

Content Online Course

Engineering Life

Where do you draw the line?

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Lesson 1 – Fundamentals of the CRISPR-Cas System and its Applications

1.1 Introduction - Video

Welcome to the online lesson series "Engineering life: principles and ethics in modifying DNA" of the University of Groningen. This free course is a production of Science Linx and team iGEM Groningen 2020, both part of the faculty of Science and Engineering, located in Groningen, the Netherlands.

Introduction to the course

In two lessons, you will learn the basics of CRISPR-Cas genome editing, a revolutionary technique allowing scientists to precisely edit human DNA. At the end of the course you will know how this new technique works, but also which ethical questions this technique raises. The aim of this lesson series is to provide you with a theoretical background enabling you to decide for yourself where you draw the line between what is acceptable and what not.

We like to share with you the theory and questions that are considered important in the field of human genome editing. If you are, however, interested in specific details of certain concepts, we provide useful links to external resources. The course is designed for an approximate 1 - 1,5 h study-load per lesson. There will not be a live teacher available, therefore we encourage you to discuss questions that you might have with your high school teacher (or if you are not a high school student to another professional).

Before we proceed we would like to make a disclaimer for the content of the course, as a whole. Molecular biology is a rapidly evolving and changing field. Therefore, the views and facts we present in this course might sooner or later be subjected to change. Please consider this and regard this course as a snapshot of the current knowledge.

Each lesson consists of video's, short articles, quizzes and further reading. Before and after the lesson series, we also kindly ask you to take part in a questionnaire. In this questionnaire you will be asked about your opinion on genetic engineering. High school knowledge of biology should be sufficient to follow most of the content of this course. We will explain the technical terms that will be used. The entire content of the course is available immediately, so that you can browse through lessons and study at your own pace.

Outline first lesson

In this first lesson, you will learn the theory required to understand the CRISPR system. Before diving into the details of CRISPR-Cas you will get a brief introduction into the biology of the cell. After covering the basics, you will learn about the medical applications of CRISPR-Cas in humans. By the end of this lesson you will know how this new technique can be used to edit the genome and how CRISPR-Cas could be used in real life. Lesson two will discuss the ethical aspects of human genome editing.

1.2 Pre-Questionnaire Erasmus MC

During this lesson series you will get to know the CRISPR-Cas genome editing system, one of the newest techniques for modifying DNA. In addition, we will discuss the ethical dilemmas associated with modifying human DNA using CRISPR-Cas. The goal of this lesson series is to give you a strong foundation to be able to build your own opinion about modifying DNA of human embryos.

Part of this lesson series are two questionnaires developed by the Erasmus Medical Center (Erasmus MC) located in the Netherlands. The link to the first questionnaire can be found at the bottom of this page. The questionnaire will take about 10 minutes to complete. At the end of this course there will be a second questionnaire. With these questionnaires we would like to know if and how your opinion about modifying DNA changes if you learn more about the underlying technology and ethical dilemmas.

The answers to the questionnaire are anonymized and used by Erasmus MC for scientific research. The results are used by scientists to advise the Dutch government in making decisions regarding regulations about DNA modification in human embryos. Participation in the questionnaire is voluntary and as a participant you can always change your mind and stop completing the questionnaire. By participating, however, you give scientists and the Dutch government more insight into the opinion about modifying embryo DNA. It is important to mention that your data will be processed anonymously.

This questionnaire is part of the Dutch DNA dialogue. The DNA dialogue is an initiative of the Erasmus MC, Erfocentrum, NEMO Kennislink, NPV and Rathenau Instituut, and is funded by the Dutch Ministry of Health, Welfare and Sport. The initiators of the DNA dialogue have different points of view on DNA editing of human embryos. Some have a clear vision, others are neutral. But the most important thing is that they invite everyone to think about the use of DNA modifying techniques on the human genome.

NOTE:

The content of the questionnaire is in Dutch. However, if you are using google chrome as web-browser, you can have the questions auto-translated. In that way, you are still able to answer the questionnaire even if you do not speak Dutch.

QR-Code to the pre-questionnaire:



Link to the pre-questionnaire:

<https://erasmusmcsurvey.erasmusmc.nl/dna/ls/ibndex.php/871119?lang=nl>

1.3 Video Summary - Introduction to Genetics

To help you understand the concepts of the video please find below a short summary of the most important points that are discussed.

What is Genetics?

In genetics, the heredity of organisms is studied. This means that genetics looks at the expression of traits in an organism and studies how these traits are passed on to next generations.

What are genes, genome, chromosomes and DNA?

Genes are the factors that influence the expression of traits (i.e. our outer appearance). Humans have 20,000 - 25,000 genes, which together form the human *genome*. The human genome is stored in the nucleus of a cell. The molecule making up *genes* and the *genome* is called *deoxyribonucleic acid* (DNA). DNA is a long, helical molecule consisting of the nucleotides, sugar and phosphate molecules along with the bases Adenine, Guanine, Thymine and Cytosine. DNA is coiled and organized in structures called chromosomes within the nucleus of the cell. Genes are certain sections on the DNA. They store information about the inherited traits we pass on to the next generation.

The promises of Genetics

Advancements made in genetics open a lot of opportunities in agriculture, industry as well as in medicine, for instance in predicting and treating diseases. However, these promises also come with considerable (potential) risks and certainly with ethical questions that need to be answered. The evaluation and discussion of those risks and questions will be critical for the application of genetic knowledge and technology.

1.4 The Genotype Influences the Phenotype

As you have seen in animation 1.3, the genes found in the cells of our body determine how we look. The scientific term that describes the observable traits (characteristics) of an organism is the *phenotype*. Examples of phenotypic characteristics are for instance eye- or hair-colors of us, humans, but the colors of a flamingo's feathers or the horn of a rhino are also examples of phenotypic characteristics. An organism's *phenotype* is (partially) determined by its genetic information, which is defined as its *genotype* (**Figure 1**).

A phenotypic characteristic can be sometimes largely determined by one gene. For example, a gene called *OR26A* directly influences whether coriander tastes soapy to you. In other cases, a phenotype is determined by multiple genes. For instance, the color of our eyes is largely determined by two genes named *HERC2* and *OCA2* which have a major influence on the eye-color together with several other genes that play a minor role. Together, this specific set of genes determines our eye-color. However, it is not only important whether a gene is present or not, but also how its genetic code looks like (i.e. what precise nucleotide sequence it has) and how much of it is actually "used" in our body cells. Not every gene in our body cells is used equally often. This also explains why all humans have the same set of genes, but still look different. It is also important to note that there are a lot of phenotypic characteristics that we do not immediately "see" or "smell", like the blood-type you have or even your behavior (you can of course observe a behavior, but it is not a specific body trait such as hair-, eye- or skin-color) .



Figure 1: The total of phenotypic characteristics of a person (the color of the eyes, curly hair, a person's size) is determined by the person's individual genetic information (the *genotype*).

It is not Only About the Genotype

Although genes play a key role in determining phenotypes, it is important to note that the genotype does not determine the phenotype of an organism on its own. The phenotype also depends on environmental and life-style factors such as climatic conditions or nutrition. A good example is the color of flamingo-feathers (**Figure 2**). The well-known pink-reddish feather color of flamingos is not determined by the flamingo's genes, but instead it is determined by what it eats. Flamingo-food, mostly aquatic organisms, contains a lot of carotenoids, a color pigment. When flamingos digest their food, they store these carotenoids in their feathers, giving them their typical color. A flamingo with paler feathers simply has not eaten as much carotenoid-rich food as its fellows (see also Figure 2).



Figure 2: It is not only about the genotype. Flamingos who ate a lot of carotenoid-containing food have a strong, pink feather color, whereas the others have paler feathers.

Summary

After all these examples, let us summarize the terms and concepts we learned so far in this lesson:

- *Phenotype:*
Another word for all the different observable traits of an individual (such as a human being), in other words "the way we look".
- *Genotype:*
The term *genotype* describes all the genetic information of an organism, the combination of all its genes. Remember, that for a gene to have an observable effect, it is often not about having a gene or not. It is rather about how the genetic code, i.e. the sequence of nucleotides of the gene look like.

- *Genotype-Phenotype:*
Although the *genotype* of an organism strongly influences its *phenotype*, the genotype is not solely responsible for an observed *phenotype*. Environmental factors such as nutrition and, in, especially also for humans, a person's social setting, do contribute to the *phenotype* (**Figure 3**).

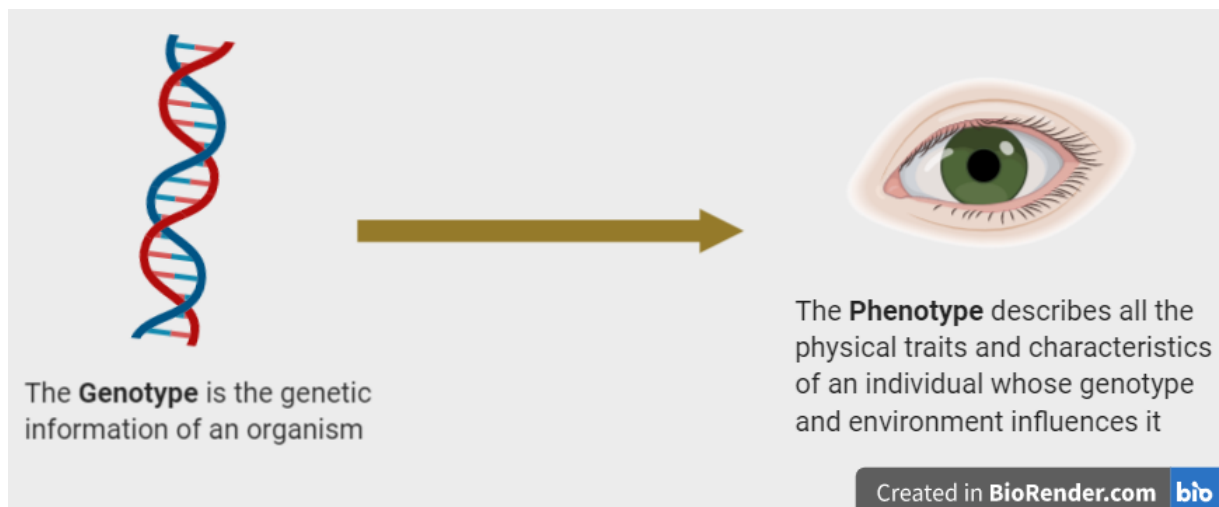


Figure 3: The genotype of an individual influences its phenotype. It is not only important what kind of genes a person has, but what specific base-sequence the genes have.

Further Genetics Resources

In this lesson, we could only touch the surface of genetics. If you are interested in how all the things around *phenotypes* and *genotypes* work in more detail, we encourage you to visit the website <https://learn.genetics.utah.edu/content/basics/> from the University of Utah (USA) and explore the content they provide.

References:

White, D. and Rabago-Smith, M. (2011) 'Genotype-phenotype associations and human eye colour', *Journal of Human Genetics*, pp. 5–7. doi: 10.1038/jhg.2010.126.

Eriksson, N., Wu, S., Do, C.B. *et al.* A genetic variant near olfactory receptor genes influences cilantro preference. *Flavour* **1**, 22 (2012). <https://doi.org/10.1186/2044-7248-1-22>.

Barros, S. P. and Offenbacher, S. (2009) 'Epigenetics: Connecting environment and genotype to phenotype and disease', *Journal of Dental Research*, pp. 400–408. doi: 10.1177/0022034509335868.

Yim, K. J. *et al.* (2015) 'Occurrence of viable, red-pigmented haloarchaea in the plumage of captive flamingos', *Scientific Reports*, 5. doi: 10.1038/srep16425

Attributions:

The image of Figure 1, Baby eye is a photo by bady abbas which is freely available on Unsplash.

The image of Figure 2, "Pink Flamingos" by Jennifavor is licensed under CC BY-NC-ND 2.0.

1.5 Heredity – How Genetic Information is Passed on

You probably already heard phrases such as “You look so much like your father” or “You’ve got your mother’s eyes” at some point (most likely on a family party). We now know from article 1.4 that our phenotypic traits such as body size and hair color are (partially) determined by our genotype. Apparently, we “get” these typical characteristics from our parents. This “getting” is called inheritance, but how does that work? How is this genetic information passed on from our parents to us? This will be discussed in the following article.

Chromosomes organize our DNA in 23 pairs

To answer the question how genetic information, the genes that are located in DNA, is passed on from parents to children, we need to take a step back and look at how our genes are organized inside our cells. Genes are localized in a large molecule called DNA. In animation 1.3, you saw that our DNA is stored inside the nucleus of our body cells (**Figure 1A**). However, it is not present there as one long piece of DNA, but rather is organized in several separate structures called chromosomes (**Figure 1A, B**). Almost all cells in our body contain 46 chromosomes. The chromosomes are divided into pairs of so-called homologous chromosomes (**Figure 1B**). This means that the 2 chromosomes in a pair are very similar (but not identical). The chromosomes in one of the pairs (number 23 in Figure 1B) are somewhat more different. These are the sex chromosomes, which determine our sex biologically. Two “X” chromosomes are present in a woman, one X and one Y chromosome make a man. For instance, the chromosome set in the example of figure 1B belongs to a male person which can be recognized by the small Y chromosome of chromosome pair 23. We will describe below how we obtain our chromosomes from our parents and how we received exactly one chromosome of each pair from our biological father and the others from our biological mother.

