# Introduction to Galaxy

# University of Rochester July 19-20, 2016

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

# y #usegalaxy @galaxyproject





# Agenda: Day 1

#### 9:00 Welcome

- 9:20 Basic Analysis with Galaxy A worked example demonstrating Galaxy Basics
- 10:45 Break
- 11:00 Integrating with other tools: BioMart & GO
- 12:20 Lunch (catered)
  - 1:20 Basic Analysis into Reusable Workflows
  - 2:50 Break
  - 3:05 RNA-Seq Analysis, Part I
  - 5:00 Done

# http://bit.ly/UR\_GXY\_2016

## 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?

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

http://galaxyproject.org

What is Galaxy?

#### Keith Bradnam's definition:

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

From

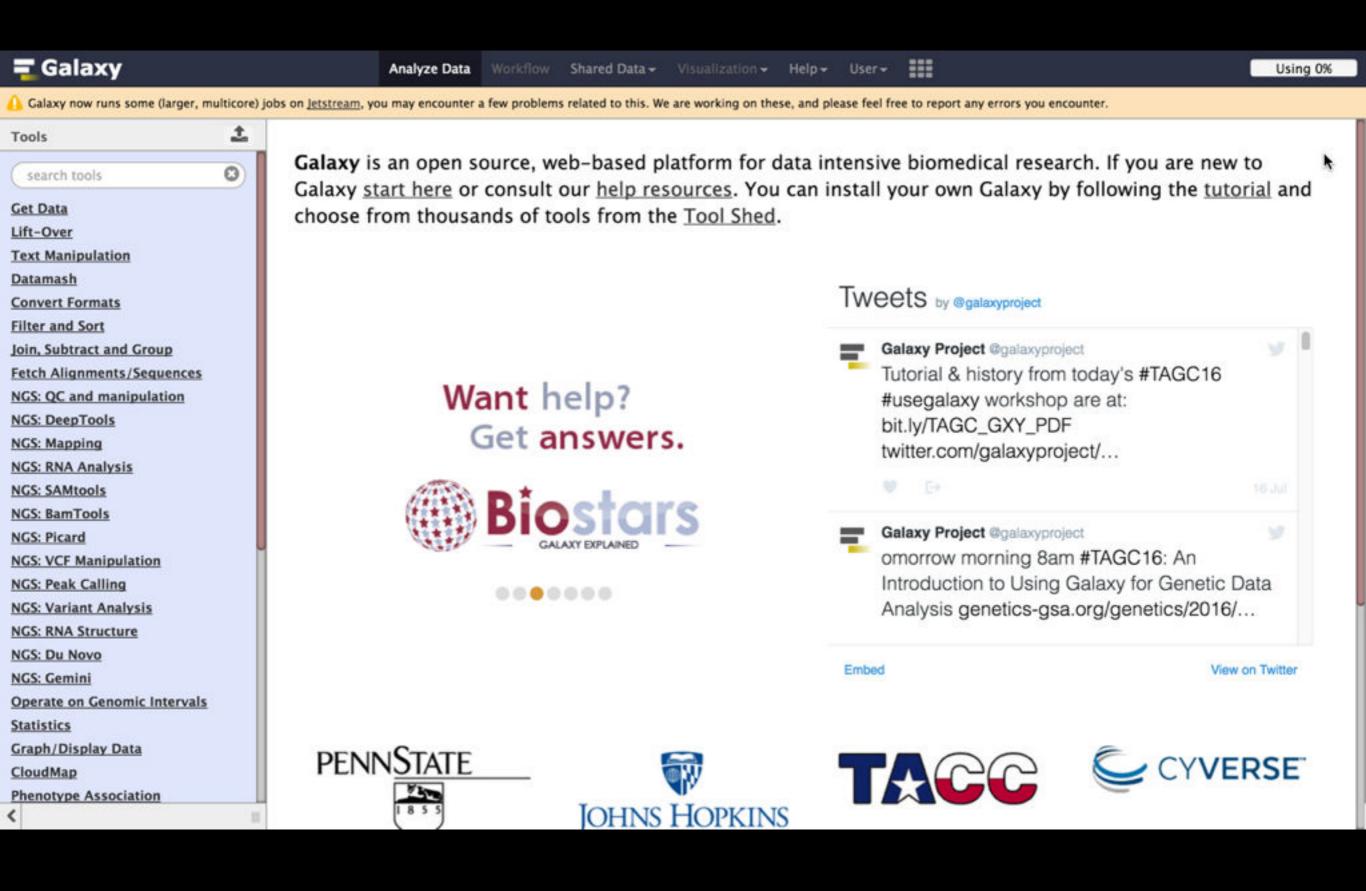
#### "13 Questions You May Have About Galaxy"

http://bit.ly/13questions

#### Galaxy is available several ways ...

http://galaxyproject.org

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



A free for everyone web service:

#### http://usegalaxy.org

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



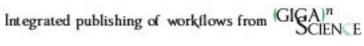
However, a centralized solution cannot support the different analysis needs of the entire world.





Welcome to the Metabiome Portal @ GMU
We have and another the MC Devidence have, a finite contract to the rest of the set of another ports, and and the information of the set o

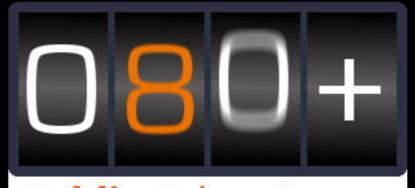




# Cistrome



A Galaxy Server dedicated to ChIP-\* analysis

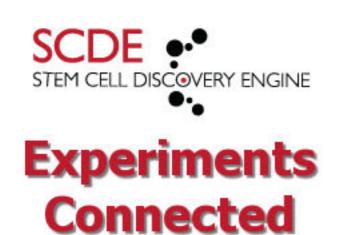


Public Galaxy Servers and still counting



The Genomic HyperBrowser

#### **Powered by Galaxy**





Whale Shark Galaxy! ×G



Genomic analysis tools for southern and Mediterranean plants

bit.ly/gxyServers

#### Galaxy is available as Open Source Software

Galaxy is installed in locations around the world.

http://getgalaxy.org

#### Galaxy is available on the Cloud







#### OpenNebula.org

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



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http://bit.ly/UR\_GXY\_2016

#### 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?

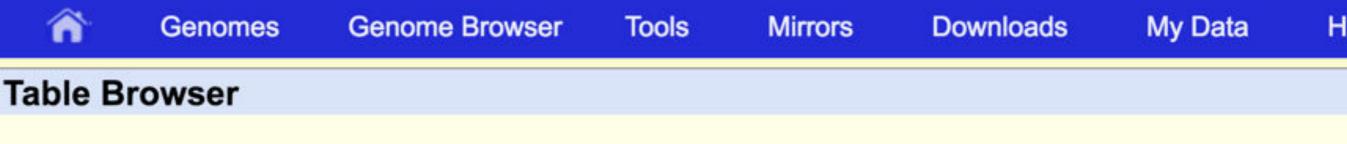
# Use Human, HG38, GENCODE v24, Chromosome 22

cloud1.galaxyproject.org
cloud2.galaxyproject.org

## 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) 🛊
<b>group:</b> Genes and Gene Predictions \$	track: GENC	ODE v24	÷	add custom tracks	track hubs
table: knownGene	describe tab	ole schema			
region:  genome  positio	nr22		lookup defin	e regions	
identifiers (names/accessions):	paste list up	load list			
filter: create					
intersection: create					
correlation: create					
output format: BED - browser extensi	ble data	\$	Send output	to 🗷 <u>Galaxy</u> 🗉	GREAT
output file:	(leave bl	ank to k	eep output ir	n browser)	
file type returned: <ul> <li>plain text</li> </ul>	gzip comp	ressed			



#### Output knownGene as BED

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

name= tb_kno	wnGene	
and the stand of the second second	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

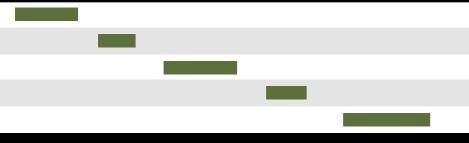
Note: if a feature is close to the beginning or end of a chromosome and upstream/downstreat in order to avoid extending past the edge of the chromosome.

bases at each end

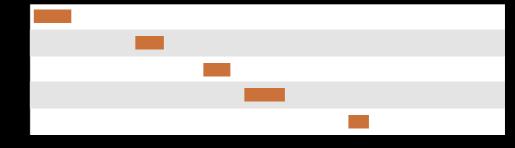
bases at each end

Send query to Galaxy

Cancel



#### **Exons**

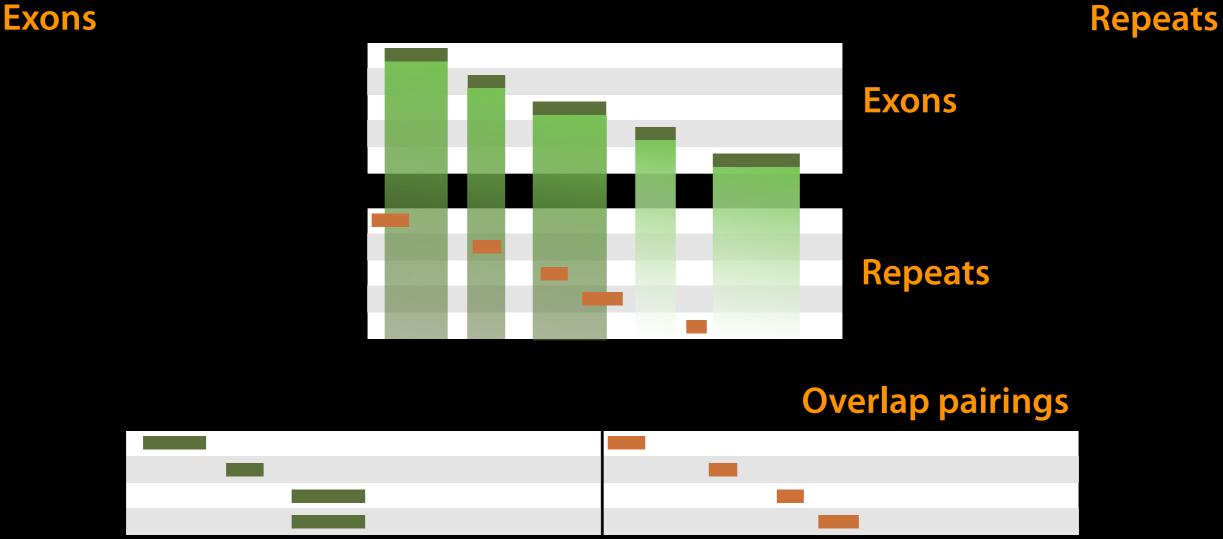


#### Repeats

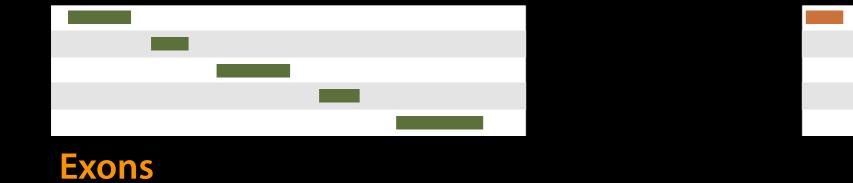
(Identify which exons have Repeats)



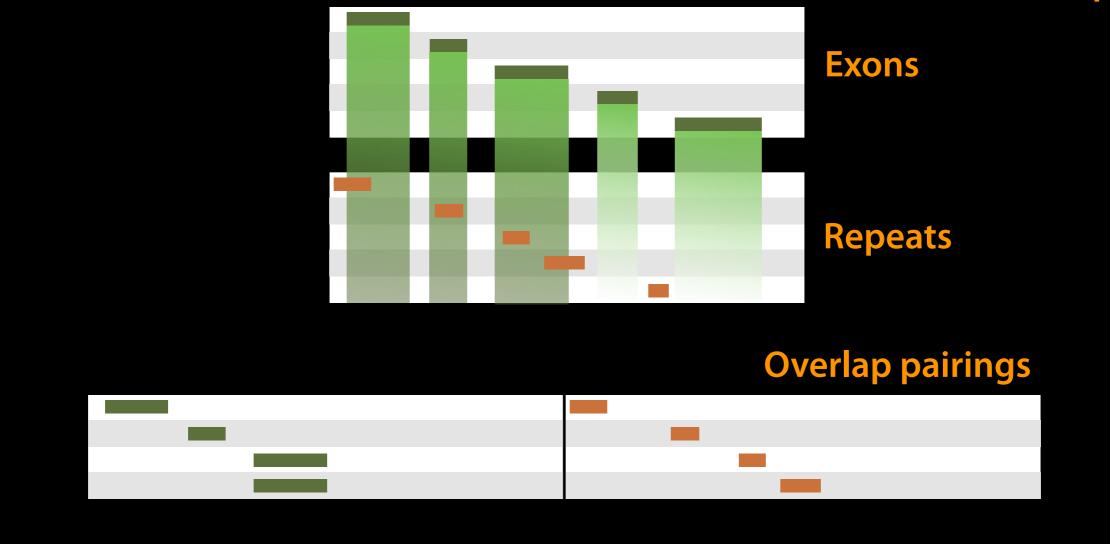




Operate on Genomic Intervals  $\rightarrow$  Join (Identify which exons have Repeats)







(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.

