

ITS2 Hymenoptera and Hemiptera

Primers:

Primer name	Primer sequence (5'- 3' orientation)	Primer used for PCR	Sequencing
PCR-Sequencing 300 bp - 800 bp of ITS2 region of Hemiptera and Hymenoptera using M13-tailed primer Cocktail			
28S_cbgp_R1	CAGGAAACAGCTATGACGATATGYTTAAATTCRGSGGGT	X	
5.8S_cbgp_F1	TGTAAAACGACGGCCAGTTCGATGAAGAACGCAGCDAHTG	X	
5.8S_cbgp_F2	TGTAAAACGACGGCCAGTTCGATGAAGAMCGCAGYTAAGT	X	
5.8S_cbgp_F3	TGTAAAACGACGGCCAGTTCGATGAAAGACGCAGCAAAYTG	X	
M13F	TGTAAAACGACGGCCAGT		X
M13R	CAGGAAACAGCTATGAC		X

Product size: 300 – 800 bp

Program: 2 min 94°C, 5x (30 sec 94°C, 1 min 45°C, 1:30 min 72°C), 35x (30 sec 94°C, 1 min 55°C, 1:30 min 72°C), 10 min 72°C, quick cooling to room temperature

Sequencing: Cycle sequencing reactions are performed using primers M13F and M13R in separate reactions.

Remarks: The M13-tailed ITS2 primer cocktail is prepared by pooling an equal volume of 10 µM of primers 28S_cbgp_R1, 5.8S_cbgp_F1, 5.8S_cbgp_F2 and 5.8S_cbgp_F3.

Master mix:

	per reaction (µl)	final concentration
DNase and RNase free water	19,125	
10x PCR buffer (incl. 15 mM MgCl ₂ , Qiagen)	2,5	1x, 1.5 mM MgCl ₂
MgCl ₂ (25 mM, Qiagen)	0,5	0.5 mM
dNTP's (10 mM each)	0,25	0.1 mM each
ITS2 primer cocktail (10 µM)	0,5	0.2 µM
Taq DNA polymerase (5 U/µl, Qiagen)	0,125	0.625 Unit
subtotal	23,0	
template	2,0	
total	25,0	