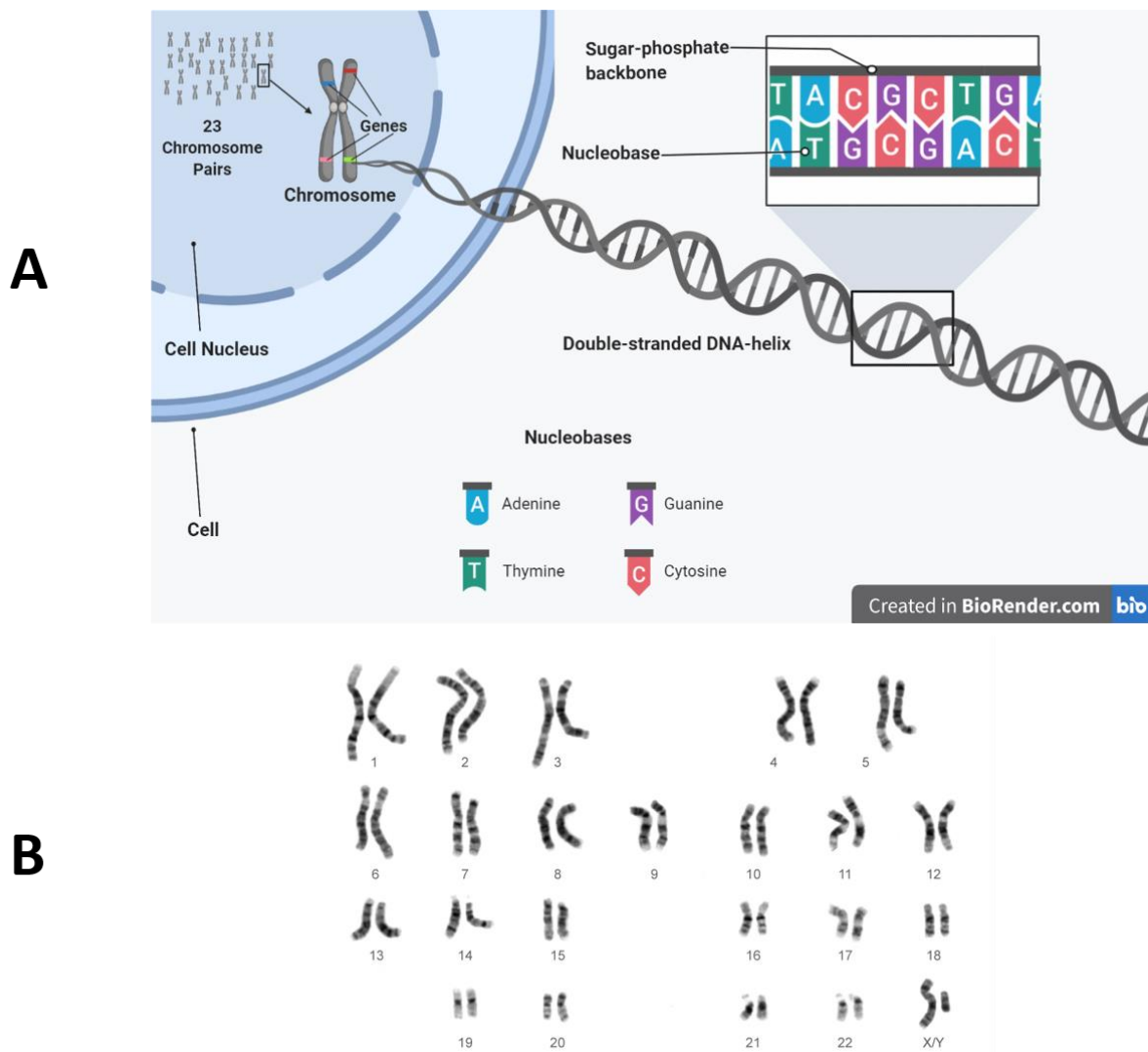


Figure 1: Organization of our DNA in the cell nucleus. **(A)** The DNA containing all our genes is stored in the nucleus of our cells in the form of 23 pairs of homologous chromosomes. The DNA consists of a sugar-phosphate backbone attached to the four nucleobases adenine, guanine, thymine and cytosine. **(B)** The 23 pairs of chromosomes can be recognized and matched by their similar shapes, sizes, and banding patterns. Note that the 23rd chromosome pair, the X and Y sex-determining chromosomes fall out of order a bit.

Homologous chromosomes are not necessarily identical

The homologous chromosomes in a pair have similar sizes and shapes and carry the same genes (Figure 1B). Although the genes on both chromosomes are similar in general, they are often slightly different among each other. Such different gene variants are called *alleles*. For instance, the two homologous chromosomes 11 each encode a gene-variant (allele) involved in determining if coriander tastes/smells soapy to you or not (see chapter 1.4). This example again shows that it is not only important that a gene is present or not, but rather how its exact genetic code looks like.

On one chromosome, the *OR6A2* *allele* might, for instance, have a genetic code for soapy coriander smell/taste, while the equivalent gene on the other chromosome encode for pleasant coriander taste/smell. This is due to slight changes in the genetic code of both alleles (**Figure 2**). Whether the gene for soapy or pleasant coriander taste/smell will eventually dominate, depends on multiple other genetic factors. Exactly how a combination of alleles leads to a phenotype, such as the coriander smell/taste is outside of the scope of this course. The take-away message is that chromosomes of our body cells, also called *somatic cells*, occur in homologous pairs that are very similar, but not identical, with respect to the genes they carry.

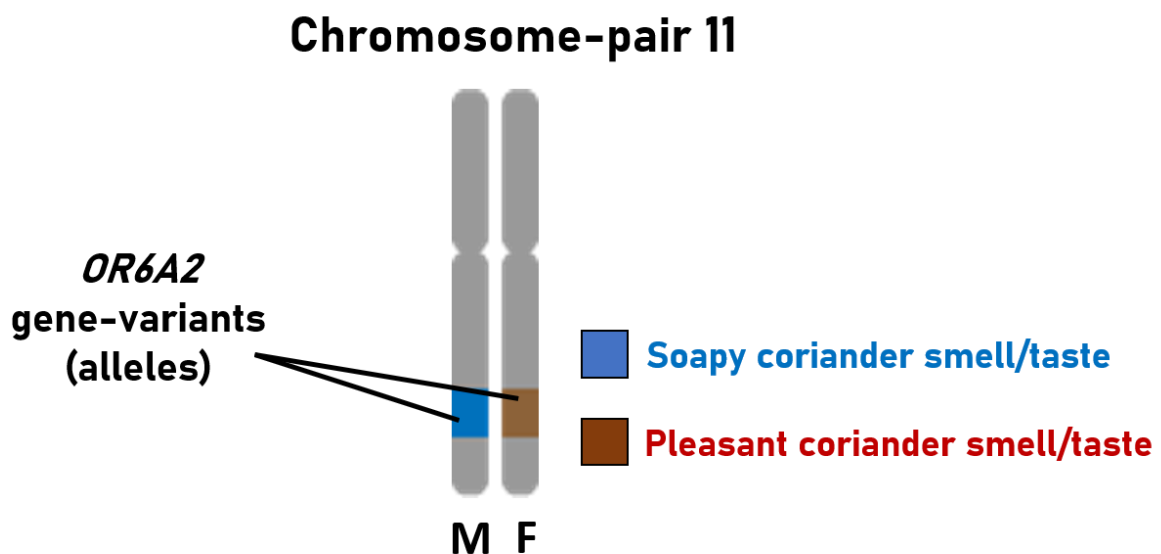
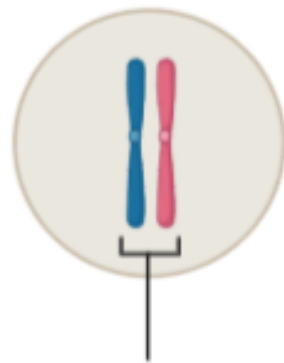


Figure 2: Gene variants (alleles) of gene *OR6A2*, which determines the smell and taste of coriander, located on homologous chromosome-pair 11. Gene variants (alleles) coding for soapy (blue) as well as pleasant (brown) coriander taste/smell are encoded on each of this exemplary chromosome-pair 11. M, chromosome from mother; F, chromosome from father.

Germ cells only carry one set of chromosomes

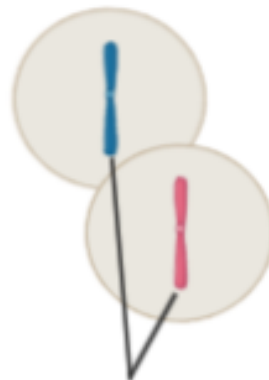
In the paragraphs above, we saw that all our *somatic cells* contain two sets of 23 chromosomes (46 chromosomes per cell in total). However, this is not true for all cells in our body. Our reproductive cells, that is, the egg cells in a woman and the sperm cells in a man, only contain a single chromosome set (only 23 chromosomes in total). Our egg and sperm cells are produced by "germ" cells (**Figure 3**). The reduction of a double chromosome set to the single chromosome set of sperm and egg cells happens during meiosis, a process that you might remember from your biology class (if not, consult our additional resources listed below). Why is it so important that sperm and egg cells only carry one set of chromosomes instead of two?

Somatic Cells





Double Chromosome Set
(23 pairs in somatic cells)

Germ Cells



Single chromosome set after
meiosis (23 chromosomes in egg
and sperm cells)

 = Fatherly chromosome set
 = Motherly chromosome set

Created in BioRender.com 

Figure 3: The reduction of 23 chromosome pairs to 23 single chromosomes in sperm and egg cells takes place during a process called meiosis which happens in germ cells. In contrast, somatic cells have 23 pairs of chromosomes stored in their nucleus.

The human life cycle

During human reproduction, one sperm cell from the father (bearing 23 chromosomes) fuses with an egg cell of the mother (also bearing 23 chromosomes) in a process called fertilization (**Figure 4**). In this way, the resulting first cell, the so-called fertilized egg-cell (zygote), contains 46 chromosomes. Without halving of the chromosome sets by meiosis, the number of chromosomes in the fertilized egg would double after each successive fertilization step per generation. Following fertilization, the fertilized egg goes through numerous rounds of cell-divisions (mitosis) and developmental processes to eventually become an embryo. After typically 9 months of development, the baby is born. As should be clear now, meiosis ensures that the number of chromosomes in the body cells of each newborn is 46 (23 pairs) instead of doubling after each fertilization. Once grown into maturity, the individual can then pass on his or her own genes through the germ cells (which produce egg and sperm), repeating the sexual reproduction cycle.

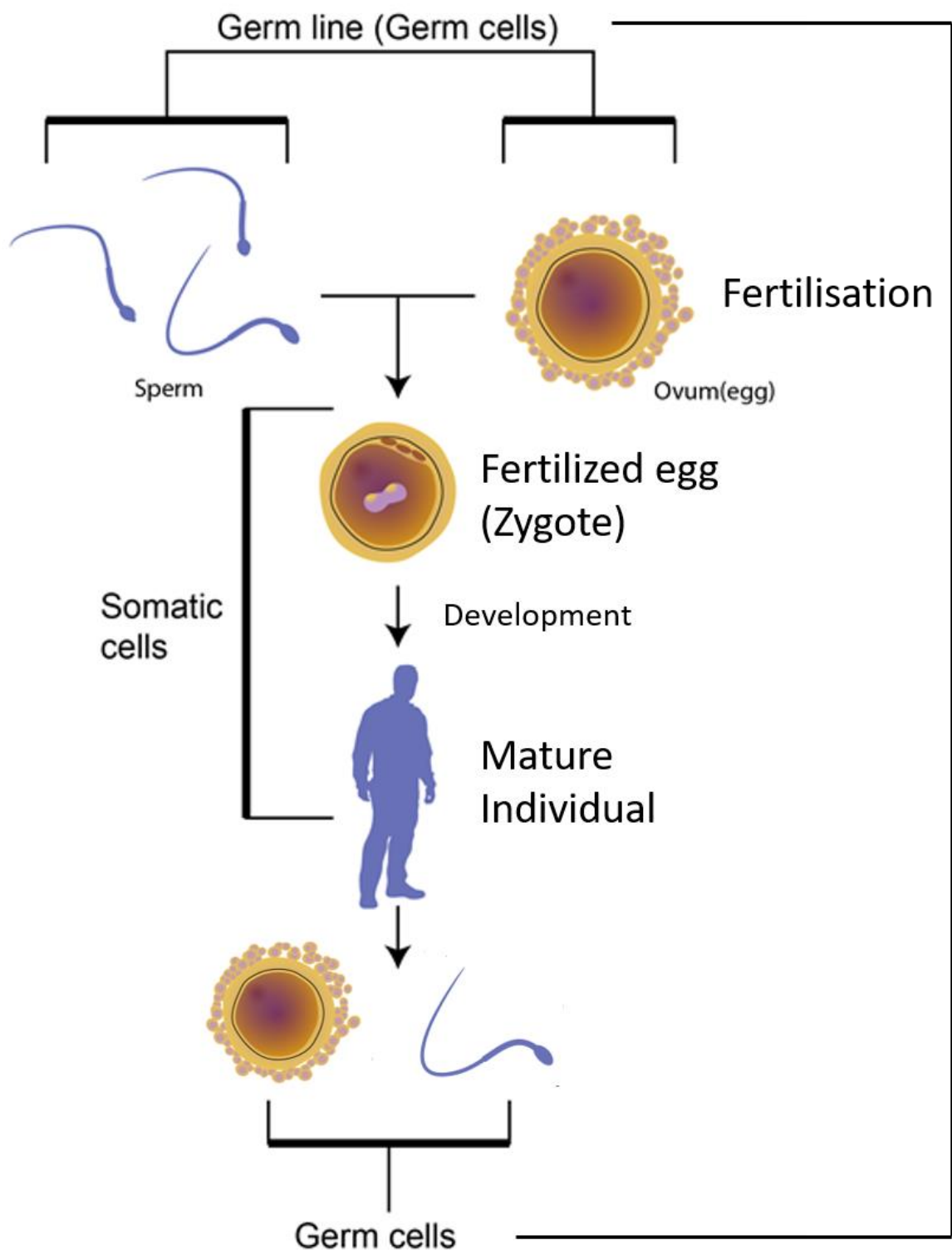


Figure 4: Human life cycle. During fertilization, an egg and a sperm cell fuse to form a fertilized egg, also called zygote, which goes through multiple divisions and developmental steps until, ultimately, becoming a mature human individual. This person will form his or her own germ cells and can produce own offspring.

Conclusion

You might now ask what all of this information about chromosomes and their distribution during reproduction tells us about how our genes are passed on to us from our parents (and how you might, in the future, give them to your children)? Let's summarize.

The basic genetic information has been passed on to us by our parents via the fusion of one sperm and one egg cell. Remember that your parents themselves received their genes from their parents, so your nose might look quite similar to that of your grandfather or -mother...! In contrast to somatic cells (46 chromosomes), sperm and egg derived from germ cells contain one set of 23 chromosomes. Upon fertilization, the fusion of a sperm and an egg cell, the fertilized egg cell, will divide and develop into a fully grown individual. Therefore, half of our chromosomes are from our father while the other half are from our mother. The combined genes of our parents result in our genotype and contribute to our phenotype.

Homologous chromosomes might carry different versions of certain genes (also called alleles), as they come from different family backgrounds (the grandparents and their ancestors). If your parents, for instance, have different gene variants determining hair color, the gene(s) determining this characteristic will combine during your procreation so you will have both gene versions (*alleles*). This genetic combination, together with environmental factors during pregnancy and later in life will lead to your specific phenotype.

Since only the germ cells (which give rise to egg and sperm cells by meiosis) carry inheritable genes, only changes in the DNA of germ cells can be passed onto the next generation. Direct changes in the DNA of somatic cells (all cells that are not germ cells), for instance in your skin cells, will not be passed onto the next generation. This is important to keep in mind for our discussion on the ethics of human germline editing later in the course. In the next chapter, you will learn more about how the DNA-sequence can (be) change(d).

Additional Resources:

Meiosis:

<https://www.genome.gov/genetics-glossary/Meiosis>

Sources:

Reece, B. R.; Urry, L. A.; Cain, M. L.; Wasserman, S. A.; Minorsky, P. V. & Jackson, R. B. (2014). *Campbell Biology, 10th edition*. Pearson.

<https://www.genome.gov/genetics-glossary/germ-line>

<https://www.genome.gov/About-Genomics/Introduction-to-Genomics>

Image-Attributions:

The image of Figure 1B, "Human Chromosomes" is provided under the public domain by [National Human Genome Research Institute](#).

The image of Figure 3 is provided under the public domain and adapted from [National Human Genome Research Institute](#).

1.6 Mutations and Genetic Modifications – Changing the Genetic Code

In the previous articles and animations you learned the basics of genetics and heredity. In the upcoming section we will see what happens when genes in an organism change. We will discuss how these changes in the genotype can arise and how they can be useful in biotechnology.

What are mutations?

