Investigating the Relaxase Behavior and Replication Functionality of the Mobilization Protein mobV in the Plasmid pBBR1

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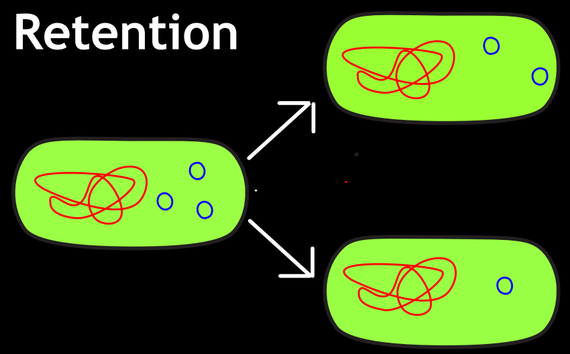
Plasmid Maintenance in Non-Model Organisms

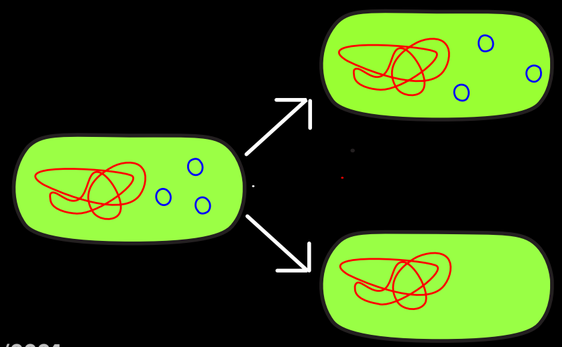
Understanding plasmid retention is important for future genetic engineering applications.

What is Plasmid Retention?

Retention



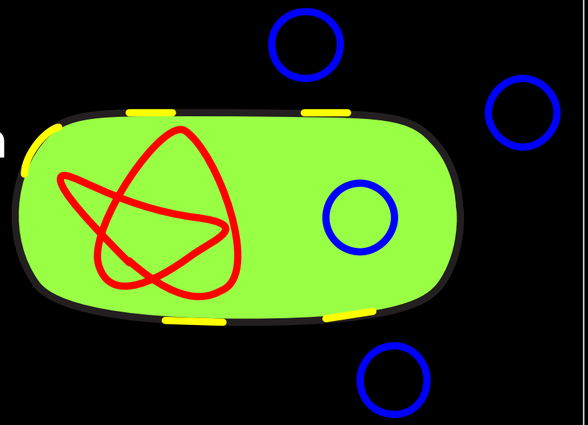
Plasmid Loss



Relevant Genetic Engineering Methods

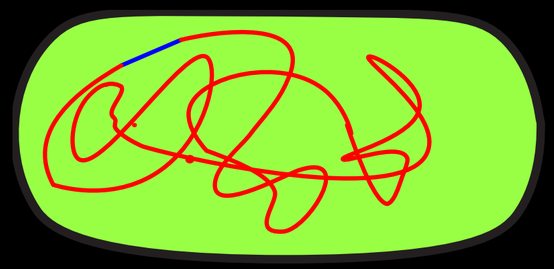
Transformation

- Electroporation
- Heat shock



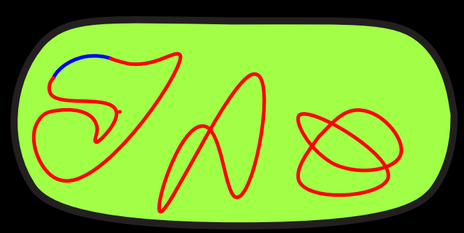
Genome Editing

- CRISPR
- TALEN

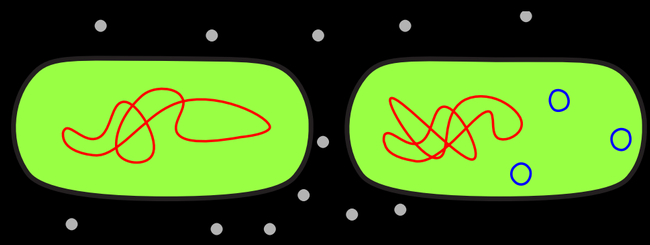


What are the Challenges?

