RECOMBINANT DNA TECHNOLOGY AND ITS APPLICATION IN FISHERIES SCIENCE

1. INTRODUCTION

Genetic recombinant is the word used to describe the exchange of genetic data through DNA fragments belonging to the similar species. Nevertheless, because to technological advancements, it is now possible to artificially transfer genes between different species.

Genetic engineering, which is a developing area of biotechnology, produces recombinant DNA molecules by separating DNA from various species, breaking it apart, and merging it together. Aquaculture and fisheries utilize recombinant technology successfully in many different types of applications. For instance, the production of fish vaccines against other pathogenic bacteria, recombinant therapeutic proteins and antigens that rarely occur in living organisms, and the manufacturing of genetically engineered fish with a rate of development that is greater than that of typical fish are all examples of products that fall under this category. The development within the fishing industry for the blue revolution is effectively supported by modern technology. According to its definition, recombinant DNA technology is "the joining together of DNA fragments of different organisms and inserting them into a genome of an organism that hosts them to produce new genetic combinations". DNA fragments often originate from a wide range of biological scenarios [1].

One of the most significant elements of biotechnology is recombinant DNA technology. In 1973, Boyer and Cohen created it.

2. RECOMBINANT DNA TECHNOLOGY TOOLS

Restriction enzymes, also referred to as "molecular scissors," are essential in order to improve recombinant DNA technologies. They make cut the DNA molecule after identifying particular sequences. An example EcoR1 cuts at GAATTC and BamH1 cuts at GGATCC. A different enzyme used to join a pair of divided DNA molecules together is DNA Ligase, also referred to as "molecular glue". Other enzymes that are used for recombinant DNA technology include kinase, topoisomerase, alkaline phosphatase, methylase, and Taq polymerase. The gene of interest is transmitted by the DNA molecule vector, which is utilized to introduce foreign genetic information into another cell. The rDNA copy or expression has been carried out in a host organism like *E. coli* [2].

Examples – restriction enzymes, cloning vector, host organisms.

1. **Enzymes for Restriction**

Helps with cutting, ligases in binding, and polymerases in synthesis. The site at which the target gene is inserted into the vector genome is determined in large part by the

restriction enzymes used in recombinant DNA technology. The two types are exonucleases and endonucleases. While endonucleases make cuts inside the DNA strand, exonucleases remove nucleotides off the leading ends of the strands. The restriction endonucleases cleave DNA at specified locations and are sequence-specific. Many times, these sequences are palindrome sequences. They measure the length of the DNA and cut it at a specific spot called the restriction site. This causes sticky ends in the sequence. Complementary sticky notes are produced by cutting the required genes and the vectors using the same restriction enzymes. This facilitates the desired gene's attachment to the vector via the ligases [3].

2. **Vectors**

Since they are the last carriers of the target gene inside the host organism, they are a crucial component of the instruments used in recombinant DNA technology. Plasmids and bacteriophages are the most often utilised vectors in the field of recombinant DNA due to their large copy numbers. The origin of replication, a series of nucleotides from which replication starts, a selectable marker made up of genes resistant to particular antibiotics, such as ampicillin, and cloning sites, which are sites where needed DNAs are inserted and recognised by restriction enzymes, are the components of the vectors [3].

3. **The Host organism**

where recombinant DNA is introduced. Recombinant DNA technology's perfect tool is the host cell, which takes the vector made with the suitable DNA using enzymes [3].

RECOMBINENT DNA TECHNIQUE IN FISHRESES SCIENCE

There are five significant uses for the technology [4]

1.Gene therapy: isolating the desired gene (DNA sequence).

2. Isolating that specific gene

3. The Isolated Gene is Inserted into a Vector;

4. The Modified Vector Is Introduced into a Host

5. Transformed Host Cell Selection

6. The host-introduced gene's expression

1. Isolation of Gene Interest (DNA Sequence) - Gene Therapy

The primary objective of recombinant DNA technology is to develop the gene (DNA sequence) containing genetic combinations or information that may be used, among other things, in agriculture, medicine, and other fields. Researchers in the field of medicine could be interested in producing insulin to treat individuals with diabetes mellitus.Therefore, it is necessary for scientists to detect and isolate the gene (INS gene)

that causes humans to produce insulin. To isolate the necessary genes or a particular sequence of DNA, it is essential to extract the organism's DNA and isolate it from among the other macromolecules, which including lipids and proteins. In basic terms, this indicates that the cell has to be ruptured in order to get to the DNA. Depending on the type of tissue, different enzymes can be employed [4].

While proteases assist in removing proteins connected to DNA, cellulase is utilised to break down the inner cell walls of plants. Lysozyme is often employed to dissolve the cell walls of bacteria. In the process of purifying DNA, other treatment techniques are also applied (fig. 1).

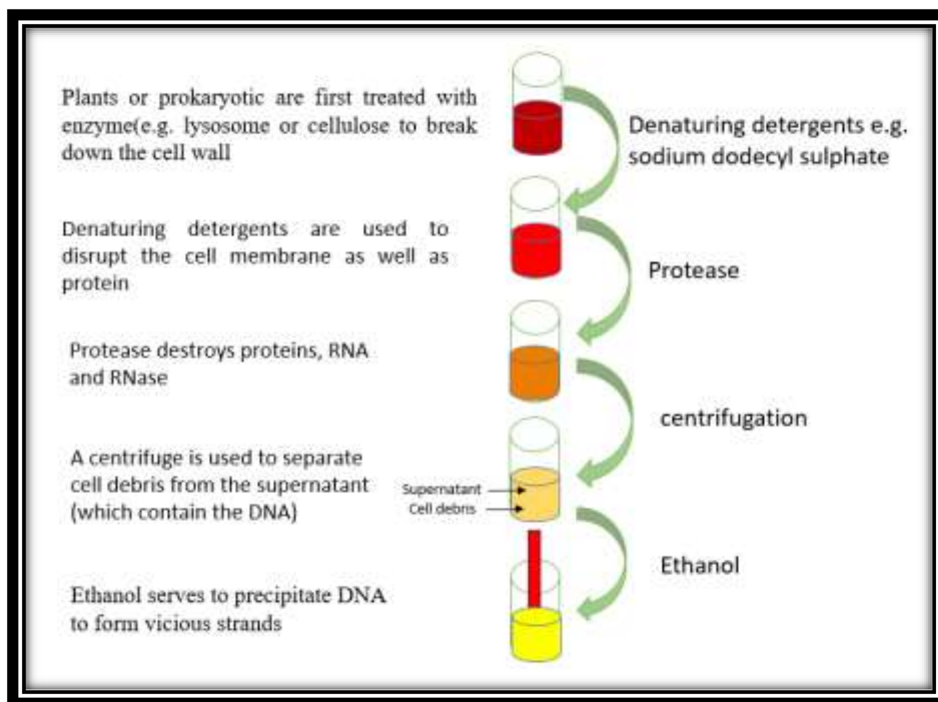

Figure 1: Overview of the separation and purification of DNA

As demonstrated in picture, the cell membrane that covers the cell membrane is broken down using enzymes like cellulase and lysozyme. The denaturation of surfactants is used to disintegrate cells without a cell membrane, for example, the majority of cells in eukaryotes, in order to eliminate their contents. Cell membranes are ruptured by detergents that act on the surface of cells, such as ethyl trimethyl ammonium bromide and sodium dodecyl sulphate, releasing the contents of the cell (organelles, etc.).Protease, an enzyme that acts on and breaks down proteins, RNA, and RNAs, is used to treat cells when the cell wall is broken, liberating the contents of the cell. It has been demonstrated that proteases destroy nucleases and enzymes. As it helps to purify

the DNA, this is especially significant. In a healthy state, the DNA is linked to these macromolecules.

After different enzymes are employed to break down macromolecules (proteins, for example), cell debris is filtered in a centrifuge so that it settles at the bottom of the tube and the resulting solution, which contains the DNA, can be retrieved. Finally, ethanol is used to precipitate DNA and retrieve it [4].

2.Isolation of the gene of interest

Using restriction enzymes, the desired gene is extracted from the purified DNA. In its most basic form, a restriction enzyme is a type of enzyme that only breaks the DNA strand at a certain site (the region with a specific nucleotide sequence) when it identifies a particular sequence.The enzymes act like molecular scissors because they may effectively cut the DNA at certain locations to extract the target gene (DNA sequences similar to the INS gene).This is often achieved by allowing a particular restriction enzyme to incubate on DNA (purified DNA), cutting the DNA molecule at predefined locations, and encouraging enzymatic activity.Due to restriction enzyme digestion, a single-strand tail can grow at both ends of the fragment, producing uneven ends (sticky ends). These ends, which are also called cohesive ends, are composed of base pairs that ultimately connect to the complementary base pairs of the vector.[4]

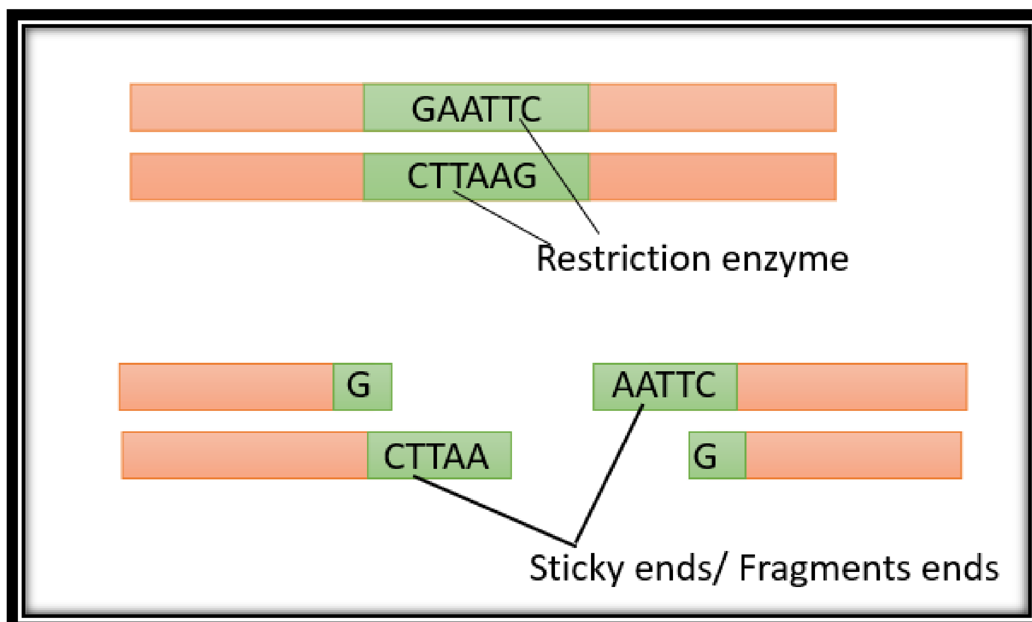

Figure 2. sticky ends are shown

If two DNA fragments were created via the same constraint nuclease or digestive enzyme, they could be linked together via cohesive ends.Using the polymerase chain reaction, an amplification technique, additional copies of the target gene can be produced when there are less copies than are required for cloning. This technique makes it possible to multiply a small number of gene copies into thousands or even millions of copies.They can also be obtained from: besides laboratory isolating genes of interest [4].

- cDNA library,
- genomic library.

