Bi 10 Prelab #1

Any questions regarding this prelab should be addressed to Yicheng Luo at ycluo@caltech.edu

1) **What is the role of each essential component in a PCR reaction? (10 points)**
2) **What are the basic steps of PCR? And what is the purpose of each step? (6 points)**
3) **A. Please design forward and reverse primers for the sequence blew. Label each primer direction with 5' to 3' and use a size range from 21nt to 25nt. (2 points)**

5'_ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGG
ACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTA
CGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCC
TCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG
CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCT_3'

B. Design a set of primers that would allow you to ligate the PCR product into a vector using BAMH1 on the 5' end and Xba1 on the 3' end. The sequences that BamH1 and Xba1 recognize are show below. (label direction as 5' to 3', size range is from 21nt to 25nt) (3 points)

Restriction enzyme	Sequence
BamHI	GGATCC
XbaI	TCTAGA

C. What is the purpose of adding additional nucleotides when you design primers for restriction enzyme digestion? (2 points)

D. For cloning certain genes from cDNA or genome, what kind of polymerase should you use to avoid point mutation during PCR reaction? Please list at least TWO specific polymerases (hint look at specific characteristics of different brands). (2 points)