Exploring Plasmid Distribution in Bacterial Communities through Single-Cell Sequencing

High-Throughput Barcoding and Preliminary Data Processing

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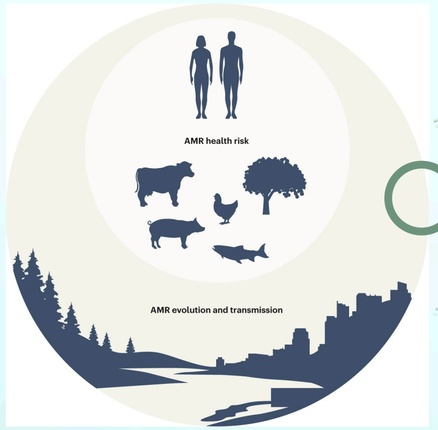
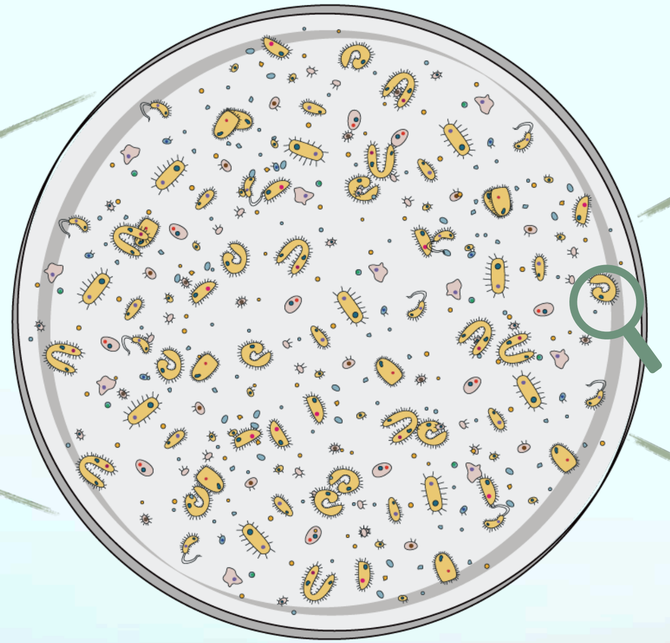
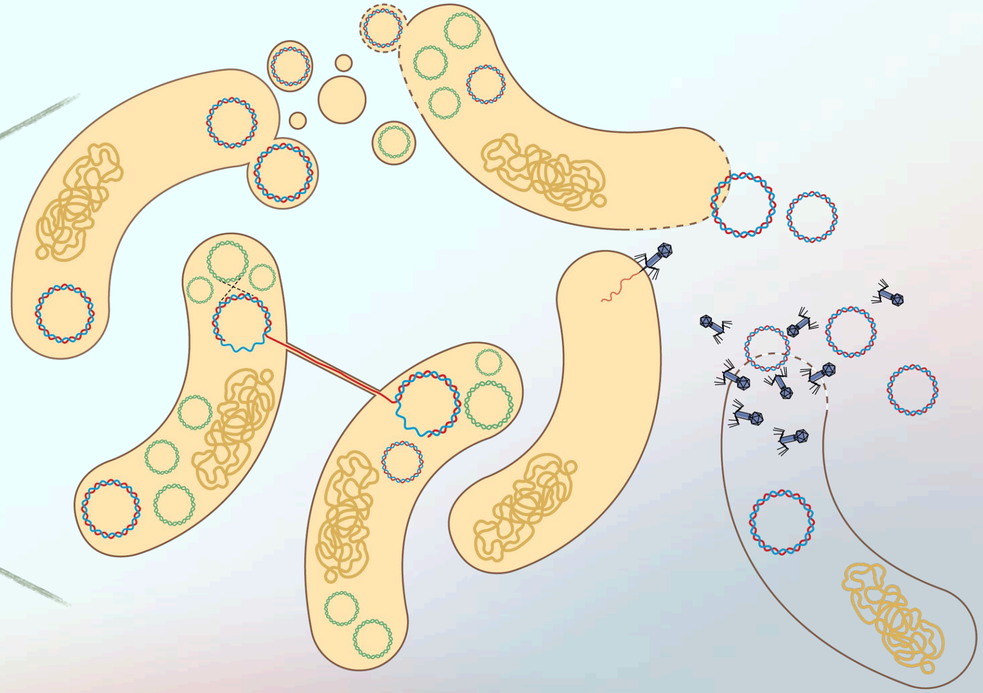
AMR, One Health and the environment

Larsson, D. G. J., et al. "AMR, One Health and the environment." Nature Microbiology 8.5 (2023): 754-755.

AMR spread







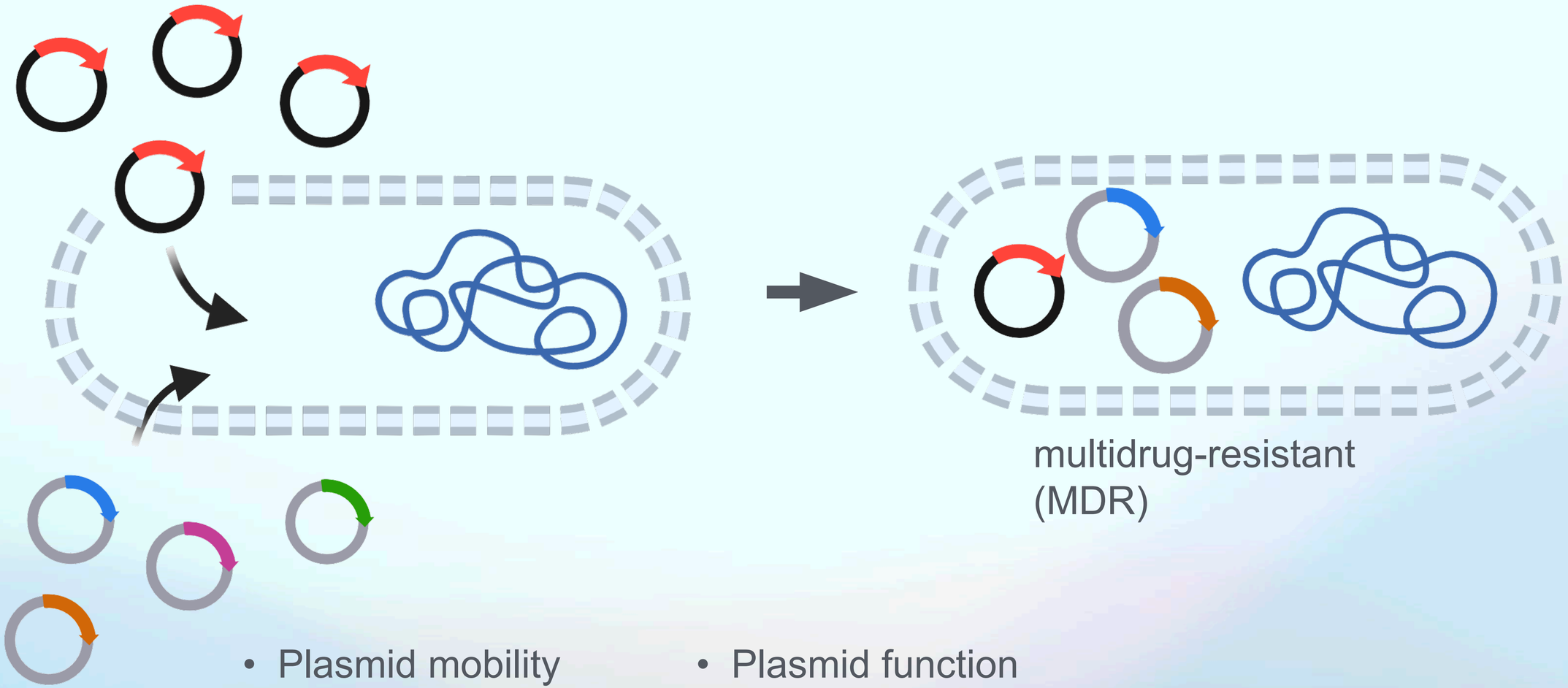
One health

Bacterial communities

Horizontal transfer of AMR

★ Antibiotic-resistance plasmids

How plasmid help spread AMR among microorganisms?



