SHORT COMMUNICATION

Identification of RAPD Primers That Reveal Extensive Polymorphisms between Laboratory Strains of Zebrafish

STEPHEN L. JOHNSON,*,[1] CLARE N. MIDSON,† ERIC W. BALLINGER,† AND JOHN H. POSTLETHWAIT†

*Institutes of *Neuroscience and †Molecular Biology, 1254 University of Oregon, Eugene, Oregon 97403*

Received June 28, 1993; revised September 26, 1993

The zebrafish has recently emerged as a useful system for understanding vertebrate developmental genetics, despite the lack of a linkage map. To identify DNA-based genetic polymorphisms for constructing a genetic map, we have screened a collection of RAPD primers for their utility in identifying genetic polymorphisms between two laboratory strains of zebrafish. Here, we report 116 primers that identify 721 strain-specific genetic markers and show how they can be used in haploid genetics of zebrafish. © 1994 Academic Press, Inc.

A genetic map is essential for making rapid progress in dissecting developmental processes by mutations. The zebrafish (*Brachydanio rerio*) has begun to contribute to our understanding of vertebrate developmental biology (2, 5) because its embryos are easy to observe and manipulate and because haploid or gynogenetically derived diploid fish (6) can be rapidly screened for new mutations (Kimmel, 1989; S. Johnson, unpublished results). Unfortunately, the genetic map of the zebrafish is rudimentary—only a few mutations with visible phenotypes have been located relative to their centromeres (7), and no loci have yet been shown to be linked. To facilitate the analysis of mutant genes that disrupt interesting developmental processes, we have begun to develop a molecular genetic map for the zebrafish. Because the zebrafish has 25 metacentric chromosomes and the genetic map of zebrafish may be quite large, a large number of genetic markers will be required to span the genome evenly. Preliminary results suggest a map about 4000 cM long (C. Midson and J. Postlethwait, unpublished results); thus, 400 evenly distributed genetic markers would be required for a 10-cM resolution map.

Several attributes characterize the types of genetic markers most useful for a detailed map. DNA-based genetic markers should be numerous, widely distributed throughout the genome, and readily cloned and sequenced. Moreover, to take full advantage of haploid zebrafish embryos that facilitate gene mapping, many genetic loci should be easily and accurately assessed from the small amount of material available in individual haploid embryos before they die at approximately 5 days. Genetic markers identified as random amplified polymorphic DNAs (RAPDs) from polymerase chain reactions (PCR) using decamer primers seem to fit these criteria (8). A single decamer primer in a PCR amplifies several bands from genomic DNA of each individual due to short inverted repeats located 50 to 3000 bp apart. Mutations at the primer binding sites or insertions and deletions between binding sites can create recessive, or less frequently, codominant genetic variants (8). Furthermore, RAPD markers have been used to successfully construct linkage maps in various plants (3, 4).

Because a RAPD primer may identify polymorphisms between one pair of strains but not others, it is important to use standard strains in the construction and application of a RAPD map. Because most zebrafish mutations have been isolated in the partially inbred AB strain (C. Kimmel, pers. comm.) or its clonal derivative, C32 (S. Johnson, unpublished results), we chose this genetic background as one of the reference strains for the zebrafish map. To identify a second reference strain, we used RAPD primers to screen several wildtype strains of zebrafish for genetic dissimilarity with AB and C32. Tested strains included Darjeeling, Hong Kong, Singapore, and Indonesia (courtesy of D. Stanier, M. Halpern, and M. Westerfield). These experiments showed that the Darjeeling strain was most polymorphic relative to the two laboratory strains AB and C32 (data not shown). Moreover, since a limited set of SSR (simple sequence repeat) markers has already been used to identify extensive polymorphism between the Darjeeling and AB strains (1), we have chosen to focus on Darjeeling or its partially inbred derivative, DA (S. Johnson, unpublished results), as the second reference strain for this project.

