NGS Data Analysis With GenomeSpace & Galaxy, Part 2

FGED 15th International Conference

Seattle, Washington June 22, 2013

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









Agenda

- 9:00 Welcome
- 9:15 GenomeSpace
- 10:15 Galaxy: First Steps and Data Quality
- 11:00 Break
- 11:30 RNA-Seq Analysis and Galaxy Project
- 12:30 Done

Link to slides on http://bit.ly/gxyEvents

Goals

- 1. Introduce Galaxy
- 2. Introduce bioinformatics concepts and formats
- 3. Hands-on experience

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.



What we actually cover depends on participants' interests.

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: First Impressions

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

RNA-seq Exercise

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

Shared Data → Published Pages → RNA-Seq Analysis Exercise

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

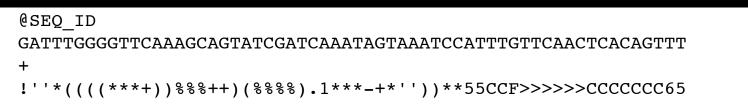
• Get input datasets; will mostly map to chr19

- 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



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

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				NOPQRSTUVWXYZ[\]^_`abcdefghijklmno 	
33	59	64	73	104	126
-	Phred+64, 62	2 values	(0, 62)	(0 to 60 expected in raw reads) (0 to 40 expected in raw reads) (-5 to 40 expected in raw reads)	

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

Look at quality Options 1 & 2:

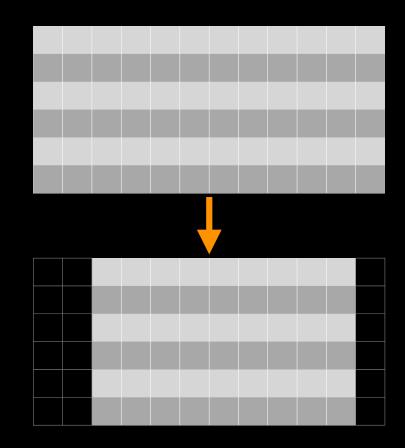
1. NGS QC and Manipulation \rightarrow Compute Quality Statistics NGS QC and Manipulation \rightarrow Draw quality score boxplot No control over how it is calculated or presented. 2. NGS QC and Manipulation \rightarrow FastQ Summary Statistics, Graph / Display Data \rightarrow Boxplot of quality statistics Lots of control over what the box plot looks like, Stats in text and graphic formats

• Get input datasets; will mostly map to chr19

- 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.

