

A conceptual illustration featuring a fish, possibly a salmon, positioned inside a large, stylized DNA double helix. The fish is oriented horizontally, with its head to the right and tail to the left. The DNA structure is composed of thick blue ribbons forming the sugar-phosphate backbones, with thinner blue rods representing the nitrogenous base pairs. The background is a solid teal color. A white rectangular box with a drop shadow is centered over the image, containing the text 'Transgenic fish'.

Transgenic fish



TRANSGENIC FISH

- ➡ Transgenic fish means fish that have genes or groups of genes that have been transferred from another organism through the process of genetic engineering
- ➡ Transfer of transgene into the nucleus of a target cell where integration into the host genome takes place.




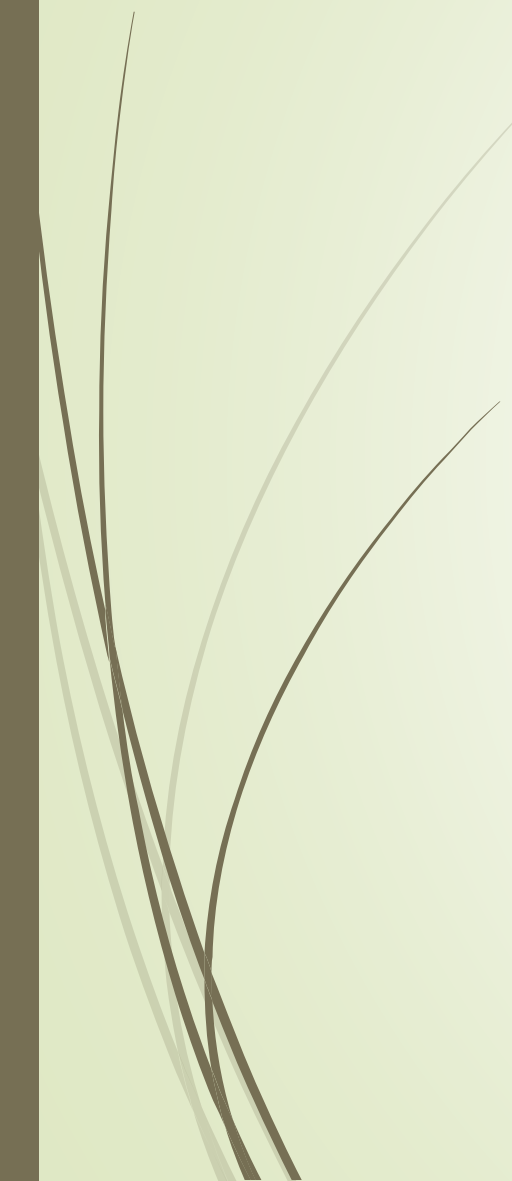
GOALS

- The primary goals for genetic manipulation of fish species used in aquaculture are to
 - a) Intensify the growth and efficiency of food conservation
 - b) Increase tolerance to environmental variables, such as temperature and salinity
 - c) Bring out new colour variants of ornamental fishes
 - d) Develop disease resistant fishes



