(To be submitted in 5 copies for each project)

The Development and Optimization of Processes For The Expression of Sialylated Recombinant Human Therapeutic Glycoprotein in Insect Cell-Baculovirus System.

Application Form For R & D Funding Under IRPA

RM8

Department of Bioprocess Engineering
Faculty of Chemical Engineering and Natural Resources Engineering,
Universiti Teknologi Malaysia,
81310 Skudai, Johor

Table 2. Summary of Project Application For Experimental Applied Research Project.

Ref	Project No.	Project Title	Project Leader	NRIC/Passport No.	Type of Research	SEO Group	Code for SEO Group	Primary FOR Group	Code for Primary FOR Group	Research Theme	Code for Research Theme	Duration
		The Development and Optimization of Processes For The Expression of Sialylated Recombinant Human Therapeutic Glycoprotein in Insect Cell-Baculovirus System.	Badarulhisam bin Abdul Rahman	671211-06-5005	Product/process development (design end engineering)	Human Pharmaceutical Products	(S20604)	Chemical Engineering	F10702	Development of Pharmaceutical Products	EA 30801	3 years

Table 2. – Continued

GRANT REQUESTED YEAR 1 (2003)							LIST OF MAJOR EQUIPMENTS REQUESTED				Status	Remark
Temporary & Contract Personnel J400	Travel & Transportation J500	Rentals J600	Research Materials & Supplies J700	Minor Modifications & Repairs J800	Special Services J900	Special Equipments & Accessories J1000	Equipment 1	Equipment 2	Equipment 3	Equipment 4		
20,000	7,000	3000	28,000	4000	5000	40000	HPLC Columns and gel chromatography resins	Vacuum evaporator				

Table 2. – Continued

GRANT REQUESTED YEAR 2 (2004)							LIST OF MAJOR EQUIPMENTS REQUESTED				Status	Remark
Temporary & Contract Personnel	Travel & Transportation	Rentals	Research Materials & Supplies	Minor Modifications & Repairs	Special Services	Special Equipments & Accessories	Equipment 1	Equipment 2	Equipment 3	Equipment 4		
J400	J500	J600	J700	J800	J900	J1000						
20,000	5,000	3000	22,000	4,000	3000	0						

Table 2. – Continued

GRANT REQUESTED YEAR 3 (2005)							LIST OF MAJOR EQUIPMENTS REQUESTED				Status	Remark
Temporary & Contract Personnel	Travel & Transportation	Rentals	Research Materials & Supplies	Minor Modifications & Repairs	Special Services	Special Equipments & Accessories	Equipment 1	Equipment 2	Equipment 3	Equipment 4		
J400	J500	J600	J700	J800	J900	J1000						
20,000	3000	1000	13,000	0	3000	0						

(To be submitted in 5 copies for each project)

The Development and Optimization of Processes For The Expression of Sialylated Recombinant Human Therapeutic Glycoprotein in Insect Cell-Baculovirus System.

Application Form For R & D Funding Under IRPA

RM8

Department of Bioprocess Engineering
Faculty of Chemical Engineering and Natural Resources Engineering,
Universiti Teknologi Malaysia,
81310 Skudai, Johor

CHAPTER

1

APPLICATION GUIDELINES AND FORMS

A. PURPOSE

Application Form should be used for applying research grant under IRPA mechanism.

B. INFORMATION REQIRED

All application for IRPA research grant should be submitted using the standard IRPA Application Form. Each form should consist only one project.

C. RESPONSIBILITY

Institution IRPA Coordinator is required to submit to MOSTE IRPA Secretariat one hard copy of application form together with the Institutional Summary Application in single merge electronic file in excel format.

D. SUBMISSION PROCEDURE

One copy of the application form is to be mailed to:

IRPA Secretariat
Ministry of Science, Technology and the Environment
Block C5, Parcel C
Putrajaya

I. Project Identification

A. Programme/Project number (Please refer to the Guidelines)

B. Programme title Programme title should be filled for Prioritised & Strategic research proposal only and repeated in all application form for each project under the same programme.

B1. Project title (for Experimental Applied, Prioritised and Strategic research proposal)

The Development and Optimization of Processes for the Expression of Sialylated Recombinant Human Therapeutic Glycoprotein in Insect Cell-Baculovirus System.

