**NGS RNA-Seq: Expression Analysis, Differential Expression Analysis**

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| **Tutorials, Training, Workshops** |
| |  | | --- | |  | | **Gene Expression**  <https://www.ebi.ac.uk/training/online/course-subject-area/gene-expression>  **Introduction to dierential gene expression analysis using RNA-seq** Nov 14 2019  <https://chagall.med.cornell.edu/RNASEQcourse/Intro2RNAseq.pdf> | |

**How to analyze RNA-Seq data? Find differentially expressed genes in your research.**

<https://www.youtube.com/watch?v=xh_wpWj0AzM>

Video 57:34

Oct 6, 2016

● Chu, C.P., Hokamp, J.A., Cianciolo, R.E. et al.

RNA-seq of serial kidney biopsies obtained during progression of chronic kidney disease from dogs with X-linked hereditary nephropathy.

Sci Rep 7, 16776 (2017).

● Brinkmeyer-Langford C, Chu C, Balog-Alvarez C, Yu X, Cai JJ, et al. (2018)

Expression profiling of disease progression in canine model of Duchenne muscular dystrophy. PLOS ONE 13(3): e0194485.

This is a class recording of VTPP 638 "Analysis of Genomic Signals" at Texas A&M University.

No RNA-Seq background is needed, and it comes with a lot of free resources that help you learn how to do RNA-seq analysis. You will learn:

(1) The basic concept of RNA-sequencing

(2) How to design your experiment: library prep, sequencing depth, budgets, statistical power.

(3) The analysis pipeline

Up-to-date RNA-Seq Analysis Training/Courses/Papers (Dec 2017)

<http://www.biostars.org/p/174376/>

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| **Relevant journal publications** |
| |  | | --- | | **RNA-seq workflow: gene-level exploratory analysis and differential expression**  2015 Oct 14  <http://master.bioconductor.org/packages/release/workflows/vignettes/rnaseqGene/inst/doc/rnaseqGene.html> | | Abstract  Here we walk through an end-to-end gene-level RNA-Seq differential expression workflow using Bioconductor packages. We will start from the FASTQ files, show how these were aligned to the reference genome, and prepare a count matrix which tallies the number of RNA-seq reads/fragments within each gene for each sample. We will perform exploratory data analysis (EDA) for quality assessment and to explore the relationship between samples, perform differential gene expression analysis, and visually explore the results.  Keywords  RNA-seq, differential expression, gene expression, Bioconductor, statistical analysis, high-throughput sequencing, visualization, genomics |      |  | | --- | | **Introduction to differential gene expression analysis using RNA-seq**  September 2015 updated November 14, 2019  <https://chagall.med.cornell.edu/RNASEQcourse/Intro2RNAseq.pdf> |  |  | | --- | | **RNA-seq: Basic Bioinformatics Analysis**  Curr Protoc Mol Biol. 2018 Oct  <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6168365/> | | The workflow includes three parts:  (a) mapping sequencing reads to a reference genome or transcriptome;  (b) quantifying expression levels of individual genes and transcripts;  (c) identifying specific genes and transcripts that are differentially expressed between samples.  Alignment/Mapping:  the sequence of each read to a reference genome, annotation of genes  - **STAR** alignment tool  Quality Assessment:  After mapping reads to the genome, it is important to survey the quality of the RNA-seq data  - **Picard**  - **SAMTools**  Quantification of Mapped Reads:  - **HTseq** to quantify sequencing reads mapped to each gene  (a) identify genes that are differentially expressed between conditions (sample groups),  (b) derive gene expression values for each individual transcript  Approaches for normalization include  CPM (counts per million reads),  RPKM (reads per kilobase per million reads),  FPKM (fragments per kilobase per million reads),  TPM (transcripts per million reads).  Differential Gene Expression Analysis:  **EdgeR** (Robinson et al., 2010)  **DESeq2** (Love et al. 2014)  Download and install required tools:  STAR: <https://github.com/alexdobin/STAR>  Picard: <https://broadinstitute.github.io/picard/>  HTseq: <https://htseq.readthedocs.io/en/release_0.9.1/install.html>  R: <https://www.r-project.org> | |