Introduction to Galaxy and preprocessing of sequences

Bioinformatics Course UEB-VHIR
November 2020

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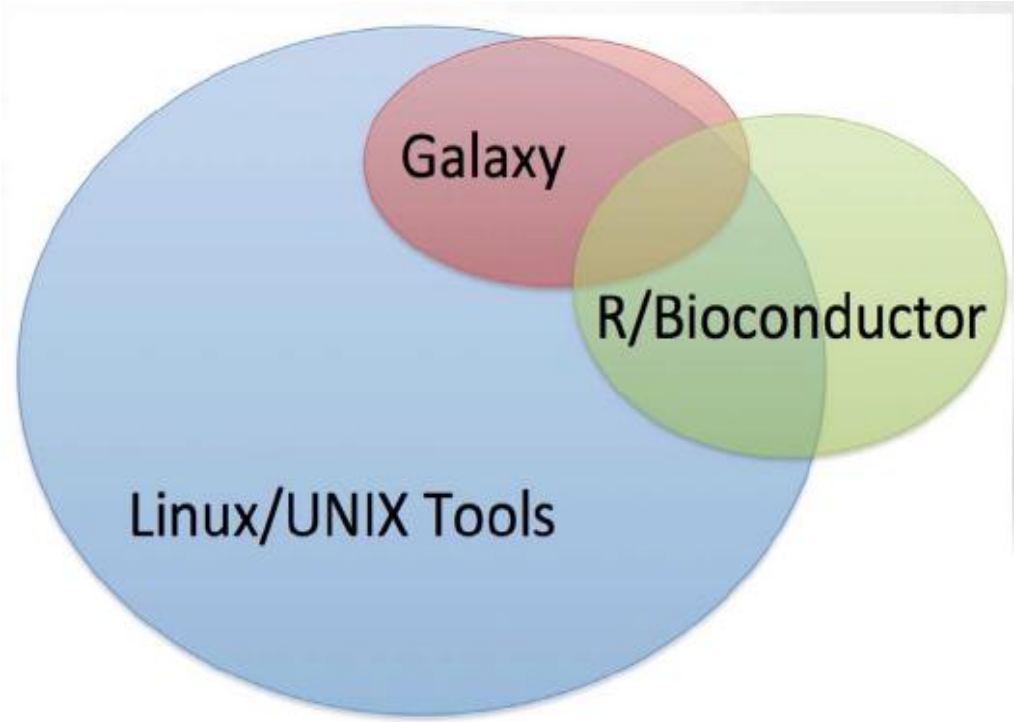
Highly efficient and fast processing tools are required to handle large volume of datasets



Introduction to Galaxy

Introduction to Galaxy

Galaxy Project
https://galaxyproject.eu/

- An open, web-based platform integrating many popular tools and resources for intensive biomedical research.
- **What can be done?**
  - Obtain data from many data sources like UCSC Table Browser, Biomart, WormBase, or your own data
  - Prepare data for further analysis by rearranging or cutting data columns, filtering data and many other options
  - Analyze data by finding overlapping regions, determining statistics, preprocessing NGS data and much more
  - Share data and workflows

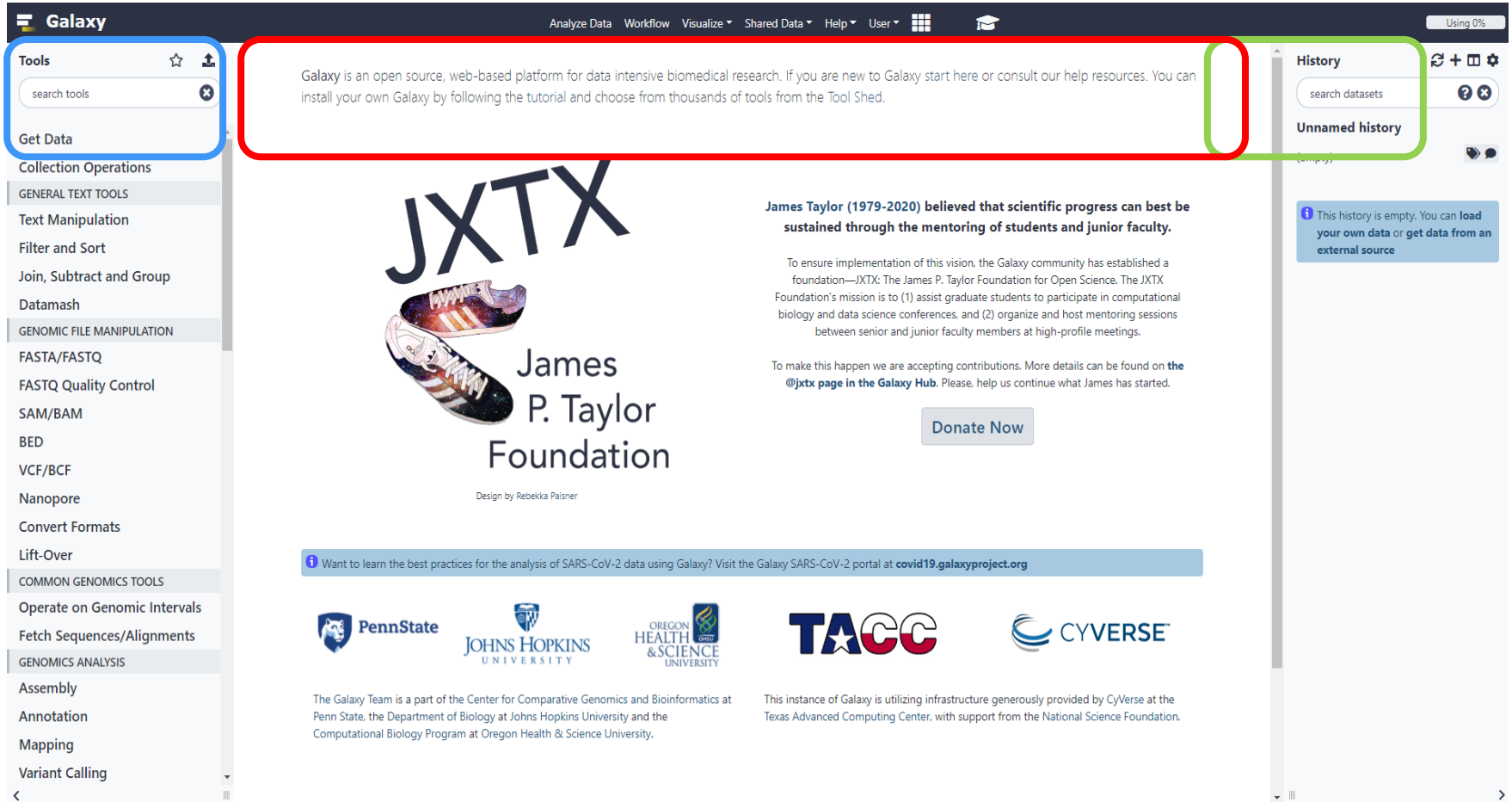
Introduction to Galaxy

- The Galaxy page is divided into three panels:

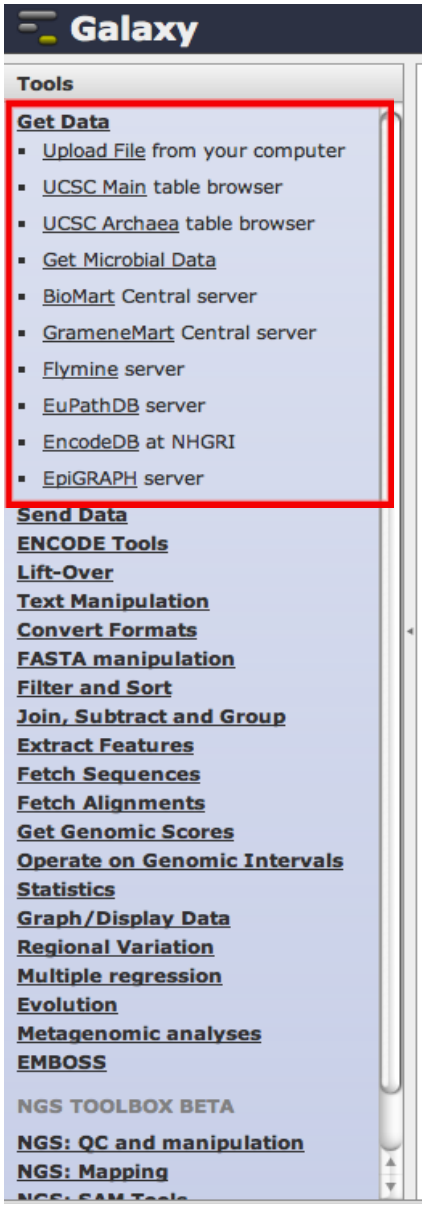
Tools for uploading, processing and analysis

Viewing panel (menus, data, results)

History of analysis steps and datasets



Introduction to Galaxy



Tools for data analysis

Get Data
- From databases (UCSC Table Browser, ...)
- From uploaded files
- From urls

