Quantifying to an annotation model

RNA-Seq uses the number of sequencing reads per gene or transcript to quantify gene expression. Once reads are aligned to a reference genome, we need to assign each read to a known transcript or gene to give a read-count per transcript or gene.

- Click the Aligned reads data node
- Click Quantification in the task menu

We will use *Partek E/M* to quantify reads to an annotation model in this tutorial. For more information about the other quantification options, please see the Quantification user guide.

• Click Quantify to an annotation model (Partek E/M) (Figure 1)

Analyses	Metadata	Log Proje	ct settings	Notebook	Data viewer	Attachments						🔊 Venn diagram
GT mRNA	Trim bases									× Aligned reads		
		-(Åc))—————						✓ Task results			
		Trimmed reads	STAR	Aligned reads	Post-alignment QA/QC					Data summary report		
										> Partek developme	nt	
									> QA/QC			
											> Post-alignment to	ols
											✓ Quantification	
											Quantify to annotation	model (Partek E/M)
											Quantify transcript abunda aligned to a genome.	nce from reads
											HTSeq	
											> Filtering	
											> Exploratory analys	is

Figure 4. Invoking Quantify to an annotation model (Partek E/M)

We will use the default options for quantification. To learn more about the different options, please see the Quantify to annotation model (Partek E/M) user auide or mouse over the innext to each option.

- Choose the latest RefSeq Transcripts 95 annotation from the Gene/feature annotation drop-down menu (you may need to download it first, via Li brary File Management)
- Click Finish (Figure 2)

Select Annotation file

Assembly

Homo sapiens (human) - hg19

Annotation model

RefSeq Transcripts 95 - 2020-08-03 (Matt Luberti) 🗸

Quantification options

Strict paired-end compatibility

If not checked, then paired end reads will count as exonic even if their mate is not compatible with the transcript (--require_proper_pair)

Require junction reads to match introns

If not checked, then junction reads will count as exonic even if their skipped regions don't match with an intron of the transcript (--check_junctions)

Minimum read overlap with feature

Percent of read length

Number of bases overlapped with feature / read length

100 🗘

Number of bases

Minimum number of bases of read that overlap with feature

50

Min reads

The sum of reads across all samples must be greater than or equal to this to be reported



Figure 5. Configuring Quantify to annotation model (Partek E/M)

The Quantify to annotation mode/task node outputs two data nodes, Gene counts and Transcript counts (Figure 3).

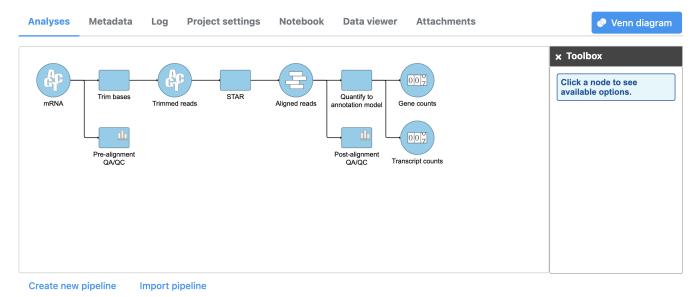


Figure 6. Gene counts and Transcript counts data nodes

To view the results of quantification, we can select either data node output.

• Double-click the Gene counts data node to view the task report

The task report details the number of reads within exons, introns, and intergenic regions. For detailed information about the quantification results, see the Q uantify to annotation model (Partek E/M) user guide.

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« Running post-alignment QA/QC Filtering features »
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Additional Assistance

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

