

Opsin primers for bees/wasps (1/6/2005)

Forward Primers:

Opsin For (=LWRhFor)	5'-AAT TGC TAT TAY GAR ACN TGG GT-3'
Opsin For3 (=LWRhFor3)	5'-AGA TAC AAC GTR ATC GTS AAR GGT-3'
Opsin For3 (mod)	5'-TTC GAY AGA TAC AAC GTR ATC GTN AAR GG-3'
Opsin For4	5'-GAG AAR AAY ATG CGB GAR CAA GC-3'
Opsin For4n	5'-CAC GAR AAR AAY ATG MGN GAR CAR GC-3'
Opsin For4i	5'-CAC GAR AAR AAY ATG MGI GAR CAR GC-3'
Opsin For5	5'-ATG CGN GAR CAR GCN AAR AAR ATG AA-3'

Reverse Primers:

Opsin Rev (=LWRhRev)	5'-ATA TGG AGT CCA NGC CAT RAA CCA-3'
Opsin Rev (mod)	5'-ATA NGG NGT CCA NGC CAT GAA CCA-3'
Opsin Rev4	5'-GGT GGT GGT RCC GGA RAC GGT G-3'
Opsin Rev4a	5'-GGT GGT RCC GGA RAC GGT GGA DGT-3'
Opsin Rev4b	5'-GGT RCC GGA RAC GGT GGA DGT NGC RTC-3'

PCR conditions:

Opsin For/Opsin Rev: 94°C 1 min, 52°C 1 min, 72°C 1 min (35 cycles)
 Opsin For3/Opsin Rev: 94°C 1 min, 54°C 1 min, 72°C 1 min (35 cycles)
 Opsin For3(mod)/Opsin Rev(mod): 94°C 1 min, 54°C 1 min, 72°C 1 min (35 cycles)
 Opsin For4/Opsin Rev4: 94°C 1 min, 58°C 1 min, 72°C 1 min (35 cycles)
 Opsin For4/Opsin Rev4a: 94°C 1 min, 52°C 1 min, 72°C 1 min (35 cycles)
 Opsin For5/Opsin Rev4: 94°C 1 min, 56°C 1 min, 72°C 1 min (35 cycles)
 Opsin For5/Opsin Rev4b: 94°C 1 min, 55°C 1 min, 72°C 1 min (35 cycles) [best one]

Opsin For4n and For4i are not very good primers. Use Opsin For5 instead.

Note: Opsin For/Rev and For3/Rev produce an approximately 700 bp fragment. You may get multiple bands (there are three copies of opsin in bees), but the LW opsin (=green) paralog is roughly 700 bp in length and can be separated from the other copies on low-melting point gel. The Opsin For4/Rev and For4/Rev4a fragment is approximately 600 bp in length and overlaps broadly with the upstream fragment (see attached map). The overlapping region includes an intron, so if you have mixed up paralogs you will know it right away.

For more information on opsin see:

Danforth, B.N., S.G. Brady, S.D. Sipes & A. Pearson (2004). Single copy nuclear genes recover Cretaceous age divergences in bees. *Syst. Biol.* 53(2): 1-18.