Text manipulation

Filter and Sort

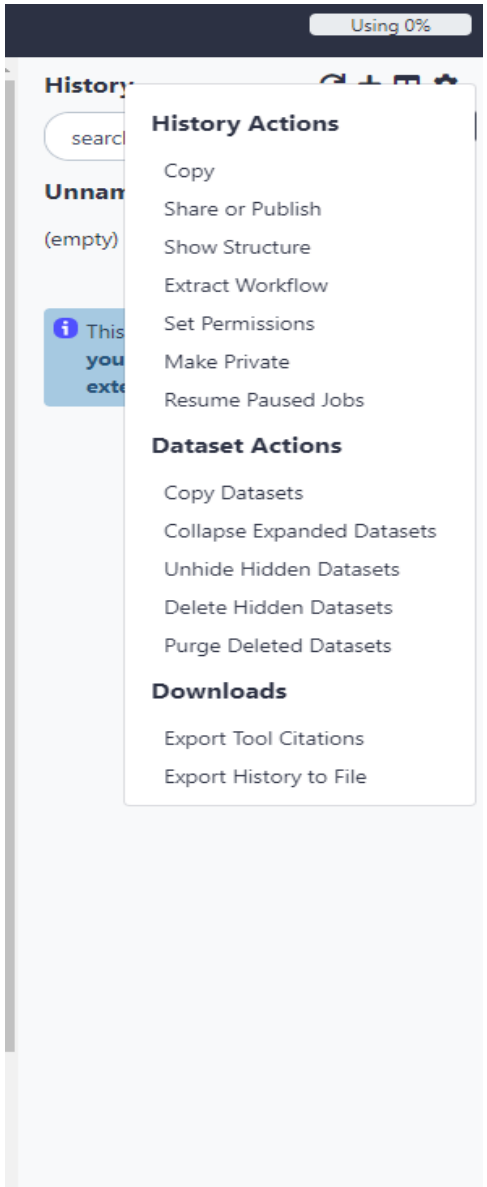
Operate on Genomic Intervals

FASTA manipulation

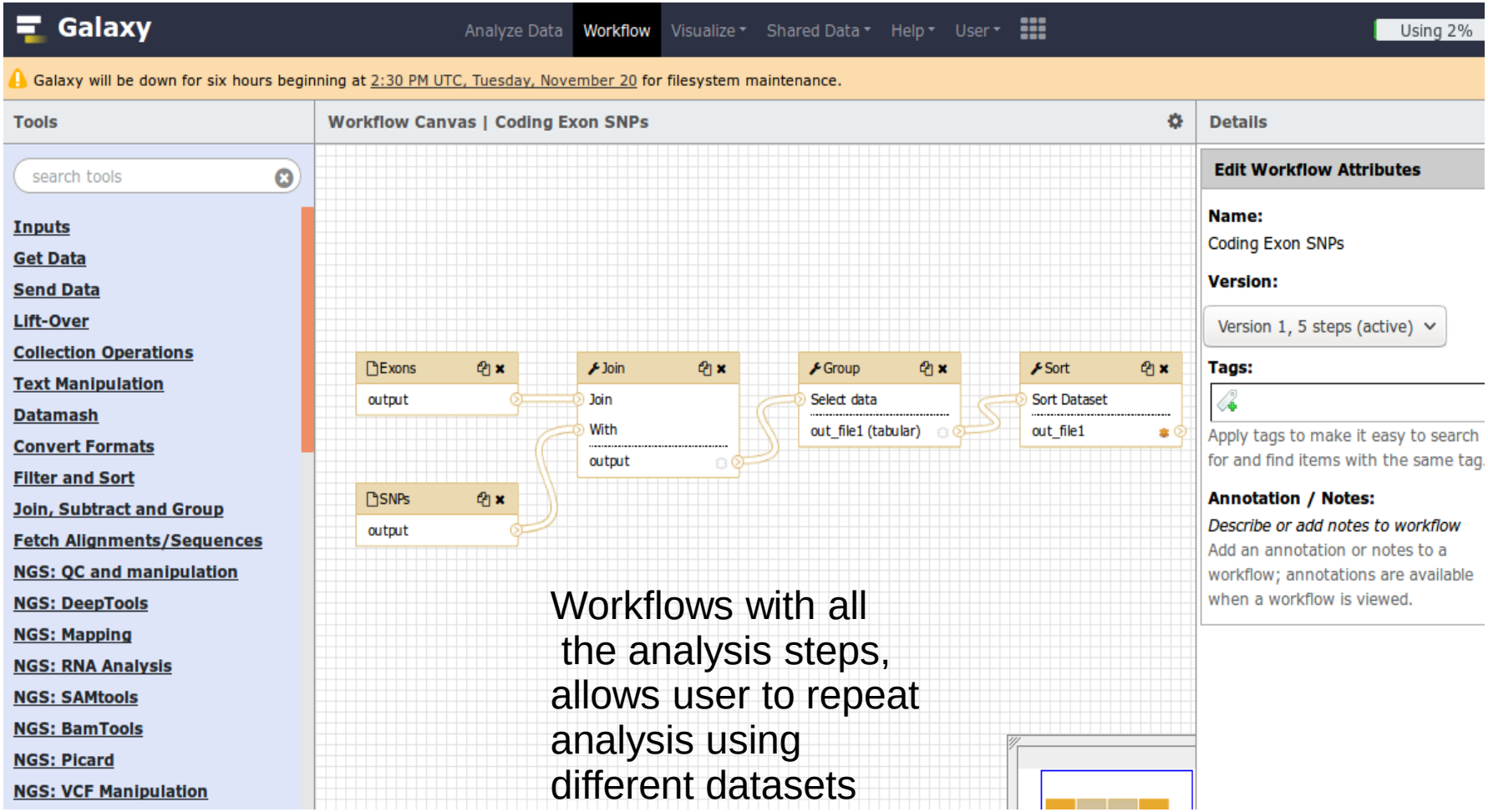
NGS analysis
- QC
- Fastq file pre-processing
- Read Alignment / Mapping
- SAM tools

Histories

List saved histories and shared histories.
Work on Current History, create new, clone, share, create workflow, set permissions, show deleted
datasets or delete history.



Workflows



Workflows with all the analysis steps, allows user to repeat analysis using different datasets

Introduction to Galaxy

Register for a Galaxy account

This will only take a moment, and will allow all the work that you do to persist between sessions and allow you to name, save, share, and publish Galaxy histories, workflows, datasets and pages.

It allows you to store up to 250GB of data on this public server.

https://usegalaxy.eu/

Introduction to Galaxy

Training Infrastructure as a Service

We want to help you conduct your training seminars. You provide the training, we provide you training infrastructure *at no cost*.

Why use UseGalaxy.eu training infrastructure?

- Free
- Private queue, no wait times
- No Galaxy Maintenance
- No Galaxy Administration
- Official Galaxy Training Materials guaranteed to work

TIaaS

Simply fill out the infrastructure request form and we'll get back to you shortly.

Find out more

After registration in European Galaxy server

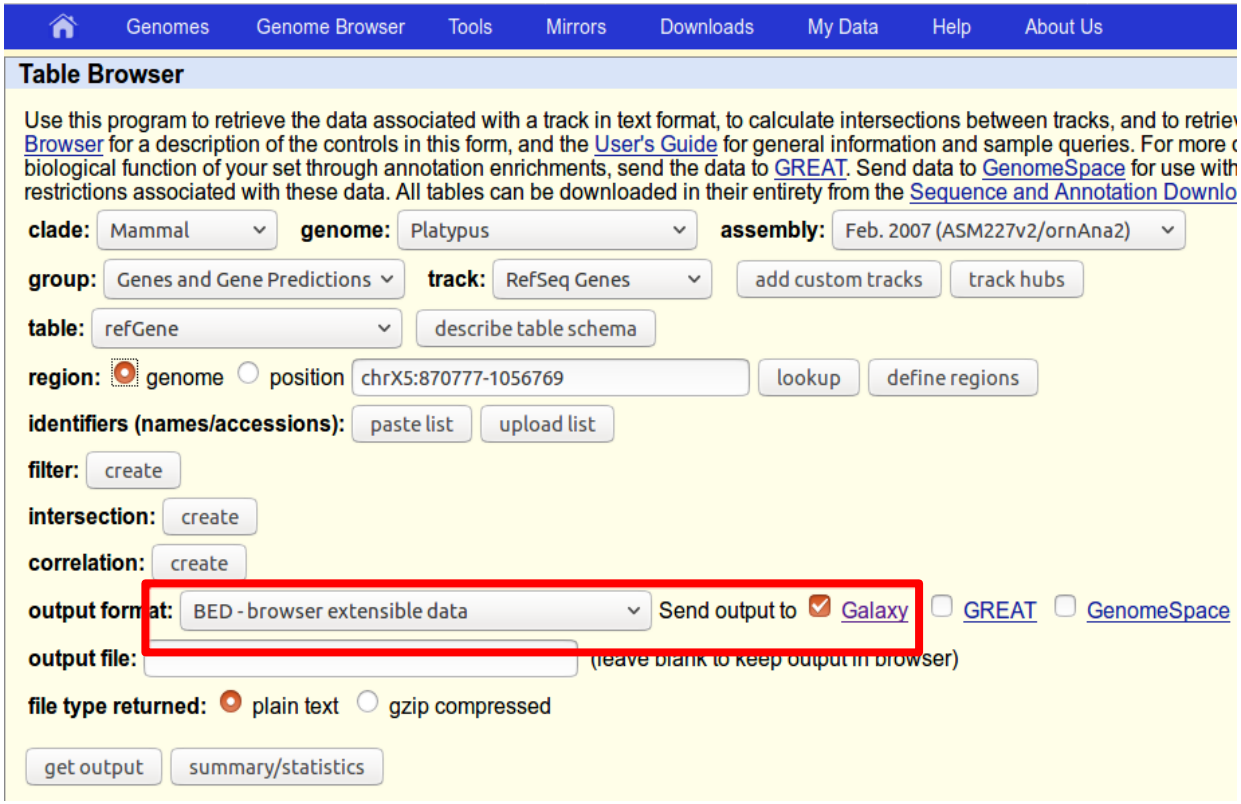
https://usegalaxy.eu/join-training/ueb_bi2020

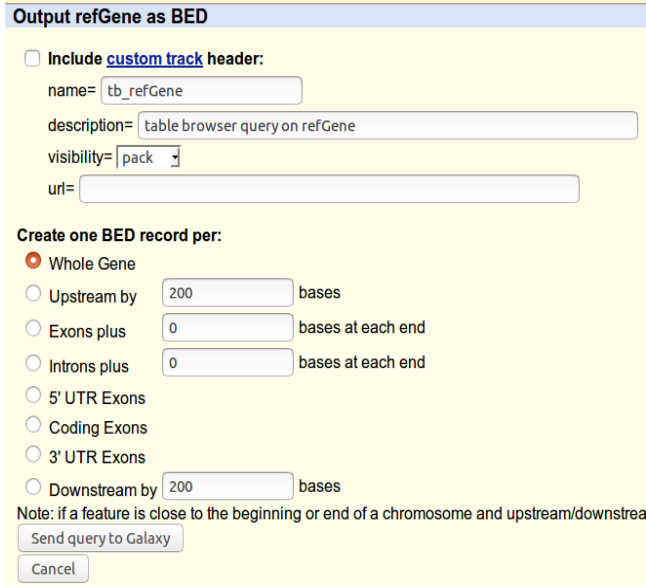
Importing data into Galaxy

1. From **database** queries: eg. obtain a BED-formatted dataset of all RefSeq genes from platypus using the UCSC Table Browser.

