Synthetic Biology - Discover the Laboratory of Life

Protocol for lecturers

1.Safety first and foremost (up to 20 minutes)

In addition to the common safety guidelines for working in the laboratory, it is necessary to mention the safety conditions for working with genetically modified organisms (GMOs).
A genetically modified organism is an organism (other than humans) whose genetic information has been modified in certain ways specified by law. In general, these are changes that would not occur in nature. Working with GMOs is regulated by law. GMOs are divided into four groups according to potential risk *(our E. coli* strain is in Group 1, no risk for human health). Every laboratory that works with GMOs must be licensed to do so, and staff must be trained. The authorisation always applies to the particular organisms and risk group.

Rules for working with GMOs:
1. In each room for working with GMOs, post an Emergency Plan and Operating Rules signed by the advisor.

2. Access by trained personnel only, BIOHAZARD marking on doors (mandatory for category 2-4)

3. Lab coats marked BIOHAZARD, gloves, bag for used gowns

4. Disinfection (Ajatin, SAVO) in the contaminated place

5. Proper inactivation of GMOs by autoclaving or disinfection

6. Labelled waste containers and safe disposal

7. Logbook: Plan and conclusion, protocols, checks

8. List of trained persons + training certificate once a year

9. Records of inspections of the area in logbook

10. Secure windows (non-opening or with net)

Answer to the question about security in the logbook:

1. I will mark the place
2. I will tell the person in charge
3. Dry the place with paper towels and then decontaminate (SAVO, Ajatin)
4. Place the generated waste in a bin designated for GMO waste.

In this course, students will also do electrophoresis, and use UV light and MIDORI dye. UV light is a valuable tool in the biology lab. It is used to sterilize rooms or just to visualize biomolecules. It excites molecules of dye that bind to DNA. UV radiation is a mutagen.

Instruct students not to look directly into the source of radiation, as a point of interest, when cutting a fragment out of the gel, work as quickly as possible to avoid damaging the DNA that is being cut. MIDORI dye is safe, non-toxic and non-mutagenic (unlike e.g. EtBr, see below.).

2. Introduction to synthetic biology

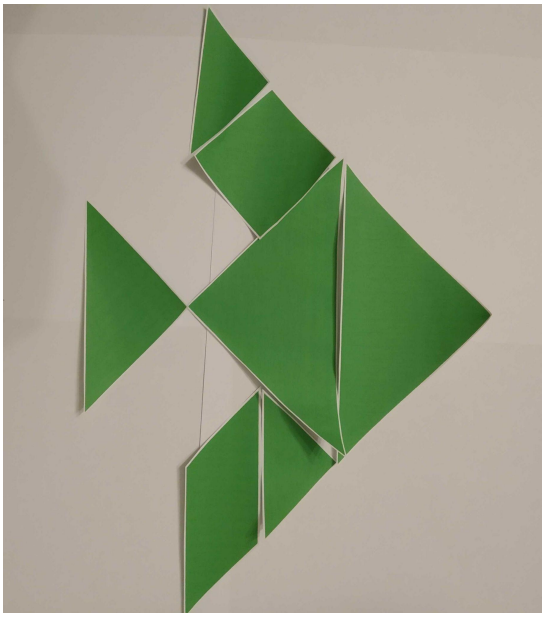
Activity: Tangram (25 minutes)

Students are given laminated pieces of different colors and build different shapes together. The instructor shows the students the original monochrome pictures of animals.

Comment: Each student is given biological pieces from different, often unrelated organisms. However, modern biology allows these pieces to be put together to create something completely new. To create a combination that would not have arisen in nature on its own (a glowing mouse, a goat producing spider silk in its milk, bacteria able to detect buried mines). The field that does this is synthetic biology. It describes and tests the pieces and puts them together. A synthetic biologist works much like an engineer. He builds large assemblies from smaller pieces. We'll talk about what these biological parts are in this course.

Templates for creating tangrams can be found at the end of the document.