So far, we considered our genotype to be constant and unchangeable. However, our genome is far from static. There are many factors that can cause our DNA-sequence to change. As a reminder, the DNA is a double helix made up from the 4 chemical units containing the bases Adenine, Guanine, Cytosine and Thymine, which we refer to as the letters A, G, C, and T, respectively (see also animation 1.3). Therefore, the DNA is represented as a sequence of the 4 letters A, G, C and T. Changes in this 4-letter DNA-sequence are called mutations. Although the term mutation may seem scary at first glance, mutations are quite common in the DNA of our cells. Mutations happen approximately 10,000-100,000 times per cell and per day. They occur mostly due to errors during cell division. Only a very little number of these mutations stay behind, since they are quickly repaired by our cells. And even if the mutations are not repaired, they mostly cause no problems. However, exposure to harmful substances or to, for instance, too much UV light (by lying in the sun for too long without sunscreen for example), also causes mutations and may increase the total amount of mutations that end up in your DNA.

Types of mutations

There are different types of mutations. Here, we will focus only on deletions, insertions, and substitutions (**Figure 1**). Deletions remove one or (many) more bases from the DNA. Insertion mutations add one or more additional bases or even larger DNA-sequences to the DNA. Lastly, during substitution mutations, single bases or larger DNA sequences are exchanged between two DNA molecules. In **Figure 1**, these 3 types of mutations are just illustrated as events in which single bases are changed. However, all mutations can also occur on larger stretches of DNA, and thereby delete, insert, or substitute whole genes or even larger genomic regions.

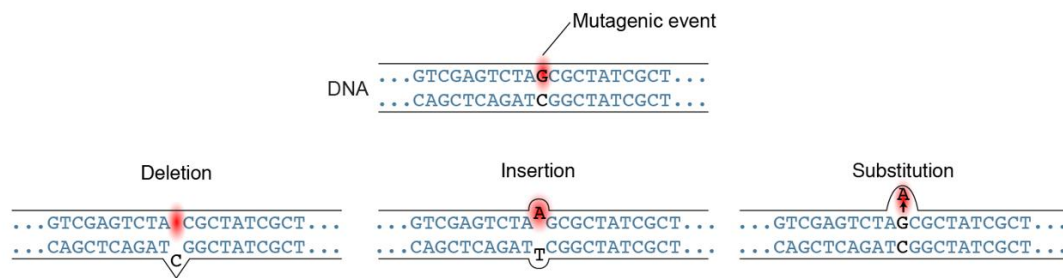


Figure 1: Deletion, Insertion and Substitution mutations. DNA is made up from the 4 chemical units Adenine, Guanine, Cytosine and Thymine, which we refer to as the letters A, G, C, and T, respectively. Changes in the sequence of A, G, C and T as shown in the picture are called mutations. Courtesy: National Human Genome Research Institute.

Genetic Modifications

Apart from naturally occurring mutations, there are also specific methods by which scientists can introduce mutations into the DNA of an organism. The process of intentionally changing an organism's genome is called genetic modification or also genetic engineering. Organisms whose genome has been altered by genetic modification/engineering techniques are defined as genetically modified organisms (GMOs; **Figure 2**). In contrast, if DNA mutates spontaneously or in a non-directed (random) way, the organism carrying the mutation(s) is considered a **non-GMO**. If this was not the case, then virtually every person would need to be considered as GMO, since spontaneous mutations happen relatively often (see above paragraph "What are mutations?"). In the next section, we will look at two different techniques to genetically modify the genome of an organism, by random genetic modification and alternatively, by using specific genome editing techniques.

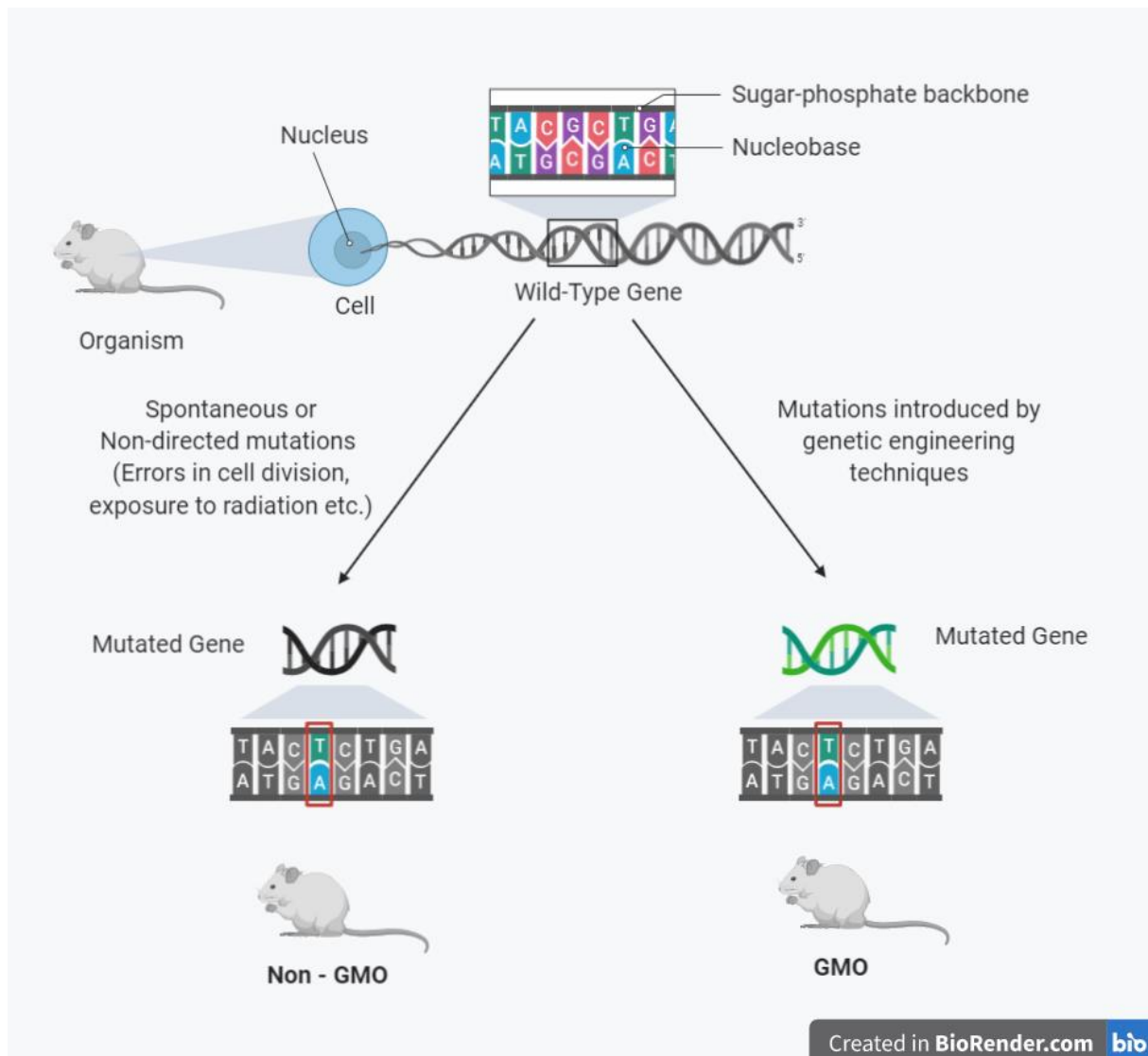


Figure 2: Genetic Modification and Genetically Modified Organisms. When mutations in a gene happen spontaneously or in a non-directed (random) way, the organism carrying the mutated gene is called a non-GMO. Conversely, if mutations are introduced into the genome in a laboratory using genetic engineering techniques, the organism is a GMO. Wild type: this term means “as occurring in nature” or “without prior editing”.

Random genetic modification

The DNA of an organism can be modified in many ways. A possible option is to expose the DNA to radiation or mutation-causing (mutagenic) chemicals to introduce mutations at random positions in the DNA. In this way, mutations are introduced into DNA in a nonspecific manner: one cannot anticipate where the genetic code will be altered, since the mutations occur randomly. This kind of random genome editing has been used a lot to develop new crop varieties and many of the flowering plants that we now have in our houses and gardens. Interestingly, crops that have been modified by random genetic modification are not considered as GMOs, although the mutations were introduced on purpose (compare **Figure 2**). The reason for this is that the mutations introduced are non-specific and (could have) happened naturally.

Specific genome editing

For scientists it is of course very interesting to be able to change the genome in a more specific way. Therefore, it should come as no surprise that various precise genome editing techniques have been developed. It is, for instance, possible to specifically modify single bases in the genome and to remove or insert whole genes at specific positions. One recent revolutionary technique for precise genome editing, which is also largely topic of this course, is the so-called CRISPR-Cas gene editing. The scientists who developed the CRISPR-Cas method, Emmanuelle Charpentier and Jennifer Doudna, were even awarded with the Nobel price in chemistry for their discovery in 2020! We will come back to the specifics of the CRISPR-Cas method later in the course. For now, we will look at why one would want to modify an organism's genome on purpose and how this is already done in our society.

Genetic Modifications – Where are we now?

There are several ways in which genetic modifications are already being used. For instance, scientists genetically modify bacteria, human cells and even organisms such as mice to study and find treatments for human diseases. GMOs also have great potential for industrial production of medication. A genetically modified variant of the bacterium *Escherichia coli* is currently used to produce human insulin (**Figure 3**). Insulin is used to treat persons suffering from diabetes. To have *E. coli* produce human insulin, scientists inserted the human gene for insulin in the genome of the bacteria. Before genetic engineering techniques existed, insulin had to be isolated from the pancreas of pigs, taken from carcasses in slaughterhouses. To isolate only ~230 g of insulin, up to 2000 kilos of pig pancreas material had to be used! In contrast, using genetically engineered *E. coli* bacteria as producers of human insulin is much more efficient and economic.

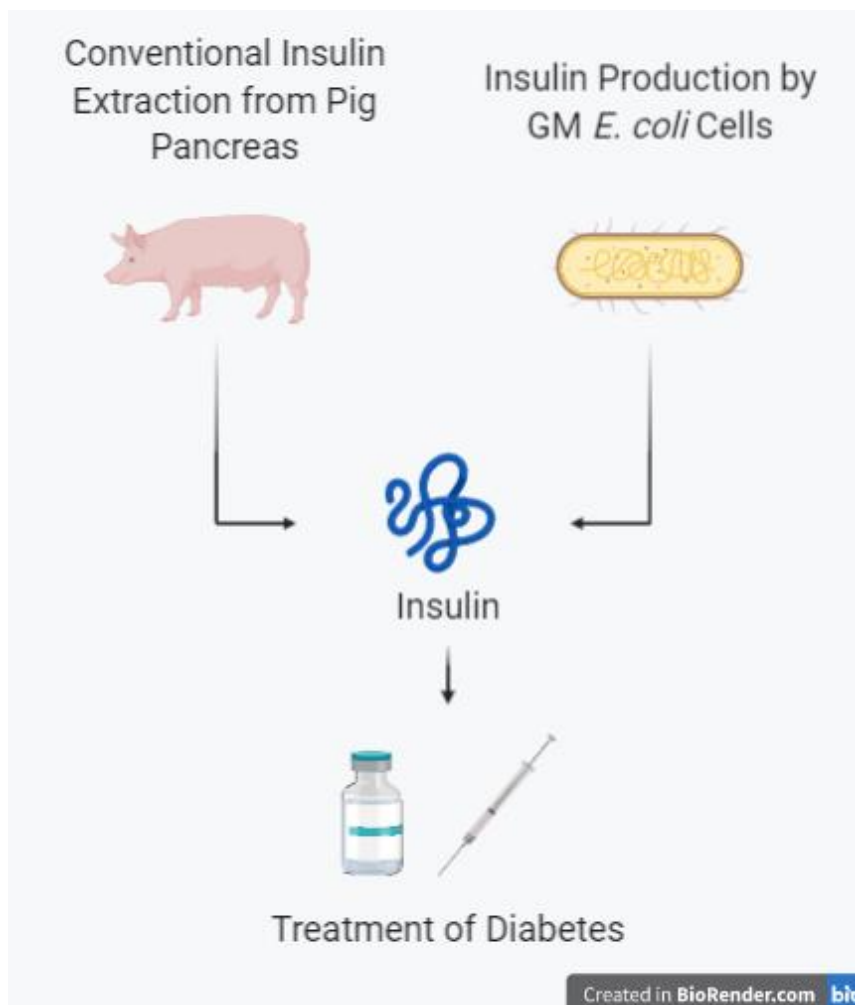


Figure 3: Genetic engineering of *E. coli* to produce insulin. By genetically engineering the bacterium *E. coli*, it is possible to produce insulin in a much more resource-sparing and profitable way than conventional insulin extraction from animal pancreas of slaughterhouse offal. GM: Genetically modified.

Outlook Next Chapters

There are many more fields in which genetic modification and genetically modified organisms are or could potentially be used in biotechnology. If you are interested, we provide resources for further reading. In this course we will focus mostly on the possible use of genetic modifications in humans using the CRISPR-Cas gene editing technique to which you will be introduced in the following articles and animations.

Further Resources:

On the use and possible risks of GMO-use:

<https://www.nature.com/scitable/topicpage/genetically-modified-organisms-gmos-transgenic-crops-and-732/>

References:

Reece, B. R.; Urry, L. A.; Cain, M. L.; Wasserman, S. A.; Minorsky, P. V. & Jackson, R. B. (2014). *Campbell Biology*, 10th edition. Pearson.

<https://www.genome.gov/About-Genomics/Introduction-to-Genomics>

<https://www.fda.gov/food/agricultural-biotechnology/science-and-history-gmos-and-other-food-modification-processes>

<https://www.nature.com/articles/d41586-020-02765-9>

<https://americanhistory.si.edu/blog/2013/11/two-tons-of-pig-parts-making-insulin-in-the-1920s.html>

1.7 Quiz: Test Your Knowledge on Genetics and Biology! (Multiple-Choice)

1. Which statement(s) about the phenotype is/are correct?

1. The phenotype is completely determined by the genotype.
2. The phenotype describes the observable traits of an organism.
3. Phenotypic traits are always inheritable.
4. Phenotypic traits are **not** influenced by the environment.

2. Which statement(s) about the genotype is/are correct?

1. The genotype always completely determines the phenotype.
2. The genotype is defined as the complete set of DNA in our cells.
3. The genotype and phenotype are not connected at all.
4. Only a few genes make up the genotype.

3. Which statement(s) on mutations is/are correct?

1. Mutations are rather unusual events.
2. Mutations mostly result in a change of an organism's phenotype.
3. Naturally occurring mutations can be repaired by the cell.
4. Mutations can be introduced into an organism on purpose.

4. Which statement(s) on genetic modification is/are correct?

1. Genetic modification is defined as introducing mutations at precise spots in the genome.
2. CRISPR gene editing is a recently developed technique to edit the genome of an organism with high precision.
3. Non-specific genetic modification is **not** a form of genetic modification.
4. An organism whose genome has been modified using genetic modification techniques is called a genetically modified organism.

5. Which statement(s) about human chromosomes is/are correct?

1. Our (somatic) body cells contain 46 chromosomes in total.
2. Most chromosomes occur in homologous pairs.
3. Homologous chromosomes and the genes they encode are completely identical.
4. We inherit one set of our chromosomes from our mother and another set from our father.

6. Which statement(s) about germ cells is/are correct?

1. Germ cells contain the same number of chromosomes as somatic cells.
2. Germ cells arise from somatic cells by meiosis.
3. Germ cells contain only a single set of chromosomes.
4. Germ cells contain a double set of chromosomes.

