Galaxy 2011

Community Conference

25-26 May Lunteren, The Netherlands

Dank u NBIC!

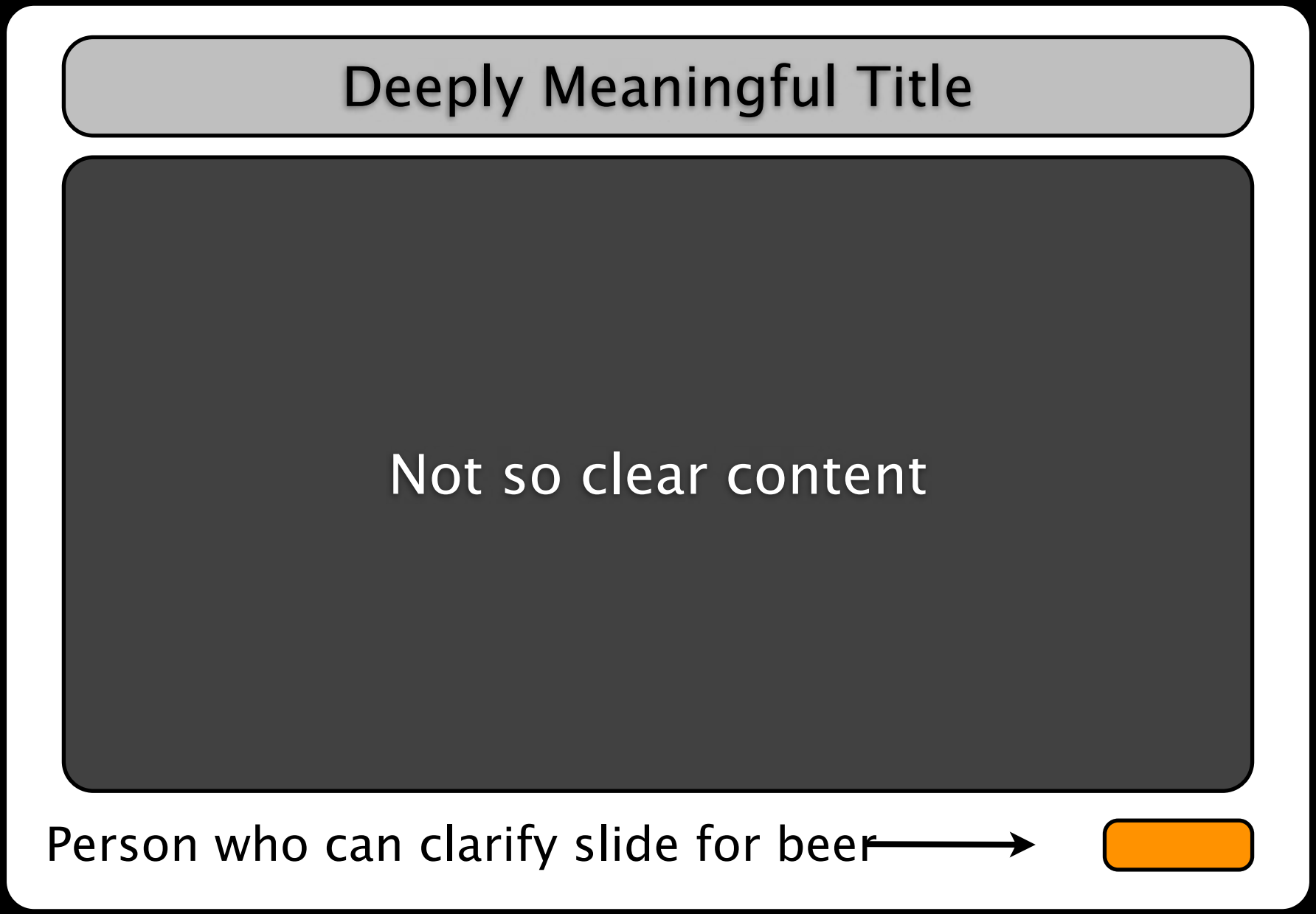
- Hailiang Lai
- Femke Francissen
- Freek de Bruijn
- Anita Radstaat
- Works at De Werelt
- Marc van Driel
- Rob Hooft
- Mons Barend

The 2011 Galaxy Community Conference is generously sponsored by the US National Science Foundation and the Netherlands Bioinformatics Centre (NBIC). Galaxy is developed by Galaxy Team and funded by NSF, NHGRI, Penn State, Emory University, Penn State Institute for CyberScience and the Huck Institutes of the Life Sciences. Galaxy is free for all.

Introduction to Galaxy and GCC

The Team

Slide Organization



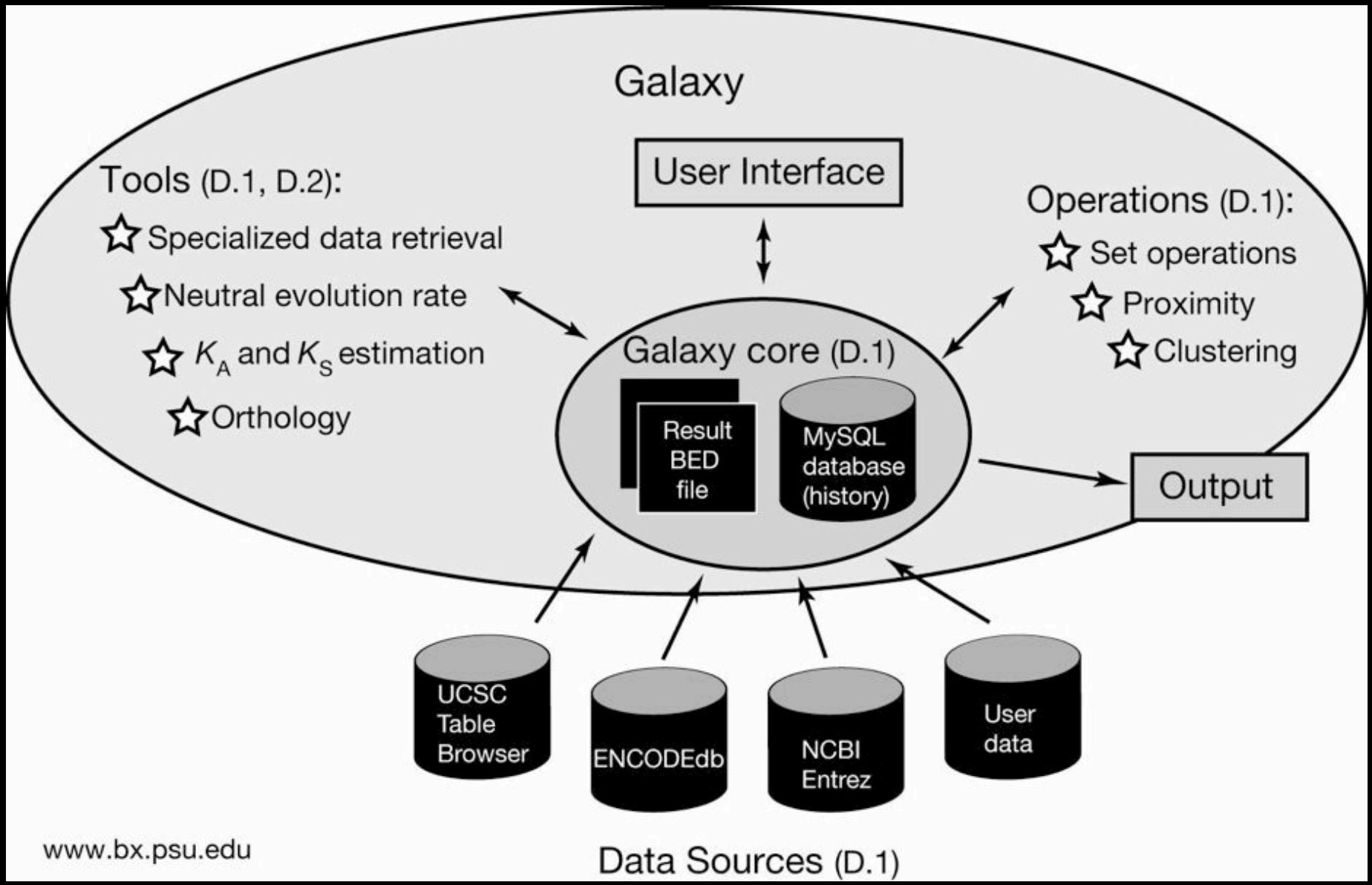
Talk Organization
The next 90 minutes

- History (2005 – 2010)
- Present (2010 – 2011)
- The “Vision” (2011 – ∞)
- The Community
- Beer

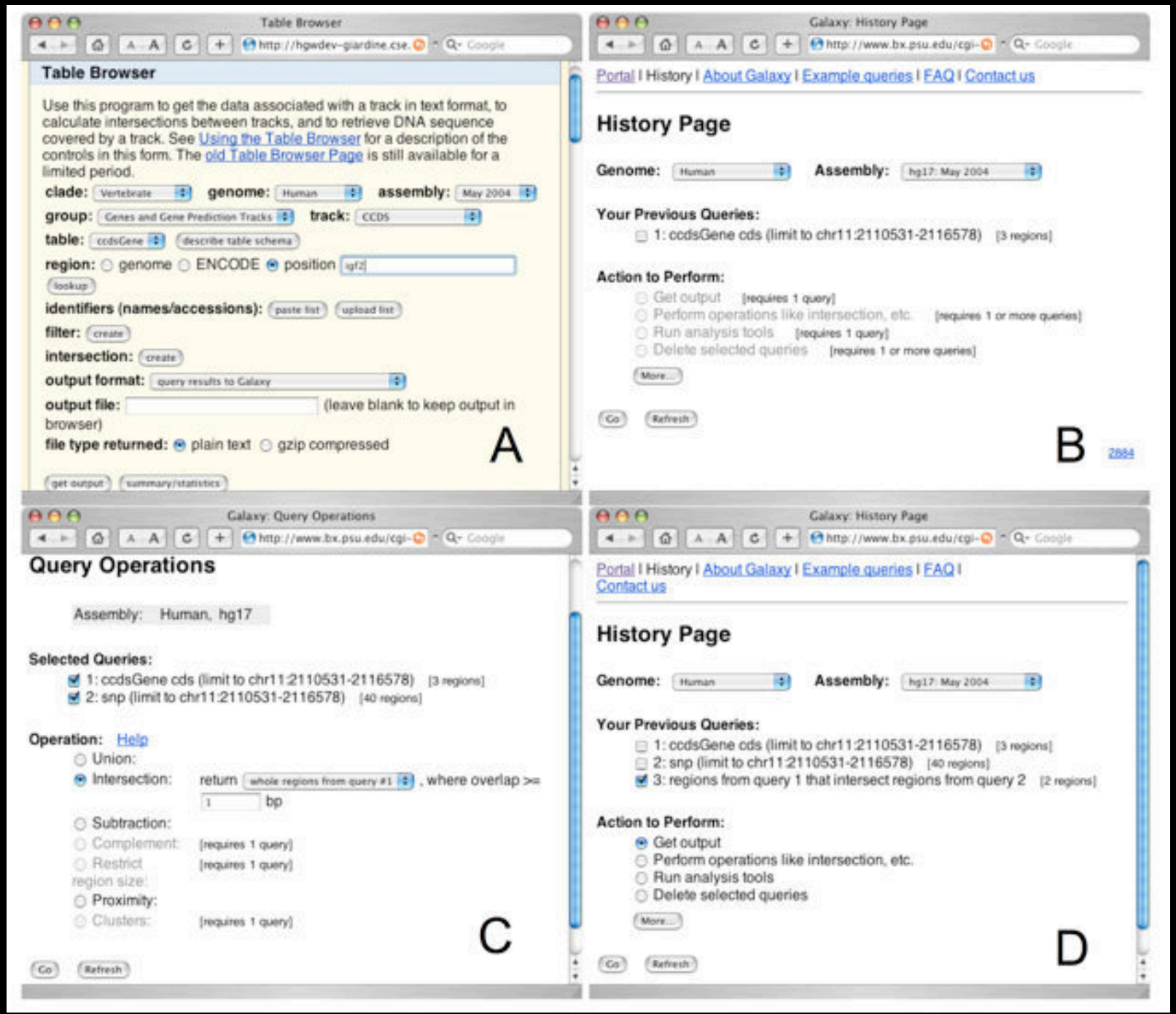
The next 89 min & 59 seconds

- History (2005 – 2010)
- Present (2010 – 2011)
- The “Vision” (2011 – ∞)
- The Community
- Beer

Galaxy as a single Perl script (!)



Galaxy as a single Perl script (!)



