

## Introduction

The possibility of human cloning, raised when Scottish scientists at Roslin Institute created the much-celebrated sheep "Dolly" (*Nature* 385, 810-13, 1997), aroused worldwide interest and concern because of its scientific and ethical implications. The feat, cited by *Science* magazine as the breakthrough of 1997, also generated uncertainty over the meaning of "cloning" --an umbrella term traditionally used by scientists to describe different processes for duplicating biological material.

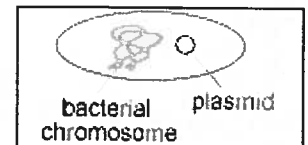
## What is cloning? Are there different types of cloning?

When the media report on cloning in the news, they are usually talking about only one type called reproductive cloning. There are different types of cloning however, and cloning technologies can be used for other purposes besides producing the genetic twin of another organism. A basic understanding of the different types of cloning is key to taking an informed stance on current public policy issues and making the best possible personal decisions. The following three types of cloning technologies will be discussed: (1) recombinant DNA technology or DNA cloning, (2) reproductive cloning, and (3) therapeutic cloning.

## Recombinant DNA Technology or DNA Cloning

The terms "recombinant DNA technology," "DNA cloning," "molecular cloning," and "gene cloning" all refer to the same process: the transfer of a DNA fragment of interest from one organism to a self-replicating genetic element such as a bacterial plasmid. The DNA of interest can then be propagated in a foreign host cell. This technology has been around since the 1970s, and it has become a common practice in molecular biology labs today.

Scientists studying a particular gene often use bacterial plasmids to generate multiple copies of the same gene. Plasmids are self-replicating extra-chromosomal circular DNA molecules, distinct from the normal bacterial genome (see image to the right). Plasmids and other types of cloning vectors were used by Human Genome Project researchers to copy genes and other pieces of chromosomes to generate enough identical material for further study.



To "clone a gene," a DNA fragment containing the gene of interest is isolated from chromosomal DNA using restriction enzymes and then united with a plasmid that has been cut with the same restriction enzymes. When the fragment of chromosomal DNA is joined with its cloning vector in the lab, it is called a "recombinant DNA molecule." Following introduction into suitable host cells, the recombinant DNA can then be reproduced along with the host cell DNA. See a diagram depicting this process.

Plasmids can carry up to 20,000 bp of foreign DNA. Besides bacterial plasmids, some other cloning vectors include viruses, bacteria artificial chromosomes (BACs), and yeast artificial chromosomes (YACs). Cosmids are artificially constructed cloning vectors that carry up to 45 kb of foreign DNA and can be packaged in lambda phage particles for infection into *E. coli* cells. BACs utilize the naturally occurring F-factor plasmid found in *E. coli* to carry 100- to 300-kb DNA inserts. A YAC is a functional chromosome derived from yeast that can carry up to 1 MB of foreign DNA. Bacteria are most often used as the host cells for recombinant DNA molecules, but yeast and mammalian cells also are used.

## Reproductive Cloning

Reproductive cloning is a technology used to generate an animal that has the same nuclear DNA as another currently or previously existing animal. Dolly was created by reproductive cloning technology. In a process called "somatic cell nuclear transfer" (SCNT), scientists transfer genetic material from the nucleus of a donor adult cell to an egg whose nucleus, and thus its genetic material, has been removed. The reconstructed egg containing the DNA from a donor cell must be treated with chemicals or electric current in order to stimulate cell division. Once the cloned embryo reaches a suitable stage, it is transferred to the uterus of a female host where it continues to develop until birth.

Dolly or any other animal created using nuclear transfer technology is not truly an identical clone of the donor animal. Only the clone's chromosomal or nuclear DNA is the same as the donor. Some of the clone's genetic materials come from the mitochondria in the cytoplasm of the enucleated egg. Mitochondria, which are organelles that serve as power sources to the cell, contain their own short segments of DNA. Acquired mutations in mitochondrial DNA are believed to play an important role in the aging process.

Dolly's success is truly remarkable because it proved that the genetic material from a specialized adult cell, such as an udder cell programmed to express only those genes needed by udder cells, could be reprogrammed to generate an entire new organism. Before this demonstration, scientists believed that once a cell became specialized as a liver, heart, udder, bone, or any other type of cell, the change was permanent and other unneeded genes in the cell would become inactive. Some scientists believe that errors or incompleteness in the reprogramming process cause the high rates of death, deformity, and disability observed among animal clones.

