(To be submitted in 10 copies for each project)

Cloning and expression of pullulanase gene from locally isolated *Bacillus* sp.

Application Form For R & D Funding Under IRPA

RM8

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

In collaboration with

Bioprocess and Chemical Technology Centre,
SIRIM Berhad,
P.O. Box 7035, Section 2,
40911 Shah Alam

&

School of Chemical Sciences & Food Technology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor

CHAPTER

1

APPLICATION GUIDELINES AND FORMS

A. PURPOSE

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

B. INFORMATION REQUIRED

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

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
	09-02-06	Cloning and expression of Pullulanase gene from locally isolated *Bacillus* sp	Nik Azmi Nik Mahmood	730309-01-5599	Experimental research	Applied Science And Technologies	S50100	Biological Sciences	F10807	Advanced technology in Biological Sciences	EA 50406	3 years

Table 2. – Continued

GRANT REOUESTED YEAR 1 (2003)							LIST OF MAJOR EOUIPMENTS REOUESTED				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	3 000		40 000	5 000	5 000	20 000	Protein purification chromatography set					

Table 2. – Continued

GRANT REOUESTED YEAR 2 (2004)							LIST OF MAJOR EOUIPMENTS REOUESTED				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	3 000		40 000	5 000	5 000							

Table 2. – Continued

GRANT REOUESTED 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	3 000		20 000	5 000	5 000							

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)

Cloning and expression of pullulanase gene from locally isolated *Bacillus sp.*

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

Name: Nik Azmi Nik Mahmood

NRIC: 730309-01-5599

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

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

Department of Bioprocess Engineering
Faculty of Chemical and Natural Resources
Universiti Teknologi Malaysia
81310 Skudai, Johor.
Tel : 07-5505271
Fax : 07-5581463

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)

Molecular Cloning
Expression
Recombinant pullulanase enzyme

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 clone and sequence pullulanase gene
2) To develop expression system for pullulanase gene
3) To purify and characterize expressed pullulanase

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) [/] New [ ] Modification to previous project [ ] Extension of existing project
- Literature review summary

Bacterial pullulanase represents one of the starch-degrading enzymes that are widely used in the starch processing industry along with amylases. Amylases hydrolyze α-(1,4)-glycosidic linkage in starch to produce a mixture of glucose , maltooligosaccharide and limited a-dextrin. All the remaining α-(1,6)-glycosidic branches in the products are hydrolyzed by pullulanase. This is an advantage to improve glucose production by coupling pullulanase and amylase in the process. As such, many pullulanase enzyme has been isolated and one has been showing optimum pH of 10-10.5 which is suitable for use in dishwasher detergent additive in removal of starch stain.

We have recently isolated a few bacterias that have shown potentially pullulanase producers by the holo-zone in pullulan-plate assay. One of them, we named Bacillus –1 shows a bigger holo-zone among others, Bacillus-1 is highly active in pH more than 7. The enzyme also shows a moderate activity towards starch that may be indicates beside hydrolyzes α-(1,6)-glycosidic linkage in starch, it also hydrolyzes α-(1,4)-glycosidic similar to α-amylase. Unfortunately the enzyme from wild-type bacteria is in lower yield and in this studies, we intend to clone and sequence the pullulanase gene and also expressed the gene in a high expression system to be able to produce in a high yield before characterizing expressed protein.

Reference:

1. Hideto T. , Koki H. (2000). Analysis of the genome of an alkaliphilic *Bacillu*s strain from an industrial point of view. Extremophiles 4: 99-108.
2. Tae-Jip K., Woo-Seok P., Shin-Kweon C., Young-Jun Y., Kwan-Hwa P. & Jung-Wan K. (2000) Molecular Cloning and Characterization of Thermostable Pullulanase from a *Thermus* strain IM6501. Food Science and Biotechnology 3: 184-194.
3. Teija T. K., Harri H., Raimo P., Mervi S. & Ilkka P.(1993) Cloning and sequencing of a gene encoding acidophilic amylase from *Bacillus acidocaldarius*. Journal of General Microbiology 139: 2399-2407.

Related research:

1) Screening for amylases and pullulanases from soils in Malaysia

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

[ ] 1. Scientific research (fundamental research)

[/] 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:S50100
- SEO Group and Code
  S50106 Applied Sciences and Technologies.

