Detailed Comments from "Coalition for a GM-free India" on Draft Document on "Genome Edited Organisms: Regulatory Framework and Guidelines For Risk Assessment"

Content Title	Comments
2. Indian Biosafety Regulatory Frameworks (Page 8)	The definitions cited from EPA 1989 Rules aptly capture the fact that genome editing deployment and products therefrom come under regulatory purview. Under Point (4) which lists out applicability of the rules, Research has been omitted and this has to be included as reflected in Point (1) on page 8.
4. Application of Genome Editing Technologies in the Indian Context (Page 10-11)	Whilst the earlier section '3. Other Applicable Laws, Acts and Procedures Governing Gene Editing' (Page 8,9) briefly mentions on genome editing having implications for Biological Weapons Convention, and Weapons of Mass Destruction and Their Delivery System (Prohibition of Unlawful Activities) Act, 2005 – 'Section 4. Application of Genome Editing Technologies in the Indian Context' does not highlight its connection to national defence at all which must be done. In United States' Director of National Intelligence 2019[1] report on Worldwide Threat Assessment of the US Intelligence Community, gene editing was mentioned as a technology with a potential to be developed as a 'novel biological warfare agent' and as a threat to 'food security.' A recent article in Hindustan Times[2] elaborated at length how an Insect Allies programme by DARPA of USA is 'working towards creating genetically modified viruses to be carried by insects to fully mature crops of agricultural importance'. 'The viruses, on dispersal by insects, would modify the genes of the target crops'. These illustrate the fact that genome editing has to be considered from a perspective of national security as well.
5. General considerations for Risk Analysis of GEd Organisms and Products Derived Thereof	a) On Page 12 – the line 'Genome editing is a precise molecular method of mutation leading to deletion or addition or substitution of target base pair(s) in the native genes/ nucleic acid sequences'**is scientifically incorrect and must be corrected**. Genome editing is imprecise, this has been rightly acknowledged on Page 13 as well – where this has been written 'currently available nucleases used for genome editing experiments are not completely error-free and therefore exhibit some off-target effect(s) /un-intentional genetic changes at other than the target location during the genome editing process'. This acknowledgement must be done in each sentence wherever genome editing is referred to as a precise method so as not to leave any confusion whatsoever. There is an increasing body of scientific research showing how genome editing results in lot of off-target effects as well in animals, humans and plants. This includes unintended insertions/deletions/mutations/DNA rearrangements as well. The references [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19] [20] [21] [22] [23] in this document shownumerous examples on the short history of genome editing

	technology which show instances on when this has happened as well. **Hence the above-mentioned text must be reworded to –** Genome editing is an imprecise molecular method of mutation leading to precise deletion or addition or substitution of target base pair(s) in the native genes/ nucleic acid sequences and can also lead to unintended effects as well. b) On the same page, for the same above-mentioned reasons, 'the site of integration is predetermined in GEd organisms unlike in GE organisms where site of foreign gene integration in the genome is random' **must be reworded to** 'whilst some of the sites integration in GEd organisms are predetermined however like GE organisms there is random genome integration which can be seen as well.' c) On Page 12, the **below text is similarly misleading and must be corrected** "In addition, genome editing also facilitates the introduction of a foreign gene(s) to introduce a new trait(s), which is similar to GE organisms, but the site of integration is predetermined in GEd organisms unlike in GE organisms where site of foreign gene integration in the genome is random". **Suggested Corrected Text -** "In addition, genome editing also facilitates the introduction of a foreign gene(s) to introduce a new trait(s), which is similar to GE organisms, but one of the sites of integration is predetermined in GEd organisms unlike in GE organisms where site of foreign gene integration in the genome is entirely random. However it has been seen that in addition to target sites, GEd also takes place at off-target sites too." d) On Page 12 and 13, the text "As a consequence of highly specific site of modification/integration, genome editing technologies may lead to products that might be undetectable and/or indistinguishable from the naturally occurring mutants and from organisms produced from conventional breeding and/or artificial/induced mutagenesis (e.g., chemical, radiation)"**is incorrect and must be corrected**. **Suggested Corrected Text –** "Genome editing technologies may lead to products that might be detectable and/or distinguishable from the naturally occurring mutants and from organisms produced from conventional breeding and/or artificial/induced mutagenesis (e.g., chemical, radiation)." **Rationale –** Above shows that genome editing technologies action can result in off-target

