Detection of Cryptosporidium in stool sample by PCR-RFLP

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Nested PCR-RFLP adapted from Nichols, R.A.B., Campbell, B.M. and Smith, H.V., 2003. Identification of *Cryptosporidium spp.* oocysts in United Kingdom noncarbonated natural mineral waters and drinking waters by using a modified nested PCR-restriction fragment length polymorphism assay. *Applied and environmental microbiology*, *69*(7), p.4183.

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- GoTaq MasterMix from Promega
- Primers

Nested PCR 12h 45m

1 Extract whole genomic DNA from stool samples. 3h

Positive control DNA for *Cryptosporidium parvum* may be obtained from ATCC. Alternatively you can extract genomic DNA from wild type Cryptosporidium parvum purchased from Bunchgrass Farms (Deary, Idaho, USA), Waterborne Inc (New Orleans, Louisiana, USA), or Sterling Labs (Tucson, Arizona, USA).

2 First round of PCR to amplify 18SrRNA

4h

2.1 Combine the following in a PCR tube:

30m

5 µl of DNA (from positive control, experimental sample, or water for negative control)
0.1 µM Forward Primer ("out NDIAGF2": CAATTGGAGGGCAAGTCTGGTGCCAGC)
0.1 µM Reverse Primer ("out NDIAGR2": CCTTCCTATGTCTGGACCTGGTGAGT)
1x GoTaq Master Mix (Promega)
Ultrapure water to bring total volume to 25 microliters

2.2 Thermocycler program:

3h 30m

95 ºC -5 minutes,
30 cycles:
94 ºC -30 seconds
58 ºC - 1 minute
72 ºC - 30 seconds

3 Second round of PCR to amplify 18SrRNA

4h 30m

3.1 Combine the following in a PCR tube:

30m

1 µl of PCR product from Step 2
0.1 µM Forward Primer ("DIAGF": AAGCTCGTAGTTGGATTTCTG)
0.1 µM Reverse Primer ("DIAGR": TAAGGTGCTGAAGGAGTAAGG)
1x GoTaq Master Mix (Promega)
Ultrapure water to bring total volume to 25 microliters

3.2 Thermocycler program:

4h

95 ºC -5 minutes,
45 cycles:
94 ºC -30 seconds
58 ºC - 1 minute
50 ºC - 1 minute

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4 Run 7 µl of PCR product from Step 3 on a 2% agarose gel and visualise by staining with ethidium bromide. 45m

RFLP 4h 45m

5 Combine the following and incubate at 37 ºC for 4 hours: 4h

5 µl PCR product from step 4 above
20u Restriction enzyme (Ssp1, Vsp1, Ase1)
1x final concentration Restriction enzyme digestion buffer (corresponds to enzyme used)
1x final concentration BSA

6 Run digested PCR product from Step 5 on a 2% agarose gel and visualize by staining with ethidium bromide. 45m

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