1、請利用下面遺傳工程技術之步驟製造人類胰島素。(16分)

___ → I →___ →___ →___ → A →___ →___ →___ →___

A. Growing bacteria
B. Constructing the plasmids with A-chain or B-chain mini-genes fused to the back of *lacZ* gene, respectively
C. Forming the disulfide bonds linking A and B chains
D. Cloning human insulin cDNA
E. Transforming 2 plasmids into *E. coli*, respectively
F. Purifying A and B chains, respectively
G. Making 2 mini-genes for insulin A-chain and B-chain
H. Splitting fused proteins with cyanogen bromide
I. Genetic engineering of insulin gene to stop formation of hexamers
J. Purifying fused proteins

2、自細菌抽取出 plasmid 後先進行DNA定量，將檢體稀釋 20倍後測得$OD_{260}=0.25$，今欲以 1 μg plasmid 進行限制酶切割反應，請問應取多少體積的檢體？(4分)

3、已知 B型肝炎病毒的 S抗原可以在人體內引起保護性抗體，如果你已獲得全長的S基因片段，請設計一個 plasmid 作為 DNA疫苗。(5分)

4. Please describe the role of σ factor in bacteria transcription cycle. (7 分)

5. Please describe how does FACT (facilitates chromatin transcription) facilitate the transcription of RNA polymerase through the histones. (7 分)

6. What is mutually exclusive splicing? (4 分) Please briefly describe the mechanisms responsible for that. (7 分)

見背面

7. 大腸桿菌中有多種 DNA methylase 會對 DNA 進行甲基化 (methylation)，請問有那些鹼基會受到 methylase 的修飾？這些甲基化的修飾有那些生理意義?(7 分)

8. 人類細胞中有那些特定的核酸序列容易被 DNA methylase 修飾，這些甲基化的修飾有何生理意義？研究顯示這些被甲基化的序列也是突變熱點 (mutational hotspot)，請說明為什麼?(6 分)

9. 請描述一個大腸桿菌的 replication fork 並標示出參與 DNA replication 的各種酵素及蛋白質。(6 分)

10. 請描述在大腸桿菌中 homologous recombination 的過程，並說明參與反應的酵素及蛋白質。(6 分)

11. 名詞解釋: (15%)
1). Genetic imprinting
2). Enhancer
3). Histone code

12. 配對題: 請於選項 A-M 中選擇<u>其一最適的答案</u> (10%)
1). Histone acetyl transferase
2). Peptidyl transferase
3). Aminoacyl tRNA synthetase
4). DNA maintenance methyltransferase
5). RNA helicase

A. Responsible for tRNA charging
B. Removes the amino acid from charged tRNA
C. Functions in RNA secondary structure unwinding
D. Plays a role in mRNA half-life
E. Catalyzes peptide bond formation during translation
F. Links two polypeptides covalently
G. Functions in chromatin remodeling; transfers phosphate groups to histone proteins
H. Functions in chromatin remodeling; acetylates conserved lysine amino acids on histone proteins
I. Functions in chromatin remodeling; removes acetyl groups from lysine amino acids on histone proteins
J. Removes the formyl group from the N-terminus of methionine
K. Removes the N-terminal methionine as well as one or two additional amino acids from a polypeptide
L. Functions in hemi-methylated DNA; adds methyl groups to DNA when one strain is already methylated
M. None of the above