Animal Biotechnology:
Where to from here?

Alison Van Eenennaam, Ph.D.
Cooperative Extension Specialist
Animal Biotechnology and Genomics
University of California, Davis
alvaneenennaam@ucdavis.edu

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Genetically-modified animals

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]*

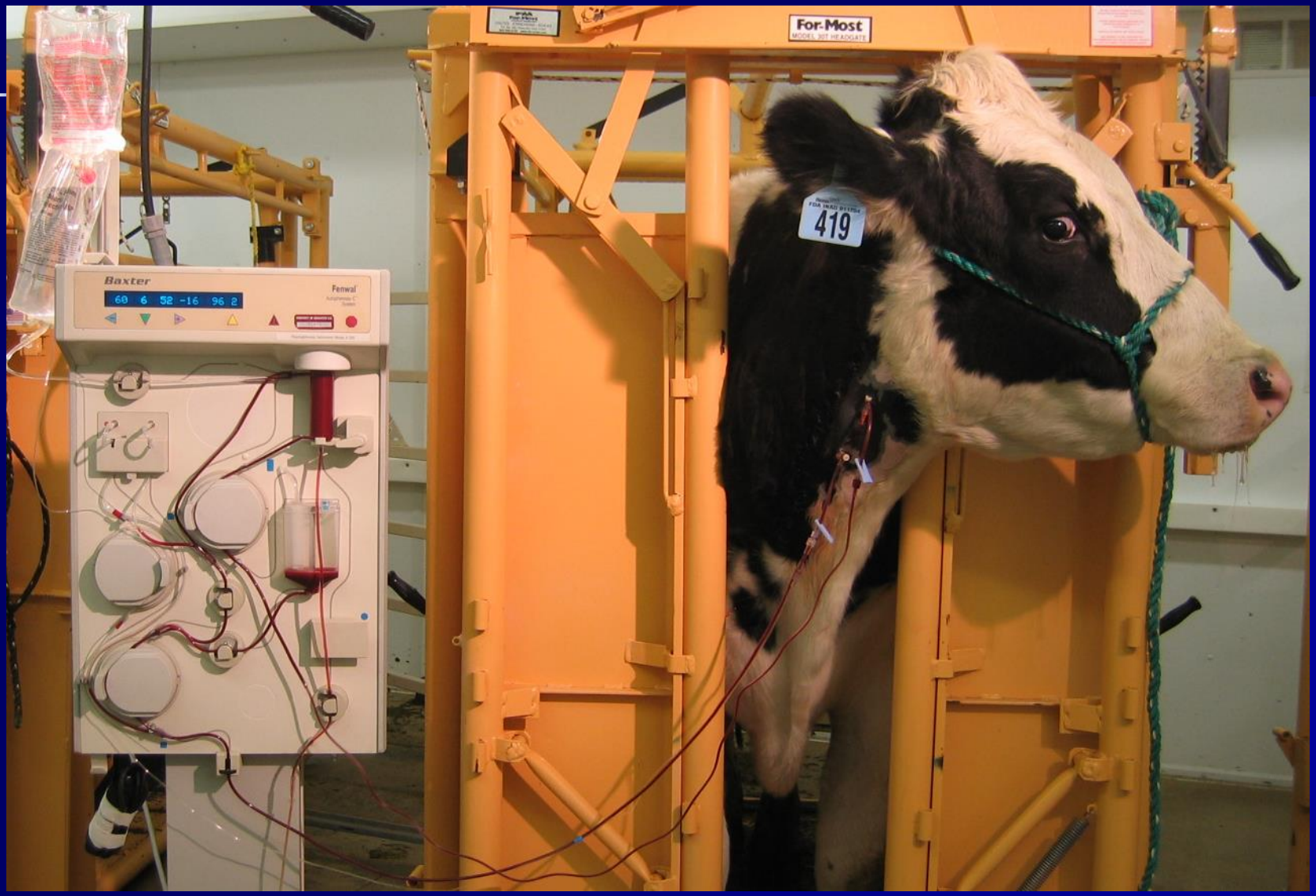
Published online: 12 August 2002, doi:10.1038/nbt727

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 (h*Ig*) 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 h*Ig* 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.

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



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

Animal Genomics and Biotechnology Education

Transgenic avian-derived recombinant human interferon-alpha2b (AVI-005) in healthy subjects: an open-label, single-dose, controlled study. International Journal of Clinical Pharmacology and Therapeutics, 2007 Mar;45(3):161-168

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Developing Improved and Affordable Bio-Therapeutics

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AviGenics, Inc. is a bio-pharmaceutical company developing improved and cost-efficient bio-therapeutics for the treatment of cancer, infectious diseases, organ-dysfunction, genetic disorders and autoimmune diseases. The Company's unique proprietary technology is designed to produce naturally glycosylated bio-therapeutics and allows rapid and efficient product development. Using this technology, AviGenics can achieve commercially feasible levels of production and purification for a wide array of bio-therapeutics.

AviGenics' proprietary technology can be applied to manufacture multiple categories of bio-therapeutics such as cytokines, human monoclonal antibodies, therapeutic enzymes, and fusion proteins. AviGenics has demonstrated success in using its proprietary technology in a number of key areas, including:

Production of several bio-therapeutics
Bio-therapeutics that feature natural glycosylation
US-FDA approved INDs for two bio-therapeutic products
Safety and efficacy of bio-therapeutic products in clinical trials
Significant capital cost reduction compared to mammalian cell processes

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BioPharm INTERNATIONAL
The Science & Business of BioPharmaceuticals

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August 1, 2006

Production of Recombinant Therapeutic Proteins in the Milk of Transgenic Animals

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

European panel now recommends GTC anti-clotting drug's surgical use

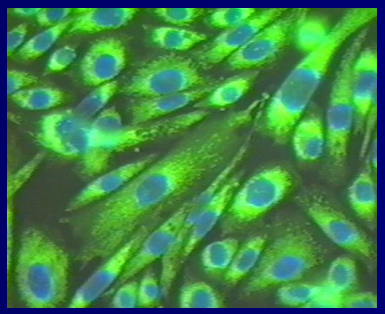
OCTOBER 15 2006 FRAMINGHAM — A European drug regulation committee reversed itself yesterday and recommended that a GTC Biotherapeutics Inc. anti-clotting drug (human antithrombin) drawn from the milk of genetically altered goats be approved for use in surgical patients

http://www.gtc-bio.com

Cell culture-based manufacturing facility for a single therapeutic protein can cost more than $US500 million

$US100,000 per patient annually for Avastin (a humanized monoclonal antibody produced in a CHO cell line) to treat breast or lung cancer

CHO Cells ☛



The production of therapeutic proteins in transgenic animals offers a significant capital cost reduction compared to mammalian cell culture processes.

PharmAthene Awarded $213 Million Department of Defense Contract for Advanced Development of Protexia

September 25, 2006

PharmAthene, Inc., announced today that it has been awarded a multi-year contract valued at up to $213 million from the Department of Defense (DoD) U.S. Army Space and Missile Command, for advanced development of the Company's broad spectrum chemical nerve agent prophylaxis, Protexia(R).

http://www.pharmathene.com

Recombinant proteins in the milk of transgenic BELE® goats from which they are extracted.

Agricultural applications of animal biotechnology

Selected Participants

- ABS Global
- Columbus Farming Corp.
- Food and Agriculture Organization of the UN
- NZ Ministry of Agriculture
- DeKalb Poultry Research
- Hy-Line International
- AgResearch (NZ)
- MetaMorphix
- National Institute of Animal Industry, Japan
- PIC Group
- Victorian Institute of Animal Science, Australia

Transgenic Research 7, 73–75 (1998)

