

UNIVERSITI SAINS MALAYSIA

First Semester Examination  
Academic Session 2005/2006

November 2005

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

Duration 3 hours  
*[Masa 3 jam]*

Please ensure that this examination paper contains SIX printed pages and ONE page of Attachment before you begin the examination

Answer FIVE out of SIX questions, in English or Bahasa Malaysia

Each question carries 20 marks

*Sila pastikan bahawa kertas peperiksaan ini mengandungi ENAM muka surat yang bercetak dan SATU muka surat Lampiran sebelum anda memulakan peperiksaan ini*

*Jawab LIMA daripada ENAM soalan yang diberikan dalam Bahasa Inggeris atau Bahasa Malaysia*

*Tiap-tiap soalan bernilai 20 markah*

. 2 .

- 1 [a] An organism believed to be from the planet Venus has been successfully isolated. Its genomic DNA was found to be similar to earth's eucaryotic organism but its mRNA was modified by having a poly-G tail at its 3' end. Describe in detail how you would construct a cDNA library from this organism.
- (12 marks)
- [b] You had cloned the cDNA gene for beta-galactosidase from the organism above and it had an optimum temperature of 98°C. Describe the necessary steps needed to highly express this cDNA gene and obtain a maximum amount of enzyme.
- (8 marks)
- 1 [a] *Satu organisma yang dipercayai berasal dari bintang Zuhrah telah berjaya dipencil. DNA genomnya didapati sama seperti organisma eukariot bumi tetapi mRNANYA didapati termodifikasi dengan mempunyai ekor poli-G dibahagian hujung 3'. Terangkan langkah terperinci bagaimana anda boleh membina perpustakaan cDNA daripada organisma ini.*
- (12 markah)
- [b] *Anda telah berjaya mengklon gen cDNA beta galaktosidase daripada organisma di atas dan ia mempunyai suhu optimum 98°C. Terangkan langkah yang perlu diambil supaya anda boleh mengekspresikan gen cDNA dengan tinggi dan dapat amaun enzim yang maksimum.*
- (8 markah)
- 2 [a] Describe the polymerase chain reaction process with the help of a diagram. Explain the basic principle for every step and label (PCR) every component in your diagram.
- (10 marks)
- [b] Describe the basic principle for every step in a Southern analysis. Label each component in your diagram.
- (10 marks)

2 [a] *Dengan menggunakan gambarajah, terangkan proses tindakbalas rantai polimerase (PCR) Terangkan prinsip asas setiap langkah dan labelkan setiap komponen dengan jelas dalam gambarajah anda*  
(10 markah)

[b] *Terangkan prinsip asas setiap langkah analisis Southern Labelkan setiap komponen dalam gambarajah anda*  
(10 markah)

3 Describe the techniques and steps needed for the following

- [i] Identification of a trisomy 21 case (also known as Down's syndrome)
- [ii] Comparative analysis of the growth hormone peptide cellular concentration between a dwarf and a normal individual
- [iii] Comparative analysis of the growth hormone mRNA cellular concentration between a dwarf and a normal individual
- [iv] Identification of whether a point mutation in the BRCA1 or BRCA2 genes has caused breast cancer in a patient

(20 marks)

3 *Terangkan teknik serta langkah yang perlu diambil untuk perkara berikut*

- [i] *Mengidentifikasi kes trisomi 21 (juga dikenali sebagai sindrom Down)*
- [ii] *Menganalisis perbezaan kepekatan dalam sel peptida hormon pertumbuhan individu kerdil dengan normal*
- [iii] *Menganalisis perbezaan kepekatan dalam sel mRNA gen hormon pertumbuhan individu kerdil dengan normal*
- [iv] *Mengidentifikasi sama ada mutasi titik dalam gen BRCA1 atau BRCA2 telah menyebabkan kanser payudara dalam pesakit*

(20 markah)

- 4 [a] Draw and label the main components of a fermentor  
(5 marks)
- [b] Explain the role of the stirrer in the control of dissolved oxygen in a fermentor  
(5 marks)
- [c] Discuss the advantages and disadvantages of shake flask and fermentor as a bioreactor in the culture of bacteria  
(5 marks)
- [d] Explain the following
- [i] Batch culture
  - [ii] Continuous culture
  - [iii] "Plug Flow" in the saccharification of starch into sugars in a column
  - [iv] "Completely mixed" in a fermentor
- (5 marks)
- 4 [a] *Lakar dan labelkan komponen utama sebuah fermentor*  
(5 markah)
- [b] *Terangkan peranan pengaduk fermentor dalam pengawalan oksigen terlarut*  
(5 markah)
- [c] *Bincangkan kebaikan dan keburukan kelalang goncang dan fermentor sebagai bioreaktor pengkulturan sel bakteria*  
(5 markah)
- [d] *Terangkan perkara berikut*
- [i] *Kultur kelompok*
  - [ii] *Kultur selanjat*
  - [iii] *Ciri "Plug Flow" dalam sakanfasi kanji kepada gula dalam turus*
  - [iv] *Ciri "Completely mixed" dalam fermentor*
- (5 markah)

