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Identification and characterization of an AFLP marker associated with carcass composition in the pig (Brief report)

(Identifizierung und Charakterisierung eines AFLP-Markers für Schlachtkörpermerkmale beim Schwein)

Background: Amplified Fragment Length Polymorphism (AFLP) is a DNA fingerprinting technique that allows genotyping of DNA polymorphisms, mainly single nucleotide substitutions (SNP), across the whole genome with high multiplex ratio and without the need of their prior knowledge. We have successfully combined AFLP with selective genotyping for mapping of quantitative trait loci (QTL) in a F_2 resource population based on Duroc and Berlin Miniature pig (DUMI) (WIMMERS et al., 2002). Here we report application of this approach for the identification of loci associated with carcass composition in a herd of commercial German Landrace pigs.

Procedures:

Primer sequences:

Vec15: 5´-AGG CAC ATG ACA ACG CTG AG-3´

Aspec2: 5´-AGT TGT GGG GTT TGA CCT GTG-3´

Animals and AFLP analysis:

Samples and phenotype records (n=239) for AFLP and association analysis were obtained from castrates of the German Landrace (LR) kept at the performance test station Frankenforst, University of Bonn. Selection of animals for AFLP selective genotyping was based on loin muscle area (LMA). The population mean LMA was 42.5 ± 4.5 cm^2, whereas animals in the low group (n=20) had a mean LMA of 35.6 cm^2 and animals in the high group (n=20) had a mean LMA of 49.7 cm^2. Other traits included in the association analysis were carcass lean content estimated using "Bonner formula" (LEANP), lean content estimated using Fat-O-Meater (FOM) and loin fat depth (BFLO). The AFLP analysis was performed using TaqI- and EcoRI-adapters and primers with one and three selective nucleotides for pre- and selective amplification, respectively, essentially as described before (WIMMERS et al., 2002). Conversion of AFLP to sequence tagged site (STS) markers was done by vectorette PCR (Sigma, Taufkirchen, Germany).

Genotyping, mapping and association analysis:

For genotyping of the marker *STS-Bo*2 a PCR (T_A=60 °C) was performed using the primers Vec15 and Aspec2 and restriction digestion using 6U of *TthHB8I* (Amersham PharmaciaBiotech, Braunschweig, Germany) at 65 °C overnight. Physical mapping of *STS-Bo2* was achieved by screening of the IMpRH panel using the same primers in a stepdown PCR (T_A=72-60 °C) and by analysis of the results using multipoint analysis option of the IMpRH mapping tool (http://www.toulouse.inra.fr). For linkage mapping 17 informative families (n=287) of the DUMI F_2 resource population were genotyped. Multipoint linkage map was established using the BUILD option of the CRIMAP 2.4

package. Distribution of the AFLP markers was analyzed by Fisher exact test in a 2 × 2 contingency table (Proc FREQ, SAS V6.12) and the association of *STS-Bo2* with carcass traits in the whole LR herd was analyzed using a mixed linear model (Proc MIXED) including genotype as a fixed effect, sire and dam nested within sire as random effects and slaughter weight as a covariate.

Results: Using 8 AFLP primer combinations the distribution (presence/absence) of 62 AFLP markers between the high and low LMA groups was analyzed. Distribution of three markers was significantly different between the high and low group at the level $p<0.05$. The most significant ($p=0.000013$) marker *E-AGT/T-AAC1*, enriched in the high group, was converted to a codominant marker *STS-Bo2* (AccNr.DQ787147). Using vectorette PCR a C>T SNP underlying the *E-AGT/T-AAC1* polymorphism was identified and on its basis a *TthHB8I* PCR-RFLP was designed. The wild type C allele, enriched in the high group (high=0.65 vs. low=0.20), segregated with a frequency of 0.44 in the whole LR herd. On the level of the whole herd the association with LMA could not be confirmed, however a trend corresponding to the selective genotyping was observed with the CC homozygous animals having 1.3 cm^2 larger LMA compared to TT homozygous animals ($p=0.27$). A similar tendency was observed for lean content (CC vs TT: LEANP +0.78%, $p=0.15$; FOM +1.6%, $p=0.06$). The positive trend in lean content associated with the C allele is explained by its significant negative association with BFLO (CC vs TT: -0.17 cm, $p=0.04$). The sequence of *STS-Bo2* shows homology (78% over 332bp) to an intergenic sequence on human chromosome 11 (~34.8Mb). Based on current human-pig comparative maps the predicted location of *STS-Bo2* is on porcine chromosome 2 (SSC2), interval SW1686-SW942. This is in perfect agreement with the assignment of *STS-Bo2* to marker-interval SW1686-SW1564 on SSC2 using IMpRH panel (Vector: 0000000000 0010010101 1000000101 1000000000 0100100000 0100000001 0101000000 0000000001 0000000001 0000000101 0010000100 01011010). This assignment is further supported by the linkage mapping of *STS-Bo2* to the interval SW240-(23)-*STS-Bo2*-(32.1)-SW1564 (in parentheses the map distance in Kosambi cM) in the DUMI population. Intriguingly, a QTL for the diameter of fast twitch muscle fibers, which to a large extent determine LMA, was mapped to this region by WIMMERS et al. (2006). Furthermore, several QTL for carcass composition traits were mapped in divergent pig crosses in this region as well (http://www.animalgenome.org/QTLdb). Our results indicate, that the gene(s) underlying these QTL segregate in LR pigs and may be exploited in marker assisted selection.

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