Study of Division of Labor in Pseudomonas through single-cell RNA-seq

From population-level to single-cell analysis

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Introduction

Division of Labour (DoL) :

The specialization of tasks within a group, optimizing resource use and enhancing collective performance.

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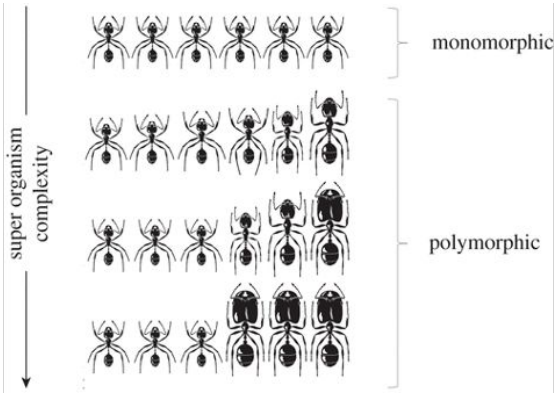
- Biological concepts
  - **Social insects** exhibit task specialization among individuals

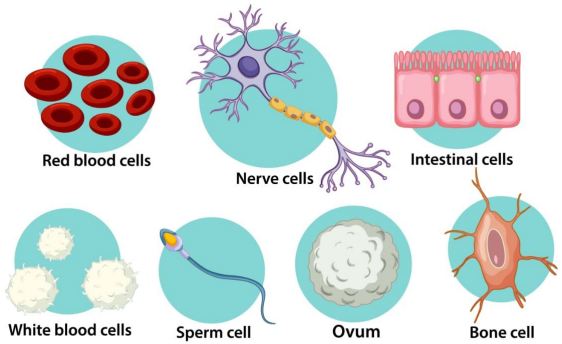


Division of labor in ant colonies

Division of Labour (DoL) :

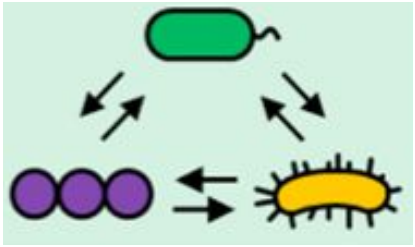
The specialization of tasks within a group, optimizing resource use and enhancing collective performance.

- Economic origin introduced by Adam Smith in 1776
- Biological concepts
  - **Social insects** exhibit task specialization among individuals
  - Specialization of **organs**, **tissues** and **cells in multicellular organism**



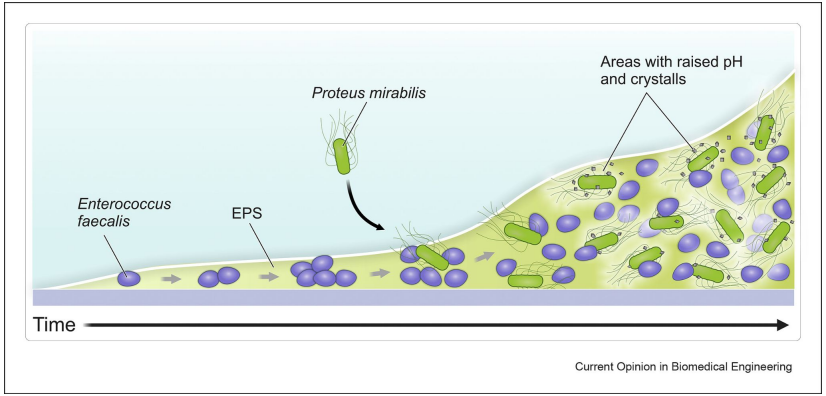
Cells specialization in multicellular organism

DoL in Microbial Communities



(Giri et al, 2019)

- **Interspecific DoL**:
  - Different microbial species engage in mutualistic interactions, such as **cross-feeding**

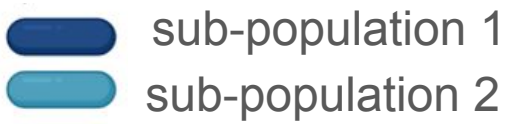


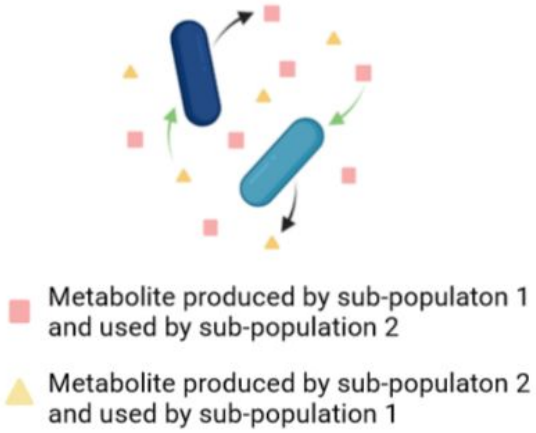
Interspecific DoL inside Biofilm

(Ramstedt and Burmølle, 2022)

Introduction

Isogenic bacterial





Intraspecific DoL Hypothesis :

DoL between cells may exhibit **functional specialization within a population**

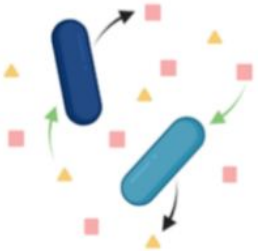
Introduction

Intraspecific DoL Hypothesis :

Isogenic bacterial

sub-population 1

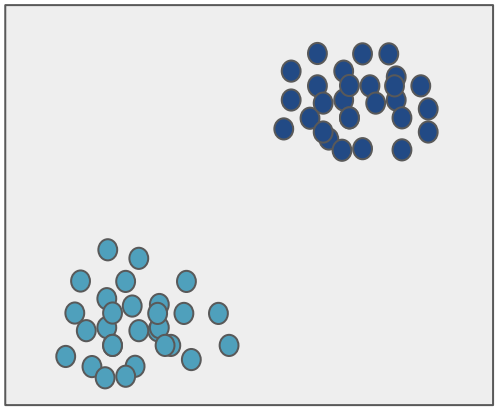
sub-population 2



Metabolite produced by sub-populaton 1 and used by sub-population 2

Metabolite produced by sub-populaton 2 and used by sub-population 1

DoL with cells may exhibit **functional specialization within a population**

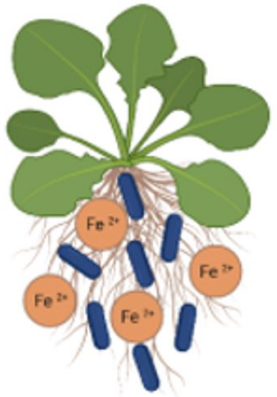


Theoretical expectations from transcriptomic analysis with sub-population specialization

Introduction

Pseudomonas brassicacearum R401 (PsR401)

- **Root colonizer** of ***Arabidopsis thaliana***
  - **Products 3 costly compounds**
    - **Phytotoxin**
    - **Antimicrobial**
    - **Siderophore**
      - **Enhances competitiveness by sequestering iron**



PsR401 a good model to study DoL

(Getske et al, 2023)
(Getske et al, 2024)
(Chesneau et al, 2025)

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Hypothesis in pure isogenic culture :

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Hypothesis in pure isogenic culture :

1°) "no production" of phytotoxin and antimicrobial *(low genes expression)*

2°) production of siderophore under low iron condition (high genes expression)

Use bacterial single-cell RNA-seq

Many technical challenges

- **No polyA tail on mRNA**
- **Cell wall makes lysis difficult**
- Very Low RNA content per cell
- High rRNA content
- …

(Nishimura et al, 2025)

Materials and Methods

2 Culture Medium

No stress:

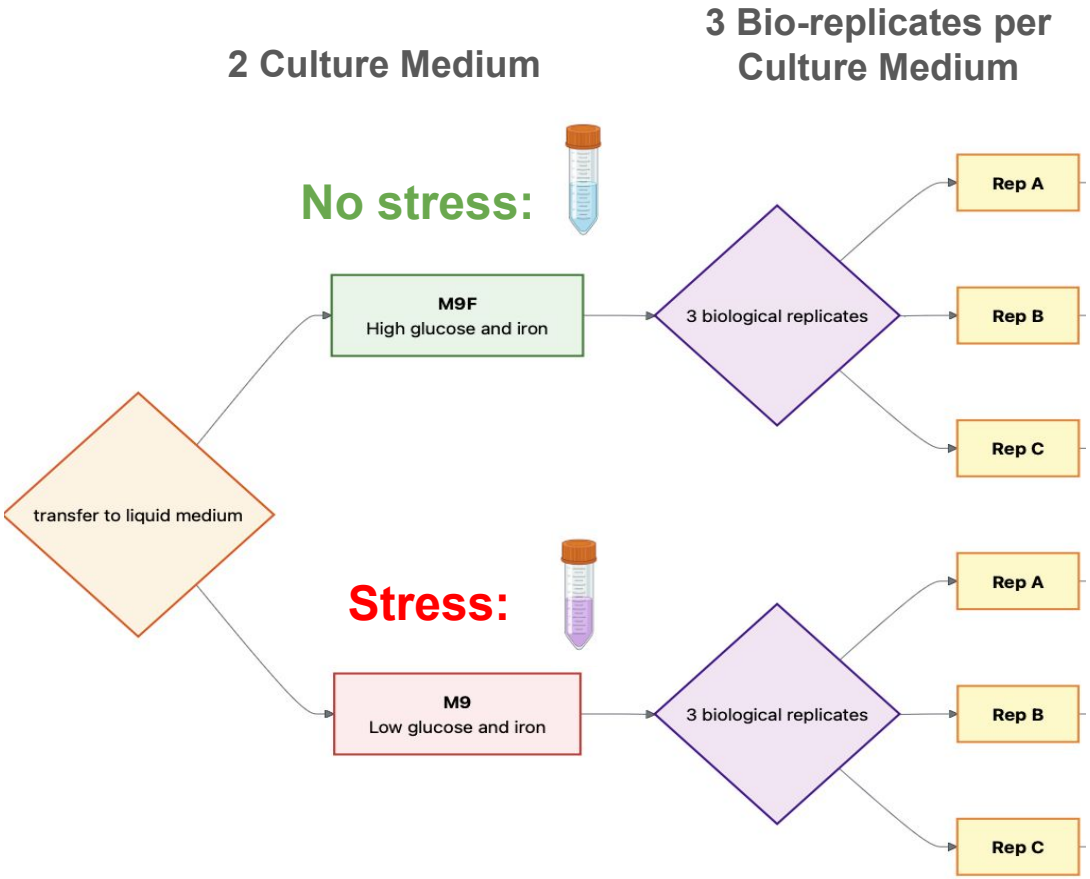
M9F
High glucose and iron

transfer to liquid medium

Stress:

