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Biotechnology — Nucleic acid synthesis —

Part 1: Requirements for the production and quality control of synthesized oligonucleotides

Biotechnologie – Synthèse des acides nucléiques —
Partie 1: Exigences relatives à la production et au contrôle qualité des oligonucléotides synthétisés

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Contents

Page

Foreword

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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).

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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 276, *Biotechnology*.

A list of all parts in the ISO 20688 series can be found on the ISO website.

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

Single stranded, linear bio-polymers made up of nucleotides which are called "synthetic oligonucleotides" or 'oligos' are indispensable components for biotechnology. For example, they are used as polymerase chain reaction (PCR) amplification primers, microarray, real time PCR or next generation sequencing (NGS) capture probes, and as input starting materials for the creation of entire target genes.

Control of quality in production is important in the synthesis of oligonucleotides. The quantification of the size range, concentration and contaminants is necessary to ensure that quality requirements are met for end-use applications. Considering that oligonucleotides are used in biologically active applications, their quality, particularly sequence and conformation, will affect fitness or function, for example molecular recognition of cognate binding site, chemical behaviour. The specific requirements for each end-use application can differ.

This document defines common quality attributes of synthetic oligonucleotides and addresses their quantification and assessment for end-use.

It is intended to help improve quality management and demonstrate product quality.

International, national or regional regulations or requirements can also apply to specific topics covered in this document. For example, when synthesized oligonucleotides are used as investigational drugs or pharmaceutical agents, regional regulations and/or good manufacturing practices (GMP) may need to be considered.

Biotechnology — Nucleic acid synthesis —

Part 1: Requirements for the production and quality control of synthesized oligonucleotides

1 Scope

This document specifies minimum requirements for the production and quality control of synthesized oligonucleotides (nominally up to 250 bases).

This document also describes general quality attributes for synthesized oligonucleotides as well as common methods for evaluating quality attributes.

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:

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

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

3.1
certified reference material
CRM
reference material (3.4) characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

[SOURCE: ISO Guide 30:2015, 2.1.2, modified — Notes have been deleted.]

3.2
performance
<synthetic oligonucleotides> ability of oligonucleotides, which are synthesized for the specific intended use including biological assays, to fulfil the requirements for the specific use

Note 1 to entry: In the case of oligonucleotides synthesized as primers for a PCR, it is the ability of such synthetic oligonucleotides to function as primers.

Note 2 to entry: In the case of oligonucleotides synthesized as probes for use in DNA microarrays, real time PCR or NGS, it is the ability of such synthetic oligonucleotides to hybridize as probes with target oligonucleotide sequence.

Note 3 to entry: Performance is confirmed by testing that evaluates full functioning of oligonucleotides in their respective uses.

3.3
purity
<synthetic oligonucleotides> ratio between the amount of expected-sequence and/or length synthetic oligonucleotides and the total amount of oligonucleotides

Note 1 to entry: Purity of synthetic oligonucleotides is the ratio of absorbance peak area corresponding to synthetic oligonucleotides of expected sequence and/or lengths in comparison with total peak area of oligonucleotides. The measurement of absorbance at 260 nm (OD 260) is calculated by high performance liquid chromatography (HPLC), or capillary electrophoresis (CE). The purity is calculated with area normalization method from the result of HPLC or CE or mass spectrometer.

3.4
reference material
RM
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

[SOURCE: ISO Guide 30:2015, 2.1.1, modified — Notes have been deleted.]

3.5
synthetic oligonucleotides
chemically synthesized DNA (deoxyribonucleic acid) or RNA (ribonucleic acid)

Note 1 to entry: Various compounds such as modified bases, base analogues, end-labelling reagents, fluorescent compounds, etc., can be introduced into the synthetic oligonucleotides.

Note 2 to entry: In this document, a synthetic oligonucleotide is considered single stranded, linear, and in length from approximately 10 nucleotide-bases to 250 nucleotide-bases.

4 Design and selection of suitable oligonucleotides that are fit for purpose

The quality and consistency requirements of synthetic oligonucleotides depend on their intended uses. For example, the quality requirements of primer and probe in a polymerase chain reaction (PCR), or in a microarray or NGS are significantly different.

In general, users are responsible for specifying quality requirements of the oligonucleotides for their specific application.

A quality grade list can be provided by the producer from which the user can select the grade that fits the intended use, for example “genomic grade” or “antisense grade” (see 5.2).

