SABBATICAL LEAVE REPORT FOR RESEARCH ATTACHMENT (VISITING SCIENTIST) AT

DEPT OF CHEMICAL AND BIOMOLECULAR ENGINEERING

HENRY SAMUELLI SCHOOL OF ENGINEERING AND APPLIED SCIENCE

UNIVERSITY OF CALIFORNIA LOS ANGELES (UCLA)

17TH SEPTEMBER 2012 – 16TH MARCH 2013

TOWARDS HETEROLOGOUS PRODUCTION OF MONOTERPENE INDOLE ALKALOIDS IN MICROBIAL SYSTEM

PRINCIPAL INVESTIGATOR

PROFESSOR YI TANG

DR ROSLINAH MOHAMAD HUSSAIN

JABATAN TEKNOLOGI MAKMAL PERUBATAN

FAKULTI SAINS KESIHATAN

KAMPUS PUNCAK ALAM

7TH MARCH 2013

Table of Contents

	Page
Acknowledgements	1
Background	2
Overall Experimental Work	3
Gantt Chart of Research Related Activities	12
Research Outcomes	13
SpinOffs/Gains from Sabbatical Attachment	13
Post Sabbatical Related Activities	14
Attachments (Copies of)	
Exit Reference from Principal Investigator	
Final Report Form (JbPSM)	
Three month Progress Form (JbPSM)	
Dean (FSK) Review	
Acceptance Letter	
Copy of State of California Oath and Patent Allegiance	

ACKNOWLEDGEMENTS

Alhamdullilah, thanks be to Allah for granting me the strength and will to complete my research during my sabbatical research attachment at the Chemical and Molecular Engineering Dept, University of California Los Angeles (UCLA).

My gracious appreciation to the Principal Investigator, Professor Yi Tang and all the postdocs and postgraduates in the dept. for their assistance and guidance in helping me complete the assigned project with a tremendous learning curve.

My sincere thanks to the Dean of Faculty of Health Sciences, Dr Hamzah Fansuri Hassan and all Deputy Deans and staff for their continued support in allowing this leave for research advancement and knowledge gain. I thank En Zed Zakari Abdul Hamid, En Nur Hisham and all staff in the Medical Technology Department for taking up the slack in teaching load and final student supervisions during my absence. May Allah grace you and return your kind gestures. I thank Dr Maria for her diligence in monitoring my postgraduate students during the period.

I thank UiTM for the funding and to JbPSM and Bendahari for their efforts in making the paperwork and monetory transactions a smooth one. I wish to express my gratitude to Prof Abu Bakar (RMI) for the trust and confidence that enabled me the attachment. I hope to share the knowledge learned with the Faculty and UiTM community the best I can, especially through research and related activities.

To my children, my heartfelt thank yous for your sacrifice and for having to bear with life trials in my absence. I pray that you, as I did, learned to appreciate family and how worthy we are of each other. I am glad to be reunited with you again, alhamdullilah.

FINAL REPORT (CONFIDENTIAL/RIGHTS OWNED BY PROF YI TANG; UCLA): VISITING SCIENTIST ATTACHMENT AT UNIVERSITY OF CALIFORNIA LOS ANGELES (UCLA), U.S.A. 17TH SEPTEMBER 2012 – 16TH MARCH 2013

Name: Roslinah Mohamad Hussain PhD, MT(ASCP)

Project Title: Toward Heterologous production of monoterpene indole alkaloids in a microbial system.

PI: Professor Yi Tang
Site of Research: Chemical and Biomolecular Engineering Dept,
Henry Samueli School of Engineering and Applied Science,
Boelter Hall,
University of California Los Angeles (UCLA)
U.S.A

Background

Plant secondary metabolites such as alkaloids, terpenoids and polyketides and others have immense medicinal, pharmaceutical and agricultural applications. Many are sought for their therapeutic potentials against various cancers and tumors. In plants, secondary metabolites are products of complex biosynthetic pathways that take years to produce and even then, at miniscule levels. Recognising these constraints, bioengineering technologies attempt to yield productions of these valuable compounds in robust systems such as in bacteria or yeast.

This project attempts to reconstruct the biosynthetic pathway VIS bioengineering and molecular methods towards production of a monoterpene alkaloid that is normally produced in plants in a microbial system such as *Saccharomyces cerevisiae*. The undertaken project represents **the initial pathway**, in a myriad of biosynthetic pathways leading towards production of Vinblastine, an expensive commercialized antitumor drug that is currently used.

The following outlines a summary of the procedural experimental work that I performed in attempt to obtain the desired outcome.

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Noteworthy, this project and all its contents is **CONFIDENTIAL** with all rights wholly belonging to Professor Yi Tang at the University of California Los Angeles (UCLA).

Overall experimental work

Part I. Gene mutagenesis

A. Splicing Overlap Extension (SOE) for gene (erg20) mutagenesis in *S.cerevisiae*.

1. Isolation of genomic DNA from *S.cerevisiae* BJ5464 (>80% homology with desired strain).

2. Four SOE primers designed to introduce mutation in erg20 gene by replacing lysine with glutamate at position 254.
   Primers:
   i. erg20 F
   ii. erg20*SOE R
   iii. erg20*SOE F
   iv. erg20 R

3. PCR to generate erg*20 SOE fragments containing desired mutagenesis.

4. DNA gel electrophoresis and gel extraction of fragments, each containing full copy of gene.
   i. Forward fragment – 717bp (mutated gene)
   ii. Reverse fragment – 313bp (normal gene)

5. Sequencing (Laragen). Ligation of erg*20 fragment into PCR Blunt Vectors followed by transformation into Top10competent cells.
   i. Mini Prep to recover plasmids.
   ii. Restriction digest with EcoRI to verify inserts.
   iii. Plasmids cleaned for sequencing – sequence confirmed correct.

