BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE PILANI, HYDERABAD CAMPUS
rDNA Technology (BIO F311) 2022-23 I SEM; Quiz2 (Closed_book) Total Marks: 20
Time 2:00 PM AM to 2:30 PM Duration: 30mins

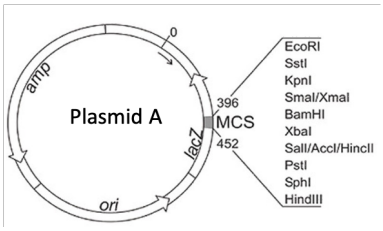
Student Name:

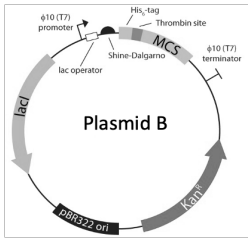
Student ID:

(A)

Write your answers legibly in the space given below. No overwriting is allowed.

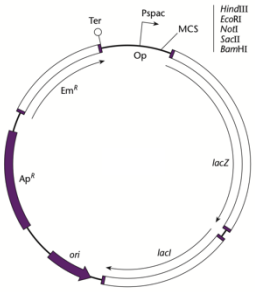
1. At the enzyme level how Golden Gate differs from Gibson assembly reactions? **1M**
2. Mention any two major limitations of TOPO TA cloning. **1M**
3. Below are the two plasmids





a. Sarthak wants to do cloning of a gene of interest(GOI). Which one can be used? Answer with one line justification. **1M**
b. Akshat wants to check the expression of the same gene used by Sarthak. Which one is the plasmid of choice for Akshat ? Justify. **1M**

4. Why DpnI enzyme is important during Site-Directed Mutagenesis? **1M**
5. Only diagrammatically show the cDNA cloning steps in phage **λ** vectors. **1.5M**
6. RACHITT is an important gene shuffling technique. Which enzyme is not involved in this method
   a. DNA ligase b. DNase c. DNA polymerase d. endonuclease. **0.5M**
7. What does it mean "DIN 8.0"? **1M**
8. We use phosphatase during Restriction enzyme mediated cloning of gene of interest. Why? **1M**
9. Function of PilQ and PilN with respect to cloning. **1M**
10. Utilizing the following plasmid Aahan wants to express protein X in the human embryonic kidney (HEK) cells. Can Aahan able to express his protein? To express protein X what alternative Aahan can do? Give one or two line justification. **2M**



11. For Lenti viral mediated transfection(1st generation) what are the three plasmids are important and why? **1M**
12. What is polyplexes and why it is used during cloning? **1M**
13. What is the difference between transduction vs transformation? **1M**
14. What is phage display? **1M**
15. In the lab you want to clone the following cDNA in the given plasmid (see below) which has two restriction enzyme site (*EcoR*I and *Pst*I). Can you diagrammatically show how will you clone the cDNA. **4M**
    cDNA sequence "ATGCCCGAATTCGCCAATTCGGATCCAAA"

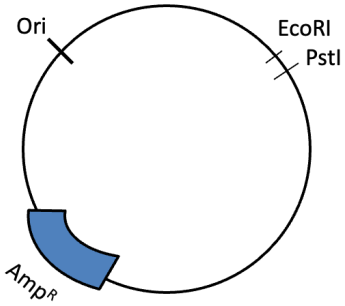


Table 4.1. Recognition sequences and cutting sites for some restriction endonucleases

Enzyme	Recognition sequence	Cutting sites	Ends
*Bam*HI	5′-GGATCC-3′	G↓GATCC CCTAG↑G	5′
*Eco*RI	5′-GAATTC-3′	G↓AATTC CTTAA↑G	5′
*Hae*III	5′-GGCC-3′	GG↓CC CC↑GG	Blunt
*Hpa*I	5′-GTTAAC-3′	GTT↓AAC CAA↑TTG	Blunt
*Pst*I	5′-CTGCAG-3′	CTGCA↓G G↑ACGTC	3′
*Sau*3A	5′-GATC-3′	↓GATC CTAG↑	5′
*Sma*I	5′-CCCGGG-3′	CCC↓GGG GGG↑CCC	Blunt
*Sst*I	5′-GAGCTC-3′	GAGCT↓C C↑TCGAG	3′
*Xma*I	5′-CCCGGG-3′	C↓CCGGG GGGCC↑C	5′

Note: The recognition sequences are given in single-strand form, written 5′→3′. Cutting sites are given in double-stranded form to illustrate the type of ends produced by a particular enzyme; 5′ and 3′ refer to 5′- and 3′-protruding termini, respectively. The point at which the phosphodiester bonds are broken is shown by the arrow on each strand of the recognition sequence.