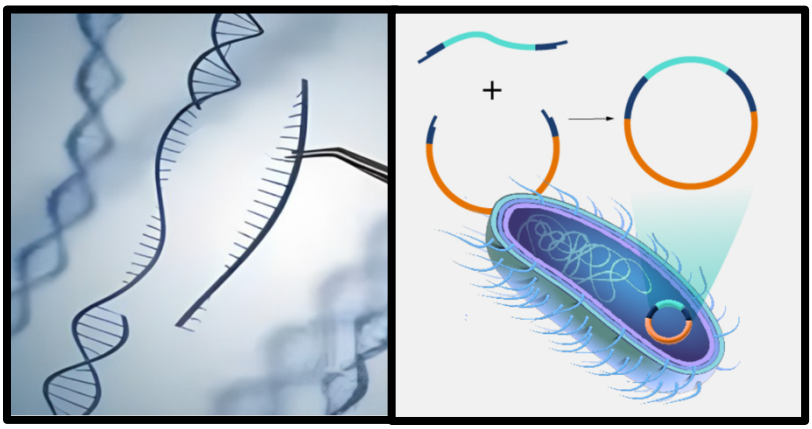
Department of Biological Sciences, KBCC, CUNY



Biology 5800: Recombinant DNA Biotechnology

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Course Information:

Catalog description: (4 credits, 6 hours.)

It is one semester upper-level course. This cutting-edge DNA technology course is for students interested to major in A.S. in Biotechnology.
Topics introduce key principle of molecular biology about recombinant DNA technology, gene cloning, genetic engineering, gene expression in both prokaryotes and eukaryotes systems. The laboratory component provides in-depth experimentation with the techniques and tools used in the study of molecular and cell biology. After completing this course students will have the option to transfer to other colleges and complete their B.S. or B.A. in Biology.

This course satisfies the elective credit requirement for Biology Majors

Prerequisites: : BIO 1400 and CHM 1100 or Department permission

Course overview:

- The Recombinant DNA Technology is a four credit one-semester course.
- It emphasizes and teaches the theories and techniques for gene cloning by transferring genes from one species to another.
- Construction of a recombinant DNA molecule using molecular tools that allow cutting genes and relocate them on DNA of different species like bacteria, animal cells or plant cells.
- Cloning a gene that produce a specific protein has a tremendous impact for therapeutic treatments.
- A cloned gene into bacteria or eukaryotic cells can produce millions of that specific protein, i.e. insulin, interferons, human growth hormone and many others.
- Your knowledge of general biology, like structure of a cell, nucleic acids structure and function will be applied to understand the process of cloning and gene expression.
- Your knowledge of chemistry will be applied to understand the biochemical reactions relevant to the process of cloning.
- If you are unsure about the basic principles of biology and chemistry, you can review them and meet with your instructor.
- Make sure to ask your professor issues that requires further clarification.

Course Goals for Student Outcomes

1. Learn the relationships between genes, DNA, RNA and proteins. Regulation of gene expression.
2. Vector DNA (plasmids) and their roles in gene cloning and transfer.
3. Methods of DNA extraction, purification, and quantification from living cells.
4. Gene cloning: cut, isolate, insert and ligate DNA fragments into unrelated DNA.
5. Transfer and introduction of DNA into living cells.
6. Identification of cloned DNA genes using Polymerase Chain Reaction (PCR) to confirm and identify the correct cloning into a different system like bacteria.
7. Use of PCR to amplify DNA for forensics, DNA fingerprinting and DNA barcoding purposes.
8. DNA sequencing methods and their applications.
9. Scope of recombinant DNA technology biotechnology: medicine, forensics, and agriculture.

Recommendations to students:

- Read the textbook prior to coming to class.
- Be responsible for all material, announcements, or assignments mentioned in class whether you are present or not.
- Write down the name of your instructor, the office, and office hours.
- It is advised to get contacts of classmates who may be contacted if you are absent, to discuss material, and to form study group.
- Check Brightspace for announcements, links and schedules.
- Take notes and make notecards of topics discussed in lecture and laboratory class.
- Do not wait until last moment to study before exam or quiz.
- Study slide decks, your notes and book chapters to study for each exam or quiz.
- Attendance to class is essential. If you miss class, you are responsible to making up the missing work.
- Best wishes to students to have a successful course. Please feel free to contact your instructor anytime by email or attending office hours.

Access - Ability Service (AAS):

- Access-Ability Services (AAS) serves as a liaison and resource to the KCC community regarding disability issues.
- Promotes equal access to all KCC programs and activities and makes every reasonable effort to provide appropriate accommodations and assistance to students with disabilities.
- Please contact this office if you require such accommodations and assistance.
- Your instructor will be glad to make the accommodations you need, but you must have documentation from the Access-Ability office for any accommodations.

Academic Integrity:

Your instructor upholds the KCC policy on academic honesty (see Student Handbook online). There are consequences for cheating on exams or plagiarizing somebody's else work (i.e. turning in work copied form another source). These include a reduced grade or zero, suspension, or dismissal.

You may find the CUNY and KCC's Academic Integrity Policies are in:

1. Kingsborough Community College Catalogue
2. Kingsborough Community College Student Handbook
3. www.kingsborough.edu/Academic_Integrity_Policy.pd

Plagiarism as a violation of academic integrity is the intentional use of another's intellectual creation(s) without attribution. Determination and penalty ranging from grade reduction to course failure is at the sole discretion of the faculty member.
Plagiarism, intentional or unintentional, is considered academic dishonesty and in all instances can be reported to the Academic Judiciary.

Grade computation:

Lecture:

Three Lecture Exams	X3	=30%
One Final Exam @	X1	=20%
Total		=50%

Laboratory:

Laboratory Quizzes	x 5	=30%
Laboratory reports	x 2	=20%
Total		= 50%

Final grade is Lecture (50%) + Laboratory (50%) = 100% translated in a letter grade
Exams and quizzes grading could be changed during the semester

Textbook:
Gene Cloning and DNA Analysis: An Introduction, 8th edition, 2020.
Author: Terence A. Brown. Blackwell Science Ltd. ISBN: 978-1119640783

Laboratory Manual:
DNA Science: A First Course, Second Edition, 2003.
Mark V. Bloom, Greg A. Freyer with David Crotty. Cold Spring Harbor Laboratory Press.
ISBN: 978-1936113170

BIO58 Lecture outline and reading assignment:

Session 1: Introduction. Study of genes, DNA, RNA, and proteins. Gene transcription and translation.
Reading assignments: Chapter 17 Campbell

Session 2: Application of recombinant DNA technology in gene cloning and ethics of gene cloning.
Reading assignments: Chapter 1: pages 1-12.

Session 3: Gene cloning vectors: plasmids and viruses.
Reading assignments: Chapters 2 & 6: pages: 14-27 and 107-131

Session 4: Methods of DNA extraction, purification and quantification from living cells.
Reading assignments: Chapter 3: pages: 28- 53.

Session 5: Tools for gene manipulation: Restriction enzymes and Ligase.
Reading assignments: Chapter 4: pages: 54-86.

Session 6: Tools for gene manipulation: Restriction enzymes and Ligase -continued.
Reading assignments: Chapter 4: pages: 54-86.

Session 7: Transfer and introduction of DNA into living cells.
Reading assignments: Chapter 5: pages: 87–106

Session 8: How to obtain a recombinant DNA specific gene clone.
Reading assignments: Chapter 8: pages: 158-180.

Session 9: Polymerase Chain Reaction (PCR) and DNA sequencing.
Reading assignments: Chapters 9 & 10: pages: 181-195 and 199-218**.**

Session 10: Molecular means for genetic identification. Restriction enzyme mapping, DNA fingerprinting, and DNA Barcoding.
Reading assignments: Chapter 8 & 11: pages: 239-250.

Session 11: Recombinant DNA cloning in Medicine.
Reading assignments: Chapter 14: pages: 302- 322

Session 12: Recombinant DNA in agriculture, forensics and archeology.
Reading assignments: Chapter 15; pages: 323-345.

OBJECTIVES:
The objectives listed can be used as guidelines for each topic discussed during in the course.

Study of genes, DNA, RNA, and proteins. Gene transcription and translation:
1. Define and describe a gene, DNA, RNA and protein
2. The flow of genetic information, molecular biology dogma: DNA $\rightarrow$ RNA $\rightarrow$ protein.
3. How a gene is transcribed, translated into proteins.
4. One gene $\rightarrow$ one protein (polypeptide).
5. Understand processing of messenger RNA, triplet codes, codon dictionary, start and stop codons and a reading frame.
6. Describe the degenerative natures of codons, nearly universal nature of codons with differences between eukaryotic and prokaryotic codons.

7. How gene expression is controlled: promoter, a terminator, and a transcription of a gene
8. Post transcriptional modifications of proteins.
9. Mutations and the different kind of mutations: a point mutation, insertion and deletion, base pair substitution, missense and nonsense mutations

Recombinant DNA technology for gene cloning and ethics of gene cloning:

1. Understanding what cloning is and gene cloning.
2. Definition of different types of cloning
3. Explanation of recombinant DNA technology (DNA cloning).
4. Learn several cloning technologies are used.
5. History of animal cloning, Dolly the first sheep cloned.
6. Advantages and disadvantages of cloning
7. Ethical question if humans can or should be cloned.
8. Cloning and its biological, environmental, economic and social implications.

Gene cloning vectors: plasmids and viruses.

A-Prokaryotic Vectors for Gene Cloning: Plasmids and Bacteriophages.

a) Plasmids: basic features, size and copy number,
b) Conjugation and compatibility. Classification of plasmids and non-bacterial plasmids.
c) Bacteriophages. Basic features of bacteriophages, lysogenic phages, DNA organization, linear and circular forms.

B-Cloning Vectors for Eukaryotes:

1-Vectors for yeast and other fungi.

2- Cloning vectors for higher plants:

a) *Agrobacterium tumefaciens* – nature's smallest genetic engineer.
b) Ti plasmid to introduce new genes into a plant cell.
c) Production of transformed plants with the Ti plasmid.
d) The Ri plasmid. Limitations of cloning with *Agrobacterium* plasmids.

3- Cloning vectors for animals:

Insects:

a) p-elements, baculovirus.

Mammals:

b) adenovirus, adeno-associated virus, papillomavirus, retrovirus, and simian virus-40

Methods of DNA extraction, purification, and quantification from prokaryotic and eukaryotic cells:

Prokaryotic cells:

1. Grow and harvest bacteria in culture.
2. DNA Extraction from bacterial culture.
3. Purification of DNA from contaminant
4. Measure yield (amount) of the extracted DNA and measure concentration.

Plasmid DNA preparation (miniprep):

1. Alkaline denaturation of DNA
2. Plasmid extraction
3. Measure yield and concentration of plasmid preparation.

Eukaryotic cells:

1. Isolation and purification of nuclear DNA from eukaryotic cells.

Tools for gene manipulation: Restriction enzymes and Ligase:

1. The discovery and function of restriction endonucleases.

2. Type II restriction endonucleases: blunt ends and sticky end cutters.
3. Frequency of recognition sequences in a DNA molecule.
4. Separation of cut DNA molecules by gel electrophoresis.
5. Visualization of DNA molecules by gel staining.
6. Ligation: joining DNA molecules together, the mode of action of DNA ligase.

Transfer and introduction of foreign DNA into living cells:

1. Introduction of DNA into living cells.
2. Transformation to uptake of DNA in bacteria.
3. Preparation of competent cells for transformation.
4. Selection of positively transformed cells and identification of recombinants.
5. Identification of positive clones base on an antibiotic resistance on agar plates.
6. Transfection of mammalian cells with foreign DNA by using possibly several techniques like liposomes, electroporation, and microinjection.

Identification of a recombinant DNA specific gene clone into either bacteria or eukaryotic cells:

1. Basic strategies to identify the positive clone.
2. Identification of a clone from a gene library.
3. Gene libraries; expression of genes in the library by isolating whole mRNAs.
4. Hybridization of complementary nucleic acid strands.
5. Colony and plaque hybridization
6. PCR of the recombinant inserted DNA in cells

Polymerase Chain Reaction (PCR) and DNA sequencing:

1. Learning principle of Polymerase Chain Reaction (PCR).
2. Use of different DNA polymerases
3. Learn how to design primers.
4. Determine of correct PCR reaction temperatures.
5. Analyze PCR products by electrophoresis.
6. DNA sequencing.
7. The Sanger method: chain-terminating nucleotides.
8. The Maxam–Gilbert method: chemical degradation of DNA.
9. Design specifc primers for sequencing.
10. Next generation sequencing (NGS)
11. Analysis of sequenced DNA using software

Molecular means for genetic identification. Restriction enzyme mapping, DNA fingerprinting, and DNA Barcoding:

1. Principles of Southern hybridization and Northern hybridization.
2. Techniques rationale of restriction enzyme mapping.
3. Principles of DNA finger printing.
4. Microsatellite DNA as a means of population identification.
5. Mitochondrial DNA for barcoding and species identification
6. PCR analysis

Recombinant DNA cloning in Medicine:

<u>Production of recombinant pharmaceuticals</u>.

1. Recombinant insulin. Synthesis and expression of artificial insulin genes.
2. Synthesis of human growth hormones in *Escherichia coli*.
3. Recombinant factor VIII.

4. Synthesis of other recombinant human proteins.
5. Recombinant vaccines.
    a. Producing vaccines as recombinant proteins
    b. Recombinant vaccines in transgenic plants
    c. Live recombinant virus vaccines
    d. mRNA vaccines

Identification of genes responsible for human diseases.

1. How to identify a gene for a genetic disease.
2. Locating the approximate position of the gene in the human genome.
3. Identification of candidates for the disease gene.

Gene therapy:

1. Gene therapy for inherited diseases.
2. Gene therapy and cancer.
3. Ethical issues raised by gene therapy
4. CRISPR/Cas9 technology

Recombinant DNA in agriculture, forensics and archeology:

1. Gene Cloning and DNA Analysis in Agriculture.
    a. plant genetic engineering.
    b. Plants that make their own insecticides.
    c. Cloning □-endotoxins of *Bacillus thuringiensis* in maize, and chloroplasts,
    d. Countering insect resistance to d-endotoxin crops.
2. Herbicide resistant crops.
    a. Roundup Ready' crops.
    b. A new generation of glyphosate resistant crops.
3. Problems with genetically modified plants.
    a. Safety concerns with selectable markers.
    b. The possibility of harmful effects on the environment.
4. Gene Cloning and DNA Analysis in Forensic Science and Archaeology.
    a. DNA analysis in the identification of crime suspects.
    b. Genetic fingerprinting by hybridization probing.
    c. DNA profiling by PCR of short tandem repeats.
    d. Studying kinship by DNA profiling.
    e. Identification of missing children and sex identification by DNA analysis.
5. Archaeogenetics: using DNA to study human evolution.
    a. The origins of modern humans.
    b. DNA analysis challenged the multiregional hypothesis.
    c. Neanderthals are not the ancestors of modern Europeans.
    d. DNA can also be used to study prehistoric human migrations.
    e. The spread of agriculture into Europe.
    f. Mitochondrial DNA depicts past human migrations into Europe.

Laboratory Outline

Session	Activities
1	Measurements, pipetting, micro pipetting and sterile techniques.
2	Isolation and enumeration of bacteria Culture of bacterial cells in plates and broth. Plasmid identification using restriction enzymes
3	Agarose Gel Electrophoresis and Transformation of Bacteria
4	Isolation and purification of plasmid DNA from transformed bacteria (Miniprep)
5	Restriction digestion analysis of pGLO (Miniprep) purified in week 4, agarose gel to analyze cut plasmid
6	Isolation and purification of Green Fluorescent Protein (GFP)
7	Polymerase Chain Reaction (PCR)
8	Restriction digestion of pcDNA plasmid and GFP PCR product
9	Inoculum of recombinant bacterial colonies from transformation Isolate DNA from plant tissue and food products to detect GMO product
10	Miniprep of recombinant pcDNA plasmid containing GFP gene Restriction enzyme digestion to confirm GFP cloning Agarose gel to check presence of recombinant GFP DNA
11	Barcoding of life
12	Analysis of sequencing results from barcoding experiment