Department of CLINICAL AND EXPERIMENTAL MEDICINE

College of MEDICINE (and Biology post-doc)

University of FOGGIA (ITALY)(POST-DOC) AND UNIVERSITY OF SALAHADDIN-ERBIL

Subject: ADVANCE BIOMOLECULAR METHODS APPLIED IN MEDICINE (Theory COURSE)

Course Book – (DOCTORATE STUDENTS-FOGGIA UNIVERSITY)

Lecturer's name:

Assist. Prof. Dr. Ari Qadir NABI (Ari Nabi)

Ph.D. in Molecular Microbiology at Universita` di Foggia-Italy.

Academic Year: 2023-2024

Course Book

1. Course name	**MOLECULAR TECHNIQUES-THEORY**
2. Lecturer in charge	**Assist. Prof. Dr. Ari Qadir NABI**
3. Department/ College	**BIOLOGY DEPT. / COLLEGE OF SCIENCES**
4. Contact	**e-mail: ari.nabi@su.edu.krd**
5. Time (in hours) per week	**Theory: 2 hrs**
6. Office hours	**To be Return to the schedule on the office door**
7. Course code	
8. Teacher's academic profile	• **Dr. Ari got the Doctorate (PhD.) degree in Molecular-Microbiology at Foggia University in Italy in 2011. From that time, as a Lecturer, he is in charge in teaching Molecular Techniques Theory for 4th class students, Molecular Biology Theory for 3rd class, Advance Molecular biology, Advance Molecular Techniques, Advance Gene Analysis to Master (M.Sc.) students, also Scientific and Biology-English for 1st year Biology students.** • **In 2005 he got the M.Sc. degree and start as Assistant Lecturer Teaching Practical Zoology at Science College, Practical Genetics at Agricultural college, and Hematology Theory at Shaqlawa Medical Institute.** • **He graduated from Salahaddin University in 2001 (Ranked Top 7th on 107 biology students and 11th at the collage). Dr. Nabi worked with the United Nations UN- F.A.O in Agro-Industrial Project Monitoring, then from 2002 until getting his M.Sc. degree he worked as Laboratory Technician in Biology Dept. at Science College teaching Practical Molecular Biology.**
9. Keywords	**DNA, RNA, Genes, mRNA, Q. PCR, rt PCR, Electrophoresis**

10. Course overview:

• The importance of studying the subject:

There have been numerous advances made in many fields of the biosciences in recent years, and perhaps the most dramatic advances have been in our ability to investigate and define cellular processes at the molecular level. These insights have been largely owing to the development and application of powerful new techniques in molecular biology—nucleic acid, protein, and cell-based methodologies, in particular. The importance and goals behind this course program is to provide the students a clear overview about important subject areas in Applied Molecular Biology, but at a level that is suitable for undergraduate students. By the end of this course, students will be able to conduct many Novel Molecular Techniques which have been invented recently and are applicable in many well-constructed laboratories in our country, to study many clinical problems and genetically

disorders. Therefore, they will find a good chance to work in many Governmental and Private sector laboratories.

- **Understanding of the fundamental concepts of the course:**

To provide a detailed discussion of each topic in the restricted time available means, it has been necessary to assume a very fast fresher review of students understanding of Basic Molecular Biology, Basic Genetics, Chromosomal Structure and DNA replication, Transcription and Protein Synthesis. This course book is therefore intended to complement your knowledge in this area of Bio-sciences.

- **Principles and theories of the course:**

This course tries to address topics that enhance students understanding the importance applying Molecular Techniques and Bioinformatics to study Biology in general and Molecular Biology, Biochemistry, Immunology and Microbiology in Particular via studying the Molecular and Genetic Revolution and a meaningful understanding of how living organisms functioning's including an appreciation of how cells operate at the molecular level from cell division to DNA doubling then to Protein Synthesis.

- **A sound knowledge of the major areas of the subject:**

In writing this course book, I have attempted to combat the frustration because I and many others have faced difficulties when reading papers, reviews and other books, in finding that essential points are often spread over many pages of text and embellished to such an extent that the salient information is difficult to extract. In accordance with these aims, I have presented the below inter-related Topics for Molecular Techniques covering most recent Separation and Visualization methods, Nucleic Acids Amplifications, Fluorescent Hybridizations and Probing Methods, and also joining Bioinformatics with Biology.

- **Sufficient knowledge and understanding to secure employment:**

In order to get a comprehensible understanding of the course topics and objectives, students must have a basic knowledge about Molecular Biology, Chromosomes, Genes and Biochemistry.

11. Course objective:

This course will give students hands-on experience in modern molecular techniques for obtaining and analysing data in population genetics and systematic biology. Following completion of this course the successful student will have practical experience in modern molecular techniques used for evolutionary genetics including molecular systematics, genomics, molecular microbiology, molecular diagnostic tools, and population/landscape genetics. Laboratories will give students hands-on experience performing techniques including polymerase chain reaction (PCR) and real-time PCR, DNA sequencing, molecular cloning, cDNA library construction, microsatellite library construction and screening, microsatellite analysis, single nucleotide polymorphisms (SNPs), RFLP, AFLP and DGGE among others. Lecture will focus on experimental design, data collection and analysis.

