Universitat Autònoma de Barcelona

2017/2018

Integrated Laboratory Class 5

Code: 100924
ECTS Credits: 3

Degree	Type	Year	Semester
2500253 Biotechnology	OB	3	1

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

Teachers

Pau Ferrer Alegre

Prerequisites

Students should be taken simultaneously or have already completed the theorical subjects related to the contents of this lab: Recombinant DNA Technology, Chemistry and Protein Engineering, Process Engineering Fundamentals, Bioreactors, and Separation and purification processes.

To attend the sessions, the student must have passed the Biosafety and Security tests found in Campus Virtual. In addition, the student should be aware and accept the functioning guidelines of the laboratories of the Faculty of Biosciences.

Objectives and Contextualisation

This practical course integrates key concepts and techniques from different fields of biotechnology. In particular, this practical course combines topics from the recombinant DNA technology, protein chemistry and bioengineering (including the growth of microorganisms in bioreactors, and recovery and purification of proteins) in the designing and development of a biotechnological process. In this way, the overall objective of this course is to obtain an overview of three of the most important parts of biotechnological processes and has the following specific objectives:

• Learn the different stages which comprise the extraction of total RNA from eukaryotic tissues, as well as the key points of the reaction of RT-PCR to amplify the specific sequences of cDNA including the basic criteria used in the designing oligonucleotides for RT-PCR.

• Know the main features of the expression vectors used for the production of recombinant proteins.

• Know the different steps of standard molecular cloning process consisting in: digestion with restriction enzymes, ligation reaction and transformation in a bacterial host, as well as the criteria used for the recognition of clones bearing vector and foreign DNA.
• Know the key parameters for heterologous expression of proteins in bacteria, as well as to design experiments of recombinant expression according to the characteristics of the protein to be expressed.

• Design and apply protocols for the purification of recombinant proteins.
• Characterize expressed proteins both at structural and functional levels
• Learn the basic sequence of operations, their distribution over time, and the key points associated with the growth of microorganisms in bioreactors and the purification of recombinant proteins at the laboratory scale.
• See the interactions between the different stages of a process of production of recombinant proteins, the characteristics of the protein to be produced, theexpression system used in the fermentation process, and the recovery and purification of the recombinant protein .
• Characterize at the laboratory scale the key parameters of the production process -growth in bioreactor, recovery and purification- of the recombinant protein of interest.

• Integrate all the results obtained, evaluate the production system of the recombinant protein globally and discuss and design strategies for optimization.

Content

This practical course is carried out entirely in the laboratory and includes fourteen sessions:

- **Session 1: Introduction.** Review of the biotechnological methods used in this course.General characteristics the of cloning vectors for the expression of recombinant proteins. Using databases to fetch the coding sequence of the human dihydrofolate reductase (hDHFR) cDNA. Designing the primers to amplify the hDHFR cDNA by RT-PCR.

- **Session 2: Obtaining the coding sequence of DHFR gene:** Purification of total RNA from a small sample of human blood. Reverse transcription using polyA as primer. PCR amplification of the cDNA from hDHFR gene.

- **Session 3: Cloning de DHFR gene into a prokaryotic expression vector (I) and recombinant DHFR purification.** Purification of PCR products. Preparing the ends of insert and cloning vector. Inactivation of restriction enzymes. Ligation reaction. Strategies to maximize the ligation of the insert to cloning vectors. Preparing E. coli cultures for production of recombinant proteins in a small scale. Induction of the expression of the recombinant hDHFR.

- **Session 4: Cloning de DHFR gene into a prokaryotic expression vector (II) and recombinant DHFR purification (I):**E. coli transformation with the ligation obtained at the end of session 3. Affinity chromatography column setup. Obtaining a cell extract and isolation of soluble and insoluble fractions. Purification by affinity chromatography of recombinant hDHFR. Interpreting the chromatographic profile obtained and identifying the samples of interest.

- **Session 5: Recombinant DHFR purification (II) and identification of the clones bearing the recombinant vector (I):** Analysis of the affinity purified hDHFR samples by SDS-PAGE. Enzyme activity characterization. Purification table. Colony selection and preparation of small scale E. coli cultures.

- **Session 6: Characterization of recombinant DHFR (I) and identification of the clones bearing the recombinant vector (II)**: Confirming the identity of hDHFR by mass spectrometry fingerprint (i). Determining the molar extinction coefficient (molar absorptivity) of hDHFR. Isolation of plasmid DNA from E. coli cultures. Digestion plasmid DNA with restriction enzymes.

- **Session 7**: **Characterization of recombinant DHFR (II) and identification of the clones bearing the recombinant vector (III)**: Confirming the identity of hDHFR by mass spectrometry fingerprint (ii). Molecular mass of the hDHFR by MALDI-TOFF. General discussion of the results obtained in the hDHFR purification and characterization experiments. Electrophoresis of the DNA samples and identification of the positive clones. General discussion of the results obtained in the cloning procedure.

- **Session 8: Designing a process to produce recombinant proteins:** Bioreactor preparation and operation. Preparation of starter cultures. Experimental design proposal as a tool to improve and redesign the production process.

- **Session 9: Bioreactor culture (I):** Bioreactor inoculation and checking the culture parameters (temperature, pH, pO_2 , cell mass, substrates subproducts, plasmid stability and hDHFR product) when operating in batch. Calculation of growing velocity, substrate utilization, subproduct production, yields and productivity. Inducing recombinant hDHFR expression.

- **Session 10: Bioreactor culture (II) and the DHFR recovery (I):** Bioreactor stop and cell mass recovery using centrifugation. Cleaning bioreactors.

- **Session 11: DHFR recovery (II):** Selection of a recovery system: implications and consequences of the selection steps when recovering and purifying the recombinant protein. Cell disruption by enzymatic and mechanical procedures. Recovery of the soluble fraction by centrifugation and microfiltering.

- **Session 12: DHFR purification (I): Preparative p**urification of recombinant hDHFR from the cell mass by affinity chromatography. Recovery of the different chromatographic fractions.

- **Session 13: DHFR purification (II):** Analysis of the chromatographic purification (total protein by Bradford, purification level by SDS-PAG and enzyme activity of hDHFR purity and activity). Consistency of the results. Performance, puirification tables and purification factor.

- **Session 14: Global analysis of the results.** Evaluation of experimental data. Obtaining information to improve and redesign the production process