



**International
Standard**

ISO 20688-2

**Biotechnology — Nucleic acid
synthesis —**

Part 2:

**Requirements for the production
and quality control of synthesized
gene fragments, genes, and
genomes**

**First edition
2024-03**

[ISO 20688-2:2024](https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024)

<https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024>

iTeh Standards
(<https://standards.itih.ai>)
Document Preview

[ISO 20688-2:2024](https://standards.itih.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024)

<https://standards.itih.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Requirements for quality management	3
4.1 General requirements.....	3
4.2 Control of documents.....	4
4.3 Quality management system.....	5
4.4 Biorisk management and safety control.....	5
5 Requirements for resource management	5
5.1 Facilities and environmental condition.....	5
5.2 Equipment and instruments.....	5
5.3 Raw materials.....	6
5.4 Personnel.....	6
6 Biosafety and biosecurity requirements	6
6.1 General.....	6
6.2 DNA sequence screening mechanism.....	6
7 Requirements for quality control in production	7
7.1 General.....	7
7.2 Quality control in synthetic gene fragments production.....	7
7.2.1 General.....	7
7.2.2 Sequence design.....	7
7.2.3 Assembly.....	8
7.2.4 Purification.....	8
7.2.5 Product preservation.....	8
7.3 Quality control in synthetic gene production.....	8
7.3.1 General.....	8
7.3.2 Colony screening.....	8
7.3.3 DNA preparation from the host cell.....	8
7.3.4 Sequence verification.....	9
7.3.5 Product preservation.....	9
7.4 Quality control in synthetic genome production.....	9
7.4.1 General.....	9
7.4.2 Assembly.....	9
7.4.3 Sequence verification.....	9
8 Requirements for product quality	10
8.1 Synthetic gene fragments.....	10
8.1.1 General.....	10
8.1.2 Yield.....	10
8.1.3 Purity.....	10
8.1.4 Size.....	10
8.1.5 Gene cloning accuracy.....	10
8.2 Synthetic genes.....	10
8.2.1 General.....	10
8.2.2 Yield.....	10
8.2.3 Purity.....	11
8.2.4 Sequence.....	11
8.2.5 Integrity.....	11
8.2.6 Residual impurities analysis.....	11
8.2.7 Supercoiled plasmid.....	12
8.2.8 Bioburden.....	12

ISO 20688-2:2024(en)

8.2.9	Specific quality indicators for synthetic DNA libraries	12
8.3	Synthetic genome	12
8.3.1	General	12
8.3.2	Sequence	12
9	Delivered/synthesized material specifications	13
9.1	Main information	13
9.2	Other information	13
Annex A (informative) Example equipment and device list and their control requirements		14
Annex B (informative) Exemplary quality measurement methods		16
Annex C (informative) Electrophoretogram		18
Annex D (informative) Sanger sequencing		22
Annex E (informative) Massively parallel sequencing		23
Annex F (informative) Additional quality control options for synthetic gene fragments		26
Annex G (informative) Example ranking of the risk posed by DNA products		28
Bibliography		29

iTeh Standards (<https://standards.iteh.ai>) Document Preview

[ISO 20688-2:2024](https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024)

<https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024>

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 document 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 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.

[ISO 20688-2:2024](https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024)

<https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024>

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 fragment, gene and genome 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.

[ISO 20688-2:2024](https://standards.iteh.ai/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024)

<https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024>

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 the 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 [ISO 20688-2:2024](https://standards.iteh.ai/catalog/standards/iso/aa35bc42-cf4f-4bc9-9c03-707c0c639e23/iso-20688-2-2024)

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 have been 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

sequence 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, the 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 the 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 protocol;

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 the 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, SOC;
- c) notify customers and end-users when their order contains SOC.

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.

The equipment and instruments shall be operated by suitably trained and qualified personnel.