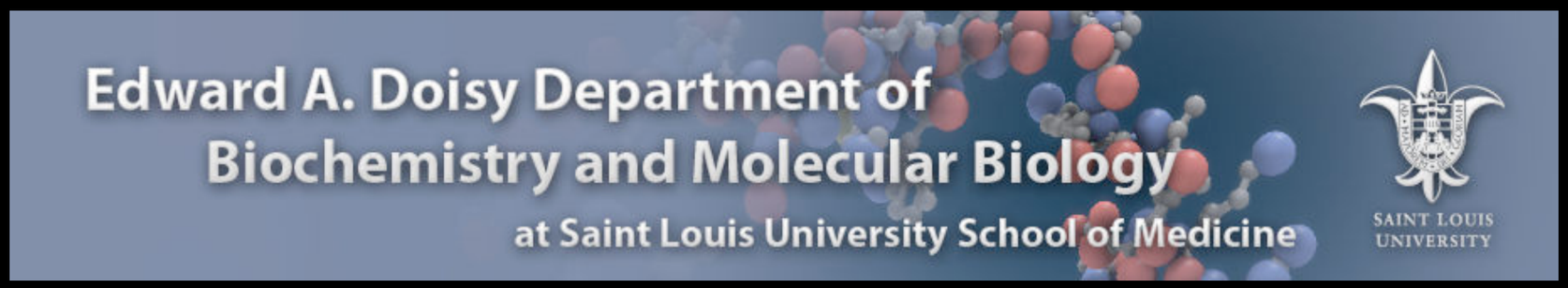
Introduction to Galaxy

Saint Louis University

St. Louis, Missouri

April 30, 2013

Dave Clements, Emory University
http://galaxyproject.org/



Agenda

9:00 Welcome
9:20 Basic Analysis with Galaxy
10:30 Basic Analysis into Reusable Workflows
11:00 RNA-Seq Example Part I
11:30 Lunch (on your own)
1:00 RNA-Seq Example Part II
1:30 Galaxy Project Overview
2:00 RNA-Seq Example Part III
2:30 Sharing, Publishing and Reproducibility
2:50 Break
3:10 ChIP-Seq Example
5:00 Done

Introductions

In 60 seconds or less tell us

- your name
- your affiliation(s)
- something about your research
- something about what you want to learn

Goals

1. Introduce Galaxy
2. Introduce bioinformatics concepts and formats
3. Hands-on experience
    - Load and integrate data
    - Perform bioinformatic analysis with Galaxy
    - Save, share describe and publish your analyses
    - Visualize your results
    - Set up your own Galaxy server in the cloud

This workshop will not cover details of how tools are implemented, or new algorithm designs, or which assembler or mapper or ... is best for you.

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Basic Analysis

On human chromosome 22,
which coding exons have the most
repeats in them?

http://cloud1.galaxyproject.org/
http://cloud2.galaxyproject.org/
http://cloud3.galaxyproject.org/

(~ http://usegalaxy.org/galaxy101)

Exons & Repeats: A General Plan

- Get some data
  - Coding exons on chromosome 22
  - Repeats on chromosome 22
- Mess with it
  - Identify which exons have repeats
  - Count repeats per exon
  - Save, download, ... exons with most repeats

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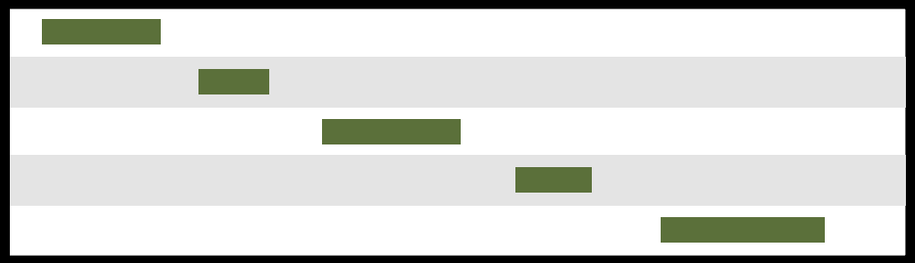
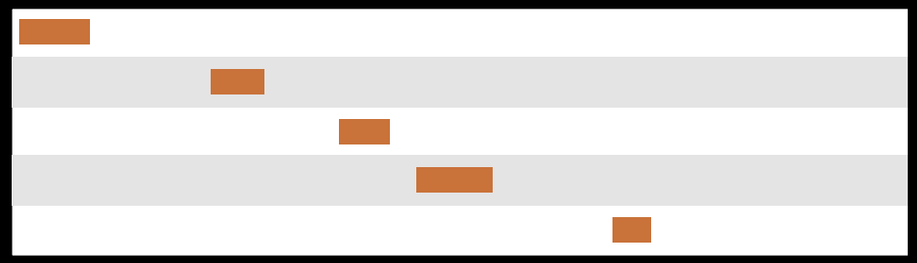
http://cloud3.galaxyproject.org/

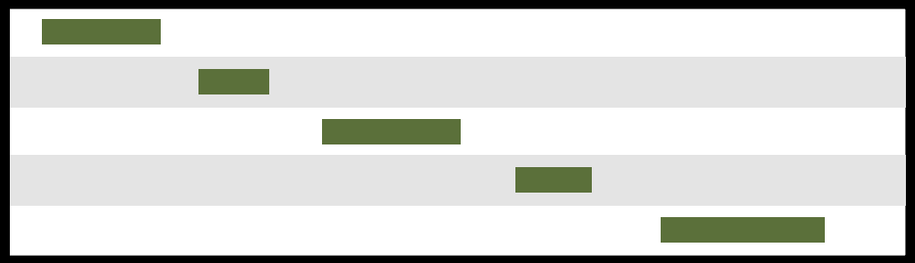
(~ http://usegalaxy.org/galaxy101)



Exons, from UCSC



Repeats, from UCSC



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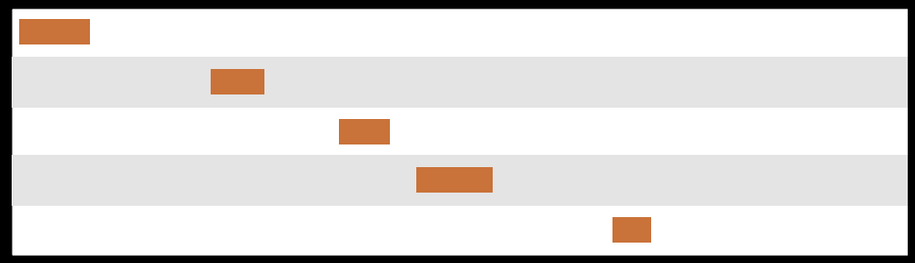
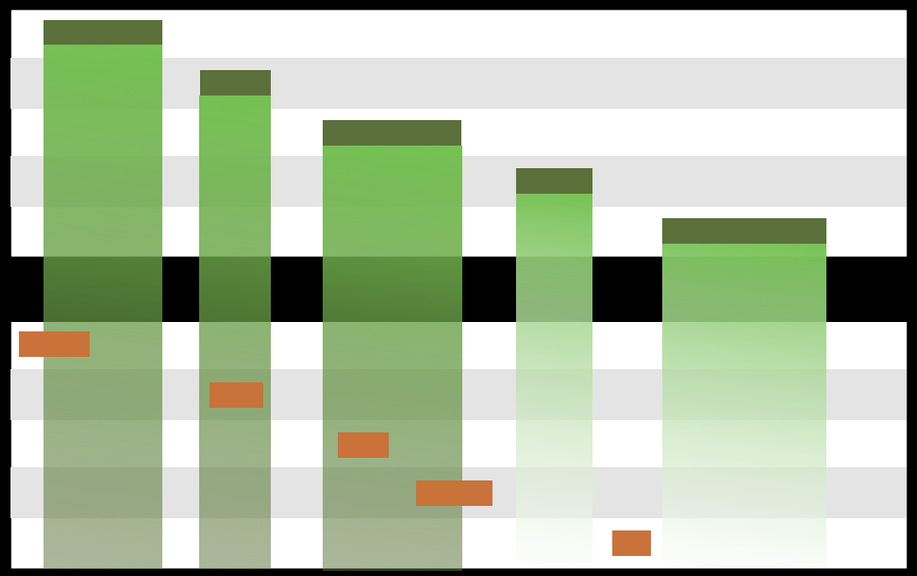


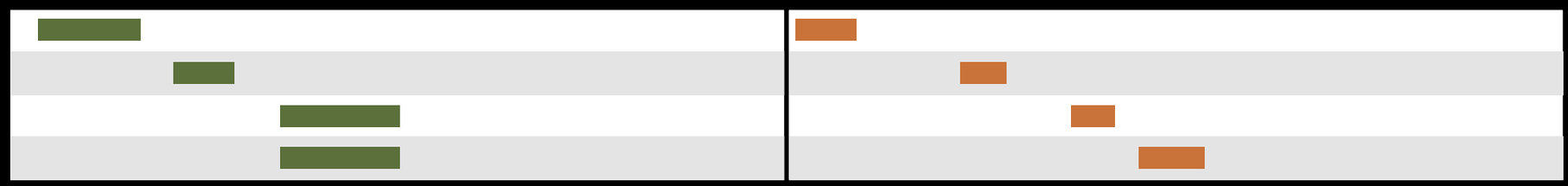
Repeats, from UCSC

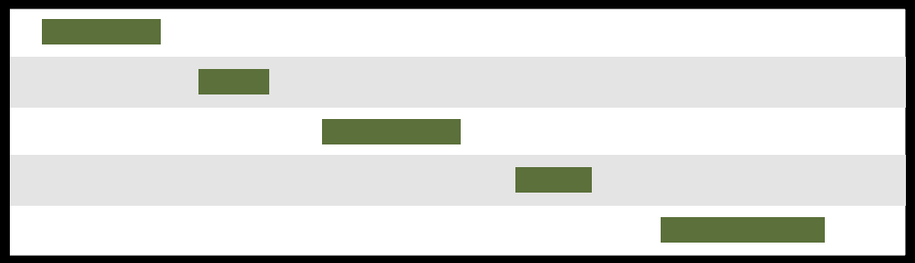


Exons, from UCSC

Repeats, from UCSC

Overlap pairings





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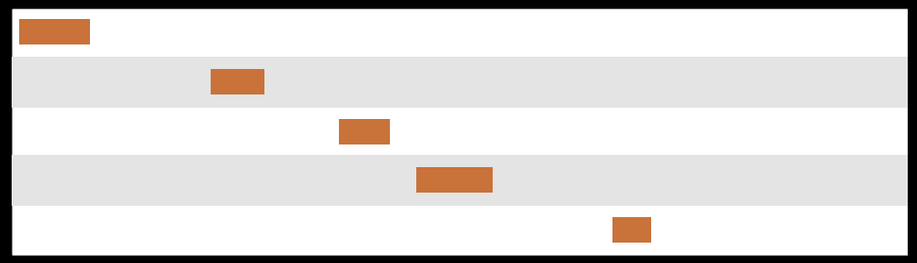
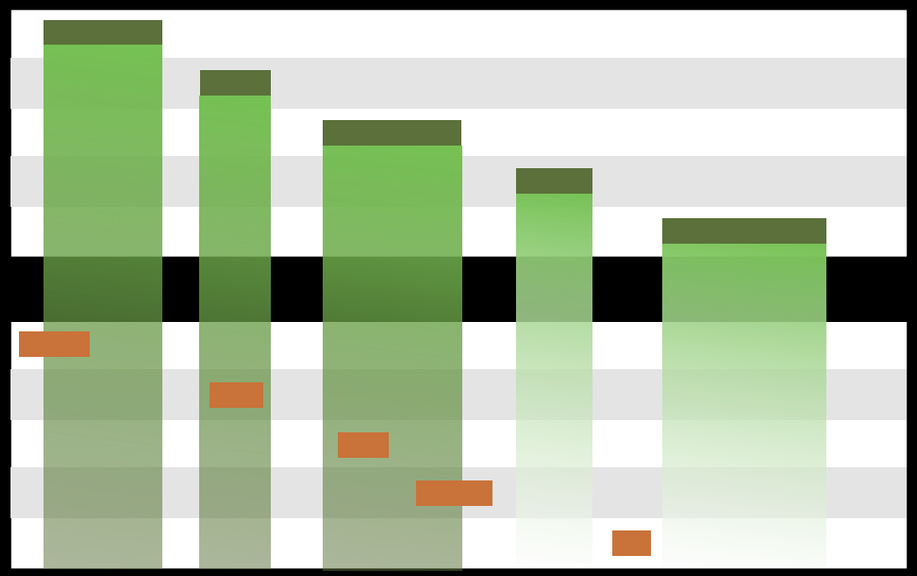


Repeats, from UCSC



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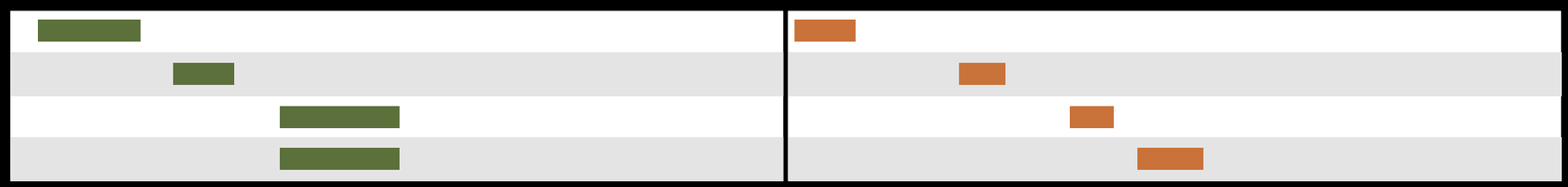
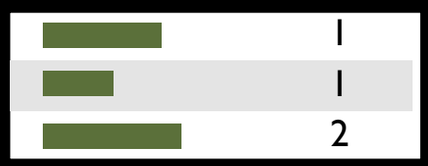
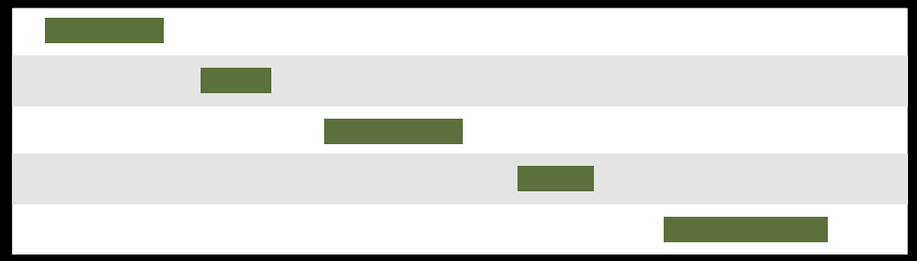




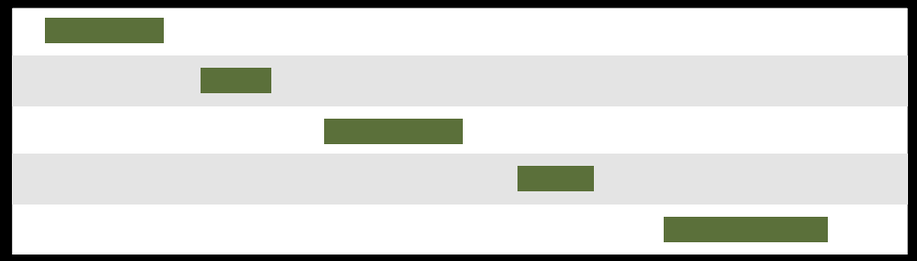
Exon overlap counts

Exon overlap counts



Exons, from UCSC

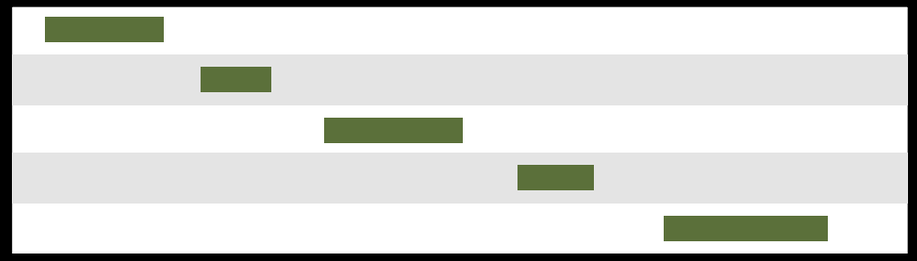
Exon overlap counts



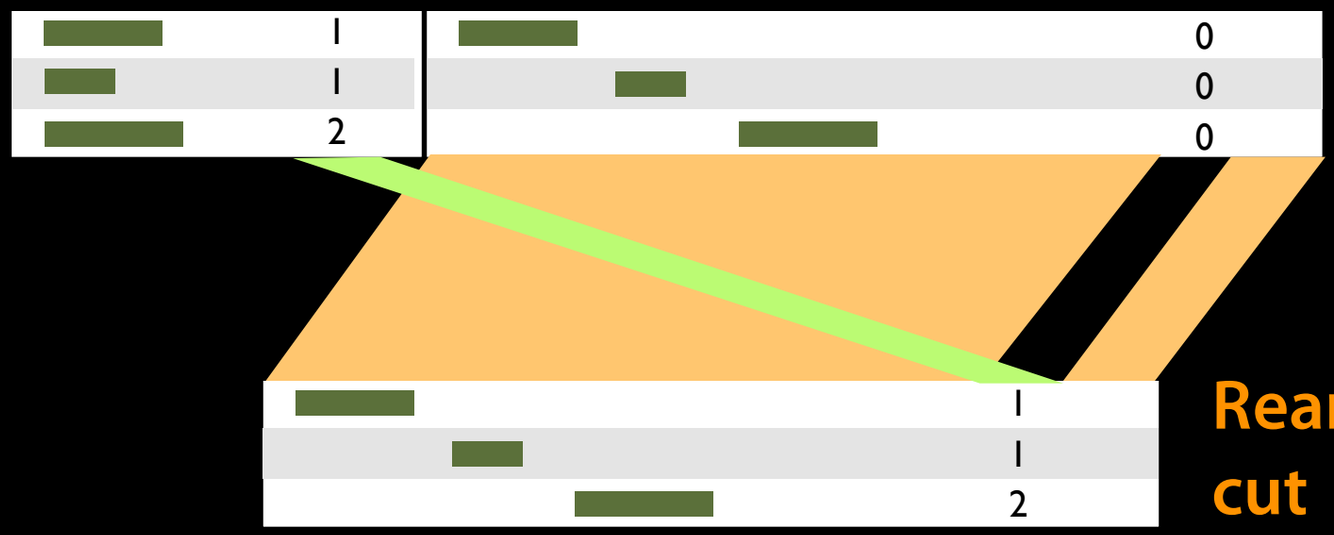
Exons, from UCSC

Join on exon name

Exon overlap counts



Exons, from UCSC



Join on exon name

Rearrange columns w/ cut

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Some Galaxy Terminology

Dataset:

Any input, output or intermediate set of data + metadata

History:

A series of inputs, analysis steps, intermediate datasets, and outputs

Workflow:

A series of analysis steps

Can be repeated with different data

Exons and Repeats *History* → Reusable *Workflow?*

- The analysis we just finished was about
  - Human chromosome 22
  - Overlap between exons and repeats
- But, ...
  - there is nothing inherently in the analysis about humans, chromosomes, exons or repeats
  - It is a series of steps that sets the score of one set of features to the number of overlaps from another set of features.

Create a generic *Overlap* Workflow

Extract Workflow from history

Create a workflow from this history.

Edit it to make some things clearer.

Run / test it

Guided: rerun with same inputs

On your own:

Count # CpG islands in each exon

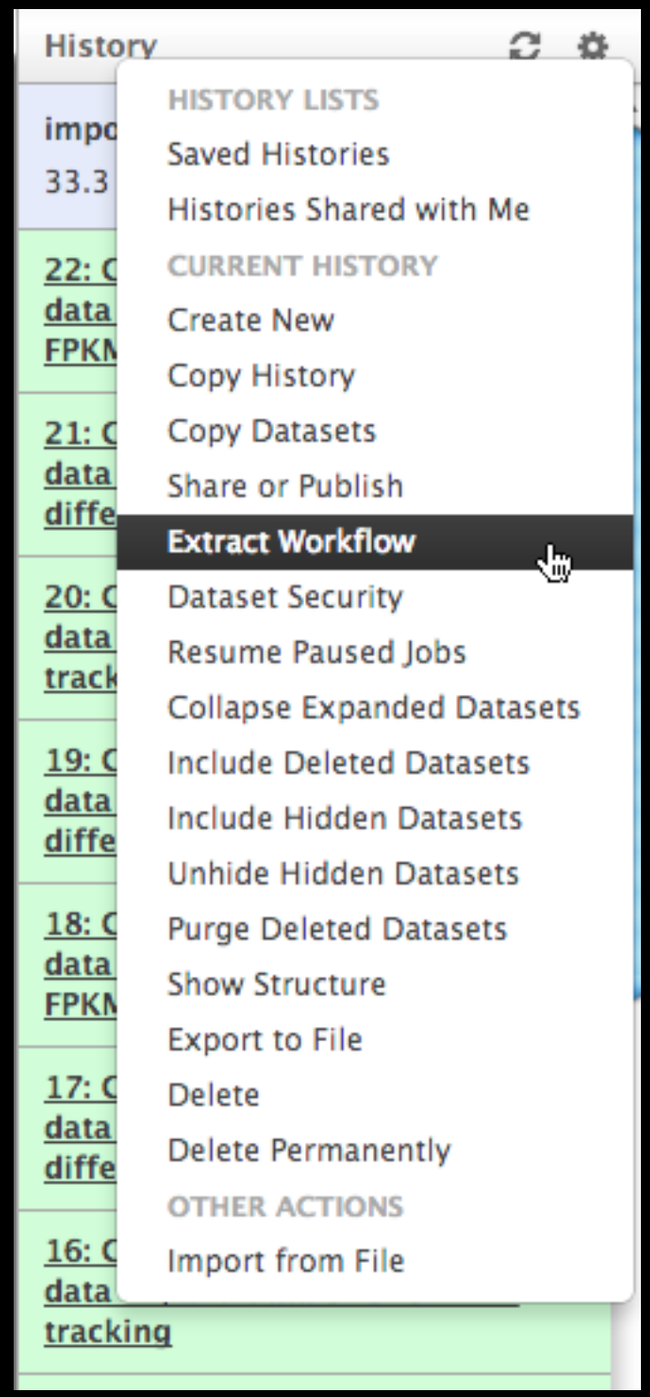
Did that work?

On your own:

Count # of exons in each repeat

Did that work? *Why not?*

Edit workflow: doc assumptions



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RNA-seq Exercise

Shared Data → Published Pages

→ RNA-Seq Analysis Exercise

RNA-seq Exercise: A Plan

- Get input datasets; hg19, will mostly map to chr19
- Look at quality
- Trim as we see fit.
- Map the reads to the human reference using Tophat
- Run Cufflinks on Tophat output to assemble reads into transcripts
- Visualize it

RNA-seq Exercise: A Plan

- Get input datasets; hg19, will mostly map to chr19
  - All datasets are FASTQ and from the Body Map 2.0 project
  - Shared Data → Published Pages → RNA-Seq Analysis Exercise
- or Shared Data → Data Libraries → RNA-Seq Example

What is FASTQ?

- Specifies sequence (FASTA) and quality scores (PHRED)
- Text format, 4 lines per entry

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

- FASTQ is such a cool standard, there are 3 (or 5) of them!

```
  SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
  ..............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
  ..........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
  !"#$%&'()*+,-./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)
```

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

RNA-seq Exercise: A Plan

Look at quality Options 1 & 2:

1. NGS QC and Manipulation → Compute Quality Statistics and then Draw quality score boxplot

   No control over how it is calculated or presented.

2. NGS QC and Manipulation → FastQ Summary Statistics, Graph / Display Data → Boxplot of quality statistics

   Lots of control over what the box plot looks like, but no additional information; stats in text and graphic formats

RNA-seq Exercise: A Plan

- Look at quality: Option 3
  - NGS QC and Manipulation → **FastQC**
  - Gives you a lot a lot more information but little control over how it is calculated or presented.
- This is what we have done

RNA-seq Exercise: A Plan

RNA-seq Exercise: A Plan

"*For the love of all that is holy, please trim your reads!*"

Chris Mason, ABRF NGS Study Report, March 4, 2013

RNA-seq Exercise: A Plan

"*For the love of all that is holy, please trim your reads!*"
Chris Mason, ABRF NGS Study Report, March 4, 2013

- Trim as we see fit: Option 1
  - **NGS QC and Manipulation → FASTQ Trimmer by column**
  - Trim same number of columns from every record
  - Can specify different trim for 5' and 3' ends

RNA-seq Exercise: A Plan

- Look at quality
- ~~Trim~~ Filter as we see fit: Option 2
  - NGS QC and Manipulation → **Filter FASTQ reads by quality score and length**
  - Keep or discard whole reads at a time
  - Can have different thresholds for different regions of the reads.
  - Keeps original read length.

http://bit.ly/GxyRNASeqEx

RNA-seq Exercise: A Plan

- Look at quality
- Trim as we see fit: Option 3
  - NGS QC and Manipulation → **FASTQ Quality Trimmer by sliding window**
  - Trim from both ends, using sliding windows, until you hit a high-quality section.
- Produces variable length reads

Trim? *As we see fit?*

- Introduced 3 options
  - One preserves original read length, two don't
  - One preserves number of reads, two don't
  - Two keep/make every read the same length, one does not
  - One preserves pairings, two don't
  - Options are not mutually exclusive!

Trim? *As we see fit?*

- Choice depends on downstream tools
- Find out assumptions & requirements for downstream tools and make appropriate choice(s) now.
- How to do that?
  - http://biostars.org/
  - http://seqanswers.com/
  - http://galaxyproject.org/search

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RNA-seq Exercise: A Plan

- Get input datasets; hg19, will mostly map to chr19
- Look at quality
- Trim as we see fit.
- Map the reads to the human reference using Tophat
  - *Imagine pages and pages of discussion on the intricacies and pitfalls of RNA-seq mapping here.*

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What is Galaxy?

- **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

- These options result in several **ways to use Galaxy**

http://galaxyproject.org

Galaxy is available ...

- As a free (for everyone) web service

  http://usegalaxy.org

  However, ***a centralized solution cannot scale to meet the analysis needs of the entire world.***

Galaxy is available ...

- As a free (for everyone) web service

  http://usegalaxy.org

- As open source software

  http://getgalaxy.org

As Open Source Software: Local Galaxy Instances

- Galaxy is designed for local installation and customization
  - Easily integrate new tools
  - Easy to deploy and manage on nearly any (unix) system
  - Run jobs on existing compute clusters
- Requires a computational resource on which to be deployed

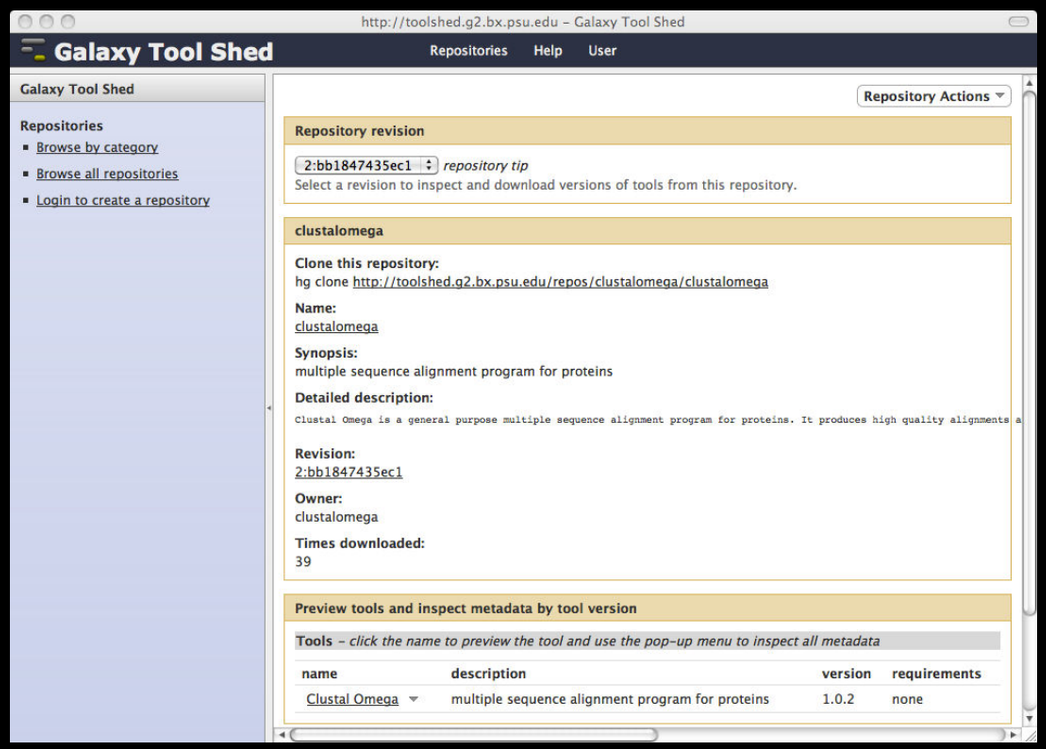
http://getgalaxy.org

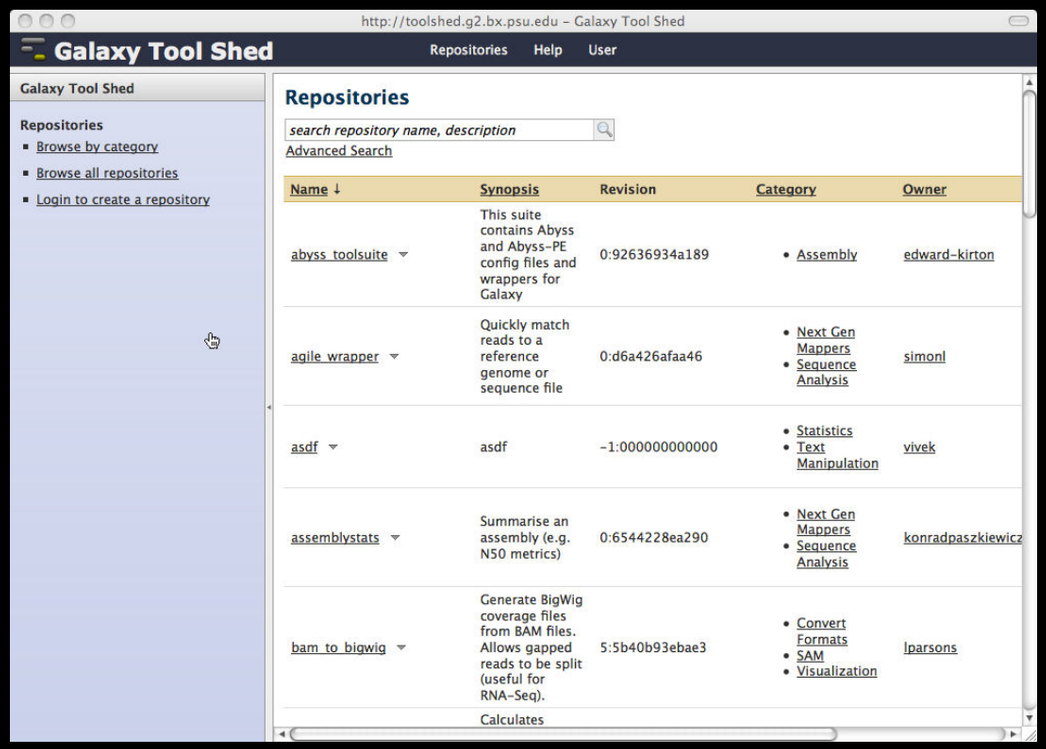
Encourage Local Galaxy Instances

- Encourage and support Local Galaxy Instances
  - Support increasingly decentralized model and improve access to existing resources
  - Focus on building infrastructure to enable the community to integrate and share tools, workflows, and best practices

Galaxy Tool Shed

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





Encourage Public Galaxy Instances

http://bit.ly/gxyServers

Interested in:

ChIP-chip and ChIP-seq?
- ✓ Cistrome

Statistical Analysis?
- ✓ Genomic Hyperbrowser

Protein synthesis?
- ✓ GWIPS-viz

de novo assembly?
- ✓ CBIIT Galaxy

Reasoning with ontologies?
- ✓ OPPL Galaxy

Repeats!
- ✓ RepeatExplorer

Everything?
- ✓ Andromeda

Plus many more

As Open Source Software: Local Galaxy Instances

- Galaxy is designed for local installation and customization
  - Easily integrate new tools
  - Easy to deploy and manage on nearly any (unix) system
  - Run jobs on existing compute clusters
- Requires a **computational resource** on which to be deployed

http://getgalaxy.org

Got your own cluster?

- Galaxy works with any DRMAA compliant cluster job scheduler (which is most of them).
- Galaxy is just another client to your scheduler.

CLUSTER RESOURCES
TORQUE

GRID ENGINE

Platform Computing

DRMAA
Distributed Resource Management Application API — www.drmaa.org

Galaxy is available ...

- As a free (for everyone) web service

  http://usegalaxy.org

- As open source software

  http://getgalaxy.org

- *On the Cloud*

  http://usegalaxy.org/cloud

  We are using this right now, and you will set up your own instance today

  http://aws.amazon.com/education

Galaxy Resources and Community

Mailing Lists (very active)
Unified Search
Issues Board
Events Calendar, News Feed
Community Wiki
GalaxyAdmins
Screencasts
Tool Shed
Public Installs
CiteULike group, Mendeley mirror
Annual Community Meting

http://wiki.galaxyproject.org

Galaxy Resources and Community: Mailing Lists

http://wiki.galaxyproject.org/MailingLists

Galaxy-Announce

Project announcements, low volume, moderated

Low volume (42 posts, 1600 members in 2012)

Galaxy-User

Questions about using Galaxy and usegalaxy.org

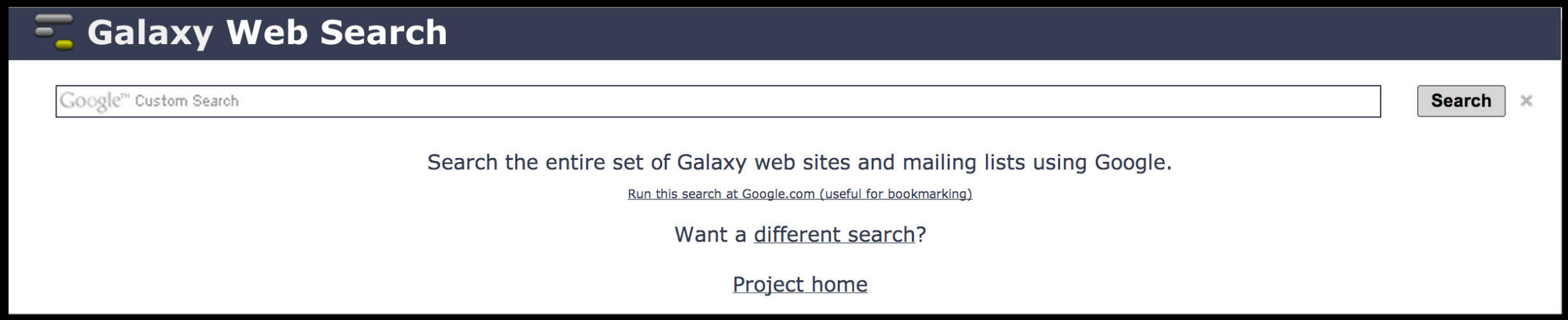
High volume (2900 posts, 2700 members in 2012)

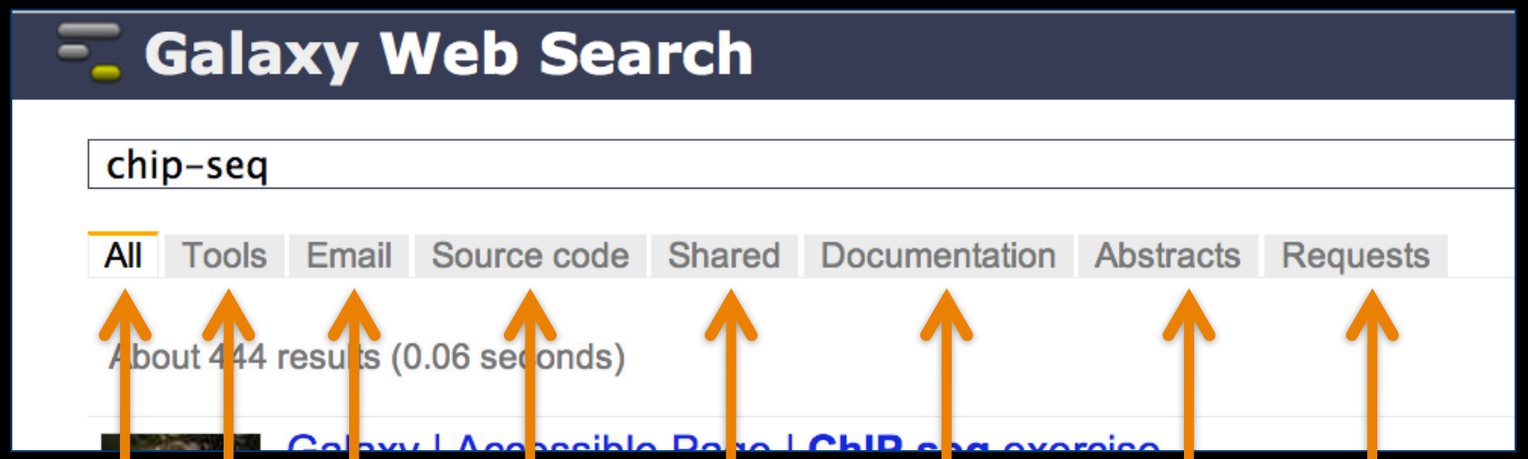
Galaxy-Dev

Questions about developing for and deploying Galaxy

High volume (4500 posts, 850 members in 2012)

Unified Search: http://galaxyproject.org/search





Find

Everything on …

Tools for …

Email about …

Source code for …

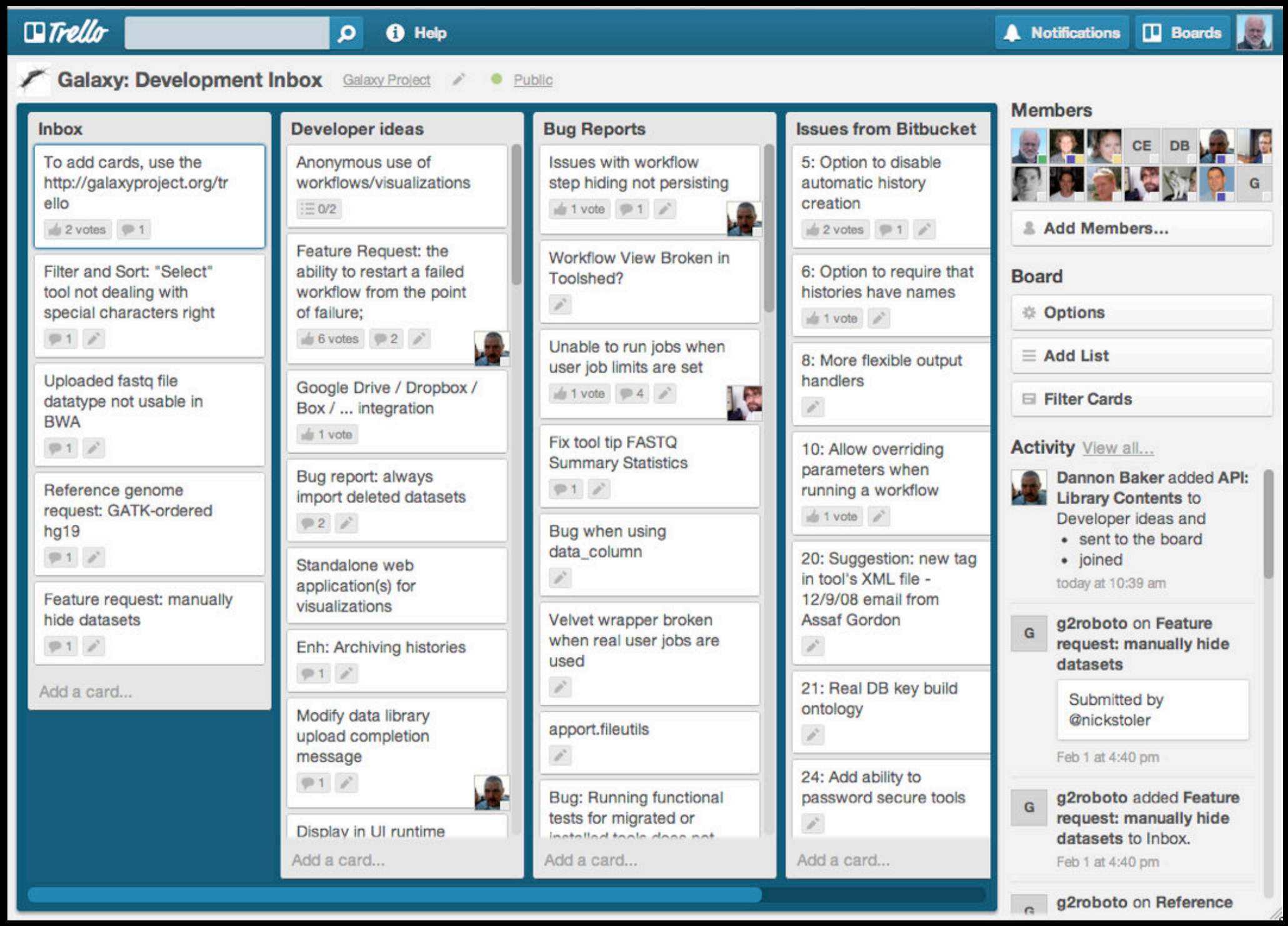
Published Histories, Pages, Workflows, about …

Documentation on …

Papers using Galaxy for …

Related feature requests

Community can create, vote and comment on issues



http://bit.ly/gxyissues

http://wiki.galaxyproject.org

Galaxy Wiki

Login | Search: Titles Text

FrontPage

Locked History Actions

Galaxy

Galaxy is an open, web-based platform for *accessible*, *reproducible*, and *transparent* computational biomedical research.

- **Accessible:** Users without programming experience can easily specify parameters and run tools and workflows.
- **Reproducible:** Galaxy captures information so that any user can repeat and understand a complete computational analysis.
- **Transparent:** Users share and publish analyses via the web and create Pages, interactive, web-based documents that describe a complete analysis.

This is the Galaxy Community Wiki. It describes all things Galaxy.

Use Galaxy

Galaxy's public service web site makes analysis tools, genomic data, tutorial demonstrations, persistent workspaces, and publication services available to any scientist. Extensive user documentation (applicable to any public or local Galaxy instance) is available on this wiki and elsewhere.

usegalaxy.org

Deploy Galaxy

Galaxy is open source for all organizations. Local Galaxy servers can be set up by downloading and customizing the Galaxy application.

