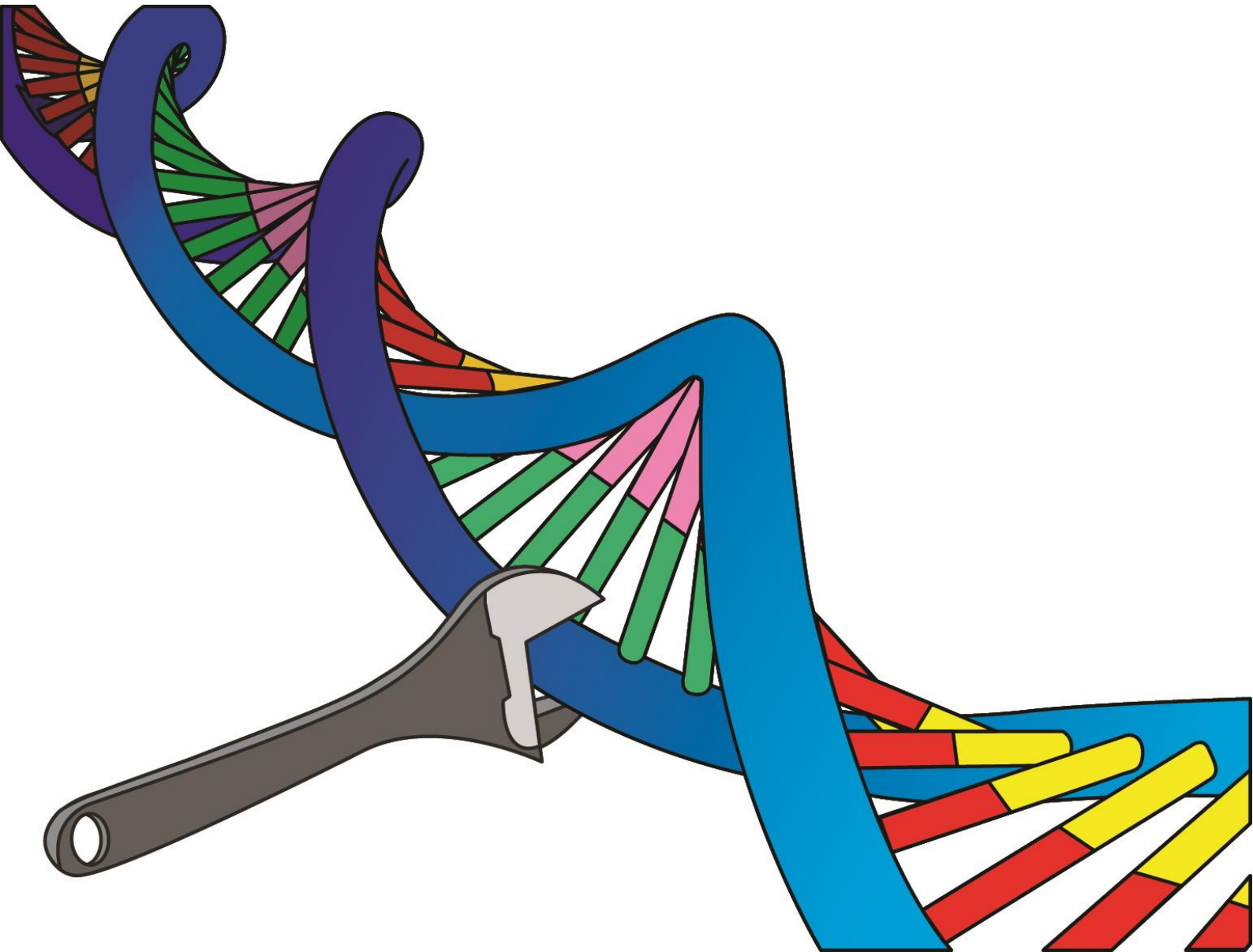
Teacher Guide

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

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Authors
Miranda Overbeek, Marie-Christine Knippels, Dirk Jan Boerwinkel, Liesbeth de Bakker, Vera Weetzel, Michiel van Harskamp
Special thanks to Wouter Spoor.

English translation
Nathalie Kuijpers; Michiel van Harskamp

Illustrations
Jenty Heijstek

Design
Miranda Overbeek

Please contact the Freudenthal Institute at fisme@science.uu.nl if you have any questions or comments.

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Content

Level and learning goals

Level	Upper secondary havo/vwo [pre-academic, age 16-18]
Subject	Biology
Required prior knowledge	Before this lesson module pupils should have completed the following themes: • Genetics • DNA • Bio technology (pupils should be familiar with techniques such as recombinant DNA technology)
Learning goals	After completing the module, pupils should be able to: • Describe what synthetic biology (synbio) is. They can indicate two aspects: designing and subsequently adapting or building either existing or new biological systems, and the interdisciplinary character of synbio; • Name and recognize (iGEM-)applications of synbio; • Consider the advisability of synbio applications from five different perspectives; • Give their considered opinion about synbio (applications); • Explain and apply gene expression and regulation within the context of synbio
End terms (Dutch curriculum)	This module supports among others the following (sub)domains of the Dutch biology exam program [*translations of end terms provided*]: • A1 Informatievaardigheden gebruiken [*Using information skills*] • A2 Communiceren [*Communicating*] • A9 Waarderen en oordelen [*Valueing and evaluating*] • A14 Systeemdenken [*System thinking*] • B1 Eiwitsynthese [*Protein synthesis*] • C1 Zelforganisatie van cellen [*Self organization of cells*] • C3 Zelforganisatie van ecosystemen [*Self organization of ecosystems*] • D1 Moleculaire interactie [*Molecular interaction*] • D2 Cellulaire interactie [*Cellular interaction*] • E2 Levenscyclus van de cel [*Cell cycle*]
Duration	3 x 50 minutes

Introduction

Synthetic biology (synbio) is a new interdisciplinary field that is experiencing rapid growth. Synbio has a lot of potential to solve problems which relate to for example health, food and energy, but there are also potential risks. In this module pupils are supported in forming an opinion about synbio. They learn what synbio is and what its possibilities are. They will also, working in groups, work on an application of synbio and give a whole-class presentation. The module ends with a dialogue on the advisability of the applications. Over the course of the module the pupils apply their knowledge of gene expression and regulation.

Background information for the teacher

The interdisciplinary scientific field of synbio has emerged over the last ten years or so, through cooperation between biologists, engineers and information scientists. Synbio further develops existing techniques from biotechnology, such as recombinant DNA technology and DNA sequencing, so that existing and new biological systems can be adapted, designed and constructed. This is also referred to as *engineering* biological systems. While in classic recombinant DNA technology the necessary DNA sequence had to be cut from existing DNA, with synbio DNA sequences can be synthetized, and DNA can be ordered online. The DNA can be selected from a database, or be designed. Another option is BioBricks, standardized DNA parts with a specific function. These allow adapting existing systems as well as creating new ones.

