SHORT PROTOCOL No. 13 I June 2015

Automated DNA Extraction from Oil Palm Leaves using QIAGEN® DNeasy® 96 Plant kit on the ep*Motion*® 5075vt/VAC-TMX

Introduction

Unlike other plants, oil palm leaves are rich in polyphenols, polysaccharides and secondary metabolites, which are often co-purified with DNA. These compounds could potentially inhibit downstream applications.
In this short protocol, we describe the integration of the QIAGEN DNeasy 96 Plant kit on the epMotion 5075vt automated pipetting system. The kit uses silica-gel-column matrices for purification in a 96-well plate format.

The purification procedure can be completed in two hours while generating high DNA yields and excellent DNA quality. The purified DNA is ideal for common downstream applications eg: restriction analysis, Southern Blotting, or PCR.

Material and Methods

Required equipment
> ep*Motion* 5075vt or ep*Motion* 5075 VAC/TMX
> TM1000-8 dispensing tool
> Gripper
> Reservoir rack
> Vac Frame 2
> Vac Holder
> Elution Plate Adapter for QIAGEN Tube Strips
> Channeling Plate
> Tissue Lyser/mixing mill

Required consumables
> epT.I.P.S.® Motion 1000 µL Filter
> ep*Motion* reservoir 30 mL
> ep*Motion* reservoir 100 mL
> Eppendorf 2 mL Deep Well Plate
> Eppendorf 400 mL reservoir
> QIAGEN DNeasy 96 Plant kit
> QIAGEN 1.2 mL Collection Microtube Racks

Sample Material
> Oil palm leaves (punches)

Worktable Layout

Position	Item
A2	1000 µL Filtertips
A3	1000 µL Filtertips
B1	1000 µL Filtertips
B2	Reagent Reservoirs
	Position 1: Buffer AP1 + RNase A + Buffer DX (100 mL)
	Position 2: Buffer P3 (100 mL)
	Position 3: Buffer AW1 (100 mL)
	Position 4: Buffer AW2 (100 mL)
	Position 5: Ethanol 96 –100% (100 mL)
	Position 6: empty
	Position 7: Buffer AE (30 mL)
B3	QIAGEN 1.2 mL Collection Microtube Racks
VACUUM	Top: DNeasy 96 plates
	Middle: Vacuum Frame 2
	Bottom: Reservoir 400 mL with Channeling Plate
C1	QIAGEN Elution Microtubes with Collection Plate Adapter for QIAGEN Tube Strips
C3	Leaf samples in Collection Microtubes, 1.2 mL
C4	Vac frame holder

