# RNA-Seq Differential Gene Expression analysis (Galaxy Server)

**GBIO0002**

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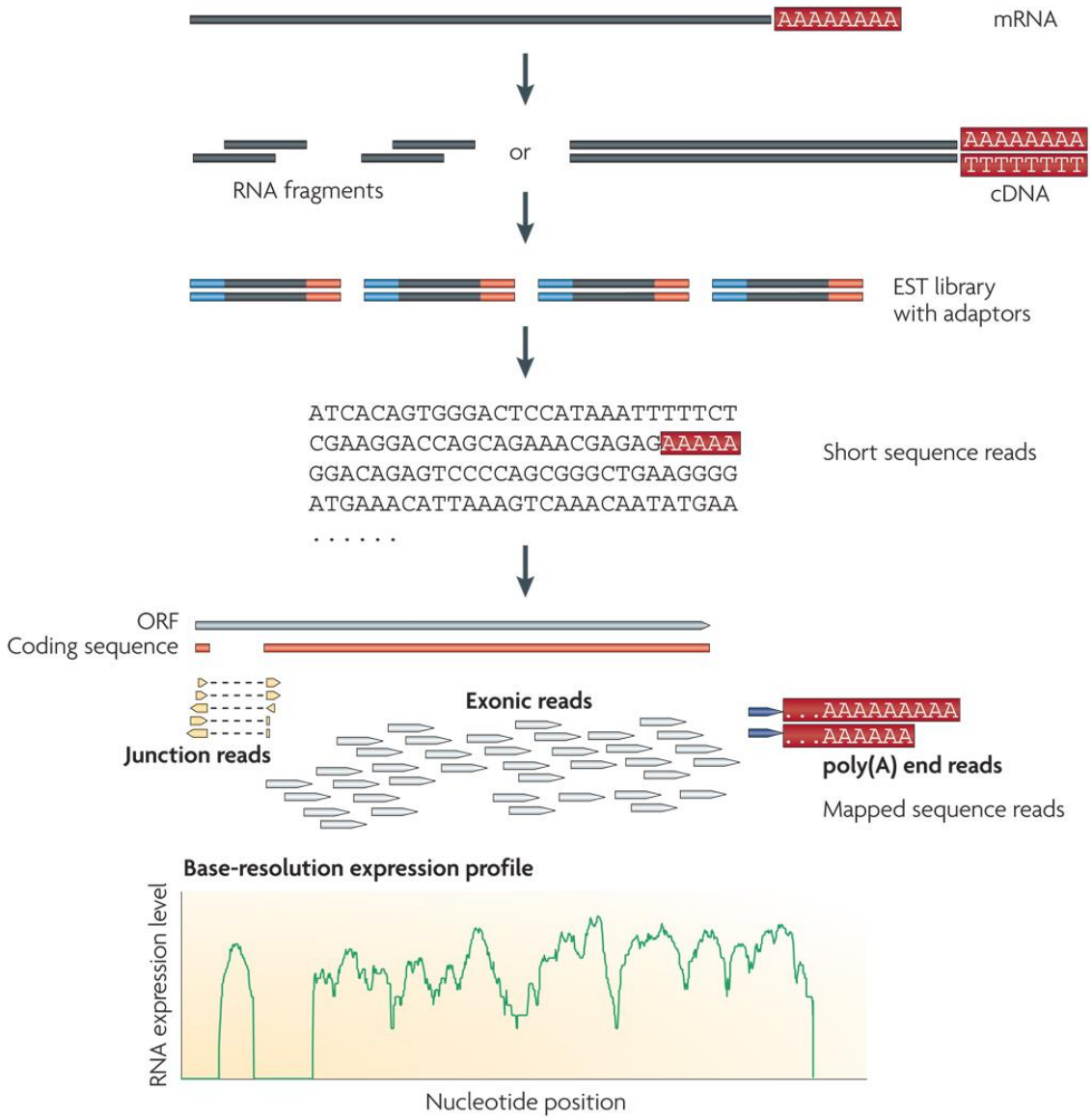
# Typical RNA-Seq Experiment



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# RNA-Seq: a revolutionary tool for transcriptomics

**Zhong Wang**, **Mark Gerstein**, and **Michael Snyder**
Zhong Wang and Michael Snyder are at the Department of Molecular, Cellular and Developmental Biology, and Mark Gerstein is at the Department of Molecular, Biophysics and Biochemistry, Yale University, 219 Prospect Street, New Haven, Connecticut 06520, USA.

## Abstract

RNA-Seq is a recently developed approach to transcriptome profiling that uses deep-sequencing technologies. Studies using this method have already altered our view of the extent and complexity of eukaryotic transcriptomes. RNA-Seq also provides a far more precise measurement of levels of transcripts and their isoforms than other methods. This article describes the RNA-Seq approach, the challenges associated with its application, and the advances made so far in characterizing several eukaryote transcriptomes.

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The transcriptome is the complete set of transcripts in a cell, and their quantity, for a specific developmental stage or physiological condition. Understanding the transcriptome is essential for interpreting the functional elements of the genome and revealing the molecular constituents of cells and tissues, and also for understanding development and disease. The key aims of transcriptomics are: to catalogue all species of transcript, including mRNAs, non-coding RNAs and small RNAs; to determine the transcriptional structure of genes, in terms of their start sites, 5′ and 3′ ends, splicing patterns and other post-transcriptional modifications; and to quantify the changing expression levels of each transcript during development and under different conditions.

# Mapping and quantifying mammalian transcriptomes by RNA-Seq

Ali Mortazavi[1,2], Brian A Williams[1,2], Kenneth McCue[1], Lorian Schaeffer[1] & Barbara Wold[1]



**We have mapped and quantified mouse transcriptomes by deeply sequencing them and recording how frequently each gene is represented in the sequence sample (RNA-Seq). This provides a digital measure of the presence and prevalence of transcripts from known and previously unknown genes. We report reference measurements composed of 41–52 million mapped 25-base-pair reads for poly(A)-selected RNA from adult mouse brain, liver and skeletal muscle tissues. We used RNA standards to quantify transcript prevalence and to test the linear range of transcript detection, which spanned five orders of magnitude. Although >90% of uniquely mapped reads fell within known exons, the remaining data suggest new and revised gene models, including changed or additional promoters, exons and 3′ untranscribed regions, as well as new candidate microRNA precursors. RNA splice events, which are not readily measured by standard gene expression microarray or serial analysis of gene expression methods, were detected directly by mapping splice-crossing sequence reads. We observed $1.45 \times 10^5$ distinct splices, and alternative splices were prominent, with 3,500 different genes expressing one or more alternate internal splices.**

The mRNA population specifies a cell's identity and helps to govern its present and future activities. This has made transcriptome approaches to large-scale RNA analysis are serial analysis of gene expression (SAGE)[4,5] and related methods such as massively parallel signature sequencing (MPSS)[6], which use DNA sequencing of previously cloned tags 17–25 base pairs (bp) from terminal 3′ (or 5′) sequence tags. These sequence tags are then identified by informatic mapping to mRNA reference databases or, for longer tag lengths, to the source genome. A strength of SAGE and SAGE-like methods is that they produce digital counts of transcript abundance, in contrast to the analog-style signals obtained from fluorescent dye–based microarrays. However, SAGE-family assays provide no information about splice isoforms or new gene discovery, and fully comprehensive measurements of lower-abundance-class RNAs have not been achieved owing to cost and technology constraints. Expressed sequence tag (EST) sequencing of cloned cDNAs has long been the core method for reference transcript discovery[7–9]. It has both qualitative and quantitative limitations, imposed partly by historic sequencing capacity and cost issues, and more crucially by bacterial cloning constraints that affect which sequences are represented and how sequence-complete each clone is. Recently, dense whole-genome tiling microarrays have been developed and applied to transcriptomes for measuring expression and for transcript discovery[10–14]. In contrast to expression arrays, these tiling arrays can discover new genes and exons, but they require large amounts of input RNA and have

# What Can You Actually Do With RNA-Seq?

- ✓**RNA-seq is a powerful and versatile tool published widely over the last few years.**

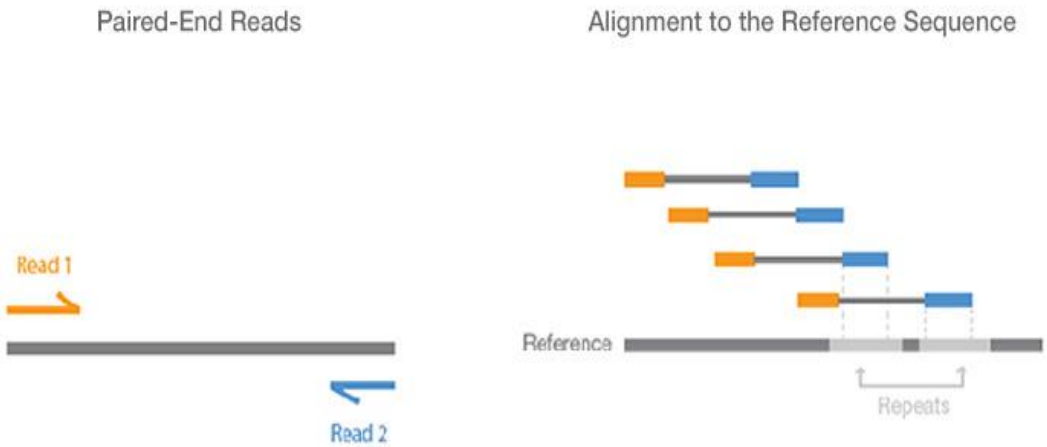
- ✓**RNA-seq used to investigate complex diseases and find new genes for functional analysis.**

- ✓**RNA-seq used in one of the study to look at the conservation of RNA Polymerase III binding in mammals.**

- ✓**RNA-seq and microarray-based capture used to identify and characterize rare transcripts, which are normally undetectable.**

# Paired end sequence

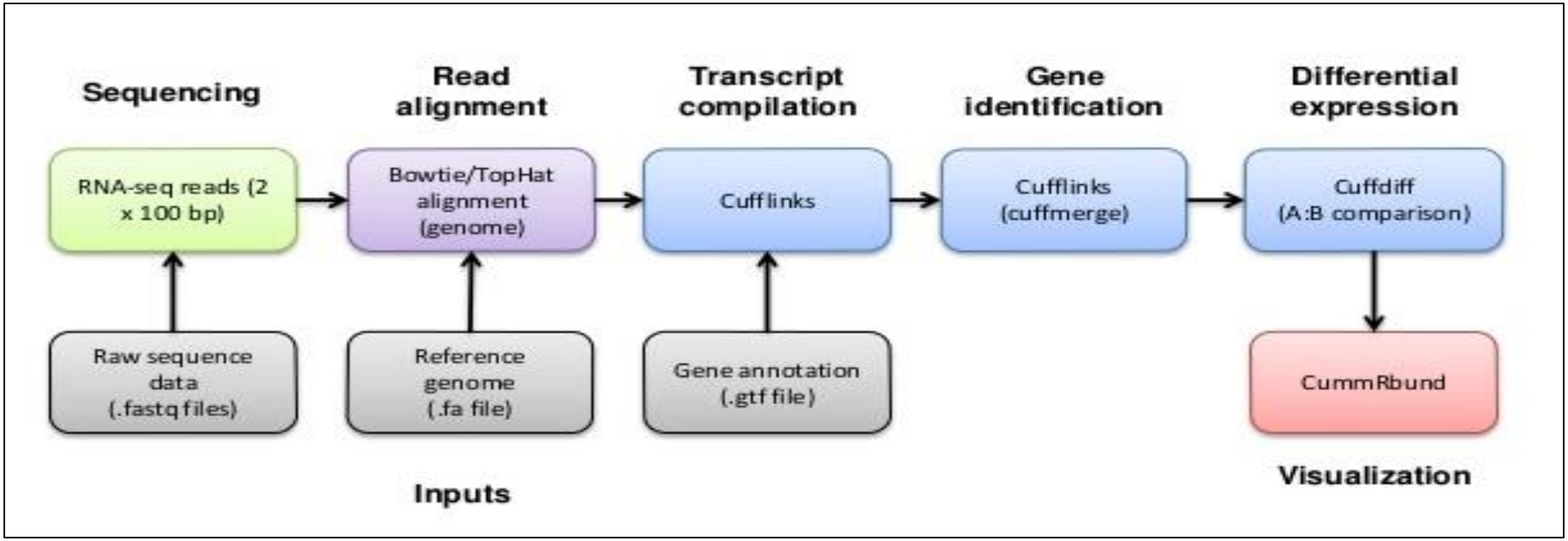
**The term 'paired ends' refers to the two ends of the same DNA molecule. So you can sequence one end, then turn it around and sequence the other end. The two sequences you get are 'paired end reads'.**




**Paired-end RNA sequencing (RNA-Seq) enables discovery applications such as detecting gene fusions in cancer and characterizing novel splice isoforms.**

# Protocol for RNA Seq Data Analysis

- ✓ RNA Seq analysis is multi step procedure.
- ✓ Different tools are required at each step.




✓We will use one of the galaxy server to perform RNASeq Data analysis

# Galaxy Community

Galaxy COMMUNITY HUB | Use | Community | Education | Deploy & Develop | Support | Search Galaxy

## Publicly Accessible Galaxy Servers


The Galaxy Project's public server (usegalaxy.org) can meet many needs, but it is not suitable for everything (see Choices for why). 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 publicly accessible Galaxy servers. To be included here a server must be accessible to any academic researcher anywhere in the world. Some servers may require logins and enforce quotas.

If you maintain a public instance of Galaxy it is recommended to sign up for the public servers mailing list to receive security fixes with priority.

**To add** your public Galaxy server to this list describe the server here and we'll post it this directory.

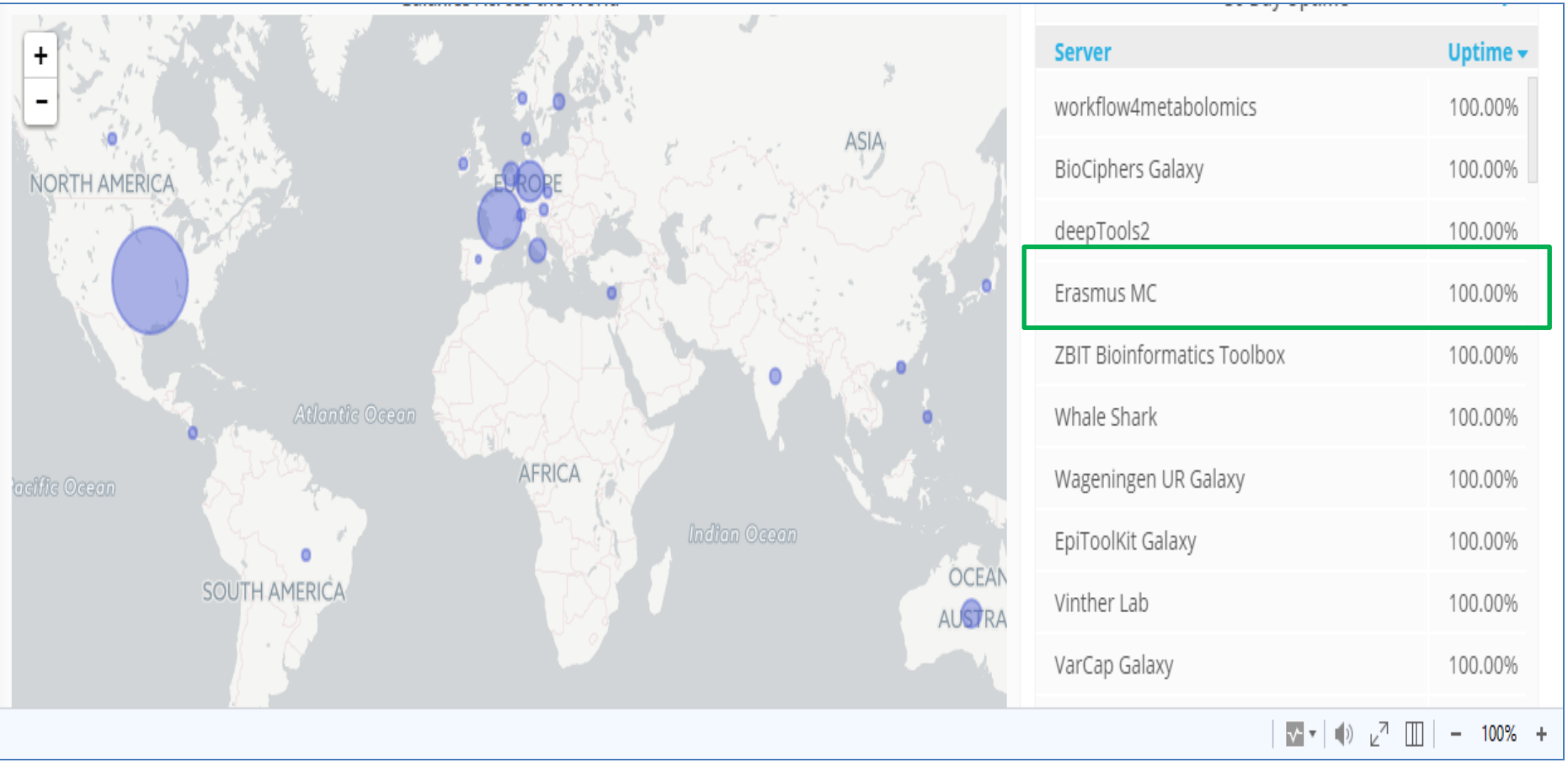
### Galaxy Services

There are also a number of Galaxy services that make Galaxy available to research communities, sometimes restricted on a geographical or domain basis. See the Galaxy services list.

