Universitat Autònoma de Barcelona

2017/2018

Recombinant DNA Technology

Code: 100856
ECTS Credits: 3

Degree	Type	Year	Semester
2500252 Biochemistry	OB	3	1

Contact

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Use of languages

Principal working language: spanish (spa)
Some groups entirely in English: No
Some groups entirely in Catalan: No
Some groups entirely in Spanish: Yes

Prerequisites

There are no official prerequisites. However, it is assumed that the student has acquired the basic knowledge of Molecular Biology explained in previous subjects of the degree of Biochemistry.

Objectives and Contextualisation

Recombinant DNA technology includes diferents methodologies developed from 1970-1980. These methodologies are now a basic tool in many biochemistry laboratories and have allowed in recent years a very important advance in the knowledge of the structure and function of biomolecules. In this subject the fundamentals of this technology will be presented. The general objective of the subject is to provide the knowledge that allows the student working with these methodologies during his professional future.

Specific objectives of the course:

- Know and apply the basic techniques of recombinant DNA: nucleic acid labeling, Southern and Northern blots, in situ hybridization, arrays, sequencing, restriction enzyme use, PCR reaction, CRISPR based technology.
- Describe the main cloning vectors in Escherichia coli, know their characteristics and know how to apply them in the different strategies for the cloning of DNA fragments.
- Understand strategies for the construction of libraries and their use for the study of genes and genomes.
- know the methodology for the expression of recombinant proteins and for the directed mutagenesis.

Content

Unit 1.- Techniques in recombinant DNA technology. Basic Tools of Recombinant DNA: restriction enzymes, polymerases, exonuclases, ligases, reverse transcriptase. cDNA synthesis. DNA sequencing (Sanger). Technologies based on the CRISPR system.

Unit 2. Hybridization techniques. Tm concept. DNA and RNA Labelling. Southern, Northern blot and their applications. Dot-Blot. in situ hybridization. Fish. Microarrays.

Unit 3: Polymer chain reaction (PCR) Introduction. Design and optimization of the reaction. RT-PCR. Quantitative PCR (real-time PCR). Applications.

Unit 4.-Molecular Cloning. Blunt and cohesive extreme. Adaptors and Linkers. DNA Ligation. Bacterial transformation. Detection of recombinant clones. Characteristics of the cloning vectors: plasmids and bacteriophages. Some examples of plasmid. Specific vectors by alternative cloning systems: recombination integration systems, topoisomerase-based cloning systems.

Unit 5: cDNA libraries. Synthesis of cDNA. Strategies for the construction of cDNA libraries. Abundance and complexity. Vectors for cDNA libraries (insertion lambda). Screening cDNA Libraries. RT-PCR with alternative to cDNA banks. Other methods for the detection and study of mRNAs: "Expressed sequence tags (ESTs)" and / or RNAseq.

Unit 6: Genomic DNA libraries vs Genome projects (high-throughput approaches to DNA sequencing) .Strategies for obtaining genomic DNA libraries. Vectors used in genomic DNA libraries: replacement lambda, cosmids, BACS and YACS. Screening of genomic DNA libraries. "Walking" and obtaining probes (inverse PCR). High-throughput DNA sequencing technology (NGS).

Unit 7: Expression of recombinant proteins in E. coli. Kind of vectors used. Optimization of recombinant protein expression. "Phage display". Fusion proteins. In vitro translation systems. Site-directed mutagenesis.

Unit 8: Cloning in Yeast (S. cerevisae). Transformation. Typology of vectors. Expressions of recombinant proteins in yeast. "Double-hybrid" method for detecting protein-protein interactions.