3. Integration of the Separated Gene into the Vector

The genes that were isolated in the first step of recombinant DNA technology are inserted into a suitable vector in the second step. A vector is essentially a carrier that can deliver the desired gene into a specific cell, where it is duplicated during cell division.It's important to remember that the gene of interest cannot be injected straight into a cell after being separated from the DNA molecule. This is so that it won't be mistaken for anything foreign and destroyed. Because of this, a vector (such as a plasmid) is crucial in delivering the gene to the cell so that it may be duplicated during regular cell division. DNA molecule which replicates by itself is referred to as a vector. As a result, DNA has the ability to reproduce on its own. A plasmid is a nice illustration of a vector. Circular DNA, also referred to as plasmid DNA, is present in the cytoplasm [4].

Given that it contains genes for resistance to antibiotics, for bacteria, this double-stranded circular DNA is essential. Apart from plasmids, another form of vector is called a bacteriophage lambda.

- For any given vector to be used in recombinant DNA technology, it has to fulfil the following criteria:
- Possess the capacity to self-replicate.
- Have a promoter region, which is a section of the DNA that helps the target DNA's transcription.
- A selectable marker is necessary because it enables the bacterial identification carrying the desired gene, which is transported by the plasmid. It also allows for the separation of bacteria from non-recombinant cells.
- Possess antibiotic-resistant genes, which allows for identification of the target cells as well as conferring antibiotic resistance to the bacteria.
- include a cloning site, which is the location where an exogenous DNA segment is added following a restriction endonuclease.

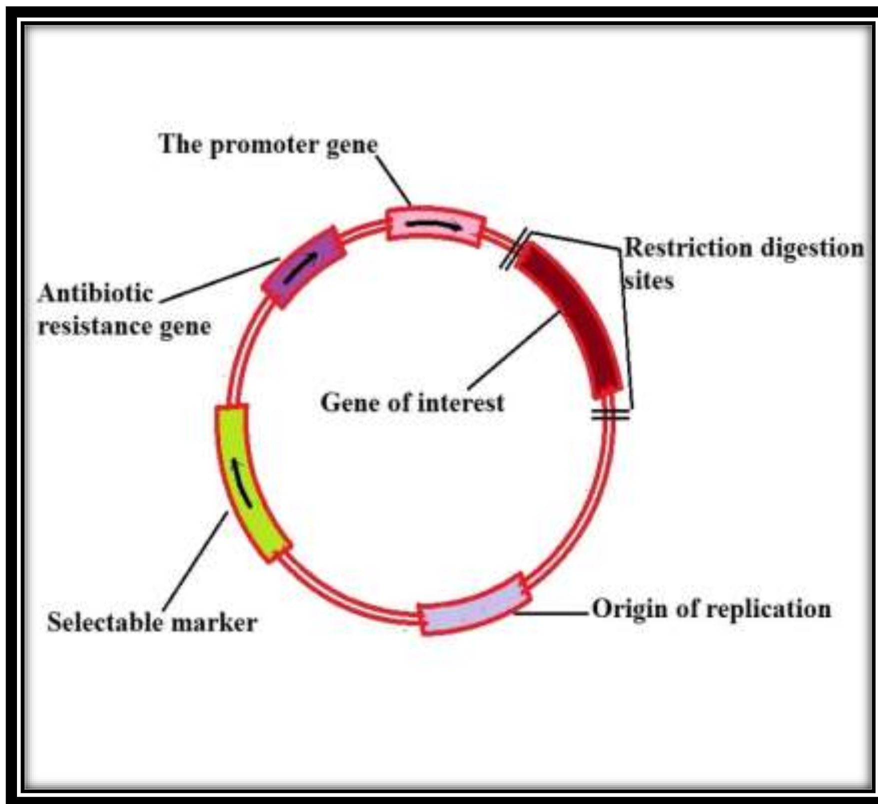

Figure 3. Diagrammatic Structure of a Plasmid for Recombinant DNA Technology

4. Transformation - Introduction of Modified Vector into a host

The third stage of recombinant DNA technology, called transformation, is inserting the modified plasmid, or recombinant DNA, into the host cell, such a bacterium. The primary objective of this step is to recover significant amounts of the DNA molecule.One of the most often utilised hosts is the bacteria E. coli. Their quick growth (doubling time of 20 minutes or less) and inexpensive cost of usage are the reasons behind this. As a result, reaching the stationary phase is quick.It's crucial to remember that while E. Coli and other bacteria are easy to work with, they are not receptive to foreign genetic material. Because of this, handling these cells with extra caution is necessary to ensure that the new DNA is effectively inserted [4].

5. Transformed Host Cell Selection

Host cells that already have the modified recombinant vector (modified plasmid) are selected for this step of the recombinant DNA process. As mentioned before, certain cells pick up plasmids that are self-ligated, whereas others take up plasmids that carry undesirable genes (none of the plasmids are taken up by other cells). If you want the desired results, you must choose only cells that carry plasmids containing the target gene. Here, many methods may be applied to select modified cells. One of the most often utilized methods is the procedure of growing the cells on antibiotic (such as tetracycline) agar plates. Using this method, E. Coli may be cultured at 37 °C and pH neutrality for an entire night on medium such as MacConkey-Sorbitol Agar. The appropriate antibiotic is also supplied to the plates.Because of the antibiotics, some of the cells cannot grow as quickly as cells whose plasmid does not include antibiotic resistance genes. On the other hand, the proportion of cells with plasmids containing antibiotic resistance genes is rising.Although this method enables the growth of cells with altered genes, some of the cells also carry plasmids containing undesirable genes. To distinguish between the host cells that have mutated plasmids (containing the gene of interest) and the others (without the gene of interest), another technique is required.Gel electrophoresis is frequently employed to isolate DNA fragments, making it easy to identify which plasmids have the desired gene [4].