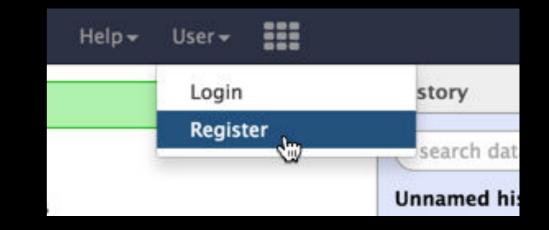
# Create a login

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

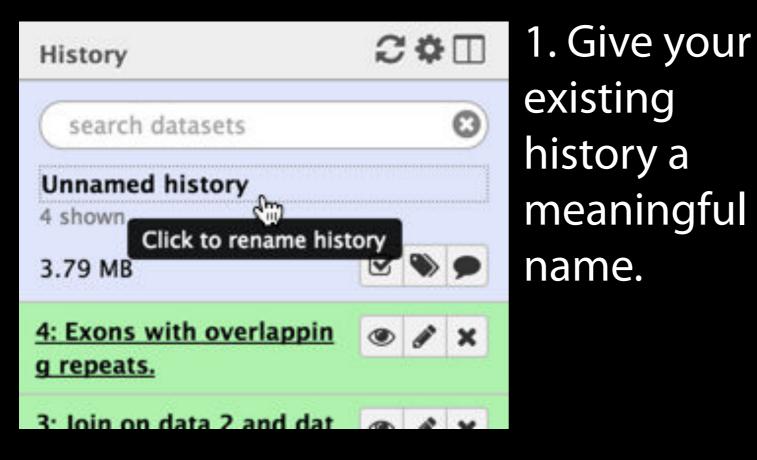
Use an email address you can remember.

Use a low security password.

This account will go away on Wednesday night.



d to g t thre racte
t



2. Create a copy of your history

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

Becomes your new current history.

Histo	O * T
matt	HISTORY LISTS
se	Saved Histories
UR 1	Histories Shared with Me
10 sh	HISTORY ACTIONS
11.2	Create New
	Copy History
<u>12: g</u>	Share or Publish
ping numl	Show Structure
2281	Extract Workflow
form	Delete
	Delete Permanently
Constant of the	DATASET ACTIONS
1 CECRE	Copy Datasets
CECK	Dataset Security
<u>11: C</u>	Resume Paused Jobs
ping	Collapse Expanded Datasets
228 I form	Unhide Hidden Datasets
torm	Delete Hidden Datasets
Gi	Purge Deleted Datasets
	DOWNLOADS
1	Export Tool Citations
AC007	Export History to File
neoon	OTHER ACTIONS
<u>10: Ju</u> n dat	Import from File
623 lin	105

## Exons & Repeats: Pick an Exercise

- 1. Include exons with no overlaps in the exon name and score list. Set the score for these to 0.
- 2. Create a list of exons with overlapping repeats, in BED format, with the score column replaced by the number of overlapping repeats.

Everything you need will be in these toolboxes

- Text manipulation
- Operate on genomic intervals
- Join, subtract and group
- Filter and sort

## All exons, even those with no overlap

Can take advantage of fact that scores are already 0. Join, subtract and group not a bad place to start.

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

# 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 <b>0</b> 🗆	
Attributes Convert Format   Datatype Permissions   Search datasets Exons with overlapping repeats BED 6 shown 3.92 MB 6 Shown 3.92 MB 6 Shown 3.92 MB 792 regions format: interval, database: hg34 Score column is the number of	0		
This will change the datatype of the existing dataset but not modify its contents. Use	BED 6 shown		
	ing repeats, in BED		
	Score column is the number of repeats that overlap with this exon.		
	B 6 2 m	۰ ا	
	1.Chrom 2.Start 3.End	4.Name	

#### Published History: Exons with overlapping repeats, in BED

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## Yay! But, a wee challenge

#### We have exon names and counts

# We really want genes (or transcripts) and counts across the whole gene (or transcript)

# What we have: Computer generated Exon IDs

uc002zmb.3\_cds\_0\_0\_chr22\_17119391\_r

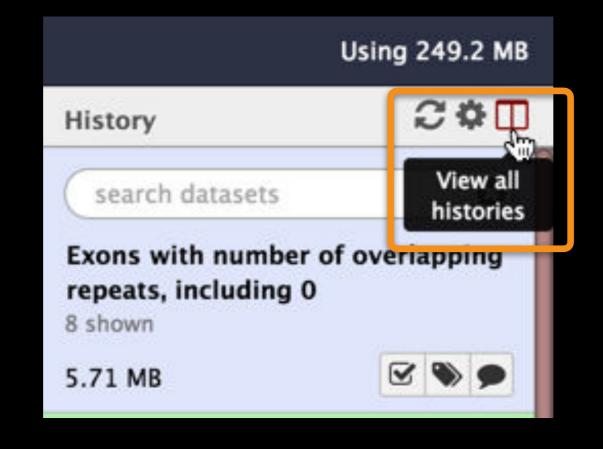
Transcript ID is embedded in Exon ID.\*

How can we extract the Transcript ID from the Exon ID?

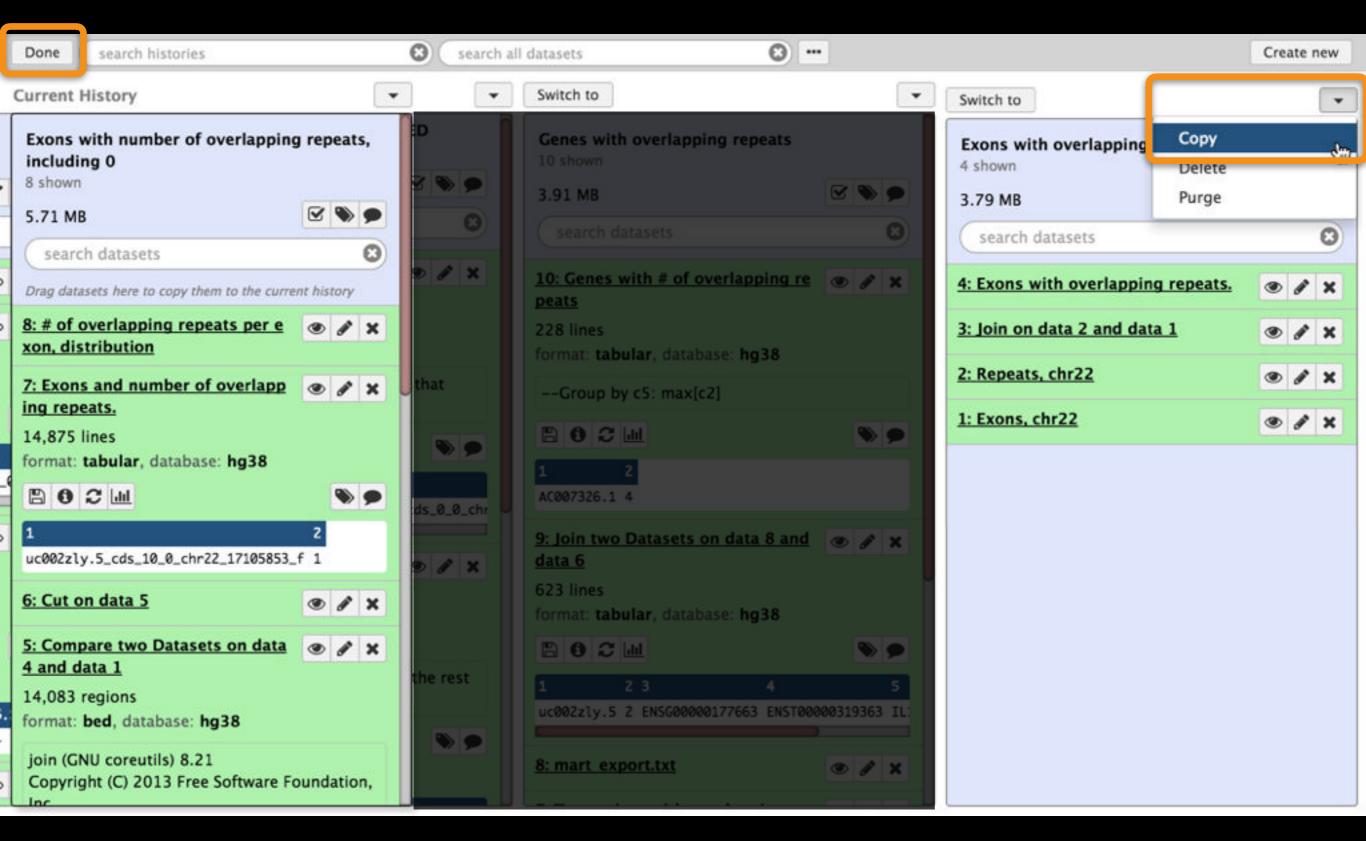
(With the transcript ID we can summarize counts for each transcript and/or get the gene ID.)

\* How do we know that's a transcript ID?

