



Lexogen Artic Panel for SARS-CoV2

User Guide

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CONTACT INFORMATION

Lexogen GmbH

Campus Vienna Biocenter 5 1030 Vienna, Austria www.lexogen.com E-mail: info@lexogen.com

Support

E-mail: support@lexogen.com Tel. +43 (0) 1 3451212-41 Fax. +43 (0) 1 3451212-99

Table of Contents

1.	Overview
2.	Kit Components and Storage Conditions
3.	User-Supplied Consumables and Equipment
4.	Detailed Protocol ARTIC Panel
	4.1 ARTIC Panel PCR
	4.2 Indexing PCR with UDI 12 nt Unique Dual Indices 11 $$
5.	Short Procedure ARTIC Panel
6.	Appendix A: Quality Control
7.	Appendix B: Sequencing
8.	Appendix C: Library Reamplification
9.	Appendix D: Data Analysis
10	Appendix F: Revision History



1. Overview

Lexogen's SARS-CoV-2 ARTIC Panel for Illumina enables sequencing of the complete viral genome and allows mutational analysis and variant detection. The panel is specifically designed for Illumina Next Generation Sequencing (NGS).

The input material for the ARTIC panel is single or double stranded cDNA. We recommend cDNA generation by a combination of random hexamers and oligo(dT) primers using SuperScriptTM IV Reverse Transcriptase in 10 μ l reaction volume. For detailed recommendations on cDNA synthesis for the ARTIC panel please refer to Supplementary Protocol **SR9042SP359**. The product of each cDNA synthesis reaction is split and inserted into two parallel PCR reactions with 2 different primer pools for 49 amplicons.

Following PCR, the amplification products generated with Primer Pool 1 and Primer Pool 2 are combined again and purified.

The purified PCR product is then inserted into Library Amplification PCR to introduce full-length Illumina adapters and indices for multiplexing and NGS sequencing.

Once unique dual indices are added to each sample the PCR products can be purified as pool, quality controlled and NGS sequenced.

Lexogen's ARTIC panel generates library insert sizes of ~400 bp and is compatible with PE250 sequencing on Illumina machines for full coverage.

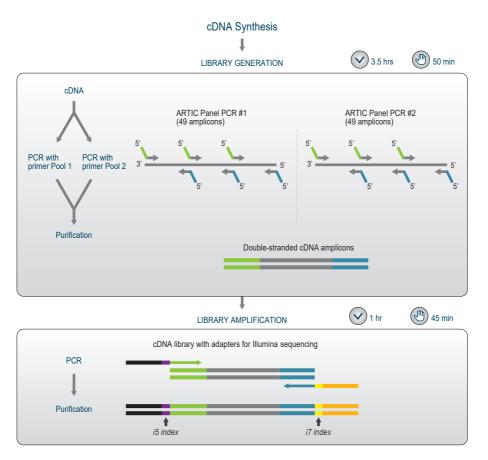


Figure 1. Schematic overview of the ARTIC panel workflow.

2. Kit Components and Storage Conditions

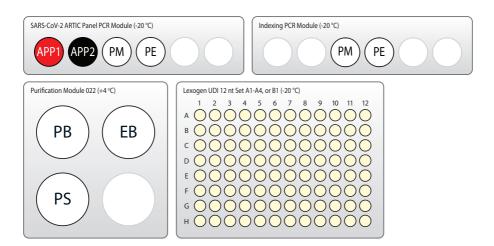


Figure 2. Location of Kit components. The SARS-CoV-2 ARTIC Panel is delivered with a Purification Module (Cat. No. 022), an Indexing PCR Module, and one of the Lexogen UDI 12 nt Index Sets. Available sets are Set A1 (UDI12A_0001-0096); Set A2 (UDI12A_0097-0192); Set A3 (UDI12A_0193-0288); Set A4 (UDI12A_0289-0384); and Set B1 (UDI12B_0001-0096).