12. Student's obligation

***Exam policy:** Student should engage in 2 exams during the course. From the 3 exams, one will be collected from the weekly quizzes and assignments. Students will have to decide which one to be chosen for correction by the teacher before. There will be no make-up exams for absences students without medical report. By the end of the 2nd semester, students must do a final examination regarding this Material.

***Classroom polices:**

1- Attendance: You are strongly encouraged to attend class on a regular basis, as participation is important to your understanding of the material. This is your opportunity to ask questions. **You are responsible for obtaining any information you miss due to absence.**

2- Lateness: Lateness to class is disruptive.

3- Electronic devices: All cell phones are to be turned **silent** at the beginning of class and put away **(NOT USED)** during the entire class.

4-Talking: During class please refrain from side conversations. These can be disruptive to your fellow students and your professor

5- No Disrespectful to both the professor and to your fellow students.

6- **Every week Quizzes for previous lectures are obligatory.**

13. Forms of teaching

As in the Course book Text and Power Point Lectures. Different forms of teaching will be used to reach the objectives of the course: real-time teaching via white board, student integrations strategy, power point presentations for titles, sub- titles, figures, flow charts and summarizing the lecture main topic. Daily quizzes, students tutorials (15 minutes at least) and assignments will be established and will have their impact on the students final Marks.

14. Assessment scheme

Component	Date	Percent (theory)%
Exam 1st attempt	December 2023	10%
Exam 2nd attempt	March 2024	10%
Exam 3rd attempt	April 2024	10%
Exam 4th attempt	Weekly quizzes and assignments	07 %
Respecting Classroom Policy		03%

Final Exam	**60%**
Total (Semesters average summation + the final exam)	**100%**

15. Student learning outcome:

The importance and goals behind this course program is to provide the students a clear overview about important subject areas in Applied Molecular Biology, but at a level that is suitable for undergraduate students. By the end of this course, students will be able to conduct many Novel Molecular Techniques which have been invented recently and are applicable in many well-constructed laboratories in our country, to study many clinical problems and genetically disorders. Therefore, they will find a good chance to work in many Governmental and Private sector laboratories. Following completion of this course the successful student will have practical experience in modern molecular techniques used for evolutionary genetics including molecular systematics, genomics, molecular microbiology, molecular diagnostic tools, and population/landscape genetics. Laboratories will give students hands-on experience performing techniques including polymerase chain reaction (PCR) and real-time PCR, DNA sequencing, molecular cloning, cDNA library construction, microsatellite library construction and screening, microsatellite analysis, single nucleotide polymorphisms (SNPs), RFLP, AFLP and DGGE among others. Lecture will focus on experimental design, data collection and analysis.

16. Course Reading List and References:

1. John M. Walker. 2008. Molecular Bio methods, Handbook . Second Edition
2. M. Tevfik Dorak . 2006. Real-time PCR. School of Clinical Medical Sciences (Child Health)
3. Gerald Karp. 2013. Cell and Molecular Biology, Concepts And Experiments. 7th Edition.
4. Nalini Chandar. 2010. Ippincott's Illustrated Reviews: Cell And Molecular Biology.
5. David Clark. 2015. MOLECULAR BIOLOGY. *Southern Illinois University.* Elsevier Academic Press
6. Eberhard Passarge. 2007. Colour Atlas Of Genetics.
7. Andreas D. Baxevanis. 2001. BIOINFORMATICS, A Practical Guide To The Analysis Of Genes And Proteins. SECOND EDITION

Useful websites (electronic sites):

1. U.S. National Center for Biotechnology Information Pub-Med (http://www.ncbi.nlm.nih.gov/pubmed/)
2. University of California (http://mcb.berkeley.edu/)
3. Biomedical Centre: (http://www.biomedcentral.com/bmcmolbiol).

17. The Topics (Theory):

Weeks / Lecturer's Name	Topics
Week 1	Course Introduction, historical view, Disciplinary use of Molecular techniques in Biological studies.
Week 2	Molecular techniques based on DNA replication Principals
Week 3	I. Nucleic Acid Amplification: 1. Polymerase Chain Reaction (PCR)
Week 4	2. PCR Calculations and components
Week 5	Modifications in Nucleic Acid Amplification: 1. Hot start PCRs and modifications
Week 6	2. Multiplex, Nested, Touch down Amplifications
Week 7	Primer Design, software Alignments and BLAST and online verification
Week 8	3. loop mediated isothermal amplification LAMP
Week 9	1st Examination
Week 10	4. The rRNA Ribotypings
Week11	5. Colony PCR
Week 12	6. Freeze Thaw method Bacterial DNA-PCR
Week 13	7. HAD (Helicase Dependent Amplification)
Week 14	8. Polymorphism and RAPD-PCR
Week 15	Separation Methods of Biomolecules 1- AGE
Week 16	2- The Denaturing Gradient Gel Electrophoresis (DGGE)
Week 17	3- Pulse Field Gel Electrophoresis- Pulsotypings (PFGE)

