RCB0300 - Tópicos em Biotecnologia III

Changing Technologies of RNA Sequencing and Their Applications in Clinical Oncology

Wang et al.

Leticia Lima Kaiser (13658057)
Beatriz Cassiano Michelon (13780836]
Gabriel Henrique Caceres da Silva (13658099)

"Mudanças nas tecnologias de sequenciamento de RNA e suas aplicações em oncologia clínica"

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Introduction

Leticia

Introduction

- **RNA-seq:** one of the most commonly used techniques in life sciences, widely used in cancer research

Progress:

- ➔ Bulk RNAseq
- ➔ Laser-captured micro-dissected RNAseq
- ➔ Single-cell RNAseq

- ➔ Digital spatial RNA
- ➔ Spatial transcriptomics
- ➔ Direct in situ sequencing

- **Goal:** discuss each of these technologies to guide cancer researchers to select the most appropriate RNAseq technique

Bulk RNAseq

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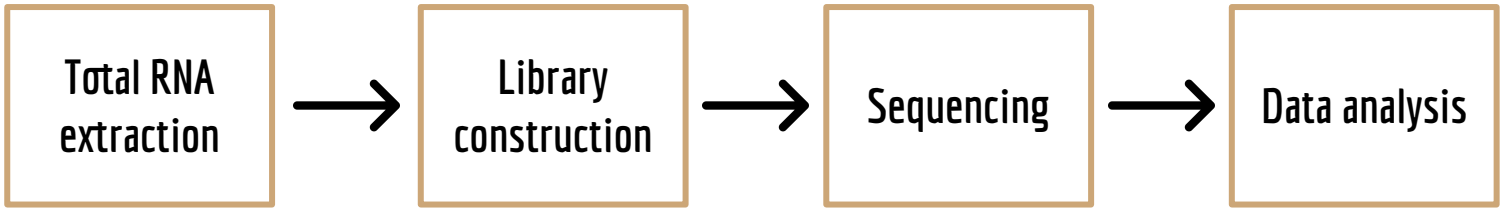
Bulk RNAseq

- was developed over **a decade** ago
- is used in **>60%** of all next-generation sequencing projects
- is the most widely used for studying **altered molecular pathways** in human cancers

4 key steps:



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Bulk RNAseq

2 types:

1. Simple RNAseq analysis

- ➔ Differentially expressed genes or markers
- ➔ Single-read sequencing (1 × 50 or 1 × 75)
- ➔ 20–30 million reads/sample
- ➔ The majority of the libraries are prepared using the poly-A RNA selection approach

2. Transcriptome sequencing

- ➔ Alternative splicing, point mutations, lncRNAs, fusion transcripts
- ➔ Paired-end sequencing (2 × 100 or 2 × 150)
- ➔ 40–50 million reads/sample
- ➔ The libraries are usually prepared using the rRNA depletion approach

