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CSIRO MLA Project BFGEN.007 Identification of the genetic factors for marbling

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FINAL REPORT

CSIRO MLA Project BFGEN.007 Identification of the genetic factors for marbling

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Primary Objectives:

Identify genes for marbling on bovine chromosomes 3, 5 and 14 in the regions identified in project SBEF.018 and develop commercial tests for these genes.

Examine how these genes may affect marbling in beef cattle through a study of their gene expression.

Milestones

1. Completion of a radiation hybrid map for chromosome 3. Complete ahead of schedule.
2. Identification of a new gene for marbling and preliminary identification of a second new gene for marbling. Complete.
3. Completion of the experimental panels to determine the temporal and spatial expression of candidate genes for marbling. Complete.
4. Completion of a radiation hybrid map for chromosome 14. Complete ahead of schedule.
5. Evaluation of gene expression of a marbling candidate gene RORC. Complete
6. Evaluation of temporal and spatial expression of candidate genes. Complete.
7. Completion of a third new direct test for marbling. Complete.
8. Completion of *in-situ* localisation of expression of candidate genes. Unlikely to be completed due to technical difficulty.
9. Completion of genotyping of candidate genes for marbling for a genomic region not covered by CS.254/SBEF.018. Complete ahead of schedule.
10. Completion of DNA sequencing of single nucleotide polymorphisms. Exceeded ahead of schedule.

Summary of Achievements

1. Radiation Hybrid maps of bovine chromosomes 3 and 14 were constructed near the marbling QTL in addition to the map on bovine chromosome 5. This completes the Radiation Mapping.
2. 91 SNP were identified in 46 genes near marbling QTL. This exceeds the number of SNP and genes that were contracted from the project.
3. In addition, the full cDNA sequences of RORC and its alternative splice variant as well as THRSP were obtained. 4.2 kb of continuous RORC genomic sequence were examined for SNP and all useful SNP were extracted, and the full cDNA sequence for THRSP was checked for SNP.
3. RORC, a positional candidate gene for marbling on bovine chromosome 3 was discovered to have consistent effects on marbling. Nine SNP for RORC have been identified and were genotyped in conjunction with the SNP in the adjacent gene LRRN6D. Of these SNP, the RORCEG3950T SNP showed the strongest association with marbling.
4. RARG, a positional candidate gene on bovine chromosome 5, was discovered to have consistent effects on marbling. In addition, a SNP in the adjacent gene ITGB7 showed some association also to marbling.
5. TG5, a confirmed predictor of marbling score, was found to be closer to the marbling QTL than originally thought. TGH, a SNP 19.5 kb from TG5 showed no association to marbling, therefore the marbling QTL must be in the close vicinity of TG5, or may even be TG5.

6. RNA was extracted from 16-17 tissues at 3 life stages of development which had been obtained in conjunction with the CRC groups in functional genomics, adipogenesis and cell and tissue biology of marbling (S. Lehnert and G. Harper).
7. The alternative transcripts of the RORC gene was examined in a range of tissues and quantified using to two house keeping genes. Some differences of gene expression were found in LD versus SC fat, although this needs to be confirmed.
8. 13 genes on bovine chromosome 5, 3 genes on bovine chromosome 3 including the RORC alternative transcripts and SCD were compared on a range of foetal and adult tissues. Greater gene expression occurred for most of these genes in foetal rather than adult tissues. Several of the genes showed no expression in LD muscle and so are unlikely to play a muscle specific role in marbling.
9. DNA variation at the THRSP gene, a gene showing differential expression in Japanese Black versus Holstein cattle, showed preliminary evidence of an association to marbling scores. This SNP was in the 3'UTR of the THRSP gene. A SNP in the coding sequence did not show associations to marbling. The THRSP gene was identified by Y.-H. Wang et al (2005) for further analysis based on the gene expression pattern. 5 other differentially expressed genes were selected for testing but no other genetic associations to marbling were seen.
10. The igenity-L SNP of the Leptin gene on chromosome 4, identified by the Schmutz group in Canada, was genotyped on Australian cattle and found not to be associated with marbling. This work has been published (Barendse *et al*., 2005, Anim Genet 36,86). Subsequent re-analysis of the P8 rump fat and marbling phenotypes indicated an effect of the Leptin SNP on P8 fat thickness, but still not on marbling.
11. A SNP in the Stearoyl-CoA Desaturase gene, identified by the Tsuji group in Japan, was genotyped in Australian cattle and found not to be associated with marbling. This work has not yet been published.
12. The Leu127Val SNP in the Growth Hormone gene on bovine chromosome 19, widely studied by many groups and offered by Chikuni and Mitsuhashi as a marbling test performed in Japan, was found to be associated with marbling. This confirms a gene not part of the CS254 and SBEF018 intellectual property. The results have been accepted for publication (Barendse *et al*., 2006 Animal Genetics in the press)
13. A SNP in the porcine marbling gene, HMGA2, a homologue of which is on bovine chromosome 5 and so is a candidate for a cattle marbling QTL on that chromosome, was not found to be associated with marbling in cattle.
14. SNP in the genes GDF11, RORC/LRRND6 and LEP are associated with P8 fat thickness in the combined sample of breeds as well as in a particular breed. In addition, GH1 showed an association with marginal significance to P8 fat. In all cases where these genes affected marbling, i.e., GH1 and RORC, the allele causing an increase in marbling caused a decrease in P8 rump fat.

Commercialisation

The RORC gene was evaluated extensively for commercialisation by us and also by Genetic Solutions P/L. These evaluations are the subject of reports prepared under the Collaborative Research and Commercialisation Agreement among the Beef CRC parties, MLA and Genetic Solutions. As MLA is aware, although Genetic Solutions was granted a licence for this technology, the parties are presently negotiating to terminate this licence because the incremental increase in revenue will be largely offset by the high patenting costs so it is likely that none of the parties will benefit from its continuation. This technology was published as part of the patenting process, and further publication in the scientific literature will occur.

The GH1 Val127Leu SNP is now a validated test for Australian cattle and can be used with some confidence. Publication of this result and dissemination of the consequences should occur.

The TG5 test is still being offered as the GeneSTAR ® marbling test, and our results indicate that it will not be replaced soon, as a better predictor for that marbling QTL is unlikely to occur.

None of the other markers associated with the project have been commercialised.

Detailed Milestone Report

Milestones 1 and 4

Construction of Radiation Hybrid Maps of Bovine Chromosomes 3 and 14.

Milestones 1 and 4 were achieved. The main aims in constructing the radiation hybrid maps was 1) to determine whether there was much rearrangement of gene order on the chromosomes that contained marbling QTL and 2) to locate some positional candidate genes which might otherwise be difficult to locate accurately to gene maps. The degree of conservation of synteny is related to the probability with which the human and cattle gene order differs on a chromosome, and when this project was proposed, only the human order was sufficiently well known to be of great use in describing gene order and location at high map densities. The radiation hybrid maps for chromosomes 3 and 14 were constructed by genotyping the Texas A&M University 3000 Rad radiation hybrid panel of Womack et al. (1997) using methods reported earlier (Barendse et al. 2000).

The chromosome 3 radiation map. The map for chromosome 3 does not show many large scale rearrangements between cattle and humans near where the marbling QTL occur, although this does not preclude some rearrangements in gene order below the level of resolution of the analysis. To construct the map of chromosome 3 (Table 2) the anonymous DNA markers RM19, RME23 and ILSTS96 as well as the gene FCGR1 were selected as the core of the map since these are close together and FCGR1, ILSTS96 and RM19 are associated with marbling in SBEF.018 and CS.254 respectively (see Final Reports for CS.254 and SBEF.018). Candidate genes that might affect adipocyte growth and differentiation, such as LEPR (Leptin Receptor), and genes, such as CD3Z antigen (zeta polypeptide, TiT3 complex), NAK (ATP1A1, ATPase, Na+/K+ transporting, alpha 1 polypeptide) and OSG (Oviduct specific glycoprotein) known to map to bovine chromosome 3 or its homologue, human chromosome 1, were added. TGLA127 and TGLA76 were added to provide an extended coverage of the chromosome. The DNA fragments were genotyped in duplicate on the clones of the Womack Texas A&M University radiation hybrid panel using standard methods (Barendse et al., 2000) and the map was constructed using the RHMAPPER software (Stein et al., 1995). LEPR does not map near the DNA markers associated with marbling. The genes FCGR1 and CD3Z, which are near to the marbling QTL, indicated a region of the human genome bounded by the co-ordinates (Hsa 1q [MB:165161690-175169214], UCSC, 2000). These co-ordinates are centred on FCGR1. A full listing of these genes can be found at http://genome.ucsc.edu/. One of these genes is an obvious candidate; PIK4CB (phosphatidylinositol 4-kinase, catalytic, beta polypeptide), is a lipid kinase found expressed in heart, pancreas and skeletal muscle (Nakanishi et al., 1995; Meyers and Cantley, 1997; Toker, 1998). PIK4CB (see below) and several other genes nearby were sequenced for single nucleotide polymorphisms and these were genotyped to test for associations to marbling.

The chromosome 5 radiation map. Bovine chromosome 5 has undergone significant evolutionary events including several inversions as well as a being split over 2 human chromosomes, Hsa 12 and 22. The radiation map for bovine chromosome 5 has been published by us (Barendse et al., 2000) and contains the following genes. In the region near ETH10, near the candidate gene RDH5 (retinol dehydrogenase 5), is a potential candidate DGKA (diacyl glycerol kinase alpha): DGKA phosphorylates diacylglycerol to phosphatidic acid (OMIM, 2002), thereby regulating the concentration of DAG, a regulator of RAS and VAV and an activator of PKC (protein kinase C). DGKA is in the same series of pathways as PIK4CB. Other positional candidates are RARG (retinoic acid receptor, gamma), identified earlier (Barendse, 1997) and genes near to it. Some of these genes, such as WNT10B (wingless-type MMTV integration site family member 10B; Ross et al., 2000), have been implicated in

adipocyte differentiation and are also being researched using Microarray gene expression experiments (Lehnert et al., 2004) in the CRC for Cattle and Beef Quality Gene Expression project.

The chromosome 14 radiation map. The radiation hybrid map for bovine chromosome 14 (Table 3) showed no evidence of significant gene rearrangment, unlike the map for bovine chromosome 5. DNA fragments for the genes DGAT1 (Diacylglycerol O-acyltransferase 1), CYC1 (Cytochrome C 1), TG (Thyroglobulin), HAS2 (Hyaluronan synthase 2), PENK (Proenkephalin), CRH (Corticotrophin releasing hormone) and IL7 (Interleukin 7) were genotyped as well as DNA microsatellites derived from the framework linkage map of Bta 14, consisting of IDVGA76, CSSM66, BMS1747, RM180, RM11, BM2934 and INRA92. These markers cover the length of the linkage map. We were not able to get satisfactory results out of RM180 and the first trial of TG5 resulted in the hamster DNA amplifying as well. TG was repeated. All scores were obtained in duplicate. When all the fragments are analysed, the order of these genes in cattle was not statistically different from the order in humans: 8cen - PENK - IL7 - CRH - HAS2 - TG - CYC1 - DGAT1 - 8qter. In comparison with other cattle maps, the location of CSSM66 in one set of non-significant analyses was closer to IDVGA76 than DGAT1. However, it is known that DGAT1 is more proximal to the centromere than CSSM66 (e.g. Grisart et al., 2002) and an order reflecting that has been reported in Table 3. The similarity of gene order indicated that inferences of gene order in cattle based on comparative information from human chromosome 8q were likely to be sound over a large genomic region since the region of conserved synteny was represented by genes from one end to the other of the bovine chromosome 14 linkage map. We have chosen genes adjacent to TG in humans to determine the extent of allele association between SNP around TG5 and marbling.

Milestone 10

Completion of DNA sequencing of single nucleotide polymorphisms

Milestone 10 was achieved. Genes for DNA sequencing were chosen mainly from bovine chromosomes 3, 5 and 14, containing the marbling QTL, as well as some candidates located in other parts of the genome, including some that have been claimed as affecting marbling in cattle or other livestock. DNA fragments were sequenced *de novo* for 46 genes for several purposes and 91 SNP were found. The purposes of sequencing were 1) to identify SNP (single/simple nucleotide polymorphisms) for genetic testing, 2) to characterise fragments for the Radiation Hybrid Map to improve the comparative map and 3) to characterise fragments for the gene expression experiments. Sequencing confirmed that the fragments represented the genes for which the primers were synthesized.

Almost all of the SNP were obtained by sequencing introns amplified using primers located in exons, in 10-12 Angus and Shorthorn cattle. Inclusion of exons made it easy to identify genes. Introns made it easy to identify SNP. Moreover, these amplicons could be used in gene expression studies since the cDNA would be a different size to the genomic DNA, due to the presence of introns. Furthermore, the same region of the gene in which the SNP occurs was then examined for gene expression. In the first few years of the project, PCR primers to amplify DNA fragments for these genes were obtained by using human and mouse genomic sequence, and the location of the primers in the cDNA was determined by using information on the intron/exon DNA structure of the human genome. In the last two years, with more bovine sequence available from the genome sequencing project, the majority of the current primers are derived from bovine sequence. The raw comparative information is contained in databases at NCBI (www.ncbi.nlm.nih.gov) and the Human Genome Browser (genome.ucsc.edu) and, initially, was integrated visually. More recently, the human genome browser has been adapted by the CSIRO Livestock Industries Bioinformatics group to include all new genome sequence and this tool has become the entry point of choice.

As might be expected SNP were not discovered in all DNA fragments. Of 67 amplified fragments (Table 1), 5 had equivocal SNP, which could be investigated further. They do not form part of the count of SNP discovered so far. Of the 62 other fragments, 17 of them had no SNP, 21 had a single SNP and 24 of the fragments had multiple SNP or 53% of those fragments with SNP have more than one SNP. Except for DGKA and PIK4CB that were initially screened for SNP using SSCP analysis (known genotypes of these SSCP were sequenced), the sequences were obtained without knowing whether SNP were present in the fragments. The preliminary step of SSCP proved to be time consuming and did not add to the efficiency of finding SNP. SNP discovery was made more efficient by increasing the size of the DNA fragment.

Milestones 2, 7 and 9.