### Public ToolSheds

In addition to the the main Galaxy and Test ToolSheds, several groups have made their tools available through publicly accessibly ToolSheds.

# Galaxies across the World




| Server | Uptime |
|---|---|
| workflow4metabolomics | 100.00% |
| BioCiphers Galaxy | 100.00% |
| deepTools2 | 100.00% |
| Erasmus MC | 100.00% |
| ZBIT Bioinformatics Toolbox | 100.00% |
| Whale Shark | 100.00% |
| Wageningen UR Galaxy | 100.00% |
| EpiToolKit Galaxy | 100.00% |
| Vinther Lab | 100.00% |
| VarCap Galaxy | 100.00% |

# GALAXY Server : Why to Use

- ✓The Galaxy Project's public server (usegalaxy.org) can meet many needs
- ✓General Purpose / Genomics Galaxy Servers
- ✓Domain Specific Galaxy Servers
- ✓Tool Publishing Galaxy Servers

# Genomics Galaxy Server

These servers implement a broad range of tools and aren't specific to any part of the tree of life, or to any specific type of analysis. These are servers you can use when want to do general genomic analysis.

| Name | Links | Summary |
|---|---|---|
| ABiMS | ABiMS Galaxy<br>Request an account | General purpose genomics analysis, featuring many standard tools plus many additional tools. Howerver, we are specialized in RNASeq with reference and RNASeq denovo |
| Biomina | Biomina Galaxy | A general purpose Galaxy instance that includes most standard tools for DNA/RNA sequencing, plus extra tools for panel resequencing, variant annotation and some tools for Illumina SNParray analysis. |
| CBiB Galaxy | CBiB Galaxy | A general purpose Galaxy instance that includes EMBOSS (a software analysis package for molecular biology) and fibronectin (diversity analysis of synthetic libraries of a Fibronectin domain). |
| DBCLS Galaxy | DBCLS Galaxy | Adds text mining tools, DBCLS DBSearch Tools, semantic web tools |
| Erasmus MC | Erasmus MC Bioinformatics Galaxy Server | General purpose genomics analysis, featuring many standard tools plus many additional tools. |
| GalaxEast | GalaxEast<br>Request an account | Integrative 'omics data analysis |
| Galaxy Main | Main | The Galaxy Project free public server; biomedical research |
| Galaxy Test | Galaxy Test | Beta version of Galaxy Main |
| Galaxy@GenOuest | Galaxy@GenOuest<br>Request a GenOuest account | A general purpose Galaxy server Includes tools developped by Dyliss and GenScale bioinformatics research teams in Rennes, France. |
| Galaxy@Pasteur | Galaxy@Pasteur | General purpose genomics analysis server. |
| Galaxy@PRABI | Galaxy@PRABI<br>PRABI Galaxy Tool Shed | Includes bioinformatics tools developed by the research teams working in the perimeter of the PRABI core facility, including *kissplice/kissDE, TETools, SEX-DETector,* and *priam*. |
| GigaGalaxy | GigaGalaxy | Standard Galaxy tools set plus SOAPdenovo and SOAPsnp for *de novo* assembly and SNP calling. |
| GVL MEL | Galaxy Melbourne | General purpose Galaxy based on the Genomics Virtual Lab platform. |
| GVL QLD | Genomics Virtual Lab GVL-QLD | General purpose Galaxy based on the Genomics Virtual Lab platform. |
| GVL Tutorial | Genomics Virtual Lab GVLTut | Small Galaxy for Training purposes. Loaded with Histories and Tools for Next Gen Sequencing tutorials. |

# Domain specific Galaxy Server

Domain servers specialize in either a particular branch of the tree of life or in particular types of analysis. However, within their specializations, domain servers offer a wide variety of tools.

| Name | Links | Summary |
|---|---|---|
| ballaxy | ballaxy Galaxy server<br>ballaxy using Docker | Hosts the BALL (Biochemical Algorithms Library) Project tools, i.e. computer aided drug design and molecular modelling based on protein and ligand structure data. |
| BIPAA (BioInformatics Platform for Agroecosystem Arthropods) | BIPAA Galaxy Server<br>BIPAA home page | Insect genomics (aphids, parasitoïd wasps, lepidopterans) |
| Center for Phage Technology (CPT) | Center for Phage Technology (CPT) Galaxy Server<br>CPT home page | Phage biology and automated annotation. |
| Cistrome Analysis Pipeline | Cistrome Analysis Pipeline | ChIP-chip/seq and gene expression data |
| CoSSci | CoSSci Complex Social Science Gateway | Tools for solving Galton's problem in Comparative Research and complex network problems in Social Science. |
| Dintor | Dintor: Data Integrator Tool Suite | GWA and NGS tools and modules for functional annotation of genes and gene products |
| Galaxy Integrated Omics (GIO) | GIO Server | Proteomics Informed by Transcriptomics (PIT) methodology, and selection of surrogate peptides for targeted proteomics. |
| Galaxy PGTB (Virtual Biodiversity Lab) | PGTB Galaxy - Virtual Biodiversity Lab<br>Plateforme Genome Transcriptome de Bordeaux | This is a standard Galaxy instance implemented with specific tools for Biodiversity (Biodiversity Virtual Lab) and NGS (Ion Torrent from the PGTB facility) analysis. |
| Galaxy-CEFAP | Galaxy-CEFAP | Galaxy-CEFAP offers a set of tools to perform RNA-Seq and miRNA analysis. |
| Galaxy-P | Use Galaxy-P | Galaxy-P is a multiple 'omics' data analysis platform with particular emphasis on mass spectrometry based proteomics. Galaxy-P is developed at the University of Minnesota, deployed at the Minnesota Supercomputing Institute. |
| Genomic Hyperbrowser | Genomic Hyperbrowser | statistical methodology and computing power to handle a variety of biological inquires on genomic datasets |
| GrAPPA | Graph Algorithms Pipeline | GrAPPA is a web-based interface constructed on the Galaxy framework for graph theoretical tools. It contains novel |

# Galaxy Services : Example

- ✓ Geography Based
- ✓ Domain Based

Australia: Genomics Virtual Lab (GVL)

Canada: GenAP

United Kingdom: CLIMB

Poland: PL-Grid

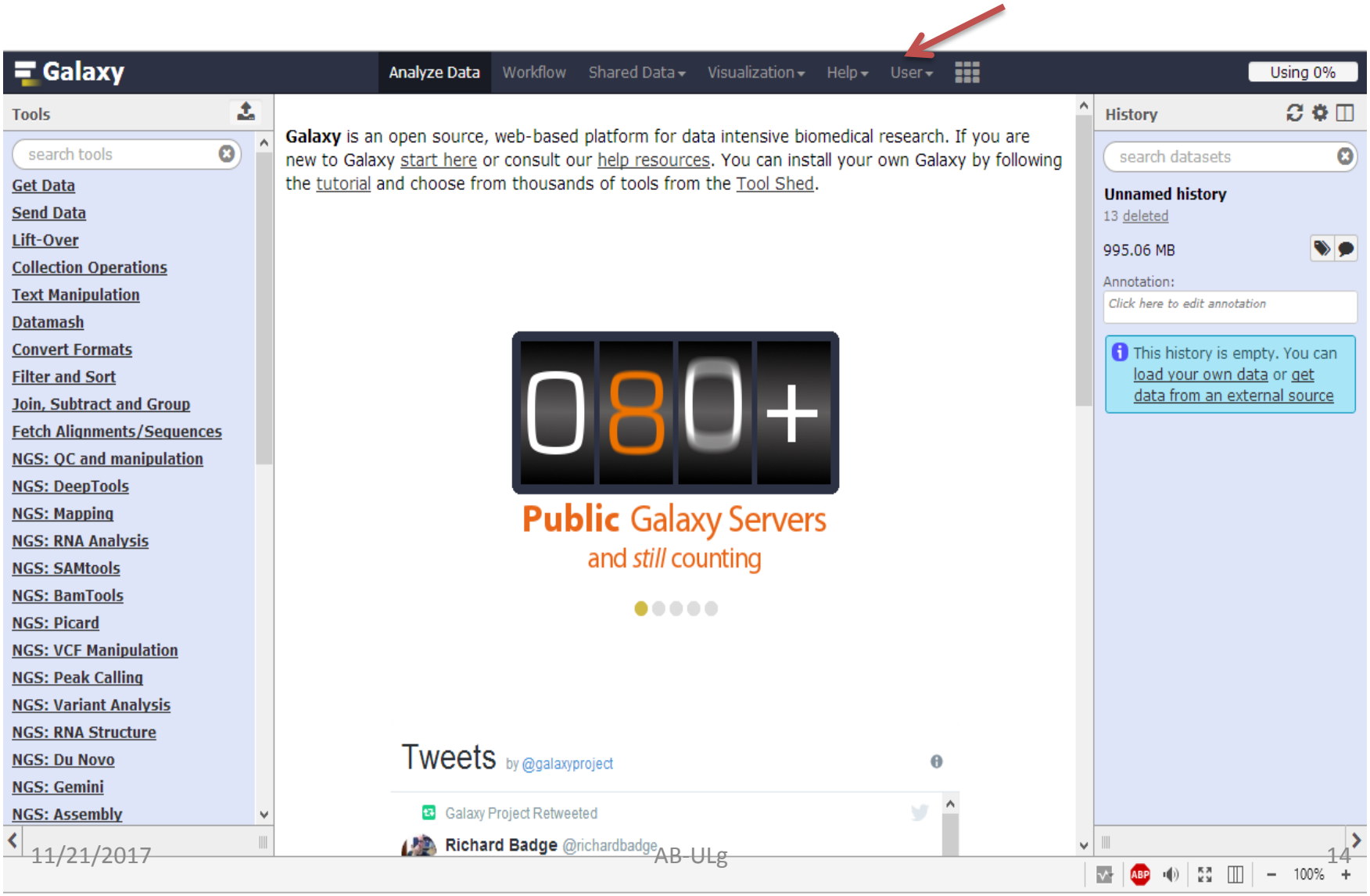
Norway: NeLS

Cancer: Cancer Computer

Galaxy Main Tool Shed

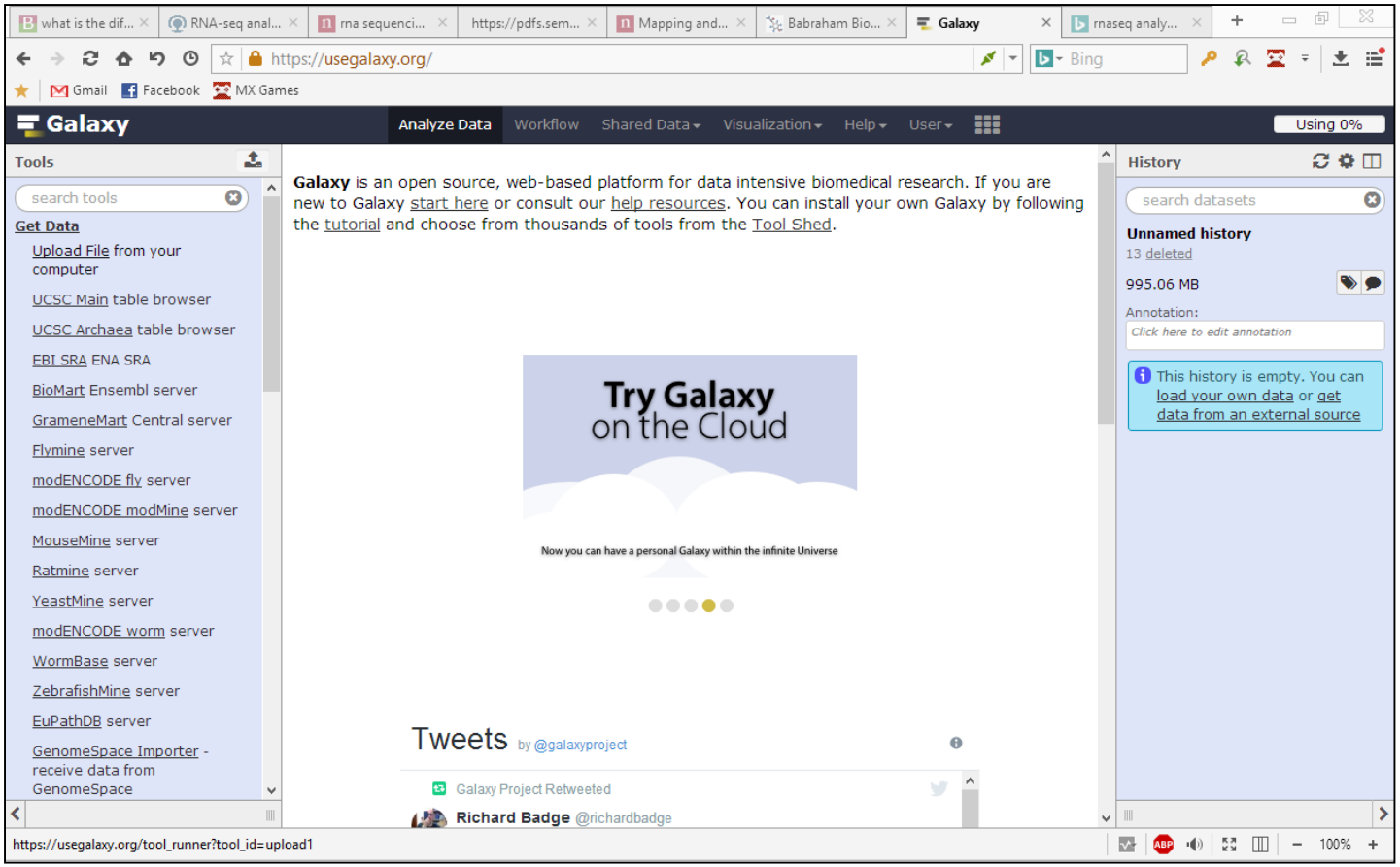
# Let Us Use Public GALAXY Server

## Go to https://usegalaxy.org/ and create login



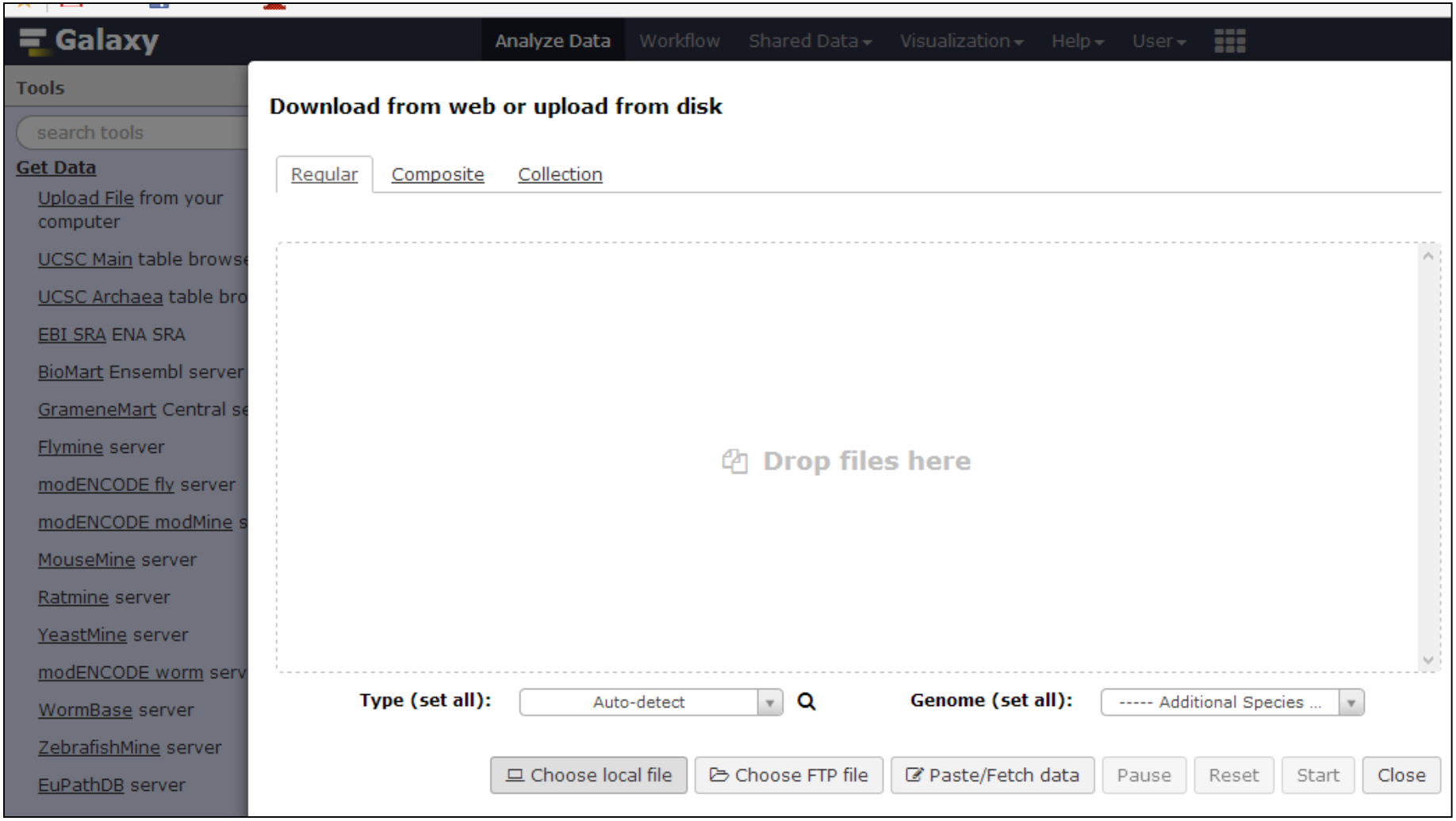
# GALAXY Server : Upload Data (I)

