



# Genetic Modification in Livestock

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December 2003

# Topics

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- **Breeding Methods to Achieve Genetic Modification**
- **Molecular Biology Methods to Achieve Genetic Modification**
- **Healthcare Applications**
- **Agricultural Applications**

## Breeding

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- Livestock have been genetically modified for centuries using breeding schemes.
- Examples of current **Breeding** goals in livestock:
  - Selection for genetic mutations in sheep that confer resistance to Scrapie (Transmissible Spongiform Encephalopathy (TSE))
  - Selection for mutations in cattle that produce polled, or hornless, cattle
  - Selection for mutations that confer “Red” versus “Black” coat color in cattle

# Molecular Approaches

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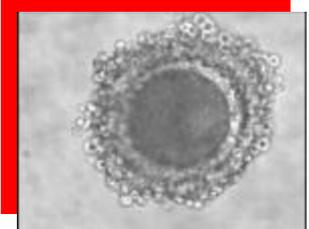
## Methods Used Prior to Nuclear Transfer:

- **Introduction of new DNA randomly into the genome via:**
  - **Injection of new DNA into oocytes and embryos**
  - **Infection of oocytes and embryos using viral vectors**
  - **Transfection of mouse embryonic stem cells**
  - **Sperm-mediated gene transfer**
- **Introduction of changes in DNA at specific locations via:**
  - **Homologous recombination in mouse ES cells**
  - **Only available in mice until the advent of nuclear transfer technologies**

# Genetic Modifications Introduced Using Nuclear Transfer Methods

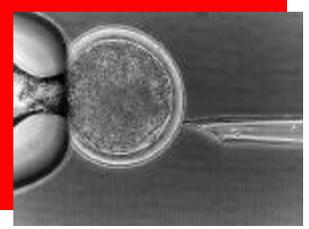


Aspirate Oocytes (Eggs) From Ovaries



Immature Oocyte

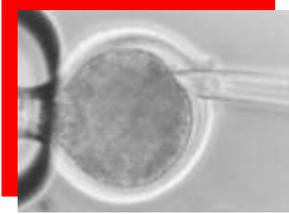
Mature 44 hrs.



Mature oocyte ready to remove nucleus



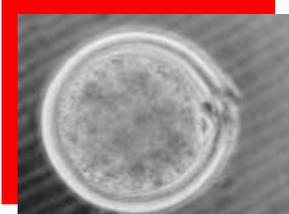
Remove Nucleus



Insert Single Donor Cell



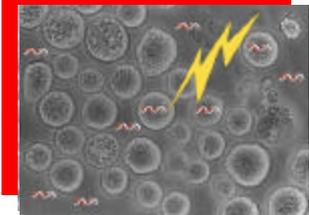
Electrofusion



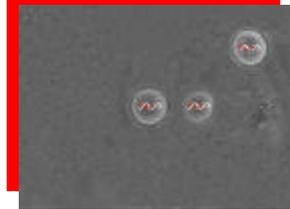
Activate



Original Animal



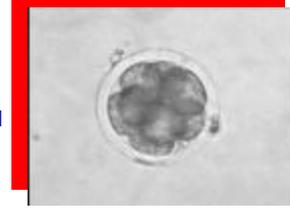
Introduce Minor Changes in DNA of Cultured Cells



Select Cells For Nuclear Transfer



Day 7 Embryos



Cell Division



Embryo Transfer



Animal with Genetic Change

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# Healthcare Applications

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# Healthcare Applications: Infigen, Inc.

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## ◆ ***Therapeutic Proteins Produced in Cloned Cow's Milk***

- Ability to Produce Complex Proteins in Large Quantity
- High Demand for Protein Production Capacity

## ◆ ***Xenotransplantation Using Pig Organs, Tissues & Cells***

- High Demand Due to Shortage of Human Donors

## ◆ ***Animals Models of Human Disease***

- Supplement to Mouse Models which Often Give Poor Results
- Effective Models to Screen Drugs before Expensive Clinical Trials

# Nuclear Transfer Results at Infigen

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## **211 Cattle**

- 70 Transgenic Cows Shipped

## **155 Pigs from 48 litters**

- 33 Gene-Targeted Miniature Swine Shipped

## **3 Sheep**

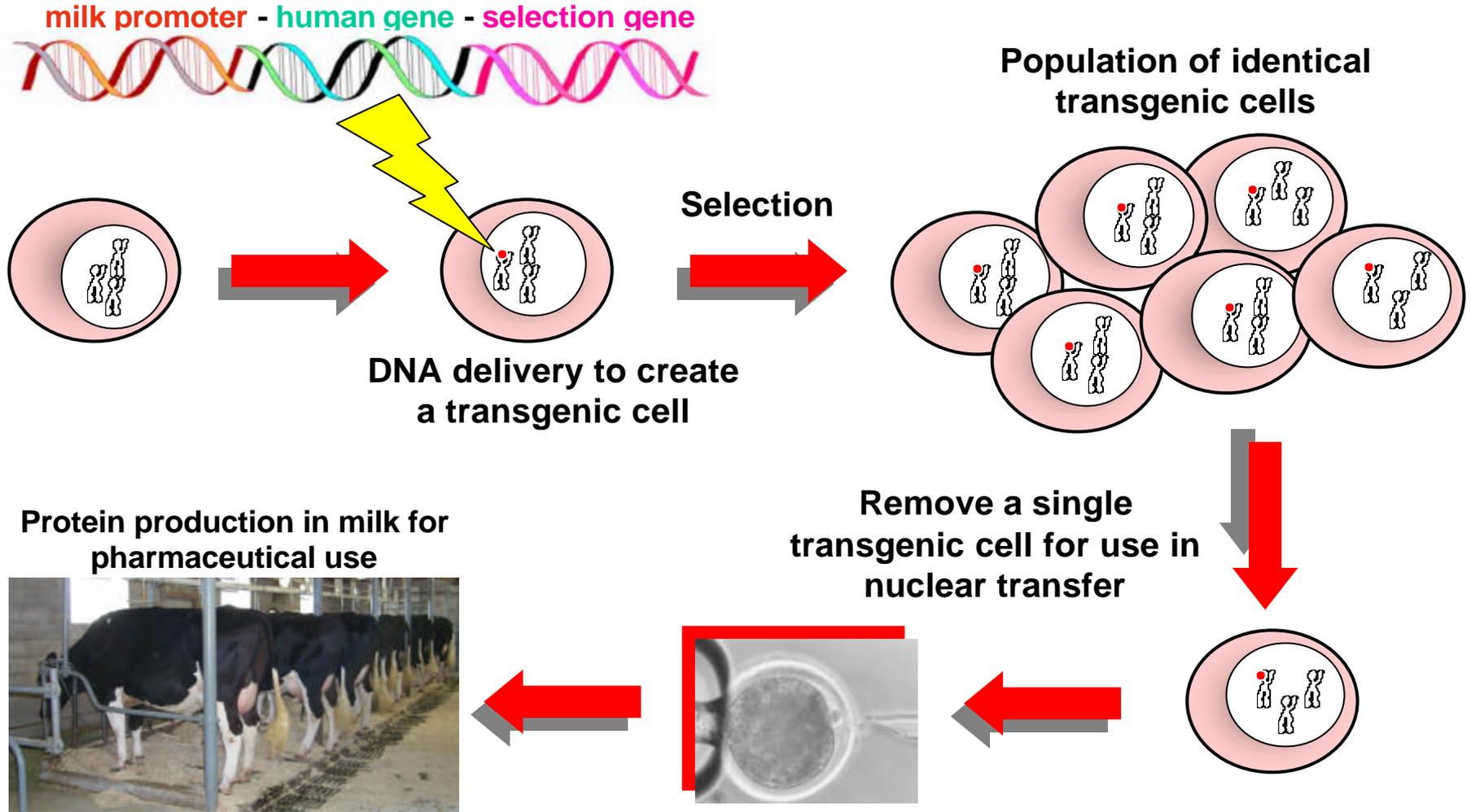
- 2 Lambs Shipped

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# **Therapeutic Proteins in Cow's Milk**

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# Therapeutic Proteins - Transgenesis Process:



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# **Xenotransplantation**

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# The Lack of Whole Organs is Provoking A Crisis Among Chronic Disease Sufferers

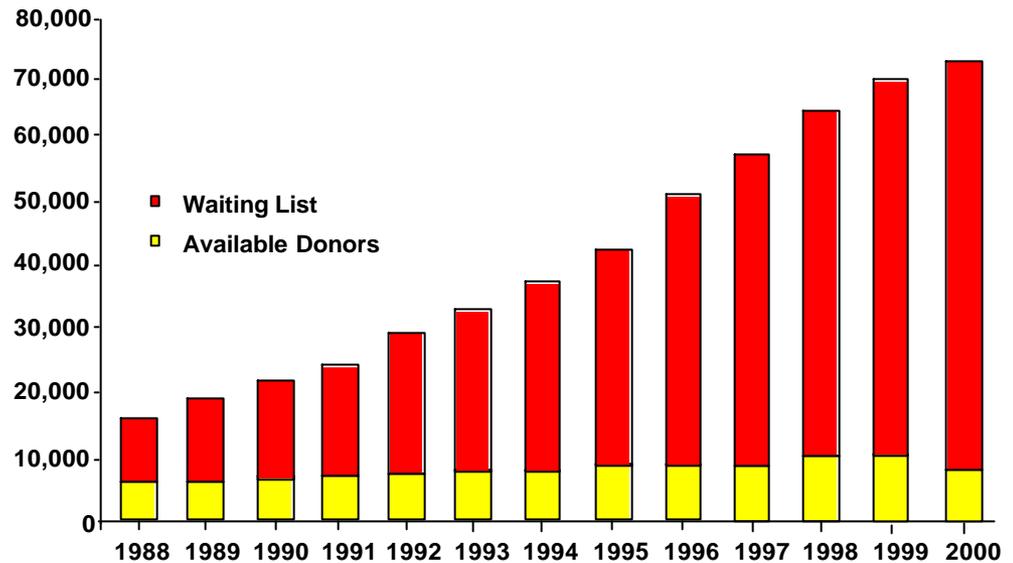
## A \$5-\$10 Billion Market Potential

### Whole Organ Transplant Needs

Type of Transplant	Patients Waiting for Transplants
Kidney transplant	54,944
Liver transplant	17,843
Pancreas transplant	1,328
Pancreas islet cell transplant	301
Kidney-pancreas transplant	2,651
Intestine transplant	183
Heart transplant	4,182
Heart-lung transplant	208
Lung transplant	3,849
<b>Total</b>	<b>85,489</b>

Source: United Network for Organ Sharing, May 2000

### Major Organ Needs



Source: United Network for Organ Sharing, Scientific Registry, March 2000

**Fifteen People Die Every Day While Waiting for a Whole Organ**

# Barriers to Xenotransplantation

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## 1) Hyperacute Rejection

- Human antibodies to galactose- $\alpha$ -1,3-galactose carbohydrates on pig glycoproteins and glycolipids



Blood  
Group A

Blood  
Group B

Gala1-3Gal

- Solution: Knockout the  $\alpha$ -1,3-galactosyl-transferase gene

## 2) Delayed Xenograft Rejection (acute vascular rejection)

## 3) Cell mediated rejection

# GGTA1-Null Miniature Swine for Xenotransplantation



1/22/03 photo



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# **Animal Models of Disease**

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## Animal Models of Disease

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- Genetic modifications, particularly gene targeting, have been limited primarily to mice
- Hundreds of genetically modified mice models exist
- Nuclear transfer allows genetic modification in any species that can be cloned
- Non-rodent disease models may be particularly relevant for research and drug testing for:
  - Cardiovascular Diseases
  - Neurological Diseases
  - Cancer
  - Cystic Fibrosis

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## **Other Health Care Applications**

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# Production of Human Antibodies in Cattle Blood

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## Hematech and Kirin

Human polyclonal antibodies (hPABs) are useful therapeutics, but because they are available only from human donors, their supply and application is limited. To address this need, we prepared a human artificial chromosome (HAC) vector containing the entire un-rearranged sequences of the human immunoglobulin (hlg) heavy-chain (H) and lambda (lambda) light-chain loci. The HAC vector was introduced into bovine primary fetal fibroblasts using a microcell-mediated chromosome transfer (MMCT) approach. Primary selection was carried out, and the cells were used to produce cloned bovine fetuses. Secondary selection was done on the regenerated fetal cell lines, which were then used to produce four healthy transchromosomal (Tc) calves. Human immunoglobulin proteins were detected in the blood of newborn calves. The production of Tc calves is an important step in the development of a system for producing therapeutic hPABs.

***Nat Biotechnol. 2002 Sep;20(9):889-94.***

Cloned transchromosomal calves producing human immunoglobulin.

Kuroiwa Y, Kasinathan P, Choi YJ, Naeem R, Tomizuka K, Sullivan EJ, Knott JG, Duteau A, Goldsby RA, Osborne BA, Ishida I, Robl JM.

Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., Japan.

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# Prevention of BSE/TSE by Knockout of the Prion Gene

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**NIH Grant Abstract:** Bovine spongiform encephalopathy is an emerging prion disease of cattle. Mounting evidence indicates that **BSE is transmissible to humans** in the form of a new, deadly variant of Creutzfeldt-Jakob disease (vCJD). Consumption of BSE-tainted beef has been implicated as the most likely mode of transmission. BSE thus represents a threat to human health via the food supply and other bovine-derived products. As no vaccine, diagnostic test or therapy exists for either vCJD or BSE, protection depends on preventative measures. The pathogenesis of prion disease requires expression of host-encoded prion protein. In mice, prion gene knockout confers resistance to prion disease. Knockout of the prion gene in cattle should similarly render the bovine resistant to BSE. The long-term goal of this work is to test the hypothesis that **cattle bearing bi-allelic prion knockouts are resistant to BSE**. Offspring with mono-allelic prion knockouts will be bred in future work to generate cattle with bi-allelic prion knockouts.

**NIH Grant Number:** 1R21NS045908-01

**PI Name:** EYESTONE, WILLARD H.

**Project Title:** Generation of Prion Knockout Cattle



# Production of Spider Silk in Goat's Milk

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**Nexia Biotechnologies** lead product, BioSteel® (BioSteel®-M, BioSteel®-I), is recombinant **spider silk extracted from the milk of transgenic goats** which is then spun into fibres. Spider silk has long been admired by material scientists for its unique combination of high-performance mechanical properties including toughness, strength, lightness, and flexibility. Unfortunately, no one has been able to produce this wonder material in commercial quantities. Nexia is the only company which has successfully produced fibres from recombinant spider silk and is currently in the process of developing commercial quantities of BioSteel® using its proprietary transgenic goat technology. Medical device applications of BioSteel®-M include wound closure systems like **microsutures, surgical meshes, and artificial ligaments** such as anterior cruciate ligaments in the knee (ACL). Industrial BioSteel®-I applications may include consumer products such as biodegradable **fishing line, tennis strings and military applications** (soft body armour).

# Genetically Modified Animals in Agriculture

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## Examples of Applications:

- **Environmentally Friendly Pigs “Enviropig™”**
- **E. Coli Resistant Pigs**
- **Mastitis Resistant Cows**
- **Improvements in Milk Composition: Caseins**
- **Improvements in Milk Composition:  $\alpha$ -Lactalbumin**
- **Hornless (Polled) Livestock**
- **Improvements in Wool**
- **Animals That Produce Single-Sex Offspring**

## Environmentally Friendly Pigs “Enviropig™”

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- Researchers in Cecil Forsberg’s lab at the University of Guelph have developed a new breed of Yorkshire pigs trademarked Enviropig™ that use plant phosphorus more efficiency.
- These transgenic pigs carry a parotid secretory protein promoter linked to the E. coli appA phytase gene that produces phytase, a bacterial enzyme, in the saliva. Phytate phosphorus is indigestible to the pig and composes 56 to 81% of the total phosphorus in common swine feeds. The phytase excreted in the saliva of the transgenic pigs releases phytate phosphorus from animal feed, reducing the need for dietary phosphorus supplementation and reducing phosphorus excretion in the manure.

## E. coli Resistant Pigs

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Serguei Golovan at the University of Guelph is interested in resistance to deadly E. coli infections in piglets. By introducing the genetic changes that contribute to piglets' resistance, he hopes to one day **eliminate E. coli-related illnesses from pork herds** using genetic technology.

# Mastitis Resistant Cows

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Transgenic mice that secrete a potent anti-staphylococcal protein into milk, lysostaphin, are **resistant to mammary infections**. These results clearly demonstrate the potential of genetic engineering to combat the most prevalent disease of **dairy cattle**.

***Nat Biotechnol. 2001 Jan;19(1):66-70.***

David E. Kerr, Karen Plaut, A. John Bramley, Christine M. Williamson, Alistair J. Lax, Karen Moore, Kevin D. Wells & Robert J. Wall

Department of Animal Sciences, University of Vermont, Burlington, VT

## Improvements in Milk Composition: Caseins

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To enhance milk composition and milk processing efficiency by increasing the casein concentration in milk, we have introduced additional copies of the genes encoding bovine beta- and kappa-casein (CSN2 and CSN3, respectively) into female bovine fibroblasts. Nuclear transfer with four independent donor cell lines resulted in the production of 11 **transgenic calves**. The analysis of hormonally induced milk showed substantial expression and secretion of the **transgene-derived caseins into milk**. These results show that it is feasible to substantially alter a major component of milk in high producing **dairy cows** by a transgenic approach and thus to **improve the functional properties of dairy milk**.

***Nat Biotechnol. 2003 Feb;21(2):157-62.***

Brophy B, Smolenski G, Wheeler T, Wells D, L'Huillier P, Laible G.  
AgResearch, Ruakura Research Centre, Hamilton, New Zealand.

## Improvements in Milk Composition: $\alpha$ -Lactalbumin

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The goal of this study was to determine whether the presence of the bovine alpha-lactalbumin transgene in first-lactation **gilts** enhances lactational performance and litter growth. Expression of the transgene was associated with **increased milk production in lactating gilts and increased growth of transgenic-reared piglets**. Increased lactose synthesis in response to the presence of the transgene may result in increased milk production in early lactation, leading to increased milk component intake by transgenic litters, and ultimately to increased growth of litters reared by first-parity transgenic **gilts**.

***J Anim Sci. 2002 Apr;80(4):1090-6.*** Lactational performance of first-parity transgenic gilts expressing bovine alpha-lactalbumin in their milk.

Noble MS, Rodriguez-Zas S, Cook JB, Bleck GT, Hurley WL, Wheeler MB.

Department of Animal Sciences, University of Illinois, Urbana

# Hornless (Polled) Livestock

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## Cattle

At present the gene for polled has not been specifically identified. However the position of the gene is known to be on the top of cattle chromosome 1. Based on this information, several DNA markers near the gene, called "linked" markers, can be used to test for homozygosity of polled in an individual **if suitable family members are also available.**

If a genetic variant is found that accounts for polled cattle, **this genetic trait can be introduced into a genetic line using nuclear transfer.**

## Improvements in Wool

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To grow wool, sheep need large amounts of cysteine, a sulfur-rich compound that is an essential building block for keratin, which they obtain from gut-dwelling bacteria that help them digest their food.

CSIRO scientists reasoned that if they could endow sheep with the ability to synthesise their own cysteine, they should be able to increase wool growth. They have tested the concept by transferring two **cysteine-synthesis genes from a microbe, *Escherichia coli* (*E. coli*)**, found in sheep gut, into laboratory mice. The transgenic mice are healthy, and **grow more fur**.

Two different approaches have been taken to increase fleece weight in sheep. The expression of insulin-like growth factor 1 (IGF-1) in the wool follicle has resulted in a 6.5% increase in fleece weights at yearling shearing in transgenic sheep. The introduction of the cysteine biosynthesis pathway into mammalian organisms has shown promising results in the mouse model but is yet to be successfully applied to sheep. The prospects for future manipulation of wool growth and characteristics are discussed.

L. N. Jones, G. E. Rogers, S. M. Bawden, M. G. Huson and P. S. Turner. CSIRO, Textile & Fibre Technology, Belmont, Vic, Australia and SARDI, Adelaide, SA, Australia

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# Animals That Produce Single-Sex Offspring

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## Infigen, Inc.

“Use a spermatogenesis-specific and haploid-specific promoter to direct the expression of a toxic gene whose product would express and remain in Y (male) or X (female) sperm and disrupt the normal development or function of the Y or X sperm.”

### Example: Approach and Implications for the Dairy Industry

1. Transfect a cell line from a desired bull to produce gene targeted cells carrying the transgene in the bovine Y chromosome only.
2. Use the targeted cells in nuclear transfer to make transgenic cloned males producing normal female sperm.
3. **Only females will be produced with this semen and they will not be transgenic.**
4. Unwanted bulls will not be produced, therefore increasing reproductive efficiency and reducing production costs for dairy farmers.

# Genetically Modified Animals in Agriculture

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## Issues:

**Public Acceptance**

**Regulations**

**Safety**

**Containment**

**Do the Benefits Confer to Consumers, Producers  
or the Environment?**