

# 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	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	29
18S_EukBr	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	29

## PCR Mixture

Reagents	Concentration	1 reaction (25 L)
DNA Template	30 ng	1 L
10X LA PCR Buffer	1X	2.5 L
MgCl <sub>2</sub>	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	∞	-