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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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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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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.

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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.

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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.

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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.

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