#### Create another copy of your original history



#### Create another copy of your original history



Put the word Gene in the history name

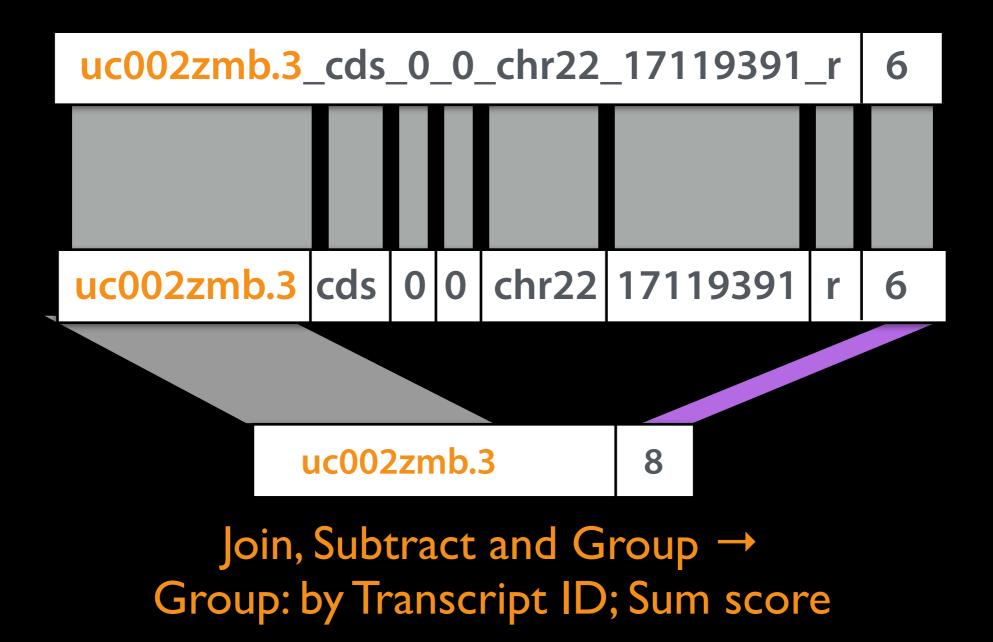
## Extract the transcript ID

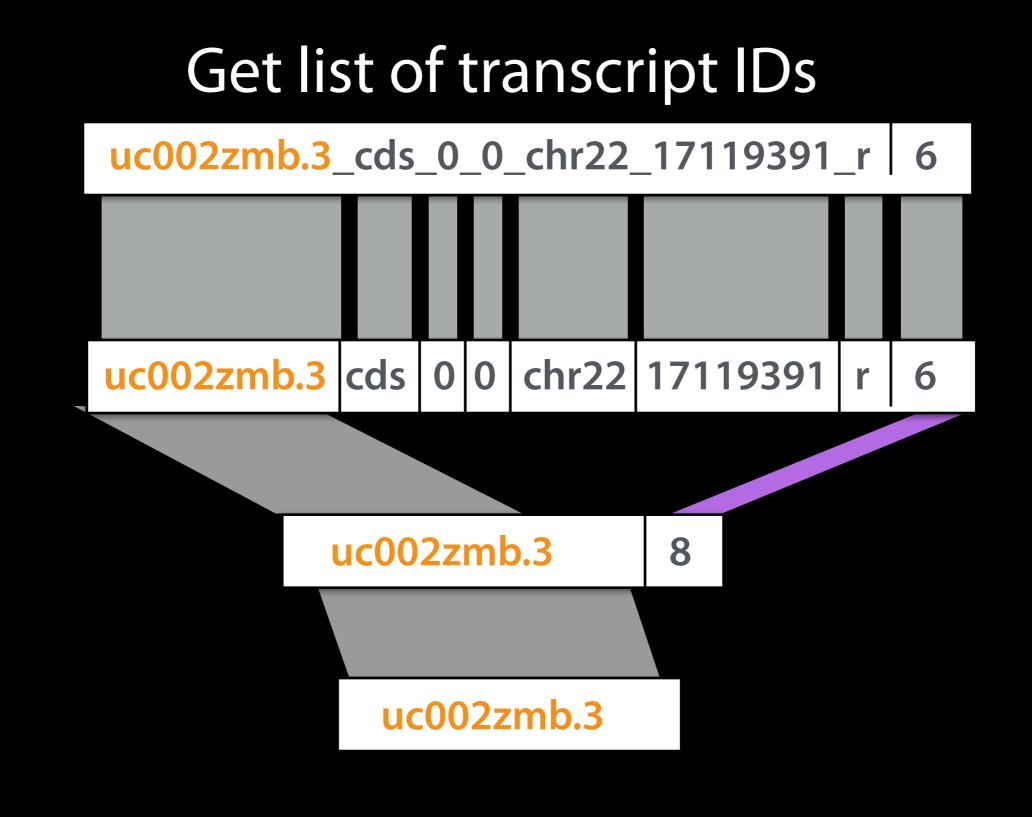
Split the exon ID into its constituent parts.

uc002zmb.3_cds_0_0_chr22_17119391_r						6	
				-122	17110201		
uc002zmb.3	cds	U	U	chr22	1/119391	r	6

Text Manipulation → Convert delimiters to TAB (convert underscores to tabs)

## Sum the scores for all exons in each transcript





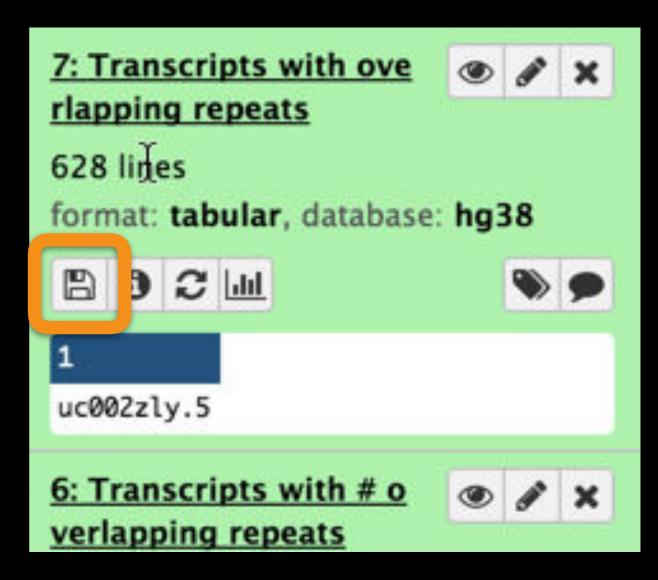
Text Manipulation  $\rightarrow$  Cut

Published History: Transcripts with # of overlapping repeats

## Have Transcripts, now get Gene IDs

## Save list of Transcript IDs to a file.

## We'll upload it to Ensembl BioMart



Published History: Transcripts with # of overlapping repeats

## Ensembl BioMart www.ensembl.org/biomart/martview Specify Ensembl Genes 84, GRCh38.p5

CEnsembles BLA	ST/BLAT   BioMart   Tools   Downlo	ad
New Count Result	ilts	
Dataset [None selected]	✓ - CHOOSE DATABASE - Ensembl Genes 84 Ensembl Regulation 84 Vega 64	Ensembl Genes 84       ✓         ✓       - CHOOSE DATASET -         Danio rerio genes (GRCz10)       Gallus gallus genes (Galgal4)         Homo sapiens genes (GRCh38.p5)       ✓         Mus musculus genes (GRCm38.p4)       ✓         Rattus norvegicus genes (Rnor_6.0)       ✓         Ailuropoda melanoleuca genes (ailMel1)       Anas platyrhynchos genes (BGl_duck_1.0)         Anolis carolinensis genes (AnoCar2.0)       ✓

New Count Results	🖕 URL 🛛 🗛 XML	Perl ③ Help
Dataset Homo sapiens genes (GRCh38.p5)	<ul> <li>Features</li> <li>Structures</li> <li>Variant (Germline)</li> <li>Structures</li> <li>Variant (Somatic)</li> <li>Homologues</li> <li>Sequences</li> </ul>	
Filters	□ GENE:	
[None selected] Attributes Ensembl Gene ID Ensembl Transcript ID Associated Gene Name	Ensembl Censembl Gene ID Ensembl Transcript ID Ensembl Protein ID Ensembl Exon ID Description Chromosome Name	APPRIS annotation Associated Gene Name Associated Cene Cource Associated Transcript Name Associated Transcript Source Transcript count
Dataset	Gene Start (bp) Gene End (bp)	<ul> <li>GC content</li> <li>Gene type</li> </ul>
[None Selected]	<ul> <li>Strand</li> <li>Band</li> <li>Transcript Start (bp)</li> <li>Transcript End (bp)</li> <li>Transcription Start Site (TSS)</li> <li>Transcript length (including UTRs and CDS)</li> <li>Transcript Support Level (TSL)</li> <li>GENCODE basic annotation</li> </ul>	<ul> <li>Transcript type</li> <li>Source (gene)</li> <li>Source (transcript)</li> <li>Status (gene)</li> <li>Status (transcript)</li> <li>Version (gene)</li> <li>Version (transcript)</li> </ul>
	Phenotype	

#### Specify attributes to put in output report

🦻 New 📓 Count 📓 Results	🖕 URL 🛛 💀 XML 🔄 Peri 💿 Help				
Deterat	GOSIim GOA Accession(s)	GOSIim GOA Description			
Dataset Homo sapiens genes (GRCh38.p5) Filters [None selected] Attributes Ensembl Gene ID Ensembl Gene ID Ensembl Transcript ID Associated Gene Name UCSC ID	External References (max 3) ArrayExpress ChEMBL ID(s) Clone based Ensembl gene name Clone based Ensembl transcript name Clone based VEGA gene name Clone based VEGA transcript name CCDS ID Database of Aberrant 3' Splice Sites (DBASS3) IDs DBASS3 Gene Name Database of Aberrant 5' Splice Sites (DBASS5) IDs	<ul> <li>MIM Gene Description</li> <li>miRBase Accession(s)</li> <li>miRBase ID(s)</li> <li>miRBase transcript name</li> <li>PDB ID</li> <li>Protein (Genbank) ID [e.g. AAA0248</li> <li>Reactome ID</li> <li>Reactome gene ID</li> <li>Reactome transcript ID</li> <li>RefSeq mRNA [e.g. NM_001195597</li> </ul>			
	<ul> <li>DBASS5 Gene Name</li> <li>EMBL (Genbank) ID</li> </ul>	RefSeq mRNA predicted [e.g. XM_0 RefSeq ncRNA [e.g. NR_002834]			
Dataset [None Selected]	<ul> <li>Ensembl Human Transcript IDs</li> <li>Ensembl Human Translation IDs</li> <li>LRG to Ensembl link gene</li> <li>LRG to Ensembl link transcript</li> <li>EntrezGene ID</li> </ul>	<ul> <li>RefSeq ncRNA predicted [e.g. XR_'</li> <li>RefSeq Protein ID [e.g. NP_001005</li> <li>RefSeq Predicted Protein ID [e.g. XI</li> <li>Rfam ID</li> <li>Rfam transcript name</li> </ul>			
	<ul> <li>EntrezGene transcript name ID</li> <li>Human Protein Atlas Antibody ID</li> <li>VEGA gene ID(s) (OTTG)</li> <li>VEGA transcript ID(s) (OTTT)</li> </ul>	UCSC ID Unigene iD UniParc			

#### Specify attributes to put in output report

New Count Results	y URL 💽 XML 🔄 Peri 💿 Help						
Dataset Homo sapiens genes	(If filter values are truncated in any	our query using criteria below lists, hover over the list item to see the full text)					
Filters	REGION:						
[ID-list specified] Attributes	GENE: Limit to genes (external references)	with HGNC ID(s) Only Excluded					
Ensembl Gene ID Ensembl Transcript ID Associated Gene Name UCSC ID	Input external references ID list [Max 500 advised]	2 UCSC ID(s) [e.g. uc002cqj.3]					
Dataset	Limit to genes (microarray probes/probesets)	with Affymetrix Microarray huex 1 0 st v2 probeset ID(s)					
[None Selected]	Input microarray probes/probesets ID list [Max 500 advised]	Codelink probe ID(s) [e.g. GE550734]					

#### Specify which genes we want this information for

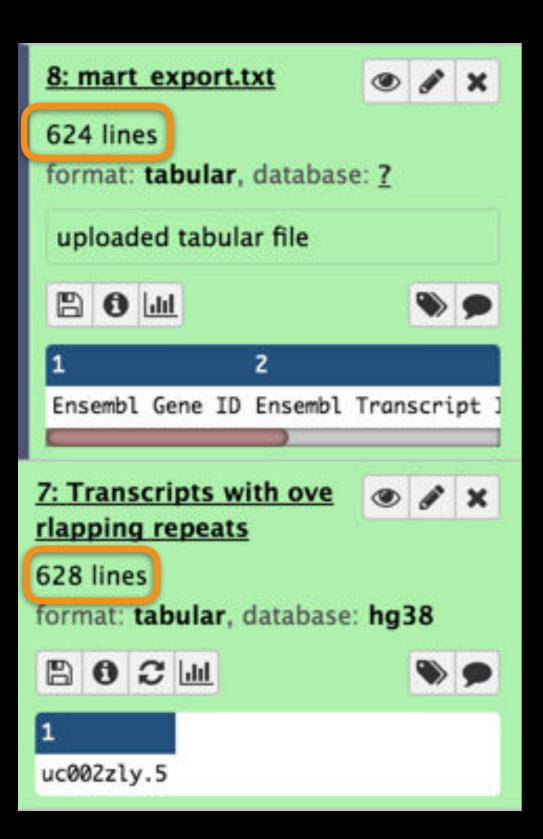
New Count Results	🐈 URL 🛛 🗗 XML 🔄 Perl 💿 Help					
Dataset	Export all results	to File		C TSV	🔉 🗆 Unique results only	Go
Homo sapiens genes (GRCh38.p5)	Email notification	to	)			
Filters	View	10	rows as html 😒 🗆		ulte only	
UCSC ID(s) [e.g. uc002cqj.3]:	VICW	10 🗸		Unique lest		
[ID-list specified]	Ensembl Gene ID	Ensembl Transcript ID	Associated Gene Name	UCSC ID		
Attributes	ENSG00000177663	ENST00000319363	IL17RA	uc002zly.5		
Ensembl Gene ID	ENSG00000183307	ENST00000331437	CECR6	uc002zmb.3		
	ENSG0000099968	ENST00000317582	BCL2L13	uc002zmw.5		
Ensembl Transcript ID	ENSG0000099968	ENST00000543133	BCL2L13	uc002zmx.4		
Associated Gene Name	ENSG0000099968	ENST0000355028	BCL2L13	uc002zmy.5		
UCSC ID	ENSG0000099968	ENST00000418951	BCL2L13	uc002zmz.4		
	ENSG0000243156	ENST00000441493	MICAL3	uc002zng.5		
	ENSG00000184979	ENST00000215794	<u>USP18</u>	uc002zny.4		
Dataset	ENSG0000100056	ENST00000252137	DGCR14	uc002zou.4		
[None Selected]	ENSG0000100075	ENST00000451283	SLC25A1	uc002zoy.5		

