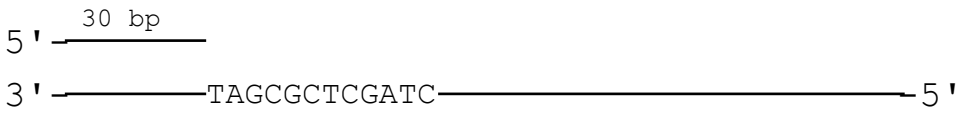
7.02/10.702 Recombinant DNA Methods
Spring 2005
Additional Exam Study Questions

Here are two more study questions to help you prepare for the RDM exam on April 26th, 2005!

Question 13

You are given a 300 nucleotide long DNA template and a 30 nucleotide (bp) long primer that anneals to the 3'end of the template DNA as shown below:



a) What will be the length(s) of the primer extension product(s) if you provide DNA polymerase, buffer needed for DNA synthesis, and the following nucleotides:

1. all four dNTPs (dATP, dCTP, dTTP, dGTP): _____
2. all four dNTPs (dATP, dCTP, dTTP, dGTP) and ddGTP: _____
3. only three dNTPs (dGTP, dATP, dCTP) and ddTTP: _____

b) Imagine that 4 nucleotide primers would work for PCR. Show where each of the following 3 primers would anneal on the denatured strands below and indicate the direction of DNA synthesis (with an arrow):

Primer 1: 5'—GTTC—3' Primer 2: 5'—GCCC—3' Primer 3: 5'—TATT—3'

5' ACTTCGTTCGCCGGGGCTCGATCGATATTTGGAAT 3'

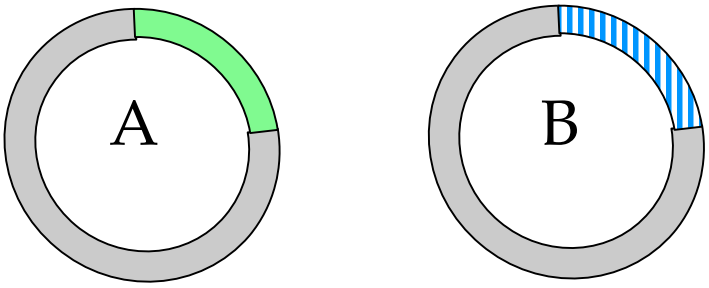
3' TGAAGCAAGCGGCCCCGAGCTAGCTATAAACCTTA 5'

c) What is the product of a PCR reaction using Primers 2 and 3 above? Primers 1 and 3 above? Explain your answer.

d) Four nucleotide primers like the ones shown in part b) would NOT be the best choice for PCR. Why not?

Question 14

Oh no! The tubes containing the two plasmids you have been given to work with have lost their labels! Plasmids A and B (shown below) were constructed using the same vector, pUC19, but contain different inserts of unknown sequence. To complicate things, each insert was cloned into the same restriction sites in pUC19 and each insert is the same length (2.5 kb).



To determine what insert is in each plasmid, you decide to use PCR to amplify the insert-containing region and then send the two PCR products to be sequenced.

a) On the figures above, draw the binding sites for the primers you will synthesize to amplify the insert-containing region in plasmid A and plasmid B. Clearly indicate what strand the primers will bind to and indicate the direction of DNA synthesis.

b) What five reagents (not counting buffer) are required for a given reaction (A,T,C,G) in **DNA sequencing**?

1. _____
2. _____
3. _____
4. _____
5. _____

c) Which of these five is **NOT** used for DNA synthesis *in vivo*? _____
Explain why not.

To learn more about the gene inserted in plasmid A, you decide to BLAST a portion of the sequence of your PCR product, and obtain the following scores and E values:

Sequences producing significant alignments:	Score (bits)	E Value
gene 1	44	0.19
gene 2	52	8e-04
gene 3	40	2.9
gene 4	40	2.9

d) Which gene (1, 2, 3, or 4) do you think is cloned into plasmid A? Why?

Question 14 (continued)

You next decide to BLAST the entire sequence of your PCR product to see if any other organisms have this particular gene. You are excited to find that *Mus musculus* (house mouse) has a gene that is very similar to your *Drosophila melanogaster* gene, and you decide to clone the mouse gene. The GenBank entry for this gene (eyes absent 2 or *eya2*) is attached.

e) Using the attached sequence, design two primers that will allow you to amplify the coding sequence of *eya2*. Make each primer less than 30 nucleotides long. Be sure to indicate the 5′ and 3′ ends of the primers and indicate which parts of the *eya2* sequence your primers are specific for (i.e. nucleotide numbers).

f) What will the **size** of your PCR product be using these two primers? _____

1: NM_010165. Mus musculus eyes...[gi:33859549] Links

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KEYWORDS    .
SOURCE      Mus musculus (house mouse)
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REFERENCE   1  (bases 1 to 2378)
  AUTHORS   Rayapureddi,J.P., Kattamuri,C., Steinmetz,B.D., Frankfort,B.J.,
            Ostrin,E.J., Mardon,G. and Hegde,R.S.
  TITLE     Eyes absent represents a class of protein tyrosine phosphatases
  JOURNAL   Nature 426 (6964), 295-298 (2003)
   PUBMED   14628052
REFERENCE   2  (bases 1 to 2378)
  AUTHORS   Clark,S.W., Fee,B.E. and Cleveland,J.L.
  TITLE     Misexpression of the eyes absent family triggers the apoptotic
            program
  JOURNAL   J. Biol. Chem. 277 (5), 3560-3567 (2002)
   PUBMED   11700312
REFERENCE   3  (bases 1 to 2378)
  AUTHORS   Zimmerman,J.E., Bui,Q.T., Steingrimsson,E., Nagle,D.L., Fu,W.,
            Genin,A., Spinner,N.B., Copeland,N.G., Jenkins,N.A., Bucan,M. and
            Bonini,N.M.
  TITLE     Cloning and characterization of two vertebrate homologs of the
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  JOURNAL   Genome Res. 7 (2), 128-141 (1997)
   PUBMED   9049631
REFERENCE   4  (bases 1 to 2378)
  AUTHORS   Xu,P.X., Woo,I., Her,H., Beier,D.R. and Maas,R.L.
  TITLE     Mouse Eya homologues of the Drosophila eyes absent gene require
            Pax6 for expression in lens and nasal placode
  JOURNAL   Development 124 (1), 219-231 (1997)
   PUBMED   9006082
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
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go_function: protein-tyrosine-phosphatase activity [goid 0004725] [evidence IDA] [pmid 14628052];
go_process: metabolism [goid 0008152] [evidence IEA];
go_process: development [goid 0007275] [evidence IEA];
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