A little about the lab certification...

\[ \text{Avg} = 83.9 \]

\[ \text{No DNA} = \text{No AB} \]

\[ \text{Unknown DNA} = \text{Other Cells} \]
M2D1: Testing an engineered biological system

10/11/12 13:14:15 pm
Butterstick
Mod2: System Engineering
Bacterial Photography

Butterstick
Mod2: System Engineering
Bacterial Photography

Butterstick

“What manner of sorcery is this?”

“wow! there is just so much awesome all over this!”
What is the manner of sorcery involved?

An engineered biological light sensing device.

Input: Light

Black Box

Output: Color change

\[ \text{LacZ} \]
A little deeper into our black box:

**Light Sensor**

- Cph1 = bacterial photoreceptor
- EnvZ = cytoplasmic responder (gene regulation!)

**Color Generator**

- PCB = photoresponsive protein from *Synechocystis*

Two Components

PCB → pPCB

PCB = photoresponsive protein from *Synechocystis*
A little deeper into our black box:

<table>
<thead>
<tr>
<th>Sensor</th>
<th>“Wild Type”</th>
<th>20.109</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK</td>
<td>EnvZ</td>
<td>Cph8</td>
</tr>
<tr>
<td>Responder</td>
<td>OmpR</td>
<td>OmpR</td>
</tr>
<tr>
<td></td>
<td>Osmolarity</td>
<td>Light</td>
</tr>
</tbody>
</table>
How do we measure the efficiency of our engineered system?

LacZ catalyzed formation of precipitate
How do we measure the efficiency of our engineered system?

Limitations of our equipment and our assay:

ONPG

\[
\text{β-galactosidase} \quad \begin{array}{c}
\text{H}_2\text{O} \\
\rightarrow \\
\text{Yellow Color}
\end{array}
\]

Abs = 420 nM

Yellow Product

Galactose

Yellow Color

Active β-Galactosidase

Thursday, October 11, 2012
How do we measure the efficiency of our engineered system?

β-Gal Assay:

1. Measure [cell]
2. Lyse cells
3. Start Reaction
4. Stop Reaction
5. Get rid of debris
6. Measure yellow product
7. Calculate activity

1 Miller Unit = 1000 * \[
\frac{(\text{Abs}420 - (1.75 \times \text{Abs}550))}{(t \times v \times \text{Abs}600)}
\]
Plan for today & Mod2 Assignment #1

1. Small liquid cultures of NB466 - quantification
2. Plates - visual
3. β-gal - NB5

Pick your paper!