C. Project leader (Please indicate the name and identification number of the project leader)

Name: Dr. Badarulhisam bin Abdul Rahman

NRIC: 671211-06- 5005

C1. Programme Head (Prioritised & Strategic research proposal is required to fill this section and should be repeated in all project under this programme)

Name:

NRIC:

D. Organisation (Please indicate the name, address, telephone and fax of the organisation in which the project leader is based)

Bioprocess Engineering Department
Faculty of Chemical and Natural Resources Engineering
Universiti Teknologi Malaysia
81310 UTM, Skudai. Johor

E. Key words (Please provide a maximum of 5 key words that describe the research of the project. The key words will be incorporated in a database on Malaysian research)

Therapeutic Product
Metabolic engineering
Insect Cell-Baculovirus
Glycoprotein
Bioprocessing

II. Objectives of the Project

A. Specific objective of the project (Please describe the measurable general and specific objectives of the project and define the expected results. Use results-oriented wording with verbs such as "to define ...", "to determine ...", "to identify ...")

1) To determine the optimal parameters (culture conditions, transferases and sugar nucleotides content) for the expression of complete sialylation of recombinant human glycoprotein.
2) To develop an optimal processing condition for the production of human like glycoprotein in an artificial system by the manipulation of metabolic engineering and process engineering approach.

B. Research background of the project (Please indicate if the project is new, modified or extended. Give a summary of your literature review to indicate the originality of the proposed research, and describe related research to assist in assessing the research rationale and the potential for success)

- Project status (please indicate) [x] New [ ] Modification to previous project [ ] Extension of existing project

- **Literature review summary**

The completion of the human genome sequence is expected to increase the demand for effective treatment using protein-based therapies (cancer, HIV, autoimmune disease, neuromuscular, organ rejection and allergic diseases). Most human proteins, glycoproteins with 'complex type' oligosaccharide moieties attached to an asparagine residue, usually terminated with sialic acid, an important determinant for biological activity, solubility and blood circulation time. Insect cells-baculovirus system, an excellent artificial systems for the expression of recombinant glycoproteins, offers a very promising alternative to the mammalian cell lines which suffer from high cost of operation, as well as slow growth and low yield. Unfortunately the *N*-glycan structure of the recombinant glycoprotein produced in insect cell culture is slightly different from the ones naturally produced in human, usually unsialylated and sometimes nongalactosylated, suggesting the nonexistence of several genes in insect cells. Metabolic engineering is one of the approaches to mimic the biosynthesis pathway that is naturally occurring in human. The used of stably transformed cells with human enzymes or co-infection with baculoviruses carrying mammalian β-(1-4)galactosyltransferase (**1**) and human CMP-Neu5Ac sugar nucleotide (**2**) were able to push the pathway towards human-like biosynthesis pathway. The addition of nucleotide sugar precursor into culture media also influences the final oligosaccharide structure (**3**). Apart from that, the existence of β-*N*-acetylglucosaminidase in native cell lines is believed to be another reason for the deviation from human glycoprotein synthesis pathways (**4**). Recently, we have cloned, expressed and characterized three *D. melanogaster* genes for β-*N*-acetylglucosaminidase and the inhibition study of these enzymes is underway (**5**). Since the complete pathway involves several enzyme and sugar nucleotides biosynthesis, process optimization for the expression of sialylated (human-like) recombinant glycoprotein is to be carried out in this project, specifically integrating molecular biology and chemical engineering approach. Missing enzymes will be introduced by means of metabolic engineering, in combination with viral co-expression, culturing condition and bioreactor design. Statistical approaches with the utilization of Response Surface Methodology and Taguchi Method will be applied to analyze several important parameters for this process.

References:

1. Ailor, E., Takahashi, Y., Tsukamoto, K. Masuda, **B. Abdul-Rahman**, D.L Jarvis, Y.C Lee and M. J Betenbaugh (2000). N-glycan patterns of human transferin produced in *Trichoplusia ni* insect cells: effect of mammalian galactosyltransferase. Glycobiology 10, (8): 837-47.
2. **B. Abdul-Rahman,** D.L Jarvis, Y.C Lee and M. J Betenbaugh β-1,4-Galactosyltransferase Activity in Native and Engineered Insect Cells Measured with Time-Resolved Eu^{3+}-Fluorescence. Journal of Carbohydrate Research Sept **2002**-In Press.
3. Lawrence, S.M., K.A.Huddleson, L.R.Pitts, N.Nguyen, Y.C.Lee, and M.J betenbaugh. Expression of the human *N*-acetylneuraminic acid phosphate synthase gene with 2-keto-3-deoxy-D-glycero-D-galacto-nonionic acid biosynthetic ability. J Biol Chem 275 (23): 17869-77.
4. Tomiya, N. E. Ailor, S.M .Lawrence, MJ Betenbaugh and YC Lee (2001). Determination of nucleotides and sugar nucleotide involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells. Anal Biochem 293(1): 129-37.
5. **B. Abdul-Rahman**, D.L Jarvis, Y.C Lee, K Palter and M. J Betenbaugh. Cloning, Expression and Characterization of *Drosophilla melanogaster* β-*N*-Acetylglucosaminidases. (Manuscript: in preparation).

