UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

COURSE SYLLABUS

Molecular Biology II

2122-3-E0201Q062

Aims

The course aims at elucidating and increasing knowledge of some expression systems in Prokaryotes and Eukaryotes; in addition to the molecular characterization of the different expression systems, applications in the biotechnological field will be considered. Particular attention will also be addressed to different molecular biology techniques that are required for the analysis of gene expression and for the in vivo study of macromolecule interactions.

Knowledge and understanding.
Students will get acquainted with some expression systems in Prokaryotes and Eukaryotes, as well as, their biotechnological applications. In parallel, they will gain knowledge of biomolecular methods required for the study and the experimental approach of gene expression and macromolecule interactions.

Applying knowledge and understanding.
Students will be able to apply the acquired knowledge in subsequent courses in the field of the molecular biology and in laboratory activities. In addition, they will be able to understand scientific papers and will have the biomolecular knowledge required to discuss biotechnology-related subjects.

Making judgments.
Students will be able to elaborate on what they have learned, as well as, to face and critically discuss scientific works in the field of the biomolecular topics covered.

Communication skills.
Students will be able to express themselves appropriately in oral reports concerning the biomolecular topics faced.

Learning skills

Students will have the skills to face autonomously the subsequent studies that require the biomolecular knowledge acquired by applying the acquired study method.

Contents

Qualitative and quantitative transcription analyses. In vivo analyses of macromolecule interactions. Prokaryotic and eukaryotic expression systems.

Detailed program

Qualitative and quantitative transcription analyses. Relative Northern analysis, Dot blot (ASO probe/diagnostic tool for thalassemia), relative RT-PCR, RT-PCR applications, RACE. The microarray technology: an outline. cDNA libraries by RT-PCR. Tagged random primers-PCR.
Analyses of macromolecule interactions. One-hybrid system. Two-hybrid system and its modifications (reverse and split, Split-ubiquitin, Sos recruitment). Three hybrid system.
Prokaryotic expression systems. Protein expression in Escherichia coli. Inducible promoters. Fusion proteins and protein purification (Ubiquitin, IMPACT).
Eukaryotic expression systems. Protein expression in yeast. Selection markers. Episomal and integrative vectors. YACs. The biology of the 2 micron plasmid. Gene targeting. Gene inactivation. Pop-in and Pop-out. Inducible and constitutive promoters. The GAL system. Plasmid shuffling. Autoselection systems. Expression and secretion of proteins (the secretory pathway and the co/post- translational modifications of proteins). The cell wall. Yeast-based screening. Yeast surface display.
Protein expression in mammalian cells. Mammalian cells: transfections. Transient and stable expressions. Selection markers (tk,dhfr and dominant markers). Constitutive and inducible (Tet-on and Tet-off) promoters.
Protein expression in insect cells: the baculovirus system.

Prerequisites

Background: Molecular biology.
Specific prerequisites: Molecular Biology I.
General prerequisites: Students can take the exams of the third year after having passed all the exams of the first year of the course.

Teaching form

Classroom lessons supported with slides.
Teaching language: italian.

Textbook and teaching resource

All teaching material is available at the e-learning platform of the course.

Recommended textbooks:
- T.A. Brown “Biotecnologie molecolari” Zanichelli
- M. Maccarrone “Metodologie biochimiche e biomolecolari” Zanichelli
- R.J. Reece “Analisi dei geni e genomi” EdiSES
- J.W. Dale and M. von Schantz “Dai geni ai genomi” EdiSES

Semester

First semester

Assessment method

Written examination (2 h).
The examination presents open questions on topics spanning the course program. Mainly, the skills of “interconnection” among the different topics will be assessed.

Office hours

Contact: on demand, upon request by mail to lecturer.