FAQ on Genome Editing and CRISPR/Cas9

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Genome editing is a generic term for new methods enabling targeted modifications to the genetic material (genome) of a cell. The CRISPR/Cas9 method in particular holds great promise for new application methods. The German Federal Institute for Risk Assessment (BfR) observes these scientific developments in the interest of consumer health protection. Here the BfR answers the most important questions on the topic of genome editing and notably the CRISPR/Cas9 method.

In November 2016, the Federal Government issued a statement entitled “Classification and management of new genetic engineering techniques”.
http://dip21.bundestag.de/dip21/btd/18/103/1810301.pdf

A group of scientific experts from the European Commission published an estimation of new techniques in agricultural biotechnology in April 2017.
http://ec.europa.eu/research/sam/pdf/topics/explanatory_note_new_techniques_agricultural_biotechnology.pdf

What is genome editing?

“Genome editing” means processing genetic material. This term denotes different new biomolecular methods which enable targeted modifications to genetic information. This includes the following techniques, amongst others: mutagenesis with the help of zinc finger nucleases or TALEN (Transcription Activator-like Effector Nuclease), oligonucleotide-directed mutagenesis (ODM) and CRISPR/Cas9. By means of genome editing processes, specific changes to the genome of the target organism can be induced. For this purpose, two components are required: a protein (nuclease) which cuts the DNA of the target organism and a “navigator” that guides this nuclease to the relevant position in the DNA. As part of the process, the “navigator” (depending on the technique used, this can be a piece of DNA, an RNA, or a protein) is customised in such a way that it “identifies” the relevant location in the genome of the target organism. The nuclease can be introduced into the cell either from the outside (CRISPR/Cas9, TALEN, zinc finger nuclease) or it is naturally contained in the cell (ODM).

These procedures can give rise to a point mutation (replacement of an individual DNA building block) or a deletion (elimination of one or several DNA building blocks). However, it is also possible to add one or more building blocks (insertion). Scientists can even introduce a larger piece of synthetic DNA into the cell which is then integrated into the genome during DNA repair.

What are the differences and similarities of genome editing and conventional plant breeding methods?

Conventional plant breeding (procedures not covered by the genetic engineering act) makes use of spontaneous or chemical- or radiation-induced changes in the plant genome as well as the natural cross-breeding of plants. As part of this process, the breeders do not know exactly where in the genome the changes have occurred. A subsequent selection process is therefore needed whereby the treated cells or plant clones are selected from a multitude to identify and select those that contain the desired modification(s). These techniques have already been used successfully over 3,000 times for the cultivation of new plant varieties. Certain barley varieties, for example, were produced with the help of gamma rays.

In contrast, genome editing induces targeted modifications in genes. What form the

modification takes at this defined location depends on how the tools are used as part of the genome editing process (see above). In some cases, it is not possible to determine on the basis of the result (DNA sequence) whether a mutation has taken place naturally or through a new technique. However, genome editing also enables the creation of genetic variants which do not occur naturally.

The genetic information, i.e. the construction plan for forming and maintaining an organism, is memorised as a specific sequence of DNA modules, the nucleotides (nucleotide sequence). Changes to the genetic information are known as mutations. They can affect individual modules or larger DNA sections and they also occur naturally. The natural mutation rate with thale cress (*Arabidopsis thaliana*), a frequently occurring wild herb, is roughly one mutation per 150,000 kilobase pairs of DNA modules (kbp). Natural mutation rates of 1-3 mutations per 100,000 DNA modules (kbp) per germ cell and generation were found with mice. Changes to genetic information are therefore a part of life.

Methods to increase the natural mutation rate (e.g. radiation and mutagenic chemicals) thereby achieving genetic variability for the breeding selection of new varieties or strains have been used in conventional plant cultivation and livestock breeding for decades. It is estimated that classical mutagenesis has produced more than 3,000 crop varieties. A newly created variety or species, regardless of whether through natural selection, breeding or genetic modification, is therefore distinguished by genetic change(s) to the original organism.

Established methods and guidelines to estimate the possible health risks (risk assessment) exist in the EU which permit testing on the basis of the available scientific information and data in line with the valid legal provisions.