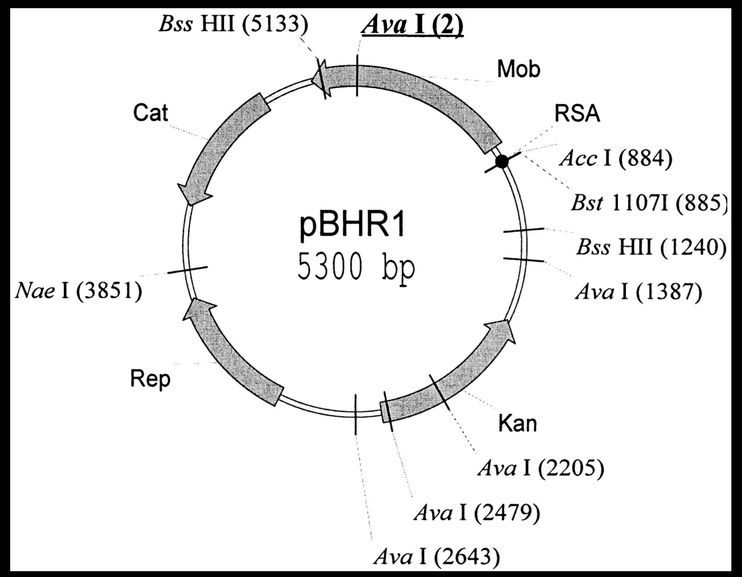
Polyploidy



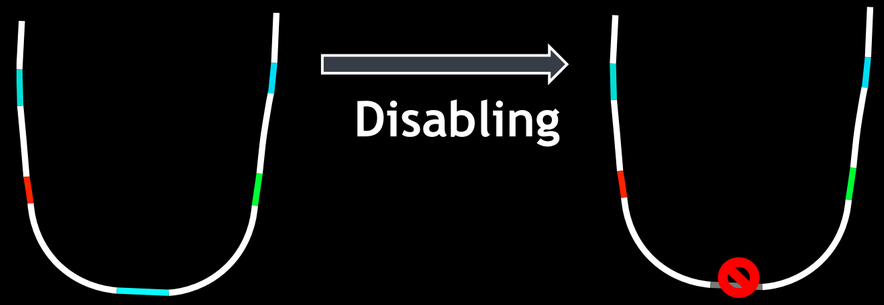
Natural Antibiotic Resistance



Understanding Mobilization Gene Relaxase Functionality



Map of pBHR1 (Szpirer et al., Journal of Bacteriology, 2001)

