UNIVERSITI SAINS MALAYSIA

Second Semester Examination
Academic Session 2004/2005

March 2005

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

Duration: 3 hours
[Masa : 3 jam]

Please ensure that this examination paper contains NINE printed pages.

Answer FIVE out of SIX questions. Candidates are allowed to answer all question in English **or** Bahasa Malaysia **or combination of both.**

Each question carries 20 marks.

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

Jawab LIMA daripada ENAM soalan yang diberikan, dalam Bahasa Inggeris ***atau*** *Bahasa Malaysia* ***atau kombinasi kedua-duanya.***

Tiap-tiap soalan bernilai 20 markah.

1. Answer the following questions based on the information extracted from the NCBI database. Phage T4 is a double-stranded DNA virus.

```
LOCUS       NC_000866              168903 bp    DNA     linear   PHG 13-JAN-2004
DEFINITION  Enterobacteria phage T4, complete genome.

     gene            complement(9410..10729)
                     /gene="dda"
                     /locus_tag="T4p017"
                     /note="synonym: sud"
                     /db_xref="GeneID:1258784"
     CDS             complement(9410..10729)
                     /gene="dda"
                     /locus_tag="T4p017"
                     /note="DNA-dependent ATPase, DNA helicase"
                     /codon_start=1
                     /transl_table=11
                     /product="Dda DNA helicase"
                     /protein_id="NP_049632.1"
                     /db_xref="GI:9632724"
                     /db_xref="GeneID:1258784"
    9301 cagtttcacg atttttgcta taagctattt cataaatttc gcttacttta tcttgaagaa
    9361 gataaaactg atctttagtt atttccacga atagcttcct caaatttaat catacataaa
    9421 acacatcata acgaccacgg gtgacaccaa cataaagaag ttgttgagct aattcaacat
    9481 ctgcataatg aatacaaggc gtataaatga aagcacggtc tacagacata ccctgcgctt
    9541 tatggaatgt tgatgcagga agtgctttca ctttactaaa ctgtgattta gcatcccaaa
    9601 aatcactcca cggagctttt ccgcctttgt tccaattttt ataagtttct gctgttttag
    9661 ctaaaaatag gttaaactta tacaattctt cgtcagatga aattatttta atctttttcac
    9721 gataatattc atcatcgcca taagtttcta ctgttaaatc ccaatgacga attagatatt
    9781 ctccaggaac accacgggct ttaacaaacg ttgatgtata ctctgcttct ataatacgaa
    9841 ctaattgtcc gttattaaaa ataatttctg acacaggctt tccatcaatt ttatatgttt
    9901 taaataatgg ttcctgcatt acaataattt caccgacaat aaaatcttta tcagtttcaa
    9961 aaatcttttt acgaataatg ctatttaact tgtcaacaga tttattcgta aatgccatta
   10021 cgcgattttc aaacaaatca tctagtgatt tgacgattga aaaataattt accataaaat
   10081 cgcgtaaagc ggtatcacca gtaaatccac gtactccatg cccgtcaaca actttatcat
   10141 aattccactt accgttgcga acgtcagtag ctacatcaat aataggagca ttactgcgtt
   10201 taacttcagt gagttcacac tgataaaaat ctttatgtgt aaagaatgga ctgatataag
   10261 cagtattttc tcctggttca acaggtctga tttgcttatt atcccctatt ccaattatag
   10321 tacaccaagg tggaatagtt gaaagcagaa ttttaaatag ctttctatca tacattgaca
   10381 cttcgtcgca gattaatact ctgcatttgg ctaaatcagg tacttctttt tgttcaaaaa
   10441 gaacattttc ttcatatgtt actgggttaa ttttaagaat actatgaata gtactcgctt
   10501 ctttccctga tagttttgaa agaatctttt tagctgcatg tgtaggagct gctaaaataa
   10561 taccagttcc acccgtagat attaaagctt caatgatgaa cttagtaaga gtagtcttac
   10621 cggtaccagc aggtccatta atagttacat gatgtttctt ttctttaata gccttcataa
   10681 caatgttaaa ggcatttttc tggccttcgg tcaaatcatc aaatgtcatc gtaaattccc
   10741 tgcaattggt atactaacaa tacgcccagt atctaaaatt cgctgatata atctttgcgt
```

(a) Design a pair of primers to amplify the helicase gene by PCR. Be sure to specify the forward and the reverse primers and the bases where they are complemented. You are also required to introduce *NdeI* (CATATG) site at the beginning of the gene and *BamHI* (GGATCC) site at the end of the gene.

