SHORT COMMUNICATION

Centromere-Linked Microsatellite Markers for Linkage Groups 3, 4, 6, 7, 13, and 20 of Zebrafish (*Danio rerio*)

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A large number of interesting mutations affecting development and organogenesis have been identified through genetic screens in zebrafish. Mapping of these mutations to a chromosomal region can be rapidly accomplished using half-tetrad analysis. However, knowledge of centromere-linked markers on every chromosome is essential to this mapping method. Centromeres on all 25 linkage groups have been mapped on the RAPD zebrafish genetic map. However, species specificity and the lack of codominance make RAPD markers less practical for mapping than microsatellite-based markers. On the microsatellite-based genetic map, centromere-linked markers have been identified for 19 linkage groups. No direct evidence has been published linking microsatellite markers to the centromeres of linkage groups 3, 4, 6, 7, 13, and 20. Therefore, we compared the microsatellite-based genetic map with the RAPD map to identify markers most likely linked to the centromeres of these 6 linkage groups. These candidate markers were tested for potential centromere linkage using four panels of half-tetrad embryos derived by early-pressure treatment of eggs from four different female zebrafish. We have identified microsatellite markers for linkage groups 3, 4, 6, 7, 13, and 20 to within 1.7 cM of their centromeres. These markers will greatly facilitate the rapid mapping of mutations in zebrafish by half-tetrad analysis.

Genetic screens have identified over 600 genes in zebrafish that are critical in early development and organogenesis (2, 6, 7). To facilitate the mapping and positional cloning of these genes, zebrafish genetic resources such as genetic maps (3, 10, 13, 17), radiation hybrid maps (4, 8), and YAC (20), BAC (Genome Systems, Inc.), and PAC libraries (1) are available. Mapping of the mutant genes is typically accomplished by bulked segregant analysis as the first step in positional cloning. In bulked segregant analysis, DNA pools of mutant and wildtype embryos are genotyped with markers representing each of the 25 linkage groups. In one approach, genome scanning, the pooled DNA is genotyped with 150 to 230 markers that span the genome, with each marker separated by 10 to 15 cM. In the second approach, half-tetrad mapping, the DNA pools obtained from gynogenetic half-tetrad mutant and wildtype embryos are genotyped with markers linked to each of the 25 centromeres of zebrafish (9, 10, 15). The distance of the mutant locus from its centromere is estimated from the frequency of mutant half-tetrads (9, 10, 12, 18). In both mapping methods, potential linkages are confirmed by genotyping individual mutant embryos using progressively closer markers. However, for polymorphic markers, initial mapping of one mutant locus by genome scanning requires 300–460 PCRs while the half-tetrad procedure requires only 50 PCRs. Thus, when a mutant phenotype can be reliably scored in half-tetrad embryos, half-tetrad mapping is an efficient way to place a zebrafish mutation on the genetic map.

Our laboratory has identified a number of mutations that affect genomic instability and cell differentiation in two screens of gynogenetic half-tetrads in zebrafish, (J. Moore, G. Tsao-Wu, K. Cheng, *et al.*, to be published elsewhere). Knowledge of markers linked to each of the 25 centromeres is essential to mapping these mutations using half-tetrads (12). On the RAPD map, which is a female-biased, haploid meiotic map, centromere-linked markers for all 25 linkage groups (LG) are known (10). However, on the microsatellite map, which is a sex-averaged, diploid meiotic map, markers tightly linked to centromeres have not been identified for 6 linkage groups: LG 3, 4, 6, 7, 13, and 20 (13, 17). In most instances, including ours, the use of RAPD markers is not possible because mutations were scored in

