CAD primers for bees (7/11/2011)

Foward Primers:
apCADfor4 5' -- TGG AAR GAR GTB GAR TAC GAR GTG GTY CG --3'
[*29 mer, Tm = 63.1C*]

apCADfor1 5' -- GGW TAT CCC GTD ATG GCB MGW GC --3'
[*23 mer, Tm = 62.1C*]

ap787for2 5' -- TGC TTY GAR CCD AGY CTH GAT TAY TG --3'
[*26 mer, Tm = 60.0C*]

apCADfor2mod 5' -- GAT GGG AYC TNR GNA ART TYC -- 3'
[*21 mer, Tm = 53.1C*]

apCADfor3 5' -- CTC HGT KGA RTT YGA TTG GTG YGC --3'
[*24 mer, Tm = 60.9C*]

Reverse Primers:
ap835rev1 5' -- GCA THA CYT CHC CCA CRC TYT TC --3'
[*23 mer, Tm = 59.8C*]

apCADrev1mod 5' -- GCC ATY RCY TCB CCY ACR CTY TTC AT --3'
[*26 mer, Tm = 62.2C*]

apCADrev4a 5' -- GGC CAY TGN GCN GCC ACY GTG TCT ATY TGY TTN ACC --3'
[*36 mer, Tm = 68.6C*]

ap1098rev2 5' -- ATA TTR TTK GGC ARY TGD CCK CCC --3'
[*24 mer, Tm = 61.1C*]

apCADrev3a 5' -- CAR GGR TAR CCR ACY TCY TCR CAA AAT TC -- 3'
[*29 mer, Tm = 60.3C*]

PCR conditions

apCADfor1 to **ap835rev1** 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 800 bp fragment, the upstream portion of the gene. It contains a single intron.

apCADfor1 to **apCADrev1mod** 94C, 1min; 55C, 1min; 72C, 1min; 35 cycles
Produces a roughly 800 bp fragment, same as above. May amplify a wider range of taxa.

ap787for2 to **ap1098rev2** 94 C, 1min; 52 C, 1min; 72 C, 1min30sec; 35 cycles
Produces a roughly 1500bp fragment. It contains two introns.

apCADfor2mod to **ap1098rev2** 94C, 1min; 52-55C, 1min; 72C, 1min30sec; 35 cycles
Produces a roughly 1500 bp fragment, same as above. May amplify a wider range of taxa, but greater multiple banding pattern.

apCADfor3 to **apCADrev3a** 94 C, 1min; 52C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 500bp fragment. Bands may be somewhat faint, since PCR conditions have not yet been optimized.

apCADfor4 to **apCADrev4a** 94C, 1min; 52C, 1min; 72C, 1min30s; 35 cycles

Amplifies for a wider range of taxa, both the upstream (for1 to rev1) and most of the downstream (for2 to rev2) regions. Produces a roughly 1500bp fragment.

apCADfor4 to **apCADrev1mod** 94C, 1min; 58C, 1min; 72C, 1min; 35 cycles

Amplifies for a wide range of taxa, band is usually sharp, but faint smears are also usually seen. Elongation time should be extended when screening because the first intron in CAD varies greatly in length, sometimes exceeding 600bp.

apCADfor2mod to **apCADrev4a** 94C, 1min; 52C, 1min; 72C, 1min30sec; 35 cycles

Amplifes for a wider range of taxa. Produces a roughly 1000 bp fragment.