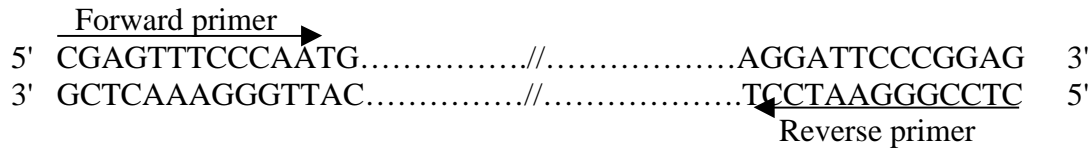


1. You would like to amplify the fragments shown below.



a) What will be the sequence of the forward primer? (indicate 5' and 3') (3 pts.)

5' CGAGTTTCCCAATG 3'

b) What will be the sequence of the reverse primer? (indicate 5' and 3') (3 pts.)

5' CTCCGGGAATCCT 3' or 3' TCCTAAGGGCCTC 5'

2. How would you clone a gene if the only information that you have is the following? (also indicate whether you would use a non-expression cDNA library, expression cDNA library, or genomic library) (6 pts. ea)

a) protein sequence of this gene

Design degenerated primers based on the protein sequence of this gene to PCR amplify corresponding cDNA clones from the cDNA library. If the cDNA clone is not full-length, one can use this cDNA clone as a DNA probe to further isolate the full-length clone from the cDNA library.

b) antibody against the protein encoded by this gene

Use the antibody as a probe to screen a cDNA expression library for the gene

c) a DNA fragment from the promoter of this gene

Using this DNA fragment from the promoter region as a DNA probe to screen for this gene from a genomic library. After one gets the genomic clone, then can use this genomic clone as a DNA probe to screen for its full-length cDNA clone from a cDNA library.

d) chromosomal map of a mutation that affects the activity of this gene (you also know the genomic sequences of two genes that locate at the left and right side of this gene, respectively)

Using the chromosome walking technique, start from a genomic DNA clone corresponding to one of the known genes, use this clone as a DNA probe to isolate overlapping genomic clones from a genomic library, and thus walk towards to mutant locus. Check if one of the genomic DNA clones can rescue the mutant phenotype.

4/29/02

3. Ras is a very important signaling protein that regulates appropriate differentiation and proliferation of mammalian cells. It has been known for a long time that Ras needs to physically associate with its downstream effectors to exert its effects on cells. Describe how you would isolate the downstream effectors of Ras. (10 pts)

Use radioisotope to label the Ras protein and then use the labeled Ras protein as a probe to screen for Ras-interacting proteins using a cDNA expression library.

4. A scientist has recently isolated a full-length cDNA clone encoding a nuclease involved in degrading chromatin during the cell death process. He suspected that this nuclease may be toxic to the bacterial cells. Please advise him how to express and purify this nuclease using bacteria as the host cells. (10 pts).

**Subclone the full-length cDNA clone into an inducible protein expression vector
Attach a fusion tag such as His tag to facilitate its purification**

Bonus (3 pts)

You have just made an expression library (with inserts directionally cloned) using a lambda vector. You have both an antibody and a fragment of the gene. You screened the library with the antibody first, but got only two positive clones. You repeated your screen of the same library, this time with the probe made from your DNA fragment. This time you obtained seven positive clones. Explain why you get that many more clones when you screen with a DNA probe.

In an expression library, on the average only one out of three clones will be inserted in the correct reading frame. Therefore, you will obtain approximately three times more clones when screening with a DNA probe compared to when screening with an antibody.

MCDB 3500 EXAM #6
4/29/02

Name: _____