browser, not on server
- View your data from within Galaxy
  - No file transfers to third party
  - Use it locally, even without internet access
- Fast, responsive, interactive UI

Wig, Bedgraph (Line Tracks)



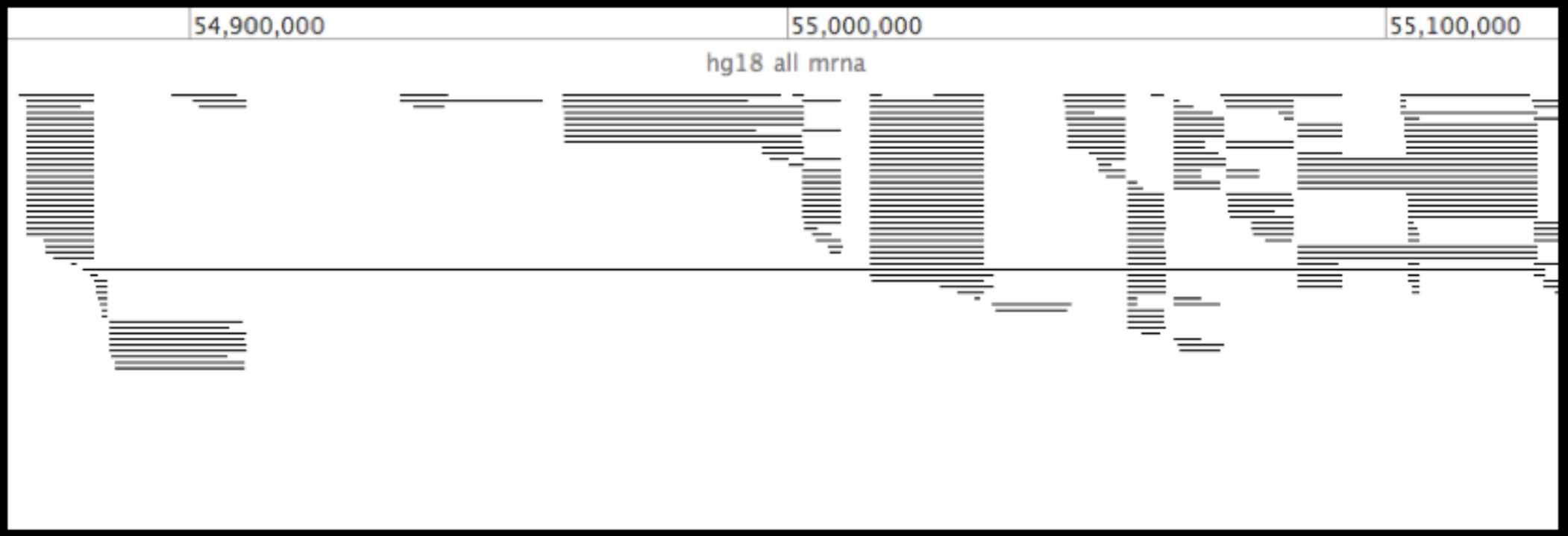
Regular line graph display

Intensity display (shades of gray)



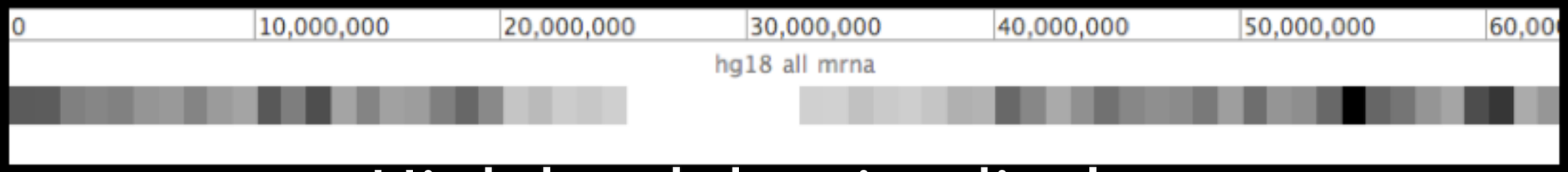
Filled line graph display

Bed (Feature Track)

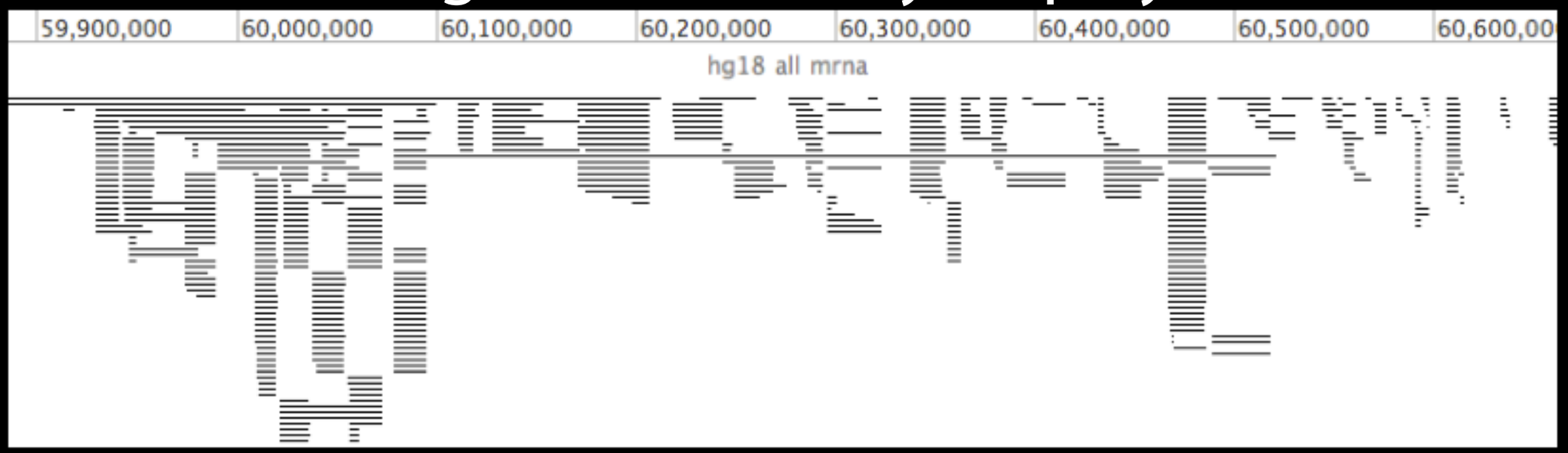


Snippet of hg18 all_mrna feature track

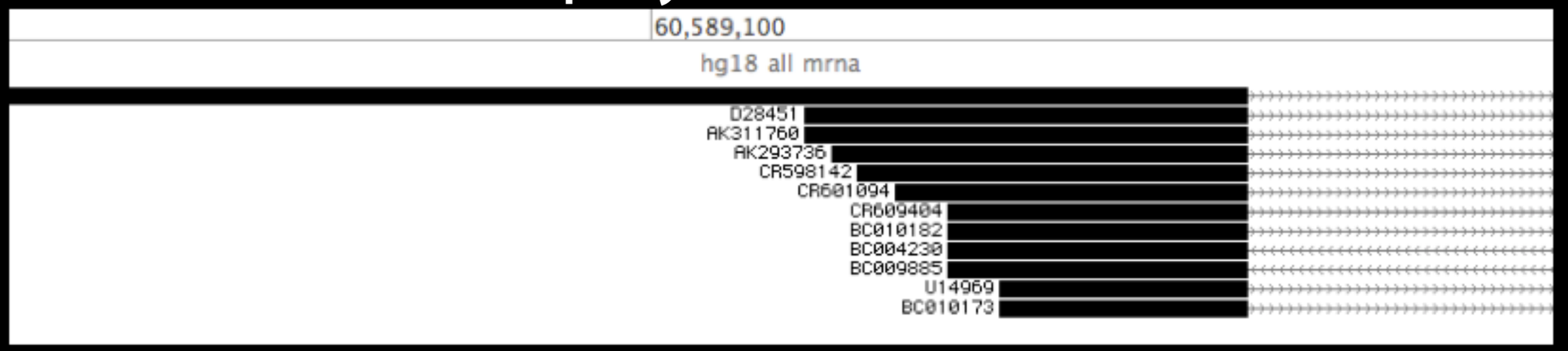
3 levels of detail: automatically adjusts based on what can fit on the screen



