µSPLiT:

A High-Throughput, Single-Cell RNA-Sequencing Technology for Microbes

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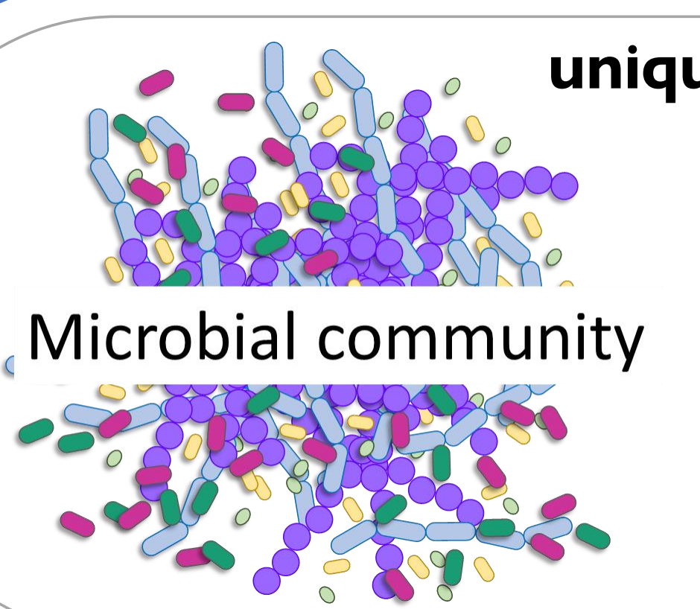
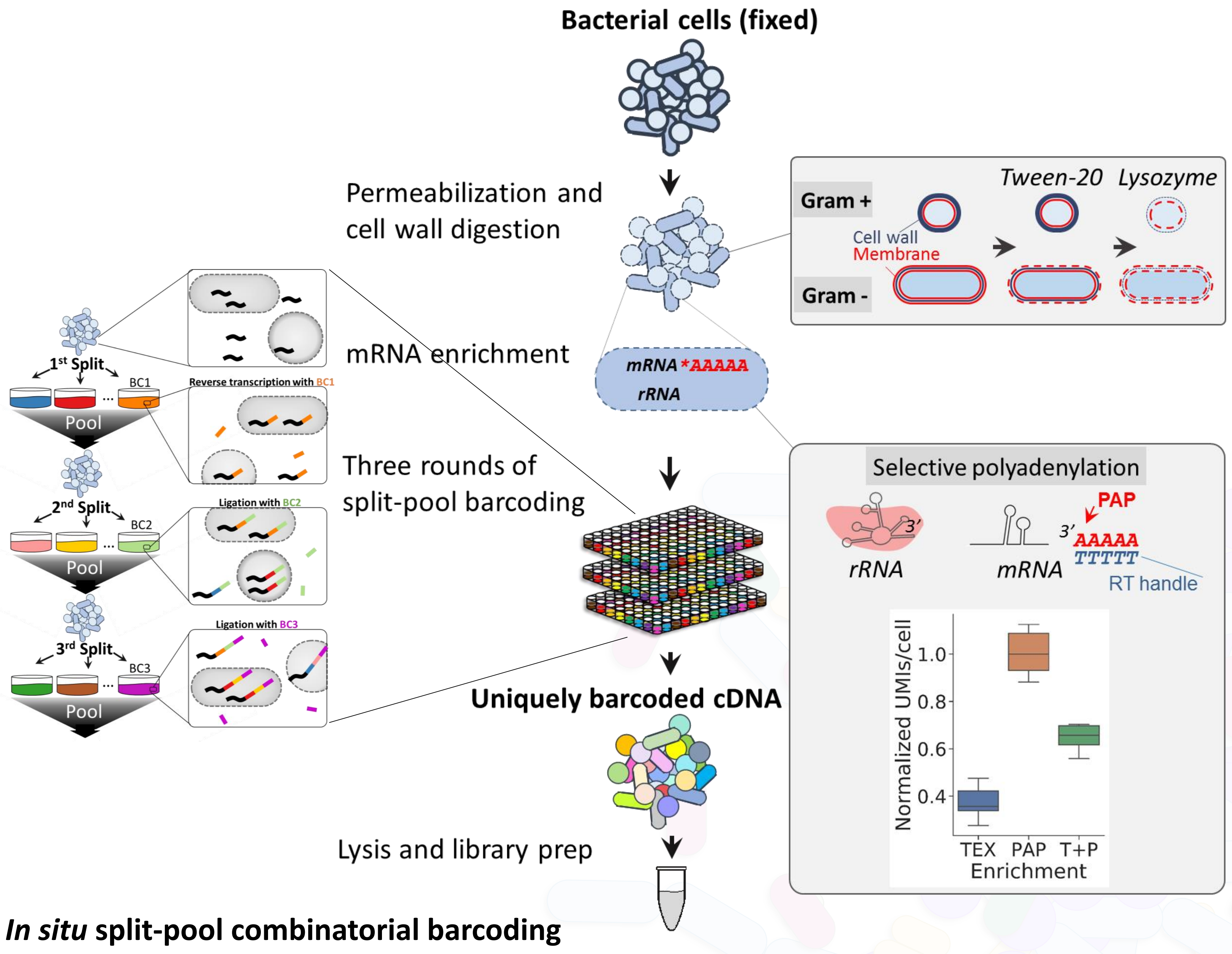
unique challenges posed by bacteria:



- Gram-positive & gram-negative cell wall architectures
- Widely varying shapes and sizes
- Up to 98%+ ribosomal RNA
- Low total transcript number
- Unknown genomes

µSPLiT method:


Bacterial cells (fixed)

Permeabilization and cell wall digestion

mRNA enrichment

Three rounds of split-pool barcoding

Uniquely barcoded cDNA

Lysis and library prep

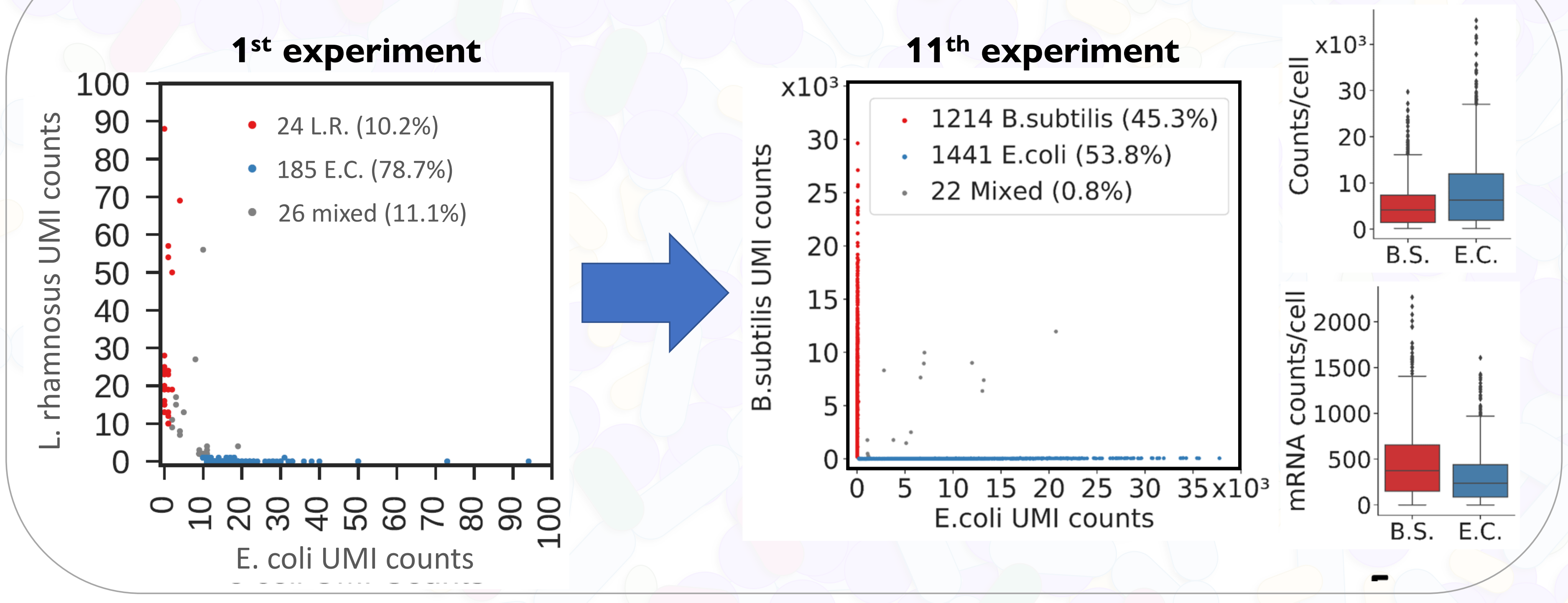
In situ split-pool combinatorial barcoding

