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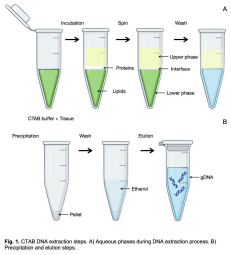
Optimized CTAB DNA extraction for different tissues



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We use this protocol and it's working

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Abstract

CTAB (cetyltrimethylammonium bromide) DNA extraction is a method for isolating genomic DNA from different tissues. This method employs a cationic detergent to break down cell membranes and contain contamination or interference from compounds such plant secondary metabolites. We have modified the CTAB protocol to suit DNA isolation from different tissue types. We found that by adjusting the percentage of CTAB in combination with modifying subsequent washing steps, total DNA recovery is optimized and increased. For plants, this protocol has been used with different solanaceous plants such as *Capsicum annuum*, *Nicotiana benthamiana*, *Nicotiana tabacum*, *Solanum lycopersicum*, and *Solanum tuberosum*, whose phenolic acid content is usually an obstacle to obtaining genomic DNA free of contamination. This protocol has also been used with *Arabidopsis thaliana*, *Marchantia polymorpha*, *Oryza sativa*, and different Citrus and Populus species. For animals, this protocol has been used with root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Globodera* and *Heterodera* spp.), aphids, mites, and thrips. This protocol has also been used with mammalian hair follicles and sperm. For fungi, this protocol has been used with mycelia of different fungal genera. This protocol allows for standardization of DNA extraction, optimization with regard to tissue type, and improved repeatability across studies.

Image Attribution

Figure 1 was designed to provide guidance during the DNA extraction procedure.

Guidelines

This methodology has been developed from the original method described by Doyle and Doyle (1987) with modifications for use in different tissues, including plant vegetative and reproductive tissue, plant seeds, fungal mycelia, nematodes, arthropods, and freeze-dried tissue.

Materials

Lab coat (the protocol involves working with toxic solvents)
Goggles (or glasses)
Mortar and pestle
Liquid nitrogen (LN_2)
CTAB buffer at 2, 3, and 4%
Isoamyl alcohol: chloroform (solution 24:1 vol/vol)
Isopropanol absolute
Ethanol absolute
Ethanol at 70%
DNase- and RNase-free water
Eppendorf Safe-Lock tubes 1.5 mL
Plastic beaker
Paper towels
Water bath or Thermoblock at 65 ºC
Centrifuge

Safety warnings

Remember to take precautions before working with detergents and solvents, such as wearing closed-toe shoes, appropriate clothing, protective goggles, and gloves.

Before start

Before beginning, be aware that the materials and Water bath or Thermoblock equipment are available.

Consider selecting the correct percentage of CTAB buffer to be used, preparing it in advance, and keeping it warm. Prepare the Cetyltrimethylammonium bromide (CTAB) buffer as follows: 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 3% CTAB, and 0.2% β-mercaptoethanol.

PREPARATION OF THE LABORATORY SPACE

1 Clean around the bench or work space with ethanol 70% and lay paper towels near of the work area.

PROCEDURE

2 Prepare fresh CTAB buffer. The percentage of CTAB depends on the type of tissue to be processed:
Use 2% CTAB for freeze-dried tissue (e.g., freeze-dried fungal mycelia).
Use 3% CTAB for plant vegetative and reproductive tissue (e.g., leaves and roots), plant seeds, fresh fungal mycelia, nematodes, and arthropods.
Use 4% CTAB for mammalian hair follicles and sperm.

3 Warm up the fresh CTAB buffer to a temperature of 60-65 ºC (Notes 1 and 2).

4 When working with non-freeze-dried tissue, grind 100 mg of tissue with LN2 to obtain a powder. When working with freeze-dried tissue use warm, fresh CTAB buffer to grind the tissue (Note 3). Add 300 µL of warm, fresh CTAB buffer and incubate samples with light shaking for 60 min at 60-65 ºC (Figure 1A). If the water bath or Thermoblock does not allow for shaking, then shake the samples by hand every 15 min.