Get Data > UCSC Main – Table Browser tool

- Set genome, RefSeg Genes, and BED output format (send to Galaxy)

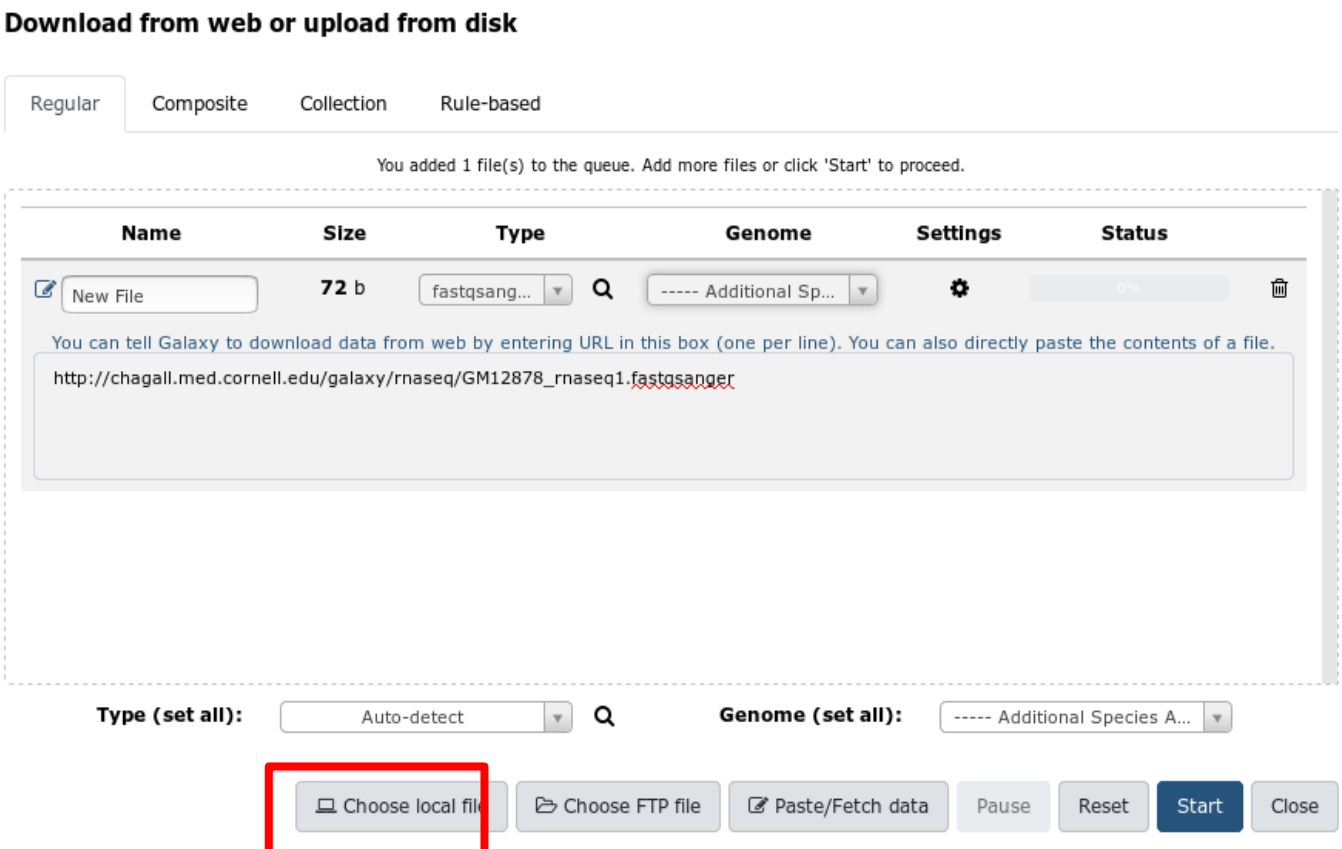




Importing data into Galaxy

2. From a **File** on your computer / FTP file:

Get Data > Upload File

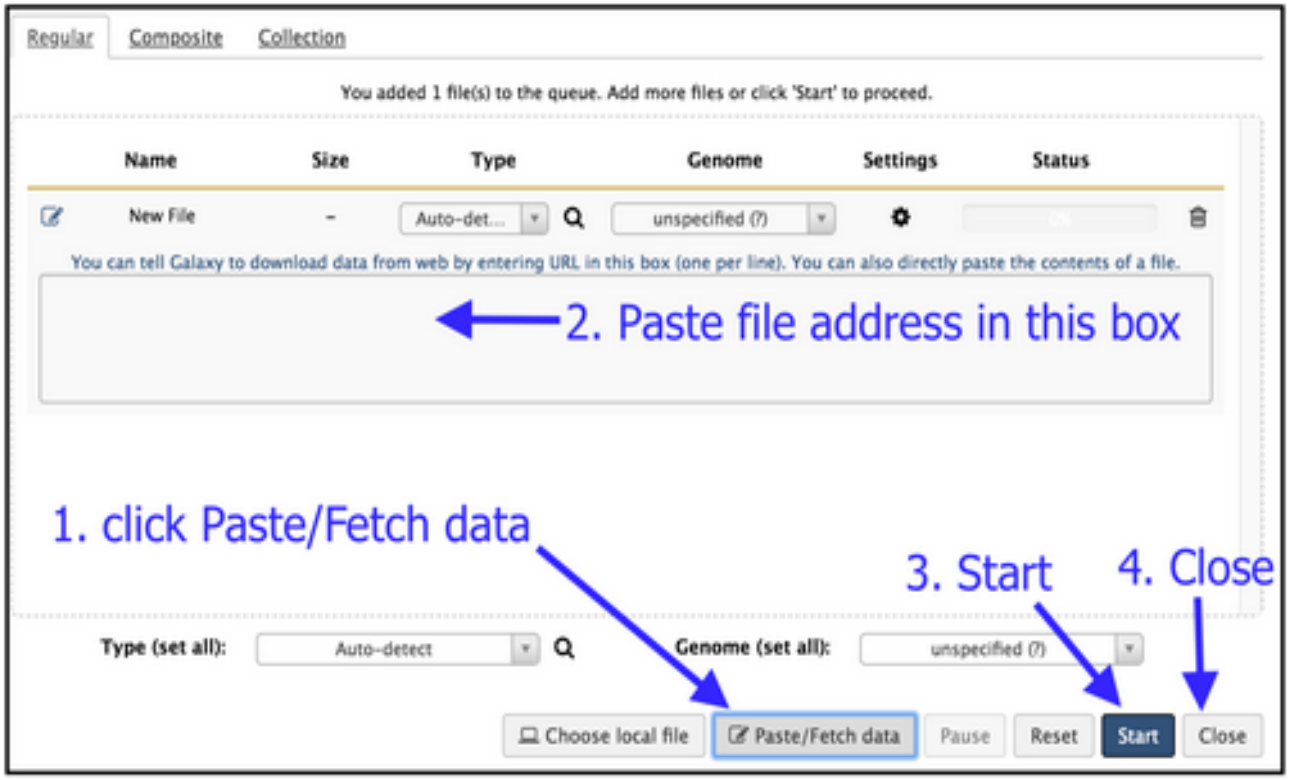


Importing data into Galaxy

3. From **a website**:

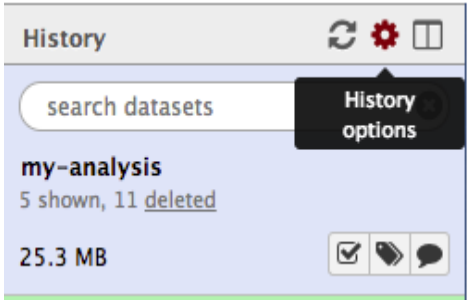
Get Data > Upload File

- Copy this URL into the text-entry box:
  https://zenodo.org/record/582600/files/mutant_R1.fastq

