

Running pre-alignment QA/QC

With attributes added, we can begin building our pipeline.

- Click the **Analyses** tab

In the *Analysis* tab, data are represented as circles, termed data nodes. One data node, *mRNA*, should be visible in the *Analysis* tab (Figure 1).

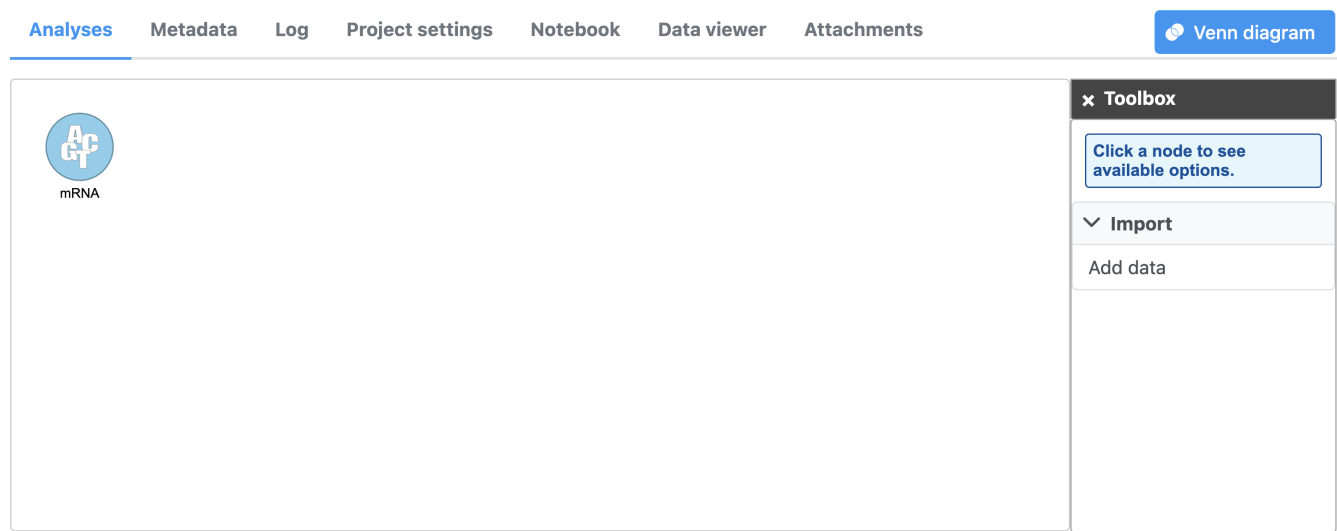


Figure 7. Data are represented as circles

- Click the *mRNA* node

Clicking a data node brings up the context-sensitive task menu with tasks that can be performed on the data node (Figure 2).

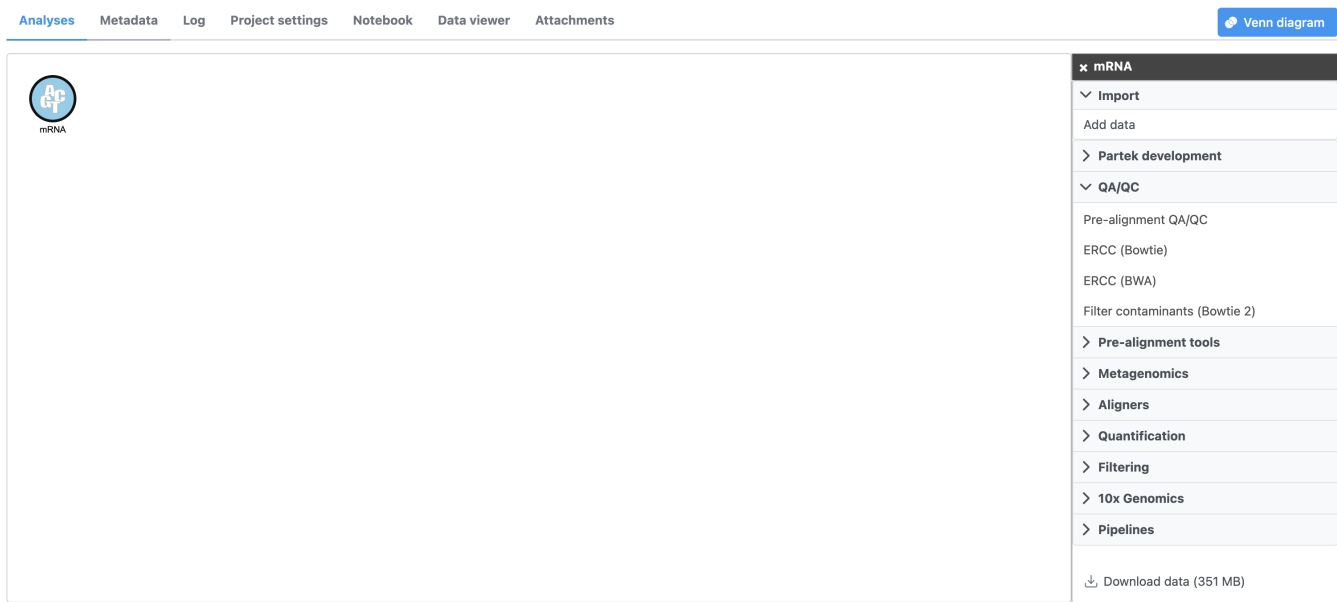


Figure 8. Select a data node to open the context-sensitive task menu

Pre-alignment QA/QC assesses the quality of the unaligned reads and will help us determine whether trimming or filtering is necessary.

- Click **Pre-alignment QA/QC** in the *QA/QC* section of the task menu

- Click **Finish** to run the task with default settings

Running a task creates a task node, e.g. the blue rectangle labeled *Pre-alignment QA/QC* (Figure 3), which contains details on the task and a report. While tasks have been queued or are in progress they have a lighter color. Any output nodes that the task will generate are also displayed in a lighter color until the task completes. Once the task begins running, a progress bar is displayed on the task node.

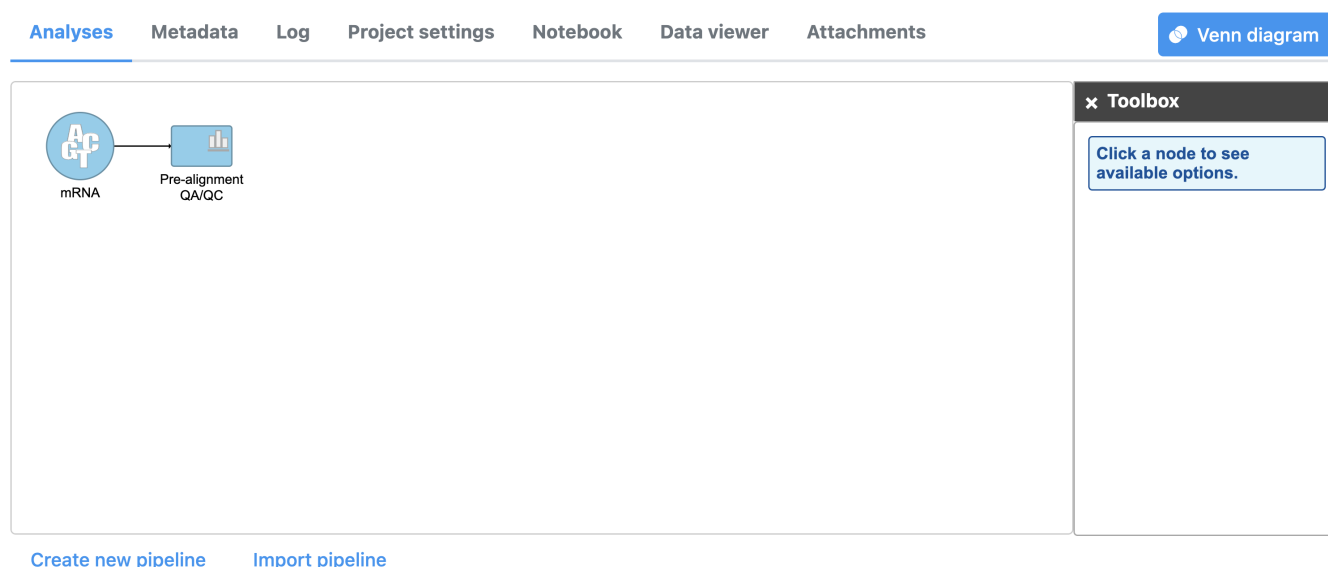


Figure 9. Tasks are represented as rectangles

- Click the **Pre-alignment QA/QC** node

The context-sensitive task menu (Figure 4) shows the option to view the *Task report* and the *Task details*. You can also access a task report by double-clicking on a task node.

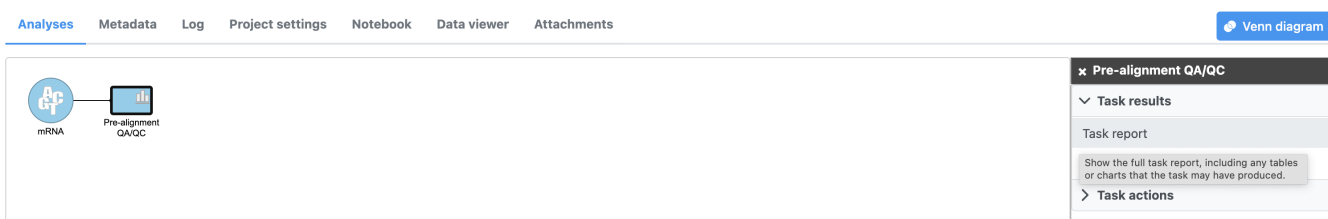


Figure 10. Navigating to the task report using the context-sensitive menu

- Click **Task report**

Pre-alignment QA/QC provides information about the sequencing quality of unaligned reads (Figure 5). Both project level summaries and sample-level summaries are provided.

[Home](#) > [RNA-Seq 5-AZA](#) > [Pre-alignment QA/QC report](#)

Optional columns

Sample name ↑⇅	Total reads ↑↓	Read length ↑↓	Avg. read quality ↑↓	% N ↑↓	% GC ↑↓
SRR592573	116,350	98.00	32.10	0%	53.74%
SRR592574	173,849	98.00	32.07	0%	53.61%
SRR592575	242,360	98.00	32.04	0%	53.25%
SRR592576	281,368	98.00	31.80	0%	52.95%
SRR592577	251,571	98.00	31.78	0.01%	52.02%
SRR592578	293,754	98.00	31.77	0%	52.89%
SRR592579	141,924	98.00	31.79	0%	51.96%
SRR592580	239,377	98.00	31.59	0.01%	53.06%
SRR592581	206,711	98.00	31.59	0%	51.98%

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Figure 11. Viewing the pre-alignment QA/QC report

- Click sample **SRR592573** in the data table of the report to open its sample-level report

The *Average base quality score per position* graph in the upper right-hand panel (Figure 6) gives the average Phred score for each position in the reads.

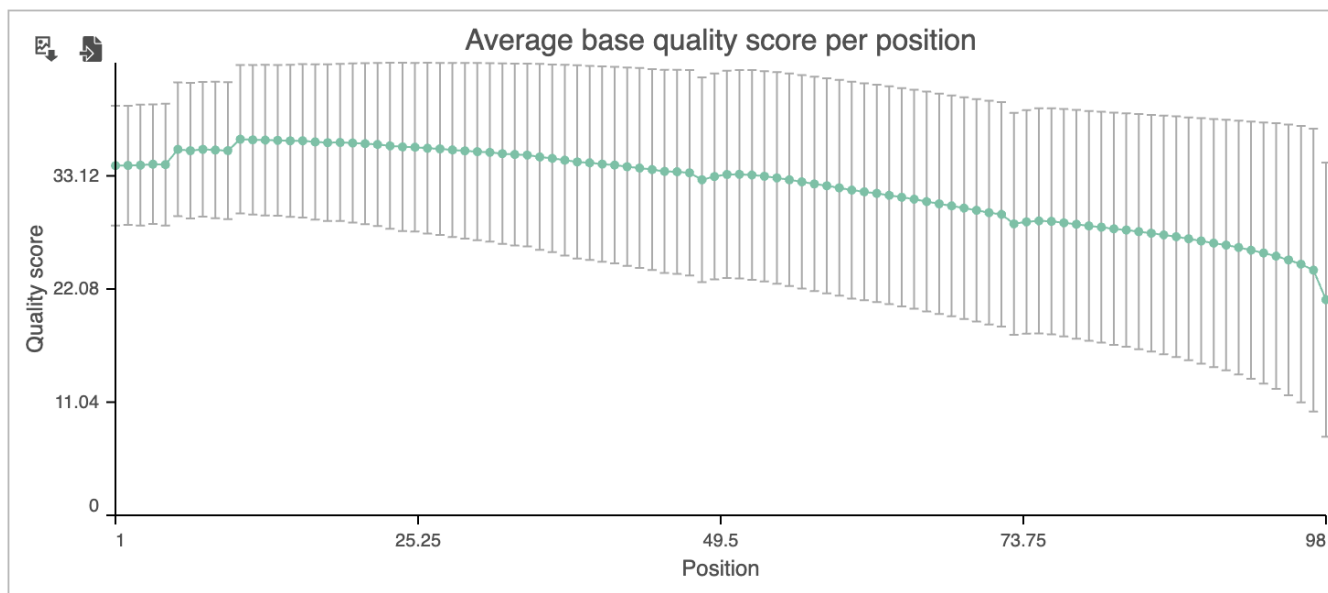


Figure 12. Average base quality score per position for sample SRR592573

A Phred score is a measure of base call accuracy with a higher score indicating greater accuracy.

Phred Quality Score	Probability of incorrect base call	Base call accuracy
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

By convention, a score above 20 is considered adequate. As you can see, the standard error bars in the graph show that some reads have quality scores below 20 for some of their base pair calls near the 3' end.

Based on the results of *Pre-alignment QA/QC*, while most of the reads are high quality, we will need to perform read trimming and filtering. For more information about the information included in the task report, please see the [Pre-alignment QA/QC](#) user guide.

- Click **RNA-Seq 5-AZA** to return to the *Analyses* tab

« [Adding sample attributes](#) [Trimming bases and filtering reads](#) »

Additional Assistance

If you need additional assistance, please visit [our support page](#) to submit a help ticket or find phone numbers for regional support.



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32 rates