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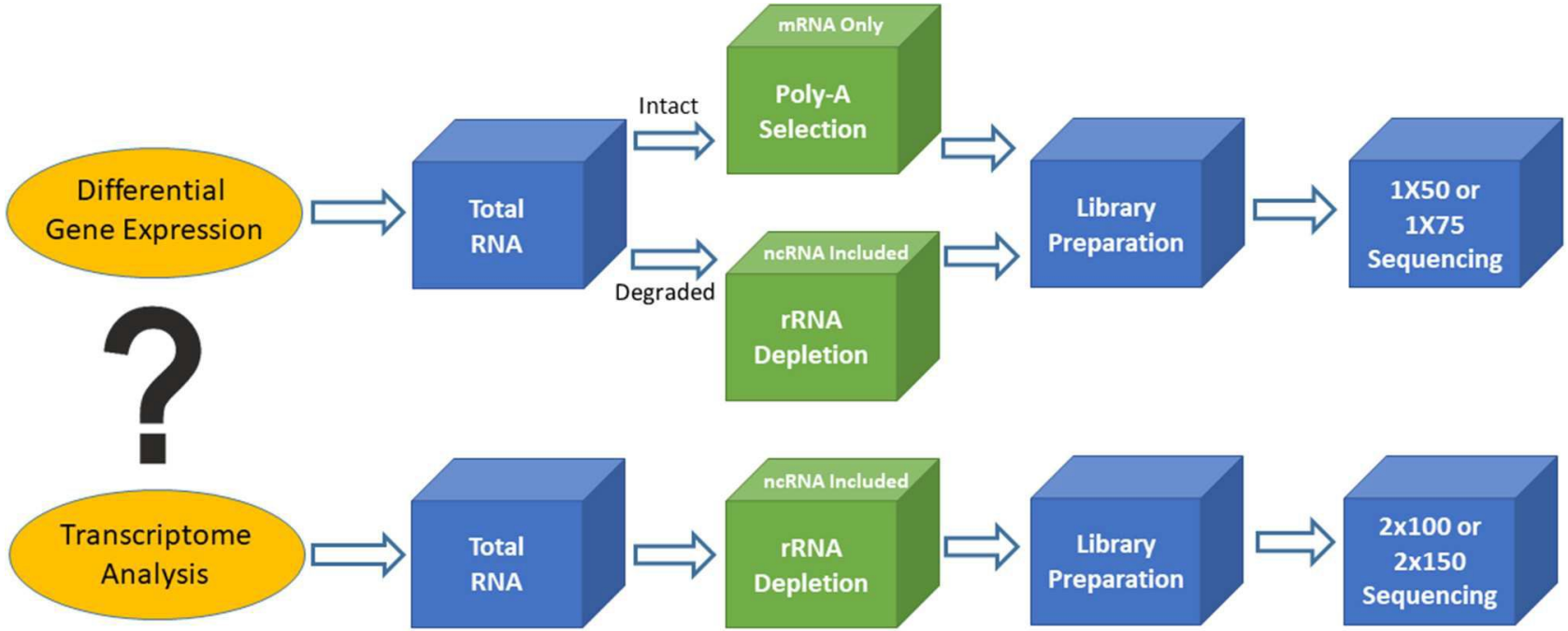
Bulk RNAseq

2 types:



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Bulk RNAseq

Applications:

1. Gene fusions detection
2. Discovery of biomarkers (signatures)
3. Guidance of therapeutic treatment

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Bulk RNAseq

Advantages

- mature technology
- cost effective
- can be applied to all tumor sample types (tumor cell lines, tumor tissues, FFPE tumor tissues, liquid biopsy samples)

Disadvantages

- average gene expression profile
- can potentially weaken the signals from a specific cell type