(5 marks)

(b) With a help of a diagram, explain the principles of PCR.

(10 marks)

(c) What would be the expected size of the amplified PCR fragment?

(1 marks)

(d) If the genome was RNA, instead of DNA, what would you do in order to amplify the helicase gene?

(4 marks)

[BTT 202E/3]

1. *Jawab soalan-soalan berikut berdasarkan maklumat dari pengkalan data NCBI. Phage T4 adalah virus yang mempunyai benang DNA ganda dua.*

```
LOCUS       NC_000866              168903 bp    DNA     linear   PHG 13-JAN-2004
DEFINITION  Enterobacteria phage T4, complete genome.

     gene            complement(9410..10729)
                     /gene="dda"
                     /locus_tag="T4p017"
                     /note="synonym: sud"
                     /db_xref="GeneID:1258784"
     CDS             complement(9410..10729)
                     /gene="dda"
                     /locus_tag="T4p017"
                     /note="DNA-dependent ATPase, DNA helicase"
                     /codon_start=1
                     /transl_table=11
                     /product="Dda DNA helicase"
                     /protein_id="NP_049632.1"
                     /db_xref="GI:9632724"
                     /db_xref="GeneID:1258784"
    9301 cagtttcacg atttttgcta taagctattt cataaatttc gcttacttta tcttgaagaa
    9361 gataaaactg atctttagtt atttccacga atagcttcct caaatttaat catacataaa
    9421 acacatcata acgaccacgg gtgacaccaa cataaagaag ttgttgagct aattcaacat
    9481 ctgcataatg aatacaaggc gtataaatga aagcacggtc tacagacata ccctgcgctt
    9541 tatggaatgt tgatgcagga agtgctttca ctttactaaa ctgtgattta gcatcccaaa
    9601 aatcactcca cggagctttt ccgccttttgt tccaattttt ataagtttct gctgttttag
    9661 ctaaaaatag gttaaactta tacaattctt cgtcagatga aattatttta atcttttcac
    9721 gataatattc atcatcgcca taagtttcta ctgttaaatc ccaatgacga attagatatt
    9781 ctccaggaac accacgggct ttaacaaacg ttgatgtata ctctgcttct ataatacgaa
    9841 ctaattgtcc gttattaaaa ataatttctg acacaggctt tccatcaatt ttatatgttt
    9901 taaataatgg ttcctgcatt acaataattt caccgacaat aaaatctttа tcagtttcaa
    9961 aaatcttttt acgaataatg ctatttaact tgtcaacaga tttattcgta aatgccatta
   10021 cgcgattttc aaacaaatca tctagtgatt tgacgattga aaaataattt accataaaat
