Unveiling the Mysteries of Synthetic Biology

with

cornell iGEM

Our Team

- **40 undergraduates**
- **5 subteams**: Wet Lab, Product Development, CS/ECE Design, Policy and Practices, and Business
- Compete against **280 multidisciplinary teams** from all around the world at the iGEM World Jamboree

What is synthetic biology?

Synthetic Biology

- Apply standardized engineering techniques to biology and thereby create organisms or biological systems with novel or specialized functions to address countless needs
- Designing and constructing biological devices, networks and pathways for useful purposes
- Creating synthetic components or re-assembling pre-existing genes
- Wiring biological circuitry to make:
  - Biological switches, oscillators, toggle switches
  - Logic gates, pulse generators
- Biosensing, therapeutic treatment, biofuels…. and much more

So...

is synthetic biology the same as genetic engineering?

Synthetic Biology versus Genetic Engineering

- The difference lies in the approach Synthetic Biology uses
  - Control of biological circuits/regulation
  - Biological standardisation, modulation, and reusability
  - Need for traditional engineering approaches such as computer modeling
- “Using synthetic biology to build upon synthetic biology”
  - iGEM standard registry - library of standard biobricks
- Synthetic Biology is Genetic Engineering 2.0

Applications of Synthetic Biology

There is a potential for synthetic biology in many applications:

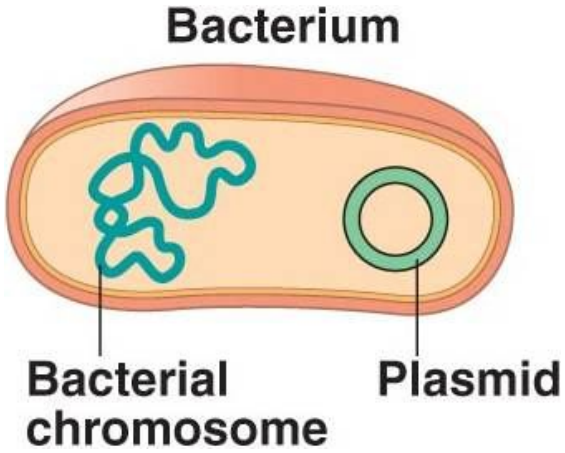
- Medical (Producing medicine (insulin), biomedical sensors and implants)
- Environmental (Environmental remediation, sensing pollutants)
- Industrial (Optimizing processes)
- Bio-based chemicals
- Vaccine and antibody production
- Energy (Biofuels)
- And Much More!

How is synthetic biology actually done?

One of the basic techniques of synthetic biology is DNA cloning with plasmid vectors.

Wait what? Cloning?

