

RNA-Seq Analysis: Align and Quantify

Alignment and Quantification using Salmon

- We will provide to salmon the reference, the collection of fastq files and the mapping between transcripts and genes.

Salmon quant Perform dual-phase, reads or mapping-based estimation of transcript abundance from RNA-seq reads (Galaxy Version 1.3.0+galaxy1) ☆ Favorite 🔗 Versions ▼ Options

Select salmon quantification mode:
Reads

Select a reference transcriptome from your history or use a built-in index?
Use one from the history

Built-ins were indexed using default options

Salmon index

Transcripts fasta file
31: gffread on data 21 and data 29: exons.fa

Kmer length
31

Select the reference we processed before

Data input

Is this library mate-paired?
Single-end

FASTQ/FASTA file
233: Trimmed NrlKO

FASTQ file.

Specify the strandedness of the reads
Not stranded (U)

Select the collection with the Trimmed KO or WT reads

File containing a mapping of transcripts to genes
74: TranscriptToGeneIDs

If this file is provided Salmon will output both quant.sf and quant.genes.sf files, where the first contains the transcript abundance estimates. The transcript to gene mapping should be provided as either a GTF file, or a in a tab-separated file with the name of a transcript and the gene to which it belongs separated by a tab.

Perform sequence-specific bias correction
Yes No

Perform fragment GC bias correction
Yes No

Select the dataset containing the mappings of transcripts to genes

Switch on the bias correction

- Leave the rest of the parameters as default. Usually the tools are setup with default options that suits most of the analysis problems. Do not change then unless you now what they mean.
- Repeat the process for the other samples.

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- Salmon produces several outputs: Transcript and Gene quantification level.

1	2	3	4	5
Name	Length	EffectiveLength	TPM	NumReads
ENSMUST00000193812	1070	820.000	0.000000	0.000
ENSMUST00000082908	110	4.000	0.000000	0.000
ENSMUST00000162897	4153	3378.824	2.859154	340.095
ENSMUST00000159265	2989	2399.420	1.542124	130.264
ENSMUST00000070533	3634	2981.014	2.121509	222.641
ENSMUST00000195335	2819	2484.967	0.022862	2.000
ENSMUST00000192336	2233	1983.000	0.000000	0.000
ENSMUST00000194099	2309	1764.923	0.016094	1.000
ENSMUST00000161581	250	20.000	0.000000	0.000
ENSMUST00000192973	2057	1807.000	0.000000	0.000
ENSMUST00000195166	3012	2496.259	0.669989	58.878

Transcript Level Quantification

1	2	3	4	5
Name	Length	EffectiveLength	TPM	NumReads
ENSMUSG00000096768	1156.96	660.115	32.6979	759.865
ENSMUSG00000095366	595.513	350.154	9.79608	120.756
ENSMUSG00000099399	444.5	195	0	0
ENSMUSG00000096178	844	594	0	0
ENSMUSG00000102011	437	188	0	0
ENSMUSG00000100608	931	681	0	0
ENSMUSG00000101402	967.5	718	0	0
ENSMUSG00000100492	963.5	714	0	0
ENSMUSG00000100067	967	717.5	0	0
ENSMUSG00000101984	802.5	553	0	0
ENSMUSG00000099649	397	148	0	0

Gene Level Quantification

- Salmon produces two quantifications TPMs and NumReads:
 - TPMs is a normalized quantification by the total number of transcripts produced in the experiment.
 - NumReads are just the counts, how many sequences mapped to that particular transcript or gene.