Supplementary Material

Figure S1. Measurement of the extent of barring using the grey value CV. Each panel (**a-d**) represents a single fish. A cropped portion of the midline, encompassing 10 body segments (myomeres), is shown underneath a plot of the average grey value for each pixel column across the image (solid line). A dashed line represents the best-fit straight line that was used for calculating the grey value CV, as described in the Methods. The resulting CV value is reported above each panel. Panels (**a,b**) represent barred fish and panels (**c,d**) represent unbarred fish.

Figure S1

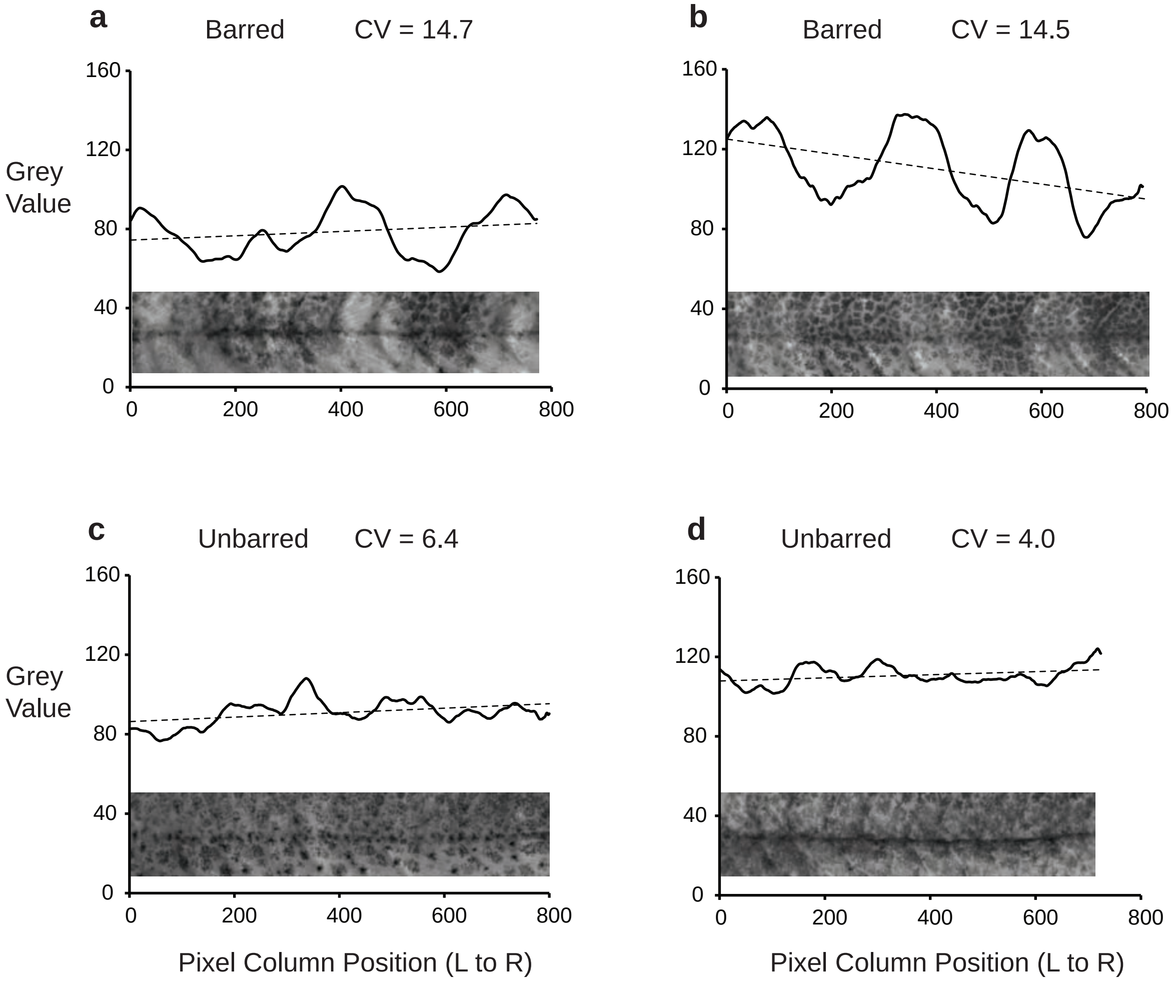
a
Barred
CV = 14.7
Grey Value
160
120
80
40
0
0
200
400
600
800
b
Barred
CV = 14.5
160
120
80
40
0
0
200
400
600
800
c
Unbarred
CV = 6.4
Grey Value
160
120
80
40
0
0
200
400
600
800
Pixel Column Position (L to R)
d
Unbarred
CV = 4.0
160
120
80
40
0
0
200
400
600
800
Pixel Column Position (L to R)

Figure S2. Persistence of barred pattern underneath iridophores in an older Hotel Lake fish. (**a**) shows a photograph of the flank of a live 32-mm Hotel Lake fish; (**b**) shows a photograph of the same fish following immersion in formalin to dissolve iridophores. Scale bar is 2 mm.

Figure S2

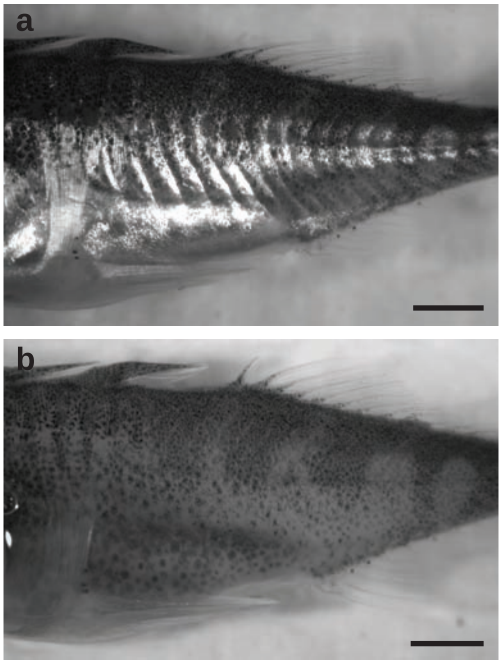

Table S1.
SNP markers used for linkage mapping.

LG	*Map position (cM)*	*Marker name (chromosome: position)*	*NCBI assay ID (ss#)*
1	0	chrI:675926	244222765
1	0.254	chrUn:37631434	244223001
1	1.145	chrI:881806	244222766
1	11.151	chrI:1549902	244222767
1	30.837	chrI:3310077	244222768
1	36.768	chrI:4219350	244222770
1	38.132	chrI:4816374	244222771
1	40.471	chrI:7820850	244222772
1	40.593	chrI:8072483	244222773
1	43.594	chrI:11963492	120258415
1	62.21	chrI:23569502	244222776
1	65.909	chrI:22361077	120258417
1	66.857	chrI:21762138	244223024
1	67.028	chrI:21909727	244223036
1	67.264	chrI:21663978	244222774
1	68.298	chrI:21641897	244223012
1	90.938	chrI:27721035	244222778
2	0.366	chrII:919438	244222781
2	1.006	chrUn:23753128	244222989
2	2.102	chrII:418094	244222780
2	2.171	chrII:411522	244223041
2	2.187	chrII:409087	244223040
2	2.187	chrII:377479	244222779
2	9.363	chrII:3384330	120258421
2	14.398	chrII:4530808	120258423
2	22.505	chrII:6475468	244222782
2	26.071	chrII:12292176	120258425
2	27.637	chrII:13353603	244222783
2	28.246	chrII:14611516	244222784
2	45.933	chrII:19985741	244222785
2	61.075	chrII:21231538	244222786
2	66.734	chrII:22443700	244222787
3	0	chrIII:706165	244222789
3	22.626	chrIII:10376395	244222790
3	30.32	chrIII:13582181	244222791
3	31.555	chrIII:13929118	244222792
3	33.955	chrIII:14307973	244222793
3	51.6	chrIII:15933767	244222795
3	57.676	chrIII:16463929	244222796
4	0	chrIV:1541964	244222797
4	4.091	chrIV:1942641	244222798

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4	40.592	chrIV:8576794	244223068
4	41.361	chrIV:10812344	244223042
4	42.149	chrIV:12810099	244223044
4	42.496	chrIV:12815271	244223046
4	42.501	chrIV:12817401	244223048
4	43.367	chrIV:13850026	244223049
4	43.659	chrIV:13920409	244223053
4	44.849	chrIV:15052901	244222804
4	45.709	chrIV:15721538	244222806
4	48.118	chrIV:26193487	244222808
4	49.309	chrIV:23968803	244223066
4	50.253	chrIV:19906553	244223064
4	76.196	chrIV:31740478	244222809
4	80.243	chrIV:32033500	244222810
4	82.324	chrIV:32133665	244222811
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4	96.33	chrUn:27478064	244222993
4	96.952	chrUn:32765800	244222999
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5	0.582	chrUn:12390868	120258569
5	16.367	chrV:2528528	244222814
5	16.452	chrV:2489835	244223075
5	16.462	chrV:2489551	244223074
5	18.7	chrV:4852958	244222815
5	26.53	chrV:9157076	244222818
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5	28.233	chrV:8562218	244222817
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5	53.305	chrUn:25946639	244222990
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6	2.882	chrVI:218630	244222820
6	5.492	chrVI:3116218	244222825
6	13.981	chrVI:1440771	244222823
6	20.454	chrVI:5777528	244222826
6	25.077	chrVI:8169471	244222827
6	46.876	chrVI:15041940	244222829
6	48.354	chrVI:15274689	244222830
6	48.942	chrVI:15390272	244222831
6	51.003	chrVI:15586470	244222832
6	52.499	chrVI:15780594	244222833
6	60.095	chrVI:16870159	244222834
7	0	chrUn:28671327	244222995
7	18.762	chrVII:1728753	244222835
7	38.988	chrVII:5936068	120258457

7	43.982	chrVII:13452516	244222836
7	45.113	chrVII:17369940	244222838
7	45.404	chrVII:18353106	244222839
7	56.728	chrVII:24331980	244222840
7	63.125	chrVII:25257754	244222841
7	68.716	chrVII:25662266	120258460
7	79.262	chrVII:26538823	244222842
8	0	chrVIII:1929053	244222843
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8	24.462	chrVIII:13412707	244222846
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8	40.352	chrVIII:16826029	244222852
8	42.147	chrVIII:16954373	244222853
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9	39.316	chrIX:5568375	244222863
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9	40.51	chrIX:5403530	120258474
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9	41.434	chrIX:5109672	244222860
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10	16.388	chrX:11252137	244222875
10	16.431	chrX:11317926	244222876
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11	3.636	chrXI:3120961	244222880
11	7.552	chrXI:5652622	244223078
11	8.205	chrXI:5752298	244223080
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13	7.581	chrXIII:2632698	244222901
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13	45.28	chrXIII:18043261	244222905
13	62.569	chrXIII:19824333	244222906
13	88.166	chrUn:55480965	244223073
14	0	chrUn:36334731	244223000
14	14.619	chrXIV:1383447	244222908
14	17.911	chrXIV:1713227	120258513
14	25.205	chrXIV:2345947	244222909
14	55.04	chrUn:21213332	120258571

15	1.574	chrXV:1029767	244223088
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15	17.975	chrXV:2377782	244222913
15	18.302	chrXV:2507809	244222914
15	27.216	chrXV:3568577	244222915
15	33.275	chrXV:5929959	244222916
15	35.182	chrXV:7200442	244223089
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15	54.495	chrXV:15139567	244222918
15	57.629	chrXV:14693722	244222917
15	57.837	chrXV:16193822	244222920
16	0	chrXVI:2392758	244222921
16	2.324	chrXVI:2650854	244222922
16	8.335	chrXVI:11849396	244223090
16	9.708	chrXVI:3206769	244222923
16	15.486	chrXVI:5562355	244222924
16	15.973	chrXVI:6451650	244222925
16	20.744	chrXVI:9428786	244222926
16	21.61	chrXVI:9857539	244222927
16	21.61	chrXVI:9981125	244222928
16	22.429	chrXVI:12111717	120258526
16	24.102	chrXVI:12996432	244222929
16	25.319	chrXVI:13588796	244222930
16	25.635	chrXVI:14093156	244222931
16	25.944	chrXVI:14283264	244222932
16	28.055	chrXVI:14963879	244222933
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16	31.055	chrXVI:15826700	244222935
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16	36.632	chrXVI:17773420	244222937
16	41.949	chrXVI:17347559	244223091
16	42.85	chrXVI:17236926	244222936
16	51.401	chrUn:26389255	244222992
17	0	chrUn:56537801	244223003
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17	18.733	chrXVII:2664810	244222940
17	20.617	chrXVII:3094026	244222941
17	27.542	chrXVII:3906379	244222942
17	29.946	chrXVII:4846889	244222943
17	30.222	chrXVII:4909843	244222944
17	34.203	chrUn:498491	244222983
17	35.291	chrXVII:8770036	244222945
17	35.591	chrXVII:9024413	244222946
17	37.109	chrXVII:9697366	244222947
17	39.234	chrXVII:10176745	244222948

17	41.325	chrXVII:10925100	244222949
18	0	chrXVIII:2251951	244222950
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18	17.782	chrXVIII:9955470	244222951
18	20.125	chrXVIII:11086837	120258542
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18	20.69	chrXVIII:11641450	244222953
18	21.961	chrXVIII:12146976	244222955
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18	22.615	chrXVIII:12501504	244222956
18	22.62	chrXVIII:12818939	120258545
18	22.697	chrXVIII:13193140	244222957
18	23.111	chrXVIII:11896010	244222954
18	29.645	chrXVIII:14076408	244222959
19	0	chrXIX:910003	244222960
19	17.254	chrXIX:2467932	244235759
19	18.201	chrXIX:2464070	244235760
19	18.211	chrXIX:2477173	244235761
19	18.213	chrXIX:2459466	244235762
19	18.374	chrXIX:2526286	244223087
19	27.369	chrXIX:14798132	244223086
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19	27.963	chrXIX:13466609	244222961
19	33.886	chrXIX:7129928	244235763
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20	36.101	chrXX:12622695	244222966
20	37.508	chrXX:9279241	244222965
20	37.766	chrXX:8905625	244222963
20	37.801	chrXX:8918466	244222964
20	47.348	chrXX:3608355	244223093
20	58.211	chrXX:1758783	244222962
20	61.45	chrXX:19020370	244222972
21	0	chrUn:5488093	244222984
21	1.772	chrUn:6720054	244222987
21	2.125	chrUn:6889743	244222988
21	8.063	chrXXI:7544041	244222978
21	8.353	chrXXI:5791519	244222974
21	8.479	chrXXI:5737465	244222973
21	8.653	chrXXI:6037992	244222976
21	8.653	chrXXI:5793103	244222975
21	8.657	chrXXI:7002178	244222977

21	22.381	chrXXI:10236129	244222980
21	29.974	chrXXI:10969152	244222981
21	31.076	chrXXI:11060209	120258566
21	34.162	chrXXI:11179443	244222982
21	52.397	chrXXI:11414383	120258567
21	52.689	chrUn:28234702	244222994
21	52.689	chrUn:28091692	244223072
21	54.145	chrUn:31339987	244222998

Marker name and associated NCBI assay ID (ss#) are shown by linkage group (LG). The position of each marker on the linkage group is shown in centimorgans (cM). The marker name indicates the chromosomal position of the SNP marker in the initial stickleback genome assembly (Broad S1, Feb 2006). Positions on unassembled portions of the genome are listed in reference to the composite “ChrUn” found on the UCSC genome browser. The NCBI assay ID can be used to retrieve SNP marker information at: http://www.ncbi.nlm.nih.gov/projects/SNP/.

Table S2.
List of pigmentation candidate genes, their positions in the stickleback genome, and their functions in pigmentation.

Gene	**Gene name**	**Chr**	**Ensembl predicted gene**	**Function**	**References**
Asip	Agouti signaling protein	ChrXVII: 1.1 ChrXXI: 1.9	Not available Not available	Melanin synthesis; Mc1r antagonist	(Cerda-Reverter *et al.*, 2005; Hoekstra, 2006)
Ap3d1	Adaptor-related protein complex 3, delta 1 subunit	ChrVIII: 14.4	ENSGACG00000011421	Melanosome biogenesis; Mouse *Mocha* mutant	(Navarro *et al.*, 2008)
Ap3s1	Adaptor-related protein complex 3, sigma 1 subunit	ChrXIII: 4.6	ENSGACG00000006172	Melanosome biogenesis	(Navarro *et al.*, 2008)
Atp6ap2	ATPase, H+ transporting, lysosomal accessory protein 2	ChrXVI: 2.3	ENSGACG00000001684	Melanosome biogenesis	(Navarro *et al.*, 2008)
Bnc2	Basonuclin-2	ChrIX: 7.8	ENSGACG00000017398	Chromatophore development; Zebrafish *bonaparte* mutant	(Lang *et al.*, 2009)
Bloc1s1	Biogenesis of lysosome related organelle complex-1 subunit 1	ChrXVII: 4.1	ENSGACG00000005870	Melanosome biogenesis	(Navarro *et al.*, 2008)
Bloc1s3	Biogenesis of lysosome related organelle complex-1 subunit 3	ChrI: 7.1	ENSGACG00000008332	Melanosome biogenesis; Mouse *Reduced pigmentation* mutant	(Navarro *et al.*, 2008; Starcevic and Dell'Angelica, 2004)
Cxcl12 (Sdf1)	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	ChrVI: 8.1 ChrIX: 14.2	ENSGACG00000006921 ENSGACG00000018739	Melanophore development and patterning; Zebrafish *choker* mutant	(Svetic *et al.*, 2007)
Cno	Cappuccino	ChrX: 5.5	ENSGACG00000003558	Melanosome biogenesis; Mouse *Cappuccino* mutant	(Navarro *et al.*, 2008)
Csf1r (Fms)	Colony-stimulating factor-1 receptor	ChrIV: 10.9 ChrVII: 27.9	ENSGACG00000018007 ENSGACG00000020913	Xanthophore development and chromatophore patterning; Zebrafish *panther* mutant	(Parichy *et al.*, 2000b)
Dct (Tyrp2)	Dopachrome tautomerase	ChrXVI: 9.2	ENSGACG00000004101	Melanin synthesis	(Braasch *et al.*, 2007)
Dhpr	Dihydropteridine reductase	ChrIV: 2.7	ENSGACG00000016641	Pteridine synthesis	(Braasch *et al.*, 2007)
Ednrb1	Endothelin receptor B	ChrIII: 0.4 ChrXVI: 9.0	ENSGACG00000012901 ENSGACG00000004048	Melanophore development; Zebrafish *rose* mutant	(Parichy *et al.*, 2000a)
Erbb3	EGF-like receptor tyrosine kinase	ChrXII: 11.3 ChrUn: 2.0	ENSGACG00000009144 ENSGACG00000000747	Chromatophore development; Zebrafish *picasso* mutant	(Budi *et al.*, 2008)
Fbxw4	F-box/WD repeat-containing protein 4	ChrVI: 3.8	ENSGACG00000003785	Chromatophore patterning; Zebrafish *hagoromo* mutant	(Kawakami *et al.*, 2000)
Foxd3	Forkhead box protein D3	ChrVII: 7.7	ENSGACG00000006933	Melanophore and iridophore development; Zebrafish *mother superior* mutant	(Curran *et al.*, 2009; Montero-Balaguer *et al.*, 2006)
Gch1	GTP cyclohydrolase 1	ChrII: 21.8 ChrV: 1.8 ChrVIII: 9.4	ENSGACG00000017325 ENSGACG00000002770 Not available	Pteridine synthesis	(Braasch *et al.*, 2007)
Gchfr	GTP cyclohydrolase I feedback regulator	ChrXV: 2.6	ENSGACG00000006183	Pteridine synthesis	(Braasch *et al.*, 2007)
Gna11	Guanine nucleotide binding protein, alpha 11	ChrVIII: 14.5 ChrXV: 8.0	ENSGACG00000011473 ENSGACG00000009827	Melanocyte development; Mouse *dark skinned 7*	(Kelsh *et al.*, 2009)

Gnaq	Guanine nucleotide binding protein, q polypeptide	ChrVII: 0.2 ChrXIII: 7.9 ChrXIV: 3.3	ENSGACG00000018567 ENSGACG00000007821 ENSGACG00000016186	Melanocyte development; Mouse *dark skinned 1/10*	(Kelsh *et al.*, 2009)
Gja5 (cx41.8)	Gap junction membrane channel protein alpha 5	ChrVI: 15.6 ChrXVI:8.4	ENSGACG00000011699 ENSGACG00000003669	Melanophore and xanthophore patterning Zebrafish *leopard* mutant	(Watanabe *et al.*, 2006)
Hps3	Hermansky-Pudlak syndrome 3	ChrVIII: 6.0	ENSGACG00000006016	Melanosome biogenesis; Mouse *Cocoa* mutant	(Navarro *et al.*, 2008)
Kcnj13 (Kir7.1)	Potassium inwardly-rectifying channel subfamily J member 13	ChrUn: 26.7	ENSGACG00000000413	Melanophore and xanthophore patterning; Zebrafish *jaguar/obelix* mutant	(Iwashita *et al.*, 2006)
Kit	Kit receptor	ChrVIII: 18.7 ChrIX: 0.50	ENSGACG00000014273 ENSGACG00000015944	Melanophore development; Zebrafish *sparse* mutant	(Parichy *et al.*, 1999)
Kitlg	Kit ligand	ChrXIX: 10.5 ChrIV: 18.0	ENSGACG00000009373 Not Available	Melanophore development; Associated with pigmentation differences in sticklebacks	(Miller *et al.*, 2007)
Ltk	Leukocyte tyrosine kinase	ChrXV: 6.0	ENSGACG00000008800	Iridophore development; Zebrafish *shady* mutant	(Lopes *et al.*, 2008)
Mc1r	Melanocortin 1 receptor	ChrII: 12.0	ENSGACG00000015952	Melanin synthesis (melanosome dispersal in fish); Associated with overall pigment variation	(Gross *et al.*, 2009; Hoekstra, 2006)
Mchr	Melanin concentrating hormone receptor	ChrIV: 13.5 ChrXI: 17.0	ENSGACG00000018390 ENSGACG00000019387	Pigment dispersal	(Kawauchi and Baker, 2004)
Mib	Mind bomb	ChrIII: 13.6 ChrXII: 5.6	ENSGACG00000017309 ENSGACG00000005209	Chromatophore development; Zebrafish *white tail* mutant	(Kelsh *et al.*, 1996; Svetic *et al.*, 2007)
Mitf	Micropthalmia transcription factor	ChrXII: 2.1 ChrXVII: 14	ENSGACG00000003425 ENSGACG00000011875	Melanophore and iridophore development; Zebrafish *nacre* mutant	(Lister *et al.*, 1999)
Myo5a	Myosin Va	ChrII: 15.6	ENSGACG00000016512	Melanosome movement	(Aspengren *et al.*, 2009)
Oca2	Ocular and cutaneous albinism 2	ChrUn: 60.4	ENSGACG00000001245	Melanin synthesis; Associated with albinism in cavefish; Medaka *pink-eyed dilution* mutant	(Fukamachi *et al.*, 2004a; Protas *et al.*, 2006)
Mycbp2 (Pam)	MYC binding protein 2	ChrXVI: 2.4	ENSGACG00000001746	Pteridine synthesis; Zebrafish *esrom/tofu* mutant	(Le Guyader *et al.*, 2005)
Pax3	Paired homeobox 3	ChrIII: 2.7	ENSGACG00000014017	Xanthophore and melanophore development;	(Minchin and Hughes, 2008)
Pax7	Paired homeobox 7	LG12: 17.01 ChrUn: 5.05	ENSGACG00000012890 ENSGACG00000001703	Xanthophore development; Associated with color variation in cichlids	(Minchin and Hughes, 2008; Roberts *et al.*, 2009)
Pcbd1	Pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha	ChrVI: 2.9	ENSGACG00000003259	Pteridine synthesis	(Braasch *et al.*, 2007)
Pcbd2	Pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha 2	ChrUn: 47.1	ENSGACG00000000164	Pteridine synthesis	(Braasch *et al.*, 2007)
Pmch	Pro-melanin concentrating hormone	ChrXIV: 10.7	ENSGACG00000018017	Pigment dispersal	(Kawauchi and Baker, 2004)
Pomc	Pro-opiomelanocortin	ChrX: 14.7 ChrXVIII: 10	ENSGACG00000009521 ENSGACG00000010098	Precursor protein for melanocyte stimulating hormone (MSH) peptides	(Logan *et al.*, 2003)

Prdm1	PR domain containing 1, with ZNF domain	ChrXVIII: 9 ChrUn: 27.8	ENSGACG00000009358 ENSGACG00000001740	Chromatophore pigmentation; Zebrafish *u-boot/narrowminded*	(Hernandez-Lagunas *et al.*, 2005; Kelsh *et al.*, 1996)
Prl	Prolactin precursor	ChrXI: 3.1	ENSGACG00000006561	Pigment dispersal	(Oshima and Goto, 2000)
Prlr	Prolactin receptor	ChrXIII: 17.3 ChrXIV: 4.0	ENSGACG00000013765 ENSGACG00000016473	Receptor for prolactin hormone	(Oshima and Goto, 2000)
Pts	6-pyruvoyltetrahydropterin synthase	ChrV: 12.1	ENSGACG00000010227	Pteridine synthesis	(Braasch *et al.*, 2007)
Silv (Pmel17)	Silver protein	ChrXII: 15.3 ChrUn: 1.13	ENSGACG00000011605 ENSGACG00000000544	Melanosome biogenesis; Zebrafish *fading vision* mutant	Schonthaler et al. 2005
Slc24a5	Solute carrier family 24, member 5	ChrII: 18.3	ENSGACG00000016752	Melanosome biogenesis; Zebrafish *golden* mutant	(Lamason *et al.*, 2005)
Slc45a2 (Aim1)	Solute carrier family 45, member 2	ChrXIV: 3.5	ENSGACG00000016297	Melanosome biogenesis; Medaka *b* mutation	(Braasch *et al.*, 2007; Fukamachi *et al.*, 2001)
Sl	Somatolactin	ChrI: 3.5	ENSGACG00000006593	Leucophore and xanthophore development; Medaka *color interfere* mutant	(Fukamachi *et al.*, 2004b)
Sox10	Sry box containing gene 10	ChrIX: 0.8 ChrXI: 4.2	ENSGACG00000016017 ENSGACG00000007318	Chromatophore differentiation; Zebrafish *colourless* mutant	(Dutton *et al.*, 2001)
Spr	Sepiapterin reductase	ChrXIII: 5.1 ChrXIV: 14.5	ENSGACG00000006643 ENSGACG00000018502	Pteridine synthesis	(Braasch *et al.*, 2007)
Trim33	Tripartite motif-containing 33	ChrXII: 5.5	ENSGACG00000004973	Iridophore development; Zebrafish *moonshine* mutant	(Kelsh *et al.*, 1996; Ransom *et al.*, 2004)
Txndc17 (Clot)	Thioredoxin domain containing 17	ChrI: 12.1	ENSGACG00000010961	Pteridine synthesis	(Braasch *et al.*, 2007)
Tyr	Tyrosinase	ChrI: 6.3 ChrVII: 20.7	ENSGACG00000007960 ENSGACG00000020587	Melanin synthesis; Zebrafish *sandy* mutant Medaka *i* mutant	(Koga *et al.*, 1995; Page-McCaw *et al.*, 2004)
Tyrp1	Tyrosinase-related protein 1	ChrVII: 5.6 ChrIX: 0.3	ENSGACG00000019503 ENSGACG00000015912	Melanin synthesis;	(Braasch *et al.*, 2007)
Vps33a	Vacuolar protein sorting 33A	ChrXIII: 12.9	ENSGACG00000011308	Melanosome biogenesis	(Navarro *et al.*, 2008)
Vps33b	Vacuolar protein sorting 33B	ChrII: 22.0	ENSGACG00000017403	Melanosome biogenesis	(Navarro *et al.*, 2008)
Xdh	Xanthine dehydrogenase	ChrI: 27.5	ENSGACG00000015453	Pteridine synthesis	(Braasch *et al.*, 2007)

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