2012 iGEM Competition:
Carnegie Mellon University

Promoter Characterization via Fluorescence-based Biosensor

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Background: Synthetic Biology

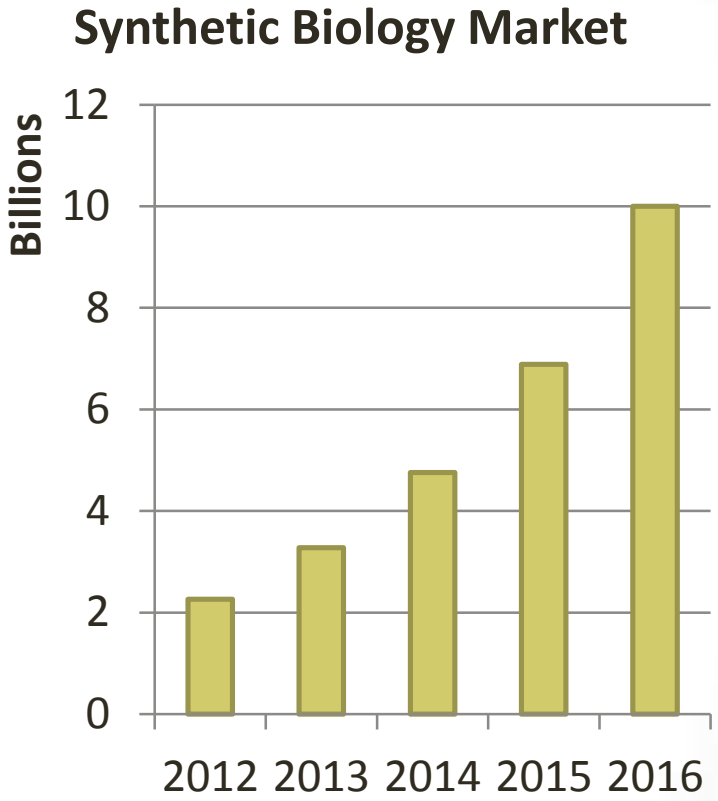
- Definition –
  - "Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature."

    -National Center Biotechnology Information

Background: Synthetic Biology

- "The global market for synthetic biology has been estimated at just over $10Bn in 2016 (with a compound annual growth rate of 45% between 2011 and 2016) spread across a wide range of product areas.

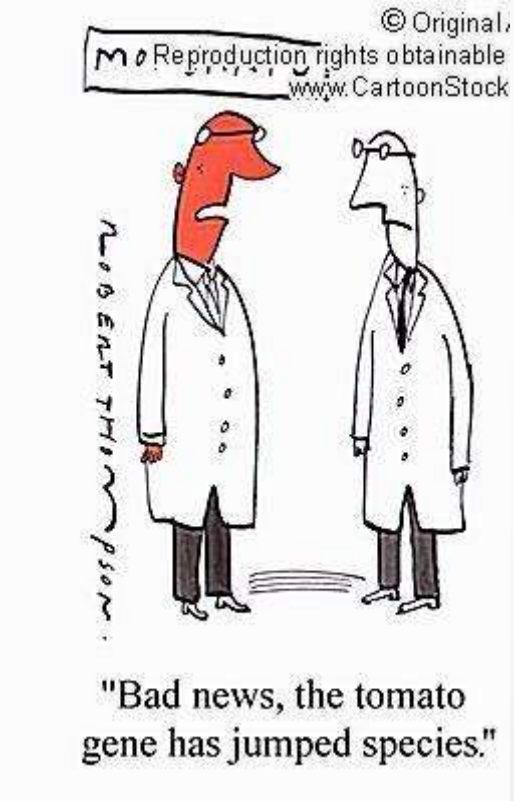
  -Biotechnology & Biological Sciences Research Council



Background: Synthetic Biology

- History –
  - *Nobel Prize in Medicine*, 1978: Awarded to Arber, Nathans & Smith
    - Scientists recognized “the new era of synthetic biology” had arrived.
  - *Nature* Journal, 2000: 1st examples of biological circuits published
    - Bacterial toggle switch in E. coli:

