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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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Soil quality - Direct extraction of soil DNA

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1.Structure and Drafting Rules of Standardization Documents"

drafted.

This document is equivalent to ISO 11063.2020 "Method for Direct DNA Extraction from Soil Quality Soil Samples".

The following minimal editorial changes have been made to this document.

---The mass calculation of potassium acetate in 5.3.7 of ISO 11063.2020 is incorrect, and the value is corrected from 176.5 to 294.4.

Please note that the content of this document may involve patents. The issuing agency of this document assumes no responsibility for identifying patents.

1 Scope

This document describes a method for the direct extraction of DNA from soil samples, and these extracted DNAs are used to

Analysis of soil bacterial community abundance and composition by biological techniques [including quantitative real-time PCR (qPCR)]. This method is mainly suitable for agricultural and

forest soil. This method may not be suitable for soils rich in organic matter (such as peat), soils heavily contaminated with organic pollutants or heavy metals.

Direct DNA extraction from soil samples provides a unique perspective for studying microbial community alpha and beta diversity. by soil DNA

Next-generation sequencing of PCR-amplified amplicons will facilitate the development of routine tools for microbial community monitoring in soil environments.

2 Normative references

The contents of the following documents constitute essential provisions of this document through normative references in the text. Among them, dated citations

documents, only the version corresponding to that date applies to this document; for undated references, the latest edition (including all amendments) applies to

this document.

ISO 18400-206 Soil quality sampling Part 206.Soils for laboratory determination of microbial processes, biomass and diversity

Guidelines for aerobic collection, handling and storage (Soilquality-Sampling-Part 206.Collection, handling and storage of

thelaboratory)

Note. GB/T 32725-2016 Guidelines for aerobic collection, treatment and storage of soils for laboratory determination of microbial processes, biomass and diversity (ISO 10381-6.

20091), IDT)

1) ISO 10381-6.2009 has been replaced by ISO 18400-206.2018.

3 Terms and Definitions

The following terms and definitions apply to this document.

ISO and IEC maintain terminology databases for standardization at.

3.1

soil DNA soilDNA

DNA extracted from living soil microorganisms and residual DNA of dead microorganisms.

4 Principles

Follow the extraction procedure below to directly extract DNA from 1 g soil samples (equivalent dry weight). Soil with extraction buffer and glass beads added

Soil samples were subjected to mechanical and chemical lysis. Lysis steps, such as bead shaking, are also very useful for DNA extraction from difficult-to-lyse microorganisms.

crucial step. Then, the samples were incubated at 70 °C for 30 min for chemical lysis. Centrifuge briefly to remove soil debris and collect supernatant

liquid. Potassium acetate was added to the supernatant fraction to precipitate proteins. After centrifugation, the supernatant was collected again, and cold isopropanol was added to

precipitate nucleic acids. centrifugal, so

The nucleic acid precipitate was washed with 70% ethanol and dissolved in molecular biology grade ultrapure water or TE buffer. Electroporation through agarose gel