Manual

NxSeq SARS-CoV-2 Whole Genome Library Kit, 96 reactions

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT
-20 ºC storage required for most components
immediately upon receipt

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Manual

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Contents

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1. Product description

The NxSeq™ SARS-CoV-2 Whole Genome Library Kit supplies the targeting primers, enzymes and other reagents required to build overlapping, sequenceable, targeted amplicons across the SARS-CoV-2 genome for whole genome next generation sequencing on Illumina sequencers. Indexing plates must be purchased separately (Cat. No. GEN-9754-001 thru GEN-9754-015), and 15 different indexing plates are available with 96 different combinatorial dual indexes (CDI) per plate for a total of 1440 CDI. This kit is compatible with single- or double-stranded cDNA input made from SARS-CoV-2 positive samples. We recommend choosing a cDNA synthesis system that uses a random-primed synthesis method, exhibits RT processivity >1 kb, and is designed to be used in two-step RT-PCR workflows.

The general kit protocol proceeds as follows:

1. Single- or double-strand cDNA synthesis of SARS-CoV-2 positive RNA samples (not included in this kit).
2. Multiplex PCR in a single tube using the cDNA as input and the SCV2 Targeting Primers and high fidelity PCR Master Mix included in this kit to produce overlapping amplicons covering the SARS-CoV-2 genome.
3. Addition of indexed Illumina-compatible sequencing adaptors to each amplicon.
4. qPCR-based library quantitation followed by library normalisation and sequencing (not included).

The targeting primers and subsequent 341 SARS-CoV-2 amplicons were validated using synthetic SARS-CoV-2 genomes from Twist Biosciences (Cat. no. 102024). As an example of successful cDNA synthesis, we used the Superscript™ IV First-Strand Synthesis System (ThermoFisher, Cat. no. 18091050) and an input RNA sample composed of the 50 ng of Universal Human Reference RNA (Agilent, Cat. No. 740000) spiked with 1000 copies of the synthetic viral genome. The recommended Superscript IV protocol was followed, except the RT incubation at 50 °C was increased from the recommended 10 minutes to ≥30 minutes. The RNase H step was not performed, and 10 µL of the final cDNA was used directly as input into the targeted amplicon protocol outlined in this manual.

2. Product designations and kit components

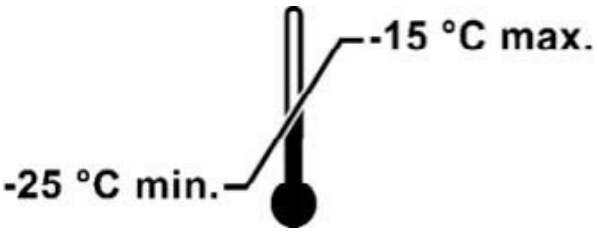
Product	Kit size	Catalogue number	Reagent description	Part numbers	Volume
NxSeq SARS-CoV-2 Whole Genome Library Kit	96 reactions	GEN-SCV2-0096	SCV2 Targeting Primers (STP)	F816145-1	212 µL
			Amplification Primer (AP)	F816146-1	320 µL
			PCR Master Mix (MM)	F816147-1	1600 µL
			Sample Dilution Buffer	F816148-1	2.4 mL
			Indexing Buffer	F816149-1	3274 µL
			Indexing Enzyme 1 (E1)	F816150-1	106 µL
			Indexing Enzyme 2 (E2)	F816151-1	106 µL
			Indexing Enzyme 3 (E3)	F816152-1	212 µL
			Elution Buffer	F816153-1	2.4 mL
			PEG/NaCl Solution	F816154-1	5 mL

