Using Galaxy for High-throughput Sequencing (HTS) Analysis

The Galaxy Team
http://UseGalaxy.org

Overview

High-throughput Sequencing (HTS) Data

Using Galaxy to Analyze HTS Data

- Prepare, quality control and manipulate reads
- Read Mapping
- SNP & INDEL analysis
- Binding sites analysis and peak calling
- Transcriptome analysis

Galaxy for Sequencing Facilities

Galaxy exercises: ChIP-seq, RNA-seq, Variant Detection

HTS Data

From the Sequencer:

- reads and quality scores (FASTQ)

In the Analysis Pipeline / Workflow:

- alignments against reference genome (SAM, BAM)
- annotations (GFF, BED)
- genome Assemblies (FASTA)
- quantitative tracks, e.g. conservation (WIG)

FASTQ Quality Scores

```
@UNIQUE_SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''')**55CCF>>>>>>CCCCCCC655
```

```
  SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
  ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
  ...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII......................
  .................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ......................
  !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
  |                         |    |        |                              |                     |
 33                        59   64       73                            104                   126

 S - Sanger        Phred+33,  raw reads typically (0, 40)
 X - Solexa        Solexa+64, raw reads typically (-5, 40)
 I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
 J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
```

http://en.wikipedia.org/wiki/FASTQ_format

Galaxy tools generally use Sanger format

- Need to convert quality scores to Sanger using Groomer tool

Getting Your Data into Galaxy

Cannot upload any file larger than 2GB via Web browser

- Galaxy does not currently support compressed files

Use FTP client, e.g. FileZilla: http://filezilla-project.org/

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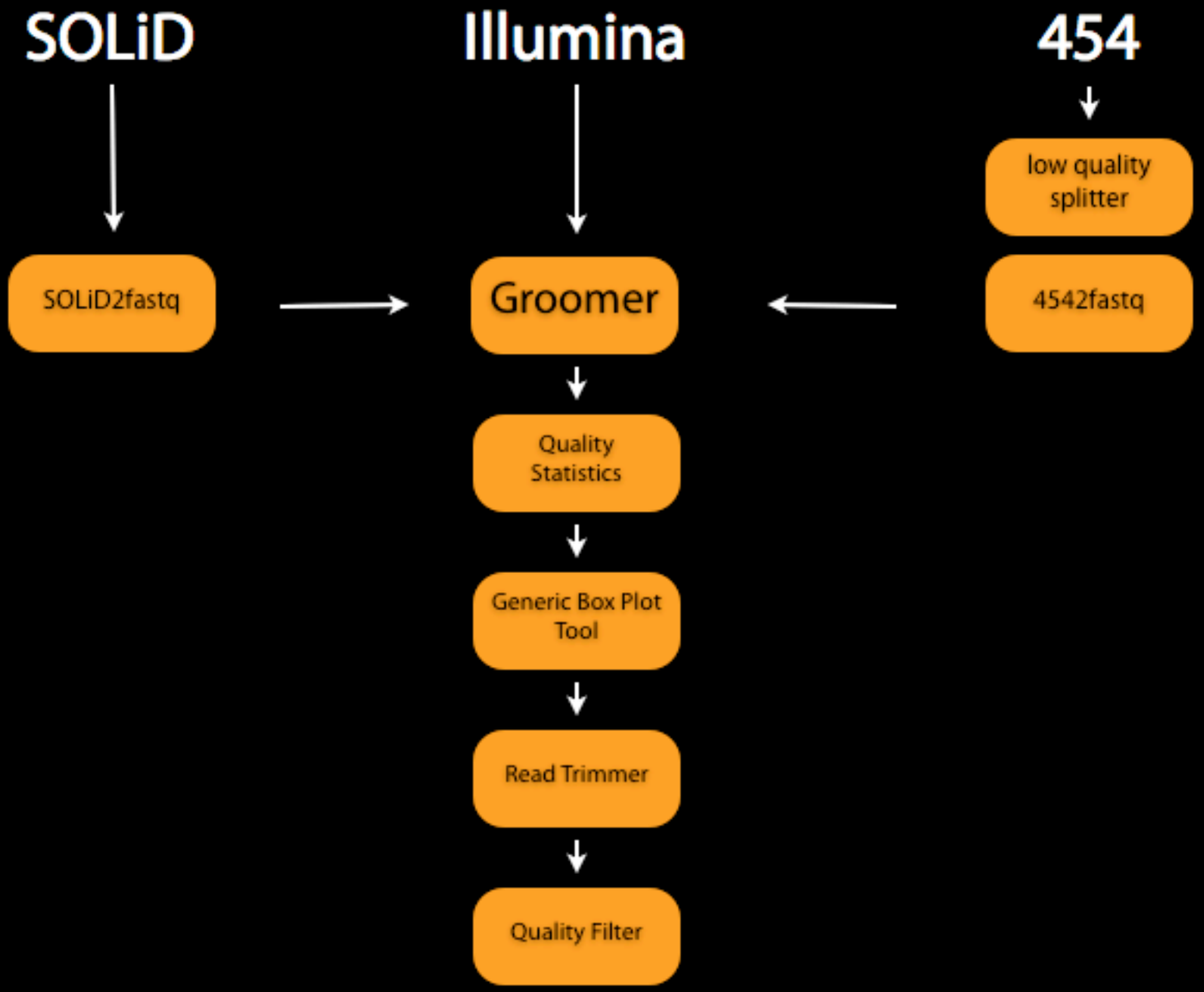
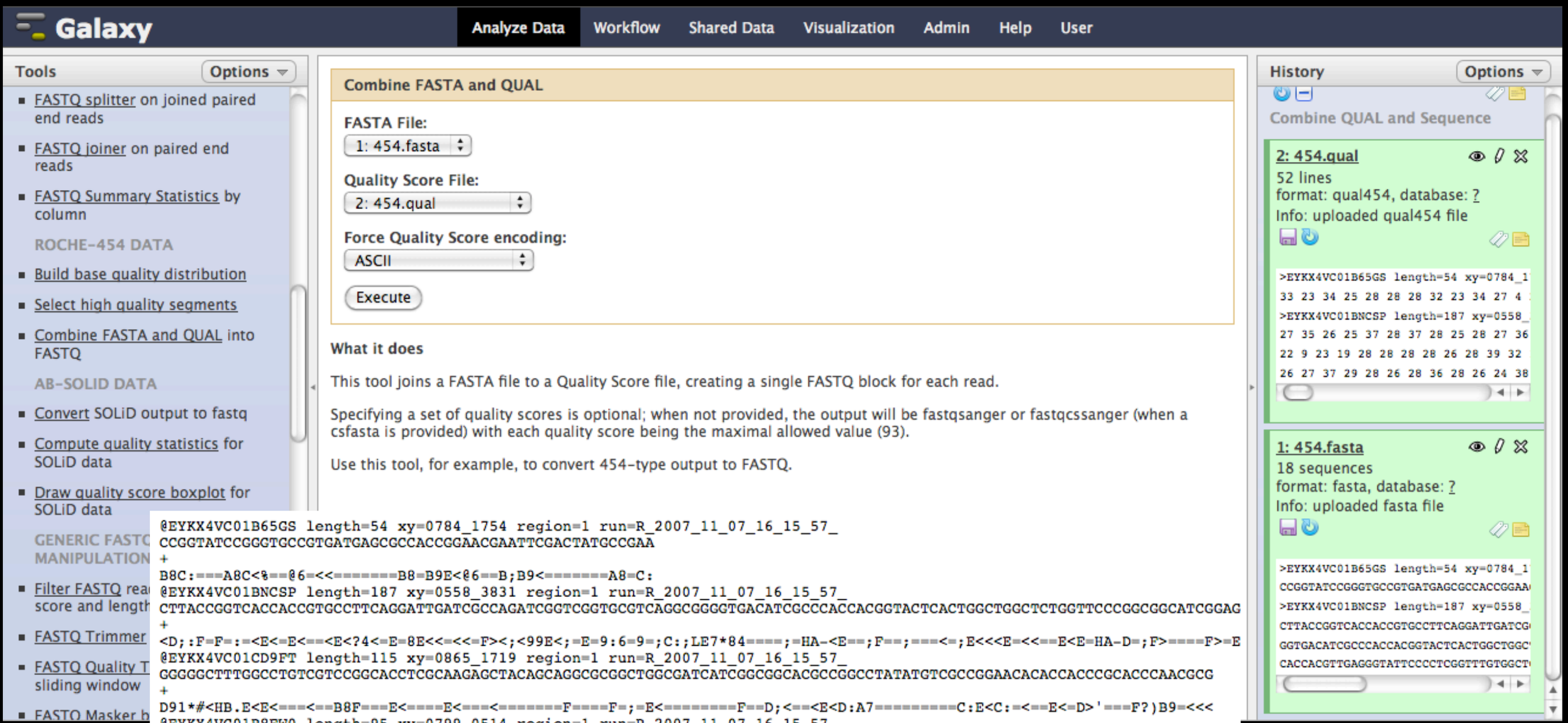
Galaxy exercises: ChIP-seq and RNA-seq

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.

Combining Sequences and Qualities



Grooming --> Sanger

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

Tools Options

NGS TOOLBOX BETA

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

FASTQ Groomer

File to groom:
3: Combine FASTA and.. and data 2

Input FASTQ quality scores type:
Sanger
Solexa
Illumina 1.3+
Sanger
Color Space Sanger
Execute

What it does

This tool offers several conversions options relating to the FA

When using *Basic* options, the output will be *sanger* formatte Space Sanger).

When converting, if a quality score falls outside of the target able value (i.e. the minimum or maximum).

When converting between Solexa and the other formats, qual scales using the equations found in Cock PJ, Fields CJ, Goto N, Heuer ML, quality scores, and the Solexa/Illumina FASTQ variants. Nucle ces with

When converting between color space (csSanger) and base/se adapter bases are lost or gained; if gained, the base 'G' is used as the adapt space if there is no adapter present in the color space sequence. Any masked or ambiguous nucleotides in base space will be converted to 'N's when determining color space encoding.

4: FASTQ Groomer on data 3

18 sequences
format: fastqsanger, database: ?
Info: Groomed 18 sanger reads into sanger reads.
Based upon quality and sequence, the input data is valid for: sanger
Input ASCII range: '!'(33) – 'L'(76)
Input decimal range: 0 – 43

```
@EYKX4VC01B65GS length=54 xy=0784_1
CCGGTATCCGGGTGCCGTGATGAGCGCCACCGGAA
+
BBC:===A8C<%==@6=<<=======B8=B9E<@6
@EYKX4VC01BNCSP length=187 xy=0558_
CTTACCGGTCACCACCGTGCCTTCAGGATTGATCG
```

History Options

Combine QUAL and Sequence

3: Combine FASTA and QUAL on data 1 and data 2

18 sequences
format: fastqsanger, database: ?
Info:
Combined 18 of 18 sequences with quality scores (100.00%).

```
@EYKX4VC01B65GS length=54 xy=0784_1
CCGGTATCCGGGTGCCGTGATGAGCGCCACCGGAA
+
BBC:===A8C<%==@6=<<=======B8=B9E<@6
@EYKX4VC01BNCSP length=187 xy=0558_
CTTACCGGTCACCACCGTGCCTTCAGGATTGATCG
```

2: 454.qual

52 lines
format: qual454, database: ?
Info: uploaded qual454 file

```
>EYKX4VC01B65GS length=54 xy=0784_1
33 23 34 25 28 28 28 32 23 34 27 4
>EYKX4VC01BNCSP length=187 xy=0558_
27 35 26 25 37 28 37 28 25 28 27 36
```

Quality Score Comparison

```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
 ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
 ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
 |                         |    |        |                              |                     |
33                        59   64       73                            104                   126

S - Sanger       Phred+33,  93 values  (0, 93) (0 to 60 expected in raw reads)
I - Illumina 1.3 Phred+64,  62 values  (0, 62) (0 to 40 expected in raw reads)
X - Solexa       Solexa+64, 67 values (-5, 62) (-5 to 40 expected in raw reads)
```

Diagram adapted from http://en.wikipedia.org/wiki/FASTQ_format

Quality Statistics and Box Plot Tool

NGS TOOLBOX BETA

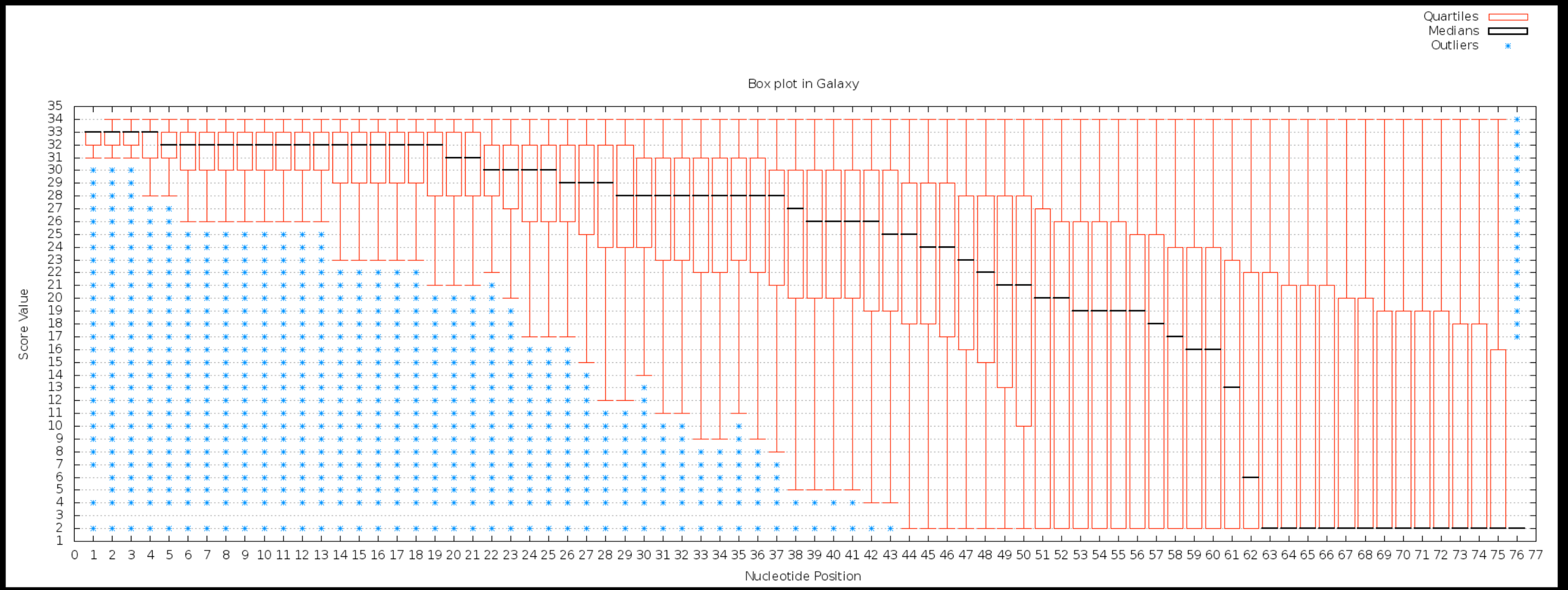
NGS: QC and manipulation

ILLUMINA DATA

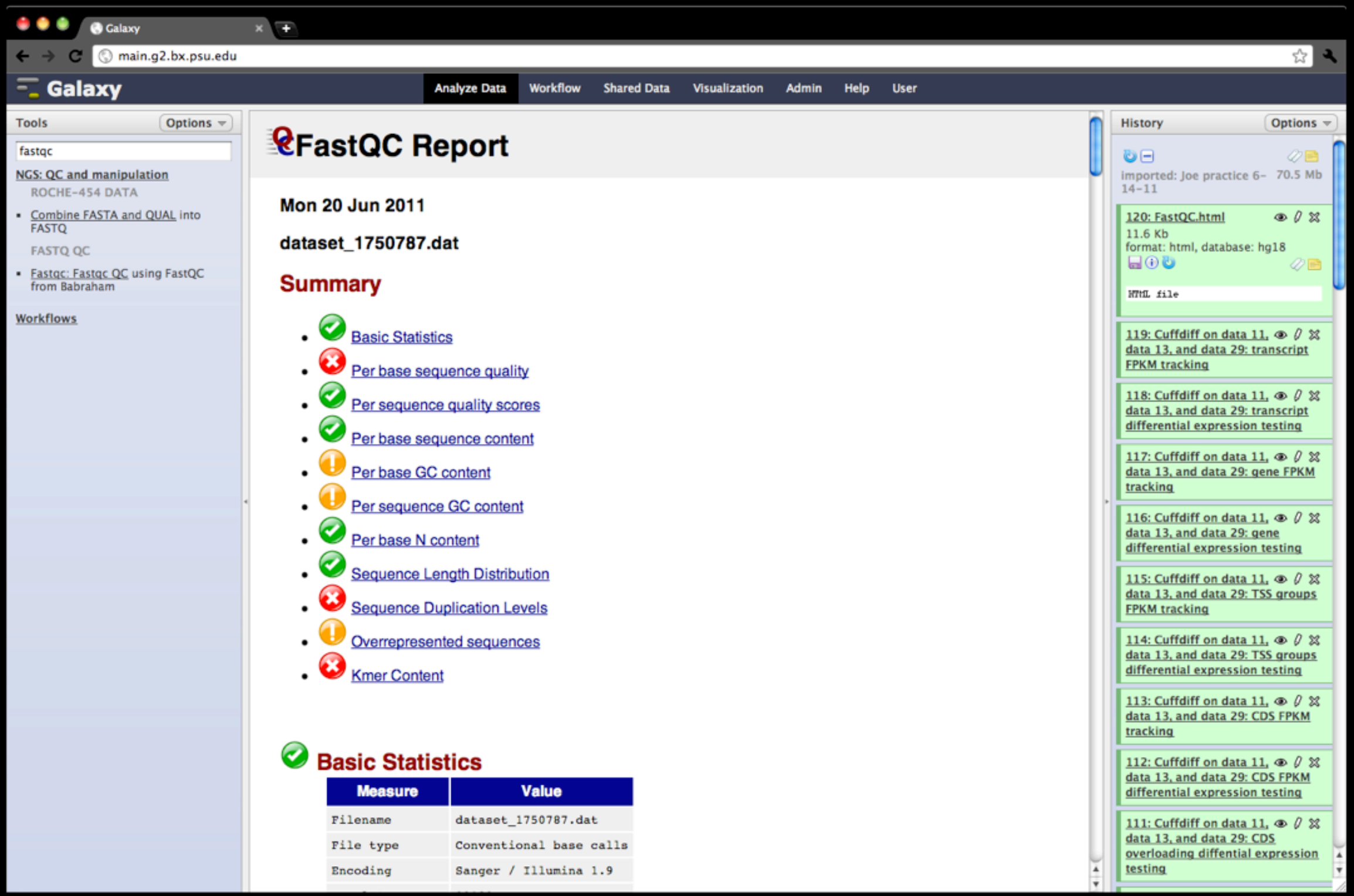
- FASTQ Groomer convert between various FASTQ quality formats
- FASTQ splitter on joined paired end reads
- FASTQ joiner on paired end reads
- FASTQ Summary Statistics by column

