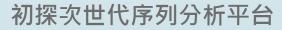


Galaxy for Next Generation Sequencing



蘇聖堯

2013/9/12





What's Galaxy?

Bringing Developers And Biologists Together. Reproducible Science Is Our Goal

- An open, web-based platform for data intensive biomedical research.
- Whether on this free public server or your own instance, you can perform, reproduce, and share complete analyses.
- The Galaxy team is a part of BX at Penn State, and the Biology and Mathematics and Computer Science departments at Emory University.
- The Galaxy Project is supported in part by NSF, NHGRI, The Huck Institutes of the Life Sciences, The Institute for CyberScience at Penn State, and Emory University.



More About Galaxy

- A platform/interface for popular NGS software.
- A data integration and analysis framework for biomedical research. It allows nearly any tool that can be run from the command line to be integrated into it.
- NO need of programming experience.
- Keeps track of all the steps performed and results throughout the analysis.

💳 Galaxy

Available Tools Pre-installed in Galaxy

Allows biologists to perform complex genomic analyses

 Analyze multiple alignments
 Compare genomic annotations
 Profile metagenomic samples
 Examine human genomic variation
 Operate on next generation sequencing data

Get Data Send Data **ENCODE Tools** Lift-Over Text Manipulation Filter and Sort Join, Subtract and Group Convert Formats Extract Features Fetch Sequences Fetch Alignments Get Genomic Scores **Operate on Genomic Intervals** Statistics Wavelet Analysis Graph/Display Data **Regional Variation** Multiple regression Multivariate Analysis Evolution Motif Tools Multiple Alignments Metagenomic analyses FASTA manipulation

NGS: QC and manipulation NGS: Picard (beta) NGS: Methylation Mapping NGS: Mapping NGS: Indel Analysis NGS: RNA Analysis NGS: SAM Tools NGS: GATK Tools (beta) NGS: GATK Tools (beta) NGS: Peak Calling NGS: Simulation SNP/WGA: Data; Filters SNP/WGA: OC; LD; Plots SNP/WGA: Statistical Models Phenotype Association VCF Tools



Portal of Galaxy

🗧 Galaxy	分析数据 工作流 共享的数据 帮助 User-	Using 5.6 Gb
工具 🍄	This is the Galaxy server, packaged for Bio-Linux.	历史 🍄
search tools	To customize this page edit /etc/galaxy/static/welcome.html then sudo restart galaxy.	35: Tophat for Illumina ● ℓ × ∧ on data 17, data 16, and data 31: accepted hits
<u>Get Data</u> Send Data		34: Tophat for Illumina @ 🖉 🕱
ENCODE Tools		on data 17, data 16, and data
Lift-Over	To find out more about Galaxy, <u>start here.</u>	31: splice junctions
Text Manipulation	WWFSMD?	33: Tophat for Illumina @ 0 🛛
Filter and Sort	grow noodly appendages	on data 17, data 16, and data
Join, Subtract and Group	Imper dataset 20 Imperiations 20	31: deletions
Convert Formats	(vdp)d supp.t	32: Tophat for Illumina @ 🖉 💥
Extract Features	Film John 32 Auto film Anno 21	on data 17, data 16, and data
Fetch Sequences	with being filled (menual) being data and the state of th	31: insertions
Fetch Alignments	Bant III Sun Quay	<u>31:</u> ● ℓ %
Get Genomic Scores	out, first Sein two Queries II Jan.	http://hgdownload.cse.ucsc.ed u/goldenPath
Operate on Genomic Intervals	with the state of	/hg19/chromosomes/chr1.fa
Statistics	ton aut, first	
Wavelet Analysis Graph/Display Data	usogalaxy org	<u>30:</u>
Regional Variation	usegalaxy.org	sd-ftp.illumina.com
Multiple regression		/Homo_sapiens/UCSC/hq19
Multivariate Analysis		<u>/Homo_sapiens_UCSC_hq19.ta</u> r.gz
Evolution	This project is supported in part by NSF, NHGRI, and the Huck Institutes of the Life Sciences.	
Motif Tools		Solution 29: Map with Bowtie
Multiple Alignments	The core Galaxy server and dependencies were packaged for Bio-Linux by the <u>NEBC Team</u> as part of a project under the <u>NERC</u> Biomolecular Analysis Facility in collaboration with the Bergman Lab at the University of Manchester.	16: mapped reads
Metagenomic analyses	biomolecular Analysis Facility in conaboration with the berginan cab at the oniversity of Manchester.	
FASTA manipulation		S 28: Tophat for ● Ø X Illumina on data 17 and data
NGS: QC and manipulation		16: accepted hits
NGS: Picard (beta)		`
<		III >



Computer Use Experience

Traditional: command mode execution

Modern: click , select file & Execute



Have You Experience These Commands On Linux?

- tail tail tophat_out_SRR039999_1/accepted_hits.sam
- head head tophat_out_SRR039999_1/accepted_hits.sam
- cat cat file1 file2 > file3
- sort sort file
- diff diff file1.sam file2.sam
- sed sed '1,2d' tophat_out_SRR039999_1/accepted_hits.sam
- awk awk '{print \$1 "\t" \$3 }' tophat_out/accepted_hits.sam
- join combines two files based on the matching content lines found
- paste merge contents of two files side by side
- split split file into smaller files



Text Manipulation

	- <u>Galaxy</u>	分析数据 工作流 共享的数据 → Visualization → Cloud → 帮助 → 账号 →
	Т具	Merge Columns (version 1.0.1)
/ :	Text Manipulation ^ Add column to an existing dataset ^ Compute an expression on every row ^ Concatenate datasets tail-to-head ^ Condense consecutive characters ^ Convert delimiters to TAB ^ Merge Columns together =	Select data:
	<u>Create single interval</u> as a new dataset <u>Cut</u> columns from a table <u>Change Case</u> of selected columns <u>Paste</u> two files side by side	Execute TIP: If your data is not TAB delimited, use Text Manipulation->Convert
	<u>Remove beginning</u> of a file <u>Select random lines</u> from a file	What it does This tool merges columns together. Any number of valid columns can be merged in any order.
	<u>Select first</u> lines from a dataset <u>Select last</u> lines from a dataset <u>Trim</u> leading or trailing characters <u>Line/Word/Character count</u> of a dataset	Example Input dataset (five columns: c1, c2, c3, c4, and c5): 1 10 1000 gene1 chr 2 100 1500 gene2 chr merging columns "c5,c1" will return:
	<u>Secure Hash / Message Digest</u> on a dataset	1 10 1000 gene1 chr chr1 2 100 1500 gene2 chr chr2
	Convert Formats FASTA manipulation Filter and Sort	A Note that all original columns are preserved and the result of merge is added as the rightmost column.



Galaxy Can Help Analyses On

- RNA-seq (Tuxedo pipeline)
- ChIP-seq/ChIP-chip
- Variant detection
- Mass Spectrometry based proteomics(Galaxy-P)
- Multivariate GWAS analysis (GWIS)
- Prediction of metagenomic taxonomy (MGTAXA)
- Gene Ontology
- Protein synthesis (GWIPS-viz)





Learning Garden

laxy 101: The first thing you should try

Galaxy Wiki

登入 | 搜尋:

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標題內文

Locked History Actions

-Galaxy web search

Use Galaxy

Use Main *(about)* Use Others! • Learn Share • Search

Communication

Support • News Events • Twitter Mailing Lists (search)

Learn

Screencasts

FAQ

Interval Ops

Datasets

Pages

Share

FTP Upload

Accounts

Support

Security

Search

Deploy Galaxy Get Galaxy • Cloud

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



Contribute Tool Shed • Share

Salasy Augustan Banda Banda Salasa Salasa

Aftat are we trying to do?

Descripted Locked File Reported Locked File R

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		stand rank and Mitamet Jasch		-	5
		barn	Assessment	dent.	
		Search and	Competition of Galaxy II, HECKA painting		
	Advanced fastQ manipulation	PL LANS	Contraction contraction of the particular of the particular of the particular of the particular of the particular of the Allowed Contraction Allowed Contraction and Contraction of the second of the	Aug 10 10	
		Independent		441	
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Learn Galaxy

目錄

Learn

- 1. Galaxy 101
- 2. Screencasts
- 3. Shared Pages, Histories & Workflows
- 4. Other Tutorials
- 5. Datasets
- 6. Tools
- 7. Visualization
- 8. User Accounts

9. Other

http://wiki.galaxyproject.org/Learn

There are many approaches to learning how to *use* Galaxy. The most popular is probably to just dive in and use it. Galaxy is simple enough to use that you can do many analyses just by exploring the interface. However, you may miss much of the power this way.