Examples of tangram animals:



3. What are the individual biological parts? What is their function? - Theory

What you know about DNA (10 minutes)

To begin with, we need to refresh students' knowledge about DNA. The main points that are important to mention are that DNA is like the building plan for a cell, DNA defines the form and characteristics of an organism. This information is stored in DNA in the order of nucleotides. How does the order of nucleotides translate into the functioning of the cell? Through the processes of transcription and translation, the transfer of information from DNA to RNA and then to proteins. Proteins are the cellular machines that physically carry out the necessary processes in the cell.
The second very important thing is that DNA is universal, so all organisms store information into DNA in the same way. This means that if you take a gene from a human and put it into a yeast, it will know what to do.

In this part, students should be involved, answering questions. In their lab journal, they will complete these two questions.

a) ***Why can we say that DNA is universal?***
All organisms work with it in the same way. The genetic code is universal.

b) ***What does this feature of genetic code allow us to do?***
To create GMOs, to put genes from native organisms into other organisms and get the same product.

Different forms of DNA: chromosome vs plasmid (5 minutes)

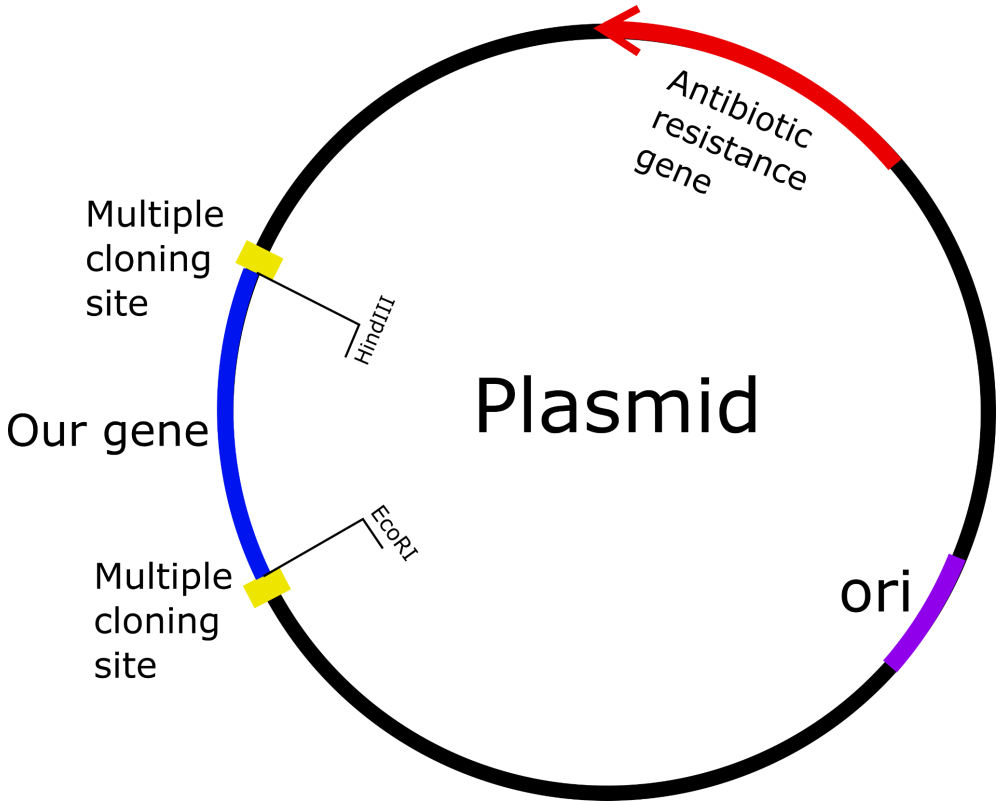
DNA can take many forms. The two basic ones are the chromosome and the plasmid. Every organism has a chromosome. It is the main location of DNA. You've probably heard of chromosomes, which are found in the nuclei of eukaryotic cells. Prokaryotes don't have nuclei, but their main circular DNA (nucleoid) is also called a chromosome - the bacterial chromosome.
The plasmid, by contrast, is not found in all organisms. In eukaryotes, plasmids are rarely found in yeast. Plasmids are mainly found in bacteria. But they also may not be found in all. Plasmids usually carry a gene for some additional function. Often it's something that gives the bacteria an advantage, like resistance to an antibiotic. A plasmid is much smaller than a chromosome (1000 times smaller in *E. coli*) and is a circular DNA molecule. Example: if you had a plasmid - a circle with a circumference of 1 metre, the chromosome of *E. coli* would be 1 kilometre long.
The genes themselves can be primarily isolated from both plasmids and chromosomes. It is the small size of the plasmid that makes it easier to manipulate and thus to use for gene transfer between organisms. Such a plasmid, serving as a carrier for our genes, must have certain regions. Ori is where the plasmid begins to replicate. The gene for antibiotic resistance is important because it then helps you to determine which bacteria contain the plasmid (due to resistance, they survive on soil with ATB). The cloning site is very important - it´s a sequence that is cut and our gene is inserted into it.