To learn more about synbio, you can use the following links:

- The virtual learning platform about synthetic biology of the Freudenthal Institute, containing among other things background information and materials: http://www.fi.uu.nl/synenergene/index.php?language=1&category=0
- There is a theme page about synbio on NEMO Kennislink with background information, applications and interviews: https://www.nemokennislink.nl/kernwoorden/synthetische-biologie
- The magazine of the Stichting Biowetenschappen en Maatschappij [*Foundation for Bio Sciences and Society*] offers extensive (background) information about synbio: http://assets.kennislink.nl/system/files/000/230/891/original/Synthetische_biologie.pdf
- A social reflection on the rise of synthetic biology by the Rathenau Institute: https://www.rathenau.nl/nl/files/leven-makenpdf

Structure of the module

For an extensive overview of the lesson module, including the expected learning effects of the various components, see the hypothetical learning trajectory in appendix 2 (pg. 20).

Lesson 1

1	Introduction	5 min	Activate pupils with question
2	Video: https://youtu.be/UHBdEwNbXI0	10-15 min	Introductory video about synbio and filling in technique/application chart
3	Lesson module part 1: 'Synthetic biology: what is it and what can you do with it?'	20-25 min	Content-based whole-class item on the history, techniques and applications of synbio
4	Lesson module part 2: 'Selecting an iGEM-application of synthetic biology'	10 min	Choosing which of the four iGEM-applications they want to work on and form a group
5	End of lesson	5 min	Homework: finding information about iGEM-application

Les 2

1	iGEM-application	5 min	
2	Lesson module part 3: 'Working on the iGEM-application'	15-20 min	In groups, pupils work on the iGEM-application based on their homework and part 3
3	Lesson module part 4: 'How advisable is your iGEM-application?'	15-20 min	In groups, pupils consider the advisability of their iGEM-application
4	Preparing presentations	10 min	Time to work on presentations in class
5	End of lesson	5 min	Homework: finish presentation

Les 3

1	iGEM-application	5 min	
2	Presentations	20-25 min	Every group has 5 minutes to present their iGEM-application. Pupils make notes in part 5 of the module
3	Short discussion on the content of the iGEM-applications	5 min	Based on the types of BioBricks used
4	Dialogue	15-20 min	Dialogue on the advisability of the iGEM-applications and synbio in general. Afterwards, pupils write down arguments in part 5 of the module
5	Writing down opinions about synbio and end of lesson	5-10 min	Pupils write down their opinion about the advisability of synbio in module part 5 and whether they changed their mind since lesson les 2

Instructions per lesson

Lesson 1

1	Introduction	5 min	Activate pupils with question
2	Video: https://youtu.be/UHBdEwNbXI0	10-15 min	Introductory video about synbio and filling in technique/application chart
3	Lesson module part 1: 'Synthetic biology: what is it and what can you do with it?'	20-25 min	Content-based whole-class item on the history, techniques and applications of synbio
4	Lesson module part 2: 'Selecting an iGEM-application of synthetic biology'	10 min	Choosing which of the four iGEM-applications they want to work on and form a group
5	End of lesson	5 min	Homework: finding information about iGEM-application

Needed

- Smartboard or projector for video (duration: 6.30 min).
    - Dutch subtitles are available by clicking the 'CC' icon on the right in the YouTube video.
- Module (1 per pupil), printed in color (for legibility of illustrations).

1. Introduction (5 min)

- Start with a question to activate the pupils' (pre-existing) knowledge:
    - If synbio has been discussed in the class before, for example: 'Do you remember what synthetic biology is, …?' or 'Does anyone know an application of synbio?'.
    - If synbio hasn't been discussed before in the class, for example: 'Has anyone heard of synthetic biology?' or 'Do you have have an idea what synthetic biology could be, …?'.
- Hand out the module, give a short introduction, and have pupils read assignment 1.

2. Video (10-15 min)

- Show the video until 2.50. Now, pupils make assignment 1. Afterwards, show the remainder of the video, after which pupils fill in the technique/application chart (also assignment 1).

3. Lesson module part 1: 'Synthetic biology: what is it and what can you do with it?' (20-25 min)

- Make part 1 with the whole class or in groups/individually (see pg. 11 for the answers).
    - Also pay attention to the different representations of DNA / genome in the figures.

4. Lesson module part 2: 'Selecting an iGEM-application of synthetic biology (10 min)*

- Short whole-class introduction of part 2.
    - Indicate what form the presentation should have.
    - Explain the homework and indicate that the homework assignment should be divided up.
    - Have the pupils select an iGEM-application.
- Have pupils with the same iGEM-application form a group. Ensure that every group of pupils is more or less the same size.
- Have the groups of pupils discuss their approach for the homework.

5. End of lesson (5 min)

- Emphasize the importance of mutually dividing and making the homework.

* Another option is to have pupils design their own synbio application, according to appendix 1 on pg. 18.

Lesson 2

1	Introduction	5 min	
2	Lesson module part 3: 'Working on the iGEM-application'	15-20 min	In groups, pupils work on the iGEM-application based on their homework and part 3
3	Lesson module part 4: 'How advisable is your iGEM-application?'	15-20 min	In groups, pupils consider the advisability of their iGEM-application
4	Preparing presentations	10 min	Time to work on presentations in class
5	End of lesson	5 min	Homework: finish presentation

Needed

- Perspective chart on an A3 sheet of paper (1 per group).
- Post-it notes (10 per group).
- Tables in group formation.
- Lesson module (pupils already have this).

1. Introduction (5 min)

- Short repeat of the previous lesson and introduce this lesson.

2. Lesson module part 3: 'Elaborating the iGEM-application (15-20 min)

- Short whole-class introduction of part 3.
- Pupils work through part 3 in groups.
- See pg. 13 for examples of elaborations of the four iGEM-applications.

3. Lesson module part 4: 'How advisable is your iGEM-application?' (15-20 min)

- Short whole-class introduction of part 4.
- Have the pupils do part 4. The first question should be answered individually, the others by each group as a whole.

4. Preparing presentations (10 min)

- The pupils are given time to work on their presentations in class.

5. End of lesson (5 min)

- Homework: finish presentation.

