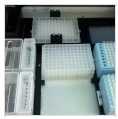

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Plant and fungal DNA extraction using a QIAGEN QIAxtractor

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1 Works for me dx.doi.org/10.17504/protocols.io.bc2fiybn

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ABSTRACT

Here we describe a semi-robotic plate DNA extraction method that we have used at the Royal Botanic Garden Edinburgh (RBGE) to process samples for PCR and Sanger sequencing. This protocol was developed at RBGE in conjunction with QIAGEN for DNA extraction from a broad range of plant and fungal tissue.

The RBGE QIAxtractor has been updated to a QIAcube; this protocol remains the same.

GUIDELINES

Ensure sampling is compliant with the Nagoya Protocol (Buck & Hamilton, 2011), which came into force on the 12th October 2014

MATERIALS TEXT

	Corbett Cat	QIAGEN Cat	QIAGEN Name
Consumable Pack, QIAxtractor	2270	950007	Consumable Pack, QIAxtractor
Plastic ware Pack, DX, QIAxtractor	2260	950017	Plastic ware Pack, DX, QIAxtractor
Lysis Plate, 96-well (5)	2271	990600	Lysis Plate, 96-well (5)
Capture Plate, standard yield (5)	913	950901	Capture Plate, standard yield (5)
Elution Plate (10)	2147	990602	Elution Plate (10)
Elution Plate 8-Strip Caps (120)	1636	990604	Elution Plate 8-Strip Caps (120)
Capture Plate, DX (5)	2273	950911	Capture Plate, DX (5)
Reagent Pack, DX	2220	950107	Reagent Pack, DX
Tissue Digest Reagent (DXT) (250 ml)	2228	950183	Tissue Digest Reagent (DXT) (250 ml)

1 Spin the homogenized tissue samples, in plates of 1.2 ml QIAGEN collection microtubes, for c. 10 mins at full speed in order to remove as much of the loose tissue powder as possible from near the tube lids. If only extracting one plate of samples instead of two, the centrifuge can be balanced by shifting half the strips of microtubes into a new rack (alternate strips and empty rows across the racks).

2 Lysis pre-treatment: in a trough mix 4 µl DX enzyme (kept at 4° C in the fridge) and 416 µl Tissue Digest (DXT) per sample (master mix 40.4 ml DXT and 392 µl DX enzyme per 96-sample plate); if extracting from fresh tissue rather than silica-dried or herbarium samples, substitute 4 µl DXT with 4 µl RNase A.

3 Separate each row of strips by at least one empty row before removing lids to mimimize the possibility of contamination. Carefully remove the lids from the strips, avoiding transferring any loose powder between tubes or contaminating gloves. Using a multichannel pipette, add 400 µl of the lysis mix to the centrifuged homogenized tissue samples in the 1.2 ml QIAGEN collection microtubes, shake the tubes to collect any powder from the lids and sides, then incubate for 1 hr to 1 ½ hrs in an Eppendorf ThermoMixer, increasing from room temperature to 70° C, with 700-800 rpm shaking.

4 Centrifuge plate of 1.2 ml QIAGEN collection microtubes at maximum speed for 10 min to pellet the sample debris. With a single plate, ensure centrifuge is fully balanced by splitting the strips of microtubes across two racks.

5 Using a multichannel pipette, transfer 220 µl of each sample from the plate of 1.2 ml QIAGEN collection microtubes into a 96-well Lysis Plate, avoiding disturbing the pellets.

6 Remove the tungsten beads that were used to homogenize the samples from the collection microtubes, and clean them for reuse: Flush with running water, sonicate in Ultraware ultrasonic bath for c. 2 mins to remove any stray sample fragments from the beads, then incubate in 0.4 M Hydrochloric acid for 1 min. Flush again with running water to remove the acid, and air dry.

7 Put the Lysis plate into the QIAxtractor.

In the machine, per plate of 96 samples, also add 66 ml DX Binding (DXB) (kept in the fridge at 4° C), 122 ml DX Wash (DXW), 62 ml DX Final Wash (DXF) and 12.4 ml Elution buffer (E).

The machine has been programmed to robotically add 440 µl DXB to each 220 µl sample in the Lysis Plate and incubate for 5 min. It then transfers 600 µl of this mixture into the vacuum plate, with a 5 min vacuum to pull the liquid through and dry the membrane. The 4 wash steps involve: 200 µl DXB followed by a 5 min vacuum, 600 µl DXW followed by a 1 min vacuum, another 600 µl DXW followed by a 1 min vacuum, then 600 µl DXF followed by a 1 min vacuum, followed by a 5 min vacuum. The vacuum plate is transferred to above an elution plate, and 100 µl DXE is added and incubated for 5 min, followed by a 1 min vacuum to elute the DNA from the membranes.

8 Transfer the extracted DNA from the elution plate into new labelled plates or tubes, and store at -20° C or 4° C.