Purdue University

Bacterial Warfare

The Rock Paper Scissors Approach

The Concept

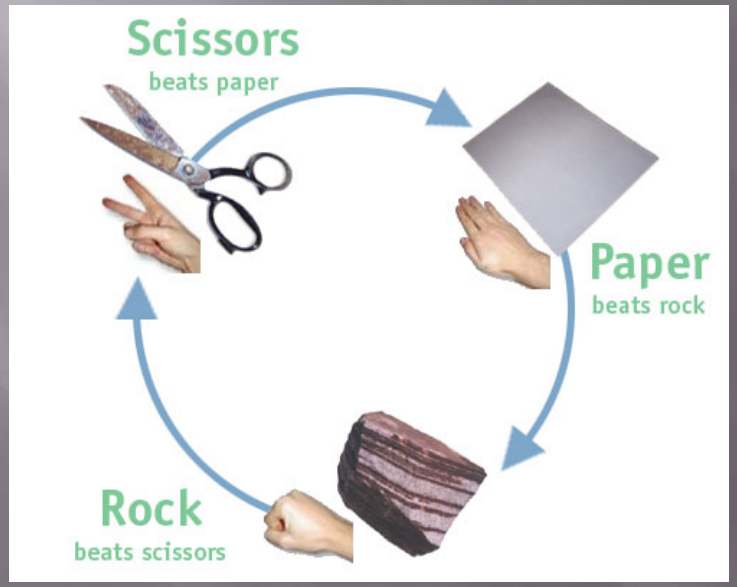
Brainstorming Ideas

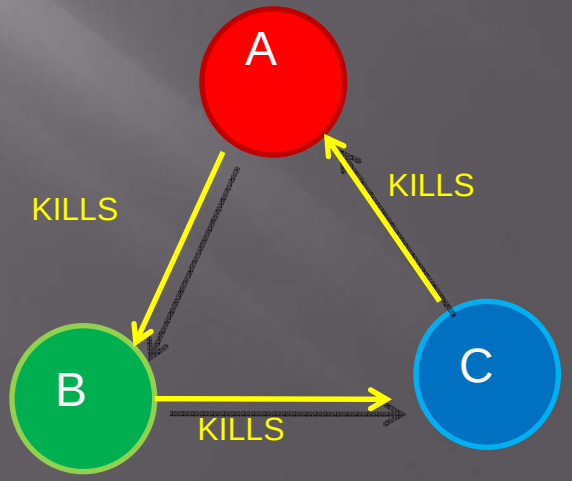
Microbe board game?

Chess?

Risk?

Some kind of microbe warfare?





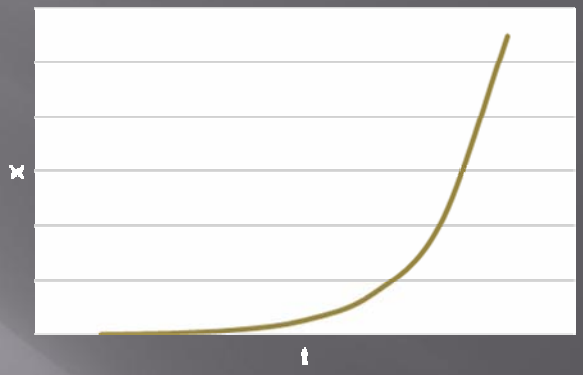
Background

- How is diversity maintained in nature?
- Environmental factors prevent equilbrium.
- Competing Species utilize common resources.
- Darwinistic, survival of the fittest, but what defines “fittest?”
- If one organism can kill another one better, does that mean that organism will survive?
- What happens to the weakest of the organisms present?

