COURSE

MASTERING SINGLE CELL ANALYSIS WITH TRAILMAKER™

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Introduction to single cell RNA-seq

What is single cell RNA-seq?

How does single cell compare to bulk RNA-seq?

How does droplet-based single cell RNA-seq work?

How does split-pool combinatorial barcoding RNA-seq work?

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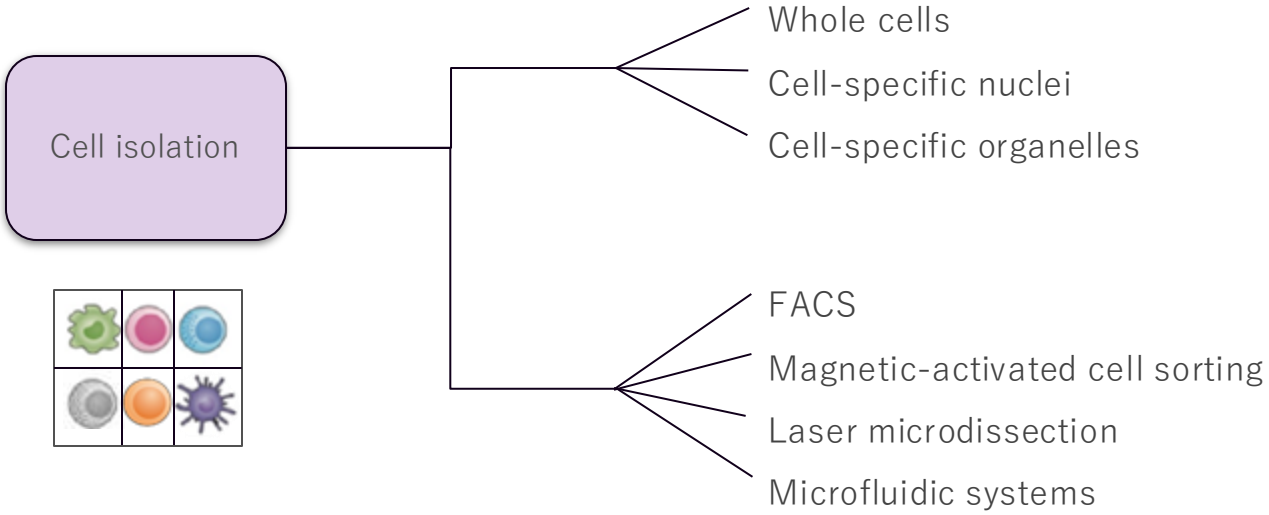
What is single cell RNA-seq?

Cutting edge technology that allows researchers to investigate gene expression at the level of individual cells



FACS = fluorescence-activated cell sorting

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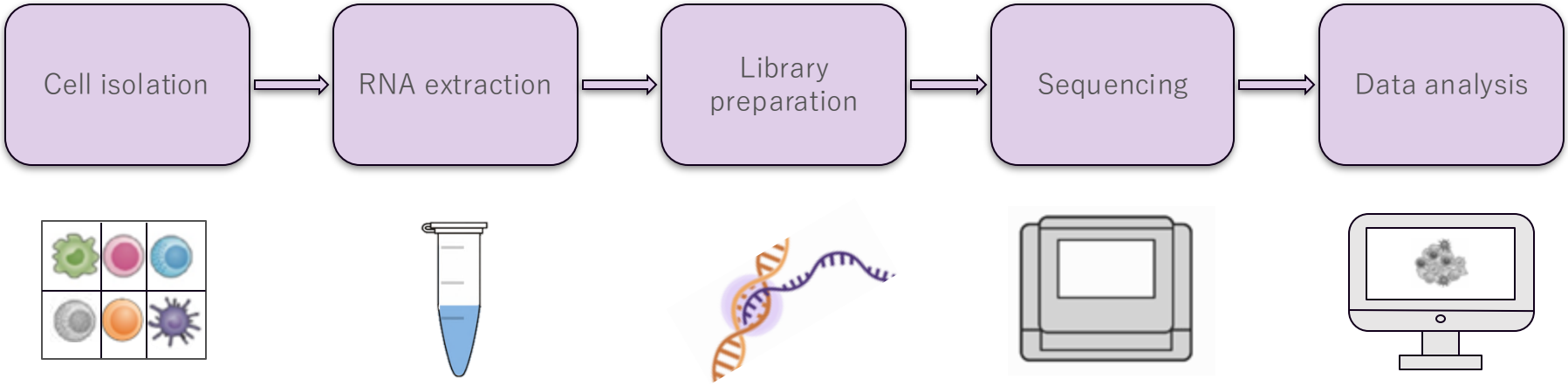

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What is single cell RNA-seq?

- Identify rare or novel cell types
- Study differentiation/development
- Investigate molecular mechanisms of disease states
- Identify biomarkers

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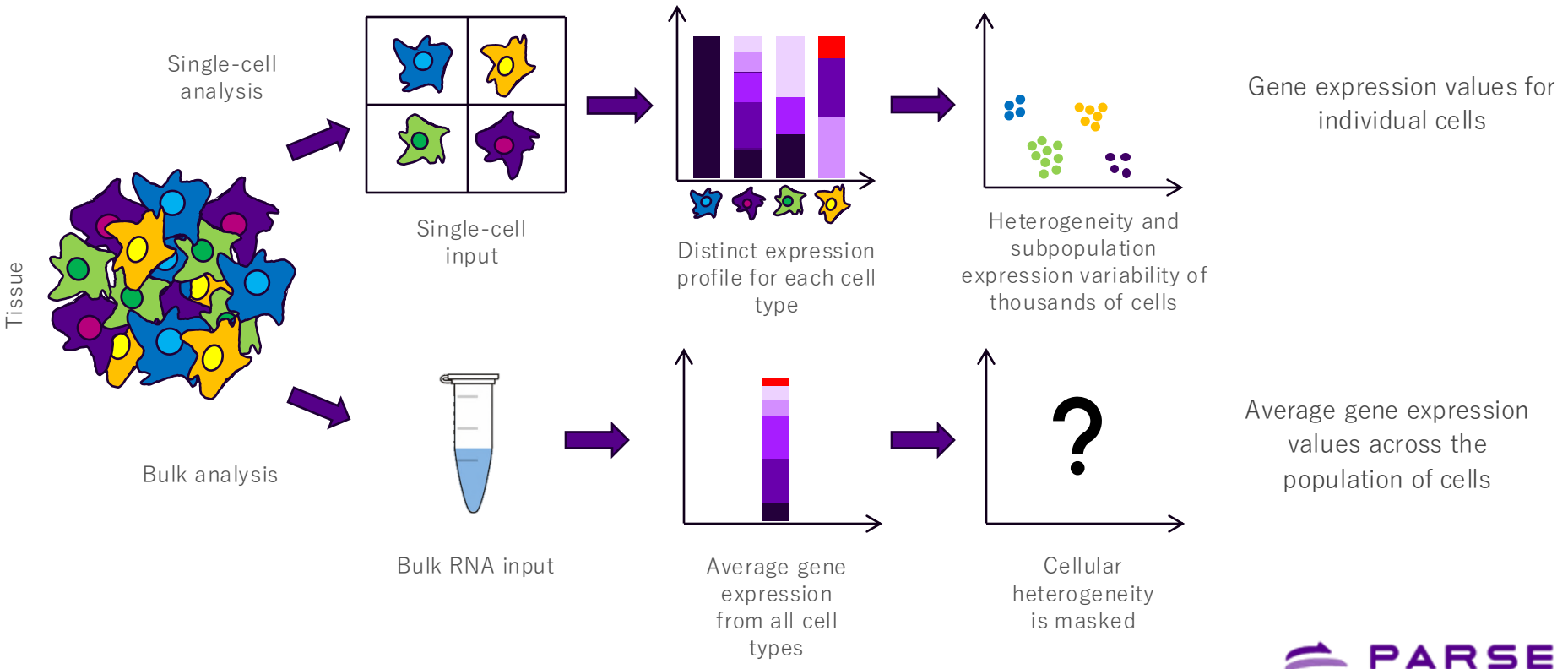
How does single cell compare to bulk RNA-seq?



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How does single cell compare to bulk RNA-seq?