Product	Size	Catalogue number	Indexing grid per plate*	Volume per well
Indexing plate 1	96 reactions	GEN-9754-001	i5 L501-508 × i7 L701-712	18 µL
Indexing plate 2	96 reactions	GEN-9754-002	i5 L501-508 × i7 L713-724	18 µL
Indexing plate 3	96 reactions	GEN-9754-003	i5 L501-508 × i7 L725-736	18 µL
Indexing plate 4	96 reactions	GEN-9754-004	i5 L501-508 × i7 L737-748	18 µL
Indexing plate 5	96 reactions	GEN-9754-005	i5 L501-508 × i7 L749-760	18 µL
Indexing plate 6	96 reactions	GEN-9754-006	i5 L509-516 × i7 L701-712	18 µL
Indexing plate 7	96 reactions	GEN-9754-007	i5 L509-516 × i7 L713-724	18 µL
Indexing plate 8	96 reactions	GEN-9754-008	i5 L509-516 × i7 L725-736	18 µL
Indexing plate 9	96 reactions	GEN-9754-009	i5 L509-516 × i7 L737-748	18 µL
Indexing plate 10	96 reactions	GEN-9754-010	i5 L509-516 × i7 L749-760	18 µL
Indexing plate 11	96 reactions	GEN-9754-011	i5 L517-524 × i7 L701-712	18 µL
Indexing plate 12	96 reactions	GEN-9754-012	i5 L517-524 × i7 L713-724	18 µL
Indexing plate 13	96 reactions	GEN-9754-013	i5 L517-524 × i7 L725-736	18 µL
Indexing plate 14	96 reactions	GEN-9754-014	i5 L517-524 × i7 L737-748	18 µL
Indexing plate 15	96 reactions	GEN-9754-015	i5 L517-524 × i7 L749-760	18 µL

Each indexing plate contains 96 combinatorial dual indexes in an (8) i5 × (12) i7 matrix. See the appendix for index details.

3. Component storage conditions and warnings

Store all components at -20 °C except the PEG/NaCl Solution, which should be stored at room temperature.



***IMPORTANT**: Separate the key reagents and keep the multiplex PCR reagents in a pre-PCR area, and indexing reagents in a post-PCR area.*

4. Additional materials and equipment needed

Material and equipment needed	Vendor
First-strand cDNA synthesis kit	Various
AMPure® XP Beads or equivalent	Beckman Coulter (Cat. #A63880, A63881, A63882)
Ethanol (high purity)	Various
Nuclease-Free Water	Thermo Fisher Scientific (Cat. #AM9938)
Magnetic rack	Various
0.2 mL thin wall PCR tubes, strips or plates	Various
qPCR-based Library Quantification Kit	Various
Qubit® dsDNA HS Assay Kit	Thermo Fisher Scientific (Cat. # Q32851 or Q32854)
Qubit® Fluorometer	Thermo Fisher Scientific
Aerosol-resistant, low retention pipettes, tips (2–1000 µL)	Various
PCR thermal cycler	Various
Minifuge	Various

5. Protocol

General notes:

- Before starting the protocol, remove enzymes from -20 °C storage and place on ice for ≥10 minutes to allow each enzyme to equilibrate to 4 °C prior to use.
- Remove PCR Master Mix from -20 °C storage and allow to thaw on ice. Mix by inversion until all components in the master mix are dissolved.
- After thawing the other, non-enzyme reagents, briefly vortex to mix thoroughly, centrifuge each tube to collect contents in the bottom of the tube and place on ice.
- Always add components to each reaction or master mix in the order listed in the protocol. The dual index adaptors are the only reagents added individually to each reaction.
- We recommend using at least 24 reactions from the 96 reaction kit each time in order to prevent running out of reagents due to the 5-10% overage required per set of samples.
- **Assemble all reagent master mixes and reactions ON ICE. Scale all reagent volumes according to the number of reactions needed and include 5% excess volume to account for any pipetting losses.**
- **Prepare your SARS-CoV-2 cDNA samples before beginning this protocol.**
- 10% overfill of each reagent is included to enable automation and accommodate master mix formulations. If greater volumes or bulk dispenses are required for your automation platform or workflow, please contact us.

Avoiding cross-contamination notes:

- **Since this kit produces targeted amplicons, it is critical to avoid contaminating samples or initial reactions with amplicons produced during previous experiments. As such, physically separate laboratory space, equipment, and supplies between areas where pre-PCR and post-PCR processes are performed.**
- Clean lab areas with 10% bleach (0.5% sodium hypochlorite) between experiments.
- Use barrier pipette tips.
- Always change tips between samples.
- Move the multiplex PCR reactions after amplification to the post-PCR area before opening.
- When preparing No Template Control (NTC) reactions, dispense and seal those reactions prior to opening SARS-CoV-2 cDNA samples in the area.

