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SEQUENCE ANALYSIS OF PMEL17 AS CANDIDATE GENE FOR CAUSING RAT-TAIL SYNDROME IN CATTLE.

by

Benjamin C. Hecht

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the degree requirements for

Master of Science

Department of Plant and Animal Sciences

Brigham Young University

August 2006



BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Benjamin C. Hecht

This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

Date

Emilie M. G. Campbell, Chair

Date

Eric N. Jellen

Date

Roy W. Silcox



BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the thesis of Benjamin C. Hecht in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and departmental style requirements; (2) its illustrative material including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

Date

Emilie M.G. Campbell Chair, Graduate Committee

Accepted for the Department

Von D. Jolley Graduate Coordinator

Accepted for the College

Rodney J. Brown Dean, College of Biology and Agriculture



ABSTRACT

SEQUENCE ANALYSIS OF PMEL17 AS CANDIDATE GENE FOR CAUSING RAT-TAIL SYNDROME IN CATTLE

Benjamin C. Hecht

Department of Plant and Animal Sciences Master of Science

Congenital hypotrichosis in cattle is commonly referred to as "rat-tail" syndrome and is characterized by a dilution of black coat color and morphological changes to the hair shaft and tail switch. Two loci are involved in the inheritance of the rat-tail phenotype, the "extension locus" (MC1R) and an unknown locus. In order to express the rat-tail phenotype the animal must inherit at least one black allele at MC1R and be heterozygous at the unknown locus. The rat-tail locus was previously mapped to an 8.7 cM region of *Bos Taurus* autosome (BTA) 5. Pmel17 is known to be involved in the expression of pigmentation and maps to the same region of BTA5 as the rat-tail locus. Cattle from a population segregating for the rat-tail syndrome were sequenced at Pmel17 in order to identify putative causative mutations. Two mutations were detected, a three base pair (bp) deletion in exon 1 at codon 18 removing a leucine residue, and a single nucleotide polymorphism (SNP) at codon 612 resulting in an amino acid substitution ($A\rightarrow E$). The



3-bp deletion in exon 1 of Pmel17 is in 100% concordance with the rat-tail phenotype in this research population and may be causative of the rat-tail phenotype.



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SEQUENCE ANALYSIS OF PMEL17 AS CANDIDATE GENE FOR CAUSING RAT-TAIL SYNDROME IN CATTLE.



Introduction

To improve the performance of British cattle, Continental cattle are often used in cross breeding programs. When some of these Continental cattle are bred to black-hided British breeds, such as Angus and Holstein, a congenital disorder manifested itself in the offspring. This disease causes a dilution of the dominant black coat color as well as negative morphological changes to the hair shaft, which affects the animal's performance and results in an economic loss. This disorder is a form of congenital hypotrichosis, commonly referred to as "rat-tail" syndrome (Ayers *et al.* 1989).

Rat-tail syndrome is expressed as a result of an epistatic gene interaction between the extension locus (MC1R) and an unknown locus. MC1R controls red versus black coat coloration, with black coat color being dominant. For the rat-tail syndrome to be expressed, an individual must inherit a dominant allele at MC1R and be heterozygous for the rat-tail allele at the unknown locus (Schalles & Cundiff 1999).

The rat-tail locus has been mapped to *Bos Taurus* autosome 5, a region syntenic to human chromosome 12 (Singrey 2002). The focus of this study is to determine candidate genes in this region and to sequence and analyze a candidate gene for causative mutations of the rat-tail syndrome. Once a mutation is found to be causative of the rat-tail syndrome it may be possible to develop a molecular diagnostic test to aid in marker-assisted selection of this syndrome.



Literature Review

Description of the Rat-tail Syndrome. Several breeds of Continental cattle introduced into the United States during the 1960s and 1970s allowed cattle producers to maximize heterosis and improve the performance of British cattle by cross breeding to larger Continental cattle. However a congenital condition results when certain Continental breeds are crossed with black-hided cattle, such as Angus or Holstein. The disorder is a form of hypotrichosis commonly referred to as "rat-tail syndrome" (Ayers *et al.* 1989).

Rat-tail syndrome in cattle is characterized by short, curly, malformed, sometimes sparse hair and the lack of normal tail switch development. Affected animals have enlarged, irregularly distributed, and clumped melanin granules in the hair shafts, which are asymmetrical, short, curled, and small (Ayers *et al.* 1989). The syndrome results in a dilution of black coat color yielding a charcoal grey coloration. Any white patches of hair on an otherwise rat-tail individual are not affected and exhibit normal characteristics within the white patch. Animals affected with the syndrome are often discriminated against at market. Rat-tail calves have lower average daily gain during winter months from weaning to yearling, which results in a lighter animal at slaughter (Schalles & Cundiff 1999). Both of these characteristics result in an economic loss.

Genetic Aspects of Rat-tail Syndrome. An epistatic interaction between the extension locus (MC1R) and an unknown locus is involved in the inheritance of the rat-tail syndrome (Schalles & Cundiff 1999). The MC1R locus determines the inheritance of either black, or red, coat color. A dominant allele at MC1R (E_) will result in black coat color, while the inheritance of two recessive alleles (ee) will result in a red coat



(Klugland *et al.* 1995). For the rat-tail syndrome to be expressed, the animal must inherit at least one dominant allele at MC1R and be heterozygous (Cc) at the unknown locus (Fig. 1A). Animals that inherit recessive red alleles (ee) at MC1R do not show any phenotypic variation when a rat-tail allele is inherited at the unknown locus. If the animal is homozygous dominant (CC) at the unknown locus and carries at least one black allele (E_) at MC1R, the animal's coat will be diluted to a light grey color with fine hair and no rat-tail characteristics (Fig. 1B). If the animal carries at least one black allele at MC1R and is homozygous recessive (cc) at the unknown locus, normal black coat coloration is expressed (Schalles & Cundiff 1999) (Fig. 1C). The combinations and resulting phenotypes from the interaction of these two loci are illustrated in Table 1.

Genetic Linkage to the Rat-tail Syndrome. Singrey (2002) used 103 informative microsatellite markers to map the rat-tail locus to BTA5 in cattle. Two microsatellite markers BMS1617 and BR2936 are in linkage disequilibrium with the rat-tail locus having LOD (Log of the odds) scores of 4.93 and 4.82 respectively (Table 2). The putative gene therefore lies in an approximately 9cM region between those two markers (Fig. 2). This region of BTA5 exhibits conservation of synteny with MMU10 and HSA12 (represented as region III in Fig. 3). This region of the human genome contains SILV (Si), a gene important in mammalian pigmentation (Liu *et al.* 2003; Clark *et al.* 2006) (Fig. 3).

