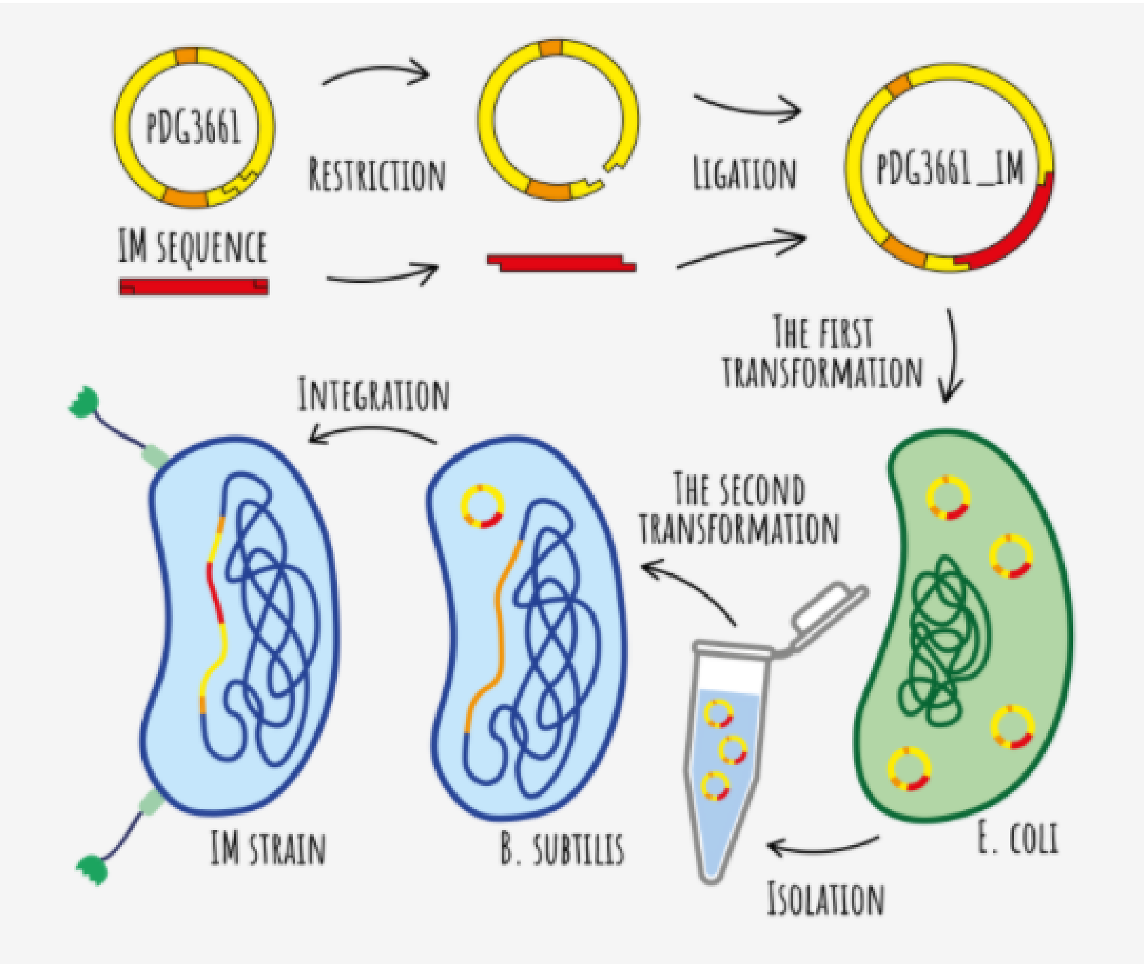
Laboratory Notebook

Synthetic Biology - Discover the Laboratory of Life

In today's course, we would like to introduce synthetic biology - a rapidly progressing scientific field that you might have already heard about, or will most likely encounter in the future. Synthetic biology combines biology and biochemistry while also looking at living organisms through the lens of engineering. As scientists figure out more and more about how certain characteristics are encoded and determined, they start to think about altering these characteristics, improving them, or using them elsewhere.



Name..............................   Date.................................

1. Safety first and foremost

a. What do I do if my GM culture spills on the ground?

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2. Synthetic Biology

"Synthetic biology is the combination of the tools of a biologist and the mindset of an engineer."

Definition: Synthetic biology is a new type of science that aims to redesign basic natural organisms and use them for artificial purposes. It creates new biological components and processes that do not exist in nature, or uses already existing ones differently while enhancing their importance and raising their yields.