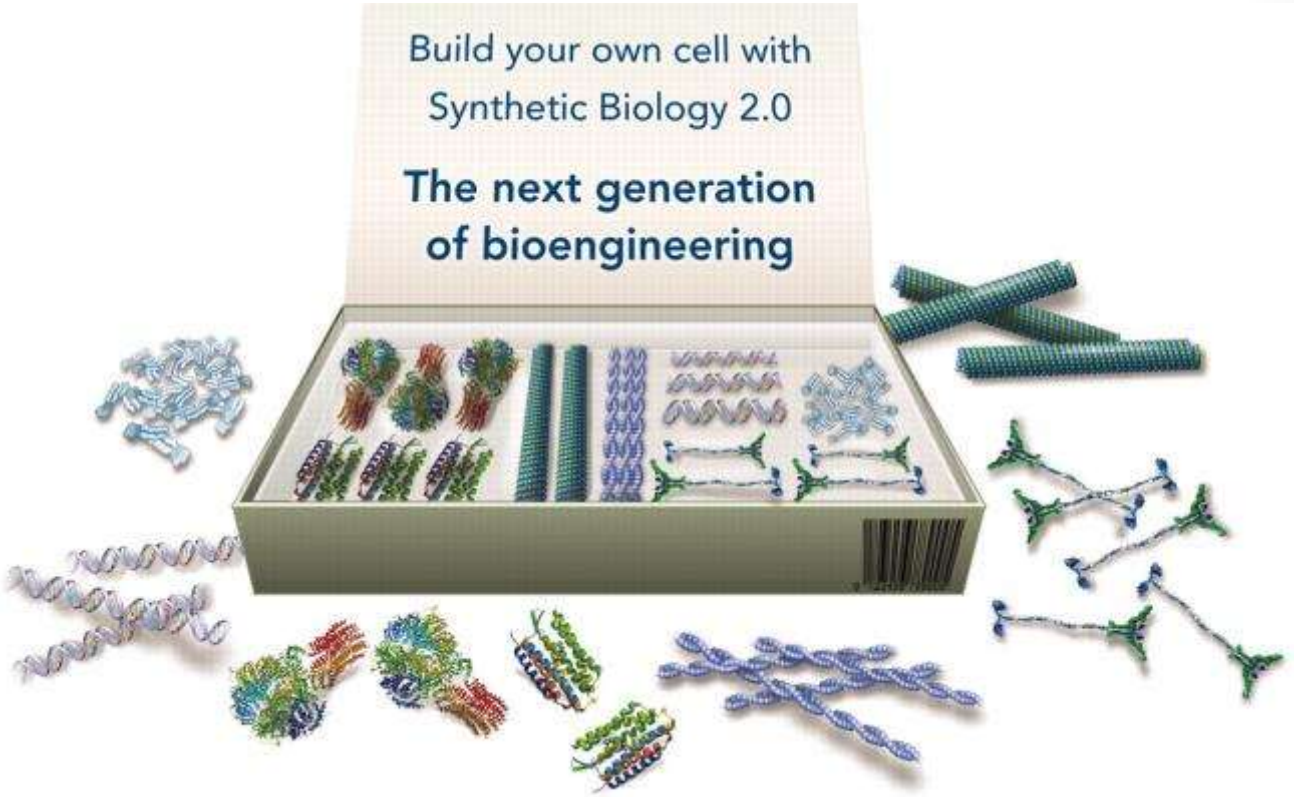
      turn on and off using heat



"Bad news, the tomato gene has jumped species."

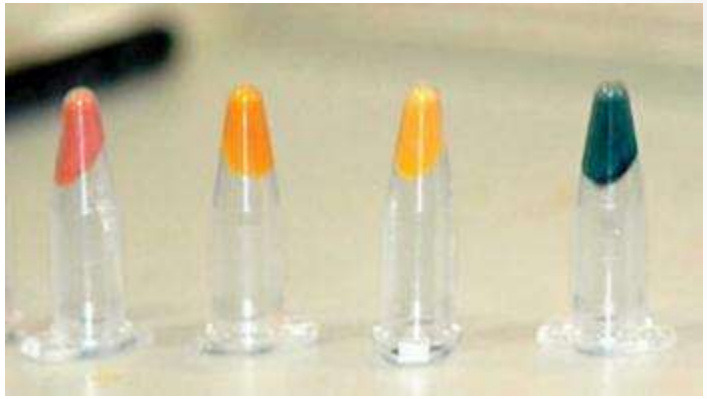
Background: Synthetic Biology

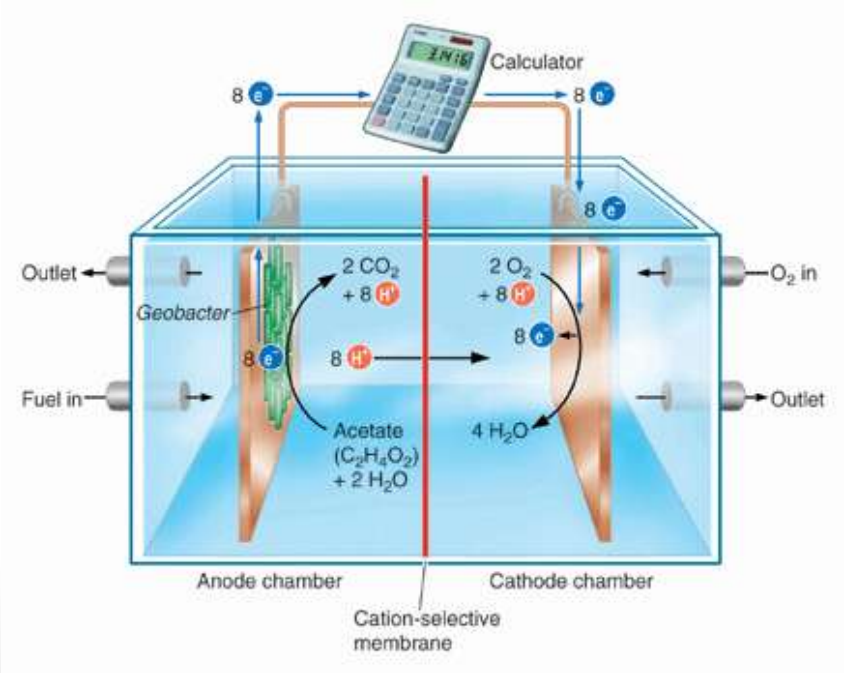
- Synthetic biologists come from different disciplines and contribute in different ways:
  - Electrical/Computer Engineering – Bio-computation
  - Chemical Engineering – Metabolic engineering
  - Biologists – Artificial cells



What Can Synthetic Biology Do for You?

