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Mapping of microsatellite markers developed from a flow-sorted swine Chromosome 6 library

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Abstract. Swine Chromosome (Chr) 6-enriched libraries, generated with size-fractionated DNA isolated from chromosomes sorted by flow cytometry, have been used to develop new Chr 6 microsatellite markers. Chromosome isolation procedures were established to reproducibly prepare high quality chromosomes from phytohemagglutinin (PHA)-stimulated swine peripheral blood lymphocytes and to sort individual chromosomes after staining with Hoechst 33258 and chromomycin A3. Chromosome purity was verified by specific staining of swine Chr 6 with fluorescence *in situ* hybridization (FISH) by use of painting probes generated by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) amplification of as few as 300 sorted Chr 6. For library construction, DNA was extracted from flow-sorted pools representing Chr 6, amplified, size selected for fragments from 300 to 700 bp, and ligated into pBluescript SK II+ or Lambda ZAP Express. The libraries were then screened with a radiolabeled poly-(dCA) DNA probe. Of 107 (CA)_n repeat-containing clones verified by sequencing, 21 were polymorphic and used to genotype the University of Illinois swine reference families. Linkage analysis was then performed with CRIMAP 2.4 (LOD > 3.0), and the results showed that 15 of the microsatellites mapped to swine Chr 6. At least three of these new markers map to locations where there were gaps in the consensus Chr 6 map. Another four markers, because of their PIC values, should provide more informative markers in other areas of the map. Most of the new markers can also be used for automated genotyping with fluorescent labeling. This set of 15 new Chr 6 markers will, therefore, be useful in helping to define QTL associated with swine Chr 6.

Introduction

Microsatellite sequences have been shown to be highly abundant and randomly distributed in many mammalian genomes, including the pig (Stallings *et al.* 1991; Wintero *et al.* 1992; Ellegren *et al.* 1993; Fredholm *et al.* 1993). Because they also exhibit a large number of alleles that can be detected by PCR, microsatellites have become the method of choice for developing linkage maps by genotyping reference families with PCR. A genome-wide effort to generate microsatellite markers in the domestic pig has led to the

production of porcine linkage maps by several groups (Echard *et al.* 1992; Andersson *et al.* 1993; Ellegren *et al.* 1994; Rohrer *et al.* 1994; Archibald *et al.* 1995). These efforts assigned approximately 80 Type I loci and over 400 Type II loci to the porcine linkage map (Rohrer *et al.* 1994; Archibald *et al.*, 1995). Recent updates to these efforts have dramatically increased the number of assigned loci, and a comprehensive porcine-linkage map now exists that includes 1042 linked loci (Rohrer *et al.* 1996). The porcine genetic map now contains linkage groups that have been assigned to all 18 autosomes and the X Chr, and spans approximately 23 Morgans (sex-averaged; Rohrer *et al.* 1996; Archibald *et al.* 1996).

The production of high-density linkage maps has been aided by chromosome-specific approaches to generate more markers. Although broad panels of somatic cell hybrids have recently become available (Rettenberger *et al.* 1994; Zijlstra *et al.* 1996; Robic *et al.* 1996), their early absence in the pig along with a clear porcine flow karyotype made chromosome sorting by flow cytometry an ideal approach. All of the pig chromosomes have been identified by bivariate flow karyotyping (Schmitz *et al.* 1992; Langford *et al.* 1993). Chromosome-enriched libraries have been constructed and characterized for Chr 1, 11, 13, and 18, and a strategy for using pools of several swine chromosomes has also been used to assign loci to the swine map (Miller *et al.* 1992; Davies *et al.* 1994; Dear and Miller 1994; Ellegren and Basu 1995; Riquet *et al.* 1995, 1996). In addition, through the use of coincidence cloning, region-specific probes have been generated from Chr 6 (Frengen *et al.* 1994). Recently, the First International Chromosome 6 Workshop resulted in the integration of the swine Chr 6 linkage maps to produce a consensus map of this chromosome (Paszek *et al.* 1995).