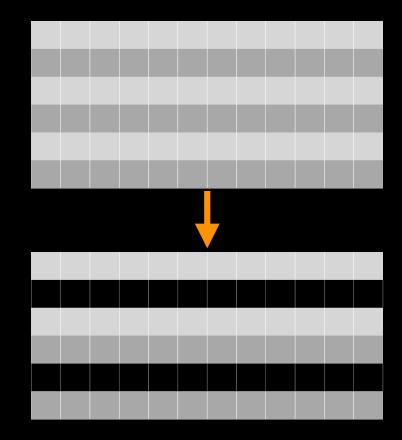
• Look at quality

- 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

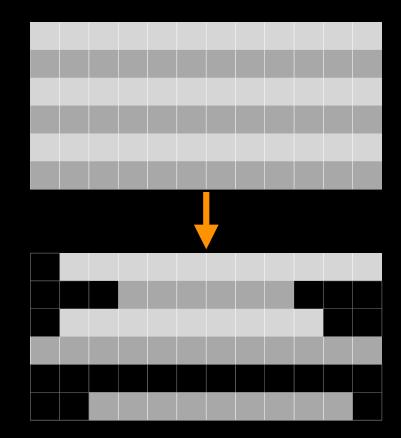


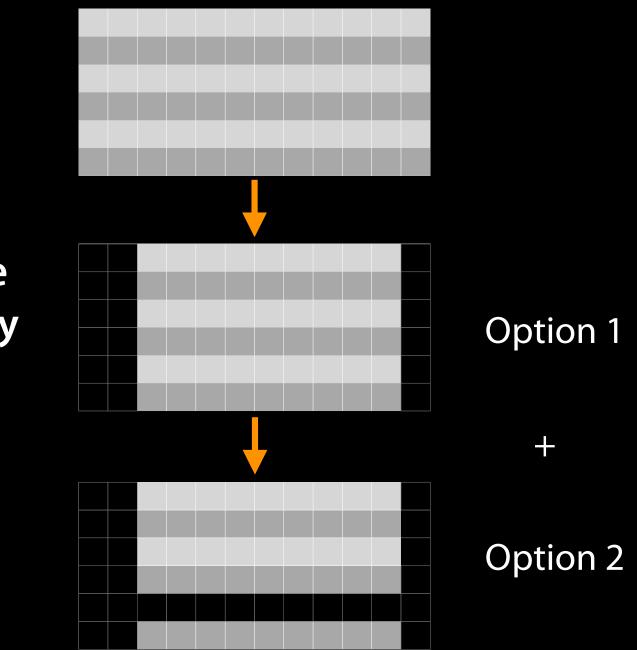
• Look at quality

- 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
 - Can have different thresholds for different regions of the reads.
 - Keeps original read length.



- 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





Options are not mutually exclusive

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

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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GMOD.org: purveyors of fine genomics software



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 - Imagine pages and pages of discussion on the intricacies and pitfalls of RNA-seq mapping here.

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• ...

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• Visualize it

Visualizing Genomics

Supported external browsers

- UCSC
- Ensembl
- GBrowse
- IGB
- IGV

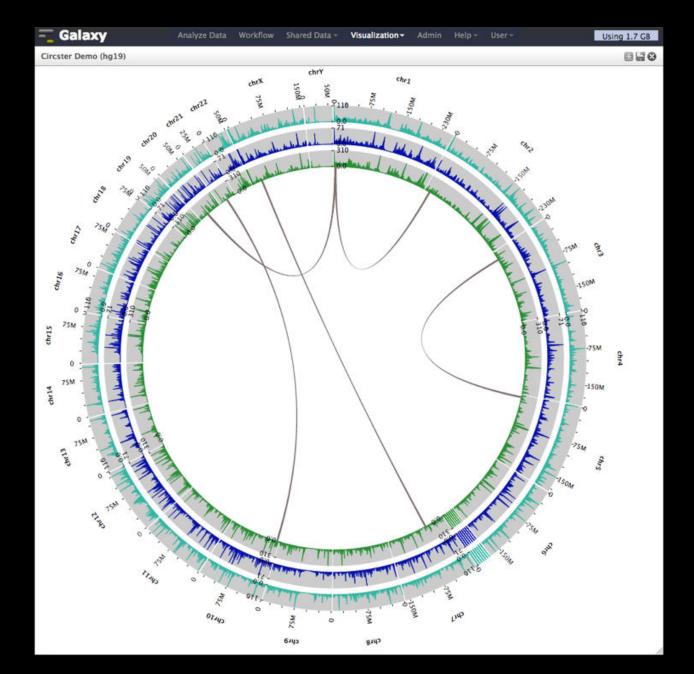
Traditional browser strengths:

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

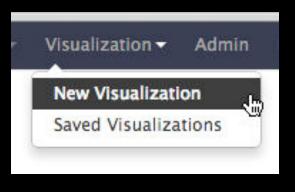
Trackster: Galaxy's embedded track browser

Analyze Data Workflow Shared Data → Visualization → Admin Help → User → chr19 \$3,221,594 - 3,301,240 \$9	\$ 3,221,594 - 3,301,240 ₽ ₽	221,594 - 3,301,240 🔎 🏓	Ð		Using 593.	0
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assembled transcripts from Cufflinks						
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drenal accepted hits from TopHat		e.			1	-
rain: accepted_hits from TopHat						
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Circster



Create a visualization in Galaxy



or

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Vizualization inside Galaxy

- Levarges visualization to evaluate and refine analyses
- Exposes basic analyses in visualization to make it more informative
- Makes the analyze-visualize-refine loop seamless and fast
- Enables learning tools and exploring their parameter space
- Supports custom genome browsers, without a predefined reference genome

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- 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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positories	Repository revision							
irowse by category irowse all repositories ogin to create a repository	2:bb1847435ec1=: repository tip Select a revision to inspect and download versions of tools from this repository.							
	clustalomega							
	Clone this repository: hg clone <u>http://toolshed.g2.bx.psu.edu/repos/clustalomega/clustalomega</u> Name:							
	clustalomega Synopsis: multiple sequence alignment program for proteins							
•	Detailed description: Clustal Omega is a genera	l purpose mul	tiple seq	uence alignment program for proteins.	It produces h	igh quality alignme	ents a	
	Revision: 2:bb1847435ec1							
	Owner: clustalomega							
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	1	Calculate	5			Ĩ				

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

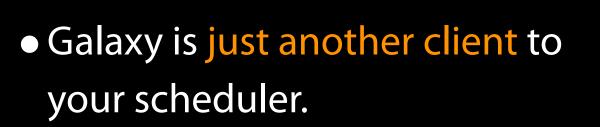
As Open Source Software: Local Galaxy Instances

- Galaxy is designed for local installation and customization
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 - 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).







