SLOVENSKI STANDARD
SIST-TP CEN/TR 17244:2019

01-julij-2019

Kakovost vode - Tehnično poročilo o upravljanju z barkodami kremenastih alg

Water quality - Technical report for the management of diatom barcodes

Wasserbeschaffenheit - Technischer Bericht zum Management von Diatomeen-Barcodes zur Bewertung des ökologischen Zustands

Qualité de l'eau - Rapport technique relatif à la gestion des codes barres Diatomées pour l'évaluation du statut écologique

Ta slovenski standard je istoveten z: CEN/TR 17244:2018

ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

SIST-TP CEN/TR 17244:2019 **en**

TECHNICAL REPORT

RAPPORT TECHNIQUE

TECHNISCHER BERICHT

CEN/TR 17244

September 2018

ICS 13.060.70

English Version

Water quality - Technical report for the management of diatom barcodes

Qualité de l'eau - Rapport technique relatif à la gestion des codes barres Diatomées pour l'évaluation du statut écologique

Wasserbeschaffenheit - Technischer Bericht zur Erstellung und Verwaltung von Diatomeen Barcodes

This Technical Report was approved by CEN on 14 May 2018. It has been drawn up by the Technical Committee CEN/TC 230.

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Ref. No. CEN/TR 17244:2018 E

Contents

Page

European foreword

This document (CEN/TR 17244:2018) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

Introduction

WARNING — Persons using this technical report should be familiar with normal laboratory practice. This European technical report does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

Diatoms are unicellular microalgae present in all types of water bodies. They are an important component of aquatic ecosystems and have been used widely for ecological assessment required by the Water Framework Directive (2000/60/EC) and Urban Waste Water Treatment Directive (91/217/EEC) in addition to other EU Directives and international agreements. The use of diatoms as indicators of water quality is based on observations that diatom taxa have distinct preferences for particular environmental conditions such as nutrients, organic pollution and acidity. Polluted waters will tend to support higher proportions of those taxa whose optima correspond with the levels of the pollutant in question. Conversely, certain species are intolerant of elevated levels of one or more pollutants, whilst others may occur in a wide range of water qualities.

Methods using diatoms to assess water quality and ecological status based on optical microscopy have been developed in several European countries. Methods for sampling and preparation are similar [4], [8] leading to the development of European Standards which, in turn, facilitated the harmonization of ecological assessment approaches [5], [6], [1]. More recently, however, molecular biology has presented new opportunities for assessment of ecological status using diatoms (e.g. [7], [10]. Such procedures, however, are not covered by existing standards.

A database of validated barcodes is an essential foundation of any assessment system that uses molecular data to determine the identities of organisms. This technical report covers the steps that should be taken if a barcode is to be correctly assigned to the appropriate taxon in a manner that can be readily checked and authenticated by other users. These instructions will enable method developers to ensure that they have provided all of the metadata necessary to validate the identity of a barcode and, if followed, will ensure that reliable identifications can be made from environmental samples.

According to the precise usage to which this technical report is to be put it is essential for specifiers and users to mutually agree on any necessary variations or optional procedural details prior to use.

All numerical values given in this technical report are approximate.

1 Scope

This technical report specifies the data and metadata necessary to validate the identity of a diatom barcode used for ecological assessment along with recommendations for storage of the barcode and metadata to ensure access to this information.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

3.1
barcode
stretch of a DNA sequence at least 100 base pairs in length that uniquely identifies a specific taxon

3.2
base pair
pair of complementary cross-linked nucleotides that is the building block of the DNA double helix

3.3
cultivator
conservator Person responsible of the cultivation of the diatom strain

3.4
diatoms
group of unicellular algae, some of which form chains or colonies, with cell walls made of silica. They are major contributors to primary productivity worldwide and are often used in ecological assessment

3.5
DNA marker
name of the coding or non-coding region (e.g. gene, spacer region) within the genome from which the barcode has been amplified. The naming of the coding or non-coding regions should follow standard scientific practice

3.6
ecological status
measure of the structure and functioning of aquatic communities

3.7
frustule
cell wall of diatoms, composed of silica and consisting of two valves linked by two or more girdle bands

3.8
habitat
specific environment in which an organism lives

3.9
isolate
population or populations of diatom cells in axenic or unialgal culture, derived from a single cell

3.10
isolator
person responsible of the isolation of the cell from which the clonal culture was established

3.11
pherogram
graphical account of the results from Sanger sequencing where each nucleotide is represented by a single peak and the sequence of peaks correlates to the DNA sequence

3.12
primer
strand of nucleic acid that serves as starting point for DNA replication

3.13
PCR
Polymerase Chain Reaction: process used for amplification of a given region of DNA

3.14
taxa
taxonomic units, for example families, genera or species

3.15
Taxonomic Backbone
index of published taxon names which is used by databases to automatically cross-reference name entries

3.16
valve
structural component of the diatom frustule (3.7)

3.17
voucher
physical record of a sample deposited in a collection

4 Procedure

4.1 DNA Harvesting

DNA is extracted from cultures that derive from a single algal cell but do not need to be axenic. The culture needs to have a strain identifier given by the conservator. It is also possible to obtain barcodes by other means (e.g. extraction of DNA from a single cell) but methods are still under development and the difficulties of providing adequate voucher material make these methods suboptimal for establishing barcode references. The procedure described here remains the most reliable approach. The DNA (or a portion of it) may be stored in a biological specimen collection, e.g. an institution affiliated to the DNA bank network.

4.2 Storage of barcode

The DNA barcode shall be stored digitally in publicly accessible databases. Examples include EMBL/Genbank, Algaterra, Rsyst.

4.3 Storage of voucher specimens

A physical voucher of the strain from which the DNA was taken in order to produce the barcode should be deposited in a recognized natural history collection. Duplicate of this voucher should be deposited in a different recognized collection. The physical voucher shall include a permanently labelled microscope slide of the cultivated strain, comprising frustules and/or valves obtained from the strain at the time of DNA extraction. The voucher may also include loose dried material and other preparations (e.g. scanning electron microscopy stubs) used to identify the taxon. It may also contain a slide of the original source community.

Pictures are considered part of the voucher and should be deposited along with the voucher specimen. The pictures should show details important for identification. Photographs should be taken from voucher material and labelled permanently via burnt-in annotation giving the strain name.

5 Associated metadata

5.1 Metadata – general remarks

If a sequence is to be used as a normative barcode for a particular taxon it shall be accompanied by a minimum set of metadata. This has to be stored in a digitally in publicly accessible database such as in EMBL/Genbank, Algaterra, R-Syst. The metadata have to be linked to the barcode via a unique identifier assigned by the database where the sequence is stored (e.g. accession numbers of EMBL/Genbank). If strain numbers are available these should be used to establish links with other databases.

Metadata should include information, as detailed below, on the DNA marker, strain cultivation details, taxon name, identification, sample location, voucher location and barcode authors.

5.2 Categies of metadata

5.2.1 DNA Marker

5.2.1.1 Obligatory data

— Sequencing technology

— Name of DNA marker

— Name of barcode

— Origin of DNA (whether DNA was extracted from a culture or a single cell)

— When more of the DNA marker than the barcode region has been sequenced (i.e., a longer sequence):

  — All primers used (Names and sequences (citations of first publication, if applicable). They should be given in 5'->3' direction.

— When only the barcode region has been sequenced:

  — Forward Primer (Name and sequence (citation of first publication, if applicable) of primer at the 5' end). It should be given in 5'->3' direction.

  — Reverse Primer (Name and sequence (citation of first publication, if applicable) of primer at the 3' end). It should be given in 5'->3' direction.