1.7 Quiz: Test Your Knowledge on Genetics and Biology! (Open Questions)

Open Questions

Take out a piece of paper or open a word processor (such as Microsoft Word) and write down the answers to the following open questions:

1. Explain briefly why genetic modification of *E. coli* made insulin production easier.
2. Briefly explain how germ cells combine to develop a new individual. Also indicate how the chromosome numbers in the different cell types change during this process.
3. What would happen if one changed the DNA of a fertilized egg cell (zygote) with respect to the developing individual? In contrast, what is the difference when DNA of somatic cells in a grown individual is changed?

1.7 Quiz: Open Questions

1. Explain briefly why genetic modification of *E. coli* made insulin production easier.

Answer: By genetically engineering E. coli to produce the diabetes therapeutic insulin, insulin production became much more profitable and resource-sparing than before. Before the use of genetically modified E. coli, insulin had to be isolated from cattle pancreas in an exhaustive and uneconomic way.

2. Briefly explain how germ cells combine to develop a new individual. Also indicate how the chromosome numbers in the different cell types change during this process.

Answer: A sperm cell (1 set of 23 paternal chromosomes) fuses with an egg cell (1 set of 23 maternal chromosomes) to yield the fertilized egg cell, also known as zygote (two sets of 23 chromosomes, 46 in total). The zygote proliferates and goes through many developmental stages to eventually give rise to all the organs and tissues of the baby that is born after typically 9 months.

3. What would happen if one changed the DNA of a fertilized egg cell (zygote) with respect to the developing individual? In contrast, what is the difference when DNA of somatic cells in a grown individual is changed?

If the DNA of the zygote is changed, the developing individual will have these changes in all its body cells (including germ cells), since all body cells are derived from the zygote. Thus, the changes in the DNA would be passed on to future generations. In contrast, if DNA of somatic cells in a grown individual is changed, it will only locally affect the modified cells and not all cells of the organism. Changes in the DNA are not passed on to future generations in this case

1.8 Video Summary: Genome editing with CRISPR-Cas

To help you understand the concepts of the video, please find below a summary of the most important points that were discussed as well as additional explanations of important concepts of CRISPR-Cas gene editing.

What does the natural CRISPR-Cas system do?

CRISPR is an abbreviation for Clustered Regularly Interspaced Short Palindromic Repeats and is a technique to modify DNA. It is a technique inspired by the natural defence mechanism of bacteria to protect themselves against bacterial viruses (bacteriophages).

In nature, the CRISPR-Cas system is used by bacteria as a kind of immune system. When a bacterium is attacked by a bacteriophage, specific RNAs in combination with the Cas enzyme find the target DNA sequence (the genome of the attacking bacteriophage) by base-pairing. Once the target DNA is found, the Cas enzyme cuts the DNA thereby disabling the multiplication and further spread of bacteriophages. Researchers have modified this system to selectively introduce changes into the DNA of any organism. In the next paragraph, you will learn more about the basic components of the modified CRISPR-Cas system and the detailed CRISPR-Cas reaction mechanism.

What are the components of CRISPR-Cas in gene editing?

The CRISPR-Cas gene-editing system consists of 2 key components: (1) A guideRNA (gRNA) and (2) a DNA-cutting enzyme called Cas 9. In the following, the most important characteristics of the two components are outlined.

- Guide RNA - Ribonucleic acid (RNA) is a molecule made up of ribonucleotides, whose function it is to convert genetic information into proteins. Unlike the DNA, RNA is mostly found in a single-stranded form. (**Figure 1**). Instead of thymine (T), RNA carries uracil (U). In the CRISPR-Cas system, RNA plays a key role in binding to the target DNA by recognizing the complementary bases that are paired (e.g. CG, AT or AU). The binding guides the second key element, Cas9, to this target location. This is why it is called guide RNA (gRNA) because it guides the Cas protein to a specific target DNA sequence.

- Cas9 - The Cas9 protein is an enzyme that acts as molecular scissors. It forms a complex with the gRNA and is guided to the target DNA. Once the sgRNA binds the target DNA and Cas9 is appropriately positioned, it will cut the target DNA at this specific location.

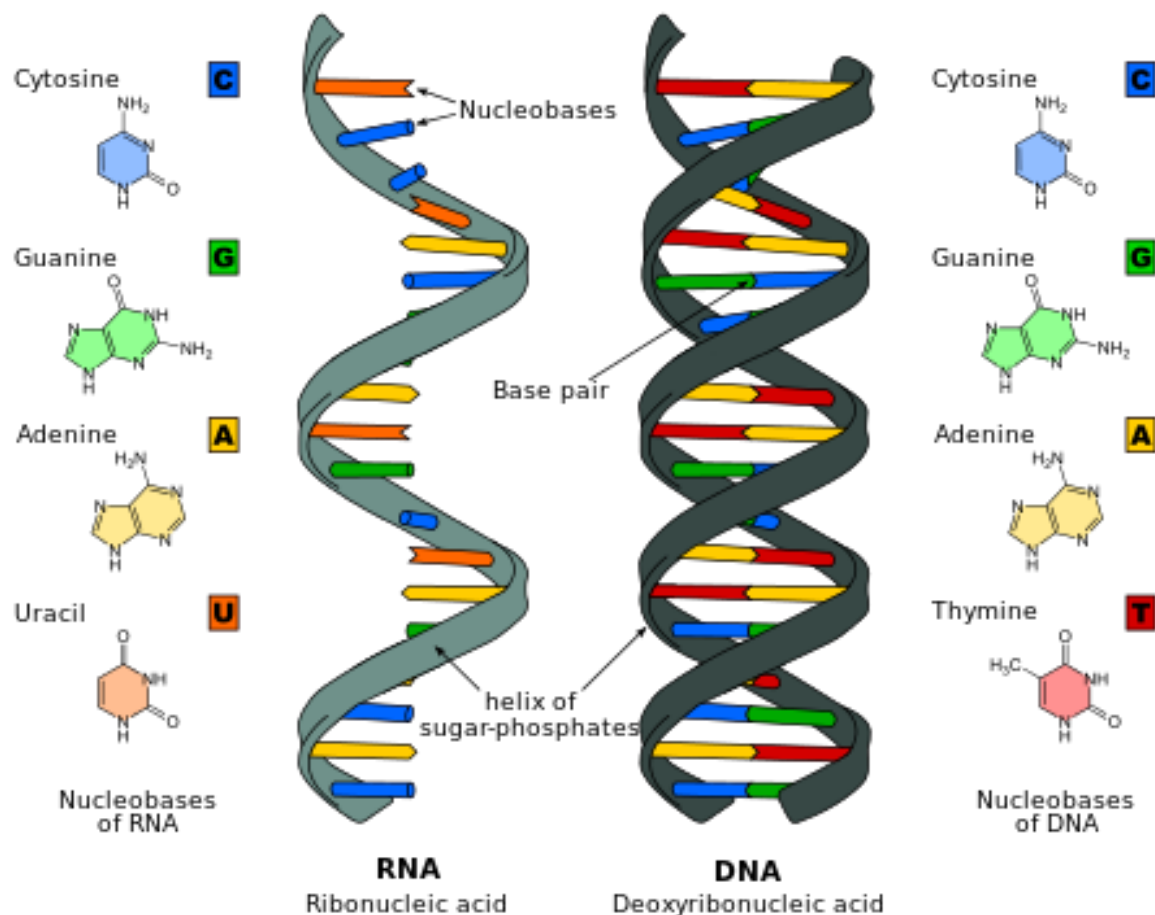
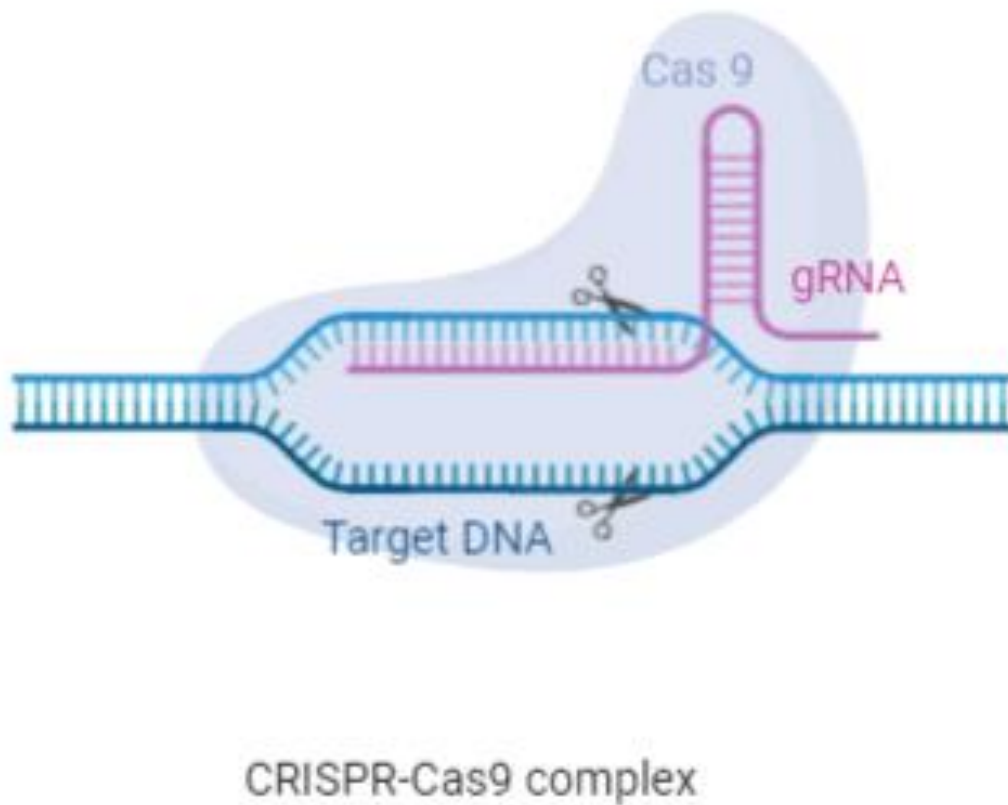


Figure 1: Structure of RNA and DNA, highlighting the differences between the 2 molecules (left) RNA is a single-stranded molecule with uracil as one of the 4 bases, whereas (right) DNA is a double-stranded molecule where uracil is replaced by thymine. The other 3 bases are the same.




Created in BioRender.com 

Figure 2: The gRNA (top strand, shown in red) and the Cas9 enzyme form a complex in which the gRNA pairs with the target DNA and thereby guides the Cas9 enzyme to its specific cutting sequence in the target DNA (shown in blue). This image was created using Bio Render.

The mechanism of CRISPR-Cas

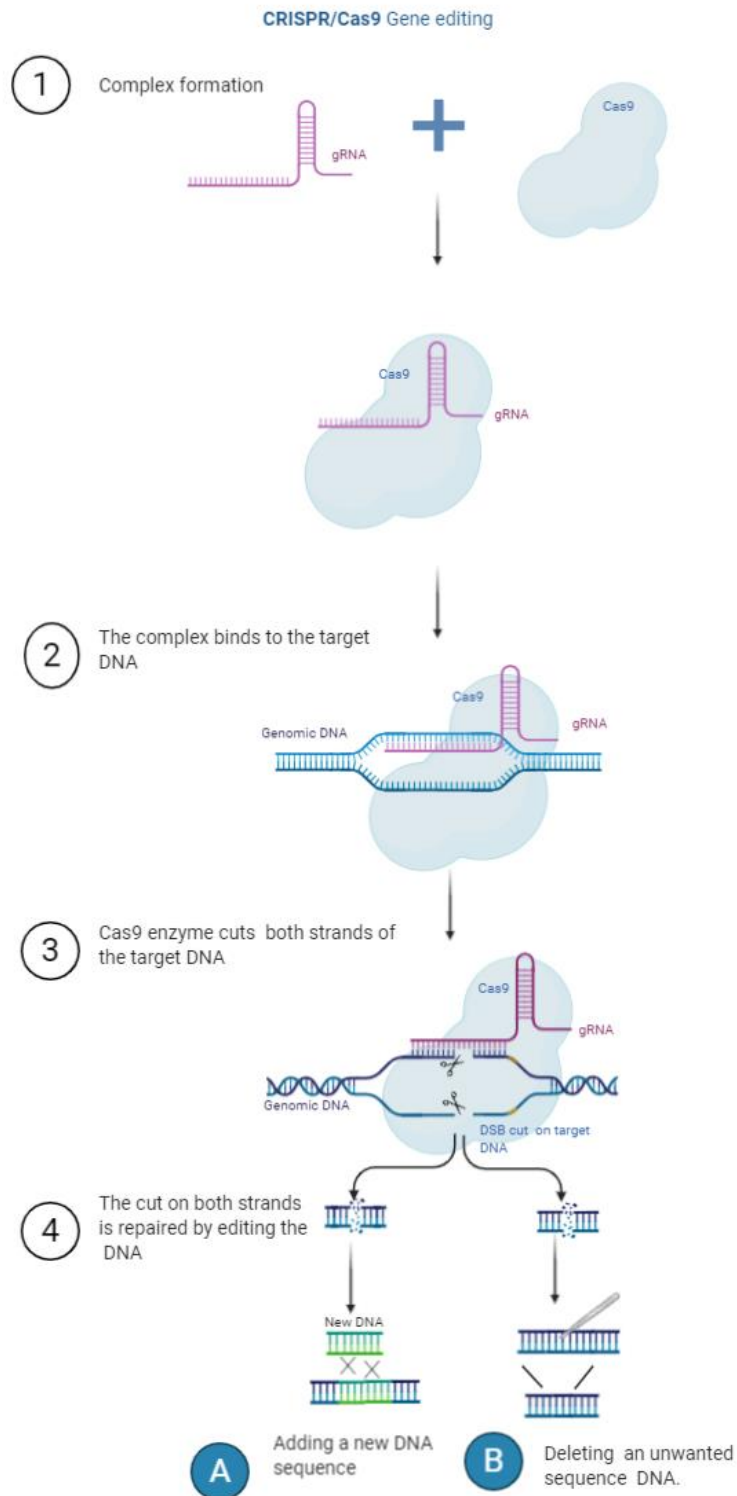
Together, Cas9 and the gRNA can be used to edit the genome. The stepwise mechanism of the CRISPR-Cas9 system for genome editing is described below (see also **Figure 3**):

- Complex formation - The guide RNA (gRNA) sequence is specifically designed to precisely match a certain sequence in the target DNA. This gRNA also forms a complex with Cas9.

- The gRNA-Cas9 complex binds to the target DNA - The gRNA finds and binds the target DNA by its complementary sequence.
- Double-stranded break in the target DNA - Once the Cas9 complex is bound to the target DNA, the Cas9 enzyme acts as molecular scissors and cuts both strands of the DNA of interest. This cut results in a so-called double-strand break (DSB).
- Editing the target DNA - Once a DSB is introduced in the target DNA, this is the location where the DNA can be repaired and edited (changed at will) at the same time. This can be done in two ways:

A: A new DNA sequence is added at the site of the DSB to repair the break. This will lead to a DNA insertion or addition (see also Chapter 1.6).

B: A part of the DNA sequence in the genome that is unwanted can be removed from the target DNA at the site of the DSB. This is known as a DNA deletion.