Bacterial Growth 101

- Exponential Model

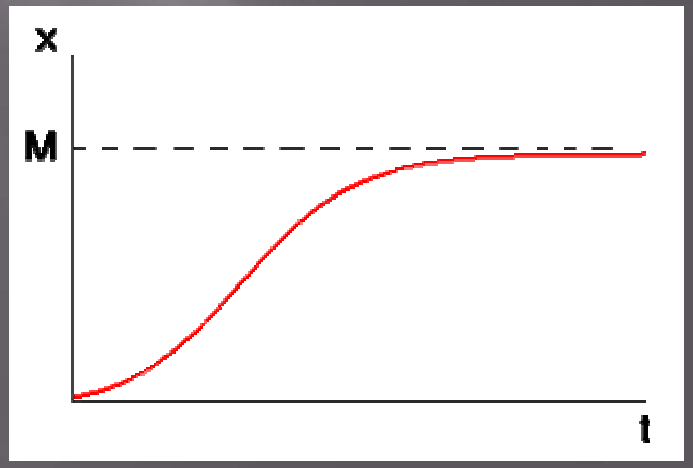
$$X = X_o * e^{U*t}$$



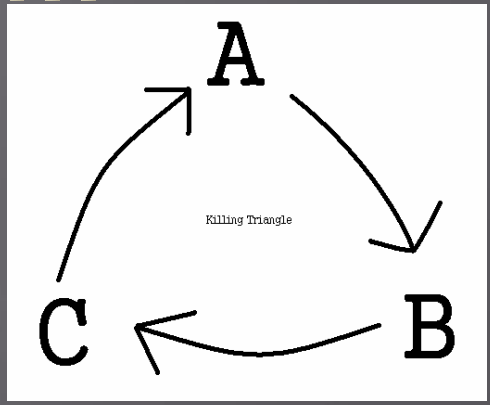
- Logistic Growth Model

$$\frac{\partial X}{\partial t} = X * U - X^2 * \delta$$

U=growth const, δ=death coef



Killing Mechanism



- A kills B
- B kills C
- C kills A
- Kill Term Must be Accounted for in Model
- Therefore:

Natural Growth Rate, Natural Death Rate, Murder Rate

$$\frac{\partial X_a}{\partial t} = X_a * U_a - X_a^2 * \delta_a - M_{ca} * X_c$$

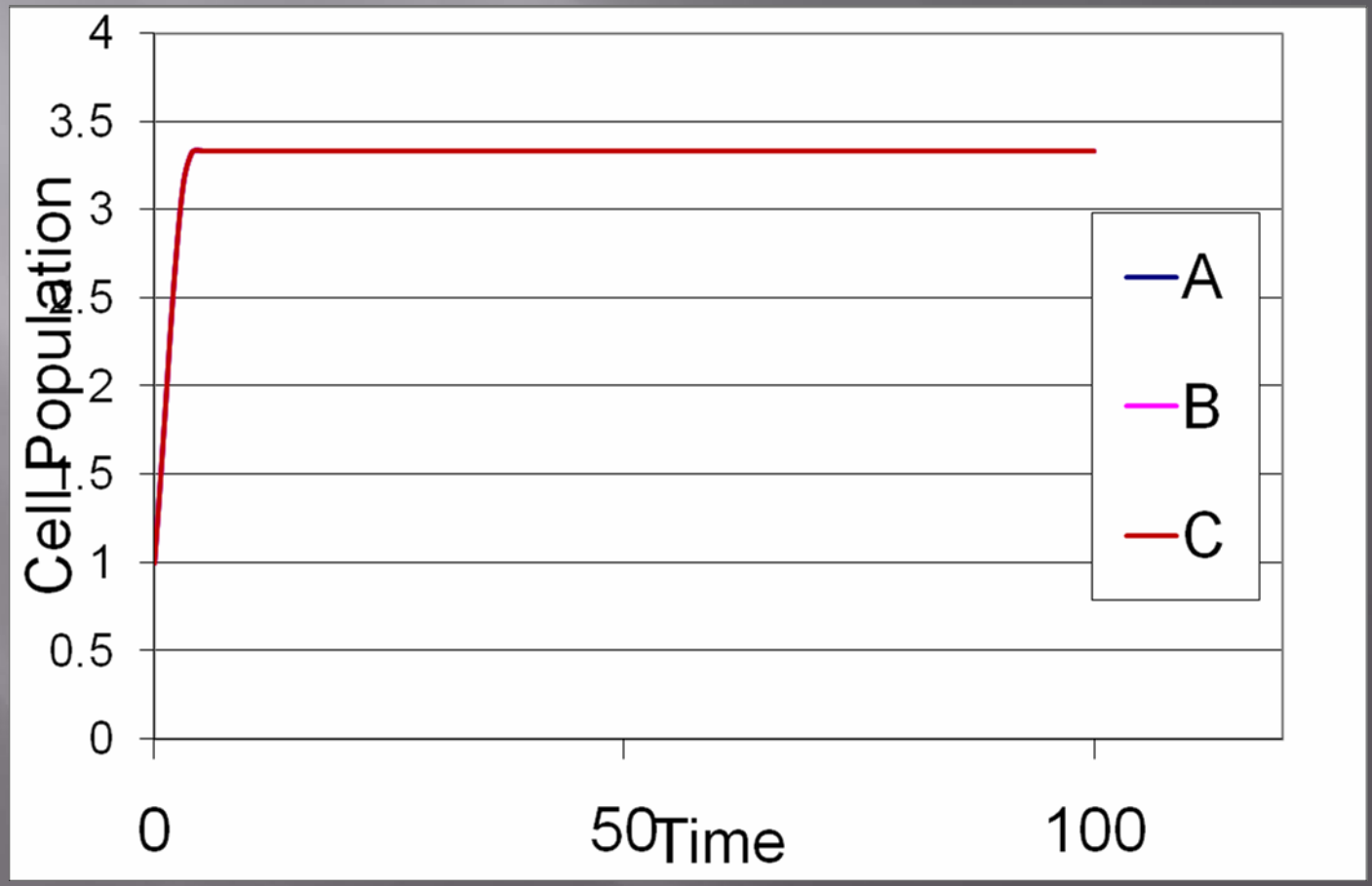
$$\frac{\partial X_b}{\partial t} = X_b * U_b - X_b^2 * \delta_b - M_{ab} * X_a$$

