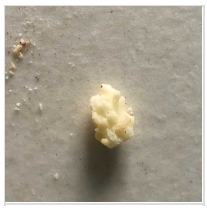

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Coral DNA Extraction - Modified DNeasy PowerSoil Pro Kit

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Luigi Colin

DNeasy PowerSoil Pro Kit modified extraction protocol to improve yield with inhibitor heavy hard corals.
Tested and developed on Acropora corals. Optimised for all coral DNA extraction that with high quantity of inhibitors

(Step 1 to 8 modified protocol; step 9 to 18 manufacturer's protocol.)

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Luigi Colin 2021. Coral DNA Extraction - Modified DNeasy PowerSoil Pro Kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bww6pfhe

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Colin L, Yesson C, Head CEI, Complete mitochondrial genomes of three reef forming corals (,) from Chagos Archipelago, Indian Ocean. Biodiversity Data Journal doi: 10.3897/BDJ.9.e72762

DNA extraction, Acropora, Scleractinian, Qiagen, coral, DNA

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- DNeasy PowerSoil Pro Kit
- Proteinase K
- Mortar and pestle
- Sharp scalpel
- Microcentrifuge (up to16,000 xg)
- Pipettor (50–1000 µl)
- Vortex-Genie2

- Sterilise all the equipment that is not single use.
- Ensure that the sample are as dry as possible before starting the protocol, taking particular care of removing any buffer they may have been kept in (Especially if RNALater was used)

Sample preparation 2h 33m 20s

1 Gently crush a small piece of coral in a mortar and pestle (~ **100 mg** , in order not to overload the column though). And transfer in a PowerBead Pro Tube (or 2 ml Microcentrifuge).
Note: For improved yield focus on adding more tissue and less carbonate from the skeleton, a scalpel

2 Add **800 µL** of buffer CD1 and vortex briefly.

Cell lysis 2h 33m 20s

3 Add **83 µL** of Proteinase K (2 ml) **Qiagen Catalog #19131** and vortex briefly.
Note: quality of Proteinase K can be tweaked depending on the quality and type of samples

4 Incubate at 56 degrees for 60 minutes. Vortex for **00:00:10** every **00:15:00** (Ideally, do this on a shaking heat block, with shaking on full) 2h 15m 10s

Note: If on orbital incubator at a minimum of 300rpm or more, can skip the vortexing.
Add an extra hour of incubation to increase yield. More than **02:00:00** will results in shearing of the longer DNA fragments

Inhibitor's removals 2h 33m 20s

5 Add **200 µL** buffer CD2 and vortex for **00:00:05** 5s

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6 Microfuge a **16000 x g, Room temperature, 00:01:00** . 1m

7 Transfer supernatant to new tube (Max **700 µL**).

Expect ~ **650 µL**

Bind DNA 2h 33m 20s

8 Add **600 µL** of buffer CD3, vortex for **00:00:05** 5s

9 Load **650 µL** of the lysate onto an MB Spin Column and centrifuge at **15000 x g, Room temperature, 00:01:00** 1m

10 Discard the flow-through and repeat step 9 to ensure that all the lysate has passed through the MB Spin Column.

11 Carefully place the MB Spin Column into a clean 2 ml Collection Tube. Avoid splashing any flow-through onto the MB Spin Column.

Wash 2h 33m 20s

12 Add **500 µL** of Solution EA to the MB Spin Column. Centrifuge at **15000 x g, Room temperature, 00:01:00** 1m

13 Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

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14 Add **500 µL** of Solution C5 to the MB Spin Column. Centrifuge at **15000 x g, Room temperature, 00:01:00** 1m

15 Discard the flow-through and place the MB Spin Column into a new 2 ml Collection

16 Centrifuge at up to **16000 x g, Room temperature, 00:03:00** . Carefully place the MB Spin Column into a new 1.5ml Elution Tube. 3m

Elute 2h 33m 20s

17 Add **50 µL** – **100 µL** of Solution C6 to the centre of the white filter membrane. 10m

Note: To improve the yield use the lower volume and add a **00:10:00** incubation at room temperature

18 Centrifuge at **15000 x g, Room temperature, 00:01:00** . Discard the MB Spin Column. The DNA is now ready for downstream applications. 1m

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