August 2009

Transgenic Animals

Matthew J. Connor
Worcester Polytechnic Institute

Nicholas Tsitsilianos
Worcester Polytechnic Institute

IQP-43-DSA-5446
IQP-43-DSA-9805

TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By:

Matthew Connor

Nicholas Tsitsilianos

August 27, 2009

APPROVED:

Prof. David S. Adams, Ph.D.
WPI Project Advisor

ABSTRACT

This project examines the controversial topic of transgenic animals, and describes the effect of this new technology on society. Chapters-1 and 2 describe how transgenic animals are created and categorizes their uses. Chapters-3 and 4 investigate the ethics and legalities surrounding this controversial technology. Based on our research, the authors of this project conclude that most types of transgenic experiments should continue, provided that strong IACUC and FDA oversights are in place to reduce animal suffering. However, experimentation in mammalian growth factor transgenesis should be discontinued due to the strong negative effects on the animals with no strong benefit to society. Patenting of transgenic animals should be continued, and will provide incentive for further investment in this kind of research.

TABLE OF CONTENTS

PROJECT OBJECTIVES

The main objective of this project was to research and examine the topic of transgenic animals, and to discuss the effect of this controversial new technology on society. This report contains a clear description of what transgenic animals are, how they are created (Chapter 1), the various categories of the types of transgenic animals created to date (Chapter 2), the bioethics surrounding this controversial technology (Chapter 3), and the legal guidelines and patent laws regulating the creation and use of transgenic animals (Chapter 4). This research is rapidly growing, producing new and exciting results. It provides new possibilities for the identification and alleviation of some of the most destructive diseases that plague humanity. Experiments in transgenic technology contain moral and ethical, questions. The ensuing chapters present information to assist the reader of this IQP, to formulate their own opinion on the value of transgenic technology.

Chapter-1: Transgenic Technology

Nicholas Tsitsilianos

The term transgenic animal refers to an animal into which a foreign segment of DNA has been intentionally placed. Their foreign DNA produces a new characteristic not possible with traditional breeding, such as the production of a human therapeutic protein as a medicine. Creating transgenic animals has played a vital role to facilitate scientific research in understanding human biology, to help understand human diseases, and to test new treatments. The implications of this scientific idea and method are infinite, yet controversial. This first chapter aims to explain the common methods of creating and screening transgenic animals to gain a better understanding of this revolutionary technology.

What is DNA?

Deoxyribonucleic acid (DNA) makes up who we are. This nucleic acid holds all the genetic information in each cell. DNA is configured into a double helix (**Figure-1**) made up of two strands (blue in the figure) connected by four nucleotide bases: adenine, thymine, guanine and cytosine (colored in the figure).

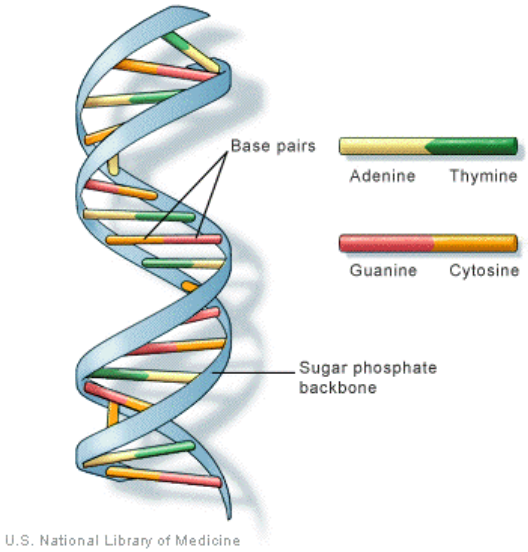

Figure 1: Structure of the DNA Double Helix. Shown are the two strands of DNA (blue) connected by four types of base pairs (color coded). (Genetic Home Reference, 2007)

DNA basepairs are held together by hydrogen bonds (**Figure-2**). The creation of hydrogen bonds between these base pairs on opposite sides of the double helix leads to the specific pairing of adenine with thymine, and guanine with cytosine (Cooper and Hausman, 2004). There are two different classifications of bases: purines and pyrimidines. Only purines can pair with pyrimidines, this is the reason adenine pairs with thymine, and cytosine with guanine. Purines are slightly larger than prymidines, so by pairing one of each type, the length between each basepair remains constant. The sequence or order in which these base pairs are configured determines an individual's hereditary characteristics. These characteristics are passed on to the next generation because the DNA is split and each strand is copied in a semi-conservative manner. These copies are passed to the daughter cells along with the genes they represent.

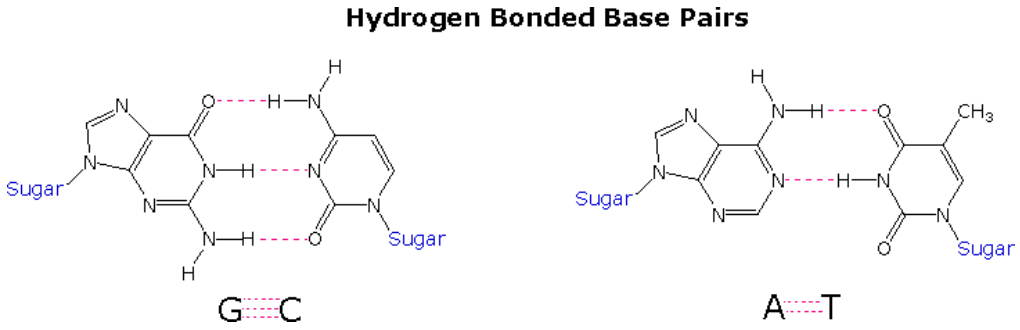

Figure 2: Complementary Base Pairing. Cytosine and guanine basepairs have three hydrogen bonding sites, while adenine and thymine basepairs have two bonding sites. (Cooper and Hausman, 2004)

RECOMBINANT DNA TECHNOLOGY

In classic experiments, Werner Arber (a Swiss scientist) investigated the process of a virus infecting a bacterium, and developed the idea that viral replicative success was limited to

rapidly replicating organisms. Simultaneous studies on viruses by Joshua Lederberg at Stanford University and Salvador Luria at MIT discovered that in some instances, the bacteria had some resistance to the viruses. This discovery excited Arber, and upon his return to Switzerland he focused on this aspect of study. He recognized that for the virus to multiply, the virus had to add its genetic material into the bacteria, which would cause the new host to copy it to allow viral replication. During his research, Arber also recognized that during viral replication restriction enzymes encoded by bacteria provided a defense against foreign DNA (in this case against viral DNA) that entered the bacteria. These restriction enzymes have the ability to cleave DNA at specific sequences.

In the 1970s, Hamilton Smith furthered this research at John Hopkins University. He was able to isolate one of the restriction enzymes (named EcoRI) from *E. coli* and discovered it could cleave DNA at specific sites (which we now know is the sequence GAATTC) without destroying that segment of DNA (**Figure-3**).

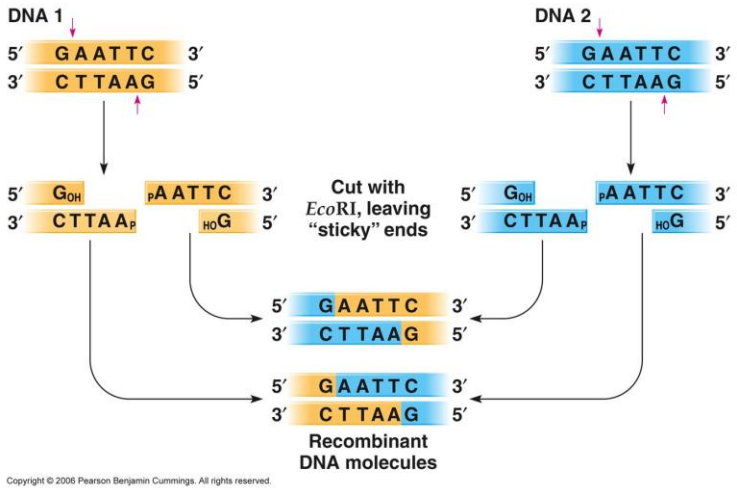

Figure 3: Cutting DNA With EcoRI Restriction Enzyme. This DNA cleavage process does not destroy the DNA, but creates fragments with sticky ends, that allow DNA fragments to be rejoined in new combinations. (Spencer, 2006)

In a related finding, Smith recognized the manner in which the cuts were made. The cuts are not cleanly vertical made from the beginning of the GAATTC sequence, but are diagonal cuts between the G and A, that leave AATT overhangs, as shown in Figure 3.

Paul Berg, in 1972, recognized the importance of restriction enzymes to cut the DNA molecule at specific points to allow the rejoining of the loose tails with the complementary sequence on the ends of the fragments. Different DNA fragments cut with one type of restriction enzyme could be annealed to create a new DNA. When the annealed DNAs were inserted into bacteria, the bacterial ligase could seal the breaks. Ligase is an enzyme that can join segments of DNA together. This discovery of using restriction enzymes to create compatible ends between different DNA fragments, and to use ligase to seal them, was the beginning of genetic engineering (Berg, 2004). The new DNA is termed recombinant DNA. Essentially recombinant DNA is DNA that has been engineered to combine two DNA fragments that do not normally recombine in nature.

Polymerase Chain Reaction

One way that DNA cloning (making copies of DNA) is made possible is through the process of polymerase chain reaction (PCR). First developed in 1985 by Kary Mullis who earned a Nobel Prize in 1993 for its discovery, PCR isolates a specific desired DNA sequence, called the target sequence, and makes it possible to make billions of copies of that sequence without having to purify it before hand. It is an extremely efficient method. To begin amplification of the target sequence (such as a human gene needed for cloning), the two strands of DNA are separated by heating the DNA to 95°C to allow the DNA molecules to be melted. After the strands have separated, the temperature is dropped to 60°C to allow a pair of short

oligonucleotide primers (one sense primer and one antisense primer) to attach to each end of the target sequence. The temperature is then raised to 72°C, the optimum temperature of Taq polymerase. Taq is an enzyme that uses the primers to initiate DNA replication. This cycle of denaturation, primer annealing, and DNA replication is usually repeated 30-35 times to create billions of amplified fragments of the target sequence for a variety of uses, including creating transgenic animals.

DNA Cloning In Vivo

A different method for cloning DNAs involves the use of vectors, such as plasmids to help copy the DNA (**Figure-4**). This process begins when the DNA that you wish to clone (such as a human gene) is cut with a restriction enzyme (i.e EcoRI) (diagram upper right). A plasmid is cut with the same enzyme (diagram upper left). A plasmid contains three elements: a cloning site (where the foreign DNA can be inserted), a drug resistance gene (which is used to degrade antibiotics to allow selective growth of positive cells), and a replication origin (to allow the plasmid to replicate in the host cell). The cut DNAs are mixed to allow annealing of compatible sticky ends (diagram center), then inserted into bacteria (lower left). The *E. coli* bacteria have been treated with calcium chloride to allow them to be more permeable to DNA molecules. Through the process of transformation the plasmids enter the bacterial cell, and the bacteria make copies of the new DNA. Cells containing the new DNA are screened by plating the bacteria on agar plates containing an antibiotic that normally kills bacteria. If the new DNA is present in the cell (diagram lower right) the antibiotic is degraded and the cells grow on the plate. The amplified DNA can then be isolated and used for a variety of purposes including making transgenic animals (see below).

