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Working

DNA extraction and Nested PCR

PLOS Neglected Tropical Diseases

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ABSTRACT

This is the protocol for the Nested PCR assay

EXTERNAL LINK

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PDF

ITS (410bp.pdf

GUIDELINES

All primer sequences are listed below:
The first round of PCR was carried out using EbGeno-fe (5'-TTC AGA TGG TCA TAG GGA TG-3') as the forward primer and EbGeno-re (5'-ATT AGA GCA TTC CGT GAGG-3') as the reverse primer,whichtogether amplify a 465-bp specific fragment.
A second round of PCR was performed using the forward primer EbGeno-fi (5'-TCG GCT CTG AAT ATC TAT GG-3') and the reverse primer EbGeno-ri (5'-ATT CTT TCG CGC TCG TC-3') in order to amplify a 410-bp internal fragment used for genotype specification.

MATERIALS TEXT

DNase/RNase-Free Deionized Water, primer, TaKaRa Taq DNA Polymerase (TaKaRa Bio Inc., Tokyo, Japan), 10×PCR Buffer, 10 mmol dNTP, 1.5 % agarose gel, GelStrain

1 DNA extraction
Genomic DNA of E. bieneusi was extracted directly from 180 to 200 mg of each fecal specimen using the recommended procedures and the provided reagents by the manufacture of QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). To obtain high yield of DNA, the lysis temperature was increased to 95 ° C according to the manufacturer's suggestion. DNA was eluted in 200 µL of AE and stored at −20°C before it was used for PCR analysis.

2 PCR amplification
Amplification was accomplished in final volume of 25 µL, containing 17 µL of DNase/RNase-Free Deionized Water, 0.5 µL of each primer, 0.5 µL of TaKaRa Taq DNA Polymerase (TaKaRa Bio Inc., Tokyo, Japan), 2.5 µL of 10 × PCR Buffer, 2 µL of 10 mmol dNTP and 2 µL of DNA .

3 The target DNA undergoes the first run of PCR with the first set of primers.

00:05:00 Initial denaturation

95 °C

4 00:00:40 Denaturation

94 °C

5 00:00:45 Annealing

53 °C

6 00:00:45 Extension/elongation

72 °C

7 go to step #4 35 cycles

8 00:04:00 Final extension

72 °C

9 The product from the first reaction undergoes a second run of PCR with the second set of primers.

00:05:00 Initial denaturation

95 °C

10 00:00:35 Denaturation

94 °C

11 00:00:40 Annealing

55 °C

12 00:00:40 Extension/elongation

72 °C

13 go to step #10 30 cycles

14 00:05:00 Final extension

72 °C

15 Final hold

The final step cools the reaction chamber to 4°C for an indefinite time, and may be employed for short-term storage of the PCR products.

16 All secondary PCR products were subjected to electrophoresis in a 1.5 % agarose gel and visualized by staining the gel with GelStrain (TransGen Biotech., Beijing, China).