

A Microsatellite Linkage Map of the Porcine Genome

Gary A. Rohrer, Leeson J. Alexander, John W. Keele, Tim P. Smith and Craig W. Beattie

USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center (MARC), Clay Center, Nebraska 68933

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ABSTRACT

We report the most extensive genetic linkage map for a livestock species produced to date. We have linked 376 microsatellite (MS) loci with seven restriction fragment length polymorphic loci in a backcross reference population. The 383 markers were placed into 24 linkage groups which span 1997 cM. Seven additional MS did not fall into a linkage group. Linkage groups are assigned to 13 autosomes and the X chromosome (haploid $n = 19$). This map provides the basis for genetic analysis of quantitative inheritance of phenotypic and physiologic traits in swine.

SPECIES-SPECIFIC, high-density linkage maps comprised of highly polymorphic markers are essential to conduct comprehensive searches for loci that affect phenotype(s) of interest (FRIES 1993). The discovery of microsatellites (MS), abundant, multiallelic, codominant markers uniformly distributed throughout the genome (LITT and LUTY 1989; WEBER and MAY 1989; WINTERO, FREDHOLM and THOMSEN 1992), provided the technology required to rapidly produce linkage maps useful in identifying segregating loci of interest (LUONGO *et al.* 1993). Since MS are typed by amplifying DNA via the polymerase chain reaction (PCR) and then electrophoresed to separate fragments based on length, the procedure is easily automated (DIETRICH *et al.* 1992). Microsatellites, as sequence tagged sites (STS), are easily distributed anywhere in the world by publishing or submitting sequences of primers to public access databases further facilitating map construction.

One focus of genetic efforts to maintain dietary meat as a major protein source centers on identifying markers segregating with rapid lean growth, improved reproductive performance and disease resistance using a marker-assisted selection strategy. Unfortunately, current maps of major livestock species are cytogenetic in nature with few MS assignments (FRIES, EGGEN and WOMACK 1993). This has limited identification of loci associated with phenotypic or quantitative traits (GEORGES *et al.* 1993a, 1993b). Comparative genome mapping (WOMACK 1987; FRIES 1993) has assigned genes (type I markers) selected from human:mouse maps (O'BRIEN *et al.* 1993) using somatic cell hybrid panels (WOMACK and MOLL 1986) or *in situ* hybridization (CHOWDHARY *et al.* 1989). Linkage groups anchored by restriction fragment length polymorphisms (RFLPs) within type I markers are few (FRIES, EGGEN and WOMACK 1993) as they are often uninformative or only slightly polymorphic

within or between livestock breeds (FRIES 1993). In cattle, only 27% of the mapped type I loci have reported polymorphisms compared with 87% of anonymous type II markers (FRIES, EGGEN and WOMACK 1993). FRIES, EGGEN and WOMACK (1993) tabulated ~350 loci organized into 32 linkage groups that span 13 chromosomes and 26 syntenic groups in cattle (haploid $n = 30$). Type I markers have now been assigned to 20 of 26 sheep autosomes (haploid $n = 27$) (ANSARI, PEARCE and MAHER 1993).

An accurate assessment of total cM covered in the swine genome is difficult when only ~120 markers have been placed in 25 linkage groups (12 chromosomally assigned) (ANDERSSON *et al.* 1993). Only 38 of 73 MS loci published to date are linked (ANDERSSON *et al.* 1993). The most extensive individual reports are by FREDHOLM *et al.* (1993), who linked 14 markers into six linkage groups (67 total cM) and ELLEGREN *et al.* (1993), who placed 59 (total) markers in 13 linkage groups covering ~288 cM. The problem is compounded by a lack of markers on 5 of 18 autosomes (ANDERSSON *et al.* 1993).

In spite of the paucity of markers, swine represent a livestock species of choice for mapping quantitative trait loci (QTLs). Global production of pork as a dietary alternative to beef is at an all-time high (FOWLER 1992). The amount of muscle relative to fat is a heritable trait (WARWICK and LEGATES 1979). For mapping purposes, generation interval is relatively short and progeny number high. As omnivores, with a cardiovascular and gastrointestinal physiology similar to humans, swine also make excellent models for human disease (HODSON 1985). Genetic lines for such diverse human diseases as obesity (MERSMANN, POND and YEN 1982) and cancer (TISSOT, BEATTIE and AMOSS 1987) are readily available for mapping purposes.

Our results based on 383 informative DNA markers

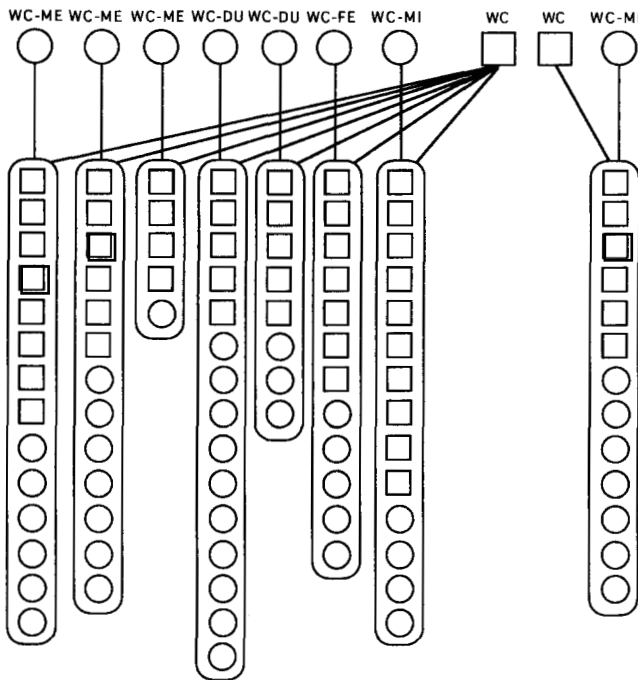


FIGURE 1.—Backcross family of two WC (1/4 Chester White, 1/4 Large White, 1/4 Landrace and 1/4 Yorkshire) boars mated to eight F₁ sows. DU, Duroc; FE, Fengjing; ME, Meishan; MI, Minzhu.

assigned to 13 autosomal and the X chromosome and 9 unassigned linkage groups spanning 1997 cM represents the first linkage map in swine sufficient to initiate a genetic analysis for any heritable trait. It represents our first step to create a high-density linkage map and initiate a systematic search for loci affecting phenotypes of interest (FRIES 1993).

MATERIALS AND METHODS

Generation of GT:CA dinucleotide microsatellites: Procedures were performed essentially as described in SAMBROOK, FRITSCH and MANIATIS (1989). Porcine genomic DNA (20 µg) was digested with *Mbo*I restriction enzyme, the products were fractionated on a 1% agarose gel and the gel section corresponding to 200- to 500-bp excised. Size fractionated DNA (80 ng) was ligated into 500 ng of *Bam*HI-digested, dephosphorylated M13 mp18 RF DNA in a 100 µl reaction at 4° overnight. The ligation mixture was transformed into competent *Escherichia coli* (XL1-Blue, Stratagene, La Jolla, Calif.) cells and the resulting library (100,000 plaques approx.) plated at 2,000 plaques per 150-mm agar plate. Plaques were transferred onto nylon membranes and sequences were screened with 5'-[³²P]-labeled (GT)₁₁ and (CA)₁₁ oligonucleotides (T4 polynucleotide kinase; [³²P]-ATP 5,000 Ci/mmol). Filters were then washed with 2×SSC (0.3 M NaCl, 0.03 M Na₃ citrate), 0.1% SDS at 65° for 30 min, positive plaques purified and rescreened with the labeled (GT)₁₁ and (CA)₁₁ oligonucleotides. Positive phage were grown, single stranded DNA (ssDNA) extracted and sequenced (SANGER, NICKLEN and COULSON 1977) using Sequenase (USB, Cleveland, Ohio). The program PRIMER (Version 0.5; M. J. DALY, S. E. LINCOLN and E. S. LANDER, unpublished data) was used to design primer pairs for PCR based genotyping. Although where possible primers were only made from unique sequences of these clones, 14% of

MS used in this study contained a short porcine repetitive element (SINGER, PARENT and EHRlich 1987) adjacent to the dinucleotide repeat. Primer pairs with one oligonucleotide designed from nonrepetitive sequence and the other oligonucleotide possessing a high level of similarity to the repetitive element are denoted as *Swr* and loci not associated with the repetitive element are designated *Sw*. Approximately 200–300 primer pairs were obtained from each ligation reaction. Oligonucleotide pairs for 14 loci were identified by scanning porcine sequences in GENBANK and EMBL databases (GCG Corp., Madison, WI). Only those MS containing at least eight simple sequence repeats were selected.

Data collection and analysis: The genetic linkage map was constructed by genotyping 104 animals from two generations of a divergent, intraspecific backcross between the commercial meat producing White Composite swine (1/4 Chester White, 1/4 Large White, 1/4 Landrace and 1/4 Yorkshire:WC) and Duroc (DU; a North American breed) or the phenotypically different Chinese breeds: Fengjing (FE), Meishan (ME) and Minzhu (MI) (Figure 1). Microsatellites were genotyped by adapting previously reported procedures (JOHANSSON, ELLEGREN and ANDERSSON 1992) to 10-µl reactions. A total of 12.5 ng of genomic DNA, 5 pmol of each primer and 0.45 units of Taq DNA polymerase were used in each reaction. Concentration of dNTP was reduced to 30 µM each and a few markers required MgCl₂ concentrations greater than 1.5 mM. Samples were heated to 92° for 2 min, 30 cycles of: 30 sec at 94°, 30 sec at annealing temperature and 30 sec at 72° followed with a 5 min extension at 72°. PCR products were radioisotopically labeled, by either end-labeling a primer or by incorporating ³²P, and electrophoresed between 2 and 5 hr (based on product size) at 40 V/cm on 7% acrylamide gels. When radioisotope was incorporated directly the concentration of dATP was reduced to 15 µM and 0.1 µCi of [³²P]dATP was included into the reaction. Allele size was approximated by comparison to M13 mp18 ssDNA sequencing reactions.

Direct incorporation of ³²P into amplified products increased sub-banding but was more economical to produce than end-labeled PCR products. End-labeled primers were used when sub-banding hindered accurate scoring, *e.g.*, *Swr* markers. This strategy permitted genotyping MS which otherwise could not be scored. Multiplexing two, three and occasionally four sets of primers enhanced data acquisition, improved scoring accuracy and reduced costs.

For those markers in which one parent and some of its offspring had an allele that would not amplify (null allele), the situation was rectified by reducing the specificity of primer annealing or markers that retained a null allele were coded as such (fragment size of 0 in Table 1). Animals whose genotypes were ambiguous (*e.g.*, homozygous 129/129 vs heterozygous 129/0) were not scored.

Traditional RFLP were produced by standard Southern blotting of 10 µg of digested genomic DNA and hybridization (SAMBROOK, FRITSCH and MANIATIS 1989) with a radiolabeled probe. Genetic variability at the major histocompatibility complex was mapped with RFLPs for the class I locus *PD6* (EHRlich *et al.* 1987) and class II loci *DQα* (*DQA*) (HIRSCH *et al.* 1990) and *DRβ* (*DRB*) (PRATT *et al.* 1990) developed in our laboratory (T. P. SMITH and C. W. BEATTIE, unpublished data). Two other loci were investigated with polymorphisms found with *Bgl*II for *kappa-casein* (*CASK*) (LEVINE *et al.* 1992) (R. STONE, unpublished results) and reported for *glucose phosphate isomerase* (*GPI*) (DAVIES *et al.* 1992a). Two RFLP were assayed by digesting PCR-amplified products. *Growth hormone* (*GH*) was amplified as described (KIRKPATRICK 1992a) and analyzed by three re-

