„De-Extinction“ with the CRISPR/Cas9

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Introduction:

The subject of the following text is about „De-Extinction“. De-Extinction is the progress of reviving extinct animal species, mostly by genetic engineering. Recently, a lot of articles about reviving extinct animal species are circulating. There were many attempts, but almost none of them were really successful. Still, scientists are searching different ways to achieve that goal. Methods like breeding back were used, but one of the newer methods is called the « CRISPR/Cas9 –Method ». With that method, the genome of an organism can be edited, more precise, more effective and way easier than before: « Genome-Editing ».

We came across an article like this and were surprised, that there is a possibility, to bring species that do not exist anymore, back to earth. In the article, they wrote about different animal species, that could be revived in the future and one of them is the «mammoth». The imagination, that we could see mammoths walking around some when, was so impressing for us, that we decided to choose this topic for this paper. How this method works, whether it is expensive and what problems could occur with this method were the first questions that came into our minds, after reading the article. We think it`s interesting to try to bring back species to earth, that we, humans, eradicated and we think it`s also important to have such an attitude.

Description of engineering technique

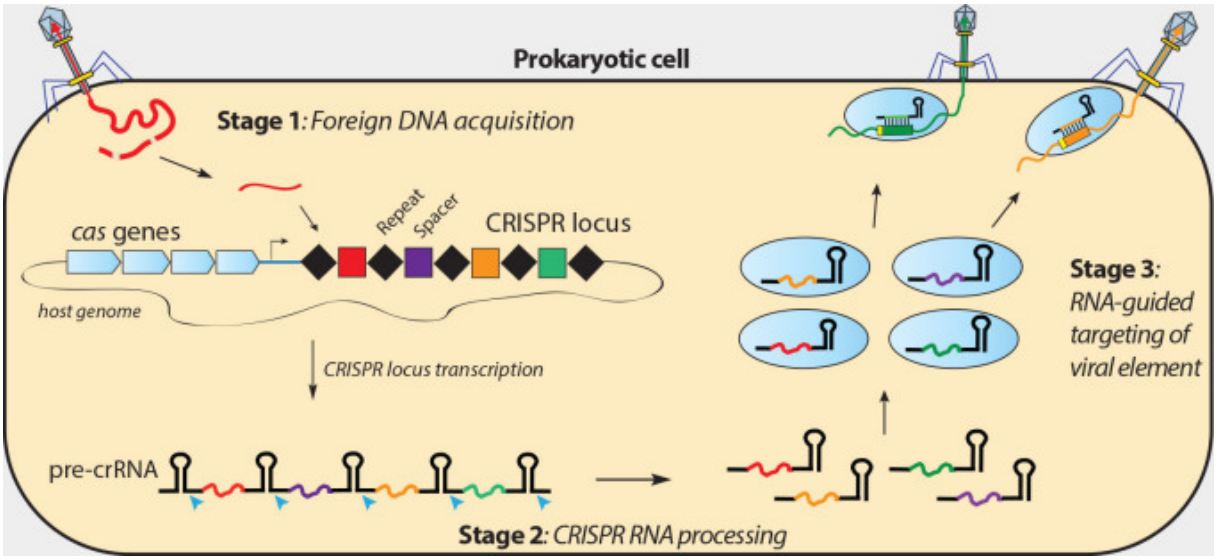
The engineering technique of our Topic is Genome Editing with CRISPR/Cas 9. This method has been discovered a few years ago by Emmanuelle Charpentier and her colleague Jennifer Doudna. The Method was discovered and not invented because

prokaryotes have been using it to protect themselves from viruses. Scientists discovered, that in the DNA of prokaryotes, there are repeating sequences of nucleotides. These were found in many different species like for example E. coli, Haloferax volcanii, Haloferax, etc. Today, scientist know that there is at least one CRISPR structure in the genome of 45% of the sequenced bacteria and in 83% of the archaea. The next step was the discovery of the cas-genes (CRISPR associated genes). They were always located near the locus of the CRISPR sequence. From then on scientist knew that it would be possible to use this in gene engineering, but they didn't know how to attach a cas-protein to a RNA sequence.

How did prokaryotes (archaea, bacteria, etc.) use CRISPR?

When a microbe is attacked by a virus the first reaction is to capture the DNA of the virus and put it into the CRISPR locus. Then, if the same virus is going to attack again the information in the CRISPR locus is copied multiple times and carried around by Cas-Proteins. They are like scissors that can cut DNA, so as the Cas-Protein travels around it searches for DNA that matches the DNA of the virus and if it finds any, the Cas-Protein can just cut through the DNA strand and therefore destroy it. Like that, bacteria can easily avoid being killed by viruses. (Fig 1)

Fig 1:



Source: https://bipolar1blog.com/2016/03/26/editing-hiv-out-of-our-genome-with-crispr/

How does CRISPR/Cas-9 Work?

The first thing you need to know to use CRISPR/Cas-9 is a unique sequence of at least 20 base pairs in the DNA molecule which you want to cut. This has to be archived. That's called the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). The next step is to arrange a RNA sequence of these 20 base

pairs in the laboratory. Then the Cas-9 Protein has to be attached to a loop on the CRISPR RNA. Because the RNA can only attach to the matching DNA sequence, the Cas-9 protein will automatically cut at the right spot. After that cut, the DNA can be edited. For example by adding an extra base pair or by adding a stop codon or even by adding new complete sequences. (Fig 2)

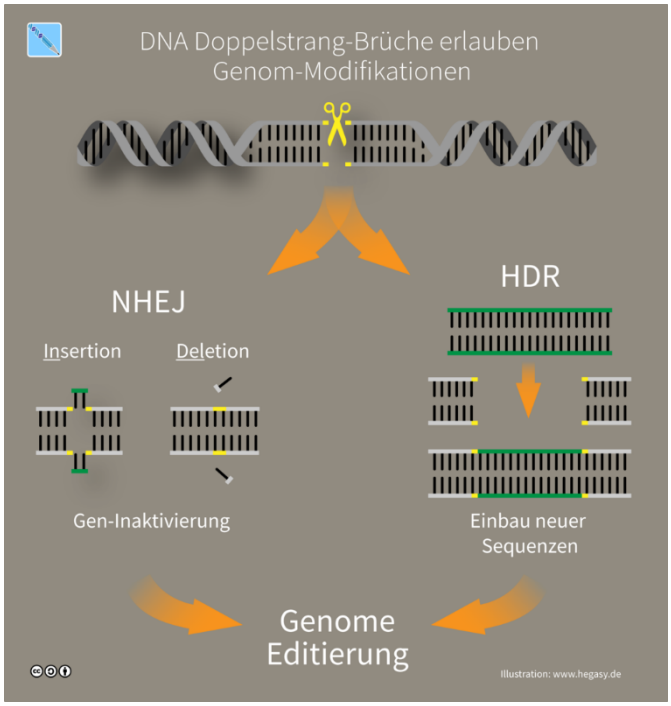
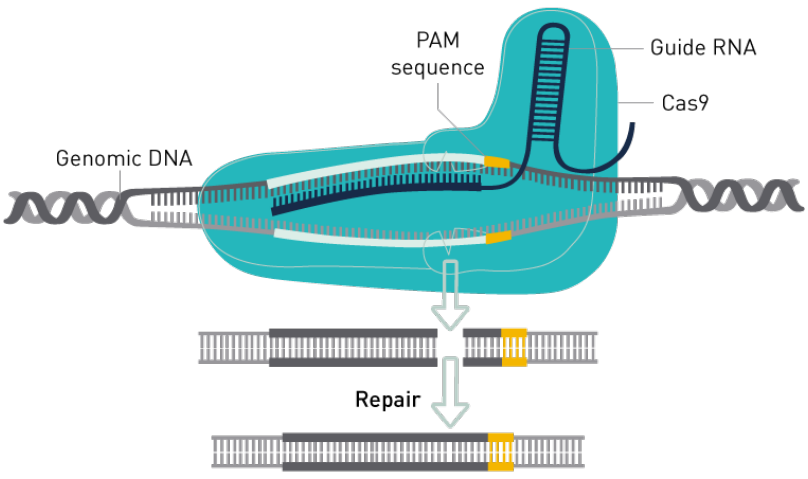


Fig 2:

Source: https://de.wikipedia.org/wiki/CRISPR/Cas-Methode

Before CRISPR/Cas9 was discovered, scientists were working on a method to manipulate genes for decades. Then after 20+ years the ZN-Finger method finally showed some results and they were able to sell thousands of their products, but right after, it was replaced by the more advanced and also simpler system TALEN. The problem with both of these systems was, that they didn't know, how to attach the Cas-protein to the guiding RNA. Then they realised that it's possible to attach the protein to a loop in the RNA and this system was called CRISPR/Cas9. (Fig 3)

Fig 3:



How is this used in “De-Extinction”?

To be able to “resurrect” extinct species by this method, the DNA has to be available, at least a big part of it. Then the DNA of a fertilized egg cell of a descendant has to be changed by using CRISPR/cas9 so that the DNA matches the DNA of the original species. Next, the changed egg cell has to be put into a female, fertile descendant which then gives birth to the new, old species.

This is the theory behind it, but practically it brings many problems with it. For example, an elephant can´t give birth to a huge mammoth. That´s why in the case and in other, more complicated cases, scientist try to do it over many generations. So first, they change up the most important DNA strands, then they wait for the mammophant to grow up and to be fertile, then they change the DNA further and so on, until we actually have a mammoth.

Other ways for De-Extinction are for example cloning or back-breeding.

Criticisms and Promoters:

George Church

Georg Church is a Professor for genetics. He is one of the biggest promoters of “de-extinction”, because he thinks that without these extinct species, detrimental consequences would occur for the environment. For example the mammoth would prevent the escalation of the greenhouse effect.

Critics:

- The results of de-extinction with this method would be unnatural.
- Even though the de-extinction with CRISPR/Cas9 is much cheaper than with older methods, it costs a lot to revive many species. That money could be invested in the maintenance of living species.
- There is a reason why the extinct species died out. They wouldn`t be able to adapt to today`s world.
- The risk would be high, that the revived species suffer, because of mistakes in the DNA reconstruction.

Ethnic questions

The genome editing technique CRISPR brings up some ethnic questions.

CRISPR could be the solution for many problems on the world, like for example diseases, but some people would also „abuse“ that ability and would start to create funny or sweet looking animals, so they would „play“ with nature and play god. It

would even be possible, to completely modify your children, so that they will match one's preferences. For example, changing the gender or the eye colour.

In Switzerland, there are a lot of laws and rules that prevent scientists from experimenting a lot with CRISPR. All experiments can only be done, if the SNF (Schweizerischer Nationalfonds) consents it. In China and the U.S.A. for example, the laws and rules aren't that strict and that's why China is number one in the race with CRISPR. With private funding, you still have to follow the ethnic rules of China/U.S.A., but you have more freedom

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Applications and future plans

Future plans:

A lot of scientists are working with CRISPR and are experimenting with it. It is basically a tool for directed gene editing. It is used in all fields, where we want to optimize or change a gene of an organism. Because CRISPR is that precise, cheap and easy to use, it could be possible for the researchers to find a way to be able to change every organism's genome with it, but at the moment, there are a lot of hurdles until getting there. The researchers are still at the beginning of finding all the potential uses of CRISPR that is why there are a lot of future research steps. Here are some uses of CRISPR, that researcher are trying to achieve:

Researchers are trying to:

1. find a way to correct genetic errors in humans, that cause diseases.
2. use CRISPR to fully destroy microbes in humans, which are responsible for diseases like HIV.

To the question, which diseases could be treated this way, Irina Conboy of the University of California answered: „Absolutely everything!"

3. **revive extinct animal species with CRISPR**

Planned extinct species:

- Passenger Pigeon
- Mammoth (Woolly mammoth)
- Aurochs
- Dodo
- Caspian Tiger
- Pyrenean ibex

Applications:

At the moment, CRISPR is used in different fields, like:

Researchers are able to:

1. edit the genome of plants for the improvement of crops, so more, healthier, more robust or even new foods can be produced.
2. change a gene of an organism to for example specifically cause a mutation for breeding. (This is especially dominant in China where they breed dogs with special features like blue eyes or a small height.)
3. find out, where specific genes are located.
4. find out, what specific genes are used for.

Summary:

CRISPR/Cas9 revolutionized the world of “Genome-Editing”, because it is very precise, effective and cheap in comparison to past methods. The attachment of the Cas protein to the guiding RNA made it possible to edit DNA just the way you want to. At the moment, CRISPR/Cas9 is mostly used for research, but in the future they plan to fight diseases, counter errors in the human DNA and for “De-Extinction”. Even tough, there are many criticisms and ethnic questions, people like George Church and Craig Venter are investing a lot of time and money into the research and achieve according results. So the hopes are big that we will see mammoths walking around again.

Interview with Professor Affolter

After asking many people for an interview, we finally got to Professor Affolter who was ready, to give an interview. We had a really interesting conversation about our subject. We talked and discussed for about 60 minutes.

Instead of writing down the interview word by word, we summarized the most important aspects:

Mario: „So our first question is, what you’re working on at the moment and what you dealt with in the past?”

Prof.: „I am a developmental biologist. I basically look at how a human, bird or a fly arise from an egg. In the past, people just looked at an organism and observed it. They studied it only from an outer point of view. Today it’s totally different. We started to analyse, how an embryo is formed out of an egg and how an adult is formed out of an embryo. Through this development we’re now at the point where we analyse which gene is responsible for which development in an organism.

I personally looked at mutations in the ‚Drosophila' fly. We irradiated their genome to form different mutations. And in that way you're able to analyse where the genome of this mutant is different and so we can see, which gene is responsible for the mutation that fly's got.

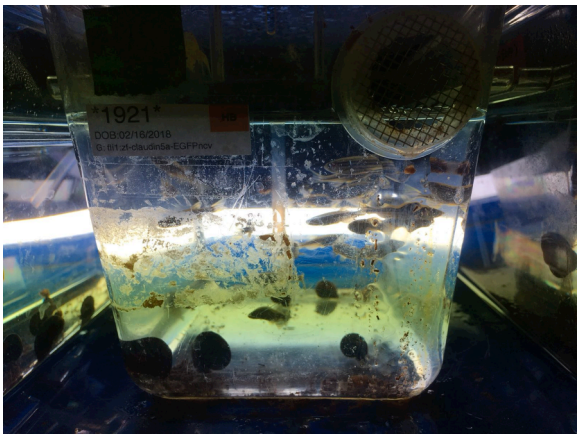
Later on, we switched to the Zebrafish which is much more complex than flies. “

(All these studies are based on the developmental biologist Christiane Nüsslein-Volhard.)

Jan: “Are you using CRISPR in the Biozentrum?”

Prof: “We are using it every day. We are doing genome editing in zebrafishes. As an example, we take a gene of interest in a zebra-fish and we know what protein it produces. In that sequence, we use CRISPR/cas9 to cut the sequence, so it loses its function, a mutation.”

On the day we made the Interview with Prof. Affolter, he told us, that one of the students was able to put a specific nucleotide in a specific spot of the genome of a



zebra-fish by using CRISPR/Cas9 for the first time in the Biozentrum!

Here we can see how the zebra-fishes are stored in the “Biozentrum”. The age of the fishes is increasing from the bottom to the top of the shelf.

Mario: „Did you already hear about scientists that are trying to use CRISPR to revive extinct animals? “

Prof.: „Yes. “

Jan: „How would you do this for example in a mammoth? “

Prof.: „I mean I don't know how they exactly want to do, or are doing it, but theoretically you can insert every mutation that the mammoth went under into an elephant. Of course, you need well obtained mammoth sequences. But then we're not talking about 20 to 50 changes in the genome anymore, then we're talking about let's say 10^9 changes. It would be sophisticated but possible. I mean when it's possible to do it 75 times, like George Church did it in pigs (*…explanation…),* it has to be possible to do it 1000 or billions of times. “

Eric: „Is it possible to do that process in a living organism, or do you have to do it in the embryo or egg cell? “

Prof.: „Basically, in humans you want do it in an adult one. You can take stem cells, change them and then put them back. In a mammoth you would probably do it in the embryo, where you first make 100 changes, which you think are the most important ones, and then repeat this over generations.

Or Craig Venter (has crazy ideas) would maybe sequence the whole genome and let the computer correlate it again. Then you can put it into an egg cell without a nucleus, like it is made in therapeutic cloning. But then we had the possibility to get a huge mammoth baby, which wouldn't be possible for an elephant. "

Mario: „So you were talking about America, where they do a lot of things like that. Are they the only ones? "

Prof.: „No they aren't. Especially China is dominant in doing crazy things, like cloning and so on. You can basically send cells of your "100 year old" dog to them, they clone it and you have the same dog again, just new as a baby. They would also create for example a mini pig, what they can give their children for Christmas. Crazy, really crazy.

Jan: „And what about Switzerland? "

Prof.: „No chance. We have too much ethical laws to do something like that. But this isn't bad at all! "

Eric: „So do you think that in Switzerland it would be illegal to revive animals? "

Prof.: „If you do experiments in Switzerland, you have to get a permission from the "SNF". And I personally think no one wants to even do something like that. We had no profit from it. And I mean we are a small country. America is huge and has more people with crazy ideas

Eric: "What other applications of CRISPR are there in Switzerland?"

Prof: "With CRISPR, they are doing models of diseases on mice for example. If the scientists know what point-mutation in the genome is causing a disease in a human, they are trying to make that same point mutation in the genome of the mouse. If the mouse has a disease with the same characteristics, they can try to make screens of it, for a drug/medicine, which could heal the disease in humans."

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