These directed cloning approaches will ultimately be beneficial for the identification of genes that contribute to quantitative traits of interest in the pig. A few quantitative trait loci (QTL) have already been detected in pigs; for example, the one for growth rate and fat deposition by Andersson and associates (1994). Several other loci for quantitative traits have been mapped in swine; for example, the *ESR* locus for reproduction traits (Rothschild *et al.* 1996) and the *RN* locus for muscle glycogen content (Mariani *et al.* 1996; Milan *et al.* 1996). Another such locus is *RYR1*, the ryanodine receptor (or *CRC*, calcium release channel, or *HAL*, halothane), the gene responsible for Porcine Stress Syndrome (PSS) (Fujii *et al.* 1991; Otsu *et al.* 1991). PSS is characterized by an increased likelihood of sudden death in response to stress and is the result of a single point mutation in the *RYR1* gene (Fuji *et al.* 1991; Hughes *et al.* 1992). It is also known that animals susceptible to PSS have superior lean meat content, ham content, loin eye muscle area, and decreased back fat depth (Jensen and Barton-Gade 1985; Simpson and Webb 1989; W. Zhang *et al.*, 1992). The genes responsible for the positive carcass traits in PSS-susceptible swine remain to be identified. Therefore, a high-resolution linkage map of the region close to the *RYR1* gene will aid in the identifi-

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cation and separation of the desirable alleles for the gene(s) responsible for the positive carcass traits from the stress-susceptible mutant *RYR1* allele.

Since the *RYR1* gene has been physically mapped to Chr 6 (6p11- > q21); Davies *et al.* 1988; Harbitz *et al.* 1990), this chromosome is a good candidate for the use of a directed cloning approach. We report here the production of Chr 6 libraries with flow-sorted Chr 6 material and the detection of 15 new microsatellite markers from these libraries.

Materials and Methods

Chromosome isolation and staining. Porcine peripheral blood lymphocytes were isolated from blood drawn aseptically from NIH minipigs (population maintained at USDA, ARS, Beltsville, MD). Lymphocytes were separated by Dextran sedimentation followed by a Ficoll density gradient and cultured at 2.5×10^6 cells/ml in RPMI containing 5 µg/ml phytohemagglutinin (PHA), 10% fetal bovine serum, 2 mM L-glutamine, 1 mM nonessential amino acids, 1 mM sodium pyruvate, and 2×10^{-5} M beta-mercaptoethanol. Chromosomes were isolated according to the polyamine protocol (Cram *et al.* 1990). Briefly, blastogenic cells grown for 72 h were blocked in metaphase by incubation in 0.1 µg/ml Colcemid for 20 h. The cells were harvested and resuspended in 1 ml of 75 mM KCl with 100 µM spermine and 250 µM spermidine, and incubated at room temperature for 45 min to allow for swelling. Following centrifugation, the pellet was resuspended in 1 ml of Chromosome Isolation Buffer (75 mM KCl, 7.5 mM Tris-Cl, 1 mM EDTA, 10 mM NaCl, 250 µM EGTA, 100 µM spermine, 250 µM spermidine, and 1 mM digitonin), and the chromosomes were released by vortexing for 20–50 s. The chromosomes were stained for bivariate flow cytometry by incubation in chromomycin A3 (75 µg/ml) overnight at 4°C. Hoechst 33258 was then added (14 µg/ml), and the chromosomes were incubated on ice for over 1 h before sorting. Dual color flow cytometry (laser one was tuned to 358–361 nm, laser two to 458 nm) was performed on the Coulter Epics V FCM with guidance from Glenn Welch and Larry Johnson (Germplasm and Gamete Physiology Laboratory, USDA, ARS, Beltsville, MD).

FISH painting. Metaphase chromosomes were obtained from pokeweed-stimulated pig peripheral blood lymphocytes according to the standard treatment of colcemid arrest, hypotonic treatment, and methanol/acetic acid fixation. The protocol used for FISH has been described in detail (Lichter and Cremer 1992). For each slide, 100 ng of biotinylated DOP-PCR product (Telenius *et al.* 1992), was combined with 4 µg of sonicated pig genomic DNA and 6 µg of sheared salmon sperm DNA as a competitor in a 10-µl hybridization volume. Probes were hybridized overnight, followed by a post-hybridization wash performed in $2 \times$ SSC at 43°C for 5 min. Hybridization was detected through fluorescein isothiocyanate-labeled avidin (avidin-FITC; Oncor) staining and amplified with biotinylated anti-avidin and avidin-FITC. Signals were visualized through a Zeiss microscope with appropriate filters.

Library construction and screening. DNA from 1000 flow-sorted Chr 6 (sort #C0034) was extracted in 100 µl of Tris:NaCl:EDTA (TNE 10:80:10 mM), 1% sarcosyl, and 100 µg/ml Proteinase K at 55°C for 5 h. The DNA was precipitated in ethanol in the presence of Quik-Precip (Advanced Genetic Technologies Corp., Gaithersburg, Md.) and then digested with *Sau3AI* (Gibco-BRL) in a total volume of 15 µl at 37°C for 5 h. Following digestion, the DNA was ligated into *Bam*HI (Gibco-BRL)-digested alkaline phosphatase (Boehringer Mannheim)-treated pBluescript SK II+ (Stratagene) overnight at 15°C in a total volume of 20 µl [15 µl digest, 2 µl of vector (100 ng), 2 µl of 10× ligation buffer, and 1 µl T4 DNA ligase (Boehringer Mannheim)]. The cloned DNA was then amplified by PCR with the T7 and T3 primers (Stratagene). PCR was performed on 2 µl of the ligation in a 60-µl reaction volume with 50 ng of each primer, 5 µl of 2 mM dNTP mix, 6 µl of 10× PCR buffer, and 0.5 µl Amplitaq DNA polymerase (Perkin Elmer). A preliminary denaturation step (94°C for 2 min) was followed by 30 cycles of 94°C for 1 min, 52°C for 1 min and 15 s, and 72°C for 1 min and 30 s. The last cycle is followed by a 72°C elongation step for 5 min. The amplified DNA was precipitated in ethanol and then digested with *Xba*I and *Pst*I (Gibco-BRL) in a 40-µl volume, and 300–700 bp fragments were isolated from a 1% low-melt agarose gel (Promega Wizard PCR Preps). These DNA fragments were then ligated into *Xba*I/*Pst*I-digested pBluescript SK II+ in a 20-µl volume

at 15°C overnight. Following ligation, 2 µl of the reaction was used to transform DH5- α *Escherichia coli* (Gibco-BRL) and plated out in the presence of IPTG and X-gal (Sigma).

This same procedure was also used to construct a library in Lambda ZAP Express (Stratagene). For this library, a later sort (#C0067), performed under more stringent sorting conditions, was used as the source of DNA. The amplified DNA was digested with *Xba*I and *Xho*I (Gibco-BRL), and 300-bp to 1500-bp fragments were isolated and ligated into Lambda ZAP Express linearized with the same enzymes. Following ligation, the DNA was packaged, stored, and propagated in the presence of IPTG and X-gal according to the manufacturer's protocol.

Colony blots and plaque lifts were transferred to nylon membrane filters (Schleicher & Schuell, Keene, NH) and screened with an [α - 32 P]-dATP (Amersham) nick translated poly-d(CA) DNA probe (Pharmacia). Hybridization was performed overnight at 43°C in the presence of 5X SSC (sodium chloride/sodium citrate), 0.1% Sarcosyl, 0.02% SDS, 50% formamide, and 2% blocking reagent (Boehringer Mannheim). Washes were performed once at 42°C and once at 65°C for 30 min each in 2X SSC/0.2% SDS, followed by 1 wash at 65°C for 15 min in 0.2X SSC/0.2% SDS. Blots were developed by autoradiography, and positive colonies and plaques were subjected to a second round of screening before sequencing.

Cloning and sequencing of porcine microsatellite sequences. DNA from positive clones was isolated with the Wizard Plasmid Mini-Preps or Wizard Lambda DNA preps (Promega). The cloned DNA was then amplified with the T7 and T3 primers and purified from a low-melt agarose gel. The isolated insert DNA was then amplified for cycle sequencing with the T7 or T3 primers according to the standard Applied Biosystems (ABI) protocol. The purified extension products were then sequenced with the ABI Model 373A DNA sequencer. Sequences were checked for uniqueness with respect to the current swine linkage map by Lee Alexander [Roman L. Hruska United States Meat Animal Research Center (US MARC), Clay Center, Nebr.]

Genotyping and linkage analysis. Unique clones were isolated and used to generate primer pairs by analysis with the program PRIMER 0.5 (Lincoln *et al.* 1991). Primer pairs were chosen to have similar annealing temperatures and were compared with the PRE1 and ARE 1 and ARE 2 swine repeat sequences (kindly provided by Lee Alexander). The primer pairs that proved successful in amplifying genomic DNA by PCR and were polymorphic are listed in Tables 1 and 2.

PCR reactions were performed in 10 µl total volumes as described above except that the annealing step was performed at the temperature listed in Tables 1 and 2 and 12.5 ng of each DNA sample was used. Prior to PCR, the forward primers were end labeled with [γ - 32 P]ATP with T4 PNK according to the Gibco-BRL exchange reaction protocol. After PCR, the reactions were run at 50°C on a 6% denaturing polyacrylamide gel with 25% formamide. Allele sizes were determined by comparison with an M13 sequence ladder.

As indicated in Tables 1 and 2 most of the markers were also fluorescently labeled and run on 6% polyacrylamide gels on the ABI 373A DNA sequencer. These gels were analyzed with the Genescan software (ABI), and the markers were genotyped with the Genotyper 1.1 software (ABI).

The reference families of the University of Illinois, PiGMap and US MARC populations, were used for genotyping, as well as ten unrelated individuals used in a US population study (kindly provided by Max Rothschild, Iowa State University). For S0441-S0444, 94 offspring of the US MARC reference population, along with five PiGMap reference families (127 animals), were genotyped. The microsatellites were assigned to chromosomes on the basis of the results of two-point (LOD > 3.0) and multi-point linkage analyses (CRIMAP 2.4; Green *et al.* 1990). All markers (including S0441-S0444) were genotyped across 96 animals from the University of Illinois reference population (families 3840, 2800, 5820, 3800, 1800, 6800, and 3820). These data were also subjected to two-point (LOD > 3.0) and multi-point linkage analyses (CRIMAP 2.4) with the other Chr 6 markers typed in the University of Illinois swine population.

Results and Discussion

Chromosome sorting. We have established chromosome isolation procedures to reproducibly prepare high-quality chromosomes from PHA-stimulated swine peripheral blood lymphocytes. These

Table 1. Summary of characterized microsatellites on Chromosome 6.

MS	Accession number	CA repeats ^a	Number of alleles ^b	Allele sizes (bp) ^b	Heterozygosity ^b	Primers (5'-3'; For/Rev)	Anneal (°C)	Fluorescent ^c
S0443	U24285	14	6	230–252	0.50	ATGACATCCCAGATGGAGCAAC ACCCAAGGAGGTAGAGGGAC	57	Y
S0444	U24286	19	9	139–161	0.60	ATGTCTAGCTCCAAGCTGGG GGAATCTTTGGCTGTAGTTGG	57	Y
DG30	U62948	21	6	81–103	0.75	CTATACCACTCGTGGGAGTG CCAGTGTCCACTGGAGGGG	51	Y
DG32	U62947	16	5	158–184	0.60	GGGTAACATTCCATTGTGGG CACTCCAGTGTATGCAGTGC	53	Y
DG53	U62953	19	6	101–119	0.70	GCAACCTCATCGTTTCTAGTTGG CCTTACCATTGGTTTCAGAGTTGC	56	Y
DG79	U62952	22	7	219–251	0.70	CTCACAATCAAAGATGGAAAGC GGGATCAATCCATACTAATGAAGC	55	Y
DG81	U62956	21	4	243–249	0.55	GGGTTTGAGATACAACATTGC CACAGTGGGAAATCCACAACG	54	Y
DG87	U62954	26	9	92–130	0.85	GGTTTCTCCATGATAGCTTGC GCAAAACCAATGCACCATTTAC	54	N
DG93	U62957	18	7	147–167	0.80	GTTGAGTGAACATCCACTCTAGCC GACCTCTTCTCCCCACTCTG	54	Y
DG94	U62959	20	8	151–187	0.85	GCCAAGGTATGGAAGCAATC CCATGACATTGCAAAATGGGAG	52	N
DG98	U62955	21	6	81–107	0.65	CACCCAGAAAGCATAATCAGTGG GAACTCGCCACTAAAATCTGG	55	N
DG136	U62944	16	6	108–146	0.70	CCCGAATGTCCATTGATGGG GGTTTCTTCTCTTTGTTAAGGC	54	N
DG154	U62945	18	5	162–194	0.75	GTAATTGCTGTGCAAAATGTTAGC GAGGTTGTACACAGACAGATG	56	Y
DG179	U66700	22	5	237–253	0.75	GGGTTTGAATAGAACATTCG CCACAGTGGGAAATCCAGAACG	54	Y
DG195	U62946	17	6	96–136	0.6	CGGGGTGCTTAGCATTTGGG GAACTCCATGAGCTTTTGACATG	54	N

^a Represents the size in the original clone.^b Obtained from ten unrelated US pigs and the reference families U of IL, US MARC, and PiGMap.^c (Y) Successfully genotyped using fluorescently labeled forward primers; (N) unsuccessful fluorescent genotyping.**Table 2.** Summary of other characterized microsatellites.

MS	Accession number	CA repeats ^a	Number of alleles ^b	Allele sizes (bp) ^b	Heterozygosity ^b	Primers (5'-3'; For/Rev)	Anneal (°C)	Fluorescent ^c	Chrom. assign. ^d
S0441	U24283	20	13	129–166	0.75	CTGAGGGGGGAGATAGCTG CTGCTATCATATTGCATAATTGTC	57	N	2
S0442	U24284	15	9	111–133	0.65	GGGAGCACAAATCTCTGATTG CTGCTATAGAGAGAATAGATGAACATG	57	N	8
DG26	U62958	21	4	201–247	0.50	GATTCCCTCTTTTGTAGTGAATG GTCCAGAGCTCTCTCTTTGGG	53	Y	3
DG33	U62951	15	4	157–169	0.75	CTGAGCCTACAAATGACAGG TATCTGCTATAGAGAGCATAGATG	52	Y	8
DG36	U62949	25	6	84–109	0.75	GAGTTCCATGAAGCTATGCTG TTGCTTCCTAAAAACAACACC	53	Y	10
DG50	U62950	26	4	107–117	0.50	CAATATACTGGGCCCATTCG CGTTGTTTCTAAGTGCTTTGGG	55	Y	3

^a Represents the size in the original clone.^b Obtained from ten unrelated US pigs and the reference families U of IL, US MARC, and PiGMap.^c (Y) Successfully genotyped using fluorescently labeled forward primers; (N) unsuccessfully fluorescent genotyping.^d Determined by genotyping the US MARC swine reference families.

procedures have allowed us to sort individual swine chromosomes after staining with Hoechst 33258 and chromomycin A3. A typical bivariate flow karyotype of our preparation of swine chromosomes is shown in Fig. 1. Chromosome peaks were assigned according to those previously described (Schmitz *et al.* 1992; Langford *et al.* 1993). Chromosome 6 is in a position that is readily identifiable in the flow karyotype and therefore can be easily gated around for sorting. Several sorts of Chr 6 have been performed, along with individual sorts of every other swine chromosome.

FISH verification of chromosome sort purity. The purity of our chromosome sorts was verified by fluorescence *in situ* hybridization (FISH). Painting probes generated by DOP-PCR amplification of 300 sorted chromosomes were used to perform FISH. The results of Chr 6, X, and 13 paints are shown in Fig. 2A, 2B and 2C, respectively. Specific staining of swine Chr 6 was evident with the

biotinylated DOP-PCR products (Fig. 2A). The Chr 6 paints also gave a strong, discrete signal near the centromere of a medium-sized telocentric chromosome, possibly Chr 14 or 15. This staining pattern was not limited to the Chr 6 paints as the X Chr paints gave a similar, although less intensive, staining pattern (Fig. 2B). To identify which chromosome had this signal, metaphase chromosomes from a porcine cell line with a 1;14 translocation {38XY t(1;14)(q2.12;q2.2)} were used with the Chr 6 painting probe (T.Q. Zhang *et al.* 1992). Figure 2D shows that the truncated Chr 14 as well as the normal Chr 14 carries the centromeric staining pattern. Therefore, the extra staining pattern observed is on Chr 14.