3. DNA as a building blueprint

a. Why can we say that DNA is universal?

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b. What does this feature of genetic code allow us to do?

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c. List 3 properties that distinguish a plasmid from a chromosome:

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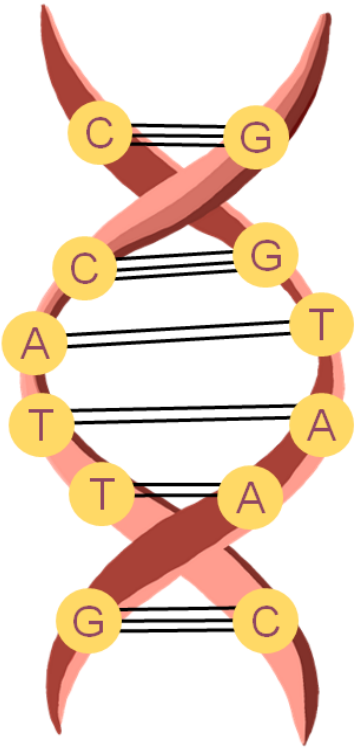
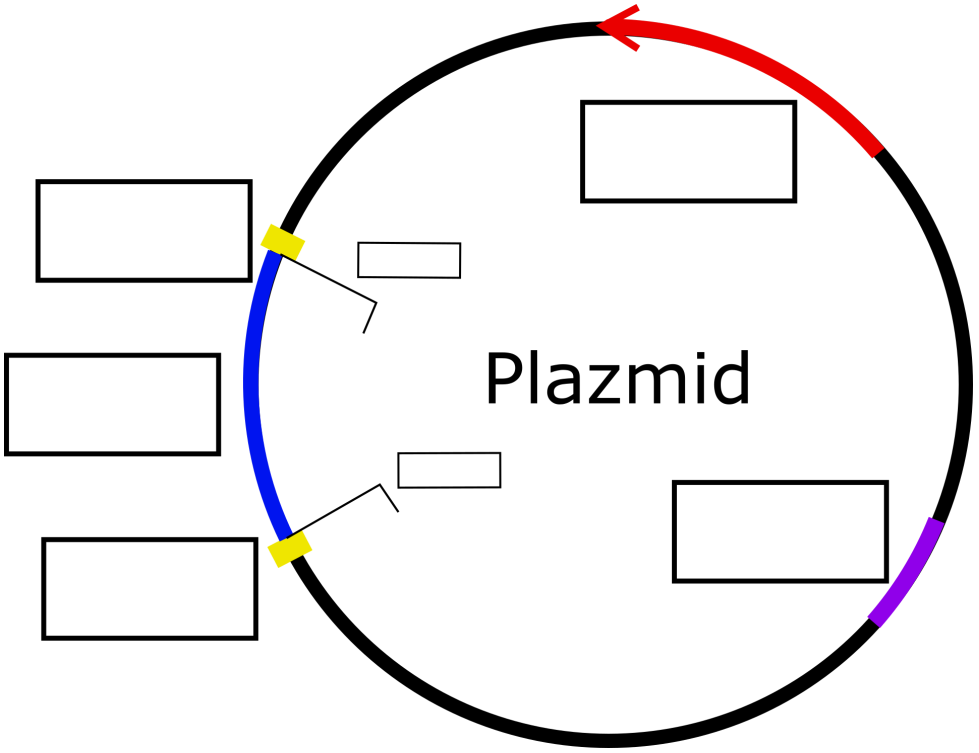


d. Describe the plasmid, filling in the correct names of its components in the empty squares.

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



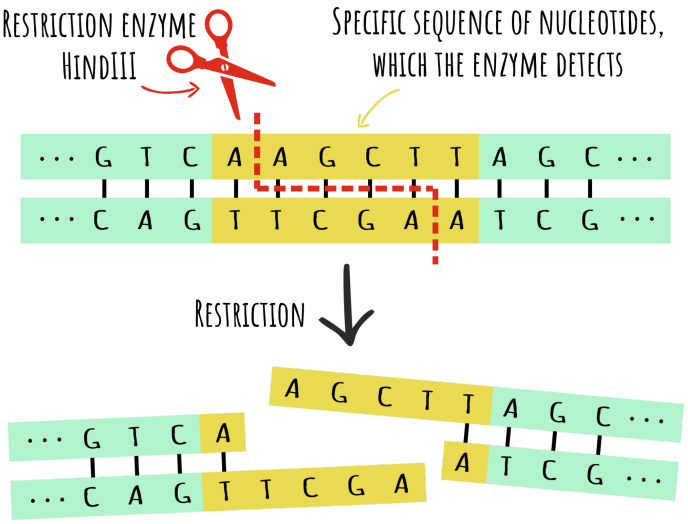
4. BioBricks

Fill in the names of the following parts in the table and add their functions:

Part of vector (Part)	SBOL symbol	Function
		
		
		
		

5. *Practical part: How we work with biological parts - GMO step by step*

In order to manipulate genetic information, we need to know in what form and in which molecules in the body this information is hidden. The important discovery of the structure of DNA in 1953 contributed to this understanding. Subsequent discoveries and the first successful experiments have shown that manipulation of DNA is possible.



A key step was the elucidation of the function of certain enzymes acting on DNA. ***Restriction endonucleases***, which allow us to cleave a DNA molecule at a precise location, are a tool not only in synthetic biology.

How to prepare the response:

1. Preparation of the reaction mixture:

		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							

- Calculate how many µl of H2O should be added to the reaction mixture to give a final volume of 20 µl.
  Calculation procedure .....
  .....
  .....

- Calculate how many µl of DNA from the **stock sample** will need to be added to the reaction mixture to give a reaction mixture containing approximately 1 µg.

  Calculation procedure .....
  .....
  .....

2. Preparation of samples:

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		

Procedure:

a) Reaction mixture:

1) Prepare a sterile microtube
2) Pipette the reagents in the following order: H2O, Buffer, BSA.
3) Vortex briefly
4) Add the restriction enzymes and **mix lightly by tapping the tube**

b) Sample:

1) Add plasmid DNA to the tubes.
2) Place the microtubes in a thermowell or water bath for 60 min at 37 °C
3) Stop the graft reaction by placing the microtubes in a thermoblock preheated to 80 °C for 10 min

3. Visualization = Agarose gel electrophoresis

a. ***What charge does DNA have?***

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b. ***Which part of the DNA carries this charge?***

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c. ***What makes the DNA on the gel visible?***

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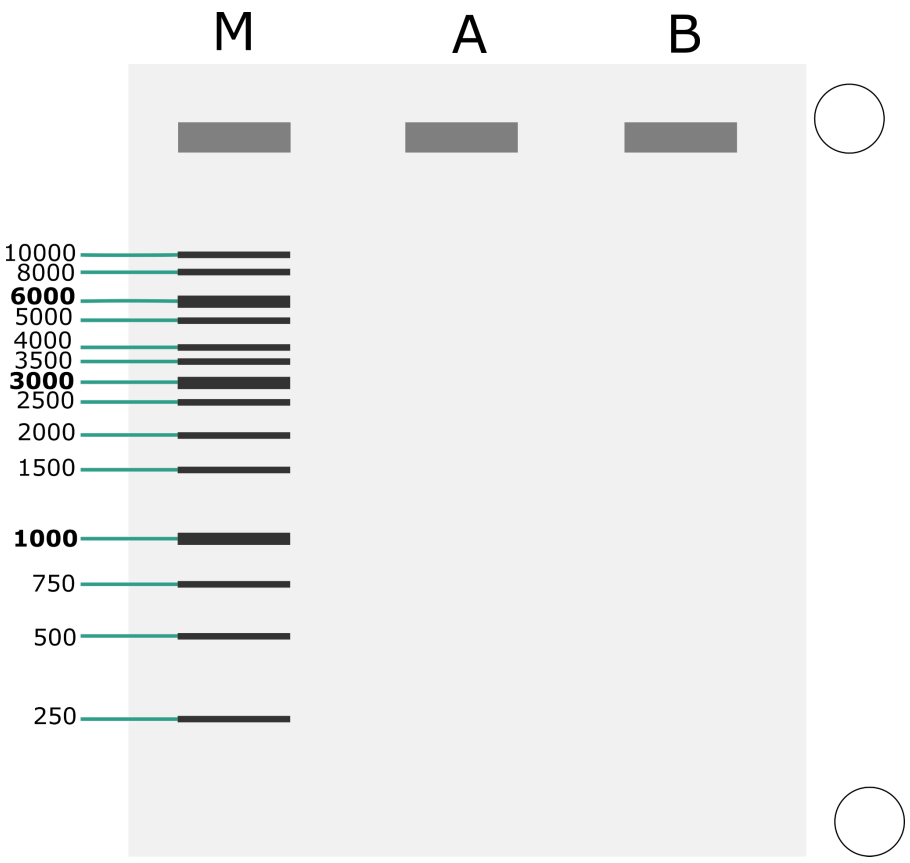
d. Describe the electrophoretic bath:

i. where the sample is pipetted
ii. add the electrode charge to the circles
iii. use an arrow to plot the direction of travel of the DNA

e. Plot the expected position of the restriction digestion products on the gel. Again, plot the direction of travel of the DNA and add electrode charge to the circles. Indicate which fragments you would work with next. *(M indicates Marker - in our case 1kb GeneRuler NEB)*



How is agarose gel prepared?

1) Weigh 1 g of agarose per 100 ml of 1x TAE and heat in the microwave
2) After dissolving, add 5 µl MIDORI and pour the liquid gel into the form
3) Insert the comb and let it solidify in the dark

Procedure for performing DNA electrophoresis:

1) Mix the digested DNA from the previous reaction with 5 µl of the loading buffer
2) Apply the entire volume (25 µl) to the selected well in the agarose gel
3) Set the power source to 150 V for 30 minutes and start
4) After electrophoresis, illuminate the gel with the blue light on the transilluminator
5) Decide which bands you would like to work with next (purification)

Ligation = joining of DNA molecules by a special enzyme

a. ***What is this enzyme called?***
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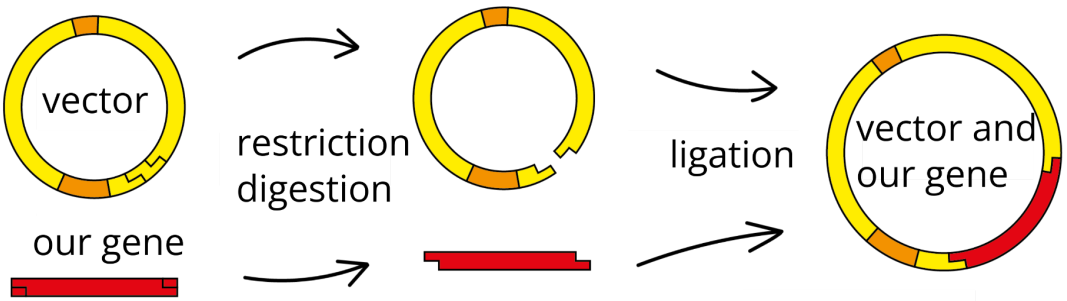

Transformation = acceptance of foreign DNA by the organism

b. ***How do we ensure that we only grow successfully transformed bacteria?***

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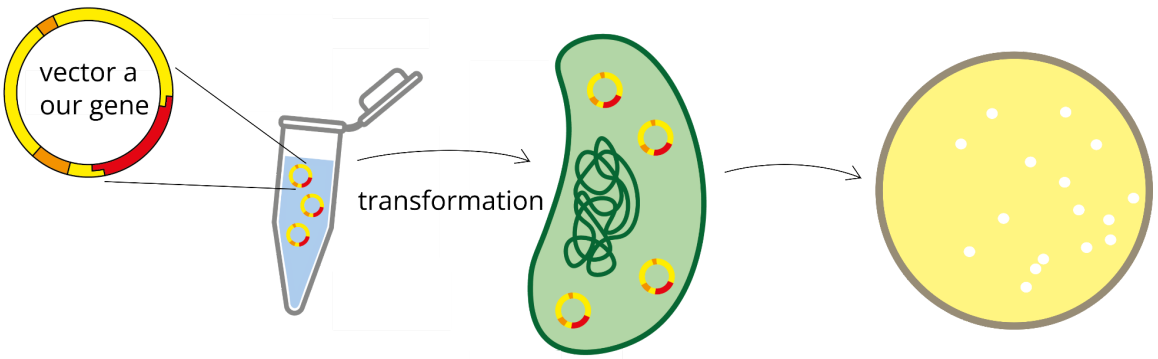

6. Design your own Genetically Modified Organism

a. ***Engage your imagination. What organism would I like to modify and how?***

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Design a functional fusion protein as follows:

PROMOTER---RBS---GEN1---GEN2---TAG---TERMINATOR

Tag/Signal: peptide tag determining the localization or further 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 mitochondrial surface protein sequence.