

Supplemental Text

Supplemental Figure 1 Vista plot for the *so* eye enhancer genomic region. The three Ey binding sites are marked by red arrows.

Binding Site Sequences for constructing new Ey PWM (Figure 2)

Dmel	shf1	tccatcacgtatgaatgggt	+
Dyak	shf1	tccatcacgtatgaatgggt	+
Dpse	shf1	cccatcacgtatgaatgggc	+
Dmel	shf2	gatataacacttgagtgggc	+
Dyak	shf2	taaataacacttgagtgggc	+
Dere	shf2	gccttcacgtgttggtgc	-
Dmel	vha1	tatgttcacttgactgcgg	+
Dere	vha1	tgtgttcacttgactgcgg	+
Dyak	vha1	tatgttcacttgactgcgg	+
Dana	vha1	ttattaatgctcgactgcaa	+
Dpse	vha1	ttattgccgcttgactgcgg	+
Dmoj	vha1	ttgtagcacttgactgcgc	+
Dvir	vha1	ttgtagcacttgactgcgc	+
Dmel	optix2	tctttgcagcctgaatgggt	+
Dere	optix2	tctttgcagcctcaatgggt	+
Dmoj	optix2	tctttgcagcctgaatgggt	+
Dana	optix2	ctttgcagcctgaatgggt	+
Dpse	optix2	cttctgcagcctgaatgggt	+
Dvir	optix2	ctctgcagctgaataagt	+
Dyak	optix2	tctttgcagcctgaatgggt	+
Dmel	optix3	atttcacgcacgaatggag	+
Dyak	optix3	tggggcacacacgaatggag	+
Dana	optix3	attttacactgaatggat	+
Dpse	optix3	acttttgggctccattggga	+
Dmoj	optix3	gtgttacgcttcattgaat	+
Dere	optix3	ctccattcgtgtgtaa	-
Dvir	optix3	ctccaagcctttgttttg	-
Dmel	eya3	tgtcgtccactgattgaac	+
Dyak	eya3	tgtcgtccactgattgaac	+
Dana	eya3	tgtcgtccactgattgaac	+
Dpse	eya3	tgtcgtccactgattgaac	+
Dvir	eya3	tgtcgtctatctaattgaac	+
Dmoj	eya3	tgtcgtctacctgattgaac	+
Dere	eya3	tgtcgtccactgattgaac	+
Dmel	so1	ctccacacgaatgagtgaaa	+
Dsim	so1	ctccacacgaatgagtgaaa	+
Dvir	so1	ctccacacgaatgagtgaaa	+
Dyak	so1	ctccacacgaatgagtgaaa	+
Dere	so1	ctccacacgaatgagtgaaa	+

Dana	so1	ctccacacgaatgagtgaaa	+
Dmoj	so1	ctccacacgaatgagtgaaa	+
Dpse	so1	ctcgaacaaaggtatgaaa	+
Dmel	so2	ctcttgccacgtgagtcage	+
Dsim	so2	tccttgccacgtgagtgage	+
Dvir	so2	tccttgccacgtgagtgage	+
Dyak	so2	tccttgccacgtgagtgage	+
Dere	so2	tccttgccacgtgagtgage	+
Dana	so2	tccttgccacgtgagtgage	+
Dmoj	so2	tccttgccacgtgagtgage	+
Dpse	so2	tccttgctacgtgaataagc	+
Dmel	so3	ttaattccccctcactgggc	+
Dsim	so3	ttaattccccctcactgggc	+
Dvir	so3	ttaattccccctcactgggc	+
Dyak	so3	ttaattccccctcactgggc	+
Dere	so3	ttaattccccctcaatgggc	+
Dana	so3	ttaattccccctcactgggc	+
Dmoj	so3	ttaattccccctcactgggc	+
Dpse	so3	ttaattccccatcaatgggc	+

Oligo Sequences for Ey Gel Shift Assay (Figure 5)

so1 wt	TTTAACTTT	CACACGAATGAGTG	GAGAACT
so3 wt	TTAAAATTA	ATTCCCCCTCACTG	GGCACAA
shf1 wt	TTCCCGTCC	ATCACGTATGAATG	GGTTGAT
shf2 wt	TTTGGGGAT	ATAACACTTGAGTG	GGCTTTT
vha1 wt	TTCAC TTAT	GTTCCACTTGACTG	CGGAAAA
optix2 wt	TTGCATTCT	TTGCAGCCTGAATG	GGTTTAA
optix3 wt	TTGGCCATT	TTCACGCACGAATG	GAGCGAC
eya3 wt	TTCAAGCTG	TCTGCCACTTGATT	GAACCGG

so1 mut	TTTAACTTT	CGCAGGAAGGTGGA	GAGAACT
so3 mut	TTAAAATTA	ACTTGCCCACCCAC	GGCACAA
shf1 mut	TTCCCGTCC	AGCAGGTAAGTAGC	GGTTGAT
shf2 mut	TTTGGGGAT	ACAAGACTAGCGGA	GGCTTTT
vha1 mut	TTCAC TTAT	GATCGACTGGTCGA	CGGAAAA
optix2 mut	TTGCATTCT	TCGCGGCCAGTAGC	GGTTTAA
optix3 mut	TTGGCCATT	TCCAGGCAGGTAGC	GAGCGAC
eya3 mut	TTCAAGCTG	TCCGCGACTAGTTG	CAACCGG