6.Expression of the Gene introduced into the host

The ultimate goal of recombinant DNA technology is to produce more of the intended product. Usually, the goal of recombinant DNA technology is to produce more copies of a certain gene in order to improve the output of that product. Consequently, the host cells behave as factories producing the finished good.The host cell keeps dividing in the presence of light, which permits improved gene expression as well as recombinant DNA replication and synthesis. Here, bioreactors are used to achieve large-scale manufacturing [4].

3. GENE TRANSFER TECHNIQUES

i) Microinjection

Microinjection is a versatile technique used to transfect challenging cells, including mesenchymal stem cells, with applications in cloning and generating transgenic animals. Typically, a small number of cells, ranging from 100 to 200, are injected, providing statistical significance even in large cultures. Its applications include creating transgenic animals, in vitro fertilization, studying transduction-challenged cells, and assessing the effects of injected materials in mixed cell cultures. However, challenges

include potential stress on cell viability and limitations in conducting larger-scale protein studies due to the restricted number of transfected [5][6].

Nuclear transfer, a method developed from single-cell microinjection, involves dissecting the original nucleus from the somatic cell and transferring the desired nucleus at a single-cell level. The efficiency of this process is influenced by the differentiation stage of donor cells [7].

In comparison with reproductive cloning, nuclear transplantation produces an autologous embryonic stem cell line derived from a cloned embryo, aiming for therapeutic cloning to generate functional embryonic stem cells for cell replacement [8]. Although microinjection is limited to injecting 100–200 cells per treatment, this number is typically sufficient for statistical significance, even in a million-cell culture. Larger-scale protein studies may face limitations due to the small number of transfected cells. It is crucial to implement proper controls to ensure that the injection does not adversely affect cell viability, as the injection itself imposes physical stress on cells. Additionally, when transfecting dividing cells, non-genome-integrated injected material is diluted with each cell division [9].

Microinjection has been commonly used in research and clinical fields such as: (1) creation of transgenic animals; (2) *in vitro* fertilization [10] (3) studies on transduction-challenged cells; and in (4) studies on distinguish effects of injected materials in a mixed cell cultures [6][11].

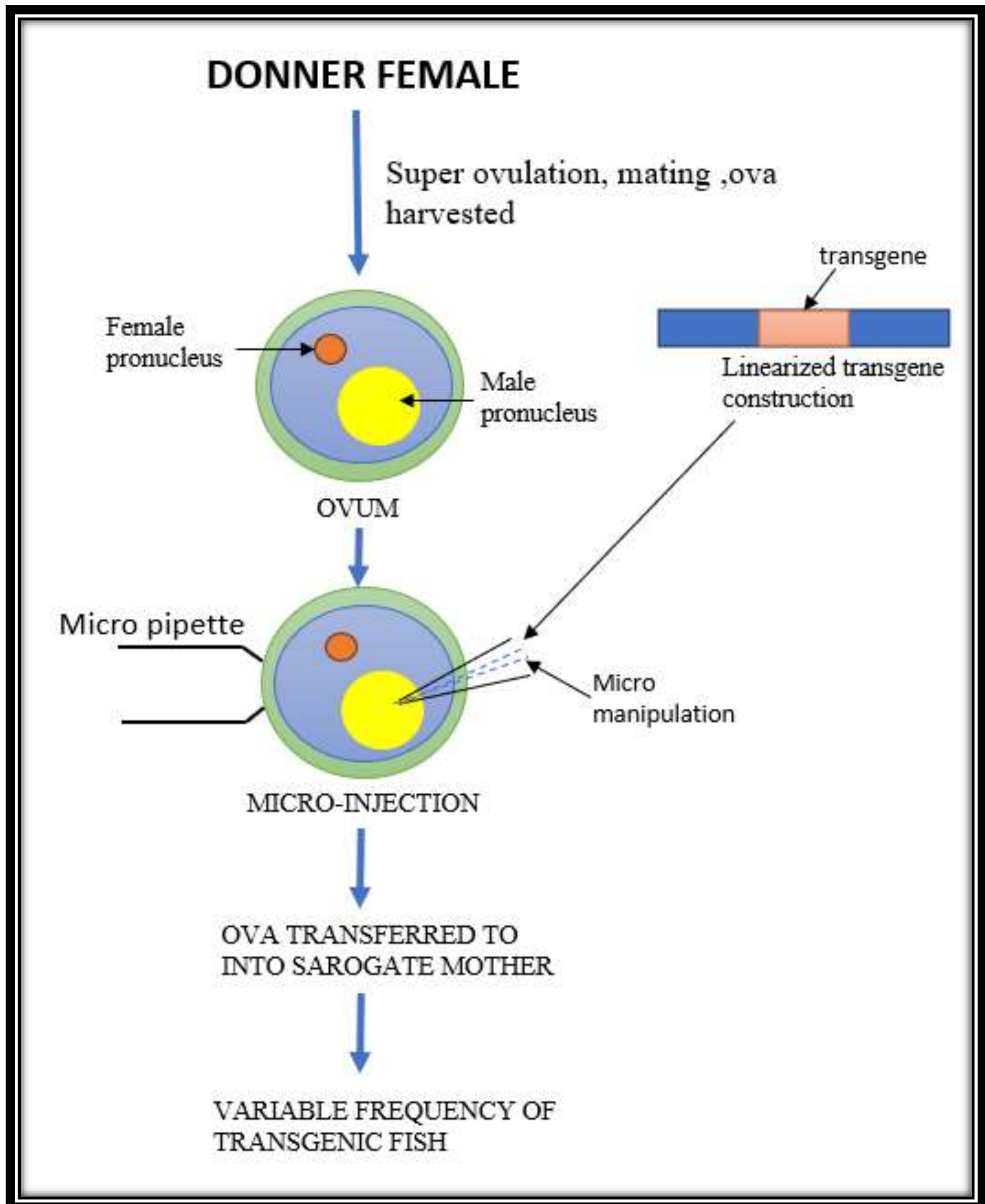

Figure 4. Microinjection technique

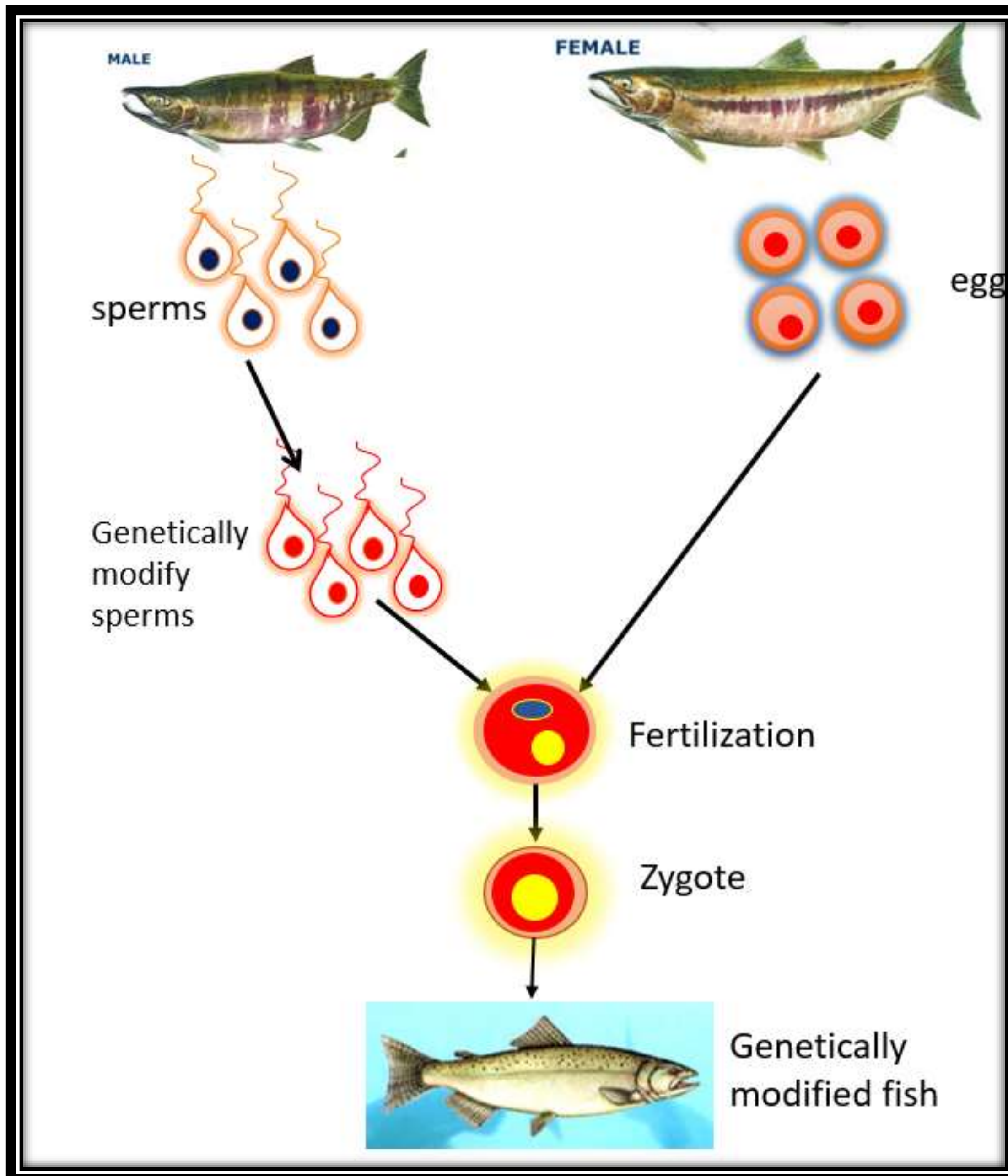

Figure 5. Genetically modified salmon.

ii) Electroporation

"Electroporation" is the technique of using short, high-voltage pulses to break through a cell membrane. By applying an external electric field that just slightly exceeds the membrane's permeability, it is feasible to cause both temporary and permanent disintegration of the cell membrane. [12]

It uses a series of brief electrical pulses to create pores in the membrane that allow DNA to pass through. Microinjection might be less effective than electroporation, which can have integrating rates as high as 30% to 100% [13].

Channel catfish embryos electroporated rather than micro-injected had more accurate hatching rates [14].

Electroporation and microinjection methods do not significantly differ when it comes to gene transfer effectiveness or integration rate.

