Biotech breeding goes bovine

Dairy farmers are rapidly adopting molecular profiling to accelerate the process of siring cows. But this seismic shift in breeding practices is raising new questions and translating more slowly to the beef industry. Stephen Strauss reports.

Following last year's publication of the *Bos taurus* genome sequence[1], the dairy industry has wasted little time in assimilating cattle genomics into its working practices. Only a few months ago, Illumina of San Diego announced the creation of a new bovine single-nucleotide polymorphism (SNP) chip with ten times the coverage of an earlier version. The chip's predecessor had already been leapt upon by breeders keen to integrate the new genomic information into their siring practices. But although uptake of the technology has been rapid, questions remain concerning the ability of marker-assisted breeding programs to ultimately predict complex traits, such as meat quality or even milk composition and yield, and the long-term effects of such tests on the meat and dairy industries remain unclear.

The genetic cream

In the summer of 2008, a group of senior managers at the Shawano, Wisconsin–based artificial insemination (AI) company Genex Cooperative huddled together to discuss the possible effects on their future business of the recently released 54,000-SNP cattle genome chip known commercially by the rather awkward name BovineSNP50 BeadChip.

The question of the day was, would their dairy farmer customers buy semen from Genex's bulls who hadn't first been 'proven'? Obtaining an accurate prediction of genetic value—what is called a sire proof—is a half-century-old procedure. In it, upwards of 100 randomly selected cows are artificially inseminated, give birth, and—when their calves grow old enough to produce milk—the offspring are tested to see if they and their milk exhibit desirable traits. A genetic prediction based on roughly 100 daughters will generally result in >90% predictive accuracy. The drawback is that the process is expensive and time-consuming, taking 5 or 6 years to complete and costing up to $50,000. Even more disconcerting is that only about one bull in ten that go through this process is eventually judged genetically superior enough to qualify as a high-quality stud.

The Illumina BovineSNP50 BeadChip had only a ~65–70% accuracy rate in trait inheritance prediction. Even so, it allowed markers associated with high-quality traits to be viewed at birth, providing substantial benefit over the traditional sire proof procedure. The Genex debate was about how many clients would value genomics' greater speed over the greater accuracy of 'being proven'. The consensus, says Roy Wilson, technology development manager for Genex, was that the intrinsic conservatism of farmers meant that, at best, in the short term, only 15% of the company's business would switch over to unproven, but genomically highly promising bulls.

They were not even close

"In the year to date, around 40–45% of our sales are from sires with no milking daughters," said Wilson in mid-December of 2009.

What this number doesn't capture is the dramatic reconfiguration of the breeding business that took place from being able to get semen to market in one-third the time.

In 2008, Genex was progeny-testing bulls in 2,000 herds. By the end of last year, this had shrunk to 160 herds. Two years ago, 300 bulls were sent through progeny testing; with the advent of the Illumina SNP profile predicting those sires with a greater likelihood of carrying desired traits, only 180 to 200 bulls were judged candidates worthy of being further tested. And the one in ten bulls who potentially might bear the title 'superstud' had through genetic prescreening grown to become one in five bulls.

A revolution waiting to happen

The uptake and implementation of genetic profiling by breeders is nothing short of astonishing, particularly to many working in or with the dairy industry. "In dairy cattle, the rate of adoption of this technology has been breathtaking," says Stephen Moore, a University of Alberta professor of agricultural genetics, who has been working with agricultural genomics companies to identify SNPs that contain genetic traits that are important for farmers. "No sooner was the chip designed than it was being used," he adds.

"We are in the front end of a major change in raising cattle. Some people are using words like 'disruptive' and 'quantum leap' to describe what is happening," says Ronnie Green, senior director of global technical services, at Pfizer Animal Genetics in Kalamazoo, Michigan, which has been selling a DNA screening test for desirable traits in cattle (**Table 1**). "Whatever word you use, we are in a time of real upheaval."

The reasons the technology has proven revolutionary are varied. In part, the change has taken place in dairy farming, because the infrastructure was already in place to rapidly integrate genomic data. Because a single bull is tremendously valuable in terms of siring many cows—and therefore has a massive impact on the genetics of a dairy herd—for several decades farmers, breeding organizations and AI companies have been collecting as many bull surface (phenological) traits as they could (e.g., vigor and haunch size) that might be linked to subsequent milk production and other useful traits in their daughters.