Graph/Display Data

- Histogram of a numeric column
- Scatterplot of two numeric columns
- Plotting tool for multiple series and graph types
- Boxplot of quality statistics



FastQC


Galaxy
main.g2.bx.psu.edu
Galaxy
Analyze Data
Workflow
Shared Data
Visualization
Admin
Help
User
Tools
Options
fastqc
NGS: QC and manipulation
ROCHE-454 DATA
Combine FASTA and QUAL into FASTQ
FASTQ QC
Fastqc: Fastqc QC using FastQC from Babraham
Workflows
FastQC Report
Mon 20 Jun 2011
dataset_1750787.dat
Summary
Basic Statistics
Per base sequence quality
Per sequence quality scores
Per base sequence content
Per base GC content
Per sequence GC content
Per base N content
Sequence Length Distribution
Sequence Duplication Levels
Overrepresented sequences
Kmer Content
Basic Statistics
Measure
Value
Filename
dataset_1750787.dat
File type
Conventional base calls
Encoding
Sanger / Illumina 1.9
History
Options
imported: Joe practice 6-14-11
70.5 Mb
120: FastQC.html
11.6 Kb
format: html, database: hg18
HTML file
119: Cuffdiff on data 11, data 13, and data 29: transcript FPKM tracking
118: Cuffdiff on data 11, data 13, and data 29: transcript differential expression testing
117: Cuffdiff on data 11, data 13, and data 29: gene FPKM tracking
116: Cuffdiff on data 11, data 13, and data 29: gene differential expression testing
115: Cuffdiff on data 11, data 13, and data 29: TSS groups FPKM tracking
114: Cuffdiff on data 11, data 13, and data 29: TSS groups differential expression testing
113: Cuffdiff on data 11, data 13, and data 29: CDS FPKM tracking
112: Cuffdiff on data 11, data 13, and data 29: CDS FPKM differential expression testing
111: Cuffdiff on data 11, data 13, and data 29: CDS overloading diffential expression testing

Read Trimming

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

Tools | Options

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

FASTX-TOOLKIT FOR FASTQ DATA

- Quality format converter (ASCII-Numeric)
- Compute quality statistics
- Draw quality score boxplot
- Draw nucleotides distribution chart
- FASTQ to FASTA converter
- Filter by quality
- Remove sequencing artifacts

FASTQ Trimmer

FASTQ File: 2: imported: GM12878..ple Dataset

Define Base Offsets as: Absolute Values

Use Absolute for fixed length reads (Illumina, SOLiD
Use Percentage for variable length reads (Roche/45

Offset from 5' end: 0

Values start at 0, increasing from the left

Offset from 3' end: 16

Values start at 0, increasing from the right

Keep reads with zero length: ☐

Execute

This tool allows you to trim the ends of reads.

You can specify either absolute or percent-based offs
trimmed. When using the percent-based method, offs

For example, if you have a read of length 36:

```
@Some FASTQ Sanger Read
CAATATGTNCTCACTGATAAGTGGATATNAGCNCCA
+
=@@.@;B-%78>CBA@>7@7BBCA4-48%<;;%<B@
```

And you set absolute offsets of 2 and 0:

FASTQ Quality Trimmer

FASTQ File: 7: FASTQ Trimmer on data 2

Keep reads with zero length: ☐

Trim ends: 5' and 3'

Window size: 1

Step Size: 1

Maximum number of bases to exclude from the window during aggr

0

Aggregate action for window: min score

Trim until aggregate score is: >=

Quality Score: 0.0

Execute

Filter FASTQ

FASTQ File:

7: FASTQ Trimmer on data 2

Requires groomed data: if your data does not appear here try using the FASTQ groomer.

Minimum Size:

0

Maximum Size:

0

A maximum size less than 1 indicates no limit.

Minimum Quality:

0.0

Maximum Quality:

0.0

A maximum quality less than 1 indicates no limit.

Maximum number of bases allowed outside of quality range:

0

This is paired end data:

Quality Filter on a Range of Bases

Add new Quality Filter on a Range of Bases

Execute

Quality Filter on a Range of Bases

Quality Filter on a Range of Bases 1

Define Base Offsets as:

Absolute Values

Use Absolute for fixed length reads (Illumina, SOLiD)
Use Percentage for variable length reads (Roche/454)

Offset from 5' end:

0

Values start at 0, increasing from the left

Offset from 3' end:

0

Values start at 0, increasing from the right

Aggregate read score for specified range:

min score

Keep read when aggregate score is:

>=

Quality Score:

0.0

Remove Quality Filter on a Range of Bases 1

Add new Quality Filter on a Range of Bases

Execute

Manipulate FASTQ

Manipulate FASTQ

FASTQ File:
7: FASTQ Trimmer on data 2
Requires groomed data: if your data does not appear here try using the FASTQ groomer.

Match Reads

Add new Match Reads

Manipulate Reads

Add new Manipulate Reads

Execute

Manipulate FASTQ

FASTQ File:
7: FASTQ Trimmer on data 2
Requires groomed data: if your data does not appear here try using the FASTQ groomer.

Match Reads

Match Reads 1

Match Reads by:
Sequence Content

Sequence Match Type:
Regular Expression

Match by:
N

Remove Match Reads 1

Add new Match Reads

Manipulate Reads

Add new Manipulate Reads

Execute

Manipulate FASTQ

FASTQ File:
7: FASTQ Trimmer on data 2
Requires groomed data: if your data does not appear here try using the FASTQ groomer.

Match Reads

Match Reads 1

Match Reads by:
Sequence Content

Sequence Match Type:
Regular Expression

Match by:
N

Remove Match Reads 1

Add new Match Reads

Manipulate Reads

Manipulate Reads 1

Manipulate Reads on:
Miscellaneous Actions

Miscellaneous Manipulation Type:
Remove Read

Remove Manipulate Reads 1

Add new Manipulate Reads

Execute

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Mapping HTS Data

Collection of interchangeable mappers

- accept fastq format, produce SAM/BAM

Mappers for

- DNA
- RNA
- Local realignment

Mappers

DNA

- short reads: Bowtie, BWA, BFAST, PerM
- longer reads: LASTZ

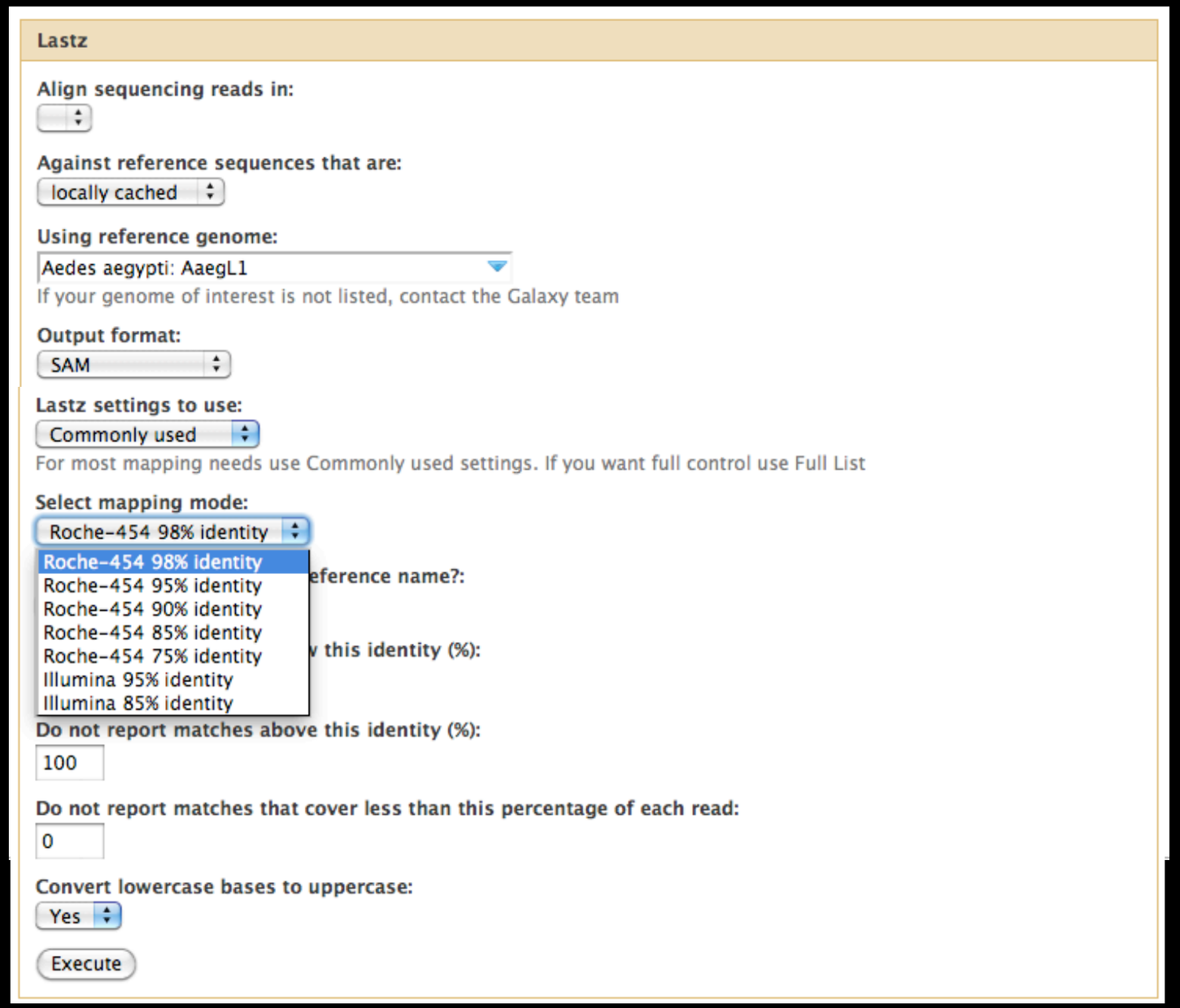
Metagenomics

- Megablast

RNA / gapped-reads mapper

- Tophat

Commonly Used/Default Parameters



Full Parameter List

Lastz

Align sequencing reads in:
53: FASTQ to FASTA on data 7

Against reference sequences that are:
locally cached

Using reference genome:
Aedes aegypti: AaegL1
If your genome of interest is not listed, contact the Galaxy team

Output format:
SAM

Lastz settings to use:
Full Parameter List
Commonly used
Full Parameter List
use Commonly used settings. If you want full control use Full List

Which strand to search?:
Both

Select seeding settings:
Seed hits require a 19 bp word with matches i
allows you set word size and number of mismatches

Select transition settings:
Allow one transition in each seed hit
affects the number of allowed transition substitutions

Perform gap-free extension of seed hits to HSPs (high scoring segment pairs)?:
No

Perform chaining of HSPs?:
No

Gap opening penalty:
400

Gap extension penalty:
30

X-drop threshold:
910

Y-drop threshold:
9370

Set the threshold for HSPs (ungapped extensions scoring lower are discarded):
3000

Set the threshold for gapped alignments (gapped extensions scoring lower are discarded):
3000

Involve entropy when filtering HSPs?:
No

Do you want to modify the reference name?:
No

Do you want to modify the reference name?:
No

Do not report matches below this identity (%):
0

Do not report matches above this identity (%):
100

Do not report matches that cover less than this percentage of each read:
0

Convert lowercase bases to uppercase:
Yes

Execute

What it does

LASTZ is a high performance pairwise sequence aligner derived from BLASTZ. It is written by Bob Harris in Webb Miller's laboratory at Penn State University. Special scoring sets were derived to improve runtime performance and quality. This Galaxy version of LASTZ is geared towards aligning short (Illumina/Solexa, AB/SOLiD) and medium (Roche/454) reads against a reference sequence. There is excellent, extensive documentation on LASTZ available here.

Input formats

LASTZ accepts reference and reads in FASTA format. However, because Galaxy supports implicit format conversion the tool will recognize fastq and other method specific formats.

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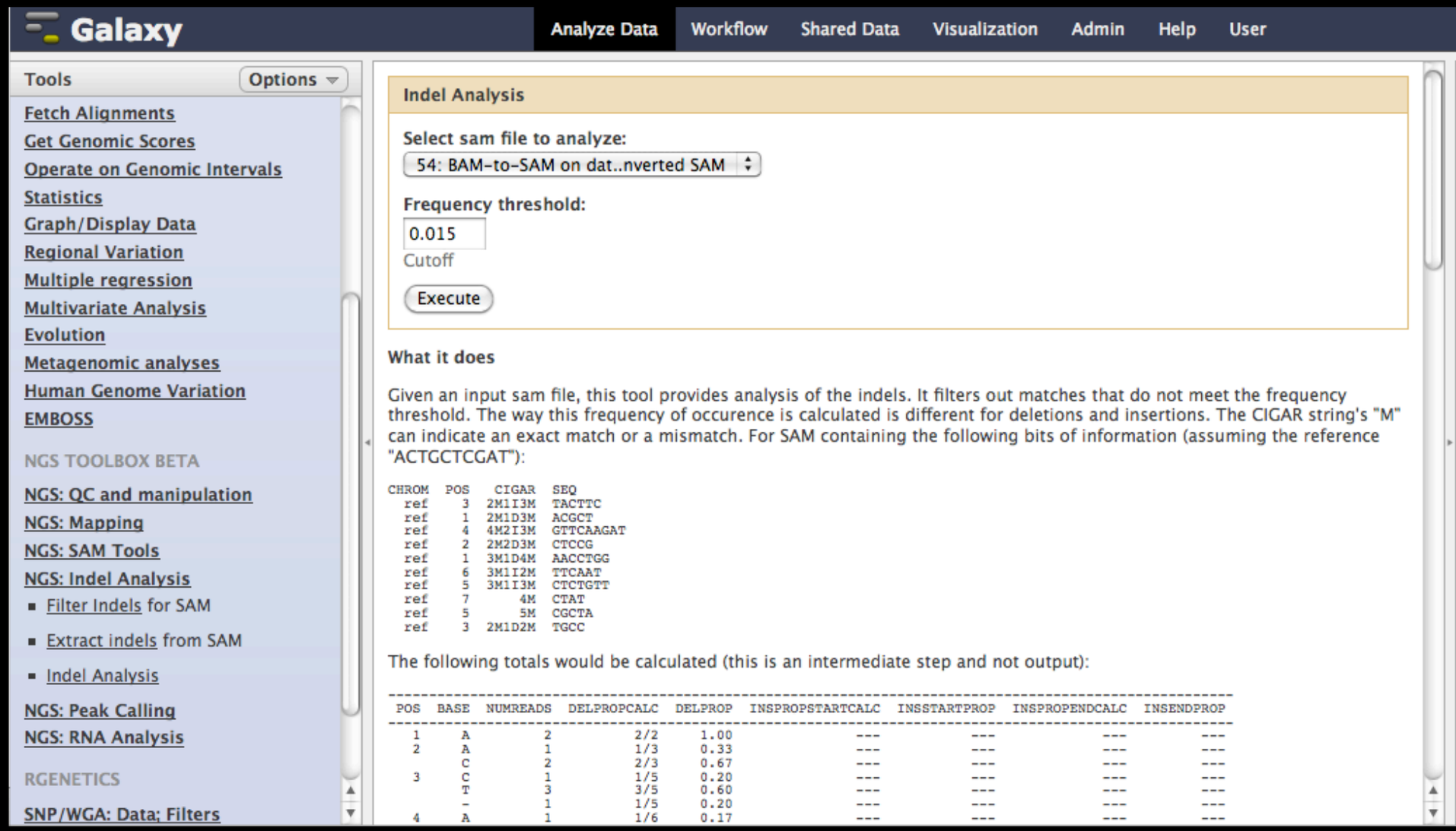
SNPs & INDELs

SNPs from Pileup

- Generate
- Filter

NGS: SAM Tools

- Filter SAM on bitwise flag values
- Convert SAM to interval
- SAM-to-BAM converts SAM format to BAM format
- BAM-to-SAM converts BAM format to SAM format
- Merge BAM Files merges BAM files together
- Generate pileup from BAM dataset
- Filter pileup on coverage and SNPs
- Pileup-to-Interval condenses pileup format into ranges of bases
- flagstat provides simple stats on BAM files



GATK Tools

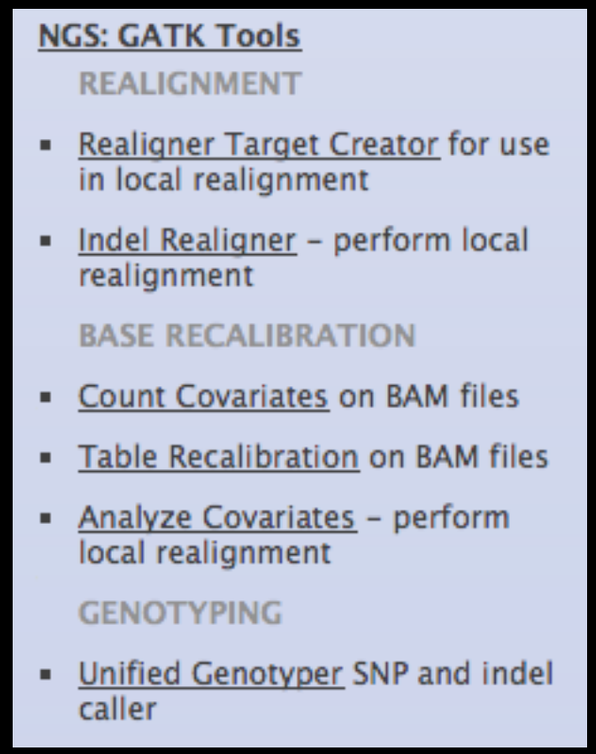
Local re-alignment

Base re-calibration

Genotyping

Alpha status

- please try, report bugs
- available on test server: http://test.g2.bx.psu.edu/



NGS: GATK Tools

REALIGNMENT

- Realigner Target Creator for use in local realignment
- Indel Realigner – perform local realignment

BASE RECALIBRATION

- Count Covariates on BAM files
- Table Recalibration on BAM files
- Analyze Covariates – perform local realignment

GENOTYPING

- Unified Genotyper SNP and indel caller

Unified Genotyper

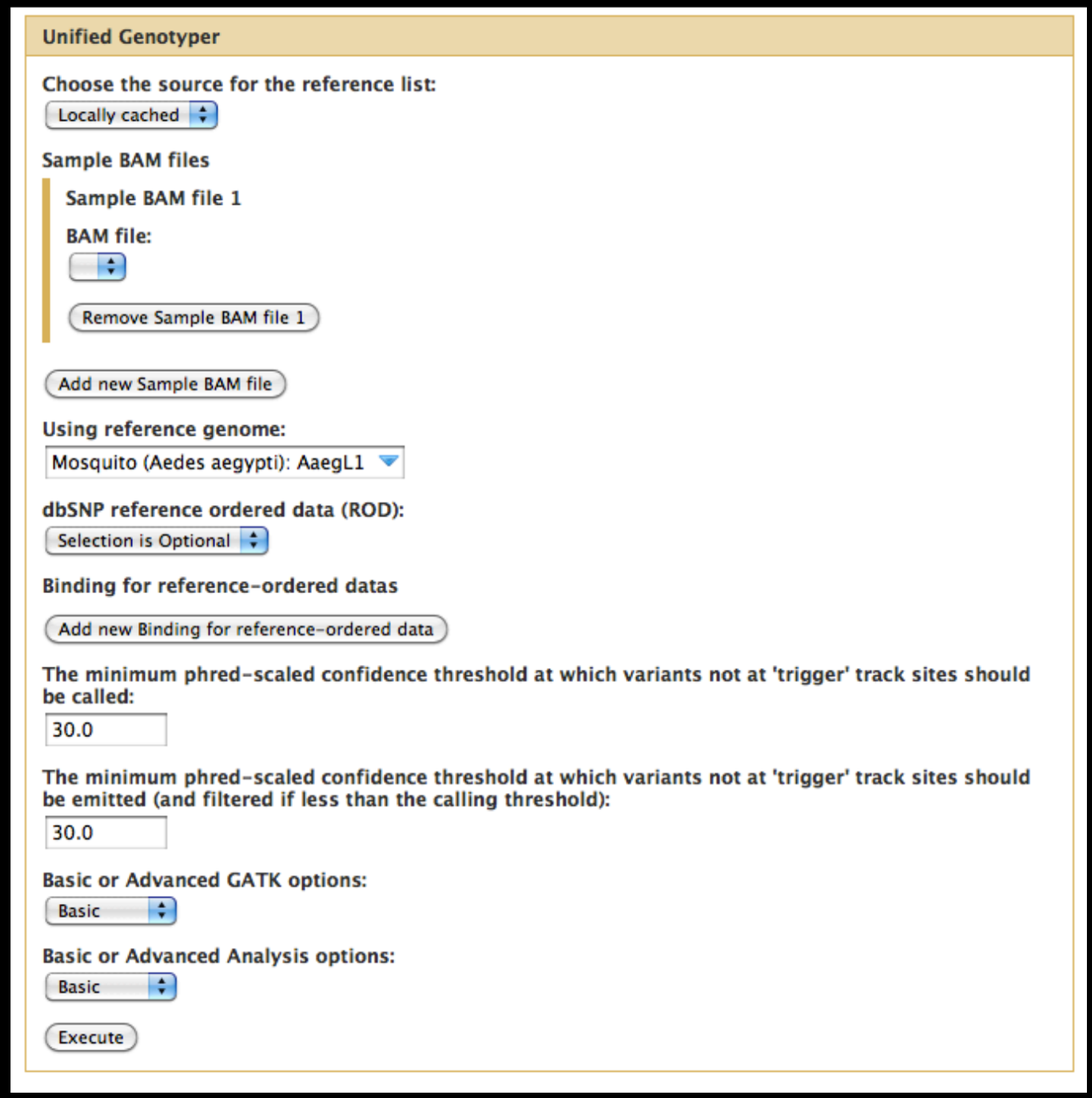
Inputs

- BAM files

Lots of possible parameters

Output

- VCF file(s)



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Peak Calling / ChIP-seq analysis

Punctate binding

- transcription factors

Diffuse binding

- histone modifications
- PolII

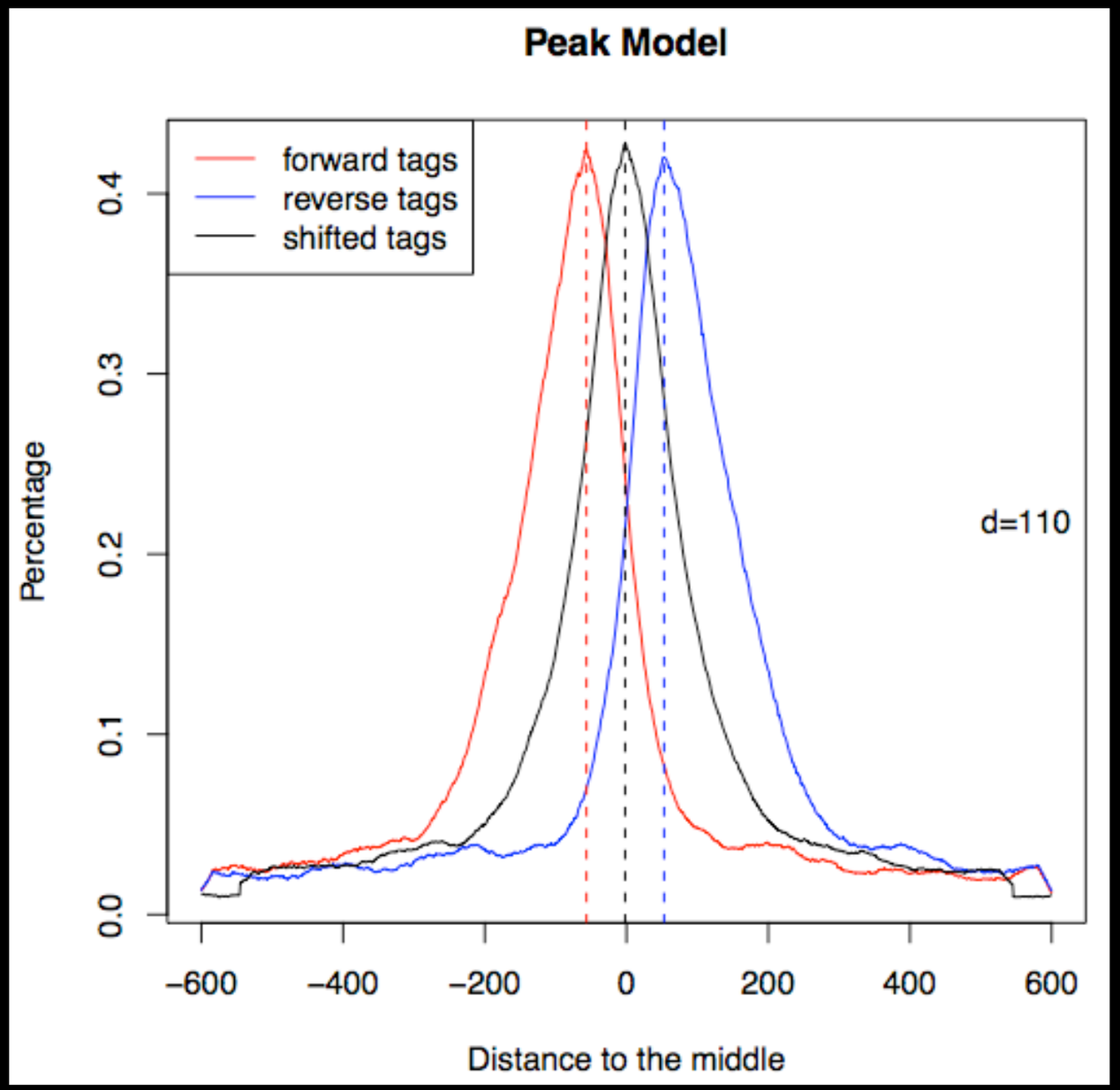
Punctate Binding --> MACS

Inputs

- Enriched Tag file
- Control / Input file (optional)

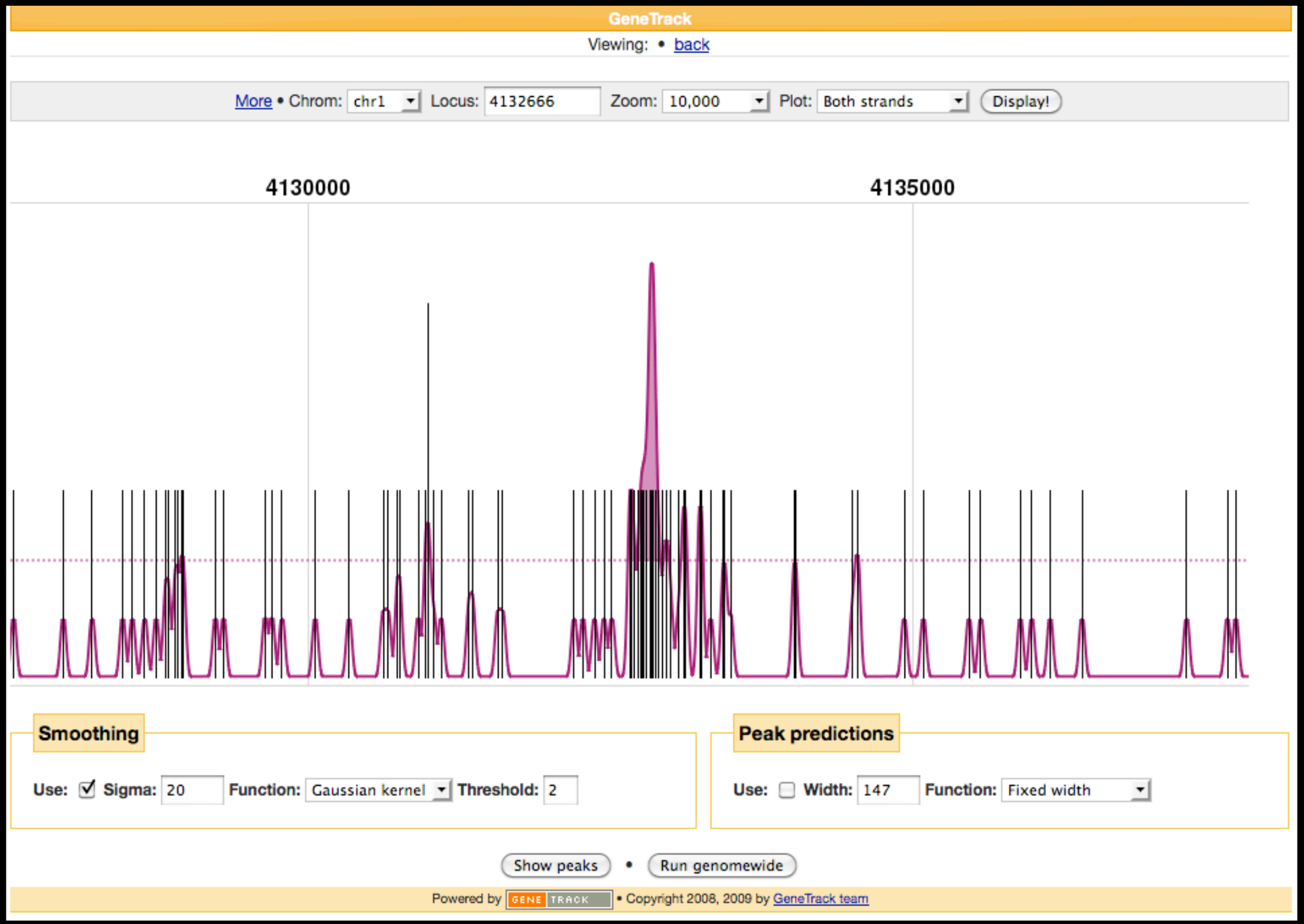
Outputs

- Called Peaks
- Negative Peaks (when control provided)
- Shifted Tag counts (wig, convert to bigWig for visualization)



Zhang et al. Model-based Analysis of ChIP-Seq (MACS). Genome Biol (2008) vol. 9 (9) pp. R137