Kit Component	Tube / Plate Label	Volume* (96 preps)	Storage
SARS-CoV-2 ARTIC Panel PCR Module			
ARTIC Primer Pool 1	APP1	317 μΙ	-20 °C
ARTIC Primer Pool 2	APP2 ●	317 μΙ	-20 °C
PCR Mix	PM O	740 μΙ	-20 °C
PCR Enzyme Mix	PEO	106 μΙ	-20 °C
Lexogen UDI 12 nt Sets			
Lexogen UDI 12 nt Set A1, A2, A3, A4, or B1	UDI12A_0001-0096, UDI12A_0097-0192, UDI12A_0193-0288, UDI12A_0289-0384, UDI12B_0001-0096	10 µl ∕ reaction	-20°C
Lexogen UDI 12 nt Sets A1-A4	UDI12A_0001-0384		-20 °C
Indexing PCR Module			
PCR Mix	PM O	740 µl	-20 °C
PCR Enzyme Mix	PEO	106 μΙ	-20 °C
Purification Module			
Purification Beads	РВ	4,646 µl	+4 °C
Purification Solution	PS	5,704 μΙ	+4 °C
Elution Buffer	ЕВ	10,560 μΙ	+4 °C

* including ≥10 % surplus

Before use, check the contents of **PS** which may precipitate during shipping. If a precipitate is visible or the content appears milky, incubate at 37 °C until solution components dissolve completely. Equilibrate to room temperature again before use.

3. User-Supplied Consumables and Equipment

Check to ensure that you have all of the necessary materials and equipment before beginning with the protocol. All reagents, equipment, and labware must be free of nucleases and nucleic acid contamination.

Reagents

- Reagents for cDNA generation including random hexamer and OligodT primers.
- 80 % fresh ethanol (washing of Purification Beads, **PB** O).

Equipment

- Magnetic rack or plate e.g., for 1.5 ml tubes: BcMag Magnetic separator-24, article# MS-03 from Bioclone; for 96-well plates: 96S Super Magnet Plate, article# A001322 from Alpaqua, or Dynamag[™]-96 Side/Side Skirted. Magnets, article # 12331D/12027 from Thermo Fisher.
- Benchtop centrifuge for spinning down liquids.
- Vortex mixer
- Calibrated single-channel pipettes for handling 1 μl to 1,000 μl volumes.
- Thermocycler
- UV-spectrophotometer to quantify RNA or DNA.

Labware

- Suitable low-binding pipette tips (pipette tips with aerosol barriers recommended).
- 1.5 ml tubes, low binding, certified ribonuclease-free.
- 200 µl PCR tubes or 96-well plates and caps or sealing foil.
- Benchtop cooler or ice pellets in ice box (for short-term storage of RNA).

Optional Equipment and Solutions

- Automated microfluidic electrophoresis station (Agilent Technologies 2100 Bioanalyzer).
- Benchtop fluorometer and appropriate assays (for library quality control and quantification).
- Agarose gels, dyes, and electrophoresis rig (for library quality control).
- RNaseZap.
- RNase inhibitor

8

4. Detailed Protocol ARTIC Panel

4.1 ARTIC Panel PCR

The cDNA generated previously is amplified in two parallel PCR reaction with 2 different primer pools for 49 amplicons (two parallel PCR rxn with 4.5 µl of cDNA as template). The amplicons on those two primer pools are shifted along the viral transcriptome/qRNA to enable variant tracing. For a cDNA synthesis protocol, see the Supplemental Protocol cDNA Synthesis for ARTIC Panel.

Preparation

PCR	Purification (Cat. No. 022)
PM ○ - thawed at RT APP1 ○ - thawed at RT APP2 ○ - thawed at RT PE ○ - keep on ice or at -20 °C spin down before opening!	PB - stored at +4 °C PS - stored at +4 °C 80 % EtOH - provided by user prepare fresh! EB - stored at +4 °C
Thermocycler 95 °C, 60 sec 95 °C, 15 sec 63 °C, 5 min 72 °C, 60 sec 10 °C, ∞	Equilibrate all reagents to room temperature for 30 minutes prior to use.

PCR with either APP1 or APP2

ATTENTION: Use only one primer pool per PCR reaction! Do not mix ARTIC Primer Pool 1 (APP1 ●) and ARTIC Primer Pool 2 (APP2 ●)! This protocol is optimized for use with cDNA generated in 10 µl volume, see Supplemental Protocol SR9042SP359 for more information on cDNA Synthesis for ARTIC Panel.

