General Information	BACELOR DEGREE IN BIOTECHONOLOGIES
Title of the subject	Molecular Biology
Degree Course (class)	Industrial and Agri-food Biotechnologies (L-2)
ECTS credits	8
Compulsory attendance	yes
Language	Italian
Academic year	2020/2021

Subject Teacher	
Name and Surname	**Carmela Gissi**
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Place and time of reception	Campus di Via E. Orabona, 4 - Palazzo Dipartimenti Biologici; 1° floor From Monday to Friday by email appointment

ECTS credits details	Discipline sector (SSD)	Area
	BIO/11 Molecular Biology	---

Study plan schedule	Year of study plan	Semester
	2	**2**

Time management	Lessons	Laboratory	Exercises	Total
CFU	**7**	**1**		**8**
Total hours	**175**	**25**		**200**
In-class study hours	**56**	**12**		**68**
Out-of-class study hours	**119**	**13**		**132**

Syllabus	
Prerequisites / Requirements	Basic knowledge of general chemistry, organic chemistry, biological and genetic chemistry
Expected learning outcomes (according to Dublin descriptors)	
Knowledge and understanding	Acquisition of knowledge of molecular biology for the understanding of basic biological mechanisms.
Applying knowledge	The laboratory activity will allow to know how to use molecular biology and genetic engineering techniques for the study of cellular systems and components of biotechnological interest
Making informed judgments and choices	Students will acquire the ability to evaluate and interpret experimental data in terms of scientific value and methodological rigor; ability to express a critical evaluation of the aspects of research in the biotechnology field
Communicating knowledge	Students will acquire skills and tools of written and oral communication aimed at exchanging ideas, information, data and methodologies with specialist and non-specialist interlocutors on problems that can be analyzed through biotechnological methods and approaches.

Capacities to continue learning	Students will acquire learning and in-depth skills through consultation of bibliographic material and updating on advances in knowledge and methodologies in the biotechnology field.
Study Program	
Content	**The structure of nucleic acids** DNA as the molecule responsible for the transmission of genetic information. Nitrogen bases, nucleosides, nucleotides. Chemical composition and three-dimensional structure of DNA. The DNA double helix: forms A, B, Z. The flexibility of the DNA double helix: curved DNA, cruciform structures and "base flipping". The topology of DNA (Preferred source: Watson et al. "Molecular biology of the gene"): topoisomers and topological bond number; separation of topoisomers by agarose gel electrophoresis; DNA topology changes catalyzed by type I and type II topoisomerase and their functional importance; notes on the mechanisms of action of topoisomerases. DNA denaturation and absorbance. RNA: chemical composition, secondary structures and three-dimensional structure. **The genetic code** Degeneration and universality. Effects of the structure of the genetic code for DNA mutability and translation efficiency. Codon-anticodon interaction and vacillation **The packaging of DNA** Packaging of DNA into viruses, eubacteria and eukaryotes. Eukaryotic chromosomes: structure of centromeres and telomeres. Secondary structures that stabilize telomeres. The structure of interphase chromatin: 10 nm and 30 nm fibers. Composition and structure of nucleosomes. Histone proteins and their characteristics. Modifications of the N-terminal tails of histones. Positioning of nucleosomes. Changes in chromatin structure: function of histone variants, post- translational modifications of histones and chromatin remodeling complexes. Enzymes responsible for post-translational modifications of histones. DNA replication and nucleosome assembly. Propagation of histone modifications to duplicated chromatin and epigenetic inheritance **DNA replication** (Preferred source: Watson et al. "Molecular biology of the gene") Semi-conservative DNA replication: Meselson and Stahl experiment. The different prokaryotic and eukaryotic DNA polymerases and their characteristics (processivity, fidelity, proofreading activity). The right-handed structure of the catalytic nucleus of DNA polymerases. Details of prokaryotic DNA polymerase III: hypothesis on the dimeric and trimeric structure of DNA polymerase III holoenzyme. The replisome and its components: DNA primase, SSB, DNA helicase, DNA ligase, "sliding clamps" and "sliding clamps" magazine. Action of topoisomerases in DNA replication of prokaryotes. The replicon model. Beginning and termination of replication in *E. coli*. Replication of the ends of eukaryotic chromosomes: telomerases and the regulation of their activity.

Recombinant DNA technology

Restriction enzymes: function in bacteria and their use in recombinant DNA techniques. Creation of flat, sticky and compatible ends. Gel electrophoresis for the separation of DNA fragments and of supercoiled circular DNA. DNA intercalating for DNA visualization on agarose gel.

DNA cloning: ligation reaction and optimization strategies. Plasmid-type cloning vectors and hints on other categories of vectors. Transformation of bacterial cells.

The DNA polymerase chain reaction (PCR): properties of primers for PCR and construction of heterologous primers, nested PCR, cloning strategies of amplicons, characteristics of various types of thermostable DNA polymerases, Real time PCR.