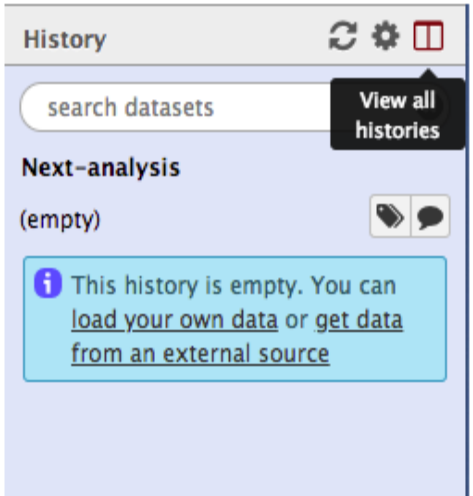


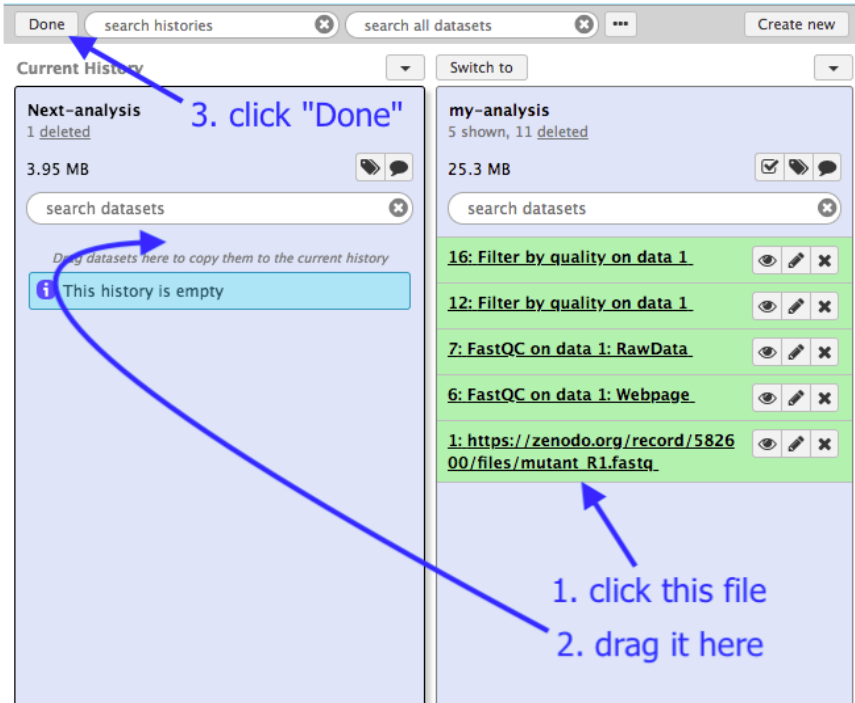
Managing histories

- Name your current history
- Create new history and rename it



- Manage datasets and histories:
- View all histories
- Drag files between histories (new history must be set to current)



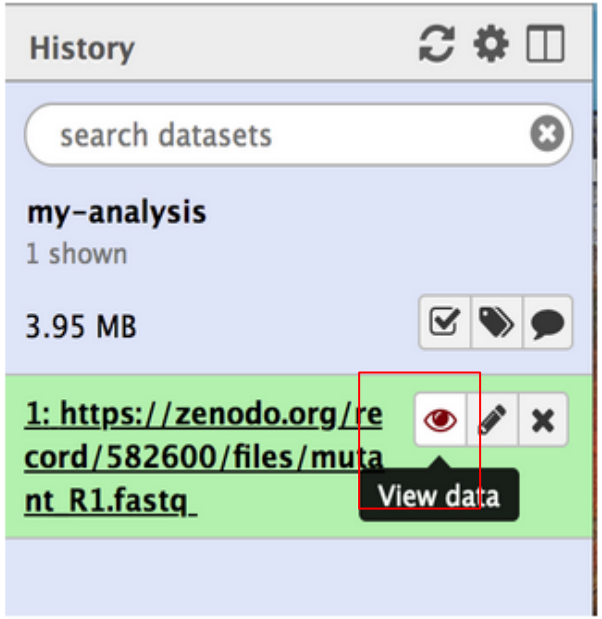


Visualizing the dataset

- You can view file content clicking the eye icon in history.

The mutant_R1.fastq file contains DNA sequencing reads from a bacteria, in FASTQ format:

```
@mutant-no_snps.gff-24960/1                                read 1 sequence
AATGTTGTCACTTGGATTCAAATGACATTTTAAATCTAATTATTCATGAATCGAACTAGTACGAAATGCAATGAG
+                                                          read 1 quality scores
5??A9?BBBDDDBEDDBFF+FGHHIIHHHEIHIIHIIAHDHIIHIG#IIHIFHHHFGIII*IHHHIHFIIHGICI
@mutant-no_snps.gff-24958/1
CAAAGTCGTTGGTCATATAAAAAACCGCGTACAGTCAACTATAGATACAATCAAGATAAACTCATGCACAGATTG
+
?A????@?DDDABDE9FGGGFGICFHIIIBGHIIIGICHHIFH=IHAFIHIHHHHIFCIIEIHAIFGIHIDDIHE
@mutant-no_snps.gff-24956/1
TATAAATTCAACTTTGCAACAGAACCATCTAATCTTCAACAAACTGGCCCGTTTGTTGAACTACTCTTTAATAAA
+
?????BBADD5DDDDDGFGCFFEECFBBICIII,IIHIICHIHIIFHHHHHIHIIIIIIIAHHIHHH5FHDHHHH
```



Create workflow from history

- From history options: Export workflow

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

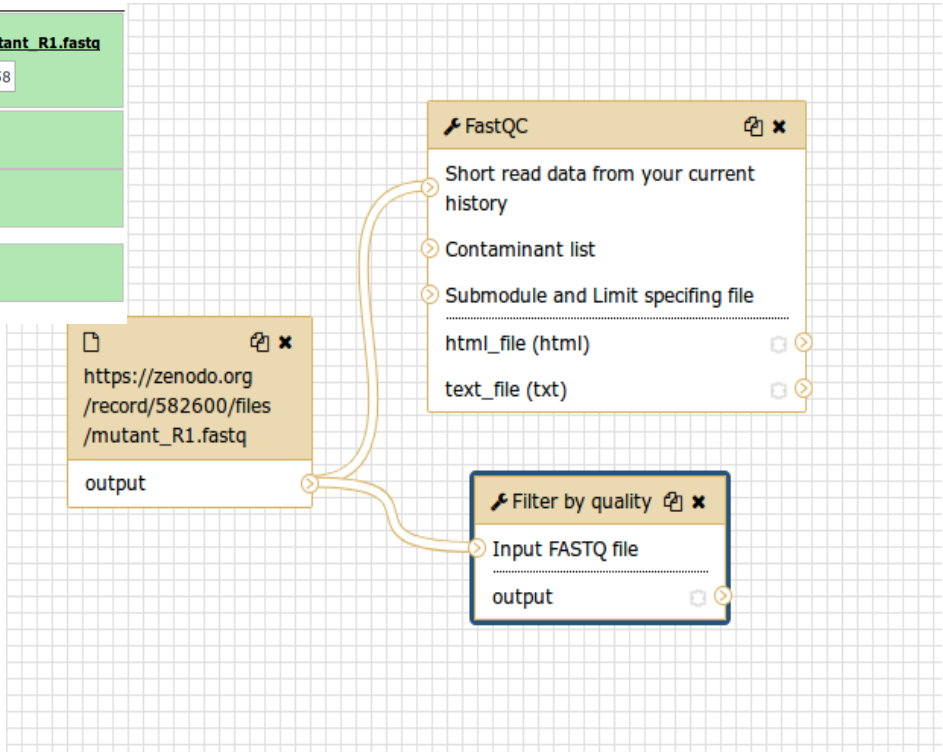
Tools which cannot be run interactively and thus cannot be incorporated into a workflow will be shown in gray.

Workflow name

Workflow constructed from history 'prova'

Create Workflow | Check all | Uncheck all

Tool	History items created
Upload File *This tool cannot be used in workflows*	1 https://zenodo.org/record/582600/files/mutant_R1.fastq Treat as input dataset https://zenodo.org/record/58
FastQC Include "FastQC" in workflow	2 FastQC on data 1: Webpage 3 FastQC on data 1: RawData
Filter by quality Include "Filter by quality" in workflow	4 Filter by quality on data 1



Introduction to Galaxy

- https://galaxyproject.org/learn/

Learn Galaxy

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.

Have you created or know of a resource that is useful for teaching with Galaxy? Then please share it! This will help others and also help get the word out about your resource. Use this Google form to describe your resource. **Also**: consider joining Galaxy Training Network and contributing your tutorial as described here!

Tutorials by Galaxy Training Network

Thanks to a large group of wonderful contributors there is a constantly growing set of tutorials maintained by the Galaxy Training Network. These include:

Introductory Tutorials

- Introduction to Galaxy Analyses
- Data Manipulation
- User Interface and Features

Scientific Analyses

- Assembly
- Computational chemistry
- Ecology
- Epigenetics
- Genome Annotation
- Imaging
- Metabolomics
- Metagenomics
- Proteomics
- Sequence analy
- Statistics and m
- Transcriptomics
- Variant Analysis

Material

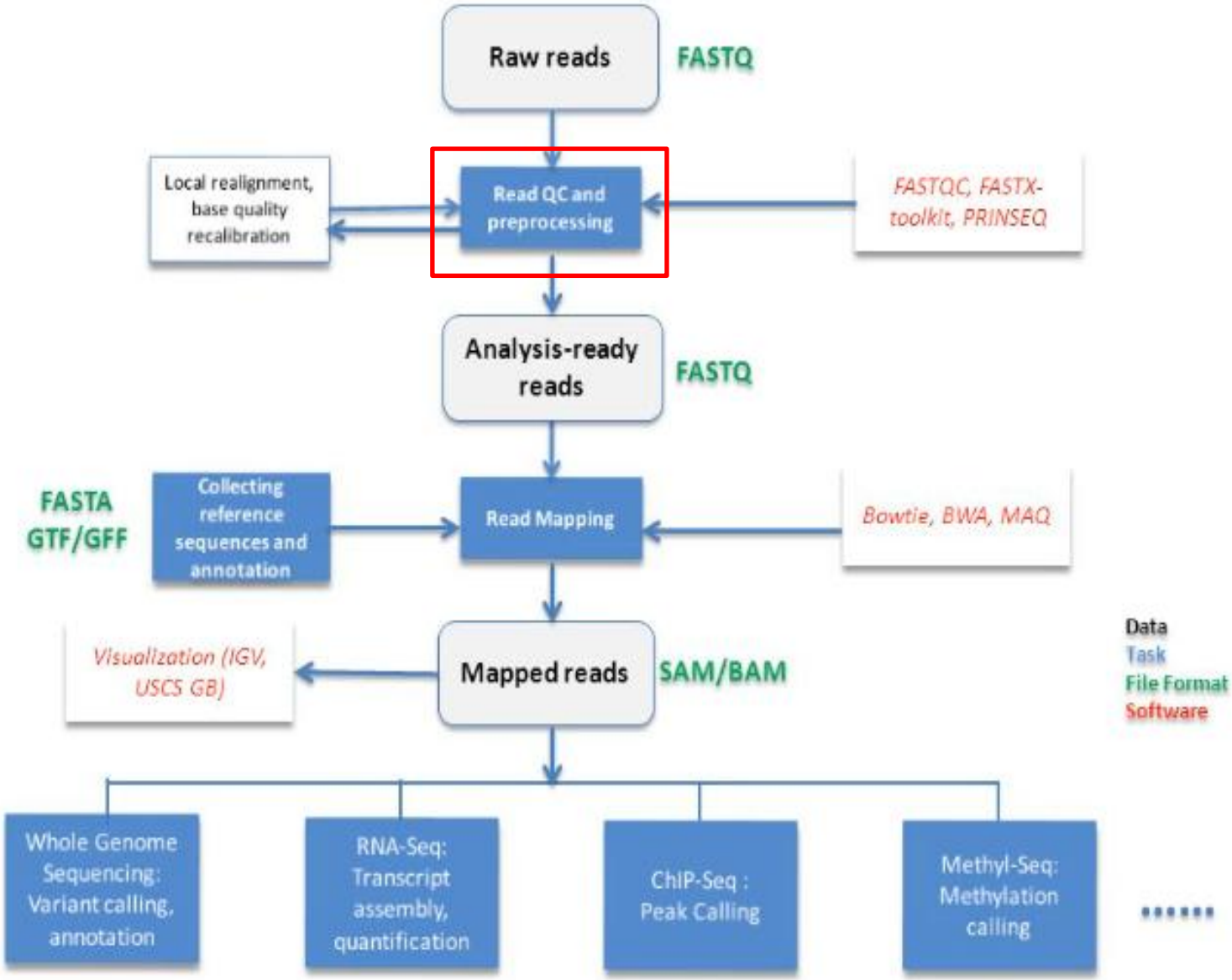
Search

Lesson	Slides	Hands-on	Input dataset	Workflows	Galaxy tour	Galaxy instances
Introduction to metagenomics						
16S Microbial Analysis with mothur (extended)						
16S Microbial Analysis with mothur (short)						
Analyses of metagenomics data - The global picture						
Antibiotic resistance detection nanopore plasmids						
Metatranscriptomics analysis using microbiome RNA-seq data metatranscriptomics						
Metatranscriptomics analysis using microbiome RNA-seq data (short) metatranscriptomics						

First steps in NGS analysis with Galaxy:

Quality Control and preprocessing of reads

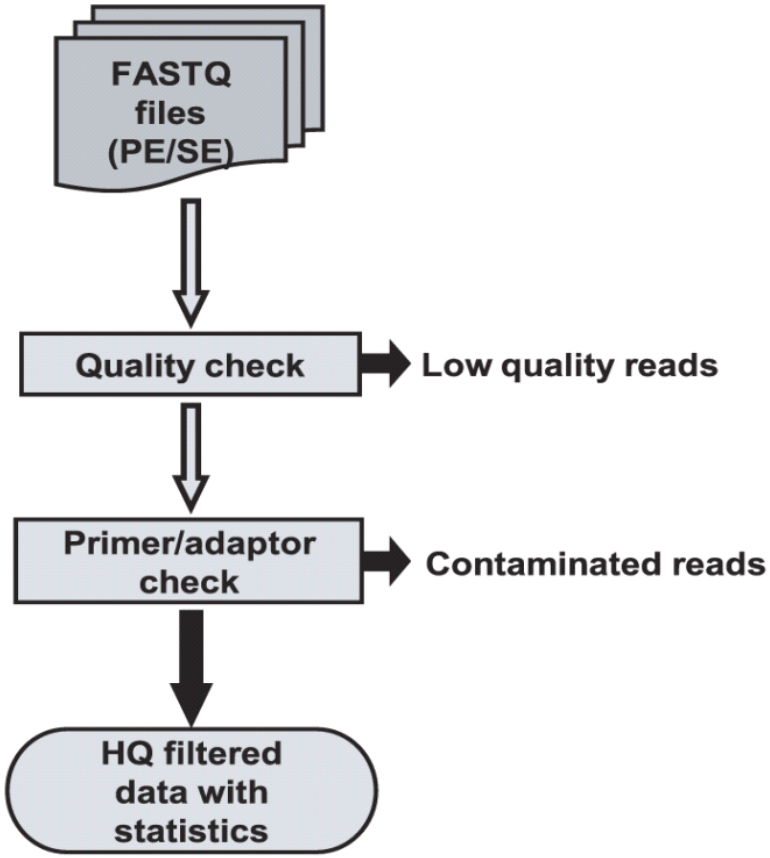
Steps in NGS analysis



Steps in NGS analysis

Quality Control

- Quality Control analysis of sequence data is extremely important for meaningful downstream analysis
  - To analyze problems in quality scores/ statistics of sequencing data
  - To check whether further analysis with sequence is possible
  - To remove redundancy (filtering)
  - To remove low quality reads from analysis
  - To remove adapter contamination



Highly efficient and fast processing tools are required to handle large volume of datasets

Quality Control

FastQC tool

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

- Basic statistics
- Quality- Per base position
- Per Sequence Quality Distribution
- Nucleotide content per position
- Per sequence GC distribution
- Per base GC distribution
- Per base N content
- Length Distribution
- Overrepresented/ duplicated sequences
- K-mer content

Quality Control

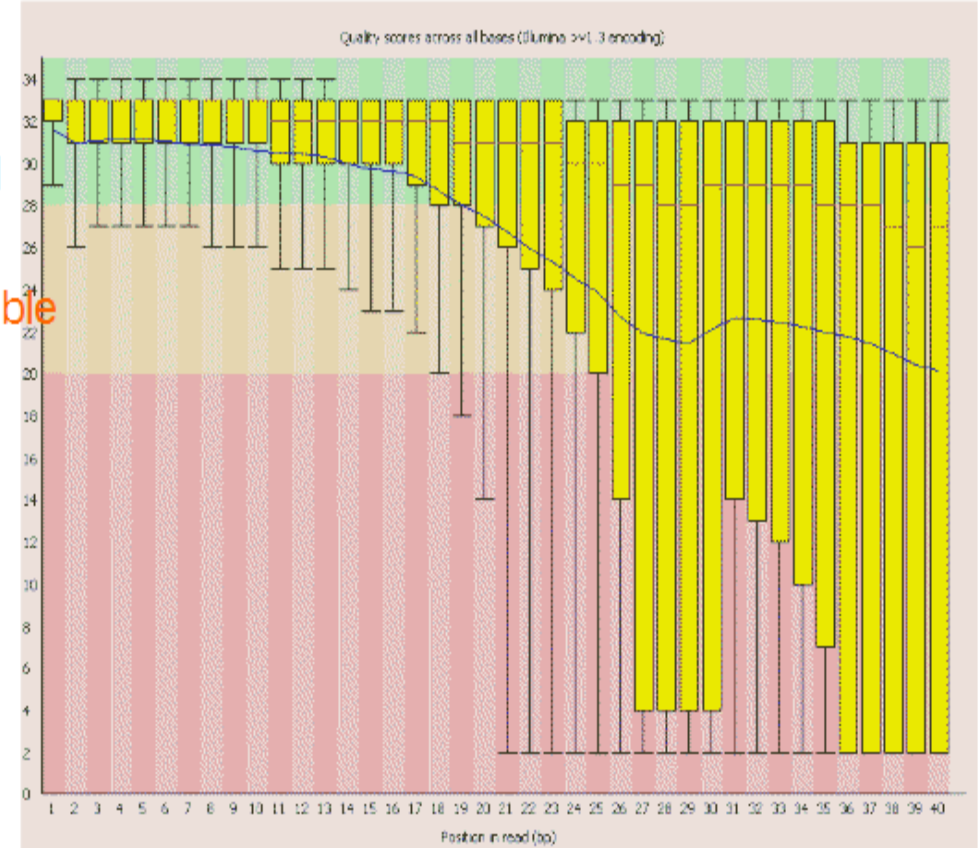
FastQC

Per base sequence quality (Boxplot)

Good

Acceptable

Bad



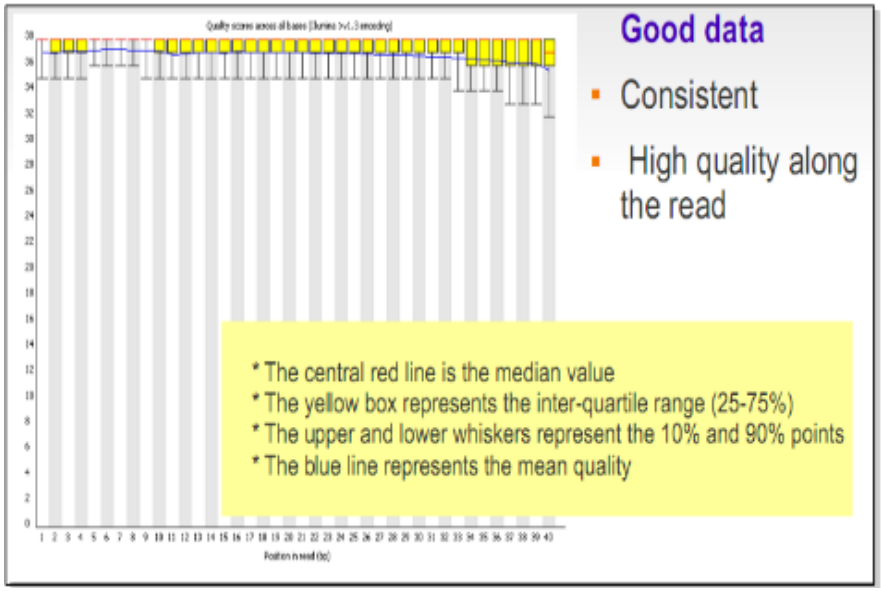
shows an overview of the range of quality values across all bases at each position in the FastQ file

