Unit III TOPIC 5 (Additional material)

**Gene Transfer Technology for Development of Transgenic Fishes:**

The most commonly used methods in fish biotechnology are chromosome manipulation and hormone treatments, which can be produced triploid, tetraploid, haploid, gynogenetic and androgenetic fish.

Other popular methods of gene transfer in fish are microinjection, electroporation of sperms, electroporation of eggs and incubation of sperms. Following are the main steps in gene transfer for development of transgenic fish.

***A. Preparation of DNA Construct:***

Desired transgene should be a recombinant gene or DNA construct, which is constructed in plasmid that contains an appropriate promoter-enhancer element and a structural DNA sequence.

The foreign genes are typically introduced with strong genetic signals, promoters and/or enhancers, which enable the foreign genes to be expressed at very high levels continuously (or constitutively), effectively placing those genes outside the normal metabolic regulation of the cell, and of the transgenic organism resulting from the trans­formed cell.

There are three main types of transgenes:

(1) Gain-of-Function:

These transgenes are able to increase particular function in transgenic individual after their expression. For example growth hormone genes from mammal and fish linked to appropriate promoter-enhancer element and a structural DNA sequence to produce GH transgene.

This GH transgene when express in transgenic individuals increases production of growth hormone leading to enhanced growth of transgenic animal.

(2) Reporter Function:

These transgenes are able to identify and measure the strength of promoter-enhancer element.

3) Loss of Function:

This transgene is not yet used for modification of transgenic fish. Such transgenes are used for interfering with the expression of host genes. The promoter-enhancer elements of transgenes are linked to a growth hormone gene of fish.

Hence transgenic fish contain extra DNA sequences that are originally derived from same species. Gene construct is then introduced into fertilized egg or embryo, so that transgene be linked to genome of each cell of egg or embryo.

***B. Gene Transfer by Microinjection:***

Microinjection is most successfully and widely used technique for gene transfer in fish. One method of microinjection technique involves the use of fine injection needle for introducing DNA into cut site in the cell. While doing so it destroys those cells that are in direct contact with the injected DNA.

To ensure the integration of the DNA it should be injected to intact cells close to the cut site. The injection apparatus consists of a dissecting stereomicroscope and two micromanipulators, one with a glass micro-needle for delivering transgene and other with a micropipette for holding fish embryo in place

The success of microinjection technique depends on the nature of egg chorion. The soft chorion facilitates the microinjection while the thick chorion limits the ability to visualize the target for injection of DNA. In many fishes (Atlantic salmon and rainbow trout) the egg chorion gets tough and hard just after the fertilization or to contact with the water and provides a difficulty in injecting the DNA.

But using the following methods can solve this problem:

(1) By using the micropyle (an opening on the egg surface for sperm entry during the fertilization) for inserting the injecting needle.

(2) By using microsurgery for making an opening on the chorion.

(3) By digesting the chorion with enzymes.

(4) By using 1mM glutathione for initiating fertilization and reducing hardness of chorion.

(5) By direct injection to the unfertilized eggs.

Another technique of gene transfer is intra-nuclear microinjection, which involves direct physical approach using a fine needle to deliver DNA into cell or even nuclei.

To facilitate rate of microinjection protoplast with partially reformed cell wall may be attached to a solid support with artificially bound substrate -without damaging the cells. Solid support may be of either glass cover slips or slides.

**Steps of Microinjection Technique:**

(1) Desired eggs and sperms are stored separately at the optimum conditions.

(2) Add water and sperms and initiate the fertilization.

(3) Ten minutes after the fertilization, eggs are dechorionated by trypsinization.

(4) Fertilized eggs are microinjected with desired DNA just within a few hours of fertilization. DNA is released into the centre of the germinal disc to the first cleavage in dechorionated eggs. The time available for microinjection is first 25 minutes and that too between fertilization and first cleavage.

(5) After microinjection the embryos are incubated in water until hatching takes place.

Survival rates of microinjected fish embryos is seem to be about 30-80% depending the fish species.



**Advantages of Microinjection Technique:**

This technique has the following merits:

(1) Optimum quantity of DNA can be delivered per cell, increasing chances for integrative transformation.

(2) The delivery of DNA is precise, even into nuclei of target cell again improving chances for integrative transformation.

(3) The small structure can be injected.

(4) It is a direct physical approach, hence it is a host range independent.

**Disadvantages of Microinjection Technique:**

(1) A single cell can be injected at a time, hence it is time consuming process.

(2) It requires sophisticated instruments and specialized skills.

(3) Limited embryonic time restricts injection to more eggs and a low transformation rate.