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Soil quality — Method to directly extract DNA from soil samples

Qualité du sol — Méthode pour extraire directement l'ADN d'échantillons de sol

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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 11063 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

DNA (deoxyribonucleic acid) is an essential component of any living organism coding for enzymes responsible for any biological activities. The study of DNA sequences from DNA sources extracted from different matrixes, by means of numerous molecular approaches, provides molecular markers that can be used to sharply distinguish and identify different organisms (bacteria, archaea and eucaryotes).

Up to now, most of the studies aiming to develop microbial soil quality indicators applicable to complex environments, such as soil, were biased by the unculturability of many microorganisms and the lack of sensitivity of traditional microbiological methods [16]. The recent development of numerous molecular biology methods based primarily on amplification of soil-extracted nucleic acids have provided a pertinent alternative to classical culture-based microbiological methods, providing unique insight into the composition, richness, and structure of microbial communities [15], [18], [26], [27], [36]. DNA-based approaches are now well-established in soil ecology and serve as genotypic (= molecular genetic) markers for determining microbial diversity.

The results of molecular analyses of soil microbial communities and/or populations rely on two main parameters:

a) the extraction of DNA representative of the indigenous bacterial community composition;

b) PCR bias, such as the choice of primers, the concentration of amplified DNA, errors in the PCR, or even the method chosen for analysis [23], [26], [38], [40]. Recently, numerous studies have investigated new methods to improve extraction, purification, amplification, and quantification of DNA from soils [20].

The aim of this International Standard is to describe the procedure used to extract DNA directly from soil samples. The reproducibility of this soil DNA extraction procedure was assessed in an international ring-test study (Annex A). The reproducibility of this soil DNA extraction procedure was successfully evaluated on both quantitative (q-PCR) and qualitative (A-RISA) approaches.

Soil quality — Method to directly extract DNA from soil samples

1 Scope

This International Standard specifies a method for direct extraction of DNA from soil samples to analyse the global structure and the abundance of soil bacterial communities using PCR-based technologies. This method is mainly dedicated to agricultural and forest soils. This method can possibly not be suitable for soils rich in organic matter (e.g. peat soils) or soils heavily polluted with organic pollutants or heavy metals.

The direct extraction of DNA from soil samples provides unique insight into the richness and structure of microbial communities which are key parameters to estimate the biodiversity of soil microbiota. Molecular approaches based on PCR (polymerase chain reaction) amplification of soil DNA constitute a promising domain and can contribute in the near future to the development of routine tools to monitor the microbiota of soil environments.

Users of the method ought to be aware that although soil submitted to the DNA extraction procedure is sieved thoroughly (2 mm mesh, procedure described in 5.1), plant residues can still remain in soil samples and, as a result, traces of plant DNA can contaminate the soil DNA extract.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
soil DNA
DNA extracted from soil-living microorganisms and remaining DNA from dead microorganisms

4 Principle

DNA is directly extracted from 0,25 g soil samples using the following extraction procedure. This method reliably allowed analysing the global structure of bacterial and archeal communities and could be adapted (extraction from a 1 g soil sample) to assess the global structure of fungal communities[32]. Soil samples added with an extraction buffer are submitted to mechanical and chemical lyses. The lysis step, e.g. by bead beating, is a crucial step to also extract DNA from microbes that are difficult to lyse. After a brief centrifugation, soil debris are removed and proteins are precipitated with potassium acetate. After centrifugation, the supernatant is recovered and nucleic acids are precipitated with ice-cold isopropanol. After centrifugation, the nucleic acids pellet is washed with 70 % ethanol and suspended in sterile ultra-pure water. DNA quality is then checked by electrophoresis on an agarose gel and the DNA quantity is estimated using a spectro-fluorimeter. A schematic overview of the procedure is given in Figure 1.

5 Test materials

5.1 Soil

Soil samples should be collected and sieved (2 mm mesh). If samples are not immediately processed, they should be stored for up to two years at –20 °C or up to 10 years at –80 °C or in liquid nitrogen (–180 °C) as specified in ISO 10381-6. If soil samples are frozen, they may be thawed only once. Some of these storage conditions are currently under testing.

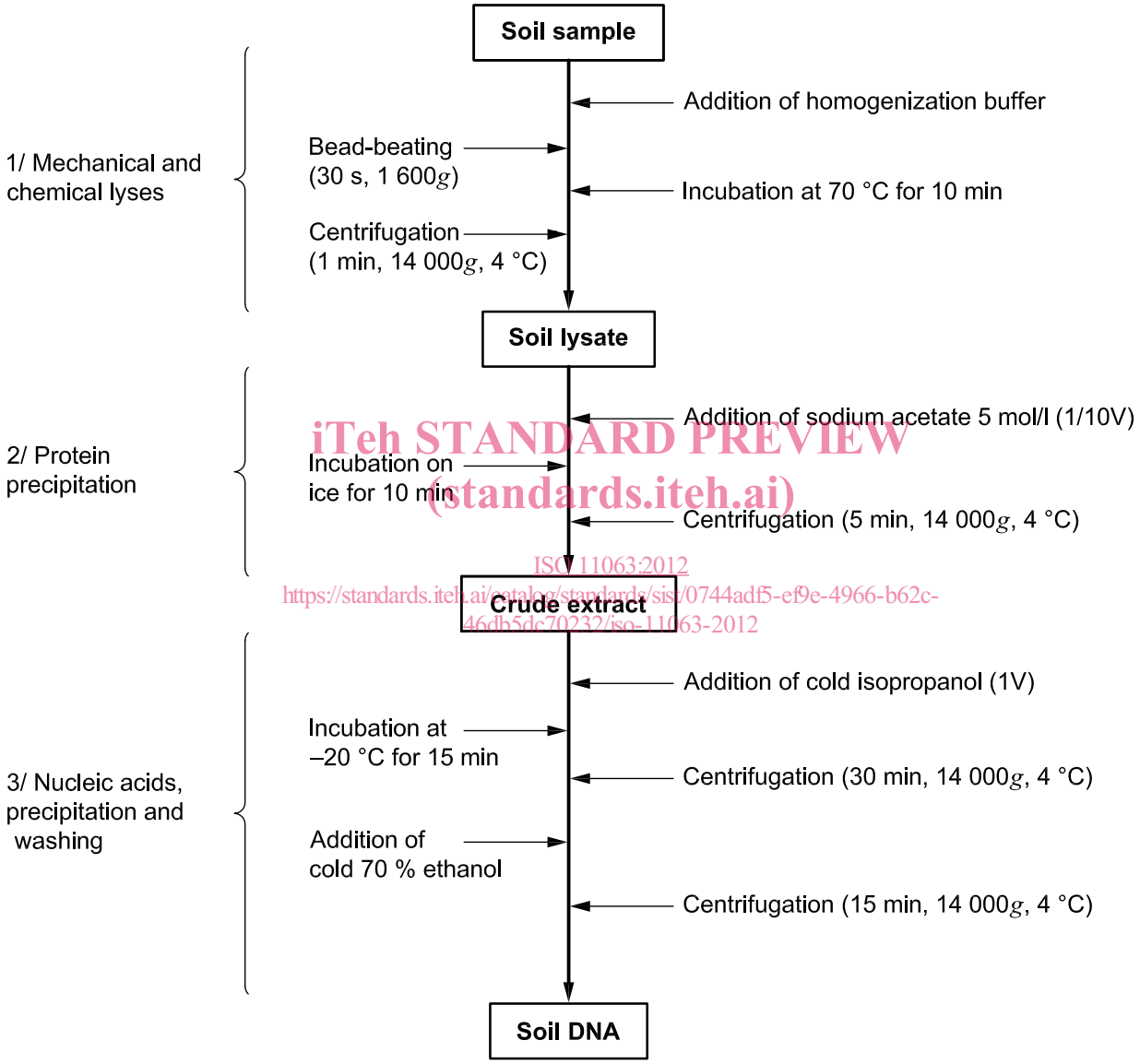

Figure 1 — Schematic overview of soil DNA extraction procedure

5.2 Chemicals

5.2.1 **Tris[hydroxymethyl]aminomethane**, $C_4H_{11}NO_3$ (CAS No. 77-86-1).

5.2.2 **Ethylenediaminetetraacetic acid disodium salt** (EDTA), $C_{10}H_{14}N_2O_8Na_2 \cdot 2\,H_2O$ (CAS No. 6381-92 6).

5.2.3 **Sodium chloride**, NaCl (CAS No. 7647-14-5).

5.2.4 Sodium dodecyl sulfate (SDS), $CH_3(CH_2)_{11}OSO_3Na$ (CAS No. 151-21-3).

5.2.5 Polyvinylpyrrolidone (PVP), $[C_6H_9NO]_n$ (CAS No. 9003-39-8).

5.2.6 Sodium acetate, CH_3COONa (CAS No. 6131-90-4).

5.2.7 Acetic acid or glacial acetic acid, CH_3COOH (CAS No. 64-19-7).

5.2.8. Isopropanol, $CH_3CHOHCH_3$ (CAS No. 67-63-0).

5.2.9 Ethanol, CH_3CH_2OH (CAS No. 64-17-5).

5.2.10 Molecular-biology-grade water, H_2O.

5.3 Buffers and reagents

Buffers and reagents (except intercalent molecules) used for soil DNA extraction are sterilized (120 °C for 20 min) and stored at room temperature. Ethanol and isopropanol are stored at –20 °C.

5.3.1 Tris-HCl, **1 mol/l**, 121,14 g of tris in 1 000 ml of H_2O, adjusting with 4 mol/l HCl to pH 8,0.

5.3.2 EDTA, **0,5 mol/l**, 186,10 g of EDTA in 1 000 ml of H_2O, adjusting with NaOH (10 mol/l) to pH 8,0.

5.3.3 NaCl, **1 mol/l**, 58,44 g of NaCl in 1 000 ml of H_2O.

5.3.4 PVP 40, **20 %**, 200 g of PVP in 1 000 ml of H_2O.

5.3.5 SDS, **20 %**, 200 g of SDS in 1 000 ml of H_2O.

5.3.6 Homogenization buffer (newly prepared just before being used), 100 ml of 1 mol/l tris-HCl (pH 8,0), 200 ml of 0,5 mol/l EDTA (pH 8,0), 100 ml of 1 mol/l NaCl, 50 ml of 20 % PVP 40, 100 ml of 20 % SDS in 450 ml of H_2O.

5.3.7 Sodium acetate, 5 mol/l (pH 5,5), 410,15 g of CH_3COONa in 800 ml of H_2O. Add 120 ml of acetic acid and then adjust the pH to 5,5 with glacial acetic acid. Add water to make up to 1 000 ml.

5.3.8 Ethanol, 70 %, 700 ml of pure ethanol in 300 ml of H_2O.

5.3.9 TE buffer, pH 8,0, 10 mmol/l tris-HCl, 1 mmol/l EDTA.

5.3.10 Glass beads (106 µm).

5.3.11 Glass beads (2 mm).

5.3.12 Ethidium bromide, 5 mg of ethidium bromide in 1 000 ml of H_2O.

5.3.13 Fluorescent nucleic acid stain, excitation at 480 nm and emission at 520 nm.

5.3.14 Pure DNA (100 ng/µl)

5.3.15 TBE buffer × 10, pH 8,0, 108 g of tris base, 55 g of boric acid, 40 ml of 0,5 mol/l EDTA (pH 8,0) in 1 000 ml of H_2O.

5.3.16 TBE buffer × 1, 100 ml of TBE buffer × 10 in 900 ml of H_2O.

6 Apparatus

Use standard laboratory equipment including pipettes, a centrifuge, fume hood cabinet, horizontal electrophoresis system and the following.

6.1 Mini-bead beating apparatus, with a beating frequency varying from, for example, 100 min^{-1} to 2 600 min^{-1} and a 16 mm amplitude of agitation.

6.2 Spectro-fluorimeter, allowing the quantification of double-strand DNA at 520 nm with a fluorescent nucleic acid stain excited at 480 nm.

7 Procedures

7.1 Preparation of soil samples

Weigh 0,25 g of soil (equivalent dry mass) in 2 ml micro-tubes just before extracting, or immediately freeze the soil sample in liquid nitrogen and keep it frozen at −80 °C until its use.

7.2 Mechanical and chemical lyses

Add 0,5 g of 106 µm glass beads (wear a mask for protection) and two glass beads (2 mm diameter) to the soil sample. Add 1 ml of homogenization buffer (composition given in 5.3.6). Agitate the soil samples 1 600*g* for 30 s (16 mm of amplitude) using a bead-beating system (tube support previously placed at −20 °C). Incubate at 70 °C for 10 min. Centrifuge for 1 min at 14 000*g* (4 °C). Carefully recover the supernatant and transfer it to a new 2 ml microtube.

7.3 Protein precipitation

To the supernatant obtained in 7.2, add 5 mol/l sodium acetate (pH 5,5) (composition given in 5.3.7) of an amount that is 1/10 of the volume of the supernatant. Mix by vortexing and incubate on ice for 10 min. Centrifuge for 5 min at 14 000*g* (4 °C). Carefully recover the supernatant and transfer it to a new 1,5 ml microtube.

7.4 Nucleic acid precipitation and washing

Perform all these steps below a fume hood because of dangerous isopropanol vapours. Liquid and solid wastes shall be evacuated as chemical waste.

To the supernatant obtained in 7.3, add cold isopropanol (-20 °C) of an amount that is 1/1 of the volume of the supernatant. Incubate the samples at −20 °C for 15 min. Centrifuge for 30 min at 14 000*g* (4 °C). Carefully eliminate the supernatant. Wash the nucleic acids pellet with cold 70 % ethanol (do not resuspend the pellet). Centrifuge for 15 min at 14 000*g* (4 °C). Eliminate any traces of ethanol and let the nucleic acid pellet dry for 15 min at 37 °C. Suspend the pellet in 100 µl of ultra-pure water or TE buffer (pH 8) (composition given in 5.3.9).

7.5 Nucleic acid storage

Aliquot the soil DNA (4 × 25 µl) and store the DNA samples at −20 °C until their use. Repeated freezing and thawing of the DNA extracts should be omitted.

8 Estimation of soil DNA quality and quantity

8.1 Soil DNA quality and purity

The quality and the size of the soil DNA are checked by electrophoresis on 1 % agarose gels in TBE buffer. Gels are stained with appropriate staining (e.g. ethidium bromide, 5 mg/l). The purity of the soil DNA is assessed by spectrophotometry at 260 nm for the DNA analysis and at 400 nm for humic acid substances [11].

The step of chemical and mechanical lysis is critical, and it should be adequate to lyse a representative portion of microbes but avoid fragmentation of the DNA [39].

DNA extracts which are still slightly brownish need a further DNA purification.

8.2 Soil DNA quantity

The soil DNA content is determined using a fluorescent nucleic acid stain (5.3.13) which fluoresces when intercalated within the double helix of DNA. A calibration curve relating the amount of standard DNA (5 ng, 10 ng, 20 ng, 50 ng, 100 ng, 150 ng and 200 ng of pure DNA) to the amount of fluorescence quantified is established and used to estimate the amount of DNA extracted from the soil. Measurements are performed using a spectro-fluorimeter (6.2). The analysis is carried out by relevant software.

Alternatively, the soil DNA content can be determined by resolving soil DNA extracts by electrophoresis in a 1 % agarose gel, stained with ethidium bromide and photographed under a camera. Dilutions of pure DNA were included in each gel and a standard curve of DNA concentration (1 000 ng, 500 ng, 250 ng, 125 ng, 62,5 ng to 31,25 ng). The ethidium bromide intensity was integrated to establish a standard curve used for estimating soil DNA concentration as described previously by Reference [32].

Alternatively, the soil DNA content can be determined by spectrophotometry at 260 nm when soil DNA is lowly contaminated with humic acid substances (400 nm) and proteins (A260/A280 averaging 1,6).

9 Validation of the extraction procedure

The laboratory can validate the procedure of soil DNA extraction by processing the reference soil and comparing the obtained yield of soil DNA extraction to the expected one.

10 International ring test

This method for extracting soil DNA was evaluated through an international ring test involving nine different laboratories working on six different European soils. The report of this ring test is provided in Annex A.

11 Test report

The test report shall include the following information:

a) a reference to this International Standard: ISO 11063:2012;

b) soil collection, including date and place (GPS coordinates) of collection;

c) treatment and storage of soil sample (e.g. sieving method, conditions and length of storage);

d) physical and chemical characteristics of the soil;

e) quantity of soil used for DNA extraction;

f) date(s) of extraction;

g) duration of nucleic acids storage (if appropriate);

h) tables of results including concentration of soil DNA extracts and amount of DNA extracted per gram of soil (dry weight equivalent);

i) any details not specified in this International Standard or which are optional, as well as any effect which may have affected the results.