What the bovine genome project means to New Zealand

J.C. McEWAN and A.M. CRAWFORD

Animal Genomics, AgResearch, PB 50034, Invermay Agricultural Centre, Mosgiel, New Zealand

ABSTRACT

The sequencing of the bovine genome commenced in December 2003 and sequencing is planned to achieve a seven-fold coverage by November 2005. This along with previous physical mapping efforts will allow a high quality genomic assembly to be produced. The sequencing is being undertaken by Baylor College of Medicine in Texas. Separately, the Michael Smith Genome Science Centre in Vancouver will sequence 10,000 full length non-redundant bovine mRNAs. The assembled genome will be annotated in collaboration with the Ensembl project at the Sanger Institute, UK, and will be publicly available on genome browsers such as NCBI, Ensembl and UCSC. In the interim the raw sequence information is being made available at NCBI within days of being generated. As well as providing DNA sequence, the program will identify more than 50,000 microsatellites and one to two million SNPs. The availability of this information will transform and accelerate ruminant genomics research. Similar genome sequencing efforts in human and laboratory species have greatly increased the rate of gene variant discovery underlying quantitative trait loci. It will also aid our understanding of gene expression control. New Zealand stands to benefit directly from this work, because of its strong reliance on grazing livestock for export income and its internationally competitive beef and dairy industries. The project is funded by an international consortium involving CSIRO Australia, Genome Canada, New Zealand, National Human Genome Research Institute of the U.S. national Institutes of Health (NHGRI), U.S. Department of Agriculture (USDA), the State of Texas, The Kleberg Foundation, U.S. Beef Council, and Texas and South Dakota Beef Councils. Significantly, the National Institutes of Health have strongly supported the project as a consequence of its human health implications. This project benefited greatly from a previous international genomic collaboration to physically map the bovine genome, and it provides an excellent example of benefits obtained from international research consortia co-investing in public domain infrastructural projects.

Keywords: bovine genome; DNA; map; Baylor; QTL.

INTRODUCTION

Biological research of New Zealand's forage-based ruminant livestock, underpins the current competitive edge of these industries in the international marketplace. Over 30% of New Zealand's current export income is derived from cattle, sheep and deer derived exports. Their productivity has been rapidly increasing over the last decade as a result of rapid technology uptake. Increasingly, biological research in animals and plants depends on a knowledge of and access to the genome sequence of the species. It is only 4 years ago since the first draft of the human genome was publicly released in June 2000. The benefits of access to this information have been immense, and it has greatly aided all mammalian research. However, it is obvious that many of the high throughput genomic technologies developed for human and associated laboratory species will require access to the genome of the species of interest if they are to operate efficiently in farmed species. Of the major livestock species: chicken, pig, cattle, and sheep, both chicken and cattle are currently being sequenced and pig is likely to be sequenced in the near future. The objective of this brief review is to outline the history of bovine genomics research, describe the current work including the bovine genome sequencing project, and identify what will be the immediate research outcomes and the likely longer term benefits to New Zealand's forage based livestock industries.

HISTORY

Genomic cattle research has vastly expanded from it humble beginnings in the mid 1980s. Perhaps the first organised international efforts to provide genomic tools in cattle were the creation of linkage maps in the mid 1990s based largely on anonymous microsatellite markers (Barensde *et al*., 1994; Bishop *et al*., 1994). These have been followed by radiation hybrid based physical maps (e.g. Band *et al*., 2000), which eased the process of comparative mapping of genes while also ordering and orientating closely spaced markers. More recently, even finer scale physical maps are being created using restriction fragment fingerprinting of bovine BAC libraries (Schibler *et al*., 2004) and BAC end sequencing (Larkin *et al*., 2003). These initiatives were accompanied by a number of EST sequencing projects (e.g. Smith *et al*., 2001) and subsequent creation and use of bovine expression arrays with the ability to measure the expression level of many genes at once from a given tissue sample (e.g. Band *et al*., 2002).

Within New Zealand, over the past five years, there has been a considerable investment in proprietary resources, largely bovine ESTs with separate initiatives by Via Lactia and a consortium of AgResearch, Genesis Research and Development Corporation and Victoria Department of Primary Industries. These resources currently involve several hundred thousand ESTs, their

clones, and associated expression arrays derived from them (e.g. McEwan *et al*., 2002; 2003a; 2003b). Associated, but preceding this work was quantitative trait loci (QTL) experiments in cattle, sheep and deer. This work was undertaken by AgResearch and also Livestock Improvement Corporation in conjunction with Via Lactia. QTL are polymorphic variants that affect continuously variable traits like growth rate or carcass composition. Inclusion of genetic markers in breeding programs to trace: identity, parentage and genetic performance of these traits is likely to be one of the first major outcomes of genomic research.