$$\frac{\partial X_c}{\partial t} = X_c * U_c - X_c^2 * \delta_c - M_{bc} * X_b$$

Simulations

Murder Rates
$M_{ab}=0.2$, $M_{bc}=0.2$, $M_{ca}=0.2$

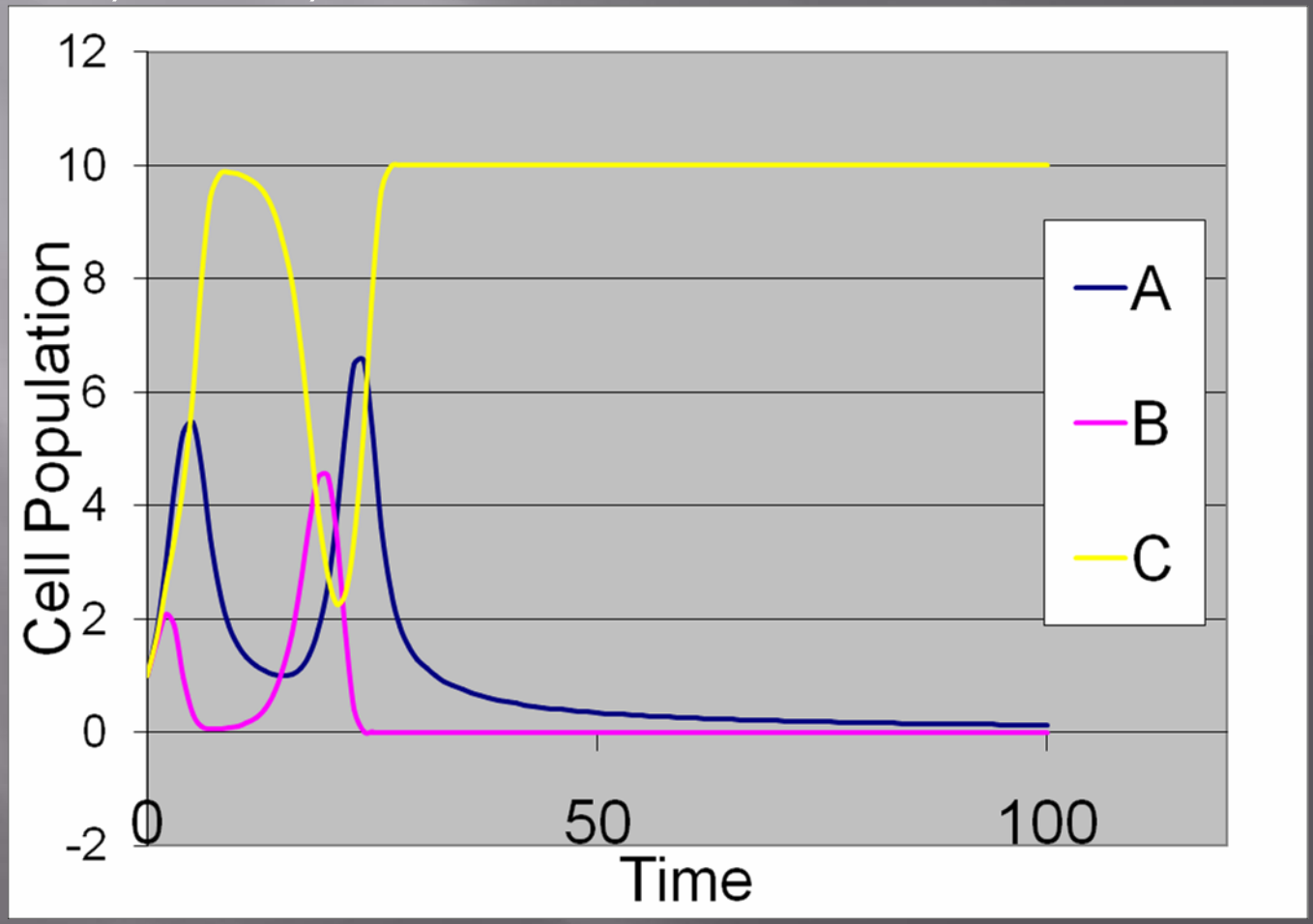
Initial Population
$A_o=B_o=C_o=1$



C is the weakest but dominates?