   10081 cgcgtaaagc ggtatcacca gtaaatccac gtactccatg cccgtcaaca actttatcat
   10141 aattccactt accgttgcga acgtcagtag ctacatcaat aataggagca ttactgcgtt
   10201 taacttcagt gagttcacac tgataaaaat ctttatgtgt aaagaatgga ctgatataag
   10261 cagtattttc tcctggttca acaggtctga tttgcttatt atcccctatt ccaattatag
   10321 tacaccaagg tggaatagtt gaaagcagaa ttttaaatag ctttctatca tacattgaca
   10381 cttcgtcgca gattaatact ctgcatttgg ctaaatcagg tacttctttt tgttcaaaaa
   10441 gaacattttc ttcatatgtt actgggttaa ttttaagaat actatgaata gtactcgctt
   10501 ctttccctga tagttttgaa agaatctttt tagctgcatg tgtaggagct gctaaaataa
   10561 taccagttcc acccgtagat attaaagctt caatgatgaa cttagtaaga gtagtcttac
   10621 cggtaccagc aggtccatta atagttacat gatgtttctt ttctttaata gccttcataa
   10681 caatgttaaa ggcatttttc tggccttcgg tcaaatcatc aaatgtcatc gtaaattccc
   10741 tgcaattggt atactaacaa tacgcccagt atctaaaatt cgctgatata atctttgcgt
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(a) Reka sepasang pencetus untuk mengamplikasi gen helikase dengan menggunakan PCR. Pastikan anda tentukan pencetus kehadapan dan pencetus kebelakang dan juga bes di mana pencetus-pencetus tersebut berkomplimen. Anda juga dikehendaki mewujudkan tapak pembatasan NdeI (CATATG) pada permukaan gen dan tapak pembatasan BamHI (GGATCC) pada penghujung gen.

(5 markah)

(b) Terangkan prinsip PCR dengan bantuan gambarajah.

(10 markah)

(c) Apakah size produk PCR yang dijangkakan terhasil?

(1 markah)

(d) Sekiranya genom adalah RNA dan bukannya DNA, apakah yang patut anda buat untuk mengamplikasikan gen helikase? .

(4 markah)

2. (a) Describe the functions and the applications of the following enzymes:

(i) T4 DNA ligase
(ii) T4 polynucleotide kinase
(iii) Ribonuclease
(iv) Terminal deoxynucleotidyl transferase
(v) Dnase I

(10 marks)

(b) How would you label the following:

(i) Oligonucleotide
(ii) 10 kb double-stranded DNA

(10 marks)

2. *(a) Terangkan fungsi dan kegunaan enzim-enzim berikut:*

(i) T4 DNA ligase
(ii) T4 polynucleotide kinase
(iii) Ribonuclease
(iv) Terminal deoxynucleotidyl transferase
(v) Dnase I

(10 markah)

(b) Bagaimana anda melabelkan yang berikut:

(i) oligonuklutida
(ii) 10 kb DNA ganda dua

(10 markah)

3. (a) Explain the principles of ethanol precipitation in plasmid DNA preparation protocol.

