Introduction to Galaxy

University of Utah November 16, 2016

Dave Clements Galaxy Team Johns Hopkins University http://galaxyproject.org/



Biomedical Informatics





y @galaxyproject

Agenda

9:00 Welcome

- 9:20 Basic Analysis with Galaxy
- 10:45 Break
- 11:00 Basic Analysis into Reusable Workflows
- 12:20 Lunch (on your own)
 - 1:20 RNA-Seq Analysis, Part I
 - 2:50 Break
 - 3:05 RNA-Seq Analysis, Part II
 - 5:00 Done

Goals

Provide an 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.

This workshop does cover RNA-Seq but you won't be an expert at the end of the workshop. You will know enough to get started, and how to use Galaxy to learn more. What is Galaxy?

Keith Bradnam's definition:

"A web-based platform that provides a simplified interface to many popular bioinformormatics tools."

From

"13 Questions You May Have About Galaxy"

http://bit.ly/13questions

Galaxy is available several ways ...

http://galaxyproject.org

As a free for everyone service on the web: usegalaxy.org



Galaxy is available as Open Source Software

Galaxy is installed in locations around the world.

http://getgalaxy.org





Welcome to the Metabiome Portal @ GMU We have also in the MMC Metablicity Parally, a finalize and contrastation we have a with the arm of a repliciting on



香港中文大學 - 華大基因跨組學創新研究院

Integrated publishing of workflows from (GIGA)ⁿ SCIENCE

Cistrome



A Galaxy Server dedicated to ChIP-* analysis



Public Galaxy Servers and still counting



The Genomic HyperBrowser

Powered by Galaxy

SCDE •• STEM CELL DISCOVERY ENGINE

Experiments Connected



Whale Shark Galaxy! SG



Genomic analysis tools for southern and **Mediterranean plants**

bit.ly/gxyServers

Galaxy is available on the Cloud







The Open Source Toolkit for Cloud Computing



We are using this today

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

Galaxy on the Cloud: Galaxy CloudMan http://usegalaxy.org/cloud

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



Galaxy on the Cloud: CloudLaunch https://launch.usegalaxy.org/

- Directly launch a Galaxy instance on AWS or Jetstream
- Uses CloudMan

Galaxy Cloud Launch

Easily launch your own cloud servers for use with Galaxy and CloudMan. See this page for detailed instructions on how to get started.

	Amazon - Tokyo (AWS EC2)						
Cloud	✓ Amazon - Virginia (AWS EC2)						
	Amazon - Ireland (AWS EC2) Jetstream (development) (OpenStack)						
Access key							
100000000000000	Your cloud account API access key. For the Amazon cloud, available from the security credentials page.						
Secret key	Your cloud account ADI secret key. For the Amazon cloud, also available from the convity credentials page						
	Four cloud account API secret key. For the Amazon cloud, also available from the security credentials page.						
Cluster name	Specify a new name or Choose a saved cluster						
	Name of your cluster used for identification and restarting. If creating a new cluster, type any name you like.						
Password							
	Your choice of password, for the CloudMan web interface and accessing the server via ssh.						
Instance type	Compute Optimized large (c3.large) (2 vCPU / 3.75GB R 👻						
2007-2019 (2017), 2019 (2017) (2019)	Type (ie, virtual hardware configuration) of the server to start.						

Galaxy on the Cloud: Jetstream https://wiki.galaxyproject.org/Cloud/Jetstream

Jetstr	eam		
H Images	Help	Login	l
Q SEARCH	TAGS		
← Ga	laxy Standalo	ne	
	Created:	3/30/2016 09:08 am MDT	
	Created by:	admin	
	Description:	Galaxy 16.01 Standalone - based on Ubuntu 14.04.4 LTS	
		This is a standalone Galaxy server that comes preconfigured with hundreds of tools and commonly used reference datasets: just launch and use.	е
		It is necessary to launch an instance type of Large or larger.	
		See https://wiki.galaxyproject.org/Cloud/Jetstream for information about this image and using it.	
		For additional information see:	

US based researchers can request an XSEDE allocation and then run Galaxy on Jetstream

U XSEDE Champion: Anita Orendt

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Quick Poll: Are you ...