TABLE 1

Microsatellite marker names, primer oligonucleotide sequences and PCR conditions

Marker name ^a	Forward primer	Reverse primer	PCR temp. ^b	Frag. Size ^c			Alleles ^d
				Max	Min		
PGHAS	GTCACAGTGGATGGCATTTC	ACATCCCCTAAGGTGCTGGC	65	366	322	12	
ATP2	GCTGCATAGGAGCTGTAGG	TAATGATGGTGGTGGTGTAGTC	62	252	246	4	
DAGK	CTATTTCCCATGAACCCATG	TCCCAGTGGGAAAAAAAAGT	58 ^{3.0}	132	110	5	
IFNG	ATTAGACCCTAGCCTGGGA	GTTGGTCCCTGTTCTCCAATAGG	62	243	0	6	
GRC	GGGCAGCTAAGGTGAGGAG	TGAAGGCCACCACGGGTACAG	55 ^{3.0}	331	270	4	
IGF1	GCTTGGATGCACCATGTTG	CACCTTGAGGGC AAATGATT	58	237	223	7	
OPN	CCAATCCTATTCACGAAAAAGC	CAACCCACTTGCTCCCAC	58	164	142	7	
TNFB	CTGGTCAGCCACCAAGATTT	GGAAATGAGAAGTGTGGAGACC	60 ^{3.0}	213	174	7	
CH13	TTGGCATCCTTGTTGAAAACC	TTCATATGCTGCAGGTGTGGC	62	137	125	7	
S0001	TGGATGGTCTCATTCTCAG	TGATTCCTAGCCTGAGAAGC	50	189	175	6	
S0002	GAAGCCCAAAGAGACAACCTGC	GTTCTTTACCCACTGAGCCA	62	209	189	5	
S0003	GAAGTGTTAAGGAAGCCCTT	AGCCTCAGTTTCTCTACCTA	60	162	131	6	
S0004	GATTATGGACAGGAAGGAT	GTCTTATTTCTGCACAGTC	55	172	164	4	
S0005	TCCTTCCTCCTGGTAACTA	GCACTTCCTGATTCTGGCTA	60	241	203	8	
S0007	TTACTTCTGGATCATGTC	GTCCTCCTCATAATTTCTG	55	197	155	11	
S0008	GAGGCAGTGTGTTCTATTTCA	GGCATGTGTAAAGTGTGGCT	58	191	177	6	
S0009	AAACATACCAAGAAGCCGAG	TAATCTTTGCCATCCCTTGT	62	132	122	5	
S0010	TTAACATGGCTGTCTGCACC	GTCCTGTCCAACCTAAGA	60	124	102	9	
S0061	AAGCAGAAGGGATCTCTCTA	GCTGTTTTCTGGGTTCTCTTA	55	187	167	6	
S0062	AAGATCATTTAGTCAAGGTACAG	TCTGATGGGAACATAGGATAAAT	55	196	146	7	
S0063	ACCCCTAGCCTGAAAATTC	GGCAGTGGCAGGAGTTTATC	66	221	186	9	
S0064	TGAGCTGGAGTTAGCTACC	TGTCAGAAAGACTGCTTGGC	58	160	93	10	
S0066	ACATTTAAGGTGAAGCAGCAAGTG	TGTCATCAACATTGAGAATTGGTG	62	158	136	4	
S0067	GGGAGCCACAACAAAGAAGG	GGCCTGGAGTGTGGGACTAG	65	113	0	7	
S0068	AGTGGTCTCTCCCTCTTGCT	GCTTCAACCTTTGAGCAAGAAC	62	260	211	10	
S0069	TGCAAACTAATGTTTTGTTGGC	CATATGCCACAGGTGTGACCTAAA	62	171	0	9	
S0070	GGCGAGCATTTTATTCACAG	GAGCAAAACAGCATCGTGAGC	62	293	0	8	
S0073	ACTGAAACAGGAATTCAGATCC	TGAAGTATTATGCCATCATGGA	55	123	105	9	
S0071	GACATGGAATCAGGTTGCTCAA	CCAGAAGCAGGTTTGAGATGA	65	200	168	7	
S0081	AACAGAATACAAAGCATAGTATAC	CCTCTTACTCTTAATTTCTTGAC	60	184	172	5	
S0082	CAGAAAAATAACTTGTCTAACTTG	AACCCGTTTTCATATCATTAAAGCC	58	180	154	6	
S0083	AGCTGCCGTATATGAAAACCTCCA	CTGCAACAGAGATGAGGAAA	58	194	0	8	
S0084	AACTCAGCCACTTGCTGGGCTGTA	TTCCATTTCAAGATGTATTCAAAG	56	120	102	9	
S0086	GCACAGTCTATTGATACTGGCGTC	CTGAGAACTTCCATATGCTCCTGG	62	184	154	5	
S0087	GACAAGCTCCAGGAAGCTTTCCTG	ATTGCCTTGTGATCCCAAGGGGCA	58	201	161	8	
S0088	AGTGCATTTTGAAAGCAGTGCTC	AGTCACCTCTAGGCGTATCAGCT	58	164	148	5	
S0089	CATGTACTTGTAAATAGGTAAGTA	CTGTAGTCTGCTGGTCTCTGAGA	55	164	142	9	
S0090	CCAAGACTGCCTTGTAGGTGAATA	GCTATCAAGTATTGTACCATTAGG	58	253	243	6	
S0091	TCTACTCCAGGAGATAAGCCAGAT	CAGTGACTCCATGCACAGTTATGA	55	168	148	8	
S0092	GGGAAACACTAAATCACACTGCCAT	GGGATCAAGACTTCCACTCCCAT	60	150	130	7	
S0094	AGTTCTCAGGGAGTTCCTCATGC	CGAGCTCGCCTATCTATCAATTCC	62	211	169	7	
S0097	GACCTATCTAATGTCTATATAGT	TTCTCCTTAGAGTTGACAAACTT	58	244	208	8	
S0099	CTGCCAGAGAGGCTTCTCTCAA	CATCCGCCTGGTTCCTCCCTAT	68	177	159	6	
S0100	CCTCTAGGAAGCTGTGTA	AGCCATGACAGGAACGCCAGTAG	55	179	165	5	
Sw2	TGCCAATGGTGTGGCTATAA	CCCTGAAGGCTCAGATGGT	55 ^{2.25}	126	88	9	
Sw5	TTCAAGTTCATCCTTGTGTC	AGTGTCCACAGATGGATGAATG	58	142	0	7	
Sw7	TAACCATGCTTTTCTAGGTGG	CCAGAGCTGAGTAAAAAGGTCA	65	112	89	7	
Sw11	CTTTTTTGTCTAACCCAGTAACC	AACACATGAGCATCGAGGTG	62	102	98	2	
Sw13	TCTTAGCCAGTGCAGGCAC	GATCAATCTCTAAACTGAAGGTG	58	161	145	8	
Sw14	TTCTGCACCAAAGTTATTTTG	AAAAGCAAAAACAAAACAAACC	58	179	153	7	
Sw15	GGTGGCGCCTAAAAGTAC	CTCAAATCTTGCCAAACTATCCC	62	162	150	4	
Sw16	CATACACCCAGATGTGGC	CTGTGGGAGTGTAGCATCTTTG	60	172	119	7	
Sw17	GTTTAAAGCCACTGGTGTATGG	ATCCTGACTTGCTTATGGCTG	58	155	143	4	
Sw24	CTTTGGGTGGAGTGTGTC	ATCCAAAATGCTGCAAGCC	58	112	92	6	
Sw29	AGGGTGGCTAAAAAAGAAAAGG	ATCAAATCCTTACCTCTGCAGC	61	173	131	9	
Sw35	TCAACTTGGAGAGTCTGAGGC	AAGACTGCCACCAATGAG	58	137	129	5	
Sw37	CTTTGTAGACGGTGGTCCT	GAAGCCACCCACAAATCA	60	226	212	4	
Sw38	ACGCTCTGTGCTGGTCCCT	GAGGCTCCTGATAGCAGCC	60	139	128	8	
Sw45	TATGACCTGGTGGTATGTTGG	TGTTTTCTCCCTCAGATTACC	58	194	174	7	
Sw54	TCCACCTTTTCTGCTCC	TCACAGCAACAGAGCTGTGG	63	124	112	3	
Sw57	GGTTCCCTAACCTGGCTGTGC	ATATGCCTTGGGTGCAGC	62	103	97	3	
Sw58	TCCTACCAGAAATCCTACCACA	ATGGGAAGAGAATCTGACAAGG	58	222	204	7	
Sw60	TCCGTATGCTGTGGATGTATC	CATGTGCTGCAAATGGC	58	152	116	8	
Sw61	GAGAGGGATGAGCACTTGG	AGAGATTTCCAGGCTTCTA	62	262	238	8	
Sw63	GAGAAAGGCAACCGCTG	GTTGGTGTGGTGTAGGCC	62 ^{3.0}	160	148	5	
Sw64	AGACCAAGGGCCATGAGAG	TTCCACGTGATGTGGGATAG	58	152	136	6	
Sw65	AAGAAATGTGACACCATCCAGC	GCTGTAGTCTGTGATTTGACCCC	65	285	251	4	
Sw66	AAACCGAGAAAGGTGGGTG	GATCTTGAAGTGCCTCCG	62	127	91	8	
Sw67	GTCCTCATGGAGACTGTTGGG	TCCATGCCATGGACACAG	60	147	125	6	
Sw68	TTGACCCTAGCTGGGAAC	TTTTCTGGGCTTAGTGGC	63	253	249	3	
Sw69	TCAGCTTCGTGAACAAGGG	ACCTCCCTCATCAGCTATTCC	55	166	146	5	
Sw70	CCCGTACAGTCAACCCAC	CTTTTTCTGGGTTGAGCAAC	62	148	120	7	
Sw71	GATCACCTTATCCCAATTC	TAGAAACACCATCATCCATTCA	62	111	93	5	
Sw72	ATCAGAACAGTGGCCGT	TTTAAAAATGGGGTGTTC	58	113	101	5	
Sw77	ATCAGACCCAGGGTTGCC	GAAATCTGCATGGTCTCAGATG	62	146	119	6	
Sw80	TGACAGCAACGTGACCAG	TGGATTGGAATAAGCAATGAGG	62	176	160	8	
Sw81	GATCTGGTCTCGACAGGG	GGGGCTCTCAGGAAGGAG	60	142	0	9	
Sw84	TTCACTATTCAGAGCCACTCTG	ATCTGACATTGCTGTGGCTG	58	178	162	7	

