NGS Data Analysis and Galaxy

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This Week

Monday Welcome, Project Intro, Basic Galaxy Usage NGS QualityControl

- TuesdayRNA-Seq Mapping and Transcript PredictionRNA-Seq: Differential expression andAlternative Pipelines; SNP & Variant Analysis
- Wednesday SNP & Variant Analysis Chip-Seq Analysis
 - Thursday Genome Assembly Install your own Galaxy on Amazon Cloud
 - Friday Customizing Galaxy, Galaxy Tool Shed, and Wrapping Tools for Galaxy

Monday Agenda

- 9:00 Welcome and Intro
- 9:40 Basic Analysis with Galaxy
- 11:00 Break + an Excerise
- 12:00 Basic Analysis into Reusable Workflows
- 13:00 Lunch
- 14:00 NGS Data Quality Control I
- 15:30 Break
- 16:00 NGS Data Quality Control II
- 16:30 Galaxy Community
- 17:00 Done

Goals

- 1. Introduce Galaxy
- 2. Introduce bioinformatics concepts and formats
- 3. Hands-on experience
 - Load and integrate data
 - Perform bioinformatic analysis
 - Evaluate different analysis options
 - Save, repeat, share describe and publish analyses
 - Visualize your results
 - Set up a 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.

Introductions

In 50 seconds or less tell us

- your name
- your affiliation(s)
- something about your research
- something about your goals for today

What is Galaxy?

• A free (for everyone) web service

• Open source software

• These options result in several ways to use Galaxy

http://galaxyproject.org

Galaxy is available ...

As a free (for everyone) web service integrating a wealth of tools, compute resources, terabytes of reference data and permanent storage 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
 - 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.





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://wiki.galaxyproject.org/Cloud We are using this right now, and you will set up your own Galaxy on AWS on Thursday

http://aws.amazon.com/education

Galaxy is available ...

- As a free (for everyone) web service
- As open source software
- On the Cloud
- With Commercial Support



A ready-to-use appliance (BioTeam) Cloud-based solutions (Appistry, ABgenomica, AIS) Consulting & Customization (Arctix, Deena Bioinformatics)

Galaxy Project: Further reading & Resources

http://galaxyproject.org http://usegalaxy.org http://getgalaxy.org http://wiki.galaxyproject.org/Cloud http://bit.ly/gxychoices

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Basic Analysis

Which genes have most overlapping Repeats? HG19, chr22

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

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

Genes & Repeats: A General Plan

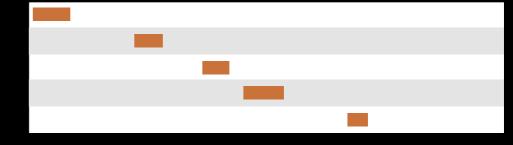
- Get some data
 - Get Data → UCSC Table Browser
- Identify which genes/exons have Repeats
- Count Repeats per exon
- Visualize, save, download, ... exons with most Repeats

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

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



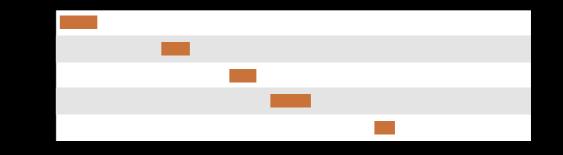
Exons



Repeats

(Identify which genes/exons have Repeats)





Exons Repeats Exons Repeats Overlap pairings

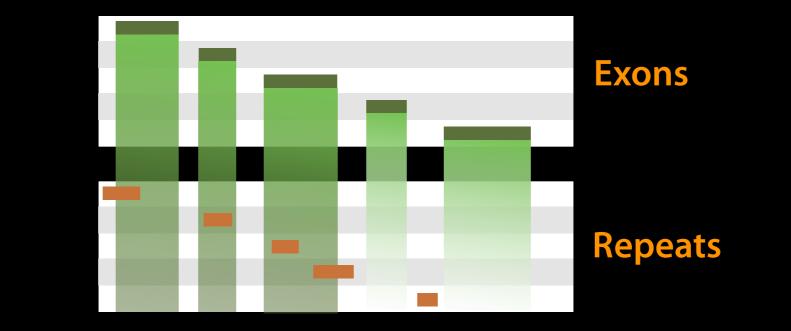
Operate on Genomic Intervals → Join (Identify which genes/exons have Repeats)



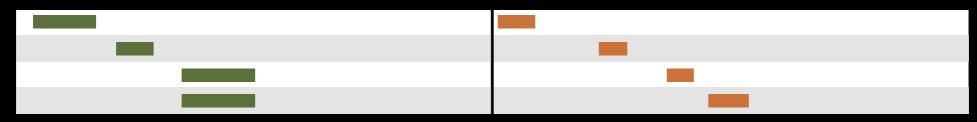


Exons





Overlap pairings





Join, Subtract, and Group → Group (Count Repeats per exon)





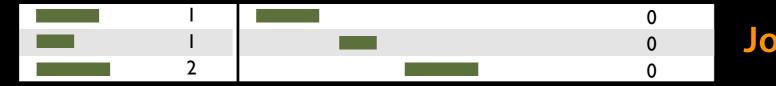
Exon overlap counts

We've answered our question, but we can do better. Incorporate the overlap count with rest of Exon information





