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1. Describe two methods applied to study apoptosis and explain some general backgrounds of these methods (10%).
2. A newly cloned gene is ubiquitously expressed in various tissues and knockout of this gene in mice is embryonic lethal. What will you do if you need to study functions of this gene in the liver in adult mice (10%)?
3. Please 1) define what a high-throughput technology is, 2) name a method based on this technology and provide the basic principle for this technique, 3) and then list a biological or clinical question which can be successfully addressed by this experimental approach (10%).
4. A novel protein X with nuclear localization signal was recently identified by Dr. Li and suspected to be a transcriptional modulator of p53 expression. Assuming Dr. Li has all the antibodies he needs, please 1) propose two complementary approaches for him to pinpoint the DNA binding region of X protein on the promoter of p53 gene and 2) describe the theoretical basis for each approach (10%).
5. In producing and purifying recombinant proteins, most scientists have taken advantage of *E. coli* as the host to express a desired gene product. Please answer the following questions and show how you will plan to obtain a desired recombinant protein with good quantity and quality (20%)
   1) How do you clone a gene of interest in order to have it properly expressed in *E. coli*? (5%)
   2) After your gene is cloned, how do you ensure *E. coli* express this gene product to a desired quantity? (5%)
   3) During protein production (i.e., translation), you may find your protein is not soluble in *E. coli*. What causes protein insolubility in *E. coli* and how are you going to solve this problem? (5%)
   4) If the protein is soluble, how will you purify your recombinant protein from *E. coli*? (5%)
6. One scientist is searching genes that are associated with genetic susceptibility to one type of disease. By positioning cloning, he/she is able to narrow down the location of diseased gene on chromosome. Subsequent sequencing and primer walking within that region, he/she encounters one gene that is significantly associated with the disease he/she is studying. As the candidate gene is a novel gene (not known nor reported in literatures), he/she suspects that the candidate gene is a noncoding RNA. Please list at least four different methods or experimental strategies that can help him/her to decide that the candidate gene that he/she is studying is a noncoding RNA (20%).
7. To express a bacterial gene A (encoding protein A) in a bacterial expression plasmid, the DNA fragment containing the A gene has been amplified by PCR. The 5'-end and 3'-end

(背面仍有題目,請繼續作答)

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of the coding strand sequences of the DNA fragment are shown in **Figure A**. The sequence of the cloning region of the expression plasmid is shown in **Figure B**. Please answer the following the questions (20%).

1) Which of the following primer sets was used to amplify the gene A-containing DNA fragment? (4%)
   a.5'-GGATCCATATGGAATTCT-3' and 5'-GTCGACTAATCGAGATCT-3'
   b.5'-AGAATTCCATATGGATCC-3' and 5'-GTCGACTAATCGAGATCT-3'
   c.5'-AGAATTCCATATGGATCC-3'and 5'-AGATCTCGATTAGTCGAC-3'
   d.5'-GGATCCATATGGAATTCT-3'and 5'-AGATCTCGATTAGTCGAC-3'
   e.5'-GGATCCATATGGAATTCT-3'and 5'-TCTAGAGCTAATCAGCTG-3'
2) The start codon of the A gene is AUG. Please show the sequence of the first five N-terminal amino acids of the protein A (4%). The codon usage table is shown in **Figure C**.
3) Please show the sequence of the last five C-terminal amino acids of the protein A (4%).
4) If we want to express the protein A as a recombinant protein with its N-terminal fused with a 6xHistidine tag (His-Tag), which of the restriction enzyme sets could used during the process of cloning? (4%)
   a. NdeI and SalI
   b. EcoRI and BamHI
   c. EcoRI and BglII
   d. BamHI and SalI
   e. BglII and Sal I
5) If we want to express the protein A as a recombinant protein with its C-terminal fused with a 6xHistidine tag (His-Tag). Which of the restriction enzyme sets could be used during the process of cloning? (4%)
   a. NdeI and SalI
   b. EcoRI and BglII
   c. NdeI and BglII
   d. BamHI and SalI
   e. XhoI and Sal I

Figure A. The PCR product containing the A gene to be cloned in the expression plasmid. The restriction sites above the sequence are unique cutting sites.

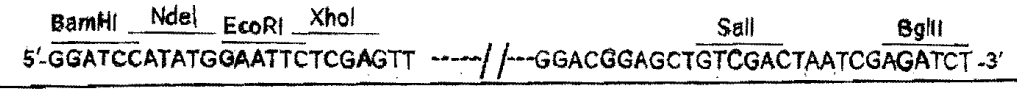

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Figure B. The cloning region of the expression plasmid. The restriction sites above the sequence are unique cutting sites.

Ribosome binding site — NdeI — XhoI — His Tag — EcoRI

5'-AAGAAGGAGATATACATATGCTCGAGCATCATCATCATCATCATGAATTC

BglII — BamHI — SalI — His Tag

AGATCTGGATCCGTCGACCATCATCATCATCATCATTAA-3'

Figure C. RNA codon table

Ala	GCU, GCC, GCA, GCG	Leu	UUA, UUG, CUU, CUC, CUA, CUG
Arg	CGU, CGC, CGA, CGG, AGA, AGG	Lys	AAA, AAG
Asn	AAU, AAC	Met	AUG
Asp	GAU, GAC	Phe	UUU, UUC
Cys	UGU, UGC	Pro	CCU, CCC, CCA, CCG
Gln	CAA, CAG	Ser	UCU, UCC, UCA, UCG, AGU,AGC
Glu	GAA, GAG	Thr	ACU, ACC, ACA, ACG
Gly	GGU, GGC, GGA, GGG	Trp	UGG
His	CAU, CAC	Tyr	UAU, UAC
Ile	AUU, AUC, AUA	Val	GUU, GUC, GUA, GUG
Stop codon	UAG, UGA, UAA		