7.02 Recombinant DNA Methods
Spring 2005
Exam Study Questions
Answer Key

Note: we've only provided the answers here. Please refer to the Study Questions document for the questions!

Question 1

a) Here are the steps you'd want to carry out:

1. **Miniprep plasmid DNA from JAF1 overnight culture**
2. **Transform this DNA into competent DAK2 cells**
3. **Plate transformed cells on LB Amp plates**

b) colonies on LB Amp plates

c) Here are the best controls, and how you'd use them to interpret the data:

Positive control:
- **transform pET into the competent DAK2 cells; plate on LB Amp plates**
- **colonies on LB Amp plates tell you that your DAK2 cells are competent**

Negative control:
- **transform ligation buffer into competent DAK2 cells; plate on LB Amp plates**
- **if you don't see any colonies, confirms that your plates had Amp, that your cells were really AmpS to begin with, you had no contamination, etc.**

d) **P1 transduction**

Question 2

a)

A concatamers B nicked C supercoiled

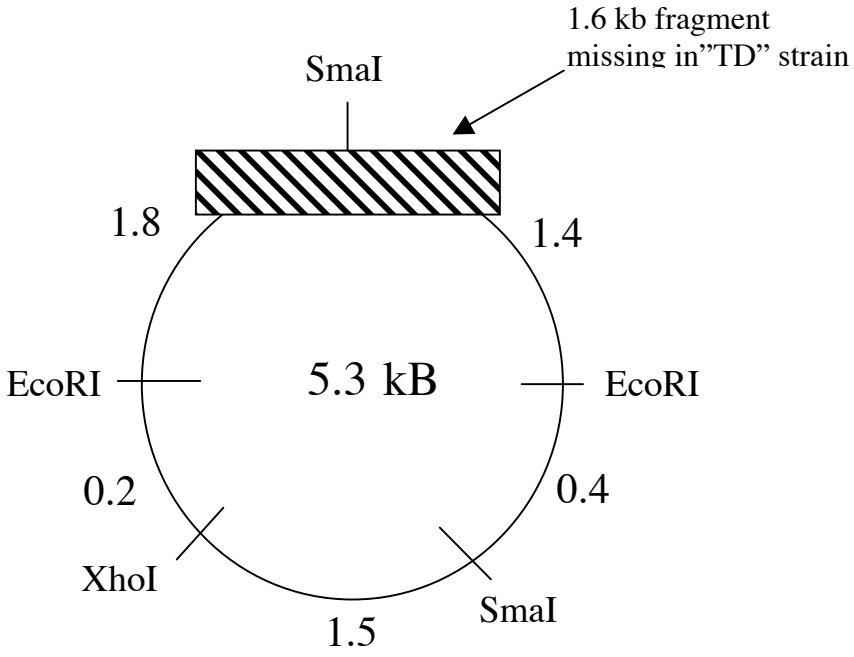
b) Once. You obtain one band that migrates between the nicked and supercoiled forms of uncut DNA; this corresponds to linearized, full-length DNA

c) Zero. SspI digested DK15 DNA has the same banding pattern (supercoiled, nicked, concatamers) as "uncut" DK15 DNA.

d) Your SmaI digest was a partial digest. The top band is likely to come from DNA molecules that were cut at one of the two SmaI sites. You know this because this DNA migrates at the same position as the linear DNA seen in the XhoI lane. The bottom two bands are likely to come from DNA that has been cut at both SmaI site. You know this because cutting a circle twice gives rise to 2 linear fragments, and both of these fragments are smaller than the total plasmid size.

Question 2 (continued)

e) Here is a restriction map that is consistent with all the data provided:



f) 1.6 kb

g) see map.

Question 3

a)

Strategy 1: Cut pEX1 with **C**
Cut pKan with **Z**

Strategy 2: Cut pEX1 with **A + B**
Cut pKan with **X + Y**

Question 3 (continued)

b) Here are four acceptable answers—choose two:

1. phosphatase (CIP) the vector **2. gel purify the vector fragment**

3. use more insert than vector **4. choose "sticky" vs. "blunt" ligation**

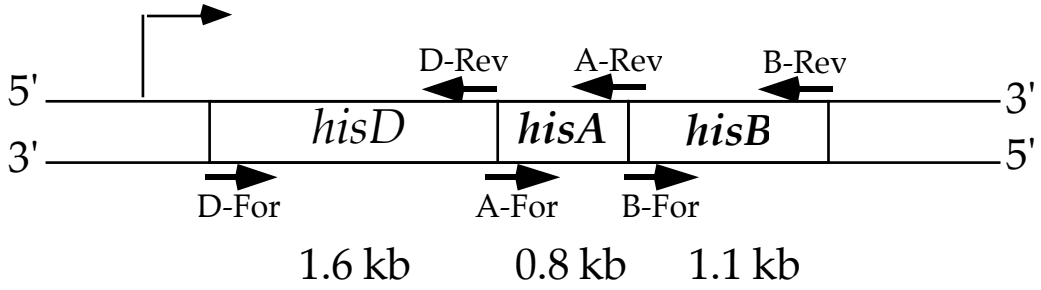
c) Your insert may ligate in the wrong orientation, as any blunt end can ligate to any other blunt end (there is no directionality).

d) Here is the experiment you should perform:

1. **Transform into BL21 cells, which express the T7 RNA polymerase required for transcription of genes under the control of the T7 promoter.**
2. **Select for cells that have taken up the plasmid by growing on LB Amp plates (both vector alone and vector plus insert will grow here).**
3. **Patch or replica plate the colonies onto LB Kan plates (1 point for LB Kan).**
4. **Any colonies that grow on both LB Amp and LB Kan have the pEX1-KanR fusion.**

Question 4

a)



b) Based on the data in lanes 5-7, it appears that the transposon is inserted in HisA. However, since this is a negative result, it is hard to have a lot of confidence in it. You can say that it is NOT is HisB/D. If it was really in HisA, you would have expected a band in lane 9 with the LacZ primer.

Question 4 (continued)

c) There are two inverted repeat sequences in the miniTn10 transposon—one on each end. Thus, your primer could bind to either/both sites (rather than a unique site) making your analysis complicated. (Also, you may get primer dimers (primers annealing to each other) formed, depending on how the primer was designed. This would lower your PCR efficiency.)

d) You could use the three Rev primers (HisD-Rev, HisA-Rev, and HisB-Rev) with the KanR-For primer in PCR. (Based on results in part b, you could also choose to use HisA3′ to confirm your initial suspicion!). The size of the PCR product would tell you how far from the 3′ end of the gene that your transposon inserted.

Question 5

a) NO. RE Buffer does not contain ATP, and the formation of the phosphodiester bond by DNA ligase is an ATP-dependent reaction.

b) YES. Both buffers have similar amounts of salt (NaCl) and MgCl2 (cofactor for these enzymes) and are both @ ~pH 7.0.

c) NO. GTE contains EDTA. ETDA chelates Mg+2 ions, which is a required cofactor for RE function. (You could also get credit for saying that GTE lacks Mg+2, and the reasoning would be the same.)

d) NO. CIP removes 5′ phosphates from both the vector and the insert; a successful ligation between vector and insert requires a 5′ phosphate and a 3′ OH to be joined by a phosphodiester bond.

e) The GFP in pET-GFP is under the control of a T7 promoter, and AG1111 cells do not express the T7 RNA polymerase required for transcription from this promoter.

Question 6

a and b)

5′-AATCGATT-3′
3′-TTAGCTAA-5′

c)

5′-AA-OH P-TCGATT-3′
3′-TTAGCT-P OH-AA-5′

Question 6 (continued)

d)

5′-AA-OH OH-TCGATT-3′
3′-TTAGCT-OH OH-AA-5′

Question 7

a) Group 2 obtained the expected results.

b)

Group #	Explanation
1	**AG1111 cells were not competent for transformation**
3	**ligation failed (ligase bad, CIP'd insert and vector)**
4	**cells were already AmpR (contaminated somehow)**

c) **To calculate transformation efficiency, you need to start with a known quantity of DNA. Thus, you needed to use the numbers from transformation e) (uncut pET at 0.5 ng/μl). Also, you'd want to use numbers from an experiment where the data turned out as expected (no contamination)—like #2 above.**

$$\frac{\text{975 Amp}^R \text{ transformants}}{\text{10 } \mu\text{l uncut pET x } \frac{\text{0.5 ng uncut pET}}{\mu\text{l}}} = \frac{\text{975 transformants}}{\text{5 ng DNA}} \times \frac{\text{1000 ng}}{\text{1 } \mu\text{g}}$$

$$= \frac{\text{1.95 x } 10^5 \text{ transformants}}{\mu\text{g of DNA}}$$

Question 8

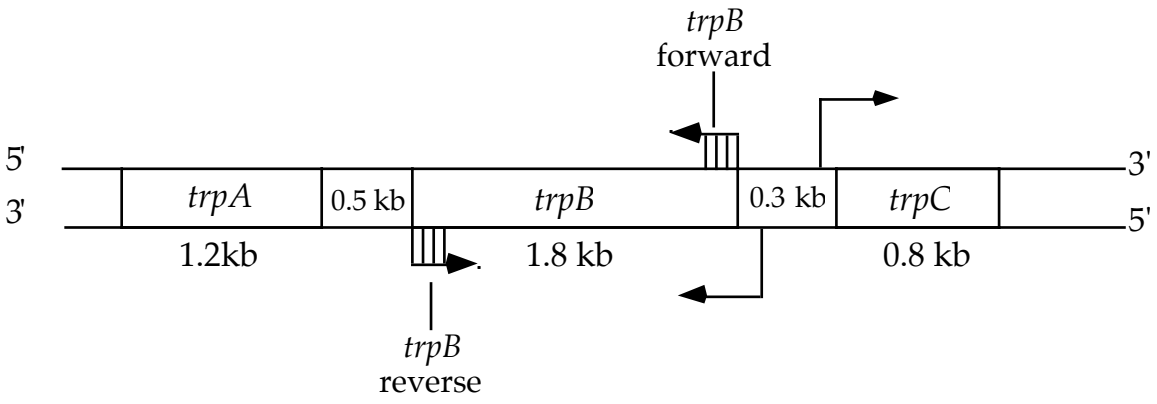
You should DISAGREE with the suggested cloning strategy for the following reasons:

1. **There is an SspI site in the middle of the maize heat shock gene ORF. If you incorporate an SspI site in your primer, then cut with SspI to clone the insert into pGAL, you will cut your ORF in half! Thus, you will not clone the whole ORF into pGAL.**

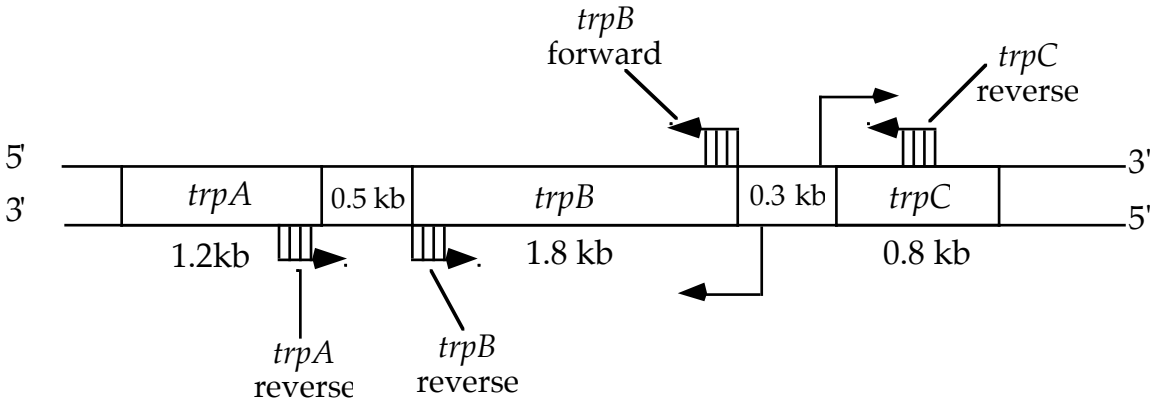
2. **Using XbaI in the forward primer and SspI in the reverse primer would also clone your ORF into pGAL in the incorrect orientation. Thus, no heat shock protein would be made.**

Question 9

a) This is identical to the binding pattern of the *araC* primers we used in the 7.02 lab!



b)

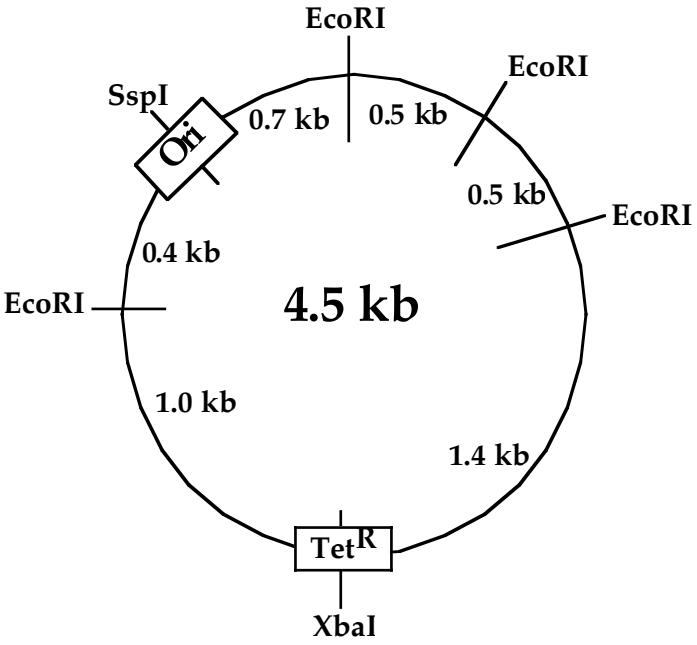


If the primers drawn above were combined in a PCR reaction with the *trpB* For and *trpB* Rev primers, there are two different sized PCR products that would form (1.8 kb and 2.5 kb). These two products would result from polymerization from three sets of primers. The 1.8 kb product would come from polymerization from the *trpB* For and *trpB* Rev primers. The 2.5 kb product would come from both polymerization from *trpB* forward and *trpA* reverse AND *trpB* reverse and *trpC* reverse.

