NAME_____TA_____ SEC____

7.012 Problem Set 4 FRIDAY October 15, 2004
Answers to this problem set must be inserted into the box outside

Problem sets will NOT be accepted late. Solutions will be posted on the web

Question 1

NASA astronauts found a mysterious gene from the H zone of outer space. They needed your help to know more about the gene, so they cloned it into a plasmid vector and told you they would send you the plasmid, that they named *p*SET (plasmid "Sequence Extra-Terrestrial"). When the package arrived, however, you found two unlabeled tubes.

Not sure of what they sent you, you decide to make restriction maps of what's in each tube, assuming that there is plasmid DNA in each.

You perform digestions with the restriction enzymes *Bgl*II (B), *Not*I (N) and *Sal*I (S) either alone or in combination and obtain the following gel-electrophoresis data.
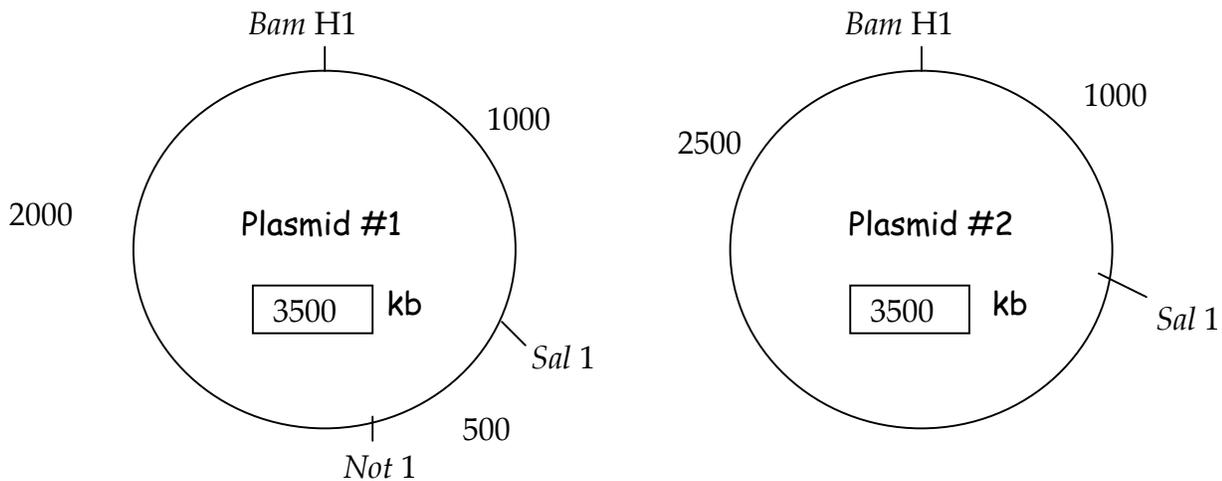
a) Draw the restriction maps of the plasmids in the space provided below. Write the distances between the restriction sites. Also write down the total sizes of the plasmids in the boxes below, in kilobases.

Bam H1

1000

2000

Plasmid #1

3500 kb

Sal 1

500

Not 1

Bam H1

1000

2500

Plasmid #2

3500 kb

Sal 1

b) Confused, you call NASA ana ask them what's going on and why they sent you two tubes. They do not know why you got two tubes, but they know that the gene of interest had been cloned into the *Not*I and *Sal*I restriction sites of the vector. Which of the plasmids has the gene? Circle your answer.

Plasmid #1   Plasmid #2

c) What is the length of the cloned gene in bases?

500

d) NASA also told you that the plasmid they used is a bacterial expression vector. Which one of the following elements should be in the vector? Circle all that apply.

centromere	multiple cloning sites	nuclear localization signal
telomere	inducible terminator	bacterial origin of replication
selectable marker	nucloid insertion sites	cytosolic targeting sequences
inducible promoter	topoisomerase gene	gene encoding restriction enzyme

Question 2

You rename the gene *GOSH* for *"gene from the outer space H."* For some reason, NASA wants you to study the effect of expressing *GOSH* in amphibians. To express *GOSH* in amphibian cells, you have to clone it into an amphibian expression plasmid (*pEA*) with an amphibian promoter. Below is the map of *pEA*.

5'GGATCC 3'	*Bam*HI recognition sequence
5'CTCGAG 3'	*Xho*I recognition sequence
5'GCGGCCGC 3'	*Not*I recognition sequence
5'GTCGAC 3'	*Sal*I recognition sequence

a) Since *pEA* does not contain *Not*I and *Sal*I sites, you decide to PCR-amplify *GOSH* with primers that will replace the *Sal*I and *Not*I sites with **Bam*HI and *Xho*I sites**. You know the sequences of the 5' and 3' ends of *GOSH* as they are in the bacterial plasmid:

```
        Start                                           Stop
         ___                                             __
5'GTCGACatggtcgccatgcga………………………..tgctcgatatcgtaaGCGGCCGC3'
_____                                         _____

   SalI                                                NotI
```

Which of the following primer pairs would you use to successfully amplify the gosh gene with the desired restriction sites at either end? **All** primer sequences are written in the 5' → 3' direction. Circle **all** that apply.

Pair1: GTCGACATGGTCGCCATGCGA   and   GCGGCCGCTTACGATATCGAG

Pair2: TCGCATGGCGACCATGTCGAC   and   GGATCACTACGAGATCGAGCA

Pair3: GGATCCATGGTCGCCATGCGA   and   CTCGAGTTACGATATCGAGCA

Pair4: TCGCATGGCGACCATCTCGAG   and   TGCTCGATATCGTAAGGATCC

Pair5: CTCGAGATGGTCGCCATGCGA   and   GGATCCTTACGATATCGAGCA

Both pair 3 and pair 5 will successfully amplify the *GOSH* gene with *Bam*HI and *Xho*I sites at the end; however, only one pair, pair 5, will produce a PCR product that can be correctly inserted into *pEA* such that the *GOSH* gene will be expressed from the amphibian promoter.

Now you try to express the new construct (*pEA-GOSH*) in frog (Xenopus) embryos. Hmm... Something seems wrong and the expected peptide is not expressed. Since PCR can introduce errors, you sequence *pEA-GOSH* from its 5' end and obtain the following sequencing gel:

b) What is the sequence in the gel? (in 5' → 3' direction)   **ATGGTCGCCATAGCGA**

ddA	ddC	ddG	ddT
▬			
		▬	
	▬		
		▬	
▬			
			▬
▬			
	▬		
	▬		
		▬	
	▬		
			▬
		▬	
		▬	
			▬
▬			
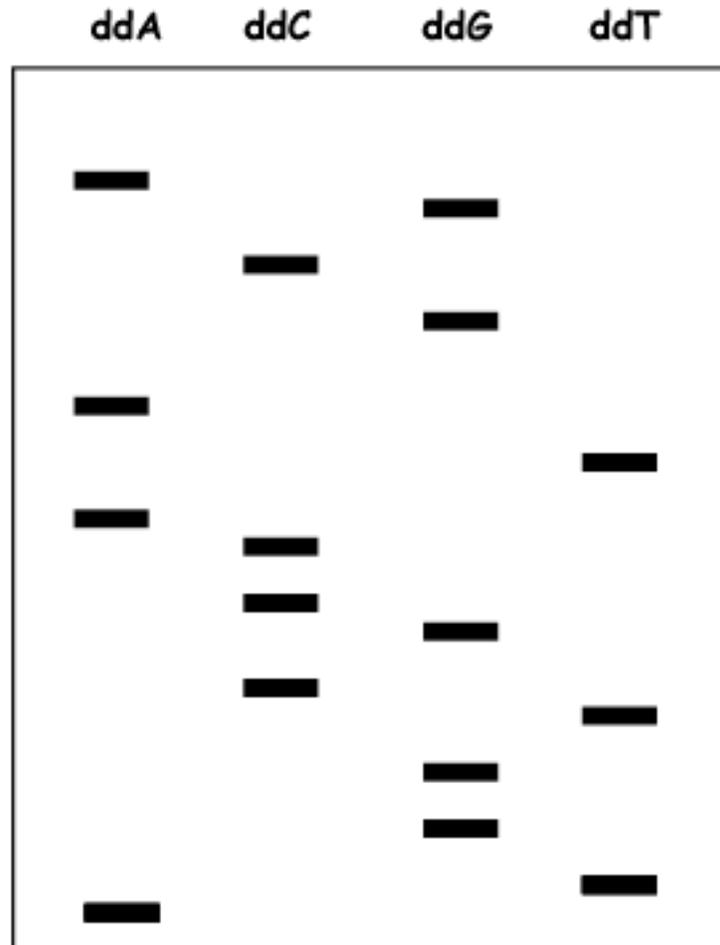
c) Based on the sequence data, why is *GOSH* not expressed? Circle all that apply.

i) Restriction enzyme recognition site is lost, resulting in an immature, nonfunctional peptide.

ii) A nonsense mutation is introduced, introducing an early stop codon.

iii) The promoter in the plasmid is lost, so *GOSH* cannot be expressed.

iv) A frameshift mutation is introduced, so the encoded protein has a different primary structure from *Gosh*.

Question 3

After fixing the mutation, you express *pEA-GOSH* in frog embryos. The transformed frogs show abnormal eye-tongue coordination. You call this phenotype *Helpless*. (See an artist's depiction below.)

frogs transformed with *pEA* vector only:

frogs transformed with the *pEA-GOSH* construct:

How to keep *GOSH*-expressing frogs alive in the laboratory. Place image like so. (Otherwise they would starve to death.)
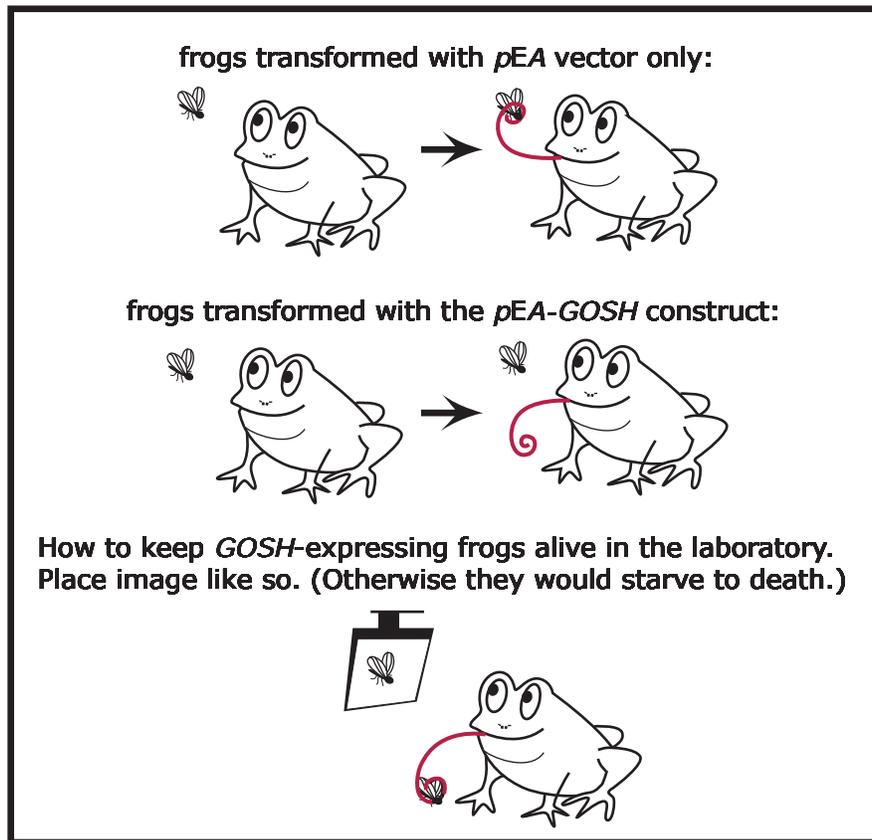
Figure by MIT OCW.

