Cellular Multiverse in a Nutshell: Advanced Single Cell Multi-Omics Solutions

Julie Laliberte, PhD
Solutions for Single Cell Sequencing

Connecticut, U.S.A.
- North American Sales
- New Technology Development

Cologne, Germany
- Product Development
- EMEA Sales
- Service Laboratory

Suzhou, China
- Instrument Development
- GMP Manufacturing
- Clinical Laboratory

Nanjing, China
- Reagent Development
- GMP Manufacturing
- Clinical Laboratory

Singapore
- Pharma CRO
- APAC Sales

From single cell multi-omics to precision medicine
Why the need of info at single cell level?

Organs and tissues contain a high diversity of cell types.

- To identify the different cell types present in each tissue and gain insights in their role in the context of the tissue.
- Understand the changes in expression level in response to disease at single cell level.
- Understand differences in patient heterogeneity to drug responses.
- Study how cells respond to each other and to their microenvironment.

From single-cell multi-omics to precision medicine
From single-cell multi-omics to precision medicine

Single-cell Sequencing in Cancer Research

Heterogeneity of cancer cells

Tumor immune microenvironment

Immune checkpoint inhibitors

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What is a UMAP?

Is a method used to visualize high dimensional datasets in 2 dimensions.

https://pair-code.github.io/understanding-umap/
How to identify your cells clusters?

Gene 1
Gene 2
Gene 3
Singleron’s Technologies
GEXSCOPE scRNAseq Workflow

Tissue → Single-cell suspension → Cell Partitioning (Cell Lysis, mRNA Capture) → RT (cDNA Amplification, Library Construction) → Sequencing → Bioinformatic Analysis

PythoN Jr Matrix NEO
Single-Cell Sequencing Solutions: SCOPE-chip®
Tissue Preservation Solution

- can effectively preserve the tissue for 72 hrs at 4 °C. (contains no chemical fixatives)

QC:
- Necrosis
- Presence of Blood
- Quantity/Quality

Tissue Preservation Solution:
- Tissue Preservation Solution
- QC:
  - Necrosis
  - Presence of Blood
  - Quantity/Quality

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Example of the mouse skin

% Cell Viability

- fresh: 98%
- 24h: 97%
- 48h: 93%
- 72h: 88%

Pearson correlation of average expression of top 2000 variable genes, comparing with fresh samples

- 0h
- 24h
- 48h
- 72h

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**Tissue Dissociation Mix**
- can dissociate different types of tissues with high efficiency within 30-60 min

QC:
- Cell aggregation
- Debris
- Red blood cells
- Dead cells
- Accurate cell counting

Cell Viability (%)
Single Cell Partitioning

1. Tissue
2. Single-cell suspension
3. Cell Partitioning
4. Cell Lysis
5. mRNA Capture
6. RT cDNA Amplification
7. Library Construction
8. Sequencing
9. Bioinformatic Analysis

Cells are loaded into the chip. Based on size, only 1 barcode bead can occupy 1 well.

Barcode Beads are loaded into the chip.

The cells are lysed.
Capture of polyadenylated RNA with a unique Cell Barcode

Bead

PCR Handle

Cell Barcode 27 bases

UMI 12 bases

Poly T

3’

AAAAAAAAAA

3’UTR

Polyadenylated RNA

5’UTR

UMI: Unique Molecular Identifier
Single-Cell Sequencing Solutions: SCOPE-chip®
Reverse transcription, cDNA amp and Library Prep

1- The poly-A sequences from the mRNA binds the poly T

1- During the RT, the Cell barcodes are added to the mRNA
2- cDNA Amplification

~ 1500 bp

~ 450 bp
Barcode Structure For Pre-Processing

<table>
<thead>
<tr>
<th>P5</th>
<th>Barcode</th>
<th>UMI</th>
<th>poly(dT)</th>
<th>Insert</th>
<th>P7</th>
</tr>
</thead>
</table>

Read 1
150 cycles

Read 2
150 cycles

From single-cell multi-omics to precision medicine
CeleScope® - Barcode Structure For Pre-Processing

Read 1

5’-Adapter-BBBBBBBBBATCCACGTGCTTGAGABBBBBBBBBTTCAGCATCGGCTACGBBBBBBBBBBLNNNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3’

Cell Barcode
Linker
UMI
polyT

Chemistry | Pattern | Corresponding kit version
--- | --- | ---
scopeV3.0.1 | C9L16C9L16C9L1U12T18 | Magnetic Bead Kit V2
Data Analysis and Interpretation