High level density display

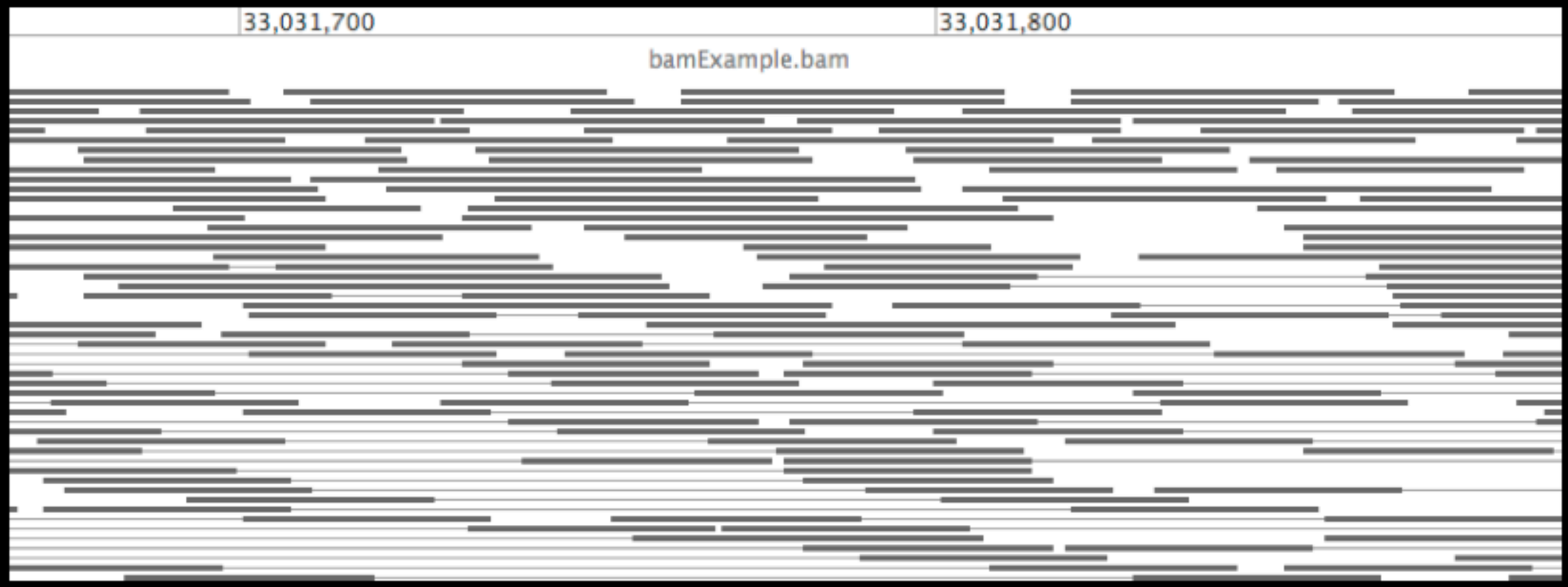


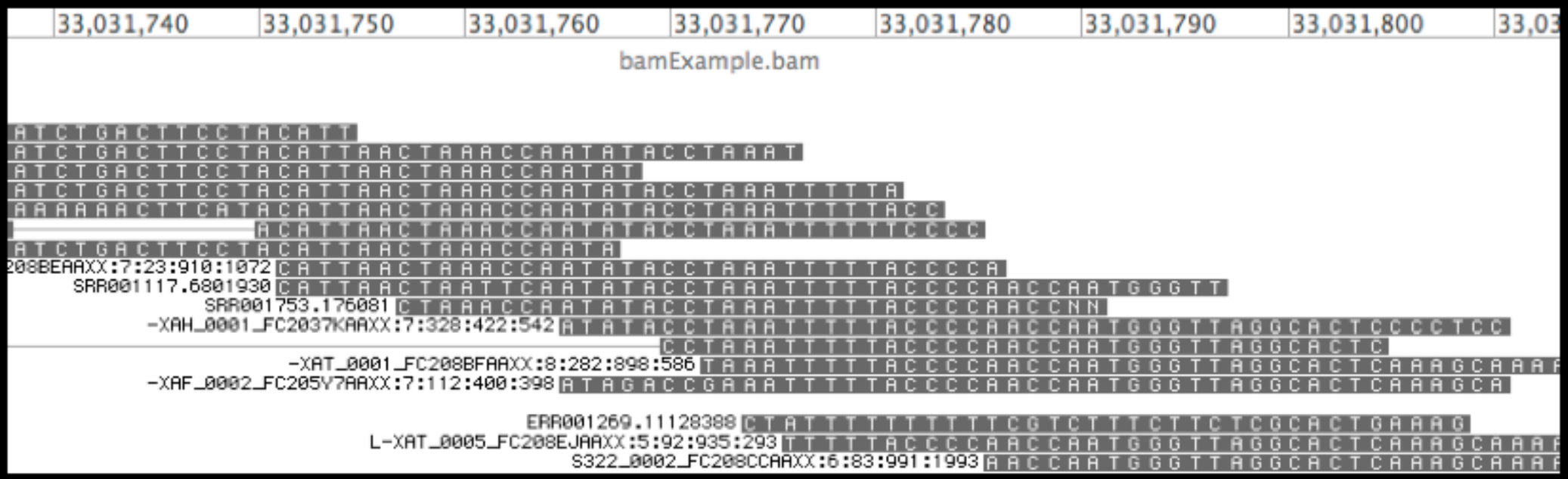
Feature display with no labels/detail



Feature display with labels, intron indicators, exon indicators

BAM (Aligned Reads)





Data Libraries

Data Library "Bushman"

Library Actions

These are the data underlying the analyses reported in the paper "Complete Khoisan and Bantu genomes from southern Africa" by S. C. Schuster et al., published in the journal Nature, February 18, 2010. Each data set can be downloaded and/or imported into a Galaxy history. Data will be updated as the project progresses.

Name	Information	Uploaded By	Date	File Size
All SNPs in personal genomes	Summary table of SNPs in all individuals	greg@bx.psu.edu	2010-01-28	676.8 Mb
Alu insertions in KB1		greg@bx.psu.edu	2010-02-10	14.9 Kb
Alu insertions in NB1		greg@bx.psu.edu	2010-02-10	6.5 Kb
KB1_microsatellites.txt		greg@bx.psu.edu	2010-02-15	3.5 Mb
NB1_microsatellites.txt		greg@bx.psu.edu	2010-02-15	828.5 Kb
amino acid differences with functional predictions		greg@bx.psu.edu	2010-02-05	1.1 Mb
gene copy numbers in KB1 and other personal genome		greg@bx.psu.edu	2010-02-15	2.1 Mb
indels in ABT		greg@bx.psu.edu	2010-02-03	105.3 Kb
indels in KB1		greg@bx.psu.edu	2010-02-03	14.2 Mb
indels in MD8		greg@bx.psu.edu	2010-02-03	109.8 Kb
indels in NB1		greg@bx.psu.edu	2010-02-03	519.5 Kb
indels in TK1		greg@bx.psu.edu	2010-02-03	123.2 Kb
novel SNPs in ABT		greg@bx.psu.edu	2010-02-09	9.4 Mb
novel SNPs in KB1		greg@bx.psu.edu	2010-02-09	16.9 Mb
novel SNPs in MD8		greg@bx.psu.edu	2010-02-09	594.1 Kb
novel SNPs in NB1		greg@bx.psu.edu	2010-02-09	4.1 Mb
novel SNPs in TK1		greg@bx.psu.edu	2010-02-09	722.6 Kb
sequenced exon-containing intervals		greg@bx.psu.edu	2010-02-03	3.1 Mb

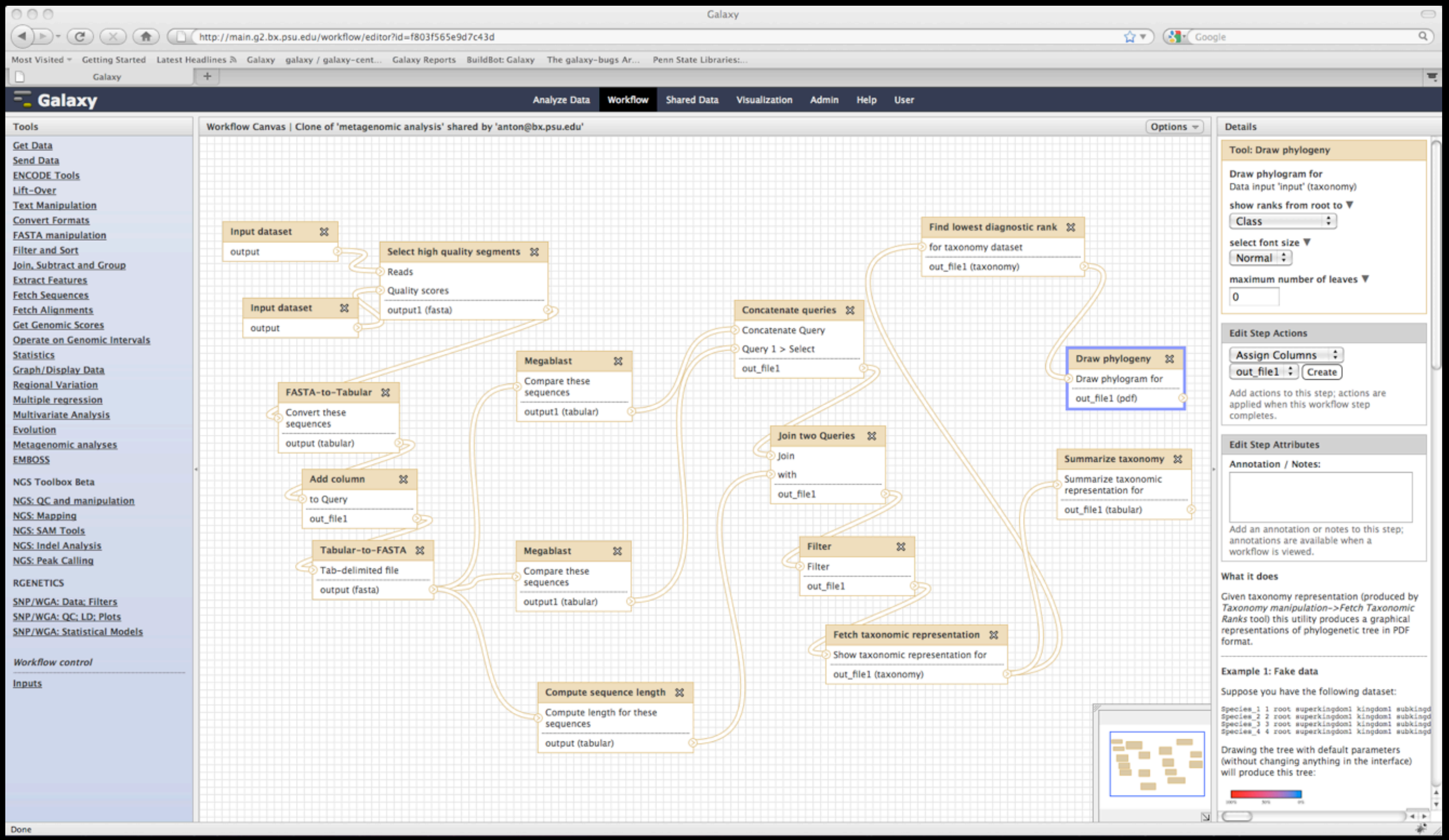
For selected items: Import into your current history Go

http://usegalaxy.org/bushman

Managing Libraries

- Loading Data
  - Upload a single file
  - Import datasets from a Galaxy history
  - Upload a directory of files
  - Directly from Sequencer using Sample Tracking System
- Accessing Data
  - Data contents on disk are not copied
  - Dataset security
    - Public
    - Role-based access control (RBAC)
- Annotating Library Data: Library Templates
  - Build user fillable forms
  - Associate at Library, Folder or Dataset level

Workflows



http://main.g2.bx.psu.edu/u/aun1/w/metagenomic-analysis

Pages

Windshield splatter analysis with the Galaxy metagenomic pipeline: A live supplement

SERGEI KOSAKOVSKY POND[1,2,*], SAMIR WADHAWAN[3,6*], FRANCESCA CHIAROMONTE[4], GURUPRASAD ANANDA[1,3], WEN-YU CHUNG[1,3,7], JAMES TAYLOR[1,5], ANTON NEKRUTENKO[1,3] and THE GALAXY TEAM[1,*]

Correspondence should addressed to SKP, JT, or AN.

How to use this document

This document is a live copy of supplementary materials for the manuscript. It provides access to the **exact** analyses and workflows discussed in the paper, so you can play with them by re-running, changing parameters, or even applying them to your own data. Specifically, we provide the two histories and one workflow found below. You can view these items by clicking on their name to expand them. You can also import these items into your Galaxy workspace and start using them; click on the green plus to import an item. To import workflows you must create a Galaxy account (unless you already have one) – a hassle-free procedure where you are only asked for a username and password.

This is the Galaxy history detailing the comparison of our pipeline to MEGAN:

Galaxy History | Galaxy vs MEGAN
Comparison of Galaxy vs. MEGAN pipeline.

This is the Galaxy history showing a generic analysis of metagenomic data. (This corresponds to the "A complete metagenomic pipeline" section of the manuscript and **Figure 3A**):

Galaxy History | metagenomic analysis

10: Concatenate queries on data 8 and data 7	Merge Megablast runs to produce a single dataset for reads compared to both WGS and NT
11: Join two Queries on data 9 and data 10	Combine sequence length data with results from Megablast runs
12: Filter on data 11	Filter suboptimal sequence alignments using the expression (sequence_alignment_length/sequence_read_length > 0.5); sequences that do not meet this criterion are filtered out
13: Fetch taxonomic representation on data 12	Get taxonomic representation for filtered, aligned sequences
14: Find lowest diagnostic rank on data 13	Get reads specific to ranks below Kingdom level
15: Summarize taxonomy on data 13	Tabulate list of taxonomic groups contained in reads from dataset 14
16: Draw phylogeny on data 14	Build and draw phylogenetic tree from ranks in dataset 14

This is the Galaxy workflow for generic analysis of metagenomic data. (This corresponds to the "A complete metagenomic pipeline" section of the manuscript and **Figure 3B**):

Galaxy Workflow | metagenomic analysis
Generic workflow for performing a metagenomic analysis on NGS data.

Supplemental Analysis

Comparison between Galaxy pipeline and Megan

(*Use this link to see Galaxy history representing this analysis. Individual elements of this history are referred to as* **History item1, 2** *and so on using* **bold** *typeface*)

The first step of a homology-based metagenomic analysis is to contrast a collection of sequencing reads against a database whose entries are assigned to taxonomic ranks. Following the procedure of (Huson et al. 2007) we used the non-redundant protein database (NR) from the National Center for Biotechnology Information. There are several avenues for importing large sets of alignments into Galaxy. First, alignments can be generated directly within Galaxy (see the following section). Alternatively, alignments generated elsewhere (e.g., using local

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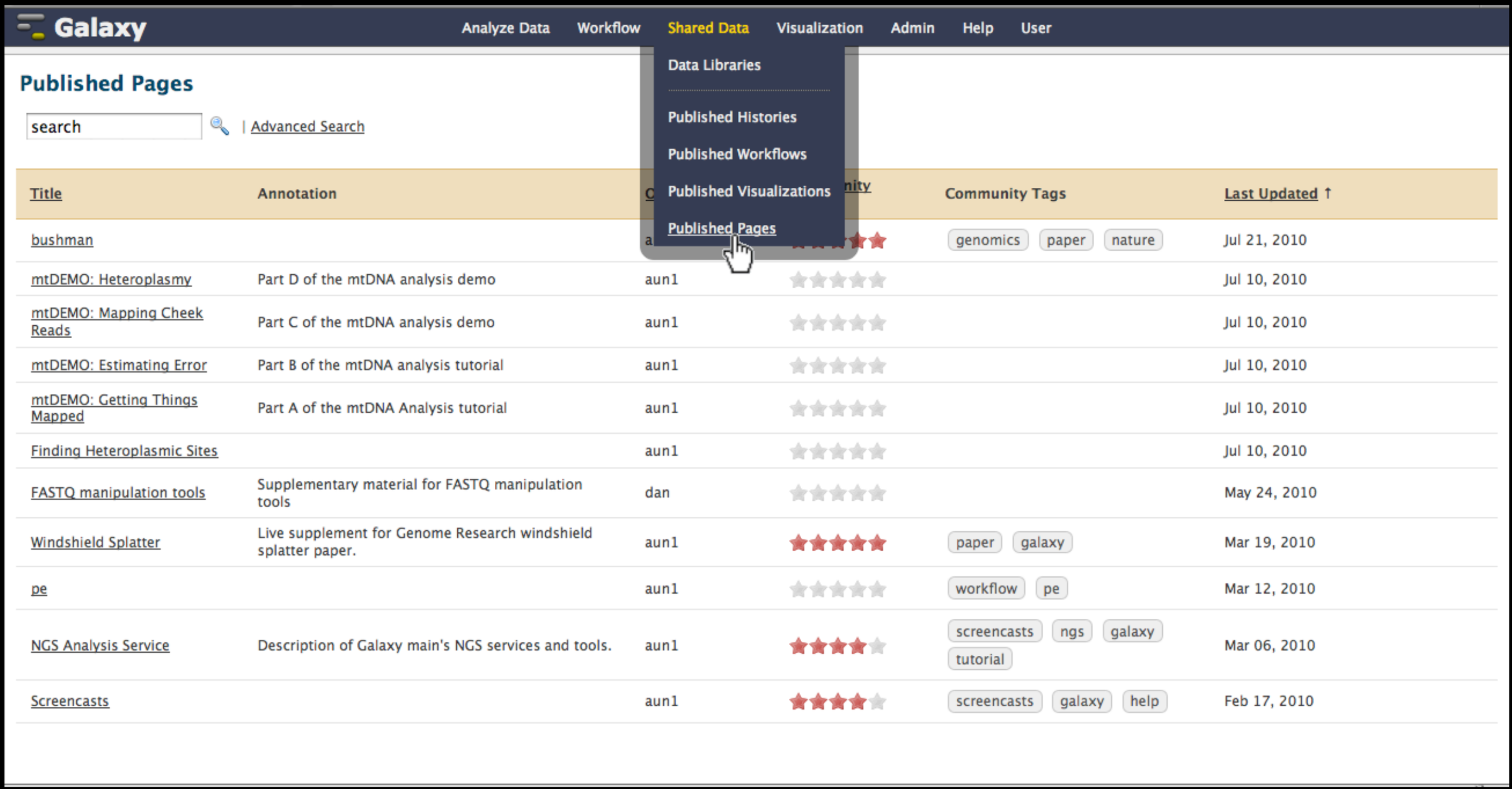
http://main.g2.bx.psu.edu/u/aun1/p/windshield-splatter

Sharing



Goecks, J, Nekrutenko, A, Taylor, J and The Galaxy Team. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 2010 Aug 25;11(8):R86.

Overview

- What is Galaxy?
- Galaxy for Experimental Biologists
- Galaxy for Bioinformaticians

Galaxy: the instant web-based tool and data resource integration platform

- Open Source downloadable package that can be deployed in individual labs
- Zero Configuration, but highly configurable
- Painlessly
  - Run your own private Galaxy Server
  - Add new Tools
  - Integrate new Data Sources
- Secure your private instance for working with sensitive data
- Modularized
- Easy to plug in your own components

The Problem

- You have written a Python script to analyze genomic data and you want to share it with command-line averse colleagues

The Galaxy Solution

- Solution: Integrate the script as a new Tool into your own Galaxy server
- Steps:
  - Obtain and install Galaxy source code (GetGalaxy.org)
  - Write an XML file describing the inputs and outputs and how to execute the script
  - Instruct Galaxy to load the tool

Quick Install

1. Get the latest copy from the repository:

The latest source code can be downloaded from the anonymous Mercurial repository with this command:

```
% hg clone http://www.bx.psu.edu/hg/galaxy galaxy_dist
```

If you don't have Mercurial, tarballs can be downloaded instead: zipped, bzipped or gzipped. However, this makes it more difficult to stay updated in the future since there's no simple way to update your copy.

2. Enable configuration files and download eggs:

Once the source code is downloaded, cd to the `galaxy_dist` directory and run the `setup.sh` script. This will copy sample configuration files and download the proper eggs for your platform:

```
% cd galaxy_dist
% sh setup.sh
```

This step requires Internet access to download the eggs. If the system on which you are installing Galaxy does not have Internet access, please follow the instructions for offline systems on Config/Eggs before attempting this step.

3. Start it up:

At this point Galaxy is ready to run. Simply run the following command:

```
% sh run.sh
```

This will start up the server on localhost and port 8080, so Galaxy can be accessed from your web browser at http://localhost:8080 . To stop the Galaxy server, just hit `Ctrl-c` in the terminal from which Galaxy is running.

GetGalaxy.org http://localhost:8080

Cluster

Cluster intervals of: 6: UCSC Main on Human: knownGene

max distance between intervals: 1 (bp)

min number of intervals per cluster: 2

Return type: Merge clusters into single intervals

Execute

TIP: If your query does not appear in the pulldown menu -> it is not in interval format. Use "edit attributes" to set chromosome, start, end, and strand columns

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Screencasts!

See Galaxy Interval Operation Screencasts (right click to open this link in another window).

Syntax

- **Maximum distance** is greatest distance in base pairs allowed between intervals that will be considered "clustered". **Negative** values for distance are allowed, and are useful for clustering intervals that overlap.
- **Minimum intervals per cluster** allow a threshold to be set on the minimum number of intervals to be considered a cluster. Any area with less than this minimum will not be included in the ouput.
- **Merge clusters into single intervals** outputs intervals that span the entire cluster.
- **Find cluster intervals; preserve comments and order** filters out non-cluster intervals while maintaining the original ordering and comments in the file.
- **Find cluster intervals; output grouped by clusters** filters out non-cluster intervals, but outputs the cluster intervals so that they are grouped together. Comments and original ordering in the file are lost.

Example

cluster.xml

```
<tool id="gops_cluster_1" name="Cluster">
  <description>[[Cluster]] the intervals of a query</description>
  <command interpreter="python2.4">
    gops_cluster.py $input1 $output -1 $input1_chromCol,$input1_startC
                   -d $distance -m $minregions -o $returntype
  </command>
  <inputs>
    <param format="interval" name="input1" type="data">
      <label>Cluster intervals of</label>
    </param>
    <param name="distance" size="5" type="integer" value="1" help="(bp
      <label>max distance between intervals</label>
    </param>
    <param name="minregions" size="5" type="integer" value="2">
      <label>min number of intervals per cluster</label>
    </param>
    <param name="returntype" type="select" label="Return type">
      <option value="1">Merge clusters into single intervals</option>
      <option value="2">Find cluster intervals; preserve comments and
      <option value="3">Find cluster intervals; output grouped by clus
      <option value="4">Find the smallest interval in each cluster</op
      <option value="5">Find the largest interval in each cluster</opt
    </param>
  </inputs>
  <help>

.. class:: infomark

**TIP:** If your query does not appear in the pulldown menu -> it is n

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**Screencasts!**

See Galaxy Interval Operation Screencasts_ (right click to open this l

.. _Screencasts: http://www.bx.psu.edu/cgi-bin/trac.cgi/wiki/GopsDesc

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**Syntax**

- **Maximum distance** is greatest distance in base pairs allowed betw
- **Minimum intervals per cluster** allow a threshold to be set on the
- **Merge clusters into single intervals** outputs intervals that span
- **Find cluster intervals; preserve comments and order** filters out
- **Find cluster intervals; output grouped by clusters** filters out n
```

Line: 87 Column: 8 XML Soft Tabs: 2

Get and Add Contributed Tools

Galaxy Tool Shed / (beta)

Tools | Help | User

Community

Tools

- Browse by category
- Browse all tools
- Login to upload

Categories

search | Advanced Search

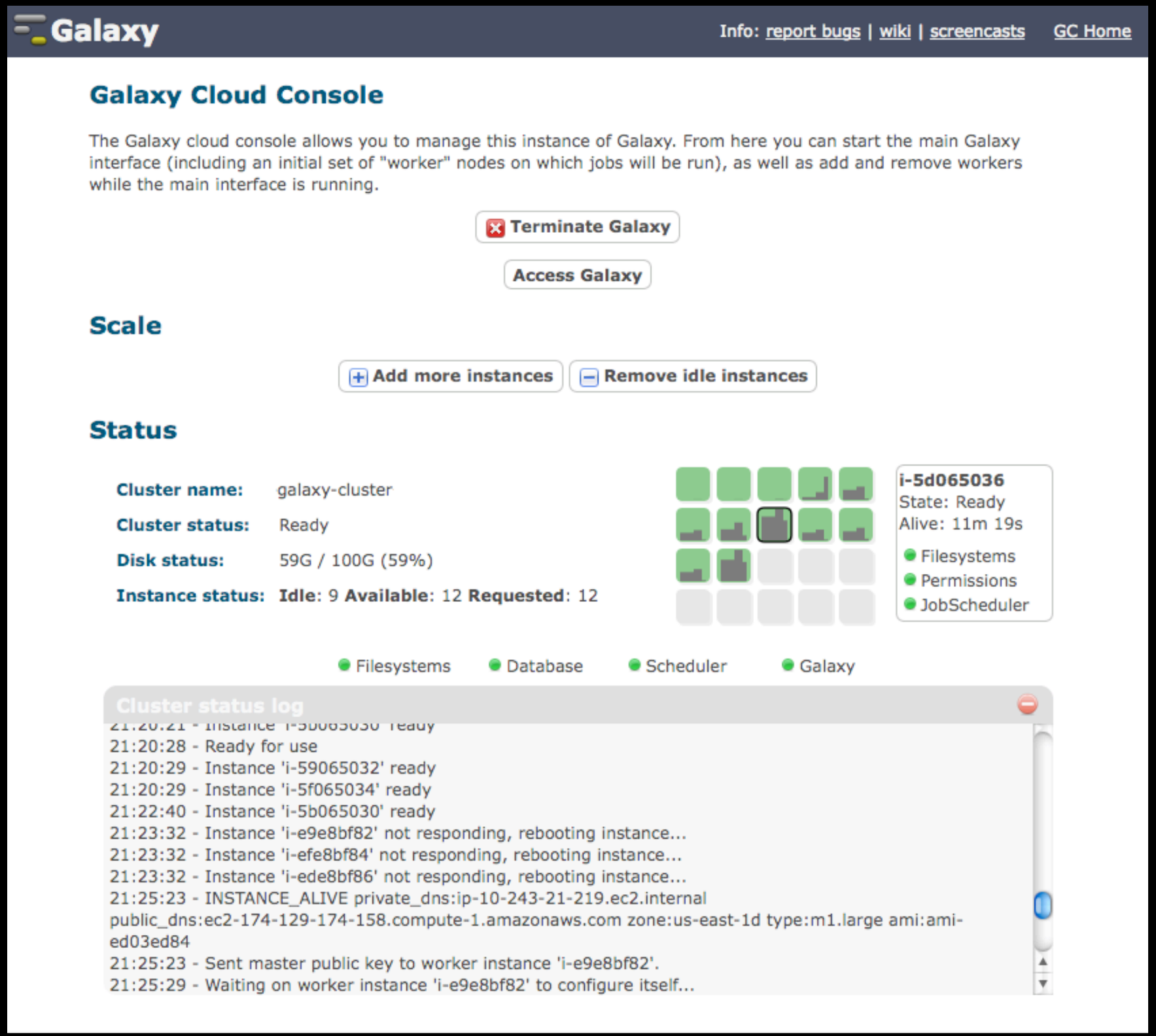
Name ↓	Description	Tools
Convert Formats	Tools for converting data formats	4
Data Source	Tools for retrieving data from external data sources	1
Fasta Manipulation	Tools for manipulating fasta data	5
Next Gen Mappers	Tools for the analysis and handling of Next Gen sequencing data	5
Ontology Manipulation	Tools for manipulating ontologies	1
SAM	Tools for manipulating alignments in the SAM format	0
Sequence Analysis	Tools for performing Protein and DNA/RNA analysis	7
SNP Analysis	Tools for single nucleotide polymorphism data such as WGA	1
Statistics	Tools for generating statistics	1
Text Manipulation	Tools for manipulating data	3
Visualization	Tools for visualizing data	1

http://usegalaxy.org/community

Galaxy on the Cloud

- Availability of Resources are not a Problem
  - Virtually unlimited resources: storage, computing, services
    - No need to maintain machines or personnel
    - Only pay for what you use
- Amazon Elastic Compute Cloud (EC2) and Eucalyptus
- Web-based Galaxy instantiation