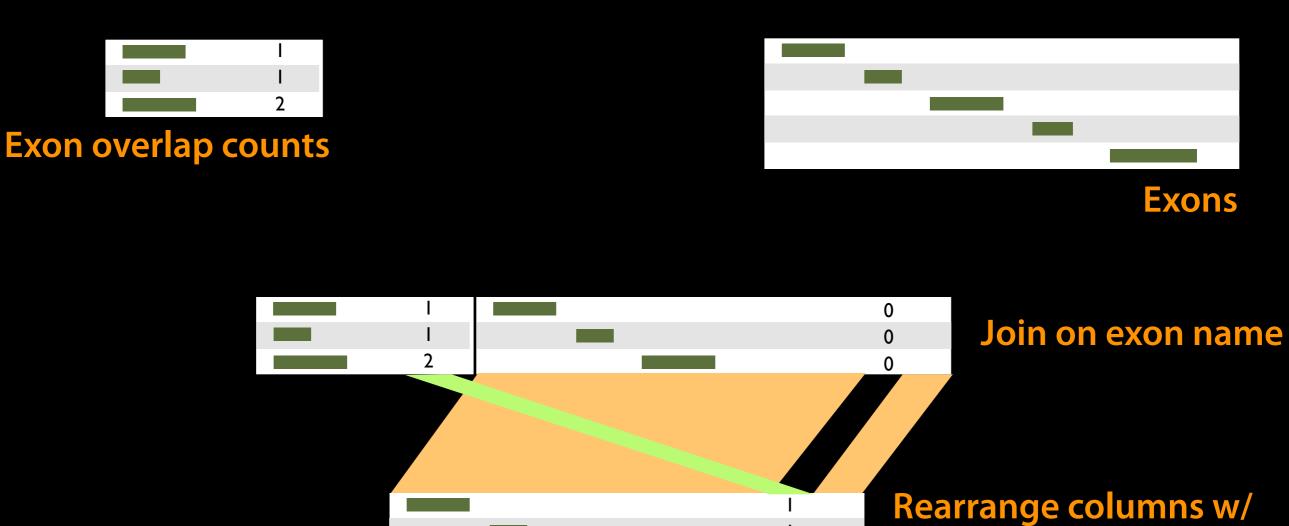




Join on exon name

Join, Subtract, and Group \rightarrow Join

(Incorporate the overlap count with rest of Exon information)



Text Manipulation \rightarrow Cut

cut

(Incorporate the overlap count with rest of Exon information)

Basic Analysis: Further reading & Resources

http://usegalaxy.org/galaxy101 https://vimeo.com/76343659

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Genes & Repeats: Exercise

Include genes/exons with no overlaps in final output. Set the score for these to 0.

Everything you need will be in the toolboxes we used in the first Gene/Exon-Repeats exercise.

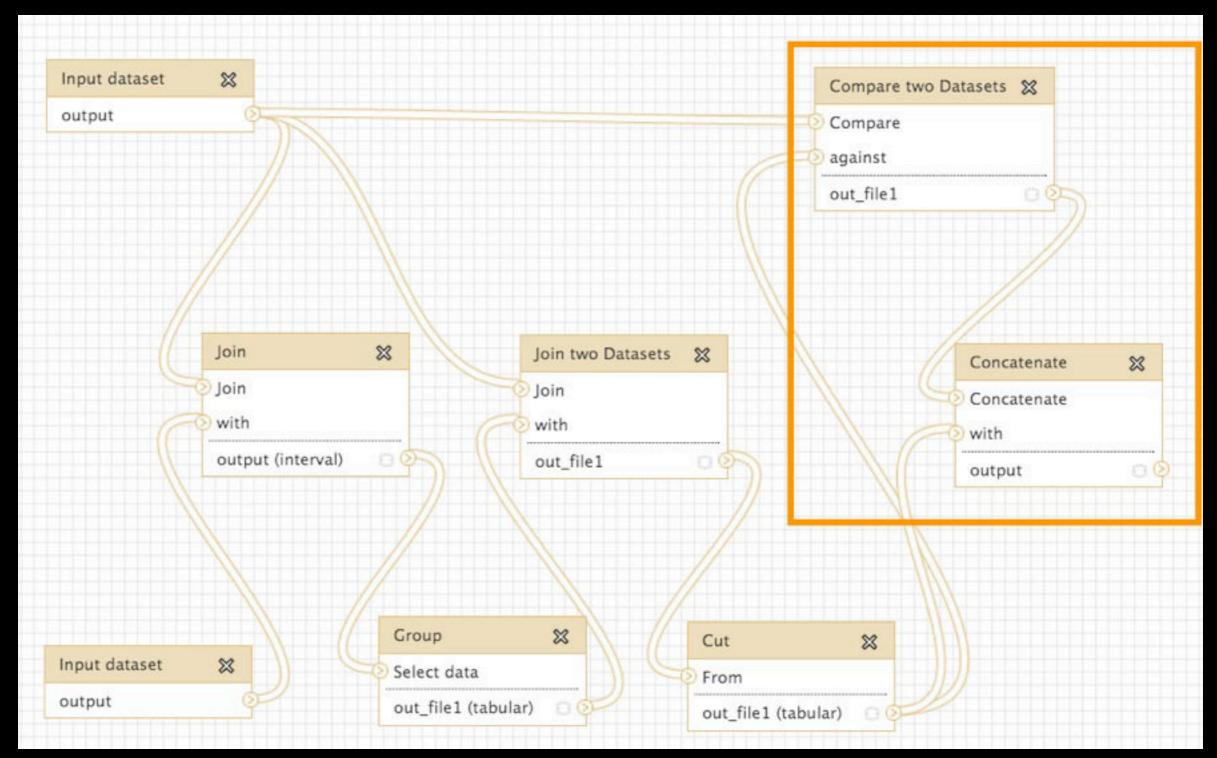
http://cloud1.galaxyproject.org/ http://cloud2.galaxyproject.org/ http://cloud3.galaxyproject.org/

Your Friend: http://galaxyproject.org/search

Coogle" Custom Search Search × Search the entire set of Galaxy web sites and mailing lists using Google. Run this search at Google.com (useful for bookmarking) Want a different search? Project home



One Possible Solution



Solution from Stanford Kwenda and Caron Griffiths in Pretoria. Takes advantage of the fact that Exons already have 0 scores.