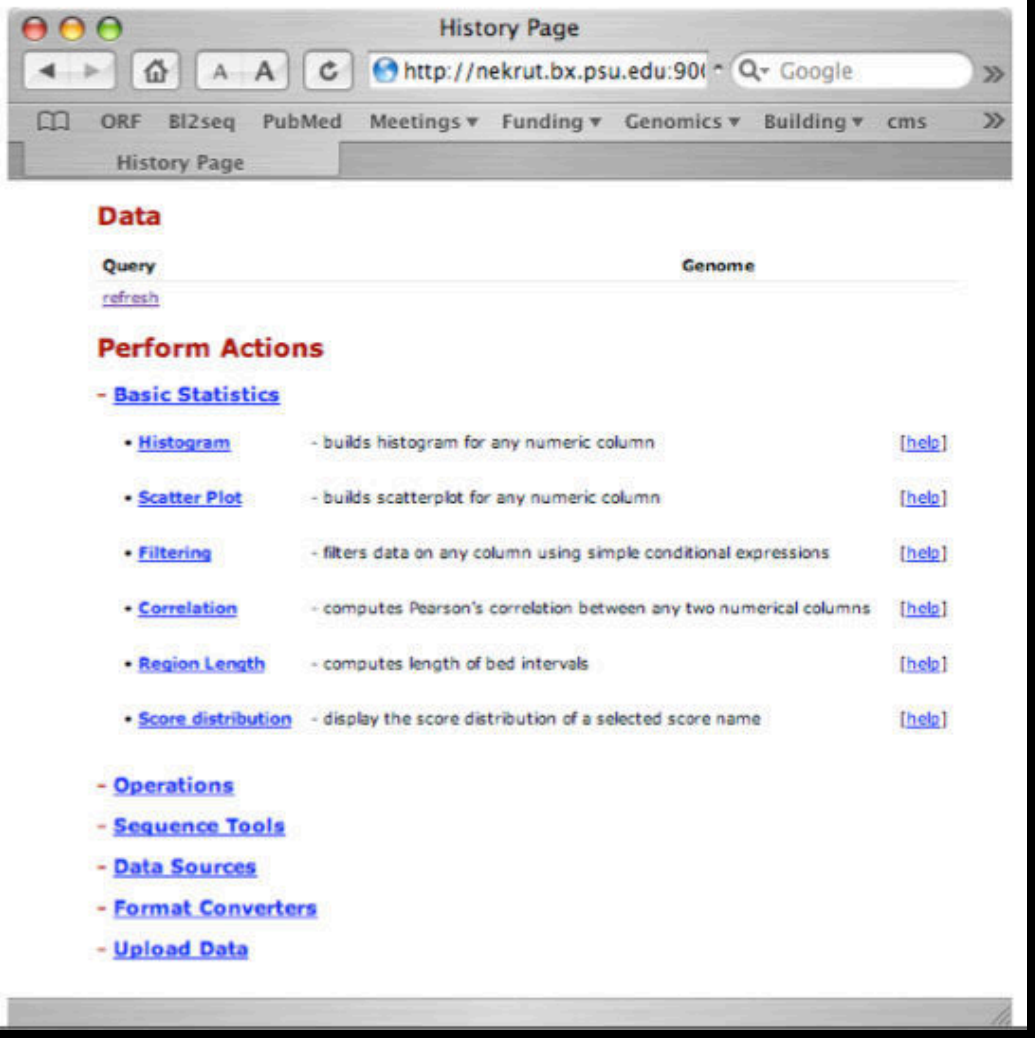
Etymology

- Galaxy = Gala + XL (Bob Harris, author of Lastz)
- GALA = Genome Alignment and Annotation Database
- Brainchild of Ross Hardison
- Taking over the universe was not our original intension

Ross Hardison

Pythonic Age (mid 2005)

```
Basic Statistics:
    Histogram: histogram.tool
    Scatter Plot: scatter.tool
    Filtering: filtering.tool
    Correlation: correlation.tool
    Region Length: region_length.tool
    Score distribution: scoreGraph.tool
Operations:
    Complement: complement.tool
    Restrict: restrict.tool
    Merge overlapping regions: merge.tool
    Cluster: cluster.tool
    Union: union.tool
    Intersect: intersect.tool
    Subtract: subtract.tool
    Proximity: proximity.tool
    Join Lists: joinLists.tool
    Vicinity: vicinity.tool
    Join Same Coordinates Region: joinSameCoor.tool
Sequence Tools:
     Extract sequences: fasta-subseq-wrapper.tool
     Extract blastZ alignments: extractAxt_wrapper.tool
Data Sources:
    UCSC query: ucsc.tool
    Genbank: genbank.tool
    Encode DB: encodedb.tool
    Featured datasets: import.tool
Format Converters:
    BED and xBED converter: bed_convert.tool
```



2006

Galaxy

http://g2.bx.psu.edu:9000/

Galaxy

columns | stack | tools | history

- Data
- Edit
- Filter and Sort
- Format
- Sequences and Alignments
- Scores
- Interval Functions
- Statistics
- Graph
- EMBOSS
- PHYLIP

Cluster regions of a single Query

Cluster and Merge regions within specified distance of each other

Complement a single Query

Coverage density of the regions of two queries

Difference find non-overlapping segments between two Queries

Intersect return overlapping segments of two Queries

Cluster

Cluster regions of Query: 3: UCSC: knownGene (chr7:12747119

Regions per cluster: 2

Within distance: 10

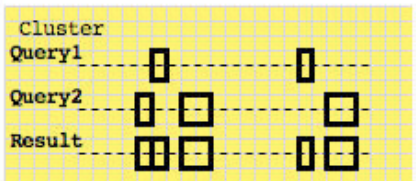
Execute

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

Syntax

- **Regions per cluster** sets the minimum number of regions to start a cluster
- **Within distance** sets the maximum separation (in base pairs) at which two regions are still considered to be within the same cluster

Example



refresh | collapse all

3: UCSC: knownGene (chr7:127471196-127495720), running, bed, hg17

2: Maf to concatenated FASTA on data 1, 8 sequences, fasta, hg17

1: UCSC: multiz8way (chr22:23153675-23162878), 511 lines, maf, hg17

History

Done

Sometime before today

Galaxy

http://main.g2.bx.psu.edu/

Galaxy

Info: report bugs | wiki | screencasts Account: create | login

Get Data
- Upload File from your computer
- UCSC Main table browser
- Get Microbial Data

Get ENCODE Data
ENCODE Tools
Edit Queries
Filter, Sort, Join and Compare
Convert Formats
Fetch Sequences and Alignments
Get Genomic Scores
Operate on Genomic Intervals
Statistics
Graph Data
EMBOSS
HYPHY

Tools

for a description of the controls in this form. For more complex queries, you may want to use Galaxy or our public MySQL server. Refer to the Credits page for the list of contributors and usage restrictions associated with these data.

clade: Vertebrate genome: Human
assembly: Mar. 2006
group: Variation and Repeats track: SNPs (126)
table: snp126
describe table schema
region: genome position
chr7:113265469-114910668 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
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.

refresh | collapse all

1: UCSC Main on Human: knownGene (chr7:113265469-114910668)

History options...

You must be logged in to store or switch histories.
- share current history
- delete current history

Done

The First Galaxy Developer Conf

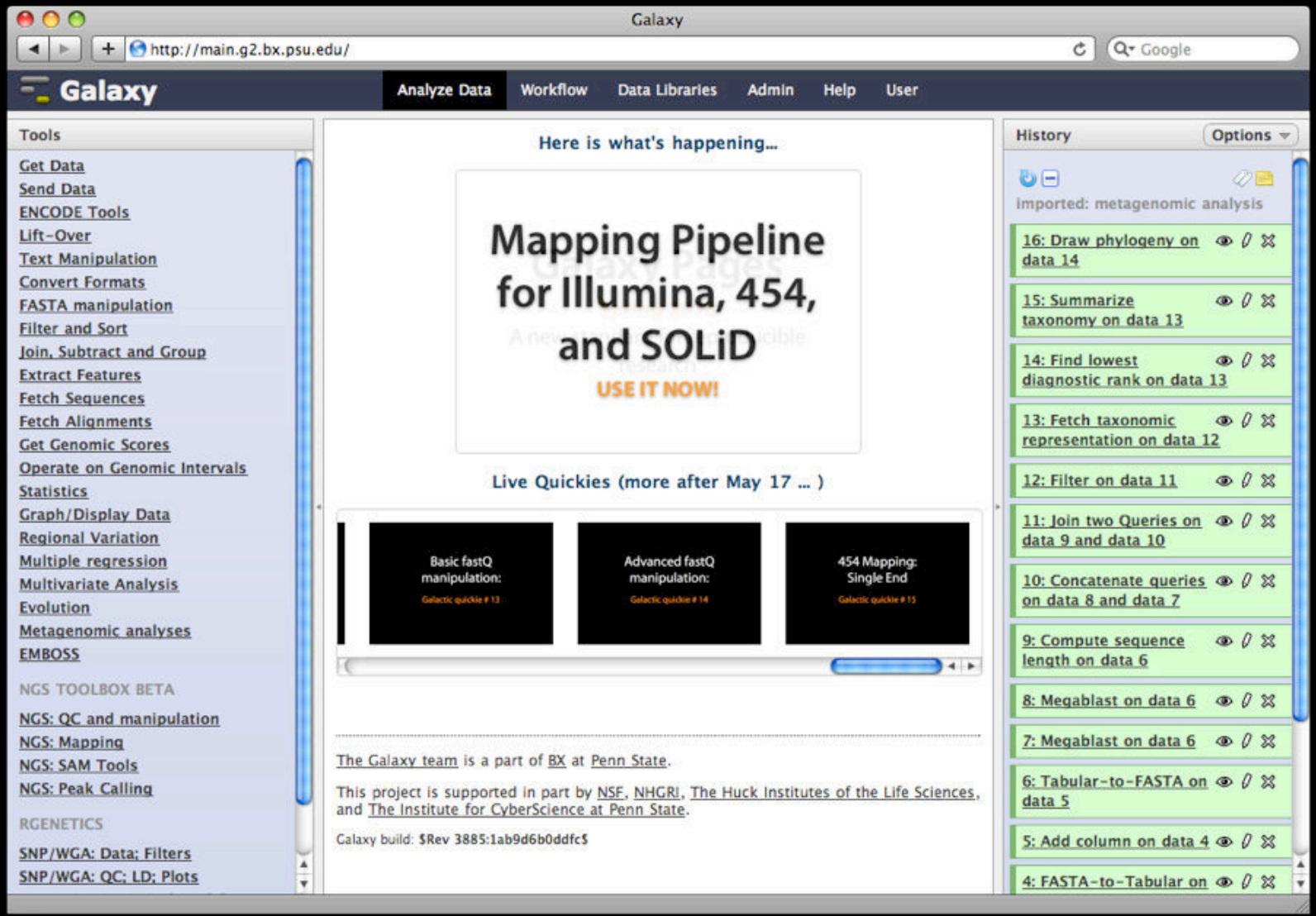
The next 90 – x minutes

- History (2005 – 2010)
- Present (2010 – 2011)
- The “Vision” (2011 – ∞)
- The Community
- Beer

Galaxy Today

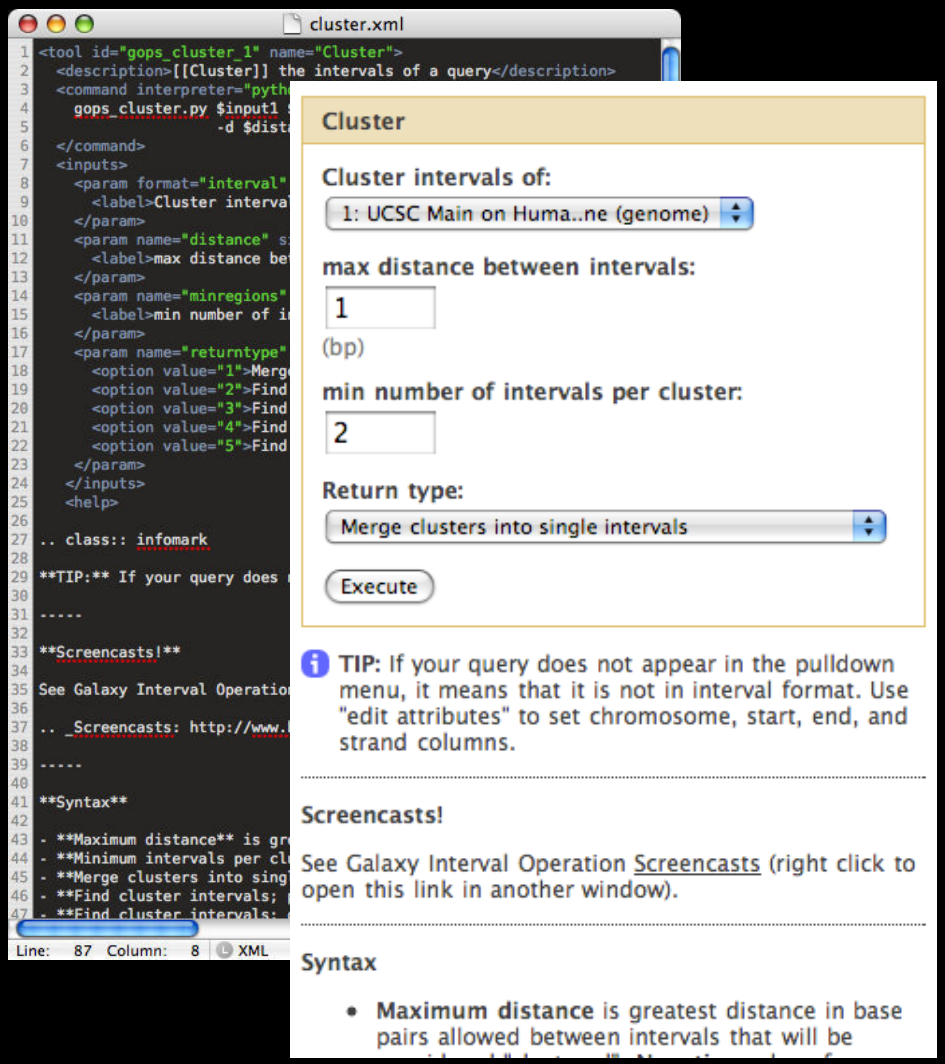
- **A free (for everyone) web service** integrating a wealth of tools, compute resources, terabytes of reference data and permanent storage
- **Open source software** that makes integrating your own tools and data and customizing for your own site simple

Galaxy: accessible analysis system



Tools

Integrating existing tools into a uniform framework



- Defined in terms of an abstract interface (inputs and outputs)
  - In practice, mostly command line tools, a declarative XML description of the interface, how to generate a command line
- Designed to be as easy as possible for tool authors, while still allowing rigorous reasoning

Dan Blankenberg, Guru Ananda, Kelly Vincent, Ross Lazarus

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

Evolution

Metagenomic analyses

Human Genome Variation

EMBOSS

NGS TOOLBOX BETA

NGS: QC and manipulation

NGS: Mapping

ILLUMINA

- Map with Bowtie for Illumina
- Map with BWA for Illumina

ROCHE-454

- Lastz map short reads against reference sequence
- Megablast compare short reads against htgs, nt, and wgs databases
- Parse blast XML output

AB-SOLID

- Map with Bowtie for SOLiD

NGS: SAM Tools

NGS: Indel Analysis

NGS: Peak Calling

NGS: RNA Analysis

RGENETICS

SNP/WGA: Data; Filters

SNP/WGA: QC; LD; Plots

SNP/WGA: Statistical Models

Workflows

NGS TOOLBOX BETA

NGS: QC and manipulation

NGS: Mapping

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

NGS: Indel Analysis

NGS: Peak Calling

NGS: RNA Analysis

RGENETICS

SNP/WGA: Data; Filters

SNP/WGA: QC; LD; Plots

SNP/WGA: Statistical Models

Workflows

NGS: SAM Tools

NGS: Indel Analysis

- Filter Indels for SAM
- Extract indels from SAM
- Indel Analysis

NGS: Peak Calling

- MACS Model-based Analysis of ChIP-Seq
- GeneTrack indexer on a BED file
- Peak predictor on GeneTrack index

NGS: RNA Analysis

RNA-SEQ

- Tophat Find splice junctions using RNA-seq data
- Cufflinks transcript assembly and FPKM (RPKM) estimates for RNA-Seq data
- Cuffcompare compare assembled transcripts to a reference annotation and track Cufflinks transcripts across multiple experiments
- Cuffdiff find significant changes in transcript expression, splicing, and promoter use

FILTERING

- Filter Combined Transcripts using tracking file

Dozens of tools for different NGS applications packaged with Galaxy

Galaxy Tool Shed

http://community.g2.bx.psu.edu/

Google

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	5
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	7
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	10
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

The Galaxy Tool Shed allows the community to contribute, share, and evaluate tools

Greg Von Kuster

Galaxy Tool Shed

http://community.g2.bx.psu.edu/

Galaxy Tool Shed / (beta)

Tools Help User

Community

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

View Tool

This is the latest approved version of this tool suite

Tool Actions

Mothur Metagenomics

Tool Id:
Mothur_toolsuite

Version:
1.15.1

Description:
Mothur metagenomics commands as Galaxy tools

User Description:
Provides galaxy tools for the commands in the Mothur metagenomics package: http://www.mothur.org/wiki/Main_Page

Uploaded by:
jjohnson

Date uploaded:
about 22 hours ago

Categories:
- Sequence Analysis

Tool Contents

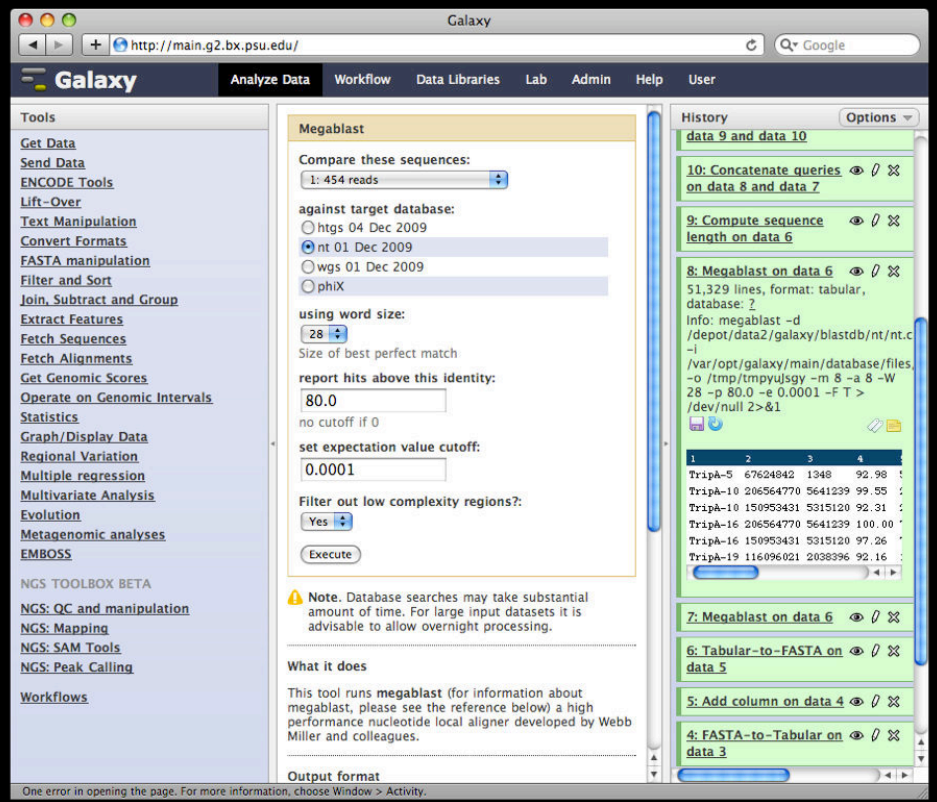
Mothur_toolsuite_1.15.1.tar.gz
- mothur/
- mothur/tools/
- mothur/tools/mothur/
- mothur/tools/mothur/split.abund.xml

For example, complete wrappers for the Mothur metagenomics suite from Jim Johnson (UMN)

Jim Johnson, Konrad Paszkiewicz, Peter Cock, Vipin Sreedharan

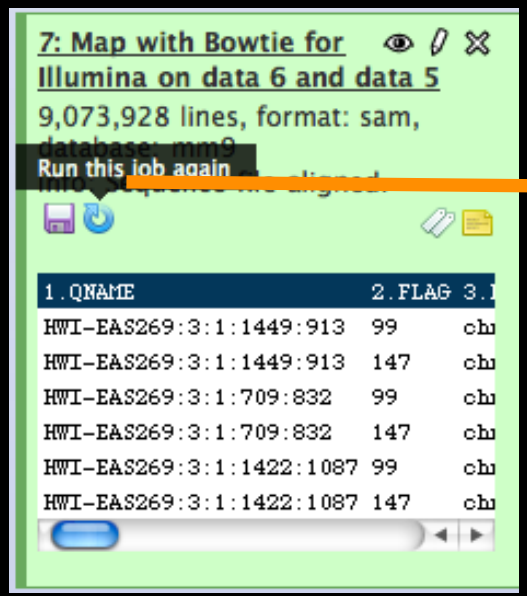
Analysis environment

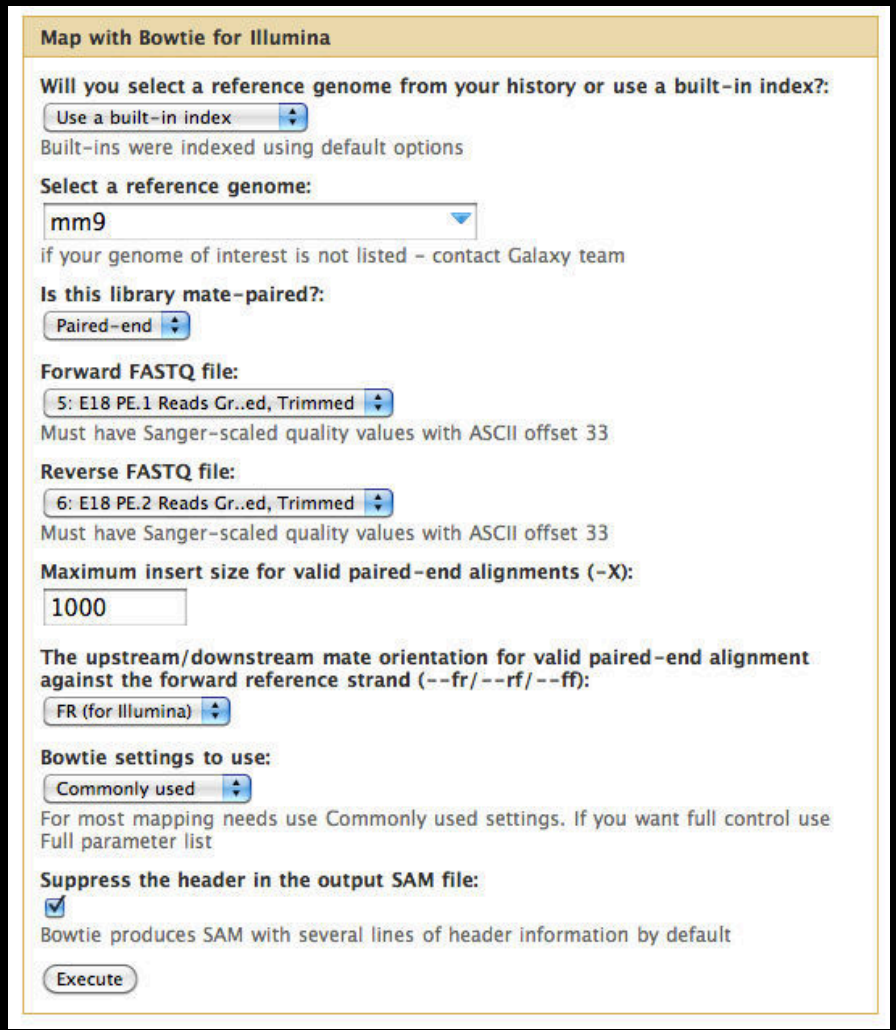
Galaxy analysis interface



- Consistent tool user interfaces automatically generated
- History system facilitates and tracks multistep analyses

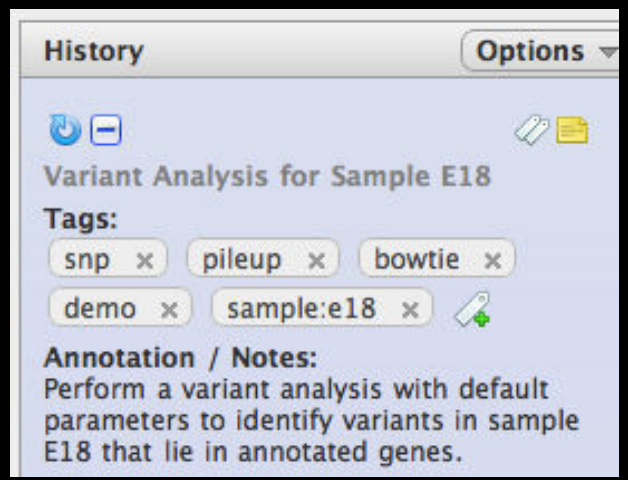
Automatically tracks every step of every

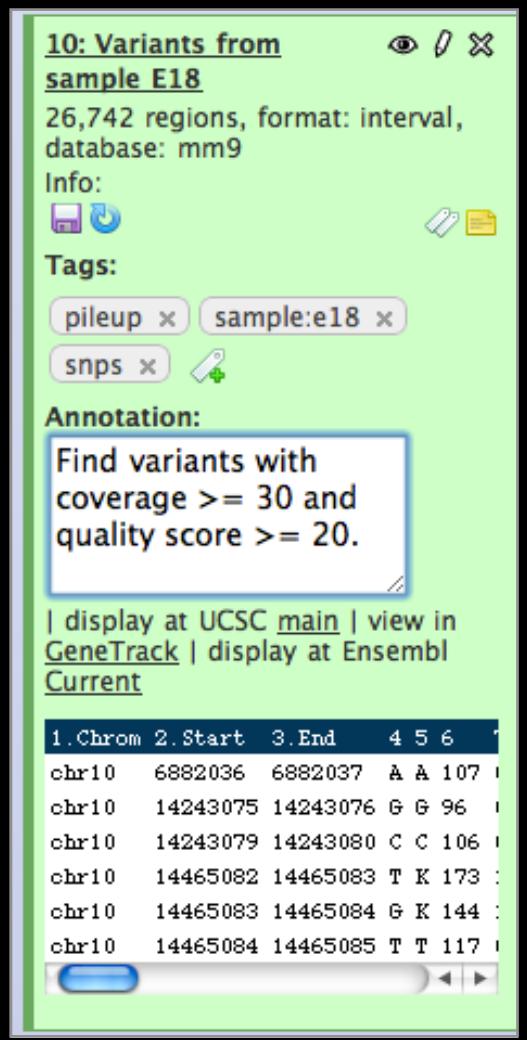




Dan Blankenberg

As well as user-generated metadata and annotation...

