

Sodium-Potassium ATPase (NaK) primers for bees (3/15/2005)

Forward primers

NaKfor1

5' - GGY GGT TTC GCS WTG YTG YTG TGG ATC GG - 3'

Tm = 67.3 C, Length = 29

NaKfor2

5' - GCS TTC TTC TCB ACS AAC GCC GTY GAR GG - 3'

Tm = 66.6 C, Length = 29

Reverse primers

NaKrev1

5' - GCG ACG ATG ATA CCG ATC ARG AAG ATG ACA GC - 3'

Tm = 63.5 C, Length = 32

NaKrev1a

5' - CCG ATN ARR AAG ATR TGM GCG TCN AGC CAA TG - 3'

Tm = 63.5 C, Length = 32

NaKrev2

5' - ACC TTG ATR CCG GCY GAW CGG CAC TTG GC - 3'

Tm = 69.0 C, Length = 29

Conditions

NaKfor1 to NaKrev1

94 C, 1min; 52 C - 55 C, 1min; 72 C, 1min

This primer pair amplifies an approximately 800 bp fragment of the upstream region and overlaps about 300 bp of the downstream fragment. It contains no introns. Unfortunately, these primers don't amplify in bees very well, and we've had some problems getting a consistently bright band - some work still needs to be done to optimize the annealing temperature.

NaKfor2 to NaKrev2

94 C, 1min; 58 C, 1min; 72 C, 1min30s

This primer pair amplifies an approximately 1100 bp fragment of the downstream region and overlaps the upstream region by about 300 bp. It contains no introns. At these conditions, the primers amplify fairly consistently, although there are hints of multiple bands in some taxa, so it's necessary to gel purify the product.

NaKfor1 to NaKrev1a

94 C, 1min; 54 C, 1min; 72 C, 1min

Amplifies the same upstream region with slightly more consistency than NaKfor1 to NaKrev1.