BBL737, Sem I, 2020-21

Minor Exam

Duration: **1 h 20 min + 10 min** extra for scanning and uploading,
Start time: **8.30 am** (10th Nov 2020) Max marks: **50**

- *Answers have to be hand written*
- *Each part of the question to be answered together*
- *Every question is to be attempted on a fresh page*
- *On every page mention name, entry number, page number*

Q. 1 **1+1.5+1+1.5+1+1**

a) What is albumin sponge effect?

b) Describe briefly the basic principle of Blue Native PAGE.

c) What are Chaotropes? Give examples.

d) Describe the technique you will use for separation of lysozyme, pI 11 and other basic proteins by 2D gel electrophoresis.

e) Write the method of cell disruption used in each case

i) bone and cartilage
ii) Gram-negative bacteria
iii) Gram-positive bacteria
iv) Spinach leaves

f) What is a negative stain? Give examples and write their applications

Q. 2 **2+2+3 [+2 bonus marks for (c)]**

a) List some of the mass spectrometry (MS) techniques in routine use today.

b) What are two commonly used ionization techniques in MS of polymers and other biological molecules that have led to rapid progress in their structural characterization? Describe them within 50 words each.

c) A simple harmonic potential $V(z) = kz^2$, where k is a constant is applied to obtain ion confinement in the z-direction of a Mass Spectrometry set-up with a Penning ion trap. Derive the form of the compensating potential, $V(x, y)$ in the x and y directions that must be applied in order to achieve the desired confinement.

Hint: If the field of electrical potential in three Cartesian coordinates, x, y, and z is denoted by Ψ, it needs to satisfy Laplace's equation, i.e. $\nabla^2\Psi(x, y, z) = 0$.

Q. 3 **2+2+1+2**

a) What is the difference between microsatellites and minisatellites?

b) Why did fingerprinting shift from employing minisatellites to microsatellites?

c) GC clamp is not a necessity for the technique of DGGE. Comment

d) For bacterial community fingerprinting a marker of ~750 bp of 16S rRNA gene is to be used to generate profiles. Which of the two techniques (DGGE and tRFLP) will you prefer and why?

Q. 4 **1+1+1+1+1+1+1**

a) Based on the photonic processes, differentiate between fluorescence and phosphorescence.

b) What is the significance of stokes shift?

c) How can one alleviate the post-filter effect in spectrofluorimetry?

d) Which fluorescent probe(s) is/are used to obtain the information about the thickness of the membrane?

e) How do you measure the steady state anisotropy of a membrane?

f) What information is obtained from FRAP technique?

g) Which technique may be employed to measure the distance between the two domains in the same protein?

Q. 5 **2+2+2+1**

a) What is the working distance of an objective means?

b) What is the diffraction limitation in microscopy means?

c) What is quantum efficiency means for a photo-detector and fluorescent molecule?

d) What is choice of fluorescent probe for live cell imaging of protein molecules?

Q. 6 **5+2**

a) Explain briefly why a GC is operated in the vicinity of optimum linear velocity of mobile phase (u_{opt}). Obtain an expression for u_{opt} using the equation described in class?

b) Schematically, show the spectrum for a sample with 2 components A (k_A = 4) & B (k_B = 6) (analyzable by GC-FID) in relation to the mobile phase peak?

Q. 7 **2+1+2+1+1+1**

I kept cells under control conditions or heat stress. After 24 hours, I isolated total RNA followed by reverse transcription and SyBr green based real-time PCR for following genes. Following are the Ct values of genes in the experiment:

	Control Condition (Ct value)	**Heat Stress (Ct value)**
Gene A	**25**	**27**
Gene B	**21**	**21**
Gene C	**16**	**20**
Housekeeping gene	**20**	**22**

a) What is Ct value and how SyBr green dye helps in the quantification of the transcripts?

b) Which of the following genes A, B or C are affected (induced, repressed) or remain unaffected by the heat stress?

c) What formula would you use to calculate the relative fold change for all genes under heat stress?

d) Why I needed to use the housekeeping gene for this experiment?

e) Why do all genes have different Ct values under the control condition?

f) In which of the two- Taqman probe or Molecular Beacon the probe is degraded by the exonuclease activity of the DNA polymerase?