Point, Click, Cloud



http://usegalaxy.org/cloud

ChIP-seq Example

- Premise:
  For the pilot phase of a study you received next generation sequencing data for a ChIP experiment on a transcription factor

- Goal:
  Using the pilot data, create a generic ChIP-seq analysis pipeline that will be used to process many ChIP experiments

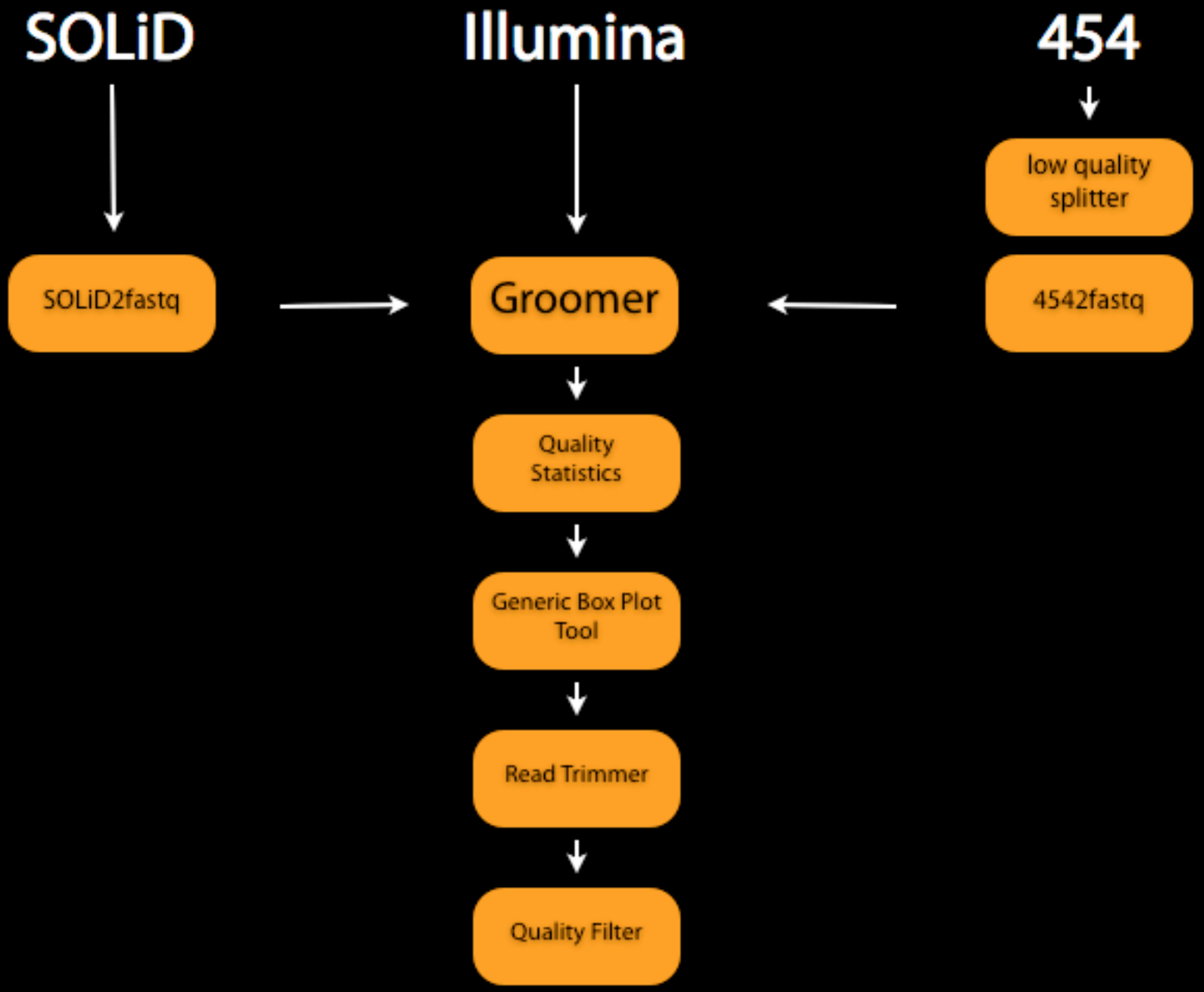
A plan

- Interactively analyze the first set of data
- Create a reusable Workflow from the interactive analysis
- Share analysis Results, History and Workflow

Interactive Analysis

- Prepare and Quality Check the raw sequencing reads
- Map sequencing reads to the target genome
- Call Peaks
- Provide primary results to collaborators or community
- Visualization and secondary analysis

Prepare and Quality Check



Blankenberg D, Gordon A, Von Kuster G, Coraor N, Taylor J, Nekrutenko A; Galaxy Team. Manipulation of FASTQ data with Galaxy. Bioinformatics. 2010 Jul 15;26(14):1783-5.

Prepare and Quality Check

NGS: QC and manipulation

ILLUMINA DATA

- FASTQ Groomer convert between various FASTQ quality formats
- FASTQ splitter on joined paired end reads
- FASTQ joiner on paired end reads
- FASTQ Summary Statistics by column

ROCHE-454 DATA

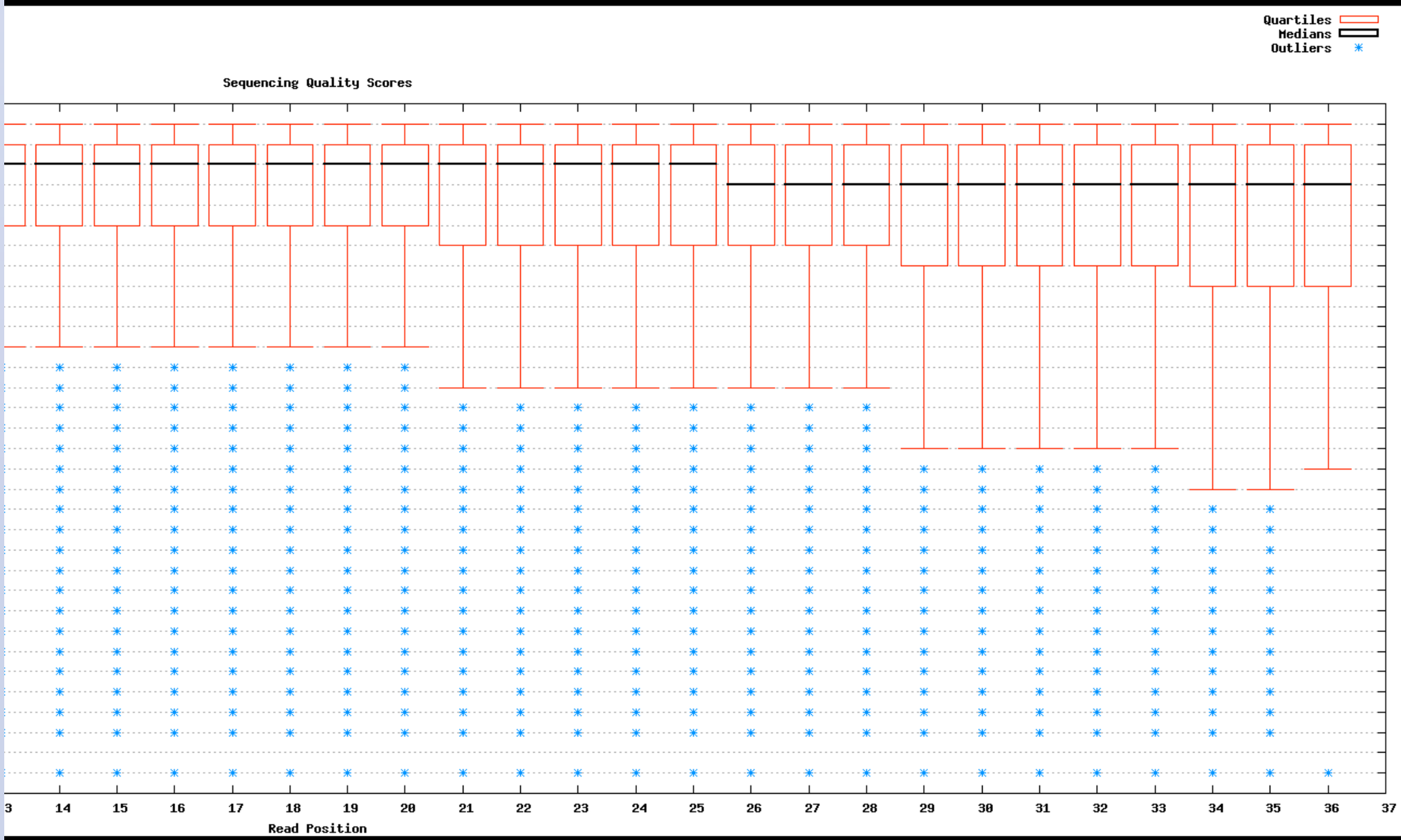
- Build base quality distribution
- Select high quality segments
- Combine FASTA and QUAL into FASTQ

