Lecture 10: Single-Cell RNA Sequencing

BIOINF3005/7160: Transcriptomics Applications

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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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- Most experiments have **highly** heterogeneous cell populations, e.g.
  - 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

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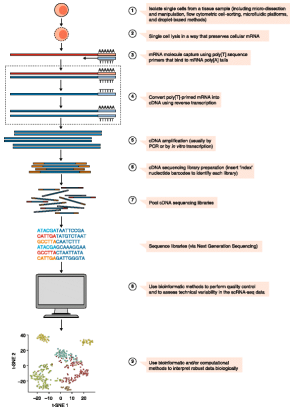
- Reality is much trickier than this
- How do we characterise which cell is which cell-type?
- How do we capture as many transcripts from each cell as we can?
  - Missing values are a huge issue in scRNA-seq
- How do we compare within the same cell-types between experimental groups?
  - E.g., treated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

Workflow Outline



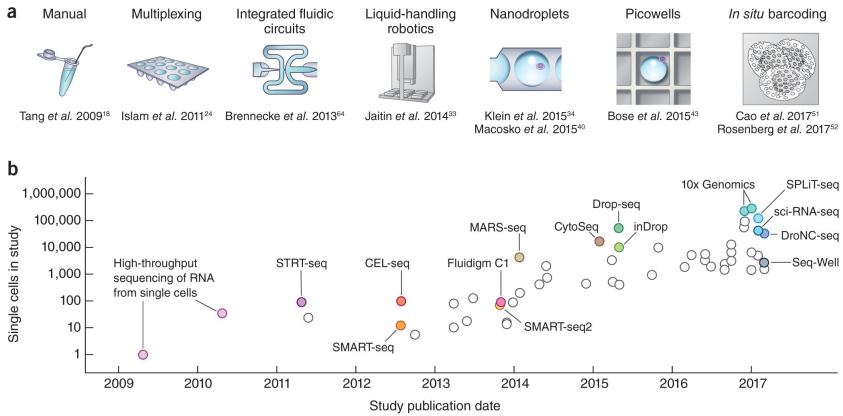
Taken from A. Haque et al. "A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications". In: *Genome Med.* 9.1 (Aug. 2017), p. 75

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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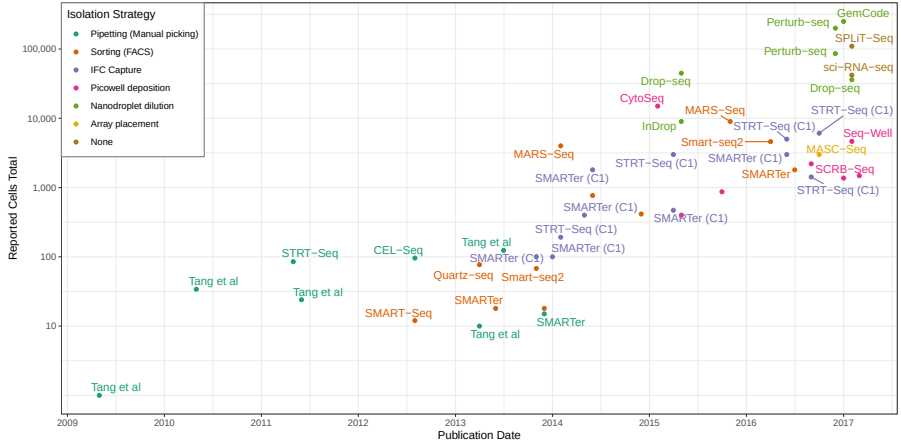

Data taken from Svensson, Vento-Tormo, and Teichmann, "Exponential scaling of single-cell RNA-seq in the past decade"

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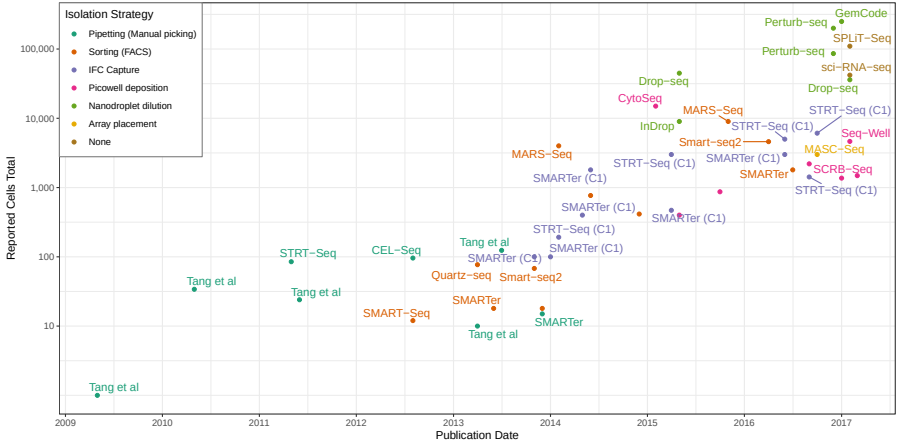
IFC Capture