Identification of new genes for marbling

Milestones 2, 7 and 9 were achieved. These gene associations to marbling are summarised here and expanded in the paragraphs below. Population associations were discovered between SNP and marbling for chromosomes 3 and 5, the population association on chromosome 14 was refined, and a population association was confirmed to a gene outside these chromosomes. The term allelic association (e.g., Edwards, 1980) is used in preference to linkage disequilibrium, since allelic association is in fact what is measured, linkage disequilibrium presumes a genetic process, which needs to be demonstrated. Allelic association is a neutral expression.

1) SNP in the RORC gene, located on chromosome 3, showed an association with marbling and P8 rump fat.
2) A SNP in the RARG gene, located on chromosome 5, showed an association with marbling but not to P8 rump fat.
3) A new SNP in the TG gene, located on chromosome 14, did not show an association with marbling, but the SNP TG5, located in the 5' leader sequence to TG, continued to show an association with marbling in new analyses, and that association has been confirmed in independent studies. This analysis refined the location of the QTL.
4) SNP in the GH1 gene, located on chromosome 19, showed association to marbling and P8 rump fat. This SNP test has therefore been validated for Australian cattle.
5) Other SNP, associated with the genes SCD, HAS2, and HMGA-2 and other candidates, were tested and found to show no associated to marbling.
6) SNP in the genes GDF11, RORC/LRRND6 and LEP are associated with P8 fat thickness in the combined sample of breeds as well as in a particular breed. In addition, GH1 showed an association with marginal significance to P8 fat. In all cases where these genes affected marbling, i.e., GH1 and RORC, the allele causing an increase in marbling caused a decrease in P8 rump fat.

The effective resolution of QTL mapping in this study is between 5-10 kbp as determined from our detailed research using the RORC and TG genes (see below and Table 7 showing Linkage Disequilibrium values between SNP), and these results showed the advantage of using data sets that contain a wide range of sires and breeds since it allows allelic association to be detected in a very narrow genetic region even in livestock (see Barendse and Fries, 1999; Barendse, 2005 for a discussion of the principles involved). All genotypes were analysed using standard methods as detailed in the Materials and Methods (see also Barendse, 1997, Barendse, 2001, Barendse et al., 2004, 2005).

Detail of associations between RORC and marbling on chromosome 3. The RORCEG3950T SNP showed the best association to marbling. Several of the SNP in this region showed association to marbling or P8 fat. The marbling QTL was first associated with the STRP FCGR1A and RM19, and 3 genes near these, PIK4CB, PRKAB2 and RORC, were tested one after the other. The RORCGHA693G SNP

showed an association to marbling. Several more SNP in RORC were identified and tested and then the area between RORC exons 3 and 7 was identified as a possible location of the QTL. The RORC genomic sequence was obtained between exon 3 and exon 7 as described in the Materials and Methods. A contig of 4219 bp consisting of 135 sequence reads was constructed. The error rate was 0.6902 per 10 kilobase pairs or an average phred quality score of 41.6 (10,000/0.6902 = 14488.55, log(14488.55) ~ 41.6). The confidence in the consensus sequence is therefore high and each SNP is well verified. A total of 7 SNP were identified in this fragment (Table 4), two of which correspond to RORCE-A232G and RORCGH-A693G. The RORCEA2486G was in the coding sequence but is silent, in a Glu amino acid. The RORCEA2303G SNP occured in a part of an intron that is conserved in human and cattle genomic sequences, which could be of interest. Three SNP occured near repeat elements, the RORCEC3075T SNP occured near a repeat motif found in the Xist gene and the other SNP, RORCEC3543T and RORCEG3950T, flanked a SINE element. Further bioinformatic analysis may recognise other motifs in this sequence.

The location of a causative mutation is most likely to be between RORCE3950 and RORC3-478. Using the Haploblock software (Table 8; Greenspan and Geiger, 2004), all intervals except those between these two loci can be ruled out. Examining the individual associations between SNP and phenotypes, the RORCE3950T allele increased marbling in all breeds and decreases P8 fat in the Angus. Not all the SNP were associated with marbling or P8 fat. More of the SNP had effects that are significant in the Shorthorn, but only RORCEG3950T had effects in both breeds and the combined sample. Although several SNP showed effects on marbling and P8 fat, the effects on P8 fat are only seen in the Angus cattle. Nevertheless, it is only the 3 SNP, RORCEC3075T, RORCEC3543T and RORCEG3950T, in introns 5 and 6 flanking the repeat motifs, which show effects on marbling not only in individual breeds but also in the combined sample of breeds. However, none of these SNP had associations to P8 fat in the combined sample. Only RORCGHA693G, the next SNP along in the gene showed an association with P8 fat in the combined sample as well as in the Angus cattle. Further away from this centre, the SNP in LRRND6 showed an association to P8 fat in the combined sample although not to the component breeds. These differences suggest that there may be more than one causative mutation affecting fatness traits in cattle in this region, because each SNP had slightly different effects in each breed and different SNP are important for each trait.

The linkage disequilibrium between the RORC SNP was calculated using both the Lewontin D' as well as the genetic correlation r^2 (Devlin and Risch, 1995). Although the D' values were uniformly high, the r2 values were mostly low. Only 1 of 45 pairwise r^2 comparisons was greater than 0.9, and 33 were less than 0.1. The r^2 values indicate that linkage disequilibrium is at background levels for most of the gene, and is consistent with the high precision of QTL mapping. This was unexpected since the largest distance between SNP is only 25 kbp. The high D' values, and D' has a value of 1 whenever one of the haplotypes is missing, in combination with the low r^2 values, suggests that the haplotype frequencies are high skewed. This is consistent with a genetic bottleneck due to domestication but it might also be preliminary evidence of a selective sweep since it applies across a group of haplotypes.

Details of the associations to marbling on chromosome 5. The RARGBFA212G SNP was associated with marbling score in this experiment. The STRP CSSM34 was associated with marbling (Barendse, 1997) and a QTL in this part of bovine chromosome 5 has been confirmed (Casas et al. 2003). Analyses of YAC clones containing CSSM34 (Barendse, 1997) indicated that these also contained the RARG gene. However, finding SNP in RARG has been difficult. The 3'UTR and all introns except number 1 were sequenced either in part or completely, and only 2 SNP were found, separated by 33 bp. RARGBFA212G SNP, which had the higher minor allele frequency (MAF = 0.25, compared to MAF = 0.1), was genotyped. Individual sequence traces of the draft bovine genome sequence have been examined but no SNP were found in RARG, not even the two found by sequencing, in a region that extends 20 kb on either side of RARG.

Although the RARG gene was the primary candidate, several genes were also genotyped that were expected to map near to RARG, based on the human sequence. These are SNP in the genes LALBA, IGFBP6, ARF3, WNT10B, SOAT2, ITGB7, GDF11 and STAT6. However, although these flanked RARG on the human map, now that the draft bovine sequence is available, all of these genes form a different gene order in cattle and all are telomeric to RARG on this chromosome. RARG was known to be located in a region of the human genome that had been extensively rearranged in cattle (Aleyasin and Barendse, 1999; Barendse et al., 2000), so the lack of conservation of gene order was a known challenge in this region. RARG is located to Bovine Scaffold 5.39 (a section of 529.5 kb), which includes ITGB7, AAAS and PFDN5 of the selected genes. PFDN5 and AAAS have not been genotyped, and their location near RARG has only been appreciated very recently. As marbling work continues in the Beef CRC, these SNP may be genotyped and tested for associations to marbling, although neither is an obvious candidate for the effect. The ITGB7E6 SNP showed an effect on marbling in Shorthorn cattle and showed a marbling:breed:genotype interaction when marbling was analysed as a qualitative trait. None of the other genes showed evidence of associations to marbling in large samples.

The gene GDF11 appeared to have an effect on P8 fat thickness. Both of the SNP in GDF show effects on P8 fat, the GDF11B SNP has an effect in the overall sample with all groups showing a consistent effect while the GDF11A SNP shows an effect in Shorthorn. GDF11B showed a high relative risk for marbling in the CS.254 sample, but otherwise there is no statistically significant association to marbling.

Details of the associations to marbling on chromosome 14. The TG5 gene effect on marbling has been confirmed in several, independent, international studies (e.g. Thaller *et al*., 2003; Pollak, 2005) since the project began so during the project our main interest became whether other SNP, either in the TG gene or in adjacent genes, also showed associations to marbling. Unfortunately, the SLA gene, which is located in the largest TG intron, showed no SNP in our tests. This was consistent with SNP in the TG gene itself, which is well known to have low numbers of SNP, and most of those that have described (Georges *et al*., 1987) have very low minor allele frequencies. We did, however, find one SNP in the 8th intron of the TG genomic sequence and tested it for associations to marbling. The TGH allele frequency is f(c) = 0.21 in the SBEF018 sample which compares to f(3) = 0.19 for TG5 in the same sample. The allele association is D' = 0.357 between TGH and TG5. This is low given the physical distance of 19 kbp between these two SNP, and when compared to that found in RORC (Tables 7) for SNP that are less than or equal to 25 kb apart. There was no association between marbling and TGH genotypes. Given our results with RORC, both for the degree of Linkage Disequilibrium between SNP and the distance at which associations were found, this lack of association to marbling for a gene with the same allele frequency as TG5, halted the search for SNP further away from TG5. These results also imply that TG5 must be close to the marbling QTL, or perhaps it is one of the causal mutations for a marbling QTL.

Details of associations to marbling on other parts of the bovine genome. Four SNP were evaluated for association with marbling in Australian cattle because they have been claimed to be associated with marbling in studies in other countries. Two of these are commercially available DNA tests. These are 1) a missense mutation in the Leptin gene, marketed as the igenity-L ® DNA test for marbling and other economically important traits, 2) a SNP in the SCD gene, which has been associated with marbling and fat composition in Japanese cattle (Tsuji et al., 2004), 3) HMGA2 which is a marbling QTL in pigs also located on Bta 5 (Rothschild et al., 2003), and 4) the Leu127Val missense mutation in the Growth Hormone 1 gene (Mitsuhashi and Chikuni, 2001). Three of these SNP did not show associations to marbling in the CS254 and SBEF018 samples (Table 9), and the Leptin SNP was also tested on fat traits in the SBEF018 data and the Beef CRC I DNA bank (Barendse et al., 2005). The leptin SNP does show an effect on P8 rump fat thickness, after new residual P8 fat thicknesses were calculated, but still shows no association with marbling. Since the SNP have been reported to be associated with marbling or fat traits in other studies, our failure to find associations to marbling will

not be due to our markers being too far away from the SNP that cause the QTL. One of these genes, SCD, is differentially expressed in Holstein versus Japanese Black cattle (Wang et al., 2005), but clearly genetic variation at this gene does not appear to be responsible for differences in marbling in our samples.

One of these SNP, the *GH1:c.457C*>G (Leu127Val) SNP is associated with marbling (Barendse *et al.* 2006). The allele frequency of the *GH1:c.457C* allele was 0.77 in Angus and 0.76 in Shorthorn. The *GH1:c.457C* allele was associated with lower marbling (P = 0.0472) and the average effect of allele substitution is -0.22 of a phenotypic standard deviation (S.D.). This allele was also associated with slightly higher rump fat (P = 0.0541) and the average effect of allele substitution was +0.11 S.D. Marbling and rump fat thickness were not strongly correlated (r=0.097, P < 0.01) in this dataset. This mutation had no significant effect on eye muscle area or hot dressed carcass weight (P > 0.1). Given these relationships, the differences between these GH1 alleles could be the result of differential deposition of fat in fat depots.

Details of associations to marbling of candidate genes that are differentially expressed. Six of the genes identified as being differentially expressed in Holstein versus Japanese black (Wang et al., 2005), i.e., CACNB3, CALB3, FABP4, LPL, SCD and THRSP, were tested for SNP. Four of these had SNP. THRSP is a small gene and, due to it being responsive to thyroid hormone, the entire coding sequence was tested for SNP in a panel of 14 taurine and zebu cattle. FABP4, SCD and LPL showed no association to marbling. Two SNP in THRSP, one a missense mutation in the coding sequence and one some distance away in the 3'UTR were tested. The THRSP SNP in the 3'UTR shows suggestive linkage to marbling in the CS.254 sample (Table 9).

The gene HAS2 has effects on connective tissue and is hypothesized to affect marbling (P. Allingham pers.comm.). A microsatellite in HAS2 was tested for association with marbling in the CS254 sample but none was found.

Milestones 3, 5, 6 and 8

Gene expression of candidate genes for marbling

Milestones 3, 5 and 6 were completed but milestone 8 is incomplete due to technical failure. 16-17 tissues of 3 life stages were collected, RNA was extracted and gene expression for 17 genes was measured, but *in situ* hybridisations of the RORC gene to LD muscle tissue have not been successful.

Gene expression patterns of positional candidate genes for marbling. The foetal expression of the 17 genes was usually greater than the adult expression, with a few exceptions, and the house keeping genes are different in their foetal versus adult gene expression. 13 genes were tested from bovine chromosome 5, 3 genes from bovine chromosome 3 including alternative transcripts for RORC, and SCD (Stearoyl CoA Desaturase) on a panel of cDNA from 14 adult and 8 foetal tissues. Comparison of the adult and foetal gene expression indicated that the foetal expression of these genes is usually greater than that of the adult gene expression, except in Kidney Fat. In addition, some genes were active in foetal or in adult tissues but not in both. For example, in the kidney, WNT10B is not expressed in the adult tissues while ADCY6 is not expressed in the foetal tissues. The only example for LD muscle, the site of marbling score measurement, is that PFKM is not expressed in foetal LD muscle. These comparisons also depended upon which housekeeping gene was used for the comparison. GAPDH provided more of the examples where the adult expression is greater than the foetal gene expression. This could mean that there are differences in the level of the house keeping genes in adult versus foetal tissues, in which the GAPDH expression is relatively lower than the 18S ribosomal protein gene expression in the adult. Another difference between the two housekeeping genes is that in the brain and muscle tissues there is often a significant decreased expression of GAPDH compared to Ribosomal Protein.

Differential expression of the RORC alternative transcripts. The RORC ex1ex2 transcript was not expressed in LD fat while the RORC-Insu2 transcript was not expressed in SC fat or ST muscle of adult tissues. The expression of these transcripts was otherwise without major difference, and both were expressed in all foetal tissues tested. This suggests that there may be functional differences between these transcripts in relation to fat deposition. Since RORC is associated to marbling or intramuscular fat in the LD muscle, these gene expression differences may influence marbling. The differences between the transcripts, where one is active in the SC fat and one in the LD fat is interesting, however, since very few of the genes showed expression in the LD fat sample, including SCD, it is possible that this cDNA sample was partly degraded, since the expression of the house keeping genes was lower in this tissue.

Gene expression characterisation of gene on chromosome 5. On chromosome 5, many of the genes were confirmed as potential genes affecting marbling. GDF11, ITGB7, AAAS, ARF3, IGFBP6, PRKAG1, SOAT2, VDR and PFKM are all expressed in the LD muscle. These are all also expressed in fat tissue. Of these, only AAAS is expressed in LD fat, but there are questions about the quality of the LD fat data given the SCD results. In addition, PFDN5 and WNT10B are expressed in fat, although there is no expression specifically in LD muscles. These genes can only be further discriminated as affecting breeding values for marbling through tests of genetic polymorphisms. Nevertheless, their activities in particular tissues would indicate the likely modes of action they might employ. For example, if WNT10B did affect marbling, it would be through direct effects on fat cells rather than through effects on muscle cells, while if PFKM affected marbling it would be through impacts on muscle cells rather than through effects on intramuscular fat cells.

In situ hybridisation of marbling genes. Finally, we were interested in examining the location of gene expression within muscle tissues by studying *in situ* hybridisation of candidate genes. Only one of the genes met the criteria we set, viz. 1) clear evidence of association with marbling and 2) gene expression localised to the tissue of interest, the RORC gene. However, despite many efforts by our collaborators, including staff ill health and departure but also failure of the methods, successful *in situ* hybridisations of the RORC gene to LD muscle spreads have not been achieved. This is the part of milestone 8 that is incomplete.

Materials and Methods

Cattle Samples, Traits and DNA. The CS254 and SBEF018 cattle samples used in this study have been reported previously (Barendse, 1997; Barendse, 2003; Barendse *et al*., 2004, 2005) and the phenotypes and DNA samples were used in the same way as before. The phenotypes available for these cattle are feed lot measurements as well as a small range of standard chiller assessments, such as AUSMEAT marbling score, ema eye muscle area, p8fat P8 fat thickness, hdw hot dressed weight, inweight entry weight to the feedlot, livewt carcass slaughter weight, dof number of days on feed.

Phenotype Analysis. Marbling was analysed in two ways, firstly as a categorical trait as before (Barendse 1997; Barendse *et al*. 2004, 2005) and second as a pseudo-quantitative trait with numerical values 1 to 5. For marbling as a pseudo-quantitative trait, and for eye muscle area, hot dressed weight and P8 rump fat, the trait measures were first adjusted to account for fixed and random effects and covariates using general linear mixed models implemented using ASREML (Gilmour *et al*., 1995). For each of the traits, the slaughter day (day), the breed and the vendor (random) were always fitted, and the statistically significant covariates among the following were also included, number of days on feed (dof), entry (inweight), hot dressed weight (hdw), rump fat at the P8 position (p8fat), marbling (marb) and eye muscle area (ema) were fitted depending upon the trait. Some of these models were explored using Aikake's Information Criterion (AIC) (Venables and Ripley, 2000). The residuals were obtained and then these were analysed for associations to genotypes. Differences in the residual trait values for each genotype were compared using t tests. The differences between means were used to calculate the average effect of allele substitution of the polymorphism (Lynch and Walsh, 1998).

Summary statistics such as variances and correlation coefficients were calculated using S-Plus. This method of analysis has been reported (Barendse *et al.* 2006)

DNA sequencing for SNP identification. SNP were primarily identified using DNA sequences obtained from a panel of 10 animals of Angus and Shorthorn ancestry from the SBEF.018 sample. These sequences were obtained by direct sequence of PCR products using the Big-Dye 3.1 terminator kit from Applied Biosystems Inc. (Foster City, CA), using the manufacturer's instructions. The genomic DNA sequence was obtained in parallel for both forward and reverse sequence using primers derived from exons and the amplification of intronic sequence between the exons. These sequences were checked for quality and were blasted back against GenBank to confirm the identity (Altschul et al., 1980). Then the sequence traces were assembled in contigs using phred and Phrap and viewed using consed. Then polyphred was used to identify variable bases, and variable bases with a phred quality score (PQS) of 20 was deemed to be of sufficiently high quality (www.phrap.org; Nickerson et al., 1997; Ewing et al. 1998; Gordon et al., 1998). SNP were preferentially chosen for genotyping if homozygotes of both alleles were present in the sequence traces. Some early SNP were obtained by subjecting the PCR amplicons to single strand conformational analysis (Barendse et al., 1993) before sequencing, but this method is no longer used as it is less efficient and more costly.

SNP genotyping. SNP were genotyped using the allele discrimination method of the *Taqman* ™ MGB system of Applied Biosystems Incorporated (Foster City, CA; Livak et al., 1995; Livak, 2003). The probes and primers were almost all designed manually using the Primer Express program, although the last few SNP systems were designed using the Assays By Design pipeline. The genotypes were obtained using the Taqman Universal Mastermix following the manufacturer's instructions. Two individuals checked the data for integrity including checking that the controls were in the correct places, then scored the allele discrimination plots, and with improvements in the Sequence Detection Software v 2.2 (ABI, Foster City, CA), checked the scoring after initial automated genotype calling. Genotypes were transferred to a Unix server and text output tables were processed to check that repeat samples had the same genotype. Genotypes were then translated into base calls and then associated with phenotypes prior to analysis. Scoring was always performed in ignorance of the phenotypes. The genes tested for SNP are in Table 1. Two SNP were genotyped using single strand conformational analysis (SSCP; Barendse et al., 1993) and DNA microsatellites (STRP, simple tandem repeat polymorphisms) were genotyped using ABI DNA sequencers. Initially, STRP were used to locate QTL to parts of chromosomes but SNP are preferred where large numbers of polymorphisms are required (Barendse, 2005).

DNA sequence of part of the RORC gene. The RORC genomic DNA sequence was obtained using primers derived from exons and the amplification of intronic sequence between the exons. The primers were designed using a comparative DNA sequence derived from human and mouse exons for the gene. Sufficient exonic material was included in the PCR amplicons to allow comparison of the DNA sequence to the human and mouse sequences. Initially, some disparate fragments of the gene were identified, around the 3rd, 5th and 7th and 8th introns, but some of the introns were too large for such a piecemeal approach. So the entire fragment from the 3rd intron to the 8th intron was obtained by walking along the chromosome. Overlapping PCR amplicons were obtained for the entire region using DNA sequence that had already been obtained. A total of 20 individuals were sequenced in this process, and both forward and reverse sequences for each amplicon were obtained, although some amplicons were so large that the DNA sequence from them did not overlap. The gaps in the sequence were filled in using PCR primers derived from the new sequence for the amplicon. For each amplicon in this region, at least 10 individuals were sequenced. DNA sequences were obtained from an ABI 377 DNA sequencer using methods described above, and the raw sequence was analysed using phred, Phrap and polyphred and assembled using consed (www.phrap.org; Nickerson et al., 1997; Ewing et al. 1998; Gordon et al., 1998).

Radiation Hybrid Map of bovine chromosomes 3 and 14. SNP and DNA microsatellites for bovine chromosomes 3 and 14 genotyped on the Texas A&M University Radiation Hybrid panel to map the DNA markers, with the primary aim of determining the degree with which synteny between humans and cattle had been rearranged. The methods used to construct the RH maps were reported in Barendse *et al*., 2000. The list of SNP used is included in Table 1.

Gene Expression using cDNA from bovine tissue. Tissues for mRNA extraction were obtained from adult, juvenile and foetal tissue of cattle, in collaboration with the groups of S. Lehnert and G. Harper at CSIRO Livestock Industries. Foetal tissues were obtained from two stages (132 and 194 day) with the aid of the group of H. Hearnshaw and P. Greenwood at NSW Department of Primary Industries. RNA was extracted from skin, kidney, brain, lung, liver, LD muscle, ST muscle, subcutaneous, cardiac, ommental, and kidney fat, mammary gland, testis, heart, spleen, pancreas and thymus using a method based on Chomczynski and Sacchi (1987). The tissue was dissected, immediately frozen in liquid nitrogen, wrapped in aluminium foil, disrupted with a hammer, homogenised in TRIzol ® (Invitrogen, Carlsbad, CA, USA) using an ultrasonic homogeniser (IKA-Ikasonic, Staufen, Germany) and RNA was extracted using the manufacturers instructions. All tissues were not available on all samples. Some tissues or organs cannot be recognised easily in the foetus. The translated cDNA from these mRNA samples were then stored at -80C until required.

Gene fragments were amplified from the cDNA using the primers used to sequence genomic DNA for SNP. The primers were ideal for examination of gene expression because they are complementary to exons but amplified introns and some part of the exon so gene identity was confirmed using BLAST. Fragments from genomic DNA could easily be discriminated from that amplified from cDNA due to the size of the fragment. Moreover, the primers had already been tested to determine whether they were efficient in amplifying DNA.

Gene expression was measured via quantitative PCR on cDNA from a range of tissue samples on the ABI Sequence Detector System 7900HT using SYBR green following the manufacturer's instructions (ABI, Foster City, CA). Gene expression was calibrated against two standard genes and Ct values were calculated using the SDS version 2 software. The Ct value for each gene was compared to the house keeping genes for each combination of tissue and life stage. These differences between genes were then tabulated (Table 11).

The gene expression comparisons have the following conventions. The comparisons are differences to the house keeping genes. Since these latter genes have high levels of expression, a small difference means a high level of expression for the target gene. Therefore, smaller values in the table mean greater gene expression. Empty cells in the table mean the gene failed to amplify in that tissue. In one tissue (SC fat: subcutaneous), the expression for SCD was greater than for the house keeping gene GAPDH, generating a negative value.

Gene Expression at the RORC gene. Full-length cDNA sequences were obtained for the RORC gene using the method of random amplification of complementary ends (RACE). Both the 5' and the 3' untranslated region were isolated. The cDNA sequence was obtained in two steps, 1) primers were synthesized for sequences in human exons 3 and 8 and, using cDNA translated from muscle mRNA with the Invitrogen Superscript Rnase H-Reverse Transcriptase kit following the manufacturer's instructions, a cDNA fragment of the RORC gene was amplified and sequenced and 2) this exon3-8 sequence was then used in 5′ and 3′ RACE to obtain the additional exons as well as the flanking 5′-UTR and 3′-UTR. The cDNA sequence obtained in this way was compared to the human and mouse sequences for the RORC gene. An alternatively spliced transcript of the RORC gene was documented (Table 5). Primers were designed to amplify up the main and the alternative splice variants to determine if there was a difference in gene expression in different organs. The gene expression was examined as described above.

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Tables

Table 1. SNP results from sequencing of DNA fragments of positional candidate genes.

Locus	Chromosome	Fragment	SNP	Name and Type
AAAS	Hsa 12 [Bta 5]	aaase9*	no SNP	achalasia, adrenocortical
		aaase12*	SNP:C-G	insufficiency, alacrimia
				comparative map
ADCY6	Hsa 12 [Bta 5]	adcybf	SNP:T-C	adenylate cyclase 6
			SNP:A-G	positional comparative
ADCY8	Hsa 8 [Bta 14]	adcy8b	SNP:G-A	adenylate cyclase 8 (brain)
				comparative map
ADRB3	Bta 27 (Hsa 8)	adrb3	no SNP	adrenergic receptor, beta 3
				candidate
ALDH9	Hsa 1 [Bta 3]	aldh9bc	in process	aldehyde dehydrogenase 9
				comparative
ARF1	Hsa 1 [Bta 3]	arf1	no SNP	ADP-ribosylation factor 1
				comparative
ARF3	Hsa 12 [Bta 5]	arf3e	no SNP	ADP-ribosylation factor 3
		arf3b	SNP:A-G	positional comparative
ATPASE6	mtDNA	atpase6	SNP:C-T	Atpase 6 (mtDNA)
			SNP:C-T	differential expression
			SNP:A-G	
CACNB3	Hsa 12 [Bta 5]	cacnb3bcd	no SNP	Calcium channel, voltage
				dependent, beta 3 subunit
				positional comparative
CALB3	Hsa X [Bta X]	calb3-3	no SNP	Calbindin 3, (vit D-dependent
				calcium binding protein)
				differential expression
DGAT1	Bta 14 (Hsa 8)	dgata	SNP: del	diacyl glycerol acyl-
				transferase
			SNP:G-C	milk fat qtl?
			SNP:C-T	
			SNP:C-T	
			SNP:G-A	
ENSA	Hsa 1 [Bta 3]	ensab	no SNP	endosulfine alpha, comparative
FABP4	Hsa 8 [Bta 14]	fabp4bc	SNP:G-T	Fatty acid binding protein 4,
			SNP:A-G	adipocyte, differential
			SNP:A-G	expression
			SNP:C-G	
LRRND6	Hsa 1 [Bta 3]	flj31810	SNP:A-G	LRRND6 similar to FLJ31810
			SNP:A-G	adjacent RORC, function
			SNP:A-C	unknown
			SNP:C-T	
			SNP:C-T	
			SNP:C-T	
GDF11B	Hsa 12 [Bta 5]	gdf11b	SNP:T-C	growth differentiation factor
			SNP:G-C	11 positional candidate
HAS2	Hsa 8 [Bta 14]	has2c	SNP:A-C	Hyaluronan synthase 2
			SNP:A-G	candidate gene
			SNP:C-G	
HMGA2	Hsa 12 [Bta 5]	hmga2-3	SNP:C-G	High mobility group AT-hook 2
				marbling gene in pigs
IGFBP6	Hsa 12 [Bta 5]	igfbp6cd	SNP:A-G	Insulin-like growth factor
			SNP:C-T	binding protein 6
			SNP:C-G	positional candidate
		igfbp6-3	SNP:C-T	
ITGA10	Hsa 1 [Bta 3]	itga103	no SNP	integrin, alpha 10
		itga10st	SNP:G-A	positional comparative
ITGB7	Hsa 12 [Bta 5]	itgb7e6*	SNP:C-T	integrin, beta 7
				comparative
KCNQ3	Hsa 8 [Bta 14]	kcnq3j	poor SNP	potassium voltage-gated channel
				KQT-like subfamily, member 3