✓**Click on Get Data and select Upload File from your computer. Download samples files from course website.**



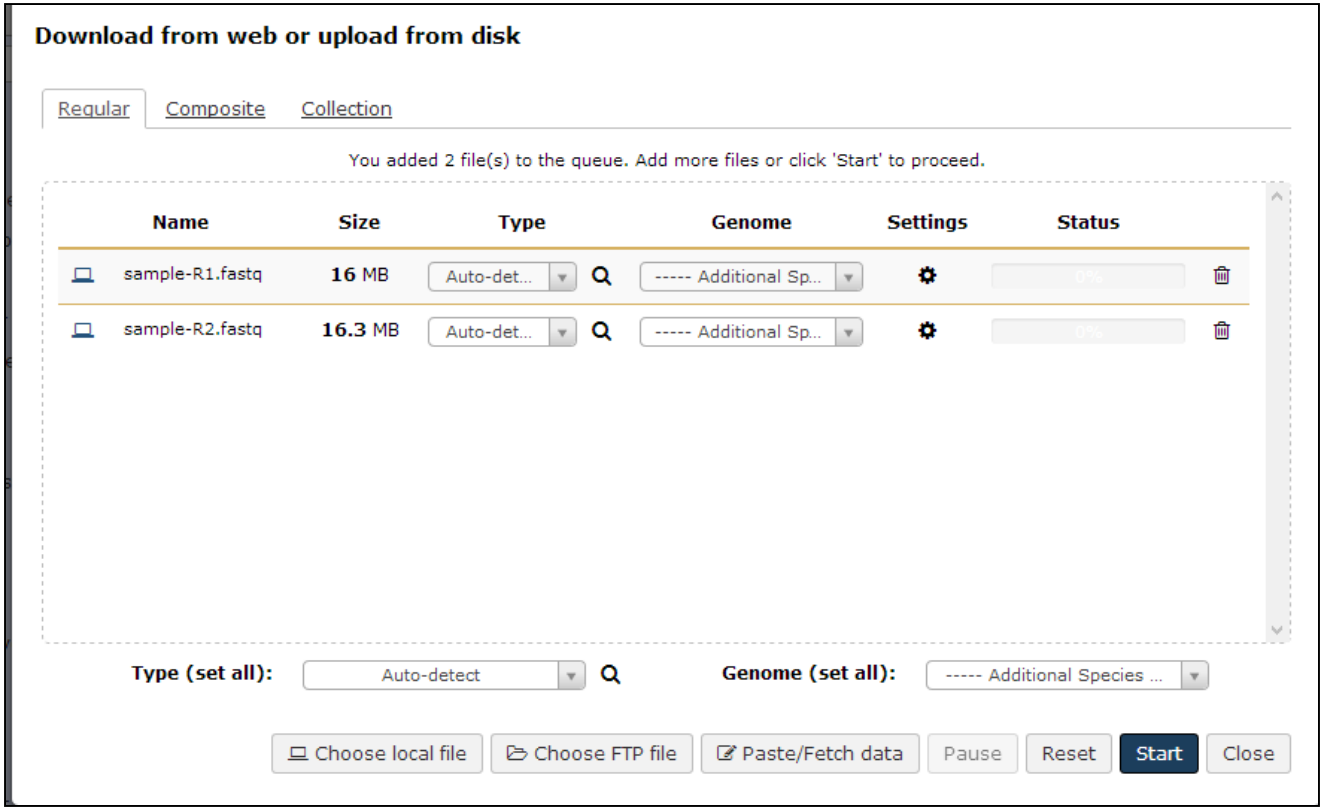
# GALAXY Server : Upload Data (II)

## New Window will appear. Now, Click option “Choose local file”



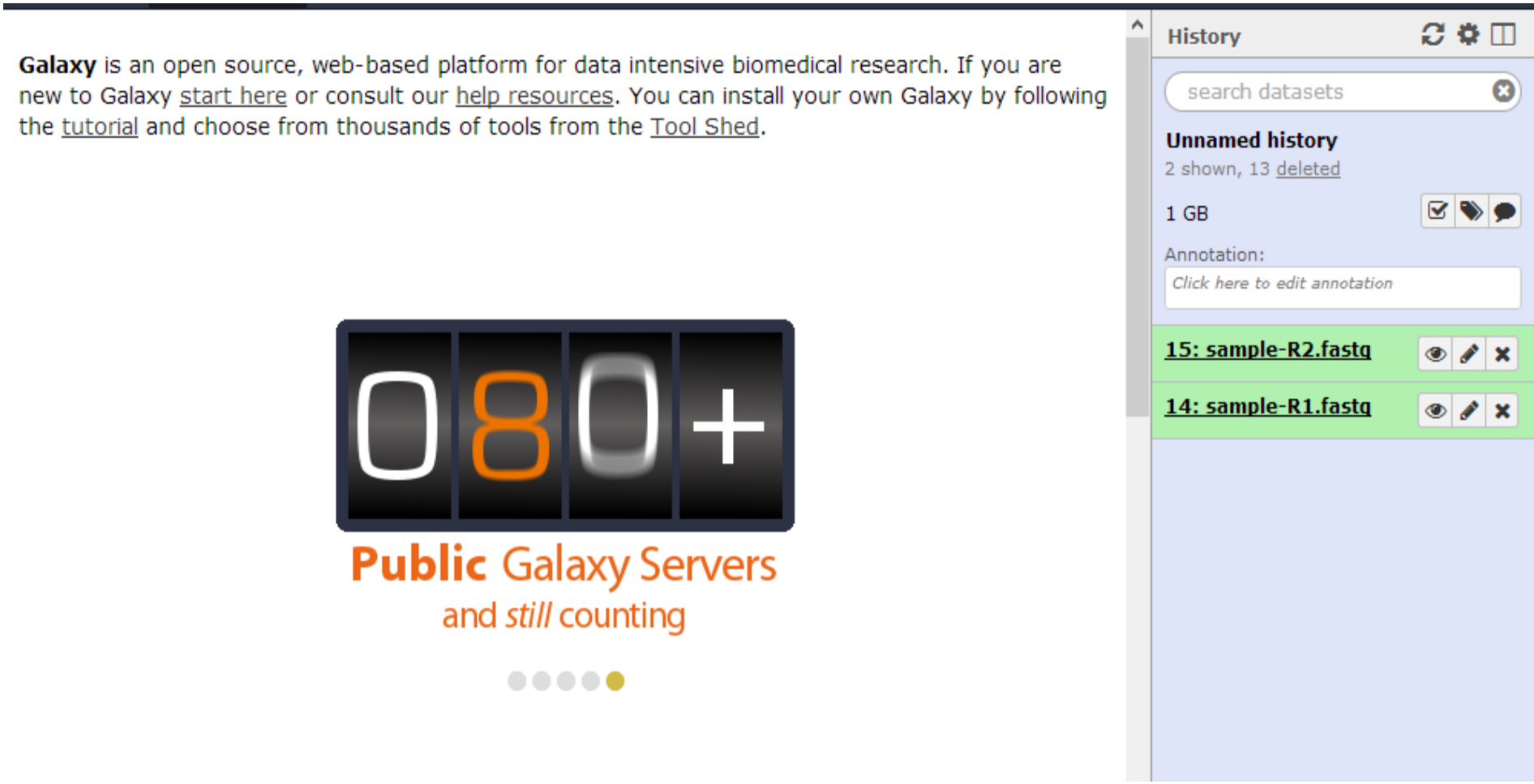
# GALAXY Server : Upload Data (III)

✓ **Now, Click option "Start". It will upload file to server.**




✓ **Now wait for 10-20 seconds.**

# ✓Files will be uploaded successfully and appears with green colour .



✓Galaxy consist of collection of Multiple Tools . Today's session, We will use

✓NGS: QC and manipulation
✓NGS Mapping
✓NGS:RNA analysis
✓SAMtools

# Protocol for RNA Seq Data Analysis

1. Pre-processing

2. Quality Filtration

3. Mapping or assembly

4. Expression analysis

# Quality Assessment

✓It is important to check the quality of your sequenced reads


✓FASTQC: free program that reports quality profile of reads

# Quality Assessment

✓**Modern high throughput sequencers can generate hundreds of millions of sequences in a single run.**

✓**Before analysing this sequence to draw biological conclusions you should always perform some simple quality control checks to ensure that**

**(I) the raw data looks good and**

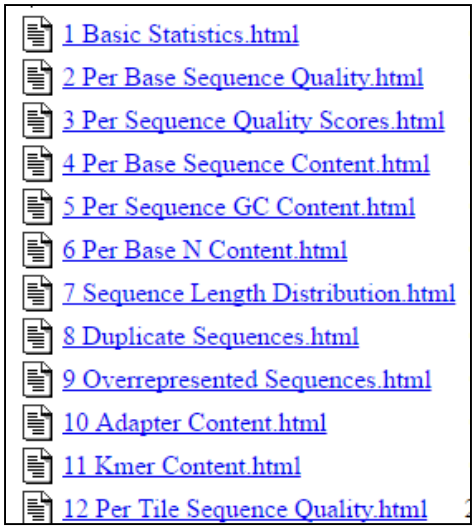
**(II) there are no problems or biases in your data**

**which may affect how you can usefully use it.**

# FASTQC tool

✓**Providing a quick overview to tell you in which areas there may be problems**

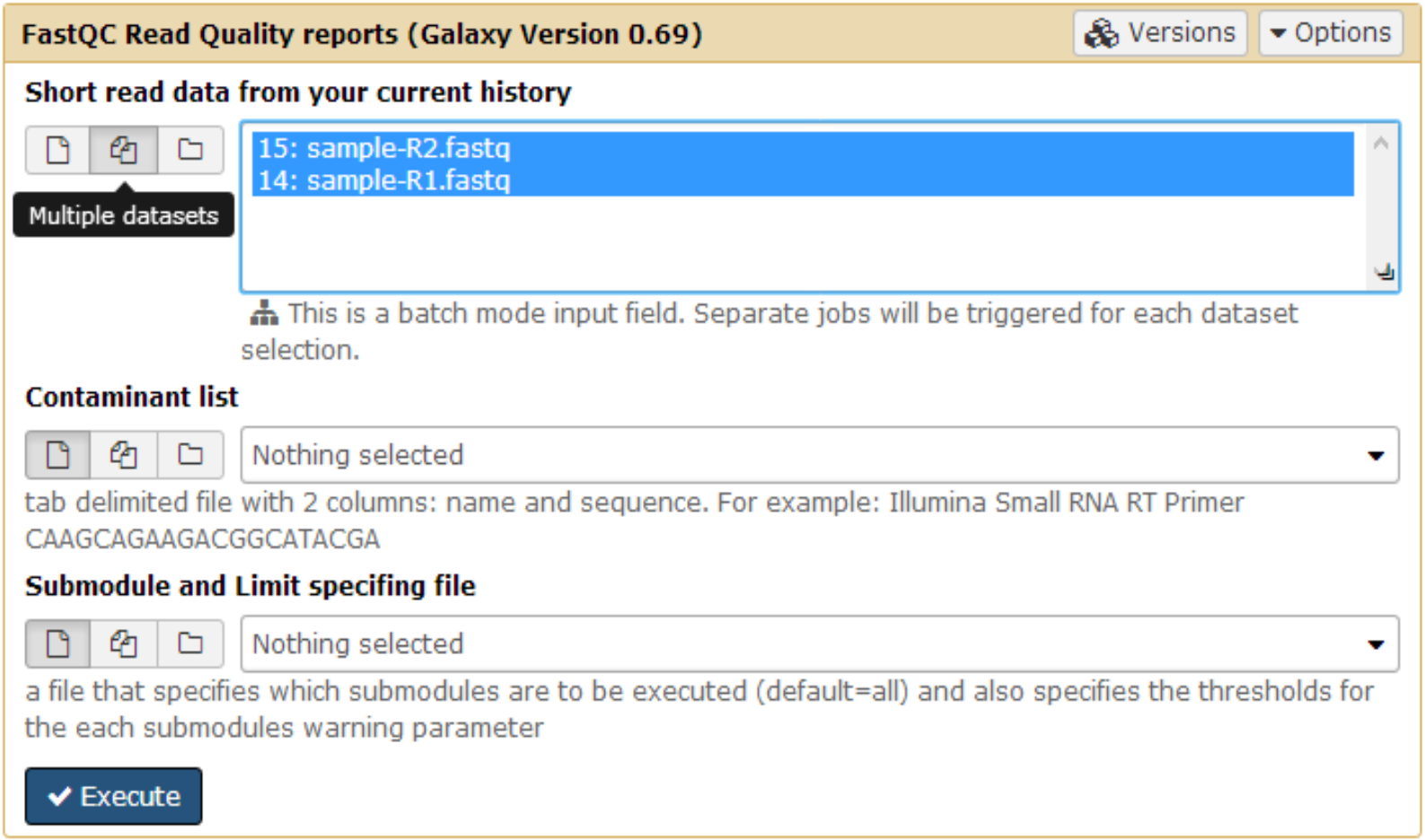
✓**Summary graphs and tables to quickly assess your data**




✓**Double click on NGS: QC and manipulation**

✓**Select application Fastqc in Galaxy**

# ✓Select Multiple Dataset to run multiple files and press “Execute”

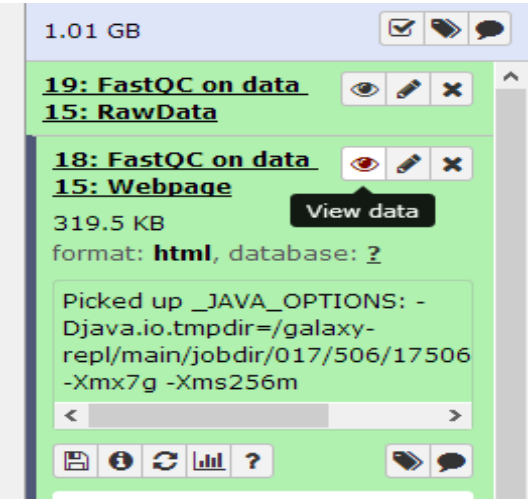


✓You will get two types of output files :

(I) Raw data – It consist of text description

(II) Web page – It consist of detail graphical representation of your fastq data.