Select Sires, Plain City, Ohio

Genomic gold. Observer, deemed the top Holstein bull according to his genetics, is the gold standard for bulls in the US.

According to Curt Van Tassell, a US Department of Agriculture (USDA) research geneticist based in Beltsville, Maryland, who has actively worked with companies trying to make use of the new genomic information, literally millions of data points have been gathered in the US and Canada since records were started 40 years ago. Data on 16 million dairy cows are part of a collection that

Table 1 Selected companies with a focus in agricultural genomics

Company	Technology	Product	Status
Affymetrix	10,000 SNPs from bovine genome sequencing project (92%) and Australia's Commonwealth Scientific and Industrial Research Organisation (8%)	GeneChip Bovine Mapping 10K	On the market
Igenity	Measures genetic component of 15 desirable traits	Ingenity Profile (dairy)	On the market
		Angus Genetics	
Illumina	54,001 highly informative SNPs uniformly distributed across the entire genomes of major cattle breed types	BovineSNP50 DNA Analysis BeadChip	On the market since 2007
	500,000 to 800,000 SNPs with information gathered from >20 different breeds	High-density Bovine BeadChip	Announced in January of 2010, first shipments in second quarter of the year
Pfizer Animal Genetics	50,000 SNPs for 14 traits	HD 50K for Angus, GeneStar MVP, GeneStar Black, GeneStar Tender Elite, SireTRACE, SureTRAK and genetic defect testing	Launched January 2010
Metamorphix (Calverton, MD, USA)	Genomic services offered for DNA-based genetic parent verification, diagnostic testing	Tru-Marbling	On the market
		Tru-Tenderness	
		DNA certified beef programs	
		Horned polled diagnostics	
Quantum Genetics (Saskatoon, Saskatchewan, Canada)	Genome manipulation to control obesity and fat deposition	Quantum Management Protocol	Under development
Genetic Visions (Middleton, Wisconsin)	Services to test for genes influencing coat color, animal health and viability, production traits	Genetic marker tests, various tests	Launched
DNA Genotek (Kanata, Ontario, Canada)	Sample collection services	Performagene Livestock	Launched

has been in existence since the 1960s at the Animal Improvement Programs Laboratory in Beltsville. The data, which are available to breeders, researchers and AI companies alike, provides a pedigree proofing-based scale that shows how much more money offspring from one bull might earn than offspring from an inferior one based on its genetics (**Box 1**). Each year, more is collated into the collection from 40–50% of US dairy cattle. Thus, a huge database of 100 or so genetically linked traits has been amassed onto which the SNPs from genome sequencing efforts can be associated.

Equally importantly, in an effort to link milk volume and quality with bull genetics, AI has become the method of choice for dairy farmers. Thus, it is employed by >80% of farmers breeding dairy herds, whereas only 7% of farmers use it for beef.

What also drove the appeal of genomic testing for dairy farmers was the fact that the majority of cows in North America and in the developed world are Holsteins. In North America, Van Tassell says, Holsteins once accounted for >95% of milk cows; even today, its herd prevalence is still >90%. This means that any anomalies due to different SNP trait locations in different breeds are eliminated.

The existing infrastructure within the dairy industry also made collaborative genomic research between companies, university scientists and the USDA easy to get off the ground. Specifically, seven AI companies, two in Canada and five in the US, joined with the USDA, the University of Alberta, the University of Missouri and Illumina to correlate SNP locations to phenotypic data. In Canada, the University of Guelph and the Canadian Dairy Network, which is in charge of national evaluations for dairy cattle, also participated.

In exchange for their financial participation and the providing of both DNA data and semen, the AI organizations were given a five-year exclusivity on the use of genomic evaluations for young bulls.

Thus, another impetus for swiftly applying the genomic findings in dairy cattle is that the AI companies had an intrinsic stake in using the milk cattle genomic information quickly while their monopoly still could convey a business advantage.

Finally, there was a Moore's Law factor. As Jacques Chesnais, senior geneticist for Semex Alliance (a dairy breeding consortium owned by four AI cooperatives in Canada) points out, the Illumina chip contained twice as many traits and sold for half as much as its main competitor from Affymetrix of Santa Clara, California.