The extra signals observed with both the Chr 6 and X paints have been previously noted by Langford and colleagues (1993). The Chr 14 signals are not sort specific or DOP-PCR specific since painting probes generated with (GAG)₇ primers from different Chr 6 sorts also painted the centromeric region of Chr 14 (Ambady *et*

Fig. 1. Bivariate flow karyotype of the male swine chromosomes isolated from PHA-stimulated peripheral blood lymphocytes. The linear fluorescence intensities were quantified with a dual beam flow cytometer after staining with Hoechst 33258 and Chromomycin A3. The location of each of the separated chromosomes is indicated.

al., 1997). However, a Chr 6 painting probe that was generated by chromosome microdissection and PCR amplification with unique primers did not paint this region of Chr 14 (Ambady *et al.*, 1997). A few possibilities exist that may explain this staining on Chr 14. First, the Chr 14 staining may represent a high concentration of swine repetitive elements that are shared by Chr 6, X, and 14. It seems unlikely that this represents a repetitive element that is common to all the swine chromosomes, since the painting probes generated from our Chr 4 and 13 sorts do not stain Chr 14 (data not shown). This would seem to be the most likely explanation, although the Chr 14 staining could also be the result of preferential amplification of certain sequences with some primers during PCR. The procedures used to generate the painting probes could account for this. Preferential amplification of repetitive elements shared by Chr 6, X, and 14 would account for this staining. It seems unlikely that the signal would be due to contamination of the Chr 6 and X sorts with a small number of copies of Chr 14. Even though Chr 14 is near Chr 6 on the bivariate flow karyotype (Fig. 1), only a discrete region of Chr 14 is stained and not the entire chromosome.

Library production and screening. The results of our FISH experiments show that we have clearly isolated individual swine chromosomes by flow cytometry, and they would therefore be useful for the production of swine chromosome libraries. With Chr 6 sorts, two Chr 6 libraries were developed as outlined in Materials and methods. The library in pBluescript SK II+ had 52% recombinants and a titer of only about 7,000 cfu. The lambda library proved to be more efficient, containing 95% recombinants and a titer of approximately 140,000 pfu.

Approximately 145,000 clones were screened from both libraries with a radiolabeled poly-d(CA) probe. From the plasmid library, seven positive clones were picked from an initial screen of about 5000 clones. Six of these were positive after a secondary round of screening. All six clones were sequenced and found to contain unique (CA)_n repeats. However, one clone contained a repeat that was too close to the vector for primer design, and one clone contained a repeat surrounded by the PRE 1 swine repetitive element. Therefore, only four primer pairs were designed for clones from this library. From the lambda library, 195 clones were picked from approximately 140,000 clones screened. 138 of these clones were positive after a second round of screening. All 138

clones were sequenced, and 101 contained unique (CA)_n repeats. There were 18 duplicate clones found, and 19 clones did not contain (CA)_n repeats. Of the 101 (CA)_n repeat-containing clones, 23 had repeats that were either too small (<8 repeat units) or too close to the vector to have primers designed. An additional 26 clones contained swine repetitive elements surrounding their (CA)_n repeat and were therefore not used for primer design. The remaining 52 clones from the lambda library were suitable for primer design.

A total of 56 potential microsatellites were isolated from both libraries produced with flow-sorted chromosomes that were suitable for primer pair synthesis. We focused our efforts on the lambda library because it had the higher recombinant titer, yielding a higher number of positive clones. We also screened our libraries with (AT)₁₀, (TCC)₆, (GTG)₆, and (GGAT)₅ repeat-containing probes, but no positive clones have been detected to date with these other probes.

Primer pairs were generated for all 56 microsatellites and used to amplify genomic DNA from ten unrelated pigs. Twenty-one of these primer pairs failed to amplify the expected size fragment from pig genomic DNA in spite of several attempts to optimize PCR conditions and redesign primers. Two of these 21 were isolated from the plasmid library. Fourteen of these primer pairs were not polymorphic in our 10 unrelated US pigs and the F₀ and F₁ samples used for genotyping. The other 21 primer pairs (two from the plasmid library, S0441 and S0442) were successful in amplifying pig genomic DNA, were polymorphic, and are listed in Tables 1 and 2.

Genotyping studies. The aforementioned 21 markers were used for subsequent genotyping studies on 96 animals from the University of Illinois reference population. These animals represent a mini-scan of seven families from this population selected for their litter sizes and informativeness with framework markers on Chr 6. An autoradiograph of one microsatellite, DG179, genotyped on family #3840 from the University of Illinois reference population, is shown in Fig. 3. A summary of the data collected from all the microsatellites is shown in Tables 1 and 2. As indicated in the tables, some of our markers were also successful in genotyping these families fluorescently. The first four microsatellites isolated, S0441-S0444, were also used to genotype the reference families of the US MARC and PiGMap populations.

