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Member State questionnaire on new genomic techniques to contribute to a Commission study requested by the Council

Fields marked with * are mandatory.

Questionnaire on new genomic techniques to contribute to the study requested by the Council

endorsed in the Joint Working Group of GMO competent authorities on new genomic techniques on 15 January 2020

Introduction

With this questionnaire the Commission is collecting contributions from Member States competent authorities to respond to the Council's request[1] for "a study in light of the Court of Justice's judgment in Case C-528/16 regarding the status of novel genomic techniques under Union law" (i.e. Directive 2001/18 /EC, Regulation (EC) 1829/2003, Regulation (EC) 1830/2003 and Directive 2009/41/EC). The scope of the study goes beyond new mutagenesis techniques, as there are other new techniques, for which the Council seeks clarification. Therefore, the study covers all new genomic techniques, which have been developed after 2001.

For the purpose of the study, the following definition for new genomic techniques (NGTs) is used: techniques, which are capable to alter the genetic material of an organism and which have emerged or have been developed since 2001[2].

Unless specified otherwise, the term "NGT-products" used in the questionnaire covers plants, animals, micro-organims and derived food and feed products obtained by NGTs for agri-food, medicinal and industrial applications and for research. GMO competent authorities are invited to seek input from other competent authorities when appropriate.

The questionnaire is meant to provide information primarily, but not exclusively, at national level. Please substantiate your replies with explanations, data and source of information as well as with practical examples, whenever possible. If a reply to a specific question only applies to a specific NGT, please indicate this in the reply. With regard to agri-food applications, replies may include considerations on specific sectors, such as the organic sector.

Please indicate which information should be treated as confidential in order to protect the commercial

interests of a natural or legal person. Personal data, if any, will be protected pursuant to Regulation (EU) 2018/1725 [3].

[1] Council Decision (EU) 2019/1904, OJ L 293 14.11.2019, p. 103-104, https://eur-lex.europa.eu/eli/dec/2019/1904/oj
[2] Examples of techniques include: 1) Genome editing techniques such as CRISPR, TALEN, Zinc-finger nucleases, mega nucleases techniques, prime editing etc. These techniques can lead to mutagenesis and some of them also to cisgenesis, intragenesis or transgenesis. 2) Mutagenesis techniques such as oligonucleotide directed mutagenesis (ODM). 3) Epigenetic techniques such RdDM. Conversely, techniques already in use prior to 2001, such as Agrobacterium mediated techniques or gene gun, are not considered NGTs.
[3] Regulation (EU) 2018/1725 of the European Parliament and of the Council of 23 October 2018 on the protection of natural persons with regard to the processing of personal data by the Union institutions, bodies, offices and agencies and on the free movement of such data, and repealing Regulation (EC) No 45/2001 and Decision No 1247/2002/EC, OJ L 295, 21.11.2018, p. 39–98

Instructions

Please note that the survey accepts a maximum of 5000 characters (with spaces) per reply field. You might be able to type more than 5000 characters, but then the text will not be accepted when you submit the questionnaire. You will also receive a warning message in red colour below the affected field.

You have the option to upload supporting documentation in the end of each section. You can upload multiple files, up to the size of 1 MB. However, note that any uploaded document cannot substitute your replies, which must still be given in a complete manner within the reply fields allocated for each question.

You can share the link from the invitation email with another colleague if you want to split the filling-out process or contribute from different locations; however, remember that all contributions feed into the same single questionnaire.

You can save the draft questionnaire and edit it before the final submission.

You can find additional information and help here: https://ec.europa.eu/eusurvey/home/helpparticipants

Participants have until 30 April 2020 (closure of business) to submit the questionnaire via EUsurvey.

QUESTIONNAIRE

* Which Member State are you representing?

Denmark

A - Implementation and enforcement of the GMO legislation with regard to new genomic techniques

* **1. Have you been consulted by companies/organisations/research institutes for regulatory advice or another issue on products developed or to be developed by NGTs ?**

- (●) Yes
- ( ) No

* Please provide details on the request

Yes, the Danish authorities have been consulted for legal advice under all three legislative frameworks.

In relation to the GM food-feed regulation (EU) 1829/2003 (Competent authority: Danish Veterinary and Food Administration, DVFA):

The DVFA and the Danish Agricultural Agency (the DAA) have both prior to and after the European Court of Justice Decision of July 2018 been consulted by several companies, organizations and research institutes regarding NGTs, e.g. regarding:

a) the development of microorganisms by gene-editing (new mutagenesis techniques by e.g. CRISPR /Cas or ODM) for use in specific areas within the food sector,

b) the development by cisgenesis of barley and wheat with increased phytase activity for use as animal feed,

c) the use of gene-editing for breeding of crop plants, e.g. cereals.
The stakeholders were concerned about the legal status of the techniques. Biotech, food-producing companies, plant breeders and universities see great potential in these techniques for development of new crops and micro-organisms for the agri-food sector, but only if the techniques are outside of the scope of Regulation 1829/2003 as most food producers do not wish to be linked in any way to the use of GMOs. If gene-editing and cisgenesis were defined as not being GMO's, there is an interest in using these techniques in product development.

d) Concerns have also been raised as to whether Member States, following the ECJ-decision, would make national legislation on organisms obtained by traditional mutagenesis.

e) An NGO has been interested in the lack of official control of gene-edited products in food, feed and seed.

In relation to the Deliberate release directive 2001/18/EC (Competent authority: Danish Agricultural Agency, DAA):
As described in question 13, the DAA has had extensive discussions with a broad range of stakeholders about the use of gene-editing on plants. Furthermore, DAA has been consulted by companies/research institutes wishing to use gene-editing to develop low-risk microorganisms for protection/growth stimulation of crops in agriculture. If gene-editing continues to be regarded as GMOs under the deliberate release directive, the stakeholders see no possibilities to develop and use such new microbial products in the EU.

In relation to the Contained use directive 2009/41/EC (Competent authority: Danish Environmental Protection Agency, the DEPA):

The DEPA has been consulted by:
a) Companies regarding the regulation of microorganisms modified by gene-editing using CRISPR/Cas for use as production organisms. The question raised was whether a GMM obtained by this mutagenesis technique is to be interpreted as GMO under the Danish implementation of Directive 2009/41/EC.
b) By foreign companies (USA) which want to sell and use their products in Denmark. The question was whether products formed by fermentation of gene-edited production organisms are covered by the EU legislation.

* **2. Have you taken specific measures (other than inspection) related to the application of the GMO legislation to NGT-products?**

- (●) Yes
- ( ) No

* Please describe the measures and, if possible, their effectiveness

There is not a concensus between agencies for this reply:
Deliberate release directive: Yes
GM food-feed regulation: No (see below)
Contained use directive: No (see below)

Deliberate release directive:
DAA has asked the national authority responsible for variety testing (Tystoftefonden) to inform its stakeholders of the ECJ-decision and its implications. This is done at the Tystoftefondens website. In the application form for new varieties, applicants shall answer the question: «Is the variety genetically modified (GM) or does the variety descend from a genetically modified variety subject to GM-regulation, according to Directive 2001/18/EC annex 1A part 1 and the European Court of Justice Decision (C-528/16) of 25 July 2018?» Therefore, relevant stakeholders are now well aware of the legislation relating to gene-editing.

If no, please explain why not.
GM-food and feed regulation:
No specific measures have been taken by the DVFA and the DAA as the legal status of NGTs has for many years been uncertain. The ECJ ruling has now brought legal clarity on the status of gene-editing, but has raised practical questions on:
a) how to ensure compliance with the GMO-legislation since the current control methods do in many cases not allow a distinction between products (plants) obtained by means of new mutagenesis techniques and products resulting from conventional mutation, and,
b) how to ensure the equal treatment between imported products and products from within the Union.
Thus, the DVFA and the DAA has concerns regarding the lack of possibilities for implementing and enforcing the legislation and controlling food, feed and seed developed by gene-editing following the court ruling. The concerns question the adequacy of the current legislative framework.

Contained use directive:
No specific measures have been taken by DEPA as the application and approval of research facilities within the directive on contained use is a simple process and free of charge. DEPA's experience with gene-editing within research is that scientists apply for approval of laboratory facilities and research projects with the same rate as for other research projects. We therefore don't find it necessary to change procedures for the applications in this area of the Danish GMO legislation.

The approval of facilities and production microorganisms for contained use for production is a non-

problematic process. We have a lot of experience in these matters. Furthermore, the Danish companies using GMOs for contained use also have experiences with these types of applications. However, as a consequence of the ECJ ruling, we experience a high increase in applications of these matters.

* What best practices can you share?

None.

* 2 bis. Have you encountered any challenges or limitations, including administrative burden or costs?

- (●) Yes
- ( ) No

* Please describe

The DVFA and the DAA has encountered limitations regarding the lack of possibilities for implementing and enforcing the legislation and controlling food, feed and seed developed by gene-editing following the court ruling.

* How could these challenges or limitations be overcome?

They cannot be overcome since the current control methods in many cases do not allow a distinction between products (plants) obtained by means of new mutagenesis techniques and products resulting from conventional mutation.

* **3. Have you adapted your inspection practices to cover all NGT-products and to ensure the enforcement of traceability requirements?**

- ( ) Yes
- (●) No

* Please explain why not

No, the Danish inspection practices have not been adapted.

GM food-feed regulation:
The official control in Denmark of authorized and non-authorized GMOs (primarily plants) in food and feed (the DVFA) and seed and propagating material (the DAA) only cover contents from transgenic GMOs. We have not adapted our inspection practices under the GM food-feed regulation to cover gene-edited products. If an NGT-crop is to be authorized for food, feed or cultivation in the EU, the applicant shall provide a quantitative PCR detection method to be used a.o. for control of the GM-labeling of products. So far, no such crops have been authorized. According to the European Network of GMO Laboratories (ENGL), it is unlikely that a detection method can be developed for gene-edited crops.

As concluded at the Joint WG Meeting of 25 April 2019 on the follow-up on the ECJ ruling on the new mutagenesis trechniques, many Member States including Denmark have concluded that – based on the ENGL report and current scientific knowledge and because the same type of modifications can be introduced through genome editing, conventional techniques or occurring naturally – there is no possibility to identify the technique used. Under these circumstances, we are not able to fulfil our obligations to carry out controls and therefore cannot in all cases be held responsible for deficiencies in enforcement. For some of

the other NGT's there is an unclear legal situation as to whether these techniques are in fact GMO's, or not. Plant products in which a new gene is inserted (e.g. using CRISPR/Cas) would in some cases be covered by the GMO screening methods, which we use today for the GMO control of food.

For the DAA, the key question relating to inspection and control of seed is how to ensure that non-authorized GMO-products are not imported into Denmark. For gene-edited crops where no detection methods are available, or are expected to be developed in the coming years, we have considered the possibility to establish a control system based on mandatory «Guaranteed free from gene-edited products» certificates followed by subsequent control by the authority of these documents. In other words to require that any importer of a product which potentially could contain gene-edited products) should be able to provide a guarantee that the product in question is not obtained by the use of non-authorised gene-edited product. This in turn implies that the importer should require such a guarantee from the provider of the product in the exporting country.

The DAA has some experience with the use of «Free from GMO certificates» as elements in our control of imported plants for traditional GMOs. However, traditional GMOs can be detected analytically and they are regulated as GMOs in their country of origin. A system based on "Guaranteed free from gene-edited products certificates" would have to be used on a completely different background. It is our impression that such a system would demand substantial financial and human resources to establish and run since it in principle should be applied to all products that potentially could contain gene-edited products and that are being imported to Denmark. Furthermore, given that analytical detection is not possible, one could question the validity of the required guarantees that form the very core of the system. The system would also require that the exporter of the product – located in a third country – is able to provide a «Guaranteed free from gene edited products» certificate to the Danish importer. This could prove difficult or impossible, particularly in the many countries that have decided not to regulate products obtained by gene-editing as GMOs. For these reasons, we do not believe that the described system is a feasible solution and have not developed it any further.

Deliberate release directive:
DAA's inspection practices have not been adapted for the reasons described above.

Contained use directive:
The DEPA has not adapted its control in the contained use area as a result of the ECJ ruling due to the fact that inspection in this area is not based on the GM itself, but rather on maintenance of physical barriers, efficient GMO-inactivation and GMO-free products (vaccines, enzymes, biomass etc.). We use the same method to all GMO work, where we inspect and hold meetings where we discuss the issues. Therefore, we find no need to change our control.

* 3 bis. Have you encountered challenges or limitations, including administrative burden or costs?

- (●) Yes
- ( ) No

* Please describe

Contained use directive:
The DEPA faces an increase in applications for production activities due to the fact that the gene-editing techniques (CRISPR) make it easier and faster to create new production strains. Hence, we expect an increase in the administrative burden in this area.

Some companies also face an increased administrative burden as a consequence of the ECJ ruling. The companies see the risk of the modifications introduced by gene-editing as low compared to the administrative burden.

* How could these challenges or limitations be overcome?

No suggestion.

* **4. Do you have experience or information on traceability strategies, which could be used for tracing NGT-products?**

- ○ Yes
- ◉ No

* 4 bis. Have you encountered any challenges or limitations, including administrative burden or costs?

- ○ Yes
- ◉ No

* Please explain why not

GM food-feed regulation and deliberate release directive:
The DVFA has no experience or information on traceability strategies to share which could be used for tracing gene-edited products in food or feed.
The DAA has (as mentioned under question 3) some experience with document-based control of traditional GMOs, typically plant seeds. However, we do not believe a similar system can ensure that non-authorized products obtained by new mutagenesis techniques are not imported to Denmark.
For stakeholders/companies/farmers who want to promote transparency, it might be possible to share information about the exact breeding method for a crop within the seed legislation. It has, however limitation and is reliant on the industry's willingness to share information.
Contained use directive:
The DEPA has no relevant experience or information on traceability strategies to share regarding contained use of gene-edited organisms.

GM food-feed regulation:
While traceability is an important parameter in the official control of food and feed containing or consisting of traditional (transgenic) GMOs, the DVFA do not see this as a possibility for products developed by the new mutagenesis techniques as such products are not considered to be GMOs in many exporting countries.
If there were to be introduced a traceability system, e.g. similar to the control of organic production, this would require world-wide control organs based to a large extent on document control to ensure that a given product is not produced by means of one or the other NGT. At least for products developed by the new mutation techniques, we do not think this is a viable solution, or a way forward for the EU.
Contained use directive:
The DEPA do not trace specific genetic modifications, but look at the overall organism (plant, microorganism, animal) in the validation of risk assessments. When we approve production facilities, we set conditions for the companies' own-control. Due to the problems with detection of changes by gene-editing, the required test can be problematic to perform by the companies. We are not aware of any useful method for tracing a genetic change introduced by gene-editing vis-a-vis a change which has occurred in nature or by traditional mutagenesis techniques. However, scientists usually register their animals modified by gene-editing in databases, and e.g. mark the cages which hold gene-edited animals.

* **5. What other experience can you share on the application of the GMO legislation, including experimental releases (such as field trials and clinical trials), concerning NGT-products in the:**

- [x] **Agri-food sector?**
- [x] **Industrial sector?**
- [ ] **Medicinal sector?**

Agri-food sector

GM food-feed regulation:
The DVFA has no experience to share regarding the application of the GM food-feed legislation on gene-edited products (see our answer to questions 3 and 4).
Deliberate release directive:
Since the DAA in 2015 became Competent Authority for the deliberate release directive, we have not received any applications for experimental releases of organisms developed by gene-editing or other NGTs, probably because the barriers caused by the current legislation deter the operators in the agri-food sector.

Industrial sector

Contained use directive:
The DEPA experiences an increase in the number of applications for approval of new strains of production organisms from the industrial sector as a consequence of the recent development of the new mutagenesis techniques (CRISPR), which makes it easier to create new production organisms.

* **6. Have plant varieties obtained by NGTs been registered in national catalogues?**

- ( ) Yes
- (•) No

* **7. Do you require specific information in national catalogue when registering plant varieties obtained by NGTs?**

- (•) Yes
- ( ) No

* Please specify

Yes, we require information in national catalogues.
When registering a GM-variety in the national catalogue, we require that the GM-event in question is authorized for cultivation in the EU. No such varieties have yet been registered.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

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B - Information on research and innovation

*

8. Have you supported with national funding programmes NGT-related research projects/programs (ongoing or finalised in the last 5 years), including on identification or traceability?

