Gene Mapping – USDA Effort

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Abstract

The recent availability of skeletal genetic linkage maps for cattle (Bishop et al., Genetics 136:619-639, 1994; Barendse et al., Nature Genetics 6:227-235, 1994) and swine (Rohrer et al., Genetics 136:231-245) provide a base for implementation of marker-assisted selection or MAS. Map development was facilitated through the use of highly polymorphic repetitive elements or microsatellites (ms), diverse crosses which increased marker heterozygosity (polymorphism), and our development of a sequence tagged site (STS) public data base capable of integrating genetic linkage and physical mapping data. The incidence of ms markers in each of the current livestock maps suggests minimal difficulty in combining individual species maps into a single comprehensive map. In addition, the significant homology between the 5' and 3' sequences flanking bovine and ovine ms translates into reciprocal marker heterozygosity. This has proven extremely useful in linkage mapping across genomes. We are, in effect, creating a comparative linkage map for bovidae suitable for dissection of those multigenic quantitative and qualitative traits which have been refractory to standard genetic manipulation.

Introduction

Recent reports of low resolution genetic linkage maps for the cattle and swine genomes (Bishop et al., 1994; Barendse et al., 1994) represent a first step in the development of high resolution, comprehensive maps and their subsequent use in Marker-Assisted Selection (MAS). Any strategy designed to rapidly improve map resolution must consider maximizing marker coverage across a genome. This requires an approach that simultaneously integrates available genetic linkage and physical mapping data for a species. In livestock, a physical map can also be considered as a comparative map where genes, whose location may be known in the human or mouse genome, are physically placed into syntenic (similar) groups or assigned to a chromosome. Inherent in this strategy is the assumption that increasing marker coverage over a genome is an essential component of map development, but not necessarily requisite to marker use in a selection process. Linked, well-anchored polymorphic markers provide the basis for MAS, which has the potential for radically transforming the way traditional animal breeding for improved production efficiency is conducted.

Our approach at MARC has been to utilize Polymerase Chain Reaction (PCR) amplifiable, highly polymorphic microsatellites (ms) to simultaneously construct genetic linkage and physical maps for the major livestock species and merge them into skeletal or low-resolution comprehensive maps. This strategy links randomly generated microsatellites cloned from bacteriophage or plasmid libraries with informative microsatellites assigned to a chromosome, either by direct amplification (Troyer et al., 1994) or through the use of larger DNA inserts in cosmid vectors and fluorescence in-situ hybridization (FISH) (Yerle et al., 1994). The essential element was the simultaneous development of a data base(s) at MARC capable of integrating genetic linkage, physical and comparative mapping data as it is developed (Keele et al., 1994).

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Results and Discussion

Current Status of Livestock Genetic Linkage Maps

First reports of genetic linkage maps for cattle (Bishop et al., 1994) and swine (Rohrer et al., 1994) were complemented by an additional map for cattle (Barendse et al., 1994) containing a number of identical markers which have the potential to refine marker interval(s). The loss of marker polymorphism within and between populations of "westernized" breeds of livestock was overcome to a large extent through the use of diverse crosses within mapping populations which significantly increased marker heterozygosity (Bishop et al., 1994; Barendse et al., 1994; Rohrer et al., 1994) (Table 1). In cattle, initial syntenic and physical assignments were used to anchor an essentially microsatellite linkage map to 24 of 29 autosomes and the sex chromosomes (haploid n=30) which covered ~2500 cM or 85% (Bishop et al., 1994) of the deduced size of the bovine genome (Logue and Harvey, 1978) at an average marker interval of 8.9 cM. A recent update of the MARC map links over 400 markers covering > 2600 cM or 93% of the bovine genome at an average interval of 7.4 cM. Linkage groups have been assigned to 28 of 29 autosomes with 72% of intervals less than or equal to 10 cM (Kappes et al unpublished). Marker heterozygosity for *Bos taurus* purebreds to *Bos taurus x Bos taurus* and *Bos indicus x Bos taurus* crosses, respectively are illustrated in Table 1. A second map has 171 linked loci covering ~1800 cM (sex averaged) and average interval distance of 15 cM (Barendse et al., 1994). Currently, the two published cattle linkage maps have ~530

Table 1. Marker Heterozygosity in Sire and Dam Breeds Comprising Map Reference Populations of Cattle and Swine.

Sex	*Breed*	*Heterozygosity (%)*
Cattle Sire(s)	Gelbvieh:Simmental	59.8
	Gelbvieh:Simmental	60.1
	Brahman[a]:Angus	73.7
	Brahman:Hereford	76.8
Cattle Dam(s)	Piedmontese:Angus	57.4
	Piedmontese:Hereford	61.9
	Longhorn:Angus	63.1
	Longhorn:Hereford	60.5
	Nelore[a]:Hereford	76.7
	Angus	46.3
	Hereford	45.0
Across all Western Breeds		49.5
Across indicus:taurus		75.7
Swine Sire(s)	WC[b] (White composite)	56.8
Swine Dam(s)	WC:Duroc[c]	64.5
	WC:Fengjing	78.2
	WC:Minzhu	80.3
	WC:Meishan	82.1

[a] Indicus breed
[b] WC = 1/4 Chester White, 1/4 Large White, 1/4 Landrace, 1/4 Yorkshire
[c] Western breed

markers in well-anchored linkage groups. Forty-three markers (8%) linked on the two maps are identical.

The MARC swine linkage map is at a similar stage of development with an initial 383 (376 ms) markers placed into 24 linkage groups with assignments to 13 autosomes (haploid n=18) and the X chromosome covering ~2000 cM of the porcine genome at an average interval of 5.5 cM. Recent additions to this map link and anchor ~500 markers to 17 of 18 autosomes with 70% of marker intervals less than or equal to 5 cM and only 3% > 20 cM. Marker heterozygosity among North American breed crosses was similar to that of *Bos taurus x Bos taurus* crosses and in excess of 80% for Chinese x North American breed crosses (Table 1).

The preponderance of ms markers in each of the livestock maps immediately suggests that there would be minimal difficulty in combining individual species linkage maps. Development of consensus linkage maps with identical markers screened across diverse mapping pedigrees should improve resolution within individual linkage groups. However, as only indirect estimates of genomic size are available for any of the major livestock species (Andersson et al., 1994; Logue and Harvey, 1978) and the informative telomeric and centromeric markers that would provide a more rigorous analysis are not yet available, consensus linkage maps are not likely to significantly improve estimates of overall coverage.

Comprehensive Livestock Maps

The paucity of qualified livestock cytogeneticists devoted to molecular hybridization techniques suggests that physical mapping should be focused on developing anchors for genetic linkage maps that would provide more robust estimates of genome coverage and refine marker interval and order. As the ultimate objective of all livestock linkage maps is directed toward providing a framework for MAS, directed physical placement of informative markers makes the development of low-resolution comprehensive maps suitable for initiating a systematic search for loci regulating economically important traits (ETLs), a realizable goal.

The as yet embryonal nature of livestock genomic maps and their ultimate use as a framework for MAS also provides an opportunity to develop a parallel approach to integrating genetic linkage and physical maps. The strategy suggested by O'Brien (1991) of simultaneous development of Type I (coding sequences) and Type II (species-specific, highly polymorphic ms or minisatellites) anchor loci maps recognized that the current labor and DNA-intensive strategies to merging genetic linkage and physical maps would limit progress. However, a strategy which combines the concept of assigning an informative Type II "framework" marker either by direct amplification (Troyer et al., 1994) on a metaphase chromosomal spread or in-situ hybridization of cosmid (~40kb) or genomic lambda (~15kb) DNA clones containing polymorphic ms (Yerle et al., 1994), while continuing to add large numbers of random ms, permits rapid development of a well-ordered linkage map(s). Our approach, which sequences the ms after high stringency $(CA/GT)_n$ oligo screening of cosmid libraries, has made the initial suggestion of random assignment of cosmid clones containing polymorphic ms in sufficient numbers to cover each chromosome at least once (Fries, 1993), tenable in the short term. Physical assignment of a cosmid-derived or directly-amplified ms rapidly creates additional linkage anchors. Concomitant assignment of Type I markers within synteny groups from extant comparative maps (O'Brien et al., 1993) rapidly provides information on the interval between coding loci, relative gene order among species and syntenic/linkage boundaries, particularly when the coding sequence or an associated Type II marker is polymorphic. This directed strategy of physically assigning informative "framework" loci is particularly useful in species from closely related families, e.g., *bovidae*, where comparative linkage maps could rapidly be constructed (Table 2). The significant homology between the 5' and 3' sequences flanking bovine and ovine ms (Moore et al., 1992) translates into reciprocal marker polymorphism and facilitates linkage mapping across genome. At MARC we are, in fact, creating a comparative linkage map for *bovidae* suitable for dissection of those multigene quantitative traits which have been refractory to standard genetic manipulation. Polymorphic framework loci provide the opportunity for rapid inte-

Table 2. Reciprocal Amplification of Bovine and Ovine ms.

Marker	*N*	*Number amplified Bovine*	*Number amplified Ovine*
Bovine	76	62 (82)	47 (76)[a]
Ovine	35	12 (44)	27 (77)
Total	111		

[a] (%) ~45% overall polymorphic

gration of species-specific physical and genetic linkage maps to create skeletal comprehensive maps and significantly enhance any search for candidate loci. The improvement in genome coverage and marker resolution also significantly increases map usefulness in MAS for economically important traits.

The human and murine genome maps have benefited significantly from the development of databases to store, assemble and disseminate map information (Buetow et al., 1994). Continued development of livestock genome maps would obviously benefit from a similar approach. Several data bases are currently in various stages of planning and development with their main focus on providing an overview of the linkage/physical map(s) of each species. The MARC livestock database can be accessed at three levels through Internet. The latest linkage maps and development information (e.g., sequences) on public markers is available on the World Wide Web (WWW). The document URL is: http:11sol.marc.usda.gov. Certain predefined ("canned") reports may be run from the operating system level. A minimal knowledge of UNIX, but no knowledge of ORACLE, is required. A terminal emulator may be required. This level requires a user ID and password. Full read access is available to those researchers wishing to submit ad hoc queries to the data base. This requires a user ID, password, knowledge of UNIX, the ORACLE RDBMS and ORACLE Report Writer. A terminal emulator may be required. Training sessions at MARC are currently being conducted for those wishing this level of access. Two notable points of this level of access is that outside users of the data base will be able to extract data and do their own linkage analysis, and the presence of a table (shell) for a consensus map for each species.

Marker-Assisted Selection for Economically Important Traits

Although genetically-linked markers promise the potential to dissect the loci responsible for expression of quantitative (e.g., lean growth) and qualitative (e.g., disease resistance) traits which have been refractory to standard genetic manipulation in livestock, it should be noted that it is neither essential to identify the gene(s) residing at a marker-identified locus nor have a demonstrable polymorphism within the locus to account for significant genetic variance in single or multigenic traits with medium (25-40%) to low (10-25%) heritability. Markers flanking a locus do ultimately provide the entry portals for a molecular approach, often referred to as positional cloning or reverse genetics, for walking through the gene of interest. However, a cluster of highly polymorphic markers, tightly linked to the locus, is all that is necessary to identify appropriate individuals to initiate introgression of favorable alleles into a breeding population. However, it should be noted that clusters of markers with relatively high polymorphism information content (PICs) within/between breeds are likely to be necessary prior to making intrabreed crosses as marker number and heterozygosity impact not only genome coverage but the efficiency of MAS. The level of marker heterozygosity is lower between western breeds used in meat production (Table 1, 2) and can be lower for an individual marker within breeds. This immediately suggests that not all markers will be useful in all families. Therefore, a systematic approach to MAS might consider screening a template of markers which uniformly cover the genome across unrelated animals within breeds to be crossed prior to initiation of large-scale genotyping of a resource population of several hundred animals or reviewing that type of information within a data base (Keele et al., 1994). Breed-specific alleles are also likely to fall out of such an approach. Ultimately, the producer must have some *a priori* knowledge as to which marker(s) alleles are likely to be useful, if any, within their population or even semen from a well-characterized sire.

This overall approach is the strategy being implemented at MARC for MAS in resource populations. Major breeds within these populations include Nellore x Hereford, Piedmontese x Angus, and Belgian Blue x MARC III (Hereford, Angus, Red Poll, Pinzgauer) sires to evaluate product quality.

Conclusion

The MARC strategy of directed linkage mapping and continued addition of microsatellites located randomly throughout the genome is intended to hasten merging the genetic linkage and physical species maps into low-resolution comprehensive maps. Improved resolution of livestock genome maps facilitate a systematic marker-assisted selection approach to dissection of single and multigenic economically important traits.

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