Bovine Genomic Sequencing Initiative

Cattle-izing the Human Genome

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Rationale and Objective. Completion of the human genome sequence provides the starting point for understanding the genetic complexity of man and how genetic variation contributes to diverse phenotypes and disease. It is clear that model organisms have played an invaluable role in capturing this information. It is also noted that additional species must be sequenced to resolve the genetic complexity of human evolution and to effectively extrapolate genetic information from comparative (veterinary) medicine to human medicine. The selection of animals representing evolutionary clades distinct from primates or rodents provides considerable power for analysis of the human genomic sequence. The domesticated, eutherian mammals that have co-evolved with man (cows, pigs, dogs and cats), represent taxa with diverse selected phenotypes and have in place strong scientific and veterinary medical communities to support and utilize genome sequence information. Thus, this "white paper" provides support for sequencing the bovine genome (6X coverage) to identify new genes and novel regulatory elements in humans, mice and rats. The bovine genome will serve as a reference non-primate, non-rodent, eutherian genome.

The bovine genome is uniquely positioned for genomic sequencing because of the advanced stage of the reagents. An international bovine BAC map with a 15X coverage will be fingerprinted and all fingerprinted clones end-sequenced by February 2003. A minimum tilling path of BAC clones will be sequenced with a whole genome shotgun sequencing effort using random insert libraries. This will increase the efficiency, saving time and money, and yielding a better product because the BAC map will be completed prior to genomic sequencing. The human, mouse, and rat sequencing efforts were done in parallel with the BAC map development. The authors are not aware of any other animal species that will have the BAC map completed prior to genomic sequencing.

Biological considerations.

Improving human health-

During its domestication, the cow has undergone intense selection pressures for various phenotypes. Selection has provided two very distinct phenotypes associated with either meat or milk production and in some parts of the world, breeds that are specialized for pulling plows, carrying loads at high altitude or thriving in tropical environments and tolerating specific pathogens. These selective pressures have differentiated subpopulations and produced phenotypes extremely relevant to current and future human health research. Clearly, understanding genetic interactions with environmental factors will be a major focus of future biomedical research. The bovine model is also relevant to human health research priorities such as obesity, female health and communicable diseases. The cow provides a valuable

biological model in these priority areas because of the vast amount of research that has been conducted with respect to genetic and environmental interactions associated with complex, multi-genic physiological traits. The domesticated cow has also played an extensive role as a source of biological material in physiological and biochemical research. Use of the cow for biochemistry, enzymology, endocrinology, reproduction, and nutrition research has contributed significantly to the continual improvement of human health.

2. Informing human biology-
The animal sciences have contributed greatly to the basic understanding of human endocrinology and physiology. Classical endocrinology studies in farm animals led to the current understanding of several reproductive and pituitary hormones. The composition of insulin was first determined for bovine insulin (Sanger et al., 1955; Sanger, 1959) and it was used for several decades to treat human diabetes. Warfarin (coumarin), an anti-coagulant, was first identified in the blood of cattle with sweet clover disease (Stahmann et al., 1941). Parathyroid hormone was first identified in extracts of bovine thyroid/parathyroid (Collip, 1925) and the luteotrophic effect of luteinizing hormone was first demonstrated in cattle (Wiltbank et al., 1961). The growth promoting effects of growth hormone were first identified by administering extracts of bovine pituitaries into the rat (Evans and Long, 1921). Administration of bovine somatotropin (bST) to dairy cattle has provided an abundancy of information on lactational effects of GH.

The bovine model provided the fundamental research platform for developing human reproductive techniques and for studying reproductive diseases. Current reproductive techniques used in humans such as superovulation, oocyte culturing, in-vitro fertilization, and embryo maturation, transfer, and freezing, are based upon many years of research with bovine embryos (Brackett et al., 1982; Robl et al., 1987; Iritani and Niwa, 1977). Research with cattle semen (Polge et al., 1949; Phillips, 1939; Johnson et al., 1987) contributed extensively to using frozen semen for fertilization of human embryos and sexing semen. Stem cell research with farm animals has provided critical information needed to develop human stem cell research. Ethical concerns over stem cell and other developmental biology type research is very minor for farm animals relative to humans.

Research comparing different cattle breeds has identified genetic differences in fat disposition of different organs. Such information will provide an experimental model for understanding obesity and nutrition. Resource bovine populations have been selected for phenotypic variation in bone density [osteoporosis], sex-expressed nutritional and reproductive characteristics, and growth and development [embryonic, pre- and post-natal]. The bovine model will also be invaluable to study host-pathogen interactions for food safety (i.e. E.coli O157-H7, Salmonella, Listeria) , potential biological warfare agents (i.e. Foot and Mouth Disease), and agents that affect food security and human health (i.e. Mad Cow Disease and other zoonotic diseases).