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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 chr22
 - Overlap between exons and Repeats
- But, ...
 - there is nothing inherent in the analysis about humans, 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 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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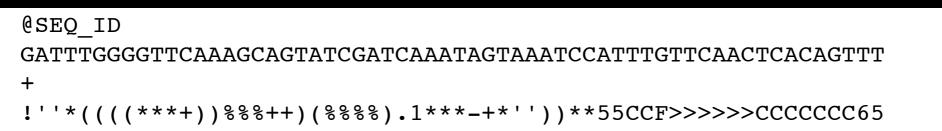
NGS Data Quality Control

- Introduce FASTQ format
- Examine quality in an RNA-Seq dataset
- Trim/filter as we see fit
- Fix some things we just broke

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!

	••••••	••••IIIII	IIIIIIIIII	SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS	IIIIIIIIIIIIIIII
				NOPQRSTUVWXYZ[\]^_`abcdefghijklmnc 104	
I - Illumina 1.3	Phred+64, 6	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

NGS Data Quality Exercise

Create new history $(cog) \rightarrow Create New$ Get some data Shared Data \rightarrow Data Libraries → UC Davis RNA-Seq Human* \rightarrow Select MeOH_REP1_R1, MeOH_REP1_R2 and then Import to current history

RNA-Seq example datasets from the 2013 UC Davis Bioinformatics Short Course. http://bit.ly/ucdbsc2013 NGS Data Quality: Assessment tools Options 1 & 2:

- 1. NGS QC and Manipulation → Compute Quality Statistics NGS QC and Manipulation → Draw quality score boxplot No control over how it is calculated or presented, statistics in text and graphic formats.
- 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, statistics in text and graphic formats

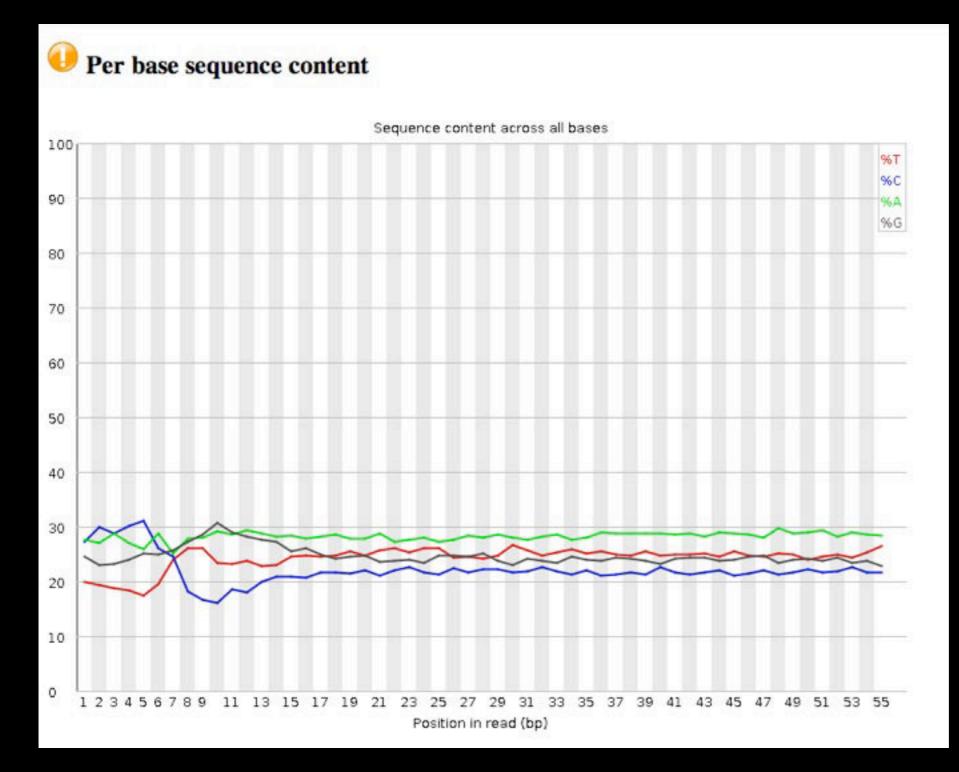
NGS Data Quality: Assessment tools

• 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.

http://bit.ly/FastQCBoxPlot

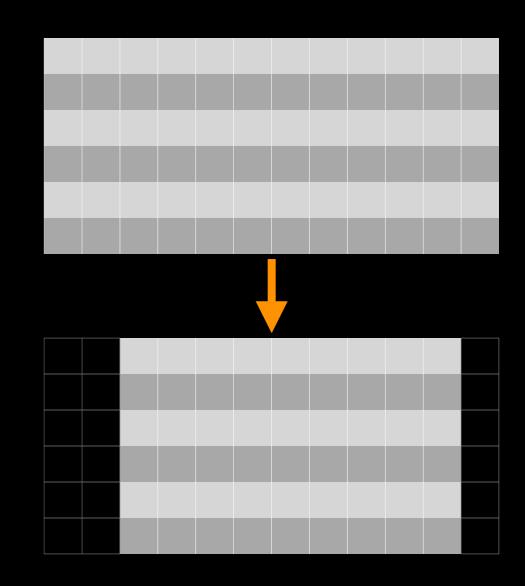
NGS Data Quality: Sequence bias at front of reads?



From a sequence specific bias that is caused by use of random hexamers in library preparation.

Hansen, et al., "Biases in Illumina transcriptome sequencing caused by random hexamer priming" *Nucleic Acids Research*, Volume 38, Issue 12 (2010)

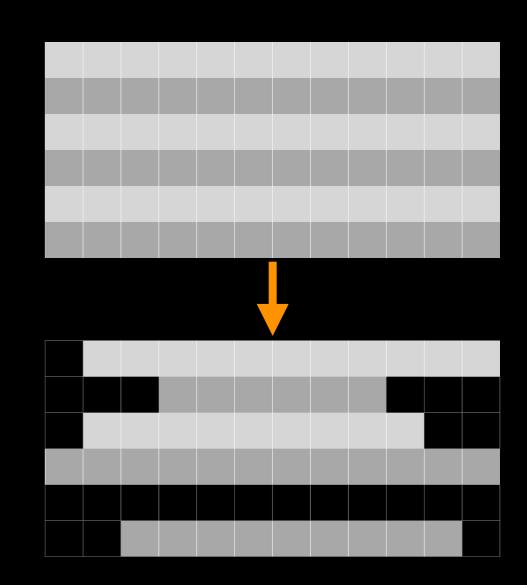
- Trim as we see fit: Option 1
 - NGS QC and Manipulation →
 FASTQ Trimmer by column
 - Trim same number of columns from every record
 - Can specify different trim for 5' and 3' ends

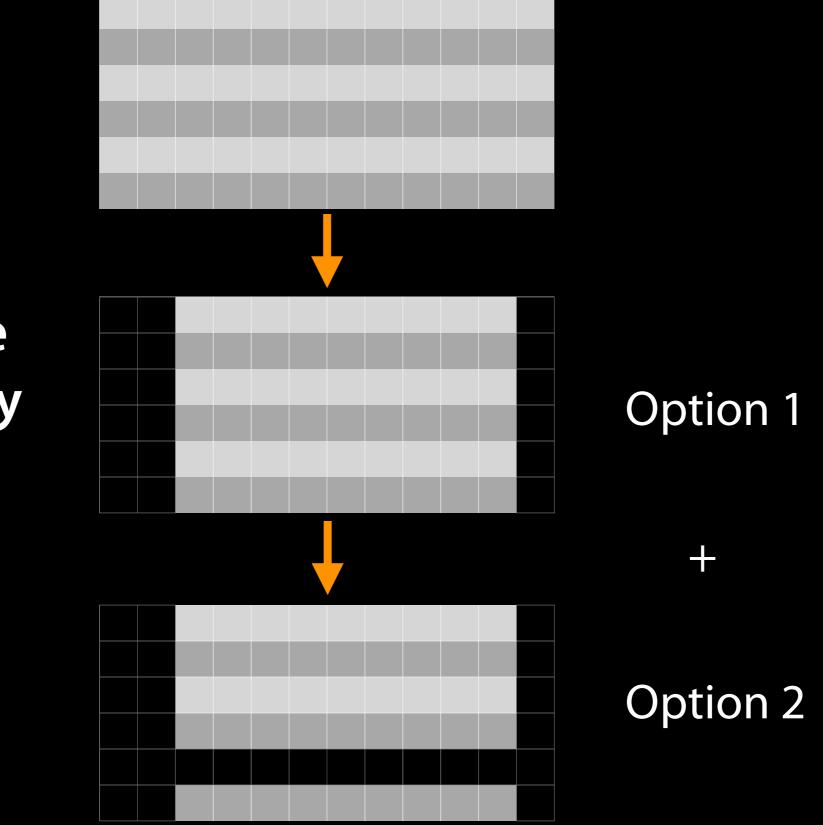


- Trim Filter as we see fit: Option 2
 - NGS QC and Manipulation →
 Filter FASTQ reads by quality
 score and length
 - Keep or discard whole reads
 - Can have different thresholds for different regions of the reads.
 - Keeps original read length.

			7			

- 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
 - Can also trim aggressively and then restore pairings

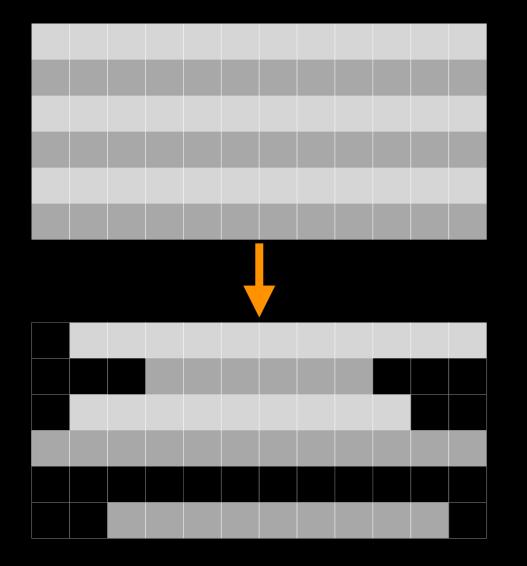
Trim? As we see fit?

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





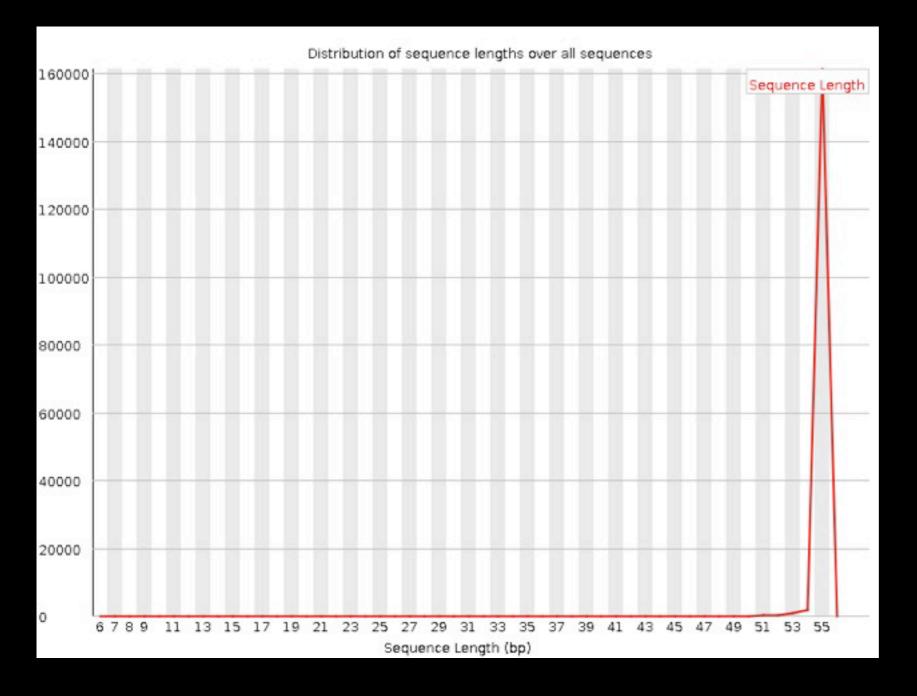


I'll use Option 3

NGS QC and Manipulation →
 FASTQ Quality Trimmer by
 sliding window

Run

● NGS QC and Manipulation →
 FastQC on trimmed dataset



New Problem: Now some reads are so short they are just noise and can't be meaningfully mapped Option 2!

NGS QC and Manipulation → **Filter FASTQ reads by quality score and length** NGS QC and Manipulation → **FastQC** on trimmed dataset

NGS Data Quality: Sequencing Artifacts

Repeat this process with MeOH Rep R2 (the reverse reads) ... and there's a new problem in Overrepresented sequences:

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
CTGTGTATTTGTCAATTTTCTTCTCCACGTTCTTCTCGGCCTGTTTCCGTAGCCT	590	0 3541692929220167	No Hit
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	342	0.2052981325073385	No Hit
CGGCCACAAATAAACACAGAAATAGTCCAGAATGTCACAGGTCCAGGGCAGAGGA	325	0.19509325457568719	No Hit
CTGCATTATAAAAAGGACAGCCAGATATCAACTGTTACAGAAATGAAATAAGACG	230	0.13806599554587093	No Hit
CGGCCGCAAATAAACACAGAAATAGTCCAGAATGTCACAGGTCCAGGGCAGAGGA	199	0.11945710049403614	No Hit
GTCAGCTCAACTTGTAGGCCCCCAAAAGAAAACAGCGTCTTACTGGGGAGGGA	197	0.11825652661972422	No Hit

NGS QC and Manipulation → **Remove sequencing artifacts**

NGS Data Quality: Done with 1st Replicate!

Now, only **5** 3 more to go...

Exercise:

Load the MeOH_REP2, R3G_REP1, and R3G_REP2 replicates into your history, and

Create a workflow that runs a single FASTQ file through all the quality steps.

Or ...

Create a workflow that runs a pair of FASTQ files through all the quality steps

NGS Data Quality: Restoring Pairings

"Mixing paired- and single- end reads together is **not** supported." Tophat manual

"Dang."

Dave C

Is that really true? Running Tophat on *no-longer-cleanly-paired* data *does map the reads*, ...

But, it no longer keeps track of read pairs in the SAM/BAM file.

Run the "Re-pair paired end reads after QC may have deleted some of them" workflow on each set of paired end reads.

Each workflow run takes the raw and trimmed versions of the forward and reverse reads for each replicate.

NGS Data Quality: Further reading & Resources

FastQC Documenation

Read Quality Assessment & Improvement by Joe Fass From the <u>UC Davis 2013 Bioinformatics Short Course</u>

Manipulation of FASTQ data with Galaxy by Blankenberg, et al.

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Community: 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					17
	Clone this repository: hg clone http://toolshed.g2.bx.g	sa.edu/res	os/clustalomega/clustalomega			
	Name: clustalomega					
	Synopsis: multiple sequence alignment pro	gram for p	robeins			
	Detailed description:					
	Closed Steps is a provid putpose	erischie se	pence alignment program for proteins.	Di produces i	high quality alignee	
	Revision: 2:bb1847435ec1					
	Owner: clustalomega					
	Times downloaded: 39					
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Community: 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

Galaxy Resources and Community: Mailing Lists http://wiki.galaxyproject.org/MailingLists

