

**7.02/10.702 Recombinant DNA Methods
Spring 2005
Exam Study Questions**

The following pages contain RDM exam questions from the past few semesters of the course. These questions are not meant to be exhaustive, but to give you an idea of what types of questions might be asked on your RDM exam on April 26th, 2005.

Don't forget to complete the BLAST tutorial before the exam!

Question 1

You have two *E. coli* strains, JAF1 and DAK2. JAF1 is phenotypically Amp^R, and DAK2 is phenotypically Amp^S. You suspect that the Amp^R gene is carried on a **plasmid** in JAF1, rather than on JAF1's chromosome, and want to design an experiment to test this hypothesis.

In designing your experiment, you may assume that you have access to the following materials:

1. all reagents, plates, plasmids, and equipment used in the 7.02 RDM module
2. Overnight cultures of JAF1 and DAK2
3. Competent cells of JAF1 and DAK2

a) Describe a **simple** experiment you could perform to test the hypothesis that the Amp^R gene is carried on a plasmid in JAF1 and not on JAF1's chromosome.

(Note: there is no need to describe the details of a particular procedure you might use, simply stating what you would do is sufficient—e.g. ligate A + B together).

b) What **specific** experimental result will tell you that your hypothesis is correct?

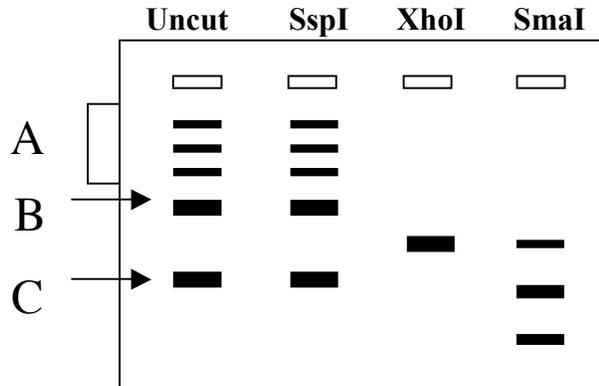
c) Identify one **positive** and one **negative** control you would include in your experiment above, and how you will use these controls to help you interpret your "experimental" results.

(d) If the Amp^R gene was carried on JAF1's chromosome, name a technique that would allow you to transfer this gene to DAK2? (Hint: GEN module!) _____

Question 2

You are studying a virus called DK15, which is known to cause tumors in mice. The genome of DK15 is a 5.3 kb, double-stranded, circular DNA molecule. You are interested in learning more about DK15, so you decide to map its DNA using restriction enzymes.

As a first step in your analysis, you digest DK15 DNA with three enzymes: SspI, XhoI, and SmaI. You run the digested DNA on an agarose gel, along with an uncut sample of the DK15 DNA as a control. You obtain the following pattern of bands**:



**You may assume that all the digests worked (i.e. all enzymes were "active")

a) Fill in the blanks with the identities of the uncut DNA bands labeled A, B, and C:

A _____ B _____ C _____

b) How many times does XhoI cut the DK15 DNA? Justify your answer **in one sentence**.

c) How many times does SspI cut the DK15 DNA? How do you know?

You are a bit puzzled by the SmaI result, and show your gel to Kate to get her input. Kate concludes that the DK15 DNA contains **two** SmaI sites—even though you observed **three** bands on your gel.

d) Assuming that Kate is correct, propose a simple explanation for your observation of three bands. Explain your answer by stating the probable identity of each band you observed, and how you deduced this.

Question 2 (continued)

To determine the restriction map of DK15 DNA, you perform the following **complete digests** with XhoI, SmaI, and a third enzyme, EcoRI. You run the digested DNA on an agarose gel, and obtain the following fragments (all sizes are in kilobases):

| <u>EcoRI</u> | <u>SmaI</u> | <u>XhoI</u> | <u>EcoRI +SmaI</u> | <u>EcoRI+XhoI</u> | <u>SmaI +XhoI</u> |
|--------------|-------------|-------------|--------------------|-------------------|-------------------|
| 3.2 | 3.5 | 5.3 | 1.8 | 3.2 | 2.0 |
| 2.1 | 1.8 | | 1.7 | 1.9 | 1.8 |
| | | | 1.4 | 0.2 | 1.5 |
| | | | 0.4 | | |

e) Draw a restriction map of the DK15 DNA that is consistent with all the data provided. Be sure your map includes: 1) the location of all restriction sites; 2) the distances between restriction sites; 3) the total DNA size.

In addition to the "wild type" DK15 DNA you mapped above, you have a second tube of DNA from a "tumor deficient" strain of DK15. Genetic evidence suggests that the *virR* gene has been deleted from the genome of the "tumor deficient" DK15. To locate the *virR* gene, you digest "tumor deficient" DK15 DNA with the same set of restriction enzymes as above, and obtain the following pattern of bands after **complete digestion**:

| <u>EcoRI</u> | <u>SmaI</u> | <u>XhoI</u> | <u>EcoRI +SmaI</u> | <u>EcoRI+XhoI</u> | <u>SmaI +XhoI</u> |
|--------------|-------------|-------------|--------------------|-------------------|-------------------|
| 2.1 | 3.7 | 3.7 | 1.7 | 1.9 | 2.2 |
| 1.6 | | | 1.6 | 1.6 | 1.5 |
| | | | 0.4 | 0.2 | |

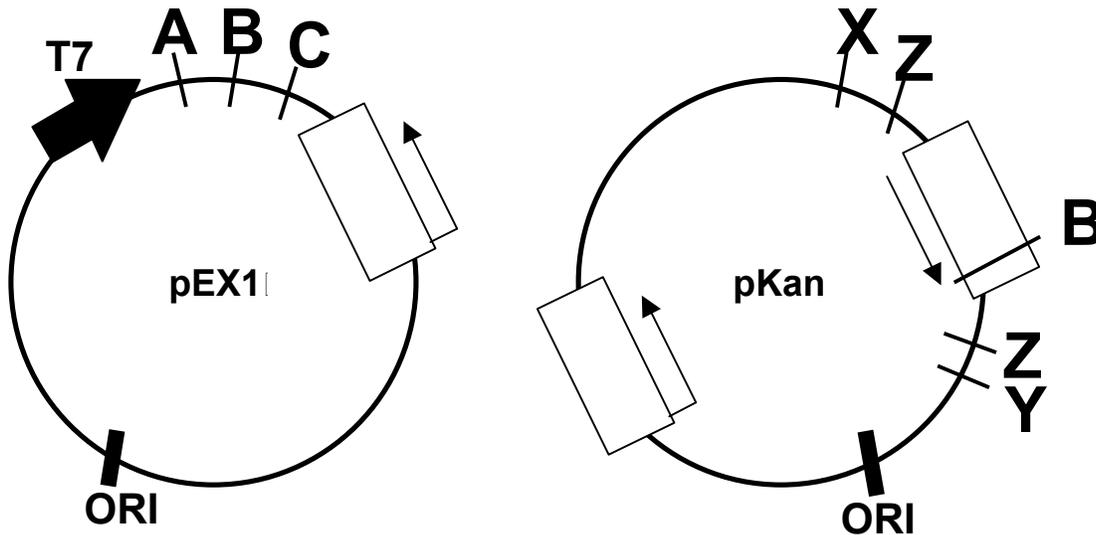
f) What **size** fragment is missing from the "tumor deficient" DK15 DNA? _____

g) On your restriction map above, indicate the **position** of this fragment as accurately as possible.

Question 3

As part of a cloning project in your laboratory, you want to create a plasmid that expresses two antibiotic resistance genes: AmpR and KanR.

As starting material, you have two plasmids: pEX1 and pKan. A diagram of each plasmid and a description of each plasmid's features are provided below:



pEX1 contains:

- AmpR gene (with its own promoter);
- origin of replication (ori)
- T7 promoter (T7)
- restriction sites for enzymes A, B, C

pKan contains:

- AmpR gene (with its own promoter)
- origin of replication (ori)
- **promoterless** KanR gene
- restriction sites for enzymes B, X, Y, and Z

**For each plasmid, an arrow indicates the direction of transcription of AmpR and KanR.

Here are the restriction enzyme recognition sequences for enzymes A, B, C, X, Y, and Z and where each enzyme cuts within its recognition sequence:

Enzyme A:

5'—GACGTC—3'
3'—CTGCAG—5'

Enzyme B:

5'—GGTACC—3'
3'—CCATGG—5'

Enzyme C:

5'—AGCGCT—3'
3'—TCGCGA—5'

Enzyme X:

5'—TACGTA—3'
3'—ATGCAT—5'

Enzyme Y:

5'—AGTACT—3'
3'—TCATGA—5'

Enzyme Z:

5'—ATCGAT—3'
3'—TAGCTA—5'

Question 3

After careful examination of all the restriction enzyme recognition sites, you realize that there are **two** possible subcloning strategies that would allow you to ligate the KanR gene into pEX1 (such that KanR is under the control of the T7 promoter).

a) For each possible subcloning strategy, identify which enzyme(s) you would use to cut each plasmid:

Strategy 1: Cut pEX1 with _____

Cut pKan with _____

Strategy 2: Cut pEX1 with _____

Cut pKan with _____

In your subcloning experiment, you want to avoid having pEX1 religate to itself without an insert (KanR), as this will increase your "background."

b) Name two experimental steps you should take to limit the number of vector religations that will occur.