- Plasmid mobility
- Plasmid function
- Plasmid compatibility
- Fitness cost

- Use qPCR to get the average plasmid copy number
- Use droplet digital PCR (ddPCR)

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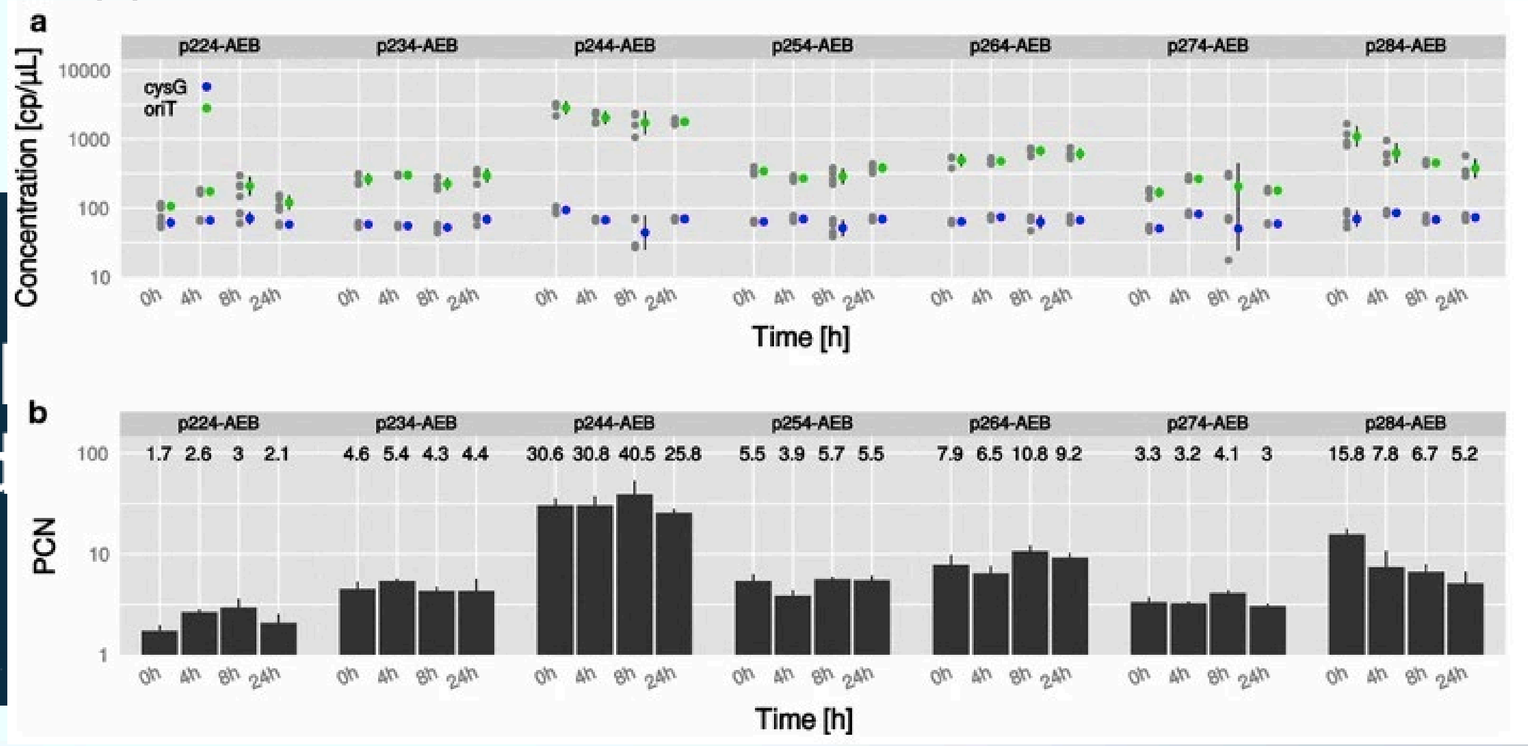
Copy number variabi plasmids determined Droplet Digital PCR

