MICRODISSECTED CHROMOSOME LIBRARIES FOR LIVESTOCK SPECIES

GENOTECAS PROCEDENTES DE MICRODISECCIÓN CROMOSÓMICA EN ESPECIES DOMÉSTICAS

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SUMMARY

An alternate approach to random library screening for the identification of molecular genetic markers is the generation of whole chromosome or subchromosomal DNA libraries. We have adapted the strategy proposed by Saunders *et al.* (1989) for chromosome microdissection and have generated whole chromosome and subchromosomal DNA libraries. The strategy is based on down scaling the DNA purification procedure of microdissected chromosomes or chromosome fragments to a one nanoliter volume. Purified DNA is digested with Sau3A I and ligated to adaptor molecules. The adaptor sites are then used for polymerase chain reaction (PCR) amplification of chromosomal DNA fragments. Chromosomal PCR products have been used to develop chromosome paints and are being used to assess interspecies chromosome homologies and chromosome rearrangements. Similarly, chromosomal PCR products have been used to generate chromosome-specific DNA libraries. Screening of these libraries for identification of clones containing microsatellite sequences has enable the generation of <3cM resolution linkage maps for cattle chromosomes. We have generated chromosome-specific DNA libraries spanning about 30 p. cent, 50 p. cent and 8 p. cent of the bovine, chicken and swine genomes, respectively.

RESUMEN

La obtención de genotecas a partir del ADN de cromosomas completos o de regiones subcromosómicas es una alternativa a la búsqueda al azar de marcadores moleculares en genotecas construidas al modo tradicional. Hemos adaptado la metodología propuesta por Saunders *et al.* (1988) para la microdisección cromosómica y hemos producido genotecas a partir de cromosomas o de regiones subcromosómicas. La estrategia se basa en reducir el procedimiento de purificación de cromosomas microdiseccionados o de fragmentos cromosómicos al volumen de un nanolitro. El DNA purificado se digiere con Sau3A I y se liga a moléculas adaptadoras. Los sitios de adaptación se utilizan entonces para la amplficación por Reacción en Cadena de Polimerasa (PCR) de los fragmentos de DNA cromosómico. Los productos de estas reacciones cromosómicas de

PCR se han usado para la identificación específica de cromosomas y para confirmar las homologías cromosómicas interespecíficas y las reorganizaciones cromosómicas. de manera similar, los productos de PCR cromosómico se han utilizado para construir genotecas específicas de un cromosoma. La búsqueda en estas genotecas con objeto de identificar clones con secuencias de microsatélites, ha hecho posible la producción de mapas de ligamiento de cromosomas bovinos con una resolución de <3 cM. Hemos producido genotecas de ADN que describen aproximadamente 30 p. cien, 50 p. cien y 8 p. cien del genoma bovino, de la gallina y porcino respectivamente.

INTRODUCTION

Major goals of livestock genome research include identification of quantitative trait *loci* (QTL) controlling important economic traits and, the eventual use of markers closely linked to these *loci* in marker assisted selection (MAS) schemes. Although, enough markers are available for whole genome scans and identification of chromosomal regions containing QTL, sufficient genetic markers essential to fine map QTL regions and assure interbreed heterozygosity for MAS, are not available (Beattie, 1994). The problem is compounded in purebred populations where the heterozygosity of markers, developed across interbred map reference families, is significantly reduced (Bishop *et al.*, 1994; Beattie, 1994). Increasing the resolution of QTL regions with additional markers will facilitate the use of interval analysis which depends on: 1) localization of the QTI, based on the physical location of the genetic markers flanking the QTL region and the genetic distance between them, 2) the ability to develop new polymorphic markers within the region containing the QTL, 3) sufficient available meioses to be able to order markers while reducing the interval about the QTL. The eventual development of contig maps using large insert libraries (P1, BAC and YAC), when the interval between markers is significantly reduced, is also greatly facilitated (Zoghbi and Chinault, 1994).

