Supplementary Table 1. List of all opsin sequences used in this analysis and their associated Genbank accession numbers

Taxon		NCBI accession number				
Genera	Species	SWS1	SWS2	RH2	LWS	RH
Alligator	mississippiensis					U23802
Ambystoma	tigrinum	AF038948	AF038946		AF038947	U36574
Anguilla	anguilla					L78008
	japonica					AJ249202
Anolis	carolinensis	AF134192	AF133907	AF134191	U08131	L31503
Aotus	trivirgatus				AB081277	
Astyanax	mexicanus		AF134766	S75255	U12024 + 5 M90075	U12328
Atherina	boyeri					Y18676
Bos	taurus	NM_174567			AF280398	AH001149
Bufo	bufo					U59921
Bufo	marinus					U59922
Callithrix	jacchus	L76201			AB046546-8	
Canis	familiaris					X71380
Capra	hircus				U68004	
Cavia	porcellus				AF132042	
Carassius	auratus	D85863	L11864	L11865 + 6	L11867	L11863
Columba	livia	AJ238856	AF149242	AF149233	AF149248	AF149231
Conger	myriaster					AB043817
Cottus	gobio		AF430489			
Cynops	pyrrhogaster	AB052889	AB040148		AB043891	AB043890
Cyprinus	carpio	AB113669	AB113668	AB110602 + 3	AB055656	Z71999
Danio	rerio	AF109373	AF109372	AF109369 + 70 AB087805-8	AF109371	AF109368
Delphinus	delphis				AY228451	
Dimidiochromis	compressiceps	AF191220	AF247113 + 17	AF247130	AF247128	Carleton et al
Equus	caballus				AF132043	
Felis	catus				AF132040	AJ417432
Gambusia	affinus					Y11146
Galeus	melastomus					Y17586
Gallus	gallus	M92039	M92037	M92038	M62903	D00702
Gecko	gecko	AY024356		M92035	M92036	
Geotria	australis	AY366495	AY366492		AY366491	
Globicephala	melas				AY228446	AF055315
Gobius	niger					Y18675
Hippoglossus	hippoglossus	AF156264	AF316497	AF156263	AF316498	AF156265
Homo	sapiens	NM_001708			NM_020061 NM_000513	NM_000539
Latimeria	chalumnae			AF131262		AF131257
Lucania	goodei	AY296735	AY296736 + 7	AY296739	AY296740	AY296738
Macaca	fascicularis	AF158977			AF158968 + 75	S76579
Macropus	eugenii	AY286017			AY286018	
Melopsittacus	undulatus	Y11787		AF021241		AF021242
Mesoplodon	bidens					AF055316
Metriaclima	zebra	AF191222	AF247114 + 8	AF247122	AF247126	Carleton et al
Miopithecus	talapoin	L76226				
Mus	musculus	NM_007538			NM_008106	NM_145383
Nonnospalax	ehrenbergi				AF139726	
Odocoileus	virginianus				AF132041	
Oncorhynchus	mykiss	AF425074	AF425075	AF425076	AF425073	AF425072
Oreochromis	niloticus	AF191221	AF247116 + 20	AF247124	AF247128	Carleton et al
Oryctolagus	cuniculus					U21688
Oryzias	latipes	AB001605	AB001602	AB001603	AB001604	AB001606
Phelsuma	madagascariensis	AF074045		AF074044	AF074043	
Phoca	vitulina				AY228448	AF055317
	groenlandica					AF055318
Poecilia	reticulata					Y11147
Raja	erinacea					U81514
Rana	catesbeiana	AB001983	AB010085			S79840
	pipiens					S49004
	temporaria					U59920
Rattus	norvegicus	U63972			NM_053548	Z46957
Sardina	pilchardus					Y18677
Sciurus	carolinensis				AF132044	
Scyliorhinus	canicula					Y17585

Continued

Supplementary Table 1. Continued

Taxon		NCBI accession number				
Genera	Species	SWS1	SWS2	RH2	LWS	RH
Serinus	canaria	AJ277922				
Sminthopsis	crassicaudata					AY159786
Solea	vulgaris					Y18672
Sus	scrofa	AY091587				AF008947
Taeniopygia	guttata	AF222331	AF222332	AF222330	AF222333	AF222329
Takifugu	rubripes		Genome scaffold 5	AF226989	Genome scaffold 5	AF201471
Trichechus	manatus				AY228447	AF055319
Tursiops	truncatus				AF055457	AF055456
Xenopus	laevis	U23463	AY177405		U90895	L04692
Zosterisessor	Ophiocephalus					Y18678

Supplementary Table 2. Amino acids present at each site in the opsin alignment. The bovine RH1 site number and amino acid are given in the first two columns. The sites which are unanimous in each opsin class are listed. The list of all occurring amino acids is given on the right. The – indicates that some opsins have a gap in the alignment at that site. Comparisons between classes identifies sites which are unique to a particular class. These signature sites are highlighted by color in the unanimity site columns

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
1	M	M	M	M	M	M	M	M	M	M	M	M
								A-	-	-	-	-
								G-	-	-	-	-
								T-	-	-	-	-
			-	-	-	-		AGTV-	-	-	-	-
			-	-	-	-		ADEGHQSTY-	-	-	-	-
			-	-	-	-		AEGHQRST-	-	-	-	-
			-	-	-	-		ALVW-	-	-	-	-
			-	-	-	-		ADEGNSW-	-	-	-	-
			-	-	-	-		DEKLPRSTV-	-	-	-	-
			-	-	-	-		AGHPQV-	-	-	-	-
			-	-	-	-		AFGIKMRSV-	-	-	-	-
			-	-	-	-		AFLVY	-	-	-	-
			-		-	-		AFLST	-	N-	-	-
			-		-	-		ADG	-	QSTY-	-	-
					-	-		AEGRVW	AV-	KMPQ-	-	-
					-	-		HQR	W-	GHKMRSV-	-	-
					-	-		AHKPQRSTY	DE-	AKPQRS-	-	-
						-		DGHLNQRY	G-	GHKNPQRSV-	S-	-
						-		ADEHNPQT-	G-	ADEGKNQRSTV	K-	-
						-		ADGHNS-	ISY-	AILMPQRS	MRS-	-
						-		DEFHLNY-	EG-	DILMPQRTV	KPR-	-
						-		DE-	ELPQ-	ADEHIMPQTV	EKLMS-	-
2	N		N			N		DE	N	DEN	EGLMSV-	N
3	G		G			G		ST	G	FLM	DEGMS-	G
4	T	T				T		T	FT	FHPQS	DEGQ-	T
5	E		E			E		HKQR	E	DE	ADEK	E
6	G		G			G		ADEGS	G	DEG	DEHWY	G
7	P							ADST	DFIKMNSV	FY	AFT	DELPQT
8	N		N					AILMSV	N	FHWY	HLVY	ADFMNY
9	F	F	F			F		F	F	IMV	LQ	F
10	Y		Y	P	-			AIRTV	Y	P	-	NY
11	V				-			FY	IV	ILMV	-	IV
12	P	T	P		-	P		T	P	APST	-	P
13	F				-			DN	LMV	LM	-	FMY
14	S				-			AENST	NS	DEN	-	LSV
15	N	N	N			N		N	N	AITV	FY	N
16	K							AHNST	KR	ADKNPST	AEGKQT	AHIKQTV
17	T	-	T	N	N			-	T	N	N	ST
18	G	-	G			G		-	G	ILMV	AGILQV	G

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
19	V	-			S			-	ILV	ST	S	ILV
20	V	T						T	AV	AEST	DIKLNSV	GV
21	R		R					KR	R	FLY	GIPV	KR
22	S			S				DG	DNS	S	DGKRS	NST
23	P	P	P	P	P	P	P	P	P	P	P	P
24	F	F						F	FY	FY	FWY	FY
25	E							DE	DELV	LS	DE	DE
26	A	G	Y	V	G			G	Y	V	G	AEFQY
27	P	P		P		P		P	EPQST	P	EP	P
28	Q	N	Q	Q	Q	Q		N	Q	Q	Q	Q
29	Y							HY	FY	DSTV	FY	HY
		-	-	-		-		-	-	-	P-	-
30	Y	H	Y	H		Y		H	Y	H	HY	Y
31	L	I		L		L		I	LM	L	IL	L
32	A	A						A	AV	AG	AP	AGV
33	E	P			P			P	DEP	DGHNST	P	ADENPS
34	P	R						R	PS	LPQST	AIKMPQRSTV	HP
35	W	W			W			W	IMW	AFGLMST	W	AW
36	Q							IMV	FIKMQ	ILMTV	ATV	AGKMQ
37	F			F				FY	FY	F	FY	FY
38	S							HIN	KRSY	KMRY	HRTY	AGST
39	M							FILMV	ACFILV	AGISTV	CFIL	ACGILMV
40	L				Q			ASTV	LV	ILM	Q	LM
41	A							ST	ACGS	AST	AT	ACGS
42	A							ACFILSTV	ACFILTV	AFGIV	AILV	AC
43	Y		Y		F	Y		FW	Y	FLY	F	Y
44	M	M			M	M		M	ILMT	MT	M	M
45	F		F		G	F		FILTV	F	FL	G	F
46	L							FILV	FL	AFILV	FILMSTV	FLMT
47	L	V	L		V	L		V	L	IKLTV	V	L
48	I					I		LV	IMV	FILVW	FL	I
49	M							AFIMTV	ACFILS	AILTV	AFILMV	ILMV
50	L							AL	AFLMTV	AFGILV	ACITV	ALTV
51	G	S	G	G	G	G		S	G	G	G	G
52	F							CILTV	FLMT	AFGITV	FLT	FL
53	P		P		P	P		FLV	P	AFGIPSV	P	P
54	I	T						T	IL	IL	FLM	IV
55	N	N	N	N	N	N	N	N	N	N	N	N
56	F					F		GS	AFGILSV	AFILTV	AFGT	F
57	L			L				LP	FL	L	ILMTV	LM

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
58	T	V	T	T		T		V	T	T	IV	T
59	L		L		L	L		IL	L	IV	L	L
60	Y							AV	FILVY	AFILV	FILVW	FY
61	V	A				V		A	CV	CV	AV	V
62	T		T	T		T		ST	T	T	IT	T
63	V							ALMVW	AF	AFILV	AFILMV	ILV
64	Q							KR	KQT	KLQR	HKR	EQ
65	H	F			Y			F	HN	FHNY	Y	HN
66	K		K			K		EK	K	KR	KR	K
67	K	K	K	K		K		K	K	K	KR	K
68	L	L	L	L	L	L	L	L	L	L	L	L
69	R			R	R	R		QR	QR	R	R	R
70	T	H	Q					H	Q	ST	AHQV	QST
71	P	P	P	H	P			P	P	H	P	AP
72	L	L	L		L	L		L	L	FL	L	L
73	N	N	N	N	N	N	N	N	N	N	N	N
74	Y	W		Y		Y		W	FY	Y	FY	Y
75	I		I	I		I		IM	I	I	IV	I
76	L	L	L	L	L			L	L	L	L	LP
77	L		V		V	L		SV	V	LV	V	L
78	N	N	N	N	N			N	N	N	N	DN
79	L		L			L		LM	L	LM	IV	L
80	A	A	A					A	A	AS	CST	AGV
81	V							FW	AFV	AFILV	AFLV	FIV
82	A		A					AV	A	AGS	ACGS	ACS
83	D	D		N	G			D	DGNS	N	G	DN
84	L			L				ILM	ALMST	L	FL	HL
85	F							AGLV	CFILV	FILW	ILMTV	CF
86	M	E		V		M		E	MT	V	ACFILMSY	M
87	V	T						T	ACV	AISTV	CDV	IV
88	F							ILV	ACFILV	CFGILMSTV	ILTV	FIL
89	G							FILV	CF	CFIMTV	FILV	CGM
90	G	A	G			G		A	G	AG	ACS	G
91	F	S	F			F		S	F	PS	INV	F
92	T		T					GT	T	FPST	FS	FPT
93	T							IV	IV	LTV	IPQSTV	ISTV
94	T	S			V	T		S	CST	ACGS	V	T
95	L							IV	FT	CFS	FS	ILMV
96	Y							CFITV	STY	CILVY	FILMV	IVWY
97	T	N						N	ACST	ACST	ACNST	ST

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
98	S	Q				S		Q	ACS	FN	AST	S
99	L							FILMV	FILMVW	AFILMSTY	ACLMSVW	LM
100	H							FHSY	NVY	CFHKNQVY	HKNQRY	HN
101	G	G			G			G	AG	KMR	G	AG
102	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
103	F	F				F		F	FMY	FM	FY	F
104	V							IV	AISVY	AFISV	FILSV	IV
105	F	L						L	FLW	FLMPV	FL	FL
106	G	G	G	G	G	G	G	G	G	G	G	G
107	P	H						H	APST	APST	HKPQRY	AEPQRSTV
108	T	P						P	LMTV	ILTV	ADFHIRT	ALMSTV
109	G					G		LM	AFG	AFG	AFILMV	G
110	C	C	C	C	C	C	C	C	C	C	C	C
111	N							IV	AGTV	KQR	AEGRS	LNY
112	L							FILV	FIMV	IMV	FILM	AFILPV
113	E	E	E	E		E		E	E	E	DE	E
114	G	G	G	G		G		G	G	G	AGS	G
115	F		F	F				FWY	F	F	ACF	FY
116	F							ILTV	FM	AILMSTV	AFLMV	FS
117	A	V	A		G	A		V	A	ASV	G	A
118	T		T			T		AS	T	AGT	ACST	T
119	L							ALSTV	IL	ILV	AHILTV	FHLMT
120	G	C	G	G		G		C	G	G	AGST	G
121	G	G	G	G	G	G	G	G	G	G	G	G
122	E	I						I	EQ	IM	LM	EIQ
123	I				V			AST	IV	ILMV	V	IMNV
124	A							AG	AS	GS	ST	AGS
125	L	L	L	L		L		L	L	L	AGS	L
126	W	W	W	W	W	W	W	W	W	W	W	W
127	S	S	S		S			S	S	CS	S	ACS
128	L	L	L	L	L	L		L	L	L	L	L
129	V		V		A	V		AST	V	ACS	A	V
130	V		V	V				IV	V	V	FV	AV
131	L	I	L		L	L		I	L	ILV	L	L
132	A		A	A				AS	A	A	AS	AS
133	I							FW	IV	FL	AFILM	FIMV
134	E	E	E	E	E	E	E	E	E	E	E	E
135	R	R	R	R	R	R	R	R	R	R	R	R
136	Y	W	Y					W	Y	CFWY	FY	WY
137	V			L				FILMV	IV	L	ILV	ILMV
138	V	V	V	V		V		V	V	V	IV	V

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
139	V	V			I			V	IV	IV	I	IV
140	C	C	C	C	C	C	C	C	C	C	C	C
141	K	K	K	K	K			K	K	K	K	EK
142	P	P	P	P	P	P	P	P	P	P	P	P
143	M	F	M					F	M	FLMV	FLM	IMV
144	S	G	G	G	G			G	G	G	G	AST
145	N							NS	NS	GNST	ANST	DN
146	F					F		ILMV	FL	FIV	FI	F
147	R					R		KR	KRT	AIQSTV	KNRT	R
148	F	F	F	F	F	F	F	F	F	F	F	F
149	G	D						D	AST	KR	ADGNST	GST
150	E							AEGS	AGS	EGPST	ANQS	ES
151	N	K						K	ANST	DENPST	GKNRS	NQST
152	H		H	H		H		LMVW	H	H	HQ	H
153	A	A		A		A		A	AS	A	AS	A
154	I							AILMSTV	AFGILMS	ILMTV	ALMSTV	FILMV
155	M							AGILMTV	AGIMV	AFGILMV	AGILMTV	AIM
156	G		G			G		GL	G	CFGR	AIV	G
157	V			C	V			IV	CIV	C	V	LV
158	A							AILTV	AGLS	AGIMV	AGILV	AGIMSV
159	F	F						F	FS	FILV	ACFILT	CFLMVW
160	T		T		T			AST	T	APT	T	ST
161	W	W	W	W	W	W	W	W	W	W	W	W
162	V							AILTV	FIV	ACFIMV	AFILMTV	FILTV
163	M	W						W	AM	ACFILMSV	ILM	AGM
164	A				G	A		AS	AS	AG	G	A
165	L							ACIW	CFILMS	FILV	FILTV	ACLMNS
166	A							AFGILV	ACST	ACFILSTV	AGSV-	AST
167	C	W	C			C		W	C	AI	CV	C
168	A							CST	AG	AGST	AGRS	AST
169	A							AT	AGV	AGILSTV	CILSTV	AEFGLMV
170	P	P	P	P				P	P	P	GP	AP
171	P	P	P	P	P	P	P	P	P	P	P	P
172	L		L	L				ILMV	L	L	FLWY	IL
173	V	F						F	FLV	FLV	FLVW	AFLV
174	G	G	G	G	G	G	G	G	G	G	G	G
175	W	W	W	W	W	W	W	W	W	W	W	W
176	S	S		S	S	S		S	PS	S	S	S
177	R	R	R	R	R	R	R	R	R	R	R	R
178	Y			Y		Y		FY	FY	Y	FY	Y
179	I	W		I		I		W	ILM	I	ILMV	I

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
180	P	P	P	P		P		P	P	P	AP	P
181	E		E	E	E	E		HY	E	E	E	E
182	G	G	G	G	G	G	G	G	G	G	G	G
183	M	L						L	ILM	FLM	IL	LM
184	Q	K	Q			Q		K	Q	HQ	GQ	Q
185	C	T		C				T	CTV	C	CT	CS
186	S	S	S	S		S		S	S	S	AS	S
187	C	C	C	C	C	C	C	C	C	C	C	C
188	G	G	G	G	G	G	G	G	G	G	G	G
189	I	P	P	P	P			P	P	P	P	IV
190	D	D	D	D	D	D	D	D	D	D	D	D
191	Y	V	Y	W	W	Y		V	Y	W	W	Y
192	Y	F	Y	Y	Y	Y		F	Y	Y	Y	Y
193	T		T	T	T	T		GS	T	T	T	T
194	P	G						G	HL	ATV	HKNV	LMPRS
195	H							NST	ANS	DEGN	CDGNS	AHKNPRST
196	E			N				DEGNSTV	EP	N	EQT	EPQ
197	E			K				DEY	DEGK	K	EKQ	ADEG
198	T							LP	FY	FWY	FLY	FITVY
199	N	G						G	HN	HIN	GHKNRSY	HNY
200	N		N			N		CV	N	NT	CST	N
201	E		E	E				AKLQ	E	E	AEST	EV
202	S	S	S			S		S	S	ST	HSTY	S
203	F				Y	F		FY	FY	FY	Y	F
204	V	M	V	V		V		M	V	V	IMST	V
205	I							ILMV	ILM	ILMW	HKNWY	IV
206	Y		Y		F	Y		ATV	Y	FY	F	Y
207	M	L				M		L	LM	IL	IL	M
208	F		F	F		F		LM	F	F	FILM	F
209	V							ILSTV	GGILSTV	CFGIT	IV	ILTV
210	V							ST	CITV	CF	FLST	CV
211	H	C	H		C	H		C	H	CGHS	C	H
212	F	C	F	F		F		C	F	F	FY	F
213	I							FI	CFILTV	AGST	CILMV	ACFILMSTV
214	I							FILT	FILTV	FTV	CFILMV	CITV
215	P	P	P	P	P			P	P	P	P	PR
216	L	L						L	IMV	FL	ILM	ILM
217	I							AFGST	ACFITV	AFGILST	AMST	AFIMSTV
218	V							ILV	ILTV	CILTV	IL	IV
219	I		I		I			IT	I	IL	I	IV

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
220	F							FILMV	AF	ILV	CFITV	FGST
221	F		F			F		FILV	F	FV	FY	F
222	C					C		C	ST	CSY	CS	C
223	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
224	G		G			G		ILV	G	AGS	AFGST	G
225	Q							AFHQ	RS	KQR	QRS	LNQR
226	L	V	L	L	L	L		V	L	L	L	L
227	V				L			WY	FIV	IL	L	LV
228	F							LMNSW	CLM	FILV	GRS	CF
229	T						AT	AT	KT	AMT	APST	AT
230	V	I	V			V		I	V	LM	CL	V
231	K					K		HR	KR	HKR	GKR	K
232	E				A			AQST	AES	ALMS	A	ADE
233	A	V	A		V			V	A	AGV	V	AS
234	A	A	A		A			A	A	AV	A	AR
235	A		A		A	A		AKLMQR	A	AKR	A	A
236	Q	Q						Q	APQ	AQ	AQ	AQ
237	Q		Q	Q	Q			QT	Q	Q	Q	HQ
238	Q	K	Q					K	Q	AEQ	AQT	EQ
239	E				E	E		DE	DE	DEQ	E	E
240	S	S	S	S	S	S	S	S	S	S	S	S
241	A	E		A				E	AE	A	AEV	AE
242	T							ST	FST	ST	ST	ST
243	T	T	T	T	T	T	T	T	T	T	T	T
244	Q		Q	Q	Q	Q		QR	Q	Q	Q	Q
245	K	K	K	K	K			K	K	K	K	KR
246	A		A	A	A	A		AV	A	A	A	A
247	E	E			E	E		E	EQ	DE	E	E
248	K							KR	KR	KR	KR	KR
249	E		E	E	E	E		DE	E	E	E	E
250	V		V	V	V	V		GV	V	V	V	V
251	T		T					ST	T	ST	ST	ST
252	R					R		HR	KR	KR	HR	R
253	M	M	M	M	M	M	M	M	M	M	M	M
254	V	V		V		V		V	CV	V	IV	V
255	I							LMV	FIV	IV	IV	IV
256	I	V	L					V	L	FILMV	FMV	ILMV
257	M		M	M	M			MV	M	M	M	LM
258	V		V		V			FILV	V	IV	V	FV
259	I							AFILMV	FILMRV	AFGLMV	ACGV	IV

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
260	A	A	G		S			A	G	AG	S	AFGS
261	F		F					FY	F	FY	FY	FY
262	L			L		L		CI	LM	L	CIV	L
263	I							FILV	FILMTV	ILV	LMTV	AIV
264	C		A	C	C			CR	A	C	C	CS
265	W	W	W		Y	W		W	W	WY	Y	W
266	L	G						G	TV	AGLMSV	AGTV	CGILTV
267	P	P	P	P	P	P	P	P	P	P	P	P
268	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
269	A		A		A	A		AT	A	ACST	A	A
270	G							ACFISV	AMSTV	ACIS	AILSV	GSY
271	V					V		CF	AFLTVY	FL	AFLMT	V
272	A							AV	AGS	AS	AG	AS
273	F							CGST	ACFGI	ILV	LMQV	AFLMW
274	Y	F		W				F	WY	W	WY	FWY
275	I		I			I		AS	I	AIV	FIMY	I
276	F		F			F		AT	F	ITV	AGISV	F
277	T							AFLV	CFLMNT	FNQST	HNTY	CFILNST
278	H		N					HNS	N	HN	ANSTY	HN
279	Q	P		R		Q		P	KR	R	DEPQRST	Q
280	G	G	G	G		G		G	G	G	DENS	G
281	S	Y						Y	AISV	AEHQRST	EHPSTV	CFST
282	D							APS	ADES	EILPST	GN	DENST
283	F		F	F		F		FW	F	F	IKLV	F
284	G	H						H	HST	DE	DTY	GT
285	P	P				P		P	APV	LPV	LY	P
286	I	L			R			L	KLMQT	GKRW	R	FILPV
287	F							KMTV	AFLST	FLM	FLM	FL
288	M	A			V	M		A	AIM	AGS	V	M
289	T					T		AS	AST	ST	AT	T
290	I							ILM	ILV	ILV	IV	AILV
291	P	P			P	P		P	CP	PR	P	P
292	A							AS	AS	APS	AS	AS
293	F		F			F		FY	F	CGIV	FL	F
294	F	F	F		F	F		F	F	FLV	F	F
295	A	A				A		A	AS	CS	PS	A
296	K	K	K	K	K	K	K	K	K	K	K	K
297	T	S						S	AST	AS	AS	AST
298	S	A	S	S				A	S	S	AS	AS
299	A	T						T	ACS	AT	CS	AS

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
300	V	I		V				I	ILV	V	IV	FILV
301	Y			Y	Y	Y		WY	FY	Y	Y	Y
302	N	N	N	N	N	N	N	N	N	N	N	N
303	P	P	P	P	P	P	P	P	P	P	P	P
304	V							IV	IV	FILV	IL	ACILMV
305	I	I	I	I	I	I	I	I	I	I	I	I
306	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
307	I							IV	IV	IV	ACSTV	IV
308	M	F			F			F	LM	FLV	F	CFLMV
309	M	M			M			M	LM	FLM	M	LM
310	N	N	N	N	N	N	N	N	N	N	N	N
311	K		K		K			KR	K	KR	K	KRS
312	Q	Q	Q		Q	Q		Q	Q	LQ	Q	Q
313	F		F	F	F			FS	F	F	F	FL
314	R		R	R		R		QR	R	R	HKNQR	R
315	N							NSTV	GNS	LST	AG	HNT
316	C	C	C			C		C	C	CS	CH	C
317	M	I	M		I			I	M	IM	I	LM
318	V							LMY	ILV	KLMRV	LM	ILV
319	T							HKQ	ANST	AKNQT	EK	MT
320	T		T			T		LM	T	LMT	LMT	T
321	L	F			V			F	ILV	ILMV	V	IL
322	C	G						G	CFG	CFG	CFL	CF
323	C	K						K	CM	CGLMN	GR	CL
324	G					G		AEKQ	GK	GS-	KRT	G
325	K							EV	GK-	ADGKR	AKPQT	KR
326	N					N		DE	NS-	ADGNS	ILM	N
327	P	-						-	P-	P-	ADEST	LP
328	L	-			-			-	AFLM	FL-	-	FL
329	G	-			-			-	EGV	GI-	-	AEGQ
330	D	D						D	DE	DE	DE	DE
331	D							AGST	DE	DE-	ADEGST	DE
332	E				S			ST	DE	DE	S	DE
333	A							DE	V-	DE	DESTV	AGSTV
								ALMV	ASTV	AESTV	AILMSTV	AST-
		S	S					S	S	ST	ACS	AST
334	S							ST-	ST-	AGSTV-	ST-	AGST
335	T							AGSTV	ASTV	ST	AGST-	AST-
								AS	SV	AGQS-	AS-	G-
336	T	-			-			-	QS-	QST-	-	ST-

Continued

Supplementary Table 2. Continued

		Unanimous sites						Amino acids present in each class				
Site	Bovine	M/LWS	RH2	SWS2	SWS1	RH	Inv	M/LWS	RH2	SWS2	SWS1	RH
337	V	-			-			-	AQT-	S-	-	AGSTV-
338	S	-						-	S-	ASTV	NQST	ST-
339	K		K	-		K		KRT	K	-	KR	K
340	T	T	T	T	T	T	T	T	T	T	T	T
341	E	E			E	E		E	DE	EQ	E	E
342	T		V	V				AV	V	V	ATV	AST
343	S	S	S	S	S			S	S	S	S	ST
								ST	ST-	KS-	ST	ST-
								AV-	AIV-	AV-	ADV	AV-
								S-	AS-	GS-	S-	CS-
								NS-	S-	GST-	AHST-	ST-
								S-	S-	AS-	STV-	S-
		-	-			-		-	-	S-	Q-	-
		-	-			-		-	-	A-	NV-	-
		-	-			-		-	-	S-	G-	-
		-	-	-		-		-	-	-	P-	-
		-	-	-		-		-	-	-	S-	-
344	Q							S-	QS-	HKQ-	KQ-	QS-
345	V					V		V-	V-	IV-	MV-	V
346	A							AST-	AGS-	AGS-	GIS-	AS
347	P	P				P		P	P-	P-	P-	P
348	A							A-	A-	AES-	AGHNST-	A-
			-			-		G-	-	K-	AQ-	-
								N-			PS-	
								D-			R-	
								A-			M-	

Supplementary Table 3. Likelihood ratio test results for pairwise comparisons of the rod opsin class with each of the cone opsin classes

A) Test for rate heterogeneity comparing M0 and M3. P-values are determined from a χ^2 distribution with 4 degrees of freedom.

Cone opsin class	M0	M3	LRT	P-value	Largest ω	Fraction of sites
SWS1	−7617.74	−7481.7	272.1	1e−57	0.122	0.092
SWS2	−8267.6	−8062.8	409.6	2e−87	0.20	0.132
RH2	−6811.5	−6657.5	308.0	2e−65	0.223	0.134
LWS	−7546.6	−7407.0	279.3	3e−59	0.178	0.155

B) Test for positive selection comparing M1 and M2. P-values are determined from a χ^2 distribution with 2 degrees of freedom.

Cone opsin class	M1	M2	LRT	P-value	Largest ω	Fraction of sites
SWS1	−8439.6	−7512.7	1853.8	0	0.036	0.72
SWS2	−8870.4	−8108.3	1524.2	0	0.069	0.65
RH2	−7188.5	−6668.3	1040.5	0	0.096	0.54
LWS	−8387.0	−7456	1862.1	0	0.049	0.72

C) Test for positive selection comparing M7 and M8. P-values are determined from a χ^2 distribution with 2 degrees of freedom.

Cone opsin class	M7	M8	LRT	P-value	Largest ω	Fraction of sites
SWS1	−7488.7	−7482.2	12.94	0.0015	4.15	0.0000
SWS2	−8065.7	−8065.7	0	1	7.74	0.0000
RH2	−6663.6	−6663.6	0	1	1.83	0.0000
LWS	−7412.0	−7409.2	5.6	0.06	1.6	0.0000

Supplementary Table 4. Comparison of invariant ET sites with previous mutational studies. Sites marked with * have one sequences which differs from all other 187 sequences and so are included as invariant. Sites which are listed as ''Other G protiens'' have been shown to be important in mutation studeis of other G proteien coupled receptors (Madabushi et al 2004)

Location	Site	Mutation	Importance	Reference
N terminus	M1		Start codon	
	P23	Del21-29	Retinal binding; glycosylation	Doi et al 1990
TM-I	N55	N55C	Chromophore regeneration	Klein-Seetharaman et al 2001
C-1	K67*	K66A/K67A K67C	No effect; Gt activation	Shi et al 1995; Klein-Seetharaman et al 1999
	L68	L68A/R69A/T70A L68C	No effect; Gt activation	Shi et al 1995; Klein-Seetharaman et al 1999
TM-II	L72*	L72A	Arrestin binding	Raman et al 2003
	N73	N73A	Arrestin binding	Raman et al 1999; 2003
	L76*		Other G proteins	
	N78*		Other G proteins	
E-1	Y102	Del101-108	Retinal binding	Doi et al 1990
TM-III	G106	Del101-108	Retinal binding	Doi et al 1990
	C110	C110S; C110A	Protein folding	Karnik et al 1988 Davidson et al 1994
	E113*	E113Q	Schiff base counterion	Sakmar et al 1989
	G121	G121A,S,T,V,I,L,W	Pigment stability	Han et al 1996
	W126			
	L128		Other G proteins	
	E134	E134D E134A/R135A	Gt activation	Franke et al 1992
	R135	R135Q	Gt activation	Franke et al 1992 Shi et al 1998
C-2	C140			
	K141*			
	P142			
	F148	R147A/F148A/G14 9A	Phosphorylation	Shi et al 1995
TM-IV	W161	W161L	No effect in RH Other G proteins	Nakayama and Khorana 1991
	P171	Del171-172	Retinal binding	Doi et al 1990
E-2	G174	Del173-174	Retinal binding	Doi et al 1990
	W175	W175A,C,H	Chromophore regeneration	Madabushi et al 2004
	S176*	Del 175-176	Retinal binding	Doi et al 1990
	R177	Del 177-178	Retinal binding	Doi et al 1990
	P180*	Del 179-180	Retinal binding	Doi et al 1990
	G182	Del 181-182	Retinal binding	Doi et al 1990
	C187	C187S	Protein folding	Karnik et al 1988
	G188			
	D190	D190A	Retinal binding	Doi et al 1990
TM-V	P215*		Other G proteins	
	Y223		Other G proteins	
C-3	S240	S240A	Gt activation	Franke et al 1992
	T243	T243V; T243C	Gt activation	Franke et al 1992; Yang et al 1996
TM-VI	A246*			
	E249			
	V250*			
	M253			
	P267	P267A(N,S)	Gt activation	Nakayama and Khorana 1991
	Y268			
TM-VII	K296		Schiff base binds to retinal	Wang et al 1980
	N302		Other G proteins	
	P303		Other G proteins	
	I305		Other G proteins	
	Y306	Y306C	Gt activation	Cai et al 1999
TM-VIII	N310	N310C	Gt activation	Cai et al 1999
	Q312			
C terminus	T340	T340A	Arrestin binding	Zhang et al 1997

Supplementary Table 5. Comparison of disease states resulting from point mutations in the opsin genes. Amino acid mutations identified in OMIM: rhodopsin (#180380), red (LWS) opsin (#303900), green (MWS) opsin (#303800), and blue (SWS1) opsin (#190900). The site numbers listed are those for the corresponding human gene. For the rod opsin, this is the same as the bovine RH1 amino acid numbers. For the cone opsins, the corresponding bovine RH # is also given. Opsin invariant sites are identical in all five opsin classes sequenced to date. Class unanimous sites are conserved in the corresponding opsin class for the human disease gene being examined. For variable sites, the other amino acids which occur at this location are listed

Gene	Site	Bovine RH #	Mutation	Disease #	Opsin invariant	Class unanimous	Variable/Other AA
Rhodopsin (RH1)	15		N15S	0.0029		1	
	17		T17M	0.0006			S
	23		P23H	0.0001	1		
			P23A	0.0043			
	45		F45L	0.0007		1	
	51		G51R	0.0034		1	
	53		P53R	0.0024		1	
	58		T58R	0.0004		1	
	87		V87D	0.0008			I
	89		G89D	0.0009			C
	90		G90D	0.0032		1	
	94		T94I	0.0042		1	
	106		G106W	0.0010	1		
			G106R	0.0025			
	110		C110Y	0.0035	1		
	114		G114D	0.0036		1	
	135		R135L	0.0011	1		
			R135W	0.0012			
	150		E150K	0.0033			S, T
	164		A164E	0.0037		1	
	171		P171S	0.0038	1		
	178		Y178C	0.0013		1	
	182		G182S	0.0021	1		
	190		D190G	0.0014	1		
			D190N	0.0017			
			D190Y	0.0027			
	207		M207R	0.0028		1	
				0.0030			
	211		H211P			1	
	267		P267L	0.0022	1		
	292		A292E	0.0031			S
	296		K296E	0.0016	1		
	345	V345L		0.0040		1	
		V345M		0.0044			
	347		P347L	0.0002		1	
			P347S	0.0003			
			P347R	0.0020			
			P347Q	0.0041			
Red opsin (LWS)	180	164	S180A	0.0002			S, A
	203	187	C203R	0.0003	1		
	338	322	G338E	0.0004		1	
Green opsin (MWS)	94	78	N94K	0.0003		1	
	203	187	C203R	0.0001	1		
				0.0002			
	330	314	R330G	0.0004		1	
Blue opsn (SWS1)	79	82	G79R	0.0001			A C G S
	214	217	S214P	0.0002			A S M T
	264	267	P264S	0.0003	1		