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	Drag and drop						
CLR							
CPM (counts per million)							
Divide by							
FPKM							
Log							
Logit							
Lower bound							

Back Finish



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.



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

Count normalization								
Transform on								
Samples O Features								
Available methods	~	Selected methods	🖞 Use recommended					
Absolute value		1. Median ratio (DESeq2 only)						
Add								
Antilog								
Arcsinh								
CLR	Drag							
CPM (counts per million)	and drop							
Divide by								
FPKM								
Log								
Logit								
Lower bound								
	-							

Figure 7. Recommended normalization settings

Back

• 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 Attach	ments		🔗 Venn diagram
MRNA	Trim bases	Trimmed reads	Aligned reads Country to Controlation model	Filter features	Normalize counts	★ Toolbox Click a node to see available options.
	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

« Filtering features Exploring the data set with PCA »

Additional Assistance

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

