

Collibri™ ES DNA Library Prep Kit

USER GUIDE

For sequencing SARS-CoV-2 with PCR enrichment

for use with:

Illumina® next-generation sequencing (NGS) platforms

With enzymatic fragmentation

With library amplification (optional)

Catalog Numbers A38607196, A38605024, A38606024, A43605024, A43606024, A43607024, A38607096, A38607096W, A38605024W

Publication Number MAN0025323

Revision B.0



Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0025323

Revision	Date	Description
B.0	22 February 2022	SKUs were updated in the Kit configuration table.
A.0	10 August 2021	New Colibri library prep workflow for sequencing SARS-CoV-2

EARLY ACCESS DISCLAIMER:

Customer acknowledges that this product is not a commercially released product and is still under development. THIS PRODUCT AND ASSOCIATED MATERIALS ARE SUPPLIED "AS IS" WITHOUT ANY WARRANTY, EXPRESS OR IMPLIED, AND ALL WARRANTIES ARE EXPRESSLY DISCLAIMED, INCLUDING WITHOUT LIMITATION THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF DEALING OR USAGE OF TRADE. Nothing herein shall be deemed to be or imply a duty for Life Technologies and/or its affiliate(s) to further develop this product or to release a commercial version thereof.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

TRADEMARKS: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Illumina and NextSeq are registered trademarks of Illumina, Inc. iSeq, MiniSeq, NovaSeq, HiSeq, and MiSeq are trademarks of Illumina, Inc. Bioanalyzer and Agilent are trademarks of Agilent Technologies, Inc. Eppendorf LoBind is a trademark of Eppendorf AG.

©2022 Thermo Fisher Scientific Inc. All rights reserved.

Contents

■ CHAPTER 1	Product information	5
	Product description	5
	Contents and storage	6
	Kit configurations	6
	Color scheme of Collibri™ ES DNA Library Prep Kit	6
	Required materials not supplied	7
	Product specification	9
	Procedure overview	10
	Protocol information	11
■ CHAPTER 2	Methods	12
	Important procedural guidelines	12
	Input DNA requirements	12
	Guidelines for cDNA quality	12
	Guidelines for DNA fragmentation	12
	Guidelines for adaptor ligation	13
	Guidelines for library cleanup	13
	Guidelines for evaluation of successful library construction	14
	Before you begin	15
	Reverse transcription of SARS-CoV-2 RNA	16
	Required materials	16
	Before you begin	16
	Prepare SARS-CoV-2 cDNA	16
	Amplicon enrichment of SARS-CoV-2 cDNA	19
	Overview	19
	Required materials	19
	Amplicon enrichment of SARS-CoV-2 cDNA	19
	Amplicon cleanup and pooling	21
	Overview	21
	Required materials	21
	Before you begin	21

Purify the amplified SARS-CoV-2 genome	22
Normalize amplified SARS-CoV-2 genome	23
Prepare 96 indexed libraries	24
Fragmentation and dA-tailing of input DNA	24
Dual indexed adaptor ligation	26
Post-ligation cleanup	28
Library PCR amplification (<i>optional</i>)	31
Purify amplified DNA libraries	32
Verify quality and quantity of prepared DNA libraries	34
Sequence on an Illumina® platform	36
■ APPENDIX A Troubleshooting	38
■ APPENDIX B Adaptor index sequence and plate layouts	39
Adaptor index sequences	39
Index sequences used for CD adaptors	39
Index sequences used for UD adaptors	40
Adaptor plate layouts	46
Combinatorial Indexed Adaptor Sets	46
Unique Dual Indexed Adaptor Sets	47
■ APPENDIX C Safety	49
Chemical safety	50
Biological hazard safety	51
■ APPENDIX D Documentation and support	52
Customer and technical support	52
Limited product warranty	52



Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

Invitrogen™ Collibri™ ES DNA Library Prep Kits for Illumina® are designed for the construction of high-efficiency DNA fragment libraries for whole-genome sequencing on Invitrogen™ next-generation sequencing (NGS) platforms. The kits support library preparation from a wide range of DNA samples and inputs (1 ng–500 ng) starting from intact DNA.

In the Collibri™ ES DNA Library Prep Kit workflow, the enzymatic DNA fragmentation step is combined with dA-tailing in a one-vial protocol. Subsequent adaptor ligation reaction is carried out in the same vial followed by a size-selection step. Reduced number of sample transfer steps used in the workflow minimizes handling errors, and saves time and valuable sample. The entire library prep takes less than 3 hours with PCR, and does not require expensive equipment to shear DNA mechanically. For convenience, the kits provide color-coded components for visual tracking of library preparation progress. Inert dyes in the reagents do not interfere with enzymatic reactions and do not compromise library prep or sequencing results.

The Collibri™ ES DNA Library Prep Kits contain all the necessary reagents required to prepare up to 96 uniquely indexed DNA libraries, including enzyme mixes, dual-barcoded plate-format adaptors and cleanup beads.

The following protocol defines a step-by-step library preparation process for whole-genome sequencing of SARS-CoV-2 with the Collibri™ ES DNA Library Prep Kits for Illumina®. Follow the recommendations in this user guide when using Collibri™ kits and other products listed in the Protocol information section along the library preparation process of SARS-CoV-2 samples.

Contents and storage

Kit configurations

The Collibri™ ES DNA Library Prep Kits for Illumina® are available in two sizes, providing sufficient reagents to prepare DNA fragment libraries for 24 or 96 samples.

Kit configuration	Kit size	DNA index type ^[1]	Cat. No.
Collibri™ ES DNA Library Prep Kit	24 preps	CD	A38605024
	96 preps		A38607096
	24 preps	UD Set A (1–24)	A38606024
		UD Set B (25–48)	A43605024
		UD Set C (49–72)	A43606024
		UD Set D (73–96)	A43607024
	96 preps	UD Set A (1–96)	A38607196
	24 preps	Without	A38605024W
	96 preps		A38607096W

^[1] CD = combinatorial dual indexes, UD = unique dual indexes

Color scheme of Collibri™ ES DNA Library Prep Kit

Component	Cap/Reagent color	24 preps	96 preps
Collibri™ ES DNA Library Prep Kit (Store at –20°C)			
5X Fragmentation and dA-tailing Enzyme Mix	White ○	250 µL	1 mL
10X Fragmentation and dA-tailing Buffer	Blue ●	125 µL	500 µL
7X Ligation Master Mix for ES	Red ●	250 µL	1 mL
2X Library Amplification Master Mix for ES	Blue ●	1.25 mL	2 × 1.25 mL
Primer Mix	Yellow ●	500 µL	2 × 500 µL
Collibri™ DNA Library Cleanup Kit (Store at 2°C to 8°C.)			
IMPORTANT! Do not freeze.			
DNA Cleanup Beads	Orange ●	10 mL	30 mL

(continued)

Component	Cap/Reagent color	24 preps	96 preps
Wash Buffer (Concentrated)	Blue ●	4.5 mL	18 mL
Elution Buffer	White ○	5 mL	20 mL
Colibri™ DNA CD^[1] or UD^[2] Indexes^[3] (Store at –20°C)			
Dual Index Adaptors (7 µM)	—	10 µL/well (24 wells)	10 µL/well (96 wells)

^[1] Combinatorial Dual-Indexed Adaptors (CD) are available with Catalog Nos. [A38605024](#), [A38607096](#)

^[2] Unique Dual-Indexed Adaptors (UD) are available with Catalog Nos. [A38606024](#), [A43605024](#), [A43606024](#), [A43607024](#), and [A38607196](#).

^[3] For the Adaptor index sequences and plate layouts, see page 39.

Required materials not supplied

For the Safety Data Sheet (SDS) of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through thermofisher.com.

MLS: Fisher Scientific™ (fisherscientific.com) or other major laboratory supplier.

Item	Source
Thermal cycler with heated lid, such as:	
VeritiPro™ 96-well Thermal Cycler	A48141
ProFlex™ 96-well PCR System	4484075
ProFlex™ 3 × 32-well PCR System	4484073
QuantStudio™ PCR System	thermofisher.com
StepOnePlus™ Real-Time PCR System	thermofisher.com
Applied Biosystems™ 7500 Fast Real-Time PCR System	thermofisher.com
Additional instruments:	
Agilent™ 2100 Bioanalyzer™ instrument ^[1]	Agilent, G2938A
Agilent™ High Sensitivity DNA Kit ^[1]	Agilent, 5067-4626
Savant SpeedVac™ system	thermofisher.com
Magnetic rack, such as:	
Invitrogen™ DynaMag™ -2 Magnet (for 1.5-mL tubes)	12321D
Invitrogen™ DynaMag™ -96 Side Magnet (for PCR strips or 96-well 0.2-mL plates)	12331D

(continued)

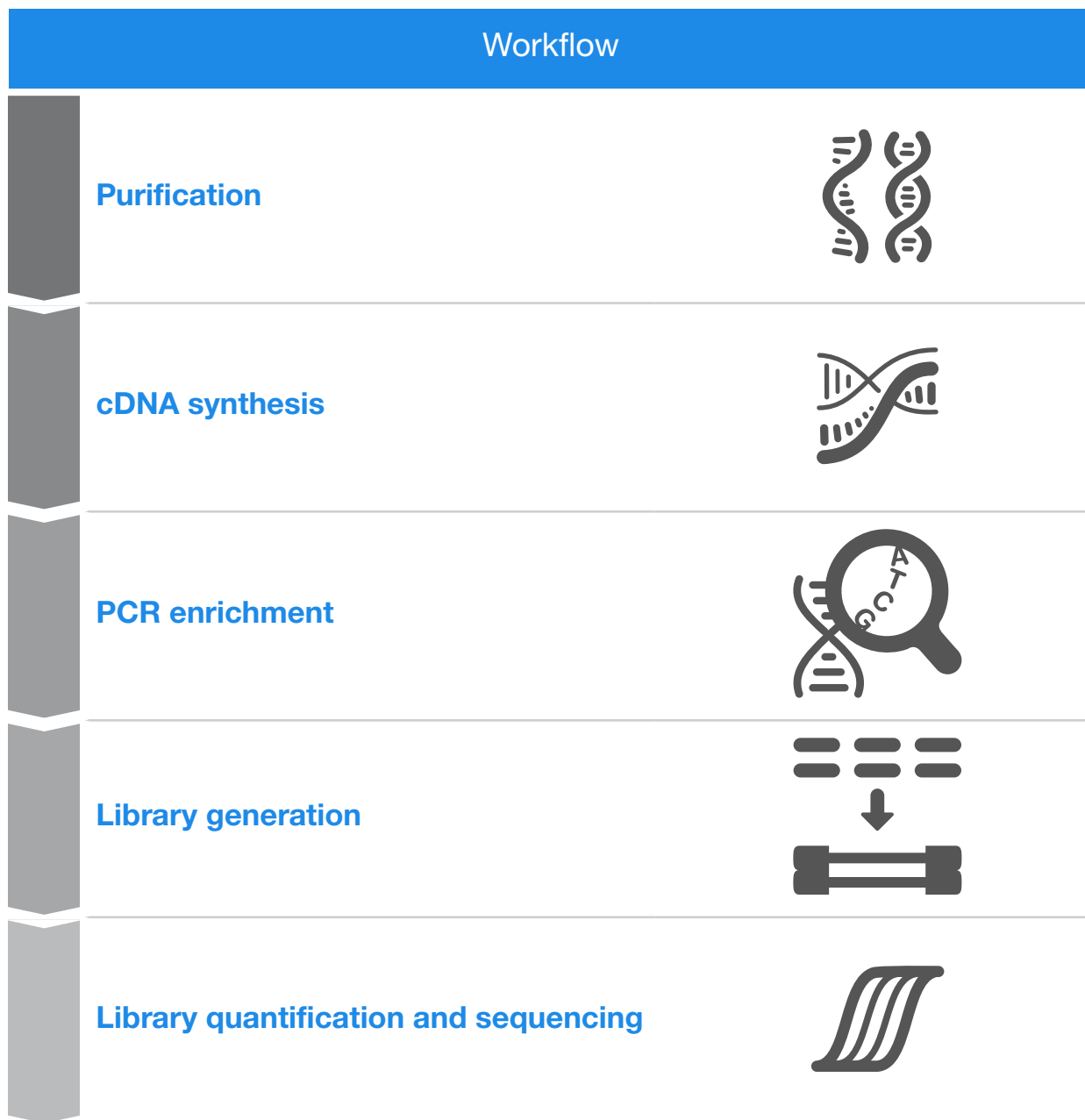
Item	Source
Consumables and instruments	
Benchtop microcentrifuge	MLS
Vortex mixer	MLS
Heating block and/or thermomixer	MLS
96-well 0.2-mL PCR plates	MLS
Nuclease-free 1.5-mL tubes, such as Eppendorf™ DNA LoBind™ Tubes	Eppendorf™, 022431021
0.2-mL or 0.5-mL thin-wall PCR tubes or plates	MLS
Cooling rack for 0.2-mL PCR tubes/plates	MLS
Calibrated single-channel or multi-channel pipettes, (1 µL–1,000 µL)	MLS
Nuclease-free pipette tips	MLS
Disposable gloves	MLS
10 mM Tris-HCl buffer, pH 7.5–8.5	MLS
Ethanol 96–100%, molecular biology grade	MLS

[1] You can also use comparable method to evaluate the quality of prepared library.

Product specification

Total time	~8–9 hours (without sequencing time)
Hands-on time	5 hrs: 1. RT prep & incubation: 1.5 hr (0.5 hr hands-on) 2. PCR: 3 hrs (0.5 hr hands-on) 3. Measuring and pooling (Pool 1 and 2): 1 hr 4. Colibri™ ES: 1.5 hrs (0.5 hr hands-on) 5. Measure and diluting for sequencing: 1 hr
Sample type	SARS-CoV-2 pool of amplicons
Sample input amount	50 ng
Fragment size range	• <300 bp of insert • <370 bp of indexed amplicon
Multiplexing	• For the highest throughput use A38607096 (CD) and A38607196 (UD) together (192 preps) • Mid-throughput (120 preps) can be achieved using these combinations: – A38605024 (CD) and A38607196 (UD) – Any UD set of 24 preps with A38607096 (CD) • Do not pool together different sizes of kits containing the same type of indexed-adaptors (CDs or UD only). For more details of 24-prep and 96-prep indexed-adaptors sequences and plate layouts, see page 39.
System compatibility	iSeq™ Hi-Seq™ 1000, Hi-Seq™ 1500, Hi-Seq™ 2000, Hi-Seq™ 2500, Hi-Seq™ 3000, Hi-Seq™ 4000, MiSeq™, MiniSeq™, NextSeq™ 500, NextSeq™ 550, NovaSeq™ 6000, NextSeq™ 1000, NextSeq™ 2000
Sequencing application	Whole-genome sequencing of SARS-CoV-2

Procedure overview



Protocol information

Step	Kit	Cat. No.
Purify total RNA	MagMAX™ Viral/Pathogen Binding Solution	A48310 , A42352
Reverse transcribe RNA into cDNA	SuperScript™ IV Reverse Transcriptase	18090050
	RNaseOUT™ Recombinant Ribonuclease Inhibitor	10777019
	dNTP Mix (10 mM ea)	18427013
	Random Hexamer Primer	SO142
PCR Enrichment	ARTIC v3 primer pools	See website for the latest releases.
	Platinum™ SuperFi™ U Multiplex PCR Master Mix	A5140024 or A5140096
Prepare NGS libraries	Collibri™ ES DNA Library Prep Kits for Illumina® Systems	Combinatorial Dual indexes (CD): A38605024 (24 prep) or A38607096 (96 prep)
		Unique Dual Indexes (UD): A38606024 , A43605024 , A43606024 , and A43607024 (24 prep) or A38607196 (96 prep)
Quantify libraries	Collibri™ Library Quantification Kit (Recommended)	A38524100 , A38524500
	Qubit™ dsDNA HS (High Sensitivity) Assay Kit	Q32851 , Q32854

Note: We strongly recommend choosing Collibri™ kits with library amplification. However, a PCR-free option is also available: [A38545024](#), [A38603096](#), [A38602024](#), [A43602024](#), [A43603024](#), [A43604024](#), [A38608196](#).

After purchasing a PCR-free option, you can perform additional PCR cycles where necessary by additionally purchasing Collibri™ Library Amplification Master Mix (Cat. No. [A38539250](#), [A38539050](#)) or Collibri™ Library Amplification Master Mix with Primer Mix ([A38540050](#), [A38540250](#)).

Important procedural guidelines

Input DNA requirements

The recommended DNA input for SARS-CoV-2 library preparation is 50 ng of PCR-enriched viral cDNA.

Guidelines for cDNA quality

- The success of SARS-CoV-2 library preparation and reliable cDNA sequencing results strongly depend on the quality and quantity of input of viral RNA used. Proper sample handling and appropriate RNA isolation method are essential for successful sequencing.
- Residual traces of contaminating proteins, organic solvents, and salts can degrade the RNA and cDNA or decrease the activity of enzymes that are necessary for efficient DNA library preparation. Ensure that your input RNA is free of such contaminants.
- Single-stranded cDNA, RNA, or free nucleotides can interfere with accurate quantification of purified DNA, especially when UV spectrometry-based methods are used for measurement. For best results, we recommend using fluorometric-based methods for input DNA quantification, such as the Invitrogen™ Qubit™ dsDNA HS Assay Kit with the Qubit™ 4 Fluorometer (or a similar instrument).
- For high-quality RNA purification from various human biological samples use specialized commercial kits.

Guidelines for DNA fragmentation

The fragment size distribution after enzymatic fragmentation depends on the fragmentation reaction time. For additional considerations, see Appendix A, “Troubleshooting”.

Guidelines for adaptor ligation

- Indexed adaptors are used to uniquely label sequencing libraries that are generated from individual biological samples. This allows pooling of indexed libraries before cluster generation and enables multiplexed sequencing, which simplifies sample preparation and reduces sequencing costs.
- Pooling applications on Illumina® sequencing platforms require the use of specific index combinations. For optimal results, we recommend following Illumina® multiplexing guidelines.
- Depending on the Collibri™ ES DNA Library Prep Kit, the Collibri™ Dual-Indexed Adaptor plate contains a set of 24 or 96 adaptors, each carrying two 8-nucleotide indexes (barcodes). For the names and sequences of the indexes and the adaptor plate layouts for 24- and 96-prep kits, see [page 39](#).
- Collibri™ Dual-Indexed Adaptors are supplied in fully skirted PCR plates, which are sealed with non-pierceable, non-porous, Easy-Peal™ seals to minimize cross-contamination during handling. Adaptors are provided at a concentration of 7 µM, and each well of the plate contains 10 µL of adaptor required for one library prep (plus a generous excess volume required for automated preps).
- Collibri™ Dual-Indexed Adaptors are duplexed oligonucleotides. Do not expose the adaptors to temperatures above room temperature to prevent denaturation.
- Use appropriate laboratory practices to avoid cross-contamination of indexed adaptors. Wipe the seal surface with 70% ethanol before each use, and use new, sterile pipette tips for every well of the adaptor plate.
- To ensure equal read distribution when multiplexing libraries, carefully quantify individual libraries and normalize before pooling. We recommend using the Collibri™ Library Quantification Kit (Cat. No. [A38524500](#)) as the preferred qPCR-based method to accurately and reproducibly quantify sequenceable molecules; the only method providing information sequenceable library quantity.

Guidelines for library cleanup

- Post-ligation library cleanup is required to remove unligated adaptors and/or adaptor-dimer molecules from the library before the library amplification or cluster generation steps.
- Post-amplification of pre-capture and post-capture library cleanups are required to remove primers and primer dimers from the library before the downstream reactions.
- The Collibri™ DNA Library Cleanup Kit (included in the Collibri™ ES DNA Library Prep Kit) eliminates unused adaptors and adaptor dimers very efficiently.
- Equilibrate the DNA Cleanup Beads to room temperature before use and carry out all library cleanup steps at room temperature. This is essential for achieving the specified library size distribution and yields.
- DNA Cleanup Beads tend to gradually settle at the bottom of the tube. Before each use, thoroughly resuspend the cleanup beads by pipetting up and down several times or by vortexing. When properly resuspended, the bead solution has a uniform color with no visible clumping on the walls or at the bottom of the tube.
- To ensure optimal cDNA recovery, it is critical that you mix the cDNA and the cleanup beads thoroughly by vortexing or extensive pipetting.

- The beads are superparamagnetic and are collected by placing the reaction plate or tube in a magnetic stand. The time required for complete separation varies depending on the strength of your magnet, tube thickness, viscosity of the solution, and the proximity of the tube to the magnet. Optimize the bead capture times accordingly.
- To ensure the best cDNA yields, do not lose any magnetic beads during the cleanup procedure. Always verify that you do not discard or transfer any beads when removing or transferring the supernatant.
- Supplement the Wash Buffer with the appropriate volume of 96% ethanol, as noted on the bottle.
- You can adjust the volume of Wash Buffer used to accommodate various reaction vessels, but it is important that cleanup beads are entirely submerged during the wash steps.
- Remove all traces of ethanol before proceeding with subsequent reactions. However, over-drying makes the beads difficult to resuspend, which can result in considerable cDNA loss.
- The volume of Elution Buffer used to elute the library DNA depends on the downstream workflow and is indicated in the protocol.
- You can store the purified DNA in Elution Buffer at 2°C to 8°C for 1–2 weeks, or at –20°C for long-term storage.

Guidelines for evaluation of successful library construction

- Verify the size distribution of the prepared DNA library by an electrophoretic method, such as performing an analysis with the Agilent™ High Sensitivity DNA Kit on the Agilent™ 2100 Bioanalyzer™ Instrument (or similar).
- To achieve the highest quality sequencing data, it is essential to create optimal cluster densities across the flow cell. Optimization of cluster densities requires accurate quantification of DNA libraries, and the best quantification methods are based on qPCR.
- We recommend the Colibri™ Library Quantification Kit for qPCR-based quantification of prepared libraries before sequencing. qPCR is the only method that can provide accurate estimates of library concentration ready for sequencing (inserts containing both adaptors). This is in contrast to fluorimetry, which does not discriminate between adaptor having and lacking fragments.

Before you begin

- Read the entire protocol before you begin. Take into account the safe stopping points where you can store the samples frozen at -20°C , and plan your workflow accordingly.
- Use good laboratory practices to minimize cross-contamination of nucleic acid products. Use filtered pipette tips and, if possible, perform library construction in a separate area or room.
- Ensure that the Collibri™ ES DNA Library Prep Kit components have been fully thawed on ice and thoroughly mixed before use.
- Keep all enzyme components on ice as long as possible during handling.
- Reaction mixtures prepared from the enzyme mixes (5X Fragmentation and dA-tailing Enzyme Mix, 7X Ligation Master Mix for ES, and 2X Library Amplification Master Mix) are very viscous and require special attention during pipetting. Pipet viscous solutions slowly, and ensure complete mixing of the reaction mixture by vortexing or pipetting up and down several times as indicated in the protocol.
- You can safely pause the library construction process after the completion of post-ligation cleanup, and the post-amplification cleanup steps. These safe stopping points are marked accordingly in the protocol.
- Purified, adaptor-ligated library DNA can be stored at 2°C to 8°C for 1–2 weeks or at -20°C for one month. When possible, minimize the number of freeze-thaw cycles.
- Enriched amplicons, their pools, and indexed SARS-CoV-2 libraries can be stored at -20°C . When possible, minimize the number of freeze-thaw cycles.

Reverse transcription of SARS-CoV-2 RNA

Required materials

Component for the reverse transcription reaction:

- Nuclease-free water
- dNTP Mix (10 mM ea) (Cat. No. [18427013](#))
- Random Hexamer Primer (100 µM) (Cat. No. [SO142](#))
- 5X SuperScript™ IV Reverse Transcriptase buffer
- SuperScript™ IV Reverse Transcriptase (200 U/µL) (Cat. No. [18090050](#))
- RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. [10777019](#))
- DTT 0.1 mM

Other materials and equipment:

- 1.5 mL Eppendorf™ DNA LoBind™ Tubes
- Vortex mixer
- Cold rack for 96-well plate
- Microcentrifuge
- 96-well plate
- PCR-compatible 96-well plate seals
- Plate centrifuge

Before you begin

Thaw and keep the reagents on ice before use.

Prepare SARS-CoV-2 cDNA

1. Prepare the master mix for 96 samples of total RNA to anneal with random hexamers:

Component	Volume
dNTP Mix 10 mM	50 µL
Random Hexamers 100 µM	50 µL
Total volume:	100 µL

Mix the resulting solution by vortexing and pulse spin to collect the liquid at the bottom of the tube.

2. Aliquot 1 µL of the master mix to each well of the 96-well plate.
3. Keep the plate on a cold rack, and add 5.5 µL of purified total RNA of SARS-CoV-2 sample to each well of the 96-well plate.

Note: It is recommended to add SARS-CoV-2 research sample RNA in BSL-2 laboratory to minimize the possibility of exposure to intact virus that may be remaining after total RNA

purification. The subsequent sample should be treated as biohazardous until after RNase treatment step.

4. Transfer the 96-well plate containing total RNA and random hexamers to a thermal cycler. Set up and run the following program with the lid temperature set at 105°C:

Step	Temperature	Time
Pre-heat the block	65°C	As required
Annealing	65°C	5 minutes

IMPORTANT! After 5 minutes of annealing, immediately transfer the plate to a cold rack to provide a rapid temperature drop.

5. Prepare a reverse transcription component master mix:

Component	Volume
5x Superscript IV RT buffer	200 µL
Superscript IV Reverse Transcriptase (200 U/µL)	50 µL
DTT (100 mM)	50 µL
RNaseOUT™ Recombinant Ribonuclease Inhibitor	50 µL
Total volume:	350 µL

6. Add 3.5 µL of reverse transcription component master mix to each well with the annealed template RNA from step 4. Mix well when pipetting and centrifuge the plate to ensure all liquid resides at the bottom of the wells.
7. Set up and run the following program on a thermal cycler to incubate the reverse transcription reaction:

Step	Temperature	Time
Reverse transcription	42°C	50 minutes
Inactivation	70°C	10 minutes
Hold	4°C	Hold

8. Prepare the RNase H and RNase A/T1 solutions:

Component	Volume
RNase H	50 µL
RNase A/T1	50 µL
Total volume:	100 µL

9. Add 1 µL of RNase mix to each sample in the 96-well plate and mix thoroughly while pipetting.

10. Set up and run the following program in a thermal cycler to incubate the 96-well plate containing the reverse transcription reaction mix:

Step	Temperature	Time
RNase digestion	37°C	10 minutes
Inactivation	80°C	10 minutes
Hold	4°C	Hold

STOPPING POINT (Optional) Store the cDNA at -20°C.

Amplicon enrichment of SARS-CoV-2 cDNA

Overview

Two 96-well plates are prepared for PCR such that each contains a master mix with the corresponding primer pool and cDNA from each biological sample.

Required materials

Components for the multiplex PCR reaction:

- 4X Platinum™ SuperFi™ U Multiplex PCR Master Mix (Cat. Nos. [A5140024](#), [A5140096](#))
- ARTIC v3 amplification primer pools

Other materials and equipment:

- 1.5 mL Eppendorf™ DNA LoBind™ Tubes
- Vortex mixer
- Cold rack for 96-well plate
- Microcentrifuge
- 96-well plate
- PCR-compatible 96-well plate seals
- Plate centrifuge
- Nuclease-free water

Amplicon enrichment of SARS-CoV-2 cDNA

1. Make the primer pool #1 and primer pool #2 master mixes with the following reagents:

Component	Volume
Nuclease-free water	1494.5 µL
ARTIC v3 primer pool 1 or 2	98 µL
4X Platinum™ SuperFi™ U Multiplex PCR Master Mix	612.5 µL
Total volume	2205 µL

Vortex until mixed and briefly centrifuge to collect all the droplets at the bottom of the tube.

2. Aliquot 22.5 µL of the resulting master mixes for pool 1 and pool 2 in two 96-well plates, labelled Pool #1 and Pool #2.
3. Add 2.5 µL of cDNA of each biological sample from step 2 to both Pool #1 and Pool #2 96-well plates. Mix by pipetting.
4. Seal the plates and centrifuge briefly to collect all liquid at the bottom of the wells.

Note: After aliquoting the PCR master mix, keep the plates on a cold rack to prevent amplification reaction from commencing.

5. Set up and run the following program in a thermal cycler to incubate the Pool #1 and Pool #2 96-well plates containing the amplicon enrichment PCR reaction mix:

Stage	Number of cycles	Temperature	Time
Initial denaturation	1 cycle	98°C	30 seconds
Denaturation	30 cycles	98°C	15 seconds
Annealing and extension		63°C	5 minutes
Hold	1 cycle	4°C	Hold

IMPORTANT! We strongly recommend performing a gradient PCR to determine the optimal annealing temperature for your thermal cycler. Subtle differences in thermal cycler calibration can result in specific amplicons dropping out. For example, reducing the annealing temperature from 65°C to 63°C for identical cDNA input can recover the amplicon.

STOPPING POINT (*Optional*) Store the amplified pools at -20°C.

Amplicon cleanup and pooling

Overview

Amplified SARS-CoV-2 genome fragments need to be cleaned up before proceeding to prepare an indexed sequencing library.

Required materials

Components from the Collibri™ DNA Library Cleanup Kit:

- DNA Cleanup Beads
- Wash Buffer (supplemented with 96% ethanol)
- Elution Buffer

Other materials and equipment:

- 1.5 mL Eppendorf™ DNA LoBind™ Tubes
- Vortex mixer
- Magnetic rack
- Microcentrifuge

Before you begin

- Equilibrate the DNA Cleanup Beads, Wash Buffer, and Elution Buffer to room temperature before use.
- Thoroughly resuspend the DNA Cleanup Beads by pipetting up and down several times or by vortexing before use.
- Ensure that appropriate volume of 96% ethanol (as noted on the bottle) was added to the Wash Buffer before first use.

Purify the amplified SARS-CoV-2 genome

1. Add 50 μ L of DNA Cleanup Beads to each amplicon sample and mix by pipetting until you obtain a homogenous suspension.

Note: Reagent reservoir can be used to aspirate the DNA cleanup bead solution with a multichannel pipette.

2. Incubate for 5 minutes at room temperature.
3. Place the tubes in the magnetic rack for at least 2 minutes or until the beads have formed a tight pellet.
4. Keep the tubes in the magnetic rack, and carefully remove and discard the supernatant using a pipette.
Ensure that all of the supernatant is removed.

Note: If the pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the side of the tube on the magnet again.

5. Keep the tube on the magnet, and add 200 μ L of Wash Buffer (pre-mixed with ethanol), then incubate for 30 seconds at room temperature.

IMPORTANT! Do not resuspend the magnetic beads in Wash Buffer.

6. Carefully remove and discard the supernatant using a pipette.
7. Repeat step 5 and step 6.
8. To remove the residual ethanol, briefly centrifuge the plate, place it back in the magnetic rack, then carefully remove any remaining supernatant with a pipette without disturbing the pellet.
9. Keep the tube on the magnet, and air dry the magnetic particles for 1 minute at room temperature or until there are no droplets of ethanol left on the walls of the tube.

IMPORTANT! Do not over-dry by prolonged incubation for more than 5 minutes. Over-drying significantly decreases the DNA yield.

10. Remove the tube from the magnetic rack, then add 23 μ L of Elution Buffer directly to the pellet to disperse the beads. Mix the suspension thoroughly by pipetting up and down.

Note: Reagent reservoir can be used to aspirate the Elution Buffer solution with a multichannel pipette.

11. Briefly centrifuge the tube, then place it back in the magnetic rack for at least 2 minutes or until the beads have formed a tight pellet.
Wait for the solution to clear before proceeding to the next step.

12. Keep the tube on the magnet, and collect and transfer 20 μL of the supernatant containing the eluted DNA to a new 96-well plate without disturbing the pellet.

Note: If the pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the side of the tube on the magnet again.

IMPORTANT! The supernatant contains the eluted DNA. Do not discard.

STOPPING POINT (Optional) Store the DNA at -20°C .

Normalize amplified SARS-CoV-2 genome

1. Determine the amplicon-enriched, purified sample concentration using a Qubit[™] or NanoDrop[™] instrument.
2. Combine 25 ng of each Pool #1 and Pool #2 in 10 mM Tris-HCl pH 7.6–8.5 in a total volume of 35 μL .

STOPPING POINT (Optional) Store the combined pools at -20°C .

Prepare 96 indexed libraries

Fragmentation and dA-tailing of input DNA

Overview

This section describes the fragmentation and dA-tailing of the input DNA to prepare it for ligation with Illumina®-compatible NGS adaptors. The Collibri™ ES DNA Library Prep Kits combine the enzymatic fragmentation of input DNA and the addition of 3' dA-overhangs in a single one-vial reaction.

Required materials

Components from the Collibri™ ES DNA Library Prep Kits:

- 5X Fragmentation and dA-tailing Enzyme Mix
- 10X Fragmentation and dA-tailing Buffer

Other materials and equipment:

- 10 mM Tris-HCl Buffer, pH 7.5–8.5
- 1.5 mL Eppendorf™ DNA LoBind™ Tubes
- 0.2-mL sterile, thin-wall PCR tubes
- Vortex mixer
- Microcentrifuge
- Thermal cycler with a heated lid set to 80–85°C
- Ice or cooling block set to 4°C

Before you begin

1. Thaw the reaction components on ice.
2. Before use, mix the 5X Fragmentation and dA-tailing Enzyme Mix by inverting the tube several times, then briefly centrifuge to collect all the droplets at the bottom of the tube. Keep on ice.
3. Mix the 10X Fragmentation and dA-tailing Buffer by vortexing to avoid any localized concentrations, then briefly centrifuge to collect all the droplets at the bottom of the tube. Keep on ice.
4. Dilute the intact genomic DNA in 10 mM Tris-HCl, pH 7.5–8.5, if needed.
5. Set incubation program on the thermal cycler before mixing reaction components so that incubation block and heating lid will have required temperature when reaction mixtures are ready.

Fragment the DNA and add dA-tails

1. On ice or a cooling rack, assemble the fragmentation and dA-tailing reaction for each DNA sample in a sterile 0.2-mL thin-wall PCR tube. Add the reagents in the order given.

IMPORTANT! Do not scale up reaction volumes or prepare master mixes.

Component	Volume
Combined amplicon pools (50 ng)	35 μ L
10X Fragmentation and dA-tailing Buffer (blue ●)	5 μ L
Total volume (light blue mixture ●):	40 μL

2. Mix the contents by vigorously pipetting for 5 seconds, then centrifuge briefly to collect the liquid at the bottom of the tube.
3. Add 5X Fragmentation and dA-tailing Enzyme Mix to the sample.

Component	Volume
Buffer-DNA mixture from step 1 (light blue mixture ●)	40 μ L
5X Fragmentation and dA-tailing Buffer (clear ○)	10 μ L
Total volume (light blue mixture ●):	50 μL

Note: 5X Fragmentation and dA-tailing Mix is very viscous. Pipette slowly to ensure accurate pipetting

4. Mix the sample by rapidly pipetting up and down (avoid creating bubbles) until the mixture becomes homogeneous.
5. Set up the pipette volume to 25 μ L and mix the sample by pipetting up and down 5 times (avoid creating bubbles), then centrifuge briefly to collect the liquid at the bottom of the tube.
Samples can be mixed while adding 5X Fragmentation and dA-tailing Enzyme Mix using the same pipette tips. The order of reagent addition can be different: first add the enzyme mix to the 35 μ L of combined pools and then add 5X buffer and mix by pipetting up and down. The rationale: there is excess of the buffer, not the enzyme mix, so you can use a reagent reservoir for the buffer.
6. Place the samples back on ice or a cooling rack.

IMPORTANT! Ensure that the reaction components are mixed well. Incomplete mixing results in uneven DNA fragmentation.

- Seal the plate and incubate the mixture in a thermal cycler with the heated lid set to 80–85°C, the block pre-cooled to 4°C, and programmed as outlined in the following table.

IMPORTANT! Heated lid is required for this step (set to 80–85°C).

Step	Temperature	Time
Pre-cool the block	4°C	As required
Fragmentation	37°C	15 minutes
dA-tailing	65°C	10 minutes
Hold	4°C	Hold

- When the thermal cycler program is complete and the sample block has cooled to 4°C, immediately remove the samples and place them on ice.

IMPORTANT! Proceed immediately to the next step, “Dual indexed adaptor ligation”.

Dual indexed adaptor ligation

Overview

This section describes the ligation of the Illumina®-compatible NGS adaptors to the fragmented and dA-tailed DNA sample. For optimal results we recommend following Illumina™ indexed adaptors multiplexing guidelines.

Note: The color of the reaction mixture changes as each reaction component is added. Mixing the Dual-Indexed Adaptors (yellow) with the fragmented, dA-tailed DNA sample (blue) produces a green mixture. If the correct amount of the Ligation Master Mix for ES (red) is added to this mixture, the final ligation reaction becomes purple.

Required materials

Components from the Collibri™ ES DNA Library Prep Kits:

- 7X Ligation Master Mix for ES

Components from the Collibri™ CD or UD Indexes

- Collibri™ Dual-Indexed Adaptor plate

Other materials and equipment:

- Fragmented and dA-tailed DNA samples (from step 8 on page 26)
- Microcentrifuge
- Thermomixer or thermal cycler with a heated lid off
- Ice or cooling block set to 4°C

Before you begin

Before use, mix the 7X Ligation Master Mix for ES by vortexing thoroughly, then briefly centrifuge to collect all the droplets at the bottom of the tube. Keep on ice.

Ligate the adaptors

1. Remove the seal from the wells of the 24-well or 96-well Collibri™ Dual-Indexed Adaptor plate that you plan to use, then transfer 10 µL of Dual-Indexed Adaptor from one well to each 50 µL fragmented and dA-tailed DNA sample (from step 8 on page 26), using a new adaptor for each DNA sample. Keep the Adaptor-DNA mixture on ice.

Component	Volume
Fragmented, dA-tailed DNA sample (light blue ●)	50 µL
Dual-Indexed Adaptor (yellow ●)	10 µL
Total volume (green mixture ●):	60 µL

IMPORTANT! Keep track of the indexes from each adaptor well used for each DNA sample.

2. Seal the used wells of the Collibri™ Dual-Indexed Adaptor plate with Easy-Peal™ seal (provided with the kit) cut to the appropriate size and shape, then store the unused adaptors frozen at -20°C. The Collibri™ Dual-Indexed Adaptor plate is stable for at least 10 freeze-thaw cycles.

Note: Do not reuse the same adaptor wells.

3. To prepare the ligation reaction mix, add the 7X Ligation Master Mix for ES to the Adaptor-DNA mixture on ice (from step 1), then mix well by vortexing.

Component	Volume
Adaptor-DNA mixture from step 1 (green ●)	60 µL
7X Ligation Master Mix for ES (red ●)	10 µL
Total volume (purple mixture ●):	70 µL

IMPORTANT! Observe the color change as each reaction component is added. If the appropriate component is added, the ligation mix should be purple.

4. Incubate the ligation reaction mixture in a heating block or a thermomixer set to 20°C for 30 minutes in a thermomixer or thermal cycler with the heated lid off.

IMPORTANT! Do not use a thermal cycler with heated lid. Ligase is a thermo-sensitive enzyme and it can be inactivated if the temperature increases above 25°C, resulting in lower library yields.

5. Proceed to (“Post-ligation cleanup” on page 28).

Note: You can store the Adaptor-ligated DNA samples at -20°C. However, this can result in lower yields.

Post-ligation cleanup

Overview

This section describes bead-based post-ligation purification of adaptor-ligated DNA library.

Required materials

Components from the Colibri™ DNA Library Cleanup Kit:

- DNA Cleanup Beads
- Wash Buffer (diluted with 96% ethanol)
- Elution Buffer

Other materials and equipment:

- Dual Index Adaptor-ligated DNA sample (from step 5 on page 27)
- 96% ethanol, molecular biology grade (used for diluting the Wash Buffer before first use)
- 0.2 ml PCR 8-tube strips with attached caps or 96-well plate
- Plate centrifuge
- Magnetic rack

Before you begin

1. Ensure that the appropriate volume of 96% ethanol (as noted on the bottle) was added to the Wash Buffer before first use.
2. Ensure that the DNA Cleanup Beads, Wash Buffer, and Elution Buffer are at room temperature.
3. Gently vortex the DNA Cleanup Beads to completely resuspend the magnetic beads in the solution.

Purify the adaptor ligated library

1. Mix the Dual Index Adaptor-ligated DNA library (70 μ L) (from step 5 on page 27) with 56 μ L of DNA Cleanup Beads by pipetting until you have obtained a homogeneous suspension.

Note: Reagent reservoir can be used to aspirate the DNA cleanup bead solution with a multichannel pipette.

2. Briefly centrifuge the plate to collect all the droplets at the bottom, then incubate for 5 minutes at room temperature.

IMPORTANT! Do not extend the binding step to more than 5 minutes. Over-incubation can result in greater amount of adaptor and adaptor dimers in the final library.

3. After incubation, briefly centrifuge the plate to collect all the droplets at the bottom, then place it on the magnetic rack for 2 minutes or until the beads have formed a tight pellet.

Note: The time required for the complete capture of the cleanup beads can vary depending on the reaction vessel and the magnet used. Optimize the capture time accordingly.

4. Keeping the reaction plate on the magnetic rack, carefully remove and discard the supernatant using a pipette. Ensure that all the supernatant is removed.

Note: If the pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the side of the plate on the magnet again.

5. Keeping the reaction plate on the magnet, add 200 μ L of Wash Buffer (pre-mixed with ethanol) to each well, then incubate for 30 seconds at room temperature.

IMPORTANT! Do not resuspend the magnetic beads in Wash Buffer.

6. Carefully remove and discard the supernatant using a pipette.

7. Repeat step 5–step 6.

8. To remove the residual ethanol, briefly centrifuge the reaction plate, place it back in the magnetic rack, then carefully remove any remaining supernatant with a pipette without disturbing the pellet.

9. Keeping the reaction tube on the magnet, air dry the magnetic beads for 1 minute at room temperature or until there are no droplets of ethanol left on the walls of the plate wells.

IMPORTANT! Do not over-dry by prolonged incubation for more than 5 minutes. Over-drying significantly decreases the elution efficiency.

10. Remove the tube from the magnetic rack, add 70 μ L of Elution Buffer, then vortex to mix thoroughly.

Note: Reagent reservoir can be used to aspirate the Elution Buffer with a multichannel pipette.

11. Briefly centrifuge the plate to collect all the droplets at the bottom, then incubate for 1 minute at room temperature.
12. Add 70 μL of fresh DNA Cleanup Beads directly to the bead suspension in Elution Buffer, then mix by pipetting until you have obtained a homogeneous suspension.
13. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 5 minutes at room temperature.
14. After incubation, briefly centrifuge the tube to collect all the droplets at the bottom, then place it in the magnetic rack for 2 minutes or until the beads have formed a tight pellet.
15. Keeping the reaction plate on the magnetic rack, carefully remove and discard the supernatant using a pipette. Ensure that all the supernatant is removed.

Note: If the pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the bottom of the plate on the magnet again.

16. Keeping the reaction tube on the magnet, add 200 μL of Wash Buffer (pre-mixed with ethanol), then incubate for 30 seconds at room temperature.

IMPORTANT! Do not resuspend the magnetic beads in Wash Buffer.

17. Carefully remove and discard the supernatant using a pipette.
18. Repeat step 15–step 16.
19. To remove the residual ethanol, briefly centrifuge the reaction plate, place it back on the magnetic rack, then carefully remove any remaining supernatant with a pipette without disturbing the pellet.
20. Keeping the reaction plate on the magnet, air dry the magnetic beads for 1 minute at room temperature or until there are no droplets of ethanol left on the walls of the plate wells.

IMPORTANT! Do not over-dry by prolonged incubation for more than 5 minutes. Over-drying significantly decreases the elution efficiency.

21. Remove the plate from the magnetic rack, add 23 μL of Elution Buffer, then pipette up and down to mix thoroughly.
22. Briefly centrifuge the plate to collect all the droplets at the bottom, then incubate for 1 minute at room temperature.
23. Place the plate on the magnetic rack for 2 minutes or until the beads have formed a tight pellet. Wait for the solution to clear before proceeding to the next step.

24. Without removing the plate from the magnetic rack, collect 20 μ L of the supernatant to a new 96-well plate for storage.

Note: If the pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the bottom of the plate on the magnet again.

STOPPING POINT Store the eluted DNA library at 4°C for up to 1–2 weeks or at –20°C for long-term storage, or immediately proceed to the next step.

Library PCR amplification (*optional*)

Post-adaptor ligation PCR amplification can benefit the specificity of sequencing and increase the overall target library amount.

IMPORTANT! PCR amplification is recommended for low-yielding samples or samples that demonstrated patchy coverage in previous sequencing.

Overview

This section describes the PCR-based amplification of the purified adaptor-ligated SARS-CoV-2 genome libraries.

Note: The color of the reaction mixture changes as each reaction component is added. Mixing the purified adaptor-ligated DNA library (clear) with the 2X Library Amplification Master Mix (blue) and the Primer Mix (yellow) produces a green PCR mixture.

Required materials

Use components from the Collibri™ ES DNA Library Prep Kit with Library Amplification:

- 2X Library Amplification Master Mix
- Primer Mix

Other materials and equipment:

- Purified, adaptor-ligated DNA library (step 24 on page 31)
- 0.2-mL sterile, thin-wall PCR tubes
- Thermal cycler with the heated lid set to 105°C
- Ice or cooling block set to 4°C

Before you begin

1. Thaw the 2X Library Amplification Master Mix and the Primer Mix on ice.
2. After the reagents have thawed, mix thoroughly by vortexing to prevent localized concentrations of reagent components, then return to ice until ready to use.

Amplify the DNA library

1. To the 20 μL of the DNA library (from step 24 on page 31) then add the following reagents in the given order, mix while pipetting:

Component	Volume
Adaptor-ligated and size-selected (clear ○)	20 μL
2X Library Amplification Master Mix (blue ●)	25 μL
Primer Mix (yellow ●)	5 μL
Total volume (green mixture ●):	50 μL

2. Centrifuge the PCR mixture briefly to collect all the droplets at the bottom.
3. Run the reactions in a thermal cycler with the lid temperature set to 105°C:

Stage	Number of cycles	Temperature	Time
Activate the enzyme	1 cycle	98°C	30 seconds
Denature	4 cycles	98°C	15 seconds
Anneal		60°C	30 seconds
Extend		72°C	30 seconds
Final extension	1 cycle	72°C	1 minute
Hold	1 cycle	4°C	Hold

4. After the PCR is completed, see “Purify amplified DNA libraries” on page 32.

Purify amplified DNA libraries

Overview

This section describes post-amplification cleanup of the DNA library using the DNA Cleanup Beads.

Required materials

Components from the Collibri™ DNA Library Cleanup Kit:

- DNA Cleanup Beads
- Wash Buffer (diluted with 96% ethanol)
- Elution Buffer

Other materials and equipment:

- PCR-amplified DNA library (from step 4 on page 32)
- 1.5 mL Eppendorf™ DNA LoBind™ Tubes or 96-well plate
- Microcentrifuge
- Magnetic rack

Before you begin

1. Ensure that the appropriate volume of 96% ethanol (as noted on the bottle) was added to the Wash Buffer before first use.
2. Ensure that the DNA Cleanup Beads, Wash Buffer, and Elution Buffer are at room temperature.
3. Gently vortex the DNA Cleanup Beads to completely resuspend the magnetic beads in the solution.

Purify the amplified DNA library

Perform all cleanup steps at room temperature.

1. Mix the amplified DNA library (50 μ L) (from step 4 on page 32) with 60 μ L of DNA Cleanup Beads by vortexing until you have obtained a homogeneous suspension.

Note: The reagent reservoir can be used to aspirate the DNA cleanup bead solution with a multichannel pipette.

2. Briefly centrifuge the plate containing the amplified DNA libraries and bead mixture to collect all the droplets at the bottom, then incubate for 5 minutes at room temperature.

IMPORTANT! Do not extend the binding step to more than 5 minutes. Over-incubation can result in lower DNA yields.

3. Briefly centrifuge the plate to collect all the droplets at the bottom, then place it on the magnetic rack for 2 minutes or until the beads have formed a tight pellet.

Note: The time required for the complete capture of the DNA Cleanup Beads can vary depending on the reaction vessel and the magnet used. Optimize the capture time accordingly.

4. Keeping the plate on the magnet, carefully remove and discard the supernatant using a pipette. Ensure that all the supernatant is removed.

Note: If the pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the side of the tube on the magnet again.

5. Keeping the tube on the magnet, add 200 μ L of Wash Buffer (pre-mixed with ethanol), then incubate for 30 seconds at room temperature.

IMPORTANT! Do not resuspend the magnetic beads in Wash Buffer.

6. Carefully remove and discard the supernatant using a pipette.
7. Repeat step 5–step 6.
8. To remove the residual ethanol, briefly centrifuge the plate, place it back in the magnetic rack, then carefully remove any remaining supernatant with a pipette without disturbing the pellet.

9. Keeping the plate on the magnet, air dry the magnetic beads for 1 minute at room temperature or until there are no droplets of ethanol left on the walls of the tube.

IMPORTANT! Do not over-dry by prolonged incubation for more than 5 minutes. Over-drying significantly decreases the elution efficiency.

10. Remove the plate from the magnetic rack, add 23 μ L of Elution Buffer, then mix the suspension thoroughly by pipetting up and down.

Note: The reagent reservoir can be used to aspirate the Elution Buffer with a multichannel pipette.

11. Briefly centrifuge the plate to collect all the droplets at the bottom, then incubate for 1 minute at room temperature.
12. Place the tube in the magnetic rack for 2 minutes or until the beads have formed a tight pellet. Wait for the solution to clear before proceeding to the next step.
13. Without removing the tube from the magnetic rack, transfer 20 μ L of the supernatant (i.e., the eluate) to a new tube for storage.

Note: If the pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the side of the tube on the magnet again.

14. Proceed to the assessment of the DNA library size and yield.

STOPPING POINT After purification, you can store the amplified DNA library 4°C for 1–2 weeks. For longer term, store the library at -20°C until ready for sequencing.

Verify quality and quantity of prepared DNA libraries

Overview

Verify the size distribution and quality of prepared DNA library by performing capillary electrophoresis analysis on Agilent™ 2100 Bioanalyzer instrument (or any similar instrument) using the Agilent™ High Sensitivity DNA Kit and Qubit™ dsDNA HS Assay Kit.

Required materials

- Agilent™ 2100 Bioanalyzer™ instrument (Agilent, G2938A)
- Agilent™ High Sensitivity DNA Kit (Agilent, 5067-4626)
- Qubit™ 4 Fluorometer (Cat. No. [Q33238](#))
- Qubit™ dsDNA HS Assay Kit (Cat No [Q32851](#), [Q32854](#))
- Nuclease-free water

Analyze the size distribution of the amplified DNA libraries

1. Remove 1 μL from each prepared DNA library (i.e., purified and amplified DNA from step 13 on page 34), and dilute it 10-fold in nuclease-free water.

2.

Options	Description
Agilent™ Bioanalyzer	Analyze 1 μL of the diluted DNA library using the appropriate chip on the Agilent™ 2100 Bioanalyzer™ instrument with the Agilent™ High Sensitivity DNA Kit.
Agilent™ Fragment Analyzer	Analyze 2 μL of the diluted DNA library using HS NGS gel matrix with appropriate 1-6000 bp DNA analysis program parameters.

3. Use the 2100 Expert software and perform a smear analysis to determine the average library length using a size range of 150–1000 bp. An ideal electrophoresis trace is shown in Figure 1. Verify that the electropherogram shows the peak of DNA fragment size positioned between 300 to 400 bp for high quality DNA (See Figure 1).

The appearance of a low molecular weight (100-150 bp) sharp peak indicates the presence of adaptor-dimers in the library (Figure 2). The appearance of secondary high molecular weight (450-550 bp) peaks indicates incomplete enzymatic shearing (Figure 2). It is acceptable to proceed with the library samples for which adaptor-dimers or incompletely sheared amplicon peaks are observed in the electropherograms as indicated in the Figure 2. See Troubleshooting information for additional considerations.

Note: For instructions on how to perform the smear analysis, refer to the *Agilent™ 2100 Bioanalyzer™ Expert User's Guide* (Agilent, Pub. No. G2946-90004).

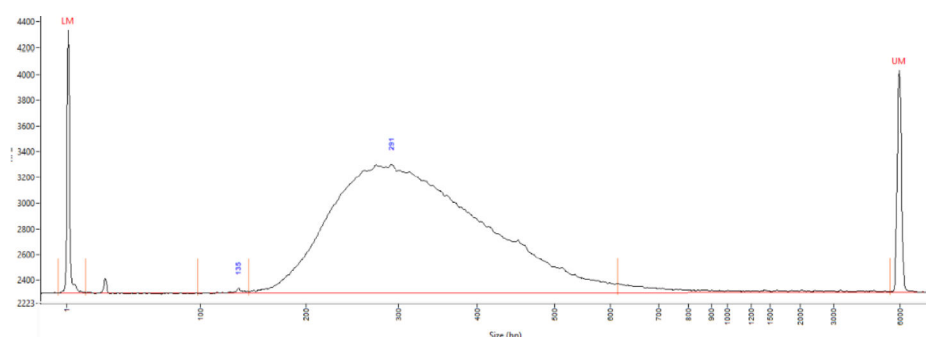


Figure 1 Library prepared from a high-quality RNA sample analyzed on NGS Gel matrix, 1-6000 bp DNA assay on Agilent™ Fragment Analyzer.

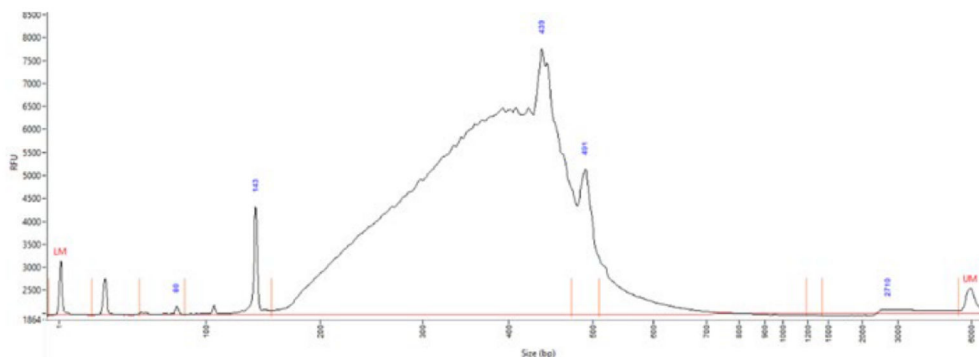


Figure 2 Library with the presence of adaptor-dimers (peak ~140 bp) and incomplete fragmentation of amplicons (peaks at 439 bp and 491 bp) prepared from a standard RNA sample analyzed using NGS Gel matrix , 1-6000 bp DNA assay on Agilent™ Fragment Analyzer.

Determine the concentration of the amplified library

Measure the DNA library concentration using Thermo Fisher Scientific Qubit™ fluorimeter software or NanoDrop™. Ideally, final concentration value should be $\geq 50 \text{ ng}/\mu\text{L}$.

The library preparation step may yield more material than needed for the next step. Excess product may be stored at -20°C for later use.

STOPPING POINT You can store the purified DNA libraries at 4°C for up to 1 month. For longer term, store at -20°C .

Sequence on an Illumina® platform

Before you begin

Before pooling of libraries for sequencing, ensure that an appropriate combination of dual-indexed SARS-CoV-2 genome libraries is selected to avoid index read failure.

Sequence the prepared library

1. Using the calculated conversion of DNA concentration, quantitative qPCR data, or the results from the Colibri™ Library Quantification Kit (preferred), pool equimolar amounts of dual-indexed SARS-CoV-2 libraries for sequencing. Fewer library dilutions and fewer replicates per sample can be performed to quantify 96 samples in two qPCR plates.

Convert Qubit™ or NanoDrop™ concentration data (read in $\text{ng}/\mu\text{L}$) to nanomolar concentration by using the following formula:

$$\frac{[\text{concentration in ng } \mu\text{L}^{-1}] \times 10^6}{660 \text{ g mol}^{-1} \times [\text{average library size}]}$$

Average library size can be estimated from Agilent Bioanalyzer or Fragment Analyzer traces using appropriate software.

2. Use 10% PhiX in the final library pool.
3. Denature, dilute, and load the libraries according to the standard guidelines appropriate for the Illumina® Hi-Seq™, NextSeq™ or NovaSeq™ platforms using the cycle settings shown in the table:

Run stage	Cycle number
Read 1	76 or 101
Index 1 (i7)	8
Index 1 (i5)	8
Read 2	76 or 101



Troubleshooting

Observation	Possible cause	Recommended action
Low PCR pool yield	RT reaction was inefficient. This can happen with difficult samples.	Prolong the incubation to 1–1.5 hr at 42°C
High unresolved base percent	PCR thermal cycler temperature calibration is off.	Re-calibrate or run temporary gradient to determine the right temperature for enrichment PCR amplification.



Adaptor index sequence and plate layouts

Adaptor index sequences

Index sequences used for CD adaptors

Index sequences used for Combinatorial Dual-Indexed Adaptors (CD) in Collibri™ ES DNA Library Prep Kits are listed in Table 1. Indexes D501–D508 and D701–D712 correspond to the respective Illumina® adaptor indexes. 24-prep and 96-prep CD adaptor plate layouts are shown in Figure 3–Figure 4.

Table 1 Indexes used in Collibri™ DNA Library kits to generate Combinatorial Dual-Indexed (CD) Adaptors.

D70X index name	i7 Bases for entry on sample sheet	D50X index name	i5 bases for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	i5 bases for entry on sample sheet (iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
D701	ATTACTCG	D501	TATAGCCT	AGGCTATA
D702	TCCGGAGA	D502	ATAGAGGC	GCCTCTAT
D703	CGCTCATT	D503	CCTATCCT	AGGATAGG
D704	GAGATTCC	D504	GGCTCTGA	TCAGAGCC
D705	ATTCAGAA	D505	AGGCGAAG	CTTCGCCT
D706	GAATTCGT	D506	TAATCTTA	TAAGATTA
D707	CTGAAGCT	D507	CAGGACGT	ACGTCCTG
D708	TAATGCGC	D508	GTACTGAC	GTCAGTAC
D709	CGGCTATG	—	—	—
D710	TCCGCGAA	—	—	—
D711	TCTCGCGC	—	—	—
D712	AGCGATAG	—	—	—

^[1] Sequencing on the iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, and Hi-Seq™ X systems follow a different dual-indexing workflow than other Illumina® systems, which require the reverse complement of the i5 index adaptor sequence.

Index sequences used for UD adaptors

Index sequences used for Unique Dual-Indexed Adaptors (UD) in Collibri™ ES DNA Library Prep Kits are listed in Table 2–Table 5.

Plate layouts of 24-prep UD Adaptor Sets A–D are shown in Figure 5–Figure 8. Plate layout of 96-prep UD Adaptor Set is shown in Figure 9.

Table 2 Indexes used in Collibri™ DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set A.

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X)[1]
Set A			
UDI001	CCGCGGTT	AGCGCTAG	CTAGCGCT
UDI002	TTATAACC	GATATCGA	TCGATATC
UDI003	GGACTTGG	CGCAGACG	CGTCTGCG
UDI004	AAGTCCAA	TATGAGTA	TACTCATA
UDI005	ATCCACTG	AGGTGCGT	ACGCACCT
UDI006	GCTTGTC A	GAACATAC	GTATGTTC
UDI007	CAAGCTAG	ACATAGCG	CGCTATGT
UDI008	TGGATCGA	GTGCGATA	TATCGCAC
UDI009	AGTTCAGG	CCAACAGA	TCTGTTGG
UDI010	GACCTGAA	TTGGTGAG	CTCACCAA
UDI011	TCTCTACT	CGCGGTTC	GAACCGCG
UDI012	CTCTCGTC	TATAACCT	AGGTTATA
UDI013	CCAAGTCT	AAGGATGA	TCATCCTT
UDI014	TTGGACTC	GGAAGCAG	CTGCTTCC
UDI015	GGCTTAAG	TCGTGACC	GGTCACGA
UDI016	AATCCGGA	CTACAGTT	AACTGTAG
UDI017	TAATACAG	ATATTCAC	GTGAATAT
UDI018	CGGCGTGA	GCGCCTGT	ACAGGCGC
UDI019	ATGTAAGT	ACTCTATG	CATAGAGT
UDI020	GCACGGAC	GTCTCGCA	TGCGAGAC

Table 2 Indexes used in Colibri DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set A. (continued)

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
UDI021	GGTACCTT	AAGACGTC	GACGTCTT
UDI022	AACGTTCC	GGAGTACT	AGTACTCC
UDI023	GCAGAAATT	ACCGGCCA	TGGCCGGT
UDI024	ATGAGGCC	GTTAATTG	CAATTAAC

^[1] Sequencing on the MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, and Hi-Seq™ X systems follow a different dual-indexing workflow than other Illumina® systems, which require the reverse complement of the i5 index adaptor sequence.

Table 3 Indexes used in Colibri™ DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set B.

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
Set B			
UDI025	ACTAAGAT	AACCGCGG	CCGCGGTT
UDI026	GTCGGAGC	GGTTATAA	TTATAACC
UDI027	CTTGGTAT	CCAAGTCC	GGAAGTGG
UDI028	TCCAACGC	TTGGACTT	AAGTCCAA
UDI029	CCGTGAAG	CAGTGGAT	ATCCTACTG
UDI030	TTACAGGA	TGACAAGC	GCTTGTCA
UDI031	GGCATTCT	CTAGCTTG	CAAGCTAG
UDI032	AATGCCTC	TCGATCCA	TGGATCGA
UDI033	TACCGAGG	CCTGAACT	AGTTCAGG
UDI034	CGTTAGAA	TTCAGGTC	GACCTGAA
UDI035	AGCCTCAT	AGTAGAGA	TCTCTACT
UDI036	GATTCTGC	GACGAGAG	CTCTCGTC
UDI037	TCGTAGTG	AGACTTGG	CCAAGTCT
UDI038	CTACGACA	GAGTCCAA	TTGGACTC

Table 3 Indexes used in Colibri DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set B. (continued)

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
UDI039	TAAGTGGT	CTTAAGCC	GGCTTAAG
UDI040	CGGACAAC	TCCGGATT	AATCCGGA
UDI041	ATATGGAT	CTGTATTA	TAATACAG
UDI042	GCGCAAGC	TCACGCCG	CGGCGTGA
UDI043	AAGATACT	ACTTACAT	ATGTAAGT
UDI044	GGAGCGTC	GTCCGTGC	GCACGGAC
UDI045	ATGGCATG	AAGGTACC	GGTACCTT
UDI046	GCAATGCA	GGAACGTT	AACGTTCC
UDI047	GTTCCAAT	AATTCTGC	GCAGAATT
UDI048	ACCTTGGC	GGCCTCAT	ATGAGGCC

^[1] Sequencing on the MiniSeq™, NextSeq™, HiSeq™ 3000/4000, and Hi-Seq™ X systems follow a different dual-indexing workflow than other Illumina® systems, which require the reverse complement of the i5 index adaptor sequence.

Table 4 Indexes used in Colibri™ DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set C.

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
Set C			
UDI049	ATATCTCG	ATCTTAGT	ACTAAGAT
UDI050	GCGCTCTA	GCTCCGAC	GTCGGAGC
UDI051	AACAGGTT	ATACCAAG	CTTGGTAT
UDI052	GGTGAACC	GCGTTGGA	TCCAACGC
UDI053	CAACAATG	CTTCACGG	CCGTGAAG
UDI054	TGGTGGCA	TCCTGTAA	TTACAGGA
UDI055	AGGCAGAG	AGAATGCC	GGCATTCT
UDI056	GAATGAGA	GAGGCATT	AATGCCTC

Table 4 Indexes used in Colibri DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set C. (continued)

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
UDI057	TGCGGCGT	CCTCGGTA	TACCGAGG
UDI058	CATAATAC	TTCTAACG	CGTTAGAA
UDI059	GATCTATC	ATGAGGCT	AGCCTCAT
UDI060	AGCTCGCT	GCAGAATC	GATTCTGC
UDI061	CGGAACTG	CACTACGA	TCGTAGTG
UDI062	TAAGGTCA	TGTCGTAG	CTACGACA
UDI063	TTGCCTAG	ACCACTTA	TAAGTGGT
UDI064	CCATTCGA	GTTGTCCG	CGGACAAC
UDI065	ACACTAAG	ATCCATAT	ATATGGAT
UDI066	GTGTCGGA	GCTTGCGC	GCGCAAGC
UDI067	TTCCTGTT	AGTATCTT	AAGATACT
UDI068	CCTTCACC	GACGCTCC	GGAGCGTC
UDI069	GCCACAGG	CATGCCAT	ATGGCATG
UDI070	ATTGTGAA	TGCATTGC	GCAATGCA
UDI071	ACTCGTGT	ATTGGAAC	GTTCCAAT
UDI072	GTCTACAC	GCCAAGGT	ACCTTGGC

^[1] Sequencing on the iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, and Hi-Seq™ X systems follow a different dual-indexing workflow than other Illumina® systems, which require the reverse complement of the i5 index adaptor sequence.