				comparative map
LALBA	Bta 5 (Hsa 12)	lalba3u	SNP:T-C	lactalbumin, alpha
			SNP:A-G	linked to marbling QTL
			SNP:C-T	
LPL	Bta 8 (Hsa 8)	lpl3	SNP:C-T	Lipoprotein lipase
			SNP:A-G	differentially expressed
			SNP:A-C	
			SNP:A-G	
			SNP:A-G	
			SNP:C-T	
PFDN5	Hsa 12 [Bta 5]	pfdn5e	SNP:C-T	Prefoldin, positional
			SNP:C-T	comparative, cytoskeletin
			SNP:A-C	
PFKM	Hsa 12 [Bta 5]	pfkmde	poor SNP	phosphofructokinase, muscle positional comparative
PIK4CB	Bta 3 (Hsa 1)	pik4cb	SNP:C-T	phosphatidylinositol 4-kinase catalytic, beta, positional candidate
PPARD	Bta 23 (Hsa 6)	pparde	SNP:C-G	Peroxisome proliferator-activated receptor delta (beta), candidate gene
PRKAG1	Bta 5 (Hsa 12)	prkag1i3	no SNP	Protein kinase, AMP activated
		prkag1jk	SNP:C-T	gamma 1 non-catalytic subunit
			SNP:A-G	positional comparative
PRKAB2	Hsa 1 [Bta 3]	prkab2cd	SNP:A-C	protein kinase, AMP-activated
			SNP:A-G	beta 2, positional candidate
PVMK	Hsa 1 [Bta 3]	pmvkd	poor SNP	phosphomevalonate kinase comparative
RAP1B	Hsa 12 [Bta 5]	rap1b	no SNP	RAP1B, RAS oncogene family comparative
RARG	Bta 5 (Hsa 12)	rarge3	no SNP	retinoic acid receptor, gamma
		rarge8	no SNP	positional candidate
		rargf3u	no SNP	
		rargsj	SNP:unverified	
		rargbf	SNP:A-G	
			SNP:C-T	
RORC	Hsa 1 [Bta 3]	rorcc	no SNP	RAR-related orphan receptor C
		rorcgh	SNP:A-G	positional candidate
		rorca	SNP:A-G	
		rorcd	no SNP	
		rorce	SNP:A-G	
		rorcf	SNP:A-G	
		rorcex6	SNP:C-T	
			SNP:G-T	
RXRB	Hsa 6 [Bta 23]	rxrb3	SNP:C-T	Retinoid X receptor beta,
		rxrbefg3	SNP:G-A	candidate gene
S100A10	Hsa 1 [Bta 3]	s100a10u3	SNP:A-G	S100 calcium binding protein A10 (calpactin) adjacent RORC
SCD	Bta 26 (Hsa 10)	scd3	SNP:C-A	Stearoyl-CoA desaturase
			SNP:G-T	(delta-9-desaturase)
			SNP:G-C	candidate gene
		scde	SNP:C-T	
			SNP:C-T	
			SNP:G-A	
SLA	Hsa 8 [Bta 14]	slaf	no SNP	SRC-like-adaptor, adjacent TG
SLC11A2	Hsa 12 (Bta 5)	slc11a2u3	SNP:A-G	Solute carrier family 11 member 2, NRAMP2, comparative map
SOAT2	Hsa 12 (Bta 5)	soat2bcd	SNP:C-G	Sterol O-acyltransferase 2
			SNP:C-T	positional candidate
			SNP:A-T	
SREBF1	Bta 19 [Hsa 17]	srebf1-3	SNP:C-T	Sterol regulatory element binding transcriptn factor 1 differentially expressed
STAT6	Bta 5 [Hsa 12]	stat6ij	SNP:A-G	Signal transducer activator

			SNP:A-G	of transcription 6 interacts with leptin, contains ETH10 positional candidate gene
TENC1	Bta 5 [Hsa 12]	tenc1zad	SNP:A-G	Tensin like C1 domain containing phosphatase comparative mapping
TG	Bta 14 [Hsa 8]	tgh	SNP:A-G	thyroglobulin, marbling QTL
			SNP:T-C	fine-mapping
THRSP	Hsa 11 [Bta 15]	thrs	SNP:A-G	Thyroid hormone responsive
			SNP:C-T	(SPOT14 homolog, rat)
			SNP:C-T	differential expression
TNF	Bta 23 [Hsa 6]	tnfa	SNP:A-G	Tumor necrosis factor
			SNP:A-G	comparative mapping
		tnfa5	SNP:A-G	
			SNP:G-T	
			SNP:C-T	
			SNP:C-T	
VDR	Hsa 12 (Bta 5)	vdrg	SNP:A-G	Vitamin D receptor, positional candidate
VDUP1	Hsa 1 [Bta 3]	vdup1d	no SNP	TXNIP Thioredoxin interacting protein, upregulated by 1,25-dihydroxyvitamin D-3 positional candidate
WNT10B	Hsa 12 [Bta 5]	wnt10bd	SNP:C-T	wingless-type MMTV integration
			SNP:C-A	site family member 10B
			SNP:C-T	positional candidate
		wnt10bx4	SNP:G-A	
46 loci		67 amplicons	91 SNP	

*: SNP reported in IBISS database

Table 2. Radiation Hybrid Map for Bta 3

NAME	BREAK FREQ	cR
TGLA127	0.283	33.3
LEP-R	0.072	7.5
TGLA76	0.280	32.9
OSG	0.077	8.0
NAK	0.246	28.2
CD3Z	0.175	19.2
FCGR1	0.044	4.5
RME23	0.116	12.3
ILSTS96	0.132	14.2
PIK4CB	0.136	14.6
RM19		

LIKELIHOOD = -203.316356
MAP LENGTH = 174.69

Table 3. Radiation Hybrid Map of bovine chromosome 14

NAME	BREAK FREQ	cR
IDVGA76	0.310	37.1
DGAT1	0.011	1.1
CYC1	0.124	13.2
CSSM66	0.272	31.8
TG5-RPT	0.380	47.8
HAS2	0.294	34.8
BMS1747	0.301	35.8
RM11	0.158	17.2
CRH	0.138	14.8
IL7	0.234	26.7
BM2934	0.367	45.7
PENK	0.384	48.5
INRA92		

LIKELIHOOD = -421.393289
MAP LENGTH = 354.52

Table 4 Sequence of the RORC gene between exon3 and exon 7.

>RORCCH|Bta|contig1 consensus of rorcc-rorcgh good sequence bp 660-4879 snp at a1252g=rorce-a232g, a2303g, a2486g, c3075t, c3543t, g3950t, a4644g=rorcgh-a693g

AGAGAGAAGAGGGTATGACGTTAAGCGGTAGGGGAGGTGAGAGGAGATAT
GCGGGAGACGAGGAGAGGTGGACGTGAGGTGAGAGGAGCACAGCGGCGAC
ATGAGGTGGATTGAAGGGATGGCTGGGGAACAAAGAAGTCGACGATGGCG
GAAGAGAGTGAAGGGAAGTCCGAGGTGTATCGGATAAACGATAGGAACGT
GGACGTGGAGAGAGAACGACTCACCGCGGAGTGAGAGGGCGTTCACGTGT
GTGCGGAGTGAATAGTAGAGAAGAAGTGTGGAGGTGATGTGACGGGGTGA
TGGAGACAGAGATAAGAGAGTCGAAGGAAGGAGGTCGTACAGAGCATGCA
GAGAAAGAGAGGCGGTAGGGTGAGAGAAGAAGGAGACGTCGTGGTATTGT
CTGAGAGAGCGCGGAATCGCGAGAGTGTGGTGTGAGAAGCAAGCGTGGGT
GGATACAGATACAGAGGTGGAGAGGAGAGAACAGAGTGTTGGAGTTAGAA
GACGTGGGATCGGGCGGGAGCGGACGTGGACGATAGGGTATGGCGGGGCG
GGACGGGAAAGTGGAGAGAGGCGTAGCGGTAGAGATGAGAAAGGCGGAAG
GACAAGTCGTGCTGAAGGGAAGTGTAGAAGGTACGGGAGAGAGGATGAAG
AGGAGAGAGTTGGGATCCACTACGGGGTTATCACCTGTGAGGGGTGCAAG
GTGAGTGCTCGCTCATACACAGTCTCTTCAGAGGATAGGAGAGGGGGCTC
GGCAGCCTCCGTACTGCCTGCAGGGCTGGTCTCCTGAGACGGCTAGGGAG
CTGCCCGGTGGCGCCTTGCCTGGAGAGCCCTGTGCCAGAGGGCCTTGTCT
TCAACTTGGGAAGCGACTGCAGGACAGATTATAATGGAGGAGAGAAAACT
GAGGGGTCCTACGATCTGGAGGTCCTCAGACTGATCTCGGCCCTGCCAGG
GCCAGCAGCCATGGTCTTCACCTGCCTTCCCCAGGGCTTCTTCCGCCGGA
GCCAGCAGTGCAACGTGGCCTACTCCTGCACCCGCCAGCAGAACTGCCCC
ATCGACCGCACGAGCCGCAACCGATGCCAGCACTGCCGCCTGCAGAAGTG
CCTGGCCCTGGGCATGTCCCGAGATGGTGAGGCCGAGTGGGCAGCCCCCC
AGGGCTTCAGTCGCCAGAGCGGCGGCCTGACCAGACGGGTTCAGCGGCAC
CAGACCGGGGTTCAGCAGCCTTCCGGGTCTCCGCCCTCCAAACTTCCCTC
TGGCAGGCTCTGGTCTCTTCCACTGTCCAGCGTGGCGGTGGGCAGGCTGC
CCTCATCAAAGGTCTTCACAGTCCCCAGCTTCAGGCTCTGCAACAACAAC
CCTTGTTTCCTCAAACTCCTAGGCCCTTTTCCCCTTCCTTGGGAGGGACT
CTCCTCACCCGACTCGCCTCCAGCCCAGATCCCATCCCCATCCACAGTCC
ACATCTTTCCCCAGCTGCCCCCTTGCTCCAACTCCACCTGGCTCCCCGTA
CCCCTATCTCCAGCTTGGGACAAAGGGCTGTGACTATGAATGGGACCACA
GTCACCCTGAGAACAGCCCGGAAGGCTGAGGTTCACAAGGGCGGCTGTTC
CTGGTGCCTTTCTCCCTTTCTCAGCCACTGCCCCCTTGGCAGCCCCCAAA
CTGCTCCCCCCTCCAACACCACAGAAGGAGCCCAGGGTGGGGCTGGGGGA
GGCATGAGGTGATGGGGAGCCAGAAGGAGCCTGTCAGCACTTTTCAGCGC
CCAAAATAACAAAGTGAAAGGAAACACGCAGGGTTGCAAAGGGGCAGGCG
GGGTGAGGGGCTGTGCCCCCACACCTGGGAGGGGCGGTGGGGCGAGTGAA
AAGGCAGGAAAGAGAGAGCAGAAGAGGATGTTCAGAAACAAGCCGCGGAG
CCTGGGTTGGGCTGTGGTGAGTATCTAGGTCACCAGGGAGCCTGTAGGCC
TGACCACAGGGAGACCTGTGTTCTCGGCTCTCCTCTTCCTCCCGACCCTC
CTAGATTGGGCGAGGTGACCCCGATACAGCTTGAGGCTCCCTCCCCAGCC
ATCCCCAGCCCCATAGCAGGAGGCCGGGCTCCTCAGCCCAGACTCATTGC
TCAAATTCTGCCATTTAGTCCAAGCTGGTTTCAAGCAGGGAGAGTTTGCT
CAGAAGACTGATACGTCTGCTGATACATCTGGGCTGGACAGAGCCCAGAA
GCAGACAGGAGTCGGCAGGAGTGGGTCTTGAACAGAAATGCCTGGAAGGC
AAGCTCCCGGGCCAGACTGGAGAAGCTCTGCTCGGAGTGAGCTCTTCAAG
GAGCAGCAAGTTAATCCTGTAATGGTCACAATGCCTATTGACTCTGCCCT
GTGCCATGTCCTCCCGCCTCCAAAACTCCTGATCCCCCGACCTCTTTCAG
CTGTCAAGTTTGGCCGCATGTCCAAGAAGCAAAGGGACAGCCTGCATGCA
GAGGTGCAGAAACAGCTGCAGCAGCGGCAACAGCAGCAACGGGAACAAGC
GGCCAAGACCCCTCCCATGGGAGCCCAAGGAGCAGACACCCTCGCCTGCA
CCTTGGGGCTCCCGGATGGGCAGCTACCCCTGGGCTCCTCGCCTGACCTG
CCAGAGGCCTCAGCCTGTCCCCCTAGTCTCCTGAGGGCTCCAGGCTGTGG
GCCCTCCTACTCCAACAGCCTGGCCAAGACCGGGCTCAACGGGGCCTCGT
ACCACCTGGAATACAGCCCTGAGCGGGGCAAGGCTGAGGGCAGGGAGAAC
TTCTATGGCATAGGCAGCCAGCTGGCCCCAGACAGGGGTGGACTTCACTC
TGAGGACCCCAGGCGTCCTGGGCTTGGGGAGCCAGGACGGGGCCTGGACA
GCTACTTCACCCCCAGTTTCCGCAGCACCCCAGAGGTGCCTTATGCTTCC
CTGACGGAGATTGGTAAGCAGCTGGGGAGGCGGAGAGTGTAGAAAGATGA
GGGAGGAGCTTTCAAGAAAGGGGCCCTGTGGGACTTCCCTGGTGGTTAAA
AGTCCACGTTTCCACTGCAGGGACATGGATTCTATCCCTGGTCAGGGAAC

TAAGATCCCACATACTGTGCAGTGTGGGAAAAAAAATGGAGTGGGGGGCC
CTAGTCAGCATCACATGTAAACCAAGCATACACAAGGGTCTAGGGGGAGG
CAGAGCAGGTCTAGGCCAGGCAGAGGAGCGCCCTTGGTATGCGAAAGGTA
GGAGCTGGCTGAGATGGAACCTCTGCCTTCCTCCCTTGACCCCAGAGCAC
CTGGTGCAGAACGTTTGCAAGTCCTACCGGGACACGTGTCAGCTGCGGCT
GGAGGACCTGCTCCGTCAGCGCTCCAATATCTTCTCACGAGAGGAGGTGG
CTGGCTACCAGAGGAAGGTGAGGCCAGGACACCGCGCCGGGGAGGAAAGA
CTCCTGCCACTACCCAGGGAGTCCAGAGACAGCAACCTGCACACATGAGT
GGGCTGGGCCGAGGCAGGGTATCCCAGAGAGACACAGTGTGCACCTGCTT
TTAAGTACGCACCCTAGCAGATGGGTGAGAGCTTCTCCTTAGCTCCTGCC
TATGGTTGTTTTACCTGGTCTTCATCGGTAAAGAATTTGCCTGCAATGGG
GGAGACCTGGGTTCGATCCCTGGGTCAGAAAAATCCCCTGGAGAAGGCAA
TGGCAACCTACTCCAGTATTTTTGATCCCATGGACAGAAGAGGCTGGAGG
GCTGTAGTCCATGGGGTCACAAAGAGTTGAACACCAATGAGTAACACACA
CACACATATACACACACACACCCTATGTCCTTCACCACCCAGACCTAGAA
GTACAGACCTCAGCATACTCTCCTTCCTCCTCCTCTATCAACAGTAGTCA
GACTTCTCATTAGCATCTGTTTCAACACTCTTTCTCTGCCAGGCACTGTG
CTGAACACTTTACATAAATTACCCTTGACCCTTACAACAACCACCCTATT
AGGCTGCCACTCCTGCTTTTAATGGACTGAGAAAGTGAGCACAGAGGAGT
TCGTAGACGTACTCCACGCCACAAGGTAGTGGAGCCAAAGGCAAATTTAG
ATCCACTGGGCCCCAAAGTGCATTGTGCTAAGAACAGGCTAAAAACACAA
GTGCTGAGAACGTGGGGGGTACTTGCCGTTTTGTCTGGGCTCCCAGGGCA
TCAGTGGGGTCCCGGGGGTCCTAAAAGACAGCTCCAGTGGGACCTCCCTC
CTCCTCTGCAGTCGATGTGGGAGATGTGGGGACGCTGTGCCCACCGACTC
ACCGAGGCCATTCAGTATGTGGTGGAGTTCGCTAAGAGGCTCCCGGGCTT
TATGGAGCTCTGCCAGAACGACCAGATCGTGCTACTCAAAGCAGGTGCCC
AGGGAGAGCGGGTGGGCTGCGGGGTAGGGGGCACCAGTGTGGAATGGGCG
GGGCTGTGTGGAGGGAGGTGCCTTAGGGACCCAGGACACTGAAGGGAGAC
AGAGACAGATTCCTGGCTCTGTGTGACAACGCGCCTGCATTTCTCCCCAC
TCCCCAGGAGCCATGGAAGTGGTGCTGGTCAGGATGTGCCGGGCCTACAA
CGCTGACAATGACACAGTCTTTTTTGAAGGCAAATACGGTGGCGTGGAGC
TGTTCCGAGCCTTGGGTGAGGGGCAGGGGGAGATGAGCAAGAGAAGTCCG
AGGCCAACCCCATCCGAGGCTTCCAGACCCAGGGCGTCCTCTTTTCAGGA
CAGATTGCCCGCTCTGCTCCAGACACCAAGGGGGTGGGTGTCCTTGGGCA
CCATGGCCTCAGCCGCCTTGCTCACTATTTTGTTTCCACCCTCCCAGGCT
GCAGTGAACTCATCAGCTCCATCTTTGACTTCTCCCGCTCCCTGAGTGCC
TTGCGCTTTTCAGAGGATGAGATTGCCCTCTTCAAAAGA

Table 5. Sequence of the 38 bp insert into the RORC gene

>RORC normal transcript at exon1 exon2 boundary
TCGCAGG|AGCTGCcGG

>RORC alternative transcript with 38 bp insert between exon1 and exon2 note sequence difference in exon2
TCGCAGGcgacggaggaagctgtcctgcctctaaagatgacaaagAGCTGCtGG

Table 6. Sequence of part of the RARG gene.

>RARG|Bta| BF fragment reverse complement contig1 5' to 3' snp bp 212 c/t opp homs snp bp 180 a/t 4 hets, poor sequence between <>
<GCCCCCGGTACT>AGTGCTGACATAGAGATCTGTGCTTAGTCACTCAGTCG
TGTCCGACTCTTTGTGACCCTATGGACTGTAGCCCGCCAGGCTCCTCTGT
CCATGGGGATTCTCCAGGCAAGAATACTGGAGTGGGTTGCCATGCCCTCC
TCCAAGGGGTCTTCCCAACCCAGGGATTAaACCCAAGTCTCCCGCCAGGT
CTCCCGCATTGcGGGCAGATTCTTTTATTGACTGAGCCACCAGGGAAGTC
CTGGGCAGGCTTTACCAGAGAAAACCACTTGCCAGAGAGGGGATGGTATA
GGGATTGGAGCAGGGGAGATGGTCTGAGTGGGGCTCATGGTCAGGGTTTG
GGGCCTGGAGGGCCATAGGGCAGGGCACCTTGGGATCAGCCCCTCATCAC
TGTCTTCCATGTCTCTCTCACTCACCTGTGCTTATCCCCAAAGACCGCAT
GGACCTGGAGGAGCCTGAGAAAGTGGACAAGCTGCAGGAACCGCTGCTTG
AAGCCCTGAGGCTCTATGCCCGGCGCCGGCGGCCCAGTCAGCCCTATATG
TTCCCCAGGATGCTCATGAAGATCACTGACCTCCGGGGCATCAGCACCAA
GGGTTAGTCGGGAGCAAGCCTCCCCTCTGTCTTCTCGGAGCTGCCGGTCT
CCCAGGTCAGGCAGAGACAAGAGCAGAGTGGGGTAGAATCAGGCAGCCTG
CACTCGCATCCTCGCTCCGCTGCATGCTAGTGGGAACACTTGGTGCAAAA
TA<CCTTTCCTTCTGCCAAATTA>

Table 7. Linkage Disequilibrium estimates between SNP at the RORC and LRRND6 genes.

Locus	Locus	h00	h01	h10	h11	f(a0)	f(b0)	D'	r^2
RORCA322	RORCE232	79	154	2052	743	0.08	0.70	0.5182	0.0532
RORCA322	RORCE2303	82	38	0	2708	0.04	0.03	1.0000	0.6739
RORCA322	RORCE2486	0	385	218	2733	0.12	0.07	1.0000	0.0091
RORCA322	RORCE3075	377	0	2725	236	0.11	0.93	1.0000	0.0097
RORCA322	RORCE3543	0	375	232	2719	0.11	0.07	1.0000	0.0095
RORCA322	RORCE3950	185	25	687	2065	0.07	0.29	0.8313	0.1264
RORCA322	RORCGH693	27	162	2326	429	0.06	0.80	0.8213	0.1842
RORCA322	RORC3-478	335	1	2504	238	0.11	0.92	0.9617	0.0095
RORCA322	LRRND6-1004	0	277	825	1992	0.09	0.27	1.0000	0.0358
RORCE232	RORCE2303	56	2083	141	762	0.70	0.06	0.5957	0.0582
RORCE232	RORCE2486	153	2071	13	999	0.69	0.05	0.7496	0.0138
RORCE232	RORCE3075	2125	60	875	80	0.70	0.96	0.3841	0.0158
RORCE232	RORCE3543	59	2124	77	868	0.70	0.04	0.3784	0.0150
RORCE232	RORCE3950	222	1605	395	226	0.75	0.25	0.5179	0.2659
RORCE232	RORCGH693	1806	163	423	312	0.73	0.82	0.5287	0.1596
RORCE232	RORC3-478	1946	73	798	77	0.70	0.95	0.3024	0.0115
RORCE232	LRRND6-1004	608	1381	55	726	0.72	0.24	0.7058	0.0615
RORCE2303	RORCE2486	0	338	221	2771	0.10	0.07	1.0000	0.0080
RORCE2303	RORCE3075	329	1	2760	244	0.10	0.93	0.9588	0.0080
RORCE2303	RORCE3543	0	327	238	2755	0.10	0.07	1.0000	0.0084
RORCE2303	RORCE3950	170	0	704	2094	0.06	0.29	1.0000	0.1456
RORCE2303	RORCGH693	0	147	2364	441	0.05	0.80	1.0000	0.2107
RORCE2303	RORC3-478	297	3	2553	249	0.10	0.92	0.8769	0.0073
RORCE2303	LRRND6-1004	0	237	837	2042	0.08	0.27	1.0000	0.0302
RORCE2486	RORCE3075	217	0	2906	253	0.06	0.93	1.0000	0.0056
RORCE2486	RORCE3543	0	217	247	2900	0.06	0.07	1.0000	0.0055
RORCE2486	RORCE3950	0	180	1030	2064	0.05	0.31	1.0000	0.0267
RORCE2486	RORCGH693	201	0	2325	782	0.06	0.76	1.0000	0.0200
RORCE2486	RORC3-478	207	0	2642	259	0.07	0.92	1.0000	0.0065
RORCE2486	LRRND6-1004	0	180	886	2162	0.06	0.27	1.0000	0.0223
RORCE3075	RORCE3543	0	2952	33	3	0.99	0.01	1.0000	0.9157
RORCE3075	RORCE3950	815	2130	95	0	0.97	0.30	1.0000	0.0755
RORCE3075	RORCGH693	2323	761	203	1	0.94	0.77	0.9788	0.0191
RORCE3075	RORC3-478	2688	24	3	31	0.99	0.98	0.9100	0.5079
RORCE3075	LRRND6-1004	884	2159	0	201	0.94	0.27	1.0000	0.0247
RORCE3543	RORCE3950	93	0	814	2125	0.03	0.30	1.0000	0.0741
RORCE3543	RORCGH693	201	0	2317	762	0.06	0.77	1.0000	0.0198
RORCE3543	RORC3-478	0	31	2684	27	0.01	0.98	1.0000	0.5292
RORCE3543	LRRND6-1004	0	197	886	2147	0.06	0.27	1.0000	0.0246
RORCE3950	RORCGH693	266	388	1867	3	0.26	0.85	0.9896	0.5133
RORCE3950	RORC3-478	742	93	1938	17	0.30	0.96	0.7794	0.0584
RORCE3950	LRRND6-1004	0	770	640	1324	0.28	0.23	1.0000	0.1198
RORCGH693	RORC3-478	2125	207	689	5	0.77	0.93	0.8972	0.0180
RORCGH693	LRRND6-1004	720	1602	1	581	0.80	0.25	0.9931	0.0816
RORC3-478	LRRND6-1004	813	1982	1	196	0.93	0.27	0.9813	0.0254

These 10 SNP occur in a region of approximately 25 kb of genomic sequence.

Table 8. Haploblock analysis of genotypes of 9 SNP from RORC and LRRND6 for the most likely location of the marbling QTL.

SBEF018 sample

PP|XS| Interval	Posterior Probability	Normalized Density
1 - 2	0.01380939 +/- 0.00000000	0.00161802 +/- 0.00000000
2 - 3	0.00098068 +/- 0.00000000	0.00160866 +/- 0.00000000
3 - 4	0.00000015 +/- 0.00000000	0.00000024 +/- 0.00000000
4 - 5	0.00000005 +/- 0.00000000	0.00000009 +/- 0.00000000
5 - 6	0.24091135 +/- 0.00000000	0.39517960 +/- 0.00000000
6 - 7	0.30381314 +/- 0.00000000	0.49836073 +/- 0.00000000
7 - 8	0.44042226 +/- 0.00000000	0.10320684 +/- 0.00000000
8 - 9	0.00006298 +/- 0.00000000	0.00002583 +/- 0.00000000

Loci code

1. RORCA322
2. RORCE232
3. RORCE2486
4. RORCE3075
5. RORCE3543
6. RORCE3950
7. RORCGH693
8. RORC3-478
9. LRRND6

Table 9. Comparison of genotypes or alleles for SNP compared to marbling.

CS254 SAMPLE

	Classes				Gadj	P	RR	D'	Chromosome
SNP v Marbling									
PRKAB2	59	35	53	37	0.288	0.591	1.18	0.04	3 (Un.3976)
RORCA	24	17	64	65	0.984	0.321	1.43	0.14	3.43
RORCE232	51	32	31	50	8.806	0.003**	2.57	0.23	3.43
RORCE2303	24	15	70	67	1.324	0.249	1.53	0.17	3.43
RORCGH	50	59	40	29	2.460	0.116	0.61	0.14	3.43
RORC3	73	75	17	9	2.277	0.131	0.52	0.28	3.43
LRRND6	17	18	73	64	0.244	0.621	0.83	0.07	3.43
PIK4CB	61	67	31	21	2.105	0.146	0.62	0.17	3.46
LALBA	15	26	53	42	4.206	0.040*	2.19	0.26	5.53
IGFBP6	71	61	21	23	0.480	0.488	1.27	0.09	5.40
ARF3B	11	17	73	69	1.360	0.243	0.61	0.20	5.44
WNT10B	47	36	29	40	3.191	0.074	1.80	0.15	5.44
SOAT2	72	60	20	24	1.079	0.298	1.44	0.13	5.40
RARG	14	23	68	61	2.531	0.111	1.83	0.23	5.39
ITGB7	32	40	65	59	1.151	0.283	0.73	0.10	5.39
GDF11A	81	66	9	16	3.084	0.079	2.18	0.31	5.87
GDF11B	8	2	80	82	3.606	0.057	4.10	0.59	5.87
HMGA2	74	69	16	15	0.000	0.989	1.01	0.00	5.75
STAT6IJ	43	42	43	40	0.025	0.875	0.95	0.01	5.79
TGH	23	18	65	64	0.402	0.526	1.26	0.08	14.16
HAS2	microsatellite				0.984	0.805			14 (Un.2331)
LEP	47	45	50	36	0.408	0.346	0.75	0.08	4.120
GH1	14	28	68	52	6.784	0.009**	0.38	0.34	19.100
TNFA	59	52	29	32	0.491	0.480	1.25	0.07	23.57
SCD	67	66	21	14	1.020	0.321	0.61	0.19	26.39
THRSP	8	16	38	28	4.101	0.042*	0.37	0.34	29 (Un.2843)

SNP haplotypes v Marbling

	Classes	Gadj	P	RR	D′	Chromosome
RORCA-E	18 28 28 6 14 38	10.41	0.005**	4.07	0.48	3.43
RORCE-GH	30 14 26 11 7 34	9.73	0.007**	3.57	0.39	3.43
RORCA-GH	17 14 43 6 8 46	5.64	0.059	3.03	0.43	3.43

Classes: Contingency table
Gadj: Heterogeneity between marbling and alleles
RR: Relative Risk
D': Lewontin D' measure of allelic association

SBEF018 SAMPLE

	Gadj	P	Dev	P	Int	P	Chromosome
SNP v Marbling							
PIK4CB	3.75	0.878	9.066	0.336	11.30	0.790	3.46
RORC	36.92	0.000***	18.853	0.015*	18.66	0.286	3.43
LRRND6	6.64	0.576	8.864	0.354	19.53	0.242	3.43

PRKAB2	7.68	0.465	6.064	0.640	26.56	0.046*	3 (Un.3976)
LALBA	23.90	0.002**	9.897	0.272	21.50	0.159	5.53
WNT10B	15.97	0.042*	6.281	0.615	20.93	0.181	5.44
SOAT2	10.30	0.244	10.420	0.236	22.93	0.115	5.40
RARG	21.36	0.006**	7.468	0.487	12.88	0.681	5.39
ITGB7	18.92	0.015*	12.581	0.127	31.15	0.012*	5.39
GDF11A	5.56	0.696	8.046	0.429	18.49	0.296	5.87
GDF11B	3.09	0.928	3.017	0.933	15.49	0.489	5.87
DGKA	2.84	0.943	4.638	0.795	19.01	0.267	5.79
TGH	13.30	0.101	11.241	0.188	15.51	0.487	14.16
LEP	8.23	0.410	4.465	0.812	15.51	0.487	4.120
GH1	10.32	0.243	11.063	0.198	10.91	0.815	19.100
SCD	18.87	0.015*	7.660	0.467	12.33	0.720	26.39
THRSP	6.974	0.539	5.222	0.734	15.61	0.48	29 (Un.2843)

Gadj: overall heterogeneity for the contingency table
Dev: deviation between genotypes and marbling
Int: deviation between breed, genotype and marbling

Table 10. Tests for associations between DNA markers and marbling or P8 rump fat with marbling analysed as a pseudo-quantitative trait

Chromosome 3													
Marbling													
Locus[1]	**mean_0**[2]	**SE**[3]	**mean_1**	**SE**	**mean_2**	**SE**	**N**[4]	**Freq**[5]	**a**[6]	**k**[7]	**α**[8]	**tmax**[9]	**PermP**[10]
rorcaa322g	0.00	0.12	0.02	0.04	0.00	0.02	1675	0.11	-0.00	-94.50	-0.01	0.40	0.7090
rorcaa322g-ANG[11]	0.07	0.12	-0.00	0.05	0.00	0.03	816	0.20	0.03	-1.10	0.06	0.55	0.5805
rorcaa322g-SHO[12]	-0.69	NaN	0.11	0.13	-0.00	0.03	757	0.02	-0.35	-1.33	-0.79	0.80	0.4263
rorcaa322g-OTH[13]	-0.89	0.44	0.10	0.18	0.01	0.10	102	0.17	-0.45	-1.22	-0.82	1.50	0.1079
rorcea232g	0.04	0.03	-0.03	0.03	-0.04	0.04	1676	0.69	0.04	-0.89	0.03	1.78	0.0790
rorcea232g-ANG	0.06	0.04	-0.04	0.04	0.01	0.05	826	0.63	0.03	-2.73	0.01	1.74	0.0895
rorcea232g-SHO	0.02	0.04	-0.02	0.05	-0.11	0.09	746	0.76	0.06	0.43	0.08	1.35	0.1786
rorcea232g-OTH	0.08	0.15	-0.05	0.12	-0.11	0.18	104	0.62	0.10	-0.40	0.09	0.74	0.4552
rorcea2303g	0.02	0.12	0.04	0.05	-0.00	0.02	1674	0.10	0.01	2.85	-0.01	0.79	0.4251
rorcea2303g-ANG	0.06	0.12	0.02	0.05	-0.00	0.03	818	0.18	0.03	-0.05	0.03	0.52	0.5978
rorcea2303g-SHO	-0.69	NaN	0.14	0.22	0.00	0.03	754	0.01	-0.35	-1.38	-0.82	0.52	0.5961
rorcea2303g-OTH	-0.46	NaN	0.12	0.17	-0.03	0.10	102	0.14	-0.21	-1.73	-0.48	0.77	0.4405
rorcea2486g	0.15	0.14	0.05	0.05	-0.00	0.02	1679	0.07	0.08	-0.26	0.09	1.03	0.3050
rorcea2486g-ANG	0.10	0.10	0.05	0.11	0.01	0.03	826	0.03	0.05	-0.12	0.05	0.39	0.6959
rorcea2486g-SHO	0.16	0.17	0.04	0.06	-0.02	0.03	751	0.11	0.09	-0.35	0.12	0.86	0.3945
rorcea2486g-OTH	NaN	NaN	0.31	0.30	-0.01	0.09	102	0.04	NaN	NaN	NaN	1.03	0.3074
rorcea3075g	**0.02**	**0.02**	**-0.10**	**0.05**	**-0.10**	**0.17**	**1699**	**0.92**	**0.06**	**-1.01**	**0.01**	**2.21**	**0.0270**[14]
rorcea3075g-ANG	0.01	0.03	-0.04	0.07	0.08	0.55	834	0.93	-0.03	2.56	-0.11	0.73	0.4571
rorcea3075g-SHO	***0.03***	***0.03***	***-0.17***	***0.06***	***-0.12***	***0.17***	***759***	***0.91***	***0.07***	***-1.71***	***-0.03***	***2.64***	***0.0090***[15]
rorcea3075g-OTH	-0.01	0.09	0.12	0.26	-0.55	NaN	106	0.94	0.27	1.48	0.63	0.47	0.6403
rorcea3543g	**-0.07**	**0.18**	**-0.11**	**0.05**	**0.02**	**0.02**	**1690**	**0.08**	**-0.04**	**2.01**	**0.03**	**2.53**	**0.0120**
rorcea3543g-ANG	0.08	0.55	-0.07	0.07	0.01	0.03	828	0.07	0.03	-3.57	0.13	1.11	0.2756
rorcea3543g-SHO	***-0.12***	***0.17***	***-0.17***	***0.06***	***0.03***	***0.03***	***757***	***0.09***	***-0.07***	***1.72***	***0.03***	***2.66***	***0.0077***
rorcea3543g-OTH	NaN	NaN	0.07	0.24	-0.01	0.09	105	0.06	NaN	NaN	NaN	0.29	0.7787
rorceg3950t	**-0.01**	**0.05**	**-0.07**	**0.03**	**0.05**	**0.03**	**1667**	**0.32**	**-0.03**	**2.97**	**0.00**	**3.06**	**0.0020**
rorceg3950t-ANG	***0.01***	***0.05***	***-0.05***	***0.04***	***0.10***	***0.05***	***821***	***0.47***	***-0.04***	***2.53***	***-0.03***	***2.47***	***0.0141***
rorceg3950t-SHO	***-0.14***	***0.14***	***-0.15***	***0.05***	***0.04***	***0.03***	***741***	***0.14***	***-0.09***	***1.14***	***-0.02***	***2.79***	***0.0060***
rorceg3950t-OTH	-0.15	0.22	0.04	0.12	-0.01	0.14	105	0.38	-0.07	-1.59	-0.10	0.69	0.5003
rorcgha693g	0.02	0.02	-0.05	0.03	0.02	0.06	1686	0.76	0.00	-30.72	-0.03	1.67	0.1010

rorcgha693g-ANG	0.06	0.04	-0.05	0.04	0.04	0.06	829	0.61	0.01	-12.47	-0.01	1.90	0.0575
rorcgha693g-SHO	0.00	0.03	-0.05	0.08	-0.42	0.12	754	0.95	0.21	0.76	0.36	1.13	0.2683
rorcgha693g-OTH	0.05	0.13	-0.03	0.13	-0.16	0.31	103	0.68	0.10	0.28	0.11	0.64	0.5280
rorc3a478g	0.01	0.02	-0.09	0.05	-0.17	0.17	1600	0.91	0.09	-0.11	0.08	1.94	0.0600
rorc3a478g-ANG	0.02	0.03	-0.06	0.07	-0.24	0.33	810	0.92	0.13	0.35	0.17	1.17	0.2329
rorc3a478g-SHO	***0.01***	***0.03***	***-0.17***	***0.07***	***-0.15***	***0.21***	***688***	***0.90***	***0.08***	***-1.31***	***-0.00***	***2.31***	***0.0196***
rorc3a478g-OTH	-0.07	0.09	0.38	0.23	-0.02	0.54	102	0.92	-0.03	-16.11	0.32	1.80	0.0794
lrrn6da1004g	0.05	0.06	-0.00	0.03	0.00	0.02	1660	0.28	0.02	-1.11	0.03	0.69	0.4830
lrrn6da1004g-ANG	***0.17***	***0.08***	***-0.04***	***0.04***	***0.01***	***0.04***	***814***	***0.32***	***0.08***	***-1.64***	***0.12***	***2.38***	***0.0179***
lrrn6da1004g-SHO	-0.16	0.11	0.03	0.05	-0.01	0.03	743	0.24	-0.08	-1.52	-0.14	1.52	0.1228
lrrn6da1004g-OTH	-0.10	0.33	0.08	0.16	-0.02	0.11	103	0.27	-0.04	-3.69	-0.10	0.54	0.5883
P8fat													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**α**	**tmax**	**PermP**
rorcaa322g	0.24	0.92	0.11	0.33	0.06	0.15	1697	0.12	0.09	-0.46	0.12	0.21	0.8340
rorcaa322g-ANG	-0.00	1.03	0.26	0.38	-0.13	0.23	815	0.20	0.06	5.11	-0.14	0.92	0.3586
rorcaa322g-SHO	0.86	NaN	-1.66	1.00	0.09	0.22	745	0.02	0.38	-5.58	2.43	1.58	0.1160
rorcaa322g-OTH	-4.28	3.77	-0.29	1.07	0.51	0.67	102	0.17	-2.40	-0.66	-3.46	1.19	0.1794
rorcea232g	-0.16	0.20	0.31	0.23	0.46	0.36	1699	0.68	-0.31	-0.53	-0.25	1.58	0.1220
rorcea232g-ANG	-0.43	0.30	0.33	0.31	0.35	0.47	825	0.63	-0.39	-0.95	-0.29	1.75	0.0783
rorcea232g-SHO	-0.07	0.27	0.20	0.39	0.51	0.68	734	0.76	-0.29	0.07	-0.30	0.78	0.4376
rorcea232g-OTH	0.45	0.97	-0.10	0.82	0.08	1.06	104	0.62	0.19	-1.95	0.10	0.44	0.6581
rorcea2486g	-0.42	1.77	0.15	0.41	0.02	0.15	1702	0.07	-0.22	-1.56	-0.52	0.33	0.7590
rorcea2486g-ANG	***7.63***	***0.38***	***-0.57***	***0.81***	***-0.05***	***0.20***	***825***	***0.03***	***3.84***	***-1.14***	***7.90***	***2.43***	***0.0173***
rorcea2486g-SHO	-3.11	1.46	0.44	0.48	-0.06	0.24	739	0.11	-1.53	-1.32	-3.09	1.78	0.0765
rorcea2486g-OTH	NaN	NaN	-0.66	2.23	0.11	0.59	102	0.04	NaN	NaN	NaN	0.36	0.7184
rorcea3075g	0.06	0.15	0.13	0.38	-0.62	1.46	1722	0.92	0.34	1.20	0.69	0.51	0.5730
rorcea3075g-ANG	-0.07	0.21	0.51	0.53	-1.48	1.69	833	0.93	0.71	1.81	1.81	0.99	0.3229
rorcea3075g-SHO	0.09	0.23	-0.28	0.56	-0.12	2.01	748	0.91	0.10	-2.59	-0.11	0.62	0.5336
rorcea3075g-OTH	0.01	0.57	0.19	2.18	-3.18	NaN	106	0.94	1.59	1.11	3.15	0.10	0.9247
rorcea3543g	-0.46	1.55	0.21	0.38	0.05	0.15	1715	0.08	-0.26	-1.63	-0.61	0.45	0.6580
rorcea3543g-ANG	-1.48	1.69	0.76	0.53	-0.08	0.21	827	0.07	-0.70	-2.20	-2.03	1.44	0.1534
rorcea3543g-SHO	-0.12	2.01	-0.30	0.56	0.07	0.23	747	0.09	-0.10	2.85	0.13	0.63	0.5245
rorcea3543g-OTH	NaN	NaN	-0.09	2.01	0.06	0.57	105	0.06	NaN	NaN	NaN	0.09	0.9321
rorceg3950t	0.69	0.36	0.10	0.24	-0.11	0.19	1689	0.32	0.40	-0.46	0.47	1.96	0.0550
rorceg3950t-ANG	***0.78***	***0.40***	***0.14***	***0.30***	***-0.77***	***0.34***	***820***	***0.46***	***0.78***	***0.17***	***0.77***	***2.97***	***0.0026***

rorceg3950t-SHO	-0.33	1.42	-0.18	0.47	0.12	0.24	729	0.14	-0.23	0.32	-0.17	0.59	0.5638
rorceg3950t-OTH	-0.28	0.91	-0.08	0.87	0.26	0.88	105	0.38	-0.27	0.27	-0.25	0.33	0.7386
rorcgha693g	**-0.14**	**0.18**	**0.31**	**0.25**	**0.82**	**0.45**	**1710**	**0.76**	**-0.48**	**0.07**	**-0.50**	**1.99**	**0.0470**
rorcgha693g-ANG	***-0.64***	***0.31***	***0.28***	***0.29***	***0.77***	***0.49***	***828***	***0.61***	***-0.71***	***-0.31***	***-0.66***	***2.46***	***0.0150***
rorcgha693g-SHO	0.05	0.22	0.19	0.73	1.35	1.42	743	0.95	-0.65	0.79	-1.11	0.39	0.6982
rorcgha693g-OTH	-0.03	0.89	-0.01	0.84	-0.30	1.25	103	0.68	0.13	1.15	0.19	0.14	0.8830
rorc3a478g	-0.07	0.15	0.21	0.38	-0.47	1.35	1589	0.91	0.20	2.48	0.59	0.73	0.4820
rorc3a478g-ANG	***-0.22***	***0.21***	***0.94***	***0.53***	***-1.25***	***1.53***	***809***	***0.92***	***0.51***	***3.25***	***1.92***	***2.04***	***0.0382***
rorc3a478g-SHO	0.12	0.24	-0.37	0.57	-0.13	2.39	676	0.90	0.12	-2.94	-0.17	0.83	0.3961
rorc3a478g-OTH	0.02	0.59	-0.84	1.92	1.00	4.17	102	0.92	-0.49	2.77	-1.61	0.51	0.2517
lrrn6da1004g	**-1.00**	**0.46**	**0.22**	**0.22**	**0.11**	**0.19**	**1684**	**0.28**	**-0.56**	**-1.19**	**-0.84**	**2.32**	**0.0190**
lrrn6da1004g-ANG	-1.02	0.62	0.08	0.30	0.17	0.29	813	0.32	-0.59	-0.85	-0.77	1.77	0.0757
lrrn6da1004g-SHO	-1.21	0.83	0.13	0.34	0.07	0.29	732	0.24	-0.64	-1.09	-1.00	1.45	0.1513
lrrn6da1004g-OTH	-1.32	1.29	1.44	1.12	-0.67	0.69	103	0.27	-0.32	-7.53	-1.45	1.70	0.0916
Chromosome 5.													
Marbling													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**α**	**tmax**	**PermP**
rargbf	**0.10**	**0.05**	**-0.02**	**0.04**	**-0.01**	**0.02**	**1640**	**0.27**	**0.05**	**-1.21**	**0.08**	**1.95**	**0.0491**
rargbf-ANG	0.17	0.15	-0.12	0.08	0.02	0.03	808	0.08	0.08	-2.69	0.26	1.64	0.0972
rargbf-SHO	***0.09***	***0.06***	***-0.01***	***0.04***	***-0.07***	***0.05***	***732***	***0.50***	***0.08***	***-0.23***	***0.08***	***2.13***	***0.0288***
rargbf-OTH	0.04	0.36	0.16	0.20	-0.07	0.10	100	0.18	0.06	2.90	-0.05	1.12	0.2646
itgb7e6	0.11	0.09	-0.03	0.04	0.01	0.02	1651	0.15	0.05	-1.87	0.11	1.28	0.1920
itgb7e6-ANG	0.14	0.10	-0.01	0.04	-0.01	0.03	808	0.24	0.07	-1.00	0.11	1.31	0.1890
itgb7e6-SHO	***-0.52***	***0.19***	***-0.19***	***0.08***	***0.02***	***0.03***	***747***	***0.05***	***-0.27***	***-0.22***	***-0.33***	***2.15***	***0.0316***
itgb7e6-OTH	0.08	0.35	0.05	0.20	0.02	0.10	96	0.20	0.03	-0.13	0.03	0.18	0.7130
soat2b	-0.00	0.02	0.01	0.04	0.08	0.10	1685	0.85	-0.04	0.54	-0.06	0.78	0.4240
soat2b-ANG	-0.02	0.03	0.03	0.04	0.09	0.11	827	0.76	-0.05	0.04	-0.06	0.99	0.3225
soat2b-SHO	0.01	0.03	-0.10	0.09	-0.59	0.13	754	0.95	0.30	0.64	0.47	1.38	0.1709
soat2b-OTH	-0.04	0.10	0.05	0.19	0.25	0.29	104	0.77	-0.15	0.39	-0.18	0.99	0.2680
wnt10bd	0.01	0.04	0.00	0.03	-0.01	0.03	1427	0.49	0.01	0.12	0.01	0.39	0.6970
wnt10bd-ANG	0.15	0.09	0.00	0.04	0.01	0.04	731	0.31	0.07	-1.13	0.10	1.58	0.1207
wnt10bd-SHO	-0.02	0.04	-0.01	0.05	-0.06	0.13	595	0.74	0.02	1.39	0.03	0.33	0.7470
wnt10bd-OTH	-0.08	0.19	0.11	0.13	-0.12	0.13	101	0.35	0.02	8.82	-0.04	1.25	0.2199

lalba3	0.03	0.05	0.03	0.04	-0.03	0.02	1572	0.24	0.03	1.02	0.02	1.60	0.1080
lalba3-ANG	0.26	0.17	-0.06	0.07	-0.01	0.03	738	0.08	0.13	-1.38	0.29	1.39	0.1667
lalba3-SHO	0.03	0.06	0.04	0.04	-0.08	0.05	736	0.41	0.06	1.08	0.04	1.82	0.0696
lalba3-OTH	***-0.33***	***0.34***	***0.36***	***0.19***	***-0.09***	***0.10***	***98***	***0.18***	***-0.12***	***-4.63***	***-0.48***	***2.23***	***0.0286***
dgka	0.01	0.04	-0.01	0.04	-0.03	0.06	819	0.64	0.02	-0.40	0.02	0.50	0.6310
dgka-ANG	0.02	0.06	0.02	0.05	-0.05	0.07	532	0.56	0.03	0.93	0.04	0.79	0.4168
dgka-SHO	-0.06	0.06	-0.07	0.08	0.36	0.21	233	0.81	-0.21	1.08	-0.34	1.98	0.0554
dgka-OTH	***0.47***	***0.19***	***-0.13***	***0.18***	***-0.29***	***0.31***	***54***	***0.65***	***0.38***	***-0.59***	***0.31***	***2.28***	***0.0232***
gdf11a	0.01	0.02	-0.06	0.04	0.04	0.06	1664	0.79	-0.02	5.74	-0.07	1.58	0.1040
gdf11a-ANG	0.00	0.03	0.00	0.09	0.02	0.11	814	0.90	-0.01	1.27	-0.01	0.12	0.9041
gdf11a-SHO	0.03	0.04	-0.08	0.05	0.05	0.07	746	0.67	-0.01	10.34	-0.05	1.75	0.0801
gdf11a-OTH	0.02	0.11	-0.03	0.13	0.06	0.39	104	0.79	-0.02	3.24	-0.06	0.26	0.7938
gdf11b	-0.24	0.31	-0.00	0.06	0.01	0.02	1612	0.05	-0.13	-0.91	-0.23	0.83	0.4110
gdf11b-ANG	-0.66	NaN	0.04	0.08	0.02	0.03	754	0.04	-0.34	-1.06	-0.67	0.21	0.8316
gdf11b-SHO	-0.31	0.44	-0.08	0.09	0.01	0.03	753	0.05	-0.16	-0.44	-0.22	0.93	0.3371
gdf11b-OTH	0.42	NaN	0.17	0.25	-0.03	0.09	105	0.10	0.23	-0.11	0.25	0.90	0.3712
P8 fat													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
rargBF	0.08	0.37	0.32	0.27	-0.06	0.18	1663	0.26	0.07	4.65	-0.08	1.16	0.2512
rargBF-ANG	-0.58	1.10	0.32	0.56	-0.02	0.21	807	0.08	-0.28	-2.23	-0.80	0.70	0.4895
rargBF-SHO	0.12	0.40	0.35	0.32	-0.56	0.42	720	0.49	0.34	1.67	0.33	1.74	0.0876
rargBF-OTH	0.91	2.92	-0.33	1.06	-0.15	0.70	100	0.18	0.53	-1.35	0.98	0.48	0.6286
itgb7E6	0.15	0.73	0.01	0.30	0.10	0.16	1673	0.15	0.02	-4.91	0.10	0.27	0.7867
itgb7E6-ANG	0.77	0.71	0.25	0.34	-0.23	0.26	807	0.24	0.50	-0.05	0.51	1.19	0.2281
itgb7E6-SHO	***-10.07***	***1.63***	***-1.93***	***0.70***	***0.24***	***0.22***	***735***	***0.05***	***-5.16***	***-0.58***	***-7.86***	***2.93***	***0.0037***
itgb7E6-OTH	-0.56	3.18	1.52	1.17	-0.25	0.69	96	0.20	-0.16	-12.41	-1.32	1.36	0.1790
soat2B	0.17	0.16	-0.07	0.29	-0.43	0.66	1708	0.85	0.30	0.19	0.34	0.77	0.4344
soat2B-ANG	-0.07	0.25	0.05	0.34	0.65	0.65	826	0.76	-0.36	0.68	-0.48	0.82	0.4162
soat2B-SHO	***0.24***	***0.22***	***-1.30***	***0.73***	***-10.36***	***0.99***	***742***	***0.95***	***5.30***	***0.71***	***8.71***	***3.20***	***0.0009***
soat2B-OTH	-0.04	0.70	0.71	1.09	-1.79	1.53	104	0.77	0.88	1.85	1.75	1.08	0.2300
wnt10B	0.11	0.28	-0.04	0.24	0.25	0.27	1451	0.48	-0.07	2.98	-0.07	0.80	0.4207
wnt10B-ANG	-0.33	0.60	-0.10	0.34	0.21	0.30	730	0.31	-0.27	0.13	-0.26	0.79	0.4227
wnt10B-SHO	0.24	0.33	-0.02	0.37	-1.20	1.20	586	0.74	0.72	0.64	0.94	1.36	0.1690
wnt10B-OTH	-1.13	1.15	0.77	1.01	-0.04	0.84	101	0.35	-0.55	-2.48	-0.95	1.16	0.2565

lalba3	0.06	0.47	0.02	0.26	0.06	0.18	1595	0.24	0.00	-81.67	0.02	0.11	0.9104
lalba3-ANG	1.33	2.43	-0.35	0.53	-0.04	0.22	737	0.08	0.68	-1.46	1.52	0.89	0.3628
lalba3-SHO	0.03	0.49	-0.01	0.32	0.06	0.37	724	0.41	-0.01	3.89	-0.00	0.14	0.8929
lalba3-OTH	-1.50	1.55	0.64	1.04	0.07	0.75	98	0.18	-0.79	-1.71	-1.64	0.86	0.3840
dgka	0.09	0.31	0.09	0.31	-0.28	0.48	821	0.64	0.19	0.98	0.24	0.65	0.5144
dgka-ANG	0.16	0.41	-0.11	0.37	-0.22	0.53	532	0.56	0.19	-0.47	0.18	0.58	0.5631
dgka-SHO	0.10	0.48	0.65	0.71	-1.18	1.75	233	0.81	0.64	1.86	1.37	0.92	0.3508
dgka-OTH	0.16	1.70	0.44	0.99	-0.03	1.38	54	0.65	0.09	3.98	0.21	0.19	0.8397
gdf11A	0.07	0.17	-0.14	0.29	0.63	0.43	1686	0.79	-0.28	1.73	-0.56	1.48	0.1358
gdf11A-ANG	-0.01	0.21	0.22	0.70	-0.40	0.70	813	0.90	0.19	2.18	0.53	0.60	0.5369
gdf11A-SHO	***0.01***	***0.31***	***-0.27***	***0.34***	***1.00***	***0.56***	***734***	***0.67***	***-0.49***	***1.57***	***-0.76***	***1.98***	***0.0490***
gdf11A-OTH	0.05	0.74	0.01	0.90	-0.05	1.72	104	0.79	0.05	0.28	0.06	0.05	0.9629
gdf11B	**2.67**	**1.88**	**-1.27**	**0.45**	**0.22**	**0.15**	**1635**	**0.05**	**1.22**	**-2.22**	**3.67**	**3.02**	**0.0026**
gdf11B-ANG	8.81	NaN	-1.21	0.72	0.11	0.22	753	0.04	4.35	-1.30	9.55	1.73	0.0857
gdf11B-SHO	-0.21	0.70	-1.07	0.68	0.19	0.22	741	0.05	-0.20	5.25	0.75	1.73	0.0860
gdf11B-OTH	8.05	NaN	-2.22	1.13	0.41	0.62	105	0.10	3.82	-1.69	9.04	1.81	0.0722
Chromosome 14													
Marbling													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
tg5	**-0.01**	**0.02**	**0.01**	**0.03**	**0.20**	**0.11**	**1669**	**0.81**	**-0.11**	**0.84**	**-0.16**	**1.98**	**0.0410**
tg5-ANG	***-0.01***	***0.03***	***0.01***	***0.04***	***0.27***	***0.16***	***823***	***0.80***	***-0.14***	***0.83***	***-0.21***	***1.98***	***0.0439***
tg5-SHO	-0.00	0.03	-0.01	0.05	0.15	0.17	746	0.82	-0.08	1.13	-0.13	0.99	0.3287
tg5-OTH	-0.07	0.11	0.09	0.14	0.01	0.40	100	0.78	-0.04	-2.68	0.02	0.87	0.3773
tgh435	-0.04	0.08	-0.02	0.03	0.01	0.02	1646	0.21	-0.03	0.29	-0.02	0.85	0.4120
tgh435-ANG	-0.06	0.09	0.01	0.04	0.01	0.04	822	0.27	-0.03	-0.79	-0.05	0.69	0.4917
tgh435-SHO	-0.02	0.18	-0.07	0.05	0.01	0.03	720	0.15	-0.01	4.73	0.03	1.26	0.2037
tgh435-OTH	0.11	0.46	-0.02	0.14	0.00	0.11	104	0.22	0.05	-1.50	0.10	0.30	0.7520
P8fat													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
tg5	0.09	0.17	-0.24	0.25	-0.21	0.66	1658	0.81	0.15	-1.16	0.04	1.10	0.3035
tg5-ANG	0.04	0.25	-0.16	0.34	-0.48	1.01	822	0.80	0.26	0.23	0.30	0.48	0.6351
tg5-SHO	0.11	0.26	-0.14	0.40	0.05	0.99	734	0.82	0.03	-7.50	-0.11	0.55	0.5804
tg5-OTH	0.39	0.73	-1.09	1.00	0.15	1.68	100	0.78	0.12	-11.50	-0.64	1.20	0.2303

