# Introduction to Galaxy

Northwestern University February 27, 2015

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Northwestern University















# Agenda

```
Welcome
9:00
      Basic Analysis with Galaxy
9:30
       Break
10:45
      Basic Analysis into Reusable Workflows
11:00
      RNA-Seq Example Part I
11:30
       Lunch (on your own)
12:20
 1:35 RNA-Seg Example Part II
       Break
2:45
      Sharing, Publishing, and Reproducibility
3:00
      Setting up your own Galaxy Cluster on Amazon
3:25
4:00
       Done
```

## Goals

Provide a basic introduction to using Galaxy for bioinformatic analysis.

Demonstrate how Galaxy can help you explore and learn options, perform analysis, and then share, repeat, and reproduce your analyses.

## **Not** Goals

### This workshop will not cover

- details of how tools are implemented, or
- new algorithm designs, or
- which assembler or mapper or peak caller or ... is best for you.

This workshop does cover RNA-Seq. However, our emphasis today is on learning general NGS and Galaxy principles.

It will still be enough to get you started with RNA-Seq.

# Want more RNA-Seq?

Transcriptome Analysis with RNA-seq -- Mar. 19, 2015

Speaker: Matthew Schipma, NUCATS Next-Generation Sequencing Core

Direct registration link

Next generation sequencing has made it possible to sequence the entire transcriptome of a cell or tissue type. This allows researchers to evaluate the expression of each gene in the genome, including specific isoforms. RNA-seq also has the potential to identify novel splice variants and even novel genes. The ability to gain useful information from an RNA-seq project depends of several experimental factors including the number of replicates and the sequencing depth. Here we will discuss the best practices in designing an RNA-seq experiment as well as the information that can be obtained through RNA-seq.

# Part of the Computational Skills for Informatics Seminar Series

http://www.galter.northwestern.edu/News/computational-skills-for-informatics-seminar-series http://bit.ly/CSIGalter

## What is Galaxy?

Data integration and analysis platform that emphasizes accessibility, reproducibility, and transparency

A free (for everyone) web server

Open source software

These options result in several ways to use Galaxy

http://galaxyproject.org

## Galaxy is available online, for free

http://usegalaxy.org

As a free (for everyone) web server integrating a wealth of tools, compute resources, petabytes of reference data and permanent storage



However, a centralized solution cannot support the different analysis needs of the entire world.

## Galaxy is available as Open Source Software

Galaxy is installed in locations around the world.

Some of them are free for anyone to use too.

http://getgalaxy.org

bit.ly/gxyServers

## Galaxy is available on the Cloud









http://aws.amazon.com/education http://globus.org/ http://wiki.galaxyproject.org/Cloud

We are using the cloud today.

# Galaxy is available with Commercial Support

A ready-to-use appliance (BioTeam)

Cloud-based solutions
(ABgenomica, AIS,
GenomeCloud)

Consulting & Customization
(BioTeam, Deena
Bioinformatics)

Training (OpenHelix)



## 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 exons have most overlapping Repeats?

Use Human, HG19, Chromosome 22

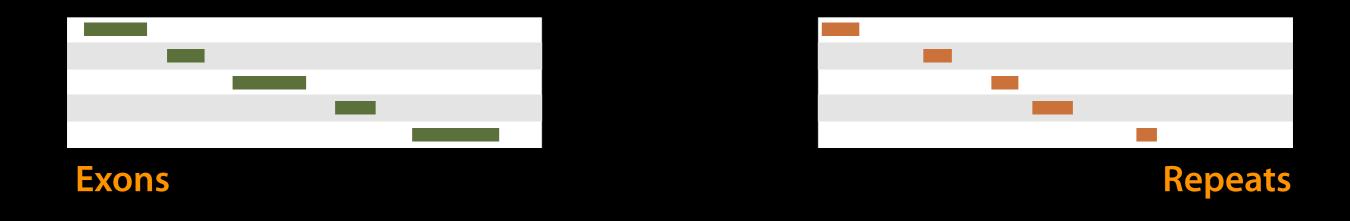
cloud1.galaxyproject.org cloud2.galaxyproject.org cloud3.galaxyproject.org

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

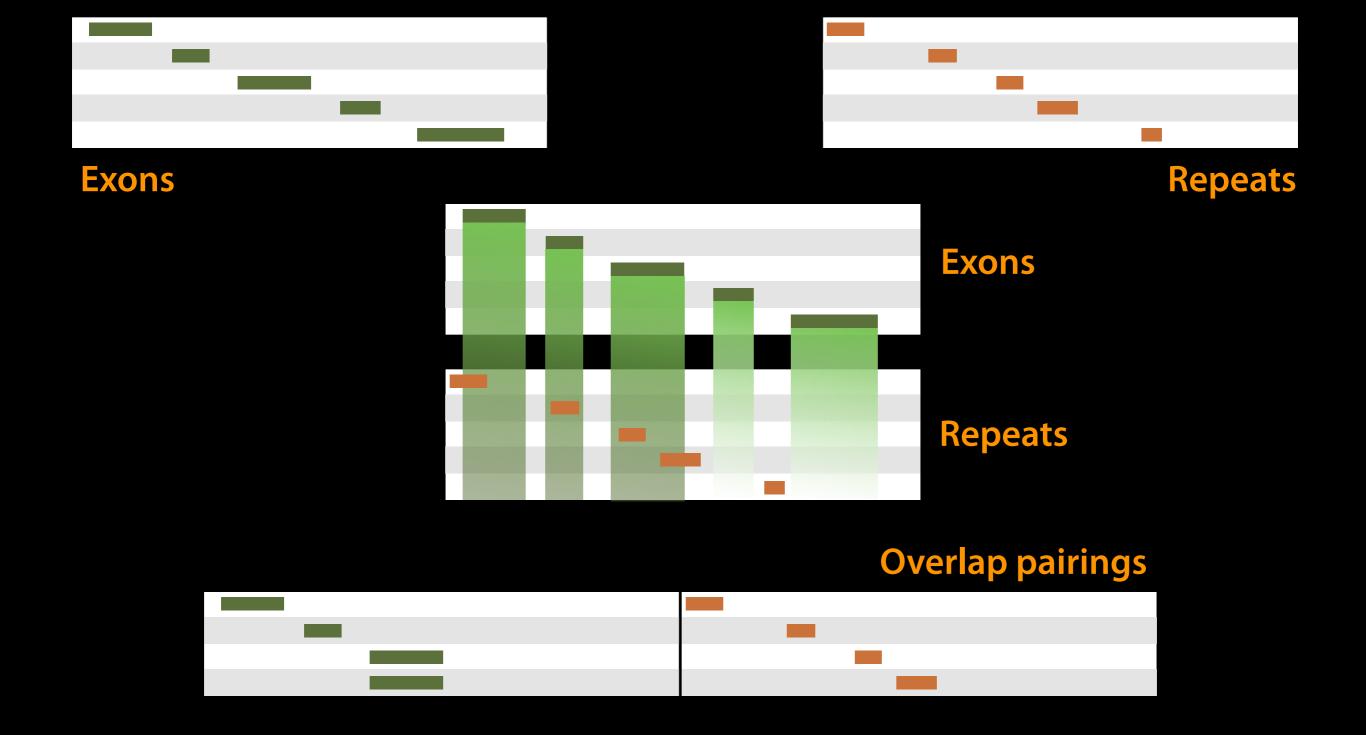
# Exons & Repeats: A General Plan

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

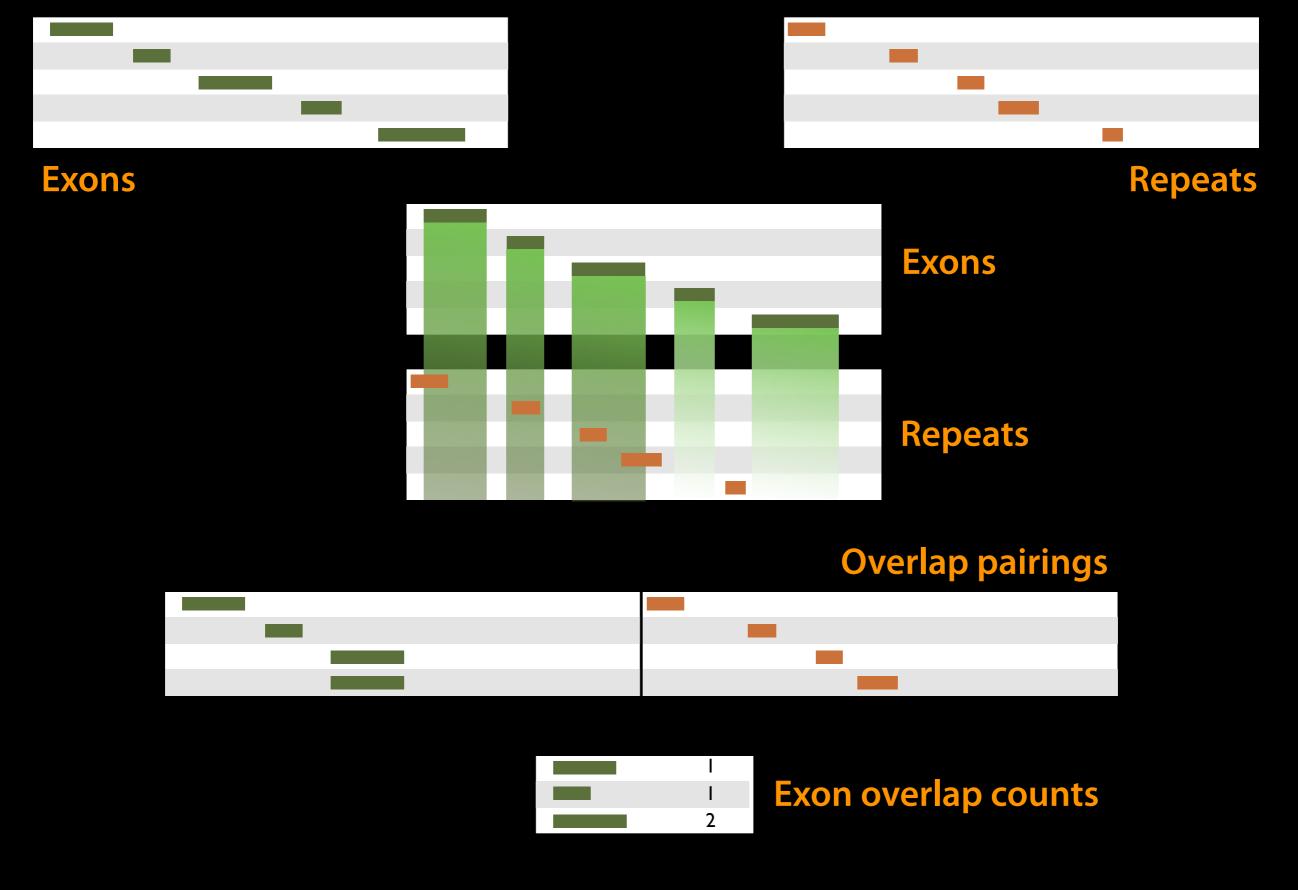
(~ http://usegalaxy.org/galaxy101)



(Identify which exons have Repeats)



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



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





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





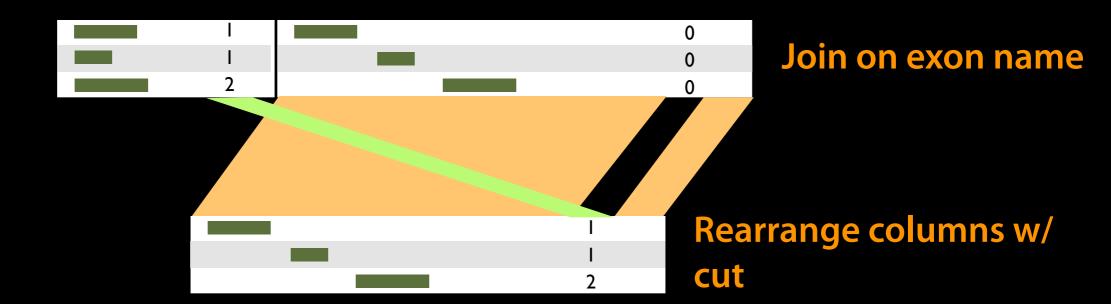
		0	Join on exon name
1	_	0	
2		0	

## Join, Subtract, and Group → Join

(Incorporate the overlap count with rest of Exon information)







## Text Manipulation → Cut

(Incorporate the overlap count with rest of Exon information)

# Exons & Repeats: Exercise

Include 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 Exon-Repeats exercise.

