Pilot: Single-Cell mRNA-seq

- Fluidigm C1 platform (Kelley lab)
- “Barnyard” experiment - mixing of human and mouse cells to test ability of platform to isolate single cells
  - Human: islets that are de-differentiated into mesenchymal stem cells, then induced to form islet-like clusters
  - Mouse: INS1 cells (mouse beta cell line)
Experimental Set-Up

- Medium-sized (10-17 um) Fluidigm 96-site IFC
  - Full-length mRNA-seq
    - *Before* doublet problem was addressed
- Cells stained for live/dead + species-specific markers
- ERCC spike-ins
- Batch 1: Smart-seq v2 chemistry
- Batch 2: Smart-seq v4 chemistry
Analysis

• Lori and Mike (Kelly) each scored each well by eye (from microscopy) - number of cells, species

• Reads quantified using pseudo-count method in Kallisto against merged human-mouse-ERCC transcriptome

• Pseudo-counts filtered to remove ECs with both mouse and human transcripts and with high missingness (47k human and 18k mouse ECs remaining)
Next Steps

• Sub-clustering of human and mouse cells
  • INS1 should be fairly homogeneous
  • Human ICAs may subdivide? - identify marker genes for each cluster, compare to known
  • Next experiment: single-nucleus sequencing using small IFC (5-10 um) to solve cell size heterogeneity problem
Neuronal subtypes and diversity revealed by single-nucleus RNA sequencing of the human brain

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RNA-sequencing from single nuclei

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