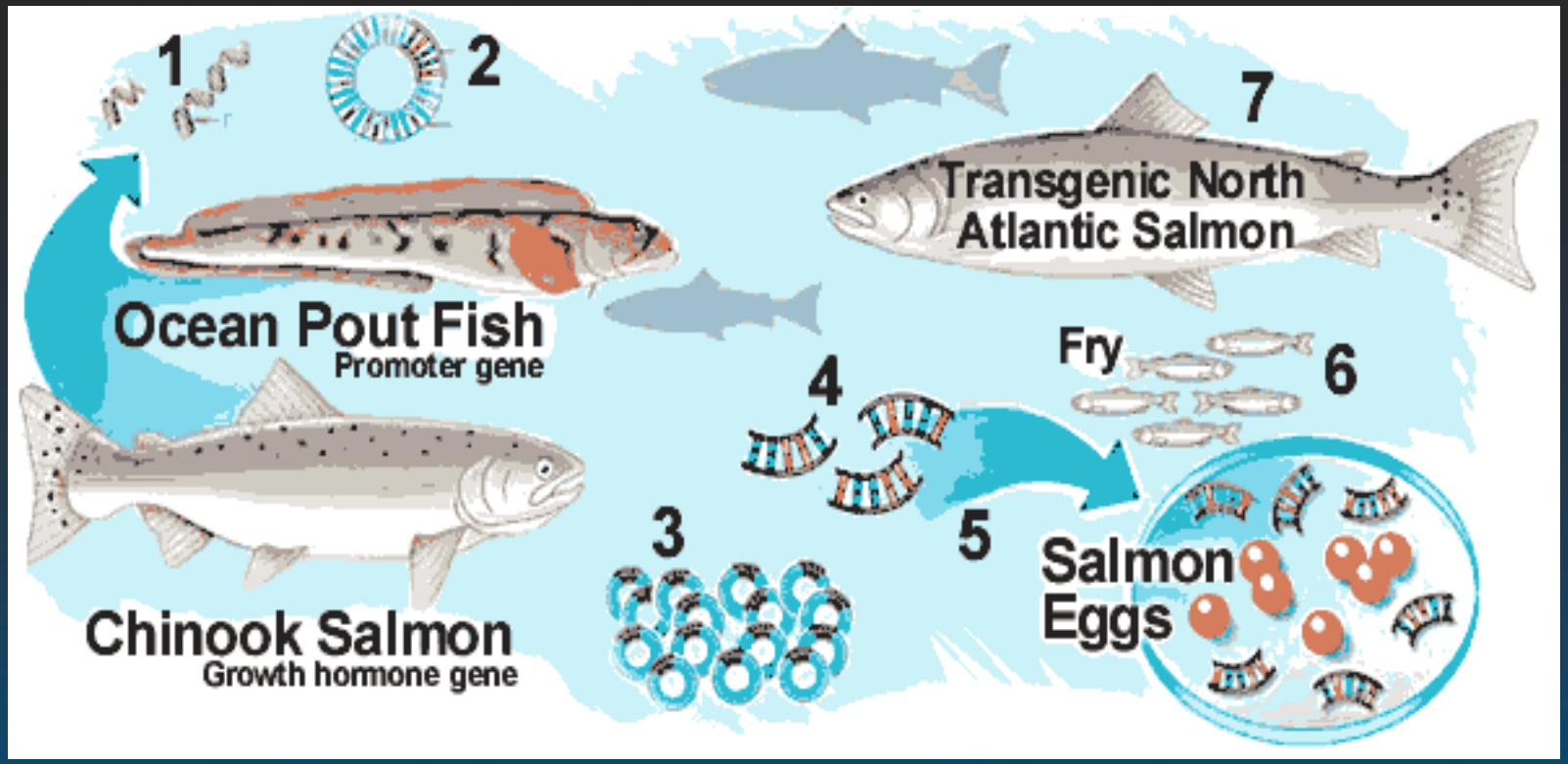
Transgenic farm animals get off the ground

Transgenic Animals in Agriculture, Conference Tahoe City, California, USA
August 24–27, 1997, sponsored by the University of California at Davis

H. Niemann (1998)

"For the next 10 years it is estimated that transgenic technology will be mainly directed towards increase in basic biological knowledge, particularly in the field of gene regulation and expression"

"This will increase the reliability and success of gene transfer methods and this should lead to transgenic farm animals in the field by the next 10-15 years."



http://www.aquabounty.com

18 month old AquaAdvantage™ salmon

U.S. Regulation of GE animals
FDA Center for Veterinary Medicine

- The U.S. Food and Drug Administration (**FDA**) has asserted jurisdiction over genetically engineered animals on the grounds that the transgene and any expressed proteins, affect the "structure and function" of the receiving animal analogous to the modalities of alternative veterinary drug formulations.
- Aqua Bounty AquaAdvantage™ growth-enhanced salmon submitted to the FDA over 10 years ago
- Hopes to launch in 2009

Regulation of SCNT cloned animals

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

- The FDA currently has a voluntary moratorium on marketing products from adult SCNT clones and their progeny that has been in effect for over 6 years.

Despite the fact that.....

- Thousands of embryo split and embryo nuclear transfer "clones" have been going into the food supply for over 20 years, since the early 1980s.
- The FDA's 678-page draft risk assessment released in December 2006 found that "***food products derived from animal clones and their offspring are likely to be as safe to eat as food from their non-clone counterparts, based on all the evidence available***"
- It is not known when FDA will give the go ahead for SCNT clones and their progeny to enter the food supply

Labeling?

Several pending state and federal labeling bills would require livestock producers to disclose to buyers that an animal **is cloned or is the progeny of a cloned animal***, as specified. They also require **food for human consumption that contains any product from a cloned animal or its progeny to be labeled to indicate that the food includes the product of a cloned animal or its progeny**.

