CleanPlex® SARS-CoV-2 FLEX Research and Surveillance Panels

User Guide

This user guide is for the following products for sequencing on Illumina® NGS platforms:

- CleanPlex® SARS-CoV-2 FLEX Research and Surveillance Panel

For other Ready to use Panels not list, see product document page for appropriate user guides

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

Revision History

Document	Date	Description of Change
UG4002-01	October 2020	• Initial version

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Overview

Product Information

CleanPlex® SARS-CoV-2 FLEX Research and Surveillance Panels are a fast, robust, and versatile solution for target enrichment and library preparation for next-generation sequencing (NGS) on Illumina® or DNBSEQ™ sequencing platforms. The CleanPlex SARS-CoV-2 FLEX panel generates highly accurate data from minimal RNA input using a fast and simple workflow. The CleanPlex SARS-CoV-2 FLEX Panel is as compared to the original SARS-CoV-2 panel has improved performance for some lower performing amplicons and a more robust design to maintain high coverage as the SARS-CoV-2 virus mutates with time. The CleanPlex SARS-CoV-2 FLEX Panel is preemptively created with expertly designed degenerate primers added to the existing optimized primers for potentially more comprehensive coverage of polymorphic regions. This enables researchers to generate valuable insights in the identification of viral infection and, active and robust surveillance of acquired genetic variability through the high mutation rate of the virus.

CleanPlex SARS-CoV-2 FLEX Panel is powered by Paragon Genomics' proprietary CleanPlex Technology, which combines an advanced primer design algorithm and an innovative background cleaning chemistry to generate best-in-class target enrichment performance and efficient use of sequencing reads. The patented CleanPlex background cleaning chemistry effectively removes nonspecific PCR products to enable ultra-high multiplexing of amplicons. More than 20,000 amplicons can be multiplexed per primer pool to build large NGS panels that can interrogate megabase-size genomic regions to unlock new applications using a streamlined workflow.

CleanPlex SARS-CoV-2 FLEX Panels feature a fast and simple workflow that can be completed in less than 6 hours from extracted RNA to indexed NGS libraries ready for sequencing. The workflow involves just 3 (MGI version) or 4 (Illumina version) steps, each consisting of a thermal-cycling or incubation reaction followed by a library purification using magnetic beads.

The first step of the CleanPlex SARS-CoV-2 FLEX workflow is cDNA synthesis and purification from purified RNA sample. The second step is a multiplex PCR reaction that uses target-specific primers to amplify targets of interest, thus covering the entire SARS-CoV-2 genome with the 2-pool design. Supplied additionally is an internal human (host) housekeeping RNA control primer pair to be added to the mPCR mix. This amplicon visualizes at 470bp separate from the library at 300bp. The third step is a digestion reaction that achieves background cleaning by removing nonspecific PCR products. The last step is a PCR reaction that uses CleanPlex Indexed PCR Primers for the sequencer of choice to amplify and add sample-level indexes to the generated libraries. CleanMag® Magnetic Beads are recommended for library purification. Please see the workflow section for a detailed depiction of the CleanPlex workflow.

CleanPlex Target Enrichment and Library Preparation Workflow for Illumina Sequencing

5.5 hours of total assay time, 60 minutes of hands-on time

Applications

The CleanPlex SARS-CoV-2 FLEX Research and Surveillance Panels can be used to effectively amplify and detect SARS-CoV-2 RNA in samples using our multiplex PCR technology, enabling reliable results from even low copy numbers. Our assay produces clean characteristic target peaks of defined sizes, which allows for direct detection of the presence of viral genome from the *Coronaviridae* family by electrophoresis. Sequencing is then used to provide confirmation of the species as well as phylogenetic information for specific strain discrimination. The primer design was specifically directed against SARS-CoV-2 but can amplify conserved regions of other coronaviruses in a sample, giving additional insight. These multiplex PCR based panels with degenerate primers are mutation tolerant to allow pinpointing sequence variability due to evolution within the SARS-CoV-2 genome, therefore providing a powerful solution for more in-depth research and surveillance purposes.

The FLEX panel is a two-pool design to cover the entire SARS-CoV-2 genome. However, for applications that do not require such in-depth study of the entire genome, just one of the two pools can be used for faster identification and to save on sequencing space. The single-pool application allows a straight forward one mPCR workflow, allowing for faster turn around and reduced magnetic bead use.

Simplified Workflow

- Single primer pool protocol (1 mPCR per sample)
- Confirm by sequencing (half of the genome)
- Identification and potential strain analysis only

Complete Workflow

- 2 primer pool protocol (2 mPCR per sample)
- Confirm by complete viral genome sequencing
- Identification, strain/mutation analysis, and surveillance studies

Organisms	Sample Types	Applications
• Virus	• Purified RNA from bronchoalveolar lavage, tracheal aspirate, sputum, NP/OP swab, nasopharyngeal wash/aspirate or nasal aspirate	• Identify viral RNA from the *Coronaviridae* family • Confirm specific SARS-CoV-2 strain(s) present in samples through sequencing • Phylogenetic / Surveillance studies through in-depth sequence data analysis • High-sensitivity detection

Positive control

The provided control human housekeeping primer pair is only necessary in one of the two mPCR pools. This control allows visual (via fragment analysis) confirmation of sample quality and library preparation success- especially for confident negative sample calling. The human RNA amplicon is ~470 bp whereas the SARS-CoV-2 amplicons are ~350bp, allowing an easy sequencing free confirmation. For samples with high viral loads, the human amplicon may not be present in a fragment analysis trace due to the overwhelming amplification of the SARS-CoV-2 product. However, this is not an issue as the control is meant for more confident negative sample calling only, by removing concerns about library preparation or sample input concerns.

The use of the control human housekeeping primer in the mPCR step is optional, but suggested for applications that commonly run negative samples.

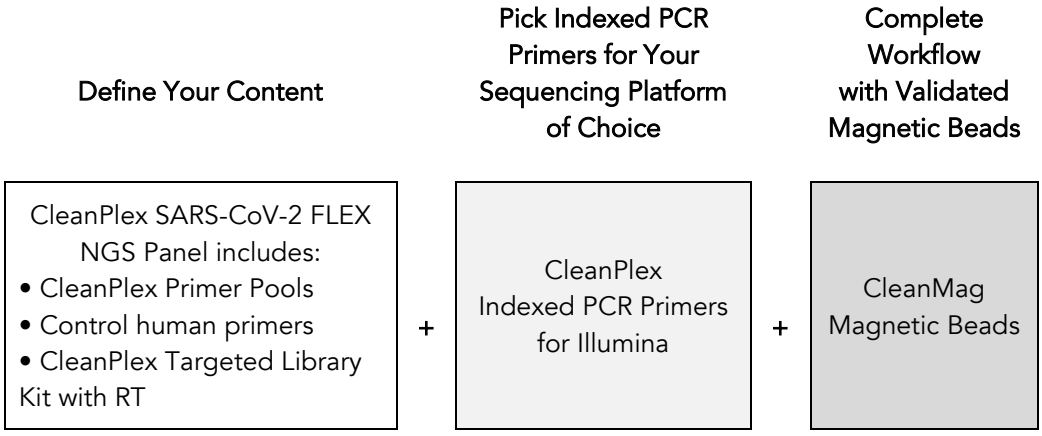
Compatible Sequencing Instruments

CleanPlex Indexed PCR Primers for Illumina are used to generate CleanPlex target-enriched NGS libraries that are compatible with Illumina sequencing platforms, including NovaSeq® 5000/6000 Systems, HiSeq 3000/4000 Systems, HiSeq 2500 System, NextSeq® Series, MiSeq® System, MiniSeq® System, and iSeq® System.

Kit Contents

The protocol outlined in the CleanPlex SARS-CoV-2 FLEX Panel User Guide requires the following components, which need to be ordered separately:

- CleanPlex SARS-CoV-2 FLEX Panel + control human housekeeping primers
- CleanPlex Indexed PCR Primers
- CleanMag Magnetic Beads (or equivalent)



Panel Specifications

Panel	Number of Primer Pools	Primer Pool Concentration	Number of Amplicons	Average Amplicon Length	Average Library Length
CleanPlex SARS-CoV-2 FLEX Panel	2	10X	343	149 bp	285 bp

CleanPlex NGS Panel — Kit Contents, Store at –20°C

Panel	SKU	Size (Reactions)	Components		
			Primer Pool 1	Primer Pool 2	CleanPlex Targeted Library Kit
CleanPlex SARS-CoV-2 FLEX Panel	918013	8	8 µl	8 µl	2-pool, 8 rxns
	918014	96	96 µl	96 µl	2-pool, 96 rxns
	918015	384	384 µl	384 µl	2- pool, 384 rxns

A CleanPlex Targeted Library Kit with RT is included in every CleanPlex SARS-CoV-2 FLEX Kit. Please note that the CleanPlex Targeted Library Kit with RT is **not** sold separately.

