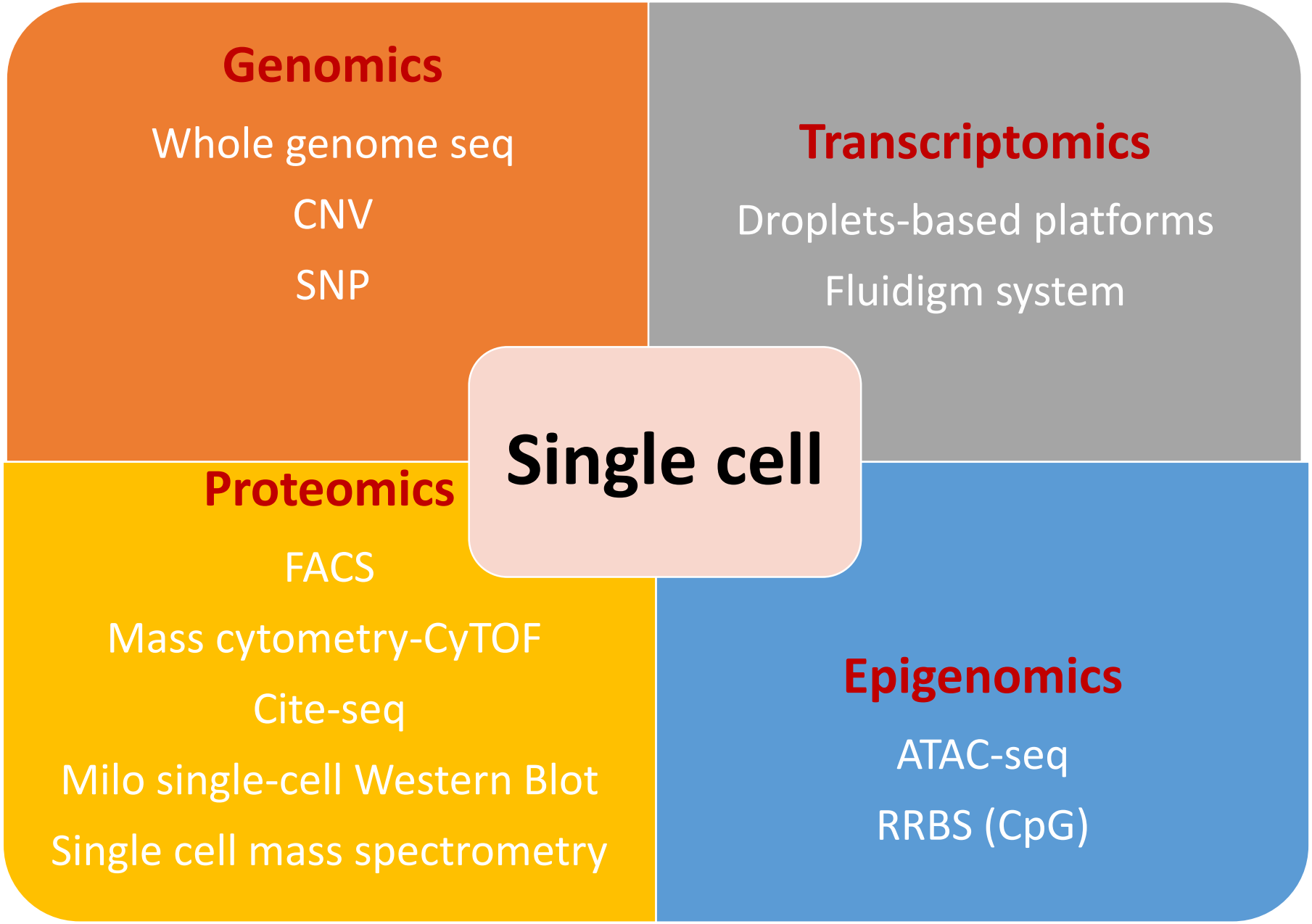
Platforms of Single Cell Analysis

Shanrun Liu

UAB CFCC Single Cell Core

Classification of single cell analysis

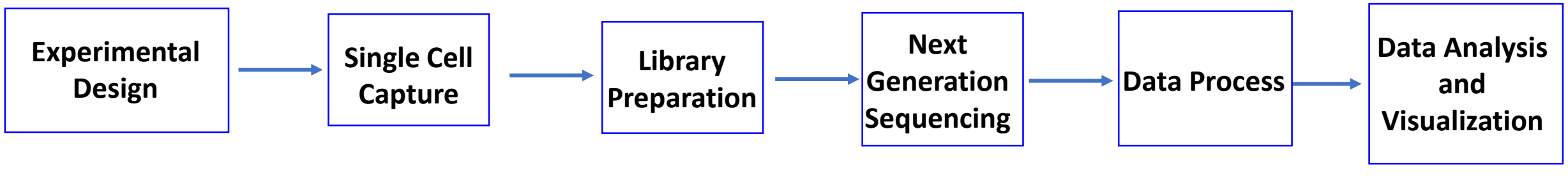


Objectives

- Understand single cell capture mechanisms of Fluidigm, BD Rhapsody and iCell8

- Understand the single cell analysis principle of Droplet-based systems

Single Cell Analysis Workflow



Investigators

aim, which platform to use, cell number, cost,

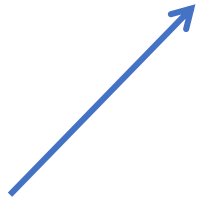
cell preparation

Facility

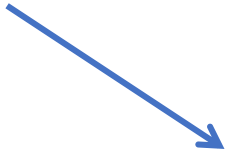
Bioinformatic people

Investigators and/or Bioinformatic people

Platforms for Single-Cell Transcriptomics

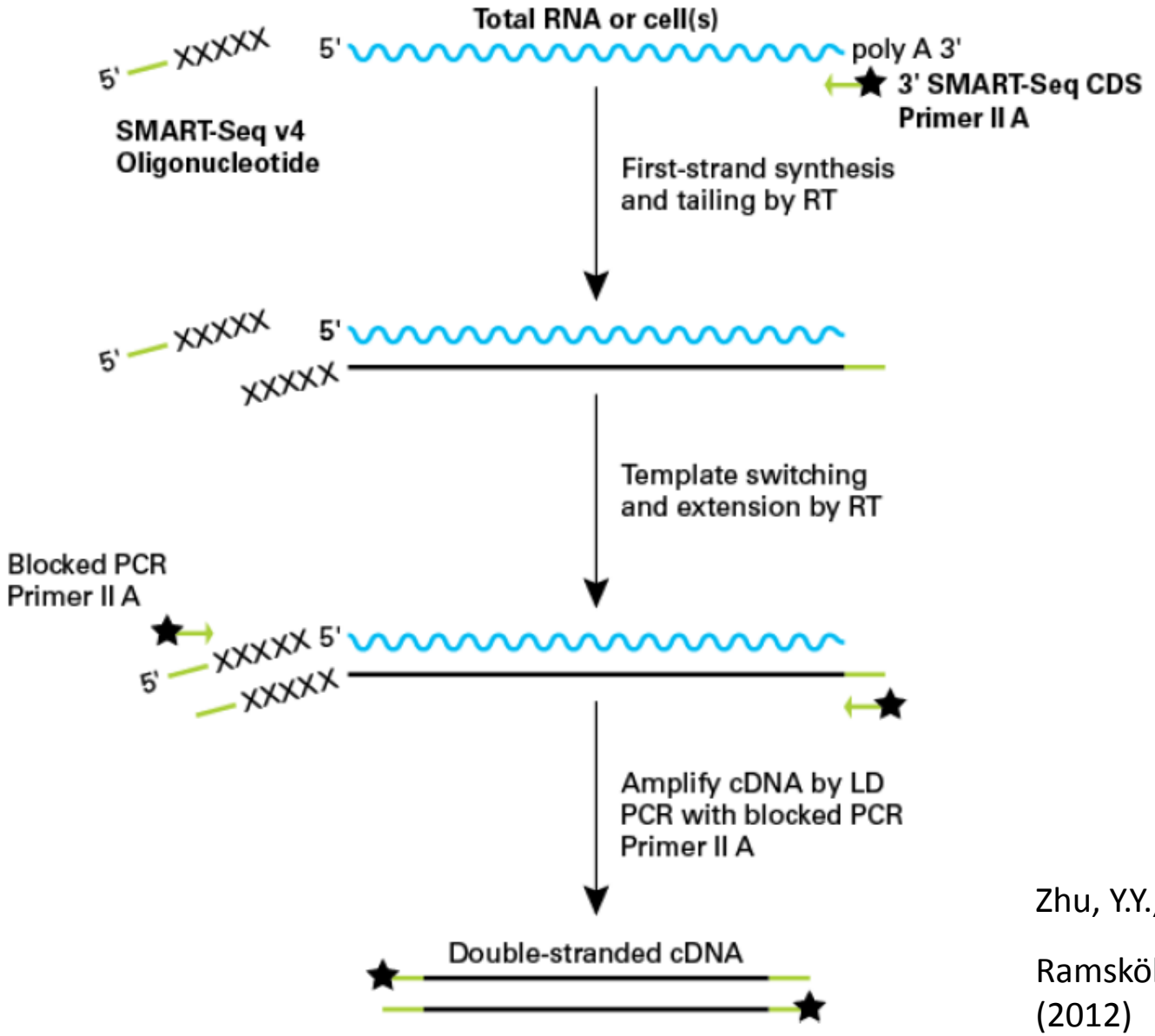


Oil Droplets: Drop-seq, Indrop, ddSeq, Dolomite, 10xGenomics



Microwells or Microchambers: Fluidigm, iCell8, BD Rhapsody, VyCap, Celsee, CellenOne, DEPArray

Template Switch



Takara SMART-Seq

Zhu, Y.Y., et al. *Biotechniques* 30, 892–897 (2001)

Ramsköld, D. *et al. Nat. Biotechnol.* 30, 777–782 (2012)