Pmel17 as Candidate Gene. Linkage-mapping of the rat-tail syndrome narrowed the search for candidate genes to the region of HSA12 syntenic to BTA5. Genes important in



pigmentation are of great interest. The SILV gene codes for the protein Pmel17, which may promote the conversion of 5,6-dihydroxyindole-2-carboxylic acid to melanin (Kwon 1995). Melanin is further converted to eumelanin or pheomelanin to give the coat and skin black or red-pigmented coloration respectively. Furthermore, Pmel17 is necessary for the formation of the fibril matrix upon which melanin intermediates are deposited late in melanosome maturation (Theos *et al.* 2005).

In the human the primary structure of the Pmel17 protein includes a 24-residue Nterminal signal peptide sequence followed by an approximately 578-residue variable lumenal domain. The C-terminal end is made first of a 25-residue transmembrane domain and terminated with a 45-residue cytoplasmic domain (Fig. 4B). Pmel17 is composed of 11 exons, with exon 6 being the largest and having variable splice sites in humans. Exon 1 encodes the signal peptide sequence, while exons 2-10 comprise the lumenal domain. The transmembrane and cytoplasmic domains are coded by exon 11 (Theos *et al.* 2005) (Fig.4). Pmel17 encodes a melanosomal protein important in pigmentation which maps to HSA12q13-14 (Kubota 1995), a region syntenic to BTA5.

Kwon *et al.* (1995) reported that an insertion mutation in the 3' region of Pmel17 in mice causes an extension of the protein by 12 residues resulting in a silver phenotype against a black background. This mutation has a negative effect on melanocytes, coat color pigment producing cells, and their viability. The silver mutation in mice primarily affects the production of the black pigment eumelanin (Silvers 1979). A decrease in the production of eumelanin causes a dilution of the black coat, and a silver phenotype results.



Mutations in Pmel17 correlate with the dominant white, dun and smoky plumage phenotypes in chicken. The dominant white allele is a result of a synonymous substitution at position 1836 in exon 6 and a 9-bp insertion in exon 10. The smoky allele was identical to the dominant white allele with the exception of a unique 12-bp deletion in exon 6. The dun allele was distinct from the dominant white allele, and possessed 13 unique single nucleotide polymorphisms (SNPs) and a 15-bp deletion in exon 10. These mutations either block or hinder the production of black pigment but allow the production of red pigment (Kerje *et al.* 2004).

An approximately 253 bp retrotransposon insertion in the 3' carboxyl terminus of Pmel17 is responsible for the merle coat patterning in domestic dogs. Merle patterning is characterized by patches of diluted pigment interspersed with normal melanin pigmentation. It is inherited in an autosomal, incompletely dominant fashion. Shetland sheepdogs that inherit the merle allele in a heterozygous condition exhibit a blue-grey merle phenotype, while those that inherit the merle allele in the homozygous condition exhibit a predominately white phenotype. Shetland sheepdogs homozygous for the normal allele exhibit a tricolor black, sable and white phenotype (Clark *et al.* 2006).

Mutation in Pmel17 causes dilutions of black coat coloration in mice and domestic dog, and also affect the production of black pigment in the plumage of chickens. Cattle that exhibit the rat-tail phenotype have a diluted black coat. This dilution may be caused by the inhibited production of the black pigment eumelanin. Inheritance of the rat-tail genotype does not seem to affect cattle that have a red coat. The merle allele in domestic dog is inherited in an incomplete dominant fashion exhibiting dilution effects of coat coloration, as observed in cattle with the rat-tail allele



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at the rat-tail locus. The similarities between the rat-tail phenotype and the phenotypes of mutant Pmel17 mice, chicken, and domestic dog provides justification for investigating Pmel17 for its potential role in the rat-tail phenotype.

Mutations within the coding region of Pmel17 may cause the dilution of the black coat color as well as the other phenotypic characteristics of the rat-tail syndrome. The focus of this study is to sequence and analyze Pmel17 from normal and rat-tail cattle to discover mutations, which may be causative of the disorder.

Materials and Methods

Research Population. The 99 cattle used in this study were composed of three different breeds: Simmental, Hereford, and Angus. This South Dakota State University (S.D.S.U.) population segregated for the rat-tail syndrome. A pedigree outline of the population is shown in Figure 5. A purebred Simmental bull (bull 211) was bred to 2/3 Hereford and 1/3 Simmental cows producing 2 bulls that were 2/3 Simmental and 1/3 Hereford (966128 & 966130). These two bulls were bred to black, 2/3 Angus, 1/3 Hereford cows, producing 1/3 Angus, 1/3 Hereford, and 1/3 Simmental F1 calves. There were no black calves and 19 rat-tail F1 calves sired by 966128. Bull 966130 sired both rat-tail and black F1 calves. Therefore, bull 966128 was assumed to be homozygous for the allele that causes the rat-tail syndrome (CC), and bull 966130 was heterozygous (Cc). One of the F1 offspring, bull 988042, also sired both rat-tail and black calves, and therefore was assumed to be heterozygous (Cc) for the allele that causes rat-tail syndrome. Black and rat-tail calves sired by two Simmental crossbred bulls, 966130 and 988042, were used in this study. Additionally black (E_cc), rat-tail (E_Cc), and light grey (E_CC) calves out



of rat-tail cows sired by either 966128 or 966130 were included. Red calves (eeC_ or eecc) were not included in this study because their genotype could not be determined at the rat-tail locus before sequencing (Singrey 2002).

DNA Extraction. Whole blood, collected from cattle of interest as described above, was transferred to 15 mL polypropylene conical tubes and centrifuged at 3000 rpm for 30 minutes at 4°C. Buffy coat was transferred to a clean 15 mL conical tube and DNA was extracted following a saturated salt procedure described by Miller *et al.* (1988). DNA was quantified by a spectrophotometer and working stock was diluted to 50 ng/µl in Low TE (10 mM Tris pH 8.0, 0.1 mM EDTA) and stored at 4°C.

Amplification and Sequence of Pmel17. Primers used to sequence Pmel17 were developed by "blasting" bovine SILV mRNA (NCBI accession number M81193) against the bovine genome sequence data available from the Baylor College of Medicine (http://www.hgsc.bcm.tmc.edu/projects/bovine/). The software program Primer3 (http://frodo.wi.mit.edu) was used to design primers around the exons of Pmel17. A total of 12 sets of primers were designed in order to amplify the 11 exons of Pmel17 in their entirety (Table 3). One set of primers was developed for exons 1-5 and 7-11, while exon 6 was so large that it required two sets of primers. Primers were developed in sequences flanking the exon of interest. Primers were amplified using a 30 μl polymerase chain reaction (PCR) recipe consisting of 28.0 μl of reaction mix (22.3 μl sterile ddH₂O, 3.3 μl *Taq* DNA Polymerase 10X Buffer w/ 15 mM MgCl₂, 0.6 μl dNTPs (5mM), 1.5 μl forward and reverse primer (20μM), and 0.3 μl *Taq* Polymerase 1:1) and 2.0 μl Genomic DNA (50 ng/μl). PCR was performed under the thermal cycling program: one cycle at



95°C for 5 minutes, and 35 cycles at 94°C for 1 minute, 30 seconds at annealing temperature (varied from 58°C to 70°C depending on the primer pair; Table 3), 72°C for 1 minute, followed by one cycle at 72°C for 5 minutes, and held at 4°C. Amplicons were visualized using electrophoresis run on 2% agarose gels stained with ethidium bromide. Amplicons were purified and eluded in preparation for sequencing using a Qiagen QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA, U.S.A.) following the manufacturers instructions. A 13.5 μ l preparation consisting of 12 μ l purified amplicon and 1.5 μ l of corresponding forward or reverse primer (20 μ M) was submitted to the Brigham Young University DNA Sequencing Center (http://dnasc.byu.edu/) for cycle sequencing.

