Seminars Program

On Genetic Engineering and Biosafety

Seminar 1. Main approaches and methods of molecular biotechnology

1. What is Genetic Engineering?
2. What is the purpose of Genetic Reengineering?
3. Give three examples of Genetic Engineering
4. What is the difference between altered and recombinant DNA?
5. What is meant by gene splicing?

Seminar 2 Methods of extraction of nucleic acids from different biological materials

Detergents used in biomedical laboratories are mild surfactants (surface acting agents), used for cell lysis and the release of intracellular materials.

* They are amphiphilic molecules, containing both hydrophilic and hydrophobic regions. This amphiphilic property allows detergents to break protein-protein, protein-lipid and lipid-lipid associations, denature proteins and other macromolecules, and prevent nonspecific binding in immunochemical assays and protein crystallization.

There are many types of detergents in cell membrane:

* Detergents play an essential role in the extraction, purification, and manipulation of membrane proteins;
* Their amphiphilic nature allows them to interact with hy- drophobic membrane proteins to keep them water-soluble outside of their native bilayer environment . Unfortunately, solubility does not always translate to native structure and stability; a detergent that is useful for extraction may not be compatible with purification and/ or biochemical studies .
* Furthermore, a detergent that works for one membrane protein may not be suitable for a different membrane protein . While there is not a set *of “golden rules”* for the uses of detergents for membrane protein applications, understanding the physical-chemical properties associated with different classes of de- tergents may be useful for deciding which detergent may work best for a particular application .

Seminar 3 Analysis of DNA

* To evaluate DNA purity by spectrophotometry, measure absorbance from 230nm to 320nm in order to detect other possible contaminants present in the DNA solution . The most common purity calculation is determining the ratio of the absorbance at 260nm divided by the reading at 280nm. Good-quality DNA will have an A260/A280 ratio of 1.7–2.0. A reading of 1.6 does not render the DNA unsuitable for any application, but lower ratios indicate more contaminants are present. However, the best test of DNA quality is functionality in the application of interest (e.g., real-time PCR).
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Seminar 4. MicroRNA Cloning from Cells of the Immune System. Use of nucleases, exonuclease, restrictase in molecular biotechnology.

Seminar 5. Analysis of different types nuclear acids

Seminar 6. DNA Separation Techniques for different types of DNA. Main principles of electrophoresis for analysis of nucleic acids.

Seminar 7. The SDS-PAGE based DNA Separation Techniques

Seminar 8. Genome mapping, genetic mapping, physical mapping, mapping distance.

1. Gene Splicing Introduction
2. Gene splicing is a post-transcriptional modification in which a single gene can code for multiple proteins. Gene Splicing is done in eukaryotes, prior to mRNA translation, by the differential inclusion or exclusion of regions of pre-mRNA. Gene splicing is an important source of protein diversity. During a typical gene splicing event, the pre-mRNA transcribed from one gene can lead to different mature mRNA molecules that generate multiple functional proteins. Thus, gene splicing enables a single gene to increase its coding capacity, allowing the synthesis of protein isoforms that are structurally and functionally distinct. Gene splicing is observed in high proportion of genes. In human cells, about 40-60% of the genes are known to exhibit alternative splicing.
3. Gene Splicing Mechanism
4. There are several types of common gene splicing events. These are the events that can simultaneously occur in the genes after the mRNA is formed from the transcription step of the central dogma of molecular biology.
5. Exon Skipping: This is the most common known gene splicing mechanism in which exon(s) are included or excluded from the final gene transcript leading to extended or shortened mRNA variants. The exons are the coding regions of a gene and are responsible for producing proteins that are utilized in various cell types for a number of functions.
6. Intron Retention: An event in which an intron is retained in the final transcript. In humans 2-5 % of the genes have been reported to retain introns. The gene splicing mechanism retains the non-coding (junk) portions of the gene and leads to a demornity in the protein structure and functionality.
7. Alternative 3' Splice Site and 5' Splice Site: Alternative gene splicing includes joining of different 5' and 3' splice site. In this kind of gene splicing, two or more alternative 5' splice site compete for joining to two or more alternate 3' splice site.

Define Genetic Engineering

1. Outline the process of genetic engineering involving some or all of the following: isolation, cutting, transformation, introduction of base sequence changes and expression
2. Know three applications: one plant, one animal, one micro-organism

Genetic Engineering Technique

The process for genetic engineering begins the same for any organism being modified (see Figure 3 for an example of this procedure).

