Genetic Linkage Mapping of Zebrafish Genes and ESTs

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Genetic screens in zebrafish (*Danio rerio*) have isolated mutations in hundreds of genes essential for vertebrate development, physiology, and behavior. We have constructed a genetic linkage map that will facilitate the identification of candidate genes for these mutations and allow comparisons among the genomes of zebrafish and other vertebrates. On this map, we have localized 771 zebrafish genes and expressed sequence tags (ESTs) by scoring single-stranded conformational polymorphisms (SSCPs) in a meiotic mapping panel. Of these sequences, 642 represent previously unmapped genes and ESTs. The mapping panel was comprised of 42 homozygous diploid individuals produced by heat shock treatment of haploid embryos at the one-cell stage (HS diploids). This "doubled haploid" strategy combines the advantages of mapping in haploid and standard diploid systems, because heat shock diploid individuals have only one allele at each locus and can survive to adulthood, enabling a relatively large quantity of genomic DNA to be prepared from each individual in the mapping panel. To integrate this map with others, we also scored 593 previously mapped simple-sequence length polymorphisms (SSLPs) in the mapping panel. This map will accelerate the molecular analysis of zebrafish mutations and facilitate comparative analysis of vertebrate genomes.

[A table of the mapped genes and ESTs is provided online at http://www.genome.org.]

Lethal mutations in zebrafish (*Danio rerio*) define the functions of hundreds of essential genes in the vertebrate genome (Driever et al. 1996; Haffter et al. 1996). Powerful methods for the analysis of mutant phenotypes can provide an understanding of the function of these genes at the cellular level (Kimmel 1989; Schier and Talbot 1998). More than 25 genes defined by mutations have been cloned, and these molecular studies demonstrate the ability of forward genetic approaches in zebrafish to reveal new genes and new gene functions that are conserved among vertebrates (Postlethwait and Talbot 1997; Talbot and Hopkins 2000). More than 1800 additional mutations have been identified in genetic screens, and developing genetic maps and other resources to accelerate the molecular analysis of these mutations is an important objective.

Beginning in 1994, several maps of the zebrafish genome have been produced to facilitate the identification of mutated genes by positional cloning and the candidate gene approach. The first map (Postlethwait et al. 1994) was constructed by scoring randomly amplified polymorphic DNA (RAPD) markers in a panel of haploid embryos. The use of haploid embryos simplified the analysis of RAPDs, which usually segregate as dominant markers for which the two alleles are scored as the presence or absence of an amplified fragment. Another mapping strategy employed simple-sequence length polymorphisms (SSLPs) and a standard diploid breeding protocol (Knapik et al. 1998; Shimoda et al. 1999). SSLPs segregate typically as codominant markers and they are more useful in diploid crosses than are dominant markers such as RAPDs. An advantage of the diploid protocol is that diploid zebrafish survive to adulthood, providing much more genomic DNA for mapping studies than haploids, which survive for only a few days after fertilization. These genetic maps benefit positional cloning projects by increasing the likelihood that a mutation will lie near a mapped DNA sequence.

To facilitate the identification of candidate genes for mutations, genes have been mapped within frameworks of RAPDs or SSLPs by scoring polymorphisms in haploid mapping panels (Postlethwait et al. 1998; Gates et al. 1999). These gene maps have also been useful in comparative studies, which have identified

groups of genes that are syntenic in zebrafish and other vertebrates.

The recent advent of two radiation hybrid (RH) maps provides a complementary approach for mapping zebrafish genes (Geisler et al. 1999; Hukriede et al. 1999). RH maps assign positions to markers based on their retention in particular members of a panel of cell lines constructed by fusing irradiated zebrafish cells to rodent cells. As such, markers that tend to be present in the same hybrid cell lines are closer together than those that are coretained infrequently. One limitation of this approach is that RH maps tend to have more uncertainty in the order of closely spaced markers than genetic maps, but an important advantage is that mapped sequences need not be polymorphic.

To expand the zebrafish gene map, we have localized single-stranded conformational polymorphisms (SSCPs) corresponding to 642 previously unmapped genes and expressed sequence tags (ESTs) in a meiotic mapping panel comprised of 42 homozygous diploid individuals. To facilitate comparisons with other maps, we also scored in the mapping panel 593 previously localized SSLPs, which were used widely in genetic linkage and RH maps, as well as 129 previously mapped genes and ESTs. This map will facilitate the molecular analysis of zebrafish mutations and provide an important resource for comparative mapping analysis.

RESULTS

Construction of Homozygous Diploid Mapping Panel

We have assembled a mapping panel comprised of gynogenetic homozygous diploid individuals (also called heat shock diploids), which are produced by heat shock treatment of haploid embryos during the one-cell stage (Fig. 1). The heat shock treatment is applied after the S-phase of the first zygotic cell cycle so that chromosomes have replicated, but because heat shock blocks the subsequent cytokinesis, the haploid chromosome set becomes doubled (Streisinger et al. 1981; Westerfield 1995). This produces diploid embryos that are homozygous at every locus. Many embryos subjected to heat shock treatment are inviable, but a substantial fraction develops normally and some individuals grow to fertile adults. Because heat shock diploids are doubled haploids, the use of this protocol in mapping studies provides the key advantages of haploid embryos, simplifying linkage analysis and facilitating the use of dominant markers (Young et al. 1998). An additional advantage is that heat shock diploids can survive beyond embryonic stages, allowing genomic DNA to be collected in relatively large quantities from juveniles and adults, whereas haploid embryos survive for only ~4 days.

The mapping panel (HS panel) was comprised of 42 heat shock diploid F_2 progeny obtained from two
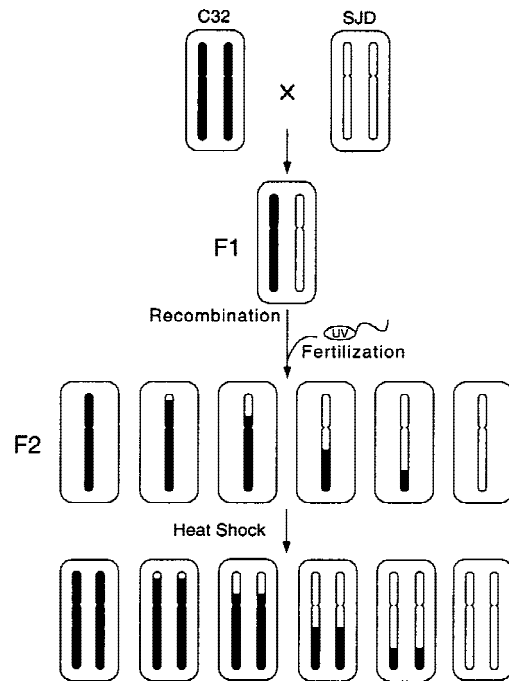
Figure 1 Production of the homozygous diploid mapping panel. C32 and SJD fish were crossed to produce a heterozygous F_1 generation. Eggs from two F_1 females were fertilized with UV-irradiated sperm, which makes no genetic contribution to the progeny. The resulting haploid embryos were subjected to heat shock treatment, which doubles the haploid chromosome set. DNA for the mapping panel was prepared from homozygous diploid F_2 adults.

C32 × SJD F_1 females. C32 and SJD are inbred mapping strains (Streisinger et al. 1981; Nechiporuk et al. 1999) and F_1 females were obtained from natural crosses of C32 and SJD adults (Fig. 1). Nineteen of the F_2 individuals in the HS panel were progeny of one C32 × SJD female and 23 were progeny of the second C32 × SJD female. The HS panel contains products of 42 meioses and the panel therefore provides an average resolution of 2.4 cM. The C32 and SJD strains are highly polymorphic with respect to one another, with ~90% of markers showing different alleles in a previous comparison of the two strains (Nechiporuk et al. 1999). Both strains are inbred, and only ~10% of SSLP markers reveal heterogeneity among different individuals of the same strain (Nechiporuk et al. 1999). Thus, we expected most markers that were heterozygous in one F_1 female also to be heterozygous in the other, such that F_2 progeny of the two females should segregate identical alleles. Accordingly, ~10% of SSLPs (21 of 200 for which we tracked allele differences) showed differences between the two families in the heat shock panel (HS panel). Most markers displaying allele differences were polymorphic in both families in the panel and only 14 of 1364 markers that we scored in the HS panel were polymorphic in one family but monomorphic in the other. The positions of these 14 markers were assigned

according to the genotypes of the family in which the marker was informative.

Markers and Linkage Analysis

To construct a genetic linkage map, we scored 1364 polymorphic markers in the HS panel (Figs. 2 and 3; Table 1, available online at http://www.genome.org). These markers included 593 previously mapped SSLPs and 771 SSCPs derived from zebrafish ESTs and fully sequenced cDNAs. Locus names, accession numbers, and primer sequences for the genes and ESTs scored in the HS panel are shown in Table 1. The genes and ESTs represented 482 different UniGene clusters (four of which were mapped in duplicate, see below) and there were 285 sequences that were not assigned to UniGene clusters in the current release (UniGene Build 6; http://www.ncbi.nlm.nih.gov/UniGene/Dr.Home.html). Sequence comparisons with TIGR Assembler (Sutton et al. 1995) and the BLAST algorithm (Altschul et al. 1997) did not reveal overlap among these 285 sequences, suggesting that most correspond to different genes.

Of the SSCPs scored in the HS panel, 642 correspond to previously unmapped genes and ESTs (Table 1, available online at http://www.genome.org). In addition, we scored 59 SSCPs corresponding to genes localized in previous genetic maps (Postlethwait et al. 1998; Gates et al. 1999) and an additional 25 genes that were localized in RH maps (Geisler et al. 1999; Hukriede et al. 1999). Forty-five of the ESTs scored in the HS panel were localized in previous maps.

Linkage analysis assembled the 1364 polymorphisms into a map with 25 linkage groups, each representing one zebrafish chromosome (Fig. 3). The markers occupied 598 unique map positions. All map positions in the 25 linkage groups were supported by lod scores of ≥3 and there were no gaps >22 cM.

The 25 linkage groups in the HS panel map spanned 3011 cM, using the Kosambi mapping function to estimate the occurrence of double crossovers. Previous estimates put the total length of the female meiotic map at 2900 cM (Johnson et al. 1996), suggesting that the HS panel map covers nearly the entire genome. Accordingly, we were able to assign map positions to all of the polymorphic markers scored in the HS panel, because all markers in the data set showed significant linkage (lod ≥3) to at least one other marker.

As one measure of the error frequency in genotype data, we examined apparent double crossovers occurring in short intervals, which are uncommon and thus likely to reflect erroneous genotype assignments. Among the 53522 individual genotype assays in the data set (an average of 39.2 individuals scored per marker), there were only two double crossovers in intervals <20 cM. This suggests that the frequency of genotyping errors in the data set is low.

As another measure of the accuracy of the map, we compared the positions of markers mapped in the HS panel and in previous work. The HS panel map assigned 591 of 593 SSLPs to the same linkage groups as Shimoda et al. (1999). Of these 591 markers, there were eight cases where the order of closely spaced markers differed between the two maps. Because closely spaced markers are separated by few recombinants, these differences may reflect a small number of genotyping errors in one or both data sets. It is also possible that these discrepancies were caused by differences in the strains used to construct the two mapping panels. Two SSLPs (Z3054, Z9559) were assigned to different linkage groups; we repeated the genotype assays and confirmed the assignment of these two markers in the HS panel. In addition, the positions we assigned for these two SSLPs matched those obtained from RH mapping (M. Ekker, pers. comm.). Thirty-nine genes localized in our map were analyzed previously in a C32 × SJD haploid mapping panel (Postlethwait et al. 1998) and all of these were assigned to the same linkage group in both maps. There was one discrepancy among the 49 genes and ESTs common to our map and that of Gates et al. (1999). We mapped EST AI544741 to LG 3, and Gates et al. (1999) localized AA606157, another EST assigned to the same UniGene cluster (Dr.1344), to LG 1. One EST that we mapped (AA495459) was assigned to a different linkage group in the T51 RH panel. The assignments of the discrepant markers AI544741 and AA495459 in the HS panel were confirmed by a second set of genotype assays. In addition, there were four cases, all confirmed in duplicate genotype assays, in which sequences assigned to the same UniGene cluster were mapped to different linkage groups in the HS panel (Dr.261, Dr.274, Dr.345, Dr.1339). These may represent genes with pseudogenes or they may be pairs of duplicate genes generated by chromosomal duplications.

Figure 2 Examples of polymorphisms scored in 23 individuals in the heat shock (HS) mapping panel and C32. An SSCP in the *wnt8* gene and the SSLP Z10663 are shown.

LG 1

Z9362 AA658718
Z728 AI497435
Z4593 AI584384
Z3054 AI496961
Z10551
AI477343
Z9217
Z3706
AA566393 Z9250
AI330838 AI331774
AI416348 bactin1
AI478005
AA658651 Z5508
AI353653
AI722480
Z7573 AI497476
AI415993

Z6643

AI354201 Z6974
AI353547
tolloid
AI384693 Z7476
AA495024 AI353543
AA606174 Z6911
Z22986
Z9409
ednrb1
Z6403
AI544873
Z21548
AI477360
AI884233 msxb

Z10888 lef1

otx3

AA606111 Z6802
Z1173 AI477673
AA542650 AI477970

AI106232 Z6283
AI397028 Z1368
AA495139
AI353541
Z22347

Z1781 Z6223
Z450 Z13724

LG 2

Z1484 Z10764
AA494741
AI397435
AI384739

AI477599

AI558333
AI723230
Z4469

AA658589

AI496880
AI478011
AI497465
Z4662

AI477087 tfa
AI332201 Z3430
AA542497

AI444487
Z11410 rxra

AI477379
AI477517
AI384734
AI497169

Z13475

Z22527 Z8451
AI331037
Z4585
AI497441 elra
AI477608 Z11023
AI477208 AI477702
Z21490
AI415987
AI496737
AI437332 alk8
Z1406 Z17291
Z3754 Z6617
AA542447 fz8b

AI384212
Z6863

Z13579
Z7358

Z11863

Z21046 Z1241
Z6818 Z6452
Z644 Z1703
Z20550
Z6090 Z46
AI477779

LG 3

Z1183 AI477082
AI353515 AI522697
AI444338 Z8492
AI416333 Z872
AA658677

Z15479

nog1
AI476936
Z1185

Z3439

AI477382 Z6068

AA494789 Z9843
AI322225 AI416334
AI477524 Z15457
AI397260 Z6566
Z1140 Z5623
AI477031 eve1
hoxb1a hoxb10a
Z5197 hoxb7a
AI384205 Z9257
AI437465 ntn1
Z10805 Z963
AI384244
AI436945 AI545106
AI384954 AI397400
AI476841 AI105815
AI544741 Z9964 Z10934
AA494813 Z21827
AI497236 AI330817
AI477336 Z8680 pyy
AI477437
AI330671 AI477936
AI396695 AI477313
Z11227 AI397338

AA494848 AI722355
bactin2 nar Z25956

Z22555

AA495179 Z3725
AI477652 AI477044
AW019806 elrc

AA495135 notch3

mta

AI477872 ba1globin
AA494750

Z20058

Z7486 AI106227

Z9463 Z20317

Z8681 Z6019
Z1401 Z3100

LG 4

Z7629 Z6580
Z1389 Z1366
Z5112 Z9319
AA495191
AI331576
Z5095 Z9590
Z4951
Z20533
Z7104 Z9920
Z7490 Z21636
Z9750 Z7191

Z10164 AI601629
Z13469
Z3602 AI397032
AI437402
AI477251 Z11876
AI497193

Z10280 mdm2
AI331981 Z17278
AI330806 Z7931
AA805850
AA658685
AI558288
AA606015
AI496930
AI545760
GOF29

Z3293

AI545283

Z1416

AI558272
Z984
Z15751

Z3275

LG 5

AI588409 Z15414
AA495371 Z3902
Z3314 Z13883
AI477039

AI397367 Z11496

Z6323

Z10663
bmpr1b
Z8921

AI497451

Z7351 pes

Z7257

AI477060
Z10517
AI461302 AI396697
AI385017 tkleif1
AI477602 AI106141
AI477929 Z22523
AI415815 p53
Z20951

AA495341

Z9856 AI437348
AA606082 AI437080

AI384951 Z4760
AI332141 Z3334
AI354205 Z1390
AI397246
AI496715 terra
Z13641 epd
AA494838 Z9871
AI497174
AI354203
AA494744 Z3804
AA542521 AA495404
AI106082 lim6
AA495294 Z5538
AI496757 AI436892

Z6880
AI396819 Z6803
AI496705 rxrg
Z22250

AI477057

AA495476
AI477436
Z4299

Z1454 Z13442
Z1813 Z22208
Z1419 Z22152
Z11349 Z13279
Z1677
GOF22

10 cM

Figure 3 (*See pages 561–565.*) Genetic linkage map of the zebrafish genome. Positions of 1364 polymorphic markers scored on the HS panel are shown. GenBank accession numbers, UniGene numbers, and primer sequences for genes and ESTs are shown in Table 1 (available online at http://www.genome.org). SSLP markers were described by Shimoda et al. (1999).

LG 6

Z10657 Z7303
Z1265 Z15493
Z13275
Z20411
Z11466
Z8342
Z22467
Z11310 Z9142
AI437331 AI722360
AA495324 Z17212
AI477769
Z10914
Z22712
AI437455
pou1
Z6767 AI942799
AI477229 Z20932
AI496951 Z13538
AI477896 AI353094
AA494864
AA494737 Z10183
AI496760
AI396612 AI496939
AI497510 AI477081
AI477543
AA494621 AI384749
Z265
Z4950 Z1050
AI722396
AI545290
contact
AI497528 AI496703
AI477996 Z7666
Z9679
Z21489 GOF2
Z21901 Z10581
GOF20

LG 7

Z9067
Z6523
AI477708
Z10785
Z8252
Z1206
Z20963
AI330927
AI106288 Z3668
AI396618
Z4999 Z4706
AI496851 AI477916
AI544525 AI477924
AI477457 AA495446
AI396864 AI476926
AI497107
AI397271
AI106294
Z3445 Z3008
AI477236
AA542481 Z1182
AI497309 AI330984
AI444505
shh AI477042
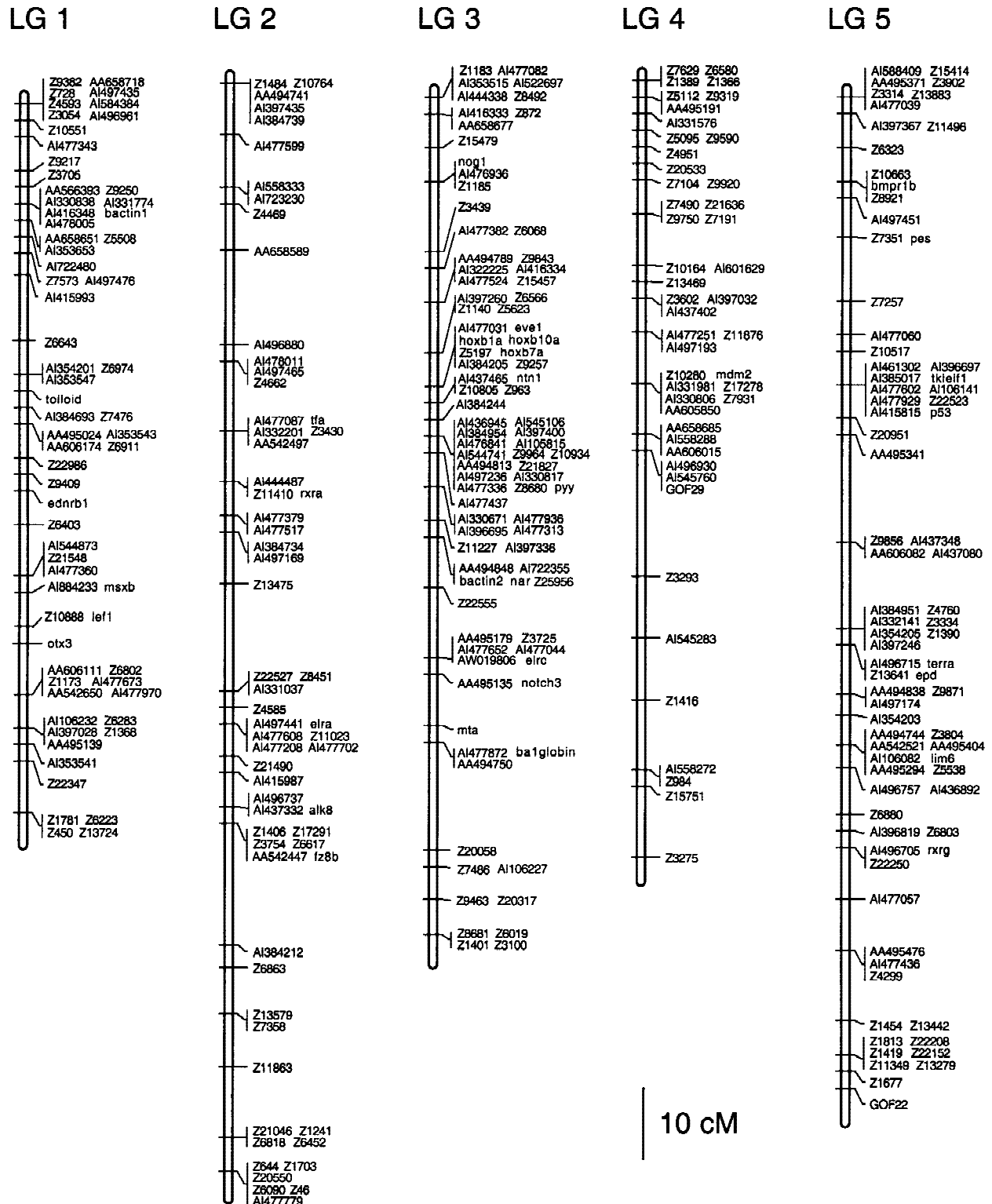AI437490 Z1059
AI384862
cyce
Z22040
AI477387 ashb
Z8604
AI496749 AI497370
AI722254 AI626634
AI497006 AI477501
AA494747
Z1239
Z21689
Z13286
AI415997
Z10281 Z5563

LG 8

Z140 Z1402
tkral1
Z11623
Z5763
Z9420 es1
AI477327
Z7819
AI477329
Z4323
AA494588
Z11001 nras
AI544571 AI444388
AI496933 notch2
AI477855 AI497520
AI496758 AA497292
AW281664 Z1589
AI477685 AI106036
AI331280 GOF9
Z6764 AI497074
AI722399 AI436884
AI477323
AI330823
AI106245
AI478014 Z11237
AI444404 GOF13
AI332283
AI354212
Z11948
AI461341
Z789 AA494708
Z7370 Z20113
Z15786
Z9279
nkx2.7 Z1052
AI496840
Z21390
AI478004 Z870
AI558436
Z9637 AI461316
Z4637

LG 9

Z13289 Z13450
Z20660 Z11781
AA566760
AI416340 Z9224
AI416005 Z1830
Z15449
pou12
hha
Z4673
AI354217 Z3124
AI416383
Z4168 Z5060
AI332227 AI545381
AI477207
AA605750
AA495423
AI496855 Z13213
AI384870
AI522386
actbb
AI385079
Z1273
AI497447
Z8363
dermo1
Z10789 cx43.4
AA494831
AI496822
Z5564
Z10166
AI353222
Z22141
AI397432 Z106
AI396874

LG 10

AA494792 Z9208
AI330852
Z7316 Z13875
Z8146 Z355
AI477308 oep
Z13632
AI497549
AA495157
AI477279
AI496711
Z13685 Z22422
AI477013 AI477212
AI397208 AI477312
AI522344 AI384675
AI396692
AA605690 Z6195
AI883146
AI544949 Z1145
AI496698 AA494784
AI353118
Z9574 Z10765
hsc70 AI384258
wnt11r Z11602
AI332033 Z11229
AA606141
Z13219 Z6183
AI436922
AA606219
Z14193 Z21976
AI641470
Z9701 mef2c
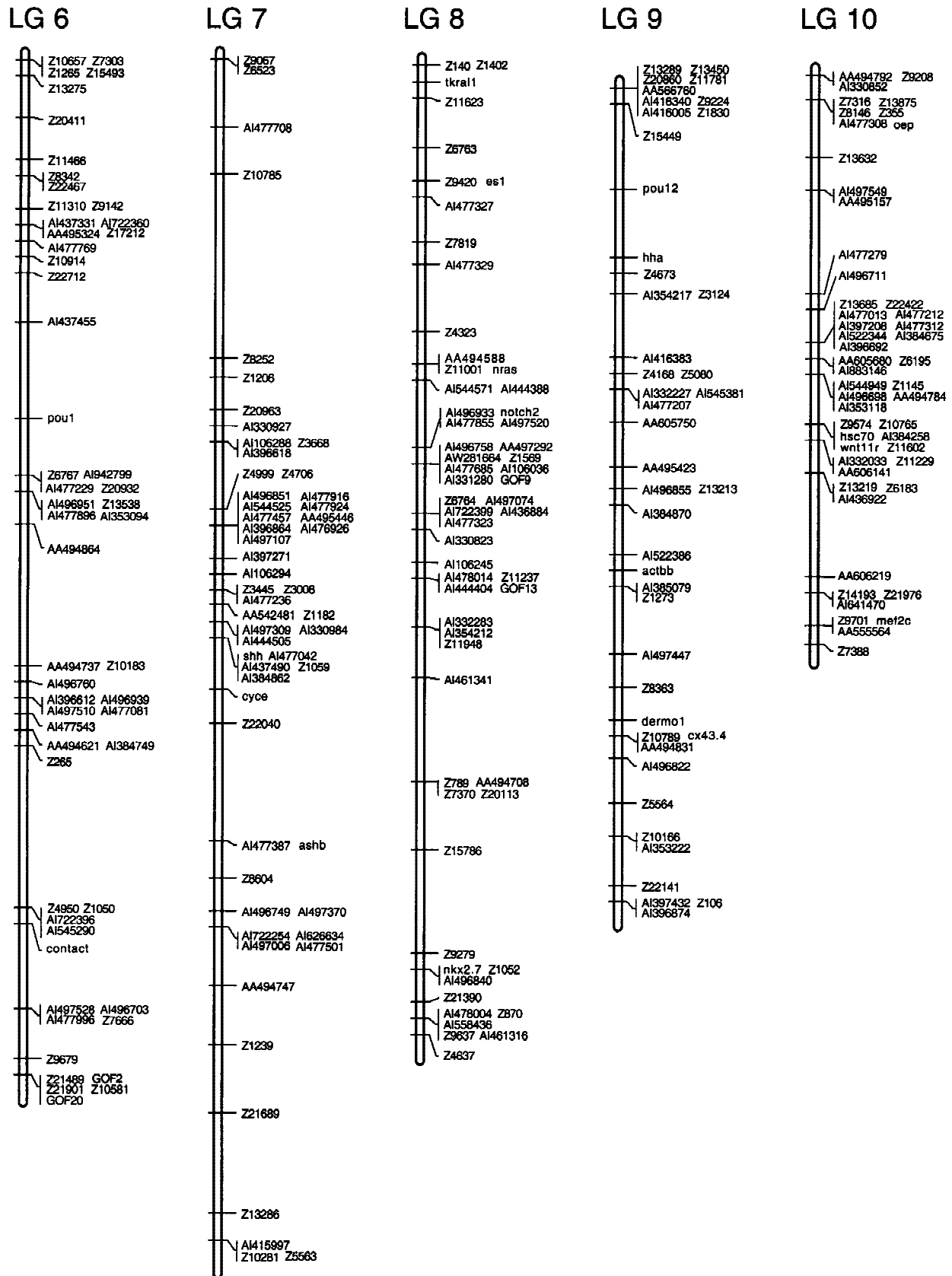AA555564
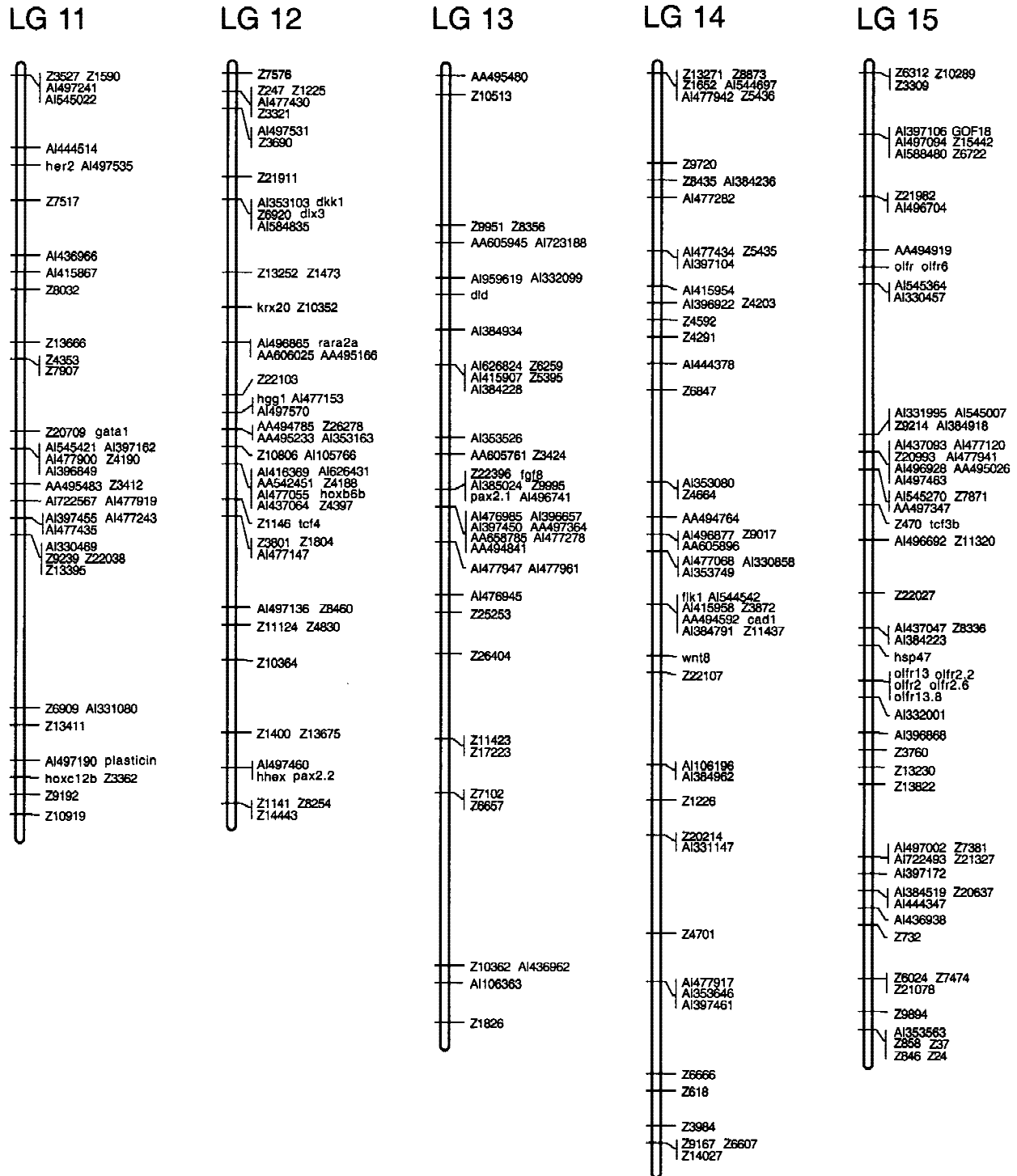Z7388

Figure 3 (*See p. 561 for legend.*)

Figure 3 (*See p. 561 for legend.*)

DISCUSSION

We have mapped 771 zebrafish genes and ESTs by scoring SSCPs in a meiotic mapping panel comprised of homozygous diploid individuals. In previous genetic linkage maps, ~150 genes were analyzed in each of two haploid mapping panels (144 in Postlethwait et al. 1998; 157 in Gates et al. 1999). In RH mapping projects, Geisler et al. (1999) and Hukriede et al. (1999) mapped 355 and 148 genes and ESTs in the T51 and LN54 panels, respectively. In total, the sequences
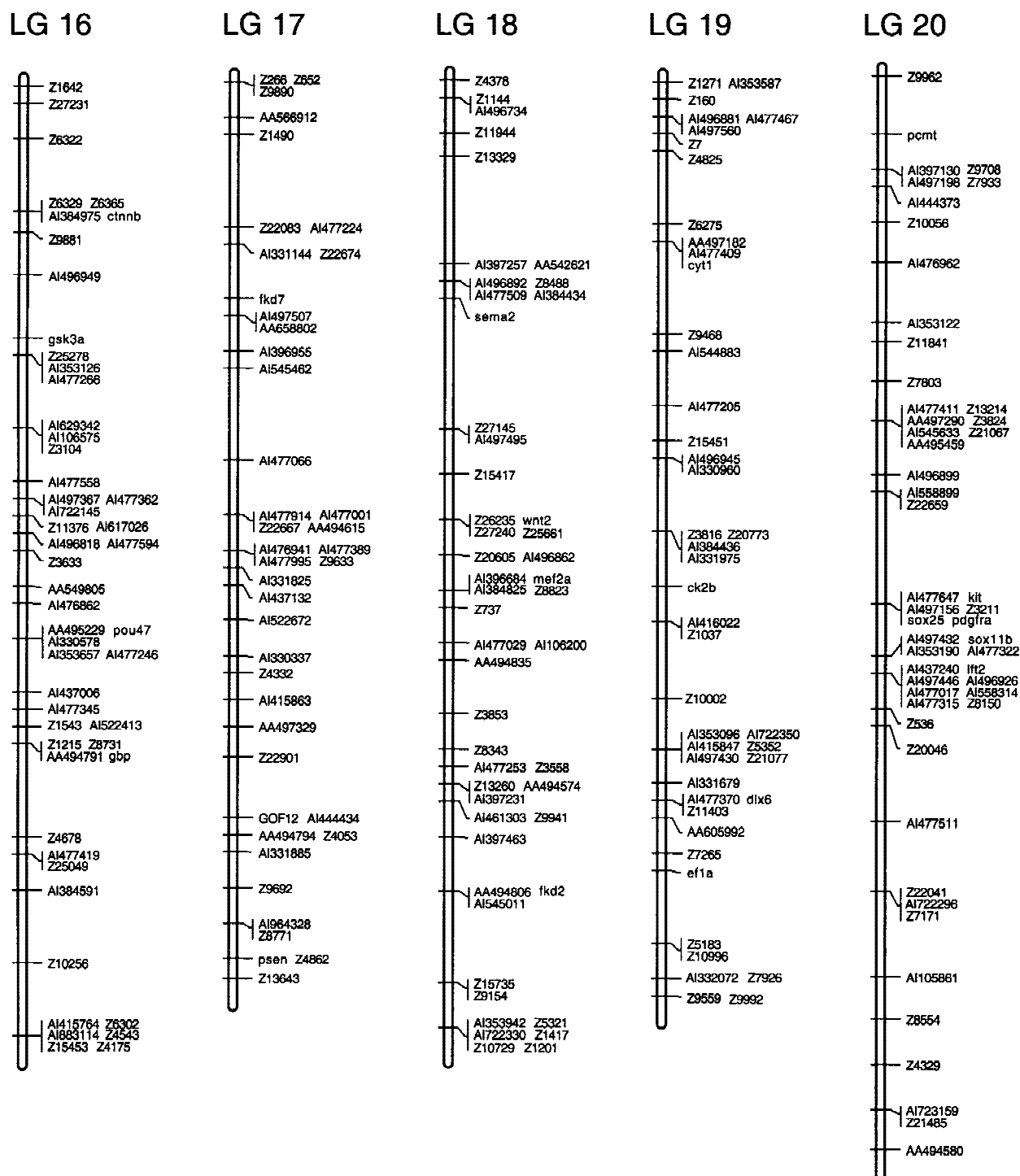
LG 16

- Z1642
- Z27231
- Z6322
- Z6329 Z6365
- AI384975 ctnnb
- Z9881
- AI496949
- gsk3a
- Z25278
- AI353126
- AI477266
- AI629342
- AI106575
- Z3104
- AI477558
- AI497367 AI477362
- AI722145
- Z11376 AI617026
- AI496818 AI477594
- Z3633
- AA549805
- AI476862
- AA495229 pou47
- AI330578
- AI353657 AI477246
- AI437006
- AI477345
- Z1543 AI522413
- Z1215 Z8731
- AA494791 gbp
- Z4678
- AI477419
- Z25049
- AI384591
- Z10256
- AI415764 Z6302
- AI683114 Z4543
- Z15453 Z4175

LG 17

- Z266 Z652
- Z9890
- AA566912
- Z1490
- Z22083 AI477224
- AI331144 Z22674
- fkd7
- AI497507
- AA658802
- AI396955
- AI545462
- AI477066
- AI477914 AI477001
- Z22667 AA494615
- AI476941 AI477389
- AI477995 Z9633
- AI331825
- AI437132
- AI522672
- AI330337
- Z4332
- AI415863
- AA497329
- Z22901
- GOF12 AI444434
- AA494794 Z4053
- AI331885
- Z9692
- AI964328
- Z8771
- psen Z4862
- Z13643

LG 18

- Z4378
- Z1144
- AI496734
- Z11944
- Z13329
- AI397257 AA542621
- AI496892 Z8488
- AI477509 AI384434
- sema2
- Z27145
- AI497495
- Z15417
- Z26235 wnt2
- Z27240 Z25661
- Z20605 AI496862
- AI396684 mef2a
- AI384825 Z8823
- Z737
- AI477029 AI106200
- AA494835
- Z3853
- Z8343
- AI477253 Z3558
- Z13260 AA494574
- AI397231
- AI461303 Z9941
- AI397463
- AA494806 fkd2
- AI545011
- Z15735
- Z9154
- AI353942 Z5321
- AI722330 Z1417
- Z10729 Z1201

LG 19

- Z1271 AI353587
- Z160
- AI496881 AI477467
- AI497560
- Z7
- Z4825
- Z6275
- AA497182
- AI477409
- cyt1
- Z9468
- AI544883
- AI477205
- Z15451
- AI496945
- AI330960
- Z3816 Z20773
- AI384436
- AI331975
- ck2b
- AI416022
- Z1037
- Z10002
- AI353096 AI722350
- AI415847 Z5352
- AI497430 Z21077
- AI331679
- AI477370 dlx6
- Z11403
- AA605992
- Z7265
- ef1a
- Z5183
- Z10996
- AI332072 Z7926
- Z9559 Z9992

LG 20

- Z9962
- pcmt
- AI397130 Z9708
- AI497198 Z7933
- AI444373
- Z10056
- AI476962
- AI353122
- Z11841
- Z7803
- AI477411 Z13214
- AA497290 Z3824
- AI545633 Z21067
- AA495459
- AI496899
- AI558899
- Z22659
- AI477647 kit
- AI497156 Z3211
- sox25 pdgfra
- AI497432 sox11b
- AI353190 AI477322
- AI437240 lft2
- AI497446 AI496926
- AI477017 AI558314
- AI477315 Z8150
- Z536
- Z20046
- AI477511
- Z22041
- AI722296
- Z7171
- AI105861
- Z8554
- Z4329
- AI723159
- Z21485
- AA494580

Figure 3 (*See p. 561 for legend.*)

mapped in these previous studies define ~500 different genes. Sequence comparisons and analysis of UniGene cluster assignments indicate that 642 of the 771 polymorphisms we localized correspond to previously unmapped sequences. Consequently, our map more than doubles the number of mapped zebrafish genes and ESTs.

The strategy we used in constructing the HS panel has a number of advantages for mapping experiments. First, the inbred mapping strains C32 and SJD are di-
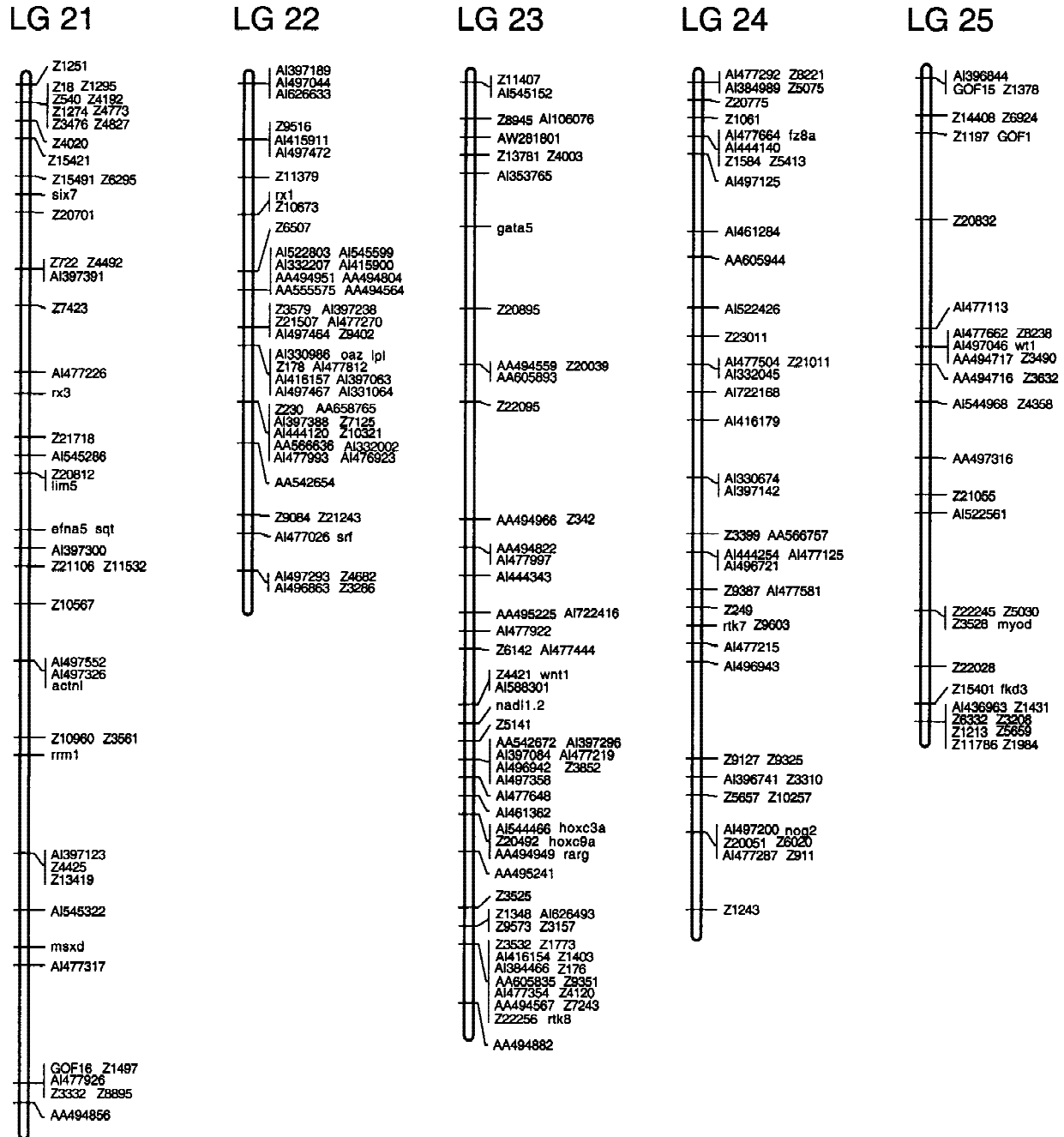
LG 21

Z1251
Z18 Z1295
Z540 Z4192
Z1274 Z4773
Z3476 Z4827
Z4020
Z15421
Z15491 Z6295
six7
Z20701

Z722 Z4492
AI397391

Z7423

AI477226
rx3

Z21718
AI545286
Z20812
lim5

efna5 sqt
AI397300
Z21106 Z11532

Z10567

AI497552
AI497326
actnl

Z10960 Z3561
rrm1

AI397123
Z4425
Z13419

AI545322

msxd
AI477317

GOF16 Z1497
AI477926
Z3332 Z8895
AA494856

LG 22

AI397189
AI497044
AI626633

Z9516
AI415911
AI497472

Z11379

rx1
Z10673

Z6507

AI522803 AI545599
AI332207 AI415900
AA494951 AA494804
AA555575 AA494564

Z3579 AI397238
Z21507 AI477270
AI497464 Z9402

AI330986 oaz lpl
Z178 AI477812
AI416157 AI397063
AI497467 AI331064

Z230 AA658765
AI397388 Z7125
AI444120 Z10321
AA566636 AI332002
AI477993 AI476923

AA542654

Z9084 Z21243
AI477026 srf

AI497293 Z4682
AI496863 Z3286

LG 23

Z11407
AI545152

Z8945 AI106076
AW281801
Z13781 Z4003
AI353765

gata5

Z20895

AA494559 Z20039
AA605893

Z22095

AA494966 Z342

AA494822
AI477997
AI444343

AA495225 AI722416
AI477922
Z6142 AI477444

Z4421 wnt1
AI588301
nadl1.2
Z5141
AA542672 AI397296
AI397084 AI477219
AI496942 Z3852
AI497358
AI477648
AI461362

AI544466 hoxc3a
Z20492 hoxc9a
AA494949 rarg
AA495241

Z3525

Z1348 AI626493
Z9573 Z3157
Z3532 Z1773
AI416154 Z1403
AI384466 Z176
AA605835 Z9351
AI477354 Z4120
AA494567 Z7243
Z22256 rtk8

AA494882

LG 24

AI477292 Z8221
AI384989 Z5075
Z20775
Z1061
AI477664 fz8a
AI444140
Z1584 Z5413
AI497125

AI461284

AA605944

AI522426

Z23011

AI477504 Z21011
AI332045
AI722168

AI416179

AI330674
AI397142

Z3399 AA566757

AI444254 AI477125
AI496721

Z9387 AI477581
Z249
rtk7 Z9603
AI477215
AI496943

Z9127 Z9325

AI396741 Z3310
Z5657 Z10257

AI497200 nog2
Z20051 Z6020
AI477287 Z911

Z1243

LG 25

AI396844
GOF15 Z1378

Z14408 Z6924
Z1197 GOF1

Z20832

AI477113

AI477662 Z8238
AI497046 wt1
AA494717 Z3490
AA494716 Z3632

AI544968 Z4358

AA497316

Z21055
AI522561

Z22245 Z5030
Z3528 myod

Z22028

Z15401 fkd3
AI436963 Z1431
Z6332 Z3208
Z1213 Z5659
Z11786 Z1984

Figure 3 (*See p. 561 for legend.*)

vergent and many markers exhibit polymorphisms in C32 × SJD crosses (Nechiporuk et al. 1999). Second, because homozygous diploid fish can survive to adulthood, we were able to collect enough genomic DNA to map >10,000 markers in future mapping studies with the HS panel. This is a substantial advantage over mapping panels comprised of haploid embryos, which survive only a few days and therefore yield much less genomic DNA. The ability to map many markers in a single cross diminishes the need to integrate maps produced in different mapping panels. Third, the use of homozygous diploids simplifies linkage analysis in comparison to sexual diploid protocols, because no heterozygotes are present in a population of homozygous diploid fish. This is useful particularly in the analysis of SSCPs, because these polymorphisms can be subtle, so that it can be difficult to distinguish heterozygotes from both classes of homozygotes.

The map presented here will accelerate the molecular analysis of zebrafish mutations by the positional cloning and candidate gene approaches. Positional cloning projects are accelerated as the density of mapped polymorphisms grows and the number of possible entry points for chromosomal walks increases. Gene maps augment the candidate approach because a comparison of map positions is a straightforward initial test to determine if a mutation might disrupt a particular gene. The polymorphisms corresponding to the genes we have mapped allow one to test candidate genes near a mutation of interest for direct linkage in a mutant mapping cross. Finally, this gene map will advance comparative mapping resources by allowing the identification of groups of syntenic genes that are conserved among vertebrates (Postlethwait et al. 1998).

METHODS

Production of Homozygous Diploids

Heat shock diploids were prepared from F_1 females that were heterozygous for C32 and SJD genomes according to the procedures in Westerfield (1995) and raised to maturity. DNA was extracted from fully grown adult heat-shocked fish as described for the isolation of high molecular weight DNA from mammalian cells (Sambrook et al. 1989). Animals varied in mass between 0.19 g and 0.64 g and yielded ~100–500 µg of genomic DNA.

Data Collection and Linkage Analysis

PCR was performed in 12.5-µl volumes as described previously (Gates et al. 1999). In typical PCR assays, 0.6 ng of genomic DNA was used as a template. SSLP markers were electrophoresed on denaturing acrylamide gels and SSCP markers were analyzed on nondenaturing acrylamide gels as described previously (Gates et al. 1999). Because SSCP analysis can generate complex banding patterns and because some primer pairs amplify multiple, unlinked fragments, polymorphisms were scored for major bands on SSCP gels.

Map data were collected and analyzed using Map Manager 2.6.5 (Manly 1993; http://mcbio.med.buffalo.edu/mapmgr.html). The complete genotype data set in Map Manager format can be obtained at http://zebrafish.stanford.edu. Each locus was placed initially at the position that maximized its lod score as indicated with the "Links" feature of Map Manager. Markers were then placed manually to minimize the number of double recombinants. In cases where double recombinants remained, autoradiographs were reexamined, and if an individual was again scored as a double recombinant, the genotype assay was repeated. Map graphics were drawn with MapMaker software (Lander et al. 1987) using the Kosambi mapping function and modified for clarity with Canvas 5.0.

Primer Design

D. rerio sequences from the NCBI nonredundant sequence database (NR) are housed in a MySQL relational database system (http://www.mysql.com), accessible through in-house-developed web query interfaces. The database was updated monthly with new ESTs (M. Clark and S. Johnson, Washington University Zebrafish Genome Resources Project; http://zfish.wustl.edu) deposited in the NR database. Genes and ESTs were clustered into groups of overlapping sequences using TIGR Assembler (Sutton et al. 1995). Primers used to develop SSCPs were designed from these contigs using Primer 3.0.6 (Rozen and Skaletsky 1997) and synthesized at the Stanford DNA Sequencing and Technology Center. SSLP primer sequences were obtained from Shimoda et al. (1999; http://zebrafish.mgh.harvard.edu/) and primers were synthesized at the Stanford DNA Sequencing and Technology Center or obtained from Research Genetics.

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