Murder Rates
A=0.3, B=0.2, C=0.1

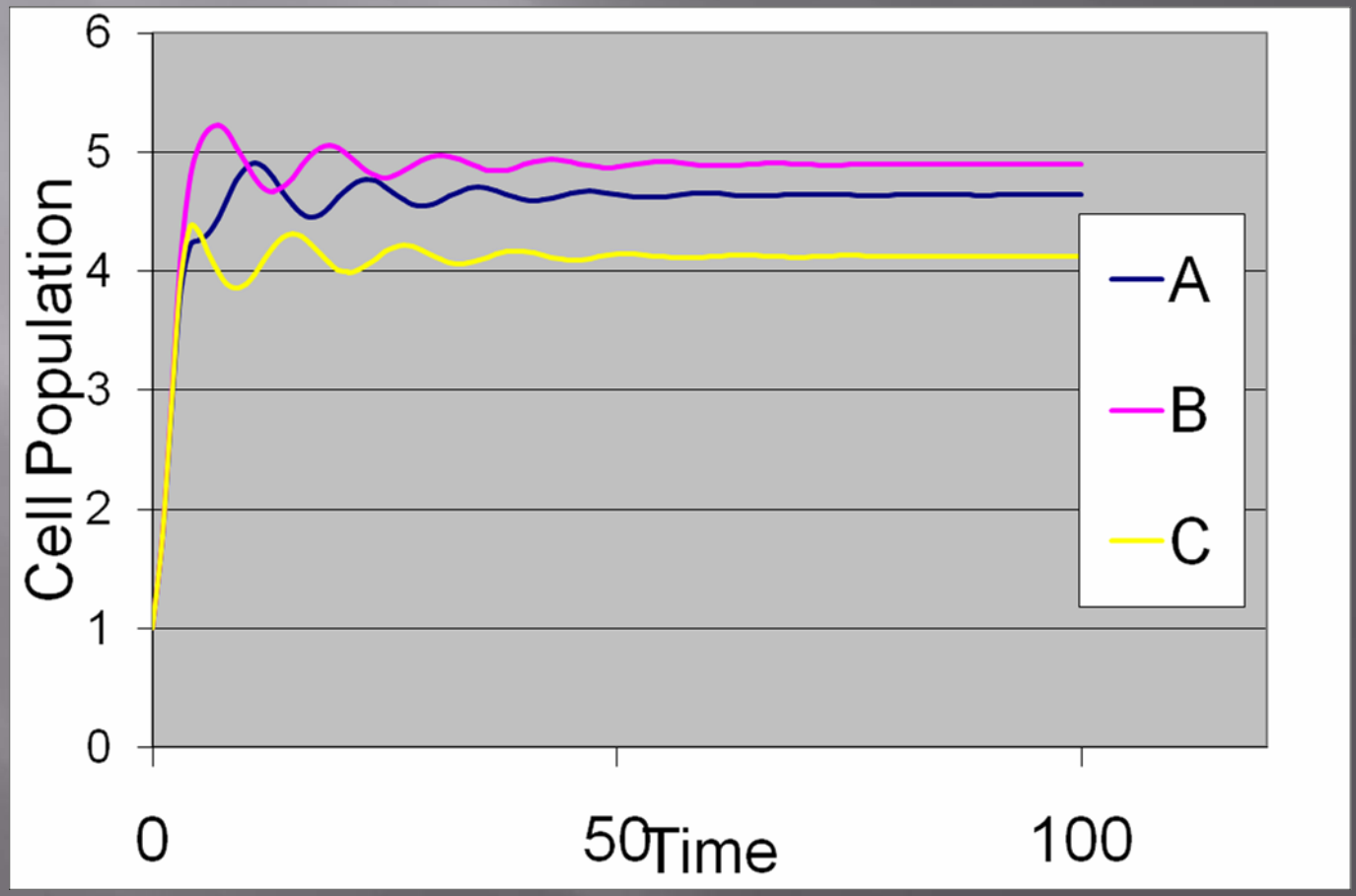
Initial Population
$A_0=B_0=C_0=1$



Oscilations

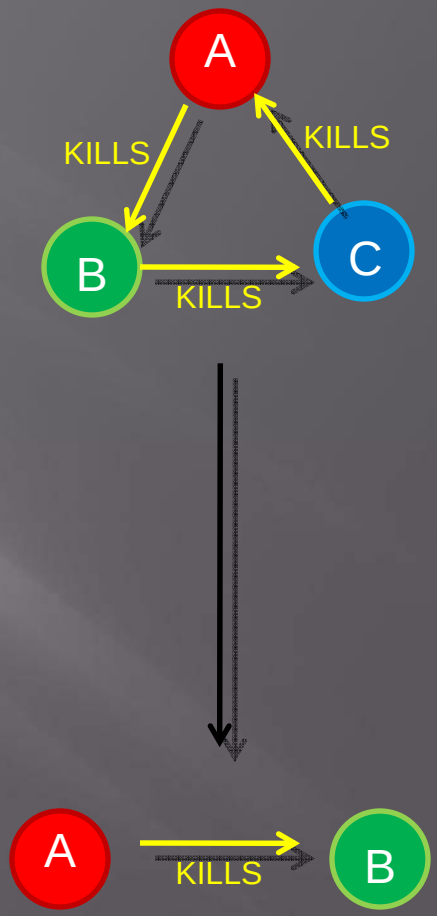
Murder Rates
A=0.11, B=0.12, C=0.13

Initial Population
$A_0=B_0=C_0=1$