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 Biology
- Research Theme Advanced technology in biological sciences.
- Research Theme Code EA50406
- Programme Genetic engineering

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
    Biological Sciences
  - FOR Group and Code
    Genetic Engineering F10807
  - FOR Area
    Gene isolation and Gene sequencing
- Secondary Field of Research
  - FOR Category
    Biological Sciences
  - FOR Group and Code
    F10808 Biotechnology

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)

Scientist at research institute and universities:

- Exchange of technical expertise, idea and information.

Industries

- Application of detergent enzymes (Novo, Nordisk).
- Linkages starch industries: Will benefit the local starch industries in using local starch such as sago for processing.

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

- Pullulanase gene.
- Bioinformation on the gene.
- Recombinant pullulanase enzyme.
- Recombinant pullulanase biochemical characteristic.

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)

Collaborative work with research institutes and companies through publication, seminars, training and workshop.

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

- Expertise Development. PhD (1) BSc (4)
  Skilled staff (2)
- Improvement of infrastructures: - new equipment
  - improved laboratory facilities
- Development of enzyme biotechnology expertise and facilities for starch degrading enzyme.

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

- Linkages between local and international research organization.
- Linkages with related industries and companies.
- Full recovery of natural resources, mostly to enhance national economic by bioresources and biotechnology.

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)

Universiti Teknologi Malaysia

- Gene isolation
- Gene sequencing
- Gene expression

SIRIM

- Pullulanase assay
- Pullulanase reaction mechanism & modelling
- HPLC purification

Universiti Kebangsaan Malaysia

- Purification of enzyme
- Enzyme assay

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

Linkages with biotechnology company in the future such as Novo Nordisk .

C. Project Team

Name[1]	Organisation	Man-months[2] on project
Project Leader (Please provide name) Nik Azmi Nik Mahmood	UTM	3 x 3 = 9
Programme Head (Please provide name)		
Researchers (Please provide names or numbers of researchers) Dr Rosli Md. Illias Dr Madihah Md Salleh PM Dr Osman Hassan En Kamarulzaman Kamaruddin Dr. Neelam Shahab	 UTM UTM UKM SIRIM SIRIM	 2 x 3 = 6 2 x 3 = 6 1 x 3 = 3 1 x 3 = 3 1 x 3 = 3
Support Staff (Please indicate how many) One laboratory Technician	UTM	1 x 3 = 3
Contract Staff (Please indicate how many) One Research Assistant	UTM	12 x 3 = 36
	Total	69

[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)

The following are the strategies to be adopted.

1) Literature Review
2) Isolation and cloning of the pullulanase gene.
   (a) Genomic DNA isolation
   The genomic DNA will be isolated from bacterial using alkaline lysis method. Pure DNA will then be subjected to PCR amplification

   (b) Genomic library method
   Pure DNA will then be subjected to partial digestion using Hind III and EcoRI. The partially digested DNA is the electrophoresis and elute from agarose gel with fragment between 5 to 6 kb. This fragment will be ligated to digested pUC 19 and transformed into *E.coli* TG1. The recombinant *E. coli* then be plated on pullulan containing agar.

   (c) PCR amplification
   Computer analysis will be carried to look for a highly conserved amino acid region sequence using BLAST. Two degenerate oligonucleotide will be designed based on the most conserved region. PCR amplification will be carried out using purified genomic DNA as a template with annually temperature between 35 - 60°C. Amplified fragment will be cloned into PGEM PCR cloning kit for sequencing.

   (d) Colony hybridization
   Genomic DNA will be digested with several restriction endonuclease such as Eco RI, hind III PSt I and Sac I. Then southern analysis on the digested DNA will be carried out using amplified PCR fragment in (c). The suitable digested genomic DNA will then be used to form a genomic library using PUC 19. Colony hybridization will then be carried out to fish out recombinant containing pullulanase gene.

3) Sequencing and gene analysis
   Isolated pullulanase gene will be sequenced using a standard method by Sanger for confirmation. The nucleotide sequence will be translated into amino acid sequences using BLAST. Amino acid comparison will be done using DNAsis programme.