HISTORY

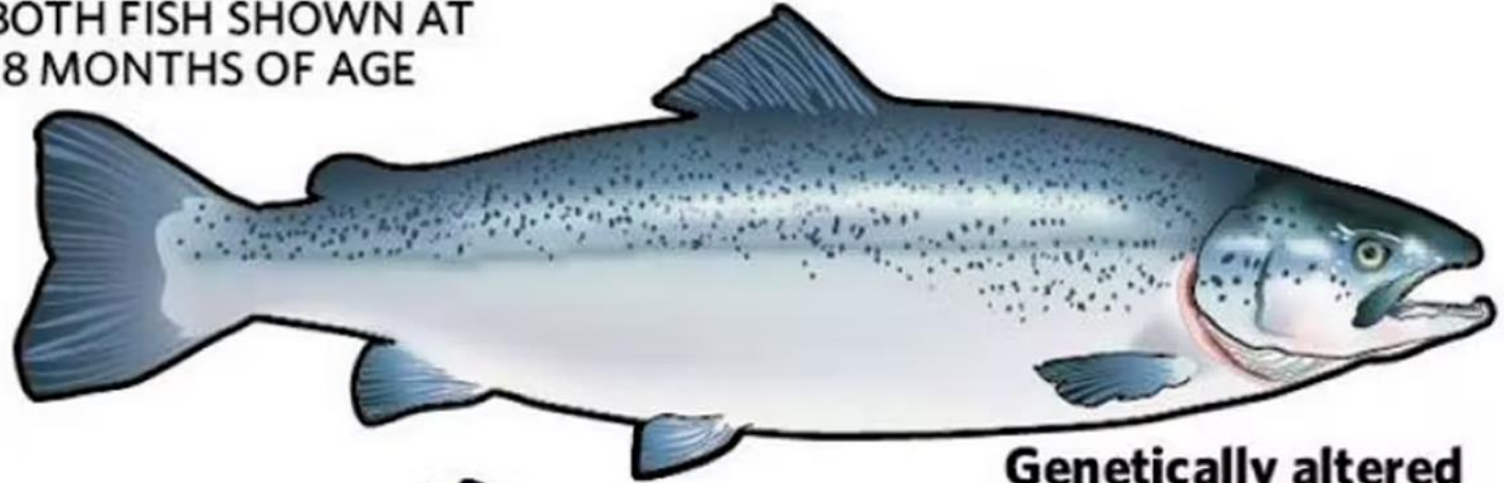
- The first report of the generation of transgenic fish in the aquaculture field was in goldfish (Zhu et al. 1985) and rainbow trout (*Onchorhycus mykiss*)
- Growth hormone gene has been targeted as a transgene aiming to increase the growth rate in more than 20 teleost species.
- Transgenic fish show better gross food conversion, the increase in fish weight per unit of food fed

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- U.S. Food and Drug Administration (FDA) approved the request to market GM fish as pets in 2003
 - The first GM animal commercialized was **GloFish**, a zebrafish with a fluorescent gene to glow in the dark under UV light
 - In 2015, the U.S. FDA approved the first edible commercial product, the genetically modified **AquAdvantage Salmon**
 - However, the commercial use of genetically modified fish, particularly in aquaculture, is still under debate in many countries



The value-added aquarium fishes have already been commercialized, such as „GloFish“ with six attractive fluorescent color combinations, including Starfire red, cosmic blue, electric green, galactic purple, sunburst orange and moonrise pink.

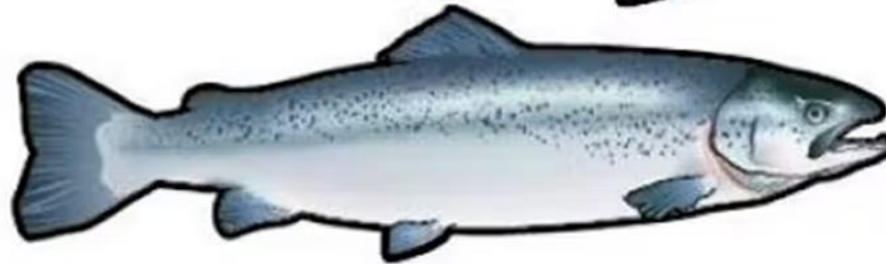
BOTH FISH SHOWN AT
18 MONTHS OF AGE



Genetically altered

Length: 24 in. (61 cm)

Weight: 6.6 lb (3.0 kg)



Normal DNA

Length: 13 in. (33 cm)

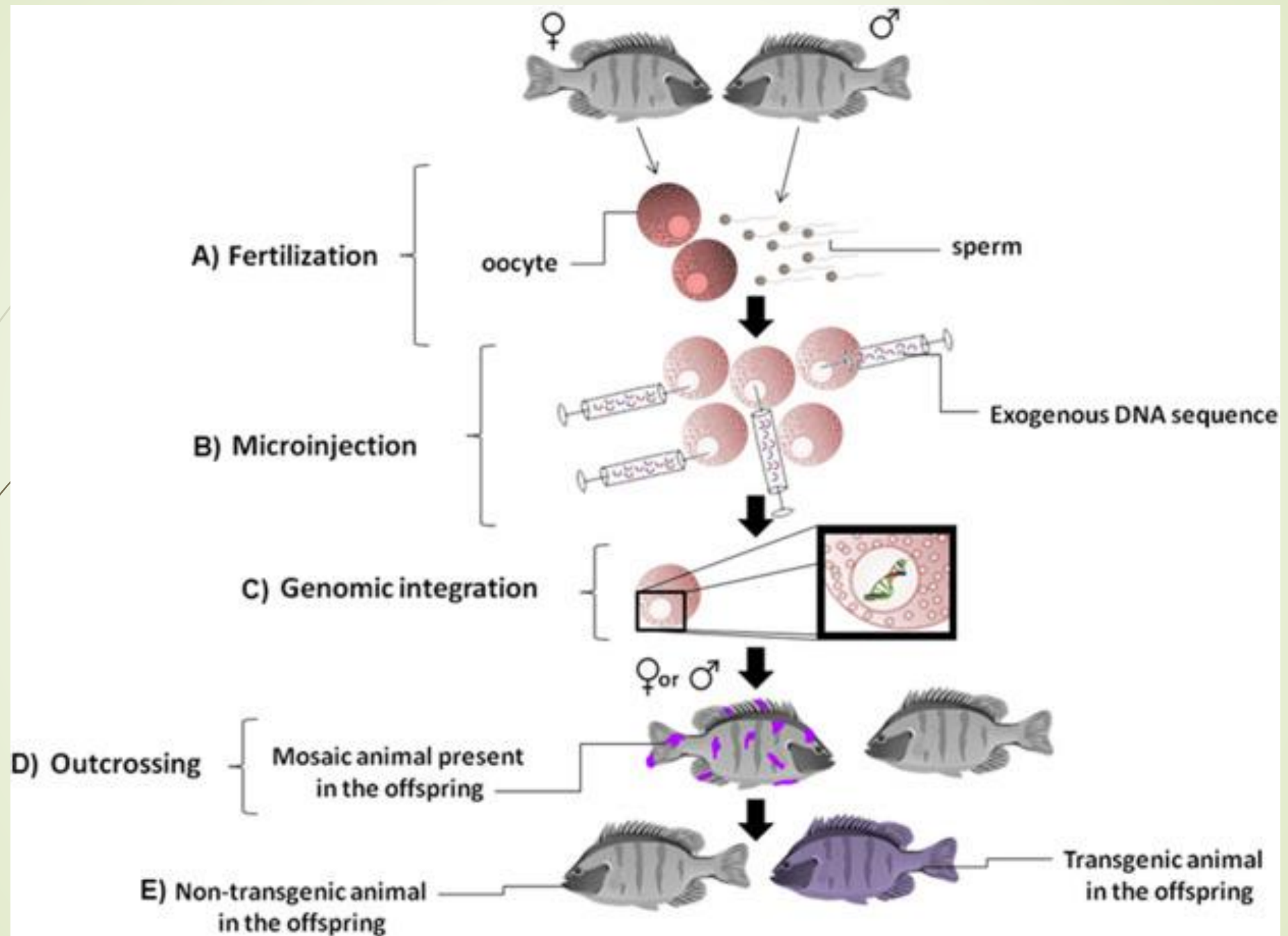
Weight: 2.8 lb (1.3 kg)

- AquaBounty AquAdvantage salmon can reach adult size in 16 to 28 months instead of 36 months for regular Atlantic salmon. These transgenic salmon eat 25 per cent less feed and are about 20 per cent more efficient at converting that food to flesh



GENETICALLY MODIFIED FISH PREPARATION VIA GENE TRANSFER

- (1) selection of the appropriate parental fish line**
- (2) construction of a functional expression vector including a promoter and target gene**
- (3) transferring the target DNA into fish embryos;**
- (4) screening and identification of transgenic F1 individuals**
- (5) Selection of F2 individuals with the same genotype**



Steps 1 + 3: Preparation of injection solution
10 ng μl^{-1} plasmid A (fluorescent reporter)
10 ng μl^{-1} plasmid B (gene of interest)
with 7.5 units *I-SceI*
in Yamamoto/*I-SceI* buffer

Step 2: Preparation of fish for spawning
Setup zebrafish matings or separate
medaka males from females the day before

