**Bio111 Week 2**

Before you come to lab:

1) Read this [abstract of this paper](http://www.jbc.org/content/280/16/15921.full.pdf).

2) View the [6 movies of cells moving.](http://www.bio.davidson.edu/people/macampbell/111/weekly_Labs/cell_movies.html)

3) Create an account on the [Campbell lab wiki page](http://ung.igem.org/Lab:Campbell_M_Lab)

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

A) Why did the swimming behavior of the bacteria vary depending on what solution they were

in?

B) What is a plasmid?

C) What are oligonucleotides (often referred to as oligos)?

D) What is a restriction enzyme?

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

In Lab:

1) Collect your plates and record your results.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **antibiotic** | ***E. coli* (Gram-)** | | ***B. subtilis* (Gram+)** | | **Mode of action** |
| ring? (y/n) | radius (mm) | ring? (y/n) | radius (mm) |
| none (control) |  |  |  |  |  |
| ampicillin |  |  |  |  |  |
| tetracycline |  |  |  |  |  |
| chloramphenicol |  |  |  |  |  |
| erythromycin |  |  |  |  |  |
| streptomycin |  |  |  |  |  |

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

In Lab:

2) Search for papers using terms such as “inducible promoter escherichia coli” or “repressible promoter escherichia coli” or “constitutive promoter escherichia coli”. Save the PDF files for use in lab.

3) Working in your lab group, find your gene’s promoter by looking upstream from the start transcription site. You might find the promoter described in a published paper which would be better than just guessing its location.

Resources you can use to find promoter sequences:

* [*E. coli* genome browser](http://microbes.ucsc.edu/cgi-bin/hgGateway?org=Escherichia+coli+K12&db=eschColi_K12&hgsid=581372).
* [List of *E. coli* gene promoters](http://margalit.huji.ac.il/promec/prom.seq.final.html)

4) Watch [movie of restriction enzyme](http://www.bio.davidson.edu/misc/movies/cutDNA.mov) cutting DNA

See the [structure of a restriction enzyme bound to DNA](http://molvis.sdsc.edu/fgij/fg.htm?mol=1ERI).

Discuss sticky ends.

5) Design your first promoter using this online tool to [make oligos that will self-assemble](http://gcat.davidson.edu/iGem10/index.html). Make sure the oligo length is no longer than 59 bases.

6) Take a screen shot of your output. Click on the “DOWNLOAD” button to copy your oligo sequences. Send me ([macampbell@davidson.edu](mailto:macampbell@davidson.edu)) an email containing your sequences and the name of the gene your promoter came from. In addition, tell me in the email what type of information is encoded in your promoter (*i.e.* inducible, repressible, constitutive) .

7) Make a [new **basic** part in the Campbell Lab](http://partsregistry.org/Add_a_Part_to_the_Registry) web page and document your design. Include the screen shot of the Oligator output and document how you learned about the promoter and its predicted behavior. Our part numbers range from BBa\_J100000 to BBa\_J100999

8) Go to the [oligo assembly protocol](http://www.bio.davidson.edu/courses/Molbio/Protocols/anneal_oligos.html) and calculate how to prepare a 20 µL solution of your oligos so they will self-assemble into a functional promoter ready for ligation.

9) [Take lab skills quiz #1.](http://checkboxweb.davidson.edu/Survey.aspx?s=88bd4b93f15542118a8faf392e01e401http://checkboxweb.davidson.edu/Survey.aspx?s=88bd4b93f15542118a8faf392e01e401)

**References**

<http://www.mun.ca/biochem/courses/3107/Topics/promoter_bacterial.html>