

Solutions for Recombinant DNA Unit Exam

Question 1

Restriction enzymes are extensively used in molecular biology. Below are the recognition sites of two of these enzymes, BamHI and BclI.

a) BamHI, cleaves after the first G. Does cleavage by BamHI result in a 5' or 3' overhang? What is the sequence of this overhang?



Cleavage by BamHI results in a 5' overhang as seen here: $\begin{array}{c} 5' \quad \text{G} \\ 3' \quad \text{CCTAG} \end{array}$

b) BclI cleaves after the first T. Does cleavage by BclI result in a 5' or 3' overhang? What is the sequence of this overhang?

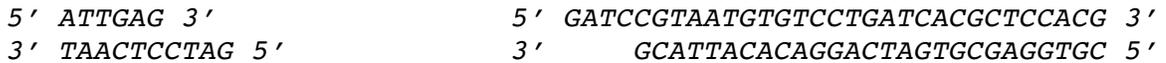


Cleavage by BclI results in a 5' overhang as seen here: $\begin{array}{c} 5' \quad \text{T} \\ 3' \quad \text{ACTAG} \end{array}$

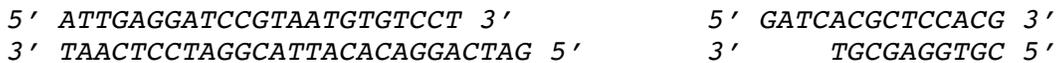
c) You are given the DNA shown below.



i) If this DNA was cut with BamHI, how many DNA fragment would you expect? Write out the sequence of these double-stranded DNA fragments.



ii) If the DNA shown above was cut with BclI, how many DNA fragment would you expect? Write out the sequence of these double-stranded DNA fragments.

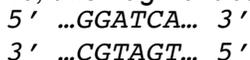


d) You can ligate the smaller restriction fragment produced in (c, i) to the smaller restriction fragment produced in (c, ii). Write out the sequence of the resulting recombinant fragment.



e) Could you cut the fragment from (d) with either BamHI or BclI? Explain.

No, this fragment cannot be cut with either enzyme. The junction site is now:



Question 2

You have isolated two different yeast strains, strain 1 and strain 2, each of which fails to grow in the absence of arginine. You want to clone the wild type copy of the gene or genes that are mutated in strain 1 and strain 2. To do so you plan to:

- 1) Obtain fragments of the entire yeast genomic DNA
- 2) Cut the chosen vector and ligate each fragment into a vector
- 3) Use this pool of original and recombinant vectors to transform *E. coli* cells
- 4) Select for *E. coli* cells that have obtained an original or recombinant vectors
- 5) Screen for *E. coli* transformed with a recombinant plasmid
- 6) Obtain recombinant plasmids from the library and transform yeast
- 7) Clone by complementation the gene that can restore the yeast to arginine prototrophy.

a) To construct a yeast genomic library in *E. coli* that will allow you to successfully complete step 7 above, what would be the **phenotype** of the yeast you would choose as the donor for the genomic DNA?

You would choose wild-type yeast or a yeast arginine prototroph.

b) You choose the vector pBlue, shown below. Note that the cloning site lies within *lacZ*, the coding region of the gene that encodes β -galactosidase. A cell that expresses β -galactosidase can take a substrate called X-gal and cleave the β -1,6 linkage to form a product that is bright blue. For each of the following sequences found on pBlue, list the step or steps (1-7 above) for which that sequence is needed and explain the role that sequence plays.

Yeast ori:

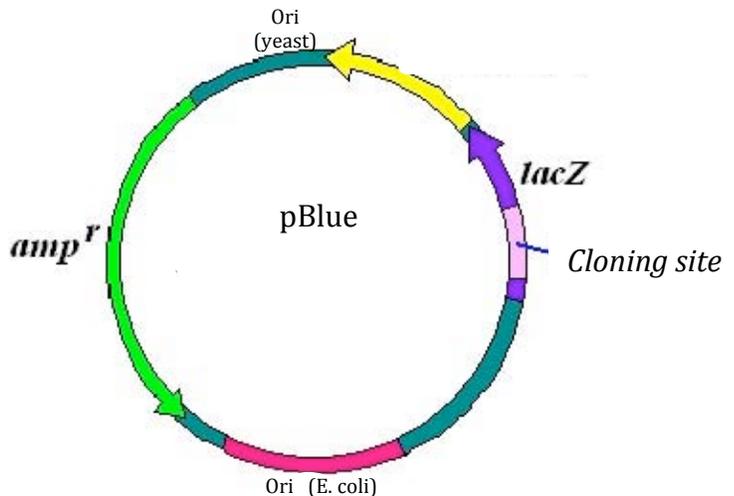
Step 6, 7. Require the yeast origin for the vector to propagate in yeast.