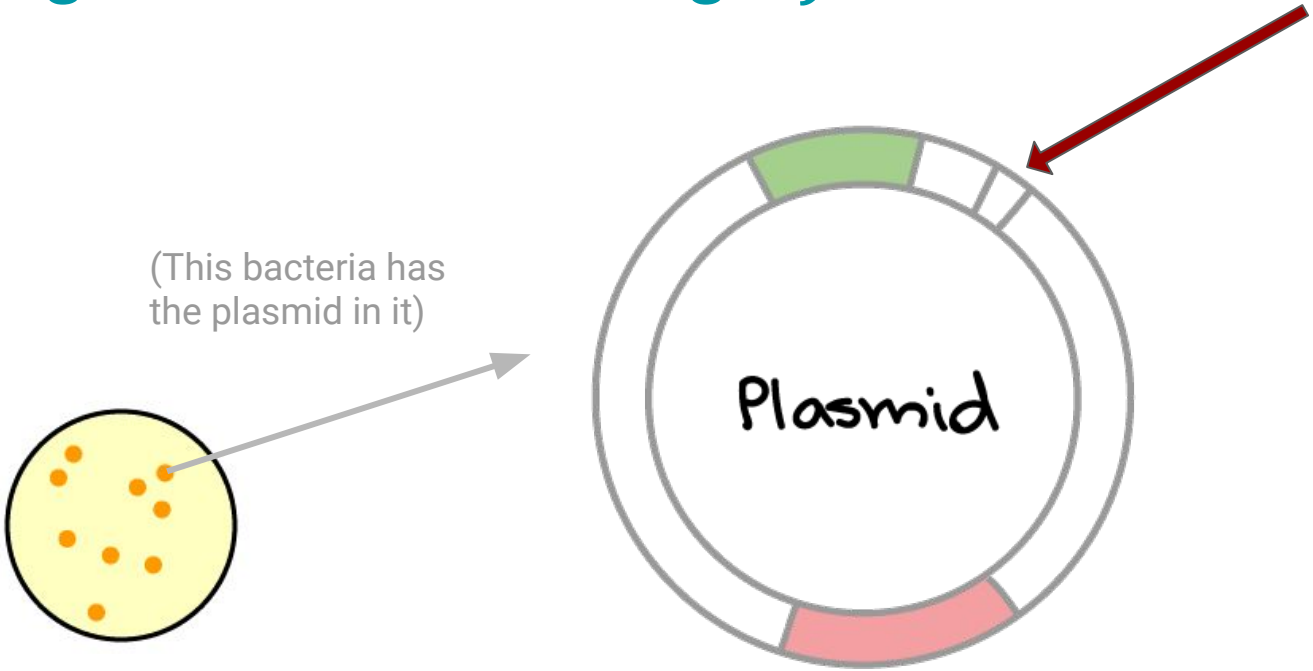
- The process of piecing recombinant DNA pieces together
- Bacteria have plasmids. We can use bacteria to grow up the plasmids we want!



Definitions

- Recombinant DNA = DNA formed from combining DNA of different organisms
- Plasmid = short circular piece of DNA that is transcribed and replicated separately from the chromosome

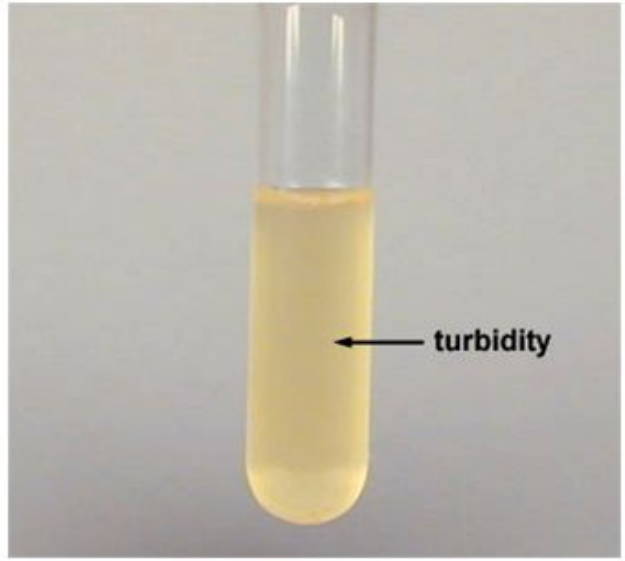
We have this plasmid. We want to insert a gene right where the two gray lines are.



First: grow up a lot of your plasmid!