CleanPlex Targeted Library Kit with RT — Kit Contents, Store at –20°C (not sold separately)					
		Configuration	2-Pool		
		Size	8 Rxns	96 Rxns	384 Rxns
Component	**Cap Color**	**SKU**	918010	918011	918012
RT Primer Mix DP	Purple Striped		24 µl	288 µl	1152 µl
RT Enzyme	Blue Striped		8 µl	96 µl	384 µl
100X Control Human Primers	Lavender		8 µl	50 µl	100 µl
RT Buffer DP	Green Striped		40 µl	480 µl	1920 µl
5X mPCR Mix	Green		32 µl	384 µl	1536 µl
CP Reagent Buffer	White		16 µl	192 µl	768 µl
CP Digestion Reagent	Yellow		8 µl	96 µl	384 µl
Stop Buffer	Red		64 µl	768 µl	3072 µl
5X 2nd PCR Mix	Blue		64 µl	768 µl	3072 µl
TE Buffer	Clear		500 µl	4 ml	16 ml
Water (DNase and RNase-free)	Clear		500 µl	4 ml	16 ml

Required Materials and Equipment Not Included

- CleanPlex Indexed PCR Primers (please visit www.paragongenomics.com/store for more indexing options)

CleanPlex Dual-Indexed PCR Primers for Illumina — Store at –20°C			
	SKU	Size (Reactions)	Format
CleanPlex Dual-Indexed PCR Primers for Illumina Set T	716021	9	Random 3 i7 indexes + 3 i5 indexes (9 tubes)
CleanPlex Dual-Indexed PCR Primers for Illumina Set A	716006 716017	96 384	12 i7 indexes + 8 i5 indexes (20 tubes)
CleanPlex Dual-Indexed PCR Primers for Illumina Set B	716018 716019	96 384	12 i7 indexes + 8 i5 indexes (20 tubes)
CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set C	716037	96	96 i7 indexes + i5 indexes (96-well plate)
CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set D	716038	96	96 i7 indexes + i5 indexes (96-well plate)
CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set E	716039	96	96 i7 indexes + i5 indexes (96-well plate)
CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set F	716040	96	96 i7 indexes + i5 indexes (96-well plate)

- CleanMag Magnetic Beads, or equivalent — eg. Agencourt™ AMPure™ XP Kit (Beckman Coulter, A63880, A63881, or A63882)

CleanMag Magnetic Beads — Store at 2–8°C			
	SKU	Size (Volume)	Reactions 2-Pool panels
CleanMag Magnetic Beads, 1 ml	718001	1 ml	~5
CleanMag Magnetic Beads, 5 ml	718002	5 ml	~25
CleanMag Magnetic Beads, 20 ml	718005	20 ml	~100
CleanMag Magnetic Beads, 60 ml	718003	60 ml	~300
CleanMag Magnetic Beads, 450 ml	718004	450 ml	~2,250

- For PCR tubes or strips, CleanMag Magnetic Rack (SKU 719001) or equivalent magnetic racks designed for PCR strip workflows
- For 96-well PCR plates, CleanMag Magnetic Plate (SKU 719002) or equivalent magnetic plates designed for PCR plate workflows

CleanMag Magnetic Rack & Plate		
	SKU	Compatibility
CleanMag Magnetic Rack	719001	2 rows of 12 tubes each for PCR strip tubes
CleanMag Magnetic Plate	719002	96-well PCR plates, full or semi- skirted compatible

- 70% ethanol (freshly prepared)
- Nuclease-free, low bind, thin-wall PCR strip tubes with attached caps, or 96-well PCR plate with adhesive film
- Pipettors and low-retention filtered pipette tips
- Thermal cycler
- Mini-centrifuge, or 96-well plate centrifuge
- Qubit® Fluorometer, RNA HS (High Sensitivity) and dsDNA HS Assay Kits, or equivalent
- Agilent® 2100 Bioanalyzer® Instrument and Agilent® High Sensitivity DNA Kit, or equivalent

Storage, Handling, and Usage

CleanPlex SARS-CoV-2 FLEX Panels and CleanPlex Indexed PCR Primers are shipped on blue ice (ice packs). Upon receipt, immediately store both at -20°C in a constant-temperature freezer. Do not store in a freezer with auto-defrost or frost-free features. Do not store at -80°C.

CleanMag Magnetic Beads solution is shipped at room temperature or on blue ice (ice packs). **CleanMag Magnetic beads may be frozen during transit**. This single freeze thaw will not affect the performance of the beads. Upon receipt, immediately store CleanMag Magnetic Beads at 2°C to 8°C . Do not store at -20°C.

Always ensure that all frozen components such as primer pool and CP Digestion Buffer are fully thawed and have been vortexed and spun down to bring all liquids to the bottom of the tubes prior to use.

The 100x Human Control primers are best stored in aliquots to prevent frequent freeze thawing as the Human controls are at a much lower concentration than primer pools, which is not sensitive to reasonable numbers of freeze thaw cycles.

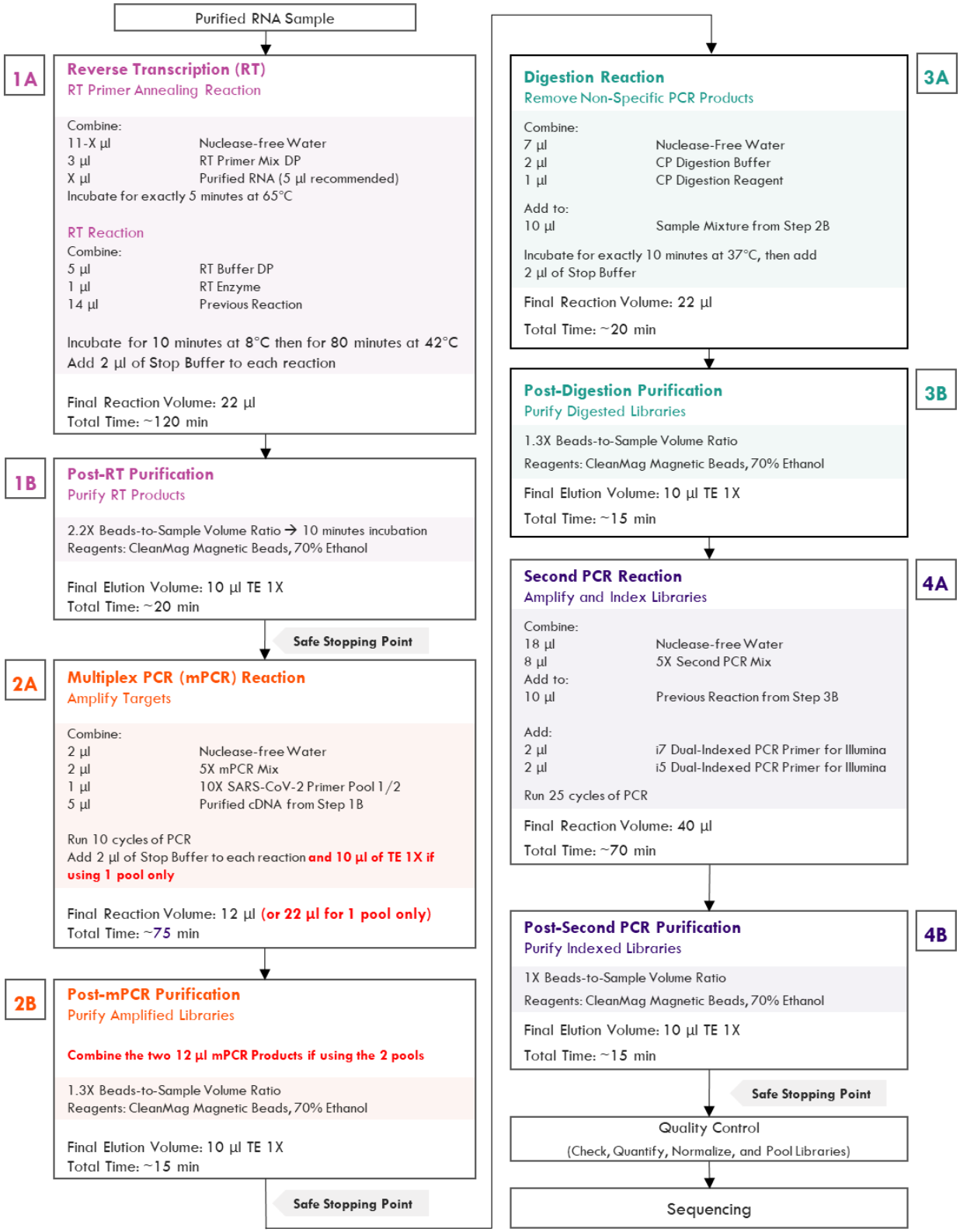
The components containing enzymes are viscous (10X SARS-CoV-2 pools, RT Enzyme Mix, 5X mPCR Mix, CP Digestion Reagent, 5X 2nd PCR Mix) and specially formulated for storage at -20°C without freezing to avoid freeze-thaw cycles to retain their full activity. Please avoid storing these components below -23°C.

CleanPlex SARS-CoV-2 FLEX Panels are developed, designed and sold exclusively for research use only. None of the products or their individual components have been tested for use in diagnostic procedures.

For hazard information, please refer to the Safety Data Sheet (SDS), which is available upon request.

Workflow

The following diagram illustrates CleanPlex NGS Panel's targeted NGS library preparation workflow.



Protocol

General Best Practices

- When using the kit for the first time, briefly vortex and spin the tubes in the kit to bring the liquid to the bottom of the tubes. Store the tubes containing enzymes (10X SARS-CoV-2 FLEX Pool(s), RT Enzyme Mix, 5X mPCR Mix, CP Digestion Reagent, 2nd PCR Mix) on ice during their respective procedures. All other components, including primer pools, may be thawed at room temperature, mixed thoroughly by vortexing and spun-down before use.

- Use good laboratory practices to minimize cross-contamination. If possible, perform PCR setup in an isolated area or room to minimize cross-contamination between samples, multiplex PCR (mPCR) primers, or indexed PCR primers. Always change pipette tips between samples and change gloves frequently. Clean all workstations and tools with 10% bleach followed by water, then alcohol at the end of each work day.

- Use a calibrated PCR thermal cycler as specified by the manufacturer's user guide. Validated thermal cyclers include Bio-Rad C1000, Eppendorf Mastercycler series, and Applied Biosystems GeneAmp PCR System 9700. Generally, a thermal cycler set with the highest ramp speed, such as 5°C/second and higher, is not recommended. For thermal cyclers with adjustable ramp speed, we recommend 3°C/second up and 2°C/second down speed, or use the default setting (no ramp adjustment).

- To ensure accurate assembly of reactions, withdraw viscous solutions (such as 10X SARS-CoV-2 FLEX Pools, RT Enzyme Mix, 5X mPCR Mix, CP Digestion Reagent, 2nd PCR Mix) slowly from containers and dispense it slowly into the reaction mixtures. A good practice is to remove excess from outside of tip and rinse the tip by pipetting up and down several times after dispensing viscous solutions into aqueous mixture. Thoroughly mix each assembly to ensure solutions are homogenous prior to PCR and incubations. Remember to briefly spin the PCR tubes or 96-well PCR plate after mixing.

- Always keep working solutions and PCR products on ice until needed. Combine PCR mixes just immediately prior to use and do not prolong storage of combined PCR mixes and PCR products.

- The protocol is designed to minimize the number of tube-to-tube transfer in order to avoid or reduce sample loss. Except for the RT reaction step and pooling of mPCR products, the entire protocol is performed following a single-tube workflow, with no tube-to-tube transfers.

- When working with 96-well PCR plates, take extra care to ensure thorough mixing of all samples and proper sealing to avoid cross-contamination between samples.

- Magnetic bead purification steps should be performed carefully to minimize residual supernatant and ethanol washes, and to minimize bead loss. Using a strong magnetic rack or plate specifically designed for manual handling of PCR tubes or 96-well PCR plates is critical for a successful bead purification.

- Always pre-warm thermal cyclers, pre-warm a water bath or heat block to 65°C or 37°C as described in the protocol.

- Assign sample indexes to specific samples before starting the protocol.

- Always prepare a master mix of reagents when working with multiplex reactions. Prepare ~5-10% excess of each master mix to allow for pipetting losses. A master mix calculation sheet can be downloaded from the Product Documents page on the Paragon Genomics website at www.paragongenomics.com/product_documents/

RNA Best Practices

- RNA is a single-stranded polynucleotide that is incredibly susceptible to degradation by base- or enzyme-catalyzed hydrolysis. This means that working with RNA can be more demanding than its double-stranded counterpart, DNA, due to the chemical instability of RNA and because of the pervasive presence of RNases. Furthermore, unlike DNases, RNases have no requirement for metal ion co-factors, and can remain active even after prolonged boiling or autoclaving.

- When working with RNA, wear gloves at all times. After putting on gloves, avoid touching contaminated surfaces and equipment with the gloved hands. Even if all the reagents have been decontaminated, RNases can be reintroduced by contact with ungloved hands or with unfiltered air.

- Use sterile, disposable plasticware whenever possible. These require no treatment and are considered to be RNase-free.

  Note: *Autoclaving without DEPC/DMPC treatment is insufficient for inactivating RNases.*

- If possible, designate a special area for RNA work only. Treat surfaces of benches, glassware, and pipettes with commercially available RNase-inactivating agents such as RNase Zap™.

- In addition, wipe benches with a three-wash method, using 10% bleach, followed by water, and finish with 70-100% ethanol **each time** prior to use, in order to rid the area of microorganisms.

- Whenever possible, purchase reagents that are free of RNases. Be sure to separate reagents used for RNA work from "general use reagents" in the laboratory, and use only for RNA processing.

- Prior to processing, store RNA at -70 to -80°C, as aliquots in ethanol, isopropanol or lysis buffer as indicated by extraction methods. Most RNA is relatively stable at this temperature. Centrifuge the RNA and resuspend in an appropriate RNase-free buffer before use. Use autoclaved DEPC water to elute and resuspend RNA or alternatively use molecular grade water certified as RNAse- and DNAse-free.

- Although DNA is relatively stable at elevated temperatures (+100°C), most RNA is not. Therefore, avoid high temperatures (above +65°C) since these can affect the integrity of RNA. This means it is critical to handle solutions of RNA on ice when using, including for thawing and processing RNA samples and related reactions.

Input RNA Requirements

Refer to the following chart for the amounts of input RNA.

Panel	RNA Input Range	Recommended RNA Input
CleanPlex SARS-CoV-2 FLEX Panel	50–100 ng Or 5-11 µl of extracted RNA	50 ng Or 11 uL of extracted RNA

- The maximum volume of RNA input per RT Reaction is 11 µl. An input of more than 5 µl of extracted and purified RNA (10-15ng/ µl concentration) is recommended per pool. When working with low viral load samples or unknown viral load samples, use the full 11 µl RNA input or best chances of detection.
- Qubit RNA HS Assay Kit (Thermo Fisher, Cat. No. Q32851 or Q32854) or an equivalent fluorometric method is recommended for measuring RNA concentration. UV spectrophotometry methods (e.g. NanoDrop™ spectrophotometer) are not recommended because it can significantly overestimate the RNA concentration.
- If sample quality is questionable, more RNA can be used for best results.
- Some recommended RNA extractions kits include: QIAamp® Viral RNA Mini Kit, QIAamp® MinElute Virus Spin Kit Thanksor RNeasy® Mini Kit (QIAGEN), EZ1 DSP Virus Kit (QIAGEN), Roche MagNA Pure Compact RNA Isolation Kit, Roche MagNA Pure Compact Nucleic Acid Isolation Kit, Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit.
- After RNA extraction, avoid diluting RNA samples to < 10 ng/µl when possible for prolonged storage, since RNA is less stable in solution at lower concentrations.
- Avoid freeze-thawing RNA samples when possible. Use freshly extracted RNA when possible.
- RNA Input is based off of tested conditions using Universal Human Reference RNA; actual optimal input may vary based on actual RNA sample quality and viral load.
- Compatible RNA buffering systems are Tris HCl or TE.

Set up and Preparations

- When working with multiple samples, prepare a master mix of RT Primer Annealing Reaction Mixture for RT. Mix well, aliquot into individual tubes, then add the recommended RNA input to each reaction.
- Bring magnetic bead solution to room temperature for at least 30 minutes before use. Replace in 2-8 °C storage at the end of the day.
- Freshly prepare 12mL (per 8 reactions) of 70% Ethanol by combining 8.4mL of 100% ethanol and 3.6mL nuclease-free water at volume ratios of 7 to 3 respectively. Do not top off one liquid with the other in a volumetric container because the volumetric ratio will not be accurate. When water and ethanol are mixed, the final volume will be less than the sum of individual volumes. Lower concentration of ethanol will affect final library yield.
- For all samples to be sequenced together, assign a specific index combination (CleanPlex Dual-Indexed PCR Primers for Illumina) to each sample.
- Note: Use the 1-Pool method for quick detection, reduced hands on time, and reduced total sequencing reads. However, for complete genome sequencing use the 2-Pool method for in-depth analysis.
- Note: There are safe stopping points after Post-RT Purification, Post-mPCR Purification, and Post-2nd PCR Purification. After starting Digestion Step, samples must be carried through to the 2nd PCR reaction without stopping. Plan accordingly.

1A. Reverse Transcription (RT) Reaction

1A.1. Thaw RT-Primer Mix and RT Buffer, vortex vigorously to mix, quick spin to collect liquid, then store on ice. Keep RT enzyme on ice when not in use. Using thin-wall PCR strip tubes (or a 96-well PCR plate), prepare the Reverse Transcription Annealing Reaction Mixture by adding components in the following order on ice or a cold block.

Note: *When working with multiple samples, prepare a master mix of RT Primer annealing reaction Mixture. Mix well, aliquot into individual tubes, then add RNA samples to each reaction.*

Note: *Pre-warm a thermal cycler or heating unit to 65°C before beginning the RT Primer Annealing Reaction.*

Note: *Thaw and keep Stop Buffer and Magnetic Beads at room temperature for use at the end of step 1A and beginning of 1B.*

Important! Use RNA Best Practices by performing all reactions on ice or a cold block, and diligently avoid contamination and introduction of RNases until RT Reaction (1A) and RT Purification (1B) are complete.

RT Primer Annealing Reaction Mixture

Reagent	Cap Color	Volume per reaction
DEPC Treated Nuclease-Free Water	Clear	11 – X µl
RT Primer Mix DP	Purple Striped	3 µl
Purified RNA	—	X µl
Total Volume per reaction		**14 µl**

1A.2. Close the caps of the PCR tubes or seal the PCR plate with adhesive film, spin briefly to bring down the liquid, mix thoroughly by pipetting up and down or by vortexing vigorously for at least 5 seconds until homogenous. While ensuring thorough mixing, avoid unnecessarily prolonged vortexing. Spin briefly for 3 seconds to collect the liquid.

Note: *It is crucial that the reaction mixture is homogenous prior to thermal cycling. Incomplete mixing can cause decreased yield and increase nonspecific product formation.*

Note: *If using a PCR plate, use an applicator tool to firmly secure each reaction well and around the perimeter of the plate to prevent evaporation during thermal cycling.*

1A.3. Preheat the thermocycler to 65 °C then load the tubes/plate in the thermal cycler and run the following thermal cycling protocol to anneal RT Primers.

RT Primer Annealing Reaction Incubation Protocol

Step	Temperature	Time	Preheat
Incubation	65 °C	5 min	65 °C
Hold	4 °C	∞	-

Important! Do not stop and store samples. Immediately after RT primer annealing, continue to the Reverse Transcription Reaction.

1A.4. Place the tubes/plate on ice or cold block immediately after incubation. Open the tubes or carefully remove the adhesive film from the PCR plate. Prepare the Reverse Transcription (RT) Reaction Mixture by adding components in the following order on ice or a cold block.

Note*: When working with multiple samples, prepare a master mix of RT Reaction Mixture. Mix well and aliquot into individual tubes.*

RT Reaction Mixture

Reagent	Cap Color	Volume per reaction
RT Buffer DP	Green Striped	5 µl
RT Enzyme	Blue Striped	1 µl
Reaction from Step 1A.3	—	14 µl
Total Volume per reaction		**20 µl**

1A.5. Close the caps of the PCR tubes or seal the PCR plate with adhesive film, spin briefly to bring down the liquid, mix thoroughly by pipetting up and down at least 5 times or by vortexing vigorously for at least 5 seconds until homogenous. While ensuring thorough mixing, avoid unnecessarily prolonged vortexing. Spin briefly for 3 seconds to collect the liquid.

Note: *It is crucial that the reaction mixture is homogenous prior to thermal cycling. Incomplete mixing can cause decreased yield and increase nonspecific product formation.*

Note: *Avoid over vortexing, RNA is single-stranded and susceptible to manual shearing and excessive fragmentation.*

1A.6. Prechill the thermocycler to 8 °C, then load the tubes/plate in the thermal cycler and run the following thermal cycling protocol.

Step	Temperature	Time	Preheat
Equilibration	8 °C	10 min	8 °C
Incubation	42 °C	80 min	
Hold	4 °C	∞	

1A.7. When thermocycling is complete, immediately add 2 µl of Stop Buffer (red cap) into each tube/well and mix by spinning briefly then vortexing. Spin again briefly to collect the liquid. The volume of each sample is now approximately 22 µl.

Important! Do not stop and store samples after the RT Reaction. Add Stop buffer then proceed to Step 1B, Post-RT Purification immediately.

1B. Post-RT Purification

Important! Ensure the Magnetic Bead Solution has come to room temperature before use.

Important! Use freshly prepared 70% ethanol. Lower concentration ethanol may result in lower yields.

1B.1. Vortex the magnetic beads suspension vigorously until homogenous. Perform a **2.2 X** bead-based purification by adding 48 µl of magnetic beads to each sample as described in the table below. Mix by pipetting up and down at least 5 times or vortex vigorously for at least 5 seconds until homogenous.