NOTE: At this point we recommend placing the Purification Module (PB, PS, and EB) for step 7 at room temperature to equilibrate for at least 30 minutes.



- Prepare a mastermix 1 containing 2 µl PCR Mix (PM O), 3 µl of ARTIC Primer Pool 1 (APP1 ●), and 0.5 µl PCR Enzyme (PE O) per reaction.
- Prepare a mastermix 2 containing 2 µl PCR Mix (PM O), 3 µl of ARTIC Primer Pool 2 (APP2 ●), and 0.5 µl PCR Enzyme (PE O) per reaction.
- For each sample, split a 10 µl RT reaction from one sample for ARTIC PCR. Mix 4.5 µl of the 10 µl RT reaction with 5.5 µl PM / APP1 ● / PE Mix.

- 4 Mix the remaining 4.5 μl of the same 10 μl RT reaction with 5.5 μl **PM / APP2 / PE** Mix.
- Conduct 35 cycles of PCR with the following program: Initial denaturation at 95 °C for 60 seconds, 35 cycles of 95 °C for 60 seconds, 63 °C for 5 minutes, and 72 °C for 60 seconds, hold at 10 °C.
 - **©** Safe stopping point. Libraries can be stored at -20 °C at this point.

Purification

The amplicon library is purified to remove PCR components that can interfere with quantification. The Purification Reagents (**PB**, **PS**, **and EB**) should equilibrate for 30 minutes at room temperature before use. The Purification Beads (**PB**) must be fully resuspended before use. Thorough mixing by pipetting or vortexing is recommended.

ATTENTION: If the libraries were stored at -20 °C, ensure that they are thawed and equilibrated to room temperature before restarting the protocol.

- Combine the Primer Pool 1 and Primer Pool 2 PCR reaction from the same cDNA, resulting in a total volume of 20 µl.
- Add 16 μ l of thoroughly resuspended Purification Beads (**PB**) to each reaction. Mix well, and incubate for 5 minutes at room temperature.
- Place the plate onto a magnet and let the beads collect for 2 5 minutes, or until the supernatant is completely clear.
- Remove and discard the clear supernatant without removing the plate from the magnet. Do not disturb the beads!
- Add 30 µl of Elution Buffer (**EB**), remove the plate from the magnet and resuspend the beads fully in **EB**. Incubate for 2 minutes at room temperature.
- Add 27 μ l of Purification Solution (**PS**) to the **PB** / **EB** mix to reprecipitate the library. Mix thoroughly and incubate for 5 minutes at room temperature.
- Place the plate onto a magnet and let the beads collect for 2 5 minutes, or until the supernatant is completely clear.
- Remove and discard the clear supernatant without removing the PCR plate from the magnet. Do not disturb the beads!
- Add 120 μ l of 80 % EtOH and incubate the beads for 30 seconds. Leave the plate in contact with the magnet as beads should not be resuspended during this washing step. Remove and discard the supernatant.
- Repeat this washing step once for a total of two washes. **ATTENTION:** Remove the supernatant completely.

- Leave the plate in contact with the magnet and let the beads dry for 5 10 minutes or until all ethanol has evaporated. **ATTENTION:** Do not let the beads dry too long!
- Add 20 µl of Elution Buffer (**EB**) per well, remove the plate from the magnet, and resuspend the beads fully in **EB**. Incubate for 2 minutes at room temperature.
- Place the plate onto a magnet and let the beads collect for 2 5 minutes or until the supernatant is completely clear.
- Transfer 17 μl of the supernatant into a fresh PCR plate. Do not to transfer any beads.

 **Safe stopping point. Libraries can be stored at -20 °C.

4.2 Indexing PCR with UDI 12 nt Unique Dual Indices

This section describes unique dual indexing PCR for multiplexing up to 384 libraries using the Lexogen UDI 12 nt Unique Dual Indices included in these kits.

