UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

SYLLABUS DEL CORSO

Cell and Molecular Biology II

2627-1-H4104D002-H4104D00202

Aims

The course provides theoretical knowledge and practical training on some widely used molecular biology techniques that are relevant for medical research.
The course provides theoretical knowledge and practical training on some molecular biology techniques relevant to medical research.
At the end of the practical course, students should be able to:
Understand the theoretical and practical basis of nucleic acid isolation
Master the principle of reverse transcription
Plan and set up a PCR reaction
Apply electrophoretic analysis techniques
Analyze and interpret experimental results

Contents

Introduction to biotechnology applications in medicine.
The course offers hands-on experience focusing on the complete workflow of cellular gene expression analysis (RT-PCR). Students will follow the molecular pathway, step by step, from RNA extraction to gene product visualization. The course is structured in two interrelated laboratory phases: Phase 1: Extraction and purification of total RNA, quantitative and qualitative measurement by spectrophotometry (Nanodrop), and reverse transcription reaction for cDNA synthesis. Phase 2: Calculation and preparation of samples for PCR, thermal cycler programming, agarose gel preparation, and electrophoresis for separation and identification of amplified fragments, followed by analysis of the results.

Detailed program

PHASE 1 – Total RNA Extraction and Reverse Transcription
The first phase is dedicated to RNA isolation and its conversion into cDNA, with particular attention paid to preventing degradation by RNases.
• Theoretical introduction: The central dogma of molecular biology, the chemical-structural differences between DNA and RNA, the experimental workflow, and RNase-free safety regulations.
• RNA extraction: Cell lysis, genomic DNA (gDNA) removal, purification on a silica column with on-column enzymatic digestion (DNase I), and elution.
• Quality control: Quantification of the extracted RNA using a Nanodrop and assessment of purity through A260/280 and A260/230 absorbance ratios.
• Reverse transcription (RT): Setting up reactions using the LunaScript RT SuperMix kit or similar to synthesize cDNA, while preparing the negative control (RT-) in parallel.

PHASE 2 – PCR Amplification and Electrophoresis The second phase focuses on the targeted amplification of the gene (e.g., GAPDH) and the physical separation of the biological fragments for subsequent visualization.
• PCR setup: Guided review of the workflow, volume calculations, and preparation of a common Master Mix (Taq polymerase, forward/reverse primers for GAPDH, dNTPs, and buffer).
• Thermal amplification: Aliquoting the mix, adding the templates (cDNA, RT- controls, positive and negative controls), and running the thermal cycles (denaturation, annealing, and extension).
• Gel preparation: Dilution of the TAE buffer from 50X to 1X, melting the agarose in a microwave (1.5% w/v gel), and adding the safe intercalating dye EuroSafe.
• Electrophoresis run and analysis: Loading the samples and the DNA Ladder, electrophoretic migration, and visualization of the results to verify the expected band.

Prerequisites

To actively participate in the lab and understand the experimental workflow, the following knowledge and theoretical requirements are recommended:
Theoretical knowledge of Molecular Biology and Genetics: In-depth understanding of the Central Dogma of Biology (the flow of genetic information from DNA to RNA and protein synthesis).
Nucleic Acid Biochemistry: Knowledge of the differential chemical structures of the nucleotides that make up DNA and RNA.
Basic Principles of Biotechnology: Theoretical knowledge of the Polymerase Chain Reaction (denaturation, annealing, and extension steps) and the separation of molecules by charge and sieve effect in an electric field (electrophoresis).
Fundamental familiarity with the concepts of molecular concentration, stock solutions, working solutions, and linear dilution calculations.

Teaching form

Practical laboratory activities for a total of 12 hours carried out in interactive mode in person

Textbook and teaching resource

The introductory slides and protocols used during the pratical training will be made available.

Semester

First semester

Assessment method

The topics will be part of the final integrated oral examination (further details are reported in the Syllabus of the entire course).

Office hours

On appointment, by arrangement with the professor (emanuele.azzoni@unimib.it)

Sustainable Development Goals

GOOD HEALTH AND WELL-BEING | QUALITY EDUCATION