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What is cloning?

Cloning in biotechnology refers to processes used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. It describes the processes used to create an exact genetic replica of another cell, tissue or organism. The copied material, which has the same genetic makeup as the original, is referred to as a clone.

When is cloning done?

Cloning is done to amplify the DNA fragments containing the gene of interest and ensures pure sample of gene. When the concentration of the required gene products (proteins) is very low, the production of multiple clones makes it easy to obtain large quantity of the gene products.

What all can be cloned?

Cloning is a powerful techninque that can be implemented to obtain clones of genes (molecular cloning), cells (cell cloning) and organisms(organism cloning). There are three different types of cloning: Gene cloning, which creates copies of genes or segments of DNA Reproductive cloning, which creates copies of whole animals Therapeutic cloning, which creates embryonic stem cells. Researchers hope to use these cells to grow healthy tissue to replace injured or diseased tissues in the human body.

What are the requisites for cloning?

In biotechnology, cloning requires a gene of interest, a vehicle (vectors) to carry the gene, a host to provide an environment for multiplication of genes and a medium for growth of the host strain.

What is DNA cloning?

DNA cloning is the procedures to produce multiple copies of a single gene or segment of DNA. 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.

What are the steps involved in cloning?

The major steps involved in cloning a gene are:
1. Preparation of carrier DNA (vector DNA).
2. Isolation of the desired gene.
3. Insertion of the isolated gene into the vector (which results in the rDNA).
4. Transformation of rDNA into a suitable host.
5. Expression of rDNA(cloned gene).

CLONING VECTORS-

Cloning vectors are self replicating DNA , that is used to carry our gene of interest into a host system where the gene is then expressed. There are many cloning vectors used in cloning procedures. Usually, the following are used :

1) Plasmids.
2) Bacteriophages
3) Bacterial artificial chromosomes
4) Yeast artificial chromosome.

Plasmids:
Plasmids are self replicating, double stranded, extra chromosomal , covalently linked circular DNA. They range in size from 5000 to 400000 bp. They can be introduced into bacterial cells by a process called transformation. The usual strategy is to use a plasmid that includes a gene that the host cell requires for growth under specific conditions, such as a gene that confers resistance to an antibiotic. Only cells transformed by the recombinant plasmid can grow in the presence of that antibiotic, making any cell that contains the plasmid “ selectable” under those growth conditions. We can insert and clone effectively upto 15000 bp sized DNA fragment.

Bacteriophage λ:
Bacteriophage λ has a very efficient mechanism for delivering its 48, 502 bp of DNA into a bacterium, and it can be used as a vector to clone somewhat larger DNA segments . Two key features contribute to its utility:

1) About one-third of the λ genome is nonessential and can be replaced with foreign DNA. 2) DNA is packaged into infectious phage particles only if it is between 40, 000 and 53, 000 bp long, a constraint that can be used to ensure packaging of recombinant DNA only. Bacteriophage λ vectors permit the cloning of DNA fragments of up to 23, 000 bp.

Bacterial Artificial Chromosomes (BACs):
Bacterial artificial chromosomes are simply plasmids designed for the cloning of very long segments (typically 100, 000 to 300, 000 bp) of DNA . They include specific marker-like sites such as resistance to the antibiotic chloramphenicol (CmR), as well as a very stable origin of replication (ori) that maintains the plasmid at one or two copies per cell. DNA fragments of several hundred thousand base pairs can be cloned into the BAC vector.

Yeast Artificial Chromosomes (YACs):
The genome of the most commonly used yeast, Saccharomyces cerevisiae, contains only 14 x 106 bp (less than four times the size of the E. coli chromosome), and its entire sequence is known. It helps us to the study of many aspects of eukaryotic cell biochemistry. YAC vectors contain all the elements needed to maintain a eukaryotic chromosome in the yeast nucleus: a yeast origin of replication, two selectable markers. Those with inserts of more than 150, 000 bp are nearly as stable as normal cellular chromosomes, whereas those with inserts of less than 100, 000 bp are gradually lost during mitosis.

\*\*HISTORY\*\*
Cloning in Nature
Cloning has been going on in the natural world for thousands of years. A clone is simply one living thing made from another, leading to two organisms with the same set of genes. In that sense, identical twins are clones, because they have identical DNA. Sometimes, plants are self-pollinated, producing seeds and eventually more plants with the same genetic code. Some forests are made entirely of trees originating from one single plant; the original tree spread its roots, which later sprouted new trees. When earthworms are cut in half, they regenerate the missing parts of their bodies, leading to two worms with the same set of genes. However, the ability to intentionally create a clone in the animal kingdom by working on the cellular level is a very recent development. Early Progress

The first cloned animals were created by Hans Dreisch in the late 1800’s. Dreich’s original goal was not to create identical animals, but to prove that genetic material is not lost during cell division. Dreich’s experiments involved sea urchins, which he picked because they have large embryo cells, and grow independently of their mothers. Dreich took a 2 celled embryo of a sea urchin and shook it in a beaker full of sea water until the two cells separated. Each grew independently, and formed a separate, whole sea urchin. In 1902, another scientist, embryologist Hans Spemman, used a hair from his infant son as a knife to separate a 2-celled embryo of a salamander, which also grow externally. He later separated a single cell from a 16-celled embryo. In these experiments, both the large and the small embryos developed into identical adult salamanders. Spemman went on to propose what he called a “ fantastical experiment” — to remove the genetic material from an adult cell, and use it to grow another adult. In this way, he theorized, he would be able to prove that no genetic material was lost as cells grew and divided. New Advances

There were no major advances in cloning until November of 1951, when a team of scientists in Philadelphia working at the lab of Robert Briggs cloned a frog embryo. This team did not simply break off a cell from an embryo, however. They took the nucleus out of a frog embryo cell and used it to replace the nucleus of an unfertilized frog egg cell, completing the “ fantastical experiment” of nearly 50 years before. Once the egg cell detected that it had a full set of chromosomes, it began to divide and grow. This was the first time that this process, called nuclear transplant, was ever used, and it continues to be used today, although the method has changed slightly. False Hopes

In 1977, a German scientist shocked the world, claiming to have cloned three mice from embryos. Although embryos had been cloned before, no one had been able to do the experiment with mice because the cells were so small and the tools so large that the cells were traumatized and would eventually die after a few divisions. He instantly became famous, telling the world how he cloned his mice. However, he refused to actually demonstrate any of his techniques, and when other scientists couldn’t replicate his work, he came under suspicion. He was challenged — repeat his work or be discredited. He accepted. He claimed to work nights and mornings when no one was around, but the equipment was never disturbed. He showed off his mouse embryos’ growth daily, even though a malfunction in the water purification system left other scientists at his lab unable to grow other embryos.

Later, in his cabinet, test tubes were found with mouse embryos in them, each at a different stage of development. Most scientists do not believe that this scientist was ever able to clone adult mice. In 1978, a science fiction writer published a book claiming that a millionaire (known to the readers only as Max) had come to him because of his connections as a writer, and asked the him to arrange for Max to be cloned. The author eventually agreed, as the story goes, and Max was cloned. The book was ranked in the Top 10 list of popular books. Scientists who read his book, however, noticed discrepancies between the book and scientific data. One man who was quoted in the book was angry enough to sue. The publisher admitted that the book was a hoax, but the author maintains his claim to this day. Within these two years, two front-page advances in cloning were discovered to be, most likely, frauds. As a direct result, many scientists began to claim that cloning of mammals was impossible. Funding and interest dropped, and cloning returned to the realm of science fiction for several years. First Cloned Mammals

A breakthrough came in 1986. Two teams, working independently but using nearly the same method, each on opposites side of the Atlantic, announced that they had cloned a mammal. One team was led by Steen Willadsen in England, which cloned a sheep’s embryo. The other team was led by Neal First in America, which cloned a cow’s embryo. Many advances were made during the course of these experiments, including progress in keeping tissue alive in lab conditions. However, neither team believed that it was possible to clone from an adult’s differentiated cells. With no progress in sight, the prospect of cloning fell by the wayside, and little research was done on the matter. Dolly

Ian Wilmut at the Roslin Institute in Scotland was assigned to a project in 1986. His goal was to create a sheep that produced a certain chemical in its milk. He chose to alter adult cells, which held up well in laboratory conditions, and then clone them, producing animals with the altered gene all throughout their bodies. He began the paperwork in 1987, and began research in 1990. One of Wilmut’s colleagues, who had experience with cloning from early embryo cells, suggested that the reason so many cloning attempts failed was that the cells were in incompatible stages of life. In one stage, the cells are adding to the DNA, in another, they are proofreading it, and in another, splitting it. The cells, he theorized, could not always start over. Wilmut’s team learned that by starving the cells, they could be forced into what is called the G0 phase, similar to cellular hibernation.

This advance increased the survival rate of the cloned cells; Megan and Morag, two lambs, were cloned from sheep embryos. Wilmut’s team now realized that differentiation did not matter in cloning. More work was done, and on July 5, 1996, a lamb was born, cloned from a frozen mammary cell from another adult sheep. Wilmut, who names his animals very creatively, named her Dolly after Dolly Parton. Although Dolly was just a step in a long experiment, the press descended upon the first animal cloned from an adult. The Roslin Institute was overrun with journalists and reporters. However, other scientists were critical — Dolly took 277 tries to create, and other labs were unable to reproduce the results. In addition, it took over a year for the institute to test Dolly’s DNA to make sure that it was indeed the same as that of the frozen mammary cells. Science, although temporarily impressed, demanded a better way. Herd of Mice

Oct 3, 1997, the Honolulu Technique created Cumulina the cloned mouse. She was cloned from cumulus cells (cells which surround developing egg cells) using traditional nuclear transfer. The nucleus was taken from the cumulus cell and implanted in an egg cell from another mouse. The new cell was then treated with a chemical to make it grow and divide. The scientists repeated the process for three generations, yielding over fifty mice that are virtually identical by the end of July, 1998. The Honolulu Technique’s success rate of 50: 1 is almost six times better than that of the Roslin Institute’s success rate, 277: 1. As cloning technology improves, more and more applications will be seen in everyday life. Mainstream Cloning

How much do you love your dog? Is your dog so perfect that you would pay over $2. 3 million dollars to have another just like it? One couple thinks their 11-year-old dog is just such an animal. Wishing to remain anonymous to avoid run-ins with the press, this couple has contracted Texas A&M University to clone their dog, Missy. Scientists are hailing this for its scientific achievement; no dogs have been cloned before because their reproductive system is rather complicated. If the cloning of dogs can be achieved, perhaps exceptional animals like rescue animals can be reproduced. In addition to the pure scientific appeal of cloning a dog, the attempt to clone Missy has another interesting addition to make to the history of cloning. A private couple wants their dog cloned. They are, of course, spending millions to have her cloned, but consider the possibilities. Could cloning the family pet one day become a normal alternative to buying a new one? Applications

Reliable cloning can be used to make farming more productive by replicating the best animals. It can make medical testing more accurate by providing test subjects that all react the same way to the same drug. It can allow mass production of genetically altered animals, plants, and bacteria. It may settle once and for all what part of personality is dependent on genetics and what part on environment. In short, it can be beneficial to almost every area of biological science.

\*\*STATE THE PROBLEM\*\*
Since March 1997, the birth of a cloned sheep, named Dolly, has caused a great sensation around the world. Though it was not the first time that the experiment using cloning succeeded, the reason why Dolly shocked the world was that she was the first clone from a cell of an adult mammal, something previously thought to be impossible? This meant that the possibility of cloning human beings was increased. For fear of realizing human cloning, a lot of countries have taken necessary measures to regulate the study of it. Some people, such as scientists in this field and certain infertile couples, are now arguing against banning it. But I think we should ban human cloning because it has a possibility to reduce the value of our life, to take away individuality in our society, and to destroy the moral and social systems human have long cultivated.

Richard Seed, a physicist who supports human cloning, said in a radio interview that he would open a clinic of human cloning for infertile people and make a big profit (Hotwired Japan). If human cloning is allowed, there would be so many people such as Seed, who use cloning technology to make money. The business operating the technology looks so profitable because according to a report, there could be 100, 000 or so women in the U. S. who would like a similar chance to use cloning to have their own babies (Watson). Probably, they do not care how much money they pay to have their own blood-related babies. To allow human cloning creates a world where we could get life with money. Buying life reduce the value of it because we cannot respect things which we can get easily. It could lead to the increase of murder. And it would be possible that someday at a department store we can buy life that is labelled, “ ON SALE!”

There are many people in the world who do anything for the sake of money. As a result, cloning technology is in danger of being abused. First, I think I should explain how to clone humans. In the simplest language, cloning is the process in which the DNA from some cell of a body is put by electrical shock into a female egg cell whose DNA has been removed. Then the egg cell is implanted into a womb, and after that it grows as same as a natural baby does. The problem is that there is absolutely no reason why the egg cell need be implanted into the same woman who offers DNA. Hence it might be connected with abuses, such as a black market of egg cells or a black company offering surrogate mothers. Watson pointed out that because many women suffer from anatomical complications which prohibit successful childbearing, they might try to find a suitable surrogate mother. And he also insists that women who just do not like the discomforts of pregnancy might use cloning technology. In this point, human cloning is no longer developed for only infertile people but also for lazy women.

As I explained in the previous paragraph, cloning needs the DNA of only one person. Dr. Ian Wilmut in Roslin Institute, where Dolly was produced, claims that we should use the word, “ copying,” for reproduction of a human instead of “ cloning”. He has investigated cloning technology in order to produce milk which contains a human protein for premature babies, not to clone humans (Begley 47). Human cloning would create duplication of humans, and the individuality of each human would be lost. Someone may oppose the idea saying that the environment in which a person grows largely contributes the formation of his personality; however, his appearance would be completely the same as the original. Someone may contend that twins have the same appearance and are accepted. However, making twins restricts the individuality artificially, not naturally. Natural and artificial are very different. We should separate them.

I doubt whether a human conceived by cloning will be treated as same as the others. In a present school situation where bullying is an everyday occurrence, the fact of being a clone may be a factor of being bullied. And it is possible that a cloned human would be dependent and inferior existence to the original. We would most likely think a clone is kind of a copy of the original rather than a kind of twin because of the disparity in age. Thus human cloning is an invasion of privacy for the human conceived by cloning. We should consider human cloning in that light. We do not have a right to take away the originality of any human – even a clone.

Of course, there are advantages to develop cloning technology. Ian Wilmut demands that the study of it not to be banned, emphasizing that the technology would be useful for the development of treatment for genetic diseases and of mass production of good cattle which will be surely needed in the future when population is larger than today. Meanwhile, he opposes human cloning for moral reasons. Human cloning will make family system even worse than it is at the present. Because cloning needs only one DNA, and it does not matter whether a man or a woman offers it, we can easily imagine that there will be much more single-parent families. If a man wants to have a baby by himself, he can have one, offering his DNA to a surrogate mother. In other words, children conceived by cloning cannot have both blood-related father and mother. Even if it is okay for a parent, it is very hard for the child. We may anticipate that juvenile delinquents would increase at higher rate than now.

Some religions would be badly damaged for the reason that the belief that God give a birth to human would be destroyed. Mark D. Eibert, an attorney at law, argues that religious thinking should not be an excuse of banning things since all people in a country do not believe one specific religion. I agree with this point; however, we should not ignore religions completely. In fact there are some countries where one religion plays an important role. Likewise, I suppose that human cloning would create a society where science technology controls everything even our birth. According to Watson, cloning technology might allow us to choose which baby we have, a boy or a girl. The technology will influence so many things in so many fields.

By permitting cloning of human, too many problems would occur. I can understand that the research of human cloning will help infertility. But I attach more importance to the value of our lives, individuality, and bond of a family than curing minor number of people. Eibert strongly insists that the government should not make cloning of human illegal. He claimed that everyone has the right to have his baby and it is a freedom which the government cannot infringe. I opposed this opinion and the government should intervene in this kind of dispute. I wonder who else except the government can make the scientists think calmly and tell them to stop improving the technology that has a possibility of breaking rules in our society. I insist that cloning of human should not be done

\*\*CONCLUSION\*\*
I conclude that cloning have many negative impact, effect and problems in ones living thing. It may affect the donor of the DNA (deoxyribonucleic acid). Researchers, scientists specifically, contributed many things here in our community today. But do cloning really upgrades the nature’s wealth? It may have the advantages and disadvantages of course.

As a student studying biology, I have tried to approach both sides and approach them with an unbiased opinion. I personally think that the world of genetics is fascinating, but after learning of what is now possible through technology, I changed my mind about pursuing a career in the field. I see cloning as a wonderful advancement in technology and knowledge. I do not think it should be used to reproduce humans though. I do not believe that we should try to develop other ways beside the natural way to bring life into this world. I strongly believe that God created us and that we are subjected to His laws and must obey. The laws of God that have the worst punishment deal with bringing life into the world and taking life out of the world. I believe that cloning people would fall under these laws also.

Cloning tissues and organs falls under a different category that cloning human beings. I think it would be advantageous to science and medicine to clone tissues and organs. However, the research in this involves fetal tissue which is a completely different ethical discussion. I do not know enough about the procedure be against it. So, with my present understanding I would allow cloning for tissues and organs. Cloning can revolutionize the world and the way we live or it may be so minimal that it would not affect us at all if it is allowed. [Two sentences taken out during update.] Is this the world you want to live in? Each person individually must decide for himself or herself if they believe that cloning should be allowed or if the governments should intervene with it.