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Identification of 29 Rat Genetic Markers by Arbitrarily Primed Polymerase Chain Reaction

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The number of genetic markers for the rat is still limited, in spite of its wide use in cancer research. To facilitate accurate mapping of both established and novel rat genetic markers, we constructed a linkage map by genotyping 105 F_2 rats from ACI/N (ACI) and BUF/Nac (BUF) crosses. This map consists of 120 genetic markers that had been previously reported, mainly by two research groups, but had not been integrated. To find new genetic markers, the arbitrarily primed polymerase chain reaction (AP-PCR) was applied to detect polymorphic bands between ACI and BUF rats. After testing 56 single primers and 12 combinations of primers, we found 36 bands produced by 16 single primers and two combinations to be reliably polymorphic between ACI and BUF rats. The 36 bands were typed in the 105 F_2 rats, and 29 of them could be linkage-mapped. AP-PCR is thus useful to detect new genetic markers in laboratory strains of rats.

Key words: Polymorphism — Chromosomal markers — Genome — Rat — AP-PCR

Important phenotypic traits, such as cancer susceptibility, are generally controlled by multiple genes, and these genes often have low penetrance. To map the genes responsible for these traits, a large family is necessary in human studies, and this is a major limitation. Studies using experimental animals have the great advantage that crosses can be made as often as necessary. Because of the density of known genetic markers and the small body size, mice are most frequently used for this purpose.[1,2] With the rat, less than 1,000 genetic markers have been reported. Furthermore, the two comprehensive rat genetic maps so far reported by two research groups[3,4] have not been integrated with each other, causing inconvenience. Since there are various good models for cancer susceptibilities in the rat,[5–7] development of an accurate and dense linkage map in this species is important.

While microsatellite markers are the major genetic markers presently applied, the arbitrarily primed polymerase chain reaction (AP-PCR) is a simple approach to identify new genetic markers.[8–10] Using a single primer or combined primers, five to 10 cycles of PCR are performed under low stringency, followed by 20–35 high-stringency PCR cycles. This method usually produces tens of DNA fragments from various regions of chromosomes that can be detected reproducibly by polyacrylamide gel electrophoresis. Almost any available oligonucleotides can be used as the PCR primer(s), and no previous information on genome structure is necessary to detect polymorphic DNA fragments.

In the present study, we first constructed an accurate rat linkage map, using 105 F_2 rats and integrating genetic markers that had been reported mostly by two research groups,[3,4] and then mapped polymorphic markers newly detected by AP-PCR.

Moderately distant laboratory strains of rats, ACI/N (ACI) and BUF/Nac (BUF),[11] were selected as founders. Thirteen pairs of female ACI and male BUF rats were mated, and 86 F_1 progeny were produced. By mating 36 pairs of the F_1 progeny, 340 F_2 progeny were produced. Among the F_2 progeny, 105 randomly selected male rats were provided by CLEA Japan (Tokyo). DNA was extracted by the standard phenol-chloroform method from the livers of the 105 F_2 rats, as well as four ACI and four BUF animals.

To integrate the maps that had been reported separately, we typed 114 microsatellite markers from two major rat genetic maps (64 reported by Yamada *et al.*[3]) and 46 reported by Jacob *et al.*[4]) and from 4 other reports,[12–15] using the conditions reported. When details of PCR conditions were not available, the following conditions were tested: annealing at 55°C or 60°C; $MgCl_2$ concentrations of 1.5 m*M*, 2.0 m*M* or 3.0 m*M*. Polymorphisms of the PCR products between ACI and BUF were first examined by electrophoresis in 5% polyacrylamide gels containing 7 *M* urea or with 30% formamide, or without these denaturants. For markers which were separable even by electrophoresis in agarose gels (3% NuSieve; FMC, Rockland, ME), genotyping of F_2 rats

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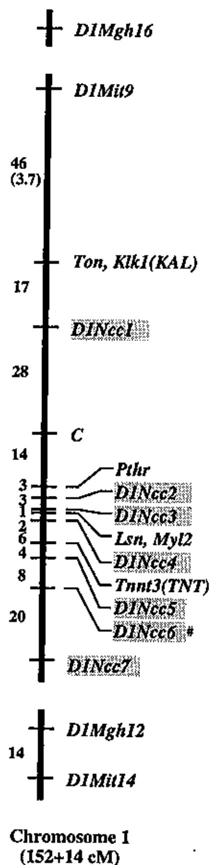
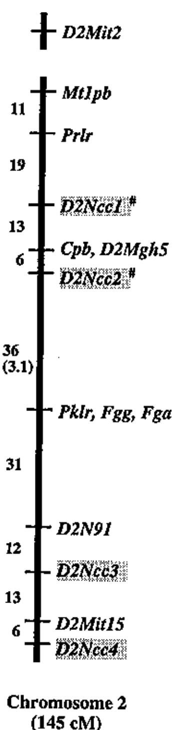
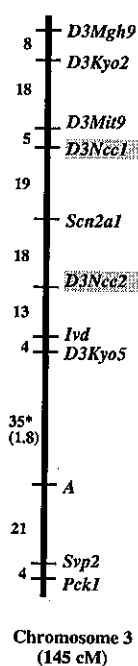
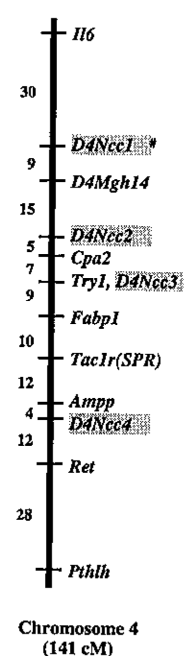
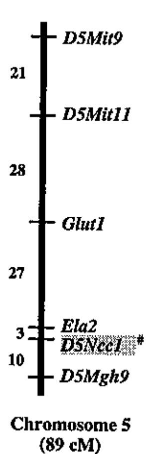
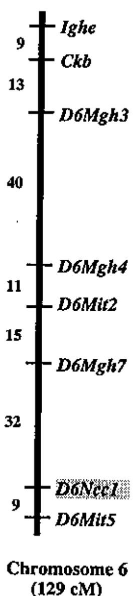

D1Mgh16
D1Mit9
46
(3.7)
Ton, Klk1(KAL)
17
D1Ncc1
28
C
14
Pthr
D1Ncc2
D1Ncc3
Lsn, Myl2
D1Ncc4
Tnnt3(TNT)
D1Ncc5
D1Ncc6 #
3
3
1
2
6
4
8
20
D1Ncc7
D1Mgh12
14
D1Mit14
Chromosome 1
(152+14 cM)

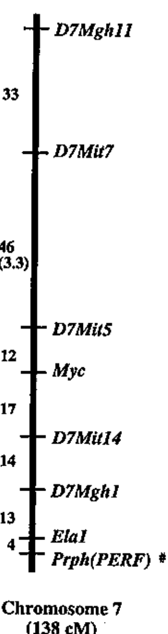

D2Mit2
Mt1pb
11
Prlr
19
D2Ncc1 #
13
Cpb, D2Mgh5
6
D2Ncc2 #
36
(3.1)
Pklr, Fgg, Fga
31
D2N91
12
D2Ncc3
13
D2Mit15
6
D2Ncc4
Chromosome 2
(145 cM)

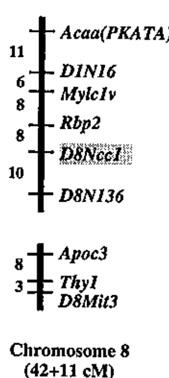

D3Mgh9
8
D3Kyo2
18
D3Mit9
5
D3Ncc1
19
Scn2a1
18
D3Ncc2
13
Ivd
4
D3Kyo5
35*
(1.8)
A
21
Svp2
4
Pck1
Chromosome 3
(145 cM)

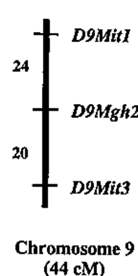

Il6
30
D4Ncc1 #
9
D4Mgh14
15
D4Ncc2
5
Cpa2
7
Try1, D4Ncc3
9
Fabp1
10
Tac1r(SPR)
12
Ampp
4
D4Ncc4
12
Ret
28
Pthlh
Chromosome 4
(141 cM)