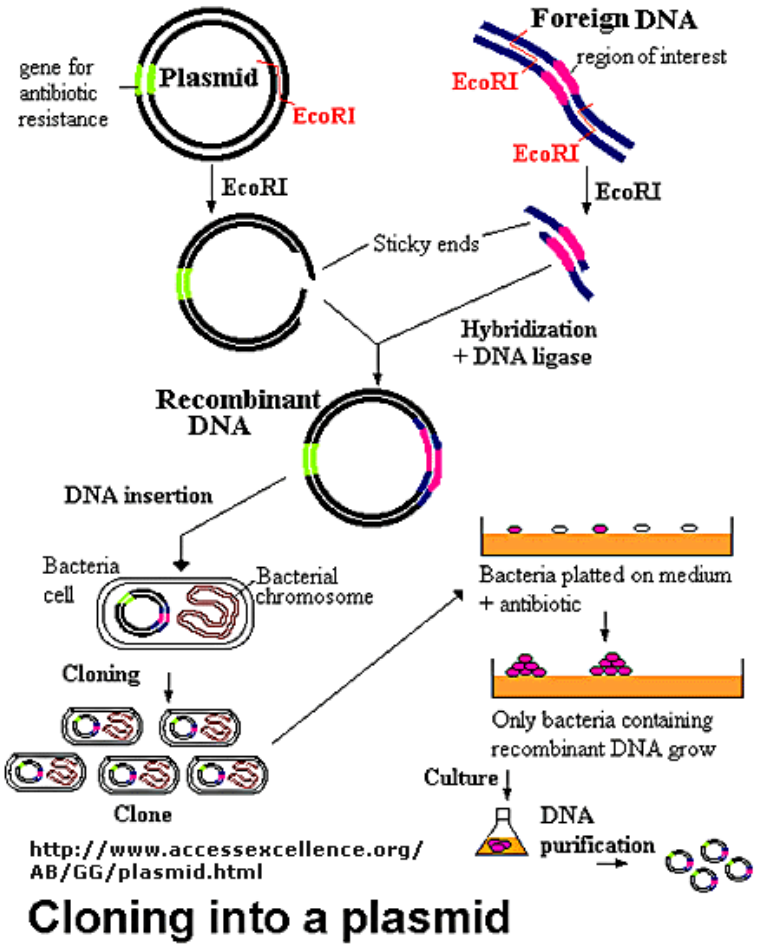

Figure 4: Creation of a Transgenic Bacterium Using a Vector. Diagram shows the process in which two DNAs (foreign DNA upper right) and a plasmid vector (upper left) are cut with EcoRI then recombined (diagram center). When the DNA is inserted into a bacterium, it gives the bacteria new properties such as antiobiotic resistance (lower right). (Access Excellence: The National Health Museum, 2009)

CREATING TRANSGENIC ANIMALS

There are several methods of creating transgenic animals—some are more popular than others. Years of research on how to make genetic changes in a genome has resulted in a number of technologies for this purpose: microinjection of DNA into embryonic stem (ES) cells,

microinjection into the pronucleus of a newly fertilized egg, DNA homologous recombination (also known as gene targeting), and somatic cell nuclear transfer (SCNT).

Microinjection into the Male Pronucleus

The earliest technique for creating transgenic animals, and one of the more popular methods, is microinjection into the male pronucleus of a newly fertilized egg during *in vitro* fertilization (IVF) (**Figure-5**, left side). A pronucleus refers to the male nucleus of a sperm cell that has entered the ovum at fertilization but prior to its fusion with the female pronucleus. The desired gene for insertion into the animal (termed a transgene) is cloned into a plasmid and then purified. A high purity of the DNA is important to the success of the procedure. Newly fertilized eggs are screened to insure that nuclear fusion has not occurred. The cloned DNA is then injected with the use of a microsyringe into the male pronucleus. The egg is then cultured *in vitro*. The pronuclei fuse to make the nucleus of a newly formed zygote. The zygote begins to divide, and after about 5 days a blastocyst is formed. To prepare the pseudopregnant foster mother for implantation of the blastocyst, she is mated with a vasectomized male. At this point the blastocyst is implanted into the uterus, and following pregnancy the pups are born. Screening is then performed to determine which pups have incoroprated the transgene (discussed below) (Transgenic Animals, 2003)

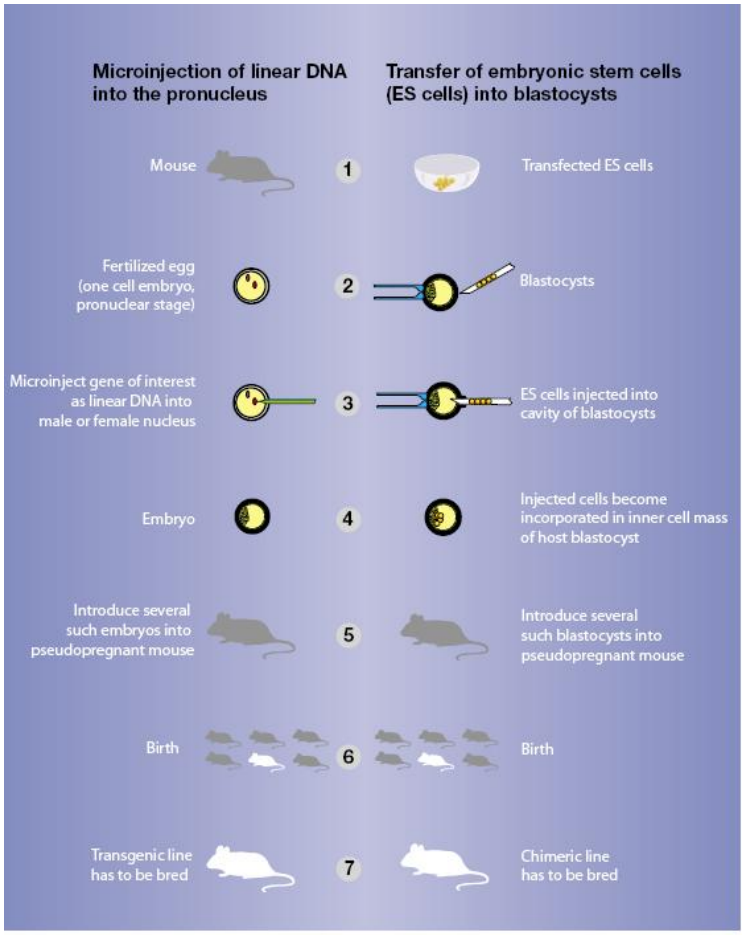

Fig. 2: Microinjection techniques

Figure 5: Two Microinjection Techniques for Creating Transgenic Animals. Shown are the microinjection of foreign DNA into the pronucleus of a newly fertilized egg (left side), and microinjection of embryonic stem (ES) cells into a blastocyst. (Sonja, 2009)

Microinjection Into Embryonic Stem Cells

The second method for creating a transgenic animal is microinjection into embryonic stem (ES) cells (**Figure-5**, right side). Specific properties of ES cells make them ideal for this process. As opposed to other cells in the body, stem cells have the ability to divide for an extended period of time, and can either renew themselves or differentiate into more specialized cells. ES cells are located in the inner mass cell of a blastocyst, a hollow ball of cells at day-5

post-fertilization. ES cells are pluripotent, meaning they can produce all cells of an animal (except extra-embryonic tissue such as the placenta). ES cells are injected with cloned purified plasmid DNA containing the transgene of interest, and in some instances the DNA integrates into the ES cell DNA. Because ES cells can be grown to large quantities *in vitro*, they can be pre-screened for transgene uptake using PCR or Southern blots (described below). ES cells positive for the transgene are microinjected into a blastocyst, and reintroduced into the uterus of a foster mother as described above (Stem Cell, 2006). Because not all of the inner cell mass ES cells contain the transgene, the founder animals are chimeras, some of whose cells contain the transgene and other cells do not. So when using the ES technique, often founder animals are subsequently bred with other founders to eventually select for pure transgenics.

Gene Targeting

The first two techniques mentioned allow the transgene to be randomly incorporated into the genome. In some cases the transgene incorporates into an inactive area of the chromosome, so the transgene is not expressed. This inactivation can be avoided using gene targeting. Homologous recombination is a biological process that occurs during mitosis in which chromosome strands can exchange between sister chromatids. This natural exchange process can be exploited to incorporate a transgene at a specific location in the gemome by simply flanking the transgene with pieces of chromosomal DNA, which carry the transgene to the same site in the chromosome containing the chromosomal sequences. An exchange between the host cell's chromosome and the DNA of the transgene occurs to allow the transgene to insert at a known location (Bronson and Smithies, 1994).

Somatic Cell Nuclear Transfer

The final method for creating transgenic animals that we are going to discuss is somatic cell nuclear transfer (SCNT). This method is believed to be a more efficient and safer way of creating these animals, as the technique requires fewer embryos. As portrayed in **Figure-6**, a skin cell is taken from a normal animal (diagram upper left). The nucleus of that cell is removed, and is microinjected with transgene DNA (not shown). The nucleus is then injected into an enucleated egg (diagram lower left). Electric current stimulates the cell to begin dividing (diagram center), and after 7 days the embryo is implanted into the uterus of a surrogate mother. The result of this process is a new born cloned animal (diagram right) with the same DNA as the skin cell donor, although it is transgenic if it was injected with a transgene (Somatic Cell Nuclear Transfer, 2006).

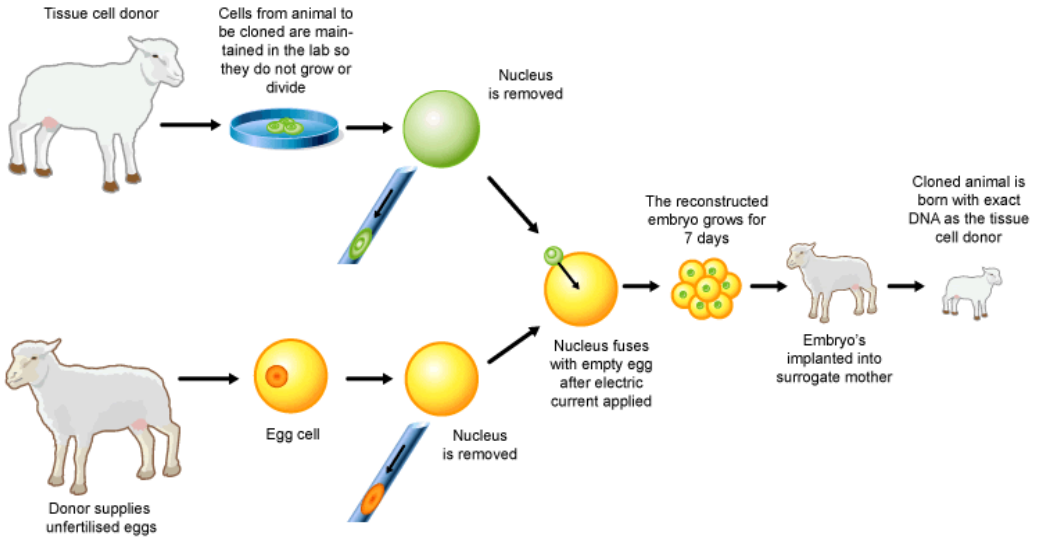

Figure 6: The Somatic Cell Nuclear Transfer Method of Making a Transgenic Animal. The nucleus is removed from an egg (lower left) and replaced with the nucleus of a tissue donor (upper left). The egg is developed about 7 days, then implanted into the uterus of a recipient. (Somatic Cell Nuclear Transfer, 2006)

METHODS FOR SCREENING TRANSGENIC ANIMALS

The creation of transgenic animals is an extremely valuable technology, but it is inefficient and many pups are born that are not transgenic. Thus the pups are screened to determine whether the transgene has incorporated, and whether it is being expressed. Methods such as the Southern Blot test and the Western Blot test provide scientists with a visual means to verify the success of their transgenic methodology.

Southern Blot Test

To determine whether the transgene has integrated into the DNA of the host animal, scientists can utilize a method known as the Southern Blot. This test has a number of useful applications, including the ability to identify the number of copies of integrated transgenes, how many chromosomal sites the transgene has inserted into, and whether the transgene remains unharmed. This test is usually performed within the first 6 weeks of the offspring's life. Scientists usually hope that 5 to 10 copies of the transgene have inserted. DNA is isolated from a test sample from the pup. The DNA is cut with restriction enzymes (mentioned above), then the DNA fragments are separated by electrophoresis in which a current is applied to a gel containing the samples. DNA is negatively charged, so it moves to the positive anode. The short fragments move faster through the gel than the larger fragments, providing a means to separate the fragments. The DNA in the gel is then treated with an alkaline solution to separate the strands of DNA to allow them to hybridize to a complementary DNA probe to the transgene. The denatured DNA in the gel is blotted to a membrane, so the pattern of separated DNA remains the same on the membrane. The membrane is then hybridized to a transgene probe that

is labeled to allow its visualization. If the film appears dark, a DNA fragment containing the transgene has been identified. (Brinton and Lieberman, 2007)

Western Blot Test

Simply because a transgene is present in an animal's DNA does not mean it is being switched on to produce the transprotein, so a Western blot test is used to determine whether the transprotein is being produced. This test is similar to the Southern blot test, except cellular protein is electrophoresed instead of DNA, and the probe used is an antibody to the transprotein, instead of a DNA probe. By using antibodies directed against the transgene protein, the western blot test can identify proteins made by that transgene. If these transproteins are identified, the transgene has been switched on (Western Blot Activity, 1998).

Real Time Reverse Transcriptase PCR

The process of PCR was described earlier, and it can be applied to screening transgenic animals. If primers flanking the transgene are used to screen DNA, PCR can rapidly tell whether the transgene has integrated in the DNA of the host animal. So PCR is sometimes used in place of Southern blots for this purpose. Alternatively, a modification of PCR termed reverse transcriptase PCR (RT-PCR) can be used to assay for the presence of mRNA produced from the transgene. RT-PCR is similar to PCR except RNA is used as template not DNA. Reverse transcriptase enzyme is used to prepare a complementary DNA template from the cellular RNA, then that DNA is tested by PCR using fluorescent transgene primers. Thus scientists can amplify mRNA encoding the trans-protein. Reverse transcriptase is an enzyme that makes many copies of DNA from RNA. The DNA that is formed is the complement of the original DNA, known as

the complementary DNA (cDNA). By amplifying the complement of this cDNA, scientists can verify whether the transgene was transcribed into RNA (Hunt, 2006).

Chapter-1 Bibliography

Access Excellence (2009) The National Health Museum. http://www.accessexcellence.org/RC/VL/GG/plasmid.php

Berg, Paul (2004) *Genetics and Genomics Timeline 1972 Paul Berg creates first recombinant DNA molecule*. Genome New Network. Accessed July 2009. http://www.genomenewsnetwork.org/resources/timeline/1972_Berg.php

Brinton, Kate, and Lieberman, Kim-An (2007) Southern Blot Testing. Basics of DNA Fingerprinting. http://protist.biology.washington.edu/fingerprint/dnaintro.html

Bronson SK, and Smithies O (1994) Altering mice by homologous recombination using embryonic stem cells. *J. Biol. Chem.* **269**: 27155-27158.

Cooper, Geoffrey, and Robert Hausman (2004) *The Cell: A Molecular Approach*. 3rd. Washington, D.C.: The American Society for Microbiology Press, 2004.

Genetic Home Reference (2007) *What is DNA?* US National Library of Medicine. http://ghr.nlm.nih.gov/handbook/basics/DNA

Hunt, Margaret (2006) "Real Time PCR". *Microbiology and Immunology* (Sept. 2006). http://pathmicro.med.sc.edu/pcr/rea;time-home.htm

Somatic Cell Nuclear Transfer (2006) Advance Access Human Reproduction . http://humrep.oxfordjournals.org/cgi/reprint/21/10/2564

Spencer, Charlotte (2006) *Genes, Aging and Immortality*. San Francisco: Pearson-- Benjamin Cummings, 2006.

"Stem Cell Basics: What are Embryonic Stem Cells?" Stem Cell Information. (2006) http://stemcells.nih.gov/info/basics/basics3.asp

" Transgenic Animals" (2003) http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/TransgenicAnimals.html

"Transgenic Animal Science: Principles and Methods" (1991) Charles River Laboratory. http://www.criver/cpm/tecjdpcs/trangen.htnl

Voss Sonja, and Eppendorf AG (2009) Transgenic Animals. Hamburg, Germany. http://www.biocompare.com/Articles/TechnicalArticle/1649/Transgenic-Animals-from-Eppendorf-AG.html&usg.

"Western Blot Activity" (1998) The Biology Project/Immunology. http://biology.arizona.edu/IMMUNOLOGY/activities/western_blot/west3.html

Chapter-2: Transgenic Technology Applications

Matthew Connor

Transgenic animals are used for a variety of applications, but most of the applications share one common thread, they exist for the sole purpose of benefitting society. Transgenic disease models are used as tools to better understand disease and its process, especially genetic diseases, some of which remain to this day incurable and untreatable. Scientific models aid scientists to understand how genetic content affects all facets of life, and how its alteration can aid in human and animal development. Transgenic applications also serve to refine the process by which medicine is produced and distributed, and to efficiently produce animals as food sources, and can even provide a source for new tissue and organ donations. The purpose of this chapter is to review the main applications of transgenic animals as a prelude for discussing their ethics and legalilties.

Disease Models

One of the most important applications of transgenic technology is the modeling of human diseases. Disease models aid scientists in examining the pathology and progression of a specific disease along with the environmental, physiological and developmental triggers. What causes a disease to have an early onset with some people, while the majority only contract it in the later years of their life? This is the kind of question that can be better examined with a transgenic disease model, especially one that is easily manipulated in a lab environment. Transgenic animals possess a shorter life span and a greatly increased rate of maturation which

can be used to examine over a period of weeks or months events that may take years or decades in a human host. By creating a transgenic animal possessing a human gene for a genetic disease the stages of development and progression of the disease can be better understood. "In turn, these studies will provide critical information concerning potential pharmacological targets and approaches for therapy that can [also] be tested in transgenic models" (Price et al., 1998).

Huntington's Mouse

Huntington's disease (HD) is a neurodegenerative autosomal disorder that typically onsets between 30 and 50 years old, but can start at any age. Similar to other neurodegenerative diseases, it is characterized by chlorea (involuntary muscle spasms and loss of motor control) and dementia. The disease being autosomal means that the gene causing it lies on an autosomal chromosome, and its contraction is entirely genetic. At this point the only proven prevention is by genetic testing of potential parents before conception. Huntington's neural degeneration is caused by a mutation in the huntingtin gene with CAG triplet nucleotide duplication, which causes a build up of polyglutamine amino acids in neurons. The extent of the CAG nucleotide buildup on the chromosome has been shown to be directly tied to the age of onset, greater build up being equivalent to an earlier onset. The degeneration also leads to expressed weight loss in the brain and the body of the affected. This weight loss is one of the advantages of using transgenic mice, the scientist can compare the affected animal with its healthy littermates to easily check the progression of the disease.

A study in 1998 showed that mice expressing the mutated human Huntington protein also showed nuclear inclusion bodies (Price et al., 1998). This observation was then confirmed in the tissue of human HD patients, and has helped led to our current understanding of how this

inclusion precedes the polyglutamine buildup, which in turn leads to neuron degeneration. This is a good example of the invaluable asset transgenic animals add to research, in this case on observation was discovered in the mouse carrying the human Huntington's gene, and then subsequently confirmed with human testing. A researcher could spend years trying to examine the CAG buildup in a human host only to discover that the subject, though carrying the Huntington's gene, does not happen to contract the disease invalidating all the research and any hypothesis or conclusions drawn on said research.

In another Huntington's study, specifically aimed at understanding the cause of body weight loss and muscle atrophy in human patients, 4 different transgenic mouse lines (R6 lines) were created containing the *Huntingtin* gene, and 2 more lines were kept as controls. All four transgenic lines integrated the transgene, but one did not express it. The three expressing lines all showed signs of the CAG buildup (**Figure-1)**. The non-expressing line likely failed to express due to integration at an inactive area of the chromosome.

transgenic line	integration site	CAG repeat size	transgene expression	phenotype
R6/1	single copy	113	+	+
R6/2	one intact copy	144	+	+
R6/5	four intact copies	128–156	+	+
R6/0	single copy	142	—	—
HDex6	*ca.* 20 copies	18	+	—
HDex27	*ca.* seven copies	18	+	—

Figure-1: Summary of Four *Huntingtin* Transgenic Mouse Lines and Two Controls. (Sathasivam et al., 1999)

The advantages of using the transgenic mice in this study are quickly evident, the mice can be discerned form their littermates in only 5 weeks with a test of their motor skills. Most of the mice were only studied through the 15th week, but the important fact is that 4 months after birth, degeneration in skeletal muscle could also be studied by the researchers seeking the

nuclear inclusions previously identified in the central nervous system. **Figure-2** shows a comparison of the cross section of tissue from the R6/1 and R6/2 mice, and corresponding controls. The transgenics show a decrease in muscle fiber thickness. The researchers went on to state that they were continuing work to identify a molecular solution to prevent and slow the polyglutamine aggregation that caused the degeneration. They hope to test these therapies on the R6 line.

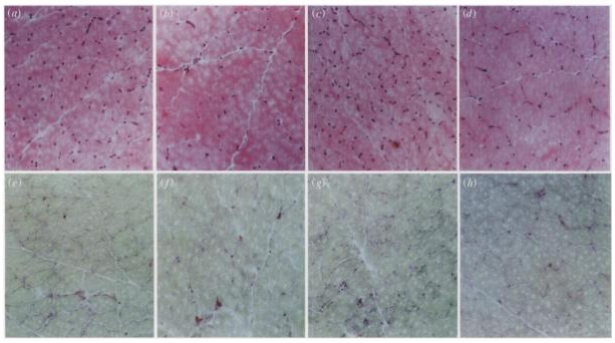

Figure-2: Analysis of Muscle Atrophy in Four R6 Transgenic Lines. Transverse sections from quadricep muscle were stained with haematoxylin and eosin (upper row) and for acid phosphatase activity (lower row). Samples from left to right are 15-month-old R6/1 transgenic animal; 15-month-old non-transgenic control; 14 week old R6/2 transgenic animal; 14-week-old non-transgenic control. The only difference between the transgenic animals and their normal littermate controls is a uniform shrinkage across all fibre types. (Sathasivam et al.,1999)

This Huntington's disease model is just a minor sampling of the scientific knowledge gained from transgenic mice. Other models have also been constructed for Alzheimer's disease, cancer, AIDS, and Parkinson's disease.

Transpharmers

Transpharmers' are transgenic animals engineered to produce therapeutics, such as insulin, in their milk, and have been sought to create abundant resources to treat deficiencies such as diabetes. The proteins are produced in the mammary glands and harvested through the

animals milk. Some initial work experimented with other means such as carrying the protein in the animals blood, but expressing the foreign proteins there caused too many complications when interacting with the animal's physiology, so subseqent experiments have focused on expression in the milk. The process can yield between 1 and 10 grams of protein per liter of milk (Ledford 2006). The goal is the large capacity inexpensive production of proteins that until now have been cultured only in cells in Petri dishes. If more of a given protein needs to be produced, the solution is as simple as breeding more animals, not scaling up an intricate manufacturing process. Also much of the processing that must occur post harvest from current methods occurs naturally in the animals physiological process. Another advantage is the low cost of the production, once an animal is created and bred into a functional population of animals with targeted yields, the largest cost incurred will simply be in animal feed and quarters. For the most part though, "the low level of transgene integration, the highly variable levels of expression and germline transmission in these animals, and the fact that it takes up to three years to generate a production flock from a transgenic male founder goat, have been until now serious drawbacks in realizing the full promise of [transpharming]" (Cloned Genes…1999).