Amp^r:

Step 4, 5. Require Amp^R to select for cells that have taken up a vector.

E. coli ori:

Step 3-6. Require the bacterial origin for the vector to propagate in bacteria.



c) You digest both the yeast genomic DNA and many copies of the vector with the BamH1 restriction enzyme. You mix the genomic fragments with the cut vectors and add DNA ligase. You then transform *E. coli* cells with the ligation mix and plate on solid agar medium. Describe what medium you could use to distinguish the bacterial colonies that carry a non-recombinant vector from the ones that carry a new recombinant vector. Explain how this media would allow you distinguish the bacterial colonies that carry a non-recombinant vector from the ones that carry a new recombinant plasmid.

You would plate on medium containing ampicillin which will kill any cell that did not obtain an original or recombinant vector. In addition, the medium should contain X-Gal. If the cell has an original vector (i.e., no insert in the BamHI site), the β -galactosidase gene will be functional and β -galactosidase will be created, resulting in blue colonies. If the cell has a vector with an insert in the BamHI site, the β -galactosidase gene will be disrupted, and no β -galactosidase is made. These colonies will be white.

Question 2, continued

d) You successfully create a yeast genomic library in *E. coli* cells, and obtain a clone that can restore the yeast of strain 1 to arginine prototrophy. Would it be possible to use the same **library** to clone by complementation the gene that can restore the yeast of strain 2 to arginine prototrophy? Explain.

Yes. If the library is a yeast genomic library, it will likely contain the gene that may complement the mutation in strain 2.

e) You successfully identify a recombinant vector that restores **yeast** strain 1 to arginine prototrophy (clone 1). You are curious as to whether this recombinant vector can also rescue a **bacterial** cell that is arg⁻ (i.e., it is also an arginine auxotroph). Give 2 reasons why this recombinant vector will NOT work to rescue the arg⁻ bacterial cell.

Many possible reasons:

- 1. Yeast promoters don't work in bacteria.*
- 2. Bacteria cannot splice out introns (though some yeast strains have almost no introns).*
- 3. The yeast pathway may not be the same as the bacterial pathway. Or, different genes are required for the same job in different species.*
- 4. Many eukaryotic proteins have post-translational modifications.*
- 5. The mutation may be in a different gene.*
- 6. Proteins may be structurally different between yeast and bacterial version, despite being homologs (evolutionarily related).*

f) Your friend suggests that you use her yeast cDNA library to attempt to restore an arg⁻ bacterial cell to arginine prototrophy.

i) Briefly describe how a cDNA library is different from a genomic library.

Possible answers:

- 1. cDNA library is generated from mRNA, so introns have already been spliced out.*
- 2. cDNA libraries lack the endogenous promoters found in the genome.*
- 3. One may have multiple cDNAs for the same gene due to alternative splicing.*
- 4. cDNA libraries will not contain genes that are not expressed in the cells used to generate the cDNA library.*

ii) You transform arg⁻ bacterial cells with your friend's yeast cDNA library and find a clone, clone 2, that restores the cells to arginine prototrophy. What sequence NOT found on pBlue would have been present on the vector that your friend used to create this library? Explain why this sequence is required.

pBlue does not have a yeast promoter and one was not needed since the genomic insert already contains the promoter for the endogenous gene. On the other hand, when making a cDNA library we need to include a yeast promoter since cDNA fragments don't contain promoters.

iii) You transform a different arg⁻ bacterial strain with clone 2 and find that clone 2 does NOT restore these cells to arginine prototrophy. What does this suggest about synthesis of arginine in bacterial cells? Explain.

The gene in clone 2 cannot complement the missing gene in the arg⁻ bacterial strain.

Question 3

Millions of children that depend primarily on rice as a food staple become blind each year due to vitamin A deficiency. Humans cannot synthesize vitamin A and must have a supply of vitamin A or vitamin A precursor like beta-carotene in their diet. You want to create a strain of yeast that has the complete beta-carotene pathway so you can easily study the biochemistry of beta-carotene and vitamin A synthesis. You have a yeast strain that has 5 of the required 7 enzymes for beta-carotene synthesis. You need to provide these yeast cells with the two missing enzymes, *crt1* from bacteria and *psy2* from daffodils.

To clone the gene encoding *crt1* into yeast, you plan to

1. Cut bacterial genomic DNA.
2. Clone it into an appropriate expression vector (vector 1) to create a library.
3. Transform your **yeast** cells with the library.
4. Select for **yeast** cells that have obtained any vector.
5. Screen the selected colonies for production of the *crt1* protein using an antibody.

a) The cloning vector chosen for this experiment has the gene *leu1*⁺, which encodes an enzyme needed to synthesize the amino acid leucine and can be used as a selectable marker. To successfully complete step 4 above, what genotype and phenotype would your yeast strain be prior to transformation?

The yeast strain would need to be leu1⁻ and be unable to grow on media lacking leucine.

b) List the minimum features, in addition to the *leu1*⁺ gene, required in the vector for all steps 1-5 outlined above to be successful. For any DNA sequence(s) listed, indicate what type of organism it would come from.

Restriction enzyme sites, origin of replication from yeast, promoter from yeast

You are also given some DNA sequence from the *psy2* locus as shown below. To complete the production of the yeast strain capable of synthesizing beta-carotene, you decide to use Polymerase Chain Reaction (PCR) to amplify the *psy2* coding sequence based on the flanking sequence shown below.

```
5' TCCGGCGGAATTCCAAGGCCT psy2 CGTCGACTCCGGC 3'
3' AGCCGCCTTAAGGTTCCGGA GCAGCTGAGGCCG 5'
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c) Circle the set of primer(s) could you use to amplify the entire *psy2* coding sequence.

Set 1: 5' AGGCCG 3' 5' GCCGGA 3'

Set 2: 5' TCCGGC 3' 5' ACCGGG 3'

Set 3: 5' TCCGGC 3' 5' GCCGGA 3'

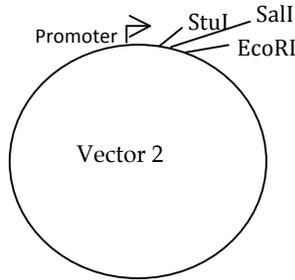
Question 3, continued

You successfully amplify the *psy2* coding sequence (repeated below for you) and plan to clone the PCR fragment into vector 2. The cloning sites available on this vector are shown below.

StuI: 5' -AGG|CCT-3'
3' -TCC|GGA-5'

SalI cuts: 5' -G|TCGAC-3'
3' -CAGCT|G-5'

EcoRI: 5' -G|AATTC-3'
3' -CTTAA|G-5'



Start codon
5' TCCGGCGGAATTCCAAGGCCT|CGTCGACTCCGGC 3'
3' AGGCCGCCTTAAGGTTCCGGA|GCAGCTGAGGCCG 5'

psy2

d) There are two different ways to insert the amplified *psy2* coding sequence into vector 2. Give the restriction enzyme(s) that you could use to cut the vector **and** the *psy2* coding sequence for each of these.

	Cut plasmid with:	Cut <i>psy2</i> loci with:
Option 1:	<i>StuI and Sal I</i>	<i>StuI and Sal I</i>
Option 2:	<i>EcoRI and Sal I</i>	<i>EcoRI and Sal I</i>

e) Which of these options would you use to create a recombinant vector that could express *psy2* in yeast? Why?

StuI and Sal I. This allows the insert to be positioned in the correct orientation with respect to the promoter.

Question 4