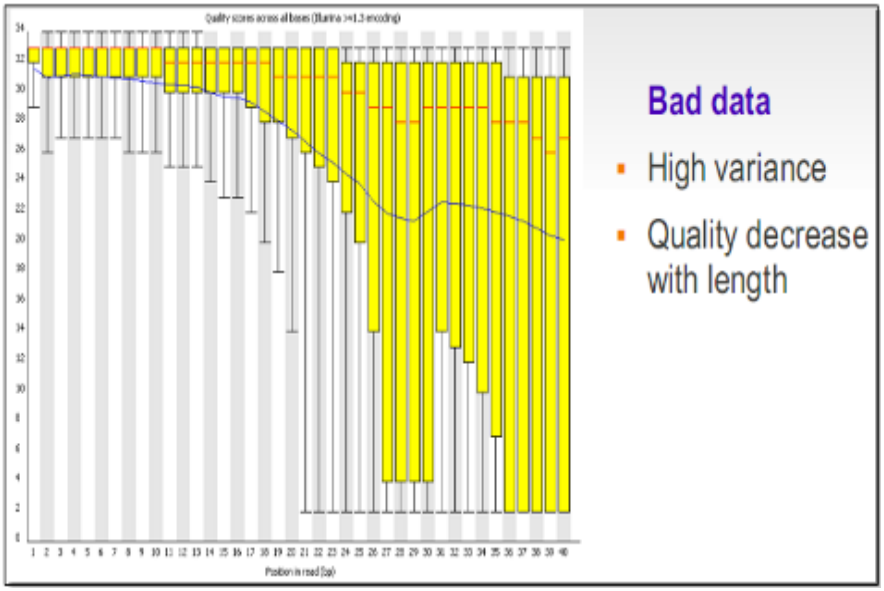
Y axis- Quality Score
X axis- Base position

Quality Control

FastQC

Per base sequence quality (Boxplot)



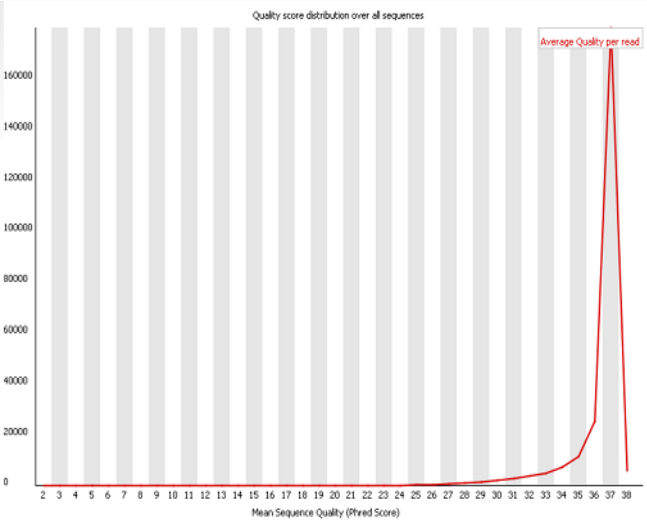


Quality Control

FastQC

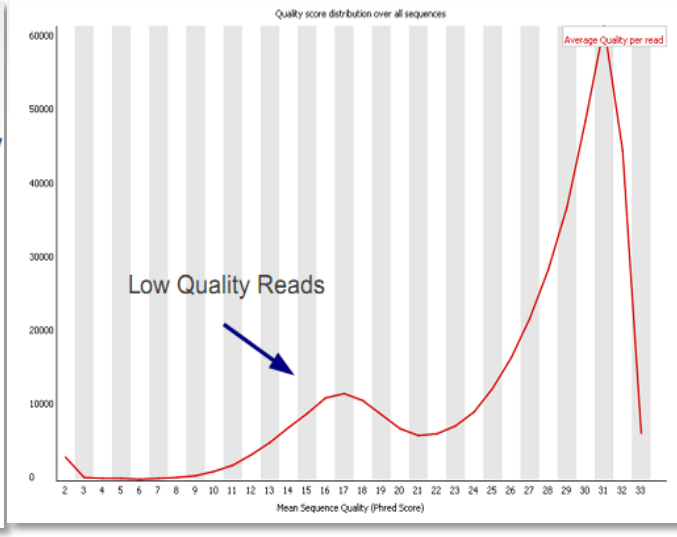
Per sequence quality scores

allows you to see if a subset of your sequences have universally low quality values.



Good data

- Most are high-quality sequences



Bad data

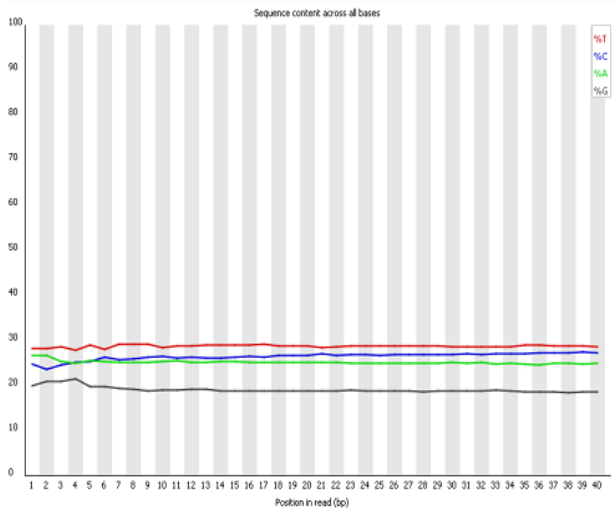
- Not uniform distribution

Quality Control

FastQC

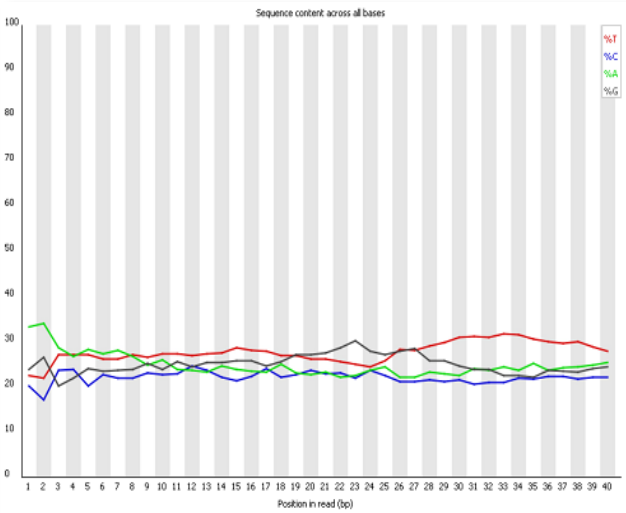
Per base sequence content

proportion of each base position in a file for which each of the four normal DNA bases has been called



Good data

- Smooth over length
- Organism dependent (GC)



Bad data

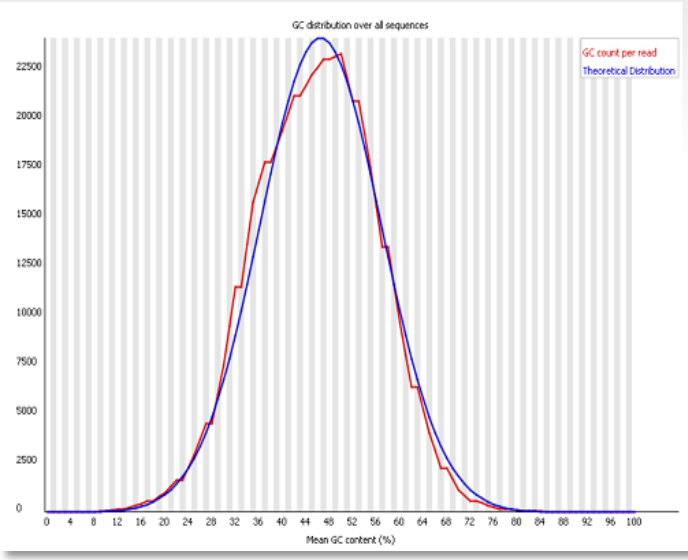
- Sequence position bias

Quality Control

FastQC

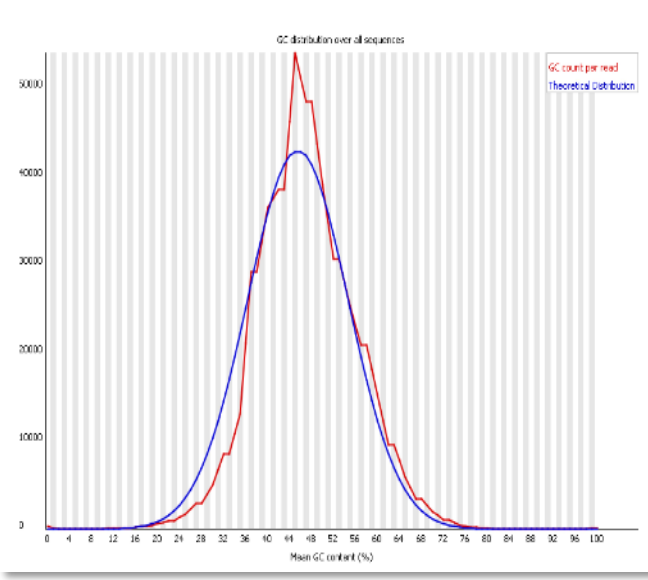
Per sequence GC content

measures the GC content across the whole length of each sequence in a file and compares it to a modelled normal distribution of GC content



