Hands-on stochastic profiling workshop

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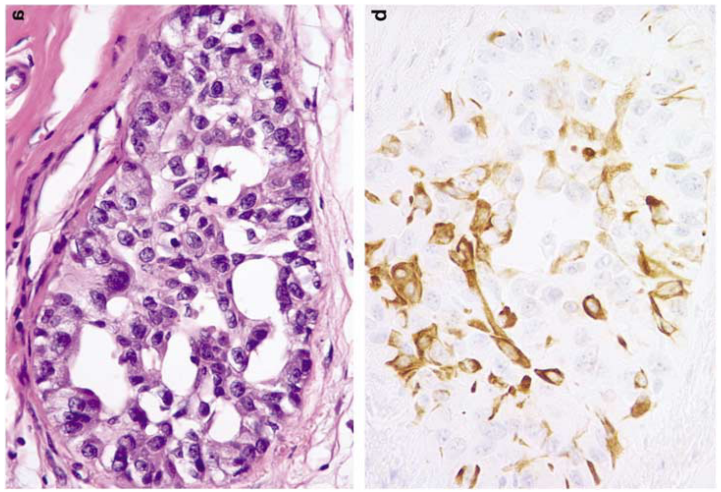
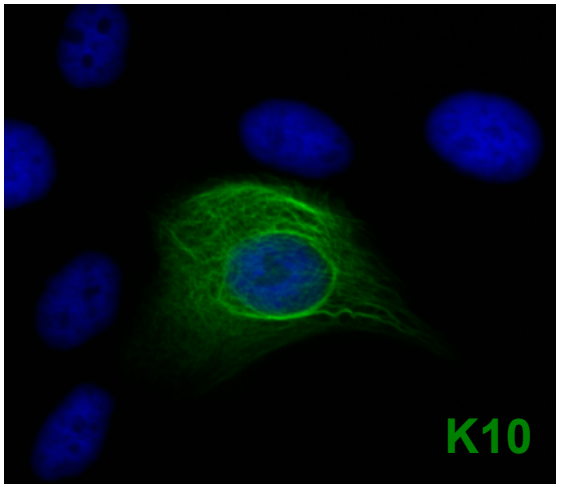
Cells are awash in heterogeneity

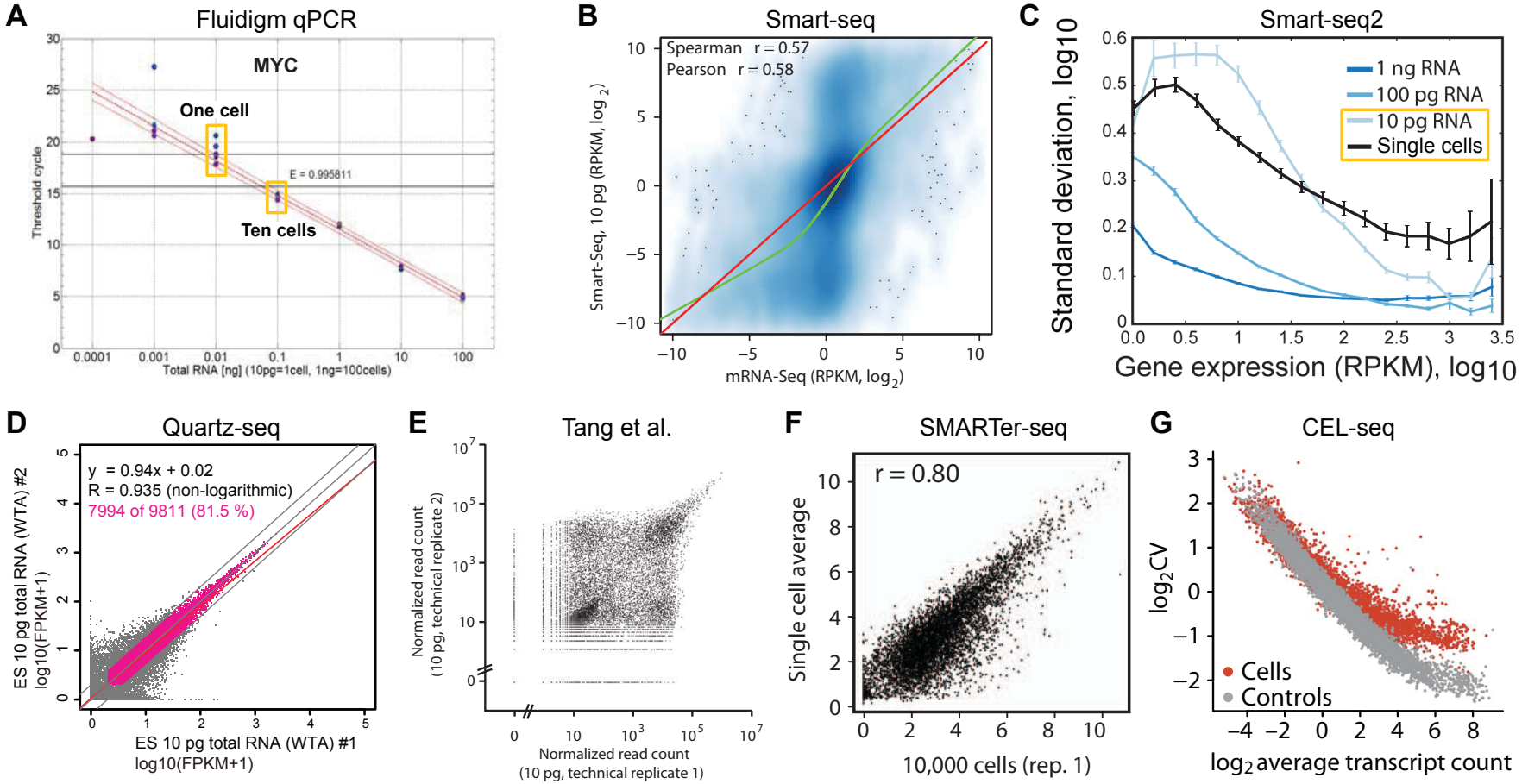


H&E KRT5 IHC



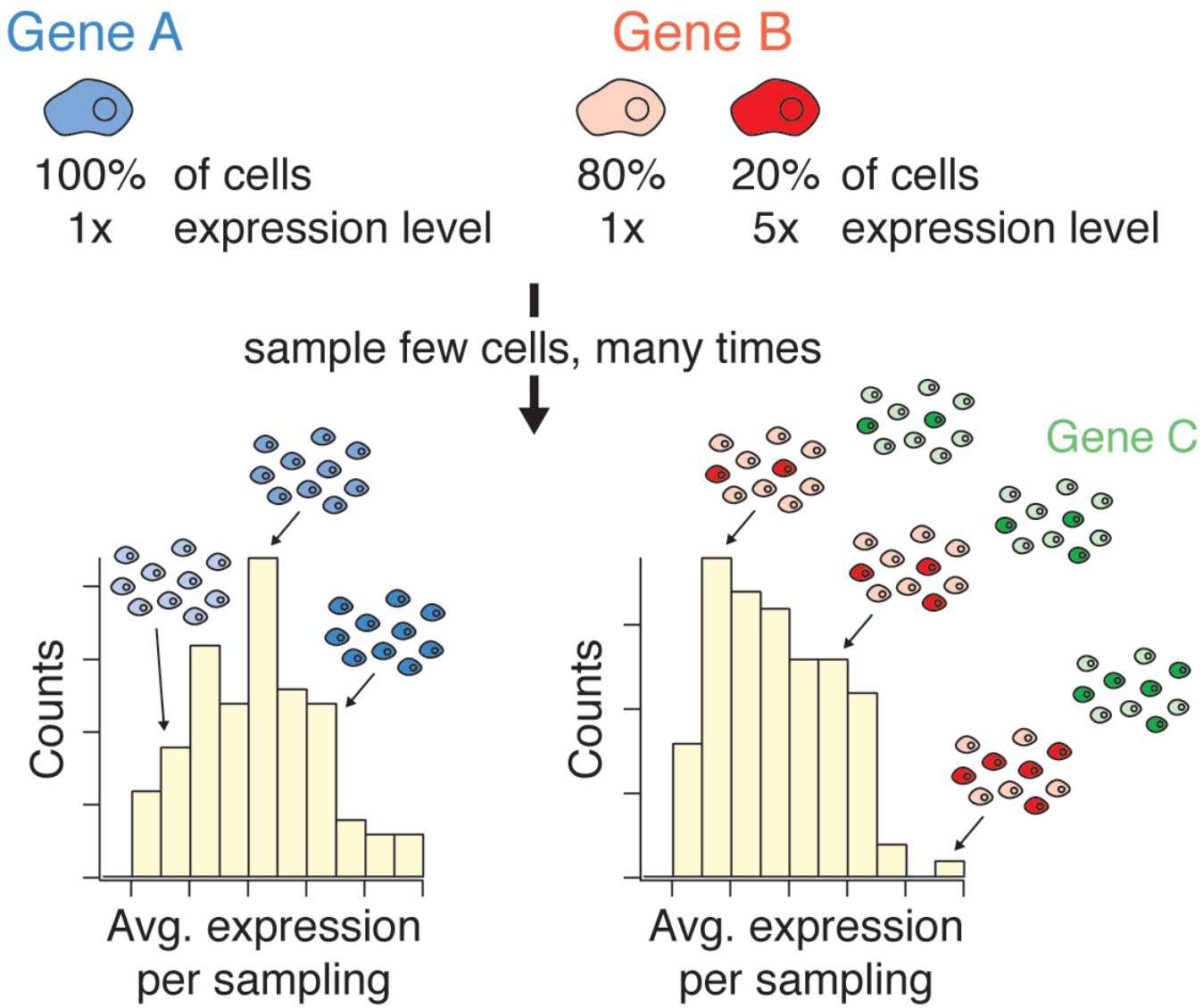
- Only ~30% of human proteome has monospecific antibodies (*Nat Biotechnol* **28**:1248-50 [2010])
- Spectral overlap beyond five colors for IF, smFISH (*Nat Methods* **5**:877-9 [2008])
- Multiparameter single-cell assays (flow, mass cytometry) are designed for suspension cells