Created in BioRender.com 

Figure 3: Overview of the CRISPR-Cas reaction mechanism. This image was created using Bio Render.

Using CRISPR-Cas9 for genome editing

CRISPR-Cas9 is a powerful genome-editing tool because of its specificity and simplicity. By changing the guide RNA sequence, CRISPR-Cas9 can be used to target virtually any region in the DNA, to cut it out or to add a new DNA sequence. There are numerous implementations for CRISPR-Cas9 in agriculture, drug development or even to cure life-threatening diseases caused by a mutation in the genome. In the next chapter, we will provide possible future applications of CRISPR-Cas9 gene editing including how to use it for treating diseases or even eradicate malaria.

Sources

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Attributions

Image 1 - "[File:Difference DNA RNA-EN.png](#)" by [Guku235](#) is licensed under [CC BY-SA 4.0](#)

Image 2 - Created with [BioRender.com](#)

Image 3 - Created with [BioRender.com](#)

1.9 Quiz: CRISPR-Cas

1. What does the acronym CRISPR stand for ?

1. Clustered Regularly interspaced short palindromic repeats.
2. Cut Reservative interspaced small palindromic recounts.
3. Controlled regular interspaced special palindromic repeats.
4. Cut recognised Intron Scissors Polygonal repeats.

2. In what type of organism was CRISPR discovered?

1. Plants
2. Marine organisms
3. Viruses
4. Bacteria

3. What is the role of the guide RNA?

1. It helps the cell repair the DNA.
2. Makes a cut across the DNA strands .
3. Ensure that the Cas enzyme cuts at the right point in the genome.
4. Cut the invading pieces of RNA.

4. The guide RNA is –

1. Complementary to the DNA sequence of interest.
2. An exact copy of the DNA sequence of interest.
3. Complementary to the Cas protein.
4. An exact copy of the Cas proteins.

5. What does the Cas protein do?

1. Cut the guide RNA.
2. Binds and cuts the guide RNA.
3. Cuts DNA.
4. Binds and cuts the DNA.

1.10 CRISPR-Cas as a remedy against malaria?

CRISPR-Cas has the potential to eradicate various life threatening diseases. One example could be freeing the world of malaria. More than 600,000 persons die of malaria every year, the majority of which are children. By inserting specific genes into the genome of the mosquito that hosts the malaria parasite, we might prevent the death of thousands of children and grown-ups. However, is it okay for us, humans, to change the course of evolution in such a drastic way?

Understanding the mechanism of malaria

Malaria is a disease caused by a parasite that is transferred to humans via a mosquito. This parasite infects a certain type of female mosquitoes, called Anopheles. The Anopheles do not suffer from the infection and are immune to the parasite. If the infected mosquito bites a human, it will inject the parasite into the bloodstream. Once inside the human body, the parasite travels through the bloodstream to the liver. Here it infects and multiplies inside the red blood cells, which then burst after a few days, releasing hundreds of new parasites per red blood cell into the bloodstream. The malaria parasite is transferred from human to human by the mosquito, which acts as a carrier. When a mosquito bites a someone infected with the malaria parasite, the parasite will stay in the mosquito's salivary glands where it is ready to infect the next person bitten by the mosquito (**Figure 1**). Symptoms of malaria can vary from a severe headache, nausea and vomiting, to a coma caused by blocked blood capillaries. This can, when untreated, result in death.

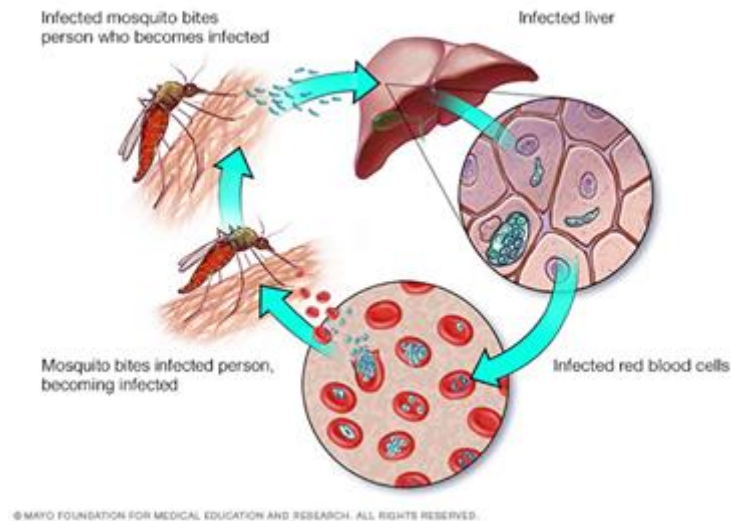


Figure 1: Malaria transmission cycle. Copyright by Mayo Foundation for medical education and research.

Apart from malaria, mosquitoes are host to other parasites and viruses that are responsible for potentially deadly diseases such as dengue fever, yellow fever and the zika-virus. In addition, mosquitoes are present in enormous numbers worldwide and can lay up to 200 eggs at a time. This makes mosquitoes one of the deadliest animals on our planet. Therefore, there is an urgent need to invent an efficient solution to this problem.

Role of CRISPR-Cas in controlling malaria

Current drugs to kill the malaria parasite are harmful to the human body. In addition, the cost of treatment is often too high for families in developing countries without constant access to laboratory equipment.

This is where CRISPR comes into play. In 2018, researchers from the Malaria Research Institute at Johns Hopkins Bloomberg School of Public Health used the CRISPR-Cas system to delete a gene called *FREP1* from the genome of mosquitoes. This gene encodes a fibrinogen-related protein that is necessary for the malaria parasite to survive inside the mosquito. Deletion of this gene drastically reduced the number of mosquitoes infected with malaria because the parasites were unable to survive.

Another CRISPR-based strategy to combat malaria is to introduce genes coding for anti-malaria proteins into the genome of the mosquitoes. These proteins, called antibodies, will then be produced by the mosquito thereby "curing" themselves of the parasite.

Ethics of using CRISPR-Cas to alter mosquito genome

Although the promise of using CRISPR-Cas against malaria seems wonderful, it opens up several ethical questions and concerns. One of the biggest worries entails the uncontrolled spread of genes that are artificially introduced into the mosquito genome. When the modified mosquitoes are released into the wild they will breed with normal mosquitoes and pass on their anti-malaria genes to future mosquito generations. Once released, there is no way of stopping the mosquitoes spreading the anti-malaria genes. Therefore this technology has to be used carefully and all the risks and ethics should be thoroughly evaluated. Should we really interfere with natural processes and drive the evolution of mosquitoes in a certain direction? On the other hand, because thousands of malaria deaths could be prevented, we should also ask the question "Is it ethical to not use this technology to help millions of persons?"

Ethical questions like these often arise when talking about possible CRISPR-Cas applications. In the following parts of this course, we will deal with many more situations that require critical ethical evaluation of the CRISPR-Cas technology and its applications. Especially, a dialogue about ethics is inevitable when considering the editing of the human genome.

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1.11 Video Summary

To help you understand the concepts discussed in the video please find below a short summary.

CRISPR-Cas and cystic fibrosis

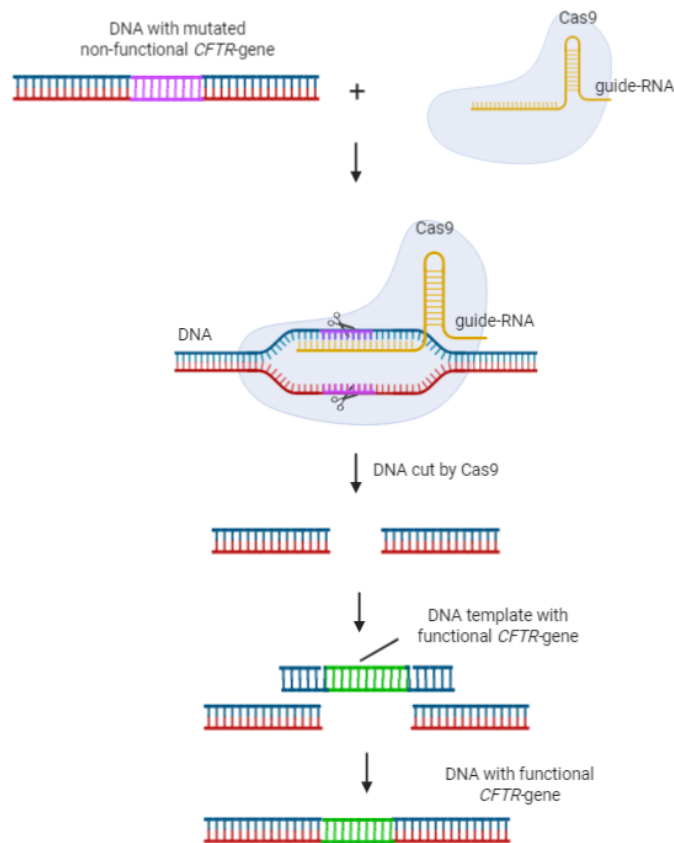
Patients with cystic fibrosis have thick sticky mucus that can clog the lungs and obstruct the pancreas. Cystic fibrosis can therefore be life threatening. This hereditary disease is caused by mutations in the CFTR gene, encoding for the CFTR protein.

CRISPR-Cas could be used to replace the mutated gene with a functional one. The Cas9 protein can unwind a section of DNA to see if the guide RNA sequence it carries matches. If there is a match, Cas9 binds and cuts the DNA to create a break. The DNA break will be repaired by the cell. At this point the mutated CFTR gene can be replaced.

Replacing the mutated gene

To replace the mutated gene with a functional one, we use our cell's own repair process that precisely edits the DNA. This repair process uses a short DNA template that contains the sequence of a functional CFTR gene. The cell uses this DNA fragment as a template and copies this sequence as it repairs the broken DNA. This results in the mutated gene sequence being replaced by the nonmutated one (see Figure1). The cell then uses the corrected DNA strand to produce a functional CFTR protein. This will restore normal mucus viscosity, so the mucus is not sticky anymore.

Using CRISPR-Cas, the mutated gene encoding nonfunctional CFTR protein could be replaced by a one that produces functional CFTR protein. Therefore, CRISPR-Cas could improve the quality of life of cystic fibrosis patients. How this technique can be used for various human applications will be discussed in more detail in the next chapter.



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Figure 1: CRISPR-Cas can be used to replace the mutated CFTR gene (purple) with a functional CFTR gene (green).

Should we engineer human DNA?

CRISPR-Cas could greatly improve the quality of life of cystic fibrosis patients, but is it desirable to modify human DNA? Should we have hereditary disorders removed from the DNA? And can one also use CRISPR-Cas to make children smarter or stronger? These and other ethical questions about modifying human DNA are discussed in the second lesson in this series.

1.12 Somatic and Germ Cell Engineering

As you have just seen in animation 1.12, CRISPR-Cas genome editing can be potentially applied to treat patients suffering from genetic diseases. Generally, there are two major approaches to cure genetic diseases. Firstly, by engineering *germ cells* and secondly, by engineering *somatic cells*. In this chapter we will look at the basic difference between CRISPR-Cas genome editing of somatic cells and germ cells. To do this, we look at specific examples to illustrate both approaches.

What are somatic and germ cells and what is the difference in genetically modifying them?

Let us begin by refreshing our knowledge on what *somatic cells* and *germ cells* are. We already came across these terms when discussing human heredity in chapter 1.6. *Germ cells* are reproductive cells, in males they produce the sperm cells and in females they produce the egg cells. Upon conception, a sperm and an egg cell fuse to form a fertilized egg cell (zygote). This process is called fertilization. The zygote divides and ultimately develops into a human being. In other words, *germ cells* give rise to future generations. Any genome editing in these cells will thus be passed onto the next generations (**Figure 1**). *Somatic cells*, on the other hand, are all the cells in our body (except the germ cells, of course). Cells making up the eyes, the brain, or any other organ or tissue are therefore *somatic cells*. Genetic changes in *somatic cells* are not passed on. Changes in somatic cells only affect specific tissues of the individual receiving gene-editing therapy.

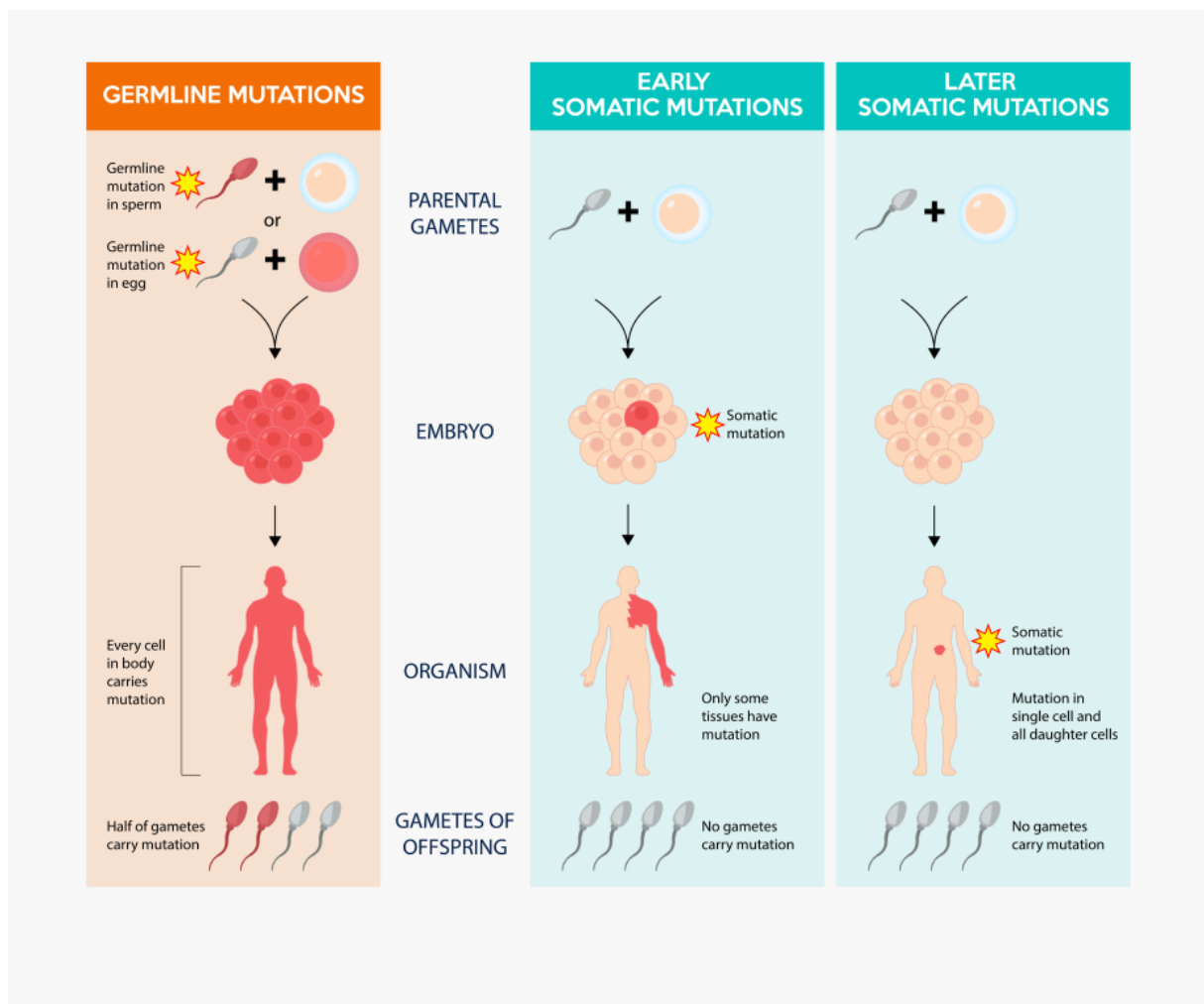


Figure 1: Difference between germ line and somatic cell genome editing.

Somatic Cell Editing - Using CRISPR-Cas to Cure Blindness

First, we will take a look at an example of somatic cell editing to cure a disease called Leber congenital amaurosis (LCA). LCA is a rare, inherited eye disease that affects the retina. This specialized tissue is located at the back of the eye and is responsible for detecting light and color and thereby producing vision. People suffering from LCA have almost no vision from childhood onward. The most common cause of LCA is a mutation in the *CEP290* gene which causes problems in retina cells (note that the *CEP290* gene is present in all cells of the body but are only relevant for LCA patients in the retina). Clinical researchers at the Casey Eye Institute of the Oregon Health & Science University (OHSU) in the USA have started a clinical trial in which they aim to remove the LCA-causing mutation in the *CEP290* gene using CRISPR-Cas genome editing. They do this by directly injecting the CRISPR components into the (somatic) cells of the retina of LCA patients. In this way, the light sensing cells of the retina should be reactivated and enable LCA patients to see clearly again.

It is important to note that in this example of treating LCA with CRISPR-Cas genome editing, no changes in germ cells are made. The changes in the DNA are restricted to retina cells and the patients receiving the treatment still carry the mutation in their germ cells. Therefore, even after successful treatment of their retina using CRISPR-Cas, the cured patients may still pass on the mutation to their children (also see **Figure 1**).

Germ Cell Editing - Eradicating Hereditary Diseases

The CRISPR-Cas genome editing technique could be used to eradicate hereditary diseases. A good example is the hereditary disorder called Beta thalassemia. Patients with this disorder cannot produce sufficient amounts of the biomolecule "hemoglobin" in their red blood cells. Hemoglobin is required for the uptake of oxygen. Therefore, beta-thalassemia patients do not take up enough oxygen into their blood and show malformed red blood cells (**Figure 2**). Patients often have a pale skin, and suffer from general weakness, fatigue or even more serious complaints up to death. In beta thalassemia, the *HBB* gene encoding an important component of the oxygen uptake system of red blood cells is mutated. Since it is not possible to fix the *HBB* mutation in all blood cells (there are simply too many), researchers in 2015 have attempted to remove the *HBB*-mutation in a single-celled embryo (zygote) using CRISPR-Cas. In this way they wanted to make sure that all cells of the later body would carry the repaired and functioning gene version. The modified "healthy" *HBB* gene would then be passed onto offspring who also would not suffer anymore from beta thalassemia. The researchers performed these experiments only in the lab on embryos that could not result in a live birth. Since the genetic modifications did not completely work as expected, the experiments were terminated at a certain point. Although these first attempts of modifying the *HBB* gene in the germ line showed that the CRISPR-Cas technique still has a long way to go before it can be used in the clinics, the authors of this study have shown that it is possible to modify disease-carrying genes in a zygote.

In the example above, the fundamental difference between germ cell and somatic cell engineering becomes apparent once again. In somatic cell engineering the genetic changes are confined to a certain tissue and cannot be inherited, whereas germ cell engineering results in the generation of heritable genetic changes (also see **Figure 1**).

Thalassemia

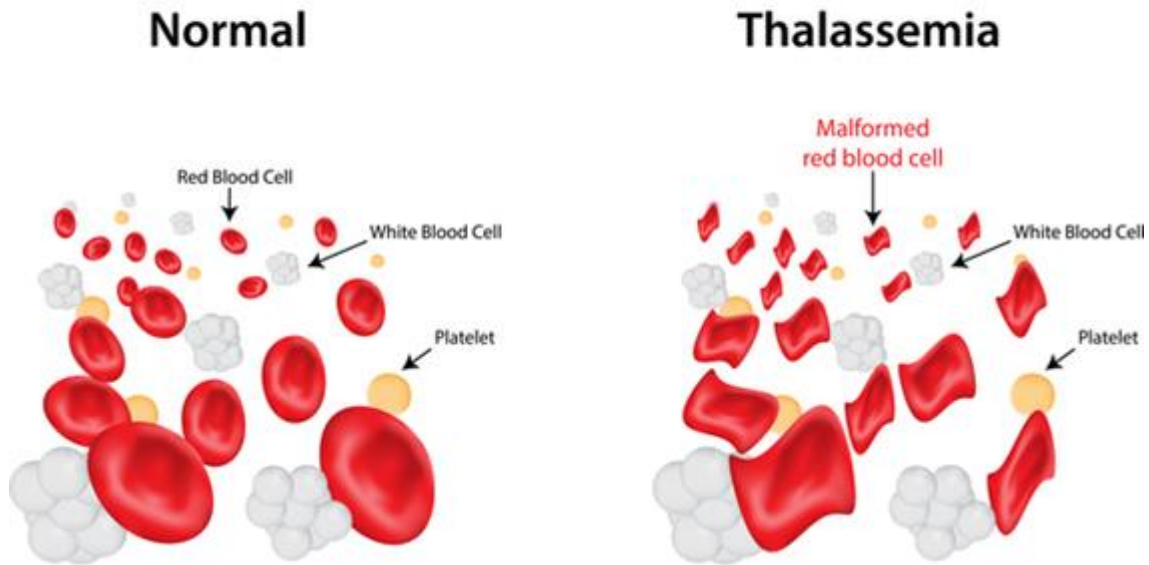


Figure 2: Schematic illustration of healthy blood cells (left) and malformed blood cells of thalassemia patients.

CRISPR-Cas genome editing in Humans - A powerful technology posing ethical questions

As we have seen in the examples above, CRISPR-Cas genome editing can be used to cure various diseases. However, changing DNA in human cells raises many ethical questions that have to be considered: "Should we allow genetic modification that can be passed onto (an) offspring?"; "Would genetic engineering of disease-related genes in germ cells be a slippery slope towards enhancing the functionality of genes?". It is important to take questions like these into account when using techniques as powerful as CRISPR-Cas. Therefore, we devote lesson two of this lesson series to the ethical aspects of using genetic engineering techniques such as CRISPR-Cas.

Sources:

Somatic cell editing - Curing LCA

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<https://clinicaltrials.gov/ct2/show/NCT03872479>

1.13 Treatment of Cystic Fibrosis Using CRISPR-Cas

In this exercise you get the chance to apply your knowledge on CRISPR-Cas gene editing. You will treat a cystic fibrosis patient using CRISPR-Cas based gene therapy.

The exercise:

[EXERCISE: Be a CRISPR-Gene Engineer Yourself](#)

The solution:

[SOLUTION: Be a CRISPR-Gene Engineer Yourself](#)

1.14 Quiz - Medical applications of CRISPR/Cas

1. Adjustments in germ cells can be passed to future generations.

- a. True
- b. False

2. Which of these options are considered germ cells ?

- a. Estrogen and testosterone
- b. Intestine and heart
- c. Sperm cells and egg cells
- d. Uterus and fallopian tubes

3. How could CRISPR be used to eradicate Malaria?

- a. The female Anopheles mosquito carrying the malaria parasite is genetically edited, such that the parasite does not survive in the mosquito
- b. The genome of the malaria parasite is edited using CRISPR, such that the mosquitoes don't carry the parasite.
- c. CRISPR is used to make drugs for quick recovery from the Malaria .
- d. Humans are genetically edited using CRISPR so the parasite does not survive in the our body.

4. Why is it currently not allowed to relieve genetically modified mosquitoes into the wild?

- a. There is a danger of malaria parasite mutating.
- b. There is a risk that genetically modified mosquitoes spread(maybe multiply) uncontrolled in the wild, which may lead to unknown consequences.
- c. The entomologists (scientists who specialize in the study of insects) have banned the genetic modification of insects.
- d. All of the above.

5. What is the difference between treating LCA patients with CRISPR and preventing development of Thalassemia?

- a. Treating LCA patients with CRISPR is a quick process, whereas, preventing development of Thalassemia takes a very long time.
- b. LCA treatment takes place in germ cells, whereas treatment of Thalassemia takes place in somatic cells.
- c. Treating LCA patients with CRISPR takes a very long time, whereas preventing development of Thalassemia is a quick process.
- d. LCA treatment takes place in the somatic cells, whereas treatment of Thalassemia takes place in germ cells.

1.15 Conclusion Lesson 1

In lesson 1 of our lesson series “Engineering Life: Principles and ethics in modifying DNA” we covered many different topics ranging from the basics of genetics up to engineering human cells. In this lesson, you have learned what our genotype and phenotype are, how genetic information is stored. Furthermore, you have learned how our genome can mutate and how these mutations can be inherited by our offspring. Lastly, you have learned how we, using the CRISPR genome editing technique, can introduce mutations ourselves to, for example, remove a disease-causing mutation.

The aim of lesson 1 was to give you background on genetics, the CRISPR genetic engineering technique and a brief glimpse into medical applications as well as the ethical questions that emerge from using genome editing in humans.

As humans, we all descend from a single cell carrying the genetic roadmap that impacts our unique identity. This roadmap can encode genes for many inherited diseases. With genome editing techniques such as CRISPR at hand, we now theoretically have the tools to change our DNA and cure many genetic diseases which had no treatment before. The question we now have to answer is, should we do it? Is it ethically and morally correct to change human DNA? Do we want to interfere with nature? Should genome editing be used to make humans smarter and happier? Or should it be only allowed to cure life-threatening diseases? Questions like these will be addressed in the next lesson “Ethics of Human Genome Editing”.

Lesson 2 – The Ethics of Human Genome Editing

2.1 Introduction Video

Welcome to the second lesson of the online course “Engineering life: principles and ethics in modifying DNA” of the University of Groningen.

In the first lesson you learned that CRISPR-Cas can be used as a molecular scissor that enables scientists to precisely edit the human genome. With this new technology at hand, it is easier than ever to make changes in DNA. You learned various medical applications of CRISPR-Cas and how this technique could be used in our daily lives to find a cure for malaria. But do we really want to engineer DNA?

This week the ethical dilemmas associated with adjusting human DNA will be discussed. You will explore different ethical standpoints regarding human genome editing using CRISPR-Cas and discuss possible future scenarios in which this is allowed. By the end of this lesson you will be familiar with the ethics of genetic engineering and have the background to build your own personal opinion on this topic.

You will also be asked to take part in the second questionnaire of this lesson series. In this questionnaire, that is part of the Dutch DNA dialogue, you will be asked about your opinion on the genetic engineering of the human genome. The results are used by scientists to advise the Dutch government in making decisions regarding regulations about DNA modifications in human embryos.

2.2 Introduction to the Ethics of Human Genome Editing

Nowadays, scientists study various applications of CRISPR - Cas in humans, animals, plants and bacteria. Several studies are being conducted to find a cure for life-threatening diseases such as various cancers, muscular dystrophy and cystic fibrosis. However, many laboratories are also conducting research on how to use gene editing for improving our daily life. For example, using CRISPR-Cas to genetically modify coffee plants to produce coffee without caffeine (decaf coffee), or to genetically engineer racehorses to make them stronger and faster.

Due to the potential of CRISPR-Cas to perform genome editing in the human germline and the impact that it may have on the individuals involved, as well as on society as a whole, several bioethical issues and concerns have arisen. In this chapter, we will discuss what ethics is and why it is important to talk about the ethical dilemmas especially with special regard to (CRISPR-Cas) genome editing.

What are ethics and why are they important?

Ethics are a set of moral principles that aim to guide an individual's behaviour. Ethics address concepts of right and wrong with respect to human behaviour. These principles serve as a compass that guides people on how they should behave among each other and in society as a whole. Consider you are trying to park your car and you accidentally bump into another car, breaking the headlight. If nobody noticed, what would you do? Stick a note on the car apologising and offering to pay for the damages? Or drive away before anyone sees you? The framework of a thought process that one applies in such scenarios to make a decision is called ethics.

With every scientific advancement, it is important to also consider the ethical aspects of the research. We do not want our research to harm anyone. Because of this, there are independent ethics committees to test the impact of our research, both on humans as well as on animals.

Ethical dilemmas of Genome Germline editing

Currently, around the globe, there are many public and worldwide discussions as to whether genome editing on human germ cells should be allowed. But why is human germline editing actually questionable? What are the ethical concerns with genome editing on human germ cells?

In the following sections, we will discuss some of these ethical dilemmas.

Consent of the unborn child

One of the most important questions regarding germline editing is about informed consent. For prospective parents with a genetic variation that carries an inheritable life-threatening disease, germline intervention might be the only solution to have a healthy child. It is important to realize that this child will be an individual affected by the choice of the parents to edit his or her genome. Giving parents the choice of having “genetically edited” children can prevent a lot of suffering from life-debilitating diseases. However, as it is not possible to ask the unborn child for the consent should we allow the genome editing?

Where should we draw a line?

Once genome editing has been proven to be safe and effective for curing life-threatening diseases, CRISPR-Cas might be used for upgrading an individual's quality of life. A medical condition can be defined as a state that may cause mild discomfort, whereas disease is defined as a clinical pathological ailment that may cause pain, discomfort. In addition to treating serious health problems, CRISPR-Cas could also potentially be used for mere enhancement purposes.

To illustrate the difference between therapy and enhancement, consider two boys with short stature. Boy A is short because both his parents are short, while boy B is short due to a deficiency in human growth hormone production (HGH) gene. Both boys desire to be taller because of the current perception of beauty. But are short due to the “genetic lottery”, that they cannot do anything about. Using gene editing for boy A would be considered enhancement since there is no clear identifiable clinical pathology. Employing gene editing for boy B would be considered gene therapy because he has a known genetic defect. This example clearly illustrates that it is difficult to decide where to draw the line between what is acceptable and what not. Also, it is important to remember that the genetic changes made will be a permanent decision for the boys and their future generation. If genome editing is used to treat conditions and enhancement, many critics believe that it will be a slippery slope

towards even more non-essential edits. This might lead to an even wider disparity between the rich and the poor leading to social inequalities.

International policies on germline/germ cell - editing

Germline editing is fairly new technology and still involves a lot of risks, which is why there is a global freeze on its clinical use. Having this moratorium provides time for discussion of ethical, societal and scientific issues and establishing proper international regulations. The EU and 30 other nations ban employing germline editing in the clinic. However, this moratorium does not apply to germline editing for research purposes as long as the studies do not involve implantation of the embryo into a uterus.

As you can see, there has been a lot of public discussion on allowing human germline editing. These discussions have led to a ban on clinical applications. In the following chapter, we will discuss current methods of genome selection.

2.3 Where are we now: Genome selection

CRISPR-Cas may seem like a new technology that could be used to engineer embryo-DNA to give birth to healthy children. However, there is already a clinically established selection method used to select healthy embryos today. In this chapter, we will discuss how this technique works and what is already being used to select for healthy children.

In-vitro Fertilization

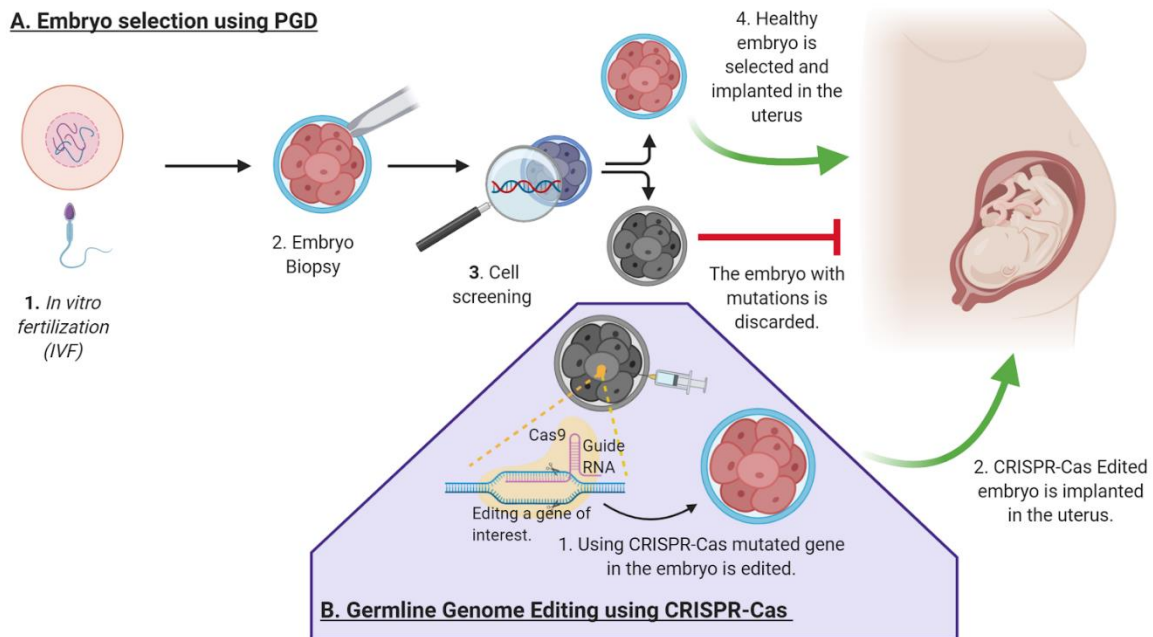
In vitro fertilization (IVF) is a routine technique used in hospitals to help couples with fertility problems or prevent children from inheriting genetic diseases. In IVF, an egg cell taken from the prospective mother is fertilized with sperm from the prospective father outside a body in the laboratory. The fertilized egg cell then develops into an early embryo (see chapter 1.6) still in the laboratory. Using a technology called "Preimplantation Genetic Diagnosis" (PGD), the embryos are screened for the desired genotype, for instance not carrying certain disease-prone alleles. The selected embryos are then implanted back into the uterus of the prospective mother to initiate a pregnancy.

Preimplantation Genetic Diagnosis

PGD (nowadays) and CRISPR-Cas (potentially in the future) are two techniques that pursue the same goal, namely to improve the chance of offering parents genetically related, healthy children (**Figure 1**). The main difference between CRISPR and PGD is that in the latter, all the embryos from prospective parents are screened for genetic mutations and the healthy embryo is selected. Embryo screening is done by taking some cells (biopsy) from the developing embryo. These cells are tested for chromosomal abnormalities like Down's Syndrome or monogenic diseases like cystic fibrosis, predisposition to cancer and even minor disabilities like deafness. Thus, PGD is a genetic selection process that can lower the risk of a child inheriting a genetic disorder, whereas, CRISPR-Cas is used to actively remove the mutation from the embryo's DNA.

It is interesting to note that, two decades ago when PGD selection technique was introduced, it met public opposition as well. The society opposed PGD on the grounds of moral and / or religious grounds, since the embryos discarded in the process are not given a choice between life and death. But over the years, the public perception has evolved. The society has accepted the elimination of embryos that are predisposed to certain cancers and diseases.

PGD versus CRISPR-Cas



Created in **BioRender.com** **bio**

Figure 1: Brief comparison between (A) PGD embryo selection and (B) CRISPR-Cas germline editing. Created with BioRender.com

Selection versus Therapy

The currently used selection technique (PGD) has limitations when both prospective parents carry the same genes for a certain disease because in those cases the genetic mutations would then be present in all their children. Here, germline intervention could be used to remove the mutation from the embryo. Critics argue that editing is not yet precise enough and may cause unwanted mutations elsewhere (called as off-target mutations) in the genome of the embryo. Once genome editing was proven to be safe and effective, using CRISPR-Cas in embryos may have a moral advantage over PGD embryo selection. In PGD a healthy embryo, the one without mutations is used to induce pregnancy, but several embryos with genetic mutations are discarded. By contrast, germline intervention using CRISPR-Cas, at least in theory, can 'repair' the mutation(s) in an affected embryo.

Gene therapy research involving embryos

To approve using CRISPR-Cas treating life-threatening diseases, it is clear that further research is needed (see introductory video of Jennifer Doudna in 2.1). Since that research has to be done on human embryos, there is a lot of discussion whether this should be allowed. Some believe that all unborn life needs to be protected on moral and/or religious grounds. In contrast, countries like Sweden and the United Kingdom have allowed genome editing research on non-viable embryos that are formed when two sperm cells fertilize the same egg. If one wants to use CRISPR-Cas for future gene therapy, using non-viable embryos with unnatural genomes will not show realistic results. Viable embryos created exclusively for research purposes would provide a realistic understanding.

CRISPR-Cas has opened doors to many applications some of which might still seem far-fetched today, but others have come closer to reality. Therapeutic application of CRISPR-Cas promises to solve many serious diseases for future generations. Important questions like consent risks involved and the possible impact on future generations all need to be discussed.

In the next chapter, we will look into a real-life case study of the world's first babies in which CRISPR-Cas gene therapy has been used.

Sources:

If you are interested to read more about this topic, please visit the following links.

<https://dev.biologists.org/content/144/1/3>

<https://onlinelibrary.wiley.com/doi/full/10.1111/bioe.12635>

<https://www.genome.gov/about-genomics/policy-issues/Genome-Editing/ethical-concerns#13>

2.4 The He Jiankui affair

In the previous chapter, we learnt about methods currently used for genome selection and the advantage of using CRISPR- Cas in germline editing. However, use of CRISPR has currently not been proven to be safe and it is not clear whether it is ethical to edit the germline/germ cells. There is an international ban on germline editing. Despite the ban, in November 2018 Chinese scientist He Jiankui made claims that shocked the world. He claimed to have created the world's first twin babies with edited genomes, making them more resistant to infection with the human immunodeficiency virus (HIV). In this chapter, we will discuss the ethical implications of this real-life case.

Understanding He Jiankui's "experiment"

The so-called "CRISPR twins" Lulu and Nana, were genetically edited when they still were embryos. The goal was to make them more resistant to HIV. HIV is a virus that invades the immune cells. HIV is a non-symptomatic infection but left untreated it can develop into the syndrome known as Acquired Immunodeficiency Syndrome (AIDS). Because AIDS infects immune cells, patients have a greatly weakened immune system and are highly susceptible to many bacterial infections and diseases that healthy people resist, and many common infections are deadly for AIDS patients. Currently, there is no cure or vaccine to treat HIV or AIDS, leaving patients no choice but to be completely dependant on expensive medicines that stop the progression of HIV into AIDS and prevents the transmission of HIV from patients to healthy people.

Previous research has shown that HIV uses the CCR5 gene to recognize immune cells and infect them. He Jiankui and his team used CRISPR-Cas to modify the CCR5 gene in a human embryo . The genetically modified fertilised egg cell/zygote of the twins was placed back into the uterus of the mother for embryonic development. After 9 months, this resulted in the birth of "HIV resistant" twin girls. However, research has shown that the "HIV resistant CCR5" is involved in other important functions in the body. Therefore, even though the twins could be resistant to HIV, they are more susceptible to other serious infections.

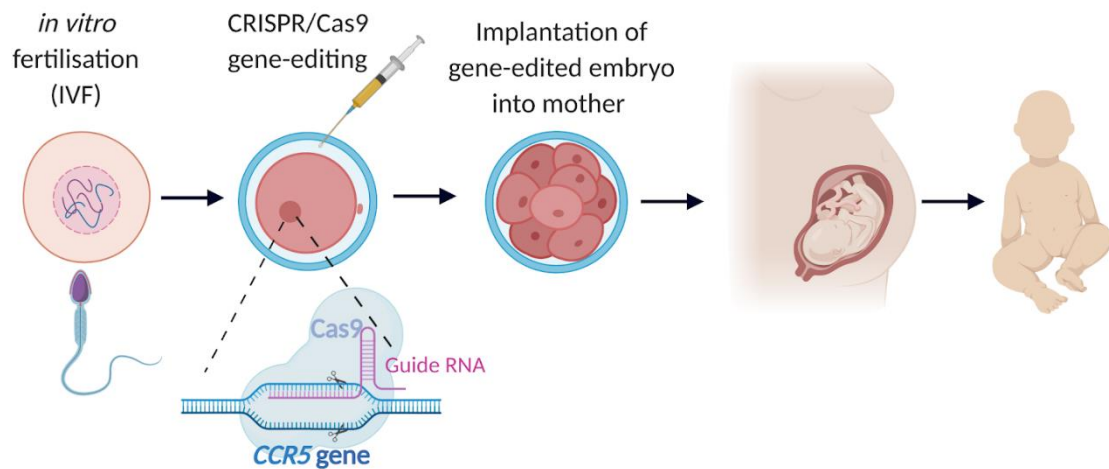


Figure 1: Overview of He Jiankui's experiment (The image is created on bio render).

The parents of the twin girls were not aware of the germline editing. As soon as He Jiankui made his experiment public he was widely criticized and condemned by the global scientific community. Even though one may argue that He Jiankui had good intentions, not following ethical and legal rules can have dangerous outcomes. In this case, Lulu and Nana have to live with the permanent changes made because of He Jiankui's experiments.

Ethical Implications

He Jiankui's experiment has violated several ethical principles and raised legal concerns. In this part of the article, we will discuss some of the most important ethical and legal concerns of the He Jiankui affair.

Informed Consent

As discussed in the previous chapter, informed consent is one of the basic rules of the ethics behind germline editing. Persons that are affected by germline editing have to know enough about the procedure to give their consent for it. A general problem of informed consent in germline editing is that unborn embryos cannot make decisions for themselves. That means that they cannot say if they want to be genetically modified or not. This decision has to be taken by others, most often by the parents. The scientific community debates on whether human genome editing violates the dignity of the future generation. Also, it is deemed important that prospective parents can make informed consent for their offspring. In the example of the He Jiankui affair, the parents of Lulu and Nana did not know about the germline editing of their children and could therefore not give informed consent to the gene editing.

Legal concerns

In spite of a global ban, He Jiankui edited the genome of the twin girls Lulu and Nana. After this experiment, the rules and guidelines have been strengthened in 2017 to prevent further violation and unethical use of the CRISPR-Cas technology.

Conclusion and Outlook

In conclusion, this case study again highlights the importance of ethics in science. It is equally important to protect the rights of people that may, now and in the future be affected by the advancement of science and technology. Violation of legal and ethical regulations could permanently affect the lives of the people.

In this and the last articles you have been introduced into some important ethical aspects in science. After finishing this article, you can test your understanding of moral principles and why ethics in science are important, especially when dealing with employing genome editing techniques in humans in a short quiz.

Resources:

If you are interested in this affair, you can find the He Jiankui's youtube videos and some articles related to this topic here:

The CRISPR baby scandal:

<https://www.nature.com/articles/d41586-019-00673-1>

Designer babies - The problem with China's CRISPR experiment:

<https://www.youtube.com/watch?v=kFFyeHJDI50&list=PL21A203F181B07EBE&index=13>

2.5 The Netherlands in 2039:

Will modification of embryo DNA be allowed or prohibited?

New techniques such as CRISPR-Cas enable us to make changes in human DNA. (Inter)national regulations currently forbid modifying the DNA of germ cells or embryos. It is possible, however, that in the future certain countries will allow the modification of embryonic DNA. If so, scientists in those countries could remove genes that cause certain hereditary diseases from the DNA of embryos or replace them with "healthy" copies. Those children will then not get that specific hereditary disease. However, genome editing techniques would also offer the opportunity to, for instance, endow children with characteristics such as extra muscle growth or a certain color of eyes.

Because of this, use of genome editing could influence human evolution. Modifying the human DNA in the germ line, does not only affect that child, but also their children and grandchildren and so on. Therefore, allowing embryo DNA modification by CRISPR-Cas could profoundly influence humanity and human society. What will happen if we would routinely allow the genetic modification of embryonic DNA? Which ethical and social issues would this raise? Are we going to have a different view on having children and the prevention of diseases? These and other questions will be discussed in the future scenarios of "The Netherlands in 2039" in the next chapters.



Courtesy of Jonathan Borba from Unsplash

Three different future scenarios

Based on literature studies and discussions with experts, the Rathenau Institute has developed three possible future scenarios. In these scenarios it pictures the Netherlands in 2039 either when it is or when it is not allowed to modify the DNA of embryos. Will the government encourage the modification of embryonic DNA to prevent illness in children? Or has the government actually banned the technology and are persons who want to have children traveling abroad to get “genetic treatment”? Researchers use these scenarios to investigate how a new technology can influence the norms and values in a society. And vice versa: they also examine how (changes in) norms and values influence the acceptance of new technologies.

In the coming section, we will travel into time and look around in the Netherlands in 2039 in these three different future scenarios. The scenarios are not intended to predict the future, but they can help you form an opinion about DNA modification in embryos more easily. All scenarios differ from each other with respect to two major points:

1. How quickly the technique for modifying DNA develops.
2. How socially accepted the use of CRISPR-Cas human genome editing is.

The three scenarios will illustrate that there are many ethical dilemmas associated with modifying embryonic DNA. Several questions will be asked after each scenario. The purpose of examining the different scenarios is to make you think about different ethical questions: Do I want genome editing to become a common practice? How would I decide in each scenario? What are the limits of human intervention in natural processes such as human reproduction?

2.6 Video Scenario 1: Adjusting DNA is very normal

DNA is central to healthcare in 2039. Most people have now had their complete DNA sequence read and healthcare providers can view this data. Based on a person's genes, they can give specific advice on suitable nutrition, the amount of exercise to be done, and more, in order to stay healthy.

Technology

Knowledge about DNA also plays a major role in having healthy children. That starts already very early: Via "DNA-Tinder" someone can find the right partner, looking at multiple factors including whether the combination of their own and their partner's DNA would be a good match, should they want children of their own. Of course, everyone wants their (future) child(ren) to live as long and healthy as possible.

When a couple discovers that one is a carrier of a serious hereditary disease, the germline can be genetically modified and the child is prevented from getting that illness. If parents opt for IVF treatment, doctors can modify the DNA of the embryo and remove or repair the piece of gene that causes the disease. Since the DNA of the embryo has been adjusted, its progeny will also no longer carry the disease causing gene. Old-fashioned embryo selection following IVF is still a common way to prevent hereditary diseases. Practised since the 1990s, a doctor selects the healthiest embryo and places it in the uterus.

