

NC, USA) was used. Therefore, these polymorphisms in *CRYGC* can be excluded as causative mutations for CAT in Entlebucher mountain dogs.

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Supplementary Material

The following supplementary material is available online at: <http://www.blackwell-synergy.com>:

Table S1 PCR primers for the amplification of canine *CRYGC* exons.

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Annotation of the Affymetrix¹ porcine genome microarray

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Overview: The Affymetrix porcine genome microarray (<http://www.affymetrix.com/products/arrays/specific/porcine.affx>) is minimally annotated. Less than 10% of the probe sets on this array are described with gene names, posing a challenge to biological interpretation of data. Lack of annotation is likely due to the limited availability of full-length porcine cDNA sequence. Presented here is a strategy for improving the annotation of this microarray.

Sequence sources: Sequences were obtained from Ensembl BioMART (human cDNA and genomic sequence), The Institute for

Genomic Research (TIGR) *Sus scrofa* Gene Index and Affymetrix Porcine Target Sequences FASTA.

Annotation: Each probe set was annotated via the following method. The Affymetrix Porcine target sequence was retrieved and extended, if possible, with the TIGR assembly.¹ These extended sequences were compared by BLAST against the Ensembl human cDNA sequence library. If the BLAST bit score was <50, extended sequences were further compared by BLAST against the Ensembl human genomic sequence library. These results were summarized into a final annotation.

Using this method, we putatively identified 19 675 of 24 123 transcripts on the Affymetrix Porcine microarray, representing 11 265 unique genes. Bit scores and sequence sources are included in Appendix S1 as a measure of annotation uncertainty, which has been recommended in previous reports² and allows investigators to establish a confidence threshold of their choice.

Concordance: We observed >96.9% concordance when comparing annotations based on original and extended target sequences in cases where we believed unextended annotations to be reliable. This level of concordance increased when the bit score threshold for unextended sequences was raised, suggesting that our strategy improved the sensitivity of identifying homologous human genes.

Probe set analysis: In order to better understand the cause of discordance in cases where comparisons by BLAST using original and extended sequences yielded different results, we manually examined 15 discordant probe sets. Detailed analysis of these sets indicated that unextended target sequences failed to match a homologous human transcript when they were derived from the porcine transcript 3' untranslated region. Affymetrix gene expression arrays are 3'-biased by design. From this, we suggest that in most cases correct annotation will be obtained more reliably from extended sequences.

Availability: This annotation is available for download as Table S1 and at <http://www4.ncsu.edu/~stai2/annotation>.

Comment: We have developed an improved annotation for the Affymetrix porcine microarray that describes approximately 82% of the probe sets. This annotation will greatly increase the usefulness of Affymetrix porcine arrays for gene expression studies.

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Supplementary Material

The following supplementary material is available online at <http://www.blackwell-synergy.com>:

¹Mention of trade names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the same by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Appendix S1 This annotation presents putative human orthologues to porcine sequences incorporated in the Affymetrix Porcine genome array. The basis and bit score of each entry is provided to allow the choice of an annotation confidence threshold appropriate to the intended application.

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Sequence variation and evaluation of *inhibin α -subunit* and *steroidogenic acute regulatory protein* for reproductive traits in swine

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Source/description: Ovulation rate is an important trait influencing litter size in swine and contributes to reproductive success in pig production. Several quantitative trait loci (QTL) for ovulation rate have been identified in the pig. One region on chromosome 15 was associated with ovulation rate in a Meishan cross and in lines selected for components of litter size.^{1–2} The porcine *inhibin α -subunit* (*INHA*) and *steroidogenic acute regulatory protein* (*STAR*) genes were previously mapped to this region by polymerase chain reaction-restriction fragment length polymorphism.^{3–4} Inhibin α -subunit is a gonadal glycoprotein that binds to the inhibin β A and β B subunits to form heterodi-

mer complexes known as inhibin A or inhibin B respectively; it also regulates the secretion of pituitary follicle stimulating hormone (FSH).⁵ Plasma inhibin is correlated with higher ovulation rates in pigs.⁶ The transportation of cholesterol from the outer to the inner mitochondrial membrane is the rate-limiting step for steroidogenesis,⁷ is conducted by *STAR* in testis, ovary and adrenal⁸ and is regulated by FSH and insulin-like growth factor 1 (IGF1).⁹ Therefore, we considered *INHA* and *STAR* as positional candidates for this QTL and evaluated their sequence for potential causative genetic variation.

