

SEED Academy, Spring 2009
Synthetic Biology Module

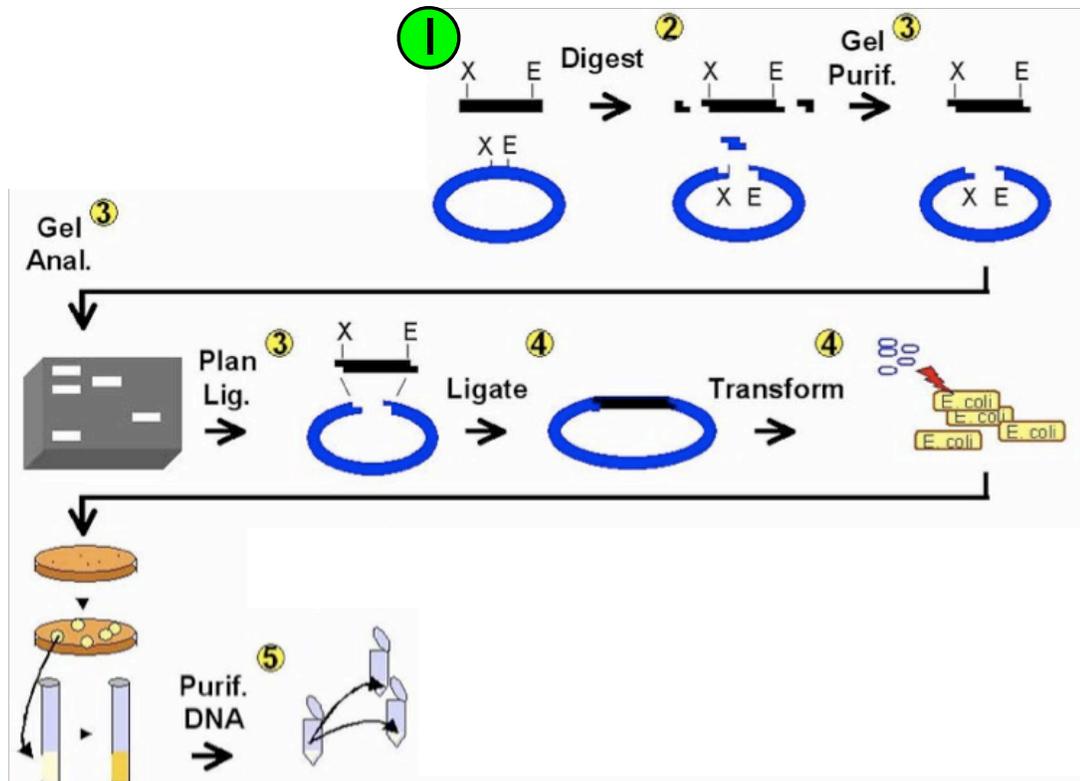
Homework #7
Due April 4, 2009

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) For steps 1-3, only consider the “blue” piece of DNA that you worked with (we KNOW that you know the name of this DNA by now...)
- 2) Consider “Gel Purif.” and “Gel Anal.” one step; disregard “Plan Lig.”
- 3) Remember: You ligated *3* pieces of DNA together at the same time. We gave two pieces to you already restriction digested. What were those pieces (describe them at the appropriate point in your summary below)? With which enzymes was each piece digested (Hint: use your notes or Homework 4, problems 3/4 to answer this question)?
- 4) If you need help, REFER TO THE WIKI!!!



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 $1/4^{\text{th}}$ 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, $1/10^{\text{th}}$ of the protein in existence is degraded.

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

4) **DNA Sequencing**

In our last class you prepared your own (or the instructor's) ligation products for sequencing and will be analyzing those results this Saturday. Thus, we'd like you to delve a bit more deeply into the method of sequencing. As you learned from the video, DNA sequencing is used to read out the bases from DNA. Many methods have been developed but the currently most common method for sequencing is known as the dideoxynucleotide method or Sanger sequencing. Look up some information about both the classical method involving radioactively labeled nucleotides and the current method using fluorescently labeled nucleotides. Answer the following questions:

- a) What does ddNTP stand for? How is it different from dNTP?

- b) What does it mean when we refer to ddNTPs as chain terminators?

- c) In the classical Sanger sequencing method, 4 separate reactions are run. Each reaction contains all of the dNTPs, DNA polymerase, the DNA to be sequenced, and a sequencing primer. What else is added to the 4 reactions?

- d) Suppose we ran a sequencing reaction with only the nucleotides ddATP, dTTP, dCTP, and dGTP. Explain what would happen during the sequencing reaction and why this is not useful for sequencing.

- e) Consider the following sequence. Suppose we use the primer 5'-TCAGT-3' and run a classical Sanger sequencing reaction. We set up 4 reactions and then run a gel to separate the bands. Draw a gel and the locations of the bands from the sequencing run. Be sure to label the four lanes.

5'-TCAGTAGCGTAGCACCTAG-3'
3'-AGTCATCGCATCGTGGATC-5'

- a) Write one paragraph summarizing your project, what it will accomplish and why you want to do it.
- b) Draw a schematic diagram of your system. Show all of the relevant inputs and outputs as well as the critical components you have identified so far, including fundamental parts to make your system, and any devices that you know you will need. Include both the specific parts that you know exactly how they will function and the abstract devices which you don't have fully defined yet.
- c) Summarize the possible states of your system. What inputs (cues) will lead to what outputs (signals). Put this data in a table.