Partitioning of the bovine genome into small units to concentrate mapping efforts in small regions of the genome becomes an important task, particularly when QTL regions are identified. Fragmenting genomes by individual chromosomes and/or chromosomal regions to construct chromosome-specific and/or chromosome-region-specific DNA libraries has proven to be a logical approach. Human and mouse chromosome specific libraries have been constructed from somatic cell hybrids carrying single human or mouse chromosomes, flow sorting of chromosomes and combination of both (Gray *et al.*, 1987, Fisher *et al.*, 1985). Likewise, chromosome painting probes and chromosome-specific libraries have been developed from flow sorted pig chromosomes (Langford *et al.*, 1992; Yerle *et al.*,1993) and from microdissected bovine chromosomes using the t(14;20) translocation (Schmutz *et al.*, 1994). The latter approach was based on the microisolation of 100 copies of the t(l4;20) marker chramosome and direct ligation into the cloning site of a lambda phage vector, resulting in 1,500 clones (Schmutz *et al.*, 1994). Microdissection of specific regions within a chromosome from metaphase spreads has resulted in the cloning of DNA fragments specific

for the selected regions (Ludecke *et al.*, 1989). Of particular importance is the strategy developed by Saunders *et al.* (1989), which is based on the ligation of an adaptor molecule to chromosomal inserts and the use of the adaptor sequence as a priming site for PCR amplification. We adopted this strategy to microclone the bovine X-chromosome (Ponce de León and Robl, 1992).

For the past five years my laboratory has been developing chromosome-specific libraries for some livestock species including cattle, pig, horse and chicken, and chromosome-region-specific libraries for cattle. This report describes de chromosome microdisection and microcloning procedures as well as the utilization of microdisected products for genetic analysis.

MATERIAL AND METHODS

CELL LINES

For microisolation of bovine X- and Y- chromosomes and swine chromosome 6, we used normal bovine or swine lymphocyte cell cultures, respectively. For bovine chromosomes 1, 4, 14, 20 and 29, we used a transformed fibroblast cell line containing the t(1;29) and t(20;14) translocations (provided by Mr. Lance Buoen, University of Minnesota, Minneapolis, Minnesota, USA) and for bovine chromosome 11 we used a fibroblast cell line containing the t(X;23) and (11:?) translocations (provided by Dr. P. Basrur, University of Guelph, Ontario, Canada and Dr. D. Gallagher, Texas A&M University, College Station, Texas, USA). For the microisolation of chicken chromosomes we used fibroblast cell cultures obtained from 10 day old embryos of broiler type birds.

PREPARATION OF METAPHASE SPREADS

Metaphase spreads were obtained by an adaptation of the method of DiBerardino and Ianuzzi (1982). Briefly, cells were synchronized by addition of 10^{-4} methotrexate to exponentially growing cells. After 15 hours of incubation, thymidine (20µg/ml) was added and cells were further incubated for 6 hours before harvesting. Colcemid (0.05 µg/ml) was added to the culture dishes 1.5 hours prior to harvesting. Harvested cells were resuspended in a hypotonic solution (0.056 M KCl) for 30 minutes at 37°C and fixed in 9: 1, 5: 1 and 3: 1 methanol: glacial acetic acid for 10 min each. Cell suspensions were dropped onto pre-cleaned coverslips and air dried at room temperature. Coverslips were kept at -20°C until use.

SAU3A I ADAPTOR

We have developed an adaptor molecule (Ponce de León *et al.*, 1996), based on the strategy outlined by Saunders *et al.* (1989). Specifically, the adaptor molecule was prepared by constructing a 28mer that has a Sau3A I site at its 5' end, and a 24mer, homologous to the 28mer, starting from the second thymidine at the 5' end. Annealing of both oligomers yields a double stranded adaptor molecule with a Sau3A I sticky end, and an EcoRI site close to the 3' end, starting at nucleotide 19. Phosphorylation of the 28mer oligonucleotide and annealing of the 28mer and 24mer oligonucleotides to form the double stranded adaptor was carried out as described by Wu *et al.* (1987) with some modifications. Briefly, 1.5 nmols of the 28mer oligo was resuspended in 25 ml of a kinase reaction

