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

Max. Marks: 60Time: 150 minsDate: 05/05/17Note:• 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 H₂SO₄, 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?

Q5. Explain why:

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

[2x6=12M]

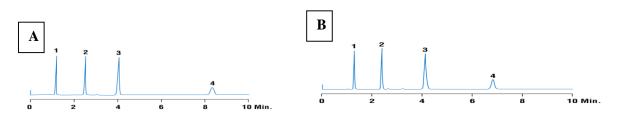
[2M]

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

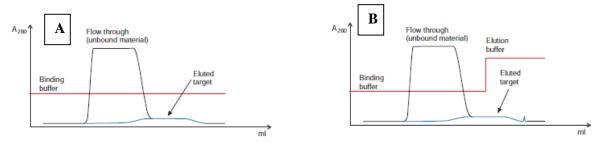
Q8. What are inclusion bodies and when are they generally formed? [2M]

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]



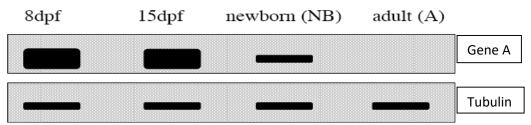
Part B (Open Book) 15M

- 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

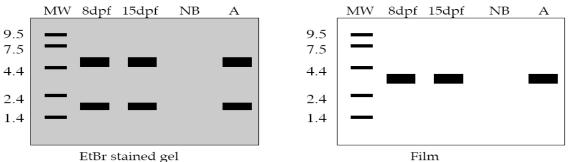
protein, tubulin. (Tubulin is a cytoskeletal protein expressed at constant levels throughout development and in the fully developed mouse.) You observe the following on your blot:

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:





- 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 <u>AAG CTT</u> GCC CC<u>G GAT CC</u>G TTT ATT <u>GGT ACC</u> GGG AAC <u>CAG CTG</u> 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:

	T7 promoter													
21	AATACGACTC ACTATAGGGA GACCACAACG GTTTCCCTCT AGAAATAATT TTGTTTAACT TTAAGAAGGA													
	Polyhistidine (6xHis) region													
91	GATATACAT ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr													
	Met Rig Gry Ser his his his his his dry het Rid Ser het hit													
	T7 gene 10 leader Xpress [™] Epitope Bam													
1 4 0														
148	GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT C <u>GA TGG GGA</u>													
	Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp Arg Trp Gly													
	EK recognition site EK cleavage site													
	Xho Sac Bgl II Pst Pvu II Kpn Nco EcoR BstB Hind III													
205	T <u>CC GAG</u> CTC GAG ATC TGC AGC TGG TAC CAT GGA ATT CGA AGC TTG ATC CGG CTG CTA													
	Ser Glu Leu Glu Ile Cys Ser Trp Tyr His Gly ile Arg Ser Leu Ile Arg Leu Leu													
	T7 reverse priming site													
262	ACA AAG CCC GAA AGG AAG CTG AGT TGG CTG CTG CCA CCG CTG AGC AAT AAC TAG CAT													
	Thr Lys Pro Glu Arg Lys Leu Ser Trp Leu Leu Pro Pro Leu Ser Asn Asn *** His													

Plasmid B

T7 promoter

RBS

AATACGACTC ACTATAGGGA GACCACAACG GTTTCCCTCT AGAAATAATT TTGTTTAACT TTAAGAAGGA 21

Polyhistidine (6xHis) region

GATATACAT ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT Met Arg Gly Ser His His His His His Gly Met Ala Ser Met Thr 91

	T7 ge	ene 10 leader			Xpre	ss [™] Epitop	e	BamH I	Xho Sac		
148	GGT GGA	CAG CAA ATO	GGT CGG GA	CTG	TAC GA	C GAT GA	C GAT AA	G GAT QCG	AGC TCG		
	Gly Gly	Gln Gln Met	: Gly Arg Asp	Leu	Tyr As	p Asp As	p Asp Ly	Asp Pro) Ser Ser		
						EK recog	nition site	EK cleavag	je site		
	Bgl II	Pst Pvu K	pn I Nco I EcoR	I BstB	I Hind III						
205	AGA TCT	GCA GCT GGT	T ACC ATG GA	TTC	ga'a go	T TGA TO	CGGCTGCI	AACAAAGO	ccc		

Arg Ser Ala Ala Gly Thr Met Glu Phe Glu Ala ***

T7 reverse priming site

GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA ACTAGCATAA 261

Plasmid C:

T7 promoter

RBS

21 AATACGACTC ACTATAGGGA GACCACAACG GTTTCCCTCT AGAAATAATT TTGTTTAACT TTAAGAAGGA

91 GATATACAT ATG CGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT Met Arg Gly Ser His His His His His Gly Met Ala Ser Met Thr

	T7 gene 10 leader							Xpress [™] Epitope									BamH I		
148	GGT	GGA	CAG	CAA	ATG	GGT	CGG	GAT	CTG	TAC	GAC	GAT	GAC	GAT	AAG	GAT	d GA	TGG	ATC
	Gly	Gly	Gln	Gln	Met	Gly	Arg	Asp	Leu	Tyr	Asp	Asp	Asp	Asp	Lys	Asp	Arg	Trp	Ile
											EK recognition site					EK cle	avage	e site	

Pst | Pvu || Kpn | Nco | EcoR | BstB | Hind ||| Xho I Bgl II

205 CGA CCT CGA GAT CTG CAG CTG GTA CCA TGG AAT TCG AAG CTT GAT CCG GCT GCT AAC Arg Pro Arg Asp Leu Gln Leu Val Pro Trp Asn Ser Lys Leu Asp Pro Ala Ala Asn

T7 reverse priming site

262 AAA GCC CGA AAG GAA GCT GAG TTG GCT GCC ACC GCT GAG CAA TAA CTA GCA Lys Ala Arg Lys Glu Ala Glu Leu Ala Ala ALa Thr Ala Gln Gln ***