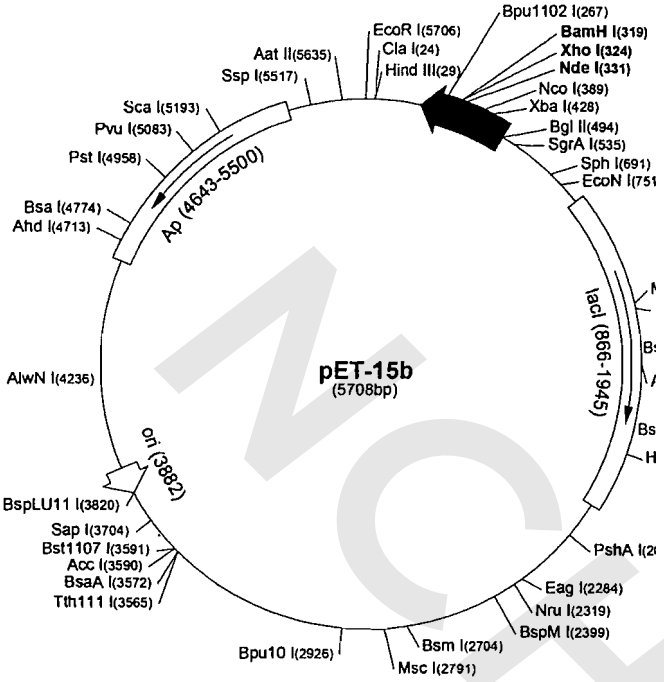
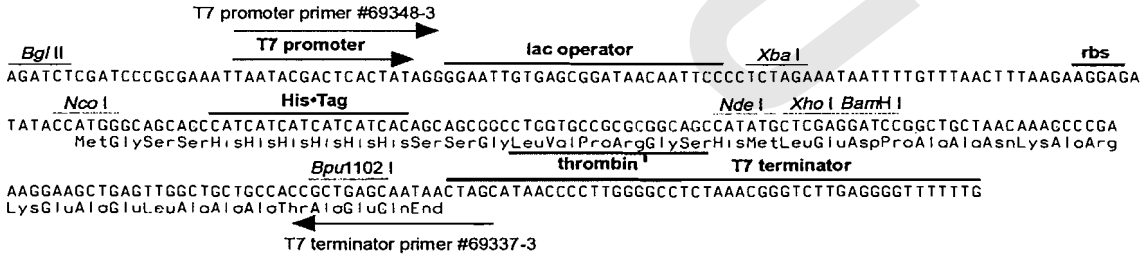
1. David is assigned to clone the gene of green fluorescent protein (GFP) into protein expression vector pET15b. The plasmid map and parts of the nucleotide sequence of GFP gene are shown below. (total 40 pt)





pET-15b cloning/expression region

The coding sequence of GFP (only the sequences at the both ends are shown):

5′-atggtgagcaagggcgaggagctgtt…(N)$_{668}$…acggcatggacgagctgtacaagtaa-3′

Please help David to answer the following questions in order to have the job done.

1.1. There is an arrow indicated by "ori" in the plasmid map. What does it mean? (2 pt)

1.2. There is a box (open reading frame) indicated by "*lacI*" in the plasmid map. What is its function? (2 pt)

1.3. There is a sequence marked by "rbs" in the cloning region of pET15b. What is its function? (2 pt)

背面有題，請繼續作答。

1.4. Because there is no *Nde*I and *Bam*HI cutting site in GFP gene, David decides to insert the GFP gene into pET15b by using the two cutting sites. To do so, he must first amplify the GFP gene engineered with an *Nde*I cutting sequence at the 5' end and a *Bam*HI site at the 3' end. Please design the pair of primer for the PCR for David. (6 pt)

1.5. What enzyme needs to be used in PCR? (2 pt)

1.6. After PCR, David wants to check the production of DNA product. So he performs an agarose gel electrophoresis. What dye can be used in the electrophoresis to visualize the DNA band? (2 pt)

