himac APPLICATION
No.163
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Extraction of Genomic DNA from Rice Leaf by using a 5-ml Tube

High-speed micro centrifuge CF15RN / CF16RN / T15A45 / T15A46 fixed angle rotor

It can extract about 3.5 times as much genomic DNA [in small-scale equipment (using a 1.5-ml micro-tube)].

5-ml tube | 1.5-ml tube

[Equipment]

5-ml Tube	Eppendorf Tubes® 5.0ml (Eppendorf)	5-ml sampling tube (ST-500) (BIO-BIK)
Rotor	T15A46 fixed angle rotor  (5-ml tube × 12)	T15A45 fixed angle rotor  (5-ml tube × 12)
Centrifuge	High-speed micro centrifuge CF15RN / CF16RN	

Bio-safety enhanced by using a rotor cover (optional)

[Operation procedure]

(1) Dispense 0.8 ml of 1.5 x CTAB extraction buffer into a 5-ml tube.
(2) Crush 0.3 g of rice leaf, put it into the 5-ml tube, turn it upside down, and then mix it by shaking the tube.
(3) Incubate (10 minutes, room temperature).
(4) Add 0.8 ml of chloroform and mix well.
(5) Incubate (30 minutes, room temperature).
(6) Centrifuge (10,000 x g, 10 minutes, 20 °C).
(7) Pour the top layer into a new 5-ml tube.
(8) Add 0.8 ml of precipitated buffer and mix by pipetting.
(9) Incubate (30 minutes, 55 °C).
(10) Centrifuge (10,000 x g, 10 minutes, 20 °C).
(11) Precipitate (remove the supernatant).
(12) Add 0.8 ml of 1M NaCl-TE and re-suspend.
(13) Incubate (2 hours, 55 °C).
(14) Centrifuge (10,000 x g, 10 minutes, 20 °C).
(15) Pour about 0.8 ml of supernatant into a new 5-ml tube.
(16) Add 0.8 ml of isopropanol and mix well.
(17) Centrifuge (10,000 x g, 10 minutes, 20 °C).
(18) Precipitate (remove the supernatant).
(19) Add 1.5 ml of 70% EtOH.
(20) Centrifuge (10,000 x g, 5 minutes, 20 °C).
(21) Precipitate (remove the supernatant).
(22) Centrifuge (10,000 x g, 2 minutes, 20 °C).
(23) Remove all supernatant by using a pipetman and dry it.
(24) Dispense 100 µl of TE buffer and completely dissolve it by using a vortex.
(25) Pour the mixture into a 1.5-ml tube and store it in a freezer.

[Reference]

・M.G.Murray and W.F.Thompson (1980) Rapid isolation of high molecular weight plant DNA, Nucleic Acids Res. ***8***:4321-4325.
・himac APPLICATION No.147 (http://www.hitachi-koki.co.jp/himac/application/pdf/life/orenge147.pdf)

If you have any inquiry of this application or products, please contact us through our web site.
http://centrifuges.hitachi-koki.com/

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