

Tips and tricks of the trade

Homemade Protocol Instead of DNA-Extraction Kit

It's not always necessary to order the latest kit when planning the extraction of DNA.

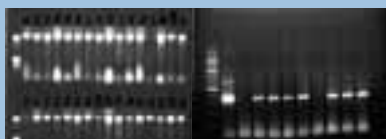
Eduardo Daniel Souza-Canada has developed a simple protocol for the isolation of genomic DNA from leaves.

Lab Hint

Dear Editors,

I have tried and compared several approaches for extraction of genomic DNA from leaves, until I found a method that perfectly meets our needs.

The extraction method I have established is reliable and fast and works with leaves from wheat, tobacco, and *Arabidopsis thaliana*. There is no need to add cetyl trimethyl ammonium bromide (CTAB) and polyvinylpyrrolidone (PVP) to the extraction buffer. You may also forget about adding beta-mercaptoethanol, phenol or proteinase A. The genomic DNA extracted according to our protocol is ready for use in PCR or restriction digests.



Agarose gel of genomic DNA extracted from wheat leaves (left figure). PCR products using genomic DNA extracted from wheat leaves as template.

Reagents and solutions (final concentrations):

100 mM Tris-HCl
700 mM NaCl
50 mM EDTA pH 8,0
RNase 10 µg/µl
pH value of extraction buffer: 8.0

Protocol (You can conduct the single steps of the DNA-extraction in 2 ml tubes)

1. Mince 60 to 100 mg of leaves in liquid nitrogen (We use a ball mill for grinding the leaves. This preserves them from being in direct contact to the nitrogen).
2. Mix the crushed leaves with 1330 µl of prewarmed (65°C) extraction buffer and incubate for 15 minutes at 65°C. Invert the reaction tube from time to time.
3. Add 650 µl Chloroform/Isoamylalcohol after cooling the sample to room temperature for one minute and gently shake for 5 minutes.
4. Centrifuge 2 minutes (14000 rpm) at room temperature. Transfer the upper layer containing the DNA into a fresh tube. (Option: add 10 µl RNase at this step and incubate 10 minutes at 37 °C).
5. Mix with 700 µl isopropanol and incubate 2 minutes at room temperature to al-



Uses his one protocol for DNA-extraction: Eduardo Daniel Souza-Canada

low the DNA to precipitate. Centrifuge for 5 to 10 minutes (14000 rpm) at room temperature.

6. Wash the pellet 5 minutes in 1 ml ethanol (70%)
7. Air-dry the pellet before dissolving it in 100 µl distilled water at room temperature (or at 65°C) for 10 minutes.

(Eduardo Daniel Souza-Canada, Institute of Plant Physiology, University Bayreuth, Germany)

Do you have any useful tips?

Contact us at:

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