1. A bioinformatics novice

2. A bioinformatics apprentice

3. A bioinformatics guru

Yes, those are your only choices.

http://galaxyproject.org

Basic Analysis

Which exons have most overlapping Repeats?

Use Human, HG38, GENCODE v24, Chromosome 22

bit.ly/utah_cloud1 (54.224.69.148) bit.ly/utah_cloud2 (54.83.150.22) bit.ly/utah_cloud3 (54.144.233.255)

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)



Use this program to retrieve the data associated with a track in text format, to calculate intersecti DNA sequence covered by a track. For help in using this application see <u>Using the Table Browse</u> this form, the <u>User's Guide</u> for general information and sample queries, and the OpenHelix Table presentation of the software features and usage. For more complex queries, you may want to us To examine the biological function of your set through annotation enrichments, send the data to <u>G</u> for use with diverse computational tools. Refer to the <u>Credits</u> page for the list of contributors and these data. All tables can be downloaded in their entirety from the <u>Sequence and Annotation Dov</u>

clade: Mammal \$ genome:	Human	\$	assembly:	Dec. 2013 (GRCh38/	hg38) 🛊
group: Genes and Gene Predictions \$	track:	GENCODE v24	÷	add custom tracks	track hubs
table: knownGene	\$ descri	be table schema			
region: genome positio	hr22		lookup defin	e regions	
identifiers (names/accessions):	paste list	upload list			
filter: create					
intersection: create					
correlation: create					
output format: BED - browser extensi	ble data	\$	Send output	to 🗷 <u>Galaxy</u> 🗉	GREAT
output file:	(leav	ve blank to k	eep output ir	n browser)	
file type returned: plain text 	gzip c	ompressed			



Output knownGene as BED

Include <u>custom track</u> header:

name= tb_kno	wnGene	
description=	table browser query on knownGene	
visibility= pa	ck 🛊	

Create one BED record per:

- Whole Gene
- O Upstream by 200 bases

0

- Exons plus
- Introns plus
- 5' UTR Exons
- Coding Exons
- 3' UTR Exons
- Ownstream by 200 bases

Note: if a feature is close to the beginning or end of a chromosome and upstream/downstreat in order to avoid extending past the edge of the chromosome.

bases at each end

bases at each end

Send query to Galaxy

Cancel



Exons



Repeats

(Identify which exons have Repeats)







Operate on Genomic Intervals \rightarrow Join (Identify which exons have Repeats)







(Count Repeats per exon)



Join, Subtract, and Group → Group Published History: Exons with overlapping repeats, basic

We have exon names and counts!

We are now going to extend that work.

Let's create a copy of this history that we will extend.

But first, create a login

Don't need to login to use Galaxy, but do need one to use all its features

Use an email address you can remember.

Use a low security password.

This account will go away on Wednesday night.



Create accoun	t
Email addres	5:
Password:	
Confirm pass	word:
Public name:	
Your public na you share pub only lower-ca	me is an identifier that will be used to licly. Public names must be at least thre se letters, numbers, and the '-' characte

Second, name your existing history

History	C	\$		
search datasets			0	Give your
Unnamed history 4 shown Click to rename hist 3.79 MB	tory	•	•	existing history a meaningful
<u>4: Exons with overlappin</u> <u>g repeats.</u>	۲	ø	×	name.
3. Join on data 2 and dat				

3rd, make a copy of your history



(cog) → Copy History
 Name the copy based on the exercise you pick

Becomes your new current history.



Exons & Repeats: Pick an Exercise

- Report the number of overlapping repeats that each exon has (what we just did), but also include exons with 0 overlapping repeats in the output.
- Create the list of exons with overlapping repeats, in 6column BED format. Set the score column to be the number of overlapping repeats that exon has.