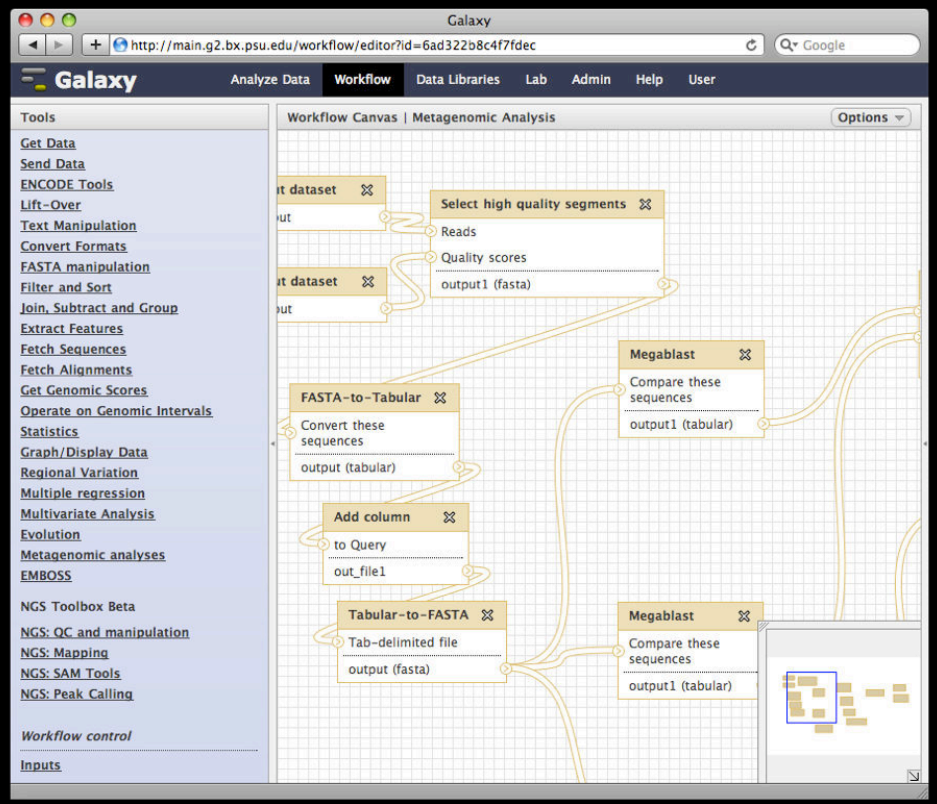




Jeremy Goecks

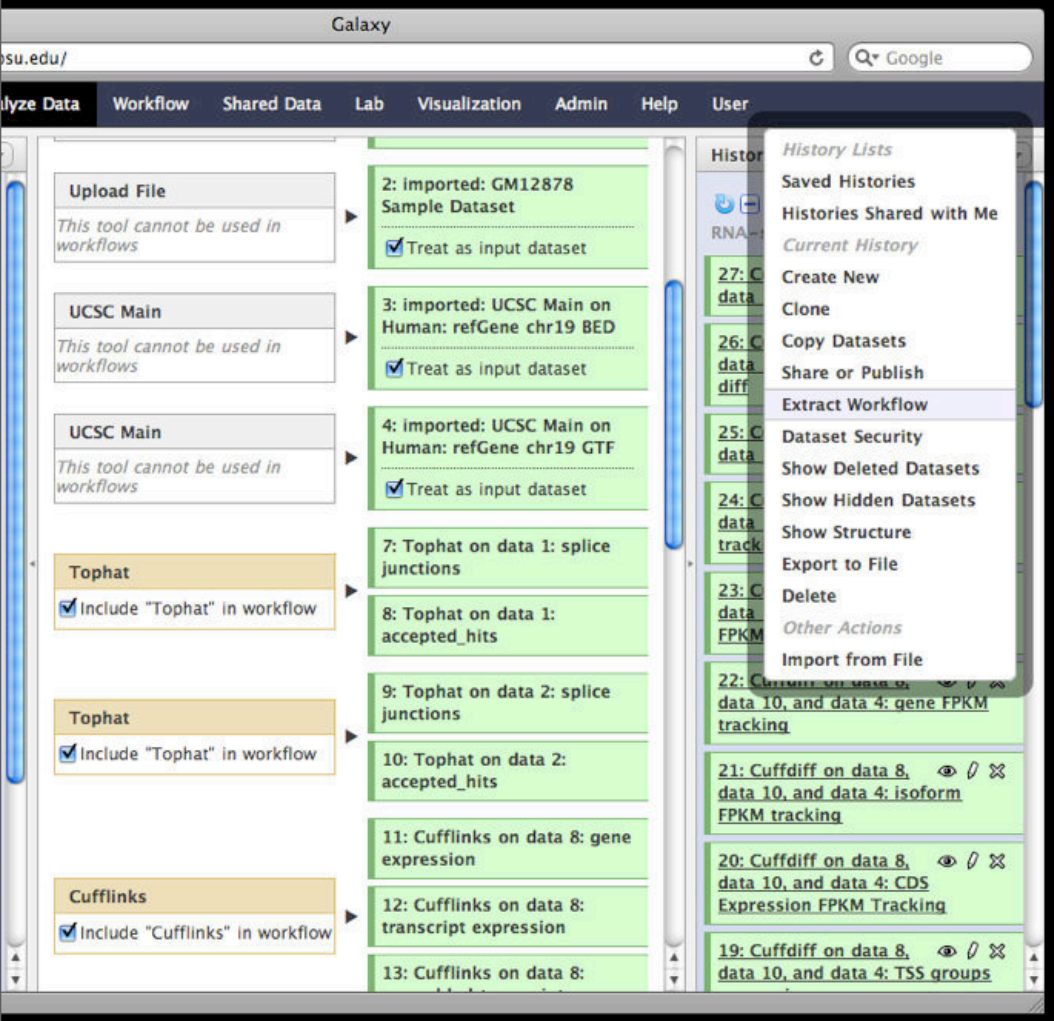
Workflows

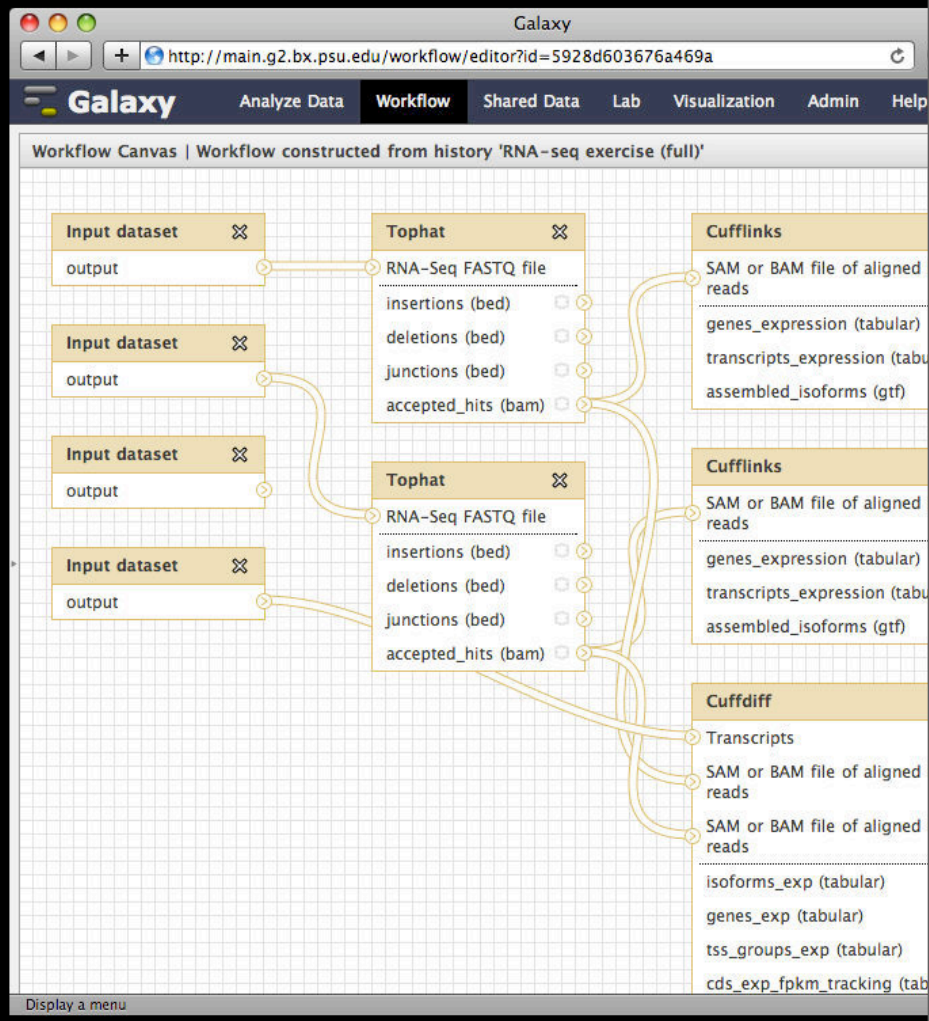
Galaxy workflow system

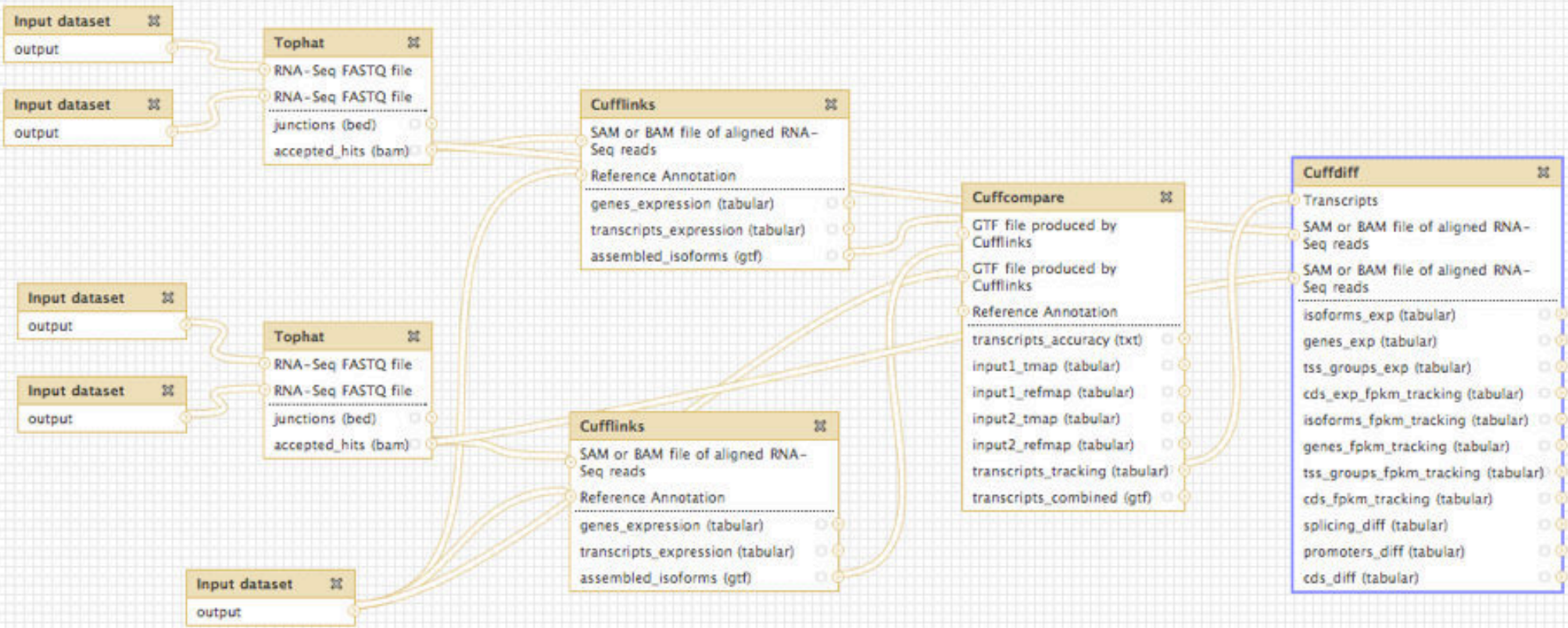


- Workflows can be constructed from scratch or extracted from existing analysis histories
- Facilitate reuse, as well as providing precise reproducibility of a complex analysis

Dannon Baker


Galaxy
Google
Analyze Data
Workflow
Shared Data
Lab
Visualization
Admin
Help
User
Upload File
This tool cannot be used in workflows
2: imported: GM12878 Sample Dataset
Treat as input dataset
UCSC Main
This tool cannot be used in workflows
3: imported: UCSC Main on Human: refGene chr19 BED
Treat as input dataset
UCSC Main
This tool cannot be used in workflows
4: imported: UCSC Main on Human: refGene chr19 GTF
Treat as input dataset
Tophat
Include "Tophat" in workflow
7: Tophat on data 1: splice junctions
8: Tophat on data 1: accepted_hits
Tophat
Include "Tophat" in workflow
9: Tophat on data 2: splice junctions
10: Tophat on data 2: accepted_hits
11: Cufflinks on data 8: gene expression
Cufflinks
Include "Cufflinks" in workflow
12: Cufflinks on data 8: transcript expression
13: Cufflinks on data 8:
History Lists
Saved Histories
Histories Shared with Me
Current History
Create New
Clone
Copy Datasets
Share or Publish
Extract Workflow
Dataset Security
Show Deleted Datasets
Show Hidden Datasets
Show Structure
Export to File
Delete
Other Actions
Import from File
data 10, and data 4: gene FPKM tracking
21: Cuffdiff on data 8, data 10, and data 4: isoform FPKM tracking
20: Cuffdiff on data 8, data 10, and data 4: CDS Expression FPKM Tracking
19: Cuffdiff on data 8, data 10, and data 4: TSS groups


Galaxy
http://main.g2.bx.psu.edu/workflow/editor?id=5928d603676a469a
Galaxy
Analyze Data
Workflow
Shared Data
Lab
Visualization
Admin
Help
Workflow Canvas | Workflow constructed from history 'RNA-seq exercise (full)'
Input dataset
output
Input dataset
output
Input dataset
output
Input dataset
output
Tophat
RNA-Seq FASTQ file
insertions (bed)
deletions (bed)
junctions (bed)
accepted_hits (bam)
Tophat
RNA-Seq FASTQ file
insertions (bed)
deletions (bed)
junctions (bed)
accepted_hits (bam)
Cufflinks
SAM or BAM file of aligned reads
genes_expression (tabular)
transcripts_expression (tabu
assembled_isoforms (gtf)
Cufflinks
SAM or BAM file of aligned reads
genes_expression (tabular)
transcripts_expression (tabu
assembled_isoforms (gtf)
Cuffdiff
Transcripts
SAM or BAM file of aligned reads
SAM or BAM file of aligned reads
isoforms_exp (tabular)
genes_exp (tabular)
tss_groups_exp (tabular)
cds_exp_fpkm_tracking (tab
Display a menu


Input dataset
output
Input dataset
output
Tophat
RNA-Seq FASTQ file
RNA-Seq FASTQ file
junctions (bed)
accepted_hits (bam)
Cufflinks
SAM or BAM file of aligned RNA-Seq reads
Reference Annotation
genes_expression (tabular)
transcripts_expression (tabular)
assembled_isoforms (gtf)
Input dataset
output
Input dataset
output
Tophat
RNA-Seq FASTQ file
RNA-Seq FASTQ file
junctions (bed)
accepted_hits (bam)
Cufflinks
SAM or BAM file of aligned RNA-Seq reads
Reference Annotation
genes_expression (tabular)
transcripts_expression (tabular)
assembled_isoforms (gtf)
Input dataset
output
Cuffcompare
GTF file produced by Cufflinks
GTF file produced by Cufflinks
Reference Annotation
transcripts_accuracy (txt)
input1_tmap (tabular)
input1_refmap (tabular)
input2_tmap (tabular)
input2_refmap (tabular)
transcripts_tracking (tabular)
transcripts_combined (gtf)
Cuffdiff
Transcripts
SAM or BAM file of aligned RNA-Seq reads
SAM or BAM file of aligned RNA-Seq reads
isoforms_exp (tabular)
genes_exp (tabular)
tss_groups_exp (tabular)
cds_exp_fpkm_tracking (tabular)
isoforms_fpkm_tracking (tabular)
genes_fpkm_tracking (tabular)
tss_groups_fpkm_tracking (tabular)
cds_fpkm_tracking (tabular)
splicing_diff (tabular)
promoters_diff (tabular)
cds_diff (tabular)

Data Libraries

Galaxy

http://main.g2.bx.psu.edu/library

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

Data Library "mtProject"