Post RT Purification — 2.2 X Beads-to-Sample Volume Ratio	
	Volume per reaction
Reverse Transcription Product	22 µl
Magnetic Beads to add	48 µl

Important! Magnetic bead volume is critical to the purification process. Always dispense slowly and carefully. Keep the outside of the pipette free from droplets**, AND** make sure the entire volume is added to the sample (residual from inside pipette tips) before discarding the tip.

Ensure the bead + sample solution is thoroughly mixed before incubation, especially when working in a 96-well PCR plate format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

1B.2. Incubate the mixture for **10 minutes** at room temperature.

1B.3. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and incubate for at least **2 minutes until the liquid is clear**. The beads will be drawn onto one side of each tube/well. While keeping the tubes/plate on the magnetic rack, carefully remove and discard the supernatant without disturbing the beads.

Note: *If the magnetic beads are aspirated into the pipette tip at any point during the purification process, dispense the solution back into tube, remove the tip, allow the beads to migrate toward the magnet again, then repeat.*

1B.4. Cap/seal the tubes/plate, and briefly spin again to bring down the remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Using a 10 µl pipette tip, carefully remove all residual supernatant from the bottom of the tube/well without disturbing the beads.

Important! Removing all residual supernatant from the RT Reaction *prior* to ethanol washing is critical to obtaining a clean, high-quality library. The above "spin and remove" step ensures complete removal of supernatant.

Note: A strong compatible magnetic rack is essential. If your magnetic rack or plate cannot collect the magnetic beads effectively, please look into a replacement.

1B.5. Add 180 µl of freshly prepared 70% ethanol to each tube/well. Reposition the tubes/plate on the magnetic rack by placing the clear side of the tubes/wells (the side without beads) against the magnet. Allow the beads to completely migrate through the ethanol to the other side. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.

1B.6. Repeat step 1B.5.

1B.7. After the second wash, briefly spin the tubes/plate to bring down all remaining liquid. Place the tubes/plate on the magnet rack to gather the beads. Carefully remove the residual ethanol in each tube/well. Keeping the tubes/plate on the magnetic rack, air-dry the beads at room temperature for 5 minutes. **Do not over- or under-dry.**

Note: Over-dried beads can dislodge from the side of the tube and lead to cross-contamination. They are also more difficult to resuspend during elution. Residual ethanol inhibits PCR and will result in reduced yield. Especially when working with 96-well PCR plates, make sure all residual ethanol is dried (may take longer than 5 minutes) before going to the next step.

1B.8. Add 10 µl TE buffer directly onto the pellet for each tube/well. Briefly spin and vortex to resuspend the dried beads completely. The cDNA will be immediately released from the beads as long as all beads are in solution. Spin briefly to collect the liquid to the bottom. There is no need to remove the beads.

1B.9. Proceed to Step 2A. Multiplex PCR Reaction.

Safe Stopping Point. Purified products may be stored at 10°C for up to 1 hour or at –20°C for up to 24 hours.

2A. Multiplex PCR (mPCR) Reaction

Important: Each primer pool requires one mPCR and 5 µl of the purified RT reaction product. When working with a 1-pool workflow, the remaining 5 µl of the purified RT reaction can be stored at -20°C for future use or discarded. When working with the 2-pool workflow, split the 10 µl of the purified RT reaction into two 5 µl tubes (1 for each pool), beads included.

Important: With first use, it is best to aliquot and store 100x Human Control Primers to minimize freeze thaw cycles. The controls are provided at 100x for better stability. Before each use, perform a 1:10 dilution of the 100x Controls for ease of pipetting. If a large master mix is made, one can also pipette directly from 100x Control if the pipetting volume is manageable. Make up the volume difference in water to maintain 5uL per reaction master mix volume.

Important: Add the diluted 10X control human primer to only one mPCR tube, irrespective of doing the one or two-pool workflow. Adjust the volume of Nuclease-Free Water to make final volume of mPCR Reaction Master Mix to 5 µl. **See Applications section on page 6 and 7 for additional information on Control Human Primer use.**

2A.1. Prepare the mPCR Reaction Mixture by adding components in the following order on ice or a cold block. Mix the RT reaction product from 1B. 8 well and add 5 µl to each mPCR reaction.

Note: *Use the 1-Pool method for quick detection and reduced turnaround time. However, if in-depth sequencing is desired use the 2-Pool method for a more complete coverage. See application section for additional information.*

Note: *When working with multiple reactions, prepare a master mix. Mix well, then add 5 µl of master mix to each sample. 2-pool workflow requires 2 mPCR reactions per sample.*

mPCR Reaction Master Mix per pool

Reagent	Cap Color	Volume per reaction
Nuclease-Free Water	—	2 µl (1 µl with control)
10X diluted Control Human Primer (optional)	Lavender	(1 µl)
5X mPCR Mix	Green	2 µl
10X SARS-CoV-2 Primer Pool 1 or 10X SARS-CoV-2 Primer Pool 2	Varies	1 µl
Total Volume per reaction		**5 µl**

Important! 5X mPCR Mix and 10X Primer Pool(s) are viscous. Pipette slowly, remove any excess reagent on the outside of the pipette tip, and rinse tip in solution when handling these reagents.

mPCR Reaction Mixture	
Reagent	**Volume per reaction per pool**
Purified RT sample	5 µl
mPCR Master mix for Pool 1 or Pool 2	5 µl
Total Volume per reaction	**10 µl**

2A.2. Close the caps of the PCR tubes or seal the PCR plate with adhesive film, spin briefly to bring down the liquid, mix thoroughly by vortexing for at least 5 seconds until homogenous. Avoid prolonged vortexing. Spin briefly for 3 seconds to collect the liquid.

Note: It is crucial that the reaction mixture is homogenous prior to thermal cycling. Incomplete mixing can cause decreased yield and increase nonspecific product formation.

Note: If using a PCR plate, use an applicator tool to firmly secure each reaction well and around the perimeter of the plate to prevent evaporation during thermal cycling.

2A.3. Load the tubes/plate in the thermal cycler with a heated lid at 105 °C and run the following thermal cycling protocol.

mPCR Thermal Cycling Protocol				
Step	**Temperature**	**Time**	**Ramping***	**Cycles****
Initial Denaturation	95 °C	10 min	-	-
Denaturation	98 °C	15 sec	3 °C/s	10
Annealing/Extension	60 °C	5 min	2 °C/s	
Hold	10 °C	∞		

* For thermal cyclers without adjustable ramp speed, the default setting can be used if max ramping speed is ≤5°C/S.

** for applications strictly detecting low viral counts (<10 copies/rxn) additional 2-4 mPCR cycles can be added for increased sensitivity

2A.4. Immediately add 2 µl of Stop Buffer (red cap) into each tube/well and mix by spinning briefly then vortexing.

Note: Do not allow samples to hold at 10 °C for longer than 30 minutes before adding Stop Buffer.

2A.5. Proceed to Step 2B. Post-mPCR Purification.

Important! Do not stop and store samples after the mPCR Reaction. Add Stop buffer then proceed to Step 2B, Post-mPCR Purification immediately.

2B. Post-mPCR Purification

Important! Ensure the Magnetic Bead Solution has come to room temperature before use.

Important! Use freshly prepared 70% ethanol. Lower concentration ethanol may result in lower yields.

2B.1. Open the tubes or carefully remove the adhesive film from the PCR plate.
- When using 1-pool workflow, add 10 µl of TE Buffer to each sample for a total of 22 µl volume.
- When using 2-pool workflow, combine the 12 µl multiplex PCR products for each sample for a total of 24 µl volume.

2B.2. Quick spin the tubes/plate to collect the liquid. Open the tubes or carefully remove the adhesive film from the PCR plate.

2B.2. Vortex the magnetic beads suspension vigorously until homogenous. Perform a **1.3X** bead-based purification by adding magnetic beads to each sample as described in the table below. Mix by pipetting up and down at least 5 times or vortex vigorously for at least 5 seconds until homogenous.

Post-mPCR Purification — 1.3X Beads-to-Sample Volume Ratio		
Reagent	**1-Pool workflow**	**2-Pool workflow**
Total Sample Volume	22 µl (12 µl of sample + 10 µl of TE)	24 µl
Magnetic Beads to add	29 µl	31 µl

Important! Magnetic bead volume is critical to the purification process. Always dispense slowly and carefully. Keep the outside of the pipette free from droplets, **AND** make sure the entire volume is added to the sample (residual from inside pipette tips) before discarding the tip.

Ensure the bead + sample solution is thoroughly mixed before incubation, especially when working in a 96-well PCR plate format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

2B.3. Incubate the mixture for **5 minutes** at room temperature.

2B.4. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and incubate for at least **2 minutes until the liquid is clear**. The beads will be drawn onto one side of each tube/well. While keeping the tubes/plate on the magnetic rack, carefully remove and discard the supernatant without disturbing the beads.

Note: If the magnetic beads are aspirated into the pipette tip at any point during the purification process, dispense the solution back into tube, remove the tip, allow the beads to migrate toward the magnet again, then repeat.

2B.5. Cap/seal the tubes/plate, and briefly spin again to bring down the remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Using a 10 µl pipette tip, carefully remove all residual supernatant from the bottom of the tube/well without disturbing the beads.

Important! Removing all residual supernatant from the Multiplex PCR Reaction *prior* to ethanol washing is critical to obtaining a clean, high-quality library. The above "spin and remove" step ensures complete removal of supernatant.

Note: A strong compatible magnetic rack is essential. If your magnetic rack or plate cannot collect the magnetic beads effectively, please look into a replacement.

2B.6. Add 180 µl of freshly prepared 70% ethanol to each tube/well. Reposition the tubes/plate on the magnetic rack by placing the clear side of the tubes/wells (the side without beads) against the magnet. Allow the beads to completely migrate through the ethanol to the other side. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.

2B.7. Repeat step 2B.6

2B.8. After the second wash, briefly spin the tubes/plate to bring down all remaining liquid. Place the tubes/plate on the magnet rack to gather the beads. Carefully remove the residual ethanol in each tube/well. Keeping the tubes/plate open on the magnetic rack, air-dry the beads at room temperature for 5 minutes. **Do not over or under dry.**

Note: Over-dried beads can dislodge from the side of the tube and lead to cross-contamination. They are also more difficult to resuspend during elution. Residual ethanol inhibits PCR and will result in reduced yield. Especially when working with 96-well PCR plates, make sure all residual ethanol is dried (may take longer than 5 minutes) before going to the next step.

2B.9. Add 10 µl TE buffer directly onto the pellet for each tube/well. Briefly spin and vortex to resuspend the dried beads completely. The DNA will be immediately released from the beads as long as all beads are in solution. Spin briefly to collect the liquid to the bottom. There is no need to remove the beads.

2B.10. Proceed to Step 3A. Digestion Reaction.

Safe Stopping Point. Purified products may be stored at 10°C for up to 1 hour or at –20°C for up to 24 hours.

3A. Digestion Reaction

Note: *After starting the Digestion Reaction, the samples cannot be stored and must continue to Post-Digestion Purification, then Second PCR Reaction and Purification. Plan accordingly.*

Note: *When thawing CP Reagent Buffer, bring the tube to room temperature. Ensure all visible precipitate is dissolved by vortexing as needed.*

Note: *Pre-warm a thermal cycler or heating unit to 37°C before beginning the Digestion Reaction.*

3A.1. Prepare Digestion Reaction Master Mix and add to each purified sample from Step 2B. The magnetic beads in the sample mixture do not affect the Digestion Reaction.

Note: *When working with multiple reactions, prepare a master mix. Mix well, then add 10 µl of master mix to each sample.*

Digestion Reaction Master Mix		
Reagent	**Cap Color**	**Volume per reaction**
Nuclease-Free Water	—	7 µl
CP Reagent Buffer	White	2 µl
CP Digestion Reagent	Yellow	1 µl
Total Volume per reaction		**10 µl**

Digestion Reaction Mixture	
Reagent	**Volume per reaction**
Digestion Reaction Master Mix	10 µl
Purified Sample from Step 2B	10 µl
Total Volume per reaction	**20 µl**

3A.2. Mix by pipetting up and down at least 5 times or vortexing vigorously for at least 5 seconds until homogenous. Avoid unnecessarily prolonged vortexing. Spin briefly for 3 seconds to collect the liquid.

Note: It is crucial that the reaction mixture is homogenous prior to incubation. Incomplete mixing can cause digestion of the library or under-digestion of nonspecific products.

Note: If using a PCR plate, use an applicator tool to firmly secure each reaction well and around the perimeter of the plate to prevent evaporation during incubation.

3A.3. Incubate at 37°C for exactly 10 minutes. **Do not** incubate shorter or longer than 10 minutes.

3A.4. Immediately add 2 µl of Stop Buffer (red cap) to each tube/well and mix by spinning briefly then vortexing. Spin again briefly to collect the liquid. The volume of each sample is approximately 22µl.

3A.5. Proceed to Step 3B. Post-Digestion Purification immediately.

Important! Do not stop and store samples after the Digestion Reaction. Proceed to Step 3B. Post-Digestion Purification immediately.

3B. Post-Digestion Purification

Note: *Remember to assign a specific index or unique combination of dual index to each sample before starting this step.*

3B.1. Vortex the magnetic beads suspension vigorously until homogenous. Perform a **1.3X** bead-based purification by adding **29 µl** of magnetic beads to each sample. Mix by pipetting up and down at least 5 times or vortex vigorously for at least 5 seconds until homogenous.

Post-Digestion Purification — 1.3X Beads-to-Sample Volume Ratio

Reagent	Volume per reaction
Digestion Reaction Product	22 µl
Magnetic Beads	29 µl

Important! Magnetic bead volume is critical to the purification process. Always dispense slowly and carefully. Keep the outside of the pipette free from droplets**, AND** make sure the entire volume is added to the sample (residual from inside pipette tips) before discarding the tip.

Ensure the bead + sample solution is thoroughly mixed before incubation, especially when working in a 96-well PCR plate format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

3B.2. Incubate the mixture for 5 minutes at room temperature.

3B.3. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and incubate for at least **2 minutes until the liquid is clear**. The beads will be drawn onto one side of each tube/wall. While keeping the tubes/plate on the magnetic rack, carefully remove and discard the supernatant without disturbing the beads.

3B.4. Cap/seal the tubes/plate and briefly spin again to bring down the remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Using a 10 µl pipette tip, carefully remove all residual supernatant from the bottom of the tube/well without disturbing the beads.

Important! Removing all residual supernatant from the Digestion Reaction *prior* to ethanol washing is critical to obtaining a clean, high-quality library. The above "spin and remove" step ensures complete removal of supernatant.

3B.5. Add 180 µl of freshly prepared 70% ethanol to each tube/well. Reposition the tubes/plate on the magnetic rack by placing the clear side of the tubes/wells (the side without beads) against the magnet. Allow the beads to completely migrate through the ethanol to the other side. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.

3B.6. Repeat step 3B.5.

3B.7. After the second wash, briefly spin the tubes/plate to bring down all remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Carefully remove the residual ethanol left behind in each tube/well. Keeping the tubes/plate open on the magnetic rack, air-dry the beads at room temperature for 5 minutes. **Do not over or under dry.**

Note: Over-dried beads can dislodge from the side of the tube and lead to cross-contamination, and they are also more difficult to resuspend during elution. Residual ethanol inhibits PCR and will result in reduced yield. Especially when working with 96-well PCR plates, make sure all residual ethanol is dried (may take longer than 5 minutes) before going to the next step.

3B.8. Add 10 µl TE buffer directly onto the pellet for each tube/well. Briefly spin and vortex to resuspend the dried beads completely. The DNA will be immediately released from the beads as long as all beads are in solution. Spin briefly to collect the liquid to the bottom. There is no need to remove the beads.

3B.9. Proceed to Step 4A. Second PCR Reaction immediately.

Important! Do not stop and store samples after Post-Digestion Purification. Proceed to Step 4A. Second PCR Reaction immediately.

4A. Second PCR Reaction

Note: Remember to assign a specific index or unique combination of dual indexes to each sample before starting this step based on your sequencing needs.

4A.1 Thaw Indexed PCR Primers, vortex thoroughly, then spin briefly to collect the liquid. Prepare Second PCR Reaction Master Mix and add to each purified sample from Step 3B. Then add a unique (combination of) Indexed PCR Primer(s) to each sample.

Note: When working with multiple samples, prepare a master mix. Mix well, then add 26 µl of master mix to each sample.

Second PCR Reaction Master Mix		
Reagent	**Cap Color**	**Volume per reaction**
Nuclease-Free Water	—	18 µl
5X 2nd PCR Mix	Blue	8 µl
Total Volume per reaction		**26 µl**

Second PCR Reaction Mixture	
Reagent	**Volume per reaction**
Second PCR Reaction Master Mix	26 µl
Purified Sample from Step 3B	10 µl
i5 Dual-Indexed PCR Primer for Illumina	2 µl
i7 Dual-Indexed PCR Primer for Illumina	2 µl
Total Volume per reaction	**40 µl**

Important! When handling Dual-Indexed PCR Primers, take extra care to prevent cross-contamination by opening one tube at a time and changing pipette tips and gloves as necessary. Avoid touching the opening and inside of the tubes with your hands, pipette channel, or anything non-disposable.

4A.2 Close the caps of the PCR tubes or seal the PCR plate with adhesive film. Spin briefly to collect the liquid, then mix thoroughly by pipetting up and down at least 5 times or vortexing vigorously for at least 5 seconds until homogenous. Avoid unnecessarily prolonged vortexing. Spin briefly for 3 seconds to collect the liquid.

Note: *It is crucial that the reaction mixture is homogenous prior to thermal cycling. Incomplete mixing can cause decreased yield and increase nonspecific product formation.*

Note: *If using a PCR plate, use an applicator tool to firmly secure each reaction well and around the perimeter of the plate to prevent evaporation during thermal cycling.*

4A.3 Load the tubes/plate in the thermal cycler, and run the following thermal cycling protocol to amplify and index the libraries. Use the three tables below for thermal cycling protocol.

Note*: The Second PCR thermal cycling protocol depends on the starting RNA input amount and RNA quality. Generally, lower quality RNA, lower RNA input amount, or fewer amplicons in a panel require more PCR cycles.*

Second PCR Reaction — Thermal Cycling Protocol				
Step	**Temperature**	**Time**	**Ramping***	**Cycles**
Initial Denaturation	95 °C	10 min	-	1
Denaturation	98 °C	15 sec	3 °C/s	See table below
Annealing/Extension	60 °C	75 sec	2 °C/s	
Hold	10 °C	∞		

* For thermal cyclers without adjustable ramp speed, the default setting can be used if max ramping speed is ≤5°C/S.

CleanPlex SARS-CoV-2 NGS Panels — Second PCR Cycle Number	
Number of Amplicons	**Cycles**
172 or 173 (1-Pool workflow)	25
344 (2-Pool workflow)	24

******* *For optimal yield, the SARS-CoV-2 panels may benefit from adjustment to Second PCR cycle numbers based on the sample quality, viral input (when known), and application.*

Note*: For applications that specialize in very low viral count detection and increased mPCR cycles were used, decrease the 2nd PCR cycles by the same amount of cycles increase in mPCR.*

4A.4 Proceed to Step 4B, Post- Second PCR Purification, immediately after cycle completion. Do not allow PCR product to hold at 10 °C for more than 30 minutes.

Important! Do not stop and store samples after the Second PCR Reaction. Proceed to Step 4B. Post-Second PCR Purification immediately.

4B. Post-Second PCR Purification

4B.1. Vortex the magnetic beads suspension vigorously until homogenous. Perform a **1X** bead-based purification by adding **40 µl** of magnetic beads to each sample. Mix by pipetting up and down at least 5 times or by vortexing vigorously for at least 5 seconds until homogenous.

Post-Second PCR Purification — 1X Beads-to-Sample Volume Ratio

Reagent	Volume per reaction
Second PCR Reaction Product	40 µl
Magnetic Beads	40 µl

Important! Magnetic bead volume is critical to the purification process. Always dispense slowly and carefully. Keep the outside of the pipette free from droplets**, AND** make sure the entire volume is added to the sample (residual from inside pipette tips) before discarding the tip.

Ensure the bead + sample solution is thoroughly mixed before incubation, especially when working in a 96-well PCR plate format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

4B.2. Incubate the mixture for 5 minutes at room temperature.

4B.3. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and incubate for at least **2 minutes until the liquid is clear**. The beads will be drawn onto one side of each tube/wall. While keeping the tubes/plate on the magnetic rack, carefully remove and discard the supernatant without disturbing the beads.

4B.4. Cap/seal the tubes/plate, and briefly spin again for to bring down the remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Using a 10 µl pipette tip, carefully remove all residual supernatant from the bottom of the tube/well without disturbing the beads.

Important! Removing all residual supernatant from the 2nd PCR Reaction *prior* to ethanol washing is critical to obtaining a clean, high-quality library. The above "spin and remove" step ensures complete removal of supernatant.

4B.5. Add 180 µl of freshly prepared 70% ethanol to each tube/well. Reposition the tubes/plate on the magnetic rack by placing the clear side of the tubes/wells (the side without beads) against the magnet. Allow the beads to completely migrate through the ethanol to the other side. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.

4B.6. Repeat step 4B.5.

4B.7. After the second wash, briefly spin the tubes/plate to bring down all remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Carefully remove the residual ethanol left behind in each tubes/well. Keeping the tubes/plate open on the magnetic rack, air-dry the beads at room temperature for 5 minutes. **Do not over or under dry.**

Note: Over-drying and under-drying the beads can lead to reduced yield.

4B.8. Add 10 µl TE buffer directly onto the pellet for each tube/well. Briefly spin and vortex to resuspend the dried beads completely. The DNA will be immediately released from the beads as long as all beads are in solution. Spin briefly to collect the liquid to the bottom. There is no need to remove the beads. At this point the library is complete and can be stored with beads at –20°C.

Note: To perform QC and sequencing, use a magnetic rack to separate the beads. Avoid transferring the beads when pipetting the clear supernatant for QC or sequencing.

Safe Stopping Point. Purified products may be stored with beads at –20°C until ready to sequence. When taking the library out for QC and sequencing, vortex briefly and place the tubes or plate on a magnetic rack to pull the beads to one side. Avoid pipetting the beads, which will affect QC and sequencing.

Quality Control Prior to Sequencing

After library preparation, amplification of viral RNA can be visualized via electrophoresis. This can be accomplished with high sensitivity fragment analyzers such as Agilent® 2100 Bioanalyzer® Instrument and Agilent® High Sensitivity DNA Kit (Agilent, Part. No. 5067-4626). The presence of a peak at ~275 bp indicates successful amplification of the targeted regions. With sequencing, it can be confirmed that the viral RNA amplified is from the SARS-CoV-2 genome. The human housekeeping RNA control amplicon visualizes at ~470bp separate from the library (~275 bp) and allows visual confirmation of sample quality and library preparation - especially for negative samples.

Note: When viral copies are high in a sample, this control target is not always visible. However, this should not be an issue as the purpose of this control is to provide confident negative sample calling. This is not a sample control, but rather a library preparation control.

In additional to qualitative confirmation of amplified product, fragment analysis allows confirmation of the quality of the library produced. It is highly recommended that a high sensitivity fragment analyzer is used to visualize the peak shape, size, and potential background concentration to ensure library preparation was performed correctly prior to sequencing.

If qualitative confirmation is not required prior to sequencing, a fragment analysis is not necessary for high-throughput workflows after ensuring that high quality libraries with little nonspecific product can be consistently produced. The final library can also be quantified using a fluorometer such as Qubit™ with dsDNA HS Assay Kit (Thermo Fisher, Cat. No. Q32851 or Q32854) or using a qPCR method. However, these methods will only give you the absolute yield and not differentiate potential background from the actual library.

An example of the expected library peak size and shapes can be found in the troubleshooting guide at the end of this user guide.

After confirmation of library quality, the libraries can be normalized using library peak concentrations, not including the nonspecific product(s) concentration(s). Then, samples with unique index combinations can be pooled for sequencing.

Recommended Sequencing Length and Depth

All CleanPlex Ready-to-Use NGS Panels are designed to be compatible with PE 150 bp reads (2x150 bp).

Recommended Sequencing Depth		
Panel	**Application**	**Average Read Depth**
CleanPlex SARS-CoV-2 Panel	detection	50-200X
	Strain identification	200X-1000X
	co-infection, strain analysis, other in-depth applications	1,000X or more

Terminology

Coverage	Also known as depth of coverage, is achieved with one set of paired end reads that cover the entire length of the amplicon.
Read	One continuous act of sequencing. For example, 1 million clusters on a flow cell would produce 1 million single reads and 2 million paired-end reads. Paired end reads count as two individual reads, and required for 1x coverage.

When calculating how many samples can be sequenced in one chip, it is Important to refer to the correct total read capacity of the chip.

For example:

For **1000x** coverage of this panel with **343** amplicons, it requires a total of 343,000 cluster reads or 686,000 paired end reads per sample. With a **Miseq V2 kit** (15 million single reads/ 30 paired end reads) you can sequence about **44** samples (30 M PE reads/686 PE reads) per run.

For recommended sample multiplexing on various Illumina NGS instruments, refer to the panel-specific product sheet available for download on the product document page: https://www.paragongenomics.com/product_documents/

A sample multiplexing calculator also be download from the product document page.

Supporting Information

Dual-Indexed PCR Primers for Illumina

Primer Sequences

Each sample is indexed by a pair of Indexed PCR Primers for sequencing on Illumina platforms. XXXXXXXX denotes the 8-nucleotide index region of the primer. Index sequences are listed below.

i5 Indexed Primer
5'-AATGATACGGCGACCACCGAGATCTACAC**XXXXXXXX**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**-3'**

i7 Indexed Primer
5'-CAAGCAGAAGACGGCATACGAGAT**XXXXXXXX**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**-3'**

Set A and Set B have mutually exclusive sequences and allow use together to form 384 combinatorial index pairs for sequencing up to 384 samples on one Illumina flow cell.

CleanPlex Targeted Library Kits and Panels are not compatible with most other commercially available index primer kits. Please use CleanPlex PCR Primers for Illumina for best results.

Index Sequences

CleanPlex Dual-Indexed PCR Primers for Illumina Set T 3 x 3 indexes, 9 reactions (SKU 716021)
A section of three i5 Indexes and three i7 Indexes from Illumina Set A to create 9 unique index combinations. Index selection may vary from kit to kit.

CleanPlex Dual-Indexed PCR Primers for Illumina Set A
12 x 8 indexes, 96 reactions (SKU 716006)
12 x 8 indexes, 384 reactions (SKU 716017)

i7 Index	Index Sequence	I7 Bases for Sample Sheet All Illumina Systems	i5 Index	Index Sequence	i5 Bases for Sample Sheet MiSeq, NovaSeq, HiSeq 2000/2500	i5 Bases for Sample Sheet MiniSeq, NextSeq, HiSeq 3000/4000
A701	GTCGTGAT	ATCACGAC	A501	TGAACCTT	TGAACCTT	AAGGTTCA
A702	ACCACTGT	ACAGTGGT	A502	TGCTAAGT	TGCTAAGT	ACTTAGCA
A703	TGGATCTG	CAGATCCA	A503	TGTTCTCT	TGTTCTCT	AGAGAACA
A704	CCGTTTGT	ACAAACGG	A504	TAAGACAC	TAAGACAC	GTGTCTTA
A705	TGCTGGGT	ACCCAGCA	A505	CTAATCGA	CTAATCGA	TCGATTAG
A706	GAGGGGTT	AACCCCTC	A506	CTAGAACA	CTAGAACA	TGTTCTAG
A707	AGGTTGGG	CCCAACCT	A507	TAAGTTCC	TAAGTTCC	GGAACTTA
A708	GTGTGGTG	CACCACAC	A508	TAGACCTA	TAGACCTA	TAGGTCTA
A709	TGGGTTTC	GAAACCCA				
A710	TGGTCACA	TGTGACCA				
A711	TTGACCCT	AGGGTCAA				
A712	CCACTCCT	AGGAGTGG				

CleanPlex Dual-Indexed PCR Primers for Illumina Set B
12 x 8 indexes, 96 reactions (SKU 716018)
12 x 8 indexes, 384 reactions (SKU 716019)

i7 Index	Index Sequence	I7 Bases for Sample Sheet All Illumina Systems
Q7005	ATATTCAC	GTGAATAT
Q7006	GCGCCTGT	ACAGGCGC
Q7007	ACTCTATG	CATAGAGT
Q7008	GTCTCGCA	TGCGAGAC
Q7015	AGTAGAGA	TCTCTACT
Q7016	GACGAGAG	CTCTCGTC
Q7017	AGACTTGG	CCAAGTCT
Q7018	GAGTCCAA	TTGGACTC
Q7023	AATTCTGC	GCAGAATT
Q7024	GGCCTCAT	ATGAGGCC
Q7025	ATCTTAGT	ACTAAGAT
Q7026	GCTCCGAC	GTCGGAGC

i5 Index	Index Sequence	i5 Bases for Sample Sheet	
		MiSeq, NovaSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
Q5001	AGCGCTAG	AGCGCTAG	CTAGCGCT
Q5002	GATATCGA	GATATCGA	TCGATATC
Q5007	ACATAGCG	ACATAGCG	CGCTATGT
Q5008	GTGCGATA	GTGCGATA	TATCGCAC
Q5009	CCAACAGA	CCAACAGA	TCTGTTGG
Q5010	TTGGTGAG	TTGGTGAG	CTCACCAA
Q5013	AACCGCGG	AACCGCGG	CCGCGGTT
Q5014	GGTTATAA	GGTTATAA	TTATAACC

Unique Dual-Indexed (UDI) PCR Primers for Illumina (Set C, D, E and F)

Primer Sequences

Each sample is indexed by a pair of Unique Dual Indexed PCR Primers (10 nucleotides) for sequencing on Illumina platforms. The indexes are available in sets of 96 for a total of 4 plates of 384 unique UDI for sequencing up to 384 samples in one lane.

Important: These 10 nucleotide Unique Dual-Indexed PCR primers **cannot** be sequenced together with the 8-nucleotide CleanPlex Dual-Indexed PCR Primers for Illumina Set T, A and B.

Index Sequences

CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set C
96 reactions (SKU **716037)**

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

UDI	i7 Index Sequence	i7 Bases for Sample Sheet All Illumina Systems	i5 Index Sequence	i5 Bases for Sample Sheet MiSeq, NovaSeq, HiSeq 2000/2500	i5 Bases for Sample Sheet MiniSeq, NextSeq, HiSeq 3000/4000
UDI001	GCTGAAGATA	TATCTTCAGC	CCAATATTCG	CCAATATTCG	CGAATATTGG
UDI002	TATCCGTGCA	TGCACGGATA	CGCAGACAAC	CGCAGACAAC	GTTGTCTGCG
UDI003	TCTATCAACC	GGTTGATAGA	TCGGAGCAGA	TCGGAGCAGA	TCTGCTCCGA
UDI004	AGGCAGGAGT	ACTCCTGCCT	GAGTCCGTAG	GAGTCCGTAG	CTACGGACTC
UDI005	CGACTATCGG	CCGATAGTCG	ATGTTCACGT	ATGTTCACGT	ACGTGAACAT
UDI006	TTCGATCTTG	CAAGATCGAA	TTCGATGGTT	TTCGATGGTT	AACCATCGAA
UDI007	GAAGGAGCCT	AGGCTCCTTC	TATCCGTGCA	TATCCGTGCA	TGCACGGATA
UDI008	CTATCCGTAT	ATACGGATAG	AAGCGCAGAG	AAGCGCAGAG	CTCTGCGCTT
UDI009	TGAGGCTATT	AATAGCCTCA	CCGACTTAGT	CCGACTTAGT	ACTAAGTCGG
UDI010	CCGATTGCAG	CTGCAATCGG	TTCTGCATCG	TTCTGCATCG	CGATGCAGAA
UDI011	ATAACTCAGG	CCTGAGTTAT	GGAAGTGCCA	GGAAGTGCCA	TGGCACTTCC
UDI012	TCTGGACGTC	GACGTCCAGA	AGATTCAACC	AGATTCAACC	GGTTGAATCT

UDI013	CCGATTATTC	GAATAATCGG	TTCAGGAGAT	TTCAGGAGAT	ATCTCCTGAA
UDI014	ACACACTCCG	CGGAGTGTGT	AAGGCGTCTG	AAGGCGTCTG	CAGACGCCTT
UDI015	CGGTCGGTAA	TTACCGACCG	ACGCTTGACA	ACGCTTGACA	TGTCAAGCGT
UDI016	GGCGAACACT	AGTGTTCGCC	CATGAAGTGA	CATGAAGTGA	TCACTTCATG
UDI017	AAGAACGTAG	CTACGTTCTT	TTACGACCTG	TTACGACCTG	CAGGTCGTAA
UDI018	TTCGTGTCGA	TCGACACGAA	ATGCAAGCCG	ATGCAAGCCG	CGGCTTGCAT
UDI019	AAGTTATCGG	CCGATAACTT	CTCCGTATAC	CTCCGTATAC	GTATACGGAG
UDI020	CGATGTCCAA	TTGGACATCG	GAATCTGGTC	GAATCTGGTC	GACCAGATTC
UDI021	TCTCAACGTT	AACGTTGAGA	CGGTCGGTAA	CGGTCGGTAA	TTACCGACCG
UDI022	TTCACTGGCC	GGCCAGTGAA	TCTGCTAATG	TCTGCTAATG	CATTAGCAGA
UDI023	CCGGAGACAT	ATGTCTCCGG	CTCTTATTCG	CTCTTATTCG	CGAATAAGAG
UDI024	GAACGCCTTC	GAAGGCGTTC	CACCTCTAGC	CACCTCTAGC	GCTAGAGGTG
UDI025	TCTAGGAACA	TGTTCCTAGA	TTACTTACCG	TTACTTACCG	CGGTAAGTAA
UDI026	ACCTCGAGAG	CTCTCGAGGT	CTATGCCTTA	CTATGCCTTA	TAAGGCATAG
UDI027	TACCGTACAG	CTGTACGGTA	GGAAGGTACG	GGAAGGTACG	CGTACCTTCC
UDI028	TTGCCATAAG	CTTATGGCAA	GAGGAGACGT	GAGGAGACGT	ACGTCTCCTC
UDI029	GCTATGCGGA	TCCGCATAGC	ACGCAAGGCA	ACGCAAGGCA	TGCCTTGCGT
UDI030	AGGTGCTTGC	GCAAGCACCT	TATCCTGACG	TATCCTGACG	CGTCAGGATA
UDI031	TAGGACAGGC	GCCTGTCCTA	GAAGACCGCT	GAAGACCGCT	AGCGGTCTTC
UDI032	GATAGACAGT	ACTGTCTATC	CAACGTGGAC	CAACGTGGAC	GTCCACGTTG
UDI033	TACATGGACG	CGTCCATGTA	TAAGTGCTCG	TAAGTGCTCG	CGAGCACTTA
UDI034	TTGCAGTTAG	CTAACTGCAA	CACATCGTAG	CACATCGTAG	CTACGATGTG
UDI035	ACCACAAGCA	TGCTTGTGGT	ACTACCGAGG	ACTACCGAGG	CCTCGGTAGT
UDI036	TGTGCTTACA	TGTAAGCACA	GATGTGTTCT	GATGTGTTCT	AGAACACATC
UDI037	ACGCAACGAG	CTCGTTGCGT	AAGTGTCGTA	AAGTGTCGTA	TACGACACTT
UDI038	CACCTCTAGC	GCTAGAGGTG	GGAGAACCAC	GGAGAACCAC	GTGGTTCTCC
UDI039	TTCTCCGCTT	AAGCGGAGAA	TGTACGAACT	TGTACGAACT	AGTTCGTACA
UDI040	CAGCGTCATT	AATGACGCTG	GGATGAGTGC	GGATGAGTGC	GCACTCATCC
UDI041	CGCGTACCAA	TTGGTACGCG	TAGTAGGACA	TAGTAGGACA	TGTCCTACTA
UDI042	TTCACCTTCA	TGAAGGTGAA	ACGCCTCGTT	ACGCCTCGTT	AACGAGGCGT
UDI043	AAGCCACTAC	GTAGTGGCTT	CACCGCTGTT	CACCGCTGTT	AACAGCGGTG
UDI044	TTCTGTTACG	CGTAACAGAA	TCTATAGCGG	TCTATAGCGG	CCGCTATAGA

UDI045	TTATGGCCTT	AAGGCCATAA	CCGATGGACA	CCGATGGACA	TGTCCATCGG
UDI046	GGTCTATGAA	TTCATAGACC	TTCAACATGC	TTCAACATGC	GCATGTTGAA
UDI047	TCGGAGTTGG	CCAACTCCGA	GGAGTAACGC	GGAGTAACGC	GCGTTACTCC
UDI048	CATACTCGTG	CACGAGTATG	AGCCTTAGCG	AGCCTTAGCG	CGCTAAGGCT
UDI049	TTGGTAGCGG	CCGCTACCAA	TTACCTCAGT	TTACCTCAGT	ACTGAGGTAA
UDI050	GGAGGTTCAG	CTGAACCTCC	CAGGCATTGT	CAGGCATTGT	ACAATGCCTG
UDI051	TAACAAGGCC	GGCCTTGTTA	GTGTTCCACG	GTGTTCCACG	CGTGGAACAC
UDI052	TCTGCGTTAA	TTAACGCAGA	TTGATCCGCC	TTGATCCGCC	GGCGGATCAA
UDI053	CGCACTACCT	AGGTAGTGCG	GGAGGCTGAT	GGAGGCTGAT	ATCAGCCTCC
UDI054	AAGTTACACG	CGTGTAACTT	AACGTGACAA	AACGTGACAA	TTGTCACGTT
UDI055	CGTCACAAGT	ACTTGTGACG	CACAAGCTCC	CACAAGCTCC	GGAGCTTGTG
UDI056	CAACGCATGG	CCATGCGTTG	CCGTGTTGTC	CCGTGTTGTC	GACAACACGG
UDI057	CGCTACAAGG	CCTTGTAGCG	TTGAGCCAGC	TTGAGCCAGC	GCTGGCTCAA
UDI058	TCACGTATGT	ACATACGTGA	GCGTTACAGA	GCGTTACAGA	TCTGTAACGC
UDI059	GGATATCAAG	CTTGATATCC	TCCAGACATT	TCCAGACATT	AATGTCTGGA
UDI060	ACATCGGCTG	CAGCCGATGT	TCGAACTCTT	TCGAACTCTT	AAGAGTTCGA
UDI061	TAGCGCATGA	TCATGCGCTA	ACCTTCTCGG	ACCTTCTCGG	CCGAGAAGGT
UDI062	TGGACGGAGT	ACTCCGTCCA	AGACGCCAAC	AGACGCCAAC	GTTGGCGTCT
UDI063	CAAGGCTGTC	GACAGCCTTG	CAACCGTAAT	CAACCGTAAT	ATTACGGTTG
UDI064	CAGATAACCG	CGGTTATCTG	TTATGCGTTG	TTATGCGTTG	CAACGCATAA
UDI065	CCGTGGAGTA	TACTCCACGG	CTATGAGAAC	CTATGAGAAC	GTTCTCATAG
UDI066	TGCCGGAAGT	ACTTCCGGCA	AAGTTACACG	AAGTTACACG	CGTGTAACTT
UDI067	GCAGCTTCAC	GTGAAGCTGC	GCAATGTGAG	GCAATGTGAG	CTCACATTGC
UDI068	AGAAGAGCAA	TTGCTCTTCT	CGAAGTCGCA	CGAAGTCGCA	TGCGACTTCG
UDI069	TACGTGCGTT	AACGCACGTA	CCTGATTCAA	CCTGATTCAA	TTGAATCAGG
UDI070	CCTGCAGTAA	TTACTGCAGG	TAGAACGTGC	TAGAACGTGC	GCACGTTCTA
UDI071	CCTCAACTGG	CCAGTTGAGG	TTCGCAAGGT	TTCGCAAGGT	ACCTTGCGAA
UDI072	TTAACGCACA	TGTGCGTTAA	TTAATGCCGA	TTAATGCCGA	TCGGCATTAA
UDI073	AAGCACTAGT	ACTAGTGCTT	AGAACAGAGT	AGAACAGAGT	ACTCTGTTCT
UDI074	GTGTTCCACG	CGTGGAACAC	CCATCTGTTC	CCATCTGTTC	GAACAGATGG
UDI075	CCACTTCCAT	ATGGAAGTGG	TTCGTAGGTG	TTCGTAGGTG	CACCTACGAA
UDI076	TGTGATCTCA	TGAGATCACA	GCACGGTACA	GCACGGTACA	TGTACCGTGC

