

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"

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9301 cagtttcacg atttttgcta taagctatth cataaatttc gcttacttta tcttgaagaa
9361 gataaaactg atcttttagtt atttccacga atagcttctt caaatttaaat caticataaa
9421 acacatcata acgaccacgg gtgacaccaa cataaagaag ttgttgagct aattcaacat
9481 ctgcataatg aatacaaggc gtataaatga aagcacggtc tacagacata ccctgagctt
9541 tatggaatgt tgatgcagga agtgctttca ctttactaaa ctgtgattta gcaccccaa
9601 aatcactcca cggagctttt ccgcctttgt tccaattttt ataagtttct gctgttttag
9661 ctaaaaatag gttaaaacta tacaattctt cgtcagatga aattatttta atcttttcac
9721 gataaatatc atcatcgcca taagtttcta ctgttaaadc ccaatgacga attagatatt
9781 ctccaggaac accacgggct ttaacaaacg ttgatgtata ctctgcttct ataatacga
9841 ctaattgtcc gttatataaa ataatttctg acacaggctt tccatcaatt ttatatgttt
9901 taaataatgg ttctctgcat acaataatth caccgacaat aaaatcttta tcagtttcaa
9961 aaatcttttt acgaataatg ctatttaact tgtaacaga tttattcgta aatgccatta
10021 cgcgattttc eaacaaatca tctagtattt tgacgattga aaaataatth accataaaat
10081 cgcgtaaagc ggtatcacca gtaaatccac gtactccatg cccgtcaaca actttatcat
10141 aattccactt accgttgcca ecgtcagtag ctacatcaat aataggagca ttactgcgtt
10201 taacttcagt gagttcacac tgataaaaat ctttatgtgt aaagaatgga ctgatataag
10261 cagtattttc tcctggttca acaggtctga tttgcttatt atccccctatt ccaattatag
10321 tacaccaagg tggaatagtt gaaagcagaa ttttaaatag ctttctatca tacattgaca
10381 ctctgtegca gattaatact ctgcatttgg ctaaatacagg tacttctttt tgttcaaaaa
10441 gaacattttc ttcatatggt actgggttaa ttttaagaat actatgaata gtactcgctt
10501 ctttccctga tagttttgaa agaatctttt tagctgcatg tgtaggagct gtaaaaataa
10561 taccagttcc acccgtagat attaaagctt caatgatgaa cttagtaaga gtagtcttac
10621 cggtagcagc aggtccatta atagttacat gatgtttctt ttctttaata gccttcataa
10681 caatgttaaa ggcatttttc tggccttcgg tcaaatcadc aaatgtcadc gtaaatcccc
10741 tgcaattggg atactaacia tacgcccagt atctaaaatt cgctgatata atctttgctt
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[BTT 202E/3]

- (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)

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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.					
<u>gene</u>	complement(9410..10729) /gene="dda" /locus_tag="T4p017" /note="synonym: sud" /db_xref="GeneID:1258784"					
<u>CDS</u>	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	gataaaaactg	atcttttagtt	atctccacga	atagcttcct	caaatttaat	catacataaaa
9421	acacatcata	acgaccacgg	gtgacaccaa	cataaagaag	ttgttgagct	aattcaacat
9481	ctgcataatg	aatacaaggc	gtataaatga	aagcacgggc	tacagacata	ccctgcgctt
9541	tatggaatgt	tgatgcagga	agtgctttca	ctttactaaa	ctgtgattta	gcacccaaa
9601	aatcactcca	cggagctttt	ccgcctttgt	tccaattttt	ataagtttct	gctgttttag
9661	ctaaaaatag	gttaaactta	tacaattctt	cgtcagatga	aattatttta	atcttttcac
9721	gataaatattc	atcatcgcca	taagtttcta	ctgttaaatc	ccaatgacga	attagatatt
9781	ctccaggaac	accacgggct	ttaacaaacg	ttgatgtata	ctctgcttct	ataatacгаа
9841	ctaattgtcc	gttattaaaa	ataatttctg	acacaggctt	tccatcaatt	ttatatgttt
9901	taantaatgg	ttcctgcatt	acaataattt	caccgacaat	aaaatcttta	tcagtttcaa
9961	aaatcttttt	acgaataatg	ctatttaact	tgtaacaga	tttattcgta	aatgccatta
10021	cgcgattttc	aaacaaatca	tctagtgatt	tgacgattga	aaaataattt	accataaaat
10081	cgcgtaaagc	ggtatcacca	gtaaatccac	gtactccatg	cccgtcaaca	actttatcat
10141	aattccactt	accgttgcca	acgtcagtag	ctacatcaat	aataggagca	ttactgcgct
10201	taacttcagt	gagttcacac	tgataaaaaat	ctttatgtgt	aaagaatgga	ctgatataag
10261	cagtattttc	tcctggttca	acaggtctga	tttgcttatt	atcccctatt	ccaattatag
10321	tacacceagg	tggaatagtt	gaaagcagaa	ttttaaatag	ctttctatca	tacattgaca
10381	cttcgctcgca	gattaatact	ctgcatttgg	ctaaatcagg	tacttctttt	tgttcaaaaa
10441	gaacattttc	ttcatatggt	actgggttaa	ttttaaagaat	actatgaata	gtactcgctt
10501	ctttccctga	tagttttgaa	agaatctttt	tagctgcatg	tgtaggagct	gctaaaataa
10561	taccagttcc	accctagat	attaaagctt	caatgatgaa	cttagtaaga	gtagtcttac
10621	cggtaccagc	aggtccatta	atagttacat	gatgtttctt	ttctttaata	gccttcataa
10681	caatgttaaa	ggcatttttc	tggccttcgg	tcaaatcacc	aaatgtcacc	gtaaatccc
10741	tgcaattggt	atactaacaa	tacgcccagt	atctaaaatt	cgctgatata	atctttgcgt

[BTT 202E/3]

- (a) *Reka sepasang pencetus untuk mengamplifikasi gen helikase dengan menggunakan PCR. Pastikan anda tentukan pencetus hadapan 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 mengamplifikasikan 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)

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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)

[BTT 202E/3]

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)

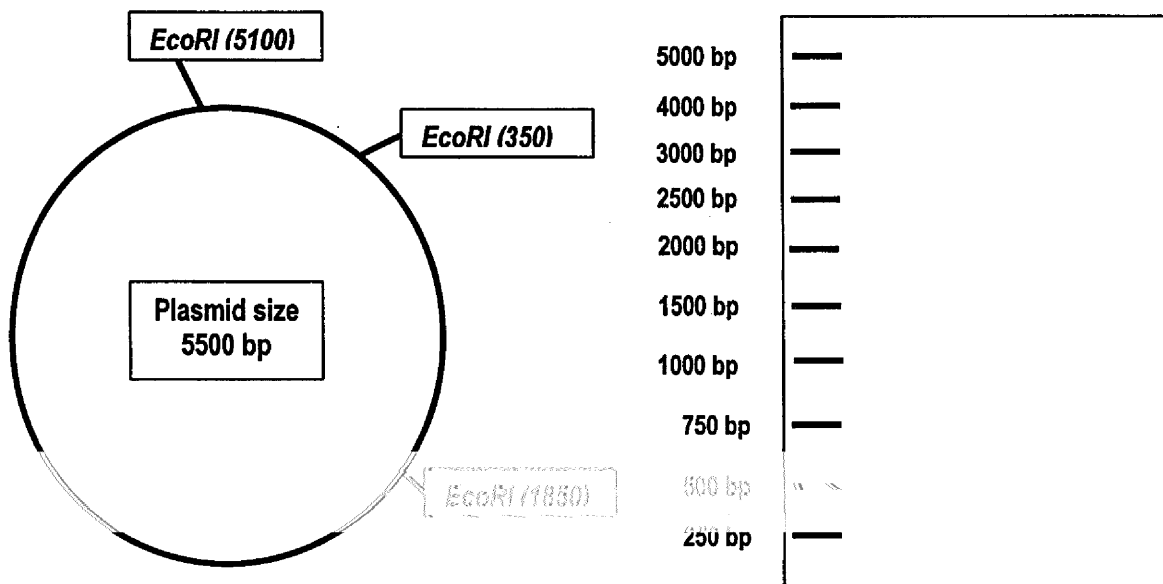
(c) After cleaning-up, the plasmid DNA sample was digested with *EcoRI* and *BamHI*. This was followed by alkaline phosphatase treatment and finally the sample was run on agarose gel electrophoresis.

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

(2 marks)

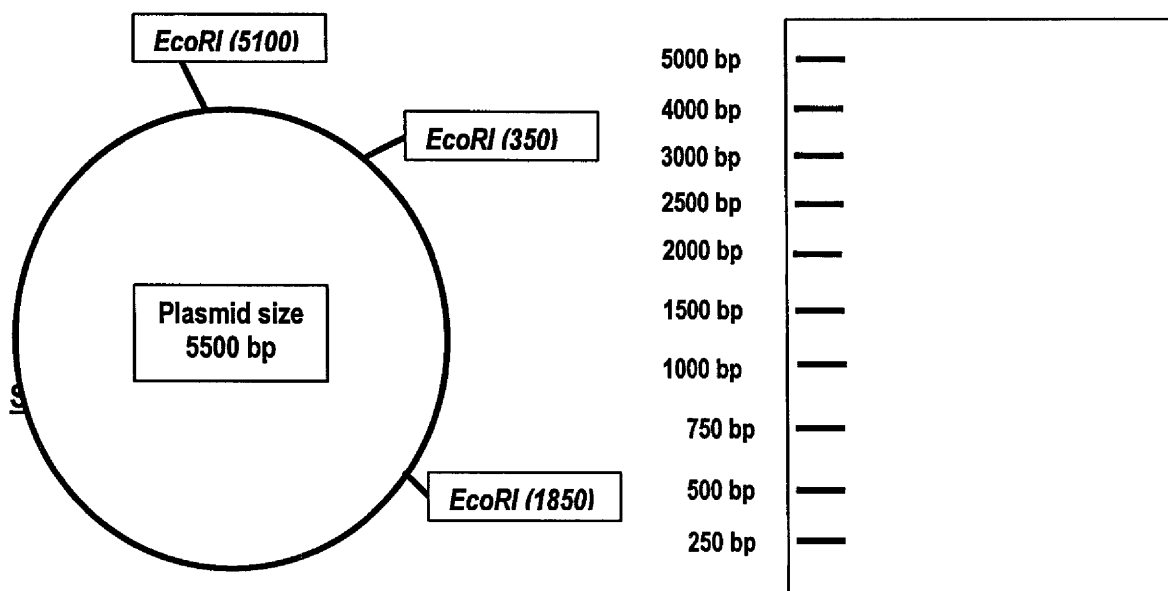
(ii) The biggest fragment was used as a vector in a ligation reaction but failed to ligate to the insert fragment. What was the problem and how would you solve it?

(6 marks)



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3. (a) *Terangkan prinsip pemendakan 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)