Linkage analysis. The reference family segregation data were then subjected to linkage analysis with CRIMAP 2.4 to assign the new markers to their chromosomal locations. The results showed that 15 of our markers mapped to Chr 6, and a summary of these markers is shown in Table 1. Two-point linkage analysis of the genotypes of markers S0441-S0444, generated on the US MARC and PiGMap reference families, confirmed their chromosomal assignments. The data from the Chr 6 markers were subsequently analyzed with multiple-point procedures of CRIMAP to determine the order of markers with the maximum likelihood. The alignment of all 15 new markers relative to other preassigned markers and their map distances is shown in Fig. 4. The information for the swine Chr 6 consensus markers listed was previously documented by Paszek and coworkers (1995). The other markers listed were previously documented by Rohrer and associates (1996).

The markers not linked to Chr 6 were also used to genotype the US MARC reference families and then analyzed by two-point linkage analysis to determine their chromosomal locations. The chromosomal locations of these six markers are shown in Table 2

Summary. Taken together, our efforts have resulted in the addition of 15 new markers to the swine Chr 6 linkage map that were isolated from our flow-sorted Chr 6 lambda library. Nineteen new markers were isolated from this library, and 15 of them map to Chr

Fig. 2. Pig metaphase spreads showing fluorescence of individual chromosomes painted by FISH with biotinylated DOP-PCR products from chromosome specific sorts: (A) Chr 6; (B) X Chr; (C) Chr 13; (D) Chr 6 paint probe with a porcine cell line with a 1;14 translocation {38XY t(1;14) (q2.12,q2.2)}.

Fig. 3. Genotyping of the University of Illinois pig reference family #3840 with DG179. The parents (24B, sire; 31B, dam) are shown in lanes 1 and 13, and the 11 offspring in lanes 2 thru 12. Three alleles were found in this family representing 239, 245, and 253 bp fragments.

6 (79%). It is interesting to note that S0441 and S0442 were the only clones isolated from the plasmid library, and they mapped to Chr 2 and 8, respectively. The two libraries were generated from different Chr 6 sorts. The sort for the lambda library was performed under more stringent conditions and most likely resulted in a purer Chr 6 sample. This is supported by the fact that 79% of our new markers isolated from the lambda library mapped to Chr 6.

We have clearly shown our ability to produce flow-sorted libraries useful for generating more polymorphic chromosome-specific markers. The detection of new microsatellites on swine Chr 6 will permit the construction of a more dense linkage map of this chromosome. There are still gaps in the existing maps that will require more markers. In addition, to perform fine mapping studies to isolate genes associated with quantitative traits, higher resolution chromosome maps will be needed. A few of our new markers may be located in regions that are marker deficient, such as *DG32*, *DG79*, *DG93*, and *DG94*. There is currently a gap of approximately 30 cM on the US MARC map (Rohrer *et al.* 1996) on the q arm of Chr 6 in the area where these four markers map. Markers such as these may therefore be useful for filling these regions and joining distant linkage groups.

Other markers such as *DG87*, *DG30*, and *S0444* may also be useful in providing new markers that have a higher percentage of heterozygosity and more alleles than existing ones in their locations. This provides additional markers that may be more infor-

Fig. 4. Cytogenetic and USDA BARC linkage map of swine Chr 6. The linkage map was developed by use of the University of Illinois reference families. Markers given in italicized font are selected framework map markers. Cytogenetic map taken from Paszek and associates (1995).

mative and/or easier to genotype in a resource population. We have also been successful in generating new markers that can be used to perform automated genotyping (Table 1), and thus have been added to the panel of existing markers that can be used fluo-

rescently. We now also have the ability to generate more Chr 6 markers in an attempt to increase the resolution for this chromosome in regions that have associated QTL, such as around the *RYR1* gene. Studies are now underway to develop region-specific markers for this area of Chr 6. The development of more genetic markers will

allow detailed mapping studies to be performed. In fact, resource families of pigs are already being developed at the University of Minnesota that have been characterized for their carcass traits as well as their inheritance of the mutant *PSS* allele of the ryanodine receptor.

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