Sequences were analyzed using Chromas Lite version 2.01 (http://www.technelysium.com.au/chromas_lite.html) to determine the genotypes of a selection of rat-tail individuals and normal non-rat-tail individuals. If rat-tail individuals were found to be heterozygous for a particular mutation while normal non-rat-tail individuals were homozygous at the same locus, sequencing was continued on the population, until all 99 animals in the population were successfully genotyped.

Results

In this study two mutations were identified in Pmel17, a 3-base pair (bp) deletion in the coding region of exon 1, and a SNP in the coding region of exon 11. The SNP in exon 11 causes a C \rightarrow A missense mutation in the second nucleotide of codon 612, substituting the residue glutamic acid for alanine. Although several of the rat-tail animals in the population were heterozygous (A/C) for the mutation in exon 11, there was not a



100% concordant association with the phenotype (Table 4). Twelve of 65 black animals sequenced were heterozygous (A/C) for the SNP and one rat-tail animal was homozygous (A/A) for the mutation. This eliminated the SNP in exon 11 as being the causative mutation for rat-tail.

The 3-bp deletion in exon 1 removes codon 18 (CTT), truncating the protein by one leucine amino acid residue. This deletion had a 100% concordant association with the rat-tail phenotype (Table 4). All 52 black animals sequenced were homozygous for the non-deleted allele and all 20 rat-tail animals sequenced were heterozygous for the deletion. Furthermore, two animals that were known to be homozygous for the rat-tail allele were homozygous for the deletion. The correlation between the 3-base pair deletion in exon 1 of Pmel17 and the rat-tail syndrome suggests this mutation may be responsible for the disorder. Genotypes and phenotypes at exons 1 and 11 of all animals sequenced can be found in Table 5.

Discussion and Conclusion

Pmel17 is the most probable positional candidate gene for causing rat-tail syndrome in cattle. Ninety-nine animals from a population segregating for the rat-tail syndrome were sequenced at Pmel17. Two mutations have been detected; a 3-bp deletion in codon 18 of exon 1, which removes a leucine residue, and a SNP missense mutation that changes the second nucleotide of codon 612 from a cytosine to an adenine base, substituting glutamic acid for alanine in exon 11. The 3-bp deletion in exon 1 of Pmel17 has displayed 100% concordance to the rat-tail phenotype in the S.D.S.U. population. All animals found to be heterozygous for the deleted allele (Cc) are of the rat-tail phenotype or known to be heterozygous carriers. And all animals that were found to be



homozygous for the non-deleted allele (cc) are black. Furthermore the two animals that are homozygous for the deletion (CC) were also known to be homozygous carriers of the rat-tail allele (Table 4). The SNP in exon 11 did not show complete concordance with the rat-tail phenotype and was eliminated as being the causative mutation.

Mutations in Pmel17 of mouse, chicken and domestic dog that cause phenotypic dilutions of black coat and plumage color are reported to occur in the c-terminal regions of Pmel17. In mouse a single bp insertion in exon 11 causes a change in the cytoplasmic domain of Pmel17 leading to a silver phenotype in an otherwise black mouse (Kwon *et al.* 1995). In chicken the dominant white phenotype is caused by a 9-bp insertion in exon 10 affecting the transmembrane region of Pmel17. Similarly a five amino acid deletion in the transmembrane region of Pmel17 causes the dun phenotype in chicken. The smoky phenotype is caused by the same 9-bp insertion in exon 10 as the dominant white allele, but also exhibits a 12-bp deletion in exon 6 (Kerje *et al.* 2004). In domestic dog an approximately 253-bp retrotransposon insertion in the exon 10 – exon 11 boundary is responsible for the merle phenotype (Clark *et al.* 2006).

The 3-bp deletion in exon 1 described in this study does not correspond to published mutations found in other species, although a personal communication between Theos *et al.* (2005) and A. Oulmouden (Limoges University, Limoges, France) suggests mutations found in the signal peptide sequence of Pmel17 is responsible for hypopigmentation in cattle. Exon 1 in humans codes for the signal peptide sequence, which is responsible for posttranslational transport of Pmel17 within the cell (Theos *et al.* 2005) (Fig.6A & 4B). The 3-bp deletion found in this study is also located in the signal peptide sequence of Pmel17.



Exon 1 is highly variable among different species, suggesting that this region is capable of experiencing mutation events and still code for a functional protein (Fig. 6). Current literature does not support the effects of an N-terminal mutation at Pmel17 playing a significant role in phenotypic variation, thus the mechanism for the effects of the mutation on the black pigment eumelanin and the hair shaft in cattle are unknown. The 3-bp deletion in exon 1 of Pmel17 in cattle may block or hinder the production of eumelanin and have no effect on the production of pheomelanin, similar to what is observed in mouse and chicken when mutations of Pmel17 occur. Although this study can not conclude with 100% certainty that the 3-bp deletion in Pmel17 is the causative mutation, the mutation has inclusive correlation with the samples from the S.D.S.U. population. It would be necessary to screen rat-tail individuals from other populations to determine if the 3-bp deletion segregates within these populations as well. If a correlation can be established outside the S.D.S.U. population the development of a simple diagnostic DNA test to determine if an individual is carrying a rat-tail allele (C) may be possible. The results of this study suggest strong linkage disequilibrium between the 3-bp deletion in exon 1 of Pmel17 and the rat-tail syndrome, however no conclusive data has been found that can suggest causation.