- (●) Yes
- ( ) No

* Please provide an overview of the project/program including title of project, a brief summary with scope and objectives, the amount of national funding received and possibly specify if the receiving entity is public or private

Yes, several research projects involving NGTs have recently received funding in Denmark. It is noteworthy that stakeholders have indicated that some of these projects could only receive funding if the application included a plan for getting products on the market that were not obtained through NGT, i.a. by using gene-editing to investigate the effect of a particular mutation that could later on be developed through traditional mutagenesis. Such «work-around strategies» are a result of the current EU-legislation.

See Appendix 1 (plant projects) and 2 (other projects e.g. with microorganisms) for a list of funded projects, and a more detailed description of some of these projects.

* 8 bis. Please highlight the potential challenges encountered when supporting/funding NGT-related research and any consequences from these challenges.

Previously, plant researchers often worked in model plants that were less relevant for plant breeders and farmers. With the new gene-editing tools it has become realistic to work in real crop plants, making such research much more relevant and interesting for plant breeders. But to get the full potential of this unique opportunity for fruitful synergy between science, plant breeders and farmers, it is crucial to have the possibility to utilize the actual plants with improved traits in breeding and cultivation which is not the case under the current EU-regulation.

* **9. How do you see NGT-related research evolving?**

GM food-feed regulation and deliberate release directive:
The DVFA finds that Danish companies and research institutes have for several years conducted NGT-related research projects in a wide range of areas in the agri-food-sector covering both plants and microorganisms (and possibly also animals), in particular by using the new gene-editing techniques (ODM and CRISPR/Cas). We expect a significant increase in CRISPR/Cas-related research in the coming years as it is the most easily applicable method of genome editing, also within the reach of small companies with limited resources for laboratory facilities. However, it will be very prohibitive for commercial applications, if the techniques continue to be kept under the GM food-feed regulation. To bring new products on the market, the techniques have to be exempted from the GMO legislation. Of the other NGT-techniques, we have only heard of cisgenesis as being of relevance for research in Denmark. Also for cisgenesis and the other genomic techniques, the future research interest for product development will depend largely on whether the techniques fall outside or inside the scope of Regulation 1829/2003.
The DAA understands from stakeholders at universities and in the agri-food sector that NGT-related research will continue, but it will not bring benefits to the EU due to current status of the EU-legislation. There are highly skilled research groups on several Danish universities and in the industry that could make important contributions to the future competitiveness of European agriculture and plant breeding industry – if they have access to utilizing the new breeding techniques. This is very important if we are to overcome the huge challenges we are facing regarding climate change and sustainable food production in the EU. However, the interest for new research initiatives has chilled following the ECJ-decision in 2018. If there is no future for breeding of crops in the EU using the new precision mutagenesis methods, the willingness

(incentives) from the industry to invest would be low. This would lead to loss of innovative solutions for plant breeding and will thus hamper the conversion of European agriculture and plant production to more climate friendly production systems. European companies are moving research activities out of the EU and thereby removing the incentive for students to enter mutagenesis-related research and product development projects.

Contained use directive:
Research based on CRISPR-techniques is also useful and has created new opportunities within the medical area, where it enables fast evolution of genomic variants (animals, mammalian cells, microorganisms), and can be used both for inactivation of genes and insertion of new genes, as well as making the identification of genomic targets easier.
Furthermore, the continuing rapid evolution of NGT-technologies like CRISPR in terms of specificity, precision, ease of use and lowering of costs will continue to create new opportunities for use. For more detailed examples, see Appendix 3 with confidential information.

*** 10. Have you identified any NGT-related research needs from private or public entities?**

- (●) Yes
- ( ) No

* Please specify which needs and how they could be addressed

The DAA:
Input to DAA from stakeholders point in two directions:
i) The need for research on the use of NGTs as a tool to adapt agriculture to major challenges such as climate change.
These stakeholders see huge potentials in gene-editing to solve challenges in the plant production and the agri-sector and point to the need to develop more robust varieties with i.a. improved nutrient utilisation and resistance against pests and diseases. However, given the current regulatory situation in the EU, they believe the potential is unlikely to be realized.

ii) The need for research on alternatives to NGTs and on risk assessment.
Stakeholders from the organic sector are sceptical about the potentials of gene-editing and recommend more research on alternatives like Genomic Selection and Marker Assisted Selection (Collard BCY and Mackill DJ (2007). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Frouin R. et al (2019). Genomic prediction offers the most effective marker assisted breeding approach for ability to prevent arsenic accumulation in rice grains).

They also see a need for more research on risk assessment of gene-editing methodologies and their application in plants and animals. Research has documented that the image of NGT as being exact and precise methods to a substantial degree is due to lack of investigations of unexpected genomic events and not least methods for their discovery (Olsen AP. et al. (2010). Analysis of illegitimate genomic integration mediated by zinc-finger nucleases: implications for specificity of targeted gene correction; Ono R. et al. (2019). Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements).

The DEPA:
DEPA has identified a need within research to create better mice models for human disease, which can establish novel disease models that can reduce cost for the pharmaceutical research and increase the chance of finding novel drugs for disease that currently cannot be treated efficiently and further to understand medical conditions. Furthermore, research needs have also been identified within agriculture and

horticulture were gene-editing is seen as tool to provide a fast development of improved crops.

However, for entities in the EU, the interest also depends on the future regulation of the different NGTs in the EU (not-regulated, or regulated less strict than now under the directive on contained use), i.e. the economical expense is at the moment too high for most private entities.

The DVFA:
We do not see any particular NGT-related research needs, as this is already a very active research area.

* **11. Could NGT-related research bring opportunities/benefits to science, to society and to the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Please find our reply in the attached supplementary file (2020-04-30 Q11 from the Danish CA on GMO.pdf).

* **12. Could NGT-related research bring challenges/concerns to science, to society and to the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Yes and no.

GM food-feed regulation:
The DVFA has no specific knowledge, but expects that parts of society – as is the case e.g. with animal cloning – may have ethical concerns when gene-editing techniques are used in live-stock breeding. No serious concerns (including ethical concerns) when the new mutagenesis techniques such as CRISPR/Cas are used for the breeding of plants and microorganisms.
The DAA has noted the following challenges/concerns among stakeholders:
Most stakeholders believe that the main challenges relating to NGT are caused by the current EU-regulation of the techniques. These challenges include the problem of detection of products made with the new mutagenesis techniques, and the competitive disadvantage faced by European plant breeders and seed companies as a result of the very expensive and uncertain approval process for GMOs in the EU. The most severe concern is that European farmers and companies are effectively prevented from utilizing the new gene-editing techniques.
One stakeholder senses a concern in society about the risk of using NGTs due to unintended effects. However, unintended effects are seen at a much higher frequency with the traditional mutagenesis methods, which fall outside the scope of the GMO legislation.
Another stakeholder finds that the wording of question 12 implies that NGT-related research as such could cause concern. However, possible challenges or concerns would not come from the type of the genomic technology used, but from the resulting organism/product and its applications.
In contrast to this, other stakeholders, including from the organic sector, find that NGTs bring new risks into the food chain. The organic market is currently displaying high annual growth rates, but the risk of undetected gene-edited varieties in the organic food production may have negative effects on this development.

Contained use directive:
DEPA has no specific knowledge of concerns regarding the use of gene-editing techniques like CRISPR/Cas to create mutations that could also be created by traditional mutagenesis techniques, or have arisen spontaneously during normal growth of the organism. We don't see an increase in risk as the modification by either methods result in the same end product (e.g. microorganism). The only difference is that use of CRISPR/Cas can speed up the process to create a desired mutation in a more controlled manner.

If no, please explain why not.

Contained use directive:
DEPA expects no serious concerns regarding modifications of microorganisms, plants and animals by the mutagenesis techniques such as CRISPR/Cas as compared to the traditional mutagenesis method, , which are excluded from the scope of directive on contained use of GMOs.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

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C - Information on public dialogues and national surveys

*** 13. Have you or other institutions/bodies/entities organised national dialogues concerning NGTs?**

- (●) Yes
- ( ) No

* Please describe briefly the content, methodology and conclusions

Yes, DAA and the Danish Council on Ethics have organized such dialogues.

As mentioned under question 1 and in more details in the presentation given by Denmark at the joint meeting of the GMO-Committees 15 January 2020, the DAA has established a working group on new breeding techniques, with focus on gene editing in plants, with representation from key stakeholder organisations and institutions, including NGO's and the consumer's organization. At meetings of this group various issues relating to regulation of these techniques have been discussed. The discussions were much more open than previous debates on GMO's. The large majority of the group found that gene editing (without insertion of foreign DNA) was different from traditional GMO's and could potentially contribute to developing a more sustainable agriculture. This majority also believed that gene-editing should be regulated differently from traditional GMOs and that this should be given priority. Other new techniques were clearly creating GMO's and should be regulated as such. Together with the working group, DAA has also arranged a well-attended public conference on gene editing in plants. Here the debates were again less polarized than previous GMO-debates.

In addition, the Danish Council on Ethics has also been involved in dialogues with the public, including students and school children. Please refer to our answer to question 15.

*** 14. Have you or other institutions/bodies/entities organised national surveys, which assessed public opinion on NGTs?**

- ( ) Yes
- (•) No

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

D Information on ethical aspects

* **15. Have any national bodies or expert groups discussed or issued opinion on the ethical aspects of NGTs?**

- (•) Yes
- ( ) No

* Please describe briefly the content, methodology and conclusions

Yes, the Danish Council on Ethics has issued such an opinion.

As mentioned in more details in a presentation given by Denmark at the joint meeting of the GMO-Committees 15 January 2020, the Danish Council on Ethics has in April 2019 issued a statement, “GMO and ethics in a new era” covering GMOs and New Breeding Techniques (http://www.etiskraad.dk/~/media/Etisk-Raad/en/Publications/DCE_Statement_on_GMO_and_ethics_in_a_new_era_2019.pdf?la=da.) 15 out of 16 members of the Council on Ethics concluded that it would be unethical not to use NGTs in light of the huge challenges – such as climate change – that mankind and the globe is currently facing.

The Danish Council on Ethics has concluded that 20 years of cultivation and experience with herbicide- and insect-resistant plants has shown no documented harmful effects on human health or nature as a result of the genetic modification itself. On this background, the Council finds it ethically problematic to reject GMO varieties if they can contribute to solving problems in agriculture and food production, and if there are no good arguments for rejecting the GMOs. The large majority of the Council members thus finds that GMOs should not be de facto prohibited (e.g. for cultivation) solely because of the technology which has been used to produce the GMO. Some GMO types, in particular organisms developed by the new mutagenesis techniques, are compatible with both the absence of particular risks and respect for natures own processes. Such GMOs should therefore not be effectively rejected by subjecting them to strict risk assessment requirements that are not imposed on similar new varieties developed by conventional mutational breeding. The Council finds that similar products should be treated under the same legislative framework, and has recommended that Denmark should work towards changing the EU authorisation procedures to a product-based system as replacement for the current process-based system.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

E - Information on opportunities and benefits from the use of NGTs and NGT-products

* **16. Could the use of NGTs and NGT-products bring opportunities/benefits to the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Yes, for all regulations, except for the organic sector on deliberate release and food-feed regulation, for which it is a no.
GM food-feed regulation and opportunities and benefits to the conventional agri-food sector: Stakeholders from the conventional farming believe that gene-editing will be one of the most important tools to develop and adapt crops and food production to challenges posed by global warming, limits on available land, increased demands on sustainability and other challenges facing the sector. Traits for which the desired genetic variation is not present in relevant genetic background can be introduced leading to better and more sustainable crop varieties. New variation can be generated and tested much faster and more efficient than what is possible with the current techniques.
Please also refer to the answers to questions 8, 10 and 11

If no, please explain why not.
The organic sector finds that NGTs could have a negative impact on organic farming, at least in the short term.
Please see the answer to question 12.

* **17. Could the use of NGTs and NGT-products bring opportunities/benefits to society in general, such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic benefits, in the short, medium and long term?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Please find our reply in the attached supplementary file (2020-04-30 Q17 from the Danish CA on GMO.pdf).

* Under which conditions do you consider this would be the case?

[Not part of the endorsed questionnaire from January 15, 2020]

* **18. Do you see particular opportunities for SMEs on the market access to NGTs?**

- (●) Yes
- ( ) No

* Please explain under which conditions

Yes, for all regulations.

According to stakeholders, NGTs pose a great opportunity for SMEs if mutation techniques are exempted from GMO-regulation. Today, the use of GMO techniques are monopolized by a few global companies, who can afford the huge GMO approval costs and who concentrate on a very limited number of characteristics in a handful of globally important crops. These companies are mainly from the chemicals sector giving them a focus on increased use of chemicals in the agri-food sector. Especially the new mutation techniques open an opportunity for European breeding companies to break this monopoly and change the focus to a more sustainable agri-food production. Breeding companies are based in the farming sector and would focus on regional/national crops and traits than the chemical sector multinational companies have done during the past 3 decades with traditional GMO techniques. This could be traits that give benefit for the farmer and consumer, see reply to question 8 (project) and 11 (research).
Therefore, European breeding companies will be able to deliver e.g. stress tolerant and disease-resistant crop varieties, which are crucial for ensuring the food and feed supply when the availability of pesticides is reduces during the coming years, see the farm-to-fork strategy by the EU Commission.
European SMEs could engage in fruitful cooperation with Universities to develop crop varieties for the market using the new techniques. Please refer to the answer to question 8, where i.a. the “ReTraQue-project” and the “KRISPS-project” are good examples of such cooperation between SMEs and Universities.
Across the EU, many SME plant breeders would be able to develop new robust and sustainable crop varieties faster. NGTs are an opportunity to strengthen the competitiveness of the smaller breeders in the EU and act faster on new demands, e.g. consumer demands, farmers needs, environmental and climate challenges.
Exemption of new mutagenesis techniques from GMO regulation will also improve the innovation at universities and benefit the emergence of new businesses from research institutions in the EU.

*** 19. Do you see benefits/opportunities in patenting or accessing patented NGTs or NGT-products?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

The Danish Patent and Trademark Office has informed that:
In general, a broad access to patenting in the biotechnology field, including patenting of NGTs/NGT products, will accelerate the development, thereby creating an economic incentive for business research and exploitation.
The existing regulatory framework makes no distinction between different areas of technology, thus the development of NGT's does not change the general applicability of regulation as provided in the Danish Patent Act or the Biotech Directive (Directive 98/44/EC).

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

F - Information on potential challenges and concerns of NGT products

*

20. Could the use of NGTs and NGT-products raise challenges/concerns for the agri-food, medicinal or industrial sector?

- (●) Yes
- ( ) No

* Please provide concrete examples/data

GM food-feed regulation and deliberate release directive:
The EU agri-food sector has been brought in a very difficult situation with the EJC Court Ruling of July 2018 because the sector to a large extent is deprived of the possibility to making use of new breeding techniques, in particular CRISPR/Cas for the development and commercial use of new robust, climate-neutral and sustainable products. The ruling has also diminished the possibilities for the sector to contribute to the required reduction in the emission of carbon to the atmosphere. In addition, Danish and European agriculture and food production has a competitive disadvantage.

The new gene-editing techniques will be used extensively in countries which provide EU with important agricultural products. This could create a serious disruption in the global free trade of agricultural commodities, which is a severe concern for the whole agri-food sector - both in terms of direct effect on availability of raw materials in the EU, but also in terms of reduced competiveness of the EU agri-food sector.

Another serious concern for the use of NGTs and NGT-products in the agri-food sector is the lack of possibilities for the companies to ensure that e.g. imported seeds, feed or food products developed by gene-editing techniques comply with the legislation.

Contained use directive:
Within research there is still concern among some researchers for creating unwanted DNA deletions. But techniques are improving rapidly reducing risks. In Denmark registered production activities with genetically modified organisms are to be traced in the surrounding environment in regard to establish whether organisms can be found in the emissions from chimneys, and whether these organisms can grow and be established in the surrounding nature. Modifications in organisms modified by NGTs such as CRISPR/Cas are not possible to detect in nature, which create a traceability problem regarding the companies self control of containment. The use of NGT products may lead to ethical concerns and debates in the public space. Potential risks/challenges are very dependent on the use and context.