Fluidigm

Biomark HD

Targeted qPCR

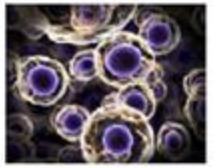
~8 hours total, 2 hours hands-on

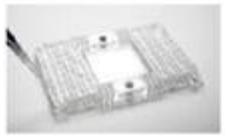
1 Enrich

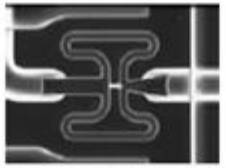
2 Load and capture

3 Wash, stain, and image

4 Lyse, amplify, and harvest







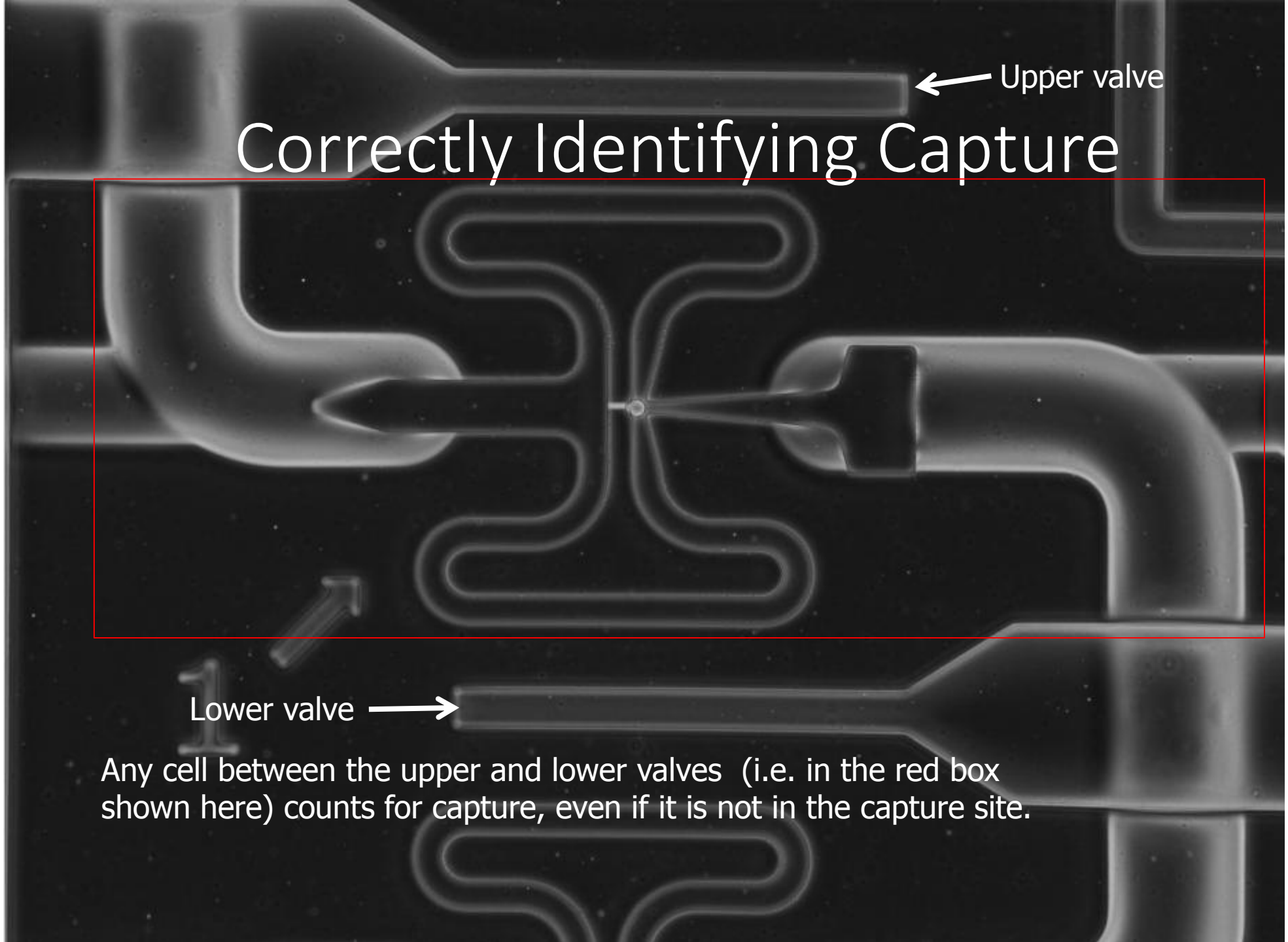
Targeted gene expression

Illumina Sequencer

Whole-transcriptome gene expression

C1 Auto-Prep

mRNA RNA-seq


Upper valve
Correctly Identifying Capture
Lower valve
Any cell between the upper and lower valves (i.e. in the red box shown here) counts for capture, even if it is not in the capture site.

BD Rhapsody System

Microwell cartridge

- Single Use
- Easy to load
- 200k+ microwells for capture of cells and beads

Complete Panel Flexibility

Pre-designed Panels

~400 targets selected and wet lab validated

- Breast Cancer – Oncology
- T-Cell Expression
- Immune Response Panels
- (Mouse and Human)
- Stem Cell
- Developmental biology, neurology and additional oncology panels to follow

Custom Base Panels

~200 – 400 targets per panel selected by users or with help from BDG

- Easy to use web tool for target selection and primer design for in silico verification
- Human and Mouse
- Additional genomes to come

Supplemental Panels

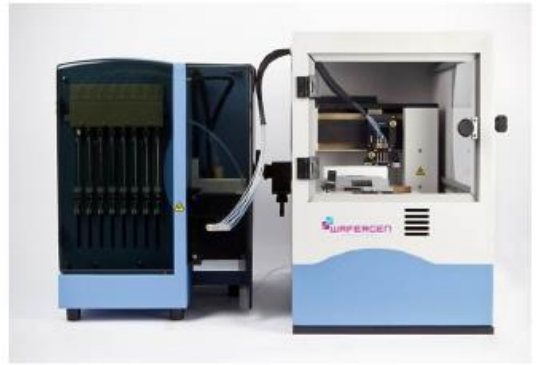
Add up to 100 additional targets as needed

- Customize pre-designed or custom base panels with additional targets
- Enhance coverage or correct issues easily and cost effectively
- Expand panels and explore unexpected targets iteratively, based on experimental results

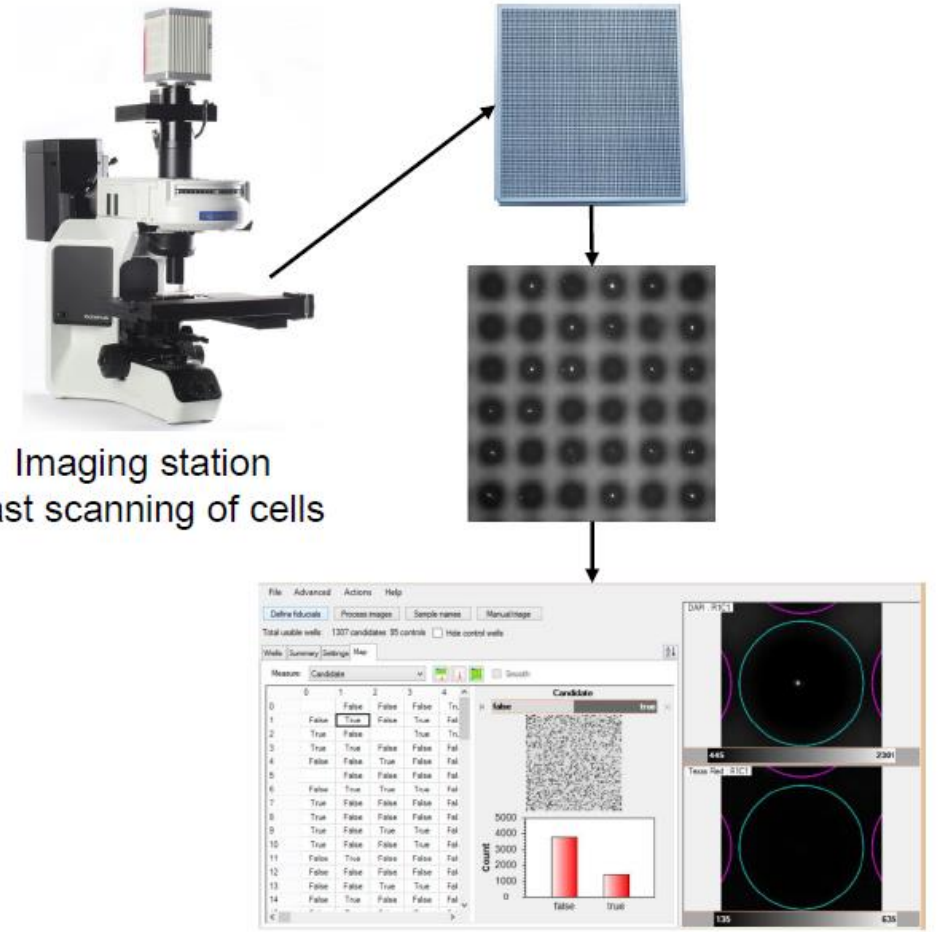
BD

ICELL8 Single-Cell System

ICELL8 chips and reagents
Pre-dispensed with 5,184 barcodes



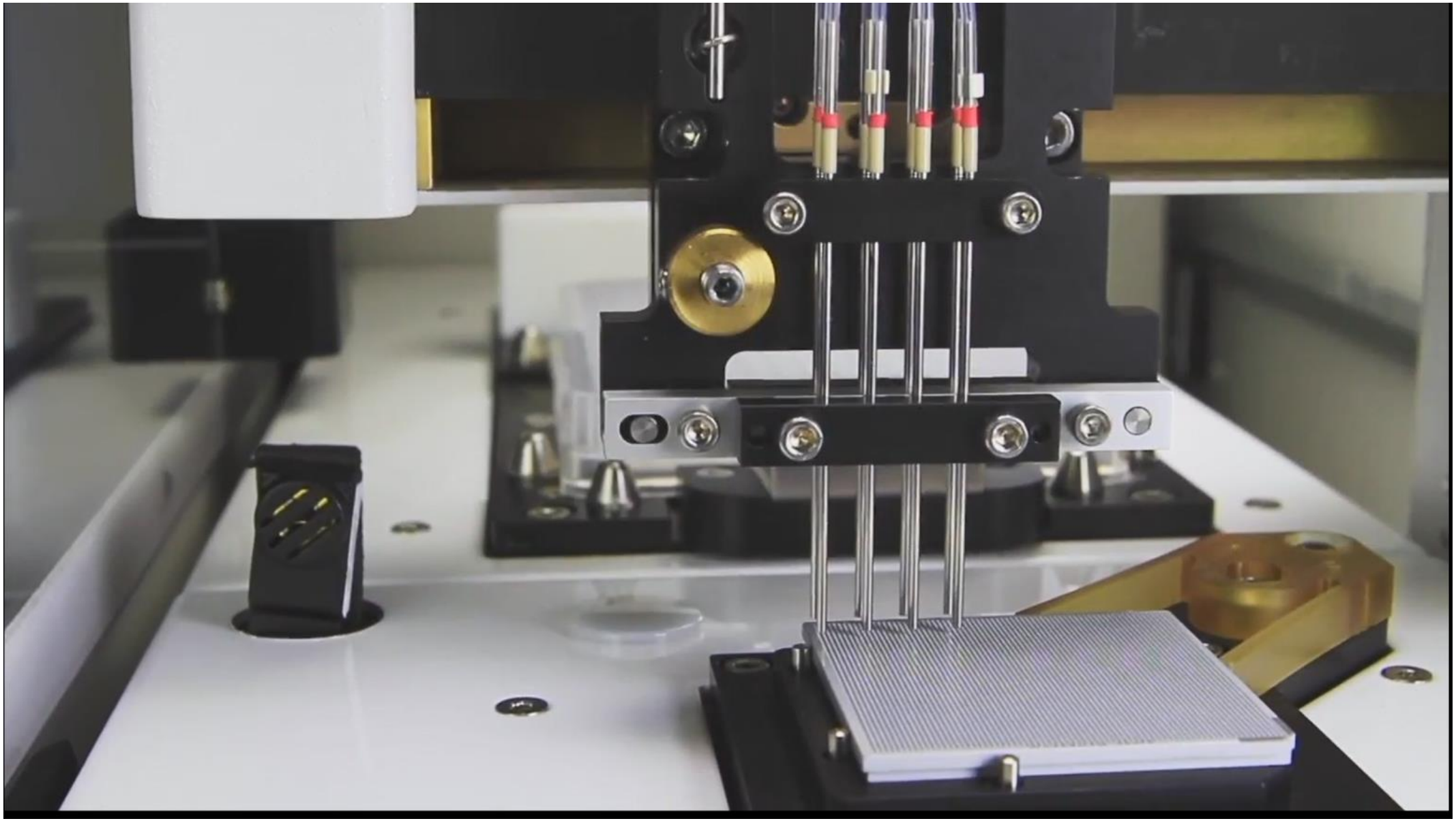
MultiSample NanoDispenser
Rapid dispensing of cells and reagents



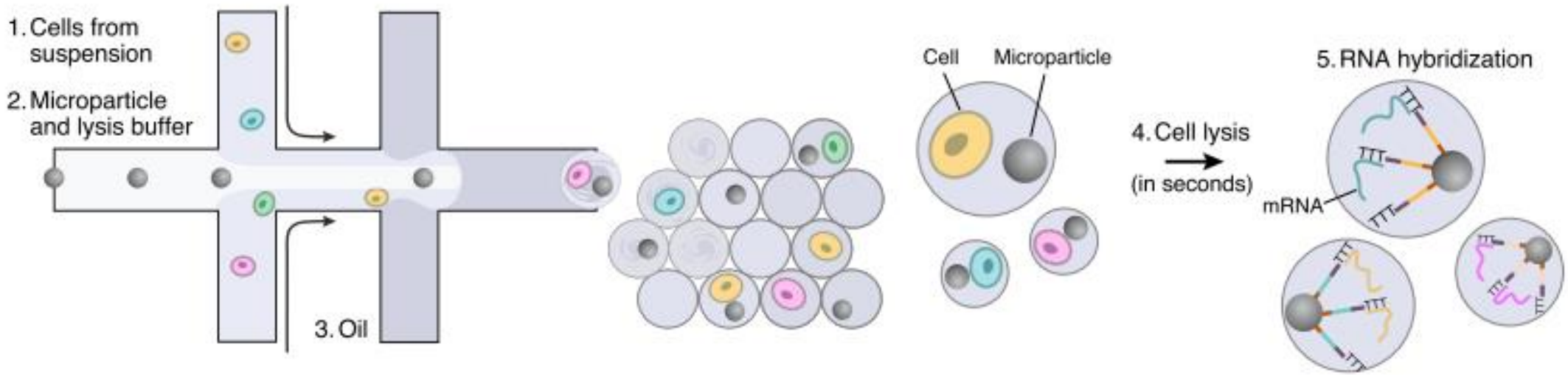
Imaging station
Fast scanning of cells

CellSelect™ software
Choose specific cells of Interest

Clontech TaKaRa cellartis

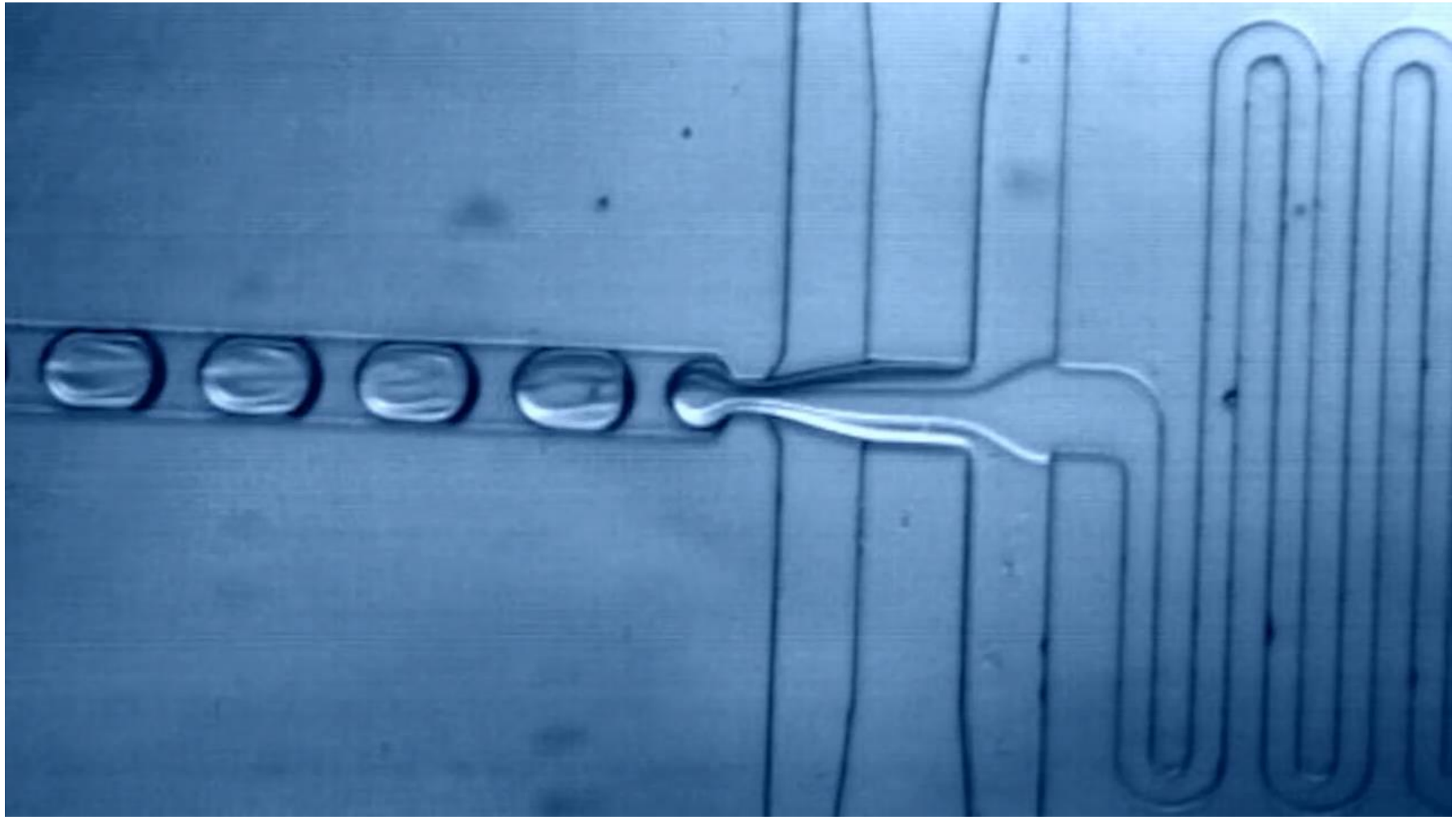


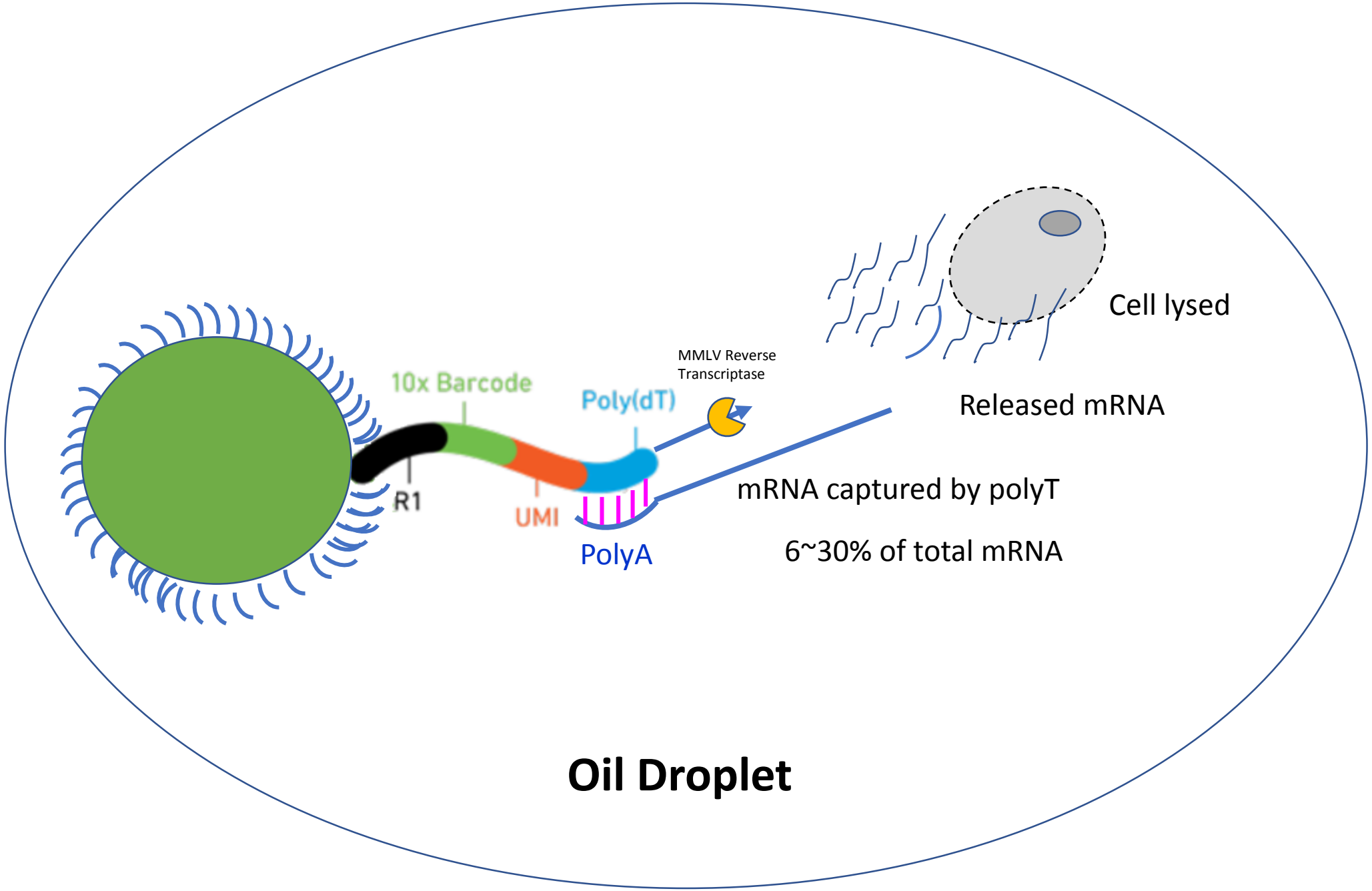
Droplets Generation



Cell, 2015, McCaroll *et al*

Droplets Generation




Cell lysed
Released mRNA
MMLV Reverse
Transcriptase
10x Barcode
Poly(dT)
R1
UMI
PolyA
mRNA captured by polyT
6~30% of total mRNA
Oil Droplet

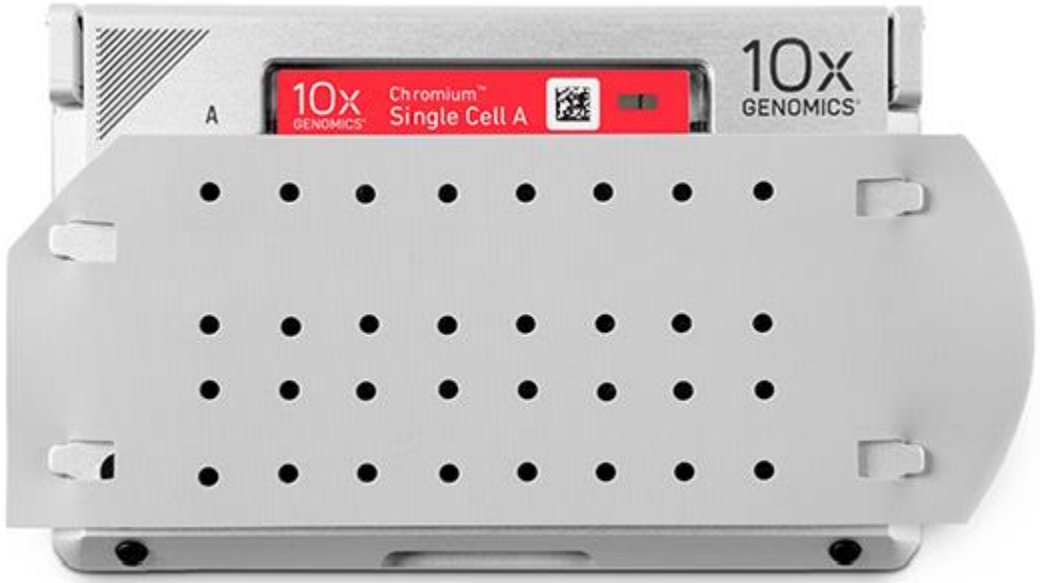
10xChromium Chip

100ul output GEMs

40ul Gel Beads

90ul master mix+cells

270ul Oil

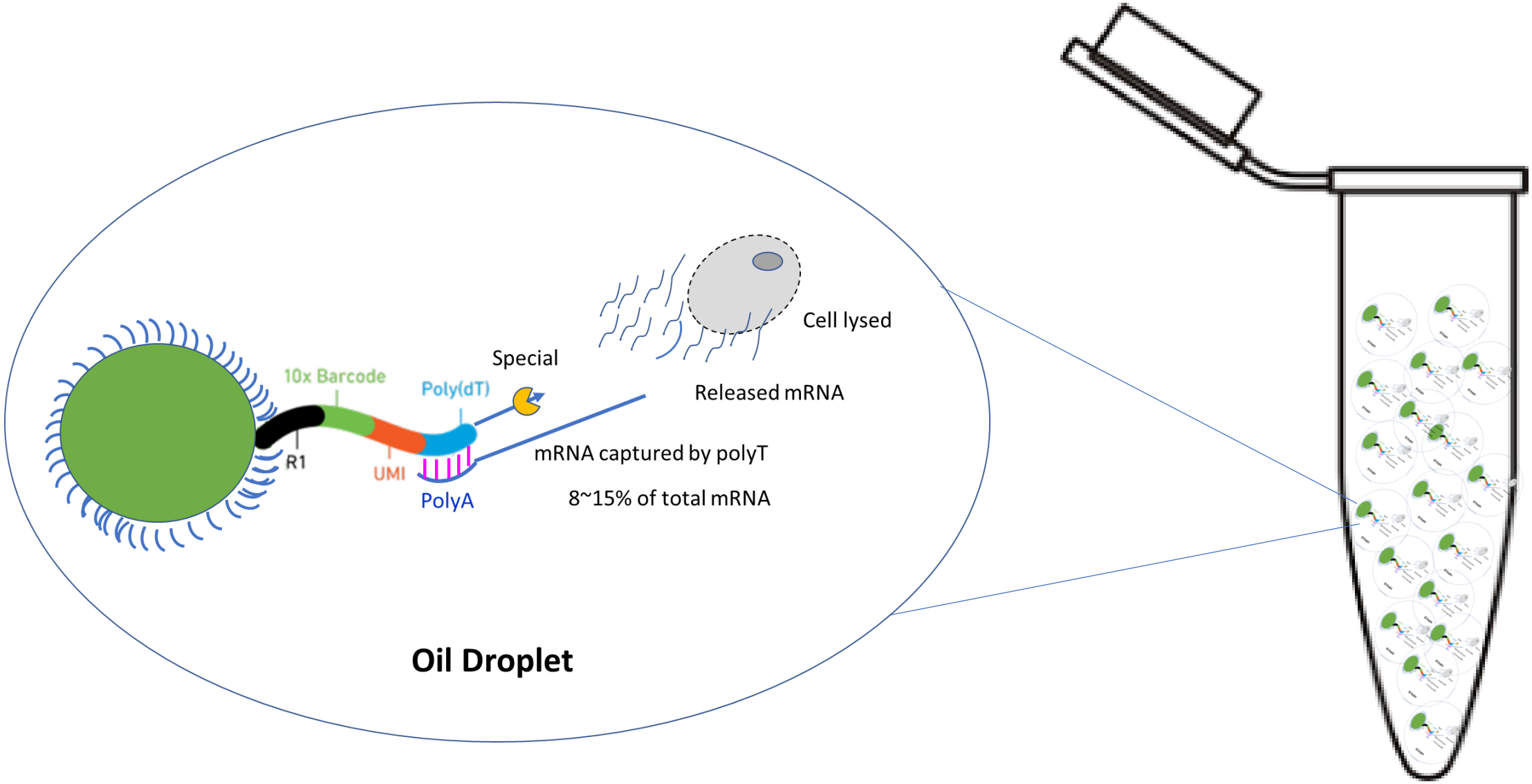


10xChromium Controller

Place the assembled Chip, 10x Chip Holder and 10x Gasket in the tray and press the button on the touchscreen to retract the tray

