Ref. Ares(2023)7389545 - 30/10/2023

Deliverable D5.3

List of barriers to implementation of genome editing technology *in vitro* and *in vivo*

Grant agreement no°: 101094718

Due submission date

2023-10-31

Actual submission date

2023-10-30

Responsible author(s)

Emily Clark (UEDIN); emily.clark@roslin.ed.ac.uk

Noraly van Hemert (EFFAB); noraly.vanhemert@effab.info

Geena Cartick (EFFAB); geena.cartick@effab.info

Confidential: No

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101094718. The content of this report reflects only the author's view. The European Commission is not responsible for any use that may be made of the information it contains.

DOCUMENT CONTROL SHEET

Deliverable name	List of barriers to implementation of genome editing technology *in vitro* and *in vivo*
Deliverable number	D5.3
Partners providing input to this Deliverable	EFFAB, UEDIN, WUR, FBN, EMBL-EBI, INRAE
Draft final version circulated by lead party to: On date	All partners in WP or task on 2023-10-20
Approved by (on date)	FBN as Coordinator (2023-10-30)
Work package no	5
Dissemination level	Public (PU)

REVISION HISTORY

Version number	Version date	Document name	Lead partner
Vs1	2023-10-20	D5.3 – List of Barriers and Opportunities for GE	UEDIN
Vs2	2023-10-20	D5.3 – List of Barriers and Opportunities for GE	UEDIN
Vs3	2023-10-30	D5.3 – List of Barriers and Opportunities for GE	UEDIN

Changes with respect to the DoA (Description of Action)

The first version of this deliverable meets the requirements of the DoA. As the EuroFAANG RI project progresses, and more barriers and opportunities related to genome editing in farmed animals are identified the deliverable will be updated annually.

Table of Content

1. Summary of results

The purpose of Deliverable 5.3 is to list the barriers to implementation of genome editing technology *in vitro* and *in vivo*. Since beginning work on the different components of work package 5, including forming the 'think-tank' on genome editing and holding the first stakeholder workshops, it became apparent that in addition to barriers we should also consider the opportunities that genome editing in *in vivo* and *in vitro* systems provide. The results presented in this deliverable are the consolidated outputs from discussions held at stakeholder workshops over the past year with additional considerations added from recent reports and publications focused on genome editing in farmed animals. A list of barriers and opportunities is provided in the form of tables and some of the implications and potential considerations for application of genome editing for farmed animal breeding in Europe are also discussed. Based on these discussions some of the main barriers identified to uptake of the technology were regulatory and legislative ambiguity, societal perception, off-target effects, and a lack of suitable editing targets. Opportunities identified included the potential to improve animal welfare, build capacity in *in vitro* systems for linking genotype-to-phenotype, enhance breeding progress and advance biomedical research. This deliverable is linked to D5.2, which provides a consolidated list of labs using genome editing in farmed animals in Europe, and D5.5 which focuses on formation of the EuroFAANG Research Infrastructure (RI) 'think-tank' on genome editing. D5.3 will be continuously updated as further stakeholder workshops and discussions take place over the duration of the EuroFAANG Research RI project.

2. Introduction

The EU Horizon Europe research program funds the EuroFAANG Research Infrastructure (RI) project under *HORIZON-INFRA-2022-DEV-01.* This call focuses on developing, consolidating and optimizing the European research infrastructure landscape and maintaining global leadership, ultimately creating a world-leading, coherent, agile and attractive RI landscape in Europe. This project will look at how to develop a concept for implementing the EuroFAANG RI. RIs are facilities, resources and services used by the research communities to conduct research and foster innovation in their fields. They include major scientific equipment (or sets of instruments), knowledge-based resources such as collections, archives and scientific data, e-infrastructures, such as data and computing systems and communication networks and any other tools essential to achieve excellence in research and innovation.

