ONT-CAPPABLE-SEQ: A NEW APPROACH TO EXPLORE THE TRANSCRIPTIONAL ARCHITECTURE OF BACTERIAL VIRUSES

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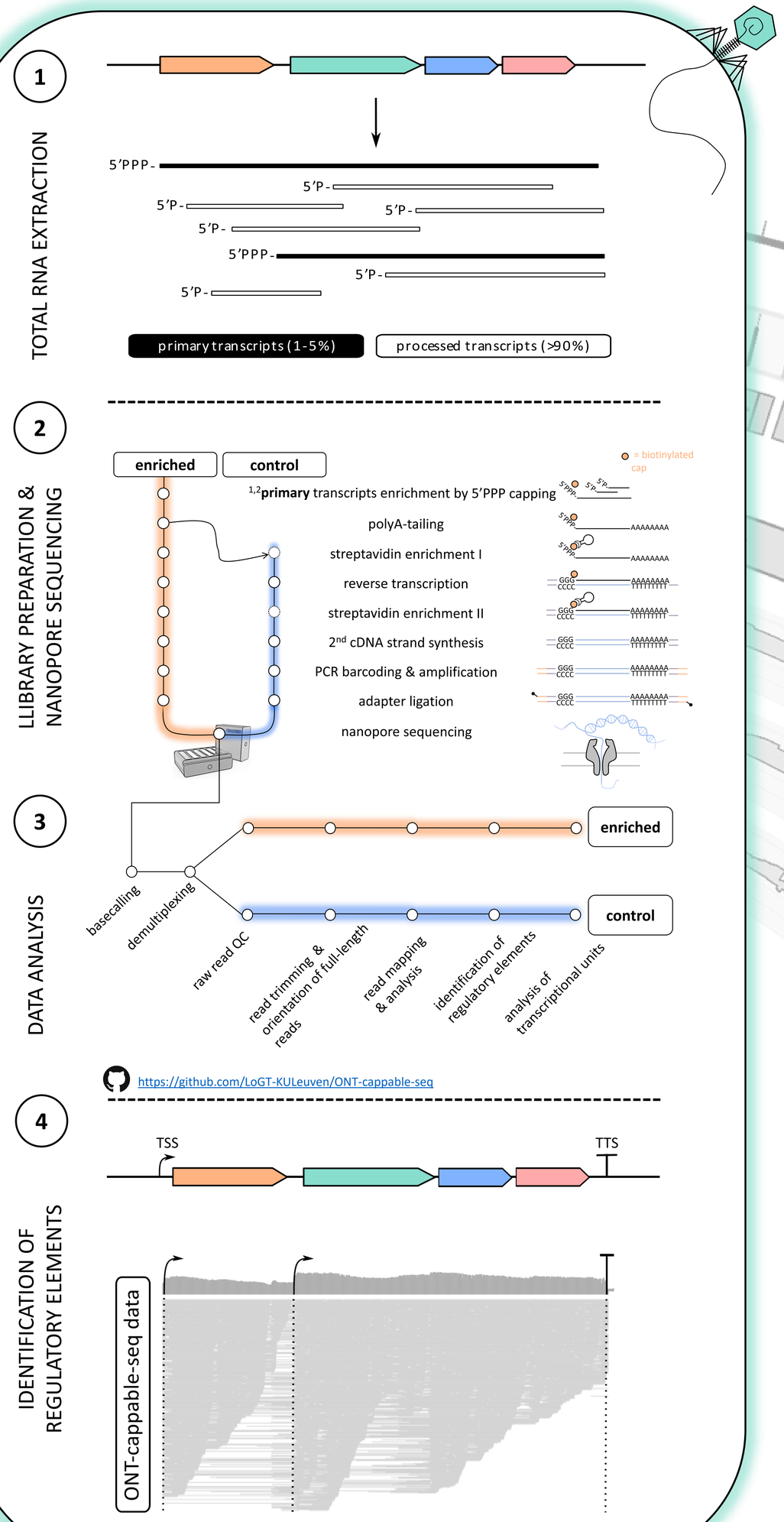
INTRODUCTION

- **Bacterial viruses** (phages) recognize their microbial host, infect it and convert the cell into a virus-producing machine within a matter of minutes.
- Classical RNA-sequencing has become the method of choice to study the transcriptional landscape of phage-infected bacteria. However, short-read RNA sequencing approaches **generally fail to capture key transcriptional features in dense viral genomes, such as operon structures and transcription start and termination sites.**
- The elucidation of these elements is fundamental to achieve a global **understanding of gene regulation mechanisms during the infection process**, to ultimately help develop alternative strategies to combat bacterial pathogens and for the development of SynBio applications inspired by phages.