1. _____

2. _____

In general, the ligation reaction between two "sticky" ends is more efficient than the ligation reaction between two "blunt" ends.

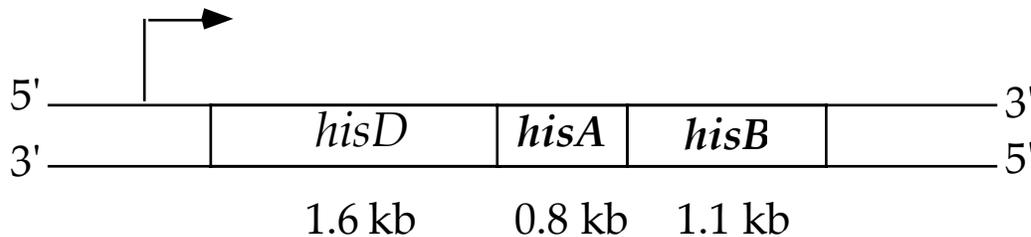
c) In addition to this difference in ligation efficiency, identify **one** complication that can arise if you try to subclone a gene using restriction enzyme(s) that leave "blunt" ends.

In 7.02 lab, you deduced that you correctly created a pET-GFP expression plasmid using two methods: one analytical (restriction digests after minipreps) and one "observational" (glowing green colonies under UV light).

d) How could you test "observationally" to see if you have successfully created a pEX1-KanR expression plasmid? Be specific about: 1) what *E. coli* cells you'd transform and **why**; 2) how you would select for cells that have taken up the plasmid; 3) how you could determine if these cells are now expressing the KanR gene.

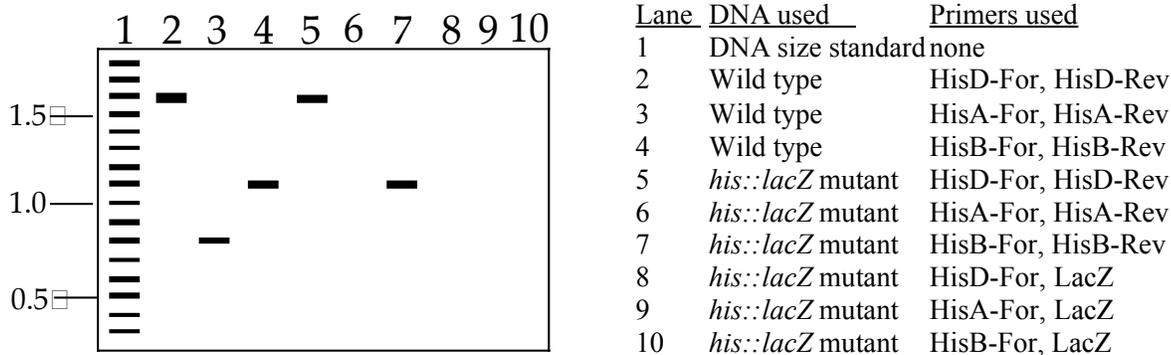
Question 4

You are interested in studying the regulation of genes in the *E. coli his* operon (histidine biosynthesis). You do transposon mutagenesis using “miniTn10,*kan*,*lacZ*” like in the GEN module, and identify a *his::lacZ* translational fusion. You think your insert is in one of three genes--*hisD*, *hisB*, or *hisA*—and decide to use PCR to pinpoint your transposon’s location. Here is a map of the *his* operon (the *his* promoter is indicated with an arrow, and each gene’s size is noted beneath it):



a) You first construct three sets of Forward and Reverse primers (HisD-For, HisD-Rev, HisB-For, HisB-Rev, HisA-For, and HisA-Rev). Indicate on the map above approximately where these primers will hybridize. (Assume that these primers correspond to the Ara primers you used in the 7.02 lab)