* **21. Could the use of NGTs and NGT-products raise challenges/concerns society in general, such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic challenges, in the short, medium and long term?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Yes, please see the answers to question 17 and 20 and the examples given hereunder.

GM food-feed regulation and Deliberate release directive:
A concern is whether NGTs and NGT-products pose a risk for the environment or for human and animal health and hence whether a risk assessment is needed prior to marketing. In this connection, it should be kept in mind that the term “NGT” covers a whole range of different techniques and that an individual technique such as CRISPR, can be used in a number of very different applications in terms of the genetic

changes introduced.

When it comes to gene-editing that only introduce genetic changes that could also occur naturally or by traditional mutation techniques, the EU Commission's Scientific Advice Mechanism (SAM) and Danish scientific experts finds that they are at least as safe as the traditional random mutagenesis techniques.

Most Danish stakeholders therefore believe that these techniques should not be subject to the full GMO-regulation of the EU.

A few stakeholders, including the organic sector, believe the potential risk of the techniques is a challenge, and therefore believe they should be subject to a thorough risk assessment before organisms produced with them are deliberately released. They support this with examples of un-intended genetic changes being introduced by eg. CRISPR (https://www.independentsciencenews.org/health/gene-editing-unintentionally-adds-bovine-dna-goat-dna-and-bacterial-dna-mouse-researchers-find/)

* Under which conditions do you consider this would be the case?

[Not part of the endorsed questionnaire from January 15, 2020]

*** 22. Do you see particular challenges for SMEs on market access to NGTs?**

- (●) Yes
- ( ) No

* Please explain under which conditions

GM food-feed regulation and deliberate release directive:
The strict EU regulation impose a heavy economic burden (estimated up to 30 mio Euros) on companies seeking EU authorisation of GMOs or derived products, as well as on management and continuous monitoring during cultivation or marketing. Only large multi-national companies can afford these costs. The present regulation therefore favors these companies, to the detriment of small and medium enterprises and public research institutions. If the new mutagenesis techniques and other NGT-techniques continue to be regulated as GMO's under the current legal framework, our SMEs and university institutes will not be able to bring new products developed using such techniques to the market (see our also answer to question 18).

*** 23. Do you see challenges/concerns in patenting or accessing patented NGTs or NGT-products?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

The Danish Patent and Trademark Office has informed that:
From a methodological perspective, there are challenges in patenting NGTs/NGT products. First of all, it can be difficult to determine whether a NGT product applied in a patent application is covered by a prior known technique, ie. whether it includes organisms that are already present in natural or breeding populations. Correspondingly, it is difficult to determine the scope of protection for any patents on such organisms.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

G - Final question

*** 24. Do you have other comments you would like to make?**

- (●) Yes
- ( ) No

Please provide your comments here

The Danish authorities on GMOs welcome that the Commission now addresses the legal status of new genomic techniques, which have been developed over the last two decades. Thus, the questionnaire covers not only the new mutagenesis techniques (gene-editing), but also many other new breeding techniques. We have also noted that the questionnaire includes use of the techniques in all areas including animal breeding and medicinal uses. We acknowledge that the Commission addresses these aspects in the study.

The gene-editing techniques seem to be particularly promising tools for the development of a green, sustainable and climate-friendly agriculture and food production in the EU¸ We therefore welcome that the Commission gives this issue the highest priority.

When it comes to making proposals for solutions, the situation with gene-editing techniques used on plants and microorganisms, requires urgent action. Notably because the ruling of the European Court of Justice from July 2018 has created severe problems regarding implementation and enforceability.

We therefore hope that the Commission as soon as possible will finalise its study of the legal options and present a proposal on the specific regulation of gene-editing techniques in the EU.
In the longer perspective, we also see a great need for looking at other breeding techniques (e.g. cisgenesis), as well as on other aspects of the GMO legislation. As to the latter, we notice that the scientific community strongly recommends that the GMO legislation should be based on a more product-oriented approach. This idea should therefore be explored.

To conclude, we would suggest a two-step procedure.

The issue of the new gene-editing techniques used on plants and microorganisms should be handled first. A simple legal solution could be to amend the GMO legislation to include a revision of the GMO definition, or the associated exemptions, in order to exempt genome-edited organisms from the scope of the legislation if (1) no foreign genetic information is inserted and/or (2) if there is a combination of genetic material that could also result naturally or through traditional breeding methods.

Beyond the short-term amendment of current genetic engineering legislation, the next step could comprise developing a fundamentally new legal framework that is detached from the previous process-based regulatory approach. The new, science-based, legal framework must link the requirements of authorisation and registration to the resulting traits.

Such an approach would be in line with the recommendations expressed by the EU-Commissions Scientific Advisory Mechanism (SAM), the Danish Council on Ethics, the European Academies Science Advisory Council (EASAC), and The Swedish Gene Technology Advisory Board.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

Contact

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Ministry of Environment and Food of Denmark
The Danish Agricultural Agency

This appendix describes five funded NGT-related research projects on plants

Related to question 8: Have you supported with national funding programmes NGT-related research projects/programs (ongoing or finalised in the last 5 years), including on identification or traceability?

Summary

Project No.	Public funding (mio DKK)	Private funding (mio DKK)	Total funding (mio DKK)
1	29	12	41
2	0	60	60
3	0	60	60
4	14.7	6.6	21,3
5	9,2	0	9,2
Total	**52,9**	**138,6**	**191,5**

Project 1

Title and timeframe:

"ReTraQue" (2018-2023)

Summary with scope and objectives :

Using NGT (CRISPR) , the project seeks to improve disease tolerance and quality parameters in cereals and potatoes, forage digestibility in grasses and alfalfa and general yield components. All traits have direct links to solving environmental and climatatical issues; improving photosynthesis will enable an increase in terms of raw production with the same input, and hence lead to less CO2 use per produced unit, increased disease resistance will secure higher and better quality of yield with less need of input of pesticides – a direct benefit for the environment and indirectly a strong driver for less used CO2 in production. Finally improving the feed quality will result in a decrease in the load on environment per produced ¨meat-unit¨, as less quantities of feedstuff is required to obtain the same growth – on top this will also increase animal welfare.

Participants (private/public):

A joint project between of Copenhagen University and Aarhus University on the public side and the private plant breeding companies Sejet, Nordic Seed, Danespo and DLF.

Funding (amount and source):

41M DKK (29M from public sources (« Innovationsfonden »), 12M from private sources)

Link:

ReTraQue - https://innovationsfonden.dk/da/nyheder-presse-og-job/ny-viden-om-planters-egenskaber-kan-oge-markedsandele-med-5-10

Project 2

Title and timeframe:

« NovoCrops: Accelerated domestication of resillient climate-change friendly crops » (from 2020 to 2026)

Summary with scope and objectives:

Plant production is facing unprecedented challenges. A growing human population will increase the demand for staple crops and livestock by 60% in 2050, while climate change is predicted to drastically limit plant production due to new and increased environmental stress factors, such as heat, drought and flooding. Modern crops have high yields, but are sensitive to environmental challenges. By contrast, wild relatives of modern crops are more resilient, but produce low yields.

The NovoCrops project aims to lay the foundation for the next, sustainable green revolution that focuses on developing new sustainable crops adapted to challenging environments and capable of meeting future agricultural production demands. This will be achieved by domesticating wild, resilient plants and developing them into future crops. The wild plants to be used include wild barley, wild potato, alfalfa, quinoa and wheatgrass.

Participants (private/public)

Aarhus University and Copenhagen University (public)

Funding (amount and source):

60M DKK from the private fund « Novo Nordisk Foundation »

Link:

https://challenge.novonordiskfoundation.com/recipients/michael-broberg-palmgren-professor-department-of-plant-and-environmental-sciences-university-

of-copenhagen-denmark/; https://novonordiskfonden.dk/da/nyheder/novo-nordisk-fonden-stoetter-forskning-i-modstandsdygtige-afgroeder-med-120-mio/; https://agro.au.dk/aktuelt/nyheder/vis/artikel/vilde-planter-klimasikrer-fremtidens-afgroeder/

Project 3

Title and timeframe:

A New Paradigm for Disease-free Crops of Tommorrow (2020-2026)

Summary with scope and objectives:

The project will work towards creating durable resistance to the fungal diseases powdery mildew and yellow rust in the major crops wheat and barley. If the project finds a way to achieve resilience, we can minimize the crop losses caused by the fungal diseases. As production becomes more efficient, it can be concentrated on fewer hectares and we can then conserve biodiversity and store CO2 on other land.

Participants (private/public :

Headed by Copenhagen University

Funding (amount and source):

60M DKK from the private fund « Novo Nordisk Foundation »

Link:

https://novonordiskfonden.dk/en/news/novo-nordisk-foundation-awards-dkk-120-million-for-research-on-crop-resilience/

Project 4

Title and timeframe:

« NaFOCo » (5 year, 2020-2024)

Summary with scope and objectives:

The project will develop natural colours for food by using i.a. new genomic techniques. Consumers all over the world are increasingly demanding food without artificial additives, just as vegetarian and vegan food solutions are gaining momentum both in Denmark and the rest of the world. It increases the need for natural and pure vegetable food colours. The project works with violet carrot as a model crop, because these carrots naturally contain colours with the potential to cover the desired colour spectrum. Furthermore, carrot is a productive crop.

Participants (private/public):

Chr. Hansen Natural Colours A/S (private) and Aarhus University (public)

Funding (amount and source :

21,3M DKK (14.7M DKK from « Innovationsfonden », (public), 6.6M DKK from private)

Link:

https://mbg.au.dk/en/news-and-events/news-item/artikel/nyt-forskningsprojekt-kan-bane-vej-for-et-farvel-til-kunstige-farver-i-mad/

Project 5

Title and timeframe:

KRISPS - Potatoes with increased resistance and innovative starch as platform for synergy between sustainable green and economic development (01-01-2020 to 31-12-2023)

Summary with scope and objectives:

Denmark has an extensive and modern potato production that is facing new challenges. Late blight control in potato require frequent pesticide applications and accounts for 20% of fungicide use. Improved resistance will meet the demands for both environmentally safe and economically sustainable production. The primary aims of this project are to reduce pesticide use and generate healthier potatoes through the targeted use of the precise and enzyme based CRISPR/Cas technology for achieving new Danish elite varieties with reduced Late blight sensitivity and reduced tuber sugar accumulation.

Participants (private/public) :

KMC amba (private), Copenhagen university, Aalborg University (both public)

Funding (amount and source):

9,2M DKK (« GUDP », public)

Link:

https://vbn.aau.dk/da/clippings/forskere-vil-udvikle-den-svampefri-kartoffel

APPENDIX 2

List of a funded NGT-related research projects in Denmark on primarily microorganisms and mammalian cells (information from the Danish Ministry of Higher Education and Science)

Related to question 8: Have you supported with national funding programmes NGT-related research projects/programs (ongoing or finalised in the last 5 years), including on identification or traceability?

Year	Title	DKK	Project description
2015	Diverse CRISPR adaptive immune systems of Sulfolobus: Molecular mechanisms and application to studying novel archaeal virus-host interactions	6.193.071	CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated) system directs the adaptive immune defense in most archaea and many bacteria. In the recent years there have been focused studies on a few model systems, which have yielded important insights into the mechanisms of the action by CRISPR systems both in DNA targeting and in RNA targeting. However, CRISPR systems show a high structural and functional diversity and many distinct types remain to be studied. In this proposal, we aim at investigating mechanisms of novel CRISPR systems, including de novo spacer acquisition by Sulfolobus type I-A, dual targeting of RNA and DNA to invasive elements by the type III-B Cmr-α, interference activity by a novel I-D system. Novel Cas accessory proteins will also be identified and investigated. Finally, CRISPR tools will be developed to study archaeal molecular virology. These studies will yield very important knowledge to this ever-fast evolving research field.
2015	DRP technology- Novel tool for evaluation of drug efficacy and genome instability	2.592.000	We seek FTP funding to develop an assay that specifically quantifies the number of DNA double strand breaks (DSBs) present in a single cell. DNA DSBs are very toxic forms of DNA lesions that threaten the integrity of chromosomes. If unrepaired, the broken DNA fragments will be lost leading to death or phenotypic changes. Strikingly, current methodologies cannot specifically detect these DNA lesions in situ. A method to accurately assess the extent and composition of DNA DSB lesions

			is of major importance as a biomarker to basic/applied research. This includes cancer therapy development and compound genotoxicity screening, because such analysis allows direct analysis of DSBs, a major genotoxic insult. We have now uncovered a specific approach to detect DNA double-strand breaks using a bacterial protein, DRP, that specifically recognizes DNA DSBs. We will target the need for DNA DSB detection by developing our DRP technology to a stage where it can be applied in laboratories.
2015	Systematic genome-wide dissection of DNA anaphase bridges using high-throughput genetics and cell biology	1.883.200	DNA anaphase bridges constitute a potential source of genome instability and have been linked to chromosomal fragile sites, which are associated with more than 50% of recurrent cancer mutations. Anaphase bridges have been observed in a wide range of organisms from yeast to human. As the mechanisms underlying anaphase bridge formation, sensing and resolution remain largely unknown, I propose to use the yeast Saccharomyces cerevisiae to perform the first systematic genome-wide dissection of anaphase bridges in a genetic model, using high-throughput genetics and cell biology to describe the molecular pathways underlying anaphase bridge biology, and to subsequently extend key findings to the hTERT-immortalized retinal pigment epithelial cell line, hTERT RPE-1, which will provide fundamental insights into the evolutionary conservation of this important biological phenomenon.
2015	Identification and characterization of lincRNAs in multiple myeloma	2.988.726	Recent data implies that a large fraction of the mammalian genome is transcribed into thousands of long noncoding RNAs (lncRNAs). Long intervening noncoding RNAs (lincRNAs) are a subgroup of lncRNAs that play important regulatory roles in many biological processes. Emerging evidence implies that lincRNA dysregulation is prevalent and associated with the pathogenesis of cancer. Multiple myeloma (MM) is the second most common hematologic cancer in Denmark, and characterized by clonal expansion of malignant plasma cells in the bone marrow. The aim here is to identify and study the functions of lincRNAs associated with the pathogenesis of MM. In addition, the therapeutic potential of locked nucleic acid (LNA)-

			modified gapmer oligonucleotides in silencing of candidate oncogenic lincRNAs will be assessed by inhibition in MM cell lines, in a 3D myeloma model and in a murine model of MM. The results are expected to provide new insights into the role and therapeutic potential of lincRNAs in MM.
2015	DOES ASSISTED REPRODUCTION AFFECT THE EPIGENOME OF THE NEWBORN?	172.800	In Denmark 7,6% of children are born after Assisted Reproductive Technology(ART). Recent studies show that ART children are at higher risk of developing metabolic complications later in life, which is reflected by an altered metabolic profile and cardiovascular function. We hypothesize that different ART procedures change the epigenetic programming of the embryo which, in turn, modifies the risk of metabolic complications later in life. The study population will be singletons divided in 4 subgroups each based on the ART procedure used: ICSI or IVF with a fresh or a frozen embryo transfer. We aim to analyze small non-coding RNA expression and the DNA methylation pattern of pure mesenchymal stem cell populations to determine if specific ART procedures alter the epigenetic signature of the newborns' cord blood cells. We anticipate the results obtained from this study will generate novel knowledge in order to optimize ART protocols and improve the health of children born after ART.
2015	A road map of clonal evolution and therapy-resistant subclones in patients with acute myeloid leukemia (AML)	2.282.990	Cancer emerges through clonal evolution propagated by accumulation of genetic aberrations. Genome sequencing facilitated by next generation sequencing (NGS) represents apowerful tool for tracking of genetic markers of clonal evolution. However, high cancer heterogeneity hampers quantification of clonal populations if the cancer is sequenced in bulk. Hence, NGSpopulations (HSCs/HPCs) from acute myeloid leukemia (AML) patients and subject these populations to NGS to delineate the clonal evolution in individual cancer patients. Significantly, analysis of HSCs/HPCs obtained from AML patients over time willstudies on cancer subpopulations purified from the tumor bulk are instrumental for investigation of clonal evolution in cancer. In this project I will purify hematopoietic stem and progenitor allow for identification of populations containing therapy-resistant subclones and thereby define bona fide leukemic stem cells in the human setting.