Week 18	4- RFLP TYPING Genetic Mapping With RFLPs
Week 19	2nd Examination
Week 20	I. Quantitative and Fluorescent based AMPLIFICATIONS 1. Fluorescent Insitu Hybridization (FISH)
Week 21	2. LCR (Ligase Chain Reaction)
Week 22	3. Real time PCR (q PCR)
Week 23	3.1- TaqMan and Probe based q PCRs
Week 24	3.2- Molecular Becons Real time PCR
Week 25	3.3. Absolute Quantification in q. Real time PCR
Week 26	3.4. Dissociation curves in Dye based Real time PCR
Week 27	4. Reverse Transcriptase PCR (RT-PCR)
Week 28	5. Nucleic Acids Sequencing
Week 29	Quantification and Detection of DNA and RNA Component
Week 30	DNA Quality estimation using 230 and 280 ratios
Week 31	3rd Examination

19. Examinations:

Samples of last year's examinations:

Q1: Indicate the **True-False** statements**. The false** statements **MUST be corrected.** **(15 Marks)**

1. The computer based program that analyse the results of Real Time-PCR is called by Bionumerics.
   F-PFGE
2. In PFGE procedure, the use of restriction enzymes is for the digestion of the cells.
   F-DNA
3. Doing a PFGE reaction, we need to increase the number of cycles to about 45 cycles.
   F-RAPD-PCR
4. In DGGE, if the fragment completely denatures, then the migration again becomes a function of size.
   Y
5. Homoduplex DNA is less stable than completely complementary Heteroduplex DNA strands and it has a lower melting point.
   F- MORE STABLE; HIGHER OR INVER THE WORDS.
6. The T_m above 65°C should be generally avoided in primer designs, because of the potential for secondary annealing.
   Y
7. In DGGE, the denaturing environment is created by a combination of uniform Temperature, typically between 54 and 72 °C and a linear denaturant gradient formed with a solution of 100% chemical denaturant consists of 4 M Urea and 70% Formamide.
   F-60-65 THEN 7 AND 40%
8. The RAPD PCR method can detect more than 95% of the single-base differences in PCR products that are 1500bp or less in length.
   F-DGGE-600
9. In the PFGE Techniques, whenever 2 attached primers pointing opposite toward each other with distance of 600 bps, the amplification will succeed.
   F-RAPD-PCR
10. Based on the principle that "as DNA melts (become single stranded), intercalating-fluorescent dyes will bind and fluoresce"
    F-WILL NOT

Q2. Fill in the **Gaps** with suitable **word(s).** **(15 Marks)**

1. The master mix of a standard Real-time PCR is different from a normal PCR reaction by containing ___FLUOROPHORE PROBES OR INTERCALATING DYE_____.
2. PFGE is used to analyse very large DNA molecules from ____10KBP_____ to __10 MBP_____.
3. If a gene which has a single nucleotide mutation from G to T, when analysed with DGGE Technique, it will be separated at the _____ of the vertical Gel.
4. _____ is any DNA region consisting of tandem repeats that vary in number from individual to individual, including microsatellites, and other tandem repeats.
5. The standard pH for the PCR master mix is about _____.
6. Primer dimers are formed by _____ between the sense and antisense primers or within the sense or antisense primers.
7. *Pfu* is a DNA polymerase enzyme which is extracted from _____.
8. If long or genomic DNA to be synthesized by PCR, so the DNA polymerase enzyme must _____.

9. In the _____ reaction, the Primers are shorter and the cycle numbers are more in comparing with other standard PCRs.

10. Additional cycles of _____can tell us what Real-time PCR products are in the reaction test tubes.

Q3: If a Touchdown-PCR run has the following protocol, then calculate the Total Time required for performing the run knowing that the ramping time (Shifting) between every two successive steps was about 2 seconds? The PCR protocol was performed as follow:

(Initial denaturation at 95°C for 10 min; then 10 cycles of denaturation at 95°C for 20 s, annealing at 68 to 59°C (-1°C per cycle) for 20 s, and extension at 72°C for 30 s; Followed by 25 cycles of denaturation at 95°C for 20 s, annealing at 59°C for 20 s, and extension at 72°C for 30 s; and a Final extension at 72°C for 15 min).
(12 Marks)

Q4:
A- Differentiate (explain with figures) the Real Time-PCR and the conventional PCR Techniques. (10 Marks)
B- Count and Describe Primer designing Rules regarding the Primers Sequences Characters. (10 Marks)

Q5: Explain the reasons behind the followings (answer only 3): (18 Marks)
1. In the TaqMan Based Real Time-PCRs, destruction of one probe molecule equals to synthesis of 1 more copy of target DNA or cDNA, why?
2. To perform a successful RAPD-PCR reaction, the Annealing Temperature must be lowered to around 36°C, Why?
3. It is important to know the Extension Rate/Speed of a selected DNA-Polymerase enzyme, while doing any PCR, why?
4. For a successful PCR reaction, the Annealing temperature must be lowered by 1°C to few Centigrade from the Melting temperature, explain why?

20. Extra notes: