

# Galaxy for virologist training

## Exercise 2: Quality control and trimming

Despite the improvement of sequencing methods, there is no error-free technique. A correct measuring of the sequencing quality is essential for identifying problems in the sequencing, thus, this must be the first step in every sequencing analysis. Once the quality control is finished, it's important to remove those low quality reads, or short reads, for which a trimming step is mandatory. After the trimming step it is recommended to perform a new quality control step to be sure that trimming worked.

## 1. Illumina Quality control and trimming

	Title	Pre-processing
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:		<ul style="list-style-type: none"><li>• How do I check whether my Illumina data was correctly sequenced?</li><li>• How can I improve the quality of my data?</li></ul>
Objectives:		<ul style="list-style-type: none"><li>• Perform a quality control in raw Illumina reads</li><li>• Perform a quality trimming in raw Illumina reads</li><li>• Perform a quality control in trimmed Illumina reads</li></ul>
Estimated time:	25 min	

### 1. Quality control

To run the quality control over the samples, follow these steps: 1. [Create a new history, as we explained yesterday](#) named **Illumina preprocessing** 2. [Upload data as seen yesterday](#), copy and paste the following URLs:

```
ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR531/002/ERR5310322/ERR5310  
ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR531/002/ERR5310322/ERR5310
```

1. Search for the **fastqc** tool and select **FastQC Read Quality reports** and set the following parameters:
  - Select multiple file data set in Raw read data from your current history
  - With the *Ctrl* key pressed, select the two datasets
  - Then go down and select **Execute**

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- [1. Quality control](#)
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- [2. Trimming](#)



**Galaxy Europe**

Flujo de Trabajo Visualizar Datos Compartidos Ayuda Usuario Using 0%

[UPDATE] On November 24th at 17:45 pm CET and for the next 24 hours, we have scheduled a maintenance task on the Usegalaxy.eu infrastructure. All services will be shut down and running jobs at that time terminated. Please take it into account in your job schedule.

**Tools**

fastqc 1

**FastQC Read Quality reports (Galaxy Version 0.73+galaxy0)**

Raw read data from your current history

2: ERR5310322\_2.fastq.gz  
1: ERR5310322\_1.fastq.gz

**Ctrl**

This is a batch mode input field. Separate jobs will be triggered for each dataset selection.

**Contaminant list**

No tabular dataset available.

tab delimited file with 2 columns: name and sequence. For example: Illumina Small RNA RT Primer CAAGCAGAAGACGGCATCGA

**Adapter list**

No tabular dataset available.

List of adapters adapter sequences which will be explicitly searched against the library. It should be a tab-delimited file with 2 columns: name and sequence. (--adapters)

**Submodule and Limit specifying file**

Nothing selected

a file that specifies which submodules are to be executed (default=all) and also specifies the thresholds for each submodules warning parameter

**Disable grouping of bases for reads >50bp**

No

Using this option will cause fastqc to crash and burn if you use it on really long reads, and your plots may end up a ridiculous size.

**FLUJOS DE TRABAJO**

All workflows

**History**

buscar conjuntos de datos

**Illumina preprocessing**

2 shown

29.86 MB

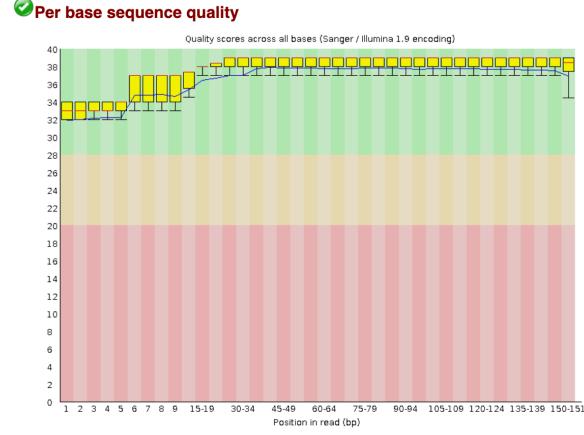
2: ERR5310322\_2.fastq.gz  
1: ERR5310322\_1.fastq.gz

**4 Execute**

To see the results we are going to open the jobs with **Web page** in their name for both data 1 and data 2.

