# Accessible, Transparent and Reproducible Analysis with Galaxy

Application of Next Generation Sequencing Technologies for Whole Transcriptome and Genome Analysis

ABRF 2013 Saturday, March 2, 2013 Palm Springs, California, United States

> Dave Clements Emory University





🚾 Galaxy

## This Workshop

Demonstrate Galaxy with a hands-on walk through of an example RNA-Seq analysis Introduce Galaxy and Galaxy Project as we go.

Complements talk on Monday:

**Galaxy for Core Facilities** 

(W6) Community Resource Solutions to Analyzing Large Genomic Data Sets

Slides are at bit.ly/ABRFgxyWS1 wiki.galaxyproject.org/Events Demonstrate Galaxy with a hands-on walk through of an RNA-Seq analysis

http://usegalaxy.org/u/jeremy/p/galaxy-rna-seq-analysis-exercise

http://bit.ly/gxyrnaseq

http://bit.ly/ABRFgxy1 http://bit.ly/ABRFgxy2 http://bit.ly/ABRFgxy3

- 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

bit.ly/gxyrnaseq bit.ly/ABRFgxy[123]

• Get input datasets; hg19, will mostly map to chr19

- All datasets are FASTQ and from the Body Map 2.0 project
- Shared Data → Data Libraries

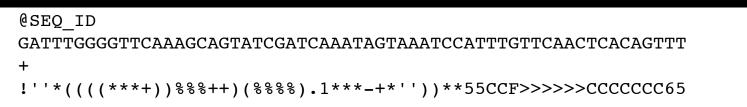




## What is **FASTQ**?

• Specifies sequence (FASTA) and quality scores (PHRED)

#### • Text format, 4 lines per entry



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

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I - Illumina 1.3 X - Solexa	•		,	(0 to 40 expected in raw reads) (-5 to 40 expected in raw reads)	

#### http://en.wikipedia.org/wiki/FASTQ\_format

- Get input datasets; hg19, will mostly map to chr19
- Look at quality: Option 1
  - NGS QC and Manipulation → Compute Quality
     Statistics
  - NGS QC and Manipulation → Draw quality score boxplot
  - Gives you no control over how it is calculated or presented.

http://bit.ly/gxyrnaseq

- Get input datasets; hg19, will mostly map to chr19
- Look at quality: Option 2
  - NGS QC and Manipulation → FastQ Summary Statistics
  - Graph / Display Data → Boxplot of quality statistics
  - Gives you a lot of control over what the box plot looks like, but no additional information

http://bit.ly/gxyrnaseq

- Get input datasets; hg19, will mostly map to chr19
- Look at quality: Option 3
  - NGS QC and Manipulation → Fastqc
  - Gives you a lot more information but little control over how it is calculated or presented.

- Get input datasets; hg19, will mostly map to chr19
- Look at quality
- 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

- Get input datasets; hg19, will mostly map to chr19
- 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/gxyrnaseq

- Get input datasets; hg19, will mostly map to chr19
- 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

http://bit.ly/gxyrnaseq

- 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.

## 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

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	clustalomega						
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## Encourage Public Galaxy Instances http://wiki.galaxyproject.org/PublicGalaxyServers

## 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

## 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?

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









## 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

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

## **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

#### 💳 Galaxy Web Search

Google<sup>™</sup> Custom Search

Search the entire set of Galaxy web sites and mailing lists using Google.

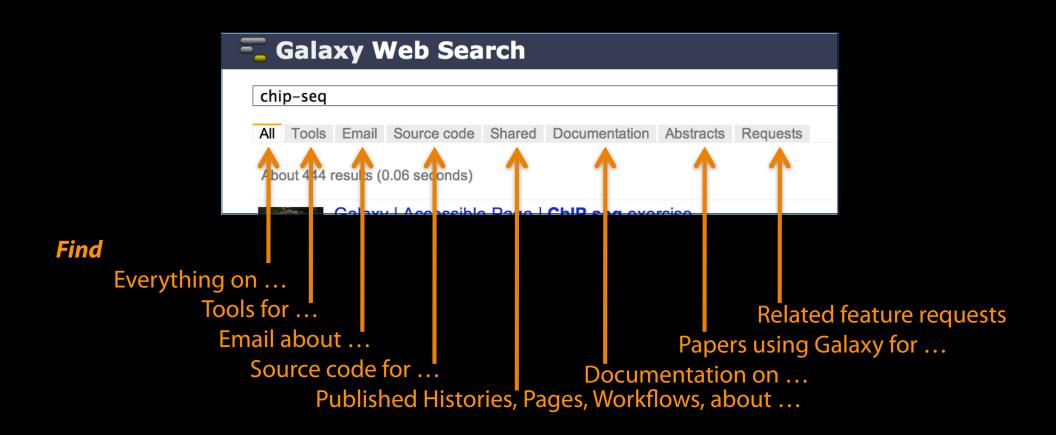
Search

×

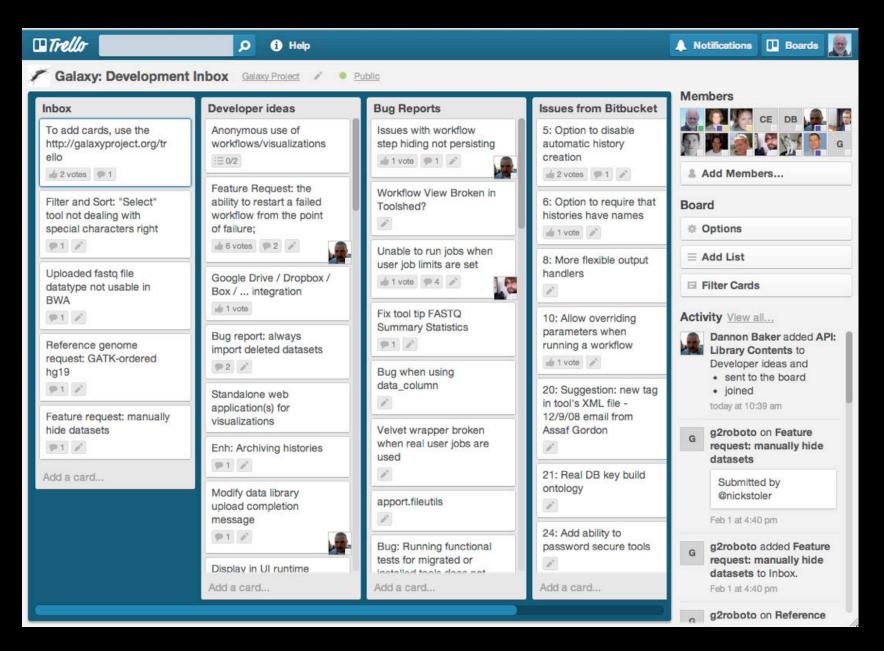
Run this search at Google.com (useful for bookmarking)

Want a different search?

Project home



### Community can create, vote and comment on issues



## http://bit.ly/gxytrello

## http://wiki.galaxyproject.org

💳 Galaxy Wiki	DaveClements Settings Logout   Search:	Titles Text
FrontPage		Edit History Actions
Ga	alaxy	GCC2013 Training Day Topic voting now open!
<ul> <li>Galaxy is an open, web-based platform for accessible, reproducible, and</li> <li>Accessible: Users without programming experience can easily spe</li> <li>Reproducible: Galaxy captures information so that any user can r</li> <li>Transparent: Users share and publish analyses via the web and ca analysis.</li> <li>This is the Galaxy Community Wiki. It describes all things Galaxy.</li> </ul>	cify parameters and run tools and workflows.	Froject Server (Use it!) Other Servers • Learn Share • Search
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.	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	Communication Support • News Events • Twitter Mailing Lists (search) Deploy Galaxy Get Galaxy • Cloud Admin • Tool Config Tool Shed • Search
Community & Project Galaxy has a large and active user community and many ways to Get Involved. • Community • News • Events • Support	<ul> <li>Contribute</li> <li>Users: Share your histories, workflows, visualizations, data libraries, and Galaxy Pages, enabling others to use and learn from them.</li> <li>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.</li> <li>Everyone: Get Involved!</li> </ul>	Contribute Tool Shed • Share Issues & Requests Support Galaxy Project Home • About Community

**Big Picture** 

Galaxy Project

#### **Events**

#### News

#### 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 5.

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 .

#### Upcoming Events

Topic/Event

Date



	<u> </u>
	Venue/Location
Galaxy Boot Camp	UC Davis Bioinformatics Core Davis, California, United States
sparent and Reproducible Analysis With Galaxy,	
ation of NGS Platforms for Whole Transcriptome e Analysis Facilities, part of unity Resource Solutions to Analyzing Large	ABRF 2013 Palm Springs, California, United States

#### 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

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

#### News Items

February 2013 Galaxy Update GCC2013 Training Day Topics: Vote! Galaxy Project Openings Jan 11, 2013 Distribution & News Brief January 2013 GalaxyAdmins January 2013 Galaxy Update Dec 20, 2012 Distribution & News Brief Galaxy Internships @ EMBL Nominate GCC2013 Training Topics Dec 3, 2012 Distribution & News Brief December 2012 Galaxy Update Nov 14, 2012 Distribution & News Brief NGS Analysis by Viz. with Trackster November 2012 GalaxyAdmins

12000			<ul> <li>About the Galaxy Project</li> </ul>	
February 4	Introduction to Galaxy Boot Camp	UC Davis Bioinformatics Core Davis, California, United States		News Archive
March 2-	Accessible, Transparent and Reproducible Analysis With Galaxy, part of SW1: Application of NGS Platforms for Whole Transcriptome	ABRF 2013	News Items	
5	and Genome Analysis Galaxy for Core Facilities, part of "W6: Community Resource Solutions to Analyzing Large Genomic Data Sets"	Palm Springs, California, United States	February 2013 Galaxy Update The February 2013 Galaxy Update is now available.	
March 26-28	RNA Technologies and Analysis Workshop	DOE JGI User Meeting	Highlights:	Galaxy <i>UPDATE</i>
April 5-6	2013 GMOD Meeting	Cambridge, United Kingdom, immediately prior to Biocuration 2013	Three new public Galaxy servers     New papers     Open Positions at five different institutions	
April 7- 10	GO Galaxy Workshop	Biocuration 2013, Cambridge, United Kingdom	GCC2013 Training Day Topic voting, Registration, and Sponsorships     January GalaxyAdmins Web Meetup slides and screencast	
April 9- 11	Workshop: Integrated Research Data Management for Next Gen Sequencing Analysis Using Galaxy and Globus Online Software- as-a-Service	BioIT World, Boston, Massachusetts, United States	Other Upcoming Events and Deadlines     Galaxy Distributions     Tool Shed Contributions	
	Talk: Integrated Research Data management and Analysis in NGS using Globus Online, Galaxy and Amazon Web Services	- United States	Other News     If you have anything you would like to see in the March <i>Galaxy Update</i> , please let us know.	
1.	Tutorial: Exploring and Enabling Biomedical Data Analysis with Galaxy	Great Lakes Bioinformatics Conference (GLBIO) 2013, Pittsburgh, Pennsylvania, United States	Dave Clements and the Galaxy Team Posted to the Galaxy News on 2013-02-01	
May 21	Initiation à l'utilisation de Galaxy			
May 29	Les deux ateliers sont maintenant complets		GCC2013 Training Day Topics: Vote!	
1.	Analyse de données issues de séquenceurs nouvelle génération sous Galaxy	Cycle "Bioinformatique par la pratique" 2013, INRA Jouy-en-Josas, France	A list of possible topics for the GCC2013 Training Day is now available. Please take a few minutes t	to review these GCC2013
May 30	Les deux ateliers sont maintenant complets		possibilities and then vote for your favorite three topics.*	Training
June 6-7	Informatics on High Throughput Sequencing Data Workshop	Toronto, Ontario, Cananda	Your votes will determine not only the topics that are offered, but also which topics should be offered, ssigned to which rooms, and which ones should not be scheduled at the same time. Your	red more than



Registration & abstract submission are now open http://galaxyproject.org/GCC2013





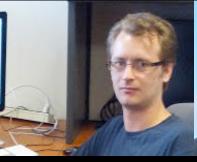
Enis Afgan



Dannon Baker



Dan Blankenberg



**Dave Bouvier** 



Dave Clements



Nate Coraor



Carl Eberhard



Jeremy Goecks



Sam Guerler



Jen Jackson



Greg von Kuster



Ross Lazarus



Anton Nekrutenko

James Taylor



You

## The Galaxy Team

http://wiki.galaxyproject.org/GalaxyTeam

• ...

- Trim as we see fit.
- 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/gxyrnaseq

• ...

- 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.

• Visualize it

http://bit.ly/gxyrnaseq

## **Visualizing Genomics**

## Supported external browsers

- UCSC
- Ensembl
- GBrowse
- IGB
- IGV

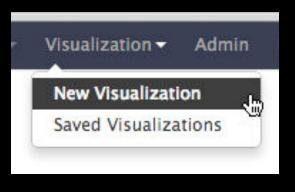
## Traditional browser strengths:

- 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?

## **Create a visualization in Galaxy**



or

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## 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?

## Acknowledgements

Nalini Raghavachari David Needleman Jim Vincent The Galaxy Team *especially* Dannon Baker

ABRF

**AWS Education Grant** 

NIH NSF Huck Institute Penn State University Emory University

## Thanks



### **Dave Clements**

## Galaxy Project Emory University

clements@galaxyproject.org

## Trackster: Galaxy's embedded track browser

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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

## 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

Let's all share...

## **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

## Sharing & Publishing enables Reproducibility

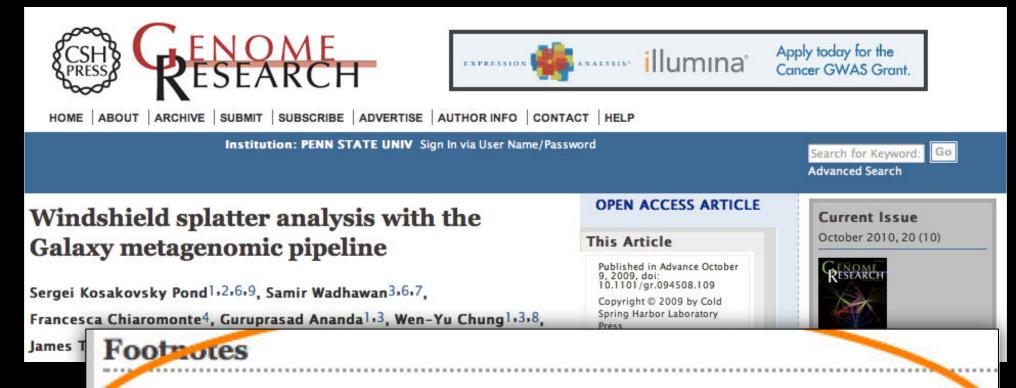
Reproducibility: Everybody talks about it, but ...

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



[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.]

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