28S primers for bees (7/18/2008)

Foward Primers:
A-28S-For [3318] 5' –CCC CCT GAA TTT AAG CAT AT -- 3'
[*20 mer, Tm = 56.0 C*] *Designed by Ward and Brady 2006*

D2-3551F (28SD2for) 5' -- CGT GTT GCT TGA TAG TGC AGC -- 3'
[*21 mer, Tm = 58.0 C*]

D2-3665F (Bel28S) 5' -- AGA GAG AGT TCA AGA GTA CGT G -- 3'
[*22 mer, Tm = 54.7 C*]

D3-4046F (28SD3for) 5' -- GAC CCG TCT TGA AAC ACG GA -- 3'
[*20 mer, Tm = 58.3 C*]

D3-4048F (28SD4for) 5' -- CCC GTC TTG AAA CAC GGA CCA AGG -- 3'
[*24 mer, Tm = 63.5 C*]

D6-4738F (28SD6for) 5' -- GGA GTG TGT AAC AAC TCA CCT GCC G -- 3'
[*25 mer, Tm = 63.1 C*]

D8-5435F (28SD8for) 5' -- CCC ATA TCC GCA GCA GGT CTC C -- 3'
[*22 mer, Tm = 62.8 C*]

Reverse Primers:
D2-4057R (28SD2rev) 5' -- TCA AGA CGG GTC CTG AAA GT -- 3'
[*20 mer, Tm = 57.0 C*]

D3-4283R (Mar28Srev) 5' -- TAG TTC ACC ATC TTT CGG GTC CC -- 3'
[*23 mer, Tm = 59.9 C*]

D3-4413R (28SD3rev1) 5' -- TCG GAA GGA ACC AGC TAC TA -- 3'
[*20 mer, Tm = 55.9 C*]

D5-4625R (28SD3rev2) 5' -- CCC ACA GCG CCA GTT CTG CTT ACC -- 3'
[*24 mer, Tm = 65.6 C*]

D5-4749R (28SD4rev) 5' -- GTT ACA CAC TCC TTA GCG GA -- 3'
[*20 mer, Tm = 55.0 C*]

D7-5482R (28SD6rev) 5' -- GAC TTC CCT TAC CTA CAT -- 3'
[*18 mer, Tm = 48.1 C*]

D10-6396R (28SD8rev) 5' -- AGT CAA ACT CCC TAC CTG GC -- 3'
[*20 mer, Tm = 57.5 C*]

PCR conditions

A-28S-For to Mar28Srev 94 C, 1min; 58 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 1000 bp fragment of the upstream D1 - D2 region.

Bel28S to Mar28Srev 94 C, 1min; 65 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 690 bp fragment of the D2 - D3 region.

Bel28S to 28SD4Rev 94 C, 1min; 58 C, 1min; 72 C, 1min 15 sec; 35 cycles
Produces a roughly 1100 bp fragment of the D2 - D3 region.

28SD4For to 28SD4Rev 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 700bp fragment of the D4-D6 region

We typically use two primer pairs to produce a 28S data set of roughly 1400 bp spanning the region between the A-28S-For and 28SD4Rev primers:

A-28S-For to Mar28Srev
Bel28S to 28SD4Rev

These are the primer pairs we have used to produce data sets for the Colletidae (Eduardo Almeida, in press) and Apidae (Sophie Cardinal, in prep.). This data set is a useful one, but alignments are difficult and results are not particularly robust.

D3-4046F to D3-4413R 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 400 bp fragment of the D3-D4 region

D3-4046F to D5-4625R 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 600 bp fragment of the D3-D5 region

D2-3551F to D2-4057R 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 500 bp fragment of the D2-D3 region

D2-3551F to D3-4283R 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 800 bp fragment of the D2-D3 region

D2-3551F to D5-4625R 94 C, 1min; 52 C, 1min; 72C, 1min30s; 35 cycles
Produces a roughly 1200 bp fragment of the D2-D5 region

D2-3665F to D5-4749R 94 C, 1min; 52 C, 1min; 72 C, 1min30s; 35 cycles
Produces a roughly 1200 bp fragment of the D2-D6 region

D6-4738F to D7-5482R 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 700bp fragment of the D6-D8 region

D8-5435F to D10-6396R 94 C, 1min; 52 C, 1min; 72 C, 1min; 35 cycles
Produces a roughly 950bp fragment of the D8-D10 region

See attached excel spreadsheet for results of PCR test reactions across a broad sample of LT bees.