Everything you need will be in these toolboxes

- Text manipulation (cut is particularly useful)
- Join, subtract and group
- Filter and sort
- Operate on genomic intervals

1. All exons, even those with 0 overlaps

Can take advantage of fact that score column of all exons is 0 to begin with.

Join, subtract and group is a good place to start.

Published History: Exons with number of overlapping repeats, including 0

2. List of exons with overlaps, in BED

Can be done in two steps, one of them a Cut, plus an edit attributes step at the end:

Attributes Convert Format Datatype Permissions	History 2 🗘 🗔
Change data type	search datasets
New Type: bed This will change the datatype of the existing dataset but <i>not</i> modify its contents. Use this if Calaxy has incorrectly quessed the type of your dataset	Exons with overlapping repeats, in BED 6 shown 3.92 MB
Save	6: Exons with overlapp ing repeats, in BED 792 regions format: interval, database: hg38
	Score column is the number of repeats that overlap with this exon.
	B 6 2 Lul 📎 🗩
	1.Chrom 2.Start 3.End 4.Name

Published History: Exons with overlapping repeats, in BED





Exon overlap counts

Exons



Join on exon name

Join, Subtract, and Group \rightarrow Join

(Incorporate the overlap count with rest of Exon information)





Exons



Text Manipulation \rightarrow Cut

(Incorporate the overlap count with rest of Exon information)

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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
- And then rolling that up to genes
- But, ...
 - is there anything inherent in the analysis about humans, exons or repeats?

Get back to the original history



Get back to the original history

= Galaxy	Analyze Data Workflow Shared Data - Visualiza	tion 🗸 🛛 Help 🗸	User 🗸 📲	Using 2.1 MB
Done search histories	Search all datasets	0 0		Create new
Current History	Switch to	-	Switch to	· _
Exons with overlapping repeats, in BED 6 shown	Exons with number of overlapping repeats, includ 7 shown	ing O	4 shown	Loading
3.92 MB	5.71 MB	2 🌒 🗩	3.79 MB	histo V
search datasets	search datasets	0	search datasets	O ries
Drag datasets here to copy them to the current history	7: Exons with # of overlapping repeats, including t	• / ×	4: Exons with number of overlapping repeats	• # ×
s, BED format	14,875 lines		3: Join on data 2 and data 1	• # ×
792 regions	format: tabular, database: hg38		2: Repeats, chr22	• # ×
	₿ 6 2 ш	•	1: Exons. chr22	
display in IGB View	1 2 uc002zly.5_cds_10_0_chr22_17105853_f 1			
1.Chrom 2.Start 3.End 4.Name chr22 11065973 11066015 uc062bdg.1_cds_0_0_c	6: Cut on data 5	• / ×		
	5: Compare two Datasets on data 4 and data 1	• / ×		
5: Join two Datasets on data 4 an <u>d data 1</u>	4: Exons with number of overlapping repeats	• / ×		
792 regions format: bed , database: hg38	3: Join on data 2 and data 1	● # ×		
B 6 2 III >> >	2: Repeats, chr22	• / ×		
display in IGB <u>View</u>	1: Exons, chr22	• / ×		
1.Chrom 2.Start 3.End 4.Name				
4: Exons with number of overlap (*) * *				
792 lines				
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

Histo-	
	HISTORY LISTS
se	Saved Histories
Exor	Histories Shared with Me
basi	HISTORY ACTIONS
4 sho	Create New
3.79	Copy History
4: Ex	Share or Publish
g rep	Show Structure
3: 10	Extract Workflow
<u>a 1</u>	Delete
2. Po	Delete Permanently
<u>2. Re</u>	DATASET ACTIONS
<u>1: Ex</u>	Copy Datasets
	Dataset Security
	Resume Paused Jobs
	Collapse Expanded Datasets
	Unhide Hidden Datasets
	Delete Hidden Datasets
	Purge Deleted Datasets
	DOWNLOADS
	Export Tool Citations
	Export History to File
	OTHER ACTIONS
	Import from File

Create a Workflow from a History: ...

The following list contains each tool that was run to create the datasets in your current history. Please select those that you wish to include in the workflow.

Tools which cannot be run interactively and thus cannot be incorporated into a workflow will be shown in gray.



Workflow editor



Published Workflow: Count Overlaps Between Feature Sets

Workflow editor: save your changes

Tools	Workflow Canvas count overlapping) features		¢	Details	
(search tools					Edit Wor	rkflow Attributes
Inputs			Help- User-			Usir
Get Data				^	Details	
Send Data				Sava	Tiarsite	
Lift-Over				Dure		orkflow Attributes
Text Manipulation				Kun 🕫		in reh
Filter and Sort				Edit Attribute	S	e tag.
NGS: QC and manipulation	🕒 Input dataset 🗙	ך Join א		Auto Re-layo	ut	verlapping features
NGS: DeepTools	output	loin		Close		low
NGS: Mapping	output					
NGS: RNA Analysis	(with			Annhu	able
NGS: SAM Tools	<u> </u>	output (interval) 🛛 🕤 🖉			Apply for any	tags to make it easy
NGS: BAM Tools						
NGS: Picard	Imput dataset					
NGS: Variant Analysis	output					
NGS: VCF Manipulation						
NGS: ChIP-seq						
Join, Subtract and Group						
Operate on Genomic Intervals						
BEDtools						
Convert Formats						
FASTA manipulation						
Extract Features						
Fetch Sequences						
Fetch Alignments						
<				N	111	>

Published Workflow: Feature Overlap Counting

Workflow Testing

Guided: rerun with same inputs Workflow → Run Did that work?

On your own: Count # of exons overlapping each repeat Did that work? *Why not?* Edit workflow: doc assumptions



Published Workflow: Count overlaps between feature sets

Workflows: Sweet spots

Short, well-defined tasks, with well-defined inputs and outputs.

Analysis pipelines for large experiments with many samples where sample and data preparation protocols are the same throughout.

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Quick Poll: Are you ...

- 1. An RNA-Seq novice
- 2. An RNA-Seq apprentice
- 3. An RNA-Seq guru
 - Yes, those are your only choices.

http://galaxyproject.org

RNA-Seq Analysis: Get the Data

Shared Data \rightarrow Data Libraries \rightarrow Training \rightarrow RNA-Seq* \rightarrow UC-Davis \rightarrow Raw Reads Select first two MeOH_REP1_R1 MeOH_REP1_R2 Import into a new history



* RNA-Seq example datasets from the 2016 UC Davis Using Galaxy for Analysis of RNA-Seq, Exome-Seq, and Variants. <u>bit.ly/ucdrnaseq2016</u>

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



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

SSSSSSSSSSSSSSSS	SSSSSSSSSSS	SSS	SSSSSSS	SSSSS	SSSS	SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS	SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS	SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
			.IIIII			IIIIIIIIIIIIIIIIIIII	IIIIIIIIIIIIIIIIIII	IIIIIIIIIIIIII
		XXX	XXXXXXXX	XXXXX	XXXX	*****	<pre>xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx</pre>	*****
!"#\$%&'()*+,/	0123456789 :;	;<=>	>?@ABCDI	EFGHI	JKLMI	NOPQRSTUVWXYZ[\]^_	abcdefghijklmno	pqrstuvwxyz{ }~
33	59)	64	73			104	126
S – Sanger	Phred+33,	93	values	(0,	93)	(0 to 60 expected	in raw reads)	
I – Illumina 1.3	Phred+64,	62	values	(0,	62)	(0 to 40 expected	in raw reads)	
X – Solexa	Solexa+64,	67	values	(-5,	62)	(-5 to 40 expected	d in raw reads)	

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

NGS Data Quality: Assessment tools

NGS QC and Manipulation → FastQC

Generates summary quality information.

