Homework MNB MocBio Lecture 5-6 (Stowell)

1) The following protein sequence is for feredoxin. From the codon table in this lecture, convert this sequence to a DNA sequence and design a single primer that will mutate met 16 to a ser. Be sure to check that your primer melting temp is reasonable.

- MERLTVVLDA SACCAMGRCA ATAPEIFDQD PETGIAVLLD ATPPPELHES ARLCAELCPC EAITVTEG

Met Glu Arg Leu Thr Val Val Leu Asp Ala Ser Ala Cys Cys Ala Met Gly Arg Cys Ala
ATG GAA CGT CTT ACT GTT GTT CTT GAT GCT TCT GCT TGT TGT GCT ATG GGT CGT TGT GCT

Ala Thr Ala Pro Glu Ile Phe Asp Gln Asp Pro Glu Thr Gly Ile Ala Val Leu Leu Asp
GCT ACT GCT CCT GAA ATT TTT GAT CAA GAT CCT GAA ACT GGT ATT GCT GTT CTT CTT GAT

Ala Thr Pro Pro Pro Glu Leu His Glu Ser Ala Arg Leu Cys Ala Glu Leu Cys Pro Cys
GCT ACT CCT CCT CCT GAA CTT CAT GAA TCT GCT CGT CTT TGT GCT GAA CTT TGT CCT TGT

Glu Ala Ile Thr Val Thr Glu Gly
GAA GCT ATT ACT GTT ACT GAA GGT

The last base of each codon can also usually be C,A,G.

met 16 is ATG and to change to Ser I need TCT. So a primer will have upstream and downstream matching sequence and a mismatch at all three bases. The primer GCT TGT TGT GCT **TCT** GGT CGT TGT GCT or the opposite strand primer AGC ACA ACG ACC **AGA** AGC ACA ACA AGC would work. Using the Tm calculator giving at the beginning of the lecture the following Tm is calculated 63.5 which is reasonable Tm for PCR.

2) Given the following DNA sequence, design **two** optimal PCR primers to amplify this DNA

- 5'ATATTTTGACGGCTGAGGACTAAGTCTGACCGAGACGTATAG CTACGTACGTTGCGGGCCCCGGCCCGGC3'

5'- ATA TTT TGA CGG CTG AGG ACT AAG TCT GAC -3' Forward primer Tm 59.5

5'- GCC GGG CCG GG -3' Reverse primer Tm 58.2