Fastq files -> trim -> alignment to genome -> feature count and matrix -> QC metrics

CeleScope Software

SynEcoSys Database

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From single-cell multi-omics to precision medicine

Automation or manual chip loading

Matrix

Manual

VS

Kidney

- Early Proximal Tubule Cells
- Proximal Tubule Cells
- Apoptotic Cells
- Kidney Loop of Henle
- Monocytes
- Collecting duct intercalated cells
- Proximal tubule cells
- Kidney Loop of Henle
- CD8+ effector T cells
- Endothelial Cells
- Erythrocytes
- Plasma cells (B Cells)
- Stroma Cells
- CD4+ memory T cells
- Granulocytes-monocyte progenitor cells

Cell Percentage per Cluster

Sample
- mean_manual
- mean_matrix
Automation or manual chip loading

Matrix

VS

Manual

Spleen

Cell Percentage per Cluster - Spleen

- B cells
- CD8+ exhausted cells
- Regulatory T cells
- CD8+ effector memory T cells
- Monocytes
- Granulocytes-monocyte progenitor cells
- Plasma cells
- Erythrocytes
- CD8s
- CD4+ memory cells
- Osteoprogenitor cells
- pDCs
- Erythroid progenitor cells
- Endothelial cells

Sample
- mean_manual
- mean_matrix
Singleron’s Service
Singleron scRNAseq Service

Bringing groundbreaking single-cell analysis technologies to clinics

3000+ research projects

- 500+ customer organizations (~80% are hospitals)
- 170,000,000+ single cell sequenced
  - Tissue samples
    - Surgical samples
    - Biopsy samples
  - PBMC
  - Cell lines/primary cell culture
- Focus on human & mouse data

400 + different sample types*

- Lung tissue
- Liver tissue
- Ovarian tissue
- Prostate tissue
- Laryngeal tissue
- Glial tumor tissue
- Breast tissue
- Bone tissue
- Blood vessel

High success rate (94%)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Success Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical samples</td>
<td>97.3%</td>
</tr>
<tr>
<td>Biopsy samples</td>
<td>87%</td>
</tr>
<tr>
<td>PBMC</td>
<td>100%</td>
</tr>
</tbody>
</table>

Success rate of tissue samples

- Succeeded: 94.00%
- Failed: 6.00%

400 + different sample types*

- Prostate tissue
- Laryngeal tissue
- Glial tumor tissue
- Breast tissue
- Bone tissue
- Blood vessel

* Updated 03. 2021
Example of sample processed by the service lab in Cologne:

Nasal biopsy
Weight: not measurable
Storage: 24 - 36 hours
Cell viability: 95%
Cell number: less than 20,000

Estimated Number of Cells: 6,501
Fraction Reads in Cells: 63.36%
Median UMI per Cell: 4,784
Total Genes: 28,169
Median Genes per Cell: 1,652
Saturation: 78.2%

6,501 cells captured
Should I perform Single cells and/or single nuclei?
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Single cell and/or Single nuclei

Mouse Liver

Level4

UMAP2

UMAP1

- B cells
- Basophils
- Dendritic cells
- Endothelial cells
- Fibroblasts
- Hepatic stellate cells
- Hepatocytes
- Kupffer cells
- Macrophages
- NK cells
- Neutrophils
- Platelets
- T cells
- T memory cells

Single cells

Single nuclei

batch
Single cell and/or Single nuclei

Mouse Brain

From single-cell multi-omics to precision medicine
Model system to study human skin
EpiSkin (human skin model)

Cells were loaded on the SD chip

Tissue dissociation into single cell suspension
Multiomics applications
Singleron’s Single Cell Multi-Omics Products

Transcriptome profiling
- Single-Cell
- Single-Nucleus
- Yeast

Transcriptome + target sequences
- Lung/ Blood Cancers
- CHIP
- EBV Virus
- Customizable

Transcriptome + Glycosylation
- Detect Surface Proteoglycans
- Cell-Level Sensitivity

Transcriptome + V(D)J profiling
- CDR3 Profiling
- Full-Length TCR/BCR

Transcriptome With Temporal Resolution
- Cell- & Gene-Level Sensitivity
- Detect Newly Synthesized Transcripts

From single-cell multi-omics to precision medicine
GEXSCOPE® Variations: More Than Just mRNA Profiling

- mRNA profiling
- Immune V(D)J Profiling
- Full-Length Immunoreceptor Profiling
- SNVs, Fusion Genes, Rare Transcripts, And Viral Genes
- Glycosylation Levels
- Nascent RNA Synthesis

From single-cell multi-omics to precision medicine
Why monitor the glycan abundance at the surface of the cell?
- Cell – cell interactions
- Control T cells response: glycosylation at the heart of immune-unbalanced diseases?

