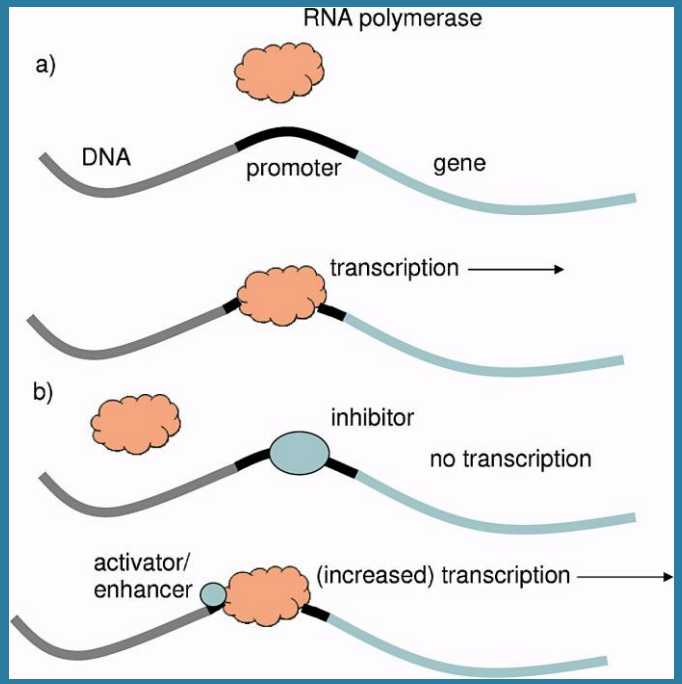
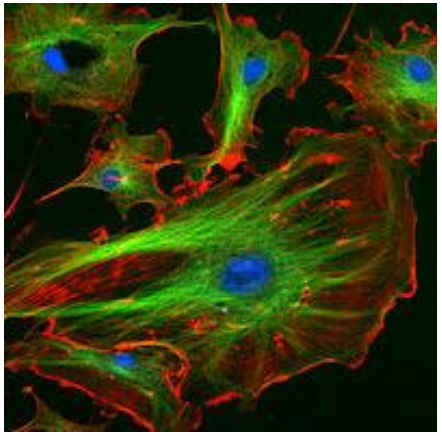




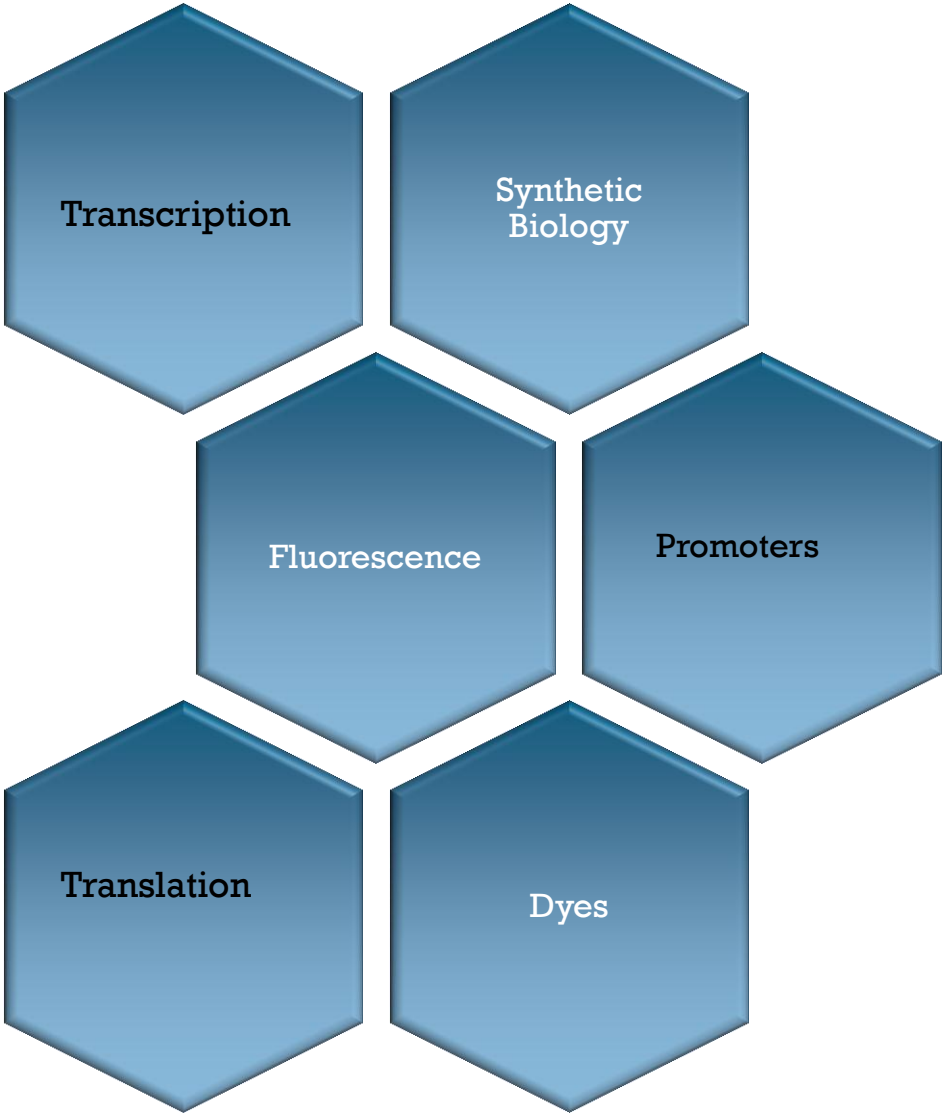
Using Technology to Analyze Promoters

BioBricks Hardware/Software Platform

Outline

- What is the science behind the kit?
- What can students do with the hardware/software?
- How does this kit relate to your curriculum?
- How can you introduce the kit to students?

Key Components



Fundamentals Already Taught

Transcription

- The process of creating a complementary RNA copy of a sequence of DNA

Translation

- When messenger RNA produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide that will later fold into an active protein

Promoters

- Regions of DNA that promote transcription

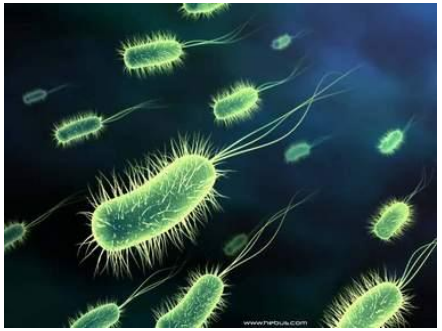
http://en.wikipedia.org/wiki/Translation_(biology)
http://en.wikipedia.org/wiki/Transcription_(genetics)

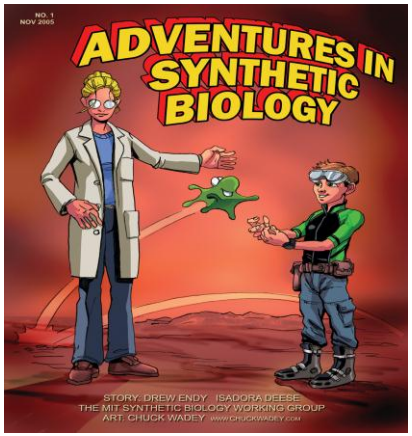
Synthetic Biology is

A) The engineering of biology

B) The design and construction of new biological parts, devices, and systems

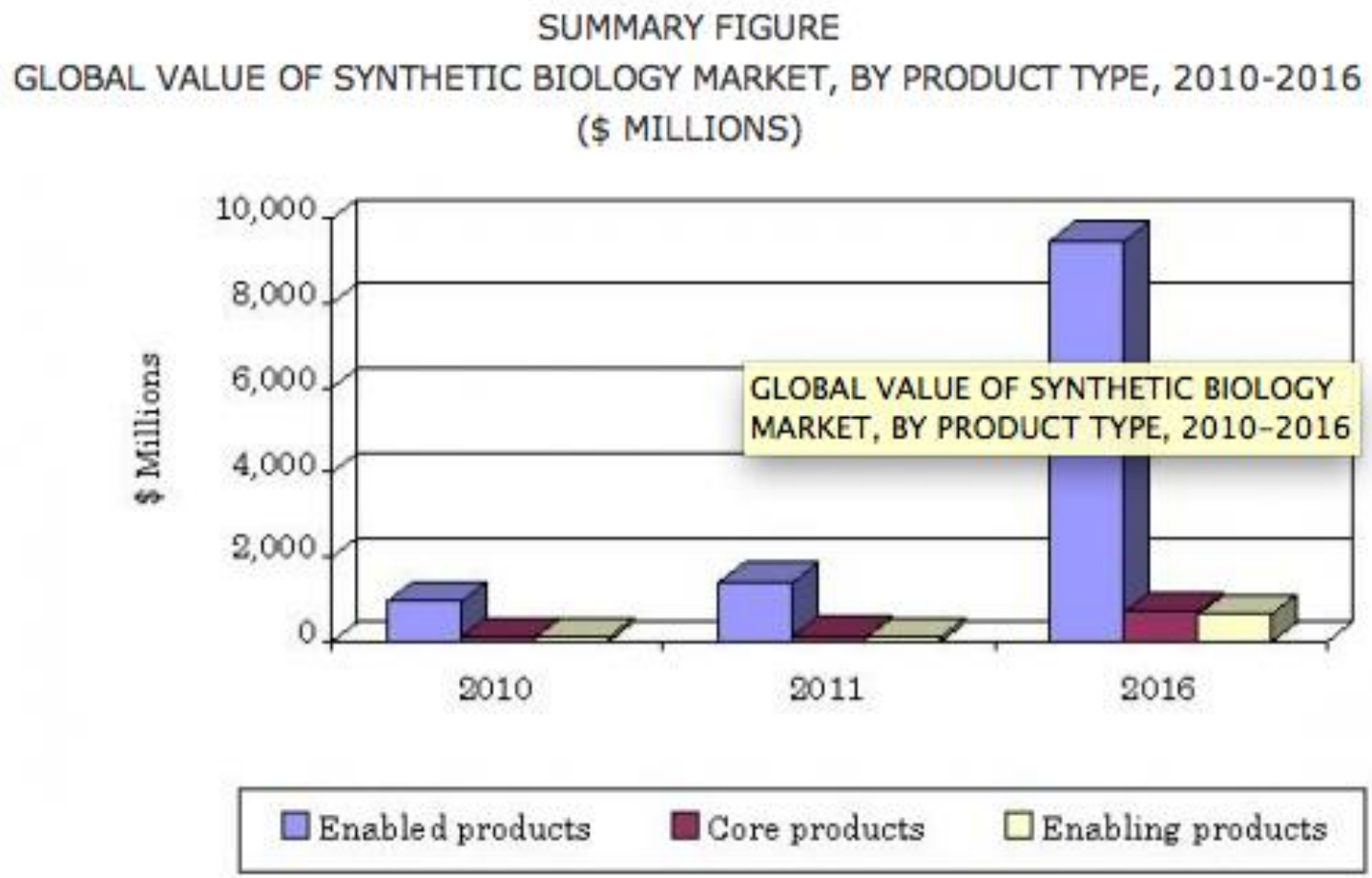
C) The re-design of existing, natural biological systems for useful purposes.





http://syntheticbiology.org

Why is Synthetic Biology Important?