Robust quality management and testing, together with a common understanding of quality attributes can provide consistent information to allow:

— users to appropriately select oligonucleotides for the intended use;

— users and producers to better communicate and agree on specification for custom oligonucleotides.

5 General quality management requirements

5.1 General requirements

The producer of synthetic oligonucleotides (hereinafter referred to as “producer”) shall establish and implement a system in which the following processes are described and documented:

a) order receipt;

b) oligonucleotide production;

c) quality control.

A quality policy and quality objectives shall be determined for the order receipt process. The quality requirements for users are likely to vary depending on their intended use, the design of their experimental method, and the potential for results that affect repeatability and reproducibility. Based on the quality policy, quality objectives, and grades of synthetic oligonucleotides, when applicable, that have been clearly stated in the process of order forms, the producer should monitor whether the production is performed in accordance with appropriate processes by such means as process checklists (see Annex A for an example). In addition, necessary actions should be taken for the production processes in order to achieve the planned results by measurements and analyses of the qualities of synthetic oligonucleotides that are produced.

5.2 Oligonucleotide grading

To reduce excessive customization of the quality requirement, grading of oligonucleotide quality can be considered. When the producer documents the grading of oligonucleotide quality, the grades should be determined based on the intended use. The purification methods should be chosen and determined to fit each grade; see 7.3.

5.3 Control of documents

The producer shall have a procedure(s) ensuring the control of documented information including records required by this document and shall ensure that unintended use of any obsolete document is prevented. When the documented information including records is retained in electronic media, the producer shall ensure the control of those electronic media.

5.4 Quality management system

The producer shall adopt a quality management system. The quality management system shall establish necessary procedures and ensure the execution of producing control based on the established procedures. The quality management system shall regularly check whether the production of synthetic oligonucleotides based on this document is performed correctly.

NOTE For labelled oligonucleotides, additional quality indicators can be considered according to the characteristics of the label, for example strength and wavelength of fluorescence.

5.5 Personnel and training

The producer shall ensure that personnel are competent for performing the procedures specified in this document and shall properly supervise the personnel. The producer shall provide appropriate training and assessment of the competence to the personnel.

5.6 Safety control

The producer shall establish a safety program considering applicable requirements in order to ensure the safety of the personnel performing oligonucleotide synthesis and purification as specified by this document.

6 Resource management

The producer shall ensure the suitable condition of the facilities as well as area for the production of oligonucleotides. The producer shall maintain equipment and instruments to produce synthetic oligonucleotides. The producer shall properly control raw material (including reagents, pure water and ancillary materials) that can potentially affect the quality of synthetic oligonucleotides.

An example of equipment and instrumentation suitable for production of synthetic oligonucleotides is listed in Annex B.

7 Requirements for production process

7.1 General

The production of a single sequence synthetic oligonucleotide generally consists of the following processes:

a) synthesis;

b) purification;

c) quality control inspection;

d) drying, when applicable;

e) formulation.

These processes shall be performed with regularly maintained instruments, for example, pipettes or dryers.

NOTE 1 Several production processes are used to produce complex oligonucleotide pools. For example, several oligonucleotides are combined to produce complex oligonucleotide pools. Alternatively, a complex oligonucleotide pool is produced via array-based synthesis. Appropriate quality control measures can be selected and implemented based on the production method.

NOTE 2 General methods to determine quality attributes are not necessarily appropriate for array-based synthesis.

NOTE 3 In some cases, single sequence oligonucleotides are synthesized with wobble bases for specific applications requiring differential annealing. In this case, bases are transversed or transitioned at a specific site.

7.2 Oligonucleotide synthesis

A quality control inspection shall be performed by using the adequately calibrated and maintained measuring instruments.

Oligonucleotide synthesizers shall be regularly maintained and controlled. Operators shall be qualified according to documented procedures. The records related to the operations shall be retained.

7.3 Purification

Appropriate purification equipment and methods shall be selected based on risks to the oligonucleotide design specifications and intended use as agreed upon with the user.

Purification options include: reverse HPLC (C8 to C18), anion exchange HPLC (SAX, WAX), polyacrylamide gel electrophoresis (PAGE), oligonucleotide purification cartridge (OPC), high purity salt free (HPSF) purification and direct precipitation (desalting). The purification method can be selected according to the intended use of oligonucleotides. The record of used method shall be retained.