→ **Development of ONT-cappable-seq to sequence primary prokaryotic transcripts in full-length using the Nanopore sequencing platform**. We applied ONT-cappable-seq to study the transcriptional architecture of phages infecting *Pseudomonas.*

WORKFLOW



RESULTS

ONT-CAPPABLE-SEQ ENRICHES PRIMARY TRANSCRIPTS

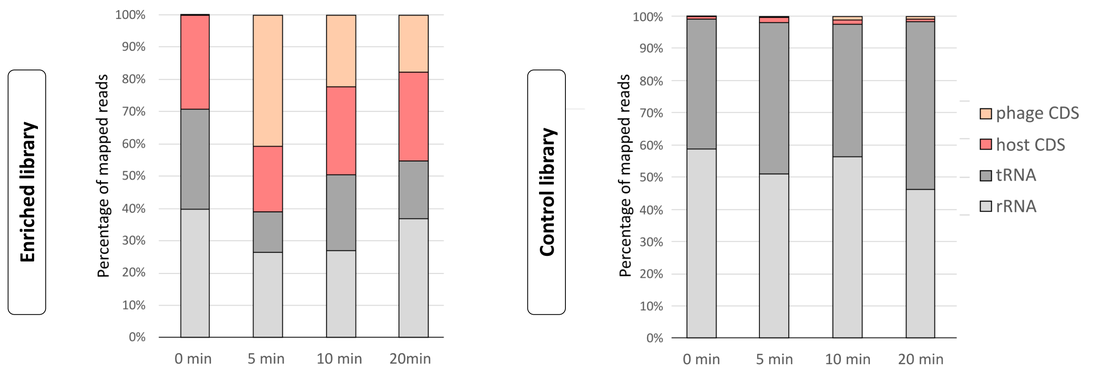


Figure 1: Distribution of reads across the genomic features of the phage and its *P. aeruginosa* host. The percentage of reads mapped to rRNA, tRNA and the coding sequences (CDS) of *P. aeruginosa* and the phage for each timepoint during infection for enriched and unenriched control libraries is presented.