2015	A genome-based analysis of factors driving assembly of pesticide-degrading bacterial communities in soil macropore hot-spots (GENOPORE)	2.746.716	In loamy soils macropores serves as preferential flow paths for downwards transport of pesticides to groundwater aquifers. With time, however, a degrading microbial community may form in the macropore region, limiting leakage of pesticides. The overall aim of GENOPORE is to clarify the evolution of catabolic potentials in macropore hotspots following pesticide exposure. The factors driving the assembly of pesticide-degrading bacterial communities in macropores will be determined, including bacterial motility, chemotaxis and horizontal transfer of catabolic genes. The research involves field work where hydraulically active macropores are determined as blue-coloured areas after application of a colour tracer. DNA will be extracted from matrix soil and macropores samples followed by sequencing of meta-genome and mobilome elements. The bioinformatics will be carried out mainly at Netherlands Institute of Ecology under supervision by Professor Jos Raaijmakers.
2015	microRNA based Chinese hamster ovary cell engineering towards improved recombinant protein N-glycosylation	3.015.403	In order to produce Chinese hamster ovary (CHO) cell-derived recombinant therapeutic proteins with ensured safety, efficacy and cost-effectiveness, developing tools that is capable of modulating protein N-glycosylation towards desired patterns is of prime importance.In this project, using RNA-seq of representative CHO cell lines based on in-house dataset and new experiment, we will try to correlate the microRNA expression dynamics with N-glycosylation related mRNA expression profiles. Selected microRNA candidates that can modulate N-glycosylation will be further screened based on experimental approaches. In this stage, we will construct plasmid library that can knock out/down or overexpress the microRNAs. State of the art Crispr/Cas9 genomic editing technology will be developed for microRNA engineering in CHO cells. Finally, we will compare N-glycosylation profiles of the recombinant protein derived from the engineered CHO cells and identify functional microRNAs for N-glycosylation.
2015	Generation of Prodrugs of Histone Deacetylase Inhibitors	216.000	Histone deacetylases (HDACs) are a class of enzymes responsible for deacetylation of modified lysine residues in histone proteins. Acetylation and deacetylation play an important role in transcriptional regulation and

			abnormalities in this mechanism can lead to a variety of diseases; e.g. cancer and neurodegenerative diseases. Being able to affect gene expression through epigenetic mechanisms, such as acetylation, therefore has the potential to improve human health. In this project, we propose to take advantage of recent structural insights to design such inhibitors based on a cyclic tetrapeptide core. The inhibitory potencies of the cyclic tetrapeptides will be conducted through full in vitro profiling in enzyme-based assays. In addition, ester and thioester analogs will be designed to achieve cellular activity. The desired outcome of the project will be to prepare cell permeable inhibitors of class-I HDACs (1–3) and profile their activities as putative anti-cancer compounds.
2015	Er resistensplasmidoverførsel hos E. coli relateret til serotyper ?	144.340	Extended Spectrum Betalactamase (ESBL) producing E.coli is spreading also in Denmark i.e. we now experience a prevalence around 8% of E.coli from bacteraemia cases. ESBL is transferred on plasmids, but in a recent study on E.coli from urinary tract infections we found that relatively few E.coli serotypes were represented among ESBL-producing isolates as compared to E.coli susceptible to all antibiotics. We therefore ask, whether conjugation of ESBL-plasmids is related to the serotype of E.coli. In the project we will test the ten most common serotypes among susceptible E.coli for ability to conjugate with a number of ESBL-E.coli donors both qualitatively and quantitatively. Interesting transconjugants will be whole-genome-sequenced (WGS) together with donor and recipient strains. Furthermore, from a collection of over 600 WGS E.coli we will study the relationship between genes for serotypes and genes involved in transfer.
2016	Extrachromosomal circular DNA - origin and impact on eukaryotic genome stability	3.203.837	Our insight in evolution relies on our understanding of variation created by mutation. We have recently found that an otherwise poorly examined class of mutations, extra chromosomal circular DNA (eccDNA), can form throughout the eukaryotic genome of yeast. This finding increase the number of known eccDNA >1kb a hundred fold and suggests that eccDNA play central yet uncharacterized roles in the eukaryotic genomes. We now suggest that eccDNA play an important role in evolution and malignancy by contributing to genetic variation through copy number variation. We plan to investigate this by screening yeast and human for the mechanisms and rates by which eccDNA form and

			explore where and how eccDNA reintegrate and amplify on the chromosomes. We will address these issues using our unique method for eccDNA mapping combined with cutting edge next generation sequencing and yeast genetics.
2016	Molecular Switches and Circuits Orchestrating Mammalian Lipid Metabolism	439.080	We present an ambitious research program that uses novel functionalbiochemical approaches to define the fundamental molecular functions of dynamic protein modifications and protein-protein interactions in the regulation of lipid metabolism with emphasis on sphingolipid metabolism. Using genome editing we will generate cell lines in which we will be able to study the regulatory function(s) of specific protein-protein interactions and phosphorylations of enzymes involved in sphingolipid metabolism, and how they affect metabolic fluxes. Studying the basal mechanisms regulating sphingolipid metabolism is a large and important part of biological research and contributes to a fundamental understanding of molecular networks and our ability to prevent or combat diseases.
2016	THE LOCUS OF EVOLUTION: Do CRE and ORF mutations contribute differently to phenotypic evolution?	2.591.548	With this proposal, we aim at addressing the unresolved questions of whether evolutionary genetic changes typically affect protein expression or protein structure, and if these distinct types of mutations contribute differently to phenotypic evolution. Answers to these questions are of critical importance for our complete understanding of the genetic basis for biological variation in Nature and for our ability to predict and control the course of evolution. Naturally, the relative importance of these different types of mutations has been the subject of considerable debate in the literature, but these discussions are typically based on a small number of examples. Here, we will establish a research program focused on resolving this debate by systematic analysis of adaptive mutations in the Bacterial domain of life. The wealth of bacterial genome sequence data available for large-scale analyses now make its possible and realistic to provide specific answers to these important questions.
2016	The role of SSX proteins in chromatin organization	1.290.240	SSX proteins are expressed in pre-meiotic male germ cells and different types of human cancer. Our preliminary results suggest that SSX proteins are a novel type of core structural element in genomic DNA

			organization. We wish to study how the SSX-DNA complexes are organized, what the structural basis of this interaction is and how SSX proteins are integrated into chromatin in cells. The proposed studies may change our understanding of structural and functional regulation of the genome in spermatogenesis and cancer development.
2016	The role of epigenetics in adaptation to environmental change	6.448.514	Response to environmental change is expected to occur by selection on standing genetic variation, however, adaptive mechanisms are more complex than so far realised, calling for integration of other 'non-genetic'(or plastic) mechanisms in broadening our understanding of adaption. I will investigate the influence of epigenetics in the response to environmental change, by examining its role in facilitating adaptation, combining genomic and transcriptomic approaches with experimental studies of spider populations in the field and lab. The objective is to link epigenetics to the expression of phenotypic traits and individual performance along a temperature gradient, to identify mechanisms underlying adaptation in specific environments. I will test hypotheses on functional relationships to provide experimental evidence for the role of epigenetic mechanisms in facilitating adaptation.
2016	The transcriptional basis for pancreatic priming in a novel human ventral foregut culture system	2.589.461	Human Embryonic Stem Cell (hESC) derived pancreatic β-cells represent a potential treatment for type I Diabetes, but hESC differentiation to functional β-cells is currently unreliable. We and others have found that ventral foregut (VFG)-like cells can be expanded from differentiating ESCs. As the embryonic VFG gives rise to the pancreas, these VFG cultures differentiate effectively into pancreatic cell types, suggesting an alternative to hESCs. In this proposal we ask how the enhanced differentiation of human VFG cells is regulated. What transcriptional and epigenetic differences specify enhanced pancreatic differentiation and how is this controlled by signalling? In mouse, Polycomb co-repressors regulate the choice of VFG to generate liver or pancreas and we ask if they can regulate VFG differentiation in vitro. Our studies will address whether inhibiting Polycomb and culture in specific cytokines can be used to enhance the production of functional β-cells.

2016	Integrated functional genomics of physical and cognitive capacity in later life – a study of monozygotic twins	2.544.480	Preservation of physical and cognitive capacity is of fundamental importance for maintenance of life quality, and thus particularly important components of healthy aging. Within the frames of the Danish Twin Registry monozygotic (MZ) twins constitute a unique resource for studying the molecular determinants of the variation in such elements of aging. The aim of this study is to use the powerful discordant MZ twin design to investigate 3 important layers of molecular information – DNA sequence variation, DNA methylation, as well as mRNA and regulatory RNA expression levels. We will make use of a large sample of MZ twins with partly available genome-wide data, and upgrade this to complete data sets for a total of 400 MZ twin pairs. Applying both separate and integrated analyses we will explore genetic, epigenetic, and transcriptional signatures to gain insight into the molecular features that may in part be responsible for individual differences in maintenance of functional capacity.
2016	In vivo characterization of mice lacking a novel immune sensor for foreign DNA	2.592.000	In recent years it has emerged that DNA stimulates innate immune responses, including the antiviral cytokines type I interferons (IFN-a/b). We previously identified IFI16 as an innate DNA sensor (Nat. Immunol. 11:997), and we have subsequently shown important roles for IFI16 in IFN production during infections with bacteria and viruses in vitro (PNAS 110:E4571, EMBO J 33:1654). We have now generated a mouse strain lacking p204 - the mouse ortholog of IFI16 - and we here propose to characterize it in vivo with respect to control of infections, immunological mechanisms, and impact on disease. The mouse colonies, incl. other relevant gene-modified mouse strains, are ready for the project, together with other reagents and expertises. Preliminary data have been generated demonstrating a clear phenotype of p204-/- mice in one infection model tested. The project will lead to fundamental new insight into how the organism mounts defense against infections by microbes carrying DNA genomes.
2016	Controlling R-loops to prevent genomic damage	2.591.526	R–loops are transcription-dependent DNA/RNA hybrids that invariably form at multiple sites in the mammalian genome. Although beneficial at physiological conditions, R-loops are also highly dangerous sources of DNA damage, causing devastating neurological diseases and cancer. Understanding the molecular mechanisms enabling cells to balance an adequate amount of R-

			loops is therefore crucial for such disease assessment and treatment. Recent data strongly implicate the ribonucleolytic RNA exosome complex in the control of R-loops, however, the mechanistic basis for this involvement is elusive. In the present project we will draw advantage of our leading role in mammalian exosome biology research to outline essential steps in exosome-dependent control of R-loops.
2016	Studies of the BIR repair pathway to unravel its role in tumorigenesis	2.465.814	One of the most frequent types of damage in our genome is a break (nick) in one of the strands in the DNA double helix. During DNA replication the nick is transformed into the most serious type of damage for a cell, a double-stranded break (DSB). The mechanism responsible for repairing this type of DSBs is called Break Induced Replication (BIR). So far BIR has only been studied outside natural context. These studies have surprisingly concluded that BIR is an imprecise mechanism leading to many mutations. It is a great paradox that a frequent occurring damage is repaired by a mechanism, which jeopardize genome stability. Thus thorough insight into the BIR pathway is of outmost importance to unravel whether BIR fuels tumorigenesis or if yet unknown control mechanisms suppresses genome instability during BIR. Using a unique cellular system designed in our lab, where BIR can be studied in a natural setting we will unravel how cells control a very critical event during BIR called resection.
2016	Defining the Molecular Mechanism of DNA Replication at Fragile Sites in Human Cells	1.594.800	Replication stress, an important driver of chromosomal instability, is characteristic of almost all cancers. Common fragile sites (CFS), which are intrinsically unstable loci of human genome, are prone to accumulate replication stress. Normally CFSs replicate late in S phase, but with replication stress this can be delayed to mitosis. A study from Ian Hickson's group showed that DNA synthesis at CFSs during mitosis helps to prevent chromosome instability in presence of replication stress, and proposed this DNA synthesis occurs by Break Induced Replication (Nature 2015, Inpress). BIR is generally employed for repair of one-ended double-stranded DNA breaks generated at collapsed replication forks or eroded telomeres in Yeast. The proposed plan aims to decipher the molecular mechanism during early mitosis which facilitate DNA synthesis at CFS during mitosis, and, in a wider perspective, define the mechanism of BIR in human

			cells, a process that we propose serves to suppress tumorigenesis.
2016	Characterization of Hepatitis C Virus RNA structure using massive parallel in-cell SHAPE probing.	2.545.867	This project will characterize the RNA structure of the Hepatitis C virus (HCV) genome in vitro, in viral particles, and inside infected cells during translation and replication. HCV is a major health problem, however, HCV research has focused mainly on the viral proteins, while the function of the extensive RNA structures of the viral genome has been impossible to study in the context of a replicating infection in a living cell. We will combine state-of-the art HCV cell culture systems and massive parallel sequencing based RNA structure probing inside cells, to identify structures in the viral RNA that differs dependent on whether the RNA is being translated or replicated. This switch between translation and replication is central for the viral life cycle and is likely to depend on RNA structures. The knowledge from this study will facilitate development of new classes of HCV inhibitors and improve the understanding of other plus stranded RNA viruses of relevance to human health.
2016	Identification and functional characterization of factors and mechanisms that coordinate helicase and polymerase activities under mild DNA replication stress	2.200.404	A replication-associated lesion can lead to gradual accumulation of mutations that results in various disorders including cancer. However, how cells deal with the physiological burden of replication stress that unavoidably occurs during normal cell proliferation remains to be defined. In particular, very little is known how key replisome activities (the helicase and polymerase subunits, respectively) are coordinated under spontaneously arising replication stress. Here, we aim to identify and understand the factors and mechanisms that coordinate helicase and polymerases activities under mild DNA replication stress and suppress the accumulation of otherwise breakable ssDNA at the fork. For this, we have designed a set of robust experiments, first to define experimental conditions to monitor slow moving replication forks that in principle rely heavily on coupling factors, and later identify key proteins and pathways operative under those conditions to suppress genome instability.
2016	A novel targeted RNA sequencing approach for	2.592.000	This project aims to generate new biological and molecular insights into prostate cancer (PC) development and progression. We will focus on long

	identification of aggressive prostate cancer		non-coding RNAs (lncRNAs), which despite their vast abundance in the human genome are still poorly annotated and their roles in cancer not well understood. To identify novel lncRNAs associated with PC development and aggressiveness, we will combine full-transcriptome sequencing with targeted deep RNAseq of known PC-associated GWAS regions (largely devoid of coding genes). By sequencing of tissue samples from our large PC biobank with clinical follow-up, we aim to identify novel deregulated lncRNAs with diagnostic/prognostic biomarker potential for PC. New top candidate markers will be validated in large independent PC patient cohorts and cancer driver functions will be analyzed for a few selected lncRNAs in preclinical PC models in vitro and in vivo after CRISPR-Cas9-based gene knockout/in.
2016	The role of accelerated nucleolar transcription in genome instability and tumorigenesis	1.776.054	We address how oncogene-induced acceleration of nucleolar transcription induces DNA damage and contributes to tumorigenesis. The nucleolus contains a highly specialized and unstable genomic domain: the ribosomal-DNA (rDNA) genes. rDNA genes encode the ribosomal RNAs (rRNAs) required for protein translation. Active rDNA genes are the most highly transcribed sequences in the genome, are tightly packed with RNA Polymerase and are low in nucleosomes. When cells experience activation of oncogenes, the rDNA transcription is accelerated further to meet the increasing demand for protein synthesis in cancer cells. This increased pressure on rDNA transcription induces DNA damage and impairs checkpoint function. We will use cell culture models with inducible oncogenes to accelerate rRNA transcription and examine the mechanisms inducing DNA damage and their correlation with the progression of tumorigenesis to understand the importance of rRNA transcription in cancer progression.
2016	Lethal mutagenesis in the control of chronic disease caused by RNA virus	2.592.000	Chronic viral disease affects large numbers of people worldwide, seriously compromising their lives and adding economic pressure on public health services. In addition to significant causes of disease (i.e. HIV, hepatitis) an increasing number of viruses establishing persistent infection have been identified in association with diverse health conditions. The lack of efficient antivirals for many of these viruses compromises the control of related chronic disease.Lethal mutagenesis

			has been proposed as a novel antiviral approach based on chemically increasing mutation rates during virus replication. As a result, virus populations mutate themselves to death. Despite vast evidence in cell culture there is limited data in vivo supporting lethal mutagenesis.We have recently demonstrated that a novel mutagenic compound can elicit lethal mutagenesis during persistent norovirus infection in mice, encouraging the present proposal in the identification of improved broad-spectrum therapies.
2016	Unravelling ash-dieback resistance: insights from single-cell analysis of tolerant and susceptible ashes across evolutionary divergent clades	5.135.471	The invasive pathogen Hymenoscyphus fraxineus causes severe damage on European ash. We hypothesize that phylogenetically related ash species with resistance against H. fraxineus share cellular defence responses that protect them against this pathogen. The aims are to explore the infection process of H. fraxineus, and compare cytological and molecular mechanisms among both resistant and susceptible species of Fraxinus and F. excelsior clones. The findings will reveal factors responsible for host resistance with the ultimate aim to strengthen future ash breeding. This is accomplished by i) in vivo comparison of cytological responses to infection by fluorescent-tagged H. fraxineus strains ii) identification of molecular defence mechanisms/pathways that moderate resistance to the fungus using single-cell transcriptomic analysis, and iii) comparison of cell wall composition using analytical chemistry.The results will increase the likelihood of identifying markers for breeding.
2016	Uncovering the epigenetic basis for hyperglycemia-induced hepatic dysfunction in diabetic late complications	1.653.200	This project aims to explore the molecular link between early exposure to hyperglycemia and the advanced progression of diabetic late complications. Poorly controlled blood glucose levels in diabetic patients accelerate the onset of detrimental sequelae, which were recently proposed to include liver dysfunction. Clinical studies have demonstrated the existence of a glycemic memory, reflecting a putative epigenetic legacy of early hyperglycemic episodes in diabetic subjects. Despite the importance of glycemic memory for the development of late complications in patients, the molecular mechanisms remain largely unknown. Here, we will investigate the epigenetic basis for hyperglycemia-induced liver dysfunction associated with type 1 diabetes using a combination of state-of-the-art genomic sequencing techniques and functional studies.

			We expect to uncover novel epigenetic regulators of the metabolic dysfunction in diabetic livers, which might serve as future therapeutic targets.
2016	Genome editing for disease resistance in cereals	6.471.864	We will design barley and wheat resistance to powdery mildew and stripe rust by exploiting our molecular insight in the interactions. We will make precise sequence changes by genome editing to improve proteins withstanding the pathogens. 1) We have discovered that high-pI plant cytosolic proteins are easily taken up by the extrahaustorial matrix, and we will alter an existing high-pI extracellular pathogen-induced chitinase and make it cytosolic to allow it to attack the fungal haustorium directly. 2) We have previously discovered two core fungal effectors, which suppress defence by interacting with plant target proteins. We have identified such targets, and will in detail study the interactions and target protein activities in defence to pinpoint amino acids that can be altered to make the targets effector-insensitive without loosing activity. This should reduce defence suppression. We will survey the occurrence of the core effectors to evaluate their importance for the pathogens.
2016	14q32 genomic variation within human cardiovascular disease	174.960	miRNA and snoRNA, previously thought to be junk-RNA, have in the last few years emerged as a new topic of research in a range of specialities. These miRNAs and and snoRNAs have shown to influence a large number of cellular processes such as cardiovascular remodelling, tissue vascularisation and cholesterol trafficking.Previous Genome Wide Association Studies at Leiden University Medical Center have shown that there are significant associations between single nucleotide polymorphisms (SNP) in the 14q32 locus and human cardiovascular disease (CVD). However, the SNPs investigated weren't a part of the miRNA coding regions of 14q32.Using genomic data from two Dutch major studies on CVD and restenosis spanning over 9000 patients, we wish to investigate the relationship between miRNA/snoRNA SNPs in the 14q32 locus and cardiovascular endpoints. As a part of our project, we'll also perform an in vitro cell phenotype study with the SNPs with the strongest association to CVDs.

2017	A protein quality control pathway that monitors exocytosis	2.145.527	As a result of stress conditions or mutations, proteins may lose their native conformation and misfold. Since misfolded proteins tend to form toxic aggregates, the accumulation of misfolded proteins represents a considerable danger to the cells. To cope with such harmful proteins, cells have evolved protein quality control (PQC) mechanisms that function to clear the cell of misfolded proteins. These rely on molecular chaperones to refold the proteins, or on degrading them via the proteasome. PQC is highly compartmentalized in the cell and in recent years a great deal has been learned about ER and nuclear quality control. In contrast, cytosolic PQC is less well defined. Recently, we found that exocytosis is regulated by PQC through degradation of an exocyst component. Here we aim at identifying components of this cytosolic PQC system using genome-wide epistasis mapping and proteomics. These data will then be paired with cellular studies and ultimately allow us to map this PQC pathway.
2017	Lysogenic conversion in marine Vibrio bacteria: Bacteriophage-driven dissemination of virulence factors in environmental bacterial communities	2.591.942	The Vibrio genus (vibrios) is an important group of marine bacteria, which includes pathogens of humans and animals. Virulence properties of vibrios are often encoded by prophages, which are phage genomes that have become integrated in the bacterial genome upon infection by a temperate phage. Here they remain dormant until conditions favor reactivation, cell lysis and reinfection of new hosts. Prophages directly influence the genetic composition of the host and potentially enrich it with new beneficial genes. The capacity of prophages to encode key virulence properties and disseminate these genes among Vibrio communities is potentially a major driver of virulence dispersal and emergence of disease in environmental marine Vibrio communities The objective of the project is to decipher the genetic mechanisms and environmental controls of phage driven virulence by quantifying phage-mediated transformation of harmless Vibrio bacteria into pathogens of humans and animals.
2017	Methylation in European eel genomes: its role in adaptation and speciation	2.572.164	Adaptation is a key concept in evolutionary biology that may now face redefinition. Hence, epigenetics and particularly methylation is important for phenotypic plasticity, and epigenetically based adaptive traits may be inherited despite not having a genetic basis. European eel shows a panmictic life history precluding genetically based local adaptation. I will analyze

			genome-wide methylation in eel larvae from the Sargasso Sea and juvenile and adults from climatically and environmentally highly divergent regions to test the hypothesis that lack of local adaptation is compensated by epigenetic variation. Moreover, I will analyze methylation in genomes of pure European and American eel and their hybrids for a) determining whether species background or the environment determines epigenetic imprints and for b) testing the hypothesis that reactivation of transposons in hybrids due to altered methylation is an important factor maintaining post-zygotic reproductive isolation between species.
2017	The role of intranuclear protein quality control in DNA replication stress recovery	2.579.863	The proposed project aims to perform a molecular genetic and cell biological dissection of the role of intranuclear protein quality control in regulating Mrc1 and Pph3 during DNA replication stress recovery using yeast Saccharomyces cerevisiae as a model system. The project combines genetics, biochemistry and fluorescence live cell microscopy to dissect the molecular mechanisms that allow DNA replication restart and cell cycle progression after genotoxic stress. The evolutionary conservation of key findings will be tested using gene editing and live cell imaging of human primary cells. The importance of intranuclear protein quality control for maintaining genome integrity will be examined by measuring mutation rates, genome-rearrangements and cell survival. The results of the project will provide the framework for understanding the role of intranuclear protein quality control in the recovery from other types of stress.
2017	Impact of DNA replication on genome architecture and function	5.901.441	Epigenetic mechanisms are critical to cell fate choices during organismal development and are often deregulated in cancer and developmental disorders. To understand these processes of broad scientific and societal interest, it is critical to understand how epigenetic states defined by chromatin are propagated during cell division. A key open question is how DNA replication and the concomitant disruption of chromatin impact on genome architecture and function, which governs cell fate choices in development. We will use our new innovative technology to map genome wide how DNA replication impact on key functional chromatin domains (enhancers/promoters, CTCF, accessibility) and gene expression. Further, we will

			address whether replication stress and changes in replication timing impact the normal restoration of genome function. We expect to bring landmark discoveries adding a new dimension to our understanding of DNA replication in the context of development and disease.
2017	Activation of ATR kinase signaling responses in health and disease	2.576.692	The ATR kinase is a master organizer of cellular responses to replication stress, a frequent yet deleterious condition caused by obstacles to DNA replication, and a major source of genome instability and severe pathologies such as cancer. Stimulation of ATR kinase activity upon replication stress is mediated by TopBP1, which contains an ATR-activating domain (AAD). My recent work revealed that ATR activation after replication stress is not exclusive to TopBP1, but that an additional AAD-containing protein, ETAA1, promotes ATR activation in parallel with but independent of the canonical TopBP1 pathway. Here, I propose a focused study that will address on a global and mechanistic level the molecular framework governing ETAA1-mediated ATR activation, and how this pathway suppresses genome instability and disease onset at the organismal level. This work will contribute important knowledge for current efforts targeting replication stress and disruption of ATR signaling in cancer therapy.
2017	Elucidating the role of potassium in the molecular function of neurotransmitter:sodium symporters	2.592.000	Neurotransmitter:Sodium Symporters (NSSs) ensure cellular function by utilizing the Na+ gradient for the uphill transport of solutes. We have recently found that the model protein for NSSs, the bacterial NSS LeuT, do not only use Na+ for transport, but also intracellular K+, a feature only previously observed for the serotonin transporter (SERT). This opens for the possibility that K+ binding is a common, yet un-investigated mechanism in NSS proteins. The purpose of the current project is to characterize the K+ binding site in LeuT and SERT in terms of its location and molecular function. We will, on purified protein, determine the K+ binding site by site-directed mutagenesis and transition metal ion FRET. Its molecular function is investigated in reconstituted systems. We will also perform crystallization trials on LeuT together with Rb+ substituting for K+. Finally, we will assess whether K+ binding is a more common feature also present in other NSSs such as the dopamine transporter.

2017	Transcriptional networks of adipocyte lineage determination	4.512.960	Adipocyte differentiation has been studied extensively in cell culture models and recent profiling of epigenomic mark and transcriptional regulators have provided detailed genome-wide insight into the transcriptional network controlling adipogenesis in vitro. However, insight into how these networks control lineage determination and differentiation in vivo is currently missing.In this project we will apply advanced genome-wide approaches combined with in vivo model systems and single-cell technologies to characterize the genomic signatures and transcriptional network that is responsible for specific preprogramming of primary adipocyte progenitor cells from different adipose depots as well as the in vivo differentiation of these into mature brown and white adipocytes. Furthermore, we will determine the role of metabolic signals derived from lipolysis in driving the transcriptional programming of mature adipocytes towards a brown-like program in response to β–adrenergic signaling.
2017	Genome integrity maintenance and its cross-talk with diverse cellular stress response pathways: Mechanisms and relevance for cancer	3.900.000	Our cells are constantly exposed to diverse external and intracellular stressful stimuli which, if not adequately dealt with, may contribute to or cause pathological changes leading to cancer or other grave pathologies. While response mechanisms to genotoxic stress (DNA damage), proteotoxic, mechanical and ribosome biogenesis stresses have been studied individually, little is known about their functional cross-talk and potential synergistic impact on cells. Taking advantage of our expertise in the DNA damage response (DDR) field and links with other stresses emerging from our recent work, here we propose to employ a range of biological models, experimental approaches and methods to examine the interplay between the DDR and other stress response pathways in normal versus cancer cells. These analyses should provide novel mechanistic insights into coordination of stress responses in human cells and identify targetable vulnerabilities and drugs potentially exploitable in cancer treatment.
2017	Inactivation of specific DNA repair pathways and treatment with chemotherapeutic agents induce characteristic,	2.592.000	DNA repair pathway aberrations significantly contribute to the genomic instability underlying cancerous development. In fact, the actual DNA repair pathway aberration in a given cancer has a profound impact on the biology of the tumor and in cases it also determines

	clinically applicable mutational signatures		the efficacy of a particular genotoxic therapy. Therefore, developing reliable methods that determine the types of DNA repair pathway aberrations and mutagenic processes present in a given tumor will have a significant impact on selecting the most effective therapy in various cancer types. Next generation sequencing offers an efficient tool to achieve this goal by detecting distinct mutational signatures in the cancer genome. In this proposal, we will create a compendium of mutational signatures induced by either chemotherapeutic agents or specific DNA repeair pathway aberrations. This work will lead to highly improved personalized cancer therapy with genotoxic agents.
2017	Genomic analysis of Metastatic Breast Cancer - Genome-MBC	2.315.520	Genomic analysis of metastatic breast cancer – Genome-MBC is a study in the field of personalized breast cancer diagnostics and treatment. Increasing access to targeted therapies calls for increased knowledge and understanding of the evolution from primary tumor to distant metastasis and for better tools for detecting and characterizing metastatic lesions. In Genome-MBC we propose to use global sequencing of circulating tumor DNA from plasma (liquid biopsy) by whole exome sequencing followed by deep targeted sequencing of suspected somatic mutations in the individual patients. By this approach we hope to achieve a more optimal detection and characterization of the metastatic process most likely leading to improved individualized diagnostics and evaluation of treatment response and likely also to better information about prognosis and choice of treatment.
2017	Unraveling a new potential tumor suppressor function for CtIP	2.592.000	Background: DNA Replication stress (RS) challenges genome stability and requires a protective response to suppress tumor development. Aim: We will characterize a new important function for the CtIP protein to counteract RS. This includes investigating breast cancer-associated CtIP variant for loss-of-function in the RS response. Supporting, preliminary data: We have recently identified a new role for CtIP to suppress RS. Our preliminary data suggest that CtIP may be required for RAD51 function, which is a crucial part of counteracting RS. Importantly, our preliminary work also suggest that breast cancer-associated genetic variant in CtIP lead to aberrant RS response, which underlies a key part of the proposal.Expected outcome:

			We will unravel the new CtIP-RAD51 pathway in RS, which is likely to display a tumor suppressive function. Further, our protein function based research strategy can be utilized for improved counseling, diagnosis and treatment for patients.
2017	Combined effects of maternal-child genetic profile and early environmental exposures on childhood obesity	1.695.507	Obesity in childhood and adulthood is hard to reverse, and we need search for possibilities for preventing obesity early in life to inform the development of future, posisbly individually targeted, prevention. We will examine the combined effects on the risk of childhood obesity of maternal and child genetic predisposition to obesity, probed by GWAS-based gene score while taking account the genetic transmission. We will further examine the interaction of these gene scores with known environmental risk factors operating in early life, such as maternal smoking during pregnancy, socioeconomic position, excessive gestational weight gain, and short duration of breastfeeding. Analyses will be based on already genome-wide genotyped data on three groups of mother-child pairs from the Danish National Birth Cohort; obese mothers and their children, obese children and their mothers, and, as controls, randomly selected mothers and their children.
2017	EcoTech: From ecology to technology - Unraveling of the bioactive potential of marine bacteria	5.882.456	To enable treatment of microbial infections in the future, we must find new compounds that can combat antibiotic resistant microorganisms. Nature continues to be our best source of new bioactive compounds and the purpose of this project is to exploit a hitherto sparsely research source, the marine microorganisms.Genome analyses show that they harbor a large number of so-called silent (unexpressed) gene clusters likely encoding bioactivity. Here we will use natural marine polymers (chitin, algal compounds) and natural marine stressors (antibiotics) to induce the silent clusters. The project is cross disciplinary and is based on expertisein bacterial physiology, marine ecology and natural product chemistry. Our tool box contains genome and transcriptome analyses as well as LC-MS/MS and NMR measurements. The project strategy and compounds found will be useful for society's future combat against infectious agents and can constitute a business area for the biotech and pharma industry

