Clone on the range: What animal biotech is bringing to the table

Biotech animals don't have the market value of biotech drugs and potential controversies loom large, but genetic screening technologies are already finding a market, and both cloned and transgenic animals may soon be on the menu. Alan Dove reports.

The EnviroPig doesn't need supplementary phosphorus in its feed, sparing costs and the environment. (Courtesy of MaRS Landing.)

Just in time for last year's winter holiday feasting season, researchers published the full genome sequence of the red jungle fowl *Gallus gallus*, the ancestor of all domestic chicken breeds[1]. As the first complete genome sequence for a major food animal, the work on the chicken, and the ongoing cattle genome sequencing project, have also inspired speculation about the future of animal biotech. With genetically engineered plants already a staple part of the food supply—more than 90% of the soybeans and half the corn grown in the US last year were genetically modified—can we expect biotech to reach the meat course soon?

An uncertain future

Even within the industry, the question is highly charged. Many academic and corporate researchers are still wary of the combustible mix of rapid scientific advancement, entrenched public ignorance and regulatory inertia that has confronted genetically engineered crops, and some worry about even greater blowback when the technology reaches food animals.

In addition to regulatory uncertainty, farm animal engineers are being held back by the shortage of funding in the field. Basic research continues to receive a trickle of funding from government agencies, and investment capital for startups is virtually nonexistent. The venture capital firm Burrill & Company, located in San Francisco, is one of the few players in this field, and spearheaded an effort to find funding for the bovine genome project. Despite the highly touted genome sequencing efforts, investor interest in barnyard biotech remains tepid.

Nonetheless, a few intrepid companies are already working their way into the field (**Table 1**). Barnyard biotechnology requires a different approach to business, regulations and technology than the more familiar turf of biologic drugs, but for better or worse, some early applications are already on the table.

A diet of chicken feed

Traditionally, agricultural science has been the poor cousin of biology, and animal biotechnology has followed that tradition. Even sequencing the genomes of chickens and cattle, arguably two of the world's most important sources of food, required a major campaign for funding. For companies hoping to profit from the new trove of genomic data, the hurdles get even higher.

"If you're producing animals for agriculture, you don't have that 15- or 20-year large return that you'd get out of a pharmaceutical application. If you look at where work is being done for agricultural animals, it's not being done in companies because they can't afford it, and it's being done at actually fewer and fewer university and government research labs," says James Murray, a professor of animal science at the University of California, Davis.

Indeed, although a riches-to-rags history is typical in biotech, work on farm animals has endured a particularly extreme shakeout. "If you look at who started trying to make animals for agriculture in 1982 or 1983, and you look at who's left, there's a lot fewer of us," says Murray.

The hardy companies that persist in the field are developing products that fall into two broad categories: genetic profiling tests for traditionally bred animals, and cloned or genetically engineered animals. The approaches are very different, but the business of selling them runs into similar problems. One of the most confounding is the wide variation in the way different animal products are produced, even from the same species.

Who's your daddy?

One thing meat producers would certainly buy is a reliable way to predict which animals will taste best. For example, it is currently hard to tell whether a particular beef cow is destined for a prime-grade steakhouse or a public school cafeteria until it has been slaughtered and sliced. In principle, genetic testing could guide breeding programs to give producers much greater control over their products.

"I think with genomics applications the big challenge is really understanding how allelic variation across many genes makes a phenotype," says Murray. Although that question will take decades to answer fully, early efforts are already yielding applications. According to Murray, "[T]here are far more people doing [genetic] typing than used to. People are willing to pay to know what their animals have."

One of the easiest traits to analyze is an animal's parentage, and in the beef industry that can be critical. As anyone who has visited a supermarket knows, the Angus breed of cattle is prized for its meat quality, but determining whether a particular cow is truly an Angus is largely a matter of educated guesswork. Austin, Texas' Viagen recently capitalized on this with a genetic test that generates a percentage-Angus score for any bovine blood or tissue sample. The beef producer Premium Gold Angus Beef, also located in Austin, recently signed a contract with Viagen, and plans to start selling genetically certified Angus steaks soon.

