Current Journal of Applied Science and Technology

Volume 42, Issue 23, Page 20-26, 2023; Article no.CJAST.103568
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Genome Editing Technology in Fishes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2023/v42i234170

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/103568

Mini-review Article

Received: 26/05/2023
Accepted: 29/07/2023
Published: 07/08/2023

ABSTRACT

Genome editing and silencing techniques can transform the biology that we understand, diseases affecting fish and other aquatic animals. Gene editing is now being tested in aquaculture, reproduction control, sterility, and disease resistance aspects. More money must be invested in innovative technology to solve these issues in this industry. So gene silencing and genomic DNA editing have the potential significant impact on aquatic animal treatment in the future. Genome editing in fish is an interested part of research that has the potential to revolutionize aquaculture and aid in the understanding of genetic diseases in fish. Genome editing has many applications in editing the genome of fish species for various uses; zinc finger nucleases (ZFNs) were used to disrupt genes in zebrafish (*Danio rerio*) both somatically and germinally. On the other side, these genetic modification techniques can cause various negative effects through multiple mutations; by that process, it's challenging to recognise the genetically modified organisms. Genome editing in fish is a complex field requiring specialized knowledge and expertise. It is always advisable to consult with experts and follow ethical and regulatory guidelines when conducting fish genome editing experiments.

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Keywords: Gene editing; CRISPR/Cas9; aquaculture; zinc finger nuclease; Transcription Activator-Like Effector Nucleases (TALENs); Fok1.

1. INTRODUCTION

Genome editing is the process of making precise, intentional changes to an organism's DNA. This can be done using different technologies such as CRISPR-Cas9, Zinc Finger Nucleases (ZFN), or Transcription Activator-Like Effector Nucleases (TALENs) [1]. Genome editing in fish is an exciting area of research that has the potential to revolutionize aquaculture and aid in the understanding of genetic diseases in fish. Gene editing uses a specific set of designed nucleases as molecule scissors to insert, remove, or replace DNA or sequences of the nucleotide at a precise location in an organism's genome. Genome editing in fish primarily aims to introduce specific genetic modifications into the fish genome [2]. This can include creating fish with enhanced traits, such as disease resistance or improved growth rates, or studying the functions of specific genes [3]. By gene editing technology aquaculture field can be revolutionized by improving growth, stress, disease and temperature. Editing genes could lead to beneficial modifications in an organism but also adverse effects. Sequence-specific programmable nucleases are used in the most frequently employed and extensively successful gene editing techniques. They act as molecular scissors to cut the DNA at a specific site [4].

1.1 Mechanism

In eukaryotic cells, the two principal forms of endogenous cellular DNA repair processes are nonhomologous end-joining (NHEJ) and homologous recombination (HR) repair, also known as homology-directed repair (HDR).

The mechanism includes the following sequential steps:

1. locating the DNA end, assembling, and stabilising the nonhomologous end-joining complex at the site of the dsDNA break bridging of the DNA ends
2. After this, the ends of the DNA are processed
3. Then, broken ends of the DNA are ligated and the dissolution of the nonhomologous end-joining complex.

Cells can use the homology-directed repair (HDR) mechanism when homologous DNA is present as a template to restore double-strand breaks in DNA. This repair mechanism can produce exact, predictable, and beneficial results for appropriately editing the genomic sequence, inducing particular deletions, insertions, or designer mutations, and inserting an external sequence in the target genome [5].

2. TYPES OF GENE EDITING

Meganuclease: The ability of endonuclease family enzymes to recognise and cut lengthy DNA sequences (14 to 40 bp) distinguishes them from other types of enzymes. The proteins in the LAGLIDADG family, to a conserved amino are the most prevalent and well-known meganuclease. They are typically present in microbial organisms [6]. They are special because they have extremely long recognition sequences (>14bp), making them naturally exceedingly specific. There is no likelihood of locating the precise meganuclease needed to function on a certain DNA sequence [6].

There are several ways to make unique meganucleases. Meganuclease creates a collection of variants for mutagenesis and high throughput screening. The combinatorial assembly enables the association or fusion of protein subunits from various enzymes. Protein units were fused to create chimeric meganucleases that can recognise the target site [7]. Two meganucleases can cleave the human XPC gene, which can lead to a high risk of skin cancer. They are less hazardous to cells due to their more exact DNA sequence recognition than zinc finger nuclease gene editing. However, creating particular enzymes for each potential sequence is expensive and time-consuming [6].