**Get Data** 

Lift-Over

**Text Manipulation** 

Filter and Sort

Join, Subtract and Group

**Convert Formats** 

**Extract Features** 

**Fetch Sequences** 

**Fetch Alignments** 

**Get Genomic Scores** 

Statistics

Graph/Display Data

**Evolution** 

**Motif Tools** 

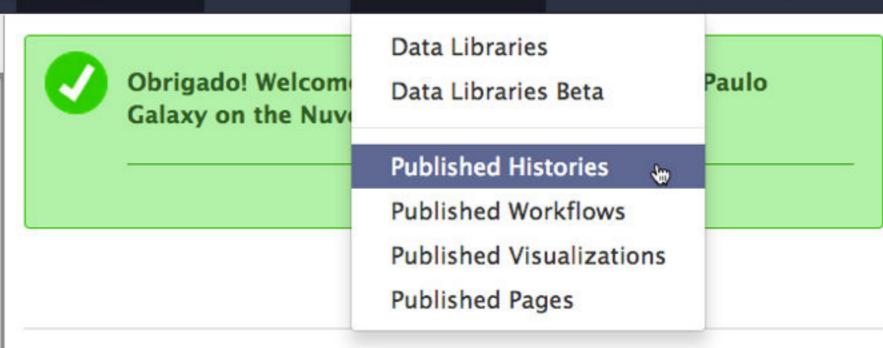
NGS: QC and manipulation

**NGS: Mapping** 

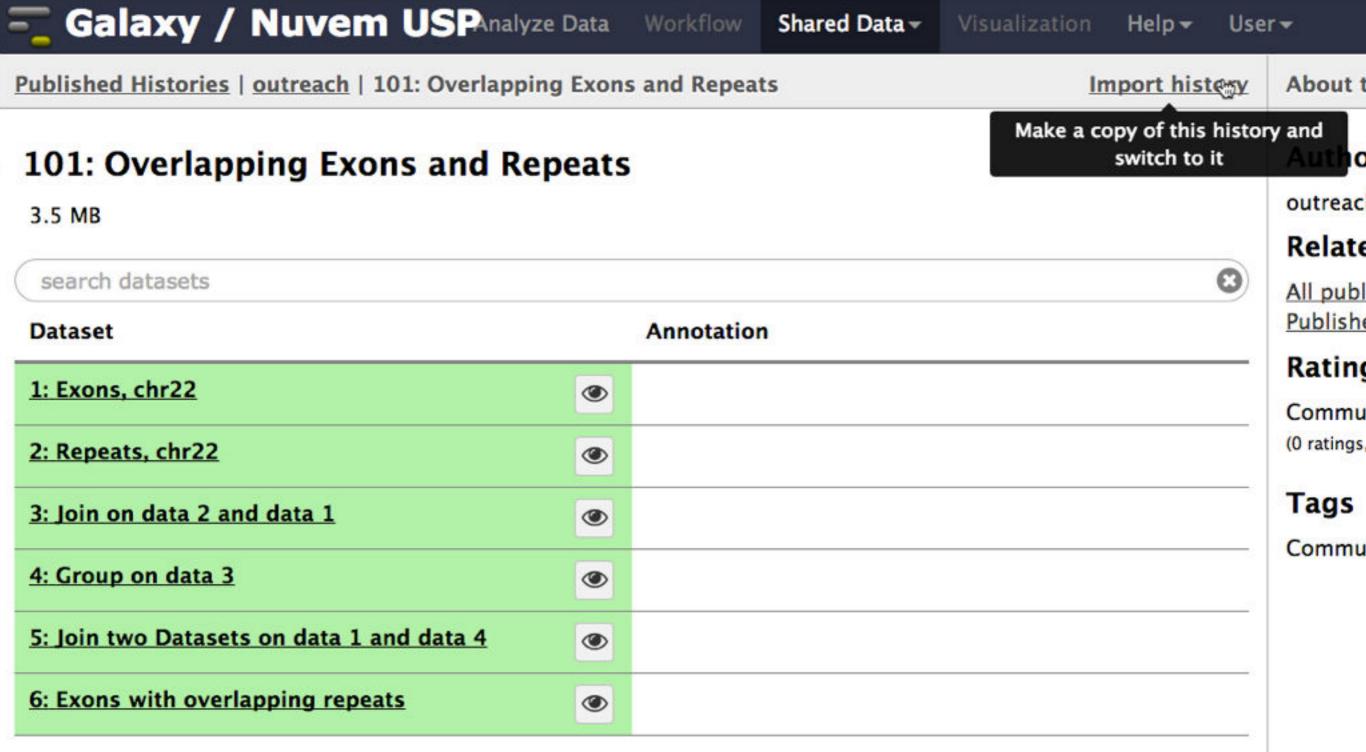
NGS: SAM Tools

**NGS: Simulation** 

Phenotype Association



Galaxy is an open, web-based platform for data intensive biomedical research. The Galaxy team is a part of BX at Penn State, and the Biology and Mathematics and Computer Science departments at Emory University. The Galaxy Project is supported in part by NHGRI, NSF, The Huck Institutes of the Life Sciences, The Institute for CyberScience at Penn State, and Emory University.

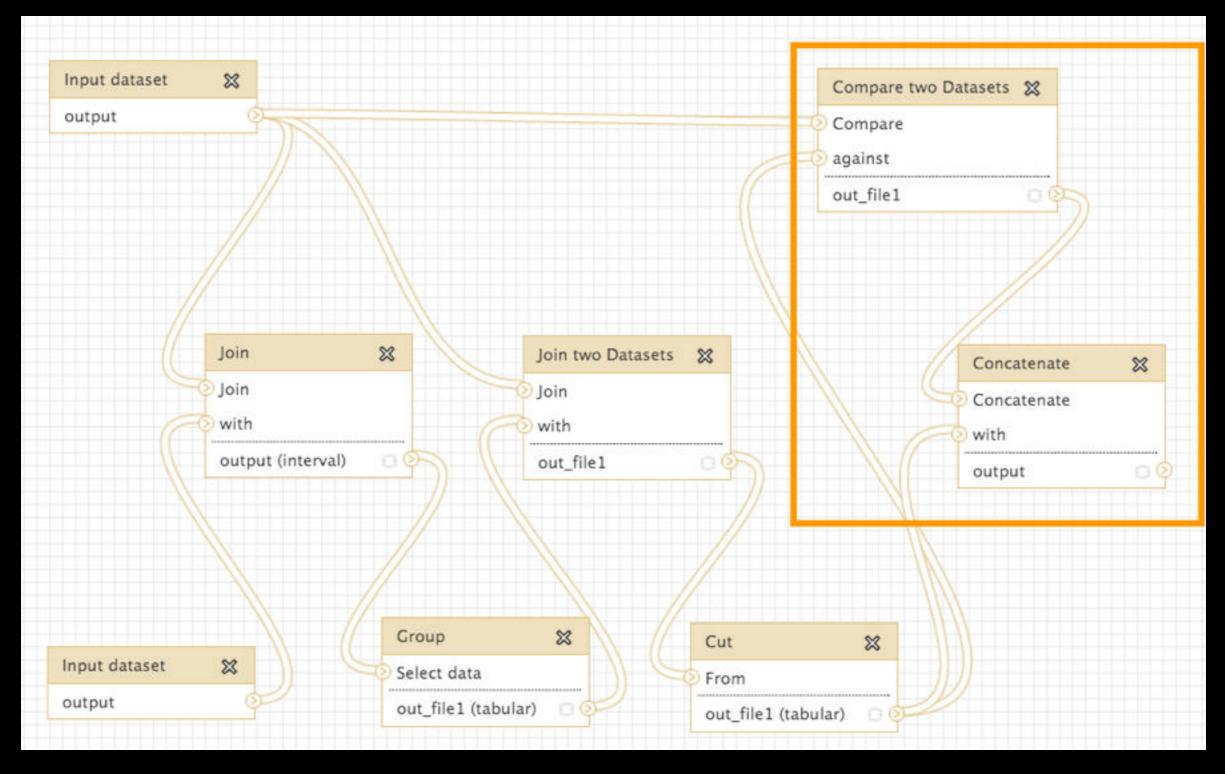


*Note:* In your solution, you can take advantage of the fact that Exons already have 0 scores.

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#### One Possible Solution



Solution from Stanford Kwenda and Caron Griffiths, 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 Workflow from a History

## **Extract Workflow from history**

Create a workflow from this history. Edit it to make some things clearer.



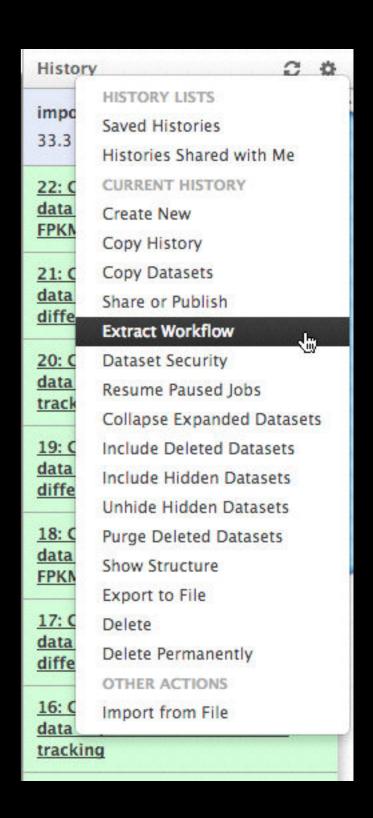
(cog) → Extract Workflow

#### 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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# RNA-Seq Analysis: Get the Data

Create new history

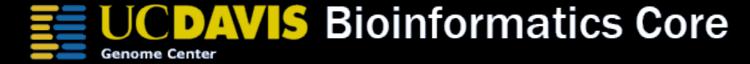


(cog) → Create New

Import:

Shared Data → Data Libraries

- → RNA-Seq UCDavis 2013 Example Data\*
  - → Unfiltered Reads
    - → MeOH\_REP1\_R1.fastq and MeOH\_REP1\_R2.fastq



\* RNA-Seq example datasets from the 2013 UC Davis Bioinformatics Short Course. http://bit.ly/ucdbsc2013

# NGS Data Quality Control

- FASTQ format
- Examine quality in an RNA-Seq dataset
- Trim/filter as we see fit, hopefully without breaking anything.