Where's the beef?

While genomic information is transforming the dairy sector, the situation is very different for beef breeders. There are several reasons for the disparity.

One is that the diversity of beef cattle is greater than that of dairy. "There are lots and lots of variations between breeds," says Moore, who is in the process of genotyping Angus and hopes to begin to do the same thing for a bull breed from the tropics. "An allele that might be a good predictor of trait in one breed might flip and actually become a negative indicator in another breed." This difference is a big stumbling block in the North American beef industry. Despite the predominance of three breeds (Angus, Hereford and Simmental make up 60% of the US beef herd), a substantial minority (40%) of the beef herd is drawn from over 80 breeds.

There is also a smaller amount of information available that associates meat product quality traits with SNP readings. And the list of qualities is much more diverse. Instead of milk quantity and fat composition, beef breeders must look at meat tenderness, fat thickness, ribeye area, marbling and yield grade among numbers of other things.

There is also a price differential growing out of the fact that while dairy cows generate income over their milk-producing lifetimes, beef cattle's value only occurs when they are slaughtered. Overall, meat breeders have paid much less attention to the genetic quality of bulls because the real money is made when animals are brought to feedlots and fattened up. This accounts for the low amount of AI usage in beef cattle and is a reason why beef cattle

Box 1 How much is your cow worth?

Researchers at the USDA's Animal Improvement Programs Laboratory (AIPL), in collaboration with academia and industry, have been turning the art of calculating the value of cattle into a science long before SNP data from chips. A cow's so-called net merit weighs traits that produce income, like milk fat and protein production, against those that cost the rancher, like the cost of feed consumed by a calf before she reaches milking age. The data come from performance records that have been collected on dairy farms for over 30 years, pedigree data provided by farmers and breed associations and, since 2009, SNP data from the Illumina BovineSNP50 BeadChip.

The average cow born in 2005 is used as a reference point, which is called the base population, and has a net merit of $0. Cows with positive values will generate more profits relative to the base population, and those with negative, less. The top Holstein bull as of April 2010 is named Observer (see photo) and has a net merit of +$848, which means that his daughters will each earn $848 more during their lifetimes (on average) than daughters of an average bull.

The US was the first to incorporate SNP chip data, according to John Cole, research geneticist at AIPL. They currently include data on 43,385 SNPs in analyzing the Brown Swiss (~1,500 genotypes), Holstein (~40,000 genotypes) and Jersey (~4,000 genotypes) breeds. The effects of each SNP are calculated for each trait, which number around 30, with some variation from breed to breed.

The SNP genotypes were originally produced by the Bovine Functional Genomics Laboratory at USDA, but that service is now provided by commercial laboratories. Owners of the animals provide a source of DNA for genotyping, and pay to have a genotype produced. The data are entered into the national dairy database and the owners of the cows and bulls receive reports about their animals. SNP effects are recalculated for each trait at AIPL as more data become available, says Cole. *Laura DeFrancesco*

growers are not interested in a genetic test that can cost somewhere between $200 and $250 dollars per animal.

Covering the bases

The high price point is one of the factors that might be addressed by ongoing innovations and improvements to the genetic tests. The race is on both to exponentially expand the number of SNPs that can be measured and lower the price.

In terms of SNP expansion, at the end of December, Van Tassell was wrestling with the problem of verifying 900,000 SNPs for Illumina in time for the company to launch a next version of their bovine chip in January. One hope is that an exponentially increased number of SNPs on a chip will allow AI and other companies to provide tracking for traits that have weaker genetic associations. "Something that has more markers has a greater statistical power in the association of traits with markers," says Mike Thompson, global manager at Illumina's animal division.

Another hope is that the chip will contain enough information to allow the disconnect between breed difference and SNP trait readings to be resolved. In January, Illumina announced it was accepting orders for a >500K chip that contains genetic data from >20 breeds of cattle.

At the same time, the San Diego–based company has let it be known that it is also going to be releasing a 3K bovine SNP chip, which is rumored to cost somewhere between $30 and $50. Here, the idea is that although there are not as many SNPs being tested, the ones that are will be of greatest interest to cattle growers and dairy farmers.