We identified primers that reveal a substantial amount of genetic polymorphisms between these genetic backgrounds by amplifying DNA from C32 and DA with individual RAPD primers (Operon Technologies, Alameda, CA), and comparing amplification products for strain-specific differences (Fig. 1). In general, primers amplified several bands from each strain. Some bands were strain-specific (for example, see primer 13B in Fig. 1A, lanes d and e), whereas other primers amplified the same bands from each strain (primer 15B; Fig. 1A, lanes

[1] To whom correspondence should be addressed. Telephone (503) 346-5188. Fax: (503) 346-4548.

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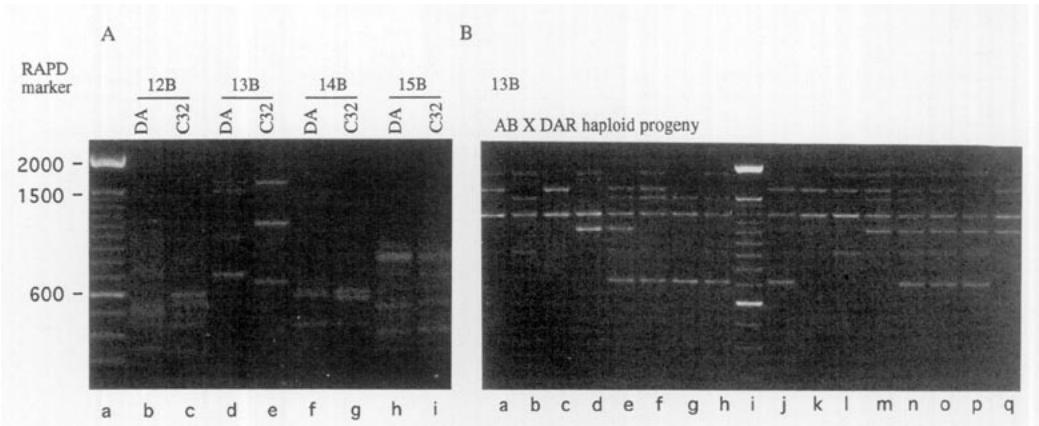

FIG. 1. Identification of useful RAPD primers. (**A**) PCR products from decamer-primed genomic DNA from the DA strain (lanes **b, d, f,** and **h**) and C32 strain (lanes **c, e, g,** and **i**) using primers 12B (lanes **b** and **c**), 13B (lanes **d** and **e**), 14B (lanes **f** and **g**), and 15B (lanes **h** and **i**). DNA was extracted individually from haploid embryos of each strain, and then the DNAs from 20 embryos were mixed together. Five nanograms of RAPD primers was used in each reaction. The sizes of standards run in lane **a** are indicated to the left. (**B**) Mendelian segregation of RAPD loci. PCR was performed with primer 13B on DNA extracted from individual haploid embryos, which were obtained from an AB/Darjeeling (DAR) hybrid female as described (6, 7) (lanes **a–h,** and **j–q**). DNA was extracted from individual embryos by incubating for 10 min at 98°C in 50 ml of 0.3% Tween, 0.3% NP-40. Proteinase K was then added to a concentration of 1 mg/ml and the mix was incubated a further 30 min at 55°C, followed by 10 min at 98°C. After centrifugation, the supernatant was diluted 50-fold in distilled water. PCR reactions were performed according to Williams *et al.* (8), except that 1.5 m*M* $MgCl_2$ was used, denaturation was performed at 92°C, and only 36 cycles were performed.

h and i). The first set of 382 RAPD primers we tested identified a total of 2445 distinct amplified bands, 1153 (47%) of which appeared to be strain-specific and, thus, potentially useful as markers for constructing a linkage map. Averaged over the entire set, each primer amplified 7.3 distinct bands, of which about three were specific to one or the other strain. Table 1 lists a selected set of 116 primers that amplify 5 or more strain-specific bands. The other 266 primers revealed fewer or no polymorphisms between these two strains. This selected set of primers (31% of those screened) identifies a total of 721 strain-specific bands, for an average of 6.2 strain-specific bands per primer.

For RAPD markers to be useful in genetic mapping, they must segregate in crosses in a Mendelian fashion. To test this possibility, we screened the haploid progeny from an individual AB/Darjeeling hybrid female for the segregation of strain-specific bands. Because each haploid embryo represents a clone of a haploid gamete, we expected to see 1:1 segregation of markers. Following amplification of individual haploid DNA with the primer 13B, most of the bands segregated to approximately 50% of the embryos. Fig. 1B shows independent segregation of bands of 720 (8/16 embryos), 910 (7/16), 1120 (7/16), 1500 (7/16), 1650 (11/16), and 1950 (11/16) bp, suggesting that each of these bands identifies a distinct genetic locus ($P = 0.2$ with a χ^2 test). The bands segregating at 720 and 1500 bp had counterparts of similar mobility in the DA-specific bands in Fig. 1A, suggesting that these markers came from the Darjeeling parent, whereas bands segregating at 910, 1120, and 1950 had their counterparts in C32-specific bands in Fig. 1A. Because the grandparents used in this cross (Fig. 1B) are different (though related) from the fish used to identify the most informative primers (Fig. 1A), some markers in the cross are not found in the amplified DNA from C32 or DA, shown in Fig. 1A. The band segregating at 1650 bp had no counterpart in either the C32 or DA strain-specific band. It is, nevertheless, found in the Darjeeling population that contributed a grandparent to this cross (not shown). Likewise, a band at 1300 bp is amplified from all haploid embryos, suggesting that the marker should be in each grandparent. Examination of Fig. 1A shows that this marker is absent from the clonal line C32; however, experiments not shown demonstrate that this marker is in fact found in individuals of the AB population used for the cross. Together, these data suggest Mendelian inheritance of RAPD markers necessary for genetic mapping.

Since our initial mapping experiments suggest a genetic map of about 4000 cM, this set of primers should allow us to construct a genetic map with average spacing of about 6 cM from 116 sets of RAPD PCR reactions. The PCR conditions employed in these investigations permit about 500 reactions (and hence up to 500 primers) with the DNA extracted from a single haploid embryo. If the average RAPD primer employed identifies five segregating polymorphisms in hybrid crosses, it should be possible to score nearly 2500 genetic loci from a single haploid embryo. Moreover, since a single hybrid zebrafish can give well over 100 eggs in a single clutch, mapping resolution of about 1 cM is readily attainable. Because most mutant screens in zebrafish are performed in the clonal C32 line or its partially inbred parent line, AB, the map derived from a single hybrid cross between C32 and DA should be informative for crosses between most mutant lines and DA. In fact, the methods described here for using RAPD primers are robust for a variety of mapping experiments. Initial experiments have utilized haploid techniques, as described here, to identify markers tightly linked to embryonic pigment pattern mutations (*albino* and *sparse;* 7), whereas DNA