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Laser capture micro-dissected RNAseq (LCM-RNAseq)

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Laser capture micro-dissected RNAseq (LCM-RNAseq)

- Developed in attempts to overcome the weaknesses of Bulk RNAseq
- The majority employs FFPE materials
  - ↳ RNA: low quantity and quality
  - ↳ the LCM procedures further reduce the RNA integrity

Proper selection of the LCM instrument with IR laser

Suitable RNAseq library construction kit

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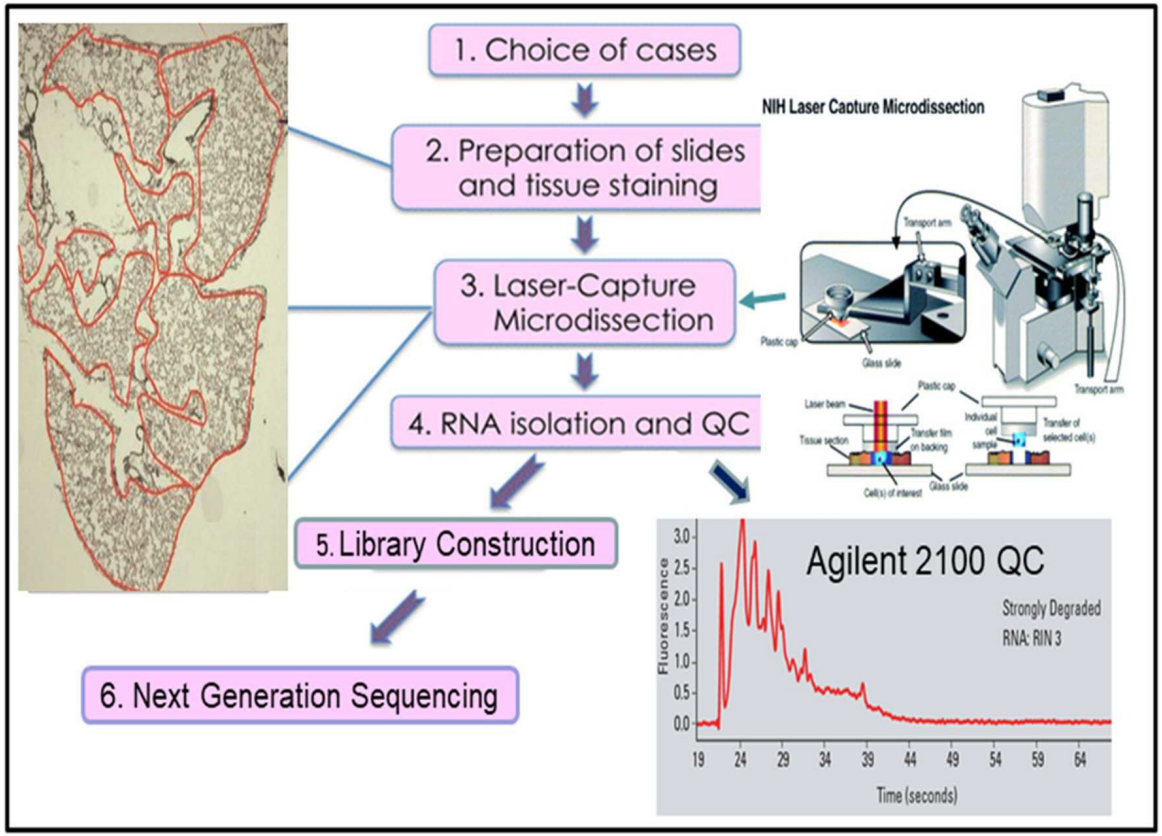
LCM-RNAseq

