Universitat Autònoma de Barcelona

2017/2018

Recombinant DNA Technology

Code: 100934
ECTS Credits: 3

Degree	Type	Year	Semester
2500253 Biotechnology	OB	2	2

Contact

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Use of languages

Principal working language: catalan (cat)
Some groups entirely in English: No
Some groups entirely in Catalan: Yes
Some groups entirely in Spanish: No

Prerequisites

No special requirements are requested.

Objectives and Contextualisation

This topic is to introduce the student to the wide range of methodologies that are commonly known as Recombinant DNA Technology. These methodologies, which were developed at the end of the last century, are one of the pillars of modern biotechnology. The general objective is to provide a solid basis allowing the student to apply these methodologies when designing biotechnological processes. In addition, it is also provided the scientific background to follow-up more specialized topics in the last courses of the degree of Biotechnology. The practical aspects of this topic are dealt in the Integrated Laboratories 4 and 5.

Objectives

• Know and apply the basic techniques of recombinant DNA: probes, Southern and Northern blots, hybridization, arrays, sequencing, use of restriction enzymes and PCR reaction.

• 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 the strategies for the construction of gene and genome libraries and their use for the study of genes and genomes.

• Know the basics and main applications of the new technologies for massive sequencing of nucleic acids (next generation sequencing).

• Know the methodology for the expression of recombinant proteins and site directed mutagenesis.

Content

Lesson 1: Basic techniques in recombinant DNA technology. What can we do with the recombinant DNA technology? Main enzymes used in recombinant DNA technology. DNA restriction. Adapters and linkers. Sanger's method for DNA sequencing. DNA denaturation and molecular hybridization. The PCR reaction. Southern Blot, Northern Blot and its applications.

Lesson 2: Cloning in Escherichia coli. Plasmids and phages as cloning vectors in E. coli. Main transformation methods. Phagemids and main host strains. Integration by recombination. Cloning of PCR products.

Lesson 3: Construction and screening of cDNA libraries. cDNA synthesis. Strategies to create representative cDNA libraries. Main vectors used to build cDNA libraries. In vivo excision system in lambda phages. Screening cDNA libraries. RT-PCR as an alternative to cDNA banks. Rapid amplification of cDNA ends. DNA subtraction libraries. Expressed Sequence Tags (ESTs). Arrays, types and quantification.

Lesson 4: Genomic DNA libraries. General concept. Rules to obtain representative libraries. Strategies to build genomic DNA libraries. Cosmid and fosmids, BACS, PACS and YACS. Screening of genomic libraries. Walking and arrangement of contigs. Next generation sequencing technologies (NGS). Main applications of NGS.

Lesson 5: In vitro mutagenesis. Concept and uses. Silent mutations. Site directed mutagenesis (SDM). Metods for SDM (cassette mutation, primer extension and PCR). Random mutagenesis. Molecular directed evolution (DNA shuffling and related technologies).

Lesson 6: Expression of recombinant proteins. Factors affecting the expression of cloned genes in E. coli. Main expression vectors. Optimization of recombinant protein expression. Synthetic genes. Fusion proteins. Phage display. In vitro translation systems.

Lesson 7: Cloning in yeasts. Cloning in Saccharomyces cerevisiae (transformation, main tors and recombinant protein expression). Two-hybrid method to detect protein-protein interaction. Other yeasts of biotechnological interest.