Watch the short Learn screencast for a learning resource overview.

Galaxy 101

Walking through the *Galaxy 101* exercise will show you the ins and outs of using Galaxy. This includes loading data (from UCSC in this example), using genome builds, the tool interface, filtering, sorting, and combining datasets, generating statistics, and Galaxy's History, Workflow and sharing support.

Galaxy 101 is available in several formats. You can start with either the Galaxy Page or the screencast.



Choice



	Main	Local	Cloud	Appliance	Other
Your data sets are moderately sized	Yes	Yes	Yes	Yes	?
Your computational requirements are moderate	Yes	Yes	Yes	Yes	?
You want to share your Galaxy objects with others	Yes	Yes	Yes	Yes	?
All needed Tools are installed on Main.	Yes	?	Yes	Yes	?
Your data sets are very large	No	?	Yes	Yes	?
Your computational requirements are very large	No	?	Yes	Yes	?
You have absolute data security requirements	No	Yes	Yes	Yes	?
No network transfer of data	No	Yes	No	Yes	Yes



CloudMan



Getting Started with Galaxy CloudMan

This page provides a step-by-step instructions on how to start your own instance of Galaxy on Amazon Web Services (AWS) Elastic Compute Cloud (EC2). More general information and instructions about Galaxy CloudMan can be found here.

AWS								
Get Started								
apacity Planning								
AMIs								
+ CloudMan								

目錄

- 1. Screencast
- 2. Step 1: One Time Amazon S
- 3. Step 2: Starting a Master Ir
- 4. Step 3: Galaxy CloudMan W
- 5. Step 4: Use Galaxy as you r
- 6. Step 5: Shutting Down

Step 1: One Time Amazon Setup

- Because AWS services implement pay-as-you-go access model for compute resources, it is necessary for every user of the service to *register with Amazon*. You will need a credit card to register. (You can apply for a AWS Education Grant after you register).
- Once your account has been approved by Amazon (note that this may take up to one business day), log into the EC2 AWS Management Console and set your AWS Region to US East (Virginia). This is the only region Galaxy CloudMan is fully supported in at this time (see screenshot 1.2).

Screencast

Here's a screencast that walks through the process of setting up your own Galaxy cloud cluster. Note, the screencast skips one step, detailed setting up of the Inbound TCP rules. See "Inbound Rules" below.

SlipStream

You are here: Home » SlipStream Appliance

SlipS	TOOLS	TASK	DATA	RUN-TIME	
	Bowtie 2	Mapping whole human genome	204 million paired-end 100bp Illumina reads	2 Hours 44 Minutes	
	SAMTools	SAM-BAM conversion	127GB SAM (41GB resulting BAM)	2 Hours 7 Minutes	
ocu: le SlipSt nsultinc	TopHat 2	RNA-Seq mapping	24 million 100bp Illumina reads	1 Hours 24 Minutes	Ð
	Cufflinks 2	Differential Expression Analysis	4.3 GB SAM File	0 Hours 11 Minutes	

The SlipStream Appliance: Galaxy Edition offers a powerful dedicated resource for data analysis. It reduces the IT and administrative burden of running a production instance of Galaxy. It offers a powerful dedicated resource and, like the Galaxy platform, is designed to lower the barrier to entry into data analysis.

SlipStream Galaxy is a hardware appliance consists of **16** Intel cores, **100 GB** of solid state drive, **384 GB** of memory, and **16 TB** of usable storage space. Galaxy is pre-installed and configured. The appliance sells for under **\$20,000**.



Where to run Galaxy

Main

http://main.g2.bx.psu.edu/

Public accessible servers

http://wiki.galaxyproject.org/PublicGalaxyServers

Local

http://your-local-site/



Public Accessible Servers

Publicly Accessible Galaxy Servers

Galaxy's public server (UseGalaxy.org, *Main*) can meet many needs, but it is not suitable for everything (see Choices for why) and cannot possibly scale to meet the entire world's needs.

Fortunately the Galaxy Community is helping out by installing Galaxy at their institutions and then making those installations either publicly available or open to their organizations or community.

This page lists such public or semi-public Galaxy servers.

To add your public Galaxy server to this list, please either just add it (hey, *it's a wiki*), or contact Galaxy Outreach Outreach AT galaxyproject DOT org>

Andromeda

• Link:

Andromeda

• Domain/Purpose:

This is a fully populated Galaxy instance.

Comments:

Support:

Andromeda is hosted at the SURFsara High Performance Computing (HPC) cloud. The installation is supported by Enis Afgan (CloudMan project) and Mattias de Hollander (NIOO).



目錄

- 1. Andromeda
- 2. ballaxy
- 3. CBiB Galaxy
- 4. Cistrome Analysis Pipeline
- 5. DBCLS Galaxy
- 6. Galaxy Main
- 7. Galaxy-P
- 8. Galaxy Test
- 9. GeneNetwork
- 10. Genboree
- 11. Genomic Hyperbrowser
- 12. Gene Ontololgy (GO)
- 13. GigaGalaxy
- 14. GWIPS-viz
- 15. GWIS
- 16. Huttenhower Lab
- 17. INRA-URGI
- 18. In Silico Galaxy
- 19. LiSIs
- 20. Martin Luther University Halle-Wittenberg
- 21. MBAC Metabiome Portal
- 22. MGTAXA
- 23. Nebula
- 24. NELLY
- 25. Netherlands Metabolomics



Data Upload

	- <mark>_ Galaxy</mark>	分析数据 工作流 共享的数据 ■ 帮助 ■ User ■
	工具 🌣	Upload File (version 1.1.3)
/	search tools <u>Get Data</u> <u>Upload File</u> from your computer <u>UCSC Main</u> table browser <u>UCSC Test</u> table browser	File Format: Auto-detect Which format? See help below File: 瀏覽・・・ 未選擇檔案。 TIP: Due to browser limitations, uploading files larger than 2GB is guaranteed to fail. To upload large files, use the URL method
	 <u>UCSC Archaea</u> table browser <u>BX main</u> browser <u>EBI SRA</u> ENA SRA <u>Get Microbial Data</u> <u>BioMart</u> Central server <u>BioMart</u> Test server CBI Rice Mart rice mart 	(below) or FTP (if enabled by the site administrator). URL/Text:
	<u>GrameneMart</u> Central server	Files uploaded via FTP:
	 <u>modENCODE fly</u> server <u>Flymine</u> server <u>Flymine test</u> server 	Your FTP upload directory contains no files. 3. Via FTP This Galaxy server allows you to upload files via FTP. To upload some files, log in to the FTP server at localhost using your Galaxy credentials (email address and password).
	 <u>modENCODE modMine</u> server <u>Ratmine</u> server 	Convert spaces to tabs:
	YeastMine server metabolicMine server	Genome:



Data Format

FASTA sequence format

NGS file formats

■fastq, sam, bam

UCSC file format specificationsbed, wig, gtf, gff



Keep In Mind The Fundamental Concept

Raw data (generated from sequencer): FASTQ

After short read mapping tools (BWA, Bowtie): SAM/BAM

Annotation file for genome browser: GTF, WIG, BED



FASTQ

FASTQ format is a text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores

Line 1 begins with a '@' character and is followed by a sequence identifier. Line 2 is the raw sequence letters. Line 3 begins with a '+' character.

Line 4 encodes the quality values for the sequence in Line 2, and must contain the same number of symbols as letters in the sequence.



