Cloning and Genetic Engineering of Animals
How, What and Why?

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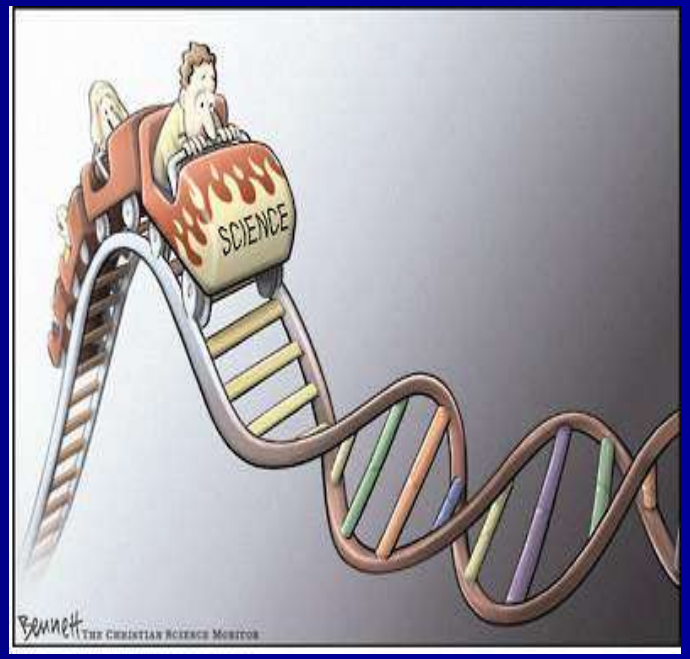
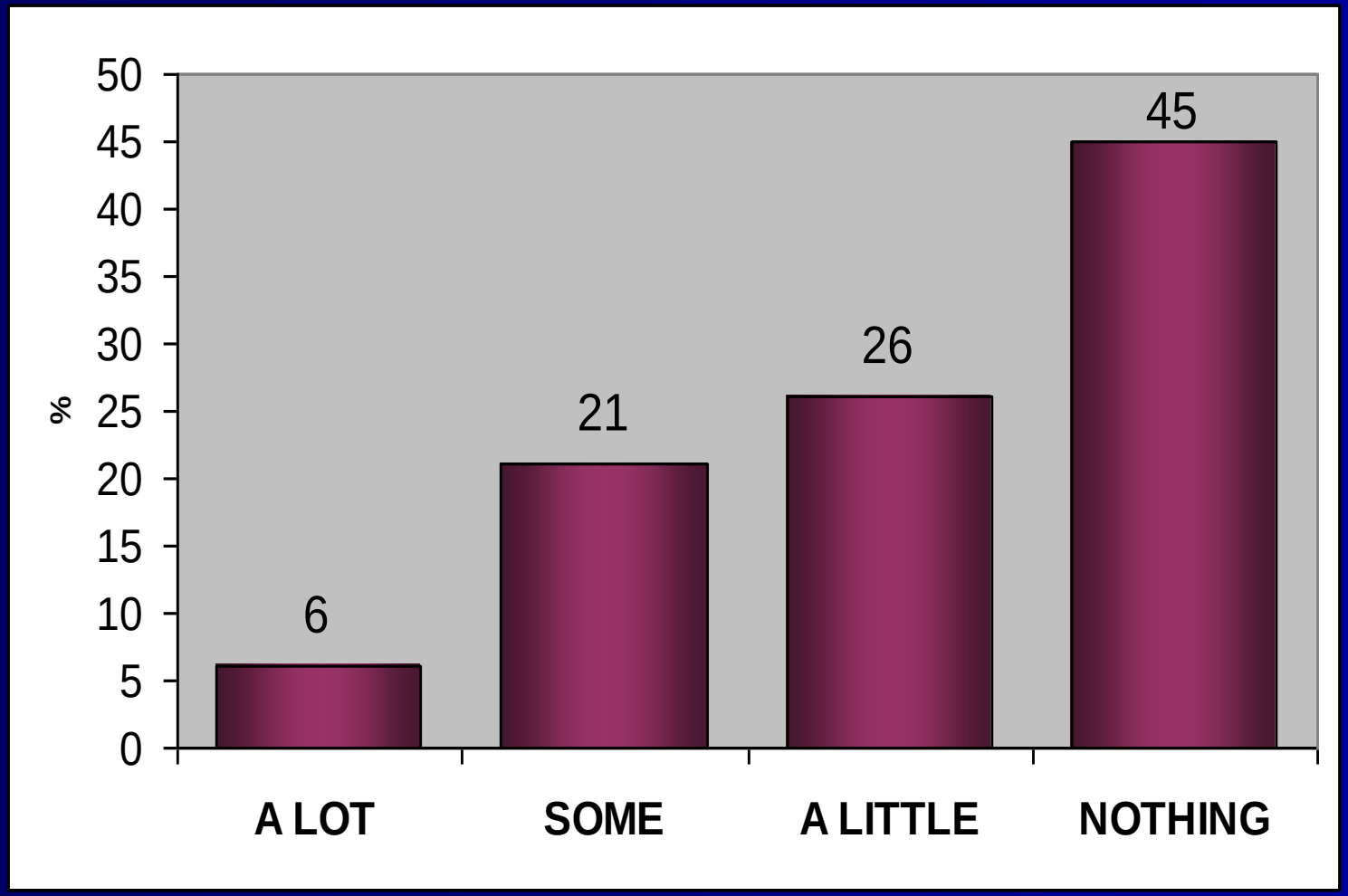


http://animalscience.ucdavis.edu/animalbiotech/

Animal Genetics 107 (1986)

US Public Attitude Surveys

How much have you heard about animal biotechnology ? (IFIC, 2005)



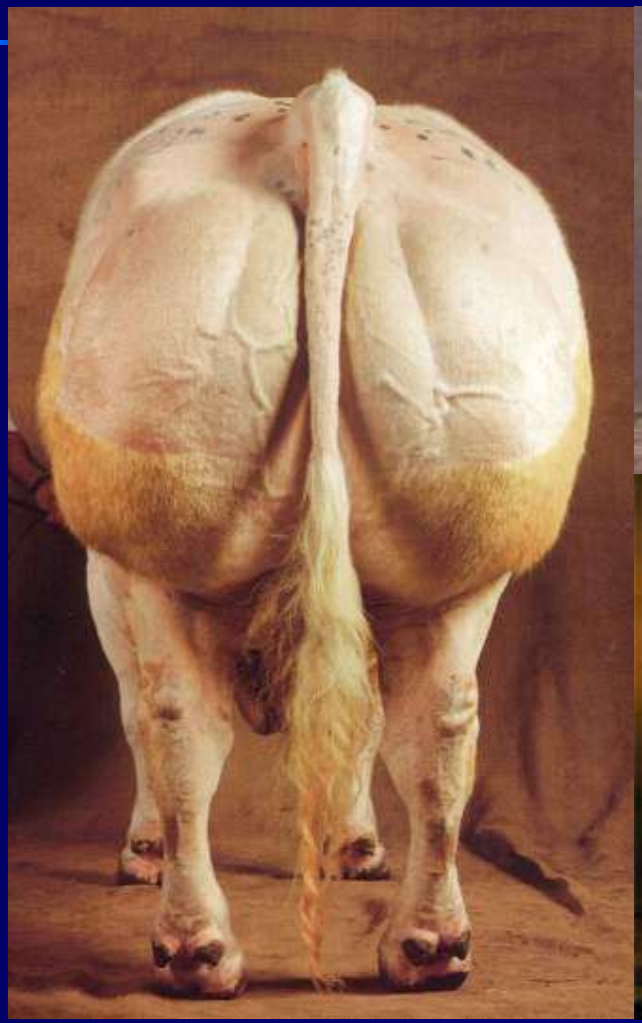
http://ific.org/research/upload/2005BiotechSurvey.pdf

"I know it when I see it"

Of the people who say they know nothing about biotechnology, genetic engineering or genetic modification; almost half (46%) disapprove of the use of genetic modification to create plant-based foods, and 66% disapprove of animal-based genetic modification.

Hallman, W. K., Hebden, W. C., Aquino, H.L., Cuite, C.L. and Lang, J.T. 2003. *Public Perceptions of Genetically Modified Foods: A National Study of American Knowledge and Opinion.* Rutgers - The State University of New Jersey.

I am not going to be talking about genetic modifications made by traditional animal breeders today……



But rather genetic modifications made by the process of cloning and genetic engineering…….