The EuroFAANG RI aims to streamline the use of interdisciplinary capabilities for Genotype-to-Phenotype (G2P) research in terrestrial and aquatic farmed animals and provide transnational access to all of the relevant facilities, expertise and knowledge to European stakeholders. This will address the need to bring together national facilities at the pan-European level in the field of animal genetic resources, phenotyping and breeding, and animal health, which was identified as a gap in the infrastructure landscape by the 2021 ESFRI Roadmap. The proposal builds on the foundation of the six current H2020 EuroFAANG projects, AQUA-FAANG, BovReg, GENE-SWitCH, GEroNIMO, RUMIGEN and Holoruminant. It connects with existing infrastructures for data management and animal agriculture in the European research infrastructure landscape.

Within this framework, work package (WP) 5 aims to develop a framework for sharing and expanding capabilities in genome editing as a route to applying FAANG data for understanding the genotype-to-phenotype link in farmed animals (1). To achieve this work package 5 has three main objectives or tasks:

1. Provide a route to the application of FAANG data in vitro by creating a framework for access to high-throughput phenotyping (HTP) platforms for the validation of candidate causal variants (D5.5).
2. Devise a framework to facilitate access to genome editing technology in vivo (D5.2) in the form of: i) animal facilities equipped to handle/raise and conserve genome-edited animal lines across generations and ii) in silico modelling studies to predict the effect of introducing an edit into a breeding population.
3. Creation of a European think-tank on genome editing in farm animals, connecting national ethics committees, projects and research infrastructures (D5.5).

Deliverable D5.3 is part of the second and third tasks of WP5. Task 5.2 and 5.3 aim to facilitate access to genome editing technology by identifying the current barriers to performing genome editing both *in vivo* in animals and *in vitro* using cell lines and organoids. The aim of D5.3 is to summarize the status quo regarding existing barriers (legal, technical, societal) to implementing genome editing *in vivo* and *in vitro* and also to highlight the opportunities that the technology provides. Creating this list of barriers and opportunities will help build a picture of the challenges and also the solution to wider uptake of genome editing technology in farmed animals in Europe. It will also determine how the EuroFAANG research infrastructure could provide transnational access (TNA) with the overarching goal of improving accessibility to genome editing technology across the European research and development space for genotype to phenotype research in farmed animals.

The results presented in this deliverable are the consolidated outputs from stakeholder workshops held over the past year, specifically the EuroFAANG World Café at the FABRE-TP Annual General Meeting in Brussels in May 2023 and the EuroFAANG Think-Tank on genome editing held virtually in October 2023. This deliverable is linked to D5.2 and D5.5 and will be continuously updated as further stakeholder workshops and discussions take place over the duration of the EuroFAANG Research Infrastructure Project.

3. Results

World Café at the FABRE-TP AGM to identify barriers and opportunities associated with genome editing in farmed animals

The first stakeholder workshop for the EuroFAANG RI project to identify the barriers to uptake of genome editing technology and the opportunities it provides took place at the FABRE-TP Annual General Meeting (AGM) in Brussels on the 30th and 31st of May 2023.

The Farm Animal Breeding and Reproduction Technology Platform (FABRE TP) promotes research and innovation for sustainable animal breeding and reproduction in Europe. FABRE TP is the main contact point for farm animal breeding and reproduction organisations in Europe, aiming to mobilise the research efforts, technological development and innovation efforts in Europe. FABRE TP brings together key stakeholders around a common vision for the development of technologies and practises around farm animal breeding and reproduction. As such the FABRE-TP (AGM) was the ideal place to have the first stakeholder workshop for D5.3 as the attendees at the meeting were a mix of academic researchers and industry stakeholders.

The session ran as a World Café, organised by EuroFAANG RI project partners in WP5 EFFAB, INRAE and UEDIN. Participants were split into two groups of up to 20 participants each and were given a brief introduction to the EuroFAANG RI project and the topic itself. One group discussed biobanking (for WP4) and the other discussed genome editing (for this deliverable) then the two groups swapped.

As primer to start the discussion each group was shown this slide.

