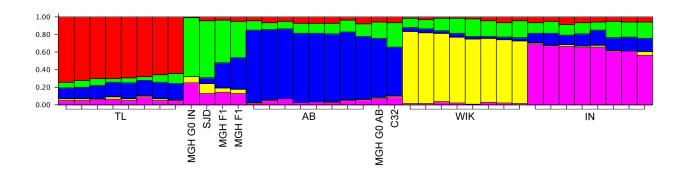


**Figure S1** Zebrafish SNP mapping panel. SNPs that were optimally informative in all potential crosses of sampled fish of strain pairs, and that were optimally distributed across the genetic map were selected to form a generic SNP mapping panel for the zebrafish. The panel is comprised of 1,536 SNPs, capturing 519 unique loci with a mean locus density of 4.0 cM. The proportion of simulated crosses in which a given SNP was informative is noted adjacent to its identifier. Further detail of panel SNPs is given in Table S3.

High resolution image available at http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.111.000190/-/DC1/FigureS1.pdf.



**Figure S2** Estimated population structure of common laboratory zebrafish strains. Individual fish were genotyped for 2,875 genetically positioned SNPs for analysis by STRUCTURE under a linkage model. Each individual fish is represented by a vertical bar, which is partitioned into K colored segments that represent the estimated membership fraction from K ancestral populations. The highest probability model is presented, K = 5. Strain membership is designated at the bottom. The G0 grandparents (one AB, one IN) and F1 parents of the MGH cross are included for comparison. Prior information of strain membership was not employed in the analysis.

			L KR K P	
Danio_rerio	1	1	MCFSVVSVVPLLFILWMLVVRFWRQSHRRTDGVAKENNKPEAVFPKLLHSLYKLFFSTVSPTISGPRSLPLLGNML	76
Tetraodon_nigroviridis	2	1	${\tt MSVEVLVAGVGAGFLLVLLLMVLKDMCVKKNHNLSFSAPPAVKRSLLQSLCGLLHRPSSPSIPGPPCRFLIGNMT}$	75
Fugu_rubripes	3	1	${\tt MVGKHLIDLDRMCSFLFCSSFSAPPAVKRNLPLSLWQLPLRPSSPPIPGPPCRFLIGNMT}$	60
Gasterosteus_aculeatus	4	1	${\tt MVIEISFITAGAAFLVVLL-MIFIGIFCQKDQSVQGDHRGKAASGLLHHLCRFLTRSSSPGLPGPPSLFLIGNMM}$	74
Oryzias_latipes	5	1	MM	2
Anguilla_japonica	6	1	${\tt MPVCMTFVGAGTAVVLCVLWLVFVYYPRQTDQKLCSQSNVVGSTLLYPLNRLLPHTLSPSLPGPPSLPLLGNML}$	74
Homo_sapiens	7	1	MLLAGARLLWNWWKLRSLHLPPLAPGFLHLL	41
Mus_musculus	8	1	MLLPGLLLLLLLLRKLHLPPLAPGFLHFL	41
			H R	
	1	77	DLAQDHLPHHLTALAKCYGNIYRLNCGSTTMVVLNNSEIIREALVKKWSDFAGRPYSYTGDIVSGGGRTISLGDFSEEWK	156
	2	76	ELMQDHLPIHLTDLAKRYGNIYRLKCGNTTMVVLSSGDVIREALVKKWSDFAGRSVSYTADIVSGGGRTISLGDYTEEWK	155
	3 4	61 75	ELMHDHLPIHLTNLAKRYGNIYRLKCGNTTMIVLNSSDIIREALVKKWSDFAGRAVSYTADIVSGGGRNISLGDYTEEWK ELTHEHLPIHLTNLARRYGNIYRLKCGNTTMVVLNSGEIIREALVKKWSDFAGRPVSYTGDIVSGGGHTISLGDYNEEWR	140 154
	5	3	ELMHDHLPIHLTSLAQRYGSIYRLKCGNTTMVVLNSGEIIREALVAXWSDFAGRPISYTGDIVSGGGRNISLGDYTEEWR	82
	6	75	ELTROHLPHHLTSLARHYGNIYRLYCGNTTIVVLSSAEMIREALVKKWSDFAGRPHSYTGDIVSGGGRNISIGDYSEAWR	154
	7	42	QPDLPIYLLGLTQKFGPIYRLHLGLQDVVVLNSKRTIEEAMVKKWADFAGRPEPLTYKLVSRNYPDLSLGDYSLLWK	118
	8	42	QPNLPIYLLGLTQKLGPIYRIRLGMQDVVVLNSNRTIEEALIQKWVDFAGRPHMLNGKMDLDLSLGDYSLMWK	114
	·	12	VINTITITION TO THE PARTY OF	
	1	157	AHRRVTHSALQRCTTDSLHSVIEKQAQHLCQVLRDYSGKAVDLSEDFTVASSNVITTLTFSKAYDKSSAELQKLQECLNE	236
	2	156	AHRRLVHSALQRCCKQSLHDVIERQALQLRKVLINYQGGAVDLSEDFTVAASNVITTLVFGKEYDKSCSELQQLHSCLNE	235
	3	141	ALRRLVHGALQRCCKHSLHNVIERQALQLRKVLVDYRGGAVDLSEDFTVAASNVIITLVFGKEYDKSSSELQQLHRCLNE	220
	4	155	AHRRLVHSALQRCCQQSLHDVIERQALHLREVLMDYRGSPVDLAEDFTVAASNVITTLAFGKEYGKSSSELQKLHSCLNE	234
	5	83	AHRRLVHGALQRCCQQSLHSVIERQALHLRKVLMGYQGSPVDLSEDFTVAASNVITTLAFGKEYDKSSSELQQLHSCLNE	162
	6	155	${\tt THRRLAHSALQRCSAQCLHAVIQKQALGLRQVLMDYNETPVDLSEDFTVAASNVITTLVFGKEYDKSSPELQRLHGCLNE}$	234
	7	119	AHKKLTRSALLLGIRDSMEPVVEQLTQEFCERMRAQPGTPVAIEEEFSLLTCSIICYLTFGDKI-KDDNLMPAYYKCIQE	197
	8	115	$\verb AHKKLSRSALMLGMRDSMEPLIEQLTQEFCERMRAQAGTPVAIHKEFSFLTCSIISCLTFGDKDSTLVQTLHDCVQD $	191
			-	
	1	237	${\tt IVSLWGSPWISALDSFPLLRKFPNPPFSRLMKEVARRDELIGKHIEEFKVRQIVQIISTESCQKFSKGGTLTSSLLKCLE}$	316
	2	236	IVALWGSAWISALDTFPLLRKFPNPVFSRLLREVTRRDEIIRKHLNQYKVQSQDNKSTDVITGSLLEGVS	305
	3	221	IVALWGSTWISALDTFPLLRKFPNPVFSRLLREVSRRDEIIRKHLNQFKVKSEGHRRTDVITGSLLEGVD	290
	4	235	IVALWGSSWISALDSFPLLRKLPNPVFARLLKEVAKRDEIIREHLNNYKSQNNKTEDAITGSLLEGLE	302
	5	163	IVSLWGSSWISALDSFPLLRKLPNPVFARLLKEVARRDEIIKQHLDNFKLQGHQSEDSITGSLLQALD	230
	6	235	IVSLWGSPWISALDSFPLLRKLPNPPFSRLLKEVARRDDIIRSHIQEYKELCQDEGTGGTITASLLQSLD	304
	7	198	VLKTWSHWSIQIVDVIPFLRFFPNPGLRRLKQAIEKRDHIVEMQLRQHKESLVAGQWRDMMDYMLQGVAQ	267
	8	192	LLQAWNHWSIQILTIIPLLRFLPNPGLQKLKQIQESRDHIVKQQLKRHKDSLVAGQWKDMIDYMLQGVEK	261
	1	317	POOGAANHTTLTDTHVHMTTVDLLIGGTETIAALLNWTVAFLLHRPEVODKVYEELCCVLDVRYPQYSDRHKLPYL	392
	2	306	KHR-KDHDVVLTDVHVHMATVDLLIGGTETTAAWLNWTVAFLLHRPEIQTKVYEELCTVLEGRYPKYSDRHRLPVL	380
	3	291	RHO-TEPGVVLTDMHVHMATVDLLIGGSETTAAWLNWTVAFLLHRPEFOTKVYEELCTVLEGRYPKYSDRORLPIL	365
	4	303	RNNNTDHKVRLTDIHVHMATVDLLIGGTETTAAWLNWTVAFLLHRPEVQAKVYEELCTVLEGRYPKYSDRRRLPVL	378
	5	231	KHONTKEGQLLTDVHVHMATVDLLIGGTETTAAWLNWTVAFLLHRPEIQDKVYEELCTVPEGRYPKYSDRQRLPVL	306
	6	305	QPLGEKERVALTETHVHMATVDLLIGGTETTAAWLGWTVAFLLHRPEVQCRVYSELCTVLDTRYPQYSDRLKLPCL	380
	7	268	PXM-EEGSGQLLEGHVHMAAVDLLIGGTETTANTLSWAVVFLLHHPEIQQRLQEELDHELGPGASSSRVPYKDRARLPLL	346
	8	262	${\tt QRD-GKDEERLHEGHVHMSVVDLFIGGTETTATTLSWAVAFLLHHPEIQKRLQEELDLKLGPGSQLLYRNRMQLPLL}$	337
		393		
	2	381	•	460
			CSLIHEVLRLRPVAPLAVPHKAIRDSSIAGYFIPRNTIIIPNLFGAHHDPEVWSDPYSFKPERFLEGGGGSTRALIPF	443
	4		CSLINEVLRLRPVAPLAVPHRAVRDSSIAGYFIPKNTVIIPNLFGAHHDPAVWSDPYSFRPERFLEGGESSTRALMPF	
	5			384
	6		SALINEVLRLRPVAPLAVPHRAIRDSSIAGYRIPKDTVIIPNLYGAHHDPAMWIDPYAFKPERFLDGGRASTRALLPF	458
			NATIAEVLRLRPVVPLALPHRTTRPSSISGYDIPEGTVIIPNLQGAHLDETVWERPHEFWPDRFLEPGKNSRALAF	422
	8	338	MATIAEVLRLRPVVPLALPHRATRASSISGYDIPKDMVIIPNIQGANLDEMVWELPSKFWPDRFLEPGKNPRTPSF	413
	1	471	GGGARLCLGEAVAKMEMFLFTAYLLREFKFLPASKEEPLPELRGVASVVLKVKPYTVIAHPREQ 534	
	2	461	e de la companya del companya de la companya de la companya del companya de la co	
	3	444		
	4	457	GGGARLCLGESVAKMELFLFTAYLLRDFHFIPPENEASLPDLRGVASVVLKVKSYTVTARPRPLT 521	
	5	385	GGGARLCLGESVAKMELFLFTAYLLRDFQFILPESNVFPPDLRGIATVVLKIKPYKVIARPRPAIGP 451	
	6	459	GGGARLCLGESIAKMELFLFTAYLLRDFQFLPSGNKETLPDLTGVASVVLKVKPFQVIVRPRPLT 523	
	7	423	GCGARVCLGEPLARLELFVVLTRLLQAFT-LLPSGD-ALPSLQPLPHCSVILKMQPFQVRLQPRGMGAHSPGQSQ 495	
	8	414	GCGARVCLGEPLARLELFVVLARLLQAFT-LLPPPDGTLPSLQPQPYAGINLPIPPFQVRLQPRNLAPQDQGERP 487	

**Figure S3** Constraint-based multiple-alignment of 21-hydroxylase (cyp21a2). The cyp21a2 orthologs of *Danio rerio* (zebrafish), *Tetraodon nigroviridis* (green spotted pufferfish), *Fugu rubripes* (Japanese pufferfish), *Gasterosteus aculeatus* (stickleback),

Oryzias latipes (medaka), Anguilla japonica (Japanese eel), Homo sapiens, and Mus musculus are aligned for comparison. The male allele of Danio rerio identified within the cross is aligned at the top, with concurrent amino acid changes of the female allele designated above. Highlighted positions are allele changes at residues conserved among fish (in blue), and additionally among humans and mice (in yellow).

4 SI

### File S1

## **Supporting Text**

## **Zebrafish SNP Mapping Panel**

Each of the 2,854 positioned SNPs was evaluated to determine the proportion of potential crosses of pairs of fish in which the SNP would have been informative, among the genotyped fish of the AB, IN, TL, TU, WIK, C32, and SJD strains. These SNPs are provided in Table S2. Each simulated cross employed a pair of fish of different strains. To identify an optimal mapping panel of 1,536 SNPs, each positioned SNP was ranked according to the proportion of crosses in which it was informative. A first selection sweep was then made, in decreasing rank order, selecting each SNP that was no closer than 3 cM to a previously selected SNP. Subsequent iterative selection sweeps were then made (again in decreasing rank order), each sweep allowing single additional SNPs to be positioned within 3 cM of those SNPs added in prior sweeps (but at least 60 bp apart, to accommodate assay). Beyond the third iterative selection, to preclude addition of uninformative SNPs to a region already populated with ≥ 3 SNPs, only SNPs informative in at least 5% of the crosses were considered. However, for region populated with ≤ 2 SNPs, even a SNP uninformative in the simulated crosses but informative in the MGH cross (a validated SNP) was considered.

The resulting generic SNP mapping panel has a mean locus density of 4.0 cM (largest gap 26.2 cM), presented in Figure S1 and Table S3. These SNPs are well distributed and most informative for crosses of pairs of the common laboratory strains, based upon the fish sampled in our study. Within the mapping panel, each locus is typically redundantly represented by several SNPs to improve the probability that at least one of them will be informative in a given laboratory cross.

#### **Zebrafish Genetic Diversity**

We used the program Structure v2.2.3 to evaluate the genetic diversity and ancestry of individuals of each strain (Falush *et al.* 2003; Pritchard *et al.* 2000). Structure can identify distinct genetic populations, assign individuals to populations, and assess individual shared ancestry. Figure S2 presents an analysis of the 2,875 SNPs positioned in the genetic map, within fish of the AB, IN, TL, WIK, C32, and SJD strains. We also included the grandparental G0 fish of the MGH cross (one AB, one IN) to assess how similar earlier samples of the AB and IN strains might be to those more recently obtained. We omitted TU from the analysis because sequence employed for SNP discovery was derived nearly exclusively from the TU strain (Bradley *et al.* 2007). Structure infers the allele frequencies of *K* ancestral populations on the basis of genotypes from a set of individuals and a user-specified value of *K*, and assigns a proportion of ancestry from each of the inferred *K* populations to each individual. The analysis was run without use of prior population information (omitting known strain identity), under a linkage model to accommodate linked

loci, varying *K* from 3 to 8. Ten replicate runs were made for each *K* to identify *K*=5 as a stable model at which the likelihood distribution reached a maximum. This was confirmed by 15 additional runs at *K*=5. The length of the burn-in period was 10,000, and the number of MCMC replications after burn-in was 10,000. The admixture burn-in length was 5,000 under the linkage model.

The resulting model is consistent with known ancestry. Present day laboratory strains of zebrafish are outbred, and are comprised of predominantly distinct genetic ancestry, shared across the strains to varying extents. The origins of the partially inbred lines C32 and SJD are visible in the data. C32 was derived from AB (STREISINGER *et al.* 1981), and SJD was derived from the *Darjeeling* line (Nechiporuk *et al.* 1999), most related to the original MGH IN strain. Based upon net nucleotide distance of the predominant ancestral cluster of each strain, the most divergent pair of strains is AB and WIK. These observations contrast with lore among zebrafish researchers that AB and IN are most divergent (the reason that these were selected for the original MGH cross). However, the IN sample evaluated appears quite distinct from the original G0 IN grandparent of the cross. Values of Fst observed for the predominant ancestral clusters of AB, TL, IN, and WIK are each relatively high (0.86, 0.79, 0.79, and 0.77, respectively), to suggest relatively greater inter- than intra-strain variability in these managed laboratory populations. Fish of the partially inbred C32 and SJD lines that were evaluated had regions of remaining heterozygosity. For each, 11% of mapped clones contained heterozygous SNPs.

# **Literature Cited**

- Bradley, K. M., J. B. Elmore, J. P. Breyer, B. L. Yaspan, J. R. Jessen *et al.*, 2007 A major zebrafish polymorphism resource for genetic mapping. Genome Biol **8**: R55.
- FALUSH, D., M. STEPHENS and J. K. PRITCHARD, 2003 Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics **164**: 1567-1587.
- Nechiporuk, A., J. E. Finney, M. T. Keating and S. L. Johnson, 1999 Assessment of polymorphism in zebrafish mapping strains. Genome Res 9: 1231-1238.
- PRITCHARD, J. K., M. STEPHENS and P. DONNELLY, 2000 Inference of population structure using multilocus genotype data. Genetics **155**: 945-959.
- Streisinger, G., C. Walker, N. Dower, D. Knauber and F. Singer, 1981 Production of clones of homozygous diploid zebra fish (Brachydanio rerio). Nature **291**: 293-296.

Tables S1-S4 are available for download at http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.111.000190/-/DC1.

Table S1 SNP and STR positions in the genetic map, and in the Zv9 physical assembly.

Table S2 SNPs of clones positioned in the genetic map, and observed alleles by strain.

**Table S3** A mean 4 cM density SNP mapping panel for zebrafish. The panel was optimally informative within zebrafish of common laboratory strains sampled in the study (described in Supporting Text). SNP identifiers, genetic map position, and physical clone position are listed. An additional column provides the proportion of all possible crosses of the sampled fish in which each SNP would have been informative.

Table S4 Count and proportion of male and female F2 progeny in each genotypic category of the chromosome 5 and 16 sexdetermination loci (see Figure 4).