Much work remains unpublished, because of commercial sensitivities, but the number of publications describing QTL in cattle is now rapidly increasing with PubMed listing 135 refereed articles relating to cattle, of which 100 have been published since January 2000. These include numerous reports of QTLs for dairy traits, (reviewed by Khatkar *et al*., 2004), reproduction (Arias & Kirkpatrick, 2004), carcass quality and meat quality traits (e.g. Stone *et al*., 1999; Kim *et al*., 2003; Casas *et al*., 2004; Li *et al*., 2004), and disease (e.g. Hanotte *et al*., 2003; Rupp & Boichard, 2003) as well as many other traits including coat colour and behaviour.

However, until recently the laboratory resources available, including the number of microsatellite and single nucleotide polymorphisms (SNPs), and the lack of genomic sequence, meant that fine mapping and identifying the underlying variants was a difficult and laborious task. Successful efforts were typically often spiced with an element of serendipity. Even given these limitations, discovery of gene variants underlying QTL has been increasing rapidly including: double muscling in cattle (Casas *et al*., 1998; Kambadur *et al*., 1997), tenderness in beef cattle (Smith *et al*., 2000; Page *et al*., 2002), and genes affecting milk composition (Grisart *et al*., 2002; Blott *et al*., 2003).

The availability of the bovine genome sequence will markedly speed up and reduce the cost of the gene variant discovery process for QTLs. This is supported by the results observed in human discoveries where Korstanje & Paigen (2002) documented an exponential increase in the discovery rate whose commencement coincided with human genome availability.

PROGRESS

The first phase of the sequencing of the bovine genome was the creation of a high quality physical BAC map. This integrates the existing linkage and radiation hybrid results with a large scale BAC fingerprinting and end sequencing initiative. The majority of this work was carried out by the International Bovine BAC Mapping Consortia (IBBMC) and preliminary results were recently presented (Chiu *et al*., 2004) and a formal publication should be available later this year. The fingerprint map consists of 294,000 BAC clones, representing a 15.8-fold coverage from three libraries and these clones have also been end sequenced and deposited in Genbank (http://www.ncbi.nlm.nih.gov/). The fingerprint map itself is available at http://www.bcgsc.ca/lab/mapping/bovine . New Zealand supported part of this work via AgResearch's involvement in BAC end sequencing at TIGR in association with Dr Shaying Zhao.

This resource was then used for the bovine genome sequencing project itself. The original proposal and justification to NIH by Gibbs *et al*. (2002) outlined the strategy to be used. Further details are available in the press release when the project commenced in December 2003 (Anon., 2003). Briefly, using the physical map previously described, a minimal tiling path was selected across the genome consisting of 19,000 clones using the BAC library CHORI-240 (http://bacpac.chori.org/bovine 240.htm) derived from an inbred Hereford bull. Subclone shotgun libraries of these BACs will be skimmed to 1-2 X sequence coverage and a 5-6X genome coverage will be derived from whole genome shotgun libraries, of varying insert sizes, from an inbred daughter of this bull. In all cases paired end reads will be used and the sequencing is being undertaken at Baylor College of Medicine in Houston, Texas. Additional sequence coverage will use whole genome shotgun libraries derived from female animals selected from a variety of breeds. The interim selection includes: Holstein, Angus, Jersey, Limousin, Brahman, and Norwegian Red. The actual depth each breed library is sequenced to will depend on the outcome of initial analyses. It is expected that one to two million SNPs will be discovered. Latest details and sequence can be obtained from the Baylor genome centre web site (www.hgsc.bcm.tmc.edu) and the NCBI cow resources portal (http://www.ncbi.nlm.nih.gov/genome/guide/cow/). The Canadian contribution will be used to identify and full length sequence of 10,000 non-redundant bovine mRNAs at the Michael Smith Genome Science Centre in Vancouver. Currently, age and physiological status and tissue selection criteria for the full length libraries are being developed. Contributors to the Bovine Genome Sequencing Project include: NHGRI, US$25 million; USDA, $11 million; the state of Texas, $10 million, including $2 million from the Kleberg Foundation and $0.4 million from the U.S. Beef Council and the Texas and South Dakota Beef Councils; Genome Canada, $5 million; The Commonwealth Scientific and Industrial Research Organization of Australia, $1 million and Agritech Investments Ltd., Dairy Insight Inc. and AgResearch Ltd., all of New Zealand, $1 million.

The amount of information being generated by this project relative to prior work is staggering. Table 1 tabulates the increase in the amount of information and shows that more than 90% of all bovine sequences have been deposited in the last 18 months. At the current rate of progress it is planned that data collection to 3 fold sequence coverage of the bovine genome will be achieved by late June 2004 and an initial genome assembly will be completed and released tothe public domain soon thereafter.

TABLE 1: Number of box Taurus DNA sequences in NCBI over time.

Date	Genbank	ESTs[1]	GSS[1]	HTGS[1]	Trace[1]	Total
Jan 2000	3759	256	440	0	0	4455
Jan 2001	4293	102121	740	0	0	107154
Jan 2002	4985	190209	1413	2	0	196609
Jan 2003	6328	213848	1512	108	415279	637075
Jan 2004	9339	331081	311296	221	798564	1360501
Apr 2004	10063	419345	311522	241	7012616	7753787

[1]EST, expressed sequence tag; GSS, Genomic survey sequence and sequence tagged sites predominantly BAC end sequence; HTGS, high throughput genomic sequence predominantly interim assembled BAC sequences, trace files are raw genomic reads predominantly from genome sequencing efforts. Note a portion of the trace file sequences are also present in GSS, and EST divisions.

FUTURE PROSPECTS

The bovine genome and the BAC physical mapping information is already making a major impact on current cattle, sheep and deer research in New Zealand. I will provide several examples using currently available information and also indicate likely future developments.

The first is that the availability of a high quality physical BAC map means that a minimal tiling path of BACs can be rapidly assembled and aligned to a corresponding human region of interest. Recently, this was completed in less than a day *in silico*, and after some brief experimental validation, selected BACs were sent for sequencing. Assembling a similar contig using laboratory methods has also been undertaken in our laboratory, using "overgo" probes designed from human and bovine EST alignments. The process took 6 months. A similar effort for the Booroola gene, prior to the availability of the human genome sequence, took several researchers two years.

The second example is that, even with the availability of many hundreds of thousands of bovine ESTs, many key bovine genes, which are expressed at low levels, in specific tissues, or at certain restricted times are not represented in these libraries. In the past month we have specifically looked for bovine sequence for some seven genes of interest not represented in these libraries, and even with the low current level of coverage have been able to find some useful bovine sequence for all of them. Obviously when the genome is finally assembled we will have not only the complete sequence but its exact location.

The third example is that people mapping QTL are constantly trying to find novel polymorphisms of known location for their work. This is because all QTL discovery efforts involve linkage or linkage disequilibrium mapping and these processes require polymorphic variants. A preliminary analysis of 180Mbp of bovine BAC end sequence in December last year identified 6100 putative dinucleotide, 1900 tri nucleotide and 650 tetra-nucleotide microsatellites or approximately 1 putative dinucleotide repeat every 29,000 bp of sequence. Even if only a fraction of these are validated they will provide a substantial increase in the number of currently available markers. Of even more importance is that these markers are on the whole, already ordered and orientated, meaning that only those of interest, i.e. those lying under QTLs, need to be validated. When the 7 fold coverage bovine genome is assembled it is estimated that at least 50,000 high quality putative ordered and orientated microsatellites will have been identified.

The project will also identify an extremely large number of putative SNPs, which are both ordered and orientated, perhaps of the order of one to two million in total, based on tentative calculations of observed polymorphism levels in cattle and the breed and animal sampling regime proposed. Validation of these SNPs and determination of breed frequency is a major task. This is intended to be a component of "Phase II" of the bovine genome sequencing programme. What are the advantages of all these SNPs? There are several benefits: the first is that for very fine mapping of QTL identified using existing methods microsatellites are too infrequent to be able to adequately resolve the location. The second is that SNPs provide a much easier and more accurate alternative for automated genotype scoring. This will be required for the extremely high throughput mapping experiments envisioned in the future. The third benefit is that genome wide linkage disequilibrium mapping approaches using panels of many thousand of SNPs will make it possible to utilise existing commercial populations, even where parentage information is not available. Examples of this could include selection of extreme individuals from a beef processing plant or sampling resistant animals after a natural disease challenge in commercial herds. However, currently there is no flexible high throughput platform that is also cost effective. Ideally SNP genotyping costs have to decline to less than 0.1 cents per genotype and currently they are typically at least 10-1000 fold higher. In the interim selected regional mapping and various pooling strategies will still be required.