Governmental policies

Couples who want to have a baby can follow a free health program. The government also reimburses DNA screening: what is the chance that two persons will pass on a hereditary disease? The government does not force future parents to match their DNA or to have their child genetically modified, but it has become the new norm to do so. When couples do not favor this artificial method of conceiving, or when they refuse preventive care, they often receive questions from family members, friends or their medical practitioner who all recommend it.

Social acceptance

When parents have a child with a hereditary disorder, they feel they have to defend themselves as to why they decided to refuse preventative care to their social environment. In addition, they notice that a decreasing amount of suitable care is available for their afflicted child, while available care is very expensive. After all, the government has mainly invested in preventive health care and less in treatment methods for increasingly rare diseases.

Video

This is the Netherlands in 2039. Healthy living is the norm. Julia and Sem want a child together. But Sem appears to be a carrier of a gene mutation that can cause breast and ovarian cancer at a young age. The doctor advises them to remove the afflicted gene with CRISPR-Cas, a fairly common procedure in 2039. This will save their child from any medical distress. But Julia struggles with the feeling that a choice is being imposed on her by the society. Which path should Sem and Julia choose for their future child?

2.7 Questions scenario 1

1. Do you agree with the following statement: If we do not want to lag behind the rest of the world, the Netherlands will have to participate in research on gene editing in embryos.

- a. Yes, these developments cannot be stopped. If it is not allowed in the Netherlands, people will go abroad and we will lose control over safety and application of gene editing techniques.
- b. No, because we should all, worldwide, decide that human embryo DNA should not be modified.
- c. Yes, but not because we follow the rest but because in the Netherlands we believe that gene editing in embryos should be possible.
- d. No, if we decide in the Netherlands that we are going too far by modifying embryo DNA then that is our moral position, regardless of what people think of this or do in the rest of the world.
- e. I don't know.

2. The money we now spend on developing treatments for hereditary diseases is better spent on research into modifying embryo DNA. Do you agree with this statement? Choose the answer that agrees most with your opinion.

- a. Yes, by modifying embryo DNA one can prevent diseases - and even remove them from the population in the long term - and that is better than trying to cure diseases.
- b. No, we do not know how safe the modification of embryo DNA is and what the consequences are in the long term.
- c. Yes, because a one-time DNA modification costs less money than a lifelong treatment for a hereditary disease.
- d. No, people who are born with hereditary disorders should also have access to adequate care.
- e. I don't know.

3. Do you agree with the following statement: If you can prevent a hereditary condition in your unborn child through DNA modification, then you owe it to your child and society to do this.

- a. Yes, everyone has the right to good health, including an unborn child.
- b. No, only you as a parent decide about the well-being of your own child.
- c. Yes, you cannot burden society with the (medical) costs of your personal decision.
- d. No, a human society not only consists of perfect people.
- e. I don't know

2.8 Video Scenario 2: 2039, the Netherlands says "NO" to modifying embryo DNA

In 2039, many countries will be fully experimenting with the genetic editing of embryo DNA, not only in the lab but also in practice. In the United Kingdom, Sweden and the United States, dozens of children with modified DNA have already been born.

Governmental policies

Only the Netherlands is not participating: modifying the DNA of embryos is prohibited. The government believes that modifying embryo DNA does not only change the genetic properties of a child, but also his or her identity. In addition, it is still unclear whether genetic modifications can cause collateral damage: if the deliberate changes in the DNA might lead to (other) diseases later in life.

The cautious attitude of the Dutch government may not be entirely unjustified. Not all treatments work well, it turns out. Some genetically modified children are hospitalized and occasionally a child dies. However, it is not certain that the poor condition of the genetically modified child is due to the genetic treatment.

Technology

Scientific research with specially bred embryos is also prohibited in the Netherlands. It has been debated several times, but no consensus was reached on whether an embryo is just a clump of cells or the start of a new life. The government therefore decided to not change the embryo law, which has been in place since 2002, and to continue to protect the unborn.

So what are the possibilities in 2039 for couples who know that they are carriers of a serious hereditary disease? They can have a number of embryos made through IVF and screen for an embryo without this disease. This disease-free embryo will later be placed in the uterus of the woman. In addition, future parents can opt for adoption, or egg/sperm cell donation, depending on who of the couple is the carrier of the disease.

Yet many Dutch couples that have the financial means travel abroad. In Belgium or the United Kingdom they have the hereditary disease removed from the DNA of their future child. It is even possible in the United States to have their child "upgraded" a little by giving it extra genes for sportsmanship, musicality and creativity. Also, in Southern European clinics it is an option to prevent autism and breast cancer.

Social acceptance

These kinds of possibilities cause a lot of commotion in the Netherlands. After all, who pays the healthcare costs for a genetically modified child that still falls ill? The health insurance company or the parents who had their child undergo a prohibited treatment abroad? Some Dutch people are also concerned about the genetically modified children who will later have children themselves. After all, they bring their genetically modified DNA into the Dutch population. What unprecedented consequences could that have?

Video

This will be the Netherlands in 2039. It is prohibited by law to genetically modify embryo DNA. A lot of research in this field is conducted abroad and dozens of babies have already been born of which the DNA was modified in such a foreign country. The demand for genetically modifying embryo DNA is also increasing in the Netherlands. Indira is 39 and would like to become a mother, but she and her partner Daan are both carriers of the cystic fibrosis gene. Should they go to a private clinic abroad, where it is allowed to engineer embryo DNA?

2.9 Questions scenario 2

1. Because modification of embryo DNA is prohibited in the Netherlands in 2039, some people will go abroad to do so. But if things go wrong, are they entitled to medical care in the Netherlands? Read the statements below and choose the one that agrees (most) with your opinion.

- a. Yes, of course: everyone is entitled to medical care when needed and the child has not been able to influence the choice of its parents, it is not to blame
- b. No, they chose to do something that they knew is illegal in the Netherlands.
- c. Yes, just because we do not allow the actual modification of embryo DNA in the Netherlands, it does not mean that we should not make use of it.
- d. No, they were aware of the risks; they should have insured themselves against this at their own expense.
- e. Yes, but only if the embryo is modified to get rid of a life-threatening disease.
- f. I don't know.

2. Do you agree with the following statement: Because too little is known about the consequences of CRISPR-Cas, we should not continue with this technique until we have ruled out all risks.

- a. No, there are always risks associated with the development of new techniques. If we were always fearful of such risks we would never have had treatments such as IVF or antibiotics.
- b. Yes, because so little is known that we cannot foresee the major consequences if things go wrong.
- c. No, because researchers go through many safety measures and protocols before they can apply the technique to humans. The risks are now so small that they are acceptable.
- d. Yes. When dealing with human research, any risk, no matter how big or small, is unacceptable.
- e. I don't know.

3. Do you agree with the following statement: In the Netherlands today, the cultivation of embryos, especially for scientific research, is prohibited by law. But if we want to do more research on CRISPR-Cas, we will have to lift that ban.

- a. Yes, because not all research into CRISPR-Cas is possible with residual embryos. For some research the use of cultured embryos is unavoidable.
- b. No, because we do not want more research into CRISPR-Cas in the Netherlands anyway.
- c. Yes, because that is not actually a matter that should be determined by law. Women can easily decide for themselves whether they want to donate their eggs to make embryos for research.
- d. No, because embryos can grow into humans, so it is unethical to use them for scientific research.
- e. I don't know.

4. Children with a hereditary condition are often treated medically after birth, so you might as well allow the disease to be prevented at the embryonic stage. Do you agree with this statement? Choose the answer that agrees (most) with your opinion.

- a. Yes, because genetic modification before birth minimizes the suffering from lifelong medical treatment and prevents high medical costs.
- b. No, you can stop or adjust a medical treatment at any time, an adjustment in the DNA, on the other hand, is irreversible for that person.
- c. Yes, because you remove the condition not only from the person treated, but also from all of his or her offspring (children, grandchildren, etc.).
- d. No, because you intervene in the genes without knowing for sure what additional consequences this could have in the long run.
- e. I don't know.

2.10 Video Scenario 3: Good genes, equal opportunities

In this third scenario of the future of "the Netherlands in 2039" it is important that everyone has equal opportunities for optimal development. Everyone must be able to fully develop their interests, competencies and talents because then you will be happiest. Mental or physical disorders such as autism, depression and diabetes are seen as a possible obstacle to a person's personal development. Doctors and care providers, therefore, consult with their patients and provide care that matches their wishes and goals in life.

Technology

The quality of care has improved enormously. Better prosthetics ensure that persons with amputations can perform any movement again. Gene therapy can modify the DNA in the body cells of the elderly with a genetic mutation to remove diseases that have arisen later in life. Persons who have fallen ill are given drugs based on their genotype, so that the drugs work optimal.

Technology has improved such that IVF is less and less of a burden for women. In addition, it is possible to modify the DNA of an embryo before it is implanted in the uterus. Genetic modification can be done for both serious and less serious conditions. The predisposition for diabetes, behavioural problems, high blood pressure or cholesterol levels are therefore a thing of the past. It is also allowed to change the appearance of a child to reduce bullying or improve chances of finding a future partner or job.

Government

In 2039, the Dutch government is of the opinion that having children is a natural right for everyone. You should not be deprived of that right because you happen to be sterile or have a partner of the same sex. Fortunately, "unwittingly childless" no longer exist, because technology has advanced to the point where it is possible to make egg or sperm cells from cells of the skin. There are also artificial wombs in which embryos can develop into healthy babies.

The Dutch government also affirms that every child deserves to be free from mental or physical illness. The government is therefore actively campaigning to reach as many people as possible who could benefit from genetic treatment. It makes these treatments available in regular healthcare. Genetically modified children are also allowed to have a regular health check to examine whether the genetic treatment is working out well, or whether extra care is required.

Social acceptance

Many future parents therefore regard hereditary diseases and an unfavorable appearance as undesirable. For that reason, more and more couples decide to have a child through IVF instead of the "natural" way as only through this treatment it is possible to alter the child's DNA.

Before parents start an IVF program, a medical practitioner will ask them questions such as: What do you know about your own health? Are you a carrier of a hereditary disease? Do you have certain characteristics that you do not want to pass on to your child?

Video

This is the Netherlands in 2039. Social equality is an important goal. Jesse and Caryn want a child together. But Jesse's family has a predisposition to autism. Fortunately, this is quite easy to adjust, and even extra traits such as "creativity" and "empathy" can be added to the hereditary package of the unborn child. Caryn, however, has her doubts: she thinks it's fine to intervene in the DNA to prevent diseases, but at the same time she believes that one should be cautious about adjustments that are not really necessary.

2.11 Questions scenario 3

1. Do you agree with the following statement: Parents owe it to their children to avoid them having an unfavorable appearance.

- a. Yes, as a parent you are entitled to a beautiful, healthy child, just like your child is also entitled to have its own beautiful, healthy children.
- b. No, parents do not have the right to determine the appearance of their child. Beauty is only subjective.
- c. Yes, your child will be less likely to be judged on his or her appearance, and therefore has more opportunities in the future.
- d. No, the appearance of a person is not important, it is the inner values that count.
- e. I don't know

2. Do you agree with the following statement: Your child deserves to have engineered DNA so that it can develop its talents properly.

- a. Yes, if your child can get the best for itself with adjustments to the DNA, you cannot deny that prospect to your child.
- b. No, there are many possibilities to develop your child's talents without having to intervene in the DNA.
- c. Yes, only then will every child have an equal start and therefore equal opportunities to develop his or her talents.
- d. No, when you as a parent choose to make adjustments in your child's DNA, you are interfering too much with your child's own developmental possibilities. You then no longer let the natural talents of your child determine his or her life.
- e. I don't know.

3. A world without hereditary diseases is a better world. Do you agree with this statement? Choose the answer that agrees (most) with your opinion.

- a. Yes, because everyone should be given the opportunity to develop fully, without the problems that come with hereditary diseases.
- b. No, because probably only the wealthy can afford removing hereditary disease-causing genes from the DNA of their offspring. Poorer people cannot, which leads to inequality and ultimately does not make the world any better.
- c. Yes, because the healthcare costs that are avoided can be used to make the world a better place.
- d. No, because people can still get non-hereditary diseases during their lifetime, so the world is not necessarily getting any better.
- e. I don't know.

1.12 Post-Questionnaire Erasmus MC

Now that we are at the end of the lesson series, we kindly ask you to complete the second questionnaire designed by the Erasmus MC in Rotterdam, the Netherlands.

The goal of this lesson series is to give you a solid foundation to be able to form your personal opinion about modifying human DNA. We would like to know if your opinion about modifying DNA has changed now that you know more about CRISPR-Cas and its ethical dilemmas. At the beginning of the lesson series, we asked you to fill in a pre-questionnaire. Now that the lesson series has ended, we kindly ask you to also complete the post-questionnaire. Completing this questionnaire takes about 10 minutes. You can still participate in the post-questionnaire if you did not participate in the pre-questionnaire.

The answers to the questionnaire are anonymised and used by Erasmus MC for scientific research. The results are used by scientists to advise the Dutch government in making decisions regarding regulations about DNA modification in human embryos. Participation in the questionnaire is voluntary and as a participant you can always change your mind and stop completing the questionnaire. By participating, however, you give scientists and the Dutch government more insight into the opinion about modifying embryo DNA. It is important to mention that your data will be processed anonymously.

This questionnaire is part of the Dutch DNA dialogue. The DNA dialogue is an initiative of the Erasmus MC, Erfocentrum, NEMO Kennislink, NPV and Rathenau Instituut, and is funded by the Dutch Ministry of Health, Welfare and Sport. The initiators of the DNA dialogue have different points of view on DNA editing of human embryos. Some have a clear vision, others are neutral. But the most important thing is that they invite everyone to think about the use of DNA modifying techniques on the human genome.

NOTE:

The content of the questionnaire is in Dutch. However, if you are using google chrome as web-browser, you can have the questions auto-translated. In that way, you are still able to answer the questionnaire even if you do not speak Dutch.

QR-Code to the post-questionnaire:



Link to the post-questionnaire:

<https://erasmusmcsurvey.erasmusmc.nl/dna/ls/index.php/959112?lang=nl>

1.13 Conclusion to Week Two and General Conclusion

With this, the two lessons of our course have come to an end. We hope you enjoyed it. The course followed two major aims. Firstly, to provide you with a theoretical background on CRISPR-Cas genome editing and its possible applications especially in medicine. Secondly, to get you to think about where you personally would draw the line between what is acceptable and what not when it comes to human genome editing. We really hope these aims could be reached.

Finally, we would like to thank you for participating and we hope that you enjoyed the course as much as we did in making it. Before closing the course, however, please take the time to go over the information on further learning we provided below and take a minute to acknowledge all the efforts that people involved in the production of this online course have made.

Further learning

Many of the topics that we covered can be elaborated on much further. If you have enjoyed this lesson series and it has sparked your interest to learn more about genetic engineering or molecular biology in general, please take a look at the Bachelor and Master degree programs offered by the University of Groningen. There are a variety of programs offered which have a strong focus on themes that we discussed also here in the lesson series. For more information on the Bachelor and master programs of the University of Groningen take a look at <https://www.rug.nl/fse/programme/bscinscience>.

Acknowledgements

This online lesson series is a team effort of many people. Without their help, the production of this lesson series would not have been possible. We would like to acknowledge their contributions here.

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Lastly, a very special thanks goes to Niels Alberts, the coordinator of this lesson series.

We are more than happy to receive your suggestions for improvement. You can reach us via the following email address: betasteunpunt@rug.nl