#### Save the results to a file for uploading into Galaxy

Tools	*			Get (	Gene	s intc	
search tools	Download from UR files from d	lisk	o Gala		Galax	У	
Get Data		following	the <u>ti</u> ualizatio	n≁ Admin Help≁	User -		
D	ownload from web or upl	oad from disk					et
1	Regular Composite						un
pul. sis atio Grc		ę	Drop files	here			pie 5) pie 5) ad file
	Type (set all):	Auto-detect	<b>₽</b> Q G(	enome (set all):	Additional Species A	A 🔻	: 51
nc	3	Choose local file	Ochoose FTP file	Paste/Fetch data	Pause Reset	Start Close	
						E. Comun	

#### Chose local file, then Start, then Close

## Get Gene IDs into Galaxy



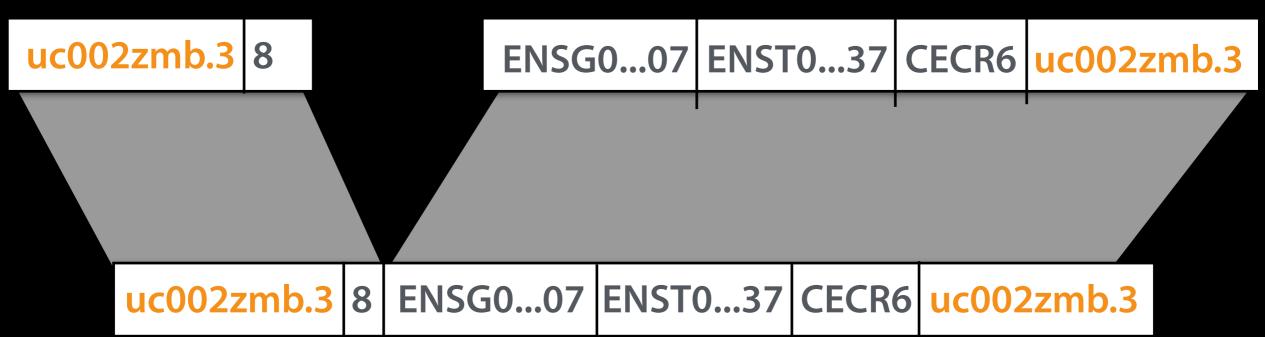
Upload file from BioMart. Note that we lost 4-5 transcripts

Do we care? Can we find out which were lost?

## Unite our Transcript Scores with Biomart info

#### Transcript Scores

#### **Biomart Info**



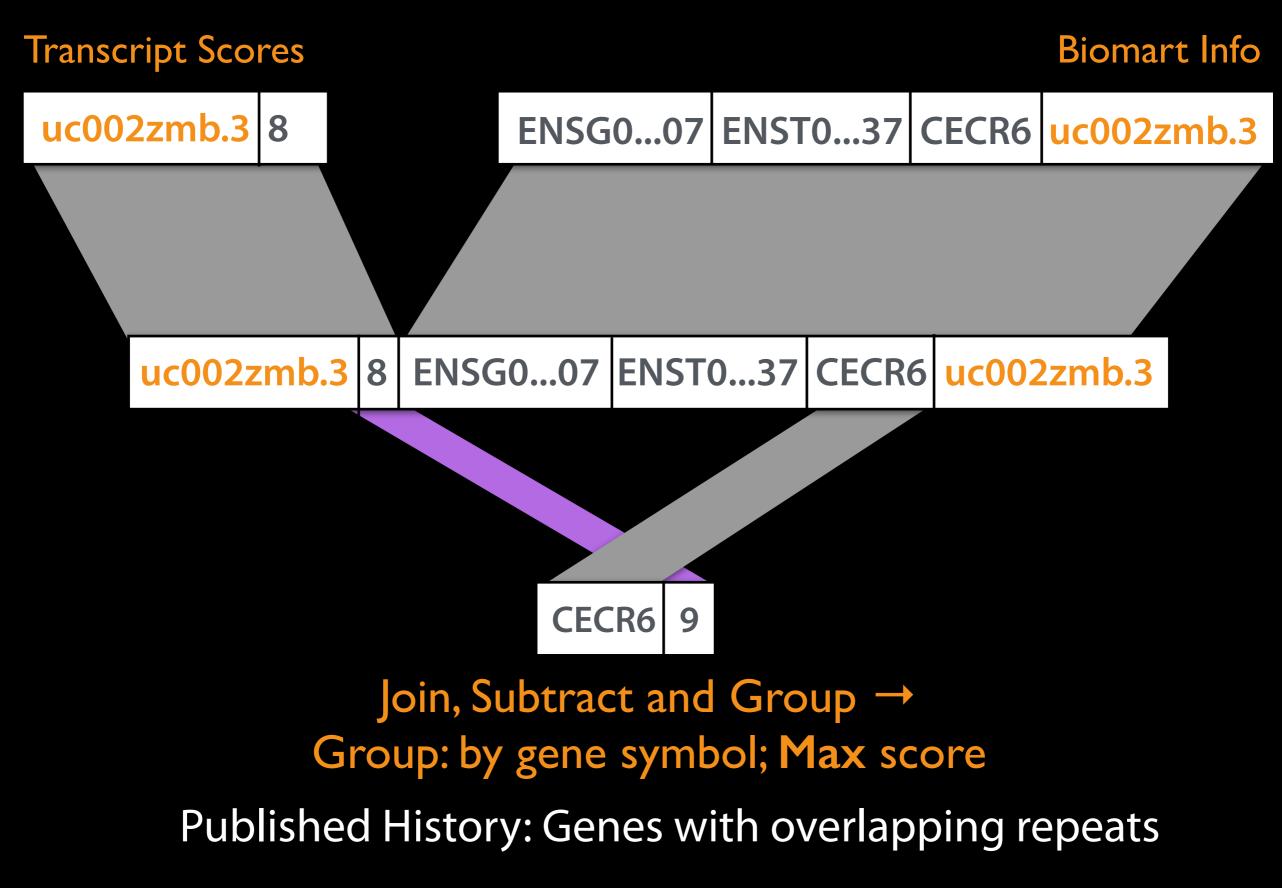
Join, Subtract and Group → Join: Transcripts with score and Biomart dataset; join on UCSC transcript ID

#### Unite our Transcript Scores with Biomart info

Join t	wo Da	ataset	s side by side on a specified field (Galaxy Version 2.0.2)	▼ Options
Join				
D	ළු		8: mart_export.txt	•
using	, colu	mn		
Colu	mn: 4			•
with				
D	ළු		6: Transcripts with # overlapping repeats	•
and c	olum	n		
Colu	mn: 1			•
Keep	lines	of firs	st input that do not join with second input	
No				•
Кеер	lines	of fir	st input that are incomplete	
No				-
Fill e	mpty	colum	ins	
No				•
✓ E	Execut	te		

#### Join, Subtract and Group $\rightarrow$ Join

## Assign scores to genes



#### Now have a list of genes with # overlapping repeats

1	2
AC007326.1	4
ACR	2
ADM2	1
ADRBK2	1
ADSL	2
ANKRD54	1
AP000349.2	1
APOBEC3B	2
APOBEC3F	1
APOBEC3H	1
APOL3	1
APOL4	2
APOL5	1
APOL6	1
ARFGAP3	1
ARHGAP8	2
ARSA	1
ASCC2	2
ASPHD2	1
ATP6V1E1	1

Published History: Genes with overlapping repeats

### Yay! We have a list of genes and overlap counts!\*

#### Now, what can we do with that?

#### All sorts of things.

\* Technically, we have a list of gene symbols, and the maximum number of overlapping repeats from any of its transcripts. We also haven't done things like normalize the scores based on gene length. Your mileage may vary. Let's not sweat the details.

## GO Term Enrichment

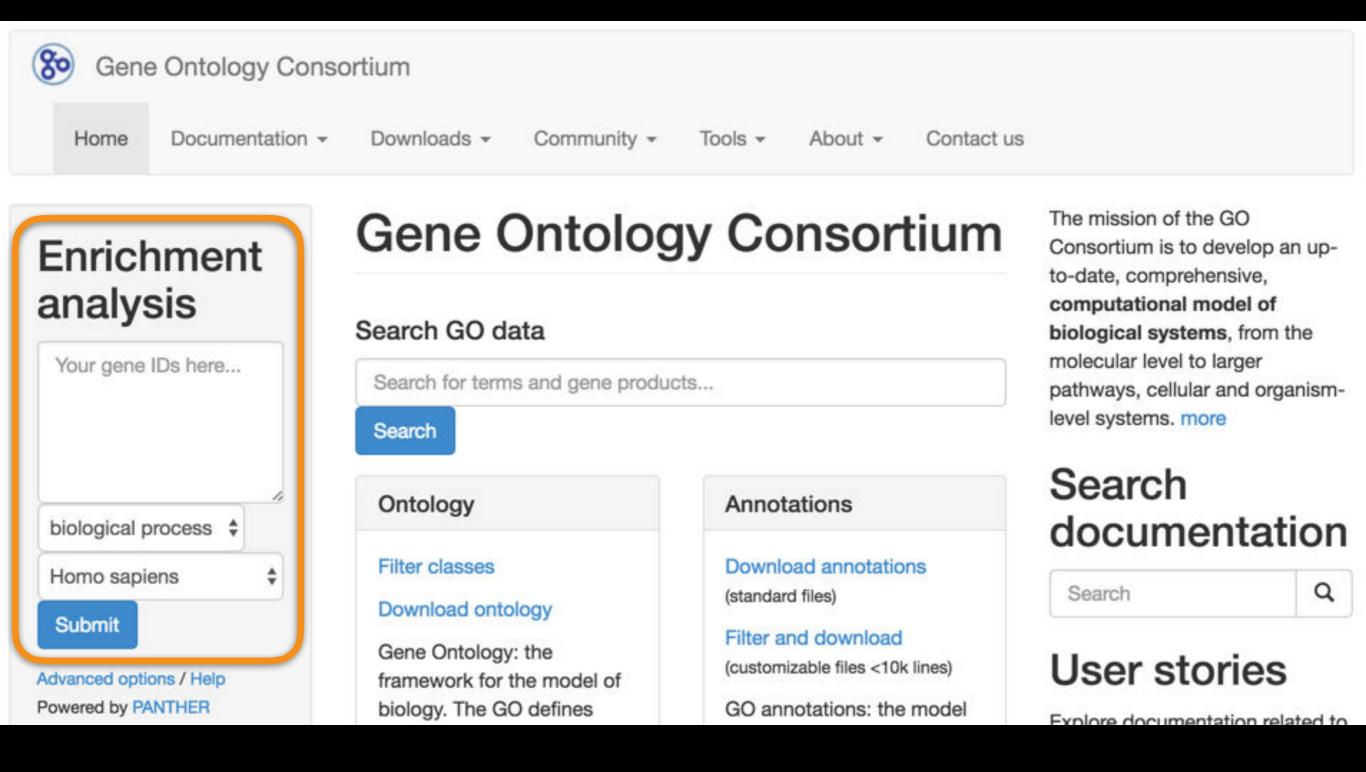
## Do genes with particular functions tend to occur in this list more often then they would by random chance?



## GO: Create a list of just the gene symbols

Remember how?

