Leveraging 4f-SAMMY-seq protocol in Prokaryotes as a high-resolution technology to study Nucleoid accessibility

Ugo Maria Iannacchero[1], Elena Chiti[2], Valentina Rosti[1,3], Andrea Vannini[2], Chiara Lanzuolo[1,3], Davide Roncarati[2], Eva Maria Pinatel[1]

[1]Institute of Biomedical Technologies, National Research Council, Segrate, Italy ; [2]Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Bologna, Italy ;
[3] INGM, National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi", Milan, Italy

• Background

The bacterial chromosome self-organizes into the nucleoid, where Nucleoid Associated Proteins (NAPs) and RNAs regulate DNA accessibility. While bacterial Hi-C protocols have recently provided insights into Nucleoid organization, their use remains technically demanding and limited to a few bacterial species, due to high-sequencing depth requirements, crosslinking biases and species-specific restriction enzymes. Moreover, the limited achievable resolution (>5 kb) fails to capture gene-level interactions, leaving key aspects of nucleoid organization unresolved.

• A novel epigenomic technology

4f-SAMMY-seq (**4**-**f**ractions **S**equential **A**nalysis of **M**acro**M**olecules accessibilit**Y**) is a high-throughput sequencing technology originally developed in mammalian systems [1]. It relies on the **biochemical separation of chromatin into four solubility-based fractions**, **each reflecting a different level of DNA accessibility**. This enables genome-wide profiling of **both open and closed regions**, and has been demonstrated to reconstruct **chromatin compartmentalization with Hi-C like resolution and accuracy at lower sequencing depth** [2].

• Why 4f-SAMMY-seq in Prokaryotes?

- Broadly **applicable across different bacterial species**.
- **Does not involve cross-linking** or **restriction enzymes**.
- **Does not rely on** the use of **antibodies**.
- **Cost-effective** and **fast experimental workflow**.
- **Works efficiently** with **only ~0.5–2M reads per fraction**, given the small size of bacterial genomes.

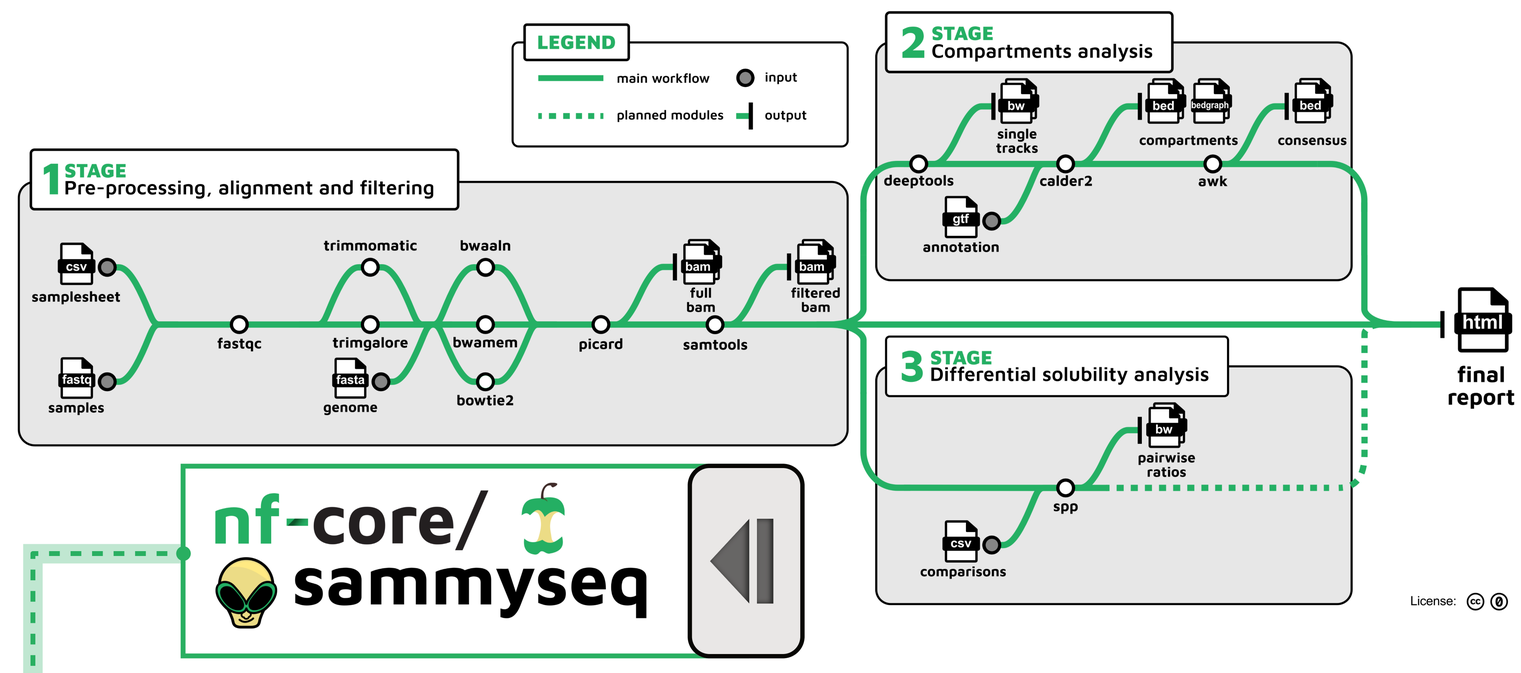
• Discussion and next steps

We adapted the **nf-core/sammyseq** [3,4] pipeline to **prokaryotes** by integrating **read processing tools better suited for bacterial genomes**, accommodating non-standard chromosome names **ensuring compatibility with Hi-C tools** and **refining genome binning strategy to support finer-scale resolution** through smaller window-sizes.

We applied it to ***Helicobacter pylori*** **in exponential growth**, where we observed **distinct signal differences between more and less accessible DNA fractions**, and **we obtained DNA accessibility domains at sub-kilobase resolution** down to the scale of gene-promoters, with as few as **500,000 reads per fraction**.

Ongoing work will focus on **finalizing the integration of the Differential Solubility module** into the pipeline, with further adaptation for prokaryotic genomes. Additionally, we aim to enhance data interpretation by **incorporating COG pathway enrichment module** to better understand the functional impact of nucleoid accessibility changes.

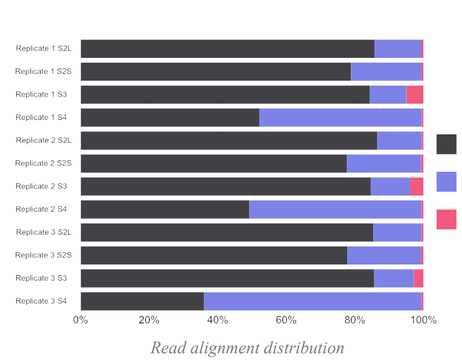
Methods of analysis



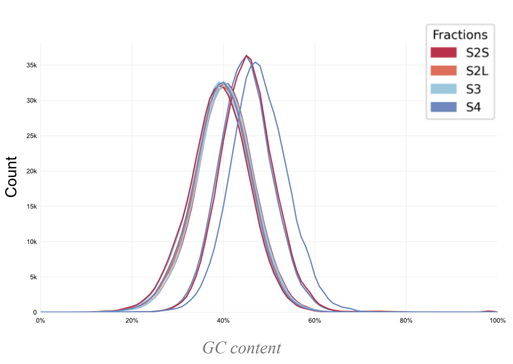
Analysis output

1 STAGE Pre-processing, alignment and filtering

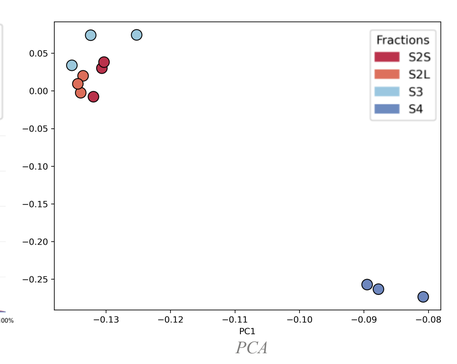
Raw sequencing reads from each solubility fraction are aligned to the reference genome, with quality control metrics including **GC content** and **read alignment distribution** assessed. To evaluate sample similarity and variability, **Principal Component Analysis (PCA)** is performed on the processed coverage profiles.



Read alignment distribution



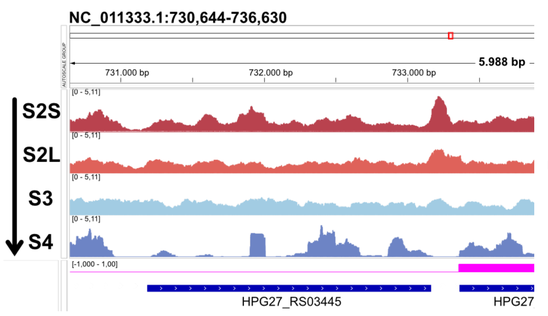
GC content



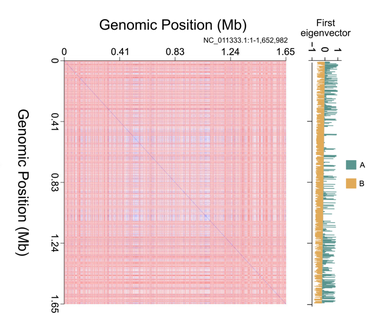
PCA

2 STAGE Compartments analysis (Hi-C like)

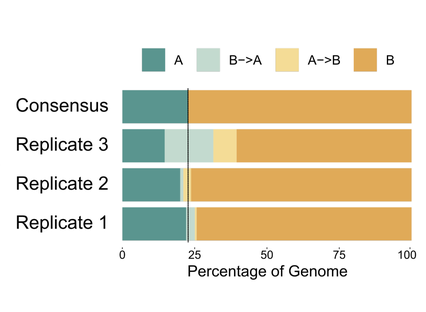
Genome-wide coverage profiles are generated for each fraction, and cross-correlation values among the four fractions are computed using Hi-C analysis tools to generate an **NxN correlation matrix** computed over user-defined genomic bin size. Eigenvector decomposition of this matrix enables identification of genome-wide compartmental organizations leading to the **generation of a consensus profile across replicates** that, when compared across conditions, can be used to individuate compartment transitions.



Genomic tracks



Correlation Matrix



Consensus profile

3 STAGE Differential solubility analysis (ChIP-seq like)

Pairwise relative ratios between the most representative fractions are calculated to detect continuous changes in DNA accessibility, enabling identification of gene-level alterations.

Pairwise comparison tracks (ratios)

References

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2. Lucini, F., Petrini, C., Salviato, E., Pal, K., Rosti, V., Gorini, F., Santarelli, P., Quadri, R., Lembo, G., Graziano, G., Di Patrizio Soldateschi, E., Tagliaferri, I., Pinatel, E., Sebestyén, E., Rotta, L., Gentile, F., Vaira, V., Lanzuolo, C., & Ferrari, F. (2024). Biochemical properties of chromatin domains define genome compartmentalization. Nucleic Acids Research, 52(12), e54–e54. https://doi.org/10.1093/nar/gkae45
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4. https://nf-co.re/sammyseq/dev/ (https://github.com/nf-core/sammyseq/tree/dev)

Contacts

ugo.iannacchero@itb.cnr.it
@ugoiann.bsky.social