Lesson 3

1	Introduction	5 min	
2	Presentations	20-25 min	Every group has 5 minutes to present their iGEM-application. Pupils make notes in part 5 of the module
3	Short discussion on the content of the iGEM-applications	5 min	Based on the types of BioBricks used
4	Dialogue	15-20 min	Dialogue on the advisability of the iGEM-applications and synbio in general. Afterwards, pupils write down arguments in part 5 of the module
5	Writing down opinions about synbio and end of lesson	5-10 min	Pupils write down their opinion about the advisability of synbio in module part 5 and whether they changed their mind since lesson les 2

Needed

- Optional smartboard or projector for PowerPoint, materials such as adhesive tape for hanging up posters or illustrations.
- Chalkboard or whiteboard for notes.
- Lesson module (pupils already have this).

1. Introduction (5 min)

- Short repeat of the previous lesson and introduce this lesson.

2. Presentations (20-25 min)

- Have each group do their presentation in 5 minutes. After each presentation a short, explanatory question can be asked.
- Have pupils write down for each presentation an application, a technique and their opinion in part 5.

3. Short discussion on the content of the iGEM-applications (5 min)

- Whole-class discussion on the similarities and differences in the techniques / BioBricks used in the iGEM-applications.

The following types of BioBricks are used in the four iGEM-applications:

1. Functional genes that produce desirable chemicals (*LactoAid, Grätzel cells, BananaGuard* and *Click Coli*).
2. Kill switch or another system that kills the bacteria with the gene construct once they are no longer needed and/or to stop horizontal transfer (*Grätzel cells* and *BananaGuard*).
3. Gene for fluorescent pigment to demonstrate production (*LactoAid and BananaGuard*).
4. Induceable promotor that responds to environmental chemicals (*LactoAid and BananaGuard*).
5. Molecule that helps with membrane penetration (*LactoAid*).
6. Receptor that recognizes chemicals in the vicinity and forms a signal for the promotor (*LactoAid*).
7. Fluorescent pigment attached to chemical that bonds with the product to be studied to determine the amount present (*Click Coli*).
8. Divergent tRNA synthetase that can insert divergent amino acid on a specific genetic code, for example a code that normally results in a stop codon (*Click Coli*).

4. Dialogue (15-20 min)

- Write down all propositions from the presentations on the board.
- Enter into a whole-class dialogue with the pupils, with the central question: "Are these iGEM-applications advisable?'
- Start with discussing the advisability of the various applications one at a time, and then continue with a discussion on the advisability of synbio in general.
- Use the perspective chart as the backbone of the dialogue, for instance by displaying the chart on the board or screen. The perspective chart can then be used in two ways:
    - Assigning arguments to a perspective.
    - Considering the dilemma more in-depth from perspectives that haven't yet been used in the dialogue.
- The purpose of the dialogue is to have pupils talk together, rather than having them defend a position. This means there is more opportunity to listen to each other and to learn from each other. Therefore it is important to keep asking questions.
- For guidance on how to guide the dialogue you can use the 'Teacher tool for holding a whole-class dialogue' in appendix 3 (pg. 24).
- After the dialogue, have pupils write down arguments in the perspective chart in part 5.

5. Writing down opinions about synbio and end of lesson (5-10 min)

- Having pupils write down their opinion on the advisability of synbio in part 5, and whether they changed their mind as a result of the presentations and dialogue.
- Briefly summarize what happened in the module. For example:
    - Learning about (applications of) synbio.
    - By not only considering the content, but also the advisability, we now have a more complete overview of synbio, and we are better able to support our view on synbio.
- Pupils who want to know more about synbio may be directed towards the NEMO Kennislink theme page. They can find more information there, and hold further online discussions about what they think of synbio and synbio applications. The link to the theme page is: https://www.nemokennislink.nl/kernwoorden/synthetische-biologie
- Pupils could also consider the advisability of synbio more in-depth in subjects like social studies, philosophy or philosophy of life.

Answers lesson module part 1

'Synthetic biology: what is it and what can you do with it?'

Assignment 1

Write down the techniques and applications of synthetic biology that are mentioned in the video.
N.B. *Pupils need only list three techniques and applications.*

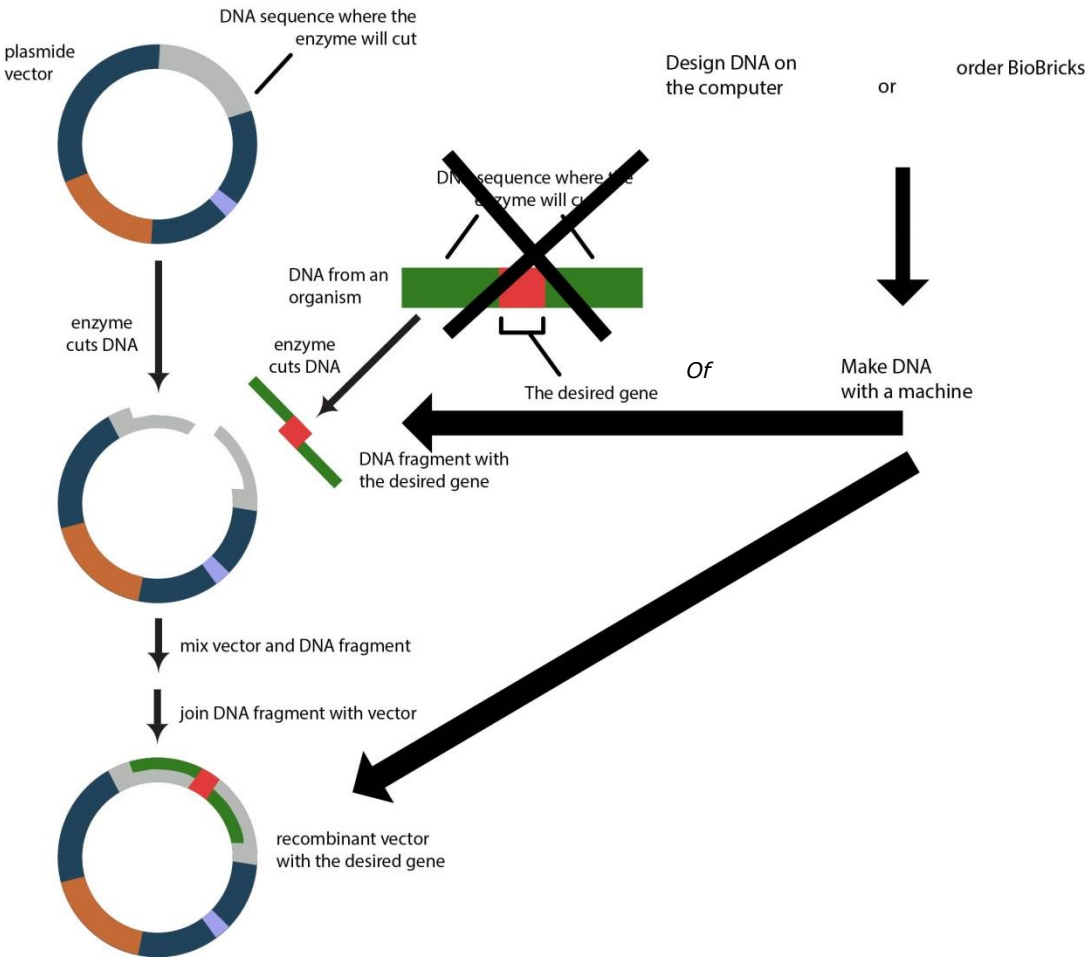
Topics that are discussed in the video and that may be given as answers here, are:

History	***Techniques***	***Applications***	***Hypothetical applications***
From descriptive to experimental biology	***EXAMPLE Cutting and pasting DNA, recombinant DNA technology***	***EXAMPLE* E. chromi*: detects various concentrations of a toxic chemical***	*Algae that eat CO_2 and produce biofuel*
Selective cultivation and breeding of plants and animals	*BioBricks: standardized DNA parts / systems with a specific function*	E. coliroid*: works like a bacterial polaroid camera*	*The 'kliver': a biological sieve-like organ that can replace liver and kidney transplants*
Discovery of DNA as carrier of inherited features	*Ordering DNA online*	*Artemisinin: a synthetic malaria drug*	*Plant seeds that can grow into a house*
DNA sequencing	*Synthetically produced DNA*	*Bacteria with a synthetic genome*	*Terraforming dead planets such as Mars*
Cutting and pasting DNA, recombinant DNA technology	*DNA as a programming language*	*Biofuel from plant waste through modified yeast*	
Decrypting the human genome			

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.

See next page.



Explanation: In synthetic biology researchers no longer have to cut the desired bits of DNA from existing DNA: they can design the desired DNA on a computer or select it from the BioBricks database. This is then produced by a machine. Next, the DNA can be inserted in the vector in the same way as with recombinant DNA. Also, an entire vector, chromosome or even genome can be designed on the computer and produced by a machine.

Assignment 3

What is the advantage of inserting a BioBrick in a minimal cell (possible future technique), compared with inserting a BioBrick in an existing organism like a yeast cell (current technique)?

Possible answers are for example:
Less risk of (negative) influence from other genes on the desired process.
More efficient production, because the cell carries out a minimum of other processes.

Answers lesson module part 3

`Elaborating the iGEM-application

Exemplary elaboration of iGEM-application LactoAid

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

- What problem does the application solve?

Burn wounds have an increased risk of getting infected and are therefore preventatively treated with antibiotics. Also the wounds are treated with bandages after skin transplantation but those need to be replaced several times a day.
Pathogens causing infections can be antibiotic resistant. Furthermore, if the pathogens enter the blood stream the patient becomes septic and will almost certainly die. Due to the increase in antibiotic resistance by pathogens, the antibiotic treatment for burn wounds is at risk of becoming more and more ineffective. Therefore a demand for new treatment methods arises.

- How does the application solve this problem?

The iGEM solution is to treat a bandage with a bacterium that does not cause infections but reacts to the presence of infective bacteria by producing substances that counteract the infection.

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

Treatment with antibiotics, treatment with bandages

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

Current treatment with antibiotics creates problems in the future by causing antibiotic resistance. In the iGEM application, bacteria themselves produce limited amounts of antibiotics which are very specific for the pathogen and are only released in the presence of the pathogen

2. What is the application?:

- What is the application called?

LactoAid

- *How does the application work*?

The LactoAid (a reference to BandAid) works as follows: Pathogens produce signal molecules with a function in communication between bacterial cells. In the iGEM solution a gene construct is inserted into Lactobacillus*, containing a promotor reacting to this signal molecule, and genes coding for substances which prevent the spread of the pathogen. By treating bandages with* Lactobacillus *containing this gene construct, anti-pathogen substances are produced when a pathogen is present.*

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

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

Techniques used to create this solution are:

a. *Creating a promotor that is switched on by the signalling molecules of the pathogenic bacteria*
b. *Coupling this promotor in a gene construct to*
   - *genes coding for antibacterial substances*
   - *genes coding for a substance that guides these substance out of the cell*
   - *a ribosome binding site*
   - *terminator sequences*
c. *Building the gene construct in a plasmid*
d. *Transferring the plasmid to* Lactobacillus*, thereby creating* Lactobacillus *capable of responding to the presence of pathogenic bacteria*

Exemplary elaboration of iGEM-application Grätzel cells

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

- What problem does the application solve?

Access to electricity is a major issue in Africa, especially in rural areas.

- How does the application solve this problem?

This project will contribute to the fight against poverty by producing solar cells based on a pigment (DSC or Grätzel cells) which can produce cheap electricity on a small scale and with simple technology.

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

(Not found in the provided text, but students can think of these themselves)
Using generators to generate electricity, wind turbines or batteries.

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

Generators need fossil fuels, wind turbines are fragile and difficult to transport, and batteries run dry and are polluting. Normal solar cells do not function during cloudy weather or sand storms, the Grätzel cell also works under darker circumstances.

2. What is the application?:

- What is the application called?

The proposal has no name of its own.

- *How does the application work*?

The project aims at the production of a pigment (Pelargonidine) that can absorb solar energy which can be transformed to electrical energy in a solar cell. Seven enzymes catalyzing the production of pelargonidine starting from tyrosine as a substrate are derived from different plant and bacteria species. The genes coding for these enzymes are built in a E coli variant BL21(DE3) in a gene construct with the promotor T7. In normal substances this promotor is switched on by lactose, but in the project a substance is used that is similar to lactose (ITPG) but is not metabolized and therefore stays within the cell and keeps the promotor switched on.
Furthermore a molecular scaffold is created in which three of the produced enzymes are bound in a fashion that speeds up the chain of reactions leading to pelargonidine.
In order to prevent survival of the transformed cells outside the reaction compartment, a 'Kill switch' is built in which will switch on when glucose becomes less abundant.

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

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

• *Isolating DNA coding for enzymes from different sources*
• *Combining these genes in a gene construct with a promotor that reacts to a substance*
• *Building this gene construct in a E coli, together with a molecular scaffold. (how this is done remains unclear from the description)*

Exemplary elaboration of iGEM-application BananaGuard

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

- What problem does the application solve?