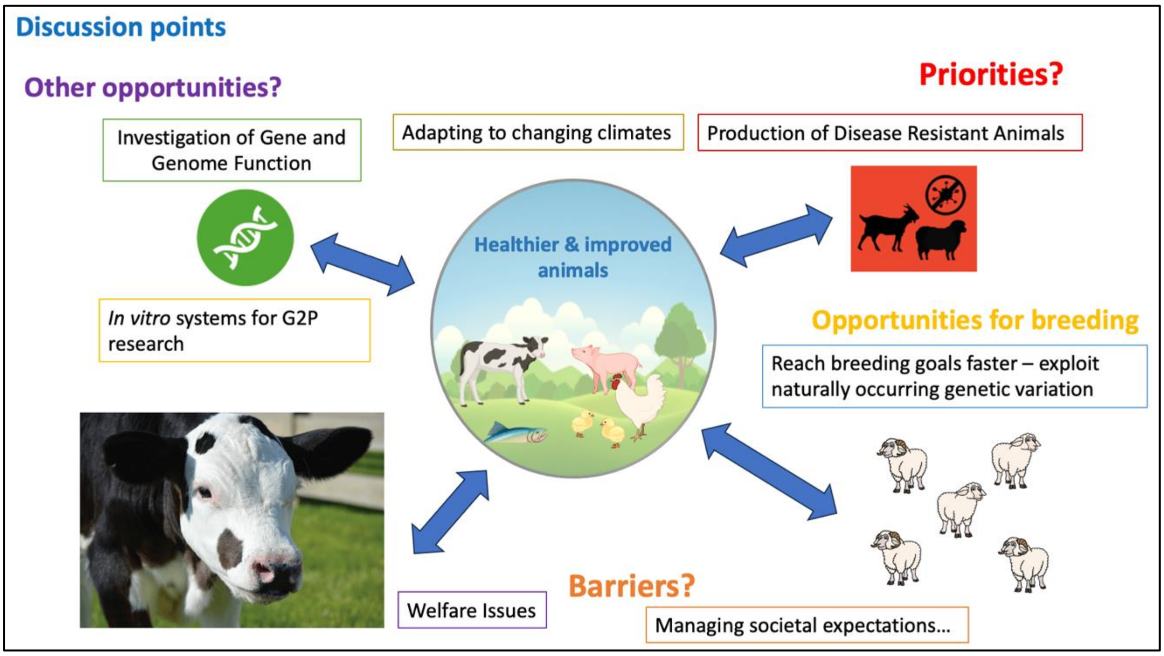

Figure 1: Primer slide used to start the World Café discussion on the genome editing at the FABRE-TP AGM.

During the FABRE-TP AGM World Café session the following opportunities and barriers were identified:

Barriers to adopting genome editing in farmed animals

- Dominant mutations that provide suitable targets for editing are rare.
- Most complex traits e.g. disease resistance traits are polygenic which requires multiplex editing.
- There needs to be a market, as competition can be created by regulatory differences across countries, and differences in legislation can impact trade deals between countries.
- Breeding programmes are expensive to set up and difficult to replicate making introducing genome edited animals into a breeding programme problematic.
- Currently the methods used for genome editing, including embryo transfer and laparoscopic surgery, are not scalable or easily applied on farm.
- Mosaicism in edited animals, particularly founder animals, is an issue, and other technological issues such as off-target effects need to be better quantified/understood.
- A lack of knowledge of fundamental biology of the genome in farmed animals is a limiting factor as often causative variants are still poorly annotated in regulatory regions making them difficult to fine map to specific editing targets.

Opportunities provided by genome editing for farmed animal breeding

- Examples of using genome editing *in vivo* where edited animals are used in a breeding context, but do not enter the food chain, may be more acceptable to regulators/consumers – e.g. surrogate hosts/sires where offspring of germ line ablated animals are not edited make it possible to disseminate desirable genetics at scale with a final product entering the food chain that is not edited.
- Genome editing will probably become socially acceptable if applied to traits related to animal welfare, and it will be most useful for traits not easy to select for e.g. resistance to infectious pathogens.
- Future potential to make multiple edits will make editing for polygenic traits e.g. disease resistance feasible.
- *In vitro* systems provide tools for functional validation of causal variants and can also be used to discover novel variants for editing and to introduce de novo alleles *in vitro*.
- There are already examples of edited farmed animals with application to the animal breeding sector e.g. SLICK cattle, polled cattle, PRRSV resistant pigs, sterility in layer hens.
- Recent successes in genome editing for human health has had a positive impact on acceptability of the technology.