WIG

The **wiggle (WIG) format** is for display of dense, continuous data such as GC percent, probability scores, and transcriptome data

variableStep chrom=chr2 300701 12.5 300702 12.5 300703 12.5 300704 12.5 300705 12.5

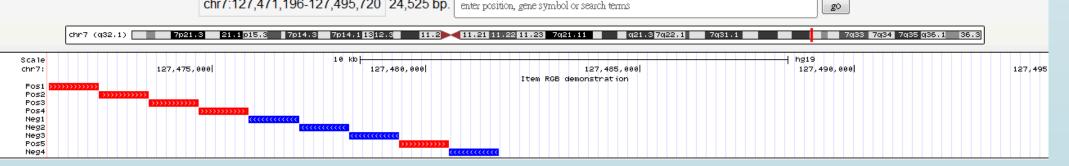
variableStep chrom=chr2 span=5 300701 12.5

0 Kb	1		115	,930 ka		ï		115,94 	0 Kb		ï	115,950 	kb	
0 - 11	ï	د. دفقد	J		7		11		1			T.	Ш	ىلە
0 - 4.001		-		1	1.1		10.1			1		dat also		alı.
> - 3.00	d				- the		1.1	1	11		an -	al.		
- 7.801			يام		-	п			1_		- 11	Lat.		
0 - 4.001		مار للله	Ψ				1.	41						ALL I
2 - 3.001	1	- 10	1.11		14	atti	and t				н	1	1	
D - 4.5DI	-	66 B	de este	1993					1				had	-

BED

BED format provides a flexible way to define the data lines that are displayed in an annotation track

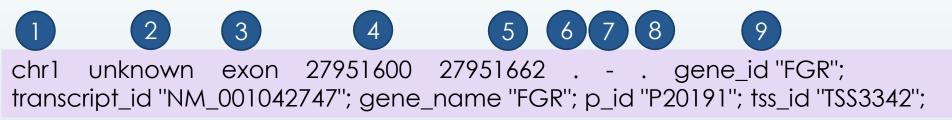
```
browser position chr7:127471196-127495720
browser hide all
track name="ItemRGBDemo" description="Item RGB demonstration" visibility=2
itemRgb="On"
chr7 127471196 127472363 Pos1 0 + 127471196 127472363 255,0,0
chr7 127472363 127473530 Pos2 0 + 127472363 127473530 255,0,0
chr7 127473530 127474697 Pos3 0 + 127473530 127474697 255,0,0
chr7 127474697 127475864 Pos4 0 + 127474697 127475864 255,0,0
chr7 127475864 127477031 Neg1 0 - 127475864 127477031 0,0,255
chr7 127477031 127478198 Neg2 0 - 127477031 127478198 0,0,255
UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
```





GFF/GTF (Gene Transfer Format)

GFF format General Feature Format is a format for describing genes and other features associated with DNA, RNA and Protein sequences.



1. seqname - Must be a chromosome or scaffold.

- 2. source The program that generated this feature.
- 3. feature The name of this type of feature. Some examples of standard feature types are "CDS", "start_codon", "stop_codon", and "exon".

4. start - The starting position of the feature in the sequence. The first base is numbered 1.

- 5. end The ending position of the feature (inclusive).
- 6. score A score between 0 and 1000. If there is no score value, enter ".".
- 7. strand Valid entries include '+', '-', or '.' (for don't know/care).
- 8. frame If the feature is a coding exon, frame should be a number between 0-2 that represents the reading frame of the first base. If the feature is not a coding exon, the value should be '.'.
- 9. group All lines with the same group are linked together into a single item.



SAM format data is output from aligners that read FASTQ files and assign the sequences to a position with respect to a known reference genome.

Coor	12345678901234 5678901234567890123456789012345
ref	AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1	TTAGATAAAGGATA*CTG
+r002	aaaAGATAA*GGATA
+r003	gcctaAGCTAA
+r004	ATAGCTTCAGC
-r003	ttagctTAGGC
-r001/2	CAGCGGCAT

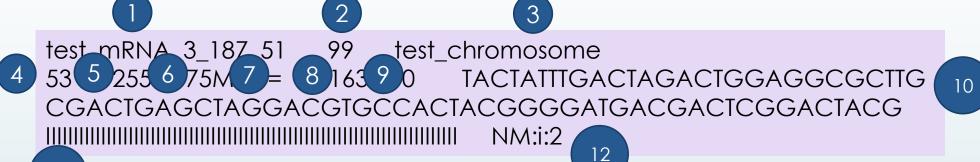
The corresponding SAM format is:

	@HD V	@HD VN:1.5 SO:coordinate											
	@SQ SN:ref LN:45												
	r001	163	ref	7	30	8M2I4M1D3M	=	37	39	TTAGATAAAGGATACTG	*		
	r002	0	ref	9	30	3S6M1P1I4M	*	0	0	AAAAGATAAGGATA	*		
	r003	0	\mathbf{ref}	9	30	5S6M	*	0	0	GCCTAAGCTAA	*	SA:Z:ref,29,-,6H5M,17,0;	
	r004	0	ref	16	30	6M14N5M	*	0	0	ATAGCTTCAGC	*		
	r003	2064	\mathbf{ref}	29	17	6H5M	*	0	0	TAGGC	*	SA:Z:ref,9,+,5S6M,30,1;	
	r001	83	\mathbf{ref}	37	30	9M	=	7	-39	CAGCGGCAT	*	NM:i:1	
1													



More About SAM Format

11

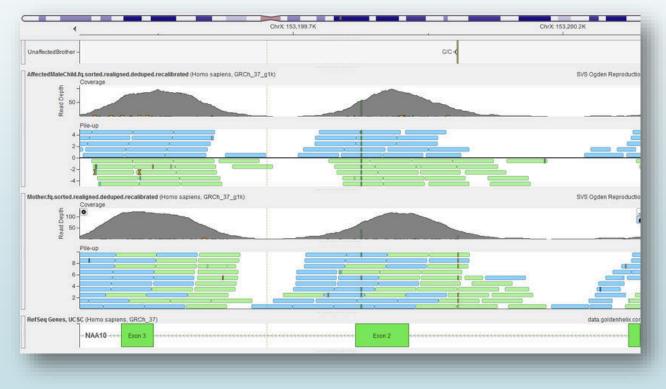


Col	Field	Description
1	QNAME	Query (pair) NAME
2	FLAG	bitwise FLAG
3	RNAME	Reference sequence NAME
4	POS	1-based leftmost POSition/coordinate of clipped sequence
5	MAPQ	MAPping Quality (Phred-scaled)
6	CIAGR	extended CIGAR string
7	MRNM	Mate Reference sequence NaMe ('=' if same as RNAME)
8	MPOS	1-based Mate POSistion
9	ISIZE	Inferred insert SIZE
10	SEQ	query SEQuence on the same strand as the reference
11	QUAL	query QUALity (ASCII-33 gives the Phred base quality)
12	OPT	variable OPTional fields in the format TAG:VTYPE:VALUE



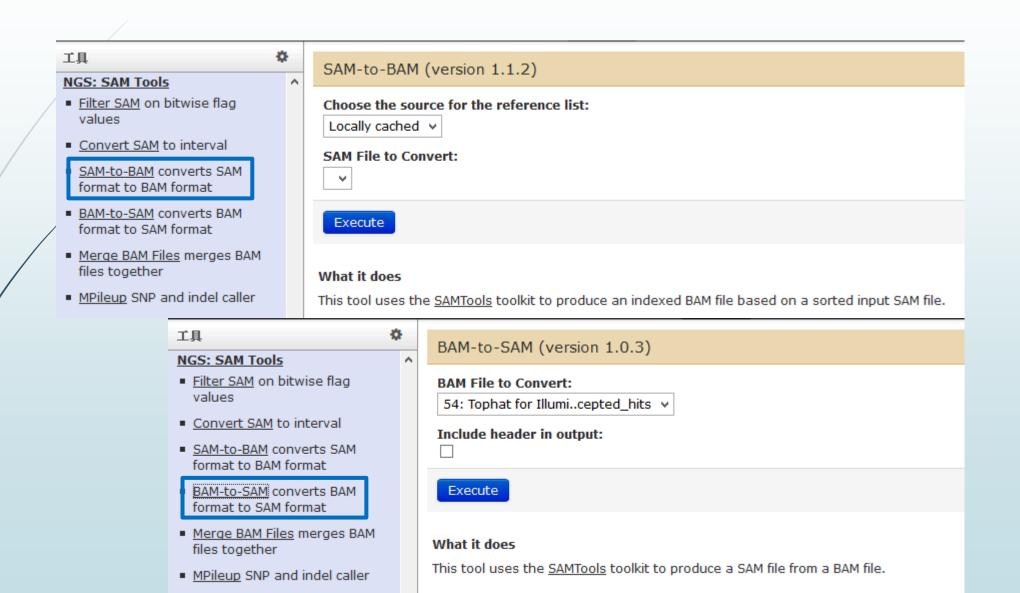
BAM

Stores the same data as SAM file in a **compressed**, indexed, binary form.