2.1 ZFN (Zinc Finger Nuclease)

Zinc finger nucleases are hybrid proteins created by artificial means that are frequently used as possible tools for gene editing.The presence of zinc ions, which are coordinated by the amino acids cysteine and histidine to form a "finger-like" structure, is what gives these domains their distinctive appearance. Different zinc fingers are used to recognise various sets of nucleotide triplets [8]. This hybrid protein was developed to target specific genomic sequences specifically and comprises particular DNA-binding domains that fuse with the endonuclease Fok I. ZFN

enzymes can cut the DNA sequences at specific target site due to the presence of zinc finger proteins (ZFPs) which recognise and bind to particular DNA sequences. Additionally, ZFN can induce a DNA double-strand break (DSB) at predetermined places [9].

Unlike many natural endonucleases, off-target cleavage is a major concern for ZFNs. ZFNs and ZFPs are divided into three main subtypes (C2H2, C4 and C6), of which C2H2 is most commonly employed in engineered ZFNs due to its simplicity [10]. This genome editing technique can maintain temporal and tissue-specific gene expression compared to conventional gene therapy [11,12].

2.1.1 Fok1

It is mainly present in Flavobacterium species and is a type II restriction endonuclease of bacteria with a DNA-binding domain at the N terminal end and a non-sequence-specific DNA cleavage domain at the C terminal [12]. Regardless of the nucleotide sequence at the cut site, the DNA cleavage domain of the protein is activated and cleaves the DNA at two places when the protein binds to duplex DNA via its DNA-binding domain at the 5'-GGATG-3' recognition site. The DNA is cut into two sticky ends with 4-bp overhangs, 9 downstream of the motif on the forward strand and 13 downstream of the motif on the reverse strand [9].

2.1.2 Transcription Activator-Like Effector Nucleases (TALENs)

The DNA-binding and Fok 1 nuclease domains are found in the TALENs. These domains function in dimer pairs, attaching to the opposing DNA strand to cause DSBs. ZFNs are replaced by TALENs for genome editing and the introduction of targeted DSBs into certain DNA locations of interest. Xanthomonas, a pathogenic bacteria that infect the cytoplasm of plant cells, secretes these particular nucleases [13]. With unique N- and C-termini designs for localization and activation, each nuclease platform has a core domain for specific DNA binding [14]. The monomers with 10 to 30 repeats that make up the DNA-binding domain bind to a single nucleotide in the target DNA sequence. They also include a general FokI catalytic nuclease [7]. With the Fok1 domains at the c-termini and cleavage taking place in the "spacer" sequence, TALENs attach to target locations in the nucleus as a dimer. After then, the error-prone NHEJ technique is used to repair DNA breaks, which predominantly happens in a manner similar to ZFNs [15].

2.2 Crispr/Cas9

One of the most recent developments in the arsenal of genome editing tools is the clustered, regularly interspaced short palindromic repeats (CRISPR) gene engineering method,This most recent technique for gene editing was found in 2012. The Cas9 endonuclease and modified single guide RNA (sgRNA/gRNA), which consists of a targeting CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA), make up the CRISPR/Cas9 system. Consequently, the Cas9 protein and sgRNA are the two crucial elements [16]. A carefully crafted 20-base pair guide RNA directs the Cas9 nuclease to its target nucleotide. A major benefits of this system is that it does not require the extensive repeating complex creation of DNA-binding arrays for target site, as in ZFN and TALEN systems. Instead, it is easy to control because it just requires a simple alteration of about 20 nucleotide sgRNA "spacer" sequences. The target sequence's protospacer-adjacent motif (PAM), which binds with Cas9, is another crucial component. PAM is a short, precise sequence (NGG trinucleotide sequence) that appears downstream of the crRNA binding site [17].

2.2.1 Different CRISPR-Cas Systems

Class 1 type I (CRISPR-Cas3) and type III (CRISPR-Cas10) CRISPR-Cas systems use a variety of Cas proteins and the crRNA. Employ a big single-component Cas-9 protein, crRNA, and tracer RNA in Class 2 types II (CRISPR-Cas9) and type V (CRISPR-Cpf1). The Streptococcus pyogenes type II system the greatest research and has been used as a powerful gene-editing tool.