4) Expression of the pullulanase gene
   Plasmid such PKK223 will be used for expression of the isolated gene. Isolated pullulanase gene will be ligated and clone into plasmid PKK223 or PRC23. The recombinant molecule will then be transformed into a suitable *E.coli* host. A volume of 5 ml of LB medium containing 100 μg/ml ampicilin will be inoculated with recombinant *E.coli* containing pullulanase gene. During lag phase, IPTG will be added to induce expression of the pullulanase.

5) Purification of pullulanase
   Ammonium sulfate precipitation and CD-affinity chromatography method will be applied to purify the expressed recombinant pullulanase.

6) Protein estimation
   Protein estimation will be carried out using the standard method by Lowry.

7) Assay of enzyme activity and characterization.
Hydrolytic activity of the enzyme is determined using DNS method using 1 % (w/v) pullulan in 50 mM sodium acetate buffer (pH 6.6). One unit of pullulan hydrolyzing activity (PU) was defined as the amount of enzyme that forms reducing sugar to give an increase of 1.0 in absorbance at 575nm. The reaction product will be analyzed by thin layer chromatography (TLC) using silica gel K6F plate (Whatmann, UK).

(8) Pullulanase Reaction Mechanism

The effect of pH temperature and metal ion ($CaCl_2$, $MgSO_4$, $FeSO_4$ etc) on pullulanase activity and stability will be carried out. Determination of the molecular weight of the enzyme will be determined by SDS page. The kinetics and performance study of the enzyme will be carried out in detail.

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. Critical Review - Collection of literature pertaining to the research.

2. Isolation and cloning of pullulanase gene.
   - Isolation of genomic DNA
   - Genomic library method
   - PCR amplification of pullulanase gene
   - Colony hybridization

3. Sequencing and gene analysis
   - Preparation of single stranded DNA
   - Sequencing of clone DNA
   - Computer analysis of nucleotide and amino acid sequence

4. Expression of the pullulanase
   - Preparation of plasmid and other expression vector
   - Cloning of pullulanase gene into expression vector

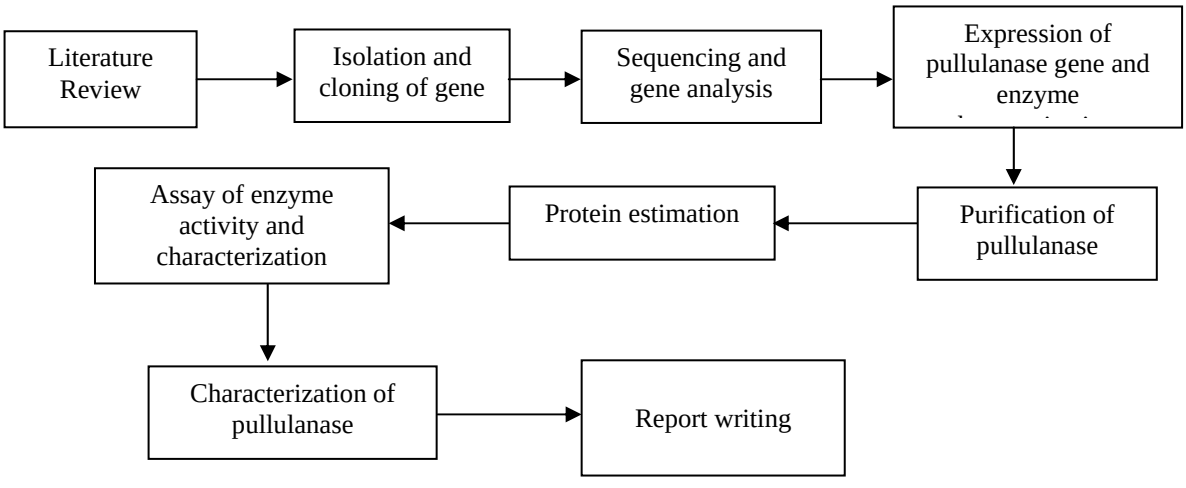
5. Purification of pullulanase
   - Ammonium sulfate precipitation
   - CD-affinity chromatography
   - Protein estimation
   - Analysis of recombinant pullulanase
   - Enzyme assay
   - Analysis of reducing sugar produced and crude pullulanase characterization.

6. Characterization of enzyme & Reaction mechanism
   - Kinetics, Km
   - Temperature, pH and ion stability

7. Discussion and review of progress of research

8. Report writing and presentation

9. Publication



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) Pullulanase gene
2) Nucleotide and amino acid sequence analysis
3) Express recombinant pullulanase
4) Purified pullulanase
5) Biochemical characterization (kinetic and reaction mechanism)

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:

	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)

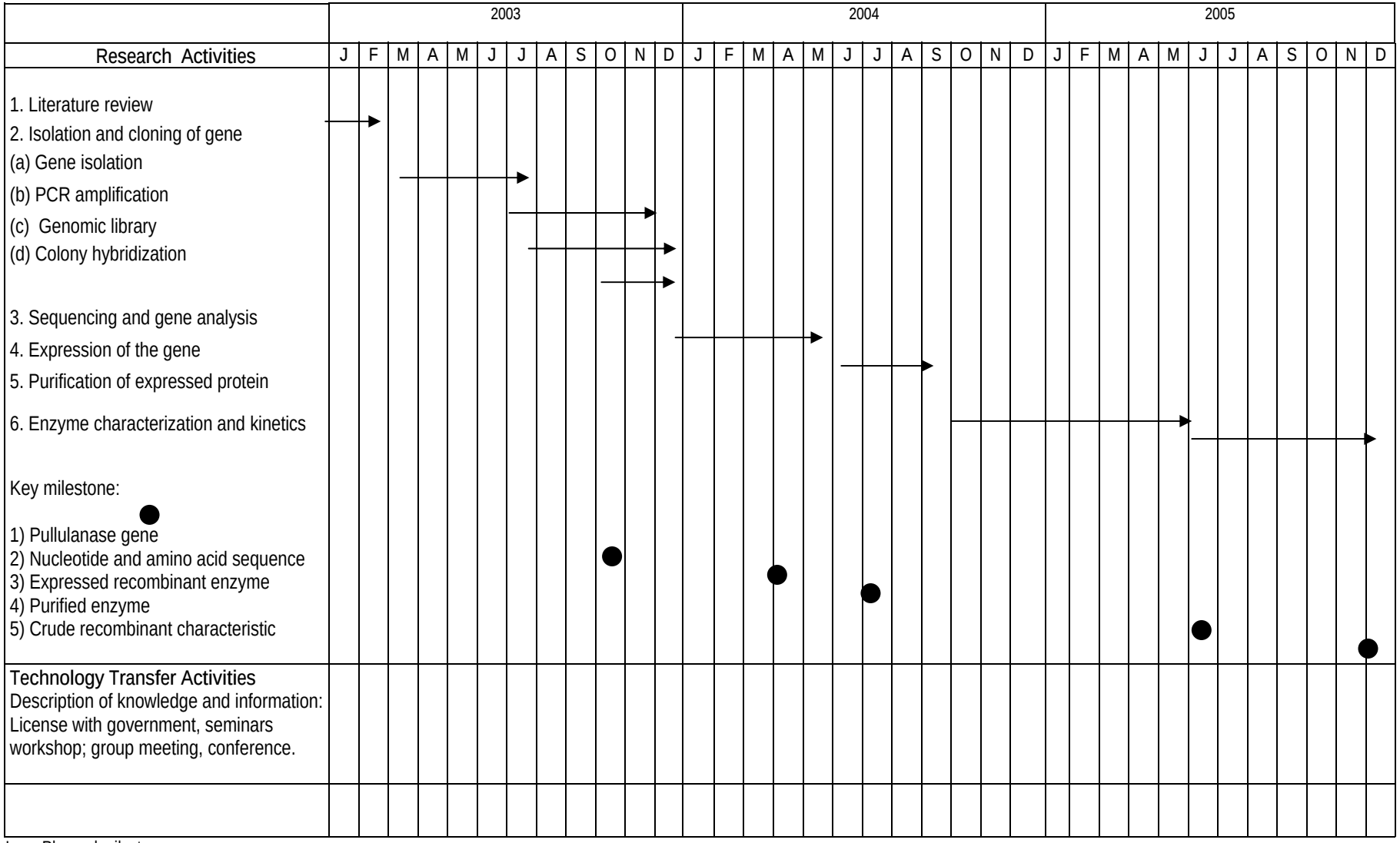
- Starting date

  Jan 2003

- Duration/elapsed time

  36 months

VI. Project Schedule



I 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 (2003)	Year 2 (2004)	Year 3 (2005)	Year 4 (…….)	Year 5 (……..)	Total
• Salaried personnel (11100)	73598	73598	73598			220794
• Temporary and contract personnel (J 400)	20000	20000	20000			60000
Sub-total staff costs	93598	93598	93598			280794

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)

Expense Category	Year 1 (2003)	Year 2 (2004)	Year 3 (2005)	Year 4(…….)	Year 5 (……..)	Total
300 00 --						
• Travel and transportation (J500)	3000	3000	2000			8000
• Rentals (J600)	--	--	--			--
• Research materials and supplies (J 700)	40000	40000	20000			100000
• Minor modifications and repairs (J 800)	5000	5000	5000			15000
• Special services (J900)	5000	5000	5000			15000
• Special equipment (J1000)	20000					20000
Sub-total staff costs	73000	53000	32000			158000

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

Year 1 (2003)	Year 2 (2004)	Year 3 (2005)	Year 4 (…….)	Year 5 (……..)	Total
165998	148398	127398			441794

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	218000	49.34
• Internal Funds	223794	50.66
• Other Sources (please specify)		
Total	441794	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 (2003)	Year 2 (2004)	Year 3 (2005)	Year 4 (2006)	Year 5 (……..)	Total
UTM	37200	31800	31800			100800
SIRIM	21800	29300	29300			80400
UKM	11000	12900	12900			36800
Total IRPA Grant	70000	74000	74000			218000

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)

A confidentiality agreement between all researchers involved in this collaboration project in order to protect any technical data generated from this project

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

Ownership of intellectual property rights will be negotiated on the basis of percentage involvement of each organizations i.e. UTM, , UKM and SIRIM on this IRPA collaboration project.

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	:	Nik Azmi Nik Mahmood
2.	IC No	:	730309-01-5599
3.	Date and Place of Birth	:	9 Mac 1973
4.	Sex	:	Male
5.	Nationality	:	Malaysia
6.	Name of Current Employer	:	UTM
7.	Address	:	Department of Bioprocess Engineering Faculty of Chemical and Natural Resources Engineering UTM Skudai Johor
8.	Telephone No	:	5505271
9.	Fax No	:	07-5581463
10.	Title of Position Held	:	Lecturer
11	Signature of Researcher	:	
12.	Date	:	5 July, 2002

B. Educational Qualifications

1. Academic Qualification

Degree	:	MSc
Field	:	Biotechnology
Year	:	2002
Name and Place of Institution	:	University of Nagasaki, Japan
(Repeat as necessary)		
Degree	:	BEng
Field	:	Chemical & Bio Engineering
Year	:	1997
Name and Place of Institution	:	University of Hiroshima, Japan

2.	Other Professional Courses Completed		
	Field	:	
	Year	:	
	(Repeat as necessary)		

C.	Research Experience		
1.	Number of Years of Experience in the Field Related to the Proposed Project	:	6 years
2.	Fields of Specialisation	:	Genetic and Protein Engineering
3.	Major Research Programmes/Projects Completed		
	Title	:	Cloning and expression of beta-hydroxybutyrate dehydrogenase from *Pseudomonas sp*.
	From	:	1999
	To	:	2002
	Position held	:	MSc Researcher
	Major output	:	1 paper in Japanese Conference
	(Repeat as necessary)		

D. Research Achievements

1.	Honours and Awards	:	i. National/Panasonic Japan Scholarship Graduate Programme (1999-2002) ii. Malaysian Government Scholarship for undergraduate oversea programme (1993-1997) Japan
2.	Major Publications	:	
3.	Number of Patents	:	
4.	Major Commercial Achievements	:	

Appendix B – Summary of Relevant Past Research

A. Project title

Cloning and expression of beta-hydroxybutyrate dehydrogenase gene from *Pseudomonas* sp

B. Relevance to proposed project

Techniques and approach of proposed research are similar especially on molecular biology and protein analysis.

