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Mapping genes in the mouse using single-strand conformation polymorphism analysis of recombinant inbred strains and interspecific crosses

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ABSTRACT **We have utilized a PCR-based analysis of single-strand conformation polymorphisms to identify polymorphisms that can be used for mapping cloned DNA sequences in the mouse. We have found that single-strand conformation polymorphism analysis of sequences that are potentially less subject to conservation (i.e., intron and 3′ untranslated regions) is a relatively efficient means of detecting polymorphisms between inbred strains. Fifty percent of the tested primer pairs were polymorphic between inbred strains and 90% were polymorphic between mouse species, which is a frequency comparable to that found for microsatellite repeat sequences. We have found that this technique can be readily used to determine the strain distribution pattern in a recombinant inbred series and is a simple and rapid means to obtain a map position for cloned sequences. When this strategy was tested on a number of previously mapped cloned genes, the strain distribution patterns obtained were consistent with that to be expected on the basis of the known map position. We also tested the utility of this approach for characterizing genes that have not been previously mapped. *Dvl*, the mouse homolog of the putative *Drosophila* dishevelled gene, and *Adfp*, encoding an adipocyte differentiation-related protein, were found to map to chromosome 4. These results were confirmed using single-strand conformation polymorphism analysis of an interspecific backcross.**

Historically, recombination analysis of cloned sequences in inbred mouse strains has been difficult, due to the relatively low frequency of detectable restriction fragment length polymorphisms. Significant progress has recently been made to overcome this difficulty. One extremely fruitful approach has been the utilization of recombination analysis in backcross progeny of interspecific crosses (1). This strategy exploits the observations that polymorphisms between mouse species are abundant and linkage relationships appear to be conserved (2). More recently, several laboratories have characterized large numbers of microsatellite sequences that are often polymorphic even between inbred strains (3–5). These microsatellite regions, which were first utilized for genetic analysis in human gene mapping (6), contain variable length stretches of short repetitive DNA sequences. These markers have been utilized to link the diabetes-susceptibility trait of the nonobese diabetic mouse to loci on mouse chromosomes 3 and 11, by using a cross between inbred strains (7).

We have been exploring an additional means of identifying polymorphisms between inbred strains. This approach involves a PCR-based analysis of single-strand conformation polymorphisms (SSCPs). It has been shown that the migration pattern of a short single-stranded DNA fragment in nondenaturing conditions is extremely sensitive to its DNA sequence, presumably due to alternative secondary structures that can be formed. Analysis of denatured PCR products can in many cases distinguish fragments with a single-base-pair difference (8). We have applied this technique for the purpose of mapping genes in the mouse, by using recombinant inbred (RI) strains (9). By focusing our analysis on regions that are potentially less subject to sequence conservation (i.e., intron and 3′ untranslated regions), we have found that SSCP analysis is a relatively efficient means of detecting polymorphisms between inbred strains, with a frequency comparable to that found for microsatellite repeat sequences. We have found that this technique can be readily used to determine the strain distribution pattern (SDP) in an RI series and is, therefore, a potentially simple and rapid means to obtain a map position for cloned sequences. With this approach we have mapped two genes, *Adfp* (adipocyte differentiation-related protein) and *Dvl* (dishevelled), to chromosome 4 and confirmed their localization using an interspecific backcross. Since it may be feasible to identify polymorphisms in almost any cloned locus (given sufficient sequence information to generate multiple primers), this strategy may also serve to complement the on-going characterization of the mouse genome by facilitating the high-resolution mapping of regions for which polymorphic microsatellite markers have not been obtained.

MATERIALS AND METHODS

PCR. Primers were designed to amplify intron or 3′ untranslated regions of cloned genes by inspection of sequence data. Sequences for *Dvl* were obtained by D.J.S. (unpublished results) and for *Adfp* were provided by D. Eisinger and G. Serrero (W. Alton Jones Cell Science Center, Lake Placid, NY). All other sequence information was obtained from GenBank. Eighteen- to 24-base oligonucleotide primers were synthesized using an Applied Biosystems model 391 DNA synthesizer. The PCR was performed using standard conditions and [^{32}P]dCTP. Specifically, 50-μl reaction mixtures contained 10 mM Tris·HCl, 50 mM KCl, 1 μg of genomic DNA, 1 μM of each primer, and 1.5 mM $MgCl_2$. This mixture was overlaid with oil, denatured at 94°C for 5 min, and transferred to an 80°C heating block. All four dNTPs were added, each to a final concentration of 0.2 mM, including 1.25 μCi of [^{32}P]dCTP [1 μl of a stock solution (3000 Ci/mmol) to eight reactions mixtures; 1 Ci = 37 GBq]. AmpliTaq DNA polymerase (Perkin–Elmer/Cetus; 0.5 μl of 5 units/μl) was added and a PCR was done as follows: 55°C annealing reaction for 1 min, 72°C extension reaction for 2 min, and 94°C denaturation for 1 min. The cycle was repeated 30 times with a final 72°C extension reaction for 5 min after the last cycle. In some instances the PCR products were digested with restriction enzymes by addition of 1 μl of the appropriate

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Abbreviations: SSCP, single-stranded conformation polymorphism; SDP, strain distribution pattern; RI, recombinant inbred.

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10× enzyme buffer and 1 μl of enzyme to 8 μl of the PCR mixture, followed by incubation for 1 hr.

SSCP. The PCR mixture (1.5 μl) was added to 8.5 μl of United States Biochemical stop solution (100% formamide containing xylene cyanol and bromophenol blue), denatured for 5 min at 94°C, and transferred to an ice bucket. A 2-μl sample was loaded on a 5% nondenaturing polyacrylamide gel containing 0.5× TBE and no glycerol (10) and electrophoresed in a 4°C cold room in 0.5× TBE at 40 W (constant power) for 2–3 hr. The gel was transferred to filter paper, dried, and autoradiographed with an intensifying screen overnight.

RI Analysis. Male AKR/J, DBA/2J, C3H/HeJ, C57L/J, C57BL/6J, and *Mus spretus* mice were obtained from The Jackson Laboratory and genomic DNA was prepared from kidneys. DNAs corresponding to individual substrain members of the AKXL, BXD, and BXH RI series were purchased from the DNA Resource of The Jackson Laboratory. SDP analysis was performed using the RI MANAGER program (Version 2.1) (11) obtained from K. Manley (Roswell Park Cancer Institute, Buffalo, NY). Map positions were confirmed in consultation with Ben Taylor of The Jackson Laboratory.

Interspecific Cross. C57BL/6J female mice were crossed with *M. spretus* (F18) males obtained from The Jackson Laboratory. Female progeny were backcrossed with male C57BL/6J animals and the offspring of these matings were sacrificed and used for DNA analysis.

RESULTS

Mapping Known Loci. To test the possible utility of SSCP analysis for the detection of polymorphisms between inbred strains, we made primers corresponding to introns or 3′ untranslated regions of several known genes. These primers were designed to generate PCR fragments of between 100 and 300 base pairs. PCR amplification of the primers was done using DNA prepared from a variety of mice that are represented in several well-characterized RI series: specifically, from AKR/J, DBA/2J, C3H/HeJ, C57L/J, and C57BL/6J mice. DNA from *M. spretus* was also analyzed. All reactions were done with an additional sample containing no genomic template DNA to control for contamination. Initially, the reaction conditions for each primer pair were tested for optimal magnesium concentration, but we have found that generally these short fragments are efficiently synthesized at 1.5 mM $MgCl_2$ and presently do not optimize conditions for individual primer pairs. The PCR was carried out in the presence of [^{32}P]dCTP. An aliquot corresponding to <1% of a 50-μl reaction mixture was denatured and analyzed by electrophoresis.

SSCP protocols will frequently result in complex band patterns, because denaturation is often incomplete and because multiple alternative single-strand conformers can be formed by the same DNA fragment. In spite of this, it was possible to detect reproducible SSCPs between inbred strains for a number of the tested loci. The polymorphic sequences included 3′ untranslated regions from the cytokeratin 8 gene (*Krt2-8*) and from N-myc (*Nmyc-1*) and intron 7 from the p53 tumor suppressor gene (*Trp53*) (Table 1). An example of the polymorphic patterns that were observed is shown for *Krt2-8* in Fig. 1. Polymorphisms between inbred strains were not detected for sequences derived from the 3′ untranslated regions of mouse protamine 1 (*MP1*), mouse protamine 2 (*MP2*), and Wingless-related int-1 (*Wnt-1*). All of the primer pairs except for one *Wnt-1* pair detected polymorphisms between C57BL/6J and *M. spretus* alleles. In some cases, restriction enzyme digestion of the amplified product facilitated resolution of the SSCP. This can be conveniently done on an aliquot of the PCR mixture. For *Trp53*, this resulted in

Table 1. Primer sequences used for SSCP analysis

Locus	Chr	Primer	Sequence	SSCP(s)			
				Allele 1	Allele 2	Allele 3	Allele 4
Krt2-8	15	DB7	GGAGTCTGGGCTGCAGAACATGAGCATTC	A, D, B	L	H	Sp
		DB8	CAACTGAATTCGGTTTGGATGGGAGGC				
Nmyc-1	12	DB35	GACTTGCTAAACGTTTCCCAC	A, H, D, Sp	L, B		
		DB36	ACAAGTGTTCCCAAGGGCATC				
Trp53	11	RH7	TGTAATAGCTCCTGCATGGGG	A, H, L, B	D*		Sp
		RH8	TTCTGTACGGCGGTCTCTCCC				
Dvl	4	Dsh4A	AAGTTCATGGGCCTCACCACCTGTC	A, H, D	B, L		Sp
		Dsh4B	TACTAGCTACCCTTCACATACC				
		Dsh5A	ACCTAGCCACTGTCTCAGTCT	D	B		Sp
		Dsh5B	ACAGAAGCAGCATTTACACAG				
Adfp	4	Dend154	TCAAGGCGTGTCCTTG	A, L, B	H, D		Sp
		Dend152	GCACAAAGCACATGTAACAAC				
		DEEX1	AGCAGTAGTGGATCCGCAACA	A, H, D, L, B			Sp
		DEEX2	AGGACACAAGGTCGTAGGTAGAGCTC				
Pnd	4	DB41	GACTAGGCTGCAACAGCTTCC	A, H, L, D	B		Sp
		DB42	CTCCTTGGCTGTTATCTTCGG				
Lck	4	DB43	CAGCCTTGATAGGCTTTCGG	A, H, D, Sp	L, B†		
		DB44	CCTGGAGACCCTCTGAAAGG				
Trpl(b)	4	DB45	CAGCAGAGAAACCTTCCAGG	A, B, H	D, L‡		Sp
		DB46	GTGCTCAGATGAAAATACAGC				
Ifα	4	DB47	CTCTGCCAATGTGCTGGGAAG	H, B, D, L	A		Sp
		DB48	CATAACTTTACCAAATTATGCTGC				
Lmyc	4	DB49	GTACCTCAGTGGCTACTAACC	A, H, B, D, L	(A)§		Sp¶
		DB50	GTGAGATTTCACGTAGGCTGC				

Chr, chromosome.
*Polymorphism is in a *Dde* I restriction site.
†Polymorphism is best resolved on a 7.5% polyacrylamide gel.
‡Polymorphism is best resolved after *Hin*fI digestion.
§Extra band in AKR/J is *Lmyc-2*, possible pseudogene.
¶Polymorphism is best resolved after *Taq* I digestion.

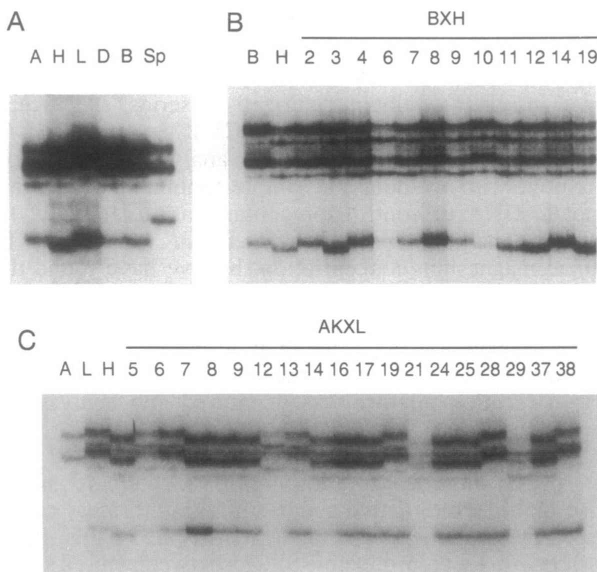

FIG. 1. SSCP analysis of cytokeratin 8 (*Krt2-8*). (*A*) SSCP pattern of *Krt2-8* in six strains of mice. Analysis was performed with the DB7 and DB8 primer pair (Table 1). Lanes: A, AKR/J; H, C3H/HeJ; L, C57L/J; D, DBA/2J; B, C57BL/6J; Sp, *M. spretus*. (*B*) The SDP of SSCPs of *Krt2-8* in the BXH RI series. (*C*) The SDP of SSCPs of *Krt2-8* in the AKXL RI series. (*B* and *C*) Numbered lanes refer to members of the RI series indicated.

the identification of a *Dde* I site in intron 7 that is polymorphic between inbred strains (Table 1).

To test the utility of the SSCP for gene mapping, we used the polymorphisms found between the inbred lines to obtain a SDP in appropriate RI series. The RI MANAGER program (11) was used to obtain a map position for these loci, based on a comparison of these SDPs with previously characterized markers in the respective RI series. We found that, although the SSCP patterns were complex, their analysis was unambiguous; i.e., a strain origin for the tested locus could be assigned for each substrain of an RI series by inspection (Fig. 1). In all cases the predicted map position corresponded to that previously reported for these loci, which were based on other methods of chromosomal localization, such as multipoint linkage analysis or somatic cell hybrids (12–14). The SDPs obtained for these loci are shown in Tables 2–4. For *Nmyc-1*, the SDP we obtained was discordant from that previously published using PCR analysis of a polymorphic microsatellite sequence (4). We synthesized the primer pair reported by Hearne *et al.* (4), repeated the analysis of the discordant RI substrains according to their protocol, and thus confirmed our original result (data not shown).

Mapping Unknown Genes by Using SSCP. To test the utility of SSCP for mapping cloned sequences using RI strains, we analyzed two previously unmapped genes. Five sets of primers corresponding to introns or 3′ untranslated regions of the *Dvl* (dishevelled) gene were synthesized. *Dvl* is the mouse homolog of the recently characterized *Drosophila Dsh* (dishevelled) gene, which likely plays a role in signal transduction of *Wnt-1* (N. Perrimon, personal communication). Four of the five primer pairs were polymorphic between inbred strains and all five were polymorphic between C57BL/6J and *M. spretus*. Primer-pair Dsh4 (Table 1) was used to determine the SDP of this locus in the AKXL and BXD RI series (Fig. 2 and Tables 2–4). In both cases, the SDP was consistent with a map position near the telomere of mouse chromosome 4, distal to *Gpd-1* (Table 5). Analysis of *Dvl* in the BXH RI series is consistent with this position, but the confidence level for this mapping analysis is <95% (15) (Table 5). To ensure that the locus identified by the Dsh4 primer pair was in fact *Dvl* (and not a different but homologous sequence), the SDP in a subset of the AKXL series was determined using two of the other *Dvl* primer pairs; these were found to be identical to that found for the Dsh4 primers (data not shown).

Table 2. SDPs of SSCPs in BXH RI strains

Locus	Chr	BXH 2	3	4	6	7	8	9	10	11	12	14	19
Adfp	4	H	B	H	B	B	B	H	B	B	H	B	B
Dvl	4	B	B	B	H	B	B	B	B	H	H	B	H
Krt2-8	15	B	H	B	B	B	B	B	H	H	H	B	H

Chr, chromosome.

A similar analysis was performed on six primer pairs derived from 5′ genomic, intron, and 3′ untranslated sequences of *Adfp* (adipocyte differentiation-related protein), a gene that is induced upon adipocyte differentiation (16). In this case only one of the primer pairs was polymorphic in inbred strains, and four were polymorphic between C57BL/6J and *M. spretus* (two primer pair sequences are included in Table 1). The 154 primer pair was analyzed in the BXD RI series, and the SDP obtained for this gene suggests it also resides on mouse chromosome 4, tightly linked to the *b* (*Trp-1*, tyrosine-related protein 1) and *Ifα* (interferon α) loci (Table 5). The colocalization of these genes on mouse chromosome 4 was unexpected but fortuitous, since it facilitated multipoint linkage analysis, as described below.

To confirm the map positions of *Dvl* and *Adfp* obtained by SSCP analysis of RI strains, we analyzed their segregation with respect to known markers on chromosome 4 in an interspecific cross. DNA was prepared from the backcross progeny of (C57BL/6J × *M. spretus*) F_1 hybrid females mated with C57BL/6J males. Primer pairs from *Dvl* and *Adfp* (differing from those previously used for the RI analysis) were analyzed by SSCP in 51 progeny. In this case, since all mice inherit a C57BL/6J allele from the paternal side, one simply scores for the presence or absence of a maternally inherited *M. spretus* allele. Again, SSCP proved useful for this purpose and is illustrated in Fig. 2. Primer pairs corresponding to reference loci on chromosome 4, including *Trp-1*(*b*), *Ifα*, *Lmyc-1*, *Lck*, and *Pnd*, were synthesized (Table 1). All loci were found to be polymorphic by SSCP analysis between C57BL/6J and *M. spretus* and were tested in the interspecific backcross progeny. No recombinants were observed between *Dvl* and *Pnd* in 51 backcross progeny. One recombinant between *Trp-1* and *Adfp* and one recombinant between *Adfp* and *Ifα* were observed in 50 progeny. The compilation of these results, which confirms the map position suggested by RI analysis, is shown in Table 5.

All five reference loci were also found to be polymorphic by SSCP analysis between inbred strains (Table 1), and

Table 3. SDPs of SSCPs in AKXL RI strains

Locus	Chr	AKXL 5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38
Dvl	4	L	A	A	L	A	L	L	L	L	A	A	A	L	A	L	L	A	A
Krt2-8	15	L	L	A	A	A	L	L	A	A	A	L	A	A	A	L	A	A	L
Lmyc-2	12	L	A	L	A	A	L	L	A	L	A	A	L	A	A	L	A	A	L

Chr, chromosome.

Table 4. SDPs of SSCPs in BXD RI strains

Locus	Chr	BXD																									
		1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Trp-1(b)	4	D	B	B	B	B	D	B	B	D	B	D	B	B	B	B	D	B	B	D	D	D	D	B	D	B	B
Adfp	4	D	B	B	B	U	D	B	B	D	U	D	B	B	D	B	D	D	B	D	D	D	D	B	D	B	B
Ifα	4	D	B	B	B	B	D	B	D	D	B	D	B	B	D	B	D	D	B	D	D	D	D	B	D	B	B
Lck	4	D	B	B	B	D	D	B	D	D	D	U	D	D	B	B	D	B	D	B	D	B	D	B	D	B	D
Dvl	4	D	D	B	B	D	D	B	D	D	D	B	D	D	B	B	D	D	B	B	B	D	D	B	D	B	D
Trp53	11	B	D	B	D	B	B	B	B	D	B	D	D	B	D	B	D	B	B	B	B	B	D	D	B	B	D
Nmyc-1	12	B	D	B	B	D	D	D	D	B	D	B	B	B	B	D	D	D	D	D	B	D	B	B	B	D	D

Chr, chromosome.

several were tested in RI series. The SDPs obtained for *Pnd* in BXD and *Trp-1(b)* in AKXL were identical to those previously reported (17–19). The SDP for *Lck* in BXD differs from that previously reported for one substrain, BXD-19, although both map to the same region of chromosome 4 (18). Additionally, the SDP we obtained corresponds to that found for *Lck* by Ben Taylor (personal communication). *Ifα* was not tested in RI strains.

