BN Danforth

Arginine Kinase primers for bees (12/30/2003)
(developed primarily based on long-tongued bees)

Foward Primers:
ArgKfor1 5' -- GTT GAC CAA GC(C/T) GT(C/T) TTG GA --3'
[*20 mer, Tm = 56.8 C*] [3*]

ArgKfor2 5' -- GAC AGC AA(A/G) TCT CTG CTG AAG AA --3'
[*23 mer, Tm = 57.7 C*] [61*]

ArgKLTfor3 5' -- TGA T(C/T)G ACG A(C/T)C ACT TCC T(A/C/G)T TCA A --3'
[*25 mer, Tm = 59.1 C*] [544*]

Reverse Primers:
ArgKrev1 5' -- CAT GGA AAT AAT ACG (A/G)AG (A/G)TG --3'
[*21 mer*] [681*]

ArgKrev2 5' -- GGT (C/T)TT GGC ATC GTT GTG GTA GAT AC --3'
[*26 mer, Tm = 60.4 C*] [627*]

ArgKLTrev1 5' -- (A/G)CC (A/C/G)CC (C/T)TC AGC CTC (G/T)GT GTG CTC --3'
[*24 mer, Tm = 68.2 C*] [942*]

ArgKLTrev2 5' -- GAT (G/T)CC ATC (A/G)T(A/G/T) CAT (C/T)TC CTT (C/G)AC (A/G)GC -- 3'
[*27 mer, Tm = 62.3 C*] [1014*]

* Numbers refer to the positions of primers based on the 5' end of the primer in the honey bee coding sequence, *Apis mellifera*. The entire *Apis mellifera* coding sequence for Arginine Kinase is roughly 1070 bp in length.

PCR conditions

ArgKfor1 and **ArgKrev1** has not effectively amplified the upstream fragment in long tongue bees.

ArgKfor2 to **ArgKrev2** 94 C, 1min; 50 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 750 to 800 bp fragment, the upstream portion of the gene. It contains a single intron that begins at approximately the 260 base of the amplified fragment.

ArgKLTfor3 to **ArgKLTrev1** 94 C, 1min; 50 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 500 bp fragment, the downstream portion of the gene. It contains a single intron that begins at approximately the 145 base of the amplified fragment. This primer pair usually produces multiple banding, and the 500 bp band must be gel purified. This fragment will overlap the upstream fragment by about 50 to 80 bp.

ArgKLTfor3 to **ArgKLTrev2** 94 C, 1min; 50 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 600 bp fragment, the downstream portion of the gene. It contains a single intron that begins at approximately the 145 base of the amplified fragment. This fragment will overlap the upstream fragment by about 50 to 80 bp.

ArgKfor2 to **ArgKLTrev1** 94 C, 1min; 50 C, 1min; 72 C, 1min 30 sec; 35 cycles
Produces a roughly 1100 to 1200 bp fragment, that spans most of the gene. It contains two introns, at approximately the 260 base and the 870 bp of the amplified fragment. This primer pair will usually produce multiple bands and must be gel purified.

The entire data set spans approximately 1300 bp.