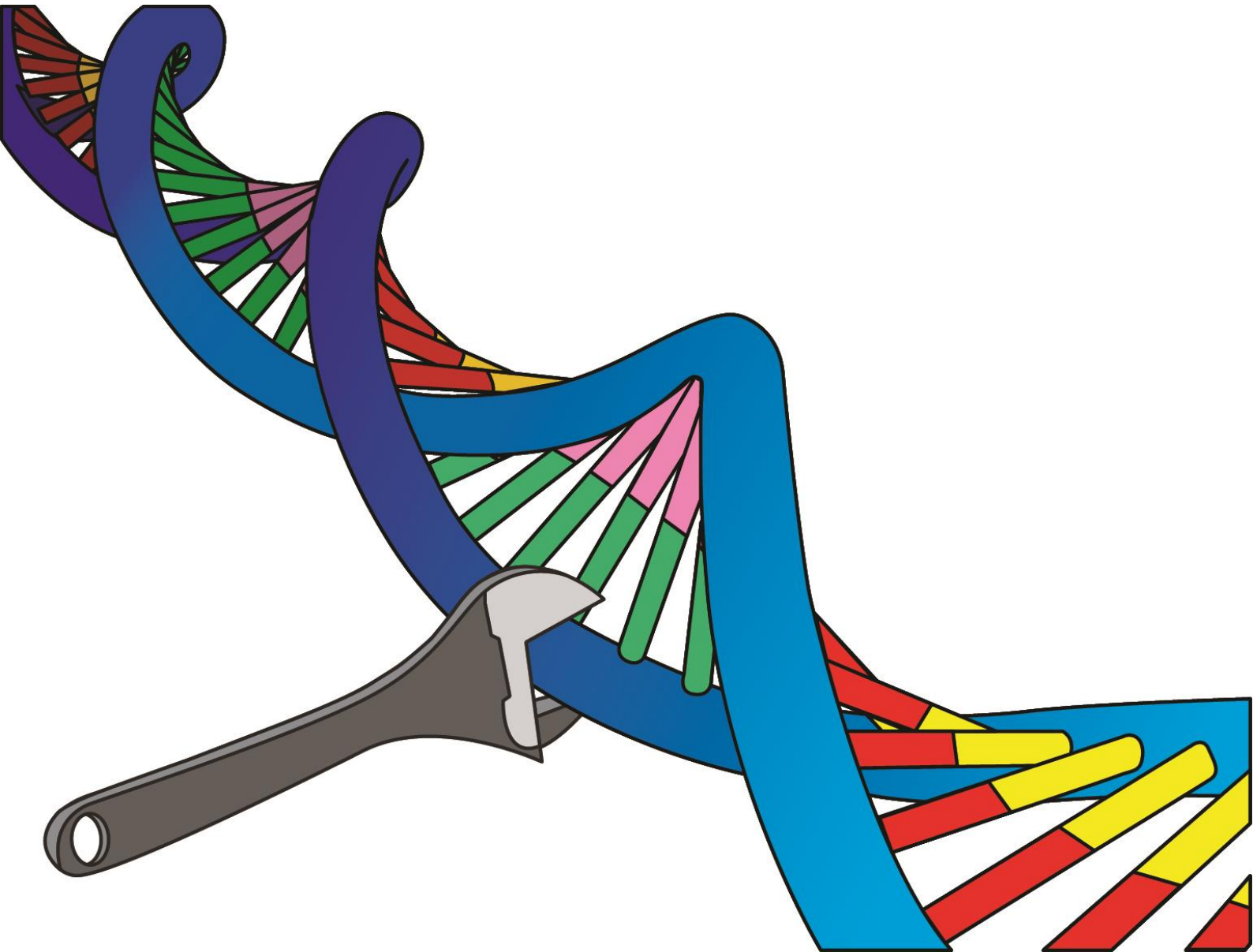
Name:
Class:

Synthetic biology

What is possible and advisable?



Colophon

Universiteit Utrecht

synenergene
Responsible Research and Innovation in Synthetic Biology

Freudenthal Instituut
voor Didactiek van Wiskunde en Natuurwetenschappen

v3.1

This teaching module was developed by the Freudenthal Institute for Science and Mathematics Education, within the framework of the European SYNENERGENE project.

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PART 1
Synthetic biology:
What is it and what can you do with it?

Video

You will be shown a video about synthetic biology. First read the following assignment.

https://youtu.be/UHBdEwNbXI0

Assignment 1

At 2.50 the video is paused. What is synthetic biology, according to the video?

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Several techniques and applications of synthetic biology are mentioned during the second part of the video. Fill in the following table after watching the video.

Technique	**Application**
DNA cutting and pasting / recombinant DNA technology	*E. chromi*: detects various concentrations of a toxic chemical

History

When biologists started working with physicists, chemists and technologists around the start of the twentieth century this led to great developments. These include the development of biotechnology and of new techniques such as recombinant DNA technology and DNA sequencing. When biologists began to cooperate with information scientists and engineers as well, this led to the rise of synthetic biology (see figure 1).

So synthetic biology (synbio) is a scientific field in which various specialisms cooperate. With using synbio, existing techniques such as recombinant DNA technology and DNA sequencing are developed further. Researchers can use these improved techniques to design and build new biological systems. They can for instance insert new functions into an existing cell, tissue or organism, or create new cells from scratch with synbio.

