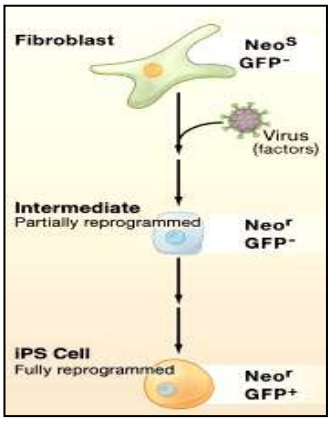
Name: **ID:**

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI
SECOND SEMESTER 2021-22
BIO G515 STEM CELL & REGENERATIVE BIOLOGY
Comprehensive Exam

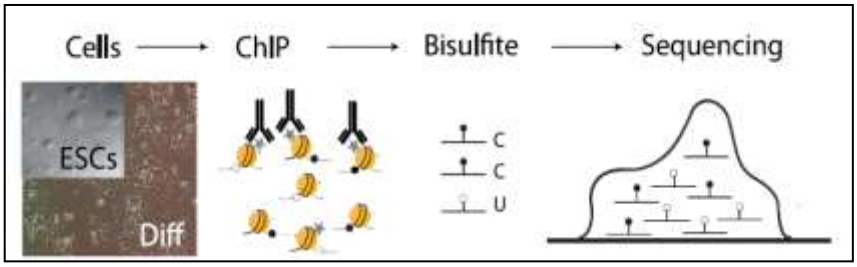
Max. Marks: 40 **Time: 180 min** **Date: 18/05/22**

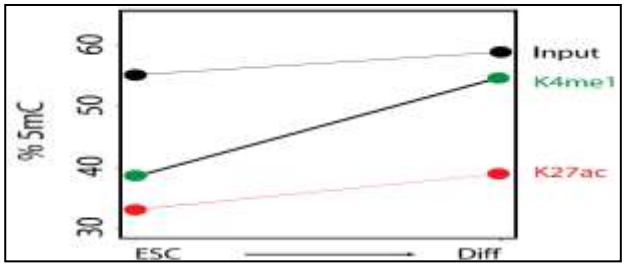
(OPEN BOOK)
Please answer in one or two lines only in the question paper itself. Out of context, long answers may not be considered for evaluation. No Justification, NO marks

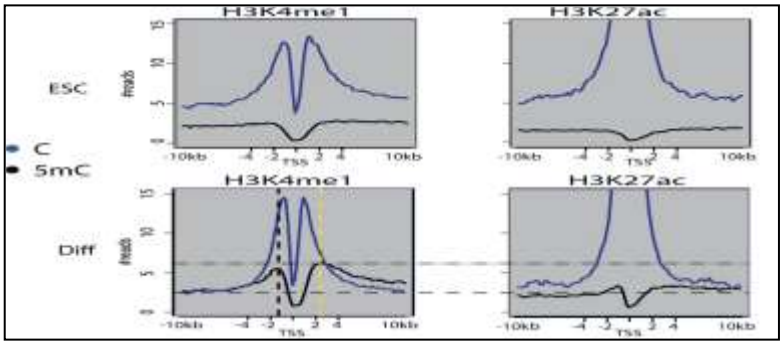


1. A. In the adjacent figure, where an experiment is conducted to generate iPSCs, why partially reprogrammed cells were Neo^r while they are GFP^-. **(2.5)**

B. If the retroviral vectors (with genes of interest) introduced into the somatic cell for iPSC generation were tagged with a GFP as well, what do you expect in terms of green fluorescence following transfection and incubation over a prolonged period of time. Justify your answer. **(2.0)**







2. In bisulphite sequencing the unmethylated cytosines are replaced with 'U', while the methylated ones stays as 'C'. The genome is then sequenced and compared with reference control genome. This allows regions of methylated CpGs (5mC) to be identified. In this particular study genomic regions occupied by H3K4me1 and H3K27Ac were pulled, as shown above, in ESC and differentiated cells, followed by bi-sulphite treatment and subsequent sequencing. The results obtained are given in the subsequent figures. Based on the above experiments answer the following questions.

A. What according to your understanding is 'Input' in Figure 2? **(2.0)**

B. In image 3, the genomic regions +/- 10kb were scanned for the enrichment of 5mC. Vertical dashed lines represent 5mC peak centers (upstream and downstream to TSS). Based on image 3 draw your conclusion on methylation pattern observed in ESC and differentiated cells. Draw your conclusion from results depicted in image 2 and 3. **(2.0)**

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Q3. Read the given research paper and answer the following questions-

A. Would there be any hindrance in reprogramming if Oct4 and Klf4 are introduced sequentially only after Myc and Sox2 in the fibroblast cells? Justify your answer citing suitable experiments performed in this regard by the research group. **(3.0)**

B. Schematically differentiate the series of events during reprogramming as observed after introduction of wild-type and mutated Oct4. **(3.0)**

C. Explain the result with justification for each lane of the blots from **Figure 5c** of the given paper. **(3.0)**

D. What conclusion can be derived from Fig. 3H and **schematically** describe the steps that might have been followed for execution of the experiment. Cite the appropriate controls used and why. **(3.0)**

E. How did the authors prove that reprogramming is achieved NOT primarily through histone acetylation and DNA demethylation. **(3.0)**

F. Based on the line of evidence can you state why Oct4 is not expressed in somatic cells. Provide meolecular reasons for your answer. **(2.0)**

Q4. A. You have discovered a molecule like Let-7 and would like to discover its mechanism of action and any role in stemness. Design experiment/s to prove the same. **(3.0)**

B. Why cancer stem cells are difficult to target? Why conventional anti-proliferative or DNA damage inducing agents are often of no use against these CSCs? **(3.0)**

C. Given that cancer cells are often targeted with antibodies that can be used as a prospective therapeutic strategy against them, you decide to target the CSCs expressing a nuclear protein (involved in cancer stemness) with a monoclonal antibody generated against it. Comment on the therapeutic success of this strategy. Provide a different idea that you think can be used to target the CSCs. **(3.0)**

Q5. A. Nanog can both activate and repress transcription of different promoters. How do you think this is possible and how would you experimentally validate the same? **(3.0)**

B. Design a microscopy based experiment with appropriate controls to prove hyperdynamicity of ES cell chromatin. **(2.5)**

********************************Have a nice summer holiday********************************