Table 5 Indexes used in Colibri™ DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set D.

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
Set D			
UDI073	CAATTAAC	CGAGATAT	ATATCTCG
UDI074	TGGCCGGT	TAGAGCGC	GCGCTCTA
UDI075	AGTACTCC	AACCTGTT	AACAGGTT
UDI076	GACGTCTT	GGTTCACC	GGTGAACC
UDI077	TGCGAGAC	CATTGTTG	CAACAATG
UDI078	CATAGAGT	TGCCACCA	TGGTGGCA
UDI079	ACAGGCGC	CTCTGCCT	AGGCAGAG
UDI080	GTGAATAT	TCTCATTC	GAATGAGA
UDI081	AACTGTAG	ACGCCGCA	TGCGGCGT
UDI082	GGTCACGA	GTATTATG	CATAATAC
UDI083	CTGCTTCC	GATAGATC	GATCTATC
UDI084	TCATCCTT	AGCGAGCT	AGCTCGCT
UDI085	AGGTTATA	CAGTTCCG	CGGAACTG
UDI086	GAACCGCG	TGACCTTA	TAAGGTCA
UDI087	CTCACCAA	CTAGGCAA	TTGCCTAG
UDI088	TCTGTTGG	TCGAATGG	CCATTCGA
UDI089	TATCGCAC	CTTAGTGT	ACACTAAG
UDI090	CGCTATGT	TCCGACAC	GTGTCGGA
UDI091	GTATGTTC	AACAGGAA	TTCCTGTT
UDI092	ACGCACCT	GGTGAAGG	CCTTCACC
UDI093	TACTCATA	CCTGTGGC	GCCACAGG
UDI094	CGTCTGCG	TTCACAAT	ATTGTGAA

Table 5 Indexes used in Colibri DNA Library kits to generate Unique Dual Indexed (UDI) Adaptors – Set D. *(continued)*

UDI Adaptor name	P7 index	P5 index for entry on sample sheet (NovaSeq™, MiSeq™, Hi-Seq™ 2000/2500)	P5 index for entry on sample sheet (iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, Hi-Seq™ X) ^[1]
UDI095	TCGATATC	ACACGAGT	ACTCGTGT
UDI096	CTAGCGCT	GTGTAGAC	GTCTACAC

^[1] Sequencing on the iSeq™, MiniSeq™, NextSeq™, Hi-Seq™ 3000/4000, and Hi-Seq™ X systems follow a different dual-indexing workflow than other Illumina® systems, which require the reverse complement of the i5 index adaptor sequence.

Adaptor plate layouts

Note: Colors of the borders in the plate layouts provided bellow match the colors of the plates containing individual adaptor sets.

Combinatorial Indexed Adaptor Sets

	1	2	3	4	5	6	7	8	9	10	11	12
A	501/701	501/702	501/703	501/704	501/705	501/706	501/707	501/708	501/709	501/710	501/711	501/712
B	502/701	502/702	502/703	502/704	502/705	502/706	502/707	502/708	502/709	502/710	502/711	502/712
C	503/701	503/702	503/703	503/704	503/705	503/706	503/707	503/708	503/709	503/710	503/711	503/712
D	504/701	504/702	504/703	504/704	504/705	504/706	504/707	504/708	504/709	504/710	504/711	504/712
E	505/701	505/702	505/703	505/704	505/705	505/706	505/707	505/708	505/709	505/710	505/711	505/712
F	506/701	506/702	506/703	506/704	506/705	506/706	506/707	506/708	506/709	506/710	506/711	506/712
G	507/701	507/702	507/703	507/704	507/705	507/706	507/707	507/708	507/709	507/710	507/711	507/712
H	508/701	508/702	508/703	508/704	508/705	508/706	508/707	508/708	508/709	508/710	508/711	508/712

Figure 3 Colibri™ 96-prep Combinatorial Indexed (CD) Adaptor plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	501/701	501/702	501/703	—	—	—	—	—	—	—	—	—
B	501/704	501/705	501/706	—	—	—	—	—	—	—	—	—
C	501/707	501/708	501/709	—	—	—	—	—	—	—	—	—
D	501/710	501/711	501/712	—	—	—	—	—	—	—	—	—
E	502/701	502/702	502/703	—	—	—	—	—	—	—	—	—
F	502/704	502/705	502/706	—	—	—	—	—	—	—	—	—
G	502/707	502/708	502/709	—	—	—	—	—	—	—	—	—
H	502/710	502/711	502/712	—	—	—	—	—	—	—	—	—

Figure 4 Colibri™ 24-prep Combinatorial Indexed (CD) Adaptor plate layout

Unique Dual Indexed Adaptor Sets

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI001	UDI009	UDI017	—	—	—	—	—	—	—	—	—
B	UDI002	UDI010	UDI018	—	—	—	—	—	—	—	—	—
C	UDI003	UDI011	UDI019	—	—	—	—	—	—	—	—	—
D	UDI004	UDI012	UDI020	—	—	—	—	—	—	—	—	—
E	UDI005	UDI013	UDI021	—	—	—	—	—	—	—	—	—
F	UDI006	UDI014	UDI022	—	—	—	—	—	—	—	—	—
G	UDI007	UDI015	UDI023	—	—	—	—	—	—	—	—	—
H	UDI008	UDI016	UDI024	—	—	—	—	—	—	—	—	—

Figure 5 Collibri™ 24-prep Unique Indexed (UD) Adaptor Set A plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI025	UDI033	UDI041	—	—	—	—	—	—	—	—	—
B	UDI026	UDI034	UDI042	—	—	—	—	—	—	—	—	—
C	UDI027	UDI035	UDI043	—	—	—	—	—	—	—	—	—
D	UDI028	UDI036	UDI044	—	—	—	—	—	—	—	—	—
E	UDI029	UDI037	UDI045	—	—	—	—	—	—	—	—	—
F	UDI030	UDI038	UDI046	—	—	—	—	—	—	—	—	—
G	UDI031	UDI039	UDI047	—	—	—	—	—	—	—	—	—
H	UDI032	UDI040	UDI048	—	—	—	—	—	—	—	—	—

Figure 6 Collibri™ 24-prep Unique Indexed (UD) Adaptor Set B plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI049	UDI057	UDI065	—	—	—	—	—	—	—	—	—
B	UDI050	UDI058	UDI066	—	—	—	—	—	—	—	—	—
C	UDI051	UDI059	UDI067	—	—	—	—	—	—	—	—	—
D	UDI052	UDI060	UDI068	—	—	—	—	—	—	—	—	—
E	UDI053	UDI061	UDI069	—	—	—	—	—	—	—	—	—
F	UDI054	UDI062	UDI070	—	—	—	—	—	—	—	—	—
G	UDI055	UDI063	UDI071	—	—	—	—	—	—	—	—	—
H	UDI056	UDI064	UDI072	—	—	—	—	—	—	—	—	—

Figure 7 Collibri™ 24-prep Unique Indexed (UD) Adaptor Set C plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI073	UDI081	UDI089	—	—	—	—	—	—	—	—	—
B	UDI074	UDI082	UDI090	—	—	—	—	—	—	—	—	—
C	UDI075	UDI083	UDI091	—	—	—	—	—	—	—	—	—
D	UDI076	UDI084	UDI092	—	—	—	—	—	—	—	—	—
E	UDI077	UDI085	UDI093	—	—	—	—	—	—	—	—	—
F	UDI078	UDI086	UDI094	—	—	—	—	—	—	—	—	—
G	UDI079	UDI087	UDI095	—	—	—	—	—	—	—	—	—
H	UDI080	UDI088	UDI096	—	—	—	—	—	—	—	—	—

Figure 8 Colibri™ 24-prep Unique Indexed (UD) Adaptor Set D plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI001	UDI009	UDI017	UDI025	UDI033	UDI041	UDI049	UDI057	UDI065	UDI073	UDI081	UDI089
B	UDI002	UDI010	UDI018	UDI026	UDI034	UDI042	UDI050	UDI058	UDI066	UDI074	UDI082	UDI090
C	UDI003	UDI011	UDI019	UDI027	UDI035	UDI043	UDI051	UDI059	UDI067	UDI075	UDI083	UDI091
D	UDI004	UDI012	UDI020	UDI028	UDI036	UDI044	UDI052	UDI060	UDI068	UDI076	UDI084	UDI092
E	UDI005	UDI013	UDI021	UDI029	UDI037	UDI045	UDI053	UDI061	UDI069	UDI077	UDI085	UDI093
F	UDI006	UDI014	UDI022	UDI030	UDI038	UDI046	UDI054	UDI062	UDI070	UDI078	UDI086	UDI094
G	UDI007	UDI015	UDI023	UDI031	UDI039	UDI047	UDI055	UDI063	UDI071	UDI079	UDI087	UDI095
H	UDI008	UDI016	UDI024	UDI032	UDI040	UDI048	UDI056	UDI064	UDI072	UDI080	UDI088	UDI096

Figure 9 Colibri™ 96-prep Unique Indexed (UD) Adaptor plate layout



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).



Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf>
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311



Documentation and support

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