Confirm the Chromium Single Cell A program shows on the screen and press the play button to start the run

Perform RT Step in Droplets in Thermocycler



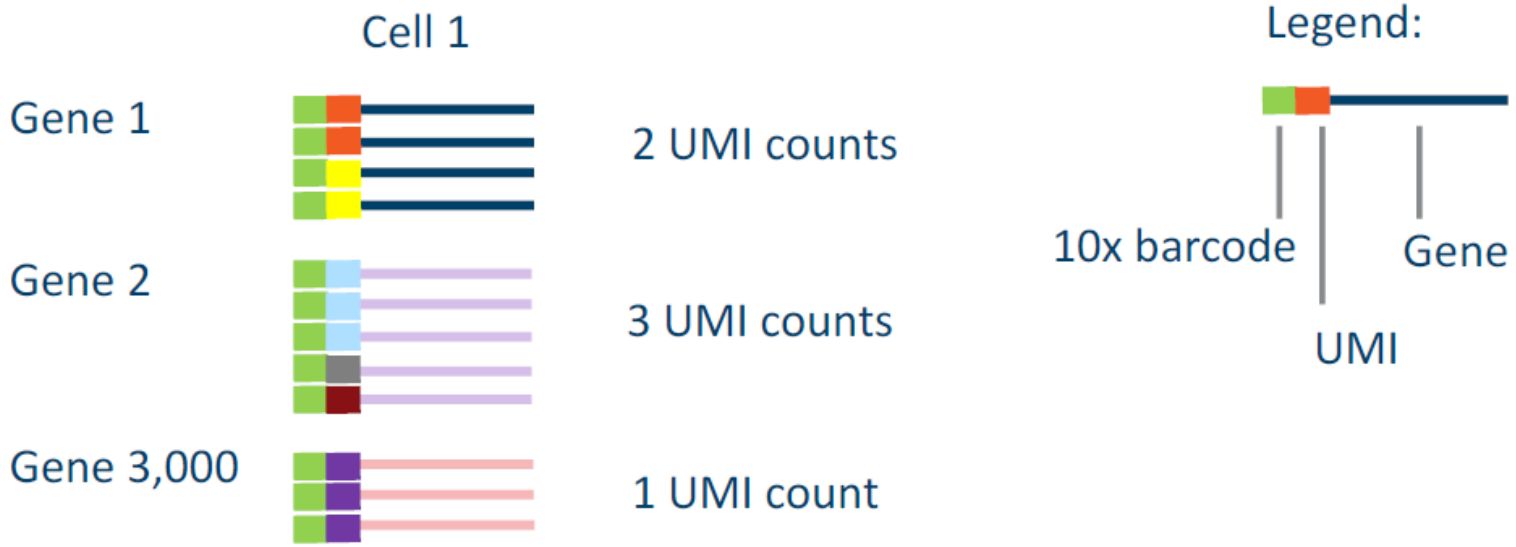
Assay Scheme for 3’ Digital Gene Expression

3’ biased

- Optimized reverse transcription for efficient generation of barcoded cDNA
- Unique molecular index (UMI) for accurate transcript quantitation

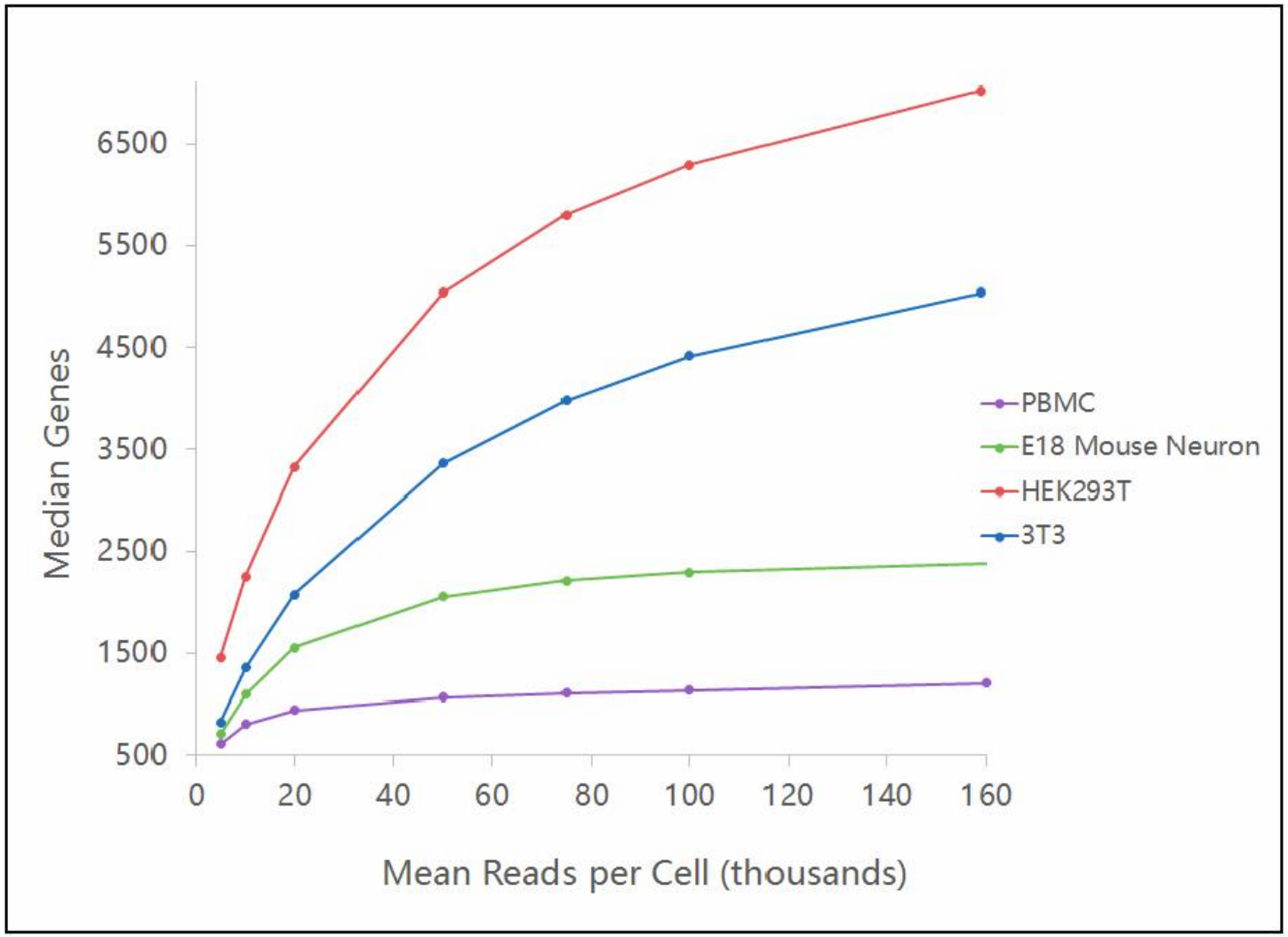
10bp UMI = Transcript Count

How many copies of the transcript are present in the single cell?



