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## 16S PCR Reaction

Modified 16S protocol from the [Earth Microbiome Project](#).

### PCR reaction mixture

Reagent	Volume
PCR-grade water	10.5 $\mu$ L
PCR master mix (2x)	12.5 $\mu$ L
Indexed Primers (should have enough of each indexed primer set for two reactions in case you need to repeat some).	1 $\mu$ L
Template DNA	1 $\mu$ L
<b>Total reaction volume</b>	<b>25.0 <math>\mu</math>L</b>

Temperature	Time, 96-well	Time, 384-well	Repeat
94 $^{\circ}$ C	3 min	3 min	Hold
94 $^{\circ}$ C	45 s	60 s	35x
50 $^{\circ}$ C	60 s	60 s	
72 $^{\circ}$ C	90 s	105 s	
72 $^{\circ}$ C	10 min	10 min	Hold
4 $^{\circ}$ C	$\infty$	$\infty$	Hold

## 12S Fish Metabarcoding

### PCR reaction mixture

Reagent	Volume
PCR-grade water	6.5 $\mu$ L
PCR master mix (2x)	12.5 $\mu$ L



Indexed Primers (should have enough of each indexed primer set for two reactions in case you need to repeat some) 1.0  $\mu$ L

Template DNA (fish DNA is much less abundant than microbes) 5  $\mu$ L

**Total reaction volume 25.0  $\mu$ L**

12S PCR program uses a lot of cycles to increase signal, which will make things even less quantitative than with fewer cycles.

Temperature	Time, 96-well	Time, 384-well	Repeat
95 °C	3 min	3 min	Hold
95 °C	20 s	30 s	40x
52 °C	20 s	30 s	
72 °C	20 s	30 s	
72 °C	5 min	5 min	Hold
4 °C	$\infty$	$\infty$	Hold