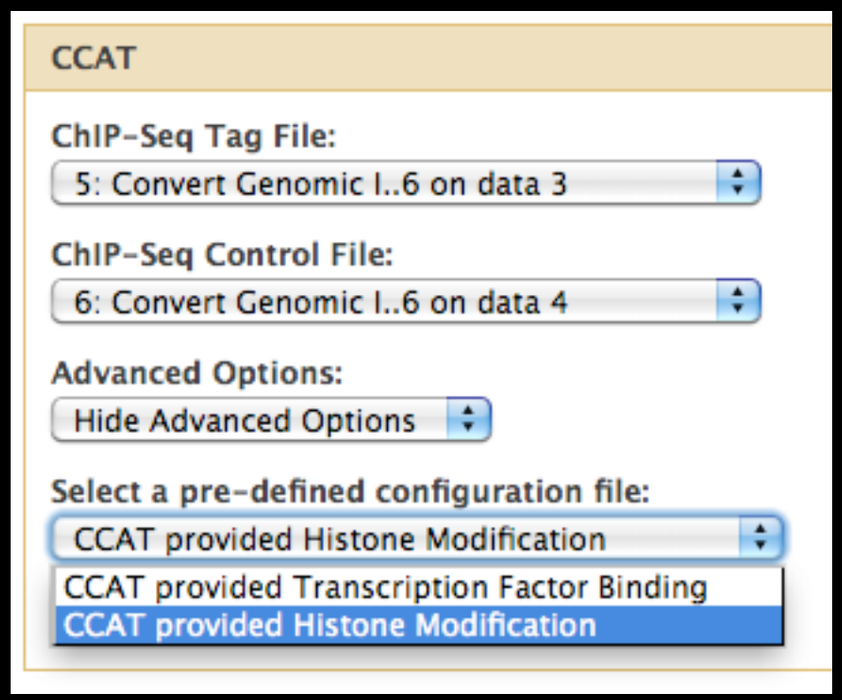
MACS --> GeneTrack

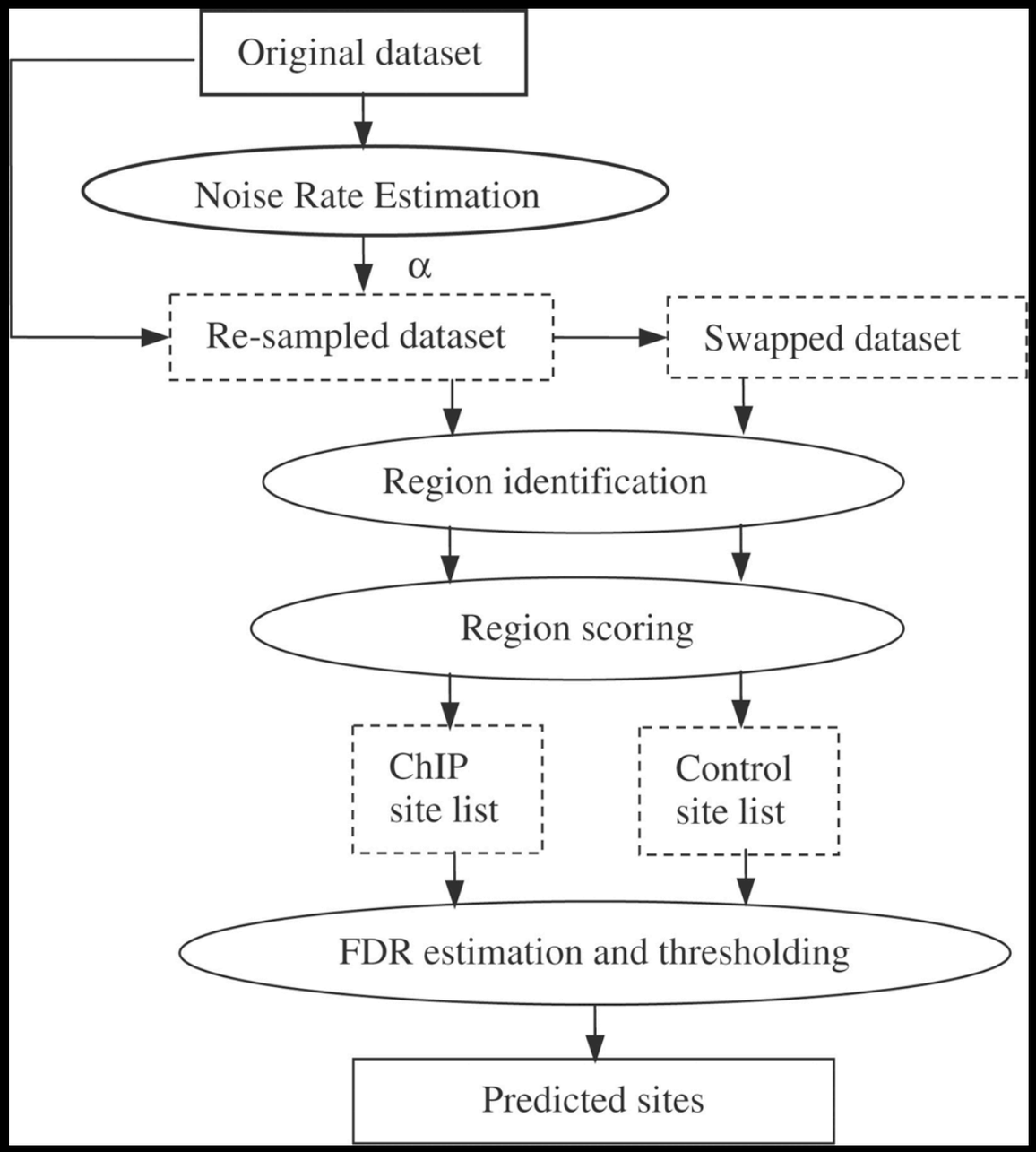


Albert I, Wachi S, Jiang C, Pugh BF. GeneTrack--a genomic data processing and visualization framework. Bioinformatics. 2008 May 15;24(10):1305-6. Epub 2008 Apr 3.

Diffuse Binding

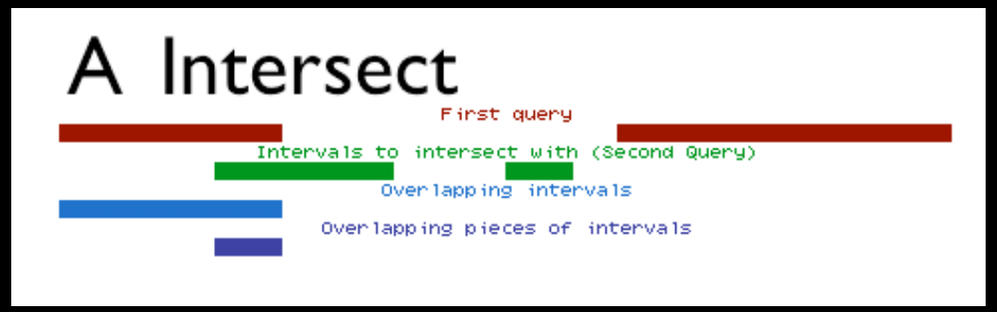
CCAT (Control-based ChIP-seq Analysis Tool)

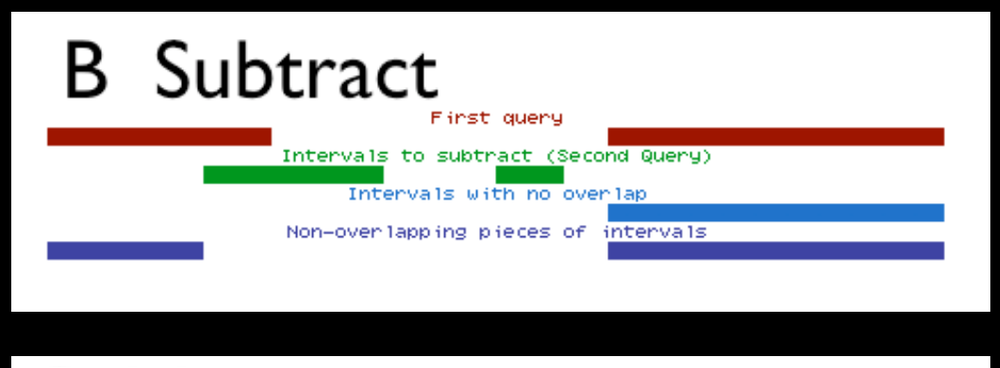


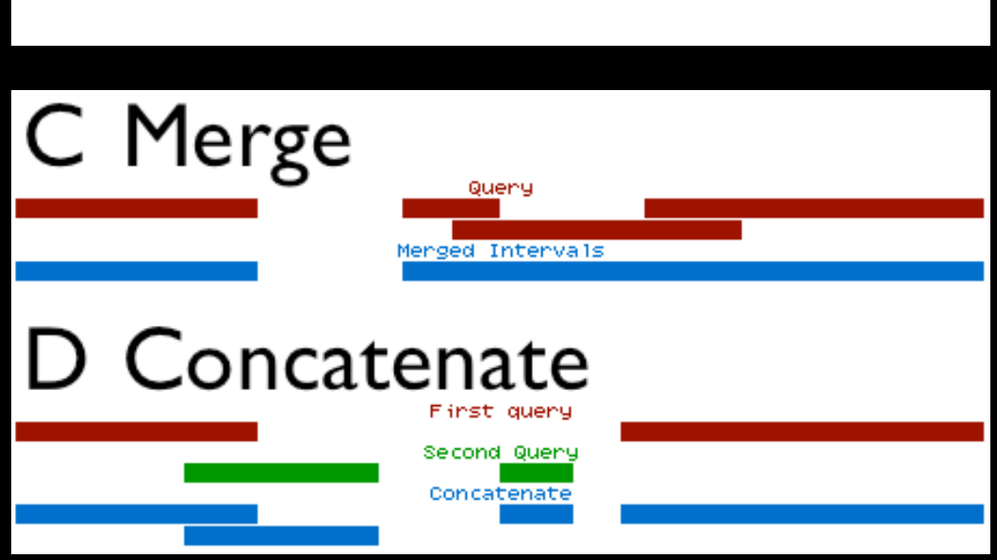


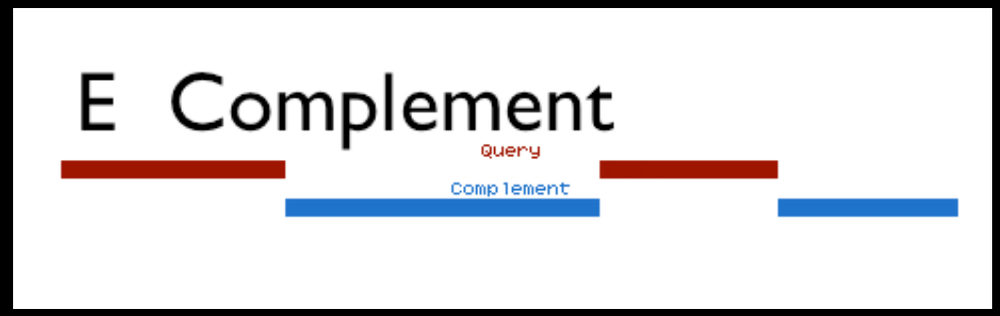
Xu H, Handoko L, Wei X, Ye C, Sheng J, Wei CL, Lin F, Sung WK. A signal-noise model for significance analysis of ChIP-seq with negative control. Bioinformatics. 2010 May 1;26(9):1199-204.

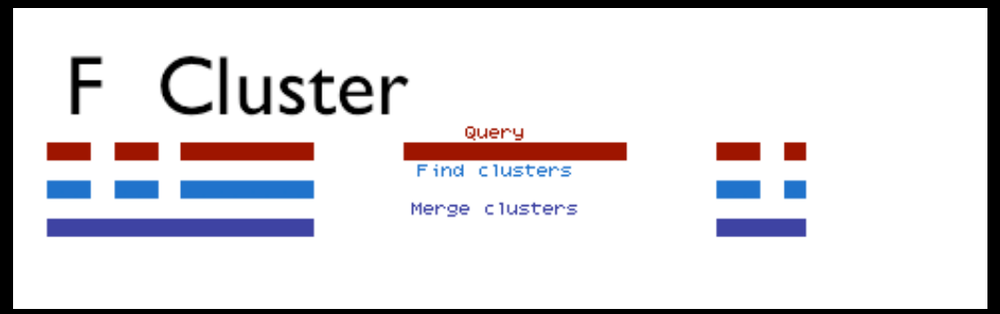
I have Peaks, now what?











Compare to other annotations using interval operations

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)

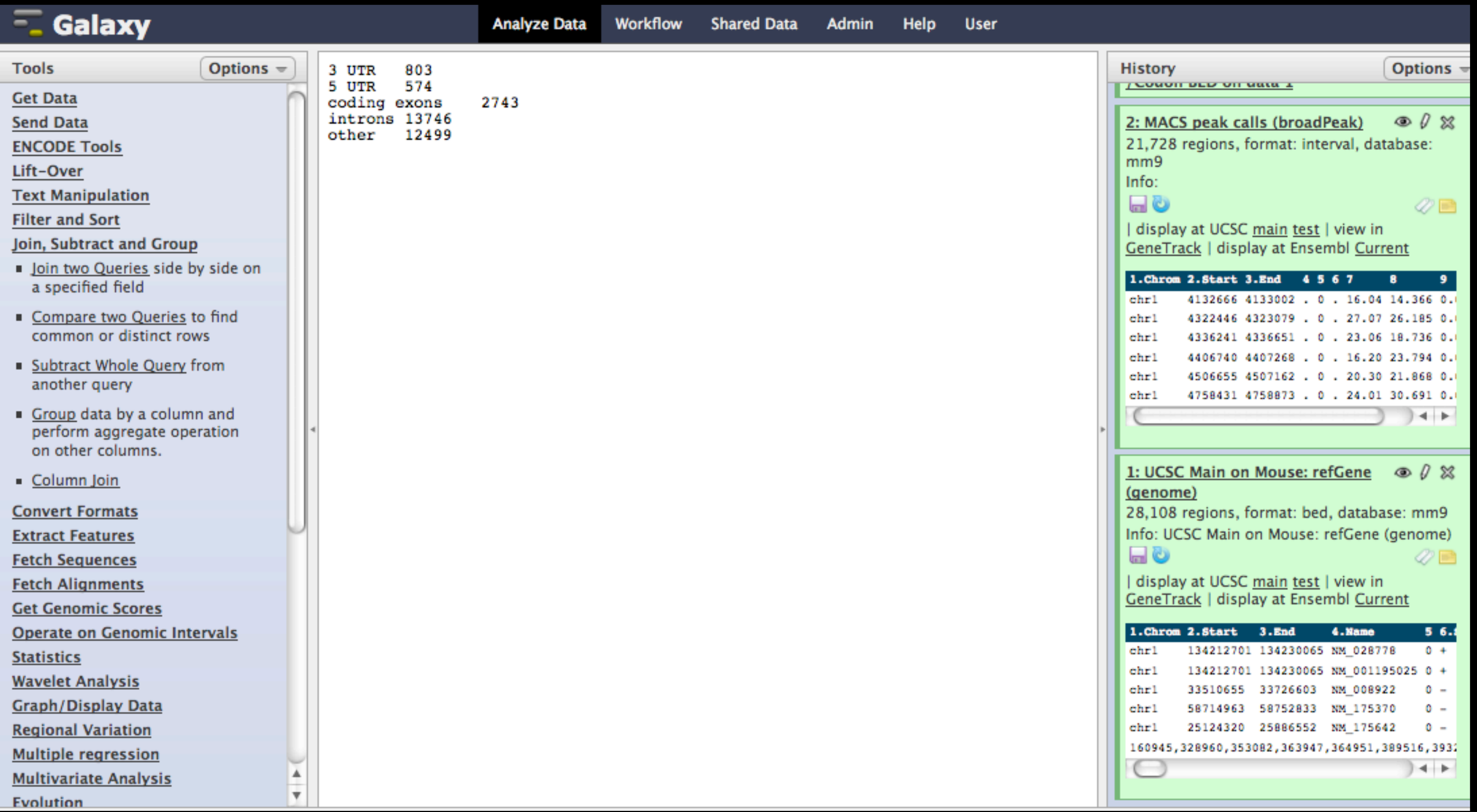
Create an Identifier column for the 'other' type (Add column)

Concatenate the exons/introns and other files

Determine which Peaks overlap the region types (Join)

Calculate counts for each region type (Group)

