amplification and sequencing of the mitochondrial gene cytochrome c oxidase subunit I (COI) in Sabellidae polychaetes

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Abstract

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Protocol

Genomic DNA extraction

Step 1.

DNA extraction was carried out following the procedure used by Capa et al. 2013 using tissue removed from the posterior end of the worms. Genomic DNA was extracted using standard protocols for the Dneasy Blood and Tissues Kit (QIAGEN Pty Ltd, Dusseldorf, Germany) and stored at -20°C until further processing.

Amplification of COI gene

Step 2.

The primer cocktail designed by Ivanova et al. 2007 was used for the amplification of the COI gene.

The amplifications were carried out in a 25 µl reaction containing 1 µl of the genomic DNA, 0.5 µl of HotStartIt Taq DNA polymerase, 1.5 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (50 mM each), 5.5 µl of ddH_2O, 12.5 µl of 10% trehalose, 2.5 µl of 10X buffer HotStartIt and 0.25 µl of each primer (10 mM).

The PCR thermal cycling profile was 94 °C for 1 min, 5 cycles of 94°C for 30s, 45°C for 40s, 72 °C for 1min followed by 35 amplifications cycles of 94°C for 30s, 50°C for 40s, 72°C for 1min with a final extension step at 72°C for 10 min.

Amplification of COI gene

Step 3.

As the PCR using the COI primer cocktail was unsuccessful for the amplification of the populations of Madeira, Veracruz, Galapagos, Tampa Bay and Hawaii, a new set of primers PolyBr_COIF/PolyBr_COIR was designed: the forward primer 5’ TCWATWAGWGTWATTATTCGKGCTG 3’ and the reverse 5’ CMGCAGGATYAAARAACCTAGTA 3’.

PCR mixture (20 µl in total volume) contained 0.5 of the genomic DNA, 0.08 µl f Platinum Taq DNA polymerase, 0.6 µl of $MgCl_2$ (50 mM), 0.4 µl dNTPs (50 mM), 15.42 µl of ddH2O, 2 µl of 10X buffer and 0.5 µl of each primer (10 mM).

The PCR thermocycler protocol was 94°C for 1 min, 5 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 40 s, 30 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 40 s, final extension at 72°C for 7 min.

Sequencing of COI gene

Step 4.

PCR products were verified by means of electrophoresis on 1% agarose gel with ethidium bromide.

The products of successful amplifications were then purified using the ExoSAP-IT PCR purification system (USB Corporation, Cleveland, Ohio, USA) and then bidirectionally sequenced using the forward and reverse primer M13 for the Mediterranean and Mazatlán populations.

For the sequencing of the samples collected from all the other populations the new set of primer was utilized. Cycle sequencing was performed using BigDye Terminator version 3.1 in a 10 µl reaction containing 5 µl of 10% trehalose, 2 µl of 5X BigDye buffer, 1 µl of BigDye, 1 µl of each primer (or primer cocktail) and 1 µl of the PCR products.

After clean-up of the sequence reactions using Zymo Sequencing Clean-Up Kit, the sequences were analysed with an ABI PRISM® 3130 Genetic Analyzer.