AB-SOLID DATA

- Convert SOLiD output to fastq
- Compute quality statistics for SOLiD data
- Draw quality score boxplot for SOLiD data

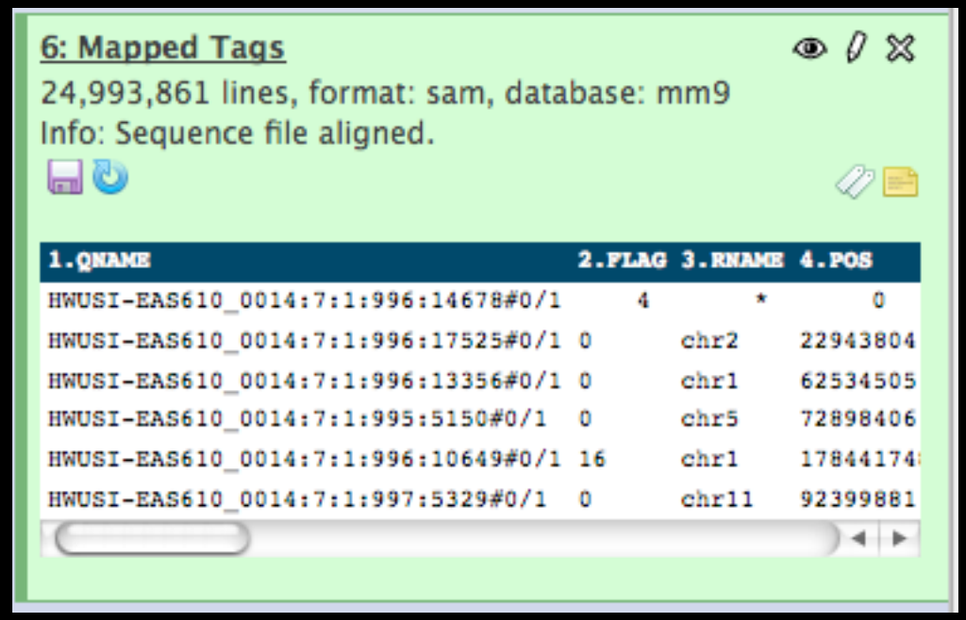
GENERIC FASTQ MANIPULATION

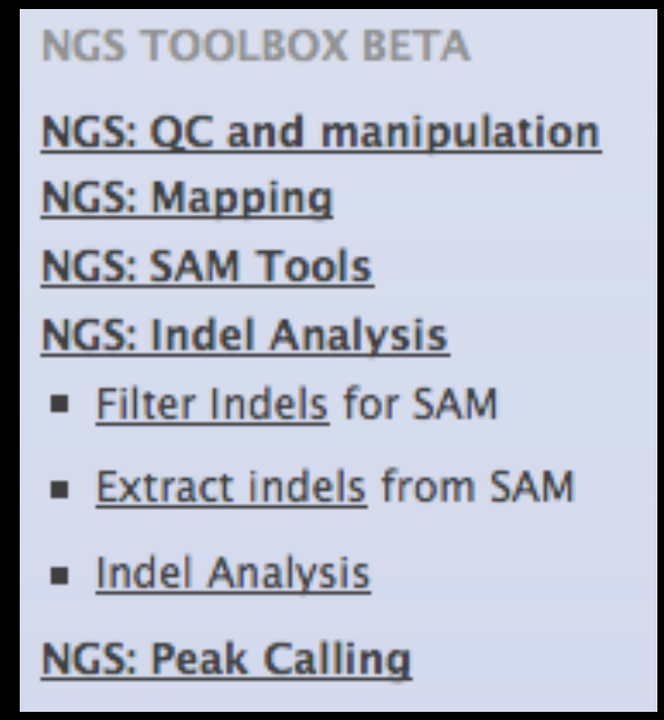
- Filter FASTQ reads by quality score and length
- FASTQ Trimmer by column
- FASTQ Quality Trimmer by sliding window
- FASTQ Masker by quality score
- Manipulate FASTQ reads on various attributes
- FASTQ to FASTA converter
- FASTQ to Tabular converter
- Tabular to FASTQ converter



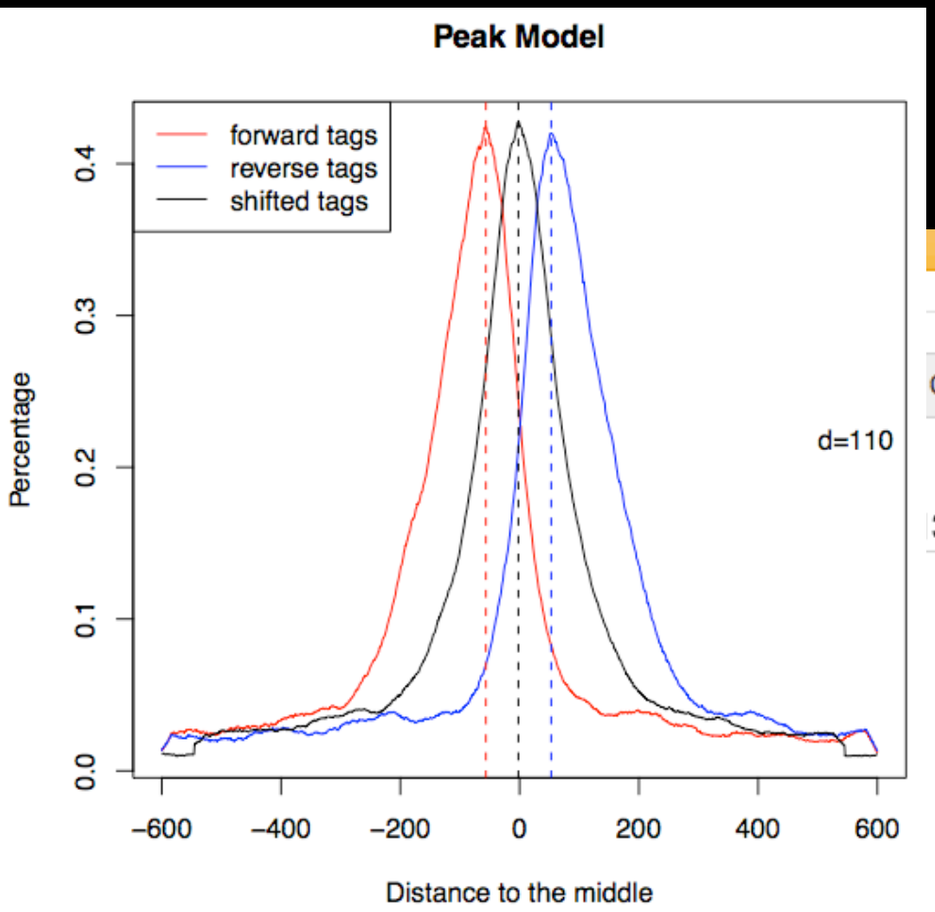
Mapping

- Collection of interchangeable mappers
  - Bowtie
  - BWA
  - BFAST
  - LASTZ
- SAM output
  - convert to BAM, BED (interval), etc
  - Peak calling
  - SNP/indel calling
    - NGS: Indel Analysis
    - Generate and filter **Pileup**