- Related research

Project Title: Evaluation of the Enzymes in the *N*-Glycosylation Pathways of Native and Genetically Engineered Insect Cells.

The proposed project will be a continuation of this project. Some stably transformed cell lines and baculoviruses carrying the genes for human transferin, (model protein), α(2-6) sialyltransferase and β-1-4 galactosyltransferase are available (Gifts from Prof. Dr. Michael J. Betenbaugh of the Department of Chemical Engineering, Johns Hopkins University, Maryland, USA). The work centered on the development of metabolic engineering approach to mimic human glycorprotein biosynthesis pathways in insect cell culture. Missing genes in cell lines were determined. By using human genome (NCBI) and Drosophila genome (FlyBASE) databases, human and Drosophila transferases genes were incorporated into suitable cell lines and used for the expression of human transferin, a model human glycoprotein. Human transferin was chosen for its simple biantennary oligosaccharide structure with terminal sialic acid. The outputs of the project: Total 7 peer-reviewed papers are expected.

1. *N*-glycan Patterns of Human Transferrin Produced in *Trichoplusia ni* Insect Cells: Effect of mammalian β(1-4)galactosyltransferase. Journal of Glycobiology vol 10, no 8 pp 837-847, **2000**.

2. *Crocus sativus* Lectin Recognizes $Man_3GlcNAc$ in the *N*-glycan Core Structure. Journal of Biological Chemistry, vol 275, 35 Sept 1, pp 26772-26779, **2000**.

3. Application of Lanthanide Fluorescence in Glycobiology. Journal of Glycobiology, vol 10,no 10, **2000**, pp 1140

4. β-1,4-Galactosyltransferase Activity in Native and Engineered Insect Cells Measured with Time-Resolved Eu^{3+}-Fluorescence. Journal of Carbohydrate Research Sept **2002**-In Press.

5.. Determination of nucleotides and sugar nucleotide involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells. Anal Biochem 293(1): 129-37, **2001**.

6. Cloning, Expression and Characterization of *Drosophilla melanogaster* β-*N*-Acetylglucosaminidases. (Manuscript: **in preparation**).

7. Exoglycosidases in Native and Genetically Engineered *Trichoplusia ni*, Snyder S2 and Sf9 Cell Lines- Activity towards natural and synthetic substrates. (Manuscript: **in preparation**).

C. Type of research (Please indicate the type of research, one only; see definition of terms in the Guidelines)

- [ ] 1. Scientific research (fundamental research)
- [X] 2. Technology development (applied research)
- [ ] 3. Product/process development (design end engineering)
- [ ] 4. Social/policy research

D. Socio-economic objective being addressed by the project (Please identify the Sector, SEO Category and SEO Group which most appropriately describe the main beneficiary of your proposed project. For definitions, please refer to the Guidelines. Refer to the R&D Priority Areas for RM8 document attached and the Malaysian R&D Classification System brochure for the SEO Group code)

- Sector: Science and Engineering
- SEO Category: Natural Sciences, Technologies and Engineering (S 50100)
- SEO Group and Code: Applied Sciences and Technologies (S50106)

E. Target Area, Research Theme, Programme being addressed (Please identify the Target Area, Research Theme and Programme under which your proposed project falls. Refer to the attached R&D Priority Areas for RM8. For definitions please refer to the Guidelines)

- Target Area: Pharmaceutical Products Resources (EA 30800)
- Research Theme: Development of Pharmaceutical Products
- Research Theme Code: EA 30801
- Programme: None

F. Fields of research (Please identify the two main FOR Categories, FOR Groups and FOR Areas which most appropriately describe the scientific discipline of your proposed project. For definitions, please refer to the Guidelines. Refer to the Malaysian R&D Classification System brochure for the FOR classification and codes)

- Primary Field of Research
  - FOR Category: Engineering Sciences (F10700)
  - FOR Group and Code: Chemical Engineering (F10702)
  - FOR Area: Other Chemical Engineering
- Secondary Field of Research
  - FOR Category: Medical and Health Sciences (F11000)
  - FOR Group and Code: Pharmaceutical Industry (F11012)
  - FOR Area: Other Pharmaceutical Industry

III. Benefits of the Project

A. Direct customers/beneficiaries of the project (Please identify clearly the potential customers/beneficiaries of the research results and provide details of their relevance, eg, size, economic contribution, etc)

1. Local Pharmaceutical industries
   eg: Inno-Biotechnology
   Cyberjaya, Selangor

2. Academic communities

3. Patients with rheumatoid arthritis, HIV infection, ulcerative colitis, sickle cell anemia and many others which requiring protein based therapeutics.

B. Outputs expected from the project (Please refer to the list of outputs in the Guidelines and give further details)

1. New processes will be developed (patentable) for the production of human-like recombinant glycoproteins for protein-based pharmaceutical.

