UNIVERSITI SAINS MALAYSIA

First Semester Examination
2014/2015 Academic Session

December 2014 / January 2015

BTT 202/3 – Techniques in Biotechnology
[Teknik-Teknik Bioteknologi]

Duration: 3 hours
[Masa : 3 jam]

Please ensure that this examination paper contains NINE printed pages before you begin the examination.

[Sila pastikan bahawa kertas peperiksaan ini mengandungi SEMBILAN muka surat yang bercetak sebelum anda memulakan peperiksaan ini.]

Instructions: Answer **FIVE** (5) out of **SIX** (6) questions, in English or Bahasa Malaysia. Each question carries 20 marks.

*[**Arahan:** Jawab **LIMA** (5) daripada **ENAM** (6) soalan yang diberikan dalam Bahasa Inggeris atau Bahasa Malaysia. Tiap-tiap soalan bernilai 20 markah.]*

In the event of any discrepancies, the English version shall be used.

[Sekiranya terdapat sebarang percanggahan pada soalan peperiksaan. versi Bahasa Inggeris hendaklah diguna pakai].

...2/-

1. [a] An organism believed to be from the planet Mars has been successfully isolated. Its genomic DNA was found to be similar to earth's eukaryotic organism but its mRNA has a poly-G tail at its 3' end. Describe in detail how you would construct a cDNA library from this organism.

[Satu organisma unik yang dipercayai berasal dari planet Marikh telah berjaya dipencil. DNA genomnya didapati sama seperti organisma eukariot bumi tetapi mRNAnya didapati mempunyai ekor poli-G dibahagian hujung 3'. Terangkan langkah terperinci bagaimana anda boleh membina perpustakaan cDNA daripada organisma ini.]

(12 marks / 12 markah)

[b] You had cloned the cDNA gene for beta-galactosidase from the organism above and it has an optimum temperature of 98^{o}C. Describe the necessary steps needed to enable you to highly express this cDNA gene and obtain a maximum amount of the enzyme.

[Anda telah berjaya mengklon gen cDNA beta galaktosidase daripada organisma di atas dan ia mempunyai suhu optimum 98^{o}C. Terangkan langkah yang perlu diambil supaya anda boleh mengekspresikan gen cDNA dengan tinggi dan dapat jumlah enzim yang maksimum.]

(8 marks / 8 markah)

2. Describe the reaction(s) performed by the following proteins and their applications in molecular biological experiments :

[Terangkan tindakbalas yang dilakukan oleh protein berikut serta kegunaannya dalam eksperimen biologi molekul :]

[a] Klenow fragment

[Serpihan Klenow]

[b] S1 nuclease

[S1 nuclease]

[c] RNAseH

[RNAseH]

[d] Reverse transcriptase

[Transkriptase berbalik]

[e] T4 DNA ligase

[T4 DNA ligase]

(20 marks / 20 markah)

3. You have isolated a gene fragment from the genome of a microorganism. The fragment contains an open reading frame (ORF). The sequencing result shows the DNA sequence of the fragment as below :