TABLE 1

Continued

Marker name ^a	Forward primer	Reverse primer	PCR temp. ^b	Frag. Size ^c		
				Max	Min	Alleles ^d
Sw102	GATCAAGATGTACACAGGCATG	AACCTTCTATCTTCTCATGCCG	58	106	96	4
Sw104	TTTGCCGGACTTATTTCAACC	ATCTGCACCCCATATTTCACTG	60	237	219	4
Sw120	TTTTAAGATGTGGCTGTGTGG	GATCACCTGCTAAGTGAAAGTCA	60	150	134	6
Sw122	TTGTCTTTTATTTTGGCTTTTGG	CAAAAAAGGCAAAAGATTGACA	58	132	110	10
Sw129	TTGTTTAAAGTTTTGGGTTGC	TTGCATGAACTTTTCAACACTG	58	129	107	7
Sw133	GGCCTGAATTACATATGTTCCC	AATGTGGCAACAAAACAAAAG	60	150	142	5
Swr136	TTCTTGCCGTCACTCATG	CTGGGACCCTCCATATGATG	58	227	191	8
Sw137	CAGCAAAGTGACCCAGCC	TCCTGCTGTGAAGCACAGAG	60	135	0	6
Sw139	CGACACCCTTGGGTTTTG	ATCTAAAAATGGGCCTTTGGG	60	128	120	5
Sw142	AGTTTGCAGCATTCGATTCC	ATTTGTTGCCATTCATGATCC	58	131	115	5
Sw147	TTGCCCTTCTCCATGTGACT	ACAACCTAACCATTTGTACAGG	58	220	208	6
Sw149	TCATGTTTACAGAACACCTTCC	AGTTACGGTGGGTCGTAATCC	58	138	108	4
Sw151	TTCCCTATGATGAGATGGC	GGTGTGGCCCTCAAAAAGG	60	207	195	4
Sw152	GGATTTTAGGGCTGAATCTGC	GATGACCTTGCAATGCC	62	182	166	5
Sw154	CAGAGGGCAGCAGAAATAC	GATCCATGAACCTGCATGTG	60	148	0	6
Sw157	GCAATGTCCGATTCTTTTATTT	ATTGCTGTGGCTGTTGTTGT	58	168	159	4
Swr158	TCCAATTCAACTCCCTGGCTC	GAATGTGCACATACCCATGCG	58	200	158	8
Sw159	GATTGGGAATTTGGGGTTG	GCATTGTTGTTTTTCATTTCGCG	58	159	131	5
Sw160	TCTTCTTGTCTACATGACCC	ACTAGACAGCCAGGCTGGG	58	132	128	3
Sw163	GCTATCCCTGAGAAACAGTGT	GATCCATAATGGGCTCAGCC	60	184	166	7
Sw168	GCACCTTTCTTCCCTTACCCC	CAGTGTAAAGCATGGAAAGATGC	62	116	102	4
Sw171	CAGATTGTTTACGCTCTGTGTG	CATCTTTTCCAATGACAACATG	60	125	101	4
Sw173	CTGGGAACCTCCATGTGC	GTCTGGGCCTTTAGCTAGG	58	216	194	6
Sw174	GCCAAAATAGCTATTGGACAGC	TCATGCTATTTGTTCCAGATG	58	131	123	5
Sw175	TCACACCTGTATAAGAGATGCA	TGTTGGGCAAAAATCTGAATT	60	126	102	6
Sw184	CTCCCTGCATATATTTTCATCC	ATCCCTAGCCCTGGAAATGTC	50 ^{3.0}	236	222	5
Sw188	ACAAACATACATCCAGTCCATG	GCTTCCCTACTGAATGCTTATG	58	150	128	5
Sw191	ATGATGGGAACCTCCCTGGCTC	AATTTCAATGTCCACCATGG	58	199	153	5
Sw193	TGCCATCCTTTCTTTCATTACG	TCACTCTGAGGGGTCCTGAC	62	109	101	4
Sw194	TGCCCTGCTTACATTAAGTGGG	CCTCACTTAAGAAGGTTCTCTGC	58	102	98	2
Swr198	TTTCATCAGCAACTTTCAGAAG	GGTCCGGCCCTAAAAAAAAG	60	110	72	6
Swr201	TGGAAACACTCTGGCATAACC	CTCCAACCTCAGCCCTAGC	62	143	123	6
Sw205	CACAGGTCCATCACTCATG	GGGTATCTAATGTACATCACGG	58	156	146	4
Sw206	TCAGTGTGTGAACCTGTGTGG	TGGTATTGGAAATGAATCTTCA	55 ^{3.0}	153	137	6
Sw207	CGCTTCACAAAATAAGTTGGG	GTTGTTACTCCAAAAGGTGC	58	188	170	6
Sw208	AACAAAATATTTAAAAACTCTGTGTG	GCAAGAAATAATCAAACAATCT	60	156	132	6
Sw210	TCATCACCATCATACCAAGATG	AATTTGCCAAGAAGAGAGGCC	60	242	218	8
Sw211	TCATCAAGAAAATTGGCTTGG	TGACCACAAGGAAGAACTGG	60	152	140	5
Sw216	ATCTGGGATAATTTGGACATCC	CCCTAATCCCAGGCTCTTTC	62	140	120	5
Sw225	AGGACCCACCAAGAGTTACC	TGCTGGTAATGGGTATTAGG	55	116	94	7
Sw236	AATGAACACTTTTATGGGGCC	GACATCTCACTGGCTGAAAGC	65	147	123	7
Sw240	AGAAATTAGTGCCTCAAAATGG	AAACCAATTAAGTCCCTAGCAAA	58	114	94	8
Sw245	TGGTGTAGCAGAACCTGTG	AAACCTGGCAACACAGCAG	60	130	106	9
Sw248	CCATCCACGTTTTTATAAATGG	CAACCTAAGTGTCCATCAATGG	58	142	114	5
Sw249	GAAAGCAGGACTTGTCTCTG	ATCATCACTCTCCCAACATGG	58	156	0	8
Swr250	CACTCAATGTCTCGAATCAAGC	CTGGGGCTGTGGTGTAGG	65	176	166	6
Sw251	CCCAATATTCATAGCAGCATTTG	TGAGTAATAGCCCATTTTGGG	58	136	130	2
Sw252	CTCTGGGTCATCCATTTTG	CTTGATGCAAAAACATGGAAAGC	62	179	149	7
Sw255	TGTACAGCTCTAGTTTGACCCC	TCTGCTTAGTGGCAAACTT	58	126	122	2
Sw256	ACAAAAGCTTTTGGAGAACTCG	TAGCATAGGAACAGGTGCAGC	62	118	92	7
Sw259	CCTTTCATGCTGTATTTAACCC	CAGAGAACAGAAGTTGGGG	55	180	0	6
Sw262	TACTTGGCTTTTGTGACCAG	TCAGCCAAAAGGGCTCTTG	62	204	200	3
Sw263	TCAGAAAATCTCTTTGCCCA	GATCTTAGCTTCAAAAACAGCA	58	149	133	6
Sw268	CTGATTCACCTTTCATTCGAGAA	AGCCCTTCCCTTAATATAACCC	60	149	121	7
Sw270	TTCCCTTACTGCTCCCCC	GAAAGGAGGGAGAGCTGCTC	60 ^{3.0}	163	139	8
Sw271	TTCCAGTGGCTTTCTGTGC	CATTCAATCCCAGTGAACCTTG	58	134	108	7
Sw274	CGCAGACGGACATCTTTTTTA	AAAGTCAGCCCTAAAAAGACA	60	145	107	5
Sw280	CTAGTTGTATCCATGCTGCTGC	TCCATATGCTGCACACAG	58	231	0	10
Swr283	TGGGACTTAATTGTGATTCTGTG	GATTTGACCCTAGCCTCG	60	158	154	3
Sw286	GGTGACAGAAATACAGTTTCCC	GATCTTCTTATTTGGCCGTGTG	62	94	74	6
Sw287	TTGAAAGTCTACCCATGTGTGG	CATCCCAATGTCACTGC	60	175	160	6
Sw288	AAAATAAAAAGCATGGCCTGC	GGGAAAAACATGTAATTGCC	60	133	103	5
Sw295	ACCTGCCAGATTGTGGC	AAGAGTTTCATTTCTCCCATCC	62	139	109	8
Sw301	AACCAAGCCACTTTCCAC	GCTGAAATGCCATCTGCG	62	156	124	7
Sw304	GATCCCTGACCTGAGAATCC	CACTGCACAGAATTTGTGGC	58	146	130	5
Sw305	AGCTTTCATTTTTTTAAACCCATC	TCACCTTTCACACCCATCACC	58	108	106	2
Sw307	CTTCCACATGTCATGGATGTG	ACCTGCTTTCATGTTTACATGG	58	135	119	4
Swr308	TCCAGTCCCTTGGTCTCTTG	TTAGCTTGGGAACCTTCCATG	65	160	119	8
Sw310	CAGAAGGATGAATATGCAAAATG	GTCTTTCAGGCTTGGAGGG	62	135	109	6
Swr312	ATCCGTGCGTGTGTGCAT	CTGGTGGCTACAGTTCCGAT	60	141	127	7
Sw314	CCTCCTTGAGCCTACCTTTC	CCCTAGCCCTGGAACTTCTG	62	118	102	6
Sw316	TTCTCCAGCCATCATGAGTG	AATGACCATTCCCTGAGGCTG	58	159	133	8
Sw317	GGGATGCTAAAGTTGGAGGG	TTAGTGTCTGGGCAAGGAG	62	168	154	5
Sw322	CATTCAACCTGGAATCTGGG	TCCCTGGAAAGGCTACACC	62	118	102	6
Sw328	GATCCAACCTGCCATTCTATTG	CAGGGTGGCGCTAGAAAG	62	182	0	5
Sw330	GTAAGTCCAGACTGTAATTTGGG	TCTACCATGCTGATAAAAATGGCA	60	116	100	4
Sw332	TTTTCAATATCACATTTCACTCATGC	TTTACAAAGTGGTAGATTAATTA	58	115	109	3
Swr334	CAAGTCAAAGACAGATACTCTGTGTG	TGTGGATAGTTTTCAGATGAGG	58	222	0	9

TABLE 1

Continued

Marker name ^a	Forward primer	Reverse primer	PCR ^b temp.	Frag. Size ^c		Alleles ^d
				Max	Min	
Sw335	GAGTATGGGAAAGCCACG	CCATCAACAAACTGTATGCACC	58	112	100	4
Sw340	CATTGGTGATTGGCATCCC	ATGGGCTGGCAGCTACAG	58	149	0	5
Sw342	GGGTTCTGTGGTAGTGACTGC	TCATCCACAACAGCAGAACC	55	127	91	10
Sw344	AGCTTCGTGTGTGCAGGAG	GTAGTGGTCCAAAGAGAGTGCC	55	174	150	6
Sw345	AACAGCTCCGATTCAACCC	TACTGAGCCTTAAAAGGAAGGG	55	166	140	5
Sw349	CCTGTTGTAGGCTCCATGAG	CTAGGAGTCGGCCCTGAAC	62	177	149	9
Sw352	GCCGCCATTCTCAATTAC	GATCAAGCTCCCTCTTCCG	55	111	107	3
Sw353	CACCCCATGCCTGAATACTG	ATGTGAAGACTCATGCTTGGG	58	164	144	8
Sw354	TGGCTTCTCAGCCTCCAC	GGTTCCTCAAACAAACATAGCC	60	222	206	5
Sw373	TTTGCTGCAAAGCAATGTTC	GGTAGGATTCATAACACCTGG	55	170	152	6
Sw374	AGTAATGCCATCCTCCCCAG	TGCTCTCCAGCCCTCAAG	62	169	141	7
Sw378	ATTATGCACCCTACTCCCC	GATTTCTTCTTTGTTTGTGCC	60	127	123	3
Sw382	CTGAGCCACAGAGAGCAGC	GAGTGTGTGCAAAGGGGG	65	144	132	3
Sw389	TTTTTTAGTGTGCTGTTTTCCG	GTAGGCCAGCAGCCACAG	62	122	120	2
Sw395	TTCCAAGGTTATGGAGATATCC	GATCCCTACCTCACACCACA	55	163	139	9
Sw398	AAGTGCCAAATGCTTTGTTC	CGGAGGAGAAATAAGGCTAGC	55	188	166	6
Sw403	GTGTATGTTTATGCTGGGTG	GTCTCTGCTTTGCTTGATG	55 ^{4,5}	114	102	5
Sw413	CAGACACACCCCAAGTGTG	AGGTCCAACCCCTCATGATG	62	174	162	5
Sw419	AATGGGAAATAGGCTTAAGCC	TCCCTCCCTATACATGTGC	55	174	154	6
Sw423	ACTCAGGTAATGGTAAACTATATGTGTG	TTCTTCCACATTATTCCTTGTGG	60	149	139	5
Sw426	CCTACATATGCCCGAGGTG	GAGAAGTGGGGAAGGGACTC	62	202	184	9
Sw428	TTCCACGTAGATTTTTGACACG	ACCTGCGTCCCTCATGGATAC	52	123	0	7
Sw435	ATCATGTGAGAAAAAGACATATGTG	TGCAAGAGAAGCTCCGGC	60	178	148	6
Sw436	GGACTTCTAGCCTCCAGAAGCTG	AATTTTCAATCACTACCACCGG	58	162	144	5
Sw439	TTCTAGCCTCCAGAAGTGAAG	CTCTCCATCTCTCACCATA	58	179	161	5
Sw443	ACAAAGGCCAAGCCACATAC	TCACCAGGTTTTCTGGGTTTC	60	134	108	7
Sw444	ATAGTTTCGGTTGGCCAG	CTTAAAGCTCAAGGTAAACAGGC	60	120	92	10
Sw445	CCTCCCTGGCACTCATTG	CACACACACAAGCAGGTGC	58	203	181	11
Sw446	GGTTTCCTGTCTGTAATAAGGG	ATCCCTGGCTGGGAAGCTC	60	164	118	10
Sw452	ACAGGAAAAGTAGCTGCC	TGGAACATGATGGAGATAATG	60	97	87	5
Sw453	TTGAAATTTTTTCATGGAAGCC	TCTGGAATGTCTGTACTGTG	51	189	173	4
Sw458	TTATGGTTTCTTGTCTGTG	CCGTTTACTGCAGGTAACC	62	123	117	3
Sw460	ATTGCACACCTATCTCATATGG	AATCTCCATGTGCCGAG	60	199	159	8
Sw467	TATACCTTTAGCCCTAGGAGC	CTCAGCCGTTGGATAACTC	55	130	114	7
Sw468	GCTTTAAAAACCCTCTGCC	TGATTTGACCTTAGCTGG	60	166	120	7
Sw472	AAAATGAACCCTCTCCAGTTTC	TCTGAACACTACAGCCCGC	58	111	95	3
Sw480	TGCCGGAAGGTTCTGC	CTTCTCTCTTGTGGCCCTG	60	144	72	6
Sw482	GGAGAAAAGAAATGATTAGCAA	CCCAGATGCTGGTTTGT	58	267	257	4
Sw485	CCATTTCAATTCATGGAAGG	CTAAGCCGCAACAGGAAGCTC	60	119	97	5
Sw486	GCAAACTTGTGGCCG	TCCATTTGCTAATAAGAGCTGA	50	160	138	6
Sw487	TGAGCACCTCTGCTTGAGTC	ACACCTCTAAAATGGCAGTGTG	58	188	148	9
Sw489	CAAGTGTGAAATTTGTGGG	CGAAGTGCTAACTATAAGCAGCA	55	174	156	6
Sw491	TTTAAAGCCACTGCACAGG	CAGGGAACCTCTCATAGTCCC	60	174	154	3
Sw492	TCCATCAGCTCACATAGTTAGC	ACCATGACAGGAAGCTCCGAG	60	146	118	7
Sw493	ATATTTAACCCATTTGCAGCATG	CTCCAGCTCCACTTAGACCC	58	194	156	8
Sw497	TTAGGAAGCTCTGGGTTTGG	TGGGAGTTCCATGTGTTG	58	116	94	9
Sw510	GTTCCATAAGCTCATCACTCA	ATATTCTGCATTGCAGCCC	60	160	150	6
Sw511	AAGCAGGAATCCCTGCATC	CCCAGCCACAGTCTGC	60	205	165	7
Sw512	TATAGTGCAGTTATATCTCAATACAAATGG	TCTGACATTAATACACCACCCC	58	157	129	7
Sw520	GCCACCGGTGTACTCTAAA	CTTTTCCCAAGTCACTTAGCA	62	124	102	5
Sw527	AGCAAGAGCCAGGACATACC	TAACTGTTGAGCCACAAAAG	62	168	128	5
Sw539	CCCATCCACGCTAAGAAGAG	TCAACGGGAACAAGTGAAG	60	150	148	2
Sw540	TCAGTGGAGGGGGAATAAAC	CCAAATCTGGCAACACAG	58	253	229	8
Sw552	AAGAGCCAGTGGGGAGG	ACTGATAAGACATGTGTGTGC	60	146	112	7
Sw557	TGTCCACTGGTAGATGAATGG	CTTTTGAATGTTCTTTTCCCC	55	250	240	3
Sw575	CTACAGCCGTTGGCTACAG	AGGAATCCATTAGCCTGG	60	157	151	3
Sw581	CCCAGATTGACTCTAGACTCG	CATGATGGAGGATAATGTGGG	57	205	199	3
Sw589	TTCAAATCTCACCACCAGTCC	CTCATCAGCAGCAACCACC	60	150	138	6
Sw590	ATTTGCTGAGAGTAAAGTGCC	GCATTGACCAGGGTCAGG	60	271	184	4
Sw605	AGCCTTCTGTGCAGAAAAGC	CCCAGGTTCTCTGCTCTC	58 ^{4,5}	131	109	5
Sw607	AGCACCTGGCAGGATAAAC	GCAAGAACTGGTTTTCCAGC	58	172	152	3
Sw617	CTGGGTTACAGTGTCTGCC	TGTGATGGAGCCTGATAGAGG	65	157	145	4
Sw619	CACTGGTAAGTATTCAGGGTGG	TATGCTTGTCTGCATCATCG	58	147	129	7
Sw632	TGGGTTGAAAGATTTCCCAA	GGACTCAGTACTTTGGCTTGA	58	173	157	6
Sw698	ATACAAACCATAGCCATGGACC	GATCCGATTTCCAGCCTTAA	58	224	194	5
Sw702	CTGCTGTTTCTGTTTCACTGC	TTGAGCTCCTGTTCAACC	65	160	152	4
Sw703	AAGATGAAGCAGGACATCAAGG	CTTATGGCTTTACTGTTCCACC	58	140	126	7
Sw705	CTGAAGTCTTGAGATGAAACGC	TGTAGAGCATTTCCAGAGGAAGC	62	163	145	5
Sw707	ACGTGCTTTCTTTGAGCTG	AAAAACGCTAAAGAACAAAGCG	58	101	0	5
Sw709	TCTCAAGGTCACACAGCAGG	AAGGACAGTGGTAGGCATG	58	143	127	3
Sw714	ATCTCTTGTTAGAATTTGTGTGTG	GAGATGAATATGGGAAAATGAAC	58	169	145	4
Sw724	TCCTAAAGGACCGAATTTAAAA	TGTGCATTAAATGCCAGGTATG	55	166	144	5
Sw726	CACCACAGAGGGAAGCTCCTC	TCATATGCCACCGGTGTC	58	155	125	7
Sw727	ATCTCTCTGGTTTTGAGATTTAAGG	GGCCTGGTTCCATTTAGGG	58	148	144	3
Sw730	ACCAAGTCCAGGCTAAATGC	GTTACAGGCTGGGAGCAAAAG	60	137	131	4
Sw732	GCAAATGAATGACCAAAAAGG	CATTTTCAATTTGGATTGTTTC	55	180	168	4
Sw741	TGCATTTCTGTGTTTTTTTTGA	GTGCCTGTGGCGTAGACC	62	144	0	6
Sw742	AATTCTACTTCTGGGAGAGGG	CTTTTGGGAACATTTCTGCC	60	224	193	9
Sw745	CTGAGTCTTCTGGGAAGTTTTCT	ACAGGGCTGGTAGTGTCCC	58	211	137	7
Sw747	TGGCCAGGAAGTTTCAG	ATCCCATATGCACCAGGC	58	153	149	3