To better understand what synbio is, we compare it to a computer: in synbio, the software (DNA) is designed and inserted into the hardware (the cell). Different software provides different programs (processes in a cell). By cutting, pasting and combining parts of the software many programs, with different functions, can be designed. You can also develop software that normally doesn't occur in your hardware. Synbio also lets you build your own hardware.

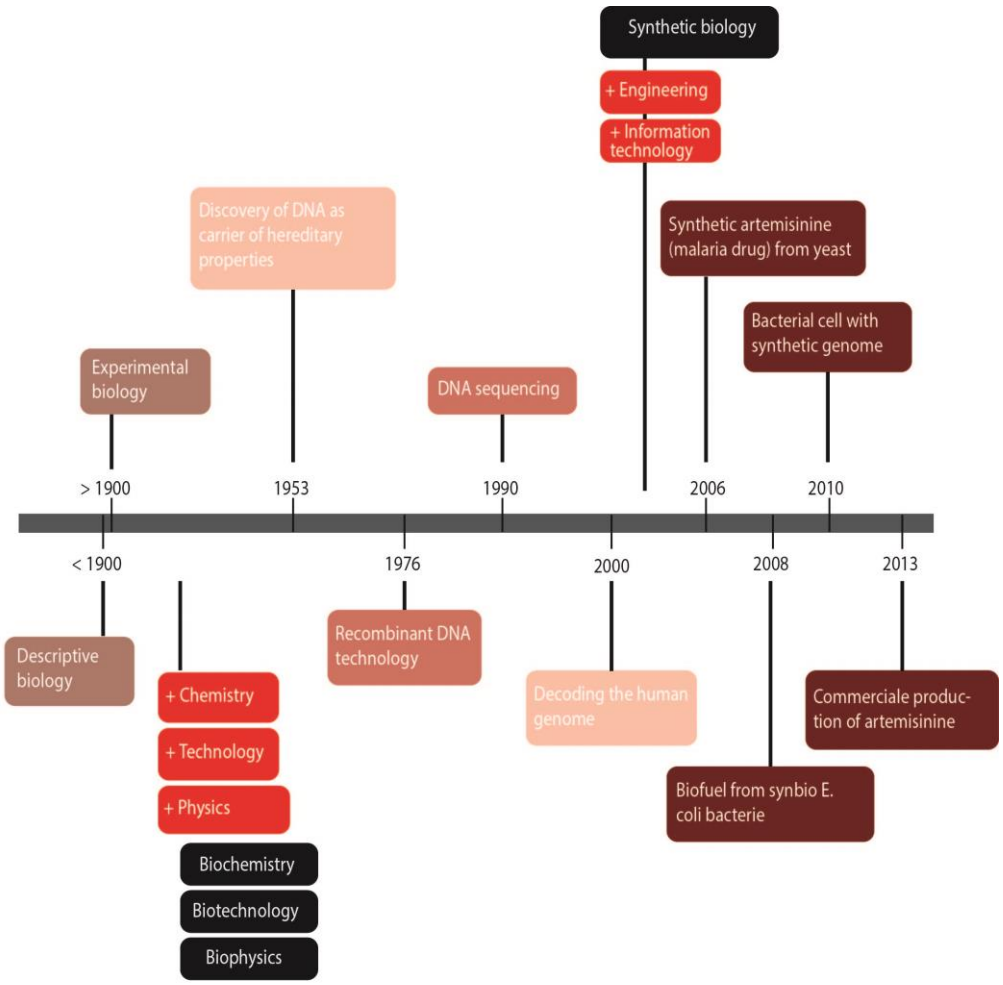

Figure 1: History of synthetic biology

Techniques

Synthetic biology is based on **recombinant DNA technology**. In figure 2 you can take another look at how this works.