TABLE 1

Selected RAPD Primers That Identify Five or More Strain-Specific Bands

RAPD primer[a]	Sequence	Common bands	C32-specific bands	DA-specific bands	Strain-specific bands	Total bands
1A	CAGGCCCTTC	4	3	3	6	10
8A	GTGACGTAGG	2	2	3	5	7
14A	TCTGTGCTGG	1	3	2	5	6
18A	AGGTGACCGT	7	4	3	7	14
1B	GTTTCGCTCC	1	7	4	11	12
4B	GGACTGGAGT	1	1	5	6	7
6B	TGCTCTGCCC	4	2	3	5	9
7B	GGTGACGCAG	7	4	1	5	12
8B	GTCCACACGG	2	3	6	9	11
11B	GTAGACCCGT	4	5	1	6	10
13B	TTCCCCCGCT	2	3	3	6	8
14B	TCCGCTCTGG	3	3	2	5	8
20B	GGACCCTTAC	1	3	2	5	6
3D	GTCGCCGTCA	3	4	1	5	8
5D	TGAGCGGACA	6	4	3	7	13
8D	GTGTGCCCCA	2	5	5	10	12
11D	AGCGCCATTG	4	3	3	6	10
1E	CCCAAGGTCC	0	3	3	6	6
2E	GGTGCGGGAA	4	3	2	5	9
5E	TCAGGGAGGT	1	5	2	7	8
7E	AGATGCAGCC	0	2	3	5	5
16E	GGTGACTGTG	3	3	2	5	8
17E	CTACTGCCGT	3	4	3	7	10
20E	AACGGTGACC	1	3	2	5	6
1F	ACGGATCCTG	8	4	2	6	14
3F	CCTGATCACC	0	2	3	5	5
5F	CCGAATTCCC	1	2	5	7	8
6F	GGGAATTCGG	1	2	3	5	6
9F	CCAAGCTTCC	0	1	9	10	10
12F	ACGGTACCAG	4	4	2	6	10
2G	GGCACTGAGG	7	3	2	5	12
4G	AGCGTGTCTG	5	6	3	9	14
9G	CTGACGTCAC	1	1	4	5	6
10G	AGGGCCGTCT	1	5	1	6	7
12G	CAGCTCACGA	1	3	2	5	6
13G	CTCTCCGCCA	2	3	2	5	7
16G	AGCGTCCTCC	6	3	2	5	11
2H	TCGGACGTGA	3	4	1	5	8
5H	AGTCGTCCCC	1	4	1	5	6
12H	ACGCGCATGT	3	7	4	11	14
20H	GGGAGACATC	4	3	4	7	11
1I	ACCTGGACAC	10	3	2	5	15
10I	ACAACGCGAG	1	4	3	7	8
11I	ACATGCCGTG	4	4	3	7	11
16I	TCTCCGCCCT	1	3	4	7	8
19I	AATGCGGGAG	2	8	0	8	10
1K	CATTCGAGCC	7	2	4	6	13
4K	CCGCCCAAAC	2	5	2	7	9
7K	AGCGAGCAAG	2	4	4	8	10
13K	GGTTGTACCC	3	3	2	5	8
16K	GGTTGTACCC	2	2	3	5	7
20K	CCTAGTCGAG	1	4	2	6	7
3L	CCAGCAGCTT	6	3	2	5	11
6L	GAGGGAAGAG	4	4	2	6	10
12L	GGGCGGTACT	3	2	5	7	10
2M	ACAACGCCTC	6	5	2	7	13
3M	GGGGGATGAG	4	3	3	6	10
4M	GGCGGTTGTC	5	1	4	5	10
5M	GGGAACGTGT	5	2	4	6	11
12M	GGGACGTTGG	5	4	1	5	10
13M	GGTGGTCAAG	6	7	4	11	17
14M	AGGGTCGTTC	1	4	2	6	7
17M	TCAGTCCGGG	1	4	1	5	6
20M	AGGTCTTGGG	4	3	2	5	9

TABLE 1—*Continued*

RAPD primer[a]	Sequence	Common bands	C32-specific bands	DA-specific bands	Strain-specific bands	Total bands
2N	ACCAGGGGCA	10	3	2	5	15
7N	CAGCCCAGAG	6	3	4	7	13
1O	GGCACGTAAG	3	2	3	5	8
3O	CTGTTGCTAC	3	4	1	5	8
4O	AAGTCCGCTC	4	5	0	5	9
6O	CCACGGGAAG	6	5	2	7	13
7O	CAGCACTGAC	8	3	2	5	13
9O	TCCCACGCAA	2	3	2	5	7
10O	TCAGAGCGCC	5	3	2	5	10
11O	GACAGGAGGT	2	4	2	6	8
15O	TGGCGTCCTT	3	4	3	7	10
18O	CTCGCTATCC	2	2	3	5	7
6P	GTGGGCTGAC	4	10	6	16	20
14P	CCAGCCGAAC	9	5	2	7	16
6Q	GAGCGCCTTG	7	3	4	7	14
15Q	GGGTAACGTG	8	2	3	5	13
1R	TGCGGGTCCT	2	4	1	5	7
2R	CACAGCTGCC	11	3	3	6	17
10R	CCATTCCCCA	1	3	3	6	7
12R	ACAGGTGCGT	4	2	3	5	9
15R	GGACAACGAG	1	5	3	8	9
20R	ACGGCAAGGA	4	3	2	5	9
3S	CAGAGGTCCC	6	5	3	8	14
9S	TCCTGGTCCC	5	4	1	5	10
10S	ACCGTTCCAG	3	4	3	7	10
11S	AGTCGGGTGG	6	3	2	5	11
13S	GTCGTTCCTG	2	3	2	5	7
14S	AAAGGGGTCC	3	4	3	7	10
1V	TGACGCATGG	4	4	3	7	11
2V	AGTCACTCCC	2	2	3	5	7
8V	GGACGGCGTT	5	3	2	5	10
15V	CAGTGCCGGT	5	3	2	5	10
17V	ACCGGCTTGT	0	1	5	6	6
20V	CAGCATGGTC	5	3	2	5	10
3W	GTCCGGAGTG	4	3	3	6	10
4W	CAGAAGCGGA	4	4	2	6	10
5W	GGCGGATAAG	0	4	3	7	7
8W	GACTGCCTCT	4	4	2	6	10
16W	CAGCCTACCA	4	6	0	6	10
3X	TGGCGCAGTG	6	2	3	5	11
4X	CCGCTACCGA	4	4	1	5	9
13X	ACGGGAGCAA	9	4	2	6	15
16X	CTCTGTTCGG	0	2	3	5	5
17X	GACACGGACC	3	5	2	7	10
19X	TGGCAAGGCA	3	2	3	5	8
1Y	GTGGCATCTC	2	3	2	5	7
3Y	ACAGCCTGCT	3	8	3	11	14
4Y	GGCTGCAATG	3	5	2	7	10
8Y	AGGCAGAGCA	1	3	2	5	6
13Y	GGGTCTCGGT	5	4	3	7	12
17Y	GACGTGGTGA	2	7	2	9	11
20Y	AGCCGTGGAA	2	2	3	5	7
Total	116	413	415	306	721	1134

[a] The listed primers are the most useful among the Operon primer sets screened: A, B, D–I, K–S, and V–Y. From these sets, 38 primers did not reliably amplify DNA and were not included in the analysis. Because Operon's primer code names resemble genetic nomenclature for zebrafish (b13, for example, is an allele of *golden;* OPB-13 is an Operon primer), we abbreviate and invert the Operon code for use in the zebrafish map. Thus, Operon primer OPB-13 becomes 13B. Specific markers amplified by a primer are designated by the transposed primer name followed by the size of the amplified product. For instance, the marker 13B720 migrates at 720 bp and segregates in the cross shown in Fig. 1B.

from diploid adults has proven useful for identifying markers linked to adult pigment pattern or fin morphogenesis mutations (S. Johnson, unpublished results). Moreover, RAPD markers are easily cloned (9), facilitating comparison of linkage relationships between test crosses and a standard map. Such a map promises to facilitate isolation and characterization of interesting developmental mutations and hence further our knowledge of vertebrate embryology.

ACKNOWLEDGMENTS

We thank Dana Africa and Heidi Howard for technical assistance and J. A. Weston for encouragement and support. This work was supported by NIH Grants HD07470 to S. Johnson, RO1 AI26734 to J. Postlethwait, and PO1 HD22486 (J. Weston, P.D.).

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