Commercially grown bananas are largely sterile clones with a triploid genome, and almost all banana plants belong to the cultivar Cavendish. This means that a banana disease such as the fungus Fusarium oxysporum *can spread rapidly and breeding for resistance is impossible. In 1992 a new variant of* F. oxysporum*, tropical race 4 (TR4) was discovered and threatens the production of bananas worldwide.*

- How does the application solve this problem?

By designing a bacterium that can eliminate this pathogenic fungus.

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

Using anti-fungal spray is ineffective against Fusarium*, cultivating resistant banana-races is impossible since bananas are sterile, so: no.*

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

N/a.

2. What is the application?:

- What is the application called?

BananaGuard

- How does the application work?

A bacterium, Pseudomonas putida *is chosen to be developed as an anti-fungus producing agent. This is done by introducing a gene construct in this bacterium consisting of*

a. a promotor-repressor system, reacting on the presence of fusaric acid produced by the fungus. Fusaric acid binds to the repressor pp1262, thereby freeing the promotor and making transcription of the genes behind the promotor possible.

b. Genes which produce substances that attack the fungus Fusarium

c. Genes that code for a molecular pump, pumping fusaric acid out of the cell

Furthermore, two systems are built in to prevent the spread of the modified bacterium:

• A 'Kill switch' that kills the bacterium in the absence of Fusarium

• A system preventing horizontal transfer between the modified Pseudomonas *and other bacteria. Two plasmids are introduced in the modified* Pseudomonas*, each of which contain the code for a different toxic substance, and the code for the antidote for the toxic substance of the other plasmid. In this way, accidental transfer of one plasmid will always kill the receiving bacterium.*

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

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

• Creating a promotor-repressor system reacting to fusaric acid

• Creating a gene construct with this promotor and genes coding for antifungal substances

• Creating two different plasmids with genes for different toxic substances and their antidotes

• Building the gene construct in a plasmid

• Introducing the plasmids in Pseudomonas *cells*

• Creating a system indicating the presence of fusaric acid in which the same promotor-repressor system is linked to a gene for a pigment. Presence of the pigment indicates the presence of fusaric acid.

Exemplary elaboration of iGEM-application Click Coli

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

- What problem does the application solve?

Genetically modified bacteria have a limited ability to survive under conditions such as industrial reactors.

- How does the application solve this problem?

Therefore a special coating for bacterial cells is developed which protects the bacteria.

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

Unknown

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

N/a

2. What is the application?:

- What is the application called?

Click Coli

- How does the application work?

The system consists of two parts; Substances covering the outside cell containing a 'hook' and a molecular 'anchor' in the membrane to which this hook can bind ('click'). The anchors consist of proteins containing azidophenylalanine, a variant amino acid which is normally not used in proteins, unless a variant (orthogonal) tRNA-synthetase is used. This variant tRNA-synthetase reads the DNA code differently; where normally TAG is a stop codon with no t-RNA's binding to it, the variant tRNA-synthetase binds to TAG and introduces azidophenylalanine in this place.
By building in a stop codon on specific places in the protein code, in the presence of the variant t-RNA a protein is produced with the molecular anchor.
To this anchor in the membrane protein, other substances such as DBCO bind specifically and form the hook that clicks to the anchor. By linking other substances to DBCO, different kind of substances can be 'clicked' to the membrane proteins containing azidophenylalanine. For example, by combining a fluorescent substance to DCBO, it can be tested to what extent the anchoring protein is built in the membrane.

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

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

• *Introducing TAG codes in a membrane protein*
• *Introducing variant (orthogonal) tRNA-synthetase, thereby building azidophenylalanine (anchors) in the membrane protein*
• *Combining DCBO with other substances*
• *Adding DCBO with linked substances to the bacterium containing the anchor, thereby clicking these substances on the outside of the bacterium*

Answers lesson module part 4

How advisable is your iGEM-application?

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.

LactoAid

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

Possible positive effects are the reduction of antibiotic use, therefore reducing antibiotic resistance. Another positive effect may be that the presence of the pathogen triggers the reaction, so when no pathogens are present, the healing may continue undisturbed.
Also, the need to replace bandages could be less, and 3rd world countries could in this way reduce costly antibiotics.

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

Possible negative effects are that the bacterium inserted in the bandage itself may cause problems, for instance in patients with severe immune response deficiencies. Other risks in general may be that the gene construct may be transferred to other bacteria, and in one way or another disturb microbiological balances in the body.

Grätzel cells

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

Positive consequences could be that electricity can be brought to isolated places where people now have no electricity or are dependent on batteries.

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

Other consequences could be that societal changes are influenced by a sudden cheap access to electricity, which can create more dependence on electricity. The question is whether these solar cells are sufficiently robust and easy to replace when broken.

BananaGuard

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

The modified bacterium may be used to protect against Fusarium.

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

However, this includes introducing a genetic modified bacterium on a large scale, with possibly unpredictable effects.

ClickColi

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

A larger production of GM bacteria because they are more heavily protected against harm, therefore, they survive longer in inhospitable situations.

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

(Cannot be read in the text, but students are able to come up with answers) The protected bacteria are possibly less easily destroyed if they might become harmful.

Appendix 1: Alternative for part 2

'Selecting an iGEM-application of synthetic biology"

PART 2
Invent your own application of synthetic biology

There are many applications of synthetic biology (synbio), such as artemisinin and bio-ethanol. What would you like to use synbio for? With your group, design an application based on synbio. In the third lesson you will present your application to the whole class.

Who are in your group? Names:

How?

A few ways to invent a new application are:

- Consider a **problem** you want to solve. One possible problem might be: the drug against malaria is rare and therefore not available for many people.
- Consider a **field** in which you would like to invent an application. Most applications are developed in the fields of **vaccines**, **drugs**, **biofuel** and **food**, but of course there are other possible fields.
- Consider **your own life**. Perhaps you have an allergy synbio might help with? Or you dislike swimming in chlorinated water and you can think of an application that would no longer make it necessary?

What else should you keep in mind?

- Consider how the application is made in practice. For example, are you inserting a gene into a cell, or removing one or more genes? Are you using a **BioBrick** or are you having DNA synthesized yourself? Will you insert the DNA into a **yeast** or another **organism**?
- What are you going to make with synbio? **Molecules**, **cells**, **organs** or **organisms**?
- How does your application work?

The presentation

In the third lesson you and your group have to give a **5 minute** presentation on your application. The following questions have to be answered in the presentation:

1. What problem does the application solve and how?

    - Is there another solution for this problem? If so, what is it? And why is your 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. 14).

This is our application:

Homework

Find information that you need to answer the following questions for the next lesson. Agree within your group who will look for what.