* "Cloned animal" means an animal that arises directly from a somatic cell nuclear transfer event.

Ag applications in the pipeline

Transgenic Research (2005) 14:563–567
DOI 10.1007/s11248-005-0670-8

Perspective

Engineering disease resistant cattle★

David M. Donovan[1], David E. Kerr[2] & Robert J. Wall[1,*]
[1]*Biotechnology and Germplasm Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705, USA*
[2]*Department of Animal Sciences, University of Vermont, Burlington, Vermont 05405, USA*

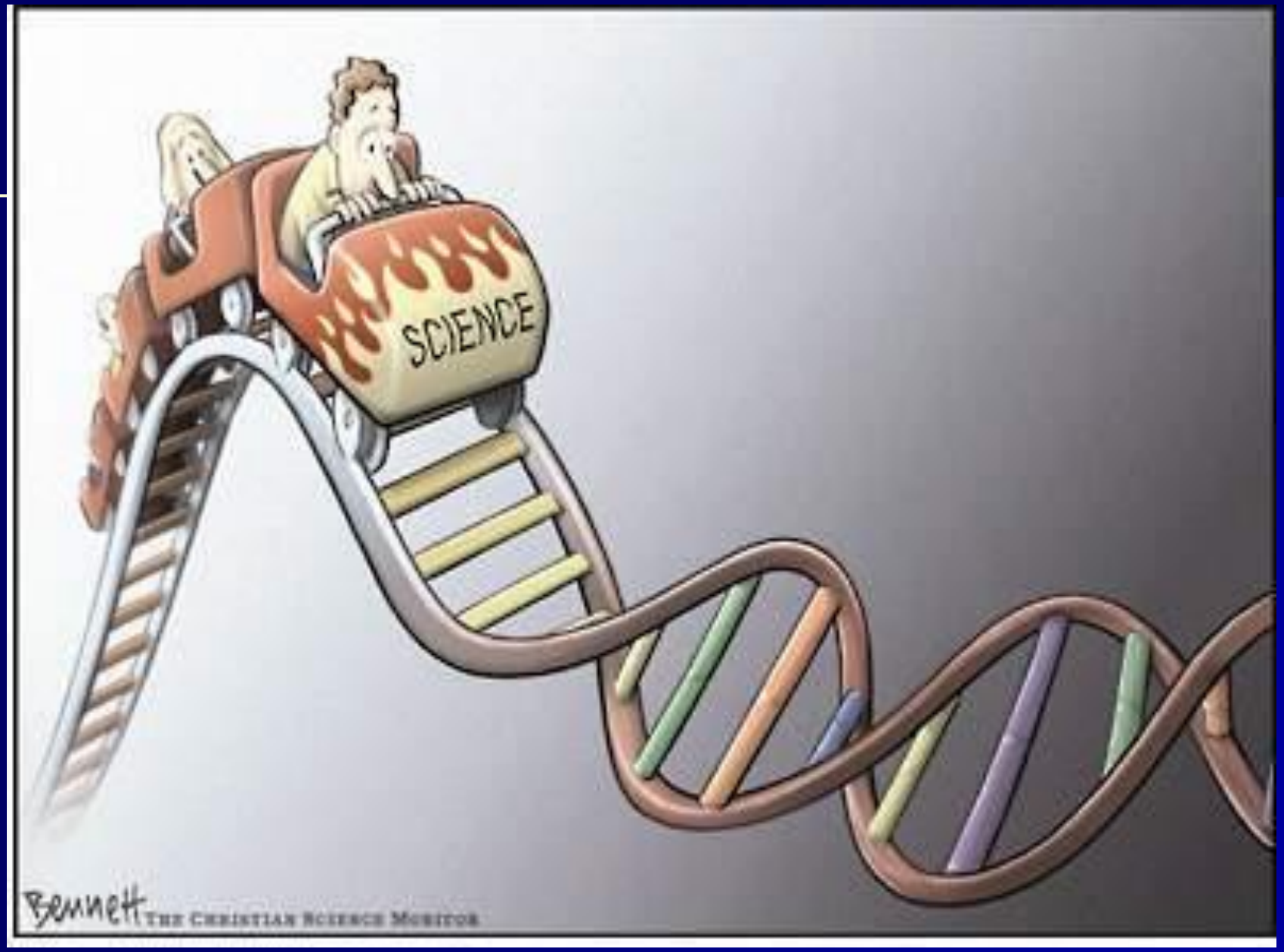
Received 7 June 2005; accepted 21 June 2005

Key words: bovine, disease resistance, mastitis, transgene

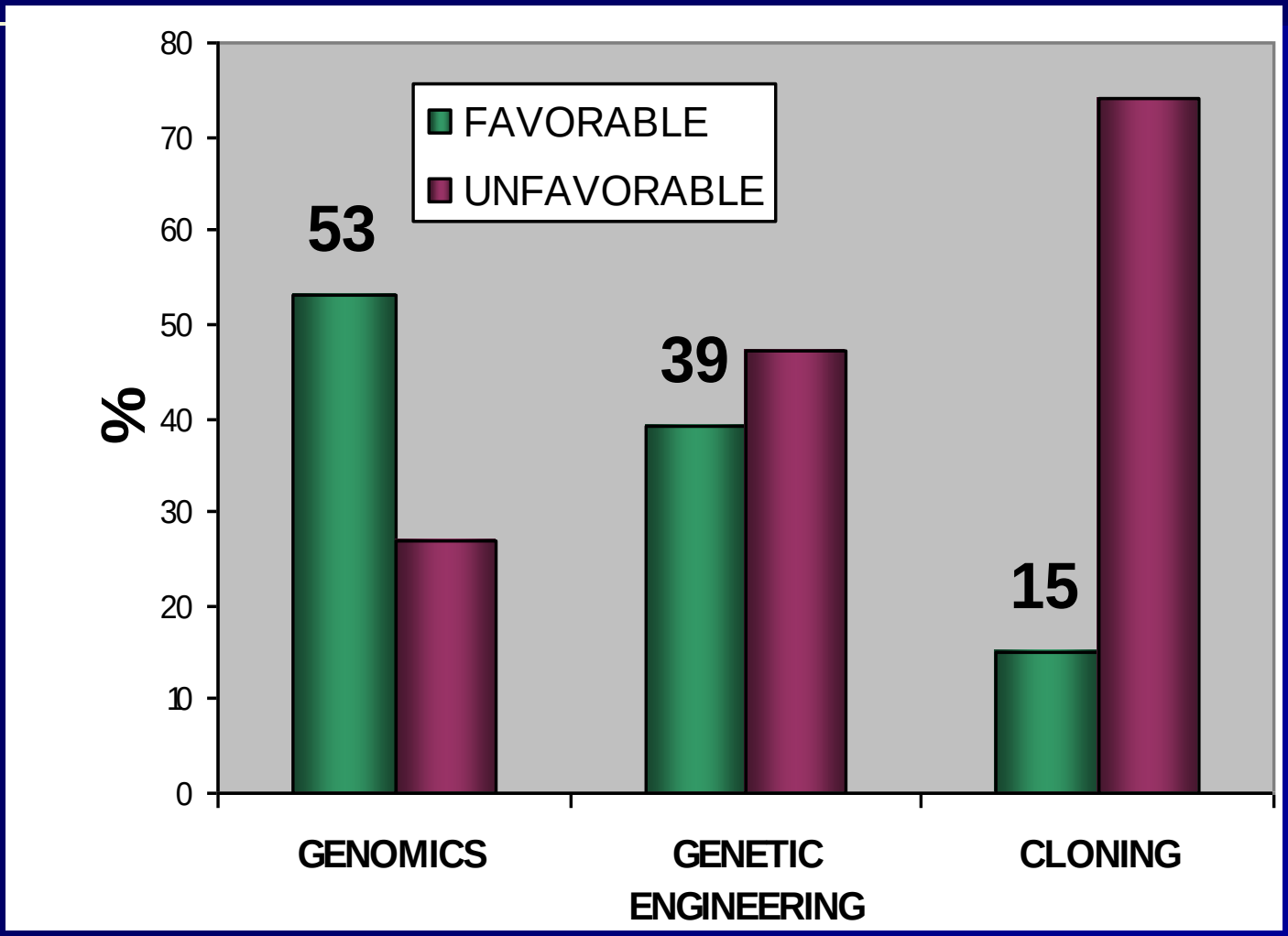
- Genetically engineered cows resistant to intra-mammary *Staphylococcus aureus* infection (2005)
- Production of transgenic goats expressing human lysozyme in their mammary gland (2006)
- Production of cattle lacking prion protein that causes "mad cow disease" (2007)

And yet GloFish™ is the only product of animal biotechnology thus far on the U.S. market

http://www.glofish.com


SCIENCE
Bennett
THE CHRISTIAN SCIENCE MONITOR

Public Attitudes Towards Specific "Animal Biotechnologies" (IFIC, 2005)



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

H. Niemann (1998)"*Transgenic farm animals get off the ground*" Transgenic Research 7:73

"An important factor that should not be underestimated is the considerable resistance of public opinion in several countries, particularly Europe, against this technology. This could affect private and public funding in these countries and thereby slow down further progress. Given the broad range of serious problems that mankind is facing in the years ahead, one can hope that rational approaches will be taken to ensure that the huge benefits of transgenic farm animals will not be an unexploited resource."