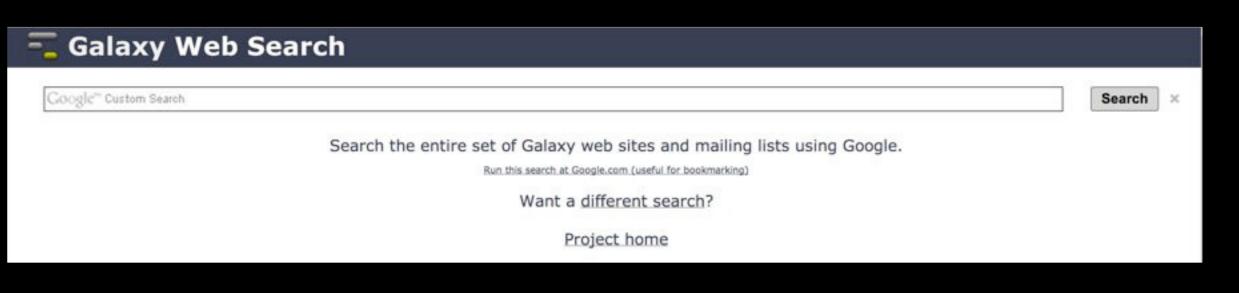
Galaxy-Announce

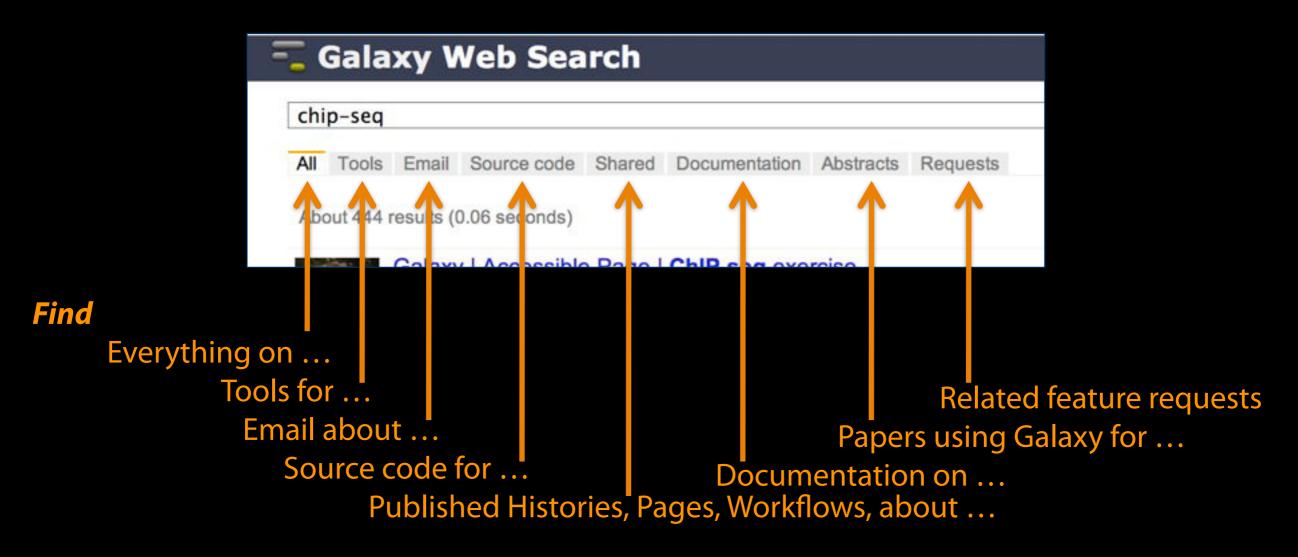
Project announcements, low volume, moderated Low volume (42 posts in 2012, 2100+ members) Galaxy-User

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

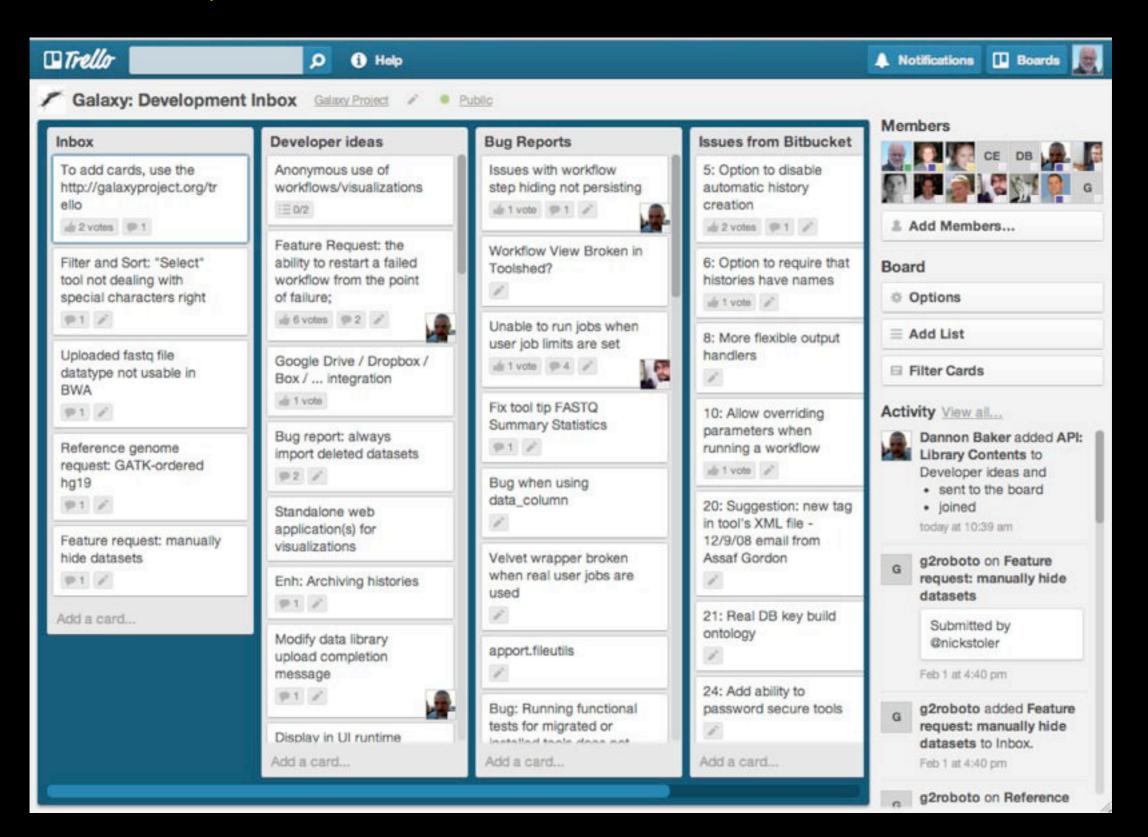
Questions about developing for and deploying Galaxy High volume (4500 posts in 2012, 900+ members)

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





Community can create, vote and comment on issues



http://bit.ly/gxyissues

http://wiki.galaxyproject.org

- Galaxy Wiki	DaveClements Settings Logout Search:	Titles Text
FrontPage		Edit History Actions
Ga	laxy	Galaxy web search Use Galaxy
 Galaxy is an open, web-based platform for accessible, reproducible, a Accessible: Users without programming experience can easily sp Reproducible: Galaxy captures information so that any user can Transparent: Users share and publish analyses via the web and o complete analysis. 	pecify parameters and run tools and workflows. repeat and understand a complete computational analysis.	Use Main (about) Use Others! • Learn Share • Search Communication
This is the Galaxy Community Wiki. It describes all things Galaxy.		Support • News S Events • Twitter Mailing Lists (search)
Use Galaxy	Deploy Galaxy	Deploy 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.	Galaxy is open source for all organizations. Local Galaxy servers can be set up by downloading and customizing the Galaxy application. • Admin • Cloud • Galaxy Appliance	Get Galaxy • Cloud Admin • Tool Config Tool Shed • Search
=usegalaxy.org	-getgalaxy.org	Contribute Tool Shed • Share

Community & Project

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

- Community
- News
- Events
- Support
- Galaxy Project

- their installations), and code to the core release.
- Everyone: Get Involved!