B. Insertion of URA3 Recyclable Marker between Mutagenized and Wildtype gene fragments.

6. SOE technique to insert marker
   i. Generate by PCR partial sequence of front erg*20 fragment (mutagenized) with front ½ of URA3 at 3' by PCR – 750bp

ii. Generate by PCR partial sequence of back erg20 fragment with back ½ of URA3 by PCR- 704bp

iii. Generate overlap full sequence erg*20 front fragment with short 3' tail containing sequence of front URA3 by PCR – 1059bp

iv. Generate by PCR overlap full sequence erg20 back fragment with short 5' tail containing sequence of back URA3- 1059bp

7. Combine, by overlap homologous extension, erg*20 front with front half of URA3
   Combine fragments from 6i. and 6iii. PCR first without primers to allow SOE recombination, and continue PCR with primersto generate expected fragment 1809bp (750bp + 1059bp)

8. Combine, by overlap homologous extension, erg*20 front with front half of URA3
   Combine fragments from 6ii. And 6iv. PCR first without primers to allow SOE recombination and continue PCR with primers to generate fragment 1763bp (704bp + 1059bp)

9. Sequencing (Laragen). Both fragments (1809bp and 1763bp) each liagted into separate PCR Blunt Vectors followed by transformation into Top10 competent cells.

   i. Mini Prep to recover plasmids.
   ii. Restriction digest with EcoRI to verify inserts.
   iii. Plasmids cleaned for sequencing – sequences confirmed correct.

10. Preparation of SOE inserts for WAT11 transformations
    i. Preparation of WAT11 competent cells.
    ii. Glycerol stock of WAT11 cells.
    iii. Generate front erg*20 URA3 and back erg20 URA3 fragments by PCR from PCRBlunt vector.
    iv. DNA gel electrophoresis for confirmation and gel extraction.

C. Generating WAT11erg*20 URA strain by SOE homologous recombinations.

1. Transformation of both fragments (1809bp and 1763bp) into *S.cerevisiae*WAT11 competent cells and confirmation of transgenic strain
    i. Chemical transformation and plated on selective media.
    ii. Selecting transformants WAT11erg20*URA3 for Mini Prep.
    iii. Isolation of genomic DNA followed by PCR screening (Green Taq
    iv. Confirmation PCR (Phusion) of screen positive transformants from Green Taq PCR.

12. Sequencing (Laragen). Ligation of front erg*20 URA3 and back URA3 erg 20 fragments each into separate PCR Blunt Vectors followed by transformation into Top10competent cells.
    i. Select transformants for Mini Prep to recover plasmids.
    ii. Plasmids cleaned for sequencing – sequences of both fragments confirmed correct.

13. Preparation of competent WAT11erg*20 URA3 cells by chemical method using S.C. Easy CompTM-Invitogen.

14. Prepared Glycerol stock of 2 confirmed transformants WAT11erg*20-5 and WAT11erg*20-12.

Part II: Cloning of 2 plant genes into *S.cerevisiae* WAT11erg*20 URA for reconstitution of biosynthesis in the heterologous yeast host.

A. Cloning of geraniol synthase version 1 (GS1) with chloroplast signal sequence) and version 2 (GS2) and G10 hydroxylase versions 1 (G100hase1) with chloroplast signal sequence and version 2 (G10Ohase2) fragments into pESC vector under control of GALI inducible promoter.

15. Generate fragments from template by PCR (Phusion) using designed primers to incorporate;

    BamHI (5') and HindIII (3') restriction sites in GS1 and GS2 repectively and
    Not1 (5') and XbaI (3') restriction sites in G10Ohase1 and G10Ohase2 respectively.

16. DNA gel electrophoresis for verification of band sizes and gel extraction, column cleaned and stored at -20OC.

17. Sequential double digestions of GS1 and GS2 with BamHI and HindIII and column cleaned

18. Ligation of linearized BamHI /HindII digested GS1 and GS2 each into separate linearized pESC vector under control of GAL1 promoter and column cleaned.

19. DNA gel electrophoresis for band size verification and gel extraction.

20. Transformation of GSI (pESC) and GS2 (pESC) each into XLI competent cells using heat shock method and plated on selective media.

21. Transformants selection for Mini Prep to recover plasmids.

22. Green Taq PCR screening to identify transformants with desired constructs.

23. Sequencing (Laragen). GS1 (pESC) and GS2 (pESC) ligated into separate PCR Blunt Vectors, transformed into Top10 competent cells, Phusion PCR to confirm insert size, column cleaned and sequenced. Both GSI (pESC) and GS2 (pESC) verified by sequence analysis.

B. Cloning of double digested G10Ohasel and G10Ohase2 fragments each into linearized GS1 (pESC) and GS2 (pESC) under control of GAL10 promoters

24. Sequential double digestions of GSI (pESC) and GS2 (pESC) with NotI and SpeI

25. Sequential double digestions of G10Ohase1 and G10Ohase2 with NotI and XbaI (XbaI used instead of SpeI because the SpeI restriction site present within fragment. XbaI and SpeI are site compatible.

26. Ligation of linearized fragments of G10Ohas1 and G10Ohase2 each with linearized GS1 (pESC) and GS2 (pESC) to yield four plasmid constructs;

GSI/G10Ohase1
GS2/G10Ohase1
GSI/G10Ohase2
GS2/G10Ohase2

27. Transformation of palsmids constructs each into separate XL1 and plated on selective media.

28. Selection of transformants for Mini Prep and plasmids recovery.
29. PCR with Green Taq screening for verification of G10Ohase 1 and G10Ohase 2 inserts in relevant constructs.

Observed bands;
1429 bp consistent with presence of G10Ohase 1 fragments in each of GSI/G10Ohase 1 and GS2/G10Ohase 1 constructs.
1425 bp consistent with presence of G10Ohase 2 fragments in each of GSI/G10Ohase 2 and GS2/G10Ohase 2 constructs.

30. Sequential double digestions of each construct with BamHI/HindIII to verify presence of GSI and GS2 in each relevant construct.

31. Clean Mini Prep (uncut plasmid) and preparation of plasmids for sequencing (Laragen) as described previously.
   i. GSI/G10Ohase2 and GS2/G10Ohase2 both sequenced correctly the first time and glycerol stocked.
   ii..Cloning and transformation procedures were repeated to generate new GSI/G10Ohase1 and GS2/G10Ohase 1 constructs verified by PCR analysis and prepared for sequencing.

iii. Only GS1/G10Ohase1 construct sequenced correctly but GS2/G10Ohase 1 failed to sequence correctly again.

32. Sequence verified plasmid constructs GSI/G10Ohasel, GS1 /G10Ohase and GS2/G10Ohase2 used in subsequent transformations.

