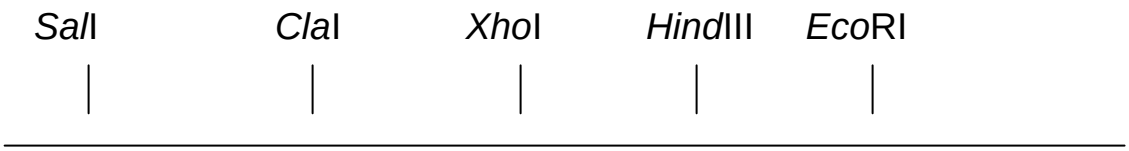
Supplementary Material

Excerpt from Take-home Exam 1

Forced cloning:

Polylinker region of pNCSU



Shanthan wants to force clone *sfg* (shanthan's favorite gene) into the plasmid pNCSU. The polylinker for pNCSU is shown above; assume enzyme sites listed are unique in the plasmid. Discuss whether each enzyme or pair of enzymes listed below can be used to force clone s*fg* into pNCSU. Assume that s*fg* can be isolated using the same enzyme(s) with which you will cut the vector and that none cut within the *sfg* sequence. Your answer should clearly indicate yes or no, include the sequence(s) of the restriction enzyme (available at www.neb.com), and discuss why forced cloning will or will not work.

A. *Eco*RI

B. *Sal*I and *Xho*I

C. *Cla*I and *Hind*III

Choosing an expression vector: Jordan is going to subclone his gene of interesting using *Sac*I and *Hind*III into a pET41 vector. The sequence of his gene of interest in show below, with the start and stop codons in bold and the restriction sites underlined. **State which vector she should chose (pET-41a, b or c; see the pdf of the vector map in the assignments folder on the class website), and write the sequence of the resulting ligated DNA molecule that contains her gene of interest, clearly indicating which parts of the resulting DNA molecule come from the vector and which come from the insert.** (you do not need to write out the entire vector sequence; just enough to clearly show the 5' and 3' junctions.)

insert DNA sequence:

5' TTCGG AGC T/CC CCC **ATG**TGGCTAGAC..rest of gene..CGAGAGAAC**TAA**AAGCTTCAG3'

Restriction Enzyme Digestion:

A. Nina needs to digest 500 ng of a 4.0kb plasmid with *Psp*GI (a unique site on the plasmid). The concentration of her plasmid is 1 µg/µL. Using the neb website, write down what she should include for a 20 µL total reaction volume. Use a five-fold excess of restriction enzyme. Be sure to include all necessary components, volumes, and incubation temperature.

B. (2 points) Nina runs some undigested plasmid alongside some digested plasmid on an agarose gel along with a molecular weight ladder. Describe what the undigested and digested plasmids would look like on her gel.

Excerpt from Take-home Exam 2

Cloning by PCR

You amplified the *egfp* gene, engineering restriction sites onto the ends, using *Taq* DNA polymerase. You created sticky ends, cloned the *egfp* gene into pET-41a, and transformed into *E. coli* strain BL21(DE3). You selected a clone that was positive by the PCR screen, the restriction digestion screen, and the antibody colony-lift screen. However, when plated on IPTG, the colony does not fluoresce. Assuming you followed the screening protocols and the IPTG protocol correctly and your positive control did fluoresce, how could you explain the occurrence? And how could you confirm your explanation?

Antibodies

Candace wants to perform a western blot using a primary antibody that was raised in pigs. Unfortunately, she can't find any secondary antibodies in the refrigerator in her lab, so she's going to have to buy some before she can do her experiment.

a. List the critical features that the secondary antibody *must* have.

b. Using the catalog for the antibody supplier www.abcam.com, give the name and catalog number of a suitable secondary antibody she could buy and use for her experiment.

cDNA libraries

Quang is interested in studying a potential *bmp*2 gene from *Meleagris gallopavo*. Despite successfully creating a cDNA library, he is unable to identify a positive clone. To screen his library, he designed a 20-nucleotide heterologous probe derived from promoter sequence from the known *bmp*2 gene from the closely-related organism *Calothorax pulcher*. He screened a sufficient number of plaques and performed the hybridization under low stringency conditions. What is the most likely reason for not being able to find a positive clone (why was this experiment doomed)? How should he change this experiment in order to find the *bmp*2 gene in *Meleagris gallopavo*?

Peer Learning Survey Questions – BIT 410/510

Demographics

1. Choose your academic level:

Undergraduate	Non-thesis Masters	Thesis Masters	Ph.D.	Non-degree studies
☐	☐	☐	☐	☐

2. What is your major or degree-granting department if applicable?

_____

3. Have you had any previous molecular biology lab experience? Yes ☐ No ☐

If so, in what context?

4. Have you had any research experience? Yes ☐ No ☐

If so, in what area and for what duration?

Attitudes

5. I consider myself a leader.

strongly disagree	disagree	neutral	agree	strongly agree
☐	☐	☐	☐	☐

6. I prefer to work on my own.

strongly disagree	disagree	neutral	agree	strongly agree
☐	☐	☐	☐	☐

7. How much did you contribute to your station's work?

Very little	less than my partner	equal to my partner	more than my partner	vast majority
☐	☐	☐	☐	☐

8. How much do you feel your partner contributed to your station's work?

Very little	less than me	equal to me	more than me	vast majority
☐	☐	☐	☐	☐

9. I feel prepared to undertake a research project in molecular biology.

strongly disagree	disagree	neutral	agree	strongly agree
☐	☐	☐	☐	☐

10a. If you are an undergraduate, do you feel you would benefit more by being lab partners with another undergraduate or with a graduate student (consider both aspects of mentorship and shared learning)?

graduate student	undergraduate student	not applicable
☐	☐	☐

10b. If you are a graduate student , do you feel you would benefit more by being lab partners with an undergraduate or with another graduate student (consider both aspects of mentorship and shared learning)?

graduate student	undergraduate student	not applicable
☐	☐	☐

Skills

Presently, I can:

11. successfully follow laboratory protocols.

strongly disagree	disagree	neutral	agree	strongly agree
☐	☐	☐	☐	☐

12. draw logical conclusions from the results I obtain.

strongly disagree	disagree	neutral	agree	strongly agree
☐	☐	☐	☐	☐

13. troubleshoot experiments.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

14. describe my lab work in a clearly written report.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

15. communicate effectively with my lab partner.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

16. accurately pipet 2ul of liquid.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

17. prepare, run, and analyze a DNA agarose gel.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

18. design PCR primers to amplify a gene of interest.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

19. perform small enzymatic reactions such as PCR, ligation, or restriction digests.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

20. successfully design and implement a cloning strategy to express a recombinant protein.

strongly disagree ☐ disagree ☐ neutral ☐ agree ☐ strongly agree ☐

21. Discussing answers to the Learning Cell questions with my classmates increased my understanding of the course material.

strongly disagree	disagree	neutral	agree	strongly agree
☐	☐	☐	☐	☐

22. Designing my own Learning Cell questions increased my understanding of the course material.

strongly disagree	disagree	neutral	agree	strongly agree
☐	☐	☐	☐	☐

23. Please leave any additional comments or suggestions regarding the Learning Cell activities.