**Topic Unit 3 /5/ Transgenic cows**

**Transgenic cows**

**What is a transgenic cow?**

Transgenic cows are genetically modified (GM) cows. They have an extra gene or genes inserted into their DNA. The extra gene may come from the same species or from a different species.

Transgenic cows produce proteins in their milk

The extra gene (transgene) is present in every cell in the transgenic cow. However, it’s only expressed in mammary tissue. This means that the transgene’s protein will only be found in the cow’s milk and can only be extracted from there.

First, the gene for the desired product is identified and sequenced. Then a gene construct containing this desired gene is created using DNA cloning, restriction enzyme digests and ligation.

The gene construct is then introduced into female bovine (cow) cells by transfection. Transgenic bovine cells are selected and fused with bovine oocytes that have had all of their chromosomes removed. Once fused with the oocyte, the transgenic cell’s chromosomes are reprogrammed to direct development into an embryo, which can be implanted into a recipient cow. After a 9-month gestation period, a female calf is born. She will only express the transgene in her milk during lactation after her first calf is born. This is because expression of the transgene is controlled by a promoter specific to lactating mammary cells.

The first transgenic cow was produced in 1997. It is a relatively new technology, but several transgenic breeds have since been developed. Cows are an attractive target for transgenesis because they naturally secrete large quantities of protein in their milk. This means that, if done correctly, the protein encoded by a transgene will be expressed in the cows milk and can easily be isolated. Thus proteins of therapeutic benefit (to treat human diseases) could be produced in large quantities, relatively inexpensively.

Examples of transgenic cows:

Betta & Kappa Casien

These two proteins are normally produced in milk. Higher concentrations of these proteins increases the value of the milk especially when used for the production of cheese (more cheese per litre is produced and the cheese is often a better quality. Scientists have managed to insert extra copies of these genes into cows to improve the value of the milk produced.

Beta Lactoglobulin (BLG)

This protein in milk has been associated with some allergic reactions, preventing some people from consuming milk products. Scientist have inserted DNA sequences that supress the production of this protein.

Lactoferrin

Lactoferrin is a valuable iron-binding protein that plays a role in preventing bacterial infections. The human lactoferrin gene has been expressed in transgeneic cows.

Scientists producing transgenic cows use a range of techniques including DNA cloning, restriction enzyme digests, ligation, polymerase chain reaction (PCR), transfection, nuclear transfer and in vitro embryo production.

Step 1. Designing the gene construct

The first step is to design a gene construct. The gene construct is a unit of DNA that includes:

an antibiotic resistance gene – to select cells that have taken up the gene construct

a tissue-specific promoter sequence – to signal the start of expression of the protein in cells of the appropriate tissue, for example, in mammary cells in lactating cows

the desired gene – for example, bovine casein or human myelin basic protein

a stop sequence – to define the end of the information for making the protein.

Step 2. Sourcing the transgene

In the past, the gene would have been extracted from the source organism’s DNA. Now, however, if the desired gene sequence is known, it can simply be synthesised in a lab. There are companies that make genes to order within a couple of weeks.

Step 3. Making the gene construct

Gene construct

A gene construct contains all the information needed for transfection into a bovine cell and expression of the desired gene in a cow. This includes an antibiotic resistance marker, a tissue-specific promoter, the transgene/gene of interest and a stop sequence.

The gene is usually supplied in a vector. A vector is a small piece of DNA, often a plasmid, into which a foreign piece of DNA can be inserted. When the gene of interest is in a vector, it can be sent from one lab to another, it can be stored, it can be manipulated or it can be used to transform bacteria to produce more copies of the gene of interest.

Vectors have multiple restriction enzymes sites (also called multiple cloning sites) so the gene can be inserted into the vector and then cut out from the vector using restriction enzymes. This article has more information on restriction enzymes.

After the gene is cut from the vector, it is then pasted into the multiple cloning site of the gene construct using a method known as ligation. This article has more information on DNA ligation.

Step 4. Transfecting bovine cells

The gene construct is incorporated into the genome of a bovine (cow) cell using a technique called transfection. During transfection, holes are made in the cell membrane that allow the DNA to enter. The holes can be made by applying an electrical pulse or by adding chemicals to the cells. Once inside the cell, the gene construct may enter the nucleus and incorporate into the cell’s genome.

Step 5. Selecting for transgene positive cells

After transfection, an antibiotic is added to select the bovine cells that have incorporated the gene construct. Transgenic bovine cells will survive treatment with an antibiotic, because they contain an antibiotic resistance gene making them resistant to the antibiotic. Cells without the gene construct will have no resistance to the antibiotic and will die. In addition to antibiotic selection, polymerase chain reaction (PCR) is used to check that the bovine cell contains the transgene.

Step 6. Making a transgenic embryo using nuclear transfer

Nuclear transfer

Nuclear transfer is used to create a whole animal from a single transgenic bovine cell.

The transgenic bovine cell is fused with a bovine oocyte that has had its chromosomes removed (called an enucleated oocyte). An electrical pulse is applied to help fuse the cells. Once fused with the oocyte, the transgenic cell’s chromosomes are reprogrammed to direct development into an embryo. After 7 days, the transgenic embryo will have about 150 cells and can be transferred into a recipient cow for further development to term.

Step 7. Confirming the cow is transgenic

If the embryo develops to full term, after 9 months, the cow will give birth to a calf. To confirm that the calf is transgenic, scientists can check using:

PCR to determine the presence or absence of the transgene

quantitative PCR (q-PCR) to determine the number of copies of the transgene

fluorescent in situ hybridisation (FISH) to visualise where the transgene is in the chromosome and whether the transgene has integrated into more than one chromosome.

When the calf is lactating (either after being induced to lactate or after having its own progeny), its milk is checked to determine if the transgenic protein is being expressed.