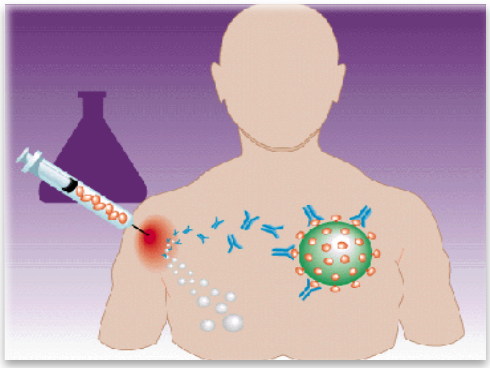


Source: BCC Research

Applications of Synthetic Biology

Engineered Bacteria

Can detect acid and chance color based on the acidity



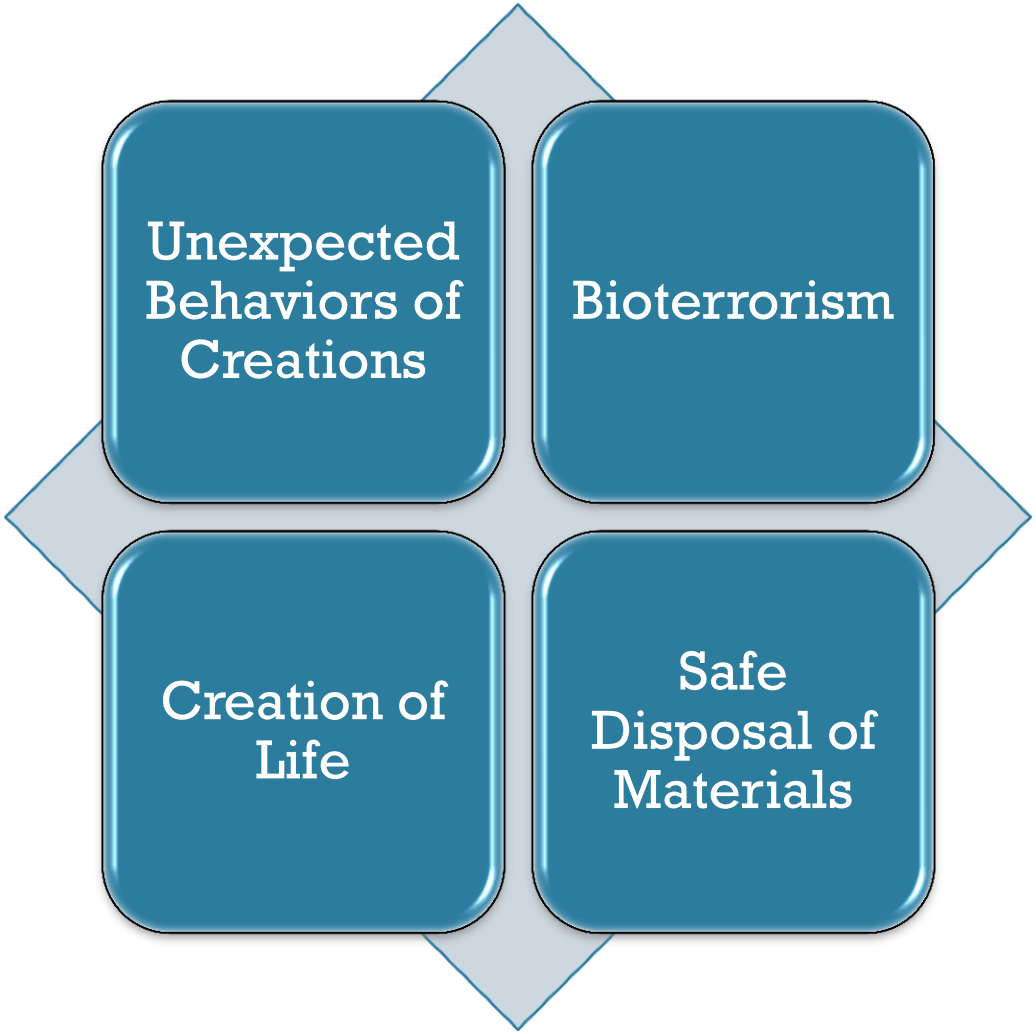
Inexpensive Vaccines

Can make cells produce cells more efficiently

Engineered Bacteria

Can turn wastewater into clean water

Ethical Concerns



Synthetic Biology

Often requires information on transcription and translation rates

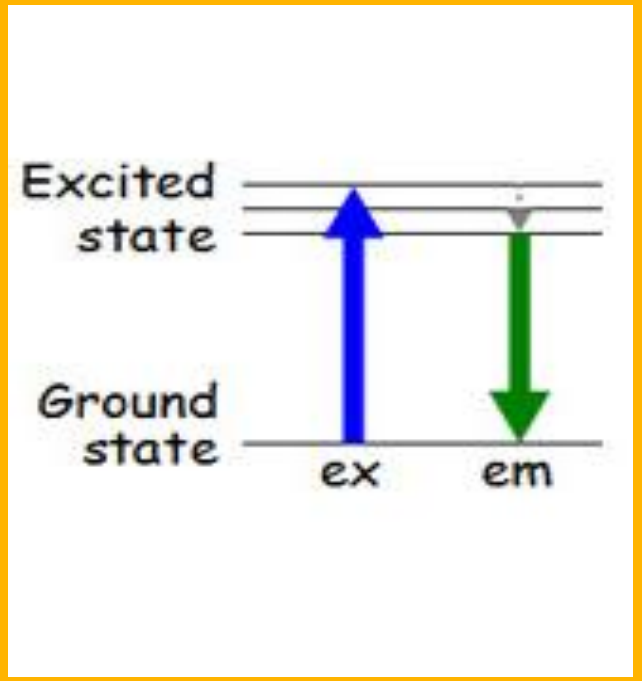
To date, quantitative information about the expression strength of a promoter is difficult to obtain

Fluorescence is

A) A property of some molecules that absorb photons at a certain wavelength and emit them at a longer, lower energy wavelength

B) The emission of light by a substance that has absorbed light or other electromagnetic radiation

Example: fluorescent molecules can take the form of organic dyes or proteins



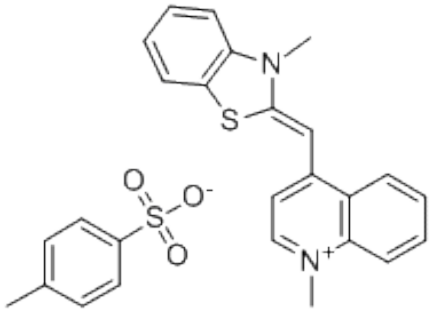
http://en.wikipedia.org/wiki/Fluorescence

Dyes are

A) Tools that can help mark the presence of a molecule or group of molecules

B) Sometimes able to penetrate cell membranes

Examples: malachite green and thiazole orange



Thiazole orange

About the Kit:
A Fluorescence-Based Sensor

- Enables researchers to characterize new promoters
- Enables researchers to look at mRNA and protein production
- Uses fluorescence to view protein and mRNA production
- Provides information about mRNA and protein degradation

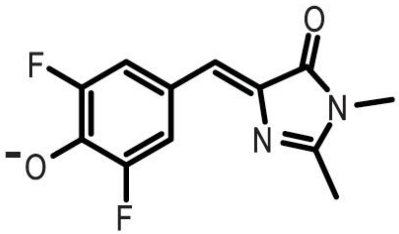
Technological Advantages of the Fluorescence-based Sensor

- Noninvasive
- Can be easily applied to a variety of promoters
- Provides results in a short time frame

Components of Biosensor

Spinach

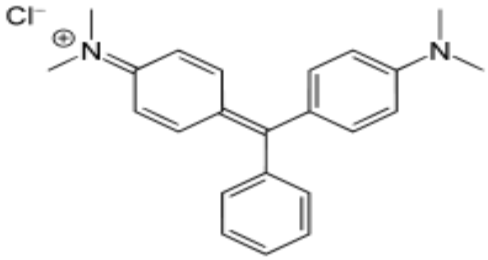
- A fluorescent RNA sequence
- Fluoresces green when DFHBI (an organic dye) is bound to it that can be used to quantify RNA concentration in a cell



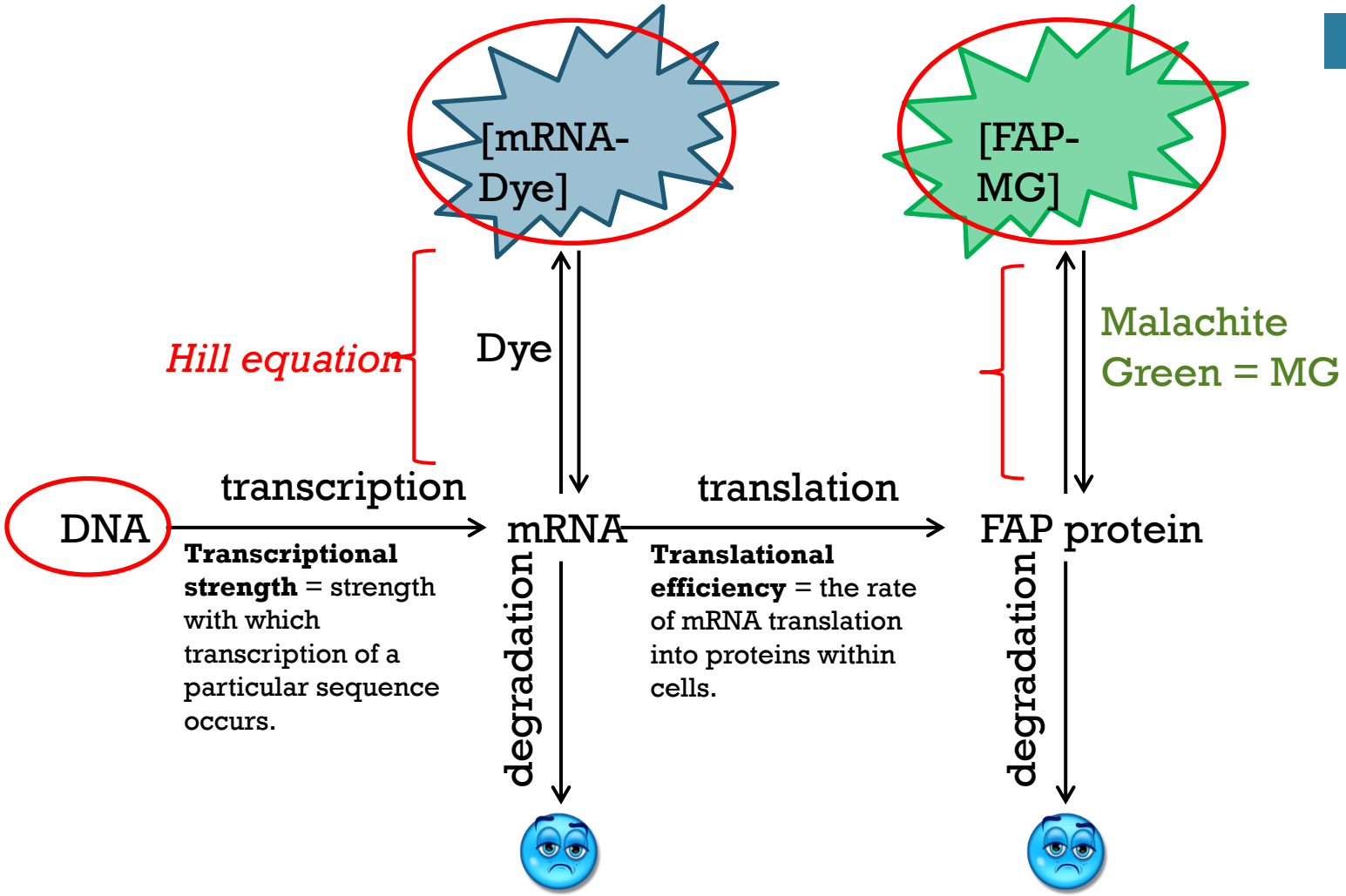
DFHBI

FAP

- A fluorogen activating protein
- Used to tag proteins
- Becomes fluorescent when bound to malachite green dye



Malachite green



Measurables identified by red circles.

This Method of Analyzing Promoters Can Quantify

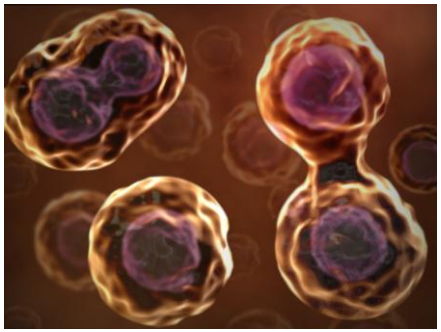
- Translational efficiency *in vivo*
- Transcription rates *in vivo*
- Promoter strength
- *In vivo* mRNA and protein half-lives in real time

Why is This Sensor Important?

> The ability to monitor protein production with fluorescence is a growing field that promises advances in drug development and improving quality control in drug manufacturing

> Promoter strength directly affects a cell's ability to perform typical functions like divide or move

> Inducible promoters are widely used in synthetic biology but many are under-characterized



Hardware/Software

- Allows the students to directly manipulate electronic components, which are formal equivalents of parts of the sensor, to affect the current and/or voltage, which are formally the equivalent of the polymerase per second and translational efficiency measured with the sensor
- Affordable, microcontroller-based, hardware platform and associated, open-source, digital simulation software
- Simulated microscope using LEDs and a photoresistor
- The software ensures that the data generated by the students is physiologically accurate

What Can Students Do With the Software?

- Interact with the components of the model and look at a table and graphical output of fluorescence of mRNA and protein over time