Table 1 Animal biotechnology companies

Name	Technology	Funding
AquaBounty Waltham, Massachusetts	Genetic screening of fish breeding stock; transgenic fish expressing growth hormone for rapid growth.	Private investors, revenue from anti-infectives.
BoviBank As, Norway	Sampling Norwegian dairy cattle and building linkage maps to identify genotypes of marketable traits.	Partnering with Center for Integrative Genetics at the Agricultural University of Norway and an unnamed American biotechnology company. Owned by two large Norwegian agricultural businesses, TINE, a dairy company, and GENO, a breeding company.
Cyagra Elizabethtown, Pennsylvania	Somatic cell cloning and preservation of cattle. Launched pig cloning business in 2002. Develops cell lines, maintains genetic banks, clones cattle as service and source of revenue.	Privately owned.
MaRS Landing Guelph, Ontario	Transgenic pigs expressing phytase in salivary glands, to break down dietary phosphorous and reduce phosphate waste.	Ontario Pork, Ontario Ministry of Food and Agriculture, Food Systems Biotechnology Center (University of Guelph), Rural Economic Development Program of the Ontario Government, The Advanced Food and Materials Network.
MetaMorphix Beltsville, Maryland	Lineage tracing of beef cattle; SNP screening to identify markers of high-quality beef.	Cargill, Cattlemen's Beef Board check-off program, American Angus Association.
Pyxis Genomics (formerly AniGenics) Chicago, Illinois	DNA traceability system allows for tracking animals and meat products from distribution to consumption. Genetic trait tools to identify in advance animals with superior production and meat characteristics.	Burrill & Company (San Francisco), Foragen Technologies (Guelph), Champaign-Urbana Venture Fund (Champaign, Illinois), AgWest Bio (Saskatoon, Saskatchewan).
ViaGen Austin, Texas	Lineage tracing of beef cattle; somatic cell cloning to propagate desirable breeds of cattle or pigs; genetic screening of shrimp to select disease-resistant strains.	Exeter Life Sciences Company (Phoenix, Arizona); strategic partnership with SmithField Foods.

"We're testing both live animals and meat," says Sara Davis, president of Viagen. She adds that "we haven't had anyone walk in with a steak and say 'I want this tested,' but we are marketing to grocery stores and high-end restaurants."

Beltsville, Maryland-based Metamorphix is Viagen's chief competition in the beef-typing business, and is also developing tests for breed identification in other species. Metamorphix is now pursuing an ambitious project to identify single nucleotide polymorphisms (SNPs) that are associated with high-quality meat, part of a contract with food giant Cargill, located in Minneapolis, to test beef cattle. Meanwhile, Pyxis of Chicago now markets tests to trace the lineage and predict the quality of pigs and dairy cattle.

Being able to separate prime beef from dog food before slaughter can obviously help a producer's bottom line, but improving the original breeding stock can have an even bigger impact. Several companies and academic researchers are already using genetic testing to augment traditional breeding programs.

At Viagen, the major breeding program is in shrimp, where the company is identifying disease-resistance genes and then selectively mating the animals that carry them. Shrimp farming is big business, particularly in the Pacific Rim, and growers are willing to pay a premium for disease-resistant strains.

Other species are also being targeted for this type of improvement. Researchers at the University of Minnesota, Minneapolis St. Paul are trying to identify genes in the turkey (*Meleagris gallopavo*) that confer such marketable traits as large breasts and resistance to *Salmonella*. Domestic turkeys are particularly attractive candidates for improvement, as current inbred strains have been selected so aggressively for large breasts that they have difficulty walking and mating. Isolating the genes responsible for good meat quality and yields could allow scientists to develop healthier breeds that are still economical to raise.

Rodeo clones

Unfortunately, efforts to improve a breed's edibility often run into a literal chicken-and-egg problem: the definitive assay of meat qua-

Box 1 What's in Soylent Green?

A 2003 meeting of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) took up the topic of genetically engineered food animals. The FAO and WHO experts recommended "... participatory deliberation by all stakeholders and the general public, starting at an early stage, including communication about potential benefits, risks and uncertainties posed by the genetic modification of animals."

Unfortunately, with the technology's early stage receding rapidly in the rear-view mirror (see main text), the general public remains almost completely ignorant about genetically engineered foods, even well-established vegetable crops. A focus-group study by the Washington, DC-based Pew Initiative on Food and Biotechnology, released in early December, underscored the problem. Some participants thought all Angus beef was genetically engineered, whereas others saw biotech crops as "foods that don't come from dirt." Many expressed suspicion and distrust of the companies developing engineered crops.

Meanwhile, biotechnologists' views have become increasingly sophisticated. "I want to make sure there's a really clear line between cloning and transgenics. Cloning is not genetic modification, [clones are] identical twins, so there's no need or reason to ever have them [labeled]," says Sara Davis, president of Viagen. The argument may persuade molecular biologists, but Davis readily concedes that such distinctions will be lost on the general public.

In reality, many experts suggest that biotechnology-enhanced meat may slip quietly into the food supply much as genetically engineered plants did, laying the groundwork for later public backlash and recriminations.

Box 2 Gene wranglers and the law

With genetically screened meats already reaching supermarkets and animal breeders pondering somatic cell cloning and transgenesis, regulators responsible for food safety have a lot on their plates.

Some of the issues are straightforward. For example, John Matheson III, senior regulatory review scientist at the US Food and Drug Administration's Center for Veterinary Medicine (FDA) says "There are no FDA regulations relating to the genetic screening of animals. Consequently, labeling depending on the results of such screening is not being contemplated." Ominously, though, Matheson adds, "You will need to ask USDA [US Department of Agriculture] if they have any jurisdiction relating to genetic screening of animals."

As anyone who followed the story of genetically engineered crops knows, having multiple agencies share responsibility for a product can produce a bureaucratic quagmire. Still, Matheson insists that "FDA is participating with other federal regulatory agencies in order to determine the most appropriate, science-based approach for regulating products derived from GE [genetically engineered] animals."

Although it is too early to tell what the approval process will be for biotech-enhanced farm animals, some experts estimate that the FDA, at least, may release a preliminary hint sometime in 2005.

lity renders the animal useless for mating, even if it was cooked rare. Biotechnology offers an obvious solution to this problem, in the form of somatic cell nuclear transfer cloning.

"The next push will be on the cloning side. The impact will be seen first in the marketplace in hogs, just because of the shorter generation interval, but we will be marketing aggressively in both beef and pork," says Viagen's Davis. Unlike most companies in the field, which are specializing in either genetic testing or genetic engineering, Viagen is working on both. Besides permitting a producer to duplicate a particularly tasty carcass, the company also plans to use somatic cell cloning to accelerate traditional breeding, "just taking the very top animals identified through traditional means and just making more of them," says Davis.

Nuclear transfer is far more expensive and far less efficient than the traditional method of making more animals, but it may reduce the number of animals required to maintain a purebred line. Hog producers, for example, typically reserve about 20% of their boars for breeding to perpetuate the line. According to Davis, strategic use of cloning technology could reduce that to 2% while still giving producers the flexibility to ramp up production in response to changing demand.

Those numbers sound promising, but animal growers will likely withhold judgment until the technology proves itself in the marketplace. So far, the reported results from animal cloning experiments provide ample cause for skepticism. With production efficiency generally below 2%, and complications ranging from oversize embryos to anatomical defects, clones may be a hard sell in some industries.

Animal growers in other industries may be more tolerant of side effects, but public perceptions will still be a concern (**Box 1**). Besides worrying about animal welfare, opponents of cloning farm animals have raised concerns about the loss of genetic diversity in the world's major food species. Companies and researchers working on the technology respond that its impact should be no greater than selective breeding or artificial insemination, but the outcome will clearly depend on how somatic cell cloning is applied.

Salmon splices and pigs without the p

Testing and propagating traditionally bred animals is useful, but biotech is best known for its ability to make specific modifications to an organism, especially by inserting useful transgenes or deleting undesirable DNA segments. For farm animals, dozens of possibilities have been suggested, from altering prion proteins to make scrapie-resistant sheep to inserting growth hormone genes to grow giant beef cattle.

Some of these schemes sound promising, but at the moment, there are only two genetically engineered food animals designed for human consumption. Their histories show the varied approaches animal biotech companies are taking in this field.

The most famous engineered food animal is the transgenic salmon developed by Waltham, Massachusetts-based AquaBounty. An outgrowth of basic marine biology research begun in the 1970s, the AquaBounty approach employs a growth hormone gene from Chinook salmon spliced to a constitutive promoter. Whereas wild-type fish induce growth hormone expression seasonally, transgenic fish carrying the new construct express the protein year-round, significantly boosting their growth rates. AquaBounty has produced salmon with the trait already, and is now applying the trait to other fish that are raised in aquaculture farms, including trout, tilapia and turbot.

It would be difficult to find a more controversial application for transgenic animals. Even ordinary fish farming is a hotly debated topic among environmentalists, who are torn between the need to reduce fishing pressure on overharvested wild stocks and the substantial problem of pollution from fish farming enclosures.

