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The "Spotted" Locus Maps to Bovine Chromosome 6 in a Hereford-Cross Population

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The spotted locus is responsible for several phenotypically distinguishable piebald patterns in cattle, including Hereford, or white face (S^H), lineback (S^P), and recessive spotting (s), in addition to nonspotted (S^+). In a backcross mapping population, the S locus has been mapped by genetic linkage to bovine chromosome 6, between microsatellite markers BM4528 and EL03. This region corresponds comparatively to a region on mouse chromosome 5 which houses several coat color mutations, among which homology is possible with Hardy–Zuckerman 4 feline sarcoma viral oncogene homologue (*Kit*), patch (*Ph*), and rump white (*Rw*). Mutations at these loci resemble mutations at the bovine S locus in both phenotype and mode of inheritance. Data are presented which show genetic linkage between the bovine S locus and microsatellite markers on chromosome 6. Candidate genes for the bovine S locus are discussed.

Spotting patterns in cattle have interested breeders for hundreds of years. Historically, piebald patterns have been favored in selection (Epstein 1971). Also, as breeds were developed, certain breed consistencies were favored as a method of rapid and convenient identification (Olson 1992). In addition to the aesthetic values attributable to coat color variation, there are other, more economical reasons for interest in coat color phenotypes. In particular, breeds that possess lighter coat color and darker skin pigmentation tend to perform better in tropical climates and increased levels of solar radiation (such as Zebu breeds; Olson 1992).

An additional interest in coat color variation has arisen recently in the form of price discrimination at the sale barn for specific coat color patterns, in some cases regardless of the actual pedigree of the animal. Animals can sell at discounts or premiums to cohorts of similar ancestry simply because of coat color variations. Although such discounts or premiums may be temporary, one cannot dispute the effect of such economic selection on the breeding decisions made by the cattle rancher.

There are several genes which are known to affect coat color and/or spotting pattern. Included in this group (as reviewed by Olson 1992) are the *E* locus (red/black), *A* locus (removal of some red and black pigmentation), *C* locus (removal of red pigment with no effect on black pigment), *D* locus (dilution series), *Br* (brindle), and *Bp* (patterned blackish). Different allelic combinations at these loci may appear to lessen or heighten the effect of a particular gene.

White-spotting mutations are present in a variety of cattle breeds (e.g., Hereford, Pinzgauer, Holstein, Simmental, etc.). Several genes have been identified as playing a role in the amount and pattern of white-spotting (as reviewed by Olson 1992), and include the *S* locus (spotting), *Cs* (color-sided), *R* (roan, may be allelic with *Cs*), *Bl* (blaze), *Bc* (brockling), and *Bt* (belted). At least four alleles at the *S* locus have been identified. The S^H allele is present in Hereford and is described as white face, belly, feet, and tail, often with a white stripe over the shoulder when homozygous. This allele shows incomplete dominance over the wild type, S^+, in that S^H/S^+ heterozygotes will have white areas generally around the face only. The S^P allele is present in Pinzgauer, Longhorn, and Florida Cracker breeds and is expressed as variable amounts of white appearing along the dorsal and ventral areas extending forward from the tail or rump. The *s* allele is recessive and is expressed as irregular areas of pigment and white, with the feet, tail, and belly usually white (as seen in the Holstein breed). The *s/s* phenotype is also classically known as "piebald" (Olson 1992).

As part of a larger study to identify quantitative trait loci (QTL) affecting traits of economic interest, a Hereford-sired bull was bred to composite (CGC; ½ Red Angus, 1/4 Tarentaise, 1/4 Charolais) and Line 1 Hereford dams to produce 77 backcross offspring. The objectives of this research were to identify the chromosomal

location of the gene(s) causing dominant spotting in Hereford cattle, and to assess the viability of candidate loci based on the available bovine-mouse comparative map. Results of linkage analysis show that the *S* locus resides on bovine chromosome 6, and also suggests that the gene causing white spotting in cattle may be homologous to one of several coat color loci on mouse chromosome 5, which produce similar phenotypic observations, and lie in a region of conserved synteny between bovine and murine genomes.

