

ITS2 Coleoptera and Lepidoptera

Primers:

Primer name	Primer sequence (5'- 3' orientation)	Primer used for	
		PCR	Sequencing
PCR-Sequencing 300 bp - 800 bp of ITS2 region of Coleoptera and Lepidoptera described by Campbell <i>et al.</i> (1993)			
ITS2F	TGTGAAGTGCAGGACACATG	X	X
ITS2R	AATGCTTAAATTTAGGGGGTA	X	X

Product size: 300 – 800 bp.

Program: 3 min 94°C, 5x (30 sec 94°C, 30 sec 45°C, 1 min 72 °C), 35x (30 sec 94°C, 1 min 51°C, 1 min 72°C), 10 min 72°C, quick cooling to room temperature

Sequencing: Cycle sequencing reactions are performed using the primers ITS2F and ITS2R in separate reactions.

Master mix:

	per reaction (µl)	final concentration
DNase and RNase free water	18,25	
10x PCR buffer (incl. 15 mM MgCl ₂ , Qiagen)	2,5	1x, 1.5 mM MgCl ₂
MgCl ₂ (25 mM, Qiagen)	0,5	1,0 mM
dNTP's (10 mM each)	0,25	0,1 mM each
ITS2F (10 µM)	0,5	0,2 µM
ITS2R (10 µM)	0,5	0,2 µM
Taq DNA polymerase (5 U/µl, Qiagen)	0,5	2,5 Unit
subtotal	23,0	
template	2,0	
total	25,0	