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Working

Universal DNA isolation protocol

Version 3

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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 5 to 6.8) 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.

EXTERNAL LINK

http://primerdigital.com/dna.html

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.

MATERIALS

NAME	CATALOG #	VENDOR
70% Ethanol		
1L TE Buffer [10X] (Tris-EDTA) (100mM Tris base, 10mM EDTA, pH 8.0)	786-034	G-Biosciences
CTAB (Hexadecyltrimethylammonium bromide)	CB0108.SIZE.500g	Bio Basic Inc.
Chloroform:isoamyl alcohol (24:1)	CB0351.SIZE.200ml	Bio Basic Inc.
HEPES, free acid	HB0264.SIZE.1Kg	Bio Basic Inc.
Isopropanol	IB0918.SIZE.500ml	Bio Basic Inc.

SAFETY WARNINGS

General rules for a laboratory safety should be followed.

BEFORE STARTING

Required solutions:

- **CTAB** solution: 1.5% CTAB, 1.5 M NaCl, 10 mM Na_3EDTA, 0.1 M HEPES/MOPS (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).

1 Eppendorf Safe-Lock microcentrifuge tube with tissue sample and glass ball (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.

2 In 2 ml tube with mechanically disrupted seeds/leaves/herbarium or DNA solution (CTAB purification) add 1 ml CTAB solution buffer with RNAse A (the sample mass should not exceed 100 mg), vortex very well and incubate the samples at 60-65°C during 60-120 min or longer (long incubation increases DNA yield).

02:00:00

65 °C

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

4 Transfer theentire clarified supernatant to a new 2 ml microcentrifuge tube contains an equal volume of chloroform. Mix well for 3-5 minutes in the MM300 Mixer Mill at 30 Hz.

5 Spin at maximum speed in a microcentrifuge for 2 minutes.

6 Transfer the entire clarified upper aqueous layer to a new 2 ml microcentrifuge tube which contains an equal or half volume of 2-propanol and vortex thoroughly.

7 Centrifuge at maximum speed in a microcentrifuge for 2-5 minutes. A whitish DNA pellet should be visible.

8 Discard supernatant and wash the pellet by adding 1.8 ml 70% ethanol, vortex thoroughly. At this stage, DNA samples can be stored at room temperature or refrigerated.

9 Centrifuge at maximum speed for 2-5 min and carefully discard the supernatant by decanting or with a micropipette. A whitish DNA pellet should be visible during discarding a supernatant.

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