FastQC Read Qua	▼ Options		
Short read data	from your current history		
C 2 C	1: MeOH_REP1_R1.fastq		•
Contaminant list			
C 2 C	Nothing selected		•
RNA RT Primer C/	AAGCAGAAGACGGCATACGA	roi example. munn	ia Sillali
C 2 C	Nothing selected		-
a file that specifie specifies the thre Execute	s which submodules are to be execute sholds for the each submodules warning	ed (default=all) and ng parameter	also

http://bit.ly/FastQCBoxPlot

NGS Data Quality: Assessment tools



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)

Common Trimming options

- Drop the first n columns from your reads
- Drop the last n columns from your reads
- Sliding window approach: only keep regions that are above a specified quality threshold
- Keep or drop whole read based on overall quality

Common Trimming Pitfalls

Broken Pairs

Often, one side of a pair passes QC, while the other does not. Broken pairings can affect results in subtle or drastic ways

Short short reads.

QC may reduce reads to a length at which their mapping is no longer meaningful.

Need help with Trimming? (and anything else)

That's a whole lotta options...

Choices you make now have impact on downstream tools NGS = a whole lotta options in general What to do? How to better understand bioinformatics & Galaxy

- Experiment. (You are already used to the idea and)
 Galaxy makes it easy
- Read tool documentation and tool and method review papers
- Get Help!
 - http://biostars.org/
 - http://seqanswers.com/
 - https://biostar.usegalaxy.org/
 - http://galaxyproject.org/search





Trimmomatic to the rescue

Frimmomatic flexible read trimming tool for Illumina NGS data (Galaxy Tool Version 0.32.3)	▼ Options
Paired end data?	
Yes No	
Input Type	
Pair of datasets	•
Input FASTQ file (R1/first of pair)	
□ 🖄 □ 1: MeOH_REP1_R1	•
Input FASTQ file (R2/second of pair)	
□	•
Perform initial ILLUMINACLIP step?	
Cut adapter and other illumina-specific sequences from the read	
Trimmomatic Operation	
1: Trimmomatic Operation	圓
Select Trimmomatic operation to perform	
Sliding window trimming (SLIDINGWINDOW)	•

Bolger, A.M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics*, doi: 10.1093/bioinformatics/btu170

Trimmomatic Operation

2

1: Trimmomatic Operation

Select Trimmomatic operation to perform

Sliding window trimming (SLIDINGWINDOW)

Sliding window trimming (SLIDINGWINDOW)

Drop reads below a specified length (MINLEN)

Cut bases off the start of a read, if below a threshold quality (LEADING)

Cut bases off the end of a read, if below a threshold quality (TRAILING)

Cut the read to a specified length (CROP)

Cut the specified number of bases from the start of the read (HEADCROP)

Trimmomatic preserves read pairing

向

Multiple filters can be run in arbitrary order

We'll use sliding window, followed by minimum length.

Run FastQC on post-Trimmatic Datasets

NGS QC and Manipulation → FastQC

Now, let's see what changed

Shared History: RNA-Seq MeOH_REPI QC

Scratchbook: View multiple datasets

Jser-	- Us	i 1g 1.3 GB	User+	Usi	ng 1.3	GB
	Enable/Disable Scratchbook	C ✿ □	History		C 🕈	
U	search datasets	8	search datas	ets		0
	RNA-Seq Example 1 6 shown		RNA-Seq Exam 6 shown	nple 1		
	57.1 MB	S D	57.1 MB		2	•
	<u>6: FastQC on data 2: Ra</u> <u>wData</u>	• / ×	<u>6: FastQC on d</u> wData	<u>ata 2: Ra</u>	•	x

And the icon turns yellow!

Poke the pre-Trimmomatic reverse read FastQC report in the eye, and then poke the post-Trimmomatic FastQC report in the eye.



And after some resizing and scrolling you see this

NGS Data Quality Assessment

Now, just 10 more datasets to go!

Your Friend: The Multiple datasets button

aired	end dat	a?		
Yes	No			
Inpu	t Type			
Pair	of datas	sets		
In	put FAS	rQ file	(R1/first of pair)	
[3 4		1: MeOH_REP1_R1.fastq	
м	ultiple da	tasets	(R2/second of pair)	
	3 0		2: MeOH_REP1_R2.fastq	

Cut adapter and other illumina-specific sequences from the read

Trimmomatic Operation

1. Trimmomatic Operation

rsion	0.32.3	3)	
aired	end d	ata?	
Yes	No		
Input	Туре		
Pair	of dat	asets	
Inp	ut FA	STQ f	ile (R1/first of pair)
Inp	ut FA	STQ f	11: R3G_REP3_R1.fastq 10: R3G_REP2_R2.fastq 9: R3G_REP2_R1.fastq 8: R3G_REP1_R2.fastq 7: R3G_REP1_R1.fastq This is a batch mode input field. A separate job will be triggered for each dataset.
) 2) C	 12: R3G_REP3_R2.fastq 11: R3G_REP3_R1.fastq 10: R3G_REP2_R2.fastq 9: R3G_REP2_R1.fastq 8: R3G_REP1_R2.fastq This is a batch mode input field. A separate job will be triggered for each dataset.

Yes No

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RNA-seq Exercise: Differential gene expression

Take samples under multiple conditions (MeOH and R3G exposure in our example)

Map them Count them Compare them

RNA-Seq Mapping: Get the Data

Import into a new history:

Shared Data → Data Libraries → Training → RNA-Seq

- → UC-Davis* → Post QC reads → Still paired reads Select first two MeOH_REP1_R1 post QC MeOH_REP1_R2 post QC
 Shared Data → Data Libraries → Training → RNA-Seq
 - → UC-Davis → Reference Select chr12.gencode.v25.basic.annotation.gtf

* RNA-Seq example datasets from the 2016 UC Davis Using Galaxy for Analysis of RNA-Seq, Exome-Seq, and Variants. <u>bit.ly/ucdrnaseq2016</u>

RNA-seq Exercise: Mapping with Tophat2

- 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: Make it quicker?

Warning: Here be dragons!

• Allow indel search \rightarrow No

Mapping with Tophat: Use Existing Annotations?

- You can bias Tophat towards known annotations
 - Supply your own junction Data? → Yes
 - Use Gene Annotation → Yes
 - Gene Model Annotation →

chr12.gencode.v25.basic.annotation.gtf

You can also restrict Tophat to known annotations

- Use Raw Junctions → Yes (tab delimited file)
- Only look for supplied junctions → Yes

Mapping w/ 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-secondaryalignments, 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
Condition

Condition
1: Condition
Filter
1: Filter
Select BAM property to filter on
mapQuality
Filter on read mapping quality (phred scale)
>=20
You can use >, <, =, and ! (not) in your expression. E.g., to select reads with mapping quality of at least 30 use ">=30"
2: Filter
Select BAM property to filter on
isProperPair -
Select properly paired reads Yes No Checked = Read IS in proper pair, Empty = Read is NOT in the proper pair
Insert Filter
Insert Condition
Would you like to set rules?

Yes No

Allows complex logical constructs. See Example 4 below.



Mapping With Tophat: What to keep?

NGS BAM Tools \rightarrow Filter

This shows two options for cleanup.

Only 5 more replicates to go!

Another way to avoid insanity is Collections RNA-Seq Differential Expression: Get the Data

Import into a new history:

Shared Data → Data Libraries → Training → RNA-Seq*

→ UC-Davis → Mapped and Filtered Select all (OK, maybe just half of them)

Shared Data → Data Libraries → Training → RNA-Seq*

→ UC-Davis → Reference Select chr12.gencode.v25.basic.annotation.gtf

* RNA-Seq example datasets from the 2016 UC Davis Using Galaxy for Analysis of RNA-Seq, Exome-Seq, and Variants. <u>bit.ly/ucdrnaseq2016</u>

Dataset collections!

Dataset Collections give Galaxy semantic knowledge about dataset relationships.

Tools can then take advantage of this knowledge.

Dataset collections





Dataset collections

Collections of datasets are permanent, ordered lists of dat	asets that can be passed to tools and workflows in More help
art over	
MeOH REP3 Mapped Filtered	Discard
MeOH REP2 Mapped Filtered	Discard
MeOH REP1 Mapped Filtered	Discard
Nam	ne: MeOH

Dataset collections



History	<i>C</i> � □
< Back to Unnamed I	history
MeOH a list of datasets	
<u>MeOH REP3 Mappe</u> <u>d</u>	ed Filtere 💿 🖋
<u>MeOH_REP2_Mappe</u>	ed Filtere 💿 🖋
<u>MeOH_REP1_Mappe</u>	ed Filtere 🗶 🖋

Differential expression with CuffDiff

Part of the Tuxedo RNA-Seq Suite (as are Tophat, Bowtie, StringTie, Cufflinks, Cuffmerge, ...)

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.

Now supports geometric normalization, the same model used by DESeq (and in fact, it's now the default). Less prone to distortion from highly expressed genes.

Cuffdiff: Which transcript definitions to use?

We'll use the official genome annotations

But there are a world of options out there for discovering and using novel transcripts. StringTie, Cufflinks, Cuffmerge, ...

- Running with 2 Groups: MeOH and R3G
- Each group has 3 replicates each
- Can take advantage of collections

Cuffdiff find significant changes in transcript expression, splicing, and promoter use (Galaxy Version 2.2.1.2)
Transcripts
C 2 C 7: chr12.gencode.v25.basic.annotation.gtf
A transcript GFF5 or on the produced by cultures, cultompare, or other source.
Omit Tabular Datasets
Yes No Discard the tabular output
Generate SOLite
Yes No
Generate a SQLite database for use with cummeRbund.
Input data type
SAM/BAM -
CuffNorm supports either CXB (from cuffquant) or SAM/BAM input files. Mixing is not supported. Default: SAM/BAM
1: Condition
Name
MeOH
Replicates
【2】 □ 8: MeOH
2: Condition
Name
R3G
Paplicatos
21 D 9: R3G

Execute it

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

test_id	gene_id	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	ACVR1B	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

Cuffdiff: differentially expressed genes

Column	Contents
test_stat	value of the test statistic used to compute significance of the observed change
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?

Are there any patterns to the identified genes?

Agenda

- 9:00 Welcome
- 9:20 Basic Analysis with Galaxy
- 10:45 Break
- 11:00 Basic Analysis into Reusable Workflows
- 12:20 Lunch (on your own)
 - 1:20 RNA-Seq Analysis, Part I
 - 2:50 Break
 - 3:05 RNA-Seq Analysis, Part II
 - 5:00 Done

The Galaxy Team



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Dannon Baker

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bit.ly/btigxy_feedback



Thanks

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5:00 Done

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Le Corum Conference centre

gcc2017.sciencesconf.org

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/

Scaling Training



Galaxy Training Network bit.ly/gxygtn











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Proteomics Metabolomics Natural Language Image Analysis Climate Change Social Science Cosmology

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

Galaxy-Dev

Questions about developing for and deploying Galaxy High volume (2336 posts in 2015, 1000+ members)

Galaxy-Announce

Project announcements, low volume, moderated Low volume (36 posts in 2015, 6500+ members)

Also Galaxy-UK, -France, -Proteomics, -Training, ...

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

= Galaxy Web Search

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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.

-usegalaxy.org

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.



Edit History Actions

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Communicate

Support • Biostar Events • Mailing Lists News S • Twitter

Deploy Galaxy

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

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Events

News

🗧 Gala	xy Wiki	DaveCie	ements Settings Logout Search: Titles Text						
Events			Edit History Actions						
Galaxy I	Event Horizon		News Items Opening at McMaster University						
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 D .			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).						
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.			From the job announcement on EvolDir:						
For events prior to this year, see the Events Archive .			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).						
Montpel			See the full announcement for details. Posted to the Galaxy News on 2014-12-05						
			December 2014 Galaxy Newsletter						
Date	Topic/Event	Venue/Location							
December 12	Introduction to Galaxy Workshop	Virginia State University, Petersburg, Virgin	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:	axy					
December 16-19	RNA-Seq and ChIP-Seq Analysis with Galaxy	UC Davis, California, United States	 Galaxy Day! In Paris! This Wednesday! Near Richmond, Virginia? There's a Galaxy Workshop at Virginia State U on December 12. GCC2015 needs sponsors! 						
		2015	Other upcoming events on two continents						
January 10-14	Galaxy for SNP and Variant Data Analysis	Plant and Animal Genome XXIII (PAG2014), States	 96 new papers, including 6 highlighted papers, referencing, using, extending, and implementing Galaxy. Job openings at 7+ organizations A new mailing list: Galaxy-Training 						
January 19-20	NGS pipelines with Galaxy	e-Infrastructures for Massively Parallel Sequ Sweden	 15 new ToolShed repositories from 10 contributors And, 10 other juicy (well maybe not <i>juicy</i>, but certainly not <i>crunchy</i>) bits of news 						
February 9-13	Analyse bioinformatique de séquences sous Galaxy	Montpellier, France	Dave Clements and the crisp Galaxy Team						
	Accessible and Reproducible Large- Scale Analysis with Galaxy	Genome and Transcriptome Analysis, pa Conference, San Francisco, Cali	Posted to the Galaxy News on 2014-12-01						
February 16-18	Large-Scale NGS data Analysis on Amazon Web Services Using Globus Genomic	Genomics & Sequencing Data Integration, of Molecular Medicine Tri-Conference, Sa	Bioinformaticians, Freiburg						
	iPenort: An Integrative "omics"	States	Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany has an opening for a Bioinformatician Max-Planck	Institute					
			tor an initial period of two years. The successful candidate will work at the interface between an in-house deep- sequencing facility (HiSeg-2500) and the various research groups at the institute. Main responsibilities include and Epigene	etics					

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

citeulike 🗉 💷

CiteULike	MyCiteULike	Group: Galaxy		P Search	Logged	in as galaxyproject	Log Out
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₽ Search	Unwatch	Copy Export	Sort	Hide Details		Filter:	
 ✓ Y eLife by M post C Vali Peer by M post 03:3 ▲ A 	-box protein 1 is e, Vol. 5 (25 August Matthew J. Shurtleff ted to methods use copy ■ My Copy dation and chara rJ Preprints, Vol. 4 Mathan K. Truelove, ted to methods use 6:18 ★★/ bstract ■ Copy	required to sort mic 2016), <u>doi:10.7554/elif</u> (Morayma M. Temoch main by galaxyproject (October 2016), <u>doi:10.</u> Loong Fai Ho, <u>Richard</u> local by galaxyproject	roRNAs into <u>e.19276</u> <u>e-Diaz, Kate V.</u> to the group <u>G</u> <u>en microsate</u> <u>7287/peerj.pre</u> <u>F. Preziosi, Ste</u> to the group <u>G</u>	exosomes in cells Karfilis, Sayaka Ri, alaxy keyed Shurtles llite markers for o prints.2559v1 aphen J. Box alaxy keyed 10.7287	s and in a Randy Sch ff2016Ybo> Jueen con 7/peerj.pret	methods workbench usemain usepublic tools isgalaxy uselocal refpublic cloud other shared	1864 1030 397 373 258 194 184 164 164 121 105
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