Quality Control is not sexy, but it is vital.

## 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!

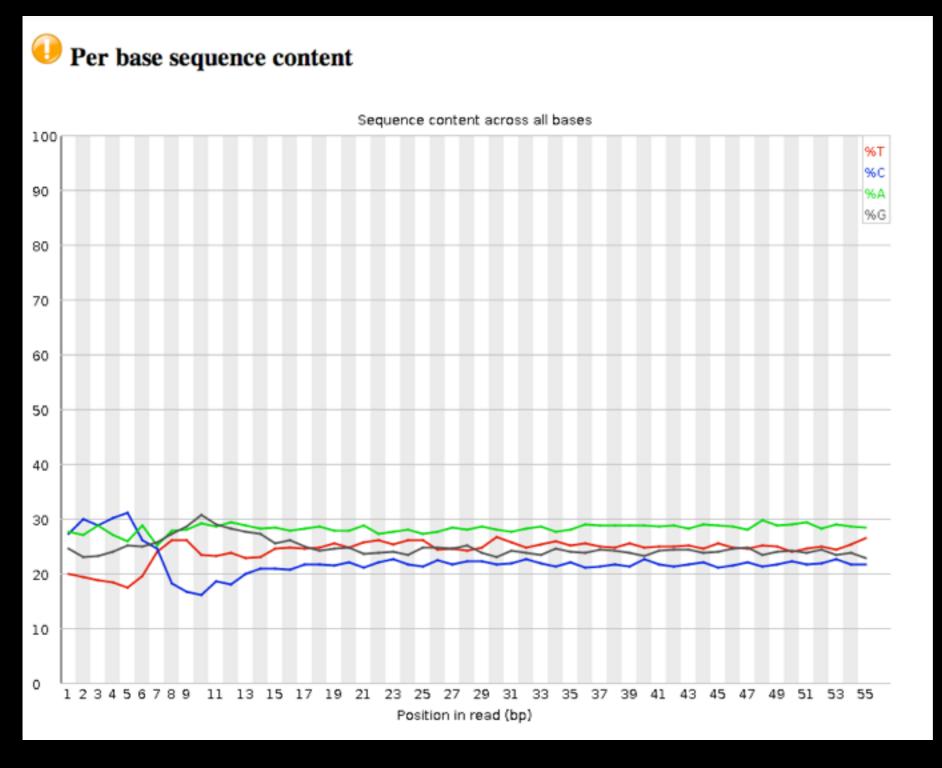
## NGS Data Quality: Assessment tools

NGS QC and Manipulation → FastQC

Gives you a lot of information but little control over how it is calculated or presented.

http://bit.ly/FastQCBoxPlot

# NGS Data Quality: Sequence bias at front of reads?

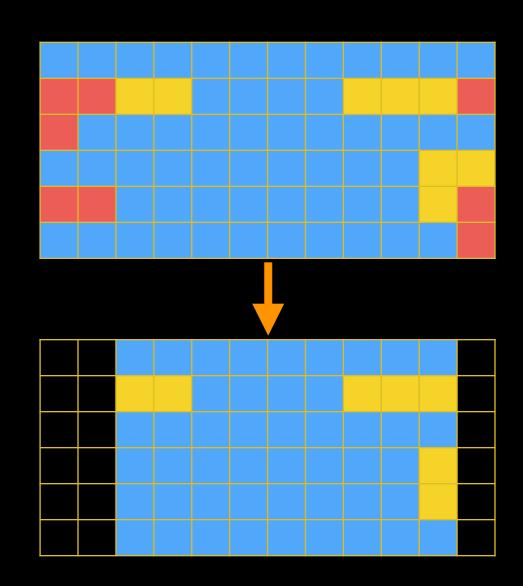


From a sequence specific bias that is caused by use of random hexamers in library preparation.

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

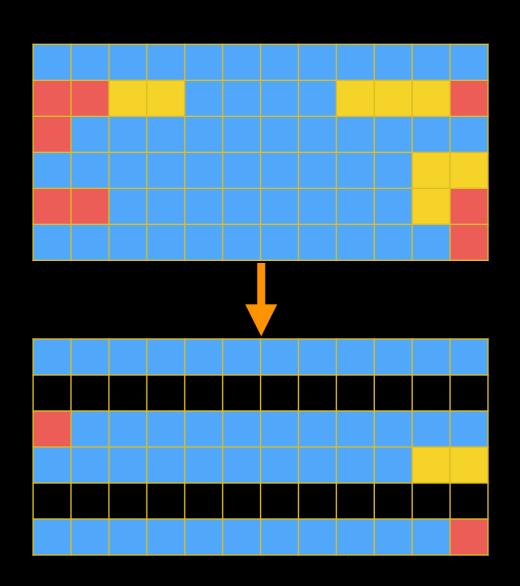
## NGS Data Quality: Trim as we see fit

- 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



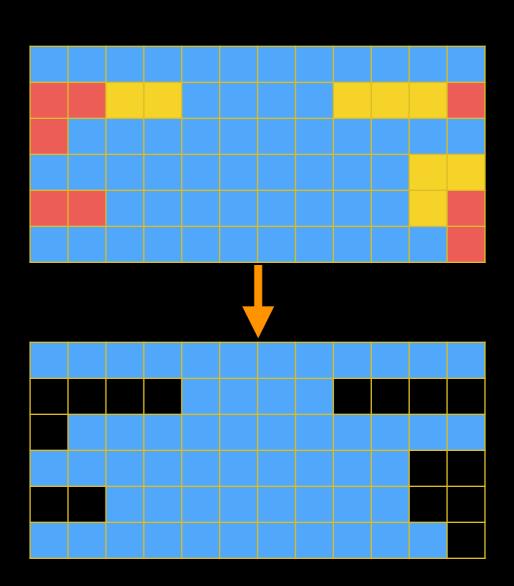
## **NGS Data Quality: Base Quality Trimming**

- 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.

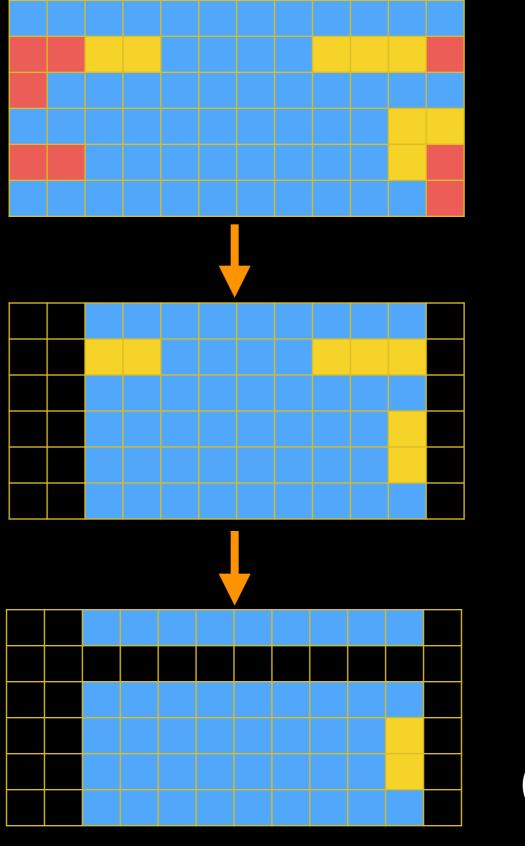


#### **NGS Data Quality: Base Quality Trimming**

- 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



Option 1 (by column)

+

Option 2 (by entire row)

#### 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?

- Read the tool documentation
- http://biostars.org/
- http://seqanswers.com/
- http://galaxyproject.org/search





#### **Tophat Overview**

TopHat is a fast splice junction mapper for RNA-Seq reads. It aligns RNA-Seq reads to mammalian-sized genomes using the ultra high-throughput short read aligner Bowtie(2), and then analyzes the mapping results to identify splice junctions between exons. Please cite: Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, and Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 14:R36, 2013.

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

Tophat Manual

"If you are performing RNA-seq analysis, there is no need to filter the data to ensure exact pairs before running Tophat."

Jen Jackson

Galaxy User Support Person Extraordinaire

Most of us

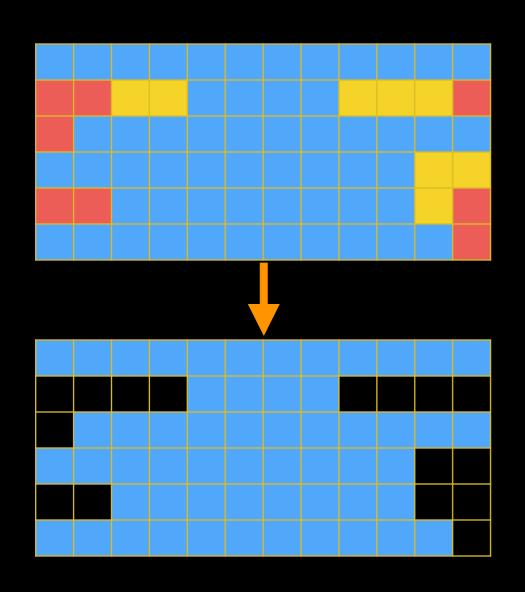
"Dang."

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.

#### Keeping paired ends paired: Things to Try

- Don't bother.
- Run a workflow (try the "Re-Pair Paired ends after QC may have broken them" workflow) that removes any unpaired reads before mapping:
- Run the Picard Paired Read Mate Fixer after mapping reads.
- Use sliding windows for QC, but keep empty reads. (This does not work with Tophat.)

## **NGS Data Quality: Base Quality Trimming**

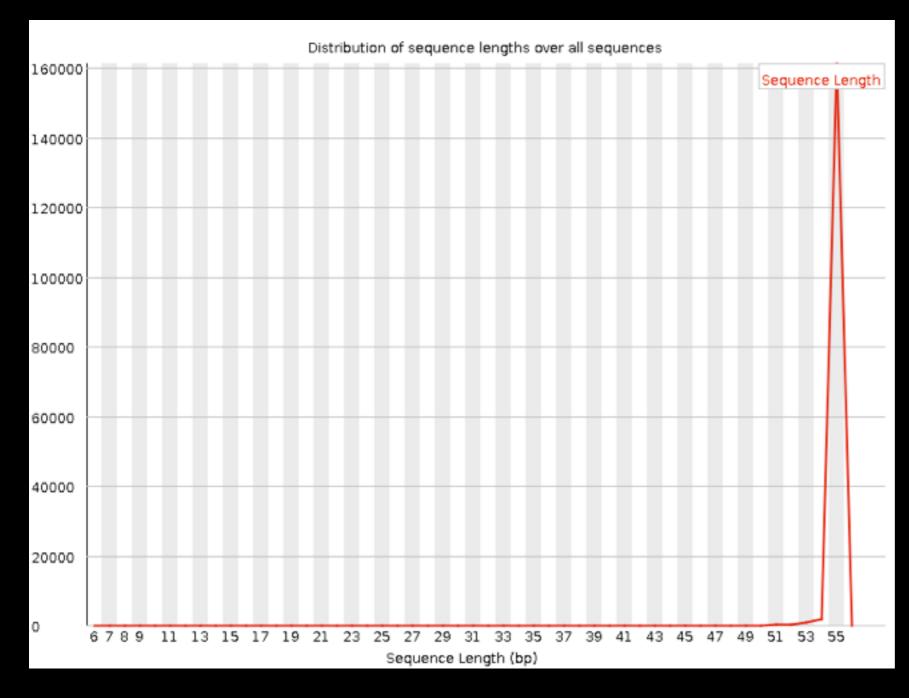


I'll use Option3, sliding windows, and run a workflow afterward to patch up pairings

- NGS QC and Manipulation → FASTQ
   Quality Trimmer by sliding window

   Run again:
- NGS QC and Manipulation → FastQC on trimmed dataset

## **NGS Data Quality: Base Quality Trimming**



New Problem?

