SLOVENSKI STANDARD
oSIST prEN ISO 17601:2024

01-oktober-2024

Kakovost tal - Ocena številčnosti izbranih sekvenc mikrobnih genov s kvantitativnim PCR analizatorjem v talnih ekstraktih DNK (ISO/DIS 17601:2024)

Soil quality - Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil (ISO/DIS 17601:2024)

Bodenbeschaffenheit- Ermittlung der Häufigkeit ausgewählter mikrobieller Gensequenzen durch quantitative PCR aus DNA-Boden-Extrakten (ISO/DIS 17601:2024)

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/DIS 17601:2024)

Ta slovenski standard je istoveten z: **prEN ISO 17601**

ICS:

13.080.30	Biološke lastnosti tal	Biological properties of soils

oSIST prEN ISO 17601:2024 **en,fr,de**

DRAFT
International
Standard

ISO/DIS 17601

Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR 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 (PCR) quantitative à partir d'ADN directement extrait du sol

ICS: 13.080.30

ISO/TC **190**/SC **4**

Secretariat: **AFNOR**

Voting begins on:
2024-08-15

Voting terminates on:
2024-11-07

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 17601:2024(en)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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*.

This second edition cancels and replaces the first edition (ISO 17601:2016).

The main changes are as follow:

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

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.

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

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 108400, *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 — 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 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

3.5
amplicon
PCR product obtained by *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 used to transform competent Escherichia coli for cloning the amplicon

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

3.8
non-template control
NTC
control, usually molecular grade water, that is used as negative control in qPCR assay to check for the absence of contaminant in the qPCR mix

3.9
cycle threshold
Ct
number of qPCR cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level)

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

4 Principle

This International Standard describes 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, 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 aims to measure the abundance of selected microbial gene sequences from soil DNA extract. 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.

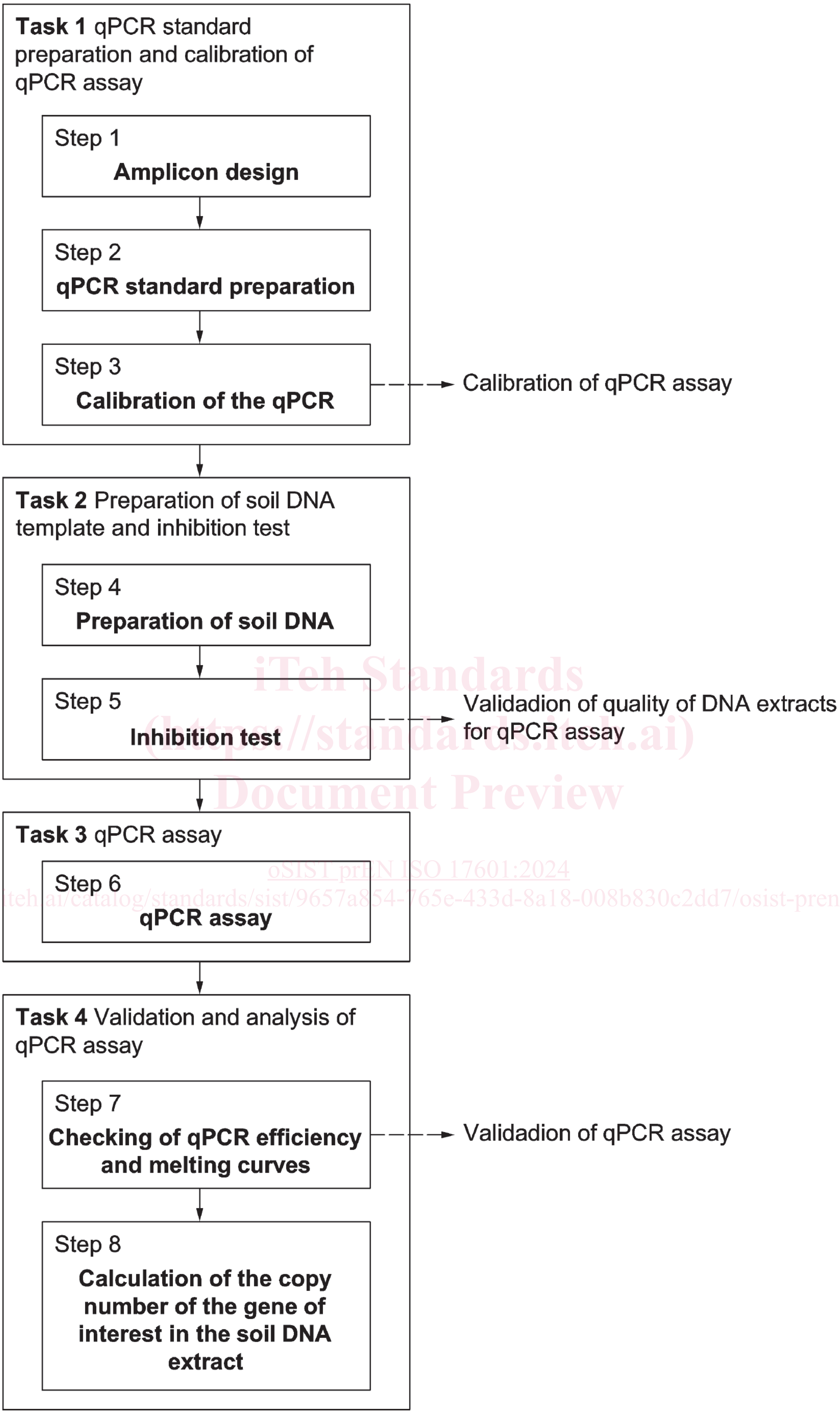

Figure 1 — Main tasks and critical steps to estimate the abundance of selected microbial gene sequences by qPCR assay

This International Standard describes qPCR assay based on the use of fluorescent DNA binding dye which has been validated by an international ring test using SYBR Green®[1] qPCR. In Annex A, information about TaqMan®[2] qPCR assay not tested in the international ring test are given. The first task is made of three steps describing the design of optimal amplicon for qPCR (step one), the preparation of qPCR standards (step two), and the procedure to calibrate the qPCR assay (step three). The second task includes two additional steps describing the procedures to prepare soil DNA samples (step four) and to test for the presence of qPCR inhibitors in soil DNA samples (step five). The third task is constituted of a single step describing the protocol to perform qPCR assay (step six). Finally, the fourth task is made of two steps, one describing the procedure to validate qPCR assays (step 7) to check the quality of qPCR assay and another one describing the different options to calculate the number of sequences of the gene of interest copy from cycle threshold (Ct) obtained from the analysis of qPCR amplification plots (step 8).

5 Test materials

5.1 DNA

5.1.1 DNA, extracted from pure bacterial and fungal isolates using classical extraction procedures or by using commercial kit to extract genomic DNA.

5.1.2 Soil DNA, extracted from aliquots of soil according to ISO 11063.

5.2 Bacteria

5.2.1 ***Escherichia coli* strain**, usually used for cloning of PCR product.

5.3 Plasmid

5.3.1 Cloning vector, usually used for cloning of PCR product in competent *Escherichia coli.*

5.4 Enzyme

5.4.1 Taq polymerase.

5.4.2 T4 DNA ligase.

5.4.3 T4 gene T32.

5.4.4 Bovine serum albumin (CAS No. 9048-46-8).

5.5 Chemicals

5.5.1 Ampicilline sodium, $C_{16}H_{18}N_3NaO_4S$ (CAS No. 69-52-3).

5.5.2 Boric acid, BH_3O_3 (CAS No. 10043-35-3).

5.5.3 Deoxynucleotide solution, dNTPs.

---

[1] SYBR Green is a registered trademark of Molecular Probes. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

[2] TaqMan is a trademark of Roche Molecular Systems, 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. Equivalent products may be used if they can be shown to lead to the same results.