Before you start:

- Prepare fresh 80% ethanol solution, approximately 1 mL per sample, using absolute ethanol and nuclease free water.
- Ensure all reagents are thawed and mixed as described above.
- Ensure PEG/NaCl Solution is at room temperature.
- Prepare reagents and master mixes in advance so there are no delays during the protocol. We recommend building master mixes with approximately 5% overage to account for any pipetting errors, etc.

5.1. Programme the thermal cycler

Multiplex PCR thermal cycler programme*

Step	Temperature	Time	Cycles	Lid heating
1	98 °C	30 seconds		ON - 105 °C
2	**98 °C**	**10 seconds**	**Repeat steps 2-4 for 4 cycles**	
3	**60 °C**	**5 minutes**		
4	**65 °C**	**60 seconds**		
5	**98 °C**	**10 seconds**	**Repeat steps 5-6 for 23 cycles**	
6	**64 °C**	**60 seconds**		
7	65 °C	60 seconds		
8	4 °C	Hold		OFF

*Note: The number of cycles can vary based on cDNA input quality and quantity.

Indexing reaction thermal cycler programme

Step	Temperature	Time	Lid heating
1	37 °C	20 minutes	OFF

5.2. Multiplex PCR to produce overlapping SARS-CoV-2 amplicons

1. Start the thermal cycler and put it on hold with a 98 °C block temperature and 105 °C lid temperature. Allow it to reach temperature before starting step 6 below.
2. Add each cDNA sample to a PCR tube or well of a 96-well PCR plate on ice as follows:

Volume per well (µL)	Component
X (≤ 10 µL)	cDNA sample
10-X	Sample Dilution Buffer
10	**Total**

3. Mix each reagent as follows before setting up the master mix.
    - Vortex the SCV2 Targeting Primer and Amplification Primer tubes.
    - Gently invert the PCR Master Mix tube.
4. Make a Multiplex PCR master mix using the following single reaction formulation and scale to the total number of reactions needed including approximately 5% overage.

Volume per well (µL)	Component
2	SCV2 Targeting Primers (STP)
3	Amplification Primer (AP)
15	PCR Master Mix
20	**Total per reaction**

5. Carefully mix the Multiplex PCR master mix by vortexing gently.
6. Combine 20 µL of the Multiplex PCR master mix with each 10 µL cDNA sample and mix by pipetting, remembering to change tips between samples.
7. Place the PCR tube(s) or 96-well plate in the preheated thermal cycler (step 1) and run the multiplex PCR programme.

   NOTE: *Near the end of the thermal cycler programme, prepare an Indexing reaction mix in the post-PCR area as outlined in the next section.*

8. After PCR amplification is complete, move samples to the post-PCR area before proceeding.

5.3. Indexing reaction master mix set-up

Make an Indexing reaction master mix on ice using the following formulation per reaction/sample and scale to the total number of reactions needed including approximately 5% overage.

Volume per well (µL)	Component
31	Indexing Buffer
1	Indexing Enzyme 1 (E1)
1	Indexing Enzyme 2 (E2)
2	Indexing Enzyme 3 (E3)
35	**Total per reaction**

5.4. Size selection and clean-up #1

1. Bring AMPure XP (or similar) Beads and multiplex PCR samples to room temperature. Briefly, vortex the AMPure XP beads to mix before use.
2. Add 36 µL of AMPure XP Beads to each 30 µL multiplex PCR sample. Mix each sample by vortexing and then do a quick spin to move contents to the bottom of the tube or well.
3. Incubate at room temperature for 5 minutes; do not use a magnetic rack.
4. Place the sample tube or plate in a magnetic rack for 5 minutes (until the supernatant becomes clear).
5. With the tube/plate in the magnetic rack, gently remove the supernatant with a pipette and discard.

6. Wash the beads by adding 180 µL of 80% ethanol to each tube/well. Wait 30 seconds, remove the ethanol with a pipette and discard the ethanol.
7. Repeat the 80% ethanol wash (step 6).
8. Quick spin the bead-cleaned samples and place back on the magnetic rack. Using a P10 pipette, carefully remove any residual ethanol present at the bottom of the tube without disturbing the bead pellet.

5.5. Indexing

1. Start the Indexing programme on the thermal cycler, and immediately put it on hold so the block can reach 37 °C.
2. Add 35 µL of the cold Indexing reaction master mix to each sample AMPure XP Bead pellet.
3. Transfer15 µL of a CDI mix from a well of an Indexing Plate to the Indexing reaction tube/well containing the amplicons and beads. Repeat this step for all samples ensuring that a different CDI pair is transferred to each sample, while recording which CDI pair is used per sample.
4. Place the Indexing reaction tubes or plate into the thermal cycler, and resume the Indexing programme (20 minutes at 37 °C) with the heated lid turned off.

5.6. Size selection and clean-up #2

1. Bring the PEG/NaCl Solution and indexed sample tubes or plate to room temperature. Briefly vortex the PEG/NaCl Solution before use.
2. Add 42.5 µL of PEG/NaCl Solution to each 50 µL indexed sample containing AMPure XP Beads from the first clean-up/size selection step. Mix each sample by vortexing and then do a quick spin to move contents to the bottom of the tube or well.
3. Incubate at room temperature for 5 minutes; do not use a magnetic rack.
4. Place the sample tube or plate in a magnetic rack for 5 minutes (until the supernatant becomes clear).
5. With the tube/plate in the magnetic rack, gently remove the supernatant with a pipette and discard.
6. Wash the beads by adding 180 µL of 80% ethanol to each tube/well. Wait 30 seconds, remove the ethanol with a pipette and discard the ethanol.
7. Repeat the 80% ethanol wash (step 6).
8. Quick spin the bead-cleaned samples and place back on the magnetic rack. Using a P10 pipette, carefully remove any residual ethanol present at the bottom of the tube without disturbing the bead pellet.
9. Immediately add 22 µL Elution Buffer to each sample pellet, remove from the magnetic rack and mix the beads and buffer gently by pipetting up and down 10 times (Do not vortex).
10. Incubate at room temperature for 2 minutes; do not use a magnetic rack during the incubation.
11. Place the tube/plate in a magnetic rack for 2-3 minutes (until the supernatant becomes clear).
12. Transfer 20 µL of supernatant containing your cleaned amplicon library to a new PCR tube or well of a 96-well plate. Be careful to avoid transferring any magnetic beads to your final tube/well.

5.7. Library quantification

Notes

- Because the final amplicon libraries are not PCR amplified after the Indexing step, these libraries are a mixture of fully adapted/sequenceable library fragments and partially adapted/unsequenceable library fragments. Therefore, qPCR library quantification must be used instead of fluorometric assays to quantify the amount of sequenceable library fragments in each sample.

1. Quantify each library using a qPCR library quantification kit according to the manufacturer's instructions.
2. We recommend quantifying a 1:10,000 dilution of each library in triplicate.
3. Use library qPCR standards of approximately 265 bp in size, and an estimated SARS-CoV-2 amplicon library size of 265 bp to calculate library molarity in combinations with the qPCR Ct values for each library.

5.8. Sequencing

- Final libraries are compatible with and can be run on any Illumina sequencer.
- Please refer to the latest version of the Illumina Experiment Manager for instructions on how to setup a sample sheet.
- Be sure to select the appropriate workflow parameters needed to sequence these amplicons:
    - Read Type: "Paired End"
    - Cycle Read 1: "151, Cycle Read 2: "151
- See the Appendix B and web resources for combinatorial dual index sequences per well of each indexing plate.
- Be certain that "Use adapter trimming" and Use adapter trimming Read 2" are selected. Failure to trim adapter sequences will result in incorrect primer trimming and inaccurate variant calling. To overcome this problem, enable automatic trimming by the Illumina sequencer software or perform adaptor trimming by Trimmomatic during data analysis.
- Each amplicon in these libraries has target regions that overlap with the target regions from adjacent amplicons. As such, the synthetic primer sequences incorporated into each amplicon should also also be trimmed from the final sequencing reads to ensure that only the target regions are being analysed for variants. Primerclip, which is publically available, can be used for this trimming step.
- Sequencing of amplicon libraries constructed using this kit have been validated for sequencing without added PhiX on both the MiSeq and the MiniSeq sequencers. For sequencing on the NextSeq 550, please consult the Illumina recommendations for PhiX spike-ins when sequencing low diversity amplicon libraries such as these.

6. Technical support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Product guarantee: LGC, Biosearch Technologies guarantees that this product will perform as specified for one year from the date of shipment.

7. Appendices

7.1. Appendix A: Sequencing depth and multiplexing options

- Biosearch Technologies recommends an average sequencing depth of 200X-700X per SARS-CoV-2 library. This depth enables accurate identification of variants within the viral genome. You can estimate the level of multiplexing possible using the following formula. We recommend some initial empirical testing prior to setting up large scale multiplexing sequencing runs.

$$\textbf{Number of multiplexed samples} = \frac{\text{number of paired end reads per run}}{341 * \text{intended average read depth}}$$

7.2. Appendix B: Indexing Plate 1 sequences and layout

- The following table lists all the combinatorial dual indexes used in Indexing Plate 1 (Cat. No. GEN-9754-001). These indexes equal the Illumina TruSeq indexes with the same i5 and i7 numbers. For a listing of the indexes present and their layout in Indexing Plates 2-15, please visit the Resources section of the NxSeq SARS-CoV-2 Whole Genome Library Kit product webpage or contact our Technical Support Team.

i5 Index name	i5 Index sequence
L501	TATAGCCT
L502	ATAGAGGC
L503	CCTATCCT
L504	GGCTCTGA
L505	AGGCGAAG
L506	TAATCTTA
L507	CAGGACGT
L508	GTACTGAC

i7 Index name	i7 Index Sequence
L701	ATTACTCG
L702	TCCGGAGA
L703	CGCTCATT
L704	GAGATTCC
L705	ATTCAGAA
L706	GAATTCGT
L707	CTGAAGCT
L708	TAATGCGC
L709	CGGCTATG
L710	TCCGCGAA
L711	TCTCGCGC
L712	AGCGATAG

Index plate ID_ basic sample ID	Plate well	i5 Index name	i5 Index sequence	i7 Index name	i7 Index sequence
Plate01_01	A01	L501	TATAGCCT	L701	ATTACTCG
Plate01_02	B01	L502	ATAGAGGC	L701	ATTACTCG
Plate01_03	C01	L503	CCTATCCT	L701	ATTACTCG
Plate01_04	D01	L504	GGCTCTGA	L701	ATTACTCG
Plate01_05	E01	L505	AGGCGAAG	L701	ATTACTCG
Plate01_06	F01	L506	TAATCTTA	L701	ATTACTCG
Plate01_07	G01	L507	CAGGACGT	L701	ATTACTCG
Plate01_08	H01	L508	GTACTGAC	L701	ATTACTCG
Plate01_09	A02	L501	TATAGCCT	L702	TCCGGAGA
Plate01_10	B02	L502	ATAGAGGC	L702	TCCGGAGA
Plate01_11	C02	L503	CCTATCCT	L702	TCCGGAGA
Plate01_12	D02	L504	GGCTCTGA	L702	TCCGGAGA
Plate01_13	E02	L505	AGGCGAAG	L702	TCCGGAGA
Plate01_14	F02	L506	TAATCTTA	L702	TCCGGAGA
Plate01_15	G02	L507	CAGGACGT	L702	TCCGGAGA
Plate01_16	H02	L508	GTACTGAC	L702	TCCGGAGA
Plate01_17	A03	L501	TATAGCCT	L703	CGCTCATT
Plate01_18	B03	L502	ATAGAGGC	L703	CGCTCATT
Plate01_19	C03	L503	CCTATCCT	L703	CGCTCATT
Plate01_20	D03	L504	GGCTCTGA	L703	CGCTCATT
Plate01_21	E03	L505	AGGCGAAG	L703	CGCTCATT
Plate01_22	F03	L506	TAATCTTA	L703	CGCTCATT
Plate01_23	G03	L507	CAGGACGT	L703	CGCTCATT
Plate01_24	H03	L508	GTACTGAC	L703	CGCTCATT
Plate01_25	A04	L501	TATAGCCT	L704	GAGATTCC
Plate01_26	B04	L502	ATAGAGGC	L704	GAGATTCC
Plate01_27	C04	L503	CCTATCCT	L704	GAGATTCC
Plate01_28	D04	L504	GGCTCTGA	L704	GAGATTCC
Plate01_29	E04	L505	AGGCGAAG	L704	GAGATTCC
Plate01_30	F04	L506	TAATCTTA	L704	GAGATTCC
Plate01_31	G04	L507	CAGGACGT	L704	GAGATTCC
Plate01_32	H04	L508	GTACTGAC	L704	GAGATTCC