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TABLE 1

Centromere-Linked Markers for Six Linkage Groups

Linkage group	Marker	Heterozygotes/total					Distance from centromere (cM)[a]
		Panel 1	Panel 2	Panel 3	Panel 4	Total	
3	Z3725[b]	0/37	0/163	0/94	0/141	0/435	<0.1
	Z10964	NP					
	Z17222	NP					
	Z20058	19/37				19/37	25.7
	Z22555	15/36				15/36	20.8
4	Z984	26/37				26/37	35.1
	Z1416	20/37				20/37	27.0
	Z3275	NP					
	Z10164	22/36				22/36	30.5
	Z11876	15/36	71/160	NP	47/140	133/336	19.8
	Z10280	NP	23/159	NP	2/141	25/300	4.2
	Z20450[b]	NP	NP	NP	0/139	0/139	<0.4
	Z22525	NP	NP	NP	NP		
6	Z4339	NP					
	Z6767[b]	NP	0/164	NP	4/125	4/289	0.7
	Z7647	NP	NP				
	Z10183	13/36				13/36	18.0
	Z10339	21/36	NP	NP	NP	21/36	29.2
	Z13538	NP					
	Z20411	34/37				34/37	45.9
	Z20932	2/37	NP	10/85	5/140	17/262	3.2
	Z22745	NP					
	Z20381	4/37	9/167	7/94	7/141	27/439	3.1
7	Z1239	NP					
	Z6852	5/37	19/163	14/93	22/141	60/434	6.9
	Z8156[b]	4/32	NP	NP	2/140	6/172	1.7
	Z8540	6/33	10/163	NP	NP	16/196	4.1
	Z8604	NP	NP	NP	NP		
	Z20576	4/37	NP	NP	NP	4/37	5.4
	Z21689	23/33				23/33	34.8
13	Z5395	NP	NP	NP	NP		
	Z9110	6/36				6/36	8.3
	Z9995	11/36				11/36	15.3
	Z10582	10/37				10/37	13.5
	Z13250	5/29				5/29	8.6
	Z13682[b]	1/34	NP	NP	3/139	4/173	1.1
	Z15438[b]	1/34	NP	NP	3/135	4/169	1.2
20	Z3824	11/35				11/35	15.7
	Z7803	4/31	3/161	9/94	9/138	25/424	2.9
	Z8809	4/35	NP	NP	NP	4/35	5.9
	Z11841	19/35				19/35	27.1
	Z20582	2/36	NP	NP	NP	2/36	2.8
	Z21067[b]	0/35	NP	2/93	NP	2/128	0.8
	Z21170	7/35				7/35	10.0
	Z22926	NP	15/167	NP	NP	15/167	4.5

Note. NP, not polymorphic.

[a] Map distance from the centromere: $x = 50y$, where y is the fraction of heterozygous half-tetrads (18).

[b] Closest to centromere.

diploid embryos using strains that differ from those used to generate the RAPD map. The utility of RAPD markers is limited by the inability to detect heterozygosity, necessitating the use of haploid embryos for genotyping. RAPD markers also cannot be used across different zebrafish strains without first cloning, sequencing and generating informative markers from new primers (13). In contrast, codominance and a high degree of polymorphism across strains make microsatellite markers ideal mapping tools (19). Recently, Kane *et al.* (11) listed centromere markers for all 25 linkage groups on the zebrafish microsatellite map but did not report either panel sizes or origins of the data. Also, for LG 4, 6, 7, 13, and 20, the centromere-linked markers reported are 5–40 cM from their respective centromeres, greatly limiting their use in half-tetrad mapping. Therefore, we embarked upon experiments to identify microsatellite markers linked to the centromeres of the 6 linkage groups lacking markers with tight centromere linkage.

Since both the RAPD and the microsatellite genetic maps have been consolidated (16), we compared the

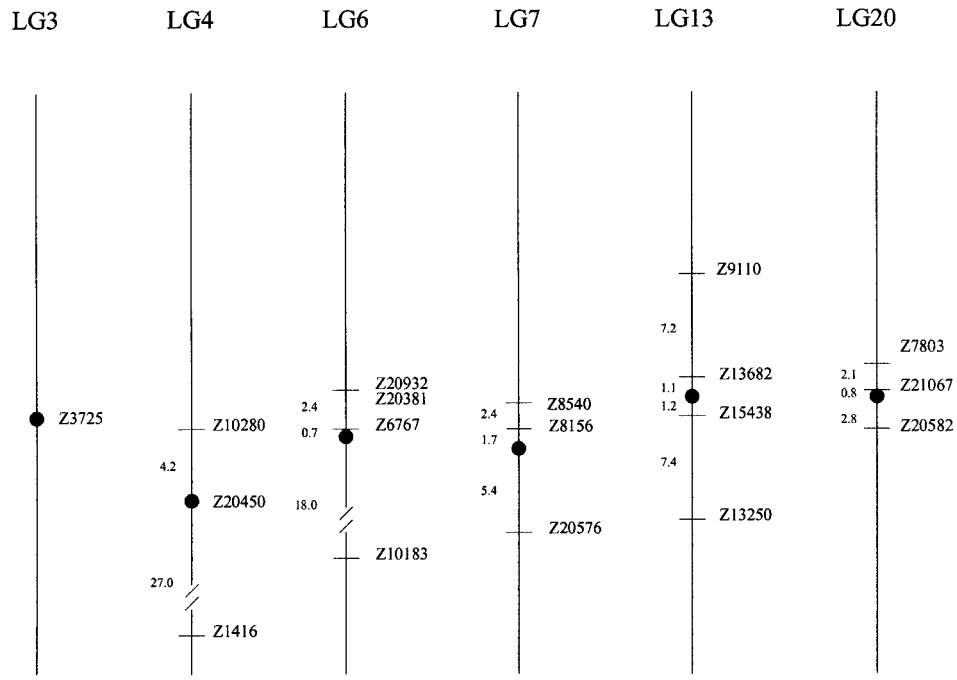

FIG. 1. Centromere-linked markers for linkage groups 3, 4, 6, 7, 13, and 20 on the zebrafish microsatellite genetic linkage map (17). Centromeres are indicated by black circles on each linkage group. The relative positions of the markers to their respective centromeres are shown. Numbers between centromeres and markers denote genetic distances in centimorgans.

two maps to deduce candidate markers on the microsatellite map to be tested for centromere linkage. By comparing the relative distances between markers and their respective centromeres on the RAPD map (10, 16, http://zebrafish.mgh.harvard.edu/map_comparison/LG03.html), taking into account the difference between a sex-averaged map and a female map length (13), we deduced, for example, that marker Z3725 is close to the centromere of LG 3. In a similar manner, potential centromere markers for LG 4, 6, 7, 13, and 20 were chosen (Table 1). These candidate markers were first screened using a gynogenetic half-tetrad panel consisting of 37 embryos (Panel 1) utilizing PCR conditions identical to those described previously (13) except that 35 PCR cycles were performed. For the closest centromere-linked markers, the analysis was expanded to three other panels (Panels 2, 3, and 4), consisting of a total of 400 additional gynogenetic half-tetrad embryos. Half-tetrad embryos were generated as described in Gestl *et al.* (5).

The use of half-tetrads in centromere-linkage analysis has been described (9, 10, 12). Briefly, for a marker that is tightly linked to its centromere, a crossover event between the marker and its centromere will occur only rarely in a half-tetrad (18). Therefore, most or all half-tetrad embryos in a panel will be homozygous for the markers that are very close to the centromere. The farther a marker is from its centromere, the greater the likelihood that a crossover will occur between the marker and its centromere, resulting in a greater proportion of heterozygous half-tetrads. The relative distance of a marker to its centromere can therefore be measured using the frequency of crossovers (i.e., heterozygotes) observed. Since each crossover is a reciprocal event yielding two heterozygous products, the distance of a marker to its centromere (x) can be determined using the equation $x = 50y$, where y is the fraction of heterozygous half-tetrads (18). This calculation assumes the high chiasma interference (i.e., one recombinational exchange completely inhibits additional crossovers) (14) that appears to occur in zebrafish (12, 18). Chiasma interference makes it practical to use tightly linked centromere markers to map a mutation to a chromosome arm independent of the distance of the mutant locus from the centromere. If all half-tetrads are homozygous for one allele of an informative centromere marker, the probability P that the mutation is not linked to that chromosome using n embryos may be approximated by the formula $P = \frac{1}{2^n}$.