- Fundamental needs:
  - Biosensors
  - Inexpensive vaccines
  - Clean water and energy





Ethics in Synthetic Biology

Ethical questions in Synthetic Biology:

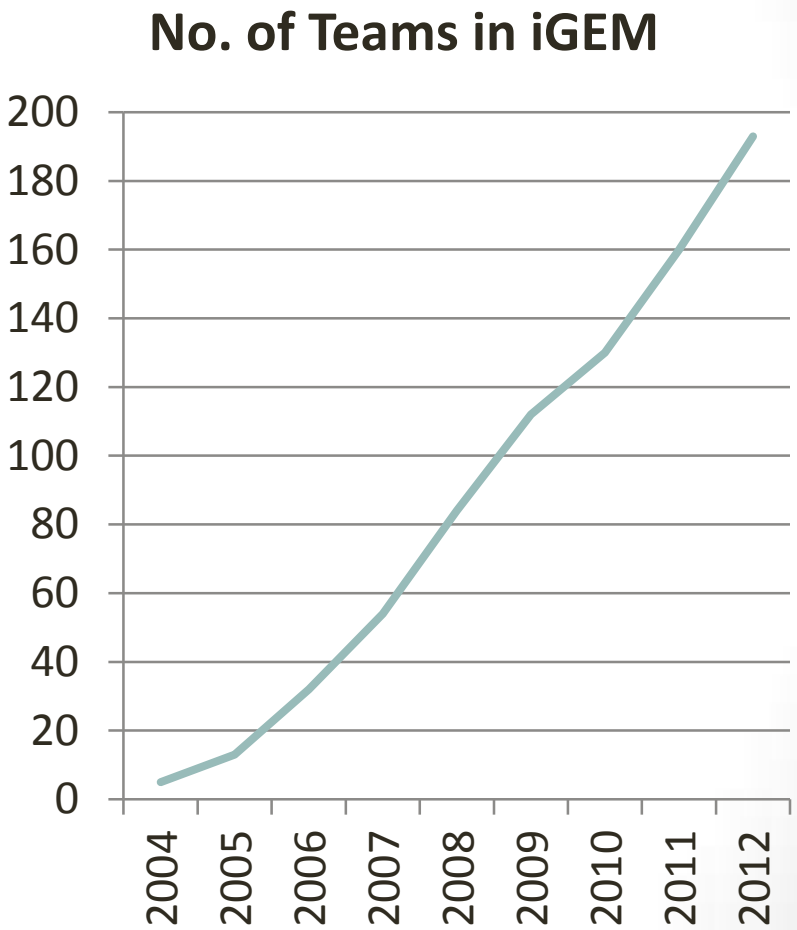
- Uncontrolled release
- Bioterrorism
- Artificial Life

- Study commissioned by the Bioscience for Society Strategy Panel

As an iGEM team, we must prove that we abide by the biological safety standards of our institution. We are also participating in a Human Practices portion for our project.

Synthetic Biology
based on standard parts

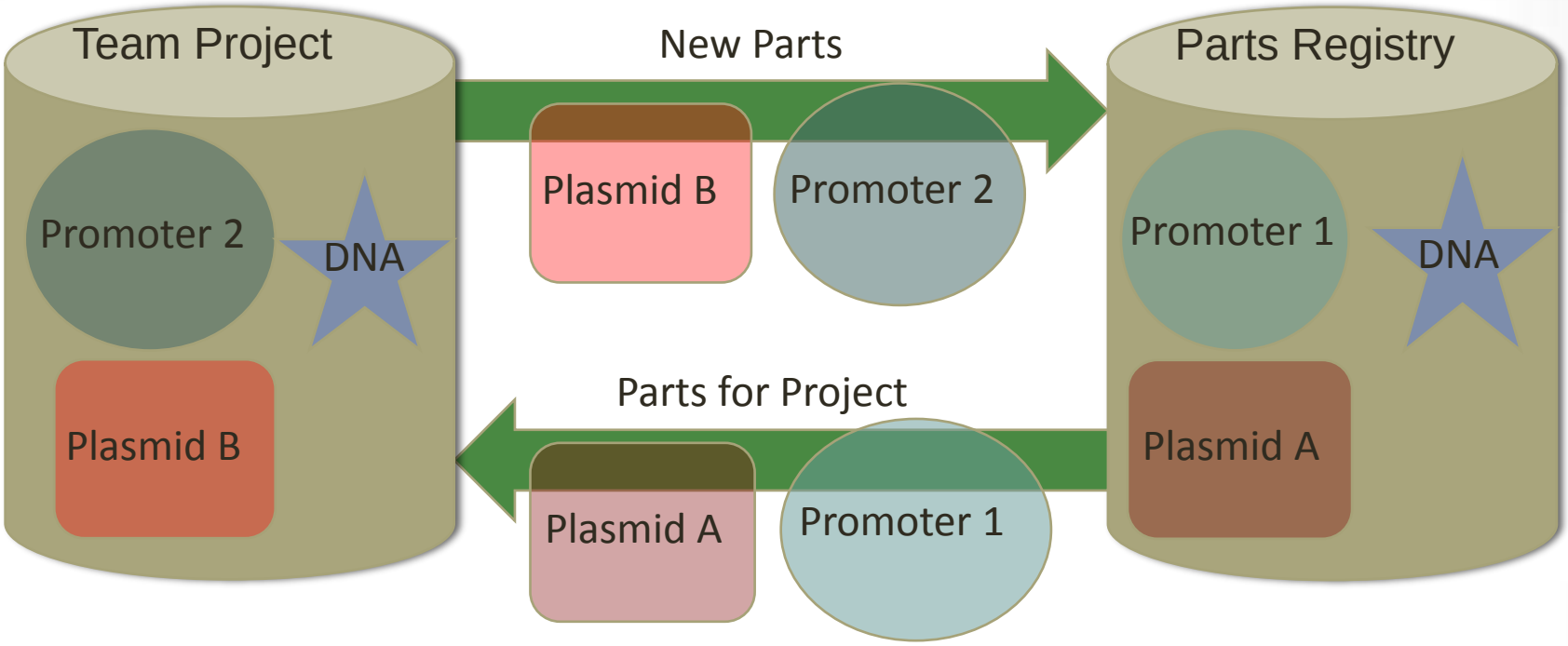
- What is iGEM?
  - International Genetically Engineered Machines
  - Independent, non-profit organization spun out of MIT.
  - Organizes and operates the iGEM Competitions
    - Premier student synthetic biology competition



- International

- Students given a kit of biological parts at the beginning of summer, and create something cool!
  - Collegiate, High School, Entrepreneurial, & Software divisions
- Submit created parts to the Registry of Standard Biological Parts
  - …a growing community collection of biological components.

iGEM Foundation: Overview



- Example Projects:
  - New E. coli strains that smell like bananas and wintergreen!
  - BactoBlood: red blood cell substitute to transport oxygen

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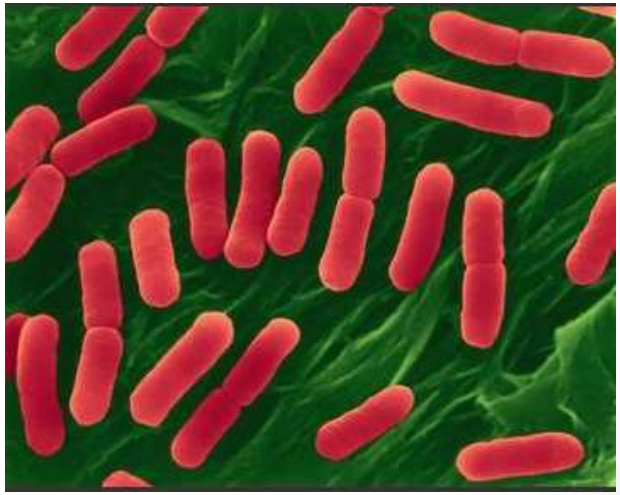
- To study cellular activity, biologists need a way to measure properties about the cellular environment.
  - Analogy: when you go to the doctor, he might:
    - Take your temperature
    - Measure your blood pressure
    - Determine your resting heart rate, etc.

What is going on inside of the cells?



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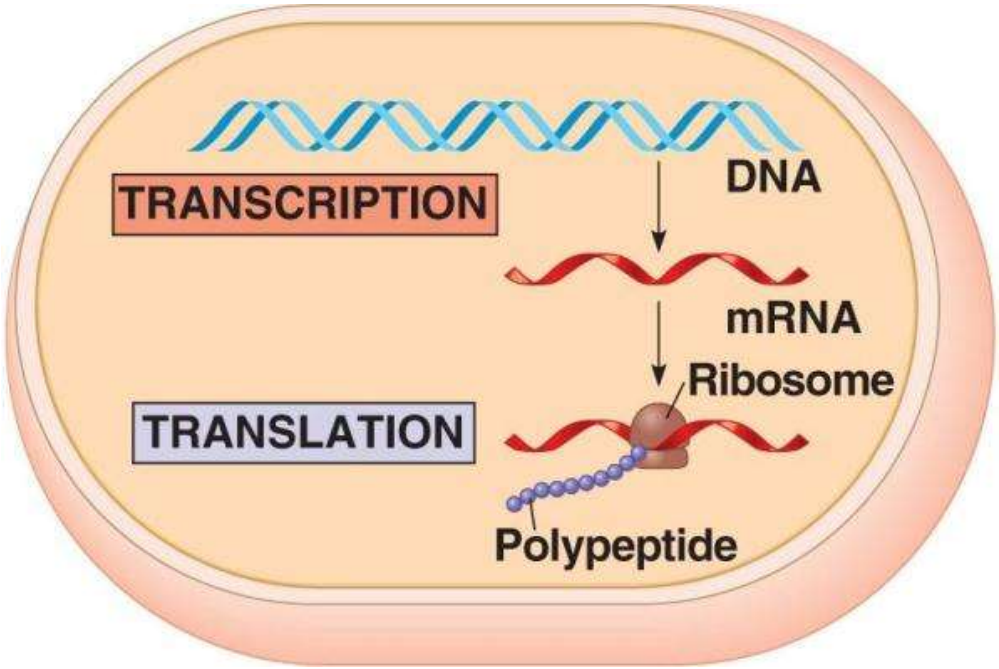

Problems…

- Time consuming
- Very expensive
- Cells do not survive
- Not easily accomplished!