Steps:



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LCM-RNAseq

Advantages

- can reveal cell population-specific gene expression profiles

Disadvantages

- time consuming
- small number of cells at a time
- the RNA yield is of low quantity and highly degraded

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Single-cell RNAseq

scRNAseq

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scRNAseq systems

- Fluidigm C1 microfluidics: one of the early technologies → this system can process ONLY 96 single cells in a SINGLE RUN over 1 day;

- Micro-droplet based single-cell sequencing have become the dominant technology:
  - **10x Genomics Chromium** - high-quality scRNAseq → enables rapid analysis - over 10,000 individual cells in one experiment.

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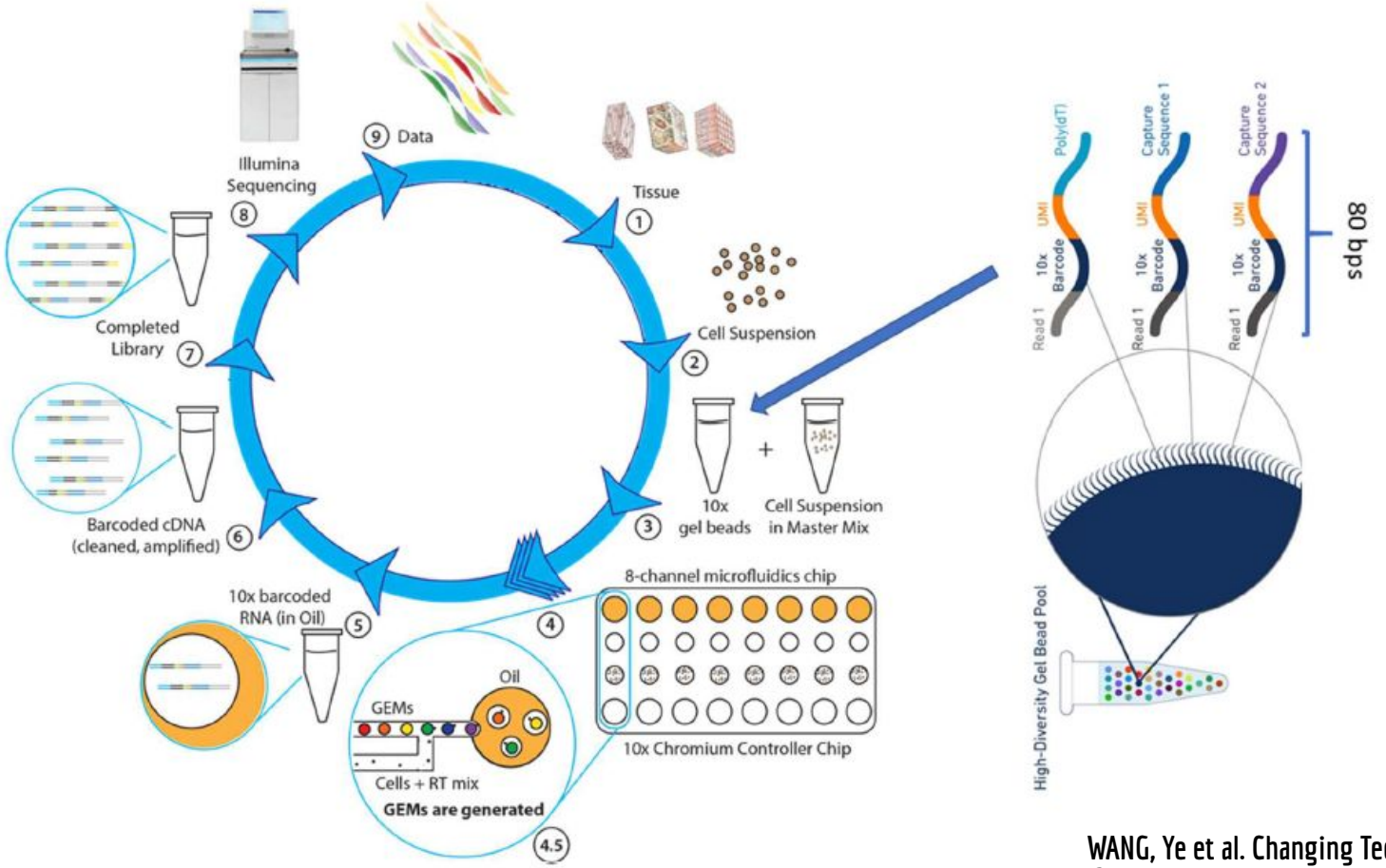