Polymorphism detection: Primers were designed in exons to amplify fragments of the entire *INHA* and *STAR* genes based on porcine cDNA sequences (X03265 and U53020⁸ respectively) and sequences generated from the current study (Table S1). The gene organization of pig *INHA* was similar to human and contained an intron of 966 bp (DQ356013). Six SNPs were identified in the gene (Fig. 1a). The complete *STAR* gene was amplified using genomic DNA from the USMARC reference population¹⁰ and consists of seven exons and six introns spanning approximately 9 kb (Fig. 1b). The genomic organization of *STAR* was similar to human except for intron 5, which was much larger in the pig than human (1500 vs. 644 bp respectively). A total of 21 SNPs in the *STAR* gene were identified in the eight parents of the MARC swine mapping family. Two contiguous SNPs (G>C and G>T) in exon 4 of *STAR* (bases 498 and 499 of U53020 respectively) were found, where the G>T polymorphism changed amino acid 126 from valine to leucine (V126L). Valine at this position is conserved in human, cattle, sheep, horse and mouse, while rat has leucine at this position.

SNP genotyping and linkage mapping: Assays were designed to genotype the *INHA* polymorphisms at nucleotides X03265:c.572G>A and X03265:c.1172G>A, as well as at the *STAR* polymorphism U53020:c.499G>T, using primer exten-

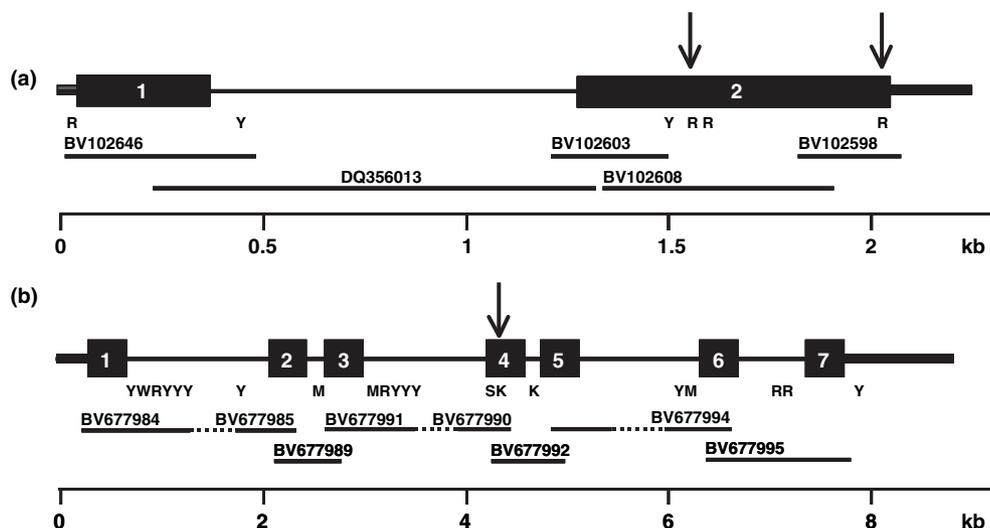


Figure 1 Genomic organization of the porcine *inhibin α -subunit* (*INHA*) (a) and *steroidogenic acute regulatory protein* (*STAR*) (b) genes. Exons are numbered as shown in boxes, and single nucleotide polymorphisms are identified in IUB code below the diagram. Arrows show the positions of the G>A polymorphisms in *INHA* (bases 572 and 1172 of X03265) and the G>T polymorphism (base 499 of U53020) in *STAR*, which were genotyped. The sequenced fragments are shown with GenBank accession numbers, and dotted lines indicate regions where sequences were not determined. Approximate sizes (in Kb) are shown below.