- 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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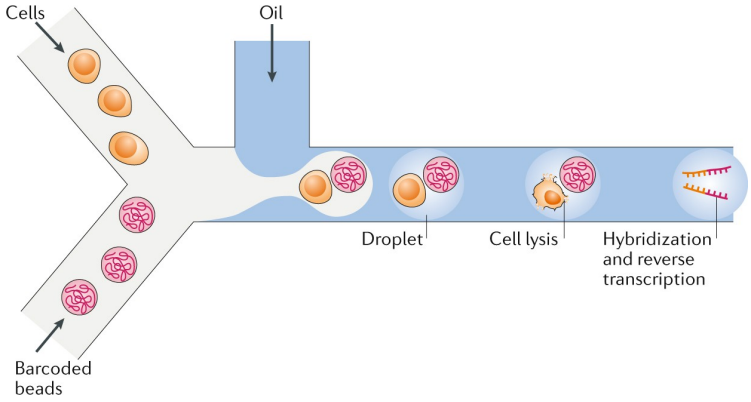
Droplet-based Approaches



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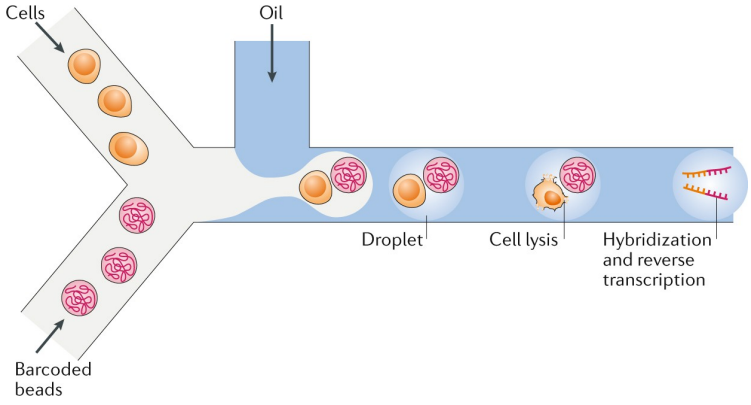
Droplet-based Approaches



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

Taken from Potter, "Single-cell RNA sequencing for the study of development, physiology and disease"

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 for cell / molecule identification
- For bulk RNA-Seq we need $0.1 - 1\mu$g of RNA ($10^5 - 10^6$pg)
  - An individual cell contains 1-50pg

SMART[1]-Seq (C1)

1. All reagents are in the IFC reaction chambers
2. Cells are lysed
3. polyA RNA reverse transcribed into **full length cDNA**
   - oligo(dT) priming and template switching
4. 12-18 PCR cycles
5. cDNA fragmentation and Adapter ligation

[1]SMART = Switching Mechanism at 5' End of RNA Template

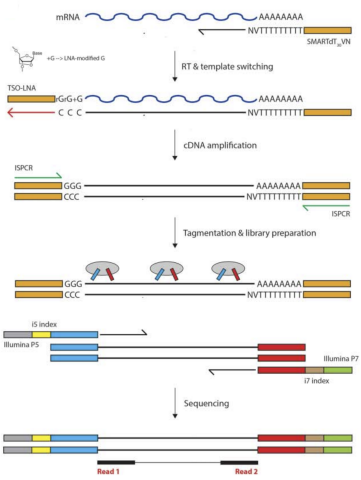


Image from S. Picelli. "Single-cell RNA-sequencing: The future of genome biology is now". In: *RNA Biol* 14.5 (May 2017), pp. 637–650