2. World-class facilities for a large-scale insect-cell expression system will be available locally.

3. Experts in protein-based pharmaceuticals (PhD, MSc graduates)

C. Technology transfer/diffusion approach (Please describe how the outputs of the project will be transferred to the direct beneficiaries/customers. Please also state if the project outputs are sustainable, ie, if they can be utilised without further external assistance)

1. Journal Publications.

2. Possibility of partnership with pharmaceutical industries.

3. Metabolic engineering is a new area in biotechnology. This project is a platform for an international collaboration with scientist from highly developed countries (USA, Japan)

D. Organisational outcomes expected (Please refer to the list of outcomes in the Guidelines and give further details)

1. Experts in the field of Metabolic Engineering especially for recombinant therapeutic processing. 1 PhD, 2 MSc. students and several final year student theses.

2. Patent right, intellectual property rights and royalty for the possible commercialization of process.

3. Organization reputation and demands for consultancy services

E. Sector/National impacts expected (Please refer to the list of impacts in the Guidelines and give further details)

1. Local technology capable of producing recombinant therapeutics will generate wealth and savings on foreign exchange for the expensive pharmaceuticals.

2. Linkages with international research institution, universities.

IV. Project Structure

A. Research organisations involved in the project (Please identify all research organisations collaborating in the project, and describe their role/contribution to the project)

1. Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering, UTM

   Contributions:

   - Experimental design.
   - Data analyses.

B. Industry linkages (Please identify any industry or end-user group involved in the project, and describe its role/contribution to the project)

Inno-Biotechnology
Cyberjaya,
Selangor.

The newly developed and optimized processes could further be tested and utilized for the production of recombinant human Erythropoietin at pilot and production scale.

C. Project Team

Name[1]	Organisation	Man-months[2] on project
Project Leader (Please provide name) Dr. Badarulhisam Abdul Rahman	Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia	6.0
Programme Head (Please provide name)		
Researchers (Please provide names or numbers of researchers) 1. Dr. Ani Idris 2. Dr. Azila Abd. Aziz 3. Prof. Dr. Michael Betenbaugh (Research Consultant-see attached letter)	Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia Chemical Engineering Johns Hopkins University Maryland USA.	4.5 4.5 0
Support Staff (Please indicate how many) 2 persons	To be appointed	12
Contract Staff (Please indicate how many) 1 person	To be appointed	35.75
	Total	62.75

[1] Please attach the curriculum vitae of key research personnel. Please follow the format included in Appendix A..

[2] Based on 24 man-days per month; please use the man-days per staff category included in the Staff Cost Estimation Form in Appendix D.

V. Research Approach

A. Research methodology (Please describe the research methodology to be followed. Identify specialised equipment, facilities and infrastructure which are required for the project, and indicate which are new)

1. Stably Transformed Cell Lines with Human β(1-2)-*N*-acetylglucosaminyltransferase II and β(1-4)Galactosyltransferase from the known sequence in human database will be generated. The genes will also be cloned into baculovirus vector together with α–2,3 sialyltransferase and CMP-Neu5Ac following established procedures. [**Ref:** Davies, A.H. 1994, Current Methods for Manipulating Baculovirus. Biotech (N.Y.) 12(1):47-50]

2. Optimization experiments to induce sugar nucleotide expression in selected cell lines. Optimal sugar concentration (*N*-acetylglucosamine, Galactose and Mannose and Mannosamine) will be evaluated. Cellular sugar nucleotides content generated will be determined and the optimal feeding conditions will be used for the next experiment. Cellular sugar nucleotides content will be determined using an HPLC with CarboPAc PA1 column or CLC-ODS column. [**Ref:** Determination of nucleotides and sugar nucleotide involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells. Anal Biochem 293(1): 129-37, **2001**]

3. Characterization of recombinat transferases activity against natural and synthetic substrates. The level of exoglycosidases activity will also be determined. Synthetic substrates of pNP-glycosides or 4-methylumbellyferyl-glycosides and pyridylaminated natural oligosaccharide will be used. [**Ref**: Procedures: See Appendix B for our past works]

4. Development of experimental design for sialylated glycoprotein production. Combination of metabolic engineering, co-expression of recombinant baculovirus with several transferases, sugar feeding will be used. The possibility of using of suitable β-*N*-acetylglucosaminidase inhibitor will also be explored. Optimal condition for the expressions of recombinant therapeutic protein (human transferin as a substrate acceptor), transferases, sugar nucleotide(substrate donor) and other parameter will all be determined. Statistical analyses (Response Surface Methodology and Taguchi Method) will be used to optimize the process.
[**Ref**: Ani,I., Ismail,A.F., Noordin,M.Y. and Shilton, S.J. (2002). Optimisation of cellulose acetate details of reverse osmosis hollow fibre membranes using Taguchi Methods. Journal of membrane Science, 5298, 1-15.]

5. Large-scale glyprotein expression with the application of several parameters and its effect on oligosaccharide structure will be carried out. Purification of recombinant protein and its oligosaccharide characterization will be carried out. [**Ref:** Appendix B for related works]

Table 1. Facilities and Equipment Required.

1. High pH Anion Exchange Chromatography	Available
2. High Performance Liquid Chromatography	Available
3. Protein Chromatography Apparatus/Accessories (HPLC Columns, ion exchange resins, affinity resin and fraction collector)	New (to be acquired from this project)
4. 2L bioreactor and cell culture facilities	Available
5. Vacuum Evaporator	New (to be acquired from this project)

B. Project activities (Please list and describe the main project activities, including those associated with the transfer of the research results to customers/beneficiaries. The timing and duration of these activities are to be shown in the Gantt chart in Form VI)

1. The determination of optimal growth and product formation conditions. These activities includes:
    i. The expression of necessary glycosyltransferases for effective glycosylation. Levels and specific activity of each enzyme will be evaluated.
    ii. Optimization experiments for sugar nucleotide expression in selected cell lines. Optimal sugar concentration (*N*-acetylglucosamine, Galactose and Mannose and Mannosamine) will be determined.

2. Development of experimental design for sialylated recombinant glycoprotein poduction. 1-2L scale glyprotein expression with the application of several parameters and its effect on oligosaccharide structures will be carried out. Optimal conditions for protein expression, sugar nucleotide level and the extent of glycosylation will be determined.

3. System improvements, process modifications and optimization.

    i. Process modifications and optimization will be carried out based on results of part 2. The extent of protein glycosylation of the purified protein will be determined using HPLC, capillary electrophoresis and mass spectrophotometer.
    ii. The use of chemical inhibitor in combination with metabolic engineering approach and the possible use of immobilized enzyme reactor will also be explored to increase the sialylation of the recombinant glycoproteins.

C. Key milestones (Please list and describe the principal milestones of the project. The timing of milestones is to be shown in the Gantt chart on Form VI. A key milestone is reached when a significant phase in the project is concluded, e.g. completion of test, review, commissioning of equipment, etc)

1. The determination of optimal growth and product formation conditions.
2. Development of the optimal processing conditions for human-like glycoprotein in an artificial system.
3. System improvements, process modifications and optimization.

D. Risks of the project (Please describe factors the that may cause delays in, or prevent implementation of, the project as proposed above; estimate the degree of risk)

Factors:

1. Protein purification and the characterization of oligosaccharide structure are relatively simple procedures but significant delays are possible under inexperienced hands

	Low	Medium	High
Technical risk:		/	
Timing risk:		/	
Budget risk:		/	

E. Duration **(State the planned starting date of the project and the elapsed time, in months, to complete this project; technology transfer activities should be excluded from elapsed time)**

- Starting date January 2003
- Duration/elapsed time 36 months

VI. Project Schedule

	2003_												2004_												2005_		200_		200_	
Research Activities	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
1. The determination of optimal growth and product formation conditions.	/	/	/	/	/	/	/	/	/	/ •																				
2. Development of the optimal processing conditions for human-like glycoprotein in an artificial system										/	/	/	/	/	/	/	/	/	/•											
3. System improvements, process modifications and optimization																	/	/	/	/	/	/	/	/	/	/•				
.																														
Technology Transfer Activities																														
1. Publications. Documentations and Patent Filing.																										/•				

- • Planned milestone

S1: First Semester
S2: Second Semester

VII. Project Cost

A. Staff costs (Please include the yearly staff costs of the project. For computation, use the Staff Cost Estimation Form in Appendix D. Numbers in parentheses refer to expense codes)

Staff Category	Year 1 (2002/2003)	Year 2 (2003/2004)	Year 3 (2004/2005)	Year 4 ( )	Year 5 ( )	Total
• Salaried personnel (11100)	30240	30240	30240	-	-	90720
• Temporary and contract personnel (J 400)	20000	20000	20000	-	-	60,000
Sub-total staff costs	50240	50240	50240			150,720

B. Direct project expense (Please include the yearly direct expenses of the project. For computation, use the Direct Expenses Estimation Form in Appendix E. Numbers in parentheses refer to expensa codes)

Staff Category	Year 1 (2002/2003)	Year 2 (2003/2004)	Year 3 (2004/2005)	Year 4 ( )	Year 5 ( )	Total
• Travel and transportation (J500)	7000	5000	3000			15000
• Rentals (J600)	3000	3000	1000			7000
• Research material and supplies (J700)	28,000	22,000	13000			63,000
• Minor modifications and repair (J800)	4000	4000	0			8000
• Special services (J900)	5000	3000	3000			11000
• Special equipment and accessories (J1000)	40000	0	0			40000
Sub-total staff costs	87,000	37,000	20,000			144,000

C. Total cost (Please add the sub-totals of A and B)

Year 1 (2002/2003)	Year 2 (2003/2004)	Year 3 (2004/2005)	Year 4 ( )	Year 5 ( )	Total
137,240	87,240	70,240			294,720

VIII. Project Funding

A. Funding sources (Please indicate funding sources for the project; see list of funding sources in the Guidelines)

Funding Sources	RM	% of Total Funding
• IRPA Grant	204,000	69.2%
• Internal Funds	90,720	30.8%
• Other Sources (please specify)	Nil	Nil
Total	294,720	100%

B. Disbursement schedule for IRPA funds, by participating research organisation (Please indicate how IRPA funding for the project will be allocated)

Organisation	Year 1 (2002/2003)	Year 2 (2003/2004)	Year 3 (2004/2005)	Year 4 (……..)	Year 5 (……..)	Total
UTM	107,000	57,000	40,000			204,000
Total IRPA Grant	107,000	57,000	40,000			204,000

IX. Contractual Matters

A. Contractual obligations under this project (Please indicate any contractual obligations with third parties that will be entered in for this project)

None

B. Ownership of intellectual property rights (Please indicate the organisation(s) that will own the intellectual property rights that may arise from this project)

UNIVERSITI TEKNOLOGI MALAYSIA

C. Approving Officer (of the organisation in which the Project Leader is based)

Name : _____

Designation : _____

Date : _____ Signature :

Appendix A – Curriculum Vitae

Please follow the following format when submitting the curriculum vitae of key research personnel

A. Personal Data

1.	**Name**	:	Dr Ani Idris
2.	**IC No**	:	630202-71-5300 (A2257141)
3.	**Date and Place of Birth**	:	2 February, 1963. Singapore
4.	**Sex**	:	Female
5.	**Nationality**	:	Malaysia
6.	**Name of Current Employer**	:	Universiti Teknologi Malaysia
7.	**Address**	:	Jabatan Kejuruteraan Bioproses Fakulti Kejuruteraan Kimia dan Kejuruteraan Sumber Asli Universiti Teknologi Malaysia Karung Berkunci 791 80990 Johor Bahru Johor
8.	**Telephone No**	:	07-5576160 ext. 5403
9.	**Fax No**	:	07-5581463
10.	**Title of Position Held**	:	Lecturer
11	**Signature of Researcher**	:	25/6/2002
12.	**Date**	:	

B. Educational Qualifications

1. Academic Qualification

i.	Degree	:	Ph.D Bioproses Engineering
	Field	:	Membrane Technology
	Year	:	2002
	Name and Place of Institution	:	Universiti Teknologi Malaysia
ii.	Degree	:	MSc. in Biochemical Engineering
	Field	:	Biochemical Engineering
	Year	:	1989
	Name and Place of Institution	:	University College London United Kingdom

iii.	Degree	:	BSc. in Chemical Engineering
	Field	:	Chemical Engineering
	Year	:	1988
	Name and Place of Institution	:	Universiti Teknologi Malaysia

C. Research Experience

1.	**Number of Years of Experience in the Field Related to the Proposed Project**	:	4 years
2.	**Fields of Specialisation**	:	Membrane Manufacturing Technology Reverse Osmosis Separation Technology/Systems Ultrafiltration Separation Systems
3.	**Major Research Programmes/Projects Completed**		
1.	Title	:	Development of Hollow Fiber Membranes
	From	:	4/99
	To	:	5/2001
	Position held	:	Principal Researcher
	Major output (Repeat as necessary)	:	Spinning rig has been fabricated, set up, improved and improvised and operated locally. A reverse osmosis test rig has been designed and also fabricated. Locally made membranes has been manufactured and tested. 12 technical papers has been published.
2.	Title	:	Production and testing of ultrafiltration membranes
	From	:	12/99
	To	:	12/2001
	Position held	:	Project leader /researcher
	Major output	:	Locally made UF membranes has been produced and tested. A UF test rig has been designed and also fabricated. 2 technical papers has been published
		:	