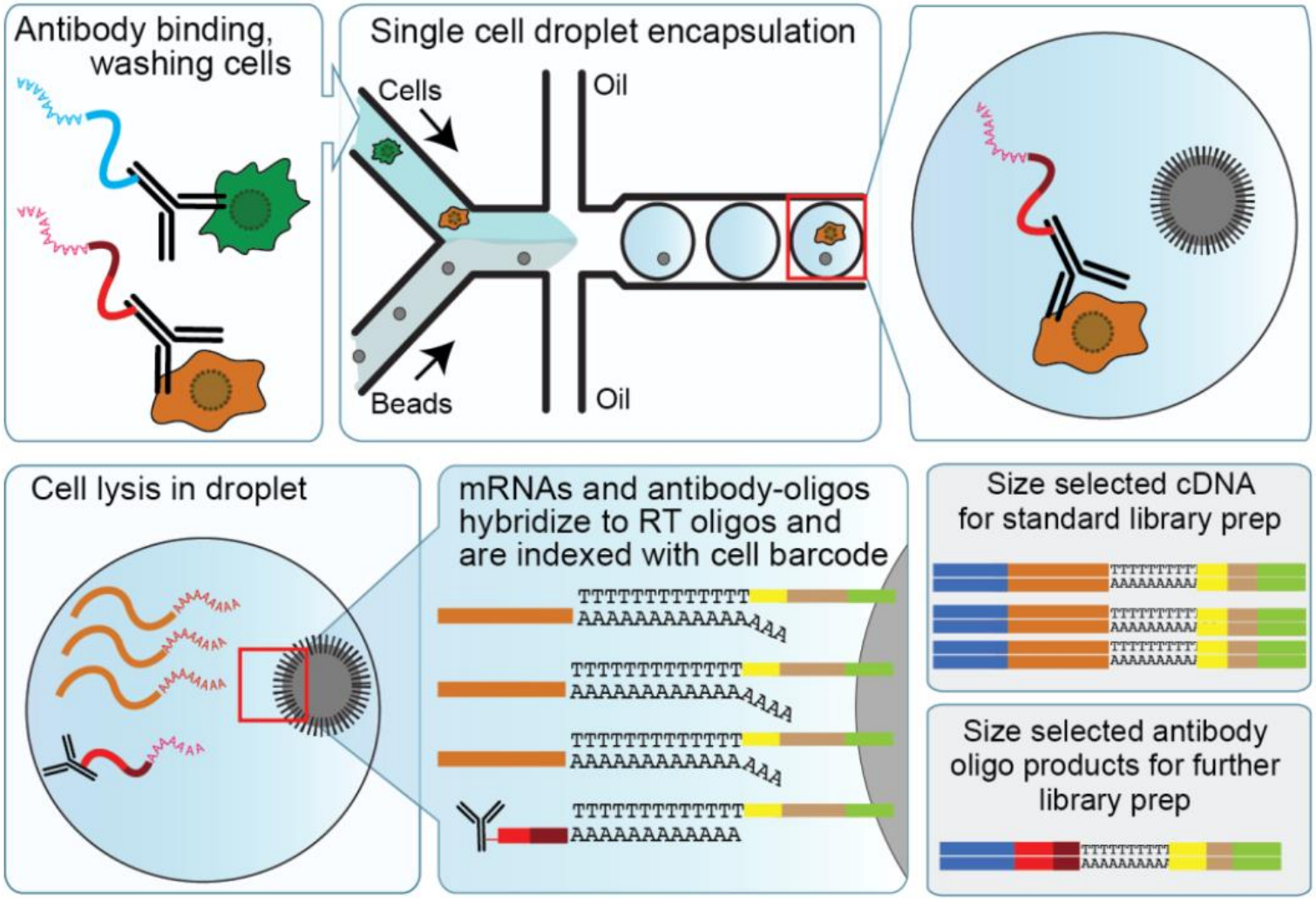
The 10bp UMI enables accurate quantitation of cell expression levels.

Sequencing Depth for Typical Samples



- 50,000 raw reads per cell is the recommended minimum sequencing depth for typical samples.
- Given variability in cell counting/ loading, extra sequencing may be required if the cell count is higher than anticipated.

Cite-seq



Ab-seq: Sci Rep. 2017 Mar, Shahi P et al.
Cite-seq: Nat Methods. 2017 Sep, Stoeckius M et al.
Reap-seq: Nat Biotechnol. 2017 Oct, Peterson VM et al.

https://cite-seq.com/

Single Nuclei mRNAseq

Detect mRNA in nuclei

Need isolate cell nuclei first

Especially useful for neural cells, frozen cells, and solid tissue

Less abundant than cytoplasmic mRNA

Enrichment of nascent, pre-processed transcripts in the nucleus

Relatively high proportion of intronic reads

Several Key Issues to Address

Sample preparation: Good single cell suspension, no fibers, no cell clumps or clusters, cell viability>90%. FACS sorting is very helpful. Do preliminary sample preparation and optimize the condition before the real experiment.

What fraction of mRNA transcripts are captured per cell?

3’ v1 kit-6.7-8.1%, v2 kit-14-15%, or v3 kit-30-32%.
Detection of a transcript is a stochastic event.

How many single cells should be analyzed?

Think about which illumine NGS system to use-for example, for NextSeq, one flowcell can generate 400x106 reads.

For 3’ V3 kit, or 5’ V2 kit, minimum 20,000 reads/per cell

One Nextseq can cover 400x106/2x104=20,000 cells

10xGenomics four-reaction kit can do four samples, so 20000 cells total/4 samples=5000 cells/sample

Cost: For four samples, 5000 cells/sample, total cost including sequencing (~$3200 depending where to sequence) is ~$10,000. This doesn’t include bioinformatic data analysis.