1.7. After treating pET15b and the PCR-amplified GFP fragment with restriction endoribonuclease *Nde*I and *Bam*HI, David wants to perform a ligation reaction by using T4 DNA ligase. What cofactor is needed in the reaction? (2 pt)

1.8. Once the construction of pET15::GFP is done, David is asked by his advisor to send pET15::GFP into *E. coli* BL21(DE3) strain, instead of *E. coli* BL21, for the protein expression. David does not know what the difference is between the two strains. Can you tell him? (2 pt)

1.9. To select the *E. coli* transformants, an antibiotic is needed to add into the selection medium. What is it? (2 pt)

1.10. David is reminded again that isopropyl-β-D-1-thiogalactopyranoside (IPTG) needs to be added into the culture medium to induce the protein expression. Can you explain the induction mechanism of this system? (4 pt)

1.11. The GFP produced by this system is actually modified by fusing a segment of amino acids, including hexahistidine, at the N terminus of GFP. Can you roughly estimate the molecular mass of this modified GFP? (please write down how the answer is figured out) (4 pt)

1.12. By taking advantage of the presence of hexahistidine, David thinks that the fusion protein can be purified by immobilized metal affinity chromatography. Can you describe the basic principle (and the protocol) of the protein binding to and subsequently being eluted from Ni^{2+}-NTA resin? (4 pt)

1.13. In theory, the concentration of a protein solution can be calculated from its optical density (OD) at 280 nm. What amino acids in the protein provide the absorbance property at 280 nm to the protein? (2 pt)

1.14. David measures the absorbance of his purified protein and finds that his protein solution has $OD_{280} = 1$. Please calculate the molar concentration (*Mr*) of the purified protein based on the fact that the extinction coefficient (ε) of the fusion protein is 22300 Mr^{-1}. (Beer-Lambert law: $OD = \varepsilon \times Mr$) (4 pt)

2. Answer the questions based on the following article. (10 pt)

High-dose injections of vitamin C, also known as ascorbate or ascorbic acid, reduced tumor weight and growth rate by about 50 percent in mouse models of brain, ovarian, and pancreatic cancers, researchers from the National Institutes of Health (NIH) report. The researchers traced ascorbate's anti-cancer effect to the formation of hydrogen peroxide in the extracellular fluid surrounding the tumors. Normal cells were unaffected.

Natural physiologic controls precisely regulate the amount of ascorbate absorbed by the body when it is taken orally. "When you eat foods containing more than 200 milligrams of vitamin C a day — for example, 2 oranges and a serving of broccoli — your body prevents blood levels of ascorbate from exceeding a narrow range," says Mark Levine, M.D., the study's lead author. To bypass these normal controls, NIH scientists injected ascorbate into the veins or abdominal cavities of rodents with aggressive brain, ovarian, and pancreatic tumors. By doing so, they were able to deliver high doses of ascorbate, up to 4 grams per kilogram of body weight daily. "At these high injected doses, we hoped to see drug-like activity that might be useful in cancer treatment," said Levine.

Vitamin C plays a critical role in health, and a prolonged deficiency leads to scurvy and eventually to death. Some proteins known as enzymes, which have vital biochemical functions, require the vitamin to work properly. Vitamin C may also act as an antioxidant, protecting cells from the damaging effects of free radicals. The NIH researchers, however, tested the idea that ascorbate, when injected at high doses, may have prooxidant instead of antioxidant activity. Prooxidants would generate free radicals and the formation of hydrogen peroxide, which, the scientists hypothesized, might kill tumor cells. In their laboratory experiments on 43 cancer and 5 normal cell lines, the researchers discovered that high concentrations of ascorbate had anticancer effects in 75 percent of cancer cell lines tested, while sparing normal cells.

2.1. Does this report suggest that the tumor cells inside a patient can be restricted by eating a lot of orange and broccoli? (2 pt)

2.2. Does this report claim that the tumor cells can be killed by vitamin C because of its antioxidant activity? (2 pt)

2.3. Does this report say that high dose of vitamin C inside the body can be achieved by taken vitamin C orally? (2 pt)

2.4. Does this report say that high dose of vitamin C triggers the generation of H_2O_2, resulting in the death of both tumor and normal cells? (2 pt)

2.5. Does this paper say that vitamin C has antioxidant activity, while ascorbate has prooxidant activity? (2 pt)

背面有題，請繼續作答。

3. Choose one BEST answer (2 points for each, total 34 points in this section)

3.1. Which of the following description about eukaryotic pre-mRNA processing is WRONG?
- A. A 5'-CAP consists of a terminal 7-methylguanosine residue linked through a 5'-5'-triphosphate is bound to the first transcribed nucleotide.
- B. The 5'-CAP is critical for recognition by the ribosome.
- C. The 5' -CAP is critical for protection of mRNA from RNase degradation.
- D. The 5' -CAP addition is coupled to transcription and occurs within nucleus.
- E. None of the above

3.2. Which of the following description about the untranslated regions (UTRs) is WRONG?
- A. UTRs refer to sections of the mRNA before the start codon and after the stop codon that are not translated.
- B. UTRs are considered as introns.
- C. UTRs may affect the stability and translational efficiency of an mRNA.
- D. UTRs may affect the cytoplasmic localization of an mRNA.
- E. None of the above

3.3. Which of the following description about mRNAs is WRONG?
- A. The lifetime of mRNAs in prokaryotes is generally much shorter than in eukaryotes.
- B. For most of the eukaryotic mRNAs, usually only a single protein is encoded.
- C. Multiple proteins with a related function can be translated from one polycistronic mRNA in prokaryotic cell.
- D. The phenomenon in "C" is known as "operon" that only a single mRNA is regulated and transcribed although multiple proteins may be produced simultaneously.
- E. In eukaryotes it is thought that mRNA molecules form circular structures due to an interaction between the cap binding complex and poly(A)-binding protein.

3.4. Which of the following about transcription factors is WRONG? Transcription factors -
- A. contain at least one DNA-binding domain.
- B. are usually modular in protein architecture.
- C. can form complexes with other proteins so to act as activators or repressors.
- D. usually read DNA sequence information in the minor groove.
- E. contain a lot of gene members in the human genome.

3.5. Which of the following is NOT the ideal characteristic for an organism to be chosen as "model" for study?
- A. It has high mutation rate in nature.
- B. It has short life-cycle.
- C. It has inbred strains.
- D. It has method of transformation.
- E. It has simple genome arrangement.

3.6. Which of he following statements about eukaryotes is WRONG?
- A. Most eukaryotes including mice, zebrafish, fruit fly, nematodes, etc. are all diploid.
- B. Polyploidy is common for both plants and animals.
- C. Totipotency is a common trait for cell of plants but not animals.
- D. Mutations in somatic cells barely can be passed on to the next generation.
- E. Somatic mutations can result in cancer.

3.7. Epigenetic changes
- A. can lead to phenotype or gene expression changes.
- B. are caused by changes other than DNA sequences.
- C. may remain through cell divisions.
- D. may pass on to progenies.
- E. All of the above

3.8. The Cre/loxP or Ac/Ds systems can be used to
- A. generate point mutations on chromosome.
- B. integrate a transgene to a designated position on chromosome.
- C. duplicate the inserted transgene on chromosome.
- D. remove the selection marker via gene excision from chromosome.
- E. generate phenotypes to facilitate screening of transgenic organisms.

3.9. Oligonucleotide chip can NOT be applied to
- A. measure gene expressions of different tissues.
- B. detect coding regions of genes.
- C. characterize gene orders on chromosome.
- D. detect nucleotide polymorphisms among alleles.
- E. detect nucleotide polymorphisms within populations.