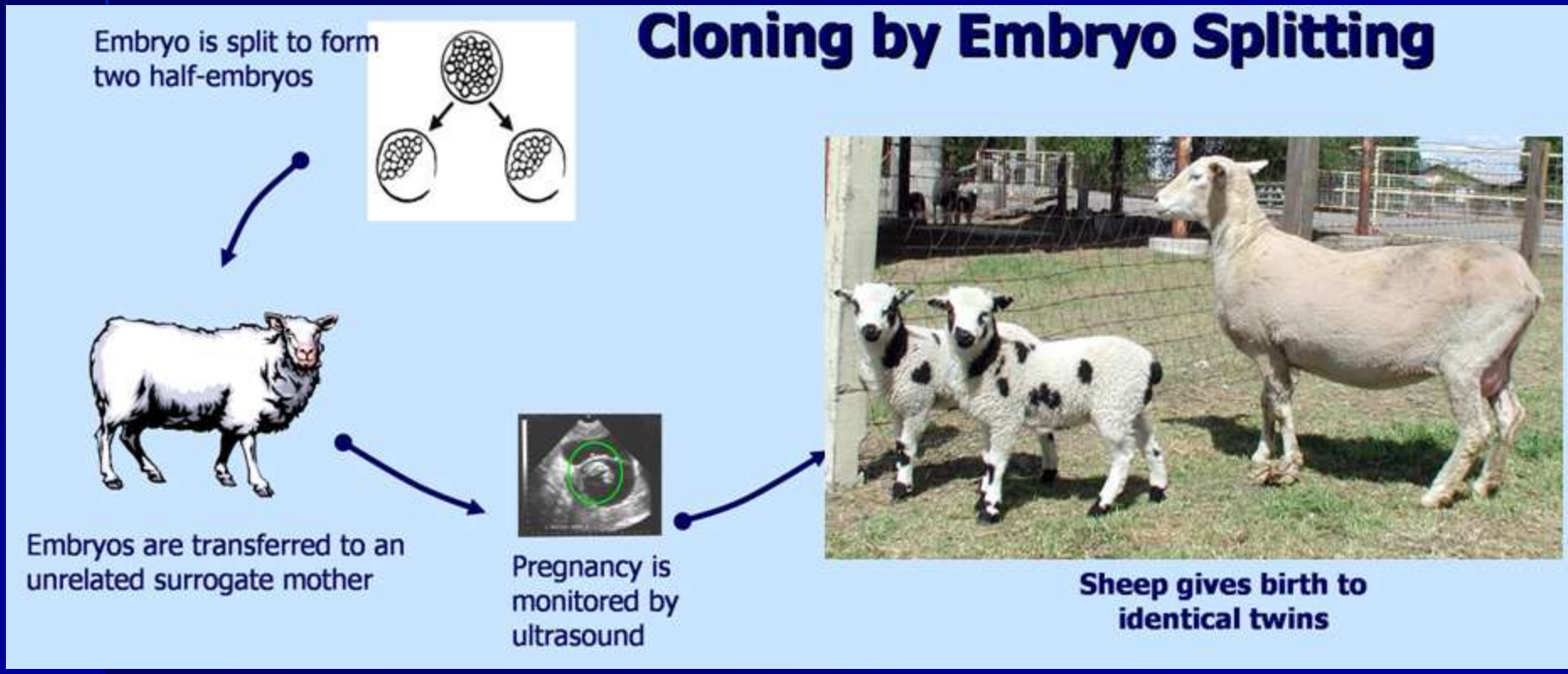
Even though the popular media likes to suggest genetically engineered animals look more like this!

Animal biotechnology

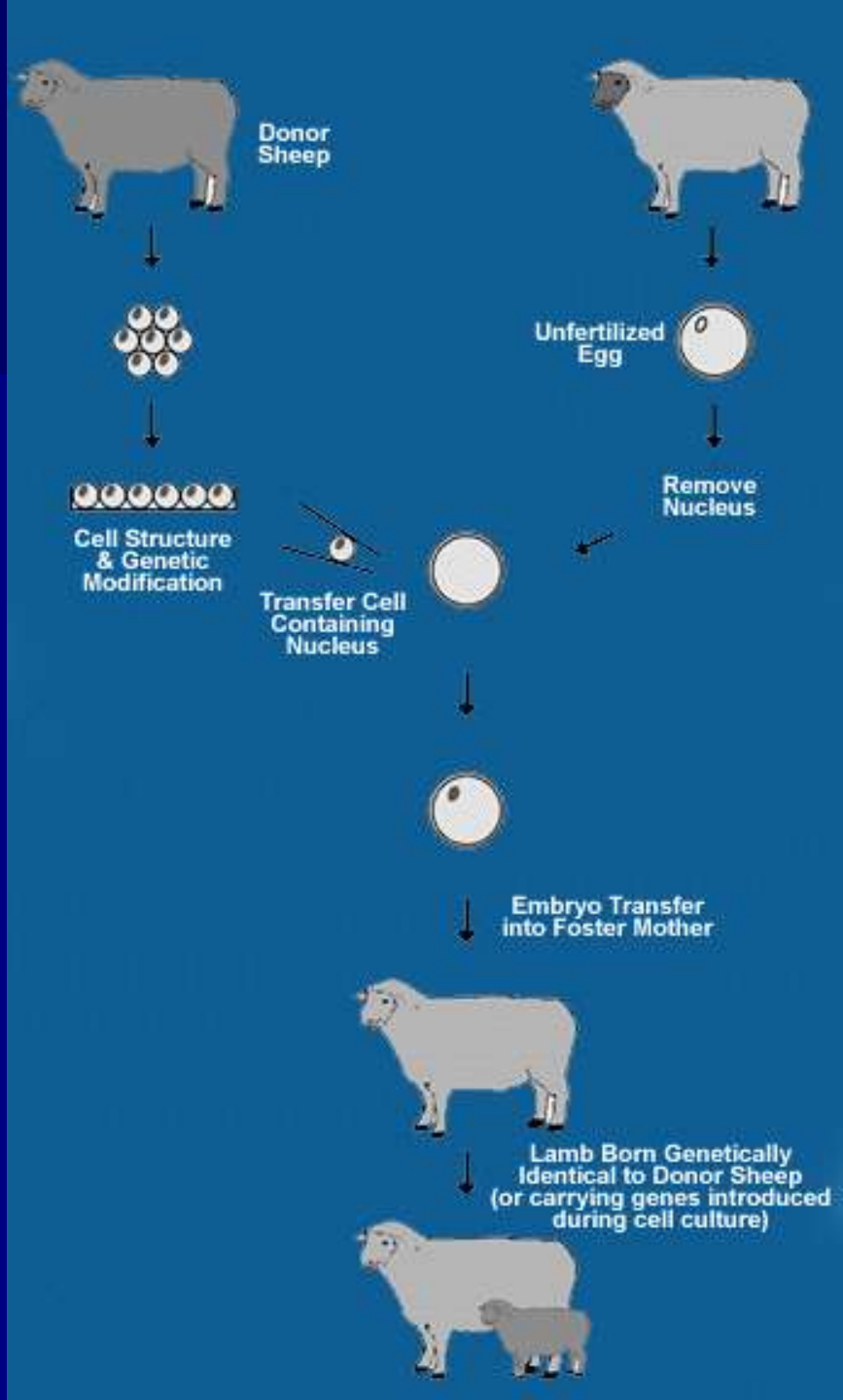
- Artificial selection (breeding programs)
- Artificial Insemination
- Hormone use
- Using DNA information for the marker-assisted selection of superior animals
- Genomics
- Cloning
- Genetic engineering

Cloning by embryo splitting

Cloning by embryo splitting



Dolly (1996), the first adult SCNT clone



Dolly rapidly became entangled with the debate over human cloning

Ensuing discussion failed to elaborate on the reasons as to why cloning was developed

Dolly the cloned sheep kills a lamb — and EATS it!

By MIKE FOSTER / *Weekly World News*

EDINBURGH, Scotland — A frightened scientist says Dolly the cloned sheep has killed a young lamb — and eaten it!

What's more, the world's first cloned mammal has exhibited other strange behavior, such as chasing a young child, biting a keeper and staring menacingly at razzled scientists.

"When you do something to nger her, she looks at you ith these

eyes full of hate," said a researcher involved in the cloning project.

Dolly's eerie antics — including the "cannibalism" episode

two months ago. "A keeper was giving her a bath, which she doesn't seem to enjoy very much," recalled the researcher. "When his back was turned, she bowled him over, then nipped his face, drawing blood.

