+ Cell culture is useful for production of cells in quantity- cancer cells for medical research, monoclonal antibodies
+ Enables production of many genetically identical copies of an animal
+ Conserves unique features
+ Useful for production of cells in quantity
+ Producing a single identical genetic line of cells with desirable characteristics maintains genetic stocks

Disadvantages

- Cross breeding reshuffles genes so desirable characteristic may be lost
- In mammals it is expensive and unreliable with a low success rate; 3% in mice
- Inadvertent selection of disadvantageous alleles is possible
- Progeny may show long term unforeseen effects such as premature ageing

Tissue culture

Cells from young animals and cancer cells can be induced to divide in vitro. Some cells retain their ability to divide in adults e.g. for healing wounds. Most cells differentiate to cells with specific functions e.g. nerve cells, muscle cells and most of these don't normally divide again.

Growing cells in a laboratory is **tissue culture.** The medium the cells grow in is closely controlled and conditions (water potential, temperature) are carefully monitored. Animal cells in tissue culture develop into mature cells of the same type from which the culture was started. All the cells are identical and contain identical genes to the parent cell.

Tissue engineering induces living cells to grow on a synthetic material framework to produce tissue

Applications:

- Skin tissue may be used for burns. Artificial skin (apligraf) widely used instead of skin grafts.
- Culture of viruses for vaccines, and producing monoclonal antibodies (pregnancy testing)
- Blood vessel replacement, bone and cartilage repair, and treatment of degenerative nerve diseases.
- **Stem cells** are undifferentiated dividing cells that are found in adult animal tissues and need to be constantly replaced. They're found in selective tissue e.g. bone marrow. They can develop into any other types of cell in the right conditions. Therefore they can help treat a variety of genetic disorders. Stem cells are also found at the earliest stages of embryo development before the cells have differentiated- embryonic stem cells
- Therapeutic stem cell cloning could be used to generate organs for transplant which would prevent immune rejection and reduce problems of organ shortages
    - A mature cell is taken from the patient and the nucleus is removed
    - The nucleus is removed from the human ovum
    - The mature nucleus is transferred to the enucleated ovum, and then divides forming a ball of stem cells
    - Stem cells are isolated and cultured with appropriate growth factors allowing them to grow into the required organ or tissue

Transfer of DNA into the host cell and the use of genetic markers

The recombinant DNA must be transferred into bacterial cells. This involves mixing the plasmids and bacteria in a medium with calcium ions allowing plasmids to pass into the bacteria.

As few as 1% of bacterial cells take up the plasmids, so these need to be identified, which involves use of antibiotic resistant genes. The low take up may be because some plasmids close without incorporating the DNA fragment.

The technique is based on that some plasmids carry a gene for antibiotic resistance and this is unaffected by the introduction of a new gene. This is a marker gene.

All bacterial cells are grown on a medium with the antibiotic, ampicillin. Bacterial cells that have taken up the plasmids have acquired the gene for ampicillin resistance. These bacterial cells can break down the antibiotic and survive.

Using the bacteria that have successfully taken up foreign DNA means the foreign DNA replicates with the rest of the plasmid every time the bacterial cell divides. Cloning of the recombinant containing bacteria causes production of multiple copies of the recombinant gene. The bacteria divide repeatedly, giving a large population of bacterial cells with replicas of the foreign DNA.

The genetically modified bacteria are cultured on a large scale using a fermenter and produce insulin which is extracted and purified.

1. Identification and isolation of the gene- The gene for insulin can be identified and isolated by starting with mRNA (made by transcription).
2. Isolation of this gene from the rest of the DNA
3. Multiplication of the gene by the polymerase chain reaction
4. Insertion of the gene into a vector to transfer it to the host cell (virus or plasmid)
5. Introduction of the gene into suitable host cells
6. Identification of host cells which have successfully taken up the gene
7. Growth of the population of host cells
8. Production of the desired protein by the host cell
9. Separation of the protein from the host cell
10. Purification of the protein for clinical use

Advantages

+ Large quantities of complex proteins can be made, not possible by other methods
+ No need to extract organs from other mammals

Disadvantages

– Technically complicated and expensive on industrial scale
– Difficult to identify the valuable genes
– Synthesis of a required protein may involve several genes each coding for polypeptide
– Treatment of human DNA with restriction endonucleases produces millions of useless fragments
– Not all eukaryotic genes express themselves in prokaryotic cells
– Hazards: