Cloning Of Plants and Animals: The Nuts in Our Head

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ABSTRACT:
The cloning of plants and animals are genetic engineering which have revolutionized medicine and agriculture. A lot of medical feats ranging from diabetes, cystic fibrosis, herpes, hepatitis to bone marrow and blood related ailments have been accomplished; as well as those in agriculture like crops modified for single, or multiple traits including lifelong tomatoes, viral resistance rice, cassava, papaya, sweet potatoes, pepper; nematode resistance in cereals; Vitamin A rice; drought or salt tolerance; nitrogen fixation; increase efficiency of use of nutrients, water, light, pharming (crops and animals for pharmaceuticals). Similarly is the cloning of animal, especially “the Dolly” and possible humans which have been very controversial. These controversies have informed the nuts in our heads in this review of cloning of plants and animals.

KEY WORDS
Genetic engineering, cloning of plants and animals, medicine, agriculture, controversies, the nuts in our heads

INTRODUCTION
The procedure of producing a line of genetically identical cells from a single altered cell is called cloning (Raven and Johnson 1999). This act has made every cell in the culture a miniature factory for producing interferon. The human insulin gene, according to these workers has also been cloned in bacteria. Today, reports these scientists, a hormone essential for treating some forms of diabetes are manufactured at relatively little expense. Beyond these clinical applications, cloning and related molecular techniques, according to Raven and Johnson ((Raven and Johnson 1999) are used to obtain basic information about how genes are put together and regulated. They mentioned specifically the interferon experiment and others like it to have marked the beginning of a new genetics, popularly called genetic engineering. The essence of genetic engineering, according to Dictionary of Biological terms (Dictionary of Biological Terms 2000) Jackson and Jackson (Jackson and Jackson 2000) and Raven and Johnson ((Raven and Johnson 1999)) are the ability to cut DNA into recognizable pieces and rearrange those pieces in different ways. In the interferon experiment, in Raven and Johnson account, a piece of DNA carrying the interferon gene was inserted into a plasmid, which then carried the gene into a bacterial cell. Most other genetic engineering approaches, according to these scientists have used the same general strategy, bringing the gene of interest into the target cell by first incorporating it into a plasmid or an ineffective virus. To make these experiment, and the plasmid DNA in such a way that the desired fragment of source DNA can be spliced permanently into the plasmid. This cutting, according to these workers is performed by enzymes that recognize and cleave specific sequences of nucleotides in DNA. These enzymes are the basic tools of genetic engineering. Hence, this episode is attempting to crack the nuts in our head in the course of cloning of plants and animals.

DISCOVERY OF RESTRICTION ENDONUCLEASES
Raven and Johnson (Raven and Johnson 1999); Haseltine (Haseltine 1997); and Anderson (Anderson 1995) narrates the scientific discoveries as inevitable origins of seemingly unimportant observations that receive little attention by researchers before their general significance is appreciated. In the case of genetic
Engineering, according to these experts, the original observation was that bacteria use enzymes to defend themselves against viruses. Eventually, most organisms started evolving means of defending themselves from predators and parasites, and bacteria were no exception. Among the natural enemies of bacteria, according to these authors are bacteriophages, viruses that infect bacteria and multiply within them. At some point, they cause the bacterial cells to burst, releasing thousands more viruses. Through natural selection, some types of bacteria have acquired powerful weapons against these viruses. They contain enzymes called restriction endonucleases that fragment the viral DNA as soon as it enters the bacterial cell. Many restriction endonucleases recognize specific nucleotide sequences, and cleave the DNA at a particular place within the recognition sequence.

**WHY DON’T RESTRICTION ENDONUCLEASES CLEAVE THE BACTERIA CELL’S OWN DNA AS WELL AS THAT OF THE VIRUSES?**

These experts (Raven and Johnson 1999) posses this question to the scientific communities and also proffers answers. The bacteria modify their own DNA, using other enzymes known as methylases to add methyl (-CH3) groups to some of the nucleotides in the bacterial DNA. When nucleotides within a restriction endonuclease’s recognition sequence have been methylated, the endonuclease cannot bind to that sequence. Consequently, the bacteria DNA are protected from being degraded at that site. Viral DNA, on the other hand, has not been methylated and therefore is not protected from enzymatic cleavage.