"Another time I brought my 8-year-old daughter to see Dolly in her pen. She was thrilled and was looking forward to

Many animal species have been since been cloned from adult cells

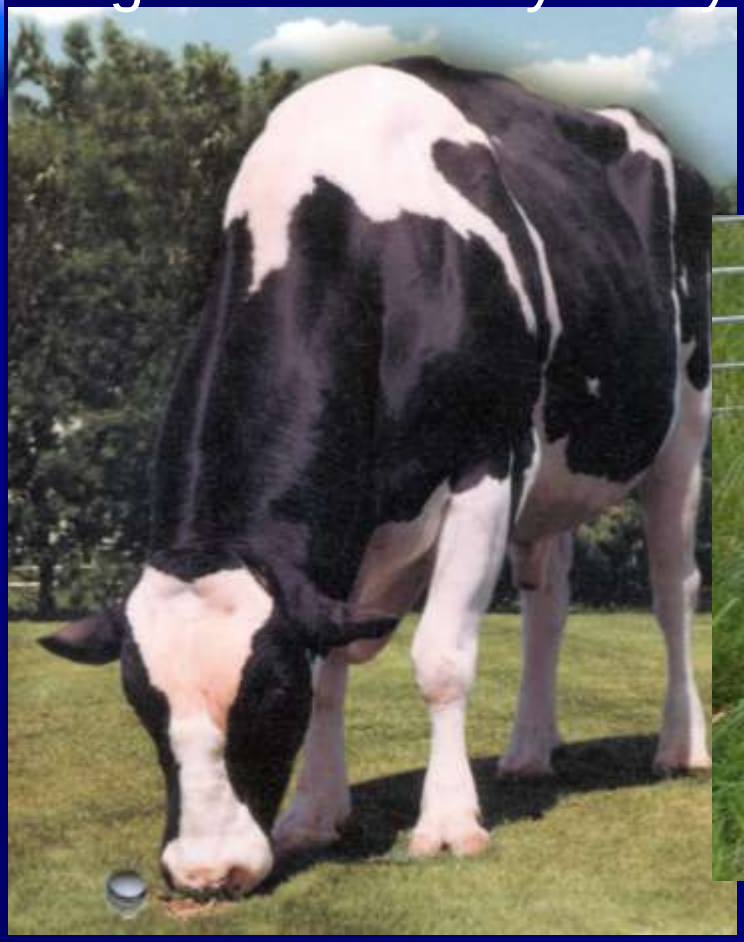
Where does cloning come into the breeders equation?

$$\Delta G = \frac{\textit{intensity of selection } X \textit{ accuracy of selection } X \sqrt{\textit{genetic variance in population}}}{\textit{generation interval}}$$

Who's Buying?

$20,000

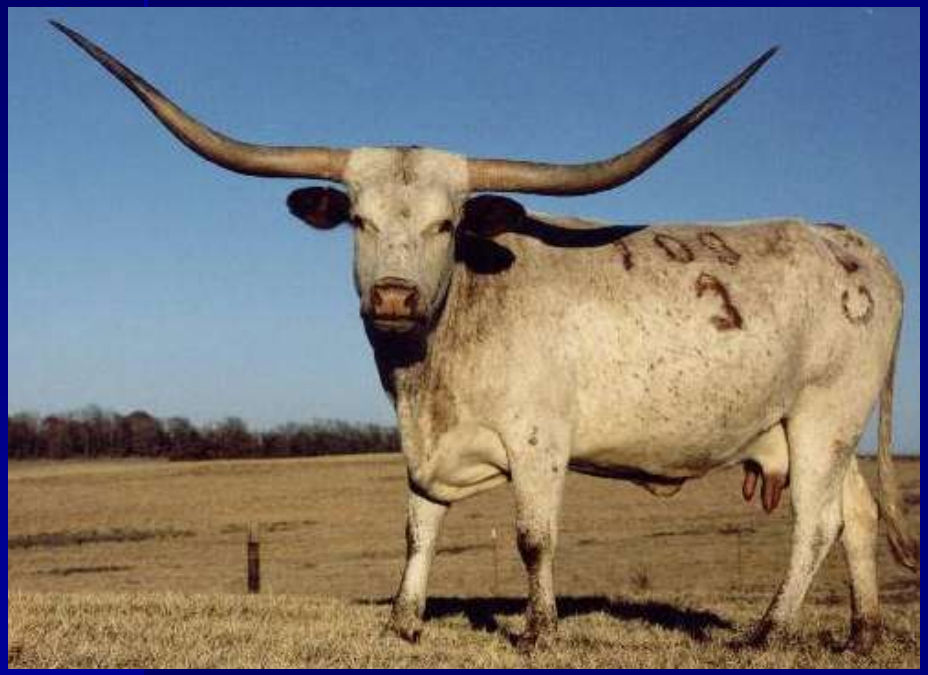
Regancrest Emory Derry died unexpectedly.



Who's Buying?

Specialty Cattle Producers

Starlight: record 77 inches 'tip to tip'

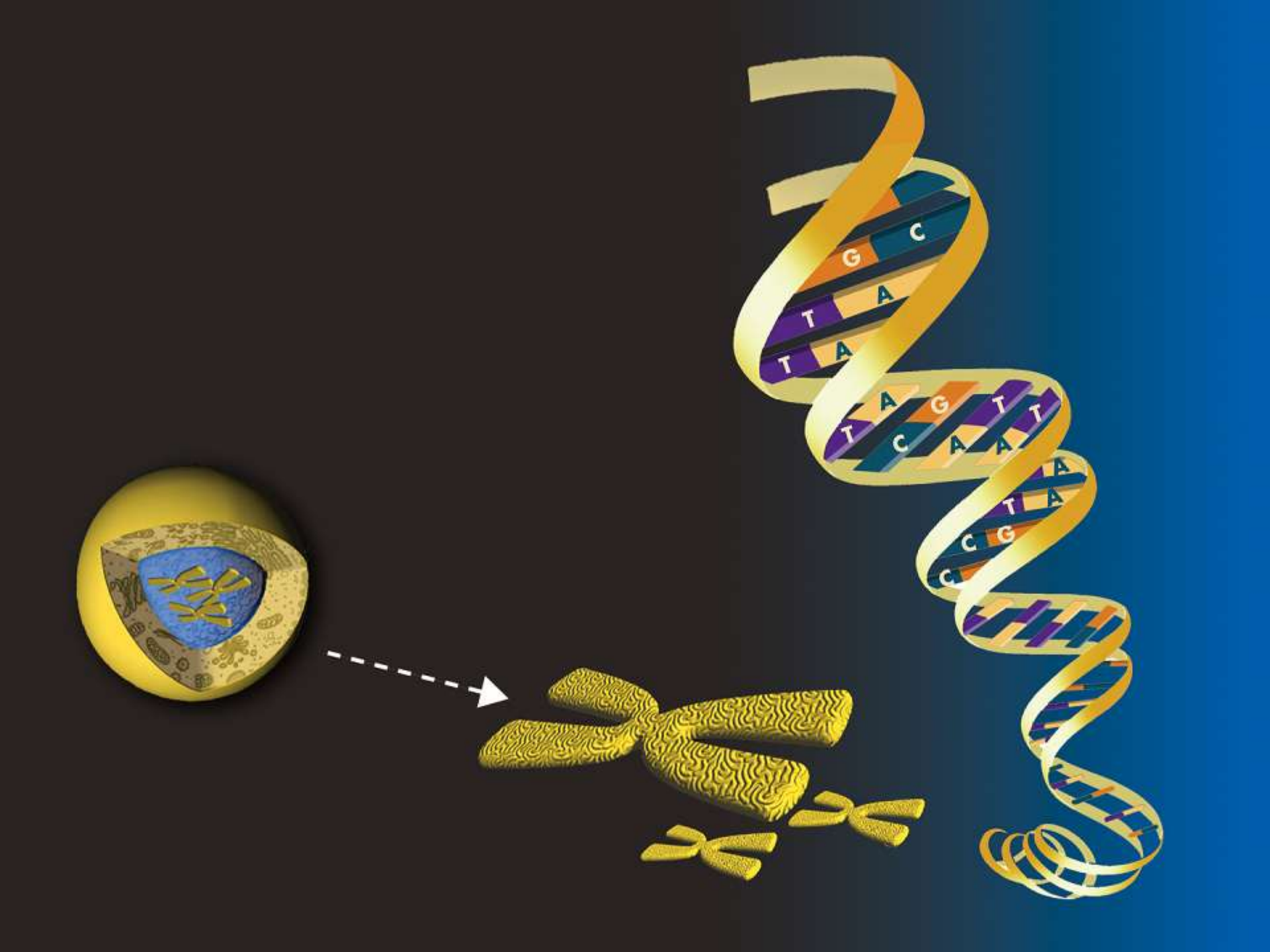


Are the milk and meat from SCNT clones safe for human consumption?

http://www.cyagra.com **http://www.viagen.com**

- In 2008 the FDA determined clones are as safe to eat as food from conventionally bred animals
- The USDA currently has a voluntary moratorium on marketing products from adult SCNT clones.

GENETIC ENGINEERNG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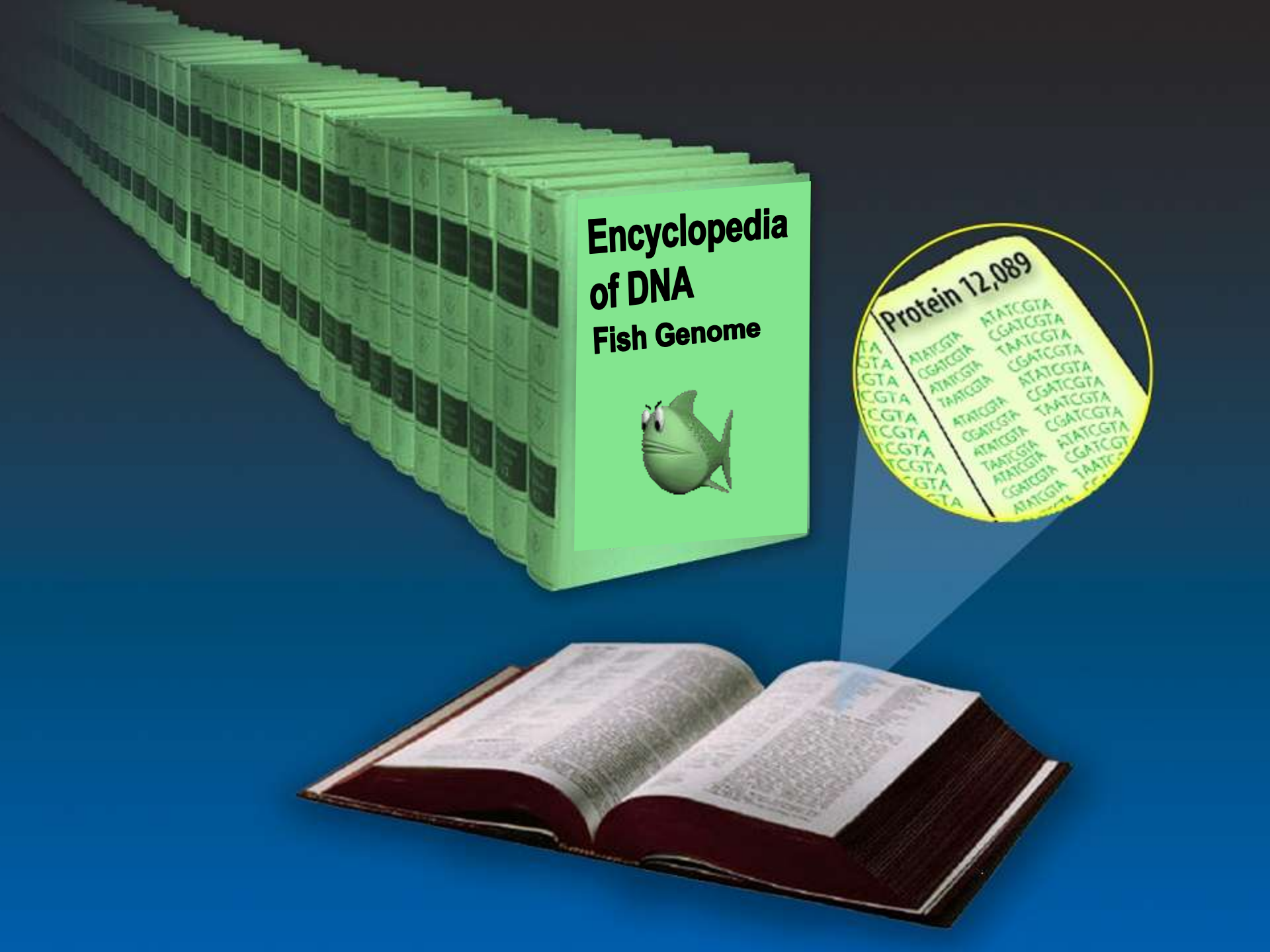
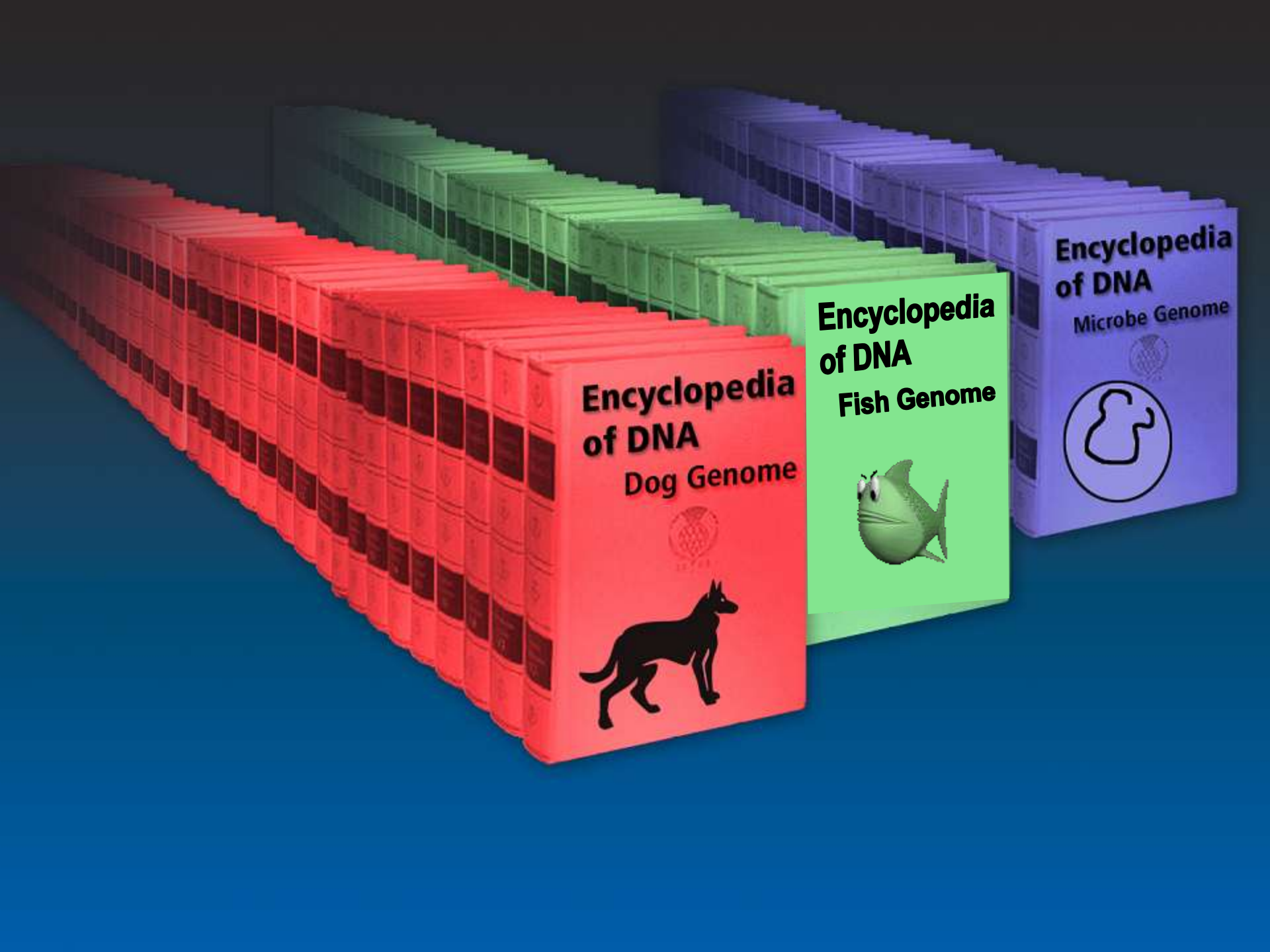
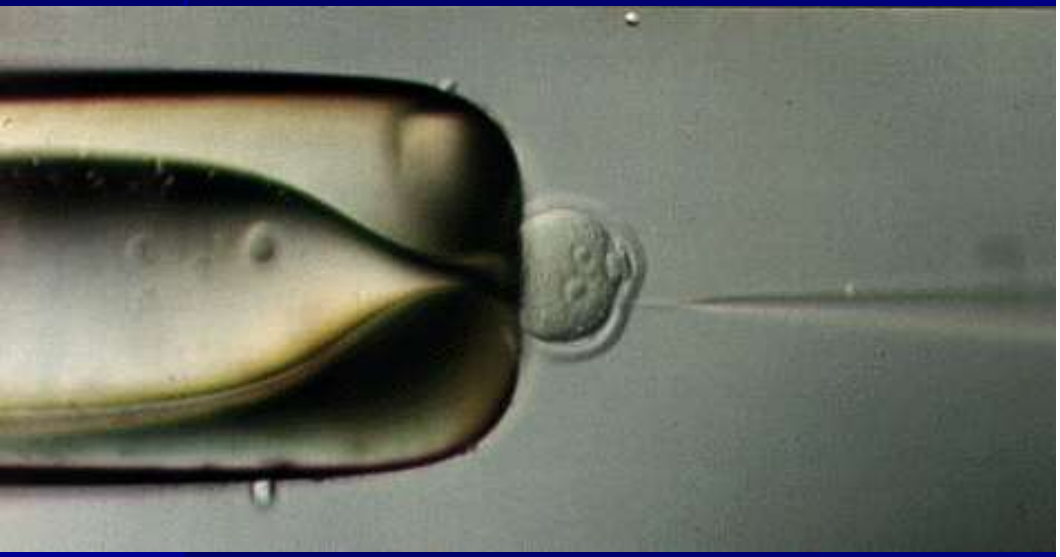
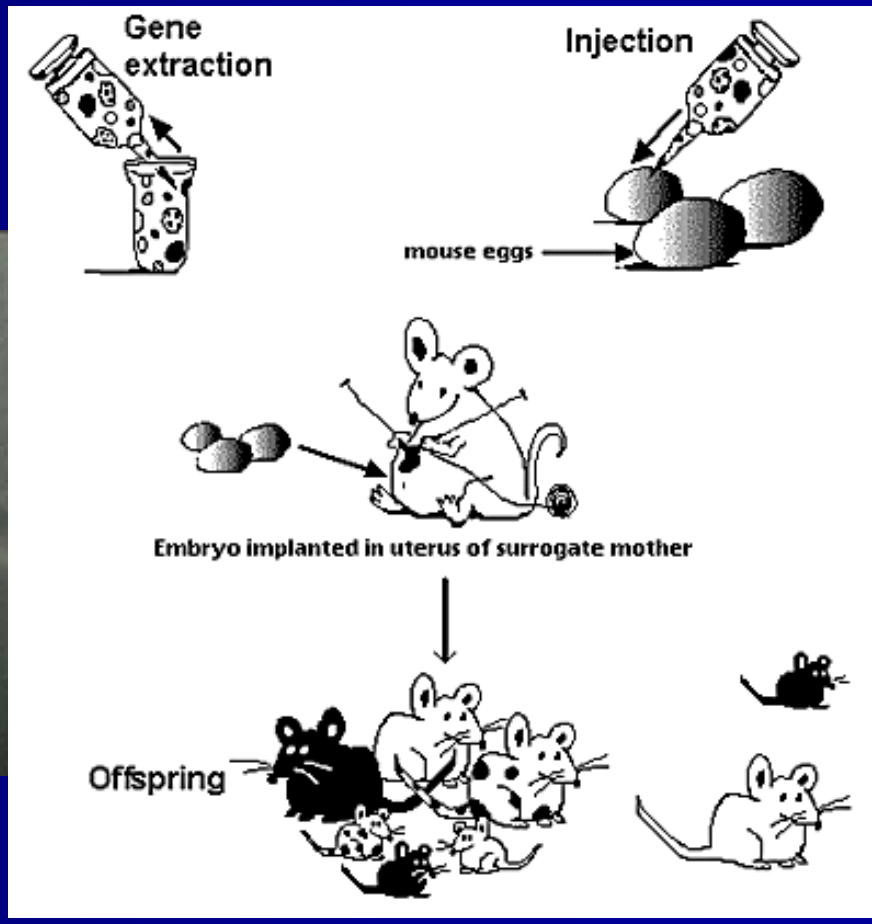

Encyclopedia
of DNA
Fish Genome
Protein 12,089


Encyclopedia
of DNA
Dog Genome
Encyclopedia
of DNA
Fish Genome
Encyclopedia
of DNA
Microbe Genome

"A genetically engineered animal carries heterologous DNA stably integrated into its genome."

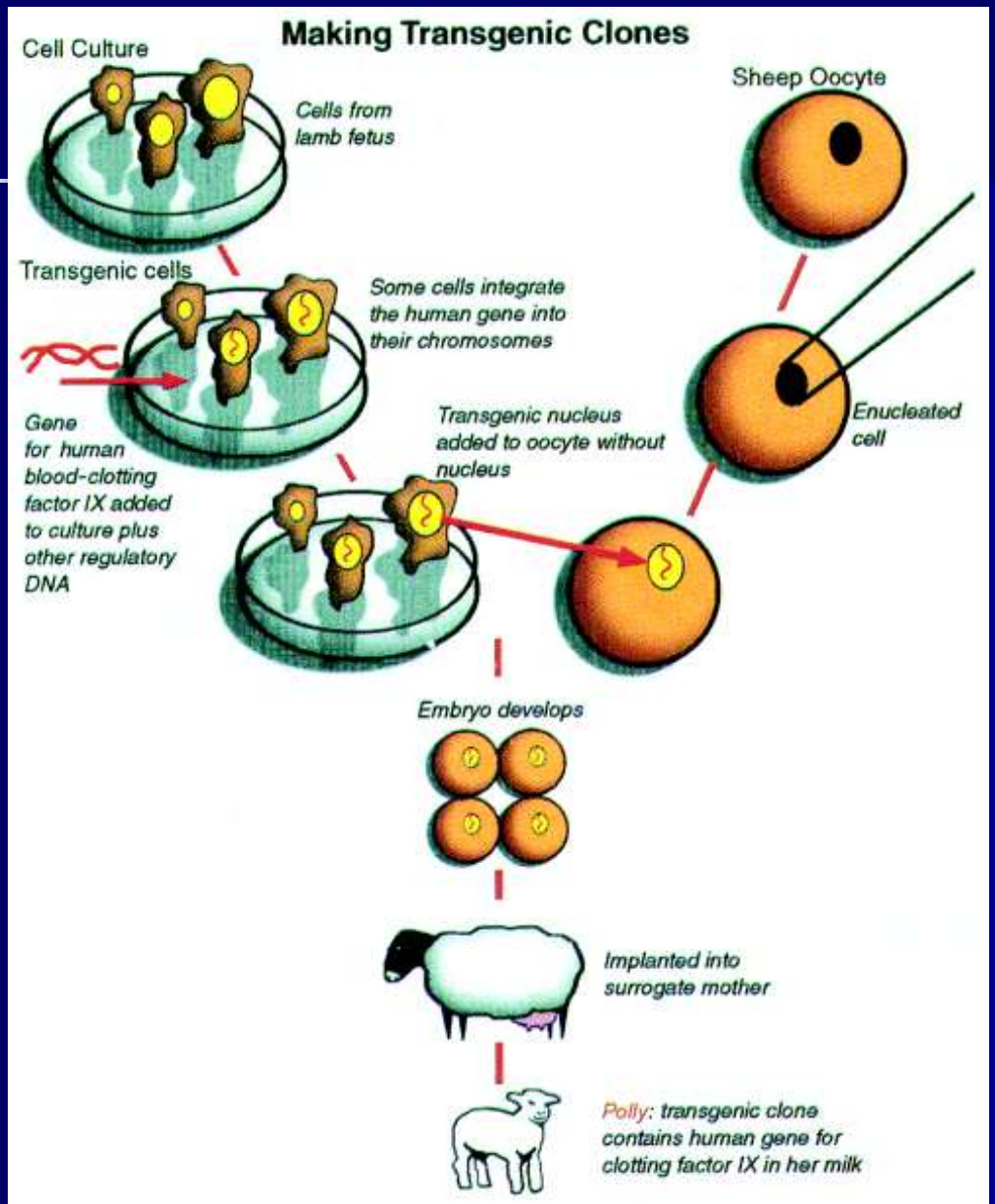




Where does genetic engineering come into the breeders equation?

