Programmed Evolution
tClone TetA: Cloning Riboswitches into Fitness Module

Erica Keffeler, Todd Eckdahl, A. Malcolm Campbell, Laurie J. Heyer, Jeffrey L. Poet, et. al.
Programming Bacterial Computers to Solve Biological Problems

- Bacteria are living computers
  - Take inputs
  - Compute solutions to problems
  - Execute programs
- Can we program bacterial metabolism?
  - Biosynthesis
  - Bioremediation
Programmed Evolution

Repeat for Successive Cycles

Combinatorics Module

Fitness Module

Biosensor Module
Theophylline

Biosensor Module

Riboswitch → GFP

GFP Fluorescence
Theophylline

Fitness Module

Riboswitch → TetA

Tetracycline Resistance

aptamer domain
expression platform

"OFF"

"ON"
Programmed Evolution Results

LB + Amp
H₂O
Disk

LB + Tet
H₂O
Disk
<table>
<thead>
<tr>
<th>Genotype Element</th>
<th>Genotypes of Starting Population</th>
<th>Genotypes of Population after Programmed Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Diagram 1" /></td>
<td><img src="image1.png" alt="Image 1" /></td>
<td><img src="image1.png" alt="Image 1" /></td>
</tr>
<tr>
<td><img src="image2.png" alt="Diagram 2" /></td>
<td><img src="image2.png" alt="Image 2" /></td>
<td><img src="image2.png" alt="Image 2" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Diagram 3" /></td>
<td><img src="image3.png" alt="Image 3" /></td>
<td><img src="image3.png" alt="Image 3" /></td>
</tr>
<tr>
<td>Promoter + RBS</td>
<td>Origin</td>
<td>Chaperone pG-Tf2</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>RBS High-High</td>
<td>High Copy</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Low Copy</td>
<td>0</td>
</tr>
<tr>
<td>RBS High-Low</td>
<td>High Copy</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Low Copy</td>
<td>0</td>
</tr>
</tbody>
</table>
Starting Population
Population after Programmed Evolution
Programmed Evolution Next Steps

- Programmed Evolution in broth
- Multistep pathways
- Improve riboswitches
tClone TetA: Cloning Riboswitches into Fitness Modules
Acknowledgements

- Todd Eckdahl, A. Malcolm Campbell, Laurie J. Heyer, Jeffrey L. Poet, 40 other undergraduate research students

- Funding
  - NSF UBM grant DMS-0733952 to Davidson College and DMS-0733955 to Missouri Western State University
  - NSF RUI grants MCB-1120558 and MCB-1329350 to Missouri Western State University and MCB-1120578 and MCB-1329351 to Davidson College
  - HHMI grants 52005120 and 52006292 to Davidson College, the Davidson Research Initiative and James G. Martin Genomics Program at Davidson College
  - Missouri Western State University Program of Research, Teaching, and Applied Learning (PORTAL)
Thank You!