## (Stop or) GO: Can do this step, or just watch



#### http://geneontology.org/

# GO: Results from whole genome, 1 or more overlapping repeats (8969 genes)

Displaying only results with P<0.05; click here to display all results							
	Homo sapiens (REF)	upload_1 (▼ Hierarchy_NEW! <sup>(2)</sup> )			)		
GO biological process complete	<u>#</u>	<u>#</u>	expected	Fold Enrichment	<u>+/-</u>	<u>P value</u>	
chromatin modification	289	<u>196</u>	123.50	1.59	+	6.66E-06	
Chromatin organization	636	376	271.78	1.38	+	5.60E-06	
La chromosome organization	984	555	420.49	1.32	+	6.19E-07	
Lorganelle organization	3133	<u>1636</u>	1338.83	1.22	+	4.94E-14	
Lecellular component organization	<u>5133</u>	2606	2193.49	1.19	+	1.25E-19	
4cellular process	14559	<u>6671</u>	6221.52	1.07	+	4.95E-22	
Lecellular component organization or biogenesis	5288	2688	2259.73	1.19	+	7.21E-21	
macromolecular complex subunit organization	<u>1983</u>	<u>1021</u>	847.40	1.20	+	4.89E-06	
peptidyl-lysine modification	314	<u>194</u>	134.18	1.45	+	4.87E-03	
4peptidyl-amino acid modification	855	<u>456</u>	365.37	1.25	+	1.34E-02	
La cellular protein modification process	2836	<u>1397</u>	1211.91	1.15	+	9.10E-05	
Leprotein modification process	2836	<u>1397</u>	1211.91	1.15	+	9.10E-05	
La protein metabolic process	4036	<u>1908</u>	1724.71	1.11	+	5.29E-03	
4-macromolecule metabolic process	7359	3685	3144.73	1.17	+	1.39E-28	
4 organic substance metabolic process	9032	<u>4308</u>	3859.66	1.12	+	7.16E-18	
4metabolic process	<u>9443</u>	4480	4035.29	1.11	+	2.03E-17	
<sup>L</sup> primary metabolic process	8601	<u>4133</u>	3675.48	1.12	+	6.53E-19	
4-macromolecule modification	3007	1480	1284.99	1.15	+	3.58E-05	

#### Published History: Gene-Repeat overlap, entire genome

# GO: Results from whole genome, 2 or more overlapping repeats (2759 genes)

Displaying only results with P<0.05; click here to display all results						
	Homo sapiens (REF)	) upload 1 (♥ Hierarchy NEW! <sup>(</sup> )			3)	
GO biological process complete	<u>#</u>	<u>#</u>	expected	Fold Enrichment	<u>+/-</u>	P value
membrane depolarization during action potential	39	<u>19</u>	5.22	3.64	+	2.01E-02
4biological regulation	<u>11384</u>	<u>1776</u>	1523.15	1.17	+	2.20E-18
4membrane depolarization	<u>61</u>	24	8.16	2.94	+	3.99E-02
regulation of histone methylation	<u>59</u>	<u>25</u>	7.89	3.17	+	7.12E-03
4regulation of histone modification	<u>129</u>	<u>43</u>	17.26	2.49	+	9.63E-04
regulation of primary metabolic process	<u>5720</u>	<u>1046</u>	765.32	1.37	+	5.02E-27
<sup>4</sup> regulation of metabolic process	<u>6087</u>	<u>1096</u>	814.43	1.35	+	2.49E-26
<sup>4</sup> regulation of biological process	<u>10767</u>	<u>1708</u>	1440.60	1.19	+	1.66E-20
regulation of macromolecule metabolic process	5730	<u>1052</u>	766.66	1.37	+	6.06E-28
<sup>La</sup> regulation of cellular metabolic process	<u>5781</u>	<u>1058</u>	773.48	1.37	+	1.21E-27
Interpretation of cellular process	<u>10292</u>	<u>1651</u>	1377.04	1.20	+	1.75E-21
4 regulation of chromatin organization	<u>152</u>	<u>50</u>	20.34	2.46	+	1.43E-04
4regulation of chromosome organization	272	<u>66</u>	36.39	1.81	+	4.52E-02
4regulation of organelle organization	1097	<u>211</u>	146.78	1.44	+	1.37E-03
Interpretation of cellular component organization	2246	409	300.51	1.36	+	1.22E-06
histone lysine methylation	<u>64</u>	27	8.56	3.15	+	2.90E-03
4-histone methylation	<u>84</u>	<u>31</u>	11.24	2.76	+	6.88E-03
histone modification	337	<u>88</u>	45.09	1.95	+	5.91E-05
Covalent chromatin modification	346	92	46.29	1.99	+	1.16E-05
macromolecule metabolic process	7359	<u>1260</u>	984.62	1.28	+	4.66E-23
4 organic substance metabolic process	9032	1384	1208.46	1.15	+	1.23E-07

# GO: Results from whole genome, 3 or more overlapping repeats (986 genes)

Displaying only results with P<0.05; click here to display all results					7	0
	Homo sapiens (REF)		upload 1 (▼ Hierarchy NEW!			3)
GO biological process complete	#	#	expected	Fold Enrichment	+/-	P value
histone H3-K4 methylation	32	12	1.55	7.75	+	7.35E-04
histone lysine methylation	<u>64</u>	<u>16</u>	3.10	5.17	+	1.41E-03
histone methylation	84	<u>19</u>	4.07	4.67	+	4.79E-04
histone modification	337	43	16.31	2.64	+	1.67E-04
La covalent chromatin modification	346	44	16.75	2.63	+	1.26E-04
4-macromolecule metabolic process	7359	<u>496</u>	356.16	1.39	+	1.25E-15
organic substance metabolic process	9032	<u>524</u>	437.13	1.20	+	2.08E-04
4metabolic process	9443	<u>535</u>	457.02	1.17	+	4.42E-03
	<u>636</u>	80	30.78	2.60	+	2.59E-10
4-chromosome organization	984	<u>93</u>	47.62	1.95	+	1.08E-05
4 organelle organization	3133	214	151.63	1.41	+	8.13E-04
4-cellular component organization	5133	319	248.43	1.28	+	2.57E-03
4cellular process	14559	773	704.62	1.10	+	9.14E-03
Cellular component organization or biogenesis	5288	329	255.93	1.29	+	1.31E-03
4-macromolecular complex subunit organization	1983	148	95.97	1.54	+	9.07E-04
primary metabolic process	8601	<u>519</u>	416.27	1.25	+	4.01E-07
4cellular macromolecule metabolic process	6693	475	323.93	1.47	+	3.28E-19
Lellular metabolic process	8525	505	412.59	1.22	+	2.32E-05

Published History: Gene-Repeat overlap, entire genome

## Agenda: Day 1

- 9:00 Welcome
- 9:20 Basic Analysis with Galaxy A worked example demonstrating Galaxy Basics
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http://bit.ly/UR\_GXY\_2016

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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?

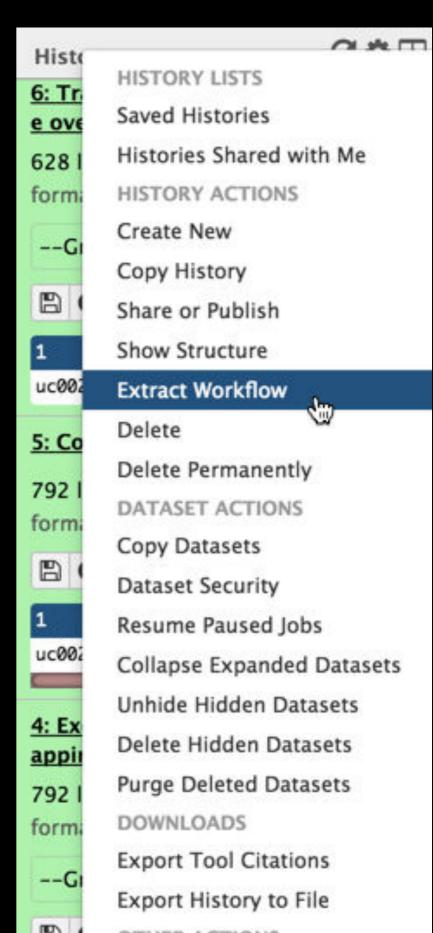
#### **Create a Workflow from a History**

#### **Extract Workflow from history**

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



(cog) → Extract Workflow



#### Create a Workflow from a History: ...

The following list contains each tool that was run to create	History 20	
those that you wish to include in the workflow.	Group by c1: sum[c8]	
Tools which cannot be run interactively and thus cannot gray.	be incorporated into a workflow will be shown in	802
Workflow name		1 2
Workflow constructed from history 'UR 101 Test Run'		uc002zly.5 2
Create Workflow Check all Uncheck all		5: Convert on data 4 💿 🖋 🗙
Tool	History items created	792 lines format: tabular, database: hg38
UCSC Main	<u>1: Exons, chr22</u>	802
This tool cannot be used in workflows	🗹 Treat as input dataset	2 3 4 5 6 7 8
UCSC Main	2: Repeats, chr22	02zly.5 cds 10 0 chr22 17105853 f 1
This tool cannot be used in workflows	🗹 Treat as input dataset	4: Exons with # of overl ( ) * *
Join		792 lines
✓ Include "Join" in workflow	3: Join on data 2 and data 1	format: tabular, database: hg38
		Group by c4: count[c1]
Group	4: Exons with # of overlapping repeats.	B02
Include "Group" in workflow		Z
Convert		002zly.5_cds_10_0_chr22_17105853_f 1
✓ Include "Convert" in workflow	5: Convert on data 4	3: Join on data 2 and d 💿 🖋 🗙
		<u>ata 1</u>
Group	6: Transcripts that have overlapping repe	911 regions format: interval, database: hg38
Include "Group" in workflow	<u>ats</u>	

## Wait ...

## Can this whole analysis be a useful workflow? (No.)

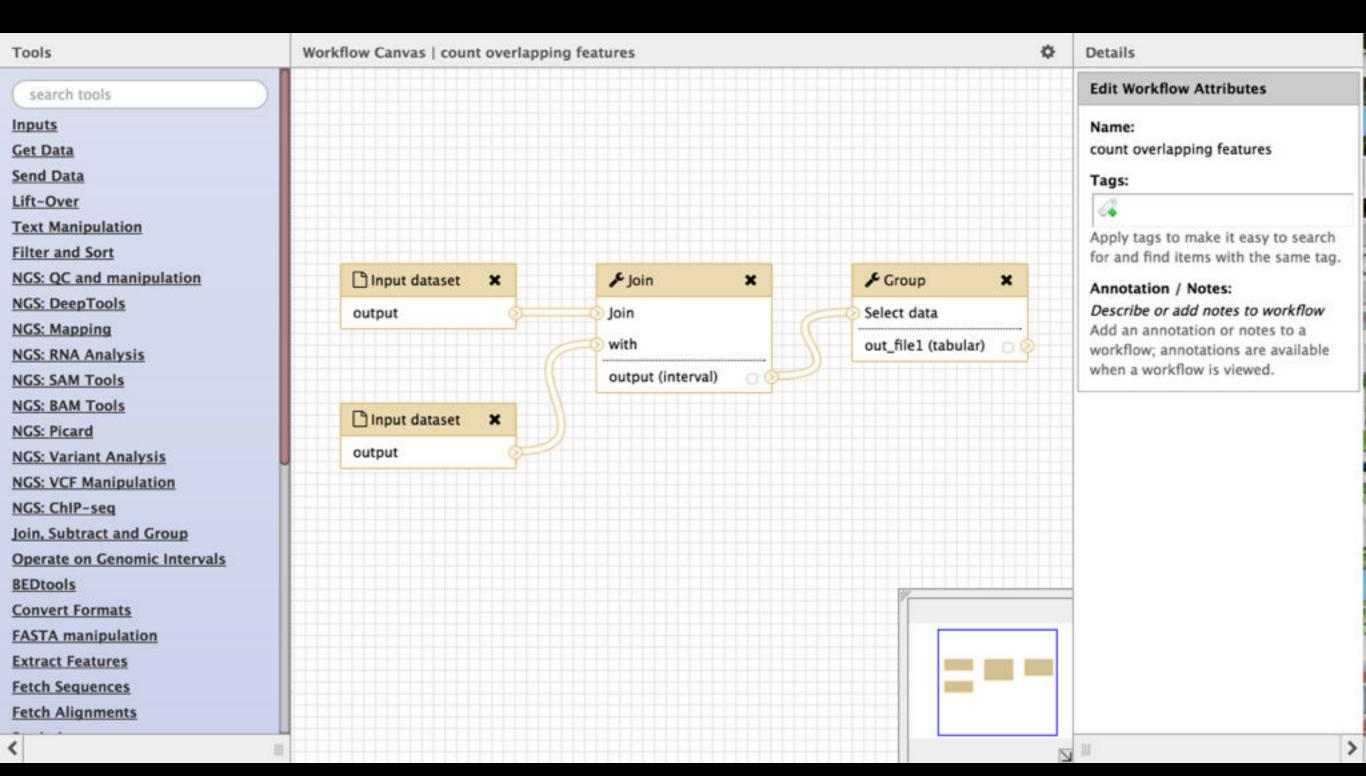
Are there parts of this analysis are a good candidate for a workflow - something to be reused on other data?