- **What problem does the application solve and how?** Look for information about the problem. Is there another solution for this problem? If so, what is it? And why is your application better?
- **What technique(s) was/were used to make the application?** Look for instance for extra information about the techniques that may be used to make your application, such as recombinant DNA technology or BioBricks, about the genes that are necessary to make your application and how you can ensure that these genes are expressed.

You could use one of the following web sites:

- https://www.nemokennislink.nl/kernwoorden/synthetische-biologie
- https://www.scientias.nl/?s=synthetische+biologie

Appendix 2: Hypothetical Learning Trajectory (HLT)

A representation of the various teaching and learning activities (TLAs) and their hypothetical learning effect, including actions by teacher and pupil.

Lesson 1

	Learning activity	**Time**	**Teacher action**	**Pupil action**	**Hypothetical learning effect**
TLA 1	Question	3 min	The teacher asks one or two short questions to the class to activate prior synbio knowledge.	A number of pupils answer the question.	The pupil is activated and will be a more active participant in the lesson afterwards.
TLA 2	Introduction of lesson 1	2 min	The teacher explains in brief what will happen in the lesson. The teacher hands out the module.	The pupil listens to the teacher's explanation and may perhaps ask something.	The pupil knows what will happen and why (attention is focused).
TLA 3	Introductory video	15 min	The teacher shows a video on synbio (https://youtu.be/UHBdEwNbXI0), pauses video at 2.50.	The pupil watches the video and afterwards fills in the technique/application chart.	The pupil's interest in synbio is raised by the video and the pupil acquires basic knowledge of synbio techniques and applications.
TLA 4	Lesson module part 1: 'Synthetic biology: what is it and what can you do with it?'	25 min	The teacher does part 1 of the module with the whole class or lets them do it in small groups/individually.	The pupils work on part 1 of the module with the whole class/in small groups/individually.	The pupil's relevant prior knowledge is activated and she links her new information about synbio to it. The pupil knows what synbio is and has a general knowledge of the history and underlying techniques of synbio. The pupil's interest is raised by concrete examples, and she has an idea of what kind of applications are part of synbio.

TLA 5	Lesson module part 2: 'Selecting an iGEM-application of synthetic biology'	10 min	The teacher introduces part 2. The teacher ensures that each pupil selects an iGEM-application and that the groups of pupils are roughly the same size.	The pupils read the four iGEM-applications and determine which one they would like to elaborate further. Pupils with the same iGEM-application form groups.	A maximum of four groups of pupils with each a different iGEM-application to work on in the rest of the module have been formed.
TLA 6	End of lesson	5 min	The teacher tells what has to be prepared for the next lesson and answers questions.	The pupil listens, writes down her homework and asks questions.	The pupil knows what to prepare for the next lesson.
HW				Look for information on their own iGEM-application based on the questions for the presentation, the website of the application and the corresponding explanation.	In lesson 2, with the information they found pupils can immediately work on part 3.

Lesson 2

	Learning activity	Time	Teacher action	Pupil action	Hypothetical learning effect
TLA 7	Introduction of lesson 2	5 min	The teacher gives a short introduction, what happened in the previous lesson and what will we do today.	The pupil listens.	The pupil knows what will happen and why (attention is focused).
TLA 8	Lesson module part 3: 'Elaborating the iGEM-application'.	20 min	The teacher introduces part 3 and walks around in the classroom to answer questions and give hints.	The pupils work on part 3 on the content of their iGEM-application in their own groups.	The pupil activates and increases her theoretical knowledge on synthetic biology and gene expression and –regulation. The pupil learns a lot about a specific synbio application and technique.
TLA 9	Lesson module part 4: 'How advisable is your iGEM-application?'	20 min	The teacher introduces part 4 and walks around in the classroom to answer questions and give hints.	The pupils work on part4 on the advisability of their iGEM-application in their own groups.	The pupil becomes aware of the perspectives you can use to consider a dilemma. The pupil considers the advantages and disadvantages of the iGEM-application.
TLA 10	Preparing the presentations	10 min	The teacher explains the required elements of the presentation and walks around in the classroom to answer questions and give hints.	The pupils work on their presentations in their own groups.	The pupil starts on the presentation.
TLA 11	End of lesson	5 min	The teacher tells what has to be prepared for the next lesson and answers questions.	The pupil listens, writes down her homework and asks questions.	The pupil knows what to prepare for the next lesson.
HW				Finish the presentation on the iGEM-application.	

Lesson 3

	Learning activity	Time	Teacher action	Pupil action	Hypothetical learning effect
TLA 12	Introduction of lesson 3	5 min	The teacher gives a short introduction, what happened in the previous lesson and what will we do today.	The pupil listens.	The pupil knows what will happen and why (attention is focused).
TLA 13	Presentations of the iGEM-applications	25 min	The teacher listens to the presentations and keeps an eye on the time (5 min per presentation). After each presentation there is a moment to ask an explanatory clarifying question about the content.	The pupil groups give a presentation about their iGEM-application, and make notes for the other presentations. After each presentation the pupils can ask an explanatory question about the content.	The pupil recognizes that the iGEM-applications from other groups are synbio applications, gains content knowledge about the iGEM-applications and increases her theoretical knowledge on synthetic biology and gene expression and –regulation.
TLA 14	Short discussion on the content of the iGEM-applications	5 min	The teacher discusses similarities between the iGEM-applications based on the kinds of BioBricks used.	The pupils think along.	The pupil gains insight into the various types of BioBricks and gene expression and –regulation.
TLA 15	Dialogue on the advisability of the iGEM-applications and of synbio in general	20 min	The teacher facilitates a dialogue on the advisability of synbio, and among other things includes the iGEM-applications in the discussion (she writes down the statements the various groups have come up with on the blackboard). The teacher may ask explicit questions, for example based on the perspectives chart.	All pupils take part in the dialogue. The pupils contribute arguments or ask questions. After the dialogue, pupils write down arguments in the perspectives chart in part 5 of the module.	The pupil recognizes the perspectives that are mentioned in the dialogue. In the dialogue the pupil can contribute points of view, arguments or questions, and connect these to the corresponding perspective.
TLA 16	Writing down opinions on synbio and end of lesson	10 min	The teacher asks pupils to write down their opinion about synbio. The teacher summarizes what was discussed in the module and ends the lesson.	Pupils write down their opinion about the desirability of synbio and whether it changed since lesson 2.	The pupil is aware of her opinion about synbio, whether it changed and why.
HW (optio-nal)			Optionally direct the pupils towards the synbio theme page on the NEMO Kennislink website. https://www.nemokennislink.nl/kernwoorden/synthetische-biologie	The pupil is able to find more information about synbio and synbio applications and enter into further discussion online.	Extra discussion on the advisability of synbio (applications).

Appendix 3: Teacher tool for having a whole-class dialogue

By: Miranda Overbeek, Marie-Christine Knippels and Arend Jan Waarlo (Freudenthal Institute, 2014)

Science and technology are constantly developing; how do you prepare pupils for decision making about *socio-scientific issues* (SSIs), i.e. complex problems as a result of scientific/technological developments that influence society? One way to do this is through a whole-class dialogue. But how do you do that? This tool gives some hints on values, since SSIs consist of a knowledge and a values component. This tool targets the values component and considers:

- Why a dialogue?
- Role of the teacher
- Techniques for asking questions
- Frames (of reference)

Why a dialogue[1]?

The goal of a dialogue is to think about a problem together and to understand each other. So a dialogue is cooperative in character[1]. In a dialogue pupils aren't made to defend a position, so that there is opportunity to learn something from the perspectives of the other pupils[2].
In a discussion or a debate the goal is to solve a difference of opinion, so that there will eventually be a winner, also if for example more points of view are possible. So a discussion or a debate is competitive in character[1]. In a discussion or a debate people tend to dig in on their own position and no longer listen to others with an open mind, so they will not learn from the perspective of the other as quickly[2].
Therefore we opted for the dialogue as a method: a dialogue is more suitable to clarify the values of pupils that are often implicitly present.

In a dialogue the following is important:

- Equality: all pupils have the same right to say or ask something.
- Mutual trust, respect, openness and understanding.
- Basing opinions on arguments and discovering the validity of these argumentations together.
- Listening to each other and trying to understand what the other is trying to say.
- Reflecting on what is said.
- Thinking along with each other on the topic.

The role of the teacher[4]

Depending on your goal– development of values through transmission (transferring certain values), through clarification (letting pupils discover their own values) or through communication (teaching pupils to communicate about values)[3] – there are different roles you can fulfil as a teacher in the dialogue. The chart below gives an overview of these different roles and the degree to which they are suitable for developing values through transmission, clarification or communication. ++ is very suitable, + is suitable and – is unsuitable.

Role teacher	Role description	Development of values through:		
		Transmission	**Clarification**	**Communication**
Participant	You are free to express ideas, opinions and emotions, like the pupils. This can be confusing for pupils, since teachers are the professionals.	+	+	+
Dedicated teacher	You disseminate your own opinion about the topic. This can lead to indoctrination.	++	-	-
Observer	You do not interfere in the dialogue.	-	+	++
Instructor	You clarify relevant information, concepts and ideas. You ask questions to assess the level of understanding. And you give positive or negative feedback to input from pupils.	-	+	+
Devil's advocate	You take on contradictory points of view to stimulate the dialogue.	-	+	++
Advocate	You present all possible points of view and conclude with your own opinion, supported by arguments.	+	-	+
Neutral chair/ Neutral facilitator	You stimulate pupils to contribute to the dialogue and keep an eye on the rules of the dialogue, but do not give your own opinion or positive/negative feedback on the input of pupils.	-	++	++
Declared stakeholder	At the start of the dialogue you give your own point of view, so that pupils will be able to assess teacher bias later on.	-	+	+

Which role do you usually take on? And would you do so after reading the descriptions of these different roles?

The premise of this tool is development of values through clarification and communication. The role of the teacher as neutral chair/ neutral facilitator in a whole-class dialogue is suitable for that. Development of values through clarification and communication requires the following from a teacher:

- Ensure an open, inviting and safe atmosphere.
- Be neutral.
- Adapt questioning techniques (described below).
- Establish the results of reasoning processes by pupils.

Questioning techniques[5]

In a whole-class dialogue it is important that:

- It is clear to everybody what a pupil is trying to say (**clarity**).
- The foundation for pupils' opinions emerges, this can be both rational and emotional/intuitive (**arguments**).
    - And examine their validity.
- There remains a focus on the original question (**structure**).

Clarity: What question can you ask when …

… it is unclear what a pupil is trying to say?

- "Can you explain this?"
- "Can you give an example?"
- "What do you mean by …?"

… you are not sure that you or the rest of the class understand correctly what a pupil is trying to say?

- "Do you mean … when you say that …?"
- "Do I understand correctly when you say that …?"

… it is unclear what something a pupil says has to do with the topic of the dialogue?

- "What does this have to do with …?"
- "How does this correspond to …?"
- "Does this help us with … in the dialogue?"

Arguments: What question can you ask when …

… a pupil doesn't support his/her point of view?

- "Why do you think that?"
- "What did you base that on?"
- "Can you prove this?"

… a pupil gives an incorrect argument?

- Ask the whole class: "Does anyone have a different idea about that?". And subsequently let them underpin their opinion (as described above).
- Ask the whole class: "If what X says is true, what could be the result of that?"
- "What did you base that on?"
- "Could the opposite be true?"
- "How does this match with what you just said?"
- Introduce your own opinion as a hypothesis: "Suppose that …, what might we be able to say about that?"

… a pupil uses a discussion breaker [6]?

- When a pupil uses a discussion breaker like "It's everybody's own decision whether or not to use the technology" or "You cannot stop/influence technological progress", explain that society and technology/science are intertwined and influence each other: society influences science and science influences society. Two examples:
    - Society influences science: society is in fact able to influence for example the use of technological innovations. When for example the company Myriad Genetics filed a patent on all medical treatments that are developed based on mutations in in BRCA-1 that they discover, breast cancer patient associations cooperated internationally to stop this.
    - Science influences society: one thing science can do is change the values of society. For example the invention of the contraceptive pill caused homosexuality to be more acceptable, because heterosexuals could now also have sex without procreation as its goal.

… a pupil gives an emotional argument?

- Stimulate pupils to look at the values behind their emotions[7]. Ask further questions / reflect on the emotion!
    - *Example of asking further questions:* A pupil says "I'm afraid of it." Ask another question: "What is it you're afraid of?" [8].
    - When reflecting on emotions it is important to include both your own emotions and those of others. *Example of reflecting on emotions:* A pupils says: "I'm afraid of the technology." To find out whether the pupil is really afraid of the technology as such (i.e. consequences for society), you can invite the pupil to take a broader perspective regarding emotions, for example: put yourself in the place of people who benefit from or are damaged by the technology. Is the technology socially acceptable or not in that case[9]?
    - Sometimes emotions are based on wrong factual information. In that case it is important to correct these at a factual level[8]. Emotions can also make us blind to quantitative considerations: for instance, plane crashes are rare, but people with fear of flying focus on them. Also correct these emotions with factual information. You should do so subtly, by acknowledging/going along with, for example: "Yes, that is terrible, but luckily it doesn't happen often." That the chance that something that someone is afraid of may actually happen is small, does not necessarily mean that the emotion 'fear' is irrational. Consider for

example nuclear energy: the chance of a nuclear disaster may be small, but the consequences are disastrous. Besides, there are alternatives for nuclear energy[9].