- Bacterial cells are fixed and permeabilized; their cell wall is degraded; mRNA is enriched
- Cells are split into wells and RNA inside is reverse transcribed with the addition of 1st barcode
- Cells are pooled and randomly split into wells two more times, where 2nd and 3rd barcodes are appended by ligation
- Cells are pooled, lysed and cDNA is amplified with the 4th barcode

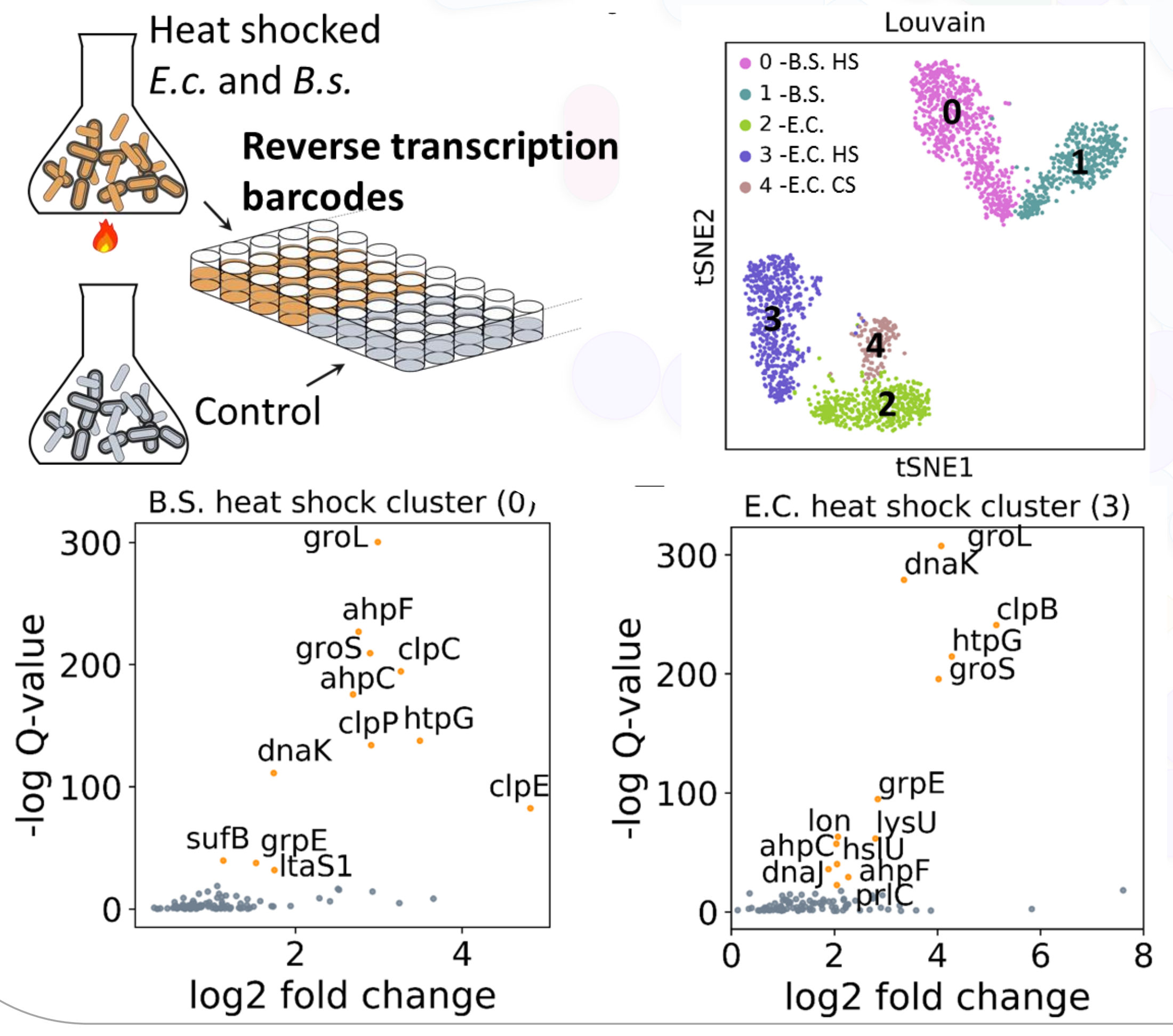
Features of µSPLiT barcoding

- cDNA in every cell is marked by a unique combination of barcodes
- Number of uniquely labeled cells increases exponentially with barcoding rounds
- Multiple samples can be frozen and multiplexed in the same experiment

optimization and validation:



detecting an environmental stimulus:



Experimental design

- *E.coli* and *B.subtilis* cultures were grown to mid-log phase, mixed and fixed
- Half of the cells were subjected to heat before mixing
- Heat shocked and control cells were distributed to separate rows in the reverse transcription plate, then pooled and processed together

Results

- We detected ~5000 transcripts\cell and >200 genes\cell in 2600 cells of both species (gram-positive and -negative)
- Distinct heat-shocked and control clusters came out of a Louvain clustering algorithm

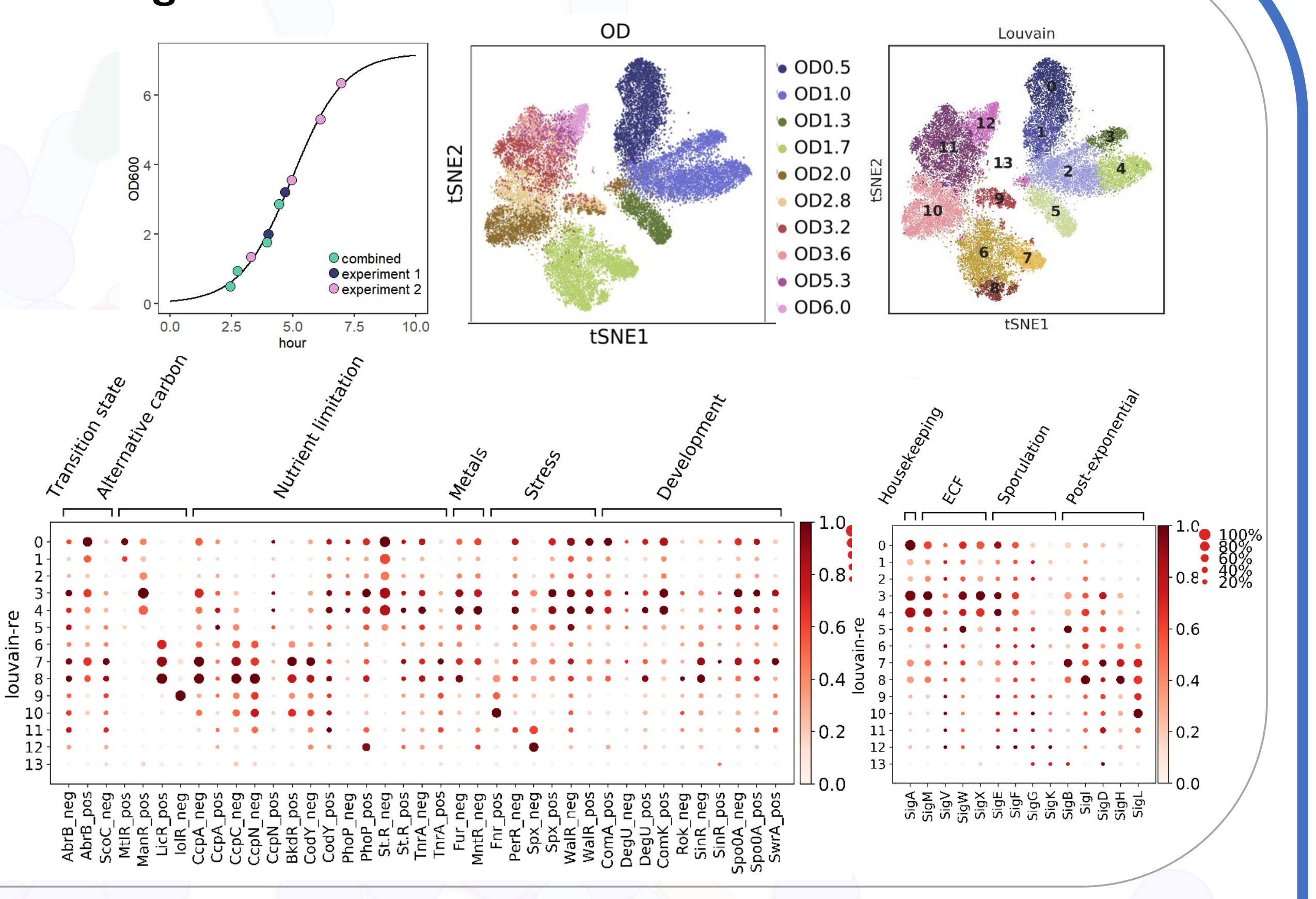
B. subtilis growth in rich media

Experimental design

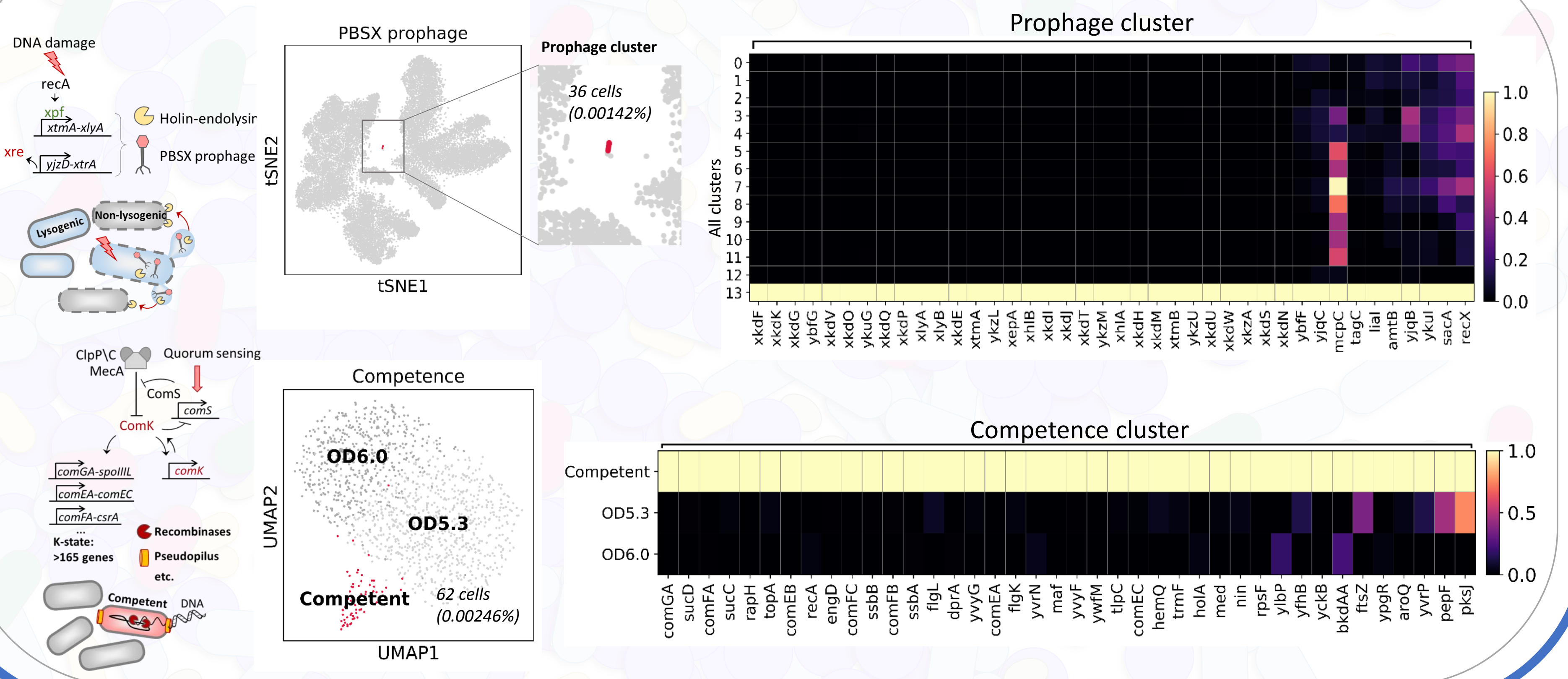
- Next, we applied µSPLiT to capture transcriptional states across the B.S. growth curve in a rich medium.
- We sampled >25k cells across two experiments