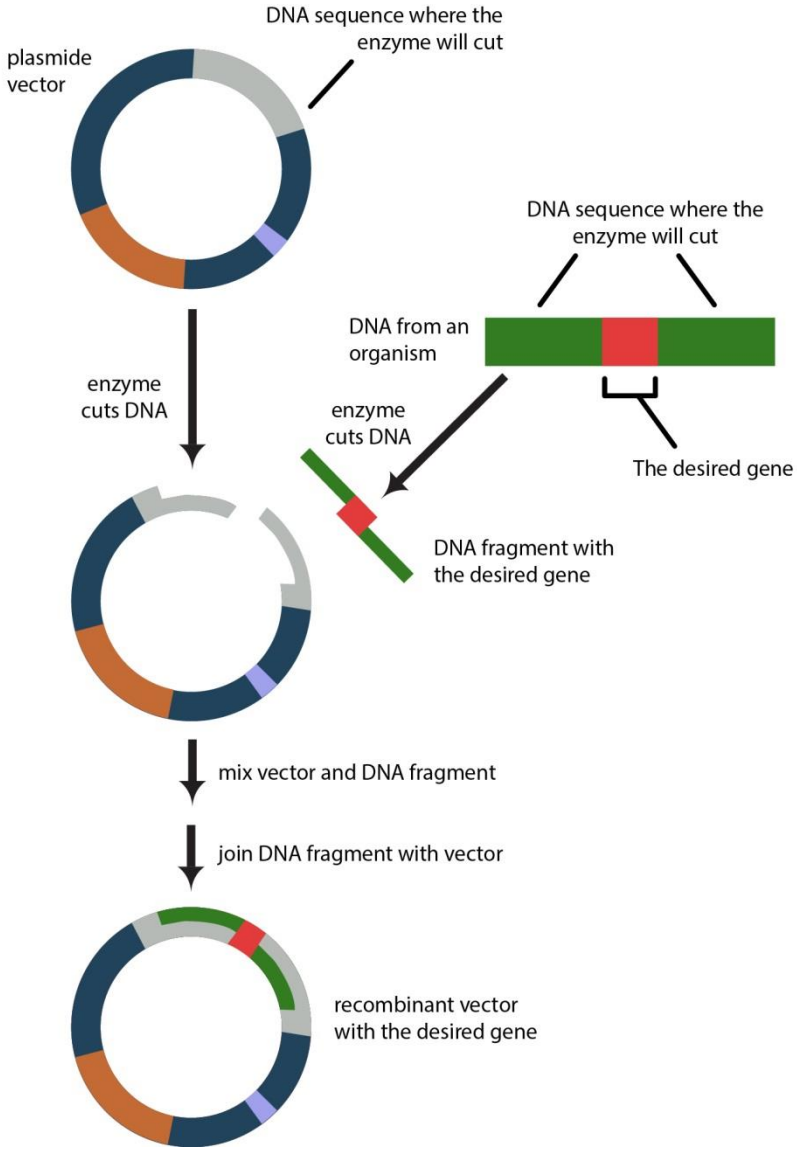

Figure 2: Recombinant DNA technology

With synthetic biology researchers no longer have to cut the desired bits of DNA from existing DNA: they can design the desired DNA themselves and order it online. The DNA is then produced synthetically by a machine, using sugar as a source material. Researchers can also order **BioBricks** from an online database. These are bits of DNA with a specific function (for instance, they code for a particular protein) that have been designed to be combined easily. BioBricks are therefore also called 'plug-and-play DNA'. There are several types of BioBricks, for example:

- BioBricks with only a coding gene or a part of the DNA that can regulate a gene.
- BioBricks that contain the coding gene as well as all parts that regulate this gene.
- BioBricks consisting of multiple genes that together perform a function.

Researchers can use BioBricks to change an existing organism, for example a yeast cell. This works as follows (figure 3):

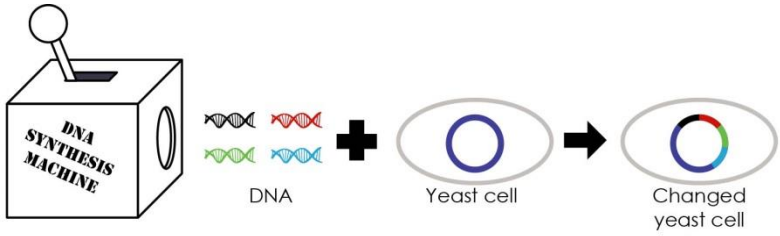

Figure 3: Changing a host with synthetic biology techniques. In this case the host is a yeast cell.

Assignment 2

Show the difference between traditional recombinant DNA technology and recombinant DNA technology as it is used in synthetic biology. To do so, change figure 2: show in this figure how synthetic biology helps you to get the desired gene. You can for example scratch out parts of the figure or add new parts.

Researchers also try to create **minimal cells**. These are cells that only contain the genes that are necessary to survive. In the future, researchers might be able to add BioBricks to these minimal cells, to have the cells perform specific functions, such as manufacturing a drug.

Minimal cells can be made in two ways: top-down and bottom-up. **Top-down** means that a researcher adapts an existing cell. To make a minimal cell, a researcher would remove as many genes as possible from the cell, until only the genes that are necessary for the cell to survive and divide are left behind (figure 4). **Bottom-up** means that the researcher builds a cell from scratch. The researcher writes the DNA, or makes use of BioBricks. By only selecting the genes the cell needs to survive and divide, you end up with a minimal cell (figure 5).

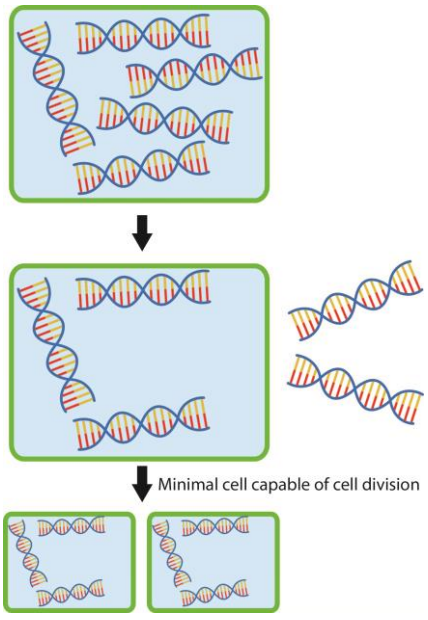

Figure 4: Minimal cell made top-down

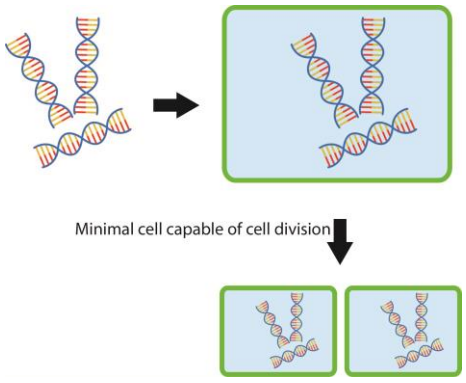

Figure 5: Minimal cell made bottom-up

Assignment 3

What is the advantage of inserting a BioBrick in a minimal cell (possible future method), compared to inserting a BioBrick in an existing organism such as a yeast cell (current method)?

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Applications

Synthetic biology has only been used for about ten years, but already an impressive number of applications has been developed.

Cheap anti-malaria drug

The malaria drug **artemisinin** was originally obtained from a plant, sweet wormwood (*Artemisia annua*). This process is expensive and there wasn't always enough of the drug available. By synthesizing the genes for the production of artemisinin with synthetic biology and inserting them in yeast (figure 6), yeasts can now produce the drug quickly and cheaply in a reactor vessel. A pharmaceutical company is using this method to produce artemisinin, resulting in about a 100 million malarial treatments a year.

Sweet wormwood

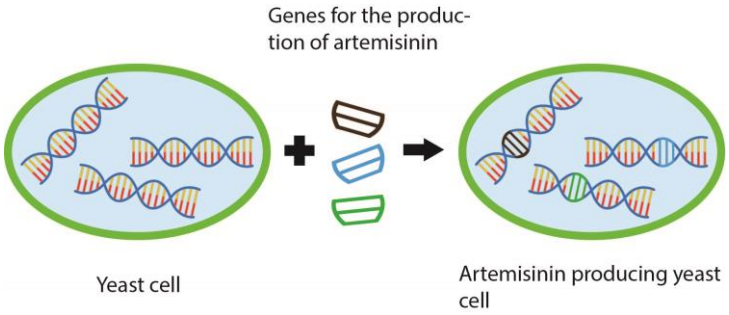
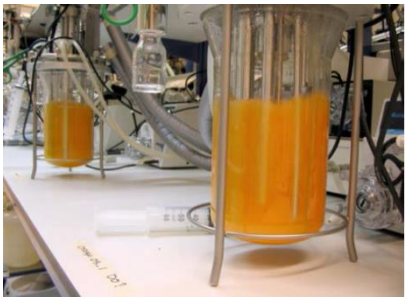


Figure 6: The synthesized genes for the production of artemisinin are inserted in the yeast DNA. The yeast can now produce artemisinin.

Sustainable fuel

Bio-ethanol is an alcohol that can be used as sustainable fuel. Bio-ethanol is made using baking yeast that can convert sugars present in corn into bio-ethanol. This can result in corn becoming too expensive as a food crop. Synthetic biology makes it possible to use agricultural waste products such as straw and corn foliage as raw materials for bio-ethanol. This has been realized by adding genes to baking yeast that can convert the sugars in waste products into bio-ethanol.



Lab with yeasts converting waste-sugars into bio-ethanol.

The first factory using this method to produce bio-ethanol opened in 2014.

PART 2
Choosing an iGEM-application of synthetic biology

In the video at the start of the lesson you heard about the iGEM-competition: an international competition between teams of students, who use BioBricks to invent and test applications of synthetic biology. With your group you will develop an iGEM-application. You will present this application to your class in the third lesson.

Who are in your group? Names:

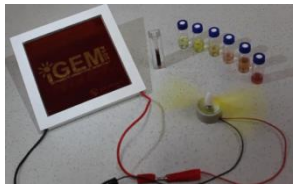
The applications

Select, in consultation with your teacher, an iGEM-application that you want to develop. You can choose from the following four applications:

1. LactoAid - a smart bandage for burns

Staphylococcus aureus and *Pseudomonas aeruginosa* infections are often the cause of complications during the treatment of burns. Therefore, in 2014 the iGEM-team in Groningen developed a new kind of bandage. This bandage prevents these infections and reduces the use of antibiotics. The bandage contains a gel with genetically modified *Lactococcus lactis*. This modified bacterium detects the abovementioned pathogens in the wound, and responds by producing the anti-microbial compound *nisin*, among other things.

2. Grätzel cells – sustainable energy

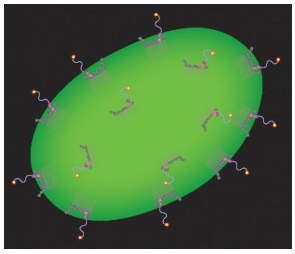


Many African countries have a limited availability of electricity, and have to cope with power failures. This is a problem for both the general population and the economy. Therefore the iGEM-team from Darmstadt developed ''Grätzel cells'' in 2014. A Grätzel cell can produce electricity like a solar cell, but can also do so under hard conditions like cloud cover or a sandstorm. The main component of the Grätzel cell is *anthocyanidin* (a plant pigment), produced by the *E. coli* bacterium.

3. BananaGuard – saving the banana from extinction

Banana plants worldwide are under threat from *Fusarium oxysporum:* a fungus that lives on banana plants, ultimately killing them. In response, in 2014 the iGEM-team from Wageningen developed the BananaGuard. The BananaGuard is a genetic system in the bacterium *Pseudomonas putida* that detects the presence of *Fusarium oxysporum* and then produces a fungicide which kills the fungus.

4. Click Coli – a coat for bacteria



A fundamental problem in using genetically modified bacteria is that they do not survive well in certain circumstances even though we would like them to, like in a reactor vessel or in the human body. That is why the iGEM-team from Eindhoven developed the Click Coli in 2014. The Click Coli is a kind of coat that can be 'clicked' on to *E. coli* bacteria. This coat enables the genetically modified *E. coli* bacteria to survive longer in the human body, so that they can be used for therapies.

The presentation

In the third lesson your group has to give a **5 minute** presentation on your iGEM-application. During the presentation you should answer the following questions:

1. What problem does the application solve and how?
    - Is there another solution for this problem? If so, what is it? And why is the iGEM-application better?
2. What is the application?
    - What is the name of the application, how does it work and what does it look like?
3. What technique(s) was/were used to make the application?
    - Provide a schematic drawing to clarify how the application was made.
    - How is the gene/BioBrick that is necessary for the application expressed?
4. What are the consequences of the application? → you will work on this in the next lesson (in part 4 'How advisable is your application?', pg. 13).

Homework

Find the information you need to answer questions 1 to 3 for the next lesson. Divide these tasks within your group.

Use one of the following websites for this:

- **LactoAid:** http://2014.igem.org/Team:Groningen,
  see appendix 1, pg. 21 for a clarification of this iGEM-application.
- **Grätzel cells:** http://2014.igem.org/Team:TU_Darmstadt,
  see appendix 2, pg. 23 for a clarification of this iGEM-application.
- **BananaGuard:** http://2014.igem.org/Team:Wageningen_UR,
  see appendix 3, pg. 24 for a clarification of this iGEM-application.
- **Click Coli:** http://2014.igem.org/Team:TU_Eindhoven,
  see appendix 4, pg. 25 for a clarification of this iGEM-application.

PART 3
Elaborating the iGEM-application

Share the information you found and write down the answers to the following questions. You have to present this to the whole class in the next lesson.

For each question an example of an answer is given using artemisinin, to give you some idea of what to fill in.

Assignment 1: What problem does the application solve and how?

- *What problem does the application solve?*

Malaria is a disease that many people suffer from each year. The current drug against malaria is expensive and in short supply. Artemisinin is a drug against malaria.

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- *How does the application solve this problem?*

Artemisinin from yeast can be produced simply, cheaply and on a large scale. As a result the drug is cheaper and in good supply.

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- *Is there another solution for this problem? If so, what is it?*

Yes, the current malaria drug is won from the plant sweet wormwood. Attempts are being made to optimize the yield from this plant.

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- *If there already is a solution why is the iGEM-application better?*

Optimizing the yield from sweet wormwood doesn't result in enough affordable artemisinin, while our solution does.

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Assignment 2: What is the application?

- *What is the application called?*

Artemisinin.

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- *How does the application work?*

The adapted yeast cell produces artemisinin.

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Assignment 3: What technique(s) was/were used to make the application?

- *What technique(s) was/were used to make the application?*

Synthesizing DNA with the most optimal nucleotide sequence to produce artemisinin in yeast, and insert it in the DNA of yeast using recombinant DNA technology.

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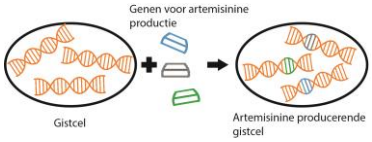
- *Provide a schematic drawing to clarify how the application was made.*



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PART 4
How advisable is your iGEM-application?

So, how advisable is your iGEM-application? What are its advantages and disadvantages? You will think about this individually and with your group.

Individually

Assignment 1

Would you like the iGEM-application to come into production? Why or why not? Write this down in short for yourself.

Opinion:

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Arguments:

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With your group

Discuss with your group whether you would want the iGEM-application to come into production. Explain to each other why you think so. Listen to what everybody is saying and try to understand what the others are trying to say. If it's unclear what someone is saying or why they think something, ask for clarification. For example:

- "What do you mean by …?"
- "Why do you think so?"

Assignment 2

Write down the conclusion. If you haven't reached agreement, write down the different points of view.

Conclusion:

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Assignment 3

Write down the arguments for your conclusion. Put each argument on one post-it note. You will need these post-its for question 5.

From different perspectives

For the following questions, you will use the **perspectives chart** (pg. 25). The chart contains five perspectives you can use to look at new developments: progress, economy, risk, ethics and globalization. You will now consider the advisability of the iGEM-application from these five perspectives.

Assignment 4

Read the descriptions of the five perspectives and the given examples. Place the post-its with arguments that you made for assignment 3 under the various perspectives in the chart.

Assignment 5
Fill in the chart further; from each of the perspectives, provide an example in favor of and against the iGEM-application.

Assignment 6
What perspective do you use in your group when you think about the iGEM-application?

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Assignment 7
Did using different perspectives change your opinions and/or arguments about why you would want the iGEM-application to come into production or not?

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The next assignment must be included in your presentation.

Assignment 8
What are possible social consequences of your iGEM- application? Include at least one positive and one negative consequence in your presentation. Make use of your completed perspective chart for inspiration.

- *What possible positive consequence(s) does the application have?*

Better control of malaria, more people being cured.

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- *What possible negative consequence(s) does the application have?*

If artemisinin is produced in yeast, there is not as much need for sweet wormwood. This is bad for the economy in the countries producing it, such as Vietnam and countries in Eastern Africa.

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- *Looking at these consequences, do you still believe the application is a good idea? Why do you think it is or isn't?*

Yes, being able to cure more people from malaria is more important than the economic risks.

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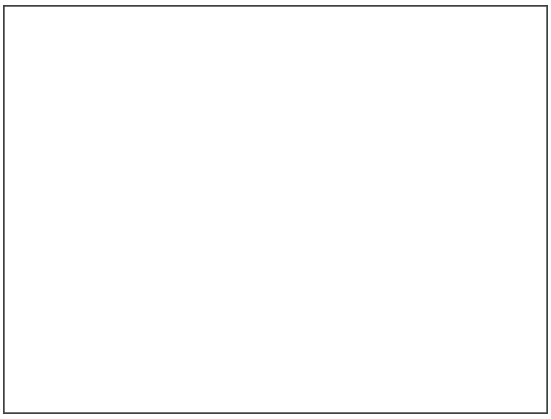
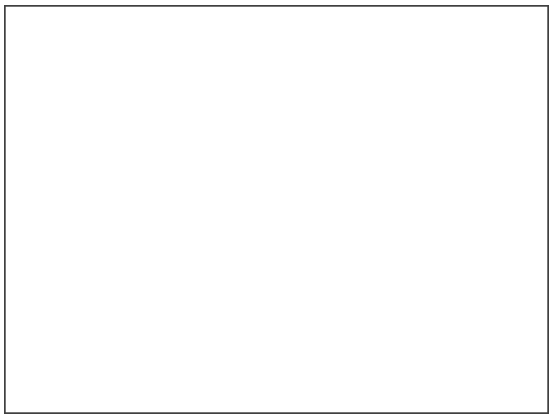
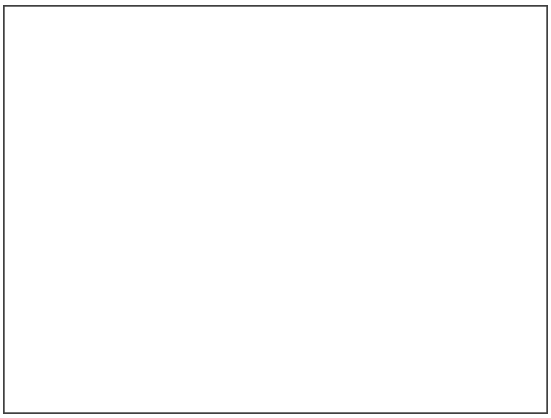
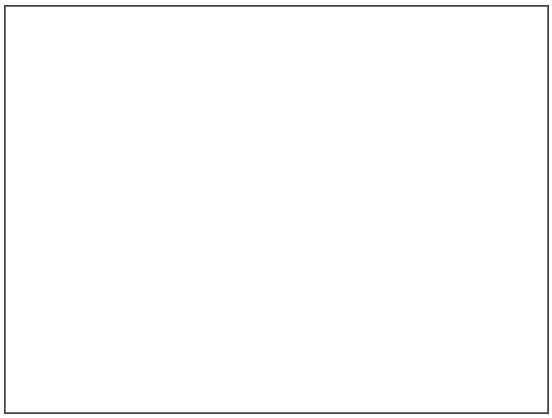
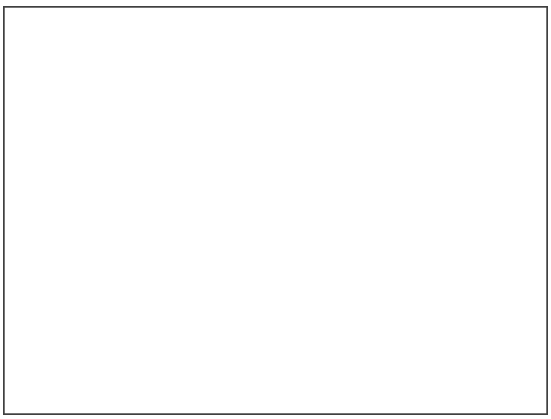
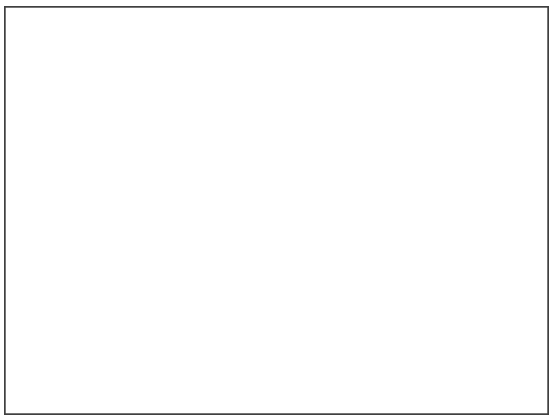
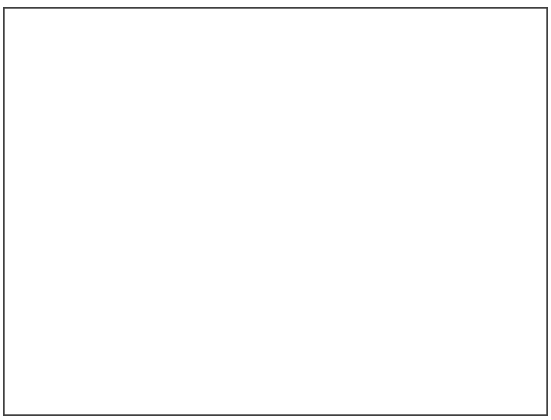
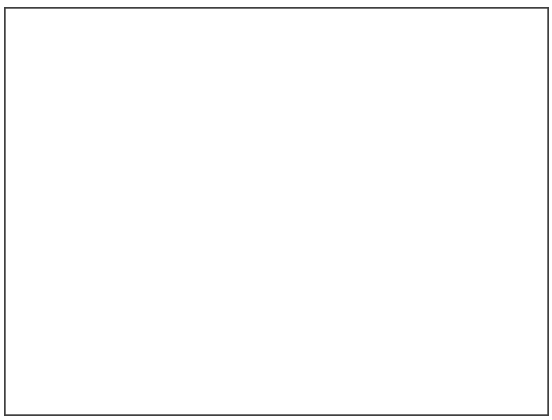
Homework

*Prepare a **5 minute** presentation for the next lesson. In the presentation, answer the four questions below (which you answered during part 3 and 4). Conclude your presentation with **a statement** on the advisability of your iGEM-application.*

1. What problem does the application solve and how?
    - Is there another solution for this problem? If so, what is it? And why is the iGEM-application better?
2. What is the application?
    - What is the name of the application, how does it work and what does it look like?
3. What technique(s) was/were used to make the application?
    - Provide a schematic drawing to clarify how the application was made.
    - How is the gene /BioBrick that is necessary for the application expressed?
4. What are the consequences of the application?
    - What are the positive consequences?
    - What are the negative consequences?
    - Do you believe the application should come into production? End your presentation with a statement on the advisability of the application.

You can use the slides found on the following page to work out the structure and content of your presentation.

PART 5
Presentations and dialogue

Presentations

For each presentation, write down which application and technique are being shown, and briefly jot down your opinion on the advisability of the application.

Presentation by:	Application:	Technique:	Opinion:

Dialogue

During the whole-class dialogue you will consider the advisability of the iGEM-applications from the various perspectives, as well as the advisability of synthetic biology.
First, go into dialogue with the class, fill in the following table afterwards.

After the dialogue, write down arguments from the dialogue below that you think are important in relation to the advisability of synthetic biology.

	Arguments IN FAVOR	**Arguments AGAINST**
Progress		
Economy		
Risk		
Ethics		
Globalization		

Your opinion about synthetic biology

Do you find synthetic biology advisable? Why?

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Did your opinion and/or your arguments about the advisability of synthetic biology change as a result of the presentations and the whole-class dialogue? Why?

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Appendix 1:
Explanation on the iGEM project LactoAid

http://2014.igem.org/Team:Groningen

The following explanation may help you to better understand the LactoAid project. In addition, it will be useful to re-read the chapter on gene expression from your textbook to understand the meaning of the terms promotor, inductor, operon and repressor.

The texts on the website mention three bacteria: *Pseudomonas*, *Staphylococcus* and *Lactobacillus*. The first two are pathogens. The iGEM-team wants to use the third (*Lactobacillus*) as the packaging ('chassis') for the gene construct they are going to make. The following explanation only discusses *Pseudomonas*; **in your presentation you can also just discuss *Pseudomonas***.

Pseudomonas aeruginosa (abbreviated as P.a. from here on) has the ability to use '**quorum sensing'**. Quorum sensing is a form of gene regulation in which the bacterium creates cell signals to which the bacterium itself responds. If the density of the bacteria increases, the concentration of the cell signal also increases and these signals serve as inductor, that is to say that they can switch on genes. Since these cell signals and genes belong to the same bacterium, we also call these signals **autoinducers**. Genes that are switched on by these signals influence the 'behavior' of P.a., including the production of toxins (virulence) and the formation of biofilms. **Biofilms** are bacterial layers that partially protect the bacterial cells laying in the layers. Through the system of quorum sensing P.a. will therefore only perform certain activities once the bacteria have reached a certain density. If you can disturb that system, you can therefore reduce the virulence of the bacteria.

The basic idea of the iGEM design is to use the cell signals/autoinducers in P.a. to switch on genes in another, harmless bacterium (*Lactobacillus*). The genes that are switched on, take part in fighting the pathogen in two ways: they code for an enzyme that breaks down the cell signal (*AHL lactonase*) and for a protein that stops the production of biofilm (*Dispersine*). The genes for these molecules have been artificially introduced in *Lactobacillus*. The iGem-team has constructed other parts around these genes that are required to make the whole construction work.

Please note:

- Sometimes the texts indicate P.a.'s cell signals/autoinducers with *PAI* (AI from Auto-Inducer) and sometimes with *AHL*.
- The cell signals cannot enter *Lactobacillus* on their own. This requires a cell membrane compound: LasR. *LasR* connects with the cell signal PAI. This connection forms the inductor that is able to bind with the promotor pLasI, thus switching on the gene.
- RBS means Ribosome Binding Site, which is the part of the DNA that codes for the RNA that connects to the ribosome in protein synthesis (so every gene has an RBS).
- To make the gene products work, they have to be able to exit the *Lactobacillus* cell, since they have to act on the pathogen. Because the gene products cannot cross the membrane themselves, this too requires a supporting molecule.

On the following page you will find a table with key concepts. If you define these concepts, the application will become more comprehensible.

Key concepts for LactoAid

Autoinducers	
Biofilm	
Inductor	
Operon	
Promoter	
Quorum sensing	
Repressor	
Ribosome binding site	

Appendix 2:
Explanation on the iGEM project Grätzel cells

http://2014.igem.org/Team:TU_Darmstadt

The following explanation may help you to better understand the Grätzel cells project. In addition, it will be useful to re-read the chapter on gene expression from your textbook to understand the meaning of the terms promotor, inductor, operon and repressor.

This project involves the production of a chemical that can be used as a pigment in a photo cell that can produce energy: *Pelargonidin*. To create this pigment from the initial amino acid *Tyrosine* six enzymes are needed, with the genes coming from different organisms. These are inserted in a special *Escherichia coli* (*E. coli*) strain: *BL21*(*DE3*). In this strain the promotor *T7* from the lactose operon has been inserted. Normally this promotor is induced by lactose, but this project uses *ITPG,* which is similar, but which isn't broken down and therefore remains present in the cell.

