Course Syllabus
MBMB 644 PCR-Based Gene Mutagenization for Protein Engineering
Academic Year 2025

Course ID and Title: MBMB 644
PCR-Based Gene Mutagenization for Protein Engineering
ชมชม ๖๔๔
การก่อกลายพันธุ์ยีนด้วยเทคนิคพีซีอาร์เพื่อการวิศวกรรมโปรตีน

Credits: 1 (0—2—1)

Curriculum: Master of Science Program in Molecular and Integrative Biosciences (Elective course)
Doctor of Philosophy Program in Molecular and Integrative Biosciences (Elective course)

Semester: 2nd Semester

Academic Year: 2025

Date and Time: March 9 — 13, 2026 (9:00 AM — 4:00 PM)

Classroom: B205 Laboratory, Institute of Molecular Biosciences, Mahidol University

Pre-Requisites: None

Course Coordinator: Assoc. Prof. Chalongrat Noree, Ph.D.
Tel. 02-441-9003 to 7 Ext. 1274, Mobile: 062-454-1556
Email: chalongrat.nor@mahidol.ac.th
chalongrat.nor@mahidol.edu
chalongrat.nor@gmail.com
Office and Lab: B205 (2nd floor, wing-B)
Institute of Molecular Biosciences, Mahidol University

Instructors:

1. Assoc. Prof. Chalongrat Noree, Ph.D.
   Email: chalongrat.nor@mahidol.edu
   Website: https://mb.mahidol.ac.th/chalongrat-profile/

2. Prof. Panadda Boonserm, Ph.D.
   Email: panadda.boo@mahidol.ac.th
   Website: https://mb.mahidol.ac.th/panadda-profile/

Support Staff:

1. Naraporn Sirinonthanawech
   (*for assistance with confocal microscope operation on the DAY 3 afternoon session*)
   Email: naraporn.sir@mahidol.edu

2. Monrudee Srisaisap
   (*for assistance with SDS-PAGE and western blotting material preparation*)
   Email: catta_w255@hotmail.co.th
3. Somsri Sakdee
   (*for assistance with the preparation of E. coli competent cells and E. coli culture material*)
   Email: somsri.sak@mahidol.ac.th

Course Learning Outcomes (CLOs):

By the end of the course, students should be able to:

1. Illustrate PCR-based techniques that can be used for gene mutagenesis and protein engineering (**Knowledge**).
2. Perform PCR-based site-directed mutagenesis and western blot analysis to solve a biological problem or question (**Skills**).
3. Comply with research ethics and scientific integrity (**Ethics**).
4. Demonstrate professional and interpersonal characteristics that can support the completion of the assigned (group/individual) works and laboratory experiments (**Character**).

Alignment of Teaching and Assessment Methods to Course Learning Outcomes:

Course Learning Outcomes	Teaching Method	Assessment Method
1. Illustrate PCR-based techniques that can be used for gene mutagenesis and protein engineering (**Knowledge** – Aligned with **PLO1**).	1. Active learning 2. Discussion 3. Assignment	1. Q&A during lecture 2. Discussion performance 3. Quiz / short exercise 4. Assignment
2. Perform PCR-based site-directed mutagenesis and western blot analysis to solve a biological problem or question (**Skills** – Aligned with **PLO2**).	1. Instructions 2. Discussion 3. Hands-on lab practice 4. Problem-based learning	1. Discussion performance 2. Lab performance 3. Problem-based learning (scientific content and inventive idea)
3. Comply with research ethics and scientific integrity (**Ethics** – Aligned with **PLO3**).	1. Discussion (about scientific integrity, responsibility, and safety practice) 2. Assignment 3. Writing lab report	1. Attendance (presence, absence, on-time?) 2. Task submission (on-time?) 3. Lab report writing (plagiarism?)

