Displacement of Bacterial Plasmids by Engineered Unilateral Incompatibility

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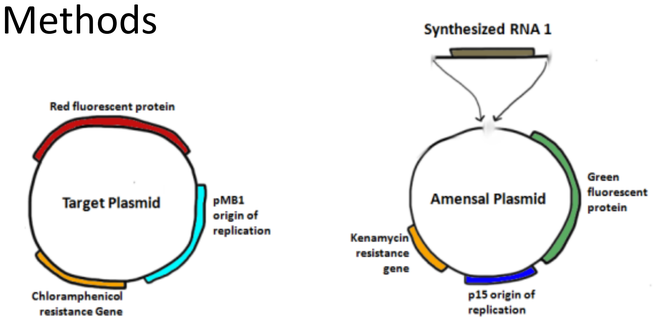
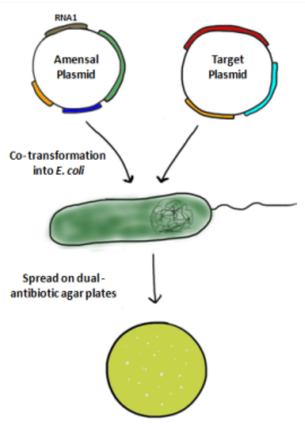
Abstract

Plasmids are self-propagating genetic elements that typically confer selective advantage to their bacterial hosts, including **resistance to antibiotics**, which pose a signficant health concern. Plasmids use copy number control systems to optimize their fitness. Plasmids whose copy number control systems interfere with one another are called incompatible: they cannot be stably maintained in a single host cell. Unilateral incompatibility is achieved by placing the self-suppressing segment of one plasmid's replication sequence onto an otherwise compatible plasmid.We are exploring the use of unilateral incompatibility for displacement of antibiotic resistance plasmids from environmental populations.

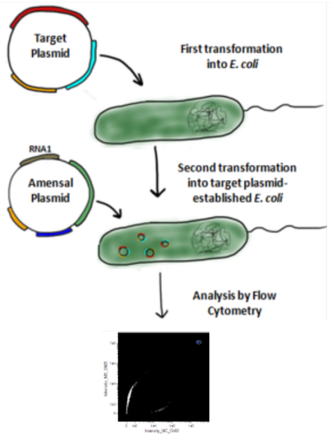
Methods



The plasmids chosen for unidirectional incompatibility experiments. Compatibility was demonstrated before the RNA1 sequence was inserted into the amensal plasmid. The RNA1 sequence inhibits replication from the pMB1 origin.

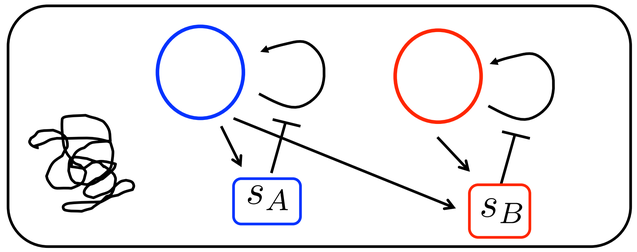


Co-transformation experiments provide evidence for unilateral incompatibility. Dual-antibiotic agar selects for cells that successfully propagate both plasmids. Only compatible plasmids are expected to produce colonies after co-transformation.



Sequential-transformation experiments permit analysis of plasmid interactions. Flow cytometry is used to detect fluorescence produced by E. coli after transformation. Levels of GFP and RFP correspond to the amounts of amensal and target plasmid respectively.