Transpharmer Cattle

Although cows produce the most milk, they are also the most difficult animal to make transgenic. Herman the bull (**Figure-3**) was genetically engineered to carry the human gene for the production of Lactoferin. Human Lactoferin is a protein essential for the immune system of infants, and is present in mother's milk, but does not naturally occur in cows milk. Although mice were the first successful case of producing a transgenic animal, Herman was the first transgenic cow. Though being a male meant he did not produce milk, Herman went on to father

his first 8 calves in 1994, all of whom received the Lactoferine gene, the females of which created and passed the protein in their lactation (Biotech Notes, 1994). Though his calves had very low yields of the target protein in their milk, they along with their father proved that transgenic feedstock was a feasible source for recombinant proteins, and that an increase of production could come from traditionally breeding the offspring.

Figure-3: Herman the Bull. Herman was the world's first transgenic cow, created to transpharm lactoferrin. His female offspring successfully produce lactoferrin in their milk. After his death, Herman was taxedermied, and placed on display at the National Musuem of Natural History in Leiden Netherlands. (Herman the Bull, 2006)

Transpharmer Goats

While mice were the first transgenic animals created, and cattle were the first large scale milk producing transgenic mammals created, transgenic goats have been the biggest success story. Transgenic dairy goats have been created that produce 5g/L of recombinant anti-thrombin III protein in their milk. An entire herd could produce up to 300 kg of marketable protein per year (Baguisi et al., 1999). The goats shown in **Figure-4** were engineered to carry the transgene for recombinant human antithrombin III (a blood thinning protein). As with Herman the bull's

offspring, the gene was expressed, but unlike Herman's calves the goats produced a high concentration of the target protein in their milk.

Figure-4: Three Transgenic Goats Expressing Recombinant Human Antithrombin Gene. The goat on the far left is eight weeks old, and the two on the right are five weeks old. (Baguisi et al., 1999)

The measured concentrations of antithrombin III per liter of milk are shown in **Figure-5**, lactation was over a period of 33 days and induced just 2 and a half months after birth. Three additional important results discerned from this particular transgenic line included a survival rate unmatched by cows or sheep, early characterization of the phenotype, and the ability to quickly produce more goats expressing the transgene from the same founder line. This drug marketed as ATryn® by Genzyme Transgenetics Corp (GTC) became the world's first FDA approved drug in 2009 (ATryn, 2009).

CFF6-1 induced lactation day	hAT concentration (g/liter)	h AT activity (U/ml)
Day 3	4.7	N/D
Day 5	5.8	20.5
Day 6	5.0	18.3
Day 7	4.7	15.6
Day 8	4.1	14.4
Day 9	3.7	14.6

Figure-5: Levels of Recombinant Human Antithrombin III Expression in a Transpharmer Goat. Shown are the expression levels in the eight week old goat (shown on left in the previous Figure) after induced lactation (Baguisi et al., 1999).

Xenotransplanters

Xenotransplanting is the process of using one species of animal as an organ donor to a different one. This process is discussed here mainly in terms of a human recipient. There is a growing shortage of human donors to meet an increasing need in America and around the world. In 1996, there were 44,000 Americans on the organ transplant waiting list. That number doubled to 90,000 on the transplant list in just over a decade in 2006. In 2005, more than 6,000 patients died due to the unavailability of donors or being too ill to receive an organ (Fabregas, 2006; Williams, 1996). As life expectancy increases, there will be an increase in the shortfall of donors available to supply recipients in dire need. Suitable animals could serve as the supply chain solution for the logistical issue of the growing need for organs. Xenotransplants have most commonly utilized baboon and pig organs. Baboons have supplied hearts, livers, kidneys, and even bone marrow to recipients. In every instance though, the patient has either died from their own rejection of the foreign organ, or from infection due to immunosuppressants being administered to counteract the rejection.

Xenotransplanter Pigs

Pigs are currently the most promising of the potential organ donors. Pigs have organs of similar size and anatomy to its human recipients, they reproduce much quicker litters, as opposed to baboons who only bear one offspring at a time. Pigs are also healthier, carrying less communicable diseases than baboons. Thus far, pigs have been limited only in that they produce a sugar alpha-1-galactose that the human body recognizes as foreign. Even with the sugar present pig livers were successfully used in 1992 as a "bridge" while awaiting a human transplant, with one patient of two surviving long enough to receive a human transplant. Initial transgenic work attempted to knockout the genes encoding the glycosyl-transferase enzymes that add the sugar to organ surfaces to counteract the rejection process (Lai et al., 2002) and are currently being tested by transplanting pig hearts into baboons. The possible future applications of transgenic pig organs and tissue could include replacing hearts, and beta cells for diabetes patients (Fabregas, 2006; Williams, 1996).

Transgenic Food Sources

Transgenic food sources attempt to remedy food shortages and our decreasing wild animal populations. Transgenic food sources aim to create animals that grow larger, mature quicker, are more nutritious and more efficiently utilize the consumed food that sustains the animal. This should lead to a more abundant food supply at a lower cost.

Super Fish

Though not yet in a full scale application due to the possibility of a severe environmental impact, transgenic fish, specifically coho salmon possessing the growth hormone (GH) transgene

appear to be the closest to market of the transgenic animal food sources. These fish contain the OnMTGH1 gene, which is a growth hormone gene under control of a strong metallothionein (MT) promoter. The fish have repeatedly positively expressed the transgene and its phenotype. "Unlike non-transgenic fish, transgenic fish fed a diet high in carbohydrates maintained strong growth rates, had increased capacity for lipid synthesis, and increased potential for biosynthetic roles of amino acids" (Leggatt et al., 2009). The transgenic coho salmon possess a faster metabolism, more efficient usage of dietary intake, and far greater growth potential than their control counterparts. One year old transgenic salmon have the developmental equivalent of controls twice their age. **Figure-6** shows a comparison of transgenic coho compared to their age-matched controls. Despite such fish being one of the major successes of this technology, the fish are still limited to research due to potential environmental impact if they escape and breed with wild type salmon.

Figure-6: Comparison of Transgenic Coho Salmon to Age Matched Controls. Four coho salmon expressing the growth hormone transgene (top four) are shown compared with four control coho salmon (bottom four).

Based on potential environmental concerns, researchers are now turning to producing an additional phenotype of sterility, so even if genetically manipulated fish were to escape the hatcheries to mingle with wild type salmon, there would be no interbreeding. One possible method is the inactivation of gonadotropin-releasing hormone which is released from the

hypothalamus to trigger the growth, differentiation, and maturation of gonads. One issue that arises though, is that this same hormone also regulates the release of the main targeted GH whose production should be maximized. Another suggested approach is to "use an antisense RNA approach in which DNA from a sterility gene to be inactivated is placed into a transgenic construct in reverse orientation such that transcription produces mRNA that is complementary to the target endogenous mRNA transcript, resulting in a knockdown in the level of target gene expression, and an increase in sterility. But a well known disadvantage associated with the use of anti-sense RNA-based technologies is they do not completely inhibit target gene expression..." (Wong 2008).

Scientific/Biological Models

Biological transgenic models are a little more varying in application than the four previous topics. These models are employed to gain a better understanding of the functionality of specific proteins and their effect on the biology and physiology of the host animal. The principle is that in some cases overexpressing a specific protein whose function is unknown can reveal its effects on the animal, exposing the mechanisms behind the genetic trait. This is one of the most interesting and wide spread applications for transgenic animals, and a place where reality can border science fiction. Hypothetically say the end goal is to give a soldier the reflex speed of a mongoose, one method of determining what part of the mongooses genetic code gives them this ability to be quick enough to catch and kill snakes would be to take the suspected section of genetic code (and its enablers) and insert them into a slower reacting mammal to try and increase reaction time. This technology offers the possibility of unlocking the functions of newly discovered proteins, and offers boundless human potentials. There are also marketable applications, by for example giving a household pet a greater communication capacity with their

owners. Transgenic biological models have been used to increase intelligence in mice, increase size in mice, and in one case to impart the green fluorescent protein of a jellyfish in a monkey.

Super Mouse

The super mouse was the first *expressing* transgenic animal ever created and was done so in 1982 (Palmiter et al., 1982). This mouse received a rat growth hormone gene, and the transgene was expressed to create very large mice relative to their non-transgenic littermates. Although the genetic trait endowed upon it (increased size) was not necessarily beneficial to society, especially when measured against some of the cases previously reviewed, it unlocked the doorway to all subsequent transgenic animal cases. It proved not only that a genetic trait from one animal could be expressed in another, it also proved that those genetic traits could be passed on to the next generation of that animal.

Smart Mouse

One of the most significant scientific model transgenic experiments ever successfully conceived was the creation of a strain of smart mice named Doogie. These mice were endowed with the gene NR2B which is a subunit of the glutamate receptor that predominates during early development when learning and memory are easier (Tang et al., 1999). Overexpression of this gene greatly increased a mouse's ability to recognize objects, learn more effectively, increase spatial learning, and reversed the slowing down of the ability to learn that usually accompanies maturation, as it does in people and most other animals. This biological model proved that the NR2B protein actually had these functions, and has much wider implications than just making mice good problem solvers. Now that the learning process is better understood, this experiment

showed that "improvement of intelligence and memory in mammals is now feasible, thus offering a striking example of how genetic technology may affect mankind and society in [this] century" (Harmon 1999).

Chapter 2 Bibliography

ATryn® (2008) Recombinant Human Antithrombin. *GTC Biotherapeutics, Inc.* http://www.gtc-bio.com/products/atryn.html

Baguisi A, et al. (1999) "Production of Goats by Somatic Cell Nuclear Transfer." *Nature Biotechnology* **17**: 456-461.

Biotech Notes (1994) Herman Becomes a Father. U.S. Department of Agriculture. http://www.accessexcellence.org/AB/BA/Herman_the_Bull.html

"Cloned Genes, Goats, and the Future of the Biopharm" (1999) *Nature Biotechnology* **17**: 405. [No authors listed]

Fabregas L (2006) Million-Dollar Pigs are Medical Marvels. Pittsburgh Tribune-Review April 9, 2006. http://www.pittsburghlive.com/x/pittsburghtrib/s_441762.html

Harmon J (1999) "Scientists Create Smart Mouse". September 1, 1999. http://www.princeton.edu/pr/news/99/q3/0902-smart.htm

"Herman the Bull Stabled in Naturalis"(2006) Naturalis, National Museum of Natural History Leiden Netherlands, http://www.naturalis.nl/asp/page.asp?alias=naturalis.en&view=naturalis.en&id=i000256&frameurl=http%3A%2F%2Fwww.naturalis.nl%2Fnaturalis.en%2Fnaturalis.en%2Fi000968.html

Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL et al (2002) Production of Alpha-1,3- Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning. *Science* **295**: 1089-1092.

Ledford, Heidi (2006) The Farmyard Drug Store. *Nature* **443**: 16-17.

Leggatt RA, Raven PA, Mommsen TP, Sakhrani D, Higgs D, and Devlin RH (2009) *Growth hormone transgenesis influences carbohydrate, lipid and protein metabolism capacity for energy production in coho salmon (Oncorhynchus kisutch)***.** Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, Vol **154**, pgs 121-133.

Palimiter RD, Brinster RL, Hammer RE, Trumbauer ME, Rosenfeld MG, Birnberg NC, and Evans RM (1982) Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* **300**: 611-615.

Price DL, Sisodia SS, and Borchelt DR (1998) *Genetic Neurodegenerative Diseases: The Human Illness and Transgenic Models.* Science, New Series, Vol. **282**, No. 5391 (Nov. 6, 1998), pp. 1079-1083. Published by: American Association for the Advancement of Science. http://www.jstor.org/stable/2897349

Sathasivam K, Hobbs C, Mangiarini L, Mahal A, Turmaine M, Doherty P, Davies S, Bates GP (1999) *Transgenic Models of Huntington's Disease. Philosophical Transactions: Biological Sciences*, Vol. **354**, No. 1386, Glutamine Repeats and Neurodegenerative Diseases: Molecular Aspects (Jun. 29, 1999), pp. 963-969. Published by: The Royal Society. http://www.jstor.org/stable/56973

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ (1999) Genetic Enhancement of Learning and Memory in Mice. *Nature* **401**: 63-69.

Williams RD (1996) "Organ Transplants from Animals: Examining the Possibilities" FDA Consumer Magazine(June 1996). http://web.archive.org/web/20071210031618/http://www.fda.gov/fdac/features/596_xeno.html

Wong, Andrew C, and Alison L. Van Eenennaam (2008) *Transgenic approaches for the reproductive containment of genetically engineered fish.* Aquaculture (Issues 1-4), Vol. **275**, pgs 1-12. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4D-4RH94RG-3&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=147bc342b3229090e47ba8a01a006671

Chapter 3- Transgenic Ethics

Nicholas Tsitsilianos

Historically, scientists have been intrigued with the challenge of understanding the immediate world around them and explain it. A scientist's primary goal has been to elucidate a better understanding of nature. In their pursuit of understanding natural phenomena, they invariably were faced with overcoming the prejudice of their time when traditionally accepted positions interfered with their ability to continue their work. When scientists were able to present and scientifically prove fundamental understanding of the rules of nature, they had achieved their primary purpose. Over the past centuries, however, scientific achievement has changed the traditional explanations, moving from describing the laws of nature, to discoving how to use the laws of nature for scientific achievement. In the 1940's and 1950's two major discoveries provided limitless possibilities for scientific achievement, the 1945 development of the atomic bomb, and Watson's and Crick's 1952 discovery of the structure of the DNA molecule. The latter discovery catapulted the development of genetics research to a new level, culminating in the ability to recombine genes and the science of genetic engineering which made scientists capable of changing nature and its laws. The technology of transgenics, the ability to insert a foreign gene into an animal's genome, was introduced in chapter-1, and a discussion of its applications followed in chapter-2. The purpose of this chapter is to discuss transgenic ethics, whether such technology *should* be pursued. Without attempting to provide a blanket approval or rejection of this technology, this chapter balances a discussion of the medical benefits of specific transgenic cases versus their detriment to society or the animal.

Transgenic Ethics Introduction

Through the studies of evolution, science has fashioned a new view of humankind. With new discoveries, science has significantly changed people's lifestyles, affecting all aspects of life. This is especially true in the science of genetic engineering, where scientists discovered methods that have a great impact on changing life, and give mankind choices to design and fulfill specific wishes. These ground-breaking scientific discoveries create challenges and raise unexplored ethical issues. At present, legislators struggle to find common ground, while safeguarding their constituent's religious freedom and basic rights, to create laws with the aim of building a society that will protect human rights. Ethics, the "investigation into the basic concepts and fundamental principles of human conduct-- including study of universal values such as the essential equality of all men and women, human or natural rights, obedience to the law of land, concern for health and safety and, increasingly, also for the natural environment", plays a significant role (Business Dictionary 2009). In the past, religious writings and cultural traditions provided the main ethical map to navigate moral dilemmas (Curran and Koszarycz, 2004). Today, theologians and ethicists coalesce to address ethical questions to make appropriate choices which will serve society's best interests. This collaboration will provide information to assist legislators in their duty to establish laws. It is then that society can assess the value and concur or object to the degree that these decisions have.

With respect to transgenics, advancements in biotechnology have been remarkable. The alteration of the genomes of plants and animals, and possibly to humans could potentially find cures for some of life's devastating diseases. In addition, this research has the potential to extend the lifespan of humans which would have a powerful effect on society (Transgenic Animals: Their Benefits to Human Welfare, 2003). Despite these possibilities, controversy surrounds its

every movement forward. Ethical issues have surfaced because genetic research has unlocked information about human genetic makeup, and uncovering possible predispositions to certain diseases. Access to such information could have serious 'privacy' concerns.

Scientific intercession, in the field on genetic engineering, is at the threshold of having the capability to modify and manipulate organisms for a variety of constructive outcomes. Attempts to develop new beneficial plants in agriculture (higher yields in productivity), medicine (research in methods controlling pathogens, as well as new medical therapies), and in manufacturing (increasing yields through disease resistance), provide humanity with unlimited possibilities. These great advancements in genetics must be scrutinized for possible environmental concerns, ethical and biological apprehensions in relation to dignity, possible harmful consequences, and legal regulations (Shannon, 1997).

Xenotransplantation Ethics

Statistics inform us of the increasing demand of organs for transplants, and the significant lack of donors. There is an extremely high demand for human organ donations-- over 150,000 hearts, livers, and kidneys in the US annually. Unfortunately it is highly unlikely that these needs can be met solely by human donations. This serious shortage of human organ donors has prompted great scientific interest in developing scientific methods for producing and harvesting animal organs suitable for human transplantation. As a result, scientists are now suggesting a new scientific alternative: xenotransplantation. Xenotransplantation is the transplantation of cells, tissue, or an organ from a nonhuman animal source (such as a pig) into a human recipient (Webster's New World Medical Dictionary, 2009). Theoretically this could result in a reprieve to the thousands awaiting human organs. The xenotransplanted organ would be similar in

physiological function as the replaced organ. Pig's heart valves are already used successfully in the human health care system. However, much is involved in preparing the animal as a good donor. For instance, the pigs must be raised in a pathogen-free environment. But more intensely, they are genetically modified to delete enzymes that encode sugar residues viewed as foreign by our immune system. Since the 1990s, scientists participating in xenotranplant research optimistically claimed treatments would be available in just a few years, but it turned out to be a far more difficult problem:

> "In closely protected organ farms, scientists are breeding genetically modified pigs whose organs they believe would be suitable for human beings. Early experiments using pig tissue implants have produced remarkable results in stroke and Parkinson's Disease sufferers. Pig to human organ transplants are within the reach of scientists and could save thousands of lives... But the risks are enormous. If pig viruses attack human cells, they could unleash a new AIDS-type epidemic against which we have no in-built defences. As science fiction becomes science fact, what price will we pay for this medical miracle? This then is the dilemma facing doctors, scientists and ethicists on a grand scale: do the benefits to the few outweigh the risks to the many?" (Bryan & Clare, 2001).

Xenotraplantation is in its early stages and is not a proven technology. As a result, animal to human transplantation has been under extensive examination due to the risks involved, ethical, philosophical, and religious questions, and public concern. The aim of xenotransplantation is to increase life expectancy and the quality of life for those who are very ill. It would alleviate the crucial shortage of organs available for transplantation. In addition, the technology would increase our knowledge of immunology and genetics. But until answers are obtained to serious questions about potential viral infection and whether genetic engineering can eliminate immunorejection, the concerns are too serious to provide a clearcut ethical approval. However, with time and further research, science might find a way to make this application successful, countless lives depend on it.

Transpharming Ethics

The technology of transgenic animals also expands to the production of pharmaceutical drugs in the milk of farm animals. By milking the animals we can mass produce a variety of human drugs cost effectively. Traditionally, human cadavers provided sources of bioproteins, such as insulin to treat diabetes, or thyroxin to treat hypothyroidism. But with the use of genetic engineering, the gene encoding a desired protein drug is transferred into another organism to produce large amounts of the drug of interest (as described in detail Chapter 1). The first drugs produced in bacteria using this technology were insulin and growth hormone, but by expanding transgenic technology to farm animals, and by changing the gene promoter to, for example a casein promoter, the downstream gene encoding a drug is produced only in the milk. Thus the animal becomes a production plant for this production. By producing the drug in the mammary gland, the drug is essentially outside of the body of the animal, so it has less physiological effect on the animal than if produced in the blood.

This technology is very useful because it allows us to make drugs in high quantity and proves to be safe for the animals. There are other advantages as well, by breeding the producing animals, expression levels can be increased. The goal of "pharming" animals in this way is to make sure the ability to produce the drug is passed on to the animal's offspring, allowing us to produce more and more of the drug with offspring animals. And one of the greatest advantages of the technology is that the drug, in most cases, is harvested directly from the animal in a very easy manner—in the milk, with no animal bleeding or sacrifice. For example, in 1994, Herman (**Figure-1**), the world's first transgenic bull carrying the human gene for lactoferrin, became the father to a litter of calves. Each one of the calves carried the gene for lactoferrin production. Lactoferrin, an iron-containing protein that is essential for infant growth, is not naturally found in

cow's milk. With scientific advancements such as this genetically engineered bull, and his descendants, nutritious milk is more readily available. Herman's example demonstrates the feasibility of producing large and complex proteins through molecular pharming.

Figure-1. Photo of Herman the Bull, The World's First Transgenic Bull. Herman is stabled in a breeding program at Gen Pharm's European laboratory in Leiden, Netherlands. Herman was the first genetically modified or transgenic bull in the world. (Naturalis, 2004)

Although this technology is groundbreaking, it not perfect. The process is not efficient. Studies have shown that only 30 percent of mouse founders produce transgenic offspring, and the efficiency worsens with larger animals. For animals such as sheep, goats, or cows, only about 5 percent of founders produce transgenic offspring. And to date, it is difficult to create an animal able to produce a desired protein with full biological activity in a cost effective quantity. Although commercial success has recently been accomplished for the first FDA approved transpharmed drug from Genzyme Transgenics (ATryn, 2008), the overall process is not easy. **Table I** shows the profit various engineered farm animals have been estimated to provide.

Drug	Animal	Value/Animal/Yr*
AAT	sheep	$15,000
tPA	goat	75,000
Factor VIII	sheep	37,000
Factor IX	sheep	20,000
Hemoglobin	pig	3,000
Lactoferrin	cow	20,000
CFTR	sheep, mouse	75,000
Human Protein C	pig	1,000,000

*Current market price of the drug and supply produced by one animal.

Drug descriptions:	
AAT	alpha-1-antitrypsin, inherited deficiency leads to emphysema
tPA	tissue plasminogen activator, treatment for blood clots
Factors VIII, IX	blood clotting factors, treatment for hemophilia
Hemoglobin	blood substitute for human transfusion
Lactoferrin	infant formula additive
CFTR	cystic fibrosis transmembrane conductance regulator, treatment of CF
Human Protein C	anticoagulant, treatment for blood clots

Table 1

Table 1: The Value of Various Transpharmer Animals. As you can see from the high profit for each animal, the drugs chosen for production so far have been carefully evaluated for cost effectiveness. The importance of each drug is shown in the lower half of the table (Biotechnology Information Series, 1995).

The authors of this IQP feel that molecular pharming, as a production method, are in its early stages and must be extensively evaluated by the Food and Drug Administration for safety, as in the ATryn drug case. Also, concerns have been voiced about the animal welfare and biotechnology's interference in the relationship between animals and humans. Because cases like Herman's are unusual, at present, this technology is very expensive.

Supporters of genetic engineering compare this process to selective breeding, conducted for centuries to enhance the development of larger, healthier farm animals. Selective breeding creates species that are more specialized than their ancestors without using any genetic engineering intervention, allowing only natural gene exchange. Selective breeding however, is not void of harmful effects on animals, as with the broiler chicken. Broiler chicken was naturally

bred to become meatier growing muscles/meat at a fast rate—1.6 kg in 37 days. But extensive health problems resulted. The fast growth rate caused leg problems and the inability to carry their own weight, which in turn caused lameness. The chicken at six weeks of age spent 76% of the time lying down because they can barely move. Cardiovascular as well as respiratory disease complications subsequently occur. Some say that genetic engineering has modified the science of selective breeding to be more specific to exchanging only one gene, and refining it for speed and accuracy.

Beltsville Pig/Super Pig Ethics

One of the most criticized transgenic experiments was the experiment of the Beltsville pig, more commonly known as superpig. By inserting the gene for growth hormone in the pig genome, scientists were able to increase the growth rate of a normal pig and produce a more lean meat, a more appealing product to consumers. Even though the scientists met their goal, the side affects greatly outweigh the benefits. Super pig suffered a great deal. The pig developed arthritis, gastric ulcers and stomach legions. It also was far less coordinated and was much weaker than other pigs (D'Silvia, 1998). Eventually the pig had to be euthanized. Ethically it was not the transgenic technology used on the pig that was objected to, but the effect it had on the pig's life. Cultural traditions can have a strong influence on a society's view of animal welfare. Because the make up of any animal's wellbeing is directed by cultural norms and ethical boundaries, it makes a simple evaluation problematic (Burghart and Herzog, 1989). As a result, considerations must include physiological and behavioral abnormalities, overall health problems, and various manifestations of pain.

Alzheimer's Mouse Ethics

In contrast to the suffering of the Beltsville pig, the transgenic animal in the Alzheimer's disease experimentation does not exhibit any signs of discomfort or suffering. This model has provided a new source of information for a terrible neurological disorder. Alzheimer's disease, an irreversible, progressive, and potentially fatal disease, destroys brain cells, in turn causing slowly eradicating thinking skills and memory. This progression is correlated to the formation of toxic beta-amyloid plaques in the brain, and tangled bundles of nerve fibers which block and degrade the connections between neurons (Duff, 1995). To date, the cause and progression of the divesting disease is not fully comprehended, but is thought to relate to the formation of the beta-amyloid toxin. The creation of a genetically modified mouse to develop dynamic AD first occurred in 1995, in part by Professor David Adams at Worcester Polytechnic Institute and his colleagues at the former Transgenic Sciences Inc (Games et al., 1995). This mouse, harbors a genetic variant of amyloid precursor protein found in an early onset pedigree in Indiana who develop the disease in their 40's instead of the usual 70's. The model has assisting scientists with uncovering the causes of aggressive early onset AD, and to subsequently design various vaccines for removing the beta-amyloid toxin. The Alzheimer's mouse model is being used for testing new medications, in hopes of getting closer to a cure. The mouse line is currently being used by Elan Pharmaceuticals to screen various vaccines for beta-amyloid removal. Although the initial vaccine trial in humans was cancelled when some patients showed brain inflammation, Elan in collaboration with Wyeth-Ayerst Laboratories has progressed to a second generation vaccine that is in Phase II of human clinical trials. It is hoped that such therapies may slow down the rate of progression of Alzheimer's disease.

Oncomouse /Harvard Mouse Ethics

Another important use for this transgenic technology is the study of the development of cancer. On April 12, 1988, Harvard University was granted a patent for two researchers: Dr. Philip Leder and Dr. Timothy Stewart, who developed "oncomouse". This team had genetically altered the strain by inserting a gene that made it unusually susceptible to developing cancer. Strains of mice based on the study of cancer have been developed, not only to study the disease process, but also to be used as controlled experimental systems to study 'gene-environment interactions'. This animal allows scientists to study 'chemoprevention strategies' in a model that is convenient to study in a research lab. Oncomouse is useful to researchers to develop drugs that can battle cancer, and to identify carcinogens in the environment. It also affords the opportunity to analyze in more detail how the genes contribute to the development of cancer (Brody, 1998). Based on new types of chemotherapies screened in oncomouse, in 2003 the number of cancer related deaths dropped from 557,271 to 556,902 (Stobbe, 2006). Other breeding techniques, such as selective breeding, have not been unable to yield the unique benefits accomplished by oncomouse experimentation.

The animal causes continuing debates on human interference in nature. The oncomouse controversy is compounded by the fact that they are genetically designed to develop cancer and suffer. Thus oncomouse presents the dilemma of providing great medical benefits to humanity while the cancer can cause high levels of suffering. In 1992, David Porter designed a pain scoring system which rated categories based on a score of 1 to 5, one being tolerant and five being intolerable (Porter, 1992). In the case of oncomouse, various methods were implemented to ease the suffering and pain incurred by the development of cancerous tumors, such as mandating the use of pain killing medication, and euthanizing the mouse when the disease

advanced to critical stages. Taking into consideration the enormous medical benefits provided by this animal, the authors of this IQP feel that experimentation using oncomouse should continue.

Transgenic Fish Ethics

The governmental regulatory agencies empowered to evaluate and approve genetically engineered animals as food move much slower than when approving genetically modified crop plants. However, increased commercial potential for animal transgenesis is evident—especially with aquatic animals. According to some scientists, genetically modified fish will probably be allowed to enter the market soon. To date, research conducted globally includes 35 species of transgenic fish (when the transgene is defined to include genes from other fish, insects, or humans). The engineering of fish with growth hormone genes has already produced superfish that mature faster, with more efficient food intake. Other transgenic modifications include the production of transgenic fish to release larger quantities of eggs per female. However, any discussion of this technology must include the impact of potential release of the transgenic fish into wild fish populations. The impact to the environment, the risks to the native ecosystems, and the potential risks to human health are unknown.

The most likely transgenic fish to first enter the commercial market will be a transgenic fish called the "super salmon". A company, Aqua Bounty Farms (Waltham, MA) has produced the first such animal for human consumption, and is seeking FDA permission to distribute it to the market. With genes donated from two different donors, this transgenic fish is able to grow year round. The super salmon reaches full growth much earlier than his wild counterpart—30 times faster. The wild type salmon only grows during warm weather, taking breaks during the

winter. The super salmon has an added gene that protects it from cold, and its growth is continual for 18 months. Supporters of the introduction of this transgenic food point out its ability to easily be aquafarmed to help alleviate world hunger. However, detractors suggest that far more data is needed prior to their consumption. For example new proteins in the fish could cause dangerous allergic reactions after consumption. Additional scientific studies at Purdue University performed on the Japanese medaka fish indicate that the introduction of only sixty transgenic fish could drive a wild type population of sixty thousand into extinction in forty generations in a process known as the "Trojan gene effect" (Muir and Howard, 2002). Perhaps a similar study should also be performed on Aqua Bounty's super salmon. Thus, it may be necessary to place safety protocols to guarantee that these expensive and important transgenic fish remain safe and protected during aquafarming. If these safeguards are in place, the authors of this IQP feel that research of genetically engineered fish should continue.

Chapter-3 Conclusions

Based on the information obtained during the course of this IQP, the authors have derived several conclusions. While it is imperative that we all remain cognizant of the potential harm transgenesis might create, we must also be cognizant of the huge benefit to society that transgenesis can provide. The ability to engineer genomes has created possibilities for helping to cure specific diseases or creating new drugs. There have always been detractors of scientific progress, even scientists or scientific academies that resisted the simple idea of hand washing before surgery. It is important that the debate includes a discussion of the benefits versus the animal suffering, as well as the important input of religious preferences and societal norms. And scientists must always design experiments in a way to reduce animal pain and suffering.

In this chapter, different transgenic models were described. In some cases, the potential benefits easily outweighed ethical concerns, while in others it is the opposite. In cases such as the Beltsville Pig and Oncomouse, experimentation resulted in higher levels of pain and suffering to the laboratory animals. For the Beltsville Pig, although the possibility of producing a large reduced fat pig to help alleviate world hunger presented a very attractive motivation, the animal suffering was bad enough to mandate euthanasia. We agree such experiments should not continue. On the other hand, in the case of Alzheimer's mouse, the model has already provided a new source of information for a terrible neurological disorder, and has provided a model to test various vaccines, while exhibiting minimal signs of animal discomfort or suffering. Thus we believe for this model the ethical issues are straightforward, and this experimentation should continue.

The experimentation involving Oncomouse, however, presents a true dilemma. The beneficial results to aiding our understanding of oncogenesis could be monumental, but the animal can suffer if the tumors grow to the advanced state. The authors of this IQP believe that for the good of society and the human race such an important scientific endeavor should be allowed to proceed, while at the same time the laboratory protocols should take into consideration methods to reduce the animal's suffering and pain. At the forefront of any experimentation the focus should remain on saving human life while reducing animal anguish.

In today's computer age, information can sometimes be disseminated with great ease. That factor gives the authors of this IQP the hope that with a very educated public in scientific matters, the public will be a positive factor to help move transgenesis forward.

Chapter-3 Bibliography

Access Excellence (2009) National Health Museum. http://www.accessexcellence.org/RC/VL/GG/plasmid.php

ATryn® (2008) Recombinant Human Antithrombin. *GTC Biotherapeutics, Inc.* http://www.gtc-bio.com/products/atryn.html

Biotechnology Information Series (1995) Pharmaceutical Production From Transgenic Animals. http://www.biotech.iastate.edu/biotech_info_series/bio10.html.

Brody, Baruch (1998) *The Ethics of Biomedical Research: An International Perspective.* New York: Oxford University Press, 1998.

Bryan J, Clare J (2001) *Organ Farm: Pig to Human Transplants: Medical Miracle or Genetic Time Bomb?* (Carlton Books: London).

Burghart and Herzog (1989) *Animal Biotechnology: Science Based Concerns. Board on Agriculture and Natural Resources (BANR), Board on Life Sciences (BLS).* *www.nap.edu/openbook.php?record_id*

Business Dictionary (2009) http://www.businessdictionary.com/definition/ethics.html

Curran, G, and Koszarycz Y (2004) Animal Transgensis and Cloning: Scientific, Religious, and Ethical Considerations. http://dlibrary.acu.edu.au/research/theology/ejournal/aejt_3/Curran_Koszarycz.html

D'Silva J (1998) Campaigning Against Transgenic Technology. *Animal Biotechnology and Ethics.* Edited by Holland A, and Johnson A. Chapman & Hall, London, pp. 92-102.

Duff, Karen, et al (1996) Increased Ambloid-Beta-1-42 (43) in the Brains of Mice Expressing Mutant Presenilin-1. *Nature* **383**: 710-713.

Games, Dora, Adams D, et al (1995) Alzheimer's Type Neuropathology in Transgenic Mice Overexpressing V717F Beta-Amloid Percursor Protein. *Nature*. **373**: 523-527.

Morrey, John D. and Sherlock, Richard (2002) Ethical Issues in Biotechnology. *A Critical View of the Genetic Engineering of Farm Animals.* Joyce D'Silva (2002)

Muir, William M. and Howard, Richard D. *Transgenic Research: Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms*. Kluwer Academic Publishers. (2002).

Naturalis National Natural History Museum of the Netherlands (2004) http://www.naturalis.nl/asp/page.asp?alias=naturalis.en&view=naturalis.en&id=i0

00256&frameurl=http%3A%2F%2Fwww.naturalis.nl%2Fnaturalis.en%2Fnaturalis.en%2Fi000968.html

Porter, David (1992) *Ethical Scores for Animal Experiments*. *Nature Magazine* **356**: 101-102.

Shannon, Thomas (1997) An Introduction to Bioethics. New Jersey: Paulist Press.

Stobbe, Mike (2006) Annual Cancer Deaths in the U.S. Fall. http://www.highbeam.com/doc/1P1-118315991.html

"Transgenic Animals: Their Benefits to Human Welfare" (2003) American Institute of Biological Sciences. http://www.actionbioscience.org/biotech/margawati.html

UC Davis Cancer Center (2003) Of Mice and Women: The OncoMouse has proved a valuable model of human breast cancer. Synthesis: A publication.of the UC Davis Cancer Center. Sacramento, California: 2003. cancer.center@ucdmc.ucdavis.edu

Webster's New World Medical Dictionary (2009) http://www.medterms.com/script/main/art.asp?articlekey=10727

Chapter-4: Transgenic Animal Legalities

Matthew Connor

Transgenic animals, and other cutting edge technologies, usually come under the scrutiny of the judicial system and the public eye when they attempt to pass through the United States Patent and Trademark Office (WIPO Magazine, 2006). The patent process, with its exclusivity to market, provides much of the motivation and drive behind the advancement of technology. But in the case of transgenesis, living animals are involved, so the debate focuses on whether life can or *should* be patented. The purpose of this chapter is to discuss transgenic legalities, as an example of the impact of technology on society.

Patent Exclusivity

Article I, Section 8, of the US Constitution states: "Congress shall have the power...to promote the progress of science and useful arts, by securing for limited times to authors and inventors the exclusive right to their respective writings and discoveries" (USPTO, 2005). This article in the Constitution shows that our forefathers believed our nation's economic prosperity was closely tied to innovation. Profit incentive constitutes a substantial portion of the drive behind the American and global economies. The Bayh-Doyle Act of 1980 expanded patent exclusivity to also allow academic research to partner with (and profit from) industry.

However, patent exclusivity is also the source of some of the controversy that arises from the production of transgenic animals. Controversy arises when the protection of the rights of industry (and inventions) limit or infringe on the interests and welfare of the public. Section 101 of the Utility Patent Act (UPA), which is the type of patent transgenic animals fall under, states

that "[any person who] invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent, [subject to the conditions and requirements of the law]" (USPTO, 2005; Quick, 2007). The patentability of transgenic animals is a major source of contention, and raises new questions on legal definitions, and classifications of the natural world.

Transgenic Patent Requirements

There are three specific requirements outlined in the Utility Patent Act which must be met in order to patent a transgenic animal. They all represent extrapolations on the "new and useful" clause in the requirement for a utility patent. John Quick gives a good summation of these requirements as:

- First, the transgenic animal in question must "have some *utility* apart from research".
- Second, it must fulfill the *novelty* requirement, or stated differently it must be something that does not already exist, the transgenic animal must differ significantly from how the animal exists in nature. There are also three underwritten conditions which must be satisfied to meet this requirement: general utility, specific utility and beneficial utility.
    - General utility requires that the animal be operable and capable of general use. (An example would be a patent wouldn't be granted on a transgenic animal that succumbed to too high of a mortality rate to satisfactorily express its phenotype.)
    - Specific utility requires that the animal must be able to solve the problem it is designed to correct. (If seeking a patent on the Huntington's mouse, it must exhibit the phenotype of Huntington's disease.)
    - Beneficial utility requires that the animal provides some societal benefit. (A patent wouldn't be granted on a mouse endowed with blue fur for no other reason than to prove it could be done.)
- The third requirement states that the transgenic animal in question must meet a *non-obvious* requirement. In establishing non-obviousness three factors are taken into account.
    - The content and scope of any relevant prior art (other similar existing discoveries or inventions).
    - How much the animal differs from the relevant prior art.
    - And that the person applying for the patent surpassed the level of ordinary skill existing in the are of the invention. A good example is that a patent would not be granted on a transgenic animal if the transgene implanted is well documented in its contribution to a phenotype and the process by which it is implanted is well

> documented and the only new work is the resultant transgenic animal. (Quick, 2007)

Another key point on patenting transgenic animals, is that patenting a specific transgenic animal also involves patenting the genetic material to be implanted. "The PTO also awards patents for isolated and purified gene *compositions* as long as the inventor discloses a specific, substantial, and credible *utility*" (Chen, 2008). This allows the patenting of the *process* by which the transgene is implanted and the resultant animal.

But even with these requirements spelled out, there still exists a lot of ambiguity about the boundaries of these patents. When patenting the process by which the transgene was inserted, is there infringement if the process is to be employed for a significantly different purpose? For the transgene itself, is it really unique if it exists in nature? If only the resultant animal is subject to a patent how broad is the scope? Is it limited to the same species or the same genus? Or how about phenotypic expression? Using Herman the bull as an example, would the incorporation of the same transgene for production of lactoferrin in a goat infringe on a patent on him? What if a different transgene could lead to the production of lactoferrin, or if the same transgene is incorporated differently to create a different phenotype, or what if the same transgene is used in the same species but lactoferrin is not reaped via the mammary gland as it was in Herman's offspring? These questions can only be answered by the rights granted or denied in each patent's claim, or in the outcomes and decisions made in response to appeals on patents granted.

Patenting Microbes: Diamond v Chakrabarty, 1980

Biological researchers sought sanctions under the breadth of a patent act created before transgenic technology or its ramifications were created, contemplated, or understood, and tried to overcome the *Product of Nature Doctrine*, which states that patents cannot be granted on something indistinguishable from nature. The landmark case that finally overcame the *Product of Nature Doctrine* was *Diamond v. Chakrabarty*. In this case, the subject was a microorganism containing a mutation that allowed it to digest oil into smaller compounds digestible by other organisms in the environment. This *Pseudomonas* variant was different than normal strains, and its utility was it could digest oil slick spills without harming the environment. Although the case was for a bacterium, it eventually paved the way for patenting animals.

The US Supreme Court in this case, as it has in many cases since, relied on their interpretation of Section 101 of Utility Patent Act as intentionally vague and broad in its scope, saying it was Congress' way of conceding that technology would eventually arise that was beyond their scope of foresight, so the court usually defaulted to siding with protecting the rights of the person seeking the patent than protecting what the patent was on. Also, the Supreme Court notes that in cases when Congress has had to enact statutes in similar situations involving genetically modified plants, the regulatory statutes from the *Plant Patent Act* (PPA) provide protection for the rights of the researchers, and are less concerned with the thing being patented.

But, regardless of the broad interpretations by the USPTO and Supreme Court, the applications for and patents granted on transgenic animals have been narrow in their scope in an attempt to satisfactorily meet ethical bounds placed on them. "Our current means of protection do not provide sufficient guidance for the future, and Congress' continued silence on the issue of patent protection for transgenic research forces the PTO and the courts to speak on its

behalf...[and this risks] a flood of research and investment potential [shifting] from the United States to other parts of the world..." (Quick, 2007).

Another key point is that protection of intellectual property in America does not ensure those rights world wide. Countries vary widely in their views on animal patentability and in rules for upholding intellectual property rights. With many large companies having international offices and laboratories, a protected transgenic disease model could be brought to another country with less oversight, and a therapy using the model could be synthesized in that country. The resultant pharmaceutical then could be brought back to the US, and marketed without ever paying a royalty to the deserving researchers who invented the disease model. Even limitations on research passed for what we as Americans deem as inhumane experimentation, risk leaving an opening for other less regulatory nations to gain the competitive edge. This has been the source of a lot of apprehension recently in biotechnology for stem cells and transgenesis. Many argue that if we don't protect the rights of our innovations, someone elsewhere will gladly pick up where we left off, stand on our shoulders, take the next step and reap all the monetary benefits. It is a constant balancing act between could we, should we, and, regardless of how we decide, will they?

First Patented Animal: Harvard/DuPont OncoMouse

A good case study that brings many of the points discussed above together, and provides some concreteness to them, is the case of the Harvard OncoMouse, the world's first patented animal. This mouse was developed at Harvard University, by Philip Leder and Timothy Stewart, subsidized by DuPont. The patent was applied for on June 22, 1984, and eventually granted in the US on April 12, 1988. A summary of the invention in the granted patent reads:

> "...a transgenic non-human eukaryotic *animal* (preferably a rodent such as a mouse) whose germ cells and somatic cells contain an activated oncogene sequence introduced into the animal, or an ancestor of the animal, at an embryonic stage....An activated oncogene sequence ...when incorporated into the genome of the animal, increases the probability of the development of neoplasms (particularly malignant tumors) in the animal..."(Leder and Stewart, 1984). The patent also extended to "the *process* and *product* in all similarly altered non-human mammals". (Ching 2003)

Although the patent took a lengthy four years to be granted in the United States, that was to be the quickest of its several trials to come in other countries, taking two decades of oppositions, appeals, and narrowing, to eventually pass through the European Patent Office, but never making it past the Supreme Court of Canada.

Oncomouse in Europe

The EPO did not grant the patent application submitted in 1985 until May 1992, where it was granted under the terms of "non-human mammalian animals". It was granted on the basis that the OncoMouse was not considered to be an animal variety and therefore did not fall under the EPO's Article 53(b), which excludes patents on "animal varieties or essential biological processes for the production of ... animals". Another hurdle of the European Patent Convention that the OncoMouse had to overcome was Article 53(A) which excludes patents on inventions "the publication or exploitation of which would be contrary to *ordre public* and morality". To address this second hurdle the EPO applied a "utilitarian balancing test", where in they measured the public interest in such a patent versus the harm and suffering done to the animal. Using this balancing test, The EPO found the possible benefits to cancer research outweighed the concern for the well being and quality of life for the animals. But this was not the end of the trial of OncoMouse in Europe. In November 2001, in response to seventeen filed oppositions to the

OncoMouse patent, the patent was further confined to include only transgenic *rodents*. The narrowing of the patent by the EPO was carried out in the belief that although they felt the potential benefit to cancer research was more relevant than duress caused to the mouse, the coverage should not necessarily apply to *all* non-human animals. Another round of appeals levied in March 2003 by six of the original seventeen opponents led to a further restriction by the Technical Board of Appeal, down to "transgenic *mice*".

Of note with respect to European patents, in 1992, when Upjohn Pharmaceuticals applied for a patent on a mouse engineered to be a disease model for baldness and wool production, the EPO found that the benefit to society did *not* outweigh the suffering the mouse would be subject to, and on that basis denied the patent application. Thus, the same utilitarian test was employed as in the OncoMouse case, but this time with a very different outcome (OncoMouse, 2004; WIPO Magazine, 2006).

Oncomouse in Canada

Perhaps even more interesting and enlightening than the EPO's utilitarian balancing test approval of OncoMouse was Canada's consideration of the same case, since it remains to this date to be the only industrialized nation to reject the patent. One of the reasons it is so interesting is that the Canadian Patent Act is extremely close in wording to the EPO's Utility Patent Act, in that it defines a patentable invention as "any new and useful art, process, machine, manufacture, or composition of matter, or any new and useful improvement in any art, process, machine, manufacture, or composition of matter." The Canadian rejection of the patent application for the end product, the OncoMouse itself, was based solely on the final assessment on the interpretation of "composition of matter". While in the US, a genetically engineered

animal no longer falls under the auspice of nature and therefore is constituted as a "composition of matter", Canada found composition of matter to reach its limitations at *lower* life forms (microbes but not including mice). It is important to note that the patent was only denied on the end product (the actual mouse), but was granted for the oncogene and the process. In 1993, the Canadian Intellectual Property Office granted two patents on both the oncogene itself and the related process by which the mouse was created.

In 1995, the Commissioner of Patents upheld the animal's denial of patentability, and was supported by the Trial Division of Federal Court in 1998. Interestingly enough, in 2000, the Federal Court of Appeal overturned the Trial Court's findings, stating that it found nothing in the Patent Act that limited the level of the life forms that could be patented, though, as with the granting of the OncoMouse patent in America, they did include a clause that patentability halted at human beings, but at only that. Then in December 2002, the Supreme Court of Canada held that a higher organism, like a mouse, was well beyond the scope of previously upheld patents on genetically engineered plants, modified human genes, and cell lines. The decision in court was 5-4 against, and much of the debate centered around the Justices' narrowing of "composition of matter". Justice Bastarache, speaking for the majority, found that "Higher life forms [including mice and humans] are generally regarded as possessing qualities and characteristics that transcend the particular genetic material of which they are composed". He also went on to state that Canadian Patent Act was simply outdated to deal with the matter at hand, and that Canada would be much better suited to err on the side of caution and allow the Parliament to enact provisions for higher life forms if they felt it to be appropriate, after sufficient debate and the ethical and legal issues were addressed (Ching, 2003).

The path that OncoMouse took through the different levels of the Canadian court system was very different than in America. The United States Patent and Trademark Office granting patentability to higher life forms so early in the life of the technology, based mainly on the outcomes of prior litigation in cases of law pertaining to plant and microbial lower life forms, bypassed a lot of the equivalent judicial and legislative process in the United States.

OncoMouse Backlash

However, the OncoMouse patents may not have been the blessing to biotechnology they initially appeared to be. Initially there was a big push and a lot of talk about the wonders to come of transgenic technology, but very little of that information made it to the general public. "Biopharmaceuticals are now a US$33-billion industry, but transgenic animals have yet to play any part in it, and pharming companies have had a difficult history" (Ledford, 2006). Simply stated, almost inherent in government is the fact that the squeaky wheel gets the grease. Transgenics may be as readily patented here as any other nation in the world, but that ease has led to a severe lack of specially tailored legislation, of knowledgeable, capable, federal bodies to manage oversight, and of products reaching the end user. Had DuPont and their IP lawyers battled the OncoMouse case in open court twenty years ago, we may have had a government and nation much better informed on the matter, an agency dedicated to protecting consumer rights in concern with transgenic products, and statutes protecting the researchers and the investors that make their research possible, leading to marketable products being present in the economy today.

FDA Guidelines on Transgenic Animals

The first steps to help ensure that transgenic animals and their products reach the consumer with federal approval were recently taken by the United States Food and Drug Administration (FDA). In September 2008, the FDA published the draft of a proposed guideline, *Regulation of GE Animals Containing Heritable rDNA Constructs*. "This guidance is intended to help industry understand the statutory and regulatory requirements as they apply to these animals [and their products], including those of the National Environmental Policy Act (NEPA), to inform the public about the process the FDA is using to regulate GE animals, and to gather input from the public and regulatory industry.." (FDA.gov, Q&A, 2009). The draft was open to a 60 day public comment period, and the finalized version was released after some revision. On January 15th, 2009, the FDA released the updated CVM GFI #178, *Guidance for Industry Regulation of Genetically Engineered Animals Containing Heritable recombinant DNA Constructs - Final Guidance* (FDA.gov, Guidance for Industry Regulation, 2009).

The FDA is going to regulate transgenic animals under the authority of the new animal drug provisions area of the 1938 Federal Food, Drug, and Cosmetic Act (FFDCA). The legal basis for the FDA's claim to regulation is that the transgene introduced into the host animal meets the definition of a new animal drug under the FFDCA's classification of a drug as "articles (other than food) intended to affect the structure or any function of the body of man or other animals", regardless of whether or not the animal's intended use is as a food, biopharmaceutical production, or any other application. This definition as a *new drug* brings transgenic animals under the New Animal Drug provisions area of the FFDCA, and will be administered by the Center for Veterinary Medicine (CVM) of the FDA. Though the finalized guidelines are non-binding, in order for a product that fits the FFDCA classification of a new animal drug to be

lawfully introduced into commerce it must possess FDA approval, so these guidelines illustrate how to get it. Though the FDA will hold non-heritable transgenes to similar regulation, these guidelines only address heritable ones, and intend to issue a separate non-heritable gene guideline at some future time. There will also be additional guidance issued to illustrate how New Animal Drug Applications (NADA) will require a division of responsibilities and coordination of other Centers along with CVM (Wadman, 2008; FDA.gov, Q&A, 2009; FDA.gov, Guidance for Industry Regulation, 2009).

Guidelines Introduction and Background Section

CVM #178 begins with an introduction and background. The introduction contains a brief explanation of the technology including where it comes under the scope of the FDA, a definition of the purpose of the document, and explains the current state of affairs in regard to transgenics within the FDA.

Guidelines Second Section

The second section of the guidelines outlines Statutory and Regulatory Authority of when the FDA will ply its trade to transgenic animals. Of note beyond the previously listed justifications, it documents that the basis for which transgenic animals are to be tested is the transformation event, or the instance of transgene insertion. This implies that a New Animal Drug Application (NADA) only need be submitted once within a single recombinant heritage, once the NADA is approved it applies to all descendents (even if crossbred with a non-transgenic animal) as long is there is no further gene introduced. It also states that the NADA should be submitted for the generation as close to those which are to be marketed as possible. Although

approval is not required for all animal descendents, there is a clause stating that separate insertions of the same gene into different animals of the same species in each case requires a separate NADA be submitted due to lack of control of gene incorporation, and the placement's effect on health and phenotype of the host animal.

The Statutory and Regulatory Authority section also outlines FDA's enforcement discretion in regards to INAD (Investigational New Animal Drug) and NADA. It states that although every transgenic animal is subject to FDA discretion, certain cases such as animals intended for strictly laboratory experimentation or insects for plant pest control which fall under the oversight of other government agencies, will not be subject to as much scrutiny unless necessarily deemed worthy.

Guidelines Third Section

Aside from touching upon animals intended for solely investigational, laboratory experimentation, in the Statutory and Regulatory Authority section, there is also an entire section, dedicated to Investigational Use of Genetically Engineered Animals. This section pertains to shipments in interstate commerce of new animal drugs for tests *in vitro* and in laboratory research animals, and for clinical investigation in animals. It outlines the specific statutes that apply to these situations, including shipping and labeling of investigational animals, animal disposition (pertaining to disposal of surplus animals and their byproducts), investigational food use authorization, environmental considerations, and how to gain exclusion for a new investigative animal.

Guidelines Fourth Section

The fourth, longest, and most important section in the guidelines is the section on how to acquire FDA Approval of GE Animals. Included in this section is New Animal Drug requirements, and a recommended process for pre-approval. The New Animal Drug requirements section essentially gives a longer explanation of what is required in the NADA, a table of contents and summary, comments etc. Much of the animal information in this subsection is reiterated in the preapproval subsection. In the animal information section is a product description where the FDA provides a list of recommended information to be included, description, number of rDNA copies, name of GE animal, poidy, zygosity, characterization of insertions site(s), and intended use. There is a completely separate molecular characterization step in the preapproval process aside from that included in the product description. This includes a description of the source(s) of the functional components, the sequence, the purpose, assembly detail, intended function of introduced DNA, and purity of the construct. There is also a step for the characterization of the lineage of the animal, and a step for the phenotypic characterization. The durability of the construct is also to be included, describing the stability of inheritance, and consistency of the phenotypic expression. The second to last step of the pre-approval process, the longest of the steps, outlines assessment of the Feed/Food and Environmental Safety. The last step is the validation and effectiveness of the claim, essentially proving that the animal does what it is intended to do.

Guidelines Fifth Section

The Fifth section outlines Post-Approval Responsibilities for industry. This includes registering the applicants name, place of business, location of facilities engaging in the

production and testing of the animal, and drug listing requirements. It also provides guidelines on requirements of record keeping, annual report submission, and supplemental information, and records and reports related to experience with the approved products.

Guidelines Sixth Section

The final section of the guideline references its import tolerances on transgenic animals and differs to the *Codex Alimentarius Guideline* for assessing food safety from rDNA Animals. Also, within its guidelines the FDA expresses its intent to increase the transparency of its deliberations, over which some concern has been expressed, by holding public advisory meetings prior to approving any animal, and posting a summary of the NADA file.

An interesting final point about these new guidelines is that they are based upon what could be viewed as the outdated 1938 FFDCA, similar to the 1908 act that Canada felt was too outdated to coherently meet the burden of regulating transgenic animals. In the Q&A section, referenced previously in this paper, the FDA states that it felt the FFDCA sufficiently contained provisions to regulate transgenic animals. The animals have actually been regulated under this act since they first came across the FDA in the 1980's, and this new guideline is only now being issued in response to the WHO June 2008 release of guidelines for assessing food safety. The FDA now felt the need to issue guidelines so developers around the world "fully understand the rigorous regulatory requirements that these animals and the products from them will have to meet in the United States" (FDA.gov, Q&A, 2009). Hopefully it's not too little, too late, to nurture this incredible technology into the amazing and beneficial giant it promised to be.

Chapter-4-Bibliography

Chen, Jim (2008) *SYMPOSIUM: Biolaw: Law at the Frontiers of Biology: ARTICLE: Biolaw: Cracking the Code.* The University of Kansas Law Review. http://www.lexisnexis.com/us/lnacademic/results/docview/docview.do?docLinkInd=true&risb=21_T7189445911&format=GNBFI&sort=RELEVANCE&startDocNo=1&resultsUrlKey=29_T7189445914&cisb=22_T7189445913&treeMax=true&treeWidth=0&csi=12159&docNo=8

Ching, Lim (2003) "Canada's Supreme Court Rules Out Patents on Higher Life Forms". ISIS: Institute of Science in Society. http://www.i-sis.org.uk/ http://www.mindfully.org/GE/2003/Canada-Patents-Life30jan03.htm

Diamond vs Chakrabarty (1980) 447 US 303-322, 1980. http://digital-law-online.info/cases/206PQ193.htm

FDA.gov (2009) General Q&A: Genetically Engineered Animals. http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/ucm113605.htm

FDA.gov (2009) *Guidance for Industry Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs-Final Gudance.* http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

Ladas.com (2003) A Brief History of the Patent Law of the United States. Ladas & Parry Intellectual Property Law. http://www.ladas.com/Patents/USPatentHistory.html

Leder, P and Stewart, T (1984) "Transgenic Non-human Mammals, The Harvard Oncomouse. US Patent and Trademark Office. Patent #4,736,866. Cambridge, MA.

Ledford, Heidi (2006) The Farmyard Drug Store. *Nature* **443**: 16-17.

OncoMouse Patent Restricted in Europe Again (2004) *Nature Reviews Drug Discovery*, 3, 728. http://www.nature.com/nrd/journal/v3/n9/full/nrd1509.html

Quick, John J (2007) Controlling Pandora's Box: The Need for Patent Protection in Transgenic Research. *University of Miami Business Law Review*, Spring / Summer, 2007, 15 U. Miami Bus. L. Rev. 303.

United States Patent and Trademark Office (2005) General Information Concerning Patents. http://www.uspto.gov/web/offices/pac/doc/general/index.html#whatpat

Wadman (2008) FDA to Regulate Genetically Engineered Animals (2008) *Nature* http://www.nature.com/news/2008/080919/full/news.2008.1120.html

WIPO Magazine (2006) Bioethics and Patent Law: The Case of the Oncomouse. http://www.wipo.int/wipo_magazine/en/2006/03/article_0006.html

PROJECT CONCLUSIONS

Based on the research performed for this project, the authors conclude that transgenic technology is extremely beneficial to society, from increasing our biological knowledge, increasing food production and efficiency, developing new disease therapies, developing new sources of transplantable organs and tissue, and gaining a better understanding of ourselves and the world around us, are all derived from this magnificent technology. The authors find that, despite the possibility of some of the animals being subjected to pain and suffering, and some succumbing to a higher rate of premature mortality, when a utilitarian test is applied, in most cases the benefit to society is too profound and unparalleled to denounce the technology.

Some cases, such as the Beltsville pig and the environmental risks of escaped transgenic salmon, definitely warrant apprehension and perhaps even outright halting by regulatory bodies. But for the most part, cases such as Huntington's mouse, OncoMouse, Alzheimer's mouse and pigs engineered for xenotransplantation, have the ability to save so many lives that would otherwise be lost, that the technology must push the envelope to do everything within safe and well defined boundaries to in the end benefit society and human life.

The FDA has recently taken a step in the right direction by producing guidelines specific for transgenic animals which help clarify and standardize the path for gaining FDA approval to scientists and companies. Still more needs to be done to create specific legislation tightly governing the technology, while nurturing its progress. Debating and eventually coming to Congressional agreement on the technology would lead to a unified approach that will foster its growth in the United States and ensure it is plied in the most humane way possible.