Research | Open access | Published: 19 December 2016



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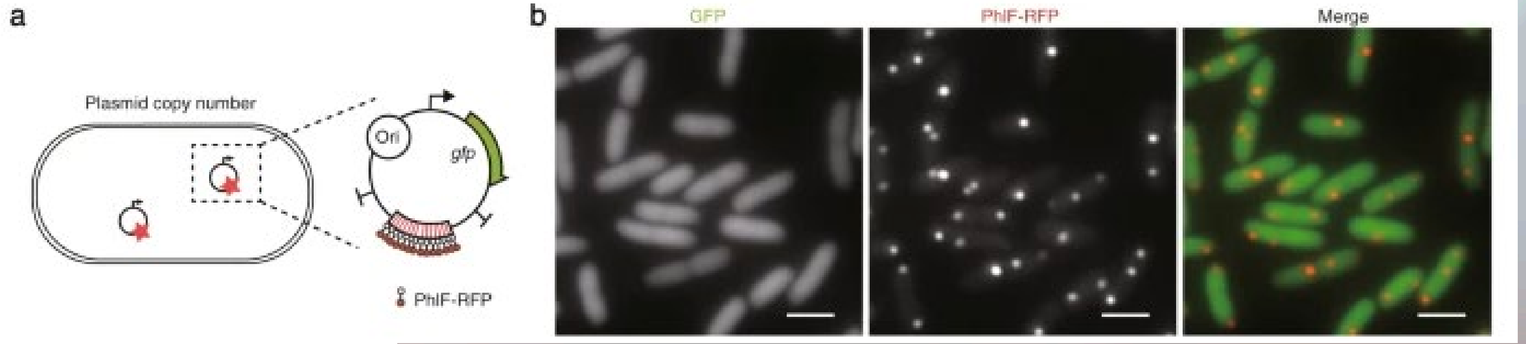
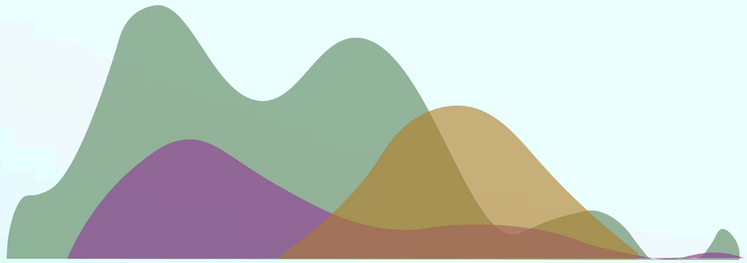
Single-cell measurement of plasmi promoter activity

Fig. 1: Measurement of plasmid copy number and transcript number.



Heterogeneity

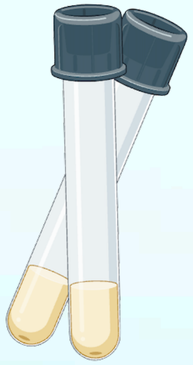


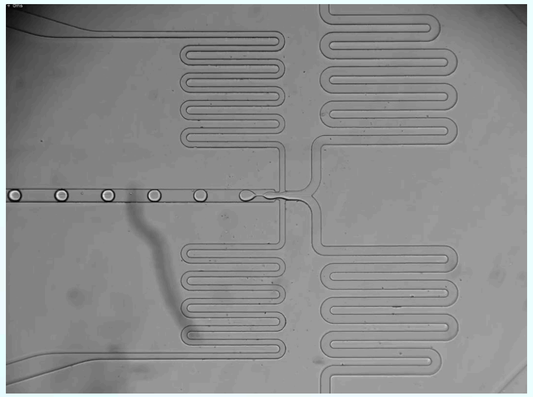
Single-Cell Sequencing

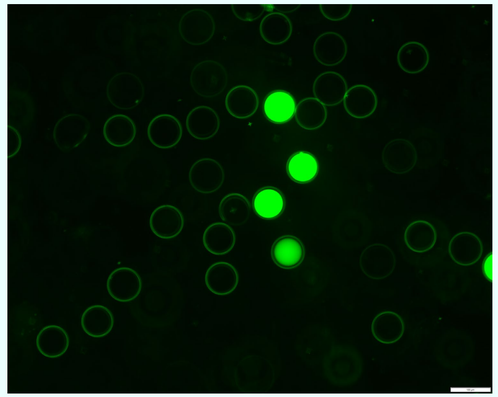
Workflow

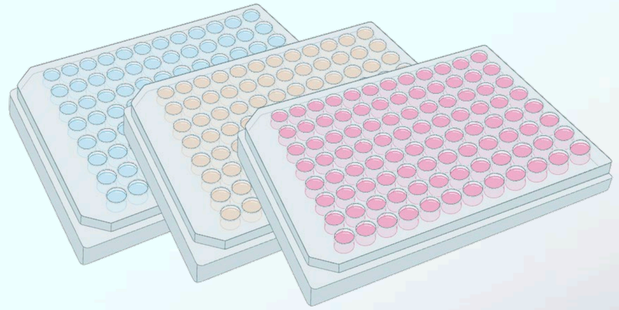
① → ② → ③ → ④ → ⑤

Microbial sample | Encapsulation | Cell lysis and Whole-genome amplification | Split and Pool Barcoding | Sequencing







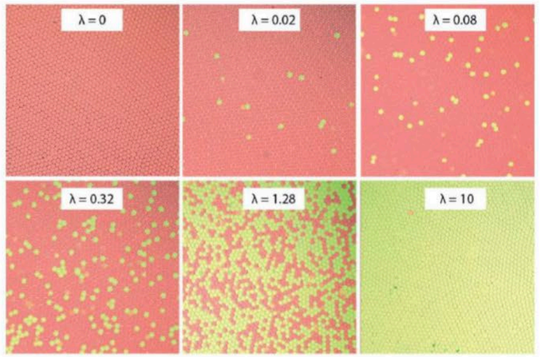


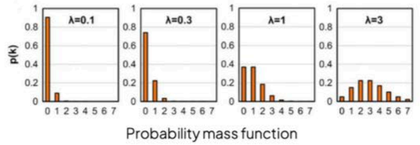
Get single cell in a droplet –Poisson distribution

$$\lambda = \frac{number\ of\ cells}{number\ of\ droplets}$$

$$P(k) = \frac{e^{-\lambda} \cdot \lambda^k}{k!}$$

λ – mean number of events in interval
k – events
P(k) – probability of events in interval
e - Euler's constant (~2.72)





Lambda value = 0.05-0.1

To get less contamination or doublets

10 x 10 x 10 =1,000

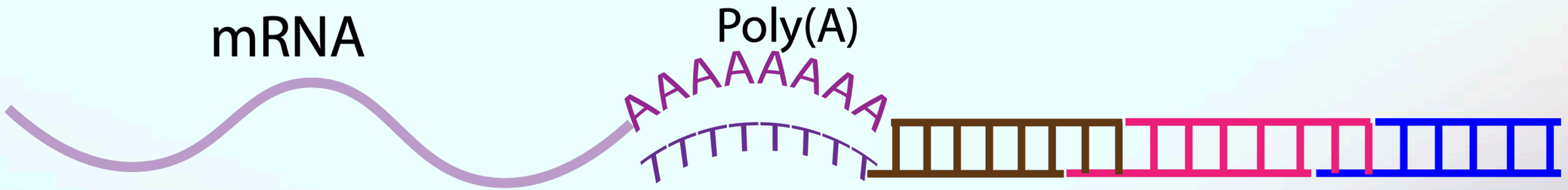
48 x 48 x 48 = 110,592

96 x 96 x 96 = 884,736

…

Barcoding for RNA

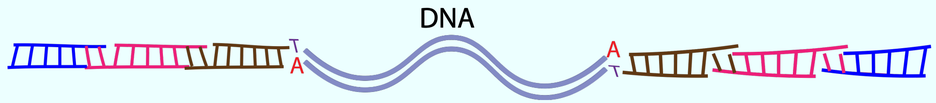
Single-stranded



Barcoding for Genome

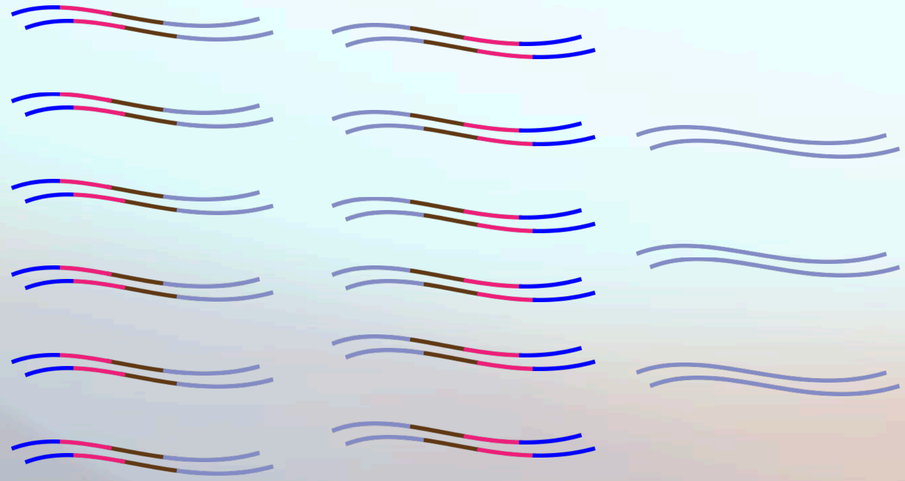
1. T4 ligation

①Barcoding via 3x ligation



②Fragmentation

③Sequencing Library



Advantage: Reduce bias caused by PCR

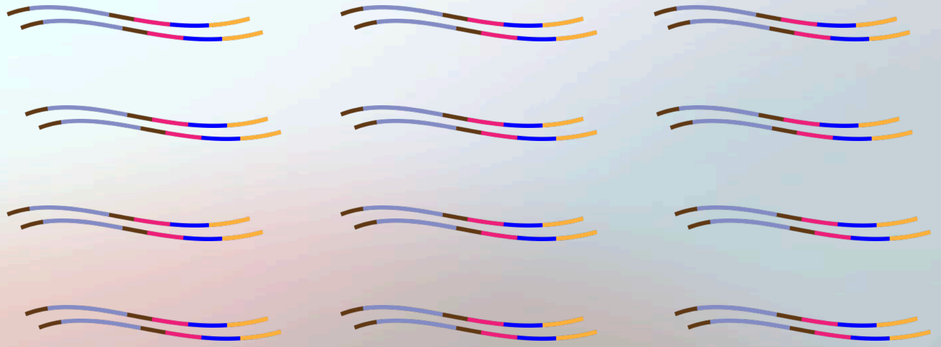
Shortcoming: Higher cost, cumbersome process,the ligation efficiency is not high