The proportions of heterozygous half-tetrads for candidate microsatellite markers and calculated map distances from their centromeres are summarized in Table 1. Markers deemed far from their centromeres in Panel 1 were not tested in the other panels. The positions of the closest markers in relation to their centromeres are diagrammed in Fig. 1. For LG 3, the marker most tightly linked to the centromere is Z3725. No heterozygotes were observed in any of the four panels tested. For LG 4, Z20450 is tightly linked to the centromere. This marker was not polymorphic in half-tetrad Panels 1, 2, or 3. Genotyping data from the fourth panel place this marker within 0.4 cM of the centromere. Z10280 and Z20450 are part of a cluster of 10 unresolved markers on LG 4 (17). These 10 markers are clustered together based upon a mapping panel of

TABLE 2

Centromere-Linked and Flanking Microsatellite Markers for All 25 Linkage Groups of the Zebrafish Genetic Map

Linkage group	Centromere-linked marker	Flanking markers	Reference
1	Z1351	Z6911, Z9409	(17)
2	Z9944, Z4300	Z4733, Z11410	(17)
3	Z3725	Z22555, Z20058	This paper
4	Z20450	Z10280, Z1416	This paper
5	Z7685, Z1167	Z13461, Z3516	(17)
6	Z6767	Z20381, Z10183	This paper
7	Z8156	Z8540, Z20576	This paper
8	Z11237	Z6764, gof13	(17)
9	Z9923, Z5080	Z6337, Z7120	(17)
10	Z13685, Z3835	Z9473, Z22422	(17)
11	Z3412	Z9239, Z4190	(13)
12	Z11521, Z4188	Z4373, Z4397	(17)
13	Z13683, Z15438	Z9110, Z13250	This paper
14	Z6847, Z4	Z4896, Z11725	(17)
15	Z21452, Z8991	Z21982, Z20993	(17)
16	Z10036	Z6921, Z11376	(17)
17	gof12, Z21194	Z1408, Z4053	(12, 17)
18	Z7256	Z9194, Z10008	(17)
19	Z13727, Z3023	Z15451, Z3770	(17)
20	Z21067	Z7803, Z20582	This paper
21	Z10432	Z7809, Z10960	(17)
22	Z13529	Z938, Z7125	(17)
23	Z7550	Z20039, Z6142	(17)
24	Z17203	Z22252, Z6239	(17)
25	Z3632, Z4198	Z15480, Z4358	(17)

44 embryos (17). The clustering of markers in centromeric regions is likely to be related to the known suppression of recombination near centromeres (10). To determine whether Z10280 was closer to the centromere than Z20450, we tested this marker on the four panels (Table 1). Genotyping data on polymorphic Panels 2 and 4 placed Z10280 4.2 cM from the centromere (Table 1). Therefore, Z20450 is closer to the centromere than Z10280. Z6767 is the closest centromere-linked marker that we were able to identify for LG 6. There were four recombinants in a total of 289 half-tetrad embryos, placing this marker 0.7 cM from its centromere (Fig. 1). For LG 7, comparison of the RAPD and SSLP maps suggested that Z1239 may be at the centromere. However, this marker was not polymorphic in any of our half-tetrad panels. Of the cluster of markers 9.9 cM proximal to Z1239 (17), we tested Z6852, Z8156, Z8540, and Z20576. It was determined that Z8156 was closest to the centromere (within 1.7 cM) and that the centromere is located between this marker and Z20576 (Fig. 1). On LG 13, Z15438 and Z13682 lie on opposite sides of the centromere at a distance of about 1 cM (Fig. 1). On the genetic map, these two markers are part of a cluster of 18 located near the middle of this linkage group (17). We have been unable to resolve which may be the closest marker because the additional markers tested (Z11695, Z20088, Z5395, and Z13571) were not polymorphic in the half-tetrad panels. The LG 20 centromere is located between Z21067 and Z20582, with marker Z21067 being the closest (Table 1, Fig. 1).

We have thus identified microsatellite markers within 1.7 cM of the centromeres of linkage groups 3, 4, 6, 7, 13, and 20. Together with the knowledge of the centromere-linked markers previously published (17), it is now possible for investigators to use half-tetrad analysis to place mutations of interest on all 25 linkage groups of the microsatellite map. A complete list of microsatellite markers for centromere-linkage analysis is provided in Table 2. We have successfully utilized these markers for centromere-linkage analysis of mutations that have been generated in our laboratory (to be published elsewhere). Also included within Table 2 are markers flanking the centromere that can be used to place mutations of interest on a specific arm after centromere linkage to a specific linkage group has been established.

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