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Bulk RNAseq x scRNAseq

- Bulk RNAseq (tumor): significant infiltration of stroma and other type of cells in the tumor;
- Difficult to deconvolve the functionally relevant signals from average signals.

- scRNAseq: complementary and powerful tool to dissect intratumoral transcriptomic heterogeneity (RNA variants related to drug-resistant tumors);
- Provide insightful clue for tumor treatment (specific RNA variants stress-tolerant cells → normal cells).

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Other applications

- To characterize known cell types, subtypes, and previously unknown cell types within and surrounding tumors (and it’s gene signature);
- Identify new cell types and biomarkers in T cell infiltration.

→ Bulk RNAseq, LCM-RNAseq, and single-cell RNAseq all suffer from a common weakness—lost **critical spatial information** due to the micro-dissection or cell dissociation at the early stage of these protocols, which impacts the understanding of cell functionality and pathological changes.

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Digital Spatial Profiling

DSP

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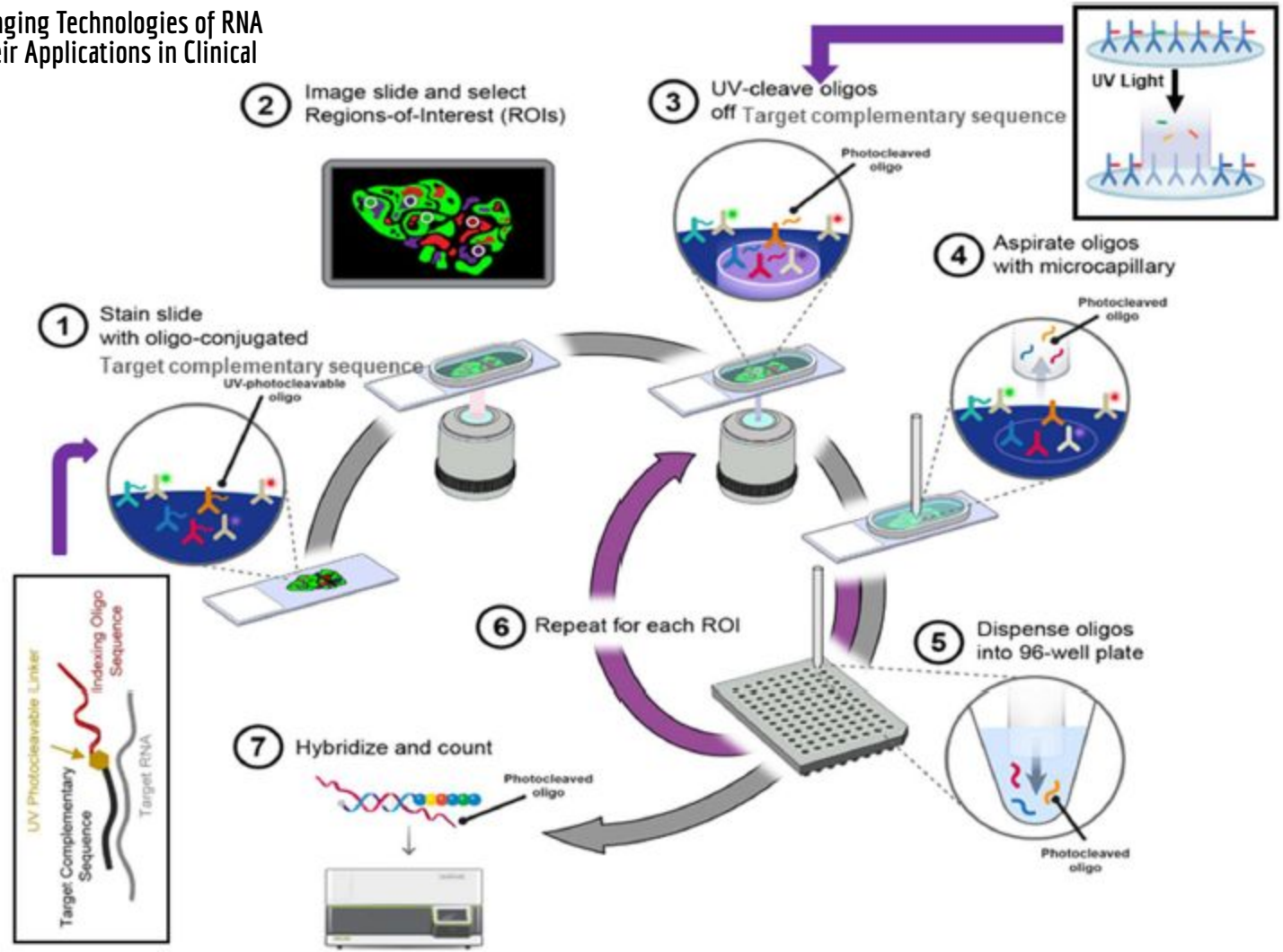

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Spatial Transcriptomics

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Spatial Transcriptomics

- A new technology has been in active development for several years.
- Overcomes the limitations of DSP technology by allowing scientists to study the whole transcriptome spatially;
- Theoretically provide information similar to bulk transcriptome analysis along with spatial content.
- The process is carried out in 8 steps that integrates the features of microarray and the barcoding system of 10x Genomics.

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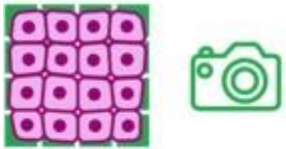
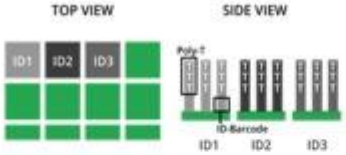
Spatial Transcriptomics

Step by step:

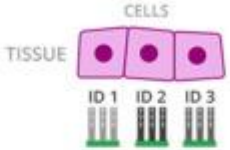
1. Histology



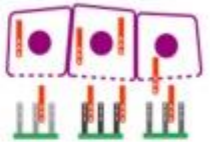
2. The Array



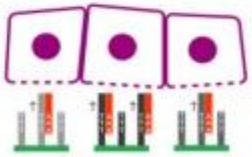
3. Tissue Fixation



4. Permeabilisation



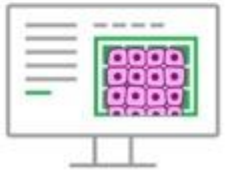
5. cDNA Synthesis



6. Library Preparation

7. Sequencing

8. Data Visualisation



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Spatial Transcriptomics

Step by step:

T7 promoter for in vitro transcription (IVT), a partial Illumina handle for the sequencing, a spatial barcode for RNA localization, a UMI for removing amplification duplicates, and oligo-dT sequences for capturing mRNA

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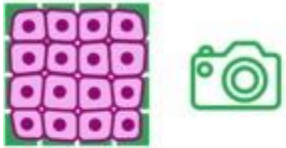

