FINAL DRAFT International Standard

ISO/FDIS 17601

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

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

Secretariat: **AFNOR**

Voting begins on:
2025-08-07

Voting terminates on:
2025-10-02

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/FDIS 17601:2025(en)

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

Page

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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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.