FINAL
DRAFT

INTERNATIONAL
STANDARD

**ISO/FDIS
20688-2**

ISO/TC **276**

Secretariat: **DIN**

Voting begins on:
2023-12-08

Voting terminates on:
2024-02-02

Biotechnology — Nucleic acid synthesis —

Part 2: Requirements for the production and quality control of synthesized gene fragments, genes, and genomes

Reference number
ISO/FDIS 20688-2:2023(E)

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Published in Switzerland

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Foreword

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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

Gene fragment, gene and genome synthesis refer to producing synthetic double-stranded DNA in the form of non-clonal fragments (that can be linear) and clonal genes in plasmids (that would be circular) by using appropriate biochemical methods.

Synthesized gene fragments, genes and genomes are important biotechnological products and are widely used in biotechnology, e.g. protein engineering, metabolic engineering, antibody and vaccine development, environmental bioremediation and natural product discovery.

The production and quality control of the synthesized gene fragment, gene and genome products are essential for ensuring the quality and their downstream applications in biotechnology. This document provides requirements for the production and quality control of synthetic gene fragment, gene and genome products, including biosecurity, purity, yield, size, gene cloning accuracy, integrity, sequences, residual impurities and other quality indicators. This document provides a uniform general guideline for the quality control of gene fragments, genes and genomes synthesis. It is intended to help to improve and ensure the quality of products and fair trade based on a unified standard.

This document is intended to be used by synthetic DNA producers during the manufacturing process for quality control to improve the quality of their products, by academic laboratories to evaluate the quality of DNA synthesized in their facilities, and by end users to verify the quality of synthesized gene fragments, genes and genomes provided by manufacturers as required.

In this document, the following verbal forms are used:

— “shall” indicates a requirement;

— “should” indicates a recommendation;

— “may” indicates a permission;

— “can” indicates a possibility or a capability.

Biotechnology — Nucleic acid synthesis —

Part 2: Requirements for the production and quality control of synthesized gene fragments, genes, and genomes

1 Scope

This document specifies the requirements for the production and quality control of synthesized double-stranded DNA. It describes requirements for quality management, resource management, biosafety and biosecurity, quality control in production, product quality, and delivered product specifications for synthesized gene fragments, genes and genomes.

This document is applicable to synthetic gene fragments, genes and genomes with a length below 10 Mbp (base pairs) in forms of non-clonal fragments (linear) and clonal genes in plasmids (circular).

This document does not provide specific requirements for materials used solely for diagnostic purposes.

When the synthesized nucleic acids are procured and used for diagnostic purposes, the user can take ISO 15189, ISO 13485 and other related clinical standards into account.

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 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
biosafety
practices and controls that reduce the risk of unintentional exposure or release of biological materials

Note 1 to entry: Biological materials refer to any material comprised of, containing, or that may contain biological agents and/or their harmful products, such as toxins and allergens (see ISO 35001:2019, 3.14).

Note 2 to entry: Biological agents refer to any microbiological entity, cellular or non-cellular, naturally occurring or engineered, capable of replication or of transferring genetic material that may be able to provoke infection, allergy, toxicity or other adverse effects in humans, animals, or plants (see ISO 35001:2019, 3.13).

[SOURCE: ISO 35001:2019, 3.22, modified — Notes to entry were added.]

3.2
biosecurity
practices and controls that reduce the risk of loss, theft, misuse, diversion of, or intentional unauthorized release of biological materials

[SOURCE: ISO 35001:2019, 3.23, modified — Notes to entry were deleted.]

3.3
colony polymerase chain reaction
colony PCR
PCR method used to screen for plasmids containing a desired insert directly from microbial colonies without plasmid extraction and purification steps

3.4
DNA assembly
joining oligonucleotides or smaller gene fragments via regions of complementarity to form a longer double-stranded DNA fragment step by step *in vitro* or *in vivo*

3.5
DNA sequencing
determining the order of nucleotide bases (adenine, guanine, cytosine and thymine) in a molecule of DNA

Note 1 to entry: Sequence is generally described from the 5′ end.

[SOURCE: ISO 17822:2020, 3.19]

3.6
gene cloning
process of introducing a particular gene or DNA sequence using genetic engineering techniques into a host cell and replicating it by asexual reproduction into many identical copies of the gene

3.7
massively parallel sequencing
MPS
sequencing technique based on the determination of incremental template-based polymerization of many independent DNA molecules simultaneously

Note 1 to entry: Massively parallel sequencing technology can provide millions/billions of short reads per run or long reads based on amplification.

[SOURCE: ISO 20397-2:2021, 3.30, modified — Note to entry was edited by adding "or long reads based on amplification.]

3.8
plasmid vector
extrachromosomal DNA molecule in cells physically separated from the chromosome and capable of autonomous replication that can be used as vehicle to carry new genes into cells

[SOURCE: ISO 16577:2022, 3.4.37, modified — “vector” added to the term, and “that can be used as vehicle to carry new genes into cells” added to the definition (from ISO 16577:2022, 3.4.58). Notes to entry deleted.]

3.9
quality score
Q score
measure of the sequencing quality of a given nucleotide base

Note 1 to entry: *Q* is defined by the following formula:

$$Q = -10\log_{10}(p)$$

where p is the estimated probability of the base call being wrong.

Note 2 to entry: A quality score of 20 represents an error rate of 1 in 100, with a corresponding call accuracy of 99 %.

