International Standard

ISO 17601

Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative polymerase chain reaction (qPCR) from DNA directly extracted from soil

Qualité du sol — Estimation de l'abondance de séquences de gènes microbiens par amplification par réaction de polymérisation en chaîne quantitative (qPCR) à partir d'ADN directement extrait du sol

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Foreword

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 17601:2016), which has been technically revised.

The main changes are as follows:

— Annex C has been expanded by adding examples of well-established qPCR systems to quantify certain microbial groups or their function.

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Introduction

DNA (DNAs) is a major component of any living organism, 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 eukaryotes).

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 analyses of soil microbial communities and 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 the 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 reported “a method to extract nucleic acids directly from soil samples” derived from Reference [10], opening a new window for developing standardized molecular approaches to estimate soil quality.[11]

The aim of this document 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 and functional groups by qPCR assays can contribute to the development of routine tools to monitor soil quality. The repeatability and the reproducibility of the qPCR procedure were assessed in an international ring test study (see Annex B). The repeatability of this procedure was successfully evaluated for 16S rRNA genes and for 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. 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.

A list of currently well-established qPCR assays to assess selected functional traits of the soil microbiome is listed in Annex C.

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Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative polymerase chain reaction (qPCR) from DNA directly extracted from soil

1 Scope

This document specifies the crucial steps of a quantitative polymerase chain reaction (qPCR) method to measure the abundance of selected microbial gene sequences from soil DNA extract. The number of microbial gene sequences quantified by qPCR provides an estimation of the abundance of selected microbial groups in soil.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 18400-206, *Soil quality — Sampling — Part 206: 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 — Direct extraction of soil DNA*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
soil DNA
DNA extracted from living and dead biota in the soil

Note 1 to entry: The soil biota includes microorganisms, plants and 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 DNA sequences targeted by a specific pair of oligonucleotide primers

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

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3.5
amplicon
PCR product obtained by *polymerase chain reaction (PCR)* (3.2) from a *template* (3.4)

3.6
cloning vector
circular DNA molecule in which the *amplicon* (3.5) is inserted by ligation and which is used to transform competent *Escherichia coli*

3.7
qPCR standard
cloned DNA target used as *template* (3.4) for *quantitative polymerase reaction (qPCR)* (3.3) to establish the standard curve relating the abundance of target sequence as a function of *cycle threshold (Ct)* (3.9) values

3.8
non-template control
NTC
control, usually molecular grade water, that is used as negative control in *quantitative polymerase reaction (qPCR)* (3.3) assay to check for the absence of contaminant in the qPCR mix

3.9
cycle threshold
Ct
number of *quantitative polymerase chain reaction (qPCR)* (3.3) cycles required for the fluorescent signal to cross the threshold (i.e. to exceed background level)

Note 1 to entry: The Ct value is inversely proportional to the abundance of the target sequence.

4 Principle

This document describes a qPCR assay using fluorescent DNA binding dye as reporter. This qPCR assay has been validated by an international ring test conducted with the SYBR Green[1)], a commonly used fluorescent DNA binding dye which binds all double-stranded DNA and can be detected by measuring the increase in fluorescence throughout the cycle.

The method comprises four tasks and eight steps as summarized in Figure 1. According to Reference [1], the three critical steps to be validated for each qPCR assay are as shown in Figure 1.

1) SYBR® Green is a trademark of Thermo Fisher Scientific Inc. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

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