2017	Non-obvious antibiotic resistance genes in therapy and diagnostics	5.384.024	Antibiotic resistance is a growing problem despite extensive research in the field. We propose that antibiotic resistance involves not only the traditional and well-characterized resistance genes but a hitherto unknown class of non-obvious resistance genes. These are bacterial genes that upon mutation allow traditional resistance elements to be establish and maintained. With massive numbers of bacterial whole genome sequences being available we now have a golden opportunity to identify such non-obvious resistance genes and study their impact. We will develop tools to identify them, validate their contribution to resistance and explore the diagnostic possibilities with the SME 1928diagnostics. The project builds on two large strain collections for which genome and resistance data are available and behind the proposal are researchers with strong expertise in antibiotic resistance, bacterial physiology and bioinformatics located at KU, DTU, Rigshospitalet and Statens Serum Institut.
2017	ActFun: Activation of Silent Gene Clusters for Secondary Metabolite Production in Fungi using a Synthetic Biology Approach based on CRISPR/Cas9	1.953.449	Fungi have the potential to produce a large number of unknown secondary metabolites, SMs. Since SMs may be toxic, we are likely exposed to unknown risks by ingesting contaminated food and by breathing air in buildings that are infested by fungi. On the other hand, unknown SMs constitute an enormous pool of potential drugs that await discovery. Uncovering the hidden potential for fungal SM production is therefore highly desirable. This task is difficult, as many genes involved in SM production remain silent under laboratory cultivations. The majority of all SMs therefore remain to be discovered, even in model fungi like Aspergillus nidulans. In this pioneering project, we propose to construct and use synthetic CRISPR/cas9 derived transcription factors to specifically activate silent genes by a method that allows multiplexing. The successful project will demonstrate that the method is feasible and versatile by identifying a number of new compounds as well as biosynthetic pathways.
2017	Methods to more efficiently analyse genetic data.	5.592.581	There is great promise in personalised medicine. For example, given an individual's genome, it should in theory be possible to accurately predict their risk of developing diabetes; or if the individual is diagnosed with epilepsy, we should be able to use their genetic information to determine best course of treatment. But despite the ever-increasing amounts of genetic data

			being produced, we currently lack statistical tools to efficiently analyse these data and make personalised medicine a reality.In this fellowship I will develop novel statistical methods for analysing genetic data, then apply these methods to large-scale datasets. I will tackle important problems in medical genetics, such as constructing models to predict disease susceptibility and severity, and classifying heterogeneous neurological diseases. Finally, I will make all my methods freely-available, so other researchers can apply them to their datasets.
2017	FUNGAL FLOW-BATTERY FOR STORING SUSTAINABLE ENERGY	5.477.743	Exploitation of renewable energy depends on fast, cheap and reliable energy storage devices and quinone based redox flow batteries is a promising technology for achieving this goal. However, the quinones that are currently being used are derived from petrochemical production, which goes against the sustainable concept of renewable energy. With the project we will demonstrate that filamentous fungi offer a solution as they produce numerous quinone pigments in high quantities. We will address the most important challenges that must be overcome before a possible commercialization. Initially we will identify the four most promising naturally occurring fungal quinones through in silico analyses and lab scale tests. Extraction procedures and flow battery conditions will then be optimized for each quinone. The responsible gene clusters will be identified through genome sequence analyses and produced heterologously in gram scale to be used in a demonstration flow battery.
2017	Data Science on the Desktop	5.867.899	Extracting knowledge from massive amounts of data is at the heart of applications as diverse as business decision making, genome analysis, or movie recommendations. The data mining models and algorithms used to extract knowledge, however, still largely assume a single-threaded processing model. This is in stark contrast to hardware surrounding us in any standard computer. Today, multi-core CPUs and powerful graphics cards (GPUs) are part of any laptop or desktop computers. In this project, we exploit the enormous potential for fast and scalable data science by creating algorithms that make use of the compute power in multi-core CPUs and GPUs: (i) we abstract principles for translating data mining problems from single-threaded to multi-threaded algorithms (ii) study

			strategies for sharing work between CPUs and GPUs, and (iii) implement and empirically evaluate prototypes to demonstrate dramatically improved runtime efficiency on standard desktop computers.
2017	Centres of Evolution: A Genomic View on Speciation in Eastern Arc	5.876.445	The Eastern Arc Mountains in Kenya and Tanzania have iconic status to evolutionary biologists. Studying the processes behind the high concentration of endemic flora and fauna in this biodiversity hotspot can provide fundamental new insights into a cornerstone in evolutionary theory: Speciation. It has been hypothesised that cycles of expansions and contractions of the ancient montane forests have acted like large-scale speciation 'pumps' by governing repeated isolation of populations overevolutionary time scales. By examining deep time genomic signatures in species distributed across the Eastern Arc, this project aims to test the principles behind this theory. Full de novo genome sequencing of 25 species representing birds, snakes, chameleons, spiders, and millipedes will allow for a detailed comparative investigation of evolutionary split times, population size trajectories, and selection processes that control speciation in this ecoregion.
2018	BACE1 cell surface proteostasis as endocrine nexus connecting metabolic disease with development of neurodegenerative disorders	2.592.000	A brief summary:Metabolic derangements like obesity and type 2 diabetes (T2D) often coincide with neurodegenerative disorders like Alzheimer’s Disease (AD) in humans and mice. Based on published reports and own unpublished findings, we and others have shown that both conditions are characterized by an overexpression of the surface protease BACE1 in peripheral organs (T2D) or the brain (AD). We hypothesize and present first evidence from CRISPR/Cas9-engineered cell systems that BACE1 cell surface proteolysis in muscle and fat leads to secretion of endocrinely acting cleavage products which in turn can deteriorate metabolism in recipient cells. This proposal aims to determine the molecular nature of these BACE1 peptides using novel proteomic approaches, assess the physiological relevance of these peptides in vitro and, via the generation of tissue-specific BACE1 overexpression mice, interrogate the consequences of BACE1 proteostasis to metabolism and AD pathogenesis in vivo.

2018	Understanding the role of TET2 in normal hematopoiesis and how its loss of function can lead to AML	2.592.000	Acute myeloid leukemia (AML) patients frequently harbor mutations in the gene encoding the DNA demethylase TET2. In this project, we will addres the molecular mechanisms by which TET2 functions in normal cells and how its loss contributes to the development of AML. We will take advantage of our recent technical advances to generate high-fidelity genome-wide datasets of TET2 binding in normal and malignant blood cells. Furthermore, we will unweil the mechanisms governing TET2 recruitment to its genomic binding sites through the delineation of the minimal region of TET2 required for DNA binding, and by the identifications of proteins required for TET2 recruitment to DNA. Finally, we will use a combination of mouse models and primary human AMLs to understand how loss of TET2 changes DNA methylation patterns and gene expression. We expect the project will yield mechanistic insights into how TET2 loss promotes AML and have implications for guiding new therapies for AML.
2018	UNDERSTANDING HOW ANEUPLOIDY DRIVES TUMORIGENESIS	1.972.800	Defects in the maintenance of genome stability underlie a number of debilitating pathological conditions in humans, including cancer, neurodegeneration, infertility and some forms of anemia. Understanding how cells maintain a stable chromosome complement is, therefore, fundamental to human health. One of the most common forms of chromosome maintenance defect is the generation of aneuploid cells that possess an abnormal chromosome number. Aneuploidy is a very common feature of solid cancers, as well as being a key cause of spontaneous abortion and birth defects. In this project, we aim to understand in mechanistic detail how defects in cell division generate aneuploidy progeny. We will build on exciting new preliminary data implicating the BLM and RIF1 proteins in controlling, via protein phosphatase 1, the checkpoint that determines whether it is safe or not for cells to complete mitotic cell division.
2018	Regulation of stem cell biology by nuclear RNA decay systems	2.591.347	Embryonic stem cells (ESCs) are central tools in the field of regenerative medicine. Consequently, a detailed understanding of mechanisms, providing ESC pluripotency, yet maintaining differentiation ability, is highly warranted. It is well established that a network of key transcription factors (TFs) and chromatin modifiers control these processes. However, with new data, we now also link nuclear RNA decay pathways: i) the

			expression of pluripotency-specific TFs, including Nanog, Sox2 and Klf4, is severely affected upon knock out of the nuclear exosome targeting (NEXT) complex, and ii) normal embryoid body differentiation is impaired in poly(A) exosome targeting (PAXT) deficient cells, showing retained expression of Oct4 and Sox2. Such regulation is unprecedented in ESC biology and we wish to combine genome-wide interrogation of RNA levels, transcription activities and chromatin states to define the molecular events linking nuclear RNA decay to ESC self-renewal and differentiation.
2018	Unravelling the role of Vitamin C deficiency in vascular remodeling	2.554.560	Vitamin C (vitC) deficiency is strongly associated with increased risk of cardiovascular disease (CVD), but a causal mechanism has not been established. VitC promotes vascular remodeling and is an important epigenetic modifier of cellular differentiation. Dysregulation of vascular smooth muscle cell (SMC) plasticity is a hallmark of CVD development. Based on our preliminary data, we propose that vitC deficiency impairs vascular SMC remodeling in three ways: 1) inadequate acute response to vessel injury, 2) attenuated proliferation and syntheses of extracellular matrix, and 3) delayed differentiation due to inactivation of epigenetic regulation of SMC differentiation. We speculate that this impaired response directly increases the risk of CVD development in the ~15% of adults with vitC deficiency. Using ex vivo and in vitro models of human vascular tissue and SMCs, as well as a guinea pig vascular injury model, this project unravels the impact of vitC deficiency on vascular remodeling.
2018	Myeloid suppressor cells in regulation of inflammatory brain disease	2.587.680	We will study mechanism underlying suppression of neuroinflammatory disease by myeloid suppressor cells, induced by phagocytosis within the central nervous system (CNS). Myeloid subsets that have phagocytosed a fluorescent particulate ligand will be defined using surface phenotype markers, and we will examine the mechanism of EAE suppression by transfer of such subsets to focal models of cortical pathology. We will use cell sorting and transcriptomic analysis to define pathways involved in disease modulation and we will screen candidate pathways by small inhibitory RNA and CRISPR technology, using particulate ligand for specific delivery to phagocytic myeloid cells in the CNS. We will track myeloid cells into the CNS and examine their

			interaction with parenchymal microglia as well as their further migration to draining lymph nodes and whether they tolerize autoimmune T cell responses. We predict that our findings will show new ways to treat and prevent autoimmune disease.
2018	Identification and functional characterization of novel regulators of genome maintenance	2.558.383	DNA is the blue print of life; thus preserving its integrity is vital for organismal development and health. The integrity of DNA is continually threatened by external and internal agents, and failure in repairing damaged DNA can have catastrophic consequences. Indeed, genomic instability is a leading cause for human diseases such as cancer, and premature aging. DNA damage repair involves local accumulation of many proteins at the sites of damage; however, our knowledge of the DNA damage repair networks is incomplete. The goals of this project are to apply innovative proteomic approaches to systematically map the networks of DNA damage repair, to discover novel factors in DNA damage repair, and to characterize their cellular functions and molecular mechanisms. A successful outcome of this project will advance our understanding of the molecular details of the genome maintenance pathways, yield new insights into the diseases mechanisms, and may discover novel therapeutic targets.
2018	Unraveling the Role of Type IV Collagen in Cardiovascular Disease	800.640	In recent years, genome-wide association studies have found the COL4A1/A2 locus encoding type IV collagen (COL4) to be associated with cardiovascular disease (CVD) and having a marked effect on disease risk comparable to loci encoding regulators of plasma cholesterol. In the proposed project COL4 quantification in tissue and plasma from patients with known COL4A1/A2 genotype and CVD status will enable us to determine the predictive value of COL4 and provide the missing link between genetic risk and CVD. Col4a1/a2+/- mice will serve to test the hypothesis that inadequate COL4 affects distinct smooth muscle cell processes resulting in build-up and destabilization of atherosclerotic plaques. Moreover, we will demonstrate proof-of-principle for a treatment strategy to counteract these detrimental effects. The proposed project will be the first to investigate causality between the highly replicated COL4A1/A2 risk locus and CVD, and unravel the role of COL4 in atherosclerosis.

2018	Deconvoluting tumour clonality to guide drug targeting in Glioblastoma	2.587.680	Glioblastoma Multiforme (GBM) is a primary brain tumor with dismal prognosis. Recent studies have shown that chances for treatment efficacy in early phase clinical trials can be increased by selecting patients based on genomic analysis. This novel approach for choosing specific targeting drugs for the individual patient has not yet been fully explored in GBM, but has the potential to improve patient outcome.In the current pilot project, we will pursue novel approaches combining a multifocal tumour sampling strategy from primary and recurrent GBM with whole-genome sequencing and state of-the-art cancer genomics, with the aim to characterize the intra-tumour heterogeneity to identify molecular targets of GBM. Our specific objectives:1) Characterize the clonal hierarchy of GBM cells2) Identify drug-targets and allocate patients to a phase I trialIt is a first step towards an individualized treatment program for GBM with the potential to improve GBM patient survival.
2018	Genetic and inflammatory links to Restless Legs Syndrome in a population of 40,000 otherwise healthy individuals	1.831.748	Restless legs syndrome (RLS) is a common neurological sensorimotor disorder linked with major comorbidities. Unfortunately, the etiology of the disorder is unknown making adequate prevention and treatment challenging.RLS is common in patients with inflammatory diseases and has been linked to iron deficiency.We have evaluated RLS symptoms in 40,000 Danish blood donors (6% with RLS). From these 40,000 we have also collected genome-wide data (650,000 genetic variants), ferritin levels, lifestyle- and demographic data. Now we need to collect data on inflammatory markers.Hypothesis: Onset of RLS is affected by host genetic background, inflammation, iron levels, and lifestyle.Specific aims - to demonstrate:1 New genetic variants associated with RLS2 The impact of inflammation on RLS3 The impact of microbial translocation on RLS4 The role of hepcidin and iron homeostasis in RLSThe interaction with genetic and environmental factors will be investigated where appropriate
2018	Elucidating the Receptor Molecular Machinery - An Atlas of Functional Sites	2.560.983	G protein-coupled receptors (GPCRs) form both the largest family of membrane proteins and drug targets. GPCRs transduce biological signals across the cell membrane via a common molecular machinery. This

	Bridging Evolution, Structure and Mutations		project will analyse sequences, 3D structures and mutations to uncover receptor functional sites ligand binding, G protein coupling or activation. These functional sites will be corroborated with in vitro receptor mutagenesis, and linked to disease-causing natural mutations. An atlas of functional sites will be made available to the wide community through the GPCRdb database, along with web-based tools to design future experiments to further our knowledge. Hence, this project will advance our understanding of receptor (mal)function, and contribute towards stabilisation of GPCRs for generation of valuable new crystal structures, and design of tool compounds and drugs with tailored activity (e.g. stimulation vs. inhibition).
2018	Femtosecond time-resolved imaging of bimolecular reactions	5.539.532	The main scientific goal of this research program is to image molecules with femtosecond time resolution during fundamental binary reactions including reactions used in organic chemistry and reactions occurring naturally in DNA. I will achieve this objective by applying femtosecond time-resolved imaging methods to molecular complexes formed inside nanometer sized liquid droplets of superfluid helium with a temperature of 0.38 K. The experimental and methodological basis for the research program has been constructed and developed during the project period of my current ERC advanced grant. This includes state-of-the-art helium droplet apparatus, several femtosecond laser sources and methods to control molecules and image their structure. The purpose of the proposal is to obtain funding for two PhD scholarships and corresponding running costs. I have already identified two very talented students with the motivation and intellectual skills needed to carry out the research program.
2018	The mutational signatures of aberrant DNA repair pathways in cancer and their potential clinical uses — an analysis of thousands of whole cancer genomes	2.584.800	Cancer is a genetic disease. It develops by random accumulation of mutations, a few of which impact cell biology. An early and limiting step in cancer development appears to often be perturbations of the DNA damage response (DDR), which is responsible for repairing DNA mutations. This induces a mutator phenotype, often including genomic instability, which allows cancer cells to evolve faster and more quickly acquire the traits needed for growth.We propose to study and characterize the mutational phenotypes associated with deficiencies in individual pathways of the system. We will study and characterize the resulting

			mutational signatures using a combination of established statistical methods and locally developed extensions and improvements. The analysis will be based on the largest available sets of whole cancer genomes, which we have access to and familiarity with through our participation in the a project under the International Cancer Genome Consortium.
2018	Plant recognition of the bacterial Type-3 Secretion System (T3SS) for activation of immunity	2.592.000	Bacterial pathogens cause serious diseases in plants and animals. Pathogenic Gram-negative bacteria share the Type-3 Secretion System (T3SS) as an essential structure for their ability to cause disease. Our unpublished findings indicate that many wheat cultivars can recognise T3SS. T3SS recognition has not previously been described in plants. We have mapped the responsible genome interval to seven genes of which two encode receptor-like kinases (RLKs), similar to receptors that recognise pathogen molecules. We wish to test which of the two that mediates the recognition. RLKs function in pairs and we will identify the other RLK partner of the complex using a pull-down assay. We have already data indicating that wheat has functional and non-functional allele variants of the RLK gene. Markers for those genes will be useful in plant breeding towards disease resistance. Therefore, we also aim to search for the T3SS recognising RLKs in other species and allele-specific markers for those.
2018	Revealing genomic composition of apple cultivars – targeting future breeding	1.609.346	To feed a growing global population and meet future climate changes there is an urgent demand for new plant cultivars. New cultivars arise from breeding that exploits important genetic variations kept in gene bank collections. Lacking knowledge of the genetic resources kept in gene bank collections is however a major obstacle for the potential utilization of germplasm resources. The aim of the study is to access genetic diversity and pedigrees in 1,000 world-wide important apples and 300 local, Danish apple cultivars. Using high-density genome-wide SNP markers we aim for identifying genomic regions in apple that have been conserved for several generations during apple breeding and are critical for successful apple cultivars. We will uncover the set of genes that each individual has inherited from each parent (haplotype) and reveal the degree of inbreeding in major apple cultivars. The findings will provide crucial knowledge for future improvement of apple cultivars.

