**Bio111 Week 3**

Before you come to lab

1) Read this evolution story: <http://www.sciencedaily.com/releases/2010/04/100411143401.htm>

2) Listen to this 6 minute information story about synthetic biology: <http://www.npr.org/templates/story/story.php?storyId=90014997>

3) Answer each of these four questions in two sentences or less.

A) Do all antibiotics work the same way? (evolution experiment)

B) What is a minimal inhibitory concentration of an antibiotic? (evolution experiment)

C) What is ligase? (information experiment)

D) What is transformation with regards to DNA engineering? (information experiment)

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**NOTE:** At 4 pm on the Wednesday before your lab, one person from each lab group MUST COME TO Dr. C’s research lab (Dana room 220). Please be on time. We need to prepare the oligos you had synthesized to build your designer promoter. See page 3 for details.

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**Evolution: Antibiotic Resistance (13 week project)**

In Lab (do this AFTER you the ligation from the information experiment – step 4 on page 3)

1) Search the internet to find the structure of each antibiotic. Use PubChem (<http://pubchem.ncbi.nlm.nih.gov/>). What effect does each antibiotic have on sensitive cells?

|  |  |  |
| --- | --- | --- |
| **antibiotic** | **structure** | **Mechanism** |
| ampicillin |  |  |
| tetracycline |  |  |
| chloramphenicol |  |  |
| erythromycin |  |  |
| streptomycin |  |  |

2) Design experiment to determine minimum inhibitory concentration (MIC). You need to choose your species of bacteria and your favorite antibiotic. Next week, you will conduct your experiment using LB liquid media (nutrient rich broth for growing bacteria). You will add the appropriate amount of antibiotic to the 2 mL aliquots of LB media.

**Information: Design and Build a New Promoter (5 week project)**

**Wednesday afternoon:**

1) Fill out this table by determining *n* and *x*:

|  |  |
| --- | --- |
| **Volume (µL)** | **Component** |
| n\* | 1 µL of each oligo |
| x = 20 – 2 – n | dH2O  |
| 2 | 10X annealing buffer |
| **20** | **final volume** |

\* n = the number of oligos you had synthesized

2) Get a fresh microfuge tube and add 1 µL of each oligo to the tube. Then add the other reagents (ingredients) to this same tube. Add the reagents in the order listed in the table above.

3) Put the tube in a beaker with ~700 mL of boiling water for 10 minutes. Then turn out the flame and let the entire beaker cool slowly overnight.

In Lab: **(Start lab at this point)**

4) Do a ligation using this protocol online:
<http://gcat.davidson.edu/mediawiki-1.15.0/index.php/Golden_Gate_Assembly_protocol>

**\*\*\*Now do the Evolution Experiment, then return to step 5\*\*\***

5) Transform cells (zippy competent JM109) with 3 different DNAs:

a) experimental ligation DNA (with your promoter)

b) ligation negative control DNA (no promoter)

c) transformation positive control DNA ([pLac promoter+RBS+RFP](http://partsregistry.org/Part%3ABBa_K315008))

6) Plate each transformation on its own LB+amp plate.

7) Discuss expression assay as a group. How will you grow the cells and how will you know if your promoter worked?

8) One person from each group will need to start the cells growing 4 pm Wednesday the day before lab. Come to Dr. C’s research lab.