NGS: SAM Tools: BAM⇔SAM





Basic Modules

- Data I/O : Get Data & Send Data
- Text Manipulation
- Convert Formats
- Statistics
- Display Data
- NGS Analysis : QC, Mapping, RNA Analysis, Methylation Mapping, Peak Calling
- SNP Analysis

Get Data Send Data ENCODE Tools Lift-Over Text Manipulation Filter and Sort Join. Subtract and Group **Convert Formats** Extract Features Fetch Sequences Fetch Alignments Get Genomic Scores **Operate on Genomic Intervals** Statistics Wavelet Analysis Graph/Display Data **Regional Variation** Multiple regression Multivariate Analysis Evolution Motif Tools Multiple Alignments Metagenomic analyses **FASTA** manipulation

NGS: QC and manipulation NGS: Picard (beta) NGS: Methylation Mapping NGS: Mapping NGS: Indel Analysis NGS: Indel Analysis NGS: RNA Analysis NGS: RNA Analysis NGS: GATK Tools (beta) NGS: GATK Tools (beta) NGS: Peak Calling NGS: Simulation SNP/WGA: Data; Filters SNP/WGA: OC; LD; Plots SNP/WGA: Statistical Models Phenotype Association VCF Tools

Must Know Your Data Types Very Well

工員

NGS: RNA Analysis

RNA-SEQ

<u>Tophat for Illumina</u> Find splice junctions using RNA-seq data

- <u>Tophat2</u> Gapped-read mapper for RNA-seq data
- Tophat for SOLiD Find splice junctions using RNA-seq data
- <u>Cufflinks</u> transcript assembly and FPKM (RPKM) estimates for RNA-Seq data
- <u>Cuffcompare</u> compare assembled transcripts to a reference annotation and track Cufflinks transcripts across multiple experiments
- <u>eXpress</u> Quantify the abundances of a set of target sequences from sampled subsequences
- <u>Cuffmerge</u> merge together several Cufflinks assemblies
- <u>Cuffdiff</u> find significant changes in transcript expression, splicing, and promoter use

DE NOVO ASSEMBLY

Trinity De novo assembly of

Tophat for Illumina (version 1.5.0)

RNA-Seq FASTQ file:

41: Galaxy5-[brain_2...fastqsanger 🗸

Nucleotide-space: Must have Sanger-scaled quality values with ASCII offset 33

Will you select a reference genome from your history or use a built-in index?:

Use a built-in index

Built-ins were indexed using default options

Select a reference genome:

hg19 ∨

¢

If your genome of interest is not listed, contact the Galaxy team

Is this library mate-paired?:

Paired-end v

RNA-Seq FASTQ file:

41: Galaxy5-[brain_2...fastqsanger \vee

Nucleotide-space: Must have Sanger-scaled quality values with ASCII offset 33

Mean Inner Distance between Mate Pairs:

20

TopHat settings to use:

Full parameter list 🗵

Use the Full parameter list to change default settings.

Library Type:

FR Unstranded





TopHat will treat the reads as strand specific. Every read alignment will have an XS attribute tag. Consider supplying library type options below to select the correct RNA-seq protocol.



Text Manipulation

\$

~

工具

Text Manipulation

- Add column to an existing dataset
- Compute an expression on every row
- Concatenate datasets tail-to-head
- Cut columns from a table
- Merge Columns together
- Convert delimiters to TAB
- Create single interval as a new dataset
- Change Case of selected columns
- Paste two files side by side
- Remove beginning of a file
- Select random lines from a file
- Select first lines from a dataset
- Select last lines from a dataset
- Trim leading or trailing characters

<

Line/Word/Character count of datacot

74: Filter of	on data 70
---------------	------------

Dataset missing? See TIP below.

Merge column:

```
c1 🗸
```

with column:

c1 ¥

Need to add more columns? Use controls below.

Columns

Add new Columns

Execute

1 TIP: If your data is not TAB delimited, use Text Manipulation->Convert

What it does

This tool merges columns together. Any number of valid columns can be merged in any order.

Example

Input dataset (five columns: c1, c2, c3, c4, and c5):

1 10 1000 gene1 chr 2 100 1500 gene2 chr

merging columns "c5,c1" will return:

1 10 1000 gene1 chr chr1 2 100 1500 gene2 chr chr2

A Note that all original columns are preserved and the result of merge is added as the rightmost column.



Extract Genomic DNA

工具

Extract Features

by two separate tools.

What it does

÷.

 \sim

This tool uses coordinate, strand, and build information to fetch genomic DNAs in FASTA or interval format. If strand is not defined, the default value is "+".

Example

If the input dataset is:

chr7 127475281 127475310 NM_000230 0 + chr7 127485994 127486166 NM_000230 0 + chr7 127486011 127486166 D49487 0 +

Extracting sequences with FASTA output data type returns:

>hg17_chr7_127475281_127475310_+ GTAGGAATCGCAGCGCCAGCGGTTGCAAG >hg17_chr7_127485994_127486166_+ GCCCAAGAAGCCCATCCTGGGAAGGAAAATGCATTGGGGAACCCTGTGCG GATTCTTGTGGGCTTTGGCCCTATCTTTTCTATGTCCAAGGCTGTGCCCATC CAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAATTGTCACCAG GATCAATGACATTTCACACACG >hg17_chr7_127486011_127486166_+ TGGGAAGGAAAATGCATTGGCGAACCCTGTGCGGATTCTTGTGGCTTTGG CCCTATCTTTTCTATGTCCAAGACGACCCTGTGCCGATCTTGTGGCTTTGG CCCTATCTTTTCTATGTCCAAGCTGTGCCCATCCAAAAAGTCCAAGATGA CACCAAAACCCTCATCAAGACAATTGTCACCAGGATCAATGACATTTCAC ACACG

Extracting sequences with Interval output data type returns:

chr7 127475281 127475310 NM_000230 GTAGGAATCGCAGCGCCAGCGGTTGCAAG 0 + NM_000230 chr7 127485994 127486166 0 GCCCAAGAAGCCCATCCTGGGAAGGAAAATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTTGG 127486011 127486166 D49487 0 chr7 + TGGGAAGGAAATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCCTTTGGCCCTATCTTTTCTATGTCCAAGCTG

Fetch Sequences Extract Genomic DNA using coordinates from assembled/unassembled genomes Fetch Alignments Get Genomic Scores **Operate on Genomic Intervals** Statistics Wavelet Analysis Graph/Display Data **Regional Variation** Multiple regression Multivariate Analysis Evolution Motif Tools **Multiple Alignments** Metagenomic analyses **FASTA** manipulation NGS: QC and manipulation NGS: Picard (beta) NGS: Methylation Mapping NGS: Mapping NGS: Indel Analysis NGS: RNA Analysis



Filter and Sort

¢

工具

Text Manipulation

Filter and Sort

- Filter data on any column using simple expressions
- <u>Sort</u> data in ascending or descending order
- <u>Select</u> lines that match an expression

GFF

- Extract features from GFF data
- Filter GFF data by attribute using simple expressions
- Filter GFF data by feature count using simple expressions
- Filter GTF data by attribute values list

Join, Subtract and Group Convert Formats Extract Features Fetch Sequences Fetch Alignments Get Genomic Scores Operate on Genomic Intervals

Filter (version 1.1.0)

Filter:

74: Filter on data 70

Dataset missing? See TIP below.

With following condition:

c1=='chr22'

Double equal signs, ==, must be used as shown above. To filter for an arbitrary string, use the Select tool.

Execute

- Double equal signs, ==, must be used as "equal to" (e.g., c1 == 'chr22')
- **TIP:** Attempting to apply a filtering condition may throw exceptions if the data type (e.g., string, integer) in every line of the columns being filtered is not appropriate for the condition (e.g., attempting certain numerical calculations on strings). If an exception is thrown when applying the condition to a line, that line is skipped as invalid for the filter condition. The number of invalid skipped lines is documented in the resulting history item as a "Condition/data issue".

1 TIP: If your data is not TAB delimited, use Text Manipulation->Convert

Syntax

The filter tool allows you to restrict the dataset using simple conditional statements.

Columns are referenced with **c** and a **number**. For example, **c1** refers to the first column of a tab-delimited file Make sure that multi-character operators contain no white space (e.g., <= is valid while < = is not valid) When using 'equal-to' operator **double equal sign** '==' **must be used** (e.g., **c1**=='**chr1**') Non-numerical values must be included in single or double quotes (e.g., **c6**=='+') Filtering condition can include logical operators, but **make sure operators are all lower case** (e.g., **(c1!='chrX' and c1!='chrY') or not c6**=='+')



Convert Formats

÷.