Incubate at room temperature for 1 h,
then on ice

Steps 4 + 5: Collection of embryos
Transfer of embryos to the injection plates
Medaka: in pre-chilled 1 \times Yamamoto's medium
Zebrafish: in 30% Danieau's medium

Step 6: Injection of embryos
Inject into cytoplasm of one-cell-
stage embryos

1–2 hours

Step 7: Screening for
reporter gene expression
Screen for expression of fluorescent reporter
gene by fluorescent binocular microscopy

1 to several d
according to
promoter used

Step 8:
Raise GFP-positive F0 founder
fish to sexual maturity

2–3 months


Step 9: Identify founders for gene of interest
Outcross F0 to wild-type and screen embryos
for expression of gene of interest

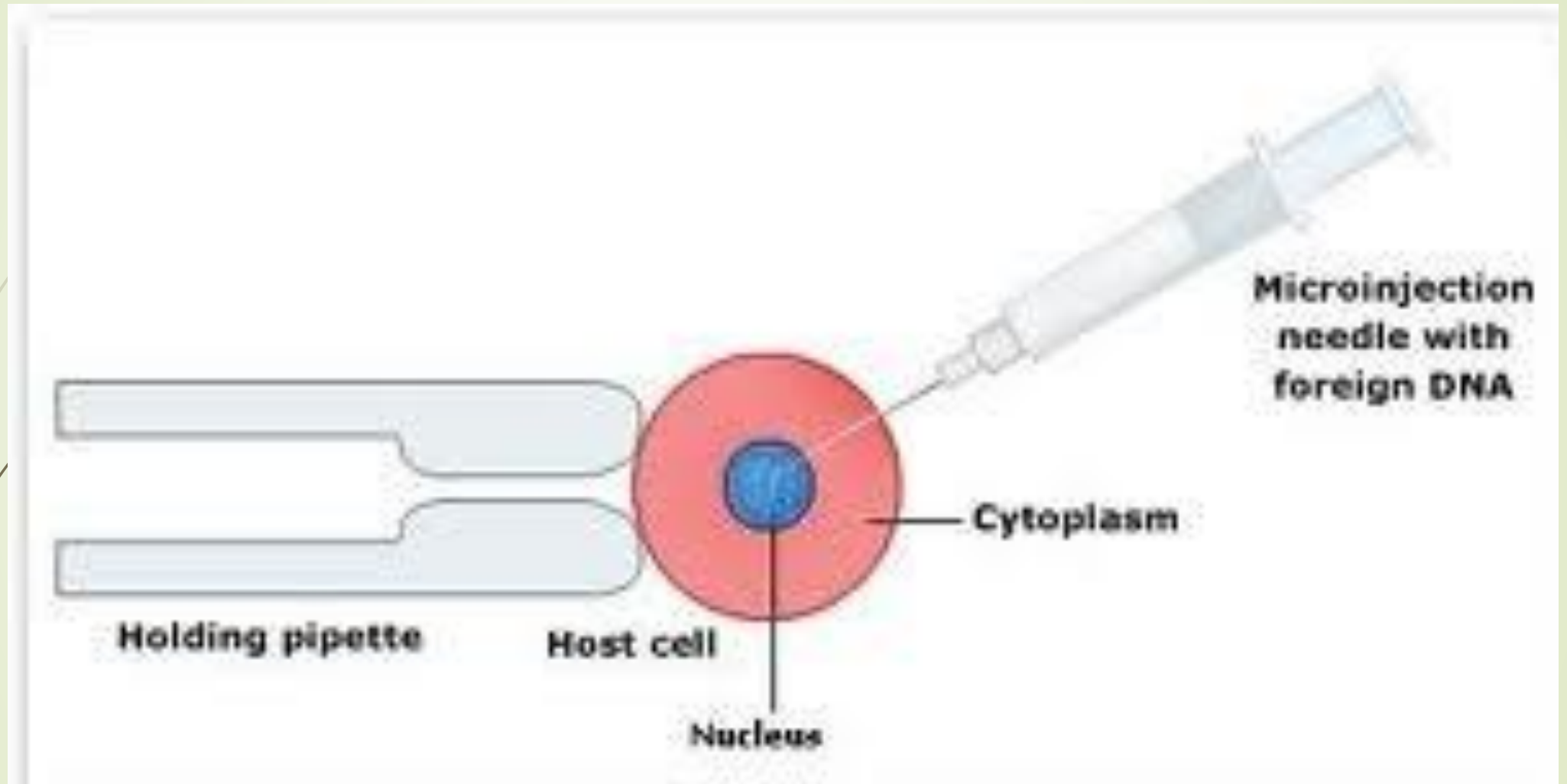
Step 10: Establish a transgenic F1 generation
Raise transgene-positive F1 embryos
identified in Step 9 to adulthood



