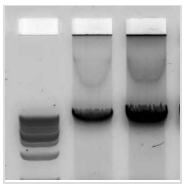

Version 4

Jul 11, 2022

Universal DNA isolation protocol V.4

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[1]PrimerDigital

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PrimerDigital

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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.

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EXTERNAL LINK

http://primerdigital.com/dna.html

PROTOCOL CITATION

Ruslan Kalendar 2022. Universal DNA isolation protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvmbx6g3pe/v4 Version created by Ruslan Kalendar

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Kalendar R, Boronnikova S, Seppänen M. Isolation and Purification of DNA from Complicated Biological Samples. Methods Mol Biol. 2021;2222:57-67. doi: 10.1007/978-1-0716-0997-2_3. PMID: 33301087.

KEYWORDS

DNA extraction, CTAB, plant tissues, blood, herbarium specimens, difficult tissues

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GUIDELINES

This protocol for DNA isolation is universal for most biological specimens. The method will effectively isolate DNA from whole blood, bones, plant samples, soil, herbarium, the mycelium of fungi, and tissues rich in secondary metabolites, polysaccharides, and pigments. DNA samples obtained using the proposed method can be used in studies where the presence of contaminants in nucleic acids is undesirable; for example, during cloning, sequencing, and genotyping.

All lab procedures are performed at room temperature. Prepare all solutions using ultrapure Milli-Q water and analytical grade reagents. Prepare and store all reagents at room temperature unless otherwise specified and away from direct sunlight.

The composition of the lysis solution contains inorganic salts (sodium chloride), within the effective concentration in the range of 1–4 M. The optimal concentration of the detergent is 1.5% CTAB. To increase the efficiency of DNA extraction, proteinase K can be added to the acidic lysing solution, which retains proteolytic activity at high ionic strength and low pH values, even in the presence of strong detergents and chaotropic agents. The subsequent extraction with chloroform increases the purity of the isolated DNA, especially from complex samples (thermally treated raw materials, blood, herbarium specimen, and soils). The effective concentration of chloroform is 1–2 volumes of the total lysate. Further, the DNA is precipitated from the aqueous phase with a water-soluble organic solvent, such as ethanol (it is preferable to use isopropanol). Depending on the biological material, DNA can be precipitated by filtration through a column with a glass microfiber filter, for example, Glass microfiber filters (Grade GF/A) or through cellulose paper. Finally, DNA is washed by precipitation or filtration in a solution of 80% ethanol and dissolved in low ionic buffered water.

MATERIALS TEXT

MATERIALS

70% Ethanol **Contributed by users**

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

CTAB (Hexadecyltrimethylammonium bromide) **Bio Basic Inc. Catalog #CB0108.SIZE.500g**

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

HEPES, free acid **Bio Basic Inc. Catalog #HB0264.SIZE.1Kg**

Isopropanol **Bio Basic Inc. Catalog #IB0918.SIZE.500ml**

SAFETY WARNINGS

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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).
- Ribonuclease A solution: 10 mg/ml in 50% glycerol, 10 mM Tris-HCL pH 8.0.
- 100% Isopropanol (2-propanol).

1 Eppendorf Safe-Lock microcentrifuge tube with tissue sample and glass ball (6 mm) freezes at -80°C, grind in the MM300 Mixer Mill for 2 min at 30 Hz. Alternatively, grind the sample in the lysis solution, so that there is enough empty space in the tube.

2 In a 2 ml tube with mechanically disrupted tissue/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 thoroughly, and incubate the samples at 55-65°C during 30-120 min or longer (long incubation increases DNA yield).

02:00:00

65 °C

3 Optionally, spin at maximum speed in a microcentrifuge for 2 minutes, and preferably transfer the entire clarified supernatant to a new 2 ml Safe-Lock microcentrifuge tube.

4 Add to the tube an equal volume of chloroform. Mix well for 1-5 minutes in the MM300 Mixer Mill at 30 Hz or vortex thoroughly for 1 minute. Spin at maximum speed in a microcentrifuge for 1-3 minutes.

5 Transfer the entire clarified upper aqueous layer to a new 2 ml microcentrifuge tube which contains half to an equal volume of 2-propanol and vortex thoroughly (5-10 secs).

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

7 Discard the supernatant and wash the pellet by adding 1.5-1.8 ml 70% ethanol, vortex thoroughly. At this stage, DNA samples can be stored at room temperature or refrigerated.

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

Do not dry the DNA pellet and immediately dissolve it in 300 µl 1xTE, pH 8.0 at 55°C for 10-20 minutes.

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