Lecture 10: Single-Cell RNA Sequencing

BIOINF3005/7160: Transcriptomics Applications

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Background

scRNA Protocols
- Cell Isolation
- Sequencing Protocols

Data Analysis
- QC
- Quantification
- Normalisation
- Clustering
- DE Analysis
- Trajectory Analysis

Spatial Transcriptomics

Background

Introduction

- scRNA-Seq is the 'latest and greatest' transcriptomic technique
- Previously all our analysis involved multiple cells per sample
- All were combined during tissue extraction, library preparation etc.
- Most experiments have **highly** heterogeneous cell populations, e.g.

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  - Different regions of the brain contain highly specialised cells
  - The immune system is highly complex
  - Cancer samples have both infiltrating and tumour cells

Introduction

- If a gene is increased 2-fold in expression:
  - Is this 2-fold in 100% of cells?
  - Or is it 4-fold in 50% of cells?
  - Or is it down 2-fold in 25% and up 8-fold in 25% and unchanged in 50%?
- Changes in gene expression can be highly specific to individual cell-types
- In general, determining heterogeneity of our samples is challenging

Introduction

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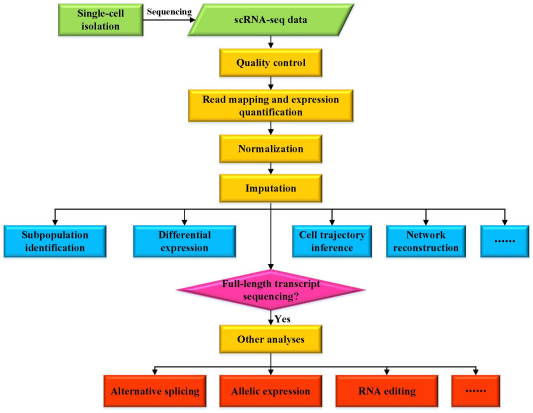
- Reality is much trickier than this
- How do we characterise which cell is which cell-type?
- How do we capture as many transcripts from each cell as we can?
  - Missing values are a huge issue in scRNA-seq
- How do we compare within the same cell-types between experimental groups?
  - E.g., treated and untreated cell types may not be assigned to the same cluster/cell-type

Workflow Outline



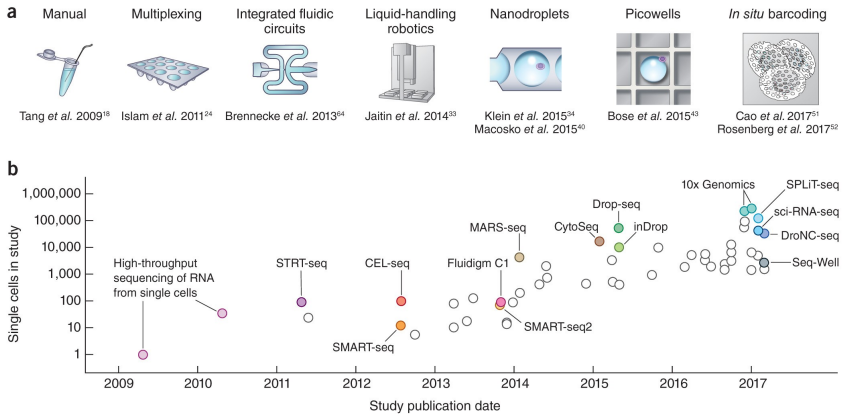
Taken from Geng Chen, Baitang Ning, and Tieliu Shi. "Single-Cell RNA-Seq Technologies and Related Computational Data Analysis". In: *Frontiers in Genetics* 10 (2019), p. 317. ISSN: 1664-8021. DOI: 10.3389/fgene.2019.00317

scRNA Protocols

Isolating Individual Cells

- Early protocols used a dilution series or manual isolation with a microscope (*micromanipulation*)
- Laser Capture Micro-dissection (LCM)
- Fluorescence-Activated Cell Sorting (FACS)
  - Labelled antibodies to specific surface markers
  - MACS is a magnetic-based approach
- Microfluidics/Droplet-based approaches

Protocol Timeline



Taken from V. Svensson, R. Vento-Tormo, and S. A. Teichmann. "Exponential scaling of single-cell RNA-seq in the past decade". In: *Nat Protoc* 13.4 (Apr. 2018), pp. 599–604

