

16S amplicon protocol

PCR specifications

Target Region and Size: V4 region (~292 bp)

Template Concentration: 30 ng/ L

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

Primers

| ID | Primer sequence | Length |
|---------|--|--------|
| V4_515F | TCGTCGGCAGCGTCAGATCGGTATAAGAGACAGGTGYCAGCMGCCG | 52 |
| V4_806R | GTCTCGTGGGCTCGGAGATGATGTATAAGAGACAGGGACTACNVGGGT | 34 |

PCR Mixture

| Reagents | Concentration | 1 reaction (25 L) |
|---------------------|---------------|-------------------|
| DNA Template | 30 ng | 1 L |
| 10X LA PCR Buffer | 1X | 2.5 L |
| MgCl ₂ | 2.5 mM | 2.5 L |
| dNTPs | 0.1 mM | 1 L |
| Forward Primer | 0.4 pmol | 1 L |
| Reverse Primer | 0.4 pmol | 1 L |
| Nuclease Free Water | - | 15.9 L |
| Takara LA Taq | 0.5 U | 0.1 L |
| Total | - | 25 L |

PCR Conditions

| Step | Temperature | Time | Cycle |
|------|-----------------------|--------------------------|-------|
| 1 | 95 °C | 3 minutes | 1 |
| 2 | 95 °C → 55 °C → 72 °C | 30 sec → 30 sec → 30 sec | 25 |
| 3 | 72 °C | 5 minutes | 1 |
| 4 | 20 °C | ∞ | - |