The first 4 items count overlaps between features. That might be useful.

#### Create a Workflow from a History: ...

The following list contains each tool that was run to cre	History 20	
those that you wish to include in the workflow. Tools which cannot be run interactively and thus canno gray.	Group by c1: sum[c8]	
Workflow name		1 2
count overlapping features		uc002zly.5 2
Create Workflow Check all Uncheck all		5: Convert on data 4 💿 🖋 🗙
Tool	792 lines format: tabular, database: hg38	
UCSC Main	<u>1: Exons, chr22</u>	B C LLI >> >
This tool cannot be used in workflows	🗹 Treat as input dataset	2 3 4 5 6 7 8
UCSC Main		02zly.5 cds 10 0 chr22 17105853 f 1
This tool cannot be used in workflows	Treat as input dataset	4: Exons with # of overl () * *
Join	3: Join on data 2 and data 1	792 lines format: <b>tabular</b> , database: <b>hg38</b>
Include "Join" in workflow		Group by c4: count[c1]
Group	4: Exons with # of overlapping repeats.	₿ 0 2 Ш >>
Include "Group" in workflow		2
Convert	E. Commentation A	002zly.5_cds_10_0_chr22_17105853_f 1
Include "Convert" in workflow	5: Convert on data 4	3: Join on data 2 and d
Group		911 regions format: interval, database: hg38
Include "Group" in workflow		

#### Workflow editor



#### Published Workflow: Feature Overlap Counting

#### Workflow editor: save your changes

Tools	Workflow Canvas   count overlapping	٥	Details			
(search tools					Edit Wo	rkflow Attributes
Inputs			Help- User-			Usir
Get Data				ő		
Send Data				8	Dotaile	
Lift-Over				Save		rkflow Attributes
Text Manipulation				Run 🔊		
Filter and Sort				Edit Attribute	s	e tag.
NGS: QC and manipulation	🕒 Input dataset 🗙	₣ Join ¥		Auto Re-layo	ut	verlapping feature:
NGS: DeepTools				Close		low
NGS: Mapping	output	- Join			12	
NGS: RNA Analysis		= with				able
NGS: SAM Tools		output (interval) 🛛 🔿				tags to make it easy
NGS: BAM Tools						
NGS: Picard	🗋 Input 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	III .	>

#### Published Workflow: Feature Overlap Counting

### Workflows

Run / test it 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: Feature Overlap Counting

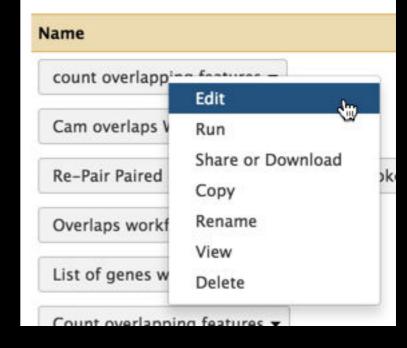
Analyze Data Workflow

Shared Data -

#### Chain tools into workflows

Galaxy is an open source, web-based platfor are new to Galaxy <u>start here</u> or consult our <u>h</u> following the <u>tutorial</u> and choose from thous

#### Your workflows



## 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.

## Agenda: Day 1

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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

Create new history

 $(cog) \rightarrow Create New$ 

Import:

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

→ UC-Davis → Raw Reads Select first two MeOH\_REP1\_R1, MeOH\_REP1\_R2



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

## NGS Data Quality Control

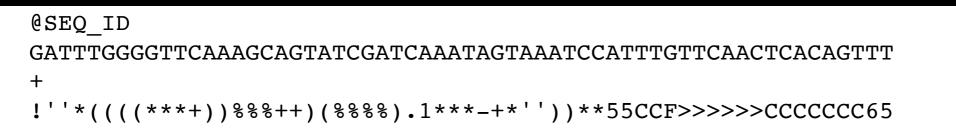
- FASTQ format
- Examine quality in an RNA-Seq dataset
- Trim/filter as we see fit, hopefully without breaking anything.

## 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



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

		IIIII XXXXXXX	IIIIIIII XXXXXXXXXX	SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS	IIIIIIIIIIIIIII XXXXXXXXXXXXXXXX
!"#\$%&'()*+,/	0123456789:;<=	>?@ABCD	EFGHIJKLM	NOPQRSTUVWXYZ[\]^_`abcdefghijklmno;	pqrstuvwxyz{ }~
33	59	64	73	104	126
•	Phred+64, 62	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: Assessment tools

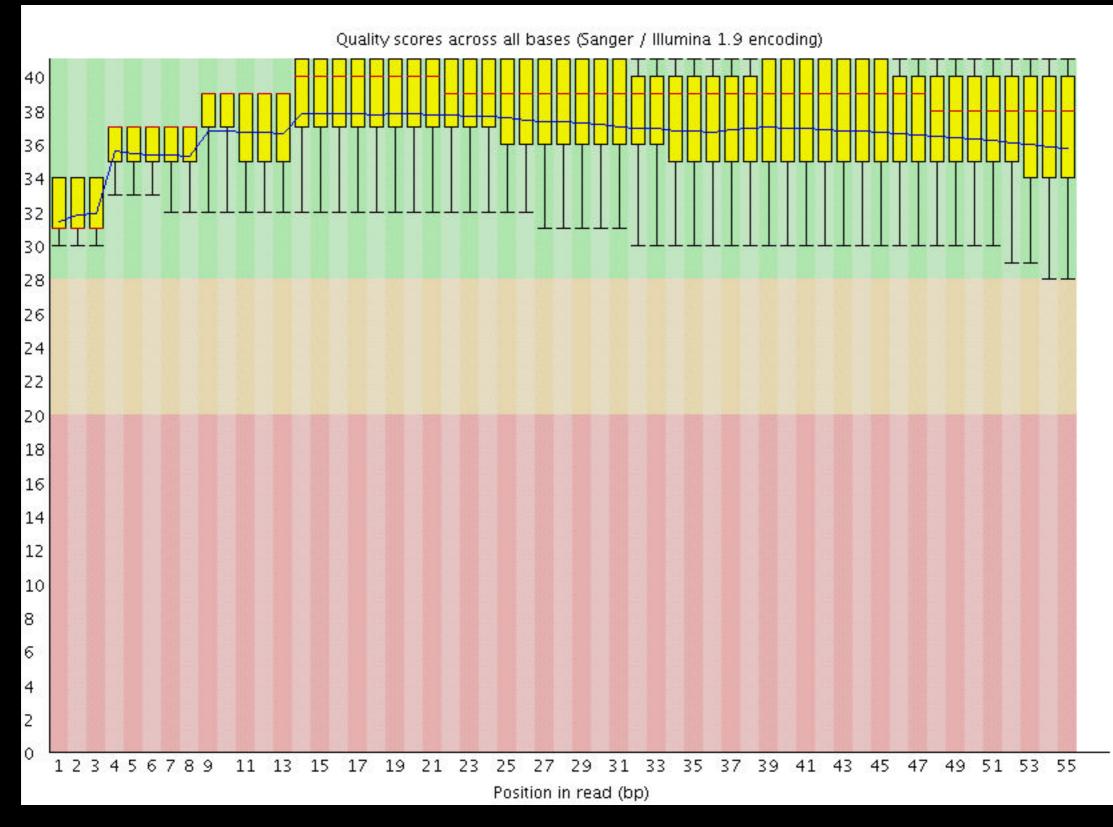
#### NGS QC and Manipulation → FastQC

#### Generates summary quality information.

FastQC Read Qua	ality reports (Galaxy Version 0.63)	& Versions	▼ Options					
Short read data	from your current history		1.2.5					
C 2 C	□ 2 □ 1: MeOH_REP1_R1.fastq							
Contaminant lis	t							
C 2 C	Nothing selected		•					
RNA RT Primer C	with 2 columns: name and sequence. I AAGCAGAAGACGGCATACGA Limit specifing file	For example: Illumir	na Small					
C 2 C	Nothing selected		•					
	es which submodules are to be execute sholds for the each submodules warning		also					

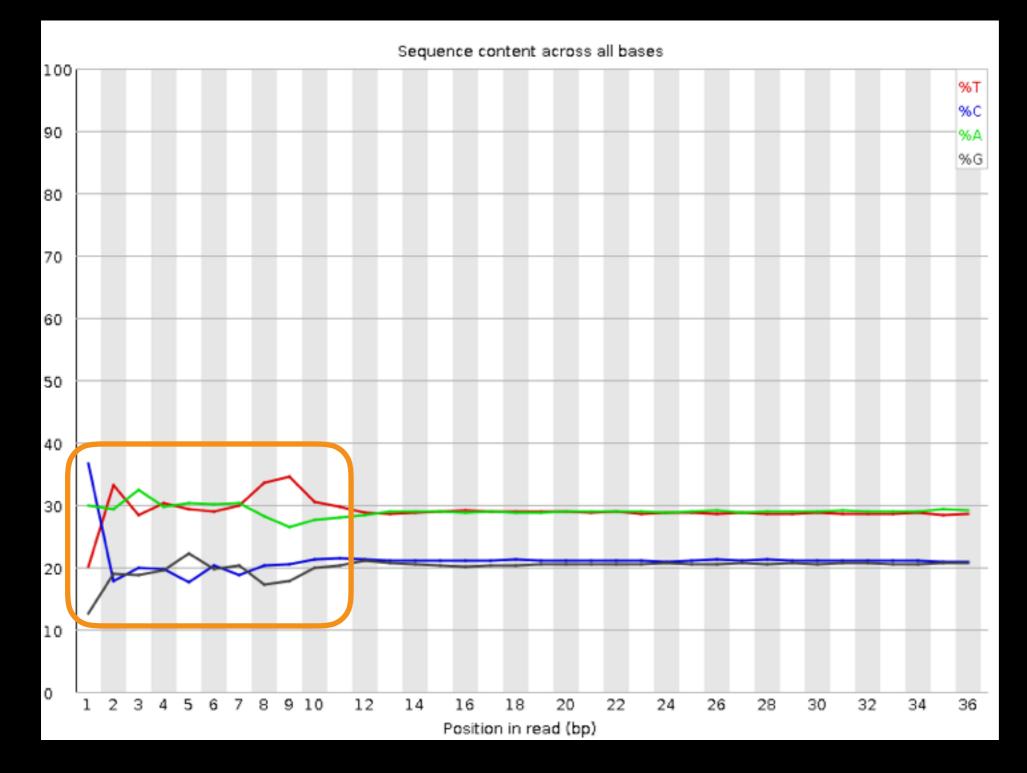
### http://bit.ly/FastQCBoxPlot

## 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)

## NGS Data Quality: Sequencing Artifacts

And only now we notice a problem with MeOH Rep1 R2 (the reverse reads)

#### 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

(But this will break pairings. More on that in a bit.)