1. Identify an organism that contains a desirable gene.
2. Extract the entire DNA from the organism.
3. Remove this gene from the rest of the DNA. One way to do this is by using a *restriction enzyme*. These enzymes search for specific nucleotide sequences where they will "cut" the DNA by breaking the bonds at this location.
4. Insert the new gene to an existing organism's DNA. This may be achieved through a number of different processes.

When modifying bacteria, the most common method for this final step is to add the isolated gene to a *plasmid*, a circular piece of DNA used by bacteria. This is done by "cutting" the plasmid with the same restriction enzyme that was used to remove the gene from the original DNA. The new gene can now be inserted into this opening in the plasmid and the DNA can be bonded back together using another enzyme called ligase. This process, shown in Figure 4, creates a *recombinant* plasmid. In this case, the recombinant plasmid is also referred to as a bacterial artificial chromosome (BAC). Refer to the associated activity [Bacteria Transformation](https://www.teachengineering.org/lessons/view/uoh_genetic_lesson01_activity1) to have students create a model to simulate and learn about the process used by genetic engineers to modify bacteria.

Once the recombinant DNA has been built, it can be passed to the organism to be modified. If modifying bacteria, this process is quite simple. The plasmid can be easily inserted into the bacteria where the bacteria treat it as their own DNA. For plant modification, certain bacteria such as *Agrobacterium tumefaciens* may be used because these bacteria permit their plasmids to be passed to the plant's DNA.

Applications and Economics

The number of applications for genetic engineering are increasing as more and more is learned about the genomes of different organisms. A few interesting or notable application areas are described below.

How many of today's crops are genetically modified? As of 2010, in the U.S., 86% of corn produced was genetically modified. *Bt*-corn is a common GMO that combines a gene from the *Bt* bacteria with corn DNA to produce a crop that is insect-resistant. The bacteria gene used contains a recipe for a protein that is toxic when consumed by insects, but safe when consumed by humans.

A number of other genes can be combined with crops to produce desirable properties such as:

* Herbicide-, drought-, freeze- or disease-resistance
* Higher yield
* Faster growth
* Improved nutrition
* Longer shelf life

The creation of genetically modified crops provides many incentives for farmers and businesses. When farmers are able to plant a crop that has a higher yield per acre, they can significantly increase production, and thus sales, with minimal cost. Disease, pest and other resistances reduce crop loss, which also helps to increase profits. Besides farmers, other benefactors from modified crops include seed, agrochemical and agriculture equipment companies as well as distributors and universities that are involved in GMO research. In 2011, the value of genetically modified seed was $13.2 billion in the U.S. alone. The value of the end products produced from these seeds topped $160 billion.

Due to their simple structures, the most commonly modified organisms are bacteria. The first modified bacteria were created in 1973. Bacteria can be modified to produce desirable proteins that can be harvested and used. One example is insulin or spider silk, which is difficult to gather naturally. Other modifications to bacteria include making changes to the cellular respiration process to alter the byproducts; typically CO2 is produced, however engineers have made modifications so that hydrocarbon byproducts such as diesel and polyethylene (a fuel and a plastic) are produced.

Seminar 9. Selection of a vector.

Splice Variant Detection Methods

Gene splicing leads to the synthesis of alternate proteins that play an important role in the human physiology and disease. Currently, the most efficient methods for large scale detection of splice variants include computational prediction methods and microarray analysis. Microarray based splice variant detection is the most popular method currently in use. The highly parallel and sensitive nature of microarrays make them ideal for monitoring gene expression on a tissue-specific, genome-wide level. Microarray based methods for detecting splice variants provide a robust, scalable platform for high-throughput discovery of alternative gene splicing. A number of novel gene transcripts were detected Seminar 10. Applications of microarrays in biotechnologyusing microarray based methods that were not detected by ESTs using computational methods. Another commonly used method for discovering of novel gene isoforms is RT-PCR followed by sequencing. This is a powerful approach and can be effectively used for analyzing a small number of genes. However, it only provides only a limited view of the gene structure, is labor-intensive, and does not easily scale to thousands of genes or hundreds of tissues.

Challenges in Microarray Design for Splice Variant Detection

Microarray based gene splicing detection poses some unique challenges in designing probes for isoforms that show a high degree of homology. In order to differentiate between these isoforms, a microarray that uses

Seminar 11. Gene targeting uses homologous recombination.