Barriers and opportunities discussed at the first meeting of the 'think-tank' on genome editing

A comprehensive report from the first meeting of the think-tank on genome editing is recorded in Deliverable 5.5. Briefly, the EuroFAANG RI project 'think-tank' on genome editing aims to connect experts in genome editing, ethics, and animal breeding across Europe to create a platform for responsible genome editing research and application in farm animals. This platform is a hub for discussions and collaboration on critical topics, such as ethics, adoption barriers, societal perspectives, defining terms, and maintaining scientific rigour in genome editing. It aims to foster dialogue, facilitate sharing of insights and practical applications, and address potential challenges related to genome editing in farm animals. The 'think-tank' will also foster collaboration between research labs to ensure Trans-National Access (TNA) to expertise and capacity for genome editing technology is both fair and equitable across Europe. The first meeting of the think-tank took place on the 5th of October 2023, with 43 participants joining the virtual meeting. The think-tank discussions emphasised the importance of effectively addressing various barriers, seizing opportunities, and setting clear priorities to navigate the complex landscape of gene editing in farmed animals.

A summary of the barriers and opportunities discussed by the think-tank are provided below:

Barriers to Genome Editing in Farmed Animals: Challenges and Hurdles

Genome editing in farm animals offers tremendous potential but faces significant obstacles. This section explores the key barriers and challenges that must be addressed for successful implementation.

- **Limited Understanding of Genome Function:** A common challenge was a need for more knowledge about genome function, resulting in few known gene editing targets.

- **Phenotypic Uncertainty:** The difficulty in predicting and validating expected phenotypes for gene-edited animals was another common challenge highlighted by participants.

- **Societal Perception and Regulatory Divergence:** Disparate societal perceptions and differing regulatory approaches between regions and countries were recognised as significant barriers to gene editing adoption.

- **Market Acceptance:** Market acceptance along the entire value chain, not just among producers, poses potential challenges.

- **Off-Target Effects:** Participants expressed concerns about the potential off-target effects of gene editing, emphasising the need for more research in this area.

- **Resource and Testing Gaps:** There is a need for improving resources and testing to validate genomic variants and enhance the efficiency of gene editing techniques.

- **Legislative Ambiguity:** The unclear and evolving legislation and regulations surrounding gene editing were identified as an additional barrier to adoption.

- **Definitions:** Refining proper definitions for key terms like "Genome Editing", "GMOs" and "Animal Welfare".

- **Historical Trust Issues:** A lack of trust in animal farming and its impacts on the environment, animal welfare, and human health emerged as a barrier to acceptance.

- **Genetic Diversity Concerns:** Technical challenges associated with spreading beneficial edits widely in populations while maintaining genetic diversity were identified as a significant challenge.

- **Traceability Issues:** Distinguishing edited animals from their descendants and addressing traceability concerns was highlighted as a critical factor to consider.

- **Scalability:** Training and capacity building is required before sufficient numbers of animals can be edited 'on-farm' in a breeding programme context.

Opportunities in Genome Editing for Farmed Animals: Unlocking Potential

Gene editing presents various opportunities to revolutionise agriculture. This section highlights the potential benefits and advancements of gene editing in farm animals.

- **Enhanced Animal Health and Welfare**: Gene editing offers the opportunity to improve animal health and welfare by conferring resistance to diseases such as e.g. avian influenza, African swine fever, and infectious bursal disease.

- **Improved Breeding Progress:** Faster breeding progress and the integration of traits without traditional breeding burden were seen as significant opportunities.

- **Societal Benefits Showcasing:** The think-tank discussions highlighted the potential for showcasing societal benefits in areas like climate adaptation and the green transition.

- **Biomedical Research Advancement:** Gene editing in animals can advance biomedical research, potentially leading to breakthroughs in human health.

- **Genomic Variant Testing:** High-throughput CRISPR screens for testing genomic variants to better understand their effects, are becoming available for farmed animals, particularly for viruses such as BVD and ASF, and have with the potential to yield valuable opportunities.

- **Cell and Tissue Culture Systems:** the development of more physiologically relevant cell and tissue culture systems for farmed animals to safely test gene edits *in vitro* before whole animal applications *in vivo* emerged as an opportunity.