$$\Delta G = \frac{\text{intensity of selection } X \text{ accuracy of selection } X \sqrt{\text{genetic variance in population}}}{\text{generation interval}}$$

SCNT cloning of genetically engineered cells



Polly – clotting factor IX milk

Pharma and industrial applications of animal biotechnology (cloning and genetic engineering)

Cloned transchromosomic calves producing human immunoglobulin

Yoshimi Kuroiwa[1], Poothappillai Kasinathan[2], Yoon J. Choi[3], Rizwan Naeem[4], Kazuma Tomizuka[1], Eddie J. Sullivan[2], Jason G. Knott[2], Anae Duteau[3], Richard A. Goldsby[3], Barbara A. Osborne[5], Isao Ishida[1]*, and James M. Robl[2]*

Published online: 12 August 2002, doi:10.1038/nbt727

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 unrearranged sequences of the human immunoglobulin (*hIg*) heavy-chain (*H*) and lambda (*λ*) 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 transchromosomic (Tc) calves. The HAC was retained at a high rate (78–100% of cells) in calves and the *hIg* loci underwent rearrangement and expressed diversified transcripts. 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.

p http://www.nature.com/naturebiotechnology

http://www.hematech.com

Plasmapheresis to extract polyclonal antibodies from the blood of cloned, transchromosomic, knockout cattle carrying human immunoglobulin

Xenotransplantation 2010: 17: 48–60
Printed in Singapore. All rights reserved
doi: 10.1111/j.1399-3089.2009.00564.x

XENOTRANSPLANTATION

Structural characterization of α1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies

Diswall M, Ångström J, Karlsson H, Phelps CJ, Ayares D, Teneberg S, Breimer ME. Structural characterization of α1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies.
Xenotransplantation 2010; 17: 48–60. © 2010 John Wiley & Sons A/S.

Mette Diswall,[1] Jonas Ångström,[1] Hasse Karlsson,[2] Carol J. Phelps,[3] David Ayares,[3] Susann Teneberg,[2] and Michael E. Breimer,[1]

[1]Department of Surgery, Institute of Clinical

www.revivicor.com

ARTICLES

nature biotechnology

Production of human monoclonal antibody in eggs of chimeric chickens

Lei Zhu[1], Marie-Cecile van de Lavoir[1], Jenny Albanese[2], David O Beenhouwer[4,5], Pina M Cardarelli[2], Severino Cuison[2], David F Deng[1], Shrikant Deshpande[2], Jennifer H Diamond[1], Lynae Green[2], Edward L Halk[2], Babette S Heyer[1], Robert M Kay[1], Allyn Kerchner[1], Philip A Leighton[1], Christine M Mather[1], Sherie L Morrison[4], Zivko L Nikolov[3], David B Passmore[2], Alicia Pradas-Monne[1], Benjamin T Preston[2], Vangipuram S Rangan[2], Mingxia Shi[1], Mohan Srinivasan[2], Steven G White[3], Peggy Winters-Digiacinto[1], Susan Wong[2], Wen Zhou[1] & Robert J Etches[1]

The tubular gland of the chicken oviduct is an attractive system for protein expression as large quantities of proteins are deposited in the egg, the production of eggs is easily scalable and good manufacturing practices for therapeutics from eggs have been established. Here we examined the ability of upstream and downstream DNA sequences of ovalbumin, a protein produced exclusively in very high quantities in chicken egg white, to drive tissue-specific expression of human mAb in chicken eggs. To accommodate these large regulatory regions, we established and transfected lines of chicken embryonic stem (cES) cells and formed chimeras that express mAb from cES cell–derived tubular gland cells. Eggs from high-grade chimeras contained up to 3 mg of mAb that possesses enhanced antibody-dependent cellular cytotoxicity (ADCC), nonantigenic glycosylation, acceptable half-life, excellent antigen recognition and good rates of internalization.

http://www.origen.com

BioPharm INTERNATIONAL
The Science & Business of BioPharmaceuticals

August 1, 2006

Production of Recombinant Therapeutic Proteins in the Milk of Transgenic Animals

By Yann Echelard,Carol A. Ziomek,Harry M. Meade

FDA Grants First-Ever U.S. Approval of GE Animal Product

Printer Friendly

For Immediate Release
2/6/2009

Contact:
Contact Karen Batra
202-449-6382

WASHINGTON, D.C. (Friday, February 06, 2009) - Advances in human health care from the genetic engineering of animals are now being realized in the United States. The U.S. Food and Drug Administration (FDA) announced today the first approval of a product derived from a genetically engineered (GE) animal.

ATryn®, a recombinant form of human antithrombin developed by GTC Biotherapeutics, was approved by the FDA for the prevention of peri-operative and peri-partum thromboembolic events in hereditary antithrombin deficient patients. It is not indicated for treatment of thromboembolic events in hereditary antithrombin deficient patients. ATryn® is the first ever transgenically produced therapeutic protein and the first recombinant antithrombin approved in the United States.

Along with the approval of ATryn®, the FDA's Center for Veterinary Medicine also approved GTC's New Animal Drug Application, the first of its kind to regulate GE animals. This is now required for a recombinant technology used to develop transgenic animals, such as the goats that produce recombinant antithrombin. GTC has granted OVATION the right to market ATryn® in the United States and pursue further clinical development.

www.gtc-bio.com

Genetically engineered food animals

Fast growing salmon

The founder female was generated in 1989 – 21 years ago

Nature Biotechnology 10:176 – 181. 1992

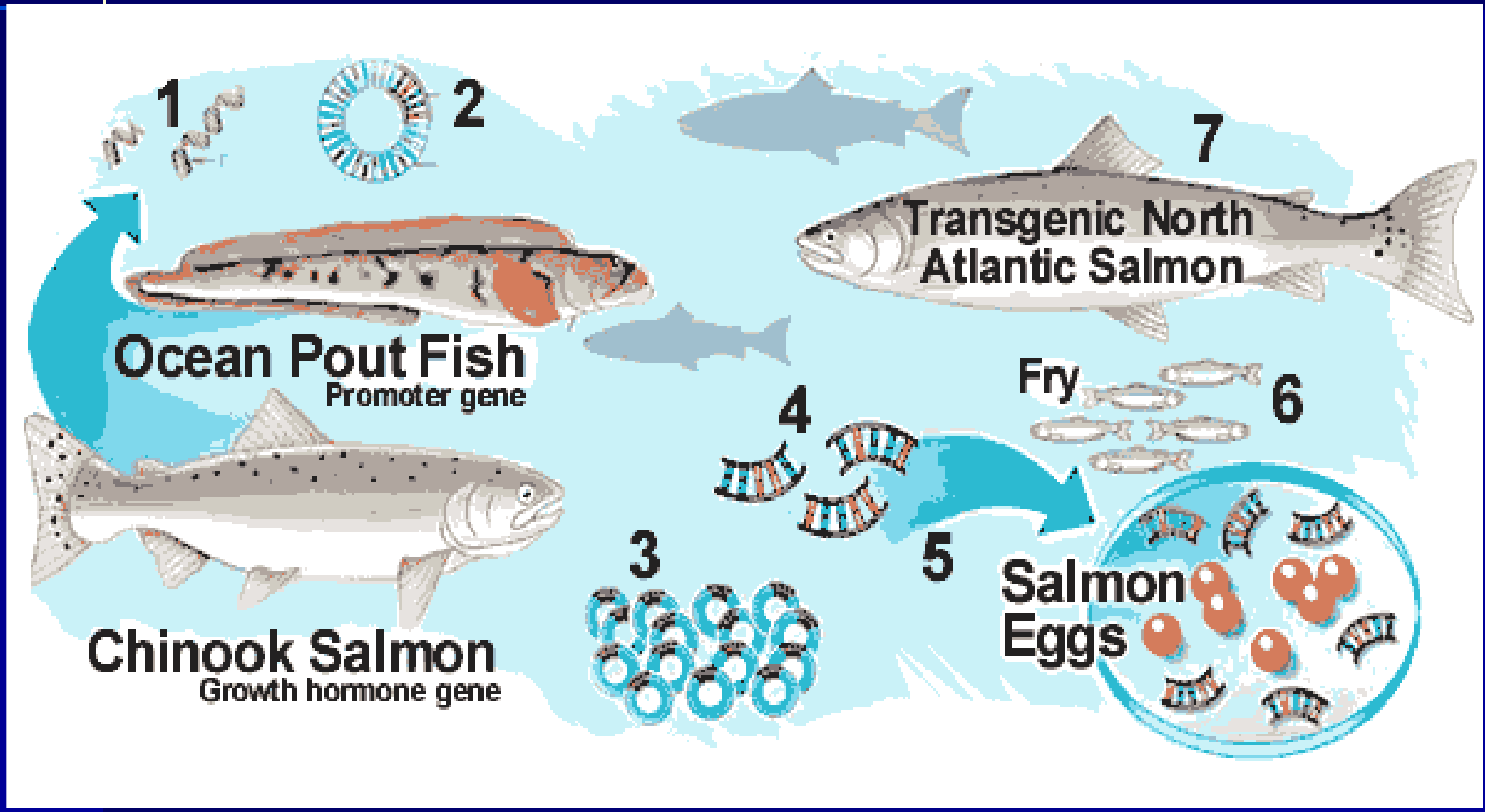
GROWTH ENHANCEMENT IN TRANSGENIC ATLANTIC SALMON BY THE USE OF AN "ALL FISH" CHIMERIC GROWTH HORMONE GENE CONSTRUCT

Shao Jun Du, Zhiyuan Gong, Garth L. Fletcher[1], Margaret A. Shears[1], Madonna J. King[1], David R. Idler[1] and Choy L. Hew*

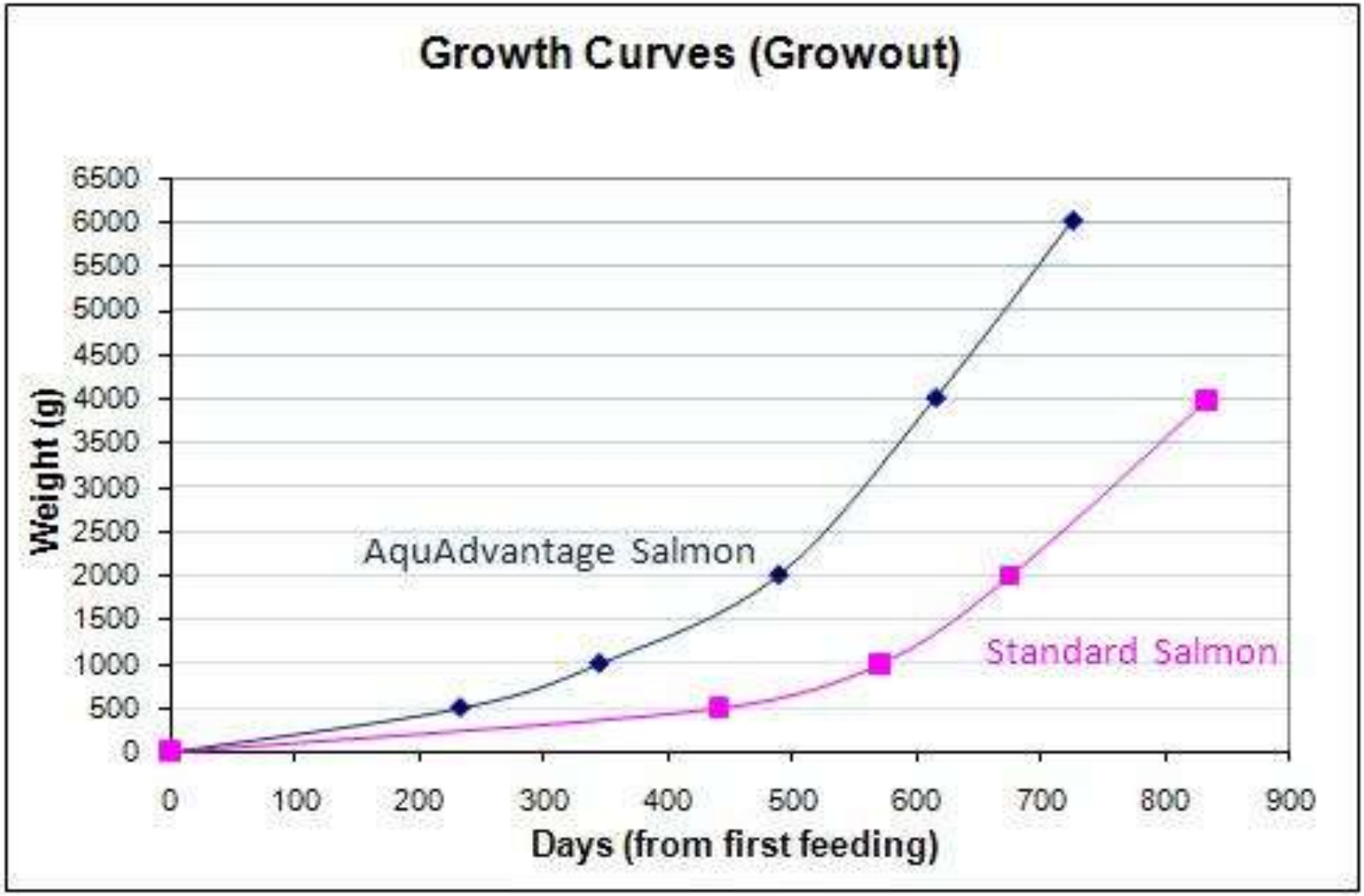
Research Institute, The Hospital for Sick Children and Departments of Clinical Biochemistry and Biochemistry, University of Toronto, Toronto, Canada M5G 1L5. [1]Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7. *Corresponding author.

We have developed an "all fish" growth hormone (GH) chimeric gene construct by using an antifreeze protein gene (AFP) promoter from ocean pout linked to a chinook salmon GH cDNA clone. After microinjection into fertilized, nonactivated Atlantic salmon eggs via the micropyle, transgenic Atlantic salmon were generated. The presence of the transgene was

What is the AquAdvantage salmon?



Fish reach adult size in 16 to 18 months instead of 30 months



Aqua bounty growth-enhanced salmon http://www.aquabounty.com/

Retrieved from "AquAdvantage" image search on web

Enviropig™ (Low-phosphorus manure)

RESEARCH ARTICLE

Nature Biotechnology 2001

Pigs expressing salivary phytase produce low-phosphorus manure

Serguei P. Golovan[1,2], Roy G. Meidinger[2], Ayodele Ajakaiye[3], Michael Cottrill[1], Miles Z. Wiederkehr[4], David J. Barney[4], Claire Plante[5], John W. Pollard[5], Ming Z. Fan[3], M. Anthony Hayes[6], Jesper Laursen[7,8], J. Peter Hjorth[7], Roger R. Hacker[3], John P. Phillips[2,*], and Cecil W. Forsberg[1,*]

To address the problem of manure-based environmental pollution in the pork industry, we have developed the phytase transgenic pig. The saliva of these pigs contains the enzyme phytase, which allows the pigs to digest the phosphorus in phytate, the most abundant source of phosphorus in the pig diet. Without this enzyme, phytate phosphorus passes undigested into manure to become the single most important manure pollutant of pork production. We show here that salivary phytase provides essentially complete digestion of dietary phytate phosphorus, relieves the requirement for inorganic phosphate supplements, and reduces fecal phosphorus output by up to 75%. These pigs offer a unique biological approach to the management of phosphorus nutrition and environmental pollution in the pork industry.

"reduces fecal phosphorus output by up to 75%"

www.uoguelph.ca/enviropig

"Mad cow"-resistant cows
(bovine spongiform encephalopathy (BSE))

nature biotechnology

Nature Biotechnology 2007

nature.com/naturebiotechnology

Production of cattle lacking prion protein

Jürgen A Richt[1,6], Poothappillai Kasinathan[2], Amir N Hamir[1], Joaquin Castilla[3], Thillai Sathiyaseelan[2], Francisco Vargas[1], Janaki Sathiyaseelan[2], Hua Wu[2], Hiroaki Matsushita[2], Julie Koster[2], Shinichiro Kato[4,5], Isao Ishida[4], Claudio Soto[3], James M Robl[2] & Yoshimi Kuroiwa[4–6]

Prion diseases are caused by propagation of misfolded forms of the normal cellular prion protein PrP^{C}, such as PrP^{BSE} in bovine spongiform encephalopathy (BSE) in cattle and PrP^{CJD} in Creutzfeldt-Jakob disease (CJD) in humans[1]. Disruption of

PrP-specific western blot analyses on fibroblasts (**Fig. 1d**), peripheral blood lymphocytes (**Fig. 1e**) and brain stem (**Fig. 1f**) from wild-type and *PRNP*$^{-/-}$ calves using the mouse anti-bovine PrP monoclonal antibody F89. We detected PrP-specific bands in the wild-type calves,

PNAS

Suppression of prion protein in livestock by RNA interference

PNAS 2006

Michael C. Golding*, Charles R. Long†, Michelle A. Carmell*, Gregory J. Hannon*‡, and Mark E. Westhusin†§

*Watson School of Biological Sciences, Cold Spring Harbor Laboratory, Howard Hughes Medical Institute, 1 Bungtown Road, Cold Spring Harbor, NY 11724; and †Department of Veterinary Physiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

Communicated by James E. Womack, Texas A&M University, College Station, TX, February 2, 2006 (received for review November 21, 2005)

Given the difficulty of applying gene knockout technology to species other than mice, we decided to explore the utility of RNA interfering RNAs enables induction of silencing by "classical" DNA expression vectors and has thus become adaptable to cell

GE Chickens That Don't Transmit Bird Flu

Breakthrough could prevent future bird flu epidemics

Suppression of Avian Influenza Transmission in Genetically Modified Chickens

Jon Lyall,[1] Richard M. Irvine,[2] Adrian Sherman,[3] Trevelyan J. McKinley,[1] Alejandro Núñez,[2] Auriol Purdie,[3]* Linzy Outtrim,[2] Ian H. Brown,[2] Genevieve Rolleston-Smith,[3] Helen Sang,[3]† Laurence Tiley[1]†‡

Infection of chickens with avian influenza virus poses a global threat to both poultry production and human health that is not adequately controlled by vaccination or by biosecurity measures. A novel alternative strategy is to develop chickens that are genetically resistant to infection. We generated transgenic chickens expressing a short-hairpin RNA designed to function as a decoy that inhibits and blocks influenza virus polymerase and hence interferes with virus propagation. Susceptibility to primary challenge with highly pathogenic avian influenza virus and onward transmission dynamics were determined. Although the transgenic birds succumbed to the initial experimental challenge, onward transmission to both transgenic and nontransgenic birds was prevented.

The diversity of avian influenza viruses (AIVs) and their propensity for interspecies transmission make them a global threat to animal and public health communities. Cross-species transmission of influenza viruses may occur directly or be facilitated by intermediate host species that amplify and diversify virus populations, notably domestic chickens, ducks, and pigs (*1*). Although control of AIV infection in its wild aquatic bird reservoir is impractical, control of AIV in domesticated hosts is possible (*2*). The diversity of viral antigenic sub-

Science 331:223-226. 2011 SCIENCE VOL 331 14 JANUARY 2011 223

http://www.roslin.ed.ac.uk/public-interest/gm-chickens

"Would there be general acceptance of transgenic technology if it could be applied to engineering resistance to influenza in poultry and therefore lessen the risk of an influenza epidemic, such as the one in 1918 that killed more than 20 million people?"

Clark,J. & Whitelaw,B. 2003. A future for transgenic livestock. Nature Reviews Genetics 4, 825-833

2001 foot-and-mouth outbreak in UK

$ 3.5 to $6 billion lost, Numerous producer suicides
Millions of animals slaughtered from 10,000 farms

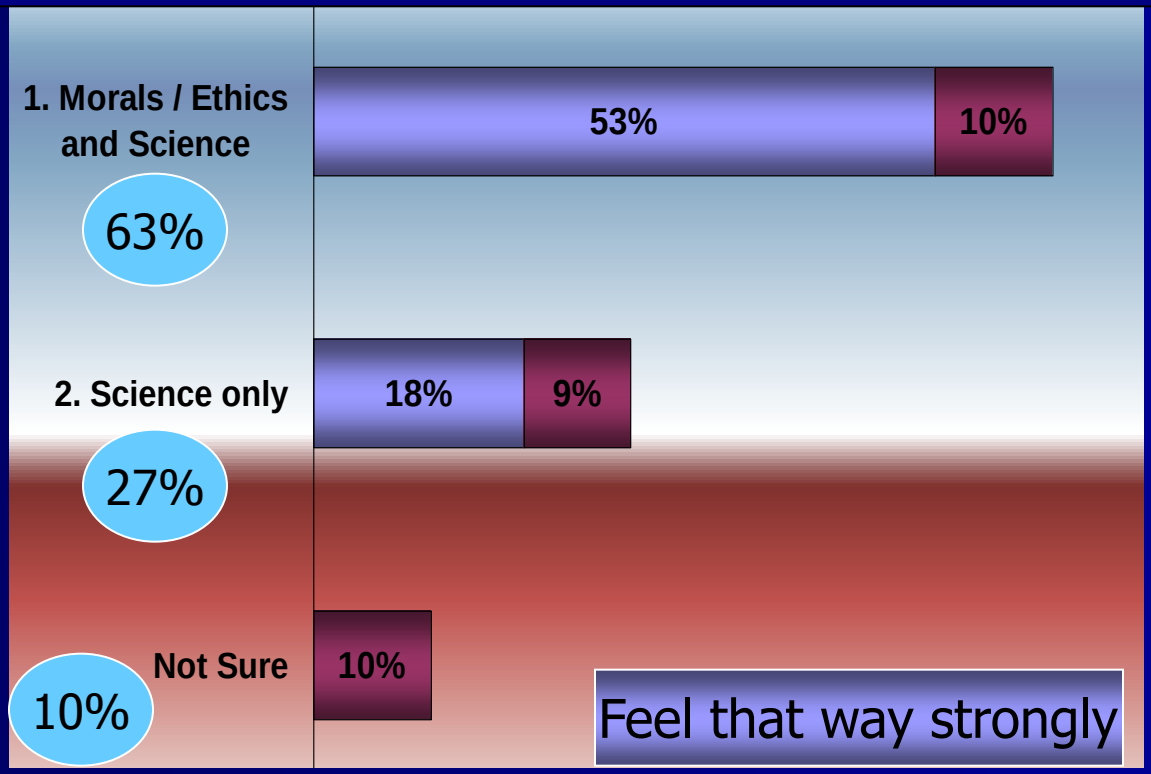
Is it possible that "playing it safe" by not pursuing research and development in genetically engineered animals might deny us a technique or products which could prevent an environmental or public health disaster in fifty years time, or could prove invaluable in the treatment of some livestock disease?

GE animals raise unique moral, ethical, and cultural questions

- Animal "integrity"
- Animal welfare
  - related to breeding goals
  - related to biotechnology
- Environmental issues with regard to gene flow from GE animals to native populations.

1. Government regulators ***should include ethical and moral considerations***, in addition to scientific evaluation of risks and benefits, when making regulatory decisions about cloning or genetically modifying animals.

2. Though ethical and moral considerations are important, government regulators ***should consider only scientific evaluation of risks and benefits*** when making regulatory decisions about cloning and genetically modifying animals.



http://pewagbiotech.org/research/2005update/2005summary.pdf

How to incorporate social and ethical issues into regulatory decisions ?

- American consumers (75%) and scientists (70%) agree that cloning and genetic engineering of animals raise some moral and ethical issues
- However public is much less likely to approve (21-25%) of these technologies than scientists (60-68%)
- How to reach a societal consensus on ***which set of values*** will ultimately be applied to decide the acceptable uses of animal biotechnology ?

Keystone Research Center (2004) – Biotechnology and ethics: a national survey of consumers and scientists. Report to the Biotechnology Industry Organization. KRC Research, Washington DC, 29pp.

"...genetic engineering is a key technology, which will be vital for meeting the world's future food needs. While animal genetics alone will not solve the world's future food problems, to fail to apply the best available technologies to the solution of contemporary and future food shortages would be morally reprehensible."

Fahrenkrug et al. 2010. Precision Genetics for Complex Objectives in Animal Agriculture. J. Anim Sci. Jul;88(7):2530-9

SUMMARY

- No GE or SCNT cloned food animals are currently on the U.S. market
- FDA regulates GE/cloned food animals in U.S.
- The future of Pharma and industrial applications of animal biotechnology looks promising
- Future of agricultural applications is less certain and regulatory process is not clear or predictable
- Yet to see if the expense of the technology and regulatory process is commercially viable
- Animal biotechnology faces some ethical questions that were not part of plant biotechnology debate


B
Red
Orange
Yellow
Green
Wild Type


GMO'S
TREATOR
TRICK?


HUMAN GROWTH HORMONE GENE
MT
PROMOTER
pBR

Chinese work on transgenic animals