Hands-on stochastic profiling workshop

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Cells are awash in heterogeneity

- Only ~30% of human proteome has monospecific antibodies \((\text{Nat Biotechnol} \textbf{28}:1248-50 \ [2010])\)
- Spectral overlap beyond five colors for IF, smFISH \((\text{Nat Methods} \textbf{5}:877-9 \ [2008])\)
- Multiparameter single-cell assays (flow, mass cytometry) are designed for suspension cells
Challenges of single-cell expression profiling

• The “conversion” of RNA to a detectable entity ranges from 20% \((Nature \textbf{510}:363-9 [2014])\) to <5% \((Nat \textit{Methods} \textbf{11}:637-40 [2014])\)

• For transcripts less than ~200 copies per cell, data are overwhelmed by technical noise \((Nat \textit{Methods} \textbf{10}:1093-5 [2013])\)

• 90% of transcripts are thought to be expressed at <50 copies per cell \((Nat \textit{Methods} \textbf{11}:25-7 [2014])\)
Identifying molecular dichotomies by stochastic sampling

Gene A

100% of cells
1x expression level

Gene B

80% 20% of cells
1x 5x expression level

sample few cells, many times

Gene C

Counts

Avg. expression per sampling

Counts

Avg. expression per sampling

Janes et al., Nat Methods 7:311-7 (2010)
Stochastic profiling uncovers a wide range of regulatory heterogeneities

Advantages of 10-cell profiling

• **Increased starting material for extraction, reverse transcription, amplification, etc.**
  – Drastically improved technical reproducibility by avoiding Poisson noise floor
  – Deeper detection of low-abundance transcripts

• **More robust and efficient sampling of the overall population**
  – Filters rare regulatory states (<2% frequency) to highlight recurrent heterogeneities
  – More cells collected per $$ measurement (20 one-cell profiles vs. 20 10-cell profiles ~ 200 cells total)
Workshop learning objectives

• To understand the theory and implementation of stochastic profiling

• To implement individual facets of stochastic profiling in different experimental contexts

• To troubleshoot customized iterations of stochastic profiling for your own applications
Schedule

• Breakfast & lecture:  8:30 am until ~10 am
• Hands on:  ~10 am until completion

• Sunday:  sample preparation (KJ and CB)
• Monday:  LCM and poly(A) amplification (KJ and LW)
• Tuesday:  qPCR of poly(A) samples (CCW)
• Wednesday:  reamplification and labeling (KJ and LW)
• Thursday:  modeling and analysis (SB)
• Friday:  free time
• Saturday:  free time