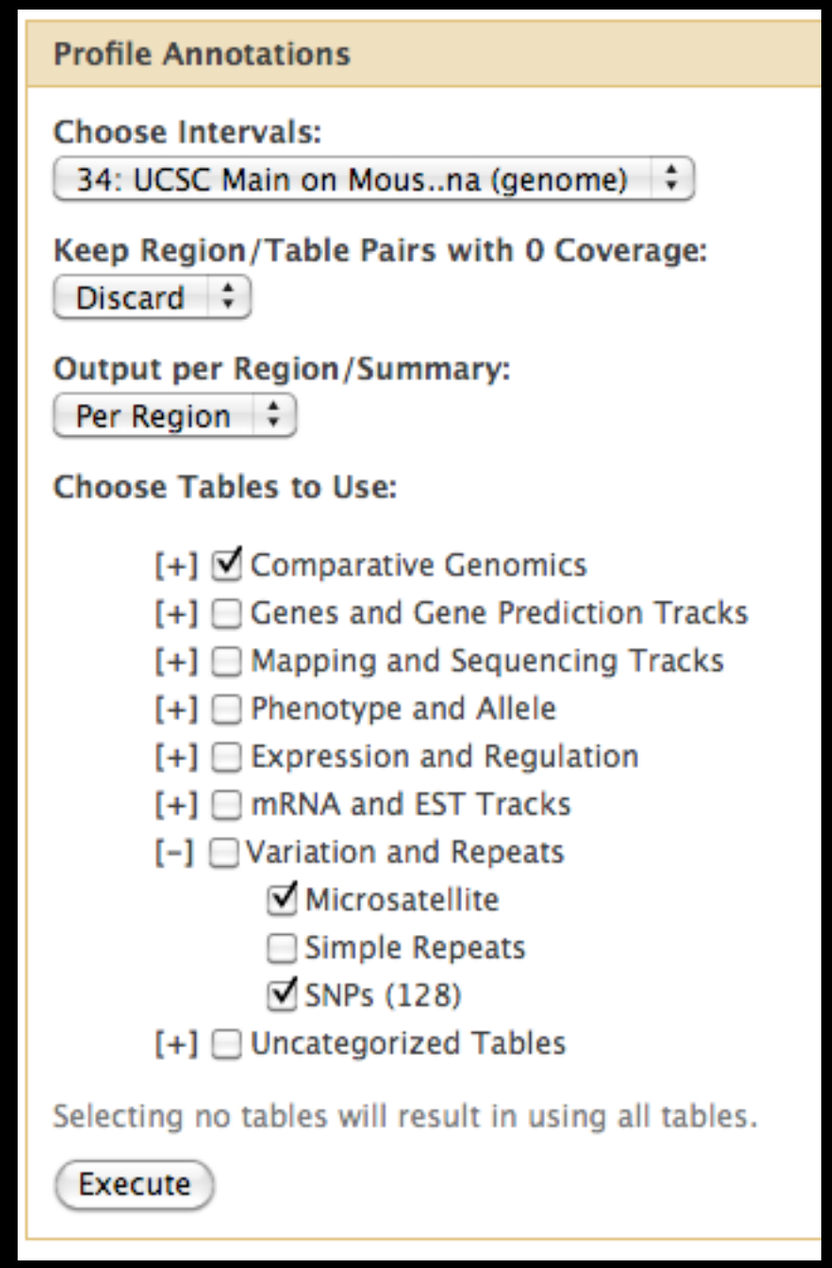
Secondary Analysis



Annotation Profiler

One click to determine base coverage of the interval (or set of intervals) by a set of features (tables) available from UCSC

galGal3, mm8, panTro2, rn4, canFam2, hg18, hg19, mm9, rheMac2



Overview

High-throughput Sequencing (HTS) Data

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- Prepare, quality control and manipulate reads
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- Transcriptome analysis

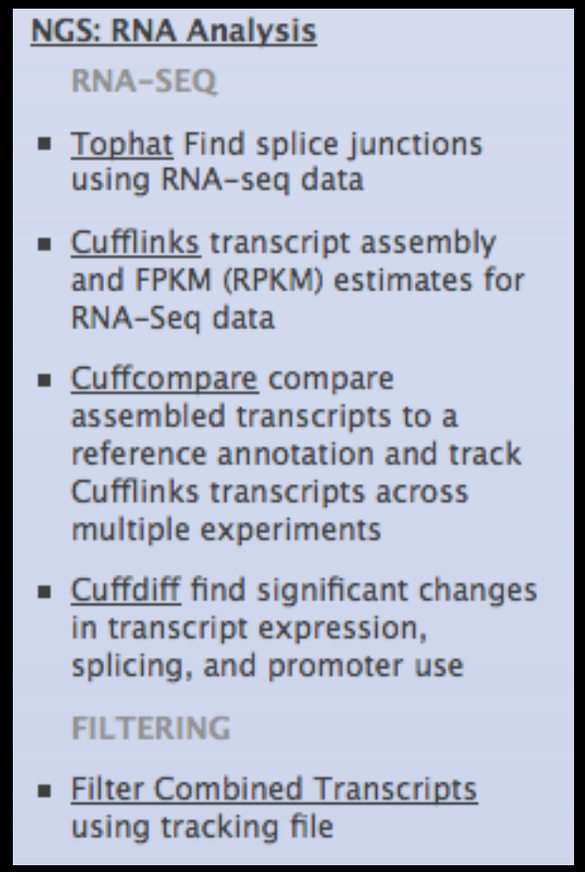
Galaxy for Sequencing Facilities

Galaxy exercises: ChIP-seq, RNA-seq, Variant Detection

Transcriptome Analysis
(with a reference genome)

TopHat

Cufflinks/compare/diff



1. Trapnell, C., Pachter, L. and Salzberg, S.L. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25, 1105-1111 (2009).
2. Trapnell et al. Transcript assembly and abundance estimation from RNA-Seq reveals thousands of new transcripts and switching among isoforms. Nature Biotechnology doi:10.1038/nbt.1621

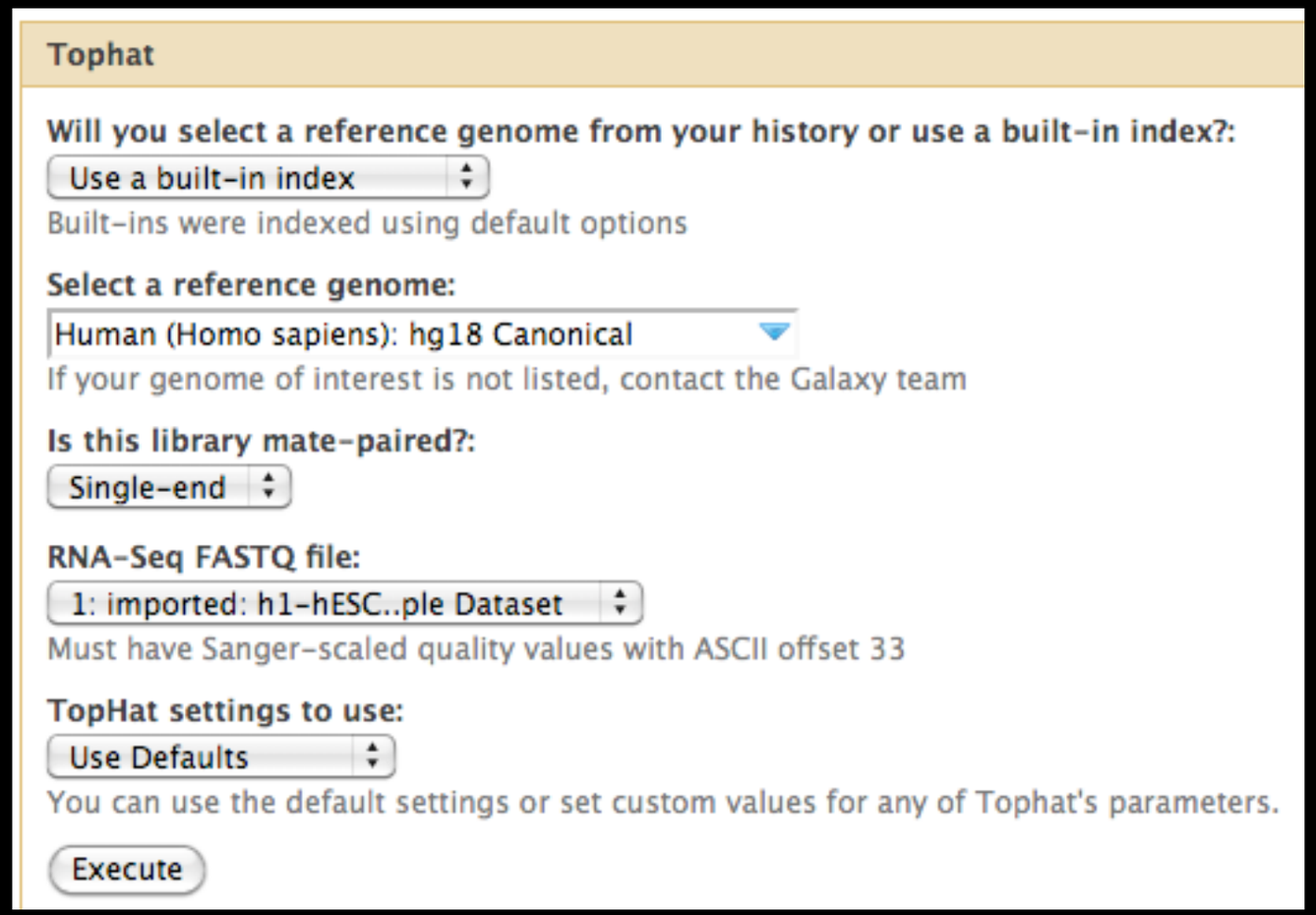
TopHat

Map RNA (FASTQ) to a reference Genome

- gapped mapper

Outputs

- BAM file of accepted hits
- BED file of splice junctions



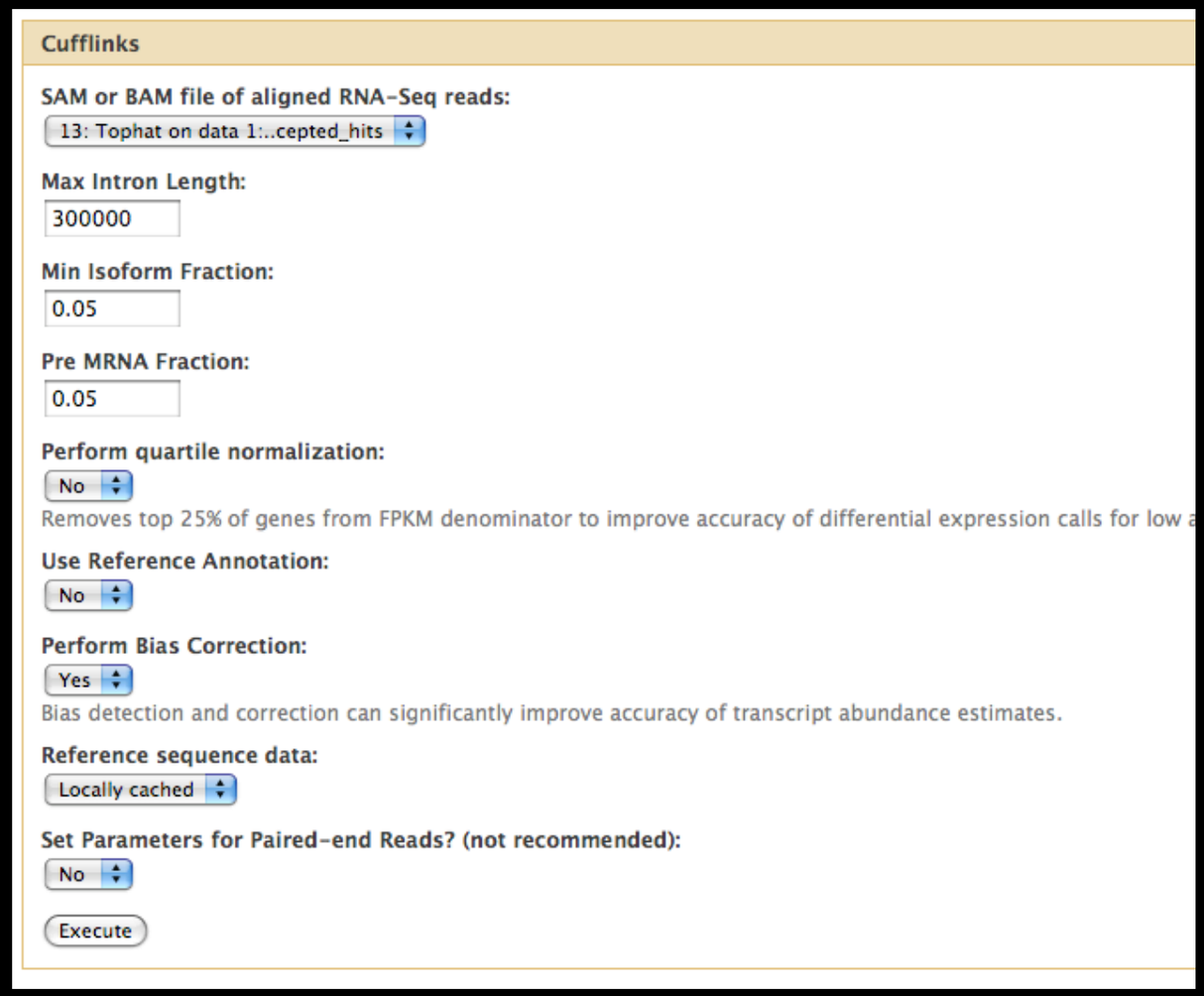
Cufflinks

Goal: transcript assembly and quantitation

Input: aligned RNA-Seq reads, usually from TopHat

Outputs

- assembled transcripts (GTF)
- genes' and transcripts' coordinates, expression levels



Cuffcompare

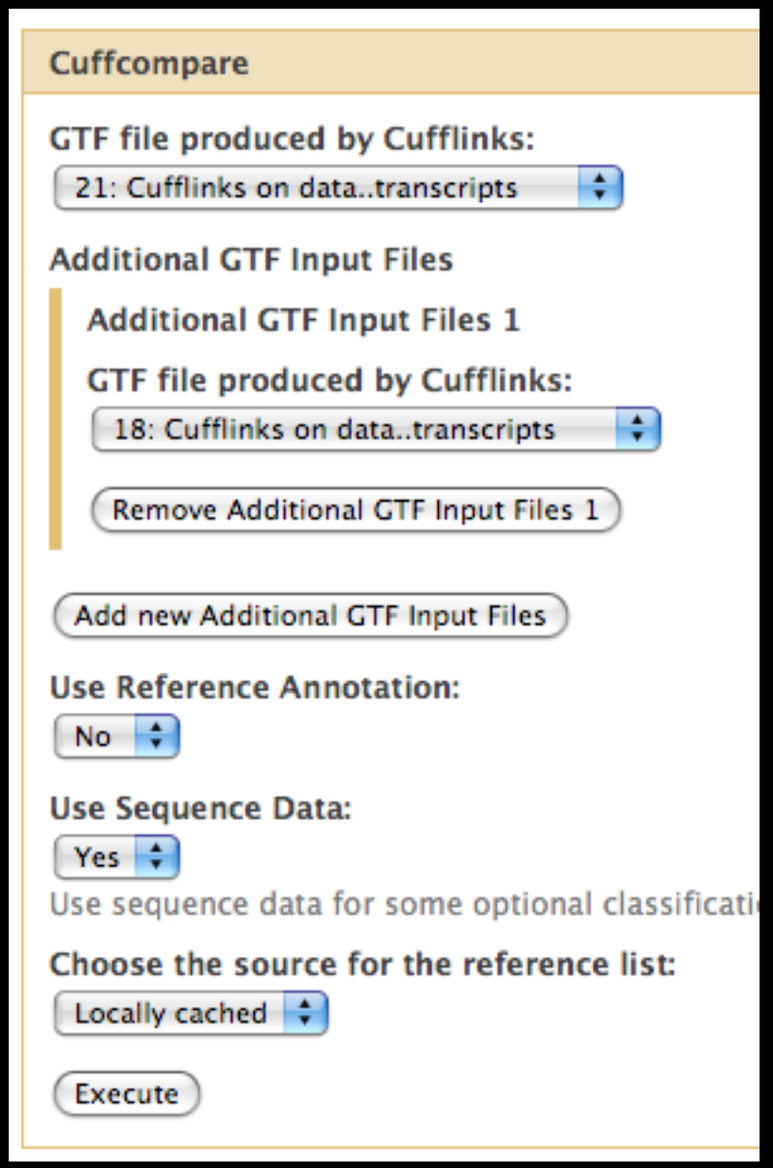
Goals

- generate complete list of transcripts for a set of transcripts
- compare assembled transcripts to a reference annotation

Inputs: assembled transcripts from Cufflinks

Outputs:

- Transcripts Combined File
- Transcripts Accuracy File
- Transcripts Tracking Files



Cuffdiff

Goals

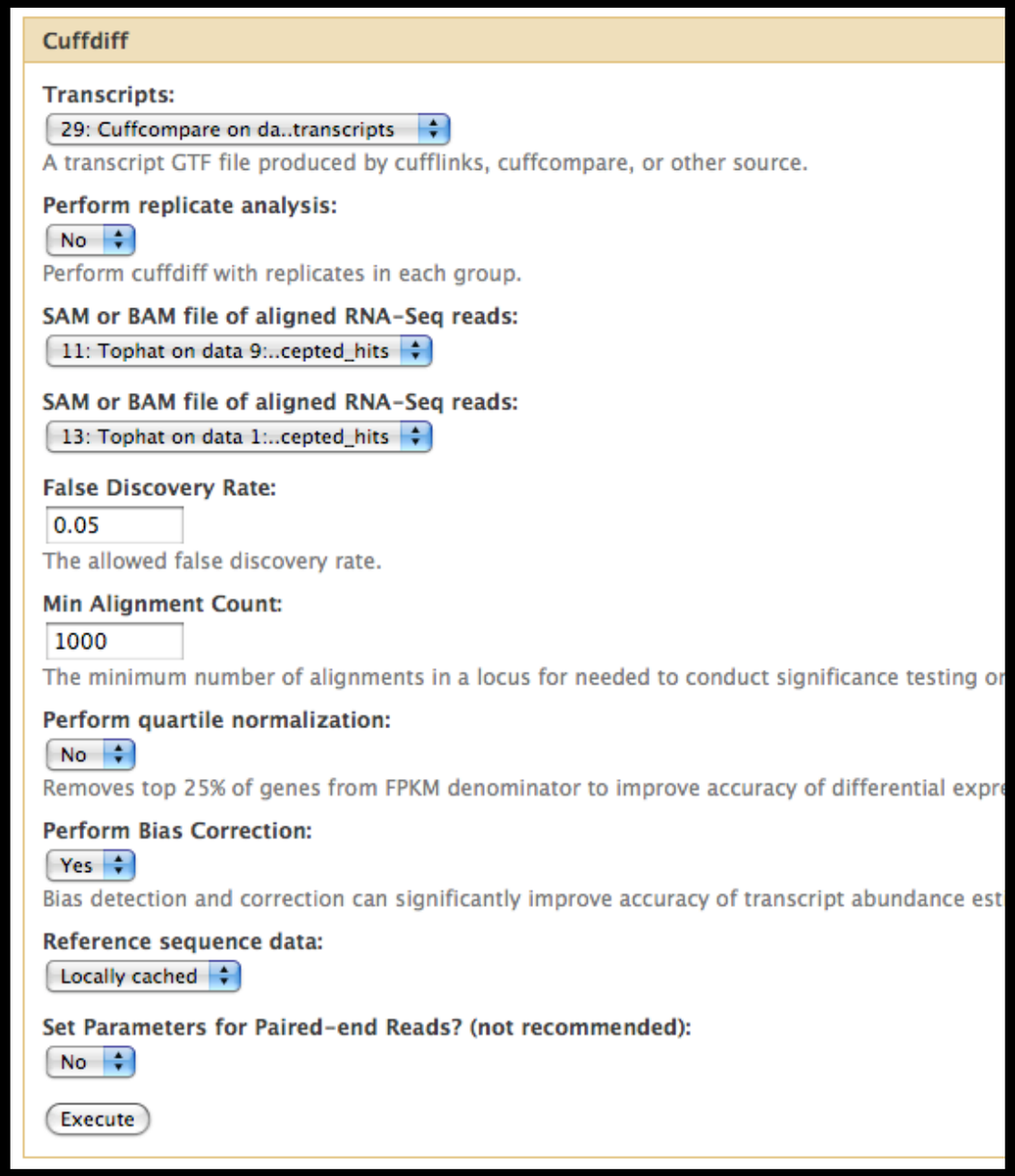
- differential expression testing
- transcript quantitation

Inputs

- Combined set of transcripts
- mapped reads from 2+ samples

Outputs

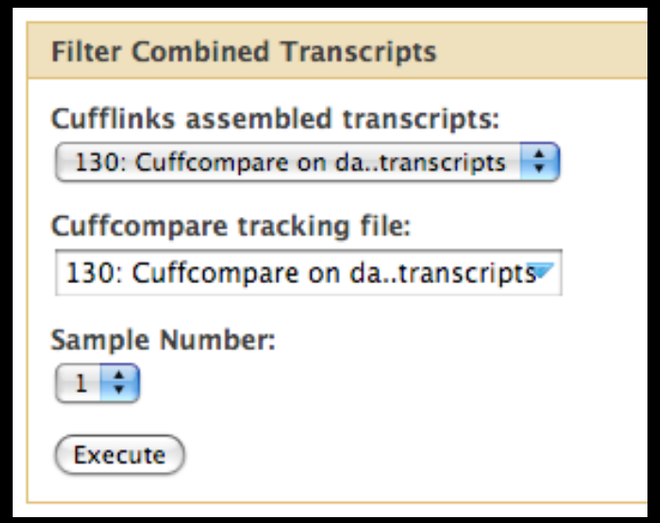
- differential expression tests for transcripts, genes, splicing, promoters, CDS
- quantitation values for most elements

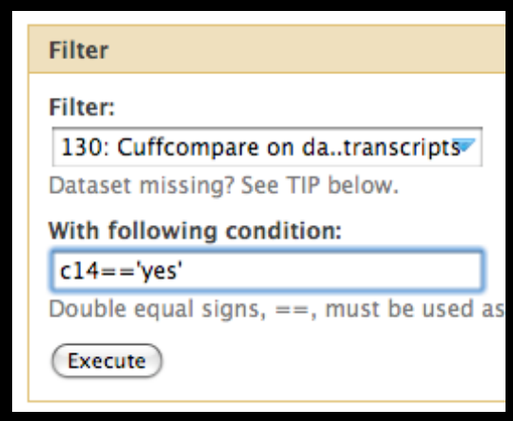


Next Steps

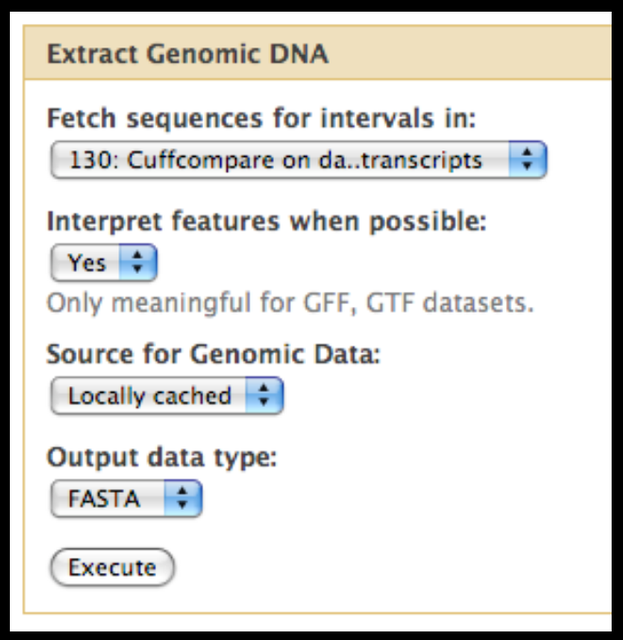
Filtering

- for differentially expressed elements
- combined transcripts (e.g. for those differentially expressed between samples)

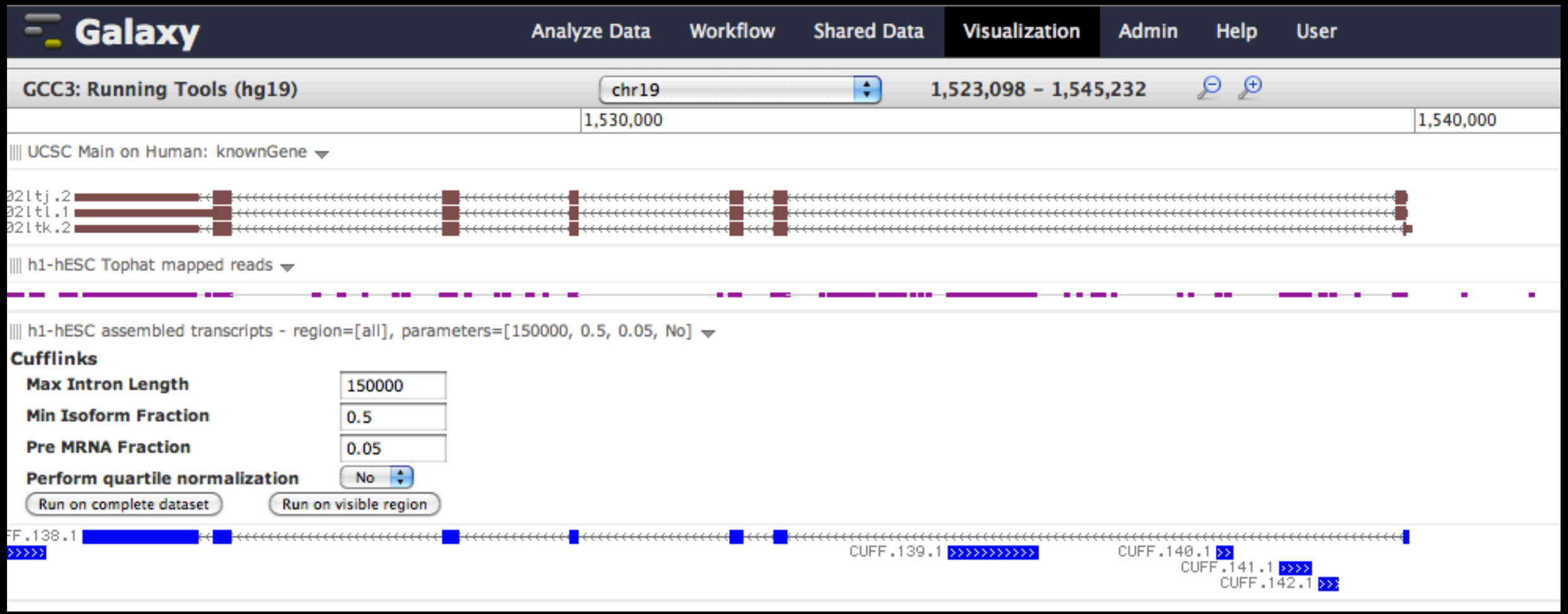




Extract transcript sequences and profile sequences for function



Integrating Tools and Visualization

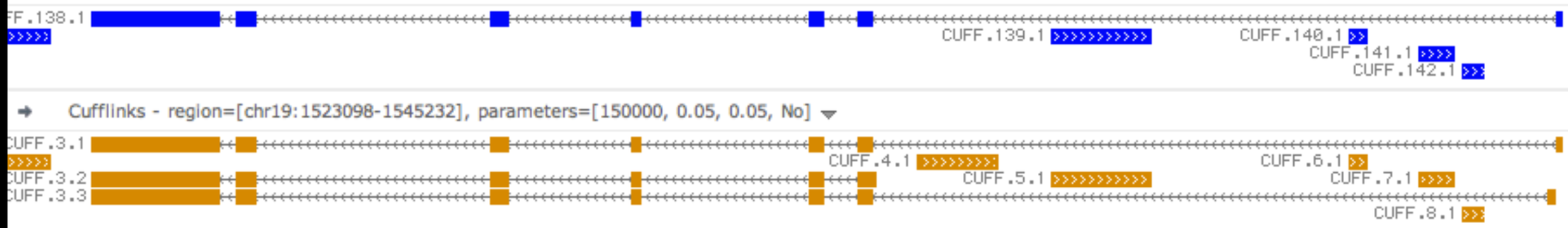


|||| h1-hESC assembled transcripts - region=[all], parameters=[150000, 0.5, 0.05, No]

Cufflinks

Max Intron Length	150000
Min Isoform Fraction	0.05
Pre MRNA Fraction	0.05
Perform quartile normalization	No

Run on complete dataset Run on visible region



Cufflinks - region=[chr19:1523098-1545232], parameters=[150000, 0.05, 0.05, No]

1,530,000 1,540,

UCSC Main on Human: knownGene

h1-hESC Tophat mapped reads

h1-hESC assembled transcripts - region=[all], parameters=[150000, 0.5, 0.05, No]

Cufflinks

Max Intron Length	150000
Min Isoform Fraction	0.05
Pre MRNA Fraction	0.001
Perform quartile normalization	No

Run on complete dataset Run on visible region

Cufflinks - region=[chr19:1523098-1545232], parameters=[150000, 0.05, 0.05, No]

Cufflinks - region=[chr19:1523098-1545232], parameters=[150000, 0.05, 0.001, No]

Working to add GATK Unified Genotyper (and more!) to Trackster as well

Working with HTS Tools

Often challenging

- many parameters
- time intensive
- evaluating results difficult

Good options

- filter early, filter often: easier to understand fewer results
- experimentation: can rerun tools, workflows
- visualization: use tools in Trackster when possible

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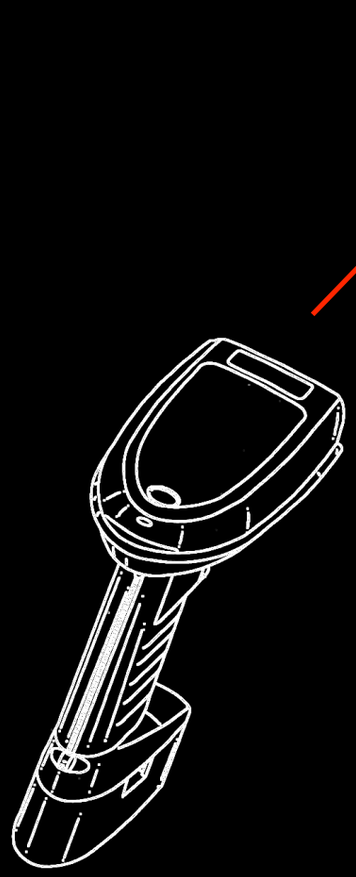
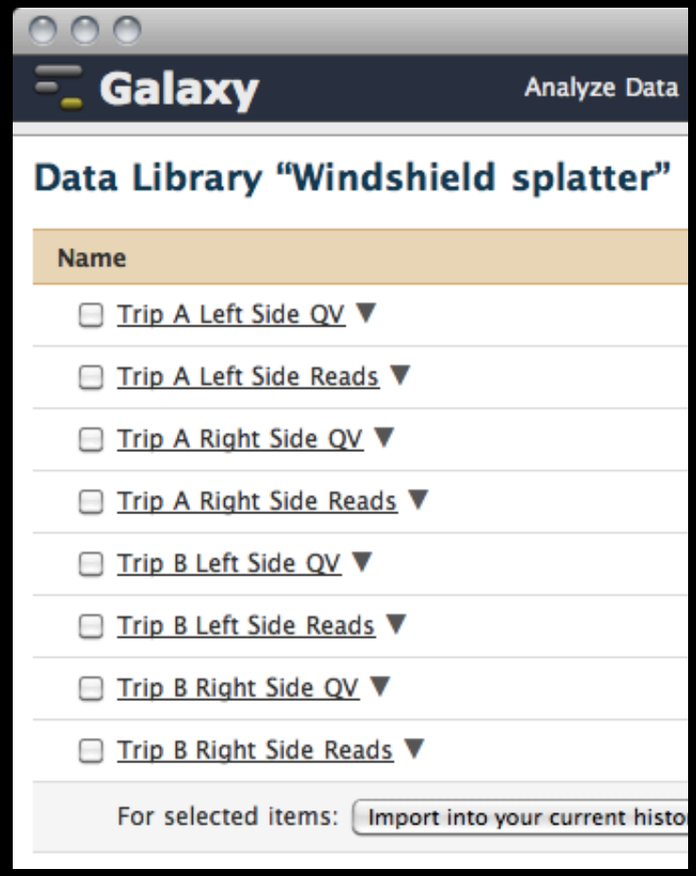


illumina

PACIFIC BIOSCIENCES™



Sample information tracked in Galaxy, state changes through laboratory workflow are captured, data is linked back to sample in user's workspace

Sample Tracking System

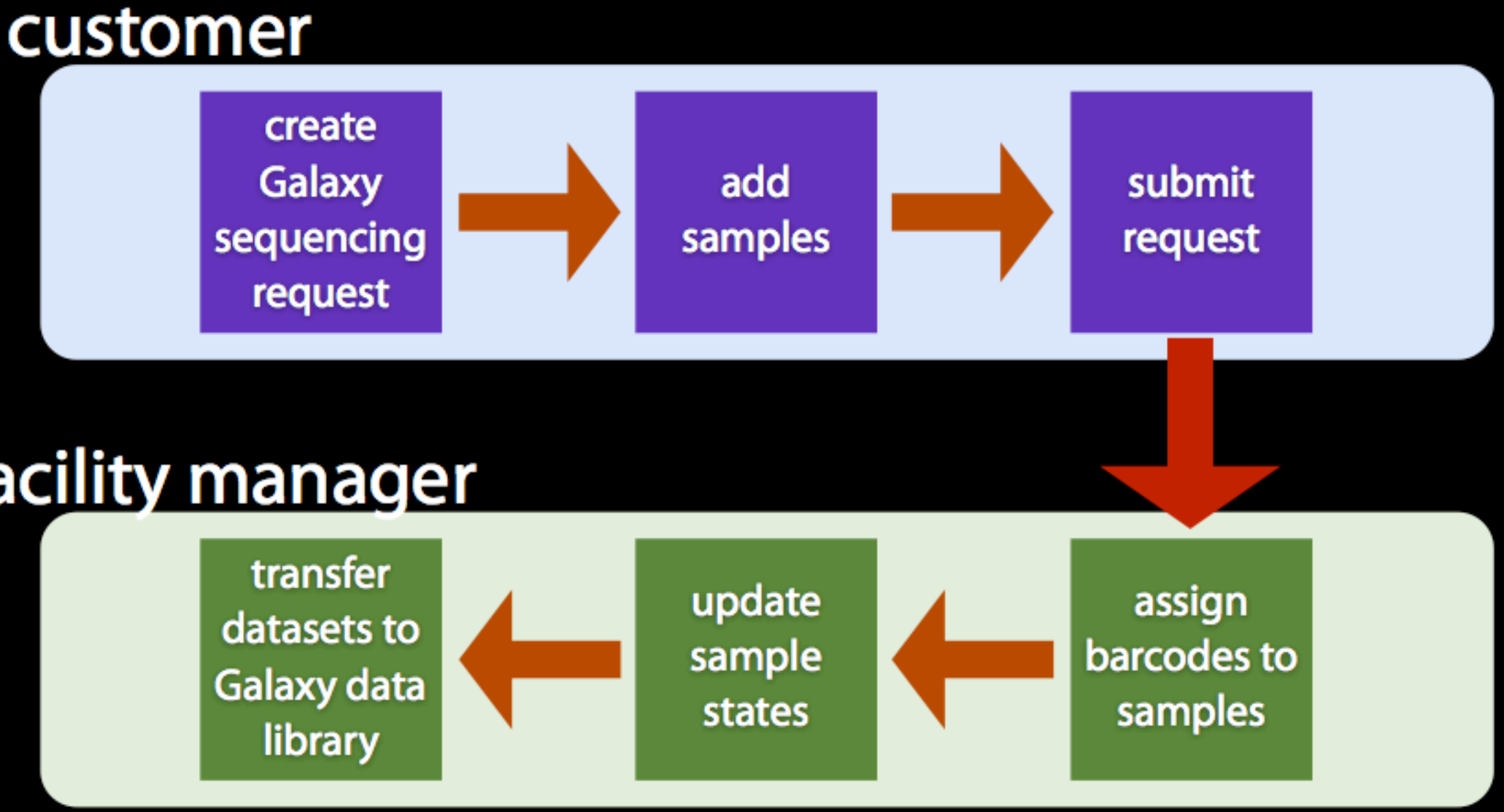
Built-in system for tracking sequencing requests

Customizable interfaces

- Sequencing Facility Managers/Administrators
- Users/Biologists

Streamlines data delivery: sequencing runs to users

How does it all work?



Sequencing Facility Managers

Setup the Galaxy sample tracking system according to the core facility workflow [Once per request type]

Create and submit a sequencing request on behalf of another user

Reject an incomplete or erroneous sequencing request

Receive samples and assign them tracking barcodes.

Setup data transfer from the sequencer

Sequencing Facility Users

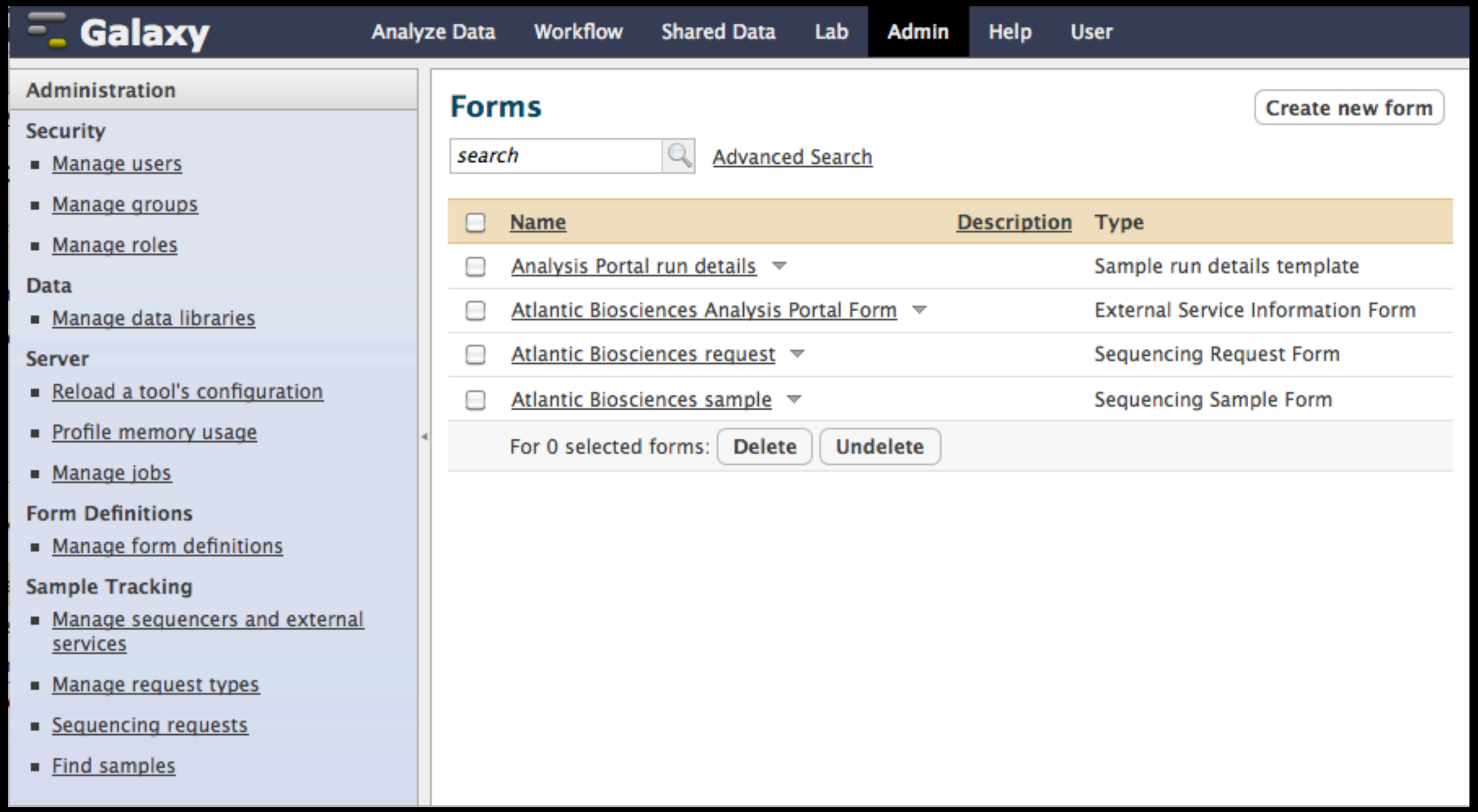
Create and submit a sequencing request

Edit and resubmit a rejected sequencing request

Obtain datasets at the end of a sequencing run

Select Libraries and Histories, and Workflows to populate and run on sequenced samples.

Configure Available Request / Sample Options



View

Edit form definition "Atlantic Biosciences request" (Sequencing Request Form)

Name

Atlantic Biosciences request

Description

Form definition fields

1. Name (TextField)

2. Scientific Contact (AddressField)

Add field

Save

Configurations can be

- custom-built
- loaded from provided configuration files

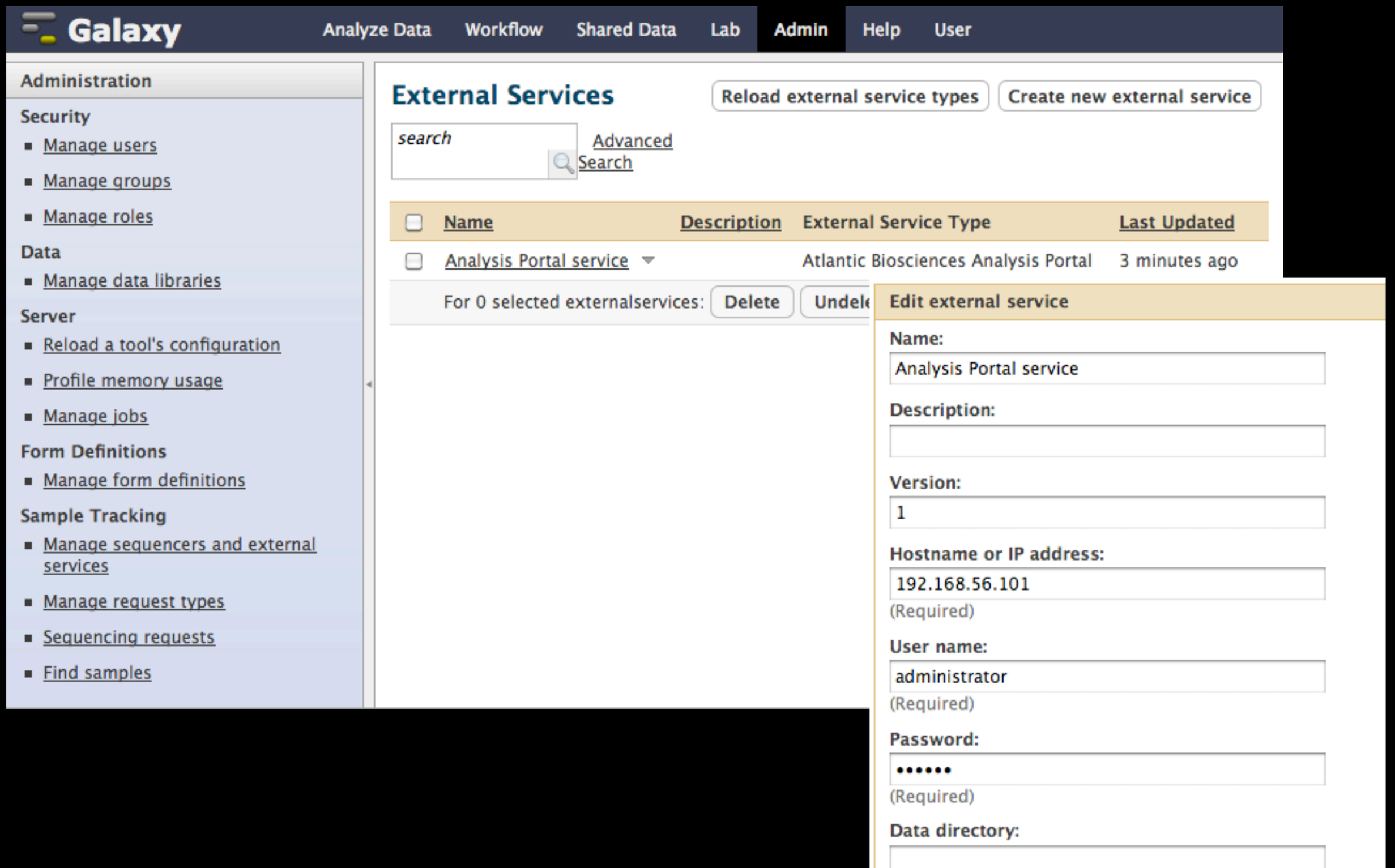
Edit

Form definition "Atlantic Biosciences sample" (Sequencing Sample Form)

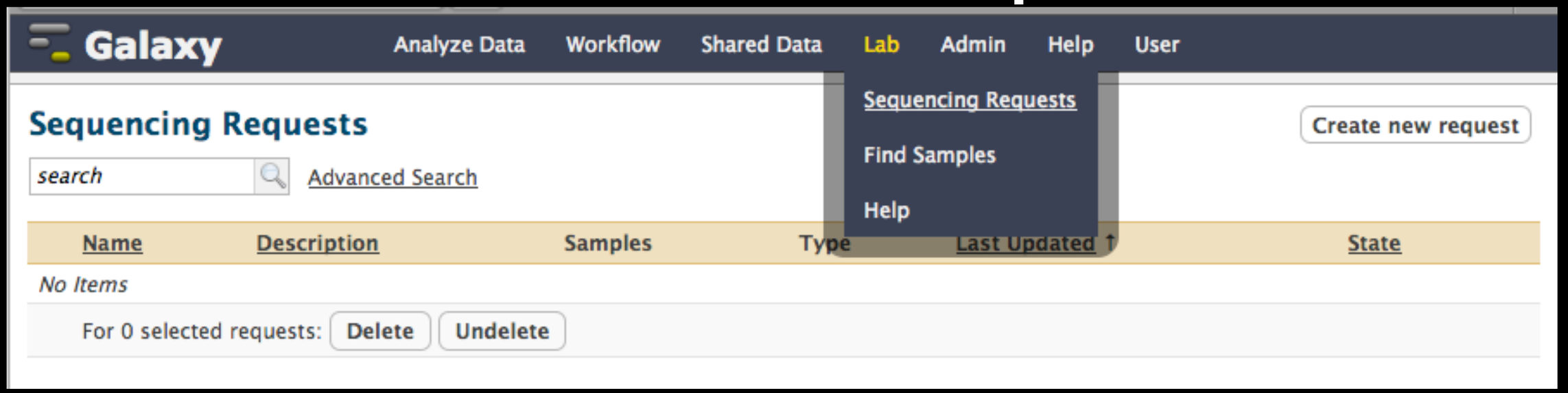
Layout1

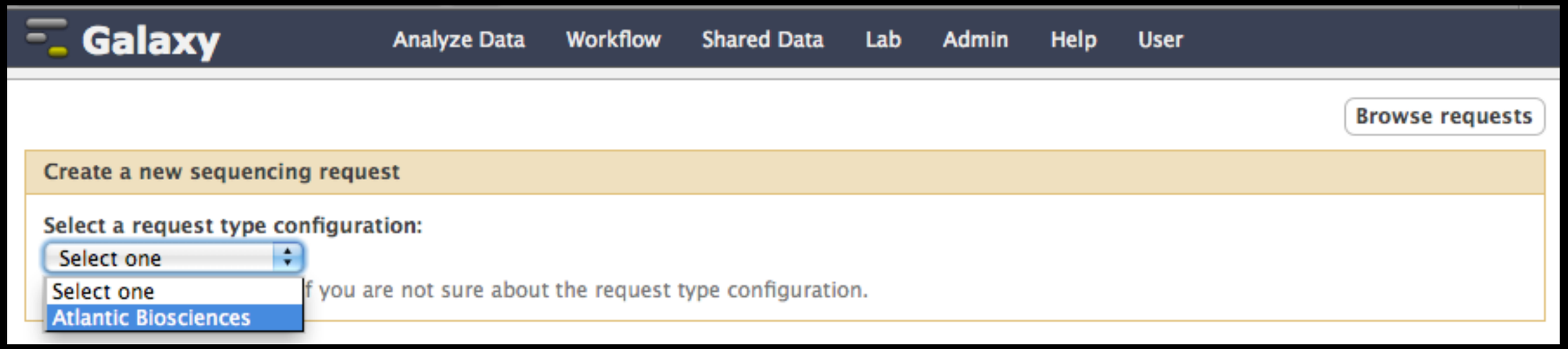
Run type	Read length	Number of Lanes	Alignment target	Processing time	Comments
SelectField: – (optional) Options: SR PE	SelectField: – (optional) Options: 36 50 75 100	TextField: – (optional)	TextField: – (optional)	SelectField: – (optional) Options: Std Rush option3	TextField: – (optional)

Configure the Sequencer



User Creates a Request





User Creates a Request

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

- Sequencing Requests
- Find Samples

Sequencing Requests

search | Advanced Search

Create new request

Name	Description	Samples
No Items		

For 0 selected requests: Delete | Undelete

Create a new sequencing request

Select a request type configuration:

Atlantic Biosciences

Contact the lab manager if you are not sure about the request type configuration.

Name of the Experiment

My first ChIP-seq Experiment

(Required)

Description

This is Experiment was performed using the protoc

(Optional)

Name

(Optional)

Scientific Contact

dan@bx.psu.edu office address

office
Penn State University
Wartik Lab
University Park PA 16803
United States
Phone: 867-5309

(Optional)

Save | Add samples

Galaxy | Analyze Data | Workflow | Sha

Create a new sequencing request

Select a request type configuration:

Select one

- Select one
- Atlantic Biosciences

f you are not sure about the request type c

User Adds a Sample

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

Request Actions

Add Samples to Sequencing Request "My first ChIP-seq Experiment"

Name	State	Data Library	Folder	History	Workflow
Sample_1 *(required)*		Dan's Sequencing Requests	ChIP-seq	My own ChIP-seq Experiment!	Dan's ChIP-seq Workflow

For each sample, select the data library and folder in which you would like the run datasets deposited. To automatically run a workflow on run datastets, select a history first and then the desired workflow.

▸ Layout1

Copy 1 samples from sample None

Select the sample from which the new sample should be copied or leave selection as **None** to add a new "generic" sample.

Add sample | Save | Cancel

Click the **Add sample** button for each new sample and click the **Save** button when you have finished adding samples.

▸ Import samples from csv file

History
Workflow to run

Samples Added, Submit Request

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

Edit samples | Submit request | Request Actions

Add Samples to Sequencing Request "My first ChIP-seq Experiment"

Name	State	Data Library	Folder	History	Workflow
Sample_1	Unsubmitted	Dan's Sequencing Requests	ChIP-seq	My own ChIP-seq Experiment!	Dan's ChIP-seq Workflow

For each sample, select the data library and folder in which you would like the run datasets deposited. To automatically run a workflow on run datastets, select a history first and then the desired workflow.

Layout1

Copy 1 **samples from sample** None

Select the sample from which the new sample should be copied or leave selection as **None** to add a new "generic" sample.

Add sample

Click the **Add sample** button for each new sample.

Import samples from csv file

Samples enter “New” state

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

Request Actions

The sequencing request has been submitted.

Sequencing request "My first ChIP-seq Experiment"

Current state:
In Progress

Description:
This is Experiment was performed using the protocol ...

User:
dan@bx.psu.edu

Request type:
Atlantic Biosciences

More

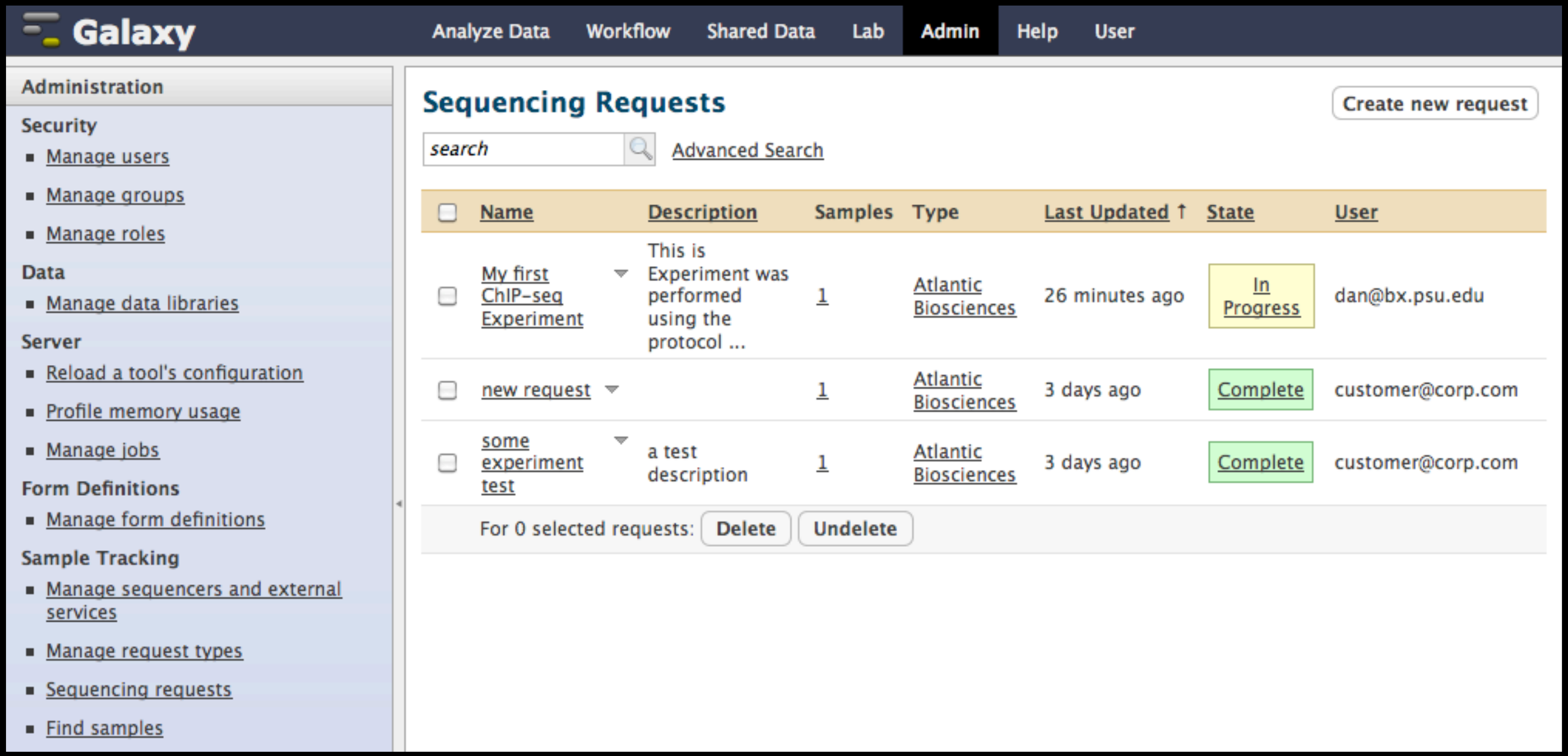
Samples

Edit samples

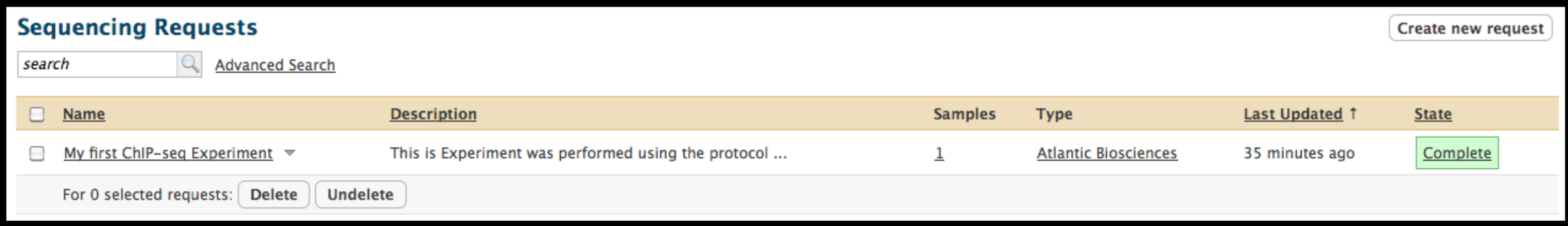
Name	Barcode	State	Data Library	Folder	History	Workflow	Run Datasets
Sample_1		New	Dan's Sequencing Requests	ChIP-seq	My own ChIP-seq Experiment!	Dan's ChIP-seq Workflow	0

Layout1

Sequencing Facility is informed of Request



Sequencing Facility Receives Samples



Facility

- assigns a barcode to sample tubes
- Scans barcode at each step to change state

User can watch progress of sequencing request

Sequencing Finished

Datasets are transferred from sequencer into Galaxy

- library
- user’s history

Galaxy Workflow is executed on Dataset

User is automatically emailed

Extending Sample Tracking with ngLims

An add-on written by community contributor Brad Chapman

http://bitbucket.org/chapmanb/galaxy-central

https://bitbucket.org/galaxy/galaxy-central/wiki/LIMS/nglims

http://bcbio.wordpress.com/2011/01/11/next-generation-sequencing-information-management-and-analysis-system-for-galaxy/

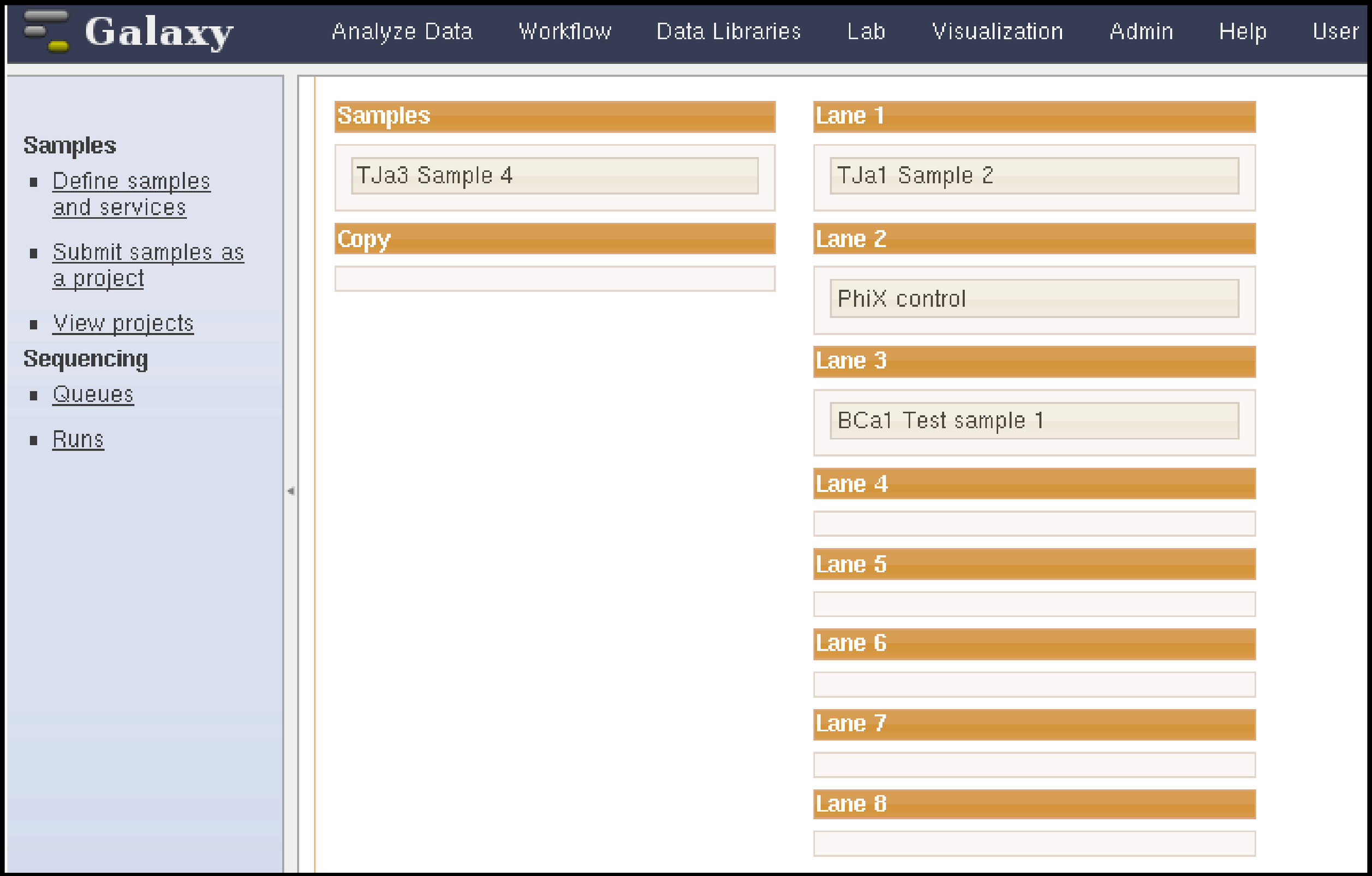
Sample tracking is completely extensible

Track manually, with barcodes, or integrate with an existing LIMS

Everything is configuration driven, capture whatever data and support whatever workflow you want

Interaction with sequence instruments and secondary analysis is completely pluggable

- For services that provide a web / REST API even easier



Example: extensions from Brad Chapman for flowcell layout, multiplexing, ...

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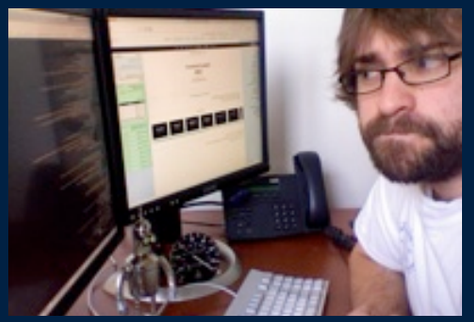
EMORY

PENNSTATE

Enis Afgan

Dannon Baker

Dan Blankenberg



Nate Coraor

Dave Clements

Jeremy Goecks

Jennifer Jackson

Greg von Kuster

Kanwei Li

James Taylor

Kelly Vincent

Anton Nekrutenko

Supported by the **NHGRI** (HG005542, HG004909, HG005133), **NSF** (DBI-0850103), Penn State University, Emory University, and the Pennsylvania Department of Public Health

Using Galaxy

Use public Galaxy server: UseGalaxy.org

Download Galaxy source: GetGalaxy.org

Galaxy Wiki: GalaxyProject.org

Screencasts: GalaxyCast.org

Public Mailing Lists

- galaxy-bugs@bx.psu.edu
- galaxy-user@bx.psu.edu
- galaxy-dev@bx.psu.edu

ChIP-seq and RNA-seq exercises

RNA-seq

- http://usegalaxy.org/u/jeremy/p/galaxy-rna-seq-analysis-exercise
  - start Tophat mapping first (second section), then look at QC (first section)

Chip-seq

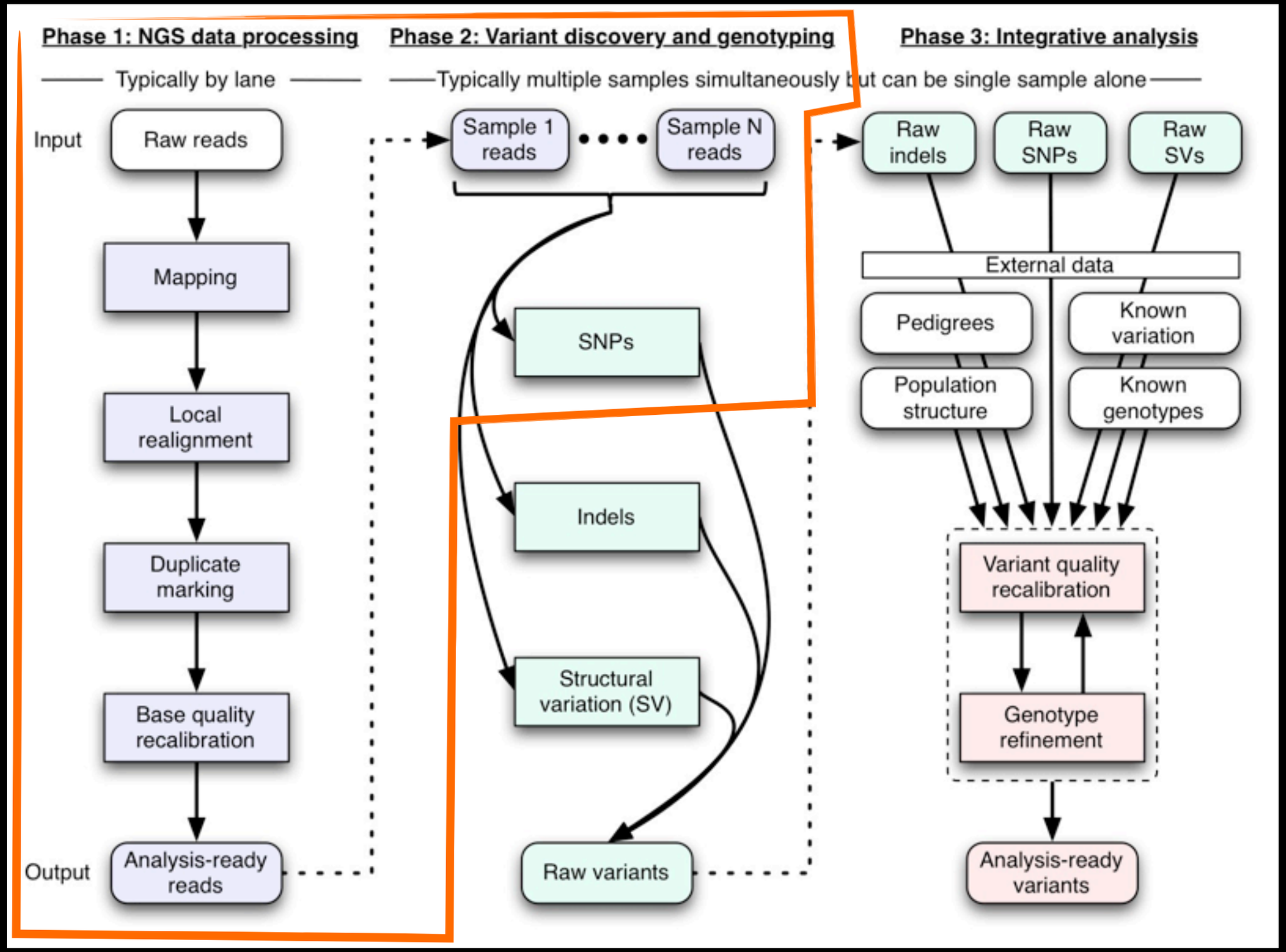
- http://usegalaxy.org/u/james/p/exercise-chip-seq

Variant Detection

- Using GATK, Picard Tools

http://ec2-50-16-165-27.compute-1.amazonaws.com/

Variant Detection



Depristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, Del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011 May;43(5):491-8.