2018	Mechanisms maintaining ribosomal DNA integrity and their role in cancer	4.099.347	We will investigate how a specialized, repetitive and highly unstable genomic region, namely the ribosomal genes, is maintained and how its instability promotes tumorigenesis. The ribosomal DNA (rDNA) cluster in the cellular organelle called the nucleolus, in which a unique DNA damage response (DDR) has developed to meet the specific requirements of these intrinsically unstable sequences. To study this unconventional DDR we have established human cell models in which we utilize the CRISPR/Cas9 technique to generate site-specific DNA damage. Using these models we will conduct in-depth analysis of signalling and repair pathways using RNAi, protein labelling and mass spectrometry to understand how rDNA integrity is preserved. We will also examine nucleolar DDR proteins in specimens from progressive stages of breast cancer to determine the nucleolar contribution to the DDR-induced anti-cancer barrier and correlate rDNA instability with clinical outcome.
2018	Intelligent and Artificial Designer Cells & Organs for Regenerative Transplant Medicine (iART)	5.892.480	One unmet need in transplant medicine is the critical shortage of organs. Pigs are of great interest for xenotransplantation whereas the barriers of human immune rejection of xenografts and risk of infection have greatly blocked the application. The aim of this starting grant application is to fully overcome xenotransplantation barriers through transformative technologies. We propose to 1) optimize and develop CRISPR gene editing technology for multiplexed and efficiently tailoring the pig genome, and define the best genetic modifications overcoming immune rejection; 2) systematically study the pig endothelium and identify new targets of the tissue-specific immune rejection barriers; 3) generate a pig model resistant to all pathogens through genome recoding. We believe that this project will generate the next generation of technologies and pigs to combat organ shortage in transplant medicine.
2018	Elucidating the evolutionary consequences of cryptic genetic variation and phenotypic plasticity for fungal host-shifts	5.900.112	Identifying the factors that promote host-shifts of fungal pathogens is fundamental for our understanding of the emergence of new fungal infectious diseases in plants, animals and humans, but little is known about the underlying genetic factors. This project aims to develop the locust and lepidopteran larvae fungal pathogens Metarhizium acridum and M. rileyi, respectively, and the generalist insect pathogen M. robertsii as eukaryote experimental models to investigate the mechanisms

			behind eukaryote pathogen host shifts. Genome sequencing, gene expression analysis and phenotypic bioassays, will be used to analyse artificial host shifts created in the laboratory. This will clarify whether pathogen plasticity after a host shift follow similar and potentially predictable evolutionary trajectories, and how standing genetic variation influence adaptive and non-adaptive expressional modifications that may lead to successful pathogen host-shifts.
2018	The role of novel susceptibility genes for insulin resistance in the pathogenesis of type 2 diabetes	103.100	The major aims of the project areAim 1: Detailed characterization of loci hypothesized to be involved in insulin resistance in human samples. We will characterize insulin resistance loci using detailed phenotypic information from large population-based samples assessed with dynamic measures of glucose and insulin metabolism, metabolomic, transcriptomic, epigenomic and proteomic profiling together with in silico data on gene regulation and transcription from public resources. Aim 2: In vitro studies of candidate genes. Based on findings from aim 1, we will prioritize potential causal genes for insulin resistance development for mechanistic studies using cell-based model systems. We will use CRISPR-Cas9 to knock out genes in adipocytes, hepatocytes and skeletal muscle cells, followed by various assays to study glucose, insulin and lipid metabolism, gene expression and metabolic pathways.
2018	TARGET:ABC – Targeted AntiBiotics for Chronic Obstructive Pulmonary Disease (COPD).A randomized, multicenter trial investigating the effect of antibiotic eradication of pulmonary infection with Pseudomas aeruginosa in patients with severe	1.322.258	Patients with severe chronic obstructive pulmonary disease (COPD) are at increased risk of pulmonary infections, and these exacerbations are the most important cause of hospital admission and mortality in COPD.Despite that Pseudomonas aeruginosa (P.aeruginosa) accounts for up to 20% of the bacterial exacerbations, it is unknown to what extent the bacteria plays a role in the clinical course of COPD.As of this, considerable uncertainty exists regarding the need for and approach to antibiotic treatment of Pseudomonas infections in thse patients.Target:ABC will be the first clinical randomized controlled trial to evaluate if targeted antibiotic treatment can eradicate P.aeruginosa and thereby reduce the risk of new exacerbations and improve the long-term prognosis of patients with COPD. Moreover, analyses of sampled Pseudomonas genomes will provide new and important

			insight and advance our fundamental understanding of infections in COPD.
2018	HappyFish: Understanding the role of the rainbow trout microbiome on growth and health in aquaculturally farmed fish	2.505.601	Aquaculture is the fastest growing food-producing sector worldwide with more than 50% of consumed fish being farmed. Key challenges for sustainable growth of the aquaculture industry are to improve fish health and feed efficiency as feed makes up >50% of the production cost, and to reduce the environmental burden of aquaculture. Recent studies in mammals have detailed how interaction between host and gut microbiota regulates metabolic and immunological pathways of the host and how such interaction can be modulated by genetics and environmental factors such as diet. The project aims 1) to establish rainbow trout as a model species for metagenome analyses in aquaculture research, 2) to establish a gene catalog of the trout gut microbiome, and 3) to decipher how feed and the gut microbiota interact in the rainbow trout with the view to develop novel feeds that improve feed efficiency and health, and thereby reduce the environmental impact of aquaculture.
2018	Were dogs and cattle domesticated in the Indus Valley? A palaeogenomic approach.	1.371.208	In early human history, the South Asian Indus valley was home to a complex social society that had an enormous impact on modern domestic animals, including dogs and the major cattle breeds of the developing world. Despite this importance, no ancient South Asian zooarchaeological materials from bovines or canids have been studied in the palaeogenomic context, leaving a large gap in our knowledge of two of the most economically important and popular animals on the planet. Through initiating a new collaboration between the Natural History Museum of Pakistan, Trinity College Dublin and The University of Oxford, this project will apply paleogenomics to the invaluable Pakistani zooarchaeological collections. By sequencing ancient nuclear genomes, I propose to both find the ancestors of zebu cattle, and clarify the role of South Asia in dog breeding and/or domestication, ultimately improving our knowledge of domestication in general, and the importance of ancient South Asia in particular.

2018	Hypothalamic plasticity in obesity	5.832.449	Genetic association studies have identified hundreds of genetic loci associated with obesity, however, our limited capacity to link these loci to underlying mechanisms has hampered efforts to translate these findings into testable biological hypotheses. This proposal leverages large-scale human genetic and mouse gene expression data to improve our understanding of the neuronal underpinnings of obesity. We hypothesize that neuronal plasticity is reduced in obesity and leverage the developing hypothalamic arcuate–median eminence complex as a robust model to characterize plasticity-driven neuronal maturation. Our overarching aim is to investigate whether altered hypothalamic plasticity contributes to obesity pathogenesis. Towards this aim, we will perform large-scale single nucleus RNA sequencing across more than 80,000 brain cells and integrate the single nuclei transcriptomes with obesity genome-wide association study results from more than 700,000 individuals.
2018	Admixture between wild and domestic bovines: implications for genetic improvement and conservation	5.887.121	I propose to use whole genome sequencing and population genetics to investigate the genome-wide landscape of introgression between a wild (banteng) and a domesticated (cattle) bovine. This will be compared with other sources of information including phenotype measurements, transcriptomics and comparative genomics to assess the potential for genetic improvement of cattle by introducing variants that already exist in wild species. We will furthermore investigate the domestication of the banteng, which occurred independently of the domestication of cattle. Lastly, the phenomenon of genetic swamping of the wild banteng and its conservation status will be assessed.
2019	Regulation of N-WASP in N-WASP dependent epigenetic control of skin inflammation	2.769.921	The Cdc42 effector N-WASP is known for its ability to polymerize actin in the cytoplasm. Recently, we demonstrated that nuclear N-WASP controls epigenetic regulation of IL-23 expression. In the absence of N-WASP in keratinocytes, a chronic IL-23 dependent skin inflammation develops in mice. In humans, N-WASP is highly phosphorylated in psoriatic lesions, which in mice corresponds to inhibition of nuclear N-WASP function and increased IL-23 expression. These data suggest that this pathway might be of relevance for psoriasis, a frequent chronic skin inflammation dependent on IL-23. Aim of this project is to better understand how nuclear

			N-WASP function is regulated (S/T and Y phosphorylation, Cdc42, UV irradiation), and to test whether the best-known function of N-WASP, actin polymerization, is involved. To this end we will generate multiple point mutants of N-WASP and analyze them with respect to epigenetic regulation of IL-23 expression in in the human HaCaT keratinocyte cell line.
2019	Molecular dissection of the prostate cancer tumor and immune microenvironment: Clinical implications for prediction of disease aggressiveness	2.851.200	We aim to develop a new molecular classification tool for improved risk stratification of prostate cancer (PC), also taking into account the tumor microenvironment (TME). We will identify stromal, immune and epithelial subtypes by unsupervised class discovery using RNAseq data of bulk PC tissue samples from 300 patients. Long clinical follow-up data allows us to delineate associations between the subtypes and PC aggressiveness. We will train new prognostic gene expression signatures and validate these in large independent PC patient cohorts using NanoString technology. Stromal, immune and epithelial cell interactions will be investigated in depth by digital pathology and single-cell RNAseq. Potential new cancer driver genes will undergo functional studies using CRISPR-cas9 and sophisticated 3D co-culture systems. We expect to identify new and more clinically relevant molecular subtypes of PC, reflecting the interplay between malignant PC cells and stromal and immune cells in the TME.
2019	Unfolding roles of B-cell receptor signaling in B-cell tolerance to cancer drugs	2.671.528	Inherent or acquired tolerance to chemotherapies is a fundamental challenge in cancer, leading to relapsed or refractory disease. Development of improved and personalized treatments rely on technologies that can unveil genes contributing to drug resistance. Using forward genetic screens based on CRISPR-based gene knockout, we have identified unique sets of candidate genes involved in acquired resistance of B-cells to several drugs that are currently used for treatment of B-cell cancer. Intriguingly, genes mapping to B-cell receptor (BCR) signaling pathways are prevalent, leading to the hypothesis that modulated BCR signaling plays a key role in B-cell drug resistance. To prove this hypothesis, we will separately knockout each of these genes (using CRISPR) in cancerous B-cell lines and study the impact on a wide range of cellular parameters including emergence of cross-resistance. Also, we will

			develop 'two-hit' CRISPR screens unveiling gene networks that contribute to drug resistance.
2019	Pseudoexon activation an underreported disease mechanism with a unique potential for tailored therapy - In vivo investigation of pseudoexons as disease cause and modifiers of gene expression.	2.880.000	We have found that introns in all genes harbor pseudoexons (PEs), which are non-functional exons that disrupt the normal mRNA, when included by splicing. This causes degradation of the mRNA and PEs are therefore not detected. Intronic SNPs/mutations can affect splicing regulatory elements (SREs) and activate PE inclusion to cause disease. We believe that this is a underreported disease mechanism. Also numerous wild-type PEs are highly included and decrease normal gene expression. Therefore gene expression can be significantly increased if PE inclusion is blocked using splice switching oligonucleotides (SSOs). We will treat patient cells and mouse models in vivo with SSOs to block inclusion of disease causing PEs and to explore the potential of wild-type PEs as regulatory switches to increase gene expression.Finally, we will establish a www searchable PE database and software tools allowing clinicians and researchers to evaluate the pathogenicity of intronic sequence variants.
2019	The role of the SOX3 topological associated domain in complex spastic paraplegia: Disease modelling using induced pluripotent stem cells	2.877.731	Hereditary spastic paraplegia(HSP) is a rare condition and more than 55 causative genes have been identified.We have identified a disruption of the topological associated domain of SOX3 on the long arm of the X-chromosome representing a novel mechanism for developing HSP.HSP and other complex genetic disorders do not fully recapitulate their phenotype in rodent models. Therefore, we will use iPS cells to model the disease without the use of transgenic animals. Preliminary data suggests that SOX3 is dysregulated in neuro-progenitor cells established from patient IPS cells and we hypothesise that such dysregulation causes failure in either neuronal differentiation or maintenance.Chromosome conformation capture techniques such as Hi-C and RNA sequencing will be used to study the impact of the genetic alteration and CRISPR/Cas9 technology will be used to correct it in order to restore normal DNA structure and possibly regain normal regulation of SOX3 and downstream targets.

2019	Deciphering the molecular mechanisms underlying the emergence of high-risk MDR/XDR clones of Pseudomonas aeruginosa	2.879.435	The increasing prevalence of nosocomial infections produced by multidrug-resistant or extensively drug-resistant Pseudomonas aeruginosa is frequently linked to a limited number of so-called epidemic 'high-risk clones´ (HiRiCs) that are widespread in hospitals worldwide. The genetic factors that enable particular clones (but not others) to emerge as HiRiCs is currently not known. The purpose of the proposal is to test the hypothesis that genetic variations in two specific genetic systems (the CRISPR-Cas system and the O-antigen biosynthesis genes) underlie the emergence of HiRiCs. We suggest that these variations alter virulence, antibiotic susceptibility, and the ability to acquire foreign genetic material thus setting the stage for HiRiC evolution and success. With a better understanding of the unique adaptations of these important clones, the medical community is more likely to stop their continuing spread and to predict/anticipate the next waves of HiRiCs.
2019	Astrocyte-related repair versus damage in the brain of patients with progressive multiple sclerosis	1.994.560	Our data suggest that astrocytes with specific gene signature contribute to remyelination in brain lesions of progressive multiple sclerosis (SPMS); and this is associated with cytokine receptor TGFb-R2 in relation to transcription factor FoxF2. By glia-enriched single nuclei RNA sequencing of brain lesions, we will examine if astrocytes expressing TGFb-R2 and FoxF2 have specific gene signatures restricted to repair. We will also examine if the absence of TGFb-R2 in astrocytes impacts experimental de- and remyelination in conditional KO mice; and investigate the effect of absent FoxF2 during de- and remyelination in FoxF2fl/fl mice. We will also examine the genome-wide binding sites of FoxF2 in astrocytes to identify its gene targets related to brain repair. Our state-of-the-art translational approach will generate important novel data about astrocyte role in CNS regeneration. The repair-related cell signatures and FoxF2 targets may identify molecules for potential SPMS treatments.
2019	RSK and MSK protein kinases: drivers and drug targets in liver fibrosis	2.872.800	Blocking activation of hepatic stellate cells is now considered a principal strategy for new treatments of fibrosis in chronic liver disease of any etiology (NASH, alcoholic/toxin, viral), a major cause of mortality world-wide. We have obtained strong evidence that stellate cell activation can be blocked by combined inhibition of the protein kinases RSK and MSK using a dual RSK/MSK

			inhibitor we have developed. We hypothesize that dual RSK/MSK inhibitors represent a novel, effective strategy for treatment of liver fibrosis and chronic liver disease, which we will test here:AIM 1. Establish RSK and MSK kinases as collaborative drivers of liver fibrosisAIM 2. Test dual RSK/MSK inhibitors for therapeutic intervention in models of liver fibrosisThis will be conclusively addressed by testing if stellate cell activation can be blocked by CRISPR knockout of RSK/MSK in mouse and human stellate cells and if RSK/MSK knockout or dual pharmacological inhibition blocks liver fibrosis in mice
2019	Defining the cellular role of K27 linkage-specific protein ubiquitylation	2.874.815	Conjugation of the polypeptide ubiquitin (Ub) to substrate proteins is a dynamic post-translational modification involved in the regulation of most cellular processes. Eight distinct Ub chain types, each of which specify important but distinct functional outcomes, are formed in cells, however the roles of some Ub chain topologies, in particular K27-linked chains, remain poorly understood. This project aims to establish unique tools allowing for the targeted disruption and selective enrichment of K27-Ub chains, which will be applied in both focused and systems-wide studies to obtain crucial first insights into how K27 ubiquitylation functions in genome maintenance and other biological responses in human cells. Representing the first foray into the cellular role of protein modification by K27-linked Ub chains, this project will lay a strong foundation for rectifying a major current knowledge gap in Ub-mediated signaling, a central regulatory principle in eukaryotic cell biology.
2019	Can evolutionary age of genes be used to predict their function?	2.854.800	We live in the age of genomics, with over 160 000 sequenced microbial genomes. And yet, over 25% of all genes are of unknown function. This means that the current methods for predicting gene functions need improvement. Here we propose that evolutionary age of genes can help to predict their function. We teamed up with one of the founders of genomic phylostratigraphy, a method that correlates emergence of new genes to macro-evolutionary transitions. In a pilot study, we predicted a role in sporulation for a number of uncharacterized genes of Bacillus subtilis. An experimental validation confirmed a role in sporulation for 43% of the predicted candidates. This is a very high score, not matched by any of the available predictors.

