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

ANSWER KEY

Question 13

a) The lengths will be:

1. **all four dNTPs (dATP, dCTP, dTTP, dGTP):** **300 base pairs**
2. **all four dNTPs (dATP, dCTP, dTTP, dGTP) and ddGTP:** **34, 36, 38, 42 bp (and others corresponding to positions of Cs in the template)**
3. **only three dNTPs (dGTP, dATP, dCTP) and ddTTP:** **32 base pairs**

b)

Primer 1: 5′—GTTC—3′
binds to: 3′—CAAG—5′

Primer 2: 5′—GCCC—3′
binds to: 3′—CGGG—5′

Primer 3: 5′—TATT—3′
binds to: 3′—ATAA—5′

```
5′  ACTTCGTTCGCCGGGGCTCGATCGATATTTGGAAT  3′
          <--3′-CGGG-5′ (primer 2)

3′  TGAAGCAAGCGGCCCCGAGCTAGCTATAAACCTTA  5′
    5′-GTTC-3′-->          5′-TATT-3′--->
      (primer 1)             (primer 3)
```

Remember: Synthesis of DNA *in vivo* ALWAYS proceeds 5′-3′. (The next nucleotide is added to the 3′ OH of the previous nucleotide.) This is an intrinsic property of the DNA polymerase!

c) **Primers 2+3: no exponentially amplified product because the primers face in opposite directions!**
Primers 1+3: no exponentially amplified product because the primers bind to the same strand!

In order to get a PCR product, you need two primers that bind to opposite strands of template and face each other (synthesis from each primer occurs in the direction of the other primer). This way, you generate binding sites for new primers in each cycle –i.e., a new set of "template DNA" molecules--and get exponential amplification!! Neither of these sets of primers meet this criteria.

d) The primer binding site will occur on average every $(1/4)^4$ base pairs, or once every 256 basepairs. Thus, four base pair primers will bind to many sites in the genome, and not to your target sequence specifically.

Question 11

a) Forward primer: binds to "inner" strand; DNA synthesis toward insert; within pUC19 sequence NOT insert. Reverse primer: binding to "outer" strand; DNA synthesis toward insert; within pUC19 sequence NOT insert. Note that the primers can/should be the same for A and B!

b) The five reagents required are: dNTPs; ddNTP; DNA polymerase; primer; template DNA.

c) ddNTPs are not used in vivo. ddNTPs cause termination of DNA synthesis because they lack the 3′ OH group required for the addition of the next nucleotide.

d) Answer: gene 2. Why: gene 2 has the highest "score" and lowest "E value"; these are hallmarks of sequences that are closely related to the sequence entered into BLAST (here, the PCR product from plasmid A!).

e) The primers you design for this problem have to have two "properties": 1) they must solve the problem that is asked (here, to amplify the *eya2* gene for cloning); 2) they must be "primerlike." Let's look at these one at a time:

Solving the problem:

> **To amplify the *eya2* gene, the forward primer has to have the same sequence as the GenBank sequence, and the reserve primer has to be the reverse complement of the GenBank sequence. (GenBank sequences are the "coding" strand read from 5'-->3'.) The primer pair you design must also amplify the entire gene (that is, the PCR product must contain both the start and stop codon of the *eya2* gene).**

"Primerlike":

> **Your primers must meet the following criteria:**
>
> 1. **Primer has 5′-3′ labeled**
> 2. **Primers have similar Tms (ideally 55-65C)**
> 3. **Primers have ~50-60% GC content**
> 4. **Primers give nucleotide #s to which they bind**

f) The size will vary based on your primers, but the size can be determined mathematically using the formula: nt# of "beginning" (5′ end) of reverse primer- nt# of "beginning" (5′ end) of forward primer + 1.