Students have the following questions in their lab notebook:

c) ***List 3 properties that distinguish a plasmid from a chromosome:***
It is smaller, not found in all organisms, carries an additional function

d) ***Describe the plasmid, filling in the correct names of its components in the empty squares.***

Think about:
In the diagram, one component is drawn twice.
Why is this?



The instructor will go over the names of the components of the plasmid with the students and make sure everyone understands their function or meaning.

4. BioBricks

The gene and its components (20 minutes)

And now we finally look at the biological parts/components themselves. A gene is a region of DNA that carries information about a particular protein (or RNA molecule). However, it does not consist of the coding sequence alone. The functioning of cells is very complex, so it must be possible to regulate **when** and **how much** a given gene is expressed.

a) **Promoter:** is the part of the sequence that controls the expression/production of a gene. RNA polymerase loads to the promoter and thereby mediates transcription of the gene into RNA. When the promoter is activated, transcription takes place. The promoter can be activated permanently - a constitutive promoter, or in response to signals - an inducible promoter. The inducible promoter is controlled by activators and repressors.

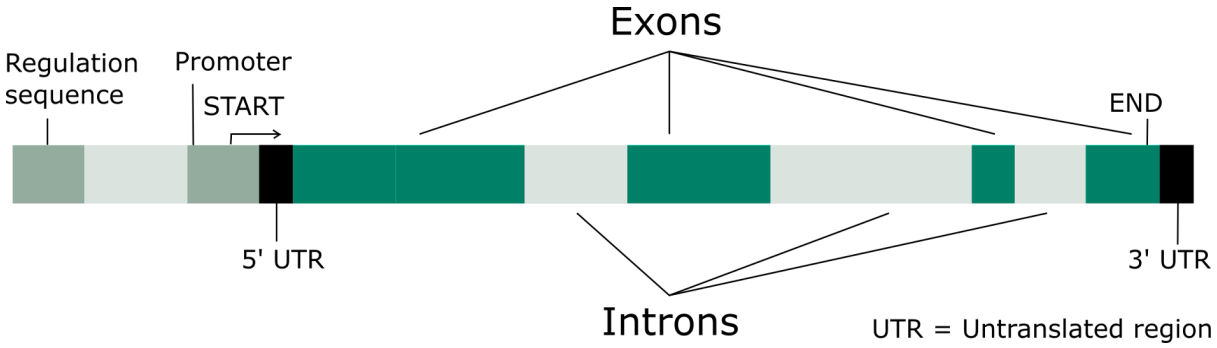
b) **Ribosome binding site (RBS):** the part of the sequence (already in the form of an RNA transcript) that the ribosome sits on during the translation process. The presence of the RBS is very important, without it translation would not take place. The form of the RBS can also affect the degree of ribosome binding and therefore the rate of translation (strong vs weak RBS).

c) **Start codon:** codon from which translation begins. It is always the codon for methionine. Thus, all proteins start with the amino acid methionine (but this can be removed in post-translational modifications).

