SEED Academy, Spring 2011
Synthetic Biology Module

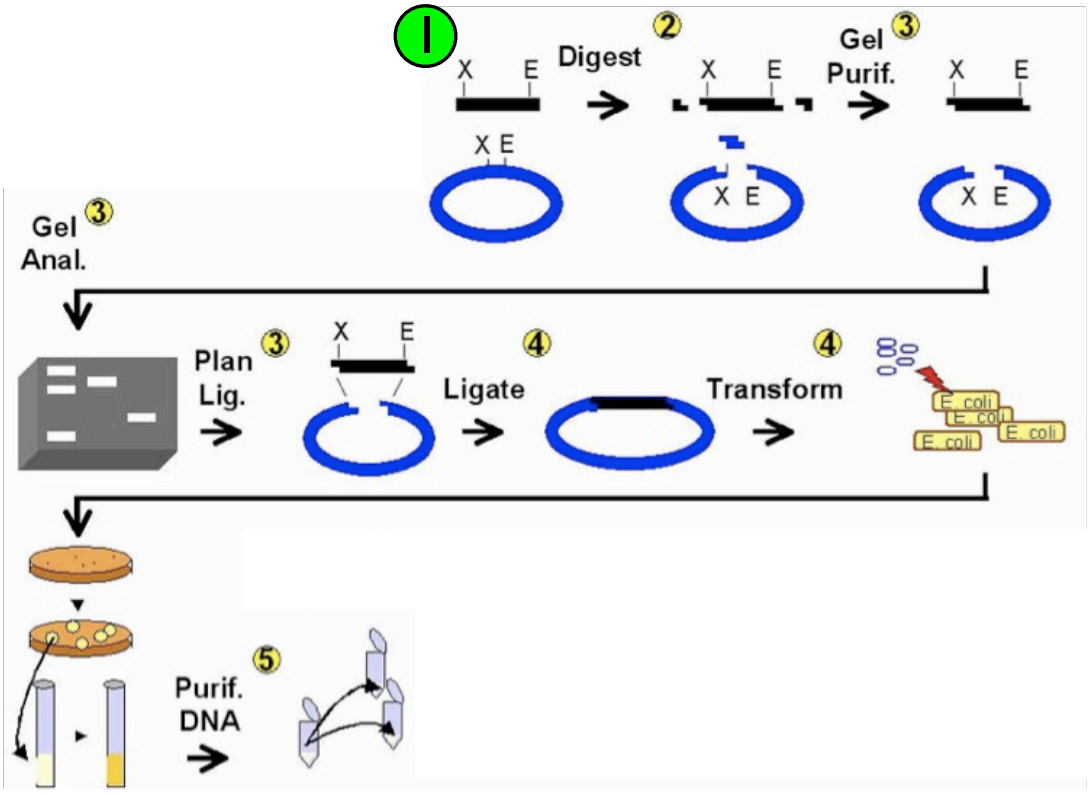
Homework #7
Due April 9, 2011

1) Cloning: *Complete* (Essentially…)

You have essentially completed the necessary steps for cloning a gene (you should remember, though, that verifying whether your product is correct, via sequencing or another method, is a crucial step in the cloning process). Thus, referring to our good friend the lab project schematic below, indicate how you got from start to finish.

Notes:

1) Treat the small black piece of DNA as the product that you PCRed.
2) This diagram suggests that you used XbaI and EcoRI for the digest. You did not. Which enzymes did you use?
3) Consider "Gel Purif." and "Gel Anal." one step; disregard "Plan Lig."



2) Transformation Efficiency

Transformation is actually an inefficient process. We seek to illustrate this point in this problem.

You note that there are 7 cells in 10 µL of medium (LB) after 5 serial 10X dilutions. You transformed 10 µL of 100 pg/µL ligation product (a plasmid) with an expected length of 5000 bp and a molecular weight of 650 Dalton per base.

<u>If you obtained 100 colonies on the plate…</u>

a. What is the transformation efficiency, i.e. the number of ligation products recovered in cells (Hint: Think about how many ligation products would you expect a single colony to represent)?

b. What fraction of viable cells obtained a plasmid with antibiotic resistance?

If you obtained 10,000 colonies on the plate…

c. What is the transformation efficiency?

d. What fraction of viable cells obtained a plasmid with antibiotic resistance?

3) Enzyme Activity

a. Expression Levels

i. mRNA

You are using a plasmid which is maintained in the cell at 5 copies per cell and a promoter which recruits RNA polymerase at a rate of 10 per second per plasmid. Assume that every second $1/4^{th}$ of the mRNA in existence is degraded.

What is the steady concentration of mRNA [#/cell] (HINT: A steady concentration will be reached when the RNA production by the polymerase equals the rate of degradation)?

ii. Protein

Use the steady mRNA concentration from above (if you did not get an answer above, use a value of 1 mRNA per cell). The ribosome binding site initiates translation of the mRNA at a rate of 2 per second per mRNA. Assume that every second, $1/10^{th}$ of the protein in existence is degraded.

What is the steady concentration of protein [#/cell]?

4) **Final Projects**

Continue researching!