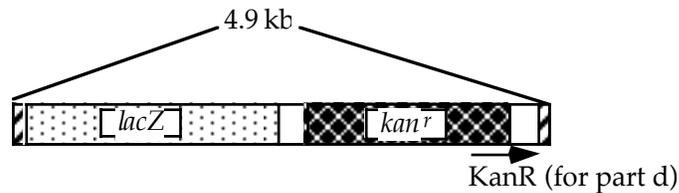
You run the following PCR reactions, and load a sample of each PCR reaction onto an agarose gel. Note that the LacZ primer is the same one you used in the RDM module, and that you allowed a 2.5 minute extension time for your PCR. You observed the following:



b) Based on this data, where do you think the transposon has inserted? How confident are you about this conclusion, and why?

Question 4 (continued)

You notice that none of the PCR reactions using the LacZ primer gave products. You decide to design a new primer specific for the transposon, and use this in a new set of PCR reactions. Here is a diagram of the miniTn10,*lacZ*,*kan* transposon:



c) You decide to design a primer that hybridizes to the inverted repeat sequence of the transposon. Your undergrad TA strongly discourages you from doing this. Why?

d) Instead, you design the primer KanR-For, which hybridizes as shown on the diagram above. What primers (of HisD-For, HisD-Rev, HisB-For, HisB-Rev, HisA-For, and HisA-Rev) could you use in PCR with KanR-For to help you determine the location of your *his::lacZ* transcriptional fusion? (Assume that you use a 2.5 minute extension time for PCR). What would the size of the PCR product you obtain tell you about where your transposon inserted?

Question 5

Your lab partner (YLP) can't keep track of all the buffers used in the RDM module, and keeps adding the wrong buffer to his reactions. Your job is to help YLP decide whether he needs to start over or not!

For each of parts a-c below, do the following:

1. Indicate whether YLP's experiments will work as described (**YES or NO**)
2. If **YES**, indicate the relevant buffer components that are consistent between the two buffers
3. If **NO**, indicate what buffer component is missing (or what component prevents it from working) and explain **why** this leads to failure.

a) YLP used RE Buffer instead of Ligase Buffer in his ligation reaction.

b) YLP used Ligase Buffer instead of RE Buffer in his restriction digests.

Question 5 (continued)

c) YLP used STET buffer instead of RE buffer in his restriction digests.

YLP finally understands the importance of choosing the right buffers, but he is still not good at following directions! You look at his notebook, and see that he added CIP to both the pET and pUGFP digests before running them on the gel, and used the isolated fragments to set up a ligation reaction.

d) Will YLP be able to successfully ligate vector and insert to create the pET-GFP plasmid? Why or why not?

YLP has found a tube containing pET-GFP DNA. He uses this DNA to transform AG1111 cells, plates them on LB-Amp, and incubates the plates overnight at 37°C. The next morning, he exposes the plates to UV light—but his colonies don't glow green!

e) Why don't YLP's AG1111 colonies glow green when exposed to UV light?

Question 6

The following is part of the sequence of an 8 base pair, palindromic restriction enzyme recognition site:

• • • • • • • •
• • • • C T A A

a) Complete the sequence of the restriction enzyme recognition sequence (draw it in the space below).

b) Label the 5' and 3' ends of each DNA strand on the drawing above.

c) Draw the products you'd expect to see if this enzyme cuts to leave a 5' overhang of 4 nucleotides. Don't forget to include the relevant reactive groups!

d) You now treat the cut DNA from part c) with Calf Intestinal Phosphatase (CIP). Draw the resulting products (with reactive groups).

Question 7

Several lab groups independently carried out the ligation/transformation steps that you performed in the 7.02 lab to join GFP and the pET vector. Reactions a-f were carried out in parallel. The results obtained by each of the groups were as follows:

| <u>Transformation into AG1111</u> | <u># of colonies on LB-Amp plates</u> | | | |
|---|---------------------------------------|----------------|----------------|----------------|
| | <u>Group 1</u> | <u>Group 2</u> | <u>Group 3</u> | <u>Group 4</u> |
| a) cut pET + GFP insert | 0 | 529 | 2 | 1125 |
| b) cut pET (CIP-treated) | 0 | 2 | 5 | 930 |
| c) GFP alone | 0 | 0 | 0 | 890 |
| d) uncut pET from gel | 0 | 852 | 900 | 1050 |
| e) 10 μ l uncut pET (0.5 ng/ μ l) | 0 | 975 | 975 | 1040 |
| f) no DNA | 0 | 0 | 0 | 925 |

a) Which group obtained the expected/desired pattern of results? _____

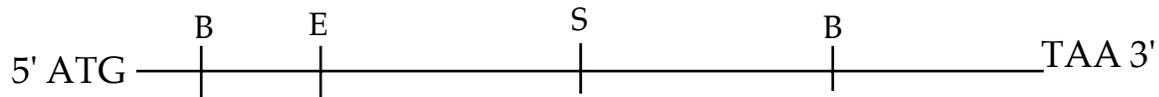
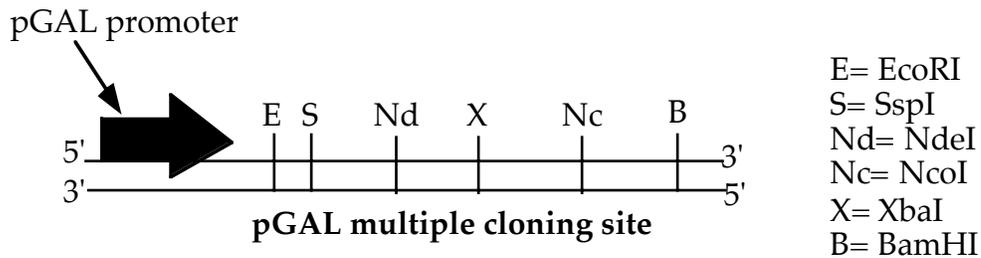
b) Provide a likely explanation for the unexpected/undesirable results obtained by each of the other three groups (**IN THE SPACE PROVIDED**).

| Group # | Explanation |
|---------|-------------|
| | |
| | |
| | |

c) Calculate the transformation efficiency of AG1111 cells. Show your work.

Question 8

You are interested in cloning the maize (corn) heat shock protein into the vector pGAL. You need to choose the restriction enzymes that you will incorporate into your PCR primers and that you will use to cut the vector (pGAL) DNA. The following are maps of the multiple cloning site of pGAL and the DNA sequence that encodes the maize heat shock protein.

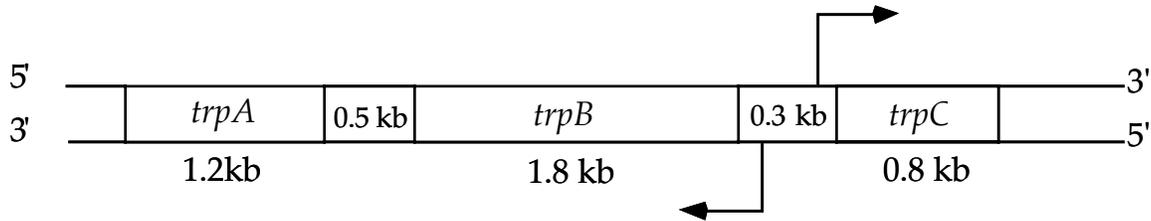


**restriction map of the open reading frame
encoding the maize (corn) heat shock protein**

Another student in the lab suggests that you clone the maize gene by including an XbaI site in your forward primer and an SspI site in your reverse primer. Do you agree or disagree with this strategy? Provide two reasons for your answer.

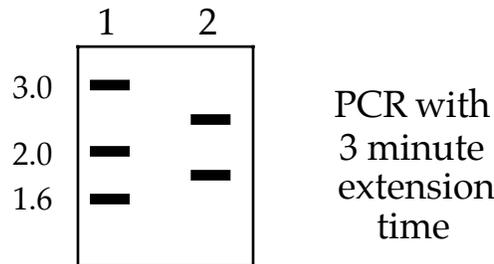
Question 9

Here is a map of the *trpB* containing region of the *B. subtilis* chromosome:



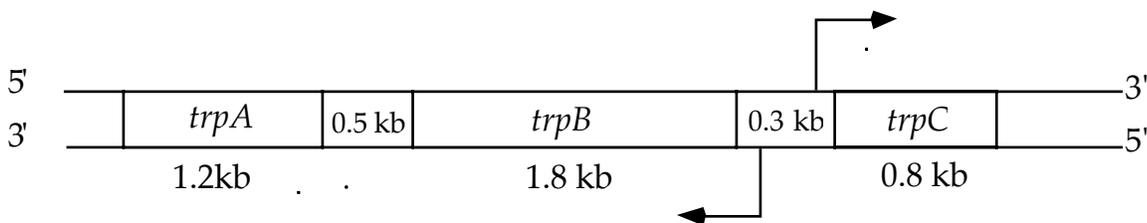
a) Indicate on the map above approximately where the *trpB* Forward and *trpB* Reverse primers will bind. (You may assume that these primers are named like the *ara* primers used in 7.02.)