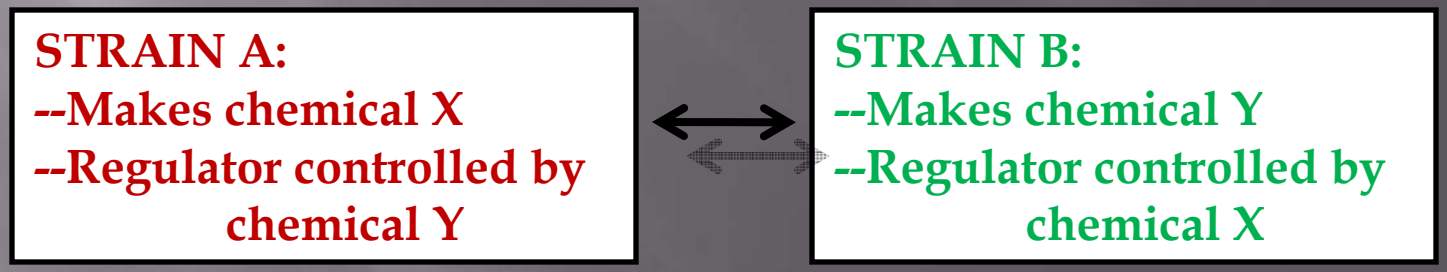
IN THE LAB

- Original concept involved 3 *e. coli* strains
- Simplified problem to a 2-strain model
- In iGEM parts registry, found 2 regulators with corresponding producer genes