## Therapeutic Cloning

Therapeutic cloning, also called "embryo cloning," is the production of human embryos for use in research. The goal of this process is not to create cloned human beings, but rather to harvest stem cells that can be used to study human development and to treat disease. Stem cells are important to biomedical researchers because they can be used to generate virtually any type of specialized cell in the human body. Stem cells are extracted from the egg after it has divided for 5 days. The egg at this stage of development is called a blastocyst. The extraction process destroys the embryo, which raises a variety of ethical concerns. Many researchers hope that one day stem cells can be used to serve as replacement cells to treat heart disease, Alzheimer's, cancer, and other diseases. See [more](#) on the potential use of cloning in organ transplants.

In November 2001, scientists from Advanced Cell Technologies (ACT), a biotechnology company in Massachusetts, announced that they had cloned the first human embryos for the purpose of advancing therapeutic research. To do this, they collected eggs from women's ovaries and then removed the genetic material from these eggs with a needle less than 2/10,000th of an inch wide. A skin cell was inserted inside the enucleated egg to serve as a new nucleus. The egg began to divide after it was stimulated with a chemical called ionomycin. The results were limited in success. Although this process was carried out with eight eggs, only three began dividing, and only one was able to divide into six cells before stopping.



### Celebrity Sheep Died at Age 6

Dolly, the first mammal to be cloned from adult DNA, was put down by lethal injection Feb. 14, 2003. Prior to her death, Dolly had been suffering from lung cancer and crippling arthritis. Although most Finn Dorset sheep live to be 11 to 12 years of age, postmortem examination of Dolly seemed to indicate that, other than her cancer and arthritis, she appeared to be quite normal. The unnamed sheep from which Dolly was cloned had died several years prior to her creation. Dolly was a mother to six lambs, bred the old-fashioned way.

Image credit: Roslin Institute Image Library

## **How can cloning technologies be used?**

Recombinant DNA technology is important for learning about other related technologies, such as gene therapy, genetic engineering of organisms, and sequencing genomes. Gene therapy can be used to treat certain genetic conditions by introducing virus vectors that carry corrected copies of faulty genes into the cells of a host organism. Genes from different organisms that improve taste and nutritional value or provide resistance to particular types of disease can be used to genetically engineer food crops. See [Genetically Modified Foods and Organisms](#) for more information. With genome sequencing, fragments of chromosomal DNA must be inserted into different cloning vectors to generate fragments of an appropriate size for sequencing. [See a diagram on constructing clones for sequencing.](#)

If the low success rates can be improved (Dolly was only one success out of 276 tries), reproductive cloning can be used to develop efficient ways to reliably reproduce animals with special qualities. For example, drug-producing animals or animals that have been genetically altered to serve as models for studying human disease could be mass produced.

Reproductive cloning also could be used to repopulate endangered animals or animals that are difficult to breed. In 2001, the first clone of an endangered wild animal was born, a wild ox called a gaur. The young gaur died from an infection about 48 hours after its birth. In 2001, scientists in Italy reported the successful cloning of a healthy baby mouflon, an endangered wild sheep. The cloned mouflon is living at a wildlife center in Sardinia. Other endangered species that are potential candidates for cloning include the African bongo antelope, the Sumatran tiger, and the giant panda. Cloning extinct animals presents a much greater challenge to scientists because the egg and the surrogate needed to create the cloned embryo would be of a species different from the clone.

Therapeutic cloning technology may some day be used in humans to produce whole organs from single cells or to produce healthy cells that can replace damaged cells in degenerative diseases such as Alzheimer's or Parkinson's. Much work still needs to be done before therapeutic cloning can become a realistic option for the treatment of disorders.

## **What animals have been cloned?**

Scientists have been cloning animals for many years. In 1952, the first animal, a tadpole, was cloned. Before the creation of Dolly, the first mammal cloned from the cell of an adult animal, clones were created from embryonic cells. Since Dolly, researchers have cloned a number of large and small animals including sheep, goats, cows, mice, pigs, cats, rabbits, and a gaur. See [Cloned Animals](#) below. All these clones were created using nuclear transfer technology.

Hundreds of cloned animals exist today, but the number of different species is limited. Attempts at cloning certain species have been unsuccessful. Some species may be more resistant to somatic cell nuclear transfer than others. The process of stripping the nucleus from an egg cell and replacing it with the nucleus of a donor cell is a traumatic one, and improvements in cloning technologies may be needed before many species can be cloned successfully.

## **Can organs be cloned for use in transplants?**

Scientists hope that one day therapeutic cloning can be used to generate tissues and organs for transplants. To do this, DNA would be extracted from the person in need of a transplant and inserted into an enucleated egg. After the egg containing the patient's DNA starts to divide, embryonic stem cells that can be transformed into any type of tissue would be harvested. The stem cells would be used to generate an organ or tissue that is a genetic match to the recipient. In theory, the cloned organ could then be transplanted into the patient without the risk of tissue rejection. If organs could be generated from cloned human embryos, the need for organ donation could be significantly reduced.

Many challenges must be overcome before "cloned organ" transplants become reality. More effective technologies for creating human embryos, harvesting stem cells, and producing organs from stem cells would have to be developed. In 2001, scientists with the biotechnology company Advanced Cell Technology (ACT) reported that they had cloned the first human embryos; however, the only embryo to survive the cloning process stopped developing after dividing into six cells. In February 2002, scientists with the same biotech company reported that they had successfully transplanted kidney-like organs into cows. The team of researchers created a cloned cow embryo by removing the DNA from an egg cell and then injecting the DNA from the skin cell of the donor cow's ear. Since little is known about manipulating embryonic stem cells from cows, the scientists let the cloned embryos develop into fetuses. The scientists then harvested fetal tissue from the clones and transplanted it into the donor cow. In the three months of observation following the transplant, no sign of immune rejection was observed in the transplant recipient.

Another potential application of cloning to organ transplants is the creation of genetically modified pigs from which organs suitable for human transplants could be harvested. The transplant of organs and tissues from animals to humans is called xenotransplantation.

Why pigs? Primates would be a closer match genetically to humans, but they are more difficult to clone and have a much lower rate of reproduction. Of the animal species that have been cloned successfully, pig tissues and organs are more similar to those of humans. To create a "knock-out" pig, scientists must inactivate the genes that cause the human immune system to reject an implanted pig organ. The genes are knocked out in individual cells, which are then used to create clones from which organs can be harvested. In 2002, a British biotechnology company reported that it was the first to produce "double knock-out" pigs that have been genetically engineered to lack both copies of a gene involved in transplant rejection. More research is needed to study the transplantation of organs from "knock-out" pigs to other animals.

## **What are the risks of cloning?**

Reproductive cloning is expensive and highly inefficient. More than 90% of cloning attempts fail to produce viable offspring. More than 100 nuclear transfer procedures could be required to produce one viable clone. In addition to low success rates, cloned animals tend to have more compromised immune function and higher rates of infection, tumor growth, and other disorders. Japanese studies have shown that cloned mice live in poor health and die early. About a third of the cloned calves born alive have died young, and many of them were abnormally large. Many cloned animals have not lived long enough to generate good data about how clones age. Appearing healthy at a young age unfortunately is not a good indicator of long-term survival. Clones have been known to die mysteriously. For example, Australia's first cloned sheep appeared healthy and energetic on the day she died, and the results from her autopsy failed to determine a cause of death.

In 2002, researchers at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, reported that the genomes of cloned mice are compromised. In analyzing more than 10,000 liver and placenta cells of cloned mice, they discovered that about 4% of genes function abnormally. The abnormalities do not arise from mutations in the genes but from changes in the normal activation or expression of certain genes.

Problems also may result from programming errors in the genetic material from a donor cell. When an embryo is created from the union of a sperm and an egg, the embryo receives copies of most genes from both parents. A process called "imprinting" chemically marks the DNA from the mother and father so that only one copy of a gene (either the maternal or paternal gene) is turned on. Defects in the genetic imprint of DNA from a single donor cell may lead to some of the developmental abnormalities of cloned embryos.

For more details on the risks associated with cloning, see the [Cloning Problems](#) links below.

## **Should humans be cloned?**

Physicians from the American Medical Association and scientists with the American Association for the Advancement of Science have issued formal public statements advising against human reproductive cloning. The U.S. Congress has considered the passage of legislation that could ban human cloning. See the [Policy and Legislation](#) links below.

Due to the inefficiency of animal cloning (only about 1 or 2 viable offspring for every 100 experiments) and the lack of understanding about reproductive cloning, many scientists and physicians strongly believe that it would be unethical to attempt to clone humans. Not only do most attempts to clone mammals fail, about 30% of clones born alive are affected with "large-offspring syndrome" and other debilitating conditions. Several cloned animals have died prematurely from infections and other complications. The same problems would be expected in human cloning. In addition, scientists do not know how cloning could impact mental development. While factors such as intellect and mood may not be as important for a cow or a mouse, they are crucial for the development of healthy humans. With so many unknowns concerning reproductive cloning, the attempt to clone humans at this time is considered potentially dangerous and ethically irresponsible. See the [Cloning Ethics](#) links below for more information about the human cloning debate.