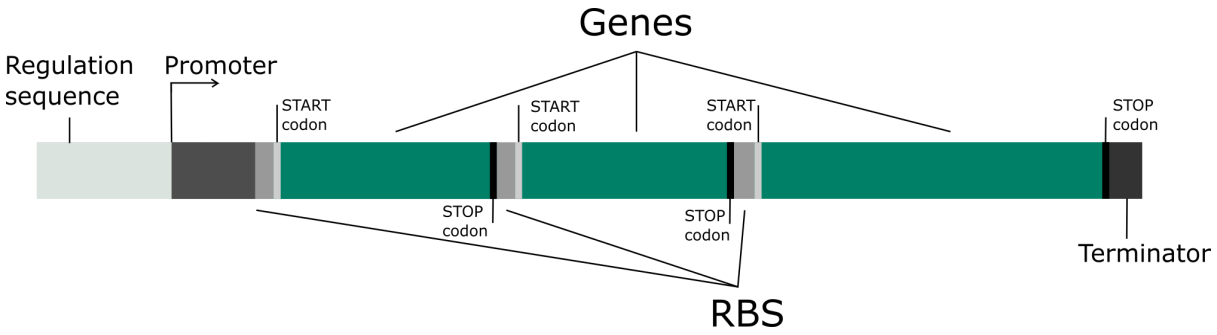
d) **Coding sequence:** a sequence in which triplets of nucleotides encode the amino acids of the target protein in the correct order. Amino acids are inserted by the ribosome according to the rules of the genetic code.

e) **Stop codon:** the last codon of the coding sequence. A sign for the ribosome not to add more amino acids and to release sequence.

f) **Terminator:** is necessary to terminate the transcription. It is a sign for RNA polymerase that the gene ends here. Again, there are different types of terminators according to their strength. E.g., if we are creating GMOs that respond to a given signal, or putting multiple unrelated constructs in a row, it is advisable to use a strong terminator to prevent the next gene from being transcribed as well.



Eukaryotic gene



Prokaryotic gene

Synthetic Biology Open Language (SBOL) standard visual symbols:

promoter	primer binding site
CDS	blunt restriction site
terminator	restriction site
ribosome entry site	5' sticky restriction site
ribonuclease site	3' sticky restriction site
operator	5' overhang
insulator	3' overhang

4. How we work with biological parts - GMO step by step (Practical part)

Obtaining the parts = isolation of the plasmid (5 minutes)

Plasmids are found in bacterial cells. If we wanted to work with them when they are inside cells, it would be very difficult and in some cases impossible. Therefore, we usually isolate them from cells into a solution that is easier to work with.
First we have to grow (expertly culture) a large number of bacteria from which we want to isolate the plasmid. This is normally done in a solution that contains all the necessary nutrients and the bacteria thrive in it. The cells are usually cultured in this way for 10 to 16 hours and then harvested from the solution.
Then we can proceed to the actual isolation of the plasmid. Nowadays, chemical kits from various companies are usually used for this. The kits contain everything you need and are easy to work with. First, the cultured cells must be broken down. Then the plasmid DNA is purified from chromosomal DNA, RNA and proteins. Then various impurities are washed off and finally the DNA is dissolved in a suitable solution.
The isolated plasmids are prepared in advance. In this course we'll break them down into their individual building blocks.

Separation of plasmids into individual parts - restriction cleavage (10 minutes)

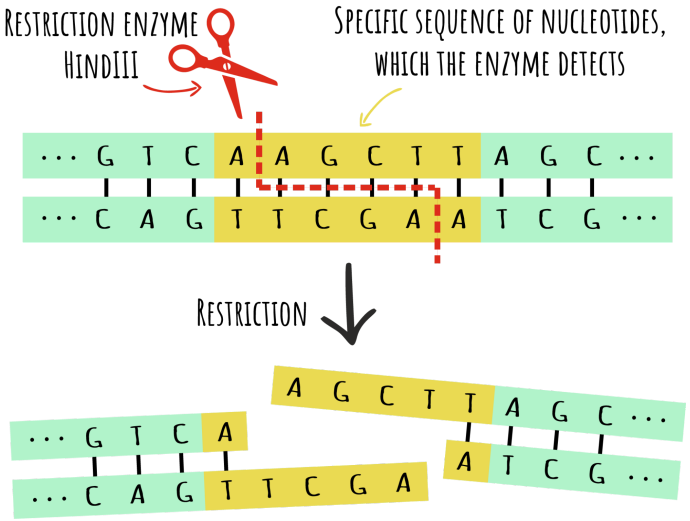
We have been showing a picture of a plasmid to show that it contains several parts - several building blocks. What often happens in practice is that we have two plasmids and we want to join together one part of plasmid A and another part of plasmid B. In our picture, for example, it would be "our gene" from plasmid A that we want to connect to the backbone of plasmid B.
In the picture, we can see that "our gene" is bounded by a short region called the cloning site. The cloning site contains special sequences called restriction sites. Restriction sites are so-called palindromic sequences - they read the same on both sides.
Students have a task in their lab notebook to identify the palindrome. For example, it's "No lemon, no melon."

Restriction sites are usually 6 base pairs long. The most common ones are, for example, EcoRI and HindIII, which we also have in the picture with plasmid. Their sequences are as follows (write on the board):

EcoRI: GAATTC
CTTAAG

HindIII: AAGCTT
TTCGAA

And what are restriction sites good for? These palindromic sequences are recognized by enzymes that can cleave the DNA at a given site,



creating so-called sticky ends. These sticky ends are then used to join multiple pieces of DNA together. Students have the following picture in their lab notebook:

Restriction cleavage - practical part (30 minutes + 20 minutes of cleavage)

Students work in pairs. They are given 2 isolated plasmids - A and B. Backbones A and B are different sizes and the inserts are also different sizes. Their task will be to cleave both samples, visualize them on a gel and determine which fragments they would use for their next work (backbone A and insert B).
On the tables there are prepared:

- A container of ice
- Stand for Eppendorf Tubes
- Eppendorf Tubes containing:
    - Plasmid A - 20 µl
    - Plasmid B - 20 µl
    - MULTI-CORE buffer from Promega - 6 µl
    - BSA from Promega - 1 µl
    - Nuclease-free water - 40 µl
    - 2 empty Eppendorf Tubes (tube A, tube B)
- Pipettes and tips
- Gloves

Students first calculate how much plasmid they will need to add to each reaction based on the plasmid concentrations and then use the table in the lab notebook to mix the Master Mix, which they then divide into two empty Eppendorf Tubes and add DNA (plasmid A → tube A, plasmid B → tube B). They then put the samples into 37 °C for 60 min to cleave.

Master Mix

		Master Mix					
Number of reactions	DNA	MULTI--CORE buffer	Restriction enzyme HindIII	Restriction enzyme EcoRI	BSA	H2O	Total volume
1		4 µl	1 µl	1 µl	0,4 µl		20 µl
2							