Plate01_33	A05	L501	TATAGCCT	L705	ATTCAGAA
Plate01_34	B05	L502	ATAGAGGC	L705	ATTCAGAA
Plate01_35	C05	L503	CCTATCCT	L705	ATTCAGAA
Plate01_36	D05	L504	GGCTCTGA	L705	ATTCAGAA
Plate01_37	E05	L505	AGGCGAAG	L705	ATTCAGAA
Plate01_38	F05	L506	TAATCTTA	L705	ATTCAGAA
Plate01_39	G05	L507	CAGGACGT	L705	ATTCAGAA
Plate01_40	H05	L508	GTACTGAC	L705	ATTCAGAA
Plate01_41	A06	L501	TATAGCCT	L706	GAATTCGT
Plate01_42	B06	L502	ATAGAGGC	L706	GAATTCGT
Plate01_43	C06	L503	CCTATCCT	L706	GAATTCGT
Plate01_44	D06	L504	GGCTCTGA	L706	GAATTCGT
Plate01_45	E06	L505	AGGCGAAG	L706	GAATTCGT
Plate01_46	F06	L506	TAATCTTA	L706	GAATTCGT
Plate01_47	G06	L507	CAGGACGT	L706	GAATTCGT
Plate01_48	H06	L508	GTACTGAC	L706	GAATTCGT
Plate01_49	A07	L501	TATAGCCT	L707	CTGAAGCT
Plate01_50	B07	L502	ATAGAGGC	L707	CTGAAGCT
Plate01_51	C07	L503	CCTATCCT	L707	CTGAAGCT
Plate01_52	D07	L504	GGCTCTGA	L707	CTGAAGCT
Plate01_53	E07	L505	AGGCGAAG	L707	CTGAAGCT
Plate01_54	F07	L506	TAATCTTA	L707	CTGAAGCT
Plate01_55	G07	L507	CAGGACGT	L707	CTGAAGCT
Plate01_56	H07	L508	GTACTGAC	L707	CTGAAGCT
Plate01_57	A08	L501	TATAGCCT	L708	TAATGCGC
Plate01_58	B08	L502	ATAGAGGC	L708	TAATGCGC
Plate01_59	C08	L503	CCTATCCT	L708	TAATGCGC
Plate01_60	D08	L504	GGCTCTGA	L708	TAATGCGC
Plate01_61	E08	L505	AGGCGAAG	L708	TAATGCGC
Plate01_62	F08	L506	TAATCTTA	L708	TAATGCGC
Plate01_63	G08	L507	CAGGACGT	L708	TAATGCGC
Plate01_64	H08	L508	GTACTGAC	L708	TAATGCGC
Plate01_65	A09	L501	TATAGCCT	L709	CGGCTATG
Plate01_66	B09	L502	ATAGAGGC	L709	CGGCTATG

Plate01_67	C09	L503	CCTATCCT	L709	CGGCTATG
Plate01_68	D09	L504	GGCTCTGA	L709	CGGCTATG
Plate01_69	E09	L505	AGGCGAAG	L709	CGGCTATG
Plate01_70	F09	L506	TAATCTTA	L709	CGGCTATG
Plate01_71	G09	L507	CAGGACGT	L709	CGGCTATG
Plate01_72	H09	L508	GTACTGAC	L709	CGGCTATG
Plate01_73	A10	L501	TATAGCCT	L710	TCCGCGAA
Plate01_74	B10	L502	ATAGAGGC	L710	TCCGCGAA
Plate01_75	C10	L503	CCTATCCT	L710	TCCGCGAA
Plate01_76	D10	L504	GGCTCTGA	L710	TCCGCGAA
Plate01_77	E10	L505	AGGCGAAG	L710	TCCGCGAA
Plate01_78	F10	L506	TAATCTTA	L710	TCCGCGAA
Plate01_79	G10	L507	CAGGACGT	L710	TCCGCGAA
Plate01_80	H10	L508	GTACTGAC	L710	TCCGCGAA
Plate01_81	A11	L501	TATAGCCT	L711	TCTCGCGC
Plate01_82	B11	L502	ATAGAGGC	L711	TCTCGCGC
Plate01_83	C11	L503	CCTATCCT	L711	TCTCGCGC
Plate01_84	D11	L504	GGCTCTGA	L711	TCTCGCGC
Plate01_85	E11	L505	AGGCGAAG	L711	TCTCGCGC
Plate01_86	F11	L506	TAATCTTA	L711	TCTCGCGC
Plate01_87	G11	L507	CAGGACGT	L711	TCTCGCGC
Plate01_88	H11	L508	GTACTGAC	L711	TCTCGCGC
Plate01_89	A12	L501	TATAGCCT	L712	AGCGATAG
Plate01_90	B12	L502	ATAGAGGC	L712	AGCGATAG
Plate01_91	C12	L503	CCTATCCT	L712	AGCGATAG
Plate01_92	D12	L504	GGCTCTGA	L712	AGCGATAG
Plate01_93	E12	L505	AGGCGAAG	L712	AGCGATAG
Plate01_94	F12	L506	TAATCTTA	L712	AGCGATAG
Plate01_95	G12	L507	CAGGACGT	L712	AGCGATAG
Plate01_96	H12	L508	GTACTGAC	L712	AGCGATAG