[Anda telah pencilkan satu serpihan gen daripada genom satu mikroorganisma. Serpihan tersebut mengandungi satu 'open reading frame' (ORF). Keputusan jujukan DNA fragmen tersebut adalah seperti di bawah :]

```
         10        20        30        40        50        60        70        80        90
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGAAATAAAGACATTGACGCATCCCGCCCGGCTAACTATGAATTAGATGAAGTAAAATTTATTAATAGTTGTAAAACAGGAGTTTCATTA
        100       110       120       130       140       150       160       170       180
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CAATTTATATATTTAAAGAGGCGAATGATTATGACTGAAATCGTTGCAGATAAAACGGTAGAAGTAGTTAAAAACGCAATCGAAACCGCA
        190       200       210       220       230       240       250       260       270
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GATGGAGCATTAGATCTTTATAATAAATATCTCGATCAGGTCATCCCCTGGCAGACCTTTGATGAAACCATAAAAGAGTTAAGTCGCTTT
        280       290       300       310       320       330       340       350       360
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAACAGGAGTATTCACAGGCAGCCTCCGTTTTAGTCGGCGATATTAAAACCTTACTTATGGATAGCCAGGATAAGTATTTTGAAGCAACC
        370       380       390       400       410       420       430       440       450
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CAAACAGTGTATGAATGGTGTGGTGTTGCGACGCAATTGCTCGCAGCGTATATTTTGCTATTTGATGAGTACAATGAGAAGAAAGCATCC
        460       470       480       490       500       510       520       530       540
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GCCCAGAAAGACATTCTCATTAAGGTACTGGATGACGGCATCACGAAGCTGAATGAAGCGCAAAAATCCCTGCTGGTAAGCTCACAAAGT
        550       560       570       580       590       600       610       620       630
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTCAACAACGCTTCCGGGAAACTGCTGGCGTTAGATAGCCAGTTAACCAATGATTTTTCAGAAAAAAGCAGCTATTTCCAGTCACAGGTA
        640       650       660       670       680       690       700       710       720
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GATAAAATCAGGAAGGAAGCATATGCCGGTGCCGCAGCCGGTGTCGTCGCCGGTCCATTTGGATTAATCATTTCCTATTCTATTGCTGCG
        730       740       750       760       770       780       790       800       810
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGCGTAGTTGAAGGAAAACTGATTCCAGAATTGAAGAACAAGTTAAAATCTGTGCAGAATTTCTTTACCACCCTGTCTAACACGGTTAAA
        820       830       840       850       860       870       880       890       900
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CAAGCGAATAAAGATATCGATGCCGCCAAATTGAAATTAACCACCGAAATAGCCGCCATCGGTGAGATAAAAACGGAAACTGAAACAACC
        910       920       930       940       950       960       970       980       990
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGATTCTACGTTGATTATGATGATTTAATGCTTTCTTTGCTAAAAGAAGCGGCCAAAAAAATGATTAACACCTGTAATGAGTATCAGAAA
       1000      1010      1020      1030      1040      1050      1060      1070      1080
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGACACGGTAAAAAGACACTCTTTGAGGTACCTGAAGTCTGATAAGCGATTATTCTCTCCATGTACTCAAGGTATAAGGTTTATCACATT
```

You have used an ORF prediction software and the results show that an ORF is located from positions 121 to 1032.

[Anda telah menggunakan satu perisian ramalan ORF dan keputusannya menunjukkan satu ORF terletak pada posisi 122 sehingga 1032.]

[a] Underline the start codon and the termination codon in the sequence above.

[Gariskan kodon pemula dan kodon penamat pada jujukan DNA di atas.]

(2 marks / 2 markah)

[b] Using the sequence above, explain how you can determine the identity of the ORF ?

[Menggunakan jujukan di atas, terangkan bagaimana anda boleh tentukan identiti ORF tersebut ?]

(8 marks / 8 markah)

[c] Design the forward and reverse polymerase chain reaction (PCR) primers of 15 nucleotides length each to amplify the predicted ORF in the sequence above.

[Reka primer ke depan dan ke belakang tindakbalas rantai polimerase (PCR) sepanjang 15 nukelotida untuk menghasilkan ORF daripada jujukan DNA di atas.]

Forward primer: 5' – 3' *[Primer ke depan: 5' – 3']*															
Reverse primer: 5' – 3' *[Primer ke belakang: 5' – 3']*															

(4 marks / 4 markah)

[d] Using the DNA sequence of the fragment above, list **THREE** (3) other analysis (apart from ORF prediction) that can be performed using online bioinformatics tools. Describe briefly each of the analysis.

*[Dengan menggunakan jujukan DNA di atas, senaraikan **TIGA** (3) jenis analisis (selain daripada 'ORF prediction') yang boleh dilakukan dengan perisian bioinformatik atas talian. Huraikan secara ringkas setiap analisis.]*

(6 marks / 6 markah)

4. You have decided to express an unknown protein using recombinant protein technology.

[Anda telah memutuskan untuk mengekspresi satu protein yang tidak diketahui dengan menggunakan teknologi protein rekombinan.]

[a] List the organisms that can be used as hosts to express the protein.

[Senaraikan organisma yang boleh digunakan sebagai hos untuk mengekspresi protein tersebut.]

(4 marks / 4 markah)

[b] Describe a tagging strategy to express and purify the target protein.

[Terangkan satu strategi penandaan untuk mengekspresi dan memencilkan protein tersebut.]

