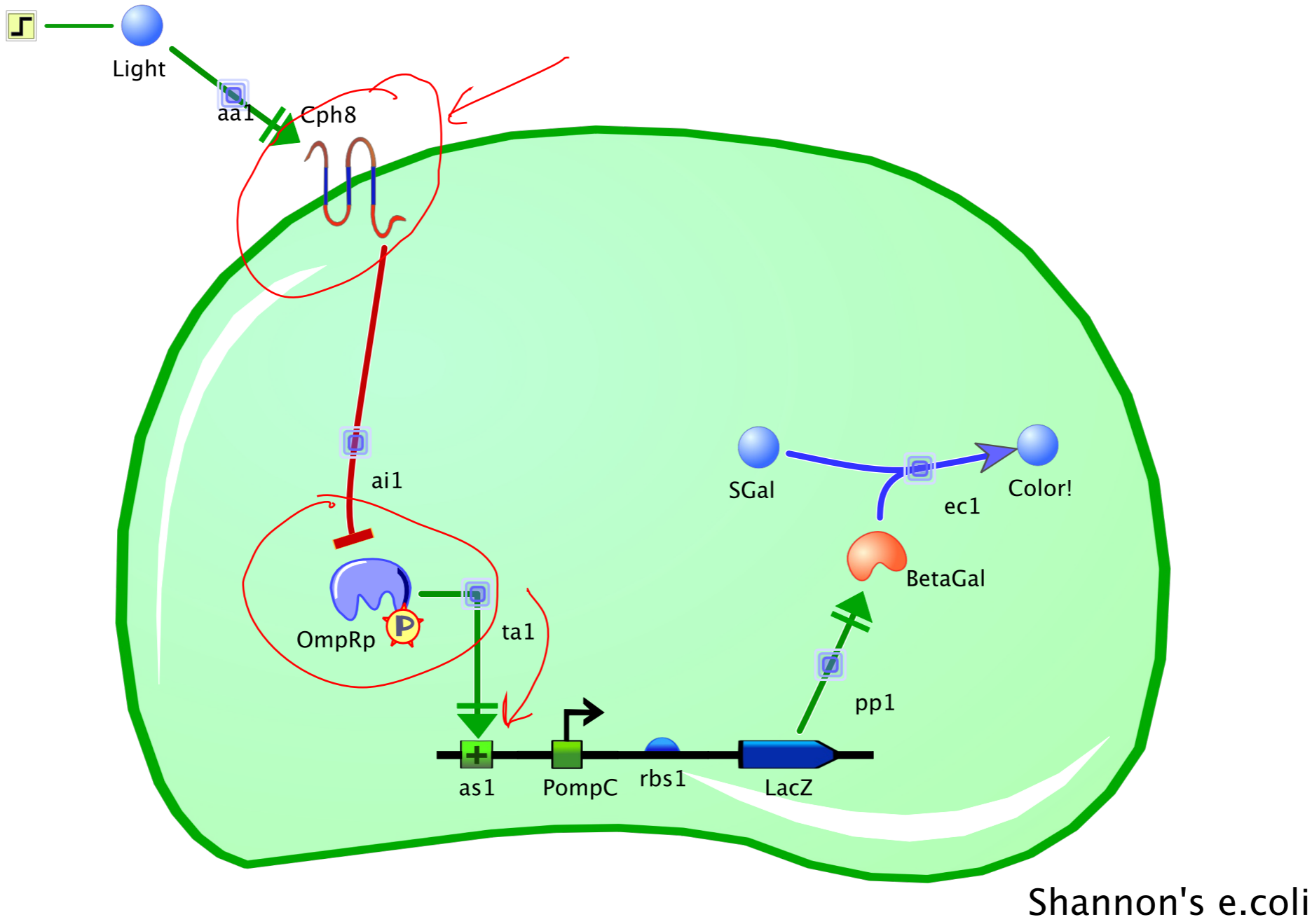


M2D3: Tools for System Engineering

10/18/12

Review of M2D2: Measuring System Performance

Inspect your photograph and document!



