Your **Name:** _____ Section & PD: _____

Opening Declaration:

DR. NEDWIDEK SBS11QGR SCARY Cloning, Sequencing & Translation Activity: Oct. 24-25, 2013

Aim: How do we clone a gene? **HW#8** to be done in advance of class 10/25/2013 (Fri).

I will be immersing you in molecular biology over the next several days to prep for your CSH field lab: this is essentially Griffiths chapters 7,8,9 & 20. Please read pp. 325-333 and pp. 731-737 by Thurs 10/24/13! Much of this material is totally unfamiliar. You need to USE book and internet, particularly for the last part. Go here to the site I set up for last year's class for an overview of concepts: www.dnai.org/lesson/go/17604

You use PCR to amplify a 45 base pair DNA fragment for gel electrophoresis, and run it on a gel.
(A) From what you know about PCR, is it necessary to first cut it with restriction enzymes before running it on an electrophoresis gel, or not?_____

(B) Using the same primer set as you did above, you sequence what you believe is both the sense antisense DNA for the region you isolated, but then you find out you ran two antisense reactions. You have a tight deadline and need the sense sequence, so you decide to generate the sense strand and the associated translation by hand on paper. With the key below, carefully carry out this task, paying attention to and indicating below the orientation of the antisense strand. Use the amino acid letter codes for the translation. To recap, transfer the antisense below from 3'-5', find the complimentary sense strand, write it out 5'-3', and translate it N to C to identify what the code says. If you are unsure of how to do this, refer to Griff 301 and Kraus 380.

Anti-sense (Anti): 5'TCAGTTGTCGTCCCAGGCTAGTAGGGCGTGATAGGGGGGGGCGTG3'

Translation Decoder:

Codon	Amino Acid	One letter amino acid (AA) code
GCC	Alanine	**A**
GAC	Glutamic Acid	**E**
CAC	Histidine	**H**
CTA	Leucine	**L**
AAC	Asparagine	**N**
CCC	Proline	**P**
TGG	Tryptophan	**W**
TAT	Tyrosine	**Y**
TGA	Stop	**!**

*NOTE: Please DO NOT EDIT the "spelling" of the AA, and please limit your answer to the provided space.

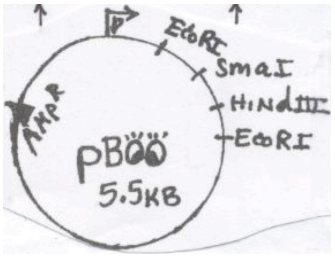
Fill in bases, 5'& 3' on both DNA (D) strands, 1-letter AA code:

Anti D: 3'----------5'

Sense D: 5'----------3'

AA Code: - - - - - - - - - - - - - - -

(C) You want to clone the 45 base pair fragment above into a 5.5 kilobase vector called peekaBOO, drawn below. Which restriction site(s) in which order would you engineer in the above sequence to orient the fragment downstream of the indicated promoter (|P →), and in the right orientation for expression? Site sequences: *EcoRI*: GAATTC / CTTAAG *HindIII*: AAGCTT / TTCGAA *SmaI*: CCCGGG / GGGCCC



(1) What must you look out for in Choosing your sites?_____
_____ (2) What site(s) do you choose and Why? ↖Write answer in space above, left. ↖

Closing Declaration: