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ARBITRARY SINGLE SHORT PRIMERS IDENTIFY POLYMORPHIC DNA MARKERS THAT DISTINGUISH INBRED STRAINS OF MICE

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The inbred mouse strains, Balb/c and SJL/J, have been widely used in biomedical research to unravel the genetic basis of susceptibility to tumors, viral diseases, autoimmune encephalitis, atopic disorders and neuro-retinopathies. In this study we attempted to identify DNA polymorphisms that distinguish them using RAPD assay. Screening of the genomic DNA of mice with a panel of 100 random decamer primers led to the identification of 36 primers which amplified 204 strain specific RAPD markers. On an average each of the selected primer amplified 11 bands of which 5.6 were strain specific. Segregation of RAPD markers in a (Balb/c x SJL/J) F1 x SJL/J backcross progeny (n=6) suggested that the markers are potentially suitable for molecular genetic linkage studies.

The last few years have witnessed a rapid progress in the genetic mapping of mouse and human genome based on the polymorphic DNA markers called simple sequence repeats (SRSs) or microsatellites, that occur frequently in most eukaryotic genomes(1,2). For DNA markers to be useful in the construction of genetic map, they should be abundant, highly polymorphic, evenly distributed and readily scored (1,3,4). In addition to SSRs, which satisfy these criteria, the recently reported random amplified polymorphic DNA (RAPD) markers fits into this category as well (3-7). The latter category of anonymous DNA markers have additional advantages viz., they are independent of target sequence information, free of radioactive hazards and an isolated RAPD marker could serve as individual specific DNA probe (3,7). A single short primer, used in the RAPD assay generates a DNA fingerprint,

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by mismatch annealing to multiple target sites in the genome flanked by perfect or imperfect invert repeats (7,8). Mutations at the primer binding sites or insertions and deletions between the binding sites can create polymorphic variants, which are identified as recessive alleles by the absence of the band(3,7). Since its discovery RAPDs have become popular genetic markers in diverse arenas including, population genetics, plant and animal breeding, microbial/parasite identification, epidemiology and genetic mapping of disease resistance loci in plants (7,9,10). Recently, the RAPD approach was used to develop a genetic linkage map for zebrafish (3). In contrast, its application to mammalian genetics, in particular, to mouse, is limited (11,12). In this study, we screened a panel of 100 decamer oligonucleotide primers and identified 204 RAPD markers that distinguish Balb/c and SJL/J strains of inbred mouse, and suggest that they are potentially suitable for genetic linkage studies.

MATERIALS AND METHODS

Genomic DNA: The tail DNA was prepared from mouse tail as follows: about 1 cm of the tail tip was cut into fine pieces in presence of 500 µl TENS buffer (0.1M NaCl, 10mM Tris-HCl, 1mM EDTA, 1% SDS). The tissue was digested with 50 µl proteinase K (10 mg/ml) for 2 hr at 37° C. The DNA was dialysed thrice against TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8). The concentration and the purity of the DNA was estimated spectrophotometrically at 260 and 280nm.
Random oligonucleotide primers: All primers used in this study were purchased as 10 mer RAPD primer set from the Biotechnology lab, the University of British Columbia, Vancouver, Canada. The primers were reconstituted in sterile water and used in the RAPD assay at a final concentration of 0.36 µM.
RAPD assay: A modified version of the protocol described before was used in the RAPD assay (13). Briefly a 25µl PCR mixture containing 1µl of the genomic DNA solution (containing 25 ng DNA) 10mm Tris HCl(pH 8.3) 50 mM KCl, 2.5mM MgCl2, 0.2 mM each of dNTPs (Pharmacia), 0.36 µm random primer and 0.75 units of Taq DNA Polymerase (Gibco BRL) was incubated in a Perkin Elmer Cetus DNA Thermal cycler. A 45 cycle program each having a 5 sec denaturation step at 94° C, a 30 sec annealing step at 36° C and a 60 sec extension step at 72° C was used. The PCR products were resolved on a 1.5% agarose gel and visualised by ethidium bromide staining. The polymorphic bands were scored by two people independently using the photographs and ambiguous, faint and smeary bands were excluded.

RESULTS AND DISCUSSION

The DNA based polymorphisms such as microsatellites, have been widely used to construct molecular genetic linkage maps for human and mouse genome (1,2). Similarly, the recently reported RAPDs have

also been exploited for the same purpose in plants and to a limited extent in vertebrates (3,7,11,12,14). In this study we demonstrate the potential of RAPD assay to generate a large number of DNA polymorphic markers between two widely used inbred strains of mice and suggest that such markers are a good source of information for constructing molecular genetic linkage maps for mouse.

Since the arbitrary primers in RAPD assay can amplify polymorphisms between certain strains but not others, selection of strains is vital. The inbred strains - Balb/c and SJL/J have been widely used in biomedical research to study the genetic basis of a number of traits by which they differ such as: (i) susceptibility to experimental autoimmune encephalomyelitis (model for multiple sclerosis), (ii) tendency to develop spontaneous tumors like reticular cell sarcoma, B cell lymphoma etc., (iii) susceptibility to viral diseases like mouse hepatitis, (iv) ability to mount a persistent IgE isotype response and bronchial hyperresponsiveness (model for atopic asthma and allergies) and (v) spontaneous retinal degeneration (15-19). Hence we attempted to develop polymorphic DNA markers that can distinguish these two strains of mouse using RAPD technique.

Initially genomic DNA from one strain was used to screen a panel of 100 decamer RAPD primers. This led to the identification of 59 primers that gave detectable amplification on agarose gels. These primers were subsequently used to identify polymorphic DNA markers. In general all primers amplified several bands from both genetic backgrounds. Some bands were specific to either Balb/c or SJL/J, while others were common to both strains (Fig.1A). Of these primers, 36 (61%) amplified polymorphic bands which were strain specific (table 1). Twenty-nine primers (33.8%) produced banding pattern without any scorable polymorhisms (eg. Fig.1, primer #15). Three primers (5%) produced banding pattern with a single strain specific band (not included in the table). The selected set of 36 primers which amplified two or more strain specific bands are listed in the table. These primers together amplified 395 bands of which 204 (51.6%) were strain specific and 191 (48.3%) were common to both strains. On an average each primer amplified 11.0 bands of which 5.6 were polymorphic between the strains. Thus nearly half of the total number of amplified bands by any of the selected primers were strain specific. By screening a set of 481 random primers, Woodward and associates identified 95 RAPD markers that distinguished inbred strains C57BL/6J and DBA/2J (11). In a recent study Johnson et.al., described 116 RAPD primers that identified

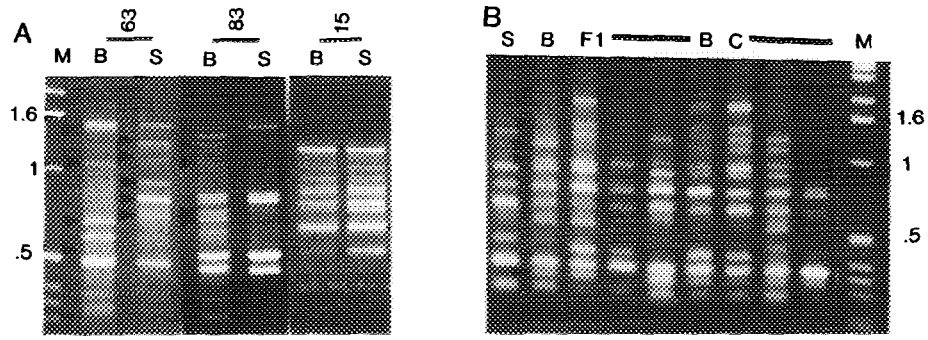

Figure 1.A. Strain specific RAPD markers for Balb/c and SJL/J strains of inbred mouse.

Genomic DNA from mouse strains was amplified using random decamer primers (#63, 83 and 15) according to the standard RAPD protocol. The PCR products were resolved on a 1.5 % agarose gel and visualized by ethidium bromide staining. The photographs were used to score polymorphic bands (arrowheads) . The primers #63, 83, and 15 amplified 9,5 and zero strain specific bands, respectively, of which 8 were Balb/c specific and 6 were SJL/J specific. Primer #15 did not amplify any polymorphic band.

B. Segregation of RAPD markers in a backcross (Balb/c x SJL/J) F1 x SJL/J progeny.

Genomic DNA from parent strains, F1 and backcross animals (n=6) was used in the RAPD assay with primer #28 . The polymorphic bands specific for Balb/c (arrowheads) segregated in the backcross progeny (see text for details).

[M: 1Kb DNA ladder; bands are marked in Kb; B: Balb/c; S: SJL/J; F1: (Balb/cxSJL/J)F1; BC: Backcross (F1xSJL/J)].

721 strain specific bands in zebrafish(4). Averaged over the entire primer set they screened, each primer amplified 7.3 distinct bands, of which about three were specific to one or the other strain of the zebrafish they used.

One of the major limitations of the RAPD technology has been the influence of a number of experimental variables on the banding pattern (7,20). Hence for a RAPD marker to be useful in genetic mapping it should be stably inherited and segregated in the

Table 1

List of RAPD primers that amplify 2 or more strain specific (SP) bands

RAPD Primer*	Sequence (5')	Common Bands	Balb/c SP Bands	SJL/J SP Bands	Total # of strain-SP Bands	Total # of Bands (common + strain SP)
5	CCT GGG TTCC	10	1	2	3	13
16	GGT GGC GGGA	5	2	0	2	7
18	GGG CCG TTTA	3	3	2	5	8
28	CCG GCC TTAA	6	5	0	5	11
29	CCG GCC TTAC	4	2	1	3	7
30	CCG GCC TTAG	5	3	1	4	9
31	CCG GCC TTCC	5	1	1	2	7
32	GGG GCC TTAA	2	7	3	10	12
33	CCG GCT GGAA	3	8	3	11	14
34	CCG GCC CCAA	4	8	1	9	13
43	AAA ACC GGGC	3	7	0	7	10
57	TTC CCC GAGG	2	2	4	6	8
58	TTC CCG GAGC	4	1	5	6	10
59	TTC CGG GTGC	5	1	2	3	8
63	TTC CCC GCCC	4	4	5	9	13
64	GAG GGC GGGA	9	5	1	6	15
67	GAG GGC GAGC	2	6	4	10	12
70	GGG CAC GCGA	6	4	2	6	12
71	GAG GGC GAGG	3	3	0	3	6
72	GAG CAC GGGA	3	3	0	3	6
75	GAG GTC CAGA	5	4	0	4	9
78	GAG CAC TAGC	6	2	1	3	9
80	GTG CTC TAGA	6	1	1	2	8
81	GAG CAC GGGG	7	5	3	8	15
82	GGG CCC GAGG	7	5	4	9	16
83	GGG CTC GTGG	11	4	1	5	16
84	GGG CGC GAGT	5	4	8	12	17
85	GTG CTC GTGC	5	3	0	3	8
86	GGG GGG AAGG	4	1	2	3	7
88	CGG GGG ATGG	4	3	5	8	12
90	GGG GGT TAGG	6	0	2	2	8
92	CCT GGG CTTT	4	2	8	10	14
95	GGG GGG TTGG	9	3	6	9	18
96	GGC GGC ATGG	4	5	2	7	11
98	ATC CTG CCAG	6	5	3	8	14
100	ATC GGG TCCG	10	3	4	7	17
Total:36		191	122	82	204	395

* The random primers were given arbitrary names # 1-100.

backcross or F2 progeny in a Mendelian fashion. Hence we investigated in to the segregation of the RAPD markers in a (Balb/c x SJL/J)F1 x SJL/J backcross progeny (n=6). The RAPD assay performed using primer #28 on the backcross progeny is shown in Fig.1B. In the backcross progeny the polymorphic bands segregated in the proportion 2/6,3/6,3/6,5/6 and 5/6. However, one polymorphic band unique for Balb/c strain was not detected in the F1 animal but segregated in the backcross progeny (3/6) (Fig.1B). Furthermore a number of bands not originating from either of the parental strains were identified in the backcross during this

screening. Appearance of a large excess of non parental bands in RAPD assay has been considered as a confounding factor for its use in the pedigree analysis in primates (21). Furthermore, the intensity of a given band varied among the backcross progeny compared to those of the parents during scoring of markers for the segregation. This may be due to- homozygous or heterozygous nature of the target loci and/or the variation in the total number of the target sites contributing to the band in the backcross progeny. So far we have studied the segregation of a selected set of RAPD markers amplified by 30 primers in this backcross. The segregation of the RAPD markers in the backcross progeny suggested that each of these bands represents distinct locus in the respective parental strains. Taken together all these studies, suggest that RAPD markers are suitable for constructing molecular genetic linkage maps for mouse genome.

The RAPD assay has been used to identify Y chromosome linked RAPD markers in mouse and construction of genetic maps of the chicken Z chromosome (12,22). Woodward et.al. constructed a molecular genetic linkage map using 76 RAPD markers that distinguish C57BL/6J and DBA/2J, using BxD series of recombinant inbred strains (11). Hence, the large number of RAPD markers we have identified here, should facilitate the construction of the genetic map of mouse. In principle these markers can then be integrated with the simple sequence repeat (SSR) based mouse maps which are being advanced at a tremendous speed (1,3). Integration of RAPD based map with the SSR based map should help increase the marker density of the targetted goal of 0.1 cM between markers in the mouse genome. Furthermore, some of the RAPD markers we have identified are potentially useful for linkage analysis of various disease traits that distinguish Balb/c and SJL/J strains.

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REFERENCES

1. Dietrich, W.F., Miller, J.C., Steen, R.G., Merchant, M., Damron, D., Nahf, R., Gross, A., Joyce, D.C., Wessel, M., Dredge, R.D.,

Marquis, A., Stein, L.D., Goodman, N., Page, D.C. and Lander, E.S. (1994) Nature Genetics 7, 220-225.
2. Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M. and Weissenbach, J. (1994) Nature Genetics 7, 246-249.
3. Postlethwait, J.H., Johnson, S.L., Midson, C.N., Talbot, W.S., Gates, M., Ballinger, E.W., Africa, D., Andrews, R., Carl, T., Eisen, J.S., Horne, S., Kimmel, C.B., Hutchinson, M., Johnson, M. and Rodriguez, A. (1994) Science 264, 699-703.
4. Johnson, S.L., Midson, C.N., Ballinger, E.W. and Postlethwait, J.H. (1994) Genomics 19, 152-156.
5. Williams, J.G.K., Kubleik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990) Nucleic acids Res. 18, 6531-6535.
6. Welsch, J. and McClelland, M. (1990) Nucleic acids Res. 18, 7213-7218.
7. Williams, J.G.K., Hanafey, M.K., Rafalski, J.A. and Tingey, S.V. (1993) *Methods in Enzymology*. (Wu, R., ed) vol.218, 704-740.
8. Venugopal, G., Mohapatra, S., Salo, D., and Mohapatra, S.S. (1993) Biochem. Biophys. Res. Commns. 197, 1382-1387.
9. Procunier, J.D., Fernando, M.A., and Barta, J.R. (1993) Parasitol. Res. 79, 98-102.
10. Williams, J.G.K., Reiter, R.S., Young, R.M., and Scolnik, P.A. (1993) Nucleic Acids Res. 21, 2697-2702.
11. Woodward, S.R., Sudweeks, J., and Teuscher, C. (1992) Mamm. Genome 3, 73-78.
12. Wardell, B.B., Sudweeks, J.D., Meeker, N.D., Estes, S.S., Woodward, S.R., Teuscher, C. (1993) Mamm. Genome 4, 109-112.
13. Yu, K., and Pauls, K.P. (1992) Nucleic Acids Res. 20, 2606.
14. Remmers, E.F., Goldmuntz, E.A., Zha, H., Crofford, L.J., Cash, J.M., Mathern, P., Du, Y., Wilder, R.L. (1993) Mamm. Genome 4, 265-270.
15. Lehmann, D., and Ben-Nun, A. (1992) J. Autoimmun. 5, 675-90.
16. Tsiagbe, V.K., Rabinowitz, J.L., and Thorbecke, G.J. (1991) Cell. Immunol. 136, 329-339.
17. Barthold, S.W., Beck, D.S., and Smith, A.L. (1993) Lab. Anim. Sci. 43, 276-284.
18. Djukanovic, R. (1994) Clin and Exptl Allergy 24, 6-9.
19. Caffe, A.R., Szel, A., Juliusson, B., Hawkins, R., Ven-Veen, T. (1993) Cell. Tissue. Res. 271, 297-307.
20. Penner, G.A., Bush, A., Wise, R., Kim, W., Domier, L., Kasha, K., Laroche, A., Scoles, G., Molnar, S.J., and Fedak, G. (1993) PCR Methods and Applications. 2, 341-345.
21. Riedy, M.F., Hamilton III, W.J., and Aquadro, C.F. (1992) Nucleic Acids Res. 20, 918.
22. Levin, I., Crittenden, L.B., and Dodgson, J.B. (1993) Genomics 16, 224-230.