- Admin
- Cloud

getgalaxy.org

Community & Project

Galaxy has a large and active user community and many ways to Get Involved.

- Community
- News
- Events
- Support
- Galaxy Project

Contribute

- **Users:** Share your histories, workflows, visualizations, data libraries, and Galaxy Pages, enabling others to use and learn from them.
- **Deployers and Developers:** Contribute tool definitions to the Galaxy Tool Shed (making it easy for others to use those tools on their installations), and code to the core release.
- **Everyone: Get Involved!**

Galaxy Community Conference 30 June - 2 July 2013 OSLO

Poster abstracts due 3 May

Use Galaxy

Use Main *(about)*
Use Others! • Learn
Share • Search

Communication

Support • News
Events • Twitter
Mailing Lists (search)

Deploy Galaxy

Get Galaxy • Cloud
Admin • Tool Config
Tool Shed • Search

Contribute

Tool Shed • Share
Issues & Requests
Support

Galaxy Project

Home • About
Community
Big Picture

Wiki

Help • All Pages

Events

Galaxy Wiki | Login | Search: Titles Text

Events | Locked H

Galaxy Event Horizon

Events with Galaxy-related content are listed here.

Also see the Galaxy Events Google Calendar for a listing of events and deadlines that are relevant to the Galaxy Community. This is also available as an RSS feed.

If you know of any event that should be added to this page and/or to the Galaxy Event Calendar, please add it here or send it to outreach@glaxyproject.org .

Contents

1. Upcoming Events
2. Other Calendars
3. Past Events
    1. 2013
    2. Archive

Upcoming Events

Date	Topic/Event	Venue/Location	Contact
April 29 - May 2	*Introduction to Galaxy Workshops* 2013 Galaxy Workshop Tour	Washington University in St. Louis Saint Louis University University of Missouri Columbia	Dave Clements
May 14-16	Tutorial: *Exploring and Enabling Biomedical Data Analysis with Galaxy*	Great Lakes Bioinformatics Conference (GLBIO) 2013, Pittsburgh, Pennsylvania, United States	Anton Nekrutenko
May 15	*GalaxyAdmins May 2013 Meetup*	GalaxyAdmins web meetup	Srinivas Maddhi, Dave Clements
May 16-17	*Galaxy Workflows for Bioinformatics Analysis*, and *Workshop 1A – Galaxy Workflows for Bioinformatics Analysis*	Workshop in Next-Generation Sequence Analysis and Metabolomics (WiNGS), UNC-Charlotte, North Carolina, United States	James Taylor
May 21 May 29	*Initiation à l'utilisation de Galaxy* Les deux ateliers sont maintenant complets	Cycle "Bioinformatique par la pratique" 2013, INRA Jouy-en-Josas, France	Sandra Dèrozier, Valentin Loux, Véronique Martin <veronique.martin AT jouy DOT inra DOT fr>
May 22 May 30	Analyse de données issues de séquenceurs nouvelle génération sous Galaxy Les deux ateliers sont maintenant complets		Jean-François Gibrat, Valentin Loux, Véronique Martin <veronique.martin AT jouy DOT inra DOT fr>
May 24 June 19	*Introduction to Galaxy*	UC Davis Bioinformatics Core Davis, California, United States	Nikhil Joshi <najoshi AT ucdavis DOT edu>
	A Genomics Virtual Lab for Cancer Research		Dominique Gorse

News

Galaxy Wiki | Login | Search: Titles Text

News | Locked History Actions

News

Announcements of interest to the Galaxy Community. These can include items from the Galaxy Team or the Galaxy community and can address anything that is of wide interest to the community.

The Galaxy News is also available as an RSS feed.

See Add a News Item below for how to get an item on this page, and the RSS feed. Older news items are available in the Galaxy News Archive.

See also

- Galaxy News Briefs
- Galaxy Updates
- Galaxy on Twitter
- Events
- Learn
- Support
- About the Galaxy Project

News Items

Environmental Metabolomics + Galaxy
Galaxy @ ASMS 2013
April 8, 2013 Galaxy Security Release
GCC2013 & Galaxy GigaScience Series
April 2013 Galaxy Update
April 1, 2013 Galaxy Distribution
Galaxy LinkedIn Group
March 2013 GalaxyAdmins Meetup
Main & Test ServerDowntime: 3/14
March 2013 Galaxy Update
GCC2013 Abstract Submission & Registration

News Archive

News Items

Environmental Metabolomics + Galaxy

A new UK-China collaboration in environmental metabolomics between the University of Birmingham, BGI and *GigaScience* has received funding from the UK's Natural Environment Research Council (NERC).

The first metabolomics project will send a developer from the University of Birmingham's School of Biosciences, to Hong Kong to work with *GigaScience* personnel on extending Galaxy for use in metabolomics data analyses.

"Metabolomics involves the detection and quantification of small molecules (metabolites) in living organisms and can provide an indication of their cellular condition and health. The toxicological responses of organisms to pollutants can be studied using environmental metabolomics, enabling researchers to discover diagnostic markers for monitoring and risk assessment of our environment. Research at Birmingham focuses extensively on the metabolic responses of the freshwater model organism, *Daphnia*, to both pollutants and engineered nanomaterials."

See the official announcement for more details.

Peter Li
GigaScience

Posted to the Galaxy News on 2013-04-22

Galaxy @ ASMS 2013

Galaxy will have a significant presence at the 61st ASMS Conference on Mass Spectrometry and Allied Topics being held in Minneapolis, Minnesota, June 9-13. Galaxy related content includes the *Galaxy Framework as a Solution for MS-based Informatics* workshop and at least 9 posters either directly about or using Galaxy.

If you do research in proteomics than please consider attending.

Galaxy Community Conference 30 June - 2 July 2013 OSLO

Poster abstracts due 3 May

Use Galaxy

Use Main *(about)*
Use Others! • Learn
Share • Search

Communication

Support • News
Events • Twitter
Mailing Lists (search)

Deploy Galaxy

Get Galaxy • Cloud
Admin • Tool Config
Tool Shed • Search

Contribute

Tool Shed • Share
Issues & Requests
Support

Galaxy Project

Home • About
Community
Big Picture

Wiki

Help • All Pages
Recent Changes
Search • Create Page

Galaxy web search

Galaxy is hiring

galaxyproject.org/GCC2013

Galaxy Community Conference

STARTING @ $125

(GIGA)n SCIENCE

GCC2013 Training Day

galaxyproject.org/GCC2013

Galaxy Community Conference

STARTING @ $125

Poster abstracts due May 3

(GIGA)n SCIENCE

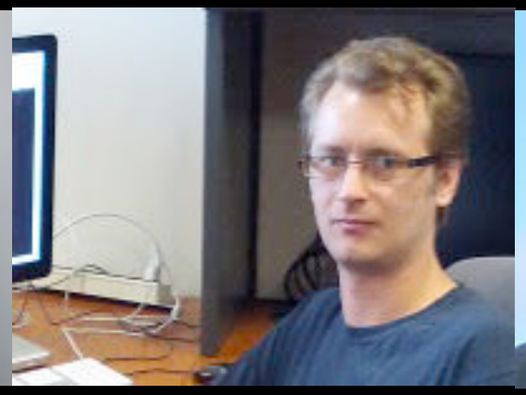
GCC2013 Training Day

The Galaxy Team

Enis Afgan

Dannon Baker

Dan Blankenberg



Dave Bouvier

Dave Clements

Nate Coraor

Carl Eberhard

Dorine Francheteau

Jeremy Goecks

Sam Guerler

Jen Jackson

Greg von Kuster

Ross Lazarus

Anton Nekrutenko

James Taylor

http://wiki.galaxyproject.org/GalaxyTeam

Galaxy is hiring post-docs and software engineers
at both Emory and Penn State.

Please help.

http://wiki.galaxyproject.org/GalaxyIsHiring

Agenda

9:00 Welcome
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11:30 Lunch (on your own)
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1:30 Galaxy Project Overview
2:00 RNA-Seq Example Part III
2:30 Sharing, Publishing and Reproducibility
2:50 Break
3:10 ChIP-Seq Example
5:00 Done

RNA-seq Exercise: A Plan

- Map the reads to the human reference using Tophat
- Run Cufflinks on Tophat output to assemble reads into transcripts
    - *Imagine pages and pages of discussion on the intricacies and pitfalls of RNA-seq transcript prediction here.*

http://bit.ly/11Qvnwh: Lance Parsons' RNA-Seq Tutorial

http://bit.ly/Y7yKzv: UC Davis (Nik Joshi?) RNA-Seq Tutorial

http://bit.ly/YfI0E0: UAB RNA-Seq Tutorial @ GCC2012

RNA-seq Exercise: A Plan

- Map the reads to the human reference using Tophat
- Run Cufflinks on Tophat output to assemble reads into transcripts
- Visualize it

