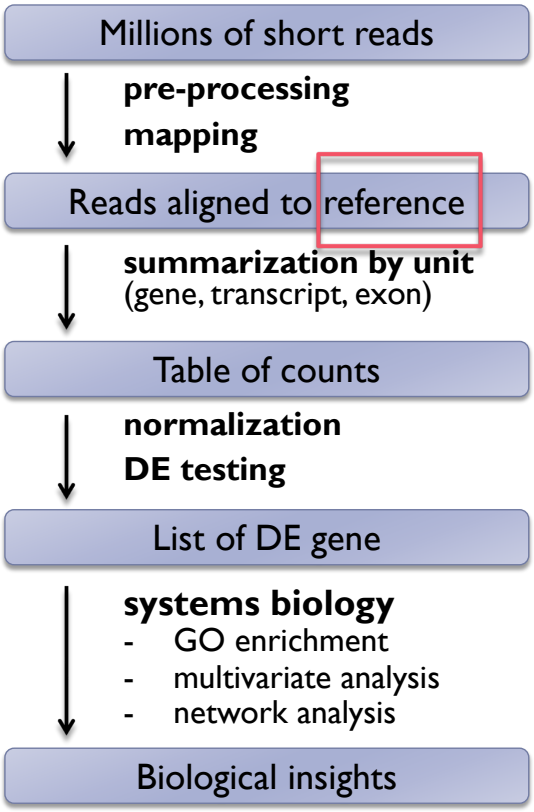
基礎生物学研究所　ゲノムインフォマティクス・トレーニングコース　2023年 3月