DNA sequencing with the Sanger method. Outline of the Maxam & Gilbert sequencing method. Primer walking sequencing strategy. Primers used for the sequencing of cloned fragments and amplicons. Optimization of the Sanger method for automatic sequencing (at the level of labeling, DNA polymerase and high resolution electrophoresis). Cycle Sequencing. Notes on pyrosequencing

DNA repair (Preferred source: Watson et al. "Molecular biology of the gene")

Mutability and DNA repair systems. Point mutations, frameshift, microsatellite mutations and chromosomal rearrangements. Damage to DNA due to endogenous and exogenous agents (hydrolytic deamination, oxidation, alkylation, action of intercalating agents, gamma rays and X rays). MMR, BER and NER repair systems in prokaryotes and eukaryotes. Repair of double-stranded DNA breaks in prokaryotes and eukaryotes. Damage tolerance and *E. coli* SOS system

Recombination

Homologous and generalized recombination. Gene conversion and site specific recombination. Transposition. Examples of sequence rearrangements for the control of gene expression

Transcription and regulation of gene expression in prokaryotes

Transcription in prokaryotes: RNA polymerase structure and conformational changes during the initiation phase. Role of the sigma factors, promoter structure and presence of conserved consensus elements. Details of the initiation and elongation phase of prokaryotic transcription. Intrinsic and Rho-dependent termination.

Regulation of gene expression in prokaryotes: positive and negative control; inducible and repressible systems. Positive and negative control of the lac operon: action of the lacI repressor and the CAP activator. Integration of the control systems. The tryptophan operon: repressible negative control at the beginning of transcription and control for attenuation of transcription termination.

The lambda phage: regulation of the lytic and lysogenic cycles. Control region of the lambda phage. Structure and function of the cI, cro, cII and cIII genes. The antitermination of pN and pQ. Weak promoters and strong promoters of the lambda phage and

	mechanisms of gene regulation. How the integration and excision of the prophage from the *E. coli* chromosome is controlled **Transcription and regulation of gene expression in eukaryotes** Transcription in eukaryotes: peculiarities of the 3 eukaryotic RNA polymerases. RNA polymerase I and the characteristics of its promoters; RNA polymerase III and the characteristics of the various categories of Pol III promoters. Maturation of rRNAs and tRNAs due to exo / endonucleolytic cuts and nucleotide modifications. Structure of the RNA polymerase II promoter and basal transcriptional apparatus. Phases of initiation and elongation of the RNA Pol II transcription. Maturation of mRNAs: capping, cut and 3 'polyadenylation. The disrupted structure of eukaryotic protein genes and the splicing process. Composition and action of the spliceosome. Alternative splicing and splicing regulation. Autosplicing introns of group I and group II and their catalytic mechanism. Introns of tRNAs of archaea and eukaryotes. mRNA editing by base conversion and by insertion / deletion. Extended editing of the *Trypanosoma* mitochondrial genome. Regulation of gene expression in eukaryotes: transcriptional activators and repressors. Modular structure of regulatory signals: RNA polymerase II promoters. Enhancer, silencer, insulators. Modular structure and domains of transcription factors. The DNA binding domains: Helix-Turn-Helix, Zn finger, leucine zipper and Helix-Loop-Helix. Post translational regulation of gene expression mediated by microRNAs and other ncRNAs with a regulatory function **Epigenetic inheritance** CpG islands and the regulation of transcription initiation through DNA methylation. The transmission of the DNA methylation state. The remodeling of chromatin **Translation** Translation in prokaryotes and eukaryotes. Structural features of tRNA and mRNA. Structure and composition of prokaryotic and eukaryotic ribosomes. Beginning, elongation and termination of translation in prokaryotes and eukaryotes. Protein factors of initiation, elongation and termination of translation. Cap-independent mechanism of initiation of translation in eukaryotes. Energy balance of translation. General translation regulation. Translational regulation of specific genes. Stability and degradation of mRNA. **Molecular Biology Laboratory: 1 CFU** 1) Digestion of DNA with restriction enzymes and electrophoresis on agarose gel 2) Plasmid DNA extraction and qualitative and quantitative analysis of the extracted DNA 3) PCR reaction and amplicon analysis by agarose gel electrophoresis
Bibliography and textbooks	1) Min text: "Biologia Molecolare" - F. Amaldi, P. Benedetti, G. Pesole, P. Plevani; Casa Editrice Ambrosiana, Terza edizione, 2017 2) For topics such as DNA replication, DNA polymerase III, DNA repair, and DNA topology: "Biologia Molecolare del Gene" Watson

	JD et al.- Settima Edizione – Zanichelli ed. 3) For recombinant DNA techniques, also: "Dai Geni ai Genomi" J. W. Dale, M. von Schantz, N. Plant (third edition) - Edises For further consultation: "Genomi 4" Brown TA, EDISES "Il Gene X" Lewin B et al., Zanichelli ed.
Notes to textbooks	None
Teaching methods	Frontal lessons with PowerPoint slides; laboratory experiences
Assessment methods (oral, written, ongoing assessment)	Written examination and ongoing tests
Evaluation criteria (describe criteria for each of the above expected outcomes)	Evaluation of the acquisition of the course contents and of the language properties in the presentation of the course contents, in all the final written test, the ongoing tests and laboratory experiences.
Further information	