3. SOME CASE STUDIES OF GENOME EDITING TECHNOLOGY IN AQUACULTURE

4. APPLICATION OF GENE EDITING IN AQUACULTURE

4.1 Monosex Culture

Through the direct disruption of the genes that determine sex through the use of targeted nucleases, it has been possible to make mono-sex and sex-reserved fish without having any appreciable effects on biodiversity. Targeting testicular development allowed for the knockout

of the fox12, sf-1, and cyp19a1a genes that are involved in defining the sex of female tilapia.

4.2 Sterility of Fish

By means of ZFN technology sterile channel catfish (*Ictalurus punctatus*) are created by deleting the subunit gene of pituitary luteinizing hormone, sterile. With the help of CRISPR-Cas9, the dead end (dnd) gene was eliminated, resulting in a salmon without germ cells.

4.3 Reproduction

The Kiss1/Gpr54 system (kisspeptin-encoding gene—Kiss1 and its G protein-coupled receptor 54—GPR54) plays a crucial role in the regulation of reproduction in most vertebrates zebrafish

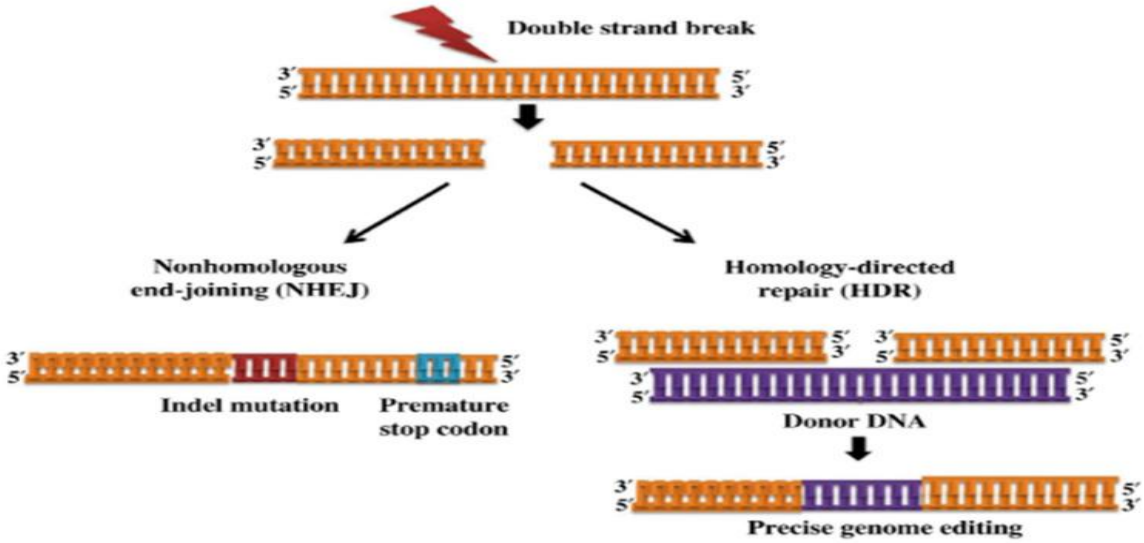

Fig. 1. DNA repair system [5]