Good data

- Fits with the expected
- Organism dependent



Bad data

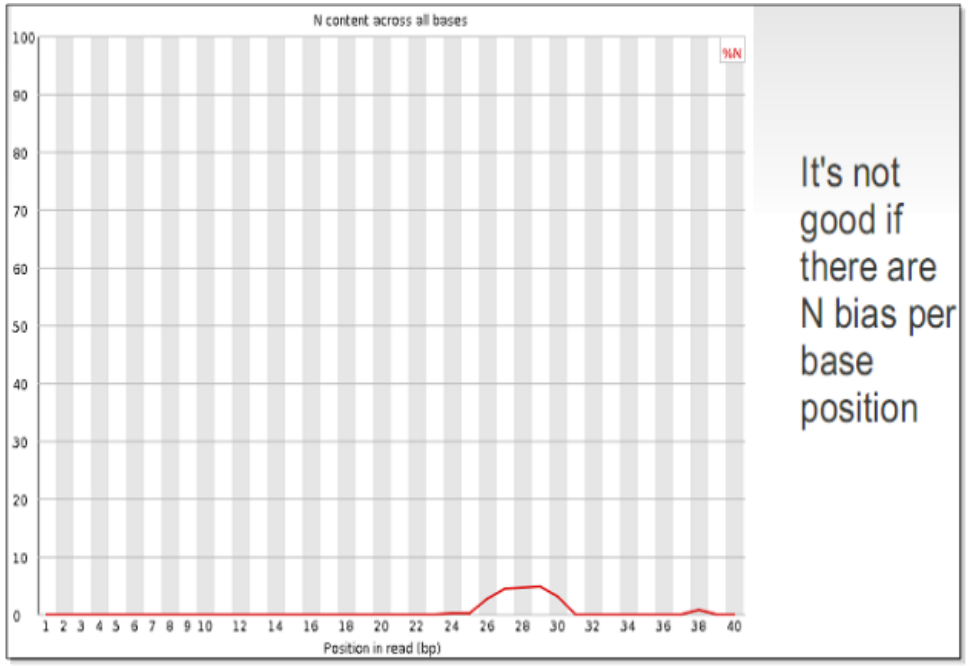
- It does not fit with expected
- Organism dependent

  Library contamination?

Quality Control

FastQC

Per base N content

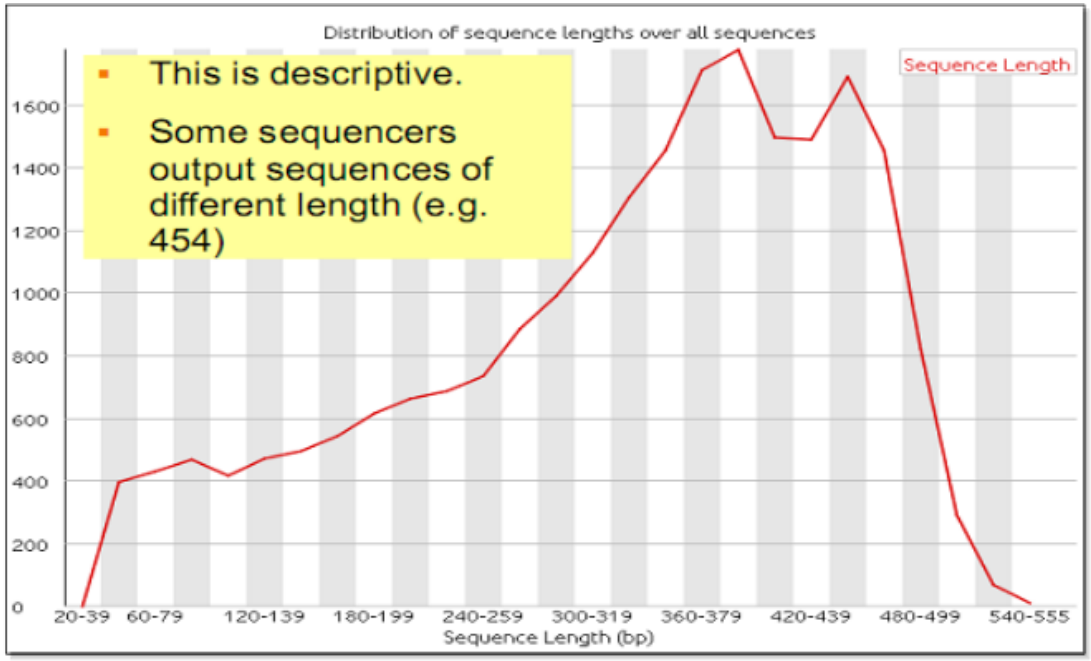


If a sequencer is unable to make a base call with sufficient confidence then it will normally substitute an N rather than a conventional base call. It plots out the percentage of base calls at each position for which an N was called.

Quality Control

FastQC

Sequence length distribution

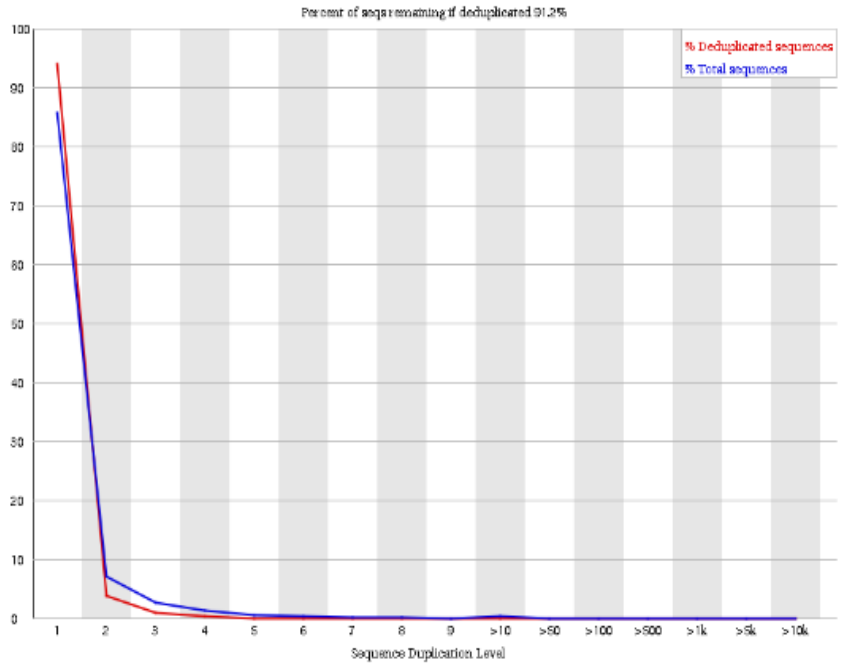


In many cases it will produce a simple graph showing a peak only at one size, but for variable length FASTQ files, it will show the relative amounts of each different size of sequence fragment.

Quality Control

FastQC

Sequence duplication level



Counts the degree of duplication for every sequence. Too many duplicate regions in the sequence may indicate contamination or technical problems

Quality Control

FastQC

Overrepresented sequences

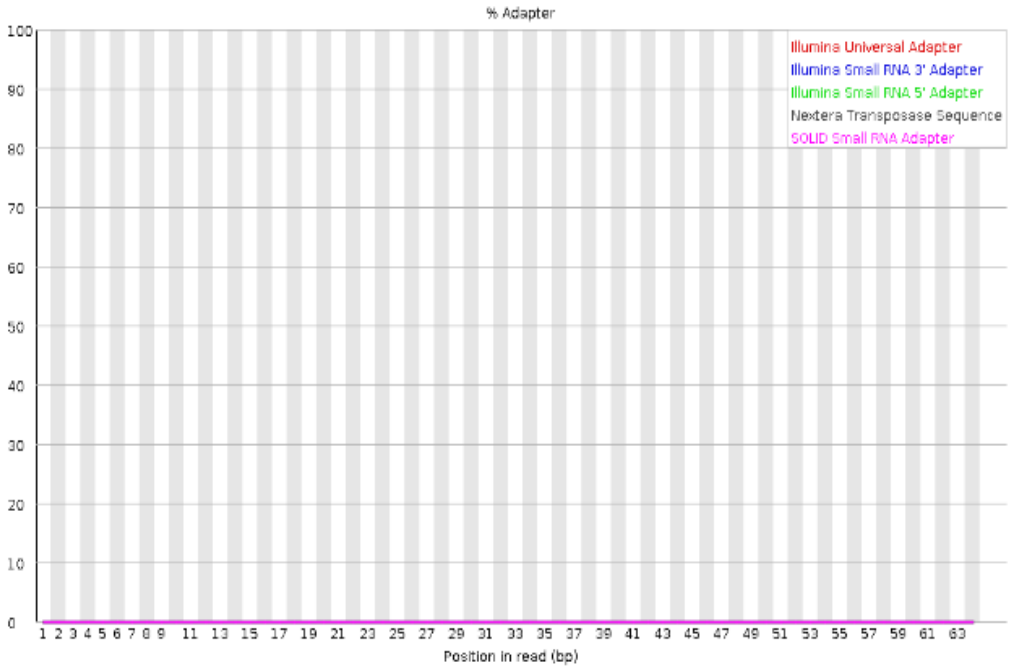
Sequence	Count	Percentage	Possible Source
AAGATCCGAGTCGTCCGGAAATCCATTGCCCGTGTTCTCACAGTTATTAA	432	0.43585733743631133	No Hit
AGATCCGAGTCGTCCGGAAATCCATTGCCCGTGTTCTCACAGTTATTAAC	335	0.33799122231750994	No Hit
TGGCAGAAGTAGAGCAGAAGAAGAAGCGGACCTTCCGCAAGTTCACCTAC	250	0.25223225546082834	No Hit
CAGAAGTAGAGCAGAAGAAGAAGCGGACCTTCCGCAAGTTCACCTACCGC	237	0.23911617817686526	No Hit
GTAGAGCAGAAGAAGAAGCGGACCTTCCGCAAGTTCACCTACCGCGGCGT	223	0.22499117187105888	No Hit
AAGAAATCTGACCCGGTCGTCTCGTACCGCGAGACGGTCAGTGAAGAGTC	204	0.2058215204560359	No Hit
AAGTAGAGCAGAAGAAGAAGCGGACCTTCCGCAAGTTCACCTACCGCGGC	151	0.1523482822983403	No Hit
CACCTGGAGATCTGCCTGAAGGACCTGGAGGAGGACCACGCCTGCATCCC	147	0.14831256621096706	No Hit
TCTGCCTGAAGGACCTGGAGGAGGACCACGCCTGCATCCCCATCAAGAAA	146	0.14730363718912376	No Hit

Lists all of the sequence which make up more than 0.1% of the total. Finding that a single sequence is very overrepresented in the set either means that is highly biologically significant, or that the library is contaminated. For each overrepresented sequence it will look for matches in a database of common contaminants.

