# REVIEW ON TRANSGENIC TECHNOLOGY IN LIVESTOCK: CURRENT STATUS AND FUTURE HORIZONS

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**ABSTRACT:** Transgenic technologies are one of the most promising arenas in the field of biotechnology. These techniques employ genetic engineering techniques to insert foreign genes of interest into genome of an individual. These foreign genes are inherited and expressed by the recipient's offspring. Transgenic livestock refers to various types of animals whose genetics have been altered to improve economically important traits such as disease resistance, growth rate, milk composition, meat quality and survival. Genetically modified livestock can be an efficient and economical source of pharmaceutical products and can also be employed to study various human diseases. Two things determine the success of any transgenic technique namely the transport of foreign piece of DNA across the recipient's cell membrane and delivery of that DNA across the nuclear membrane gaining access to the chromosomes of the recipient. Many transgenic techniques are employed for genetic manipulation like microinjection, somatic cell nuclear transfer, chemical methods, restriction enzyme mediated gene transfer, retrovirus mediated gene transfer as well as embryonic stem cell and sperm mediated DNA transfer etc. The transgenic animals can be employed in drug discovery, disease research and xenotransplantation etc. This review summarizes various transgenic techniques, applications and future prospects of the transgenic technology.

**Key words:** Transgenic technologies, transgenic livestock, human diseases, genetic modification, applications, pluripotent cells, biotechnology.

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## INTRODUCTION

The transgenic animals are those animals whose genome has been modified artificially and in contrast to spontaneous mutations, these modifications are calculated and deliberate. Genetic engineering technologies are used to integrate foreign genes into an animal's genome with the implicit purpose of expression and inheritance of these genes by the offspring. The recombinant DNA technology is used to introduce foreign DNA into the animals. This foreign genetic material is transferred through germ line such that all the cells of that animal including germ cells possess same altered genetic material. The genetic alterations of germ cells can enable us to transfer the traits into the next generations through normal reproduction. The history of genetic engineering is fascinating dating back to 1950's. Man has been using selective breeding to improve livestock for thousands of years. Various historical milestones in transgenic technology are summarized in Table.1.

An excellent control of the gene expression and transgenic efficiency are the main limiting factors encountered in the production of transgenic animals. Advanced and sophisticated studies will permit transgenic technologies to explore various possibilities for genetic improvement of animals. It will also enable us to study various disease models, gene functions and organ transplantations etc. Transgenesis can also involve complete organisms besides individual cells and body functions that can be altered in vivo. Recent advancements in the animal gene transfer technologies are gene transfer mediated by sperm, gene transfer mediated by embryonic stem cells, microinjection method and nuclear transplantation of somatic cells. These technologies can furnish us a more suiting platform for the development of transgenic and novel varieties of animals and can boost up the progress in livestock production, medicinal sciences and other sciences.

Transgenesis permits us to improve nutrient quality of animal products as well as quality and quantity

of whole food. Table.2 summarizes various gene targets for genetic manipulation in farm animals.

Gene transfer technologies can furnish us a way of enhancing nutritionally beneficial characteristics. For instance, the incidence of coronary heart diseases in humans can be decreased by increasing the omega-3 fatty acid constituents in fish meat. Transgenic pigs have been produced which possess higher levels of omega-3 fatty acids (23). Transferring of genes responsible for elevation of omega-3 type fatty acids can be a helpful tool to produce pork of better nutritional quality (23). More nutritious and low fat animal products obtained by transgenic technologies can enable us to improve public health. Changing the cholesterol or fat composition of carcass is another way of manipulating the composition of carcass. By changing the uptake or metabolism of fatty acids and cholesterol, the cholesterol and fat contents of meat, cheese and eggs can be decreased. Also, the genes of some receptors e.g. low density lipoprotein (LDL) and some hormones e.g. leptin are in the list of potential targets that may reduce cholesterol and fat content of animal products. Recently, prion suppressed (44) and prion free (57) livestock have been produced. Prions are responsible for BSE (bovine spongiform encephalopathy) or 'mad cow disease' in the cattle and CJD (Creutzfeldt-Jacob Disease) in the humans. Beef production in Brazil can be significantly improved by transferring genetic material from Holstein to Guzerat cows (50).

**Transgenic animal:** An organism having a deliberately inserted foreign gene into it is termed as transgenic animal. It is genetically modified to have the characteristics it normally does not possess. Transgenesis in animals either means changing DNA of animal or transferring a foreign piece of DNA into animal. These animals are modified genetically by introducing a gene from another species or by molecularly manipulating the indigenous DNA. The newly acquired genes are inherited by the coming generations in same fashion as the normal genes of the organism. The earlier Transgenesis involves the microinjection of DNA into fertilized egg of mouse. Since the integration site of foreign DNA cannot be controlled by this method, it is highly imprecise tool. Mice produced by this microinjection are generally referred to as "over-expressers".

Mice constitute about 95% of all the transgenic animals employed in the biomedical research. About 80% mice genes function similarly to the humans. The mice embryos can be easily manipulated and it also has a brief reproductive cycle. Mice therefore serve as human surrogate to study diseases. Hopefully, the improvement of Transgenesis technologies in mice can serve to reduce the use of primates and other higher animals. Besides mice, pigs, rats and sheep are also used in trans-genetic studies.

## METHODS TO INDUCE TRANSGENESIS

**Microinjection:** DNA microinjection is by far the most widely used method for transfer of genes in animals. First animal to undergo successful Transgenesis by this method was mice. It uses the traditional approach involving microinjecting embryos with DNA to produce transgenic livestock. The process of microinjection is illustrated in Fig.1.

For this processes i.e. cellular and pronuclear injection, first of all, position the cell (target) under microscope. Two manipulators are used one to hold the pipette and the other to hold a fine capillary needle having 0.5-5 µm diameter. The diameter of micro needle used can be increased to inject stem cells into embryo. The needle is used to penetrate cell membrane as well as nuclear membrane (12). The method is based on integrating the transgenic DNA randomly by utilizing DNA repair pathways of the cell. The process is highly inefficient with success rates ranging between 1-4% (37). In a commercial farm in USA, the microinjection technique in goats has given a result of 4.2% (15). Despite all above mentioned drawbacks, the process is still frequently employed to produce transgenic organisms (3). The method is now being improved by co-injecting the restriction enzymes along with DNA so as to facilitate the incorporation of trans-genome into the host chromosome (51).

**Retrovirus vectored gene transfer:** The term retrovirus means a virus having RNA as genetic stuff rather than DNA. The retrovirus method is more efficient primarily because of provirus integrations into each target cell. The method was used successfully in 1974 for the first time to insert a simian virus into the mice embryos. The main advantages of using retrovirus as DNA vector are the effectiveness, easiness and target cell specificity. In the cells infected with retrovirus infection, the incoming viral genome (RNA) after undergoing reverse transcription (converting to DNA) as well as integration into host cell becomes a functional constituent of the host genome for whole life of the host cell (43). Various steps of the process are explained in Fig.2.

Retroviruses are among the most widely explored viruses for gene therapy as well as treatment of genetic diseases (51). More recently, the constructs prepared from lentivirus are used to infect embryonic tissues to generate transgenic mice and rats (46). Retrovirus based methods to modify chicken genome are also progressing (20).

**Somatic cell based nuclear transfer:** Somatic cell nuclear transfer (SCNT), a laboratory based technique in developmental biology and genetics used for generating viable embryos from body and egg cells. The technique employs encapsulated oocyte (egg cells) and involves the implantation of a somatic cell nucleus in it. The famous

sheep Dolly, the first mammal to be cloned successfully was produced by this process (26). The process is explained in Fig.3.

This technique has also become the focus of stem cell research. The purpose of technique is to get pluripotent stem cells of the cloned embryo. These cells have same genetic makeup as their donor organism. This provides them the potential to generate the patient specific cells (pluripotent) which can be employed in disease research or therapies (29). Another potential utility of genetically matched stem cells could be to generate the cell lines having specific genes linked with patient's particular disease.

By this technique, a specific in vitro disease model can be generated which could be used to study the disease, probing its pathophysiology and establishing therapies (28). For example, if a patient of Parkinson's disease donates his or her body's somatic cells, the stem cells produced by SCNT will have genes contributing to the development of Parkinson's disease. These cell lines specific for the disease can be investigated to enhance our understanding of this condition. A second potentially useful application of this stem cell based research is to use special cell lines which are specific for the patient to produce tissues and even organs. These tissues and organs can be transplanted into specific patient (41). The SCNT technique can be used to produce many organisms having identical genetic makeup. Due to the recent progress in SCNT and other molecular techniques, the production of transgenic animals has become much easier. Although the cloning efficiency is low in goats, it's potential to produce genetically similar animals having a single or multiple desirable genes can be employed to enhance the productivity and economic livelihood (1).