### Seminar 12. Horizontal gene transfer.

* Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. This takes place through a [pilus](https://en.wikipedia.org/wiki/Pilus).
* Bacterial [conjugation](https://en.wikipedia.org/wiki/Bacterial_conjugation) is a mechanism of [horizontal gene transfer](https://en.wikipedia.org/wiki/Horizontal_gene_transfer) as are [transformation](https://en.wikipedia.org/wiki/Transformation_(genetics)) and [transduction](https://en.wikipedia.org/wiki/Transduction_(genetics)).

These two other mechanisms do not involve cell-to-cell

is the primary mechanism for the spread of antibiotic resistance in bacteria.

*Horizontal gene transfer* plays an important role in the evolution of bacteria that can degrade novel compounds such as human-created pesticides and in the evolution, maintenance, and transmission of *virulence*.

* *Horizontal gene transfer* often involves [*temperate*](https://en.wikipedia.org/wiki/Temperateness_(virology))[*bacteriophages*](https://en.wikipedia.org/wiki/Bacteriophage)and [*plasmids*](https://en.wikipedia.org/wiki/Plasmids).

Genes responsible for antibiotic resistance in one species of bacteria can be transferred to another species of bacteria through various mechanisms of HGT such as [*conjugation*](https://en.wikipedia.org/wiki/Conjugation_(genetics))*,* [*transformation*](https://en.wikipedia.org/wiki/Transformation_(genetics)), and [*transduction*](https://en.wikipedia.org/wiki/Transduction_(genetics))Seminar 13. Preparation of DNA chip and the experiment.

Seminar 14. Applications of horizontal gene transfer

* is the process by which foreign [DNA](https://en.wikipedia.org/wiki/DNA) is introduced into a cell by a [virus](https://en.wikipedia.org/wiki/Virus) or [viral vector](https://en.wikipedia.org/wiki/Viral_vector).
* An example is the viral transfer of [DNA](https://en.wikipedia.org/wiki/DNA) from one bacterium to another and hence an example of [horizontal gene transfer](https://en.wikipedia.org/wiki/Horizontal_gene_transfer). *Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA* (which occurs in conjugation), and it is DNase resistant (*transformation is susceptible to DNase*).
* *Transduction* is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome (both bacterial and mammalian cells).
* When viruses, including bacteriophages (viruses that infect bacteria), infect bacterial cells, their normal mode of reproduction is to *harness* (*впрягать*) the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat A bacterial artificial chromosome (BAC) is a [DNA construct](https://en.wikipedia.org/wiki/DNA_construct), based on a functional fertility [plasmid](https://en.wikipedia.org/wiki/Plasmid) (or [F-plasmid](https://en.wikipedia.org/wiki/F-plasmid)).
* *The F factor* is carried on the F [episome](https://en.wikipedia.org/wiki/Episome), the first episome to be discovered.
* Unlike other plasmids, *F factor* is constitutive for transfer proteins due to a mutation in the gene *finO*.
* The F plasmid belongs to a class of conjugative plasmids that control sexual functions of bacteria with a fertility inhibition (Fin) system.

Seminar 15. Clinical trial of gene electrotransfer.

Electroporation is increasingly being used for delivery of chemotherapy to tumors. Likewise, gene delivery by electroporation is rapidly gaining momentum for both vaccination purposes and for delivery of genes coding for other therapeutic molecules, such as chronic diseases or cancer. This chapter describes how gene therapy may be performed using electric pulses to enhance uptake and expression.

Plasmid or non-viral gene therapy offers an alternative to classic viral gene delivery that negates the need for a biological vector. In this case, delivery is enhanced by a variety of approaches including lipid or polymer conjugation, particle-mediated delivery, hydrodynamic delivery, ultrasound or electroporation. Electroporation was originally used as a laboratory tool to deliver DNA to bacterial and mammalian cells in culture. Electrode development allowed this technique to be modified for in vivo use. After preclinical therapeutic studies, clinical delivery of cell impermeant chemotherapeutic agents progressed to clinical delivery of plasmid DNA. One huge benefit of this delivery technique is its malleability. The pulse protocol used for plasmid delivery can be fine-tuned to control the levels and duration of subsequent transgene expression. This fine-tuning allows transgene expression to be tailored to each therapeutic application. Effective and appropriate expression induces the desired clinical response that is a critical component for any gene therapy. This chapter focuses on clinical trials using in vivo electroporation or electrotransfer as a plasmid delivery method. The first clinical trial was initiated in 2004, and now more than fifty trials use electric fields for gene delivery. Safety and tolerability has been demonstrated by several groups, and early clinical efficacy results are promising in both cancer therapeutic and infectious disease vaccine applications