(6 marks / 6 markah)

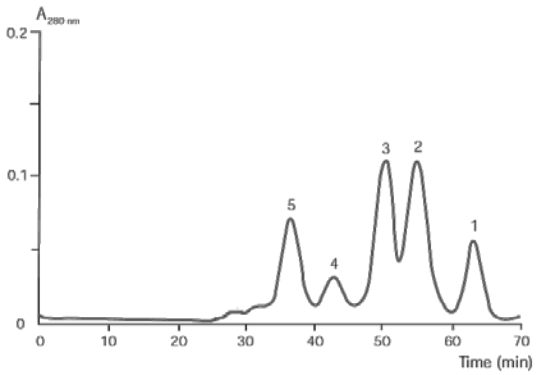
[c] After purification of your protein, you passed it through an analytical gel filtration column which you first calibrated using protein standards.

[Selepas penulenan protein anda, anda mengalirkannya melalui turus penapisan gel analitikal yang telah ditentukur dengan protein piawai.]

	Protein Standards *[Protein Piawai]*	Molecular Weight (kDa) *[Berat Molekul (kDa)]*
1.	Myoglobin	17
2.	Ovalbumin	43
3.	Albumin	67
4.	IgG	158
5.	Ferritin	440

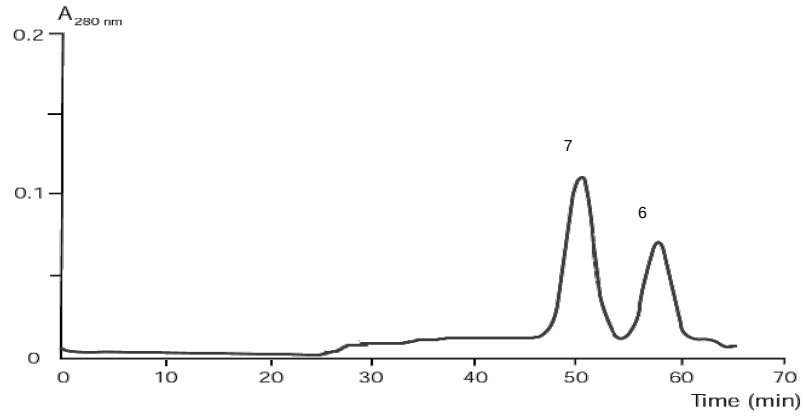
Chromatogram of protein standards.

[Kromatogram protein piawai.]



Chromatogram of your protein sample.

[Kromatogram sampel protein anda.]



Use the chromatograms and table of the protein standards above to estimate the molecular weights of peaks 6 and 7.

[Gunakan kromatogram tersebut dan jadual protein piawai di atas untuk menganggar berat molekul puncak 6 dan 7.]

Peak 6: _____

[Puncak 6: _____]

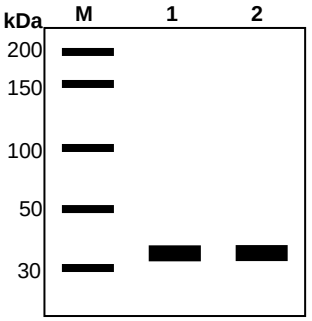
Peak 7: _____

[Puncak 7: _____]

(4 marks / 4 markah)

[d] You have analyzed a sample from the fractions representing peaks 6 and 7 by SDS-polyacryladmide gel electrophoresis. The results are as below :

[Anda telah menganalisa sampel daripada 'fraction' untuk puncak 6 dan 7 menggunakan elektroforesis gel SDS-poliakrilimida. Keputusannya seperti berikut: :]



M - Protein molecular weight marker
[Penanda berat molekul protein]

1 – Sample from Peak 6.
[Sampel daripada puncak 6.]

2 – Sample from Peak 7.
[Sampel daripada puncak 7.]

What can you conclude about your protein based on the chromatogram and SDS-PAGE results ?

[Apa yang boleh anda simpulkan tentang protein anda daripada keputusan SDS-PAGE dan kromatogram di atas ?]

(6 marks / 6 markah)

5. Discuss and differentiate between **FIVE** (5) types of fermenters that regularly used in bioprocess operation.

   *[Bincangkan dan bezakan **LIMA** (5) jenis fermenter yang digunakan dalam operasi bioproses.]*

   (20 marks / 20 markah)

6. Discuss the **FOUR** (4) required in upstream process prior to the products formation in bioreactor.

   *[Bincangkan **EMPAT** (4) langkah yang diperlukan dalam proses huluan sebelum penghasilan produk dalam bioreaktor.]*

   (20 marks / 20 markah)

- oooOooo -