[66mM Tris-HCl (pH 7.5), lmM spermidine, 10mM $MgCl_2$, 10mM DTT, lmM ATP, 200 ng/ml BSA and 16 U of T4 polynucleotide kinase] and incubated at 37°C for one hour. The reaction was stopped by adding EDTA (SmM final concentration) and incubating the reaction at 70°C for 5 min. Similarly, 1.5 nmols of the 24mer oligonucleotide were resuspended in 25 ml of kinase buffer [66mM: Tris-HCI (pH 7.5), lmM spermidine, 10mM $MgCl_2$, 10mM DTT]. Both oligonucleotides solutions were thoroughly mix by extensive vortex-shaking and placed in a beaker containing water at 80°C and allowed to cool to room temperature. Following annealing, adaptors were ethanol precipitated in the presence of glycogen (40 mg/ml) at -70°C, overnight. The precipitated adaptors were then resuspended in 15ml of sterile deionized water, and were immediately used to prepare a 2X adaptor-ligation mix [5 ml annealed adaptor, 10 ml of 10X T4 DNA ligase buffer (Promega) and 35 ml of sterile deionized water]. Mixing was done by gentle pippetting and 2 ml aliquots were stored at -20°C until used.

The PCR amplification primer is complementary to the 28mer oligo and it extends from the 5' Sau3A I sequence site to the Eco RI site. Ponce de León *et al.* (1996) reported the adaptor and PCR primer DNA sequences to be as follows:

ADAPTOR
5'GATCTCCTGTGTGATATTGAATTCCGCT3'
AGGACACACTATMCTTAAGGCGA
Eco RI

PCR PRIMER
5'GAATTCAATATCACAGACGAGATC3'

This construct offers the advantage of 1) Sticky end ligation of Sau3A I chromosomal fragments to the Sau3A I adaptor sites. 2) Adaptor dimers generated after the ligation step can be eliminated by digestion with Bgl II. 3) The amplification primer does not self amplify and does not recognize a specific target site in any of the livestock genomes.

CHROMOSOME MICRODISSECTION AND MICROCLONING

Microdissection of chromosomes was carried out by an adaptation of the procedure described by Pirrota *et al.* (1984). Approximately 10 or more chromosomes per experiment (estimated yield of 0.5-1.0 pg of DNA) were scraped from a coverslip containing metaphase spreads. To do this, two glass coverslips were placed adjacent to each other on a coverslip holder that was strapped to the stage of an inverted microscope. One was a clean siliconized coverslip and the other contained fixed metaphase spreads. A chromosome was scraped with a microneedle, lifted from the coverslip containing metaphases and placed on a pre-marked spot on the clean siliconized coverslip. This manipulation was repeated for each chromosome until all of the necessary chromosomes andlor chromosome fragments were microi-solated. To the chromosome pile, a drop (1 nl) of extraction buffer (0.5 mg/ml proteinase K, 10 mM

Tris-HCl pH 7.5, 10 nM NaCl, 0.1 p. cent SDS) was added followed immediately by a drop of heavy mineral oil, to avoid evaporation. The reaction was incubated at 37°C for two hours in a humified chamber.

The proteinase K extraction proce-

dure, as well as the subsequent phenol and chloroform extractions were performed in an oil chamber in a one nanoliter hanging droplet as described by Pirrota *et al.* (1984). All necessary micromanipulation of drops were done with a mechanical micromanipulator. Afler chloroforrn extraction to remove any residual phenol, the droplet containing the DNA was transferred to a fresh oil chamber. The restriction enzyme Sau3A I (New England Biolabs, 50 units per µl) was diluted with an equal volume of 4X restriction buffer (New England Biolabs) and an equal volume (approx. I nl) was added to the reaction drop. Digestion was carried out at 37°C for two hours in a humidified chamber. The enzyme was inactivated by incubation at 65°C for 20 minutes. An equal volume of solution containing 10 µM primer in 10 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM 2-β-mercaptoethanol, 12 mM ATP was added. One nanoliter volume of T4 DNA ligase (1 unit per µl) was also added to the reaction, followed by overnight incubation at 4°C. Since the adaptor-adaptor ligation generates a Bgl II restriction site, adaptor dimers were digested with Bgl TI in a 10 µl volume reaction.

Digested DNA was amplified by PCR using the Perkin-Elmer Cetus Amplitaq kit and thermocycler. The volume of the reaction was adjusted to 100 µl with PCR solution components (IX PCR bufer, 2.5 mM of each of the four deoxynucleoside triphosphates, 1 µM of the 24mer primer and 2.5 units of Taq DNA polymerase). The reaction was heated to 96°C for 10 min before adding Taq polymerase. Cycling conditions were: one cycle at 95°C for 1 min, 55°C for 1 min, 72°C for 2 min and 35 cycles at 94°C for 30 sec, 55°C for 1 min and 72°C for 2 min, with a final extension at 72°C for 7 min. Amplified chromosomal fragments were purified either by passing the PCR product through a Sephadex G50 column (Pharmacia) or through a Microcon 100 column (Amicon) according to the manufacturer's instruction. A fraction of the amplified chromosomal inserts was used to generate the chromosome specific DNA library and another fraction was saved to be able to amplified the pool of chromosome specific inserts when necessary.

FLUORESCENT *IN SITU* HYBRIDIZATION (FISH)

Origin and purity of chromosomal inserts (chromosome cocktail) was assessed by FISH to demonstrate their capacity to delineate (paint) the chromosome from which they originate. Labeling of chromosomal inserts with biotin-16-dUTP and/or biotin-14-dATP was carried out by PCR as described above. FISH was done as described by Ponce de León *et al.* (1996).

Chromosome specific DNA libraries: Chromosome *cocktails* were fractionated by size and the fraction containing chromosomal fragments varying from 250 to 1.000 bp were used for the production of chromosome specific DNA libraries. The fractionated chromosome cocktail was digested with Sau3Al to eliminate the adaptor molecules and generate Sau3AI sticky ends. Adaptors were removed by passing the reaction through a Microcon 100 column (Amicon). Digested chromosomal inserts were ligated to the BamHI cloning site of the BamHI-ClAP lambda Zap expression

vector (Stratagene) according to the manufacturer's protocol. Libraries were amplified and stored as described in standard protocols.

RESULTS AND DISCUSSION

The cell line containing bovine translocated marker chromosomes was particularly useful for the unequivocal identification of the chromosome of interest. However, it is well known that transformed cells undergo chromatin rearrangement and the extent of these rearrangements, for the cell line used, have not been characterized. In fact, during the course of our work and at latter passages the cell line developed new chromosome translocations, one of which allowed us to microisolate bovine chromosome 4 (Carpio and Ponce de León, 1995).

The PCR amplification product obtained from a microisolated chromosome is a complex collection of chromosomal fragments that vary between 100 to 2,000 bp in length. This complex mixture of chromosomal fragments which we refer to as *chromosome cocktail* represents, at best, 75 p. cent of the DNA content of the microisolated chromosome. The latter is intrinsic to the strategy used. Digestion of the adaptor dimers by Bgl II, a necessary step for successful PCR amplification of chromosomal fragments, also eliminates about 1/4 of the possible

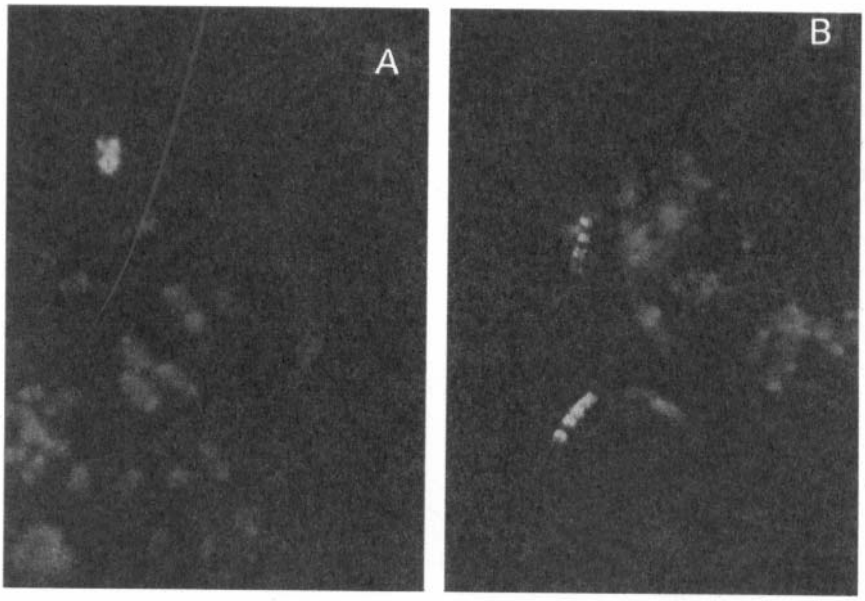

***Figure 1**. Bovine X-chromosome paint. Observe the fluorescent FITC signal on the X chromosome of male (A) and female (B) metaphase spreads.* (Marcaje e hibridación *in situ* del cromosoma bovino X. Obsérvese la señal fluorescente FITC sobre las metafases del cromosoma X del macho (A) y de la hembra (B)).

adaptor-chromosome fragment ligation events. Therefore, reducing by 1/4 the available chromosomal fragments for PCR amplification (Ponce de León *et al.*, 1996). Moreover, FISH analysis, after biotin-14-dATP labeling of the chromosome cocktail has invariably shown absence of FISH signals in centromeric chromatin, suggesting that centromeric chromosomal regions were not efficiently cloned.

Even though a chromosome cocktail does not represent all of the chromatin content of a chromosome, it has been useful for the generation of chromosome paints **(figure 1)** and assessment of inter-species chromosome homologies. Carpio and Ponce de León (1995) demonstrated the use of bovine painting probes for the identification of chromosome homologies between bovine and caprinae species. Results confirmed previous bovine/sheep and bovine/goat chromosome homologies identified by comparative analysis of chromosomal banding pattems (Hayes *et al.*, 1991).

Likewise, Ponce de León *et al.* (1996) utilizing the bovine Xp and Xq painting probes demonstrated that the bovine Xp region is interstitially localized in the Xq

Table I. *Microdissected chromosome-specific DNA libraries available for farm animal species.* (Librerías DNA para específicos cromosomas microdiseccionados de especies de animales domésticos).

Chromosome	p. cent of genome	Fragment size (bp)	# of plaques generated	Chromosome equivalents per library	p. cent of genome cloned by chromosome
BTAI[a]	5.73	700	1×10^6	3.64	
BTA4[a]	4.75	775	4.45×10^5	2.92	
BTAII[b]	3.81	400	1.15×10^6	5.60	
BTA14[a]	3-17	-	-	-	29.18
BTA20[a]	2.60	650	3.49×10^5	3.42	
BTA29[a]	2.03	500	7.49×10^5	7.17	
BTXp[c]	1.81	500	3.50×10^5	3.22	
BTXq[c]	3.51	500	4.50×10^5	2.92	
BTY[d]	1.77	675	2.53×10^5	3.84	
SSA6[e]	7.14	500	1.40×10^6	3.90	7.14
GDA1[f]	15.7	500	8.14×10^5	3.13	
GDA2[f]	11.9	500	6.60×10^5	3.30	
GDA3[f]	8.4	700	3.45×10^5	3.45	50.50
GDA4[f]	7.4	500	3.93×10^5	3.20	
GDZ[g]	7.1	I,000	9.13×10^8	14.20	

[a]Carpio and Ponce de León (1995).[b]Ambady, *et al.* (1996b). [c] Ponce de León *et al.* (1996). [d]Ponce de León and Carpio (1995). [e] Ambady *et al.*, (I996c), submitted. [f] Ponce de León, unpublished. [g]Ambady *et al.* (1996a) in press.

region of the sheep and goat X chromosome. Moreover, the use the Y-chromosome painting probe allowed the unequivocal localization of the pseudoautosomal region of the bovine X chromosome to Xq4 (Ponce de León and Carpio, 1995). Chicken chromosome painting probes have also been used to identify interspecies chromosome homologies with turkey (Ambady *et al.*, 1996) and partridge (Dias *et al.*, 1995).

Equally successful has been the generation of chromosome-specific and chromosomeregion-specific DNA libraries **(table I)**. Chromosome-specific DNA libraries for about 30 p. cent of the bovine, 7 p. cent of the swine and 50 p. cent of the chicken genomes have been constructed. Since these DNA libraries were expected to contain a high level of clone redundancy due to the preferential PCR amplification of shorter DNA fragments, their usefulness was assessed by screening the Xq library (Ponce de León *et al.*, 1996). Results showed that about 33 p. cent of all positive microsatellite carrying clones were unique, while 40 p. cent were redundant and 27 p. cent did not contain a microsatellite sequence. These findings have been confirmed during the screening ofthe bovine Xp (Sonstegard *et al.*, 1996a) and chromosome 1 (Sonstegard *et al.*, 1 996b) microdissected libraries. The use of microdissected libraries for the identification of pre-mapped microsatellite clones has accelerate the development of saturated linkage maps for bovine X-chromosome (average interval of 2.7 cM, Sonstegard *et al.*, 1996a) and chromosome 1 (average interval of 1.9 cM, Sonstegard *et al.*, 1996b).

The use of chromosome cocktails as probes for screening of large insert DNA libraries was tested with the chicken chromosome 1 cocktail. To do the latter, the chromosome cocktail was end labeled with ^{32}P, denatured and reannealed in the presence of 20 fold of biotin labeled Cot 1 chicken DNA to block the ^{32}P labeled repetitive sequences. Biotin-Cot 1 /^{32}P-repetitive double stranded products were removed by phenoVchloroform extraction. The subtracted chromosome cocktail probe was then used to screen a chicken cosmid library. Seventy percent of the positive cosmids were chromosome 1 specific and some were physically assigned to chromosome 1 (Li, 1994). However, the approach remains to be improved, as many of these clones physically hybridized to regions containing medium repetitive sequences.

Chromosome cocktails should also be useful for the screening of cDNA libraries and identification of premapped coding sequences which in turn could be developed as Type I markers (O'Brien *et al.*, 1993). Likewise, they should be useful for the screening of large insert libraries (Pl, BAC and YAC) with the objective to develop large insert chromosome specific sub-libraries that would facilitate their screening for identification of coding sequences.

REFERENCES

Ambady, S., S. Ciufo, J.M. Robl, J.R. Smyth and F.A. Ponce de León. 1996a. Development of a chicken Z-chromosome-specific DNA library. *J. of Heredity*: In press.

Ambady, S., P.K. Basrur, R.Z. Ma, C. Park, H.A. Lewin, S.M. Kappes, T.L. Smith, C.W. Beattie and F.A. Ponce de León. 1996b. A genetic and cytogenetic map for the bovine chromosome 11. In: XXV International Conference on Animal Genetics, International Society for Animal Genetics (ISAG), Tours, France, (Abstract) p 90.

Ambady, S., J.R. Mendiola, C.F. Louis, L. Buoen, L.B. Schook and F.A. Ponce de León. 1996c. Development and use of a microdissedted swine chromosome 6 DNA library. *Cytogenet. and Cell Genet.*: Submitted.

Beattie, C.W. 1994. Livestock genome maps. *Trends in Genetics* 10: 334-338.

Bishop, M.D., S.M. Kappes, J.W. Keele, R.T. Stone, S.L.F. Sunden, G.A. Hawkins, S. Solinas Toldo R. Fries, M.D. Grosz, J. Yoo and C.W. Beattie. 1994. A genetic linkage map for cattle. *Genetics* 136: 619-639.

Carpio, C.M., and F.A. Ponce de León. 1995. Generation of bovine chromosome-specific DNA libraries for autosomes 1, 4, 14, 20 and 29. In: 9th North American Colloquium on Domestic Animal Cytogenetics & Gene Mapping, Texas A&M University, Texas, USA, (Abstract) p, 18.

Dias, D., C.M. Carpio, S. Ambady, T. Rafael and F.A. Ponce de León. 1995. Heterologous painting of Red-legged partridge chromosomes with chicken chromosome painting probes. In: XXX Jornadas Luso-Espanholas de Genética, Lisbon, Portugal, (Abstract 60).

Di Berardino, D. and L. Iannuzzi L. 1982. Detailed description of R-banded bovine chromosomes. *J. Heredity* 73: 434-438.

Fisher, E.M.C., J.S. Cavanna and S.D.M. Brown. 1985. Microdissection and microcloning of the mouse X chromosome. *Proc. Natl. Acad. Sci.* USA 82: 5846-5849.

Gray, J.W., P.N. Dean, J.C. Fuscoe, D.C. Peters, B.J. Trask, G.J. Van Den Engh and M.A. Van Dilla. 1987. High speed chromosome sorting. *Science* 238: 323-329.

Hayes, H., E. Petit and B. Dutrillaux. 1991. Comparison of RBG-banded karyotypes of cattle, sheep and goats. *Cytogenet. Cell Genet.* 57: 51-55.

Langford, C.F., H. Telenius, N.P. Carter, N.G.A. Miller and E.M. Tucker. 1992. Chromosome painting using chromosome-specific probes from flow-sorted porcine chromosomes. In: XXIII International Conference on Animal Genetics, International Society for Animal Genetics (ISAG), Interlaken, Switzerland, (Abstract) p 85.

Li, Y. 1994. Cytogenetic and molecular approaches for physical mapping of the chicken genome. University of Massachusetts-Amherst, Amherst, Massachusetts, USA. Ph.D. Thesis, p, 92.

Ludecke, H.J., G. Senger, U. Clacssen and B. Horsthemke. 1989. Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. *Nature* 338: 348-350.

O'Brien, S.J., J.E. Womack, L.A. Lyons, K.J. Moore, N.A. Jenkins and N.G. Copeland. 1993. Anchored reference *loci* for comparative genome mapping in mammals. *Nature Genetics* 3: 103-1 12.

Pirrotta V, H. Hackle and J.E. Edstrom. 1984. Microcloning of Microdissected chromosome fragments. in: Genetic Engineering. Principles and Methods. eds. Hollaender, A. and J.K. Setlow, Plenum, New York. Vol. 5: 1-17.

Ponce de León, F.A., S. Ambady, G.A. Hawkins, S.M. Kappes, M.D. Bishop, J.M. Robl and C.W. Beattie. 1996. Development of a bovine X linkage group and painting probes to assess

cattle, sheep and goat X-chromosome segment homologies. *Proc. Natl. Acad. Sci.* U.S.A. 93: 3450-3454.

Ponce de León, F.A., and C. Carpio C. 1995. Identification of the bovine X chromosome pseudoautosomal region. In: 9th North American Colloquium on Domestic Animal Cytogenetics & Gene Mapping, Texas A&M University, Texas, USA, (Abstract) p, 8.

Ponce de Leon, F.A. and J. Robl. 1992. Microisolation and microcloning of the bovine X-chromosome. In: XXIII International Conference on Animal Genetics, International Society for Animal Genetics (ISAG), Interlaken, Switzerland, (Abstract) p62

Saunders, R.D.C., D.M. Glover, M. Ashburner, I. Siden-Kiamos, C. Louis, M. Monastirioti, C. Savakis and F. Kafatos. 1989. PCR amplification of DNA microdissected from a single chromosome band: a comparison with conventional microcloning. *Nucleic Acids Research* 17: 9027-9037.

Schmutz, S.M., J.S. Moker, T.G. Berryere, C.P. Schelling and D.S. Gallagher. 1994. Bovine 14;20 DNA library preparation using chromosome microdissection. In: XXIV International Conference on Animal Genetics, International Society for Animal Genetics (ISAG), Prague, Czech Republic (Abstract) p 125.

Sonstegard, T.S., N.L. Lopez-Corrales, S.M. Kappes, R.T. Stone, S. Ambady, F.A. Ponce de León and C.W. Beattie. 1996a. An integrated genetic and physical map of the bovine X chromosome. *Mammalian Genome:* In press.

Sonstegard, T.S., F.A. Ponce de León, N.L. Lopez-Corrales, C.W. Beattie, S.M. Kappes. 1996b. A chromosome specific lambda library increases density on bovine chromosome 1. *Genome Research:* Submitted.

Wu, R., Wu, T., and Ray, A. 1987. Adaptors, Linkers and Methylation. In: Methods in Enzymology 152: Guide to Molecular Cloning Techniques. (Editors Berger, S.L., and Kimmel, A.R.), Academic Press, New York, USA, pp: 343-349.

Yerle, M., A. Schmitz, D. Milan, B. Chaput, L. Monteagudo, M. Vaiman, G. Frelat and J. Gellin. 1993. Accurate characterization of porcine bivariate flow karyotype by PCR and Fluorescence in *situ* hybridization. *Genomics.* 16: 97-103.

Zoghbi, H.Y. and A.C. Chinault. 1994. Generation of YAC contigs by walking. In: YAC Libraries: A user's Guide. (Editors: Nelson DL, and Brownstein BH). W.H. Freeman New York, USA, pp: 93-112.