RNA-seq解析パイプライン上級：
de novo RNA-seq,
single-cell RNA-seq

Shuji Shigenobu
重信　秀治

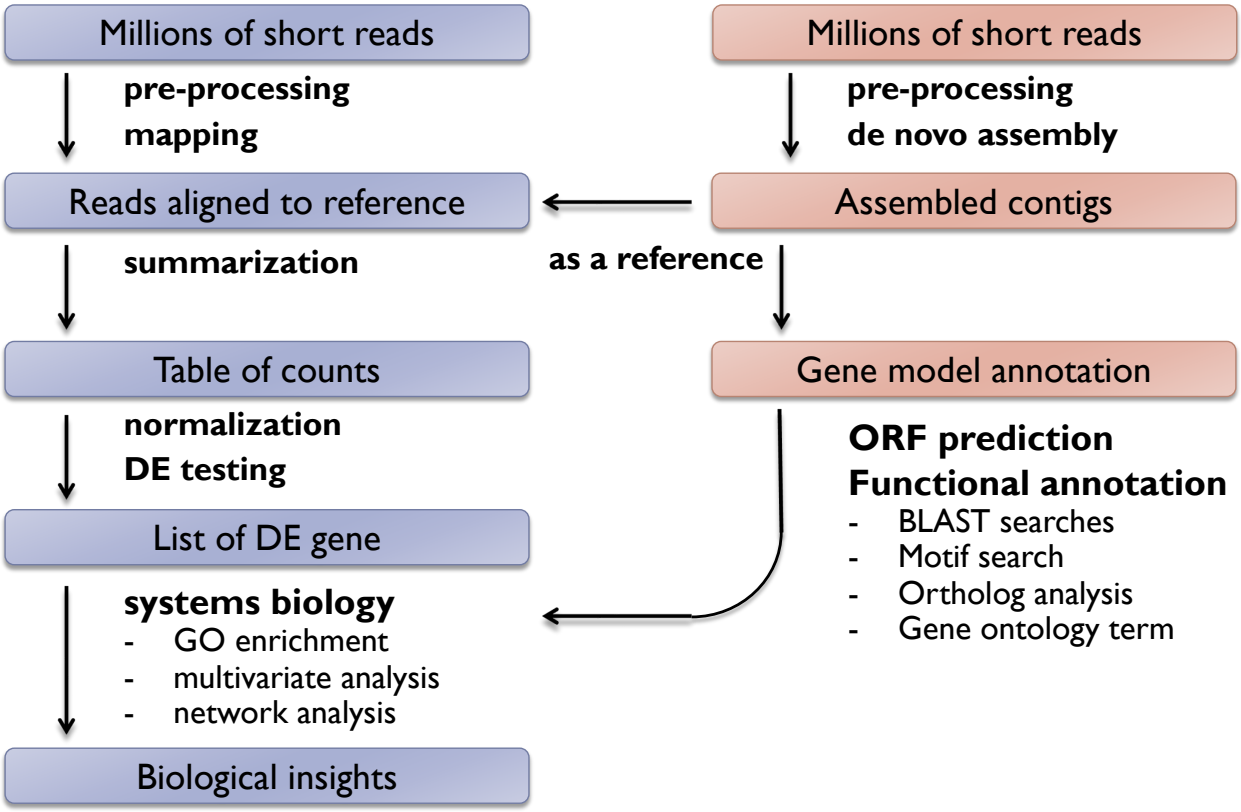
基礎生物学研究所
生物機能解析センター

de novo RNA-seq



1. **Build** reference
2. **Characterize** reference

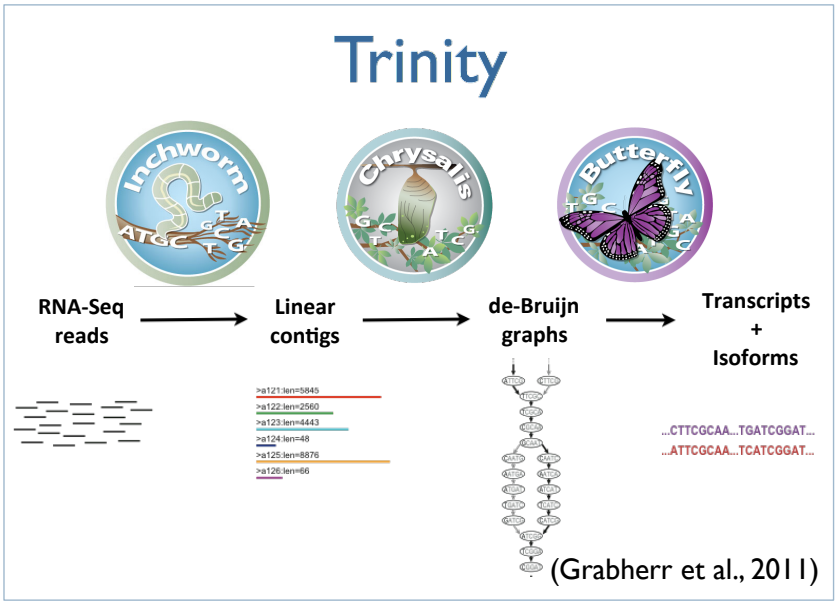
RNA-seq analysis pipeline (***de novo*** strategy)



de novo assemblers of RNA-seq

De novo assemblers use reads to assemble transcripts directly, which does not depend on a reference genome.

- Trinity
- Oases
- TransAbyss
- …



https://github.com/trinityrnaseq/trinityrnaseq/wiki

trinityrnaseq / trinityrnaseq

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Code | Issues 25 | Pull requests 0 | Projects 0 | Wiki | Insights

Home

https://github.com/trinityrnaseq/trinityrnaseq/wiki

Brian Haas edited this page on Nov 1, 2017 · 35 revisions

RNA-Seq De novo Assembly Using Trinity

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- Trinity Wiki Home
- Installing Trinity
  - Trinity Computing Requirements
  - Accessing Trinity on Publicly Available Compute Resources
  - Run Trinity using Docker
- Running Trinity
  - Genome Guided Trinity Transcriptome Assembly
  - Gene Structure Annotation of Genomes
- Trinity process and resource monitoring
  - Monitoring Progress During a Trinity Run
  - Examining Resource Usage at the End of a Trinity Run

Quick Guide for the Impatient

Trinity assembles transcript sequences from Illumina RNA-Seq data.

Download Trinity here.

