Universal DNA isolation protocol Version 2

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Abstract

The isolation of nucleic acids from a sample is an important step for many molecular biological applications and medical diagnostic assays. This protocol describes an efficient method for purification or/and isolation of nucleic acids from difficult animal tissues, plant material and other samples from which DNA extraction is generally regarded as being difficult which can contain impurities and inhibitors or interfering substances. This method is established acidic CTAB (with a pH value of 4 to 5.5) based extraction protocol that allows for reliable isolation of high molecular weight genomic DNA for removing contaminants from nucleic acids in a sample, e.g., environmental or biological samples such as soil, food, plant, animal or microorganism. DNA may quickly be extracted from samples without phenol, guanidine thiocyanate or 2-mercaptoethanol.

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Guidelines

The procedure is suitable for all types of tissues from wide variety of animal, blood and plant species. All DNA extraction steps are performed at weak acid pH (HEPES free acid) and optionally with hot chloroform for 'difficult' samples, and at room temperature.
The following protocol is designed for small and large tissue samples (tissue volume 100-200 µl). Note that isolating genomic DNA not requires gentle mixing because the DNA not be sheared by vortexing.

Before start

Required solutions:

- **CTAB** solution: 1.5% CTAB, 1.5 M NaCl, 10 mM Na_3EDTA, 0.1 M HEPES (pH ~5.3);
  100 ml: 1.5 g CTAB, 1.2 g HEPES-acid, 2 ml 0.5 M Na_3EDTA, 30 ml 5 M NaCl;
- Fresh 1xTE (1 mM EDTA, 10mM Tris-HCl, pH 8.0).

Materials

✓ 70% Ethanol by Contributed by users

1L TE Buffer [10X] (Tris-EDTA) (100mM Tris base, 10mM EDTA, pH 8.0) 786-034 by G-Biosciences

CTAB (Hexadecyltrimethylammonium bromide) CB0108.SIZE.500g by Bio Basic Inc.

Chloroform:isoamyl alcohol (24:1) CB0351.SIZE.200ml by Bio Basic Inc.

HEPES, free acid HB0264.SIZE.1Kg by Bio Basic Inc.

Isopropanol IB0918.SIZE.500ml by Bio Basic Inc.

Protocol

Step 1.

2 ml Eppendorf Safe-Lock microcentrifuge tube with tissue sample and glass ball (5 or 6 mm) freeze at -80°C, grind in the MM300 Mixer Mill for 2 min at 30 Hz. Alternatively, grind the sample in lysis solution.

Step 2.

In 2 ml tube with mechanically disrupted seeds/leaves/herbarium or DNA solution (CTAB purification) add fresh 400 µl CTAB solution buffer with RNAse A (the sample mass should not exceed 50 mg), vortex very well and incubate the samples at 60-65°C during 30-60 min.

TEMPERATURE

65 °C Additional info:

Step 3.

Add 2 volume 800 µl of chloroform, vortex very well (in the MM300 Mixer Mill for 1 min at 30 Hz); optionally: discard the lower phase (chloroform) contains dissolved polysaccharides.

Step 4.

Spin at maximum speed in a microcentrifuge for 2 minutes, transferred the upper aqueous layer to a new 2 ml microcentrifuge tube.

Step 5.

Optionally: add 800 µl of chloroform, vortex very well for 1 minute creating an emulsion (in the MM300 Mixer Mill at 30 Hz) and incubate the samples at 60-65°C during 30 min. Vortex very well and discard the lower phase (chloroform).

TEMPERATURE

65 °C Additional info:

Step 6.

Spin at maximum speed in a microcentrifuge for 5 minutes.

Step 7.

Transferred the upper aqueous layer to a new 1.5 ml microcentrifuge tube which contains of 400 µl 2-

propanol (or 1 ml 96% ethanol), vortex well and centrifuge the tubes at maximum speed in a microcentrifuge for 3 minutes.

Step 8.

Discard supernatant and wash pellet by adding 1 ml 70% EtOH, vortex well. Centrifuge at 14,000 rpm for 2 min and discard ethanol.

Step 9.

The DNA pellet do not dry and dissolved immediately in 300 µl 1xTE, pH 8.0 at 55°C for 10-20 minutes.

Warnings

General rules for a laboratory safety should be followed.