3.10. Chromatin immunoprecipitation (ChIP) assay is a technique that used to -
- A. investigate the interaction between proteins and DNA.
- B. quantify gene expressions between different alleles.
- C. detect nucleotide polymorphisms.
- D. determine the composition of microorganism in environmental samples.
- E. All of the above

3.11. What is the difference between the native Ti plasmids and binary vector systems used in today's plant transformations? In the binary vectors, -
- A. the oncogenic genes within T-DNA are absent.
- B. the virulent regions that producing type IV channel proteins are absent.
- C. the left and right borders of T-DNA are eliminated.
- D. the antibiotics selection marker is removed.
- E. the replication origin is removed.

3.12. Which of the following descriptions about *Agrobacterium tumefaciens* is WRONG? *Agrobacterium* -
- A. can transfer DNA between species.
- B. can manage trans-kingdom T-DNA transfer.
- C. is a plant pathogen that cause tumor-like growth.
- D. can provide plant with the ability of nitrogen fixation.
- E. None of the above

背面有題，請繼續作答。

3.13. In one method of creating a transgenic animal, engineered embryonic stem cells are inserted into a blastocyst with the hope that:

A. The inserted gene will be taken up by the cells of the blastocyst.
B. The engineered embryonic stem cells will differentiate into the germ cell line.
C. The engineered embryonic stem cells will fuse with the blastocyst cells.
D. a + b
E. a + c

3.14. The reason of "animal cloning" is -?

A. to be able to breed improved animals more quickly.
B. to introduce a transgene into the donor nucleus during cloning process so to create identical animals that express pharmaceutical proteins.
C. to preserve premium genetic materials for eugenics purpose.
D. to rescue rare breeds or endangered species of animals without crossbreeding.
E. All of the above

3.15. What property can be measured with a scanning probe microscope (SPM)?

A. Temperature change
B. Electrostatic force
C. Magnetism
D. Light absorption
E. All of the above

3.16. Which pair of prefix and its definition is WRONG?

A. Nano, 10^{-9}
B. Pico, 10^{-12}
C. Kilo, 10^{-3}
D. Femto, 10^{-15}
E. Micro, 10^{-6}

3.17. Metabolic engineering is the practice of optimizing genetic and regulatory processes within cells to increase the cells' production of a certain substance. According to this definition, which of the following is NOT a product of metabolic engineering?

A. beer
B. blue rose
C. biofuel
D. monoclonal antibody
E. None of the above

4. Matching (2 points for each, total 16 points in this section)

4.1. Choose one MAJOR technology listed in A~F that had been used for research and development of the following immune products.

4.1.1 monoclonal antibody
4.1.2 attenuated vaccine
4.1.3 subunit vaccines
4.1.4 DNA vaccine

A. recombinant DNA construction, heterologous protein expression and antigen purification
B. conjugation with adjuvant or carrier protein
C. cell fusion with myeloma cell
D. physical (such as heat) or chemical (such as mutagen) disruption of pathogenesis of viruses or bacteria
E. plasmid construction that place gene encoding antigen under control by a strong promoter
F. induction of immune response against DNA itself

4.2. Choose one MAJOR usage listed in A~F for each of the following biological research techniques.

4.2.1 immunohistochemical analysis
4.2.2 *in situ* hybridization
4.2.3 Enzyme-Linked ImmunoSorbent Assay (ELISA)
4.2.4 Fluorescence Activated Cell Sorting (FACS)

A. used to identify antigens that expressed only *in vivo*
B. used to detect and separate cells with different surface antigens
C. used to visualize the location of specific proteins in different tissues or cell layers
D. used to visualize the location of specific mRNA in different tissues or cell layers
E. used to identify specific chromosome
F. used to quantify the amount of antigen with specimen