GRID ENGINE



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

Sharing and Publishing

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





Apply today for the Cancer GWAS Grant.

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Windshield splatter analysis with the Galaxy metagenomic pipeline

Sergei Kosakovsky Pond^{1,2,6,9}, Samir Wadhawan^{3,6,7},

Francesca Chiaromonte⁴, Guruprasad Ananda^{1,3}, Wen-Yu Chung^{1,3,8},

James Taylor^{1,5,9}, Anton Nekrutenko^{1,3,9} and The Galaxy Team¹

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This Article

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

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October 2010, 20 (10)



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Abstract 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.]

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Using

Published Pages | aun1 | Windshield Splatter

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

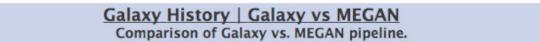
SERGEI KOSAKOVSKY POND^{1,2,*}, SAMIR WADHAWAN^{3,6*}, FRANCESCA CHIAROMONTE⁴, GURUPRASAD ANANDA^{1,3}, WEN-YU CHUNG^{1,3,7}, JAMES TAYLOR^{1,5}, ANTON NEKRUTENKO^{1,3} and THE GALAXY TEAM^{1*}

Correspondence should addressed to SKP, JT, or AN.

How to use this document

This document is a live copy of supplementary materials for <u>the manuscript</u>. 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 <u>create a Galaxy account</u> (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:



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**):

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Galaxy History | metagenomic analysis



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

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

About this Page





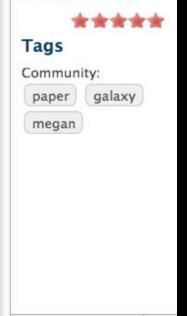
aun1

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Rating

Community (6 ratings, 5.0 average)



>

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

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 in 2012, 2100+ members) Galaxy-User

Questions about using Galaxy and usegalaxy.org High volume (2900 posts in 2012, 2700+ members) Galaxy-Dev

Questions about developing for and deploying Galaxy High volume (4500 posts in 2012, 900+ members)

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

💳 Galaxy Web Search

Google[™] Custom Search

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

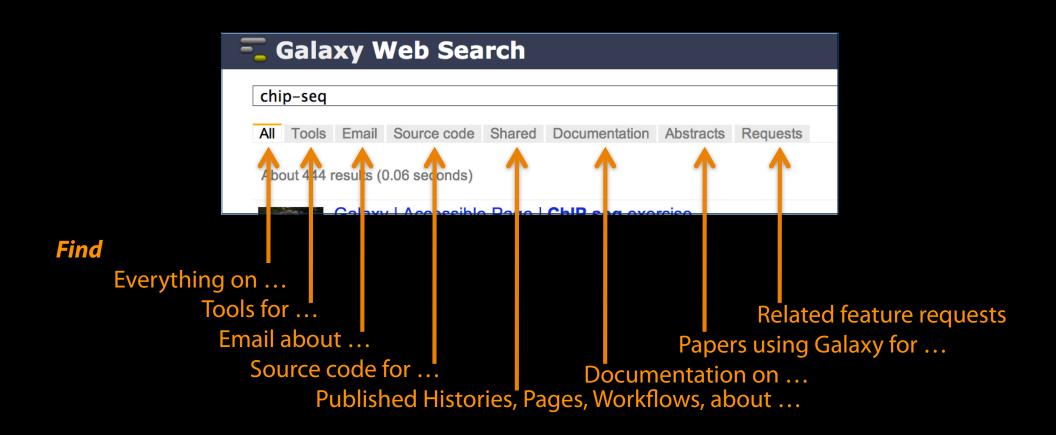
Search

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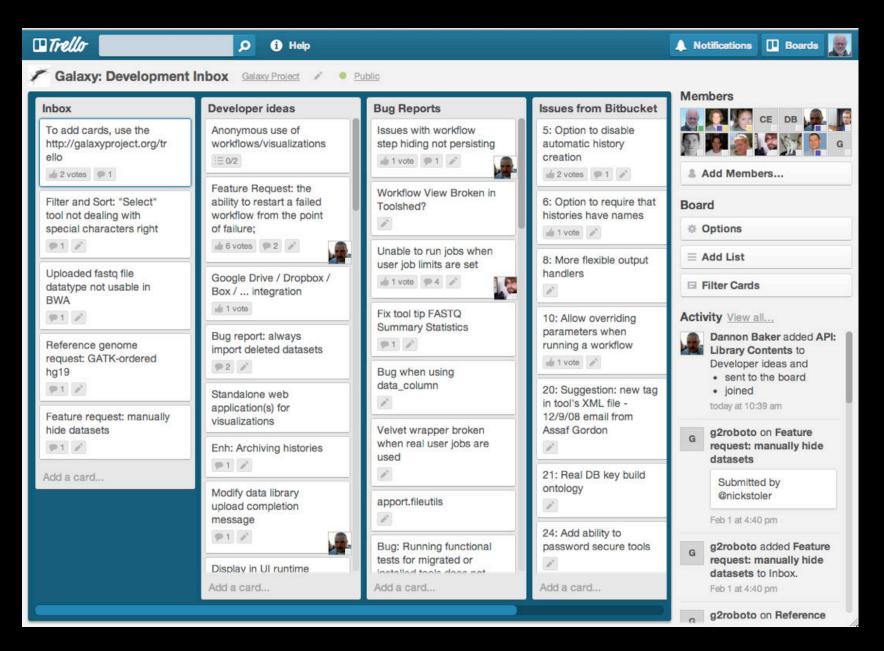
Run this search at Google.com (useful for bookmarking)

Want a different search?

Project home



Community can create, vote and comment on issues



http://bit.ly/gxyissues

http://wiki.galaxyproject.org

🗧 Galaxy Wiki	Login Search:	Titles Text
FrontPage		Locked History Actions
Galaxy is an open, web-based platform for accessible, reproducible, a • Accessible: Users without programming experience can easily spe • Reproducible: Galaxy captures information so that any user can r	Galaxy Community Conference 22013 2013 0SLO Poster abstracts due 3 May Use Galaxy	
 Transparent: Users share and publish analyses via the web and c analysis. 	Use Main (about) Use Others! • Learn Share • Search	
This is the Galaxy Community Wiki. It describes all things Galaxy.		Communication
Use Galaxy Galaxy's public service web site makes analysis tools, genomic data,	Deploy Galaxy Galaxy is open source for all organizations. Local Galaxy servers can be	Support • News Events • Twitter Mailing Lists (search)
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	set up by downloading and customizing the Galaxy application. • Admin	Deploy Galaxy
wiki and elsewhere.	• Cloud	Get Galaxy • Cloud Admin • Tool Config Tool Shed • Search
usegulaxylorg		Contribute
Community & Project	Contribute	Issues & Requests Support
Galaxy has a large and active user community and many ways to Get Involved. • Community	 Users: Share your histories, workflows, visualizations, data libraries, and Galaxy Pages, enabling others to use and learn from them. 	Galaxy Project
News Events Support	 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. 	Home • About Community Big Picture

Galaxy Project

• Everyone: Get Involved!

Wiki

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Events

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Events				Locked History	News		Locked History Actions
Events with Als de av If you know Event Cale Upcomin	adlines that are relevant to ailable as an RSS feed .	oogle Calendar for a listing of the Galaxy Community. This be added to this page and/or send it to ⊠outreach@glaxy	is also 2. Other Calendars 3. Past Events 1. 2013 2. Archive	Gelaxy Conference OSLOO Poster abst due 3 Ma Use Galaxy Use Main (abou Use Others! • L Share • Search Communic: Support • News Events • Twitte Mailing Lists (st	The Galaxy News is also available as an RSS feed Cu. 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	News Items Environmental Metabolomics + Galaxy Galaxy @ ASMS 2013 April 8, 2013 Galaxy Security Release GCC2013 & Galaxy Security Release GCC2013 & Galaxy Security Release April 2013 Galaxy Update April 1, 2013 Galaxy Update April 1, 2013 Galaxy Update Main & Test ServerDowntime: 3/14 March 2013 Galaxy Update GCC2013 Abstract Submission & Registration News Archive	Communication Support - News Events - Twitter Mailing Lists (search) Deploy Galaxy
Date 1	Fopic/Event	Venue/Location	Contact	Deploy Gal	Conclusion of Collins and Collins		Get Galaxy • Cloud
	Introduction to Galaxy Workshops 2013 Galaxy Workshop Tour	Washington University in St. Louis Saint Louis University University of Missouri Columbia	Dave Clements	Get Galaxy • C Admin • Tool C Tool Shed • Se Contribute	News Items Environmental Metabolomics + Galaxy		Admin • Tool Config Tool Shed • Search Contribute Tool Shed • Share Issues & Requests
May 14-	Futorial: Exploring and Enabling Biomedical Data Analysis with Galaxy	Great Lakes Bioinformatics Conference (GLBIO) 2013, Pittsburgh, Pennsylvania, United States	Anton Nekrutenko	Tool Shed • Shi Issues & Reque Support Galaxy Proj	of Birmingham, BGI and GigaScience has received funding fro Environment Research Council (NERC). The first metabolomics project will send a de	eceived funding from the UK's Natural oject will send a developer from the University of Birmingham's Hong Kong to work with <i>GigaScience</i> personnel on extending Galaxy	
May 15	GalaxyAdmins May 2013 Meetup	GalaxyAdmins web meetup	Srinivas Maddhi, Dave Clements	Home • About			Big Picture
May 16- a 17	Galaxy Workflows for Bioinformatics Analysis, and Norkshop 1A – Galaxy	Workshop in Next- Generation Sequence Analysis and Metabolomics (WiNGS), UNC-Charlotte, North Carolina, United States	James Taylor	Community Big Picture Wiki Help • All Page Recent Changes	molecules (metabolites) in living organisms and can provide a cellular condition and health. The toxicological responses of o be studied using environmental metabolomics, enabling resea diagnostic markers for monitoring and risk assessment of our extensively on the metabolic responses of the freshwater mon	an indication of their rganisms to pollutants can irchers to discover environment. Research at Birmingham focuses	Recent Changes S Search • Create Page
May 21 d May 29	Initiation à l'utilisation de Galaxy Les deux ateliers sont maintenant complets		Sandra Dèrozier, Valentin Loux, Véronique Martin <veronique.martin at="" dot="" fr="" inra="" jouy=""></veronique.martin>	Search • Create	Peter Li	on 2013-04-22	web search
May 22	Analyse de données ssues de séquenceurs nouvelle génération sous Galaxy Les deux ateliers sont maintenant complets	Cycle "Bioinformatique par la pratique" 2013, INRA Jouy-en-Josas, France	Jean-François Gibrat, Valentin Loux, Véronique Martin <veronique.martin at="" dot="" fr="" inra="" jouy=""></veronique.martin>	equidance and a second se	Galaxy @ ASMS 2013 Galaxy will have a significant presence at the 61st ASMS Conf Spectrometry and Allied Topics being held in Minneapolis, Min Galaxy related content includes the Galaxy Framework as a S based Informatics workshop and at least 9 posters either dire Galaxy.	Inesota, June 9-13.	, and a second
May 24 June 19	Introduction to Galaxy	UC Davis Bioinformatics Core Davis, California, United States	Nikhil Joshi <najoshi at="" dot="" edu="" ucdavis=""></najoshi>		Galaxy. If you do research in proteomics than please consider attendi Dave Clements		
	A Genomics Virtual Lab for Cancer Research		Dominique Gorse		Posted to the Galaxy News of April 8, 2013 Galaxy Security Release	on 2013-04-19	

News









Late registration ends Wednesday





The Galaxy Team



Enis Afgan



Dannon Baker



Dan Blankenberg



Dave Bouvier



Dave Clements



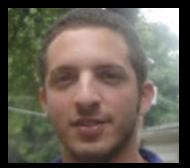
Nate Coraor



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/GalaxylsHiring

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Feedback, please: http://bit.ly/fged13w1

Acknowledgements

Michael Reich Jennifer Weller Gabriella Rustici Roger Bumgarner Marco Ocana Dannon Baker & The Galaxy Team Stephanie Johnson

The Functional Genomics Data Society

AWS Education Grant

NIH NSF Huck Institute Penn State University Emory University

http://bit.ly/fged13w1

Thanks http://bit.ly/fged13w1



Dave Clements

Galaxy Project Emory University

clements@galaxyproject.org