Plasmid A tube

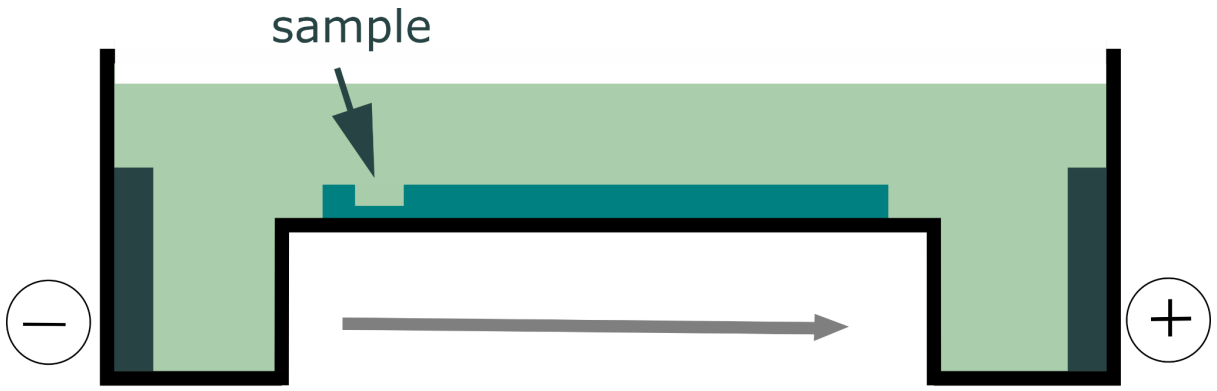
Reagents	How much to add?	Added?
Master Mix		
Plasmid DNA		

