GENETICS OF MODEL ORGANISMS

1. Introduction and history of Model Organisms

OUTLINE

- General characteristics and advantages of model organisms: ease of experimental handling, ease of genetic crossbreeding and analysis, similarity and applications to human biological processes.
- Understanding the phylogenetic relationships of classical model organisms and their evolutionary relationship to humans.

LEARNING OUTCOMES

After completing this chapter, you will have become familiar with the classical model organisms, the characteristics on the basis of which they were selected, and their evolutionary relationship to humans.

2. From genes to genomes

OUTLINE

- DNA sequencing techniques: Sanger sequencing and automation.
- Online genome databases: Visualization and annotation of the sequence.
- Genome sequencing strategies: hierarchical shotgun sequencing, and whole genome shotgun sequencing. Relationship of genetic maps with natural maps.
- Insights from sequencing the human genome and the genomes of model organisms: number of genes, low percentage of coding regions & high percentage of repeat sequences in the human genome, synteny, complex domain architecture of proteins.
- Genetic polymorphism: SNPs as disease biomarkers, genome-wide association studies (GWAS).

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the process of sequencing a genome, the contribution of bioinformatics to functional genomic analysis, the main features of the human genome, SNPs and GWAS studies.

3. *Escherichia coli* (*E. coli*) as a model organism

OUTLINE

- Overview of the bacteria: morphological and genetic characteristics of prokaryotes.
- Metabolic / phenotypic diversity in bacteria.
- Bacterial genetic analysis: Mutation identification techniques: mutagens, replicate plating, genetic screening and selection, transposable elements.
- Bacterial genomes: Bacterial chromosomes and plasmids. Comparative genomic analysis. Metagenomics.
- Horizontal gene transfer: transformation, conjugation, transduction.
- Pathogenicity islands.

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the genetics of bacteria, with an emphasis on *E. coli*, as well as techniques for horizontal gene transfer, selection of mutants and genetic screens.

4. The yeast *Saccharomyces cerevisiae* as a model organism

OUTLINE

- The genome of *S. cerevisiae*. Chromosomes, centromere, telomere, copy start position.
- Advantages of *S. cerevisiae* as a model organism.
- Life-cycle: alternation of diploid and haploid forms. Usefulness in genetic analysis of mutations. Tetrad analysis.
- The eukaryotic cell-cyle: Importance of checkpoints. Identification of heat-sensitive mutations in the cell cycle. Using genetic libraries and complementarity testing for gene cloning.
- Cellular differentiation in *S. cerevisiae* (mating types).

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the genetics of yeast, the use of haploid and diploid forms in the life cycle of *S. cerevisiae* in genetic analysis, and the identification of mutations in the cell cycle.

5. *Caenorhabditis elegans* as a model organism:

OUTLINE

- Characteristics and advantages of *C. elegans* as a model organism.
- The *C. elegans* genome. Chromosomes, lack of centromeres.
- Gene expression: operons, 5΄ splice leaders, trans splicing.
- Life-cycle and sex determination in *C. elegans*. Anatomy of the hermaphrodite and the male. Gametogenesis, fertilization.
- Embryogenesis and development. Genealogy of cell types from the embryo to the adult. Laser ablasion technique.
- Genetic analysis and modification techniques: Transgenic animals. Use of the GFP molecule as a reporter gene. RNA interference (RNAi).
- Mutagenesis, production of homozygotes through self-fertilization.
- Programmed cell death / apoptosis: role in development, identification of ced mutations (cell death). Applications to carcinogenesis.

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on *C. elegans* genetics, sex determination, the genealogy of cell types from the embryo to the adult, the techniques for producing transgenic animals, and the identification of mutations related to apoptosis.

6. *Drosophila melanogaster* as a model organism

OUTLINE

- Characteristics and advantages of *D. melanogaster* as a model organism.

- Genome structure and organization in *D.melagaster*. Polytene chromosomes.
- Life cycle in *Drosophila*
- Classical genetic approaches in *Drosophila*. Mutations and complementation test.
- Balancer chromosomes and its applications in *Drosophila*.
- Hybrid dysgenesis in Drosophila.
- Transposon P-elements as molecular tools in molecular genetics. Structure and function of P-elements-Applications of P-elements as means of transformation, genetic labels and trap enhancers.
- Production of genetic mosaics through mitotic recombinations induced either by X-ray based strategies or the FLP/FRT system.
- Ectopic gene expression in Drosophila.
- Gene knockout through homologous recombination or RNA interference technology.
- The genetic analysis of body plan development in *Drosophila*

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the genetics of *D.melanogaster*, the structure and organization of the genome, the life cycle and the use of balancer chromosomes, P-elements transposons, genetic mosaics, ectopic gene expression and targeted gene knockouts in genetic analysis of *Drosophila*.

7. *Mus musculus* as a model organism: advantages and applications

OUTLINE

- Characteristics and advantages of *D. melanogaster* as a model organism.
- Life cycle in *Mus musculus.*
- Genome structure and organization in *Mus musculus.*
- Gametogenesis in males and females, and fertilization.
- Genetic analysis and modification techniques: Design and production of transgenic animals. The microinjection method-Pros and Cons.
- Functional analysis in transgenic animals. Knockout mice.
- Applications of transgenic animals.

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the genetics of *Mus musculus*, the structure and organization of the genome, the life cycle, and the use of design and production of transgenic animals, the means of functional analysis and its applications in transgenic animal systems.

8. Zebrafish *Danio rerio*: advantages and applications

OUTLINE

- Characteristics and advantages of *D. rerio* as a model organism.
- Embryogenesis and development. Genealogy of cell types from fetus to adult. Applications in the study of the development of the cardiac valve.
- Creation of transgenic animals. RNA, DNA, morfolino.
- Mutagenesis and sorting of mutants. Gene cloning.

- Drug screening in *D. rerio*. Applications in human signalling pathways.
- Tissue regeneration studies.
- Models of human diseases.

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the genetics of *D. rerio*, the genealogy of cell types from the embryo to the adult, the techniques for producing transgenic animals which are also used as models of human disease, the identification of mutations associated with the development of the cardiac valve, and the study of tissue regeneration.

9. The plant model *Arabidopsis thaliana*: advantages and applications

OUTLINE

- Characteristics and advantages of *A. thaliana* as a model organism.
- Genome structure and organization.
- Anatomy and life cycle. Fertilization, embryogenesis, flowering, aging.
- Genetic analysis of growth and hormonal control systems.
- Genetic tools: T-DNA insertion mutation. RNA interference (siRNA).

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the genetics of *A. thaliana*, the techniques for creating transgenic plants, and the identification of mutations related to development and hormonal control systems.

10. Beyond the classic model organisms

OUTLINE

- Some important biological processes are easier to study in organisms other than the classical model organisms. New molecular techniques are now making it easier for many organisms to be used in the laboratory.
- Discovery of telomeres in *Tetrahymena thermophila*. Identification of telomerase. Applications in aging and cancer.
- The concept of regeneration in biology. Anatomy of Planaria. Regeneration of the anatomy after injury: maintaining scale, proportion and polarity. Normal regeneration and applications in the study of aging.
- Challenges and prospects in establishing new model organisms. Usefulness of sequencing techniques, RNAi, CRISP-R. Problems in cultivation and reproduction in the laboratory.

LEARNING OUTCOMES

After completing this chapter, you will have gained knowledge on the study of telomeres and telomerase in *Tetrahymena thermophila*, and the study of regeneration in planaria.

The main goal of this last chapter is to make students think about the suitability of classical model organisms for the study of all biological processes, and to become familiar with the new techniques that now facilitate the establishment of new organisms as laboratory models.

Laboratory practicals

OUTLINE

1. Genome data viewer at NCBI.
2. OMIM database at NCBI.
3. PCR primer design.
4. Sanger sequencing sample preparation and results analysis.
5. CRISPR / Cas9.
6. Managing references with the bibliography tool Mendeley.
7. Drosophila laboratory demonstration.
8. Critical reading of a scientific publication.

LEARNING OUTCOMES

Laboratory exercises aim to deepen the understanding of the concepts and applications presented in the theory.

Specifically, the exercises focus on the following skills:

- Using the Genome data viewer online tool at NCBI for analysis of genes, chromosomes, and genome comparison. Understanding the structure of a genomic database. Analysis of the genetic locus of the human gene CFTR, responsible for cystic fibrosis. Comparison of the genetic locus of the human CFTR and adjacent genes, with the corresponding genetic locus in the mouse genome.
- Use of the OMIM (Online Mendelian Inheritance in Man) database at NCBI. Browse entries related to the CFTR gene and cystic fibrosis, with the aim of understanding the structure of entry pages in the OMIM databse, to find information about phenotypes, alleles, heredity, orthologous genes in model organisms, and related scientific publications. Consolidate knowledge about different types of mutations (deletion, nonsense, missense, frameshift, destruction of a splicing site, polymorphism, or within regulatory regions of a gene).
- Designing primers for PCR using the online tools Primer3 and primerBLAST. Practice using the primer3 online tool for designing PCR primers. Tm calculation. Assessment of the specificity of the primers for use in a diagnostic PCR reaction to detect a specific deletion in the CFTR gene. Practice using the primerBLAST online tool. Evaluation of the secondary structure of the primers using the Oligo Evaluator tool provided by SIGMA-ALDRICH.
- Design and preparation of samples for Sanger sequencing, evaluation of sequencing results. Presentation of the methodologies used for the preparation and cleanup of DNA samples (plasmid DNA or PCR product) with silica nanoparticles. Practice on the strategy, design and preparation of DNA samples before sequencing. Get familiar with the process of evaluating the sequencing results, either using FASTA sequences and BLAST against NCBI databases, or by evaluating the sequencing process using the Bioedit software to look at pherograms.

- Experimental design of gene inactivation (knockout) with the CRISPR / Cas9 method through appropriate online tools. Introduction of the genome editing Crispr/Cas9 system, presentation of the experimental design and strategies used for developing a gene knout out via the CrispR/Cas9 system and defining the criteria for proper selection of pair of sgRNAs. Use and practice in the CHOP CHOP database for evaluation and suitable selection of sgRNAs.
- Laboratory demonstration of *Drosophila*. Presentation of the basic anatomic body features of an adult fly, the life cycle stages, practice on distinguishing the gender of a fly. Presentation of the basic molecular tools in *Drosophila* biology, including P-element transposons, balancers, and genetic mosaics. Practice in phenotyping of various mutant fly lines under stereoscope based on various phenotypic features: eye color, wing shape, body color and gender.
- Managing references with the bibliography tool Mendeley. Get familiar with and practice using Mendeley to create a database of bibliographic references, insert such references into text written in Word, and the ability to change the formatting of references.
- Critical reading of a scientific publication. Practice critical reading and comprehension of a scientific publication, guided by questions about the question / hypothesis posed by the publication, the methods and presentation of experimental data (images, tables, graphs), and drawing conclusions from the experimental data presented.