3&4. Informing human sequence and provide better connection between sequence from humans and other species-
Comparative genetic maps have indicated that the bovine and human genomes are more similarly

organized than when either is compared to the mouse. The mean length of conserved syntenic segments between human and cow is approximately twice as long as the average length of conserved syntenic segments between human and mouse (Band et al., 2000). Furthermore, the organizational similarities between the human and bovine genomes are reflected in similarities at the nucleotide level. In more than 600 comparisons of non-coding DNAs aligned by orthologous exonic sequences on human chromosome 7, cattle (and pigs, cats and dogs) sequences consistently grouped closer to human and non-human primate sequences than did rodent (mouse and rat) sequences (Eric Green, 2002). Furthermore, the rodent genomes are evolving at a different (faster) rate than other representative genomes. For these reasons it is necessary to produce the genomic sequence of another eutherian mammal outside the primate and rodent lineages in order to better inform the human sequence. The rich genetic history and strong molecular resources currently available clearly identify the domestic cow as the appropriate choice for a third mammalian genome sequence.

5. Expand knowledge of basic biological processes relevant to human health-
The discovery that mammalian genomes probably contain only 30,000-40,000 genes suggests that alternate transcripts and post-translational modification must play a greater role in phenotypic expression than previously appreciated. We also expect single gene products to affect different traits or disease states, dependent on temporal and spatial presence of gene products. As one example, the bovine model illustrates that many hormones thought to only affect reproduction are also found to affect growth, muscle development, or diseases not normally associated with reproduction. The phenotypic diversity of hundreds of cattle breeds distributed throughout the world provides a tremendous resource for "comparative phenomics", the application of comparative genomic principles to discovery of new genes underlying diverse phenotypes. In only a few thousand years, selective breeding has produced cattle breeds to thrive at high altitudes or to thrive in tropical environments, to convert energy to milk production or to muscle mass, and to tolerate specific pathogens as in the well documented case of the trypanotolerant N'Dama cattle of West Africa (Roelants, 1986). In many respects, breeds of cattle are similar to human ethnic groups with diverse geographic origins, except with exaggerated phenotypic diversity. There can be little doubt that the understanding of what makes bovine breeds different with respect to reproductive efficiency, bone structure, growth rates and fat deposition, altitude or heat tolerance, and resistance to specific pathogens will be important to understanding basic biological processes important to human health.

6. Provide surrogate systems for human experimentation-
The domesticated eutherian mammal clade, represented by cows, pigs, dogs and cats, has provided numerous surrogate experimental models for biomedical research. There has been a long tradition of using abbatoir tissues for the purification of enzymes and the elucidation of metabolic pathways. These tissues have also served as initial biologicals with bovine and porcine insulins providing pre-recombinant DNA therapeutics and purified enzymes used to determine crystalline structure. Bovine gamete biology has played a critical role in our understanding of stem cells and in vitro fertilization. Because of the wealth of biological information using the bovine system it has increasingly become important for studying epigenetic effects as well as unraveling such as genomic imprinting. Recent collaborations between biomedical researchers at Duke University and the Lawrence Livermore Laboratory have

focused on using the bovine model to identify imprinting mechanisms associated with growth, lactation, nutrition and health.

7. Facilitating the ability to do experiments e.g. "Direct genetics" or positional mapping-
The bovine research community has a long history in quantitative genetics, and more recently in genomics research. The genetic contribution of many multi-genic traits in cattle is well documented and this knowledge has provided the basis for the identification and mapping of a growing number of quantitative trait loci (QTL) (Georges et al., 1993; 1995; Blattman et al., 1996; Casas et al., 1998; Davis et al., 1998; Stone et al., 1999; Smith et al., 2000). The only limitation to performing direct genetic experiments and identifying genes underlying these traits is the lack of a complete genome sequence. Selection experiments, heterosis studies and breed comparisons have all been used in bovine genetic studies. Many populations have been used to map genes to large chromosomal regions but positional mapping the gene has been difficult. Sequencing the bovine genome and generating 100,000 SNP will provide additional polymorphic markers and positional candidate genes from the human and mouse map. Large populations with designed matings will be used to positional map genes. The populations can be generated by natural reproduction, artificial insemination or assisted reproductive technologies. Clones can also be generated from fibroblasts, or stem cells and cryopreserved. This technology provides the opportunity for knock-out or knock in experiments in an animal other than the mouse. Interspecies bovidae hybrids are easily produced and would be very valuable for knockout/in experiments and studying genomic imprinting (Gao and Womack, 1997).

8. Expanding the understanding of evolutionary processes-
While all the eutherian mammalian orders probably diverged 70-80 million years ago, it is evident that some have genomes that are much more highly conserved relative to primates than others. Cattle, pigs, dogs and cats represent a clade (Eric Green, 2002) with respect to sequence divergence, intermediate to and distinct from the primates and rodents. These domestic animal groups also are more conserved relative to humans than rodents with respect to total genomic structure as revealed by comparative gene mapping. They themselves are diverse taxa, of course, and over the next few years, each genome should be sequenced to reveal its evolutionary history and to facilitate the important role each animal plays in comparative medicine. The cattle genome is somewhat unique, however, in that it represents all the pecoran ruminants, a phenotypically diverse and species rich clade of animals with circumglobal distribution and conserved karyotypes. With minor exceptions, the cattle genome sequence will serve as a platform for the genomes of goats, sheep, buffalo, and other artiodactyls whose chromosomes are remarkably similar (Gallagher et al, 1994).

B. Strategic Issues

1. Demand for new sequence -
A recent CRISP search demonstrated that 600 currently funded NIH grants use domestic animals as experimental model systems. The bovine system accounts for one-third of these active RO1s with studies funded by at least six institutes. Increased genetic analysis of the bovine genome has expanded significantly as demonstrated by the number of ESTs being contributed to various public databases. Total number of bovine sequences in public databases ranks fourth, behind human, mouse and rat.

There is significant scientific community support for using the bovine genome as the initial prototype of the non-primate, non-rodent eutherian mammal. This is demonstrated by the recent establishment of a Bovine BAC Constorium that has global representation of government, university and industrial constituents [see section B.4 for list of participants]. The non-profit Alliance for Animal Genome Research has provided support for development of this "white paper" and has provided leadership for convening a NAS/NRC workshop that is being supported by the USDA, NIH, DOE, and NSF to further define scientific objectives related to this and other sequencing initiatives. During the recent Plant, Animal and Microbe Genome Meeting, the development of this "white paper" and its merits were discussed and the USDA National Research Special Project on Animal Genomics, which is the coordinating body for the USA efforts, endorsed the selection of the bovine genome as a model system. The National Cattlemens Beef Association (NCBA) strongly supports the sequencing of the bovine genome (see attached letter of support) because of potential benefits to rural America. The NCBA is a trade association with about 33,000 individual members, 45 state cattle associations and 40 national breed and industry organizations. Together these organizations represent more than 230,000 cattle breeders, producers and feeders. Thus, there is significant endorsement by the international and national research community and industry for the bovine genome sequencing initiative. Global participation and coordination of this initiative will be conducted through the International Society for Animal Genetics [ISAG] and the Comparative Genomics program of HUGO. ISAG provides a framework for workshops and exchange of information that is critical for the implementation of this initiative and has served as a bridge with HUGO in the development of comparative genomics workshops. ISAG will provide a workshop at its 2002 meeting in Germany that will engage the research community with respect to this bovine sequencing initiative.

2. Suitability of the organism for experimentation-

Domestic cattle have a long historical and economic association with human cultures. Consequently, cattle are abundant and represented as many different breeds that can be considered as biologically equivalents to human ethnic and racial groups. Large and very deep pedigrees of cattle are available for research supported by phenotypic measurements. Additional genetic diversity has been created in interspecies crosses such as cattle x bison and cattle x gaur to produce high density comparative genetic maps of type I markers. Reproductive technologies for the propagation of cattle are highly developed and historically were important in the development of assisted reproduction techniques in human medicine. Cattle have been cloned by nuclear transfer from blastocysts (Robl et al., 1987) and from somatic cells and stem cells. Experimental tissues are readily available in large quantities from abbatoirs and the utilitarian aspects of the cattle-human association minimizes ethical and social objections to the use of cattle in research. Due to their large size, cattle are anatomically appropriate for human medical experimentation and have been used to develop key methodologies in organ transplantation, and artificial hearts.

3. Rationale for complete sequence of organism-

Domestic cattle are but one of several ruminant species that are used extensively in biomedical research or serve as hosts for zoonotic diseases. The history of sheep and goats in the nutritional and reproductive sciences and the recent documentation of transmissible spongiform encephalopathy in deer

and elk in the Western states serve as examples. Karyotypic data demonstrate extremely conserved genomes in these species, suggesting that a complete sequence of the bovine genome will provide a genomic matrix for all ruminants (Gallagher et al, 1994). A 1X genomic sequencing coverage or perhaps only transcript maps will serve the others species well. The other domestic animal genomes (pig, dog, cat) are clearly diverged and we are supportive of sequencing those genomes when end sequenced BAC maps and other mapping resources are fully developed.