Imagine a scientist, trying to measure transcription and translation…

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- Proposed Solution:
  - We need to find a better way to make the cells tell us about:
    - mRNA production for a specific gene
    - Protein production for a specific gene
  - How can we do that?
    - Well, we have really good microscope equipment, but protein/mRNA are microscopic and hard to see…
    - Can we make them stand out?
      - Yes! Attach **fluorescent** components to the protein and mRNA
      - Take a very high-quality picture with a microscope or get a numerical value from a “plate-reader”.

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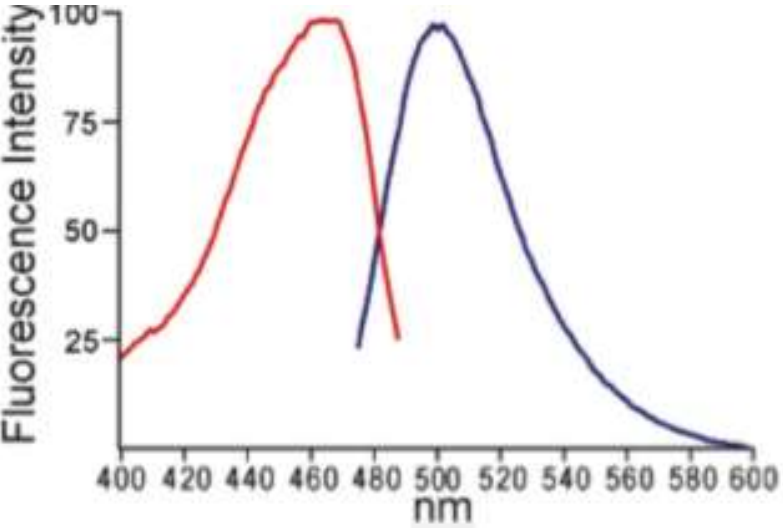
- What is fluorescence?
  - Fluorescence is a property of a molecule.
    - When the molecule is **excited**, it absorbs a photon.
    - The molecule can then *emit* a photon at a lower energy.*
  - **Excitation**: The wavelength of light shown on the dye (ideally at the top of the peak)
  - *Emission*: The wavelength of light that is emitted from the dye. Ideally, the most amount of light is emitted, resulting in a bright color