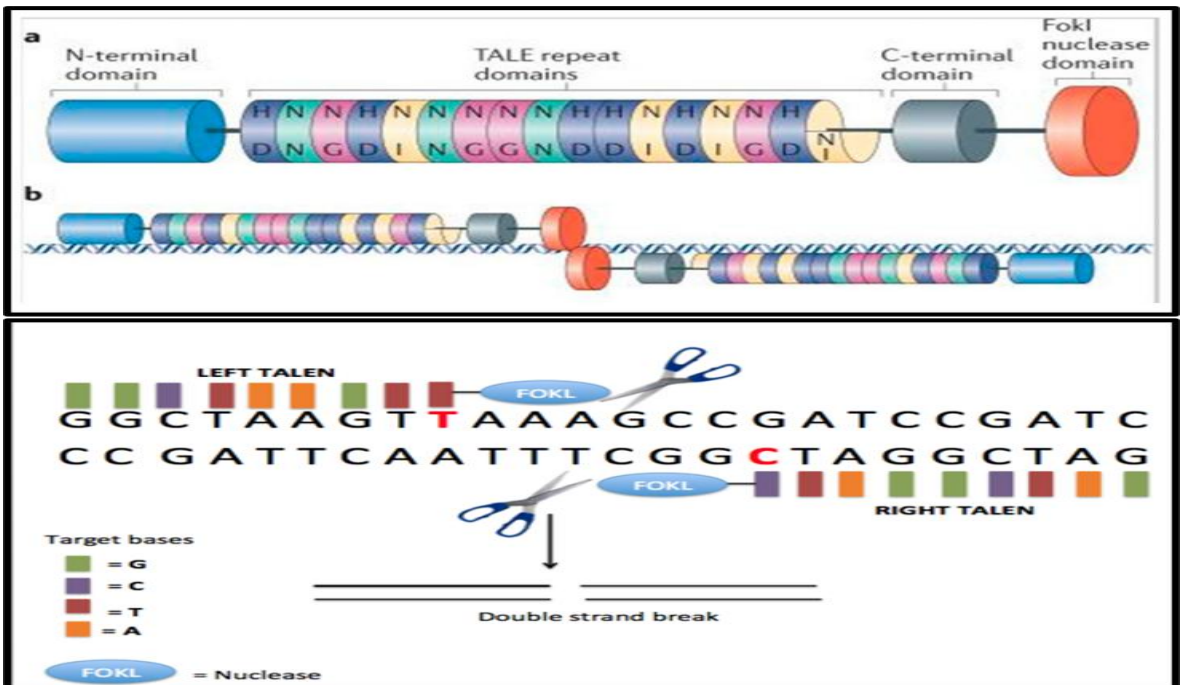

Fig. 2. (a) TALEN Mechanism (b) TALENs binds on a target DNA site and cleave as dimers [13]

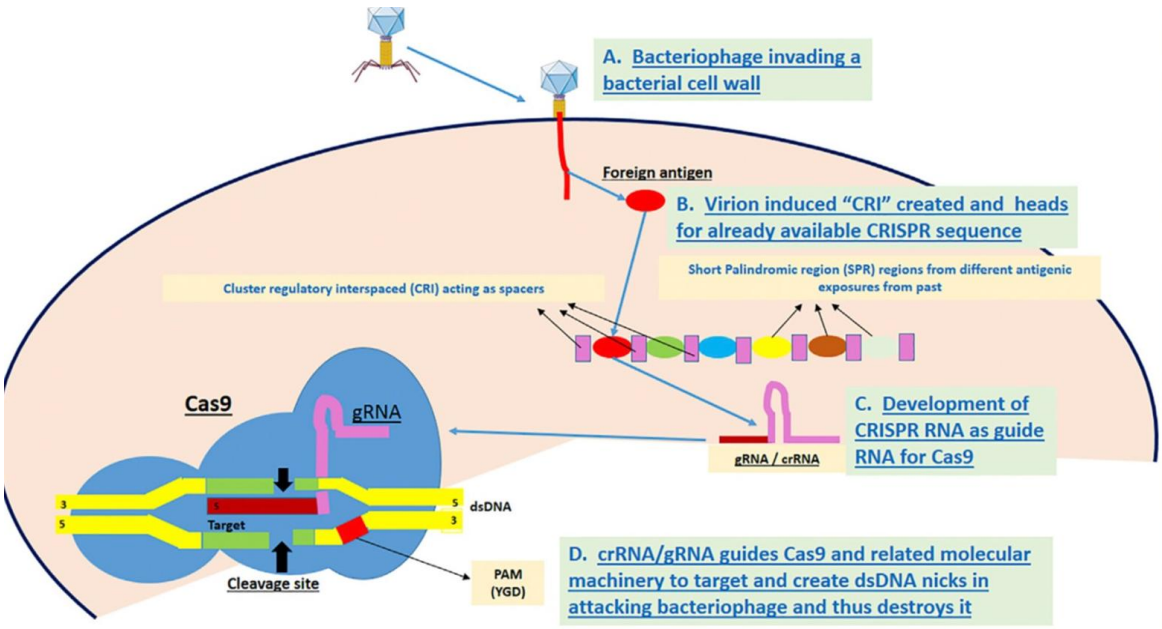

Fig. 3. Concept of CRISPR/Cas9 Interactions [15]