Trinity example

- Input: Illumina short reads in FASTQ | FASTA format
- Output: assembled contigs in FASTA format

```
# Run Trinity
$ Trinity --seqType fq --left left_all.fq --right right_all.fq \
          --CPU 8 --max_memory 20G
```

(Trinity is supported on only Linux)

Let's try Trinity assembly

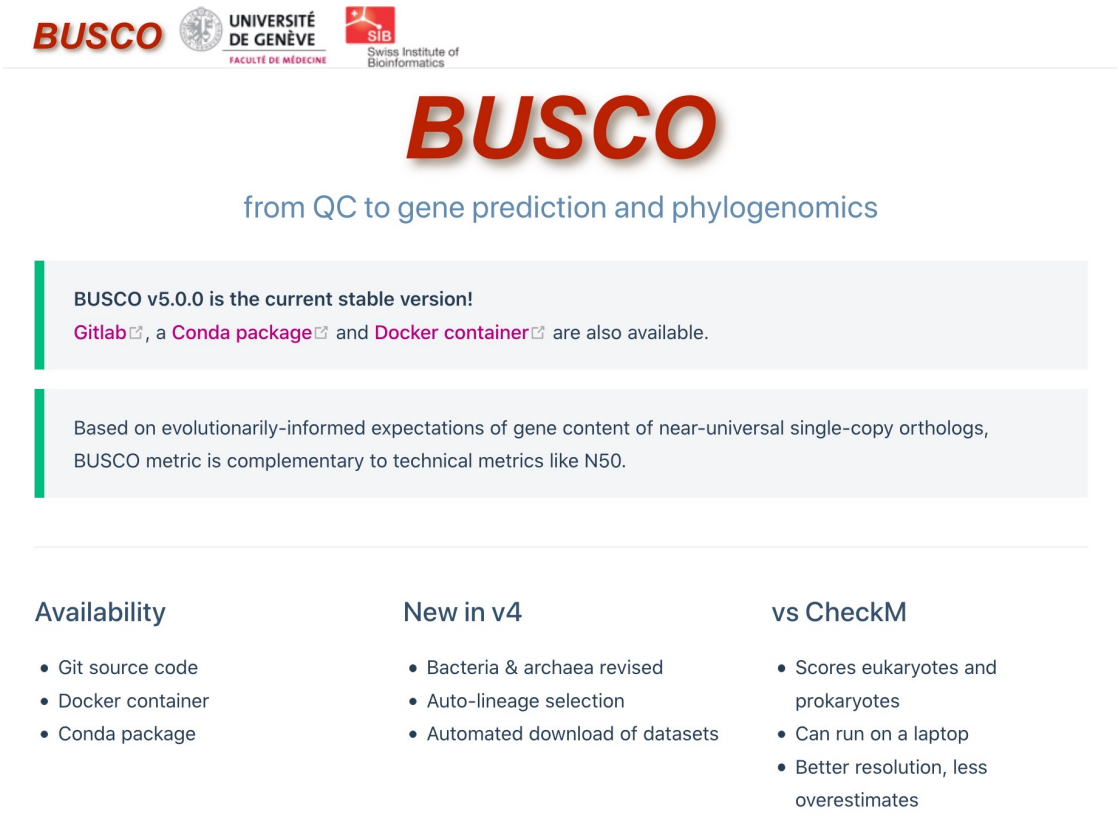
- ex701: *de novo* RNA-seq assembly using Trinity

Evaluate assembly

- Assembly stats
  - Number of contigs
  - Total length
  - mean, median, N50
- Coverage
  - BUSCO
  - Map back input reads
  - Map other RNAseq reads / known transcripts
- Contamination
  - BLAST (diamond) nr

BUSCO

https://busco.ezlab.org/



BUSCO

BUSCO provides a quantitative assessment of the completeness in terms of expected gene content of a genome assembly or transcriptome by using universally conserved one-copy gene set. The results are simplified into categories of Complete and single-copy, Complete and duplicated, Fragmented, or Missing.

```
# Run BUSCO
$ busco -m transcriptome contigs.fa -o OUTPUT -l lineage
```

```
# example of output
    (Insecta)
    C:94.5%[S:88.5%,D:6.0%],F:1.1%,M:4.4%,n:978

    925 Complete BUSCOs (C)
    866 Complete and single-copy BUSCOs (S)
    59  Complete and duplicated BUSCOs (D)
    11  Fragmented BUSCOs (F)
    42  Missing BUSCOs (M)
    978 Total BUSCO groups searched
```

練習：ex702

Advanced

Clean up reference sequences

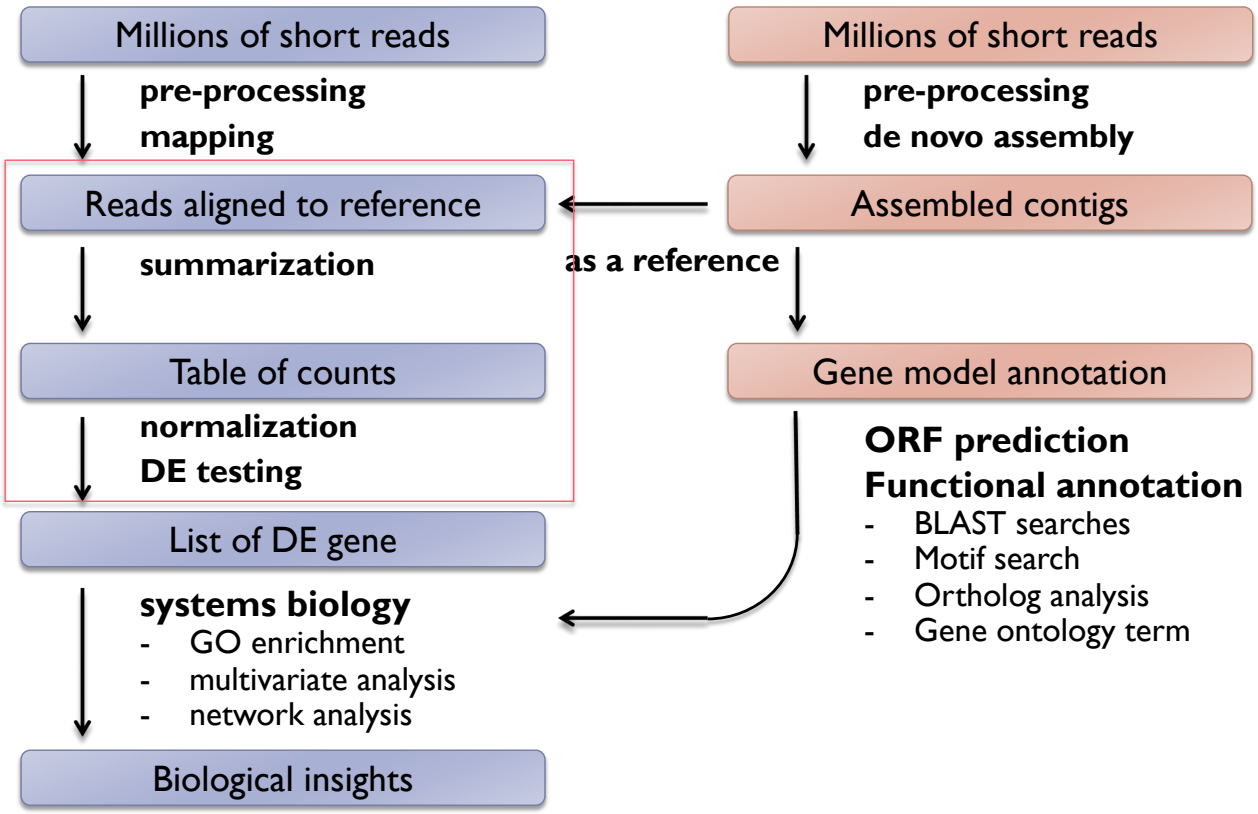
- An issue: Inflation of the number of Trinity contigs is often observed.
  - Trinity outputs splicing variants separately
  - Contaminations
  - Artifacts (bad contigs)
  - Incomplete contigs with very low expression.
- Solution
  - Filter out unwanted contigs.
  - Filter out very lowly expressed transcripts.
  - Cluster similar sequences.

Advanced

Remove redundancy in reference sequences

- Strategy and Tools
  - Choose one representative transcript from each cluster based on Trinity component information. (longest or highest expression)
  - Clustering
    - CDHIT-EST (http://weizhongli-lab.org/cd-hit/)
    - Corset (Davidson et al., 2014).
    - RapClust (https://github.com/COMBINE-lab/RapClust)
    - EvidentialGene (http://arthropods.eugenes.org/EvidentialGene/trassembly.html)
- Advantage of redundancy reduction
  - Gene-oriented analysis => easier interpretation
  - Better control of multiple comparison.

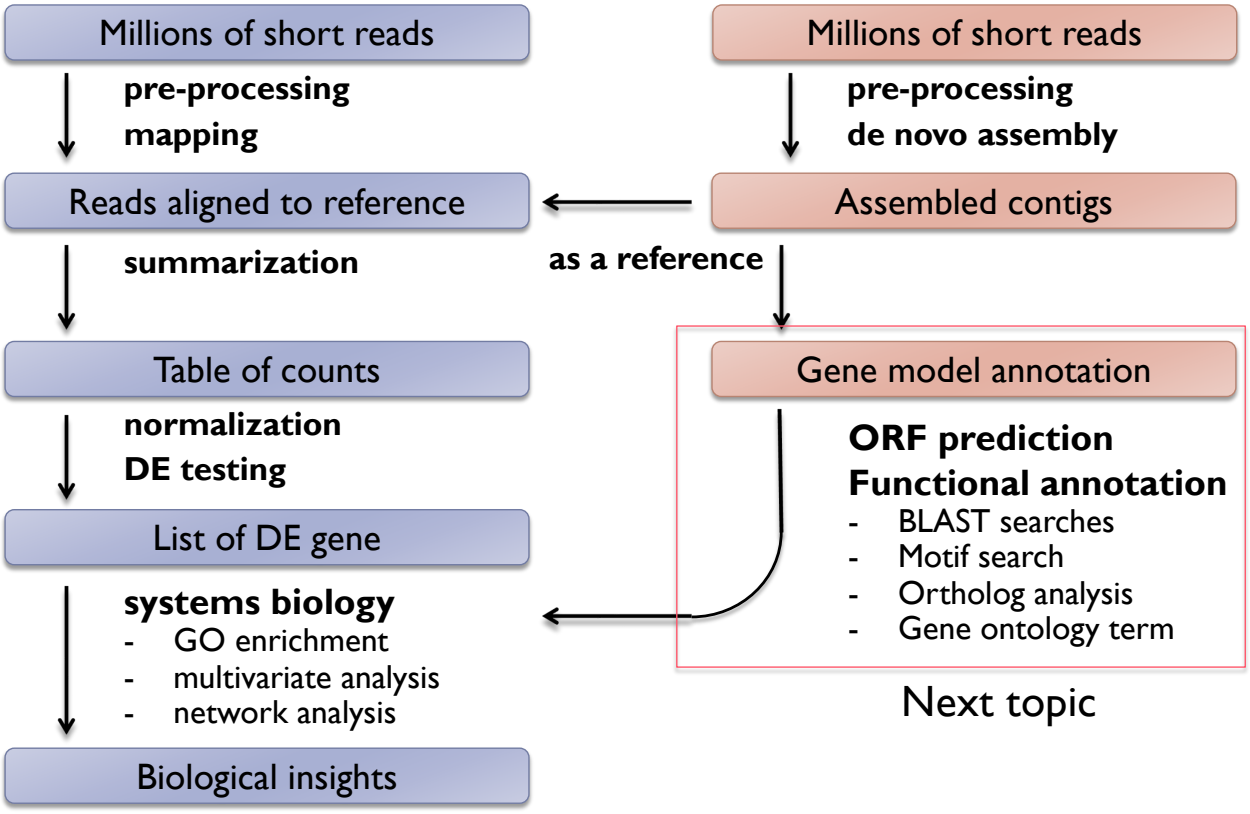
RNA-seq analysis pipeline (*de novo* strategy)



DEG analysis

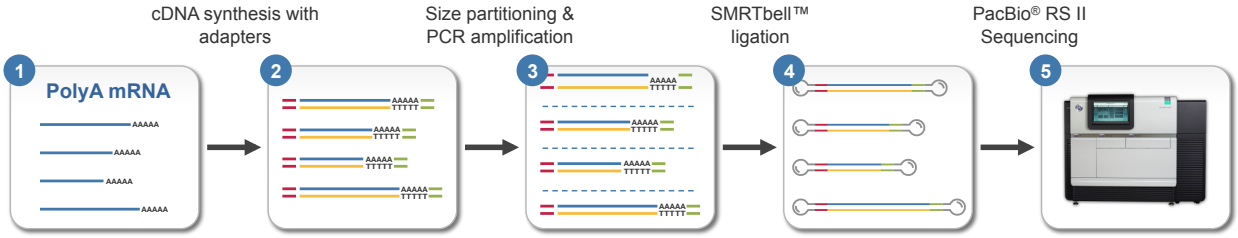
- Follow transcript-based RNA-seq pipeline

