Module Information

Module Code	Module Title	Semester	Mod. Credits
LSM2191	Laboratory Techniques in Life Sciences	1 & 2	4

Module Description

This module introduces the theory and practical applications of techniques used in molecular biology and protein biochemistry. Knowledge in recombinant DNA techniques, such as RNA isolation, reverse transcription, polymerase chain reaction, recombinant DNA construction and recombinant protein expression; and in protein purification, such as liquid chromatography, polyacrylamide gel electrophoresis and western blotting, will be integrated with laboratory practice.

Eligibility and requirements

Prerequisites (prior knowledge required): LSM1102 or LSM1106

Corequisites: NIL

Precluded modules (if any): NIL

Instructional methods

The following instructional methods will be employed:
1) Lecture
2) Tutorial
3) Laboratory (Dry/Wet)

Assessment modes

The following assessments will be employed:
1) Writing Report = 60%
2) Final Examination = 40%

Note, these weightages are subject to periodic review and therefore may change.

Contact information for Module Coordinator and other instructors

Assoc. Prof. Lu Gan (Module Coordinator, Sem 1)
Email: lu@anaphase.org

Assoc. Prof. Norbert Lehming (Module Coordinator, Sem 2)
Email: micln@nus.edu.sg

Assoc. Prof. Maxey C.M. Chung (Module Coordinator, Sem 2)
Email: bchcm@nus.edu.sg

Course content and syllabus

The entire module involves a real-world exploration of the central dogma: DNA; RNA; Protein; Function. The students will also learn how the Nobel-prize-winning discovery of the exception to the central dogma, reverse transcription, is used as an essential tool in modern biotechnological research. Using the lactate dehydrogenase system and starting with only mouse tissue, students will convert RNA to DNA. They will then use a popular cloning system to create new DNA molecules that are amenable to production and purification of enzymes. These transformation and overexpression experiments will help students get a concrete grasp of the DNA; RNA; protein information flow. The students will cement their theoretical knowledge of enzymology by observing enzyme catalysis, which further reveals the nature of the invisible proteins they have purified.

In addition to these real-world lab skills, students will learn fundamental sequence analysis skills, using popular web-based and locally installed software. They will see how nearly any DNA molecule can be manipulated at base-pair accuracy and how these DNA molecules are used in advanced fundamental and applied research.

Finally, the students will be exposed to case studies. These studies relate molecular structures and their expected phenotypes of these molecules. By understanding the biological molecules as concrete entities, the students will see how modern science uses existing knowledge to predict future experimental results.

Learning activities

The following learning activities will be employed to achieve the learning outcomes of knowledge, cognitive skills, generic skills and/or attributes development stated in the 'Intended Learning Outcomes' below:

1) Group Discussion or Discussion Forum
2) Group/Individual Project
3) Problem-based Learning
4) Laboratory Activities (Wet/Dry)
5) Hands-on Technology
6) Report/Essay Writing

Modern biotechnology is a highly collaborative and fast-paced enterprise. Well-executed biotech projects can have world-changing results, as recently demonstrated by the record-breaking conception, development, testing, and commercialization of the mRNA-based COVID vaccines. To achieve this level of success, scientists must learn to carry out fundamental experiments in the wet lab, then connect the observed data with invisible biological molecules. However, in science, it is not enough to be a pair of hands at the bench. Science requires accurate and concise communication of hypotheses, experimental design, results, and the greater context and ramifications of the results. This communication, which is done in the form a report, will add extreme value when it explains what the next steps of a research project are, either in improving a result or in obtaining a new result. It is expected that students will use these skills in undergraduate research, such as UROPS and FYPs to further cement their connections between the theory and experiments.

Intended Learning Outcomes

Knowledge development

By the end of the module, the student will have learned how to:
1. Isolate mRNA from tissue and amplify one specific gene.
2. Clone a gene into a bacterial expression plasmid.
3. Express and purify an enzyme from a bacterial over-expression system.

In addition, the student is expected to become proficient in analysis:
1. Measurement of both nucleic acid and protein molecule yields and sizes.
2. Measure enzyme activities.
3. Detect minute quantities of a specific protein by western blot.
4. Troubleshoot unexpected results.
5. Propose alternative experimental strategies and methods.

Finally, the students will learn how to communicate a science project in clear and concise writing.

