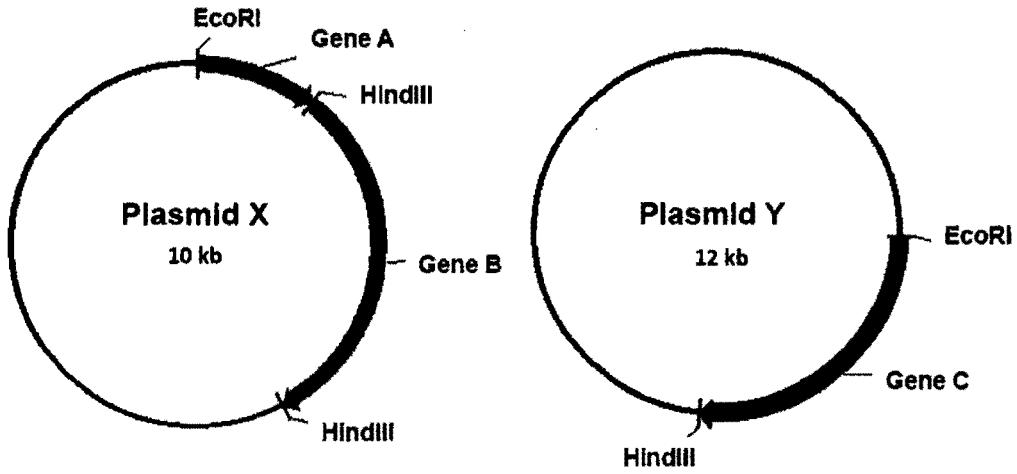
選擇題：單選（48 分，每小題 3 分）請將答案寫在答案卷上。

1~4. The 10 kb Plasmid X contains genes A and B, which are 1 and 3 kb, respectively, while the 12 Kb Plasmid Y contains the 3 kb gene C. Both plasmids contain EcoRI and HindIII restriction sites.



1. Plasmids are usually analyzed by agarose gel electrophoresis with or without restriction enzyme digestion. Which of the following statement regarding the DNA gel electrophoresis is correct? (3%)

a. The DNA fragments migrate from the anode (positive electrode) to the cathode (negative electrode) in the electrophoresis
b. The large linear DNA fragments move faster than the small linear ones
c .The gels with higher concentrations of agarose facilitate separation of larger DNAs
d. Pure water can serve as the electrophoresis buffer
e. The circular form of DNA exhibits mobility different from the linear DNA of the same mass in the electrophoresis

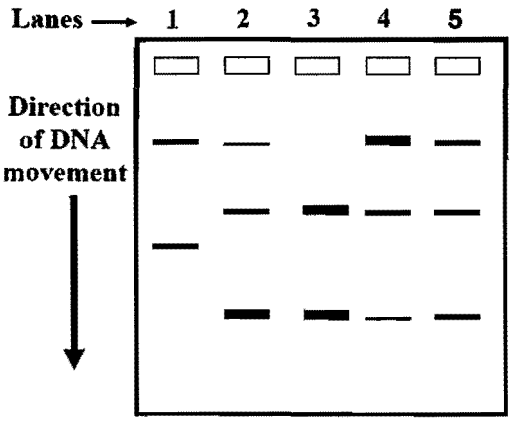
2. To visualize the DNA on the agarose gel after electrophoresis, which of the following reagent is usually used for DNA staining? (3%)

a. SDS
b. Comassie blue
c. Glycerol
d. Ethidium bromide
e. DMSO

(背面仍有題目,請繼續作答)

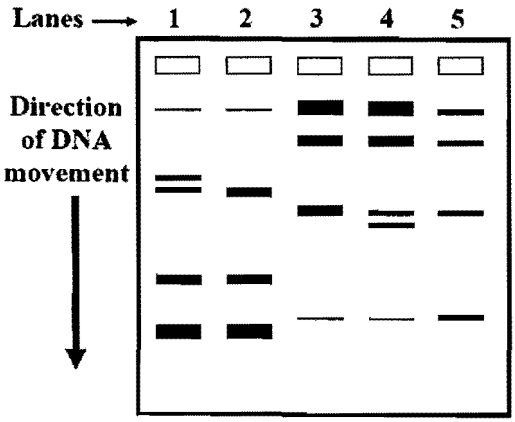
3. If the plasmid X was completely digested by HindIII and EcoRI, what would be the most possible result shown on the agarose gel after electrophoresis? (3%)

a. Lane1
b. Lane2
c. Lane3
d. Lane4
e. Lane5



4. Equal molecule numbers of the plasmids X and Y are mixed, and completely digested with both HindIII and EcoRI. What would be the most possible result shown on the agarose gel after electrophoresis? (3%)

a. Lane1
b. Lane2
c. Lane3
d. Lane4
e. Lane5



5. Which of the following statement regarding polymerase chain reaction (PCR) is NOT correct? (3 points)

a. The GC content of primers affect the annealing temperature of PCR
b. The fidelity of Taq DNA polymerase is lower at lower concentration of Mg^{+2}
c. The extension rate of Taq DNA polymerase is about 1 kb/min at 72℃
d. After hybridizing with the template DNA, the primers extend from their 3' end in PCR
e. Taq DNA polymerase has no proofreading function

6. To amplify the DNA fragment W (a double strand DNA) by PCR, which of the following primer sets could be used in the PCR reaction? (3%)