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

### **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

Trim	momati	ic flexi	ble read trimming tool for Illumina NGS data (Galaxy Tool Version 0.32.3)	<ul> <li>Options</li> </ul>
aired	end dat	a?		
Yes	No			
Input	t Type			
Pair	of datas	ets		
Ing	out FAST	rQ file	(R1/first of pair)	
	ා අ		1: MeOH_REP1_R1	
Ing	out FAST	rQ file	(R2/second of pair)	
	3 43		2: MeOH_REP1_R2	,
Yes ut ada	No	d othe	MINACLIP step? r illumina-specific sequences from the read tion	
.: Trin	nmomat	ic Ope	ration	ť
Selec	t Trimn	nomat	ic operation to perform	
Slidi	ing wind	ow tri	mming (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**

III

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 <del>v</del>	<b>4</b>	Using	1.3 G	8	User-		<b>.</b> .	Jsing	1.3 (	GB
Enable/Disal	ole Scratchboo	k C	•	Π		History		C	Ф	
search	datasets		(	3		search da	tasets			0
RNA-Seq 6 shown	Example 1					RNA-Seq Ex 6 shown	xample 1			
57.1 MB			8 🔊 9			57.1 MB		V	۲	•
<u>6: FastQC</u> wData	on data 2: R	<u>a</u> @		×		<u>6: FastQC or</u> wData	n data 2: Ra	۲	<b>B</b>	×

#### 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	۲Q file	(R1/first of pair)	
[	3 0	D	1: MeOH_REP1_R1.fastq	
м	ultiple da	tasets	(R2/second of pair)	
1	3 2		2: MeOH_REP1_R2.fastq	

Cut adapter and other illumina-specific sequences from the read

#### **Trimmomatic Operation**

1. Trimmomatic Operation

rimmo ersion			exible	read trimming tool for Illumina NGS data (Galaxy	<ul> <li>Options</li> </ul>
Paired	end	data	1?		
Yes	No				
Input	t Тур	e			
Pair	of da	atase	ets		•
Ing	out F	AST	Q file	(R1/first of pair)	
Ing		名 AST	Q file	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 with triggered for each dataset. (R2/second of pair)	ill be
		2		<pre>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 wi triggered for each dataset.</pre>	ill be

Yes No

# Your other friend:

# Another way to avoid insanity is **Collections**

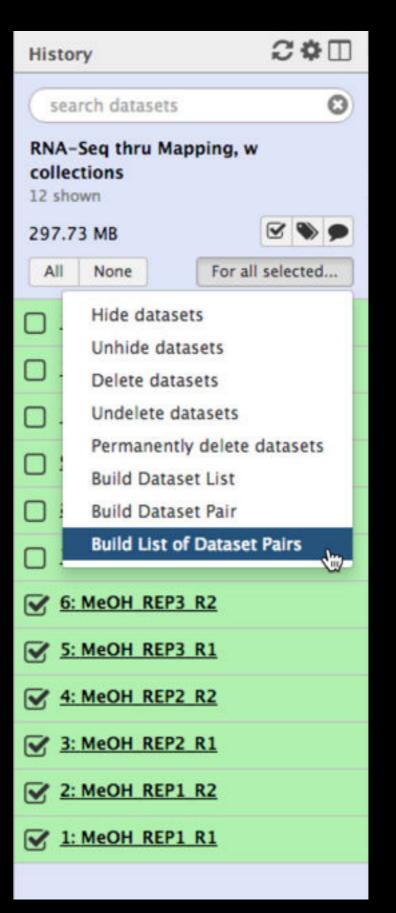
#### Dataset collections!

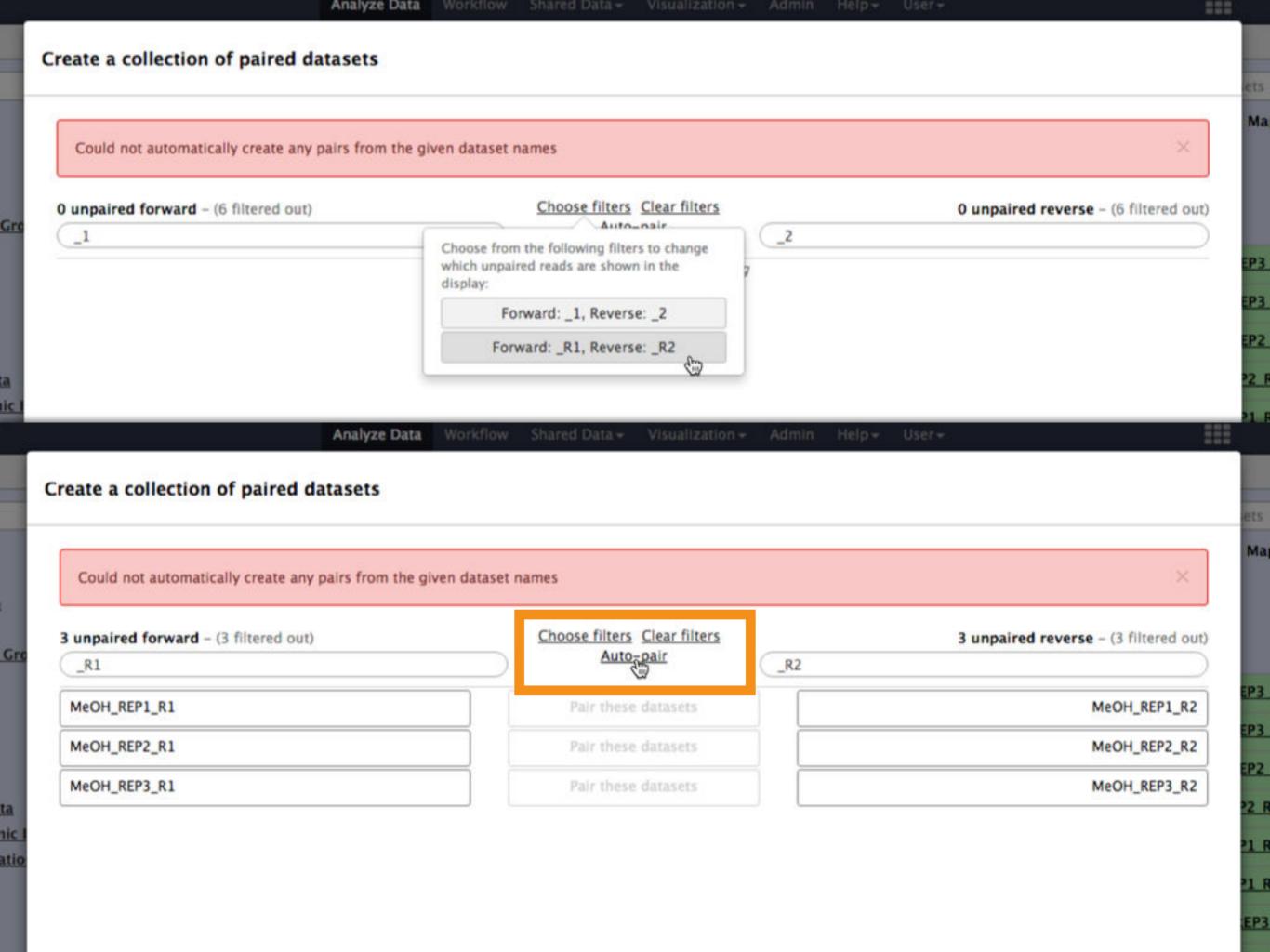
# Dataset Collections give Galaxy semantic knowledge about dataset relationships.

Tools can then take advantage of this knowledge.

## Dataset collections







Analyze Data	worknow	Shared Data +	VISUAIIZACIUN +	Aarmin	neib.* Ozer.*		==
Create a collection of paired datasets							
3 pairs created: all datasets have been successfully	y paired						×
0 unpaired forward - (0 filtered out)		Choose filters	<u>Clear filters</u>	_R2		0 unpaired reverse - (0 filtered	l out)
		3 paired L					
MeOH_REP1	_R1 🗲	MeOH_	REP1	<b>€</b> N	MeOH_REP1_R2		55
MeOH_REP2	_R1 →	MeOH_	REP2	<b>€</b> N	MeOH_REP2_R2		S
MeOH_REP3	_R1 🗲	MeOH_	REP3	<b>€</b> N	MeOH_REP3_R2		55

п

0

Cancel

		Remove file extensions from pair names? 🜌
Name:	MeOH	
		Create list



# Dataset collections



a pair of datasets

History	2¢⊡
< Back to MeOH	
MeOH_REP1 a pair of datasets	
<u>forward</u>	•
<u>reverse</u>	•



# Dataset collections Created

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5:00 Done

http://bit.ly/UR\_GXY\_2016



# Thanks

# Agenda: Day 2

- 9:00 Welcome
- 9:15 RNA-Seq Analysis Part II: Mapping
- 10:45 Break
- 11:00 RNA-Seq Analysis Part III: Differential Expression
- 12:20 Lunch (catered)
  - 1:20 RNA-Seq Analysis Part IV: Novel Transcripts
  - 2:50 Break
  - 3:05 RNA-Seq Analysis Part V
  - 5:00 Done

## http://bit.ly/UR\_GXY\_2016

# cloud5.galaxyproject.org

Just because.

Need to recreate your account

Clouds 1 and 2 are still there, but they don't work with HISAT.

#### **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 GTF for hg38, chr12 from Sanger

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

#### **RNA-seq Exercise: Mapping with HISAT2**

- HISAT looks for best place(s) to map reads, and best places to insert introns
- Does same thing as Tophat2, but HISAT2 is faster and more sensitive.
- Both HISAT and Tophat run on top of Bowtie

#### **RNA-seq Exercise: Mapping with HISAT2**

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

#### Mapping with HISAT2: basics

Single or paired: Collection of paired reads Paired reads: MeOH\_REP1

Source for reference genome: use a built-in genome Select a reference genome: hg38

### Mapping w/ HISAT2: Primary alignments

Some reads align to more than one place equally well. For such reads, how many should HISAT include? If more than the specified number, HISAT will pick those with the best mapping score.

Search for at most K distinct, primary alignments for each read. Primary alignments mean alignments whose alignment score is equal or higher than any other alignments. The search terminates when it can't find more distinct valid alignments, or when it finds K, whichever happens first. (-k)

### Mapping with HISAT2: advanced

If you expand all parameter sections, HISAT options go from 1 screen to almost 6.

Understanding all these options is the right thing to do, but it's also daunting.

One of Galaxy's strengths is that it allows you to experiment with tools and learn them incrementally.

We are going to start with setting just a few parameters.

#### Mapping with HISAT2: advanced

Spliced alignment parameters: Specify spliced ... GTF file with known splice sites: GTF for hg38, chr12 from Sanger

#### becomes

--known-splicesite-infile <path>

With this mode, you can provide a list of known splice sites, which HISAT2 makes use of to align reads with small anchors.

https://ccb.jhu.edu/software/hisat2/manual.shtml

#### Mapping with HISAT2: advanced

Transcriptome assembly reporting:

Report alignments specifically tailored for Cufflinks

Click Execute

#### .... C

ilter	
1: Filter	
Select BAM property to filte	r on
mapQuality	,
Filter on read mapping qu	uality (phred scale)
>=20	
You can use >, <, =, and ! at least 30 use ">=30"	(not) in your expression. E.g., to select reads with mapping quality of
2. Filter	តែ
2: Filter Select BAM property to filte	
	r on
Select BAM property to filte	
Select BAM property to filte isProperPair Select properly paired real Yes No	r on
Select BAM property to filte isProperPair Select properly paired real Yes No	r on .ds

Yes No

Allows complex logical constructs. See Example 4 below.



Mapping With HISAT2: What to keep?

NGS BAM Tools  $\rightarrow$  Filter

This shows two options for cleanup.

## Agenda: Day 2

- 9:00 Welcome
- 9:15 RNA-Seq Analysis Part II: Mapping
- 10:45 Break
- 11:00 RNA-Seq Analysis Part III: Differential Expression
- 12:20 Lunch (catered)
  - 1:20 RNA-Seq Analysis Part IV: Novel Transcripts
  - 2:50 Break
  - 3:05 RNA-Seq Analysis Part V
  - 5:00 Done

#### http://bit.ly/UR\_GXY\_2016

RNA-Seq Differential Expression: Get the Data

Import into a new history:
Shared Data → Data Libraries → Training → RNA-Seq\*
→ UC-Davis → Mapped
Select all
Shared Data → Data Libraries → Training → RNA-Seq\*
→ UC-Davis → Reference
Select GTF for hg38, chr12 from Sanger

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

## 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 (Same ones HISAT used for short anchors)

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 & Versions 🗸 Options
Version 2.2.1.2)
Transcripts
🗋 🙆 🗅 13: GTF for hg38, chr12 from Sanger 🗸
A transcript GFF3 or GTT me produced by cummks, curcompare, or other source.
Omit Tabular Datasets
Yes No
Discard the tabular output.
Generate SQLite
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
Condition
1: Condition
Name
MeOH
Replicates
C 62: HISAT2 on R3G
2: Condition
Name
R3G
Replicates
C 58: HISAT2 on MeOH

#### 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	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
A Destanda San Ca	Contraction of the	Shall whether a		and a state of the	10000	Contraction of the local sector	1000000		State States	Service and the service of the servi		16	

## 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?

Shared History: RNA-Seq trimmed reads to diff gene

## Agenda: Day 2

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  - 2:50 Break
  - 3:05 RNA-Seq Analysis Part V
  - 5:00 Done

#### http://bit.ly/UR\_GXY\_2016

RNA-Seq Novel Transcrpts: Get the Data

Import into a new history:
Shared Data → Data Libraries → Training → RNA-Seq\*
→ UC-Davis → Mapped
Select all
Shared Data → Data Libraries → Training → RNA-Seq\*
→ UC-Davis → Reference
Select GTF for hg38, chr12 from Sanger

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

#### RNA-Seq Novel Transcrpts: StringTie

StringTie transcript assembly and quantification (Galaxy Version 1.0.3)	▼ Options
Mapped reads to assemble transcripts from	
□         □         58: HISAT2 on MeOH	•
This is a batch mode input field. A separate job will be triggered for each dataset.	
Use GFF file to guide assembly	
Use GFF	•
Reference annotation to use for guiding the assembly process	
13: GTF for hg38, chr12 from Sanger	•
-G	
Perform abundance estimation only of input transcripts	
Yes No	
-e	
Output additional files for use in Ballgown	
Yes No	
-b	
Options	
Use defaults	-
✓ Execute	

#### Run it again on R3G

#### RNA-Seq Novel Transcrpts: StringTie

StringTie transcript assembly and quantification (Galaxy Version 1.0.3)	▼ Options
Mapped reads to assemble transcripts from	
□         □         58: HISAT2 on MeOH	•
This is a batch mode input field. A separate job will be triggered for each dataset.	
Use GFF file to guide assembly	
Use GFF	•
Reference annotation to use for guiding the assembly process	
13: GTF for hg38, chr12 from Sanger	•
-G	
Perform abundance estimation only of input transcripts	
Yes No	
-e	
Output additional files for use in Ballgown	
Yes No	
-b	
Options	
Use defaults	-
✓ Execute	

#### Run it again on R3G

RNA-Seq Novel Transcripts: CuffMerge

Now have separately discovered transcripts for each condition.