M9
Low glucose and iron

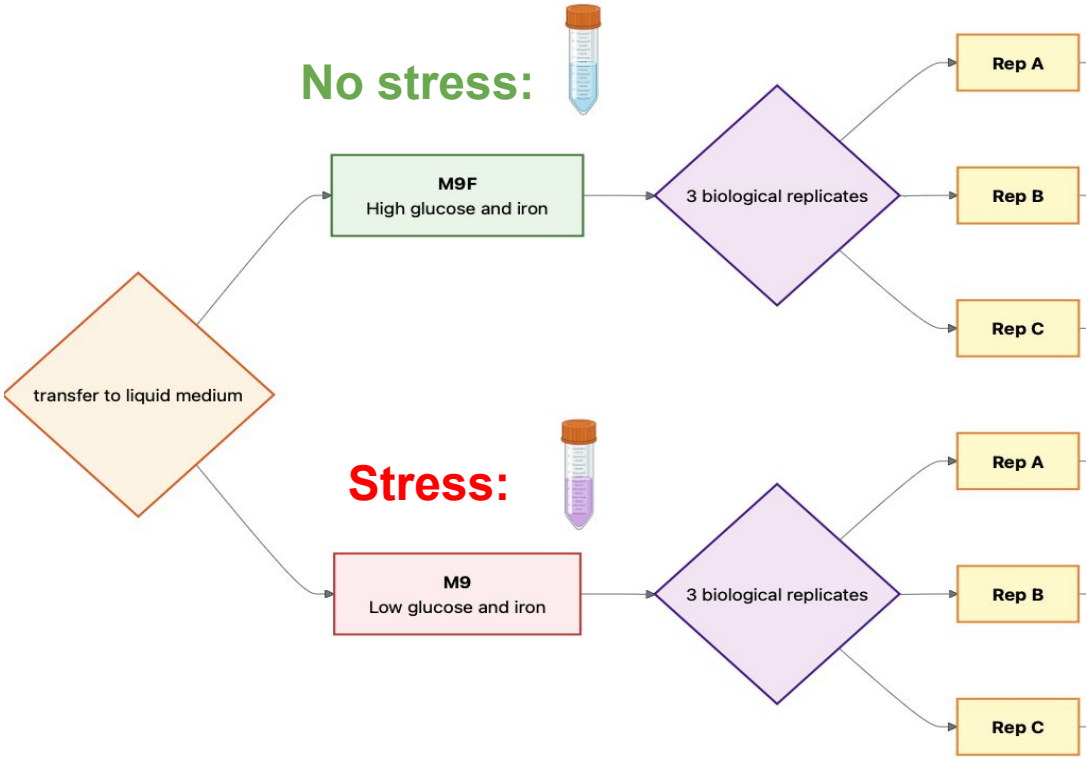
Materials and Methods



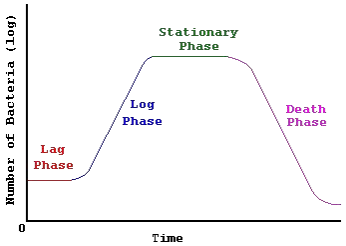
Materials and Methods

2 Culture Medium

3 Bio-replicates per Culture Medium

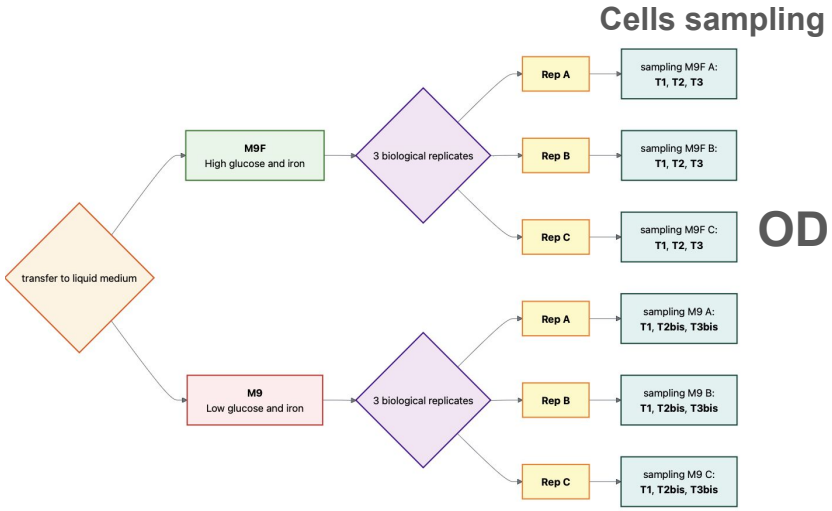


For each sample :
Growth was measured over time using optical density (OD)



Materials and Methods

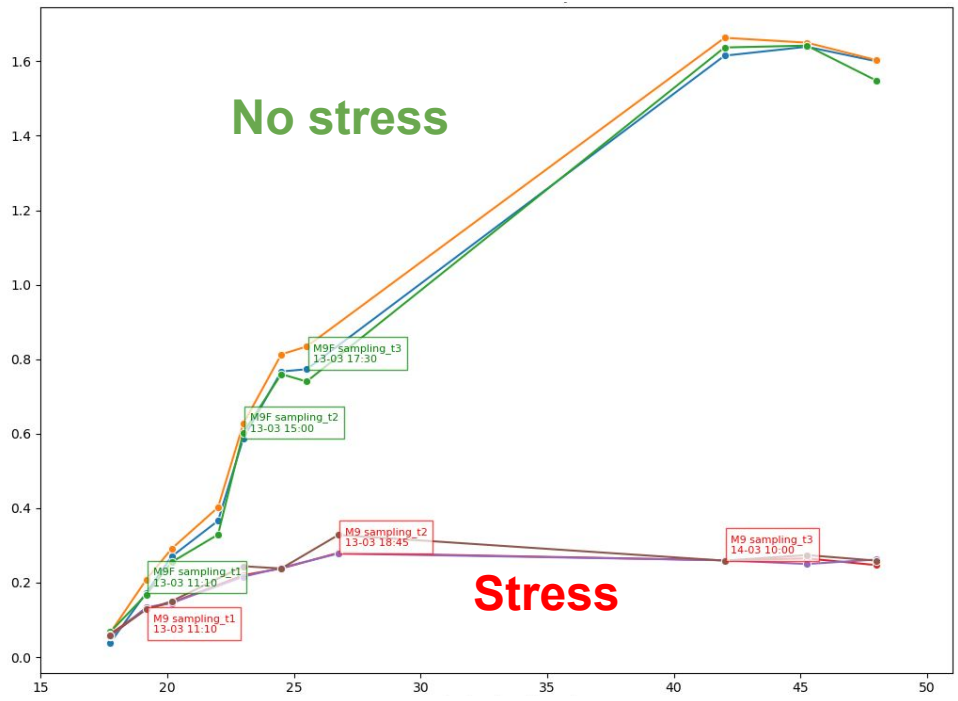
- Cells sampling over time to follow dynamics of DoL



OD

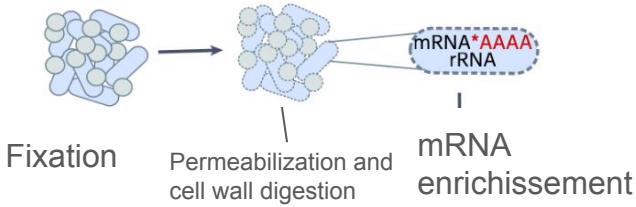
2 Culture Medium X 3 Bio-replicate X 3 sampling over time = **Total : 18 conditions**

Growth of the different PsR401 populations



Incubation time (hours)

MicroSPLiT : Split-pool barcoding



For each of 18 conditions

Materials and Methods

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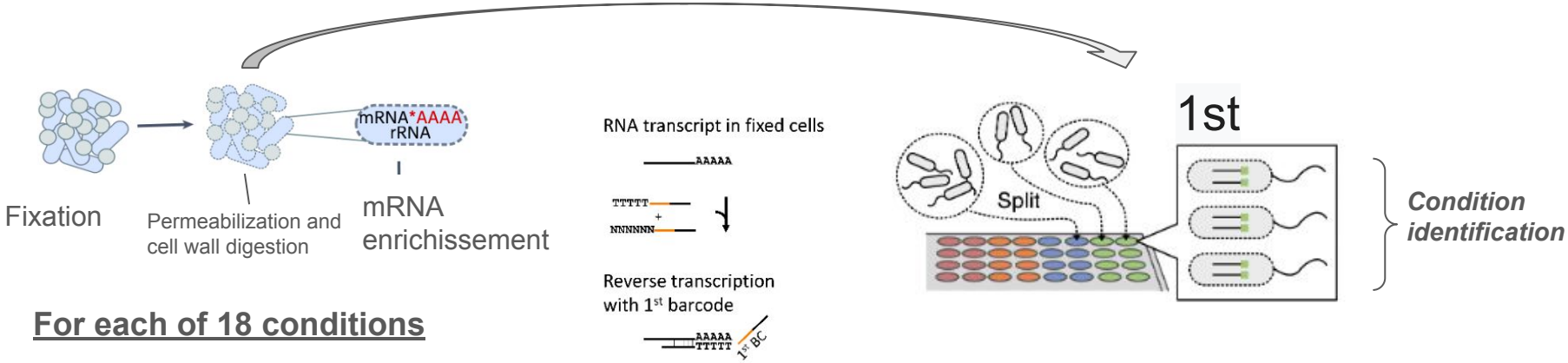

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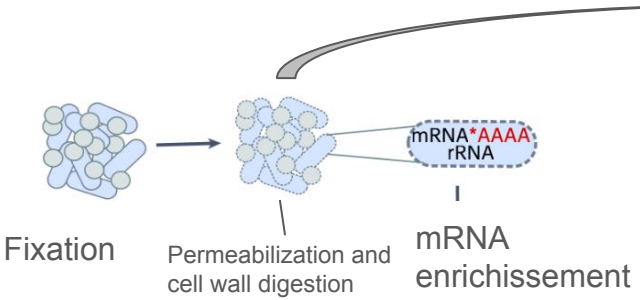

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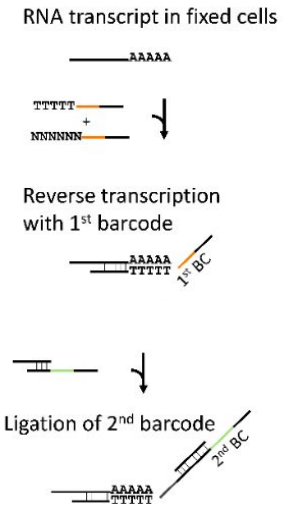

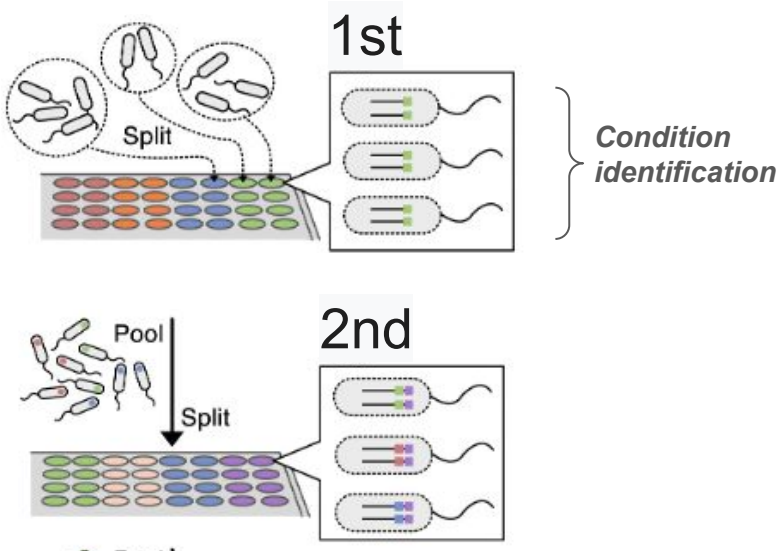

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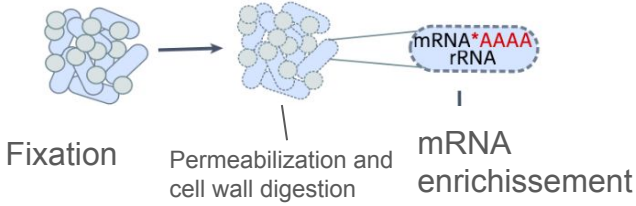
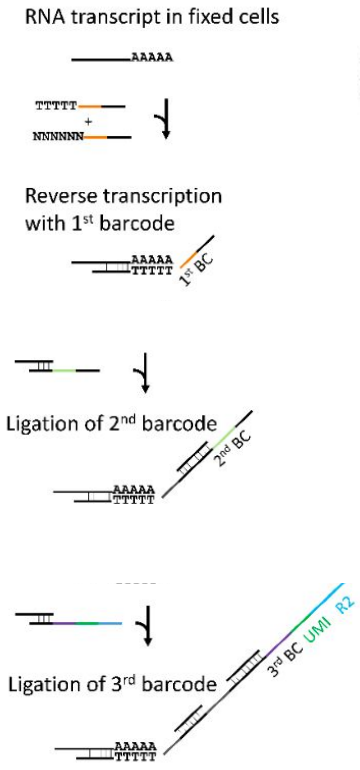
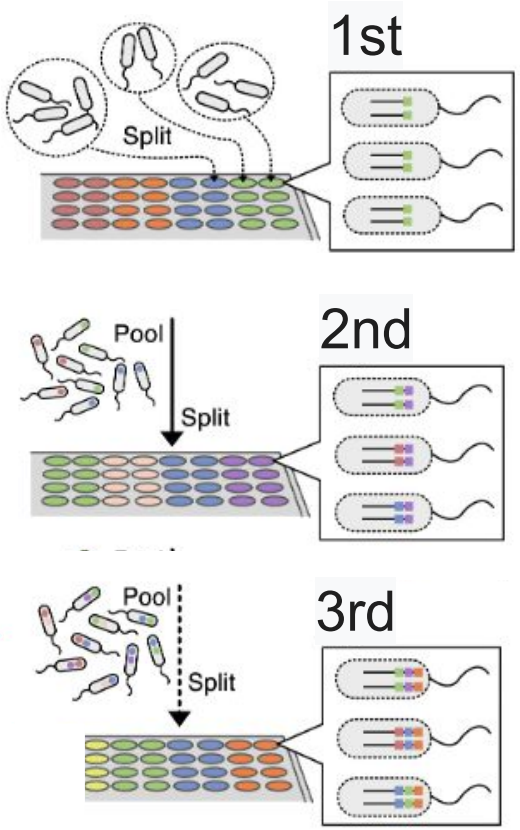


For each of 18 conditions





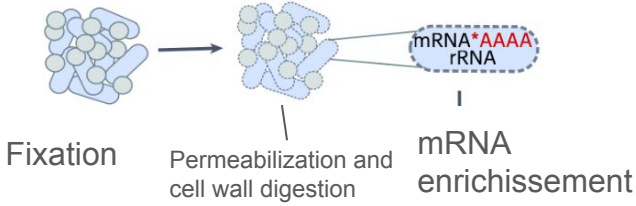
Condition identification

Unique cell and transcript identification (UMI)

more than
800 000 possible
barcode combinations

Materials and Methods

MicroSPLiT : Split-pool barcoding



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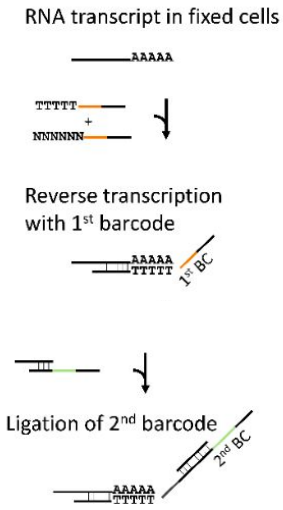

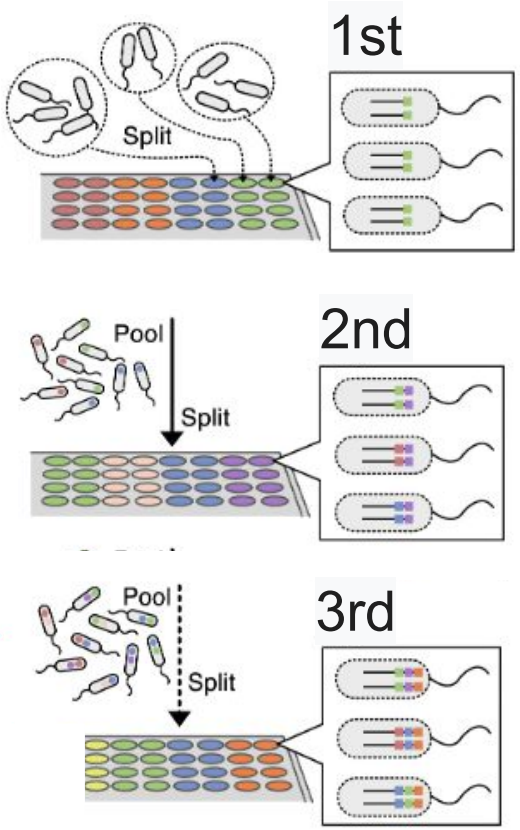

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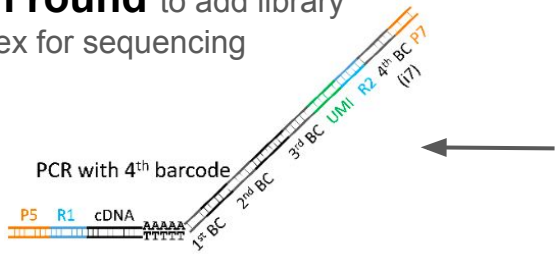
4th round to add library index for sequencing



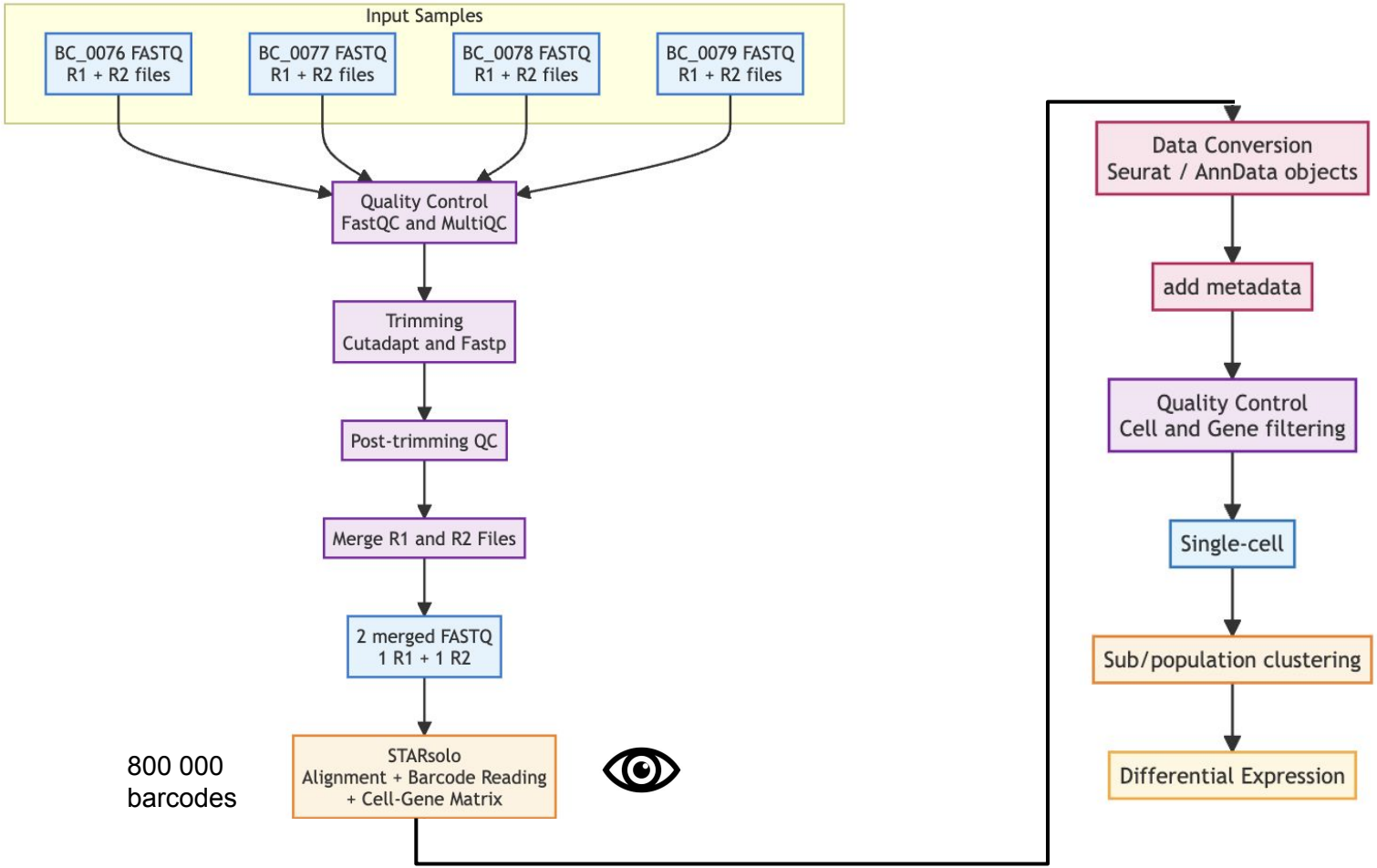
Materials and Methods

Sequencing Four sub-libraries :

- ≈ **3000 cells**
- depth: **1.5 billion reads**
  - Illumina NovaSeq™ X plus

Materials and Methods

Pipeline for microSPLiT data processing



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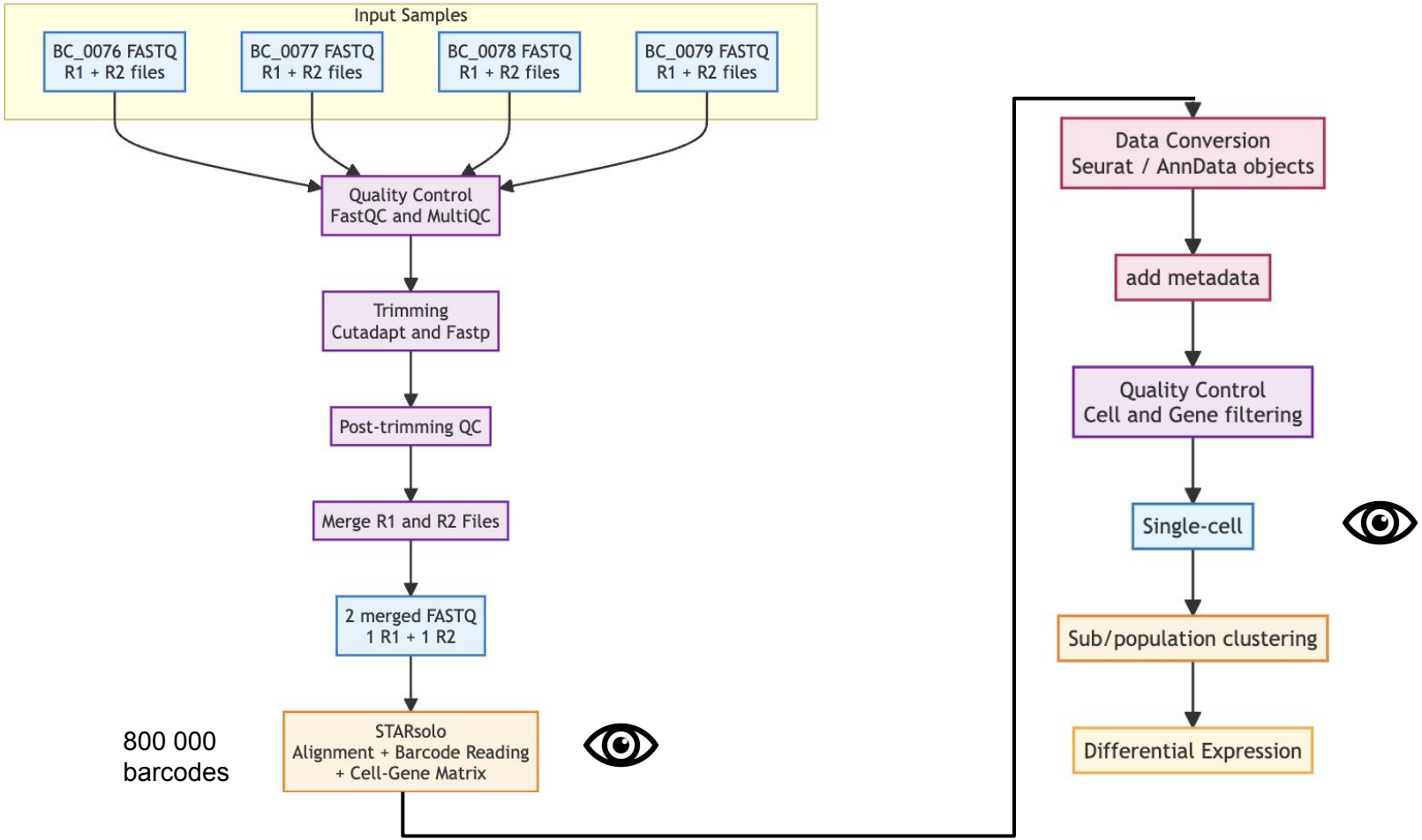

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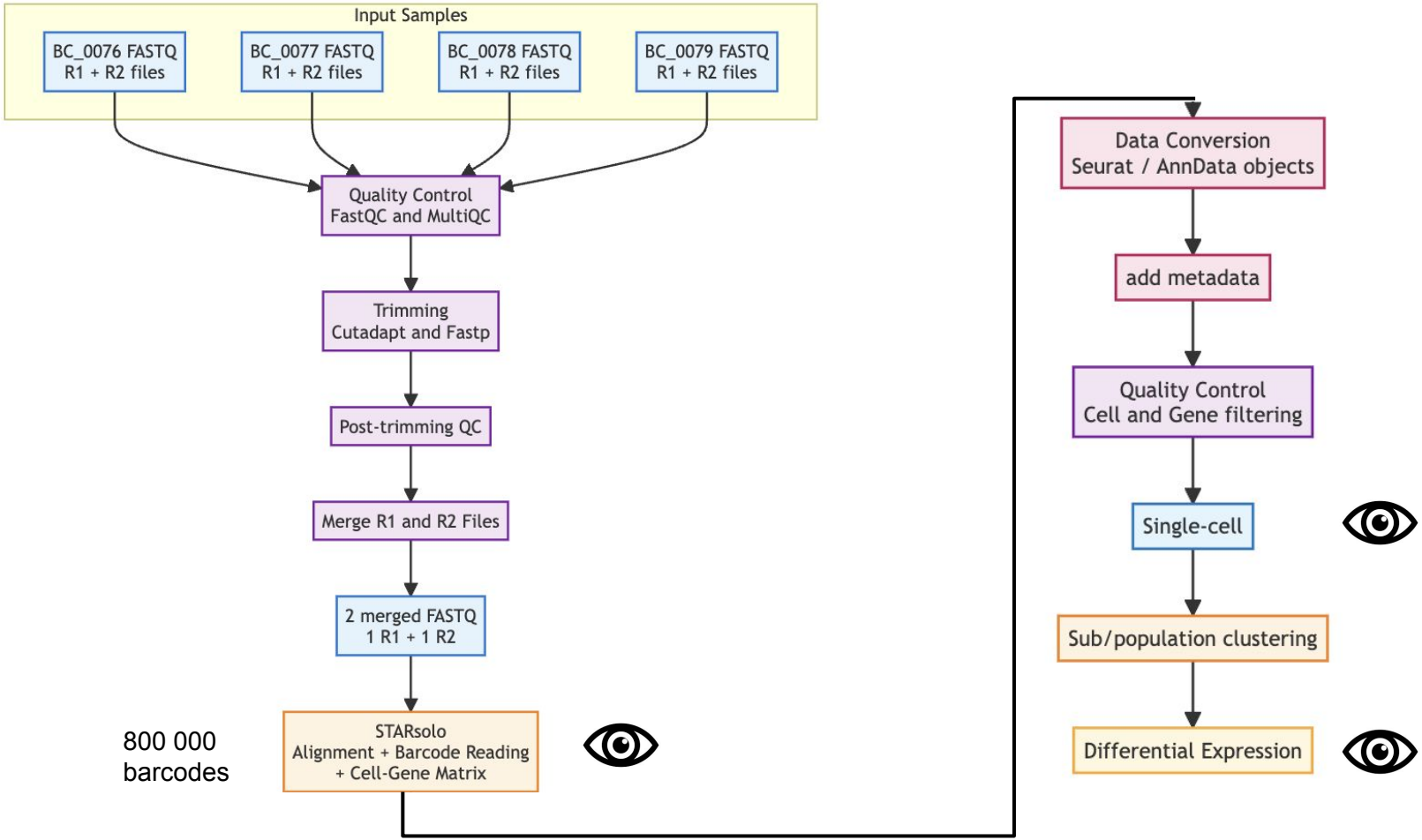

