Solid-phase DNA storage media enables comprehensive analysis of single cell transcriptomes and protein targets for elucidating genome biology

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Introduction

Limited material from single cell suspensions ➔ Multiple levels of omic analysis ➔ Consumption of material ➔ Incomplete knowledge of single cell profiles ➔ Pressing need to develop system to address challenges

APEX: Flexible DNA storage for iterative interrogation

Conceptual Framework

- Maximize molecular informativity
- Perpetual interrogation of samples
- Noise reduction of single molecule readouts

1. APEX conjugation
2. Multiple & Repeated Assays
3. Multi-omic Data Integration

Technology

- Illumina
- ONT
- PacBio

Omic Features

- Gene Expression
- Isoform Usage
- SNV analysis
- Gene Targeting
- Cell Targeting

Iterative assays without loss

Experimental framework of APEX on single-cell transcriptomes

- Rapid click chemistry conjugates DNA permanently onto magnetic beads
- Iterative interrogation by polymerases preserves original DNA
- Compatible with many DNA analytes, including single cell cDNA
- Enables rapid integration across technologies

Enabling analysis of primary tumors

- Custom Python workflow to identify nanopore reads with cellular barcodes using kmers
- Robust to errors

Pilot study: single cell RNA-Seq of PBMCs

- Initial feasibility study
- Conjugated full length cDNA of PBMCs
- Amplified full length cDNA and sequenced on Oxford Nanopore system

Gene-specific enrichment

Microfluidic manipulation

- Digital microfluidics to programatically perform assays
- Electrowetting moves APEX bead-containing droplets
- Mixing prevents settling of beads

Novel multi-omic workflows

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Bioinformatic integration

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- Robust to errors

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