

18S amplicon protocol

PCR specifications

Target Region and Size: V9 region (~145 bp)

Template Concentration: 30 ng

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

Primers

ID	Primer sequence	Length
18S_1391F	TCGTCGGCAGCGTCAGATGTGTATAAGAAACAGAGTACACACCGCCCGTC	49
18S_EukBr	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACAGTGATCCTTCTGCAGGTT	50

PCR Mixture

Reagents	Concentration	1 reaction (25 L)
DNA Template	30 ng	1 L
10X LA PCR Buffer	1X	2.5 L
MgCl ₂	0.8 mM	0.8 L
dNTPs	0.1 mM	1 L
Forward Primer	0.20 pmol	0.5 L
Reverse Primer	0.20 pmol	0.5 L
Nuclease Free Water	-	17.6 L
Takara LA Taq	0.625 U	0.1 L
Total	-	25 L

PCR Conditions

Step	Temperature	Time	Cycle
1	95 °C	5 minutes	1
2	95 °C → 57 °C → 72 °C	30 sec → 30 sec → 30 sec	30
3	72 °C	5 minutes	1
4	20 °C	∞	-