**HOW RESTRICTION ENDONUCLEASES CUT DNA**

The sequences recognized by restriction endonucleases, according to Raven and Johnson (Raven and Johnson 1999) are typically four to six nucleotides long, and they are often palindromes. This means the nucleotides at one end of the recognition sequence are complementary to those at the other end, so that the two strands of the DNA duplex have the same nucleotide sequence running in opposite directions for the length of the recognition sequence. Two important consequences arise from the arrangement of nucleotides.

First, because the same recognition sequence occurs on both strands of the DNA duplex, the restriction endonuclease can bind to and cleave both strands, effectively cutting the DNA in half. This ability to cut across both strands is almost certainly the reason that restriction endonucleases have evolved to recognize nucleotides sequences with twofold rotational symmetry.

Second, according to Raven and Johson (Raven and Johnson 1999) the bond cleaved by a restriction endonuclease is typically not positioned in the center of the recognition sequence to which it binds. Again, the DNA strands are anti-parallel; the cut sites for the two strands of a duplex are offset from each other. After cleavage, each DNA fragment has a single-stranded end a few nucleotides long. The single-stranded ends of the two fragments are complementary to each other. Some restriction endonucleases, according to these workers cleave the center of a four or six nucleotide sequence, producing fragments without single-stranded ends. Such fragments do not spontaneously reassociate, so the endonucleases that produce them are often used in genetic engineering procedures where it is important to prevent spontaneous reassociation.

**THE USEFULNESS OF RESTRICTION ENDONUCLEASES**

Raven and Johnson (Raven and Johnson 1999) asserts the hundreds of bacteria restriction endonucleases with each one having a specific recognition sequence. By chance, a particular endonuclease’s recognition sequence is likely to occur somewhere in any given sample of DNA, the shorter the sequence, the more often it will arise by chance within a sample. According to these experts, a given restriction endonuclease can probably cut DNA from any source into fragments. Each fragment will have complementary single-stranded ends characteristics of that endonuclease. Due to their complementarity, these single-stranded ends can pair with each other (consequently, they are sometimes called sticky ends). Once their ends have paired, two fragments can then be joined together with the aid of the enzyme DNA ligase, which reforms the phosphodiester bonds of DNA.

**WHAT MAKES RESTRICTION ENDONUCLEASES SO VALUABLE FOR GENETIC ENGINEERING?**

According to Raven and Johnson (Raven and Johnson 1999) any two fragments produced by the same restriction endonuclease can be joined together. Fragments of elephant and ostrich DNA cleaved by the same endonuclease can be joined to one another as readily as two bacterial DNA fragments. Genetic engineering involves manipulating specific genes by cutting and rearranging DNA. A restriction endonuclease
cleaves DNA at a specific site, generating in most cases two fragments with short single-stranded ends. Because these ends are complementary to each other, any pair of fragments produced by the same endonuclease, from any DNA source, can be joined together.

MANIPULATION OF GENES USING ENDONUCLEASES

Raven and Johnson (Raven and Johnson 1999) defines chimera as a mystical creature with the head of a lion, body of a goat, and tail of a serpent. Although no such creatures existed in nature, biologists have made chimeras of a more modest kind through genetic engineering (this is one of the nuts in super scientist’s heads). Let’s know how they do it.

CONSTRUCTING PSC101

To fully understand these super-scientists, we must be conscious of sub and super-scripts in their terminologies. Raven and Johnson (Raven and Johnson 1999) announced the manufacture of the first chimeras from a bacterial plasmid called a resistance transfer factor by American geneticists Stanley Cohen and Herbert Boyer in 1973 (they always venture first into the unknown). They used a restriction endonucleases I, or EcoRI to cut the plasmid into fragments (no harm in trial). One fragment, 9000 nucleotides in length (only God Knows how they got the exact length), according to these gurus contained both the origin of replication (incredible scientists) necessary for replicating the plasmid and a gene that conferred resistance to the antibiotic tetracycline (tet1). Because both ends of this fragment were cut by the same restriction endonuclease, they could be ligated to form a circle, a smaller plasmid, which Cohen dubbed “pSC101”. Thank you my friend (what of pSC101 to infinity?). Don’t laugh at my ignorance.

THE NEXT STEP: USING PSC101 TO MAKE RECOMBINANT DNA

Raven and Johnson (Raven and Johnson 1999) followed Cohen and Boyer very closely in this journey. The stars also used EcoRI to cleave DNA that coded for rRNA that they had isolated from an adult amphibian, the African clawed toad, Xenopus laevis (probably from Ethiopia, the ancient of human). They then mixed the fragments of Xenopus DNA with pSC101 plasmids that had been reopened by EcoRI and allowed bacterial cells to take up DNA from the mixture (All these you did in your laboratory kitchen for humanity. I wish I was there to test your delicacies. Well Done, Guys).

Some of the bacterial cells immediately became resistant to tetracycline (they are liars, no way, they must come back) indicating that they had incorporated the pSC101 plasmid with its antibiotic resistance gene. Furthermore, some of these pSC101 containing bacteria also began to produce toad ribosomal RNA! Cohen and Boyer concluded that the toad rRNA gene must have been inserted into the pSC101 plasmids in those bacteria (Super Scientists). In other words, according to Raven and Johnson (Raven and Johnson 1999) the two ends of the pSC101 plasmids produced by cleavage with EcoRI had joined to the two ends of a toad DNA fragment that contained the rRNA gene, also cleaving with EcoRI.

The pSC101 plasmid containing the toad rRNA gene is a true chimera, confirms Raven and Johnson (Raven and Johnson 1999). It is an entirely new genome that never existed in nature and never would have evolved by natural means. It is popularly called recombinant DNA. That is DNA created in the laboratory by joining together pieces of different genomes to form a novel combination.

WHAT DO YOU MEAN BY OTHER VECTOR RAVEN AND JOHNSON?

Let me tell you what these super scientists, accounted for “other vector”. It is the introduction of foreign DNA fragments into host cells that has become common in molecular genetics. The genome that carries the foreign DNA into the host cell is called “a vector”. Newer-model plasmids, with names like pUC18, pGEM® or pBluescript® can be induced to make hundreds of copies of themselves and thus of the foreign genes they contain. Entry into bacterial cells can also be achieved by using a bacterial virus, such as λ virus, as a vector instead of a plasmid. According to Raven and Johnson (Raven and Johnson 1999), not all vectors have bacterial targets. Animal viruses, for example, have served as vectors to carry bacterial genes into monkey cells, and animal genes have been introduced into plant cells.

THE FOUR STAGES OF A GENETIC ENGINEERING EXPERIMENT

Raven and Johnson (Raven and Johnson 1999) documents four stages of most genetic engineering after the procedure of Cohen and Boyer. The four stages of the experiment consist of DNA cleavage, production of recombinant DNA, cloning and screening. The stage 1 is where a restriction endonuclease is used to cleave the source DNA into fragments. Because the endonuclease’s recognition sequence is likely to occur many times within the source DNA, cleavage will produce a large number of different fragments. A different set of fragments may be
obtained by employing endonucleases that recognize different sequences. The fragments can be separated from one another according to their size by electrophoresis (Mullis 1990).

The second stage is where the fragments of DNA are inserted into plasmids or viral DNA, which have been cleaved with the same restriction endonuclease as the source DNA. The third stage is where the plasmids or viruses serve as vectors that can introduce the DNA fragments into cells, usually, but not always, bacteria. As each cell reproduces, it forms a clone of cells that all contain the fragment-bearing vector. Each clone, according to the authors is maintained separately, and all of them together constitute a clone library of the original source DNA. The stage four is where the clones containing a specific DNA fragment of interest, often a fragment that includes a particular gene, are identified from the clone library.

**COMMERCIAL APPLICATION OF GENETIC ENGINEERING IN MEDICINE**

The first and perhaps most obvious commercial application of genetic engineering was the introduction of genes that encode clinically important proteins into bacteria (Raven and Johnson 1999) According to these workers, bacteria cells can be grown cheaply in bulk (fermented in giant vats, like the yeasts that make beer), bacteria that incorporate recombinant genes synthesize large amounts of the proteins that those genes specify. This method has been used to produce several forms of human insulin and interferon, as well as commercially valuable proteins such as growth hormones and erythropoietin, which stimulates red blood cell production.

Among the medically important proteins now manufactured by these approaches, according to Raven and Johnson (Raven and Johnson 1999) are atrial peptides, small proteins that may provide a new way to treat high blood pressure and kidney failure. Another is tissue plasminogen activator, a human protein synthesized in minute amounts that cause blood clots to dissolve and may be effective in preventing heart attacks and strokes. The problem reported, for this general approach by these authors is the difficulty of separating the desired protein from the others that the bacteria make. The purification of proteins from such complex mixtures is both time-consuming and expensive, but it is still easier than isolating the proteins from the tissues of animals, for example, insulin from dog pancreases, which is how such proteins was obtained. Researchers have succeeded in producing RNA transcripts of cloned genes. They use the transcripts to produce only these proteins in a test tube containing the transcribed RNA, ribosome’s, cofactors, amino acids, tRNA and ATP (Capecchi 1994; Mullis 1990).

Jackson and Jackson (Jackson and Jackson 2000); Raven and Johnson (Raven and Johnson 1999) recorded the first researchers attempt to combat genetic defects by the transfer of human genes. This is based on the science, that when a hereditary disorder is the result of a single defective gene, an obvious way to cure the disorder is to add a working copy of the gene. This approach is being used to combat cystic fibrosis, and offers potential for treating muscular dystrophy and a variety of other disorders. One of the first successful attempts was the transfer of a gene encoding the enzyme adenosine deaminase into the bone marrow of two girls suffering from a rare blood disorder caused by the lack of this enzyme (Capecchi 1994; Cohen and Hogan 1994). Another area that has been explored is the use of genetic engineering to produce subunit vaccines against viruses such as those that cause herpes and hepatitis. In this search, genes encoding part of the protein-polysaccharide coat of the herpes simplex virus or hepatitis B virus, are spliced into a fragment of the vaccinia (cowpox) virus genome. The vaccinia virus, used by British Physician, Edward Jenner for over two centuries ago, was against smallpox. Today, it is used as a vector to carry the herpes or hepatitis viral coat gene into cultured mammalian cells. These cells, according to Raven and Johnson (Raven and Johnson 1999) produce many copies of the recombinant virus, which has the outside coat of a herpes or hepatitis virus. When this recombinant virus is injected into a mouse or rabbit, the immune system of the infected animal produces antibodies directed against the coat of the recombinant virus. It develops immunity to herpes or hepatitis virus. Vaccines produced in this way are harmless, since the vaccinia virus is benign and only a small fragment of the DNA from the disease-causing virus is introduced via the recombinant virus.

The great breakthrough of this approach, according to these experts is that it does not depend upon the nature of the viral disease. It enables similar recombinant viruses to be injected into humans, which can confer resistance to a wide variety of viral diseases. For example, in 1995, the first clinical trials was made when new DNA vaccine that depends not on antibodies, but rather on the second arm of the body’s immune defense, the so called cellular immune response, in which blood cells known as killer T cells attack infected cells. The infected cells are attacked and destroyed when they stick fragments of foreign proteins onto their outer surfaces that the T cells detect. The basis of discovery by Peter Doherty
and Rolf Zinkernagel that infected cells do so. This led to their receiving the Nobel Prize in Medicine in 1996. The first DNA vaccines spliced an influenza virus gene encoding an internal nucleoprotein into a plasmid, which was then injected into mice. The mice developed strong cellular immune responses to influenza. Though new, yet the technology, according to Raven and Johnson (Raven and Johnson 1999) and Neufeld and Colman (Neufeld and Colman 1990) offer great promise.

## COMMERCIAL APPLICATION OF GENETIC ENGINEERING IN AGRICULTURE

Another major area of genetic engineering activity is the manipulation of the genes of key crop plants (Falkner 2002; Igboji 2015). According to Raven and Johnson (Raven and Johnson 1999) the primary experimental difficulty in plants lay on identifying a suitable vector for introducing recombinant DNA. Plant cells do not possess the many plasmids that bacteria do, so the choice of potential vectors is limited. The most successful results in plants is the Ti (tumour-inducing) plasmid of the plant bacterium Agrobacterium tumefactionis which infects broadleaf plants such as tomato, tobacco and soybean. Part of the Ti plasmid integrates into the plant DNA, and researchers have succeeded in attaching other genes to this portion of the plasmid. The characteristics of a number of plants have been altered using this technique, which are valuable in improving crops and forests. The qualities interested by scientists are resistance to disease, frost and other stress, nutritional balance and protein content and herbicide resistance (Falkner 2002; Igboji 2015; Pretty 2002 et al).

Unfortunately, Agrobacterium generally does not infect cereals such as corn, rice and wheat, but alternative methods have been developed to introduce new genes into them. A recent advancement reported by Raven and Johnson (Raven and Johnson 1999) is the genetically manipulated fruit of Calgene’s “Flavr Savr” tomato, which was long approved for sale by the USDA. According to these workers, the tomato is engineered to inhibit genes that cause cells to produce ethylene. In tomatoes and other plants, ethylene acts as a hormone to speed fruit ripening. In Flavr Savr tomatoes, inhibition of ethylene production delays ripening. The result is a tomato that stay on vine longer and that resists over-ripening and rotting during transport to market.

Raven and Johnson (Raven and Johnson 1999) covered broadleaf plants genetically engineered to be resistant to glyphosate, the active ingredient in Roundup, a powerful, biodegradable herbicide that kills most actively growing plants. Pretty (Pretty 2002) Igboji (Igboji 2015) and Falkner (Falkner 2002) also gave detailed account of these novel GMOs. Glyphosate, according to Raven and Johnson (Raven and Johnson 1999) works by inhibiting an enzyme called EPSP synthetase, which plants require to produce aromatic amino acids. Humans, according to these experts, do not make aromatic amino acids. They get them from their diet, so they are unaffected by glyphosate. To make glyphosate-resistant plants, agricultural scientists used a Ti plasmid to insert extra copies of the EPSP synthetase genes into plants. These engineered plants produce 20 times the normal level of EPSP synthetase, enabling them to synthesize proteins and grow despite glyphosate’s suppression of the enzyme. In subsequent experiments, a bacterial form of the EPSP synthetase gene that differs from the plant form by a single nucleotide was introduced into plants via Ti plasmids, the bacterial enzyme in these plants is not inhibited by glyphosate.

These advances are of great interest to farmers, since a crop resistant to Roundup would never be weeded if the field were simply treated with the herbicide. Because Roundup is a broad-spectrum herbicide, farmers would no longer need to employ a variety of different herbicides, most of which kill only a few kinds of weeds. Furthermore, glyphosate breaks down readily in the environment, unlike many other herbicides commonly used in agriculture. A plasmid is actively being sought for the introduction of the EPSP synthetase gene into cereal plants, making them also glyphosate-resistant.

For Raven and Johnson (Raven and Johnson 1999) long-range goal of agricultural genetic engineering is to introduce the genes that allow soybeans and other legume plants to fix nitrogen into key crop plants. These so called nif genes are found in certain symbiotic root-colonizing bacteria. Living in the root nodules of legumes, these bacteria break the powerful triple bond of atmospheric nitrogen gas, converting N₂ into NH₃. The plants then use the ammonia to make amino acids and other nitrogen-containing molecules. Other plants lack these bacteria and cannot fix nitrogen, so they must obtain their nitrogen from the soil. Farmland where these crops are grown soon becomes depleted of nitrogen, unless nitrogenous fertilizers are applied.

Worldwide, introducing the nitrogen-fixing genes from bacteria, according to Raven and Johnson (Raven and Johnson 1999) would reduce cost if major crops like wheat and corn could be engineered to carry out biological nitrogen fixation. However, introducing the nitrogen-fixing genes, according to these experts, do not seem to
function properly in eukaryotic cells like wheat and corn. Researchers are actively experimenting with other species of nitrogen-fixing bacteria whose genes can function better in plant cells.

Another area that has received attention is insect resistant crops and animals. Many commercially important plants are attacked by insects, and the traditional defense against such attacks is to apply insecticides. Over 40% of the chemical insecticides used today, according to Raven and Johnson (Raven and Johnson 1999); Pretty (Pretty 2002) are targeted against boll weevils, bollworms and other insects that feed on cotton plants. Genetic engineers have produced plants resistant to insect pests, removing the need to use many externally applied insecticides.

The approach is to insert into crop plants encoding proteins that are harmful to the insects that feed on the plants but harmless to other organisms. One such insecticide, reported by these experts is Bacillus thuringiensis, a soil bacterium. When the tomato hornworm caterpillar ingests this protein, enzymes in the caterpillar's stomach convert it into an insect-specific toxin, causing paralysis and death. Because these enzymes are not found in other animals, the protein is harmless to them. Using the Ti plasmid, scientists have transferred the gene encoding this protein into tomato and tobacco plants. They have found that these transgenic plants are indeed protected from attack by the insects that would normally feed on them. In, 1995, the EPA approved altered forms of potato, cotton and corn. The genetically altered potato can kill the Colorado potato beetle, a common pest. The altered cotton is resistant to cotton bollworm, budworms and pink bollworm. The corn has been altered to resist the European corn borer and other moth-like insects.

Another striking discovery by Monsanto screening scientists are natural compounds extracted from plant and soil samples. Some isolation of a new insect-killing compound from a fungus, using the enzyme called cholesterol oxidase. Apparently, the enzyme disrupts membranes in the insect gut. The fungus gene, called the Bollgard gene after its discoverer, has been successfully inserted into a variety of crops. It kills a wide range of insects, including the cotton boll weevil and the Colorado potato beetle, both serious agricultural pests. The field tests, according to Raven and Johnson (Raven and Johnson 1999) started in 1996.

Some insect pests that attack plant roots and B. Thuringiensis is being employed to counter that threat as well. This bacterium does not normally colonize plant roots, so biologists have introduced the B. Thuringiensis insecticidal protein gene into root-colonizing bacteria, especially strains of Pseudomonas.

On the part of farm animals, the gene encoding the growth hormone somatotropin was one of the first to be cloned successfully (Raven and Johnson 1999). This followed the receipt of Federal approval in 1994 by Monsanto scientists to make its recombinant bovine somatotropin (BST) commercially available and dairy farmers worldwide, who began to add the hormone as a supplement to their cow diets, increasing the animal's milk production. Genetically engineered somatotropin is also being tested to see if it increases the muscle weight of cattle and pigs and as a treatment for human disorders in which the pituitary gland fails to make adequate levels of somatotropin, producing dwarfism. BST ingested in milk or meat has no effect on humans, reports Raven and Johnson (Raven and Johnson 1999) because it is a protein and is digested in the stomach. Nevertheless, BST has met with some public resistance, due primarily to generalized fears of gene technology (Falkner 2002; Pretty 2002). Some people mistrust milk produced through genetic engineering, even though the milk itself is identical to other milk. Problems concerning public perception are not uncommon as gene technology makes an even greater impact on our lives (Igboji 2015; Pretty 2002).

On the other hand transgenic animals engineered to have specific desirable genes are becoming increasingly available to breeders. Now, instead of selectively breeding for several generations to produce a racehorse or a stud bull with desirable qualities, the process can be shortened by simply engineering such an animal right at the start.

CLONING OF ANIMALS AND MAN

Experts (Jackson and Jackson 2000; Raven and Johnson 1999) admit the difficulty in using transgenic animals to improve livestock. According to Raven and Johnson (Raven and Johnson 1999) breeding produces offspring very slowly and recombination acts to undo the painstaking work of the genetic engineer. Until 1997, according to these scientists, it was commonly accepted that animals cannot be cloned. In 1997, scientists announced the first successful cloning of differentiated vertebrate tissue, a lamb grown from a cell taken from an adult sheep. This startling discovery revolutionized agricultural science.

The idea of cloning animals was first suggested in 1938 by German embryologist Hans Spemann, often called the father of modern embryology, who proposed what he called a “fantastic experiment” by which you remove the nucleus from an egg cell and put in its place a
nucleus from another cell (Raven and Johnson 1999). It was 14 years before technology advanced far enough for anyone to consider Spemann serious (Incredible man). In 1952, two American Scientists (always early adopters) Robert Briggs and T. J. King used very fine pipette to suck the nucleus from a frog egg (frog eggs are usually large), making the experiment feasible and transferred a nucleus sucked from the body of an adult frog into its place. The experiment did not work when done this way, but partial success was achieved 18 years later (1970) by the British (second early adopters) developmental biologist John Gurdon, who inserted nuclei from advanced toad embryos rather than adult tissue. The toad eggs developed into tadpoles, but died before becoming adults (mission accomplished).

For 14 years (1970–1984), nuclear transplant experiments were attempted without success (what a shame, a small British boy would exclaim). Never mind, we shall get there, he will quickly add up. Technology, according to Raven and Johnson (Raven and Johnson 1999) continued to advance, until in 1984 (victory at last), a Danish embryologist working in Texas (Bushes’ State) succeeded in cloning a sheep using nucleus from a cell of an early embryo. This exciting result was replicated by others in a host of other organisms, including cattle, pigs and monkeys. Only early embryo cells, according to these experts seemed to work. Researchers became more confident and convinced those animal cells become irreversibly committed after the first few cell divisions of the developing embryo, and after that nuclei from differentiated animal cells cannot be used to clone entire organisms. A Scottish Geneticists unravelled this mystery and unwarranted conclusions. In Scotland, Keith Campbell (super girl), a specialist in studying the cell cycle of agricultural animals, forgot about herself and the so husing “husband” of a thing to raise the Scottish flag in space. By the early 1990s, the knowledge of how the cell cycle is controlled, advanced by cancer research, had led to an understanding that cells don’t divide until conditions are appropriate. Just as a washing machine checks that the water has completely emptied before initiating the spin cycle, so the cell checks that everything needed is on hand before initiating cell division. Campbell reasoned (super reasoning) maybe the egg and the donated nucleus need to be at the same stage in the cell cycle.

This proved to be a key insight. In 1994, a researcher Neil First, and in 1995 Campbell himself working with reproductive biologist Ian Wilmut succeeded in cloning farm animals from advanced embryos by first starving the cells so that they paused at the beginning of the cell cycle at the G2 checkpoint. Two starved cells are thus held in resting phase at the same point in the cell cycle. The stage was now set for success (Raven and Johnson 1999).

Wilmut then set out to attempt the key breakthrough, the experiment that had eluded researchers since Spemann proposed it 59 years before: to transfer the nucleus from an adult differentiated cell into an enucleated egg, and allow the resulting embryo to grow and develop in a surrogate mother, hopefully producing a healthy animal (thank you very much Wilmut). Wilmut removed cells from the udder of a 6 year old sheep. Mammary cells, the origin of these cells, gave the clone its name, "Dolly" after the English singer, Dolly Parton (Excellent Tribute). The cells were grown in tissue culture, and some frozen so that in the future it would be possible with genetic fingerprinting to prove that a clone was indeed genetically identical with the cells from the 6 year old sheep (Thank you, Wilmut).

In preparation for cloning, according to Raven and Johnson (Raven and Johnson 1999), Wilmut’s team greatly reduced the 5 days the concentration of serum on which the sheep mammary cells were subsisting. In parallel preparation, eggs obtained from a ewe were enucleated, the nucleus of each egg carefully removed with a micropipette. Mammary cells and egg cells were then surgically combined in January of 1996 (Big January, of Big Year). The mammary cells inserted inside the covering around the egg cell. Wilmut then applied a brief electrical shock. A neat trick, this causes the plasma membranes surrounding the two cells to become leaky, so that the contents of the mammary cell pass into the egg cell. The shock also kick-starts the cell cycle, causing the cell to begin to divide. After 6 days, in 30 of 277 tries (a patient dog eats the fattest bone), the dividing embryo reached the hollow-ball "blastula" stage and 29 of these were transplanted into surrogate mother sheep. A little over 5 months later, on July 5, 1997 (Raven and Johnson 1999), one sheep gave birth to a lamb (the first landing in embryology space). This lamb, "Dolly" was the first successful clone generated from a differentiated animal cell. Unfortunately, it did pass to mother earth as every one of us will do one day. Thanks to the patience and ingenuity of these super brains in the science community.

THE FUTURE OF CLONING

Wilmut’s successful cloning of fully differentiated sheep cells, 18 years running is a milestone event in gene technology. Even though his procedure proved inefficient (1/277 trials succeeded), it established the way forward that cloning of adult animal cells can be done. In 1998, following their feat, researchers succeeded in advancing the
efficiency of cloning. Seizing upon the key idea in Wilmut's experiment, to clone a resting-stage cell, they returned to the nuclear transplant procedure pioneered by Briggs and King, but used 800 resting stage cumulus cells from a female mouse. From them they produced 10 healthy mouse clones, all identical to the female source, and soon after, 7 clones of the clones (Raven and Johnson 1999).

These workers, including Anderson (Anderson 1995) and Cohen and Hogan (Cohen and Hogan 1994) are optimistic on the impact of transgenic cloning on medicine and agriculture. Animals with human genes can be used to produce rare hormones. For example, the authors gave a sheep that have been genetically engineered to secret a protein called “alpha-1 antitrypsin” that are helpful in relieving the symptoms of cystic fibrosis into their milk. This has cheapened the production of the expensive drug. The experts also speculated on the possibility of cloning a human. According to Raven and Johnson (Raven and Johnson 1999) there is no reason to believe such an experiment would not be done. In addition is the ethics of human cloning which have not been fully addressed. The Western World thought is based on the concept of human individuality. Yet, human cloning engenders considerable controversy (the basis of the nuts in our heads). Many people, including influential activists and members of the scientific community have expressed concern that genetic engineers are “playing God”. Absolutely right. They are tampering with genetic material. Hence, it is more than an ethical or moral problem, with ramifications that could be quite serious. For instance, the questions are endless, what would happen if one fragmented the DNA of a cancer cell, and then incorporated the fragments at random into vectors that were propagated within bacterial cells?. Might there not be a danger that some of the resulting bacteria would transmit an infective form of cancer? What would happen if a killer virus was engineered? Could genetically engineered products administered to plants or animals turn out to be dangerous for consumers after several generations?. What kind of unforeseen impact on the ecosystem might improved crops have? Suppose a genetically superior plant was released into the wild, where it out-competed all other existing plants. Is ethical to create genetically superior organisms including “Super-Humans”. What of the aliens somewhere there in other planets?. Can we catch or beat them or clone them as well. All these are the nuts in our heads.

The debate rages on. Raven and Johnson (Raven and Johnson 1999) and Stix (Stix 1995.) argue on many public concerns about genetic engineering. According to the experts, less than 1% of known bacteria are pathogenic to humans, and almost all of the bacteria used in genetic engineering are not. In fact, according to these scientists, most of those used in genetic engineering could not live in or on a human host at all. Nor does a rot-resistant tomato appear to be threatening. In most cases, argue the workers, genetically altered crops differ by just one or a few genes, which are often obtained from other lines of the same species. Recombinant technology achieves the same kind of gene reassortment as the genetic crosses of Mendel, only faster. In that sense, according to these experts, science isn’t doing anything that nature isn’t already doing. For these workers, mutations occur naturally on the random basis, and organisms with favorable mutations are selected for, which according to the scientists, are the essence of evolution. The genetic dabbling performed by humans is minuscule, according to scientists when compared with that performed by nature. They admit that mistakes can occur and that nature cannot take something synthetically engineered and improve on it for better or worse. Still, the benefits of genetic engineering seem to far outweigh the risks. The debate is endless and only future can tell where there will be no victor no vanquished.

CONCLUSIONS
Cloning of plants and animals is a very complicated and controversial issue. On the part of scientists, researchers, marketers, consumers, citizens, government, regulators, religious and civilization. While the cure of cancer, HIV/AIDS, Ebola may be revolutionized by genetic engineering and herald the dawn of a new era in the world; similarly will provision of food, water and if possible cloned soil at little or no cost to estimated world populace of 6 billion, with over 3 billion classed as very poor, hungry and malnourished in Africa and Asia; and targeted population of over 10 billion by 2050 with same pattern of distribution; anything called food will go for a hungry world, whether genetically or naturally modified. Again, in the era of aging population higher than younger generations in the western world, and the need to retain and replicate sexes of same race in western world, and to avoid loss of generational lineage, coupled with increased problems of infertility arising from exposure to pollutants and professional stress, especially in the western countries; majority will opt for cloning of humans to take off stresses of motherhood, fatherhood and their respective responsibilities. Who will nurture such cloned humans?. Where will the breast milk come from required for conferring initial immunity? Will naturally immunity be cloned as well? How will
cloned humans behave? What will be the intelligence quotient? Will they have complete cells, tissues, organs and systems? What will be the future of cloned humans? Will it wipe out biological races of man? Will it wipe out reproductive systems of man and other animals and plants? Will cloning of humans, animal and plants give rise to a new planet on earth? What will be the faith of science and technology? Can they pilot planes, captain ships, drive cars, invent, manufacture and manipulate computers and other modern gadgets? Can they be heads of state, responsible citizens, manage nuclear stockpiles, undertake military expeditions, manage space crafts, till the land for agriculture, be genetic engineers? Can they be teachers, mentors, engineers, medical and professional doctors, lawyers, artists, scientists? The list is endless. These are the nuts in our heads. Nevertheless, the fact remains that man is highly expeditory species. As far as we remain human, massive expedition in mind, soul, spirit will continue. Man will even attempt to clone God. Man is over insatiable in our needs, wants and conquests. Man is a very delicate being. Very fragile, always intimidated and insecure. A little twig of the flower upsets man. Scientists are even at a crossroads on whom to trust – God, Nature, Man or Nothing. For eternity, cloning of plants and animals will remain the nuts on our heads.

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