This module will provide the opportunities to develop the following cognitive skills, generic skills and attributes:

Very Good Opportunities	Good/Average Opportunities
1) Understand: Question, Connect & Explain 2) Apply: Use, Execute & Implement 3) Analyze: Differentiate, Organize & Attribute 4) Written Communication 5) Analytical & Critical Thinking 6) Problem-solving & Decision-making 7) Collaboration & Teamwork 8) Planning, Organizing & Management skills 9) Adaptability & Learnability 10) Resilience	1) Evaluate: Review, Check & Critique 2) Create: Ideate, Plan, Generate & Produce 3) Quantitative Thinking 4) Creative Thinking 5) Self-Efficacy

Required and/or recommended readings

Note, the following online learning materials will be updated as needed or when a better version is found.

VIDEOS
Before entering the wetlab, learn how to use a micropipette:
https://www.youtube.com/watch?v=uEy_NGDfo_8&spfreload=1
https://www-jove-com.libproxy1.nus.edu.sg/v/5033/an-introduction-to-the-micropipettor

Principles of PCR (JoVE)
https://www-jove-com.libproxy1.nus.edu.sg/v/5056/pcr-the-polymerase-chain-reaction

DNA / molecular biology experiments
Practical 1A
RNA Extraction Tutorial
https://www.youtube.com/watch?v=MgNicWbANkA

Simplified RT – Reverse Transcription Animation
https://www.youtube.com/watch?v=0MJIbrS4fbQ

How to use Nanodrop
https://www.youtube.com/watch?v=ZodxPBlyKvQ

How to design primer for cloning
https://www.youtube.com/watch?v=-Gx08NLJLwc

Practical 1B
How to prepare an agarose gel for DNA electrophoresis (Labtricks)
https://www.youtube.com/watch?v=2UQIoYhOowM

Practical 2
Detailed explanation of an expression vector's features
https://www.embl.de/pepcore/pepcore_services/cloning/choice_vector/ecoli/vectorfeatures/

Practical 3
Linear regression using MS Excel
https://www.youtube.com/watch?v=L_a8Z0BVjyM

Linear regression using Google sheets
https://www.youtube.com/watch?v=sz7cZ92xWn0

Practical 4
How to use BLAST (for nucleic acids)
https://www.youtube.com/watch?v=rlK-5joOlyU

How to use BLAST (for proteins)
https://www.youtube.com/watch?v=HXEpBnUbAMo

Interpreting BLAST results
https://www.youtube.com/watch?v=fqcmhI34HAE

Protein experiments – These videos demonstrate the principles of LSM2191's protein experiments. Our equipment setup and parameters are different from those portrayed here, so don't take every part of these videos literally. Some of these videos are extra,

meaning that we won't actually be doing, for example, Gel filtration chromatography. However, you should try to understand these concepts because they are used routinely for protein biochemistry. The details of some of these experiments are in PDF documents (zipped on LumiNUS). Please study the included figures carefully.

Practical 5:
Bacterial cell culture (awesomesaucesable)
https://www.youtube.com/watch?v=C-x_QmUZSMg

How to make a buffer (MIT OpenCourseWare)
https://www.youtube.com/watch?v=HZFIdpThd-s
* Note that some online videos show improper technique. Good buffers are key to all subsequent experiments!

Another video with good technique (Bionetwork):
https://www.youtube.com/watch?v=S6bgIeM5wSQ

Practical 6 & 7:
Affinity chromatography (GE life sciences)
https://www.youtube.com/watch?v=FUAQKjKT99Y

Bonus: the following videos show purification methods not covered in the practicals

Gel filtration / size exclusion chromatography (GE life sciences)
https://www.youtube.com/watch?v=oV5VB5kO3tQ

Ion exchange chromatography (IEC/IEX) (GE life sciences)
https://www.youtube.com/watch?v=q3fMqgT1do8

Hydrophobic interaction chromatography (HIC) (GE life sciences)
https://www.youtube.com/watch?v=v6SPK6ZovgA

https://www.cytivalifesciences.com/en/us/solutions/protein-research/knowledge-center/protein-purification-methods/hydrophobic-interaction-chromatography

Practical 7:
How to pour an acrylamide gel (Labtricks)
https://www.youtube.com/watch?v=EDi_n_0NiF4]

Practical 8:
Western Blot, semi-dry transfer (Thermo)
https://www.youtube.com/watch?v=7SVHqK_mFtQ

Western Blot, wet transfer (BioRad)
https://www.youtube.com/watch?v=VgAuZ6dBOfs