**CHEM455 BIOCHEMISTRY LABORATORY**
**MIDTERM EXAM SAMPLE QUESTIONS**

1. Describe in detail how to isolate the protein B from the mixture of proteins A/B/C/D using a gel filtration chromatography column (G-75; fractionation range of 4,000-80,000) and an ion exchange chromatography column (Q or S column). Specify the pH of your buffer solution to be used for the ion exchanger.

| Proteins | A | ***B*** | C | D |
|---|---|---|---|---|
| pI | 7.5 | ***7.1*** | 9.2 | 7.2 |
| Molecular Weight | 11,000 | ***30,000*** | 30,500 | 56,000 |

2. The amino acid and DNA sequences of a newly discovered protein are shown below.

```
   1 - ATGCTCACGGTTCGTGTCCTGCAGGCCCATCGCCTACCCTCTAAGGACCTAGTGACCCCC  -  60
   1 -  M  L  T  V  R  V  L  Q  A  H  R  L  P  S  K  D  L  V  T  P  -  20

  61 - TCTGACTGCTACGTGACTCTCTGGCTGCCCACGGCCTGCAGCCACAGGCTCCAGACACGC  - 120
  21 -  S  D  C  Y  V  T  L  W  L  P  T  A  C  S  H  R  L  Q  T  R  -  40

 121 - ACGGTCAAGAACAGCAGTAGCCCTGTCTGGAACCAGAGCTTTCACTTCAGGATCCACTAG  - 180
  41 -  T  V  K  N  S  S  S  P  V  W  N  Q  S  F  H  F  R  I  H  *  -  60
```

a) Design two end primers to clone the above sequence into an expression vector using BamH1 (recognition sequence: 5'-ggatcc-3') and Nde1 (5'-catatg-3') as 5' and 3" restriction enzymes, respectively (15 points).

b) Assuming that your end primers have Tm of $58^0$C and $60^0$C respectively, design an optimal PCR protocol, including temperature and duration of denaturation, annealing and extension steps.

** There will be 5 to 6 questions like these in the actual exam.*