**scRNASeq 2nd Day CourseOutline**

## Hour 0-1.5

* Overview
  + What is (bulk) RNASeq
* Process to FASTQ
  + experimental design
  + chemistry of library preparation
  + sequencing chemistry
* QC
  + Duplication rate
* Methods of analysis
  + Alignment vs. non-alignment methods
  + R/FPKM & TPM vs. DGE analysis
    - What is R/FPKM and TPM
  + DGE analysis overview
    - simplification of what the analysis does
    - What can you do with it?
      * Conditional contrasts (treatment comparison)
      * Linear modeling of more complex experimental design
* Case studies
* Transition to scRNASeq

## Hour 1.5-3.5

* Where is bulk RNASeq failing
  + Contrast what bulk RnAseq measures vs. scRNASeq measures
* scRNASeq overview
  + what questions can we ask with scRNASeq
* How does scRNASeq work
  + Chemistry of library preparation
    - 3’ mRNA sequencing
  + Contrast the 3 major technologies
  + Use 10x as detailed breakdown
    - chemistry
    - sequencing
* QC considerations
  + Sequencing quality
    - breadth and depth of gene sequencing
    - number of cells
    - optimizing read counts
  + multiplets
* Methods
  + why can’t we just use bulk RNASeq methods
  + overview of major use cases (5)
* Clustering
  + Two types: supervised & unsupervised
  + dimensionality reduction
    - PCA
    - tSNE and UMAP
  + how to cluster
    - biomarker based (manual)
    - kmeans, knn, etc.
      * considerations on interpretability of these methods
      * marker identification from clusters
* Conditional testing
  + DGE b/n clusters
    - MAST
      * how’s it different from DGE in bulk
* cell type transition / differentiation
  + manual
    - needs prior knowledge
  + algorithmic
    - ex: tradeseq (in R)
* demographic shift
  + seurat’s method
* deconvolution
  + case study example
  + importance of re-using old data
  + experimental design
    - re-create bulk in vitro with scRNASeq
    - use as priors for deconvolution
  + Walk through case study again
* Wrap up scRNASeq

## Hour 3.4-4

* Case Studies