✓Click on “eye” symbol to view output files.



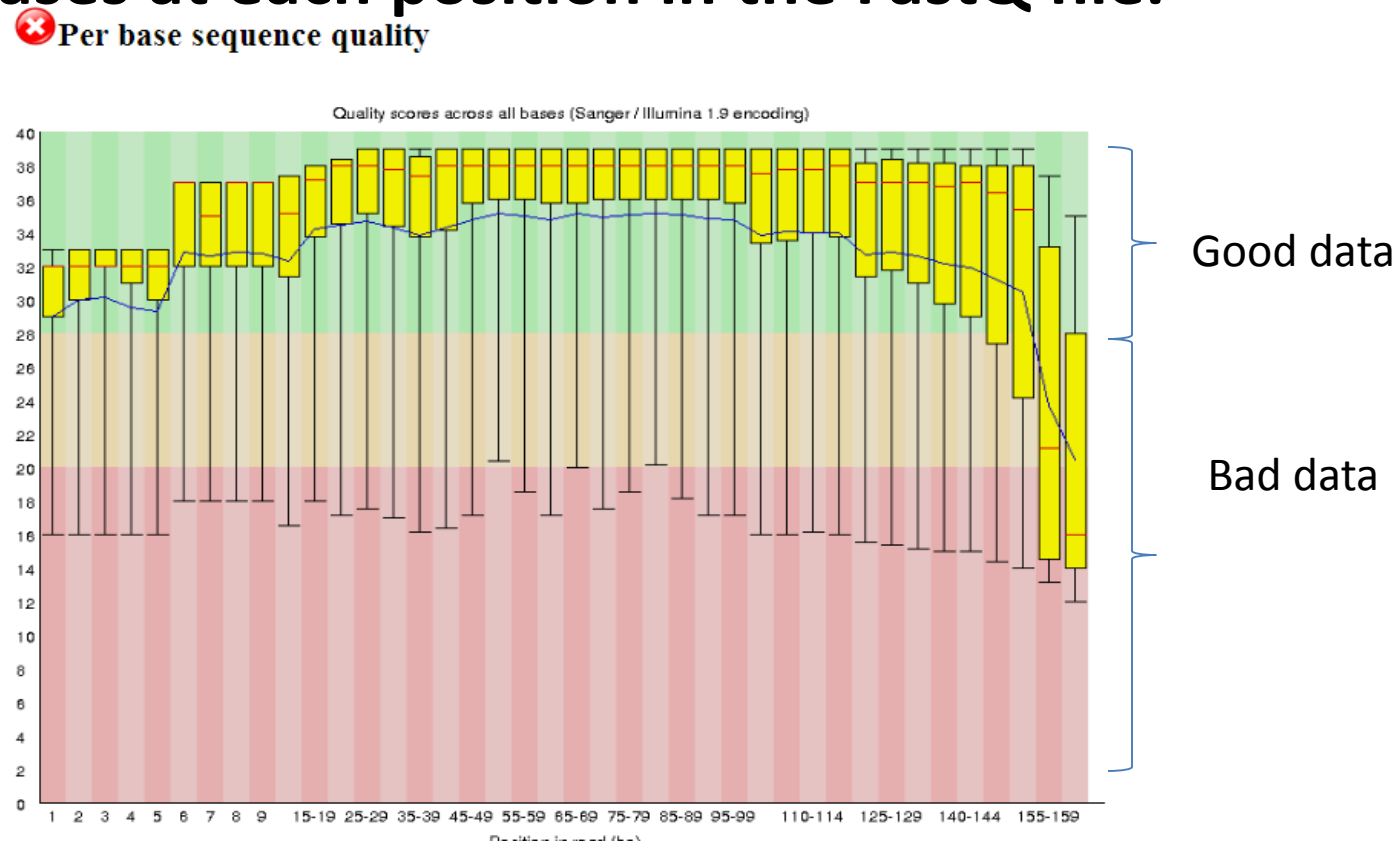
**The left hand side of the main interactive display or the top of the HTML report shows a summary of the modules as normal (green tick), slightly abnormal (orange triangle) or very unusual (red cross).**

# Basic Sample Statistics

| Measure | Value |
|---|---|
| Filename | sample-R2_fastq |
| File type | Conventional base calls |
| Encoding | Sanger / Illumina 1.9 |
| Total Sequences | 43435 |
| Sequences flagged as poor quality | 0 |
| Sequence length | 154-160 |
| %GC | 47 |

# Per Base Sequence Quality

**This view shows an overview of the range of quality values across all bases at each position in the FastQ file.**



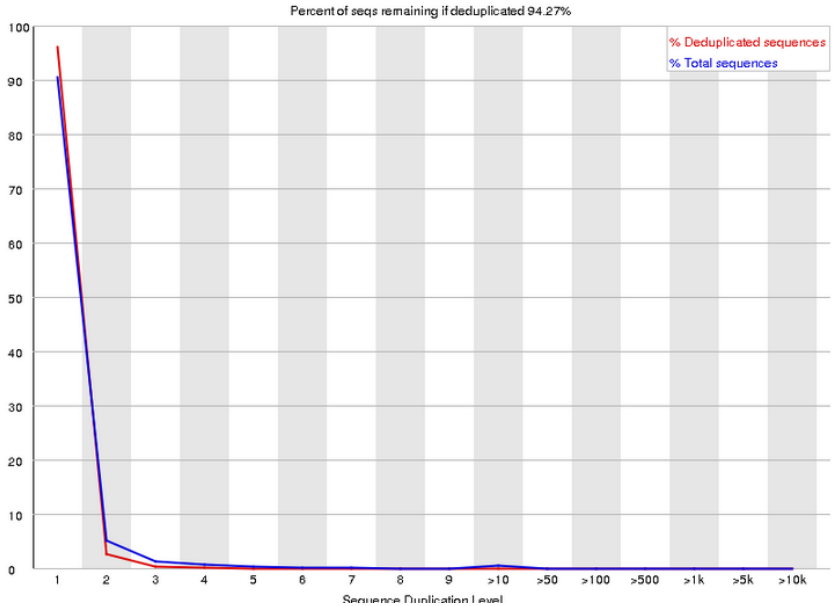

✓**We must consider threshold of Quality : Q30 or above . This graph indicate we need to perform filtration on our data.**

✓**By looking at figure, we can say that there is problem in bases in position 140-150. It can be fixed during quality filtration step.**

# Duplicate Sequences

✓A low level of duplication may indicate a very high level of coverage of the target sequence

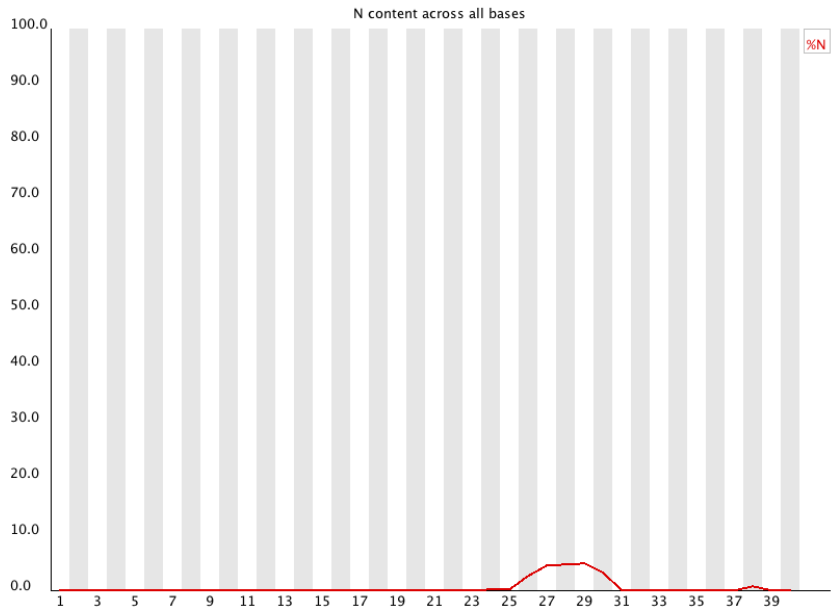
✓A high level of duplication is more likely to indicate some kind of enrichment bias (eg PCR over amplification).




✓High duplication could affect the mapping efficiency and bias your interpretation.

# Per Base N Content

✓If a sequencer is unable to make a base call with sufficient confidence then it will normally substitute an N rather than a conventional base] call.

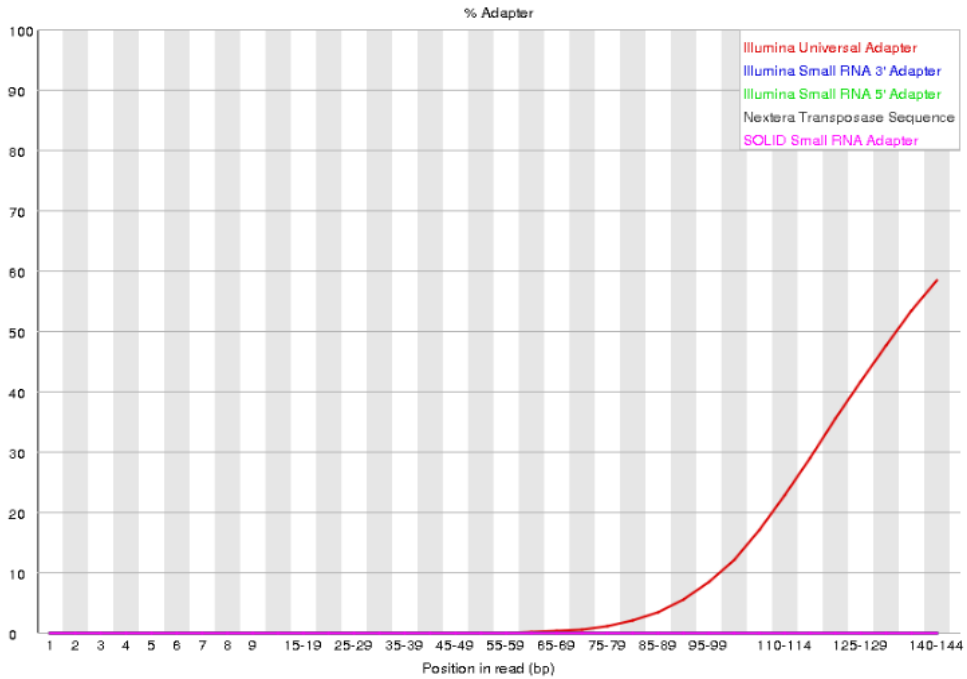



✓This module plots out the percentage of base calls at each position for which an N was called.

# Adapter : Trimming

# Adapter Content

✓If we know the adapter sequence, we can trim it using Trimmomatic tool.




✓To get the adapter sequence information, one can contact person who performed the sequencing and can get full detail of "Adapter sequences".

# Protocol for RNA Seq Data Analysis

1. Pre-processing

2. Quality Filtration

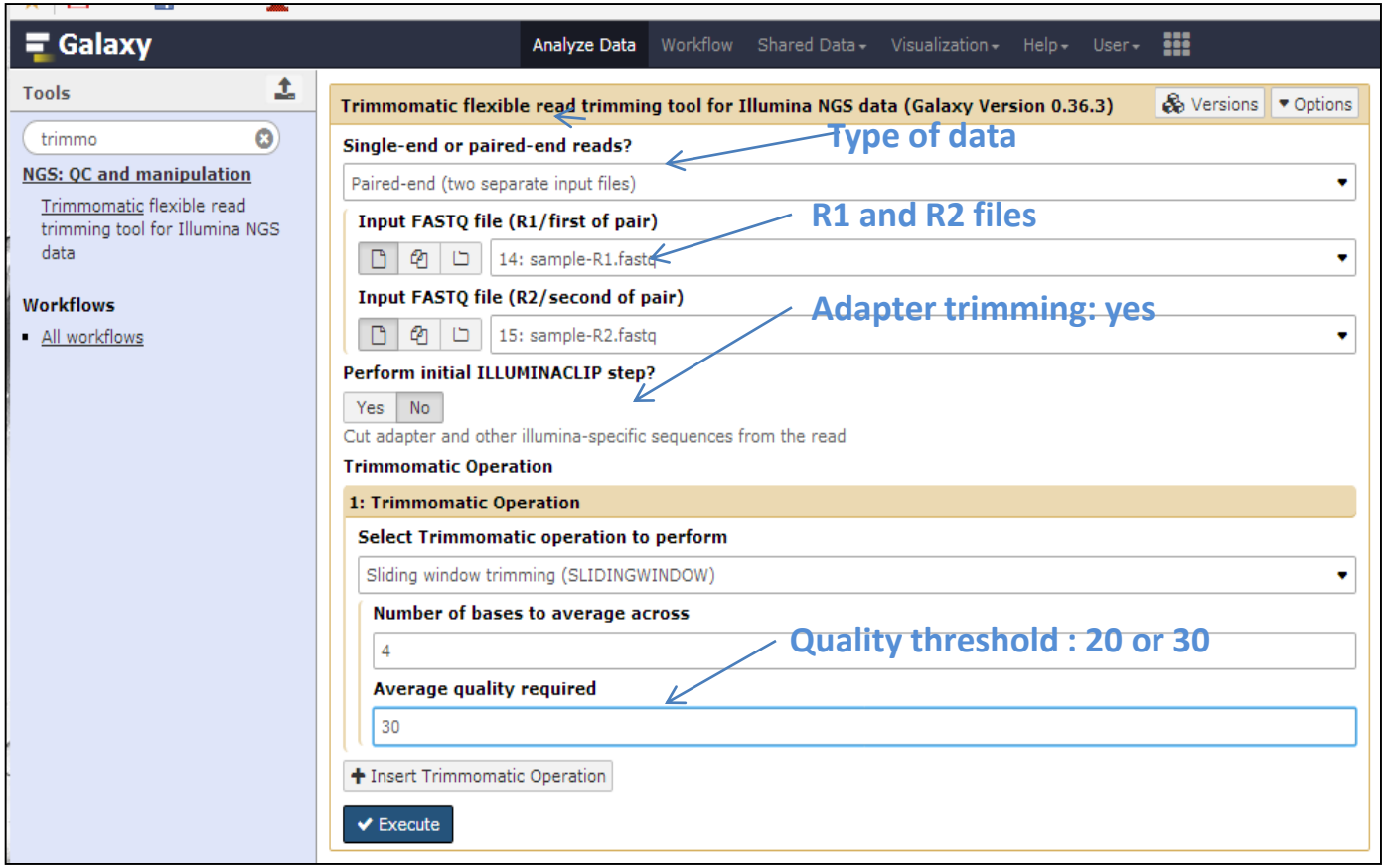
3. Mapping or assembly

4. Expression analysis

# Quality Filtration

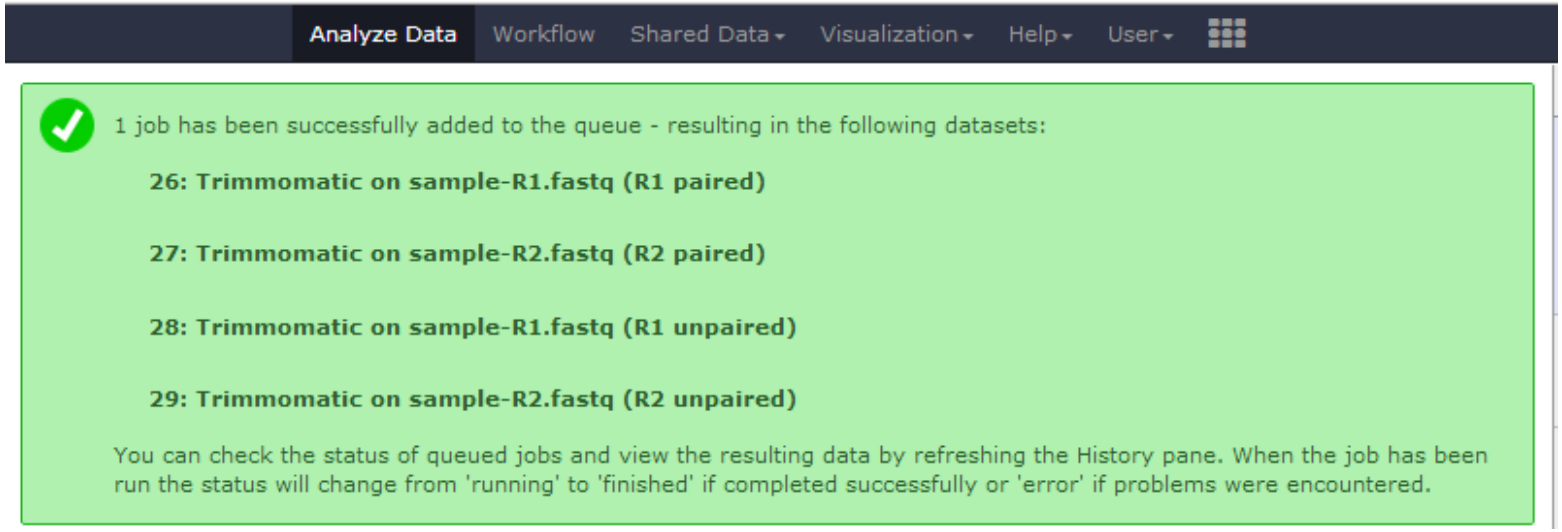
✓ **Goals : To improve the quality of Data**

## Trimmomatic :



# Quality Filtration

✓Trimmomatic will produce four output files.