Bulk RNA-seq

- Requires less cells
- Less costly
- Impossible to distinguish individual cells

scRNA-seq

- Data is noisier
- Requires more cells
- More costly
- Possible to distinguish individual cells

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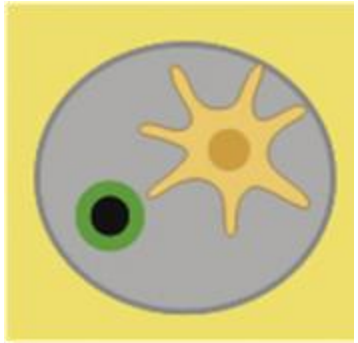
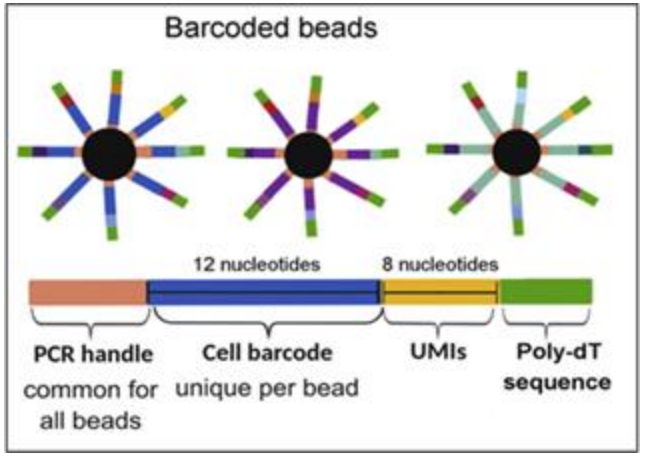
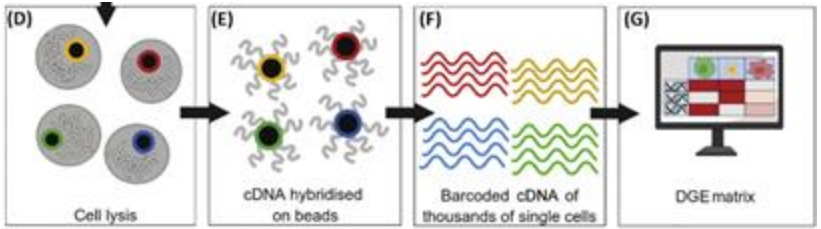
How does droplet-based single cell RNA-seq work?







DOI:https://doi.org/10.1016/j.tplants.2019.10.008

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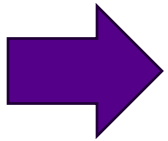
How does droplet-based single cell RNA-seq work?

Limitations

- Noise
- Dropout
- Biases/distortions introduced by PCR
- Scalability
- Requires specialized instruments



Split-pool combinatorial barcoding

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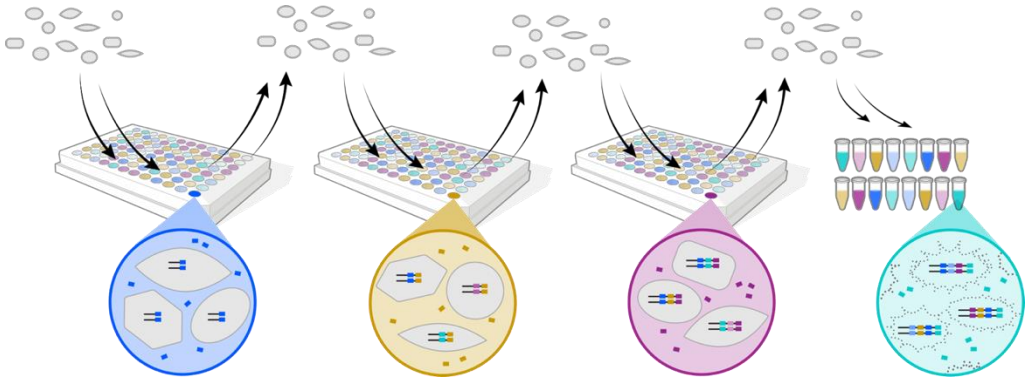
How does split-pool combinatorial barcoding work?

- No specialized instrumentation
- Multiple rounds of barcoding
- No physical isolation of single cells



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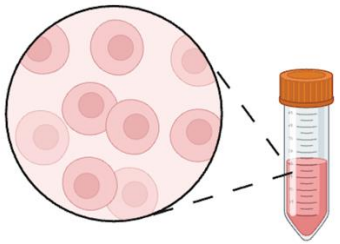
How does split-pool combinatorial barcoding work?

1) Fixation and permeabilization

Each cell is turned into its own reaction compartment



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How does split-pool combinatorial barcoding work?

2) First round of barcoding

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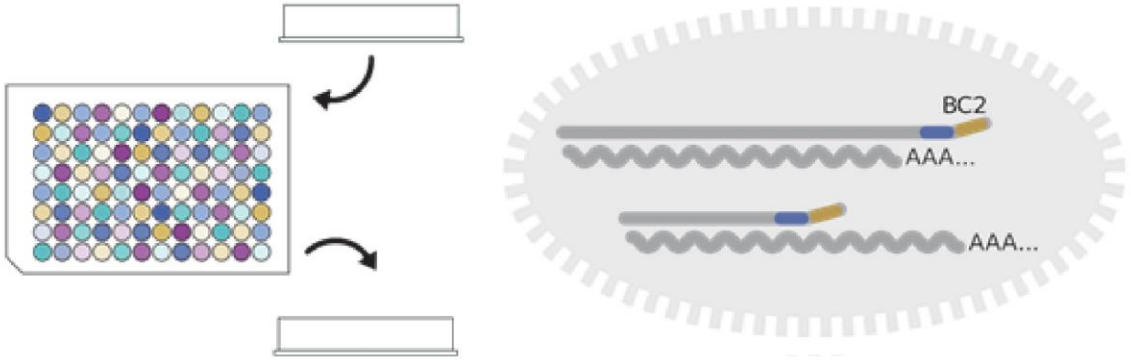
How does split-pool combinatorial barcoding work?

3) Pooling and second round of barcoding



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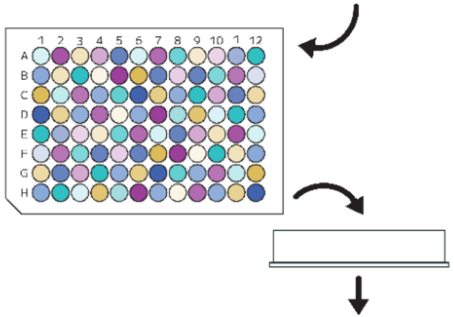
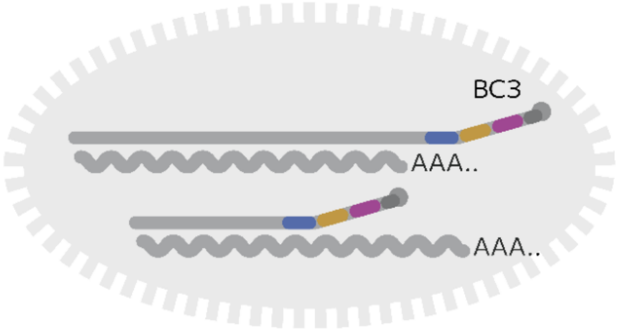
How does split-pool combinatorial barcoding work?

4) Pooling and third round of barcoding





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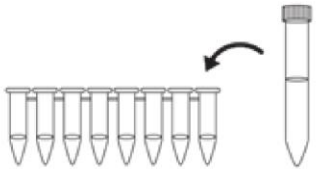
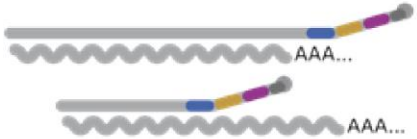
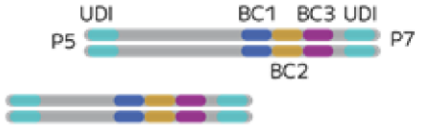
How does split-pool combinatorial barcoding work?

5) Fourth round of barcoding and sublibrary generation







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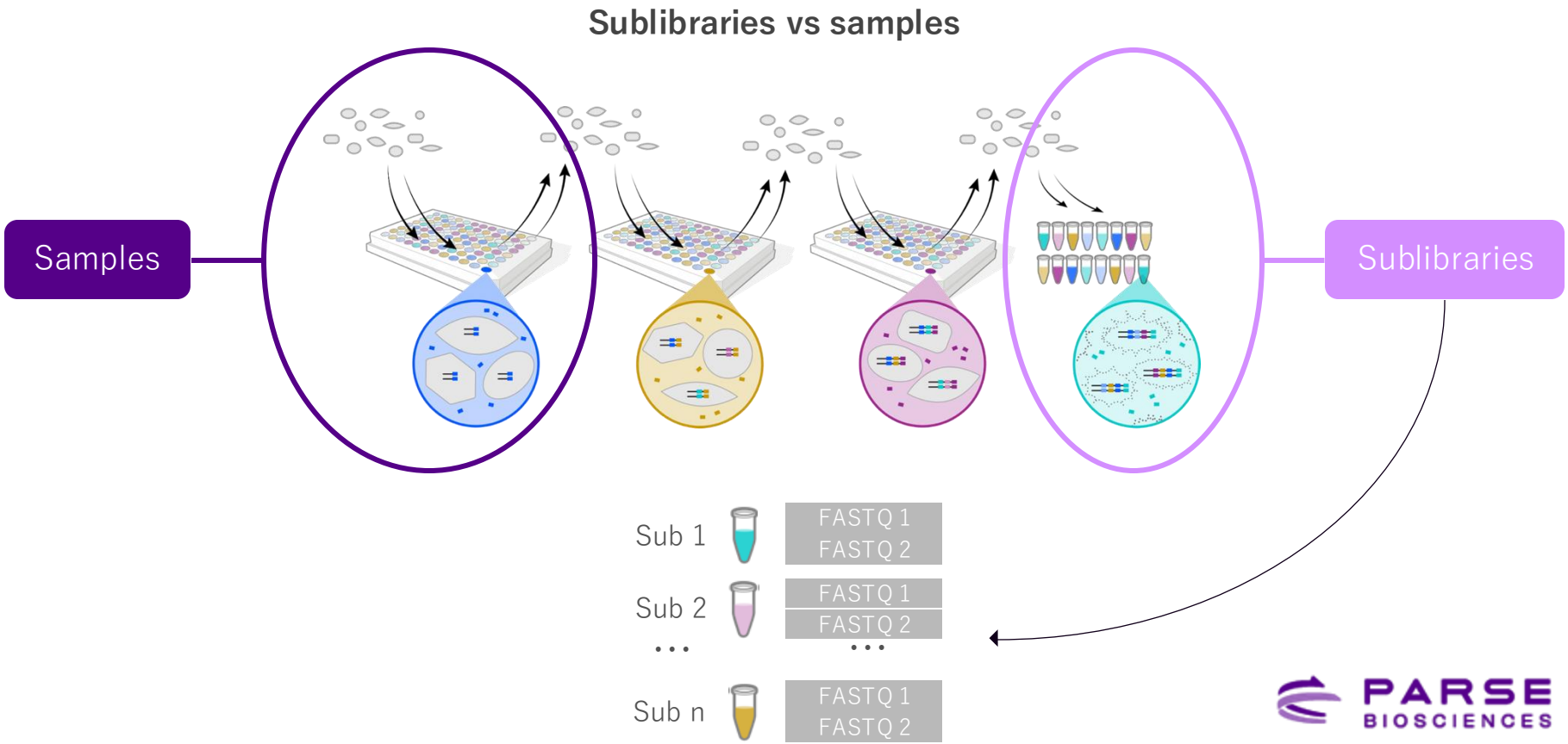
How does split-pool combinatorial barcoding work?



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How does split-pool combinatorial barcoding work?

Advantages

- Avoidance of ambient RNA contamination
- Lower doublet rate
- No physical isolation requires – high throughput
- No need for specialized equipment
- Low cost
- Unmatched data quality
- Sample multiplexing and reduced batch effects

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Summary of Key Points

- scRNA-seq is a cutting-edge technology that allows researchers to investigate gene expression at the level of individual cells
- The main steps of a typical scRNA-seq workflow involves several steps, including include cell isolation, RNA extraction, library preparation, sequencing, and data analysis
- scRNA-seq allows to explore the heterogeneity of gene expression across different cell types, states, and developmental stages, revealing cellular heterogeneity and dynamics that would be difficult or impossible to obtain using bulk RNA-seq
- Droplet-based scRNA-seq vs Split-pool combinatorial barcoding

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References

Rich-Griffin, Charlotte et al. “Single-Cell Transcriptomics: A High-Resolution Avenue for Plant Functional Genomics.” Trends in plant science vol. 25,2 (2020): 186-197. doi:10.1016/j.tplants.2019.10.008

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