TABLE 1

Continued

Marker name ^a	Forward primer	Reverse primer	PCR temp. ^b	Frag. Size ^c		Alleles ^d
				Max	Min	
Sw748	CATACATACACCATGCCCATG	TTTGCCACAGAAATGTTTAC	50	193	179	6
Sw749	TTCCCAAACCAACAAAGAG	AGGAACCTGCCAAAATCACG	58	113	107	4
Sw750	CATGGACATTAATAAAGTGCTC	GGAAGCTCCATGTGCTG	58	147	140	5
Sw752	TCAAGAAATAAGGACAGGAACC	CTACCTTCCCATTTGATGCTG	60	124	108	6
Sw761	CTTTGCTCCCCATTAAGCTG	TCTAGCAAATGCTGAGATGCC	60	161	149	6
Sw763	GGGTGCATTGTTCTCATATGG	TGCTTAGCAACACACACCC	62	187	171	4
Sw764	TAGCAGATTGTTTAGCCTCTGTG	AAGCATCTTTTCTAAGCACAACA	60	128	112	7
Sw766	AATCAATTGTCTCCACTTCAGG	AATTCTGCCTTGTCCAAAGG	60	164	142	6
Sw767	TGCGTGACTAGAACCCTGTG	TCACGCAGAACGTTTCAGAC	60	137	0	5
Sw769	GGATGACCAAAAAGTCTGGG	TCTGCTATGTGGGAAGAAATGC	60	139	106	7
Sw775	TTGCCTTTTAATTTCCCTACTTT	TGATGGAACATGATGGAGGA	58	176	170	3
Sw776	TAATCCGTTGCACCCAG	CCATATGCCACAGTTTCGG	58	117	88	7
Sw779	TACATGTGCAGAAAACAGAGCC	TGTTGGCTCCACTTCTTCAC	62	107	103	3
Sw780	TCTACCAGCTAAAATGCTCACTG	TAGGACCTGGAATATACTCCCTG	62	136	116	7
Sw781	CAACTACGTCCTTCTTTTGGC	GATCCTTGGTCTGGAACCTTG	62	198	123	8
Sw782	TCTTACATATGAGCACAACC	CGGAACAAGAGGAAGTGTG	60	99	89	4
Sw783	CATACCTGCACATCTCTTCAGC	GCAGCTATAGCTCCGATTGG	62	186	0	4
Sw787	CTGGAGCAGGAGAAAGTAAATTT	GGACAGTTACAGACAGAAGAAGG	60	161	153	5
Sw790	CTGTGGGAGTGTAGCATCTTTG	CATACACCCAGATGTGGC	62	172	118	7
Sw792	TACTGGGGTGTAGCTTGTGTG	TTCCCTCCTCTCCTCTTTCC	62	156	140	4
Sw803	GGTCACAATTGAGGGCACTC	TCCCAAGCAACAGAAGTGC	58	104	96	3
Sw811	GCGGGTATAGCTCCGATT	TCCTAAACCTGCTTATGCAATT	58	183	167	4
Sw813	AGTTGATTTAAAATGTTGTGCCA	AATATTTCAAAAAAGGAATGGG	58	110	0	4
Sw816	TTCCATACGCTGTGCTTCTG	AGGATTAGGAGGGTCTCAAGG	60	177	147	7
Sw818	TCTGATACCACAGATATGGCC	TTTAACTGTGTGAGCCATCCG	58	179	126	9
Sw824	CTAAACTTTGAATGTCTTCGCTG	ACCAAAGCTCGTTCAAGA	58	179	163	6
Sw830	AAGTACCATGGAGGGAAATG	ACATGGTTCCAAAGACCTGTG	62	189	179	4
Sw832	ACTATCCTCTTACTCTCCCTGCA	TAGCCTGGGAATGTCCATATG	62	243	227	4
Sw833	CTGACTGTTTTGCTGCAGTG	TCCACTGAGGTCCTCACTCTC	60	183	171	5
Sw835	TGGCTCAGAGTTTTCACTCTG	CAGAGGTTTTACCAAGTTTTGGC	60	240	218	8
Sw836	TCCAGTGACAATGTGAGTTTC	TTAGTCACTGTTTTGGAGCCTC	58	153	135	6
Sw839	GGAAACCAGGATAACAGGAGG	TAACCCACTGTACCACAAGG	62	166	144	6
Sw840	CCTGGAAAACAACCTAAGTGTCC	TTCCACATTAGTTCGGGGAC	55	137	121	5
Sw853	CTTTCTTCTGTCTGGGTGTGG	GGGAAAAATAGCCTCCACCTC	62	101	89	5
Sw855	TCTCTTTTTCTCAAAAGCTGCC	GGGAAACTGCTTTTACTCCAC	58	146	128	3
Sw856	AGGGGGTGGGTGATTGTG	AACCTTCCCCATGCTGCTG	62	200	160	10
Sw857	TGAGAGGTGAGTACAGAAGACC	GATCCTCTCCAAATCCCAT	58	159	145	7
Sw859	TTACAGTTTTGGGTGATGCC	CAGGTGTGGCCCTAAAAGG	60	123	85	4
Sw864	TTGCACAGATGCTAATCTTCC	TTAAGACTGTCTTGGGCAATCC	60	178	168	6
Sw866	AGTGTGGTGTGACTGATTTGG	CATGCAGGGAAGGAGAGAG	60	185	146	4
Sw871	ATCCCTGTTTTCCCTCCACCTC	AATTAAGCCATTCCACTGGGG	60	126	102	5
Sw873	TCCATCTACACTGACCCAAATG	ACAGTAGCCAAGATATGGAAGC	60	140	134	4
Sw874	AAAAGAACCCTAAGTACAGCAGC	TTTATGAGGGTATCCTGACACC	60	219	191	8
Sw878	CTGGGAGCACAACAGATAGTG	CAAGCAATCAATTCCTTAAGGG	60	120	101	3
Sw882	TGGTCTCCATCATCATGTG	TTTTCCGGGAAACAGAAC	58	135	119	4
Sw886	AATTGGTTTTGTCCAGAAATTTGG	GATCATTTCCCATTTGTTGAATT	58	174	142	8
Sw902	ATCAGTTGAAATGATGGCC	CTTGCTCAAAGAGTTGTAAGG	60	203	195	6
Sw903	TTTCTTTGACAGTTGTGCAAGG	TGAAGTACAGCAGGACCTG	58	201	195	4
Sw904	CCCTTTTCAGAAAGATGAAAA	CCTAGTGGCCAACCAAGT	60	179	163	5
Sw905	ATCCCAACCTTCTTTCAAAAGG	TCCAGTGGCAGAACAACATG	60	151	125	6
Sw906	GAGGACAATGTGAGAAAAAGAATG	TTTTTCTGTGATTAGAAGCTTAGG	55	184	158	7
Sw911	CTCAGTTCTTTGGGACTGAACC	CATCTGTGGAAAAAAAAGCC	60	173	151	7
Sw915	TTTCAATGTTCCCTATTACAGCA	GCTATAGCTCCAAATTCGACCC	60	157	139	6
Sw916	GGAGGTGGCAATAACAGG	GTGCCAGGCTGTTTAAAGG	60	142	136	4
Sw917	AATCTTGGAACTATGGCCC	CCAACAATTTCAATCAAGTTG	58	137	117	5
Sw919	TCCAAAGTCAAGATTTATTC	TCACAGACTAAATGTAAGAGCT	58	132	87	11
Sw920	CATGGAGCTGAACCTGCAAAA	ATCAAGCCCAACTAAGAATACA	60	150	142	4
Sw925	AGCTCCAATTTGACCCAG	CTCCAAATTTCTTTGCTCAAGG	62	148	123	8
Sw926	TAGCAGACCAGATTTTTTTGC	TTGACCCCTAACCTGGGAG	65	115	111	2
Sw933	ACATATACTTCGACAGCCCC	AAGAGCTTGGTGAATTGAGAGC	60	133	97	5
Sw935	GTGGTGGTTTTCCCTCTATAGC	ATATAAGGGAAAAAATCTGAAAGAGTATG	58	203	195	5
Sw936	TCTGGAGCTAGCATAAAGTCC	GTGCAAGTACACATGCAGGG	58	112	94	6
Sw937	GTGGAGAACCAAAATGGC	TGGAACCTTTGAACCTGACACC	58	226	214	6
Sw938	TTATATTTCCATTGCCATTGG	CACCTATGATGGAACATGATGG	58	157	0	5
Sw940	TACCTGTGTATGCAGCACG	TGAGCATCTCATTTCCGTCTC	58	157	0	5
Sw940	GGCTCCAGTGTACCAAGTCC	TGTTTTCCAGCTCTATCCG	60	144	136	4
Sw943	AGGAGGACTAGAGCGCTG	AGAGAGCCCAAGATAAGAACCC	62	132	118	2
Sw944	CTCCAGTTCAATTTGAGTTCC	TCTTCATGTATCACACCCTGC	58	166	164	2
Sw949	TGAGCAATGAGTTCAATGCC	TCGTTGGTGAAGGCATCC	58	204	178	8
Sw950	CTCCATGTGCTGCAGGTG	CCAAACACTCCCTGCAC	62	163	145	8
Sw951	TTTCACAACCTCTGGCAGCAG	GATCGTGCACAAATGGAC	58	136	124	5
Sw952	AACGGGCACTGCTGTATAG	GATCATTTCTGCTGCACAGC	59	149	143	4
Sw955	CTGCTCAAAGTTTATCTTTCCC	GTCACCTCACTGTCTTTCCC	65	113	103	5
Sw957	AGGAAGTGAGCTCGAAAGTGC	ATGGACAAGCTTGGTTTTCC	58	157	113	9
Sw960	TCTATGAGCCATGCTATGAAGC	AGTGGGGCCAAACATTAATTC	58	182	152	4
Sw962	TGAATCTCAAGCAGTAGAGCAC	TCAAGATGCCACTCACCTC	60	160	130	5
Sw964	GTGGTTCCCTCTATGCAGAGTCC	ATGTGATGAAACATGATGGAGG	58	248	220	5
Sw967	AGCAGACTGTTTACTCTGTTCAG	GGGGCAGCTGAAAAGTCC	58	114	95	7
Sw969	AGCCTGGAACATTTTTGAGTG	TTTTCAATTTGGTTCTGTGTCC	60	140	120	6
Sw970	AGTGGGCAACCAATAATGC	GTCTGCCACAAGCTGACTGA	58	375	227	6