The bovine information will also be of great value for other ruminant species including sheep. There is strong evidence that the majority of the bovine and ovine genomes are co-linear and their amino acid coding regions have approximately 96% sequence homology. In non coding regions it is approximately 90% for those genomic regions that can be uniquely aligned in both species. This means that almost all PCR primers designed using high homology bovine regions, will amplify a product in sheep, typically exons spanning small introns of less than 1500bp. Resequencing these in a number of individuals usually identifies sheep SNPs, the benefit of the bovine genome program is that much more sequence will be available and it can be much easier targeted to specific regions. Within our group this

process is commonly called TIPs (McEwan *et al*., 2001). However, the efficiency of this process outside high homology regions is poorer, with only 48% of primers designed in intergenic regions amplifying a product under standard conditions (de Gatori *et al*., 1997). This success rate coupled with small insertions and deletions make the process fraught for sequencing large ovine regions based on bovine primers. Unfortunately, due to the number of generations separating the species and their respective population sizes very few bovine SNPs identified by the genome sequencing project will be present in sheep, meaning that this aspect will have to be addressed in an ovine specific effort. However, there is considerable potential for using the more informative bovine microsatellites identified, with perhaps 38% able to be validated and used in sheep (de Gatori *et al*., 1997). Once again the positional information that comes with the bovine genome enhances their usefulness remarkably as only those in regions of interest need to be validated.

Another benefit of an annotated genome is that it can be used to create high quality oligonucleotide expression arrays. As stated previously, current bovine EST based expression arrays often have important genes missing, because they are not represented in the library. The bovine genome project and associated full length mRNA sequencing effort will provide resources that will allow much better arrays to be produced. In all likelihood this information can also be used to design effective arrays for use in sheep as well, albeit with some proviso's for rapidly evolving genes.

Much of the human effort in sequencing mammalian and related vertebrate genomes is in investigating the evolution and change of conserved features of the genome, in order to understand how the genome is controlled and what makes humans different from other mammals (Thomas *et al.*, 2003; Margulies *et al*., 2003). This includes detection of positive selection events on certain genes. Of course this same information can be examined from the perspective of what makes a cow unique. However, to be truly effective in bovine it will need more closely related species to be sequenced as well. If the pig is sequenced, this will be a major first step along that path.

Finally, the availability of BACs and clones derived from the full length sequencing component will allow their use in transfection studies and production of the proteins they contain for a variety of experimental and commercial uses.

CONCLUSION

All prior cattle and ruminant genomic research has been greatly hampered by the lack of annotated genomic sequence. This is the core resource upon which all genomic reagents, diagnostics and products are ultimately based. The sequencing of the bovine genome will overcome many of the previous difficulties and provide the underpinning information required for the next several decades of cattle research. Its utility will cover all research activities including: identifying and mapping genetic variations affecting health, reproduction, lactation, and meat production. It will lead to a better understanding of the mechanisms affecting disease, production and adaptation to extreme environments and produce new products and novel methods to utilise this information. It is also hoped that this will also identify the basis of the core genetic differences that make ruminants so successful, including the ability to digest forage. It is likely this information will also lead to a better understanding of what factors influence the release of greenhouse gases by ruminants, an issue which has significant potential impacts on New Zealand industry since the signing of the Kyoto protocol.

This work will also have flow on effects to human health, both directly via better and safer products for human consumption, and indirectly via DNA sequence comparison to aid annotation of the human genome and research using cattle as medical models for obesity, reproduction and communicable diseases.

The fact that the bovine genome was the first mammalian farm animal selected for sequencing was of critical importance to New Zealand, because its economy is dependent on ruminant animals grazing forage to a much more significant degree than other advanced economies. The future challenge for New Zealand scientists will now be to use these resources effectively to ensure that our industries remain efficient and competitive with the major alternatives: chicken and pork.

Finally, from a research perspective this work provides a clear demonstration of the cost-effectiveness and benefits of international groups combining to invest in core basic research infrastructure and then placing the results in the public domain. The project was too large for any individual animal research group, and significant synergies and cost savings were made possible by combining resources. The wide availability of the information via the internet and lack of intellectual property entanglements also mean that industry benefits will accrue much faster and to greater level than otherwise.

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