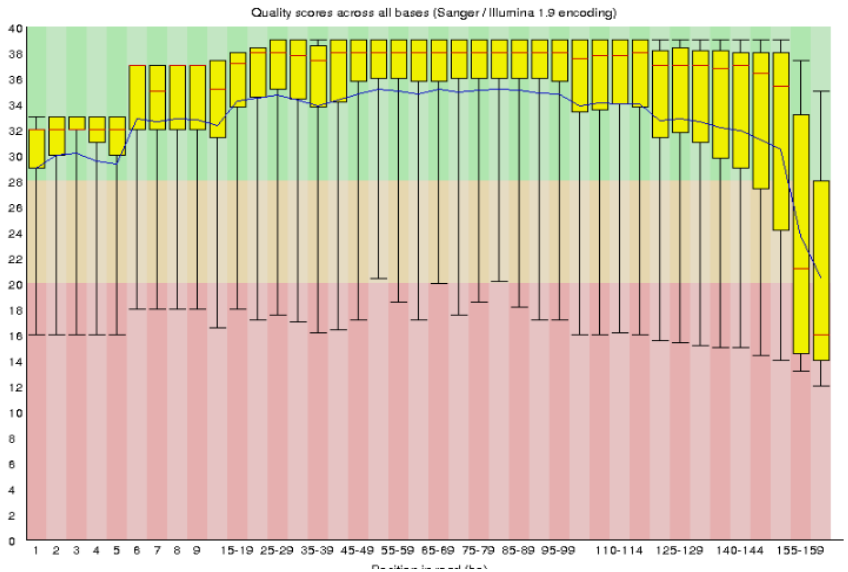



✓For next analysis, we will consider only R1 paired and R2 paired data While unpaired reads will be discarded.

✓Rerun the Fastqc on paired end R1 and R2 paired end files and check statistical output.

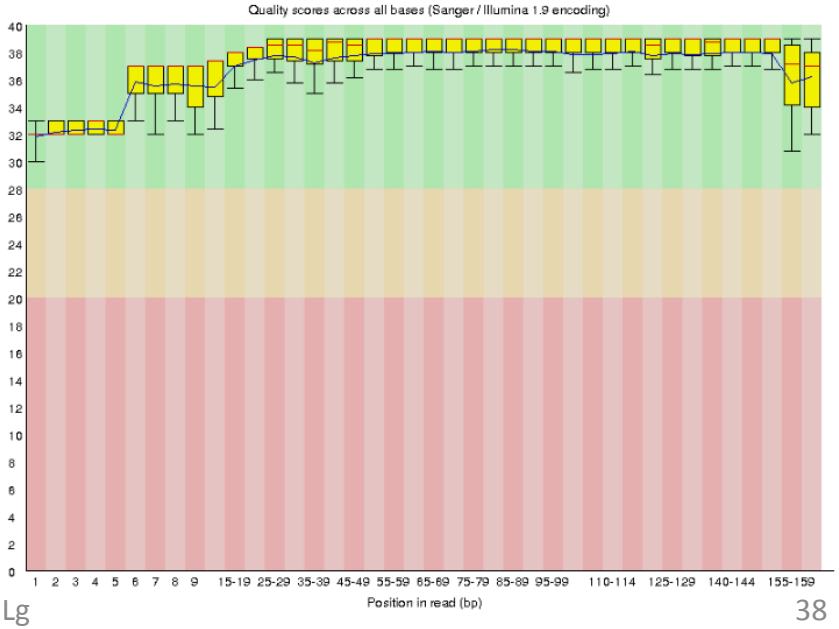
# Let us Do Comparison of dataset
# <u>Before and After Quality filtration</u>

Per base sequence quality



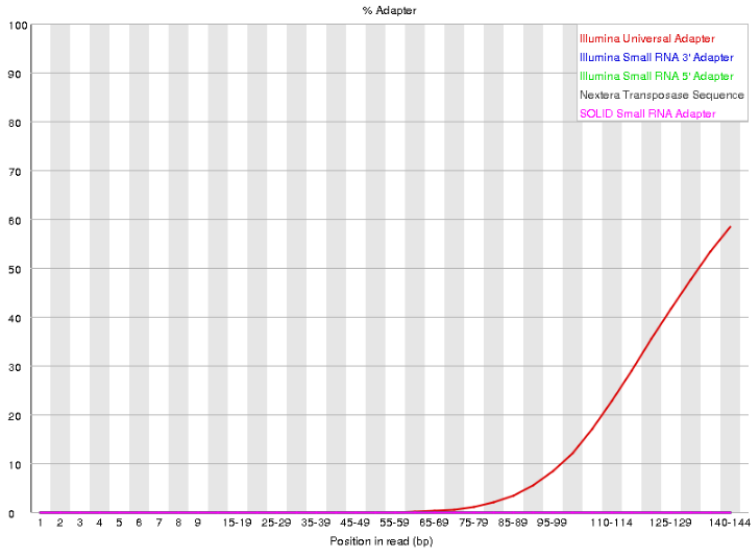

Before Quality filtration : **Bad Data**

Per base sequence quality




After Quality filtration : **Good Data**

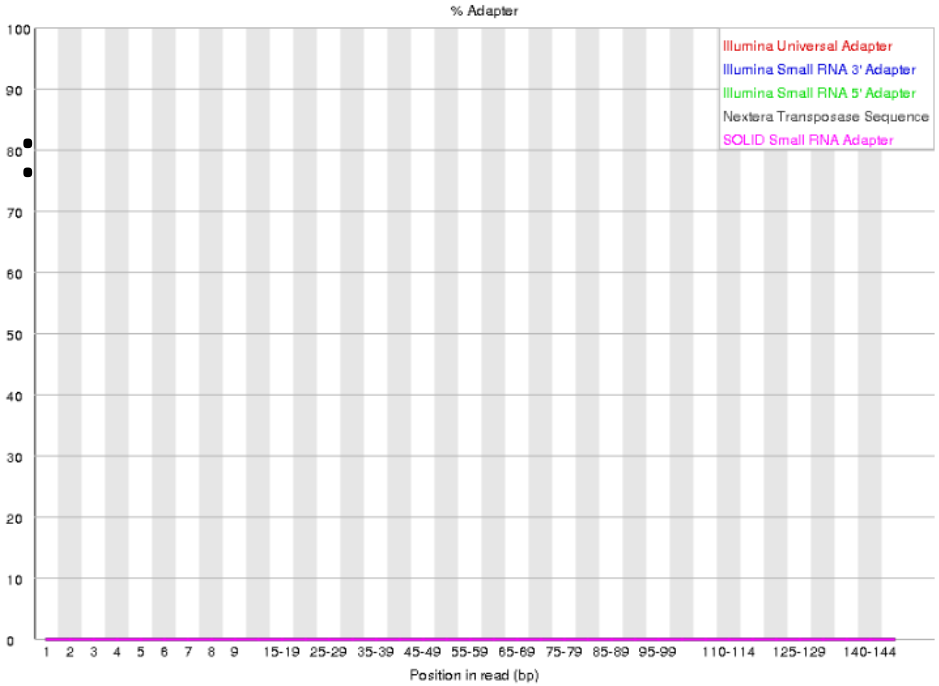
Adapter Content




Before Quality filtration : **Adapter contamination**

Before Quality filtration : **No Adapter contamination**

Adapter Content



# Questions ?

# Protocol for RNA Seq Data Analysis

1. Pre-processing

2. Quality Filtration

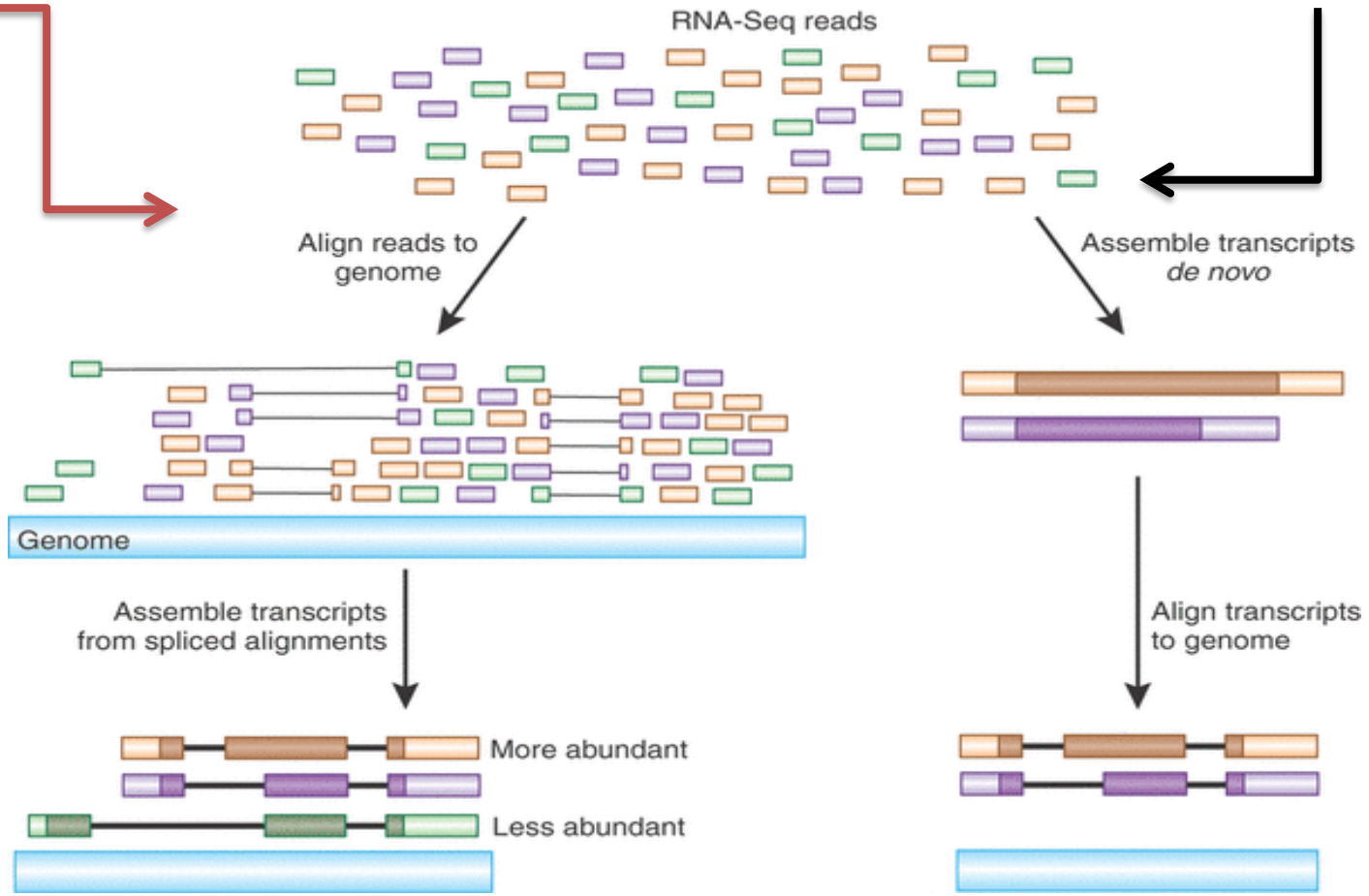
3. Mapping or assembly

4. Expression analysis

# How to decide : Mapping or assembly?

If reference genome is available

If reference genome is not available



# Mapping tool: Bowtie

- ✓**Bowtie is an ultrafast and memory-efficient tool for aligning sequencing reads to long reference sequences.**

- ✓**It is particularly good at aligning reads of about 50 up to 100s or 1,000s of characters to relatively long (e.g. mammalian) genomes.**

- ✓**Bowtie supports gapped, local, and paired-end alignment modes.**

# Bowtie : End to End Alignment

The following is an "end-to-end" alignment because it involves all the characters in the read. Such an alignment can be produced by Bowtie 2 in either end-to-end mode or in local mode.

```
Read:      GACTGGGCGATCTCGACTTCG
Reference: GACTGCGATCTCGACATCG

Alignment:
  Read:      GACTGGGCGATCTCGACTTCG
             |||||  |||||||||| |||
  Reference: GACTG--CGATCTCGACATCG
```

# Bowtie : Local Alignment

The following is a "local" alignment because some of the characters at the ends of the read do not participate. In this case, 4 characters are omitted (or "soft trimmed" or "soft clipped") from the beginning and 3 characters are omitted from the end. This sort of alignment can be produced by Bowtie 2 only in local mode.

```
Read:      ACGGTTGCGTTAATCCGCCACG
Reference: TAACTTGCGTTAAATCCGCCTGG

Alignment:
  Read:      ACGGTTGCGTTAA-TCCGCCACG
                 ||||||||| |||||
  Reference: TAACTTGCGTTAAATCCGCCTGG
```

✓**Mapping quality: higher = more unique ☺**

# Reference Mapping : Bowtie

**Bowtie2 - map reads against reference genome (Galaxy Version 2.3.2.2)** Versions Options

**Is this single or paired library**

Paired-end

**FASTA/Q file #1**

26: Trimmomatic on sample-R1.fastq (R1 paired)

Must be of datatype "fastqsanger"or "fasta"

**FASTA/Q file #2**

27: Trimmomatic on sample-R2.fastq (R2 paired)

Must be of datatype "fastqsanger"or "fasta"

**Write unaligned reads (in fastq format) to separate file(s)**

Yes No

--un/--un-conc (possibly with -gz or -bz2); This triggers --un parameter for single reads and --un-conc for paired reads

**Write aligned reads (in fastq format) to separate file(s)**

Yes No

--al/--al-conc (possibly with -gz or -bz2); This triggers --al parameter for single reads and --al-conc for paired reads

**Do you want to set paired-end options?**

No

See "Alignment Options" section of Help below for information

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

Use a built-in genome index

Built-ins were indexed using default options. See `Indexes` section of help below

**Select reference genome**

Human (Homo sapiens) (b37): hg19

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

**Set read groups information?**

Do not set

Specifying read group information can greatly simplify your downstream analyses by allowing combining multiple datasets.

**Select analysis mode**

1: Default setting only

Output from trimmomatic

Species name : Human

1 job has been successfully added to the queue - resulting in the following datasets:

**52: Bowtie2 on data 27 and data 26: unaligned reads (L)**

**53: Bowtie2 on data 27 and data 26: unaligned reads (R)**

**54: Bowtie2 on data 27 and data 26: aligned reads (sorted BAM)**

**55: Bowtie2 on data 27 and data 26: mapping stats**

You can check the status of queued jobs and view the resulting data by refreshing the History pane. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

- ✓**It will produce the multiple output files. BAM file consist of complete mapping information which stores the same data in a compressed, indexed, binary form.**

- ✓**The SAM Format is a text format for storing sequence data in a series of tab delimited ASCII columns.**

# Mapping Statistics

```
7106 reads; of these:
  7106 (100.00%) were paired; of these:
    2828 (39.80%) aligned concordantly 0 times
    2360 (33.21%) aligned concordantly exactly 1 time
    1918 (26.99%) aligned concordantly >1 times
    ----
    2828 pairs aligned concordantly 0 times; of these:
      324 (11.46%) aligned discordantly 1 time
    ----
    2504 pairs aligned 0 times concordantly or discordantly; of these:
      5008 mates make up the pairs; of these:
        2949 (58.89%) aligned 0 times
        720 (14.38%) aligned exactly 1 time
        1339 (26.74%) aligned >1 times
79.25% overall alignment rate
```

**Uniquely mapped**

**Multi mapped**

- ✓**Uniquely mapped – Reads mapped one time over the reference genome**
- ✓**Multi mapped - Reads mapped more than one time over the reference genome**

# Which Information is in SAM & BAM

- ✓Both SAM & BAM files contain an optional header section followed by the alignment section.