**Sperm-mediated gene transfer:** The ability of spermatozoa of mammals to uptake and effectively transmit foreign piece of DNA was for the first time reported by Bracket et al. in 1971. The sperm cells are given the exposure of exogenous DNA. This DNA adheres to the sperm surface via specific interactions. It is agreed that following steps in the process are reproducible and well established: (a) spontaneous interactions between foreign DNA molecules and sperm cells, and (b) the transfer of sperm bound molecules of DNA to the oocyte during fertilization. Studies have been conducted to determine the optimum conditions employed when incubating sperm and DNA molecules (25). This technique is perceived to be highly promising to produce transgenic animals.

Various approaches are being employed to enhance the uptake of DNA by sperm cells. One approach involves conjugating the recombinant molecule of DNA to the sperm head by a DNA-amalgamated antibody (9). The antibody employed in this study recognizes various surface proteins which are common to sperms from mice, chicken, pigs, sheep, cattle and humans. Many other approached including electroporation (45) method or lipofection (24) techniques are being employed to generate transgenic animals by inserting the DNA molecule into the sperm head. By improving techniques employed for the culturing as well as expanding the sperm associated stem cells, there is a rich opportunity of *in vitro* engineering of these cells to produce transgenic sperms that can be utilized to fertilize the ova and produce transgenic organisms (36). A comparison of Retrovirus-mediated Gene Transfer, DNA Microinjection, and Embryonic Stem (ES) Cell-mediated Gene Transfer Methods is given in Table.3.

**Liposome's mediated DNA transfer:** liposomes are the small cellular bodies consisting of lipid layers resembling the membranes which are surrounding the hydrous compartments. Cationic liposomes were employed to enhance the transfection capacity of sperms. The association of DNA molecules to the cationic liposomal complexes may permit transfer of DNA into the oocytes at the time of fertilization (2). However the fertilizing capability and sperm motility were less when high concentrations of liposomes were used as estimated by the microscopic observations. Recently it has been reported that BSA, an important bovine serum protein can impair cell's ability to uptake DNA/liposome complexes. More recently, improved transgenic rates were reported in mouse F1 (about 41%) and F2 (about 37%) generations by using testes mediated gene transfer. This technique utilizes plasmid DNA treated with liposomes (19). Besides this, the existence of diverse verities of liposomes makes it extremely hard to predict the likelihood of success. The situation is complicated due to lack of specially designed empirical studies.

**Linker (a receptor) based gene transfer:** This technique involves linking the foreign piece of DNA to the sperm head and was firstly reported by employing monoclonal antibody mAbC (9). This antibody is a linker protein bearing positive charge and is basic in nature. It has the ability to bind negative charge bearing DNA molecules by ionic interactions. These ionic interactions are responsible for bonding the foreign DNA to the sperm head precisely. The binding of DNA molecules to the polycations by strong monocovalent fashion results in the formation of soluble complexes. If DNA is conjugated to antibody fragments or whole antibodies, it has the ability to internalize these complexes by a receptor mediated endocytosis (55).

**Integration mediated by restriction enzymes:** This process involves transforming the cells having a blend of the plasmid DNA linearized with specific restriction enzyme and another restriction enzyme which is capable

of synthesizing compatible and cohesive ends in the genome. This procedure has proved to be a useful tool for the genetic screening as well as placing molecular and genetic markers at specific points in the genome. The plasmid DNA molecules were completely linearized by using a specific restriction enzyme resulting in the production of cohesive ends (single stranded) and finally it was transferred to de-condensed sperm nucleus in vitro (48). Shemesh *et al*. (2000) generated transgenic embryos of bovines by conjugating the above technique with liposomes. He demonstrated that the transgenic sperms produced by this method can be employed to generate genetically modified embryos and consequently live organisms by AI or IVF.

## APPLICATIONS AND USES OF TRANSGENICALLY DERIVED ANIMALS

**Human health:** One of the major potential usefulness of transgenic organisms is to produce biologically active recombinant proteins in mammary glands. This process called "gene pharming" can be employed for the benefit of humans. Mammary gland offers the opportunity to extract and purify large quantities of recombinant proteins (32, 47). Another reason of targeting mammary gland is that milk is a normal secretion produced by females of all species and it can be easily collected in large quantities without causing harm to animal.

