BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI (RAJASTHAN)
BIO F418 Genetic Engineering Techniques First Semester 2023-2024
Max. Marks: 20 Comprehensive examination (closed book) Duration: 30 min

- Provide justification and calculations wherever necessary
- Be as brief as possible. Please avoid stories in your answer

********** Best of luck**********

Q1. Match the following: **(2)**

A. Terminal transferase	1. Protruding 5′ termini
B. Exonuclease III	2. Nick translation
C. DNA pol I	3. Primer extension
D. Klenow fragment	4. homopolymer tailing

Q2. Represent diagrammatically the principle for gene knockout. At what voltage was the electroporation performed? Why, in this case, do you perform electrotransformation instead of chemitransformation? **(3 + 2 + 2 = 7)**

Q3. Study the table below and comment on which thermostable polymerase would perform best with dNTPs concentration 50 µM. Justify. (In general, 200 µM dNTPs are used for a PCR reaction). Ignore the superscripts in the table. **(2)**

	Taq	$Vent_R$	$Vent_R$ (exo-)	Deep $Vent_R$
Km dNTPs	13 µM[g]	60 µM[g]	40 µM[g]	50 µM[g]
Km DNA[d]	2 nM[g]	0.1 nM[g]	0.1 nM[g]	0.01 nM[g]
Extend RNA Primer[y]	-	-	-	+
Extension From Nick	+	+	+	+

Q4. While cloning the gene, your friend used alkaline phosphatase to prevent self-ligation of the vector. As the buffer is compatible with the ligase, to the reaction vector, she added insert molecules freshly restricted with compatible enzyme. However, she did not see any ligated product. Her supervisor suggested heating the vector and the insert mix to 70 C before adding the ligase. To her surprise, this gave her the ligated product. Based on the training in the BIO F418 course provide justification on why her experiment failed initially. **(4)**

Q5. Study the absorption spectra of pigment-producing bacteria you have just isolated from soil. Now you wish to monitor the growth of the bacteria so that you can decide on the OD value to be used for preparing competent cells and performing transformation. **(5)**

(i) What should be the wavelength (nm) used for monitoring the growth? Justify.

(ii) Which phase is generally used for preparing competent cells? Justify