To better understand the function of *Gosh*, you construct two more plasmids. The first one is *pEA-GFP*, which will express the green fluorescent protein in amphibians. The second one is *pEA-GOSH-GFP*, which will express a fusion protein of *Gosh* and GFP. You examine the impact of expressing these plasmids in frogs.
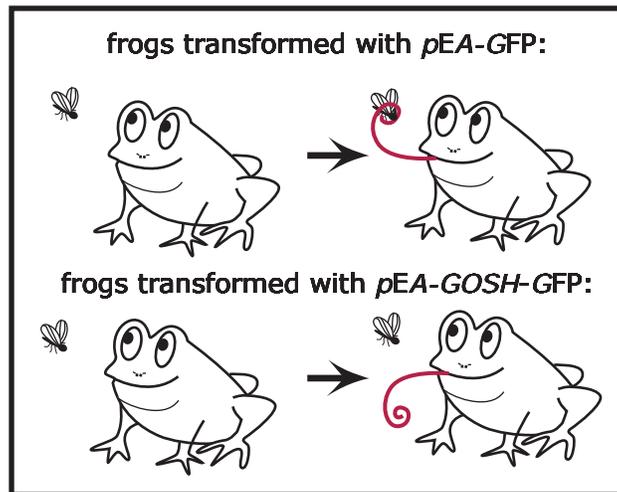
Figure by MIT OCW.

a) According to the result above, does the presence of GFP affect the function of *Gosh*? Briefly explain your answer. *No. The fusion of GFP to GOSH does not change the phenotype caused by GOSH itself. Thus GFP does not affect the function of GOSH.*

b) Under a microscope, you compare the green fluorescence signals in frog neuronal cells that express either GFP alone, or the *Gosh*-GFP fusion protein. (If your P-set is printed black and white, please look at this colored figure in the PDF file on a computer screen.)
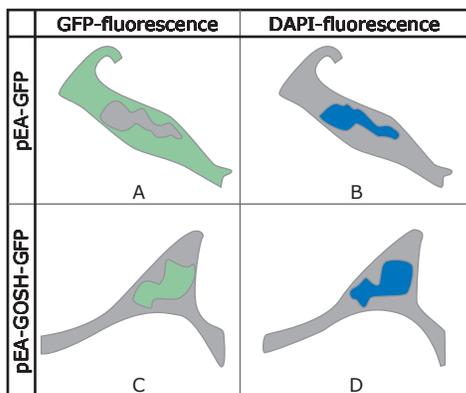
Figure by MIT OCW.

Legend:

A and B are two independent microscopy pictures of the same neuronal cell that expresses GFP.

In A, the green signal comes from GFP molecules. In B, the blue signal comes from DAPI molecules. DAPI is a blue dye that binds (and therefore stains) DNA.

Likewise, C and D are two pictures of another neuronal cell, which expresses the *Gosh*-GFP. Green signal comes from GFP molecules and blue signal comes from DAPI molecules.

c) What do these images suggest? Circle all that apply.

i) GOSH contains an NLS.
ii) GFP contains an NLS.
iii) GOSH-GFP is transported into the central vacuole.
iv) GOSH-GFP is synthesized by ribosomes on rough ER.
v) GOSH-GFP is a part of the nuclear pore complex.

d) You intend to find out which part of the *Gosh* protein causes the phenomenon seen in part B. How would you do it? (Please keep your answer within 20 words. Do NOT go into technical details.

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Just write down the <u>principle</u> of your experiment briefly.) *Either sequence and look for NLS or cut up gene in snippets and attach it in frame to gene encoding GFP.*

Question 4:

A human geneticist next door comes to you to discuss a hyperactive human mutant, who in his bone marrow can convert a small amount of adenosine into caffeine. After doing some research, you find out that this person grew up in a region where *Coffea mirus* (a coffee tree) was abundant. At age 10, he had a viral infection during a viral epidemic in *Coffea mirus*.

a) You want to find out if a known enzyme that makes caffeine in *Coffea mirus* is **expressed** in the human mutant's bone marrow. Which of the following methods would you use?

i) Create a cDNA library from the mutant's bone marrow. Then probe for the cDNA encoding the enzyme in the library.

ii) Create a genomic DNA library from the mutant's bone marrow. Then probe for the gene encoding the enzyme in the library.

iii) Create an RNA library from the mutant's bone marrow. Then probe for the messenger RNA of the enzyme in the library.

iv) Create an rDNA library from the mutant's bone marrow. Then probe for the rDNA of the enzyme in the library.

v) Create an rRNA library from the mutant's bone marrow. Then probe for the rRNA of the enzyme in the library.

b) Suppose you do find the coffee-tree enzyme expressed in the mutant's bone marrow. What is a likely vehicle that transferred the gene from the coffee tree to the human mutant? [Give a very short yet concise answer, please.] *Virus.*

We hope you liked this p-set. Please remember to hand it in by 4:40pm on Oct 21.