Improvements to single-cell mRNA sequencing

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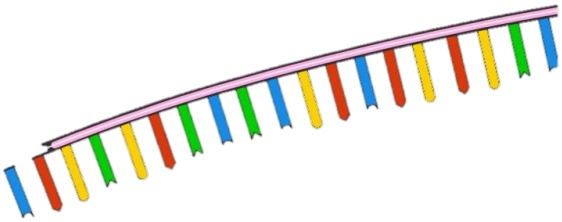

Funding sources:

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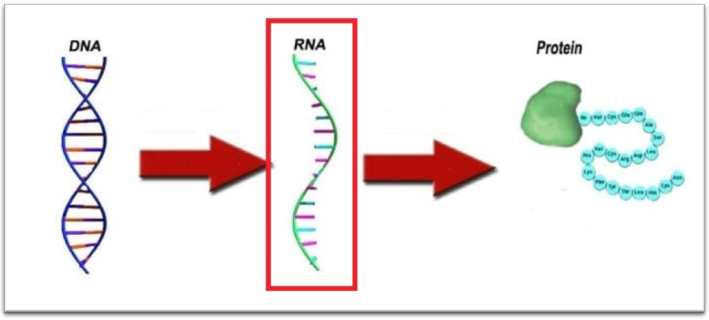
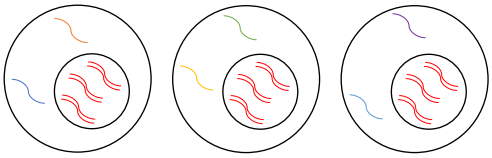
RNA Sequencing Shows Cell Functionality





heart cell kidney cell skin cell

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Single-Cell Sequencing Reveals Rare Cell Types

- RNA sequencing conducted in bulk conceals the information gained from single cells

Bulk Sequencing

Single-Cell Sequencing

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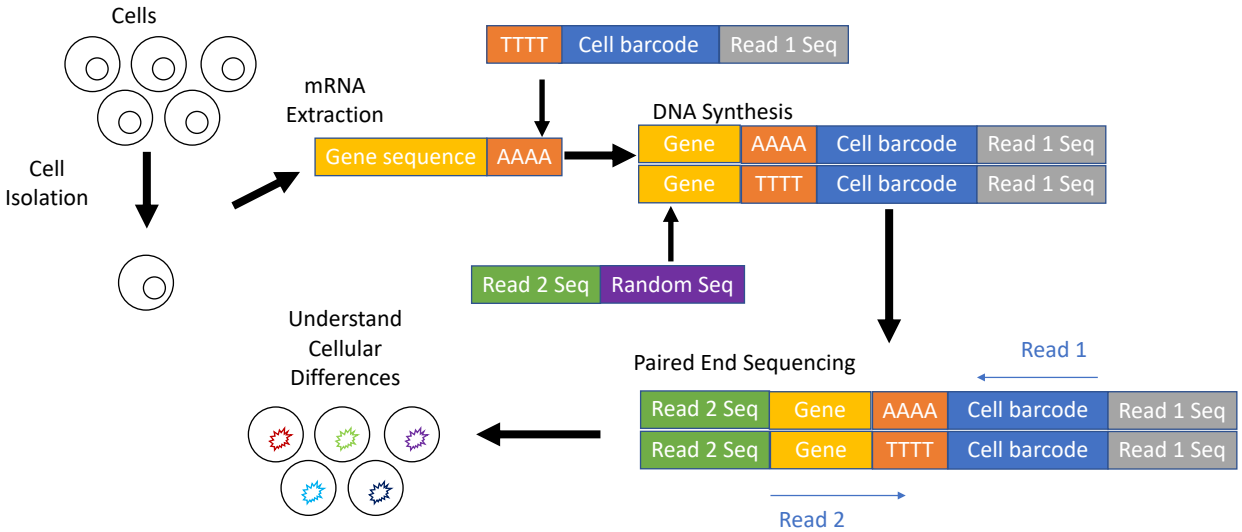
Current Method For Single-Cell mRNA-Seq



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Project Aims

Overall Goals:
1. Lower Costs
2. Maintain sensitivity

Accomplished By:
1. Sequencing Less Nucleotides
2. Reversing The Current Method

Sensitivity: the ability to accurately identify a sequence as being unique to a gene

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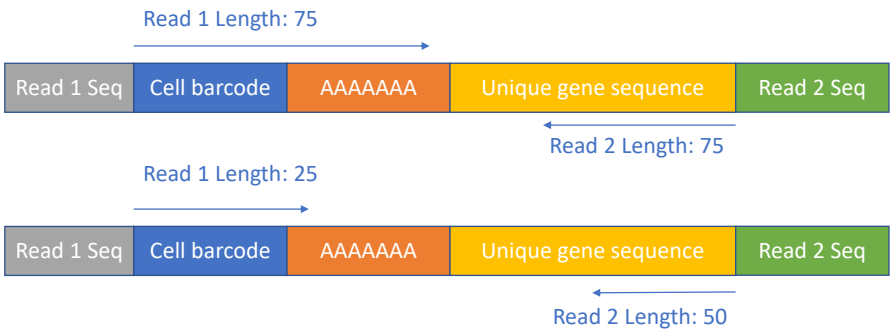
Currently 150 Bases are Read During Paired-End Sequencing But a 75 Base Kit Is Available



$3.20/cell

$1.70/cell

It is unclear if 50 base pairs of gene sequence is enough to uniquely identify a gene

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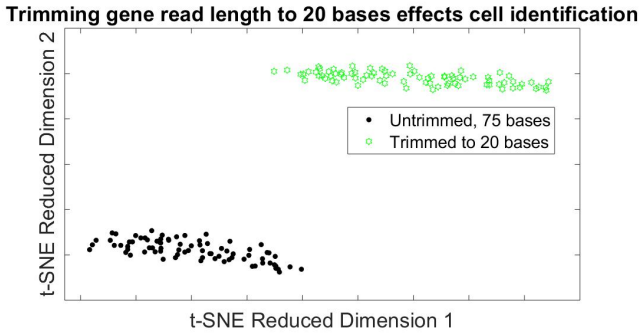
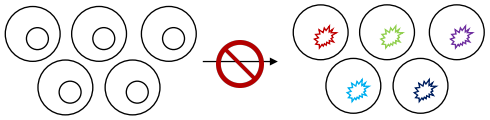
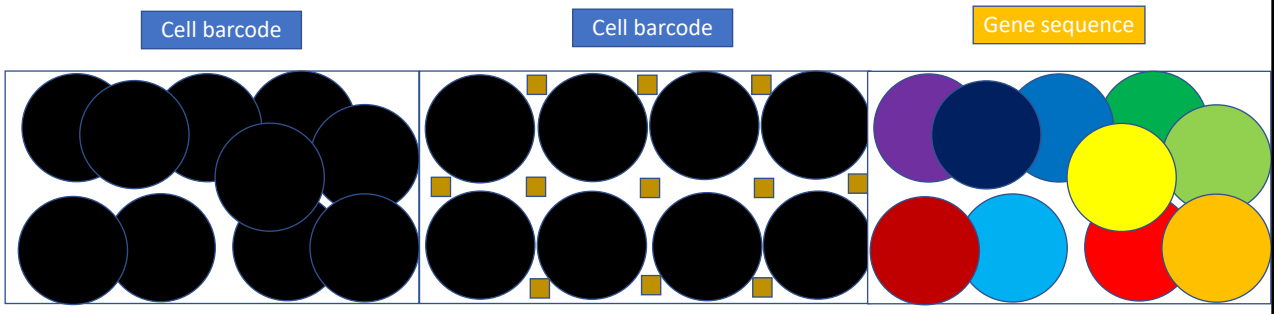
Moving To A 75 Base Kit Does Not Affect Cell Type Identification