The results we obtained with *Lmyc-1*, however, illustrate the hazard of this technique. The SDP of the apparent polymorphism we observed does not map to chromosome 4 but rather is identical with that of *Lmyc-2*, a homologous locus that is possibly a pseudogene (14). Examination of the undenatured PCR products does, in fact, reveal additional bands in AKR/J that are absent from other lines (data not shown). The major band, which corresponds to *Lmyc-1*, is not polymorphic.

DISCUSSION

We have tested the utility of SSCP analysis of PCR products from cloned genes for the detection of polymorphisms between mouse strains. This is a simple and sensitive technique that can detect a single base difference in DNA fragments (8). We have found that by focusing this analysis on 3′ untranslated regions and introns, which may be subject to less conservation pressure than coding sequences, useful polymorphisms are readily detected, even between inbred strains. Thirteen of the 30 primer pairs (43%) tested to date were polymorphic between C57BL/6J and DBA/2J or between AKR/J and C57L/J (Table 1 and data not shown). Twenty-six of 30 (86%) were polymorphic between C57BL/6J and *M. spretus*. These frequencies of polymorphism are only slightly less than those found for microsatellite markers (4, 5). The characterization of SSCPs in cloned sequences has the additional virtue that they identify polymorphic sequence-tagged sites in expressed genes.

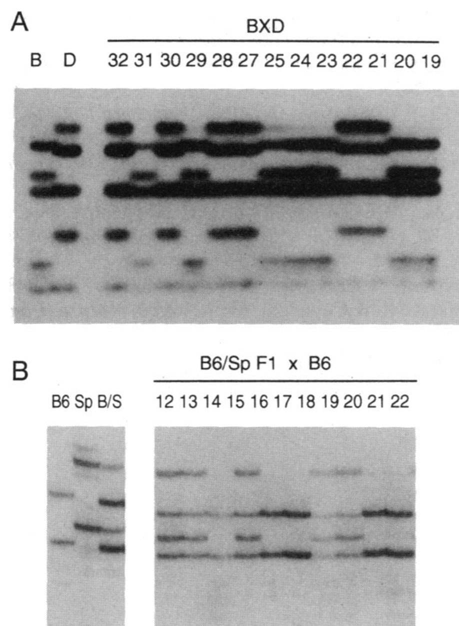

FIG. 2. SSCP analysis of dishevelled (*Dvl*). (*A*) The SDP of SSCPs of *Dvl* in 13 substrains of the BXD RI series. Analysis was performed with the Dsh4 primer pair (Table 1). Lanes: B, C57BL/6J; D, DBA/2J. (*B*) The SDP of SSCPs of *Dvl* in 11 backcross progeny of a C57BL/6J × *M. spretus* interspecific cross. Analysis was performed with the Dsh5 primer pair (Table 1). Lanes: B, C57BL/6J; Sp, *M. spretus*; B/S, (C57BL/6J × *M. spretus*) F_1. (*A* and *B*) Numbered lanes refer to members of the BXD RI series or the interspecific cross indicated.

Analysis of these polymorphisms in RI strains provides a rapid and convenient means to map cloned sequences in the mouse. We tested this strategy on a number of cloned genes whose map positions were known. Polymorphisms were detected in 7 of 11 genes tested. For *Krt2-8, Nmyc-1, Trp53, Pnd, Lck*, and *Trpl(b)*, the SDP of the SSCP either corresponded to those reported previously for these loci or is consistent with that to be expected on the basis of the known map position. For *Lmyc-1*, an apparent polymorphism identified a homologous locus present in AKR/J.

We also tested the utility of this approach for analyzing genes that were not previously mapped. Primer pairs were prepared from intron and 3′ untranslated sequence of *Dvl*, the mouse homolog of the putative *Drosophila dsh* (dishevelled) gene. In both the BXD and AKXL strains, the SDP of *Dvl*

Table 5. Recombination analysis of SDPs

Strain	Genes	Chr	×	*N*	Map	SE	95% CI
BXH	*Adfp:Ifα*	4	1	12	2.38	2.61	0.1–22.8
	Gpd-1:Dvl	4	3	12	10.00	8.00	NL
	Krt2-8:Wnt-1	15	0	12	—	—	0.0–11.0
AKXL	*Gpd-1:Dvl*	4	2	18	3.33	2.67	0.4–18.1
	*Krt2-8:Tgn**	15	10	18	—	—	NL
BXD	*Trp-1(b):Adfp*	4	2	24	2.38	1.84	0.3–11.3
	Adfp:Ifα	4	1	24	1.11	1.16	0.0–7.7
	Lck:Ahd-1	4	2	25	2.27	1.75	0.2–10.7
	Dvl:Tel4q	4	2	26	2.17	1.67	0.2–10.1
	Trp53:Zfp3	11	0	26	—	—	0.0–4.1
	Nmyc-1:D12Nyu10	12	1	26	1.02	1.06	0.0–7.0
Inter-	*Adfp-2:Trpl(b)*	4	7	51	4.32	1.91	1.6–10.8
specific	*Trpl(b):Adfp*	4	1	51	0.51	0.52	0.0–3.1
backcross	*Adfp:Ifα*	4	1	51	0.51	0.52	0.0–3.1
	Ifα:Lmyc-1	4	12	50	9.37	3.69	4.1–22.3
	Lmyc-1:Pnd	4	10	50	7.14	2.89	3.0–17.1
	Pnd:Dvl	4	0	51	—	—	0.0–1.9

Recombination between loci analyzed by SSCP and closely linked markers was calculated with the program RI MANAGER (11). Chr, chromosome; ×, crossover(s); *N*, number of strains; map, recombination distance; SE, standard error; 95% CI, 95% confidence interval; NL, not linked with >95% likelihood.

**Tgn* is the only marker mapped on chromosome 15 in the AKXL series.

predicted a map position on chromosome 4, close to the telomere. This was confirmed using SSCP analysis of a different primer pair in an interspecific backcross. Analysis of the dishevelled phenotype in *Drosophila* suggests that it likely plays a role in *Wnt-1* signal transduction (N. Perrimon, personal communication). The importance of *Wnt-1* gene in mammalian neurological development has been clearly demonstrated by "knock-out" experiments, in which its disruption has profound effects on the formation of cerebellar and midbrain structures (20, 21). Inspection of the genetic map reveals two neurological mutations, *cri* (cribriform degeneration) and *je* (jerker), close to the predicted *Dvl* map position, for which it is a potential candidate locus.

We also analyzed the *Adfp* gene in the BXD and BXH inbred strains by using SSCP. This suggested a map position for *Adfp* on chromosome 4, between the *Trp-1(b)* and *Ifα* loci. The analysis of interspecific backcross progeny by SSCP (using an independent primer pair) also confirmed this result.

Since polymorphisms could frequently be detected for 3′ untranslated sequences, the potential utility of this approach for mapping cloned cDNA sequences is obvious. There are additional advantages to SSCP analysis for genetic mapping. By using RI strains, the map density of these resources is increased, which further enhances their utility for genome analysis. Additionally, since only a small amount of DNA is required for PCRs, this approach is also useful for interspecific backcrosses, in which the DNA derived from individual progeny is a finite and increasingly precious resource as the characterization of the cross proceeds. The major qualification of this procedure is that the major bands detected may not correspond to the locus of interest, but instead to a region of homology that may be unrelated. It is therefore advisable that a putative map position obtained using a single primer pair be confirmed by additional investigation; for example, by mapping a different primer pair from the same gene, using either RI or interspecific backcrosses. Alternatively, direct sequencing of the PCR product used for RI analysis can be done to confirm that it corresponds to the locus of interest.

Finally, the power of this technique for mapping mutations and traits in the mouse should also be clear. In this regard, this strategy complements the efforts to characterize polymorphic microsatellite markers that span the mouse genome (3–5). If a mutation or trait is found to fall between such markers in a cross between inbred strains, it should be feasible to more finely map it by testing cloned sequences that are known to reside in the marked interval for single-strand conformer polymorphisms. Given sufficient noncoding sequence information and primer pairs, it is possible that polymorphisms can be detected for any cloned gene, in which case very high-density maps of specific subchromosomal regions may be readily obtainable.

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