2. PCR

①Fragmentation

②Barcoding via 3x PCR

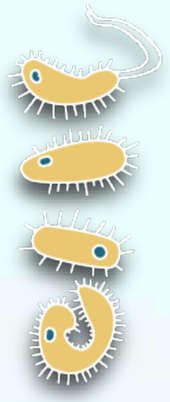
③Sequencing Library

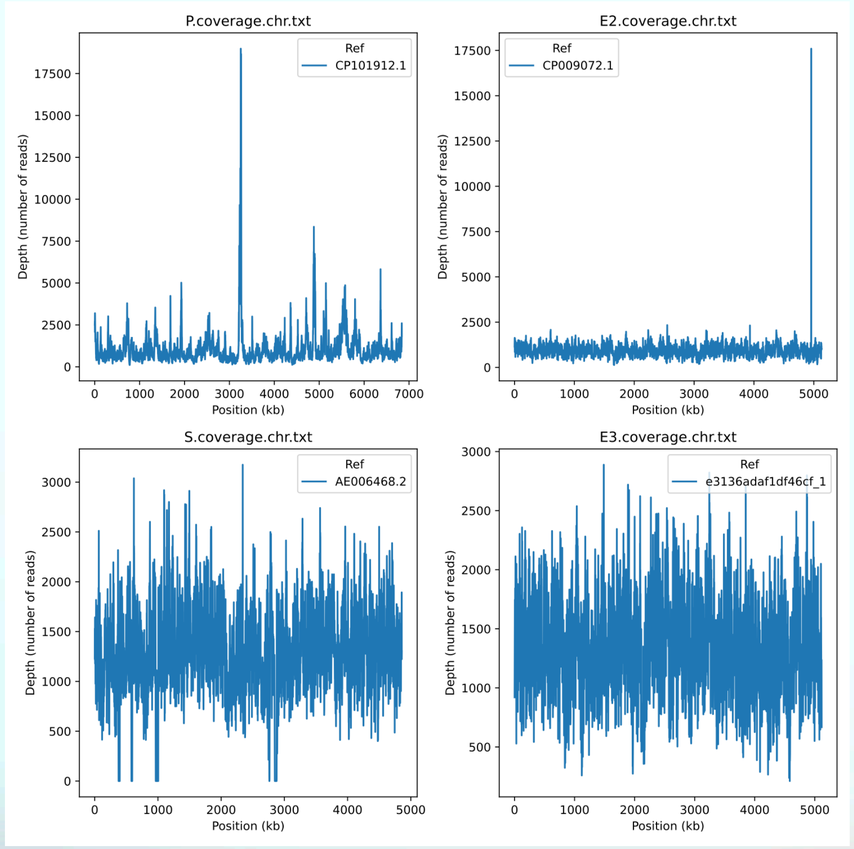


Advantage: Cost saving and high efficiency

Shortcoming: biased

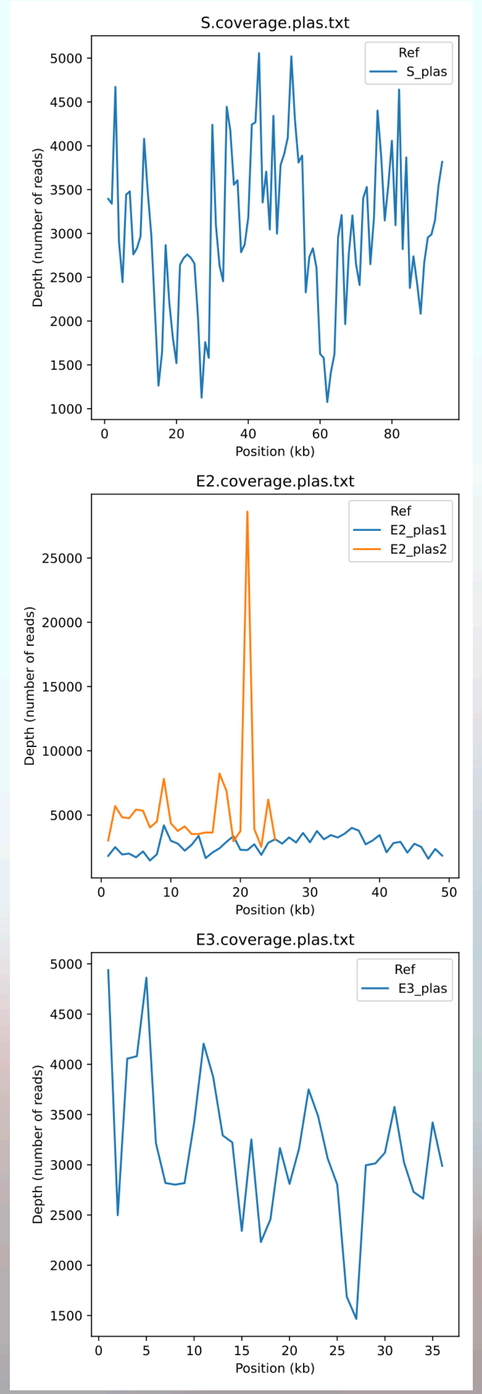
Small trial

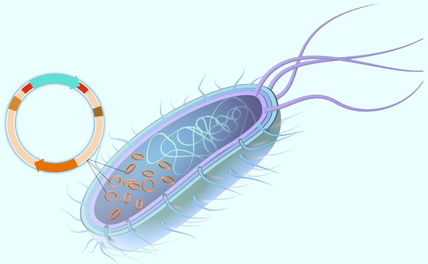




Chromosome

Plasmid





Challenge:

- Obtain more and higher quality sequencing reads
- Analysis of downstream research


Thank you for your attention