TABLE 1

Continued

Marker name ^a	Forward primer	Reverse primer	PCR ^b temp.	Frag. Size ^c		Alleles ^d
				Max	Min	
Sw973	CACAGTTTGCATTGTGGGTC	TAGGGGGCCCCGTAAGTTC	58	183	171	3
Sw974	GGTGAAGTTTTTGCCTTGAACC	GAAAGAAAATCCAAATCCAAACC	58	166	126	9
Sw977	GATCAAGGTGAGTCTGACATTA	CGTCACAAGTGACGCCCTTTA	58	104	96	4
Sw978	CCCGGTGATGTCAAGTGAC	CATATGCCGCAAGTGCAG	62	150	122	5
Sw980	CTTCAGTGTAGTCCAAGTGGC	GATGTTTTGCTGATAGGAAGGG	55	132	0	9
Sw983	GCACTCCACTCTTAGGTATATATCC	ATAATGCTGCTATGAACACTGTAGTG	60	121	95	5
Sw986	AGGAAGCAAAATCTTAAGAGGC	GGTAGCCAGGAACAAGTATG	58	164	150	5
Sw987	TTGTTATGCCACTGTGTTGG	CTCCATATGCCACAGGTGTG	58	115	93	6
Sw989	CTCATTAAATTTAATTGAGTGAGTGTG	CCCGTGGTTCTGACTGAACT	55	135	105	7
Sw995	TTAAGCACTTCATGGAGCTTTG	CATAATGGAAAATACCCGGTCC	58	164	150	6
Sw100	CAAGGAGTATCTTCTCACAGCA	CTGGAACTTCCATAAGCCA	58	125	0	4
Sw1004	TGGGAACACCTGGTTTCATTC	TCCATATGCCCAAGTGTG	60	167	147	5
Sw1008	ACAGCCACCAACAGTGGTTG	GAACTTCCATATGCTGCAAGTG	62	255	203	9
Sw1021	CGCCACAAGTGAACCTCC	CCGGGGTCCAGCTATAG	60	115	93	4
Sw1026	TGGAGAGCAATGCTGTATG	GTATTTCACTGACGCTCC	60	118	97	6
Sw1027	AGCAACCTGAGCCACAGTG	GGAACCTCCACACGCCAC	60	159	133	9
Sw1030	AACTGGGGAAAGTAGAAGAGCG	TCATCTCATGCCGTGTCTAAA	58	145	137	3
Sw1031	ATCACCCAGACAAAACAATCTC	TATGTCAACCCCAACCC	58	117	93	4
Sw1032	ATTGGGTGGACTGATATGGT	GATCTATAAAGTGTAAATGTGTGTG	58	171	153	3
Sw1038	CAGTCTCTGAACAGTCTTTTCA	GCTGTTGGTGAGAGTCAATCC	58	159	137	4
Sw1041	ATCAGAAAATGGTCAACAGTTCA	GGAGAATTTCCCAAGTTAATAGG	58	103	95	5
Sw1042	TCAAATCAGTCTTTGCGGTG	GCCTGGGAACCTCTATACC	60	107	93	6
Sw1045	GGTTTTATCTTTTTCCAAAAGG	GTGAGCCAGCCCTCAAAG	55	148	144	3
Sw1053	CCCACCCACTGACTCCTG	TGTCGGGGAGTAGACTCAGG	60	116	114	2
Sw1055	CTCTTCGCTGTGCTAACCC	CACCTGTCCCAGGCTTGG	60	97	91	4
Sw1057	TCCCTGTGTACAGATTGATG	TCCAATTTCCAAAGTTCCTACTAGC	58	188	150	7
Sw1059	TCTCATGGCCAATCTTTTAC	CCTGCAACCTTCAAGTTCAGC	60	215	133	11
Sw1065	TGTAGTGTATGTCAGCACAGG	TCAGGATGACCTAACCC	60	124	120	3
Sw1066	GCAGGATGAACCCCTG	CTCTTGAGGCACCTGCTG	62	199	161	8
Sw1067	TGCTGGCCAGTACTCTG	CCGGGGGATTAACAAAAG	60	175	144	7
Sw1070	CTTGCAGCACTACTTAGGC	TCTATGTGCTTGGAGTGAGG	60	206	168	8
Sw1071	AGTGTCTGATATCAAGCACAAGC	TCACTTCCCACCCCTTACAC	62	152	126	6
Sw1073	GGGTGCAGCCCTAGAAAAG	TCAGTACAGATTTGTTTCCCC	62	166	150	5
Sw1080	GGGAAATTTGGATTTGAAATTG	CCCTGTCACTGTAACTTGCC	60	189	187	2
Sw1081	AACTGTAGAACCAGCTGGAGC	GACCTGTAGCATTAGGACTGG	65	152	126	6
Sw1082	ATTTGTGAGATAGGTTGGTGT	CTACCCACTCTCTCTTCC	65	107	89	5
Sw1083	CCTTGTGGCCCTCCTAAC	CATACTCCAAAATTTCTATGTTGA	60	147	117	5
Sw1085	CAGGCTCCCTGACTTCAGAC	TAGGTCCATCCATGTTTCTGC	60	135	117	3
Sw1089	TTTTCCCTTCACTCACCC	GATCAAAGTCCCTTACTCCGG	58	182	142	7
Sw1092	CCTGCTATGTCTTATTGGGAGG	GATCCTGCATTGCCAAGG	58	314	224	8
Sw1094	GATCATGGTGTACCACCTTTATA	ATTTCTGATGTTGGTACATGGTG	58	150	142	4
Sw1101	AACCTCCATATGCCACAGGTG	GGCTCCTCCTCAGAAAAGTCCC	62	170	122	9
Sw1105	TTCAATTCAAAGAAGTGTTTGTG	GGTGCATGATGCTCACACC	60	139	105	8
Sw1108	GTCTTCTTACACGGAAATGC	CCCCACCTCACACATACATG	60	143	131	4
Sw1110	GATCTGATGGATTCTAATTTGTGTG	AGATCGGCTCCAATCTG	60	194	0	7
Sw1111	AGGTCTACTGTCCATCACAGG	GAAGCAGAGTTGGCTTACAGTG	65	181	165	6
Sw1112	CTGGGTTTTGTTTTCTGTTTTG	TGGCTTGGGAATCTCCATAC	60	107	101	4
Sw1113	ATGGAACCTGGGTTGGCTTC	TGCAGCTCTGATTGGCTC	60	166	150	4
Sw1117	AGGGCCATAAATGGAAATCC	AAAAACAATAAGACCCCTGTG	58	178	157	5
Sw1118	ATCACCTTCACTGCAAAATCC	CTGTGCTATGCAATGCAC	62	188	170	5
Sw1119	CAACCTCAAAAATGGAGAAAGG	GTCTTGGGCTGTTTGGC	60	160	144	7
Sw1120	CAAAATGGAACCCATTACAGTCC	ACTCCTAGCCCCAGGAGCTTC	62	173	144	6
Sw1122	TCCCATTTTACAAGAAAAGGTG	ACTGACATCCTCCAGCCTA	62	135	119	5
Sw1123	GAGTCTGCCTGCATTGTGAA	TCTGTCTTTGTTTTCTGTCTT	57	178	152	6
Sw1125	TAGATGTATATACTTCCATGTGTG	ATGTTGAGCTTTAATTTTATACA	60	141	117	10
Sw1129	GATCATATGAGGAAAAGAATGTGT	CACAGGGGAACACCTTAAT	58	155	127	6
Sw1134	TAAGTTTAGGTGCCTCATTTGATTT	GAAAACCTCTTAGTTCTTTATGCAA	58	146	134	5
Sw1135	TAAGTTTAGGTGCCTCATTTGATTT	GAAAACCTCTTAGTTCTTTATGCAA	60	192	180	7
Sw1200	AATGCAAGTATATAAGAGCTTTTTG	GGTGGTTTTGGCTCCAATTG	60	158	130	8
Sw1201	CCAACCAACCAACAGAAAAC	CGGCAGCTGGTAACCCAATT	58	212	200	4
Sw1202	TAGAGATGGTTGAGGAAAAGGC	ATGATCCCGGCTCCCTTTTC	58	132	124	3
Sw1204	ATTTTGAACATGAGTAATCCGTG	TGATGCTCTTTTCTGTATGTG	58	114	0	4
Sw1210	GGATGGAACTCCCAAGATA	AAGAAAATCTCAAGCCAAGGA	60	142	130	3
Sw1211	TAGACCCTCCTGTGTTCTTTCC	CATATGCTGTGGAAATGCC	62	85	81	4
Sw1218	TGTGACTATGGCATAGGCTCC	GCCTATTAGAAAATGTTCTCTGG	58	199	174	8
Sw1262	TTGGGGCTCACAAGTAC	TTGGTAATTTCCGATGCTGC	60	147	127	3
Sw1263	AGATGAAACTGACATCTTCTGCC	GATCAAGGAAATAACACTGCTGT	55	165	151	5

^a Markers SOXXX were produced at European laboratories with numbers S0001–S0073 contributed by FREDHOLM *et al.* (1993), numbers S0081–S0100 were contributed by L. ANDERSSON and colleagues (ELLERGRÉN *et al.* 1993; JOHNSON, ELLEGREN and ANDERSSON 1992). CH13 is from DAVIES *et al.* (1992b). Primers for markers from structural genes were developed in our laboratory from GenBank sequences. Marker names beginning with Sw and Swr were developed in our laboratory.

^b The PCR profiles are described in the text. The values refer to the annealing temperature and the superscripts refer to the [MgCl₂] (millimolar) when it was not 1.5 mM.

^c Allele sizes were determined in relation to a sequencing ladder of M13mp18 and should be considered approximate. The number 0 refers to alleles that would not amplify in some animals.

^d The number of alleles (including null alleles) that were observed in this study.

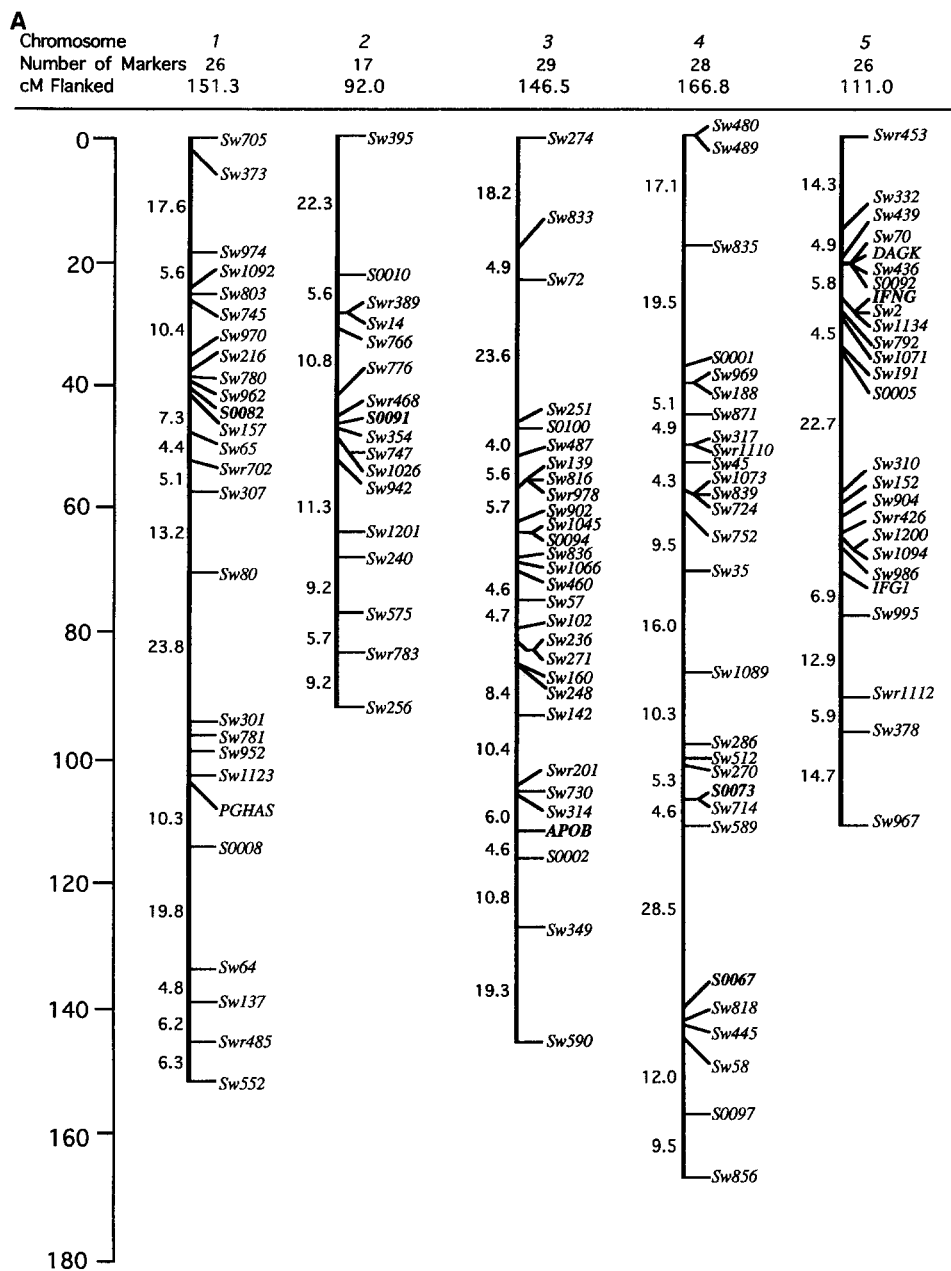


FIGURE 2.—Porcine genetic linkage map. Individual chromosomes are represented by vertical lines. The chromosome number, where known, or linkage group is indicated above together with the number of markers and length in sex-averaged Kosambi centimorgans. The interval between markers is shown on the left side when 4 cM or greater. One pair of linked markers (*Swr68/Sw983*) are not shown as no recombinants were observed. *The 56.5-cM interval on the X chromosome was not significant.

striction enzymes (*ApaI*, *HaeII* and *MspI*) (KIRKPATRICK 1992a; LARSEN and NIELSEN 1993). *Apolipoprotein B (APOB)* was amplified using primers 3 and 7 (in KAISER *et al.* 1993) and digested with *HincII*. Another fragment was amplified with primers 2 and 6 (in KAISER *et al.* 1993), digested with *HindIII* and found to be monomorphic.