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

Wiki

Help • All Pages

Issues & Requests

Galaxy Project

Home . About

Community

Big Picture

Support

Events

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Date	Topic/Event	Venue/Location					
July 18-23	Introduction to Galaxy Workshop National Institute of Environmental Health Sciences (NIEHS) Introduction to Galaxy Workshop University of North Carolina, Chapel Hill Galaxy Installation Tutorial 2013 GMOD Summer School	2013 Research Triangle Wo Tour, North Carolina, United	ivew Cloudivian Release				
	Introduction to Galaxy Workshop North Carolina State University		We just released an update to Galaxy CloudMan. CloudMan offers an easy way to get a personal and completely functional instance of Galaxy in the cloud in just a few minutes, without any manual configuration.	CloudMan			
July 19-23	ISMB/ECCB, BOSC and MS SIG 2013 Talks, posters and workshops. Lots of them.	Berlin, Germany	IMPORTANT - please read Any new cluster will automatically start using this version of CloudMan. Existing clusters will be	be given an option to do an automatic update			
July 21-25	Experiences in building a Next-Generation Sequencing Analysis Service using Galaxy, Globus Online, and Amazon Web Services A Sustainable National Gateway for Biological Computation	XSEDE13, San Diego, Call United States	once the main interface page is refreshed. Note that this upgrade is a major version upgrade and thus the migration is rather complicate The migration process has been automated but will take a little while to complete. If you have made customizations to your cluster in terms of adding file systems, upgrading the database, or similar, we do not recommend you perform the upgrade. Note that this upgrad comes with (and requires) a new AMI (ami-118bfc78), which will automatically be used when starting an instance via CloudLaunch.				
	Supporting Genomics and other Biological Research		This update brings a large number of updates and new features, the most prominent or	ies being:			
September 28 - October 1	Galaxy Workshop	The Genomic Bioinformatics Workshop, Sydney, Australia	Galaxy Tool Shed when installing tools into Galaxy. Added initial support for Hadoop-type workloads	change makes it possible to utilize the			
October 1-3	Galaxy	Beyond the Genome 2013, Francisco, California, United	 Added a new file system service for an instance's transient storage, allowing it to be used 	I across the cluster over NFS			
October 7-8	TBD Using Galaxy to Provide a NGS Analysis Platform	NGS & Bioinformatics Su Europe	Added optional Loggly based off-site logging support Added tags to all resources utilized by CloudMan For more details on the new features, see the the CHANGELOG and for even more details see,	, all 291 commit messages from 7			
		GenoToul bioinformatics faci	contributors,				
	Galaxy Training Days	INRA, Toulouse Auzeville, Fr	Enjoy and please let us know what you think, Enis Afgan				
October 22- 26	High Throughput Data Analysis and Visualization with Galaxy	ASHG 2013, Boston, Massach United States					
November 6- 12	Computational and Comparative Genomics Course Application Deadline: July 15, 2013	Cold Spring Harbor Laborato York, United States	SlipStream Appliance: Galaxy Edition				



BALTIMORE, MD | JUNE 30 - JULY 2, 2014

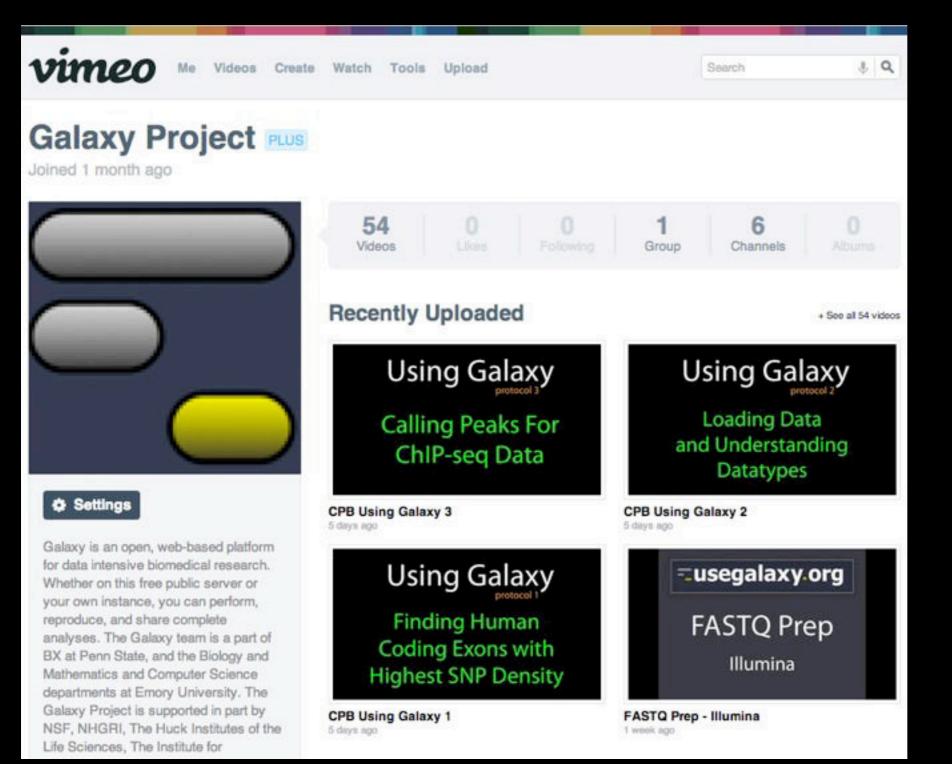
http://bit.ly/gcc2014







Galaxy Resources & Community: Videos



"How to" screencasts on using and deploying Galaxy

Talks from previous meetings.