Basic Statistics	
Measure	Value
Filename	ERR5310322_1_fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	265989
Sequences flagged as poor quality	0
Sequence length	35-151
%GC	51

Basic Statistics	
Measure	Value
Filename	ERR5310322_2_fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	265989
Sequences flagged as poor quality	0
Sequence length	35-151
%GC	51



Here, you can see the number of reads in each file, the maximum and minimum length of all reads in the sample, and the quality plots for both R1 and R2. They look quite good, but we are going to run trimming over the samples.

- ▶ How many reads do the samples have?

#### First question

- ▶ How do I check whether my Illumina data was correctly sequenced?

## 2. Trimming

Once we have performed the quality control, we have to perform the quality and read length trimming:

1. Search for **fastp** in the tools and select **fastp - fast all-in-one preprocessing for FASTQ files**

## 2. Select custom parameters:

- Single-end or paired reads > Paired
  - Input 1 > Browse datasets (right folder icon) > Select ERR5310322\_1.fastq.gz
  - Input 2 > Browse datasets > Select ERR5310322\_2.fastq.gz
- Display Filter Options
  - Quality Filtering options
    - Qualified Quality Phred = 30
    - Unqualified percent limit = 10
  - Length Filtering Options
    - Length required = 50
- Read modification options
  - PoliX tail trimming > Enable polyX tail trimming
  - Per read cutting by quality options
    - Cut by quality in front (5') > Yes
    - Cut by quality in tail (3') > Yes
    - Cutting mean quality = 30

## 3. Finally, click on Execute

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**Tools**

fastp 1

Upload Data

Show Sections

fastp - fast all-in-one preprocessing for FASTQ files 2

fastpca - dimensionality reduction of MD simulations

**FLUJOS DE TRABAJO**

All workflows

fastp - fast all-in-one preprocessing for FASTQ files (Galaxy Version 0.20.1+galaxy0)

Paired 3

Input 1 4

Input 2 4

Filter Options 5

History

buscar conjuntos de datos

Ilumina preprocessing

6: FastQC on data 2: Raw Data

5: FastQC on data 2: Web page

4: FastQC on data 1: Raw Data

3: FastQC on data 1: Web page

2: ERR5310322\_2.fastq.gz

1: ERR5310322\_1.fastq.gz

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**Tools**

fastp

Upload Data

Show Sections

fastp - fast all-in-one preprocessing for FASTQ files

fastpca - dimensionality reduction of MD simulations

**FLUJOS DE TRABAJO**

All workflows

Filter Options

Quality filtering options

Disable quality filtering

No 6

Quality filtering is enabled by default. If this option is specified, quality filtering is disabled. (-Q)

Qualified quality phred

30

The quality value that a base is qualified. Default 15 means phred quality >=Q15 is qualified. (-q)

Unqualified percent limit

10 7

How many percents of bases are allowed to be unqualified (0~100). Default 40 means 40%. (-u)

N base limit

If one read's number of N base is >n\_base\_limit, then this read/pair is discarded. Default is 5. (-n)

Length filtering options

Disable length filtering

No

Length filtering is enabled by default. If this option is specified, length filtering is disabled. (-L)

Length required

50 8

Reads shorter than this value will be discarded. Default is 15. (-l)

Maximum length

History

buscar conjuntos de datos

Ilumina preprocessing

6: FastQC on data 2: Raw Data

5: FastQC on data 2: Web page

4: FastQC on data 1: Raw Data

3: FastQC on data 1: Web page

2: ERR5310322\_2.fastq.gz

1: ERR5310322\_1.fastq.gz

**Galaxy Europe**

Flujo de Trabajo Visualizar Datos Compartidos Ayuda Usuario

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**Tools**

- fastp

**Upload Data**

**Show Sections**

**fastp** - fast all-in-one preprocessing for FASTQ files

**fastpca** - dimensionality reduction of MD simulations

**FLUJOS DE TRABAJO**

All workflows

**Read Modification Options**

**PolyG tail trimming**

Automatic trimming for illumina NextSeq/NovaSeq data

This feature is enabled for NextSeq/NovaSeq data by default. NextSeq/NovaSeq data is detected by the machine ID in the FASTQ records.