Genotypic data were independently scored and entered into the database by two individuals. Software was developed (D. BEHRENS, J. WRAY and G. A. ROHRER, unpublished data) to compare scores, verify data in concordance and report discrepancies. Discrepancies were either resolved by the scoring individuals or data eliminated from the analyses. All linkage computations were performed using CRIMAP 2.4 (GREEN, FALLS and CROOKS 1990) linkage analysis software on a DEC 5000/25 work station and based on sex-averaged recombination rates (except for markers exhibiting *X*-linked inheritance). The two sire and eight dam pedigree structure (Figure 1) prompted the selection of CRIMAP 2.4 over available software packages for linkage anal-

yses. After preliminary alignment, the CHROMPIC option in CRIMAP along with software developed on site were used to identify unlikely double crossovers (those that occur within a 40-cM region) present in the data. Data contributing to these double crossovers were reanalyzed to determine their validity by rerunning the PCR reactions and blindly scoring the results. The number of errors remaining in these data should be negligible based on the error checking system implemented.

RESULTS

Genotyping: A two generation reference population with eight full-sib families and 94 progeny was developed with two WC boars and eight F₁ sows. Two F₁ sows were WC-DU and six F₁ females (one WC-FE, two WC-MI and three WC-ME) were produced by crossing boars from one of three Chinese breeds: FE,

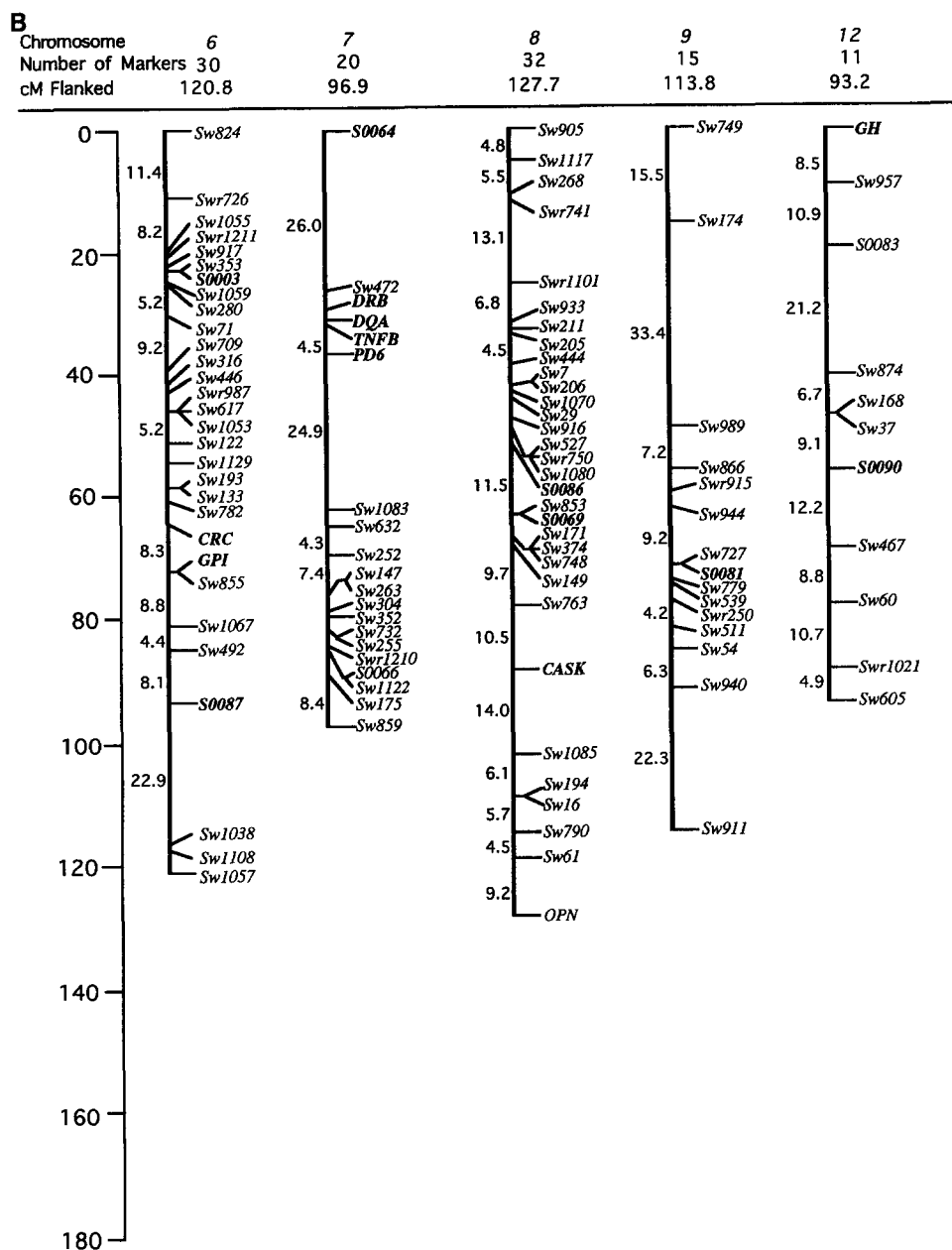


FIGURE 2.—Continued.

ME or MI with WC sows. The two DU-WC sows had litters of 15 and eight piglets; one FE-WC sow had a litter of 12; three ME-WC had litters of 14, 13 and 5; and two MI-WC sows had litters of 14 and 13. One boar sired seven of the eight litters with 81 progeny, while the other boar sired one litter (dam was MI-WC) of 13 piglets (Figure 1). The cross between North American and Chinese pigs was an attempt to design the most genetically and phenotypically diverse intraspecific cross possible in swine. The genetic diversity present in this population will increase type I marker polymorphism and facilitate development of comparative maps.

Our strategy to screen recombinant M13 swine genomic clones for GT:CA dinucleotide MS resulted in 0.24% of all recombinant M13 clones yielding

primer sequences. Eighty-five percent of all primer pairs amplified locus specific products. Only 3% (11/349) of MS developed from our M13 libraries were monomorphic in our families. Forty-nine of the 338 MS markers (14%) developed in our laboratory were adjacent to a short repetitive element (SINGER, PARENT and EHRlich 1987) (designated *Swr*; Table 1). These markers were similarly informative.

We were unable to amplify specific products from three loci (*apolipoprotein A1*, *follistatin* and *inhibin β (b)-subunit*) of 14 MS adjacent to, or within, porcine coding sequences obtained by screening the GenBank and EMBL databases. Three loci were monomorphic (*interleukin 1 α* , *growth hormone* and *apolipoprotein C3*) leaving eight (73%) informative loci for analyses: *calcium activated ATPase (ATP2)*, *diacylglycerol kinase*

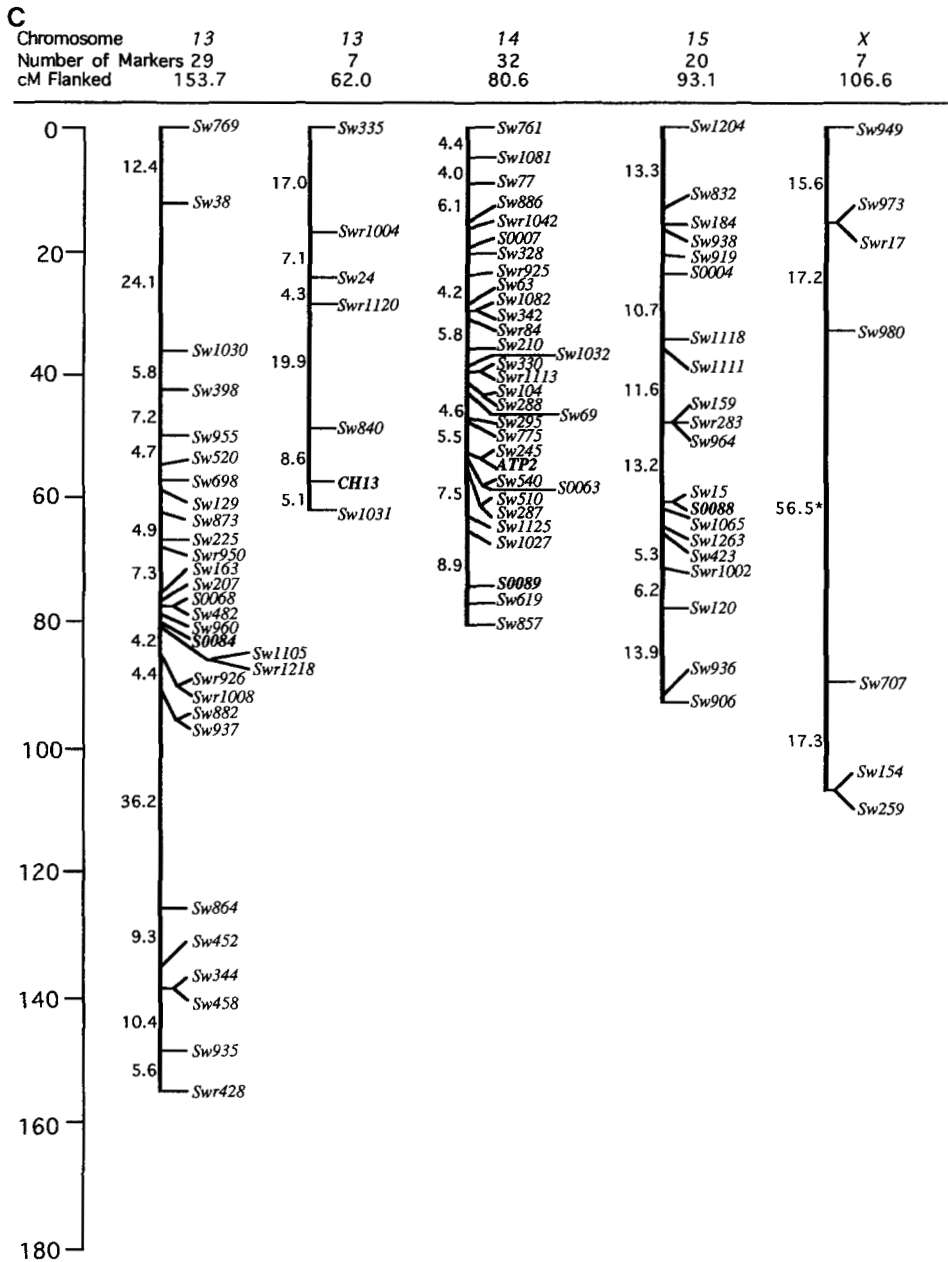


FIGURE 2.—Continued.

(DAGK), *insulin-like growth factor I (IGF1)* (KIRKPATRICK 1992b), *interferon γ (IFNG)*, *osteopontin (OPN)*, *pituitary glycoprotein hormone α -subunit (PGHAS)*, *ryanodine receptor 1 (CRC)* (BOLT, VOGELI and FRIES 1993) and *tumor necrosis factor β (TNFB)* (Table 1). As MS were more polymorphic than RFLPs, we preferred to use MS associated with genes as MS cost less to genotype and required less labor. While only 36 of 44 published MS (DAVIES *et al.* 1992b; JOHANSSON, ELLEGREN and ANDERSSON 1992; ELLEGREN *et al.* 1993; FREDHOLM *et al.* 1993) were scorable in our families, all 36 were informative. Locus name, primer sequences, PCR conditions and number and range of alleles for MS genotyped are presented in Table 1. The average number of alleles observed across all MS was 5.8.

As expected MS were more polymorphic in WC-Chinese sows. The mean level of heterozygosity was 54.4% for WC boars, 65.9% for WC-DU sows and 81.4% for WC-Chinese sows with WC-ME the most heterozygous breedtype (83.9%). Heterozygosity levels of 46–58% within breeds (ELLEGREN *et al.* 1993; FREDHOLM *et al.* 1993) and ~75% in F₁ animals from diverse crosses (COPPIETERS *et al.* 1993; ELLEGREN *et al.* 1993) of swine have been reported. The level of heterozygosity observed in North American breed composite crosses (WC and WC-DU) was similar to what has been observed in *Bos indicus* \times *B. taurus* crosses (60–65%) (S. KAPPES and M. BISHOP, unpublished data); humans (63%) (HUDSON *et al.* 1992); and in intraspecific crosses between inbred strains of mice (50%) (DIETRICH *et al.* 1992). The inclusion of WC-

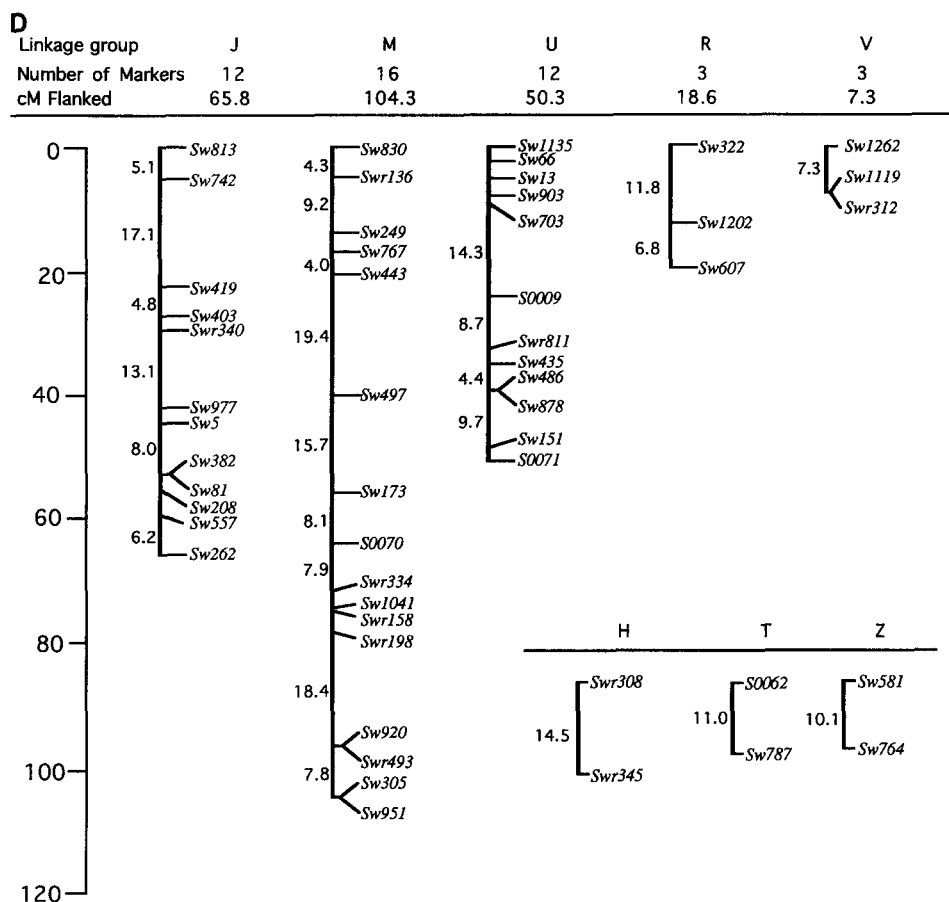


FIGURE 2.—Continued.