Part I: Library Screen -- Improving our photography system

| | | | | | | |
|-----------------------|---------------------------|-------------|-------------|-------------|----------------------------------|-------------|
| <u>EnvZ</u> wt seq | H243 CAC | D244 GAC | L245 TTG | R246 CGC | T247R (K+P-) Thr = ACG | P248 CCG |
| <u>Cph8</u> | H537 | D538 | L539 | R540 | T541 | P542 |
| to test: | Kinase Dead mutant | | | | NNY mutagenesis | |

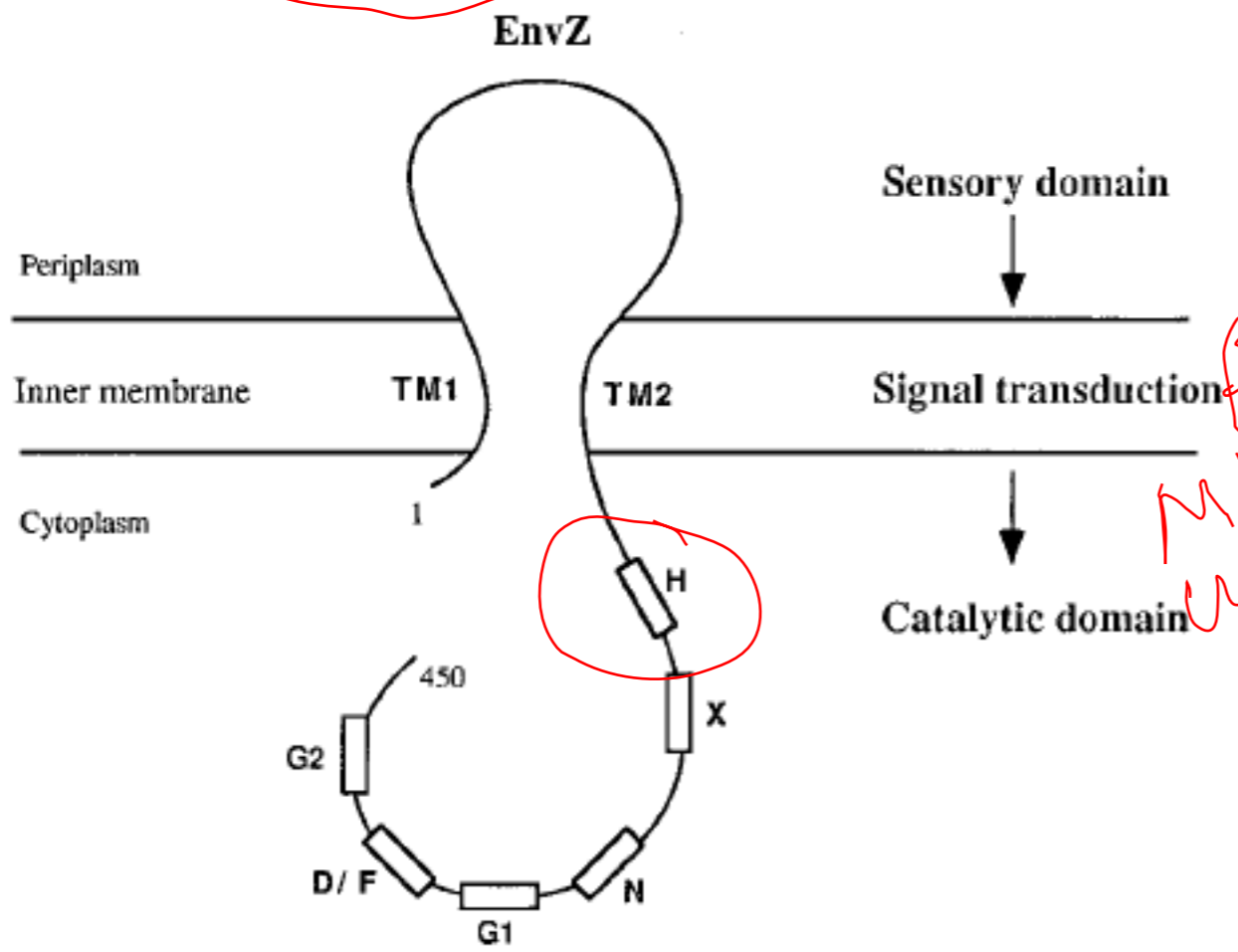


FIG. 1. Schematic presentation of the structure of EnvZ. Conserved regions in the catalytic cytoplasmic domain are indicated with lettered boxes.