The following is the DNA sequence of the **wild type allele of Gene Z** that you want to amplify using the polymerase chain reaction (PCR).

5' CTCGAGGTGAATATGAAAG-----Gene Z-----CATTTGGCGCGTAATCGATA3'
3' GAGCTCCACTTATACTTTC-----Gene Z-----GTAAACCGCGCATTAGCTAT5'

a) If you amplify a DNA sequence through PCR what are the reaction components that you would **absolutely need**? Briefly state the function of each of these components.

DNA primer – provides a 3' hydroxyl for the polymerization reaction

Thermostable DNA polymerase – enzyme to catalyze extension

DNA template – the thing we want to amplify

dNTPs – nucleotides used in the reaction

(Optional) *Buffer* – keeps the reaction conditions constant over the long time

b) Circle the **set of primers** from the options below, which you would use for PCR reaction in part (a)?

Set 1: 5'TACACTTATACTTTC3' and 3'GTAAACCGCGCATTAG5'

Set 2: 5'CTCGAGGTGAATAT3' and 3'CCGCGCATTAGCTAT5'

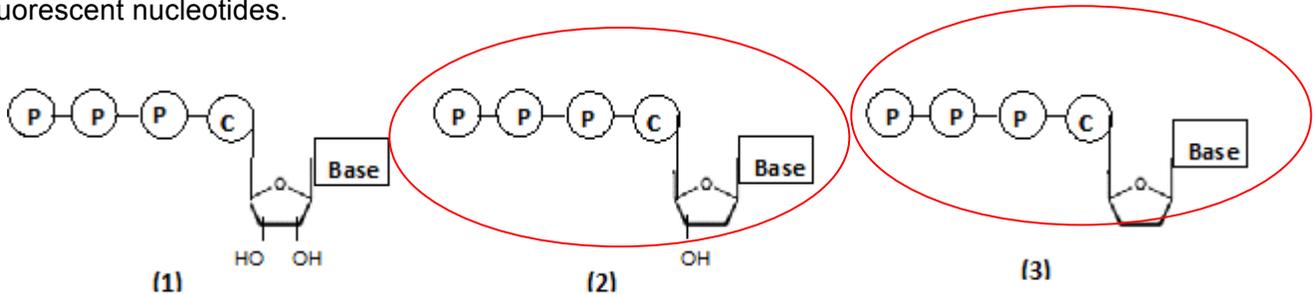
Set 3: 5'GAGTTACACTTATAC3' and 3'TGGCGAGTAATCGATA5'

Question 4, continued

c) In the PCR reaction, you need a three-step reaction cycle, which results in a chain reaction that produces an exponentially growing population of identical DNA molecules. Each step of a reaction cycle is performed at a specific temperature i.e. 95°C for Step 1, 55°C for step 2 and 70°C for Step 3. Briefly **explain** why the three steps are performed under different temperatures.

The first step at 95°C ensures that the DNA strands are melted. The second step at 55°C is meant to ensure that the template can anneal to the appropriate complementary sequence with specificity. Lastly, 70°C is the optimal temperature for the thermal-stable DNA polymerase to work.

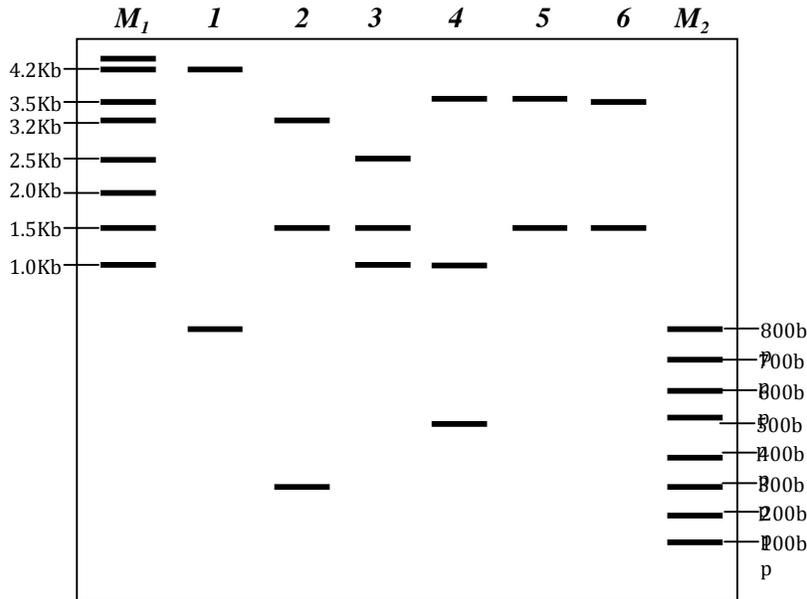
d) You decide to determine the complete nucleotide sequence of Gene Z by DNA sequencing using fluorescent nucleotides.



- Which of the above nucleotides(s) (1/2/3) are used in DNA sequencing reaction? Circle all that apply.
- Which of the above nucleotides(s) (1/2/3) would you fluorescently tag for DNA sequencing?
You would Tag nucleotide 3.

Question 5

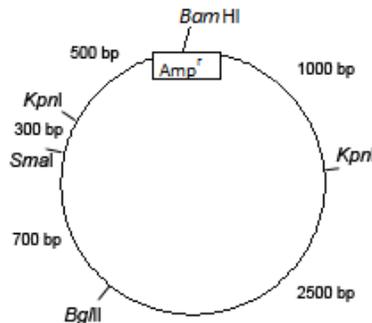
You are given a plasmid. In order to map this plasmid you set up a series of restriction digests and obtain the following results using agarose gel electrophoresis.



*M1 and M2 are DNA markers.

Lane	Digest	Size of fragments in bp
1	BamHI and SmaI	4200, 800
2	SmaI and KpnI	3200, 1500, 300
3	KpnI and BglII	2500, 1500, 1000
4	BamHI and KpnI	3500, 1000, 500
5	KpnI	3500, 1500
6	BglII and BamHI	3500, 1500

- What is the approximate size of the plasmid?
Approximately 5 kb
- Add the *SmaI*, *KpnI*, *BglII* sites to plasmid map. On your map give the distances between each of the restriction sites.



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