3.	Title	:	Model development for characterization of ultrafiltration and Reverse osmosis membranes hollow fiber membranes
	From	:	1/3/02/2002
	To	:	28/02/2003
	Position held	:	Project leader
	Major output	:	In progress.
4.	Title	:	Thermal Energy Storage
	From	:	10/94
	To	:	11/95
	Position held	:	Project leader /researcher
	Major output	:	Spiral wound heat exchanger has been designed
5.	Title	:	Pilot scale production of efficient techniques for deodorisation of cocoa butter
	From	:	3/96
	To	:	11/98
	Position held	:	Researcher
	Major output	:	1 MSc Student
6.	Title		Biodegration of lubricants /oil wastes from industries
	From		3/93
	To		8/95
	Position held		Researcher
	Major output		1 MSc. Student
7.	Title		Alternative Fuel for diesel from vegetable oil
	From		3/92
	To		8/93
	Position held		Researcher
	Major output		1 technical paper

D.	**Research Achievements**		
1.	**Honours and Awards**	**:**	
2.	**Major Publications**	**:**	Presented and published in International Journals and Conference Proceeedings. List as publications as attached overleaf.
3.	**Number of Patents**	**:**	None
4.	**Major Commercial Achievements**	**:**	None.

Appendix B – Summary of Relevant Past Research

A. Project title

Evaluation of the Enzymes in the *N*-Glycosylation Pathways of Native and Genetically Engineered Insect Cells

B. Relevance to proposed project

The proposed project will be a continuation of this project. Some stably transformed cell lines and baculoviruses carrying the genes for human transferin, (model protein), α(2-6) sialyltransferase and β-1-4 galactosyltransferase are available (Gifts from Prof. Dr. Michael J. Betenbaugh of the Department of Chemical Engineering, Johns Hopkins University, Maryland, USA).

C. Organisation(s) that were involved in the project (Please indicate the organisation that led the project)

Department of Biology & Department of Chemical Engineering, The Johns Hopkins University, Baltimore, Maryland, USA.

D. Names of senior staff

- Programme head:
- Project leader: Prof. Dr. Michael J. Betenbaugh
- Key researchers: Prof. Dr. Yuan Chuan Lee
  Badarulhisam Abdul-Rahman, PhD
  Shawn Lawrence, PhD

E. Description of the project (Please indicate project customers/beneficiaries, research approach adopted and outputs)

The work is centered on the development of metabolic engineering approach to mimic human glycorprotein biosynthesis pathways in insect cell culture. Missing genes in cell lines were determined. By using human genome (NCBI) and Drosophila genome (FlyBASE) databases, human and Drosophila transferases genes were incorporated into suitable cell lines and used for the expression of human transferin, a model human glycoprotein. Human transferin was chosen for its simple biantennary oligosaccharide structure with terminal sialic acid.

The outputs of the project are two PhD dissertation and total 8 peer-reviewed papers are expected from the project.

1. *N*-glycan Patterns of Human Transferrin Produced in *Trichoplusia ni* Insect Cells: Effect of mammalian β(1-4)galactosyltransferase. Journal of Glycobiology vol 10, no 8 pp 837-847, **2000**.

2. *Crocus sativus* Lectin Recognizes $Man_3GlcNAc$ in the *N*-glycan Core Structure. Journal of Biological Chemistry, vol 275, 35 Sept 1, pp 26772-26779, **2000**.

3. Application of Lanthanide Fluorescence in Glycobiology. Journal of Glycobiology, vol 10,no 10, **2000**, pp 1140

4. β-1,4-Galactosyltransferase Activity in Native and Engineered Insect Cells Measured with Time-Resolved Eu^{3+}-Fluorescence. Journal of Carbohydrate Research Sept **2002**-In Press

5. Expression of the human *N*-acetylneuraminic acid phosphate synthase gene with 2-keto-3-deoxy-D-glycero-D-galacto-nonionic acid biosynthetic ability. J Biol Chem 275 (23): 17869-77.

6. Determination of nucleotides and sugar nucleotide involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells. Anal Biochem 293(1): 129-37, **2001**.

7. Cloning, Expression and Characterization of *Drosophilla melanogaster* β-*N*-Acetylglucosaminidases. (Manuscript: **in preparation**).

8. Exoglycosidases in Native and Genetically Engineered *Trichoplusia ni*, Snyder S2 and Sf9 Cell Lines- Activity towards natural and synthetic substrates. (Manuscript: **in preparation**).