Purification operation shall be performed by qualified personnel. The records related to the operations shall be retained.

7.4 Quality control inspection

The quality control inspection shall be performed by using the adequately calibrated and maintained measuring instruments. Measurement operation shall be performed by qualified personnel in accordance with documented procedures. In addition, operation record shall be retained. The quality control process shall be conducted to establish identity, purity, impurity, quantity, and other important attributes of the oligonucleotide with respect to its intended use.

7.5 Drying

Drying can be performed with centrifugal evaporation, freeze drying or air drying equipment.

These operations shall be performed by qualified personnel in accordance with documented procedures, and the operation record shall be retained.

7.6 Formulation

These operations shall be performed by qualified personnel in accordance with documented procedures, and the operation record shall be retained.

8 Requirements for quality control process

8.1 General

The quality control process shall be conducted to establish identity, purity, impurity, quantify, and other important attributes of (an) oligonucleotide(s) with respect to its (their) intended use.

Appropriate method(s) shall be selected and validated for establishing the identity and quality of oligonucleotides.

The measurement shall be performed by qualified personnel.

8.2 Validation and verification of analytical methods

Analytical methods used to perform the quality control and to determine the quality attribute(s) shall be validated and verified. Instrumentation should be calibrated by utilizing certified reference materials.

NOTE An example of accuracy control of measurement instruments by using certified/standard reference materials is described in Annex E.

8.3 Identity and purity

8.3.1 Identity of the base sequence

The base sequence can be determined by appropriate methods, for example electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) as ion source, time of flight (ToF), ion trap or quadrupole mass spectrometer (MS) as mass analyser, or sequencing.

NOTE 1 An example for the confirmation of base sequences using MS is described in Annex D.

NOTE 2 MS can be used to determine the sequence for short oligonucleotides while there are limitations on accurate analysis for sequence determination depending on the length of synthetic oligonucleotides.

The determined identity of the base sequence shall be documented.

8.3.2 Purity

The purity of a given oligonucleotide for a specific intended use should be determined and agreed upon between the producer and user.

EXAMPLE The purity of a synthetic oligonucleotide can be determined as the ratio between the amount of intended oligonucleotide and the total amount of oligonucleotides. The intended oligonucleotide(s) is (are) the one(s) with the “expected-length” and/or the correct sequence.

Methods to determine the purity can include one or a combination of the following methods:

a) analytical reverse-phase or ion exchange high performance liquid chromatography;

b) capillary or slab gel electrophoresis with validation of relative linearity response;

c) MS (for example ESI or MALDI as ion source, ToF, ion trap or quadrupole MS as mass analyser);

d) sequencing.

NOTE Synthetic oligonucleotides are complex molecules and will produce different results based on the oligonucleotide design and production process.

The determined purity shall be documented.

8.3.3 Impurity

In some applications, it is important to determine the impurity.

The acceptable degree or range of impurity of a given oligonucleotide for a specific intended use should be determined and agreed upon between the producer and user. In general, impurity is the quantity of oligonucleotide that interferes with the intended use. The degree of impurity shall be determined using an appropriate method.

NOTE Impurities include but are not limited to organic solvent, mono-nucleotides, short nucleic acids and nucleic acids with incorrect sequences.

Methods to determine impurity can include one or a combination of the following methods:

a) analytical reverse-phase or ion exchange high performance liquid chromatography;

b) capillary or slab gel electrophoresis with validation of relative linearity response;

c) MS (ToF or ESI);

d) sequencing.

The determined impurity shall be documented.

8.4 Quantity

8.4.1 General

Sufficient quantity of synthetic oligonucleotides shall be provided for the intended use as agreed in advance.

The producer should confirm that the synthesized amount fulfils the user's requirements.

The agreed quantity of synthetic oligonucleotides shall be documented.

8.4.2 Concentration

The oligonucleotide concentration measurement can be used as a part of quality control during production or product testing (check). The optical density (OD) can be used to measure the concentration of oligonucleotides present in a solution. In general, the optical density at 260 nm is used.

The molar concentration is calculated based on the molar extinction coefficient and the OD. Formula (C.1) and the parameters to calculate the molar extinction coefficient are shown in Annex C.

As different assumptions are used in the calculation of molar extinction coefficient, the molar extinction coefficient data, including the used assumption, shall be documented (see Reference [7]).