Part III. Transformation of verified plasmid constructs intoWAT11erg*30URA3 for biosynthesis of geraniol and 10 hydroxygeraniol.

33. Transformation of plasmids each into WAT11 erg*20 URA3 – 5 as per procedure and plated onto selective media without uracil and leucine.

34. Transformants selected and proceeded with insert verification using colony PCR (Green Taq Screening).

35. Confirmation of GS and G10Ohase relevant inserts in transformants by DNA gel electrophoresis.

Part IV. Cultivation and induction of transgenic WAT11 erg*20 URA3-5 transformants for detection of geraniol and 10 hydroxygeraniol.

36. One colony of each transformant with confirmed GSI/ G10Ohase2, GS2/G10Ohase 2 and GSI/G10Ohase I were grown for 3 days in dextrose minimal media.

37. Cells were washed and transfered into galactose minimal media for 24 hour induction and sampled at 24, 48 and 72 hours for extraction.

Part V. Extraction procedure of samples from induced WAT11 erg*20 URA3 strains carrying plasmid constructs GSI/ G10Ohase2, GS2/G10Ohase 2 pESC-Leu

A. First extraction procedure

38.

i. Cells lysed in acetone overnight, debris pelleted and supernatant collected.

ii. Extraction with chloroform followed by three partition washes with distilled water

iii. Chlroform layer transferred into scintillation vial and vacuum dried.

iv. Resuspension of dried extract with acetonitrile and parttioned with hexane.

v. Acetonitrile and hexane layer collected into separate sciltillation vials and each vacuum dried.
vi. Dried extracts each resuspended in 500uL hexane , stored in airtight vials at 4C for two weeks.

38. GCMS analysis of samples showed no geraniol peaks in samples from GS1/G10Ohase2and GS2/G10Ohase 2 referenced against authentic geraniol standard (1:50 dilution in hexane)

B. Investigation of extraction procedure using authentic geraniol standard.

39. Extraction was procedure was repeated as follows;

i. Extraction was performed with 1 uL geraniol standard starting with chloroform and without acetone. Geraniol peaks found in both acetonitrile and hexane fractions but more abundant in acetonitrile fraction.

ii. Extraction was performed with 10ul geraniol standard starting with acetone and water followed by chloroform. Geraniol peaks were not found in acetonitrile nor hexane fractions.

C. Investigation of expression of GS versions 1 and 2 in pESC-LEU in WAT11erg*20URA3-5

40. GS1 (in pESC) and GS2 (in pESC) each transformed into XLI and plated on selective media.
41. Colonies picked and grown for Mini prep for plasmid recovery.
42. Green Taq PCR screening performed and bands verified
GS1-5 – 1701bp
GS2-15 – 1572bp

43. GS1-5 (pESC) and GS2-15 (pESC) column cleaned and each transformed into separate WAT11erg*20 URA3 -5 competent cells, plated on SDMM and incubated 30OC.Transformants GS1-5 and GS2-15 selected for cultivation and induction procedure.

D. Modified extraction procedure of WAT11erg*20URA3 strains each containing GS1/G10Ohase1 (pESC), GS1 (pESC) and GS2 (pESC)

i. One colony of each transgenic strain inoculated into SDMM (50ml GS1/G10Ohase1, 100ml GS1 and 100 ml GS2) and incubated 30OC with shaking (3 days GSI/G10Ohase1, 4 days GS1 and GS2).

ii. Cells washed with SGMM and each transferred into 50 ml SGMM and incubated shaking at 30oC. Samples were taken at 24, 48 and 72hours post induction.

iii. Cells pelleted and resuspended in dH20 and chloroform added. Mixture vortexed and allowed to sit on ice 15-30min.

iv. Cells lysed in sonicator iced water bath 15-20x until chloroform layer appears turbid.

v. Distilled water added, mixed and replaced until water layer(top) is clear.

vi. Chloroform layer (bottom) removed and filtered into clean scintillation vial and vacuum dried.

vii. Dried extract resuspended in acetonitrile and transferred to clean Scott bottle on ice.

viii. Hexane (HPLC grade) added and mixture allowed to partition on ice for 30 minutes.

ix. Acetonitrile layer (bottom) and hexane layer (top) separated into clean screw capped scintillation vials and vacuum dried.

Dried fractions each resuspended in 500ul hexane and stored airtight at 4oC until GCMS analysis performed (one week).

Part VI. URA3 popout procedure from WAT11 erg*20 URA3-5 and WAT11 erg*20 URA3-12 strains.

44. Both strains each plated on separate FOA (fluoroorotic acid) media and incubated 30oC.
45. Colonies observed after 7 days, picked and restreaked onto fresh FOA media and incubated 30oC
46. Colonies selected and grown in SDCT(A,T) with no uracil or leucine added at 30oC.

47. Isolation of genomic DNA. from cultures as per procedure.

48. PCR (Phusion) of gDNA followed by DNA gel electrophoresis and gel extraction of fragments with verified bands – 1059bp
49. Ligation. Ligation of fragments with PCR Blunt vector, transformed into Top10 cells and plated on selective media and incubated 37oC.
50. Mini Prep. Colonies were grown in selective broth and Mini Prep (Blue Lysis Buffer) performed for plasmid recovery.
51. Sequencing (Laragen). Plasmids column cleaned and stored at -20oC until sent out for sequencing.
52. Colonies each picked into fresh SDCT (A,T) without uracil or leucine and grown 30oC and glycerol stocked.
53. WAT11 erg*20 5-1 (URA3 popout) and WAT11 erg*20 12-2 (URA3popout) glycerol stocked -80oC.

Gantt Chart Of Sabbatical Attachment (Visiting Scientist) /Research Activities

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	17th – 28th Sept 2012	1st – 31st October 2012	15th October 2012	1st -30th Nov 2012	1st – 31 Dec 2012	1st -31st Jan 2013	1st-28th Feb 2013	1st-15th March 201
Mandatory Lab Safety Courses – THREE separate courses conducted by EHSS (UCLA)	Passed. Enrolled in laboratory							
Official visit to UCLA by Dato VC, HEA and MSD delegates			Meetings with various Heads of relevant Depts. and Provost (UCLA) – Facilitator for UiTM/Msian delegates					
1.Splicing Overlap Extension (SOE) for gene (erg20) mutagenesis in *S.cerevisiae*. 2. Insertion of URA3 Recyclable Marker between Mutagenized and Wildtype gene fragments.		Completed						
1.Generate WAT11erg*20 URA strain by SOE homologous recombinations 2. Cloning of geraniol synthase version 1 (GS1; with chloroplast signal sequence) and version 2 (GS2) and G10 hydroxylase versions 1 (G100hase; with chloroplast signal sequence) and version 2 (G10Ohase2) fragments into pESC vector under control of GALI and GAL10 inducible promoters.				Completed				
1.Transformation of verified plasmid constructs into WAT11erg*20URA3 for biosynthesis of geraniol and 10 hydroxygeraniol.					Completed			
1.Cultivation and induction of transgenic WAT11 erg*20URA3-5 transformants for detection of geraniol and 10 hydroxygeraniol 2. Extraction of samples from induced WAT11erg*20URA3 strains carrying plasmid constructs GS1/G10Ohase2, and GS2/G10Ohase2 in pESC-LEU 3. GC-MS Analysis of extracts 4. Investigation of extraction procedure using authentic geraniol standard.						Completed		
1.Investigation of expression of GS versions 1 and 2 in pESC-LEU transformed in WAT11erg*20URA3-5 2.Modified extraction procedure of WAT11erg*20URA3 strains each containing GS1/G10Ohase (pESC), GS1(pESC) and GS2 (pESC) 4. URA3 popout procedure from WAT11erg*20URA3-5 and WAT11erg*20URA3-12 .							Completed	
1.GCMS analysis of extracts 2. Data Analysis and Lab Notebook Updates 3. Bench Clean Up, Glycerol Stock Preparations 4.END of sabbatical attachment								Complete

Research accomplishments

1. Development of a mutagenized strain of *Saccharomyces cerevisiae* by introducing mutation in a gene in the biosynthetic pathway leading to production of fanesyl pyrophosphate.

2. Construction of **FOUR** plasmid constructs in the shuttle vector pESC-LEU, each harboring two plant gene sequences for the biosynthesis of geraniol and 10 hydoroxygeraniol. **THREE** of the constructs were correctly sequenced and transformed into mutagenized yeast.

3. Proven production of geraniol from one of transformed constructs, albeit at low yields, as determine by GCMS analysis. Work continues at increasing yields of product.

4. Development of novel biosynthetic mechanism for a plant product in a microbial system that works, thereby setting platform for intense work in pursuit of the desired compound.

5. Recognition/acknowledgement of self and UiTM in publications by Pro Yi Tang in future publication relating to work accomplished.

Spin Offs/Gains from sabbatical attachment.

1. Recognition of UiTM staff as having credible potential for research at international level with good outcome.

2. Potentially secured avenue in UCLA for future research attachment of UiTM postgraduates and staffs in related disciplines.

3. Gaining trust and willingness of a distinguished Professor from UCLA for collaborative research work in metabolic engineering through collaborative research grant.

4. Knowledge transfer of metabolic engineering that is novel in Malaysia towards production of commercially viable plant product of medicinal and pharmaceutical value in microbial systems.

5. Gaining international expertise and advise for postgraduate student research, examination and joint supervision from an internationally renowned and distinguished researcher and acadamia. Prof Yi Tang has graciously accepted invitation from the Dean as a Visiting Professor for the Faculty of Health Sciences. **Paperwork for appointment in progress.**

Postsabbatical related activities 2013

	16thMarch Saturday/LA) Last day of sabbatical leave	18thMarch Monday/Msia	19th March Tuesday	25th-27th March	28th March Friday	1st – ongoing	18th April 2013
Departure flight from Los Angeles (LAX)							
Arrival at KLIA							
Officially report for duty at JbPSM, Pejabat Pendafter							
1.Meeting with Dean Faculty of Health Science for sabbatical related follow up . 2.Invitation for appointment of Prof Yi Tang as Visiting Professor by Dean via email. 3.Sabbatical report writing (Overview)							
1.Submission of reports to JbPSM; i. Final report form with Dean's assessment ii. Copy of Three month progress report form (previously sent via email in December 2012 to JbPSM) iii.Sabbatical exit reference email from Prof Yi Tang to Dean Faculty of Health Sciences 2. Contact Prof Tang for official CV							
1.Receipt of CV from Prof Tang 2.Paperwork for appointment of Visiting Professor (in progress) 3.Discussion with Deputy Dean Industrial Linkage & Attachment for intended proposal of collaborative research in metabolic engineering. 4. Detailed Visiting Scientist Sabbatical Attachment report writing for submission (hardbound) to RMI and Faculty. 5. Collaborative research grant proposal and applications							
Submission deadline for submission of hardcopy to RMI							

UCLA Engineering
HENRY SAMUELI SCHOOL OF ENGINEERING AND APPLIED SCIENCE
Chemical and Biomolecular Engineering Department

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March 14th, 2013

Dr Hamzah Fansuri
Dean, Faculty of Health Sciences
Universiti Teknologi MARA,
Kampus Puncak Alam,
Puncak Alam 42300
Selangor, MALAYSIA

Dear Dean Fansuri:

I am writing to certify that Dr. Roslinah Hussain worked as a visiting scientist in my lab at University of California, Los Angeles, for six month during 17th September 2012 - 16th March 2013. Dr. Hussain worked on the metabolic engineering of *Saccharomyces cerevisiae* towards production of plant monoterpenes. Dr. Hussain constructed a yeast mutant strain that is partially blocked in diterpene biosynthesis and introduced new genes that can produce the important precursor geraniol. Dr. Hussain learned new techniques including working with yeast strain, molecular biology, product extraction and chemical analysis. She accomplished the goal of the 6-month project and observed geraniol production in yeast. Based on her results, my lab is currently optimizing the strain to increase the product titer.

Dr. Hussain worked hard in the lab and kept an immaculate record of her work. She was very careful in her work and very professional in her relationship with the coworkers. She participated in all laboratory functions, completed her assigned laboratory tasks and followed safety requirements diligently. It was a pleasure having her in the group.

After her return to your institution, I will be happy to collaborate with her to continue a project in this area through an international collaboration. I have told her that I am open to a formal collaboration with her and your institution. I look forward to a mutually fruitful joint venture.

Yours sincerely,

Yi Tang

2/28/2013
Pn Hasliza / Mahadu at 3pm.

UNIVERSITI TEKNOLOGI MARA

JPbSM/CS.2/2001

JABATAN PEMBANGUNAN SUMBER MANUSIA

LAPORAN CUTI SABATIKAL

BAHAGIAN I

Untuk diisi oleh staf :

1. Nama Staf : DR ROSLINAH MOHAMAD HUSSAIN
2. No. Pekerja : 109561 3. Jawatan/Gred : DM52 / PENSYARAH KANAN
4. Fakulti : SAINS KESIHATAN (PUNCAK ALAM)
5. Tujuan Cuti Sabatikal : [✓] Membuat Penyelidikan [ ] Menulis Buku Ilmiah
6. Bidang penyelidikan/ ~~penulisan~~: METABOLIC ENGINEERING/RECOMBINANT TECHNOLOGY IN MICROBIAL SYSTEMS
7. Tempoh cuti sabatikal diluluskan : Mulai 16hb SEPT 2012 hingga 16hb MAC 2013
8. Tempat cuti sabatikal : CHEMICAL AND BIOMOLECULAR ENGINEERING DEPT, UNIVERSITY OF CALIFORNIA LOS ANGELES (UCLA)
9. Bantuan luar yang diterima (jika ada) : TIADA

   Tempoh Bantuan : Dari — Hingga —
10. Rancangan cuti sabatikal yang diikuti :

Lokasi/Tempoh	Rancangan/ Program Kerja
UCLA / EHSS (ENVIRONMENTAL HEALTH SAFETY SERVICE 18hb SEPT – 30hb SEPT 2012	MANDATORY SAFETY AND HEALTH COURSES (THREE) AND SAFETY EXAMINATION FOR LAB REGISTRATION
UCLA / DCISS (INTERNATIONAL STUDENTS & SCHOLARS SERVICES 18hb SEPT – 30hb SEPT 2012	ORIENTATION FOR VISITING SCHOLAR/ J1 VISA BRIEFINGS
UCLA/BOELTER HALL (LABORATORY) 1hb OKTOBER 2012 – 28hb FEB 2013 CHEMICAL & BIOMOLECULAR ENGINEERING	EXPERIMENTAL RESEARCH AS OUTLINED IN ATTACHMENT
UCLA / BOELTER) MOLECULAR SCIENCES 1hb MAC – 15hb MAC 2013	DATA ANALYSIS (GCMS) / LAB CLEAN UP AND REPORT WRITING

11. Nama penyelia (jika berkenaan) : PROFESSOR YI TANG (yitang@ucla.edu)

    Jawatan dan alamat : PROFESSOR / PRINCIPAL INVESTIGATOR
    5531 BOELTER HALL
    BOX 95192
    LOS ANGELES, CA90095-1592
    Phone : 310-825-0375

12. Butir-butir rancangan cuti sabatikal yang diikuti :

i) Objektif	1. Introducing a gene mutation in the iridoid biosynthetic pathway creating a weakened yeast strain 2. Cloning and construction of plasmid constructs containing Two plant genes of interest for production of geraniol and 10hydroxygeraniol 3. Transformation of sequenced constructs into weakened yeast strain 4. Small scale fermentation and extraction of desired product as determined by GCMS analysis
ii) Hasil yang dicapai (Nota : Sila sertakan satu salinan laporan hasil penyelidikan atau senaskah buku yang dihasilkan)	1. Development of a mutagenized strain of Saccharomyces cerevisiae by introducing a gene mutation in the biosynthetic pathway for iridoid biosynthesis 2. Construction of FOUR plasmid constructs in the inducible vector pESC-LEU, each harboring two plant gene sequences for biosynthesis of geraniol and hydroxygeraniol THREE of the constructs were correctly sequenced and transformed into mutagenized yeast strain 3. ~~Proven production of geraniol from one transformant~~ albeit at low yields, as determined by GCMS analysis. 4. Development of a novel biosynthetic mechanism for a plant product in a microbial system that is proven to work.
iii) Masalah yang dihadapi Tempat penginapan yang mahal dikawasan hampir dgn kampus UCLA. Jangka masa 6 bulan agak pendek untuk peningkatan hasil projek. Dicadangkan attachment selama sekurang-nya 1 tahun untuk lebih berkesan.	

13. Nyatakan rancangan atau kegiatan susulan selepas cuti sabatikal ini (Contoh : menerbitkan hasil kajian/ buku, dll.)

1. Kerjasama penyelidekan dengan PI (Prof Yi Tang) untuk geran penyelidekan didalam bidang metabolic engineering
2. Hubungan atau international linkage antara UiTM/FSiE dan UCLA untuk tujuan kerjasama didalam pengelidekan, penerbitan dan peluang attachment untuk staf dan pelajar
3. Memperolehi kontek dgn UCLA untuk tujuan mendapatkan khidmat professor pelawat, external examiner dan penasihat penyelidekan dom akademik untuk tujuan meletakkan fakulti oleh UiTM diperingkat antarabangsa.
4. Menjalinkan MoU antara UiTM dan UCLA (melalui Prof Yi Tang) untuk kerjasama akademik dan lain-lain aspek seperti penyelidekan

Tarikh : 18hb Mac 2013

BAHAGIAN II

Ulasan Dekan terhadap laopran Bahagian I di atas :

1. Keterangan mengenai pencapaian staf dalam melaksanakan rancangan cuti sabatikalnya:

Pegawai telah berjaya mencapai semua "outcomes" yg. ditetapkan:
1. Penghasilan strain mutan S. cerevisiae
2. Memperkenal gen baru kedalam S. cerevisiae.
3. Menghasilkan produk gerenial dari muten yang telah dibina.

2. Hubungan/Faedah rancangan cuti sabatikalnya kepada Jabatan/ Fakulti:

1. Telah mendapat kemahiran/kepakaran terkini dlm genetik engineering peningkat antarabangsa.
2. Telah mengujudkan kolaborasi penyelidikan peningkat antarabangsa dgn. organisasi tersohor.
3. Telah mendapat kepercayaan UCLA terhadap kepakaran staf Fakulti FSK.