**Therapeutic proteins (recombinant):** A large number of new proteins have been produced and extracted from transgenic animal's mammary gland. A number of conventional methods employing yeasts, bacteria and plants etc. were employed to produce these recombinant proteins but many of these methods are deficient in post translational modification machinery of the eukaryotic cells. Hence, transgenic animals can potentially serve as bioreactors to produce valuable transgenic products. Proteins such as tissue plasminogen activator (TPA), antithrombin III (AT III) and antitrypsin etc. are being extracted from the mammary glands of transgenic goats and sheeps. The AT III which is employed to treat proteins which are resistant to heparin, is hopefully in the market (21). Glycosidase used to treat Pompes disease has been synthesized by using rabbit mammary gland as a vehicle (53). A topical antibacterial for *Streptococcus mutans,* effective against dental caries is hopefully completing clinical trials, proteins along with their sources and application are mentioned in Table.4.

**Blood substitutes:** Transgenic pigs have been produced which synthesize functional hemoglobin having almost the same oxygen carrying capacity as that of normal mammalian hemoglobin. This hemoglobin molecule can be isolated and purified from swine blood (11).

**Transgenic animals derived antibodies:** A large number of recombinant and monoclonal antibodies are derived from transgenic cattle and goat (33, 17). These antibodies can be used to target cancer cells. Transgenically produced animals can be used to produce human polyclonal antibodies for clinical purposes (22). An increasing number of novel therapeutic products containing human antibodies are being commercialized. Transgenic rodents can become a source of high affinity human monoclonal antibodies after immunization. Several strategies have been employed to produce animals which express repertoires of human antibodies. In case of rodents, genes incorporated to bacterial or yeast origin artificial chromosomes can be transferred by embryonic stem cell transfection or oocyte microinjection. The endogenous Ig loci were silenced by gene targeting to enhance optimal production of human antibodies (30).

**Disease models:** Many farm animals e.g. pigs and cattle can be employed as a suitable model to study human diseases e.g. cancer, cystic fibrosis and also various neurodegenerative disorders and also discover their controls (52, 39, 27). Pigs can serve as a useful and effective model to study growth hormone releasing hormone (GHRH) related disorders (14).

**Improving carcass composition and growth:** Transgenic animals have been produced which have been endowed with genes to improve quality of food and growth rate. The genes of insulin like growth factor and growth hormones are expressed at various levels in such animals. Transgenic salmon fish and cattle have been developed containing foreign genes. The inoculation of chicken sky gene has been associated with hypertrophy of muscles in cattle and pigs (4). The Rendement Napole (acid meat) gene has thought to be responsible for decreasing processing yields as well as quality of pork. Eliminating its expression in pigs can change postmortem pH and increase meat quality. Other genes e.g. IGF binding protein and GHRH etc. can also play a role to favorably alter the growth. The pigs endowed with human origion metallothionein promoter have also significantly better feed conversion and growth (38).

**Lactation and milk production:** The recent developments in Transgenesis provide ample opportunities to improve quantity as well as quality of milk. Transgenic animals could be prepared which can secrete certain nutriceutical products in their milk. These products can influence the growth of their progeny. Various varieties of casein are the main targets to improve the quality, physio-chemical properties and composition of milk. Transgenic cow have been cloned which produce higher quantities of kappa and beta casein in their milk. It increases the milk value to produce milk products such as yogurt, cheese and also can improve the product's shelf life (6). Transgenic animals can be produced to secrete "infant milk" having elevated levels

of human lectoferrin. It can also be used to produce milk free of lactose for people having lactose intolerance by preventing the expression of lactalbumin gene. Hypoallergic milk can also be produced by inhibiting the expression of specific B-lactoglobulin gene. The recent advances in genetic engineering particularly the development of genome editing techniques have significantly improved our ability to produce transgenic animals which produce various products in their milk at a cost significantly lower than cellular systems (49). Transgenic animals can also be developed to secrete various antibodies and antibacterial peptides (lysozyme etc.) in the milk which can improve animal's resistance to diseases e.g. mastitis. It was reported (18) that transgenic animals can be produced to secrete many growth factors in the milk which can beneficially effect the maturation and growth of offspring.

**Disease resistance:** One of the major uses of Transgenesis is to manipulate major histocompatibility complex (MHC) genes. These manipulations can be used to increase immune response and disease resistance in livestock. It was reported that Transgenesis can be employed to produce sheep having increased resistance to Visna virus (10). Prion protein can also be knocked down to prevent the spread and transmission of scrapie and bovine spongiform encephalopathy (56). Mice have been altered transgenically which can secrete various recombinant antibodies in their milk which can neutralize corona virus which is the causative agent of transmissible gastroenteritis a disease of commercial importance for pigs (8). Transgenic cattle have been produced which secrete higher amounts of lysostaphin in their milk which increases the animal's resistance to the mastitis by killing *Staphylococcus aureus* (13).

**Transgenesis in aquaculture industries:** Aquaculture species are especially amenable to trans-genetic manipulations. Fishes and shellfishes show high fecundity and produce high amounts of gametes. Many species of aquatic animals can be modified genetically to harvest sperm and eggs which can be used in in-vitro fertilization. It is useful because eggs of aquatic animals are larger in size and develop outside the body after fertilization. Hence, no further manipulations e.g. re-plantation etc. are needed. Gene transfer in fish was successfully carried out in China in 1985. A special DNA construct having human growth hormone along with mouse origin metallothionein promoter was inserted by micro-injection into germinal disc of *Carassiusauratus* (goldfish) embryo. A similar procedure was carried out on an economically important fish Nile tilapia (Orecochromisniloticus) (5). Recently, in rats, Drosophila and zebra fish, zinc finger nuclease (ZFN) which encode for DNA or mRNA has been employed to produce heritable mutations at certain loci by micro-injection method (7, 16).

**Pharmaceuticals production by transgenic animals:** The synthesis of therapeutically important proteins by transgenic animals involves the expression of their genes from promoters which are specific to mammary gland. This process will result in the secretion of transgenic product into the milk (Martin et al, 2005). An application for market authorization of a product named Atryn® was received by European Medicine Agency in January 2004. It was a recombinant human antithrombin which was isolated from milk of a transgenic goat. It was the first transgenic animal derived product which was submitted for approval. Recently, transgenic embryos of buffalo (*Bubalus Bubalis*) containing human insulin gene were produced and successfully transferred to the recipients (34). Hence in future, transgenic buffalo can be prepared secreting human insulin in their milk and can be used for commercial scale production of human insulin. Similarly a study in Brazil has found that transgenic goats expressing human lysozyme gene can be used to control gastrointestinal diseases (40). Milk is most employed vehicle for recombinant products. Seminal plasma, blood, urine and egg white are other examples of possible vehicles.

**Xenotransplantation:** The successful human to human transplantation of various vascularized organs e.g. liver, heart, pancreas, lungs and kidney etc. has saved countless lives over the past few decades. However, this process is plagued by acute shortage of donor organs. It was accepted earlier that for ethical, anatomical, physiological reasons, pig could become donor of vascularized organs for humans. However, the realization of this dream can only be possible after resolving various important immunological issues (42). The first reported pig to a primate transgenic xenograft employed a new delivery method for human regulatory complement proteins (31). Transgenic animals may soon become a source of transplant organs. This xenotransplantation is complicated by a specific pig protein which is associated with donor rejection. Recent research is aimed at removing this protein and substituting it with a human protein.

**Tissue repair:** The pluripotent stem cells were inoculated to the vitreous fluid of the injured mice eye. The stem cells grafted into retina of the eye induced repair and growth of vascular vessels (35).

**Improved welfare of animals:** Almost all applications of transgenic technologies focus on traits associated with sustainability such as disease resistance, improved production etc. Consequently, little effort is being made to influence the welfare of animals and enhance role of these animals in global agriculture. The recent advancements in gene editing technologies have considerable potential to promote characters which promote animal health and welfare (54) involving no

importation of genome from other animal species. The issues with public acceptance and regulation of transgenic products also demand an increased shift towards improving welfare of the animals. The focused traits are lower heat stress, improve consumption and livability etc.

**Ethical Concerns:** The opinion about the use of transgenic animals is highly divided. Public opinion surveys in Japan, New Zealand and USA showed that about 54%, 42% and 58% people respectively are in the favor of using transgenic animals. The opponents argue that employing animals in biotechnological research is associated with huge suffering to the animals. However, some people accept this suffering by the animal in interest of human community. This attitude is best described by the term "interest sensitive speciesism". Some people opinion that by using animals to produce transgenic products, we are reducing them to mere factories, ignoring their basic welfare. The fact that animals are also living creatures and feel pain and pleasure similarly as humans is ignored. Some animal rights propagandists' opinion that animals have same rights as humans. However, this opinion is rejected by most communities. An argument mentions that each species has right to exist as a distinct and separate species. However, most biologists disagree with this opinion and do not consider species as water-tight and fixed entities. According to most biologists, the species are constantly evolving and dynamic. Lastly, the incorporation of human genes to the animals (and vice versa) is viewed by many people as blurring the precise definition of "humanness". However, most of human genes are not strictly unique to humans and their comparable genes do exist in animals. A counter argument is that the genome of many retroviruses has been integrated into human genome without damaging our humanness.

**Limitation of Transgenesis:** Despite its enormous applications, the Transgenesis is associated with many limitations insertational mutations can alter critical biological processes and unregulation of gene expression can result in maligned gene products.There is also risk of side effects e.g. cancer, arthritis and dermatitis etc. in transgenic animals.

**Conclusion:** By mid-1980 the scientific community and pharmaceutical industry have been greatly fascinated by the introduction of transgenic technologies. At that time, transgenic technology was considered as the upcoming wave in rapidly progressing horizon of biotechnology. Now three and half decade later, we are positioned to harvest the fruits of the technological discoveries that are still in their adolescence. The advent of 21$^{st}$ century has seen the launch and marketing of various biotechnological products as well as a rising ethical concerns about these products. There are enormous challenges but equally and even more opportunities exist in the field of biotechnology. Hopefully, we will witness social acceptance of biotechnological products which bear the tremendous promise of improving human welfare and needs.

The emergence and development of Transgenesis has extended the scope and dimensions of novel molecular biotechnological techniques which can be helpful to improve farm animals. Transgenic technologies can be an answer to the enormous challenges faced by the farmers to improve animal production. The transgenic animal origin biological products should be handled with care as they can be contaminated and damaged easily. Hence, there is a need to develop safety guidelines for exploitation of the recombinant proteins at commercial level. It will ensure that animal to human transfer of pathogens is averted.

Hence, the genetically modified animals and biotechnological products can have a critical role to refine quality as well as quantity of production, environmental protection, maintenance of the genetic diversity and improving animal welfare.

**Table.1. History of Transgenesis.**

| Year | Scientist | Work |
|---|---|---|
| **1891** | Heape | Conducted first embryo transfer successfully |
| **1949** | Hammond | Developed specific culture systems capable of sustaining ova through different cleavage divisions |
| **1966** | Lin | Developed microinjection method and used it in murine zygotes |
| **1972** | Paul Berg | Developed first recombinant molecule of DNA |
| **1973** | Stanley Cohen and Herbert Boyer | First successful development of genetically modified organism |
| **1976** | Robert Swanson and Herbert Boyer | Foundation of first genetic engineering company |
| **1977** | Gurdon | Transferred DNA and mRNA into the Xenopus eggs |
| **1980** | Brinster et al. | Obtained a suitable translation product by using mRNA of rabbit globin |
| **1981** | Ruddle and Gordon | Coined the term "Transgenesis" |

**Table 2. Various Gene Targets for Genetic Manipulation in Farm Animals.**

| Species | Transgenic gene | Target trait(s) | References |
|---|---|---|---|
| **Swine** | Lysozyme, Fatty acid | Health | 1,2 |
| **Cattle** | Udder | Omega-3 and K-casein | 3,4 |
| | Health | Lysozyme | 5 |
| **Sheep** | Health | BSE | 6 |
| | Growth | IGF-1 | 7 |
| **Goat** | Udder | Fatty acid | 8 |
| | Health | Lysozyme | 9 |

**Table.3. Comparison of Retrovirus-Mediated Gene Transfer, DNA Microinjection and Embryonic Stem (ES) Cell-Mediated Gene Transfer Methods.**

| Techniques | Retrovirus-mediated Gene Transfer | DNA Microinjection | Embryonic Stem (ES) Cell-mediated Gene Transfer |
|---|---|---|---|
| **DNA Vector** | Wild type or recombinant retroviruses | Any cloned piece of DNA | Retroviruses or cloned DNA |
| **Introduction of DNA** | Only injectable after removal of zonapellucida | Microinjection of DNA into pronucleus | Retroviral infection or electroporation |
| **Embryo transfers** | Uterus | Oviduct | Into blastocoel followed by uterus |
| **No of copies of integrated DNA** | 1 | 1-200 | Variable, depending upon method of inoculation used |
| **Embryonic stage** | One cell stage or even later | One cell stage | Totipotent embryonic stem cells |
| **Percentage of recipients that are transgenic** | 5-40% | 10-30% | Up to 100% |
| **Expression of new DNA** | Poor | Average | Gene trap, enhancer trap |
| **Screening methods for newborns** | PCR or Southern blots | PCR, Southern and dot blots | Coat color, Southern bots, PCR |

**Table.4. List of Various Recombinant Proteins Derived From Transgenic Organisms.**

| Proteins | Source | Used for |
|---|---|---|
| Lactoferrin | Cow | Infectious arthritis and GIT infections |
| Monoclonal antibodies | Chicken, cow, goat | Vaccine manufacturing |
| Glutamic acid decarboxylase | Goat | Treat type-1 diabetes |
| Pro 542 | Goat | HIV |
| Serum albumin (human) | Sheep, cow | Blood volume maintenance |
| Fibrinogen | Sheep, cow | Wound healing |
| Antithrombin III | Goat | Thrombosis |
| à-antitrypsin | Sheep | Emphysema |
| à-glucosidase | Rabbit | Pompe's disease |
| Human protein C | Goat | Thrombosis |
| Factor XIII, IX | Sheep, cow, pig | Hemophilia |
| Tissue plasminogen activator | Pig, sheep | Thrombosis |

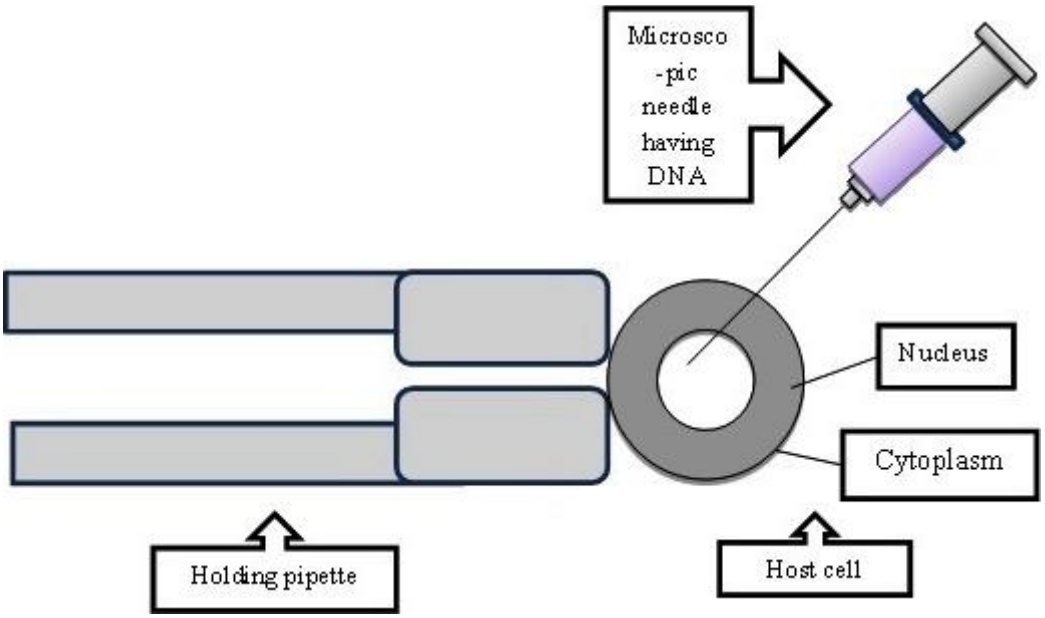


**Figure 1. Microinjection of Foreign DNA Into Host Cell.**

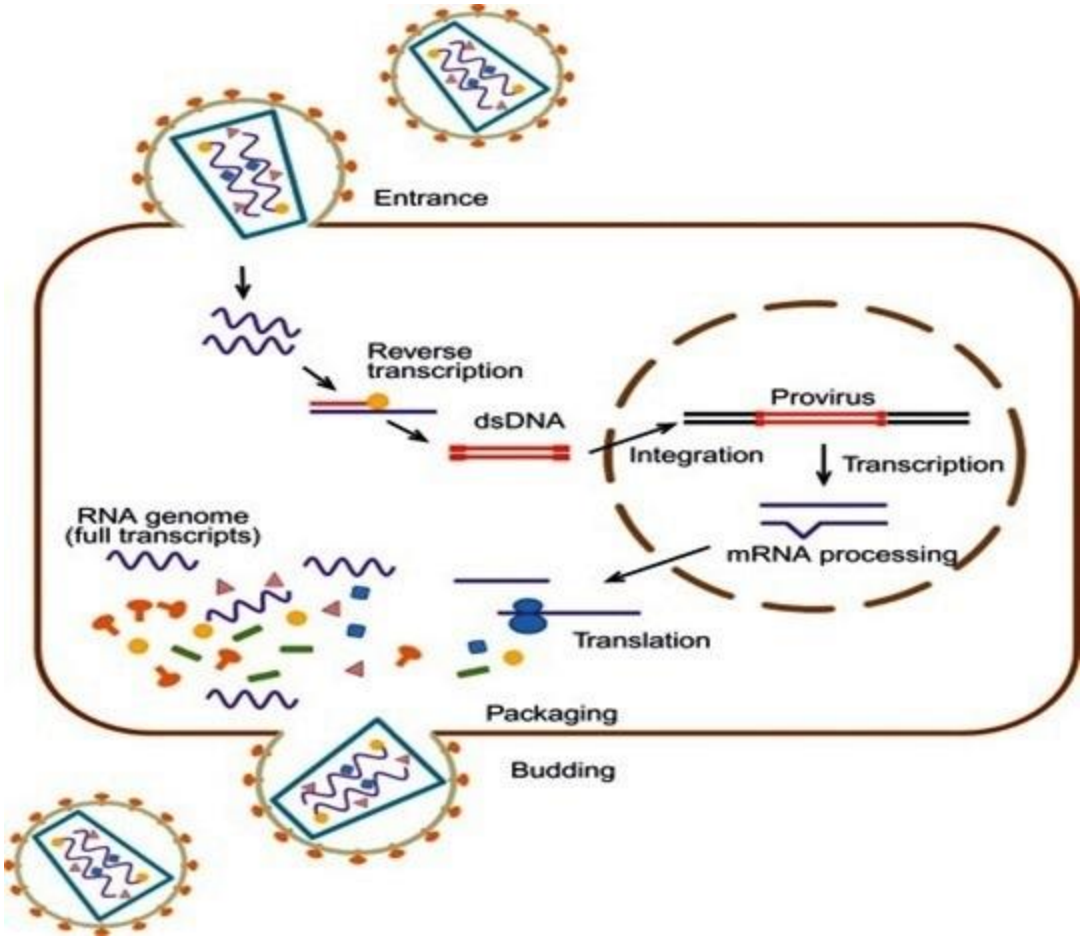


**Figure 2. Retrovirus Mediated Gene Transfer Technique.**

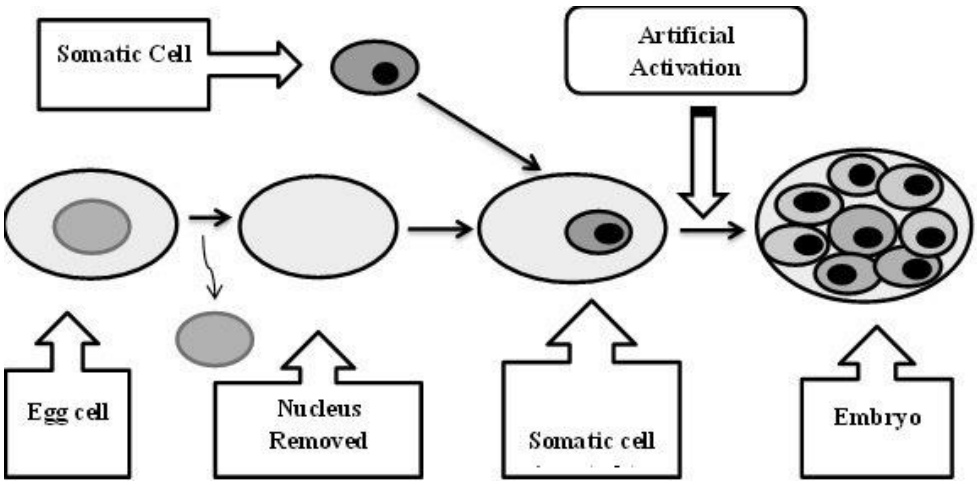


**Figure 3. Illustration of Somatic Cell Nuclear Transfer Technique**.

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