University of Alberta's Moore says he has done an as yet unpublished study using the 3K SNP chip and found "the results look a lot cleaner than the 50K one. All you do [at 50K] is increase the noise level."

But on the horizon is a holy grail of the intersection of Moore's Law and bovine genomics—a beyond cheap test. Van Tassell says he has begun working with biotech companies Fluidigm and Sequenom in pursuit of a bovine DNA test that costs $10 or less. "That's a number that resonates because it is analogous to the price of a pizza," says Van Tassell, "that value seems to be a tipping point for very large-scale adoption."

It is also a price that is low enough to encourage every cattle farmer in North America—dairy and beef—to give all of their animals a genomics profile.

Going global

To facilitate the association and mapping of traits with the increasing numbers of SNPs that appear on Illumina BeadChips, as well as to understand breed differences, many in the cattle industry believe that testing will need to expand beyond the narrowness of a locale or even a country. "One of the things that we are discovering with the application of this technology to real populations is that nobody has an adequate number of animals to characterize the sequences we are describing," says John Pollak, a Cornell University professor who is director of the National Beef Cattle Evaluation Consortium. And driven by the need to get more information, countries that formally guarded their animals' genomic qualities as a competitive advantage are coming together in the global marketplace.

For example, last October a group of European livestock associations—UNCEIA (the French National Association of Livestock & Artificial Insemination Cooperatives), CRV (an international cattle improvement organization with headquarters in Arnhem, the Netherlands), DHV and vit (a German national umbrella organization of the Holstein breeding industry and German computing center of cattle data) and VikingGenetics (Danish-Swedish cattle breeding association)—came together to form EuroGenomics. The organization is devoted to using their collective 16,000 proven Holstein bulls to increase the reliability of genomic testing.

A similar collaboration pooling the genomic information of Brown Swiss cattle found in Italy, France, Austria, Switzerland, Slovenia, Germany, Canada and the United States has recently been initiated.

A changing business

Although getting more information is good, managing all the information is another matter. One issue that has begun looming in peoples' minds is information overload. How exactly will a farmer deal with breeding and herd management decisions in a universe where complex traits are governed by hundreds of genes that may be found in numerous DNA locations?

Already there are breeding calculators that try to make this easier, but in the short term, AI companies are beginning to change their business models when speaking with farmers. Lyle Kruse, vice president of US market development for Select Sires, a Plain City, Ohio–based federation of AI cooperatives, says that increasingly they find themselves having to act as sort of genetic consiglieres for their customers.

"A lot of customers are really busy; genetics and the investment in reproduction take up a small part of day-to-day demands. They rely on us to focus on what to use and how to use it. We have a group of people who are mating evaluators. They go out and actually break down a cow into 16 traits. They will do a customized mating for a herd based on sire selection and traits farmers want to focus on," he says.

This customized genetic counseling feeds into the question of exactly what will the future of all AI companies be when the five-year monopoly they have on bull semen genomics runs out. One model sees individual farmers discovering what they didn't know before—that one of their bulls or cows by genetic chance carries a highly desirable mix of genes. The question is, would and could that farmer sell semen or flushed eggs directly to other farmers and circumvent the AI industry middle men entirely?

Part of the lure of doing that is price. In the US, companies charge $13 or $14 for a 'unit'—the amount of semen it takes to inseminate a cow. Kruse says that it only costs ~$2 or $3 to harvest that unit. Although cooling the semen with liquid nitrogen clearly raises this cost, Kruse suggests that after the semen genotype monopoly runs out, individual farmers or groups of farmers are likely to compete with existing AI corporations. "The bottom line is that a lot more private individuals will sell semen from specific bulls."

A somewhat similar challenge may arise for existing genomics companies, particularly Illumina, Affymetrix and other chip manufacturers. After one year, the SNP information that underlies the applications, which came through collaboration with the USDA, becomes a matter of public record. This means other companies could use the information to create SNP chips that undercuts their price or better their results.

"They can do it in theory," admits Rob Cohen, senior market manager for applied markets for Illumina. He says the specter of this is forcing his company to continue their innovation efforts at breakneck speed.

And then there is the possibility that the simplicity of genetic testing might undermine the programs which today link phenotypes with SNPS associated with desirable traits. If genomics tells farmers with greater reliability what traits have been passed on, there may be less incentive to gather trait information. "We can lose the tests that actually help maintain the accuracy of the genomic data," says Kruse about this paradox.

A final problem is the issue of inbreeding. If the genes from prize bulls and prize cows get into herds in a third or half of the time it previously took, then bad gene combinations can enter into breeds much faster than before. Even before wide implementation of SNP marker-assisted breeding strategies, scientists at the USDA and University of Guelph found that 30% of the Holstein's genome has been shaped by human breeding. More troubling still has been their observation that many of the same SNPs that are associated with higher milk production also seemed tied to lower cow fertility.

Although Green points out that with the new DNA tests "for the first time we actually have a way to measure inbreeding," Chesnais and others argue that knowledge isn't necessarily the same as the wisdom to do the right thing. "The competitive pressure in this industry is tremendous and farmers are used to wanting semen from the very best bulls. Unless enough caution is exercised, genomics could accelerate this trend and lead to a more rapid decline in the genetic diversity of the breeds we work with."

Whereas a restricted sire pool might in the long term decrease Holstein variability, it is difficult for any single company to simply start doing the right thing genomically speaking. "All the breeding companies are competing with one another and the way to compete is to breed the best of the best, even if it may not be the most desirable approach in the longer term," Chesnais adds.

Healthier prospects?

Although marker-assisted breeding has been the emphasis until now, the great hope is that SNP information, integrated with other genetic information, will prove useful to animal husbandry more generally. "What we are looking for and what we think has a much greater application than just breeding is what I would call marker-assisted management," says Stewart Bauck of Merial's Igenity, in Duluth, Georgia, which produces a genomic profile of both beef and dairy animals.

Here, the idea is not simply to select the best cows to breed, but to drill down further and see what an individual animal's genetic make up tells you about how to treat them. What food would make a beef cow put on weight the fastest? Are there different strains of the same breed that would thrive better in Alberta than in Arizona? Would some animals respond better to a medication than others?

Although their present lack of good predictive value means trait-specific tests haven't experienced anything like the explosion of interest that followed the Illumina BovineSNP50 BeadChip's release, companies such as Pfizer, Igenity and others have started marketing tests looking at specific traits, mainly in beef cattle.

In the meantime, marker-assisted technology is already starting to save dairy farmers money. Brad Sayles, vice president for global marketing at Semex in Madison, Wisconsin, says that semen from unproven but genomically validated bulls sells for anywhere from $15 to $30 less per dose in Canada than doses of proven bulls' semen. As it takes an average of four doses to impregnate a cow, this means that for each 100 cows, Canadian farmers can now save between $6,000 and $12,000 yearly.

It's also starting to earn those animals with good genomic profiles more money. Kruse says that when pedigrees were all breeders had to go on, they paid $3,000–4,000 to buy a promising bull. Now that it is easier to separate future winners from losers on the basis of a genetic profile, the price has gone up to somewhere between $6,000 and $14,000.

Even so, there is caution as people move ahead with a technology that is only just now beginning to bear fruit in terms of animals mature enough to produce milk. Carl Loewith, who with his brother and son, run a dairy farm with 330 milking animals and 700 cattle in toto in Ontario, has begun inseminating their cows with semen from unproven bulls. However, because the risk of a dud sire is higher than with proven semen, they have been following the cautions of the AI companies, who advise against taking all semen from the same bull, at least in the short term. "We are told because there is still a bit of unreliability you should pick groups of bulls, maybe five or so, because one or two might not live up to their genomic potential."

Nonetheless, with cheaper marker tests on the horizon, a wider piece of the genomics industry has started quite literally knocking at farmers' doors. "Just last week a person came by test marketing a DNA kit that wasn't yet on the market. You could just take a swab from the cow's nose and put it into a solution or a test tube and get a reading," says Loewith. Those knocking apparently got a positive reception; in January, DNA Genotek of Ottawa, Ontario, released a nasal swab DNA test for cattle, sheep and swine.

Stephen Strauss, Toronto

1. Anonymous. *Nat. Biotechnol.* **27**, 487 (2009).