**PolyG minimum length**

The minimum length to detect polyG in the read tail. 10 by default. (--poly\_g\_min\_len)

**PolyX tail trimming**

Enable polyX tail trimming

Similar to polyG tail trimming. When polyG tail trimming and polyX tail trimming are both enabled, fastp will perform polyG trimming first, then perform polyX trimming. Disabled by default.

**PolyX minimum length**

The minimum length to detect polyX in the read tail. 10 by default. (--poly\_x\_min\_len)

**UMI processing**

**Enable unique molecular identifier**

Yes

Enable unique molecular identifier (UMI) preprocessing. (-U)

**UMI location**

Specify the location of UMI, can be (index1/index2/read1/read2/per\_index/per\_read, default is none. (-umi\_loc)

**History**

9

buscar conjuntos de datos

**illumina preprocessing**

6 shown

34.99 MB

6: FastQC on data 2: Raw Data

5: FastQC on data 2: Web page

4: FastQC on data 1: Raw Data

3: FastQC on data 1: Web page

2: ERR5310322\_2.fastq.gz

1: ERR5310322\_1.fastq.gz

**Galaxy Europe**

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**Tools**

- fastp

**Upload Data**

**Show Sections**

**fastp** - fast all-in-one preprocessing for FASTQ files

**fastpca** - dimensionality reduction of MD simulations

**FLUJOS DE TRABAJO**

All workflows

**Per read cutting\_by\_quality options**

**Cut by quality in front (5')**

Yes

Enable per read cutting by quality in front (5'), default is disabled (WARNING: this will interfere deduplication for both PE/SE data). (-3)

**Cut by quality in tail (3')**

Yes

Enable per read cutting by quality in tail (3'), default is disabled (WARNING: this will interfere deduplication for SE data). (-3)

**Cutting window size**

The size of the sliding window for sliding window trimming, default is 4. (-W)

**Cutting mean quality**

30

The bases in the sliding window with mean quality below cutting\_mean\_quality will be cut, default is Q20. (-M)

**Base correction by overlap analysis options**

**Enable base correction**

No

Enable base correction in overlapped regions (only for PE data), default is disabled. (-c)

**Output Options**

**Email notification**

No

**History**

6 shown

34.99 MB

6: FastQC on data 2: Raw Data

5: FastQC on data 2: Web page

4: FastQC on data 1: Raw Data

3: FastQC on data 1: Web page

2: ERR5310322\_2.fastq.gz

1: ERR5310322\_1.fastq.gz

To see the trimming stats, have a look at the **fastp on data 2 and data 1: HTML report** file. You should see something like that.

# fastp report for ERR5310322\_1\_fastq.gz.fastq.gz

## Summary

### General

fastp version:	0.20.1 ( <a href="https://github.com/OpenGene/fastp">https://github.com/OpenGene/fastp</a> )
sequencing:	paired end (151 cycles + 151 cycles)
mean length before filtering:	105bp, 105bp
mean length after filtering:	113bp, 113bp
duplication rate:	19.977989%
Insert size peak:	84

### Before filtering

total reads:	531.978000 K
total bases:	56.257825 M
Q20 bases:	54.842431 M (97.484094%)
Q30 bases:	54.605191 M (97.062393%)
GC content:	50.644494%

### After filtering

total reads:	433.314000 K
total bases:	49.003611 M
Q20 bases:	48.876432 M (99.740470%)
Q30 bases:	48.825481 M (99.636496%)
GC content:	51.087943%

### Filtering result

- ▶ How many reads have we lost?