Unify them with reference annotation using CuffMerge.

Could do this with StringTie, if the Galaxy wrapper supported it.

#### RNA-Seq Novel Transcripts: CuffMerge

Cuffmerge merge together several Cufflinks assemblies (Galaxy Version 2.2.1.0)	🗞 Versions	▼ Options
GTF file(s) produced by Cufflinks		
210: StringTie on MeOH: Assembled transcripts		•
Additional GTF Inputs (Lists)		
1: Additional GTF Inputs (Lists)		Ŵ
GTF file(s) produced by Cufflinks		
234: StringTie on R3G: Assembled transcripts		•
+ Insert Additional GTF Inputs (Lists)		
Use Reference Annotation		
Yes		•
Reference Annotation		
🗋 🖄 🗀 13: GTF for hg38, chr12 from Sanger		
Requires an annotation file in GFF3 or GTF format.		
Use Sequence Data		
No		•
Use sequence data for some optional classification functions, including the addition of the p_io Cuffdiff.	d attribute requir	ed by
Minimum isoform fraction		
0.05		)
Discard isoforms with abundance below this value		
✓ Execute		

2016 Galaxy Community Conference (GCC2016)

June 25-29, 2016 Bloomington, Indiana galaxyproject.org/GCC2016

Slides & posters are now online. Video will be shortly



# 

LE CORUN

#### Le Corum Conference centre

## gcc2017.sciencesconf.org

## November 7-11



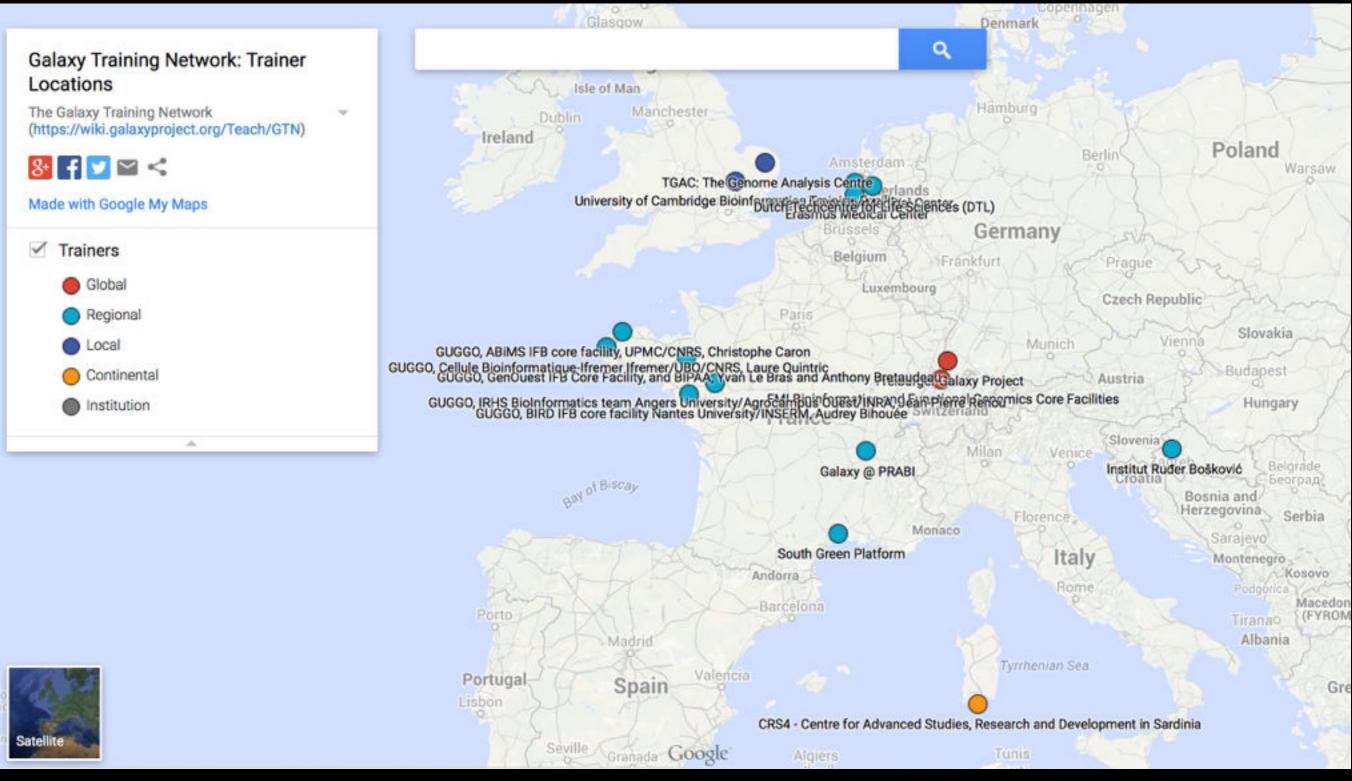
## Salt Lake City, Utah

**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

Salaxy / Dintor	Andres Data Sociality Stand Data - manipulate State - par-	=	Languite
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ENCE-IT is supported by the NEE cyberSBES program award No. 13233762

The Galaxy project a supported in part by MiR, NHOR, and the Huck matitudes of the Life Sciences.

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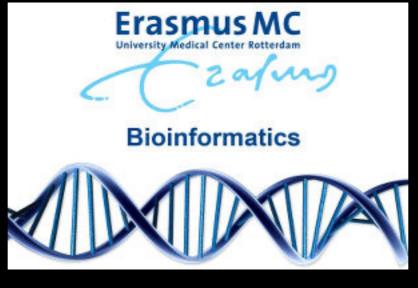
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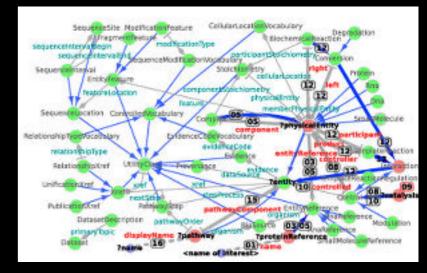
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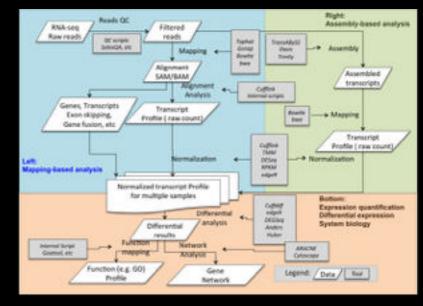
Image Analysis

Climate Change

**Social Science** 

Cosmology







Analyses and Bioinformatics for Marine Science



#### 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

# 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



#### http://wiki.galaxyproject.org

DaveClements Settings Logout |

FrontPage

💳 Galaxy Wiki



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

#### Contribute

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



Edit History Actions

#### Use Galaxy

Servers • Learn Share • Search

#### Communicate

Support • Biostar Events • Mailing Lists News S • Twitter

#### Deploy Galaxy

Get Galaxy • Cloud Tool Shed • Search

#### Contribute

Develop • Tools **Issues & Requests** Logs • Deployments Teach

#### Galaxy Project

Home • About • Cite Community **Big Picture** 

#### Community & Project

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

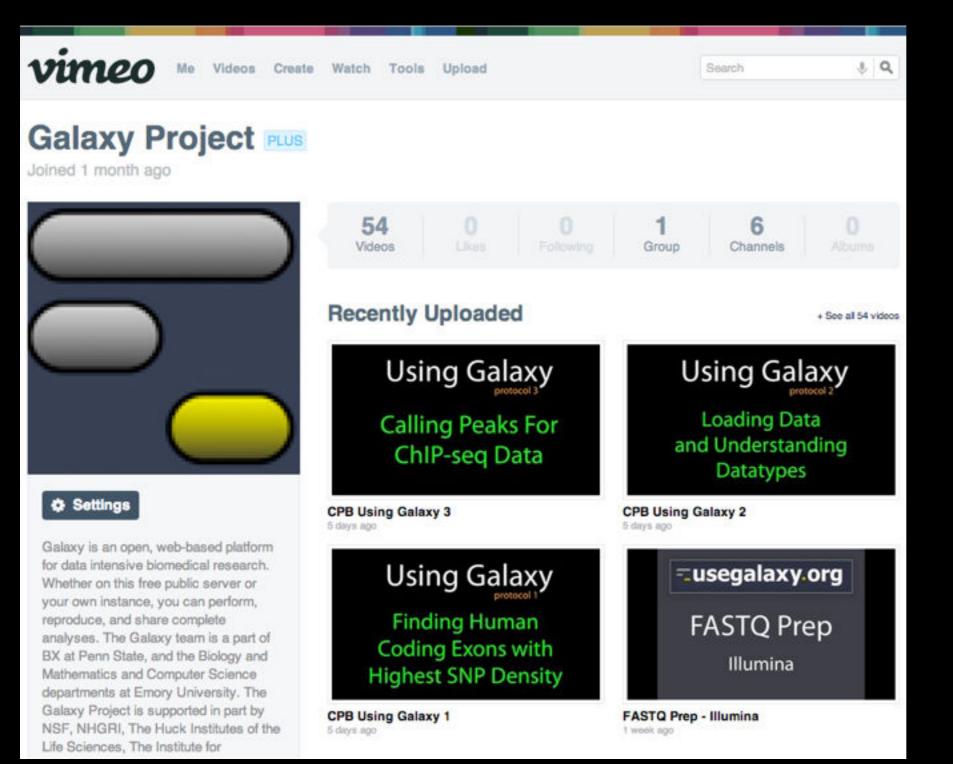
Community

#### **Events**

#### News

- Gala	axy Wiki	DaveC	lements Settings Logout   Search: Text				
Events			Edit History Actions				
Galaxy Event Horizon			News Items				
Events with Galaxy-related content are listed here.			Opening at McMaster University				
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 .			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.							
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).				
	M		See the full announcement for details. Posted to the Galaxy News on 2014-12-05				
17			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:				
	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				
	Galaxy for SNP and Variant Data Analysis	Plant and Animal Genome XXIII (PAG2014), States	<ul> <li>96 new papers, including 6 highlighted papers, referencing, using, extending, and implementing Galaxy.</li> <li>Job openings at 7+ organizations</li> <li>A new mailing list: Galaxy-Training</li> </ul>				
January 19-20	NGS pipelines with Galaxy	e-Infrastructures for Massively Parallel Sequ Sweden					
	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, pr Conference, San Francisco, Cali					
February	Large-Scale NGS data Analysis on Amazon Web Services Using Globus	Genomics & Sequencing Data Integration,					
16-18	Genomic	of Molecular Medicine Tri-Conference, Sa					

#### **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	Group: Galaxy	P Search Re	egister Log i
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A Sleeping Beauty forward genetic screen identifies new genes and pathways driving osteosarcoma development and



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