Linkage mapping of a single nucleotide polymorphism (SNP) in the porcine QDPR gene to chromosome 8\(^1,2\)


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Source/description of primers: Quinoid dihydropteridine reductase (QDPR) catalyzes the NADH-mediated reduction of quinoid dihydrobipterin and is an essential component of the pterin-dependent aromatic amino acid hydroxylation systems\(^3\). A cDNA clone containing the full coding region of the porcine QDPR (GenBank accession no. AF526879) has been isolated from the ‘Meat Animal Research Center (MARC) 2PIG’ expressed sequence tag primary library\(^2\). Primers were designed to amplify across the 3’ untranslated region of the cDNA. The forward (F3) and reverse (R1) primers correspond to bases 744–763 and 1118–1098 of the porcine QDPR cDNA. Agarose gel electrophoresis and sequencing of the polymerase chain reaction (PCR) amplicons of porcine genomic DNA indicated that the size of this product was 375 bp as expected.

PCR Primer sequences and a flanking sequence for a single nucleotide polymorphism: QDPR F3: CCAACGCAGGAAAGACAGAG
QDPR R1: CATCCCCAGCAATCTCTGACC
Sequence flanking polymorphism: ATGTGTCGCC(G/A)ATGGGGCCG

PCR conditions: Polymerase chain reactions were carried out in a 25-μl volume containing 100 ng genomic DNA, 1.5 mM MgCl\(_2\), 20 pmol of each primer, 100 μM dNTP, and 0.35 U Taq polymerase. Amplification was performed under the following PCR conditions: 2 min at 94 °C; 45 cycles of 30 s at 94 °C, annealing for 1 min at 61 °C, 1 min at 72 °C; and a final extension of 5 min at 72 °C. The amplified genomic DNA of eight parents (seven F1 sows and one white composite boar) from a subset of the MARC swine reference population\(^3\) were bidirectionally sequenced and evaluated for polymorphisms 4.

Polymorphism and chromosomal location: An A/G single nucleotide polymorphism was detected at nucleotide 1075 in AF526879. This polymorphism was heterozygous in five of the seven F1 sows and two boars from the MARC swine reference population\(^1\). An assay was designed to genotype this polymorphism using primer extension with analyte detection on a MALDI-TOF mass spectrometer (Sequenom Inc., San Diego, CA, USA)\(^5\). This marker generated 153 informative meioses in the MARC swine reference population. The QDPR gene was mapped to chromosome 8 position 25.7 cm, which is the same position as marker PEPS\(^6\) on the current MARC swine chromosome 8 linkage map (http://www.marc.usda.gov/) using CRI-MAP. The most significant two-point linkage detected was with PEPS (LOD = 29.50) at 0 recombination. The QDPR gene in human is located on chromosome 4p15.31, which shares homology with swine chromosome 8.

References

Fishing in silico: searching for tilapia genes using sequences of microsatellite DNA markers

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Description: Genetic linkage maps of some of the main cultured fish species were constructed in recent years. Maps of tilapia\(^1^,\(^2\), trout\(^3\) and catfish\(^4\) consist of hundreds of microsatellites and amplification fragment length polymorphism DNA markers, but only several genes.

Microsatellites DNA markers are short tandem repeats, usually dinucleotide (CA)n, with unique flanking sequences, providing primer binding sites for PCR amplification\(^5\). Microsatellites are highly abundant throughout the genome and appear in coding and non-coding regions. Therefore, it is likely that the flanking sequences can be part of a gene, which can be identified by similarity searches against the GenBank sequence database. This in silico data mining approach has already been used to identify genes in mice\(^7\), cattle, pigs and chickens\(^8\). In this study we utilized a similar approach to add nine anchored genes to the current 14 genes in the tilapia\(^1\) map, and suggest seven additional genes that can be anchored by mapping to their matching microsatellites.

Methods: Microsatellites sequences were downloaded in FASTA format from the GenBank database using the Entrez nucleotide query webpage (http://www.ncbi.nlm.nih.gov/Entrez). The search string used to retrieve the tilapia microsatellites was

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