	modification/integration too which shows that their products are distinguishable from products produced from conventional breeding. Differences between conventional breeding and genome editing are highlighted here as well. [24] This shows that in conventional breeding, some regions in genome undergo change less frequently than others as those regions are protected by repair mechanisms in the cell whereas CRISPR can bypass these naturally occurring processes. There are studies which show on how genome edited technologies products are detectable as well. [25] **Genome Editing products are not the same as chemical/radiation induced mutagenesis or conventional breeding products** At certain points in the document - including above points - genome editing has been compared with chemical/radiation induced mutagenesis as a technique with a long history of safe use and now it is unregulated. It was in use many years before the GM regulation was drafted and classifying it as safe requires long-term controlled nutritional/toxicological studies comparing mutagenesis-derived diet with non-mutagenesis derived diet which has not been done yet. Whereas mutagenesis has been seen having negative impacts on fruit flies [26] and plants [27] too. In addition, experiments [28] have shown that some areas of genomes are less susceptible to mutations by radiations/chemicals because of the way DNA is compacted and protected by proteins and other molecules. e) The text "Therefore, the genome editing tool can be used to create a wide range of genome modifications that includes production of 'nature-identical' traits, that is, traits that could also be derived by conventional methods, production of cisgenic and intragenic plants and animals, and introduction of exogenous genes with minimum change in the cell's/organism's genome" should be changed. Rationale – It is misleading to suggest that genome modification results in production of 'nature-identical' traits and with 'minimum change in cell's/organism's genome'. As shown above, these traits are accompanied by significant off-target actions. Similarly, minimum change in cell/organism is not a scientifically precise statement as what is minimum has not been quantified here. **Suggested Corrected Text –** "Therefore, the genome editing tool can be used to create a wide range of genome modifications with significant change in the cell's/organism's genome." f) Consequently, the line 'However, GEd organisms differ from GE organisms in many respects' is incorrect and must be replaced to GEd organisms are a sub-category of GE organisms.

6. Tiered approach for the risk assessment of GEd products/organisms (Page 14)	**We strongly disagree with this tiered approach for the risk assessment and regulation of GEd products and organisms. This is because the risks of genome editing process have been under-estimated in such a proposal, and that the approach assumes that GEd organisms resemble organisms evolved through mutations or induced mutagenesis. This is further explained by our points below. We strongly demand that such a tiered approach to regulation should not exist and that all Gene Editing should be regulated equally.**
Figure 2: Risk scenarios in genome editing (Page 14)	GEd Group I (SDN-1, ODM etc) have been assigned a risk category Low, this category is not scientifically explained and is incorrect. Whilst SDN-2 and SDN-3 appear to have higher risk than SDN-1, referring to SDN-1's risk as Low is incorrect. SDN1 which results in normal nucleotide gene sequence disruption eventually results in partial or entire gene disruption. The omnigenic model of genes which is being understood for plants and humans[29] [30] only now that genes operate in networks not through isolation. That is edits in even a single gene can alter core biochemical pathways in the plant in unpredictable ways and until scientific conclusion is not reached on it, it will be foolhardy and mistaken to categorise it as having Low risk. Not only that, tissue culture which is used in genome editing as well, have also been found to cause mutations in genome editing too. [20] The Agrobacterium infection at times used for SDN-1 has been seen to increase mutations in genome editing as well.[20] The text in Page 17 of the document 'In rare cases, single base pair mutation(s) might result in the introduction of novel trait (e.g., Herbicide tolerance) which might pose additional biosafety concerns' itself shows that this cannot be considered under Low Risk category.
Table 1: Grouping of the GEd organisms (Page 15-16)	a) This text – 'Changes leading to knockdown/knock out of protein/ RNA that result in a new trait which may be familiar with prior knowledge' must be reworded to 'Changes leading to knockdown/knock out of protein/ RNA that result in a new trait. May or may not be familiar with prior knowledge'. b) This text – 'Some countries like Japan and Australia have recently amended their regulatory approval process for GEd organisms/products involving SDN-1 type modification' must be reworded to 'Whilst some countries like Japan and Australia have recently amended their regulatory approval process for GEd organisms/products involving SDN-1 type modification, others like New Zealand and EU countries cover all types of genome editing approaches for complete regulation' so that readers of the regulation get to know perspective in countries which have adopted full coverage in their regulation as well.
GEd Group I: GEd cells/organism harbouring single or few base pair edits or small deletions like	a) However, it may not be easy to detect single base pair edited plants without prior knowledge of the modification since they are genetically indistinguishable from naturally occurring alleles. b) Changes leading to protein with new/altered functions. In rare cases, single base pair mutation(s) might result in the introduction of novel trait

