PROTEIN ENGINEERING

Time allowed: 3 hours
Answer **ALL** questions.
You are advised to spend an equal amount of time on each question.
All questions carry an equal number of marks.
Answer **EACH** question in the answer book provided.

1. Answer ALL parts (a) to (b)
The sequence of a small section of a double stranded DNA molecule is:
5′-GATATCCTTTCGGCTGTCAGG-3′
3′-CTATAGGAAAGCCGACAGTCC-5′
Assume that the first codon starts in the first base of the DNA sequence above.

(a) What is the mRNA sequence transcribed from this DNA sequence? **(10mks)**

(b) What is the amino acid sequence of the protein encoded by this DNA molecule? A codon table is presented below. **(10mks)**

First Base	Second Base: U	Second Base: C	Second Base: A	Second Base: G	Third Base
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

2. Answer **BOTH** parts.
(a) Protein engineering can be achieved by rational design, directed evolution, or using a semi-rational approach. Describe TWO of these methods, summarizing

the steps required. **[20mks]**
(b) Discuss briefly some of the advantages and disadvantages of solid-phase peptide synthesis. Include at least three of each. **[20mks]**

3. Answer ALL parts (a), (b), (c) and (d).
A single-chain enzyme of molecular weight 25 kDa has been chosen for use in an industrial process which will take place at a temperature slightly above the melting temperature of the enzyme, Tm. To increase the operational half-life of the enzyme at the process temperature, it has been decided to attempt to increase its stability by introducing a disulfide bridge. The enzyme has been cloned but its three-dimensional structure is not available. However, both the primary and high-resolution crystal structures are available for a highly homologous enzyme from the same family.

(a) state how the primary and tertiary structural information available can be used to generate a model for the structure of the target enzyme. **[20mks]**

(b) Describe how you might use this model to help decide which residue(s) to mutate so as to maximize the chance of introducing a disulfide bridge into the enzyme. **[20mks]**

END OF PAPER