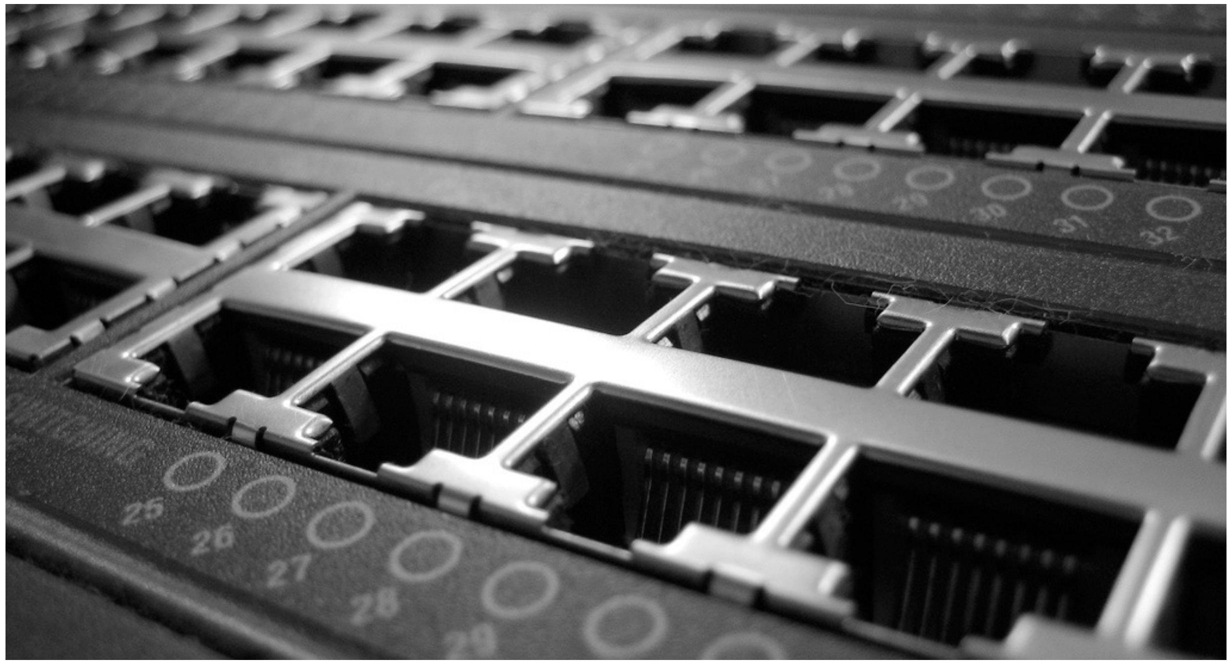



Introduction to RNA-sequencing Experimental Design and Analysis (CB321)

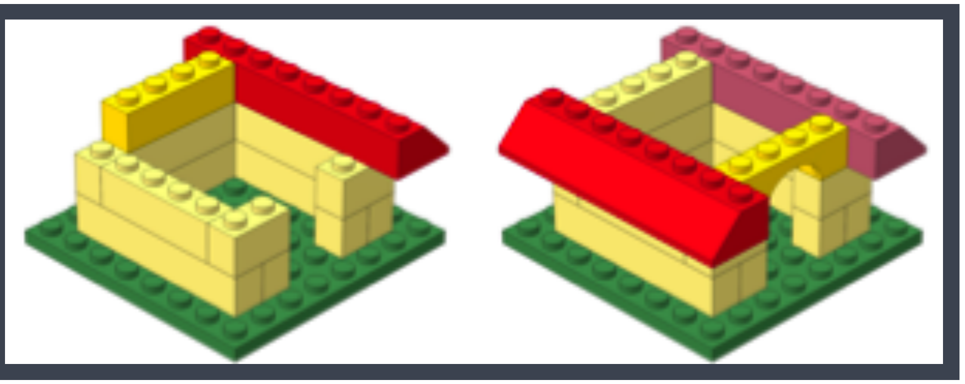
Sarah Boswell, PhD
Radhika Khetani, PhD

https://hbctraining.github.io/rnaseq-cb321/

Transcriptomics (RNA-seq)

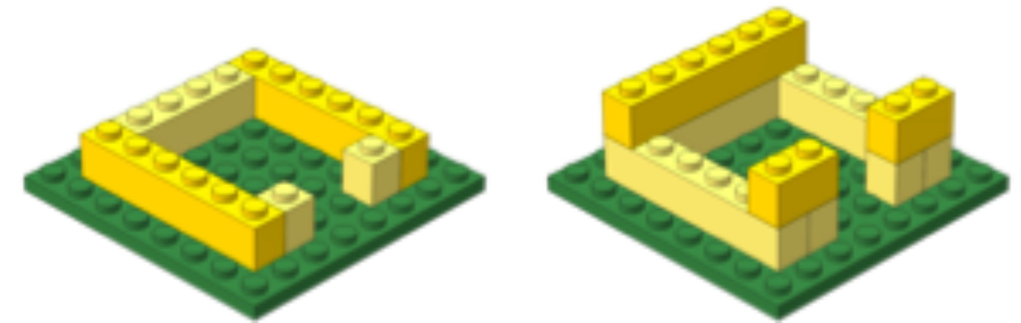
- RNA-seq is the process of sequencing the “transcriptome”
- Uses include:
  - Differential Gene Expression
  - Transcriptional profiling with single cells
  - Transcriptome assembly
  - Metatranscriptomics or community transcriptome analysis
  - Mechanisms of transcription looking at nascent transcripts
- Specific use of RNA-seq will determine experimental protocols, sequencing strategies, and analysis methods

Learning Objectives



- ✓ Describe sequencing by synthesis, including cDNA library preparation.
- ✓ Describe the design considerations to perform an **RNA sequencing experiment**.
- ✓ Describe the workflow for a standard bulk or single-cell RNA-seq data analysis.
- ✓ List computational skills necessary to implement the various steps in the analysis workflow.

Base components



- ✓ Unix/Linux shell
  - Dealing with large data files (e.g. FASTQ, BAM)
  - Using bioinformatics tools (e.g. Salmon, Trinity)
  - Accessing and using high-performance compute clusters (O2)
- ✓ R
  - Parsing and working with smaller results files
  - Statistical analysis, e.g. differential expression analysis (DESeq2) or single-cell analysis (Seurat)
  - Generating publication-quality figures from complex data (ggplot2)

Workshop webpage

https://hbctraining.github.io/rnaseq-cb321