Visualizing Genomics

Supported external browsers

- UCSC
- Ensembl
- GBrowse
- IGB
- IGV

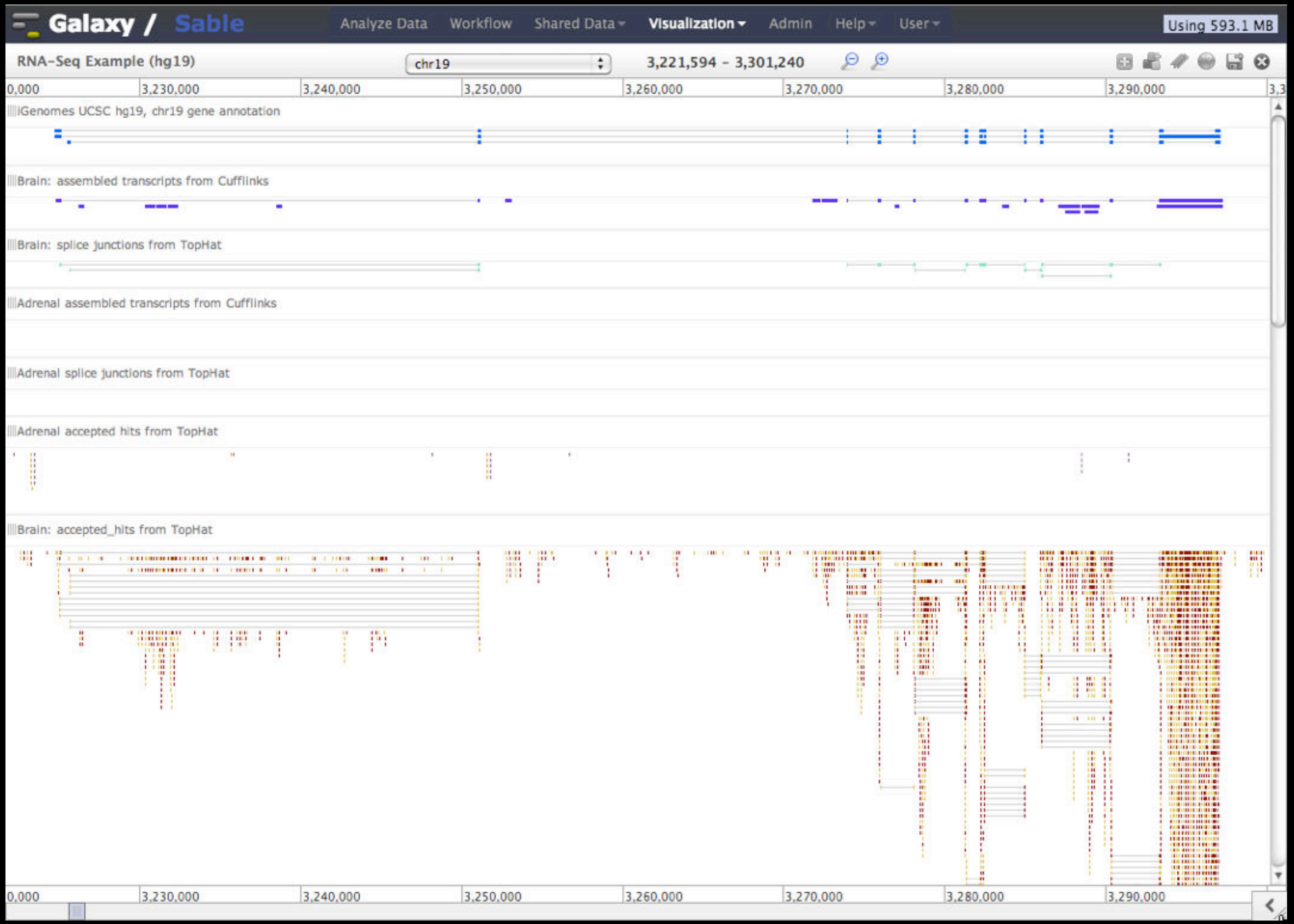
Traditional browser strengths:

- Showing what is nearby
- what else is happening here
- highlighting correlations
- integrating many datasets

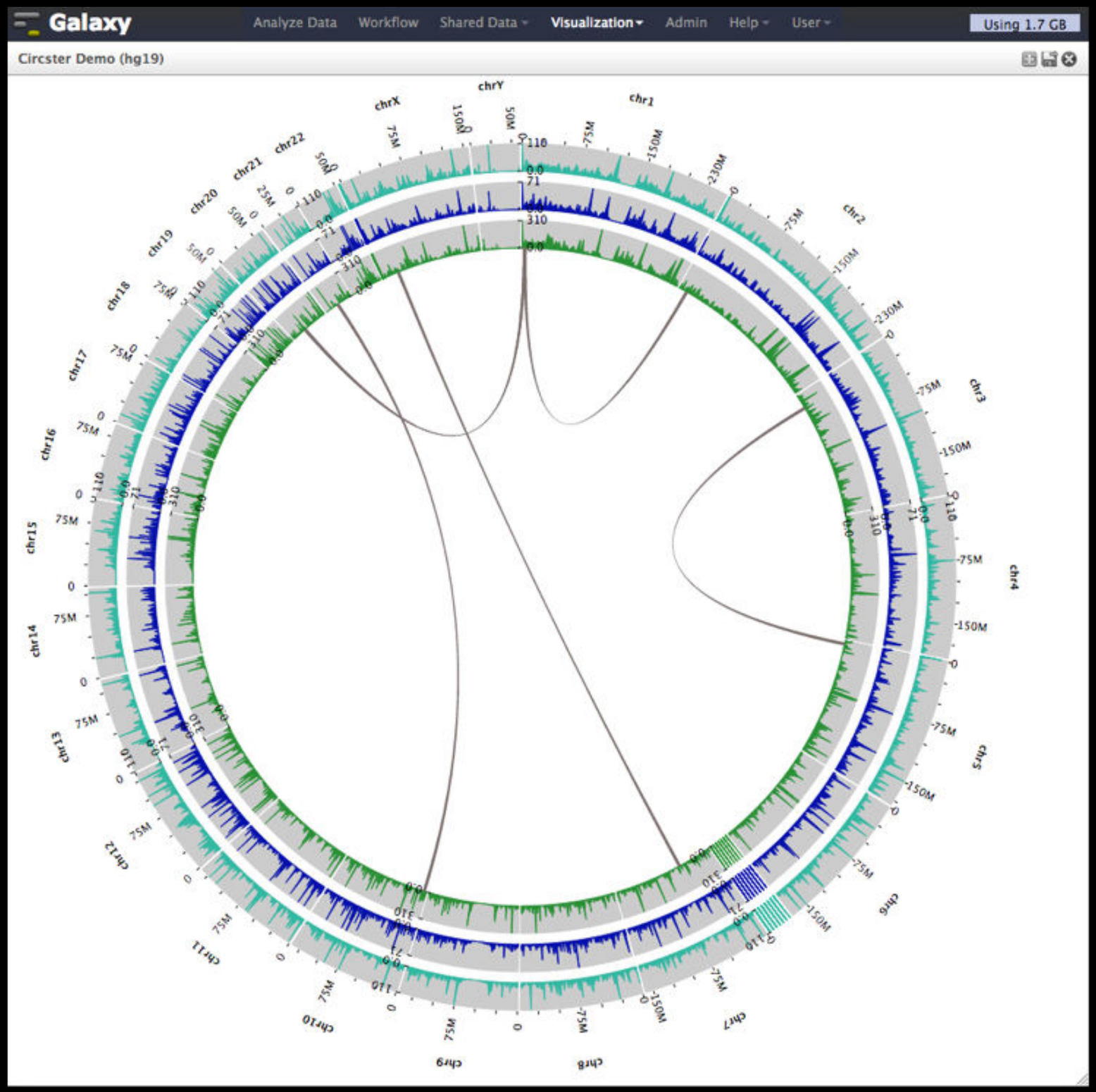
But, *wouldn't it be nice to*

- Use visualization to evaluate and refine analyses?
- Expose some basic analyses in visualization to make it more informative?
- Make that analyze-visualize-refine loop seamless and fast? That is, integrate the two?
- Use visualization to learn tools and explore their parameter space?
- Not be tied to a predefined reference genome?

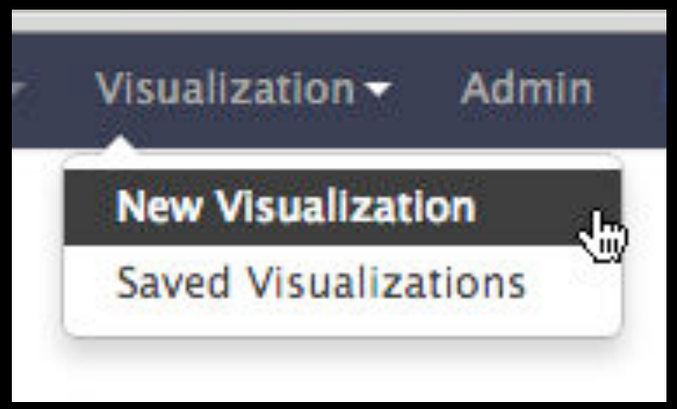
Trackster: Galaxy's embedded track browser



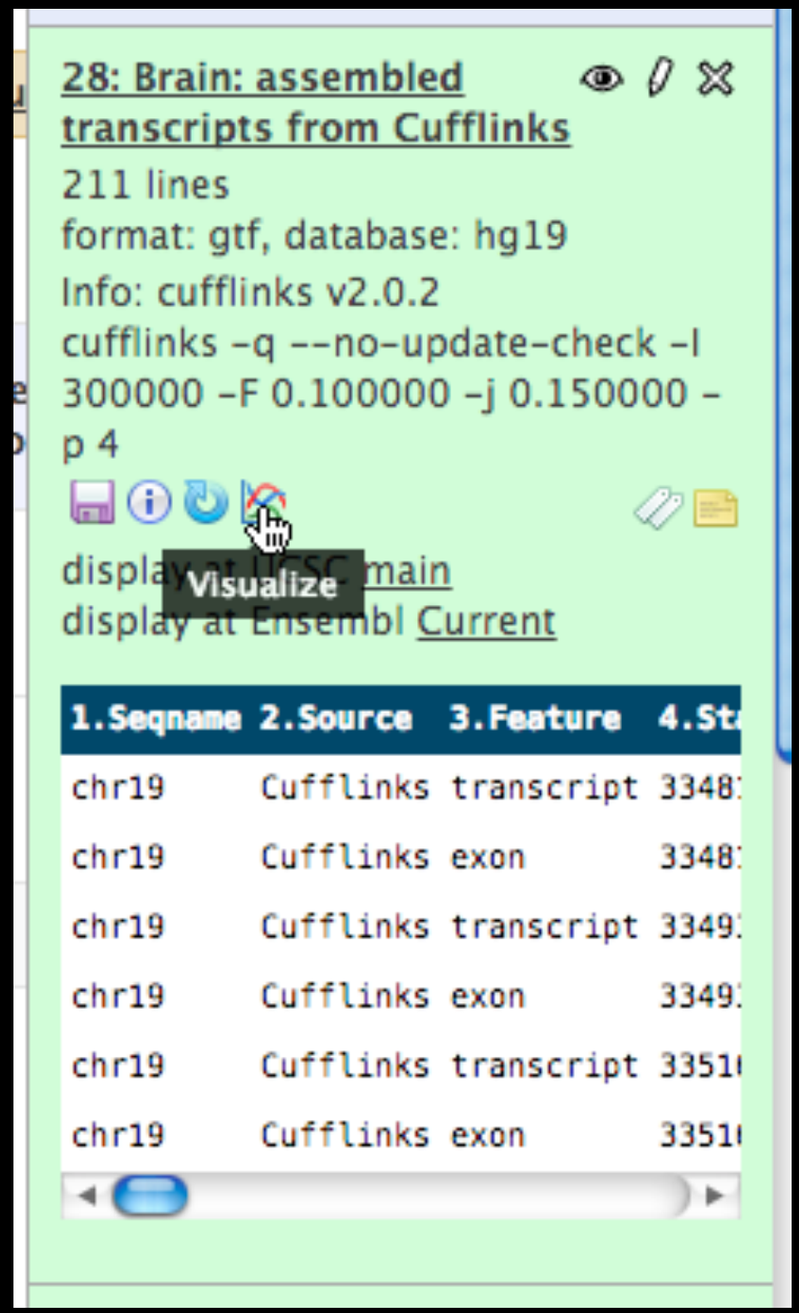
Circster


Galaxy
Analyze Data
Workflow
Shared Data
Visualization
Admin
Help
User
Using 1.7 GB
Circster Demo (hg19)
chrY
chrX
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22

Create a visualization in Galaxy



or



Isn't it nice to

- To do all those things we talked about?
  - Use visualization to evaluate and refine analyses?
  - Expose some basic analyses in visualization to make it more informative?
  - Make that analyze-visualize-refine loop seamless and fast? That is, integrate the two?
  - Use visualization to learn tools and explore their parameter space?
  - Not be tied to a predefined reference genome?

