This fact sheet describes the process of cloning and producing stem cells. It outlines the potential benefits, ethical challenges, and provides an overview of the current policies governing the technology.

In summary

- **Stem cells are cells that have not yet differentiated into a specific tissue or organ cell**
- **Cloning is the creation of an exact genetic replica of a small segment of DNA, a cell or a whole organism**
- **Reproductive cloning is used to produce a genetic duplicate of an existing organism e.g. Dolly the sheep**
- **Therapeutic cloning is used to create undifferentiated stem cells that can then be turned into a specific cell type needed to repair or replace damaged tissue**
- **In Australia, human reproductive cloning is banned. Therapeutic cloning is allowed only for medical research that is governed and overseen by the National Health and Medical Research Committee (NHMRC).**

WHAT IS A STEM CELL?

Stem cells are cells that have several important properties that allow them to repair or replace damaged tissue or organs:

- They can renew themselves through cell division
- They are unspecialised so therefore they are able to develop into different cell types for different tissues and organs.

WHAT IS CLONING?

Cloning is the creation of an exact genetic replica of a small segment of DNA, a cell or a whole organism.

- Identical twins are an example of human clones that are created naturally
- In contrast, Dolly, the cloned sheep, was created artificially in a laboratory in Scotland in 1997.

Dolly's creation led to international awareness of cloning but there are several different types of cloning that can be used for purposes other than producing a genetic replica (or identical twin) of a whole organism like humans or sheep.

TYPES OF CLONING

(a) DNA Cloning

DNA cloning is also known as recombinant DNA technology, molecular cloning and gene cloning.

It is used to produce many copies of a particular segment of DNA containing one or more genes so that the activity or function of the DNA can be studied in the laboratory.

How is DNA cloning done?

The DNA segment of interest from an organism such as a human is incorporated into the **plasmid DNA** of a bacterial cell. A plasmid is a circular self-replicating DNA molecule that is separate from the bacterial DNA (*Figure 22.1*).

The plasmid containing the genes or DNA of interest is now a piece of **recombinant DNA**, made up of human and bacterial DNA. It is then put into a cell that will act as a host: as the host cell is copied over and over again, the recombinant DNA is copied as well. Bacteria are most often the host cells but yeast and mammal cells can be used too. The end result is multiple identical copies of the same human DNA segment or gene.

Benefits of DNA cloning

Cloning produces enough copies of a gene or DNA segment to be analysed for human genetic testing or can be used for the development of drugs and treatments for genetic conditions.

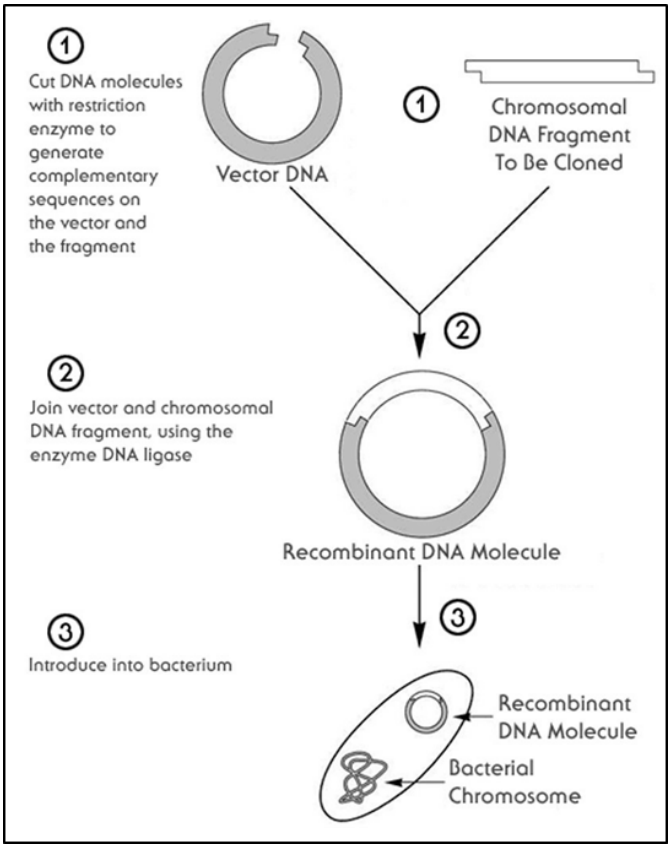

***Figure 22.1**: By fragmenting DNA of any origin (human, animal, or plant) and inserting it in the DNA of rapidly reproducing foreign cells, billions of copies of a single gene or DNA segment can be produced in a very short time. The DNA fragment of interest from an organism such as a human is incorporated into the 'plasmid DNA' of a bacterial cell Figure courtesy of https://public.ornl.gov/site/gallery/detail.cfm?id=385&topic=&citation=&general=cloning&restsection=all)*

(b) Reproductive Cloning or adult DNA Cloning

Reproductive cloning is also called adult DNA cloning. The purpose of this type of cloning is to produce a genetic duplicate of an existing or previously existing organism.

How reproductive cloning is done

Dolly the cloned sheep produced in 1997 is an example of an animal produced by cloning as shown in *Figure 22.2*. She was produced in a process called **somatic cell nuclear transfer (SCNT)**.

A somatic cell is any cell in the body other than the sperm or egg reproductive cells.

In SCNT, the nucleus of a somatic cell isolated from the donor animal is put in an egg cell that has had its nucleus removed. The egg cell is treated with an electric charge or chemicals to simulate fertilisation and cell division to produce an embryo.

The newly formed embryo can be implanted into a surrogate mother and carried to term. An embryo created by SCNT is a genetic replica of the donor animal.

The difference between an embryo created by SCNT versus an embryo created naturally is where the two sets of chromosomes that make up the embryo came from. We normally have 23 pairs of chromosomes.

We receive 1 of each pair from our mother and 1 of each pair from our father. In SCNT, all of the chromosomes come from a somatic cell from the donor. Thus the cloned embryo receives all of the chromosomes from one adult.

An important step in the development of the cloning technology of whole animals is being able to activate the genes that are needed for an embryo to grow and develop that are inactivated soon after their job has been done. Adult cells therefore have to have these genes 'turned on' again.

The discovery of how to do this embryonic gene activation enabled the cloning of Dolly and the technique has since been replicated with a number of other animals including mice, pigs, goats, cats, rabbits and cows.

It should, however, be noted that a clone such as Dolly produced by SCNT *is not an exact* replica of the donor animal.

This is because while Dolly has the same chromosomal DNA located in the nucleus as the white-faced donor sheep, the clone's mitochondrial DNA comes from the egg of another animal – in this case a black-faced sheep.

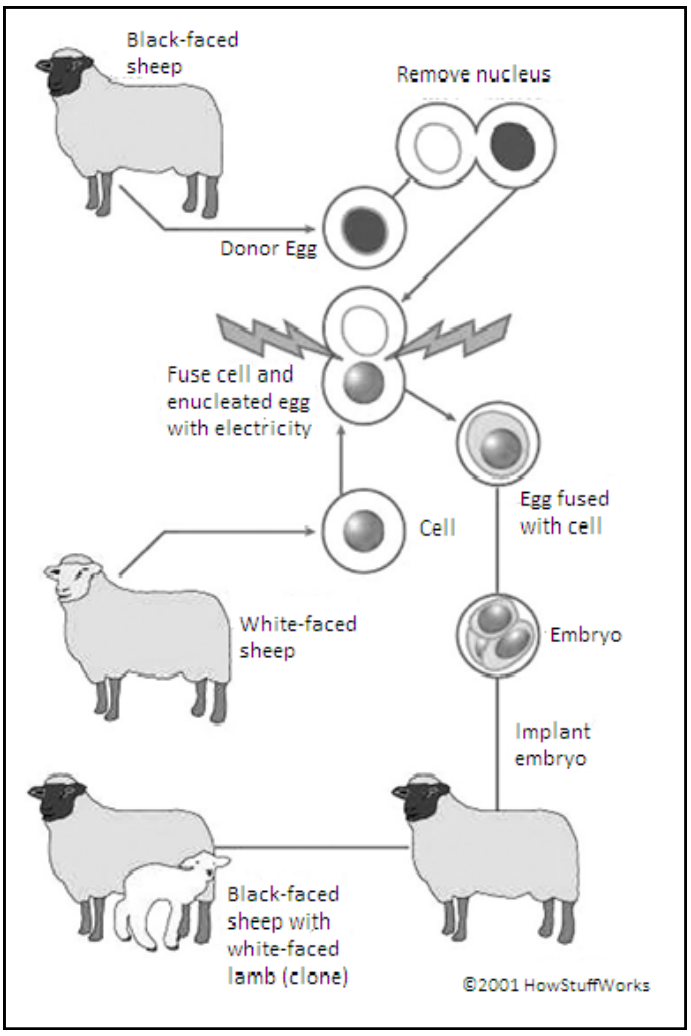

Figure 22.2: *Cloning of Dolly step by step.*
1: Skin cells removed from the udder of a white faced Finn Dorset Ewe and placed in a growth culture containing very low nutrients: the cells are starved; stop dividing and the active genes are switched off.
2: The DNA is removed from an egg cell from a Scottish Blackface Ewe leaving the egg cell cytoplasm containing mitochondria.
3: The skin cell and the egg cell are placed next to each other and subjected to an electric pulse to cause fusion. The fused cell contains 46 chromosomes from the Finn Dorset Ewe in the cytoplasm of the Scottish Blackface Ewe. The fused cell is given a 2nd electric pulse to activate cell division and turn on the genes necessary for growth of the embryo.
4: The embryo is then implanted into the uterus of a different Scottish Blackface Ewe. Dolly, a coned white faced Finn Dorset lamb, is born.
Figure courtesy of: http://science.howstuffworks.com/cloning2.htm

Chromosomes are found in the nucleus of all body cells except for red blood cells which have no nucleus and therefore do not contain chromosomes.

Mitochondrial DNA is found is in very small compartments called mitochondria (the energy centres of the cell) that are found scattered outside the nucleus (*Figure 22.3).* Mitochondrial DNA has its own genes, arranged as one long circle of DNA.

Benefits and limitations of reproductive cloning
Reproductive cloning can have many uses:

- If the low success rates and issues of safety could be improved as discussed below, the technology can be used to mass-produce animals with special qualities, such as animals that are important agriculturally or are able to produce helpful drugs for human use
- SCNT has environmental uses in that it can be used to repopulate endangered species, as has been shown with the wild ox and the gaur
- Some supporters of human reproductive cloning also see it as a way of overcoming male infertility, where other methods of assisted reproduction have failed.

The difficulties however in producing cloned animals are reflected in the number of abnormalities seen in the experiments done to date.

- More than 90% of offspring from cloning are not viable: only one or two offspring are viable for every 100 cloning attempts
- Cloned animals have higher rates of cancer and infection, and a range of other health problems
- While the animal may seem healthy at birth, problems often have appeared later in the life of the resulting clone, so that mature clones have often undergone sudden, unforeseen and unexplainable deaths.

One of the reasons for the high rates of death and abnormalities in cloned animals is perhaps in the reprogramming of the genetic make-up from an adult cell into an embryonic cell that must take place. Some genes will only be switched on or off if they are passed to an embryo through the egg or sperm. This is called **imprinting**.

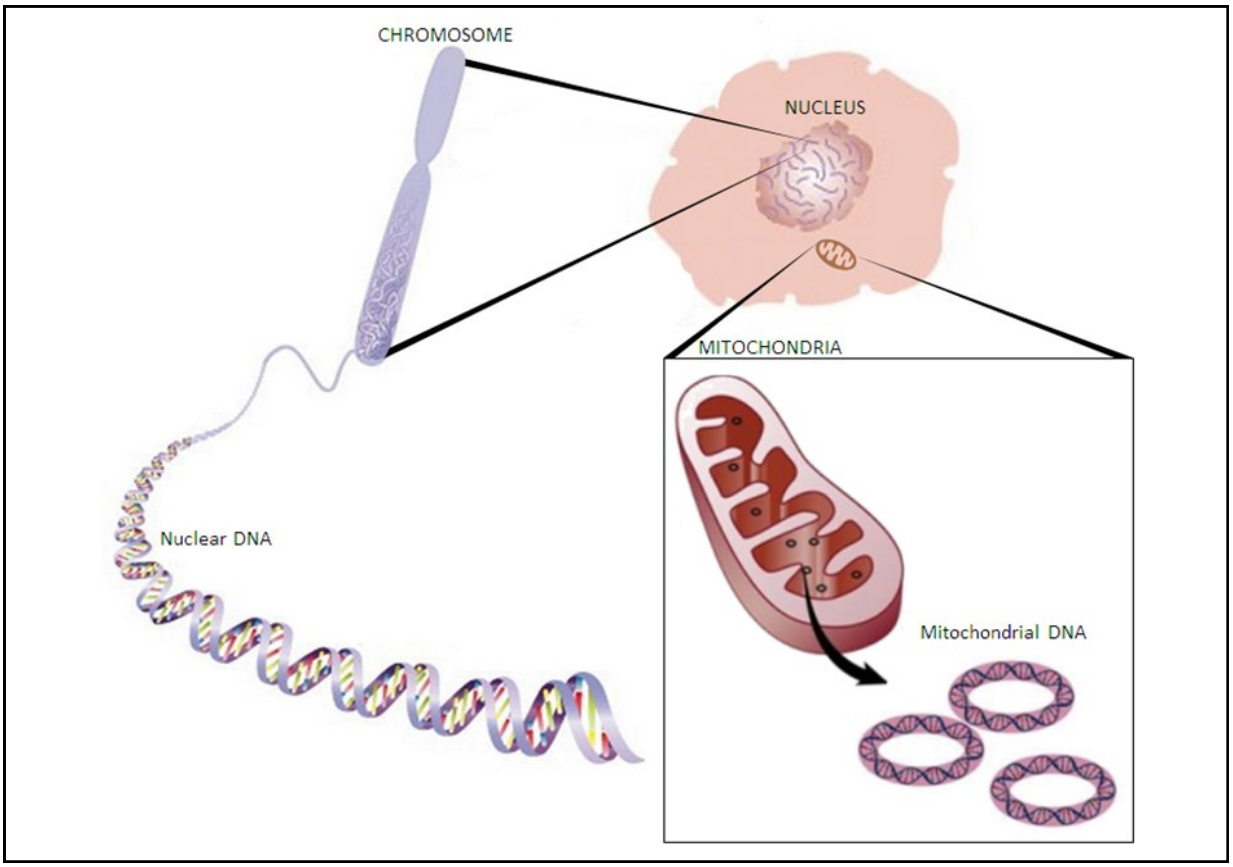

***Figure 22.3**: Diagram of a human cell showing nuclear DNA which is found on chromosomes in the nucleus of a cell and the mitochondrial DNA which is found in the energy centres of cells known as mitochondria. Figure adapted from the NHS National Genetics and Genomics Education Centre.*

Ethical considerations in reproductive cloning

Human reproductive cloning has raised many concerns, not the least of which are the safety considerations and difficulties listed above.

While these technical difficulties may be overcome in the future and may enable cloning of animals with a greater success rate, the use of reproductive cloning for humans is viewed differently.

- Intellectual and emotional development is essential for human growth and health. It is also important to realise that a person is much more than a product of their genes.
- If it were possible to produce a cloned human, the clone would not be a duplicate of that person, other than the nuclear DNA. A person is a product of their environment as well as their genetic make-up. Even identical twins have subtle differences between them.
- The concepts of parenthood, family and views of social responsibilities are all challenged by human reproductive cloning.

(c) Therapeutic cloning to produce stems cells

The aim of therapeutic cloning is to create stem cells containing the person's own DNA that could be grown in the laboratory and then transplanted into them without the risk of tissue rejection.

Diseases that might be treated by transplanting cells generated from human stem cells include Parkinson disease, diabetes, traumatic spinal cord injury, Duchenne muscular dystrophy, heart disease, and vision and hearing loss.

Therapeutic cloning using embryos

When using embryos as the source of the stem cells, this type of cloning is also known as therapeutic cloning, biomedical cloning, embryo cloning or artificial embryo twinning.

This type of cloning essentially mimics the natural process of producing identical twins or triplets.

- Identical twins are formed when the fertilised egg tries to divide into a two-cell stage but the two cells separate instead
- The two separate cells continue dividing on their own and eventually develop into two genetically identical individuals.

In embryonic cloning, cells are removed from an embryo after it has divided for about 5 days. At this stage, the embryo is called a **blastocyst**. Once the cells are removed the embryo is destroyed.

The cells of an embryo are an excellent source of stem cells because they have not yet been differentiated into the cells of specific tissues or organs of the body. While there are around 20,000 genes in each body cell, only those genes that are needed for the function of the cells of the tissue or organ are switched on. The remainder are inactive.

For example, brain cells will have different genes sending instructions to the cells than liver cells. Embryonic stem cells still have all the genes needed for the function of the body's cells to be able to be switched on and so can be differentiated into any type of body cell.

To generate cultures of specific types of differentiated cells — heart muscle cells, blood cells, or nerve cells, for example — scientists try to control the differentiation of embryonic stem cells.

They change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes. Through years of experimentation scientists have established some basic protocols or recipes for the directed differentiation of embryonic stem cells into some specific cell types.

Therapeutic cloning using adult cells

An alternative source of stem cells are adult stem cells that can be extracted from adult tissue such as the bone marrow or fat tissue without harm to the individual.

- An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ. It can renew itself and can differentiate to yield the major specialised cell types of the tissue or organ
- The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are located
- Somatic stem cell is an alternative term instead of adult stem cell
- Unlike embryonic stem cells, which are defined by their origin (the inner cell mass of the blastocyst), the origin of adult stem cells in mature tissues is unknown.

Adult stem cells occur in many more tissues than was once thought possible, and adult stem cells from bone marrow that form into mature blood cells have been used in transplants for 30 years. Tissues include brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin and liver.

There are, however, often only a very small number of stem cells in each tissue. Stem cells are thought to reside in a specific area of each tissue where they may remain quiescent (non-dividing) for many years until they are activated by disease or tissue injury.

Certain kinds of adult stem cells seem to have the ability to differentiate into a number of different cell types, given the right conditions. If this differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of therapies for many serious common diseases.

For example adult stem cells can be used in the treatment of type 1 diabetes where the damaged pancreatic cells that produce insulin are replaced, removing the requirement for daily administered insulin (*Figure 22.4)*.

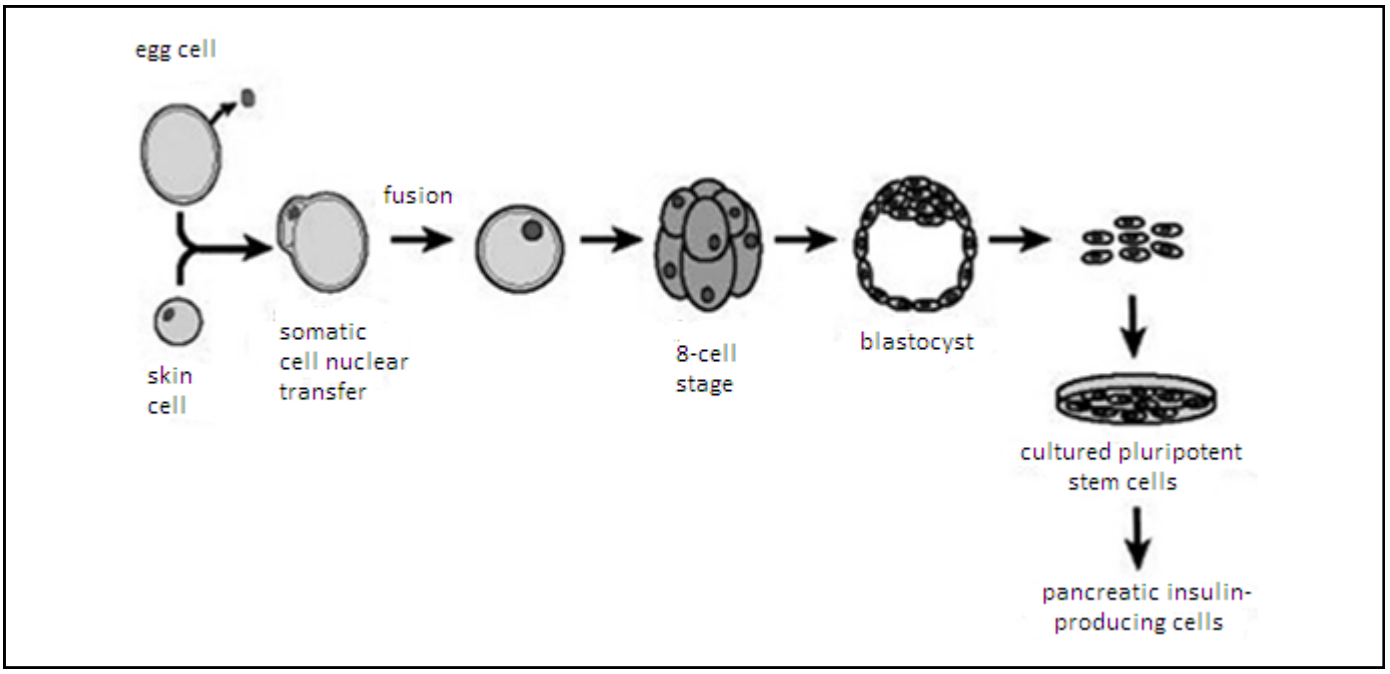

Figure 22.4: *A doctor takes a sample of skin cells from the patient and isolates their DNA. Next, a donor egg cell, emptied of its own genetic contents, is injected with the DNA from the patient. The embryo is nurtured to grow and divide into a blastocyst. Some blastocyst cells are harvested and coaxed with growth factors to mature into insulin-producing cells. Finally, millions of insulin-producing cells are injected back into the patient. In an ideal world, the patient's diabetes is temporarily 'reversed', with no side effects. Figure adapted from: Stem Cells: A Primer, US National Institutes of Health- Source: Cloning around with stem cells http://abc.net.au/science/slab/stemcells/default.htm*

Benefits and limitations of therapeutic cloning

Whatever their source, before stem cells can be used to treat health problems, a number of barriers have to be overcome.

- The stem cells have to be able to be isolated from a source and then grown in the laboratory
- The stem cells have to be able to be turned into the specific cell type needed for treatment.

These first two hurdles have been passed for most of the 220 cell types in the human body.

Overcoming the next barriers of applying the technology clinically and ensuring that the new tissue or organ poses no risk to the patient is still a matter of much research.

While one organ, the skin, is already able to be grown in the laboratory to create a self-compatible skin graft, skin is relatively easy to grow because the mature, differentiated skin cells are still able to divide and produce more cells to repair damage. The cells of other organs or tissues do not have this ability.

An alternative is to create genetically modified pigs from which organs suitable for human transplantation could be harvested.

Pig tissues and organs are the most similar to humans of the animal species that have been cloned. The transplant of organs and tissues from animals to humans is called **xenotransplantation**. One of the major concerns, however, in the transplant of pig tissues and organs to humans, is the transmission of pig viruses to humans.

While adult stem cells may overcome some of the ethical concerns, further studies are needed to determine their ability to differentiate into a variety of new types of cells.

Ethical considerations in therapeutic cloning

Cloning presents many complex ethical questions. A few to consider are who should have access to the technologies, how will the use of the technologies be monitored, who gets to decide what aspects of cloning are morally acceptable and legal to use and which are not. So even if it is possible to clone humans – should we?

The therapeutic cloning technology used to harvest stem cells ultimately results in the destruction of an embryo. The creation of embryos specifically for the purpose of destroying them causes concern for some people.

The advantage of using adult cells as a source of stem cells is that it would avoid this ethical issue of destruction of the embryo.

Currently, embryos that are used for the production of stem cells are those created by infertile couples during *in vitro* fertilisation (IVF) programs. Usually more embryos than are needed to have a baby are produced. Some couples have agreed for their excess embryos to be used for stem cell research.

CLONING POLICIES IN AUSTRALIA

In 1999, the Council of the Australian Academy of Science unanimously endorsed a position statement called 'On Human Cloning' (https://www.science.org.au/sites/default/files/user-content/clone.pdf) that recommended a ban on human reproductive cloning but recognised the potential of therapeutic cloning and considered that it should be fostered in research.

Australia has passed legislation banning human reproductive cloning (Prohibition of Human Cloning for Reproduction and the regulation of Human Embryo Research Amendment Act 2006) but has treated therapeutic cloning differently by passing the Research Involving Human Embryos Act 2002.

- The object of this Act was to address concerns, including ethical concerns, about scientific developments in relation to human reproduction and the utilisation of human embryos by regulating activities that involve the use of certain human embryos created by assisted reproductive technology (ART)
- In 2007, approval was given to use embryos (created after April 5 2002 by ART with the aim of achieving a pregnancy) that are in excess of a couple's needs, and with their informed consent, for stem cell research. This approval applies when that research is governed and overseen by a committee of the National Health and Medical Research Committee (NHMRC)
- The 2007 Act allows scientists to create embryos through therapeutic cloning and extract their stem cells for use in medical research.

Currently, therapeutic cloning research is being conducted in private laboratories in the USA and in both government and private laboratories in the UK, Japan, France, Australia, and other countries.