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WP3 DigiOmica collaborative learning in Integrated omics for environmental sustainability

Module 7: *Microbial gene transcripts in environmental samples*

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Educational goals: the aim of this module is to present knowledge about the

- Environmental transcriptomics linking genetic potential with microbial biogeochemical activity
- Basic steps in the protocol for analysis of partial environmental transcriptomes
- Different approaches and solutions to overcome the difficulties of transcriptional heterogeneity

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Summary

Environmental mRNA (environmental transcriptomics) retrieves transcriptomes of microbial assemblages lacking information on the kinds of genes expressed at the community level. It links microbial genetic potential with their biogeochemical activity. However, the vision of using this approach for various applications in environmental science at the molecular level is hampered by technical difficulties of working with mRNA, such as lacking polyadenylation mechanism, very short half-lives of mRNAs, and lack of abundance of mRNA molecules within the total RNA pool in the microbial cell resulting in poor detection signals. Protocols have been developed to overcome these difficulties and facilitate the analyses of partial environmental transcriptomes. Among the promising studies, the retrieval of community-specific sequences of functional genes essential for quantitative ecological surveys, the generation of new hypotheses for known microbial processes, or the assessment (with the aid of environmental genomics) of the genetic potential and activities patterns of natural microbial assemblages can be listed.

- **Expected learning outcomes**: Upon completion of this Module the learners will be able to:
  - Present the core of environmental mRNA (environmental transcriptomics)
  - Explain the technical difficulties of working with mRNA
  - Know and apply the basic steps in the protocol for analysis of partial environmental transcriptomes
  - Understand the major promising applications of environmental mRNA approach in microbial ecology
  - Apply the good practices in the state of art in single-cell transcriptomics and single-cell RNA-sequencing

Provisional Table of contents:

Presentation of the learning content

1. Introduction

- Principles of the technology of **environmental transcriptomics** that links genetic potential with microbial biogeochemical activity
- **Advantages and technical pitfalls** of the technology
- **Possible decisions** to overcome the technical problems

Presentation of the learning content

2. Findings

2.1 Protocol for analysis of partial environmental transcriptomes

- Library generation: samples collection from the environment and total RNA isolation, selective removal of rRNA and enrichment in mRNA, mRNA reverse transcription to generate cDNA template population which members are PCR-amplified to yield cDNA clone environmental transcript libraries.
- Environmental transcript libraries characterization
- Putative taxonomic origin of the transcripts determination and annotation

Presentation of the learning content

3. Alternatives

3.1 Approaches to overcome the difficulties of transcriptional heterogeneity

- Profiling transcriptional states in microbial communities
- Following the expression of bacterial specific transcriptional programmes through high-throughput sequencing techniques
- Studying isogenic bacterial populations for their heterogeneity

Presentation of the learning content

3. Alternatives

3.2 Promising applications of microbial gene transcripts in environmental samples

- Retrieval of community-specific sequences of functional genes important for quantitative environmental surveys
- Generation of new hypothesis for known microbial processes
- Assessment (with the aid of environmental genomics) of the genetic potential and activities patters of natural microbial assemblages

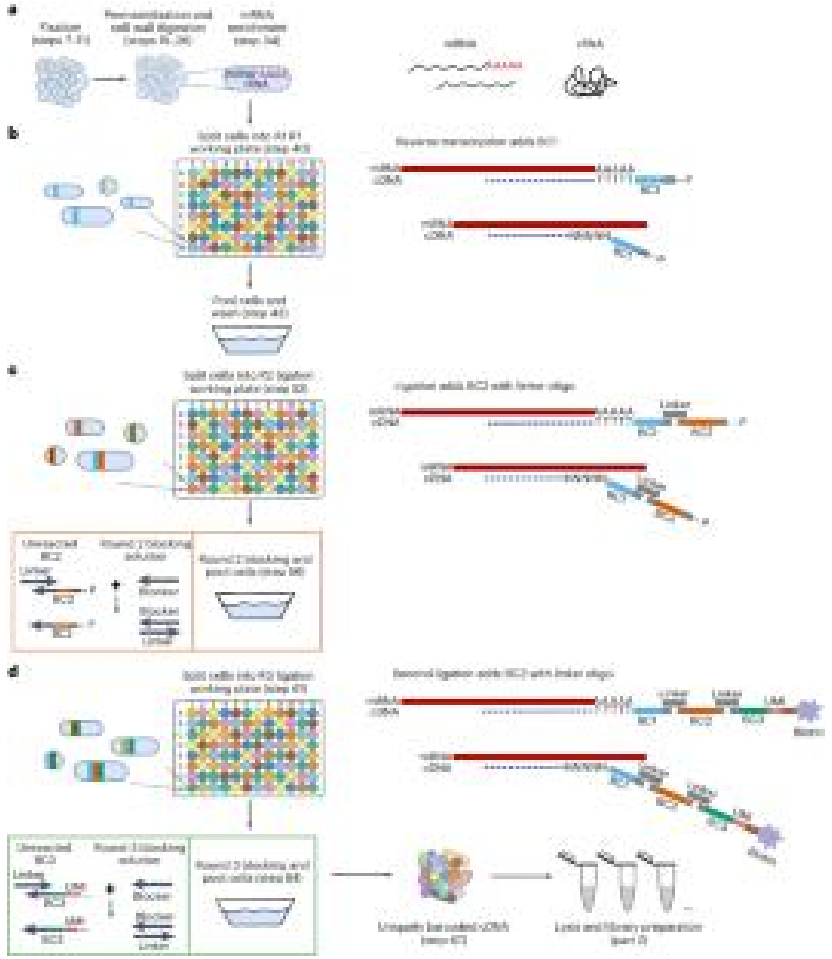
Presentation of the learning content

4. Solutions

4.1 Single-cell transcriptomics with combinatorial barcoding

- Transcriptomic analysis of individual bacterial cells and detection of phenotypically distinct subpopulations by 4-rounds of combinatorial barcoding

- Profiling huge amount of bacterial cells in a single bench-based experiment



Source: Gaisser et al., 2024 *Nat Protoc* (2024). https://doi.org/10.1038/s41596-024-01007-w

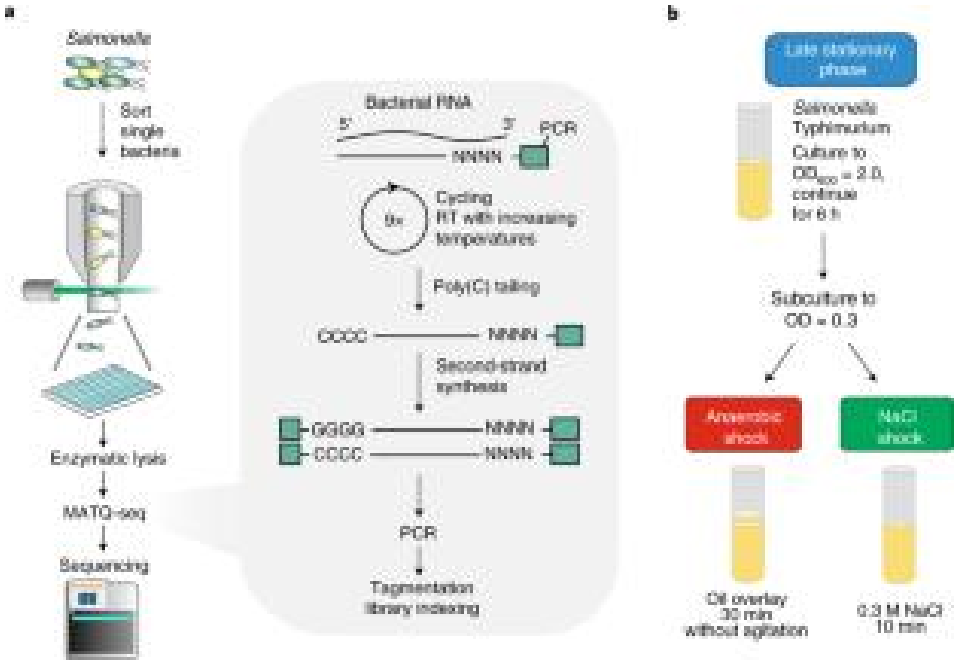
Presentation of the learning content

4. Solutions

4.2 Poly(A)-independent single-cell RNA-sequencing

- Improved poly(A)-independent single-cell RNA-sequencing protocolfor following growth-dependent gene expression patterns in individual *Salmonella* and *Pseudomonas* bacteria across all RNA classes and genomic regions