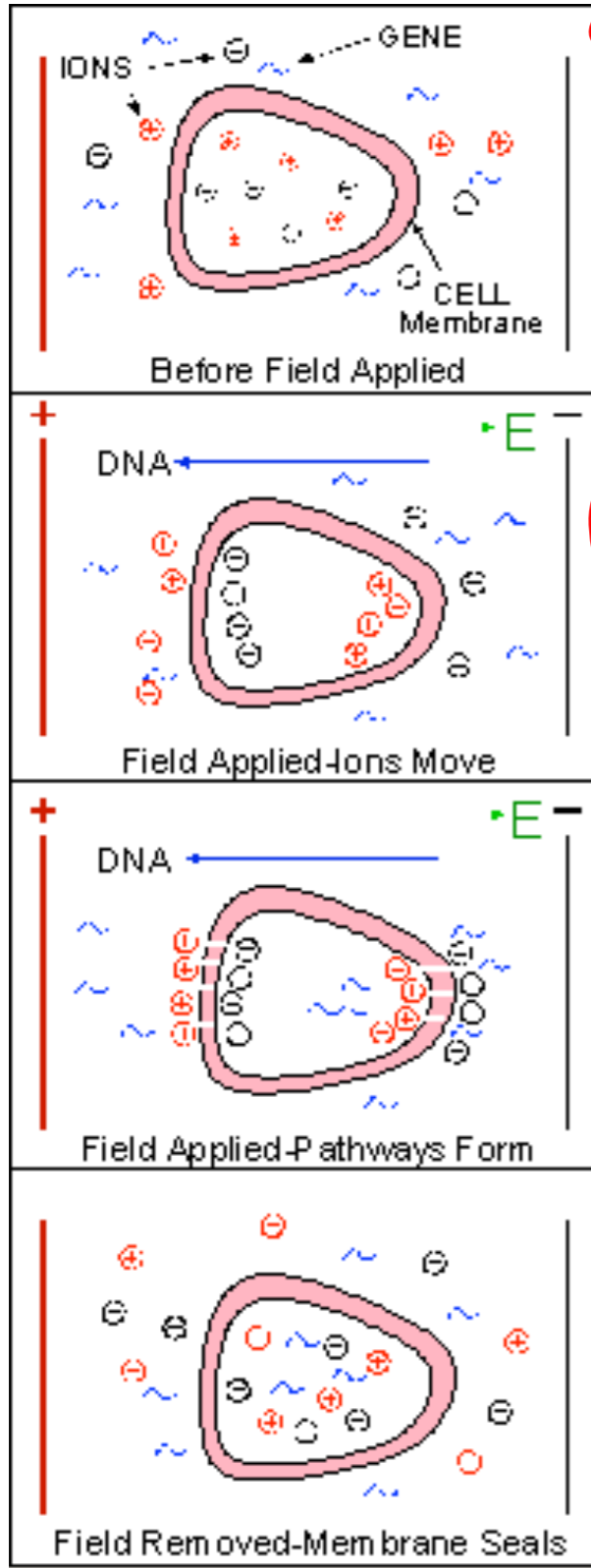
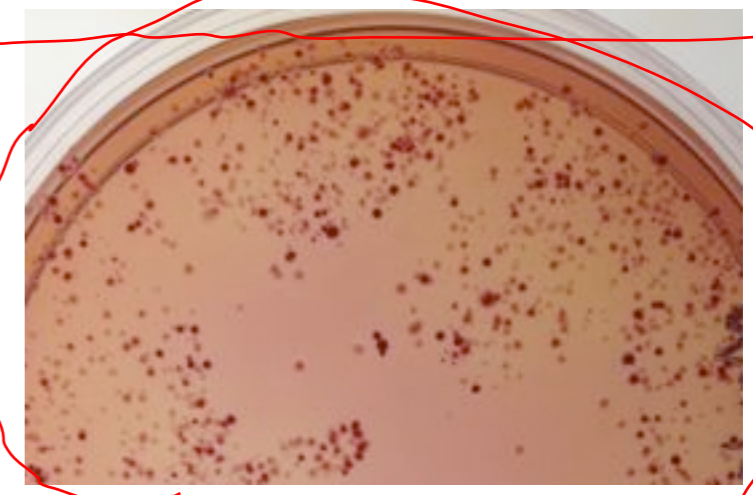
Weihong Hsing, Frank D. Russo, Karen K. Bernd and Thomas J. Silhavy
J. Bacteriol. 1998, 180(17):4538.

Handwritten notes and diagrams:

- Red circles around the **EnvZ** and **H243** entries in the table.
- Red arrows pointing from the **T247R** and **T541** entries to the right.
- Red vertical lines representing protein variants: **WT**, **H243/537**, and **T541X**.
- Red boxes labeled **L**, **D**, and **L** above the variant lines.
- Red arrow pointing up from **T541X** labeled **control**.
- Handwritten text: **β-gal** and **Miller units**.