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

University of Nagasaki, Japan
University of Nagaoka , Japan
University Technology Malaysia

D. Names of senior staff

- Programme head: Prof. Dr Yoshimoto Tadashi
- Project leader: PM Dr Kiyoshi Ito

Key researchers: Nik Azmi Nik Mahmood

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

The beta-hydroxybutyrate dehydrogenase enzyme is known and used commercially as an indicator of ketone bodies in diabetes patients. Commercially available types are from crude extract directly from microbes but the cost of production is low. The attempt of recombinant enzymes approach was to overcome the low yield production as it could be produced largely in industry. We have managed to clone the gene in *E.coli* as host and the purification from this recombinant enzyme as expected produced high yield of enzymes. The methodology and approach of this project have been patented in Japan and continuous studies have been done by the perspective institution to enhance the activity and mechanism of the enzyme through protein structure and function.

Appendix C – Staff Cost Estimation Worksheet

Role in Project	Total	Project Leader	Researchers	Support Staff	Contract Staff
Daily Rate (RM)		300	300	66.6	69.44
Research Activities			Man-Days[1]		
1. Literature review	74	12	24	-	38
2. Isolation of genomic DNA					
• Genomic library	180	20	50	10	100
• Colony hybridization	118	20	54	7	37
• PCR amplification	180	20	40	7	113
Total Year 1 (2001) Man-days	552	72	168	24	288
Total Year 1 (2001) Cost (RM)[2]	93594	21, 600 (11100)	50,400 (11100)	1,598 (11100)	20,000 (J 400)
1. Gene sequencing	187	20	56	7	104
2. Gene expression	196	32	61	7	96
3. Expressed gene purification	169	20	51	10	88
Total Year 2 (2002) Man-days	552	72	168	24	288
Total Year 2 (2002) Cost (RM)[2]	93594	21, 600 (11100)	50,400 (11100)	1, 598 (11100)	20,000 (J 400)
1. Purification of gene	187	20	56	7	104
2. Enzyme characterization					
• Kinetics	196	32	61	7	96
• Reaction mechanimsm	169	20	51	10	88
Total Year 3 (2003) Man-days	552	72	168	24	288
Total Year 3 (2003) Cost (RM)[2]	93594	21, 600 (11100)	50,400 (11100)	1, 598 (11100)	20,000 (J 400)
Total Year 4 (200_) Man-days					
Total Year 4 (200_) Cost (RM)[2]		(11100)	(11100)	(11100)	(J 400)
Total Year 5 (200_) Man-days					
Total Year 5 (200_) Cost (RM)[2]		(11100)	(11100)	(11100)	(J 400)
Total Project Man-days	1656	216	504	72	864
Total Project Staff Cost (RM)	280794	64,800 (11100)	151,200 (11100)	4,794 (11100)	60,000 (J 400)
Total Man-months[3]	69	9	21	3	36

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

A. Expense Categories and Items	Year 1 (2003)	Year 2 (2004)	Year 3 (2005)	Year 4 (2006)	Year 5 (…….)
Travel and transportation	10 000	10 000	10 000		
Conference, seminar, workshop and meeting	10 000	10 000	10 000		
Rentals (J600)					
Research materials and supplies (J700)	30 000	20 000	15 000		
•Enzyme, cloning kit, phenol for gene isolation. Agarose and general chemical for bacteria growth. • Consumable disposable tip.	25 000 5 000	15 000 5 000	10 000 5 000		
Minor modifications and repairs (J800)	10 000	10 000	5 000		
Maintenance and repair of existing equipment	10 000	10 000	5 000		
Special services (J900)	11 250	7 500	6 250		
cDNA & Primer	11 250	7 500	6 250		
Special equipment and accessories (J1000)	20 000				
Protein purification chromatography set	20 000				
Total direct expenses	81 250	47 500	36 250		

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

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

1. Description : Protein purification chromatography set.

2. Justification : The set consists of columns for affinity andgel filtration chromatography with polymeric matrix and also SDS Polyacylamide gel electrophoresis set for identification and quantification of protein/enzyme that has been produced.

3. Estimated Cost : RM 20 000

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

1. Description :

2. Justification :

3. Estimated Cost :

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

1. Justification :

2. Estimated Cost :

3. Description :