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DNA extraction protocol for corkwing wrasse (*Symphodus melops*)

Steps:1 -9 must be carried out in a fume cupboard or seal tubes tightly with parafilm before placing them into the oven.

1. In a fume hood add 500 µl of 2XCTAB solution into a new 1.5 ml tube or add 500 µl of CTAB solution and 1 µl of 2-mercaptoethanol.
2. Add a small piece of tissue (from the middle of a fin, approx. 3x3mm).
3. Add proteinase K (20 µl of 10 mg/ml conc.)
4. Leave for at least 2 hours on the oven at 56º C for digestion and vortex* every 15-20 min (or leave it overnight).
   ** You shouldn't vortex a sample extracting for PacBio. It's better to leave it in the oven and if necessary mix by inverting the tube several times every 20 min or leave it in the oven with a rotary mixing option.*
5. Add 4 µl of Qiagen RNase A (concentrated; 100 mg/ml, vortex* and leave for the next 30-60 min at 37º C and next for 10 min at 65º C)
   ** Do not vortex, mix by inverting the tube several times.*
6. Take out the tube from 65º C and let it cool down for 5 min. Add 500 µl of Chloroform (wait a bit before closing the lid).
7. Put the tube in a laboratory rotator for 10 min
8. Centrifuge for 5 min at 10,000 x g
9. Transfer the aqueous layer (top layer) into a new-labelled tube (use wide-bore tips).
10. Add 4 µl of Qiagen RNase A (concentrated; 100 mg/ml), mix gently and leave first for the next 30-60 min at 37 º C and then for 10 min at 65 º C
11. Take out the tube from 65º C and let it cool down for 5 min.
12. Pre-spin yellow Gel Lock Columns, transfer the sample using wide-bore tips into a yellow column and spin at 14,000 rpm (max speed) for 5 min at room temp.
    Carefully transfer the upper phase into a new tube. If there is a thin gel layer on top of it, remove it with a pipette tip.
13. Add two volumes of ice cold 99% ethanol. Immediately mix by inverting, incubate 5 min. DNA should precipitate.
14. Centrifuge for 20 min at 4º C at 10,000 x g*.

** If you can see loads of DNA (a fluff) retrieve it with a glass rod by spinning the rod in the fluff. Rinse the DNA on the rod in 70% ethanol two times (prepare two separate tubes with EtOH and spin the rod with DNA gently in each of the tubes. Dry the DNA fluff in air on the rod for a few minutes (until the ethanol evaporates) and place it in a new 1.5 ml tube in 30 µl of Nuclease Free Water.*

15. Decant ethanol.
16. Add two volumes of 70% ethanol and place in the rotator for 10 min.
17. Centrifuge for 5 min at 10,000 x g
18. Add two volumes of 70% ethanol and place in the rotator overnight.
19. Centrifuge for 5 min at 10,000 x g
20. Decant ethanol, use pipette if necessary.
21. Air-dry the pallet at room temp. First decant as much as possible, then spin to pipette out the rest (no longer than 10-15 min, do not over-dry it! pipette out visible drops from the tubes)
22. Add 30 µl of Nuclease Free Water.
23. Store at 4º* C for 1 day and check the DNA concentration, aliquote and then put in a fridge.

** Let the DNA dissolve overnight in the room temperature.*

Samples were cleaned with Genomic DNA Clean & Concentrator™-10 (Zymo)*
** It is better to use beads instead of columns*

REAGENTS

2 XCTAB

50 ml 1M tris-HCl, ph 8.0
175 ml 4M NaCl (or 40.9g)
20 ml 0.5M EDTA
10 g CTAB (hexadcyltrimethylammonium bromide)
add distilled H2O to make 500 ml
Heat with stirring to dissolve CTAB (be careful not to boil over)
Autoclave

Add 1 ml of 2-mercaptoethanol under fumehood (or later directly into samples)
Don't use 2xCTAB longer than one week (CTAB with 2-mercaptoethanol).

1M tris-HCl, ph 8.0

1. Dissolve 121.14 g Tris (American Bioanalytical #AB14042) in 800 ml dH2O.
2. Adjust pH to 8.0 with the appropriate volume of concentrated HCl. Bring final volume to 1 liter with deionized water.
3. Autoclave and store at room temperature.

Tissue preserving buffer (DMSO)

To prepare 500ml of DMSO 40%
Mix

DMSO	200ml
DH20	300ml

To make a liter of DMSO/0.25M EDTA
Add 500 ml of 40% DMSO
Add 500 ml of 0.5 M EDTA
Add NaCl to saturate (you may see 1 cm of salt in the bottom)

0.5M EDTA

Add 186g of EDTA to 500 ml of dH20. Stir
Adjust to correct ph using 4M NaOH or KOH.
Make to 1 L with dH20.