Part I: Library Screen -- Electroporation

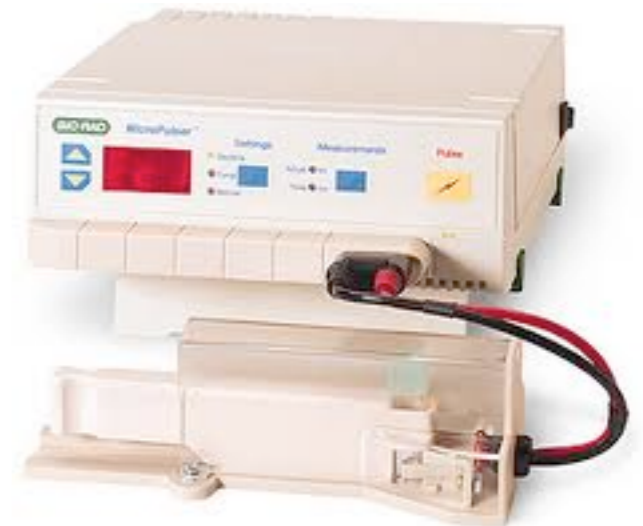
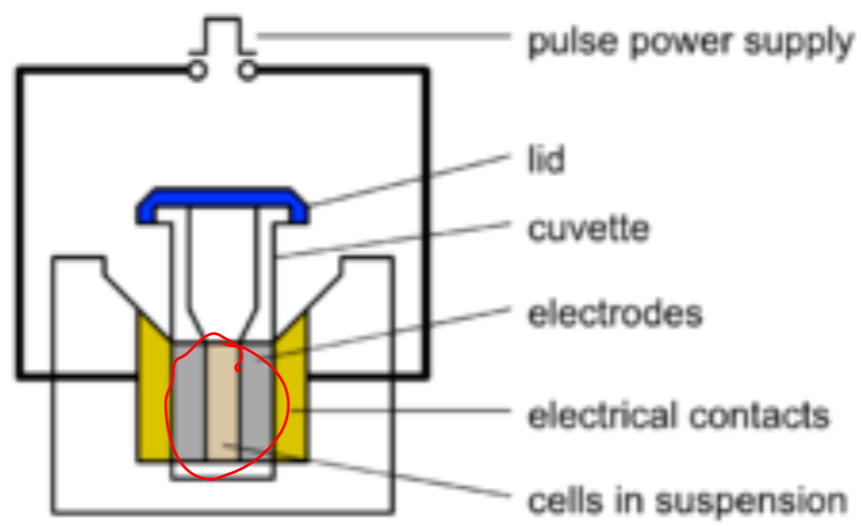
MacConkey Media - Agar plates



① Cells don't like
⇓

Read the Protocol

② Safety glasses







<http://www.onemedplace.com/blog/archives/854>

Part 2: Registry of Standard Biological Parts -- Building a protein generating system in e.coli





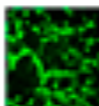
Web address: <http://partsregistry.org>

Browse parts by type

| Catalog | List |
|---|---|
|  | Promoters (?) : A promoter is a [the downstream DNA sequence. |
|  | Ribosome Binding Sites (?) : A can bind and initiate translation. |
|  | Protein domains (?) : Protein do protein coding sequence. Some protein for cleavage, or enable it |
|  | Protein coding sequences (?) : that some protein coding sequen from start codon to stop codon. C here. |

Browse parts and devices by chassis

Unless otherwise specified, most parts in the Registry work in E

| Catalog | List |
|---|---|
|  | Escherichia coli (?) : Most parts in the Reg |
|  | Yeast (?) : Yeast are simple eukaryotes. |
|  | Bacteriophage T7 (?) : Bacteriophage T7 is |
|  | Bacillus subtilis (?) : Bacillus subtilis is a n |
|  | MammoBlocks (?) : MammoBlocks are a n dozens of MammoBlocks suitable for rapid |

Browse parts and devices by standard

Unless otherwise specified, most parts in the Registry co

Assembly standard 10 (?): Assembly standard 10, or th comply with this assembly standard.

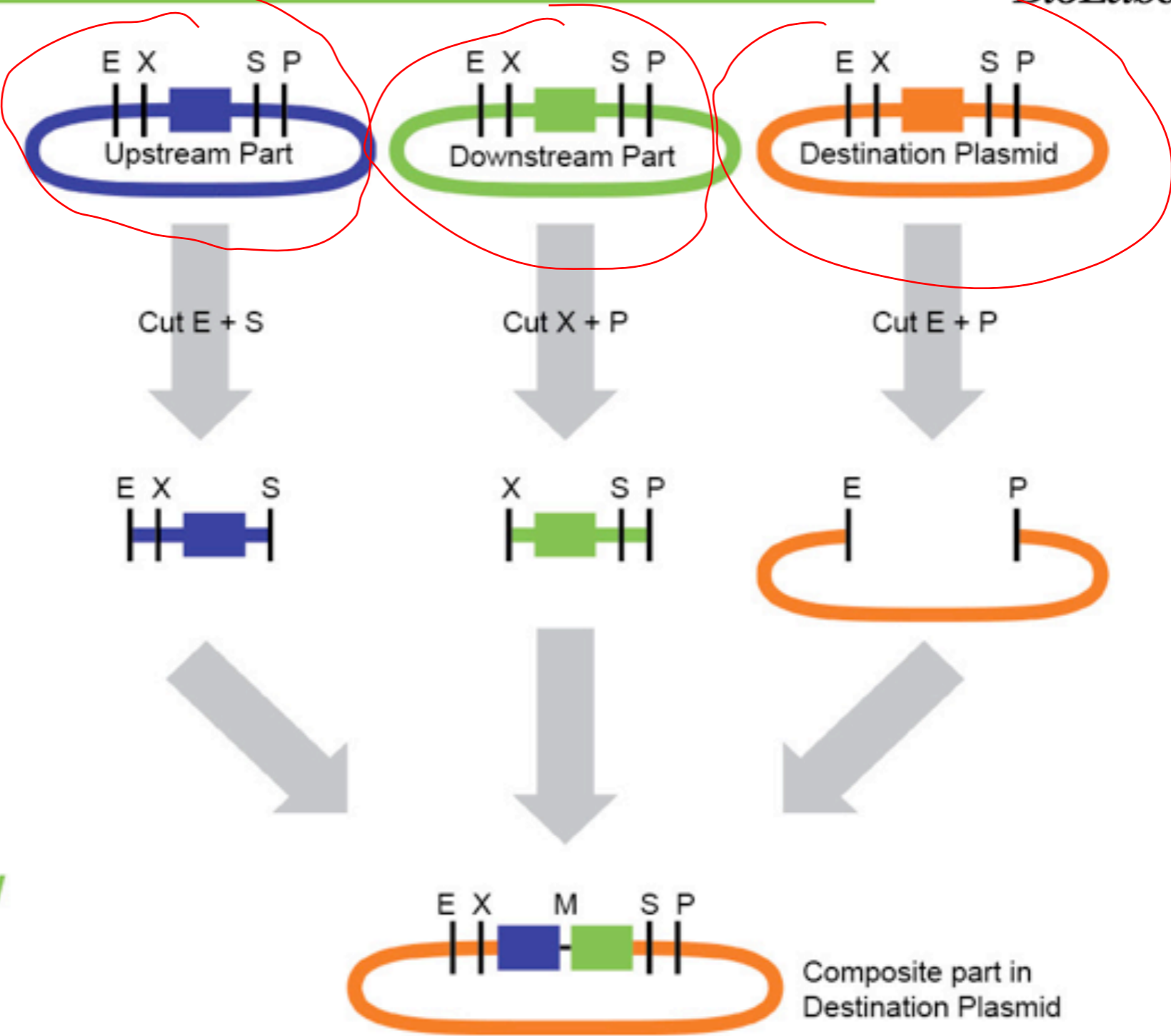
Assembly standard 23 (?): Assembly standard 23, or th protein domains.

Assembly standard 25 (?): Assembly standard 25, or th of protein domains.