Course Learning Outcomes	Teaching Method	Assessment Method
	4. Hands-on lab safety practice	4. Lab performance (follow safety practice?)
4. Demonstrate professional and interpersonal characteristics that can support the completion of the assigned (group/individual) works and laboratory experiments (**Character** – Aligned with **PLO4**).	1. Discussion 2. Writing lab report 3. Individual or group assignment/presentation 4. Problem-based learning	1. Discussion performance (active participation?) 2. Lab report writing performance 3. Performance in the team (teamwork or leadership skills)

Course Description:

PCR-based site-directed mutagenesis; plasmid construction for PCR-based gene mutagenization; primer design for PCR-based site-directed mutagenesis; bacterial transformation and selection; protein expression in *E. coli* system; western blot analysis; fluorescence microscopic analysis

(In Thai) การก่อกลายพันธุ์ที่ตำแหน่งจำเพาะโดยอาศัยเทคนิคพีซีอาร์ การสร้างพลาสมิดสำหรับใช้ในการก่อกลายพันธุ์ด้วยเทคนิคพีซีอาร์ การออกแบบไพรเมอร์สำหรับการก่อกลายพันธุ์ที่ตำแหน่งจำเพาะโดยอาศัยเทคนิคพีซีอาร์ การนำดีเอ็นเอเข้าสู่เซลล์แบคทีเรียและการคัดเลือกแบคทีเรียที่ได้รับดีเอ็นเอ การสร้างโปรตีนโดยอาศัยแบคทีเรียเป็นระบบการผลิต การวิเคราะห์ตัวอย่างโปรตีนด้วยเทคนิคเวสเทิร์นบล็อท การวิเคราะห์ตัวอย่างด้วยกล้องจุลทรรศน์ฟลูออเรสเซนซ์

Course Schedule:

	Activities	Description	Time	Instructors
		DAY 1		
1	**Active Discussion: Mutagenesis by PCR**	• To discuss the technique of site-directed mutagenesis by PCR.	9:00 AM – 4:00 PM	**CN**
2	**Lab: PCR**	• To amplify the mCherry plasmid, introducing the Y72H mutation (to the mCherry coding sequence).		
3	**Lab: Agarose Gel Electrophoresis**	• To check the PCR product (the linear mCherry plasmid with the Y72H mutation).		
4	**Lab: *Dpn*I treatment and PCR purification**	• To remove the plasmid template. • To purify the *Dpn*I-treated PCR product using a PCR purification kit.		

	Activities	Description	Time	Instructors
5	**Lab: Determination of DNA Concentration and Ligation**	• To quantify the amount and concentration of the PCR product (after *Dpn*I treatment and column purification) • To re-circularize the linear plasmid carrying the Y72H mCherry by incubation with T4 DNA ligase.		
6	**Active Discussion**	• To recap lab objectives and workflow integration (DAY 1).		
		DAY 2		
1	**Lab: Bacterial Transformation**	• To transform competent *E. coli* cells with the ligation products.	9:00 AM – 4:00 PM	**CN**
2	**Computer Lab: Primer Design and DNA Sequencing Analysis**	• To learn how to design mutagenic primers and how to perform pairwise alignment for verifying the mutagenized mCherry plasmids (isolated from selected bacterial transformants).		
3	**Active Discussion**	• To recap lab objectives and workflow integration (DAY 2).		
		DAY 3		
1	**Lab: Preparing Master Plate**	• To make copies of randomly selected transformants for further analysis and use.	9:00 AM – 4:00 PM	**CN**
2	**Lab: Inoculation**	• To grow positive bacterial transformants in liquid LB to achieve log phase growth.		
3	**Lab: IPTG Induction**	• To induce protein expression (with IPTG) in *E. coli*.		
4	**Lab: Preparing Protein Samples**	• To collect the cells from the IPTG-induced cultures and prepare whole cell extracts.		
5	**Lab: Fluorescence Microscopic Analysis of *E. coli* Expressing Wild-Type (WT) vs Y72H mCherry Proteins**	• To inspect the phenotype of WT and Y72H mCherry proteins as expressed in *E. coli*.		
6	**Active Discussion**	• To recap lab objectives and workflow integration (DAY 3).		
		DAY 4		
1	**Active Discussion: SDS-PAGE and Western Blot Analysis**	• To discuss the fundamentals and applications of SDS-PAGE and western blot analysis.	9:00 AM – 4:00 PM	**PB, CN**
2	**Lab: Preparing Acrylamide Gels**	• To prepare/cast the acrylamide gels to be used for SDS-PAGE.		

