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Extraction of genomic DNA from plant tissue using QIAGEN Kit

Table-top high-speed micro centrifuges CT15E/CT15RE and fixed angle rotors T15A61/T15A62

We would like to introduce an example of extraction of genomic DNA from plant tissue using centrifugal filter “DNeasy Plant Kit” (supplied by QIAGEN). You can extract high quality genomic DNA by a centrifuge, it is more efficient and faster than other methods.

【Instruments】

Centrifuge:	Table-top high-speed micro centrifuge CT15E or CT15RE
Rotor:	T15A61 fixed angle rotor (2ml/1.5ml x 24 tubes) or T15A62 fixed angle rotor
Extraction kit:	DNeasy Plant Mini Kit (supplied by QIAGEN)

“x g” setting is also available.

Table-top high-speed micro centrifuge CT15E

T15A61 angle rotor

【Operating procedure】

Grind plant tissue to fine powder with liquid nitrogen using a pestle and a mortar.

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Put the ground powder in a 2-ml micro tube.

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Add 400 µl of Buffer AP1 and 4 µl of 100 mg/ml RNase A in the tube. Vortex it vigorously.

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Incubate it at 65°C for 10 minutes. *Mix it by overturning 2 or 3 times during incubation.

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Add 130 µl of Buffer AP2 in the tube and mix it.

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Incubate it on ice for 5 minutes.

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Centrifuge it at 14,500 rpm (20,000xg) for 5 minutes at room temperature.

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Put QIAsherdder Mini spin column in a new 2-ml micro tube*. Pour the supernatant into the QIAsherdder Mini spin column.

*Cut the cap of the micro tube containing the spin column.

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Centrifuge it at 14,500 rpm (20,000xg) for 2 minutes at room temperature.

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Pour the filtrate fraction passed through the column into a new 2-ml micro tube.

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Add 1.5 times greater capacity of Buffer AP3/E in the tube and mix it with a pipette.

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Put DNeasy Mini spin column in a new 2-ml micro tube*. Pour 650 µl of mixed solution into the DNeasy Mini spin column.

*Cut the cap of the micro tube containing the spin column.

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Centrifuge it at 8,000 rpm (6,000xg) for 1 minute at room temperature.

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Discard the filtrate.

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Add 500 µl of Buffer AW in the DNeasy Mini spin column.

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Centrifuge it at 8,000 rpm (6,000xg) for 1 minute at room temperature.

↓

Discard the filtrate.

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Add 500 µl of Buffer AW to the DNeasy Mini spin column.

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Centrifuge it at 14,500 rpm (20,000xg) for 2 minutes at room temperature and dry the filter.

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Put the DNeasy Mini spin column used in the above procedure in a new 1.5-ml micro tube and add 100 µl of Buffer AE in the column

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Incubate it at room temperature for 5 minutes.

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Centrifuge it at 8,000 rpm (6,000xg) for 1 minute at room temperature.

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Remove the DNeasy Mini spin column from the tube. Preserve the eluted genomic DNA solution in the tube at -70°C.

Note 1): Be sure to read the instruction manual of the rotor before use.

Note 2): Read the instruction manual of QIAGEN's kit for details of the protocol.

Note 3): **Do not spin the rotor over 10,000 rpm (12,000xg)** when using the 1.5-ml micro tube with the cap opened at 4°C. Otherwise, the cap will be torn by centrifugal force.

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