Pharmaceutical Biotechnology Exam (K09J3a)

07.02.2022

Candidate name: …….

Candidate student number or Q number: ……

DO NOT TURN OVER UNTIL TOLD TO DO SO

Exam instructions:

The exam is composed of two parts.

Part I includes 16 short answer questions each worth 1 point (out of a total of 24).

Part II includes 2 essay questions each worth 4 points.

You are expected to answer all questions. The duration of the exam is 3 hours.

A copy of **Park et al. (2021) Soluble Cytoplasmic Expression and Purification of Immunotoxin HER2(scFv)-PE24B as a Maltose Binding Protein Fusion** is provided at the end of the exam and you can consult it where relevant. You are not allowed to use your personal copy of that paper.

Part I – Short answer questions

Q.1 – DNA polymerases in the absence of nucleotide triphosphates can work as exonucleases, digesting DNA from its 3'-end. In the presence of a single nucleotide triphosphate, for instance dCTP, the exonuclease function proceeds until the first C is removed, upon which it is readily re-incorporated.

a. Given the DNA sequence below, write down the opposite strand and cross out the bases that would be removed from the DNA.

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5′- GAA GTA TCG … TGT ACG CTA – 3′

3′-              …              - 5′
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b. This method can be used for DNA assembly. Explain how it can be used for cloning and compare it to Type IIS or Golden Gate cloning.

Q.2 – Using the SBOL symbols below draw a plasmid for the expression of a single-chain Fragment variable protein labelled at its C-terminus with a Strep-tag. Highlight in your construct the boundaries between tags, linkers and immunoglobulin domains.

A.	 Origin of replication	**E.**	 Terminator
B.	 Recombination site	**F.**	 Operator
C.	 Promoter	**G.**	 Translation stop site
D.	 Ribosomal binding site	**H.**	 Open Reading Frame

Q.3 – List two Critical Quality Attributes for a protein-based biotherapeutic and briefly explain how they can affect its therapeutic function.

Q.4 – Given the choice of the following available reagents, write down a viable immunoassay topology for the detection of a yeast cytoplasmic protein, called EX1.

1. EX1-containing sample

2. mouse anti-EX1 IgG

3. rabbit Fab anti-EX1

4. Streptavidin-coated immunoassay plate

5. horseradish peroxidase-Protein A fusion

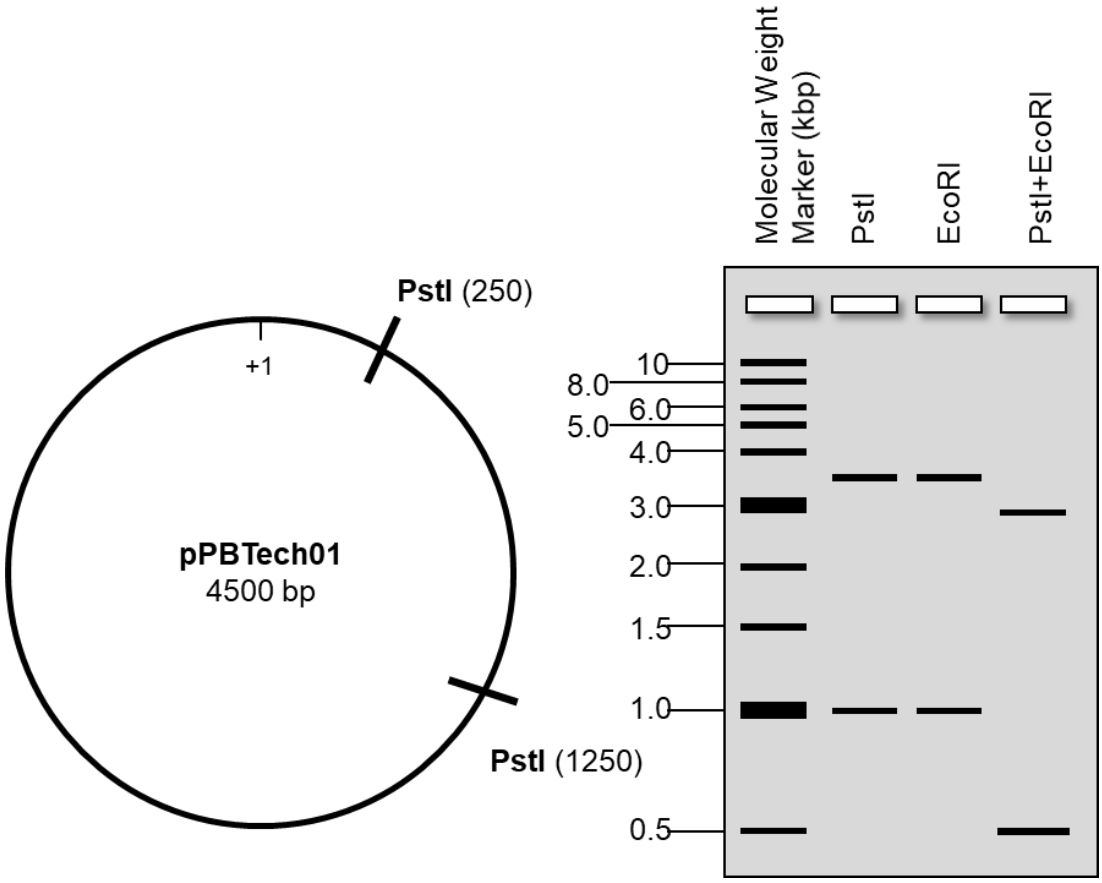
6. Bovine milk casein (typically used as a blocking agent)

7. Uncoated immunoassay plate (i.e. will bind any protein)

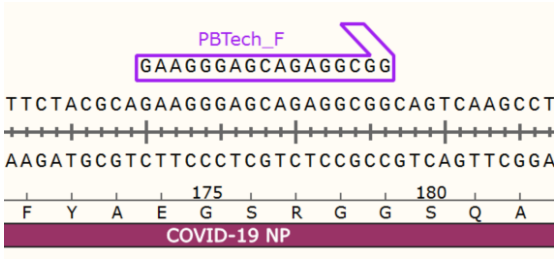
8. luciferase

Q.5 – Define a reporter gene. Explain, giving an example, how reporter genes can be used to monitor cloning. Propose a possible reporter gene for monitoring cloning in eukaryotic hosts.

Q.6 – Given the restriction patterns observed below in an agarose gel, map the positions of the cut sites to the plasmid backbone. A PstI restriction site (and its position) is already given on the plasmid map. Is the information given sufficient for unambiguous assignment? Justify your answer.



Q.7 – The following primers (PBTech_F and PBTech_R) were designed for the detection of COVID from patient samples.

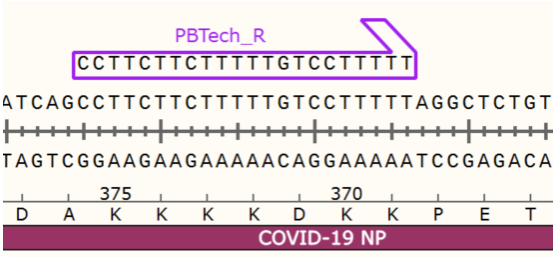


PBTech_F (17-mer):

Tm: 58.5°C

Most stable self-dimer: ΔG = - 3.61 kcal/mol

GC content: 70%



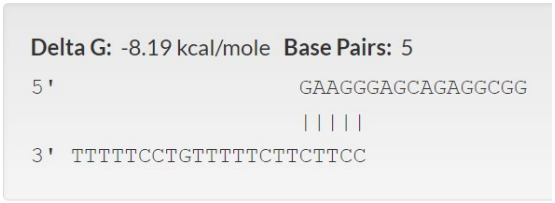
PBTech_R (22-mer):

Tm: 49.5°C

No significant stable self-dimers

GC content: 32%

Heterodimer predictions:



Unfortunately, initial experiments using the following conditions yielded only non-specific amplifications

Denaturation	5 min @ 95°C
Denaturation	1 min @ 95°C
Annealing	10 s @ 57°C
Extension	10 s @ 68°C

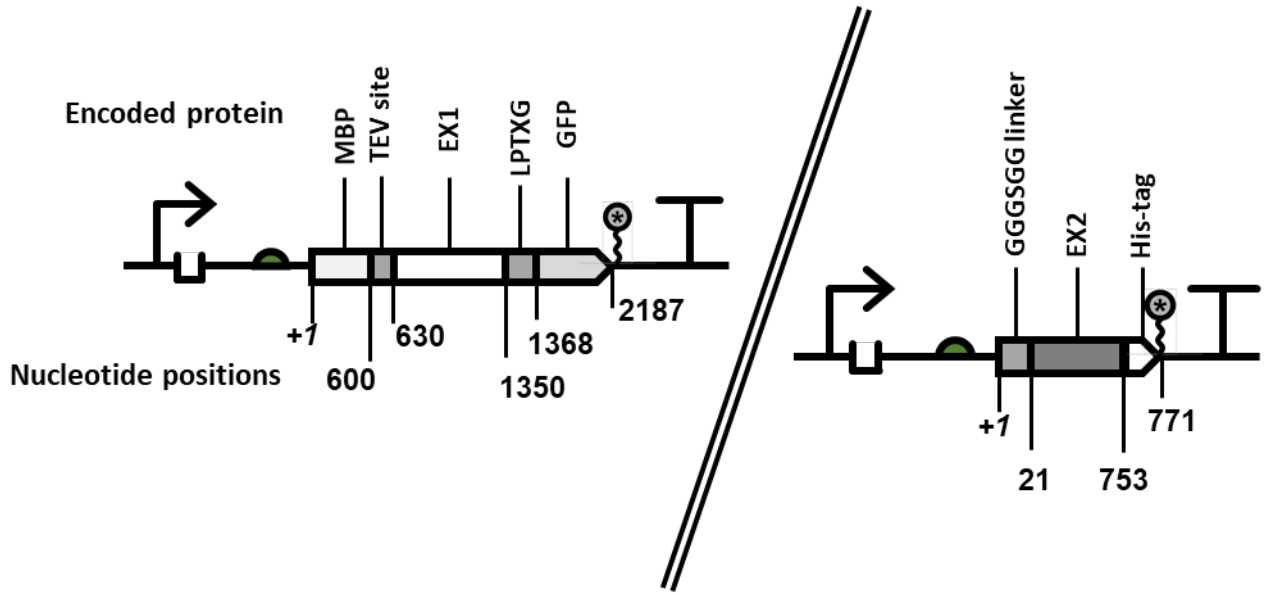
X10

Propose, and briefly justify, two possible changes to the experiment to improve its success as a diagnostic platform.