- Created artificial reduction of sequencing data in a simulation
- Simulation with 50 to 25 bases as unique gene sequence to distinguish between the real (75 base) run



Current Lack of Diversity in Read 1 Necessitates the Addition of Spacer DNA During Sequencing



Original protocol
10 genes

Original with spacer
8 genes

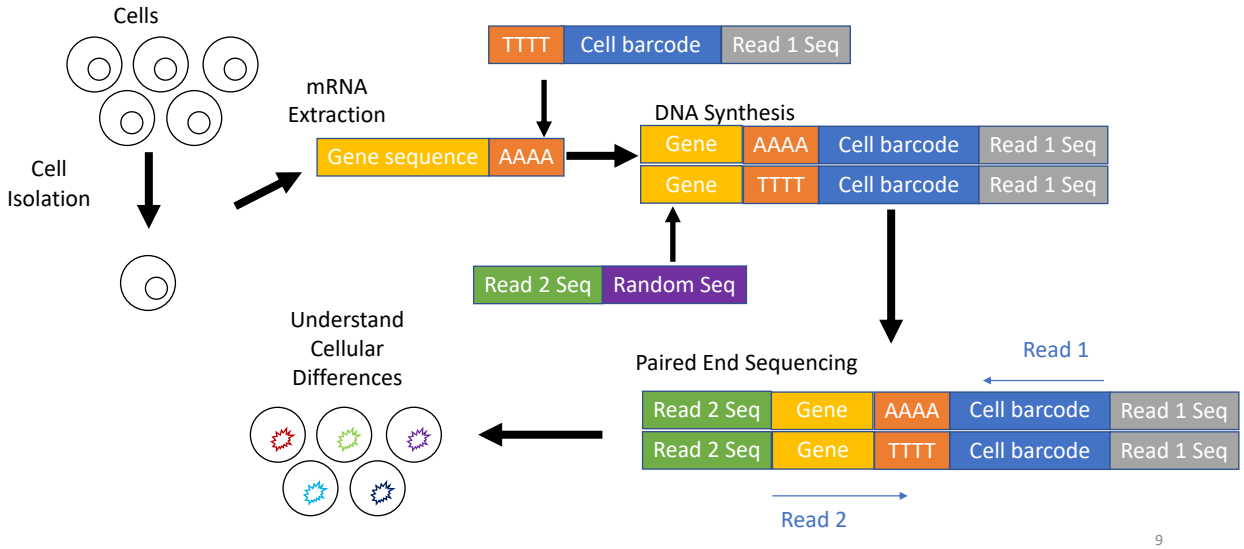
Revised protocol
10 genes

Cell barcode | AAAAAAA | Unique gene sequence

= spacer DNA

20% more sequencing area

Implementation of Reversed Protocol



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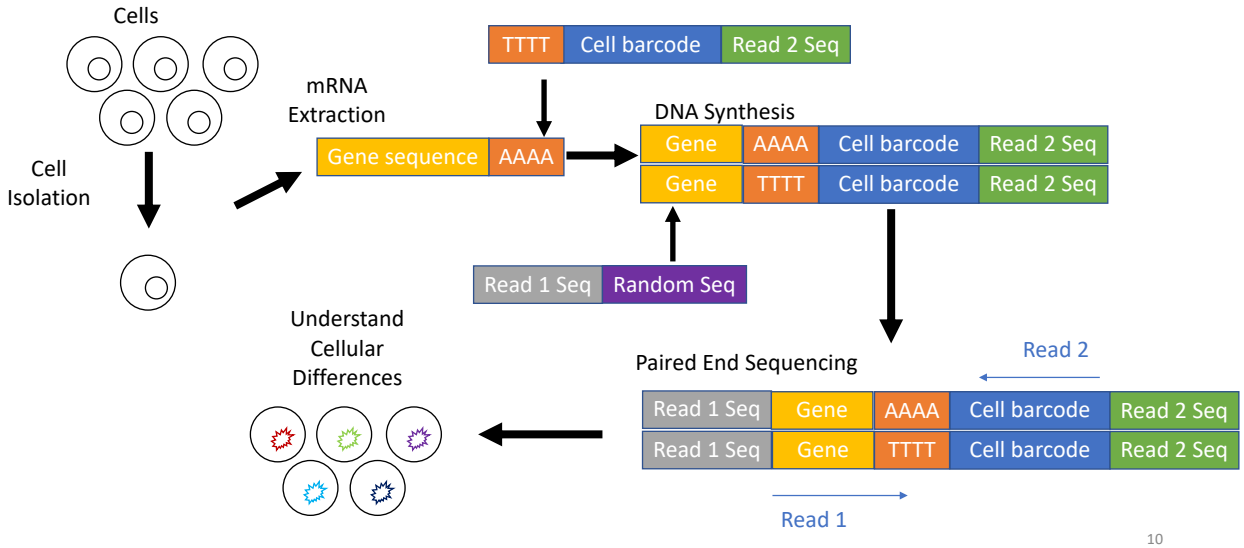
Implementation of Reversed Protocol



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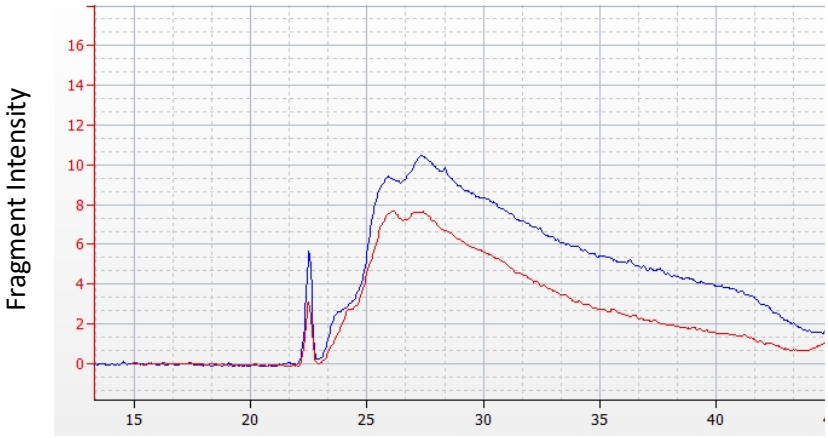
