SYNTHETIC BIOLOGY

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. With the progress made in DNA manipulation, this is no longer just speculation, but a very real possibility.

The beginnings of synthetic biology

In order to manipulate genetic information, we must first understand how this information is encoded. One of the milestones on the way towards understanding genetic code was the discovery of DNA structure in 1953. Other experiments, for example the creation of recombinant DNA, then showed that manipulating DNA is possible.

Another big step was uncovering the function of certain enzymes that interact with DNA. Restriction endonucleases are vital for not only synthetic biology as they allow us to splice a DNA molecule. Unlike exonucleases, which gradually digest DNA molecules from their ends, endonucleases can split DNA at a specific place inside of the molecule, which is marked by a short sequence, composed of 4-8 nucleotides, with which the enzyme interacts. While some restriction endonucleases splice DNA molecules in the proximity of their binding sequence, others do so exactly inside of this sequence. These enzymes are the most useful as they allow us to determine the exact place where the DNA molecule will be split. As of today, there are 3 000 known enzymes belonging to this group of endonucleases.

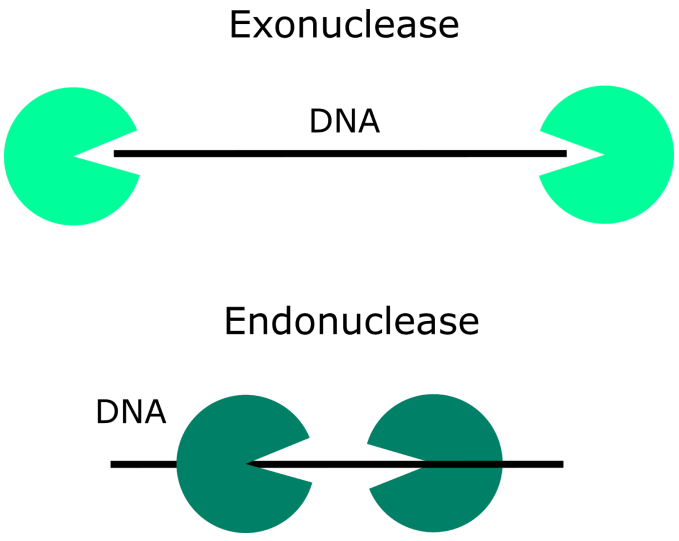

Figure 2. Endonucleases and exonucleases

Fun fact:

Some restriction endonucleases splice very common DNA sequences. Frequently used enzyme EcoRI digest the DNA at the palindrome sequence GAATTC (letters signify nucleotide bases: A = adenine, C = cytosine, G = guanine, t = thymine). Other endonucleases interact with fairly rare sequences.

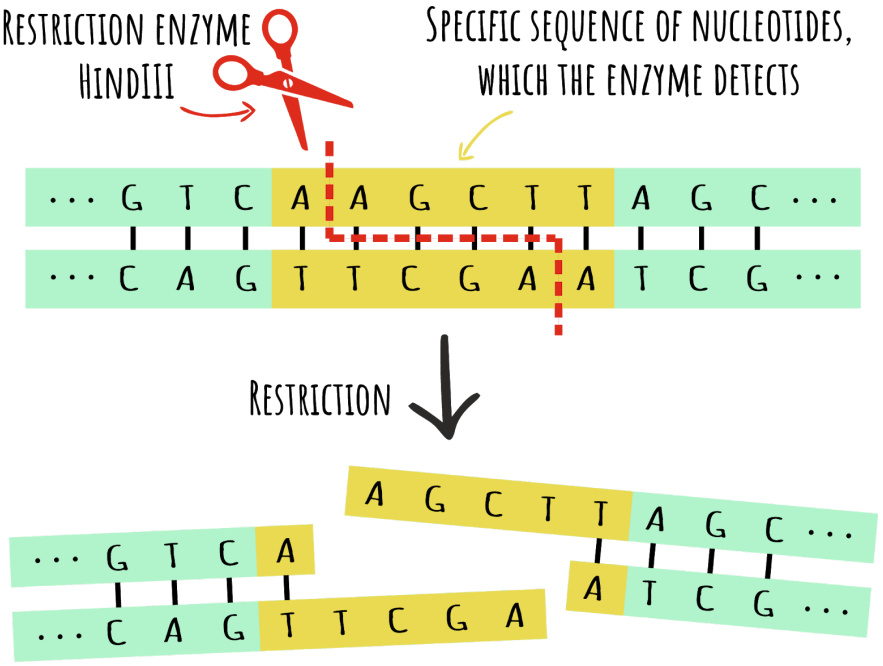

Figure 3. EcoRI binding and digestion site

Another important group of enzymes are DNA ligases, which can connect DNA molecules by their previously digested ends. DNA polymerases are also vital, as they can synthesize one DNA strand based on the sequence of the other strand or just fill in the gaps of a double stranded DNA molecule and thus fix some mistakes in the sequence.

This method of DNA manipulation has been used since the 70s. Based on this information, you might think that scientists can splice DNA molecules and then stick them together however they want and while it is a good base for understanding synthetic biology, there is much more to this process then that.

The importance of DNA sequence

So how do we know how to splice and connect the right DNA segments to achieve a specific result? In order to know what we are doing, we must first understand the function of a given DNA sequence.

First step is to uncover the order of nucleotide bases in our DNA molecule. This can be achieved by many different methods of DNA sequencing.

We should also understand the function of said sequence. This sequence can be transcribed into mRNA and then translated into a sequence of amino acids, which create a primary structure of a protein. This protein then carries out a certain function in the organism. However only 1-1.4 % of human DNA is used for protein synthesis. The rest of our DNA sequence is by no means redundant - some segments get transcribed into different RNA molecules, which then participate in a variety of processes in the cell and other sequences can interact with proteins, for example to regulate the expression of specific genes.

Regulatory DNA sequences can bind transcription factors which increase the effectivity of the transcription of a following DNA segment. Others can interact directly with DNA or RNA polymerases and their presence is thus necessary for the initiation of DNA replication of transcription. Some sequences also act as ribosomal binding sites (RBS). When transcribed into mRNA, these sequences can bind to ribosomes and thus initiate the translation of the rest of the mRNA sequence into the order of amino acids in a protein.

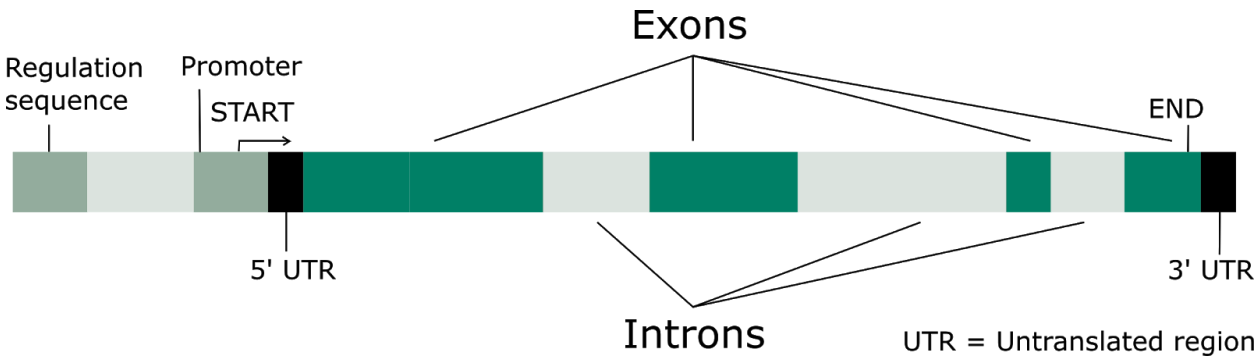

Figure 4. DNA sequence

BioBricks

The term BioBrick in synthetic biology, is a label used for any functional DNA sequence that can behave in some of the ways mentioned above. In synthetic biology, we can look at a living organism as a machine, whose characteristics are determined by its DNA - a construction plan

composed of many different parts (or BioBricks), which can be added, removed, altered or switched. There are many different kinds of BioBrics, each carrying out a specific function in the DNA sequence.

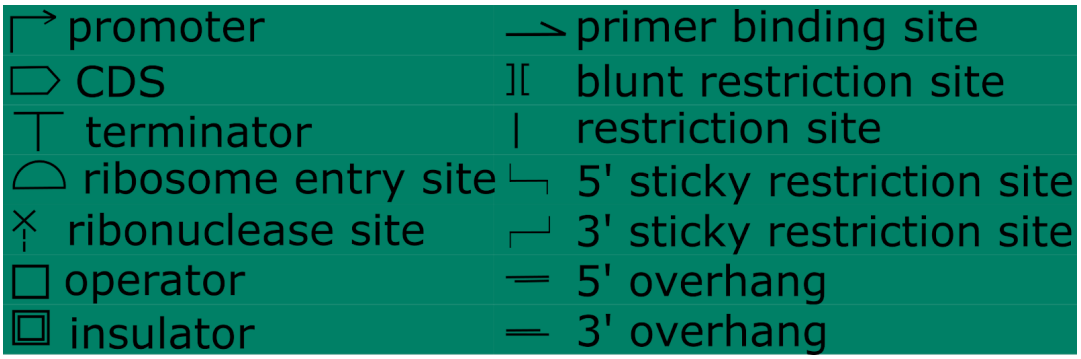

Figure 5. BioBricks

And what organisms do we work with?

At the center of most research into synthetic biology are simpler organisms with smaller numbers of genes and lesser complexity. An organism with an entirely known genome sequence is also much easier to work with. One of the most thoroughly studied organisms is *E. coli,* a gram negative bacterium, that is a natural part of human intestinal microbiome. Another group of organisms often associated with synthetic biology are yeasts such as *Saccharomyces cerevisiae.* Surprisingly, these organisms are evolutionarily closer to humans than to bacteria. These organisms are frequently used for the production of enzymes or other protein-based substances. Yeasts have the advantage of having more complex mechanisms of protein synthesis and thus allow us to mass-produce proteins which require these mechanisms for altering mRNA molecules before translation or helping fold the newly synthesised

proteins. Recently there have been some experiments with altering the known genomes of plants. Tobacco plants and *Arabidopsis thaliana* for example seem to be well suited for this area of research.

Fun fact:

Uses of synthetic biology will be further discussed in a following course - Synthetic biology II. Many additional information about this topic will also be discussed at our presentation about Genetically modified organisms. For now we can use the tobacco plant as an example. Some bacteria have enzymes which allow them to change toxic form of mercury Hg^{2+} into a nontoxic Hg^{0}. If we palace the genetic sequence coding these enzymes into the genome of a tobacco plant, these plants can also degrade toxic mercury and thus are able to grow in toxic soil, while gradually getting rid of the source of its toxicity.

You have arrived at the end of our first course looking into synthetic biology. Don't forget to try completing our quiz to test your knowledge. We will be looking forward to seeing you next week.

Sources:

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Quiz

1. **Which vital discovery was made in 1953?**
   a) The structure of DNA was described for the first time (YES)
   b) The first microscope was made
   c) Recombinant DNA was made for the first time

2. **How many nucleotides tend to be in a binding site for restriction endonucleases?**
   a) 1-2 nucleotides
   b) About 6 nucleotides (YES)
   c) 100 nucleotides

3. **The main function of DNA ligases is:**
   a) Splicing RNA molecules
   b) Connecting DNA molecules (YES)
   c) Digesting the ends of DNA molecules

4. **Polymerases can**
   a) Connect DNA molecules
   b) Splice DNA molecules in the middle
   c) Synthesize missing nucleotides of one DNA strand (YES)

5. **The use of restriction endonucleases is:**
   a) Splitting DNA on the inside of the molecule (YES)
   b) Connecting DNA molecules
   c) Synthesizing missing nucleotides of one DNA strand

6. **What is the purpose of RBS?**
   a) To bind restriction endonucleases
   b) To bind to ribosomes (YES)
   c) Digestion of food

7. **How much of the human genome is translated into a protein sequence?**
   a) Large majority
   b) 40 %
   c) Around 1 % (YES)

8. **BioBricks are**
   a) Functional "parts" of the DNA (YES)
   b) Structures present on the leaves of genetically modified plants
   c) Enzymes

9. ***Saccharomyces cerevisiae* is a:**
   a) Bacterium
   b) Yeast (YES)
   c) Plant

10. **Gene for the digestion of toxic mercury is originally from:**
   a) Tabacco plant
   b) Yeast
   c) Bacterium (YES)

CERTIFICATE

CONGRATS! YOU'VE REACHED THE LEVEL COFFEESHOP SLACKER, WHICH MEANS YOU'VE GOTTEN 0-25% CORRECT ANSWERS.

SOMEONE IS SLACKING OFF AT CAFES, INSTEAD OF ATTENDING THEIR LECTURES! IT IS CLEAR THAT YOU'VE GOT SOME GAPS IN YOU KNOWLEDGE ABOUT SYNTHETIC BIOLOGY. IT'S FINE THOUGH, EVERYONE HAS THEIR STRENGHT AND WEAKNESSES.

THANK YOU FOR BEING A PART OF #GENERATIONM

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CERTIFICATE

CONGRATS! YOU'VE REACHED THE LEVEL SLEEPING STUDENT, WHICH MEANS YOU'VE GOTTEN 25-50% CORRECT ANSWERS.

TAKING A NAP INSTEAD OF ATTENDING YOUR LECTURE? UNFORTUNATELY, IT HASN'T REALLY PAID OFF IN THIS CASE. YOUR RESULTS ARE NOT THE BEST, BUT THERE IS DEFINITELY AN OPPORTUNITY FOR GROWTH. HOW ABOUT STARTING RIGH AWAY?

THANK YOU FOR BEING A PART OF #GENERATIONM

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CERTIFICATE

CONGRATS! YOU'VE REACHED THE LEVEL HARDWORKING STUDENT, WHICH MEANS YOU'VE GOTTEN 50-75% CORRECT ANSWERS.

YOUR RESULT IS GREAT, CONGRATS! AS A HARDWORKING STUDENT YOU SURELY KNOW A TON OF FACTS, THEORIES AND EXPERIMENTS. BUT DO YOU REALLY KNOW EVERYTHING IMPORTANT? THERE IS STILL SOME JOURNEY AHEAD OF YOU. GOOD LUCK!

THANK YOU FOR BEING A PART OF #GENERATIONM

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CERTIFICATE

CONGRATS! YOU'VE REACHED THE LEVEL iGEM PARTICIPANT, WHICH MEANS YOU'VE GOTTEN 75-100% CORRECT ANSWERS.

WOW! CONGRATULATIONS FOR ACHIEVING THE BEST RESULT POSSIBLE. AS AN iGEMer, YOU SHOULD KNOW SYNTHETIC BIOLOGY AS WELL AS YOUR PROJECT LIKE THE BACK OF YOUR HAND. KEEP UP THE HARD WORK!

THANK YOU FOR BEING A PART OF #GENERATIONM

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