METHODS

MICROINJECTION METHOD

- successfully in the production of transgenic fish
 - commonly used technique due to its simplicity and reliability
 - Results in higher survival rates for manipulated fish embryos than the electroporation method
 - The most established method for gene transfer in fish is microinjection
 - Microinjection that allows delivery of the transgene directly into the nucleus.
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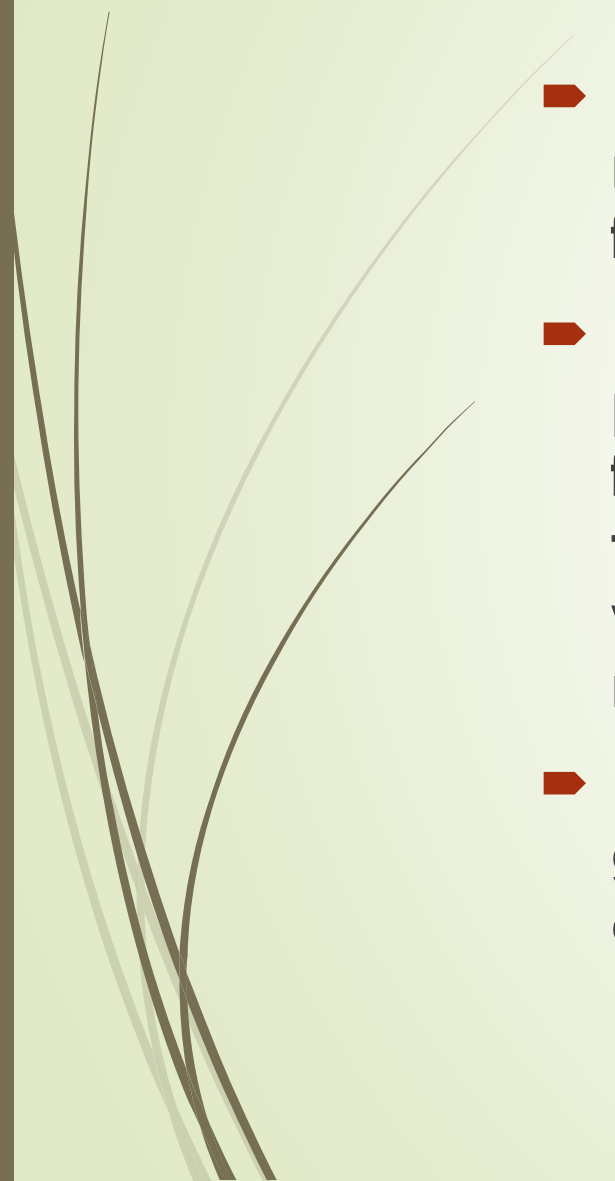