- **Genome Characterisation:** Participants emphasised the opportunity of characterising animal genomes better to identify variants that could address future challenges, such as those related to climate change impacts.

Further considerations from current reports and publications

Polygenic traits and multiplexed edits

One example of a barrier, identified by both the stakeholder workshop and the 'think tank' on genome editing, is the issue of polygenic traits and the need to perform multiplex editing.

- Application of genome editing *in vivo* is currently limited to modification of a single gene or a variant with a large effect; however, the majority of production relevant traits are polygenic (2).
- Traits that are important in future sustainable farmed animal breeding programs, such as improved feed efficiency, reduced methane emission and improved health and welfare appear to be highly polygenic, and as such will require multiple edits.
- A potential solution is that multiplexing technologies that allow for polygenic traits to be altered in a single step are under development and will become available for farmed animals in the future (3).
- These improvements in editing technologies will be required to enable multiple edits in elite breeding animals within a breeding nucleus to target multiple traits or multiple causative alleles for the same trait (4).
- The ability to generate and test multiplexed edits is much more feasible in *in vitro* systems. Organoids and cell lines, which are the focus of WP4 in the EuroFAANG RI project, provide the opportunity to perform genome wide CRISPR screens for the discovery of multiple causative variants.
- The power of these CRISPR screens was illustrated recently in a study investigating loci involved in replication of African Swine Fever (ASF) Virus in porcine cells (5).

Considerations for including genome editing in breeding programs for farmed animals

Discussion at the stakeholder workshop and 'think-tank', with representatives from industry and the animal breeding sector, both indicated that including genome edited animals in existing breeding programmes could not be straight forward. Recent publications, e.g. (2), have also raised the following points:

- Careful integration of genome-editing technologies into breeding programs is essential to ensure continuous genetic improvement whilst also conserving existing genetic diversity (6).
- Where novel genetics are created using genome editing that would not have arisen naturally, e.g. for the PRRS virus resistant pigs (7), and avian influenza resistant chickens (8), thorough phenotypic characterization of the edited animals will be required. This is because deleting all or a region of a functional protein could lead to a loss of biological function, which could have deleterious consequences for the individual animal and the breeding population itself (9).
- It will also be important to consider when applying genome editing in a breeding program if a gene or variant of interest is located within a locus that has been actively selected, which could indicate whether a potential target is associated with known production traits (10).
- Introducing edits into multiple elite animals, in a breeding program, will be required to avoid genetic bottlenecks (11).
- Editing of different breeds and lines will be essential to maintain genetic diversity, and enable structured cross-breeding (11).
- Issues of scalability of the technology ‘on farm’ and the training of skilled personnel will also need to be considered before the technology becomes widely accessible to producers (12).
- Efficient means of evaluating breeding values when genome edited animals and their offspring are included in a breeding program will be essential (13).
- Efficient and scalable means to trace genome edited animals and their progeny in a breeding program will also be required, and the further development of whole genome sequencing and other ‘omics approaches should help to facilitate this (13).
- Molecular characterization of genome edited animals and their progeny will also likely need to be expanded in any regulatory framework to detect any genomic irregularities, including off-target effects, un-intended on-target effects and effects on genome regulation (13).
- The FAANG consortium is working towards building a better knowledge of genome function in order to provide more information to link genotype-to-phenotype in farmed animals and provide more editing targets (1).
- Genome editing is a key route to application of the data from the EuroFAANG H2020 projects and the global FAANG initiative (1).

Ethical and regulatory considerations for the use of genome editing *in vivo*

The use of genome editing in farmed animal breeding also has important societal, economic, and political implications (14,15) and the following considerations have been raised in recent reports:

- In the 2021 Nuffield Council on BIoEthics report on "Genome editing and farmed animal breeding" it is stated that development and adoption of genome editing technology should be informed by public views and that regular review of policy and regulation is essential (15).
- Others recent reports and publications have indicated that approval processes and regulatory guidelines for genome edited food animals are currently lengthy, complex and require simplifying before genome editing can be widely adopted (16,17).
- Whether animal breeders will be able to employ genome editing in genetic improvement programs for farmed animals will depend largely upon global decisions around the public perception, regulatory framework and governance of genome editing for food animals (18).
- It is also clear that the disparate regulatory approaches being proposed for genome editing in farmed animals globally give rise to some uncertainty as to whether it can serve as a complementary approach to genomic selection to inform efficient and sustainable genetic improvement programs for animal breeding (11,17).
- The licencing landscape for CRISPR is also complex. Currently a licence is required for use of CRISPR technology in a commercial rather than academic capacity, if revenue will be generated from its use, making application in commercial animal breeding programs more difficult (19).