http://vimeo.com/galaxyproject

Galaxy Resources & Community: CiteULike Group

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http://bit.ly/gxycul

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Galaxy Page:

Analysis documentation within Galaxy; easy to embed any Galaxy object

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Windshield splatter analysis with the **Galaxy metagenomic pipeline**

Sergei Kosakovsky Pond1,2,6,9, Samir Wadhawan3,6,7, Francesca Chiaromonte⁴, Guruprasad Ananda^{1,3}, Wen-Yu Chung^{1,3,8}, James Taylor1,5,9, Anton Nekrutenko1,3,9 and The Galaxy Team1

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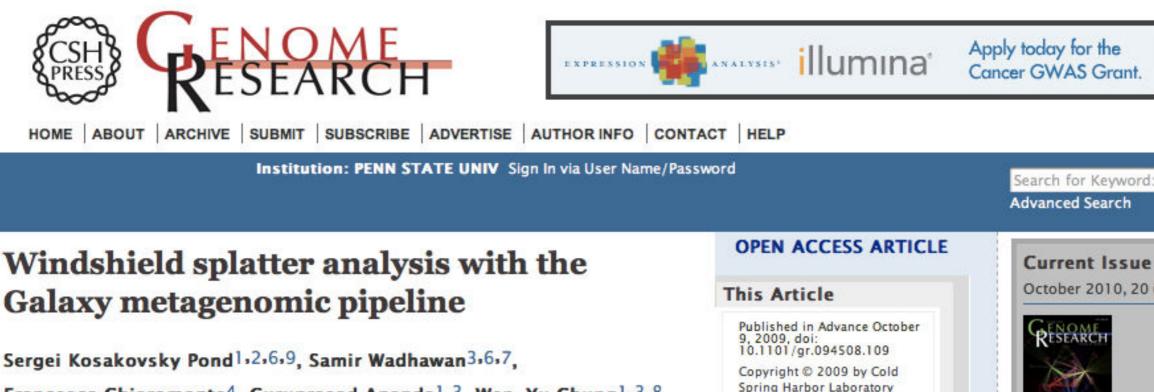
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Sharing & Publishing enables Reproducibility



Francesca Chiaromonte⁴, Guruprasad Ananda^{1,3}, Wen-Yu Chung^{1,3,8}, James Taylor1,5,9, Anton Nekrutenko1,3,9 and The Galaxy Team1

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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.]

- Galaxy

Cloud - Help - User -

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Using

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Published Pages | aun1 | Windshield Splatter

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

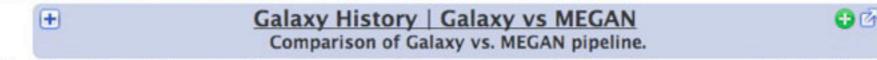
SERGEI KOSAKOVSKY POND^{1,2,*}, SAMIR WADHAWAN^{3,6*}, FRANCESCA CHIAROMONTE⁴, GURUPRASAD ANANDA^{1,3}, WEN-YU CHUNG^{1,3,7}, JAMES TAYLOR^{1,5}, ANTON NEKRUTENKO^{1,3} and THE GALAXY TEAM^{1*}

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:

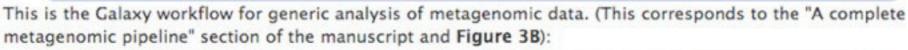


This is the Galaxy history showing a generic analysis of metagenomic data. (This corresponds to the "A complete metagenomic pipeline" section of the manuscript and Figure 3A):



+

Galaxy History | metagenomic analysis

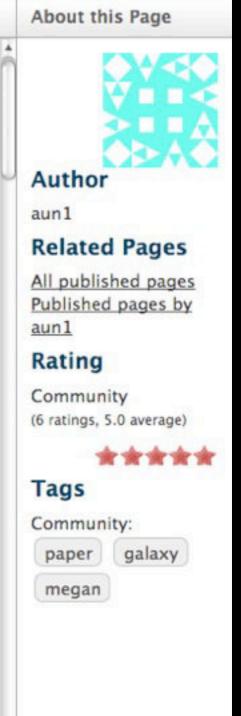


Galaxy Workflow | metagenomic analysis Generic workflow for performing a metagenomic analysis on NGS data.

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



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

The Galaxy Team



Enis Afgan



Dannon Baker



Dan Blankenberg



Dave Bouvier



Marten Cech



Dave Clements



Nate Coraor



Carl Eberhard



Dorine Francheteau



Jeremy Goecks



Sam Guerler



Jen Jackson



Greg von Kuster



Ross Lazarus



Nick Stoler



Anton Nekrutenko

James Taylor

http://wiki.galaxyproject.org/GalaxyTeam



Galaxy is hiring post-docs and software engineers



Please help. http://wiki.galaxyproject.org/GalaxyIsHiring

Community: Further reading & Resources

http://toolshed.g2.bx.psu.edu http://bit.ly/gxyServers http://wiki.galaxyproject.org/MailingLists http://galaxyproject.org/search http://bit.ly/gxyissues http://bit.ly/gcc2014 http://vimeo.com/galaxyproject http://bit.ly/gxycul

Monday Agenda

- 9:00 Welcome and Intro
- 9:40 Basic Analysis with Galaxy
- 11:00 Break + an Excerise
- 12:00 Basic Analysis into Reusable Workflows
- 13:00 Lunch
- 14:00 NGS Data Quality Control I
- 15:30 Break
- 16:00 NGS Data Quality Control II
- 16:30 Galaxy Community
- 17:00 Done

Thanks



Dave Clements

Galaxy Project Emory University

clements@galaxyproject.org