Preparation

PCR			Purification (Cat. No. 022)*
PM O Lexogen UDI 12 : PE O	nt Sets (A1 - A4, or B1)	- thawed at RT - thawed at RT - keep on ice or at -20 °C	from standard library prep kits: PB - stored at +4 °C PS - stored at +4 °C 80 % EtOH - provided by user prepare fresh! EB - stored at +4 °C
Thermocycler	95 °C, 60 sec 95 °C, 15 sec 60 °C, 15 sec 72 °C, 60 sec 72 °C, 6 min 10 °C, ∞	5-10x (depending on viral load of the sample)	Equilibrate all reagents to room temperature for 30 minutes prior to use.

PCR

The library is amplified to add the complete adapter sequences required for cluster generation and unique indices for multiplexing, and to generate sufficient material for quality control and sequencing.

ATTENTION: Important notes for Library Amplification.

- Avoid cross contamination when using the Lexogen UDI 12 nt Indexing Sets. Spin down the
 Index Set before opening and visually check fill levels. Pierce or cut open the sealing foil of
 the wells containing the desired UDIs only. Reseal opened wells using fresh sealing foil after
 use to prevent cross contamination.
- Each well of the Lexogen UDI 12 nt Index Set is intended for single use only.

NOTE: At this point we recommend placing the Purification Module (**PB**, **PS**, and **EB**) for step at room temperature to equilibrate for at least 30 minutes.

- Prepare a mastermix containing 7 μ l PCR Mix (**PM** O) and 1 μ l PCR Enzyme (**PE** O) per reaction.
- Add 8 μ l of the **PM / PE** mastermix to 17 μ l of the purified ARTIC PCR product from step 20.

Add 10 μ l of the respective Unique Dual Index Primer pair (UDI12A_0001-0384, or UDI12B_0001-0096) to each sample. Use only one UDI per sample! **ATTENTION:** Spin

down the plates containing the UDIs before opening! Pierce or cut open the sealing foil of the wells containing only the desired UDIs. Reseal opened wells of the UDI plate after use with a fresh sealing foil to prevent cross contamination!

Conduct 5 - 10 cycles of PCR (determine the required cycle number by qPCR) with the following program: Initial denaturation at 95 $^{\circ}$ C for 60 seconds, 5 - 10 cycles of 95 $^{\circ}$ C for

24 15 seconds, 60 °C for 15 seconds and 72 °C for 60 seconds, and a final extension at 72 °C for 6 minutes, hold at 10 °C.

■ Safe stopping point. Libraries can be stored at -20 °C at this point.

Purification

The final library is purified to remove PCR components that can interfere with quantification. The Purification Reagents (**PB**, **PS**, **and EB**) should equilibrate for 30 minutes at room temperature before use. The Purification Beads (**PB**) must be fully resuspended before use. Thorough mixing by pipetting or vortexing is recommended.

ATTENTION: If the libraries were stored at -20 °C, ensure that they are thawed and equilibrated to room temperature before restarting the protocol.

- Add 28 µl of thoroughly resuspended Purification Beads (**PB**) to each reaction. Mix well, and incubate for 5 minutes at room temperature.
- Place the plate onto a magnet and let the beads collect for 2 5 minutes, or until the supernatant is completely clear.
- Remove and discard the clear supernatant without removing the plate from the magnet. Do not disturb the beads!
- Add 30 µl of Elution Buffer (**EB**), remove the plate from the magnet and resuspend the beads fully in **EB**. Incubate for 2 minutes at room temperature.
- Add 27 µl of Purification Solution (**PS**) to the **PB / EB** mix to reprecipitate the library. Mix thoroughly and incubate for 5 minutes at room temperature.

- Place the plate onto a magnet and let the beads collect for 2 5 minutes, or until the supernatant is completely clear.
- Remove and discard the clear supernatant without removing the PCR plate from the magnet. Do not disturb the beads!
- Add 120 μ l of 80 % EtOH and incubate the beads for 30 seconds. Leave the plate in contact with the magnet as beads should not be resuspended during this washing step. Remove and discard the supernatant.
- Repeat this washing step once for a total of two washes. **ATTENTION:** Remove the supernatant completely.
- Leave the plate in contact with the magnet and let the beads dry for 5 10 minutes or until all ethanol has evaporated. **ATTENTION:** Do not let the beads dry too long!
- Add 17 µl of Elution Buffer (**EB**) per well, remove the plate from the magnet, and resuspend the beads fully in **EB**. Incubate for 2 minutes at room temperature.
- Place the plate onto a magnet and let the beads collect for 2 5 minutes or until the supernatant is completely clear.
- 37 Transfer 16 μ l of the supernatant into a fresh PCR plate. Do not to transfer any beads.
- At this point, the libraries are finished and ready for quality control, pooling , and cluster generation.
 - Safe stopping point. Libraries can be stored at -20 °C.

5. Short Procedure ARTIC Panel

ATTENTION: Spin down solutions before opening tubes or plates!

4 hrs ARTIC Panel PCRs

ARTIC panel PCRs
Split a 10 µl random hexamer/oligo(dT)-primed RT reaction into two parallel PCR reactions with either ARTIC Primer Pool 1 (APP1 ●) OR ARTIC Primer Pool 2 (APP2 ●). ATTENTION: Only add 1 primer pool per PCR reaction!
Prepare Mastermix 1 with 2 μ l PM O, 3 μ l of ARTIC Primer Pool 1 (APP1 •), and 0.5 μ l PE O per rxn. Mix well and spin down.
Prepare Mastermix 2 with 2 μl PM O, 3 μl of ARTIC Primer Pool 1 (APP2 •), and 0.5 μl PE O per rxn. Mix well and spin down.
Mix 4.5 μl of a 10 μl RT reaction with 5.5 μl PM / APP1 ● / PE Mix.
Mix 4.5 μ l of a 10 μ l RT reaction with 5.5 μ l PM / APP2 \bullet / PE Mix.
PCR: 95 °C, 60 sec
95 °C, 15 sec
60 °C, 15 sec 35x
72 °C, 60 sec
10 °C, ∞. ⊕ Safe stopping point.
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 µl (2x 10 µl).
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 μ l (2x 10 μ l).
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 μl (2x 10 μl). Add 16 μl PB , mix well, incubate for 5 min at RT.
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 μl (2x 10 μl). Add 16 μl PB , mix well, incubate for 5 min at RT. Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant.
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 μl (2x 10 μl). Add 16 μl PB , mix well, incubate for 5 min at RT. Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant. Add 30 μl EB , remove from magnet, mix well, incubate 2 min at room temperature
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 μl (2x 10 μl). Add 16 μl PB , mix well, incubate for 5 min at RT. Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant. Add 30 μl EB , remove from magnet, mix well, incubate 2 min at room temperature Add 27 μl PS , mix well, incubate 5 min at room temperature
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 μl (2x 10 μl). Add 16 μl PB , mix well, incubate for 5 min at RT. Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant. Add 30 μl EB , remove from magnet, mix well, incubate 2 min at room temperature Add 27 μl PS , mix well, incubate 5 min at room temperature Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant. Wash the beads twice with 120 - 150 μl 80 % EtOH, 30 sec. ATTENTION: Use 150 μl for
Purification Combine the primer pool 1 and pool 2 PCR reaction from the same cDNA sample to a total volume of 20 μl (2x 10 μl). Add 16 μl PB , mix well, incubate for 5 min at RT. Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant. Add 30 μl EB , remove from magnet, mix well, incubate 2 min at room temperature Add 27 μl PS , mix well, incubate 5 min at room temperature Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant. Wash the beads twice with 120 - 150 μl 80 % EtOH, 30 sec. ATTENTION: Use 150 μl for 1.5 ml tubes.

	Indexing PCR		
	Prepare a Mastermix of 7 μl PM O + 1 μl PE O per rxn. Mix well and spin down.		
	Add 10 μ l of one Unique Dual Index Primer pair (UDI12A_0001-0384, or UDI12B_0001-0096) to each sample. ATTENTION: Reseal opened index wells after use! Use only one UDI / sample.		
	Add 17 µl purified ARTIC panel PCR product.		
	PCR: 95 °C, 60 sec		
	95 °C, 15 sec 60 °C, 15 sec 72 °C, 60 sec 72 °C, 6 min		
	10 °C, ∞. 👉 Safe stopping point.		
	Purification		
	Add 28 μl PB , mix well, incubate for 5 min at RT.		
	Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant.		
	Add 30 μ l EB , remove from magnet, mix well, incubate 2 min at room temperature		
	Add 27 μl PS , mix well, incubate 5 min at room temperature.		
	Place on magnet for 5 - 10 min until the supernatant is clear, discard supernatant.		
00	Wash the beads twice with 120 - 150 μl 80 % EtOH, 30 sec. ATTENTION: Use 150 μl for 1.5 ml tubes.		
	Air dry beads for 5 - 10 min. ATTENTION: do not over dry the beads!		
	Add 17 µl EB , remove from magnet, mix well, incubate 2 min at RT.		
	Place on magnet for 2 - 5 min, transfer 16 µl of the supernatant to a fresh tube.		

6. Appendix A: Quality Control

The analysis of a small volume of the amplified library with microcapillary electrophoresis has become standard practice for many NGS laboratories and generates information regarding library concentration and size distribution. Several electrophoresis platforms are available fromvarious manufacturers. For low- to medium-throughput applications, we recommend the Bioanalyzer 2100 and High Sensitivity DNA chips (Agilent Technologies, Inc.). For high throughput applications instruments such as the Fragment Analyzer or 2200 TapeStation (Agilent Technologies, Inc.), or LabChip GX II (Perkin Elmer) are recommended. Typically, 1 µl of a QuantSeq library produced according to the directions in this manual is sufficient for analysis. Depending on the minimum sample loading requirements for each instrument, 1 µl of the finished library may be diluted to the required volume (e.g., 2 µl sample for TapeStation and 10 µl for LabChip GX II).

More accurate library quantification can be achieved with custom or commercially available qPCR assays. With these assays, the relative or absolute abundance of amplifiable fragments contained in a finished library is calculated by comparing Cq values to a set of known standards. While delivering a more accurate quantification, these assays do not supply the user with information regarding library size distribution. Unwanted side-products such as linker-linker artifacts are not discernible from the actual library in the qPCR assay as both will be amplified. Hence it is highly recommended to combine such an assay for quantification with microcapillary electrophoresis analysis for library size distribution.

Typical Results

Figure 3 shows the bioanalyzer trace of a typical ARTIC amplicon Library with unique dual indexing. The amplicons are \sim 400 bp long resulting in a final library of \sim 490 - 590 bp, linker-linker by-products are \sim 175 bp.

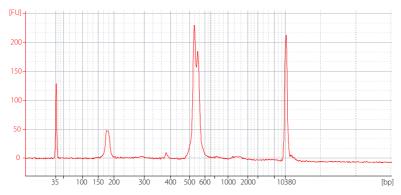


Figure 3. Bioanalyzer trace of dual-indexed ARTIC amplicon libraries generated from SARS-CoV-2 cDNA. Reverse transcription was carried out as described in SR9042SP359, cDNA was split and 4.5 μ l of the reverse transcription product was inserted in ARTIC PCRs with Primer Pool 1 and Primer Pool 2, respectively. The products were combined, purified and inserted into indexing PCR followed by the final purification. The amplicon library has an average size of ~530 bp, linker-linker by-products are visible at ~175 bp.

7. Appendix B: Sequencing

The SARS-CoV-2 ARTIC Panel generates amplicons with a size of ~400 bp. For full coverage, sequencing in paired-end mode using a read length of 250 (PE250) is recommended. Libraries are generated with 12 nt unique dual indices.

Multiplexing

The Lexogen UDI 12 nt Sets enable adjustable read-out of 8, 10, or the full 12 nucleotides long UDI sequence while maintaining superior error correction features. The longer the UDI read-out the higher is the error correction capability. The complete lists of i5 and i7 index sequences for all Lexogen UDI 12 nt Sets are available at www.lexogen.com/docs/indexing. Depending on the instrument workflow, flow cell type (paired-end, PE; single-read, SR), and chemistry, i5 indices are sequenced on the forward or the reverse complement strand. For more information on UDI Set selection, please visit our webpage, consult our FAQs, or contact www.lexogen.com.

