Human Long-wavelength cone pigment gene (OPN1LW) primers

RD2e222F	CAG CAT TGT GAA CCA GGT CTC
RD2e241F	TCT GGC TAC TTC GTG CTG G
RD2i617F	GGA GCA GTT TGT GGT TCG
RD2i1157F	ATC TGG GAA GCC TGT GGA C
RD2i1775F	ACG GGT ACT GCT AAT ACT GCC
RD3i258F	GTC TCT TTC TTG CAC ACG CTG
RD3i419R	AAA CAG TGA AAT GCT GTC CCA C
RD3i949F	CCT GGG CTC AAG TGA TCC TC
RD3i1088R	CCC AGG AGT TCA AGA CTA GCC
RD4i61R	TGA GGG CAG AGC AGC TTA G
RD4i462F	ACC CGA GAG TGC CAT TTG
RD4i609R	TCC CTT GCT CTG ACT TCC TG
RD4i1116F	AAG CCT GAG GGA AGT GTA TGC
RD5e129R	AGC AAA GCA TGC GAA GAA GGT G

All primers are presented from 5' to 3'. Other than the two underlined primers (OPN1LW-specific, see below), all primers were generated from simple primer program searches of publicly available OPN1LW nucleotide sequence. However, although all exons, and introns 2 and 4, had been previously studied, no data was publicly available for intron 3 at the time of this project and so all primers in this region were generated after initial 'chromosomal' walks of repeated sequencing reactions. Primer labels refer to their placement in their respective introns and exons and 'F' and 'R' refer to forward and reverse primers, respectively. For example, RD2i617F is a forward primer that starts with nucleotide bp 617 in intron 2. At the time of the project, an ABI 3100 (16 capillary) automated sequencer was used, and therefore, sequencing primers were placed ~660 bp apart, and resulted in sequences with ~40 bp of overlap. Note that new ABI sequencers can easily return sequences of ~900 bp from PCR products. Primary PCR fragment breakdown is as follows:

partial exon 2	:	83 bp
intron 2	:	1,987 bp
exon 3	:	169 bp
intron 3	:	1,467 bp
exon 4	:	166 bp
intron 4	:	1,554 bp
partial exon 5	:	129 bp

PCR Conditions

Primary amplification from genomic: RD2e222F / RD5e129R (5,555 bp)

** These two primers are the only ones that are specific to the OPN1LW gene and are modified from Winderickx et al. (1992) Nature 356:431-433. The conditions below are optimized for long-template PCR, which typically uses lower denaturing and extension temps to protect the sensitive enzyme over long periods. All PCR was performed on a MJ thermocycler, which takes advantage of much shorter cycle times in order to not unnecessarily cook the enzyme too long (i.e., 12 sec at 94° is more than sufficient). Conditions below are for 25 ul reactions and produces a single PCR product.

initial 94° for 1 minute
94° (12 sec)
66° (30 sec)
68° (6 min)
X 40 cycles

All PCR products were run on ~1% generic agarose gels in TAE buffer and stained in EtBr post-electrophoresis. PCR bands were quickly visualized on a LOW intensity UV-illuminator; PCR products were excised and placed in tubes of 100 ul of water for ~24 hrs at ~55° to release DNA from gel but so as not to dissolve gel in water (the latter typically happens at ~65°). Gel was not subsequently removed from the water. This 5.5 kb template was used (~2-8 ul depending on PCR success) in a second round of 25 ul PCR to re-amp smaller fragments with generic cheap Taq (doesn't do so great for fragments over 3 kb) to prepare for cycle sequencing. All re-amps were performed using the universal conditions below...quite robust because the template is highly specific now and all primers are nested (i.e., internal). PCR products were cleaned using typical SAP-EXOI protocol and directly cycle-sequenced with ABI Big-Dye. For further specificity and clean sequence reads, sequencing primers are nested as well. Fragment III was amplified and sequenced first with RD4i1116F to conservatively verify that the 5.5 kb fragment had amino acid sites in exon 5 consistent with L-cone pigments (i.e., not PCR fragments from the M-cone pigment gene OPN1MW).

initial 94 °
94 ° (12 sec)
62 ° (25 sec)
72 ° (3 min)
X 35 cycles

Fragment I : RD2e222F / RD3i419R (2,658 bp)

Sequenced product with : RD2e241F, RD2i617F, RD2i1157F, RD2i1775F

Fragment II : RD3i258F / RD4i609R (1,984 bp)

Sequenced product with : RD3i1088R, RD4i61R

Fragment III : RD3i949F / RD5e129R (2,367 bp)

Sequenced product with : RD4i462F, RD4i609R, RD4i1116F