You run a PCR reaction using the *trpB* Forward and *trpB* Reverse primers and a 3 minute extension time, and run an agarose gel on a sample of the PCR reaction. To your surprise, you observe the following:



1: DNA ladder
2: PCR product

b) Based on the gel results, you are convinced that your primers have been contaminated!! On the map below, indicate the binding sites of two additional primers that, when used with *trpB* For and *trpB* Rev, would lead to the results shown on the agarose gel. Be sure to NAME these primers correctly (as the *ara* primers were named).



Question 9 (continued)

d) You need to get this PCR reaction done today, and don't have time to order new primers or purify the *trpB* primers away from the contaminating primers. What one change could you make in the PCR protocol you used in part c) to avoid this undesired PCR product??

Question 10

You obtain a plasmid, pTet, from a colleague in another laboratory. When transformed into *E. coli*, pTet confers resistance to the antibiotic tetracycline (tet). Unfortunately, this colleague is notoriously bad at keeping a lab notebook, and you need to map this plasmid yourself.

You perform **complete** digests with the enzymes EcoRI, XbaI, and SspI in the combinations listed below, and obtain the following sized fragments when you run an agarose gel (sizes are listed in kilobases):

| <u>EcoRI</u> | <u>XbaI</u> | <u>EcoRI + XbaI</u> | <u>XbaI + SspI</u> | <u>EcoRI + SspI</u> |
|--------------|-------------|---------------------|--------------------|---------------------|
| 0.5 | 4.5 | 0.5 | 1.4 | 0.4 |
| 1.1 | | 1.0 | 3.1 | 0.5 |
| 2.4 | | 1.1 | | 0.7 |
| | | 1.4 | | 2.4 |

You also find that cloning your favorite gene (*yfg*) into the XbaI site(s) causes loss of tetracycline resistance, and cloning into the SspI site(s) prevents the plasmid from replicating.

a) Draw a restriction map of the pTet plasmid that is consistent with **ALL** the data you obtained. Be sure your map clearly indicates the following: 1) location of all restriction sites; 2) distance between restriction sites; 3) total size of the plasmid; 4) location of the TetR gene; and 5) location of the origin of replication.

b) EcoRI, whose restriction site is 5'-GAATTC-3' was originally isolated from *E. coli*. In general, how do bacteria protect themselves from their own restriction enzymes?

Question 11

You are interested in subcloning an interesting gene (*yfg*) from a storage plasmid, pYFG, into the expression plasmid pET. To start with, you need to create a restriction map of pYFG.

You perform **complete** digests of pYFG with the enzymes HindIII (H), EcoRI (E), SspI (S), and XbaI (X) in the combinations listed below, and obtain the following sized fragments when you run an agarose gel (sizes are listed in kilobases):

| HindIII | EcoRI | SspI | XbaI | HindIII + EcoRI | HindIII + SspI | HindIII + XbaI | EcoRI + SspI | EcoRI + XbaI | SspI + XbaI |
|---------|-------|------------|------------|-----------------------|----------------------|----------------------|--------------------|--------------------|--------------------------|
| 5.2 | 5.2 | 2.3 2.9 | 2.2 3.0 | 1.0 4.2 | 0.8 1.5 2.9 | 0.5 1.7 3.0 | 0.2 2.3 2.7 | 0.7 1.5 3.0 | 0.9 1.0 1.3 2.0 |

a) Draw a restriction map of pYFG consistent with the data presented above. Be sure your map includes the following information:

- location of all restriction sites
- distances between restriction sites
- total size of the plasmid

Question 12

In 7.02 so far, you have used a number of techniques or reagents that are similar in purpose, origin, or outcome in different experimental situations.

For each pair of techniques/reagents given below, briefly state how the two members of the pair are **SIMILAR** and how they are **DIFFERENT**. For example:

Mg⁺² (GEN) and Ca⁺² (GEN)

Similar: both are divalent cations important for phage binding to cells

Difference: Lambda requires Mg⁺² to bind, whereas P1 requires Ca⁺²

- a) agarose gel electrophoresis (RDM) and SDS-PAGE (PBC)

- b) transformation (RDM) and transduction (GEN)

- c) GFP (RDM) and *lacZ* (GEN)

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

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

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