Galaxy for virologist training Exercise 3: Illumina Assembly 101

Title	Galaxy
Training dataset:	PRJEB43037 - In August 2020, an outbreak of West Nile Virus affected 71 people with meningoencephalitis in Andalusia and 6 more cases in Extremadura (south-west of Spain), causing a total of eight deaths. The virus belonged to the lineage 1 and was relatively similar to previous outbreaks occurred in the Mediterranean region. Here, we present a detailed analysis of the outbreak, including an extensive phylogenetic study. This is one of the outbreak samples.
Questions:	• What is assembly? • How can I evaluate my assembly?
Objectives:	• Understand assembly concept • Learn how to interpret assembly quality control metrics
Estimated time:	40 min

1. Description

Sometimes, we don't have a reference genome to map against, or we want to reconstruct a genome without any bias caused by a reference. In such cases, we need to do a *de novo assembly*. This type of analysis tries to reconstruct the original genome without any template, using only the reads. Some considerations:

- When we assemble, the longer the reads are and the longer the size of the library fragments the easier it gets for the assembler. That's why pacbio or nanopore are recommended for assembly. Think of it like a puzzle, the bigger the pieces, the easier it is to form the image.
- It's almost imposible to reconstruct the entire genome of a large-genome microorganism with only one sequencing, although it can be done for smaller ones, like viruses.
- Assembly is not recommended for amplicon based libraries due to the depth of coverage uneveness and the amplicons intrinsic bias.

2. Upload data to galaxy

Training dataset

- Experiment info: PRJEB43037, WGS, Illumina MiSeq, paired-end
- Fastq R1: ERR5310322_1 - url : `ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR531/002/ERR5310322/ERR5310322_1.fastq.gz`
- Fastq R2: ERR5310322_2 url : `ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR531/002/ERR5310322/ERR5310322_2.fastq`

`.gz`

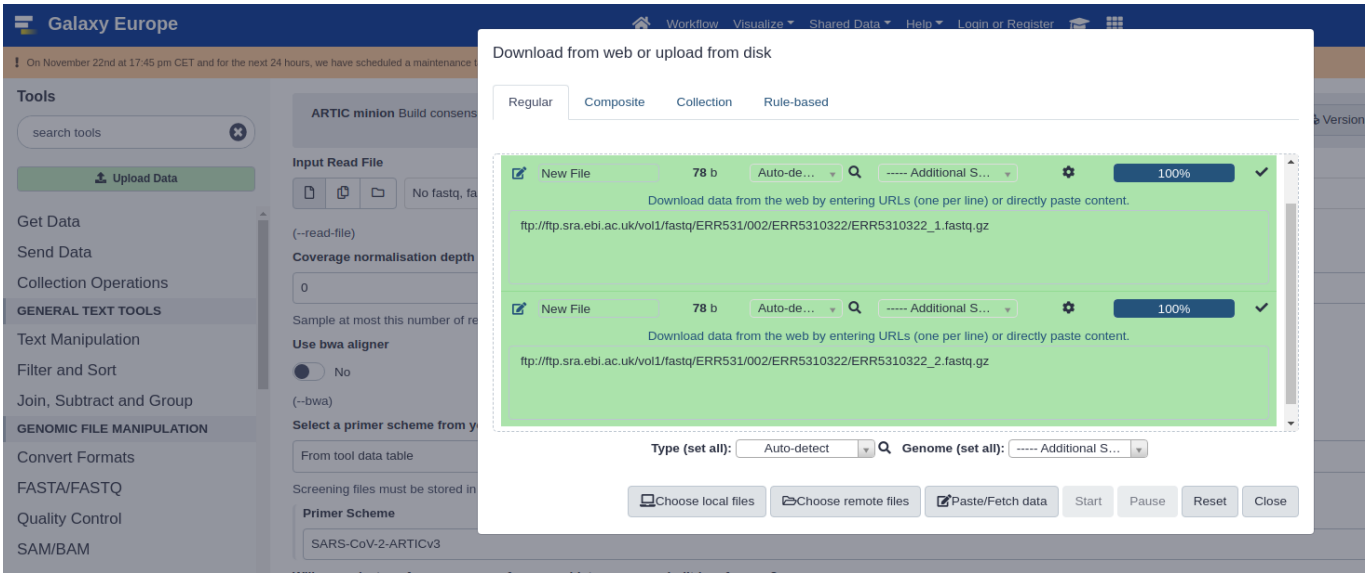
- Reference genome NC_009942.1: fasta -- gff