3. Ulasan keseluruhan oleh Dekan:

① Satu pencapaian yg. sangat baik
② Akan di rumuskan dengan kolaborasi berterusan kerana telah mendapat persetujuan pihak UCLA. Teknikal.

Tarikh : 25/3/2013

Dekan
Fakulti Sains Kesihatan
UiTM Kampus Puncak Alam
42300 Bandar Puncak Alam

PERINGATAN : Sila gunakan kertas berasingan jika ruang tidak mencukupi

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Untuk Kegunaan JPbSM

Diterima pada : ..... ***Tandatangan :*** .....

CUTI SABATIKAL
BORANG LAPORAN BERKALA (SETIAP TIGA BULAN)

Untuk diisi oleh Pegawai

BUTIR-BUTIR PERIBADI:

Nama : ROSLINAH BINTI MOHAMAD HUSSAIN

No.Pekerja: 109561 Fakulti : SAINS KESIHATAN / KAMPUS PUNCAK ALAM

Program Cuti Sabatikal : [✓] Membuat penyelidikan
(SABBATICAL LEAVE PROGRAM)

Tempoh Cuti Sabatikal : Mulai 17th SEPTEMBER 2012 Hingga 16th MARCH 2013.
(DURATION OF SABBATICAL)

Tempat : UNIVERSITY OF CALIFORNIA, LOS ANGELES (UCLA)
VENUE UNITED STATES OF AMERICA

LAPORAN KEMAJUAN PROGRAM : *(tandakan yang berkaitan sahaja)*
(PROGRAM PROGRESS REPORT)

Ini adalah laporan
- [✓] Tiga bulan pertama (FIRST THREE MONTHS REPORT)
- [ ] Tiga bulan kedua (terakhir bagi CS satu semester)
- [ ] Tiga bulan ketiga (terakhir bagi CS dua semester)

Progres penyelidikan:
(RESEARCH PROGRESS)

Progres terkini : *(tandakan yang berkaitan)*
(CURRENT PROGRESS)
- [ ] Kajian literatur LITERATURE SEARCH
- [✓] Kajian ~~tinjauan/survey~~/eksperimen/~~simulasi~~ EXPERIMENTAL WORK / RESEARCH
- [✓] Analisa data/maklumat DATA ANALYSIS
- [ ] Menulis laporan WRITE UP

Maklumat terperinci mengenai progres/ Hasil penemuan dari penyelidikan : .....
(DETAILED PROGRESS / RESEARCH FINDINGS).

ERG 20 mutagenesis in Saccharomyces cerevisiae strain using allele replacement technique.
Construction of four inducible plasmids each carrying two plant gene fragments in the biosynthetic pathway for production of the monoterpene alkaloid of interest.
Sequence confirmation and successful transformation of two verified plasmid constructs into mutated S. cerevisiae strain.
Two plasmid constructs awaiting sequence confirmation to proceed with transformation into mutated S. cerevisiae
WORK ONGOING : Cultivation and induction of transformed S. cerevisiae for production of monoterpene alkaloid of interest

Masalah yang dihadapi (jika ada) : .....

May require additional time for the production / induction phase to

Jika menulis buku ilmiah: Not applicable.

Progres terkini : *(tandakan yang berkaitan)*

- ☐ Kajian literatur
- ☐ Pengumpulan bahan-bahan/maklumat
- ☐ Menyediakan draf
  berapa bab (nyatakan) ..... dari .....jumlah bab.
- ☐ Menulis manuskrip (sila hantar satu salinan kepada Dekan, satu salinan kepada JPbSM)

Maklumat terperinci mengenai progres: .....

.....

.....

.....

(sila gunakan lampiran jika ruang tidak mencukupi)

Masalah yang dihadapi (jika ada) : .....

.....

UCLA Engineering
HENRY SAMUELI SCHOOL OF ENGINEERING AND APPLIED SCIENCE
Chemical and Biomolecular Engineering Department

Yi Tang
Professor
5531 Boelter Hall
Box 951592
Los Angeles, CA 90095-1592
(310)825-0375
(310)206-4107 FAX

Dr. Roslinah M. Hussain
Senior Lecturer
Faculty of Health Sciences, University Technology MARA
Malaysia
Tel: 06 03-32584427
roslinah561@salam.uitm.edu.my

February 28, 2012

Dear Dr. Roslinah M. Hussain:

It is with great pleasure that I write to invite you to visit my laboratory as a visiting scholar starting in September of 2012 for the duration of up to six (6) months. I anticipate you can work on a number of projects related to metabolic engineering of *E. coli* strains for biotechnology applications. One potential project can be "Towards heterologous production of plant monoterpene indole alkaloids in microorganisms." I anticipate the amount of bench cost (which includes consumables, reagents and instrumentation cost) will be ~ $1000 per month.

Please be advised that University of California requires demonstration of financial support for foreign visiting scholars (J-1 visa). The amount of support needs to be at least $28,000 per year. You will be responsible for your health care costs.

To proceed with processing paperwork and other related steps, please contact our departmental manager Mr. Alain De Vera (alain@ucla.edu). He will be able to help you with DS-2019 and other visa documents. If you have other questions, please do not feel hesitant to contact me.

Sincerely

Yi Tang

	UNIVERSITY OF CALIFORNIA STATE OATH OF ALLEGIANCE. PATENT POLICY, AND PATENT ACKNOWLEDGMENT	EMPLOYEE'S NAME (Last, First, Middle Initial)		DATE PREPARED Mo/Dy/Yr
	UPAY585 (R 11/2011) E0420 71443-180	EMPLOYEE ID	DEPARTMENT	EMPLOYMENT DATE Mo/Dy/Yr

STATE OATH OF ALLEGIANCE *I do solemnly swear (or affirm) that I will support and defend the Constitution of the United States and the Constitution of the State of California against all enemies, foreign and domestic; that I will bear true faith and allegiance to the Constitution of the United States and the Constitution of the State of California; that I take this obligation freely, without any mental reservation or purpose of evasion; and that I will well and faithfully discharge the duties upon which I am about to enter.*

Taken and subscribed before me on: _____
Mo/Dy/Yr

Signature of Authorized Official: _____

Title: _____

County: _____ State: _____

Signature of Officer or Employee: _____

(Do not sign until in the presence of proper witness.)