IN VIVO VALIDATION EXPERIMENTS CONFIRM REGULATORY ELEMENTS IDENTIFIED BY ONT-CAPPABLE-SEQ

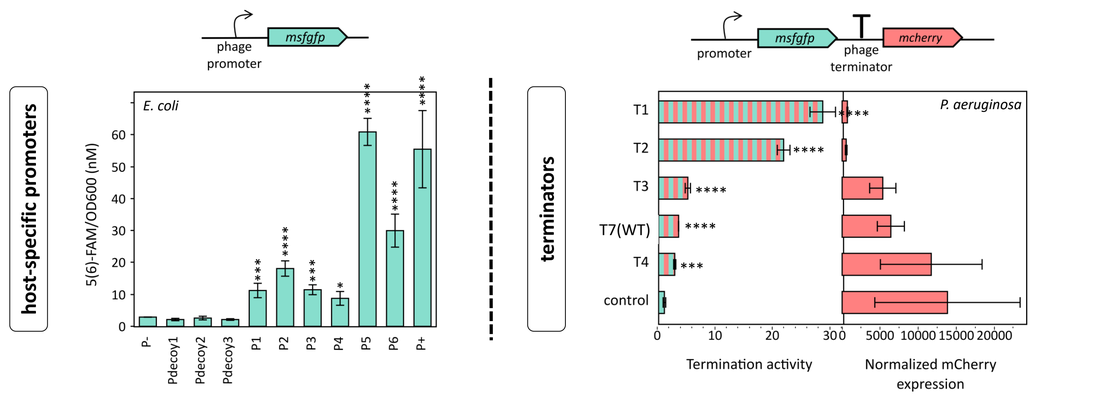
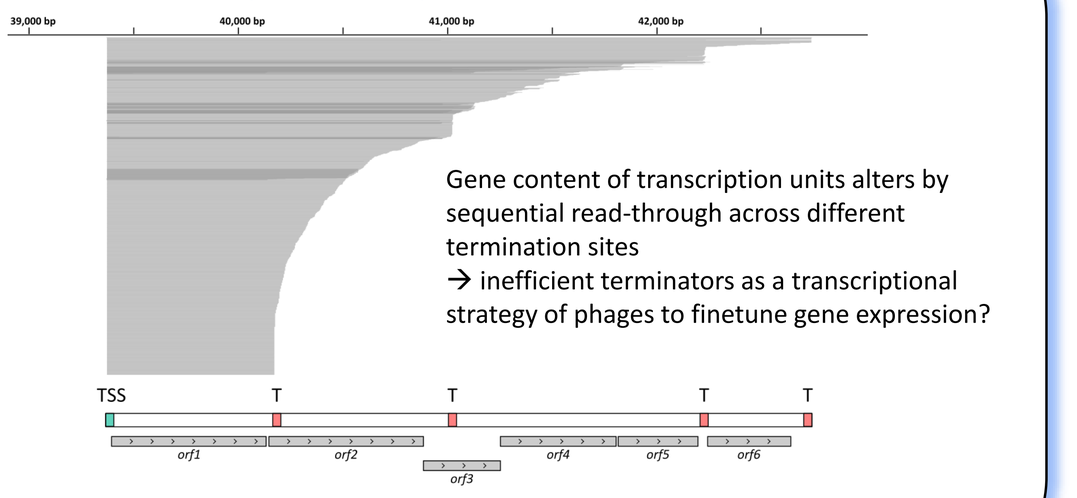


Figure 2: *in vivo* validation of phage regulators using SEVATile-based expression systems[3]. Promoter activity was quantified by normalized msfGFP expression levels (displayed in 5(6)-FAM/OD600 nM (P-: no promoter insert, P+: constitutive promoter, P_{decoy}1-3: random phage regions, P1-6: phage promoters). Termination activity was quantified by comparing the ratio of the normalized msfGFP and mCherry expression levels of phage terminators (T1-T4 and T7(WT)) with the ratio normalized msfGFP and mCherry levels in the control (no terminator insert).

Data represent mean values (n=4) and standard deviation is indicated by error bars. Regulators showing significantly fluorescence intensity or termination activity compared to the negative control are indicated with an '*' (Student's t-test, **** = p ≤ .0001)

EXPLORATION OF PHAGE TRANSCRIPTIONAL LANDSCAPES

stepwise transcription termination patterns



Gene content of transcription units alters by sequential read-through across different termination sites
→ inefficient terminators as a transcriptional strategy of phages to finetune gene expression?

Figure 3: ONT-cappable-seq data track showing the transcriptional pattern at phage genes *orf1-orf6* as an example of sequential readthrough at different termination sites (T) for reads starting at the same TSS.

CONCLUSIONS

- **ONT-cappable-seq allows full-length transcriptional profiling** and enables the simultaneous identification of both 5' and 3' transcriptional boundaries. Applied to phages, it provides a **comprehensive genome-wide map of viral transcriptional start sites, terminators and complex operons** that fine-regulate gene expression during the infection process.
- Our method enables the **exploration of dense phage transcriptional landscapes in unprecedented detail** and can provide new insights in the still cryptic biology and transcriptional regulatory features of bacterial viruses.
- Although this study primarily focused on viral transcripts, **similar analyses can be performed on the bacterial side** to shed further light on the regulatory features that govern the complex interaction between phages and their hosts.

References:

[1] Ettwiller L, Buswell J, Yigit E, Schildkraut, I. A novel enrichment strategy reveals unprecedented number of novel transcription start sites at single base resolution in a model prokaryote and the gut microbiome. *BMC Genomics*. 2016;17(1-)14).

[2] Yan, B, Boitano M, Clark TA, Ettwiller L. SMRT-cappable-seq reveals complex operon variants in bacteria. *Nat Commun*. 2018;9(1).

[3] Lammens, EM, Boon M, Grimon D, Briers Y, Lavigne R. SEVAtile: a standardised DNA assembly method optimised for *Pseudomonas*. *Microb Biotechnol*. 2022;15(1).

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