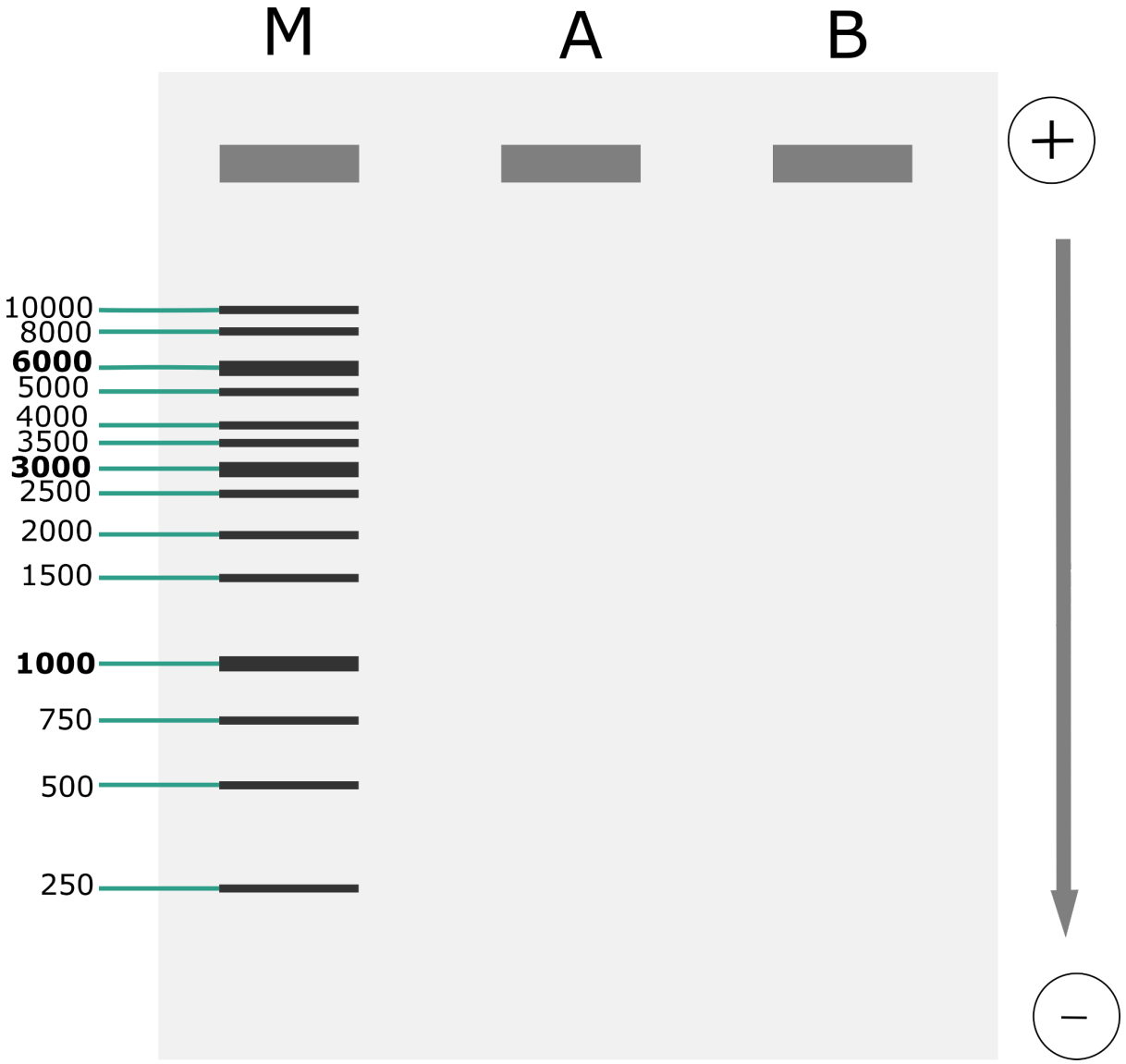
Plasmid B tube

Reagents	How much to add?	Added?
Master Mix		
Plasmid DNA		

Visualization of parts - agarose electrophoresis (5 minutes)

Electrophoresis is generally a laboratory method based on the fact that charged particles move non-randomly in an electric field. The speed at which these particles move depends on their size, shape, temperature, the amount of total surface charge, and the concentration of the substance in solution. Today, it finds its greatest application in work with nucleic acids and proteins.
Horizontal gel electrophoresis is one of the most basic and important methods used in laboratories of molecular biology. It has a number of advantages and applications when working with nucleic acids. It can be used for analytical and preparative purposes, but also as a purely informative method (whether the desired DNA fragment is present in the sample or not). This type of electrophoresis is based on the fact that nucleic acids are negatively charged macromolecules that move in an electric field towards a positive electrode - i.e. the anode. In gel electrophoresis we use an agarose or polyacrylamide gel as a matrix, but this is also another factor that affects the experiment. It acts as a sieve, is porous and, depending on the size of the pores (i.e. the concentration), influences the rate of passage of the nucleic acid particles through the gel. DNA or RNA fragments pass through these pores, and the larger ones have more difficulty passing through the porous gel and are delayed compared to smaller fragments. Thus, for a specified amount of time, nucleic acids are pulled through the gel by the electric force, and when the electric field is turned off, they stay where they arrived. This facilitates eventual separation by scalpel out of the gel, dissolution of the gel, and further work with fragments of known size or mass.

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Agarose gel electrophoresis - practical part (20 minutes preparation + 30 minutes run)

Before the course, the instructors will prepare 2 gels into which the students will pipette their samples. One gel will be prepared during the course so that students can see the procedure.

Gel preparation: 1 g of agarose per 100 ml of 1x TAE, add 5 µl of MIDORI when dissolved, pour into a gel tank, insert a comb and let it set in the dark.

After the restriction reaction, each pair mixes their samples with the loading buffer. They have 2 Eppendorf Tubes prepared on the tables, each containing 5 µl of the loading buffer. They will transfer the loading buffer into the tubes with restriction reaction and then pipette this mixture into the gel. Each person tries to pipette 1 sample into the gel, the ladder is pipetted by the lecturer. The gel is then run at 150 V for 30 minutes. After that it is observed under blue light on special transilluminators. Students are asked to show what the backbones of plasmids A and B are and what the inserts A and B are, and which they would cut and purify from the gel if they wanted to fuse plasmid A to insert B. They will determine the fragment sizes by comparing them to a ladder, the function of which will be explained by the instructor.

