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BMEG3105
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Lecture 19: Single cell RNA sequencing (short)

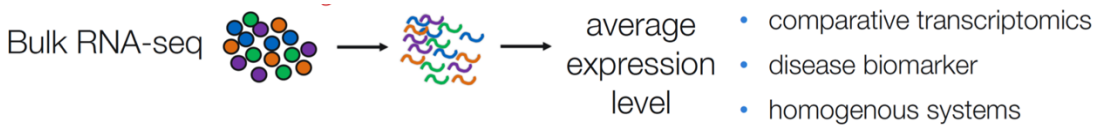
Expected outcomes:

- Understand the definition and purposes of single-cell RNA sequencing
- Method of getting single-cell RNA-seq
- Single-cell RNA-seq data analytics

Contents:

1. *Why single-cell analysis*

- Applications of bulk RNA-seq
  - Can only find disease biomarker at tissue level with lower resolution
  - Gene expression comes from different types of cells
  - Limited application to comparative transcriptomics and homogenous systems



- Applications of scRNA-seq
  - Study heterogeneity of tissues
  - Identify and analyse rare cell population
  - Study cell population dynamics (cell composition, dissection of temporal changes)

  Examples:
    - Differentiation paths
    - Cancer heterogeneity
    - Neural cell classification
    - Embryonic development
    - Drug treatment response

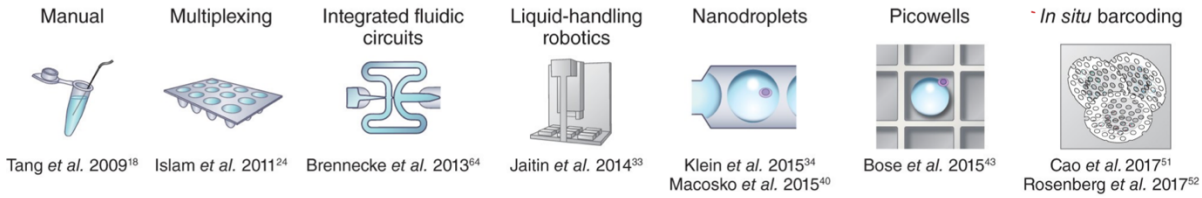
2. *Definition*

- Single cell sequencing examines the sequence information from individual cells with optimized next-generation sequencing (NGS) technologies, providing a higher resolution of cellular differences and a better understanding of the function of cellular differences and a better understanding of the function of an individual cell in the context of its microenvironment

3. *Method for scRNA-seq*

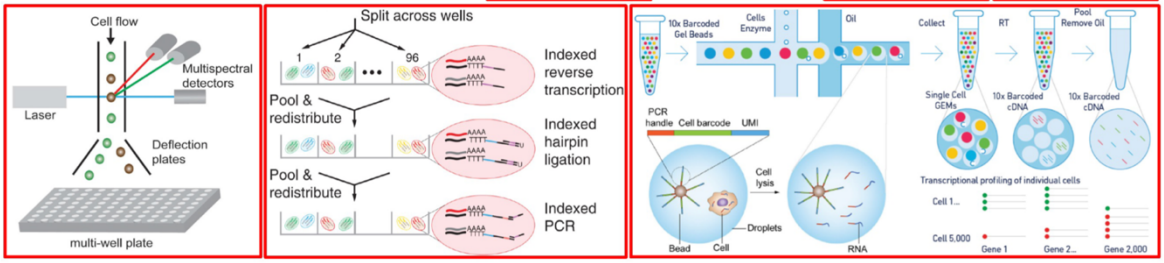
- Step 1: Single cell isolation

  Different methods



- Step 2: Sequencing

Paplexi et al. 2017	FACS	CyTOF	qPCR	Plate-based protocols (STRT-seq, SMART-seq, SMART-seq2)	Fluidigm C1	Pooled approaches (CEL-seq, MARS-seq, SCRB-seq, CEL-seq2)	Massively parallel approaches (Drop-seq, InDrop)
Cell capture method	Laser	Mass cytometry	Micropipettes	FACS	Microfluidics	FACS	Microdroplets
Number of cells per experiment	Millions	Millions	300–1,000	50–500 ★	48–96 ✖	500–2,000 ★	5,000–10,000 ★
Cost	$0.05 per cell	$35 per cell	$1 per cell	$3–6 per well	$35 per cell	$3–6 per well	$0.05 per cell
Sensitivity	Up to 17 markers ✖	Up to 40 markers ✖	10–30 genes per cell ✖	7,000–10,000 genes per cell for cell lines; 2,000–6,000 genes per cell for primary cells	6,000–9,00 genes per cell for cell lines; 1,000–5,000 genes per cell for primary cells	7,000–10,000 genes per cell for cell lines; 2,000–6,000 genes per cell for primary cells	5,000 genes per cell for cell lines; 1,000–3,000 genes per cell for primary cells



- Step 3: Data analytics
  - Pre-processing
    - Quality control
    - Normalization
    - Data correction (e.g. batch)
    - Feature selection
    - Dimensionality reduction
    - Cell-cell distances
    - Unsupervised clustering
    - …
  - Downstream analysis