## Other trimming tools

1. Search for **trimmomatic** in the tools and select **Trimmomatic flexible read trimming tool for Illumina NGS data**
2. Select custom parameters:
  - Single-end or paired-end reads? = Paired-end (two separated files)
  - Input FASTQ file (R1/first of pair) = ERR5310322\_1.fastq.gz
  - Input FASTQ file (R2/second of pair) = ERR5310322\_2.fastq.gz
  - Insert Trimmomatic Operation:
    - Select Trimmomatic operation to perform: **MINLEN**
    - Minimum length of reads to be kept = 50
3. Select **Execute**

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**Tools**

1. Trimmomatic

2. Trimmomatic flexible read trimming tool for Illumina NGS data

3. History

4. Select Trimmomatic operation to perform: Sliding window trimming (SLIDINGWINDOW)

5. Average quality required: 20

6. Minimum length of reads to be kept: 50

7. Execute

**Galaxy Europe**

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**Tools**

1. Trimmomatic

2. Trimmomatic flexible read trimming tool for Illumina NGS data

3. Trimmomatic Operation

4. Select Trimmomatic operation to perform: Drop reads below a specified length (MINLEN)

5. Average quality required: 20

6. Minimum length of reads to be kept: 50

7. Execute

8. What it does

Trimmomatic performs a variety of useful trimming tasks for illumina paired-end and single ended data.

**History**

9. fastp on data 2 and d  
ata 1: HTML report

8: fastp on data 2 and d  
ata 1: Read 2 output

7: fastp on data 2 and d  
ata 1: Read 1 output

6: FastQC on data 2: Ra  
wData

5: FastQC on data 2: W  
ebpage

4: FastQC on data 1: Ra  
wData

3: FastQC on data 1: We  
bpage

2: ERR5310322\_2.fast  
q.gz

1: ERR5310322\_1.fastq

Trimmomatic does not perform statistics over trimmed reads, so we need to perform FastQC again over the Trimmomatic results.

► Try to do it on your own.

#### Second question

► How can I improve the quality of my data?

- This hands-on history URL: <https://usegalaxy.eu/u/s.varona/h/illumina-preprocessing>

## 2. Nanopore Quality control and trimming

	Title	Galaxy
Training dataset:	The data we are going to manage corresponds to Nanopore amplicon sequencing data using ARTIC network primers for SARS-CoV-2 genome. From the Fast5 files generated by the ONT software, we are going to select the pass reads, so they are already filtered by quality.	

- Title** Galaxy
- Questions:**
- How do I know if my Nanopore data was correctly sequenced?
  - Perform a quality control in raw Illumina reads
- Objectives:**
- Perform a quality trimming in raw Nanopore reads
  - Perform a quality control in trimmed Nanopore reads

**Estimated time:** 15 min

## 1. Quality control

### 1.1. Nanoplot

To run the quality control over the samples, follow these steps: 1. [Create a new history has explained yesterday](#) named **Nanopore quality** 2. [Upload data as seen yesterday](#), copy and paste the following URLs:

```
https://raw.githubusercontent.com/nf-core/test-datasets/viralrec
https://raw.githubusercontent.com/nf-core/test-datasets/viralrec
https://raw.githubusercontent.com/nf-core/test-datasets/viralrec
```

1. Search for the **Nanoplot** tool and select **NanoPlot Plotting suite for Oxford Nanopore sequencing data and alignments**
2. Run the tool as follows:
  - o In *Select multifile mode*: **Combined** (as we are working with 3 different fastq files for the same sample, we can analyze them in batch)
  - o In the *files* part, use *Ctrl* to select the three fastq files.
  - o Display **Options for customizing the plots created**:
    - **Specify the bivariate format of the plots** > *Select all*
    - **Show the N50 mark in the read length histogram** > *Yes*
  - o Select **Execute**

Galaxy Europe

Herramientas

nanoplot 1

2

3

4

5

History

Nanopore quality

The screenshot shows the Galaxy Europe web interface. On the left, there's a sidebar with 'Herramientas' (Tools) containing 'nanoplot' and other options like 'Cargar Datos' (Load Data) and 'Show Sections'. Below that is a section for 'NanoPlot' with a brief description. To the right is the main workspace for 'Nanopore quality' analysis. A central panel shows 'Options for filtering or transforming input prior to plotting' and 'Options for customizing the plots created'. Under 'Specify a color for the plots.', it says 'Nothing selected'. Under 'Specify the bivariate format of the plots.', there are checkboxes for 'kernel density estimation (kde)' and 'dots (dot)'. A note says 'Select/Unselect all' (highlighted by step 6). Another note says 'Show the N50 mark in the read length histogram.' with a 'Yes' toggle switch (highlighted by step 7). Under 'Email notification', there's a 'No' radio button. At the bottom is a blue 'Execute' button with a checkmark (highlighted by step 8).