Create new history

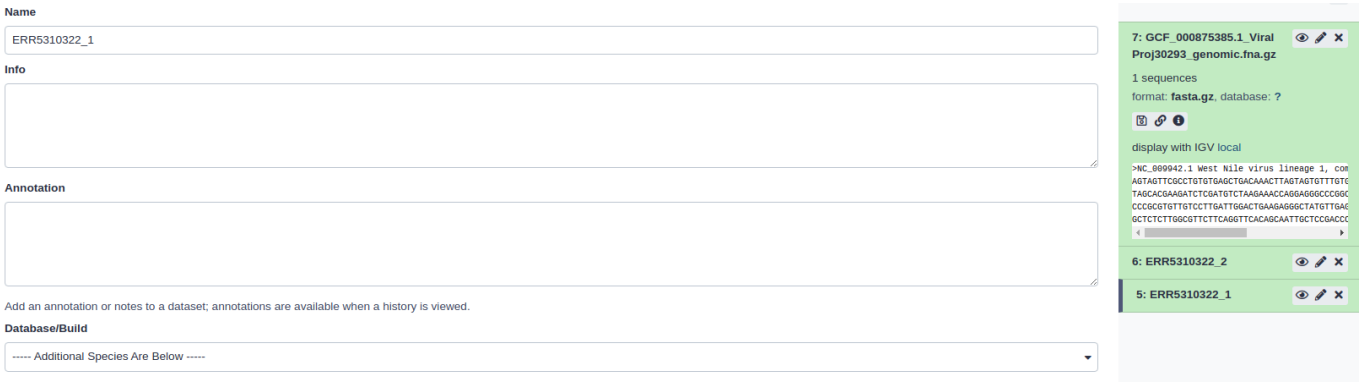
- Click the + icon at the top of the history panel and create a new history with the name `Illumina Assembly` as explained here

Upload data

- Import and rename the read files `ERR5310322_1` and `ERR5310322_2`
    1. Click in upload data.
    2. Click in paste/fetch data
    3. Copy url for fastq R1 (select and Ctrl+C) and paste (Ctrl+V).
    4. Click in Start.
    5. Wait until the job finishes (green in history)
    6. Do the same for fastq R2.