Table 1. Some case study of crispr in aquaculture

Fish	Target gene	Results	References
Zebrafish (Danio rerio)	Ovarian aromatase (cyp19a1a)	All-male phenotype	[18]
Atlantic salmon (Salmo salar)	tyr and slc45a2	Loss of Pigment	[19,20]
Red sea bream (Pagrus major)	Myostatin or GDF 8	Increase in skeletal mass	[21]
Zebrafish (Danio rerio) (TALEN)	Mesp genes (mesp aa, mesp ab, mesp ba and mesp bb)	Growth and immune function	[22]

kiss1-/-, kiss2-/- and kiss1-/-; kiss2-/- mutant lines together with kissr1-/-, kissr2-/- and kissr1 -/-; kissr2-/- by using TALEN restriction enzyme mutant lines is generated. The outcome made it quite evident that none of these mutant lines had impaired spermatogenesis, folliculogenesis, or reproductive potential.

4.4 Ornamental Fish

Zinc finger nucleases (ZFNs) were used to disrupt genes in zebrafish (*Danio rerio*) both somatically and germinally. CRISPR/Cas nuclease system in zebrafish also represents a highly successful gene knockdown technique.Four endogenous loci—tyr, golden, mitfa, and ddx19—were also successfully targeted. The high incidence of mutagenesis demonstrates that biallelic mutations were present in the majority of cells.

Some other applications are in Pigmentation, Fast growing fish, Body configuration, Medicine, Viral and parasitic disease control.

5. RISKS ASSOCIATED WITH GENE EDITING

5.1 Off-Target Effects

Tools for gene editing may unintentionally change genes that weren't their original targets, which could have unanticipated effects and endanger the organism [23].

5.2 Incomplete Editing

Gene editing techniques might not produce correct or complete changes, leading to incomplete edits or unintentional genetic changes that could have negative consequences.

5.3 Unknown Long-Term Effects

We still don't fully understand how gene editing would affect an organism's long-term health, growth, and progeny. Over time, unanticipated side effects might appear.

5.4 Ethical Concerns

Since gene editing could impact future generations and create ethical issues about enhancement, eugenics, and consent, it is especially problematic when it involves changing the human germline (sperm, eggs, and embryos).

5.5 Unequal Access and Misuse

Social inequality may increase if gene editing becomes widely available and only the wealthy can buy it. In addition, there is a risk that gene editing technologies will be misapplied, possibly [23].

5.6 Regulatory Challenges

It is difficult to create adequate regulations to control the use of gene editing technology. This may require constant attention to strike a balance between promoting scientific advancement and guaranteeing responsible and secure implementations [14].

6. CONCLUSION

With the help of genome editing tools that are being used in both model fish species and those which are having economic importance, we are going into an era that will visualise an extensive adoption of these powerful gene editing technologies in a wide range of fish species in order to study functional genome or to create new genetically modified stains with special characteristics. This research highlights the enormous potential for using genome editing in aquaculture to create novel kinds with desirable features. So gene silencing and genomic DNA editing have the potential to have a significant impact on aquatic animal treatment in the future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bharati Jaya, et al. Genome editing in animals: An overview. Genomics and Biotechnological Advances in Veterinary, Poultry, and Fisheries. 2020:75-104. Available:https://www.sciencedirect.com/science/article/pii/B9780128163528000035
2. González Castro, Nicolás, et al. Comparison of the feasibility, efficiency, and safety of genome editing technologies. International Journal of Molecular Sciences. 2021;22(19):10355. Available:https://www.mdpi.com/1422-0067/22/19/10355
3. Khalil, Ahmad M. The genome editing revolution. Journal of Genetic Engineering and Biotechnology. 2020;18(1):1-16. Available:https://jgeb.springeropen.com/articles/10.1186/s43141-020-00078-y
4. Gutási, Anikó, et al. Recent Applications of Gene Editing in Fish Species and Aquatic Medicine."Animals. 2023;13(7):1250. Available:https://www.mdpi.com/2076-2615/13/7/1250
5. Martin Lainie P, Thomas C. Hamilton, Russell J. Schilder. Platinum resistance: The role of DNA repair pathways. Clinical Cancer Research. 2008;14(5):1291-1295. Available:https://aacrjournals.org/clincancerres/article-abstract/14/5/1291/179780
6. Silva, George, et al. Meganucleases and other tools for targeted genome engineering: Perspectives and challenges for gene therapy. Current gene therapy. 2011;11(1):11-27. Available:https://www.ingentaconnect.com/content/ben/cgt/2011/00000011/00000001/art00003
7. Tröder Simon E, Branko Zevnik. History of genome editing: From meganucleases to CRISPR. Laboratory Animals. 2022;56 (1):60-68. Available:https://journals.sagepub.com/doi/abs/10.1177/0023677221994613
8. Urnov, Fyodor D, et al. Genome editing with engineered zinc finger nucleases. Nature Reviews Genetics. 2010;11(9):636-646. Available:https://www.nature.com/articles/nrg2842
9. Chen, Fuqiang, et al. High-frequency genome editing using ssDNA oligonucleotides with zinc-finger nucleases. Nature Methods. 2011;8(9): 753-755.