c) Shorten the extension time to 2 minutes (1.8 minutes < extension time < 2.5 minutes)

Question 10

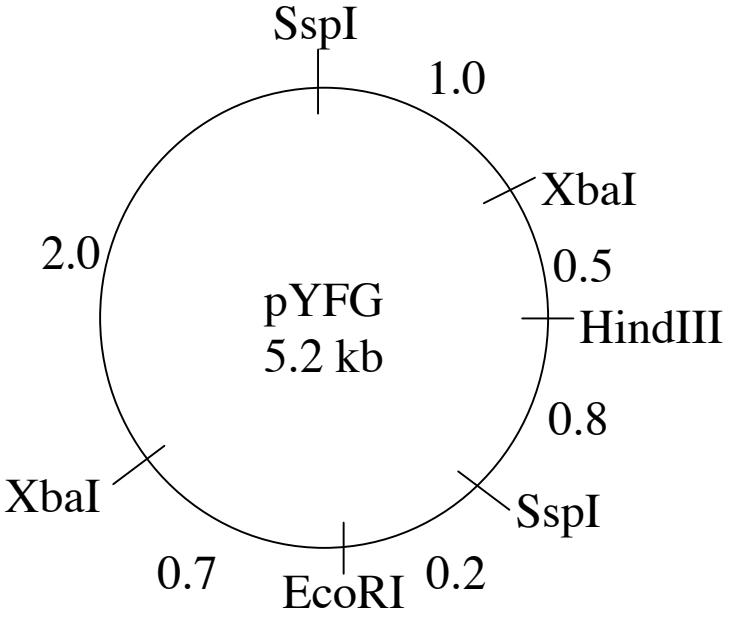
a) The following restriction map fits all the data provided:



b) In addition to restriction enzymes, bacteria also contain methylases (modifying enzymes) that methylate the chromosomal DNA at one of the bases of the RE recognition site. The restriction enzyme cannot cut at methylated RE sites, and the bacterial DNA is protected. Foreign DNA, such as that from a phage, is not methylated and thus is a target for the bacterial restriction enzymes.

Question 11

a) The following restriction map is consistent with all the provided data:



Question 12

a) agarose gel electrophoresis (RDM) and SDS-PAGE (PBC)
similar: both are gels used to separate biomolecules on the basis of size (MW)
different: agarose gels are used for DNA and the DNA's phosphate backbone provides negative charge; SDS-PAGE is for proteins and uses SDS to give all proteins a constant charge/mass ratio.

b) transformation (RDM) and transduction (GEN)
similar: both are methods of introducing a foreign DNA sequence of interest into a bacterial host
different: transformation uses plasmids to carry the sequence of interest; transduction uses phage to carry a DNA sequence of interest

c) GFP (RDM) and *lacZ* (GEN)
similar: both are used as biological reporters (a gene/protein that tells you about the expression or localization of another gene/protein that is hard to assay)
different: GFP is an autofluorescent protein that is visualized by UV light; *lacZ* encodes B-galactosidase, and a B-gal substrate like Xgal is required to "visualize" the reporter.

Question 12 (continued)

d) Ethidium bromide (RDM) and Coomassie blue (PBC)

similar: both are biological stains used to "visualize" a biomolecule
different: EtBr is an intercalating agent used to visualize DNA under UV light; Coomassie Blue is a blue dye that binds nonspecifically to proteins.

e) T7 RNA polymerase (RDM) and T4 DNA ligase (RDM)

similar: both are phage enzymes (T7 and T4 phage) that are now used in recombinant DNA experiments.
different: T7 RNA polymerase is used to express (transcribe) genes from the T7 promoter; T4 DNA ligase is used to join two pieces of DNA—like our vector and insert—together via a phosphodiester bond.

f) AG1111 (RDM) and BL21 (RDM) cells

similar: both are *E. coli* strains used in transformations during the 7.02 GFP subcloning project.
different: AG1111 has a higher transformation efficiency and no T7 RNA polymerase; BL21 has a lower transformation efficiency and expresses T7 RNA polymerase