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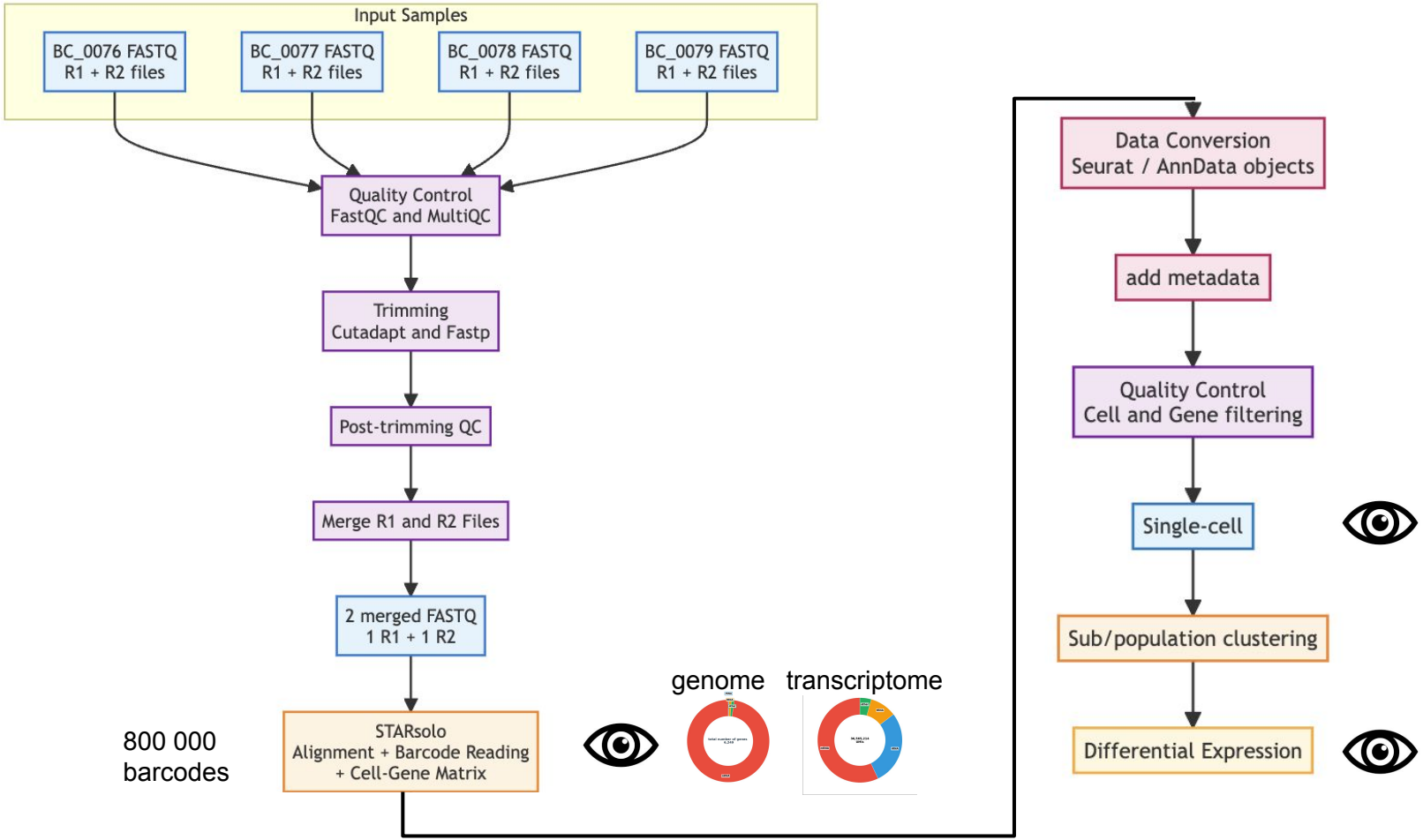
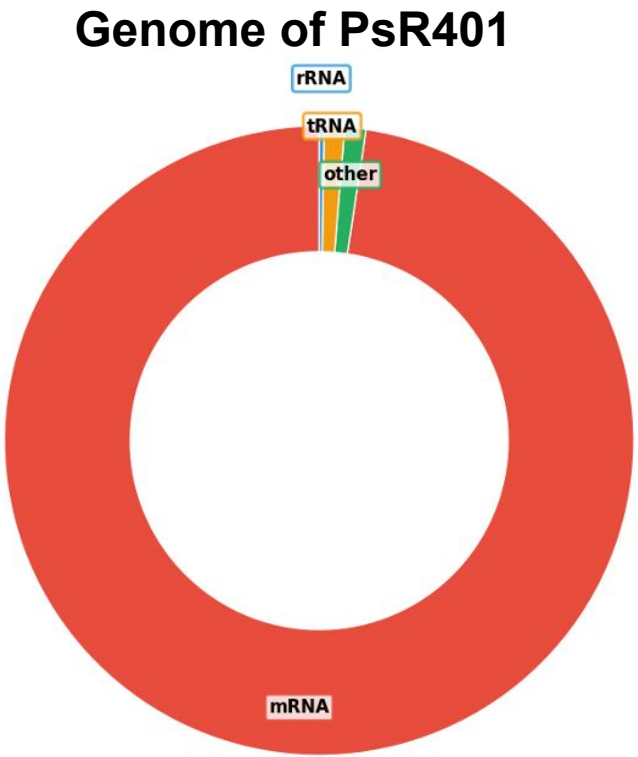


Results

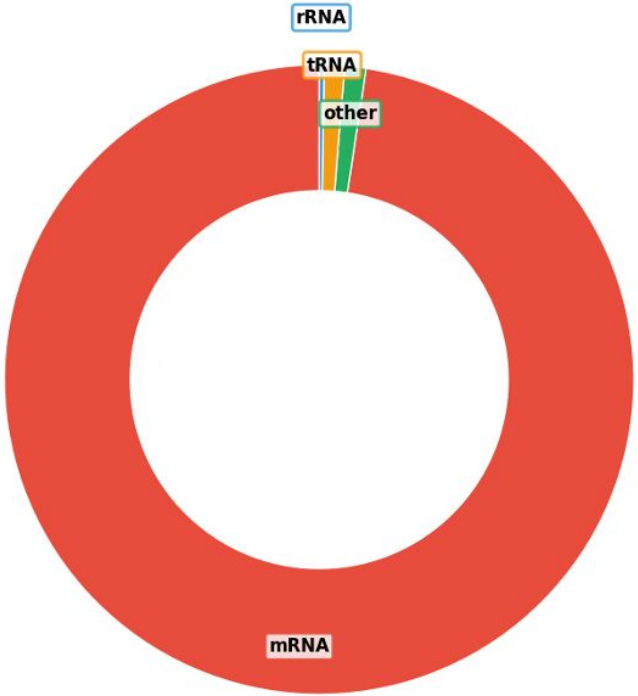


Results

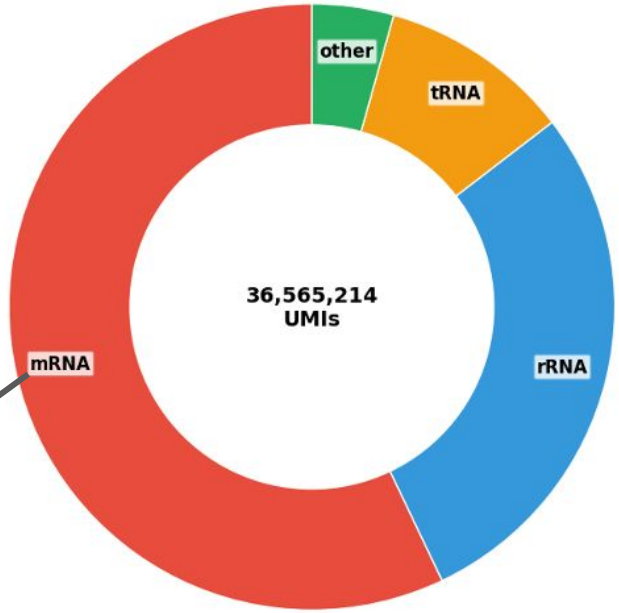
Raw Data STARsolo

Does microSPLiT scRNA-seq work effectively to uncover transcriptional heterogeneity in bacteria?

Genome of PsR401



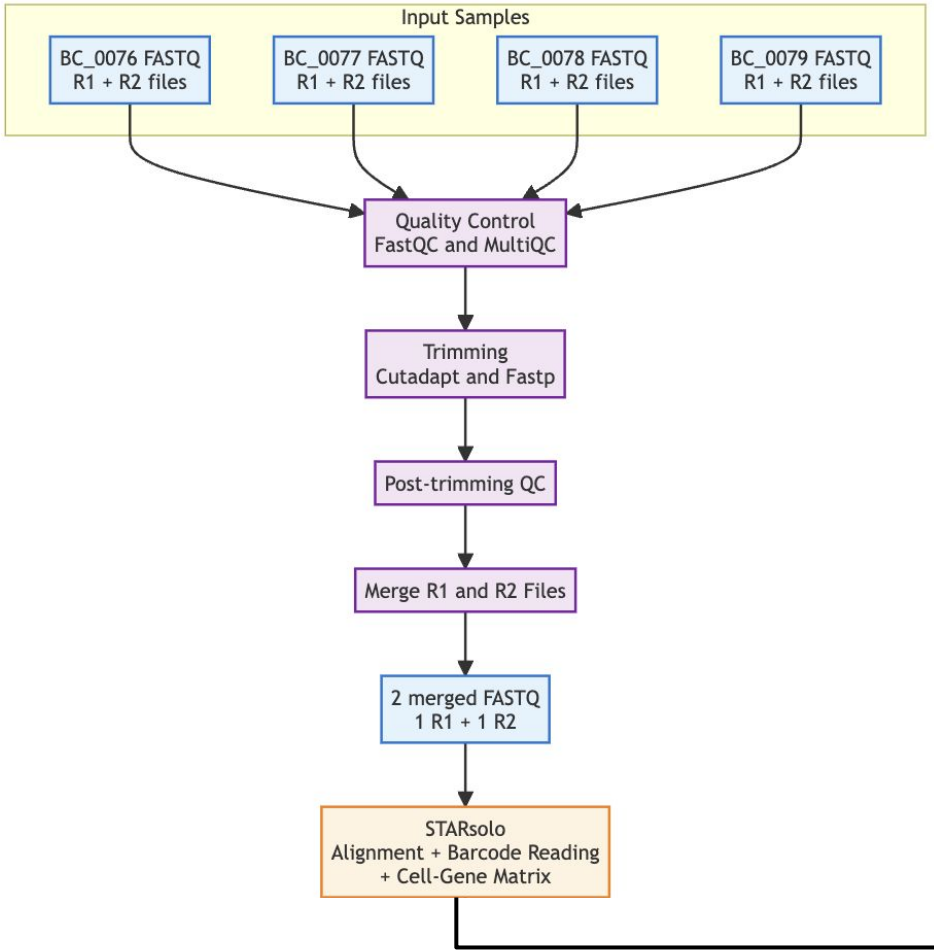
Transcriptome of PsR401

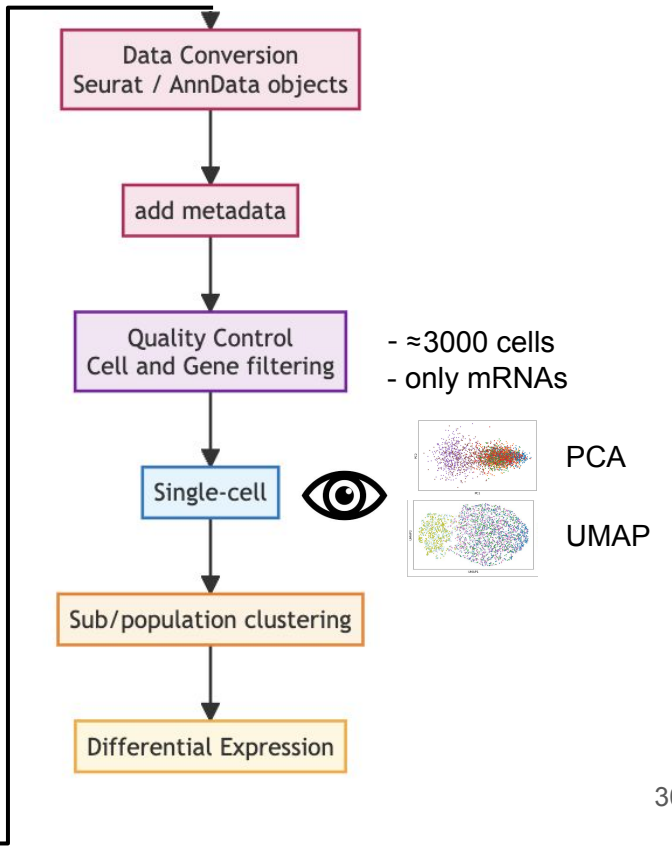


57,1% mRNAs

Materials and Methods

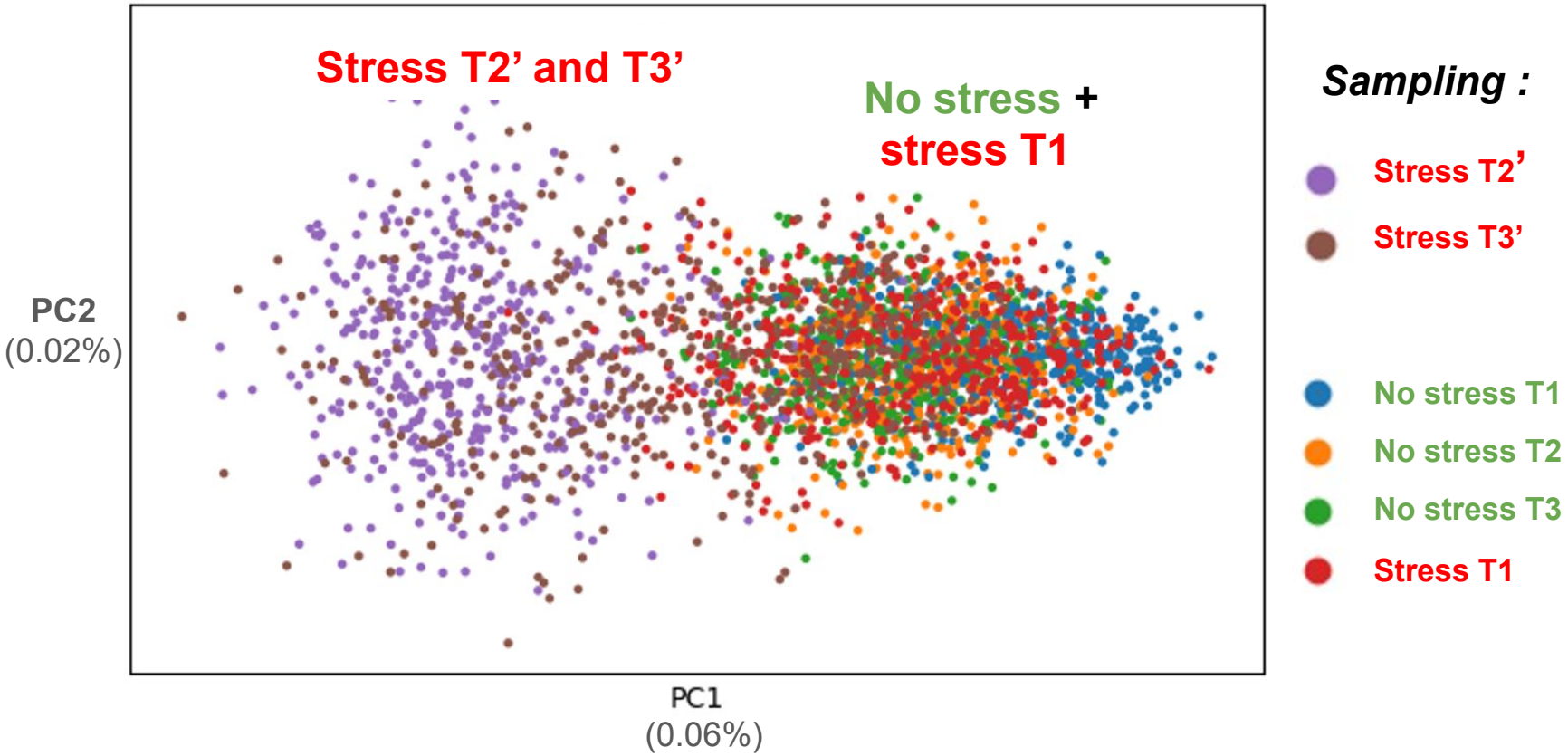
Pipeline for microSPLiT data processing





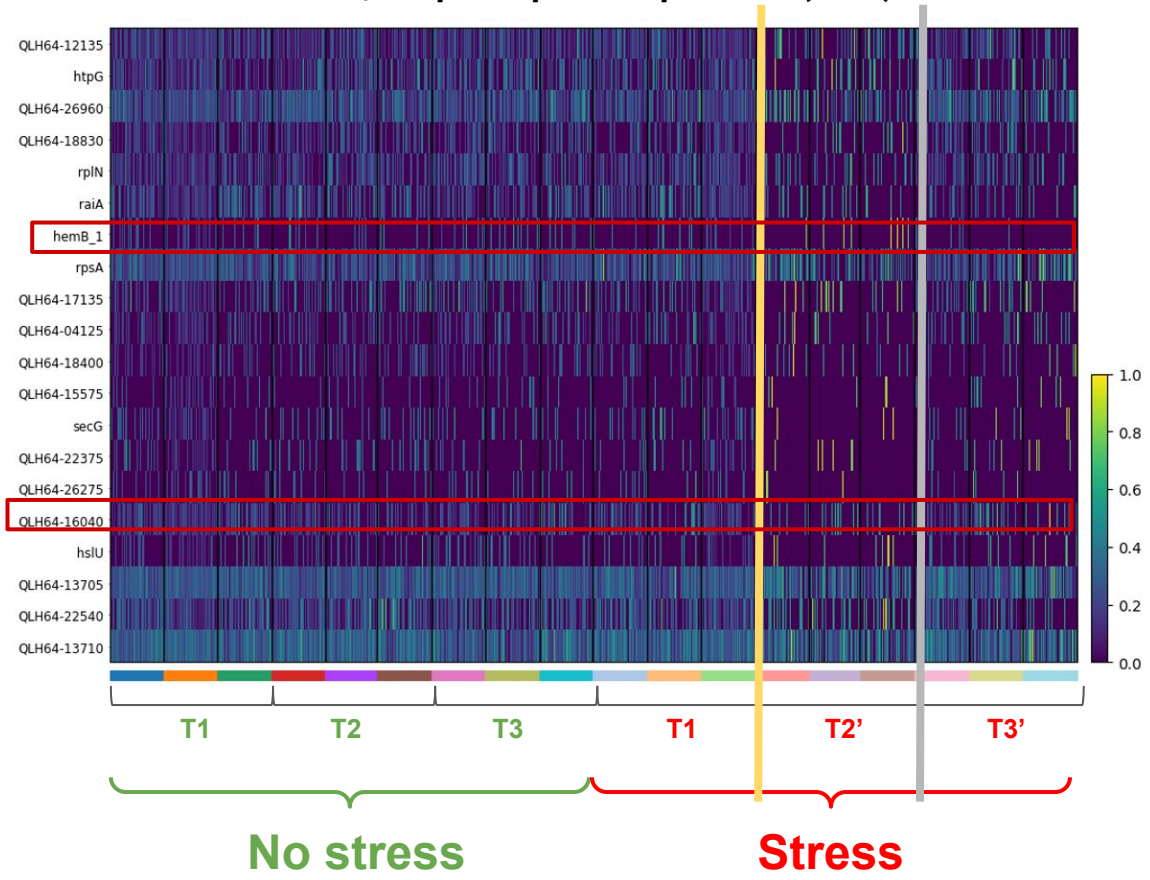
Results

PCA Analysis



Results

Heatmap of the top 20 contributing genes to the first principal components (PC1)



PCA Analysis

Hp : production of siderophore under low iron condition (high genes expression)

Iron related genes :

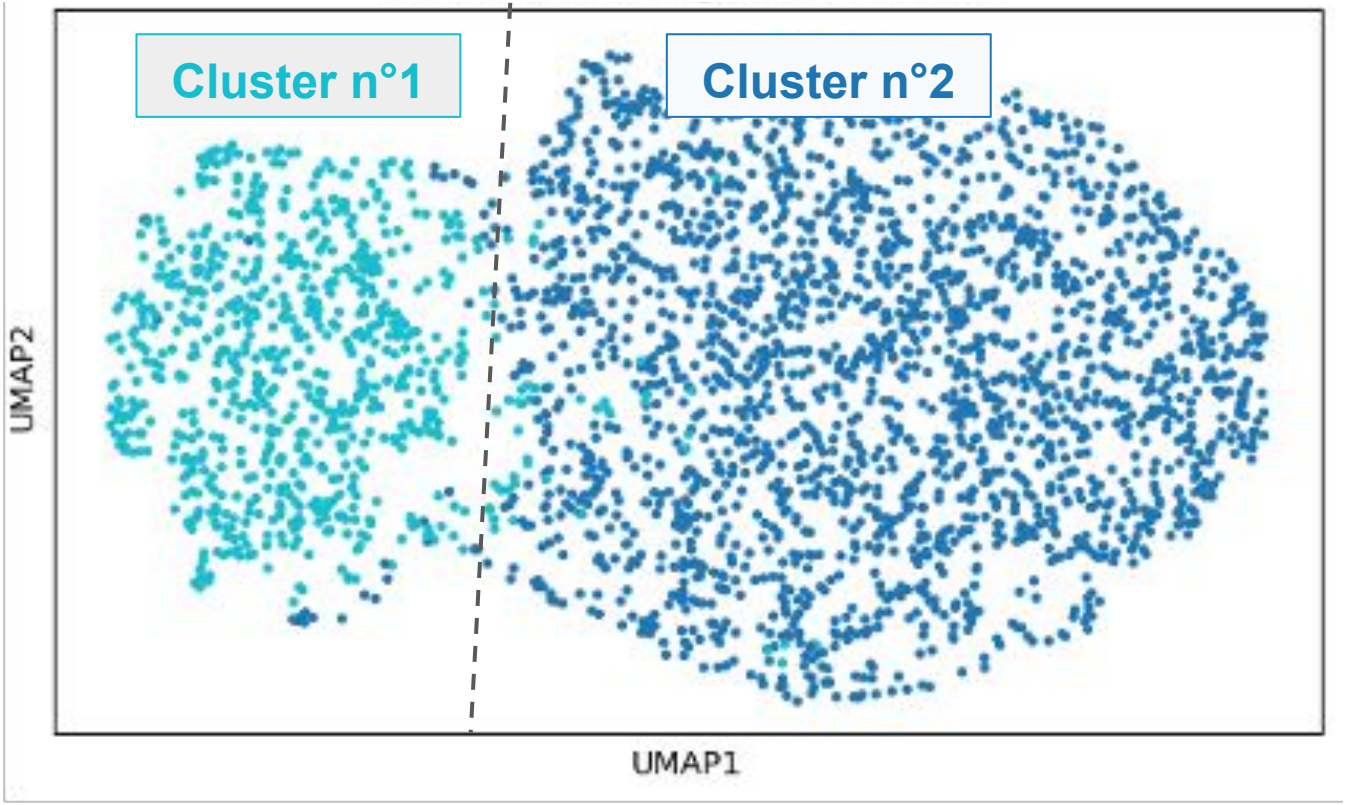
- ***hemB_1***
  - Porphobilinogen synthase
- ***QLH64-16040***
  - TonB-dependent siderophore receptors

Fujita et al, 2019)
(Moeck et al, 1998)

Results

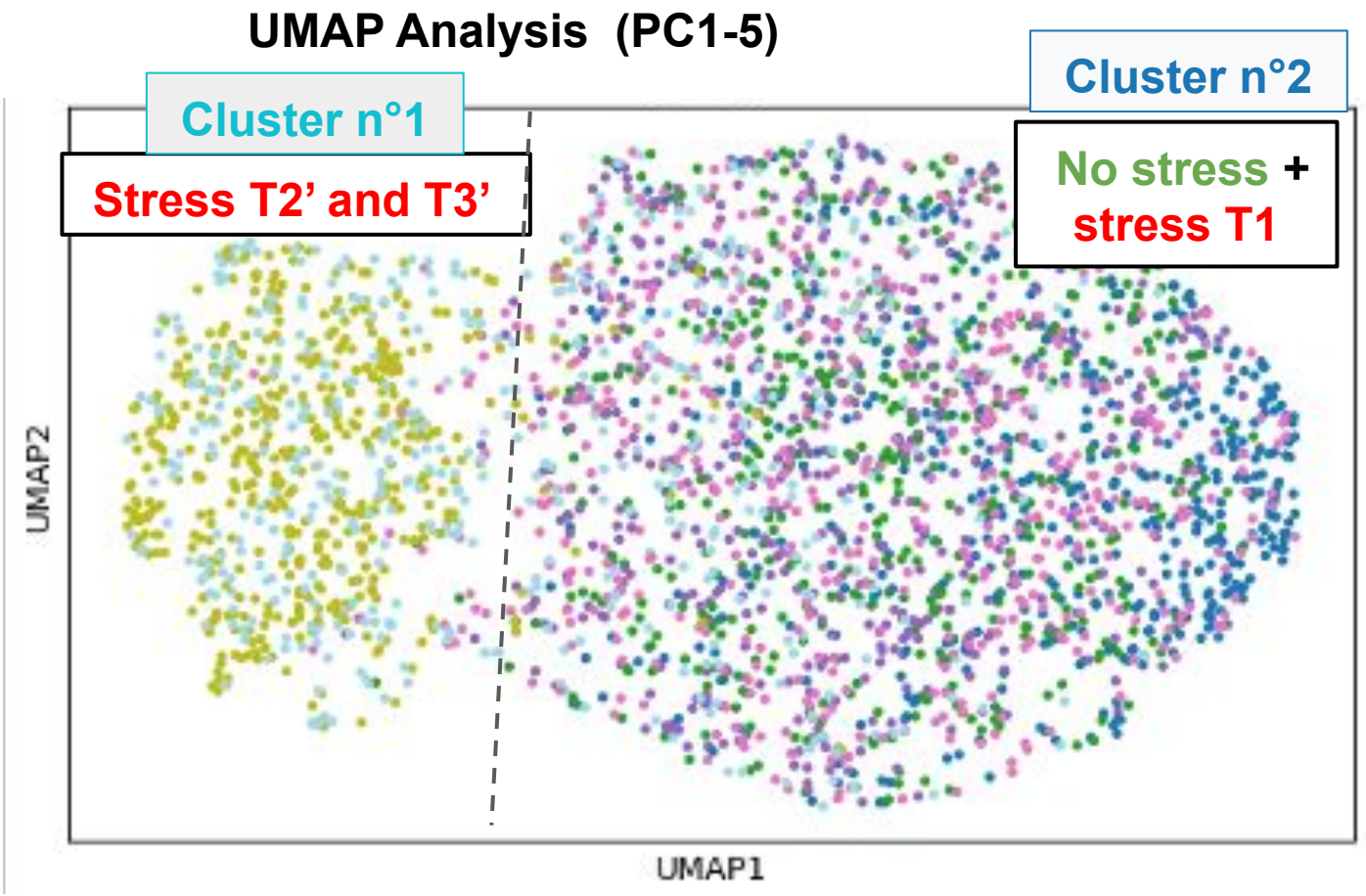
UMAP Analysis (PC1-5)

Leiden Clustering resolution 0.1



Only 2 clusters

Results

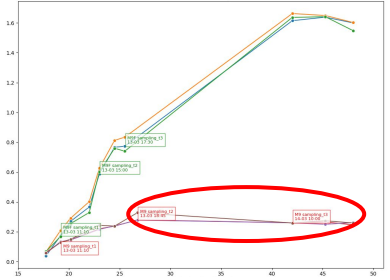


Sampling :

- Stress T2'
- Stress T3'
- No stress T1
- No stress T2
- No stress T3
- Stress T1

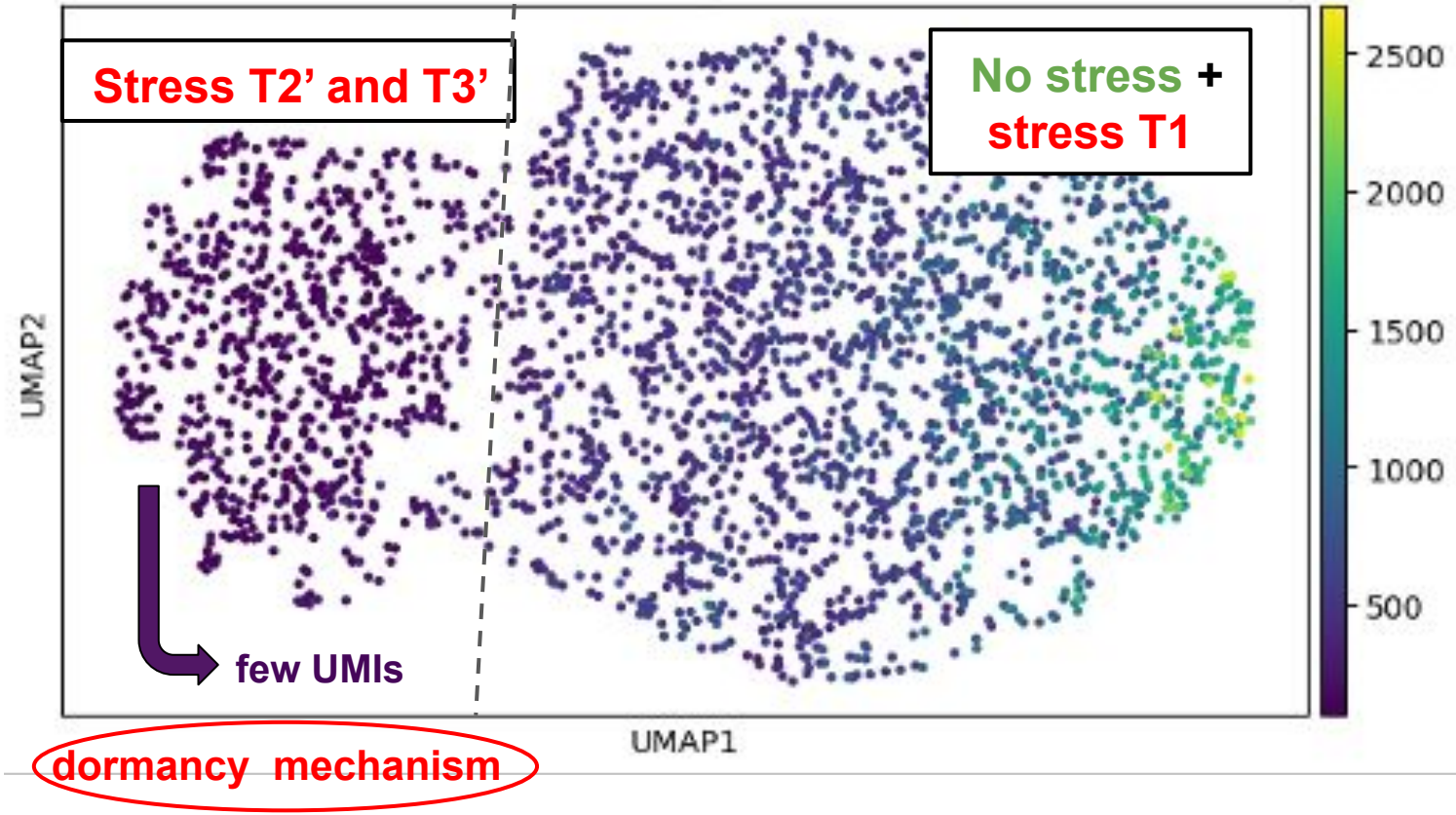
Two clusters were detected, without distinct condition-specific / or population grouping within them

Results



UMAP Analysis (PC1-5)

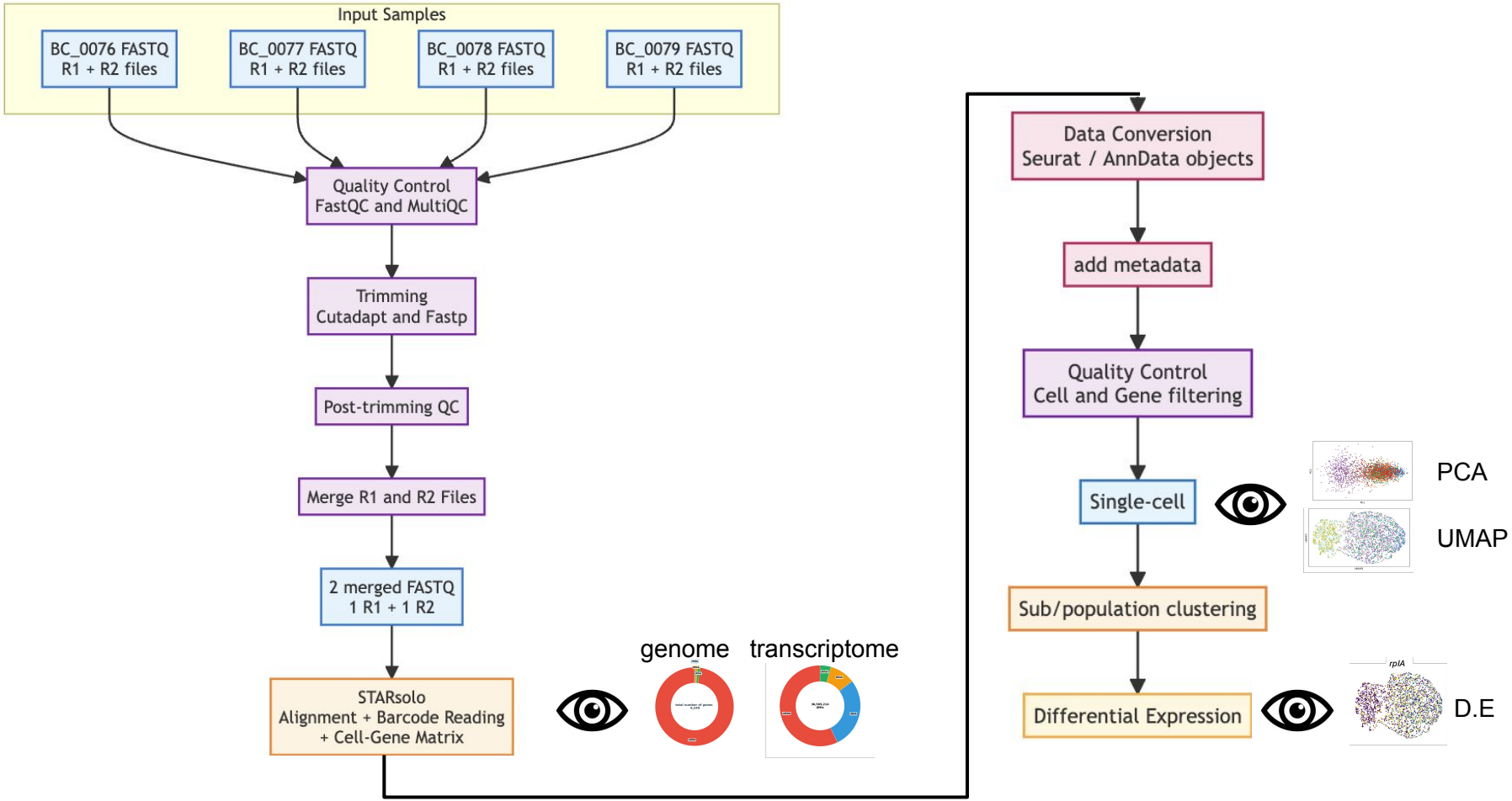
Total UMIs per Cell



dormancy mechanism

Materials and Methods

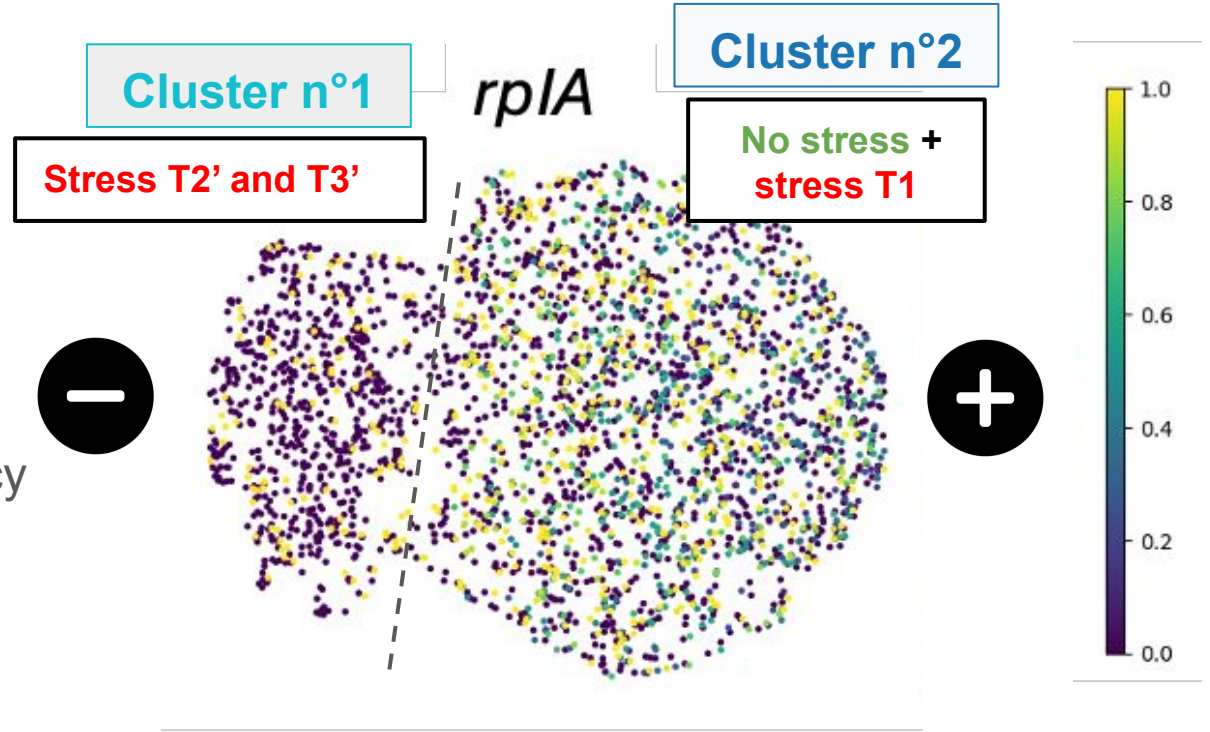
Pipeline for microSPLiT data processing



Results

RplA : 30S ribosomal protein L1

Example of differentially expressed gene between the 2 Clusters (K-W and FDR<0.05)



link to dormancy mechanism

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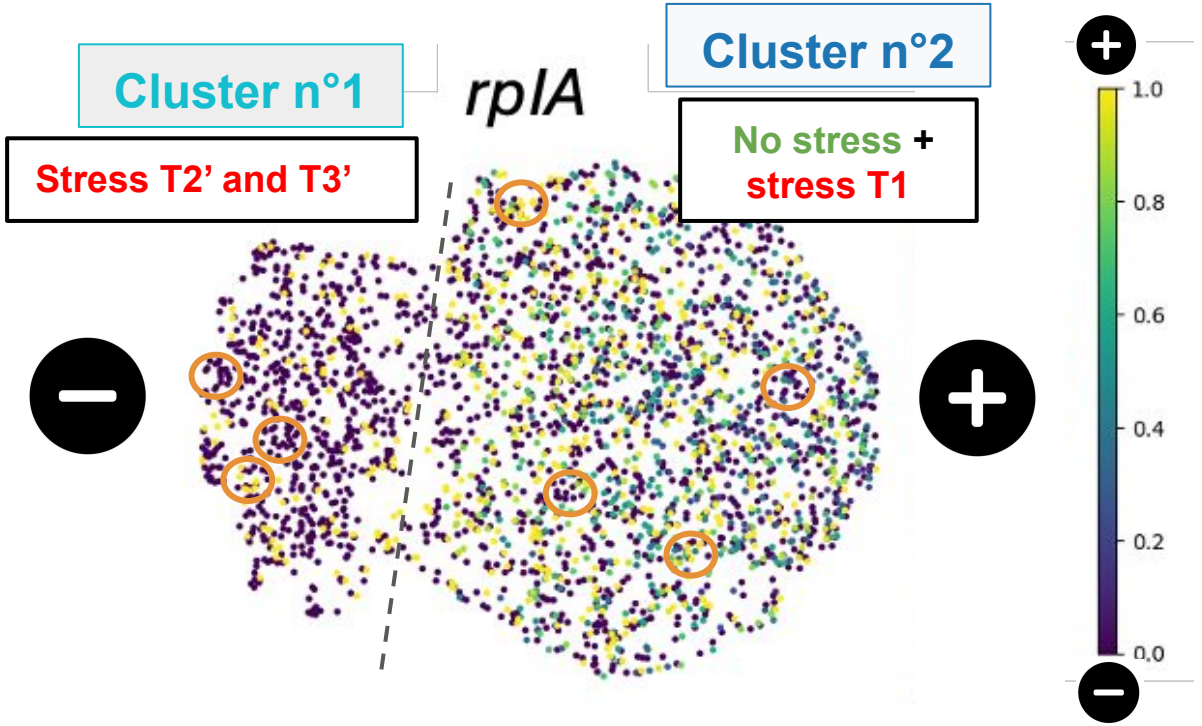

But heterogeneity inside clusters

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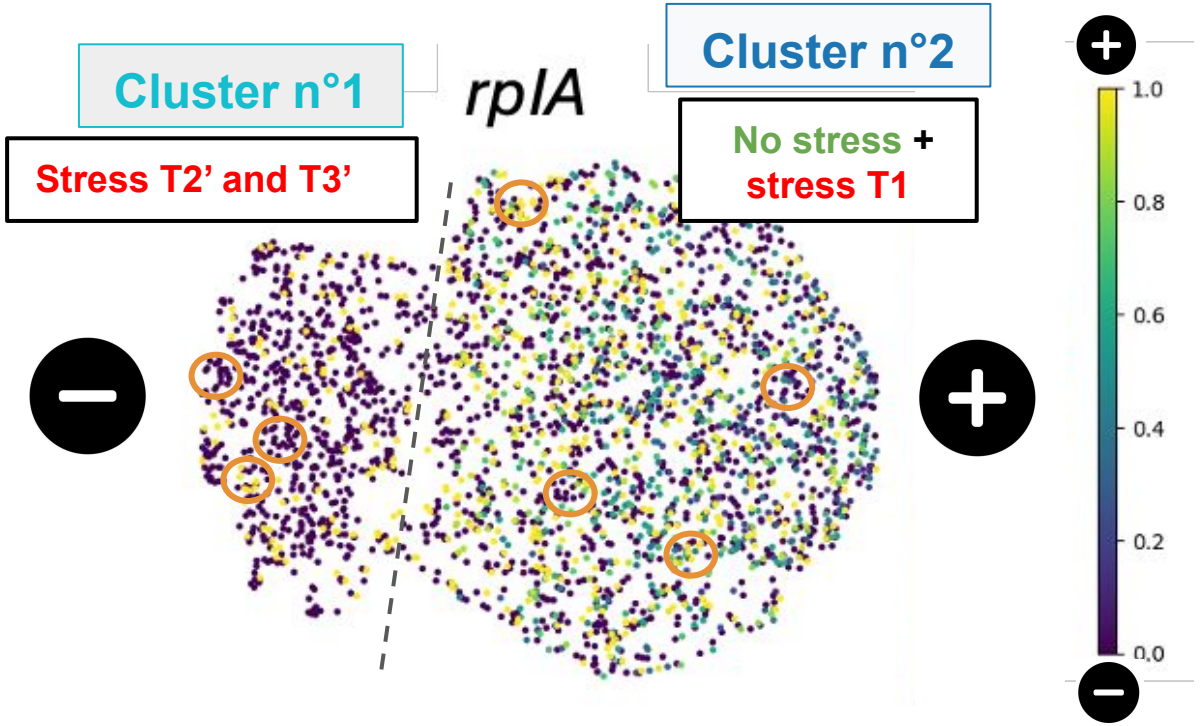


But heterogeneity inside clusters

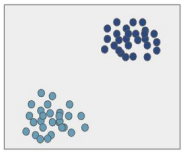
=> specialization? or ≠ conditions

=> need more investigations

Discussion and perspective

Does microSPLiT scRNA-seq work effectively to uncover transcriptional heterogeneity in bacteria?

- UMIs, mRNAs
- Distinguish stressed vs. non-stressed conditions
- siderophore



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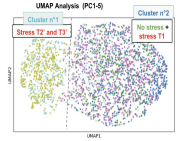

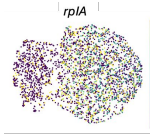

Two clusters were detected, without distinct condition-specific / or population grouping within them

=> Further investigations are needed

- **Exclude stressed T2' and T3' samples from the analysis to reduce variance**
- **Check for clustering within each condition individually**

Discussion and perspective

Bioinformatics perspectives

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Bioinformatics perspectives

- implementation of other quality QC tools

  - for contaminants detection : **FastQ Screen**, **Recentrifuge**…

Discussion and perspective

Bioinformatics perspectives

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  - for contaminants detection : **FastQ Screen**, **Recentrifuge**…
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- for better Single Cell analysis finish to test **BacSC pipeline**

  (preprint: Ostner et al, 2024)

Discussion and perspective

Bioinformatics perspectives

- implementation of other quality QC tools

  - for contaminants detection : **FastQ Screen**, **Recentrifuge**…

  - for barcoding : **BarQC** (preprint : Rossello et al, 2025)

- for better Single Cell analysis finish to test **BacSC pipeline**

  (preprint: Ostner et al, 2024)

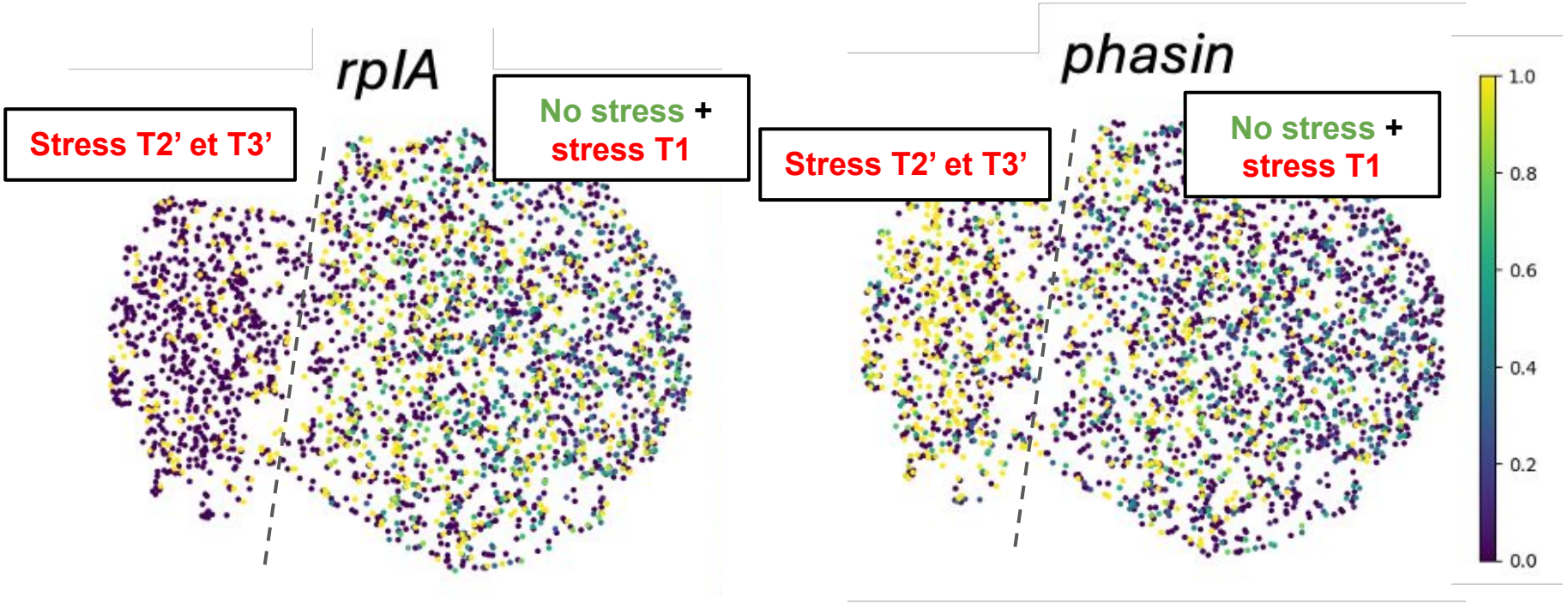
- **Gene Ontology** enrichment, **cell trajectory inference**, **metabolomic pathway** analysis, and **pseudobulk** analysis…

Thank you for your attention

M2 Bioinformatique

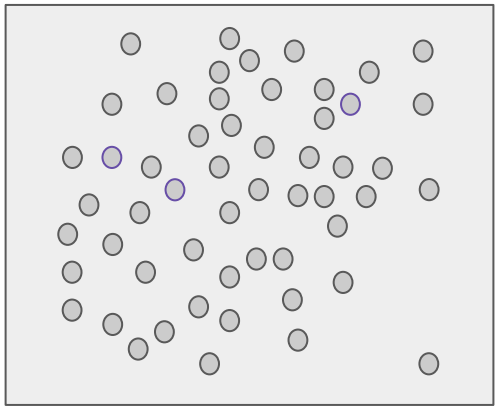
Valentin Goupille

Results


rplA
Stress T2' et T3'
No stress + stress T1
phasin
Stress T2' et T3'
No stress + stress T1
1.0
0.8
0.6
0.4
0.2
0.0

Intraspecific DoL Hypothesis :

hp n°2 : DoL through noisy gene expression (stochastic expression) (Lopez and Wingreen, 2022)

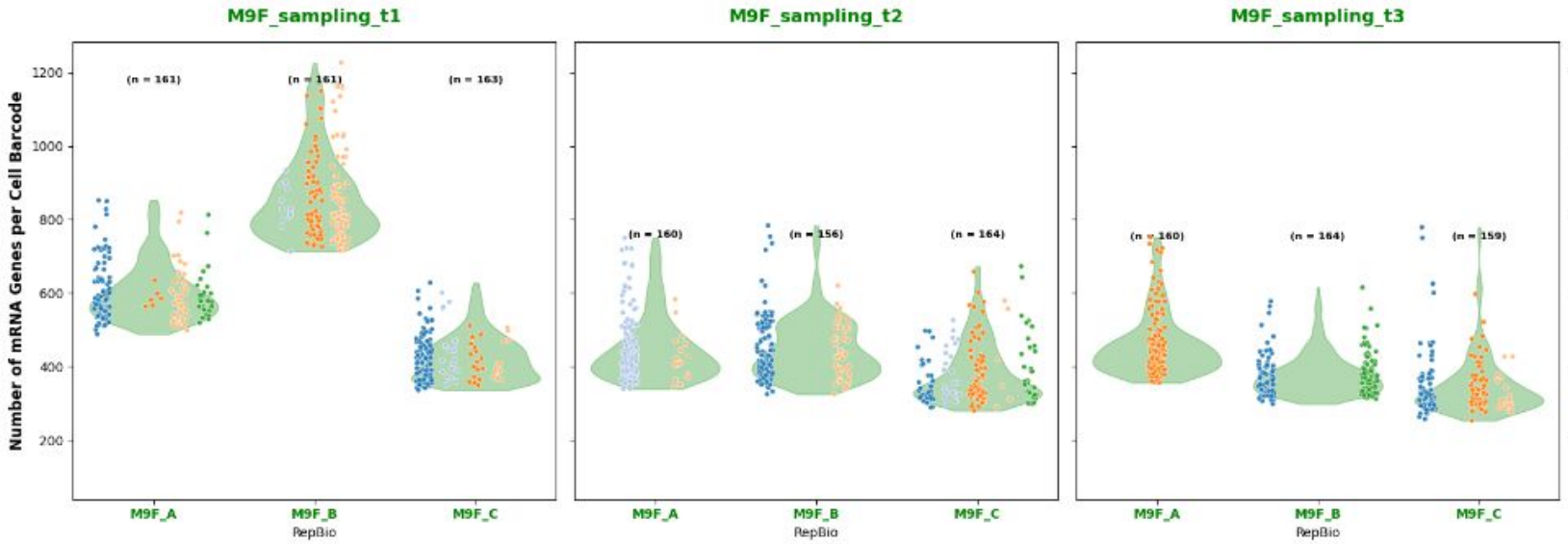


Theoretical expectations from transcriptomic analysis with Noisy genes expression

Each cell expresses only a subset of the genes

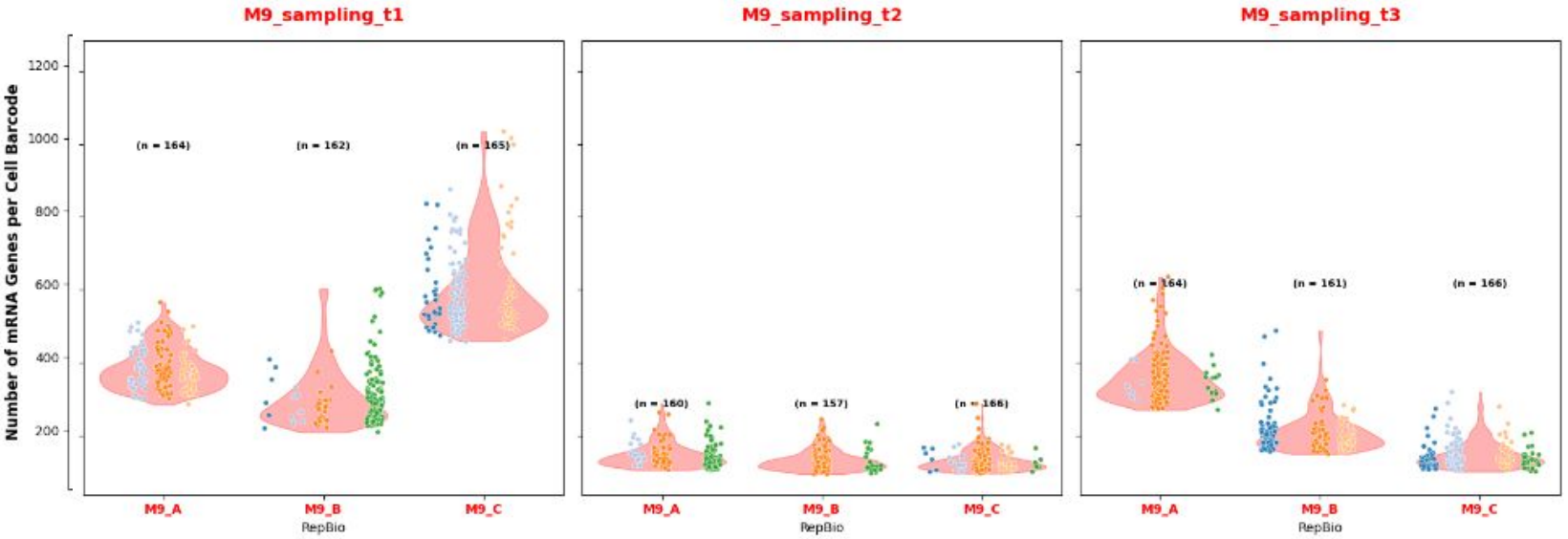
- increase growth rate by exchanging metabolites within a clonal community
- regulation at population level

Results



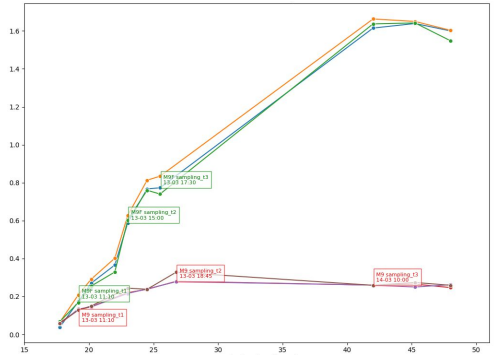
Control (M9F) : high iron

Results



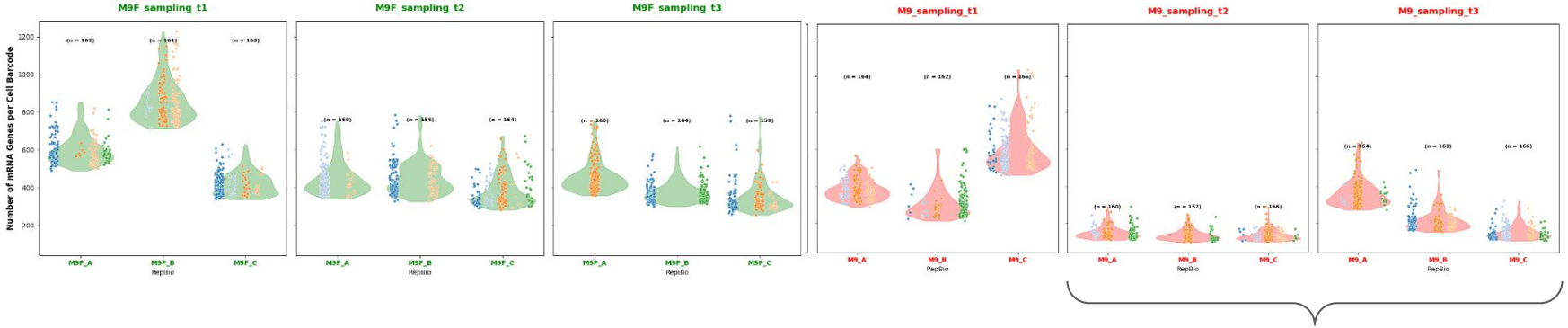
Stress (M9) low iron

Results



Control (M9F) : high iron

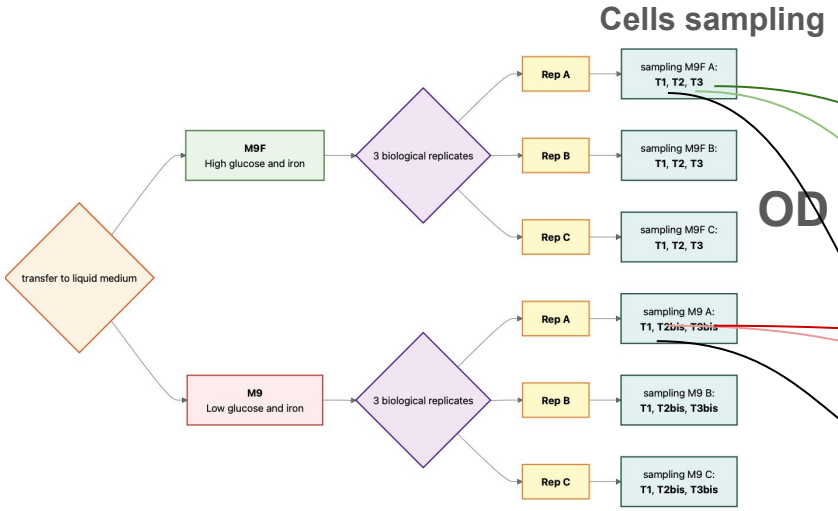
Stress (M9) low iron



Very few genes are expressed at T2 and T3 in M9F, suggesting a dormancy mechanism — possibly entering a VBNC state, with slight transcriptional reactivation at T3.

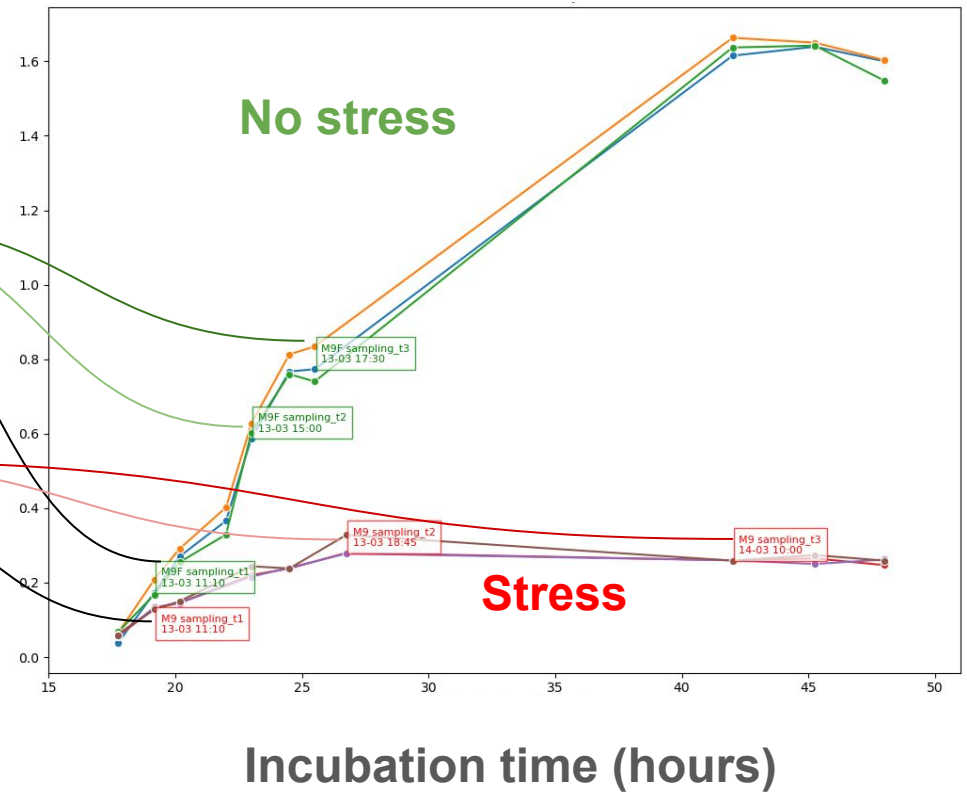
Materials and Methods

- Cells sampling over time to follow dynamics of DoL



2 Culture Medium X 3 Bio-replicate X 3 sampling over time = **Total : 18 conditions**

Growth of the different PsR401 populations



Filtering transcriptome (not detailed here)

- **only mRNAs are conserved**
- **800 000 barcodes => ≈ 3000 cells (160 cells / per condition)**