Solution key - 7.012 Recitation 10 - 2010

Questions:

1. You are interested in making many copies of a specific DNA sequence. The sequence that you want to amplify is flanked by regions with the sequence given below:

Primer 1 should bind in this region — Primer 2 should bind in this region

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5′ CGCGCGAATTCGATCGA————————————————TTAATACGTACTAG 3′
3′ GCGCGCTTAAGCTAGCT————————————————AATTATGCATGATC 5′
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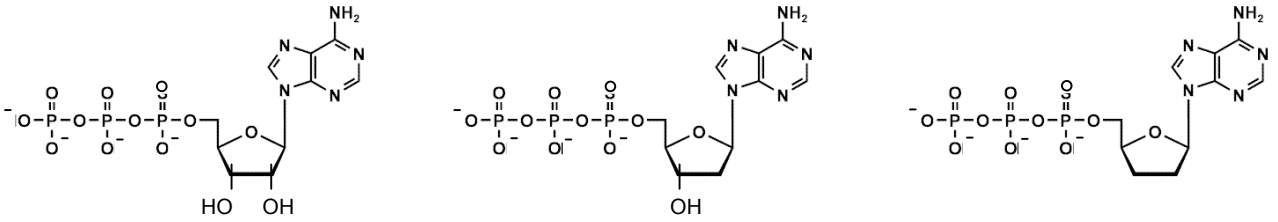
Circle the set(s) of primers that will amplify this region.

	Primer 1		Primer 2
Set A:	5′TCGATCGAATTC 3′	AND	5′TAATACGTACTA 3′
Set B:	5′GCTTAAGCTAGC 3′	AND	5′GATCATGCATAA 3′
Set C:	5′GAATTCGATCGA 3′	AND	5′CTAGTACGTATT 3′

2. Take a look at the molecules drawn below

#1 #2 #3



a) Which of the above molecules is non-physiological and is only used in DNA sequencing? *#3 since it only has H atoms at the 2′C and 3′C position of the sugar (i.e. it has dideoxyribose sugar).*

b) Which one of the above molecules is used in RNA? ? *#1 since it has OH groups at the 2′C and 3′C position of the sugar (i.e. it has ribose sugar).*

c) Which one of the above molecules is used in DNA? *#2 since it has OH groups at the 3′C and H atom at 2′C position of the sugar (i.e. it has deoxyribose sugar).*

d) Which one of the above molecules is used as the major source of energy in cells?
#1 since it is ATP, which serves as the energy currency of the cell.

3. You decide to sequence the following piece of DNA using the following primer:

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5′–…GTATAGCCTCCGT…–3′
3′–…CATATCGGAGGCA…–5′
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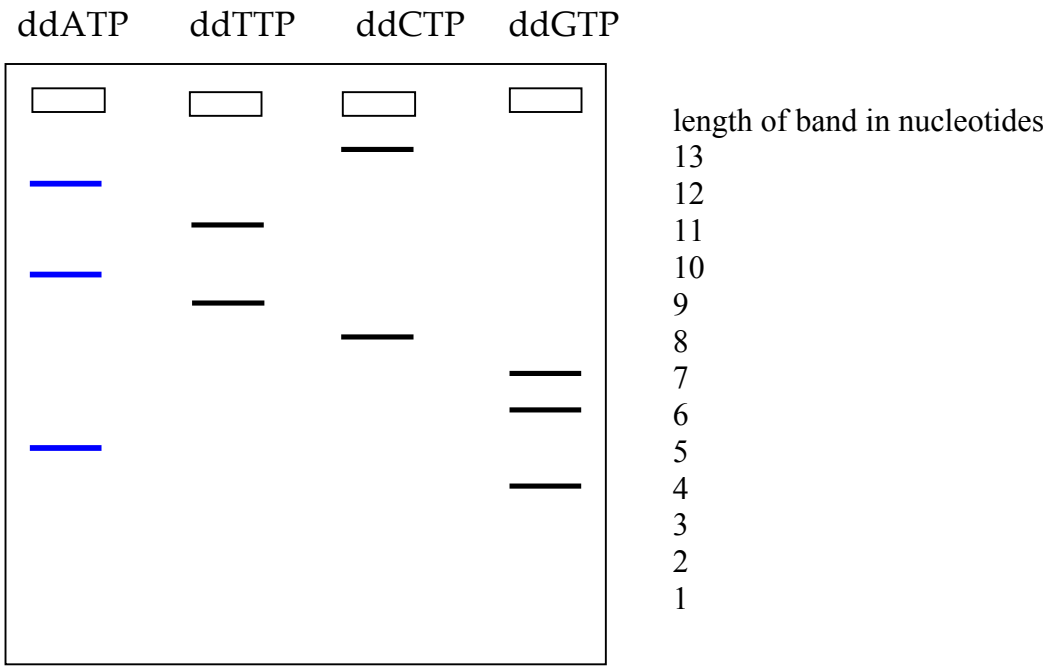
The primer you use is 5′-ACG-3′ (Note: real primers are usually 18-22 nts long). You set up a DNA sequencing reaction with this DNA, this primer, DNA polymerase, dATP, dTTP, dGTP, dCTP, and ddATP.