Disadvantages

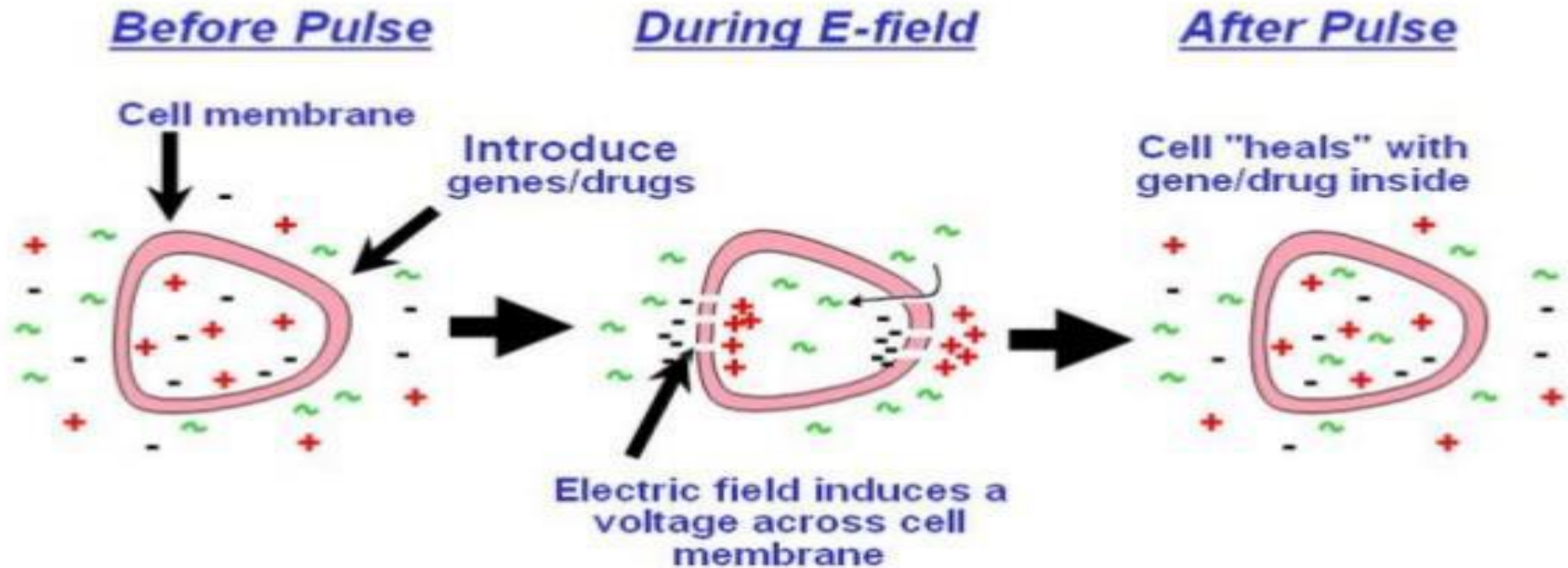
- Time-consuming and labour intensive
- The nuclei of fish eggs are small and difficult to visualize
- The chorion, hardens soon after fertilization and in many fish species the pronuclei of fertilized eggs is not visible and transgenes are usually injected into the egg cytoplasm.
- Low efficiency of generation of transgenics
- To improve the efficiency of selection of transgenics, genetic markers are co-injected with the transgene to monitor for transformed zygotes.
- The Green Fluorescent Protein (GFP) from Jellyfish (*Aequorea victoria*) has been used for this purpose in zebrafish



Electroporation

- Electroporation has been shown to be the most effective means of gene transfer in fish since a large number of fertilized eggs can be treated in a short time by this method
 - Electroporation utilizes a series of short electric pulses to permeate the cell membrane that make possible the formation of temporary pores on the surface of the target cells through which the transgene is introduced into the cytoplasm where it is then delivered to the nucleus by the cellular machinery
 - Electroporation has been preferred in many laboratories for gene transfer in fish systems because of its efficiency, speed and simplicity
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Electroporation Cell Process






