
**Biotechnology — Massively parallel
sequencing —**

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

Biotechnologie — Séquençage parallèle massif —

Partie 1: Acides nucléiques et préparation des collections



COPYRIGHT PROTECTED DOCUMENT

© ISO 2022

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 Nucleic acid sample quality evaluation	2
4.1 General	2
4.2 Sample quantification	3
4.3 Sample purity	3
4.4 Sample integrity	3
4.4.1 General	3
4.4.2 Agarose gel electrophoresis	3
4.4.3 Capillary gel electrophoresis	3
4.4.4 Microfluidic analysis system	3
4.4.5 PCR method	3
5 Nucleic acid library preparation	4
5.1 General	4
5.2 Fragmentation	4
5.2.1 General	4
5.2.2 Mechanical fragmentation	4
5.2.3 Enzymatic fragmentation	4
5.2.4 Chemical fragmentation	5
5.2.5 Fragmented nucleic acid sample quantity	5
5.2.6 Fragmented nucleic acid sample purity	5
5.2.7 Fragmented nucleic acid size distribution	5
5.2.8 Fragmented nucleic acid purification using gel electrophoresis	5
5.3 Addition of universal sequences	5
5.3.1 Repair	5
5.3.2 Ligation of adapter	5
5.3.3 Barcoding/indexing	6
5.4 Size selection	6
5.5 Amplification	6
5.6 Purification and clean up procedures	6
5.7 Library quantification	7
5.7.1 Library quantification method	7
5.7.2 Selection of quantification method	7
5.8 Library qualification	7
5.8.1 General	7
5.8.2 Methods	7
6 Validation	7
7 Reference materials or controls	8
7.1 General	8
7.2 Control samples	8
7.3 Positive control	8
7.4 Negative control	9
7.5 No-template control	9
7.6 Spike-in control	9
7.7 Reference materials	9
8 Contaminations	9
8.1 General	9
8.2 Primary sample evaluation	9

8.3	Protocol and operation procedure.....	10
Annex A	(informative) Checklist for sample quality assessment before library construction	11
Annex B	(informative) Examples of quality criteria for selected MPS platforms and applications.....	12
Annex C	(informative) Reference material list.....	14
Bibliography	15

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).

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).

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 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.