

ITS amplicon protocol

PCR specifications

Target Region and Size: ITS 1 region (~321 bp)

Template Concentration: 50 ng

Enzyme and Master mix: TaKaRa LA Taq (Cat. No. RR002A)

Primers

ID	Primer sequence	Length
ITS1F (1723-1744)	TCGTCGGCAGCGTCAGATGTCGTATAAGAGACAGCTTGGTCATTTAGACAG	55
ITS2R (2024-2043)	GTCTCGTGGGCTCGGAGATGCTGTATAAGAGACAGGCTGCGTTCTTCA	34

PCR Mixture

Reagents	Concentration	1 reaction (25 L)
DNA Template	50 ng	1.7 L
10X LA PCR Buffer	1X	2.5 L
MgCl ₂	1.8 mM	1.8 L
dNTPs	0.1 mM	1 L
Forward Primer	0.28 pmol	0.7 L
Reverse Primer	0.28 pmol	0.7 L
BSA	0.1 g	0.5 L
Nuclease Free Water	-	15.975 L
Takara LA Taq	0.625 U	0.125 L
Total	-	25 L

PCR Conditions

Step	Temperature	Time	Cycle
1	95 °C	5 minutes	1
2	95 °C → 56 °C → 72 °C	30 sec → 30 sec → 30 sec	32
3	72 °C	7 minutes	1
4	20 °C	∞	-