tgh435	-0.00	0.61	0.11	0.25	0.08	0.17	1667	0.22	-0.04	-1.74	-0.08	0.17	0.8680
tgh435-ANG	-0.14	0.71	-0.19	0.33	0.12	0.26	821	0.27	-0.13	1.38	-0.05	0.75	0.4555
tgh435-SHO	0.24	1.40	0.24	0.46	0.05	0.25	708	0.15	0.10	0.96	0.03	0.37	0.7113
tgh435-OTH	***-0.71***	***3.53***	***1.55***	***1.00***	***-0.89***	***0.67***	***104***	***0.22***	***0.09***	***26.39***	***-1.25***	***2.11***	***0.0353***
Other chromosomes													
GH1													
marbling													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
gh1:c.457	**0.24**	**0.14**	**0.05**	**0.04**	**-0.03**	**0.03**	**1027**	**0.23**	**0.14**	**-0.39**	**0.16**	**2.04**	**0.0472**
gh1:c.457-ANG	0.19	0.17	-0.00	0.06	-0.04	0.04	534	0.23	0.12	-0.67	0.16	1.53	0.1212
gh1:c.457-SHO	0.36	0.24	0.10	0.06	-0.02	0.05	426	0.24	0.19	-0.37	0.23	1.59	0.1092
gh1:c.457-OTH	NaN	NaN	0.26	0.24	0.01	0.13	67	0.13	NaN	NaN	NaN	0.95	0.3258
P8fat													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
gh1:c.457	***-0.80***	***1.00***	***-0.20***	***0.29***	***0.52***	***0.24***	***1022***	***0.23***	***-0.66***	***0.09***	***-0.63***	***1.94***	***0.0541***
gh1:c.457-ANG	-1.01	1.21	-0.21	0.39	0.40	0.32	534	0.23	-0.70	-0.14	-0.75	1.20	0.2315
gh1:c.457-SHO	-0.34	1.84	-0.23	0.43	0.80	0.39	420	0.24	-0.57	0.81	-0.33	1.79	0.0794
gh1:c.457-OTH	NaN	NaN	1.06	1.61	-0.06	0.81	67	0.13	NaN	NaN	NaN	0.67	0.4902
LEP													
Marbling													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
lepEx2	-0.01	0.03	0.02	0.03	0.01	0.05	1664	0.67	-0.01	-1.69	-0.01	0.78	0.4220
lepEx2-ANG	-0.02	0.05	0.00	0.04	0.04	0.05	821	0.52	-0.03	0.28	-0.03	0.84	0.4030
lepEx2-SHO	-0.01	0.03	0.05	0.06	-0.14	0.14	742	0.82	0.06	1.94	0.14	1.06	0.2916
lepEx2-OTH	-0.01	0.13	0.01	0.12	-0.18	0.29	101	0.67	0.09	1.22	0.12	0.67	0.5039
P8fat													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
lepEx2	**-0.02**	**0.20**	**0.37**	**0.22**	**-0.59**	**0.37**	**1686**	**0.66**	**0.28**	**2.39**	**0.50**	**2.24**	**0.0240**
lepEx2-ANG	***0.24***	***0.37***	***0.42***	***0.28***	***-0.93***	***0.40***	***820***	***0.52***	***0.58***	***1.31***	***0.62***	***2.77***	***0.0051***

lepEx2-SHO	-0.17	0.25	0.22	0.42	0.39	1.22	731	0.82	-0.28	-0.40	-0.21	0.82	0.4263
lepEx2-OTH	-0.13	0.87	-0.69	0.87	2.30	1.17	101	0.67	-1.21	1.46	-1.81	1.61	0.1191
SCDE													
marbling													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
scde	0.01	0.02	-0.04	0.04	-0.08	0.08	1569	0.85	0.05	-0.21	0.04	1.21	0.2280
scde-ANG	0.03	0.04	-0.02	0.04	-0.05	0.09	763	0.74	0.04	-0.37	0.03	0.98	0.3304
scde-SHO	-0.01	0.03	-0.18	0.15	NaN	NaN	706	0.99	NaN	NaN	NaN	1.01	0.3121
scde-OTH	0.09	0.12	-0.13	0.14	-0.65	0.31	100	0.78	0.37	0.40	0.45	1.42	0.1409
P8fat													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
scde	-0.08	0.17	0.47	0.28	0.33	0.67	1587	0.85	-0.20	-1.70	0.04	1.61	0.1020
scde-ANG	-0.34	0.27	0.45	0.32	0.16	0.70	762	0.74	-0.25	-2.19	0.01	1.87	0.0570
scde-SHO	0.04	0.22	-2.41	1.08	NaN	NaN	694	0.99	NaN	NaN	NaN	1.79	0.0706
scde-OTH	-0.32	0.78	1.46	0.74	1.08	1.78	100	0.78	-0.70	-1.54	-0.10	1.56	0.1134
THRSP													
Marbling													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
thrsp-1232	-0.02	0.02	-0.01	0.04	0.21	0.21	1582	0.91	-0.11	0.95	-0.20	1.30	0.2210
thrsp-1232-ANG	-0.01	0.03	-0.03	0.05	0.22	0.22	804	0.85	-0.11	1.17	-0.21	1.38	0.1718
thrsp-1232-SHO	-0.02	0.03	0.19	0.13	0.11	NaN	700	0.97	-0.06	-2.15	0.07	1.72	0.0830
thrsp-1232-OTH	-0.07	0.10	-0.51	0.23	NaN	NaN	78	0.94	NaN	NaN	NaN	1.63	0.1042
P8fat													
Locus	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**N**	**Freq**	**a**	**k**	**alpha**	**tmax**	**PermP**
thrsp-1232	0.18	0.16	-0.16	0.35	0.17	1.30	1602	0.91	0.01	-51.35	-0.27	0.90	0.3620
thrsp-1232-ANG	0.33	0.23	-0.46	0.38	-0.05	1.42	803	0.85	0.19	-3.21	-0.23	1.75	0.0822
thrsp-1232-SHO	-0.00	0.23	0.07	0.97	5.42	NaN	688	0.97	-2.71	0.97	-5.19	0.07	0.9455
thrsp-1232-OTH	-0.06	0.65	0.43	1.49	NaN	NaN	78	0.94	NaN	NaN	NaN	0.27	0.7848

1 SNP name
2 mean of genotype 0, which is the homozygote for allele 0

```
3 Standard Error of the mean of genotype 0
4 Sample size
5 Frequency of the 0 allele
6 half the difference between homozygote means
7 standardised dominance deviation, k = 1 is dominance, k = 2 is overdominance
8 average effect of allele substitution
9 t test between means with the largest significant difference
10 P value derived from 100,000 permutations of the data
11 Angus
12 Shorthorn
13 Other taurine cattle
14 bold for those significant across several breeds
15 bold italic for those significant with a particular breed
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Table 11. Gene expression of positional candidate genes in foetal and adult tissues.

Gene Expression in Adult tissues standardised to the house keeping genes GAPDH and Ribosomal Protein

Adult		GDF11B	ITGB7E6	PIK4CB	AAASE12	ARF3b	IGFBP6CD	PRKAGIJK	PRKAB2CD	SCDE
Kidney	Gapdh		12.579	7.426	9.182	9.530		12.776	14.742	16.114
	RibProt		13.449	8.296	9.479	9.828		13.074	15.040	16.400
Liver	Gapdh		9.903	7.854	9.043	9.517	17.097	10.081	14.051	14.913
	RibProt		9.368	7.318	7.980	8.455	16.273	9.019	12.988	14.765
Brain	Gapdh	15.242	12.029	7.770	8.456	8.316	15.245	11.107	13.568	12.193
	RibProt	12.484	9.272	5.013	6.148	6.008	11.815	8.800	11.260	9.327
Spleen	Gapdh		6.336	6.398	6.409	7.536	13.170	9.174	12.966	13.413
	RibProt		8.425	8.487	7.779	8.906	14.880	10.544	14.337	15.807
LD Fat	Gapdh				3.882				3.090	
	RibProt				4.721				3.929	
SC Fat	Gapdh	16.748	7.821	6.553	5.913	6.819	10.942	7.919	6.469	-1.244
	RibProt	18.652	9.725	8.457	7.823	8.729	13.347	9.829	8.379	0.654
Kidney Fat	Gapdh	16.233	7.915	7.333	6.344	7.762	12.659	8.749	6.211	4.932
	RibProt	17.098	8.780	8.198	6.994	8.412	13.227	9.399	6.861	5.562
Smooth Mus	Gapdh		11.197	5.611	7.086	11.972	12.335	11.044	10.315	
	RibProt		12.358	6.773	6.273	11.159	12.147	10.231	9.502	
Placenta	Gapdh	20.025	13.822	9.179	3.263	5.362		8.497	11.373	7.594
	RibProt	19.022	12.820	8.176	3.739	5.838		8.973	11.849	7.237
ST Mus	Gapdh		16.141	14.181	11.762	17.156	17.574	16.420	13.341	
	RibProt		11.959	9.999	5.759	11.152	12.508	10.416	7.338	
LD Mus	Gapdh	19.495	13.430	12.604	10.315	15.224	18.956	12.698	7.087	9.509
	RibProt	15.684	9.618	8.793	6.319	11.228	15.207	8.702	3.091	5.675
Adult		SOAT2BCD	WNT10BD	ADCY6BF	PFDN5E	PFKMDE	RXRBEFg3	VDRG	Rorc-ex1ex2	Rorc-insu2
Kidney	Gapdh			14.842				14.149	12.294	13.605
	RibProt			15.871				14.435	12.702	14.013
Liver	Gapdh	10.872		17.889					11.979	13.231

	RibProt	10.723		17.065					11.333	12.585
Brain	Gapdh						18.792	18.845	16.841	16.929
	RibProt						15.925	15.978	14.157	14.245
Spleen	Gapdh			17.493				15.001	15.402	14.816
	RibProt			19.203				17.394	17.053	16.467
LD Fat	Gapdh									19.822
	RibProt									15.969
SC Fat	Gapdh	15.940		18.035	18.261		18.924	12.340	13.243	
	RibProt	17.838		20.439	20.665		20.822	14.238	14.320	
Kidney Fat	Gapdh	16.718	18.157	18.711	19.200		19.644		14.415	14.560
	RibProt	17.348	18.517	19.279	19.768		20.274		15.283	15.428
Smooth Mus	Gapdh								17.003	16.136
	RibProt								17.125	16.258
Placenta	Gapdh	16.451	12.913	20.489	19.073			13.646	15.079	14.163
	RibProt	16.094	12.518	20.202	18.786			13.289	14.693	13.776
ST Mus	Gapdh								19.070	
	RibProt								14.477	
LD Mus	Gapdh	19.129				23.407		22.039	11.986	16.550
	RibProt	15.295				19.658		18.205	8.245	12.809

Gene Expression in Foetal tissues standardised to the house keeping genes GAPDH and Ribosomal Protein

Foetal		GDF11B	ITGB7E6	PIK4CB	AAASE12	ARF3b	IGFBP6CD	PRKAGIJK	PRKAB2CD	SCDE
LD Muscle	Gapdh	16.710	9.973	12.119	6.746	10.785	14.545	10.675	5.674	9.522
	RibProt	16.496	9.758	11.904	5.915	9.955	13.714	9.844	4.843	8.691
Kidney	Gapdh	16.372	7.421	10.959	3.986	7.081	15.307	7.961	5.865	7.964
	RibProt	16.546	7.595	11.133	4.572	7.667	15.893	8.546	6.450	8.550
Liver	Gapdh	14.810	5.073	10.448	4.110	8.732	16.717	6.816	4.931	4.815
	RibProt	17.097	7.361	12.735	5.531	10.154	18.138	8.237	6.352	6.236
Brain	Gapdh	14.635	9.898	10.367	5.185	5.449	15.651	8.530	5.762	6.563
	RibProt	14.296	9.559	10.028	4.344	4.608	14.810	7.689	4.921	5.722
Mammary	Gapdh	13.573	7.085	9.521	3.871	7.289	13.416	7.443	5.078	3.413
	RibProt	15.550	9.062	11.498	6.432	9.850	15.977	10.004	7.639	5.974
Testis	Gapdh	16.251	6.806	9.514	3.733	7.146	12.890	7.275	4.971	6.628

	RibProt	17.677	8.231	10.940	5.849	9.262	15.006	9.391	7.087	8.744
Kidney Fat	Gapdh	14.958	8.195	9.549	4.179	7.910	13.833	7.901	5.849	2.242
	RibProt	17.405	10.641	11.996	6.625	10.356	16.279	10.347	8.295	4.688
Skin	Gapdh	13.703	7.587	9.324	3.931	7.290	14.415	7.967	5.486	7.390
	RibProt	15.710	9.595	11.332	6.023	9.382	16.507	10.059	7.577	9.482
Foetal		SOAT2BCD	WNT10BD	ADCY6BF	PFDN5E	PFKMDE	RXRBEFg3	VDRG	Rorc-ex1ex2	Rorc-insu2
LD Muscle	Gapdh	18.551						20.728	12.881	16.587
	RibProt	17.720						19.823	12.331	16.037
Kidney	Gapdh	17.914			18.565			14.343	5.671	8.807
	RibProt	18.499			19.243			15.022	4.711	7.847
Liver	Gapdh	4.950			16.802			16.071	5.766	8.670
	RibProt	6.371			18.097			17.366	7.288	10.192
Brain	Gapdh	19.365			18.643			19.856	14.207	18.105
	RibProt	18.524			18.578			19.791	13.917	17.815
Mammary	Gapdh	16.423	18.388		17.483			15.658	11.369	10.033
	RibProt	18.984	20.949		19.865			18.040	12.964	11.629
Testis	Gapdh	15.880		19.272	17.961			15.936	7.901	11.513
	RibProt	17.996		21.028	19.718			17.693	9.928	13.540
Kidney Fat	Gapdh	17.840	19.709	19.823	17.808			18.447	13.508	13.025
	RibProt	20.286	22.155	21.852	19.837			20.476	15.033	14.550
Skin	Gapdh	18.522	16.809		18.388		18.166	13.358	10.273	13.228
	RibProt	20.613	18.900		20.684		20.462	15.653	12.537	15.492