ANIMAL BIOTECHNOLOGY

1. Introduction to Animal Biotechnology

CHAPTER OUTLINE

1.1 Production of pharmaceutical proteins with biotechnological methods
1.2 Transgenic technologies in animal organisms
1.3 The laboratory mouse as a model organism
1.4 Biology, Genetics and Genome of the laboratory mouse
1.5 Oogenesis, spermatogenesis and fertilization in mammals
1.6 Critical embryo stages in Animal Biotechnology

LEARNING OBJECTIVES

On completion of the course the student will:

- Have been trained in biotechnological approaches for the production of pharmaceutical proteins, and in modern techniques used for the production of transgenic animals.
- Understand the usefulness of the laboratory mouse in Biomedical Research.
- Distinguish the embryonic stages used in Animal Biotechnology.

2. Technologies for the generation of transgenic animals

CHAPTER OUTLINE

2.1 Functional Genomics Technologies: Forward and Reverse Genetics
2.2 Creation of transgenic animals through the technique of DNA microinjection into the pronuclei of fertilized egg
2.3 Creating transgenic animals with lentiviruses
2.4 Disadvantages of lentiviruses
2.5 Transgenic animal applications
2.6 Applications of transgenic animals in agricultural animals for the production of pharmaceutical proteins
2.7 Applications of transgenic animals as models of human diseases

LEARNING OBJECTIVES

On completion of the course the student will:

- Have acquired knowledge of the genetic engineering technologies used to create genetically modified animals and will have understood the differences between the approaches of Reverse and Forward Genetics.
- Have learned in detail about the process of creating transgenic animals by the method of microinjection of DNA in pronuclei of fertilized eggs but also with lentiviruses and will be able to choose the appropriate method depending on the size of the transgene and the organism.
- Have acquired knowledge about the applications of transgenic animals, such as in the production of medicinal proteins from livestock, in disease resistance and the use of transgenic mice as models of human diseases.

3. Gene targeting technology

CHAPTER OUTLINE

2.1 Embryonic stem cells
2.2 Design of gene construct

2.3 Positive and negative selection markers for the selection of embryonic stem cell clones
2.4 Gene targeting stages for the generation of knockout mice
2.5 Gene targeting applications

LEARNING OBJECTIVES

On completion of the course the student will:

- Have acquired knowledge about the use of embryonic stem cells in gene targeting through the introduction of gene construct and selection of clones in which homologous recombination has taken place.
- Have understood the stages of gene targeting to create knockout mice and its applications.

4. Genome modifications of animals with zinc-finger nucleases (ZFNs)

CHAPTER OUTLINE

4.1 ZFNs features and mechanism of action.
4.2 Target Recognition by ZFNs
4.3 Design and construction of ZFNs
4.4 Mechanisms of genomic modifications with ZFNs
4.5 Use in animal organisms and applications

LEARNING OBJECTIVES

On completion of the course the student will:

- Have acquired knowledge of ZFNs design, recognition and cutting of a target sequence while being able to understand its advantages over gene targeting.
- Understand the usefulness of ZFNs in genome modifications of animal organisms either to create knockout animals, or to introduce targeted mutations or to replace a mutated allele with a normal one.

5. Genome modifications of animals with the system CRISPR/CAS9

CHAPTER OUTLINE

5.1 The CRISPR / CAS gene locus in the streptococcus
5.2 Description of the CRISPR / CAS9 system
5.3 Biotechnological use of the CRISPR / CAS9 system in animal organisms
5.4 Strategies to minimize off-target modifications
5.5 Comparison with ZNFs and TALENs nucleases

LEARNING OBJECTIVES

On completion of the course the student will:

- Have understood the organization of the CRISPR / CAS gene loci in streptococcus but also the mode of action of the CRISPR / CAS9 system for the genome modification of animal organisms.
- Have understood why the CRISPR / CAS9 system is superior as a genetic tool for modifying animal organisms compared to other techniques such as gene targeting, ZNFs and TALENs nucleases.
- Have acquired knowledge of CRISPR / CAS9 system applications in the genome modifications of animal organisms either to create knockout animals, or to introduce targeted mutations, or to replace a mutated allele with a physiological. He will also be able

to understand the problems arising from the off-target modifications of the CRISPR / CAS9 system and will be informed about the optimization of the system.

6. Spatial and temporal control of inducible gene expression and modifications in animals

CHAPTER OUTLINE

6.1 Recombination with the Cre / loxP system
6.2 Applications of the Cre / loxP system to animal organisms
6.3 Tissue-specific gene activation
6.4 Tissue-specific gene inactivation
6.5 Inducible temporal gene modifications at the transcriptional level with the tetracycline system
6.6 Inducible temporal gene modifications at the post-transcriptional level

LEARNING OBJECTIVES

On completion of the course the student will:

- Have learned the mechanism of Cre / loxP recombination, how to design a gene construct, and how Cre / loxP is used to activate or inactivate genes in mammalian cells in combination with gene targeting.
- Be able to distinguish and select genetic tools for the application of gene modifications in mammals either in specific cell types or at specific time intervals.

7. Forward Genetics: from the phenotype to the identification of the causal gene

CHAPTER OUTLINE

7.1 Chemical mutagenesis with N-ethyl-N-nitrosourea
7.2 Phenotypic analysis
7.3 Detection of the mutated gene by mapping
7.4 Stages of mapping
7.5 Polymorphic genetic markers
7.6 Examples

LEARNING OBJECTIVES

On completion of the course the student will:

- Have understood the usefulness of Forward Genetics in the identification of genes involved in the pathogenesis of genetic diseases.
- Have acquired knowledge about the application of random mutagenesis with N-ethyl-N-nitrosourea in mice to produce new phenotypes.
- Have understood how from the phenotype with mapping and the use of polymorphic indicators we can identify the mutated gene.

8. Animal cloning

CHAPTER OUTLINE

8.1 Animal cloning methods
8.2 Nuclear transfer
8.3 Cloning stages

8.4 Re-programming in cloning
8.4 Applications of animal cloning in agriculture and biotechnology
8.5 Bioethical issues

LEARNING OBJECTIVES

On completion of the course the student will:

- Have understood mammalian cloning methods with an emphasis on nuclear transfer.
- Have acquired knowledge of how the genome is reprogrammed during cloning and what are the applications of cloning for reproductive and therapeutic purposes.
- Become familiar with bioethics issues.

9. Stem cells

CHAPTER OUTLINE

9.1 Types of mammalian stem cells and their topology
9.2 Embryonic stem cells
9.3 Adult stem cells
9.4 Induced pluripotent stem cells
9.5 Stem cell applications

LEARNING OBJECTIVES

On completion of the course the student will:

- Have acquired knowledge about the categories and the topology of stem cells in mammals.
- Have understood the differences between embryonic stem cells, adult stem cells and induced pluripotent stem cells.
- Have understood the importance of using stem cells in the treatment of human diseases and will be updated with new technologies that allow the dedifferentiation of somatic cells into stem cells.

10. Gene therapy

CHAPTER OUTLINE

10.1 History of gene therapy
10.2 Disease targets
10.3 Prerequisites and tools in gene therapy
10.4 *Ex vivo, in vivo* gene therapy applications
10.5 Vectors in gene therapy
10.6 Stages of gene therapy
10.7 Problems in gene therapy
10.8 Latest achievements

LEARNING OBJECTIVES

On completion of the course the student will:

- Have understood the stages of gene therapy, the disease targets, and tools that are used, with an emphasis on cells and viral vectors.
- Have realized the problems that delay the application of gene therapy and is updated with the latest achievements in the field.

11. Monoclonal antibodies - Vaccines

CHAPTER OUTLINE

11.1 Review of the immune system
11.2 Monoclonal, polyclonal antibodies
11.3 Techniques for the production of monoclonal antibodies
11.4 Applications of monoclonal antibodies in the diagnosis and treatment of human diseases
11.5 Types of vaccines
11.6 Vaccination mechanisms
11.7 Examples, HPV vaccines

LEARNING OBJECTIVES

On completion of the course the student will:

- Be familiar with technologies for the production of monoclonal antibodies but also with their wide application in the diagnosis and treatment of human diseases.
- Distinguish vaccine types with emphasis in vaccines produced with biotechnological methods.
- Have understood the mechanisms of action of vaccines, the importance of vaccination to protect the health of the population and will be informed about vaccines against HPV as means of preventing cervical cancer.

12. Laboratory

OUTLINE

- Generation of transgenic mice
- Design of transgene construct
- Isolation of genomic DNA from mouse tissue
- Genotyping of transgenic mice
- Animal cell culture
- Overproduction of proteins in mammalian cell lines
- Cryopreservation
- In vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI)
- Design of gene inactivation with the CRISPR / CAS9 system

LEARNING OBJECTIVES

On completion of the course the student is able:

Generation of transgenic mice: to know how to prepare DNA for microinjection, to select the mouse strains required for the procedure, to ovulate and isolate fertilized eggs, to know the stages of microinjection DNA in the nuclei of fertilized eggs and the storage conditions of the zygotes, to know how the fertilized eggs are implanted in pseudo-pregnant females.

Design of transgene construct: to know the elements needed in a transgenic construct, to use the genome compiler program to design a recombinant plasmid by creating recognition sites of restriction enzymes at the ends of cDNA with primers and PCR, to subclone the PCR products in a plasmid vector, to find the sizes of the plasmids, to select the appropriate restriction enzymes for the subcloning, the confirmation of the recombinant plasmids and the extraction of the transgene from the recombinant plasmid. Be able to adequately present the results and conclusions of the experimental process.

Isolation of genomic DNA from mouse tissue: to know which substances are needed for tissue lysis, to develop experimental skills for isolating genomic DNA, to determine the purity of DNA and to calculate the concentration of DNA samples. Be able to evaluate the experimental results and adequately present the results and conclusions of the experimental process in collaboration with other students.

Genotyping of transgenic mice: to genotype transgenic mice with PCR, to select the appropriate positive and negative controls, to know the conditions of genotyping and how the process is optimized. To develop experimental skills for creating PCR reactions, using the PCR thermocycler, loading PCR products on agarose gel, setting up electrophoresis and UV gel photography. Be able to evaluate the experimental results and adequately present the results and conclusions of the experimental process in collaboration with other students.

Animal cell culture: to distinguish the types of cell cultures, the conditions of mammalian cell growth, but also problems that arise in the process of cell culture. Is trained in the principles of cell culture and related equipment. To develop experimental skills in measuring dead and alive cells with a hematocytometer to calculate alive cells in a suspension. Be able to evaluate the experimental results and adequately present the results and conclusions of the experimental process in collaboration with other students.

Protein overexpression in mammalian cell lines: to know the vectors of protein expression in mammalian cells, the elements required in the plasmid vector, and the applications of the process. Be familiar with the cell line transfection techniques, and procedures for achieving transient or stable expression. Use the genome compiler program to design a recombinant plasmid to overexpress a chimeric protein with EGFP in mammalian cell lines. Be able to evaluate the experimental results and adequately present the results and conclusions of the experimental process in collaboration with other students.

Cryopreservation: to know the principles and applications of cryopreservation. Recognize the anatomy of mice with an emphasis on the urogenital system, isolate epididymis, monitor the process of cryopreservation of mouse sperm and evaluate sperm motility under a microscope. Be able to evaluate the experimental results and adequately present the results and conclusions of the experimental process.

In vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI): to know the stages of in vitro fertilization, and intracytoplasmic sperm injection and their applications.

Design of gene inactivation with the CRISPR / CAS9 system: to know the principles of the CRISPR / CAS9 system, to design and select sgRNAs using appropriate web tools.