What does the abbreviation CRISPR/Cas9 stand for?
The letters CRISPR stand for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats. These are repeated DNA sequences which occur in the genome of many bacteria and which play an important role in bacterial defence systems. When a virus enters a bacterium, the cell incorporates parts of the virus DNA in its CRISPR structure. If another virus with this DNA enters the bacterium, it will be recognised due to the CRISPR sequences. Cas9 is the abbreviation for **C**RISPR-**as**sociated protein **9**. This Cas9 enzyme latches onto a recognised DNA section and cuts viral DNA.

How does CRISPR/Cas9 work?
CRISPR and Cas9 were originally discovered as part of a system which protects bacteria from entry of foreign genetic material through viruses or plasmids. For a few years now, CRISPR/Cas9 has been used for genome editing and developed further:

The enzyme Cas9 merely needs to be attached to a so-called guide RNA (“navigator” function); it will then assume the role of the viral DNA, i.e. detection. If Cas9 finds the matching piece of genomic DNA, it will cut the DNA strand. This DNA fracture can subsequently be repaired again in different ways through the cell’s own processes, which can give rise to mutations (see above).

What are the application areas for genome editing?
Genome editing is comparatively easy to implement, faster and, most importantly, more precisely targeted than previous methods (including genetic engineering procedures). As a result, scientists hope that the creation of higher-yield or disease-resistant varieties and breeds, such as mildew-resistant wheat and potatoes that can be stored in cool conditions, will be possible in plant and animal husbandry with the help of genome editing. In the area of medicine it is expected that genome editing will provide fresh momentum in the development of novel therapeutic procedures for different diseases. Developments of this kind require a considerable amount of time, however, so that success cannot be expected from one day to the next.

How is it possible to assess potential health risks posed to consumers by genome editing in the area of food and feed safety?
Within the EU, the principle applies that food that is not safe must not be introduced to the market. Analogously, feed that is not safe must be neither sold nor fed to animals used in food production.

With the use of genome editing, genetic variants can be produced which could form either naturally and artificially. In principle, established procedures used in the health risk assessment of food and feed made from genetically modified plants can also be applied to the risk assessment of plants generated by means of genome editing.

In the view of the group of experts from the EU Commission, each case has to be examined individually in order to assess the risk of organisms created through new technologies.

As is the case with every method for changing the genome, scientists applying genome editing cannot exclude the possibility of other gene sections than the targeted ones being modified (off-target events). According to the current state of knowledge, genome editing techniques are, provided they are used taking into account the latest state of knowledge, seen as very specific. For this reason, the consensus in the scientific community is that so- called off-target events should occur less frequently than in conventional procedures. Due to the rapid developments in this field and the scant experience with technical applications of genome editing, there is still a need for research in this area, however.

Does genome editing count as genetic engineering?
As a scientific institution, the BfR does not make any decisions on how genome editing is to be classified from a legal point of view. The European Court of Justice (ECJ) ruled on 25.7.2018 that as a basic principle, organisms created through genome editing are genetically modified organisms (GMO) as defined by the GMO regulation of the European Parliament and of the European Council, and are therefore subject to genetic engineering rules.
It can also be taken from the ECJ ruling that products manufactured in this manner are subject to the marking and labelling requirements for GMO so that they can be recognised by consumers.

What sort of work does the BfR do in the area of genome editing?
The focus of the institute's work is on human health protection. Through its independent scientific assessments, research and transparent communication of health risks, the BfR makes an impartial contribution to the safety of food and feed, products and chemicals. Against this background, the BfR is also concerned with the scientific dimension of genome editing and as such engages in regular exchange with national, European and other international institutions.

For example, with its symposium "New Technologies for the Modification of the Genome" held on 6 December 2016, the BfR provided information on the current state of knowledge and offered a platform for discussing the numerous aspects of this topic. With this opening event, the BfR realises its legal mandate of communicating potential, identified and assessed risks in a balanced and scientifically sound way.

The decision as to whether genome editing procedures will come under the legal regulations governing genetic engineering will be taken by the risk management authorities.

What role does the BfR commission for genetically modified food and feed play?
The members of the BfR commissions provide independent and free advice on unresolved scientific issues. The BfR commissions are not involved in the BfR's risk assessments.

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. It advises the Federal Government and Federal Laender on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.

This text version is a translation of the original German text which is the only legally binding version.