Two microsatellites on pig chromosome 7

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Source/description: A short insert (250–500 bp) pig genomic DNA library was constructed in plasmid vector pSP72 and screened with a $(CA)_n$ probe as described by Coppieters *et al.*[1] S0013 was developed from a 387 bp clone (DJ2C8) and contains a PRE1-like sequence next to the $(CA)_{19}$ repeat. S0029 was developed from a 487 bp clone (DG1C11) containing a $(CA)_{24}$ repeat. The sequences of DJ2C8 and DG1C11 have been submitted to GenBank (acc. nos U66247 and U66248).

PCR conditions: Amplification was carried out in 10 µl containing 20 ng porcine genomic DNA, 1 µM each primer, 200 µM dNTPs, 2 mM $MgCl_2$ and 0·25 units *Taq* polymerase. Thermocycling was performed by initial denaturation at 94°C (1 min) followed by 30 cycles of 30 s at 94°C, 30 s at 50°C and 30 s at 72°C. PCR products were separated and analysed on an ALF sequencer (Pharmacia).

Primer sequences:
S0013 U: 5'-AAT TCT TTT CAG CCA TGA TT-3'
L: 5'-ATG TTT TAG GAT TGA TTG AG-3'
S0029 U: 5'-TGC CCA AGG AGA TGT TAC TA-3'
L: 5'-AAG GTC ATG GAG GCT GAA AT-3'

Mendelian inheritance: Co-dominant segregation of both markers was observed in the families of the PiGMaP reference populations.

Chromosomal location: Both markers were mapped to chromosome 7 by the PiGMaP consortium. Marker S0013 maps close to *TAP1* and S0029 maps between S0047 and S0115 at position 116 (Kosambi map distance).[2]

Polymorphism: Eleven different alleles for S0013 were detected in the PiGMaP families: 0, 123, 125, 131, 137, 139, 141, 143, 145, 147 and 149 bp. The polymorphism of S0029 was studied in more detail by Van Zeveren *et al.*[3] Ten alleles: 147, 151, 153, 155, 157, 159, 173, 175, 177 and 179 bp, were detected in four different breeds: Belgian Landrace ($n = 200$), Belgian Negative ($n = 213$), Large White ($n = 122$), and Piétrain ($n = 215$).

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A polymorphic porcine dinucleotide repeat *S0532* (BHT487) at chromosome 13q48

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Chromosomal location: The cosmid clone BHT487 was localized to 13q48 by fluorescence *in situ* hybridization (FISH) as previously described.[1] Biotinylated cosmid DNA (100–200 ng) was used to probe R-banded male porcine metaphase spreads.

Characterization of the CA-repeat: The cosmid DNA was digested with *Sau*3A and subcloned into pUC19. Screening of the subclones with a Poly(CA) probe revealed a positive subclone, which was isolated and sequenced (EMBL acc. no. X99434). Primers were designed on the basis of the repeats flanking sequences. The expected PCR product length was 160 bp.

Primer sequences:
Primer 1: 5'- TGG AAA GAA CTG GGT ACC CTC TG -3'
Primer 2: 5'- GAC CAC TAA ATG CAG CGA TTG TT -3'

PCR conditions: PCR was carried out on 100 ng genomic DNA in 25 µl of 50 mM KCl, 10 mM Tris (pH 8·4), 200 µM of each dNTP, 1·0 mM $MgCl_2$, 0·2 units *Taq* polymerase and 0·4 µM each primer with one of the primers end-labelled with γ-^{32}P. Thermocycling was performed using 35 cycles of 1 min at 94°C, 1 min at 52°C and 1 min at 72°C. The samples were run on a 6% denaturing polyacrylamide sequencing gel and visualized by exposure of the gel to radiographic film.

Polymorphism: Five different alleles were observed in the PiGMaP shared reference pedigrees.[2]

Mendelian inheritance: Segregation was observed and Mendelian inheritance was confirmed for the locus in the PiGMaP shared reference pedigrees.

Linkage analysis: The microsatellite locus *S0532* was genotyped in the PiGMaP shared reference pedigrees and tested for linkage to all other markers on the PiGMaP consortium linkage map.[2] *S0532* showed linkage to loci on the distal part of chromosome 13, which was in agreement with the FISH assignment.

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A polymorphic porcine dinucleotide repeat *S0531* (BHT10) at chromosome 1p22

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Chromosomal location: The cosmid clone BHT10 was localized to 1p22 by fluorescence *in situ* hybridization (FISH) as previously described.[1] Biotinylated cosmid DNA (100–200 ng) was used to probe R-banded male porcine metaphase spreads.

Characterization of the CA-repeat: The cosmid DNA was digested with *Sau*3A and subcloned into pUC19. Screening of the subclones with a Poly(CA) probe revealed a positive subclone, which was isolated and sequenced (EMBL acc. no. X99433). Primers were designed on the basis of the repeats flanking sequences. The expected PCR product length was 208 bp.

Primer sequences:
Primer 1: 5'- GAT CTT TCA CTA TGT TTT GT -3'
Primer 2: 5'- TGT GGC AAG CTA CAG CGA AA -3'

PCR conditions: PCR was carried out on 100 ng genomic DNA in 25 µl of 50 mM KCl, 10 mM Tris (pH 8·4), 200 µM of each dNTP,