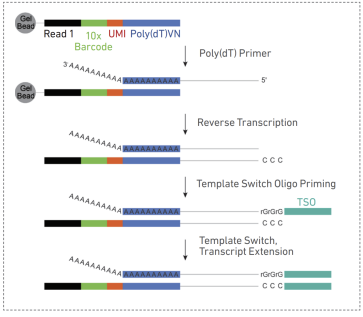
Droplet-based Methods

- Popularised by the 10X Genomics Chromium System
- Each gel bead contains the reagents
  - 30nt poly(dT) primer with 16nt 10x Barcode, 12nt UMI[2]
- Illumina primers and restriction enzymes added later

[2] Unique Molecular Identifier

10X Chromium Protocol

Inside individual GEMs

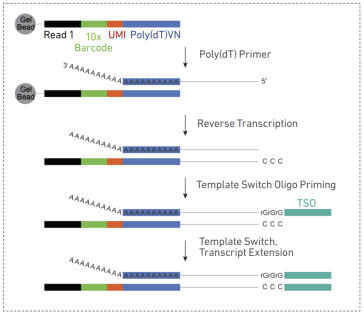


Barcoded, full-length cDNA is pooled then PCR amplified

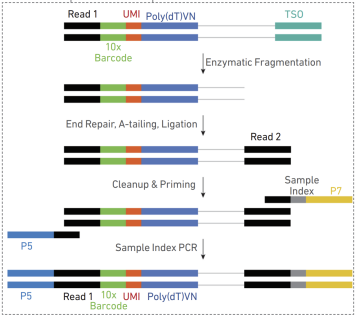
Images from 10X Genomics CG000204_ChromiumNextGEMSingleCell3_v3.1_Rev_D.pdf

10X Chromium Protocol

Inside individual GEMs



Pooled amplified cDNA processed in bulk



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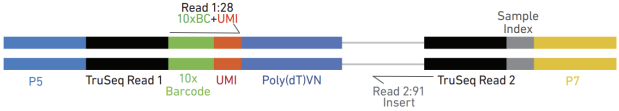
10X Chromium Protocol



- Only R2 contains the sequence information
- Only the 3' end is sequenced
- Each template RNA should have one UMI $\implies$ PCR duplicates can be identified

Other Variations

CITE-Seq[3]

- Prior to sorting cells can be 'labelled' with antibody-oligo complexes
- Oligos allow additional recognition of surface proteins
- On cell lysis these oligos are amplified along with RNA

[3] Cellular Indexing of Transcriptomes and Epitopes by sequencing

Other Variations

SPLIT-Seq[4]

- Cells are split into pools and fixed
- One barcode/pool
- Multiple rounds of pooling and barcoding
- All amplification is *in situ*
- Able to be applied to single nuclei

[4] Split-Pool Ligation-based Transcriptome Sequencing

Comparison of Methods

Protocol	**C1 (SMART-Seq)**	**SMART-Seq2**	**Chromium**	**SPLIT-Seq**
Platform	Microfluidics	Plate-based	Droplet	Plate-based
Transcript	Full-length	Full-length	3'-end	3'-end
Cells	$10^2 - 10^3$	$10^2 - 10^3$	$10^3 - 10^4$	$10^3 - 10^5$
Reads/Cell	10^6	10^6	$10^4 - 10^5$	10^4

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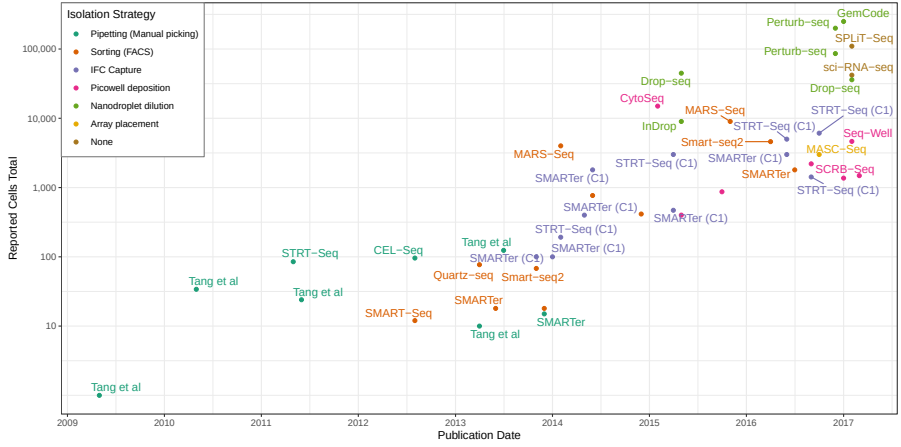

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Data Analysis

Spatial Transcriptomics