Pereira MS. Frontier in Immunology, 2018
Cell surface glycosylation combined with transcriptome
Cell surface glycosylation combined with transcriptome

mRNA capture, RT, library preparation

sequencing, data analysis
During granulopoiesis in the bone marrow, distinct neutrophil granules are successively formed. Distinct receptors and effector proteins, many of which are glycosylated, are targeted to each type of granule according to their time of expression, a process called "targeting by timing."
**Single Cell Multiomics – Detection of somatic mutations**

<table>
<thead>
<tr>
<th>Lung cancer</th>
<th>Clonal hematopoiesis</th>
<th>Blood cancer</th>
<th>Epstein-Barr Virus</th>
<th>Custom</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>DNMT3A</td>
<td>WT1</td>
<td>EBNA1</td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td>TET2</td>
<td>KRAS</td>
<td>EBNA2</td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td>ASXL1</td>
<td>IDH1/IDH2</td>
<td>EBER1</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>JAK2</td>
<td>TP53</td>
<td>EBER2</td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>TP53</td>
<td>BCRABL1</td>
<td>ZEBRA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PML_RARA</td>
<td></td>
</tr>
</tbody>
</table>

*Specific mutation sites are targeted in the genes showed above.*

From single-cell multi-omics to precision medicine
Detection of Different KRAS Mutations

**Experimental setup:** NB4, CCRF and K562 were mixed in equal proportions.

**NB4** cell line contains **KRAS (A18D)** and **TP53 (R248Q)** mutations and **PML-RARA** fusion gene.

**CCRF** cell line contains **KRAS (G12D)** and **TP53 (R248Q, R175H)** mutations.

**K562** cell line contains **BCR-ABL1** fusion gene.
Detection of Different TP53 Mutations

**Experimental setup:** NB4, CCRF and K562 were mixed in equal proportions.

**NB4** cell line contains KRAS (A18D) and TP53 (R248Q) mutations and PML-RARA fusion gene.

**CCRF** cell line contains KRAS (G12D) and TP53 (R248Q, R175H) mutations.

**K562** cell line contains BCR-ABL1 fusion gene.

Cell Annotations

**R175H TP53**

**R248Q TP53**
Detection of translocations

**Experimental setup:** NB4, CCRF and K562 were mixed in equal proportions.

**NB4** cell line contains **KRAS (A18D)** and **TP53 (R248Q)** mutations and **PML-RARA** fusion gene.

**CCRF** cell line contains **KRAS (G12D)** and **TP53 (R248Q, R175H)** mutations.

**K562** cell line contains **BCR-ABL1** fusion gene.
**Sensitivity in detecting translocations**

*K562* cell line contains **BCR-ABL1** fusion gene.

*3T3* cell line **doesn’t contain** BCR-ABL1 fusion gene.

<table>
<thead>
<tr>
<th>Input Ratio</th>
<th>Total Captured Cell Number</th>
<th>3T3 Cells</th>
<th>K562 Cells</th>
<th>Fusion Gene Detected in K562</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:1</td>
<td>6188</td>
<td>5240</td>
<td>948</td>
<td>941 (99%)</td>
</tr>
<tr>
<td>100:1</td>
<td>7498</td>
<td>7362</td>
<td>136</td>
<td>136 (100%)</td>
</tr>
<tr>
<td>1000:1</td>
<td>5826</td>
<td>5816</td>
<td>10</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

From single-cell multi-omics to precision medicine
GEXSCOPE® Variations: More Than Just mRNA Profiling

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- Immune V(D)J Profiling
- Full-Length Immunoreceptor Profiling
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- Nascent RNA Synthesis

From single-cell multi-omics to precision medicine
Learn how the immune system responds and evolves in relation to diseases.

Help decide on treatments and understand disease progression or response to drugs.

Understand the pathway of activation related to specific clonotypes.

