BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI (RAJASTHAN)
BIO G642 Experimental Techniques
Second Semester 2016-2017
Mid Semester Exam (Closed Book)

Max Marks: 40M **Duration: 90mins.** **Date: 07.03.2017**

Note: Please write precise answers only. Avoid lengthy answers.

Q1.

A) Answer the following related to RNA isolation
i) Importance of guanidium salts ii) Mechanism of RNase inhibition by DEPC iii) Removal of DEPC contamination.

B) What can be the cause and solution for a very low 260/ 230 readings? **[6+2=8M]**

Q2.

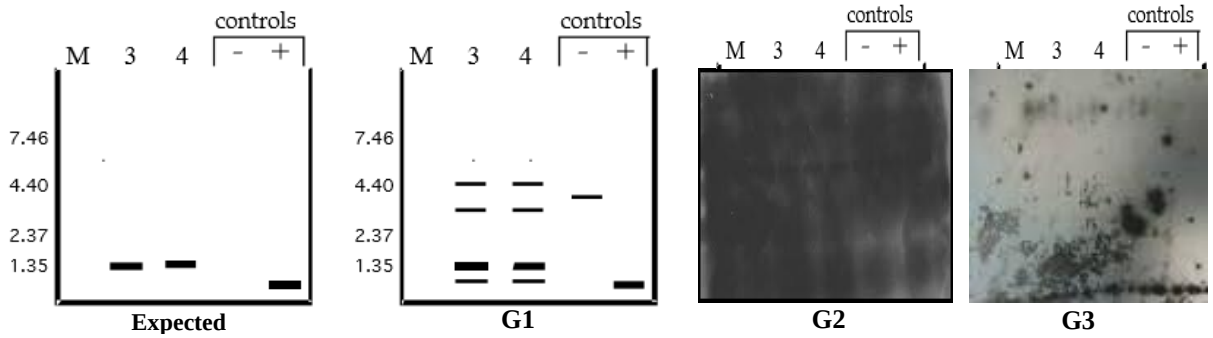
A) Leena wanted to perform a Real time PCR to check the relative expression of several non-coding RNAs in both control and patient samples. What kind of primer she should use to synthesize the c-DNA. Explain your answer with reasons.

B) What is the role of RNaseH in reverse transcriptase PCR.

C) Now she is ready to proceed for the Real time and she has an option of following fluorescent dyes to use in Real time. i) Sybr Green ii) Gene specific Taq man Probe iii) Scorpion primers. Discuss with reasons the pros and cons of each dye and its uses? **[2+2+6=10M]**

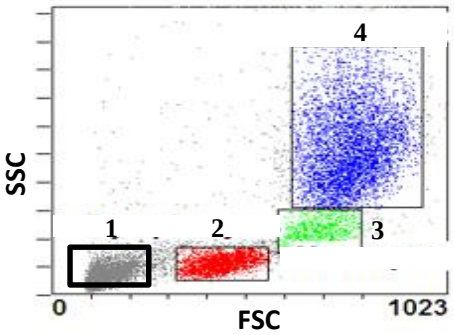
Q3.

A) Three groups G1, G2, & G3 have performed Northern Blotting, following is the result obtained at the end. For each result, briefly identify what went wrong and how do you intend to troubleshoot the issues?



B) When doing a northern blot, why you usually avoid soaking the gel in 0.5 M NaOH/1.5 M NaCl as you do in Southern blotting. **[6+2=8M]**

Q4A).Poonam is using FACS to isolate different cell populations of blood without any dye. She obtained the following result in the scatter plot. Can you identify the four populations (1-4) from the scatter plot? Explain your answer with reasons. B) From your understanding of FACS can other more specific methods be used to identify different cell populations? Justify? C) Where do you require auto-fluorescence and compensation during your FACS experiment? Explain with reasons. **[4+2+4=10M]**



Q5. Explain the effect of temperature and salt concentration on stringency of hybridization in a southern blot. Justify. **[4M]**