

PCR for T. versicolor gene amplification

Introduction

This is a protocol for PCR amplification of the Laccase_Tversicolor2 from NEB.

Materials

- › NEB HCK LTv fwd (100µM)
- › NEB HCK LTv (Xbal) rev (100µM)
- › Q5 High-Fidelity 2x Master Mix
- › Laccase_Tversicolor2
- ›

Procedure

PCR Preparation for 100µL PCR Volume

1. Dilute 100µM Primer 1:10 -> 10µM
2. Add 50µL Q5 MM
3. Add 38,5 µL H₂O
4. Add 5µL fwd Primer
5. Add 5µL rev Primer
6. Add 1,5µL Template DNA (should be: 1ng - 1g)
7. Mix and distribute 25µL per PCR tube

Q5 PCR Thermocycling Conditions

8. 98°C 30sec Initial Denaturation
9. 25-35 cycles: 98°C (5-10sec) -> 67°C (10-30sec) ->72°C (20-30sec/kb -> ca. 45 sec)
10. 72°C 2min Final Extension
11. Hold 4-10°C