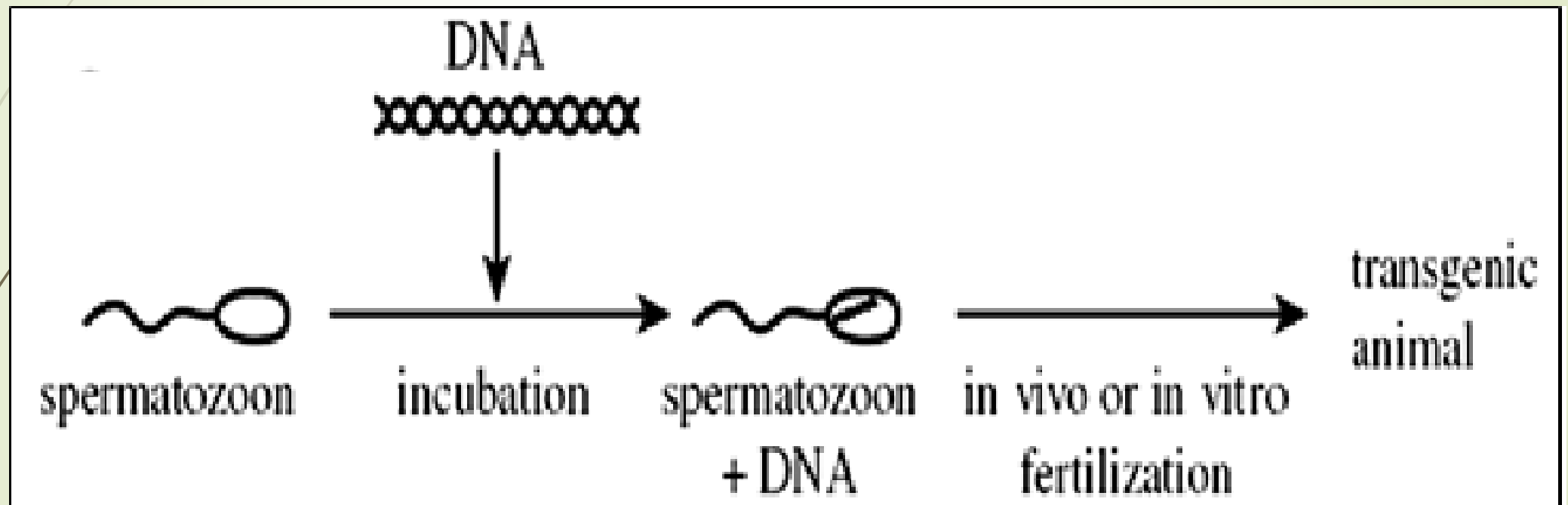
Disadvantages

- The presence of a tough chorion layer around the fish eggs reduces efficiency, removal of the chorion is a tedious procedure and introduces additional stress on newly fertilized eggs
- The efficiency of the electroporation reduced because of voltage, number and frequency of pulses



Sperm mediated gene transfer method

- **SMGT is a transgenic technique that transfers genes based on the ability of sperm cells to spontaneously bind to exogenous DNA and transport it into an oocyte during fertilization to produce genetically modified animals.**
 - **SMGT have low efficiency and it is mainly due to low uptake of exogenous DNA by the spermatozoa.**
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Retroviral infection method

- The successfully used retroviral vector or constructs reported to produce transgenic crayfish and top minnows, *Poeciliopsis lucida*.
- Retroviral vector has been used as a tool for transducing sea urchin embryos.
- This is a valuable gene transfer technique for fishes, in general.
- But, introduction of viral sequences into food fish may not be accepted by the public, but it could be used for ornamental fish development.
- **Disadvantage:** The preparation of retroviral particles including the transgene of interest is a very laborious process, increases costs and requires technology.



Current and potential applications

➤ Growth rate

- The trait of growth rate has received the greatest attention because of its importance in aquatic animal production systems
- Two- to threefold increases relative to non transgenic fish have been reported for tilapia and Atlantic salmon, and up to twofold increases in common carp



➤ Freeze resistance

- Resistance to freezing is the second major application of transgenesis to fish, but it is restricted to one species, the Atlantic salmon.
- The idea is to use “antifreeze” genes from fish that live in polar regions..

➤ Disease resistance

- If successful, developments of increased disease resistance could result in reduced production losses and less use of antibiotics to control disease
- Whereas there are promising results with channel catfish in the United States, there is still more work to be done
- Some fear that disease-resistant fish could be toxic to humans or become reservoirs for pathogens.

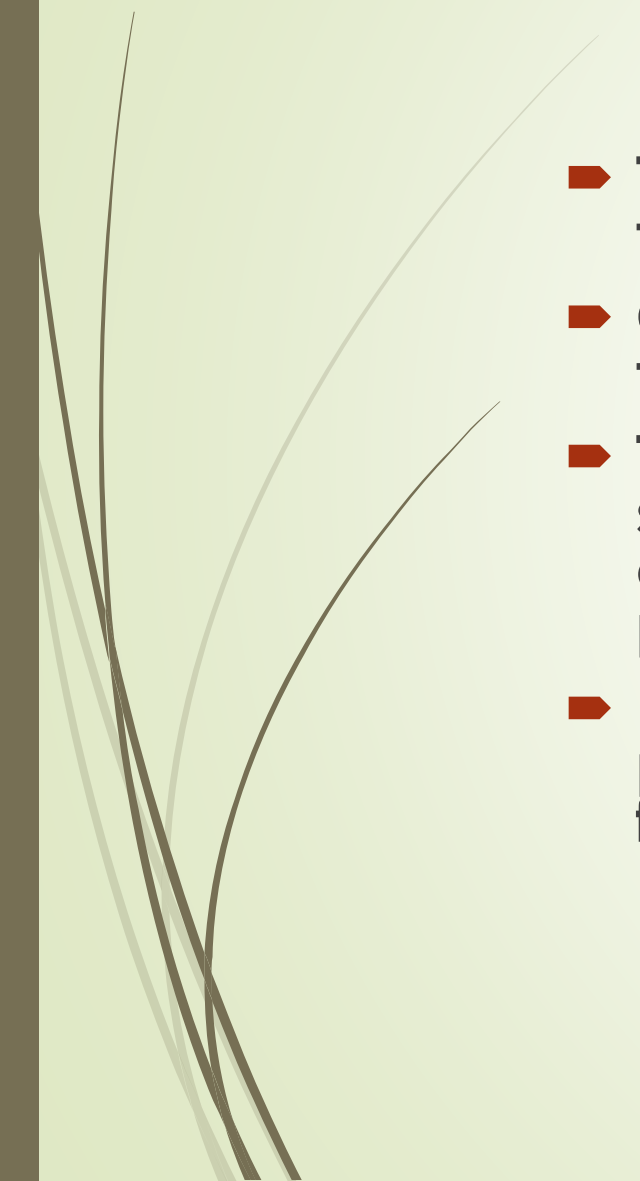




Metabolic modification

- If the carbohydrate metabolism of the fish could be improved, they could better use diets based on ingredients of land origin, such as soybean meal and vegetable oils.



Advantages of transgenic fish



- The growth rate of Transgenic fish can be increased by 400% to 600%
 - Can reduce feed input by up to 25% per unit of output, thereby improving food conversion ratios
 - Transgenic fish have been developed for various applications such as the experimental models for biological research, environmental monitoring, ornamental fish and aquaculture production.
 - Use of transgenic fish as bioreactors for the large-scale production of rare human therapeutic proteins or novel foods for specific dietary requirements.
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- Transgenic lines of tilapia engineered to produce human clotting factor VII, which is used in liver transplants and in treating injuries
 - Development of transgenic animal models represents a revolutionary advance in the study of a variety of disease processes
 - Development of transgenic fish as a model in reducing or replacing selected mammals used in toxicity testing
 - Medaka fish has several characteristics that are especially well suited for environmental toxicology, including well-characterized histopathology
 - They have been used extensively in chemical hazard testing, carcinogenesis bioassays and germ cell mutagenesis studies



Disadvantages of transgenic fish

- Mosaicism, the condition in which not all cells in embryos have the targeted transgene, can occur
- Risk of involuntary escape of transgenic fish into environment.
- Concerns of interbreeding with native fish populations and effects resulting from heightened competition for food and prey species
- Reduced milt production in the GH transgenic Nile tilapia

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- **Extremely fast-growing transgenic salmon and loach have low fitness and can die**
 - **Only about one out of every 100 eggs microinjected will stably incorporate the recombinant DNA sequence into its genome and subsequently transmit the transgene to its progeny**
 - **Transgenes is the unnatural in the sense**
 - **Transgenic fish could produce new or modified proteins that could be toxic to humans, they generally show reduced swimming ability and lower reproductive performance than non-transgenic fish**

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- **The transgenic fish are also more active and aggressive when feeding, and more willing to risk exposure to predation**
 - **The transgenic salmon pose serious ecological threats to wild populations and are not engineered for natural environments**
 - **Transgenesis can result in an unpredictable number of copies and site of integration of the insert**



THANK YOU