Universitat Autònoma de Barcelona

2018/2019

Recombinant DNA Technology

Code: 100856
ECTS Credits: 3

Degree	Type	Year	Semester
2500252 Biochemistry	OB	3	1

Contact

Name: Inmaculada Ponte Marull

Email: Inma.Ponte@uab.cat

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: Libraries of cDNA versus RT-PCR/RNA-seq. Strategies for the construction of libraries, concept of abundance and complexity of mRNA. Synthesis of cDNA. Main vectors used in the construction of cDNA libraries. Screening of cDNA libraries. RT-PCR / RNA-seq as an alternative to cDNA libraries.

Unit 6: Libraries for genomic sequencing. Construction and screening of genomic llibraries versus high throughput genomic sequence. Concept of Representativeness. Strategies for obtaining libraries for genomic sequencimg. Vectors used in genomic llibraries: Lambda, Cosmids, BACS. Screening of genomic DNA libraries. " Genomic Walking" and/or obtention of probe (reverse PCR). High throughput sequencing technologies.

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 proteinsin yeast. "Two-hybrid" method for detecting protein-protein interactions.