Normalizing counts

Because different samples have different total numbers of reads, it would be misleading to calculate differential expression by comparing read count numbers for genes across samples without normalizing for the total number of reads.

- Click the **Filtered counts** data node
- Click **Normalization and scaling** in the task menu
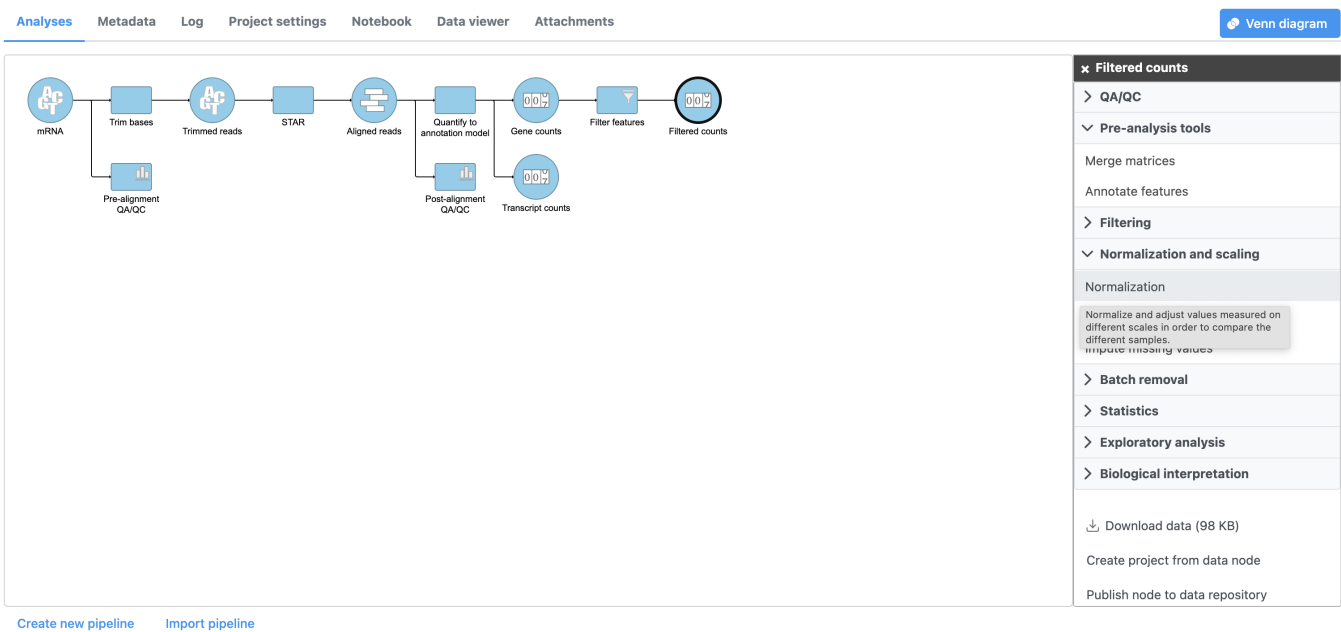- Click **Normalization** (Figure 1)

Figure 5. Invoking Normalize counts

The *Count normalization* menu will open (Figure 2).

Count normalization

Transform on

◉ Samples   ○ Features

Available methods   **Selected methods**   👍 Use recommended

Absolute value

Add

Antilog

Arcsinh

CLR   Drag
                                                       and
CPM (counts per million)   drop
                                                        ➡
Divide by

FPKM

Log

Logit

Lower bound

Back   Finish

Figure 6. Read counts normalization task menu

Normalization can be performed by sample or by feature. By sample is selected by default; this is appropriate for the tutorial data set.

Available normalization methods are listed in the left-hand panel. For more information about these options, please see the Normalize counts user guide.

For this tutorial, we will use the recommended default normalization settings.

- Select 👍 **Recommended**

This adds the *Median ratio* normalization method, which is suitable for performing differential expression analysis using DESeq2 (Figure 3).

Count normalization

Transform on

⦿ Samples ◯ Features

Available methods

Absolute value
Add
Antilog
Arcsinh
CLR
CPM (counts per million)
Divide by
FPKM
Log
Logit
Lower bound

Selected methods 👍 Use recommended

1. Median ratio (DESeq2 only)

Drag
and
drop
➡️

[Back] [Finish]

Figure 7. Recommended normalization settings

- Click **Finish** to perform normalization

A *Normalize counts* task node and a *Normalized counts* data node are added to the pipeline (Figure 4)
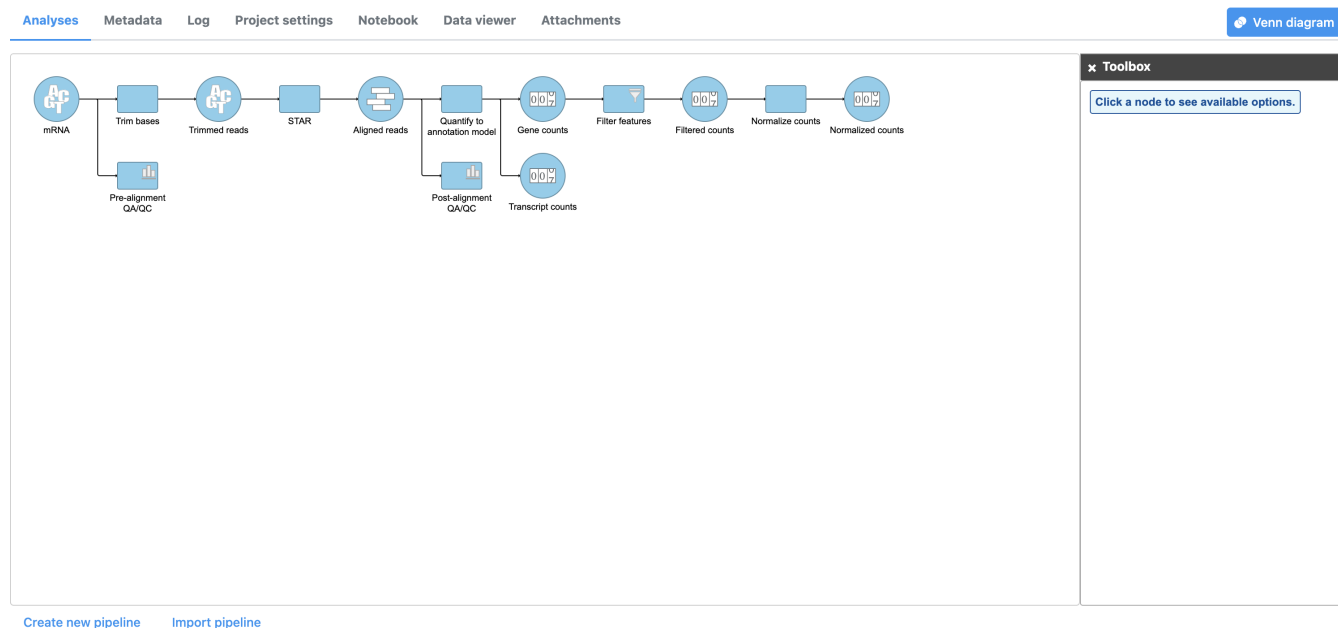

Analyses Metadata Log Project settings Notebook Data viewer Attachments   🏷️ Venn diagram

× **Toolbox**

Click a node to see available options.

mRNA Trim bases Trimmed reads STAR Aligned reads Quantify to annotation model Gene counts Filter features Filtered counts Normalize counts Normalized counts

Pre-alignment QA/QC Post-alignment QA/QC Transcript counts

Create new pipeline   Import pipeline

Figure 8. Normalize counts task node and Normalized counts data node

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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