Chinese sows accelerated the development of the map as they were nearly as informative as interspecific hybrid mice (90%) (DIETRICH *et al.* 1992).

Only seven RFLPs with previously reported, readily scorable polymorphisms and chromosomally assigned were mapped. All RFLPs developed in our laboratory (analysis of the SLA cluster will be reported elsewhere) for class I (*PD6*) (EHRlich *et al.* 1987) and class II *DQA* (HIRSCH *et al.* 1990) and *DRB* (PRATT *et al.* 1990) major histocompatibility loci were informative. Only one band per allele was observed for class I *PD6* (EHRlich *et al.* 1987) and class II *DQA* (HIRSCH *et al.* 1990) and no more than two bands per allele were found for class II *DRB* (PRATT *et al.* 1990). Restricting probes to regions of genes with low levels of interlocus homology eliminated multilocus hybridization and ensured the observed genetic variability was confined to a single locus. Two alleles of *CASK* (LEVINE *et al.* 1992) also segregated in the population. Glucose phosphate isomerase (*GPI*) was the most informative RFLP (nine alleles present) as the probe sequence is adjacent to an intronic variable number tandem repeat (VNTR) (DAVIES *et al.* 1992a).

Two additional RFLPs were characterized in restriction endonuclease-digested PCR-amplified products. Three restriction enzymes were used for *GH* to maximize the number of informative meioses for this

locus (KIRKPATRICK 1992a; LARSEN and NIELSEN 1993). When analyzed as a single locus for the linkage study, no recombinants were detected within the haplotype. Two alleles for *APOB* were detected in our reference population with *HincII* (KAISER *et al.* 1993).

Linkage analyses: Markers were placed into putative linkage groups based on two-point linkage estimates ($LOD > 3.0$). Each set of markers was then aligned based on the linear order that maximized the log likelihood (LOD) from multiple-point linkage analyses. All intervals greater than 20 cM were tested for significance by comparing the LOD of the initial analysis (LOD_M) with the LOD holding the recombination rate of the large interval to 0.5 ($LOD_{0.5}$). Linkage groups were separated by multipoint analysis using CRIMAP 2.4 if the difference ($LOD_M - LOD_{0.5}$) was less than 3.0, thus eliminating spurious two-point linkages. The average number of coinformative meioses observed between all pairs of markers was 73 (range 0–188). As only 60 coinformative meioses are required to detect linkage between markers 20 cM apart with a power of 90% (J. KEELE, unpublished data), most intervals between markers flanking 20 cM or less should be detected. The overall power of detecting linkage was reduced because only two generations of animals were available and without grandparental data the phase of linked markers had to be computed.

Linkage analyses identified 23 autosomal and one X chromosomal linkage groups. Idiograms of each linkage group are presented in Figure 2 and distances between markers are proportional to the sex-averaged rate of recombination. One pair of linked markers (*Swr68/Sw983*) is not presented in Figure 2 as no recombinants were observed. Markers are aligned in the order that maximized the LOD. However, marker order within 5-cM intervals should be considered tentative until additional linkage has been established. Linkage group orientation with respect to the centromere and telomere was arbitrary as polymorphic markers physically assigned to chromosomes are currently minimal in the porcine map. The 383 linked markers covered 1997 cM. The average distance between adjacent markers ($n = 362$ intervals) was 5.5 cM. Sixty-three percent of all intervals were less than or equal to 5.0 cM while only 3.6% of the intervals were greater than 20.0 cM. Individual linkage groups had between two and 32 markers (mean 16) and spanned from 0 to 167 cM (mean 79.5 cM). An additional seven MS were unlinked in the final analyses (*Sw11*, *Swr67*, *Sw413*, *Sw491*, *Sw943*, *S0061* and *S0099*).

Twenty-seven previously assigned polymorphic loci (20 MS and seven RFLP; Table 2) were incorporated into linkage groups anchored to 13 autosomal chromosomes (Figure 2). Five anchor loci are located on chromosome 7 and four on chromosome 6 with the remaining 11 chromosomes having between one and three anchors each. *Kappa-casein* was assigned to chromosome 8 based on the close linkage of the four casein genes (α_{s1} -casein, α_{s2} -casein, β -casein and κ -casein) in cattle (FERRETTI, LEONE and SGARAMELLA 1990; THREADGILL and WOMACK 1990) and sheep (LEVEZIEL *et al.* 1991) and the physical assignment of α_{s1} , α_{s2} - and β -casein to porcine chromosome 8 (ARCHIBALD *et al.* 1992). Fourteen linkage groups contained anchor loci. We assigned linkage groups to chromosomes when at least one member of the group had been directly or indirectly assigned to a chromosome (Table 2). Two linkage groups were assigned to chromosome 13. All five anchor loci for chromosome 7 were members of the same linkage group and all four anchors on chromosome 6 were within one linkage group. The same was true for anchors assigned to chromosome 4, 8 and 12. No linkage group could be established for chromosomes 10, 11, 16, 17 and 18. Informative markers for chromosomes 10, 11 and 16 have recently been developed but have yet to be published (B. CHOWDHARY, personal communication). Chromosomes 17 and 18 remain bereft of markers (ANDERSSON *et al.* 1993). Four randomly generated markers (*Sw154*, *Sw259*, *Sw707* and *Sw980*) exhibited X-linked inheritance in every animal in our reference population and were assumed to be located on the X

chromosome. One of these X-linked markers (*Sw980*) was not significantly linked to the other three (Figure 2). However, *Sw980* was linked to three other markers exhibiting autosomal inheritance. Presumably, *Swr17*, *Sw949* and *Sw973* are located on the pseudoautosomal region of the X and Y chromosomes. We were unable to assign nine linkage groups containing 54 MS markers to chromosomes. As additional markers for chromosomes 10, 11, 16-18 are developed and reported, it is likely that the larger unassigned linkage groups (J, M and U) will be placed on some of these chromosomes.

Our results provide the first assignment of four structural genes and 13 published MS (JOHANSSON, ELLEGREN and ANDERSSON 1992; ELLEGREN *et al.* 1993; FREDHOLM *et al.* 1993) to autosomal chromosomes in the porcine genome. *Diacylglycerol kinase* (*DAGK*) and *IGF1* are assigned to chromosome 5, *PGHAS* is assigned to chromosome 1 and *OPN* to chromosome 8. Marker *S0008* is assigned to chromosome 1, *S0010* to chromosome 2, markers *S0002*, *S0094* and *S0100* to chromosome 3, *S0001* and *S0097* to chromosome 4, *S0005* and *S0092* to chromosome 5, *S0066* to chromosome 7, *S0007* and *S0063* to chromosome 14, and *S0004* to chromosome 15. We were also able to assign four previously published linkage groups to chromosomes. The linkage group of *S0007* and *S0072* (FREDHOLM *et al.* 1993) (also reported as U6: ANDERSSON *et al.* 1993) is assigned to chromosome 14. Linkage groups X, XI and XII (U9) (ANDERSSON *et al.* 1993) established in ELLEGREN *et al.* (1993) are assigned to chromosomes 4, 5 and 3, respectively.

Coverage of the genome: While the exact size of the porcine genome remains unknown, the presence of only seven unlinked markers in our analyses initially suggests that the 1997 cM reported here covers a majority of the genome. Our results also indicate that there are at least 20 cM between groups that are currently unlinked but located on the same chromosome, *e.g.*, chromosome 13 had two linkage groups detected. There were five more linkage groups than chromosomes identified in this study (24 linkage groups; $n = 19$). As the unlinked MS are located on chromosomes for which we have other markers, the porcine genome is clearly greater than the 1997 cM reported here; however, if microsatellites are randomly distributed then our data suggest the porcine genome is approximately 2300 cM (J. W. KEELE, unpublished data). Based on length of metaphase chromosomes (ANDERSSON *et al.* 1993), our linkage groups for chromosomes 2, 3, 5, 6, 7, 14, 15 and X are not complete. Large gaps are also present in linkage groups, particularly on chromosomes 1, 7, 9 and 13. Marker distribution in the present study was similar to that expected if MS are distributed uni-

TABLE 2
References and chromosomal assignments for anchor loci

Locus name	Type of marker	Chromosome	Type of assignment ^a	Reference
<i>S0082</i>	MS	1	LG	ELLEGREN <i>et al.</i> (1993)
<i>S0091</i>	MS	2	LG	ELLEGREN <i>et al.</i> (1993)
<i>APOB</i>	RFLP	3	IS	SARMIENTO and KADAVIL (1993)
<i>S0067, S0073</i>	MS	4	LG	SOLINAS <i>et al.</i> (1992a)
<i>IFNG</i>	MS	5	IS	FREDHOLM <i>et al.</i> (1993)
<i>S0003, S0087</i>	MS	6	LG	JOHANSSON <i>et al.</i> (1993)
<i>RYR</i>	MS	6	IS	FREDHOLM <i>et al.</i> (1992)
<i>GPI</i>	RFLP	6	IS	ELLEGREN <i>et al.</i> (1993)
				HARBITZ <i>et al.</i> (1990)
				DAVIES <i>et al.</i> (1988)
				CHOWDHARY <i>et al.</i> (1989)
				YERLE <i>et al.</i> (1990)
<i>S0064</i>	MS	7	LG	FREDHOLM <i>et al.</i> (1993)
<i>TFNB</i>	MS	7	IS	CHARDON <i>et al.</i> (1991)
				SOLINAS <i>et al.</i> (1992b)
<i>PD6, DQA, DRB</i>	RFLP	7	IS	GEFFROTIN <i>et al.</i> (1984)
				RABIN <i>et al.</i> (1985)
				ECHARD <i>et al.</i> (1986)
<i>S0069, S0086</i>	MS	8	LG	FREDHOLM <i>et al.</i> (1993)
				ELLEGREN <i>et al.</i> (1993)
<i>CASK</i>	RFLP	8	SA	See text
<i>S0081</i>	MS	9	LG	ELLEGREN <i>et al.</i> (1993)
<i>S0083, S0090</i>	MS	12	LG	ELLEGREN <i>et al.</i> (1993)
<i>GH</i>	RFLP	12	IS	THOMSEN <i>et al.</i> (1990)
				YERLE <i>et al.</i> (1993)
<i>S0084</i>	MS	13	LG	ELLEGREN <i>et al.</i> (1993)
<i>CH13</i>	MS	13	CS	DAVIES <i>et al.</i> (1992b)
<i>S0089, ATP2</i>	MS	14	LG	ELLEGREN <i>et al.</i> (1993)
<i>S0088</i>	MS	15	LG	ELLEGREN <i>et al.</i> (1993)

^a Assignment abbreviations are as follows: LG, linkage analysis; IS, *in situ* hybridization; CS, chromosomal specific library.

formly and selected randomly from the genome (WINTERO, FREDHOLM and THOMSEN 1992; DIETRICH *et al.* 1992). As more informative MS derived from cosmid or lambda genomic clones are placed on the linkage and physical maps, MS distribution as well as genomic coverage can be more accurately assessed.

DISCUSSION

We have integrated 334 newly identified MS with 34 MS previously reported, eight MS and seven RFLP associated with type I markers into a skeletal genetic linkage map of the porcine genome. Although comparisons between current linkage results and those previously published are difficult due to the absence of blood typing or serum protein analyses in our study, we were able to compare six intervals in five linkage groups (chromosomes 5, 6, 7, 12 and 14) where identical markers were used (ELLEGREN *et al.* 1993). Six interval distances were comparable including the distance between the *CRC* (*RYR1*) or malignant hyperthermia locus and *S0087* (chromosome 6) (ELLEGREN *et al.* 1993). In five additional linkage groups interval distance between identical markers was significantly greater in the present study when compared with that

reported by FREDHOLM *et al.* (1993) in a smaller pedigree. The accuracy of marker interval and order will be enhanced as similar sets of markers including erythrocyte antigens and serum proteins are screened across several reference populations.

