Acid nucleic extraction from rice dried leaves

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

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ABSTRACT

Acid nucleic extraction of rice dried leaves by a CTAB method adapted from Li et al 2008.

Li R, Mock R, Huang Q, Abad J, Hartung J, Kinard G. A reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens. Journal of Virological Methods. 2008;154(1):48-55. doi: 10.1016/j.jviromet.2008.09.008.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

M. Bangratz, I. Wonni, K. Kini, M. Sondo, C. Brugidou, G. Béna, F. Gnacko, M. Barro, R. Koebnik, D. Silué, C. Tollenaere (2020) Design of a new multiplex PCR assay for rice pathogenic bacteria detection and its application to infer disease incidence and detect co-infection in rice fields in Burkina Faso. PLoS One.

MATERIALS

NAME	CATALOG #	VENDOR
TE buffer		
Isopropanol		
NaCl	S-3014	Sigma-aldrich
Hexadecyltrimethylammonium bromide (CTAB)	H9151	Sigma Aldrich
1 M Tris/HCl Stock Solution (dissolved Tris base adjusted to pH 8.0 with HCl)		
Chloroform: Isoamyl Alcohol (24:1)		
Polyvinylpyrrolidone	PVP40	Sigma – Aldrich
ethanol		
sodium bisulfite		Sigma Aldrich
Na2EDTA		

MATERIALS TEXT

Waterbath at 65°C
Qiagen TissueLyser II
Microcentrifuge at 4°C

SAFETY WARNINGS

working with chloroform : Isoamyl alcohol under a fume hood

BEFORE STARTING

Citation: Martine Bangratz, Charlotte Tollenaere (04/15/2020). Acid nucleic extraction from rice dried leaves . https://dx.doi.org/10.17504/protocols.io.bcntiven

- Put 20-50 mg of dried rice leaves sample into a safe lock tube 2.0 ml containing two stainless steel beads, 5mm.

- Prepare CTAB Extraction Buffer (warm up the buffer for the CTAB dissolution) :
For 100 ml
2 g CTAB (2% w/v)
2 g PVP-40
10 ml 1M Tris-HCl, pH8.0
8.18 g NaCl
744,48 mg EDTA
0,5 g sodium bisulfite (add just before to use)
qs 100 ml H20

1 Grind the dried leaves with the Qiagen TissueLyser II until obtaining a fine powder

2 Add 1 ml CTAB extraction buffer (see 'before start' for the buffer content) and homogenize by vortexing.

3 Incubate at 65°C for 30 min. Periodically, mix gently the tubes during the incubation. **65 °C** **00:30:00**

10000 x g, 4°C 00:10:00

4 Transfer the supernatant (650µl) to a 1.5 ml microcentrifuge tube.

Add equal volume of chloroform/isoamyl alcohol (24:1)

5 After shaking, **15000 x g, 4°C 00:10:00**

6 Transfer the supernatant (500µl) in a new 1.5 ml tube containing 350 µl of isopropanol (pre-chilled at -20°C). Mix gently
-20 °C **00:30:00**

15000 x g, 4°C 00:10:00

7 Discard the supernatant . Wash the pellet with 70% ethanol.

15000 x g 00:05:00

8 Remove the ethanol and air-dried the pellet.

9 Dissolve the pellet in 50 µl sterile water or TE buffer and conserve the DNA at -20°C.

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