APPENDIX C – Staff Cost Estimation Worksheet

Role in Project	Total	Project Leader	Researchers	Support Staff	Contract Staff
Daily Rate (RM)		**200**	**200**	**65**	**70**
Research Activities	**Man-Days[1]**				
1. Expression of necessary glycosyltransferases.	267	25	42	50	150
2. Characterization of enzymatic activity in transformed and baculovirus infected cells	165	15	20	30	100
3. Optimisation experiment for sugar nucleotide concentration	72	8	10	16	36
Total Year 1 (2003) Man-days	**504**	**48**	**72**	**96**	**286**
Total Year 1 (2003) Cost (RM)[2]	**50240**	**9600** (11100)	**14400** (11100)	**6240** (11100)	**20,000** (J 400)
4. Development of experimental design for sialylated glycoprotein production	115	5	10	20	80
5. Evaluation of processing parameters.	62	10	27	0	25
6. Optimisation of protein expression and glycosylation	141	10	15	56	60
7. System improvements, process modifications and optimization	90	10	10	10	60
8. Data collection and analysis	78	5	5	10	58
9. Publications.	18	8	5	0	3
Total Year 2 (2004) Man-days	**504**	**48**	**72**	**96**	**286**
Total Year 2 (2004) Cost (RM)[2]	**50240**	**9600** (11100)	**14400** (11100)	**6240** (11100)	**20,000** (J 400)
6(cont). Optimisation of protein expression and glycosylation	223	15	30	48	130
7(cont). System improvements, process modifications and optimization.	219	15	26	48	130
8.(cont). Data collection and analysis	46	10	8	0	28
9(cont). Publications.	16	8	8	0	0
Total Year 3 (2005) Man-days	**504**	**48**	**72**	**96**	**286**
Total Year 3 (2005) Cost (RM)[2]	**50240**	**9600** (11100)	**14400** (11100)	**6240** (11100)	**20,000** (J 400)
Total Project Man-days	**1506**	**144**	**216**	**288**	**858**
Total Project Staff Cost (RM)	**151200**	**28800** (11100)	**43200** (11100)	**18720** (11100)	**60,000** (J 400)
Total Man-months[3]	**62.75**	**6**	**9**	**12**	**35.75**

Notes
1. For each research activity, estimate the man-days required by each staff category.
2. Compute the staff cost for each year by multiplying the total man-day by the daily rate of the corresponding staff category. For daily rate computation, refer to the Guidelines.
3. Compute the total man-months required for the project by dividing the total project man-days by 24.
Numbers in parentheses are expense codes as shown in Form VII.

Appendix D - Direct Expenses Estimation Worksheet

Expense Categories and Items	Exp Code	Total RM	2003	2004	2005	2006
Travel and transportation	**J500**	**15,000**	**7,000**	**5,000**	**3,000**	**0**
1 Seminar/workshop/conference		7,000	3,000	2,000	2,000	
2 Short courses for technology updates		4,000	2,000	1,000	1000	
3 Travelling to collaborators overseas		4,000	2,000	2,000		
Rentals	**J600**	**7,000**	**3,000**	**3,000**	**1,000**	**0**
1 Analytical equipment		5,000	2,000	2,000	800	
2 Others		2,000	1,000	1,000	200	
Research materials and supplies	**J700**	**63,000**	**28,000**	**22,00**	**13,000**	**0**
1 Culture Media, serum, antibiotics and standard oligosaccharides		22,700	10,000	10,800	1,900	
2 Analytical Chemicals and Digestion Enzymes (aminopyridine, 4MU-glycosides, PNG-F galactosidase, sialidase, Glucosaminidase etc)		35,900	15,900	10,000	10,000	
3 Communication		400	100	200	100	
4 Books, journals		2,000	1,000	500	500	
5 Others		2,000	1,000	500	500	
Minor modifications and repairs	**J800**	**8,000**	**4,000**	**4,000**	**0**	**0**
1 Modification of fluorescence detector		4,000	2,000	2,000		
2 Recalibrate, service & minor repair of existing equipments.		4,000	2,000	2,000		
Special services	**J900**	**11,000**	**5,000**	**3,000**	**3,000**	**0**
1 Overseas expert advisers		9,000	5,000	3,000	1,000	
2 Filing of patents		1,000			1,000	
3 Exhibition & competition		1,000		.	1,000	
Special equipment and accessories*	**J1000**	**40,000**	**40,000**	**0**	**0**	**0**
1 Protein chromatography acessories		25,000	25,000			
2 Vacuum evaporator		15,000	15,000			
Total direct expenses		**144,000**	**87,000**	**37,000**	**20,000**	**0**

* If major equipment, please provide description on page 2 of this appendix.

Special Equipment and Accessories (please describe and provide justification for major purchases)

1. **Description**
   Protein Chromatography Apparatus/Accessories
   (HPLC Columns, ion exchange resins, affinity resin and fraction collector).

2. **Justification**
   These accessories are important in the purification of recombinant proteins works.

3. **Estimated Cost**
   RM 25,000

Special Equipment and Accessories (please describe and provide justification for major purchases)

1. **Description**
   Vacuum evaporator

2. **Justification**
   Vacuum evaporator used to vaporize acidic sample quickly and this step is essential during the preparation of sample for analysis.

3. **Estimated Cost**
   RM 15,000

Special Equipment and Accessories (please describe and provide justification for major purchases)

1. **Justification**

2. **Estimated Cost**

3. **Description**