The discovery of conserved sequences across species has proved valuable in the identification of novel genes and conserved regulatory elements. We propose that genomes of different primate species are not sufficiently diverged to identify many biologically significant elements and that human and rodent genomes are often too far removed on the molecular clock to find others. The genome of at least one non-primate, non-rodent placental mammal must be sequenced to triangulate the comparative sequencing strategy for finding biologically important sequences.

4. The state of readiness and cost of sequencing the genome-

The first linkage maps were published in 1994 (Bishop et al., 1994; Barendse et al, 1994) and current linkage maps collectively contain > 2300 markers (http://spinal.tag.csiro.au/cgd.html, http://locus.jouy.inra.fr/, http://sol.marc.usda.gov/). They have been used to identify chromosomal regions that influence numerous quantitative traits. Numerous normalized libraries have been developed from many different tissues at different physiological stages. The current number of ESTs deposited in Genbank exceeds 200,000 clones. TIGR has performed cluster analysis on 167,000 ESTs sequences and generated 58,500 non-redundant gene indices that are routinely used by the international research community. A subset of the ESTs have been selected for sequencing the predicted intron sequences (based upon human genomic sequence) to identify SNP for mapping the ESTs on the linkage map. The same set of ESTs are mapped on the RH map. The EST mapping effort is designed to improve the human-cattle and cattle-pig comparative map. A first generation radiation hybrid map of the bovine genome integrates microsatellite markers with ESTs selected from BLAST hits with human sequence (Band et al., 2000). This map also represents a 1000 marker comparative map of the bovine and human genomes and the second generation map is currently being assembled with 2000 comparative markers.

A coordinated international effort has been initiated to develop a bovine BAC map with two BAC libraries made by Pieter J. de Jong (pdejong@chori.org). The RPCI - 42 library was digested with EcoRI and it represents a 11.9X genome coverage. The CHORI-240 library was digested with MboI and it represents a 10.7X genome coverage. Additional libraries are also available from Texas A&M University (4X) and INRA (Institut National de la Recherche Agronomique, France) (4X). An international consortium was developed to fund the development of the bovine BAC map. USDA-ARS (U.S. Meat Animal Research Center, Clay Center, Nebraska), United Kingdom (The Roslin Institute, Edinburgh, Scotland and Biotechnology and Biological Sciences Research Council), Canada (University of Alberta, Edmonton, Canada and the Alberta cattleman- Alberta Livestock Genomic Initiative), and the University of Illinois have funded Dr. Marco Marra (Genome Sequence Centre, Vancouver, British Columbia, Canada) to perform the fingerprinting. Sequencing the ends of all fingerprinted BAC

clones will be performed by TIGR (The Institute of Genomic Research, Rockville, Maryland), the University of Illinois, AgResearch (New Zealand), CSIRO (Australia), Texas A&M University, University of Alberta (Canada), INRA (France), USDA-ARS, and the Roslin Institute. These laboratories will also map EST, microsatellite markers, genes and other STS onto the BAC clones to tie the linkage and RH maps to the BAC map. The final product will represent a 15X coverage of the bovine genome. The Genome Sequence Centre will maintain the database to store fingerprint, sequence and STS mapping information. The BAC map and end sequencing will be completed by February 2003. A similar international effort has recently been initiated for a pig BAC map at the Sanger Centre (Cambridge, England).

The scientists drafting this white paper met at the Baylor College of Medicne (BCM) Human Genome Center in Houston on December 20, 2001 to evaluate the readiness of domestic animal genomes for a sequencing initiative and to discuss the relative merits of bovine genomics in evolutionary and medical sciences. From this meeting emerged an enthusiastic consensus that because of its evolutionary history, its medical significance, the support of a strong research community, and the state of genomic information and resources already amassed, domestic cattle should be put forward as the first of several domestic animal genomes to be sequenced.

Collaboration between several individuals involved in this initiative demonstrates an intellectual dedication to the project and an institutional commitment to support genome sequencing. A close working relationship was established in 2001 between Drs. Weinstock and Gibbs from BCM and Drs. Womack and Skow from the College of Veterinary Medicine at Texas A&M University to produce a draft sequence of the bovine major histocompatibility complex (MHC) as a representative ruminant MHC. Approximately 5.5 megabases of raw sequence have been produced from ordered, end-sequenced BACs for an average of 3X coverage of the bovine MHC. The sequence is presently being assembled and annotated to draft status at Texas A&M and promises to serve as a useful paradigm for developing strategies for the assembly and analysis of the bovine whole genome sequence. This collaborative study was undertaken to provide sequence support for the examination of MHC gene expression as a function of locus position; the MHCs of Ruminantia appear to be unique among mammalian orders in that the complex has been disrupted by a large inversion that placed some class II loci, proteosomes and transporter loci near the centromere, more than 15 megabases removed from other MHC loci. Initial results of this study indicate strong conservation of gene content, order and orientation compared to HLA, the inversion not withstanding. Importantly, we have successfully used anchored orthologous regions of HLA to exploit conservation in non-coding intergenic DNA for the assembly of sequencing contigs of the bovine MHC. Similar attempts using mouse sequences were not successful. Such capabilities will be valuable in assembly of the whole genome sequence of the bovine. Results of this project are consistent with the premise that the bovine genome is more similar to the human genome than is the mouse and that the research community will be well served by having a complete sequence of cattle to better inform the study of the human genome.

The cost of sequencing the bovine genome is estimated to be similar or less than sequencing the rat genome (~$50 million) because the BAC map will be completed prior to genomic sequencing and the

experience gained by the Baylor College of Medicine Human Genome Sequencing Center from sequencing the rat genome. The bovine genome is similar in size to other mammalian species with an estimated size of 3 billion bases and a 6X sequencing effort is proposed.

The bovine genome project is a perfect match for the strategy being used at the Baylor College of Medicine Human Genome Sequencing Center for the rat genome project. Marco Marra's lab and TIGR are also performing fingerprinting and end sequencing for the rat genome project, and have experience working jointly with the BCM-HGSC. An important difference will be that the bovine fingerprint map and BAC end sequence information will be completed before the sequencing project starts. Thus it should be possible to determine a BAC tiling path from these two datasets, identifying a set of BACs with minimal overlap at the outset of the sequencing. The project will then be conducted as for the rat: about 25,000 BAC clones will be sequenced to about 1-2x coverage and the remaining 4-5x coverage will come from whole genome shotgun sequencing of 3kb, 10kb, and 50kb libraries. The genome will be assembled from these components using the ATLAS sequence assembly suite from the BCM-HGSC. It should be possible to reduce the number of BAC DNA preparations and shotgun libraries by using the pooled clone array approach, but this may not be necessary as there will be a pre-existing tiling path. In this approach, the BACs are distributed into arrays of dimension anywhere from 20x20 to a single large 160x160 array and then clones are pooled row-wise and column-wise. DNA preparations are made from each pool and shotgun libraries and sequencing is performed on the pools. The sequences are deconvoluted by co-assembling sequences from each row with each column and identifying those contigs formed by mixtures of row and column reads.

Given the experience gained from the rat genome project, as well as the previous success of the BCM-HGSC/Vancouver/TIGR collaboration, it can be anticipated that this project will be performed on schedule, without cost overruns, in a state-of-the-art process. Based on the current BAC preparation throughput at the BCM-HGSC (about 500 clones per week), the BAC skimming portion of the project should take about one year. The WGS component and ATLAS assembly should make the entire project take two years. In addition, the BCM-HGSC has developed tools to allow the research community to use the data before the final assembly is completed. This includes the BAC-fisher, a component of the BCM-HGSC web site that allows researchers to submit a sequence (a BAC, clone, cDNA, etc.) and be returned all of the WGS and BAC reads the are in the region of interest. In this context, it will also be important to consider a limited amount of finishing for regions of high biological interest (perhaps up to 500MB) as well as sequencing of full-length cDNAs, which is also a high-throughput activity at the BCM-HGSC.

5. Partial sources of funding-

During the past year, significant allocation of resources has occurred with respect to positioning the bovine genome sequencing initiative. This has included the establishment of the International Bovine BAC consortium in which participants have contributed over $3M towards the development of a whole genome bovine BAC fingerprint with complete BAC end-sequencing. The consortium will provide its deliverables to this bovine genome sequencing initiative. Equally critical is the mobilization of existing bioinformatics tools and efforts throughout the scientific community. The USDA-ARS bioinformatics

research effort is small but it has been leveraged by research agreements with Lincoln Stein (Cold Spring Harbor Laboratory) and the Cornell Theory Center. The USDA bioinformatics effort will continue to expand since it is a high priority research area and some of the new funds are likely to be used to develop additional research collaborations with 'experts-in-the-field'. Also, sequencing support [$1M per year] is being provided by the USDA-ARS and the University of Illinois to target regions that contain physiologically relevant trait loci.

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