5'-AGTCTTACCTCGTTAGCTGGCCTGCTTGCAATAGCAGATCAGACCATCAC -3'
Sequence of the DNA fragment W

a. 5'-AGTCTTACCTCGTTA-3' and 5'-AGATCAGACCATCAC-3'
b. 5'-AGTCTTACCTCGTTA-3' and 5'-CACTACCAGACTAGA3'
c. 5'-TCAGAATGGAGCAAT-3'and 5'-CACTACCAGACTAGA-3'
d. 5'-AGTCTTACCTCGTTA-3'and 5'-GTGATGGTCTGATCT-3'
e. 5'-TCAGAATGGAGCAAT-3'and 5'-TCTAGTCTGGTAGTG-3'

7. Which of the following assays can be used to detect mRNA? (3%)
a. Southern blot assay
b. Northern blot assay
c. Nest PCR
d. Far western blot analysis
e. Enzyme-Linked Immunosorbent Assay (ELISA)

8. Which of the followings is not involved in DNA transferring in or among bacteria? (3%)
a. Transposon
b. Intergron
c. phage
d. Plasmid
e. Flagella

9. By treatment with which of the followings would make bacterial cells become more permeable to uptake plasmids? (3%)
a. Calcium chloride
b. High pressure
c. Alkali
d. Ultrasound
e. Heat

(背面仍有題目,請繼續作答)

10. Minipreparation (Miniprep) of plasmid DNA is a rapid, small-scale isolation of plasmid DNA from bacteria. Ethanol is used in the minipreparation. What is the function of ethanol in this procedure? (3%)
    a. To remove chromosomal DNA
    b. To remove RNA
    c. To breakdown cells
    d. To remove proteins
    e. To precipitate DNA

11~14. About DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissues:

11. Which of the following is used to remove the paraffin? (3%)
    a. 100% Ethanol
    b. 75% Ethanol
    c. Xylene
    d. Distilled water

12. Which of the following is used to digest proteins in the FFPE DNA extraction procedure? (3%)
    a. Proteinase K
    b. Trypsin
    c. Chymotrypsin
    d. Protease S
    e. Collagenase

13. Which of the followings is true for the enzyme used in question #12? (3%)
    a. Raising the temperature of the reaction from 37°C to 50°C will decrease its activity.
    b. It is heat inactivated at 95°C for 10 minutes.
    c. It loses its activity in the presence of detergents, such as 0.5% (w/v) sodium dodecyl sulphate (SDS).
    d. It is inhibited by 1 mM EDTA.

14. The optimal fixation time for FFPE is: (3%)
    a. 1~6 hours
    b. 6~48 hours
    c. 2~7 days
    d. 1~4 weeks

15~16 About centrifuge balancing.

15. How to balance 5 eppendorf tubes in a 12-position rotor? Imagine a round centrifuge rotor with 12 holes like a clock with 12 numbers. One should put the tubes in positions: (3%)
    a. 12,1,4,8,9
    b. 12,2,5,8,10
    c. 12,1,4,7,8
    d. 12,2,5,7,9
    e. 1,3,6,9,11

16. How to balance 7 eppendorf tubes in a 18-position rotor? Imagine a round centrifuge rotor with evenly distributed 18 holes, clockwise-numbered from 1 to 18. One should put the tubes in positions: (3%)
    a. 18,1,6,9,10,13,16
    b. 18,1,5,6, 11,12,15
    c. 18,1,4,7,12,13,14
    d. 18,1,2,6,10,11,12
    e. 1,3,6,9,11, 13, 16

(背面仍有題目,請繼續作答)

問答題：(52 分)

1. MicroRNA is a 19~22 nt ribonucleotide that can regulate gene expression at the posttranscriptional level. As it is very short, how can you design a method to measure the miroRNA quantitatively? If necessary, you may want to diagram your idea schematically. (10%)

2. One student is asked to perform DNA cloning experiment. He or she plans to use T4 DNA ligase for his DNA ligation experiment. He or she would like to prepare his own DNA ligase buffer. Which nucleotide should be used for ligase buffer preparation, dATP or ATP, among other buffer ingredients? (4%)

3. Please describe what sort of enzymatic activity E coli DNA polymerase I have? (6%). How is Klenow enzyme related to DNA polymerase I? (4%) What sort of enzymatic activity do Klenow fragment retain? (4%) TaqMan chemistry utilize the *Thermus Aquaticus* (Taq) DNA polymerase I for real-time quantitative polymerase chain reaction, what feature of DNA polymerase I is used in this reaction? May Klenow enzyme be used for TaqMan assay? Why? (4%) (Total 18%)

4. 假設有一個基因 X，它有 50 個 exon，平均長度 300bp。今天你接到一項任務，必需設計一個方法，從腫瘤組織中偵測該基因的 exon 裡是否有突變，並且必須符合下面的條件：
   a. 該基因的突變沒有 hot spot，因此必須得到所有 50 個 exon 的基因序列。
   b. 腫瘤組織不全然是腫瘤細胞，因此該方法的敏感度必須達到 20%，也就是說帶有突變的 allele 佔所有 allele 的 20%以上的時候，必須可以被偵測到。
   c. 由於臨床檢體常常是 FFPE，RNA 的保存並不好，因此該方法一定要用 genomic DNA。
   d. 因爲臨床檢體量少，因此該方法必須在只得到 500 ng genomic DNA 的時 候，也能夠符合上述 a、b 兩點.
   e. 要得到可靠的 PCR 反應，每一管 PCR 必須加入至少 80ng genomic DNA；如果是 real-time PCR，每一管必須加入至少 2 ng genomic DNA。

   請分析困難點在哪裡，並且提出你的對策，並說明爲何你的方法可以成功。不需也不要寫出詳細的實驗步驟。(20%)