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5:00 Done

More Galaxy Terminology

Share:

Make something available to someone else

Publish:

Make something available to everyone

Galaxy Page:

Analysis documentation within Galaxy; easy to embed any Galaxy object

Sharing & Publishing enables Reproducibility

Reproducibility: Everybody talks about it, but ...

Galaxy aims to push the goal of reproducibility from the bench to the bioinformatics realm

All analysis in Galaxy is recorded without any extra effort from the user.

Histories, workflows, visualizations and ***pages*** can be shared with others or published to the world.

Sharing & Publishing enables Reproducibility

CSH PRESS 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]

OPEN ACCESS ARTICLE

This Article

Published in Advance October 9, 2009, doi: 10.1101/gr.094508.109

- » Abstract *Free*
- Full Text (PDF) *Free*

Current Issue

October 2010, 20 (10)

Sharing & Publishing enables Reproducibility

CSH PRESS 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]

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This Article

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» Abstract *Free*
Full Text (PDF) *Free*

Current Issue
October 2010, 20 (10)

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.]

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

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.

Accessing the Data

Windshield Splatter datasets analyzed in this manuscript can be accessed through this Galaxy Library. From

Author

aun1

Related Pages

All published pages
Published pages by aun1

Rating

Community
(6 ratings, 5.0 average)

Tags

Community:
paper | galaxy | megan

http://usegalaxy.org/u/aun1/p/windshield-splatter

Sharing for Galaxy Administrators Too

Data Libraries

Make data easy to find

Genome Builds

Care about a particular subset of life?

Galaxy Tool Shed

Wrapping tools and datatypes

Agenda

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ChIP-Seq Exercise

- Identify zinc-finger CTCF transcription factor tags in mouse
- Example and data from
  - Hillman-Jackson, *et al.*, "Using Galaxy to Perform Large-Scale Interactive Data Analyses" *Curr. Protoc. Bioinform*. 38:10.5.1-10.5.47;
  - ENCODE transcription factor binding experiment: http://bit.ly/QmD6Nk. Raw original data generated & analyzed at Michael Snyder's lab, Stanford University, and Sherman Weissman's Lab, Yale University.
- We'll use build mm9 and datasets that have been prescreened to mostly map to chr19
- All datasets are FASTQ

ChIP-Seq Exercise: A Plan

- Get input datasets; control and tags
- Groom the datasets into FASTQSanger format
- Look at quality
- Trim as we see fit
- Map the reads to genome using Bowtie
- Call peaks with MACS (Model-based Analysis of ChIP-seq)

ChIP-Seq Exercise: A Plan

- Get input datasets; control and tags

  Shared Data →

  Data Libraries →

  ChIP-Seq basic datasets

ChIP-Seq Exercise: A Plan

- Get input datasets; control and tags
- Groom the datasets into FASTQSanger format
  - NGS: QC and manipulation → FASTQ Groomer
    - Input FASTQ quality scores type: Illumina 1.3-1.7
    - Run on both datasets

ChIP-Seq Exercise: A Plan

- Get input datasets; control and tags
- Groom the datasets into FASTQSanger format
- Look at quality:
  - NGS QC and Manipulation → FastQC
  - Or one of the other two options we covered earlier

ChIP-Seq Exercise: A Plan

- ...
- Look at quality
- Trim as we see fit:
  - Use one or more of the options described earlier.

Read length is only used for building model to predict fragment length. So if you set fragment size by yourself, it really doesn't matter how long each read is. Also, in MACS models, only 5' ends of each read (only talking about single end sequencing here), where ultrasound or enzymes cut DNA, are informative, for both fragment size prediction and peak calling. So you can still try to let MACS predict fragment size by setting a fixed read length. I think the current cross-correlation way in MACS v2 can give a more stable result than the previous way in MACS v1 just measuring distance between plus and minus read pileup summits.

Tao Liu
https://groups.google.com/forum/?fromgroups=#!topic/macs-announcement/A_Rf0eQ_BLU

ChIP-Seq Exercise: A Plan

- Get input datasets; control and tags
- Groom the datasets into FASTQSanger format
- Look at quality
- Trim as we see fit
- Map the reads to genome using Bowtie
  - NGS: Mapping → Bowtie2
    - Library: Single-end
    - Run on both control and tag files
    - Use mm9 as the reference genome

ChIP-Seq Exercise: A Plan

- Get input datasets; control and tags
- Groom the datasets into FASTQSanger format
- Look at quality
- Trim as we see fit
- Map the reads to genome using Bowtie
- Call peaks with MACS (Model-based Analysis of ChIP-seq)

ChIP-Seq Exercise: A Plan

- Call peaks with MACS (Model-based Analysis of ChIP-seq)
  - NGS: Peak Calling → MACS
  - Set ChIP-Seq Tag File and ChIP-Seq Control File
  - Set Effective genome size: 1.87e+9
  - Set Tag size to 36 (still correct?)
  - Set Select the regions with MFOLD: 32
  - Set Parse xls files into distinct interval files
  - Save shifted raw tag count at every bp into a wiggle file
  - Resolution for saving wiggle files: 1 (or 10?)

That's a lot of knobs to set. Get used to it.

Using MACS to Identify Peaks from ChIP-Seq Data

Jianxing Feng,[1] Tao Liu,[2] and Yong Zhang[1]

[1]School of Life Sciences and Technology, Tongji University, Shanghai, China
[2]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute and Harvard School of Public Health, Boston, Massachusetts

ABSTRA

Model-bas
Shirley Li
karyotes, e
sites and
control sa
information on how to use MACS to identify either the binding sites of a transcription factor or the enriched regions of a histone modification with broad peaks. Furthermore, the basic ideas for the MACS algorithm and its appropriate usage are discussed. *Curr. Protoc. Bioinform.* 34:2.14.1-2.14.14. © 2011 by John Wiley & Sons, Inc.

Keywords: MACS • ChIP-Seq • peak-calling • cistrome • epigenome

types of histone modifications, the distribution of reads obeys a continuous property, as the epigenetic status of nearby nucleosomes tends to be similar, usually resulting in quite broad peaks. With proper parameter settings, MACS performs well to detect histone-modification-enriched regions. Similarly, MACS can also be applied in affinity enrichment-based DNA methylation studies, such as MeDIP-Seq data.

Know what you are doing

⚠ There is no such thing (yet) as an automated gearshift in short read mapping. It is all like stick-shift driving in San Francisco. In other words = running this tool with default parameters will probably not give you meaningful results. A way to deal with this is to **understand** the parameters by carefully reading the documentation and experimenting. Fortunately, Galaxy makes experimenting easy.

PROTOCOL

Identifying ChIP-seq enrichment using MACS

Jianxing Feng[1,3], Tao Liu[2,3], Bo Qin[1], Yong Zhang[1] & Xiaole Shirley Liu[2]

[1]Department of Bioinformatics, School of Life Sciences and Technology, Tongji University, Shanghai, China. [2]Department of Biostatistics and Computational Biology, Harvard School of Public Health, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. [3]These authors contributed equally to this work. Correspondence should be addressed to Y.Z. (yzhang@tongji.edu.cn) and X.S.L. (xsliu@jimmy.harvard.edu).

Published online 30 August 2012; doi:10.1038/nprot.2012.101

Method

Model-based Analysis of ChIP-Seq (MACS)

Yong Zhang¤*, Tao Liu¤*, Clifford A Meyer*, Jérôme Eeckhoute†, David S Johnson‡, Bradley E Bernstein§¶, Chad Nusbaum¶, Richard M Myers¥, Myles Brown†, Wei Li# and X Shirley Liu*

Addresses: *Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute and Harvard School of Public Health, 44 Binney Street, Boston, MA 02115, USA. †Division of Molecular and Cellular Oncology, Department of Medical Oncology, Dana-Farber Cancer Institute and Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA. ‡Gene Security Network, Inc., 2686 Middlefield Road, Redwood City, CA 94063, USA. §Molecular Pathology Unit and Center for Cancer Research, Massachusetts General Hospital and Department of Pathology, Harvard Medical School, 13th Street, Charlestown, MA 02129, USA. ¶Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, MA, 02142, USA. ¥Department of Genetics, Stanford University Medical Center, Stanford, CA 94305, USA. #Division of Biostatistics, Dan L Duncan Cancer Center, Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

¤ These authors contributed equally to this work.

Correspondence: Wei Li. Email: wl1@bcm.edu. X Shirley Liu. Email: xsliu@jimmy.harvard.edu

Published: 17 September 2008

Genome ***Biology*** 2008, **9**:R137 (doi:10.1186/gb-2008-9-9-r137)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2008/9/9/R137

Received: 4 August 2008
Revised: 3 September 2008
Accepted: 17 September 2008

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2:50 Break
3:10 ChIP-Seq Example
5:00 Done, almost

Feedback

http://bit.ly/20130430Gxy

Acknowledgements

Maureen Donlin
Kristi Holmes
Bob Engeszer

The Galaxy Team
You!

Edward A. Doisy Department of
Biochemistry and Molecular Biology
Saint Louis University

AWS Education Grant

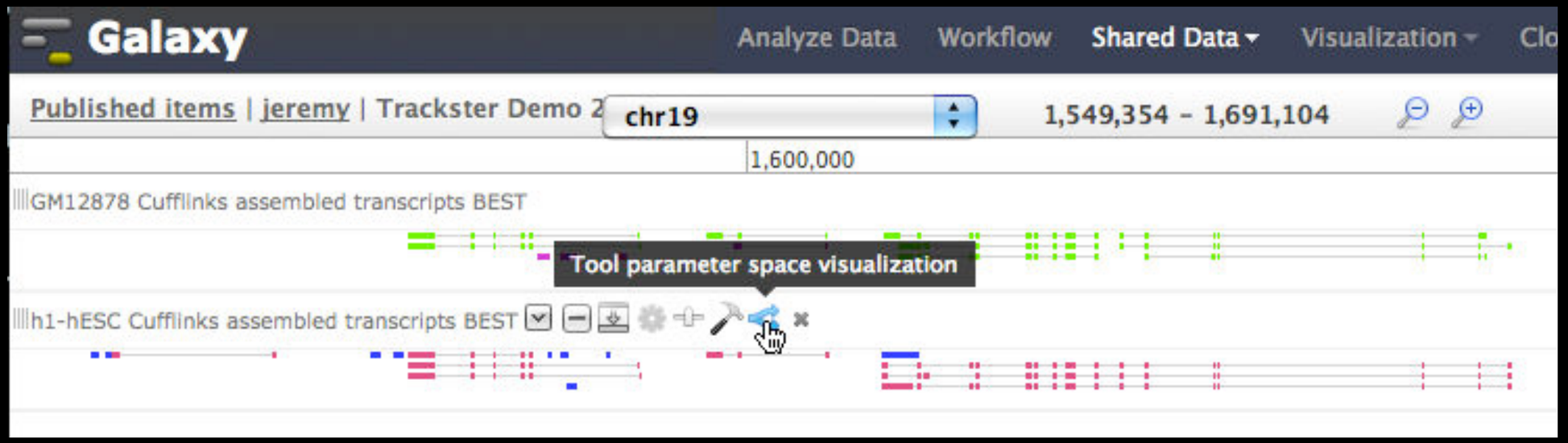
NIH NSF Huck Institute
Penn State University Emory University

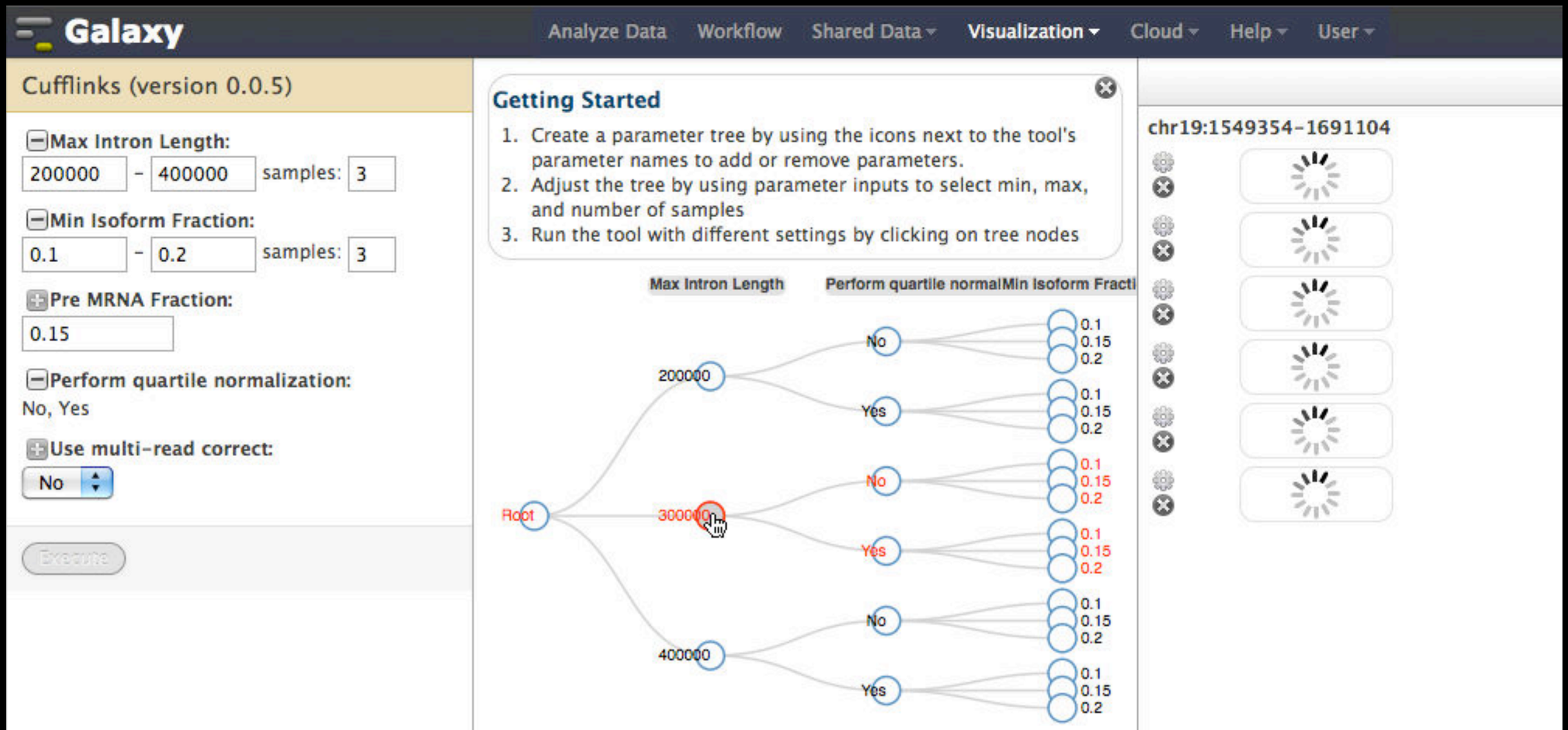
http://bit.ly/20130430Gxy

Thanks

http://bit.ly/20130430Gxy

Exploring Parameter Space with Trackster





Galaxy CloudMan
http://usegalaxy.org/cloud

- Start with a **fully configured and populated** (tools and data) Galaxy instance.
- Allows you to scale up and down your compute assets as needed.
- Someone else manages the data center.
- **We are using this today.**

- **You will set up an instance now**

http://aws.amazon.com/education

Could do this step by step, but ...

http://bit.ly/GXYAWSGetStarted

Galaxy Wiki

Login | Search:

CloudMan/AWS/GettingStarted

Getting Started with Galaxy CloudMan

This page provides a step-by-step instructions on how to start your own instance of Galaxy on Amazon Web Services (AWS) Elastic Compute Cloud (EC2). More general information and instructions about Galaxy CloudMan (GC) can be found here.

AWS
Get Started
Capacity Planning
AMIs
↑ CloudMan

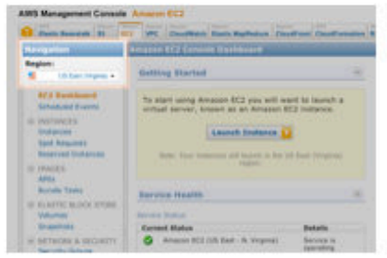
Contents

1. Step 1: One Time Amazon Setup
2. Step 2: Starting a Master Instance
3. Step 3: Galaxy CloudMan Web Interface
4. Step 4: Use Galaxy as you normally would
5. Step 5: Shutting Down

Step 1: One Time Amazon Setup

1. Because AWS services implement pay-as-you-go access model for compute resources, it is necessary for every user of the service to *register with Amazon*. You will need a credit card to register. (You can apply for a AWS Education Grant after you register).
2. Once your account has been approved by Amazon (note that this may take up to one business day), *log into the EC2 AWS Management Console* and set your AWS Region to *US East (Virginia)*. This is the only region Galaxy CloudMan is fully supported in at this time (see screenshot 1.2).
3. Click **Network & Security → Key Pairs** or **My Resources → *n* Key Pairs** (see screenshot 1.3 - if it does not look like this, then try using the Chrome browser) and then click **Create Key Pair**. Enter a memorable name for the key pair, e.g., `GalaxyCloud` and click **Create**.
4. *Save your private key!* The previous step creates the key pair and downloads a copy to your machine with the name `MemorableName.pem`. Save this file and protect it like you would your password. The key pair can be used to access started instances from

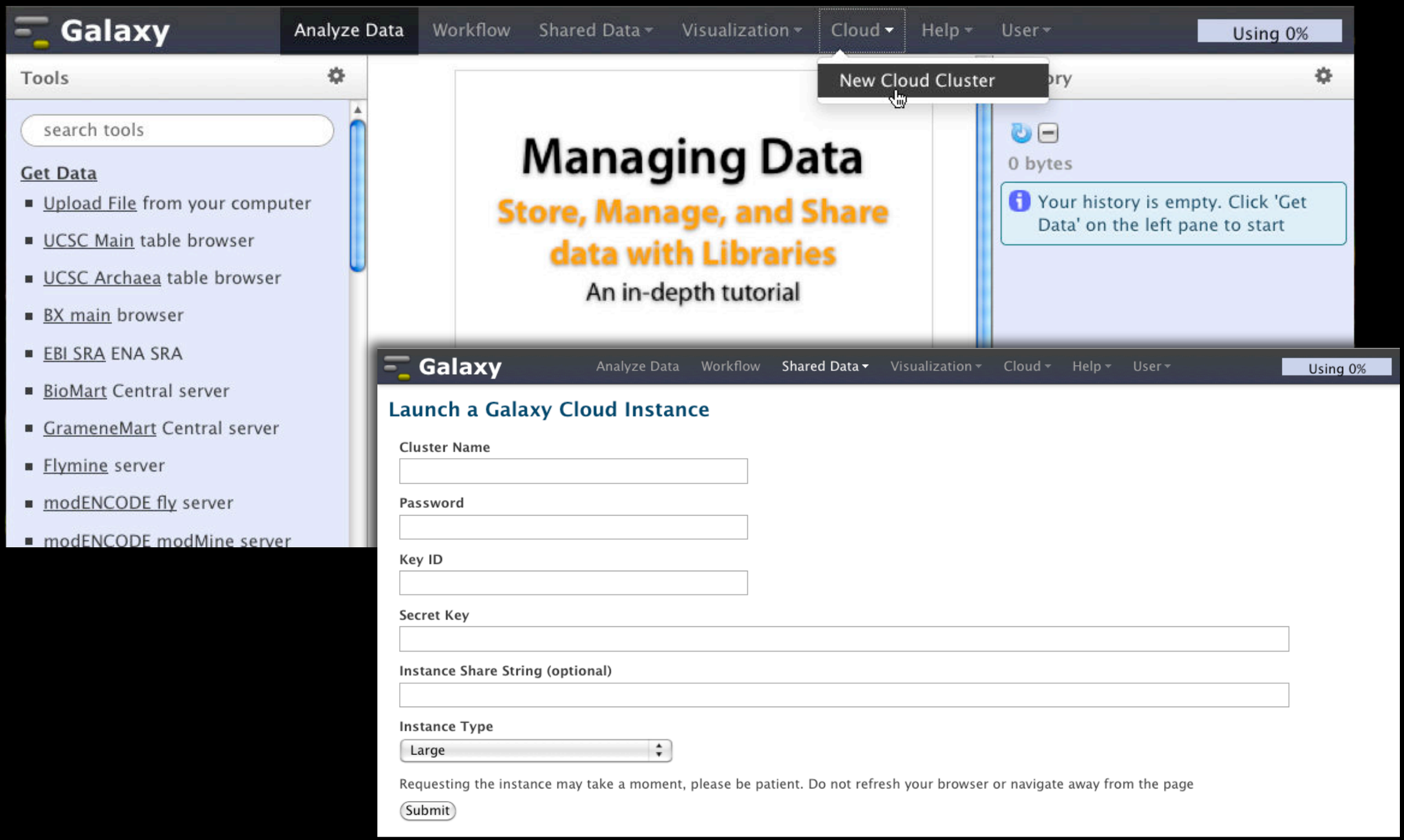
Step 1 Screenshots



1.2. Set region

Instant CloudMan

http://usegalaxy.org/cloudlaunch



Instant CloudMan

AWS Credentials

http://bit.ly/