a) How many different products would form from this reaction?
Since you are using ddATP in the reaction mixture halts whenever there is an incorporation of a ddATP instead of dATP. Since there are 3A's in the sequence (excluding the A that is a part of the primer) and therefore you may have 3 reaction products of different lengths. There is always a probability that you may have a reaction product where on dATP is incorporated and NO ddATP is incorporated at all. Combining these two together you may have 4 reaction products (5b, 10b, 12b and 13bases long).

b) How many nucleotides long would each different product of this reaction be?
They would be 5b, 10b, 12b and 13bases long).

c) The sequencing gel is shown below. Draw into the first lane what bands would appear in this lane after you run the gel. Keep in mind that the gel is a denaturing gel.



i. Write the sequence of DNA that you read from this gel below **including the primer**. Label the 5′ and 3′ ends of the molecule.

5′–…ACGGAGGCTATAC…–3′

ii. Would you be able to tell the sequence of the molecule if you had loaded into a single lane a reaction in which all four ddNTPs had been added from a lane?

Yes, but only if each ddNTP was labeled with a fluorophore of different color.

4. You are interested in what makes a certain bacterial species that lives in the deep sea produce light. You mutagenize cells and perform a genetic screen for mutants that don't produce light. You find two mutant colonies, mut1 and mut2. You transform the mut1 cells with a plasmid library (which is made up of 5000 plasmids, each of which contain one gene from the wild-type bacterial genome), so that every cell gets one plasmid. You screen the transformed cells and find one colony that now can produce light.

i. Explain why this colony of mut1 cells can produce light.

The bacterial cells that make this colony have been transformed with a recombinant plasmid that had the light producing gene as an insert.

ii. How would you identify your gene of interest (which you name "*lyeT*") now that you have this colony?

You isolate the recombinant plasmid from this colony and digest it with a specific restriction enzyme(s) that have recognition sites at the two ends of your lyet gene insert so that you end up obtaining a band that corresponds to the plasmid alone and a band that corresponds to the lyeT gene insert. You can then sequence this gene using PCR by designing primers that are complementary to the flanking regions of the lyeT gene.

iii. You try to identify the gene mutated in "mut2" by complementation and it doesn't work, but you know that your library contains every gene in the genome. You sequence the *lyeT* gene in mut2 cells and find that it contains a mutation. Propose a possible explanation for your inability to identify that there was a mutation in *lyeT* in the mut2 bacterium using this strategy.

The mutation in lyeT gene in mut2 is resulting in a dominant phenotype. Or, mut2 has some other mutation that prevents the expression lyeT gene or functioning of the corresponding protein.