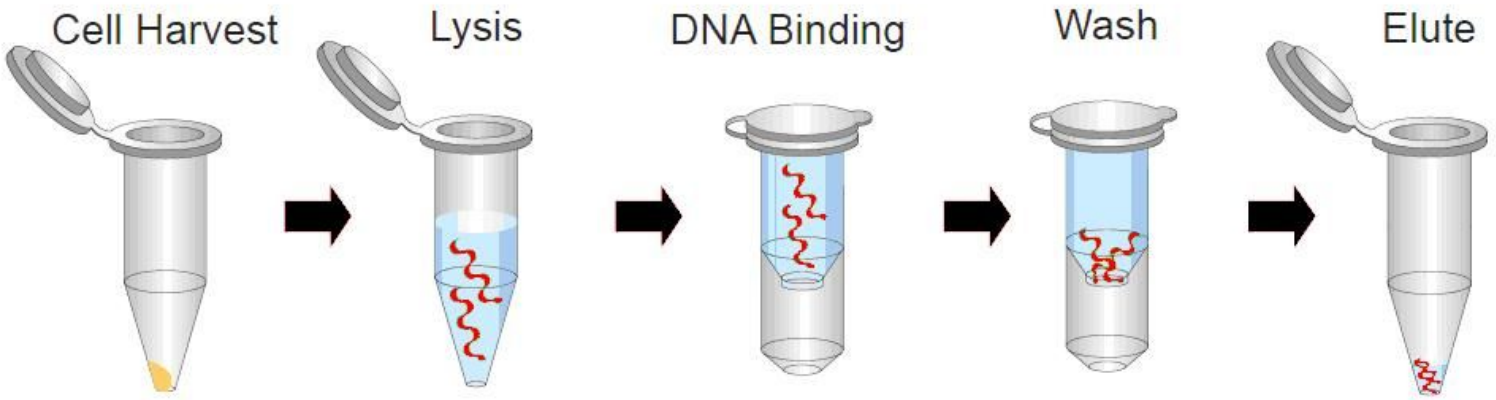
- Take one of the dots on the plate (a bacterial colony), and grow it up in broth

- Put the colony in LB broth (a solution at the right pH with all the necessary nutrients)
- Incubate at 37 degrees Celsius



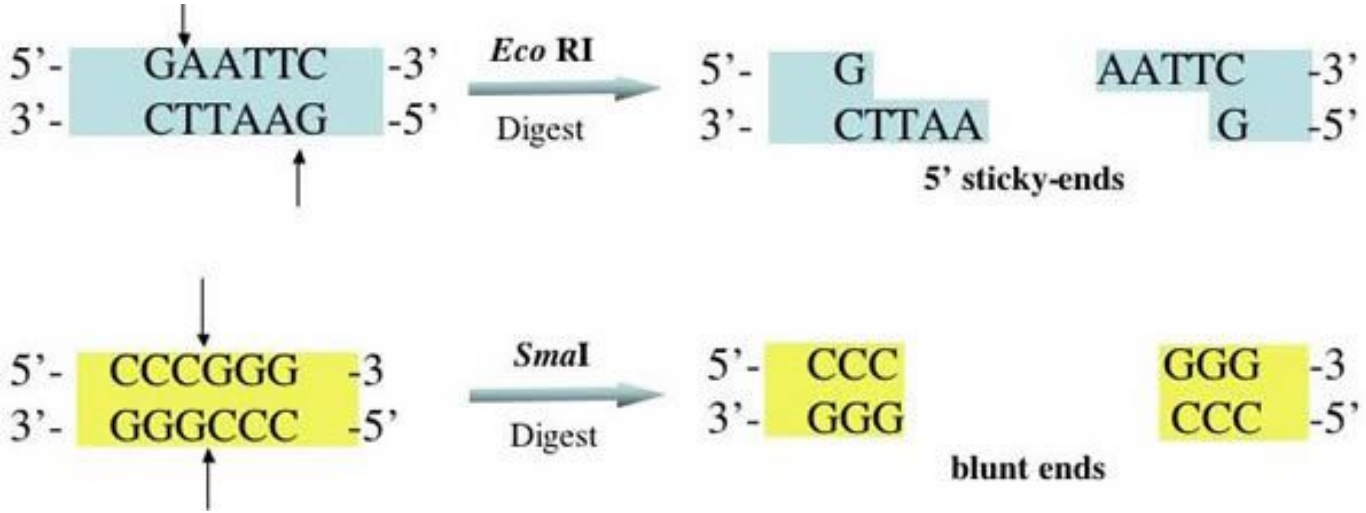
Extract the plasmid DNA with a miniprep

- We need to get the plasmid DNA out of the bacteria
- Lyse the bacteria (i.e. break it down)
- Then, clean it up to get the DNA that you want (and get rid of the debris)



Now we have plasmid DNA. Let's cut it up.

- Restriction enzymes recognize "cut sites" and then digest the DNA there
- Results in "sticky ends" or "blunt ends"
- Usually use stick ends in DNA cloning techniques



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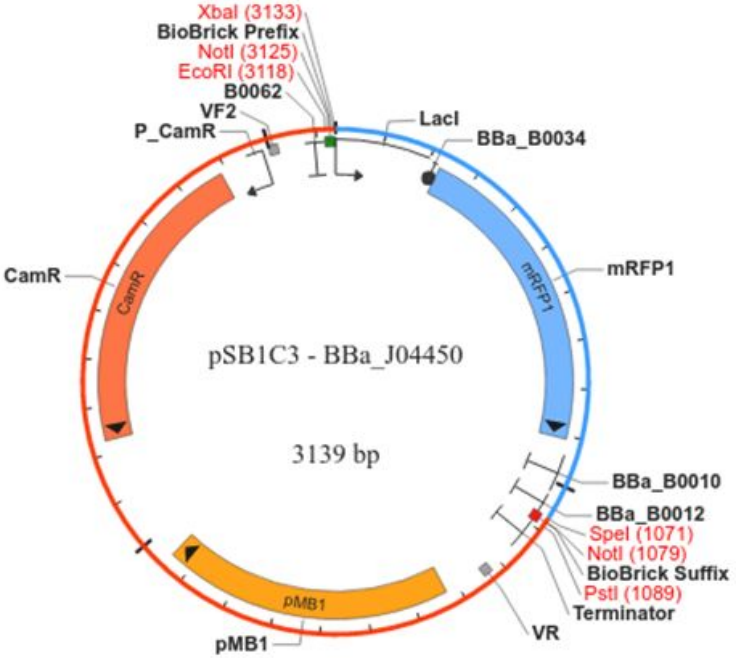
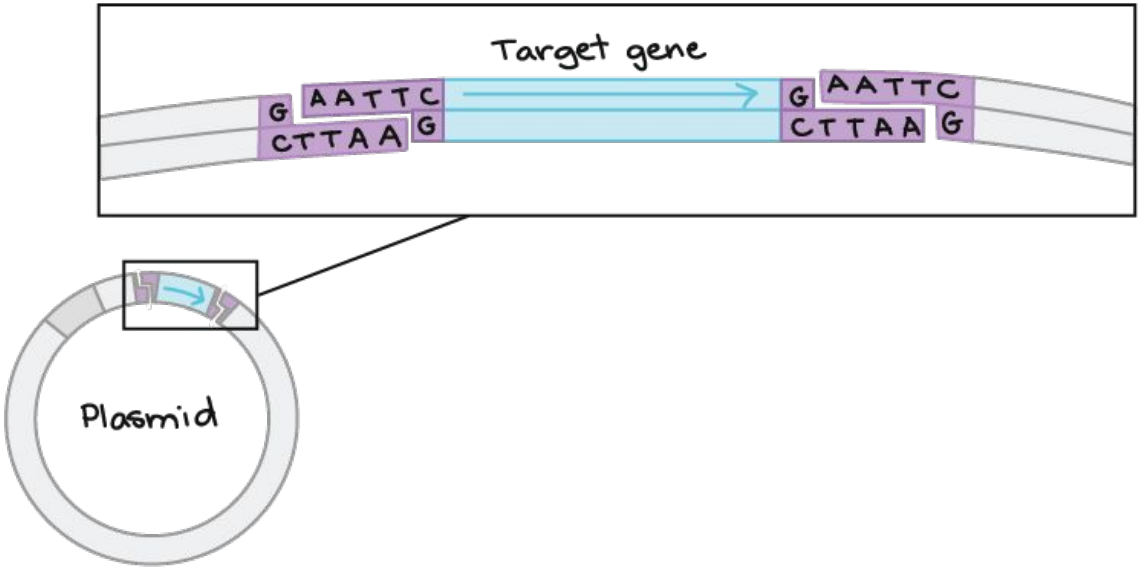


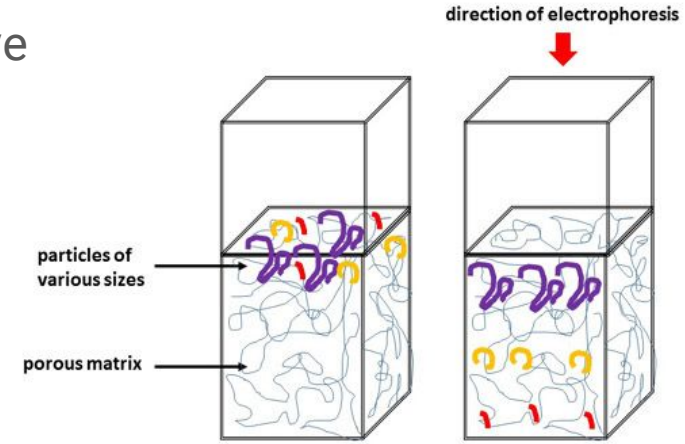
Now we have plasmid DNA. Let's cut it up.

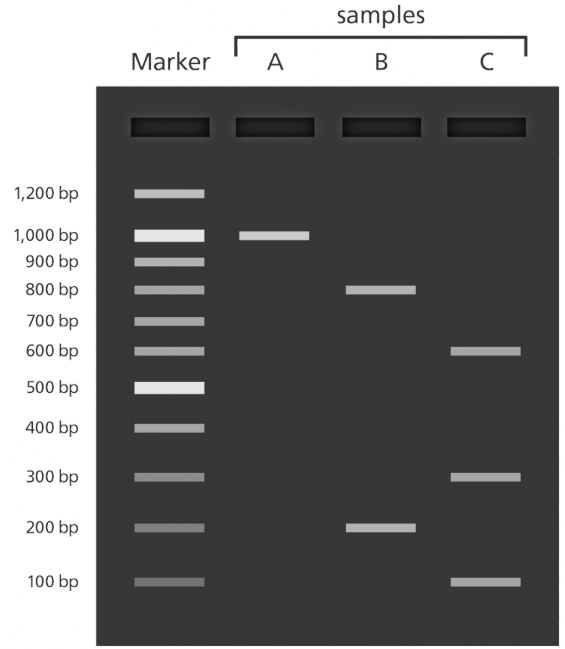
- If you cut two different plasmid DNA pieces with the same restriction enzymes, you can piece them together! (have complementary sticky ends)



Visualize with gel electrophoresis

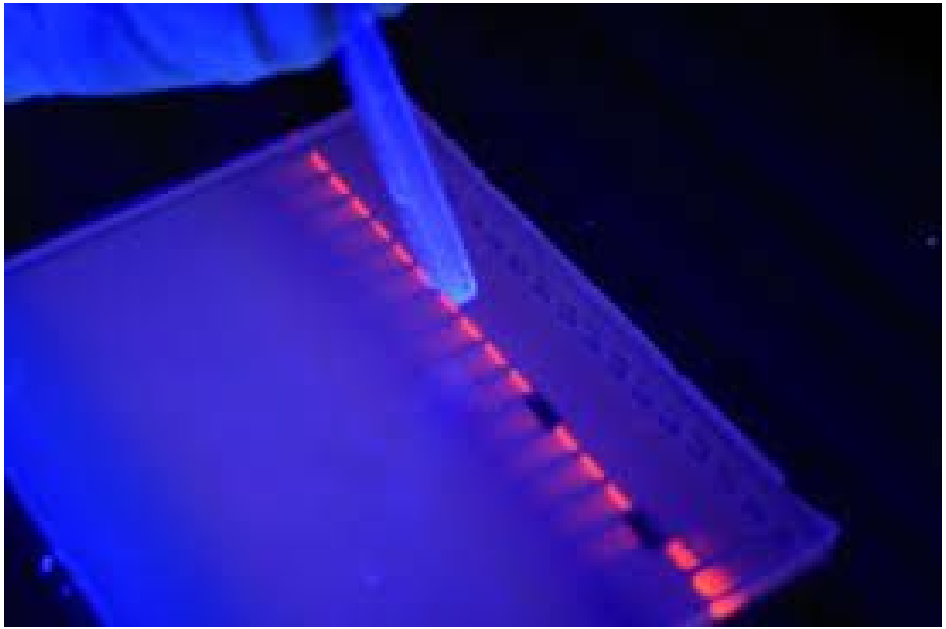
- Load DNA into a gel
- DNA is negative and travels to the positive end
- Separates by size (Smaller particles move faster)
- Check to make sure that you cut the plasmid correctly by confirming sizes





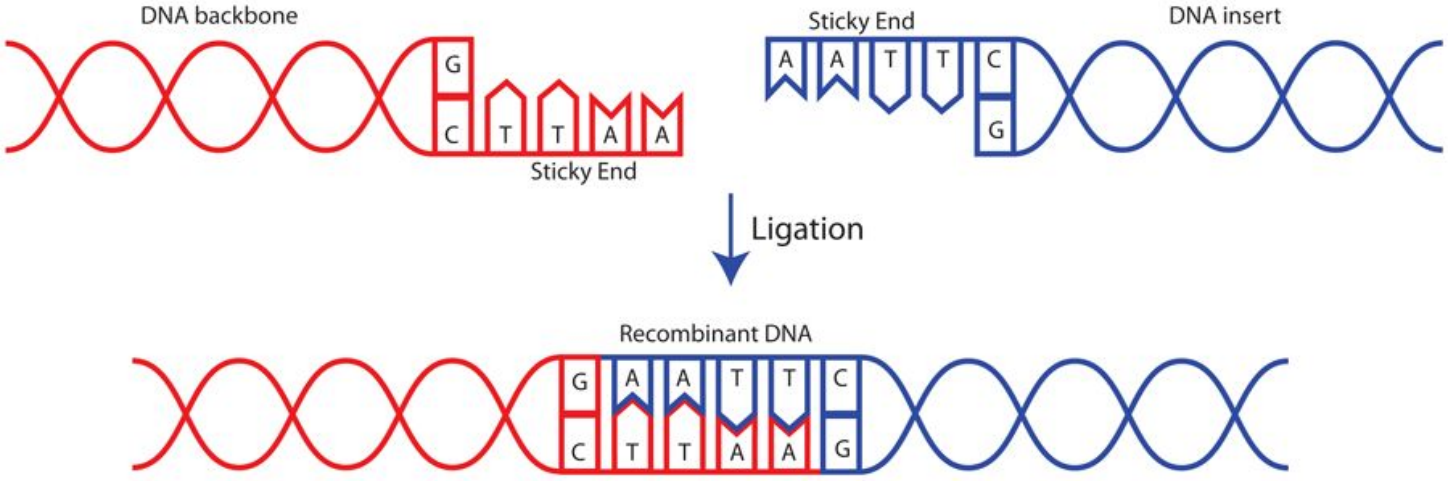
Perform a gel extraction

- Cut the band that you want out and then clean it up (similar to miniprep)
- After the miniprep, we had the entire plasmid vector.
- After the gel extraction, we have just the piece that we want now!

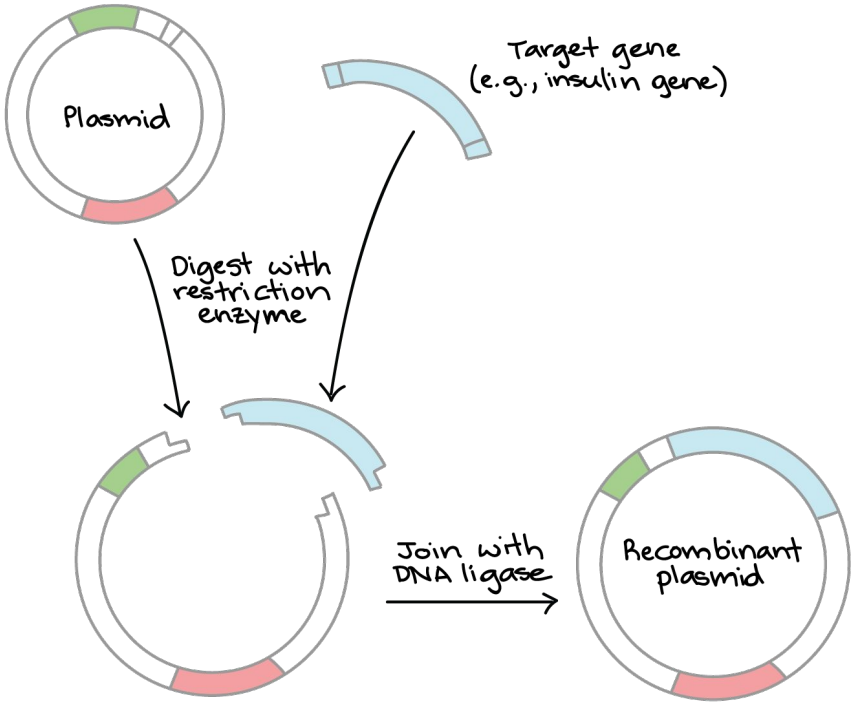


Ligate - the “gluing”

- Ligate with an enzyme called DNA ligase to make our pieces a whole



Let's take a look at it all over again.



How we used plasmids:

We used plasmids in our 2016 project, Legendairy

Treatment of Bovine mastitis using Bacteriocins

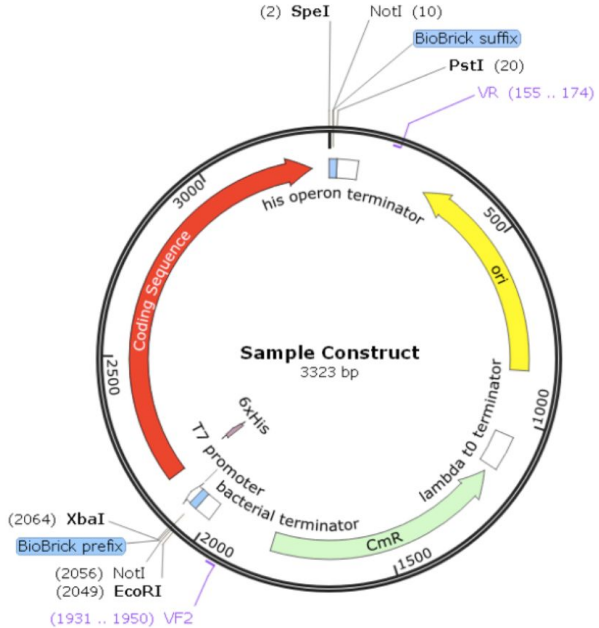
- Potentially fatal mammary gland infection
- Most common disease in dairy cows in the U.S.
- Caused by bacteria, including *E. coli, S. aureus, P. aeruginosa*

