

# TRANSGENIC FISH AND ITS PROGRESS

## Abstract

Transgenic fish serve as excellent experimental models for basic scientific investigations as well as biotechnological applications. Transgenic fish of both cold water and warm water are fast growing and disease resistant species have been produced in various laboratories throughout the world including India. From the aspect of biotechnological applications, for scientific investigations, environmental toxicology and compensate for human needs the development of transgenic fish can serve as excellent experimental models. In the field of aquaculture and to support economic efficiency GH gene transgenic fish will be of great importance in the field of future biotechnology. For the fish farmers and human being transgenic fish are more economical, efficient food source and recreation.

**Keywords:** Transgenic fish, GH gene, molecular biology, DNA

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## I. INTRODUCTION

According to Ponzoni and Nguyen [1], introduction of exogenous genetic material (DNA) into a host genome for its stable maintenance, transmission and expression is termed as Transgenesis. The first successful production of genetically modified transgenic fish was carried out with rainbow trout and goldfish during the period 1984 and 1985, and more than 35 aquatic animal species have been produced through transgenesis. Transgenic fish are fish that contain an improved variation of genes or groups of genes transferred from another organism through a process of genetic engineering. In a typical transgenic process, the desired fish can be created by injecting bacterial artificial chromosomes (BACs) or single-cell embryos containing the desired DNA, including sequences with recognition sites for DNA-modifying enzymes, which allow random insertion of the DNA by molecular techniques [2]. When the foreign genes are taken out, the transgenic organism regenerates and the transgenic variety can be bred from that organism. Transgenic fish production has expanded to focus on five general applications: to enhance traits of commercially important species, to develop fish as bioreactors to produce bio-medically important proteins, to enhance the utility of fish as indicators of adverse health effects associated with exposure to toxic substances. Aquatic environments, to develop new non-mammalian models for comparative biomedical research and for functional genomics studies. Transgenic fish have been produced that could benefit the aquaculture industry, but regulatory agencies have not yet approved them for commercial use [3] to increase aquaculture production. As genetic variation is high in fish, there are many more opportunities for cultivation and genetic improvement than in other terrestrial animals [4].

Through Auto-transgenesis scientists of India are able to develop transgenic fish involving increases the copies of GH genes, leads to increase flesh content of fish. In Auto-transgenesis process generation time is shorter whereas breeding frequency is relatively higher in most of the fish species. The advantage of this process are production of a large number of genetically identical eggs by a single female fish and external fertilization which can be easily controlled by experimentally. Most disadvantage is the scarcity of piscine transgene but more than 8500 genes and cDNA are isolated, characterized and cloned through advanced molecular biology throughout the world [5].

### **Basic Concept For transgenic fish production following steps to be taken for gene transfer**

- Isolation of desired gene sequence.
- Later on, the desired gene introduced popularly plasmid Vector by using endonuclease and ligase enzymes.
- Plasmids are take in the bacteria for production billions of copies of gene.
- Plasmids are introduced into host linear DNA including new gene (e.g., GH gene) to develop individual (e.g., fish), and finally transmitted generation to generation.
- Stable information recorded for genetic improvement.

## II. GENE TRANSFER TECHNOLOGY

Various techniques such as calcium phosphate precipitation, direct microinjection, lipofection, retroviral infection, and electroporation and particle gun bombardment have been used to introduce foreign DNA into animal cells, plant cells, mammalian germ lines, and other vertebrates [6].

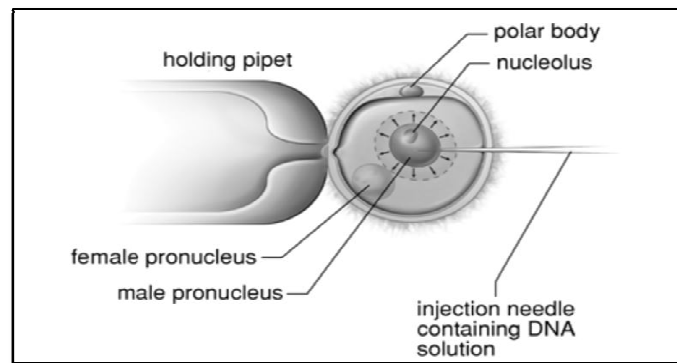
Chromosome manipulation and treatment with hormones are the most accepted methods of gene transfer technology to produce haploid, triploid, tetraploid and androgenetic and gynogenetic fish [4]. The most accepted and modern techniques for gene transfer are microinjection of sperm into fish, electroporation and incubation.

- 1. Construct of Transgene:** A transgene of the desired properties used in the production of transgenic fish for basic research or application must be a recombinant gene construct that produces the gene product at the desired level in the desired tissue at the desired time(s). Therefore, the prototype of the transgene is usually constructed in a plasmid to contain the appropriate promoter-enhancer element and structural gene sequence. Strong genetic signals are essential for the implementation of foreign genes and the expression of those foreign genes is enabled by either a promoter gene or an enhancer.

Depending on the purpose of the gene transfer study [6] transgenes are grouped into three main categories: (I) gain-of-function. (II) reporter function and (III) loss-of-function

- **Gain-of-Function Transgene:** To study gene function, the most informative ways has been to manipulate its expression in a model organism either through loss-of-function or overexpression [7]. Gain of function transgenes in transgenic individuals are designed to add new function or to facilitate the identification of the transgenic individuals if the genes are expressed properly. The structural genes containing transgenes of fish and mammalian growth hormone (GH or their cDNA) are fused to the promoter, which led to the greater production of growth hormones. Chicken and fish  $\beta$  actin gene promoters are the example of the gain of function transgenes [6]. GH transgene are produced through growth hormone genes from mammal and fish linked to appropriate promoter-enhancer element and a structural DNA sequence. The expression of GH transgene in the animals leads to increased growth of transgenic individuals.
- **Reporter Function Transgene:** A reporter gene is an exogenous coding region fused to a promoter sequence or element in an expression vector that is introduced into cells to provide a means to measure promoter activity. Once expressed in cells, the strength of the fused promoter element is assayed by directly measuring the reporter protein or by assessing its enzymatic activity, correlating the amount of reporter produced [8]. These functional genes are usually used to identify a successful gene transfer attempt. Examples of reporter function transgenes are bacterial chloramphenicol acetyl transferase (CAT),  $\beta$  -galactosidase or luciferase genes fused to functional promoters [6].

- **Loss of Function Transgene:** Missing transgenes are engineered to disrupt the expression of host genes that encode catalytic RNAs and cleave specific mRNAs, inactivating normal gene product production [5]. The engineered gene is placed in a fertilized egg or embryo and the transgene is combined with the genome of each egg or embryo cell [4].
- 2. Microinjection Procedure of Eggs and Embryo:** The most successful and widely used technique for gene transfer is microinjection in the production of transgenic fish and is a commonly used technique due to its simplicity and reliability. In this method, DNA is microinjected with a microinjection needle directly into the male pronuclei of fertilized eggs or embryos at the one-cell stage [9, 10]. It has been reported that DNA injected into the cytoplasm of fertilized eggs of zebra fish can integrate into the fish genome and be inherited in the germ line. The frequency of germline transmission of microinjected DNA can be as high as 20% in zebra fish [11]. Microinjection of foreign DNA into embryos was first used in goldfish and then applied to different fish species [9, 12].



**Figure 1:** Micro-injection Apparatus to introduce DNA

Depending on the chorion of the egg membrane, where the softness of the membrane facilitates successful microinjection and the thick membrane limits the injection of DNA. The chorion of fish eggs is tough and opaque and is recalcitrant to insertion of glass micropipettes resulting in less efficient gene transfer [ 9 , 13 ].To overcome this problem following methods can be useful:

- Insertion of the injecting needle through micropyle of the egg.
  - Opening on the chorion through microsurgery.
  - Chorion membrane digestion with enzymes.
  - Initiation fertilization and softening of chorion by using 1mM glutathione.
  - The unfertilized eggs will be injected directly.
  - Intra-nuclear microinjection.
- **Steps of Technique**
    - At the optimum conditions desired eggs and sperms are stored separately.
    - Initiation of fertilization by adding water and sperms.
    - Eggs are dechorionated by trypsinization after ten minutes of fertilization.

- Apparatus for injection contains a dissecting stereoscopic microscope and two micromanipulators, one with glass micro needle and other with a micropipette holding the embryo in proper place for delivering transgenes.
- Routinely, within first two hours of fertilization fertilized eggs are micro-injected and about 10<sup>6</sup> -10<sup>8</sup> molecules of a linearized transgenes with or without plasmid DNA (about 20nL) is injected into the egg cytoplasm [6].

So, depending upon species of fish, survival rates of microinjection seems to be 30-80%.

- **Merits or Advantages of Microinjection Technique**

- DNA can be delivered in optimum quantity per cell to increase the chances for integrative transformation.
- To improve the integrative transformation into nuclei of target cell.
- Very small fragmented DNA can be microinjected.
- Host range independent.

- **Demerits or Disadvantages of Microinjection Technique**

- Time consuming process as single cell can be injected at a time.
- Specialized skills and sophisticated instruments is required.
- Injection to more eggs is limited due to restricted time.
- Transformation rate is low.

**3. Electroporation Process of Gene Transfer:** Electroporation is the safest, easiest, fastest and most convenient way to transfer genes. In this method, high voltage electric current is used to introduce DNA and foreign genetic material into the cells of many different organisms [9, 14]. Transfection of foreign genes involves maintaining the cells in an appropriate transfection buffer. High voltage electric shocks are used to make the cells more porous so that foreign genes can easily enter the cells, then the transfected cells are diluted and grown in a non-selective medium. Appropriately selected cells were isolated and evaluated for transgene insertion [9, 14].

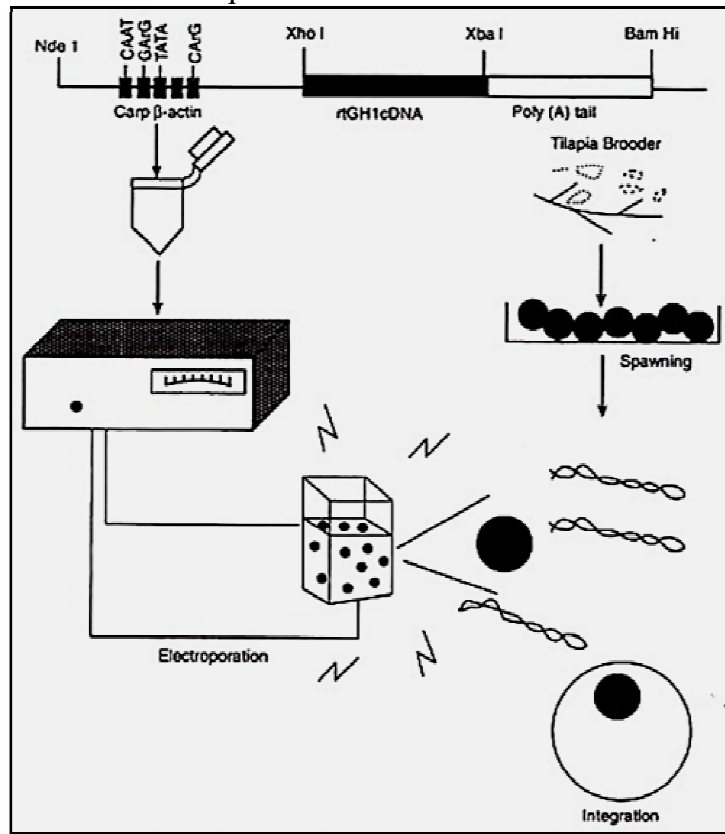
A chain of electrical impulses occurs through the cell membrane, so that Na<sup>+</sup> molecules enter the embryo. The cell membrane is temporarily exposed to DNA by exposing the cell to a short electric current. With the help of an electric current, the desired DNA fragment is placed in direct contact with the protoplast membrane. Finally, the hole can be created and stabilized by favourable dipole interaction with the electric field. The survival rate of DNA integration in the electroporated embryo is more than 25%, which is slightly higher compared to microinjections.

Electroporation can be used by introducing transgenes into spermatozoa [15] either before fertilization [16] or directly into the fertilized embryo [17]. This has been used with success in vivo results in fish [18], shrimp [19], Nile tilapia [20] and medaka and salmon, changes that are transmitted through the germline [21].

- **Advantages or advantages of the electroporation technique**

- Simultaneous input of DNA constructs.
- The microinjection process is not suitable for very small eggs, if applicable.

- Specialized skills not required.



**Figure 2:** Electroporation Gene Transfer Technique [22]

- Antifreeze Protein Gene Transfer:** Fish that live in the icy waters of the polar region produce a series of antifreeze glycoproteins (AFGP) or antifreeze proteins (AFP) to protect against freezing. Without changing the melting point, this protein acts to lower the freezing point of the solution. A unique property of these proteins is "thermal hysteresis", which binds AFGP and AFP to ice crystals and inhibits ice crystal growth. There are three types of AFP and one type of AFGP with similar anti-freeze properties, but the protein structure is slightly different. A fourth type of AFP has also been reported in longhorns.

*Salmo salar* cannot survive in sea water temperature (-0.6 ° C to -0.80 ° C) due to the lack of AFGP or AFPs genes, posing a major challenge for cage farming on the North Atlantic coast. Fletcher *et al.* [23] cold-resistant AFP or AFGP genes were created in salmon by gene transfer technology. They used a genomic clone (2A-7) from *Pleuronectus americanus* as a candidate for gene transfer encoding a major liver strain.

AFP (wflAFP-6, formerly known as HPLC-6). AFP Type I flounder AFPs are a diverse family of 80-100 copies that encode small high-alanine polypeptides and two different isoforms, liver and skin AFPs. Hepatic-type AFPs are only synthesized in the liver as precursor AFPs (wflAFP-6 or wflAFP-8 (HPLC-8), while skin-type AFPs are synthesized more peripherally as cellular mature AFPs (including wfsAFP-2). in tissues.) and wfsAFP-3).

- 5. Growth Hormone Gene Transfer:** Scientists have recently cloned and sequenced growth hormone (GH) and carbonic anhydrase (CA) genes in grass carp and common carp [24]. The grass carp CA gene promoter was ligated to GH-cDNA to generate a high-efficiency expression vector, pCAZ. Thus, the scientist was able to develop a "whole fish" model of growth hormone.

The CAT gene (pCA grass carp GH), used as a receptor gene, was inoculated into inseminated carp and immobilized by micropyle to produce transgenic "sticky fish" carp. Reverse transcriptase PCR and Northern blotting confirmed the presence of the transgene, and these transgenic fish showed a growth rate of approximately 137% of the control.

- 6. Disease Resistance Gene Transfer:** To combat grass carp haemorrhagic virus (GCHV) disease, scientists have tested genes that help fight the disease. This gene, which encodes 11 different gene fragments, was cloned and isolated using a GCHV single-gene genome fragment from in vitro translation.

Based on cDNA data of SP6 and SP7 capsid protein genes, three oligonucleotides were synthesized, joined to the SV40 MT promoter, introduced into carp cells Cytokine Inducible Killer (CIK) by the constructed expression vector, and transformed by GCHV. As a result, this newly developed gene reduces mortality from GCHV infection.

- **Applications of Transgenic Fish:** For the following purposes Transgenic Fish may be used:
  - Increasing fish production rate to compensate the demand of protein food with increasing human population.
  - Fish originated product for pharmaceutical and other industrial purposes.
  - Aquarium purposes development and propagation of transgenic native glow fish.
  - To monitor aquatic pollution as fish acts as biosensor.
  - To isolate genes, promoters and synthesize effective gene constructs.
  - Research on embryonic stem cells and in vitro embryo production.
  - To produce antifreeze proteins.
- **Transgenic Fish Growth:** In the current scenario, the development and research of transgenic fish is mainly done on salmon, trout, carp, tilapia, etc., which provide protein sources. Many of these engineered fish grow faster than their wild or conventionally farmed cousins. Today, about 40-50 laboratories around the world are working on developing genetically modified fish.

Typically, fish growth hormone genes are transferred from one fish species to another to achieve rapid growth in fish production. These fish are marketed in a shorter period of time and are fed more efficiently. Growth hormone (GH) from carp has been used to produce transgenic carp for production in mud ponds and improved feeding characteristics.

- **Transgenic Salmon:** There are two types of salmon: Atlantic salmon and Pacific salmon, and Atlantic salmon has Pacific salmon growth hormone controlled by a polar antifreeze enhancer gene. These transgenic salmon did not grow as fast when GH was introduced. As a result, research scientists created a new GH gene in which

all the genetic elements were derived from salmon and changed the growth hormone gene in Coho salmon, *Onkorhynchus kisutch* [25]. By inserting a new GH gene, transgenic coho salmon grew an average of 11 times faster than wild fish, and 37 times faster in the largest fish.

In these transgenic fish, GH levels do not decrease in winter, as in normal salmon, and GH levels remain high throughout the year [26]. Unlike conventional salmon, this transgenic salmon reaches the market after one year, while conventional farmed salmon takes at least three years.

- **Transgenic Zebra Fish:** The Zebrafish (*Bmchydanio rerio*) is an aquarium fish genetically modified to produce a fluorescent red pigment, also known as the Goldfish. This zebrafish has been sold in the USA since 05.01.2004 without regulatory approval.

A novel zebrafish transgene contains three vibrantly colored fluorescent proteins, namely green fluorescent protein (GFP), yellow fluorescent protein (YFP), and red fluorescent protein (RFP or dsRed), expressed in the muscle-specific mylz2 strong promoter [27]. These fluorescent proteins are visible to the dark eye in daylight and in ultraviolet light. Green fluorescent protein (GFP) was first isolated from jellyfish (*Aequorea victoria*). Madurai Kamaraj University (MKU), Center for Cell and Molecular Biology (CCMB), Hyderabad and National Matha College, Kollam, India have started transgenic fish research in collaboration with foreign scientists. Indian scientists from Madurai Kamaraj University (MKU) created the first transgenic fish in 1991. Recently, Indian scientists developed transgene rohu fish, zebra fish, catfish and singhi fish. Local gene origins, progenitors and vectors are now available for engineering development from rohu and singhi. Madurai Kamaraj University produces transgenic rohu.

### III. CONCLUSION

For aquaculture development and commercial production, aquatic animals are being engineered in various laboratories throughout the world. Through proper utilization with efficient and safe management the production and applications of transgenic fish technology solved major problems and drawbacks in aquaculture as well as biomedical research. Further progress and careful research work should be taken when DNA sequences of fish is utilizing to increase public acceptance and avoid sequences of bacterial or viral origin. This technology is rapidly developing but consumers and environmentalists remain cautious to ensure the safe use of transgenic technology and thus increase public blessings.

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