Now we are going to have a look to the results.

1. Select the :eye: icon in the **NanoPlot on data 3, data 2, and data 1:**  
**HTML report result.**
2. Have a look to the stats.

The screenshot shows the Galaxy Europe interface with a 'NanoPlot report' page. The left sidebar has sections for 'Tools', 'Get Data', 'Send Data', 'Collection Operations', 'GENERAL TEXT TOOLS', 'Text Manipulation', 'Filter and Sort', 'Join, Subtract and Group', 'GENOMIC FILE MANIPULATION', 'Convert Formats', 'FASTA/FASTQ', 'Quality Control', 'SAM/BAM', 'BED', 'VCF/BCF', 'Nanopore', and 'COMMON GENOMICS TOOLS'. The main content area shows a 'Summary statistics' table with various metrics like Mean read length (537.5), Median read length (516.0), and Total bases (1,612,409.0). Below this is a table for 'Number, percentage and megabases of reads above quality cutoffs' with rows for >Q5, >Q7, >Q10, >Q12, and >Q15. Further down are tables for 'Top 5 highest mean basecall quality scores and their read lengths' and 'Top 5 longest reads and their mean basecall quality score'. On the right, the 'History' panel shows several previous jobs related to Nanopore quality analysis, including 'Nanopore quality' and various 'NanoPlot' runs.

As you can see, the Mean read length is around 500 nt, which makes sense because we are using amplicon sequencing data.

- How many reads do the samples have?

## 1.2. PycoQC

To use PycoQC we need to use the `sequencing_summary.txt` provided by de Nanopore sequencing machine.

1. [Upload data as seen yesterday](#), copy and paste the following URL:  
`https://raw.githubusercontent.com/nf-core/test-datasets/viralrecon/nanopore/minion/sequencing_summary.txt`
2. Search for the **Pycoqc** tool and select it.
3. Run the tool as follows:
  - In A *sequencing\_summary file*: Select the `sequencing_summary.txt` we just uploaded
  - Leave the rest as defaults
  - Select **Execute**

The screenshot shows the Galaxy Europe web interface. On the left, there's a sidebar with 'Herramientas' (Tools) and a search bar containing 'pycoqc' with a count of '1'. Below that are buttons for 'Cargar Datos' (Load Data) and 'Show Sections'. The main area is titled 'Pycoqc' (Galaxy Version 2.5.2+galaxy0). It shows a 'sequencing\_summary\_file' input field containing '16: sequencing\_summary.txt'. Below it, a message says 'Must be generated by Albacore 1.0.0 + (read\_fast5\_basecaller.py) / Guppy 2.1.3+ (guppy\_basecaller) (--summary\_file)'. There's also a note about an alignment file and a 'No bam dataset available.' message. Under 'Output JSON Summary File?' is a checked 'Yes' button. A 'Minimum quality to consider a read as 'pass'' input field has the value '7'. A red arrow labeled '4 Execute' points to the bottom right of the interface.

Then inspect the resulting PycoQC HTML Report:

### General run summary

Status	Run Duration (h)	Active Channels	Number of Runids	Number of Barcodes
All Reads	7.98	496	1	9
Pass Reads	7.98	494	1	9

### Basecall summary

Status	Reads	Bases	N50	Median Read Length	Median PHRED score
All Reads	2.449000e+4	1.284332e+7	515	514	13.055
Pass Reads	2.435200e+4	1.276470e+7	515	514	13.067

#### Question

- ▶ Do you understand all the plots?
- ▶ How do I check whether my Nanopore data was correctly sequenced?

## 2. Trimming

When Nanopore reads are being sequenced, the MinKnown software splits Fast5 reads into quality pass and quality fail. As we will select only Fast5 pass reads, we won't need to perform a quality trimming, so even if we see that the reads have a bad Phred score, we know that the ONT software considered the reads as "good quality".

Then we will only be performing a read length trimming. As we are using

amplicon sequencing data, we won't be expecting reads smaller than 400 nucleotides, nor higher than 600, which would obviously correspond to chimeric reads.

1. Search for **artic** tool
2. Select **ARTIC guppyplex Filter Nanopore reads by read length and (optionally) quality**
3. While pressing the *Ctrl* key, select the three samples
4. Remove reads longer than = 600
5. Remove reads shorter than = 300
6. Do not filter on quality score (speeds up processing) = Yes (we had already select pass reads)

**Galaxy Europe**

Tools

artic 1

Upload Data Show Sections

ARTIC minion Build consensus sequence and call variants from amplicon-based nanopore sequence data

ARTIC guppyplex Filter Nanopore reads by read length and (optionally) quality 2

Analyze particles of binary image

WORKFLOWS

All workflows

**ARTIC guppyplex** Filter Nanopore reads by read length and (optionally) quality (Galaxy Version 1.2.1+galaxy2)

Structure of your input data

Multiple input datasets per sample 3

Partial sequencing datasets for your sample

3: FAO93606\_pass\_barcode01\_7650855b\_2.fastq  
2: FAO93606\_pass\_barcode01\_7650855b\_1.fastq  
1: FAO93606\_pass\_barcode01\_7650855b\_0.fastq 4

Multiple datasets selected here will get combined into a single output for a single assumed sample. Select a nested list to have its inner lists interpreted as data from one sample each and to obtain one output per inner list.

Remove reads longer than 5

remove reads greater than this number of base pairs

Remove reads shorter than 6

remove reads less than this number of base pairs

Eliminate reads with a mean base quality score of less than 7

Set to 0 to skip the quality check.

Email notification

No

Send an email notification when the job completes.

Execute 7

Now we are going to repeat NanoPlot on filtered data:

1. Search for the **Nanoplot** tool and select **NanoPlot Plotting suite for Oxford Nanopore sequencing data and alignments**
2. Run the tool as follows:
  - o In the *files* part, select ARTIC output file.
  - o Display **Options for customizing the plots created**:
    - **Specify the bivariate format of the plots** > *Select all*
    - **Show the N50 mark in the read length histogram** > Yes
  - o Select **Execute**

**Galaxy Europe**

Workflow Visualize Shared Data Help User

**Tools**

nanoplot 1

Upload Data Show Sections

NanoPlot Plotting suite for Oxford Nanopore sequencing data and alignments 2

WORKFLOWS

All workflows

**NanoPlot Plotting suite for Oxford Nanopore sequencing data and alignments**

Select multifile mode

batch

When supplying multiple files, batch mode will run NanoPlot on each file separately, while combined mode will run NanoPlot once on all the reads together. When only supplying a single input file, this setting does not matter.

Type of the file(s) to work on

fastq

files 3

9: ARTIC guppyplex on data 3, data 2, and data 1

(--fastq)

Options for filtering or transforming input prior to plotting

Options for customizing the plots created 4

Specify a color for the plots

**Galaxy Europe**

Workflow Visualize Shared Data Help User

**Tools**

nanoplot

Upload Data Show Sections

NanoPlot Plotting suite for Oxford Nanopore sequencing data and alignments

WORKFLOWS

All workflows

**Options for customizing the plots created**

Specify a color for the plots.

Nothing selected

(--color)

Specify the bivariate format of the plots.

5  Select/Unselect all

kernel density estimation (kde)  
 dots (dot)

(--plots)

Show the N50 mark in the read length histogram.

6  Yes  
(--N50)

Email notification

Yes  
No

Send an email notification when the job completes.

7 **Execute**

## Questions

- ▶ Did our data length and quality improve?
- ▶ How many reads did we lose during trimming step?
- This hands-on history URL: <https://usegalaxy.eu/u/svarona/h/nanopore-quality>

**NOTE:** We can't use nanofilt because it is not installed in Galaxy