- A retrovirus (adenovirus) mediated gene transfer:

They are RNA viruses having reverse-transcriptase enzyme to make DNA from RNA. They have the ability to integrate into the host DNA and copied when the cell divides. It results in the production of chimeras because not all cells can get the transgene. The pure homozygous transgenic animals may result after 10 to 20 generations through inbreeding
and can be stored for subsequent implantation.
It is suitable to make transgene in poultry and other species of animals.
Examples of viruses used for this purpose include Moloney Leukaemia Virus (causes lymphoid leukemia in mice, rats, and hamsters); Rous Sarcoma Virus (cancer formation in humans) and Avian Leukosis Virus (poultry flocks). Size limit on the amount of DNA inserted, unable to replicate in early embryonic cells, lower efficiency than the natural coupled with the danger of forming new pathogens are among the drawbacks of this method.

- Stem cell-mediated gene transfer:

According to Gordon (1996), Markkula and Huhtaniemi (1996) and Miao (2012), it involves insertion of the desired gene into totipotent stem cells and the stem cells containing the gene of interest are incorporated to the host embryos resulting in chimeric animals. It does not need a live transgenic animal to test the presence of a desired transgene and it allows testing at the cell stage. It allows gene targeting by allowing directed modification of endogenous genes

- Somatic cell nuclear transfer:

The technique involves transfer of somatic cell nucleus to the cytoplasm of enucleated egg to be reprogrammed by egg cytoplasmic factors to form a zygote. In animals, the zygote must be artificially placed into the uterus of a surrogate mother.
It starts during the mid-1980s, after 30 years of initial successful experiments with frogs and now it is practicable in different species of animals except humans. DOLLY is the best example of somatic cell nuclear transfer technique.

Preview from Notesale.co.uk
Page 7 of 8

Transgenic markers and screening of transgenesis

To test whether the cells incorporate the transgene or not, we should also incorporate transgenic markers which should be screened in different ways. This increases efficiency of transgenesis by identifying true transgenes.
β-galactosidase, firefly luciferase, secreted placental alkaline phosphatase and green fluorescent protein (GFP) are currently available transgenic markers but GFP is the most ideal marker which allows selection of transgenic embryos soon after gene transfer or prior to embryo transfer. Enzymes that inactivate aminoglycoside antibiotics such as neomycin or kanamycin are common markers used in selecting transgenic cells, which are mostly important in molecular biology when the efficiency of transferring gene constructs is poor and a pool of many cells is targeted for transfection .
The Southern blot assay is the most widely used for testing the presence of transgene in the host animals. It involves digestion of DNA with restriction enzymes and analysis is made on agarose gel electrophoresis, which uses electric current. The fragments of DNA moved towards the positive pole from the negative one and settled in the gel according to their size with the bigger fragments on the top while the smaller fragments move faster to the gel and settled on the positive pole. Then, the DNA is denatured by strong base or acid and blotted on to the membrane with hybridization of a probe with DNA of the gene of interest. If the gene of interest is present the blotted membrane picks the probe and illuminates the gene.
The other assay is **western blot**, which is used to detect the transgenic protein produced by animals. SDS-polyacrylamide gel is used for electrophoresis. If the protein is small it moves