… a pupil gives a general argument[10]?

- Ask the pupil to be more specific. For example: a pupil says "We must respect autonomy." Ask for example: "What does that mean here in this situation?"

… pupils do not (want to) understand each other's opinion/argumentation

- Invite them to take the other's perspective; let them put themselves in the other's place through emotions[3].
- Pupils can use different patterns of arguments /ways of thinking. If this is the cause of them opposing each other, make it clear to them that they are both using a different pattern of arguments /way of thinking. There are three patterns of arguments/ ways of thinking[4]:
    - *Pragmatic argumentation:* Action X may (not) be performed, because X leads to the (un)desirable consequence Y.
    - *Deontological argumentation:* Action X may (not) be performed, because X is (not) in agreement with moral principle Y.
    - *Argumentation based on justice:* Action X may (not) be performed, because action X itself or the consequences of X are (un)just.

Structure: What question can you ask when …

… you want to wind up the dialogue?

- "Do we have a better understanding of the situation?" / "Do you have a clearer view of the situation?"
- "Have some of you changed your mind from before?"

… you want to reflect on the dialogue?

- "Have we looked at all possible options?"
- "Is this dialogue completed?"

… the topic of the dialogue has moved away too far from the original question?

- "What does this have do with what we discussed first?"
- "How did we get from … to …?"

Frames (of reference)[11]

Frames are cognitive shortcuts, or rather frames of reference, that people use (subconsciously) to be able to understand complex information. Frames are based on someone's convictions, values and experiences. Frames help you to interpret and organize information. They provide a simplification of the information, by (subconsciously) filtering it: you will consider some aspects of the information as important and others you leave out of consideration because they appear irrelevant or go against your intuition. Because different people have different frames, they can interpret the same situation differently. As a result, frames can interfere with a dialogue[12]. When there is a difference of opinion in a dialogue it is therefore important to discover how pupils frame the dialogue/ what the underlying frames are. To find out, it is necessary to ask questions about underlying convictions/values and then consider the point from the other's perspective (described under questioning techniques).

In addition to the above-mentioned *personal frames*, there are also *media frames*. Media frames concern the way that information is presented in the media: aspects may be over- or underexposed (subconsciously)[13]. As a teacher, there are two ways you can use media frames in the dialogue:

- Make sure that you are not subconsciously framing the dialogue, for example by only looking at the topic from an economic perspective in the introduction.
- Considering the topic of the dialogue from various angles/ contributing new perspectives to the dialogue. You can do so through the exemplary questions from the frame schematic below, or by asking for example "But if you look at it from an economic perspective, what do you think of it then?".

Below you will find an overview of possible media frames in relation to technological innovations, with some possible questions that can be used in the dialogue for each frame. Also, some possible questions from whole-class dialogues on genetic manipulation are given(with the exception of the frame 'law and regulations' which was not considered in these whole-class dialogues). Often you will have to point out the frames 'globalization' and 'law and regulations' yourself as a teacher, while other frames are more self-evident to pupils. The various frames are not mutually exclusive. Some frames are in fact closely related, this has been indicated with arrows in the overview.

Media frame	Description and possible questions	Example
Progress	What could be the consequence of a technological innovation, in terms of progress? Are there disadvantages to this progress? Progress here can take many forms, for instance: prosperity (related to economic frame), well-being (being happier is also a form of progress), doing good (related to ethical frame) or limiting/avoiding damage (related to risk frame).	"You can lessen people's suffering (for instance, if cancer no longer existed)."
Economic	What could the technological innovation yield, in terms of economic progress? And who would profit from that? Are there other ways to gain the same economic progress? Or will the economy suffer? And who would be disadvantaged by that? Or does it have no effect on the economy?	"It doesn't have a positive effect on the economic system, because there will be overpopulation when there are no diseases. The economic crisis will only be worse."
Ethical	Is de technological innovation ethically responsible: can you do this? What might be allowed or not allowed to be done with the innovation, based on ethical principles? Where do we draw the line? Which risks of the innovation are acceptable (from an ethical point of view)? Are there consequences if we are unable to introduce the innovation, because potential advantages can't be realized (*risk of inaction*)? The ethical frame also includes *soft impacts*[14]: the technological innovation influences values (*techno-moral change)*. Example: the invention of the contraceptive pill caused homosexuality to be more acceptable, because heterosexuals could now also have sex without procreation as its goa.	"There have to be limits. For example, changing the way you look is less important than curing or preventing diseases."
Risk	The risk frame can be divided into soft impacts and hard impacts[14]. *Hard impacts* are quantifiable consequences of the technological innovation. Questions that might be asked, are: What kind of risks might the innovation carry with it? What are the risks for man, animals and nature (*bio safety*)? Can we predict the risks at all (*unknown risk*)? And can we control the risks? Will the innovation not be put to harmful use (*bio security*)? *Soft impacts* are non-quantifiable consequences of the technological innovation on individuals and on society as a whole. Questions that might be asked, are: How does a pupil see the innovation in relation to her/himself/ how does the innovation influence him or her? How would certain groups look at the innovation (stereotypes) / how does the innovation affect them? What is the effect of the innovation on people's norms and values?	"The long-term consequences are unclear, there are things that could go wrong."

Nature-nurture	Does the technological innovation influence nature/nurture? For example, are environmental influences lessened by the innovation? Will genes / genetic variation change as a result of the innovation? Two *control orientations* can be distinguished within nature-nurture: external (it is fate, this is uncontrollable = nature oriented) and internal (autonomy, you can influence it yourself = nurture oriented). In other words: is something a given or can it be controlled? Developments such as genetic testing change the external control orientation (fate becomes less uncontrollable). Synthetic biology for example results in more control, which means the internal control orientation is enlarged.	"It's natural that there are healthy and sick people, and that is what natural selection works with."
Law and regulation	Who controls the technological innovation: slow down if necessary, regulate? Should/could the populace be involved? Is society responsible? Are there different interests at personal and societal levels?	"The authorities were concerned that the therapy might be so effective that its widespread use might lead to overpopulation. That's why they limited its usage to those without children."
Globalization	What are the consequences of the technological innovation if you look at the global level? Does it improve our country's economic position? Do third-world countries gain anything from it?	"Third-world countries cannot afford it, so that the crops are only going to Europe, and third-world countries don't gain from it, and differences only become worse."

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