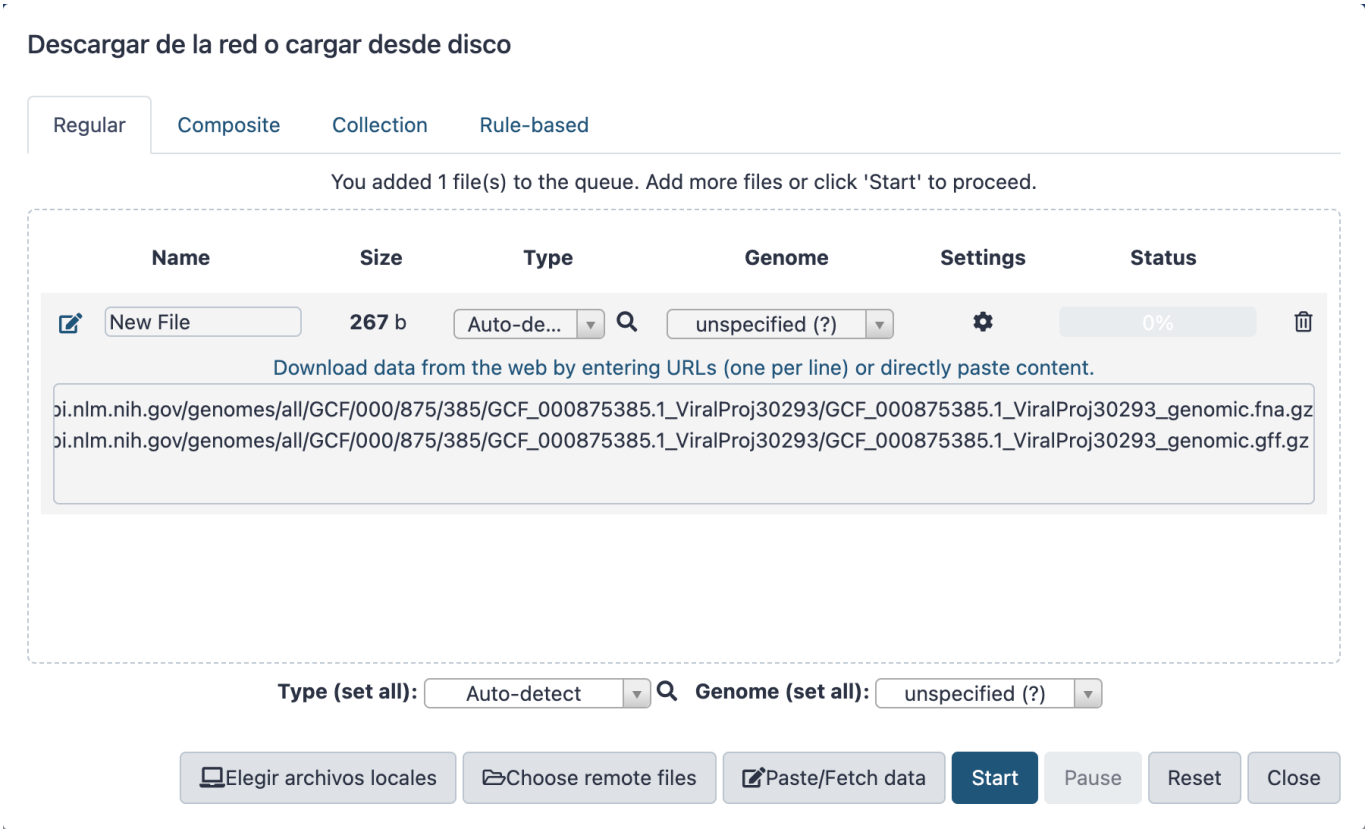


- Rename R1 and R2 files.
    1. Click in the ✏️ in the history for `ERR5310322_1.fastq.gz`
    2. Change the name to `ERR5310322_1`
    3. Do the same for R2.