- Proteinaceous toxins made by bacteria to inhibit growth of other bacteria

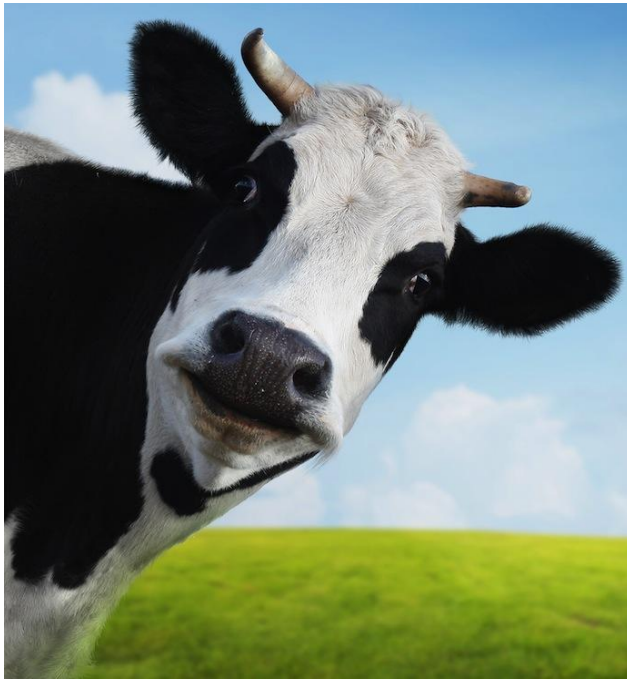
The Legendairy plasmid (Cornell iGEM 2016)

Inserted 13 bacteriocins into plasmid to target the different bacteria

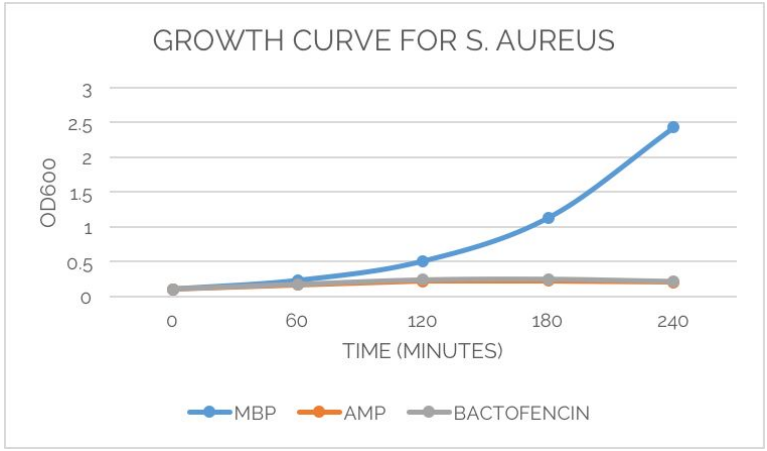
Bacteriocin	Producer	Target
Nisin U	*Streptococcus uberis*	*Streptococcus spp.*
Nisin F	*Lactococcus lactis*	*Staphylococcus aureus* *Staphylococcus carnosus* *Lactobacillus spp.*
Nisin Z	*Lactoccus lactis*	*Enterococcus spp.*
Nisin A	*Lactococcus lactis*	*Enterococcus spp.*
Subtilin	*Bacillus subtilis*	Broad spectrum Gram-positive
Subtilosin	*Bacillus subtilis*	Broad spectrum Gram-positive and Gram-negative
Epidermicin NI01	*Staphylococcus epidermidis*	*Staphylococcus epidermidis* *Staphylococcus aureus*
Colicin M	*Escheria Coli*	*Escheria Coli*
Colicin 10	*Escheria Coli*	*Enterobacter spp.*
Enterocin E760	*Enterococcus spp.*	Broad spectrum Gram-positive and Gram-negative
Microcin E492	*Klebsiella pneumoniae*	*Enterobacter spp.*
Aureocin A53	*Staphylococcus aureus*	*Staphylococcus aureus*
Lysostaphin	*Staphylococcus simulans*	*Staphyloccus aureus*



And.. it worked!!



- Displayed zones of inhibition
- Decreased bacterial growth comparable to antibiotics



Just because we can use synthetic biology for something, should we...?

Bioethics Concerning Synthetic Biology

- Just because we can use Synbio for something, should we?
- Important ethical concerns regarding synthetic biology
- Some concerns about Synthetic Biology:
  - Is it harmful to human health?
  - Is it harmful to the environment?
  - What are relevant ethical considerations?
    - Utility, Fairness

Activity

- Split into groups
- Discuss different bioethical case scenarios that arise when developing new things in synthetic biology
- Load gels for electrophoresis!