4. Conclusions

Genome editing technologies are undoubtedly important tools for genotype-to-phenotype research in farmed animals and for farmed animal breeding more broadly than in Europe. In Deliverable 5.3, through stakeholder workshops and reviewing available publications and reports, we have identified the key barriers to adoption of the technology, and considered the opportunities it provides.

The results of Deliverable 5.3 suggest that, in conjunction with well-managed efficient breeding programs, genome editing for trait improvement could provide an opportunity for improving farmed animal health, productivity and welfare (11). Both the stakeholder workshop and 'think-tank' highlighted the fact that public and regulatory perception are very important for the future adoption and application of genome editing in farmed animals (15). Recent success stories including the PRRSV resistant pigs (7) and more recently chickens that are resistant to avian influenza (8) have highlighted in the mainstream media the potential of the technology *in vivo*.

The potential of using CRISPR screens to identify potential editing targets using *in vitro* systems is also showing considerable promise for diseases that are putting significant pressure on farmed animal breeding in Europe e.g. African Swine Fever (5). A lack of suitable cell lines and organoid systems previously hindered progress, but this is rapidly changing. Work package 4 of the EuroFAANG RI project will address this and look at ways to build capacity and TNA for genome editing using *in vitro* systems across Europe.

As research and development relating to genome editing in farmed animals rapidly develops, dialogue surrounding the regulatory framework including a wide diversity of stakeholders is necessary (2) and the EuroFAANG RI project can provide through the 'think-tank' and stakeholder workshops a forum for these discussions.

It is likely that the utilization of genetic variation that could have occurred naturally, as opposed to the creation of *de novo* alleles, in genome editing strategies may be viewed more favourably from a regulatory and societal perspective. If this is the case the distinction between the two editing strategies will become increasingly important. At the next meeting of the 'think-tank' in Spring 2024 we intend to discuss this as a specific issue.

This deliverable will be continually updated as additional stakeholder workshops and meeting of the 'think-tank' take place and new reports and publications on genome editing in farmed animals become available.

5. References

1. Clark EL, Archibald AL, Daetwyler HD, Groenen MAM, Harrison PW, Houston RD, et al. From FAANG to fork: application of highly annotated genomes to improve farmed animal production. Genome Biol . 2020;21(1):285. https://doi.org/10.1186/s13059-020-02197-8

2. Clark EL. Breeding in an Era of Genome Editing. Encyclopedia of Sustainability Science and Technology. In: Meyers RA, editor. New York, NY: Springer New York; 2022. https://doi.org/10.1007/978-1-4939-2493-6_1122-1

3. McCarty NS, Graham AE, Studená L, Ledesma-Amaro R. Multiplexed CRISPR technologies for gene editing and transcriptional regulation. Nat Commun . 2020;11(1):1281. https://doi.org/10.1038/s41467-020-15053-x

4. Tait-Burkard C, Doeschl-Wilson A, McGrew MJ, Archibald AL, Sang HM, Houston RD, et al. Livestock 2.0 – genome editing for fitter, healthier, and more productive farmed animals. Genome Biol . 2018;19(1):204. https://doi.org/10.1186/s13059-018-1583-1

5. Pannhorst K, Carlson J, Hölper JE, Grey F, Baillie JK, Höper D, et al. The non-classical major histocompatibility complex II protein SLA-DM is crucial for African swine fever virus replication. Sci Rep . 2023;13(1):10342. https://doi.org/10.1038/s41598-023-36788-9

6. Gratacap RL, Wargelius A, Edvardsen RB, Houston RD. Potential of genome editing to improve aquaculture breeding and production. Trends Genet. 2019 Sep;35(9):672–84.

7. Burkard C, Lillico SG, Reid E, Jackson B, Mileham AJ, Ait-Ali T, et al. Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLOS Pathog . 2017 Feb 23;13(2):e1006206. https://doi.org/10.1371/journal.ppat.1006206

8. Idoko-Akoh A, Goldhill DH, Sheppard CM, Bialy D, Quantrill JL, Sukhova K, et al. Creating resistance to avian influenza infection through genome editing of the ANP32 gene family. Nat Commun . 2023;14(1):6136. https://doi.org/10.1038/s41467-023-41476-3

9. Proudfoot C, Lillico S, Tait-Burkard C. Genome editing for disease resistance in pigs and chickens. Anim Front . 2019 Jun 25;9(3):6–12. https://doi.org/10.1093/af/vfz013

10. Johnsson M, Ros-Freixedes R, Gorjanc G, Campbell MA, Naswa S, Kelly K, et al. Sequence variation, evolutionary constraint, and selection at the CD163 gene in pigs. Genet Sel Evol . 2018;50(1):69. https://doi.org/10.1186/s12711-018-0440-8

11. Bishop TF, Van Eenennaam AL. Genome editing approaches to augment livestock breeding programs. J Exp Biol. 2020 Feb;223(Suppl 1):jeb207159.

12. McFarlane GR, Salvesen HA, Sternberg A, Lillico SG. On-Farm Livestock Genome Editing Using Cutting Edge Reproductive Technologies . Vol. 3, Frontiers in Sustainable Food Systems. 2019. p. 106. https://www.frontiersin.org/article/10.3389/fsufs.2019.00106

13. Kawall K, Cotter J, Then C. Broadening the GMO risk assessment in the EU for genome editing technologies in agriculture. Environ Sci Eur . 2020;32(1):106. https://doi.org/10.1186/s12302-020-00361-2

14. Nuffield Council on BioEthics: Genome Editing an Ethical Review . 2016. https://www.nuffieldbioethics.org/publications/genome-editing-an-ethical-review

15. Nuffield Council on Bioethics - Genome editing and farmed animal breeding: social and ethical issues . 2021. https://www.nuffieldbioethics.org/assets/pdfs/Genome-editing-and-farmed-animal-breeding-FINAL-WEB-PDF.pdf

16. Fan Z, Mu Y, Li K, Hackett PB. Safety evaluation of transgenic and genome-edited food animals. Trends Biotechnol . 2021; https://www.sciencedirect.com/science/article/pii/S0167779921002626

17. Van Eenennaam AL, De Figueiredo Silva F, Trott JF, Zilberman D. Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay. Annu Rev Anim Biosci . 2021 Feb 16;9(1):453–78. https://doi.org/10.1146/annurev-animal-061220-023052

18. Van Eenennaam AL. Application of genome editing in farm animals: cattle. Transgenic Res . 2019;28(2):93–100. https://doi.org/10.1007/s11248-019-00141-6

19. Ledford H. Major CRISPR patent decision won’t end tangled dispute. Vol. 603, Nature. England; 2022. p. 373–4.

6. Annexes

Table 1: List of Barriers to Adopting Genome Editing Technology

Limited Understanding of Genome Function	A common challenge was a need for more knowledge about genome function, particularly regulatory regions were variants controlling traits of interest are located, to identify more target loci for gene editing.
Phenotypic Uncertainty	Difficulty in predicting and validating expected phenotypes for gene-edited animals. Thorough phenotypic characterization of the edited animals will be required because deleting all or a region of a functional protein could lead to a loss of biological function.
Societal Perception and Regulatory Divergence	Disparate societal perceptions and differing regulatory approaches between regions and countries. Regulatory differences between countries can block trade and shared markets.
Market Acceptance	Market acceptance along the entire value chain, not just among producers but also consumers.
Off-Target Effects	Potential off-target effects of gene editing, which might have potential pleiotropic or other effects.
Resource and Testing Gaps	Improved resources and testing to validate genomic variants and enhance the efficiency of gene editing techniques, including access to high-throughput CRISPR screen using in vitro systems (D5.5).
Legislative Ambiguity	Rapidly evolving and sometimes unclear legislation and regulations surrounding gene editing.
Definitions	A lack of proper definitions for key terms like “Genome Editing”, “GMOs” and “Animal Welfare”.
Historical Trust Issues	A general lack of trust in animal farming and production and their impacts on the environment, animal welfare, and human health
Genetic Diversity Concerns	Technical challenges associated with spreading beneficial edits widely in populations while maintaining genetic diversity