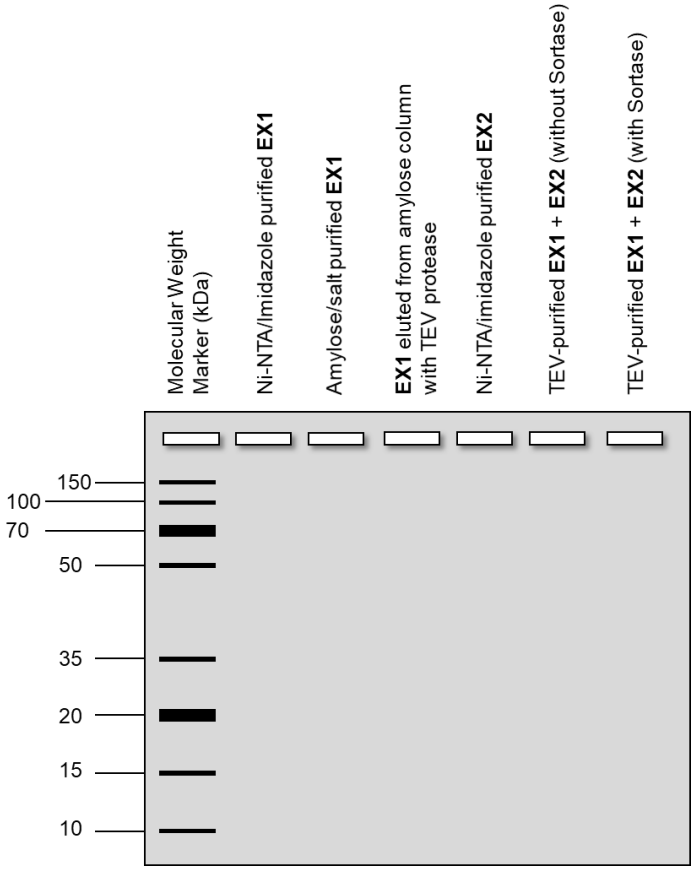
Q.8 – You have been tasked with expressing and purifying mouse IgGs from human hybridoma cells in culture for a clinical trial. With respect to other possible hosts (e.g. bacterial cells or mouse hybridoma cells in culture), is this a good choice of host for IgG expression? Briefly justify your answer.

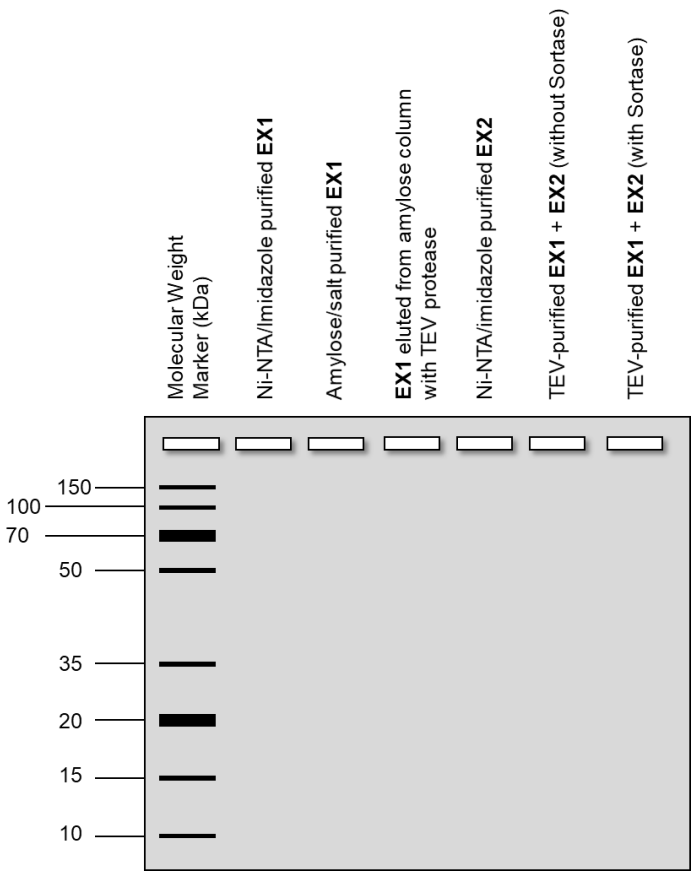
Q.8 – Preparation of a novel bivalent scFv, called EX1-EX2, requires sortase-mediated ligation of EX1 and EX2. The components are expressed (in separate cells) from the annotated genetic circuits depicted below. Using the knowledge that an average amino acid is 110 Da, complete the SDS-PAGE and Western blot. (Show your calculations)

Construct:



SDS-PAGE:



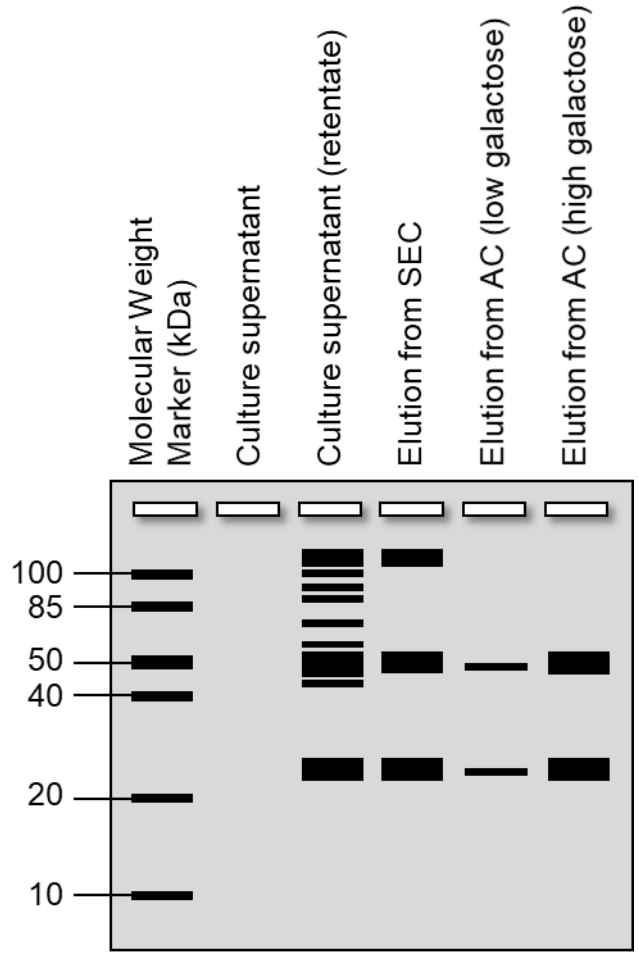


Western Blot: Developed using a rabbit anti-GFP IgG and a goat anti-rabbit IgG conjugated to HRP.

Q.10 – Continuing your task from Q.8, you express the IgGs, recovering the culture supernatant and proceeding with the following purification procedure:

1. Concentration through a 100 kDa filter
2. Size exclusion chromatography (SEC)
3. Affinity chromatography using a column containing lectins with affinity for galactose

The results are shown in the following reducing SDS-PAGE:



SDS-PAGE

a. Based on the SDS-PAGE, was purification successful? Briefly justify your answer.
b. Although you filtered the sample using a 100 kDa filter, why are there proteins lower than that cut-off in the sample?
c. Propose an alternative purification approach for the IgGs.

Q.11 – Still with regard to the IgG purification above, list two possible contaminants and briefly describe how they can be removed.

Q.12 – Explain what genotype-phenotype linkage is in directed evolution. Is it important? Briefly justify.

Q.13 – Regarding Park et al., explain the experimental rationale behind Figure 7. (What is the experiment? What is its main conclusion? What are the experimental controls?)

Q.14 – Regarding Park et al., the authors state that only 11.8% of the MCF cells were HER2 positive. How did the authors came to that conclusion? Does that agree with the findings shown in Figure 7? Justify your answer.

Q.15 – Regarding Park et al., what (molar) concentration of HER2(scFv)-GFP was used for the flow cytometry experiments? The scFv used is known to have an affinity for HER2 of approximately 20 pM. Given the definition fractional binders [$\theta = [A]/(K_d + [A])$], what fraction of the scFv is expected to be bound?

* as shown in Figure 6, the molecular weight mass of HER2(scFv)-GFP is approximately 50 kDa.

Q.16 – Regarding Part et al., what is the *E. coli* shuffle strain and why was expression in it attempted?

Part II – Essay questions

E.1 – Explain the impact of variability of personal pharmacokinetics and pharmacodynamics on biotherapeutic dosing and monitoring.

E.2 – Describe available strategies to engineer a nanobody (mass 12.5 kDa) against a cancer cell-surface marker ($k_{on} = 10^8\ M^{-1}\ s^{-1}$; $k_{off} = 10^{-1}\ s^{-1}$) to increase its therapeutic potential (i.e. better production yield, higher affinity, greater potency and longer half-life if relevant).