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PCR protocol for gene COXI neo-caledonian freshwater micro-crustaceans V.3

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We use this protocol and it's working

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Abstract

This PCR protocol has been used to amplify the Cytochrome Oxydase I gene of neo-caledonian genera *Boeckella*, *Lynceus*, *Eulimnadia* and *Streptocephalus* with several primers.

Troubleshooting

1 Prepare the mix for the PCR depending on the quantity of DNA samples you want to amplify

Note

The quantities per well and for 96 DNA samples (with a 4 volumes margin) are detailed in the sub-steps

Safety information

Be careful to take enough margin to avoid a lack of reagent and, if possible, do a positive and a negative control

1.1 For each well, mix 12.44 µL distilled water , 2 µL DNA buffer for Taq polymerase , 1 µL DMSO , 1 µL BSA , 0.8 µL DNTP and 0.12 µL Taq polymerase . This mix is hereafter named "intermediary mix".

Note

Very few micropipettes are able to dose such a small amount so you'll have to make your mix for several wells at a time.
So, for 96 DNA samples with a 4 volume margin (100 volumes), mix 1244 µL distilled water , 200 µL DNA buffer for Taq polymerase , 100 µL DMSO , 100 µL BSA , 80 µL DNTP and 12 µL Taq polymerase .

1.2 Add your primers to the mix, 0.32 µL selected H primer [M] 10 picomolar (pM) and 0.32 µL selected L primer [M] 10 picomolar (pM) for each well. This mix is hereafter named "final reagent mix".

Safety information

You may have to use different primers in your wells depending on the individual and the gene you want to amplify. If so, distribute the intermediary mix in as much tubes as primer pairs you want to use and add your primers in the proper quantity to make your final reagent mix. Don't forget to add margins in each mix for each different pair of primers you use.

Note

So, for 96 DNA samples with a 4 volume margin (100 volumes) and if you use the same primers for all 96 wells, add 32 µL selected H primer and 32 µL selected L primer

2 In each well, deposit 18 µL of the final reagent mix and 2 µL genomic DNA unless for the negative control.
For the positive control, use the DNA of the species on which the primer has been designed.

3 Close the PCR plate with a heated aluminium foil and centrifuge it enough to make sure the reagents are all in the bottom of the well.

4 To amplify targeted DNA sequences, place your plate in the thermocycler, set and launch the cycle as described in the following sub-steps

4.1 00:05:00 at 94 °C 5m

4.2 40 cycles of the following steps : 2m
- 00:00:30 at 94 °C
- 00:00:30 at 50 °C
- 00:01:00 at 72 °C

4.3 00:08:00 at 72 °C 8m

4.4 Infinite timing at 20 °C so the samples are well preserved and you have some time to pick it up

5 To know if your amplifications worked properly, you now have to proceed to a gel electrophoresis of your PCR products

5.1 To prepare the gel,

Safety information

This step is to be made under an extractor hood with gloves, a blouse and glasses as BET is highly mutagenic and carcinogenic

mix 0.8 mg agarose and 40 mL TAE buffer, heat the preparation in the microwave for a few moments until it is homogeneous (you can agitate the preparation). Wait until the preparation is cold enough to touch its container and add 0.8 µL BET.

5.2 Pour the preparation in a mold and let the gel polymerize with a bar to form wells to deposit your PCR products

5.3 Deposit the gel in the electrophoresis tank

5.4 Fill each well with 2.5 µL PCR product and 0.5 µL DNA loading dye (6x), don't forget to save a well to deposit 1.2 µL molecular weight marker

5.5 Start electrophoresis in TAE buffer for 00:15:00 at 200 Volts 15m

5.6 Look at your gel under Ultra Violet light to see the results