Now some reads are so short they are just noise and can't be meaningfully mapped. Have potential to bog down mapping.

Option 2 can fix this, but breaks pairings (if you still have them). Or, your mapper may have an option to ignore shorter reads.

## RNA-Seq Analysis

I'll use option 2, since my pairings are already broken.

NGS QC and Manipulation

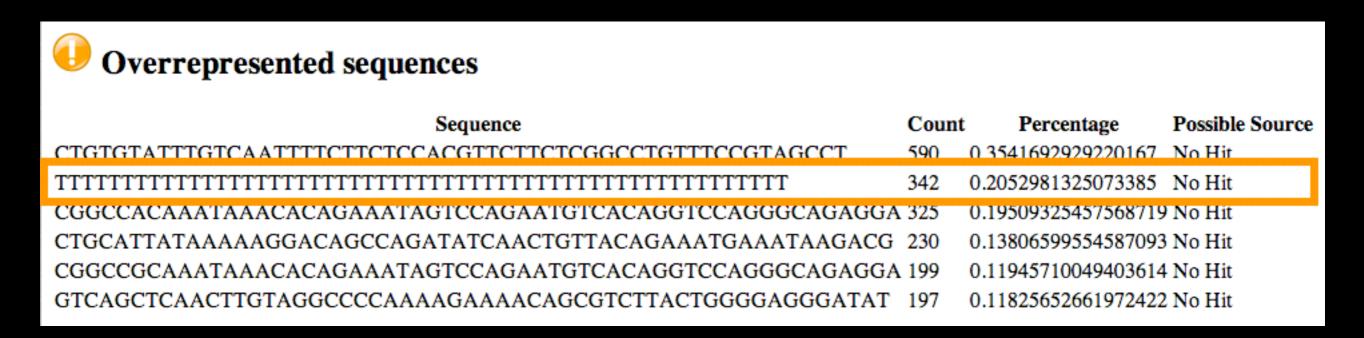
→ Filter FASTQ reads by quality score and length

Pick a minimum length. I used 32.

#### NGS Data Quality: Sequencing Artifacts

Repeat this process with MeOH Rep1 R2 (the reverse reads)

... and now we notice a problem in Overrepresented sequences:



NGS QC and Manipulation → Remove sequencing artifacts

But this will break pairings (if we still have them).

Or, can rely on mapper to just not map them.

## RNA-Seq Analysis: Restore Pairings

If your QC filters might have broken pairings, then you may want to restore them.

#### Shared Data → Published Workflows

- → Re-Pair Paired ends after QC may have broken them
  - → Import

#### Workflows

- → Re-Pair Paired ends after QC may have broken them
  - → Run

#### Re-Pair Paired ends after QC may have broken them

#### Workflow takes 4 inputs

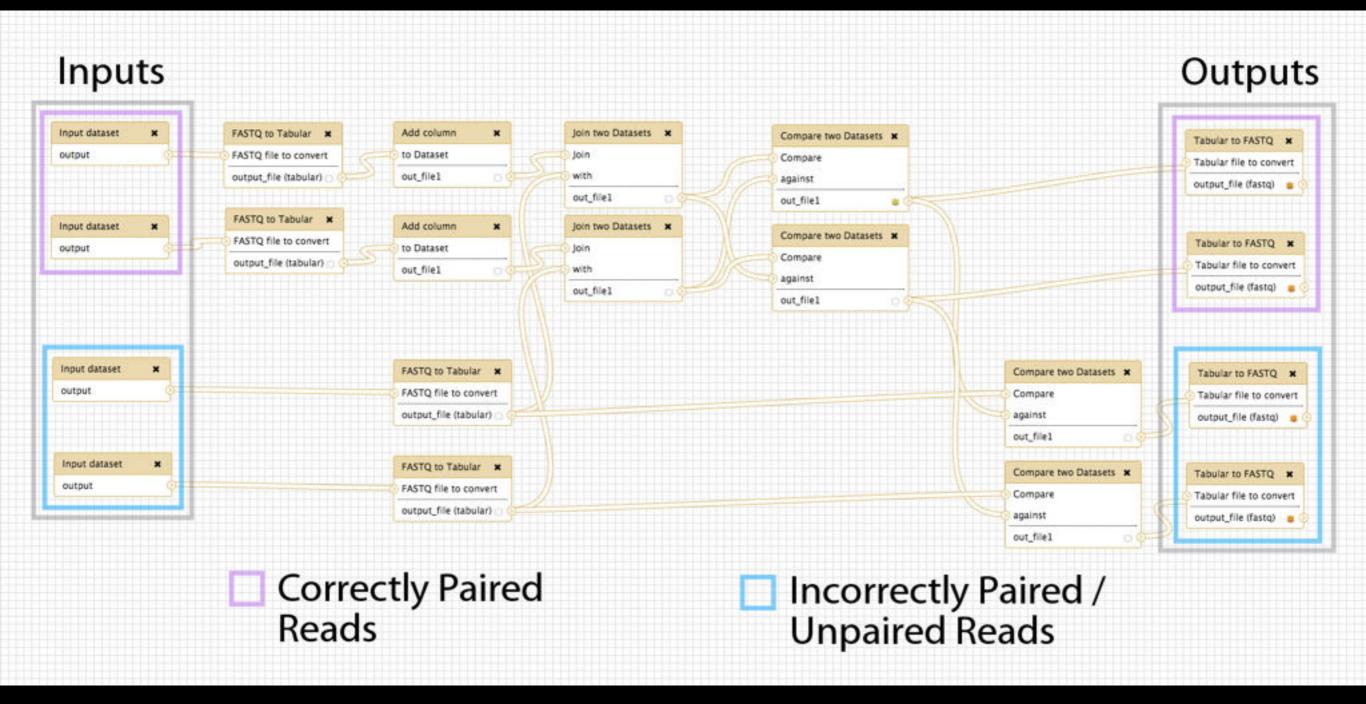
- Forward Reads, before QC
- Reverse Reads, before QC
- Forward Reads, after QC
- Reverse Reads, after QC

#### And produces 4 outputs

- Forward reads, re-paired
- Reverse reads, re-paired
- Forward reads, singletons
- Reverse reads, singletons

Workflow assumes pre-QC reads are correctly paired

#### Re-Pair Paired ends after QC may have broken them



#### NGS Data Quality: Done with 1st Replicate!

Now, only 5 more to go!

Workflows?

Create a QC workflow that does the trimming

Or, cheat and import trimmed+paired datasets from the

RNA-Seq UCDavis 2013 Example Data →

Reads, Post-QC, Re-Paired

shared data library

## NGS Data Quality: Further reading & Resources

#### FastQC Documenation

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

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

## Mapping with Tophat

## RNA-Seq: Mapping with Tophat

Create new history

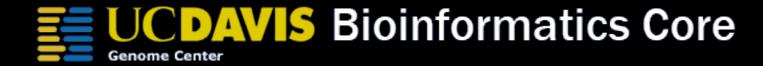


**★** (cog) → Create New

Get filtered reads

Shared Data → Data Libraries

- → RNA-Seq UCDavis 2013 Example Data\*
  - → Reads, Post-QC
  - → Select MeOH\_REP1\_R1, MeOH\_REP1\_R2 Also select genes\_chr12.gtf And then Import to current history



\* RNA-Seq example datasets from the 2013 UC Davis Bioinformatics Short Course. http://bit.ly/ucdbsc2013

## RNA-seq Exercise: Mapping with Tophat

- Tophat looks for best place(s) to map reads, and best places to insert introns
- Imagine pages and pages of discussion on the intricacies and pitfalls of RNA-seq mapping here.

#### Mapping with Tophat: mean inner distance

#### Expected distance between paired end reads

- Determined by sample prep
- We'll use 90\* for mean inner distance
- We'll use 50 for standard deviation

\* The library was constructed with the typical Illumina TruSeq protocol, which is supposed to have an average insert size of 200 bases. Our reads are 55 bases (R1) plus 55 bases (R2). So, the Inner Distance is estimated to be 200 - 55 - 55 = 90

From the 2013 UC Davis Bioinformatics Short Course

## Mapping with Tophat: Use Existing Annotations?

You can bias Tophat towards known annotations

- Use Own Junctions → Yes
  - Use Gene Annotation → Yes
  - Gene Model Annotation → genes\_chr12.gtf
- Use Raw Junctions → Yes (tab delimited file)
- Only look for supplied junctions → Yes

## Mapping with Tophat: Make it quicker?

#### Warning: Here be dragons!

- Allow indel search → No
- Use Coverage Search → No (wee dragons)

TopHat generates its database of possible splice junctions from two sources of evidence. The first and strongest source of evidence for a splice junction is when two segments from the same read (for reads of at least 45bp) are mapped at a certain distance on the same genomic sequence or when an internal segment fails to map - again suggesting that such reads are spanning multiple exons. With this approach, "GT-AG", "GC-AG" and "AT-AC" introns will be found *ab initio*. The second source is pairings of "coverage islands", which are distinct regions of piled up reads in the initial mapping. Neighboring islands are often spliced together in the transcriptome, so TopHat looks for ways to join these with an intron. We only suggest users use this second option (--coverage-search) for short reads (< 45bp) and with a small number of reads (<= 10 million). This latter option will only report alignments across "GT-AG" introns

## Mapping with Tophat: Max # of Alignments Allowed

Some reads align to more than one place equally well.

For such reads, how many should Tophat include?

If more than the specified number, Tophat will pick those with the best mapping score.

Tophat breaks ties randomly.

Tophat assigns equal fractional credit to all *n* mappings

Instructs TopHat to allow up to this many alignments to the reference for a given read, and choose the alignments based on their alignment scores if there are more than this number. The default is 20 for read mapping. Unless you use --report-secondary-alignments, TopHat will report the alignments with the best alignment score. If there are more alignments with the same score than this number, TopHat will randomly report only this many alignments. In case of using --report-secondary-alignments, TopHat will try to report alignments up to this option value, and TopHat may randomly output some of the alignments with the same score to meet this number.