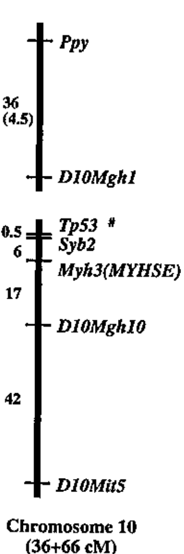

D5Mit9
21
D5Mit11
28
Glut1
27
Ela2
3
D5Ncc1 #
10
D5Mgh9
Chromosome 5
(89 cM)


Ighe
9
Ckb
13
D6Mgh3
40
D6Mgh4
11
D6Mit2
15
D6Mgh7
32
D6Ncc1
9
D6Mit5
Chromosome 6
(129 cM)


D7Mgh11
33
D7Mit7
46
(3.3)
D7Mit5
12
Myc
17
D7Mit14
14
D7Mgh1
13
Ela1
4
Prph(PERF) #
Chromosome 7
(138 cM)


Acaa(PKATA)
11
D1N16
6
Mylc1v
8
Rbp2
8
D8Ncc1
10
D8N136
Apoc3
8
Thy1
3
D8Mit3
Chromosome 8
(42+11 cM)


D9Mit1
24
D9Mgh2
20
D9Mit3
Chromosome 9
(44 cM)


Ppy
36
(4.5)
D10Mgh1
0.5
Tp53 #
Syb2
6
Myh3(MYHSE)
17
D10Mgh10
42
D10Mit5
Chromosome 10
(36+66 cM)