RNA-seq analysis pipeline (*de novo* strategy)

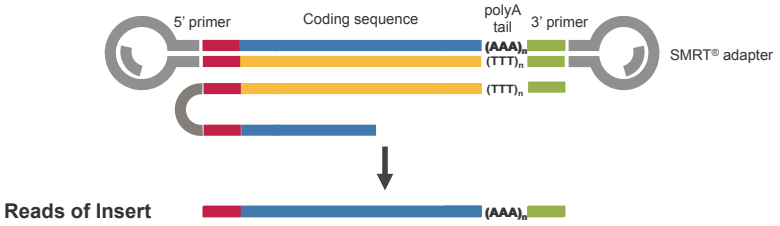


PacBio Iso-Seq for building a transcriptome catalogues

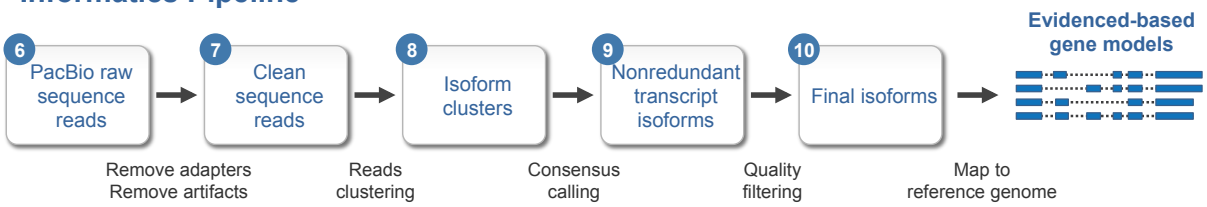
Experimental Pipeline



SampleNet: Iso-Seq Method with Clonetech® cDNA Synthesis Kit

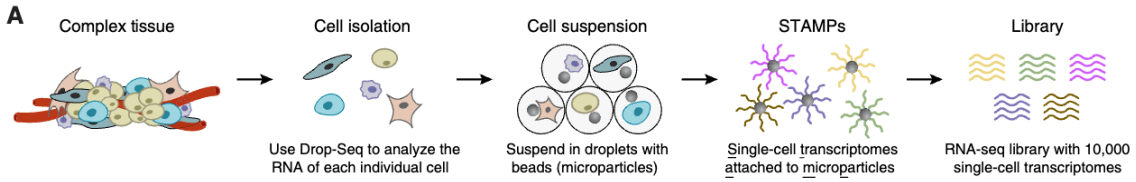


Informatics Pipeline



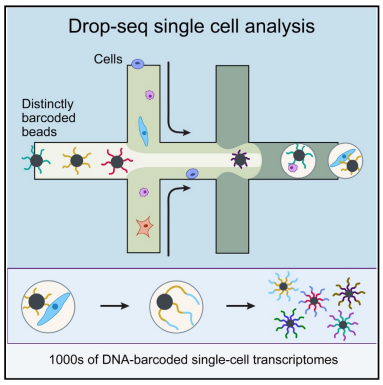
Single-cell RNA-seq

Drop-seq / Single-cell RNA-seq



Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Graphical Abstract



Authors

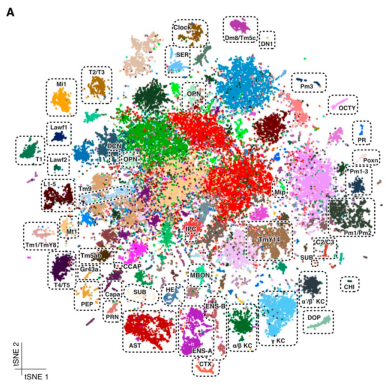
Evan Z. Macosko, Anindita Basu, ..., Aviv Regev, Steven A. McCarroll

Correspondence

emacosko@genetics.med.harvard.edu (E.Z.M.), mccarroll@genetics.med.harvard.edu (S.A.M.)

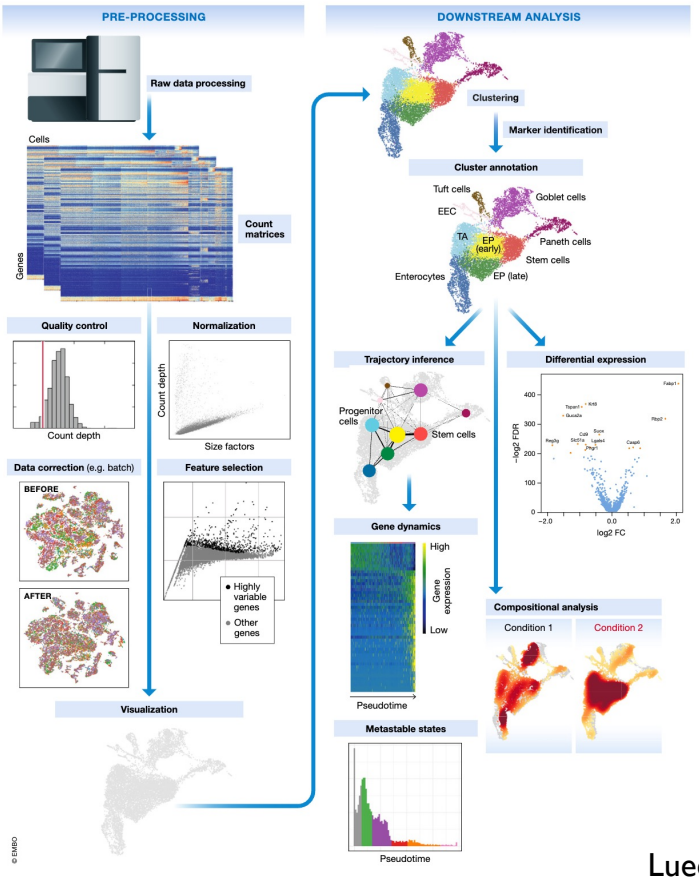
In Brief

Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers of each type.



(Macosko et al., 2015)

Typical single-cell RNA-seq bioinformatics workflow



Luecken et al., 2022

Bioinformatics of single-RNA-seq

- 代表的なプラットフォーム 10x Genomics Chromium. 数千細胞の transcriptome。
- 観測細胞が多いだけで、genes x cells のカウントマトリックスを扱う点は、Bulk RNA-seq と同じ。したがってバイオインフォマティクスの基礎は同じ。
- とはいえ、RNA-seq特有の問題も多く、scRNA-seqに特化したアルゴリズム・ソフトウェアが活発に開発されている。
- Bulk RNA-seq と異なる点
  - Sparce data (ゼロカウントの遺伝子が多い）。それゆえ、データはnoisy。
  - UMIを導入しているプラットフォームでは、生のリードカウントではなくUMIを使う。
  - 観測細胞が桁違いに多い
  - 細胞のクラスタリングに重きを置いた解析が多い
  - scRNA-seqならではの解析の例として、pseudotime 解析など
- Popular tools
  - CellRanger: 10x Genomics社純正　QC + mapping + count matrix generation
  - Seurat: integrated analysis platform (from QC to clustering)

Sparse matrix data

- scRNA-seq data matrix is "sparse" matrix (many zero count)
- Rather than the regular CSV format, sparse formats (only the nonzero entries are stored) are preferred.
- CellRanger use Market Exchange Format (MEX)

MEX

```
$ tree filtered_feature_bc_matrix
filtered_feature_bc_matrix
├── barcodes.tsv.gz
├── features.tsv.gz
└── matrix.mtx.gz

[features.tsv]
ENSG00000141510       TP53          Gene Expression
ENSG00000012048       BRCA1         Gene Expression
ENSG00000139687       RB1           Gene Expression
CD3_GCCTGACTAGATCCA   CD3           Antibody Capture
CD19_CGTGCAACACTCGTA  CD19          Antibody Capture

[barcodes.tsv]
AAACCCAAGGAGAGTA-1
AAACGCTTCAGCCCAG-1
AAAGAACAGACGACTG-1
AAAGAACCAATGGCAG-1

[matrix.mtx]
%%MatrixMarket matrix coordinate real general
%
32738 2700 2286884
32709 1 4
32707 1 1
32706 1 10
32704 1 1
32703 1 5
```

Seulat

https://satijalab.org/seurat/