Name	Message	Uploaded By	Date	File Size
F4-bM4C3	Family 4, child M4C3, blood, PCR (no replicates)	anton@bx.psu.edu	2010-09-01	2.4 Gb
F4-bM5G-1	Family 4, grandmother M5G, blood, PCR1	anton@bx.psu.edu	2010-09-01	2.0 Gb
F4-bM5G-2	Family 4, grandmother M5G, blood, PCR2	anton@bx.psu.edu	2010-09-01	2.4 Gb
F4-bM9	Family 4, sister M9, blood, PCR (no replicates)	anton@bx.psu.edu	2010-09-01	1.4 Gb
F4-cM4C3	Family 4, child M4C3, cheek, PCR (no replicates)	anton@bx.psu.edu	2010-09-01	1.5 Gb
F4-cM5G-1	Family 4, grandmother M5G, cheek, PCR1	anton@bx.psu.edu	2010-09-01	1.6 Gb
F4-cM5G-2	Family 4, grandmother M5G, cheek, PCR2	anton@bx.psu.edu	2010-09-01	1.7 Gb
F4-cM9	Family 4, sister M9, cheek, PCR (no replicates)	anton@bx.psu.edu	2010-09-01	1.6 Gb
F4-bM4C2-1	Family 4, child M4C2, blood, PCR1	anton@bx.psu.edu	2010-01-08	247.1 Mb
F4-bM4C2-2	Family 4, child M4C2, blood, PCR2	anton@bx.psu.edu	2010-01-08	426.8 Mb
F4-cM4C2-1	Family 4, child M4C2, cheek, PCR1	anton@bx.psu.edu	2010-01-08	92.3 Mb
F4-cM4C2-2	Family 4, child M4C2, cheek, PCR2	anton@bx.psu.edu	2010-01-08	157.7 Mb
F4-bM4-1	Family 4, mother M4, blood, PCR1	anton@bx.psu.edu	2010-01-08	85.7 Mb
F4-bM4-2	Family 4, mother M4, blood, PCR2	anton@bx.psu.edu	2010-01-08	132.3 Mb
F4-cM4-1	Family 4, mother M4, cheek, PCR1	anton@bx.psu.edu	2010-01-08	150.2 Mb
F4-cM4-2	Family 4, mother M4, cheek, PCR2	anton@bx.psu.edu	2010-01-08	99.8 Mb
F7-bM10C2-1	Family 7, child M10C2, blood, PCR1	anton@bx.psu.edu	2010-01-08	90.8 Mb
F7-bM10C2-2	Family 7, child M10C2, blood, PCR2	anton@bx.psu.edu	2010-01-08	156.3 Mb
F7-cM10C2-1	Family 7, child M10C2, cheek, PCR1	anton@bx.psu.edu	2010-01-08	162.3 Mb
F7-cM10C2-2	Family 7, child M10C2, cheek, PCR2	anton@bx.psu.edu	2010-01-08	170.0 Mb
F7-bM10-1	Family 7, mother M10, blood, PCR1	anton@bx.psu.edu	2010-01-08	192.9 Mb
F7-bM10-2	Family 7, mother M10, blood, PCR2	anton@bx.psu.edu	2010-01-08	118.1 Mb
F7-cM10-1	Family 7, mother M10, cheek, PCR1	anton@bx.psu.edu	2010-01-08	243.3 Mb

Greg Von Kuster

Sharing and publishing

Everything can be shared

Sharing and Publishing History 'Variant Analysis for Sample E18'

Making History Accessible via Link and Publishing It

This history **accessible via link and published**.

Anyone can view and import this history by visiting the following URL:

http://main.g2.bx.psu.edu/u/jgoecks/h/variant-analysis-for-sample-e18

This history is publicly listed and searchable in Galaxy's Published Histories section.

You can:

Unpublish History

Removes history from Galaxy's Published Histories section so that it is not publicly listed or searchable.

Disable Access to History via Link and Unpublish

Disables history's link so that it is not accessible and removes history from Galaxy's Published Histories section so that it is not publicly listed or searchable.

Sharing History with Specific Users

You have not shared this history with any users.

Share with a user

Back to Histories List

Galaxy | Published Pages

http://main.g2.bx.psu.edu/page/list_published

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

Published Pages

search | Advanced Search

Title	Annotation	Owner	Community Rating	Community Tags	Last Updated ↓
ChrY 1000 Genomes	A demo workshop project during CSHL course on Computational Genomics Nov 2010	ericy			2 days ago
Galaxy Exercises	Various exercises for learning about Galaxy	james			5 days ago
Galaxy 101: The first thing you need to try	An elementary guide to Galaxy	aun1	★★★★★	exons, snps, tutorial	Nov 03, 2010
Windshield Splatter	Live supplement for Genome Research windshield splatter paper.	aun1	★★★★★	megan, paper, galaxy	Oct 27, 2010
Galaxy RNA-seq Analysis Exercise	An exercise that illustrates how to use Galaxy for RNA-seq analyses.	jeremy	★★★★★		Oct 27, 2010
heteroplasmy		aun1	★★★★★	heteroplasmy, bwa, resequencing, illumina	Oct 26, 2010

Pervasive search allows others to find published items of interest

Galaxy | Published Page | heteroplasmy

http://main.g2.bx.psu.edu/u/aun1/p/heteroplasmy

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

Published Pages | aun1 | heteroplasmy

Dynamics of mitochondrial heteroplasmy in three families: A fully reproducible re-sequencing study

Hiroki Goto[1], Benjamin Dickins[2], Enis Afgan[3,5], Ian M. Paul[4], James Taylor[3,5], Kateryna D. Makova[1], and Anton Nekrutenko[2,5]

Correspondence should be addressed to KDM, JT, or AN.

1. How to use this document

This document is a live copy of supplementary materials for the manuscript. It provides access to all the data as well as to 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 sequencing data. 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. To make this even easier, we created several screencasts (very short movies) to help you:

- access our datasets
- re-use workflows listed on this page
- view and import histories listed on this page

In addition, we created two longer screenacasts:

- Watch the analysis of one family (F7) from start (Illumina reads) to finish (a list of variable position);
- Watch how the complete analysis can be performed on the Amazon Cloud.

If you experience any problems while using this page, please e-mail our bug report list and we will get back to you.

2. Accessing the Data

All datasets discussed in the paper can be found in two places:

- A Galaxy Library called mtProject;
- An S3 bucket on the Amazon Cloud

Galaxy Page for a recent study on mitochondrial heteroplasmy

Galaxy | Published Page | heteroplasmy

http://main.g2.bx.psu.edu/u/aun1/p/heteroplasmy

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

Published Pages | aun1 | heteroplasmy

M10, M10C2, M15, and M15C2;

- the workflow '**mt analysis 0.01 strand-specific (*fastq single*)**' was run four times on datasets that lacked PCR replicates: M9 and M4C3;

for this we created three separate histories: one for each family. Each history (F4 = Family 4, F7 = Family 7, F11 = Family 11) can be examined in detail and imported below (see a Screencast explaining how to do this):

Galaxy History | F4

Galaxy History | F7

Galaxy History | F11

Each of the histories contain original Illumina datasets and outputs of workflows.

3.3 Generating initial summary datasets

In the previous step we identified variable sites in all samples. Now we need to merge the results by generating reports for each family. To do this we first copied results workflow executions into a new history called "**F4-F7-F11 final report**" (for explanation on how to copy datasets between histories see this Screencast):

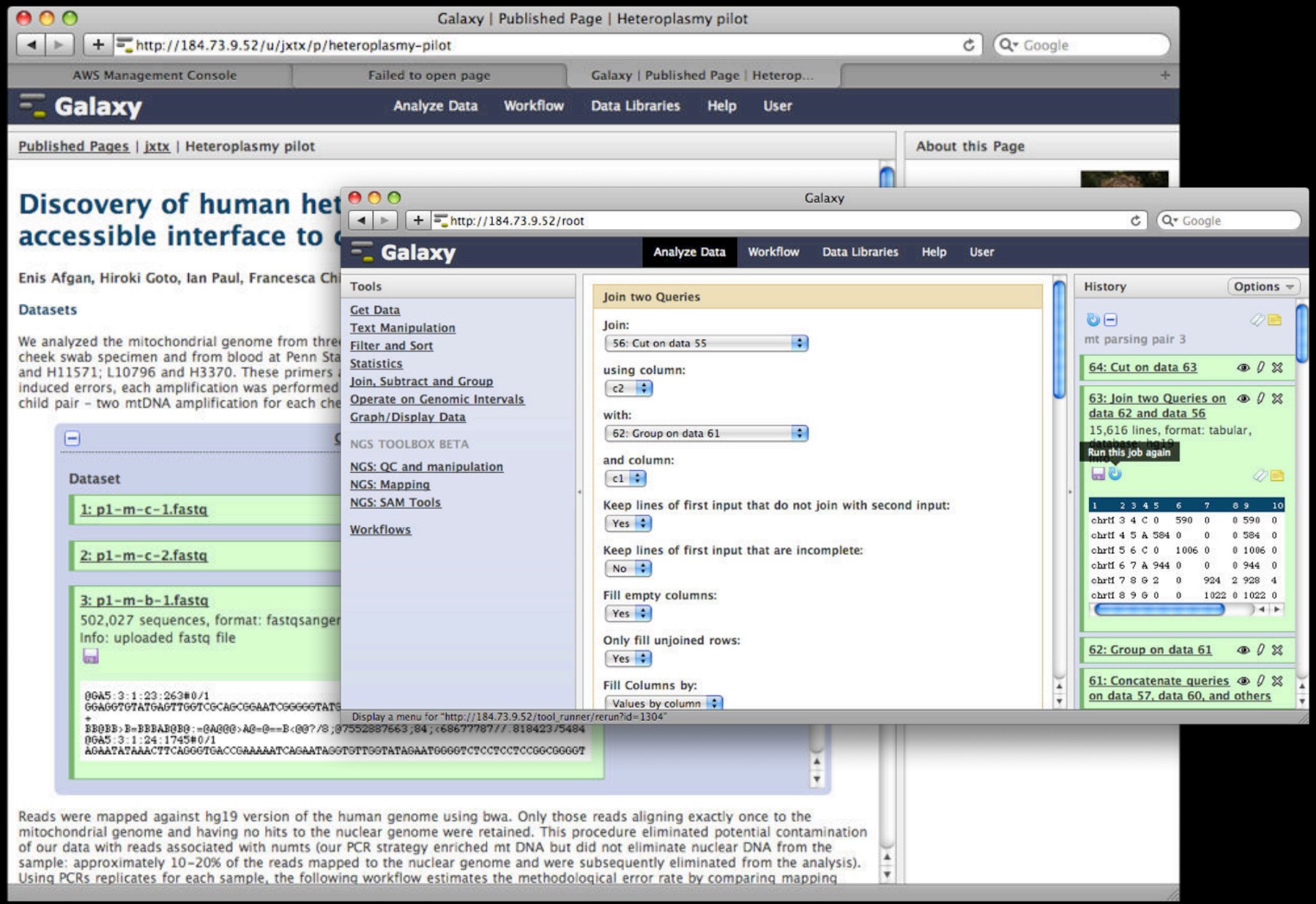
Galaxy History | F4-F7-F11 final report

Within this history individual datasets are merged into summaries generated for each family. To be more specific, datasets 1 through 10 were merged into dataset 19 called "**F4 summary**", datasets 11 - 14 were joined into history item 22 called "**F7 summary**", and, finally, datasets 15 - 18 were used to generate #24 called "**F11 summary**". Merging of datasets was performed with "***Join, Subtract, and Group -> Column Join***" tool. Let's look at datasets "**F7 summary**" to understand what this means:

Galaxy Dataset | F7 summary

Results of heteroplasmy workflow for all individuals of family 7 joined together. You can click in "rerun" button above to see the parameters.

Actual histories and datasets directly accessible from the text



Histories can be imported and the exact parameters inspected

Galaxy | Published Page | Heteroplasmy pilot

http://184.73.9.52/u/jxtx/p/heteroplasmy-pilot

AWS Management Console | Galaxy | Published Page | Heterop...

Galaxy — Analyze Data | Workflow | Data Libraries | Help | User

Published Pages | jxtx | Heteroplasmy pilot

We analyzed the mitochondrial genome from three mother/child pairs. For each mother and child pair the DNA was collected from cheek swab specimen and from blood at Penn State Medical School. mtDNA was amplified with PCR using two primer sets L2815 and H11571; L10796 and H3370. These primers are originally described in Tanaka et al. (1996). To control for possible PCR-induced errors, each amplification was performed twice. In total we generated 24 Illumina datasets (eight for each mother and child pair - two mtDNA amplification for each cheek swab and blood samples

Galaxy History | mt datasets

Reads were mapped against hg19 version of the human genome using bwa. Only those reads aligning exactly once to the mitochondrial genome and having no hits to the nuclear genome were retained. This procedure eliminated potential contamination of our data with reads associated with numts (our PCR strategy enriched mt DNA but did not eliminate nuclear DNA from the sample: approximately 10-20% of the reads mapped to the nuclear genome and were subsequently eliminated from the analysis). Using PCRs replicates for each sample, the following workflow estimates the methodological error rate by comparing mapping results between two amplifications. To do so we identified all sites where in one replicate where there were no deviant reads (all reads contained the same nucleotide; i.e. 1000 'A' bases) but the other contained such sites (e.g., 1000 As and 12 Cs). Dividing the number of deviant reads (12 in this case) by the total read coverage (1012) at such positions gave us error the rate of 1.18% (12/1012) at this position.

Galaxy Workflow | Determine threshold from PCR replicates

c1==chrM and c10 >= 200

Step 16: Filter

Filter
Output dataset 'out_file1' from step 14

With following condition
c1=='chrM' and c10 >= 200

Replicate 2: Keep only positions that map to chrM and have quality adjusted coverage greater than 200

Step 17: Join

Join
Output dataset 'out_file1' from step 15

with
Output dataset 'out_file1' from step 16

Create a joined file containing the pileup information for all positions that have sufficient quality to consider in both replicates

Histories resulting from first workflow on each pair: History 'mt replicates pair 1', History 'mt replicates pair 2', History 'mt

Display a menu

About this Page

Author

jxtx

Related Pages

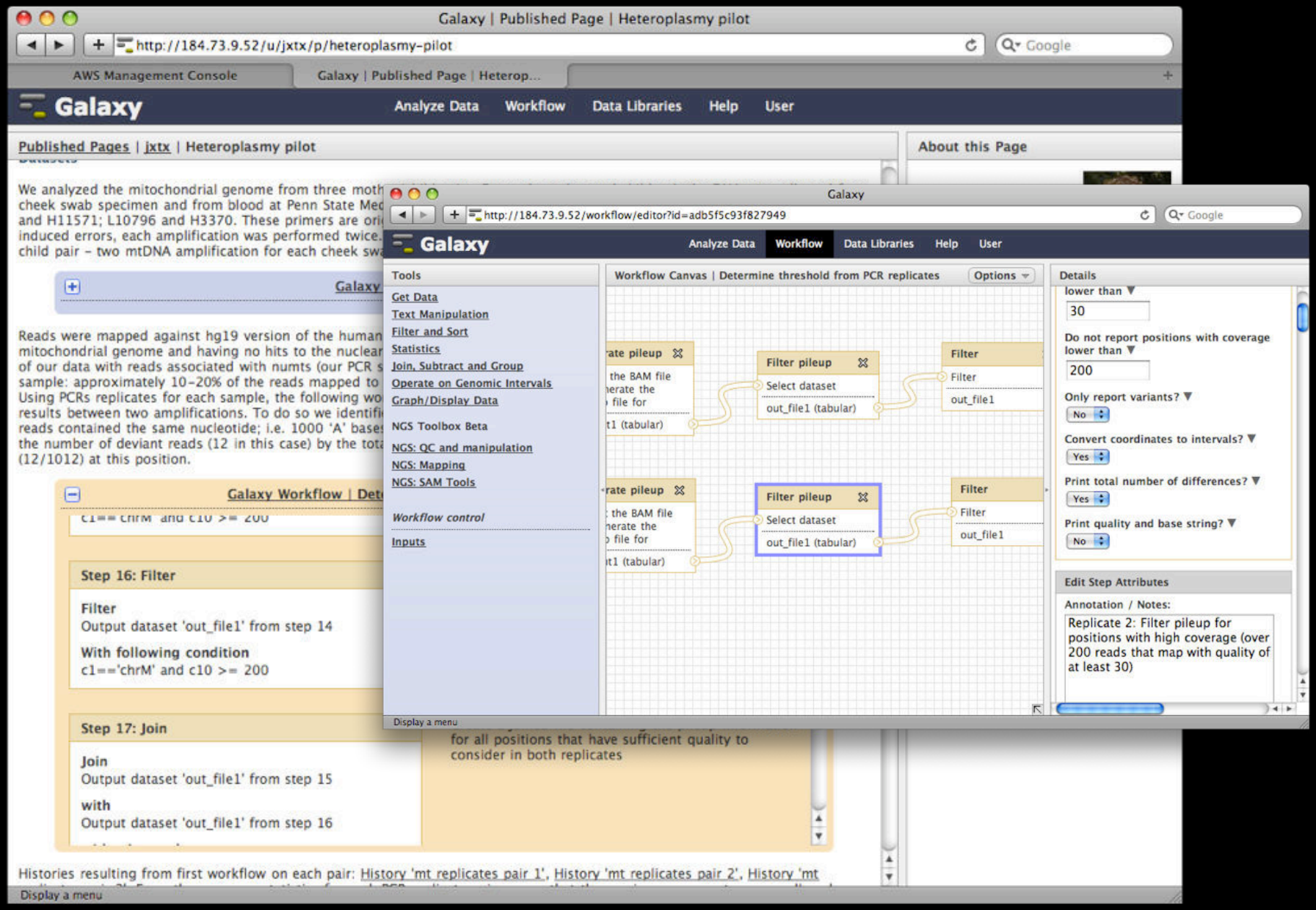
All published pages
Published pages by jxtx

Tags

Community:
cloud | heteroplasmy | ngs

Yours:
heteroplasmy × | cloud × | ngs ×

Workflows and other entities can also be embedded



And imported for inspection, verification, and reuse

The power of Galaxy publishing and

- Galaxy's publishing features facilitate access and reproducibility without any extra leg work
- One click grants access to the actual analysis you performed to generate your original results
  - Not just data access: the full pipeline
  - Annotate each step

Jeremy Goecks

Windshield splatter analysis with the Galaxy metagenomic pipeline — Genome Research

Windshield splatter analysis with the Galaxy metagenomic pipeline

Sergei Kosakovsky Pond[1,2,6,9], Samir Wadhawan[3,6,7], Francesca Chiaromonte[4], Guruprasad Ananda[1,3], Wen-Yu Chung[1,3,8], James Taylor[1,5,9], Anton Nekrutenko[1,3,9] and The Galaxy Team[1]

[+] Author Affiliations

Abstract

How many species inhabit our immediate surroundings? A straightforward collection technique suitable for answering this question is known to anyone who has ever driven a car at highway speeds. The windshield of a moving vehicle is subjected to numerous insect strikes and can be used as a collection device for representative sampling. Unfortunately the analysis of biological material collected in that manner, as with most metagenomic studies, proves to be rather demanding due to the large number of required tools and considerable computational infrastructure. In this study, we use organic matter collected by a

Footnotes

[Supplemental material is available online at http://www.genome.org. All data and tools described in this manuscript can be downloaded or used directly at http://galaxyproject.org. Exact analyses and workflows used in this paper are available at http://usegalaxy.org/u/aun1/p/windshield-splatter.]

Article published online before print. Article and publication date are at http://www.genome.org/cgi/doi/10.1101/gr.094508.109.

Galaxy deployment models

Galaxy Main (usegalaxy.org)

- ~130,000 jobs a month
- Every month is “best ever”
- Approximately 1Tb in user uploads per week
- Unsustainable in the long term

Community!

Nate Coraor

Building local Galaxy instances

- Galaxy is designed for local installation and customization
  - Just download and run, completely self-contained
  - Easily integrate new tools
  - Easy to deploy and manage on nearly any (unix) system
  - Run jobs on existing compute clusters

Scale up on existing resources

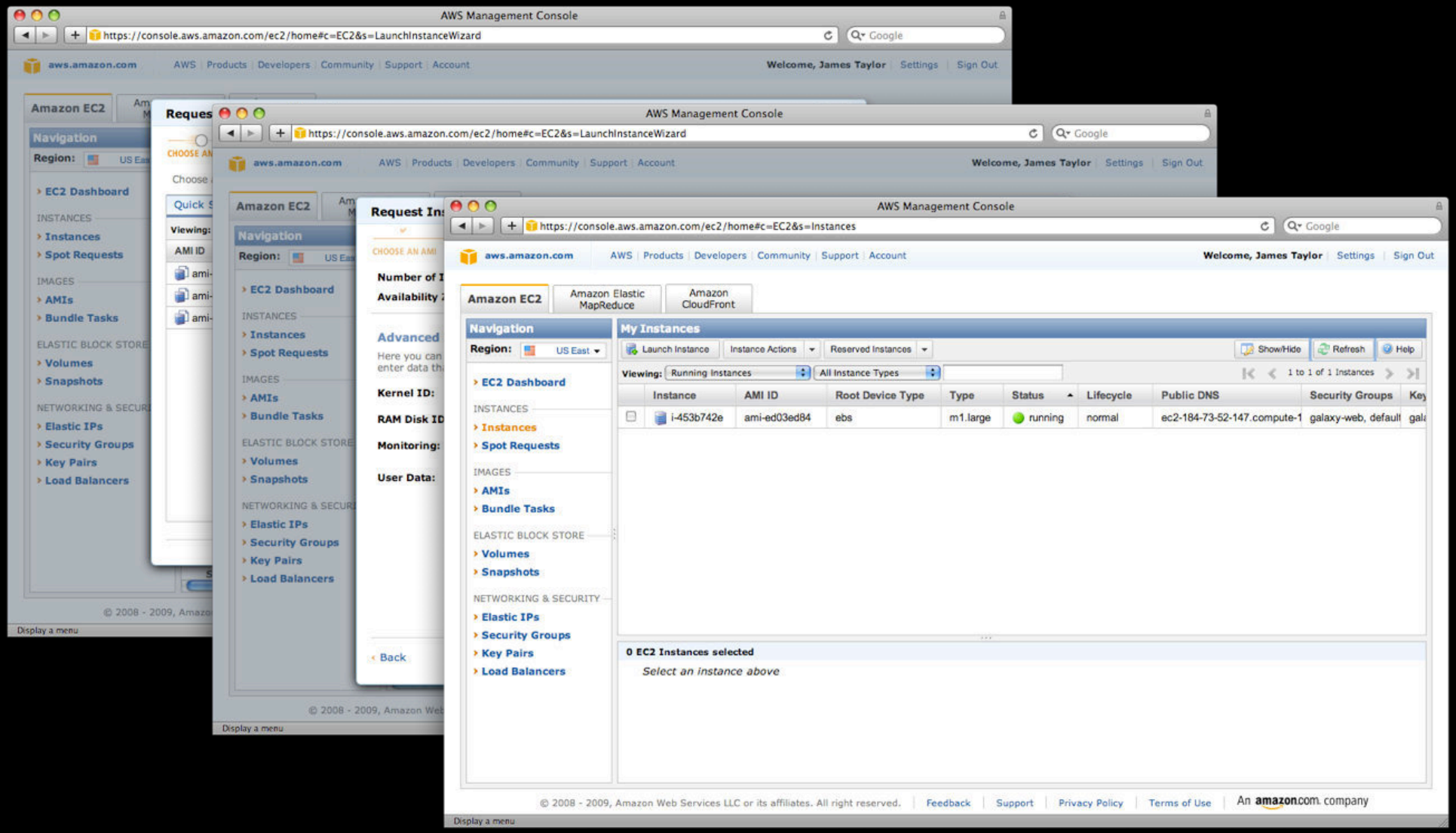
- Move intensive processing (tool execution) to other hosts
- Frees up the application server to serve requests and manage jobs
- Utilize existing resources
- Supports any scheduler that supports DRMAA (most of them)

Cloud computing

- On-demand resource acquisition fits well with the irregular resource needs of many labs working with sequence data
- Our goal is to approach the ease of use of a “software as a service” solution while maintaining the flexibility and control of an infrastructure based solution

Enis Afgan

Using Amazon EC2: Startup in 3 steps



Galaxy Cloud

http://ec2-174-129-103-83.compute-1.amazonaws.com/cloud

Galaxy

Info: report bugs | wiki | screencasts

Galaxy Cloudman Console

Welcome to Galaxy Cloudman. This application will allow you to manage this cloud and the services provided within. If this is your first time running this cluster, you will need to select an initial data volume size. Once the data store is configured, default services will start and you will be add and remove additional services as well as 'worker' nodes on which jobs are run.

Terminate cluster | Add nodes ▾ | Remove nodes | Access Galaxy

Status

Cluster name: ttt

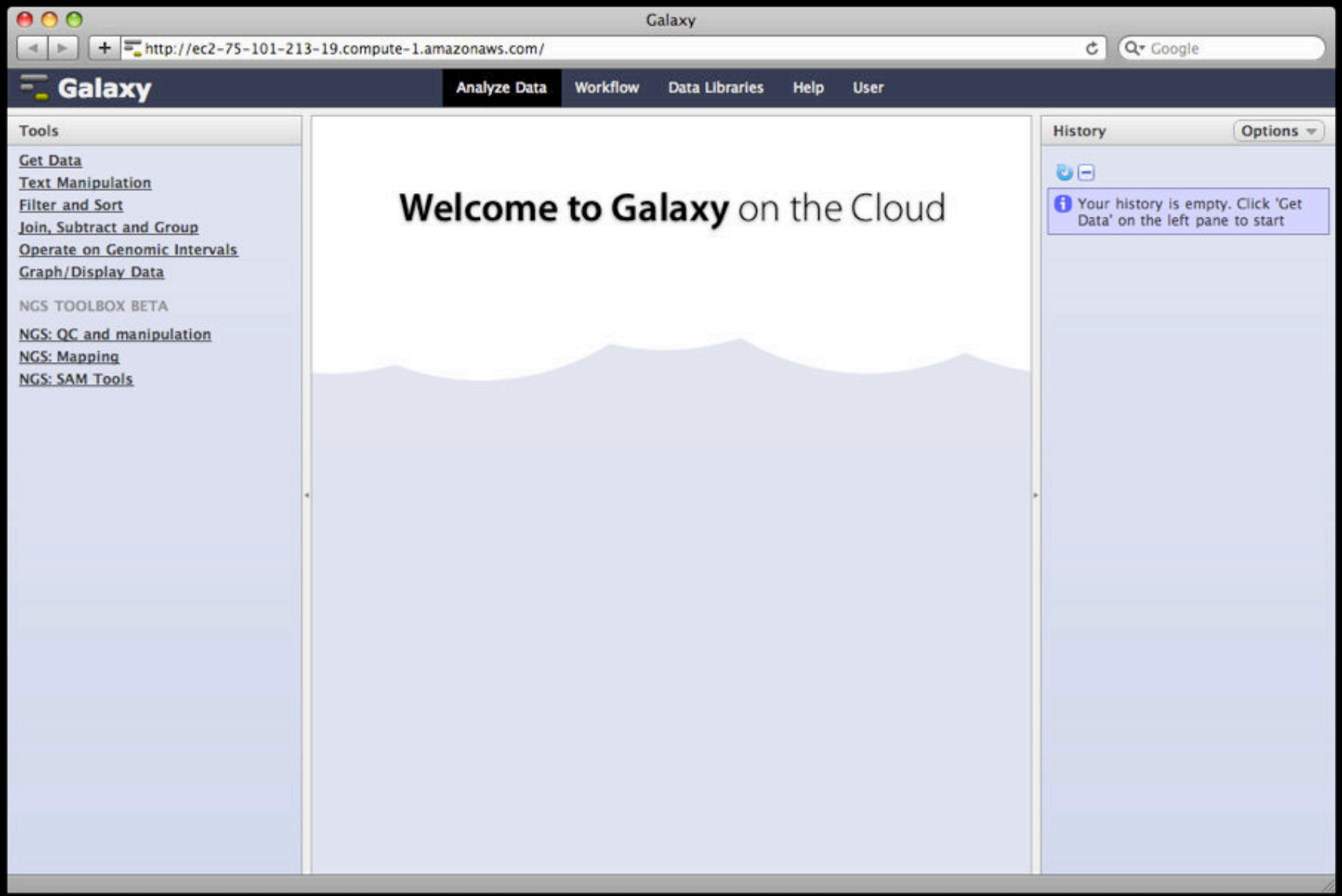
Disk status: 0 / 0 (0%)

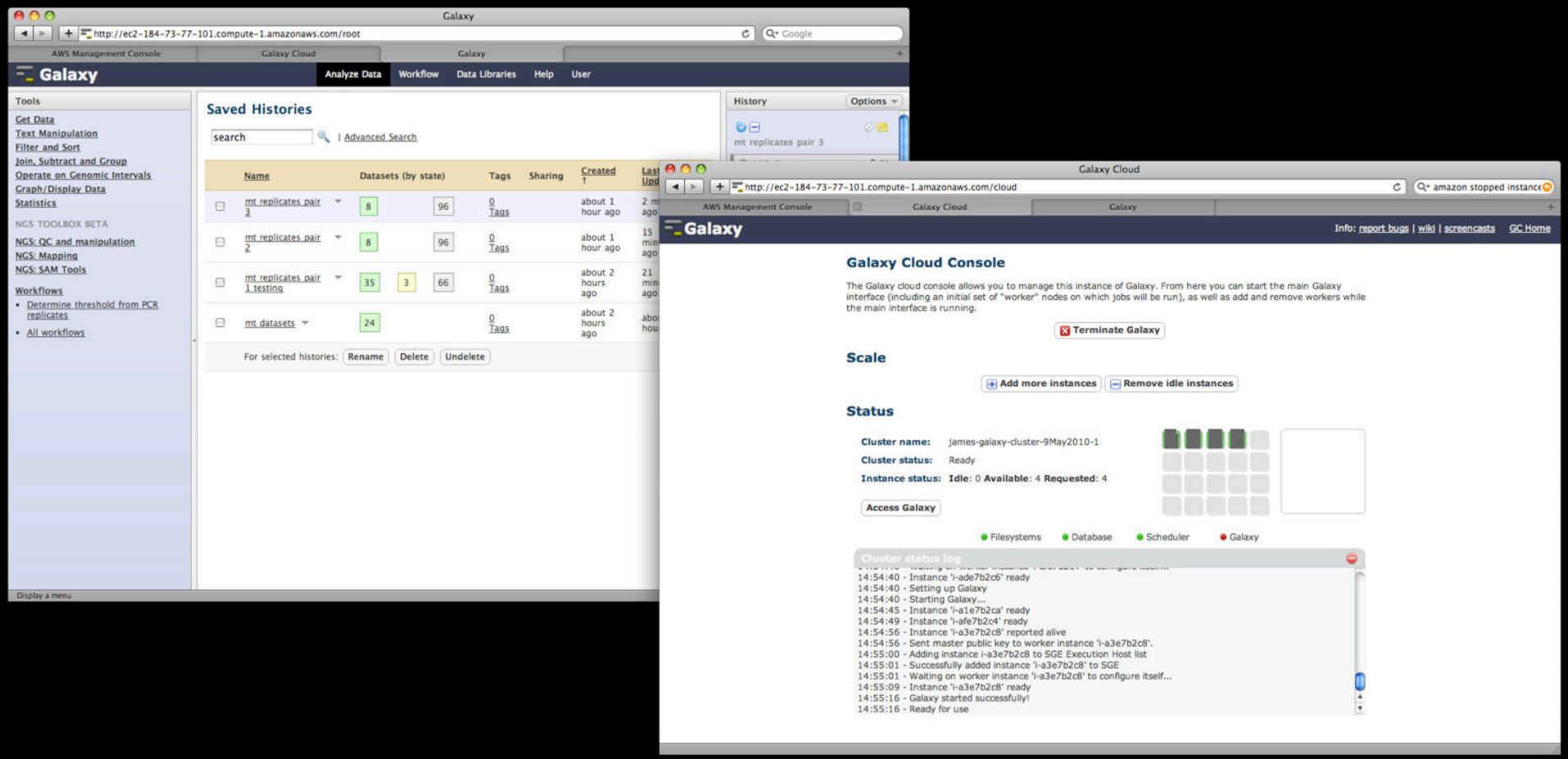
Worker status: **Idle**: 0 **Available**: 0 **Requested**: 0

Service status: Applications ● Data ●

Pending
Starting
Ready
Error

Cluster status log


Galaxy
http://ec2-75-101-213-19.compute-1.amazonaws.com/
Google
Galaxy
Analyze Data
Workflow
Data Libraries
Help
User
Tools
Get Data
Text Manipulation
Filter and Sort
Join, Subtract and Group
Operate on Genomic Intervals
Graph/Display Data
NGS TOOLBOX BETA
NGS: QC and manipulation
NGS: Mapping
NGS: SAM Tools
Welcome to Galaxy on the Cloud
History
Options
Your history is empty. Click 'Get Data' on the left pane to start



Can use like any other Galaxy instance, with additional compute nodes acquired and released (automatically) in response to usage

Galaxy Cloud

http://ec2-184-73-135-47.compute-1.amazonaws.com/cloud/

AWS Management Console | Galaxy Cloud

Galaxy Cloudman

Info: report bugs | wiki | screencast

Galaxy Cloudman Console

Welcome to Galaxy Cloudman. This application allows you to manage this instance of Galaxy CloudMan. Your previous data store has been reconnected. Once the cluster has initialized, use the controls below to add and remove 'worker' nodes for running jobs.

Terminate cluster | Add nodes ▾ | Remove nodes | Access Galaxy

Status

Cluster name: james-cm-31march

Disk status: 181M / 100G (1%)

Worker status: **Idle**: 0 **Available**: 0 **Requested**: 0

Service status: Applications ● Data ●

External Logs: Galaxy Log

Autoscaling is off. Turn on?

Cluster status log

Share a snapshot of this instance

Galaxy Cloud

http://ec2-184-73-135-47.compute-1.amazonaws.com/cloud/

AWS Management Console | Galaxy Cloud

Galaxy Cloudman

Info: report bugs | wiki | screencast

Galaxy Cloudman Console

Welcome to Galaxy Cloudn... loudMan. Your previous data store has been recon... d and remove 'worker' nodes for running jobs.

Terminate clust... | Access Galaxy

Status

Cluster name: jame...
Disk status: 181...
Worker status: Idle
Service status: Appli...
External Logs: Gala...

Autoscaling is off. Turn on?

Cluster status log...

Currently shared instances

Share-an-instance

This form allows you to share this cluster instance, at its current state, with others. You can make the instance public or share it with specific users by providing their account information below.
You may also share the instance with yourself by specifying your own credentials, which will have the effect of saving the instance at its current state.

While setting up an instance to be shared, all currently running cluster services will be stopped. Then, a snapshot of your data volume and a folder in your cluster's bucket will be created (under 'shared/[current date and time]); this folder will contain your cluster's current configuration. The created snapshot and the folder will be given READ permissions to the users you choose (or make it public). This will enable those users to instantiate their own instances of the given cluster instance. This implies that you will only be paying for the created snapshot while users deriving a cluster from yours will incur costs for running the actual cluster. After the sharing process is complete, services on your cluster will automatically resume.

Public Shared

Share-an-instance

Display a menu

Enis Afgan

The next 90 – x minutes

- History (2005 – 2010)
- Present (2010 – 2011)
- The “Vision” (2011 – ∞)
- The Community
- Beer

Where do we go from here

- Why do we want to change the World?
- How are we going to do this?

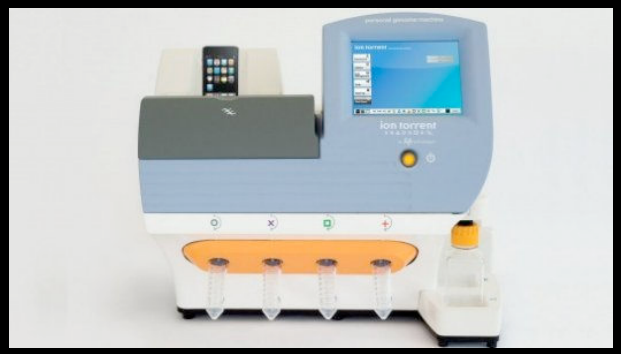
Why Changing the World?

The workhorse: Illumina

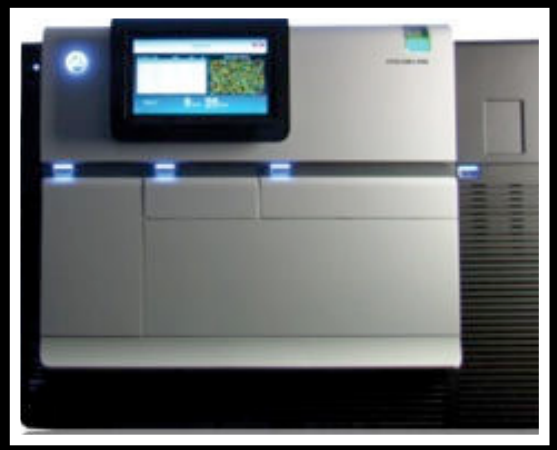
HiSeq 2000

- ~25 GB per day
  - $16k–$20k per run
  - > 1Mb per dollar
- Can multiplex 192 samples per run
  - as little as $100 per sample!

454 GS / Junior: 40-400Mb runs, but read lengths pushing 1kb



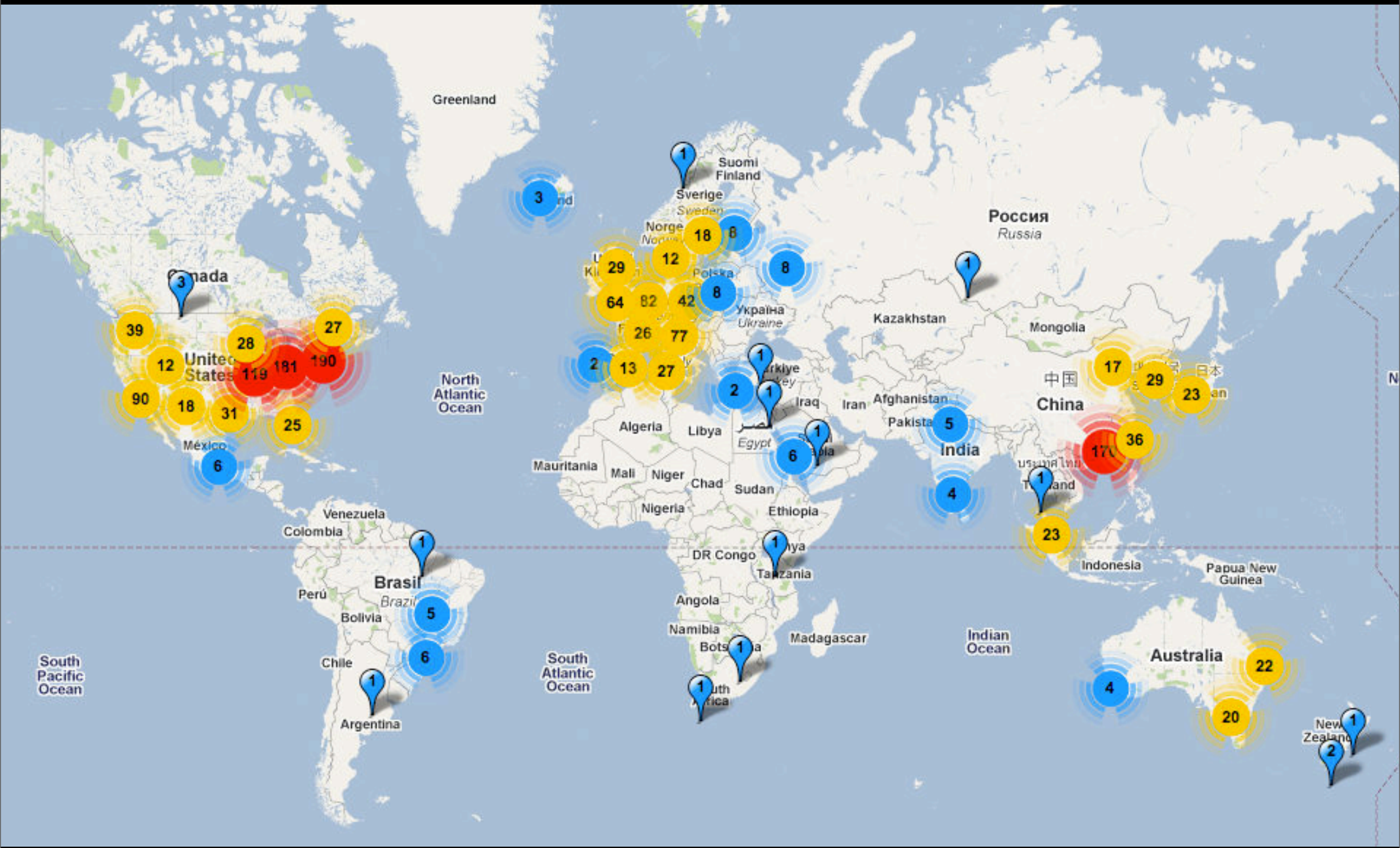
Ion Torrent PGM: 10Mb-1Gb runs, 200-400bp reads, 2 hour runtime, $500!



PacBio RS: Direct single molecule sequencing, only 35k reads, but long read lengths, 30 minute runs!

