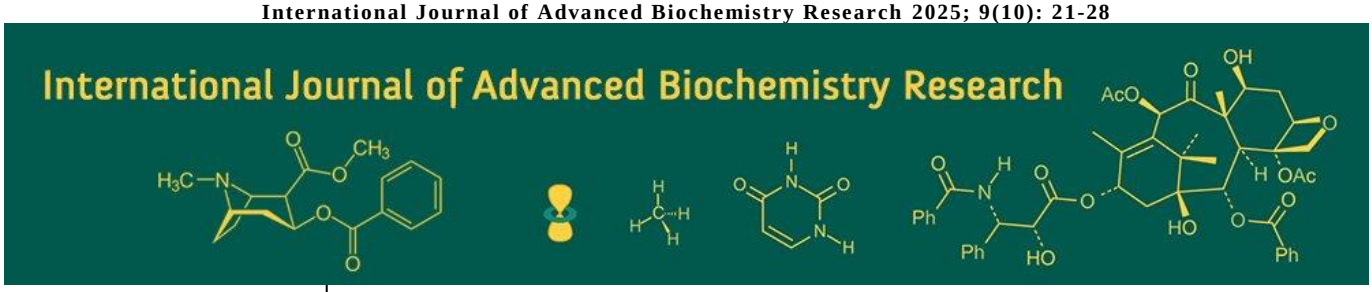

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Aquatic innovation: Genome editing routes to designer fish

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Abstract
Ornamental fish constitute a significant sector of the global aquaculture industry, contributing substantial economic value through the aquarium trade, eco-tourism, and export markets. Beyond their aesthetic appeal, they serve as important model organisms in biomedical and developmental research, while also supporting livelihoods in many developing countries. The demand for novel varieties with enhanced colouration, unique morphological traits, and improved resilience to environmental stress has increased markedly in recent years. Conventional selective breeding, although effective, is time-consuming and limited in its precision. Genome editing technologies, particularly the CRISPR-*Cas9* system, have emerged as transformative tools for accelerating trait development in ornamental fishes. CRISPR-Cas9 offers high precision, efficiency, and cost-effectiveness compared to earlier platforms such as transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs). Its application enables targeted modifications of genes influencing pigmentation (e.g., *mitfa*, *slc24a5*, *kit*), fin morphology, growth patterns, and stress tolerance. In addition to generating novel phenotypes of commercial interest, genome editing can improve disease resistance, enhance adaptation to captive environments, and produce sterile strains to mitigate risks of genetic pollution from accidental releases. This review comprises recent advancements in the application of genome editing in ornamental fish.

Keywords: Aquatic innovation, genome, CRISPR-Cas9, ornamental fish, transgenic

Introduction
The practice of raising fish in aquariums has grown to be a major hobby and interest for millions of people worldwide in recent years. After photography, aquarium maintenance is reportedly the second most popular pastime worldwide. Because of its enormous economic potential and opportunities, this industry is rapidly becoming more significant. It requires the least amount of room and care. Ornamental fish are used for a variety of purposes, including adornment, teaching for kids, entertainment, good fortune, and the collection or even propagation of uncommon species. A wide variety of ornamental fish can be found in India. More than 374 native freshwater ornamental fish species may be found in India. Additionally, there are more than 700 native species of decorative marine fish. About 9 billion dollars are traded in ornamental fish worldwide, of which 85% are freshwater species and the remainder are marine. With a global yearly growth rate of more than 10%, ornamental fisheries is one of the industries with the quickest rate of expansion. Additionally, the ornamental fish trade is growing domestically by 20% annually. Native species of freshwater ornamental fish account for 90% of India's exports. In 2023-2024, India exported 244 MT of ornamental fish valued at Rs. 28 crore (Shraborni, Mandal, & Parhi, 2024) MPEDA, 2024.
The primary ornamental species that account for over 14% of the trade include discus, angels, neon tetras, and gold fish. Together, guppy and zebra danio account for more than 14% of the trade export of ornamental fish. It is a major economic activity in over 125 countries, with Sri Lanka ranking sixth among these countries. Around US$18-20 billion is traded globally. The world's leading exporting nation, Singapore, is the EU's biggest importer and the biggest market for ornamental fish. With a high biodiversity of 374 native freshwater fish species, 700 native species, and over 300 exotic marine fish species, Indian ornamental fish are in high demand on the global market of these, 80% are traded from freshwater, with

20% coming from brackish and marine water. About 500 crore ornamental fish are traded domestically, and 8.40 crore are exported (2017-18), growing at an annual rate of 11.6%. There are 5,000 production facilities, with West Bengal accounting for 55% of the total, Tamil Nadu for 30%, Kerala for 5%, Maharashtra and other states for 7%, and the North East and islands for 3%.

Role of melanin in fish

Among the most stable and insoluble biochemicals are melanins, which are large molecular weight, polymorphous, multifunctional biopolymers (Jacobson, 2000) [23]. Primarily a pigment that absorbs light, eumelanin serves a variety of ecological purposes, including communication, camouflage, and photoprotection. Additionally, melanins' effective antioxidant and immunostimulant properties, which are consistent with their extracutaneous location, are making other physiological roles more apparent (McGraw, 2005) [38]. Fish xanthophores and erythrophores exhibit pteridine and/or carotenoid pigments; however, fish melanophores solely generate eumelanin and no pheomelanin (Bagnara & Matsumoto, 2006; Fujii, 1993; Kottler *et al*., 2015) [3, 10, 28]. In fact, melanin is the primary pigment used for visual communication, camouflage, and photoprotection. Melanophore contraction and dispersal are two physiological and environmental factors that cause color changes in aquatic organisms (Baker, 1993; Höglund, Balm, & Winberg, 2000; Nery & de Lauro Castrucci, 1997) [4, 17, 4]. All ectothermic creatures rely on melanin to regulate their body temperature. In particular, lighter bodies may be able to prevent overheating because they absorb less light, whereas darker bodies absorb more light (warmth) (Rudh & Qvarnström). Rudh and Qvarnstrom (Rudh & Qvarnström) assert that solar radiation heats darker genotypes more effectively. That explains the role of melanin in producing darker color in fishes. Melanin functions as an optical screen, reducing the amount of UV radiation that reaches the underlying tissue by absorbing wavelengths across the visible and ultraviolet spectrum (Kollias, Sayre, Zeise, & Chedekel, 1991) [26]. The scalloped hammerhead shark (*Sphyrna lewini*, Sphyrnidae), a cartilaginous fish housed in shallow water, was the first to exhibit morphological color change brought on by UV light (Lowe & Goodman-Lowe, 1996) [34]. Later, it was discovered that when subjected to artificially intense UV-B radiation starting two weeks after hatching, whitefish larvae (*Coregonus lavaretus* and *Coregonus albula*, Salmonidae) increased skin melanin concentrations (Häkkinen *et al*., 2002) [16].

Furthermore, because melanophores absorb radiation, which causes genotoxic stress, such pigmentation shields the skin from ultraviolet (UV) radiation (Lin & Fisher, 2007) [32]. Melanin pigmentation may in fact have a protective effect, as evidenced by UV trials using heavily and lightly pigmented Xiphophorus (Heckel, 1848) hybrids. Darker fish showed less UV-induced DNA damage (Ahmed & Setlow, 1993) [1]. Additionally, compared to UV-treated wild-type individuals, albino olive flounders, *Paralichthys olivaceus*, exhibited noticeably decreased survival rates after UV treatment (Fukunishi *et al*., 2017) [12]. According to (Meredith & Sarna, 2006) Pigmentation protects the skin and eyes from chemical stressors, and melanin pigments can also serve as antioxidants (Nilsson Sköld, Aspengren, & Wallin, 2013) [44]. Because of this, albino phenotypes raised in aquaculture may have worse well-being than wild phenotypes and may experience lasting health effects from exposure to direct sunlight while housed in open water, including damage to DNA, skin, or eyes or even death. When stocked in open water, these individuals should be closely watched, and species-specific shelter choices should be offered.

Role of genes in Melanogenesis

The same processes that cause physiological color change also cause changes in melanophore form and density during long-term background adaptation (Sugimoto, 2002) [54]. The peptide a-MSH, the most efficient pigment-cell dispersion agent, is believed to be the primary cause of the rise in melanophore density and dendricity through up-regulating the expression of melanogenic genes (Fujii*, 2000; Sugimoto, 2002) [11, 54]. Within 14 days, it was demonstrated that exogenous a-MSH increased the quantity and size of melanophores in Mozambique tilapia, as well as their melanin content (Van Eys & Peters, 1981) [55]. One important mechanism for vertebrate pigment phenotypes is thought to be the functional regulation of Mc1r, which is extensively expressed in fish skin and whose activation promotes melanin synthesis and melanosome dispersion in fish melanophores. Under a white background, the synthesis of the neurosecretory melano-concentrating hormone (MCH), an antagonist of a-MSH, is enhanced. This hormone not only inhibits skin melanin but also causes pigment cell aggregation. accumulation in different teleosts (Cánepa, Pandolfi, Maggese, & Vissio, 2006; Green, Baker, & Kawauchi, 1991; Nagai, Oshima, & Fujii, 1986; Sugimoto, 2002; Yamanome, Amano, & Takahashi, 2005) [8, 15, 42, 54, 58]. However, it has not yet been shown that MCH directly affects melanophore or other chromatophore apoptosis (Sugimoto, 2002) [54].

Melanogenesis Synthesis

Melanocytes

Melanogenesis synthesis starts during the embryonic stages of fish. These cells are derived from neural crest dendritic cells, present in the stratum basale layer of the epidermis of the skin. Melanocytes synthesise melanin with the help of melanosome and are involved in the transfer of melanosomes to the adjacent keratinocytes. They are present in the form of a network of dendrites and are involved in the dispersion of melanin pigment in the epidermis. Melanocytes are of two types, in which one is involved in the synthesis and transfer of melanosome (Secretory melanocytes) and the other is involved in the distribution and redistribution of melanosome from the perinuclear zone to dendrites (non-secretory melanocytes/melanophore).

Melanosome

Melanin is synthesised in cytoplasmic organelles called melanosomes, which reside in skin cells called melanocytes. Melanosomes are unique membrane-bound organelles present in the cytoplasm of melanocytes, where melanin synthesis, storage and transportation happen. Melanosomes are synthesized in four stages. The endosomal system gives rise to nonpigmented vacuoles known as stage I pre-melanosomes. After that, they develop distinctive interior striations (stage II). Stage III melanin pigment is applied to the striations, which finally results in the development of mature, fully melanized stage IV melanosomes (Raposo & Marks, 2007) [47]. Melanosome transfer to keratinocytes

occurs through 4 ways, in first the melanosome and melanocyte membrane fuses which leads to release of melanosome to intercellular space which through phagocytosis enters into keratinocytes (Exocytosis). In second way through cytophagocytosis of dendrite of melanocyte, and in other way it can occur through the space created when fusion of melanocyte plasma membrane with keratinocyte plasma membrane and lastly through shedding of melanosome filled vesicle which then gets into keratinocytes by phagocytosis.

Melanogenesis pathway

The process of producing melanin pigments, or melanogenesis, is typically carried out by cells known as melanocytes (Bonaventure, Domingues, & Larue, 2013; Borovansky & Riley, 2011) [6, 7]. Melanosomes are subcellular lysosome-like organelles found in melanocytes. They are responsible for the synthesis and storage of melanin pigments prior to their distribution to neighboring keratinocytes (Marks & Seabra, 2001) [37]. To develop and become capable of producing melanin, melanosomes need a variety of particular structural and enzymatic proteins. The Raper-Mason pathway is a sequence of spontaneous and enzymatic chemical processes that produces melanin in vertebrates from the phenolic amino acid precursor L-tyrosine (Borovansky & Riley, 2011) [7]. Mammals produce two types of melanin (from dopaquinone precursors): reddish yellow pheomelanin, which is reliant on the presence of sulfhydryl compounds in the melanosomes pheomelanin ratio, and brownish black eumelanin, which is produced from L-dopachrome (Lin & Fisher, 2007) [32]. Although melanin is crucial for skin homeostasis and tanning, which is a symptom of distress, it can also function as a photosensitizer after exposure to UV light, producing superoxide radicals that can cause fatal cellular damage (Slominski, Tobin, Shibahara, & Wortsman, 2004) [51]. Tyrosinase, a copper-containing membrane-bound enzyme found in melanosomes, catalyzes the rate-limiting step in melanin formation, the hydroxylation of L-tyrosine to L-DOPA (Borovansky & Riley, 2011; Videira, Moura, & Magina, 2013) [7, 56].

To act as the substrate for tyrosinase, phenylalanine hydroxylase (PAH) may transform L-phenylalanine in the cytosol into tyrosine (Videira *et al*., 2013) [56]. In addition to tyrosinase, melanosomes include TYRP1 and TYRP2, which are essential for catalyzing processes that produce eumelanin. Through its peroxidase function, TYRP1 has been proposed to raise the eumelanin: pheomelanin ratio and provide protection against oxidative stress (Videira *et al*., 2013) [56]. Cysteinyl dopa, a condensation product of dopaquinone and the amino acid L-cystein, is synthesized as part of the metabolic process that produces pheomelanin (Borovansky & Riley, 2011) [7]. Tyrosine and L-DOPA act as tyrosinase substrates during melanogenesis. For other cellular processes, they also operate as bioregulatory agents. Among these are the development of dendrites and the promotion of cell motility (by downregulating PKC) (Videira *et al*., 2013) [56].

Different ornamental transgenic fish

The market for ornamental fish is very large. As a result, a number of research institutions are working to create altered ornamental fish varieties by genetic and transgenic methods. In order to express certain colour genes, ornamental transgenic fishes are being created employing tissue-specific promoters or specialised colour genes. According to earlier research, stable zebrafish lines were created utilizing tissue-specific promoters like krt8 and mylz2, which were connected to color genes like RFP, GFP, BFP, YFP, and CYP (Gong *et al*. 2003) [14]. As a result, 'GloFish' has six eye-catching neon colour variations that are marketed under the names galactic purple, electrifying green, sunburst orange, moonrise pink, and cosmic blue (www.glofish.com). These model fish are utilized in numerous labs worldwide due to a variety of characteristics, including their short lifespan and external fertilization. The zebrafish-derived mylz2 promoter is used in several species for ornamental transgenesis, including rohu (*Labeo rohita*), skirt tetra (*Gymnocorymbus ternetzi*), and medaka (*O. latipes*) (Mohanta, Jayasankar, Das Mahapatra, Saha, & Barman, 2014; Pan, Zhan, & Gong, 2008; Zeng, Liu, Seebah, & Gong, 2005) [40, 46, 60].

Transgenesis and Genome editing in fishes

Zhu *et al*. (Zhu, He, & Chen, 1985) [61] introduced the rat growth hormone gene into the goldfish genome, resulting in the production of transgenic fish and establishing the goldfish as a model for investigating the expression of a gene in fish development and confirming its stability in the offspring. In a Recent study on goldfish by (H. Li, Wang, Zhang, Liu, & Zhu, 2024) [29], *TyrA* and *Tyr B* gene of goldfish disrupted using CRISPR/Cas9 for enhancing the colouration and depicting the successful potential of gene editing in goldfish. Gong *et al*. (Gong *et al*., 2003) [14] introduced *RFP*, *GFP*, *BFP*, *YFP*, *and CYP genes* in Zebrafish and resulted in the development of red, green, blue, yellow-colored transgenic zebrafishes. Thus, the fish muscle may be explored as another useful bioreactor system for the production of recombinant proteins. Later, Beirl *et al*. (Beirl, Linbo, Cobb, & Cooper, 2014) [5] use CRISPR/Cas9 to interrupt the *Oca2* gene in zebrafish melanophores, showing a reduction in the number of differentiated melanophores and hence pigmentation in the fish. Studies conducted on medaka introduced *GFP*, *RFP foreign* genes for producing a transgenic colored medaka variety for improving its ornamental trade. (Fang, Chen, Pan, & Wang, 2018) [9] introduced CRISPR/Cas9 for targeting the *TYR* gene into medaka fish. *tyr* is a key gene in the melanogenesis pathway and plays a major role in pigmentation development and coloration in fishes. This enhanced the appearance of medaka and showed the potential of CRISPR in enhancing coloration in fish. Similarly, the *mc1r* gene was targeted in Taiwanese loach and Common carp using gene editing technology CRISPR/Cas9 for enhancing color in these fish, as the *mc1r* gene is also critically associated with the melanogenesis pathway and results in alteration of pigments in fish. Similarly, Klaassen *et al*. (Klaassen, Wang, Adamski, Rohner, & Kowalko, 2018) [25] used genome editing for the *oca2* gene in Cavefish, which is also associated with the melanogenesis pathway in the fish body and results in fish colour alteration.

Ethical concerns of transgenic ornamental fish

However, ethical questions have been raised regarding the transgenic fish's sustainability, the permanence of the desired features, and the potential environmental effects of escapees. While Howard *et al*. (Howard, Rohrer, Liu, &

Muir, 2015) [18] reported that the transgenic trait was entirely eradicated after 15 generations and that the male GloFish was less competitive with the wild type for mates, Snekser *et al*. (Snekser, McRobert, Murphy, & Clotfelter, 2006) [52] reaffirmed that the social activities of GloFish were unaffected. Additionally, GloFish raised in natural headwater creeks matured earlier and laid smaller eggs (Magalhães, Brito, & Silva, 2024) [36]. Mosaicism, or the inability to achieve ubiquitous expression, was the main barrier to the development of GM fish. According to several researchers, this occurs primarily due to the transgene's random integration, delayed integration at the one-cell stage, host enzyme degradation, and inability to inherit the transgene (Moreau, Gamperl, Fletcher, & Fleming, 2014; Wu, Sun, & Zhu, 2003) [57]. Stable integration of foreign genes and DNA at the 1-2 cell stage of fertilized eggs is required for effective transgenesis in fish in order for future generations to inherit the transgene. Many genes, including GFP and RFP, have reportedly been used to track the transgene's effective integration during the early phase. Several labs are using transposable vectors to increase integration efficiency. Transposable vectors have produced effective GMOs and are being used in many labs to increase integration efficiency (Ivics *et al*., 2009) [22]. Transposable elements Tol2, which were primarily found in the genome of the Japanese medaka fish, have so far shown their utility in creating transgenic fish, including zebrafish and medaka (Ivics *et al*., 2009; Nishidate, Nakatani, Kudo, & Kawakami, 2007) [22, 45]. According to earlier research, GH transgenes in common carp impair growth and prevent consistent growth in their offspring (Lian *et al*., 2013) [31]. Concerns about ethics and the environment were mentioned as additional hazards connected to transgenic fish. Although transgenic fishes have not been shown to have any negative impacts on wild fishes, multiple research groups have warned that they could be a major hazard. The containment approach may be useful in preventing ecological harm and other ecosystem-related issues. These include creating sterile fish or confined growing transgenic fish in a specific location (Su *et al*., 2015) [53]. Common carp have been shown to produce sterile triploid fishes through intercrossing transgenic diploids with tetraploids (Zhu *et al*., 1985) [61]. Transgenic fishes could escape into the environment, breed with natural populations, and cause ecological harm, thus it's also not a good technology. Additionally, triploids may cause major issues for fish, such as phenotypic changes that impair the GMO's functionality. Several laboratories worldwide have indicated that it is fairly possible to create genetically modified organisms in aquaculture species, but due to a lack of money and support, model research facilities, including bio-safety equipment, are not accessible. The two biggest challenges facing fish transgenic research are public perception and food safety. Modern methods, including NGS and gene editing technologies, have emerged to comprehend and alter genes and genomes with the purpose of improving traits in order to allay those worries.

CRISPR Cas 9 (Gene Editing)

Gene editing has revolutionized the field of aquaculture breeding by providing precise tools to manipulate the genetic makeup of aquaculture fish species (Iqbal *et al*., 2025) [19]. Modifying key genes in fish is expected to rapidly enhance growth rate, disease resistance, and other economically important traits, leading to increased profitability, sustainability, and competitiveness of the industry (Yang *et al*., 2025) [59]. The flexible and popular gene editing tool CRISPR/Cas9 makes it possible to precisely alter an organism's DNA. CRISPR-Cas systems stand out as highly effective gene-editing tools**,** utilizing a unique RNA-based system (Jinek *et al*., 2012; Iqbal *et al*., 2023) [24, 20, 21] The benefits of CRISPR-Cas include its broad application to a variety of organisms, ease of reagent preparation, and the accessibility of reasonably priced commercial CRISPR products (Yang *et al*., 2025) [59]. For inducing specific genomic changes, the CRISPR-Cas9 system has surfaced as a potentially simple and effective substitute for ZFNs and TALENs (Gaj, Gersbach, & Barbas, 2013). CRISPR/Cas9-based genome engineering of zebrafish using a seamless integration strategy has been described (Luo *et al*., 2018) [35].

The CRISPR/Cas9 gene editing technology has shown significant potential for improving various traits in fish and aquaculture species (Iqbal *et al*., 2023) [20, 21]. This potent instrument has helped researchers learn a great deal about how critical physiological and production-related traits are genetically regulated. Researchers have used CRISPR/Cas9 to help create gene drives, which are genetic engineering tools that increase a gene's likelihood of being passed on to the following generation. This is accomplished using gene drives, which guarantee that the modified gene is inherited at much greater frequencies nearly 100% instead of the usual 50% inheritance rate. S. Li *et al*. (2021) [30] develop sterile, all-male populations of Nile tilapia using CRISPR/Cas9. According to their research, the growth rates of these genetically engineered tilapias were noticeably higher than those of typical mixed-sex populations. Numerous genetically modified fish have been created as experimental models for scientific studies in addition to the species bred for economic gain. The biological sciences and medical domains will be greatly impacted by the quick development of gene-editing technology. Functional gene knockout, gene mutation or correction, and epigenome editing are just a few of the techniques available for precise and targeted genome modification through the clustered regularly interspaced short palindromic repeats/associated nuclease (CRISPR-Cas9) platform (Liu, Yin, Wang, & Wang, 2019) [33].

Methods of introducing Foreign DNA or any other material

Microinjection

Direct-pressure microinjection using a micropipette is a crucial technique for delivering various impermeant substances into the cytoplasm or nucleus of both plant and animal cells. This method is the most straightforward way to explore the function and behaviour of intracellular components, create transgenic animals, or address male infertility issues. The article outlines the fundamental elements of a microinjection system. A standard microinjection setup consists of an inverted light microscope, a micromanipulator, a micropipette holder, a gas pressure regulator, a micropipette puller, glass capillary tubing, a micrometer syringe, and a vibration isolation table. Depending on the specific experimental requirements, more advanced systems can be built. For instance, one might enhance the system with features like a specimen incubator, a CCD camera, a shutter controller, epifluorescence equipment, digital image processing software, and a computer (Komarova, Peloquin, & Borisy, 2011) [27].

Components of a microinjection system

Microinjection is a widely used laboratory technique designed for introducing small quantities of substances like DNA, RNA, proteins, and other macromolecules into cells or embryos via a glass capillary. This process requires specialized equipment, including a microinjector, a micromanipulator, and a microscope. Researchers have employed microinjection to genetically alter various organisms, facilitating the creation of transgenic models, gene knockouts, and gene therapy approaches, all aimed at exploring the complexities of intracellular components. Microinjection continues to be a favored and effective technique for delivering DNA, RNA, and proteins into fertilized zebrafish eggs. This method is both straightforward and dependable. A microinjection pipette is filled with the solution of DNA or RNA and connected to a system that expels the solution using air pressure. A small quantity of this solution is injected into the embryo's cytoplasm before the pipette is removed, and the injected embryos are subsequently incubated for further development. Once the foreign DNA or RNA is inside the cells, it can be transcribed and/or translated by the developing embryos, allowing researchers to assess the functional impact of the resulting protein through various morphological, physiological, or molecular changes. Therefore, microinjection has become a common practice for creating transgenic fish, studying gene function through the overexpression of DNA or RNA, and tracing cell fate in early blastula embryos.

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Electroporation

Electroporation refers to the technique that employs brief high-voltage pulses to penetrate the cell membrane barrier. By applying an external electric field that slightly exceeds the cell membrane's capacitance, a temporary and reversible disruption of the membrane occurs. This induced permeable state allows for the introduction of a wide range of molecules into the cells. For smaller molecules, simple diffusion can be utilized, while larger molecules, like DNA, can enter through electrophoretic processes that exploit the destabilized membrane. Initially created for gene transfer, electroporation has expanded its applications to various molecules, including ions, drugs, dyes, tracers, antibodies, oligonucleotides, and both RNA and DNA. It has been effective in different contexts, including *in vitro*, *in vivo*, and in clinical settings, where it has facilitated drug delivery to malignant tumors. Early electroporation methods often inflicted considerable damage to cells; however, advancements over the years have led to improved equipment and refined protocols. Many laboratories could enhance their electroporation techniques with minimal adjustments.

The semipermeable characteristic of cell membranes can be momentarily disrupted by high-intensity electric pulses that last only microseconds and are measured in kilovolts/centimetre. Ion leakage, metabolite release, and improved cell absorption of medications, molecular probes, and DNA are the results of this disturbance. A frequent term for this phenomenon is "electroporation." Applying a high electric field to cell membranes can also cause cell lysis, bleb development, and membrane fusion. Studying the bioelectrochemistry of cell membranes is essential for understanding their structure and function, and this requires an understanding of electroporation and its associated effects. These occurrences are also seen in situations involving electric shocks to the heart during medical operations, electrocution, and electrical damage. Plasmids or foreign DNA can be introduced into living cells for gene transfection, cells can be fused to form heterokaryons, hybridomas, or hybrid embryos, proteins can be inserted into cell membranes, drug delivery can be improved to increase the effectiveness of chemotherapy for cancer, human cells can be fused with animal tissues to create animal models, membrane transporters and enzymes can be activated, and gene expression in living cells can be changed. Additionally, a brief synopsis of research on electroporation mechanisms is given.

Sperm mediated transfer

Transferring genes by directly soaking sperm in plasmid DNA in zebrafish is favored due to its simplicity and effectiveness for large-scale use. This approach requires no costly equipment. Sperm cells could serve as potential carriers of external DNA into the eggs following fertilization, leading to the creation of transgenic animals. The method known as sperm-mediated gene transfer (SMGT) is straightforward to implement in field studies and can be effectively used for species that reproduce through gametes. The concept of utilizing a sperm cell to introduce foreign DNA into an oocyte during fertilization is promising, but its feasibility remains uncertain. Since 1989, the literature has produced conflicting reports, and currently, there is no consensus on the matter. Considering the significant implications this technique could have for creating transgenic animals across both mammalian and non-mammalian species, this review aims to encapsulate the advancements made in this area. While certain aspects, like the attachment of DNA to sperm cells, are now well-supported by experimental evidence, the actual production of transgenic individuals still relies on contested findings. This review will critically analyze the key data available, equipping readers with the information needed to assess the effectiveness of this method objectively.

Retrovirus method

Retrovirus-mediated gene transfer is an effective approach for exploring gene functions. We have improved our capacity to generate reliable functional expression cloning methods by producing a variety of retrovirus vectors and effective packaging cell lines. In this study, we go over function-based screening techniques and retrovirus-mediated ways for examining gene functions. One useful technique for researching gene functions is retrovirus-mediated gene transfer. Our functional expression cloning techniques have greatly improved since we developed a variety of retrovirus vectors and effective packaging cell lines. In this study, we cover different function-based screening methods and investigate the retrovirus-mediated approaches used to investigate gene functions.

Recombinant retroviruses have been widely utilized for delivering foreign genes into mammalian cells. Numerous convenient packaging cell lines and vector plasmids have been made available, leading to the establishment of custom retroviral vectors as essential research tools in many labs. When compared to traditional gene transfer methods, retroviral vectors are highly efficient in gene delivery and do not pose detectable harm to target cells. Once inside the nucleus, the retroviral genetic material integrates into the chromosomal DNA, allowing for lasting incorporation and stable transmission to all descendant cells. Retroviral vectors can accommodate up to 8 kilobases of foreign gene sequences, which is sufficient for most gene therapy applications. Moreover, these vectors can be produced in large quantities while meeting stringent safety standards. Consequently, they have emerged as the preferred choice in 80% of clinical gene therapy trials that have been approved so far. Notably, there have been no reports of short-or long-term toxicity linked to their use in human gene therapy trials, which date back to 1989. Despite this strong safety record, there remains significant potential and necessity for developing enhanced retroviral vectors and packaging systems to promote further advancements in human gene therapy. The upcoming discussion will review existing retroviral vectors and highlight current areas in need of technological innovation.

Future perspectives

As we continue to investigate this technology, it is reasonable to foresee the potential application of genome editing in fish, which could significantly advance theoretical research and the genetic enhancement of preferred traits across various fish species. CRISPR holds broad potential in biomedical research; for example, it can be used to introduce or delete genes linked to diseases in fish, as well as in areas like regenerative biology, drug discovery, and toxicology. Furthermore, it can aid in developmental biology and genetics by helping us understand how specific genes contribute to development, pigmentation, and organ formation. Gene-edited ornamental fish may function as biosensors for environmental contaminants, with engineered varieties capable of fluorescing or altering their behavior in response to heavy metals, endocrine disruptors, or pesticides, thus serving as live indicators of water quality. Transgenic lines can also be utilized to observe hormone pathways and their disruptions in real time, particularly with the help of visible markers. By modifying ornamental fish, researchers can gain insights into natural processes; for instance, tweaking genes related to the melanogenesis pathway allows scientists to explore how coloration and camouflage have evolved. Altering genes that impact aggression, schooling behavior, or mating preferences provides deeper understanding of the genetic underpinnings of complex behaviors. Modifications of traits associated with sexual selection, like fin shapes or colors, enable experimental investigations into how new species might evolve based on mate choice. Additionally, ornamental fish could be used as platforms for synthetic gene circuits, where specific gene expressions are triggered by environmental changes. Lastly, engineered fish could participate in optogenetics experiments, responding to stimuli such as pH levels, temperature, or chemical exposures.

Conclusion

CRISPR/Cas9 technology shows an immense potential to advance the aquaculture ornamental industry as it improves fish genetics and promotes sustainable practices. The aquaculture sector must leverage gene editing technologies to create a more resilient, efficient, and environmentally responsible industry by decisively addressing current challenges and emphasising forward-looking research.. As we advance these developments, working together with academics, regulators, and stakeholders will be essential to achieving the long-term advantages of CRISPR/Cas9 technology in aquaculture. In the modern era, the aquaculture industry is essential to both global trade and food production. In recent years, numerous innovative techniques have successfully accomplished gene modification in a variety of fish species because of the biological advantages provided by fish models. According to these studies, gene editing techniques like the CRISPR/Cas9 approach are very effective and often used in aquaculture. Fish species with unique adaptations (like cavefish) and more evolutionarily basic species (like lamprey), as well as large, economically significant species (like Atlantic salmon), as well as model organisms (like zebrafish) and cell lines (like ZFL, SJD, and ZF4) are all

used with this technique. Future aquaculture production may undergo substantial changes as a result of the exact changes made to the genomic DNA of different fish species. These developments improve aquaculture's growth, reproduction, and disease resistance, among other qualities. Silencing gene expression also requires RNA interference, or RNAi. By providing an environmentally safe molecular tool, this novel technique enables RNAi-mediated gene suppression of certain target genes. These developments can have a major impact on the development of functional genomics and therapeutic applications in fish and crustacean species. In conclusion, it is now common practice in aquaculture to create mutant animals using particular gene editing techniques.

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