

# RNA-seq

## Style 1

Paired-ends sequencing reads were clipped for adapter sequence, trimmed for minimum quality (Q30) in 3' and filtered for minimum length of 32 bp using Trimmomatic [PMID: 24695404].

Surviving read pairs were aligned to the **INCLUDE REFERENCE GENOME** assembly using STAR [PMID: 23104886] the two-pass method.

A gene-level count-based gene quantification against Ensembl annotations was performed using HT-seq count [PMID: 25260700] in the *intersection-nonempty* mode. Exploratory analysis was conducted using various functions and packages from R and the Bioconductor project [PMID: 25633503]. Differential expression was conducted using both edgeR [PMID: 19910308] and DEseq [PMID: 20979621]. Terms from the Gene Ontology were tested for enrichment with the Goseq [PMID: 20132535] R package.

Transcript-level assembly, quantification and differential expression analysis was performed using Cufflinks [PMID: 20436464] and Cuffdiff [PMID: 23222703].

All of the above processing steps were accomplished through the GenPipes framework [PMID 31185495]

## Style 2

Raw reads derived from the sequencing instrument are clipped for adapter sequence, trimmed for minimum quality (Q30) in 3' and filtered for minimum length of 32 bp using Trimmomatic [PMID: 24695404]. Surviving read pairs were aligned to **INCLUDE REFERENCE GENOME** by the ultrafast universal RNA-seq aligner STAR [PMID: 23104886] using the recommended two passes approach. Aligned RNA-Seq reads were assembled into transcripts and their relative abundance was estimated using Cufflinks [PMID: 20436464] and Cuffdiff [PMID: 23222703].

Exploratory analysis was conducted using various functions and packages from R and the Bioconductor project [PMID: 25633503]. Differential expression was conducted using both edgeR [PMID: 19910308] and Deseq [PMID: 20979621]. Terms from the Gene Ontology were tested for enrichment with the GOseq [PMID: 20132535] R package.

All of the above processing steps were accomplished through the GenPipes framework [PMID 31185495]