(6 marks)

(b) A prepared sample of plasmid DNA could not be digested with restriction enzymes. What are the possible explanations to this problem?

(6 marks)

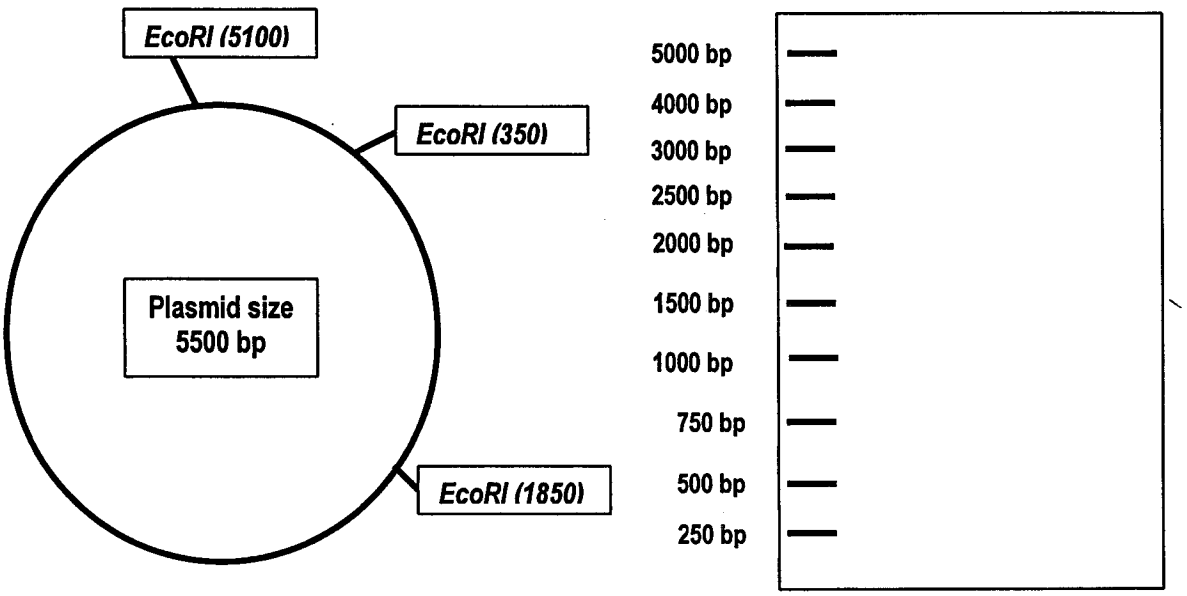
(c) After cleaning-up, the plasmid DNA sample was digested with *EcoRI* and *BamHI*. This was followed by alkaline phosphatase treatment and finally the sample was run on agarose gel electrophoresis.

(i) Draw the fragments resulting from the restriction enzymes digestion on the agarose gel provided.

(2 marks)

(ii) The biggest fragment was used as a vector in a ligation reaction but failed to ligate to the insert fragment. What was the problem and how would you solve it?

(6 marks)



3. (a) *Terangkan prinsip pemendakkan etanol dalam penyediaan DNA plasmid.*

(6 markah)

(b) *Didapati DNA plasmid yang telah disediakan tidak boleh dihadamkan oleh enzim pembatasan. Apakah penjelasan-penjelasan yang mungkin kepada masalah ini?*

(6 markah)

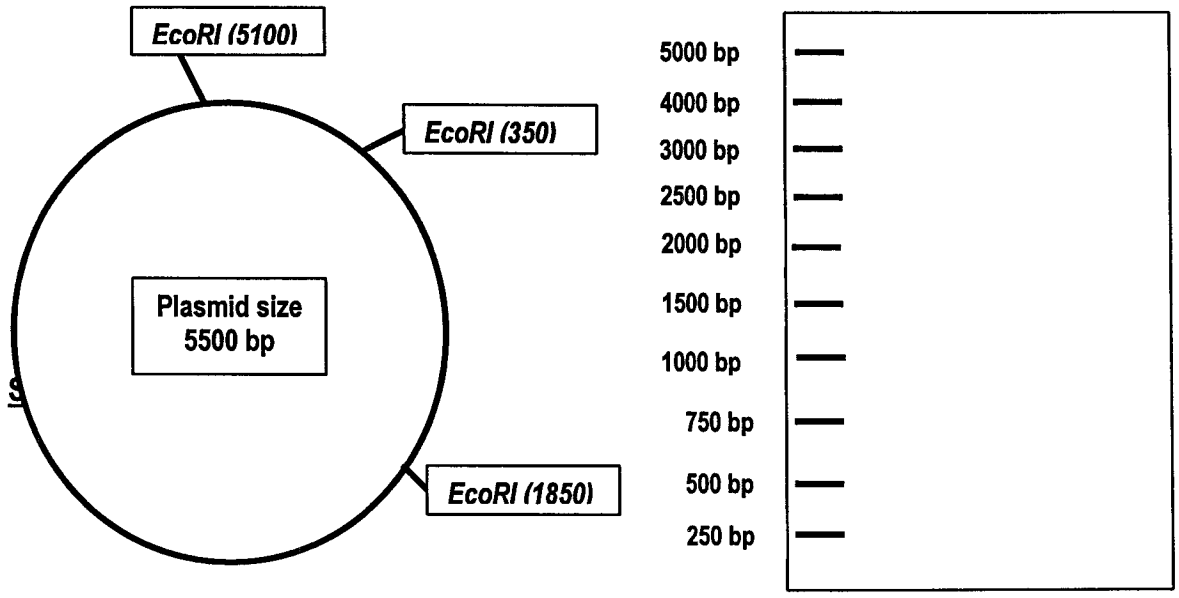
(c) *Selepas dicuci, DNA plasmid dihadamkan dengan enzim pembatasan EcoRI dan BamHI. DNA plasmid kemudiannya ditindakbalas dengan enzim fosfatase alkali dan akhirnya dilarikan di atas gel agarosa.*

(i) *Lakarkan produk-produk yang terhasil dari penghadaman enzim pembatasan di atas gel agarosa yang disediakan.*

(2 markah)

(ii) *Serpihan yang terbesar telah digunakan sebagai vektor dalam tindakbalas ligasi tetapi gagal untuk diligasi. Apakah masalah yang dihadapi dan bagaimana untuk mengatasinya?*

(6 markah)



4. Describe the ultrafiltration technology and its application in downstream processing.

(20 marks)

4. Terangkan teknologi penurasan ultra serta kegunaannya dalam proses hiliran

(20 markah)

5. Draw the flow-chart of the purification of a bacterial intracellular protease. Discuss each unit-process involved.

(20 marks)

5. Lakarkan carta-alir proses penulenan enzim protease yang dihasilkan dalam sel bakteria. Bincangkan setiap proses unit yang terlibat.

(20 markah)

6. Write notes on gel-filtration and ion-exchange chromatography.

(20 marks)

6. Tulis nota berkenaan kromatografi penurasan-gel dan pertukaran ion.

(20 markah)

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