			Now we want to check if this method can be used more generally: can it predict functions in other developmental phenomena, can it be used for different species and can it help us identify genes involved in production of new antimicrobials.
2019	Mechanisms of nuclear envelope rupture and repair in eukaryotic cells	2.000.000	The nuclear envelope of eukaryotic cells physically separates the chromosomes from the cytoplasm and shield and protect the genome integrity. Deformation of the nucleus by mechanical means such as under cell migration, can cause rupture to the nuclear envelope resulting in DNA damage and uncontrolled leakage of molecules between the nucleoplasm and cytoplasm. Cells overcome and survive nuclear ruptures by actively resealing the nuclear envelope, which involve nuclear membrane bending and scission facilitated by the Endosomal Sorting Complex Required for Sorting (ESCRT) III complex. Our recent research suggest that regulators of plasma membrane repair such as annexin proteins and actin cytoskeleton also play a role in the nuclear repair response. However, despite the essential function of nuclear repair in maintaining genome integrity, repair mechanisms are, yet, poorly characterized. This project aims at revealing fundamental mechanisms of nuclear envelope repair in eukaryotic cells.
2019	Stability and unfolding of G-rich DNA roadblocks	2.879.502	Helicase enzymes have major roles in genome maintenance and repair as they unwind structured nucleic acids, making them accessible for various cellular processes. In addition to its B-DNA form, cellular DNA is able to form other DNA secondary structures, which contribute to making DNA highly dynamic, but can act as roadblocks for DNA metabolic processes. In this project, we aim at elucidating the role of the Werner helicase, which is linked to the Werner syndrome, in resolving G-rich DNA structures, for which the protein is believed to have high affinity. Understanding transactions between these DNA structures and Werner is important for how helicases preserve genome homeostasis. As these processes are highly dynamic, we will use single molecule tools to dissect individual events and yield a direct observation at high spatial and temporal resolution of nucleic acids proteins interactions vital to cellular health.

2019	Some like it cold: industrially relevant low-temperature enzymes	2.879.921	Enzymes find widespread industrial use as sustainable biological catalysts. Enzymes from cold-active organisms show increased activity compared to meso- and thermophilic counterparts and their usage (particularly in food and laundry) would lower enzyme dosage, processing time and energy consumption and reduce inactivation costs. We will data mine recently obtained metagenomic data from >120 cryophilic bacteria isolated from permafrost and able to grow from <-10°C to >30°C to discover novel enzymes with broad activity at 0-20°C. Starting with primary metabolic enzymes as a test case, selected enzymes will be subjected to structure-function studies (mutagenesis in combination with enzymatic, biophysical and structural techniques) to elucidate the basis for cold activity and to increase their stability. The long-term goal is to establish a general approach to develop robust, cold-active enzymes to facilitate industrial processes operating at low temperatures.
2019	CRISPRcomp: Computational modeling and assessment of CRISPR off-targets	6.189.470	The CRISPR technology has opened up for a huge range of applications in health care, research, biotech and medical industries. However, off-target effects on unintended genomic loci occur and can do great harm and lead to misinterpretation of results. While finding off-targets experimentally is costly we will bring computational off-target prediction to a whole new level and overcome the limitations of current methods, which are based on machine/deep learning and ignore explicit information of the e.g. guide RNA folding. We take outset in a recent energy binding model (from nucleotide duplex interactions), integrate it with a deep learning framework, while using generated chromatin accessibility data and corresponding off-target data. In contrast to current deep learning strategies, we will include convolution over the RNA base pair probabilities as well. Our deep learning hybrid scheme for CRISPR off-target prediction will be validated in cell lines and a mouse model.
2019	Tuberculosis risk factors and transmission dynamics analyzed by nationwide register data and whole genome sequencing	2.455.037	In tuberculosis (TB) low-incidence countries such as Denmark, there are still extraordinary high rates of TB in specific vulnerable and hard-to-reach risk group populations where TB control remains challenging. We will take advantage of comprehensive nationwide Danish register data and Mycobacterium tuberculosis (Mtb) strain collections to describe the molecular

			epidemiological TB trends in Denmark focusing on risk factors and Mtb transmission dynamics. We will identify TB patients' demographic characteristics and risk factors, and causes of deaths, and specifically investigate the consequences of low socioeconomic status among TB patients. Data will be compared to a background population and we will use family cohorts for sub-analyses on socioeconomic status as an independent risk factor. Based on whole genome sequencing, changes in Mtb transmission dynamics will be described and linked to identified risk factors to point out new opportunities for TB control in Denmark.
	Sum:	**264.730.474**	

Final replies to 'Questionnaire on new genomic techniques to contribute to the study requested by the Council' (endorsed in the Joint Working Group of GMO competent authorities on new genomic techniques on 15 January 2020)

Questionnaire

Supplementary reply from the Ministry of Environment and Food of Denmark.

11. Could NGT-related research bring opportunities/benefits to science, to society and to the agri-food, medicinal or industrial sector?

The Danish authorities find that NGT-related research, especially using gene-editing, will bring opportunities and benefits to both science, to society and to the agri-food, medicinal and industrial sectors. It is our impression that, the strict legislation on GMOs in the EU prevents to a large extent the use of the new mutagenesis techniques and other NGTs.

o If yes, please provide concrete examples/data.

GM food-feed regulation and the deliberate release directive:

The DVFA and the DAA expects that gene-editing will contribute significantly to the much needed development of a sustainable and climate-neutral agriculture and food production in Europe. New green solutions are needed which will increase and optimize our food production from farm to fork with a significantly lower carbon footprint and a lower negative impact on biodiversity. NGTs such as CRISPR/Cas have the potential to develop new plants, microorganisms and animals, which will contribute to achieving these goals with a greater precision and a lower-risk profile than with traditional breeding techniques.

In plants, the new properties could be resistance against fungal, bacterial, and viral diseases in crops, such as wheat, barley and potatoes. Disease resistance is a valuable trait to reduce crop losses and chemical pesticide usage. Other possibilities are a reduced need for fertilizers, tolerance to soil salinity, drought, wet fields, and other climate stresses. Other possibilities might be perennial plants with deep roots with the ability to store larger amounts of CO_2 from the atmosphere, crops with improved food and feed quality traits such as wheat with reduced gluten content, vegetables with increased vitamin levels, oil crops with improved oil composition, and crops with a decrease of anti-nutrients. The new mutagenesis techniques are also expected to be important tools for *de novo* domestication of wild plants, breeding of semi-domesticated plants, and re-wildering of plants.

Microorganisms like fungi and bacteria are very important for a range of applications in feed and food production, e.g. as starter cultures or food supplement, as feed additives, or for a variety of fermentation purposes. Gene-editing techniques are expected to be important tools for the further development of strains with desired or optimized properties, or for inactivation of problematic genes, e.g. antibiotic resistance.

NGT-related research on microorganisms is the solution for a way forward to address some of the current challenges facing agriculture. Gene-edited microorganisms may have the potential to replace the use of harmful chemicals in agriculture, e.g. for plant protection and stimulation of growth. Targeted introduction

of phosphorus assimilation pathways in microorganisms used in agriculture can reduce the need for mineral fertilizers, and introduction of certain genetic sequences in microorganisms used in agriculture can improve crops' resistance to abiotic stress, e.g. heat.

Gene-editing in livestock breeding has the potential to improve agricultural productivity and animal health. However, some uses of gene-editing on animals may raise concerns about animal welfare or other ethics issues.

Examples involving published research on improvement of existing crops using NGT:

Genome editing has already shown a significant potential in generating disease-resistant crops and crops tolerant to drought and temperature, e.g.:

- Mildew resistance in wheat (Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu J-L. 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology 32, 947–951)
- Drought tolerance in maize (Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE. 2017. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnology Journal 15, 207–216)
- Increased N-uptake in rice (Lu Y, Zhu J-K. 2017. Precise Editing of a Target Base in the Rice Genome Using a Modified CRISPR/Cas9 System. Molecular Plant 10, 523–525)

A recent Danish report from Aarhus University ("Vidensyntese om nye planteforædlingsteknikker og deres effekt på dansk landbrug") gives an overview of the new techniques, including their potential benefits for Danish agriculture.

On-going Danish research projects on the use of NGTs in plants:

As described in the answer to question 8, a number of on-going Danish research projects use NGT to reach aims that could benefit both agriculture and society. This include projects to develop new crops by domesticating wild species and to improve existing crops.

Contained use directive

In Science genome-editing provides easy access to studies of gene function and gene phenotype relationships.

For society, The genome-editing technology may if employed right, ensure a smaller environmental foot print of the medicinal and industrial sectors. Furthermore, disease diagnostics may be revolutionised, and previously untreatable diseases may become treatable.

In the Industrial sector, the technology may minimize the polluting effects of materials as e.g. plastics, by allowing for production of biodegradable versions of otherwise very hard to degrade compounds. The technology may in addition provide means to lessen environmental footprint of production of e.g. biofuels and chemical compounds making the industrial sector less dependent on fossil fuels and chemicals. This is probably the sector with the highest potential for use of the technology.

For benefits within in the medical sector, the technology may improve treatments by improving and facilitating identification of disease related genes. The technology may also allow development of better

drugs through improvement of disease models for better translation to humans. This may lead to the use of a lesser number of animals used for pharmacological testing. The NGT technology may in addition provide new options for diagnosis and/or cure of the disease in patient groups where the disease is caused by a gene defect in a single gene, or specific smaller groups of genes. For recombinant protein production the technology may provide avenues to minimizing the environmental footprint of the production by e.g increasing yields and quality of the pharmaceutical substances. Improving CRISPR genome editing will reduce research costs and increase successful somatic gene therapy. For more information see Appendix 3 for confidential examples.

- **If no, please explain why not.**

Final replies to 'Questionnaire on new genomic techniques to contribute to the study requested by the Council' (endorsed in the Joint Working Group of GMO competent authorities on new genomic techniques on 15 January 2020)

Questionnaire

Supplementary reply from the Ministry of Environment and Food of Denmark.

17. Could the use of NGTs and NGT-products bring opportunities/benefits to society in general, such as for the environment, human, animal and plant health, as well as social and economic benefits, in the short, medium and long term?

Yes. Improvements of crops and microorganisms by traditional breeding methods have over many decades contributed significantly to increased crop yields, larger and more stable feed and food supplies, as well as a better economy for farmers, industry and society worldwide. No significant adverse effects on the environment or the health of consumers, animals or plants have been documented due to this practice.

It is estimated that to date, more than 3,200 different commercially available crop varieties and a large number of microorganisms have been developed worldwide using conventional methods of random mutagenesis. Very few (if any) of these crops and microorganisms have shown negative effects on animal or human health or the environment. Because unintended effects will occur less frequently in gene-edited products, these products are potentially safer than the products of random mutagenesis. In addition, the end-product is better characterized with respect to specific mutation(s) in the targeted position(s).

o If yes, please provide concrete examples/data.

<u>The conventional agri-food sector:</u> The increasing world population requires the development of more robust and high-yielding crops. New varieties developed using gene-editing could increase the efficiency of water and nutrient use for both crop and forage production. Stakeholders from research institutions and from the conventional agri-food sector believe that gene-editing could play a very important role for the development of a more sustainable agriculture as well as for the adaptation of agriculture to challenges posed by climate change.

Please refer to the answer to question 11 for concrete examples.

The Danish Council of Ethics holds a similar position. *Please refer to the answer to question 15 and to the examples mentioned in the statement from the Council of Ethics.*

<u>GM food-feed regulation</u>:

Gene-editing has the potential to bring new significant benefits to the environment, biodiversity within agriculture, to human, animal and plant health, as well as providing economic benefits.

Plants: Generation of plants resistant to fungi and pests will greatly reduce the use of pesticides and thereby provide healthier food and feed to the consumers (humans and animals). A potentially more

efficient utilization of water and nutrients in the soil will reduce watering and fertilization of the plants, e.g. in horticultural crops and thereby benefit the environment and the farmers economy. In addition, the development of perennial crops with deep roots that store more carbon in the soil will benefit the climate agenda.

Microorganisms: With respect to microorganisms, random mutagenesis has been used for many years to develop e.g. bacteria, yeast and fungi with optimised properties for a wide range of applications in food production. For example in the fermentation industry for producing food and feed ingredients, for beer- and winemaking, as feed additives, for baking, and within the dairy industry. Gene-editing is a very promising tool for the development of new micro-organisms in food production. Again, the risk associated with the new mutagenesis techniques is not expected to be higher than with the traditional microbial breeding method.

Animals: With respect to animals, animal research using genome editing is already covered by strict legislation and control on animal research, and is subject to the widely agreed principles of replacement, reduction, and refinement. Therefore it seems reasonable to conclude that genome editing in livestock breeding has the potential to improve agricultural productivity and animal health, if concerns about animal welfare or other ethics issues are tackled satisfactorily (European Academies Science Advisory Council (EASAC), *Genome editing: scientific opportunities, public interests and policy options in the European Union*, March 2017).

Contained use directive:

As scientists learn more about human diseases, including genetic disorders, NGTs has added a lot of value to research regarding new medical options and treatments. Currently untreatable diseases (e.g. cancer and diabetes) might be possible to cure by the use of NGTs. They are found to benefit the society, due to the new options for better treatments and give patients the opportunity to live more normal lives and reducing the need for hospitalization and care, potentially free up society resources for other uses. Also, the use of NGT to make investigational mice models reduce the amount of mice bred to bring about the same genetic modification.

If NGTs that introduce point mutations (such as CRISPR/Cas) is excluded from the GMO regulation, it will open the opportunity to use waste products from biotech companies to form biogas, as a pathway in a circular economy. Currently, such waste products containing GMO's need to be inactivated by adding large amounts of chemicals or heat. Without the need of inactivation, the waste products could be used in the future circular economy in the EU, and thereby benefit the environment.

o If no, please explain why not.

The organic sector finds that possible future benefits of NGT are to a very large extent speculative. At the introduction of GMOs in 1996 the future prospects of GMO was abounded with exactly the same promises as the NGT proponents bring forth today. The sector finds that traditional breeding, combined with Genomic- and Marker Assisted Selection, has a better track record.