Quality Control

FastQC

Adapter content



Does a generic analysis of all the Kmers in the library to find those that don't have even coverage through the length of the reads.

Quality Control

FastQC

•Good (Illumina) quality data:

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/good_sequence_short_fastqc.html

•Bad (Illumina) quality data:

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/bad_sequence_fastqc.html

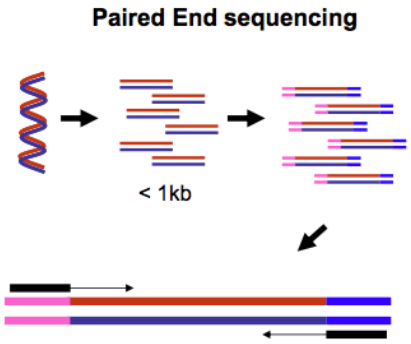
Quality Control

Your turn!

- We will analyze exome sequencing data from a study that aimed to identify genetic variants associated to a disease. The data comes from paired-end sequencing, each file corresponding to the forward or reverse, respectively:

https://zenodo.org/record/3243160/files/proband_R1.fq.gz
https://zenodo.org/record/3243160/files/proband_R2.fq.gz

Paired-end data: a single physical piece of DNA/RNA is sequenced from two ends and so generates two reads. These can be represented as separate files (two fastq files with first and second reads) or a single file were reads for each end are interleaved.



Quality Control

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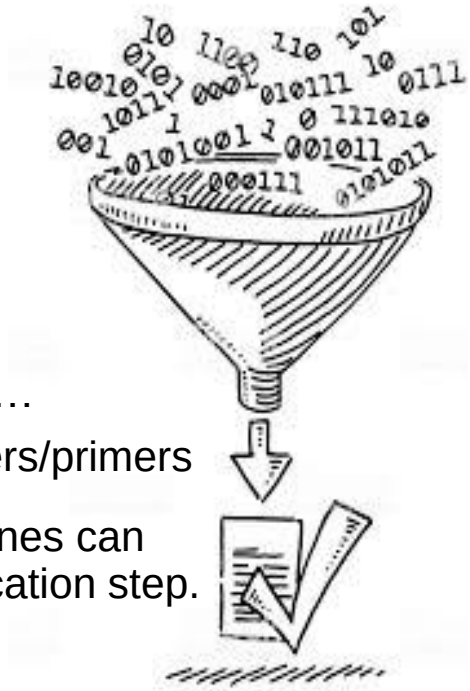
https://zenodo.org/record/3243160/files/proband_R1.fq.gz
https://zenodo.org/record/3243160/files/proband_R2.fq.gz

1. Create a new history and name it as you want (eg. Practica1)
2. Upload the fastq files into Galaxy from the urls copied above
3. Update the attributes of the two datasets (pencil icon):
   a) Rename the datasets to “sample-f.fq.gz” and “sample-r.fq.gz”, respectively.
   b) Check data type is set to “fastqsanger”
   c) Associate the dataset with the human hg38 genome in the Database/Build field.
4. Run a quality control on each dataset using the FastQC tool.
   a) What is the length of reads?
   b) Are sequences of good quality? Any adapter that should be removed?
5. What would be the next step in the analysis workflow?

Quality Control

Preprocessing of raw data

Based on the information provided by the QC graphs, the sequences may be treated to reduce bias in downstream analysis:



- **Filtering sequences**
  - with low mean quality score
  - too short
  - with too many ambiguous (N) bases
  - based on their GC content
  - Biological contamination: polyA-tails, rRNA or mtDNA sequences,…
  - Technical contamination: PhiX internal control sequences, adapters/primers
  - Removing duplicate reads is not advised since high expressed genes can have genuine duplicate reads that are not due to the PCR amplification step.
- **Cutting/Trimming sequences**
  - from low quality score regions
  - beginning/end of sequence
  - removing adapters, primers

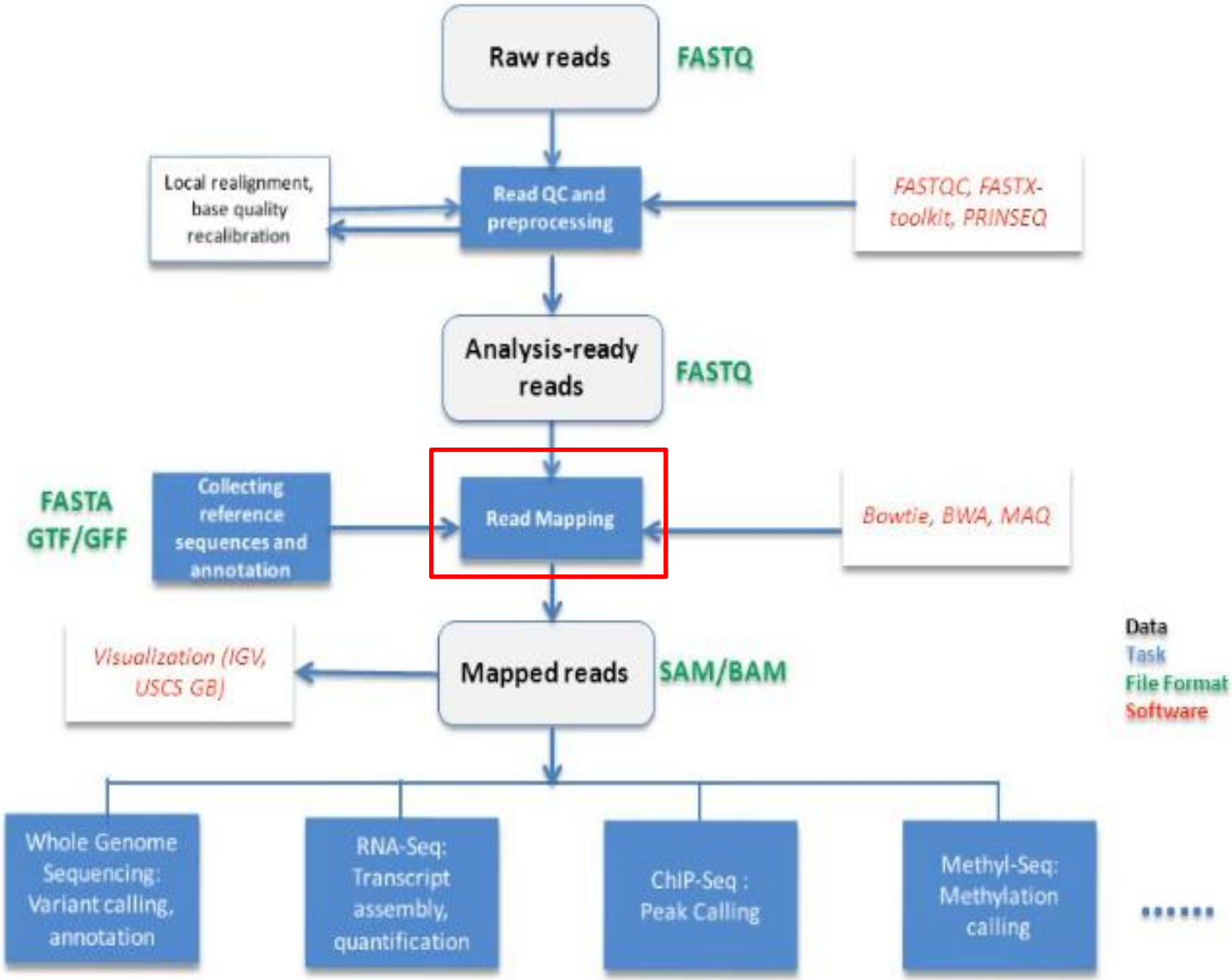
When preprocessing paired-end data coming from separate files, this must be taken into account so that reads are preprocessed “in pairs”

Quality Control

Your turn!

6. Trim the reads in each dataset using **Cutadapt** tool. Set the parameters:
   a) Paired-end data
      File 1: sample-f (forward)
      File 2: sample-r (reverse)
   b) Determine from the FastQC boxplot where the quality of the reads begins to drop off sharply. Calculate how many bases have to be trimmed from the end and use that number as the Offset from 3' end.
   c) Output options: Report=yes
7. Inspect the results:
   a) How many datasets do we get? Rename them to sample-f-trim / sample-r-trim, respectively. What is their format?
   b) Do they have the same number of reads?
8. Re-run FastQC on the trimmed data, and inspect the new FastQC report. Has the sequence quality been improved?
9. Convert your analysis history into a workflow
10. What would be the next step in the analysis workflow?

Steps in NGS analysis



References and resources

Bibliography

Goecks J, Nekrutenko A, Taylor J; Galaxy Team.*Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 2010*

Links and resources

Galaxy tutorials

https://galaxyproject.github.io/training-material/topics/variant-analysis/

https://galaxyproject.github.io/training-material/topics/variant-analysis/tutorials/exome-seq/tutorial.html