- ✓The header section may contain information about the entire file.

- ✓The alignment section contains the information for each sequence about where/how it aligns to the reference genome.

# Let us convert BAM to SAM

✓Select BAM to SAM tool under samtools

**BAM-to-SAM convert BAM to SAM (Galaxy Version 2.0)** Versions Options

**BAM File to Convert**

54: Bowtie2 on data 27 and data 26: aligned reads (sorted BAM)

**Header options**

Include header in SAM output (-h)

Allows to choose between seeing the entire dataset with the header, header only, or data only.

Execute

**What it does**

Converts BAM dataset to SAM using `samtools view` command:

samtools view -o [OUTPUT SAM] [-h|-H] [INPUT BAM]

Citations Show BibTeX

Definition of SAM/BAM format. [Link]

Li, H. and Handsaker, B. and Wysoker, A. and Fennell, T. and Ruan, J. and Homer, N. and Marth, G. and Abecasis, G. and Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. In *Bioinformatics, 25 (16), pp. 2078–2079.* [doi:10.1093/bioinformatics/btp352][Link]

Li, H. (2011). Improving SNP discovery by base alignment quality. In *Bioinformatics, 27 (8), pp. 1157–1158.* [doi:10.1093/bioinformatics/btr076][Link]

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. In *Bioinformatics, 27 (21), pp. 2987–2993.* [doi:10.1093/bioinformatics/btr509][Link]

Danecek, P., Schiffels, S., Durbin, R.. Multiallelic calling model in bcftools (-m). [Link]

Durbin, R.. Segregation based metric for variant call QC. [Link]

Li, H.. Mathematical Notes on SAMtools Algorithms. [Link]

SAMTools GitHub page. [Link]

# SAM File : Mapping information

| QNAME | FLAG | RNAME | POS | MAPQ |
|---|---|---|---|---|
| @HD | VN:1.0 | SO:coordinate | | |
| @SQ | SN:chr10 | LN:135534747 | | |
| @SQ | SN:chr11 | LN:135006516 | | |
| @SQ | SN:chr11_gl000202_random | LN:40103 | | |
| @SQ | SN:chr12 | LN:133851895 | | |
| @SQ | SN:chr13 | LN:115169878 | | |
| @SQ | SN:chr14 | LN:107349540 | | |
| @SQ | SN:chr15 | LN:102531392 | | |
| @SQ | SN:chr16 | LN:90354753 | | |
| @SQ | SN:chr17_ctg5_hap1 | LN:1680828 | | |
| @SQ | SN:chr17 | LN:81195210 | | |
| @SQ | SN:chr17_gl000203_random | LN:37498 | | |
| @SQ | SN:chr17_gl000204_random | LN:81310 | | |
| @SQ | SN:chr17_gl000205_random | LN:174588 | | |
| @SQ | SN:chr17_gl000206_random | LN:41001 | | |
| @SQ | SN:chr18 | LN:78077248 | | |
| @SQ | SN:chr18_gl000207_random | LN:4262 | | |
| @SQ | SN:chr19 | LN:59128983 | | |
| @SQ | SN:chr19_gl000208_random | LN:92689 | | |
| @SQ | SN:chr19_gl000209_random | LN:159169 | | |
| @SQ | SN:chr1 | LN:249250621 | | |
| @SQ | SN:chr1_gl000191_random | LN:106433 | | |
| @SQ | SN:chr1_gl000192_random | LN:547496 | | |
| @SQ | SN:chr20 | LN:63025520 | | |
| @SQ | SN:chr21 | LN:48129895 | | |
| @SQ | SN:chr21_gl000210_random | LN:27682 | | |
| @SQ | SN:chr22 | LN:51304566 | | |
| @SQ | SN:chr2 | LN:243199373 | | |
| @SQ | SN:chr3 | LN:198022430 | | |
| @SQ | SN:chr4_ctg9_hap1 | LN:590426 | | |
| @SQ | SN:chr4 | LN:191154276 | | |
| @SQ | SN:chr4_gl000193_random | LN:189789 | | |
| @SQ | SN:chr4_gl000194_random | LN:191469 | | |
| @SQ | SN:chr5 | LN:180915260 | | |
| @SQ | SN:chr6_apd_hap1 | LN:4622290 | | |
| @SQ | SN:chr6_cox_hap2 | LN:4795371 | | |

History

search datasets

Unnamed history
17 shown, 39 deleted

1.06 GB

56: BAM-to-SAM on data 54: converted SAM

55: Bowtie2 on data 27 and data 26: mapping stats

15 lines
format: txt, database: ?

```
7106 reads; of these:
7106 (100.00%) were paired; of th
2828 (39.80%) aligned concordantl
2360 (33.21%) aligned concordantl
1918 (26.99%) aligned concordantl
```

54: Bowtie2 on data 27 and data 26: aligned reads (sorted BAM)

53: Bowtie2 on data 27 and data 26: unaligned reads (R)

52: Bowtie2 on data 27 and data 26: unaligned reads (L)

31: FastQC on data 27: RawData

30: FastQC on dat

80%

Query ID

Header

Chromosome number

Position on chromosome

```
@SQ     SN:chrUn_gl000247       LN:36422
@SQ     SN:chrUn_gl000248       LN:39786
@SQ     SN:chrUn_gl000249       LN:38502
@SQ     SN:chrX LN:155270560
@SQ     SN:chrY LN:59373566
@PG     ID:bowtie2      PN:bowtie2      VN:2.3.2        CL:"/galaxy/main/deps/_conda/envs/mulled-v1-cf272fa72b0572012c68ee2cbf0c8f909a02f29be46918c2a23
M00991:178:000000000-BBP68:1:1106:9736:6918     99      chr10   429971  42      68M3D88M        =       430051  166     GTTCGCACCGTCCGCCACTATCAGCATTCGC
M00991:178:000000000-BBP68:1:1106:9736:6918     147     chr10   430051  42      86M     =       429971  -166    AATGCATATCCCTCGATTTCACACACGCCACTTTTGCTA
M00991:178:000000000-BBP68:1:2108:23825:17989   99      chr10   860519  42      153M    =       860666  155     ATATTAAAGGTATTTTGTACAGAAAACACAACACAGACA
M00991:178:000000000-BBP68:1:2108:23825:17989   147     chr10   860666  42      8M      =       860519  -155    GGCACGAG        BAAAABBA        AS:i:0
M00991:178:000000000-BBP68:1:2107:12293:26439   161     chr10   3142833 35      17M     chr17   71196804        0       ACAAAGTCAGCACGCGC       AAAAAFF
M00991:178:000000000-BBP68:1:1104:13558:18222   73      chr10   3201129 42      100M    =       3201129 0       GCCAAAGCCAGATTCAATCAAGGCTTTGTAAAAGGGAGA
M00991:178:000000000-BBP68:1:1104:13558:18222   133     chr10   3201129 0       *       =       3201129 0       ACCAAGCACCGTGGTGCCAGCTCAGACACCCTGGGACAA
M00991:178:000000000-BBP68:1:1103:20292:14133   97      chr10   3820991 42      155M    chr19   10776364        0       GTTGTGTTATATTTGTAAATACACAGCTTAT
M00991:178:000000000-BBP68:1:1107:17049:25808   99      chr10   3821316 42      155M    =       3821331 170     GTATACTCCTTACACACAAAACATTCAAACTACTTTTTT
M00991:178:000000000-BBP68:1:1107:17049:25808   147     chr10   3821331 42      155M    =       3821316 -170    ACAAAACATTCAAACTACTTTTTTTCCATCTCTTGCAGT
M00991:178:000000000-BBP68:1:1104:5844:11822    165     chr10   5170810 0       *       =       5170810 0       ACACATTCATGTCGTGAGTTGCTAAGGATAGCAGACAAG
M00991:178:000000000-BBP68:1:1104:5844:11822    89      chr10   5170810 1       9M      =       5170810 0       AGTATTCAG       @4FFBBBBB       AS:i:0
M00991:178:000000000-BBP68:1:1105:15914:19028   165     chr10   5494394 0       *       =       5494394 0       GACCATACGAGTCCTAGATGTCAATAACCAGTCCTTCAG
M00991:178:000000000-BBP68:1:1105:15914:19028   89      chr10   5494394 1       16M     =       5494394 0       CACCCGCCAAGAGAAG        AAAEDFFFFFFAAAA
M00991:178:000000000-BBP68:1:1101:6644:20822    165     chr10   5494395 0       *       =       5494395 0       GACCATACGAGTCCTAGATGTCAATAACCAGTCCTTCAG
M00991:178:000000000-BBP68:1:1101:6644:20822    89      chr10   5494395 1       15M     =       5494395 0       ACCCGCCAAGAGAAG ACG?FFFFFFBBBBB AS:i:0
M00991:178:000000000-BBP68:1:2114:18702:22202   163     chr10   5766266 42      158M    =       5766282 172     AAATGATAAAGGTTTCTGAGTAGTATTTCTATTCTTTCA
M00991:178:000000000-BBP68:1:2114:18702:22202   83      chr10   5766282 42      156M    =       5766266 -172    TGAGTAGTATTTCTATTCTTTCATTTTTGCAACATATAA
M00991:178:000000000-BBP68:1:1111:5298:18126    99      chr10   11616654        42      155M    =       11616696        197     CTTACTGTACTGCCAATTTTCCT
M00991:178:000000000-BBP68:1:1111:5298:18126    147     chr10   11616696        42      155M    =       11616654        -197    CCCATGAATTATTTTGACATTTT
M00991:178:000000000-BBP68:1:1104:21028:25600   163     chr10   11954572        1       4M      =       11954643        81      CCCA    AAAA    AS:i:0
M00991:178:000000000-BBP68:1:1104:21028:25600   83      chr10   11954643        1       10M     =       11954572        -81     CGAAGCTGGG      DB1FFAA
M00991:178:000000000-BBP68:1:2114:16947:22919   99      chr10   12070773        42      155M    =       12070865        188     CCTGTGGTCCCTTTTCAGGTGTT
M00991:178:000000000-BBP68:1:2114:16947:22919   147     chr10   12070865        42      96M     =       12070773        -188    CACTAGGAGGAAAACTCAAATTA
M00991:178:000000000-BBP68:1:2109:26937:12502   97      chr10   13325702        23      155M    chr16   4700341 0       CTGGGTTTTATTCTGACCAGATCCGTGGATG
M00991:178:000000000-BBP68:1:2107:14639:5173    99      chr10   13361154        31      155M    =       13361183        172     CCCGGGTGTGGGATTCACATTTT
M00991:178:000000000-BBP68:1:2107:14639:5173    147     chr10   13361183        31      143M    =       13361154        -172    CCACTTGTGGTGCGACCTCGATG
M00991:178:000000000-BBP68:1:2105:16260:9454    101     chr10   15151770        0       *       =       15151770        0       GGCAGTTCCAGAAATCATTAAAT
M00991:178:000000000-BBP68:1:2105:16260:9454    153     chr10   15151770        32      19M     =       15151770        0       AAACTTGAGTTTTTCCAAG
M00991:178:000000000-BBP68:1:1103:14887:6379    99      chr10   15834731        42      106M    =       15834858        186     GTTGCTTCCTGACATATAATTGT
M00991:178:000000000-BBP68:1:1103:14887:6379    147     chr10   15834858        42      59M     =       15834731        -186    TCTTTGGAGGTTATGGAATAAGC
M00991:178:000000000-BBP68:1:1109:7860:8475     163     chr10   17271678        42      7M      =       17271687        165     TCTCGCT B?ABABB AS:i:0
M00991:178:000000000-BBP68:1:1109:7860:8475     83      chr10   17271687        42      156M    =       17271678        -165    CCGACGCCATCAACACCGAGTTC
M00991:178:000000000-BBP68:1:1110:18848:15480   163     chr10   17271697        42      116M2D40M       =       17271740        199     CAACACCGAGTTCAA
M00991:178:000000000-BBP68:1:1110:18848:15480   83      chr10   17271740        42      73M2D81M        =       17271697        -199    CTGCAGGAGCTGAAT
M00991:178:000000000-BBP68:1:1111:17753:19097   165     chr10   17275755        0       *       =       17275755        0       CCTTGAACGCAAAGTGGAATCTT
M00991:178:000000000-BBP68:1:1111:17753:19097   89      chr10   17275755        0       4M3I7M15I126M   =       17275755        0       AGATTGCCTTTTTGA
M00991:178:000000000-BBP68:1:2107:13404:9576    81      chr10   17275761        0       5M15I136M       =       17275764        141     TCTTGAAGAAACTCC
```

- ✓ **Millions of reads mapped to genome.**
- ✓ **Is it possible to analyse it manually ?**
- ✓ **Answer is NO**
- ✓ **To estimate expression , we needed another tool.**
- ✓ **In 2010, Trapnell et al. published cufflinks and made the transcript abundance an easy task.**

nature biotechnology

LETTERS

# Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation

Cole Trapnell[1–3], Brian A Williams[4], Geo Pertea[2], Ali Mortazavi[4], Gordon Kwan[4], Marijke J van Baren[5], Steven L Salzberg[1,2], Barbara J Wold[4] & Lior Pachter[3,6,7]

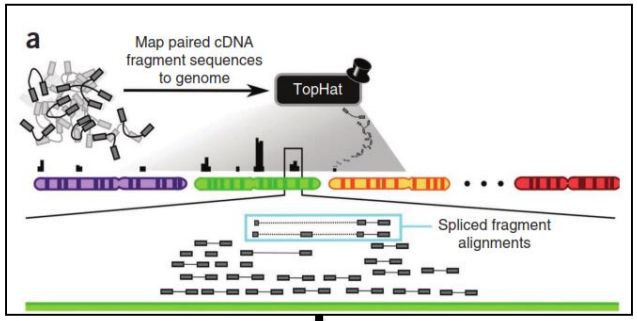


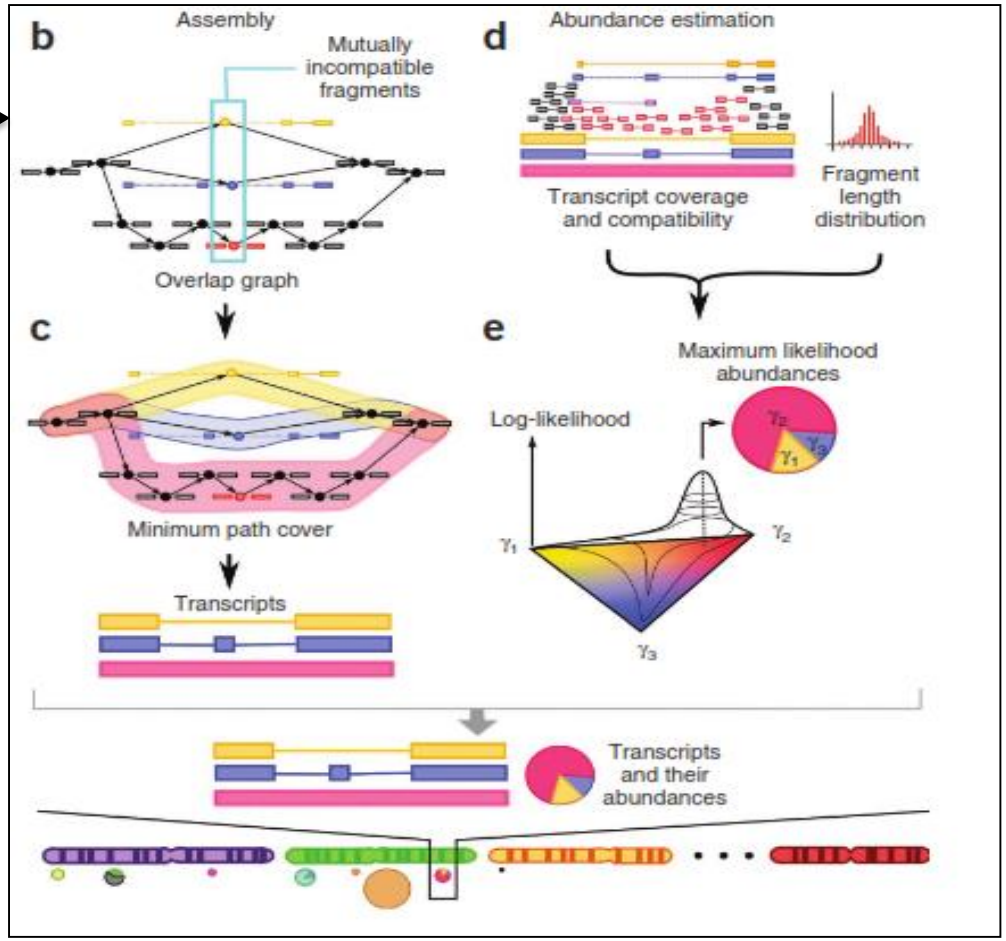
**High-throughput mRNA sequencing (RNA-Seq) promises simultaneous transcript discovery and abundance estimation[1–3]. However, this would require algorithms that are not restricted by prior gene annotations and that account for alternative transcription and splicing. Here we introduce such algorithms in an open-source software program called Cufflinks. To test Cufflinks, we sequenced and analyzed >430 million paired 75-bp RNA-Seq reads from a mouse myoblast cell line over a differentiation time series. We detected 13,692 known transcripts and 3,724 previously unannotated ones, 62% of which are supported by independent expression data or by homologous genes in other species. Over the time series, 330 genes showed complete switches in the dominant transcription start site (TSS) or splice isoform, and we observed more subtle shifts in 1,304 other genes. These results suggest that**

(75 bp in this work versus 25 bp in our previous work) and pairs of reads from both ends of each RNA fragment can reduce uncertainty in assigning reads to alternative splice variants[12]. To produce useful transcript-level abundance estimates from paired-end RNA-Seq data, we developed a new algorithm that can identify complete novel transcripts and probabilistically assign reads to isoforms.

