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**Biotechnology — Massively parallel  
sequencing —**

**Part 1:  
Nucleic acid and library preparation**

*Biotechnologie — Séquençage parallèle massif —*

*Partie 1: Acides nucléiques et préparation des collections*



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## 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 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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

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](http://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 20397 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](http://www.iso.org/members.html).

## Introduction

Massively parallel sequencing (MPS) is a high throughput analytical technology for nucleic acid sequencing. MPS methods can process thousands to billions of nucleotide sequence reads simultaneously in a single run, allowing whole genomes, transcriptomes and specific nucleic acid targets from different organisms to be analysed in a relatively short time.

MPS is used in many life science disciplines permitting determination and high throughput analysis of millions of nucleotide bases. The biological variability of deoxyribonucleic and ribonucleic acid polymers from living organisms provides challenges in accurately determining their sequences. The quality of sequence determination by MPS depends on many factors including, but not limited to, sample quality, library preparation, and sequencing data quality.

The quality of nucleic acids and libraries prepared for MPS is critical to obtaining high quality sequence data. Controlling the upstream processing steps of MPS and evaluating nucleic acid samples and libraries for their suitability for sequencing significantly improves MPS results, downstream analyses and ultimately conclusions dependent upon the MPS data.

# Biotechnology — Massively parallel sequencing —

## Part 1: Nucleic acid and library preparation

### 1 Scope

This document specifies the general requirements for and gives guidance on quality assessments of nucleic acid samples. It specifies general guidelines for library preparations and library quality assessments prior to sequencing and data generation.

### 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 20395:2019, *Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — qPCR and dPCR*

### 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 20395:2019 and the following 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 <https://www.electropedia.org/>

#### 3.1

##### **adapter**

oligonucleotides of known sequence that are enzymatically added (e.g. ligase or polymerase chain reaction) to the end(s) of a DNA/cDNA fragment

#### 3.2

##### **barcode index**

short sequence of typically six or more nucleotides that serve as a way to identify or label individual samples when they are sequenced in parallel on a single sequencing lane, chip or both

Note 1 to entry: Barcodes are typically located within the sequencing *adapters* (3.1).

#### 3.3

##### **barcoding indexing**

unique DNA sequence identification

method that enables multiple samples to be pooled for sequencing

Note 1 to entry: Each sample is identified by a unique *barcode* (3.2), which enables identification of results during the parallel analysis.