To explain DNA staining:
Ethidium bromide is chemically 3,8-diamino-5-ethyl-6-phenylphenanthridin-5-ium bromide. It is a substance often used in laboratories of molecular biology for its advantageous staining properties. It is able to bind to the structure of DNA and RNA and emit light when UV light is applied. It is therefore used to make nucleic acid samples visible. This substance is a potent mutagen and should therefore be handled with extreme caution. In laboratories, special facilities and special containers, pipettes and gloves are used only for working with ethidium bromide. Nowadays, it can already be replaced by less hazardous substances.

The lecturer will then explain that the fragments are cut out of the gel with a scalpel and purified with a set of chemicals (kit), and then they can be used for further experiments.

Combining the parts into new combinations - ligation (2 minutes)

The prepared parts of DNA (e.g. insert and backbone) can be joined together using the enzyme ligase and the sticky ends we have created during restriction digestion.

Inserting the new puzzle into the bacteria - transformation (5 minutes)

Special - competent cells are prepared so that they have a disrupted cell wall and easily take up DNA. Add the newly created plasmid to the competent cells and then force the bacterium to let it pass through its cell wall. This is done, for example, by a short heat shock - we put the cells in a 42°C water bath for a few tens of seconds. We then transfer the transformed cells to Petri dishes with an antibiotic, which ensures that only the cells into which we have managed to insert the new plasmid grow on the dishes.

And so we have cells that contain our new plasmid, and that will produce the protein that we have chosen. And now you can let your imagination run wild. What protein would you like to produce and in what organism?

6. Design your own Genetically Modified Organism (15 min)

Final activity. Students can choose from different biological parts from which they can create their own GMO with unique properties. They should not forget all the necessary parts of the gene. At the same time, their CDS will consist of a fusion protein (2 proteins+tag). Students will also consider which organism they would like to modify in this way. They will glue the result in their lab notebook.
Tag: a peptide tag that determines the localization or future fate of a protein. It often consists of a piece of sequence that is already present at the destination. For example, a tag for a mitochondrial protein will contain a piece of the mitochondrial surface protein sequence.

PROMOTER---RBS---GENE1---GENE2---TAG---TERMINATOR

All selected biological parts exist. And the creation of such organisms would be theoretically possible. But it's good to tell students that it's not as easy as it may look. Biological systems are often erratic, and what should work in theory may not work at all in practice. At the same time, ethical issues are an important aspect. For example, genetically modifying humans or human embryos is in the vast majority of cases unacceptable.

The table for this activity can be found at the end of this document.

What you need to prepare before the course

- Isolated plasmids A and B diluted to the same concentration
- Two 1% agarose gels

- **For each table**
  - A container of ice
  - Stand for Eppendorf Tubes
  - Eppendorf Tubes containing:
    - Plasmid A - 20 µl
    - Plasmid B - 20 µl
    - MULTI-CORE buffer from Promega - 6 µl
    - BSA from Promega - 1 µl
    - Nuclease-free water - 40 µl
    - 2 tubes, each containing 5 µl of loading buffer
    - 2 empty tubes
  - Pipettes and tips
  - Gloves

Signal for directing to flowers	Signal for direction to hair bulbs	Signal for targeting the genital organs
Signal to the brain	Signal for direction to seeds	Signal for direction to leaves
Signal to the eye	Signal for direction to teeth	Signal for targeting muscles
Signal to the surface of the body	Signal to target roots	Signal to.....
Gene for enlargement	Gene for increased performance	Gene for hair
Green fluorescent protein gene	Gene for higher number of offspring	Gene for exoskeleton
Gene for the production of pink fluorescent protein	Gene for reduction	Gene for venom
Gene for white spots	Gene for oxygen synthesis	Gene for resistance to water shortage
Gene for pest resistance	Gene for insulin	Gene for odour
Gene for striping	Gene for.....	Terminator
Promoter - light activation	Promoter - activation upon pathogen attack	Promoter activated all the time
Promoter - activation in the dark	Promoter - activation on maturation	Promoter - activation under stress
Promoter - dry activation	Promoter - activation in heat	RBS with high production
Promoter - wet activation	Promoter - activation during nutrient deficiency	RBS with low production