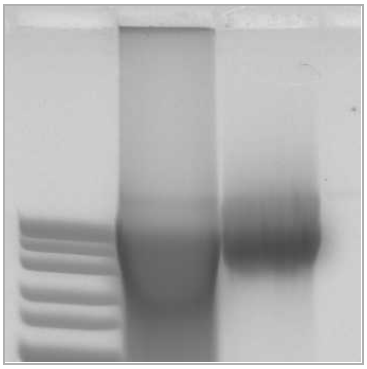

VERSION 1

JUL 11, 2023

OPEN ACCESS

**DOI:**
dx.doi.org/10.17504/protocols.io.n2bvj3x85lk5/v1

**External link:**
http://primerdigital.com/dna.html

**Protocol Citation:** Ruslan Kalendar 2023. Protocol for the purification of total DNA from soil samples. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj3x85lk5/v1Version created by Ruslan Kalendar



**Protocol status:** Working
We use this protocol and it's working

**Created:** Jul 11, 2023

**Last Modified:** Jul 11, 2023

# Protocol for the purification of total DNA from soil samples V.1

Ruslan Kalendar[1]

[1]University of Helsinki

High molecular weight DNA extraction from all kingdoms

PrimerDigital

Ruslan Kalendar
PrimerDigital

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## ABSTRACT

The protocol can also be used for other 'complex' samples (e.g. faeces) and tissues containing high amounts of polymeric molecules, pigments that cannot be separated in any other way. The scale in this example is taken to obtain DNA from 1 - 5 grams samples but can be scaled to microvolume and sample mass.
The goal of this protocol is to isolate as much total DNA from the sample as possible, without purification. The sample can be soil or any other source for which all existing approaches for isolation and purification are unsuitable. Once the DNA sample is in a soluble and concentrated form, it can be further purified by other approaches.

**PROTOCOL integer ID:**
84819

**Keywords:** DNA purification, DNA extraction, high-molecular-weight DNA, DNA sequencing, third generation sequencing, long-read sequencing, Oxford Nanopore

MATERIALS

- CTAB solution (2% CTAB, 1.5 M NaCl, 10 mM $Na_3EDTA$, 0.1 M HEPES-acid, pH 5.3); 100 ml: 2 g CTAB, 2.4 g HEPES-acid, 2 ml 0.5 M $Na_3EDTA$, 30 ml 5 M NaCl;
- 100% isopropanol (isopropyl alcohol, 2-propanol);
- 70% ethanol;
- 1xTE (0.1 mM EDTA, 10mM Tris-HCl, pH 8.0);

## Grinding

1 The sample is ground at -80°C by freezing in liquid nitrogen or at -80°C and ground in a mortar or stirrer chilled to -80 °C.

2 A sample of homogenized soil weighing no more than 5 grams was placed in a 50 ml Falcon tube and a CTAB solution (2% CTAB, 1.5 M NaCl, 10 mM $Na_3EDTA$, 0.1 M HEPES-acid, pH 5.3) was added to a final volume of 40 ml. Soil

## Lysis

3 Stir very vigorously on a stirrer and incubate overnight at 65°C. Overnight

4 Soil sample tubes are centrifuged to clarify and precipitate soil, in a large tube centrifuge, at +4°C, for 10 minutes at 10,000 rpm. 00:00:00

Equipment

**Centrifuge 5810/ 5810 R** NAME

Benchtop Centrifuge TYPE

Eppendorf BRAND

Catalog No. 022625501 SKU

https://www.eppendorf.com/us-en/eShop-Products/Centrifugation/Multipurpose-Centrifuges/Centrifuge-5810-5810R-p-PF-240994 LINK

SPECIFICATIONS

Centrifuge 5810/5810 R, with its renowned quality and reliability, offers you the most cost-effective solution for medium to high-throughput applications requiring speeds of up to 20,913 x g (14,000 rpm). This benchtop centrifuge has been designed to fit into every lab and make routine work more comfortable thanks to its quiet operation, soft-touch lid closure and low access height for easy loading and unloading. Experience true versatility with a broad selection of rotors and adapters that facilitate mixed loading of tubes, bottles and plates. A new generation of rotors increases the capacity of Centrifuge 5810/5810 R to a maximum of 4 x 750 mL (or 28 x 50 mL / 56 x 15 mL). The swing-bucket rotor and adapters accommodate tubes and bottles from 0.2 mL to 750 mL, and the fixed-angle rotor 0.2 mL to 85 mL tubes. The plate rotor is ready for you to carry out centrifugation of all types of multiwell plates, such as PCR, cell culture, or deep-well plates.

5 The clarified supernatant is separated into two equal portions of 20 ml each and transferred into new 50 ml Falcon tubes into which an equal volume of 100% isopropanol (20 ml) cooled to -20°C has already been added and mixed vigorously on a stirrer.

6 The tubes are centrifuged at +4□C for 10 minutes at 10,000 rpm and any supernatant is

discarded. A brown DNA precipitate containing soil components was formed at the bottom of the tube.

7 10 ml of 70% ethanol is added to the Falcon tubes with the pellet, and the precipitate is well mixed on a stirrer. The tubes are centrifuged at +4□C for 3 minutes at 10,000 rpm.

8 The supernatant alcohol solution is completely removed, not dried, and 1 ml of 1x TE solution is added immediately to the precipitate, stirred well, and incubated at 65□C until the DNA precipitate is completely dissolved.

9 The DNA solutions from both Falcon tubes are combined and the pooled DNA solution can be stored in 2-5 ml microtubes.