References:

Ayers J.R., Leipold H.W., Schalles R., & Cole D. (1989) Pathological studies of crossrelated congenital hypotrichosis in cattle. *Journal of Veterinary Medicine* **36**, 447-56

Bailin T., Lee S.T. & Spritz R.A. (1996) Genomic organization and sequence of D12S53E (pmel17), the human homologue of the mouse silver (si) locus. *Journal of Investigative Dermatology* **106**, 24-7

Clark L.A., Wahl J.M., Rees C. A. & Murphy K.E. (2006) Retrotransposon insertion in *silv* is responsible for merle patterning in the domestic dog. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 1376-81

Kerje S., Sharma P., Gunnarsson U., Kim H., Bagchi S., Fredriksson R., Schütz K., Jensen P., von Heijne G., Okimoto R. & Andersson L. (2004) The dominant white, dun and smoky variants in chicken are associated with insertion/deletion polymorphisms in the Pmel17 gene. *Genetics* **168**, 1507-18

Klungland H., Vage D.I., Gomez-Raya L., Adalsteinsson S. & Lien S. (1995) The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. *Mammalian Genome* **9**, 636-9

Kubota R., Wang Y., Minoshima S., Kudoh J., Mashima J., Oguchi Y. & Shimizu N. (1995) Mapping of the human gene for a melanocyte protein pmel17 (D12S53E) to chromosome 12q13-14. *Genomics* **26**, 430-1

Kwon B.S., Halaban R., Ponnazhagen S., Kim K., Chintamaneni C., Bennett D. & Pickard R. T. (1995) Mouse *silver* mutation is caused by a single base insertion in the putative cytoplasmic domain of Pmel17. *Nucleic Acids Research* **23**, 154-8

Liu Z., Hansen M., Womack J.E. & Antoniou E. (2003) A comparative map of interstitial bovine chromosome 5 with human chromosomes 12 and 22. *Cytogenetic and Genome Research* **101**, 147-54

Miller S. A., Dykes D.D. & Polesky H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**, 1215

Schalles R.R. & Cundiff L.V. (1999) Inheritance of the "rat-tail" syndrome and its effect on calf performance. *Journal of Animal Science* **77**, 1144-7

Silvers W.K. (1979) The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction. Springer-Verlag, New York

Singrey A.R. (2002) Linkage mapping of the rat-tail syndrome. *Master of Science Thesis, South Dakota State University*



Theos A.C., Truschel S.T., Raposo G. & Marks M.S. (2005) The *silver* locus product pmel17/gp100/silv/me20: controversial in name and in function. *Pigment Cell Research* **18**, 322-36



Web References:

Baylor College of Medicine, Human Genome Sequencing Center, Bovine Genome Project. <u>http://www.hgsc.bcm.tmc.edu/projects/bovine/</u>

BYU DNA Sequencing Center <u>http://dnasc.byu.edu/</u>

Chromas Lite Version 2 http://www.technelysium.com.au

Primer3

Rozen S. & Skaletsky H.J. (2000) *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press, Totowa, NJ, pp 365-386 <u>http://frodo.wi.mit.edu</u>

Qiagen. QIAquick PCR Purification Kit Qiagen Inc., Valencia, CA 91355, U.S.A., <u>http://www1.qiagen.com/Default.aspx?</u>

United States Department of Agriculture (USDA), Meat Animal Research Center (MARC), Clay Center Nebraska, <u>http://www.marc.usda.gov</u>



Appendix A: Tables

Genotype	Phenotype		
EECC	Light to modium grov cost color with fine bair		
EeCC	Light to mediam grey coat color with the han		
EECc	Chargeal cost color with rat tail phonotype		
EeCc	Charcoal coat color with rat-tail phenotype		
EEcc	Normal black coat color		
Eecc	Normal Black Coat Color		
eeCC			
eeCc	Normal red coat color		
eecc			

Table 1 Genotype and phenotype of epistatic interaction between MC1R (E) and unknown rat-tail locus (C).

Marker	Distance (cM)	Recombination	LOD score
DM(02/	/ 7		
BIVI6026	6.7	0.26	0.58
BP1	18.8	0.34	0.29
RM103	28.6	0.32	0.41
BMS1617	56.3	0.09	4.93
BR2936	65.17	0.04	4.82
BMS1248	88.4	0.28	0.5
BM315	100.1	0.26	0.96

Table 2 Linkage analysis of BTA5 with cM distance assignments from U.S.D.A. MARC(http://www.marc.usda.gov) and recombination and LOD scores from Singrey (2002). Significant linkagebetween the rat-tail syndrome and markers BMS1617 and BR2936 was found by Singrey (2002).



Primer Pair		Primer Sequence	Annealing Temperature °C	Product Size bp	
Exon 1	F	5' - GAG GGG AGG AAG GGC TAT G - 3'	67	293	
	R	5' - AAT CAA ATG GGT GGG AGA CA - 3'	01	200	
Exon 2	F	5' - GCA AGC CAC AAC TAC CTG ACC - 3'	70	359	
	R	5' - CCC ACC CAT TTC CAC CAT A - 3'	10		
Evon 3	F	5' - TAC AGG GAG GAG AAG CCA AG - 3'	70	231	
	R	5' - TCT GGA CCT TGG GAA GGA G - 3'	10	201	
Exon 4	F	5' - CCA GGG ATT GAG GGA GAC T - 3'	67	200	
	R	5' - GGG GGA AGT GAA GAT TAG GG - 3'	07	290	
Exon 5	F	5' - CCA GTA ACC CCC TTC CCT AC - 3'	68	226	
	R	5' - CTT GTC CCC TCC TCA GTC C - 3'	00	220	
Exon 6-I	F	5' - TGG GTT GGT TGA AAA ATA TGG - 3'	62	359	
	R	5' - ACA GGA GGT GAG AGG AAT GG - 3'	02	559	
Exon 6-II	F	5' - GCA CTC ACG GTC ACT CAC AC - 3'	68	540	
	R	5' - TGC CCC ACC TCT ATG AAC TC - 3'	00	540	
Exon 7	F	5' - AGC CTG CGG GTT CAA ATA C - 3'	68	651	
	R	5' - CAG CCT GTA GGA TCT CAG CA - 3'	00	051	
Evon 8	F	5' - AAG GCA GAA TGG GAT TAG GG - 3'	68	2/1	
	R	5' - GGA CAG TAG TCA CCC CAG GA - 3'	00	241	
Exon 9	F	5' - AGT GTC CCT TCC CAG ATT CC - 3'	58	310	
	R	5' - CTC CCC GTG TCT TCA TCC TA - 3'	50	512	
Evon 10	F	5' - GAA GAC ACG GGG AGA TGG TA - 3'	58	235	
	R	5' - CCA TGA GGA AGA GTG GTG GT - 3'	50	200	
Evon 11	F	5' - GAG CCA GGA TCA AGA CCA AG - 3'	66	125	
	R	5' - CCT CCA CCC CTT AAG TGA CA - 3'	00	420	

Table 3 Primer design table with primer sequence, annealing temperature and approximate product sizes.Forward primer represented by: F, reverse primer represented by: R.

		Rat-	
Exon 1	Black	Tail	Red
TTCTG/ TTCTG	0	0	2
TTCTG/ TTCTTCTG	0	20	2
TTCTTCTG/ TTCTTCTG	52	0	0
		Rat-	
Exon 11	Black	Tail	Red
A/A	0	1	2
A/C	12	29	2
C/C	53	0	0

Table 4 Intraexonic mutations and relative frequency of occurrence within population. Exon 1 normal allele represented by: TTCTTCTG, deleted allele represented by TTCTG. Exon 11 SNP represented by nucleotide base at locus (A or C).



	Genotype	Allele	Genotype	Allele	
Sample	exon 1	Exon 1	exon 11	Exon 11	Phenotype
89-002	homo	ttcttct	homo	CC	black
89-050	homo	ttcttct	homo	CC	black
91-161	homo	ttcttct	homo	CC	black
91-175	homo	ttcttct	homo	CC	black
92-089	homo	ttcttct	homo	CC	black
93-044			homo	CC	black
93-045	homo	ttcttct	homo	CC	black
94-022	homo	ttcttct	homo	CC	black
95-045	homo	ttcttct	homo	CC	black
95-060			homo	CC	black
96-066	homo	ttcttct	homo	CC	black
96-067	homo	ttcttct	homo	CC	black
96-095	homo	ttcttct	homo	CC	black
96-128	homo	tct delete	homo	AA	homozygous carrier
96-130	het	tct delete	het	AC	heterozygous carrier
96-165	homo	ttcttct	homo	CC	black
97-086	homo	ttcttct	homo	CC	black
97-092	homo	ttcttct	homo	CC	black
97-098	homo	ttcttct	homo	CC	black
97-114	homo	ttcttct	homo	CC	black
98-034	homo	ttcttct	homo	CC	black
98-042	het	tct delete	het	AC	heterozygous carrier
98-046	het	tct delete	het	AC	rat-tail
98-047	homo	ttcttct	homo	CC	black
98-049	homo	ttcttct	homo	CC	black
98-051			het	AC	black
98-052	homo	ttcttct	homo	CC	black
98-060	homo	ttcttct	het	AC	black
98-073	homo	ttcttct	homo	CC	black
98-074	homo	ttcttct	het	AC	black
98-096	homo	ttcttct	het	AC	black
98-111			homo	CC	black
98-122	homo	ttcttct	homo	CC	black
98-126			het	AC	rat-tail
98-128	homo	ttcttct	het	AC	black
98-131	homo	ttcttct	homo	CC	black
98-134	homo	ttcttct	homo	CC	black
98-148					
98-151	het	tct delete	het	AC	rat-tail
98-324	homo	ttcttct	homo	CC	black
99-040	homo	ttcttct	homo	CC	black
99-043	homo	ttcttct	homo	CC	black
99-073	het	tct delete	het	AC	rat-tail
99-095	homo	ttcttct	het	AC	black
99-098	het	tct delete	het	AC	rat-tail



99-110	homo	ttcttct	het	AC	black
99-127	homo	ttcttct	het	AC	black
99-139	homo	ttcttct	het	AC	black
99-147	homo	ttcttct	homo	CC	black
99-157			homo	CC	black
00-051	homo	ttcttct	homo	CC	black
00-056	het	tct delete	het	AC	rat-tail
00-057	homo	ttcttct	homo	CC	black
00-060	het	tct delete	het	AC	rat-tail
00-063	het	tct delete	het	AC	rat-tail
00-071			het	AC	unknown
00-077	het	tct delete	het	AC	rat-tail
00-079	homo	ttcttct	homo	CC	black
00-095	het	tct delete	het	AC	rat-tail
00-104	homo	ttcttct	homo	CC	black
00-108	het	tct delete	het	AC	rat-tail
00-115	het	tct delete	het	AC	rat-tail
00-120	het	tct delete	het	AC	rat-tail
00-123	homo	ttcttct	homo	CC	black
00-132			homo	CC	black
00-135*					
00-328	het	tct delete	het	AC	rat-tail
00-356	homo	ttcttct	homo	AC	black
01-008	homo	ttcttct	homo	CC	black
01-010			het	AC	black
01-029			homo	AC	black
01-038	homo	tct delete	homo	AA	homozygous carrier
01-048			homo	CC	black
01-058			het	AC	rat-tail
01-074	homo	ttcttct	homo	CC	black
01-090			het	AC	rat-tail
01-098			het	AC	rat-tail
01-113			homo	CC	black
01-121			homo	CC	black
01-135*					
01-141	homo	ttcttct	homo	CC	black
01-145			het	AC	rat-tail
01-163			het	AC	rat-tail
01-169			het	AC	rat-tail
02-025	homo	ttcttct	het	AC	black
02-054	het	tct delete	het	AC	rat-tail
02-096	homo	ttcttct	homo	CC	black
02-101	het	tct delete	het	AC	rat-tail
02-103	het	tct delete	het	AC	rat-tail
02-108			homo	CC	black
02-124			het	AC	rat-tail
02-201			homo	CC	black
02-218			het	AC	rat-tail



03-301	homo	ttcttct			black
03-305	het	tct delete	homo	AA	gray
03-306			het	AC	black
03-307	homo	ttcttct	homo	CC	black
03-308	het	tct delete	homo	AA	rat-tail
03-310	het	tct delete	het	AC	rat-tail
03-317	homo	ttcttct	homo	CC	black
03-318	het	tct delete	het	AC	gray
03-330	homo	ttcttct	homo	CC	black
03-333			het	AC	rat-tail
03-338	homo	ttcttct	homo	CC	black
03-353	het	tct delete	het	AC	rat-tail
03-374	het	tct delete	het	AC	rat-tail

Table 5 Genotype and phenotype of exons 1 and 11 at Pmel17 for individuals in South Dakota State University population segregating for the rat-tail syndrome (data missing for individuals at exon 1 is due to the loss of genetic material during the screening process). Homozygote represented by: homo, heterozygote represented by: het. Exon 1 normal allele represented by: ttcttct, deleted allele represented by: tct delete. Exon 11 allele represented by nucleotide bases present at SNP (AA, AC, or CC). * Samples were confused in the storage process and could not be distinguished for screening.



Appendix B: Figures



Figure 1 Phenotypes of calves: A) rat-tail (E_Cc) described as charcoal grey and lacking tail switch, B) light grey (E_CC) with normal patches of white hair, and C) normal black (E_cc).



Figure 2 Linkage map of BTA5 including rat-tail locus and linked microsatellite markers. There was strong linkage disequilibrium between the rat-tail locus and markers BMS1617 and BR2936, with LOD scores of 4.93 and 4.82, respectively (Singrey 2002).





Figure 3 Comparative map of syntenic human, mouse and cattle linkage groups. SILV (Si) locus is found in region III (Liu *et al.* 2003).





Figure 4 Human pre-melanosomal protein (Pmel17) gene and splice isoforms. A) Organization of human SILV (Pmel17) gene according to Bailin *et al.* (1996). Exons are represented as boxes and introns by lines. The numbers below each box represent the codon at the start of each exon. 5' and 3' untranslated regions (5'UT, 3'UT) are indicated in gray. Hatched regions indicate alternatively spliced exons. B) Schematic diagram of the products of the four known human Pmel17 splice isoforms. The intermediate (int.) form is the most abundant, and the short forms (short-1 and short-i) are the least abundant. ss, signal sequence; tm, transmembrane domain; cyt, cytoplasmic domain. Numbers indicate the the amino acids at the borders of the topologic domains (ss, luminal, tm and cyt.) or of the alternatively spliced regions with respect to Pmel17-long (Theos *et al.* 2005).





Figure 5 Pedigree outline of rat-tail breeding population from South Dakota State University (Singrey 2002).



A					•	•			
peptide V	¥~			Ŷ	C		Y	~	
	NTR	F	KD	RP	r 🗌	ĸ	LD	MCy	t.
1 25		215	297	315	435	516	587	623	668
в									
Homo sapiens	MDLVLKRCLLHLAV	20 IGALLAVGATKVPR	NODWLGVSROLRTK	40	60 RLDCWRGG	OVSLKVSNDGPTLIGA	80 ASFSIALNFPGS	1 QKVLPDGQVIV	.00 WVNN : 10
Rattus norvegicus Eguus caballus	:MGVQRRCFLPVLV	LGALLALGSIEGSR	NONWHEVSROLVTS	WWNKQLYFEWTE-VQ	GSNCWRGG	QVSLKVRNDGPTLVGA	TSFSIALEFPGS	OKVLPDGQVIN	WVNN : 10
Bos taurus Canis familiaris	MDLVLRKYLLHVAL	MGVLLAVGTTEGPR	DRDWLGVSROLRIK DQDWLGVPROLTTK	ANNROLYPENTE-SO	GPDCNRGG	HISLKVSNDGPTLIGA QVSLKVSNDGPTLVGA	ASPSIALEFPKS ASFSIALEFPRS	QKVLPDGQVIN	WANN : 10 WANN : 5
Coturnix coturnix Gallus gallus	: MRLHGAIVLLAALLAL : MRLHGAIVLLAALLAL	TTAQQRGGGRNRGA VTAQQRGGGRSRGG	VQGPLWGGRPTPFP VKGSAWGGRPAPFP	SWDATRYRPWKEGTAG	QSDCWRGG	DVTFDISNDAPTMAGA DVTFDISNDAPTLVGA	CATFSMLLGFPST RATFSIALRFPGT	QRALPDGRVV9 QTVLPDGRVV9	MNON : 11 MSON : 11
Xenopus laevis Xenopus tropicalis	: -MKGVFCLVVLWVFCV : -MKGVYGLVVFWVLCA	GGETQNWSQNRVQQ GAETQNWSQNRVQQ	TNQQVAGNRQSPFK MNQQVAGNRQPPFK	SWNSRMYPIWRGSEAG SWNSRMYPIWRGTEAG	KKNCWRGG	QVTFDLVNDAPTLTGM QVTFNLVNDAPTLTGM	CATFSIRLNFPNN CATFSIQLNFPKN	QTVLPDGQVV QTVLPDGQVV	NGON : 11 NGON : 11
Danio rerio Tetraodon nigroviridis	:MWTSLIFLILSL	ASGALSOSR	TRFARYP	SWNSQHYPVWRDGDPR SWNTRMYPVWRDGDPR	YRDCWKGG FGNCWTGG	EVTIEVRSDSPTLTGM EVTFDLKNDGPTLTGM	CASENIDVREPON AATENINLNEPPN	QTVLPDGQVV9 QTALSDGQVV9	narn Naon
Homo sapiens	120 : TIINGSOVWGGOPVYP	ORT-DDACIFPD	140 GGPCPSGSWSOKRS	160 FVYVWKTWGOYWOVLG	180 GPVSGLSIGTGRAMLGT	HTMEVTVYHRRGSRSY	200 /PLANSSSAFTIT	220 DOVPFSVSVS0	OLRA : 22
Mus musculus Rattus norvegicus	: TIINGSQVWGGQPVYP : TIINGSQVWGGQPVYP	QEP-DDACVFPD REP-DDACIFPD	GGPCPSGPKPPKRS GGPCPSGPKPPRRS	FVYVWKTWGKYWQVLA FVYVWKTWGQYWQVLA	GPVSRSSIATRHAKLGI GPESKLSIPTGHARLGI	HTMEVTVYHRRGSQSY HTMEVTVYHRRGSQSY	PLAHASSIFIIT PLAHSSSIFIIT	DQVPFSVSVSQ	QLQA : 22 QLQA : 27
Equus caballus Bos taurus	: TIINGSQVWGGQLVYP	QEP-DDTCIFPD	GEPCPSGPLSQKRC	FVYVWKTWDQYWQVLG	GPVSGLSIGTOKAMLGT	RGSQTY YNMEVTVYHRRGSQSY	PLANSRSAFTIT /PLANSSSAFTIT	DQVPFSVSVSQ DQVPFSVSVSQ	QLQA : QLQA : 2
Canis familiaris Coturnix coturnix	: TIIDGSQVWGGQPVYP : CTVNGTRMVQGDPVFP	QVL DDACIFPD EQL-VEGSDGVFPD	GRACPSGPWSQTRS GQPFPRSSWGKRGB	LAXAMMINCHXMÖAAI LAXAMMINCHXMÖAAI	VSGLSIVTGKAVLGT GAASKLTVGTDGVALGS	HTMEVTVYHRRESQSY YTMEVVVYHYRGRQKF	PLARSCSAFTIT	DQVPFSVSVSQ DQVPIAVDVTQ	QLEV : 20
Xenopus laevis	: CTVNGTMLQGDPVYP : RTDNGTWIPSEEPIYP	DES-TEGSECTFPD	GRPFPRGVEKKHSK	CALAND AND AND AND AND AND AND AND AND AND	GPSSNLTVETDGIPLGS	YTMEVVVYHYRGRORF YTMOVVVYHYRGRORF	PIGSISSOFTIT	DQIPVSVSIS	QLLD : 23
Danio rerio Tetraodon nigroviridis	CTVNGTSYTMGQSVYP CTVNGTSYTMGQSVYP	ESSIPODWSGVFPD	GTPLNR-DOKKRSB	YVEVNKINGRINQVAD YVEVNKINGRINQVAD	GPSSLLSIDTNSVPLGS GPSSLLSIGTDNMPLGS	YSMEVVIYHCRGKDKF YSMEVVIYHCRGKDKF	LPLGYVSTQFSIT LPLGYASTVFTIT	DQIPFAVTLS	QVGD : 21 QVND : 1
	240		260	280	300				
Jomo sapiens Mus musculus	: LDGCNKHFLENQPLTF : LDGETKHFLENHPLIF	ALQLHDPSCYLAEA ALQLHDPSCYLAEA	DLSYTWDFGDSSGT DLSYTWDFGDGTGT	LISRALWVTHTYLEDG	PVTAQVVLQAAIPLTSC SVTAQVVLQAAIPLVSC	GSSPVPGTTDG			: 3:
lattus norvegicus Iquus caballus	: LDGENKRFLRNHPLIF : LDGRNKHFLKNQPLTF	ALQLHDPSGYLAEA ALRLHDPSGYLAGA	DLSYTWDFGDSTGT DLSYTWDFGDSTGT	LISRAPDVTHTYLEPG	SVTAHVVLQAAIPLVSC PVTAQVVLQAAIPLTSC	GSSPVPGTTDG-			····· : 31
Sos taurus Canis familiaris	: LDGRNKRFLRKOPLIF : LDGGNKHFLRNHPLIF	ALQLHDPSGYLAGA ALRLHDPSGYLSGA	DLSYTWDFGDSTGT DLSYTWDFGDHTGT	LISRALTVTHTYLESG LISRALVVTHTYLESG	PVTAQVVLQAAIPLTSC PITAQVVLQAAIPLTSC	GSSPVPGTTDR- GSSPVPVTTDG-			: 3
Coturnix coturnix Sallus gallus	: ATGDGGRFVLNHPVAF : AAGDGGSFVRNRPVAF	NVRLHDPSHYLRDA NVRLHDPSHYLRDA	DISYSWDFGDOSGT DISYSWDFGDOSGT	LISRSPTVTHTYLQAG	SFAARLVLQAAIPLGSC SFAARLVLQAAIPLSSC	G-TSAAPVVDP			: 3
Tenopus laevis Kenopus tropicalis	: LDQEDQRFIQNRAVSF : LDQEDQRFIQNRAVSF	AVAIHDPSHYLQAA AVSIHDPSHYLQAA	DISFSWDFGDOSGT DISFSWDFGDOSGT	LITENTDVTHTYVSPG	VFRPKVVLQAAIPIAPC VFRPKVVLQAAIPITPC	GSTAPVATAEP-			: 3
)anio reric "etraodon nigroviridis :	: INQGDONFIONRAVAF : VNQNDONFIONQALAF	SINLHDPSSYLSSS TVKLHDPSKYLSGA	DITFNMDFGDNSGT DISFSMDFGDSTGT	LISRETTVTHTYLTPG LISRALTVTHTYLTVG	SFRPQVILMAAISTGCF TFRPQVVLMAGIPSGCG	OPTAAIPVD	DEGTALDTIVVAV	SPQPVDVIPNA	AEEG : 33
iomo sapiens	320	N				3-	10	360 OVPTTEVIST	APVO : 3
dus musculus Rattus norvegicus	· ····································	G			T	TSRQGTTTKVVGTTPG TPRQGATTK	MPTTOPS	GT1 GT1	TVVQ : 3
quus caballus los taurus	YVPTAEAP	G			T	TAGQVPTADVVNTTPG TAGQVPTTEVMGTTPG	VPTAEPSRTTAV VPTAEAP	QVPTTEVIST GT	TPVQ : 1 TVGW : 3
anis familiaris Coturnix coturnix	: HAPTARIP : -TTGSVPSLGPTATEP	G		TPTAPGTTAAF	AASGAF	TAGRVPTAEVIKPS AEPTGVSVVVPSDSAA	TEPIPDPVLSTG	AAANTDPT	ADPQ : 4
allus gallus Genopus laevis	: -TTGPVPSLGPTATOP : -VPTTVAPAOPTTAAA	8		ILTGSGTAAAPGTTAAP PPGTTAALPG	RASGAP NITEPQ	AEPTGVSVAVLSDSAA GTINGIVVTIPSDE-Q	TEPLPDPVLSTAV	ANAAAGTDP 73	ADPL : 41 AATL : 3
Tenopus tropicalis Danio rerio Fetraodon nigroviridis	: -VPSTVIPPOPTTAVA : VAADATVVANDLAVAE : SAVPAVVVIDPTAAAA	S	TSGGQVIDA- AVAEADNTAVVEG- AAGGEAAAVEV-	PTGTPAVLPG TVATEAELAA PASDATPLEA	NVTEPQ EAENTATDALATPAVIE DANTVAE	GTINVIVVTLPSEETQ AEDEAENTATDALATP ATDAVDPNRRPGRGQW	NLAAEATSSVS- AVIEAEAAAEAEN BEIPAEEKGARQ-	-ASALPDNEJ TATDALATPAN -GEAVADVEJ	NATL : 39 VIEA : 44 AAAV : 3
	380			400	420	440			
Homo sapiens Mus musculus	: MPTAESTGMTPEKVPV : MPTTEVTATTSEQMLT	SEVMGTTLA	EMSTP EVSTT	LATGMTPAEVSIVVLS	GTTAAQVIIITEWVETTA GTTVAQAIITE	RELPIPEPEGPDASSI GPDASPL	STESITGSLGP-		: 4
attus norvegicus Squus caballus	: -PTTEVTATTSEQMLT: : VPTAEDIGTTPEQVST	PESLGTTLA	EMSTP EMPLQ	ESTTPARPS RLKGIPP-EVSNSRSL	GTTVAQATITELVRTTA LGESAQVTVQSWWKP-Q	EELPTPKPEGSDASPL WRGTQPLSLRVQMPAH	PTPSSTGSVSR- SCYRRNYRSQSP-		: 4
Sos taurus Canis familiaris	: VPITEDVGTTPEQVAT	SKVLSTTPV KESVEPTAG	EMPTA EGPTP	KATGRTP-EVSTTEPS	GTTVTQGTTPELVETTA	GEVSTPEPAGSNTSSF GPDTNLF	PTEGITGSQSA-		: 4
Coturnix coturnix Sallus gallus	: SPTSVSSOGDAPGTVD: : PPTSVSSOGDAPGTVA	PTAVEGSVAAGVG- PTAVEGSVAAGVGT	ARTPGATAA AEDVAAATPGATAA	DVEVDAAGPTAGATAG DVAVDTAGATDGDAVG	TMADSTAGIMADATAGA PTAAATAESIADPTAGA	TAQSMAGATAGATAGA TDGDAVGPTAAATAES	ADPTAGATAG ADPTAGATDGDA	VGPTAAATAES	SIAD : 5
Genopus laevis Genopus tropicalis	: PEAIEDEAGVTVAGEE : PEGTEEEAGVTDAAEE	TVPEREAVPNQEQ TVPERVEAVPNQEQ	AVTVA AAEAVPSQGAVTVA	EAVP-NQEQAVTVPNQ EAVP-SQE-AEAVPSQ	EQAVTVAEAVPSQE E-AEAVAEAVPSQEAVS	EAVPSQEAVEAVTSEA	ETTEAIAELAEG- ENTEAGAELAEE-		: 4
Janio rerio Fetraodon nigroviridis :	THE R R R R R R R R R R R R R R R R R R R	PAVIZAEAAAEAEN	TATDALATPAVIEA	PAA-EAENTATDALA	TOAVIUSUDAAVASVT	T ALATPAVTEAEPAV	CAPAAEIDLAATV		: 5:
	: EAAAFAENTATDALAT : EAAADVEAAAADVEAA	ADVEPSAAADEAA-	ħ	DVAD-AAAATVDAAAD	IAAVTEAPADNIVAPAA	TETAATEEVAAADVEA	EVQAAENNVAAT-	L	: 4
lomo sapiens	: EAAAEAENTATDALAT : EAAADVEAAAADVEAA	ADVEPSAADEAA-		DVAD-AAAATVDAAAD	IAAVTEAPADNIVAPAA	460	VQAAENNVAAT-	480 DCVLYRYGSFS	SVTL : 4
Homo sapiens Mus musculus Rattus norvegicus	E EAAEAEAENTATDALAT	ADVEPSAADEAA	A	DVAD-AAAATVDAAAD	IAAVTEAPADNIVAPAA	460 LLDGTATLR LLDDTDTIM	UKROVPL UKROVPL UKROVPL	480 DCVLYRYGSFS DCVLYRYGSFS DCVLYRYGSFS	SVTL : 4 SLAL : 4 SLTL : 4
iomo sapiens fus musculus tattus norvegicus iquus caballus ios tautus	E EAAAEAENTATOALAT		A		IANTEAPADNIVAPAN	460 460 	JVKRQVPL JVKRQVPL JVKRQVPL JVKRQVPL JVKRQVPL JKRQAPL	480 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5	SVTL : 44 SLAL : 45 SLTL : 45 SLTL : 45 SLTL : 25 SLTL : 47
iomo sapiens fus musculus kattus norvegicus fguus caballus los taurus lanis familiaris oturnix coturnix	E EAAAUAENTATOALAT E EAAADVEAAAADVEAA	AIADPTAGAI			IAAVTEAPADNIVAPAN	450 450 LLDGTATLR LLDGTATLR LLDGTATLF LLDGTATLF 	VQAAENNVAAT- LVKRQVPL LVKRQVPL LVKRQVPL LKRQVPL LKRQVPL LAKRETPL	480 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5	SVTL : 44 SLAL : 43 SLTL : 45 SLTL : 25 SLTL : 45 SLTL : 35 STEL : 54
Nomo sapiens fus musculus lquus caballus Sos taurus Tanis familiaris Joturnix coturnix Tallus gallus isnopus laevis	EAAADARATATOALAT EAAADVEAAADVEAA PIVGATDGDAVGPTAA PIVGATDGDAVGPTAA		AA		IAAVTEAPADNIVAPAA	460 	VQAAENNVAAT-	480 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 -CVLYRYGSF5 -CVLYRYGTF3	SVTL : 48 SLAL : 45 SLTL : 45 SLTL : 45 SLTL : 45 SLTL : 47 SLTL : 54 STEL : 54 STEL : 54 STEL : 54
iono sapiens fus musculus tatus norvegicus jeuus caballus ios taurus lanis familiaris Joturnix coturnix lallus gallus lanopus laevis ionopus tropialis unio rerio Verradon nigroviridis	EAAADARATATOALAT EAAADVEAAADVEAA PTUGATDGDAVGPTAA TUGATDGDAVGPTAA	AUVEPSAALDERA-	ADP	DVAD-AAAATVDAAAD	IAAVTEAPADNIVAPAA	460 	VXRQVP	480 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 DCVLYRYGSF5 CVLYRYGSF5 -CVLYRYGSF5 -CLLYRYGTF3 SCHVYRYGSF5 -CVLYRYGT53	SVTL : 4 SLAL : 4 SLTL : 4 SLTL : 2 SLTL : 4 SLTL : 3 STEL : 5 STEL : 5 STEL : 5 STEL : 5 STEL : 5 STEL : 5 ATDL : 5 STEL : 5 STE
iomo sapiens Mus musculus Natus norvegicus Sos taurus Janis familiaris Oturnix coturnix Salus gallus Ganopus Laevis Ganopus Laevis Sanio rerio Petraodon nigroviridis	ERAACHENTATOLAA ERAACHENAACHENA PIVGATDGDAVGPTAA 	AIADPTAGAI ATAS STADTAGAI PSVPSQTRLADT PSVPSQTRLADT PSVPSQTRLADT -AAATGEDEVQAEV	ADP		IAAVTEA PADNIVAPAA	460 460 LLDOTATIR LLDOTATIR LLDOTATIR LLDOTATIV LLDOTATIV LLDOTATIV LLDOTATIV LLDOTATIV LLDOTATIV PARTARAESVV VPARTARAESVV	VVRQVPL VKRQVPL VKRQVPL VKRQVPL VKRQVPL VKRQVPL VKRQVPL VKRQVPL VKRQVP	480 DCVLYRYGSF DCVLYRYGSF DCVLYRYGSF DCVLYRYGSF DCVLYRYGSF CLYRYGSF CLYRYGTF CLYRYGTF CLYRYGTF SCMVYRYGSF -CTVYRYGTJ	SVTL : 4 SLAL : 4 SLAL : 4 SLTL : 4 SLTL : 2 SLTL : 3 STEL : 5 STEL : 5 STEL : 6 ATDL : 5 STEL : 6 ATDL : 5
tomo sapiens tas musculus tatus norvegicus tatus norvegicus too taurus too taurus too taurus taius gallus tanopus laevis tanopus laevis tanopus laevis tanopus toopicalis tano regio terraodon nigroviridis tomo sapiens tomo sapiens	ERAACHENTATORIAE ERAACHENARDURAA PICARDURAACHENARDURAA PICARDURAE PICARDURAE PICARDURAE PICARDURAE SOO DIVOGIESAELLANP	-ALAD PLACE ALAD PLACE ATAZ SIAD PLACE PAVESQINEL CON PAVESQINEL CON PAVESQINEL CON PAVESQUE VALOR - ALAT CEDEVQAEV SG	ADP	VYAD-AAAATVUAAAD YAEDTVIEAJAEADDAA MIIISSICOPEADAA	IAAVTEN PADNIVA PAN NALTSVYSI PRAAVTEL SQUYLBES AQQLVIQUI	460 	VXRQVP VXRQVP VXRQVP	480 CVLYRYGSF DCVLYRYGSF DCVLYRYGSF DCVLYRYGSF DCVLYRYGSF CVLYRYGSF CULYRYGF CLLYRYGF SCWYRYGSF CLLYRYGF SCWYRYGSF CLLYRYGF CULYF	SVTL : 4 SLAL : 4 SLAL : 4 SLTL : 4 SLTL : 4 SLTL : 4 SLTL : 5 STEL : 5 STEL : 5 ATDL : 5 STEL : 5 ATDL : 5 STEL : 5 ATDL : 5 STEL : 6 ATSV : 5 600 ACLG : 6 GOLG : 6
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Figure 6 Domains among premelanosomal protein (Pmel17) and their identity among vertebrates. A) Individual domains within the human Pmel17 long form are shown in different colors, with amino acid positions for start and end of each domain indicated below. TM, transmembrane domain; Cyt, cytoplasmic domain; CS, cleavage site. B) Amino acid sequence comparison of Pmel17 orthologs in indicated species. The long form of human Pmel17 is shown. Red text indicates amino acid identities among all sequenced orthologs; orange indicates amino acid homology among most orthologs. Individual domains are represented as bars over the sequence colored as in (A) (Theos *et al.* 2005 (see also for higher resolution figure)).