For our initial demonstration of Cufflinks, we performed a time course of paired-end 75-bp RNA-Seq on a well-studied model of skeletal muscle development, the C2C12 mouse myoblast cell line[13] (see Online Methods). Regulated RNA expression of key transcription factors drives myogenesis, and the execution of the differentiation process involves changes in expression of hundreds of genes[14,15]. Previous studies have not measured global transcript isoform expression; however, there are well-documented expression changes at the whole-gene level for a set of marker genes in this system. We aimed to

# Cufflink : Assembly and Abundance Estimation



# Protocol for RNA Seq Data Analysis

1. Pre-processing

2. Quality Filtration

3. Mapping or assembly

4. **Expression analysis**

# Let us run CUFFLINKS to estimate the expression of genes on genomes

**Cufflinks transcript assembly and FPKM (RPKM) estimates for RNA-Seq data (Galaxy Version 2.2.1.0)** Versions Options

**SAM or BAM file of aligned RNA-Seq reads**

Created sam file

56: BAM-to-SAM on data 54: converted SAM

**Max Intron Length**

300000

ignore alignments with gaps longer than this

**Min Isoform Fraction**

0.1

suppress transcripts below this abundance level

**Pre MRNA Fraction**

0.15

suppress intra-intronic transcripts below this level

**Use Reference Annotation**

No

**Perform Bias Correction**

No

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

**Use multi-read correct**

No

Tells Cufflinks to do an initial estimation procedure to more accurately weight reads mapping to multiple locations in the genome.

**Apply length correction**

Cufflinks Effective Length Correction

Mode of length normalization to transcript FPKM.

**Set advanced Cufflinks options**

No

**Job Resource Parameters**

Use default job resource parameters

Execute

# You will get 5 output files.

1 job has been successfully added to the queue - resulting in the following datasets:

**57: Cufflinks on data 56: gene expression**

**58: Cufflinks on data 56: transcript expression**

**59: Cufflinks on data 56: assembled transcripts**

**60: Cufflinks on data 56: total map mass**

**61: Cufflinks on data 56: Skipped Transcripts**

You can check the status of queued jobs and view the resulting data by refreshing the History pane. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

# Different Method of Abundance Estimation

✓**Counts per million : Reads counts scaled by the number of fragments you sequenced (N) times one million.**

$$\mathrm{CPM}_i = \frac{X_i}{\frac{N}{10^6}} = \frac{X_i}{N} \cdot 10^6$$

✓**Transcripts per million (TPM) is a measurement of the proportion of transcripts in your pool of RNA.**

$$\mathrm{TPM}_i = \frac{X_i}{\widetilde{l}_i} \cdot \left( \frac{1}{\sum_j \frac{X_j}{\widetilde{l}_j}} \right) \cdot 10^6$$

✓**FPKM is a unit of expression. FPKM is simply a unit of expression**

$$\mathrm{FPKM}_i = \frac{X_i}{\left(\frac{\widetilde{l}_i}{10^3}\right)\left(\frac{N}{10^6}\right)} = \frac{X_i}{\widetilde{l}_i N} \cdot 10^9$$

✓**If you have FPKM, you can easily compute TPM:**

$$\mathrm{TPM}_i = \left(\frac{\mathrm{FPKM}_i}{\sum_j \mathrm{FPKM}_j}\right) \cdot 10^6$$

# Transcript Expressions

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| tracking_id | class_code | nearest_ref_id | gene_id | gene_short_name | tss_id | locus | length | coverage | FPKM |
| CUFF.1.1 | - | - | CUFF.1 | - | - | chr11:1016561-1017335 | 774 | 9.82598 | 8303.04 |
| CUFF.2.1 | - | - | CUFF.2 | - | - | chr11:1017439-1018092 | 653 | 6.64209 | 5489.47 |
| CUFF.3.1 | - | - | CUFF.3 | - | - | chr11:62292242-62293305 | 1063 | 3.05145 | 2641.01 |
| CUFF.4.1 | - | - | CUFF.4 | - | - | chr11:65272989-65273355 | 366 | 32.6074 | 27409.5 |
| CUFF.5.1 | - | - | CUFF.5 | - | - | chr11:65266580-65270418 | 3838 | 15.0753 | 12927.1 |
| CUFF.6.1 | - | - | CUFF.6 | - | - | chr11:65270666-65272939 | 2273 | 15.1561 | 13692.1 |
| CUFF.7.1 | - | - | CUFF.7 | - | - | chr14:106207848-106208692 | 844 | 4.63947 | 4182.81 |
| CUFF.8.1 | - | - | CUFF.8 | - | - | chr15:45007636-45007912 | 276 | 43.8713 | 35014.3 |
| CUFF.9.1 | - | - | CUFF.9 | - | - | chr17:19091226-19091547 | 321 | 138.008 | 116298 |
| CUFF.10.1 | - | - | CUFF.10 | - | - | chr1:28835082-28835270 | 188 | 127.939 | 105533 |
| CUFF.11.1 | - | - | CUFF.11 | - | - | chr22:23243039-23243586 | 547 | 4.52043 | 5972.5 |
| CUFF.12.1 | - | - | CUFF.12 | - | - | chr2:89156745-89157197 | 452 | 12.2365 | 11515.3 |
| CUFF.13.1 | - | - | CUFF.13 | - | - | chr3:185135517-185136470 | 953 | 4.33674 | 3577.22 |
| CUFF.14.1 | - | - | CUFF.14 | - | - | chr3:195507749-195508870 | 1121 | 2.01657 | 2699.64 |
| CUFF.15.1 | - | - | CUFF.15 | - | - | chr3:195508955-195510544 | 1589 | 1.60876 | 2386.61 |
| CUFF.16.1 | - | - | CUFF.16 | - | - | chr3:195510927-195512268 | 1341 | 3.58167 | 4358.11 |
| CUFF.17.1 | - | - | CUFF.17 | - | - | chr3:195512377-195514082 | 1705 | 1.95635 | 2615.64 |
| CUFF.18.1 | - | - | CUFF.18 | - | - | chr7:100550701-100551060 | 359 | 17.9467 | 16894 |
| CUFF.19.1 | - | - | CUFF.19 | - | - | chr9:135894829-135895508 | 679 | 9.04212 | 7354.52 |
| CUFF.20.1 | - | - | CUFF.20 | - | - | chrM:2037-2817 | 780 | 3.91122 | 3929.93 |
| CUFF.22.1 | - | - | CUFF.22 | - | - | chrX:73047134-73047924 | 790 | 27.3414 | 22828.6 |
| CUFF.21.1 | - | - | CUFF.21 | - | - | chrX:73062299-73062927 | 628 | 6.87826 | 5810.22 |
| CUFF.23.1 | - | - | CUFF.23 | - | - | chrX:73069152-73069629 | 477 | 10.7957 | 9727.26 |
| CUFF.24.1 | - | - | CUFF.24 | - | - | chrX:139865623-139866556 | 933 | 6.06793 | 5936.29 |

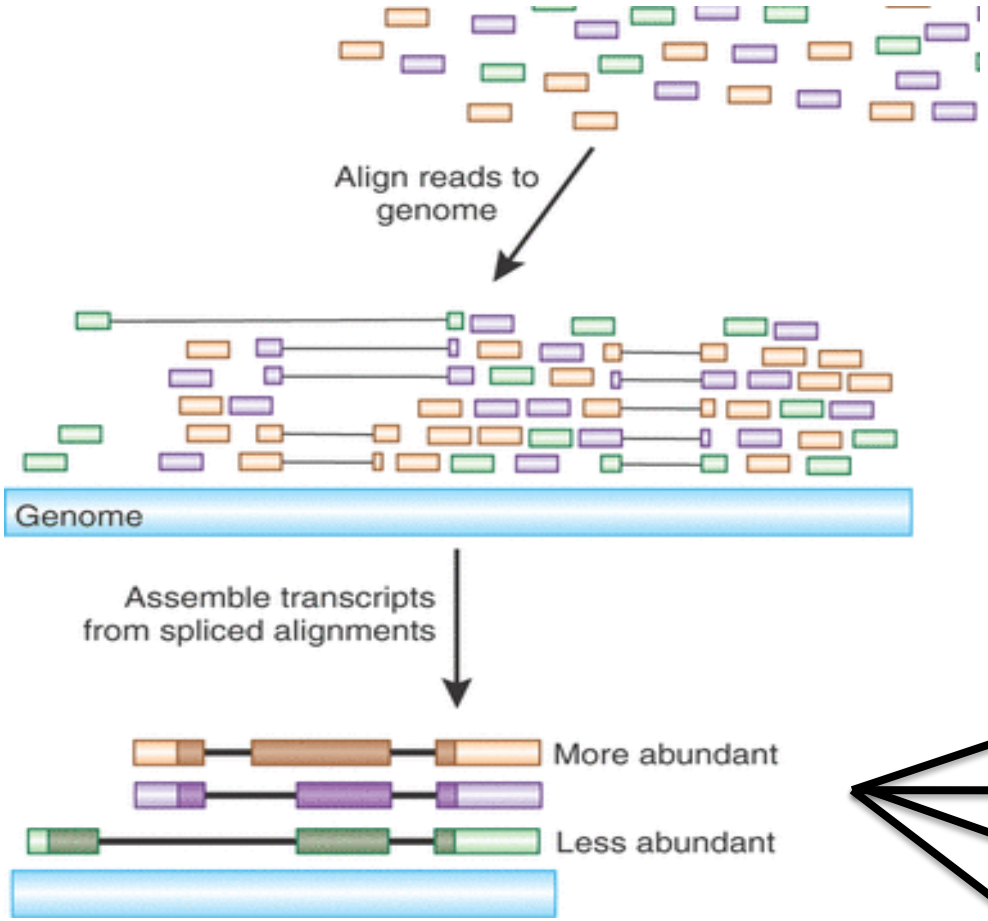
.

**Length of transcript**

**Chromosomal position on genome**

**Expression in term of FPKM**

# What if We have two different samples ??




**Differential Expression**

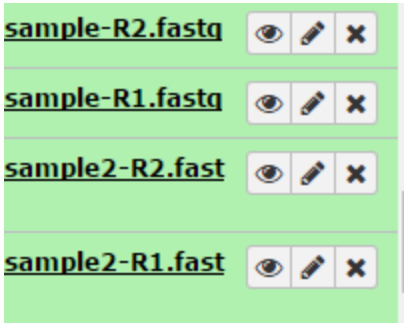
**Differential Isofom Abundance**

**SNPs , Indels**

**RNA editing**

# Sample 2: Start Analysis

✓Upload Sample2 in galaxy server




✓ Run Fastqc (Is there any issue in Quality plot ?)

✓Run Trimmomatic to fix if you find any issue

✓Run bowtie2 (Can you see mapping statistics ? If yes, how many reads mapped)

✓Run cufflink (what is minimum and maximum transcript expression?)

# Differentially expression in two different conditions

- ✓Cuffdiff is a highly accurate tool for performing sample comparisons, and can tell you which genes are up- or down-regulated between two or more conditions.

- ✓Go to NGS RNA Analysis in galaxy web server.

- ✓Select cuffdiff tool.

- ✓Select assembled transcript as input (output from cufflinks tool).

**Cuffdiff find significant changes in transcript expression, splicing, and promoter use (Galaxy Version 2.2.1.3)** Versions Options

**Transcripts**

124: Cufflinks on data 59: Skipped Transcripts
122: Cufflinks on data 59: assembled transcripts
119: Cufflinks on data 55: Skipped Transcripts
117: Cufflinks on data 55: assembled transcripts

This is a batch mode input field. Separate jobs will be triggered for each dataset selection.
A transcript GFF3 or GTF file produced by cufflinks, cuffcompare, 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**

sample1

**Replicates**

59: Bowtie2 on data 47 and data 46: aligned reads (sorted BAM)
55: Bowtie2 on data 49 and data 48: aligned reads (sorted BAM)

**2: Condition**

**Name**

sample2

**Replicates**

59: Bowtie2 on data 47 and data 46: aligned reads (sorted BAM)
55: Bowtie2 on data 49 and data 48: aligned reads (sorted BAM)

**Cufflinks output as input in cuffdiff**

**Sample 1 in condition 1**

**Mapping information**

**Sample 2 in condition 2**

**Mapping information**

# Cuffdiff Output : FPKM tracking files

| | |
|---|---|
| **isoforms.fpkm_tracking** | **Transcript FPKMs** |
| **genes.fpkm_tracking** | **Gene FPKMs. Tracks the summed FPKM of transcripts sharing each gene_id** |
| **cds.fpkm_tracking** | **Coding sequence FPKMs. Tracks the summed FPKM of transcripts sharing each p_id, independent of tss_id** |
| **tss_groups.fpkm_tracking** | **Primary transcript FPKMs. Tracks the summed FPKM of transcripts sharing each tss_id** |

# Cuffdiff Output : differential files

| | |
|---|---|
| isoform_exp.diff | Transcript-level differential expression. |
| gene_exp.diff | Gene-level differential expression. Tests differences in the summed FPKM of transcripts sharing each gene_id |
| tss_group_exp.diff | Primary transcript differential expression. Tests differences in the summed FPKM of transcripts sharing each tss_id |
| cds_exp.diff | Coding sequence differential expression. Tests differences in the summed FPKM of transcripts sharing each p_id independent of tss_id |

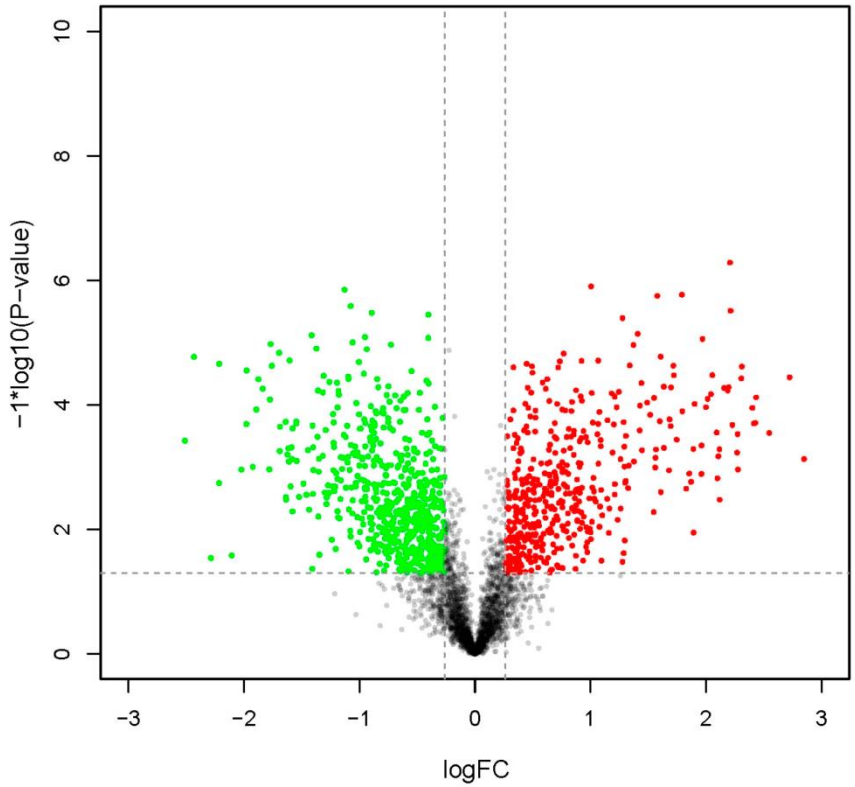
# Cuffdiff Output :

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| test_id | gene_id | gene | locus | sample_1 | sample_2 | status | value_1 | value_2 | log2(fold_change) | test_stat | p_value |
| CUFF.1 | CUFF.1 | - | chr10:98510037-98510664 | C1 | C2 | OK | 9493.98 | 32599.5 | 1.77976 | 1.48669 | 0.17545 |
| CUFF.10 | CUFF.10 | - | chr12:6619384-6619710 | C1 | C2 | OK | 25232.2 | 72273.2 | 1.5182 | 1.41818 | 0.1831 |
| CUFF.11 | CUFF.11 | - | chr12:49521782-49522637 | C1 | C2 | OK | 3189.87 | 5058.44 | 0.665194 | 0.593004 | 0.57295 |
| CUFF.12 | CUFF.12 | - | chr12:125396257-125398338 | C1 | C2 | OK | 10291.6 | 10108.2 | -0.0259507 | -0.0195277 | 0.9848 |
| CUFF.13 | CUFF.13 | - | chr12:133402275-133402691 | C1 | C2 | OK | 14701.8 | 29691.4 | 1.01406 | 0.808465 | 0.40285 |
| CUFF.14 | CUFF.14 | - | chr14:106090797-106091148 | C1 | C2 | OK | 22101.4 | 22363.8 | 0.0170267 | 0.0176527 | 0.93665 |
| CUFF.15 | CUFF.15 | - | chr14:106173453-106173905 | C1 | C2 | OK | 13261 | 1561.22 | -3.08644 | -0.0862077 | 0.25195 |
| CUFF.16 | CUFF.16 | - | chr14:106174096-106174509 | C1 | C2 | OK | 12801.5 | 22623.4 | 0.821506 | 0.65991 | 0.5265 |
| CUFF.17 | CUFF.17 | - | chr14:106207785-106208145 | C1 | C2 | OK | 70913.6 | 62761.8 | -0.176176 | -0.147622 | 0.887 |
| CUFF.18 | CUFF.18 | - | chr14:106109504-106110274 | C1 | C2 | OK | 9954.7 | 7797.09 | -0.352442 | -0.242097 | 0.8281 |
| CUFF.19 | CUFF.19 | - | chr14:106208210-106208574 | C1 | C2 | OK | 31595.9 | 71169.3 | 1.17152 | 0.8854 | 0.4042 |
| CUFF.2 | CUFF.2 | - | chr11:1016562-1018587 | C1 | C2 | OK | 5138.32 | 18878.9 | 1.8774 | 1.47427 | 0.1785 |
| CUFF.20 | CUFF.20 | - | chr14:106209105-106209429 | C1 | C2 | OK | 49411.3 | 140200 | 1.50457 | 1.16794 | 0.2812 |
| CUFF.21 | CUFF.21 | - | chr14:106110802-106111119 | C1 | C2 | OK | 23214.4 | 59909.3 | 1.36776 | 1.05483 | 0.32665 |
| CUFF.22 | CUFF.22 | - | chr14:106235622-106235928 | C1 | C2 | OK | 28273.8 | 4183.93 | -2.75654 | -0.0769921 | 0.2652 |
| CUFF.23 | CUFF.23 | - | chr15:45007610-45007908 | C1 | C2 | OK | 56496.2 | 187100 | 1.72758 | 1.33425 | 0.2249 |
| CUFF.24 | CUFF.24 | - | chr15:82664618-82665097 | C1 | C2 | OK | 11063.8 | 26506.2 | 1.26048 | 1.15576 | 0.2548 |
| CUFF.25 | CUFF.25 | - | chr15:83040991-83041657 | C1 | C2 | OK | 5466.52 | 16061.9 | 1.55495 | 1.41648 | 0.192 |
| CUFF.26 | CUFF.26 | - | chr16:2812452-2814215 | C1 | C2 | NOTEST | 2353.05 | 1611.13 | -0.546457 | 0 | 1 |
| CUFF.27 | CUFF.27 | - | chr16:2815866-2817216 | C1 | C2 | OK | 2859.11 | 0 | -inf | -nan | 0.00075 |
| CUFF.28 | CUFF.28 | - | chr16:21413502-21415549 | C1 | C2 | OK | 3972.75 | 388.56 | -3.35393 | -0.23512 | 0.2134 |
| CUFF.29 | CUFF.29 | - | chr16:21415834-21416578 | C1 | C2 | OK | 11614.4 | 3411.63 | -1.76737 | -1.32884 | 0.1962 |
| CUFF.3 | CUFF.3 | - | chr11:1265350-1265887 | C1 | C2 | OK | 7079.54 | 5677.35 | -0.318437 | -0.254093 | 0.8104 |
| CUFF.30 | CUFF.30 | - | chr16:21845954-21848154 | C1 | C2 | OK | 3665.06 | 896.135 | -2.03205 | -1.56799 | 0.14375 |
| CUFF.31 | CUFF.31 | - | chr16:21848746-21849079 | C1 | C2 | OK | 18252.1 | 6470.03 | -1.49622 | -0.104869 | 0.44675 |
| CUFF.32 | CUFF.32 | - | chr16:22544931-22547789 | C1 | C2 | OK | 11608.2 | 2150.91 | -2.43213 | -1.71478 | 0.13965 |
| CUFF.33 | CUFF.33 | - | chr16:29494914-29497219 | C1 | C2 | OK | 2996.75 | 850.891 | -1.81635 | -1.37141 | 0.17895 |
| CUFF.34 | CUFF.34 | - | chr16:30234372-30235341 | C1 | C2 | OK | 3511.86 | 0 | -inf | -nan | 0.00125 |
| CUFF.35 | CUFF.35 | - | chr16:30235457-30237120 | C1 | C2 | OK | 2936.07 | 246.171 | -3.57615 | -0.0998629 | 0.27625 |
| CUFF.36 | CUFF.36 | - | chr16:51680166-51680519 | C1 | C2 | OK | 17105.6 | 27534.1 | 0.686749 | 0.622949 | 0.5407 |
| CUFF.37 | CUFF.37 | - | chr17:18965231-18965486 | C1 | C2 | OK | 127516 | 89551.2 | -0.50989 | -0.370282 | 0.71965 |
| CUFF.38 | CUFF.38 | - | chr17:18967179-18967437 | C1 | C2 | OK | 60815.1 | 38804.1 | -0.648222 | -0.536013 | 0.5706 |
| CUFF.39 | CUFF.39 | - | chr17:19015668-19015938 | C1 | C2 | OK | 94638.2 | 71390 | -0.4067 | -0.299543 | 0.7727 |
| CUFF.4 | CUFF.4 | - | chr11:61732071-61732368 | C1 | C2 | OK | 28564.7 | 18460 | -0.629832 | -0.367058 | 0.65485 |
| CUFF.40 | CUFF.40 | - | chr17:19091317-19091593 | C1 | C2 | OK | 2.28429e+06 | 1.58952e+06 | -0.523156 | -0.530726 | 0.5975 |
| CUFF.41 | CUFF.41 | - | chr17:43591221-43592815 | C1 | C2 | NOTEST | 2349.71 | 775.744 | -1.59883 | 0 | 1 |
| CUFF.42 | CUFF.42 | - | chr17:43595213-43595732 | C1 | C2 | OK | 8195.18 | 1205.06 | -2.76567 | -0.0772472 | 0.2652 |
| CUFF.43 | CUFF.43 | - | chr17:43595898-43596821 | C1 | C2 | OK | 2879.33 | 0 | -inf | -nan | 0.0057 |
| CUFF.44 | CUFF.44 | - | chr17:78318471-78319080 | C1 | C2 | OK | 9415.36 | 922.399 | -3.35155 | -0.0936056 | 0.2514 |
| CUFF.45 | CUFF.45 | - | chr1:28833875-28834105 | C1 | C2 | OK | 264558 | 270134 | 0.0300931 | 0.0260127 | 0.97805 |

# Cuffdiff Output :

- ✓**Count genes showing log2 => 2 , known as unregulated genes** (increase in expression of a gene in Condition A as compared to B).
- ✓**Count genes showing log2 < 2 , known as down regulated genes** (Decrease in expression of a gene in Condition A as compared to B).

**By looking into figure, can you tell**
**What are unregulated genes (colour)?**
**What are down regulated (colour)?**



# Questions ?

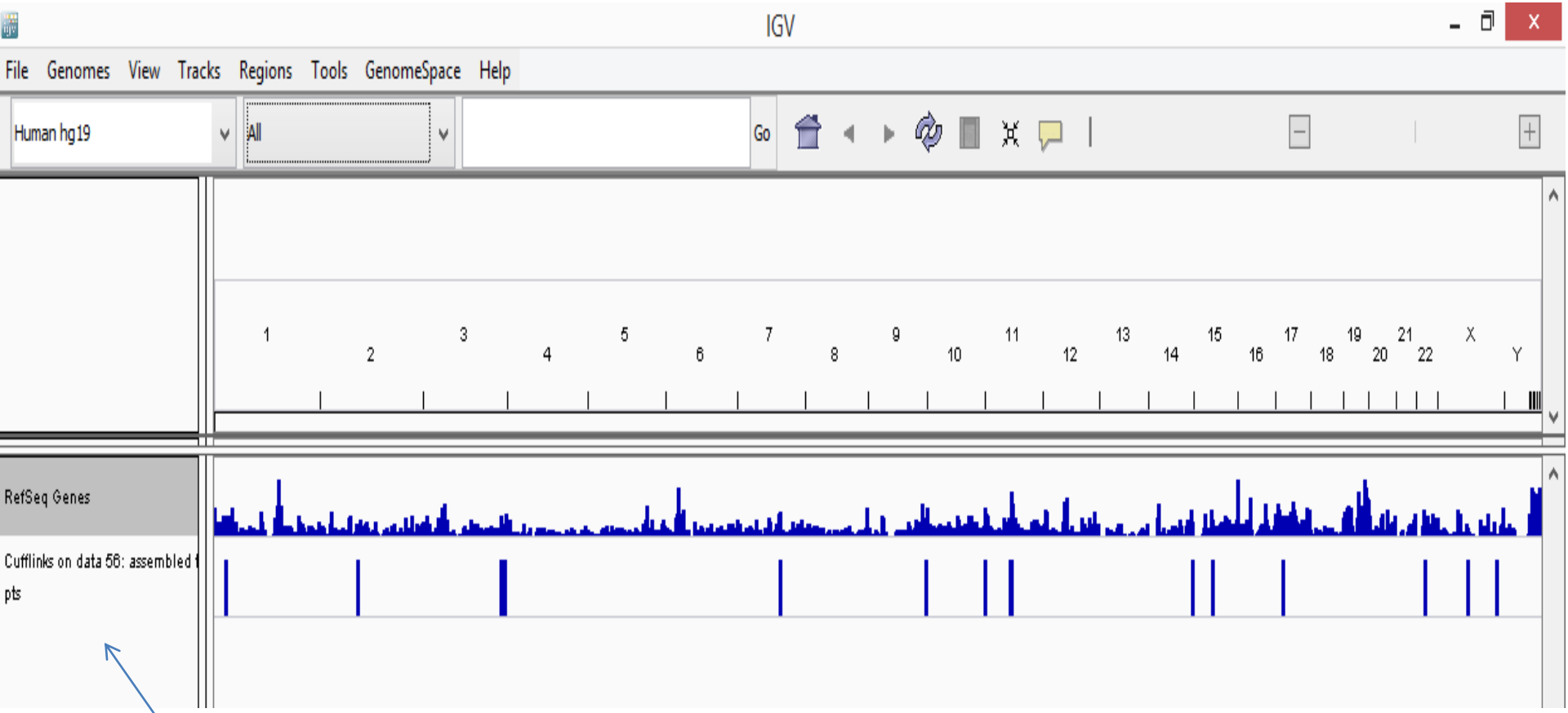
# How does mapping look on reference genome

- ✓In previous practical session, you used the association viewer tool to get idea about how well a SNP associated with a locus.

- ✓Let us visualise the mapping using IGB/IGV tool. Here you will see how well a particular locus expressing itself using RNASEQ DATA.

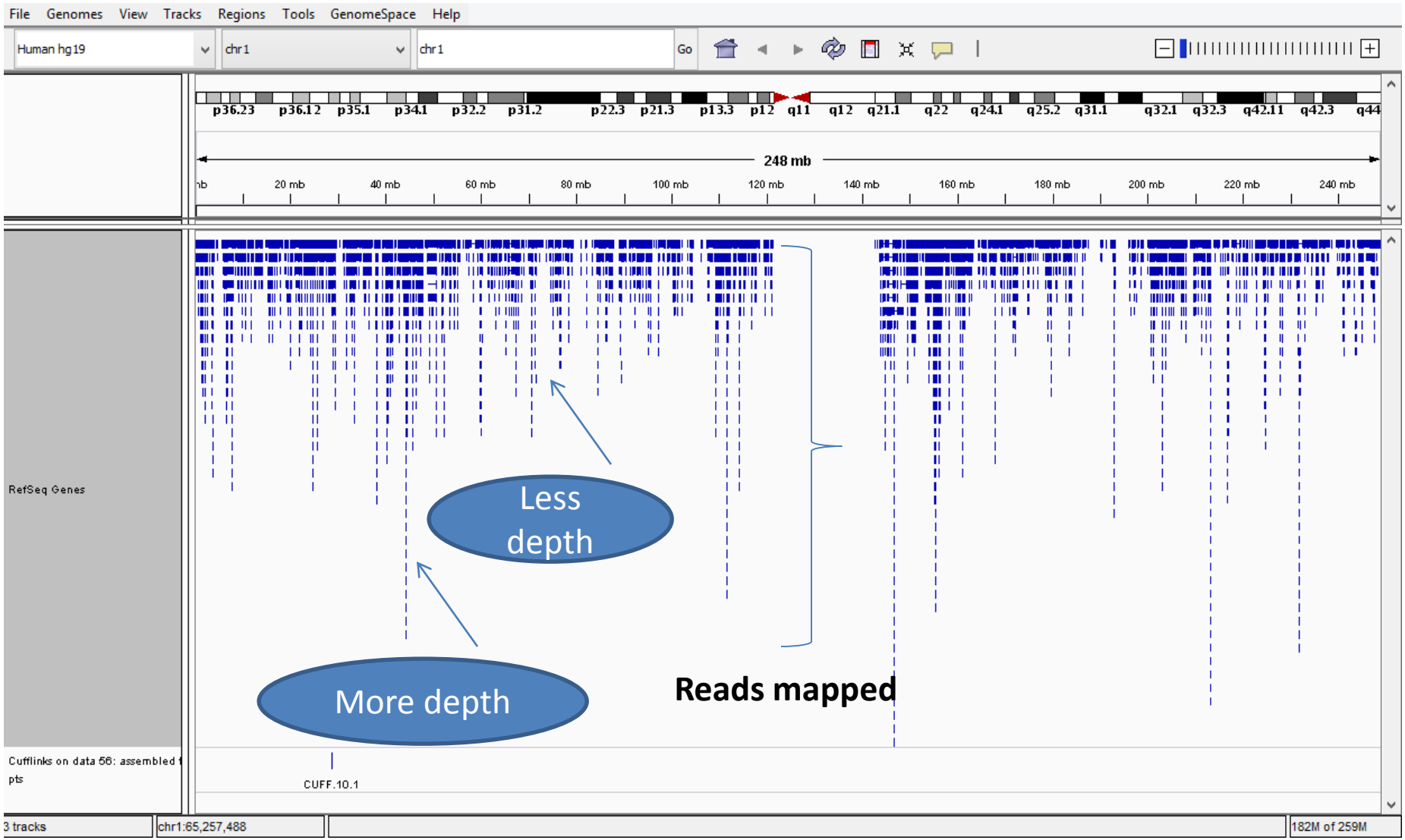
- ✓Download IGV/IGV java application and display assembled transcripts data.

# IGV : All chromosomal




Cufflink output

# IGV : Chromosome 1



# Questions ?