- Important reference point for single-cell RNA-sequencing of other bacterial species, mixed microbial communities and host–pathogen interactions



Source: Imdahal et al., 2020 *Nat Microbiol 5, 1202–1206 (2020). https://doi.org/10.1038/s41564-020-0774-1*

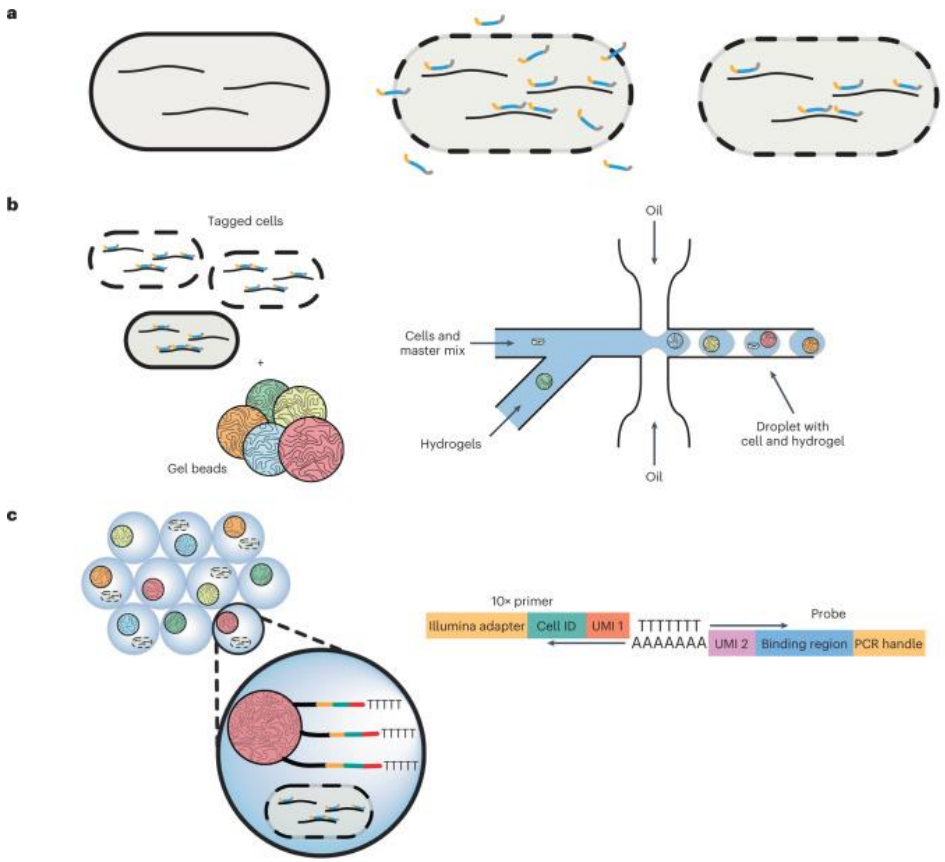
Presentation of the learning content

4. Solutions

4.3 Probe-based bacterial single-cell RNA sequencing

- Probe-based bacterial sequencing (ProBac-seq) using libraries of DNA probes and a commercial microfluidic platform to conduct bacterial single-cell RNA sequencing

- Application of the approach to *Clostridium perfringens* revealed heterogeneous expression of the bacterial toxin that can affect pathogenicity



Source: McNulty et al., 2023 *Nat Microbiol 8, 934–945 (2023). https://doi.org/10.1038/s41564-023-01348-4*

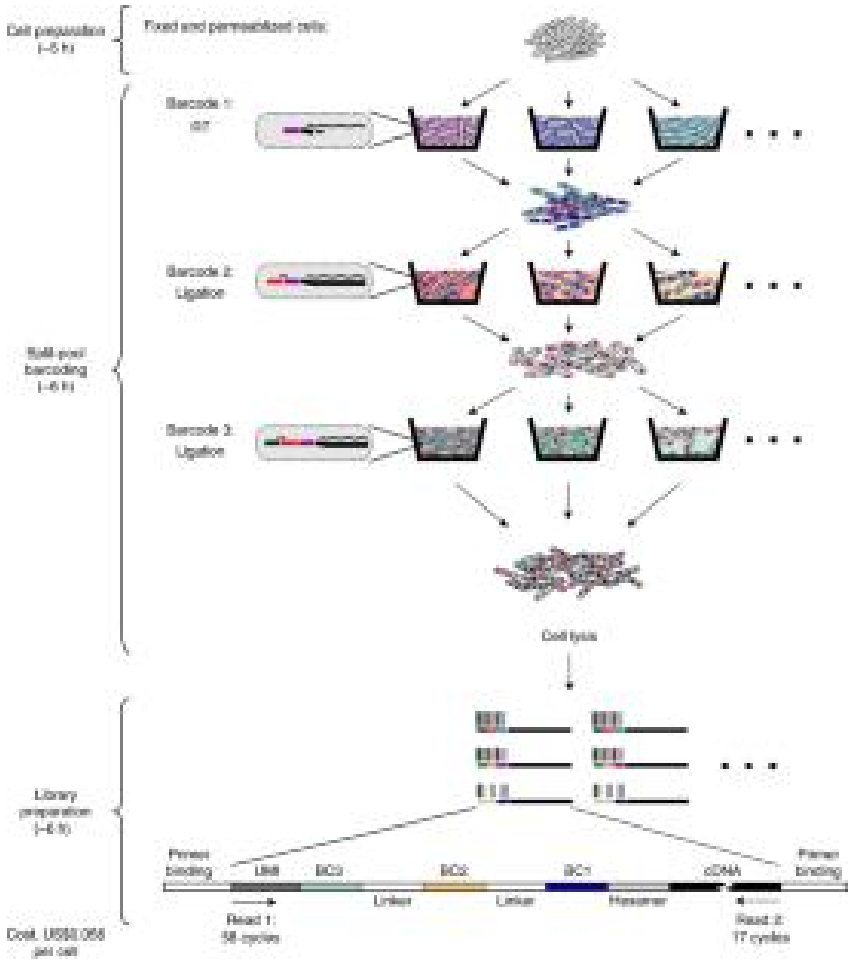
Presentation of the learning content

4. Solutions

4.4 *In situ* combinatorial indexing for bacterial single-cell RNA sequencing

- Prokaryotic expression profiling by tagging RNA *in situ* and sequencing (PETRI-seq that enables robust discrimination of cell states corresponding to different phases of growth

- Application of the approach in defining single-cell states and their dynamics in complex microbial communities



Source: Blattman et al., 2020 *Nat Microbiol 5, 1192–1201 (2020). https://doi.org/10.1038/s41564-020-0729-6*

Presentation of the learning content

6. References

- Poretsky R.S, N. Bano, A. Buchan, G. LeCleir, J. Kleikemper, M. Pickering, W.M. Pate, M.A. Moran, J.T. Hollibaugh. Analysis of microbial gene transcripts in environmental samples. Appl Environ Microbiol. 2005 71(7):4121-6. doi: 10.1128/AEM.71.7.4121-4126.2005.
- Gaisser, K.D., Skloss, S.N., Brettner, L.M. et al. High-throughput single-cell transcriptomics of bacteria using combinatorial barcoding. Nat Protoc (2024). https://doi.org/10.1038/s41596-024-01007-w
- Imdahl, F., Vafadarnejad, E., Homberger, C. *et al.* Single-cell RNA-sequencing reports growth-condition-specific global transcriptomes of individual bacteria. *Nat Microbiol* **5**, 1202–1206 (2020). https://doi.org/10.1038/s41564-020-0774-1
- McNulty, R., Sritharan, D., Pahng, S.H. et al. Probe-based bacterial single-cell RNA sequencing predicts toxin regulation. Nat Microbiol 8, 934–945 (2023). https://doi.org/10.1038/s41564-023-01348-4
- Blattman, S.B., Jiang, W., Oikonomou, P. et al. Prokaryotic single-cell RNA sequencing by in situ combinatorial indexing. Nat Microbiol 5, 1192–1201 (2020). https://doi.org/10.1038/s41564-020-0729-6

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