Results

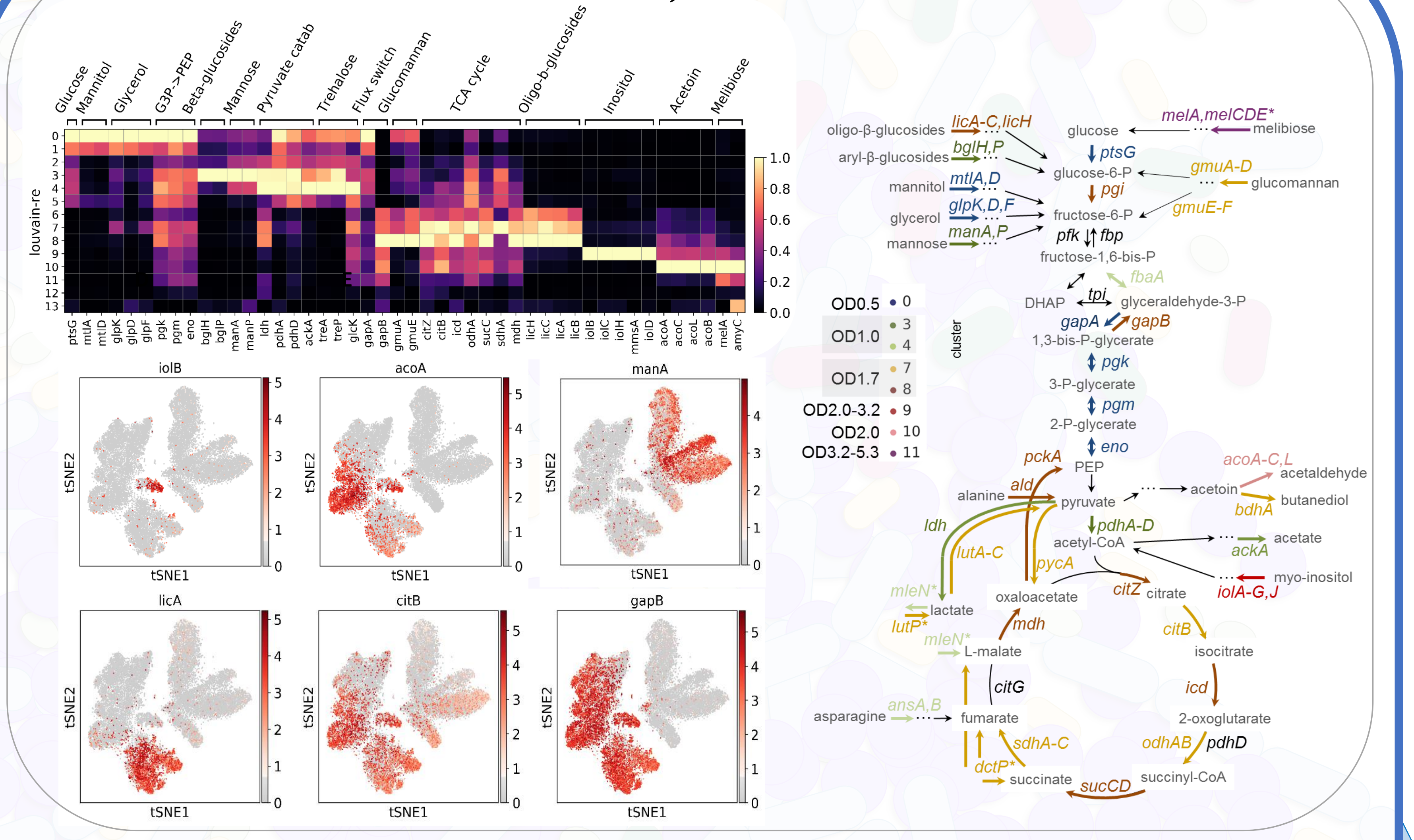
- Unsupervised clustering of the combined datasets revealed 14 clusters.
- The separate clusters differentially express a variety of regulons.
- We found small to rare states including cells expressing genes for myo-inositol utilization (cluster 9), PBSX prophage (cluster 13), and competence.



detecting rare states:



B. subtilis continued, carbon metabolism:



antimicrobials, motility, stress, metal balance: