

## Pol II primers for bees (9/19/2006)

### Forward Primers:

**polfor1** 5' -- CGT ACT GTY ATY ACW CCY GAT CC --3'  
[23 mer,  $T_m = 55.8C$ ]

**polfor2** 5' -- TGG GAY GSY AAA ATG CCK CAA CC --3'  
[23 mer,  $T_m = 61.6C$ ]

**polfor2a** 5' --AAY AAR CCV GTY ATG GGT ATT GTR CA --3'  
[26 mer,  $T_m = 58.0C$ ]

**polfor3** 5' -- CAR GTT ATY GCT TGT GTS GCY CAA C --3'  
[25 mer,  $T_m = 59.6C$ ]

### Reverse Primers:

**polrev1** 5' -- CAA ATA TGM AGY ART GAH CCR GCW G --3'  
[25 mer,  $T_m = 57.0C$ ]

**polrev2** 5' -- TTY ACA GCA GTA TCR ATR AGA CCT TC --3'  
[26 mer,  $T_m = 55.5C$ ]

**polrev2a** 5' -- AGR TAN GAR TTC TCR ACG AAT CCT CT --3'  
[26 mer,  $T_m = 57.0C$ ]

**polrev3** 5' -- GAA ARA TCT TYT GYA CGT TGG ADA TC -- 3'  
[26 mer,  $T_m = 54.3C$ ]

## PCR conditions

**polfor1 to polrev1** 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles  
Produces a roughly 900bp fragment

**polfor2 to polrev2** 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles  
Produces a roughly 800bp fragment.

**polfor2a to polrev2a** 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles  
*This is the best pol II primer pair.* It works on a wide variety of taxa and produces a sharp, bright band of roughly 700 bp.

**polfor3 to polrev3** 94 C, 1min; 52C, 1min; 72 C, 1min; 35 cycles  
Produces a roughly 600bp fragment. PCR products are faint and difficult to purify for sequencing.