Single-cell profiling of the transcriptome is technically irreproducible



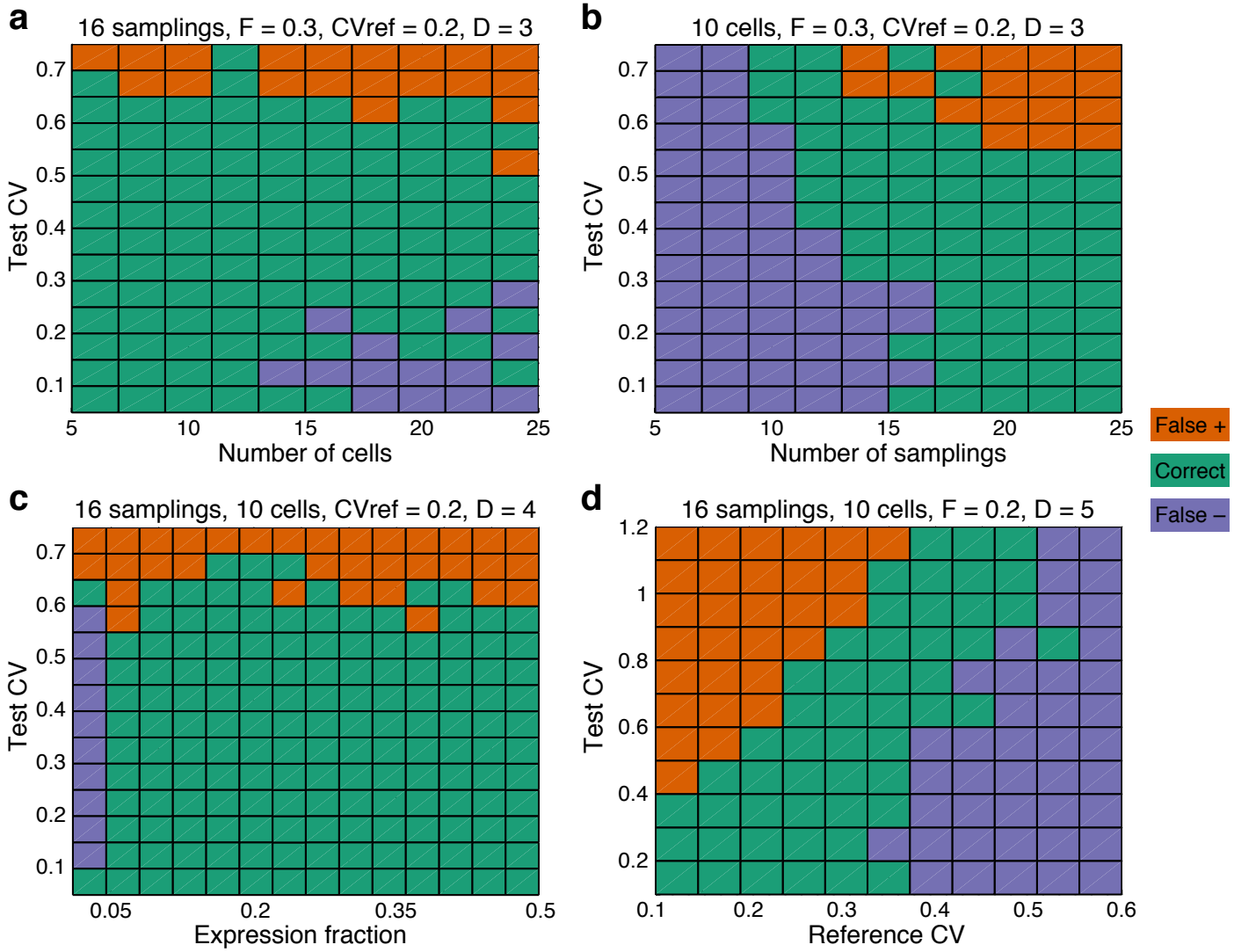
- "Conversion" of RNA to a detectable entity ranges from 20% (*Nature* **510**:363-9 [2014] to <5% (*Nat Methods* **11**:637-40 [2014])
- For transcripts <200 copies per cell, data are overwhelmed by technical noise (*Nat Methods* **10**:1093-5 [2013])
- 90% of transcripts are thought to be expressed at <50 copies per cell (*Nat Methods* **11**:25-7 [2014])

Identifying molecular dichotomies by stochastic sampling



Janes et al., *Nat Methods* **7**:311-7 (2010)

Stochastic profiling uncovers a wide range of regulatory heterogeneities



Wang and Janes, *Nat Protoc* **8**:282 (2013)

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 purification (KJ and LW)
- Thursday: modeling and analysis (SB)
- Friday: free time
- Saturday: free time