

# 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) | TCGTCGGCAGCGTCAGATCGTC       | 55     |
| ITS2R (2024-2043) | GTCTCGTGGGCTCGGAGATG         | 34     |
|                   | GTGTATAAGAGACAGGCTGCGTTCTTCA |        |

## PCR Mixture

| Reagents            | Concentration | 1 reaction (25 L) |
|---------------------|---------------|-------------------|
| DNA Template        | 50 ng         | 1.7 L             |
| 10X LA PCR Buffer   | 1X            | 2.5 L             |
| MgCl <sub>2</sub>   | 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                 | ∞                        | -     |