NOTE: No fee may be charged for administering this oath.

The oath must be administered by either (1) a person having general authority by law to administer oaths—for example, Notaries Public, Civil Executive Officers (Gov. Code Sec. 1001), Judicial Officers, Justices of the Peace, and county officials named in Gov. Code Sections 24000, 24057, such as, district attorneys, sheriffs, county clerks, members of boards of supervisors, etc., or (2) by any University Officer or employee who has been authorized in writing by The Regents to administer such oaths.

WHO MUST SIGN THE OATH: All persons (other than aliens) employed by the University, in common with all other California public employees, whether with or without compensation, must sign the oath. (Calif. Constitution, Article XX, Section 2, Calif. Gov. Code Sections 3100-3102.)

All persons re-employed by the University after a termination of service must sign a new Oath if the date of re-employment is more than one year after the date on which the previous Oath was signed (Calif. Gov. Code Sec. 3102.)

WHEN OATH MUST BE SIGNED: The Oath must be signed BEFORE the individual enters upon the duties of employment (Calif. Constitution, Article XX, Section 3: Calif. Gov. Code Sec. 3102.)

WHERE OATHS ARE FILED: The Oaths of all employees of the University shall be filed with the Campus Accounting Office.

FAILURE TO SIGN OATH: No compensation for service performed prior to his subscribing to the Oath or affirmation may be paid to a University employee. And no reimbursement for expenses incurred may be paid prior to his subscribing to the Oath or affirmation. (Calif. Gov. Code Sec. 3107.)

PENALTIES: "Every person who, while taking and subscribing to the Oath or affirmation required by this chapter, states as true any material which he knows to be false, is guilty of perjury, and is punishable by imprisonment in the state prison not less than one or more than 14 years." (Calif. Gov. Code Sec. 3108.)

PATENT ACKNOWLEDGMENT

This acknowledgment is made by me to The Regents of the University of California, a corporation, hereinafter called "University," in part consideration of my employment, and of wages and/or salary to be paid to me during any period of my employment, by University, and/or my utilization of University research facilities and/or my receipt of gift, grant, or contract research funds through the University.

By execution of this acknowledgment, I understand that I am not waiving any rights to a percentage of royalty payments received by University, as set forth in the University of California Patent Policy, hereinafter called "Policy."

I also understand and acknowledge that the University has the right to change the Policy from time to time, including the percentage of net royalties paid to inventors, and that the policy in effect at the time an invention is disclosed shall govern the University's disposition of royalties, if any, from that invention. Further, I acknowledge that the percentage of net royalties paid to inventors is derived only from consideration in the form of money or equity received under: 1) a license or bailment agreement for licensed rights, or 2) an option or letter agreement leading to a license or bailment agreement. I also acknowledge that the percentage of net royalties paid to inventors is not derived from research funds or from any other consideration of any kind received by the University. The Policy on Accepting Equity When Licensing University Technology governs the treatment of equity received in consideration for a license.

I acknowledge my obligation to assign, and do hereby assign, inventions and patents that I conceive or develop 1) within the course and scope of my University employment while employed by University, 2) during the course of my utilization of any University research facilities, or 3) through any connection with my use of gift, grant, or contract research funds received through the University. I further acknowledge my obligation to promptly report and fully disclose the conception and/or reduction to practice of potentially patentable inventions to the University authorized licensing office. Such inventions shall be examined by the University to determine rights and equities therein in accordance with the Policy. I shall promptly furnish University with complete information with respect to each.

In the event any such invention shall be deemed by University to be patentable or protectable by an analogous property right, and University desires, pursuant to determination by University as to its rights and equities therein, to seek patent or analogous protection thereon, I shall execute any documents and do all things necessary, at University's expense, to assign to University all rights, title, and interest therein and to assist University in securing patent or analogous protection thereon. The scope of this provision is limited by Calif. Labor Code Sec. 2870, to which notice is given below. In the event I protest the University's determination regarding any rights or interest in an invention, I acknowledge my obligation: (a) to proceed with any University requested assignment or assistance; (b) to give University notice of that protest no later than the execution date of any of the above-described documents or assignment; and (c) to reimburse University for all expenses and costs it encounters in its patent application attempts, if any such protest is subsequently sustained or agreed to.

I acknowledge that I am bound to do all things necessary to enable University to perform its obligations to grantors of funds for research or contracting agencies as said obligations have been undertaken by University.

University may relinquish to me all or a part of its right to any such invention, if, in its judgment, the criteria set forth in the Policy have been met.

I acknowledge that I am bound during any periods of employment by University or for any period during which I conceive or develop any invention during the course of my utilization of any University research facilities, or any gift, grant, or contract research funds received through the University.

In signing this acknowledgment, I understand that the law, of which notification is given below, applies to me, and that I am still required to disclose all my inventions to the University.

NOTICE: This acknowledgment does not apply to an invention which qualifies under the provision of Calif. Labor Code Sec.2870 which provides that (a) Any provision in an employment agreement which provides that an employee shall assign, or offer to assign, any of his or her rights in an invention to his or her employer shall not apply to an invention that the employee developed entirely on his or her own time without using the employer's equipment, supplies, facilities, or trade secret information except for those inventions that either: (1) Relate at the time of conception or reduction to practice of the invention to the employer's business, or actual or demonstrably anticipated research or development of the employer; or (2) Result from any work performed by the employee for the employer. (b) To the extent a provision in an employment agreement purports to require an employee to assign an invention otherwise excluded from being required to be assigned under subdivision (a), the provision is against the public policy of this state and is unenforceable. In any suit or action arising under this law, the burden of proof shall be on the individual claiming the benefits of its provisions.

RETENTION: Accounting: 5 years after separation, except in cases of disability, retirement or disciplinary action, in which case retain until age 70.

Other Copies: 0-5 years after separation

Employee/Guest Name (Please print): ROSLINAH MOHAMAD HUSSAIN

Employee/Guest Signature: Roslinah Hussain Date: 06/27/12

Witness Signature & University Acceptance: _____ Date: _____

PLEASE SIGN STATE OATH AND PATENT ACKNOWLEDGMENT

UNIVERSITY OF CALIFORNIA PATENT POLICY—October 1, 1997

I. PREAMBLE

It is the intent of the President of the University of California, in administering intellectual property rights for the public benefit, to encourage and assist members of the faculty, staff, and others associated with the University in the use of the patent system with respect to their discoveries and inventions in a manner that is equitable to all parties involved.

The University recognizes the need for and desirability of encouraging the broad utilization of the results of University research, not only by scholars but also in practical application for the general public benefit, and acknowledges the importance of the patent system in bringing innovative research findings to practical application.

Within the University, innovative research findings often give rise to patentable inventions as fortuitous by-products, even though the research was conducted for the primary purpose of gaining new knowledge. The following University of California Patent Policy is adopted to encourage the practical application of University research for the broad public benefit; to appraise and determine relative rights and equities of all parties concerned; to facilitate patent applications, licensing, and the equitable distribution of royalties, if any; to assist in obtaining funds for research; to provide for the use of invention-related income for the further support of research and education; and to provide a uniform procedure in patent matters when the University has a right or equity.

II. STATEMENT OF POLICY

A. An agreement to assign inventions and patents to the University, except those resulting from permissible consulting activities without use of University facilities, shall be mandatory for all employees, for persons not employed by the University but who use University research facilities, and for those who receive gift, grant, or contract funds through the University. Such an agreement may be in the form of an acknowledgment of obligation to assign. Exemptions from such agreements to assign may be authorized in those circumstances when the mission of the University is better served by such action, provided that overriding obligations to other parties are met and such exemptions are not inconsistent with other University policies.

B. Those individuals who have so agreed to assign inventions and patents shall promptly report and fully disclose the conception and/or reduction to practice of potentially patentable inventions to the Office of Technology Transfer or authorized licensing office. They shall execute such declarations, assignments, or other documents as may be necessary in the course of invention evaluation, patent prosecution, or protection of patent or analogous property rights, to assure that title in such inventions shall be held by the University or by such other parties designated by the University as may be appropriate under the circumstances. Such circumstances would include, but not be limited to, those situations when there are overriding patent obligations of the University arising from gifts, grants, contracts, or other agreements with outside organizations.

In the absence of overriding obligations to outside sponsors of research, the University may release patent rights to the inventor in those circumstances when:

1. the University elects not to file a patent application and the inventor is prepared to do so, or

2. the equity of the situation clearly indicates such release should be given, provided in either case that no further research or development to develop that invention will be conducted involving University support or facilities, and provided further that a shop right is granted to the University.

C. Subject to restrictions arising from overriding obligations of the University pursuant to gifts, grants, contracts, or other agreements with outside organizations, the University agrees, following said assignment of inventions and patent rights, to pay annually to the named inventor(s), or to the inventor(s)' heirs, successors, or assigns, 35% of the net royalties and fees per invention received by the University. An additional 15% of net royalties and fees per invention shall be allocated for research-related purposes on the inventor's campus or Laboratory. Net royalties are defined as gross royalties and fees, less the costs of patenting, protecting, and preserving patent and related property rights, maintaining patents, the licensing of patent and related property rights, and such other costs, taxes, or reimbursements as may be necessary or required by law.

Inventor shares paid to University employees pursuant to this paragraph represent an employee benefit. When there are two or more inventors, each inventor shall share equally in the inventor's share of royalties, unless all inventors previously have agreed in writing to a different distribution of such share.

Distribution of the inventor's share of royalties shall be made annually in November from the amount received during the previous fiscal year ending June 30th, except as provided for in Section II.D. below. In the event of any litigation, actual or imminent, or any other action to protect patent rights, the University may withhold distribution and impound royalties until resolution of the matter.

D. The DOE Laboratories may establish separate royalty distribution formulas, subject to approval by the President. Distribution of the inventor's share of DOE Laboratory royalties shall be made annually in February from the amount received during the previous fiscal year ending September 30th. All other elements of this policy shall continue to apply.

E. Equity received by the University in licensing transactions, whether in the form of stock or any other instrument conveying ownership interest in a corporation, shall be distributed in accordance with the Policy on Accepting Equity When Licensing University Technology.

F. In the disposition of any net income accruing to the University from patents, first consideration shall be given to the support of research.

III. PATENT RESPONSIBILITIES AND ADMINISTRATION

A. Pursuant to Regents' Standing Order 100.4(mm), the President has responsibility for all matters relating to patents in which the University of California is in any way concerned. This policy is an exercise of that responsibility, and the President may make changes to any part of this policy from time to time, including the percentage of net royalties paid to inventors.

B. The President is advised on such matters by the Technology Transfer Advisory Committee (TTAC), which is chaired by the Senior Vice President—Business and Finance. The membership of TTAC includes the Provost and Senior Vice President—Academic Affairs, the Director of the Office of Technology Transfer, and representatives from the campuses, DOE Laboratories, Academic Senate, the Division of Agriculture and Natural Resources and the Office of the General Counsel. TTAC is responsible for:

1. Reviewing and proposing University policy on intellectual property matters including patents, copyrights, trademarks, and tangible research products;

2. Reviewing the administration of intellectual property operations to ensure consistent application of policy and effective progress toward program objectives; and

3. Advising the President on related matters as requested.

C. The Senior Vice President—Business and Finance is responsible for implementation of this Policy, including the following:

1. Evaluating inventions and discoveries for patentability, as well as scientific merit and practical application, and requesting the filing and prosecution of patent applications.

2. Evaluating the patent or analogous property rights or equities held by the University in an invention, and negotiating agreements with cooperating organizations, if any, with respect to such rights or equities.

3. Negotiating licenses and license option agreements with other parties concerning patent and or analogous property rights held by the University.

4. Directing and arranging for the collection and appropriate distribution of royalties and fees.

5. Assisting University officers in negotiating agreements with cooperating organizations concerning prospective rights to patentable inventions or discoveries made as a result of research carried out under gifts, grants, contracts, or other agreements to be funded in whole or in part by such cooperating organizations, and negotiating with Federal agencies regarding the disposition of patent rights.

6. Approving exceptions from the agreement to assign inventions and patents to the University as required by Section II.A. above.

7. Approving exemptions to University policy on intellectual property matters including patents, copyrights, trademarks, and tangible research products.

PLEASE SIGN THE STATE OATH and PATENT ACKNOWLEDGEMENT on page 1