TopHat Manual

#### RNA-Seq Mapping With Tophat: Resources

RNA-Seq Concepts, Terminology, and Work Flows by Monica Britton

Aligning PE RNA-Seq Reads to a Genome by Monica Britton

both from the <u>UC Davis 2013 Bioinformatics Short Course</u>

RNA-Seq Analysis with Galaxy by Jeroen F.J. Laros, Wibowo Arindrarto, Leon Mei

from the GCC2013 Training Day

RNA-Seq Analysis with Galaxy
by Curtis Hendrickson, David Crossman, Jeremy Goecks
from the GCC2012 Training Day

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## Galaxy Community Resources

## Galaxy Community Resources: Galaxy Biostar

Tens of thousands of users leads to a lot of questions.

Absolutely have to encourage community support.

Project traditionally used mailing list

Moved the user support list to Galaxy Biostar, an online forum, that uses the Biostar platform



https://biostar.usegalaxy.org/

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

## Galaxy-Dev

```
Questions about developing for and deploying Galaxy High volume (5200 posts in 2013, 900+ members) (3246 posts in 2014, 1000+ members)
```

#### Galaxy-Announce

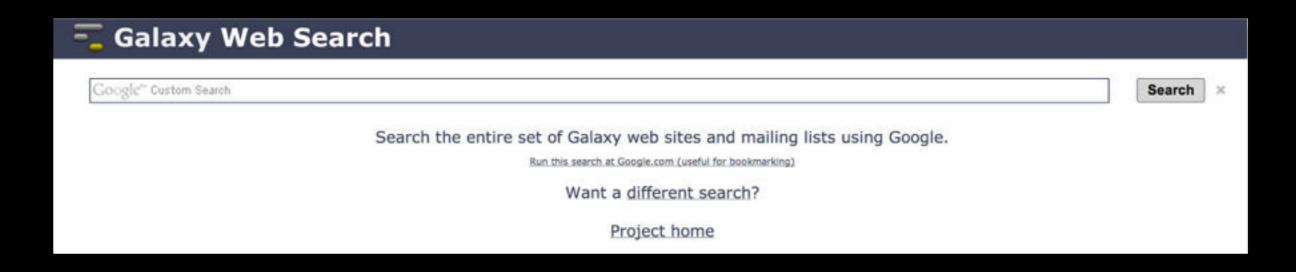
```
Project announcements, low volume, moderated Low volume ( 47 posts in 2013, 3400+ members) ( 34 posts in 2014, 4400+ members)
```

## Galaxy-User (discontinued 2014/05)

```
Questions about using Galaxy and usegalaxy.org
High volume (1328 posts in 2013, 2600+ members)

( 358 posts in 2014, 2600+ members)
```

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





## http://wiki.galaxyproject.org



DaveClements Settings Logout |

Search:

Titles



Edit History Actions FrontPage



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 web server usegalaxy.org 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.



#### Deploy Galaxy

Galaxy is a free and open source project available to all. Local Galaxy servers can be set up by downloading the Galaxy application.

- Admin
- Cloud

**=**getgalaxy.org

#### Community & Project

Galaxy has a large and active user community and many ways to get involved.

Community

#### Contribute

 Users: Share your histories, workflows, visualizations, data libraries, and Galaxy Pages, enabling others to use and learn from them.



#### Use Galaxy

Servers • Learn Main . Choices Share • Search

#### Communicate

Support • Biostar Events • Mailing Lists News 
Twitter

#### Deploy Galaxy

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

#### Contribute

Develop • Tools Issues & Requests Logs • Deployments Teach

#### Galaxy Project

Home . About . Cite Community Big Picture

#### **Events**

#### News



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Events

#### 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 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, send it to outreach@glaxyproject.org.

For events prior to this year, see the Events Archive.

#### Upcoming Events





Date	Topic/Event	Venue/Location
December 12	Introduction to Galaxy Workshop	Virginia State University, Petersburg, Virginia
December 16-19	RNA-Seq and ChIP-Seq Analysis with Galaxy	UC Davis, California, United States
		2015
January 10-14	Galaxy for SNP and Variant Data Analysis	Plant and Animal Genome XXIII (PAG2014) States
January 19-20	NGS pipelines with Galaxy	e-Infrastructures for Massively Parallel Seq Sweden
February 9-13	Analyse bioinformatique de séquences sous Galaxy	Montpellier, France
February 16-18	Accessible and Reproducible Large- Scale Analysis with Galaxy	Genome and Transcriptome Analysis, pa Conference, San Francisco, Cal
	Large-Scale NGS data Analysis on Amazon Web Services Using Globus Genomic	Genomics & Sequencing Data Integration, of Molecular Medicine Tri-Conference, St
	(Banarty An Interestive Semice)	

#### News Items

#### Opening at McMaster University

The McArthur Lab in the McMaster University Department of Biochemistry & Biomedical Sciences is seeking a Systems Administrator / Information Technologist to help establish a new bioinformatics laboratory at McMaster, plus develop the next generation of the Comprehensive Antibiotic Resistance Database (CARD).



From the job announcement on EvolDir:

The candidate will configure BLADE and other hardware for general bioinformatics analysis, development of a GIT version control system, construction of an in house Galaxy server (usegalaxy.org), and development of a new interface, stand-alone tools, APIs, and algorithms for the CARD (based on Chado).

See the full announcement for details.

Posted to the Galaxy News on 2014-12-05

#### December 2014 Galaxy Newsletter

As always there's a lot going on in the Galaxy this month. "Like what?" you say. Well, read the dang December Galaxy Newsletter we say! Highlights include:



- Galaxy Day! In Paris! This Wednesday!
- Near Richmond, Virginia? There's a Galaxy Workshop at Virginia State U on December 12.
- GCC2015 needs sponsors!
- · Other upcoming events on two continents
- · 96 new papers, including 6 highlighted papers, referencing, using, extending, and implementing Galaxy.
- Job openings at 7+ organizations
- A new mailing list: Galaxy-Training
- 15 new ToolShed repositories from 10 contributors
- . And, 10 other juicy (well maybe not juicy, but certainly not crunchy) bits of news

Dave Clements and the crisp Galaxy Team

Posted to the Galaxy News on 2014-12-01

#### Bioinformaticians, Freiburg

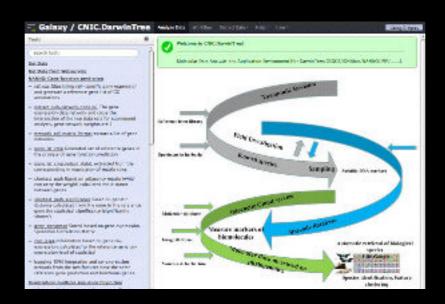
Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany has an opening for a Bioinformatician for an initial period of two years. The successful candidate will work at the interface between an in-house deepsequencing facility (HiSeq-2500) and the various research groups at the institute. Main responsibilities include