Engineer immune cells to destroy cancer cells: T cell receptor (TCR) T cell therapy

Example of TetTCR-Seq

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From single-cell multi-omics to precision medicine

T cell Repertoire

**A**

- **Vβ x 52**
- **Dβ1**
- **Jβ1x6**
- **Cβ1**
- **Dβ2**
- **Jβ1x7**
- **Cβ2**

**TRA**

**TRB**

- Germline DNA
- D-J joining
- V-D-J joining
- Rearranged DNA
- TCR β mRNA

**B**

- 5’
- **CDR1**
- **CDR2**
- **CDR3**

**TCR β mRNA**

5’

**CDR: complementarity-determining regions**

**C**

- **β Chain**
- **α Chain**

**Transmembrane domain**

**Constant domain**

**Variable domain**

**TCR library**

**Index(17) P7**

**P5**

**CCC TSO**

**C1 D1 V**
sCircle: Full length immunoreceptor sequencing

From single-cell multi-omics to precision medicine

- Transcription library construction
- cDNA Circularization & VDJ Enrichment

- Obtain Full Length cDNA
- RT-PCR
- C DNA
- Cell Barcode
- UMI
- FL-seq read 1
From single-cell multi-omics to precision medicine

**Immunoreceptor Enrichment**

- Premade primers available for human and mouse
- Primers can be customized for any species

**Library Preparation**
sCircle – human PBMC
sCircle – Mouse PBMC

From single-cell multi-omics to precision medicine
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From single-cell multi-omics to precision medicine
From single-cell multi-omics to precision medicine

DynaSCOPE®: Adding time-resolution to single-cell RNA-seq data

1.) $S^4U$ labeled cells or dissociated tissues

2.) Single cell lysis and barcoding

3.) Conversion of the $S^4U$ into a cytosine analogue

- Mismatched cytosine in nascent RNA
- Uracil in stable transcripts

4.) Reverse Transcription:
   -Mismatched cytosine in nascent RNA
   -Uracil in stable transcripts

5.) Sequencing:
   Bioinformatical separation of nascent and long-lived transcripts

Library
DynaSCOPE: Time-resolved transcriptomic dynamics

"new" and "old" variation in t-SNE plot colored by substitution rate.
Single cells for non mammalian organisms
Single cells for plant model – large well chip

Protoplasts

50 μm

From single-cell multi-omics to precision medicine
Single cells for yeast model

No cDNA was obtained when cell lysis was unmodified

After cell wall digestion, cDNA was obtained from yeasts
Single cells for yeast model

Differentially expressed genes per cluster

- ANB1
- TIR1
- AGA2
- GAL1
- GAL7
- GAL10
- RGI1
- HSP12
- EGO4

From single-cell multi-omics to precision medicine
From single-cell multi-omics to precision medicine

Single cells for yeast model

The expression of HXT2/3 and MIG1 was increased in response to glucose reduction.

Suppression of glucose transporters by MTH1.
Surface protein detection
Multiomics: the power of RNA and protein combined

Why Proteins?

Proteins are more abundant than RNA
~1-3M proteins/cell vs 60-300k RNA/cell
Increase sensitivity (targeted)

Proteins are responsible for cellular functions
Multiomics: the power of RNA and protein combined

Each antibody is linked to an DNA oligo containing a unique tag and a poly A at the 3’

mRNA and antibody tags are both captured by the poly T on the beads

Multiomics, for a better annotation
From single-cell multi-omics to precision medicine

Multiomics: CITE-Seq adaptation

CD14 protein

Monocytes

CD14 mRNA
Multiomics, for a better annotation

CD4 protein

Monocytes

CD4+ T cell

CD4 mRNA

precision medicine
From single-cell multi-omics to precision medicine

Multiomics, for a better annotation

CD25 protein

FOXP3 mRNA

CD4+ T-cell

Naïve T cells

Natural CD4+ T-cell (nTreg)
Thanks !
Single-Cell Sequencing
SCOPE-chip®

- No Need For Specialized Equipment – Use A P200
- View Cell Loading With Any Standard Microscope
- Similar Size To A Hemocytometer
Single-Cell Sequencing Solutions: SCOPE-chip®

Initial Bead Loading

PBS Push #1

Beginning Of PBS Push #2
Single-Cell Sequencing Solutions: SCOPE-chip®

Cells Only

Cells + Beads
Single-Cell Sequencing Solutions: **SCOPE-chip®**

Outlet Reservoir Cleaning

Bead Recovery
Single-Cell Sequencing Solutions: **SCOPE-chip®**

**Chip Overview**

**Before Priming**

**After Priming**

**Interior Wells**

**Peripheral Wells**
QC of cDNA and NGS library

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