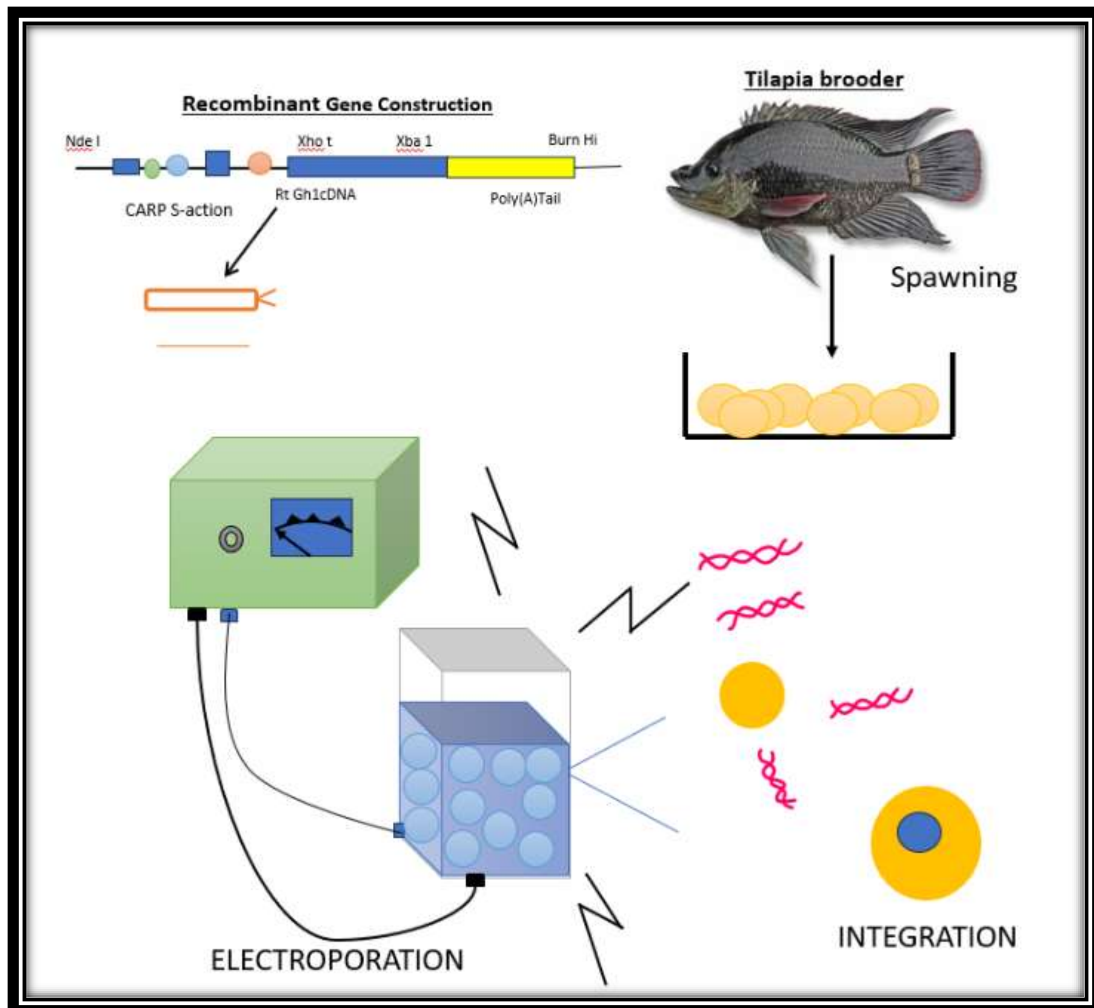

Figure 6. Transfer of foreign genes into fish embryos by electroporation

iii) Retroviral integration

Channel catfish embryos electroporated rather than micro-injected had more accurate hatching rates [15]. Electroporation and microinjection methods do not significantly differ when it comes to gene transfer effectiveness or integration rate.

4. APPLICATIONS OF GENE TRANSFER IN FISHERIES SCIENCE

In aquaculture, fish having significant economic value can be given genetic traits like accelerated growth, resilience to disease and cold temperatures, and tolerance to reduced dissolved oxygen levels. Table 1, gene sequences that were recently successfully transmitted to fish are given. Genetic traits that can speed up growth, boost resilience to disease and cold temperatures, increase feed conversion efficiency, and increase tolerance for low dissolved oxygen gases have been developed in several different species of fish and transferred to fish with significant economic worth for profitability. [16]

S.N.	**Common name**	**Gene construction**	**Results**	**Reference**
1.	Mutiara catfish	pTarget-CMV-CgGH	Growth increase	[17]
2.	Catfish	mBP-tiGH	Growth increase	[18]
3.	Mutiara catfish	PhGH (Pangasius hypophthalmus Growth Hormone)	Resistant to disease	[19]
4.	Tilapia	1α (EF1α) promoter	Increase fish colour brightness by green fluorescent protein (GFP) expression	[20]
5.	Tilapia	Growth hormone	Enhanced growth accompanied by reduced feeding rate (FR), fat and energy content in comparison to control fish.	[21]
6.	Siamese catfish	Lysozyme gene	Resistant to disease	[22]
7.	Rainbow trout	Glucose transporter and heksokinase	Carbohydrate metabolism	[23]

Table 1. Gene Construction in Several Fish Species

6. VACCINES

- The development of aquaculture is greatly aided by the vaccines created using recombinant DNA technology. The vaccination is the substance that encourages the fish's immune response to identify and eliminate the disease-causing agent as a foreign entity. Additionally, it detects and eliminates related germs that it might come into contact with later. The created vaccinations must be capable of both short-term immunity and long-term death prevention. Recombinant vaccines are those created using recombinant DNA technology [24].

6.1 PRODUCTION

1. Recognize and separate a particular genome from bacteria or a virus.
2. Ligation occurs after a gene is introduced into plasmid DNA.
3. This plasmid was produced to change into a different bacterium.
4. Permit the bacteria to multiply themselves and create the antigenic protein.
5. The vaccine is recovered from bacteria or viruses and purified [24].

a. DNA VACCINES

DNA vaccines include DNA that encodes for certain proteins or pathogen antigens. They frequently produce antigenic proteins when they are introduced into the cells. The immune system is aroused and immunological responses are triggered when these types of proteins are detected as foreign material, digested by the host cells, and exposed on the outermost layer [25]. DNA vaccines are made up of an expression plasmid that has a particular gene that codes for a chosen antigenic protein, which when expressed in the host is anticipated to stimulate an effective immune response [26].