SDN-1, ODM, etc. (Page 16-17)	(e.g., Herbicide tolerance) which might pose additional biosafety concerns.
Table 2. Regulatory Approval Pathway for GEd Organisms/ Products derived thereof (Page 20)	Given above, following changes must be made – a) RCGM/GEd Research & Product Development must be changed from – All research and product development experiments related to GEd Group II & III (Plants, Animals/ human stem cells). To All research and product development experiments related to GEd Group I, II & III (Plants, Animals/ human stem cells). b) RCGM/GEd Plants must be changed from RCGM to recommend to GEAC based on molecular characterization data and contained/confined trials data of GEd plants or product(s) of Group II and III experiments and GEd animals falling under Group I, II and III experiments. To RCGM to recommend to GEAC based on molecular characterization data and contained/confined trials data of GEd plants or product(s) of Group I, II and III experiments and GEd animals falling under Group I, II and III experiments. c) GEAC/GEd Plants must be changed from GEd organisms and products derived from Group II and III experiments on Plants and Group I, II and III experiments on Animals/human stem cells for environmental release. To GEd organisms and products derived from Group I, II and III experiments on Plants and Group I, II and III experiments on Animals/human stem cells for environmental release.
8. Data Requirement for Risk Assessment (Page 23)	• DNA fingerprinting and proteomics analysis and full characterization, both structurally and functionally, of the differences between the GEd cell and the parent organism. It is established that genetic engineering leads to a higher rate of mutation than conventional breeding. DNA fingerprinting may (of course, not always) pick up some of the mutations. Functions in a cell largely depend on its protein profile. The only way to pick up changes in cellular protein profiles is through a proteome analysis which would identify new, altered or deleted proteins. Sequence comparison with known proteins in the protein data base (coupled with the knowledge we have of structure-relationships in proteins) can give some idea of the possible function of a new or altered protein - for example allergenicity, examples for which exist in the literature • The total sequence of the gene-edited flanking regions and the transgene (if used), and identification of the site(s) of integration. The region that is sequenced should be large enough to identify the nature of the site of integration

	• Identifying changes in the glycosylation pattern of proteins, which are known to occur in GEd cells (and can affect the function of the protein). • Determination of any selective increase in transcription and translation, thus including a study of the transcriptome. Changes in transcription pattern can lead to changes in proteins and thus changes in their functioning. Changes in the relative concentration of major and important intracellular metabolites. Normal ranges are available in many cases or can be easily obtained. For example, the free amino acid profile of a cell is generally reflected in the gross amino acid composition of the total protein in the cell. A major change in the concentration of just one amino acid can lead to translational errors and changes in the protein profile (apart from influencing pathways via feedback etc.). The metabolites and precursors chosen will depend on the particular case. • Monitoring changes in surface properties that may affect normal interactions between species, and with the environment. This can be studied through techniques such as scanning electron microscopy, atomic force microscopy and fluorescence-activated cell sorting (FACS). The cell types chosen would depend on the GEd cells and its projected use. • Monitoring reproductive interference. This refers to a change in the reproductive capability of an organism consuming the GMO or a product derived from the GMO • Monitoring gene flow to other organisms • Dispersal into areas where harm could be done (as happened with water hyacinth and parthenium) • Development in India (if not already available) of a technique to determine with accuracy, even a 0.01 % contamination with GEd cells or its product. 0.01% is the level of contamination with a GMO that can be reliably detected with today's technology. It is also the limit of contamination of non-GMOs, by GMOs, permitted by the Government of India. • In the case of GEd food material, possible interaction with commonly used drugs. Drug-drug interaction is today accepted as an important issue in medical practice. A new or altered protein could have a drug-like effect • Carrying acute toxicity studies with native (not "surrogate") protein, GEd seeds and other GEd plant material that is normally ingested by animals, including cattle. These studies should be done both on experimental lab animals and on farm animals such as goat, sheep and cows) • Carrying chronic toxicity studies (including carcinogenicity) as above • Studying effect on cattle GI microflora • Looking for effects on soil micronutrients in regions (rain-fed, irrigated, semi-arid, etc.) where GEd cell is likely to be released or find its way • Development of resistance to the trait • Increasing requirements for refuge crops, if any • Increase in susceptibility to pests and infectious agents other than those that may be expected to be killed by the edited gene