3 Parts --> Protein Production

Part 2: Registry of Standard Biological Parts -- Building a protein generating system in e.coli

BioBrick™ Assembly Manual

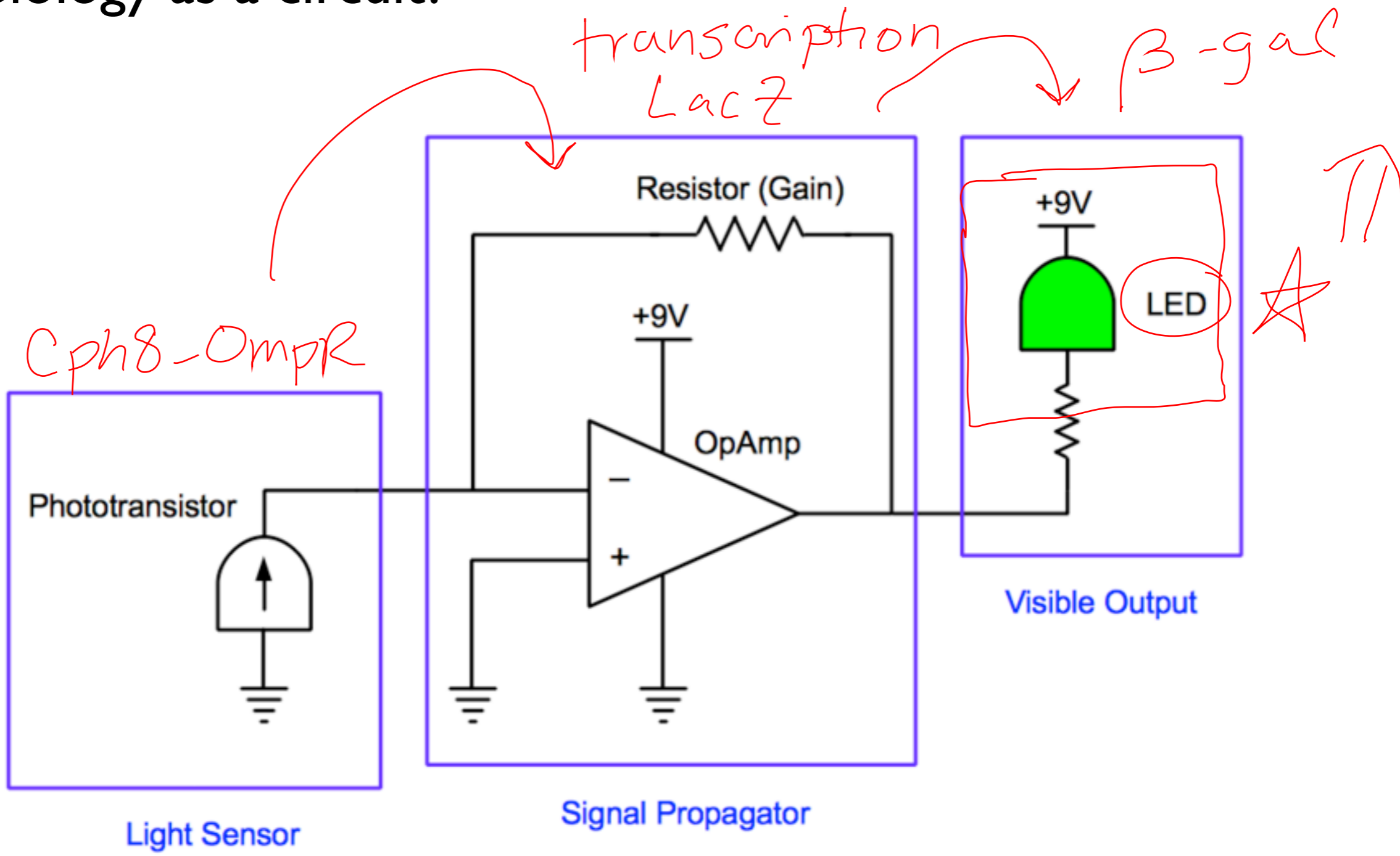


E=EcoRI-HF™
X=XbaI
S=SpeI
P=PstI
M=Mixed site

BioBrick
assembly
overview

Composite part in
Destination Plasmid

Part 3: Biology as a circuit?



Circuit designed by Steve Wasserman

Plans for today:

1. Lab



& Hand-in Lab Notebooks

2. Document your picture of Butterstick!

3. Electroporation of mutant library

4. Build a protein production system from standard parts

5. Does Electrical Engineering = Biology?

6. Finish up your TinkerCell simulations (Part 4)

Data!

| Group Color | Miller Units in Dark | Miller Units in Light |
|-------------|----------------------|-----------------------|
| Green | 1566.9 | 437.3 |
| PINK! | 1159.7 | 289.5 |
| Red | 1115.3 | 416.7 |
| Orange | 1003.8 | 312.1 |
| Blue | 1178.4 | 341.7 |
| yellow | 1137.2 | 467.8 |
| purple | 1150.33 | 601.81 |