Universitat de Lleida

DEGREE CURRICULUM

LABORATORY METHODS IN PLANT BIOTECHNOLOGY

Coordination: BASSIE , LUDOVIC

Academic year 2022-23

LABORATORY METHODS IN PLANT BIOTECHNOLOGY 2022-23

Subject's general information

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<tr><td>Code</td><td>101629</td></tr>
<tr><td>Semester</td><td>2nd Q(SEMESTER) CONTINUED EVALUATION</td></tr>
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<tr><th>Degree</th><th>Course</th><th>Character</th><th>Modality</th></tr>
<tr><td>Bachelor's Degree in Biotechnology</td><td>4</td><td>OPTIONAL</td><td>Attendance-based</td></tr>
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<tr><td>Course number of credits (ECTS)</td><td>6</td></tr>
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<tr><th>Activity type</th><th>PRALAB</th><th>TEORIA</th></tr>
<tr><th>Number of credits</th><td>5</td><td>1</td></tr>
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<tr><td>Coordination</td><td>BASSIE , LUDOVIC</td></tr>
<tr><td>Department</td><td>CROP AND FORESTRY SCIENCES</td></tr>
<tr><td>Teaching load distribution between lectures and independent student work</td><td>60 hores presencials
90 hores no presencials</td></tr>
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<tr><td>Language</td><td>Català 90%
Anglès 10%</td></tr>
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LABORATORY METHODS IN PLANT BIOTECHNOLOGY 2022-23

Teaching staff	E-mail addresses	Credits taught by teacher	Office and hour of attention
BASSIE , LUDOVIC	ludovic.bassie@udl.cat	6	

Subject's extra information

The subject of **Lab Techniques in Plant Biotechnology (TLBP)** is an optional subject that is taught during the first semester of the fourth year of the Biotechnology Degree. The teaching load is 6 credits that are distributed between theoretical and practical classes with 1 and 5 credits respectively. The subject is structured in 6 theoretical sessions of 50 minutes, 2 sessions for the preparation of the mini-project (4 hours) and 50 hours of laboratory practices.

The TLBP subject is complementary to the subject of Plant Biotechnology (BTV). The tools used in plant biotechnology and the different transformation systems including the methods of integration of a transgene have already been explained in fully details in the subject of BTV.

To begin, we will see the methodology and strategy involved in the process of gene cloning and in subcloning stages. These techniques belong to the first crucial phase of plant genetic engineering.

The second group of techniques is devoted to methods for the analysis transgene expression, a fundamental post-transformation procedure to verify whether the gene of interest introduced into the host plant's genome is functional.

Recommendation: to have passed the subject Biotecnologia Vegetal (BTV)-101621

Learning objectives

Objectives of knowledge:

The student that exceeds the subject must:

- have knowledge of elemental notions in the basic operation of a laboratory of biotechnology

- have complementary knowledge in genetic engineering strategies and adapted to the plant system

- have advanced knowledge in some relevant techniques of gene expression analysis

Objectives of capacity:

The student who exceeds the subject must be able:

- to know how to organize himself in the planning of the applied methodology

- to use efficient and reliable techniques for the analysis of gene expression

- to show a strong capacity of integration in a research team

Competences

GC1 Being able to selectively search for and use sources of information necessary to achieve the training objectives.

GC3 Working in a team, with a multidisciplinary vision and with the ability to make a rational and efficient distribution of tasks among team members.

GC4 Knowing and adequately using the scientific and technical vocabulary of the different areas of Biotechnology.

GC5 Working in the laboratory applying criteria of quality and good practice.

GC6 Knowing and knowing how to use the specific software and databases in the different fields of biotechnology.

GC7 Using the scientific method to analyze data and design experimental strategies with biotechnological applications.

Subject contents

The theoretical classes are based on the practical and strategic aspect in order to give the user the possibility to quickly evolve in the learning of the applied techniques and to provide him clear advices and explanations of procedure so that he can apply these methodologies in efficient way.

The program is structured in 3 parts. In the first part, chapter-I deals with the 'elemental notions' that must be applied and followed at the laboratory. The subject is addressed in a reminder but with insistence on the essential and delicate points. In chapter II, we explain the strategies involved at the different stages for isolating genes from both plant and bacterial origin. We see the stratagems and procedural tricks that can be used to initiate a gene cloning experiment and carry it out successfully. In the third part we describe in details the methods of analysis to verify the expression of genes at transcriptional and translational level. Although they are classical methodologies, they are used systematically and are considered to provide very reliable results. They are techniques that must be applied to confirm results obtained by transcriptomic and proteomic approaches.

Chapter I. Elemental notions

Topic 1. Generalities

Main safety measures in the laboratory: chemicals, ultraviolet light, general housekeeping- protective equipment, important safety messages, and emergency responses. Preparation and ownership of the common buffers and stock solutions. Sterilization by filtration and by autoclave. Maintenance of the laboratory notebook. The importance of following a protocol.

Topic 2. Use of basic equipment

Use of Equipment: General comments- Micropipettors- pH Meter- Autoclave operating procedures. Preparation of medium for E.coli bacterial culture. Work in sterile conditions. Working with E. coli: small scale cultures- Permanent storage.

Chapter II. PCR cloning and sub-cloning

Topic 3. Gene cloning

-Choice of nucleic acid type for amplification: cDNA or genomic DNA.

-Primer design for the coding sequence of interest by using gene sequence databases.

- cDNA synthesis reaction.

- Amplification of the sequence of interest via PCR.

- Separation, isolation and purification methods of the PCR product(s).

- Ligation in a cloning vector via TA-cloning system: pGEM-like.

- The Hanahan method for preparation of competent E. coli for high-efficiency transformation.

- Competent bacterial cell transformation with the ligation products. Cultivation in selection medium.

- Screening methods for positive bacterial clones.

- Isolation of the recombinant plasmid.

- Sequence analysis of the insert by Sanger sequencing.

Topic 4. Molecular sub-cloning

- Choosing an adequate expression vector that contains the appropriate promoter.

- Choosing compatible restrictions enzymes for the insertion of the coding sequence of the gene (insert) in the vector.

- Isolation of the digested fragments (if required): separation by electrophoresis and purification by column.

- Ligation reaction between the insert and the vector.

- Advantages of Anza restriction enzyme cloning system.

- Classical method of bacterial transformation / plasmid purification.

Chapter III. Approaches for (trans)gene expression analysis

Topic 5. Histochemical analysis

GUS staining assay: expression analysis of the reporter β-glucuronidase in non-differentiated and differentiated tissues.

Topic 6. RNA blot analysis

- Reminder of working conditions with RNA

- Methods for isolating total RNA: Trizol-like, silica-membrane based kits; LiCl precipitation.

- Checking RNA integrity.

- NaAc precipitation for concentrating RNA.

- Preparation of material for electrophoresis.

- Preparation of formaldehyde agarose gel.

- Preparation of RNA samples: denaturing buffer and thermal denaturation step.

- Electrophoretic separation and quality control.

- Wash of low salt strength followed by transfer to a solid support.

- Fixing RNA by UV-crosslinking.

- DIG-labeled probe synthesis.

- Pre-hybridization and probe hybridization steps

- Sequential washes of variable stringency.

- DIG-labelled probe detection.

Topic 7. Western blotting

- Process of protein extraction.

- Bradford assay for protein quantification.

- Preparation of the solutions required for the gel and the transfer.

- PAGE-SDS electrophoresis: acrylamide gel preparation for denaturing condition (Laemmli method).

- Coomassie blue staining and destaining.

- Semi-dry transfer to PVDF membrane followed by blocking step.

- Types and properties of antibodies used for immunodetection.

- Incubation steps with primary and secondary antibodies.

- Detection of alkaline phosphatase activity (AP-conjugated secondary antibodies)/ detection of luminescence (HRP-conjugated antibody antibodies).

Laboratory practices

Several technical procedures are organized.

The first one is devoted to protein analysis using the western blot method. Rice samples that overexpress the *gus* reporter gene controlled by the constitutive 35S CAMV promoter will be analyzed. All the stages of the methodology are carried out, from protein extraction from leaf tissue, through the preparation of the material to the final step by detecting the β-glucuronidase protein (GUS). In addition, as a complementary study, a Southern blot analysis is performed to identify the transgenic plant lines.

The second activity is dedicated to some key steps of gene cloning and sub-cloning procedures. High competent DH5α E. Coli s cells are prepared according to the method of Hanahan. Efficiency is evaluated by using different quantities of a relevant plasmid and positive clones are screened by PCR after cell lysis.

The third activity is dedicated to RNA work. Student learns how to handle RNA samples by working in special conditions to preserve RNA integrity. Total RNA will be extracted from corn leaf tissue and the quality of the samples will be checked by electrophoresis.

It should be mentioned that the procedures of the different activities are done in a discontinuous manner; throughout the day steps of different protocols are performed in parallel, therefore optimizing time use. Time management is an important skill that the student must develop.

Day1: Protein isolation

- Harvesting rice leaf samples, weighting and preservation in liquid nitrogen.

- Preparation of some buffers that are required for the two weeks of lab practices.

- Protein extraction procedure.

- Genomic DNA digestion from transgenic lines (*gus* transgene)

Day2: Western blot-I

- Quantitative analysis of protein with the Bradford assay.

- Preparation of acrylamide gels (SDS-PAGE)

- Preparation of solid and liquid LB culture medium, with and without selection agent for E. coli DH5α culture.

Day3: Western blot-II

- Sample preparation for electrophoresis + gel running

- Assembly of the material (Mini-PROTEAN, Biorad)

- Blotting procedure + blocking step

- Plating-out DH5α cells on non-selective LB medium.

- Preparation of transgenic seeds for germination

Day 4: Western blot-III + Southern blot

- Washing steps

- Incubations with primary and secondary antibodies

- Colorimetric detection with alkaline phosphatase activity

- Interpretations of the results

- Mini culture of DH5α cells in LB liquid media

- Southern blot: preparation of digested gDNA for electrophoresis and gel running.

Day 5: PAGE-SDS+ Southern blotting

-Preparation of PAGE-SDS

-Preparation of TfbI and TbfII solutions (for competent cells preparation)

-Southern blot: ethidium bromide gel staining; denaturation; neutralization and transfer set up

Day 6: Isolation of RNA-I /Preparation of competent cells

-Harvesting samples from rice leaf tissue; weighting and liquid nitrogen storage

-RNA extraction using LiCl precipitation

-Preparation of competent DH5α cells (Hanahan protocol).

Day 7: Isolation of RNA-II / Transformation of competent cells

- Continuation of the RNA isolation.

- Transformation of the competent DH5α cells with two different plasmid conditions.

-Southern blot: pre-hybridization and probe hybridization

Day 8: Southern blot detection / PAGE SDS

-Colony subcultures on selection medium (ampicillin)

-PAGE-SDS running (same conditions than day-3)

-Coomassie blue staining and destaining

-Southern blot: washing steps and procedure for probe detection

Day 9: Colony PCR screening + Qualitative RNA analysis

-Cell lysis in TE buffer

-PCR: calculations and preparation of the mastermix for the detection of transformed bacterial clones with the plasmid of interest.

-Electrophoresis gel to check RNA integrity

Day 10: PCR result

-Preparation of agarose gels + electrophoresis to check gel to check PCR products

-Revision of all the results obtained during the different sessions.

-Questions and doubts

Methodology

Tipus d'activitat	Descripció	Activitat presencial alumne		Activitat no presencial alumne		Avaluació	Temps total
		Objectius	**Hores**	**Treball alumne**	**Hores**	**Hores**	**Hores**
Lliçó magistral	Classe magistral (Aula. Grup gran)	Explicació dels principals conceptes	**6**	Estudi: Conèixer, comprendre i sintetitzar coneixements			
Problemes i casos	Classe participativa (Aula. Grup gran)	Resolució de problemes i casos		Aprendre a resoldre problemes i casos			
Seminari	Classe participativa (Grup mitjà)	Realització d'activitats de discussió o aplicació		Resoldre problemes i casos. Discutir			

Laboratori	Pràctica de Laboratori (Grup mitjà)	Execució de la pràctica: comprendre fenòmens, mesurar...	**50**	Estudiar i realitzar Examen			
Aula d' nformàtica	Pràctica d'aula d'informàtica (Grup mitjà)	Execució de la pràctica: comprendre fenòmens, mesurar...		Estudiar i Realitzar memòria			
Pràctiques de camp	Pràctica de camp (Grup mitjà)	Execució de la pràctica: comprendre fenòmens, mesurar...		Estudiar i Realitzar memòria			
Visites	Visita a explotacions o industries	Realització de la visita		Estudiar i Realitzar memòria			
Activitats dirigides	Treball de l'alumne (individual o grup)	Orientar a l'alumne en el treball (en horari de tutories)	**4**	Realitzar un treball bibliogràfic, pràctic, etc.			
Altres							
Totals			**60**				

Development plan

See 'Contents' section

Evaluation

-Laboratory report [60%]:

The assessment of the report is based on the following points:

-General organization and coherence

-Details of the calculations and volumes used

-Explanation of issues

-Explanation of delicates steps

-Description and interpretation of the results obtained

-Participation, motivation and interaction among students

-Mini project of metabolic pathways engineering in plants [40%]:

Project: find out an original idea, in the framework of plant biotechnology, for an application dedicated to agriculture or industry.

The objective is to describe the key points that are necessary for the generation of transgenic plants that express proteins of interest (enzymes and / or others) involved in the engineering of a specific metabolic pathway (endogenous or added).

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Describe the following points with examples and technical information supported by the associated references.

1- Objective: Explain the purpose of your idea: selected plan specie; study of the metabolic pathway (key enzymes)

2. Molecular construction/s: sequence analysis of the necessary gene(s) of interest; cloning strategy of the genes/s of interest; sub-cloning in appropriate vector(s) of expression.

3. The transformation method.

4. The key points for the regeneration of the transformed plants.

5. Expression analysis (RNA /protein): In order to characterize the transgenic plants, describe the most relevant method for transgene and/or endogenous gene expression.

6-Bibliography

Bibliography

-Molecular Cloning- A Laboratory Manual. Vol 1,2,3. (Third Edition). 2001. J. Sambrook, DW. Russell. Cold Spring Harbor Laboratory Press, New York.

-Molecular Plant Biology Volume1, A practical approach. 2002. Philip M. Gilmartin and Chris

Bowler. Oxford University Press.

-www.protocol-online.org (protocols i fòrums)