Wingless primers for bees/wasps (9/20/2006)

Forward Primers:

Lep wg1a	5'-GAR TGY AAR TGY CAY GGY ATG TCT GG-3'
beewgFor	5'-TGC ACN GTS AAG ACC TGY TGG ATG AG-3'
beewgFor2*	5'-GGC AGC ATY CAG TCS TGY TCC TGC GA-3'
wgColletFor**	5'-CAC GTG TCB TCB GRG ATG MGR SAG GA-3'

***** Primer developed by Sedonia Sipes (Southern Illinois University)
****** Primer developed by Eduardo Almeida (Cornell University)

Reverse Primers:

Lep wg2a	5'-ACT ICG CAR CAC CAR TGG AAT GTR CA-3'

PCR conditions:

Lep wg1a/Lep wg2a: 94^{o}C 45 sec, 54^{o}C 45 sec, 72^{o}C 45 sec (35 cycles)

beewgFor/Lep wg2a: 94^{o}C 45 sec, 58^{o}C 45 sec, 72^{o}C 45 sec (35 cycles)

Note: We know of at least three wingless paralogs in bees, so be careful to confirm that you have the right paralog. Lep wg1a/Lep wg2a produces an approximately 400 bp PCR product with multiple secondary bands. The brightest band is the wnt-1 copy. This band should be gel-purified prior to sequencing. However, even gel-purified bands may contain similar sized paralogs, so in many cases the sequencing is noisy. For better results clone the PCR products into a plasmid vector (such as pGEM) or use beewgFor/Lep wg2a, which produces a single, roughly 350 bp band that can also be gel purified and sequenced. Sedonia Sipes is currently developing wingless primers for a much larger portion of the gene.

For more information on wingless 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): 309-326.