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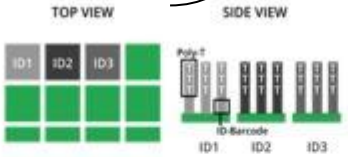

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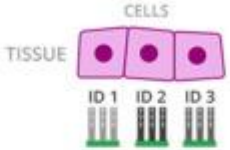

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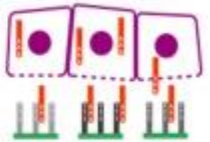

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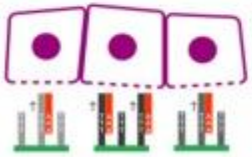

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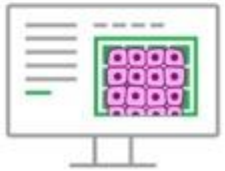

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Spatial Transcriptomics

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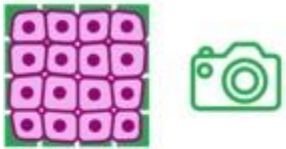

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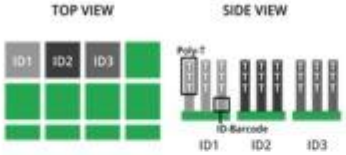

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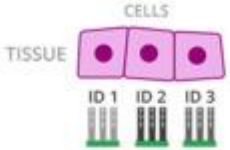

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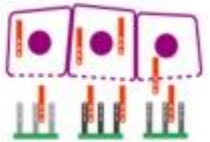

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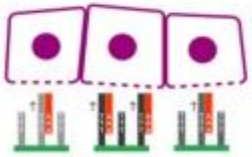

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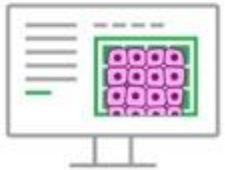

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Spatial Transcriptomics

Applications:

1. Investigation of intratumor heterogeneity

2. Cancer diagnosis

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Spatial Transcriptomics

Advantages

- Doesn't require specialized equipment or pre-knowledge of gene sequences;
- Has a throughput higher than that of digital spatial profiling methods

Disadvantages

- the currently available product is not able to offer single-cell resolution;

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Fourth-generation RNAseq

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Fourth-generation RNAseq

- The recent developments of fourth-generation sequencing technologies, such as in situ sequencing (ISS) and fluorescent ISS (FISSEQ), have potential to reach the ultimate goal of RNAseq: a simple, robust, spatially-resolved transcriptomic analysis at a single-cell resolution;
- In situ sequencing (ISS) and fluorescent ISS (FISSEQ) are the two main fourth-generation RNAseq technique ;
- The ISS method applied padlock probes combined with rolling circle amplification (RCA) to generate in situ amplified, targeted sequencing libraries that are subsequently sequenced via sequencing-by-ligation NGS chemistry;

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Fourth-generation RNAseq

- In contrast, the fluorescent in situ sequencing (FISSEQ) method uses random hexamers with a sequencing primer tag to initiate in situ RT;
- These technologies are still in their very early developmental stages and many technical aspects need to be addressed before they can be applied in cancer research and clinical applications.

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Summary

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Summary

	Strengths	Weaknesses	Suitable applications
Bulk RNASeq	High throughput, cost effective, mature technology	Average gene expression profile, lack of spatial content	Whole transcriptome-based biomarker discovery, targeted RNAseq panel for gene fusion
LCM-RNAseq	Cell type specific gene expression profile	Time consuming, low quality data, lack of spatial content	Tumor heterogeneity by dissecting cell type specific population
Single cell RNASeq	>10,000 single cell gene expression profile	High cost, a limited number of unique transcripts, lack of spatial content	Tumor heterogeneity, cell type characterization, and discovery
Digital spatial profiling	Spatial information, applicable to FFPE materials	Limited to small number of genes (gene panel only), lack of sequencing information	Tumor microenvironments, immuno-oncology biomarker discovery and optimizing immunotherapy
Spatial transcriptomics	Whole transcriptome analysis with spatial and sequencing information	Long procedures, early stage of technology	Tumor heterogeneity, tumor microenvironments, optimizing immunotherapy
Fourth generation RNAseq	*In situ* sequencing with future potential	In-matured technology	Not demonstrated yet

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Thanks!