Genetically engineered fish add the danger of gene escape, since farm-raised fish routinely get out of their enclosures and mate with their wild relatives. AquaBounty has taken precautions to minimize the risks, but the technology has nonetheless been a lightning rod for criticism of genetic engineering. Since faster-growing fish would primarily benefit the fish-farming industry rather than directly help consumers, the company faces an uphill public relations battle even if it clears the regulatory hurdles (**Box 2**).

The other transgenic farm animal is a somewhat easier sell. The EnviroPig, developed by researchers at the University of Guelph in Ontario, carries a gene for phytase driven by a salivary gland-specific promoter. Phytase breaks down phosphates in the pigs' food, reducing phosphorous excretion in the animals' waste by 30%.

"Phosphorous is the number one pollutant from pig farms generally," explains John Kelly, executive director of MaRS Landing, a consortium of the University of Guelph, the city of Guelph and Ontario Agri-Food Technologies, adding that "if you have a highly concentrated swine production area where phosphorous is an issue, this is a great technology." Cleaner pig farming would be a boon for many countries, but would be especially useful in China, where 1.3 billion people share space with half of the world's pigs.

MaRS Landing is now in preliminary discussions with regulatory agencies, and the company has analyzed the animals' meat extensively and found it indistinguishable from ordinary pork. Nonetheless, Kelly insists that the scientists will await clearance from the regulators before performing the final quality-testing experiment. Only then will the company be able to bring home the EnviroBacon.

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1. International Chicken Genome Sequencing Consortium *Nature*, **432**, 695–716 (2004).

Erratum: Agbio groups join BIO

Jeffrey L. Fox
Nat. Biotechnol. **22**, 1493 (2004)
Erratum: *Nat. Biotechnol.* **23, 117 (2005)**

The erratum incorrectly stated that "CropLife International…sometimes receives funding from CBI." In fact, CropLife International provides funding to the Council for Biotechnology Information (CBI), not the other way around.

Erratum: Chasing biotech, state by state—winners and losers

Ken Howard Wilan
Nat. Biotechnol. **23, 175–179 (2005)**

On page 178, paragraph 2, line 7, it was erroneously reported that "Rockefeller University (New York, NY, USA) still doesn't have a tech transfer office." The university has had a tech transfer office since 2000.

Erratum: Clone on the range: what animal biotech brings to the table

Alan Dove
Nat. Biotechnol. **23, 283–285 (2005)**

On page 285, last column, paragraph 2, last line, the reduction in fecal phosphorus was reported as 30%. It should have read 70%.

Corrigendum: Problems in monitoring horizontal gene transfer in field trials of transgenic plants

Jack A. Heinemann & Terje Traavik
Nat. Biotechnol. **22, 1105–1109 (2004)**

On page 1108, paragraph 1, line 7, reference 49 in the statement "*B. thuringiensis* has 'a significant history of mammalian pathogenicity'[46] and is thus not irrelevant to food safety or other environmental issues" was inappropriately cited (reference 46 states: "*Bt* does not have a significant history of mammalian pathogenecity".) The text should have read that "*B. thuringiensis* belongs to a closely related clade of bacteria, which includes *Bacillus cereus* and *Bacillus anthracis*, and which has a significant history of mammalian pathogenicity[1,2] and is thus not irrelevant to food safety or other environmental issues. Members of this group are so closely related that they may be considered members of the same species, often differing only by the presence or absence of certain plasmids[3,4]."

1. Helgason, E., Caugant, D.A., Olsen, I. & Kolsto, A.-B. Genetic structure of population of *Bacillus cereus* and *B. thuringiensis* isolates associated with periodontitis and other human infections. *J. Clin. Microbiol.* **38**, 1615–1622 (2000).
2. Økstad, O.A., Hegna, I., Lindbäck, T., Rishovd, A.-L. & Kolstø, A.-B. Genome organization is not conserved between *Bacillus cereus* and *Bacillus subtilis*. *Microbiol.* **145**, 621–631 (1999).
3. Helgason, E. *et al.* *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*—one species on the basis of genetic evidence. *Appl. Environ. Microbiol.* **66**, 2627–2630 (2000).
4. Hoffmaster, A.R. *et al.* Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax. *Proc. Natl. Acad. Sci. USA* **101**, 8449–8454 (2004).