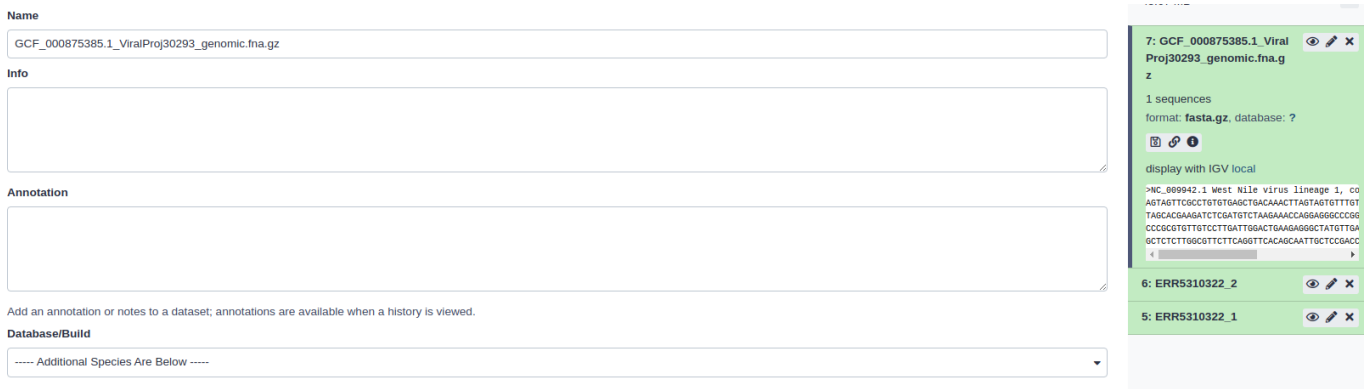


- Import the reference genome and GFF file.

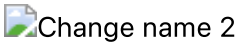
```
https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/875/385/GCF_000875385.1_V
iralProj30293/GCF_000875385.1_ViralProj30293_genomic.fna.gz
https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/875/385/GCF_000875385.1_V
iralProj30293/GCF_000875385.1_ViralProj30293_genomic.gff.gz
```



- Rename the reference genome and gff file.
    1. Click the ✏️ for the reference file in the history.
    2. Change the name to `NC_009942.1`