(plus nanopore and other single molecule techniques on the horizon)

Sequence data production capability
is
widely distributed



(http://pathogenomics.bham.ac.uk/hts/)

Sequencing applications

Genome sequencing

- Direct sequencing of genomic DNA to resolve new genome sequences
  - Direct deep sequencing + de novo assembly for novel genomes
  - Re-sequencing to identify variations with respect to a reference
    - Single-end resequencing for SNP, copy number variation

RNA-seq for transcriptomics

- The diversity of (known) functional RNAs is enormous
- Even the best understood units (protein coding transcripts) are processed in myriad ways including alternative splicing
- In RNA-seq, capture a class of RNA, sequence (directly or through a cDNA clone)
- Reconstruct (possibly overlapping) RNA sequences and quantify the level at which they

Sequencing for functional annotation

- We can turn many functional annotation problems into sequencing problems (this is only a sample)
- The genome is relatively static within an individual, sequence it once and you are done
- Transcript levels, epigenomic modifications, and chromatin structure vary based on cell type, time, condition, ...
  - Enormous potential for data generation

“Democratization of sequencing”

- Because of the diverse utility of sequencing based assays, investigators across all of biology seek to take advantage of these techniques
- Large community data production projects have become relatively rare, data production is increasingly investigator driven
- Democratization of sequencing has not yet been matched by democratization of analysis infrastructure, burden is largely on the investigator
- Use of these techniques requires sophisticated and computationally intensive approaches

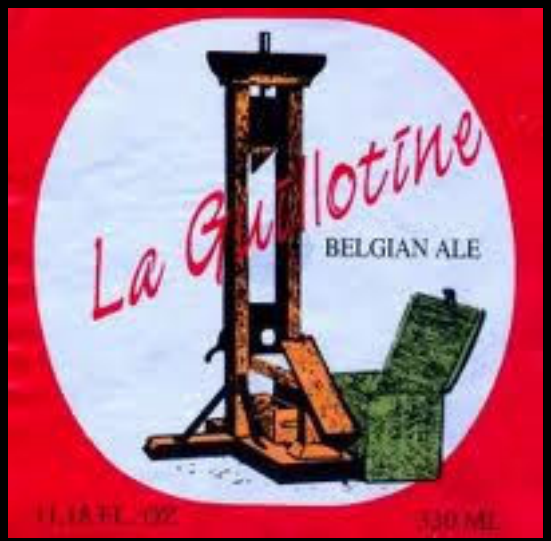
Most biologists don't write code

- High throughput data is very new to Biology, programming is not part of the training (this is not Astronomy...)
- Efforts like Bioperl and Bioconductor (R) have enabled some to pick it up, too often just enough to be dangerous

Much bioinformatics software is "research quality"

- Most software is written for a specific publication
- Poor performance and scalability, not designed with reuse in mind
- The rate with which underlying technologies and methods change makes it pointless to invest in improving
- Difficult to publish purely software papers in good journals, publish updates or improvements to existing software
- Pressure to use only software that is published

Commercial Bioinformatics Software is sustained by ignorance



Key Reproducibility Problems

- **Datasets**: not all available, difficult to access
- **Tools**: inaccessible, hard to record details
- **Publication**: results, data, methods separate

Microarray Experiment Reproducibility

- 18 Nat. Genetics microarray gene expression experiments
- Less than 50% reproducible
- Problems
  - missing data (38%)
  - missing software, hardware details (50%)
  - missing method, processing details

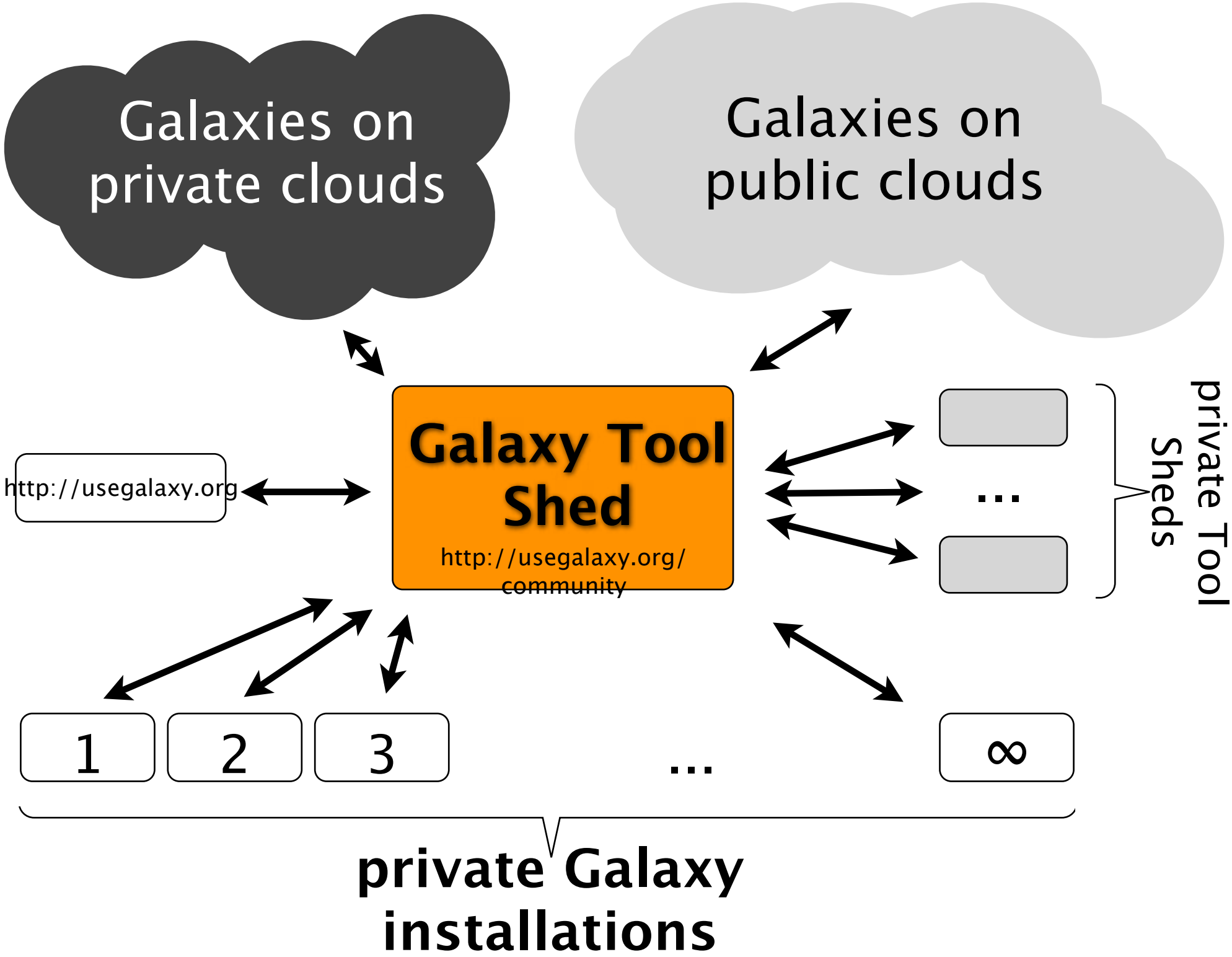
Ioannidis, J.P.A. et al. Repeatability of published microarray gene expression analyses. Nat Genet 41, 149-155 (2009)

NGS Re-sequencing Experiment Reproducibility

- 14 re-sequencing experiments in Nat. Genetics, Nature, and Science (2010)
- 0% reproducible?
- Problems
  - limited access to primary data (50%)
  - some or all tools unavailable (50%)
  - settings & versions not provided (100%)

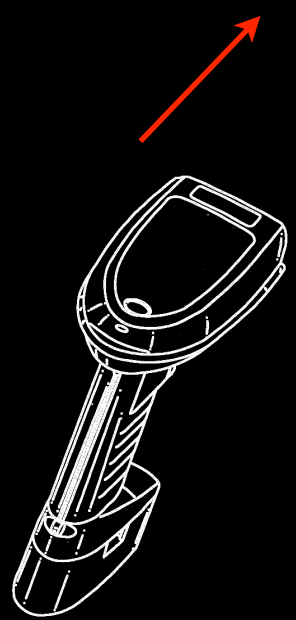
Going Forward

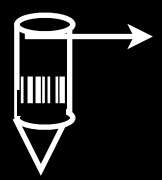
- Development of best practices
- Simplifying deployment of Galaxy and dependencies
- Tool Shed as the HUB
- Transparent publishing of analyses for reproducible research
- Tight Integration with NGS instruments


Galaxies on private clouds
Galaxies on public clouds
Galaxy Tool Shed
http://usegalaxy.org/community
http://usegalaxy.org
...
private Tool Sheds
1
2
3
...
∞
private Galaxy installations

Sample tracking and instrument integration

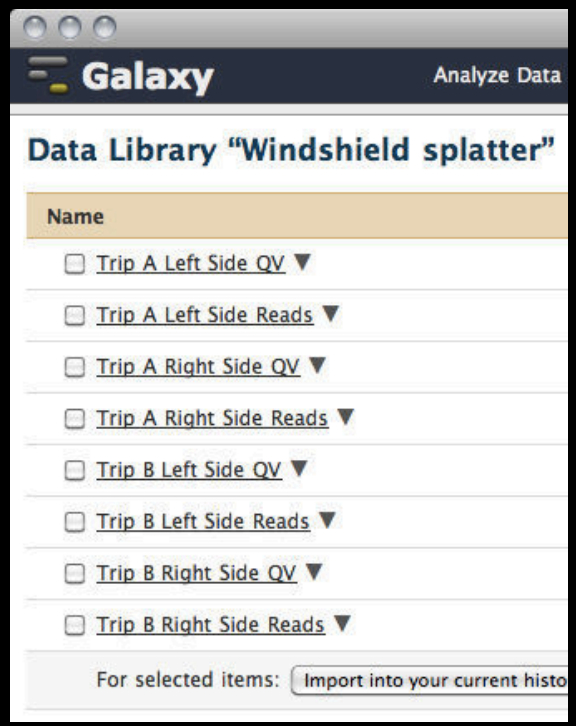
(work in progress)





PACIFIC BIOSCIENCES™

illumina®



Greg Von Kuster, Dannon Baker

Galaxy

http://localhost:8080/requests

Google

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

Request Actions

Add Samples to Sequencing Request "Snail transcriptome"

Name	State	Data Library	Folder	History	Workflow
Sample 1 (required)		Select one	Select one	Lambda History	lambda Input BAM: Coverage (gff) Reference Genome: lambda_ref.fasta

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.

Additional information

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.

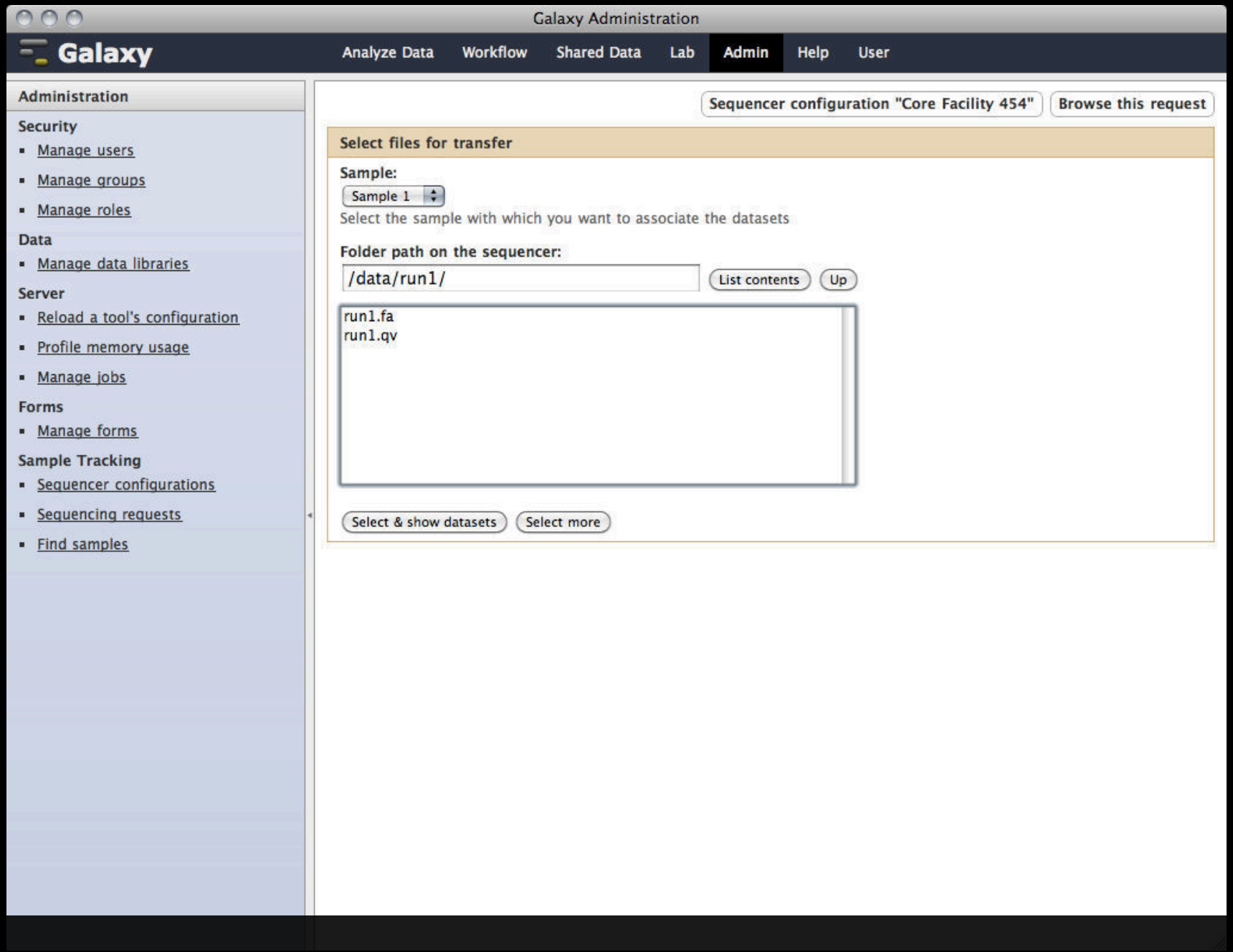
Add sample | Save | Cancel

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

Display a menu

Consumer creating sequencing requests in Galaxy interface, and



Simplest scenario, lab manager manually imports run data and

Galaxy

http://localhost:8080/requests

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

Request Actions

Sequencing request "Snail transcriptome"

Current state:
Complete

Description:

User:
james.taylor@emory.edu

Request type:
Pacific Biosciences

More

Samples

Name	Barcode	State	Data Library	Folder	History	Workflow	Run Datasets
Sample_1		Done	Pacific Biosciences	Pacific Biosciences	Lambda History	lambda	6

Additional information

Display a menu

Within instrument integration plugins, data acquired automatically and

Sample tracking is completely

- 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

ONLY AS A COMMUNITY WE CAN ACHIEVE THESE GOALS!