Traceability Issues	Distinguishing edited animals from their descendants and providing adequate methods for traceability if animals are to enter breeding programs or the food chain.
Clarity on editing strategies	Lack of clarity on utilization of genetic variation that could have occurred naturally within a population, as opposed to the creation of *de novo* alleles.
Polygenic traits	Most complex traits e.g. disease resistance traits are polygenic which requires multiplex editing, and capacity to perform multiplex edits in vivo is currently limited.
Lack of editing targets	Dominant mutations that provide suitable targets for editing are rare.
Market and trade issues caused by regulation	There needs to be a market, competition can be created by regulatory differences across countries, and differences in legislation can impact trade deals between countries.
Difficulties with integration in breeding programmes	Breeding programmes are expensive to set up and difficult to replicate making introducing genome edited animals into a breeding programme problematic.
Scalability on farm	Currently the methods used are not scalable or easily applied 'on farm'.
Mosaicism	Mosaicism in edited animals, particularly founder animals, is an issue, and other technological issues such as off-target effects need to be better quantified/understood.
Lack of suitable in vitro systems	Well characterised cell lines and organoid systems for functional validation of causal variants using gene editing are lacking for farmed animals.
Licencing landscape	Licencing for use of CRISPR technology is complex. Currently use in an academic context is allowed but if the technology is used for commercial purposes, that generate revenue, then appropriate licencing and patent fees need to be in place, and can create significant legal hurdles.
Limited Trans-National Access (TNA)	Access to genome editing technology is not distributed evenly across Europe.

Table 2: List of opportunities provided by genome editing for farmed animal breeding

Enhanced Animal Health and Welfare	Gene editing offers the opportunity to improve animal health and welfare by conferring resistance to diseases such as avian influenza, African swine fever, infectious bursal disease and PRRSV.
Improved Breeding Progress	Faster breeding progress and the integration of traits in combination with the use of slower traditional breeding methods provides the potential to reach adjusted breeding goals, as a consequence of disease or other climate pressures, more flexibly.
Societal Benefits Showcasing	Potential for moving the narrative away from food production and showcasing societal benefits in areas like climate adaptation and the green transition and prepared to tackle future pandemics.
Biomedical Research Advancement	Gene editing in animals can advance biomedical research, potentially leading to breakthroughs in human health. Successes can also help to change public perceptions of genome editing.
High-Through-Put (HTP) Tools for Genomic Variant Testing	Testing of a very large number of genomic variants at scale to better understand their effects using in vitro systems and robotic platforms.
In Vitro Systems	More relevant cell, organoid and tissue culture systems are becoming available for farmed animals for functional validation. These can be used to safely test potential edits *in vitro* before whole animal applications minimising the use of animals in application of genome editing technology.
Genome Characterisation	The FAANG consortium is working towards a better knowledge of genome function to better link G2P in farmed animals and identify variants that could address future challenges, such as those related to climate related and sustainability traits.
Surrogate Sires/Hosts	Examples of using genome editing in vivo where edited animals, are used in a breeding context, but do not enter the

	food chain, may be more acceptable to regulators/consumers.
Multiplexing Technologies	That allow for polygenic traits to be altered in a single step are under development and will become available for farmed animals in the future.
Tackling Climate Change	Edited animals can help mitigate challenges to the food production system posed by climate pressures and future pandemics more quickly.
Sustainable food production	Editing for reduced methane emission or reduced resource input could help meet European climate targets and sustainable development goals, including those outlined in the Farm to Fork strategy more quickly.
FAANG to Fork	Genome editing provides a key route to application of the data generated by the FAANG consortium to advance genotype-to-phenotype research in farmed animals in Europe.
Building Trans-National Access (TNA)	Building fair and equitable access to genome editing technology across Europe, through sharing of research capacity and expertise, will increase uptake of the technology and maximise its potential.