As the porcine physical map develops, new assignments of genes to chromosomal locations will improve the comparative map between the human, mouse and swine genomes. Our strategy to reduce the randomness of saturating the porcine genome with type II markers is to place more type I markers from established syntenic groups (O'BRIEN *et al.* 1993) in our linkage map and assign porcine cosmid clones containing informative MS by *in situ* hybridization through collaborative efforts. As MS are developed that anchor centromeric and telomeric regions, additional randomly generated MS can be rapidly included into the linkage map, expanding genomic coverage and marker density. A combination of approaches by groups mapping the swine genome should rapidly place a significant number of linked markers on the map. Continued searching of databases will provide type II markers, close to or within type I loci. This overall strategy should provide a saturated linkage

map while yielding a sufficient number of dually mapped loci to accurately assess genomic coverage and chromosomal orientation of linkage groups (FREDHOLM *et al.* 1993).

In summary, the number of MS markers linked in the present swine genetic map will allow us and other investigators to initiate a concerted effort to identify markers which can be used in MAS and provide the frame work for identifying gene(s) that contribute to production efficiency.

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LITERATURE CITED

- ANDERSSON, L., A. L. ARCHIBALD, J. GELLIN and L. B. SCHOOK, 1993 1st pig gene mapping workshop (PGM1), August 7, 1992, Interlaken, Switzerland. *Anim. Genet.* **24**: 205–216.
- ANSARI, H. A., P. D. PEARCE and D. W. MAHER, 1993 Regional assignment of anchored reference loci to sheep chromosomes. Proceedings 8th North American Colloquium on Domestic Animal Cytogenetics and Gene Mapping **8**: 59–61.
- ARCHIBALD, A. L., J. F. BROWN, C. S. HALEY, M. FREDHOLM, A. K. WINTERO, *et al.*, 1992 Linkage mapping in the domestic pig (*Sus scrofa*). *Anim. Genet.* **23**: 88.
- BOLT, R., P. VÖGELI and R. FRIES, 1993 A polymorphic microsatellite at the *RYR1* locus in swine. *Anim. Genet.* **24**: 72.
- CHARDON, P., M. NUNES, F. DEZEURE, D. ANDRES-CARA and M. VAIMAN, 1991 Mapping the genetic organization of the *TNF* genes in the swine *MHC*. *Immunogenetics* **34**: 257–260.
- CHOWDHARY, B. P., I. HARBITZ, A. MÄKINEN, W. DAVIES and I. GUSTAVSSON, 1989 Localization of the *glucose phosphate isomerase* gene to the p12-q21 segment of chromosome 6 in pig by *in situ* hybridization. *Hereditas* **111**: 73–78.
- COPPIETERS, W., A. VAN DE WEGHE, L. PEELMAN, A. DEPICKER, A. VAN ZEVEREN and Y. BOUQUET, 1993 Characterization of porcine polymorphic microsatellite loci. *Anim. Genet.* **24**: 163–170.
- DAVIES, W., I. HARBITZ, R. FRIES, G. STRANZINGER and J. HAUGE, 1988 Porcine malignant hyperthermia carrier detection and chromosomal assignment using a linked probe. *Anim. Genet.* **19**: 203–212.
- DAVIES, W., S. KRAN, T. KRISTENSEN and I. HARBITZ, 1992a Characterization of a porcine variable number tandem repeat sequence specific for the *glucose phosphate isomerase* locus. *Anim. Genet.* **23**: 437–442.
- DAVIES, W., B. HOYHEIM, B. CHAPUT, G. FRELAT and A. KEISERUD, 1992b Rapid isolation of porcine chromosome 13 specific microsatellites. *Anim. Genet.* **23**: 90–91.
- DIETRICH, W., H. KATZ, S. E. LINCOLN, H. SHIN, J. FRIEDMAN, *et al.*, 1992 A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* **131**: 423–447.
- ECHARD, G., M. YERLE, J. GELLIN, M. DALENS and M. GILLOIS, 1986 Assignment of the major histocompatibility complex to the p14-q12 region of chromosome 7 in the pig (*Sus scrofa domestica* L.) by *in situ* hybridization. *Cytogenet. Cell Genet.* **41**: 126–128.
- EHRlich, R., R. LIFSHITZ, M. D. PESCOVITZ, S. RUDIKOFF and D. S. SINGER, 1987 Tissue specific expression and structure of a divergent member of a class I MHC gene family. *J. Immunol.* **139**: 593–602.
- ELLEGREN, H., M. JOHANSSON, B. P. CHOWDHARY, S. MARKLUND, D. RUYTER, *et al.*, 1993 Assignment of 20 microsatellite markers to the porcine linkage map. *Genomics* **16**: 431–439.
- FERRETTI, L., P. LEONE and V. SGARAMELLA, 1990 Long range restriction analysis of the bovine casein genes. *Nucleic Acids Res.* **18**: 6829–6833.
- FOWLER, T., 1992 Pigmeat: situation and outlook. *Pig News and Information* **13**: 87N–89N.
- FREDHOLM, M., A. K. WINTERO, K. CHRISTENSEN, B. KRISTENSEN, P. B. NIELSEN, *et al.*, 1993 Characterization of 24 porcine (dA-dC)_n-(dT-dG)_n microsatellites: genotyping of unrelated animals from four breeds and linkage studies. *Mamm. Genome* **4**: 187–192.
- FRIES, R., 1993 Mapping the bovine genome: methodological aspects and strategy. *Anim. Genet.* **24**: 111–116.
- FRIES, R., A. EGGEN and J. E. WOMACK, 1993 The bovine genome map. *Mamm. Genome* **4**: 405–428.
- GEFFROTIN, C., P. POPESCU, E. CRIBIU, J. BOSCHER, C. RENARD, *et al.*, 1984 Assignment of MHC in swine to chromosome 7 by *in situ* hybridization and serological typing. *Ann. Genet.* **27**: 213–219.
- GEORGES, M., R. DRINKWATER, T. KING, A. MISHRA, S. S. MOORE, *et al.*, 1993a Microsatellite mapping of a gene affecting horn development in *Bos Taurus*. *Nat. Genet.* **4**: 206–210.
- GEORGES, M., A. B. DIETZ, A. MISHRA, D. NIELSEN, L. S. SARGEANT, *et al.*, 1993b Microsatellite mapping of the gene causing Weaver disease in cattle will allow the study of an associated quantitative trait locus. *Proc. Natl. Acad. Sci.* **90**: 1058–1062.
- GREEN, P., K. FALLS and S. CROOKS, 1990 *Documentation for CR1-MAP, version 2.4*. Washington University School of Medicine, St. Louis.
- HARBITZ, I., B. P. CHOWDHARY, P. D. THOMSEN, W. DAVIES, U. KAUFMANN, *et al.*, 1990 Assignment of the porcine *calcium release channel* gene, a candidate for the malignant hyperthermia locus to the 6p11-q21 segment of chromosome 6. *Genomics* **8**: 243–248.
- HIRSCH, F., D. H. SACHS, K. GUSTAFSSON, K. PRATT, S. GERMANA, *et al.*, 1990 Class II genes of miniature swine III. Characterization of an expressed pig class II gene homologous to HLA-DQA. *Immunogenetics* **31**: 52–56.
- HODSON, C. J., 1985 The pig as a model for studying kidney disease in man, pp. 1691–1709 in *Swine in Biomedical Research*, edited by M. C. TUMBELSON. Plenum Publishing, New York.
- HUDSON, T. J., M. ENGELSTEIN, M. K. LEE, E. C. HO, M. J. RUBENFIELD, *et al.*, 1992 Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms. *Genomics* **13**: 622–629.
- JOHANSSON, M., H. ELLEGREN and L. ANDERSSON, 1992 Cloning and characterization of highly polymorphic porcine microsatellites. *J. Hered.* **83**: 196–198.
- JOHANSSON, M., B. CHOWDHARY, F. GU, H. ELLEGREN, I. GUSTAVSSON, *et al.*, 1993 Genetic analysis of the gene for porcine submaxillary gland mucin: physical assignment of the *MUC* and *interferon γ* genes to chromosome 5. *J. Hered.* **84**: 259–262.
- KAISER, M. E., D. N. NEVIN, S. L. STURLEY, C. PURTELL and A. D. ATTIE, 1993 Determination of pig *apolipoprotein B* genotype by gene amplification and restriction fragment length polymorphism analysis. *Anim. Genet.* **24**: 117–120.
- KIRKPATRICK, B. W., 1992a *Hae*II and *Msp*I polymorphisms are detected in the second intron of the porcine *growth hormone* gene. *Anim. Genet.* **23**: 180–181.
- KIRKPATRICK, B. W., 1992b Identification of a conserved micro-

- satellite site in the porcine and bovine *insulin-like growth factor-I* gene 5' flank. *Anim. Genet.* **23**: 543–548.
- LARSEN, N. J., and V. H. NIELSEN, 1993 *ApaI* and *CfoI* polymorphisms in the porcine *growth hormone* gene. *Anim. Genet.* **24**: 71.
- LEVEZIEL, H., L. METENIER, G. GUERIN, P. CULLEN, C. PROVOT, *et al.*, 1991 Restriction fragment length polymorphism of ovine casein genes: close linkage between the α_{s1} , α_{s2} , β - and κ -casein loci. *Anim. Genet.* **22**: 1–10.
- LEVINE, W. B., L. J. ALEXANDER, G. E. HOGANSON and C. W. BEATTIE, 1992 Cloning and sequencing of the porcine κ -casein cDNA. *Anim. Genet.* **23**: 361–363.
- LITT, M., and J. A. LUTY, 1989 A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the *cardiac muscle actin* gene. *Am. J. Hum. Genet.* **44**: 397–401.
- LUONGO, C., K. A. GOULD, L. SU, K. W. KINZLER, B. VOGELSTEIN, *et al.*, 1993 Mapping of multiple intestinal neoplasia (Min) to proximal chromosome 18 of the mouse. *Genomics* **15**: 3–8.
- MERSMANN, H. J., W. G. POND and J. T. YEN, 1982 Plasma glucose, insulin and lipids during growth of genetically lean and obese swine. *Growth* **46**: 189–198.
- O'BRIEN, S. J., J. E. WOMACK, L. A. LYONS, K. J. MOORE, N. A. JENKINS, *et al.*, 1993 Anchor reference loci for comparative genome mapping in mammals. *Nat. Genet.* **3**: 103–112.
- PRATT, K., D. H. SACHS, S. GERMANA, M. EL-GAMIL, F. HIRSCH, *et al.*, 1990 Class II genes of miniature swine II. Molecular identification and characterization of B(β) genes from the SLA haplotype. *Immunogenetics* **31**: 1–6.
- RABIN, M., R. FRIES, D. SINGER and F. H. RUDDLE, 1985 Assignment of the porcine major histocompatibility complex to chromosome 7 by *in situ* hybridization. *Cytogenet. Cell Genet.* **39**: 206–209.
- SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- SARMIENTO, U. M., and K. KADAVIL, 1993 Mapping of the porcine *apolipoprotein B (APOB)* gene to chromosome 3 by fluorescence *in situ* hybridization. *Mamm. Genome* **4**: 66–67.
- SINGER, D. S., L. J. PARENT and R. EHRLICH, 1987 Identification and DNA sequence of an interspersed repetitive DNA element in the genome of miniature swine. *Nucleic Acids Res.* **15**: 2780.
- SOLINAS, S., J. HASLER-RAPACZ, N. MAEDA, J. RAPACZ and R. FRIES, 1992a Assignment of the pig *apolipoprotein B* locus (*APOB*) to chromosome region 3q24-qter. *Anim. Genet.* **23**: 71–75.
- SOLINAS, S., U. PAULI, P. KUHNERT, E. PETERHANS and R. FRIES, 1992b Assignment of the porcine *tumor necrosis factor alpha* and *beta* genes to the chromosome region 7p11-q11 by *in situ* hybridization. *Anim. Genet.* **23**: 267–271.
- THOMSEN, P. D., M. FREDHOLM, K. CHRISTENSEN and M. SCHWERIN, 1990 Assignment of the porcine *growth hormone* gene to chromosome 12. *Cytogenet. Cell Genet.* **54**: 92–94.
- THREADGILL, D. W., and J. E. WOMACK, 1990 Genomic analysis of the major bovine milk protein genes. *Nucleic Acids Res.* **18**: 6935–6942.
- TISSOT, R. G., C. W. BEATTIE and M. S. AMOSS, JR., 1987 Inheritance of Sinclair swine cutaneous malignant melanoma. *Cancer Res.* **47**: 5542–5545.
- WARWICK, E. J., and J. E. LEGATES, 1979 *Improving swine*, p. 467 in *Breeding and Improvement of Farm Animals*, Ed. 7. McGraw-Hill, New York.
- WEBER, J. L., and P. E. MAY, 1989 Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* **44**: 388–396.
- WINTERO, A. K., M. FREDHOLM and P. D. THOMSEN, 1992 Variable (dG-dT)_n·(dC-dA)_n sequences in the porcine genome. *Genomics* **12**: 281–288.
- WOMACK, J. E., 1987 Comparative genome mapping: A valuable new tool for mammalian developmental studies. *Dev. Genet.* **8**: 281–293.
- WOMACK, J. E., and Y. D. MOLL, 1986 A gene map of the cow: conservation of linkage with mouse and man. *J. Hered.* **77**: 2–7.
- YERLE, M., J. GELLIN, M. DALENS and O. GALLMAN, 1990 Localization on pig chromosome 6 of markers *GPI*, *APOE* and *ENO1* carried by human chromosomes 1 and 19 using *in situ* hybridization. *Cytogenet. Cell Genet.* **54**: 86–91.
- YERLE, M., Y. LAHBIB-MANSAIS, P. D. THOMSEN and J. GELLIN, 1993 Localization of the porcine *growth hormone* gene to chromosome 12p1·21Ap1·5. *Anim. Genet.* **24**: 129–131.

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