- 5 [a] Intracellular products will require cell breakage List the techniques available to break microbial cells  
(10 marks)
- [b] With reference to unit operations involved in product recovery, write short notes on techniques that can be employed to achieve
- [i] Cell separation (e.g. cells from medium),
  - [ii] Enrichment, and
  - [iii] Drying
- (10 marks)
- 5 [a] *Produk yang terhasil intrasel memerlukan sel dipecah Huraikan teknik-teknik yang boleh digunakan untuk memecah sel mikrob*  
(10 markah)
- [b] *Merujuk kepada operasi unit untuk mendapatkan product dalam pemprosesan hiran, tulis nota ringkas berkenaan teknik-teknik yang diboleh digunakan untuk tujuan*
- [i] Pemisahan sel (contohnya sel daripada medium),*
  - [ii] Pengkayaan, dan*
  - [iii] Pengeringan*
- (10 markah)
- 6 [a] In the process of protein purification, ion-exchange chromatography is often carried out before gel-filtration chromatography Explain the rationale of this practise  
(3 marks)
- [b] Explain the principle of separation in the following chromatography techniques
- [i] Ion-exchange chromatography  
(5 marks)
  - [ii] Hydrophobic interaction chromatography  
(5 marks)
  - [iii] Gel-filtration chromatography  
(5 marks)

- [c] With reference to the ammonium sulphate saturation nomogram attached, determine how many grams of ammonium sulphate is required to
- [i] Make one liter of 100 % saturation ammonium sulphate solution  
(1 mark)
- [ii] Raise the saturation of 0.2 liter of 20 % saturation, to 90 % saturation  
(1 mark)
- 6 [a] *Dalam proses penulenan protein, kromatografi pertukaran ion kerap digunakan dahulu, kemudian diikuti dengan kromatografi penurasan-gel. Terangkan rasional amalan ini*  
(3 markah)
- [b] *Terangkan prinsip pemisahan dalam jenis kromatografi berikut*
- [i] *Kromatografi pertukaran-ion*  
(5 markah)
- [ii] *Kromatografi interaksi hidrofobik*  
(5 markah)
- [iii] *Kromatografi penurasan-gel*  
(5 markah)
- [c] *Berpandukan nomogram ketepuan ammonium sulfat yang terlampir, tulis berapa gram ammonium sulfat yang diperlukan untuk*
- [i] *Menghasilkan satu liter larutan ammonium sulfat yang tepu 100 %*  
(1 markah)
- [ii] *Meningkatkan ketepuan 0.2 liter larutan 20 %, menjadi 90 % ketepuan*  
(1 markah)

**Nomogram for Ammonium Sulphate Saturation**

		Final concentration of ammonium sulfate ( % saturation )															
		10	20	25	30	35	40	45	50	55	60	65	70	75	80	90	100
		Grams solid ammonium sulfate to be added to 1 liter of solution															
Initial concentration of ammonium sulfate( % saturation )	0	56	144	144	176	209	243	277	313	351	390	430	472	516	561	662	767
	10		57	86	118	150	183	216	251	288	326	365	406	449	494	592	694
	20				59	91	123	155	189	225	262	300	340	382	424	520	619
	25				30	61	93	125	158	193	230	267	307	348	390	485	583
	30					30	62	94	127	162	198	235	273	314	356	449	546
	35						31	63	94	129	164	200	238	278	319	411	506
	40							31	63	97	132	168	205	245	285	375	469
	45								32	65	99	134	171	210	250	339	431
	50									33	66	101	137	176	214	302	392
	55										33	67	103	141	179	264	353
	60											34	69	105	143	227	314
	65												34	70	107	190	275
	70													35	72	153	237
	75														36	115	198
80															77	157	
90																79	

Nomogram for determining the amount of ammonium sulfate needed per liter to yield various percentages of saturation. A saturated solution is 4.1M and 3.9M in ammonium sulfate at 25 and 0°C, respectively. The initial and final concentrations of ammonium sulfate are found on the vertical and horizontal scales, respectively. The point of intersection of two lines drawn from these points indicates the number of grams of ammonium sulfate that must be added to each liter of solution at the initial concentration to yield one of the final concentration. (Modified from *Methods of Enzymology*, Vol 1, Academic Press, New York, 1968, p 76)