工具

Convert Formats

- <u>AXT to concatenated FASTA</u> Converts an AXT formatted file to a concatenated FASTA alignment
- <u>AXT to FASTA</u> Converts an AXT formatted file to FASTA format
- <u>AXT to LAV</u> Converts an AXT formatted file to LAV format

BED-to-GFF converter

- FASTA-to-Tabular converter
- <u>GFF-to-BED</u> converter
- <u>LAV to BED</u> Converts a LAV formatted file to BED format
- <u>Maf to BED</u> Converts a MAF formatted file to the BED format
- <u>MAF to Interval</u> Converts a MAF formatted file to the Interval format
- MAF to FASTA Converts a MAF formatted file to FASTA format
- <u>Tabular-to-FASTA</u> converts tabular file to FASTA format
- FASTQ to FASTA converter

BED-to-GFF (version 2.0.0)

Convert this query:

60: (as bed) Cuffmerge on data..transcripts 🗸

Execute

What it does

This tool converts data from BED format to GFF format (scroll down for format description).

			_	
E	xa	m	pl	e

The following data in BED format:

chr28 346187 388197 BC114771 0 + 346187 388197 0 9 144,81,115,63,155,96,134,105,112, 0,24095,261

Will be converted to GFF (note that the start coordinate is incremented by 1):

##gff-version 2 ##bed_to_gff_converter.py

(chr28	bed2gff	mRNA	346188	388197	0	+	-	mRNA	BC114771;
(chr28	bed2gff	exon	346188	346331	0	+		exon	BC114771;
(chr28	bed2gff	exon	370283	370363	0	+		exon	BC114771;
(chr28	bed2gff	exon	372378	372492	0	+		exon	BC114771;
(chr28	bed2gff	exon	377194	377256	0	+		exon	BC114771;
(chr28	bed2gff	exon	378319	378473	0	+		exon	BC114771;
(chr28	bed2gff	exon	379722	379817	0	+		exon	BC114771;
(chr28	bed2gff	exon	383182	383315	0	+		exon	BC114771;
(chr28	bed2gff	exon	387981	388085	0	+		exon	BC114771;
(chr28	bed2gff	exon	388086	388197	0	+	-	exon	BC114771;



Reverse Complement

¢

工具

- Filter by quality
- Remove sequencing artifacts
- Barcode Splitter
- <u>Clip</u> adapter sequences
- <u>Collapse</u> sequences
- Rename sequences
- Reverse-Complement
- Trim sequences

NGS: Picard (beta) NGS: Methylation Mapping NGS: Mapping NGS: Indel Analysis NGS: RNA Analysis NGS: SAM Tools NGS: GATK Tools (beta) NGS: Peak Calling NGS: Simulation SNP/WGA: Data; Filters

- SNP/WGA: QC; LD; Plots
- SNP/WGA: Statistical Models
- Phenotype Association

VCF Tools

Reverse-Complement (version 1.0.0)

Library to reverse-complement: 42: http://hgdownload..es/chr19.fa v

Execute

What it does

This tool reverse-complements each sequence in a library. If the library is a FASTQ, the quality-scores are also reversed.

Example

Input FASTQ file:

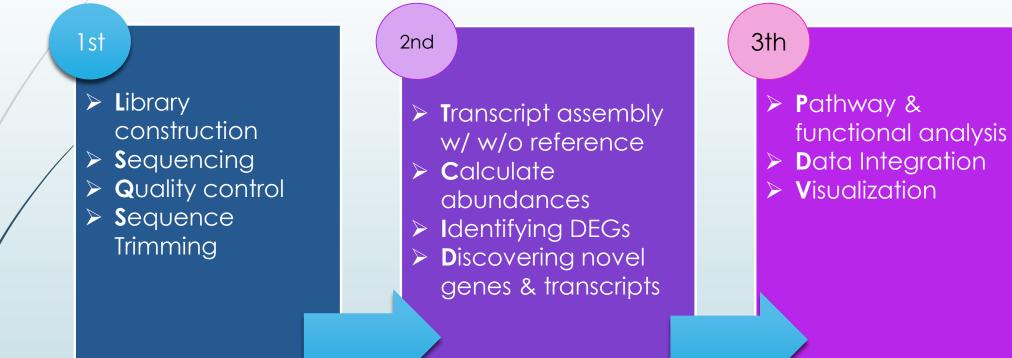
@CSHL_1_FC42AGWWWWXX:8:1:3:740 TGTCTGTAGCCTCNTCCTTGTAATTCAAAGNNGGTA +CSHL_1_FC42AGWWWXX:8:1:3:740 33 33 34 33 33 33 33 33 33 33 33 33 27 5 27 33 33 33 33 33 33 27 21 27 33 32 31 29 26 24 5 5 15 17 27 26

Output FASTQ file:

This tool is based on FASTX-toolkit by Assaf Gordon.



RNA-Seq Data Analysis





Exercise

- Adrenal & brain tissues RNA-seq data (Illumina BodyMap 2.0)
- Know its reads quality (Trim reads)
- Map the reads



- Assemble and analyze transcripts
- Identify all novel splice junctions and transcript isoforms
- Find loci that exhibit differences in TSS and splicing



FastQC: NGS Quality Control

工目

TASTA MUMPHUUM

NGS: QC and manipulation

Fastgc: Fastgc QC using FastQC from Babraham

FASTO Groomer convert between various FASTQ

FASTQ splitter on joined

FASTQ joiner on paired end

FASTQ Summary Statistics by

Build base quality distribution

ILLUMINA FASTQ

quality formats

paired end reads

reads

column

FASTQC: FASTQ/SAM/BAM

L7 GA120-6 NoIndex L007 R1 001.fastq FastQC Report

Report Wed 4 Sep 2013 L7 GA120-6 NoIndex L007 R1 001.fastq

Summary



Per sequence GC content

Sequence Length Distribution

Sequence Duplication Levels

Overrepresented sequences

Per base N content

Kmer Content



Measure

Filename

File type

Encoding

%GC

L7 GA120-6 NoIndex L007 R1 001.fastq

Filtered Sequences 0

Sequence length

- 100
- Total Sequences 4000000
- Conventional base calls Sanger / Illumina 1.9 49

Value

Select high quality segments

ROCHE-454 DATA

Combine FASTA and QUAL into FASTQ

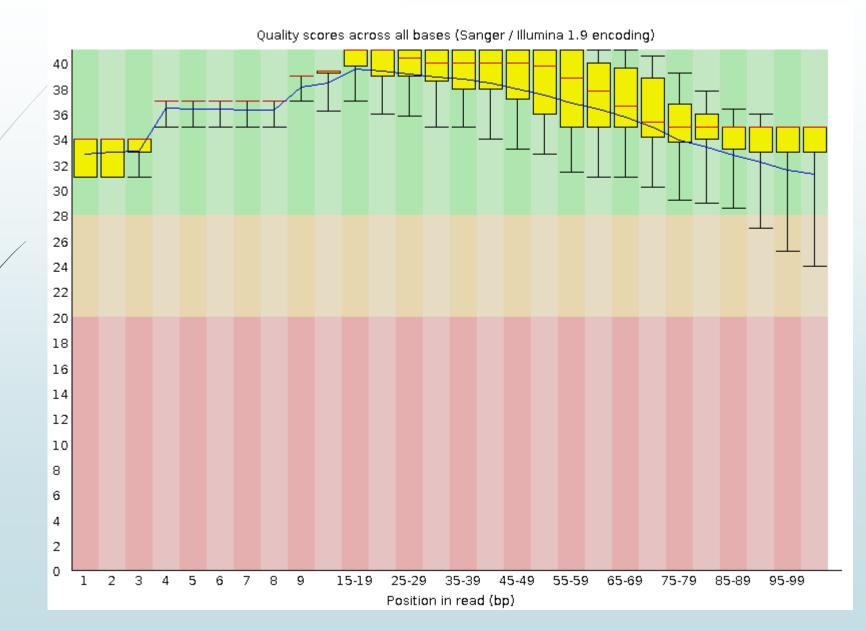
AB-SOLID DATA

- Convert SOLiD output to fastq
- Compute guality statistics for SOLiD data
- Draw guality score boxplot

v



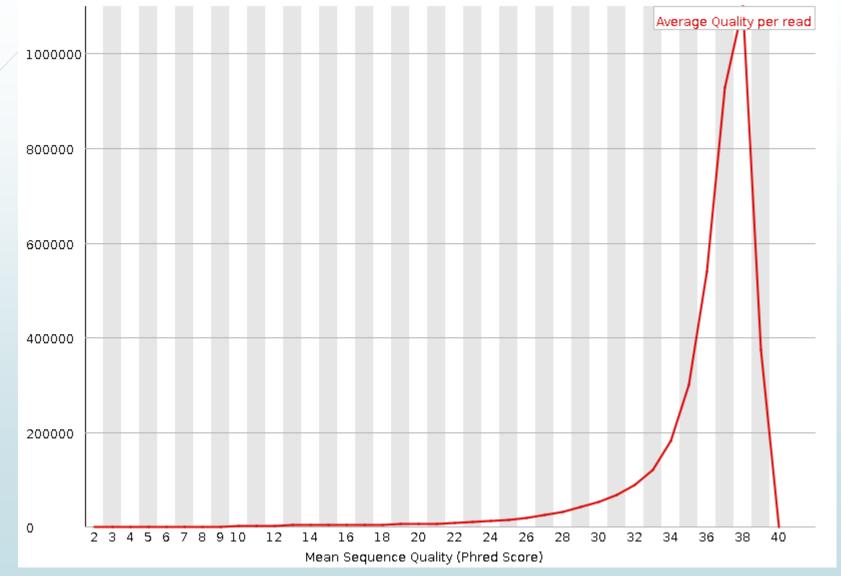
Per base sequence quality



Per sequence quality scores



Quality score distribution over all sequences





FASTQ Trimmer

	\$	FASTQ Trimmer (version 1.0.0)
 Filter FASTQ reads by quality score and length 	/	FASTQ File: 41: Galaxy5-[brain_2fastqsanger v
 <u>FASTQ Trimmer</u> by column <u>FASTQ Quality Trimmer</u> by sliding window <u>FASTQ Masker</u> by quality 		Define Base Offsets as: Absolute Values Use Absolute for fixed length reads (Illumina, SOLiD) Use Percentage for variable length reads (Roche/454)
score <u>FASTO interlacer</u> on paired end reads 	You (trimr	tool allows you to trim the ends of reads. can specify either absolute or percent-based offsets. Offsets are calculated, starting at 0, from the respective end to be ned. When using the percent-based method, offsets are rounded to the nearest integer. example, if you have a read of length 36:
 <u>Manipulate FASTQ</u> reads on various attributes <u>FASTQ to FASTA</u> converter 	@Some CAATA +	FASTQ Sanger Read TGTNCTCACTGATAAGTGGATATNAGCNCCA ;B-%?8>CBA@>7@7BBCA4-48%<;;% <b@< th=""></b@<>
 FASTQ to Tabular converter Tabular to FASTQ converter FASTX TOOL KIT FOR 	@Some ATATO +	you set absolute offsets of 2 and 9: FASTQ Sanger Read TNCTCACTGATAAGTGGATA -%78>CBA@>707BBCA4-4
		ou set percent offsets of 6% and 20% (corresponds to absolute offsets of 2,7 for a read length of 36): FASTQ Sanger Read

ATATGTNCTCACTGATAAGTGGATATN +

@.@;B-%?8>CBA@>7@7BBCA4-48%

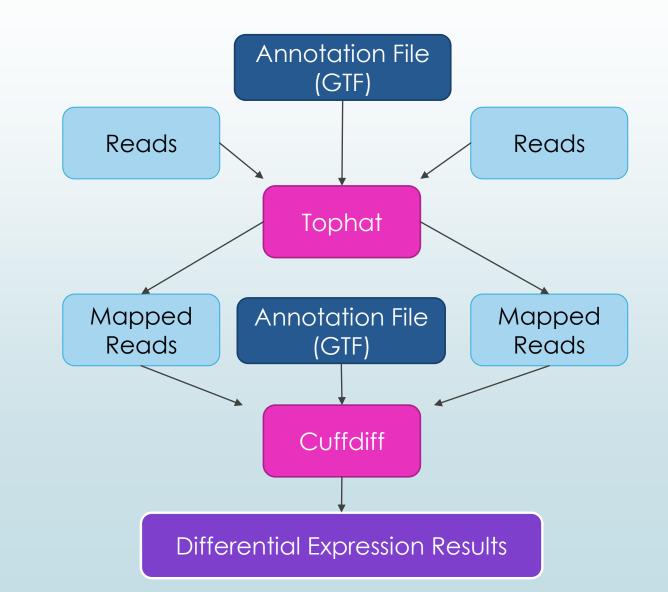


RNA-seq Analysis: Tuxedo Tools

Tool	Description
Bowtie	Ultrafast short read aligner
Tophat	Aligns RNA-seq reads to the genome using Bowtie Discovers splice sites
Cufflinks	Assembles transcripts
Cuffcompare	Compares your assembled transcripts to a reference annotation Tracks Cufflinks transcripts across multiple experiments
Cuffmerge	Merges two or more transcript assemblies
Cuffdiff	Finds significant changes in transcript expression, splicing, and promoter use

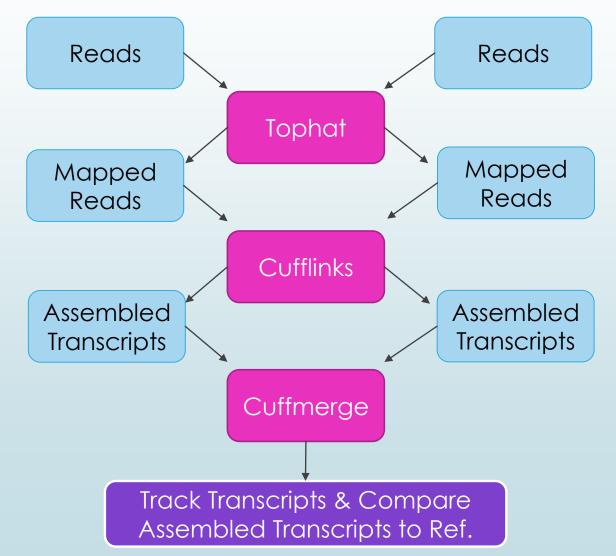


Differential Expression Analysis



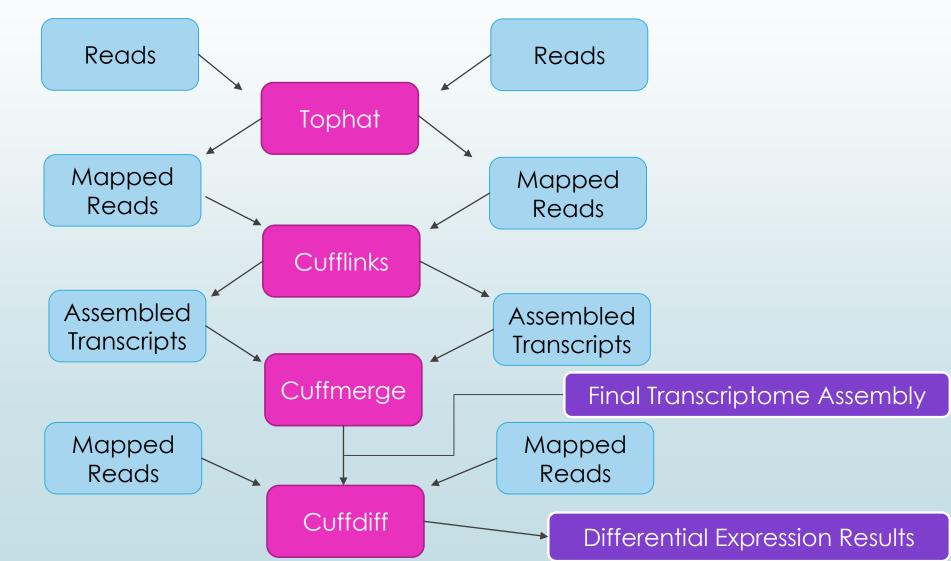


Transcript Assembly and Transcript Comparison





Transcript Assembly and Differential Expression Analysis





Map The Reads (Tophat)

¢

NGS: Mapping

工具

NGS: Indel Analysis

NGS: RNA Analysis

RNA-SEQ

 <u>Tophat for Illumina</u> Find splice junctions using RNA-seq data

- <u>Tophat2</u> Gapped-read mapper for RNA-seq data
- Tophat for SOLiD Find splice junctions using RNA-seq data
- <u>Cufflinks</u> transcript assembly and FPKM (RPKM) estimates for RNA-Seq data
- <u>Cuffcompare</u> compare assembled transcripts to a reference annotation and track Cufflinks transcripts across multiple experiments
- <u>eXpress</u> Quantify the abundances of a set of target sequences from sampled subsequences
- <u>Cuffmerge</u> merge together several Cufflinks assemblies
- <u>Cuffdiff</u> find significant

Tophat for Illumina (version 1.5.0)

RNA-Seq FASTQ file:

40: Galaxy4-[brain_1...fastqsanger ∨

Nucleotide-space: Must have Sanger-scaled quality values with ASCII offset 33

Will you select a reference genome from your history or use a built-in index?:

Use one from the history 🗸

Built-ins were indexed using default options

Select the reference genome:

42: http://hgdownload..es/chr19.fa v

Is this library mate-paired?:

Paired-end 🗸

RNA-Seq FASTQ file:

41: Galaxy5-[brain_2...fastqsanger 🗸

Nucleotide-space: Must have Sanger-scaled quality values with ASCII offset 33

Mean Inner Distance between Mate Pairs:

110

TopHat settings to use:

Default settings 🛛 🗸

Use the Full parameter list to change default settings.

Execute



Assemble Transcripts (Cufflinks)

工具

NGS: RNA Analysis

RNA-SEQ

- <u>Tophat for Illumina</u> Find splice junctions using RNA-seq data
- <u>Tophat2</u> Gapped-read mapper for RNA-seq data
- <u>Tophat for SOLiD</u> Find splice junctions using RNA-seq data

 <u>Cufflinks</u> transcript assembly and FPKM (RPKM) estimates for RNA-Seq data

- <u>Cuffcompare</u> compare assembled transcripts to a reference annotation and track Cufflinks transcripts across multiple experiments
- <u>eXpress</u> Quantify the abundances of a set of target sequences from sampled subsequences
- <u>Cuffmerge</u> merge together several Cufflinks assemblies
- <u>Cuffdiff</u> find significant changes in transcript expression, splicing, and promoter use

DE NOVO ASSEMBLY

<u>Trinity</u> De novo assembly of

Cufflinks (version 0.0.5)

SAM or BAM file of aligned RNA-Seq reads:

54: Tophat for Illumi..cepted_hits ∨

Max Intron Length:

300000

•

Min Isoform Fraction:

0.1

Pre MRNA Fraction:

0.15

Perform quartile normalization:

No 🗸

Removes top 25% of genes from FPKM denominator to improve accuracy of differential expression calls for low abundance transcripts.

Use Reference Annotation:

Use reference annotation as guide v

Reference Annotation:

1: genes.gtf			~	
O	distance of the second second	OTE	OFFO	<i>c</i>

Gene annotation dataset in GTF or GFF3 format.

Perform Bias Correction:

No 🗸

Bias detection and correction can significantly improve accuracy of transcript abundance estimates.

Set Parameters for Paired-end Reads? (not recommended):

Yes 🗸

V .



Merge Assemblies (Cuffmerge)

工具

NGS: RNA Analysis

RNA-SEQ

- <u>Tophat for Illumina</u> Find splice junctions using RNA-seq data
- <u>Tophat2</u> Gapped-read mapper for RNA-seq data
- <u>Tophat for SOLiD</u> Find splice junctions using RNA-seq data
- <u>Cufflinks</u> transcript assembly and FPKM (RPKM) estimates for RNA-Seq data
- <u>Cuffcompare</u> compare assembled transcripts to a reference annotation and track Cufflinks transcripts across multiple experiments
- <u>eXpress</u> Quantify the abundances of a set of target sequences from

Cuffmerge (version 0.0.5)

GTF file produced by Cufflinks:

57: Cufflinks on data..transcripts

Additional GTF Input Files

Add new Additional GTF Input Files

Use Reference Annotation:

Yes 🗸

¢

Reference Annotation:

1: genes.gtf

Make sure your annotation file is in GTF format and that Galaxy knows that your file is GTF--not GFF.

v

 \mathbf{v}

Use Sequence Data:

No 🗸

Use sequence data for some optional classification functions, including the addition of the p_id attribute required by Cuffdiff.

Execute

Identify Significant Changes (Cuffdiff)

工具

NGS: RNA Analysis

RNA-SEQ

- <u>Tophat for Illumina</u> Find splice junctions using RNA-seq data
- <u>Tophat2</u> Gapped-read mapper for RNA-seq data
- Tophat for SOLiD Find splice junctions using RNA-seq data
- <u>Cufflinks</u> transcript assembly and FPKM (RPKM) estimates for RNA-Seq data
- <u>Cuffcompare</u> compare assembled transcripts to a reference annotation and track Cufflinks transcripts across multiple experiments
- <u>eXpress</u> Quantify the abundances of a set of target sequences from sampled subsequences
- <u>Cuffmerge</u> merge together several Cufflinks assemblies

<u>Cuffdiff</u> find significant changes in transcript expression, splicing, and promoter use

DE NOVO ASSEMBLY

Trinity De novo assembly of

Cuffdiff (version 0.0.5)

Transcripts:

٠

60: Cuffmerge on data..transcripts 🗸

A transcript GTF file produced by cufflinks, cuffcompare, or other source.

Perform replicate analysis:

No 🗸

Perform cuffdiff with replicates in each group.

SAM or BAM file of aligned RNA-Seq reads:

50: Tophat for Illumi..cepted_hits 🗸

SAM or BAM file of aligned RNA-Seq reads:

54: Tophat for Illumi..cepted_hits 🗸

False Discovery Rate:

0.05

The allowed false discovery rate.

Min Alignment Count:

10

The minimum number of alignments in a locus for needed to conduct significance testing on changes in that locus observed between samples.

Perform quartile normalization:

No 🗸

Removes top 25% of genes from FPKM denominator to improve accuracy of differential expression calls for low abundance transcripts.

Perform Bias Correction:

No 🗸

v

Bias detection and correction can significantly improve accuracy of transcript abundance estimates.



Differential Expression Results

	test_id	gene_id	gene	locus	sample_1	sample_2	status	val ^	历史 🌣
	TCONS_0000001	XLOC_000001	DDX11L1	chr1:11873-29370	q1	q2	NOTEST		71: Cuffdiff on data 50, @ Ø 🖇 🥤
	TCONS_0000002	XLOC_000002	OR4F5	chr1:69090-70008	q1	q2	NOTEST		data 54, and data 60: transcript
	TCONS_0000003	XLOC_000003	LOC100132062	chr1:323891-328581	q1	q2	NOTEST		FPKM tracking
	TCONS_0000004	XLOC_000003	LOC100133331	chr1:323891-328581	q1	q2	NOTEST		70. 0. ((1))
	TCONS_0000005	XLOC_000004	OR4F3	chr1:367658-368597	q1	q2	NOTEST		<u>70: Cuffdiff on data 50,</u>
	TCONS_0000006	XLOC_000005	LOC643837	chr1:763063-789740	q1	q2	NOTEST		differential expression testing
	TCONS_0000007	XLOC_000006	SAMD11	chr1:861120-894679	q1	q2	NOTEST		
	TCONS_0000008	XLOC_000007	KLHL17	chr1:895966-901099	q1	q 2	NOTEST		69: Cuffdiff on data 50,
/	TCONS_0000009	XLOC_000008	PLEKHN1	chr1:901876-910484	q1	q2	NOTEST		data 54, and data 60: gene FPKM tracking
	TCONS_0000010	XLOC_000008	PLEKHN1	chr1:901876-910484	q1	q2	NOTEST		
	TCONS_00000011	XLOC_000009	ISG15	chr1:948846-949919	q1	q2	NOTEST		<u>68: Cuffdiff on data 50,</u> @ 🖉 💥
	TCONS_0000012	XLOC_000010	AGRN	chr1:955502-991499	q1	q2	NOTEST		data 54, and data 60: gene
	TCONS_0000013	XLOC_000011	LOC254099	chr1:1072396-1079434	q1	q2	NOTEST		differential expression testing
	TCONS_0000014	XLOC_000012	MIR200B	chr1:1102483-1102578	q1	q2	NOTEST		67: Cuffdiff on data 50, @ 🖉 🕱
	TCONS_00000015	XLOC_000013	MIR200A	chr1:1103242-1103332	q1	q2	NOTEST		data 54, and data 60: TSS
	TCONS_0000016	XLOC_000014	MIR429	chr1:1104384-1104467	q1	q2	NOTEST		groups FPKM tracking
	TCONS_00000017	XLOC_000015	TTLL10	chr1:1109285-1133313	q1	q2	NOTEST		
	TCONS_0000018	XLOC_000015	TTLL10	chr1:1109285-1133313	q1	q2	NOTEST		66: Cuffdiff on data 50, data 54, and data 60: TSS
	TCONS_00000019	XLOC_000016	B3GALT6	chr1:1167628-1170420	q1	q2	NOTEST		groups differential expression
	TCONS_00000020	XLOC_000017	SCNN1D	chr1:1215815-1227409	q1	q2	NOTEST		testing
	TCONS_00000021	XLOC_000017	SCNN1D	chr1:1215815-1227409	q1	q2	NOTEST		
	TCONS_00000022	XLOC_000018	PUSL1	chr1:1243993-1260067	q1	q2	NOTEST		<u>65: Cuffdiff on data 50,</u> <u>data 54, and data 60: CDS</u>
	TCONS_00000023	XLOC_000019	GLTPD1	chr1:1260142-1264276	q1	q2	NOTEST		FPKM tracking
	TCONS_00000024	XLOC_000020	TAS1R3	chr1:1266725-1269844	q1	q2	NOTEST		
	TCONS_00000025	XLOC_000021	LOC148413	chr1:1334909-1342693	q1	q2	NOTEST		<u>64: Cuffdiff on data 50,</u> @ 🖉 💥
	TCONS_00000026	XLOC_000022	TMEM88B	chr1:1361507-1363167	q1	q2	NOTEST		data 54, and data 60: CDS
	TCONS_00000027	XLOC_000023	VWA1	chr1:1370902-1378262	q1	q2	NOTEST		FPKM differential expression testing
	TCONS_0000028	XLOC_000023	VWA1	chr1:1370902-1378262	q1	q2	NOTEST		<u>testing</u>
				• • • • • • • • • • • • • • • • • • • •		-		>	
								-	



Publish Your Workflow

- Galaxy	が Step 8: Tophat for Illumina	Using 5.6 Gb	
Published Workflows search name, annotation, owner, and tags Advanced Search Calaxy Published Workflows daniel RNA-seq Analysis Exercise 20130912 Galaxy Workflow ' RNA-seq Analysis Exercise 20130912' Step Step 1: Input dataset Input Dataset select at runtime Step 2: Input dataset	RNA-Seq FASTQ file Output dataset 'output' from step 4 Will you select a reference genome from your history or use a built-in	Using 5.6 Gb Vorkflows	
Input Dataset select at runtime	TopHat settings to use Default settings	none	
Step 3: Input dataset	Step 9: Cufflinks		
Input Dataset select at runtime	SAM or BAM file of aligned RNA-Seq reads Output dataset 'accepted_hits' from step 7		
Step 4: Input dataset	Max Intron Length		
Input Dataset select at runtime	300000 Min Isoform Fraction 0.1		
Step 5: Input dataset	Pre MRNA Fraction		

Run Existing Workflow

	Your workflows	Running workflow "RNA-seq Analysis Exercise 20130912"	Expand All Collapse
	Name RNA-seq Analysis Exercise 20130912 -	Step 1: Input dataset	
/	imported: RNA-seq Analysis Exercise 20130912 RNA-seq Workflows shared with you by e	Input Dataset Image: Second state of the	
	No workflows have been shared with you. Other options Configure your workflow menu	Step 2: Input dataset	
		Input Dataset Image: Comparison of the second s	
		Step 3: Input dataset	
		Input Dataset Image: Second secon	
		Step 4: Input dataset	
		Input Dataset 41: Galaxy5-[brain_2fastqsanger v type to filter	
		Step 5: Input dataset	



iGenome

illumina				Log	in to get persor	alized account info	ormation.	Quick Order	View Cart ∰
marmina							Conta	act Us MyIllun	nina Tools
APPLICATIONS	SYSTEMS	CLINICAL	SERVICES	SCIENCE	SUPPORT	COMPANY	Searc	h	Q
Support » Sequenc	ing » Sequen	cing Software	» iGenomes					& ,	🛨 Follow us: 🕒

Ready-To-Use Reference Sequences and Annotations

The iGenomes are a collection of reference sequences and annotation files for commonly analyzed organisms. The files have been downloaded from Ensembl, NCBI, or UCSC, and chromosome names have been changed to be simple and consistent with their download source. Each iGenome is available as a compressed file that contains sequences and annotation files for a single genomic build of an organism.

For more information, see the iGenomes Overview and Change Log.

Species	Source	Build(s)		
Arabidopsis thaliana	Ensembl	TAIR10	TAIR9	
	NCBI	TAIR10	build9.1	
Bacillus_cereus strain ATCC 10987	NCBI	2003-02-13		
Bacillus_subtilis strain 168	Ensembl	EB2		
Bos taurus (Cow)	Ensembl	UMD3.1	Btau_4.0	
	NCBI	UMD_3.1	Btau_4.6.1	Btau_4.2
	UCSC	bosTau7	bosTau6	bosTau4
Caenorhabditis elegans	Ensembl	WBcel215	WS210	
	NCBI	WS195	WS190	
	UCSC	ce10	ce6	
Canis familiaris (Dog)	Ensembl	CanFam3.1	BROADD2	
	NCBI	build3.1	build2.1	
	UCSC	canFam3	canFam2	

http://support.illumina.com/sequencing/sequencing_software/igenome.ilmn

	0000	danker			
Drosophila melanogaster	Ensembl	BDGP5	BDGP5.25		
	NCBI	build5.41	build5.3	build5	build4.1
	UCSC	dm3			



UCSC

+ Mgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/

gunzip <file>.fa.gz

All the files in this directory are freely available for public use.							
Human Genome					· · · · · · · · · · · · · · · · · · ·		
Feb. 2009 (hg19, GRCh37)	<u>Name</u>		Last modified	<u>d</u> <u>Siz</u>	<u>e Description</u>		
 Full data set)irectory	00 V 0000	-			
 Data set by chromosome 		<u>).tar.gz</u>	20-Mar-2009 (
Annotation database	<u>chromFa</u>		20-Mar-2009 (
		<u>lasked.tar.gz</u>	20-Mar-2009 (
<u>GC percent data</u>		<u>t.tar.gz</u>	20-Mar-2009 (
 Protein database for hg19 		f.tar.gz	20-Mar-2009 (
 SNP137-masked FASTA files 	est.fa.g		10-Sep-2013 (
 <u>SNP135-masked FASTA files</u> 	<u>est.fa.</u>		10-Sep-2013 (
 SNP132-masked FASTA files 	<u>hg19.2b</u>		08-Mar-2009				
SNP131-masked FASTA files	<u>md5sum.</u> t		29-Jul-2009				
LiftOver files	mrna.fa		10-Sep-2013 (
	mrna.fa		10-Sep-2013 (
 Pairwise Alignments 	refMrna.		10-Sep-2013 (
 Human self alignments)	. <u>fa.gz.md5</u>	10-Sep-2013 (
• Human/Chimp (panTro4)		<u>1000.fa.gz</u>	10-Sep-2013 (
 Human/Chimp (panTro3) 		<u>n1000.fa.gz.md5</u> ₀2000_fa_gz	10-Sep-2013 (
• Human/Chimp (p http://hada							

• Human/Chimp (p http://hgd	ownl	oad.cse.ucsc.ed	u/downloads	s.html#human
 Human/Gorilla (gorGor3) 	0	upstream5000.ra.gz	10-Sep-2015 05:02	MOC
 Human/Gorilla (gorGor1) 	0	<u>upstream5000.fa.gz.md5</u>	10-Sep-2013 05:02	53
 Human/Orangutan (ponAbe2) 	0	<u>xenoMrna.fa.gz</u>	10-Sep-2013 04:42	3.8G
		xenoMrna.fa.gz.md5	10-Sep-2013 04:43	49
∘ <u>Human/Gibbon (nomLeu1)</u>	0	xenoRefMrna.fa.gz	10-Sep-2013 04:58	212M
 o Human/Rhesus (rheMac3) 	0	xenoRefMrna.fa.gz.md5	10-Sep-2013 04:58	52



Take Home Message

Galaxy is very powerful!

Its user-friendly interface allows biologists to perform complex genomic analyses. (RNA-seq, ChIP-seq, SNP analysis, etc)

The use of computing resources should be taken into account.



Literature

- Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences.
 Genome Biology 11:R86 (2010)
- Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks.

Nature Protocols 7, 562–578 (2012)

 Full-length transcriptome assembly from RNA-Seq data without a reference genome.

Nature Biotechnology 29, 644-652 (2011)

Thank you !

Galaxy for Next Generation Sequencing

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