- Compare different promoters and see which ones are the strongest
- See how all parts of the model affect the mRNA and protein production

Students Will Learn About:

- Biological systems and synthetic biology
- Teamwork in research
- The interdisciplinary nature of synthetic biology
- Gene expression and the central dogma of molecular biology
- How the software measures properties of promoters
- How synthetic biologists tackle real-world problems

The Software and Your Curriculum

Pennsylvania Academic Standards for Science and Technology and Engineering Education

- **3.2.12.D**: Analyze and use the technological design process to solve problems.
- 3.2.10.B: Apply process knowledge and organize scientific and technological phenomena in varied ways.
- Try to incorporate this lesson/s after:
  - **3.2.10.D**: Identify and apply the technological design process to solve problems.

Pennsylvania Assessment Anchors

- **S11.A.2.2.2** Explain how technology is used to extend human abilities and precision.
- **S11.A.3.2.2:** Describe advantages and disadvantages of using models to simulate processes and outcomes.
- Try to incorporate this lesson/s after:
  - **S11.B.2.2.1** Describe how genetic information is expressed (i.e., DNA, genes, chromosomes, transcription, translation, and replication).

Students Will Learn About

- transcription
- translation
- promoters
- RNA
- proteins

The Software and Your Curriculum

- Describe how STUDENTS came together to design this solution
- Explain how science and technology can be intertwined to solve problems
- Explain how the technology goes beyond what human hands alone can do

Implementing the Software in Class

1. Teach background information using the slides in this presentation, or your own materials
2. Break students into groups of 3-4
3. Assign each group a kit
4. Follow activity instructions