Testing for RNA in reversed protocol shows to be effective



Red: indicates fragments produced in ***original*** protocol

Blue: indicates fragments produced in ***reversed*** protocol

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Costs Reduced for Protocol

Original Cost: $3.20/cell

✓ Sequencing Less Nucleotides → $1.70/cell

✓ Reversing The Current Method → $1.36/cell (20%)

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Acknowledgements

The Dey group

Graduate students

Alex Chialastri
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Former graduate students

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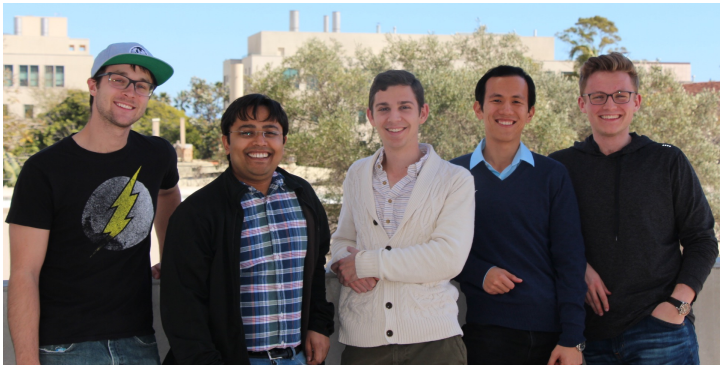
Nicole Chan (rotating)



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