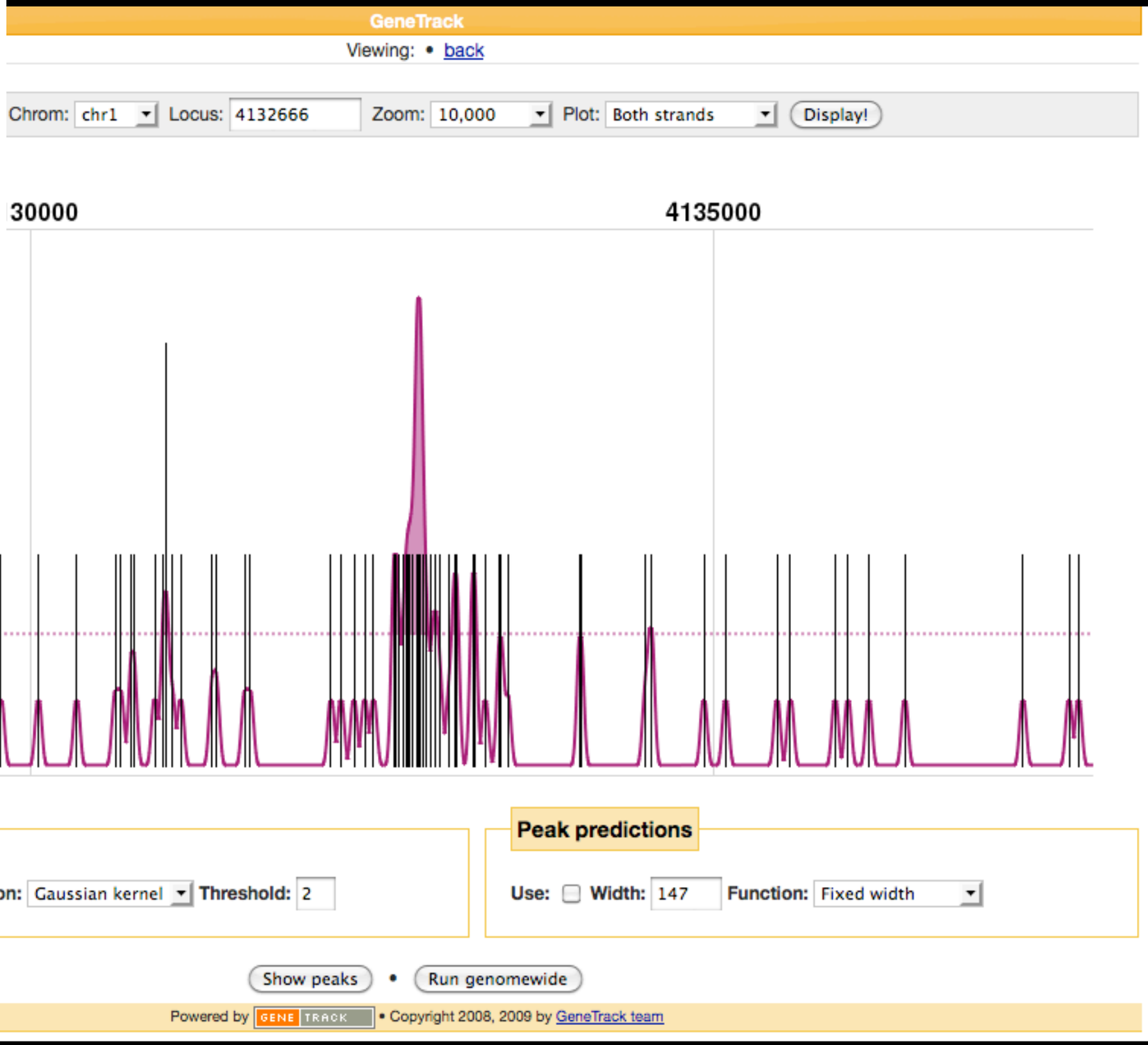
Peak Calling



MACS

*CCAT on test server and more coming

GeneTrack



Primary Results Ready

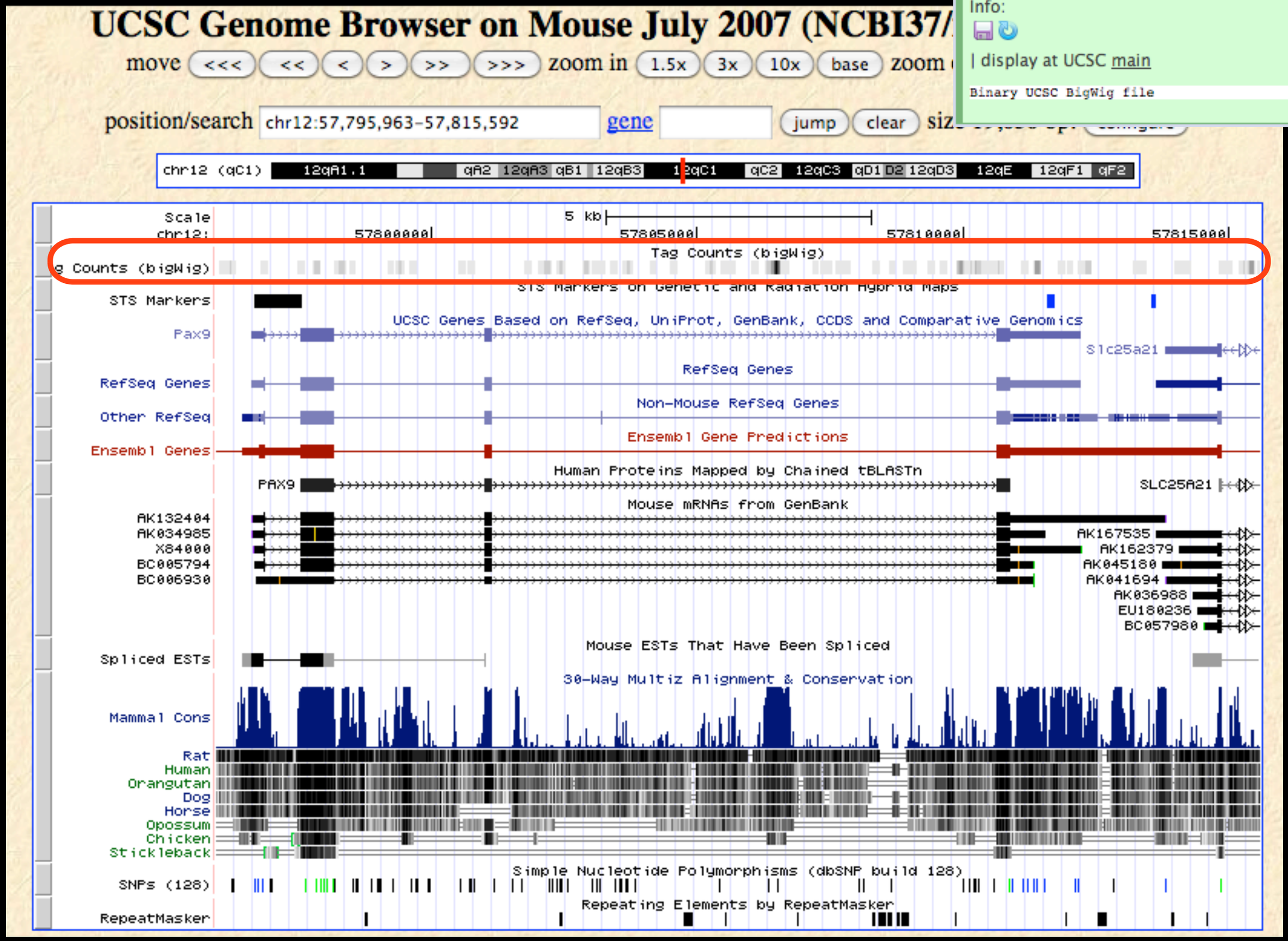
Data Library "Transcription Factor ChIP-seq"

Library Actions

Name	Information	Uploaded By	Date	File Size
ChIP-seq TFBS – *ChIP-seq (Transcription Factor Binding Sites)*				
Controls				
Enriched				
CTCF ChIP-seq				
CH12 Cells				
Replicate 1				
01Feb2010_ln7 CTCF CH12 groomed reads	None	dan@bx.psu.edu	2010-09-16	2.0 Gb
MACS peak calls (broadPeak)	None	dan@bx.psu.edu	2010-09-16	932.6 Kb
Mapped Tags (BAM)	None	dan@bx.psu.edu	2010-09-16	493.4 Mb
Tag Counts (bigWig)	None	dan@bx.psu.edu	2010-09-16	1.8 Gb
Replicate 2				
G1E Cells				
MEL Yale Cells				

For selected items: Import into your current history Go

Visualize



14: Tag Counts (bigWig)

2.4 Gb, format: bigwig, database: mm9

Info:

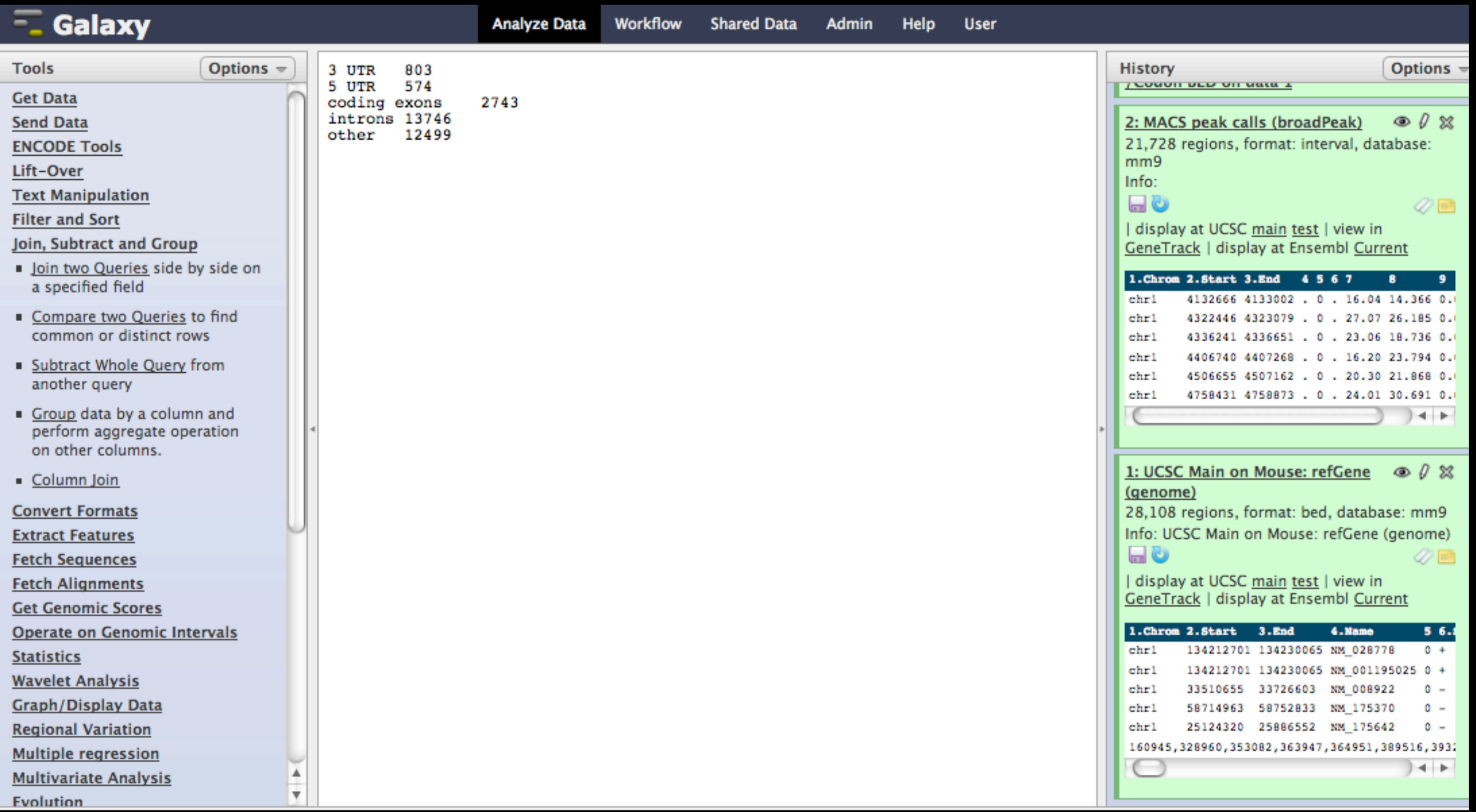
| display at UCSC main

```
Binary UCSC BigWig file
```

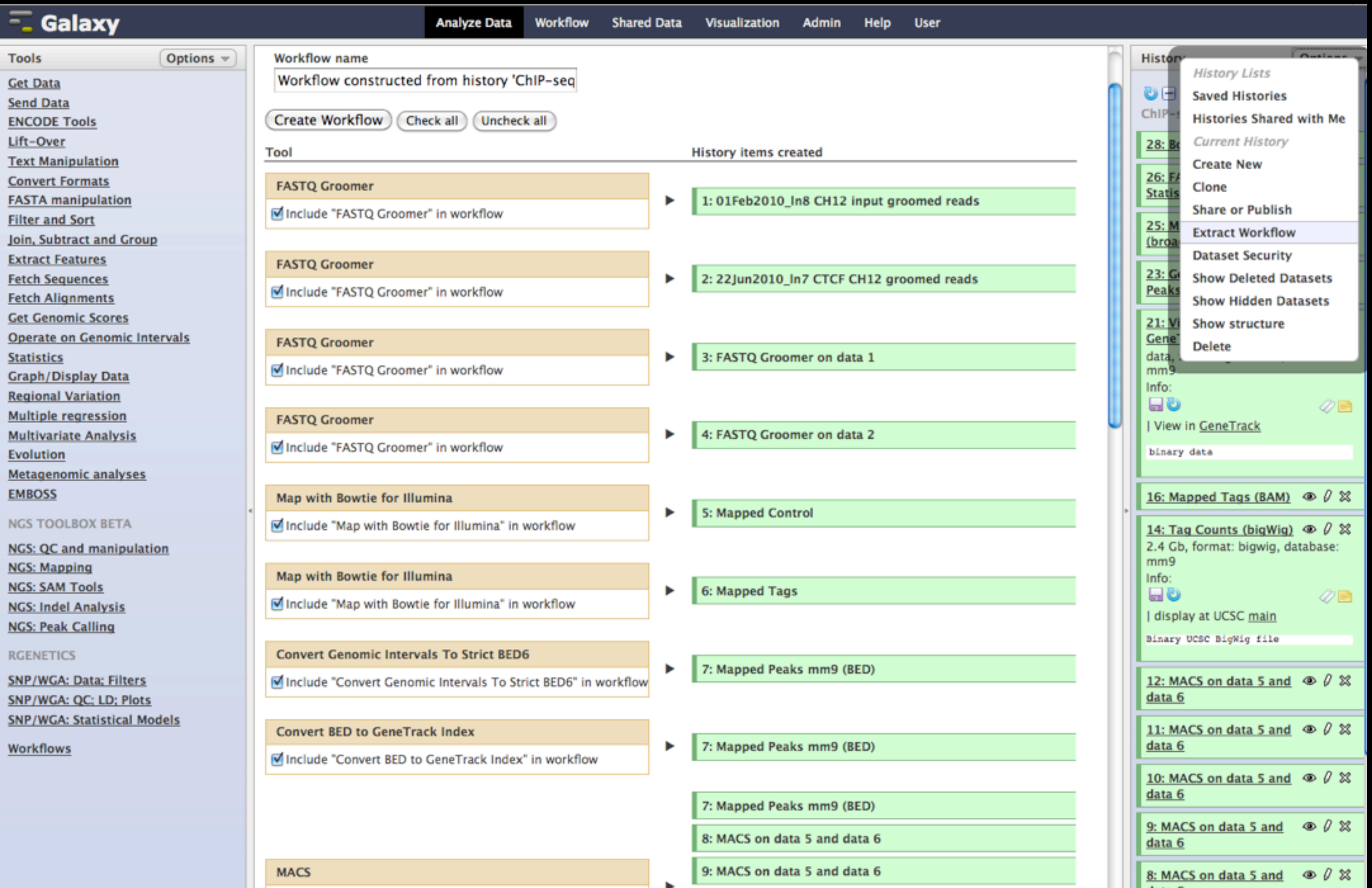
Secondary Analysis

- A simple goal: determine number of peaks that overlap a) coding exons, b) 5-UTRs, c) 3-UTRs, d) introns and d) other regions
- Get Data
  - Import Peak Call data
  - Retrieve Gene location data from external data resource
- Extract exon and intron data from Gene Data (Gene BED To Exon/Intron/Codon BED expander x4)
- Create an Identifier column for each exon type (Add column x4)
- Create a single file containing the 4 types (Concatenate)
- Complement the exon/intron intervals
- Force complemented file to match format of Gene BED expander output (convert to BED6)
- Create an Identifier column for the 'other' type (Add column)
- Concatenate the exons/introns and other files
- Determine which Peaks overlap the region types (Join)
- Calculate counts for each region type (Group)

Secondary Analysis



Create Reusable Workflow



Run new Workflow on additional data

Running workflow "Mapping and Peak Calling – mm9, 1 bp resolution"

Step 1: Input dataset

Control File
2: 22Jun2010_ln7 CTC..oomed reads

Step 2: Input dataset

Tag File
1: 01Feb2010_ln8 CH1..oomed reads

Step 3: FASTQ Groomer

File to groom
Output dataset 'output' from step 1

Input FASTQ quality scores type
Sanger

Advanced Options
Hide Advanced Options

Step 4: FASTQ Groomer

File to groom
Output dataset 'output' from step 2

Input FASTQ quality scores type
Sanger

Advanced Options
Hide Advanced Options

Scale-up and Share

Galaxy | Analyze Data | Workflow | Shared Data | Visualization | Admin | Help | User

Private Page | Transcription Factor ChIP-seq

Here, one can access histories for the ChIP-seq datasets that have been subjected to this workflow:

Galaxy Workflow | Mapping and Peak Calling - mm9, 1 bp resolution

Histories:

GATA 1 - G1E-ER4+E2

Replicate 1

Galaxy History | 12May2009_ln4_15Jun2009_ln4_ln8 GATA1 G1E-ER4+E2 combined groomed reads

- 3: FASTQ Groomer on data 2
- 4: FASTQ Groomer on data 1
- 5: Mapped Control
- 6: Map with Bowtie for Illumina on data 4
  Job is currently running
- 7: MACS on data 5 and data 6
  Job is waiting to run
- 8: MACS on data 5 and data 6
- 9: MACS on data 5 and data 6

CTCF:

G1E

Galaxy History | 12Nov2009_ln4 CTCF G1E groomed reads

G1E-ER4+E2

Galaxy History | 12Nov2009_ln3 CTCF G1E-ER4+E2 groomed reads

MEL Yale

Galaxy History | 22Jun2010_ln4 CTCF MEL groomed reads

CH12

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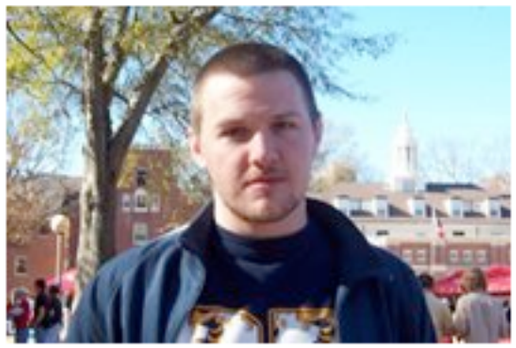

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