Single cell and spatial transcriptomics

Life Science Technology Specialist
VIB Tech Watch team
Michiel.bontinck@vib.be
VIB Tech Watch

Scout

• Scout for disruptive technologies.
• Partner with companies

De-risk

• Funding.
• Hands-on support.

Facilitate

• Increase adoption of novel technologies.
• Staying at the forefront of life science research
The premise of single cell analysis

- Single-Cell Analysis
  - Single-Cell input
  - Each cell type has a distinct expression profile
  - Reveals heterogeneity and subpopulation expression variability of thousands of cells
  - Cellularity heterogeneity masked

- Bulk Analysis
  - Bulk RNA input
  - Average gene expression from all cells

- Single cell
  - Genome
  - Epigenome
  - Transcriptome
  - Proteome
  - Metabolome

SCIENCE MEETS LIFE
Single cell transcriptomics workflow

Starting material → Sample preparation → Clean up/enrichment → Compartimentalization

Library preparation and sequencing → mRNA and barcode binding → Cell lysis

Seyfferth C, et al. (in preparation)
Single cell transcriptomics workflow

Data preparation

Cell clustering and identity calling

Developmental trajectories

Gene regulatory network
Arabidopsis root single cell atlas

Wendrich J, et al. (2020) Science

De Rybel lab
Atlassing reveals new marker genes

Wendrich J, et al. (2020) Science
Cell-type specific responses to stress (rice)

Cluster annotation

Maize ear atlas

Xu X, et al. (2021) Developmental Cell
Spatial transcriptomics

Method of the Year 2020: spatially resolved transcriptomics

Spatially resolved transcriptomics methods are changing the way we understand complex tissues.

Bayraktar et al. 2020 – Nature Neuroscience
Targeted spatial transcriptomics

- Fixed tissue section
- Stain with target probes
- Hybridize fluorescent readout probes
- Image and erase signal
- Decode RNA identity

Reconstructing spatial transcriptomics data to cell-type maps

Raw data and segmentation

Clustering on marker expression

Construct map

Targeted spatial transcriptomics

- Fixed tissue section
- Stain with target probes
- Hybridize fluorescent readout probes
- Image and erase signal
- Decode RNA identity

Superior resolution and sensitivity
Method of choice for validating scRNA-seq markers
Limited panel “budget”
Specialized equipment

Targeted spatial transcriptomics at VIB

Mouse brain cortex
100 gene panel

1mm²
2 million transcripts detected

Unpublished data
Targeted spatial transcriptomics at PSB

Maize shoot apical meristem, 90 gene panel

Laureyns R, et al. (in preparation)
Targeted spatial transcriptomics at PSB
Untargeted spatial transcriptomics

Fixed tissue section

Application of 2D barcode matrix

Permeabilisation and ligation of 2D barcodes

Bulk library prep and sequencing

Clustering and spatial reconstruction
Untargeted spatial transcriptomics

Untargeted spatial transcriptomics

Fixed tissue section

Application of 2D barcode matrix

Permeabilisation and ligation of 2D barcodes

Bulk library prep and sequencing

Clustering and spatial reconstruction

Transcriptome-wide = clustering + spatial info

Readout using illumina sequencing

Resolution/capture efficiency

Chen Ao, et al. 2021 (BioRxiv)
Additional benefits of spatial transcriptomics

- No protoplasting required
  - No protoplasting/stress-induced genes
  - No loss of cell types due to inefficient tissue dissociation or sample prep
- Throughput
  - Max output of a single 10X lane = 10k cells
  - Depending on sample, ST can interrogate 100k of cells in a single run
- Beyond marker validation
  - Spatial co-expression analysis
  - Cell-cell interactions
  - Subcellular localisation of transcripts
Take home

**Single cell transcriptomics**
- Discover novel markers for cell types and developmental states
- Study response to environment or genetic perturbation at single cell level
- Lacks spatial component

**Targeted spatial**
- Validate novel single cell markers or locate new cell-types
- Study spatial transcriptomes at (sub)cellular resolution
- Requires rational target panel design

**Untargeted spatial**
- Transcriptome-wide spatial mapping
- Limited resolution
Technology evolution forecast

- **#genes**
  - Low: 0
  - High: 10,000

- **Sensitivity and resolution**
  - Low
  - High

- **Technology:**
  - 10X Genomics VISIUM
  - BGI
  - nanoString
  - seqFISH
  - Vizgen
  - HDST
  - Slideseq_v2
  - READCOOR
  - CARTANA
  - REBUS BIOSYSTEMS
  - Molecular Instruments

- **Targeted**
- **Untargeted**