UNIVERSITY OF ALBERTA

SOMEBWHERE OVER THE RAINBOW:

SPATIAL CONTROL OF GENE EXPRESSION
The Test:

- An E. coli colour wheel on an agar plate
- A self-contained circuit
- Using commonly available parts
Putting Together the Color Wheel

1) Primary Colors and How We Control Them

2) A New Kind of Control: Plasmid Loss

3) Returning to the Color Wheel

4) Major Achievements
Putting Together the Color Wheel

1) Primary Colors and How We Control Them

2) A New Kind of Control: Plasmid Loss

3) Returning to the Color Wheel

4) Major Achievements
Our Primary Colors

1) Primary Colors and how we control them
1) Primary Colors and how we control them
Controlling the Colors Transcriptionally

1) Primary Colors and how we control them
Putting Our Controlled Colors Onto Agar Plates

1) Primary Colors and how we control them
Putting Together the Color Wheel

1) Primary Colors and How We Control Them

2) A New Kind of Control: Plasmid Loss

3) Returning to the Color Wheel

4) Major Achievements
What if we could FORCE Plasmid Loss?

2) A New Kind of Control: Plasmid Loss

- **Antibiotic Selection:** ~200 copies/cell
- **No Antibiotic Selection:** A little less
- **Repressor Induced Plasmid Loss:** A lot less!
Regulating the Origin of Replication

2) A New Kind of Control: Plasmid Loss
Our Origin of Replication Control Plasmids

LacI
(IPTG)

1) Strong
-35 __ -10

2) Medium
-35 __ -10

3) Weak
-35 __ -10

TetR
(ATC)

1) Strong
-35 __ -10

2) Medium
-35 __ -10

2) A New Kind of Control: Plasmid Loss
Inoculum Experiment

2) A New Kind of Control: Plasmid Loss

Maintenance: +Ind/+chlr
Passive loss: +Ind/-chlr
Forced loss: -Ind/-chlr

Small inoculum

+ inducer
+ chlr

Selective conditions
Plating Inoculum Samples

Weak Lac-Ori

1% Dilution Factor

10^0 10^1 10^2 10^3 10^4 10^5 10^6

indr/AB

2) A New Kind of Control: Plasmid Loss
2) A New Kind of Control: Plasmid Loss
Testing Origin of Replication Control

IPTG gradient

-35  -10  RNA II  Ori

2) A New Kind of Control: Plasmid Loss
Putting Together the Color Wheel

1) Primary Colors and How We Control Them

2) A New Kind of Control: Plasmid Loss

3) Returning to the Color Wheel

4) Major Achievements
Copy control out-performs direct gene control

2) A New Kind of Control: Plasmid Loss
Using Origin of Replication for a Color Wheel

IPTG gradient

-35  

LacO  

RNA II  

-10  

Ori  

weak wt-Pr[Lacl]  

intermediate Lacl-Pr*  

strong Lacl-Pr  

wt control

3) Returning to the Color Wheel
Our Experimental Strains

Yellow E. coli Strain

Red E. coli Strain

3) Returning to the Color Wheel
Closer to the rainbow

minus chloramphenicol
Co-culture of cells expressing either amilGFP or RFP under IPTG copy-control. amilGFP- moderate origin promoter. RFP- weak origin promoter.

plus chloramphenicol

3) Returning to the Color Wheel
A New Angle: Plasmid Loss Circuit

3) Returning to the Color Wheel
A New Angle: Plasmid Loss Circuit

Circuit Behavior

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<th>Input</th>
<th>IPTG</th>
<th>ATC</th>
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3) Returning to the Color Wheel
Major Achievements

• Submission of 5 plasmids to the registry
• Developed a novel form of regulation using the origin of replication
• Plated Color Wheel Using 2 E. coli strain mixture
• Designed novel circuits using origin of replication regulation
• High school team members and handbook
Other applications for controlled copy#

- Positive cloning selection:
  - Related: Plasmid shuffling
    - Plasmid curing
  - Regulating stubborn genes
    - (Cambridge colours)
  - Environmental containment switch

- 2-D chemical mapping:
  - Readout
  - Surface
  - Chemical A
  - Chemical B