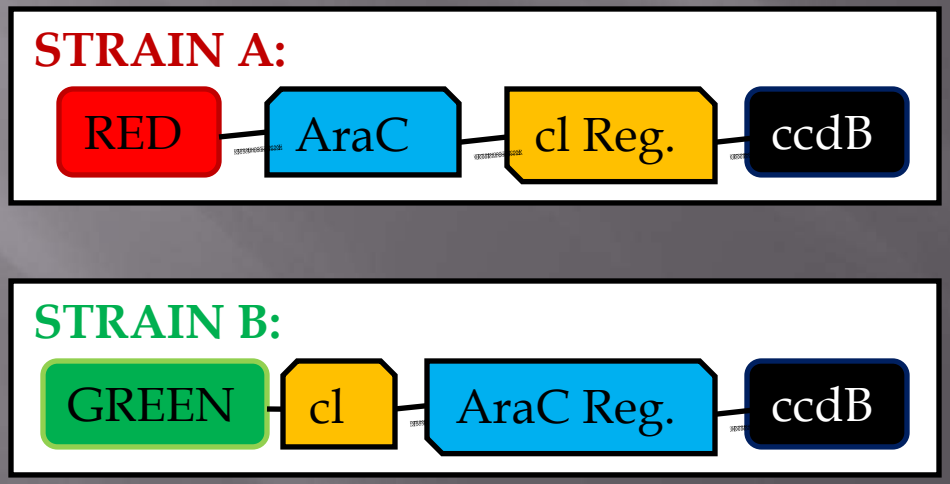
The Mechanism

- Indirect Killing:
  - Inducible regulators are normally "OFF"
  - Inducible regulator + Death gene = controlled death
  - Each strain's "weapon" is a gene producing the chemical corresponding to the opposite strain's regulator



The Parts

- Death Gene: ccdB gene
  - Usually used to regulate part-making
- Chemical Producer genes: AraC (arabinose), cl
- Inducible Regulators: cl, AraC
- GFP (colors): Red and Green
  - For distinguishing strains and measuring their growth

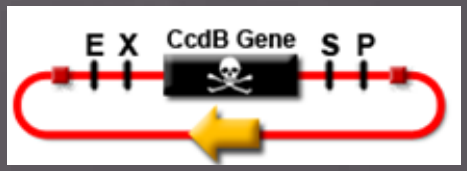


BIOLOGICAL IDEAS

- In order to make bacteria "kill" each other in a meaningful way, the death gene used had to be regulated.

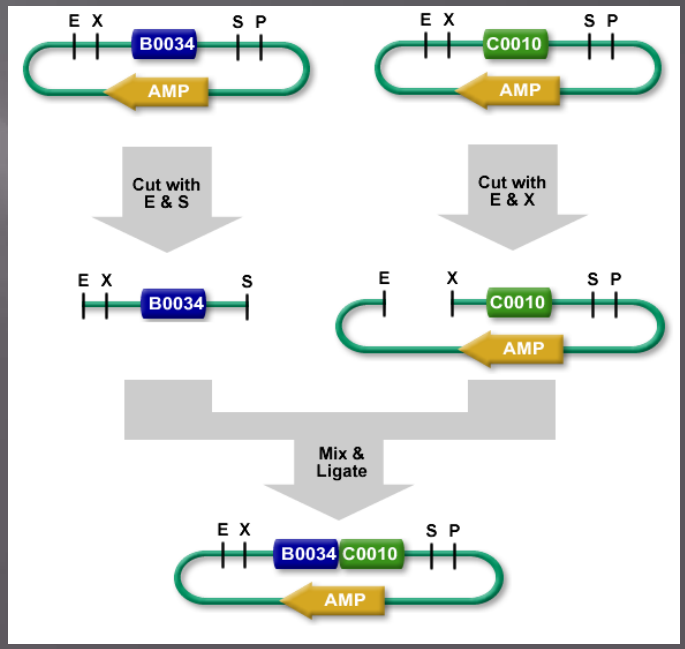
- AraC and cI regulators were used to control the activity of the death gene.

- Upon contact with AraC (or Arabinose) or cI, the death gene is activated and the cell "commits suicide."



