BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI
SECOND SEMESTER 2016-17
BIO G642 Experimental Techniques
COMPREHENSIVE EXAMINATION

Max. Marks: 60 **Time: 150 mins** **Date: 05/05/17**

Note:

- **The paper consists of two parts: Part A (Closed Book) and Part B (Open Book)**
- **Attempt both the parts in separate Answer sheets.**
- **"NO" marks would be allotted if you don't justify your answer.**

PART-A (Closed Book) (45 Marks)

Q1.

a) During ELISA, towards the end we added diluted solution of H_2SO_4, which changes the color of the solution from blue to yellow. Is there a need to convert substrate from blue to yellow in ELISA? Is the first color change from white to blue sometimes flawed?
b) Explain the mechanism for detection using HRP and ALP labels on antibodies. **[4M]**

Q2. Briefly describe how ammonium sulfate precipitates proteins. Under what circumstances you will use ammonium sulfate precipitation for your protein sample. **[3M]**

Q3. You have performed a western blot of a protein sample isolated from crude E.coli lysate. However, to your dismay instead of discrete bands you observe a high background. State the possible reasons for your results. Describe what measures you need to take to get proper results. **[3M]**

Q4. What is the role of urea in protein purification process? **[2M]**

Q5. Explain why: **[2x6=12M]**

a) Semi-dry blotting is less time consuming still less used than tank blotting.
b) Glacial acetic acid is used while staining as well as destaining of protein gels
c) SDS- PAGE can't be run horizontally.
d) Protein Purification is performed at low pressure.
e) SDS used in the gel and sample lysis buffer is important for the transfer of proteins.
f) PMSF is added to the pellet before going for protein purification.

Q6. PVDF membrane has advantages over nitrocellulose membrane to transfer proteins for Western blot analysis. However, nitrocellulose membrane is more commonly used for above purpose. Why? Can you use simple Whatmann paper instead of these membranes? Explain your choice. **[3M]**

Q7. From a small culture, you have purified the DNA of a recombinant plasmid. You have resuspended the DNA in a volume of 50uL. You dilute 20uL of the above sample into a total volume of 1000uL using distilled water. You measure the absorbance of this diluted sample at 260nm and 280nm and obtain following readings- A260 = 0.550; A280 = 0.324.

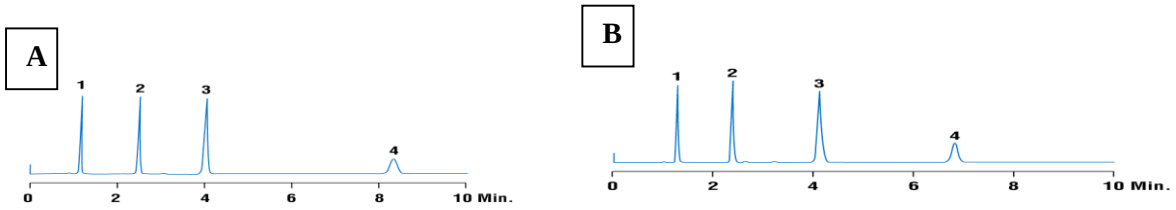
a) What is the DNA concentration of the purified plasmid?

b) Comment on the purity of DNA. **[2M]**

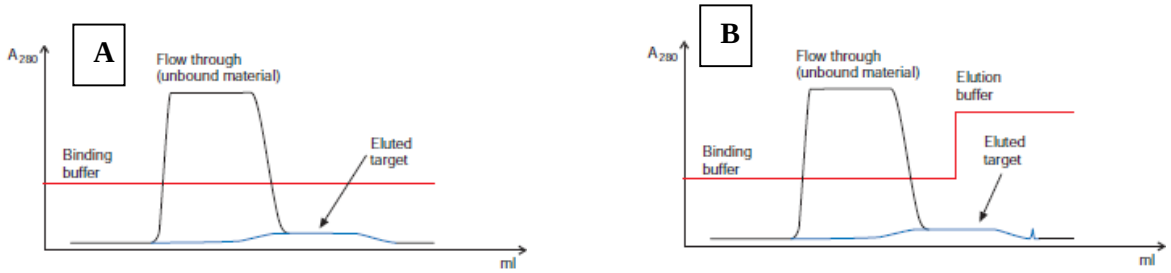
Q8. What are inclusion bodies and when are they generally formed? **[2M]**

Q9. In SDS– PAGE, give name & function of different reagents that aid in polymerization. **[2M]**

Q10. What do you understand by Capacity factor and Selectivity factor in HPLC? The following two chromatograms were generated on the same column several weeks apart. Notice the reduction in Capacity Factor of the last peak. What can be the cause for this change? **[6M]**



Q11. Heena is purifying a protein with NI-NTA affinity column.She did her purification for 2 times and obtained a result as shown in Fig A and B. Was she successful? What do you think is the problem? Can you help her troubleshoot the problem? **[6M]**



Part B (Open Book) 15M

Q12. Ashish and Kreeti are working with separation of mixtures with HPLC. Ashish is using a C-18 column whereas Kreeti is using an amino ($-C_3H_6NH_2$) column. Both are having a mixture (hypothetical) of following compounds, which they want to separate- toluene, acetaldehyde, isopropanol, propane and nitroglycerine. Will the mixtures elute out in the same way for both. Draw a pattern of elution of the compounds from the mixtures with time and comment? **[5M]**

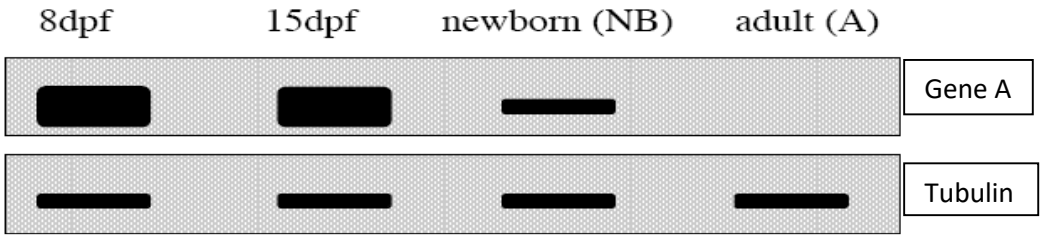
Q13. Your lab project focuses on a gene 'A' that you suspect to be involved in the embryonic development of the mouse. To study this gene, you isolate total DNA, total RNA, and total protein samples from mice at the following developmental stages:

1) Early embryos (8 days post fertilization or 8dpf)
2) Late embryos (15 days post fertilization or 15dpf)
3) Newborn pups (NB)
4) 3 month old adults (A)

To understand the regulation of Gene A, you perform Northern and Western blots for your samples. First to perform the Western blot, you probe the membrane for Gene 'A' and another

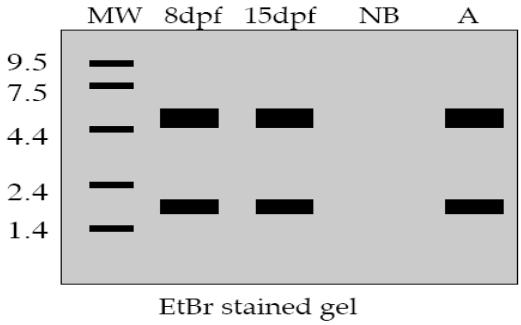
protein, tubulin. (Tubulin is a cytoskeletal protein expressed at constant levels throughout development and in the fully developed mouse.) You observe the following on your blot:

Western Blot of Gene A:

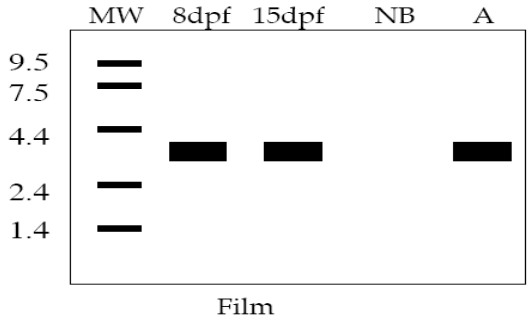


You next perform a Northern blot, probing for Gene 'A'. You develop the blot, expose it to film, and obtain the result shown below:

Northern Blot



EtBr stained gel



Film

a) Your friend suggests that Gene 'A' is **not transcribed** in newborn mice. Do the gel and Northern blot data definitively support his conclusion? Why or why not?
b) Based on the data from the Western and Northern blots, how is Gene 'A' expression regulated in mice; at RNA level or at protein level. Justify your answer referring to **specific lanes** of the blot/film that support your conclusion. **2.5+2.5=5M**