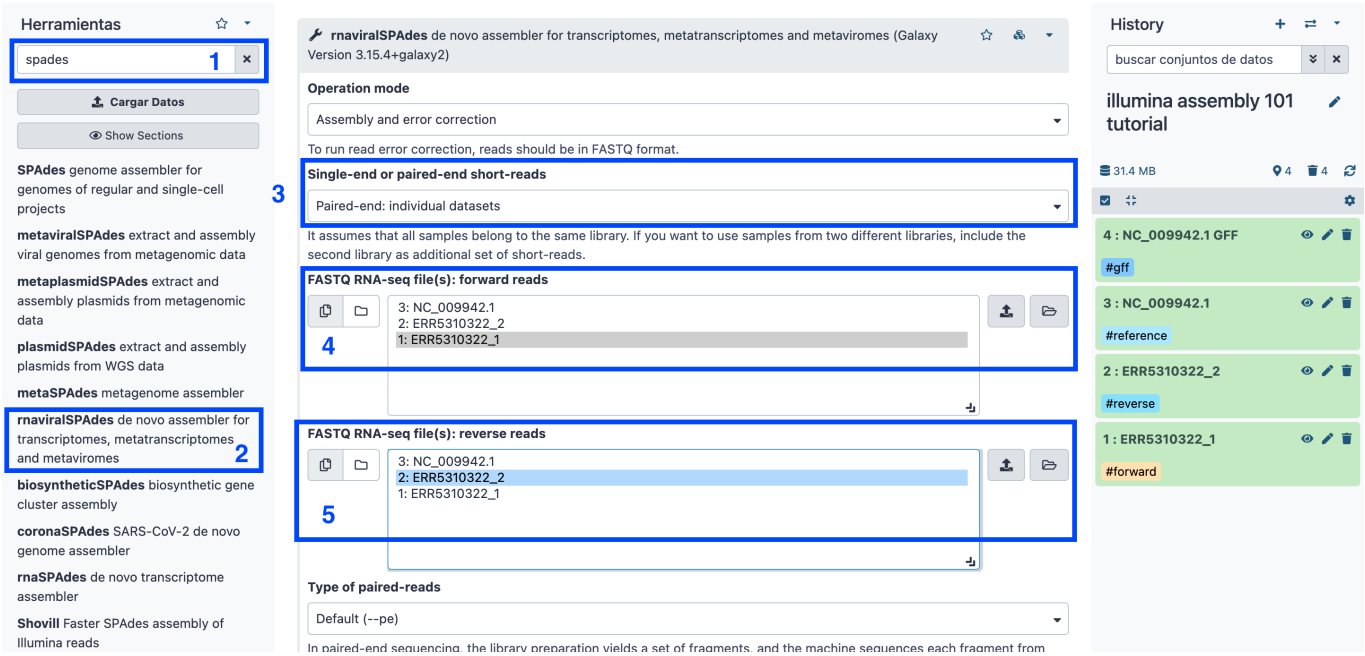
- Finally, add some usefull tags

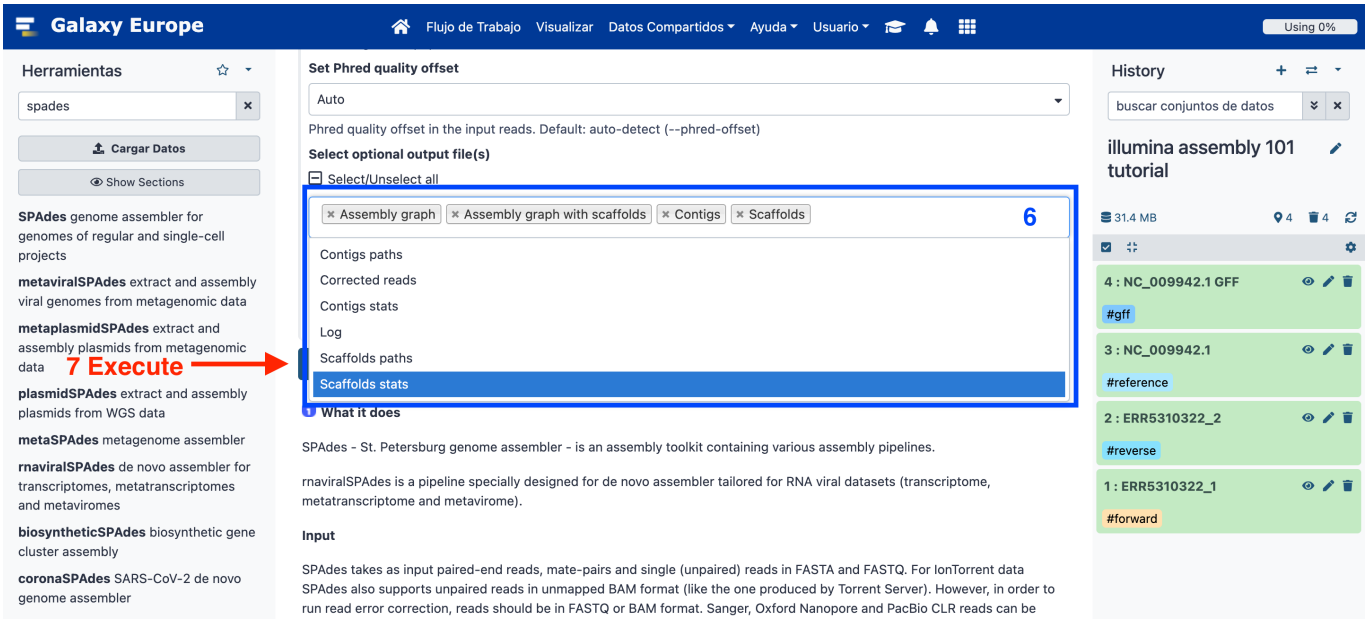


Assemble reads with Spades

1. Search `Spades` in the search tool box and select *rnaviralSPAdes de novo assembler for transcriptomes, metatranscriptomes and metaviromes*

2. Single-end or paired-end short-reads > Paired-end: individual datasets
3. *FASTQ RNA-seq file(s): forward reads*: ERR5310322_1; *FASTQ RNA-seq file(s): reverse reads*: ERR5310322_2
4. Select optional output file(s) > Scaffolds stats
5. Click execute and wait.





> **Warning** **Assembly takes time!** There is no such thing as Assembly in real time. It can take anywhere between 90 minutes and two hours.

Questions:

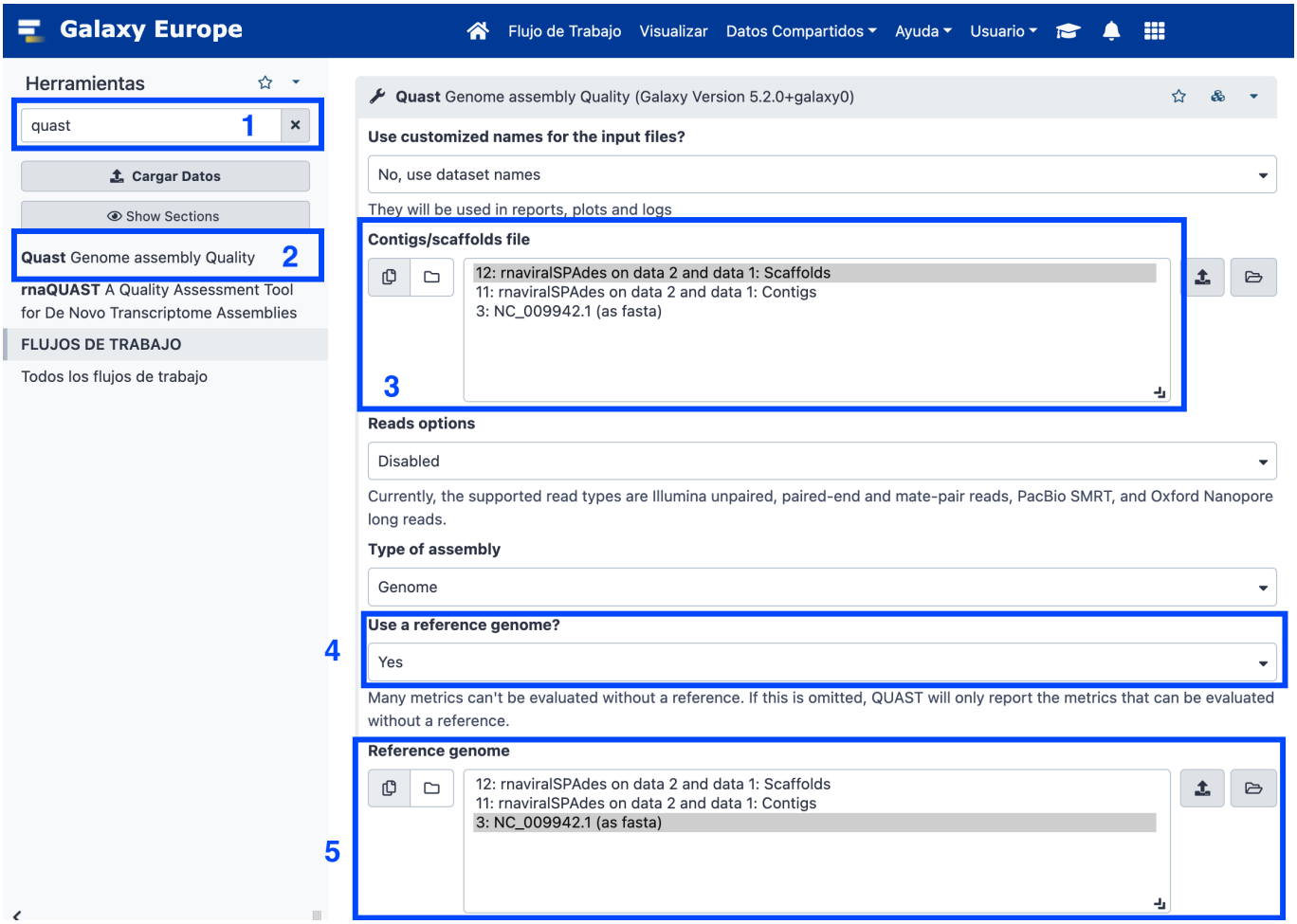
Click the :eye: icon in the history: Spades Contigs stats.

