

SVENSK STANDARD

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Markundersökningar – Uppskattning av utvalda mikrobiella gensekvensers abundans (förekomst) genom kvantitativ PCR av DNA som extraherats direkt från jord (ISO 17601:2016)

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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EUROPEAN STANDARD

EN ISO 17601

NORME EUROPÉENNE

EUROPÄISCHE NORM

February 2018

ICS 13.080.30

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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Contents

Page

European foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Test materials	4
5.1 DNA.....	4
5.2 Bacteria.....	4
5.3 Plasmid.....	4
5.4 Enzyme.....	4
5.5 Chemicals.....	4
5.6 Product for bacterial culture medium.....	5
5.7 Buffer and reagents.....	5
6 Apparatus	6
7 Procedure	6
7.1 qPCR standard preparation and calibration of qPCR assay (task 1).....	6
7.1.1 General.....	6
7.1.2 Amplicon design (task 1, step 1).....	6
7.1.3 qPCR standard preparation (task 1, step 2).....	7
7.1.4 Isolate DNA, environmental DNA, artificial DNA.....	7
7.1.5 Calibration of the qPCR (task 1, step 3).....	9
7.2 Preparation of soil DNA template and inhibition test (task 2).....	10
7.2.1 General.....	10
7.2.2 Soil DNA preparation (task 2, step 4).....	10
7.2.3 Inhibition test (task 2, step 5).....	10
7.2.4 Dilution of DNA template.....	12
7.3 qPCR assay (task 3).....	12
7.3.1 General.....	12
7.3.2 qPCR.....	12
7.4 Validation and analysis of qPCR assay (task 4).....	12
7.4.1 General.....	12
7.4.2 Validation of the qPCR assay.....	12
7.4.3 Calculation of the copy number of the gene of interest in the soil DNA extract.....	13
8 Examination of the critical steps of the qPCR assay	14
9 Expression of the results of the qPCR assay	14
10 International ring test	14
11 Test report	14
Annex A (informative) Description of principal steps of TaqMan® qPCR assay	15
Annex B (informative) International ring-test for evaluating qPCR to quantify the abundance of selected microbial gene sequences from DNA directly extracted from soil	17
Bibliography	30

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.

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