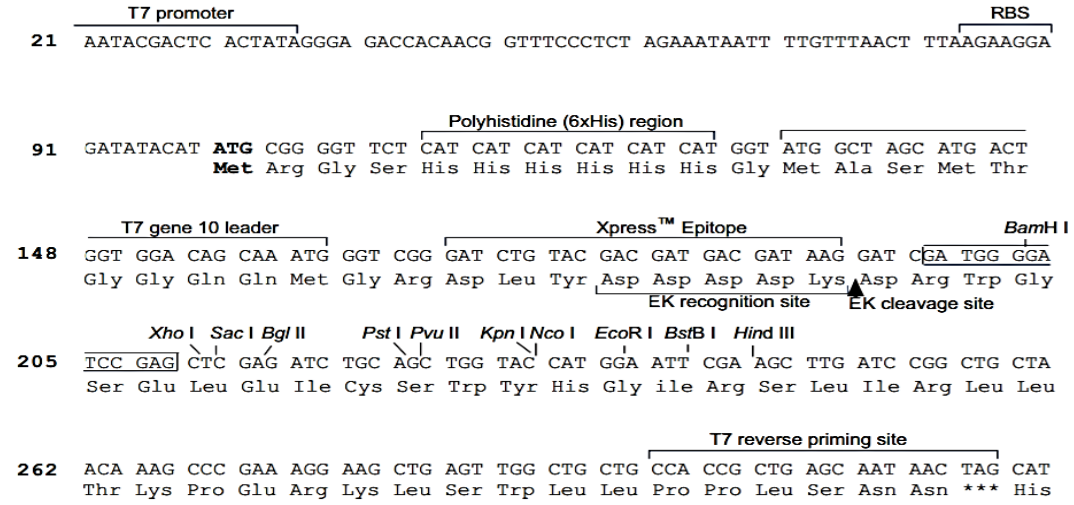
Q14. Given is the sequence of Gene Y. Design the primers for the same, keeping in mind that you have to clone the gene in a prokaryotic system for protein expression. Amongst the given plasmids (A, B and C) which one you will use to clone your gene. Justify your choice. Also, mention how you will proceed for expression and purification studies with proper justification for each step. **5M**

Sequence of Gene Y

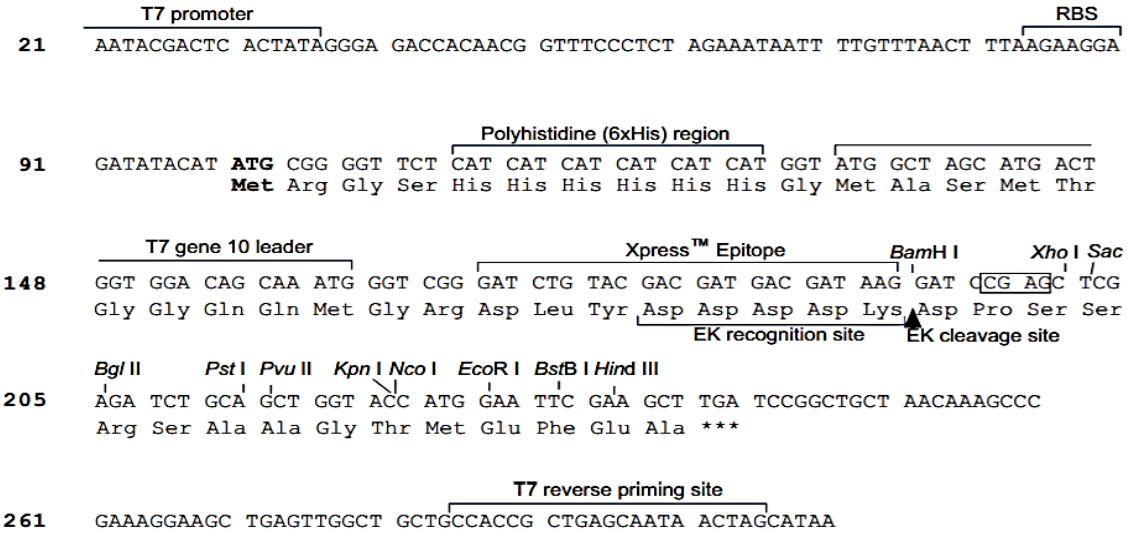
5'ATG CCA GCT AGA TAG CCT GGC ATA AAA TGC AGT AGG TAG TAT **AAG CTT** GCC CC**G GAT CC**G TTT ATT **GGT ACC** GGG AAC **CAG CTG** AAT TGC ATG GTT AAT TAT CAA AGG TAC GCT TTT GCC AAC GCG TAA 3'

Note: The restriction sites are marked in bold and underlined.

Plasmid A:



Plasmid B



Plasmid C: