EF-1alpha primers for bees/wasps (5/15/2003)

Forward Primers:

For1deg 5'-G[C/T] ATC GAC AA[A/G] CGT AC[C/G] AT[C/T]G-3' 462*
(21 mer; Tm = 70°C)

HaF2For1 5'-G GG[T/C] AAA GG[A/T] TCC TTC AA[A/G] TAT GC-3' 511*
(24 mer; Tm = 68 - 72 °C)

For3 5'-GGN GAC AA[C/T] GTT GG[T/C] TTC AAC G-3' 1496*
(22 mer; Tm = 58.8 - 76.4 °C)

For3rho 5'-GGY GAC AA[C/T] GTT GTT TT[C/T] AA[C/T] G-3' 1496*
(22 mer; Tm ~ 60 - 75 °C)
This is a modified version of For3 that seems to work better on bees

Reverse primers:

EF1-intron1(rev) 5'-GTA ATC ATG TT[C/T] TTG AT[A/G] AAA TCT CT-3'
(22-mer, Tm = 61°C)

F2-rev1 5'-A ATC AGC AGC ACC TTT AGG TGG -3' 1600*
(22 mer. Tm = 68.2 °C) <-– F2 copy specific primer

Cho10(mod) 5'-AC [A/G]GC [A/G/C]AC [G/T]GT [T/C]TG [A/T/C]C[T/G] CAT GTC-3'
(23 mer; Tm = 63.5 - 83.8 °C) 1887*

* Numbers refer to the positions of primers based on the 5' end of the primer in the honey bee, Apis mellifera (Walldorf & Hovemann 1990).

PCR conditions:

HaF2For1 to F2-rev1: 94°C, 1 min; 54°C, 1 min; 72°C, 1.5 min.
Produces a roughly 1100 bp fragment (F2-specific)

For1deg to F2-rev1: 94°C, 1 min; 52°C, 1 min; 72°C, 1.5 min.
Produces a roughly 1300 bp fragment including all of the fragment amplified with HaF2For1 to F2-rev1. Includes first intron in F2 copy, which is not very useful. I use For1deg in place of HaF2For1 when the latter primer fails for some taxa.

For3 [or For3rho] to Cho10: 94°C, 1 min; 58°C, 1 min; 72°C, 1 min.
Produces two bands: 400 bp and 600 bp. You will need to gel-purify the 600 bp fragment and sequence it. This fragment overlaps with the HaF2For1 to F2-rev1 fragment and together gives a roughly 1600 bp data set.

Do all PCR reactions with 35 cycles.