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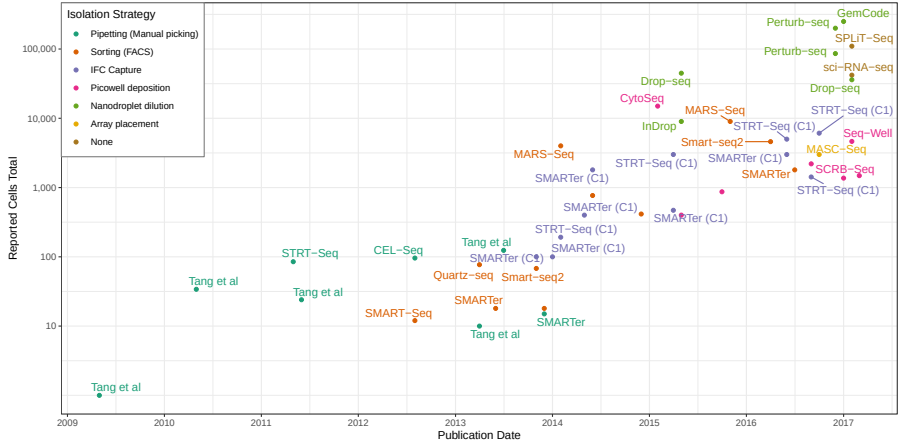
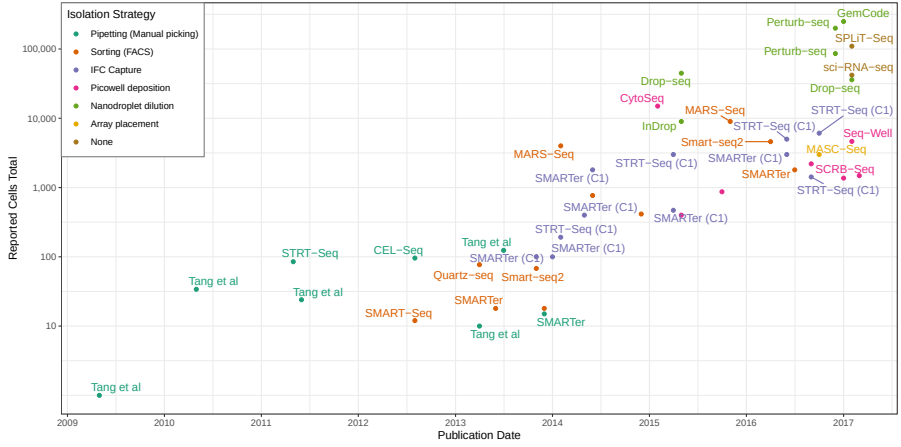


Data taken from Svensson, Vento-Tormo, and Teichmann, "Exponential scaling of single-cell RNA-seq in the past decade"

IFC Capture

- Integrated Fluidic Circuit (IFC) chips
  - Most common is the Fluidigm C1
- Deliver tiny volumes into 'reaction chambers'
- Early chips had 96 chambers $\implies$ multiple chips / experiment
- Recent chips handle $\sim$800 cells

Protocol Timeline



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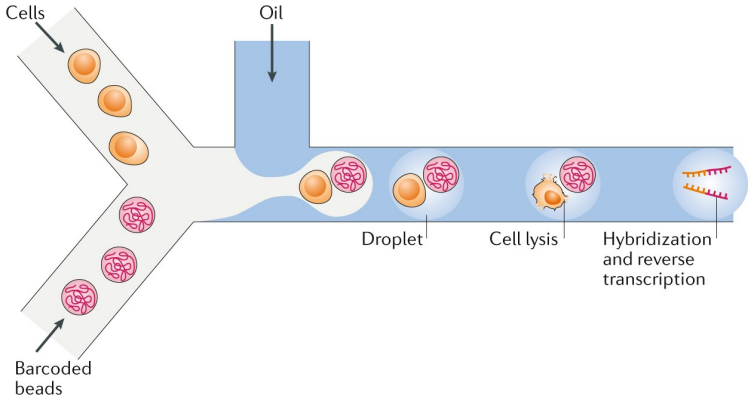
Droplet-based Approaches



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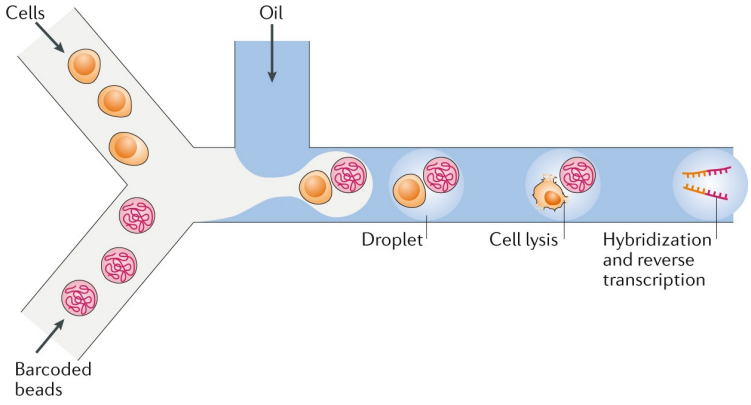

Flow rate is modelled as a *Poisson* process to minimise doublets

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Sequencing Overview

- Individual cells are isolated $\implies$ how do we sequence?
- Need a method to track which reads come from which cell
- Sequencing is performed on a standard Illumina machine, i.e. multiplexed
- Each cell is essentially an individual library prep
  - Barcodes / UMIs are used to identify individual cells

Data Analysis

Spatial Transcriptomics