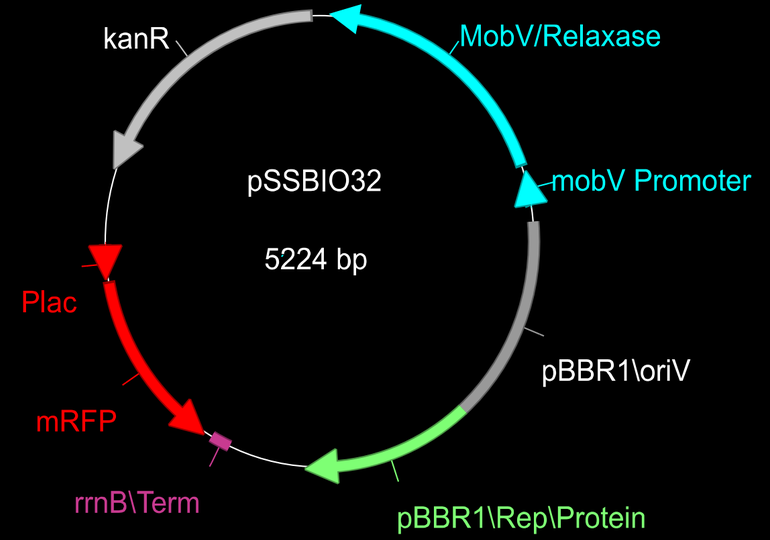


Active Site amino acid replacement

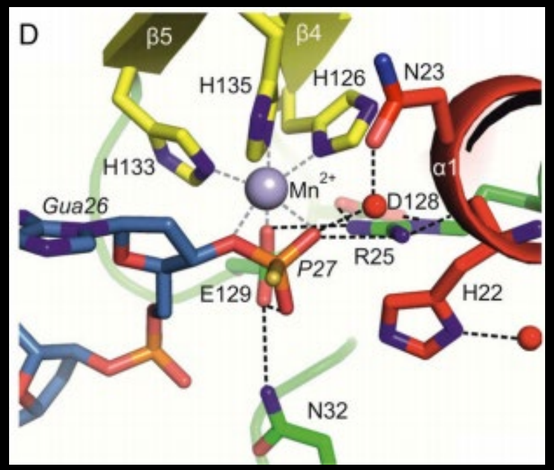
Mob Gene

Very similar amino acid sequence to mob gene found in pMV158

Hypothesis: mob protein contains **histidine** amino acids within their active sites



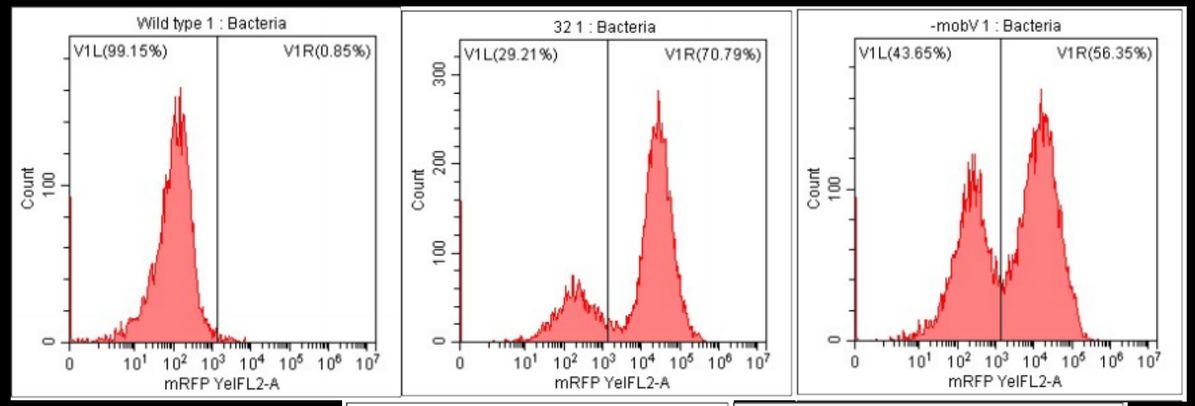
Plasmid used in transformation

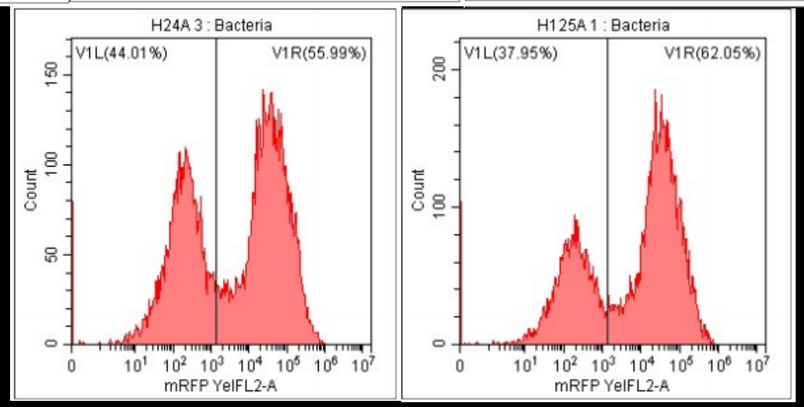


Active Site structure of mob protein (Pluta et al., PNAS, 2017)

Flow Cytometry and Future Plans

Methodology: Replace histidine (as well as other potentially crucial) amino acids in potential active sites with Alanine and observe plasmid retention effects and relaxation effects





Y axis: Count

X axis: Fluorescence Intensity

Flow Cytometry results from amino acid replacement strains transformed into *R. palustris* using mRFP as an indicator

Future work:

1. **Plasmid relaxation assay**
2. **qPCR**

Example of Plasmid Relaxation Assay (Pluta et al., PNAS, 2017)

Mob Gene → *Regulates* → **Plasmid replication protein**

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