	• Comparison of the growth characteristics of the GEd cell and the parent organism • Emergence of new dangers, for example of super weeds, following prolonged use of herbicide-resistant crops • Effect on the population density of non-susceptible pests, following at least five successive plantations – for example in the case of Bt plants. In many cases involving successive plantations of Bt crops, the density of pests that were not originally susceptible to the Bt toxin increases. The number five is used as a guideline on the basis of published data on the progressive increase in the population of pests that were initially known to be resistant to the Bt toxin • Carrying out a statistically validated programme involving the karyotyping and gross chromosomal analysis of GEd food plants and their consumers. Chromosome alterations, e.g. translocations, can lead to serious health problems. • If the GEd cell is a plant, its biomass productivity in comparison to the parent. • Comparison of inputs required for optimal growth of the GEd cell in comparison to the parent organism and comparison of the relative cost:benefit ratio (including financial inputs and social costs). • Impact on ecology in controlled field trials (for example, on population of bees, and other useful insects). This would require total mapping of insects and other living species in every region where the GEd cell is intended to be released, over a substantial period of time. • Stability of the GEd cell in the whole organism and/or parts thereof, under various conditions of storage or handling (e.g. cooking in case of an edible GEd product) • Efficacy on useful insects. • Monitoring effect on microflora of the soil • Testing for allergenicity Existing regulatory institutions that work as arms of implementation for GEAC should continue. More coordination is required between agencies and departments in other Ministries to ensure that GEd cells and products are not imported into India from elsewhere illegally and such coordination mechanisms should be included in the regulatory framework and guidelines. Such coordination does not exist as of today.
Glossary	**Targeted Genome Engineering -**Modification of the genome at a precise, predetermined locus. **Must be changed to** **Targeted GenomeEngineering -**Modification of the genome at a precise, predetermined locus with possible changes/modifications at off-target locations.

FURTHER EXPLANATION ON SOME OF THE POINTS RAISED ABOVE AND THE COVERING LETTER

Light touch regulation for GEd Group I(SDN-1, ODM, etc) and GEd Group II(SDN-2) in India must be avoided

Problem with product-based regulation as is being proposed is that it focuses only on the trait of the final product. Whereas process-based regulation looks at the uncertainities of the GEd process highlighted earlier in the document as well.

Downstream impacts of genome editing resulting in novel expressions at the level of plant biochemistry and composition will need to be tested as well. Disturbances in gene expression or functions of protein encoded by the gene must be tested for too.

In addition, performing mutagenesis is something which is not easy enough for a lay person to do. Whereas with DIY (Do-It-Yourself) kits for gene editing already starting to be available in some countries and biohackers injecting themselves attempting to make themselves more muscular, to treat herpes and home-brewed treatments for H.I.V.[31] Light-touch regulation for GEd Group I and GEd Group II must be avoided.