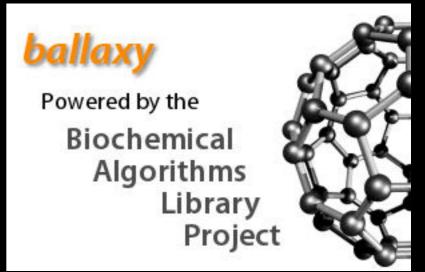


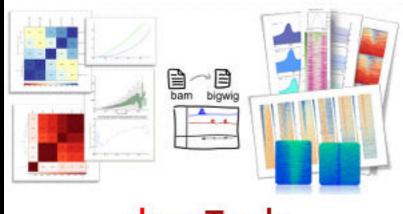
## Cistrome



A Galaxy Server dedicated to ChIP-\* analysis



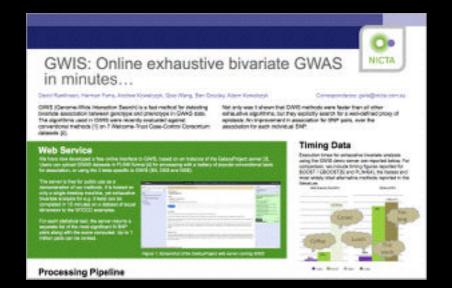




deepTools



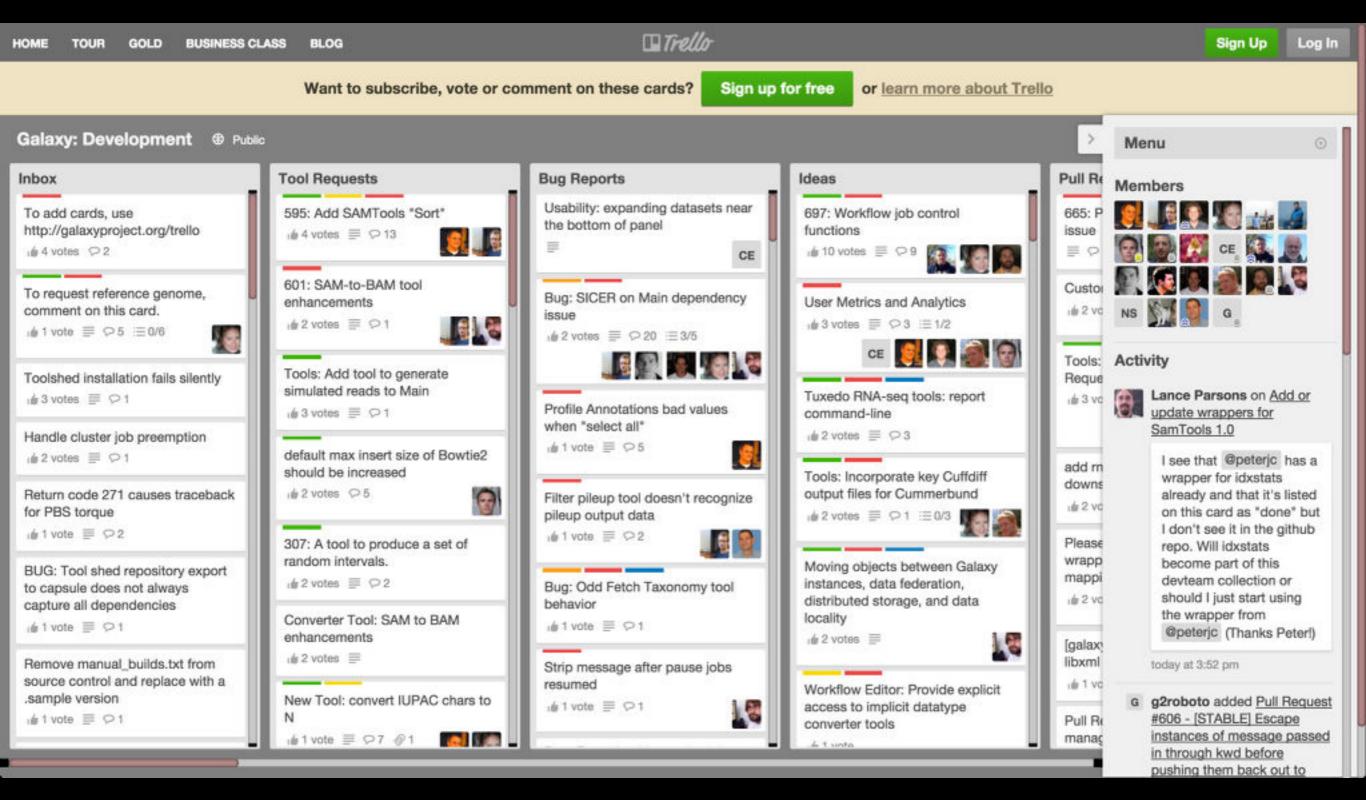








## Community can create, vote and comment on issues





## GALAXY COMMUNITY CONFERENCE

BALTIMORE, MD | JUNE 30 - JULY 2, 2014

Slides, posters & videos now online http://bit.ly/gcc2014











Norwich, UK

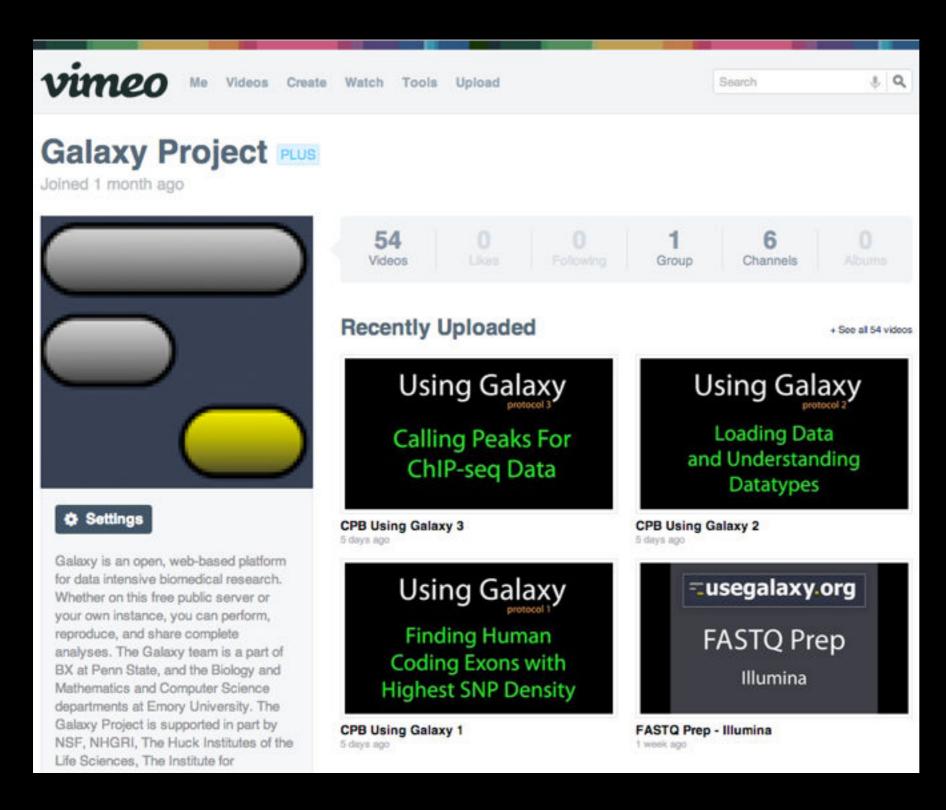
gcc2015.tsl.ac.uk

# Galaxy Australasia Workshop 20 1

We also support community organized efforts and events.



### **Galaxy Resources & Community: Videos**



"How to"
screencasts on
using and
deploying
Galaxy

Talks from previous meetings.

http://vimeo.com/galaxyproject

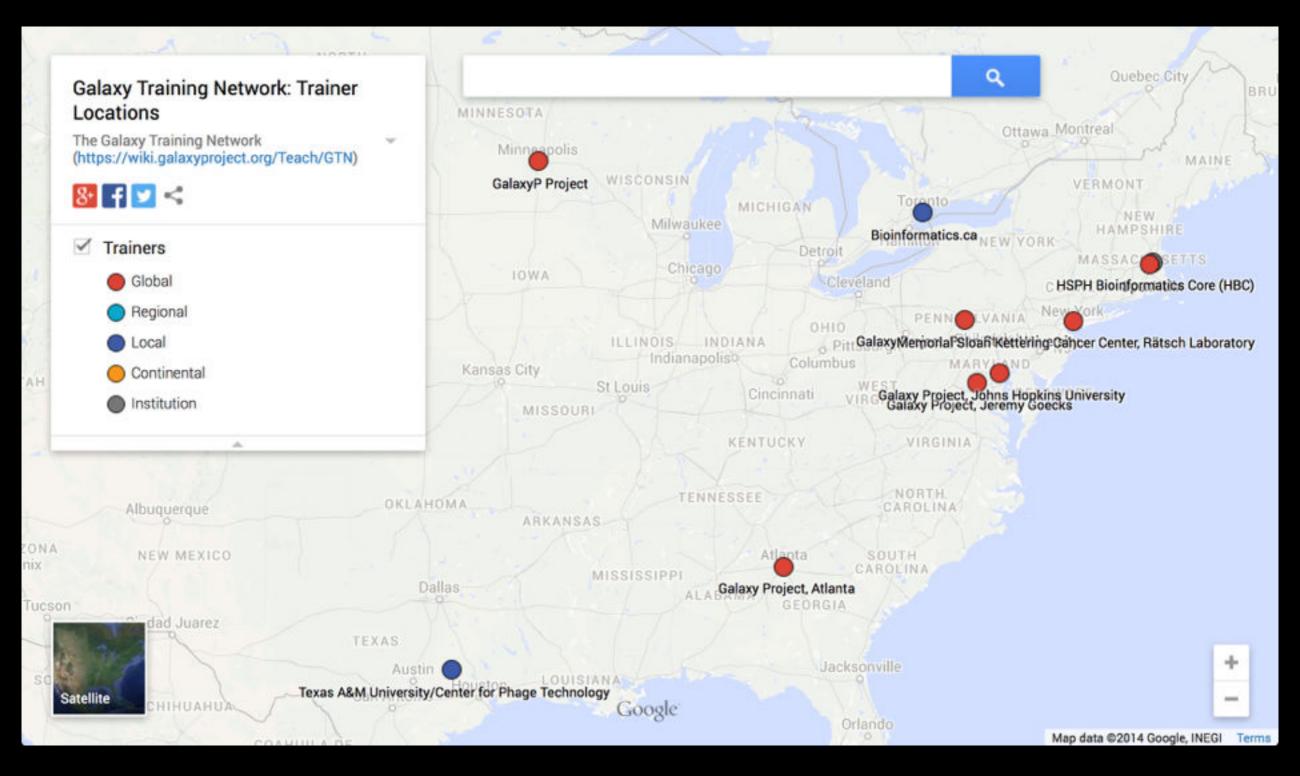
# Galaxy Resources & Community: CiteULike Group





Over 2100 papers

# **Scaling Training**



Galaxy Training Network launched In October.

bit.ly/gxygtn

# Agenda

9:00 Welcome 9:30 Basic Analysis with Galaxy 10:45 Break 11:00 Basic Analysis into Reusable Workflows 11:30 RNA-Seq Example Part I 12:20 Lunch (on your own) 1:35 RNA-Seq Example Part II Break 2:45 Sharing, Publishing, and Reproducibility 3:00 Setting up your own Galaxy Cluster on Amazon 3:25 4:00 Done

# Tuxedo Suite: Some parts of the ensemble

Bowtie	Short read mapper. Bowtie2 can do gapped alignments and emphasizes reads > 50 bases
Tophat	Intron-aware mapper for RNA-Seq data. Works with Bowtie to find best mapping locations
Cufflinks	Construct transcript predictions from mapped reads (from Tophat output)
Cuffmerge	Merges multiple sets of transcript predictions into a unified set with one coherent set of IDs.
Cuffdiff	Differential expression analysis; Can work with Tophat output directly or Cufflinks/merge, if looking for novel genes/transcripts

Used already

Will not use today

Will use next

RNA-Seq: Differential Expression with Cuffdiff

# RNA-Seq Differential Expression: Get the Data

Create new history

- (cog) → Create New
- Import:
  - Shared Data → Data Libraries
    - → RNA-Seq UCDavis 2013 Example Data\*
      - → Tophat Outputs
        - → Select all accepted\_hits datasets Also select genes\_chr12.gtf
        - And then Import to current history



\* RNA-Seq example datasets from the 2013 UC Davis Bioinformatics Short Course. http://bit.ly/ucdbsc2013

- Part of the Tuxedo RNA-Seq Suite (as are Tophat and Bowtie)
- Identifies differential expression between multiple datasets
- Widely used and widely installed on Galaxy instances

**NGS:** RNA Analysis → Cuffdiff

Cuffdiff previously used FPKM/RPKM as central statistic.

Total # mapped reads heavily influences FPKM/RPKM.

Can lead to challenges when you have very highly expressed genes in the mix.

- Running with 2 Groups: MeOH and R3G
- Each group has 3 replicates each

- Which Transcript definitions to use?
  - Official (genes\_chr12.gtf in our case)
  - MeOH or R3G Cufflinks transcripts
  - Results of Cuffmerge on MeOH & R3G Cufflinks transcripts
- Depends on what you care about

**NGS:** RNA Analysis → Cuffdiff

# Produces many output files, all explained in doc We'll focus on gene differential expression testing

test_id	gene_ld	gene	locus	sample_1	sample_2	status	value_1	value_2	log2(fold_change)	test_stat	p_value	q_value	significant
A2M	A2M	A2M	chr12:9217772-9268558	MeOH	R3G	NOTEST	3.32147	3.13694	-0.0824644	0	1	1	no
A2M-AS1	A2M-AS1	A2M-AS1	chr12:9217772-9268558	MeOH	R3G	NOTEST	7.45797	13.9413	0.902515	0	1	1	no
A2ML1	A2ML1	A2ML1	chr12:8975149-9029381	MeOH	R3G	NOTEST	4.83055	7.79884	0.691072	0	1	1	no
A2MP1	A2MP1	A2MP1	chr12:9381128-9386803	MeOH	R3G	NOTEST	2.49656	0	-inf	0	1	1	no
AAAS	AAAS	AAAS	chr12:53701239-53715412	MeOH	R3G	OK	269.035	159.23	-0.756683	-2.22857	0.0005	0.00194017	yes
AACS	AACS	AACS	chr12:125549924-125627871	MeOH	R3G	NOTEST	29.2933	35.0339	0.258178	0	1	1	no
ABCB9	ABCB9	ABCB9	chr12:123405497-123451056	MeOH	R3G	NOTEST	4.68869	1.7732	-1.40283	0	1	1	no
ABCC9	ABCC9	ABCC9	chr12:21950323-22089628	MeOH	R3G	OK	553.247	487.261	-0.18323	-2.02806	0.0004	0.00162143	yes
ABCD2	ABCD2	ABCD2	chr12:39945021-40013843	MeOH	R3G	OK	86.1377	172.795	1.00435	4.3436	5e-05	0.000246739	yes
ACACB	ACACB	ACACB	chr12:109577201-109706030	MeOH	R3G	NOTEST	8.45306	15.5772	0.881885	0	1	1	no
ACAD10	ACAD10	ACAD10	chr12:112123856-112194911	MeOH	R3G	NOTEST	21.8237	27.8326	0.350882	0	1	1	no
ACADS	ACADS	ACADS	chr12:121163570-121177811	MeOH	R3G	NOTEST	38.644	16.1739	-1.25658	0	1	1	no
ACRBP	ACRBP	ACRBP	chr12:6747241-6756580	MeOH	R3G	NOTEST	2.96987	3.26939	0.138621	0	1	1	no
ACSM4	ACSM4	ACSM4	chr12:7456927-7480969	MeOH	R3G	NOTEST	0	0	0	0	1	1	no
ACSS3	ACSS3	ACSS3	chr12:81471808-81649582	MeOH	R3G	NOTEST	0	0	0	0	1	1	no
ACTR6	ACTR6	ACTR6	chr12:100593864-100618202	MeOH	R3G	OK	475.594	421.324	-0.174799	-0.797581	0.1588	0.258406	no
ACVR1B	ACVR1B	ACVR18	chr12:52345450-52390863	MeOH	R3G	NOTEST	32.5737	38.3075	0.233922	0	1	1	no
ACVRL1	ACVRL1	ACVRL1	chr12:52301201-52317145	MeOH	R3G	NOTEST	1.27713	2.16161	0.759201	0	1	1	no
ADAM1A	ADAM1A	ADAM1A	chr12:112336866-112339706	MeOH	R3G	NOTEST	30.0162	55.2154	0.879331	0	1	1	no
ADAMTS20	ADAMTS20	ADAMTS20	chr12:43748011-43945724	MeOH	R3G	NOTEST	0.453322	0.502067	0.147346	0	1	1	no
ADCY6	ADCY6	ADCY6	chr12:49159974-49182820	MeOH	R3G	NOTEST	9.32722	17.6743	0.922135	0	1	1	no
ADIPOR2	ADIPOR2	ADIPOR2	chr12:1800246-1897845	MeOH	R3G	OK	207.468	179.333	-0.210248	-1.02392	0.09	0.158988	no
AEBP2	AEBP2	AEBP2	chr12:19592607-19675173	MeOH	R3G	OK	143.039	128.293	-0.156957	-0.688267	0.2254	0.344537	no
AGAP2	AGAP2	AGAP2	chr12:58118075-58135944	MeOH	R3G	OK	98.2385	116.302	0.243511	0.935119	0.11475	0.198086	no
AICDA	AICDA	AICDA	chr12:8754761-8765442	MeOH	R3G	NOTEST	78.1514	63.4313	-0.301077	0	1	1	no
AKAP3	AKAP3	AKAP3	chr12:4724675-4754343	MeOH	R3G	NOTEST	6.12385	7.89626	0.366731	0	1	1	no
ALDH1L2	ALDH1L2	ALDH1L2	chr12:105413561-105478341	MeOH	R3G	NOTEST	7.11374	8.11722	0.190377	0	1	1	no
ALDH2	ALDH2	ALDH2	chr12:112204690-112247789	MeOH	R3G	NOTEST	12.8033	8.05635	-0.668321	0	1	1	no
ALG10	ALG10	ALG10	chr12:34175215-34181236	MeOH	R3G	NOTEST	54.8575	59.3459	0.11346	0	1	1	no
ALG10B	ALG10B	ALG10B	chr12:38710556-38723528	MeOH	R3G	NOTEST	43.8157	63.0457	0.524952	0	1	1	no
ALKBH2	ALKBH2	ALKBH2	chr12:109525992-109531293	MeOH	R3G	OK	679.517	297.183	-1.19316	-3.34255	5e-05	0.000246739	yes
ALX1	ALX1	ALX1	chr12:85674035-85695561	MeOH	R3G	NOTEST	0	0	0	0	1	1	no
NEW WALK	100000000000000000000000000000000000000	1945		2002/02/0	ACCOUNT OF		16-20-22	PARTIE DE LA CONTRACTION DEL CONTRACTION DE LA C	DOM: DOM: DOM: DOM: DOM: DOM: DOM: DOM:	77/19	76	240	

# Cuffdiff: differentially expressed genes

Column	Contents
test_stat	value of the test statistic used to compute significance of the observed change in FPKM
p_value	Uncorrected P value for test statistic
q_value	FDR-adjusted p-value for the test statistic
status	Was there enough data to run the test?
significant	and, was the gene differentially expressed?

- Column 7 ("status") can be FAIL, NOTEST, LOWDATA or OK
  - Filter and Sort → Filter
    - c7 == 'OK'
- Column 14 ("significant") can be yes or no
  - Filter and Sort → Filter
    - c14 == 'yes'

Returns the list of genes with

- 1) enough data to make a call, and
- 2) that are called as differentially expressed.

# Cuffdiff: Next Steps

Try running Cuffdiff with different normalization and dispersion estimation methods.

Compare the differentially expressed gene lists. Which settings have what type of impacts on the results?

### RNA-Seq Differential Expression with Cuffdiff: Resources

RNA-Seq Concepts, Terminology, and Work Flows by Monica Britton

from the <u>UC Davis 2013 Bioinformatics Short Course</u>

RNA-Seq Analysis with Galaxy by Jeroen F.J. Laros, Wibowo Arindrarto, Leon Mei

from the GCC2013 Training Day

RNA-Seq Analysis with Galaxy
by Curtis Hendrickson, David Crossman, Jeremy Goecks
from the GCC2012 Training Day