A chain of enzymes in a reaction chain works better if they are also close together spatially, as you can also see in the process of oxidative phosphorylation. This is achieved by building a **scaffold** (a sort of protein bench) on which three different enzymes from the reaction chain are always present together.

Finally the project also has a **Kill switch** that kills the bacteria as soon as there no longer is an overflow of glucose. This stops the modified bacteria from surviving outside the test environment.

Please note:

- The texts on the website are careless about the difference between the gene and the gene product (the protein). Sometimes the proteins are mentioned, rather than the genes for these proteins.

Key concepts for Grätzel cells

This table contains key concepts of Grätzel cells. If you write down definitions in the table, the application will become more comprehensible.

Photo cell	
Inductor	
Kill switch	
Operon	
Pigment	
Promoter	
Repressor	
Scaffold	

Appendix 3:
Explanation on the iGEM project BananaGuard

http://2014.igem.org/Team:Wageningen_UR

The following explanation may help you to better understand the BananaGuard project. In addition, it will be useful to re-read the chapter on gene expression from your textbook to understand the meaning of the terms promotor, inductor, operon and repressor.

The bacterium *Pseudomonas putida* was selected because it isn't sensitive to the effect of *fusaric acid* from the fungus, thanks to a pumping system that can pump the chemical out of the cell. The transcription of the genes for this pump is probably switched on by fusaric acid, through binding the *repressor pp1262*. Because fusaric acid binds to the repressor, the promotor of the underlying genes is accessible and these genes are expressed. As a result the protein pump that removes fusaric acid from the cell is produced. You can also use this system to show the presence of fusaric acid, by including genes for a pigment instead of those for the pumping proteins (**reporter gene**).

The next step is to place genes coding for molecules that combat the pathogenic fungus *Fusarium oxysporum* in a gene construct with the above gene regulation (repressor that connects to fusaric acid followed by releasing the promotor).

Finally systems should be included that stop the spread of the modified bacteria and transfer of plasmids (**horizontal transfer**). There is a double safety mechanism:

1. A **Kill switch** that eliminates the bacteria when there no longer is *Fusarium* in the soil (witch activates after the fungus is eliminated!).
2. A system that stops horizontal transfer. Only bacteria that have *two* different plasmids survive, because each of the plasmids has a different toxin + the antidote for the toxin from the other plasmid. Transfer of 1 plasmid to another bacterium (horizontal transfer) is therefore always fatal.

Key concepts for BananaGuard

This table contains key concepts of the BananaGuard. If you write down definitions in the table, the application will become more comprehensible.

Horizontal transfer	
Inductor	
Kill switch	
Operon	
Plasmid	
Promoter	
Reporter gene	
Repressor	

Appendix 4:
Explanation on the iGEM project Click Coli

http://2014.igem.org/Team:TU_Eindhoven

The following explanation may help you to better understand the Click Coli project. In addition, it will be useful to re-read the chapter on gene expression from your textbook to understand the meaning of the terms promotor, inductor, operon and repressor.

This project produces a membrane protein which functions to protect the cell. Therefore the protein needs a connector on the outside of the cell, where other molecules can connect to.

The technique involves the following:

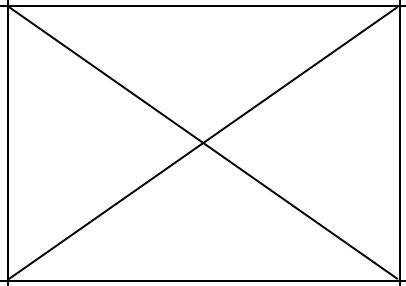
- In a specific location in the gene for the membrane protein a stop codon (TAG) is inserted. Therefore normal translation will not produce a complete protein.
- A variant (*orthogonal*) tRNA-synthetase is used, that doesn't read a 'stop' for TAG but inserts an amino acid that is also a variant (*pAzF*). So, in the presence of the variant synthetase and the variant amino acid, a protein with the variant amino acid included will be produced.
- Because other molecules (*DBCO*) can in turn bind to this variant amino acid, and those other molecules can in their turn link to a fluorescent molecule, it is possible to measure to which degree the membrane protein is present on the outside.

Key concepts for ClickColi

This table contains key concepts of ClickColi. If you write down definitions in the table, the application will become more comprehensible.

Amino acid	
Inductor	
Membrane protein	
Operon	
Ortogonal	
Promoter	
Repressor	
Stop codon	

Appendix 5: Perspective chart

	Progress	**Economy**	**Risk**	**Ethics**	**Globalization**
Description	What can the application mean in terms of progress? Are there any disadvantages to this progress?	What can the application mean in terms of economic growth? And who would profit from that? Could it lead to an unfair distribution of profit? Or will it have a negative economic effect? And who will be affected by that?	What potential risks are associated with the application? What are the risks to mankind and nature?	Is the application ethically responsible: is this allowed? Do we want this? Where do we draw the line?	What are the consequences of the application at a worldwide scale? Does it improve the position of our country in the world economy? Is there a benefit for third-world countries?
Example	Argument in favor: many patients can be cured from breast cancer with this application.	Argument in favor: selling the application can make a lot of money. This is good for the economy.	Argument against: you don't know what the consequences are if the application (with synthetic DNA) is accidentally released in nature.	Argument against: we shouldn't just use and change nature for our own advantage.	Argument against: Third-world countries cannot afford the application, leading to even greater differences.
Argument in favor					
Argument against					