DNA EXTRACTION PROTOCOL FOR PHOENIX SPECIES

Co-funded by the European Union

This protocol shows how to perform an optimal DNA extraction from any species of the genus *Phoenix* (Arecaceae). It is based on the method described by Dellaporta et al. (1983) and modified by Corniquel and Mercier (1994).

This protocol has been drawn up within the framework of the European LIFE-PHOENIX project. [https://lifephoenix.gesplan.es/es] entitled Restoration and improvement of priority habitat 9370*. Phoenix palm groves.

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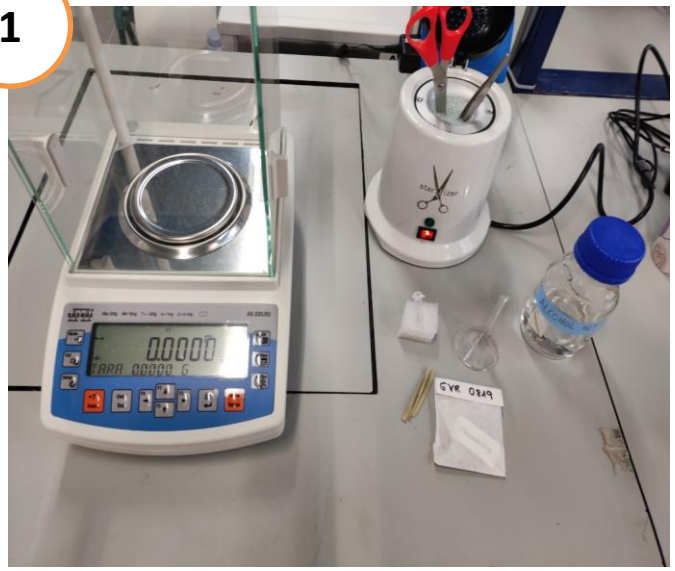
LIFE PHOENIX

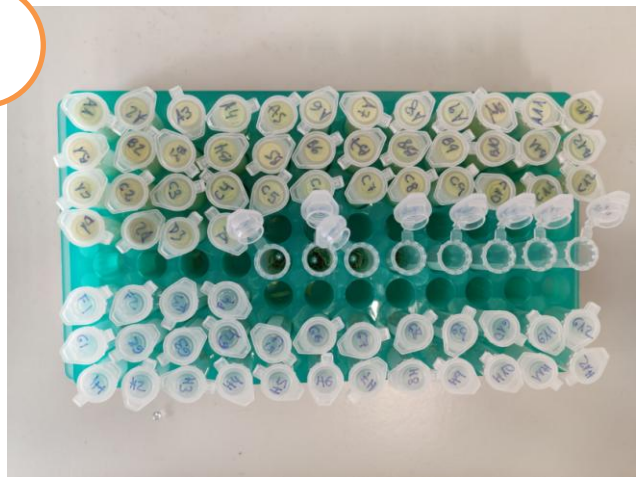
PHASE I

1



Weigh 50 mg of the palm sample (cut into small pieces) into a collection microtube labelled with your code.

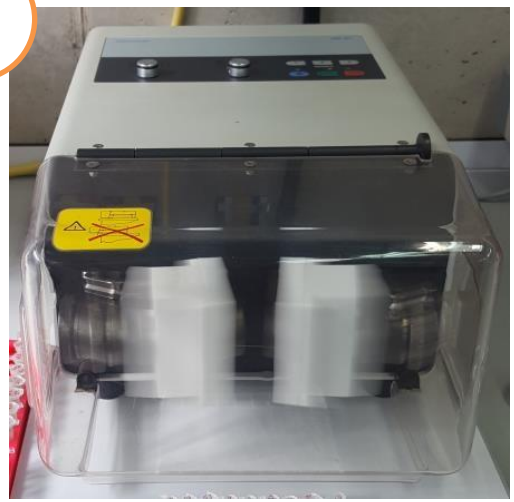
2



Place 2 steel balls in the tube for crushing the samples.

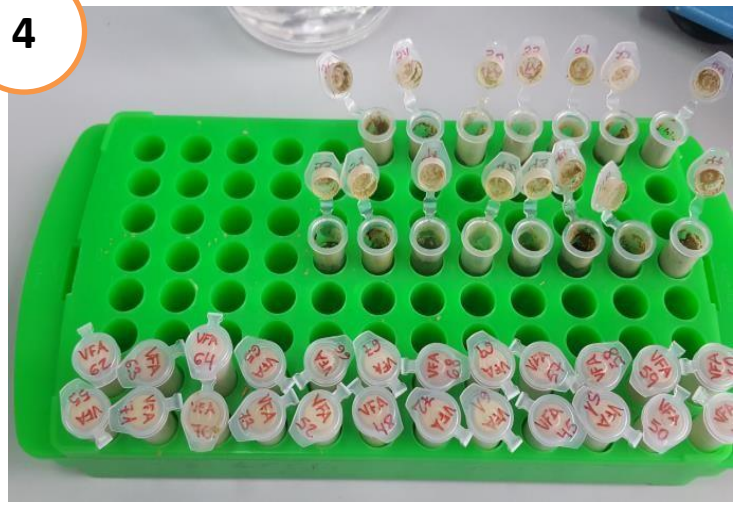
PHASE II

3



Homogenise the tube with Mixer Mill MM 300 (Retsch), using the approved tube adapters (Retsch 2x10) (3 min - 30 Hz) twice, changing the orientation of the plate).

4



Add 700 µl of extraction buffer 1 (0.1 M Tris-HCL pH 8; 50 mM EDTA; 0.5 M NaCl; 1% SDS; 2% PVP).

5

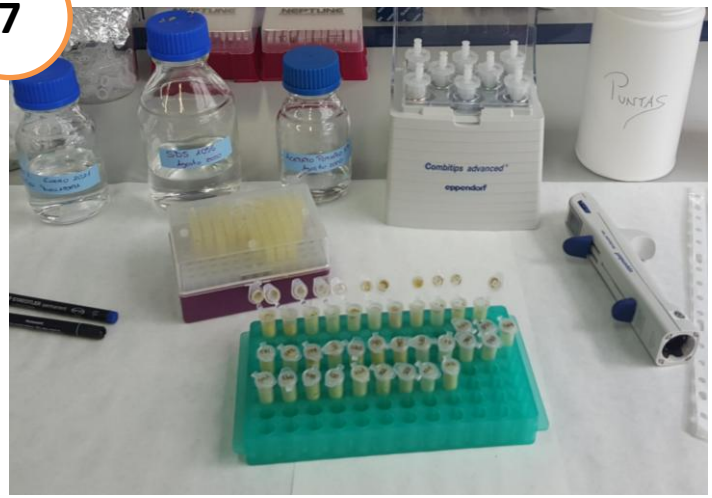
Shake the tubes with the aid of a Vortex shaker.

6

Add 300 ul of 10% SDS and place the tubes in a heating block at 65°C for 20 min.

PHASE IV

7



Add 300 ul of 5M potassium acetate and shake briefly. Place the samples on a bed of ice for 20 min.

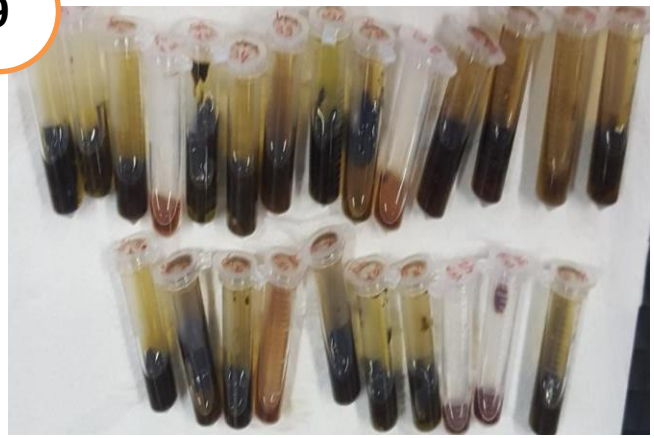
8

Centrifuge 13,000 rpm during 30 min - room temperature

LIFE PHOENIX

PHASE V

9



Collect the upper phase from the centrifugation, which contains the DNA, and transfer to a tube with 500 ul of very cold isopropanol (stored at -20°C).

10

Mix the solution by inversion and store overnight at -20°C.

11

Centrifuge at 13,000 rpm for 15 min - room temperature

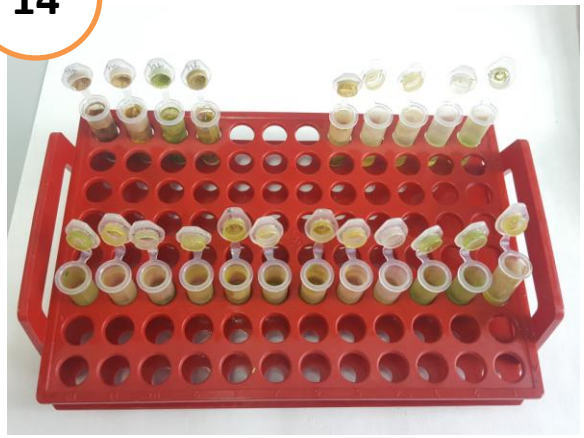
12

Discard the supernatant by pouring the liquid out of the tube and allow the sediment to dry by inverting the tube on blotting paper for 10 min

13

Resuspend the pellet with Buffer-2 (50 mM Tris-HCL pH 8.0; 10 mM EDTA) and centrifuge again at 13,000 rpm for 10 minutes. Room temperature

14



Transfer the supernatant to a new tube and add 75 ul of 3M Sodium Acetate and 500 ul of cold Isopropanol (-20°C). Mix by inversion

PHASE VIII

15

Centrifuge at 13,000 rpm. 30 min - room temperature

16

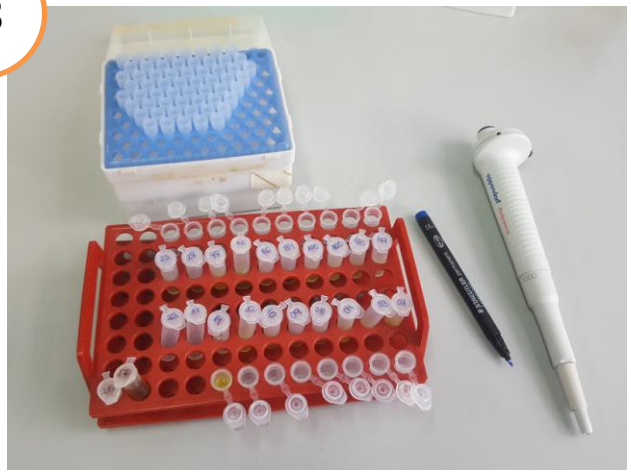
Carefully discard the supernatant and wash the sediment with one or two drops of 80% ethanol (5 min)

PHASE IX

17

Remove the ethanol by inversion on blotting paper and dry the sediment by incubating in an oven at 36ºC.

18



Resuspend DNA in 100 ul TE buffer (100 mM Tris; 1 mm EDTA).

19

Store in freezer at -20°C or -80°C.

DNA stored at this temperature and under these conditions can remain unaltered for up to 12 months

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