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Introduction to CITE-Seq

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What is CITE-Seq?

Cellular indexing of transcriptomes and epitopes

is a sequencing-based method that simultaneously quantifies cell surface protein and transcriptomic data within a single cell readout.

Studying cells concurrently at transcriptomic and proteomic levels can offer unprecedented insights into new cell types, disease states, or other conditions.



<https://emea.illumina.com/techniques/sequencing/rna-sequencing/cite-seq.html>

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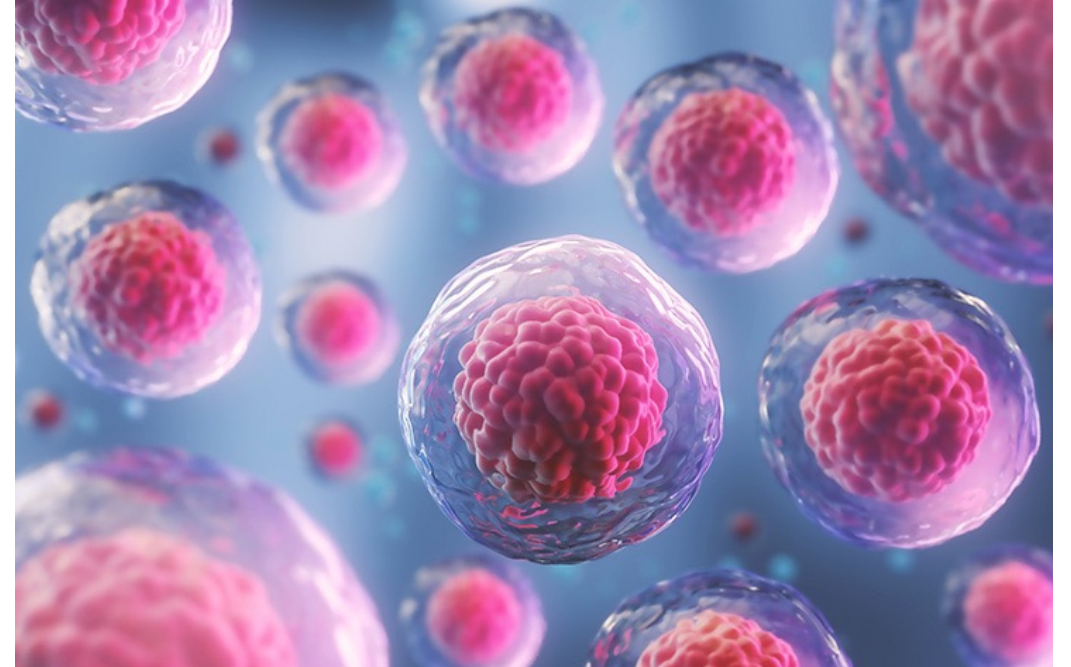
Why is CITE-Seq Useful?

Studying the transcriptome and protein expression simultaneously at the single-cell level.

Using transcriptional information to understand cell physiology is helpful, but it doesn't provide the entire picture as cell function is incomplete without protein expression information.

RNA analysis cannot accurately measure post-transcriptional and translational modifications such as protein degradation, isoform detection, and glycosylation.

A long-standing method to study surface protein expression, such as flow cytometry, is robust but it cannot simultaneously provide transcriptomic data.



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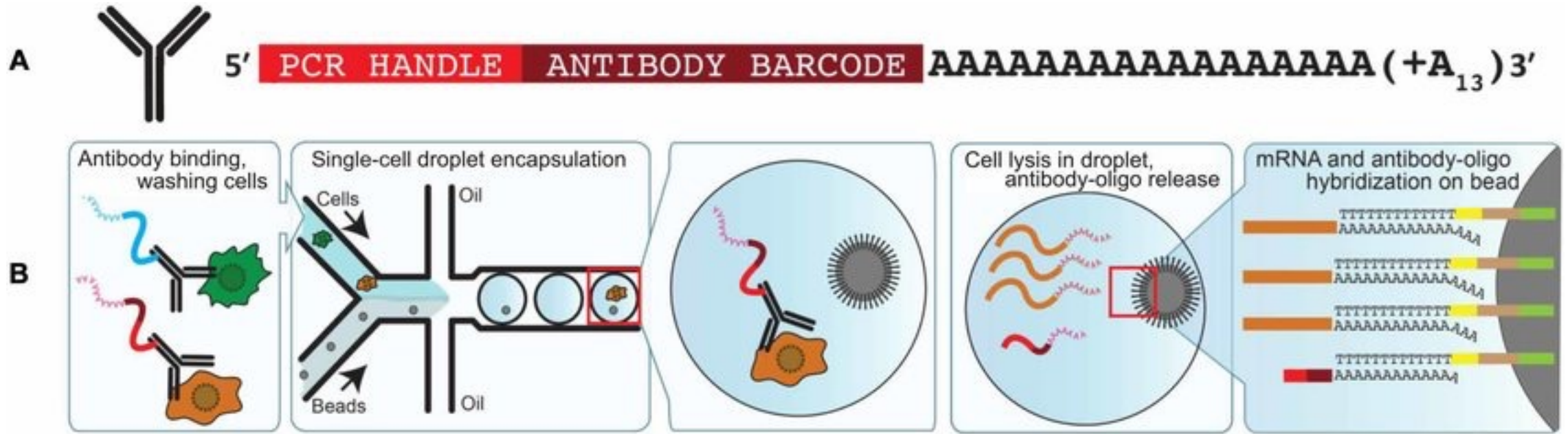
How Does CITE-Seq Work?

CITE-seq uses unique oligo-tagged antibodies to identify surface proteins, using sequencing as a readout.

The number of barcodes that can be conjugated to antibodies surpasses the number of fluorophores or heavy metal tags used in flow cytometry or CyTOF, expanding the number of proteins that can be measured simultaneously with RNA.

These barcoded antibodies are part of a unique workflow that produces protein and nucleic acid data using next-generation sequencing (NGS) technologies.

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Schematic figure of
(A) the CITE-seq antibody linked with the barcoded oligo (
(B) the CITE-seq protocol. Credit: [Winston & Gregory Timp \(2020\)](#), license: [CC-BY 4.0](#)

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Two ways to analyze CITE-Seq Data

1) Clustering – eg. Phenograph

- 1) High dimensional data
- 2) Data exploration
- 3) Advantage: - No pre-knowledge required

1) Boolean gating

- 1) 3-10 Antibodies
- 2) Recreating of Flow cytometry gating
- 3) Advantage: - Robustness of FACS
- Compare experiment to previous FACS data