7.3. Appendix C: Basic overview of the NxSeq SARS-CoV-2 Whole Genome Library Kit protocol

The following figure illustrates the basic library preparation method used in this targeted amplicon-seq protocol. Importantly, the final library will be composed of a variety of fragments. There are 341 total overlapping amplicons produced by this kit although in some cases, the forward and reverse primers from adjacent primers may produce longer amplicons that span from the 5' end of the first amplicon to the 3' end of the downstream adjacent amplicon. This figure is only showing the correct final library fragments for a few of the possible amplicons across the entire SARS-CoV-2 viral genome covered by this kit. Please note that since the final library is not PCR amplified, there will be some fragments in the library that do not have adaptors at both ends and are not sequenceable (not shown).

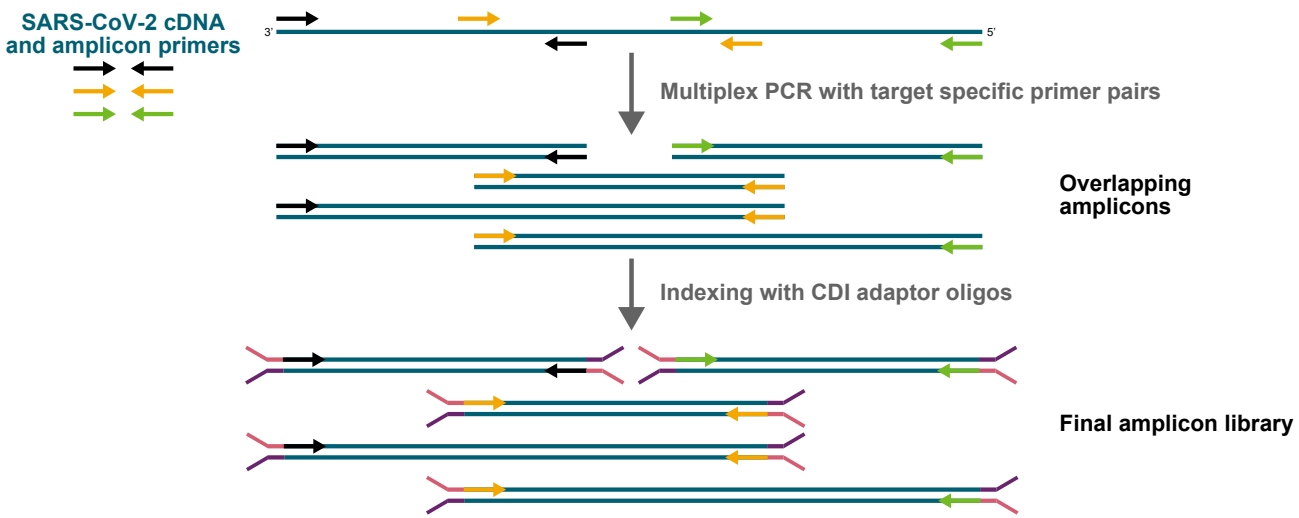


Figure 1. Overview of targeted amplicon library preparation using the NxSeq SARS-CoV-2 Whole Genome Library Kit.

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GEN/905/SW/0421

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