BIOLOGICAL PROCEDURES

1. Amplifying DNA
2. Extract and purify plasmid DNA
3. Digest DNA.
4. Gel Electrophoresis.
5. Mix and ligate.
6. Transform new plasmids with desired arrangement of genes into E. Coli



astrographics. com

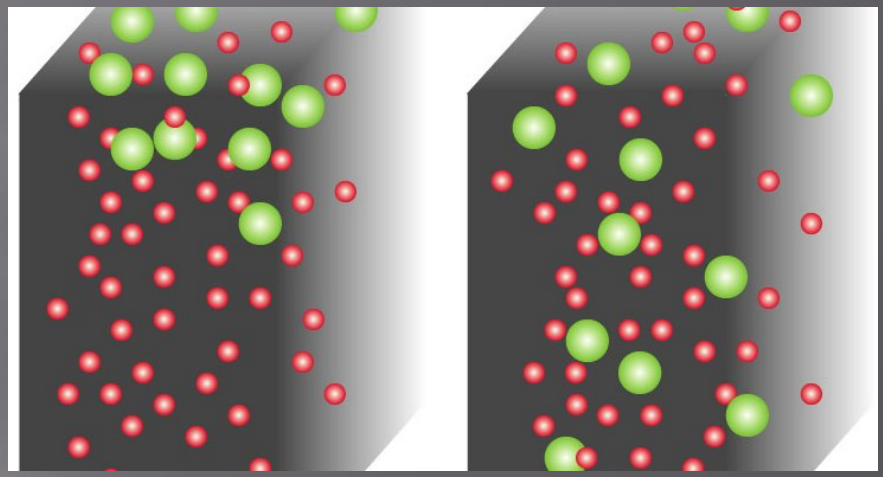
Problems With the Model

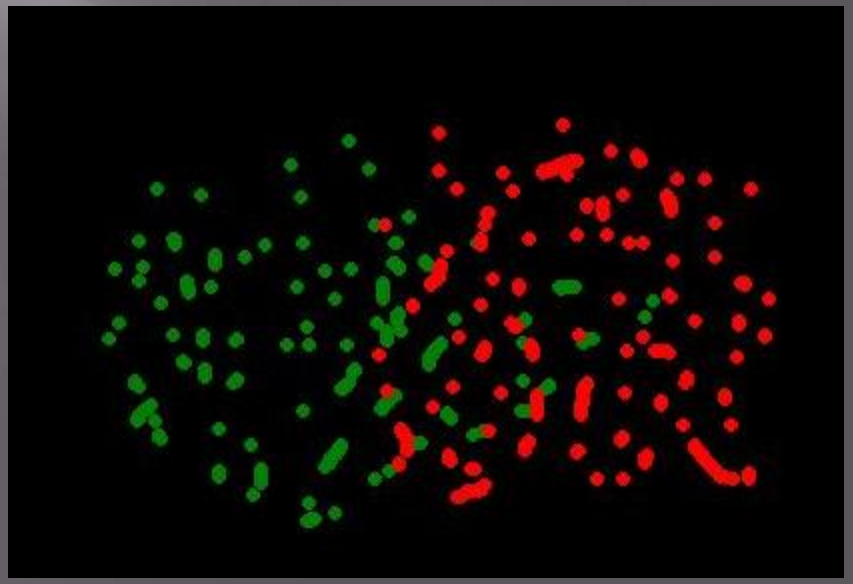
- During our presentation at the iGEM jamboree, we realized that our cI regulator would not work.
- It was brought to our attention that this regulator does not do what we assumed.
- We are currently working on rerouting the mechanism to correct this.

BIOLOGICAL PROBLEMS

How fast will the signaling protein diffuse?

- Will the protein toxin diffuse out of the dead cell and kill the nearby bacteria?





Modeling The Lab

- Does genetic engineering effect?
  - Growth rate
  - Death rate
- What are the murder rates?
  - A→B
  - B→C
  - C →A

FUTURE PLANS

- Recruit iGEM members
  - Flyers and posters
  - Go to related lectures and give short speeches
  - Let academic advisers send emails to related majors
  - Currently have 25 students interested in iGEM, a 257% increase!
- Generate plans & ideas in spring. Start experiments & modeling in summer.

iGEM PLANS

- Keep the project student run
  - Continue this project if the next generation members are interested
  - Currently entertaining more project ideas due to the large contingency of student interest.