When it comes to preventing viral infections, DNA vaccines are frequently more effective, and they are particularly effective against fish. [27]. The use of DNA vaccines has been successful in preventing fish from coming into contact with internal pathogens like Mycobacterium [28]. The first DNA vaccination was tested on rainbow trout (Oncorhynchus mykiss) and protected against IHNV. IHNV DNA vaccine that was authorized to be made in Canada. Clynav is a DNA vaccine that is sold in the European Union to prevent pancreatitis caused by the salmonid alphavirus subtype 3. Plasmid deoxyribonucleic acid, the vaccine's active ingredient, promotes active immunity. Since DNA vaccines are free of infection, they are seen as being healthier than attenuated live vaccinations. They do not express the full organism; only the antigenic protein fragments. [29].

Sl. no	Fish species	Pathogenic organisms	Genes used
1.	Rainbow trout	IHNV	IHNV-G, IHNV-G2 SVCV-G
2	Atlantic salmon	ISAV, IPNV, IHNV	Hemagglu tininesterase (ISAV) IHNV-G
3	Asian sea bass	Vibrio Anguillarum	OMP 38 gene
4	Hybrid striped bass	Mycobacterium marinum	Ag58A
5	Spotted sand bass	Aeromonas veronii	Omp 38, Omp 48
6	Common carp	SVCV	SVCV-G
7	Turbot	AHNV	VHSV-G, Capsid proteins (AHNV).

Table 1. The DNA vaccines in fish species

b. BENEFITS FROM DNA VACCINES

1. The capacity of DNA vaccines to stimulate both the humoral and adaptive immune systems is their fundamental benefit.
2. Malignancies, autoimmune diseases, and genetic disorders could all be treated with DNA vaccines.
3. Plasmid DNA encoding a tumour-associated antigen (TAA) can be synthesized to trigger CTL (Cytotoxic T Lymphocyte) activities towards malignant cells that exhibit the antigen when employed for cancer therapy [30]
4. DNA plasmids can carry immune modulatory proteins that may regulate the immune response in autoimmune diseases.
5. DNA vaccines eliminate any chance of reversion because they don't employ microbes [31].

c. DISADVANTAGES OF DNA VACCINE

1. No temporary infection occurs, just like with "killed" and subunit vaccinations.
2. Immune responses from DNA vaccinations are likely to be modest and call for repeated boosting.
3. A well-established supply chain system is necessary because DNA degradation by nuclease may occur.
4. The generation of antibodies against DNA may be stimulated.

5. Genes may be able to regulate the growth of cells [32]

6.2 RECENT ADVANCES

Successful applications of recombinant DNA technology can be found in the fishing industry. In the realm of aquaculture, the production of genetically modified fish that is improved by the application of techniques from genetic engineering produced positive outcomes. Therapeutic proteins will be produced for the treatment of fish illnesses. Through the use of gene editing technologies, new features can be bred into fish. Aquaculture disease outbreaks can be significantly reduced with the aid of DNA vaccines. Recombinant DNA technology is a tool for moving forward in the development of the nation's fisheries sector thanks to the introduction of new technologies like CRISPR cas and enhanced sequencing techniques.

The outcome of this.

(1) Despite the absence of species advancement in the general population, the transfer of genes in freshwater fish has great potential, particularly in addressing global food deficiencies and assisting in environmental preservation.

(2) Electrophoresis and the microinjection technique are two methods of gene transfer.

(3) Increasing growth, improving fish susceptibility to cold temperatures, and improving fish resistance to infections are all examples of gene transfer uses in freshwater fish farming.

7. FUTURE PERSPECTIVE

1.developing marine fish in a freshwater ecosystem as a starter.
2. Altering the duration of the cycle of reproduction in continuous breeders.
3. Increasing the species used in aquaculture's adaptability to a larger variety of environmental factors.
4. Improving taste and nutritional value.
5. Regulating sexual maturation to stop fish carcasses from decomposing with age. [33].

REFERENCES

[1] Subitsha.L., Dr.Suresh.e ., Dr Kathirvelpandian. A.,(2020)., Role of recombinant dna technology in fisheries and aquaculture development., International Research Journal of Engineering and Technology (IRJET)., Volume: 07 Issue: 05.

[2] S. A. Shinde*, S. A. Chavhan, S. B.Sapkal, V. N. Shrikhande.,2018.,Recombinant DNA Technology and its Applications: A Review., International Journal of MediPharm Research.

[3] (S. A. Shinde*, S. A. Chavhan, S. B. Sapkal, V. N. Shrikhande., (2021)., Recombinant DNA Technology and its Applications., International Journal of MediPharm Research., Vol.07, No.01, pp 79-88.

[4] Recombinant DNA Technology Steps, Applications and Gene Therapy. (http://tinyurl.com/8uh6p8kn).

[5] Siewert C., Haas H., Nawroth T., Ziller A., Nogueira S. S., Schroer M. A., et al. (2019). Investigation of charge ratio variation in mRNA – DEAE-dextran polyplex delivery systems. *Biomaterials* 192 612–620.

[6] Tiefenboeck P., Kim J. A., Leroux J.-C. (2018). Intracellular delivery of colloids: past and future contributions from microinjection. *Adv. Drug Deliv. Rev.* 132 3–15. 10.1016/j.addr.2018.06.013

[7] Stein P., Schindler K. (2011). Mouse oocyte microinjection, maturation and ploidy assessment. *J. Vis. Exp.* 53:2851. 10.3791/2851

[8] Kobayashi N., Rivas-Carrillo J. D., Soto-Gutierrez A., Fukazawa T., Chen Y., Navarro-Alvarez N., et al. (2005). Gene delivery to embryonic stem cells. *Birth Defects Res. C Embryo Today* 75 10–18. 10.1002/bdrc.20031

[9] Zhang Y., Yu L. C. (2008b). Single-cell microinjection technology in cell biology. *Bioessays* 30 606–610. 10.1002/bies.20759

[10] Malter H. E. (2016). Micromanipulation in assisted reproductive technology. *Reprod. Biomed. Online* 32 339–347. 10.1016/j.rbmo.2016.01.012

[11] Khanna P. (2012). “Cellular microinjection for therapeutic and research applications,” in *Mems for Biomedical Applications*, eds S. Bhansali and A. Vasudev (Amsterdam: Elsevier;), 432–448. 10.1533/9780857096272.4.432

[12] Gehl J.., (2003)., Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research., Acta Physiol Scand;177(4):437-47

[13] D.A. Power et.al, Molecular Marine Biology and Biotechnology, 1:301-309 (1992).

[14] S.D. Walker, Master of Science Thesis, Auburn University, AL (1993).

[15] Gehl J.., (2003)., Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research., Acta Physiol Scand;177(4):437-47

[16] Aisyah ., Ayi Yustiati ., 2022; Application of Gene Transfer in Freshwater Fish: A Review., Asian Journal of Biochemistry, Genetics and Molecular Biology ,10(1): 13-17

[17] Buwono ID. Application of Sperm Electroforation for the Production of Transgenic Mutiara Catfish (Clarias sp.). Indonesian Journal ofAquatics. 2019;1(2):109-119 Available:https://doi.org/10.24198/jaki.v1i2. 29157

[18] Gusrina, Alimuddin, Komar S, Utut W. Transfer of genes encoding growth hormone of tilapia (tiGH) in catfish (Clarias sp.) by microinjection method. J. Ris. Aquaculture. 2009;4(3):333-340. Available: http://dx.doi.org/10.15578/ma.7.2.2012.59-64

[19] Buwono ID, Latifah AU, Subhan U. Detection of Genotype Diversity of Sangkuriang Catfish Hybrids, Transgenic and Non-GMO Mutiara Catfish in First Offspring. Indonesian Journal of Biology. 2018;14(1):133–141. Available: http://dx.doi.org/10.14203/jbi.v14i 1.3671

[20] Fujimura K, Kocher TD. Tol2-mediated transgenesis in tilapia (Oreochromis niloticus). Aquaculture. 319(3-4):342-346. Available: https://doi.org/10.1016/j.aquacult ure.2011.07.021

[21] Zhong C, Song Y, Wang Y, Li Y, Liao L, Xie S, Hu W. Growth hormone transgene effects on growth performance are inconsistent among offspring derived from different homozygous transgenic common carp (Cyprinus carpio L.). Aquaculture. 2012; 356:404-411. Available: https://dx.doi.org/10.1016/j.aqua culture.2012.04.019

[22] Hadie W, Hadie LE, Lusiastuti AM, Tahapari E, Marnis H. Lysozyme gene insertion in striped catfish Pangas anodon hypophthalmus (Sauvage, 1878) to generate disease resistance breed's line. Journal Iktiologi Indonesia. 2012;15(2):119-127. Available: https://doi.org/10.32491/jii.v15i2. 66

[23] Van Eenennaam, Alison L, Olin Paul G. Careful Risk Assessment Needed to Evaluate Transgenic Fish. California Agriculture. 2006;60(3):126-131. Available: https://escholarship.org/uc/item/9 fx369v6

[24] Subitsha.L, Dr.Suresh.E , Dr.Kathirvelpandian.A (2020). Role Of Recombinant Dna Technology in Fisheries and Aquaculture Development.,International Research Journal of Engineering and Technology (IRJET). Volume :07 Issues: 05.

[25] Klinman DM, Yamshchikov G, Ishigatsubo Y. (1997)," Contribution of CpG motifs to the immunogenicity of DNA vaccines". J Immunol. 1997;158(8):3635-9.

[26] Kurath G, (2008), "Biotechnology and DNA vaccines for aquatic animals", Rev, SciTech off. Int. Des. Epizoot. 2008; 27.1.1793

[27] Holvold L.B., Myhr A.I., Dalmo R.A. (2014), "Strategies and hurdles using DNA vaccines to fish". Vet, Res 2014; 45:21. Doi:10.1186/1297-9716-45-21

[28] Pasnik D, J., Smith S.A, (2005), "Immunogenic and protective effects of a DNA vaccine for Mycobacterium marinum in fish. Vet. Immunol. Immunopathol. 2005; 103:195-206. Doi: 10.1016 /j. vetimm. 2004.08.017.

[29] Weiner D.B. (2008), “DNA vaccines: crossing a line in the sand introduction to special issue”. Vaccine. 2008; 26:5073. doi:10.1016/j. vaccine.2008.07.024

[30] Eschenburg G, Stermann A, preissner R, Meyar HA, Lode HN. (2010), “DNA vaccination: using the patient’s immune system to overcome cancer”, Clin Dev immumol (2010) Doi: 10.1155/2010/169484.

[31] subitsha., suresh.e., kathirvelpandian.a., (2020.,) Role of recombinant DNA technology in fishers and aquaculture development., International Research Journal of Engineering and Technology., Volume: 07 Issue: 05., e-ISSN: 2395-0056., p-7515.

[32] Lucy jane pope (2015), vaccine theory and practical [power point presentation]. available at https://slideplayer.com/user/15242257/.

[33] Maclean, N and Laight, R.J., (2000), “Transgenic fish: an evaluation of benefits and risks”. Fish and Fisheries, 1,2000, 146-172.

[34] Figure1,2,3=Recombinant DNA Technology - Steps, Applications and Gene Therapy. MicroscopeMaster. Available from: https://www.microscopemaster.com/recombinant-dna-technology.html#gallery[pagegallery]/0/.

[35] figure 4,5 = S. Barun., Revolution of Transgenic Fish and its impact on Aquaculture and Biodiversity (2015)., The Beats of Natural Sciences., volume2.

[36] figure 6. Thomas T. Chen., Application of Transgenic Fish Technology in Aquaculture., American Meat Science Association., P-108