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Biotechnology And The Dairy Industry

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The term biotechnology encompasses many different areas of biology that have created a set of unique approaches to enhance our current biological knowledge base. Some of these technologies are envisioned to have an important impact in the way we do things and in providing an opportunity to enhance the economic viability of animal and plant agricultural industries. I will focus on two of these areas, molecular genetics (gene mapping) and reproductive physiology (animal cloning). The aim is to describe the work that is being done and provide a glimpse to its possible applications.

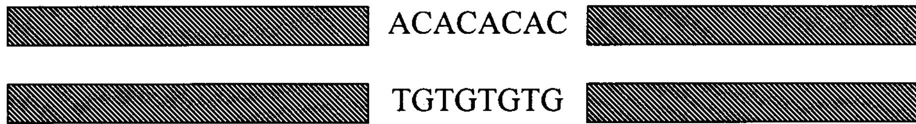
1. Gene mapping and marker assisted selection.

In broad terms, gene mapping refers to the identification and localization of genes. For instance, coat color is the phenotypic result of the expression of genes. Whether coat color is controlled by one or several pairs of genes can only be determine by evaluating the progeny of specific matings that will allow geneticists to infer the most appropriate pattern of color inheritance. In this example we have used coat color as a marker to follow its pattern of inheritance. In this way we have learned that in Holsteins, black coat color is dominant to red. While the black/red pattern of inheritance has been known for some time, it is only recently that we have learned that the gene is located in chromosome 18. The latter has been accomplished by tracing the cosegregation of DNA polymorphic markers and coat color in appropriate matings.

The composition of DNA found in one animal is not the same as in another animal of the same species and breed unless the animals are identical twins. Differences in DNA composition among individuals are called DNA polymorphisms and are heritable and, with appropriate tools, can be traced in pedigrees. DNA polymorphisms are an abundant and important resource of genetic markers. A handful of genetic markers based on DNA polymorphisms have been described in the literature (Singer and Berg, 1991). I am going to describe a specific DNA polymorphism which has become the most useful and has yield the most comprehensive genetic maps available for livestock species. Microsatellites are small stretches of DNA that contain di- tri- or tetra-nucleotide repeats. These stretches of repeats are flanked by unique DNA sequences which become the identity signature of each microsatellite locus. The polymorphic nature of the microsatellite resides on differences in the number of repeats (Fig. 1) found among animals.

Another important feature of the technology is the use of the polymerase chain reaction (PCR) to amplify (Appendix 1) the stretch of DNA containing the microsatellite sequence. This is possible because specific DNA primers (Fig 2. green and red bars) homologous to the unique microsatellite flanking regions can be synthesized for each microsatellite locus.

ANIMAL # 1 Shows 4 AC repeats. $(AC)_4$



ANIMAL # 2 Shows 5 AC repeats. $(AC)_7$

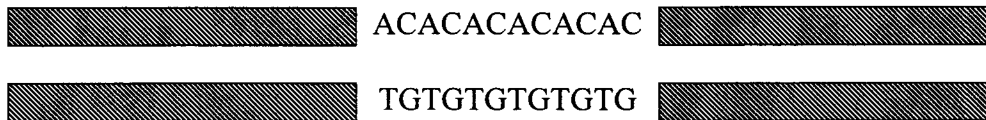
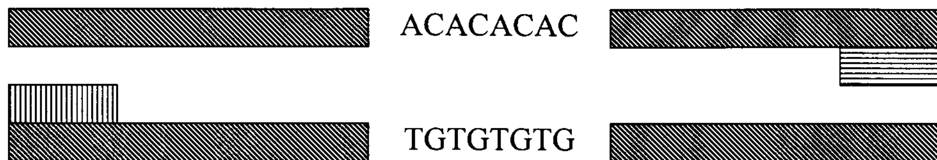


Fig. 1. Schematic representation of a microsatellite. Animal #1 contains four AC repeats and animal # 2 contains seven AC repeats. Both repeats are flanked by unique DNA flanking sequences (blue lines) which are similar in both animals.

ANIMAL # 1 Shows 4 AC repeats. $(AC)_4$



ANIMAL # 2 Shows 5 AC repeats. $(AC)_7$

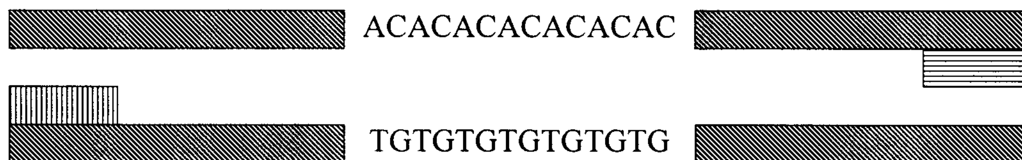


Fig. 2. PCR amplification of a microsatellite locus. For all animals the same primers (green and red bars) are used to amplify a specific microsatellite. Amplified products show different sizes depending upon the number of AC repeats. PCR product size differences can be detected

with polyacrilamide gels. In this figure animal #1 has an $(AC)_4$ allele and animal # 2 has an $(AC)_7$ allele.

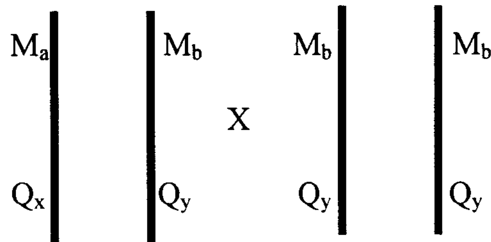
Microsatellites are abundant (~ 6 x 10^4) and randomly distributed along the bovine genome. Microsatellite sequences are screened and isolated from DNA genomic libraries using $(AC)_{12}$ oligonucleotide probes. DNA fragments containing microsatellite sequences need to be sequenced to detect the di- tri- or tetra-nucleotide repeats and to identify unique priming sites for PCR primer development. DNA libraries can be whole genome libraries or chromosome-specific libraries. For the past few years our laboratory has been involved in developing bovine chromosome-specific DNA libraries (Ponce de León et al. 1996, Ponce de León, 1996). Briefly, chromosome metaphases are placed on coverslips and with the help of an inverted microscope and micro-needles, specific chromosomes are scraped from the coverslips. A total of 10 chromosomes and/or chromosomal regions are accumulated before adding a nanoliter (10^{-9}) drop onto the chromosome pile. Chromosomal DNA is purified with Proteinase K, digested and ligated to DNA adaptor molecules that would allow PCR amplification of chromosomal fragments. Amplified fragments are cloned in the lambda Zap (Stratagene) vector and a chromosome and/or region specific DNA library is generated. These libraries have helped increase the marker density for specific chromosomes (Appendix 2. and 3).

Useful genetic markers have large numbers of alleles. For instance, a marker with a single difference, identifies two alleles A and B and therefore only three genotypes (AA, AB and BB) can be observed in the population. However, a DNA marker with 4 alleles A, B, C and D can identified 10 different genotypes. DNA markers with larger number of alleles at greater than 5% frequencies are more useful than markers with large numbers of rare alleles. About 1,500 microsatellite markers have been developed so far (National Agricultural Library, http://probe.nalusda.gov:8000/). Appropriate mapping reference populations have been used to generate a comprehensive genetic map currently being used for detection of quantitative trait loci (QTL).

Microsatellites and QTL cosegregation analysis (crossing over) is required to identify the presence of a QTL. In few words, if one allele of a microsatellite marker cosegregates with a specific QTL, then every time the allele is identified in an animal, this animal should also express the QTL. The closer the microsatellite is to the QTL the more often the microsatellite and QTL will cosegregate (Fig 3).

Essentially the objective is to develop genetic markers that are very close to and flank the QTL to avoid losing the cosegregation of marker-QTL due to recombination events. The best genetic marker of all is the gene(s) itself if it contains enough sequence polymorphism among animals. In this way every marker associated to a QTL can be used for Marker Assisted Selection (MAS). Depending on the importance of the QTL for a breeding program the marker can be assigned a specific weigh when calculating sire breeding values. The technology will have greater effect for identification and selection programs for low heritability traits, like reproductive traits and disease resistance and/or susceptibility traits.

Cross: Heterozygous x recessive



Progeny:

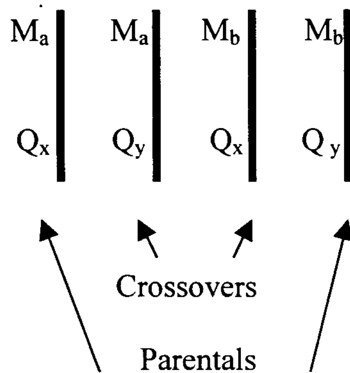


Fig 3. Cosegregation of a QTL and marker alleles. The QTL (red box) and the two marker alleles (green and light blue boxes) are shown for the backcross and progeny

2. Cloning.

The scientific journal "Science" labeled mammalian cloning the scientific breakthrough of 1997. However, cloning is not new. The newness of it is that it has been accomplished by artificial means. Cloning occurs naturally in most unicellular organisms and also in mammals. Identical twins are nature's clones. Before elaborating on its scientific significance let me briefly review the cloning procedure. First, it is necessary to start with fresh oocytes that are collected from fresh slaughter house ovaries. These oocytes are matured in vitro and are at the second meiotic division stage. Chromosomes should be found underneath the polar body and are removed with a micro-pipette. The enucleated oocyte is now perforated with a second micro-needle and a somatic cell (fibroblast) is injected between the zona pellucida and the oocyte membrane. After electrical stimulation pulses, both cells (the oocyte and the somatic cell) fuse and the nucleated oocyte is simultaneously activated. Oocytes are placed on culture media where they develop into morulas and blastocysts. These are transferred to synchronized recipient cows and eventually a live cloned animal is born (Schnieke et al. 1997). It has been postulated that cloning is successful

when somatic cells used for nuclear transfer have been synchronized by serum starvation or are in a quiescent stage.

For the past several decades biology has been working under the assumption that programmed cells have a define terminal fate. The paramount significance of cloning is that this largely accepted dogma has been chattered because nuclear reprogramming occurs in the cytoplasmic oocyte environment. It has certainly open a new approach for the study of cell programming which could lead to the possibility of in vitro tailoring of specific cell types that would be used for human cell therapies. Many other uses of the technology for the benefit of humankind can be envisioned (xenotranplantation) but will not be discussed in this manuscript.

Current technologies also facilitate the random as well as the directed integration (homologous recombination) of foreign genes into cultured embryonic stem cells and fibroblast cells. This technology has largely been used in mouse to generate "knock out" mice. "Knock out" mice are produced when a specific gene or genes are eliminated and/or obliterated by homologous recombination. In this way the study of gene function has been enhanced by observing and studying the phenotype of knock out mice. The capability then exists to replace a specific allele with an alternative allele at any given locus. Hence, identification and localization of genes becomes more relevant because it would be possible to introgressed genes without disturbing the genome at other sites. In other words genes and or alleles that are found in wild animal populations or specific breeds will be easily transferred, if necessary, to domestic populations. While this seems now possible, we are many years away from practicing it and from evaluating its practicality when compared with introgression of genes by breeding. In the mean time, for animal agriculture, the best use of the technology will be the generation of transgenic animals (particularly dairy) that will produced milk with specific proteins useful for human therapies, like human serum albumin or blood clotting factors (Schnieke et al. 1997). Other transgenic animals will be produced where specific modification of genes will be caried out to modified milk composition targeted to improvements in cheese quality (casein) and to specific consumer groups (reduced lactose), etc.

3. Strategic research initiative.

The Department of Animal Science and SES (Waseca) have maintained a control line of Holstein cows since 1964. The "control line" has not been under any form of selection for the past 34 years and represents a genetic line that differs significantly from contemporary selected Holstein cows. Estimated breeding value (EBV) differences for Milk and somatic cell score are + 7,812 pounds and .46 respectively (L. Hansen, personal communication). These differences and differences for reproductive traits, coupled to increased resistance to mastitis offer a unique opportunity for identification of these QTLs. To this end, faculty from the College of Agricultural, Food and Environmental Sciences and the College of Veterinary Medicine have developed a tentative collaborative program that is aimed at improving dairy health and production efficiency (Beattie et al., 1998). The objective of the program is to generate an F2 population that is based on a cross of the control line of Holstein cows to contemporary Holstein bulls. Programmed matings of F1 animals will generate the F2 resource population that will allow the identification of genes involved in resistance and/or susceptibility to mastitis and

Johne's disease among others. The opportunity for studying the effective use of consumed nutrients on genetic and reproductive efficiency has also been included in this project.

This is the most powerful experimental design available for QTL detection and the uniqueness of the control line of cows offers the opportunity to do it. Even though this is the most powerful experimental design, it is also the most costly. However, when one considers the amount of information that can be extracted from a project of this magnitude plus the number of collaborating researchers and institutions that need to be involved, the financial investment will not be as big as it might otherwise seem. Data on genetic markers and genetic technologies generated from this project will be publicly accessible to directly benefit producers and the dairy industry as a whole. The potential also exists for the control herd to be recognized as a National Dairy Research Herd and offer the University of Minnesota the opportunity to be the lead research institution in any national initiative for improving dairy herd health and production efficiency.

In summary, biotechnology is a promising area of investment and in the future it will be a factor in maintaining the vitality and economic viability of animal industries, particularly the dairy industry.

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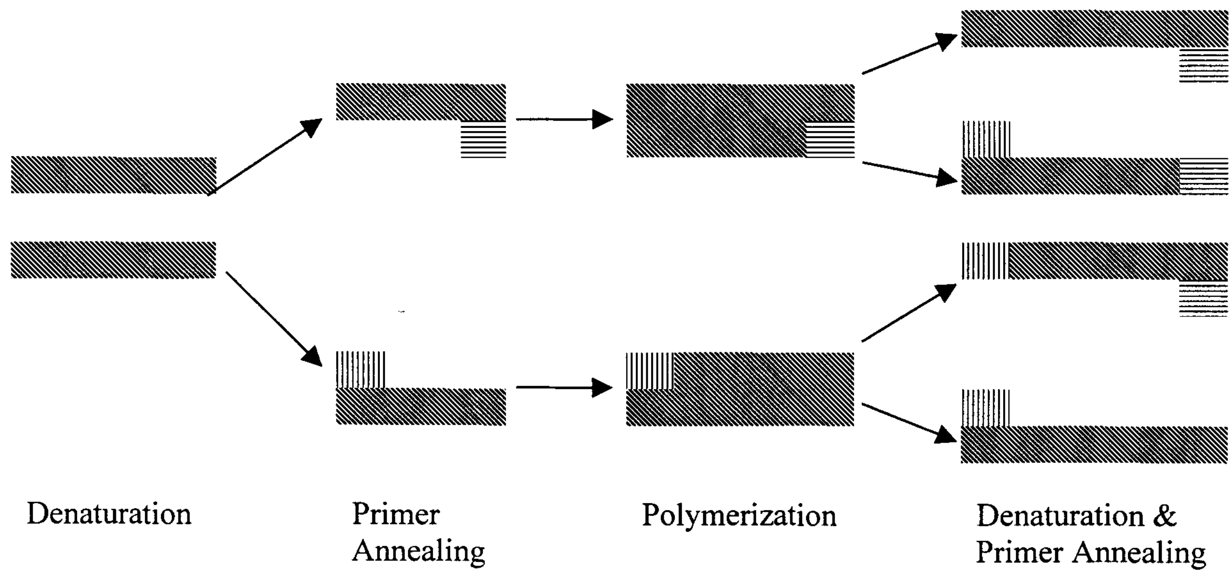
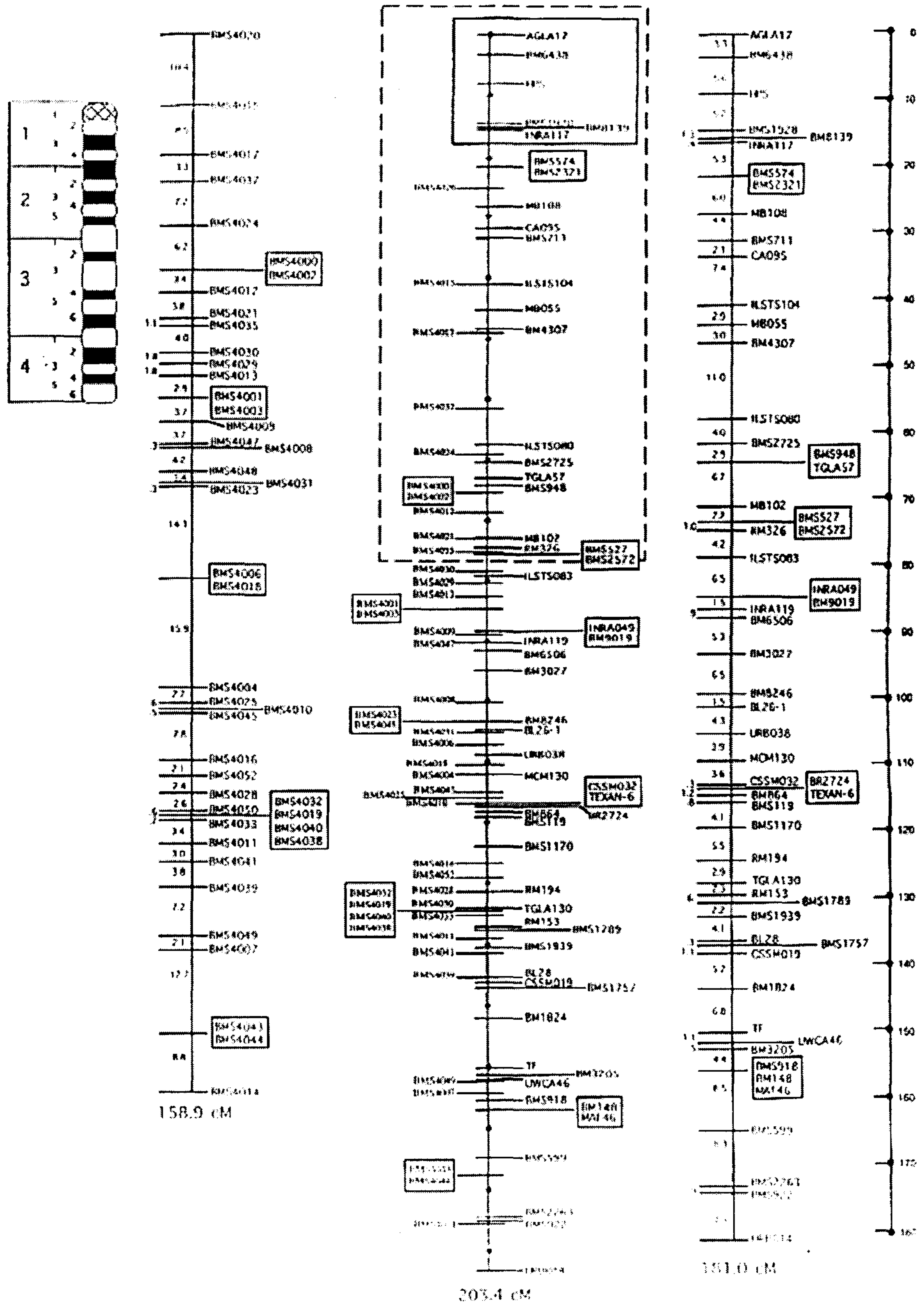
Sonstegard, T.S., N.L. Lopez, S.M. Kappes, R.T. Stone, S. Ambady, F.A. Ponce de León, C.W. Beattie (1997b). An integrated genetic and physical map of the bovine X chromosome. Mammalian Genome 8:16-20.

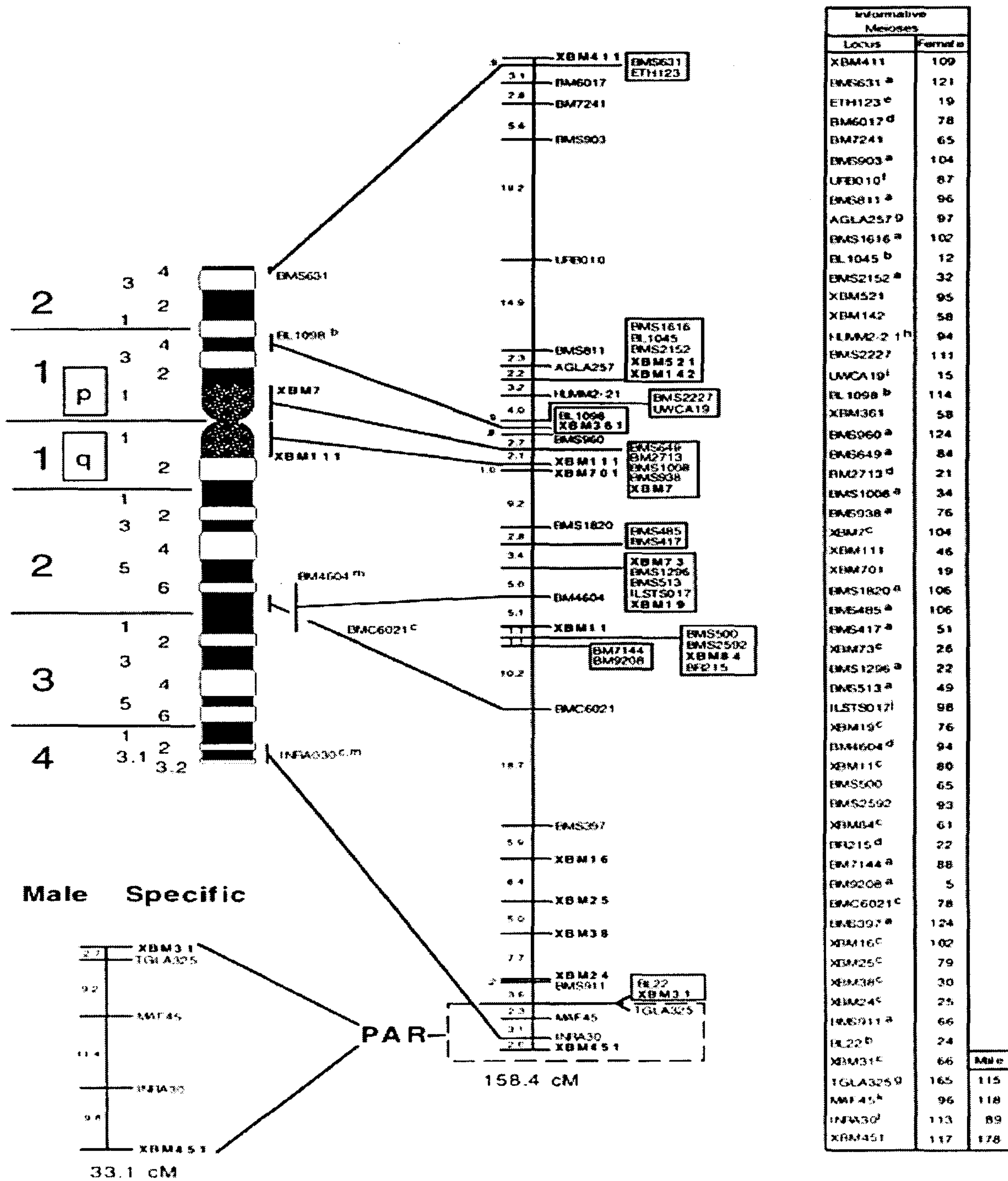
Appendix 1. Polymerase Chain Reaction amplification (PCR). After denaturation of the double stranded DNA molecule, two single stranded templates are generated. Primers (green and red bars) anneal to complementary sequences and Taq Polymerase extends the sequence by addition of complementary bases. Hence producing two copies of the original sequence. The cycle is repeated about 30 times.



Appendix 2. Genetic map for bovine chromosome 1 (BTA1) (Sonstegard et al, 1997a)



Appendix 3. Genetic map for the bovine X-chromosome (BTX) (Sonstegard et al, 1997b)



Informative Meioses		
Locus	Female	Male
XBM411	109	
BMS631 [a]	121	
ETH123 [e]	19	
BM6017 [d]	78	
BM7241	65	
BMS903 [a]	104	
UFB010 [f]	87	
BMS811 [a]	96	
AGLA257 [g]	97	
BMS1616 [a]	102	
BL1045 [b]	12	
BMS2152 [a]	32	
XBM521	95	
XBM142	58	
HUMM2-2 1 [h]	94	
BMS2227	111	
UWCA19 [i]	15	
BL1098 [b]	114	
XBM361	58	
BMS960 [a]	124	
BMS649 [a]	84	
BM2713 [d]	21	
BMS1008 [a]	34	
BMS938 [a]	76	
XBM7 [c]	104	
XBM111	46	
XBM701	19	
BMS1820 [a]	106	
BMS485 [a]	106	
BMS417 [a]	51	
XBM73 [c]	26	
BMS1296 [a]	22	
BMS513 [a]	49	
ILSTS017 [j]	98	
XBM19 [c]	76	
BM4604 [d]	94	
XBM11 [c]	80	
BMS500	65	
BMS2592	93	
XBM84 [c]	61	
BR215 [d]	22	
BM7144 [a]	88	
BM9208 [a]	5	
BMC6021 [c]	78	
BMS397 [a]	124	
XBM16 [c]	102	
XBM25 [c]	79	
XBM38 [c]	30	
XBM24 [c]	25	
BMS911 [a]	66	
BL22 [b]	24	
XBM31 [c]	66	
TGLA325 [g]	165	115
MAF45 [k]	96	118
INRA30 [l]	113	89
XBM451	117	178