Deterministic Modeling: in-host plasmid dynamics



Plasmid concentration

$$\frac{d}{dt}y_A(t) = r_A(s_A(t))y_A(t) - \delta y_A(t) \qquad \frac{d}{dt}y_B(t) = r_B(s_B(t))y_B(t) - \delta y_B(t)$$

Inhibitor concentration

$$\frac{d}{dt}s_A(t) = \alpha_A y_A(t) - \beta_A s_A(t) \qquad \frac{d}{dt}s_B(t) = \alpha_B y_B(t) + \alpha_{BA} y_A(t) - \beta_B s_B(t)$$

Steady state:

$$\bar{y}_B = \frac{\beta_B r_B^{-1}(\delta) - \alpha_{BA}\frac{\beta_A}{\alpha_A} r_A^{-1}(\delta)}{\alpha_B}$$

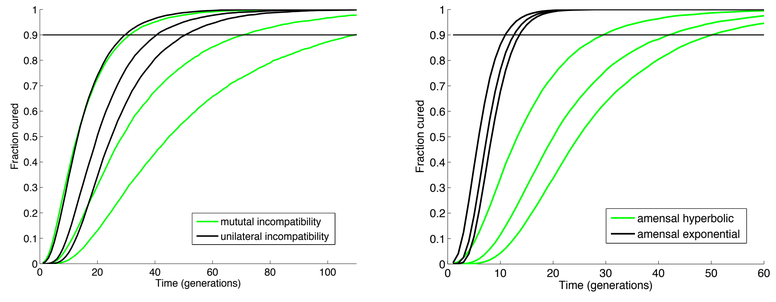
Displacement successful if either (i) engineered plasmid sufficiently abundant and/or (ii) inhibitor expression sufficiently strong

Stochastic Modeling

Plasmid replication: birth process with per-plasmid replication rate:

Hyperbolic: $\dfrac{k_{tr}}{1+\frac{p}{Ke^t}}$ or Exponential: $k_{tr}e^{-\frac{p}{Ke^t}}$

Cell growth: coordinated cell division cycle
Cell division: binomial distribution of plasmids to daughters
Population maintenance: half of newly formed daughter cells culled

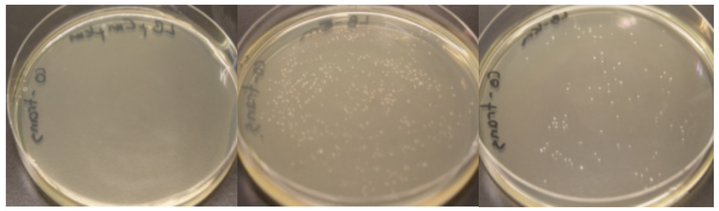


Simulations predict displacement of target plasmid

Lab Results

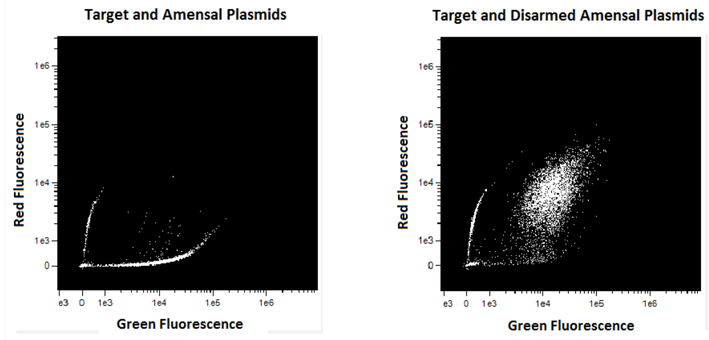
Co-transformations

Chloramphenicol + Kanamycin *Chloramphenicol only* *Kenamycin Only*



After co-transformation with the engineered amensal and target plasmids, transformants were plated onto three agar plates supplemented with antibiotics. Colonies picked from the chloramphenicol and kenamycin plates were miniprepped, digested with single-cut enzymes, and run through gel electrophoresis to confirm plasmid identity. Chloramphenicol plate colonies only hosted target plasmids and Kenamycin plate colonies only hosted amensal target plasmids.

Sequential Transformations



E. coli cells began with an established population of target plasmids and were subsequently transformed by either the amensal plasmid or a disarmed amensal plasmid that lacked the RNA1 sequence. The two flow cytometry results above were acquired after a two day incubation following sequential transformations. Transformation by the amensal plasmid produced no cells that expressed both green and red fluorescence.

References

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