5 Open the tubes carefully and add an equal volume of isoamyl alcohol:chloroform (24:1).

6 Mix the suspension well with vortexing until it turns white.

7 Centrifuge the suspension at 14,000 rpm for 10 min at room temperature.

8 Recover the upper phase (supernatant) without touching the interphase (white layer) and add it to a new Eppendorf tube.

9 Add an equal volume of isoamyl alcohol:chloroform (24:1), mix the suspension well with vortexing, and centrifuge it at 14,000 rpm for 10 min at room temperature. Repeat this step until the interface is completely transparent or clear (Figure 1A) (Note 4).

10 Recover the upper phase (supernatant) without touching the interphase (white layer) and add it to a new Eppendorf tube.

11 Add an equal volume of absolute isopropanol and incubate from 30 to 40 min at -20 ºC (Note 5).

12 Centrifuge at 14,000 rpm for 15 min at room temperature.

13 Wash the pellet with 500 µL of absolute ethanol and vortex until it comes off the bottom of the tube (Figure 1B).

14 Centrifuge at 14,000 rpm for 7 min at room temperature.

15 Wash the pellet with 70% ethanol and centrifuge at 14,000 rpm for 7 min at room temperature.

16 Decant the ethanol using the plastic beaker and leave the tubes inverted on a paper towel to dry the pellet for no more than 5 min.

17 Dissolve the pellet by adding nuclease-free water and incubating the tubes at 65 ºC for 10 min (Note 6).

18 Quantify and analyze the genomic DNA (Note 7).

NOTES

19 1- Pretreatment with proteinase K (100 µg/µL) is necessary to extract genomic DNA from mammalian hair follicles and sperm. Add proteinase K solution to cover the tissue, incubate for 60 min at 60 ºC, and proceed with step 2.

2- Cyst nematode eggs and juveniles can also be pretreated with proteinase K at 60 ºC, but for 30 min instead of 60 min. However, in the case of juvenile nematodes, they can alternatively be boiled for 30 s before proceeding with step 2.

3- Add enough powder to fill 100 µL of the Eppendorf tube's volume. The amount of tissue can be increased to 250 µL of volume, but the volume of CTAB buffer should then be adjusted to 500 µL.

4- Typically, the number of washes with isoamyl alcohol:chloroform (24:1) required depends on the type of tissue in the extraction. Nematodes, arthropods, young plant leaves, and freeze-

dried tissue require three washes; plant seeds, roots, and fungal mycelia usually require four washes, and mammalian hair follicles and sperm require five washes. Be careful not to touch the interface to avoid contaminating the DNA with proteins and/or carbohydrates.

5- If the amount of tissue used for the extraction was less than 30 mg, incubating for 3 hr or more could help to improve the DNA concentration. However, if the amount of tissue used was 100 mg or higher, incubation for more than 60 min could promote unwanted DNA contamination.

6- The amount of water used typically depends on the size of the pellet; however, to concentrate the DNA, add no more than 40 µL. If the pellet is not visible, add 20 µL.

7- Using this method, DNA concentrations in the range of micrograms per microliter are obtained starting from a volume of 100 µL of powdered tissue. Genomic DNA can be used directly in PCR or put through a trace RNA cleanup process before being used in sequencing.

Protocol references

Tao Y, ..., Godinez-Vidal D, ..., Hall ME. 2023. Enhanced photosynthetic efficiency for increased carbon assimilation and woody biomass production in engineered hybrid poplar. Forests 14:827. DOI: **10.3390/f14040827**

Sacco M, Lau J, Godinez-Vidal D and Kaloshian I. 2022. Non-canonical nematode endogenous retroviruses resulting from RNA virus glycoprotein gene capture by a metavirus. Journal of General Virology 103:001739. DOI: **10.1099/jgv.0.001739**

Godinez-Vidal D, López-Leal G, Covarrubias AA and Reyes JL. 2020. Early events leading to water deficit responses in the liverwort *Marchantia polymorpha*. Environmental and Experimental Botany 178:104172.
DOI: **10.1016/j.envexpbot.2020.104172**

Doyle JJ and Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19(1):11-15.