Table 1 Linkage analysis of S locus

	BM143	BMS1242	BM4528	*S*	EL03	BMS2460
BM143	239	1 cM, 43.99	17 cM, 13.8	24 cM, 4.17	21 cM, 6.26	27 cM, 6.43
BMS1242	172	206	17 cM, 13.5	24 cM, 4.4	23 cM, 6.31	29 cM, 5.17
BM4528	149	150	172	9 cM, 11.66	10 cM, 13.46	12 cM, 18.9
S	67	72	70	75	6 cM, 13.59	13 cM, 18.69
EL03	100	105	94	69	130	7 cM, 17.6
BMS2460	187	173	161	73	109	230

Number of coinformative meioses are given below the diagonal, linkage distances (cM) and associated LOD scores are given above the diagonal and total genotyped meioses on the diagonal. All microsatellite loci have been previously mapped to chromosome 6.

Materials and Methods

An F_1 bull derived from a cross of Miles City Line 1 Hereford bull (Line 1) and Composite Gene Combination dam (CGC; ½ Red Angus, 1/4 Tarentaise, 1/4 Charolais) was mated to CGC and Line 1 dams to produce backcross calves. The phenotype of Line 1 Herefords is the classic Hereford pattern, with white face, belly, feet, and tail, and white stripe over the shoulder. Genotypically this pattern can be defined as S^H/S^H. CGC cattle represent all coat colors present in Red Angus, Charolais, and Tarentaise breeds, including red, dilution, and brindle. None of the CGC dams (nor any members of the CGC herd) have ever presented a spotted phenotype. Therefore the CGC dams are considered genotypically S^+/S^+, and the F_1 bull is S^H/S^+. Calves produced as a result of breeding the F_1 bull to Line 1 Hereford dams were not phenotyped for the *S* locus because of the difficulty in reliably distinguishing between S^H/S^H and S^H/S^+ phenotypes. However, all calves (77 CGC backcross calves and 73 Line 1 Hereford backcross calves) were used for linkage analysis concerning microsatellite markers.

The F_1 bull was mated to CGC dams by artificial insemination to produce 77 offspring. These offspring were genotypically either S^+/S^+ (completely nonspotted) or S^H/S^+ (white spotted on or around the face). Calves were scored for the presence (S^H/S^+) or absence (S^+/S^+) of white spotting at approximately 1 month of age. Three calves were solid white, making it impossible to determine the extent of their spotting. Solid white calves were not scored for the *S* locus. Of the 74 scored calves, 38 (51.35%) where scored as white faced (S^H/S^+) and 36 (48.65%) were scored as non-white faced (S^+/S^+), which is consistent with a 1:1 ratio as expected for the *S* locus.

Microsatellite markers were prescreened by genotyping the F_1 sire by the method of Bishop et al. (1994). Markers that were informative were then used to genotype calves, sires, dams, paternal grandsires, and paternal grandams. Eighty-four of 159 markers screened (53%) were informative in the sire and were used to genotype the entire population. With 74 informative meioses, a LOD score supporting linkage would be achieved with 21 recombination events (28.4 cM, LOD = 3.11). At least 1 marker was present on each chromosome, with the exception of 25, X, and Y.

All genotypic data was entered into a relational database housed at USDA-ARS, Meat Animal Research Center, Clay Center, Nebraska (Keele et al. 1994), and linkage analysis was performed using CRI-MAP (Green et al. 1990). Two-point analyses were performed to determine maximum likelihood estimates. If the maximum log-likelihood estimate was 3.0 greater than the log-likelihood estimate of a recombination frequency of 0.5 (unlinked), then the data was interpreted as evidence for linkage. All two-point linkages between microsatellite markers and *S* with a LOD score (Z) greater than 3.0 were identified. Markers which showed a two-point LOD score of greater than 3.0 were analyzed to determine the best order of markers using the CRI-MAP BUILD and ALL functions.

Results and Discussion

Two-point linkage analysis of the dataset revealed linkage between *S* and five markers on chromosome 6, with LOD scores ranging from 4.17 to 18.69 (Table 1). This was considered strong evidence for the assignment of *S* to bovine chromosome 6. To ensure that no other linkages existed, stringency on the test for linkage was reduced to a log-likelihood estimate greater than 1.5 times the LOD at a recombination frequency of 0.5. Under these relaxed conditions, no linkage was observed between *S* and the markers not located on chromosome 6.

To determine the order of linked markers along chromosome 6, the ALL function of CRI-MAP was used. Based on our mapping population, the order of microsatellite markers from the centromeric end of chromosome 6 was observed to be BM143-1 cM-BMS1242-16 cM-BM4528-8 cM-EL03-6 cM-BMS2460-34 cM-BL1038. By using the ALL function of CRI-MAP, the most likely order (after insertion of S) is BL1038-BMS2460-*S*-EL03-BM4528-BM143 (Figure 1).

The current status of comparative maps allows for selection of candidate genes from regions known to share homology between two species. In order for two genes to be considered homologous, several criteria must be met, including conservation of function and conservation of sequence. The most effective method of identifying homologous genes across species is by first identifying regions of conserved synteny, followed by analysis at the sequence and functional levels.

The majority of genes mapped to bovine chromosome 6 also map to mouse chromosome 5. There is additional conservation of synteny between the centromeric end of BTA6 and MMU3. A comparison of loci on BTA6 and homologous mouse loci is shown in Table 2. According to the map published by Barendse et al. (1997), the distance from BM4528 to SPP1 is 30 cM. Assuming the highest likelihood order, which places *S* between BM4528 and EL03, our data show BM4528 and *S* to be separated by 6 cM, suggesting that *S* lies on the region of bovine chromosome 6 which is homologous to murine chromosome 5.

A search of the Mouse Genome Database web site (The Jackson Laboratory, http://www.informatics.jax.org/) for coat color–associated loci located on MMU5 yields five loci, including Kit oncogene (*Kit*), patch (*Ph*), rump white (*Rw*), light ear (*le*), and buff (*bf*). All five loci are positioned between 42 and 64 cM from the centromere. Light ear and buff are clearly phenotypically dissimilar to bovine spotting, which leaves *Kit*, *Ph*, and *Rw* (all

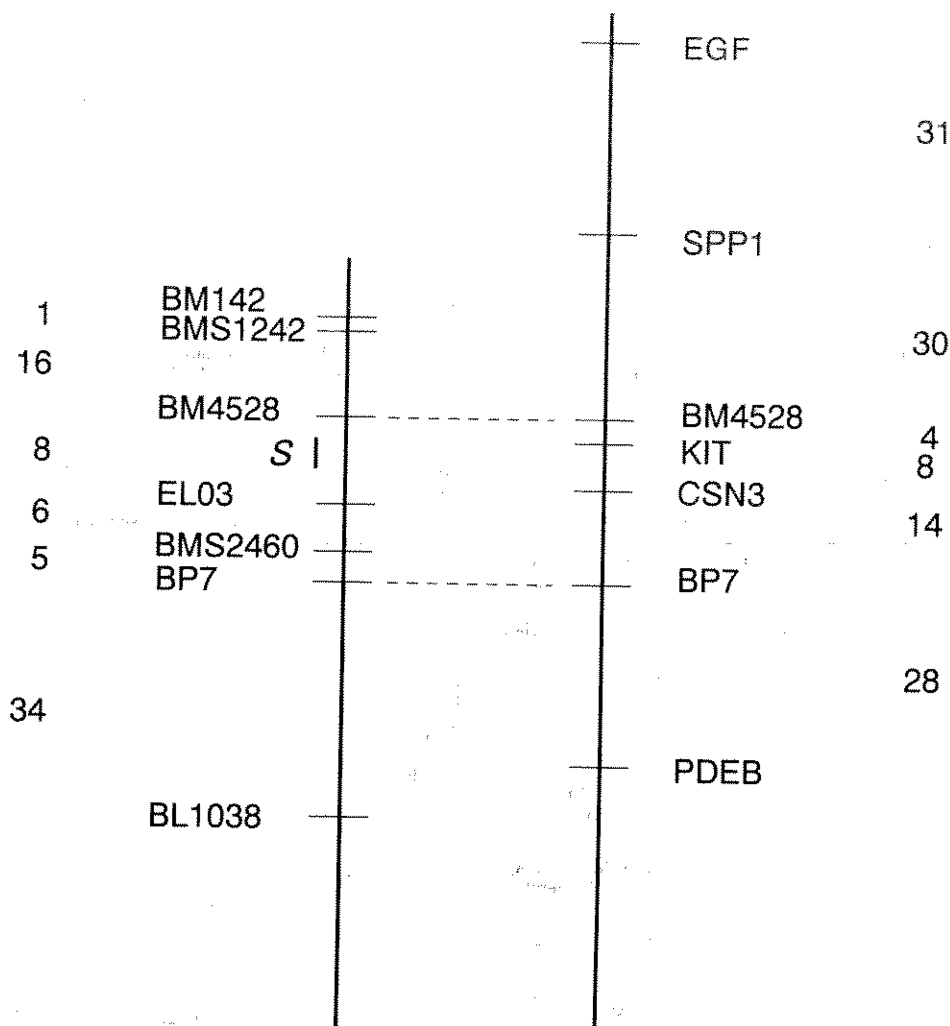

Figure 1. Linkage maps of bovine chromosome 6. Map on right represents markers used in this study (referenced in Kappes et al. 1997). Map on left is taken from Barendse et al. (1997). Linkage distances are in centiMorgans.

mapped to MMU5, 42 cM) as candidates for the bovine *S* locus.

The *Ph* mutation arose spontaneously in a C57BL strain. Homozygotes die in utero with malformations of the skull which interfere with proper development of the nose and palate. Heterozygotes survive and display variable levels of white spotting around the belly. The *Ph* locus is suggested to exert control over hematopoiesis (Loutit and Cattanach 1983). Several published reports have suggested that patch is a mutation involving the deletion of *Pdgfra* (Orr-Urtreger et al. 1992; Stephenson et al. 1991) and therefore would be more appropriately classified as an allele at the *Pdgfra* locus. However, because such a deletion may involve additional, adjacent genes (*Kit*; Duttlinger et al. 1995), *Ph* is not yet accepted as a *Pdgfra* allele.

Rump white was introduced by breeding (C3H/He × 101/H)F_1 males after exposure to low-intensity neutron irradiation. *Rw* behaves in a semidominant manner, with heterozygotes showing white spotting on the hind legs, tail, and posterior abdomen. Homozygotes die in utero at midgestation. Nagle et al. (1994) has suggested that rump white may well owe its effects on pigmentation to disruption of *Kit* and/or *Ph* regulatory sequences.

Kit is a member of the family of growth factor receptors that possess tyrosine kinase activity when bound to a specific ligand. The *Kit* gene is known to be involved with at least three populations of migratory stem cells: neural crest-derived melanocytes, hematopoietic stem cells, and primordial germ cells (Fleischman 1993). In the mouse, *Kit* and its alleles cause reduced and/or localized pigmentation, macrocytic anemia, and sterility (Geissler et al. 1988; Nocka et al. 1990).

Mutations at the *Kit* locus are semidominant and occur frequently (Schlager and Dickie 1967), with over 30 currently documented in the Mouse Locus Catalog literature. Examination of the listed phenotypes yields several general similarities. Homozygotes or compounds of two different mutations are generally white, sterile, and have severe macrocytic anemia, often causing death in utero. Animals containing one mutant allele show white spotting, are fertile, and may be slightly anemic. Mutations which affect the level of *Kit* kinase activity also tend to affect the severity of phenotypic expression. Mutations that abolish activity are homozygous lethal, while mutations with residual kinase activity are homozygous viable (Bernstein 1991).

In addition to white spotting, almost all *Kit* mutations also involve anemia. The condition is detectable at about 12 days of gestation and persists throughout life. Red cell count and hemoglobin are considerably reduced and the red cells are macrocytic (Bannerman et al. 1973; Russell 1970). This is an interesting finding, taking into account the discovery by Steffen et al. (1991) of a genetically based syndrome of anemia (along with progressive alopecia and dyskeratosis) transmitted genetically in a herd of polled Herefords.

This coincidence is compounded when it is considered that the frequency of bovine ocular squamous cell carcinoma ("cancer eye") is significantly higher in Herefords than other breeds (Anderson 1970; Blackwell 1956). Although the primary tumor type identified with *Kit* oncogene in mice is a papilloma of the forestomach and duodenum (Kitamura et al. 1980), the precise tumorigenic pathway is still poorly understood. Two hypotheses are presented: (1) during the formation of the Hereford breed, white-faced animals were combined and bred to produce true-breeding, white-faced (S^H/S^H) animals. However, these animals are only phenotypically similar. Underlying the mutation which causes spotting, there may be other mutations (in the same gene) which predispose animals to combinations of anemia, dyskeratosis, and tumors (as is seen in *Kit* mutants). (2) The presence of gene sequences which cause the phenotypic expression of S^H may also alter the expression and/or stability of nearby genes, which would predispose the animal to combinations of anemia, dyskeratosis, and tumors (as seen in *Rw* mutants).

This article has presented data which localizes the gene responsible for the Hereford white-face spotting pattern (*S* locus) to bovine chromosome 6 within a genomic region displaying conserved syn-

Table 2. Bovine chromosome 6 loci and map location in mouse and human

Gene symbol	Gene name	Mouse location (chr, cM)	Human location
EGF	Epidermal growth factor	3, 65.2	4q25
IF	I factor (compliment)	3, 66.6	4q24-q25
ADH3	Alcohol dehydrogenase 3	3, 71.2	4q21-q23
SPP1	Secreted phosphoprotein 1	5, 56	4q11-q21
GABRA2	Gamma-aminobutyric acid receptor, subunit alpha 2	5, 40	4p13-p12
KIT	Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue	5, 42	4q12
CSN3	κ-casein	5, 45	4p16.3-q21[b]
ALB	Serum albumin variant	5, 50	4q11-q13
GC	Group specific component	5, 50	4q12-q13
PDEB	Phosphodiesterase, cGMP, rod receptor, beta polypeptide	5, 57	4p16.3
QDPR	Quininoid dihydropteridine reductase	5, 30	4p15.3
PGM2[a]	Phosphoglucomutase 2	5, 38	4p14-q12
IDUA	Alpha-L-iduronidase	5, 57	4p16.3
PEP7	Peptidase 7	5, 39	4p11-q12
CNCG	Cyclic nucleotide gated channel, cGMP gated	5, 41	4p14-q13
PDGFRA	Platelet derived growth factor receptor, alpha polypeptide	5, 42	4q11-q12
GNRHR	Gonadotropin releasing hormone receptor	5, 44	4q21.2
SOD3	Superoxide dismutase 3, extracellular	Unknown	4p16.3-q21

Data obtained from Mouse Genome Database web site, The Jackson Laboratory, http://www.informatics.jax.org/, Barendse et al. (1997), Fries et al. (1993), Gallagher et al. (1992), Kappes et al. (1997), Threadgill and Womack (1990), and Zhang et al. (1992).

[a] PGM2 maps to MMU 4, 45 cM; however, PGM1 maps to MMU5, 38 cM, and most likely represents the appropriate homologous locus.

[b] 4p16.3-q21 represents the map location of CSN2, which along with CSN3 and other genes comprises the casein cluster.

teny with murine chromosome 5. Coincident with this finding are the observations of anemia and increased incidence of bovine ocular squamous cell carcinoma in the Hereford breed. Whether these observations are related through mechanisms involving the *Kit* oncogene or other genes remains to be proven. However, the prospect of a link between the bovine *S* locus and ocular squamous cell carcinoma is intriguing and warrants further study.

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References

Anderson DE, 1970. Cancer eye in cattle. Mod Vet Pract 51:42–47.

Bannerman RM, Edwards JA, and Pinkerton PH, 1973. Hereditary disorders of the red cell in animals. Prog Hematol 8:131–179.

Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, Sun HS, Eggen A, et al., 1997. A medium-density genetic linkage map of the bovine genome. Mammal Genome 8:21–28.

Bernstein A, Forrester L, Reith AD, Dubreuil P, and Rottape R, 1991. The murine W/c-kit and Steel loci and the control of hematopoiesis. Semin Hematol 28:138–142.

Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA, Solinas-Toldo S, Fries R, Grosz MD, Yoo J, and Beattie CW, 1994. A genetic linkage map for cattle. Genetics 136:619–639.

Blackwell RL, Anderson DE, and Knox JH, 1956. Age incidence and heritability of cancer eye in Hereford cattle. J Anim Sci 15:943–951.

Dickie MM, 1966. Recessive spotting. Mouse News Lett 35:31.

Duttlinger R, Manova K, Berrozpe G, Chu TY, DeLeon V, Timokhina I, Chaganti RS, Zelenetz AD, Bachvarova RF, and Besmer P, 1995. The Wsh and Ph mutations affect the c-kit expression profile: c-kit misexpression in embryogenesis impairs melanogenesis in Wsh and Ph mutant mice. Proc Natl Acad Sci USA 92:3754–3758.

Epstein H, 1971. The origin of the domestic animals of Africa, vol. I. Leipzig: German Democratic Republic.

Fleischman RA, 1993. From white spots to stem cells: the role of the Kit receptor in mammalian development. Trends Genet 9:285–290.

Fries R, Eggen A, and Womack JE, 1993. The bovine genome map. Mammal Genome 4:405–428.

Gallagher DS Jr, Womack JE, Baehr W, and Pittler SJ, 1992. Syntenic assignments of visual transduction genes in cattle. Genomics 14:699–706.

Geissler EN, Ryan MA, and Housman DE, 1988. The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. Cell 55:185–192.

Green P, Falls K, and Crooks S, 1990. Documentation of CRI-MAP, version 2.4. Washington University School of Medicine, St. Louis, MO.

Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TPL, Lopez-Corrales NL, Beattie CW, 1997. A second-generation linkage map of the bovine genome. Genome Res 7:235–249.

Keele JW, Wray JE, Behrens DW, Rohrer GA, Sunden SLF, Kappes SM, Bishop MD, Stone RT, Alexander LJ, and Beattie CW, 1994. A conceptual database model for genomic research. J Comp Biol 1:65–76.

Kitamura Y, Yokoyama M, Matsuda H, and Shimada M, 1980. Coincidental development of forestomach papilloma and prepyloric ulcer in nontreated mutant mice of W/Wv and Sl/Sld genotypes. Cancer Res 40:3392–3397.

Loutit JF and Cattanach BM, 1983. Haematopoietic role for Patch (Ph) revealed by new W mutant (Wct) in mice. Genet Res 42:29–39.

Mouse Genome Database (MGD). Mouse Genome Informatics. The Jackson Laboratory. Bar Harbor, Maine. World Wide Web (URL: http://www.informatics.jax.org/). July 1997.

Nagle DL, Martin-DeLeon P, Hough RB, and Bucan M, 1994. Structural analysis of chromosomal rearrangements associated with the developmental mutations Ph, W19H, and Rw on mouse chromosome 5. Proc Natl Acad Sci USA 91:7237–7241.

Nocka K, Tan JC, Chiu E, Chu TY, Ray P, Traktman P, and Besmer P, 1990. Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W37, Wv, W41 and W. EMBO J 9:1805–1813.

Olson TA, 1992. Qualitative genetics. In: Genetic aspects of beef cattle production in the southern region. (Tess MW and Thrift FA, eds). Auburn, Alabama: Auburn University.

Orr-Urtreger A, Bedford MT, Do MS, Eisenbach L, and Lonai P, 1992. Developmental expression of the alpha receptor for platelet-derived growth factor, which is deleted in the embryonic lethal Patch mutation. Development 115:289–303.

Russell ES, 1970. Abnormalities of erythropoiesis associated with mutant genes in mice. In: Regulation of hematopoiesis (Gordon AS, ed). New York: Appleton-Century-Crofts; 649–675.

Schlager G and Dickie MM, 1967. Spontaneous mutations and mutation rates in the house mouse. Genetics 57:319–330.

Steffen DJ, Leipold HW, Gibb J, and Smith JE, 1991. Congenital anemia, dyskeratosis, and progressive alopecia in polled Hereford calves. Vet Pathol 28:234–240.

Stephenson DA, Mercola M, Anderson E, Wang CY, Stiles CD, Bowen-Pope DF, and Chapman VM, 1991. Platelet-derived growth factor receptor alpha-subunit gene (Pdgfra) is deleted in the mouse patch (Ph) mutation. Proc Natl Acad Sci USA 88:6–10.

Threadgill DW and Womack JE, 1990. Genomic analysis of the major bovine milk protein genes. Nucleic Acids Res 23:6935–6942.

Zhang N, Threadgill DW, and Womack JE, 1992. Synteny mapping in the bovine: genes from human chromosome 4. Genomics 14:131–136.

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