Note 3 to entry: Higher quality scores indicate a smaller probability of error. Lower quality scores can result in a significant portion of the reads being unusable. Low quality scores can also indicate false-positive variant calls, resulting in inaccurate conclusions.

[SOURCE: ISO 20397-2:2021, 3.32]

3.10
sequence alignment
arrangement of nucleic acid sequences according to regions of similarity

Note 1 to entry: Sequence alignment may not require a reference genome/reference targeted nucleic acid region and its aim might not produce an assembly.

[SOURCE: ISO 20397-2:2021, 3.20]

3.11
sequence of concern
SOC
sequences of 50 bp or greater that either encode for biological functions or directly endow or enhance toxicity or pathogenicity

3.12
synthetic DNA library
double-stranded DNA fragments synthesized with targeted genetic diversity that have been inserted into a specific cloning vector(s)

Note 1 to entry: Genetic diversity refers to the number of unique sequences in a DNA library. Diverse libraries can permit high-throughput evaluation of genetic designs or functional variants.

3.13
synthetic gene
synthetic, cloned, double-stranded DNA fragment containing necessary biological parts

Note 1 to entry: Linear plasmid by restriction enzyme digestion is a kind of delivered product form of synthetic gene.

3.14
synthetic gene fragment
synthetic, non-cloned, double-stranded linear DNA fragments, assembled from synthetic oligo nucleotides

3.15
synthetic genome
synthetically-built genome containing all necessary genetic information for a living organism, produced by the assembly of oligonucleotides or smaller gene fragments *in vitro* or *in vivo*

4 Requirements for quality management

4.1 General requirements

The producer, as an entity synthesizing double-stranded DNA and distributing double-stranded DNA to one or more customer(s), shall establish and implement a system in which the following processes are described and documented:

a) order receiving process;

b) biosafety and biosecurity risk assessment process;

c) gene synthesis process;

d) final product quality control process.

A quality policy and quality objectives shall be determined in the order receiving process. The quality requirements are different depending on the synthetic production process, the form of the final synthetic double-stranded DNA, quality control method and its end application. Necessary actions should be taken in the synthetic processes in order to achieve the planned results and quality by analysis of the characteristics of synthetic nucleic acids that are produced.

4.2 Control of documents

The producer of synthetic double-stranded DNA should have a procedure ensuring the control of documented information including following points:

a) customer information:
   1) point-of-contact name;
   2) organization;
   3) address;
   4) phone number;
   5) email;

b) order sequence information:
   1) nucleotide sequences ordered;
   2) vector used;

c) sequence screening protocols;

d) sequence screening report;

e) standard operation procedure of synthesis;

f) quality control method;

g) product form;

h) data and report;

i) shipment information:
   1) date placed and shipped;
   2) shipping address;
   3) receiver name;
   4) transport storage conditions, etc.

The producer shall ensure that unintended use of any obsolete document is prevented. The producer shall ensure the integrity and security of synthetic gene order and customer information and prevent unauthorized access to these data.

When the documented information including records is retained in electronic media, the producer shall ensure the control of those electronic media. Adequate cybersecurity measures shall be implemented to protect the intellectual property and identity of customers.

4.3 Quality management system

The producer can adopt and establish a quality management system to document necessary procedures, ensure control of production processes, and regularly monitor and document the production and quality control of synthetic double-stranded DNA.

4.4 Biorisk management and safety control

The producer can establish a biorisk management system (e.g. based on ISO 35001, the World Health Organization's (WHO's) *Laboratory Biosafety Manual*[10] and the WHO's *Global guidance framework for the responsible use of the life sciences*[11]) to effectively identify, assess, control, and evaluate the biosafety and biosecurity risks inherent in its activities.

The producer can establish an occupational health and safety management system (e.g. ISO 45001) in order to reduce or eliminate possible risks associated with performing double-stranded DNA synthesis and quality control as specified by this document.

The sequence of the synthetic double-stranded DNA should be screened against a list of pathogens and toxins. The biosafety and biosecurity risk level of the synthetic gene should be assessed according to the appropriate reference standard and documents of biosafety and biosecurity. An example of ranking risk levels can be referred to in Annex G.

The producer should have a procedure to ensure the legitimacy of customers, principal users and end users of synthetic genes containing sequences of concern (SOCs). Providers and third-party vendors of synthetic genes should:

a) know to whom they are distributing a product;

b) know if the product that they are synthesizing and/or distributing contains, in part or in whole, SOCs;.

c) notify customers and end-users when their order contains SOCs.

5 Requirements for resource management

5.1 Facilities and environmental condition

Facilities, including sources of energy, lighting and environmental conditions (temperature, humidity, cleanness and atmospheric pressure) shall be functional and reliable for double-stranded DNA synthesis and quality control. Facilities and environmental conditions shall not adversely affect the synthesis and quality control of synthetic double-stranded DNA. Influences that can adversely affect the product quality can include, but are not limited to, other nucleic acid contamination, microbial contamination, dust, electromagnetic disturbances, radiation, humidity, inconsistent electrical supply, temperature and vibration.

Synthetic gene fragments, genes and genomes shall not be contaminated by other nucleic acids from the manufacturing environment and shall not be released into the exterior environment without proper treatment.

The producer shall monitor, control and record environmental conditions in accordance with relevant specifications, methods or procedures.

5.2 Equipment and instruments

Equipment and instruments used in the production and quality control of synthetic gene fragments, genes and genomes shall be properly controlled, maintained and calibrated.

The records of the control, maintenance and calibration shall be retained according to documented record retention policies.