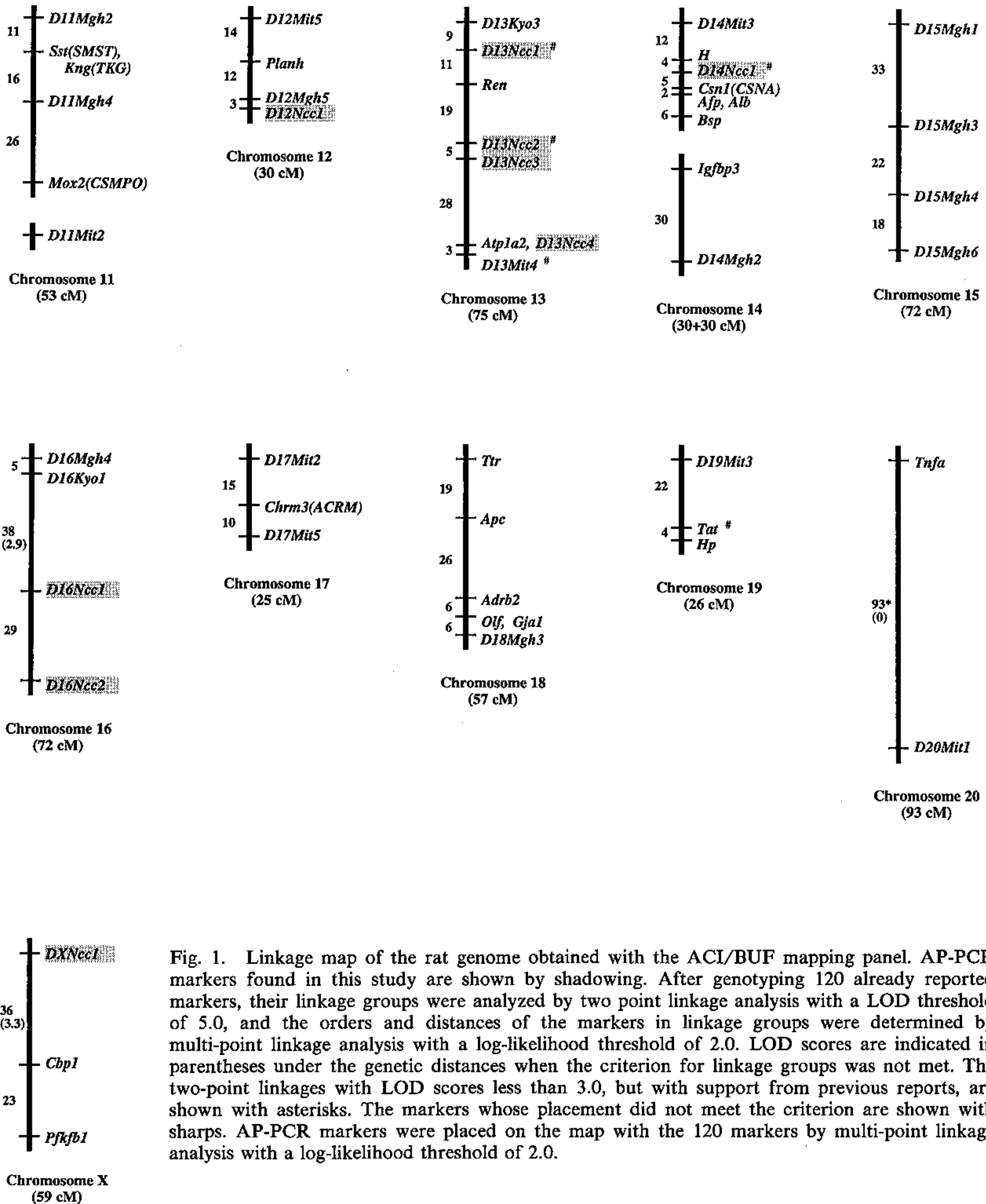


Fig. 1. Linkage map of the rat genome obtained with the ACI/BUF mapping panel. AP-PCR markers found in this study are shown by shadowing. After genotyping 120 already reported markers, their linkage groups were analyzed by two point linkage analysis with a LOD threshold of 5.0, and the orders and distances of the markers in linkage groups were determined by multi-point linkage analysis with a log-likelihood threshold of 2.0. LOD scores are indicated in parentheses under the genetic distances when the criterion for linkage groups was not met. The two-point linkages with LOD scores less than 3.0, but with support from previous reports, are shown with asterisks. The markers whose placement did not meet the criterion are shown with sharps. AP-PCR markers were placed on the map with the 120 markers by multi-point linkage analysis with a log-likelihood threshold of 2.0.

Table I. List of the Loci Typed.

Marker name	
Chrom1 (11)	
Ton (TON)[a]	104
Klk1 (KAL)[a]	105
C[a]	105
Pthr (PTHrel)[a]	102
Lsn[a]	105
Myl2[a]	105
Tnnt3 (TNT)[a]	105
D1Mgh16[b]	100
D1Mgh12[b]	98
D1Mit9[b]	103
D1Mit14[b]	101
Chrom2 (10)	
Mt1pb[a]	105
Prlr[a]	103
Cpb[a]	38
Fga[a]	105
Fgg[a]	36
Pklr[a]	38
D2Mit2[b]	104
D2N91[d]	102
D2Mit15[b]	97
D2Mgh5[b]	103
Chrom3 (9)	
D3Kyo2[a]	73
Scn2a1[a]	73
Ivd[a]	37
D3Kyo5[a]	67
A[a]	75
Svp2 (SVS2P)[a]	93
Pck1[a]	38
D3Mgh9[b]	101
D3Mit9[b]	101
Chrom4 (9)	
Il6[a]	102
Cpa2 (CPA)[a]	105
Try1[a]	105
Fabp1[a]	102
Tac1r (SPR)[a]	105
Ampp[a]	100
Pthlh[a]	87
D4Mgh14[b]	88
Ret[c]	105

Marker name	
Chrom5 (5)	
D5Mit9[b]	105
D5Mit11[b]	104
D5Mgh9[b]	98
Glut1[a]	86
Ela2[d]	105
Chrom6 (7)	
Ighe[a]	85
Ckb[a]	81
D6Mgh3[b]	105
D6Mgh7[b]	104
D6Mit2[b]	105
D6Mgh4[b]	102
D6Mit5[b]	105
Chrom7 (8)	
D7Mit5[b]	105
Myc[a]	85
D7Mit14[b]	103
Prph (PERF)[a]	38
Ela1[a]	86
D7Mit7[b]	105
D7Mgh11[b]	100
D7Mgh1[b]	105
Chrom8 (8)	
Acaa (PKATA)[a]	105
D1N64[d]	105
Mylc1v[a]	86
Rbp2[a]	105
D8N136[d]	104
Apoc3[a]	105
Thy1[a]	89
D8Mit3[b]	105
Chrom9 (3)	
D9Mit1[b]	105
D9Mgh2[b]	105
D9Mit3[b]	103

Marker name	
Chrom10 (7)	
D10Mit5[b]	105
Myh3 (MYHSE)[a]	105
Syb2[a]	105
D10Mgh10[b]	103
Ppy[a]	84
D10Mgh1[b]	105
Tp53[c]	104
Chrom11 (6)	
D11Mgh2[b]	103
D11Mit2[b]	105
D11Mgh4[b]	105
Sst (SMST)[a]	84
Kng (TKG)[a]	38
Mox2 (CSPMO2)[a]	86
Chrom12 (3)	
D12Mit5[b]	104
D12Mgh5[b]	102
Planh[a]	104
Chrom13 (4)	
D13Kyo3[a]	78
D13Mit4[b]	105
Ren[a]	105
Atp1A2[a]	105
Chrom14 (8)	
D14Mit3[b]	96
H[a]	75
Csn1 (CSNA)[a]	37
Afp[a]	85
Alb[a]	37
Bsp[a]	105
Igfbp3 (IGFBP)[a]	86
D14Mgh2[b]	93
Chrom15 (4)	
D15Mgh1[b]	99
D15Mgh3[b]	103
D15Mgh4[b]	103
D15Mgh6[b]	104

Marker name	
Chrom16 (2)	
D16Kyo1[a]	79
D16Mgh4[b]	102
Chrom17 (3)	
Chrm3 (ACRM)[a]	104
D17Mit5[b]	102
D17Mit2[b]	104
Chrom18 (6)	
Ttr[a]	105
Adrb2[a]	105
Gja1[a]	105
Olf[a]	38
D18Mgh3[b]	56
Apc[c]	105
Chrom19 (3)	
Hp[a]	80
Tat[a]	82
D19Mit3[b]	104
Chrom20 (2)	
Tnfa[a]	105
D20Mit1[b]	102
ChromX (2)	
Cbp1[a]	86
Pfkfb1 (PFKFB)[a]	84

a) Ref. 3. *b*) Ref. 4. *c*) Tumor-related genes; Ref. 16–18. *d*) *D2N91,* Ref. 12; *Ela2,* Ref. 13; *D1N64,* Ref. 14; *D8N136,* Ref. 15. A total of 114 microsatellite markers, three coat color markers and three tumor-related genes were genotyped. The names of the markers and the numbers of rats typed for each marker are listed for each chromosome. The numbers of the markers typed for each chromosome is shown in the parentheses after chromosome number.

was performed using agarose gel electrophoresis. Three coat color loci (*A, C* and *H*) were scored by visual examination of the animals. Three tumor-related genes, *Ret, Apc* and *Tp53*, were also typed by PCR-SSCP analysis (*Ret*) and PCR-RFLP analysis (*Apc* and *Tp53*) as reported earlier.[16–18] These 114 microsatellite, three coat color and three tumor-related gene markers were selected as showing polymorphism between ACI and BUF, as

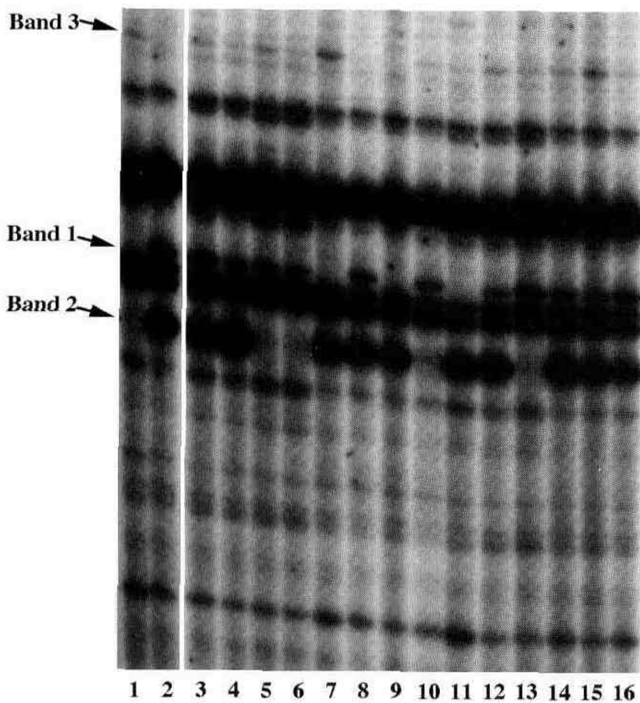


Fig. 2. Representative results of AP-PCR with primer "p53 ex8.-F." Lanes 1 and 2: parental ACI and BUF rats. Lanes 3–16: rats of the (ACI×BUF) F_2 progeny. Bands 1, 2, 3 correspond to markers *D1Ncc1, D1Ncc7,* and *D0Ncc3*, respectively. *D1Ncc1* and *D1Ncc7* were absent in ACI rats and present in BUF rats, and *D0Ncc3* was present in ACI rats and absent in BUF rats. For *D1Ncc1* and *D1Ncc7*, F_2 rats were genotyped as A (homozygote of the ACI allele) or C (heterozygote of the ACI and BUF alleles or homozygote of the BUF allele). As for *D1Ncc7*, rats in lanes 3–16 were genotyped as C, C, A, A, C, C, C, A, C, C, A, C, C, and C.

well as covering all chromosomes, and so that the intervals between the markers would be as equal as possible. The names of the markers and numbers of F_2 rats typed for individual markers are shown in Fig. 1 and Table I.

The genotypes of the 105 F_2 rats for the 120 markers were analyzed to make an integrative linkage map, using the MAPMAKER/EXP software package.[19] First, the 120 markers were grouped into linkage groups by two point linkage analysis at a LOD threshold of 5.0 and a distance threshold of 40 cM. The resultant linkage groups were essentially consistent with previous reports, except that *D1N64* (mapped on chromosome 1 in the original paper[14]) was in the same linkage group as markers on chromosome 8. Because the LOD score for linkage between *D1N64* and *Acaa* exceeded 20.0, *D1N64* was concluded to be on chromosome 8. Some linkage groups were connected even with LOD scores below 5.0 when the linkages were supported by previous reports. Such cases are distinguished by showing two point LOD scores in parentheses below the figures for genetic distances (Fig. 1). These manipulations allowed one large linkage group to be obtained for each chromosome. Linkages on a part of chromosome 3 and on chromosome 20 (shown with asterisks in Fig. 1) require re-examination because the linkage of *D3Kyo5* and *A* on chromosome 3 was supported by a LOD score of 1.8 and that of *Tnfa* and *D20Mit1* on chromosome 20 was not supported by a positive LOD score.

Next, the orders of markers within each linkage group were determined by multi-point linkage analysis with a log likelihood threshold of 2.0. When the order of markers was not conclusive with this criterion, the order consistent with previous reports was chosen. The markers whose placement was not conclusive are distinguished by sharps in Fig. 1. The total length of the map was 1,774 cM, covering most of the rat genome, 1,750 cM.[4] Since our linkage map was constructed using 105 F_2 rats, which corresponds to 210 meioses, the map has a maximum resolution of 0.5 (1/210) cM.

To obtain new genetic markers for rat chromosomes, AP-PCR was applied. Oligonucleotide primers for AP-PCR were synthesized in a 392 DNA synthesizer (Applied Biosystems, Foster City, CA). Most primers were 20 bases long, and their G+C content ranged from 40% to 70%. The reaction mixture contained 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 4.0 m*M* $MgCl_2$, 100 μM dATP, dGTP and dTTP, 40 μM dCTP, 2 mCi [α-^{32}P]-dCTP, 20 pmol of primer, 0.5 unit of AmpliTaq polymerase (Perkin-Elmer Cetus, Norwalk, CT) and 100 ng of genomic DNA. PCR was carried out in a thermal cycler (Perkin-Elmer Cetus) using 7 cycles with low-stringency conditions (92°C for 30 s, 45°C for 45 s, 72°C for 45 s), followed by 28 cycles with high-stringency conditions (92°C for 30 s, 60°C for 30 s, 72°C for 30 s). The PCR products were electrophoresed in 5% polyacrylamide gels under denaturing or nondenaturing conditions.

Differences in the presence or absence of a band between two strains of rats can be considered as a polymorphism.[8–10] In addition to length polymorphism between ACI and BUF, a search for presence or absence of polymorphism was made by amplifying DNA from ACI and BUF rats (Fig. 2). On average, one potentially polymorphic band was recognized per primer or per combination. Using 56 single primers and 12 sets of two-primer combination from these, 36 reproducible bands were selected after amplifying DNAs from the four individual ACI rats and four individual BUF rats (Table II). The 36 bands were produced by 16 single primers and two sets of two-primer combinations, and were used as genetic markers.

Table II. AP-PCR Markers Identified.

#	Name of the primer	Sequence of the primer(s) used	Band no.	Name of the marker
1	NRAS 2-6	GGTGTTTCAGAAAACATT	1	*D1Ncc5*
			2	*D2Ncc3*
2	HRAS 1-2	CAGAATACAAGCTTGTGGTG	1	*D2Ncc4*
3	APOC3.R	GTCTAGCTGCCCACAGGAG	1	*D13Ncc2*
4	APOC3.F	GATTTGAAGCGATTGTCCAT	1	*D4Ncc3*
			2	*D16Ncc1*
5	KAL.R	ACTGTTGGGTAACAAAGTTATGG	1	*D0Ncc2*
6	P53-10.3	CTTGTCCAGTGCTTCCATCT	1	*DXNcc1*
7	lacI-Y13	TTCTTTTCACCAGTGAGACG	1	*D1Ncc6*
			2	*D13Ncc4*
			3	*D1Ncc3*
			4	*D8Ncc1*
8	P53 ex.6-R	GGAGGGCCACTGACAACCA	1	*D13Ncc3*
9	P53 ex.8-F	TTGGGAGTAGATGGAGCCT	1	*D1Ncc1*
			2	*D1Ncc7*
			3	*D0Ncc3*
10	D8S206-F	ACATGCATTAGCACTACCATGC	1	*D1Ncc2*
			2	*D0Ncc4*
11	D10S175-R	CCATAGCCATTCTTCCTCCA	1	*D4Ncc4*
12	D8S205-F	ATTCTCTCGTCCTTTCTTGGG	1	*D1Ncc4*
			2	*D2Ncc2*
			3	*D0Ncc5*
13	D16S310-F	GGGCAACAAGGAGAGACTCT	1	*D12Ncc1*
14	D16S312-R	CTGTCCACCCTGTGACCC	1	*D3Ncc1*
			2	*D3Ncc2*
15	PKATA.F	GGTATGAGATCAATGCTGCC	1	*D5Ncc1*
			2	*D14Ncc1*
16	MYHSE.R	GATGAACCAGCACATGGAAG	1	*D4Ncc2*
			2	*D13Ncc1*
			3	*D0Ncc6*
17	PKATA.F	GGTATGAGATCAATGCTGCC	1	*D2Ncc1*
	MYHSE.R	GATGAACCAGCACATGGAAG	2	*D4Ncc1*
	(combined)		3	*D0Ncc1*
18	PTHLH.F	GGGAAGCAGTTACACACACAC	1	*D6Ncc1*
	IGHE.F	GTTGGGTTGTTCCATCTATGC	2	*D16Ncc2*
	(combined)		3	*D0Ncc7*

Sixteen primers (#1–16) and two combinations of primers (#17, 18) yielded 36 polymorphic bands, 29 of which were linkage-mapped.

The 36 AP-PCR markers were then typed in the 105 F_2 rats. When a band was present in ACI but not in BUF, homozygotes for the BUF allele were distinguished from heterozygotes (ACI and BUF alleles) or ACI homozygotes (see the legend to Fig. 2), and *vice versa*. The 36 AP-PCR markers underwent chromosomal assignment by two-point linkage analysis, and 29 of them showed linkage to one of the linkage groups of our map (chromosomes 1, 2, 3, 4, 5, 6, 8, 12, 13, 14, 16 and X) with a LOD threshold of 3.0. Then, the AP-PCR markers were placed within the framework of our map at a log-likelihood threshold of 2.0. Some of the marker placements were not conclusive with this threshold, as distinguished in Fig. 1.

In line with the recent report by Kuramoto *et al.* describing identification of 10 AP-PCR markers in rats,[10] our findings indicate that AP-PCR can be regarded as an effective approach to identify polymorphic markers.

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