Sets A1 - A4 (UDI12A_0001-0384) for Forward Strand Workflow (A)

For instruments using the Forward Strand workflow, the Index 2 Read (i5) is primed using the Grafted P5 Oligo on the flow cell (or the Index 2 (i5) Sequencing Primer (HP9) for SR HiSeq 2000 / 2500 flow cells).

Set B1 (UDI12B_0001-0096) for Reverse Complement Workflow (B)

For instruments using the Reverse Complement workflow, the Index 2 Read (i5) is performed after Read 2 Resynthesis, using the Index 2 (i5) Sequencing Primer.

ATTENTION: If library preps with UDI Set A (UDI12A_0001-0384) are sequenced on Illumina machines using the Reverse Complement Workflow (B), or *vice versa*, the i5 Index will be read out as reverse complement. **In this case, all 12 nt of the i5 Index must be read out if error correction shall be applied**. Additionally, the reverse complement of the i5 index read out needs to be analyzed.

EXAMPLE: i512_0001 is read as GTCTTTGGCCCT instead of AGGGCCAAAGAC. The read out in reverse complement (GTCTTTGGCCCT) shall be used for demultiplexing and error correction.

Lane Mix Preparation

Libraries should ideally be pooled in an equimolar ratio for multiplexed sequencing. It is important to ensure accurate quantification of the individual libraries prior to pooling, as well as for the library pool (lane mix). To quantify your libraries:



Measure the concentration of each library, using either qPCR or fluorescence-based assays (e.g., QuBit, Thermo Fisher Scientific Inc.).



Determine the average library size, using microcapillary electrophoresis analysis (e.g., Bioanalyzer, Agilent Technologies, Inc.). Set the range to 175 - 1,000 bp to include the whole library size distribution, and to exclude any linker-linker artifacts or overcycling bumps (>1,000 bp).

Molarity is then calculated from the average library size and the concentration (ng/ μ l) using the following equation:

Molarity = (library concentration $(ng/\mu l) \times 10^6$) / (660 x average library size (bp))

A template for molarity calculation is also available for download from www.lexogen.com.

After pooling the libraries, the prepared lane mix and any dilutions made for denaturing (e.g., 2 nM), should be reanalyzed to ensure the accuracy of the concentration. This can be performed according to steps 1 and 2 as above.

8. Appendix C: Library Reamplification

If your library yields are extremely low and insufficient for pooling, reamplification can be performed using the Reamplification Add-on Kit for Illumina (080.96). This kit is available only upon request. Please contact Lexogen at support@lexogen.com for more information. Please note that the PCR Add-on Kit (Cat. No. 020) **cannot** be used for reamplification of dual-indexed libraries.

9. Appendix D: Data Analysis

Demultiplexing can be carried out by the standard Illumina pipeline. Index sequences (UDI12A_0001-0384 and UDI12B_0001-0096) are available for download at www.lexogen.com/docs/indexing.

Additionally to the standard error-correction included in the Illumina pipeline, Lexogen's idemux tool is freely available on github: https://github.com/Lexogen-Tools and can be used for higher accuracy in error correction.

For more information on data processing please visit $\underline{\text{https://github.com/Lexogen-Tools}}$ or contact $\underline{\text{support@lexogen.com}}$.

10. Appendix E: Revision History

Publication No. / Revision Date	Change	Page
SR9042UG358V0100 July 05, 2021	Initial Release.	



Associated Products:

008, 099 (SPLIT RNA Extraction Kits)
022 (Purification Module with Magnetic Beads)



Lexogen Artic Panel for SARS-CoV2 · Short Protocol

Lexogen GmbH
Campus Vienna Biocenter 5
1030 Vienna, Austria
Telephone: +43 (0) 1 345 1212-41
Fax: +43 (0) 1 345 1212-99
E-mail: support@lexogen.com

© Lexogen GmbH, 2021

Lexogen, Inc.
51 Autumn Pond Park
Greenland, NH 03840, USA
Telephone: +1-603-431-4300
Fax: +1-603-431-4333
www.lexogen.com