Whilst the draft regulatory framework itself acknowledges that GEd Group I itself might lead to 'introduction of novel traits like herbicide tolerance leading to biosafety concerns', the proposed regulatory framework for GEd Group I and Group II does not address these biosafety concerns. The fact that genes do not function in isolation rather act as networks necessitates full spectrum of testing in all cases. As the regulatory framework document itself touches too, and numerous studies pointed earlier also showing off-target impacts of genome editing which must be duly factored to regulate GEd Group I and Group II genome editing. Biosafety must be a critical factor in shaping the form of regulatory framework which the current proposal does not even pretend to do so.

Whilst the regulation talks about some countries having light-touch regulation, countries like New Zealand and European Union countries [32] which regulate all forms of genome editing which is what India must move towards. Not only that given EU being India's largest trading partner with 12.9% [33] of total Indian trade, ahead of US and others – it makes economic sense to have regulations on par with it which will help ensure India's exports are unaffected (both to EU and around the world) as well. Otherwise India's food and animal produce exports (to EU) are likely to be harmed in fear of those falling short of EU standards of GM regulation.

Enforcing compliance

The document also touches frequently on the challenges of enforcing compliance. Initially earlier-generation GMOs were difficult to detect as they were not authorized in the world and hence information on their DNA sequence wasn't available. However in 2006 when an unauthorized GM rice

being field-tried in US was found in several continents around the world, EU Commission developed sampling and detection methods and required importers to EU to apply them so as to prove that their imports did not contain illegal contamination. This approach helped EU during the issue in regarding GM flax seed from Canada.

Detection mechanisms for genome editing products have also started to be found too. [34]

Outstanding pending improvements in current GM framework

Genome editing by its definition falls within genetic modification and consequently as a base minimum the test framework must be the same as exists for genetically modified organisms. At the same time the flaws and gaps in the current regulatory framework must be fixed in the emerging regulatory framework for genome editing too. This leads to a critical point in various gaps in the current GM regulatory framework for the gaps existing in current framework will exist in future genome editing regulatory framework as well. Many of these points have been pointed in both Supreme Court Technical Expert Committee on GM crops [35] and Parliamentary Standing Committee on 'Cultivation of Genetically Modified Food Crops – Prospects and Effects' [36].

- Hiding/blinding identity of developer to regulator should be done at the time of sample submission.
- Breaching of Confidentiality during from sampling until results are submitted directly to GEAC to be considered as a civil crime.
- Independent labs with prescribed testing parameters must be setup for genome testing – such labs should accept samples from citizens too and not just regulators.
- Testing costs should be paid by the applicant to the Government at the time of processing application/sample submission.
- Samples provided by the promoter cannot be relied upon (as evident from the cases of Bt BN and Bt NHH44). Samples must be independently collected by testing authorities.
- Conflict of interest in members involved in GM regulation existing at various levels in the current process must be tackled.
- Full transparency must be built in with public including on all genome editing research work being undertaken in the country and its status which must be pro-actively shared on a regulator's website. All biosafety dossiers must be made available to the public – unlike the case of GM Mustard where even after instructions by CIC, full biosafety dossier has yet not been shared more than 3 years after being asked to.
- Regulatory body composition must include members from AYUSH, Department of Animal Husbandry, Dairying & Fisheries, National Biodiversity Authority, Department of Consumer Affairs, Directorate General of Foreign Trade, Department of Food & Public Distribution and

Ministry of Defence. This will help in addressing various unaddressed aspects in current regulatory framework.

- All states and districts must be asked to conduct and share SBCC/DLC meeting details to regulators which must be put transparently on the website. And Agriculture being subject of the state; states should be given upper hand and supreme power to reject GEd research and commercialization in agriculture sector if they feel necessary in their state.
- Independent testing to be conducted by regulators – this should include long-term, inter-generational testing and assessment.
- Need assessment and alternative analysis to be performed by neutral regulatory agency before starting any research.
- List of tests to be conducted during biosafety assessment should be comprehensive, as listed above.
- Liability regime for contamination, import (of products/seeds/animals) and illegal cultivation must be setup – this will help tackle situations such as today when Bt brinjal, HT cotton and GM soybean have been found to be cultivated illegally in India. And large-scale import of illegal GM products is taking place in India.
- Learning lessons from the past in India, where various GM crops have been introduced and cultivated illegally without any punitive action against anybody; there is dire need for much stricter, neutral, third party, scientific and long term/ intergenerational testing should be conducted without any conflict of interest.
- A mechanism to recall any GEd product released in the environment should be set up.
- There needs to be an institutional coordination mechanism put into place to ensure that GEd organisms and products are not imported into India.
- A database accessible for public all GEd research (public, private) and its status which is updated on a quarterly basis must be done.
- Anybody applying for traditional GM/GEd must provide appropriate reference material for the GM/GEd (samples) and detection method as well – as is done in EU. [36]

References –

[1] Coats DR, 2019, Statement for the Record - Worldwide Threat Assessment of the US Intelligence Community https://www.dni.gov/files/ODNI/documents/2019-ATA-SFR---SSCI.pdf

[2] Bahadur M, 2019, The weaponisation of food is taking a new form. It could change warfare ,Hindustan Times https://www.hindustantimes.com/analysis/the-weaponisation-of-food-is-taking-a-new-form-it-could-change-warfare/story-I75w53fmLeoL50Pi8rHiqI.html

[3] Mussolino C, Morbitzer R, Lutge F, Dannemann N, Lahaye T, Cathomen T. A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity. Nucleic Acids Res. 2011;39(21):9283-9293. doi:10.1093/nar/gkr597

[4] Pattanayak V, Ramirez CL, Joung JK, Liu DR. Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. Nat Methods. 2011;8(9):765-770. doi:10.1038/nmeth.1670

[5] Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPRCas nucleases in human cells. Nat Biotechnol. 2013;31(9):822-826. doi:10.1038/nbt.2623

[6] Unckless RL, Clark AG, Messer PW. Evolution of resistance against CRISPR/Cas9 gene drive. Genetics. 2017;205(2):827-841. doi:10.1534/genetics.116.197285

[7] Braatz J, Harloff H-J, Mascher M, Stein N, Himmelbach A, Jung C. CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (Brassica napus). Plant Physiol. 2017;174(2):935-942. doi:10.1104/ pp.17.00426

[8] Devang Mehta et al (2019). Linking CRISPR-Cas9 interference in cassava to the evolution of editing-resistant geminiviruses. Genome Biology. DOI: 10.1186/s13059-019-1678-3

[9] Gabriel R, Lombardo A, Arens A, et al. An unbiased genome-wide analysis of zinc-finger nuclease specificity. Nat Biotechnol. 2011;29(9):816-823. doi:10.1038/nbt.1948

[10] Yang L, Grishin D, Wang G, et al. Targeted and genome-wide sequencing reveal single nucleotide variations impacting specificity of Cas9 in human stem cells. Nat Commun. 2014;5. doi:10.1038/ncomms6507

[11] Bortesi L, Zhu C, Zischewski J, et al. Patterns of CRISPR/Cas9 activity in plants, animals and microbes. Plant Biotechnol J. 2016;14(12):2203-2216. doi:10.1111/pbi.12634

[12] Unckless RL, Clark AG, Messer PW. Evolution of resistance against CRISPR/Cas9 gene drive. Genetics. 2017;205(2):827-841. doi:10.1534/genetics.116.197285

[13] Braatz J, Harloff H-J, Mascher M, Stein N, Himmelbach A, Jung C. CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (Brassica napus). Plant Physiol. 2017;174(2):935-942. doi:10.1104/pp.17.00426

[14] Gelinksky E and Hilbeck A (2018). European Court of Justice ruling regarding new genetic engineering methods scientifically justified: a commentary on the biased reporting about the recent ruling. Environmental Sciences Europe 30(1):52.
https://enveurope.springeropen.com/articles/10.1186/s12302-018-0182-9

[15] Kosicki M et al (2018). Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. Nature Biotechnology 36:765–771. https://www.nature.com/articles/nbt.4192

[16] Tuladhar R et al (2019). CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation. Nature Communications vol 10, Article number: 4056, 6
Sept. https://www.nature.com/articles/s41467-019-12028-5

[17] Smits AH et al (2019). Biological plasticity rescues target activity in CRISPR knock outs. Nat Methods 16, 1087–1093. https://www.ncbi.nlm.nih.gov/pubmed/31659326

[18] Sansbury BM et al (2019). Understanding the diversity of genetic outcomes from CRISPR-Cas generated homology-directed repair. Commun Biol 2, 1–10. https://www.nature.com/articles/s42003-019-0705-y

[19] Wolt JD et al (2016). Achieving plant CRISPR targeting that limits off-target effects. The Plant Genome 9: doi: 10.3835/plantgenome2016.05.0047. https://www.ncbi.nlm.nih.gov/pubmed/27902801

[20] Tang X et al (2018). A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. Genome Biology
19:84. https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1458-5

[21] Norris AL et al (2019). Template plasmid integration in germline genome-edited cattle.
BioRxiv. https://www.biorxiv.org/content/biorxiv/early/2019/07/28/715482.full.pdf

[22] Ono R et al (2019). Exosome-mediated horizontal gene transfer occurs in double-strand break repair during genome editing. Communications Biology 2: 57 https://www.nature.com/articles/s42003-019-0300-2.pdf?origin=ppub

[23] Shin HY et al. (2017). CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. Nature Communications 8, Article number: 15464.
doi:10.1038/ncomms15464. https://www.ncbi.nlm.nih.gov/pubmed/28561021

[24] Kawall K (2019). New possibilities on the horizon: Genome editing makes the whole genome accessible for changes. Frontiers in Plant Science, 10:525. https://doi.org/10.3389/fpls.2019.00525

[25] Bertheau, Yves. (2019). New Breeding Techniques: Detection and identification of the techniques and derived products. In: Melton L et al (eds.) (2019). Encyclopedia of Food Chemistry. Reference Module in Food Science. Elsevier. 320-336. 10.1016/B978-0-08-100596-5.21834-9

[26] Sawyer SA, Parsch J, Zhang Z, Hartl DL. Prevalence of positive selection among nearly neutral amino acid replacements in Drosophila. Proc Natl Acad Sci USA. 2007;104:6504-6510. doi:10.1073/pnas.0701572104

[27] Acquaah G. Principles of Plant Genetics and Breeding. Oxford, UK: Wiley-Blackwell; 2007. http://bit.ly/17GGkBG

[28] Katharina Kawall, New possibilities on the horizon: Genome editing makes the whole genome accessible for changes,Front. Plant Sci., 24 April 2019 | https://doi.org/10.3389/fpls.2019.00525

[29] Boyle et al (2017) Cell. 2017 Jun 15; 169(7):1177-1186 doi: 10.1016/j.cell.2017.05.038 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5536862/

[30] Chateigner et al, 2019,To what extent gene connectivity within co-expression network matters for phenotype prediction? Biorxiv https://www.biorxiv.org/content/10.1101/523365v3.full

[31] Emily Baumgaertner, As D.I.Y. Gene Editing Gains Popularity, 'Someone is going to get hurt', New York Times https://www.nytimes.com/2018/05/14/science/biohackers-gene-editing-virus.html

[32] Steffi Freidrichs et al, 2019, An overview of regulatory approaches to genome editing in agriculture, Biotechnology Research and Innovation, https://doi.org/10.1016/j.biori.2019.07.001

[33] India Trade Policy, European Commission, https://ec.europa.eu/trade/policy/countries-and-regions/countries/india/

[34] Siddiqui Imran et al, Supreme Court Technical Expert Committee on GM Crops Final Report, 2013, http://www.indiaenvironmentportal.org.in/files/file/TEC-Main-Report.pdf

[35] Basudeb Acharia et al, Cultivation of Genetically Modified Food Crops – Prospects and Effects, Ministry of Agriculture http://www.indiaenvironmentportal.org.in/files/file/GM_Report.pdf

[36] EU Science Hub, The European Commission's science and knowledge service, Reference Material for GMO analysis See Reg (EC) No 1829/2003 https://ec.europa.eu/jrc/en/research-topic/reference-materials-gmo-analysis