Available:https://www.nature.com/articles/nmeth.1653
10. Carroll, Dana. Genome engineering with zinc-finger nucleases. Genetics. 2011;188(4):773-782. Available:https://www.nature.com/articles/nrg2842
11. Carroll, Dana. Progress and prospects: Zinc-finger nucleases as gene therapy agents. Gene therapy. 2008;15(22):1463-1468. Available:https://www.nature.com/articles/gt2008145
12. Hongyi Li, et al. Applications of genome editing technology in the targeted therapy of human diseases: Mechanisms, advances and prospects. Signal Transduction and Targeted Therapy. 2020;5(1):1. Available:https://www.nature.com/articles/s41392-019-0089-y
13. Wright David A, et al. TALEN-mediated genome editing: Prospects and perspectives. Biochemical Journal. 2014; 462(1):15-24. Available:https://portlandpress.com/biochemj/article-abstract/462/1/15/46840
14. Capps, Benjamin, et al. Falling giants and the rise of gene editing: Ethics, private interests and the public good. Human Genomics. 2017;11:1-10. Available:https://link.springer.com/article/10.1186/s40246-017-0116-4
15. Sarasin, Alain. Progress and prospects of xeroderma pigmentosum therapy. Molecular Mechanisms of Xeroderma Pigmentosum. 2008:144-151.
16. Ran F Ann, et al. Genome engineering using the CRISPR-Cas9 system. Nature Protocols. (2013;8(11):2281-2308. Available:https://www.nature.com/articles/nprot.2013.143 Available:https://link.springer.com/chapter/10.1007/978-0-387-09599-8_16
17. Lino Christopher A, et al. Delivering CRISPR: A review of the challenges and approaches. Drug Delivery. 2018;25(1): 1234-1257. Available:https://www.nature.com/articles/nbt.3659
18. Nakamura, Masaru, Ramji K Bhandari, Mikihiko Higa. The role estrogens play in sex differentiation and sex changes of fish. Fish Physiology and Biochemistry. 2003; 28:113-117. Available:https://link.springer.com/article/10.1023/B:FISH.0000030495.99487.17
19. Edvardsen, Rolf B, et al. Targeted mutagenesis in Atlantic salmon (*Salmo salar* L.) using the CRISPR/Cas9 system induces complete knockout individuals in the F0 generation. PloS One. 2014;9(9): e108622. Available:https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0108622
20. Wargelius, Anna, et al. Dnd knockout ablates germ cells and demonstrates germ cell independent sex differentiation in Atlantic salmon. Scientific reports. 2016; 6(1):21284. Available:https://www.nature.com/articles/srep21284
21. Kishimoto, Kenta, et al. Production of a breed of red sea bream Pagrus major with an increase of skeletal muscle mass and reduced body length by genome editing with CRISPR/Cas9. Aquaculture. 2018;495:415-427. Available:https://www.sciencedirect.com/science/article/pii/S0044848617324705
22. Zhu, Bo, Wei Ge. Genome editing in fishes and their applications. General and Comparative Endocrinology. 2018;257:3-12. Available:https://www.sciencedirect.com/science/article/pii/S0016648017306445
23. Xiong, Shuting, et al. Loss of stat3 function leads to spine malformation and immune disorder in zebrafish. Science Bulletin. 2017;62(3):185-196. Available:https://www.sciencedirect.com/science/article/pii/S2095927317300087

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/103568