Soil quality - Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil (ISO 17601:2016)

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NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 17601:2018 sisaldab Euroopa standardi EN ISO 17601:2018 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 17601:2018 consists of the English text of the European standard EN ISO 17601:2018.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 21.02.2018.	Date of Availability of the European standard is 21.02.2018.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

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EUROPEAN STANDARD

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English Version

Soil quality - Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil (ISO 17601:2016)

Qualité du sol - Estimation de l'abondance de séquences de gènes microbiens par amplification par réaction de polymérisation en chaîne (PCR) quantitative à partir d'ADN directement extrait du sol (ISO 17601:2016)

Bodenbeschaffenheit - Abschätzung der Häufigkeit ausgewählter mikrobieller Gensequenzen durch quantitative PCR aus DNA-Boden-Extrakten (ISO 17601:2016)

This European Standard was approved by CEN on 14 February 2018.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

The text of ISO 17601:2016 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 17601:2018 by Technical Committee CEN/TC 444 “Test methods for environmental characterization of solid matrices” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2018, and conflicting national standards shall be withdrawn at the latest by August 2018.

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Endorsement notice

The text of ISO 17601:2016 has been approved by CEN as EN ISO 17601:2018 without any modification.

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

DNA (DNAs) is a major component of any living organisms coding for enzymes responsible for their biological activities. The study of DNA sequences from DNA sources extracted from different environmental matrices, 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 quality indicators applicable to complex environment such as soil were biased by the poor culturability of many microorganisms under laboratory conditions and the lack of sensitivity of traditional microbiological methods. The recent development of a large set of molecular biology methods based 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.[2] [3] [4] [5] [6] DNA-based approaches are now well established in soil ecology and serve as genotypic markers for determining microbial diversity. The results of molecular analyzes of soil microbial communities and/or populations rely on two main parameters: a) the extraction of DNA representative of the indigenous bacterial community composition and 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.[7] [4] [8] [9]

Numerous studies have investigated new methods to improve extraction, purification, amplification, and quantification of DNA from soils.[10] Recently, ISO 11063 reporting "a method to extract nucleic acids directly from soil samples" derived from Reference [10] is opening a new window for developing standardized molecular approaches to estimate soil quality.[11]

The aim of this International Standard is to describe the procedure used to set up and perform quantitative PCR to quantify the abundance of soil microbial phyla, as well as functional groups from DNA directly extracted from soil samples. The quantification of soil microbial phyla, as well as functional groups by qPCR assays can contribute to the development of routine tools to monitor soil quality. The repeatability and the reproducibility of the procedure of the quantitative PCR were assessed in an international ring test study (see Annex B). The repeatability of this procedure was successfully evaluated for both 16S rRNA genes, as well as genes coding a functional marker of denitrifiers (the nitrite reductase gene *nirK*). The reproducibility of this procedure revealed a laboratory effect which can be overcome by interpreting the results of the quantification of the abundance of the microbial groups by comparison, either by using an external reference (DNA extracted from a control strain) in the assay or by calculating a percentage of variations between treatments to normalize the data.

Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil

1 Scope

This International Standard specifies the crucial steps of a quantitative real-time polymerase chain reaction (qPCR) method to measure the abundance of selected microbial gene sequences from soil DNA extract which provides an estimation of selected microbial groups.

It is noteworthy that the number of genes is not necessarily directly linked to the number of organisms that are measured. For example, the number of ribosomal operon is ranging from one copy to 20 copies in different bacterial phyla. Therefore, the number of 16S rRNA sequences quantified from soil DNA extracts does not give an exact estimate of the number of soil bacteria. Furthermore, the number of sequences is not necessarily linked to living microorganisms and can comprise sequences amplified from dead microorganisms.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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*

ISO 11063, *Soil quality — Method to directly extract DNA from soil samples*

3 Terms and definitions

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

3.1
soil DNA
DNA extracted from soil of living and dead biota

EXAMPLE Microorganisms, plants, animals.

3.2
polymerase chain reaction
PCR
method allowing the amplification of a specific DNA sequence using a specific pair of oligonucleotide primers

3.3
quantitative polymerase chain reaction
qPCR
method allowing the quantification in a DNA *template* (3.4) of the number of a specific DNA sequence using a specific pair of oligonucleotide primers

3.4
template
DNA sample used to perform *PCR* (3.2) to amplify a specific DNA sequence