European project to map research and commercial activities worldwide for animal cloning and genetic modification

Animal cloning and genetic modification. A prospective study Joint Research Center Institute for Prospective Technological Studies. European Commission, Seville. *In preparation*

- Number of published papers on animal GM/GE increased over the period 1985 – 1998 and then leveled off, with most coming from the EU, followed by the USA.
- Number published papers on cloning increased from 1990 – 2000 but this has since leveled off. Most of the work was done in the USA with Europe coming third (after the Far East). The work concentrated on technical aspects and was mostly publicly funded.

United States Department of Agriculture

Agricultural Research Service

Cooperative State Research, Education, and Extension Service

Blueprint for USDA Efforts in Agricultural Animal Genomics 2008–2017

"In the long-term, animal genomics efforts will lead to efficient and economical production of human pharmaceutical proteins in animals, new technologies for manipulation of gene expression in animals (i.e., RNA interference, transgenesis, etc.), and improved methods for conserving biodiversity and unique animal germplasm. Because of the existing widespread use of quantitative genetics in animal breeding programs in the U.S. and the rapid rate at which genomic information is being discovered, ***the initial applications of genomics efforts will be the combined use of genomic data with quantitative genetics for animal improvement, management, and biosecurity***."

USDA National Research Initiative (major funding source for academic agricultural research in the U.S.)

43.0 Animal Genome (A): Translational Animal Genomics

FY 2008 Priorities for Research Projects – Applicants must address at least one of the following priorities.

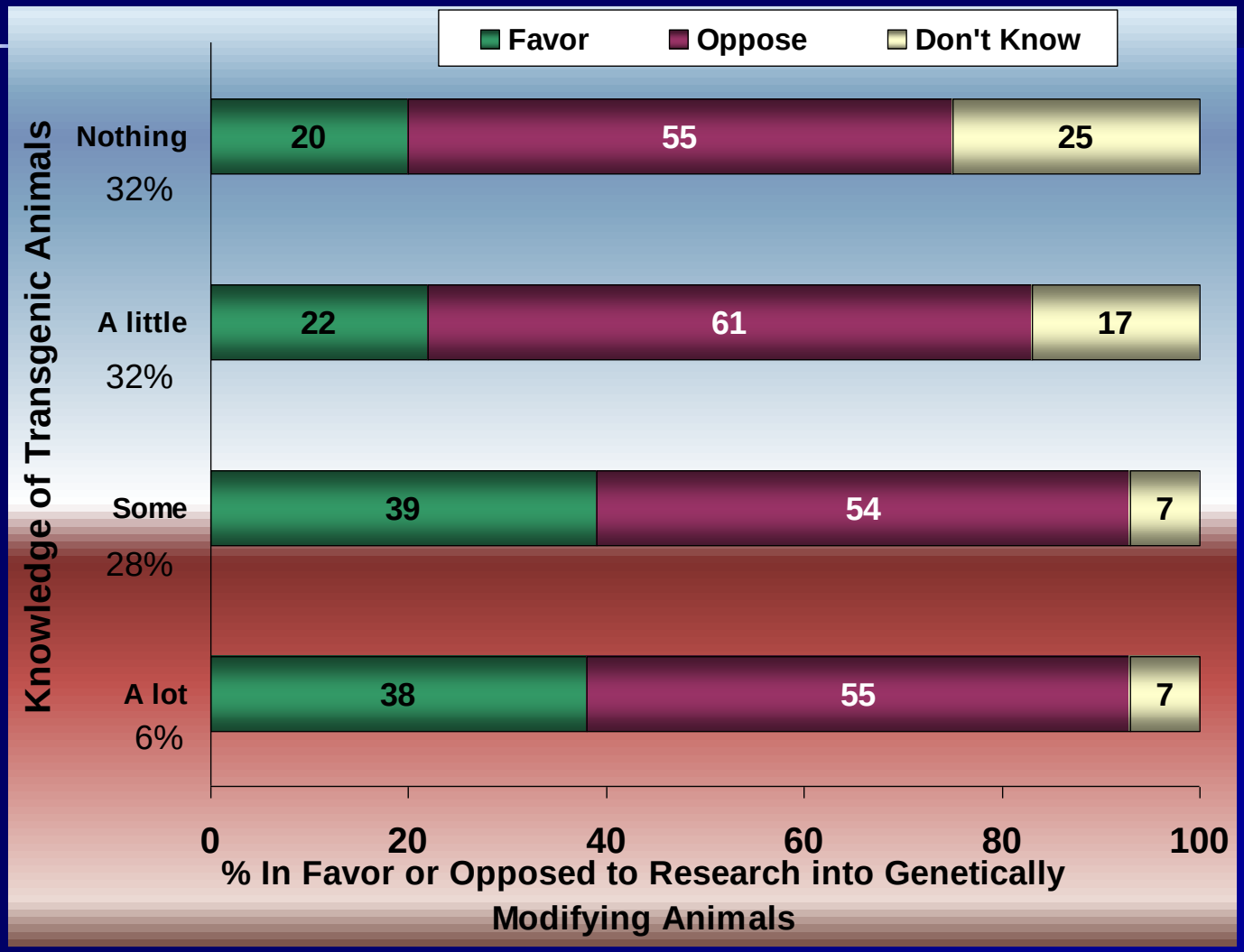
1. Identification and mapping of genomic markers, including quantitative-trait loci (QTL), economic trait loci (ETL), causative mutations, and candidate genes for traits of importance to animals in agriculture, including aquaculture species.

2. SNP-based cost-effective genotyping as it relates to whole genome enabled animal selection, genomic capabilities that enable parentage, and identity verification (traceability) and genetic diversity.

3. Development and application of methods to modify the animal genome to aid in the understanding of gene function or expression (e.g. RNAi, nuclear transfer, embryonic stem cells, and transgenics).

- Applications whose primary aim is to improve the efficiency in the production of clones or transgenic animals through manipulation of the nucleus will no longer be accepted by the Animal Genome program.

The majority of Americans oppose scientific research into genetic modifications of animals - irrespective of self-assessed knowledge level



10/2005

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

It may be difficult to know what the public has heard about animal biotechnologies….

Supercow and pigs that glow at night - an average day on the GM farm

Last updated at 10:11am on 3rd November 2006

Channel 4 is to unveil a shocking menagerie of genetically modified animals in a new show revealing the frightening leaps technology has taken.

See also:

- Swan falls in love with paddle boat
- VIDEO: Fuelish driver - woman loses control of car at petrol station
- The best viral emails doing the rounds

Among the bizarre engineered creatures from around the world is a giant cow, three times the size of ordinary cattle, reared without fat to produce gallons of milk.

But the so-called Belgian Blue - pictured

Appliance of science: the Belgian Blue is bred to produce gallons of milk

Transgenic pigs constitutively expressing an omega-3 fatty acid desaturase.

HEALTH & SCIENCE

Making Bacon That's Healthier for You

Listen by Joe Palca

Steve Morse/University of Missouri Extension and Agricultural Information

These pigs carry a gene that helps their bodies produce a healthier fat, known as omega-3 fatty acid.

NPR.org, March 26, 2006 · If you think scientists never do anything useful, consider this: A team of researchers may have found a way to make bacon that's good for your heart. This stunning achievement comes from a mixture of molecular genetics, cloning, and good old American know-how.

The key to this delicious prospect? A modified gene that changes some of the omega-6 fatty acids -- which pigs normally create -- into omega-3 fatty acids.

Omega 3 is the healthy fat you get in oily fish and flax seed. It's generally thought that a diet high in omega-3 fatty acids helps prevent heart disease.

The first animals to get this

Wall,R.J. Lai, L.X., et al. 2006. **Generation of cloned transgenic pigs rich in omega-3 fatty acids**. *Nature Biotechnology* **24**, 435-436

Clop,A., et al. 2003. **Detection of QTL affecting fatty acid composition in the pig.** *Mammalian Genome* **14**, 650-656

Nii,M., et al. 2006. **Quantitative trait loci mapping for fatty acid composition traits in perirenal and back fat using a Japanese wild boar x Large White intercross**. *Animal Genetics* **37**, 342-347.

Skim milk straight from the cow

Liz Williams
Cosmos Online

Monday, 28 May 2007

SYDNEY: A new breed of cow that produces skim milk naturally – straight from the teat – has been discovered by New Zealand scientists.

The cow's milk is low in saturated fat but high in protein, according to the researchers. It is also high in omega-3 oils, which have been linked to improved brain power and mental wellbeing, as well as decreased incidence of cardiovascular disease.

Mutant Marge

A Friesian cow called Marge is at the centre of the breakthrough. Discovered in 2001 by New Zealand-based biotech company ViaLactia, Marge has a random genetic mutation that enables her to produce milk containing significantly less fat than regular milk.

More importantly, Marge's milk also has substantially lower levels of saturated fat – a leading cause of obesity and cardiovascular disease in humans.

The researchers identified the low-fat milk in a random screening of millions of New Zealand cattle in 2001. They bought her for NZ$300 (AU$265) from

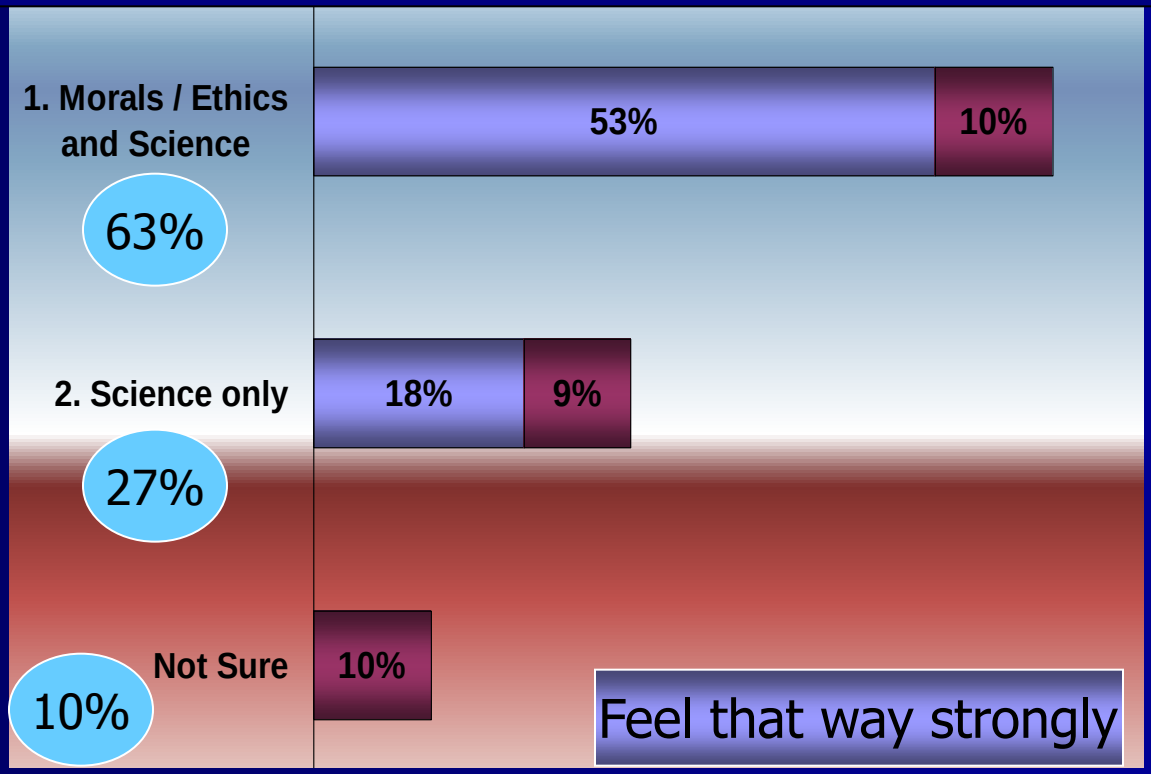
A Friesian cow that produces low-fat milk from the teat has been discovered by New Zealand scientists

Image: iStockphoto

Cloning and genetic engineering of animals is an easy target for the development of morally repugnant and powerful imagery

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.

Animal cloning regulations that included ethical considerations in Denmark and Norway resulted in the prohibition of cloning for food and ag purposes.

Ethical considerations were therefore given 100% weighting in these decisions

"Birth of cloned calf poses test for Europe's food safety regulations"

"**January 2007.** Reacting to reports that a cloned cow from the United States has birthed a calf on a British farm, virtually all major British grocery chains have pledged to boycott meat from clones or their offspring.

Tesco, Wal-Mart's Asda chain, Morrisons and Marks & Spencer were among the chains participating in the boycott, which would include meat, milk or "anything else from clones or their offspring," according to an Asda spokesperson."

How can you test/trace/verify an identical copy?

SUMMARY

- No GE or SCNT cloned food animals are currently on the U.S. market
- FDA will regulate 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 unique 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