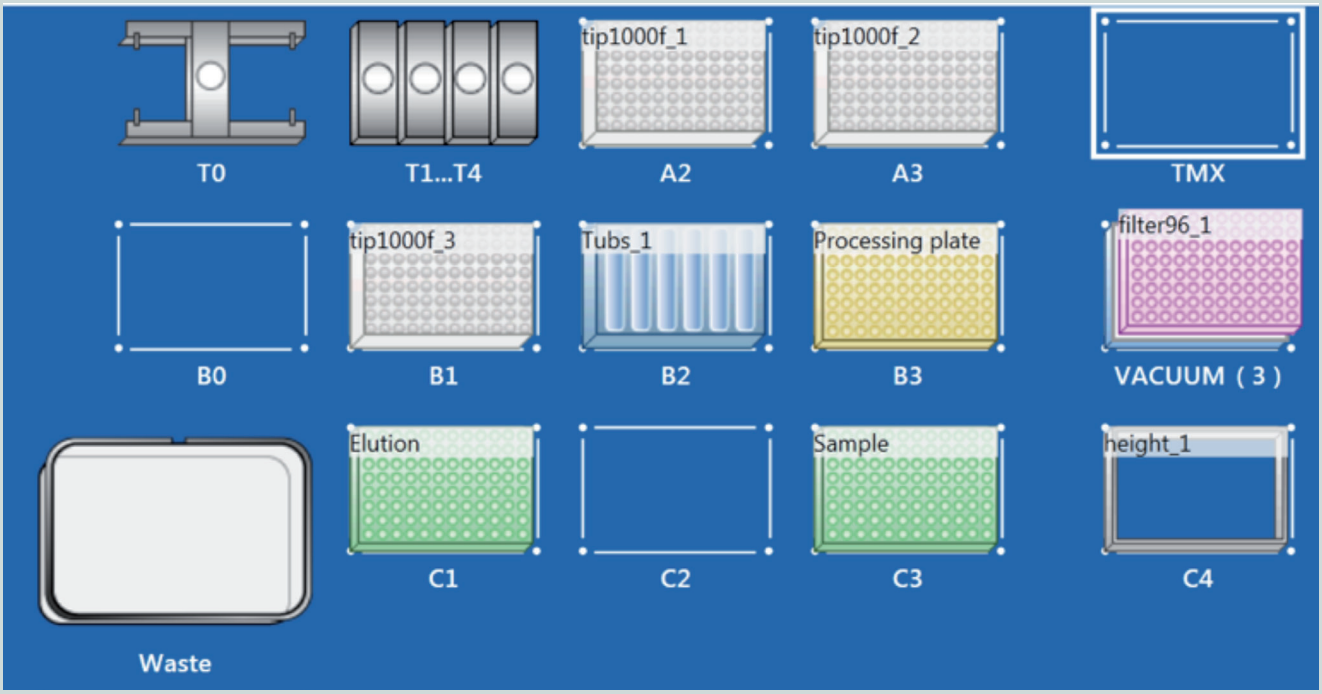

Figure 1: Screen shot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC+TMX worktable for use with the QIAGEN DNeasy 96 Plant kit.

Preparation
Fresh oil palm leaves (≤ 50 mg wet weight or ≤10 mg lyophilized tissue) are measured into each tube in 1.2 mL collection microtube racks (supplied with DNeasy 96 Plant Kit) containing one 3 mm tungsten carbide bead.

Automation
The automated procedure starts with the addition of 402 µL of the working lysis solution into each collection microtube at position C3. The working lysis solution is prepared beforehand by combining Buffer AP1, RNase A and Reagent DX, according to the instruction manual.

User intervention – sample lysis
Samples microtubes are removed from ep*Motion* and sealed with the caps provided. Samples are homogenized for 1.5 min at 30 Hz with TissueLyser II (QIAGEN) or mixing mill. The sample microtubes are then centrifuged at 1500 × *g* for 10 seconds to remove any debris/solution from the caps. The sealing caps are removed and the sample microtubes are returned to the position C3 on the epMotion.

Automation
The automated protocol continues with addition of 130 µL Buffer P3 neutralization buffer to the sample microtubes. Sample microtubes are then transported from position C3 to TMX. The samples are mixed by the TMX module at 800 rpm for 5 min at 25 ˚C. While shaking, 600 µL volume of AW1 buffer is added into each well of the purification block (Eppendorf 2 mL Deep Well Plate) at position B3.

User intervention – precipitation of protein & inhibitors
The sample microtubes and purification block are removed from the ep*Motion*. Sample microtubes are kept at -20 °C for 10 min for precipitation, followed by centrifugation at 3900 rpm for 20 min. An aliquot of cleared lysates (400 µL) is transferred carefully to purification block containing AW1 buffer, taking care to avoid carryover of any dirt or debris.

Automation
The purification block is briefly centrifuged and returned to ep*Motion* (position C2). The lysate mixture is mixed by repeated pipetting up and down, prior to a transfer into the filterplate. Vacuum step is performed for 5 min under vacuum pressure of 400 mbar. The silica membrane is subsequently washed with 800 µL Buffer AW2 (twice), followed by vacuum-drying for 15 min. Finally, the DNA is eluted in 100 µL of elution buffer.
Notes: Alternatively, the automation could be started from cleared plant lysate following a centrifugation step, which involves offline execution of lysis and neutralization steps. All further steps are performed by the instrument after clear lysate separation.

Ordering information

Description	Order no. international
ep*Motion*® 5075vt	5075 000.304
TM1000-8 Dispensing Tool	5280 000.231
Gripper (optional)	5282 000.018
Vac Frame 2 (optional)	5075 785.005
Vac Frame holder (optional)	5075 778.009
Reservoir rack	5075 754.002
Elution plate adapter	5075 785.030
Channeling plate	5075 794.004
epT.I.P.S.® Motion, 1000 µL, filtered	0030 014.499
30 mL Reservoir	0030 126.505
100 mL Reservoir	0030 126.513
400 mL Reservoir	5075 751.364

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