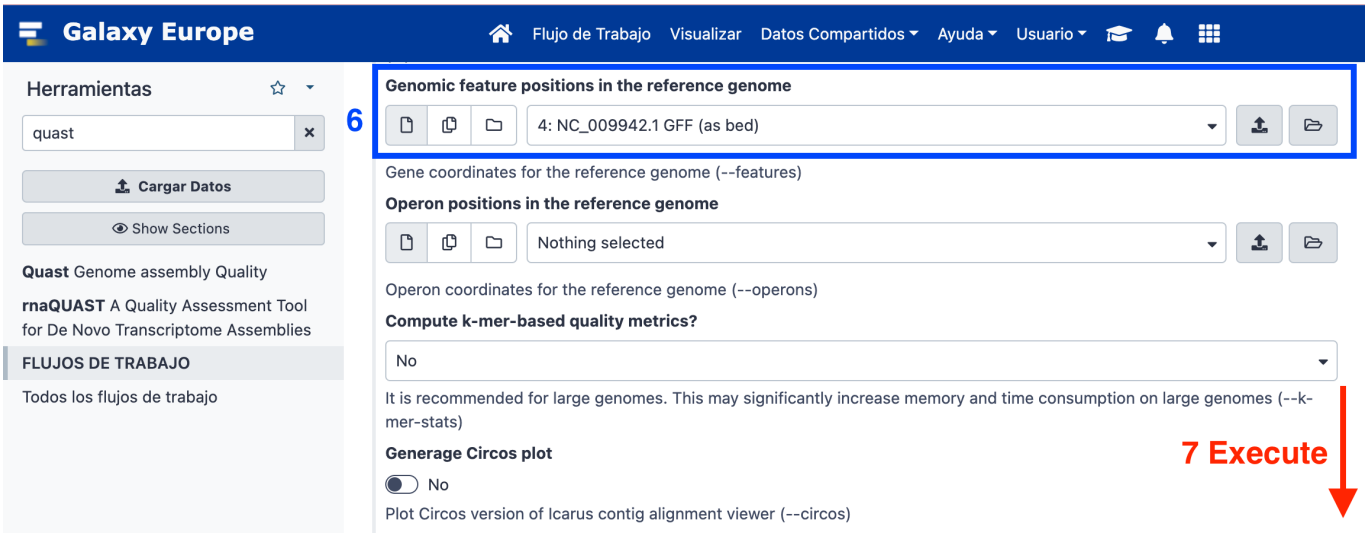
▶ How many contigs has been assembled?

Click the :eye: icon in the history: Spades scaffolds.

Assembly quality control with Quast

1. Search Quast in the search tool box.
2. rnaviralSpades Scaffolds
3. Use a reference genome: Yes. Select the NC_009942.1 fasta file previously loaded.
4. Genomic feature positions in the reference genome > NC_009942. gff file previously loaded.

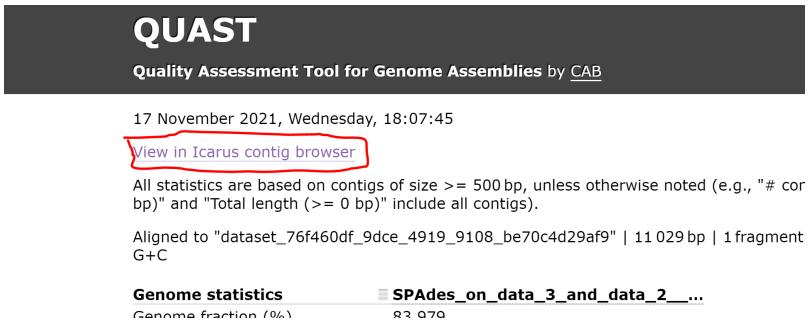




5. Click the :eye: icon Quast HTML report.

- ▶ How much of or reference genome have we reconstructed?
- ▶ How many contigs do we have greater than 1000 pb?
- ▶ How long is the largest contig in the assembly?
- ▶ Which is the N50?

6. Open the Icarus viewer in the quast report.



▶ How did the contig align against our reference genome?

> This training history is available at: https://usegalaxy.eu/u/s.varona/h/illumina-assembly-101-tutorial