	Activities	Description	Time	Instructors
3	**Lab: SDS-PAGE**	• To separate bacterial proteins on the acrylamide gel.		
4	**Lab: Western Blotting (Part I)**	• To transfer the separated proteins from the acrylamide gel to a PVDF membrane and then block the blot.		
5	**Active Discussion**	• To recap lab objectives and workflow integration (DAY 4).		
		DAY 5		
1	**Lab: Western Blotting (Part II)**	• To detect mCherry on the blot using mouse anti-mCherry and HRP-conjugated goat anti-mouse IgG.	9:00 AM – 4:00 PM	**CN**
2	**Computer Lab: ImageJ**	• To learn how to quantify protein bands on the blot.		
3	**Recap and Reflection**	• To provide students with opportunities to describe their learning experiences gained from this course and how these can be applied to their future learning.		
4	**After Action Review (AAR)**	• To collect comments and suggestions from students for further improvements of the course.		

Note: **CN** Chalongrat Noree
PB Panadda Boonserm

Assessment Criteria:

	Assessment Criteria	Description (in Details)	Scoring Rubric
1	**Class Attendance (5%)**	Showing up in the class (**5%**)	• Full attendance (**4**) • ~ 80% attendance (**3**) • ~ 60% attendance (**2**) • < 50% attendance (**1**)
2	**Lab Report (15%)**	The presence of intro, methods, results, discussion, and conclusion **with no plagiarism** (**3%**)	• Complete (**4**) • ~ 80% complete (**3**) • ~ 60% complete (**2**) • < 50% complete (**1**)
		Data presentation (**3%**)	• Complete (**4**) • ~ 80% complete (**3**) • ~ 60% complete (**2**) • < 50% complete (**1**)
		Data analysis and interpretation (**3%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**)

Assessment Criteria		Description (in Details)	Scoring Rubric
			• Need to be improved (**1**)
		English and writing skills (**3%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**) • Need to be improved (**1**)
		Report format and typing errors (**2%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**) • Need to be improved (**1**)
		On-time submission (**1%**)	• On-time (**4**) • Late (**2-3**) • Very late (**1**)
3	**Quiz / Exercise (20%)**	Depending on the correctness and completion (**20%**)	**Raw scores will be adjusted to a range of 0-20%**
4	**Discussion Performance (20%)**	Participation and performance (**5%**)	• Active (**4**) • Fairly active (**2-3**) • Inactive (**1**)
		Professional and interpersonal skills (responsibility, teamwork, and leadership) (**5%**)	• Active (**4**) • Fairly active (**2-3**) • Inactive (**1**)
		Creative and high-order thinking skills (**10%**)	• Highly expressed (**4**) • Fairly expressed (**2-3**) • Not shown (**1**)
5	**Reflection (10%)**	Knowledge sharing (**2.5%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**) • Need to be improved (**1**)
		Inventive and creative thinking skills (**2.5%**)	• Highly expressed (**4**) • Fairly expressed (**2-3**) • Not shown (**1**)
		Communication skills (**2.5%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**) • Need to be improved (**1**)
		Professional and interpersonal characters (responsibility, teamwork, and leadership) (**2.5%**)	• Active (**4**) • Fairly active (**2-3**) • Inactive (**1**)
6	**Lab Performance (30%)**	Safety practice (**5%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**) • Not solid (**1**)
		Responsibility (**5%**)	• Highly expressed (**4**) • Fairly expressed (**2-3**)

Assessment Criteria		Description (in Details)	Scoring Rubric
			• Not shown (**1**)
		Lab skills (**10%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**) • Need to be improved (**1**)
		Decision making and trouble-shooting skills (**10%**)	• Excellent (**4**) • Good (**3**) • Fair (**2**) • Need to be improved (**1**)

Student's achievement will be graded using symbols: A, B+, B, C+, C, D+, D or F, based on the criteria as follows:

Percentage	Grade	Description
80—100	A	Excellent
75—79	B+	Very Good
70—74	B	Good
65—69	C+	Fairly Good
60—64	C	Fair
55—59	D+	Poor
50—54	D	Very Poor
0—49	F	Fail

Date of Revision: November 2025