*Lower energy means longer wavelength

DFHBI Emission Spectra
Source: Lucerna Technologies

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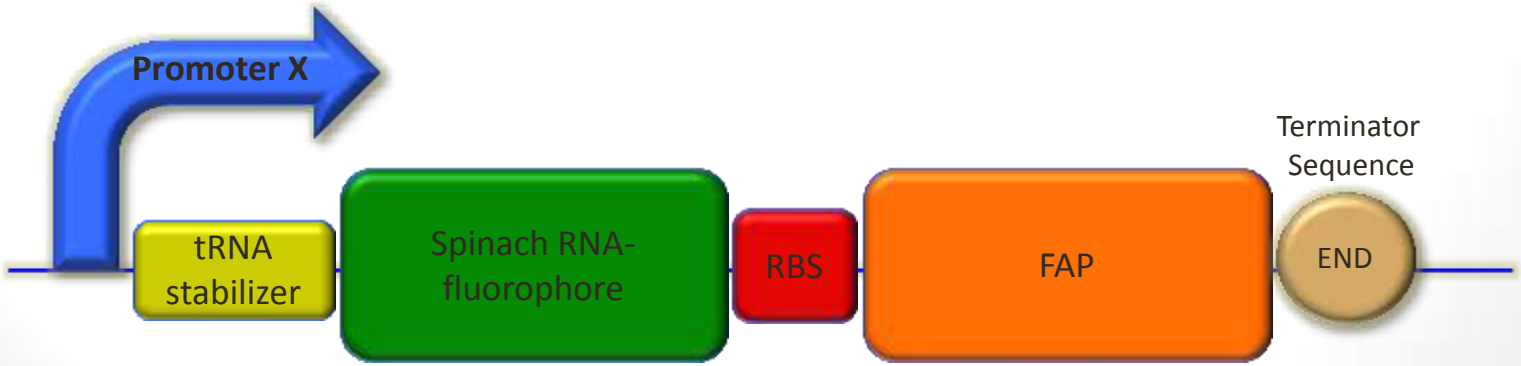
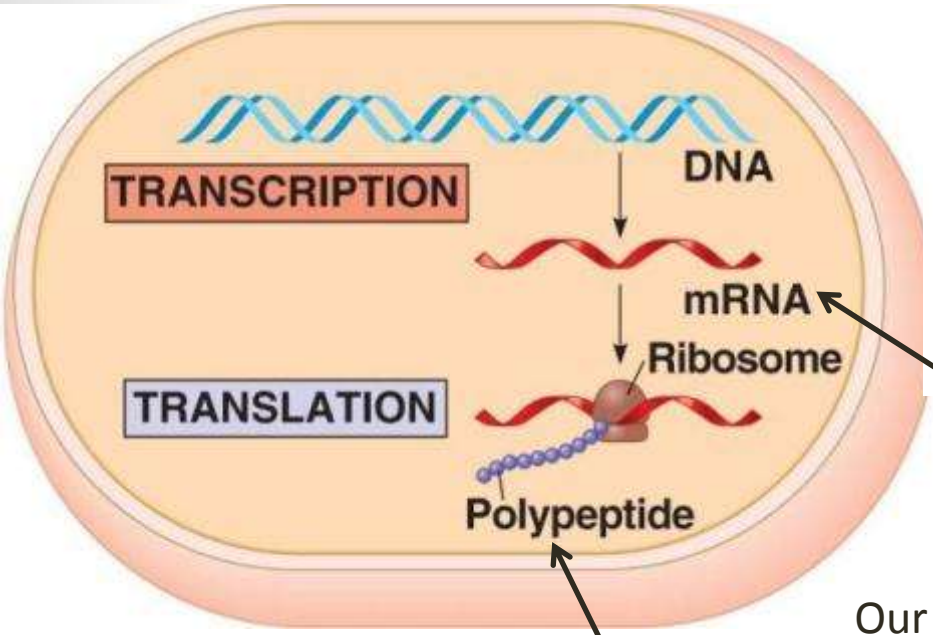
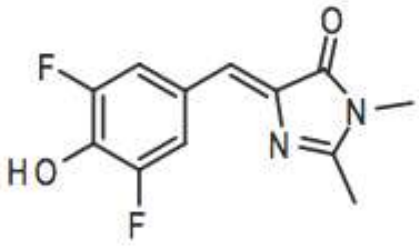
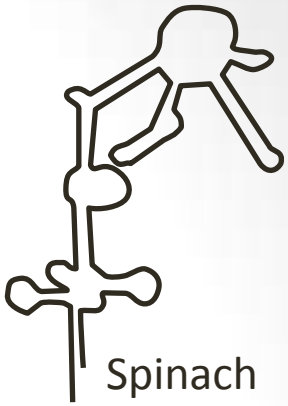
- mRNA fluorescence:
  - Insert a benign genetic sequence that happens to fluoresce when transcribed to mRNA
  - We found one! It's called "Spinach".
  - Insert Spinach between the promoter and the RBS.
- Protein fluorescence:
  - Put a fluorogen activating protein after the RBS so it is translated. => **"FAP"**
  - We found many! Not all of them will behave like we want them to, so **we must choose**.



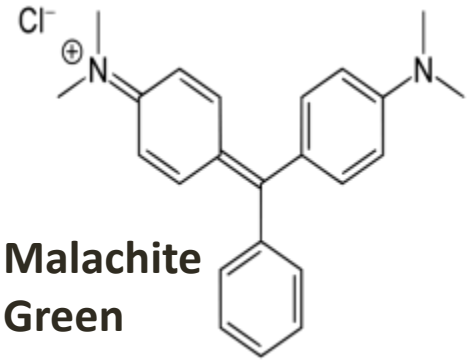




DFHBI



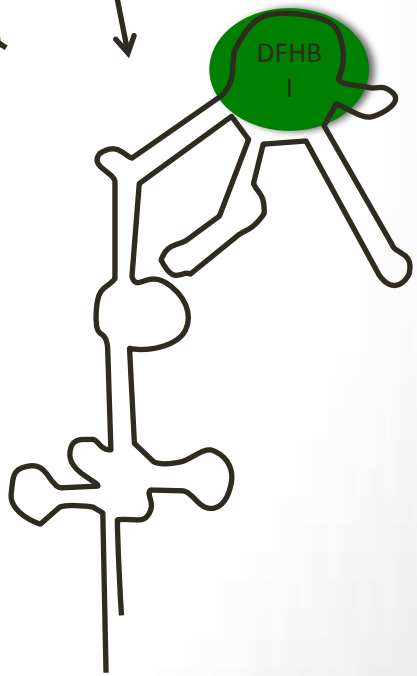
Spinach



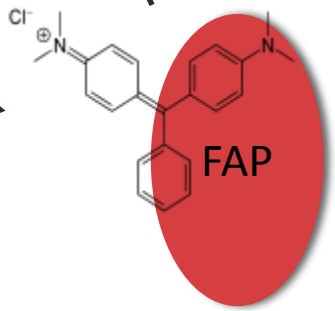
Malachite Green

Our system tags RNA and protein by adding known concentrations of specific dyes.

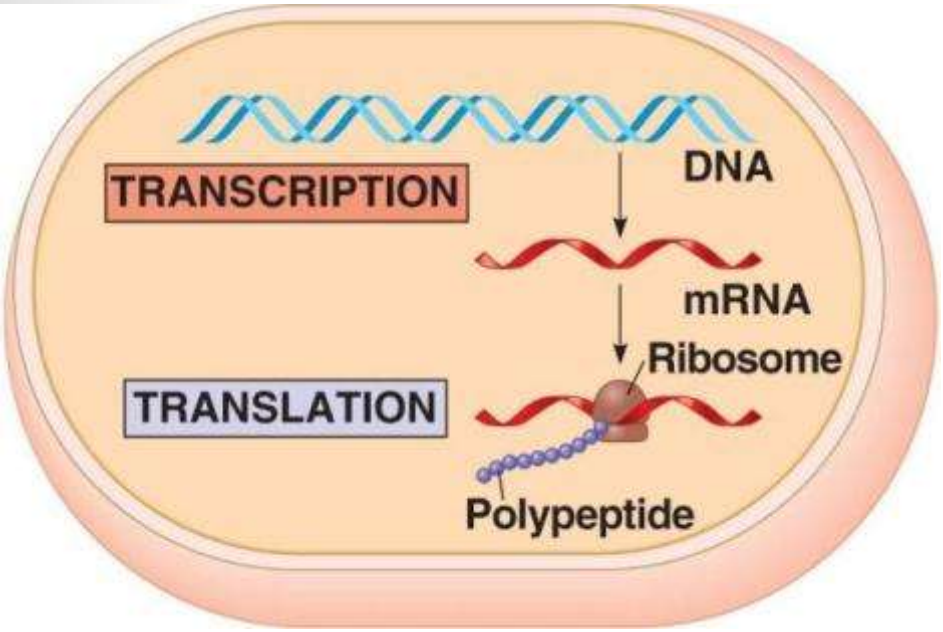
Can determine when
[Protein]=[MG]
[RNA]=[DFHBI]



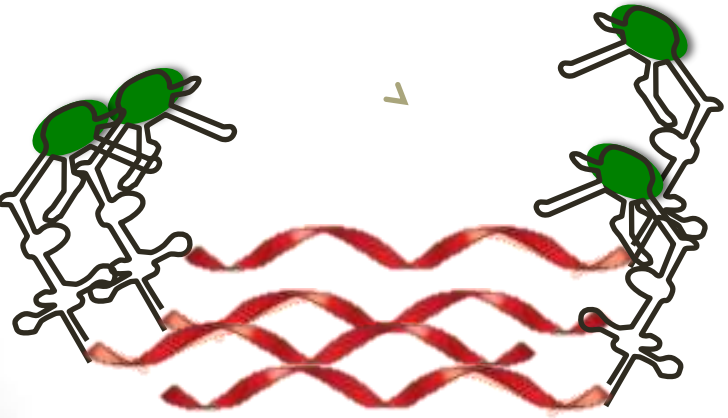
FAP



FAP

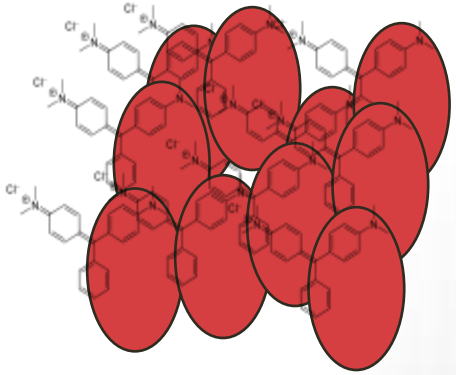


Dyes allow us to conditionally tag protein or RNA. This simplifies problems with experimental setup. This also allows to develop a way to determine concentrations of RNA and protein.



Spinach-tagged mRNAs

Translation



Fluorogen Activating Protein (FAP)

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- So is it really that simple? Just take a picture, and quantify the amount of light/fluorescence?
  - Nope, we needed to develop a mathematical model for taking more complex aspects of the project into consideration.
    - Protein degrades at a measureable rate!
    - mRNA degrades at a measureable rate!
    - Dye Concentrations are essential to make accurate calculations

*Also, laboratory procedures can be *TRICKY*!

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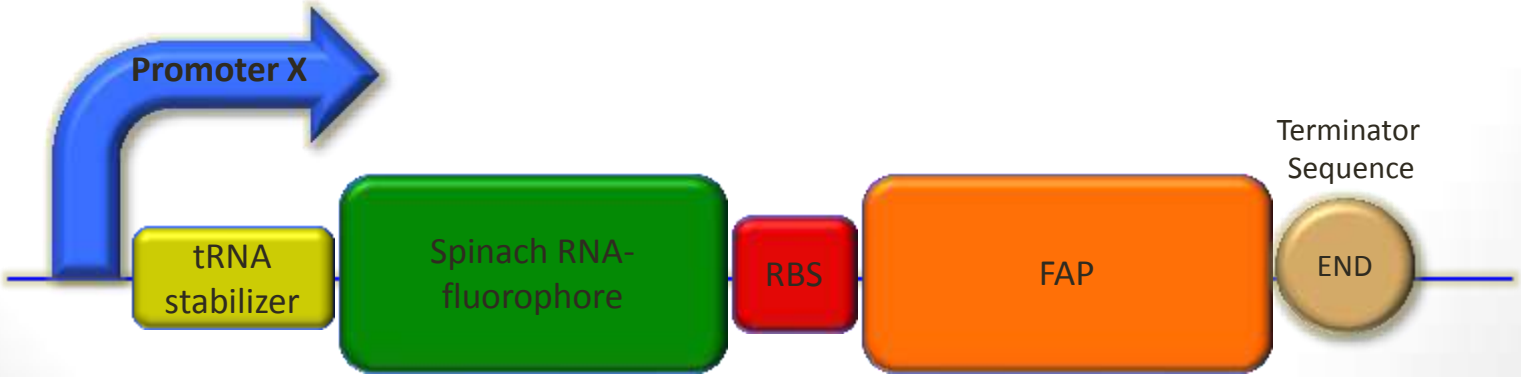
- Project:
  - We are characterizing the *promoters*!
    - Create new T7/Lac promoters (promoter X,Y etc.)
      - T7 promoters are very strong and are widely used
      - The lac operator is a short DNA sequence that binds to a protein that prevents transcription unless IPTG is present.
      - The combination of these two elements creates an “inducible-promoter”.
    - Take fluorescence measurements: mRNA & protein
    - Use our model on data to characterize the new promoters!
      - Transcription rates, translation rates and translation efficiency!



iGEM 2012:
CMU Circuit Demo

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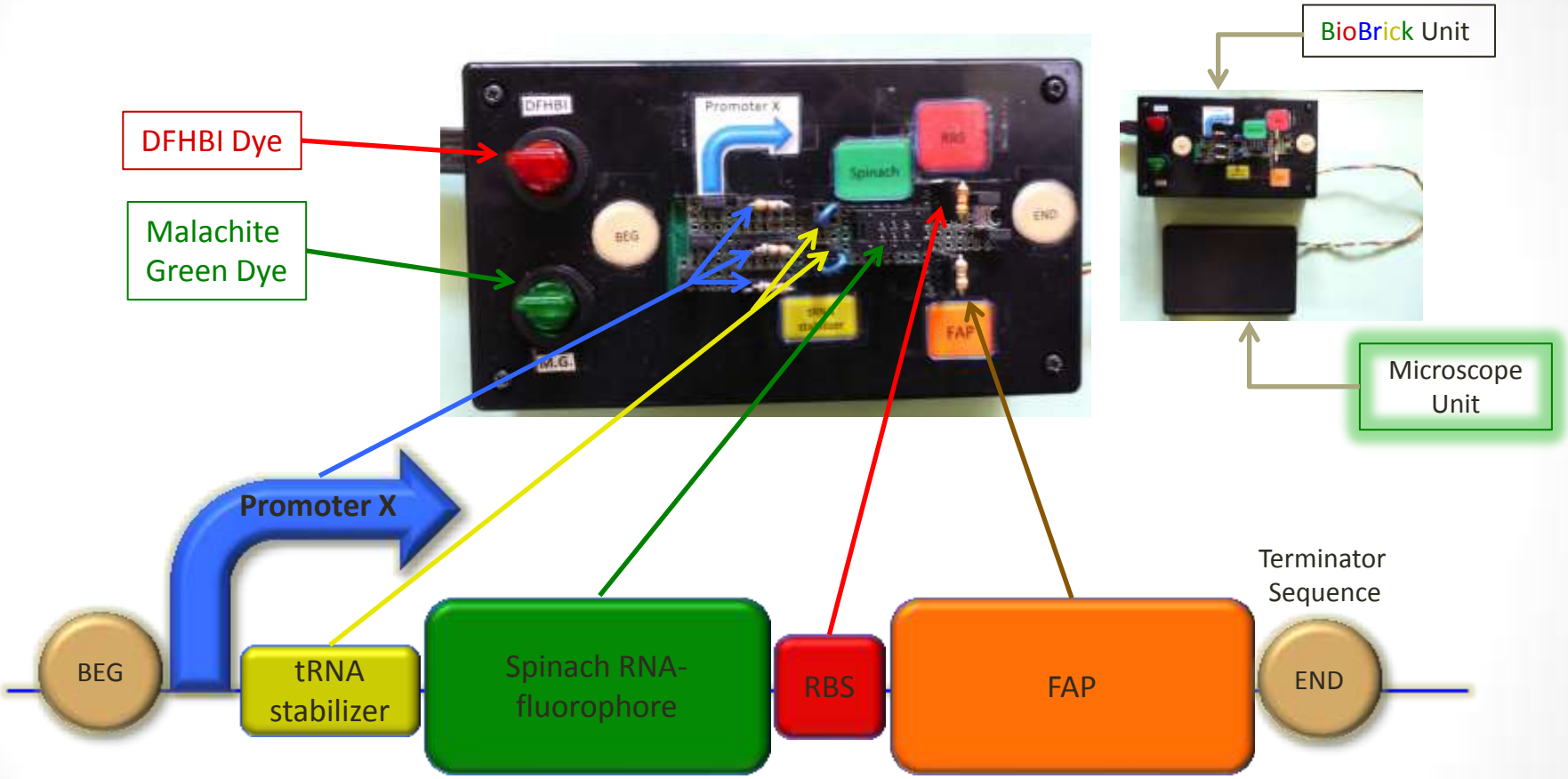

BioBrick for characterizing promoters

Sources

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