Supplementary Table S1 Sequencing Primers and Conditions

PCR #	Region Amplified	Sequence	Primer Binding Position	Length (base pairs)
1	intron 1 to exon 5, M genes only	GTCTCTGGCTTGAGGGACAG	intron 1, 180 bp upstream of exon 2	5912
		AAGCAGAATGCCAGGACCATC	M gene exon 5, codon 279	
2	intron 1 to exon 5, L genes only	GTCTCTGGCTTGAGGGACAG	intron 1, 180 bp upstream of exon 2	5911
		GCAGTACGCAAAGATCATCACC	L gene exon 5, codon 278	
3	L/M exon 1	AGTCCCAGGCCCAATTAAGAGAT	155 bp upstream of ATG start codon	301
		CAGCCACCCAGCCTCCAC	intron 1, 35 bp downstream of exon 1	
4	L/M exon 2	GGTGGGATCAGCACTGGTAT	74bp upstream of exon 2	420
		GCAGGGTGAATGAGTGGTTT	49 bp downstream of exon 2	
5	L/M exon 3	TGTCGTTTTTTCCACCTCAGTCC	intron 2, 136 bp upstream of exon 3	351
		CAGAGTCTGACCCTGCCCACT	intron 3, 46 bp downstream of exon 3	
6	L/M exon 4	TGGCTGCCGGCCCTTC	intron 3, 23bp upstream of exon 4	251
		TTGAGGGCAGAGCAGCTTAGG	intron 4, 62bp downstream of exon 4	
7	L/M exon 5	TCCAACCCCCGACTCACTATCC	intron 4, 35bp upstream of exon 5	314
		ACGGTATTTTGAGTGGGATCTGCT	intron 5, 39 bp downstream of exon 5	
8	L/M exon 6	ACCCTTCCCTGCTCTGCTCAA	intron 5, 42bp upstream of exon 6	201
		GGAGAGGTGGCCAAAGCCC	intron 6, 51bp downstream of exon 6	
9	First gene in the L/M array	CCTGGGCTTTCAAGAGAACCACATG	459 bp upstream of ATG start codon	12912
		CACCTAAGCCTTCTGCTAAGGGCCA	202 bp downstream of exon 5	
10	Nonspecific down-stream L/M genes	ATACCCTGCAAGTGGGAATCTA	736 bp upstream of ATG start codon	11747
		ACGGTATTTTGAGTGGGATCTGCT	intron 5, 39 bp downstream of exon 5	
11	Last gene in the L/M array, intron 4 to end	CCACGCCCAGTCATCAATCAAATC	intron 4, 331bp upstream of exon 5	27792
		GAATGTGCTCGCCCTGTGTCTGAA	25kb downstream of exon 6	

The L and M opsin genes were separately and specifically amplified using primer pairs 1 and 2. Specificity for L or M genes was conferred by the reverse primers because they hybridize to sequences within exon 5 unique to L or M genes. The PCR products obtained with primer pairs 1 and 2 which amplify a gene segment encompassing exons 2 through a portion of exon 5 were used in another round of PCR to amplify exons 2, 3, and 4 individually using primer pairs 4, 5, and 6 (Supplementary Table S1). For a subset of subjects, exons 1, 5, and 6 were amplified non-specifically from all genes in the array using primer pairs 3, 7, and 8 (Supplementary Table S1). Primer pairs 3 through 8 amplify individual exons including about 50 base pairs of flanking introns. Primer pair 9 specifically amplifies the first gene in the array, primer pair 10 specifically amplifies downstream genes (all genes after the first), and primer pair 11 amplifies part of the final gene in the array. For each primer pair, the forward primer is listed first and the reverse primer is underneath. All primer sequences are 5' to 3'. For the L or M specific PCRs, cycling conditions were: (1x) 94C for 3 minutes; (30x) 94C for 30 seconds, 61C for 30 seconds, 68C for 6 minutes; (1x) 68C for 20 minutes. For exon-amplifying PCRs, cycling conditions were: (1x) 94C for 3 minutes; (30x) 94C for 30 seconds, 61C for 30 seconds, 68C for 30 seconds; (1x) 68C for 3 minutes. For the first gene and downstream gene PCRs, cycling conditions were: (1x) 94C for 3 minutes; (30x) 94C for 10 seconds, 56C for 30 seconds, 68C for 11 minutes plus 20 seconds per cycle starting in cycle 11; (1x) 68C for 20 minutes. For the last gene PCR, cycling conditions were: (1x) 94C for 3 minutes; (30x) 98C for 10 seconds, 68C for 20 minutes plus 20 seconds per cycle starting in cycle 16; (1x) 68C for 20 minutes.

DNA segments smaller than 10 kilobase pairs (kb) were amplified either with the AmpliTaq Gold PCR kit or the XL-PCR kit in conjunction with AmpliWax Gems until the latter product was discontinued. The Invitrogen Platinum Taq kit was used for the remainder of the samples. DNA segments larger than 10 kb were amplified with Takara LA Taq kit (Clonetech). The final reaction volume of each PCR was 50 ul with primer concentrations of 200 nM. Concentrations of all other reaction components were those recommended by the manufacturers.
The PCR products obtained with primer pairs 3 through 8 were sequenced with the same primers using BigDye Terminator v3.1 cycle sequencing (Applied BioSystem). Reactions were analyzed on an ABI 3500 Genetic Analyzer.

The spectral class of the pigment encoded by the last gene in array was identified by selectively amplifying the last gene using primer pair 11 with a reverse primer that lies outside the repeat unit of the array, then amplifying exon 5 from the last gene using primer pair 8, and finally subjecting the PCR product to Rsa I restriction enzyme digestion.

Supplementary Table S2 MassArray Primers

SNP Location	Purpose	PCR Primer Sequences	Extension Primer Sequence
L/M opsin nucleotide +1	Characterize L/M opsin array	ACGTTGGATGTTTTAAGGTGAAGAGGCCCG ACGTTGGATGTGGCTATGGAAAGCCCTGTC	GGGGTGGCAGCCGGCCCTGG
L/M opsin codon 309		ACGTTGGATGCTTCCACCCTTTGATGGCTG ACGTTGGATGACCTGCCGGTTCATAAAGAC	CCTGCCCTGCCGGCCT
L/M opsin codon 116	Spectral tuning sites	ACGTTGGATGGTCATCGCCAGCACTATCAG ACGTTGGATGCTCCAGGACACACATAGGGT	CCCAGCACGAAGTAGCCA
L/M opsin codon 180		ACGTTGGATGTGAGATTTGATGCCAAGCTG ACGTTGGATGCTCCAACCAAAGATGGGCG	GGGACTGTCCACACAGCAG
L/M opsin codon 230		ACGTTGGATGATTGTCCTCATGGTCACCTG ACGTTGGATGCACACTTGGAGGTAGCAGAG	TCACCTGCTGCATCA
L/M opsin codon 203	Known deleterious mutations	ACGTTGGATGTTGGTTGGAGCAGGTACTGG ACGTTGGATGATCATGTAAGACTGCACCCC	CACCACGGCCTGAAGACTTCA
S opsin codon 56		ACGTTGGATGCTTCCTTATAGGGTTCCCAC ACGTTGGATGGCTGCCGCAACTTTTTGTAG	ACTCAATGCCATGGTGC
S opsin codon 79		ACGTTGGATGAGCCCCTCAACTACATTCTG ACGTTGGATGTTACAGCTGGCGACGAAGAC	GAAGAGGAGGAAGCCTC
S opsin codon 190		ACGTTGGATGGTTTGGTCCTTTGCAGGTTC ACGTTGGATGAGGACTCGCTGCGGTATTTG	GGCCCTGACTGGTACA
S opsin codon 214		ACGTTGGATGAGTCCTATACGTGGTTCCTC ACGTTGGATGTCAGCAGCTGAGTGTAGGAG	TCTGCTTCATTGTGCCTCTC
S opsin codon 264		ACGTTGGATGGTGATGGTAGGATCCTTCTG ACGTTGGATGCCATGGTTACGGTTGTTGAC	CGAAGGCCGCGTAGG
S opsin codon 283		ACGTTGGATGTCAACAACCGTAACCATGGG ACGTTGGATGGTAGATGCAAGCACTCTTGG	GAACCATGGGCTGGACTTAC

For each PCR primer pair, the forward primer is listed first and the reverse primer is underneath. All primer sequences are 5' to 3'. PCR conditions are the standard recommended condition given by Sequenom for use with the MassArray system.