# Agenda

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 3:25
 4:00
       Done
```

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# 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**

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





HOME ABOUT ARCHIVE SUBMIT SUBSCRIBE ADVERTISE AUTHORINFO CONTACT HELP

Institution: PENN STATE UNIV Sign In via User Name/Password

Search for Keyword: Go
Advanced Search

# Windshield splatter analysis with the Galaxy metagenomic pipeline

Sergei Kosakovsky Pond<sup>1,2,6,9</sup>, Samir Wadhawan<sup>3,6,7</sup>,

Francesca Chiaromonte<sup>4</sup>, Guruprasad Ananda<sup>1,3</sup>, Wen-Yu Chung<sup>1,3,8</sup>,

James Taylor1,5,9, Anton Nekrutenko1,3,9 and The Galaxy Team1

#### OPEN ACCESS ARTICLE

#### This Article

Published in Advance October 9, 2009, doi: 10.1101/gr.094508.109 Copyright © 2009 by Cold

Spring Harbor Laboratory Press

Abstract Free
 Full Toyt (BDE) 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.]

Published Pages | aun1 | Windshield Splatter

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

SERGEI KOSAKOVSKY POND<sup>1,2,\*</sup>, SAMIR WADHAWAN<sup>3,6\*</sup>, FRANCESCA CHIAROMONTE<sup>4</sup>, GURUPRASAD ANANDA<sup>1,3</sup>, WEN-YU CHUNG<sup>1,3,7</sup>, JAMES TAYLOR<sup>1,5</sup>, ANTON NEKRUTENKO<sup>1,3</sup> and THE GALAXY TEAM<sup>1\*</sup>

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

About this Page



#### Author

aun1

#### **Related Pages**

All published pages Published pages by aun1

#### Rating

Community (6 ratings, 5.0 average)



#### Tags

#### Community:

megan

paper galaxy

# Basic Analysis: Further reading & Resources

http://usegalaxy.org/galaxy101

https://vimeo.com/76343659

# Agenda

9:00 Welcome Basic Analysis with Galaxy 9:30 10:45 Break Basic Analysis into Reusable Workflows 11:00 RNA-Seq Example Part I 11:30 Lunch (on your own) 12:20 1:35 RNA-Seq Example Part II Break 2:45 Sharing, Publishing, and Reproducibility 3:00 Setting up your own Galaxy Cluster on Amazon 3:25 4:00 Done

# Galaxy is available ...







OpenNebula.org
The Open Source Toolkit for Cloud Computing

http://aws.amazon.com/education http://globus.org/ http://wiki.galaxyproject.org/Cloud

We are using the cloud today.

# **AWS in Education Grants Program**



http://aws.amazon.com/education

# What is our path?

### Today we will:

- Launch our own Galaxy server on AWS
- Make the server dynamically scalable in response to demand.
- Run some basic analysis on it.
- Make it go away.

### Full Disclosure

To use AWS you must create an AWS account with a credit card associated with it.

You must also have created a key pair.

We will use the IAM account for this workshop.

# IAM Accounts

Imagine, a link to a list of accounts, and credentials, here.

# CloudLaunch: From <u>UseGalaxy.org</u>

**Analyze Data** 

Workflow

Shared Data +

Visualization

Cloud -

Help -

**New Cloud Cluster** 

User +



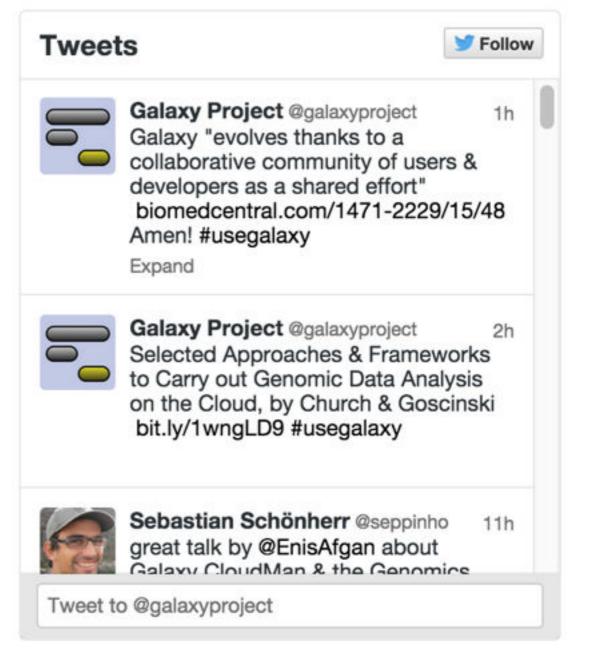


tion

itervals

Galaxy is an open source, web-based platform for data intensive biomedical research. If you are new to Galaxy start here or consult our help resources.







Analyze Data

Workflow

Shared Data -

Visualization

Cloud +

Help+

User +

#### Launch a Galaxy Cloud Instance

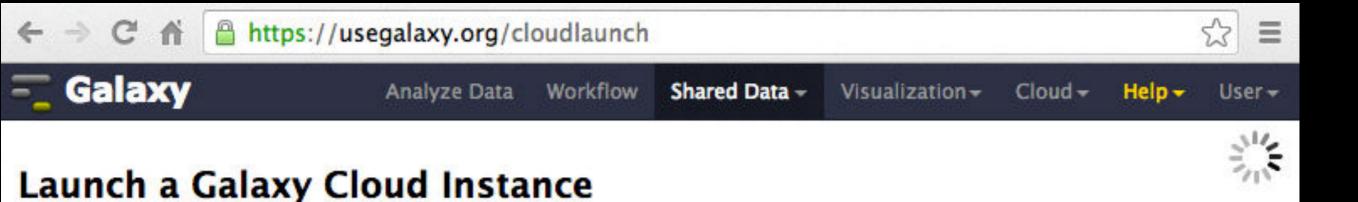
To launch a Galaxy Cloud Cluster, enter your AWS Secret Key ID, and Secret Key. Galaxy will use these to present appropriate options for launching your cluster. Note that using this form to launch computational resources in the Amazon Cloud will result in costs to the account indicated above. See <a href="mailto:Amazon's pricing">Amazon's pricing</a> for more information.

Key ID	
This is the text string that uniqu	lely identifies your account, found in the <u>Security Credentials section of the AWS Console</u>
Secret Key	
This is your AWS Secret Key, als	o found in the Security Credentials section of the AWS Console.

#### Launch a Galaxy Cloud Instance

To launch a Galaxy Cloud Cluster, enter your AWS Secret Key ID, and Secret Key. Galaxy will use these to present appropriate options for launching your cluster. Note that using this form to launch computational resources in the Amazon Cloud will result in costs to the account indicated above. See <a href="mailto:Amazon's pricing">Amazon's pricing</a> for more information.

Key ID
This is the text string that uniquely identifies your account, found in the Security Credentials section of the AWS Console.
Secret Key
This is your AWS Secret Key, also found in the Security Credentials section of the AWS Console.
Instances in your account
New Cluster
Cluster Name
This is the name for your cluster. You'll use this when you want to restart.
Cluster Password
Cluster Password - Confirmation
Key Pair
cloudman_keypair \$
Instance Type
Compute optimized Large (2 vCPU/4GB RAM) \$
Requesting the instance may take a moment, please be patient. Do not refresh your browser or navigate away from the page
Submit





Launch Pending, please be patient.

Galaxy

Analyze Data

Workflow

Shared Data -

Visualization

Cloud -

Help -

User-

#### Launch a Galaxy Cloud Instance

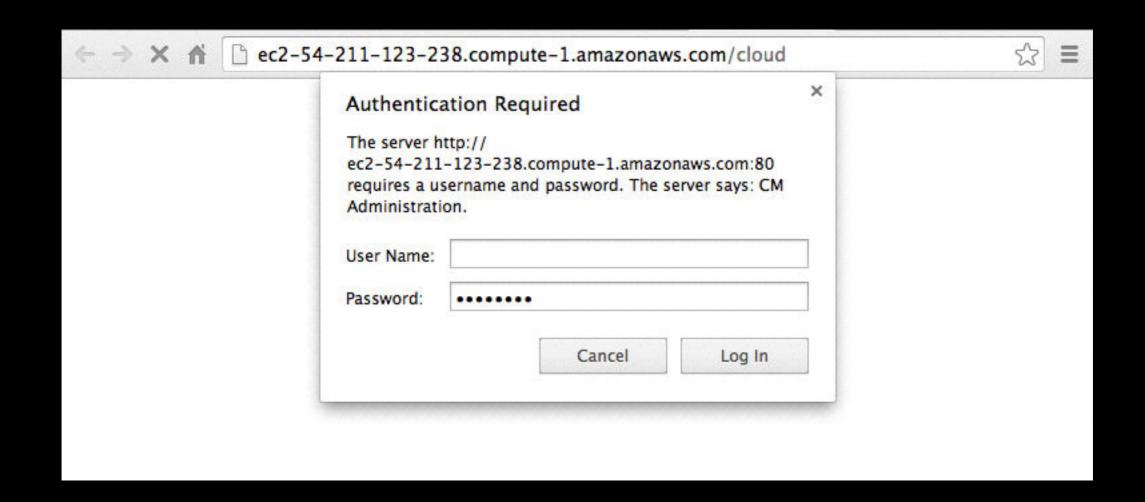
#### Access Information

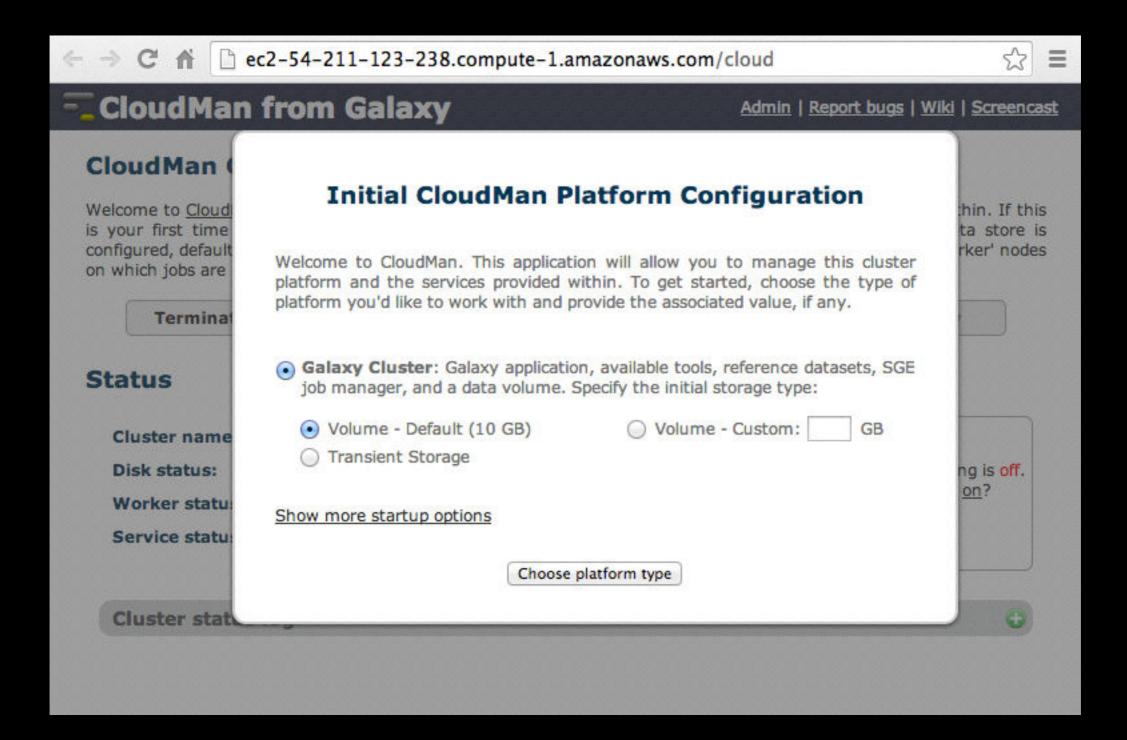
Your instance 'i-61503e9b' has been successfully launched using the 'ami-a7dbf6ce' AMI.

While it may take a few moments to boot, you will be able to access the cloud control panel at ec2-54-196-164-110.compute-1.amazonaws.com/cloud.

SSH access is also available using your private key. From the terminal, you would execute something like:

`ssh -i cloudman\_key\_pair.pem ubuntu@ec2-54-196-164-110.compute-1.amazonaws.com`







ec2-54-211-123-238.compute-1.amazonaws.com/cloud







#### Messages

Initializing 'Galaxy' cluster type. Please wait... (2014-01-15 06:48:34)

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

Terminate cluster

Add nodes ▼

Remove nodes

**Access Galaxy** 

#### Status

Cluster name: PAG\_CLOUD\_2

0 / 0 (0%) Disk status:

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

Service status: Applications 

Data

Autoscaling is off. Turn on?

Cluster status log



### Cloud Launched





← → C ↑ ec2-54-211-123-238.compute-1.amazonaws.com/cloud



×





#### Messages

Initializing 'Galaxy' cluster type. Please wait... (2014-01-15 06:48:34)

All cluster services started; the cluster is ready for use. (2014-01-15 06:53:24)

is your first time running this cluster, you will need to select an initial data volume size. Once the data store is configured, default services will start and you will be able to add and remove additional services as well as 'worker' nodes on which jobs are run.

Terminate cluster

Add nodes ▼

Remove nodes

Access Galaxy

#### Status

Cluster name: PAG\_CLOUD\_2

Disk status:

3.2G / 10G (32%) 🕝

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

Service status: Applications 

Data

Autoscaling is off. Turn on?

Cluster status log



# Cool things to do

- Create a login
- Become an admin
- Set up autoscaling
- Run ~ Galaxy 101
  - http://usegalaxy.org/galaxy101
- Shut it down

# Agenda

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# We Need Your Feedback

# The Galaxy Team



**Enis Afgan** 

Dannon Baker

Dan Blankenberg

**Dave Bouvier** 

Marten Cech

John Chilton



**Dave Clements** 

**Nate Coraor** 

Carl Eberhard

Jeremy Goecks

Sam Guerler



Jen Jackson

**Ross Lazarus** 

Anton Nekrutenko

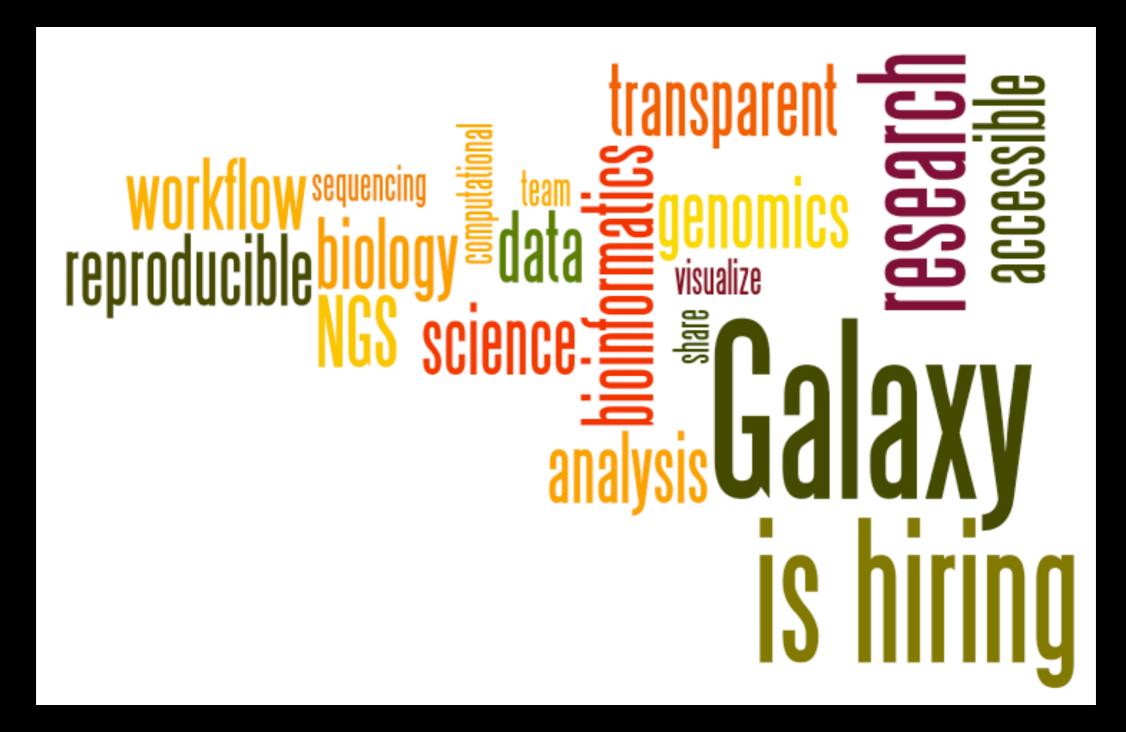
Nick Stoler

**James Taylor** 

Nitesh Turaga

http://wiki.galaxyproject.org/GalaxyTeam

# Galaxy is hiring post-docs and software engineers



Please help.

http://wiki.galaxyproject.org/GalaxylsHiring

### Also Thanks To













# Pamela Shaw Kristi Holmes

National Institutes of Health

# Agenda

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 3:25
 4:00
       Done
```

### **Thanks**



### **Dave Clements**

Galaxy Project

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# Matt Schipma

NGS Core Facility
Center for Genetic Medicine (CGM)
Northwestern University
m-schipma@northwestern.edu

### Cuffmerge

- Each Cufflinks run creates a set of transcript predictions.
- Cuffmerge unifies all those predictions into a single set.
- Makes this incredibly tedious task easy.