UDI077	CACCAAGGAC	GTCCTTGGTG	TGTCAAGAGG	TGTCAAGAGG	CCTCTTGACA
UDI078	TTCCACGCTC	GAGCGTGGAA	TCTAAGGTAC	TCTAAGGTAC	GTACCTTAGA
UDI079	ACAGCGTGTG	CACACGCTGT	GAACGGAGAC	GAACGGAGAC	GTCTCCGTTC
UDI080	TGTACAACCA	TGGTTGTACA	CGCTACCATC	CGCTACCATC	GATGGTAGCG
UDI081	TGTGAGTGAT	ATCACTCACA	TTACGGTAAC	TTACGGTAAC	GTTACCGTAA
UDI082	TCTACCTCCG	CGGAGGTAGA	TTCAGATGGA	TTCAGATGGA	TCCATCTGAA
UDI083	TTGTCAACTC	GAGTTGACAA	TAGCATCTGT	TAGCATCTGT	ACAGATGCTA
UDI084	CAAGTTCGGC	GCCGAACTTG	GGACGAGATC	GGACGAGATC	GATCTCGTCC
UDI085	TGTGAGGCCT	AGGCCTCACA	AGGTTCTGTT	AGGTTCTGTT	AACAGAACCT
UDI086	CTAACAGAGA	TCTCTGTTAG	CATACTCGTG	CATACTCGTG	CACGAGTATG
UDI087	AATCGTCGGA	TCCGACGATT	CCGGATACCA	CCGGATACCA	TGGTATCCGG
UDI088	AACATAGCCT	AGGCTATGTT	ATGTCCACCG	ATGTCCACCG	CGGTGGACAT
UDI089	CAAGAGAACG	CGTTCTCTTG	CACCAAGTGG	CACCAAGTGG	CCACTTGGTG
UDI090	CCATAGACAA	TTGTCTATGG	TTGAGTACAC	TTGAGTACAC	GTGTACTCAA
UDI091	TGTATCCATC	GATGGATACA	CGGTTCCGTA	CGGTTCCGTA	TACGGAACCG
UDI092	CGCCTAAGTG	CACTTAGGCG	GGAGGTCCTA	GGAGGTCCTA	TAGGACCTCC
UDI093	TAGCCAGTGT	ACACTGGCTA	CCTGCTTGGA	CCTGCTTGGA	TCCAAGCAGG
UDI094	CAGTGGCGAT	ATCGCCACTG	TTCACGTCAG	TTCACGTCAG	CTGACGTGAA
UDI095	TTCACGTCAG	CTGACGTGAA	AACATAGCCT	AACATAGCCT	AGGCTATGTT
UDI096	AGACGATTGA	TCAATCGTCT	TGACATAGTC	TGACATAGTC	GACTATGTCA

CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set D
96 reactions (SKU **716038)**

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

UDI	i7 Index Sequence	i7 Bases for Sample Sheet All Illumina Systems	i5 Index Sequence	i5 Bases for Sample Sheet	
				MiSeq, NovaSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
UDI097	TATAGGCGAT	ATCGCCTATA	TTGGCTCATA	TTGGCTCATA	TATGAGCCAA
UDI098	CAGGAATCCG	CGGATTCCTG	CAGAATACGG	CAGAATACGG	CCGTATTCTG
UDI099	CCACGTGTGA	TCACACGTGG	TGTATAGGTC	TGTATAGGTC	GACCTATACA
UDI100	GGAATGCTGC	GCAGCATTCC	GTATACCACA	GTATACCACA	TGTGGTATAC
UDI101	TTCACCACGG	CCGTGGTGAA	AACTGGACGG	AACTGGACGG	CCGTCCAGTT
UDI102	CCGTTCTGTG	CACAGAACGG	TGTGAGTGAT	TGTGAGTGAT	ATCACTCACA
UDI103	TTCGATCCAT	ATGGATCGAA	AACTCAGCAA	AACTCAGCAA	TTGCTGAGTT
UDI104	AGGTGAGACC	GGTCTCACCT	AGACGATTGA	AGACGATTGA	TCAATCGTCT
UDI105	TACGGTGTTG	CAACACCGTA	CGGCTTGTTC	CGGCTTGTTC	GAACAAGCCG
UDI106	CCAATATTCG	CGAATATTGG	TTCCGTGCTG	TTCCGTGCTG	CAGCACGGAA
UDI107	GCTGGAATTA	TAATTCCAGC	CGAATACGAT	CGAATACGAT	ATCGTATTCG
UDI108	TAACCGCGAC	GTCGCGGTTA	ACCTCACCAG	ACCTCACCAG	CTGGTGAGGT
UDI109	CGACGCAGAA	TTCTGCGTCG	TTCGTACACC	TTCGTACACC	GGTGTACGAA
UDI110	AAGTATGCGT	ACGCATACTT	AAGTACGAGA	AAGTACGAGA	TCTCGTACTT
UDI111	TTGTGCAGCC	GGCTGCACAA	TCGGACCTCT	TCGGACCTCT	AGAGGTCCGA
UDI112	TTGGCTTGGT	ACCAAGCCAA	CCGCCTTGTA	CCGCCTTGTA	TACAAGGCGG
UDI113	GGACAATTGG	CCAATTGTCC	GCGTATGAGC	GCGTATGAGC	GCTCATACGC
UDI114	AAGGCGTCTG	CAGACGCCTT	TTGAGCTCTG	TTGAGCTCTG	CAGAGCTCAA
UDI115	TCTGGCAATT	AATTGCCAGA	AACGTACCGT	AACGTACCGT	ACGGTACGTT
UDI116	TCTGGTATCA	TGATACCAGA	GGCCTTCACA	GGCCTTCACA	TGTGAAGGCC
UDI117	TAACAACCTC	GAGGTTGTTA	TGTGCACTGG	TGTGCACTGG	CCAGTGCACA
UDI118	CTGATACTCT	AGAGTATCAG	GGATACAGGT	GGATACAGGT	ACCTGTATCC

UDI119	CATACGCCAG	CTGGCGTATG	CCAATGTTAC	CCAATGTTAC	GTAACATTGG
UDI120	CGAGATGACC	GGTCATCTCG	GCTATGCGGA	GCTATGCGGA	TCCGCATAGC
UDI121	TTGTTCGACA	TGTCGAACAA	CCAGAATCTA	CCAGAATCTA	TAGATTCTGG
UDI122	TTCGTGCCAC	GTGGCACGAA	CCAATTAGCA	CCAATTAGCA	TGCTAATTGG
UDI123	TCTAAGGCTT	AAGCCTTAGA	CGTGTTATGA	CGTGTTATGA	TCATAACACG
UDI124	AGCCTTAGCG	CGCTAAGGCT	TGTGCCGGTT	TGTGCCGGTT	AACCGGCACA
UDI125	GGTCGTGATT	AATCACGACC	CACCAGAAGT	CACCAGAAGT	ACTTCTGGTG
UDI126	CGACAGCTAC	GTAGCTGTCG	TCTGCGTTAA	TCTGCGTTAA	TTAACGCAGA
UDI127	ACCTTACGTG	CACGTAAGGT	AGCTTAGAGG	AGCTTAGAGG	CCTCTAAGCT
UDI128	CATGAAGTGA	TCACTTCATG	TTGCGACCAC	TTGCGACCAC	GTGGTCGCAA
UDI129	AGACGCCAAC	GTTGGCGTCT	CGAAGTCTAG	CGAAGTCTAG	CTAGACTTCG
UDI130	TTGGCGTGTG	CACACGCCAA	GCTGAAGATA	GCTGAAGATA	TATCTTCAGC
UDI131	TTCACAGTGT	ACACTGTGAA	TCTGTTAGAC	TCTGTTAGAC	GTCTAACAGA
UDI132	AGAACAATCG	CGATTGTTCT	TGTACAACCA	TGTACAACCA	TGGTTGTACA
UDI133	CAGTAGCCGA	TCGGCTACTG	CTATTGTGTG	CTATTGTGTG	CACACAATAG
UDI134	CCTCTTACAA	TTGTAAGAGG	GAAGCAGCTG	GAAGCAGCTG	CAGCTGCTTC
UDI135	AACGGACTCG	CGAGTCCGTT	CCGCAGTAGT	CCGCAGTAGT	ACTACTGCGG
UDI136	TTGAGTACAC	GTGTACTCAA	AAGGTTGCTT	AAGGTTGCTT	AAGCAACCTT
UDI137	AACGTCACGC	GCGTGACGTT	CTCTCTTCTA	CTCTCTTCTA	TAGAAGAGAG
UDI138	TCAGACGCCT	AGGCGTCTGA	GGATCTTGTG	GGATCTTGTG	CACAAGATCC
UDI139	CCTCGTAAGT	ACTTACGAGG	AGCGATTAAC	AGCGATTAAC	GTTAATCGCT
UDI140	TTACGACCTG	CAGGTCGTAA	GAAGGCATAA	GAAGGCATAA	TTATGCCTTC
UDI141	AACTAGCGTA	TACGCTAGTT	AGCAGACTAA	AGCAGACTAA	TTAGTCTGCT
UDI142	GCACGACAGA	TCTGTCGTGC	AAGCACTAGT	AAGCACTAGT	ACTAGTGCTT
UDI143	TGCCAAGATC	GATCTTGGCA	TTAGACAGCG	TTAGACAGCG	CGCTGTCTAA
UDI144	TGGCTCTCCA	TGGAGAGCCA	TTAGGCACAA	TTAGGCACAA	TTGTGCCTAA
UDI145	CGAGATTGGT	ACCAATCTCG	TTCCGGCACT	TTCCGGCACT	AGTGCCGGAA
UDI146	GTGTCACGAC	GTCGTGACAC	TTGTATGGCT	TTGTATGGCT	AGCCATACAA
UDI147	CGACTAGAGA	TCTCTAGTCG	TGGATCGATT	TGGATCGATT	AATCGATCCA
UDI148	CAACCGTAAT	ATTACGGTTG	CGGAATCACC	CGGAATCACC	GGTGATTCCG
UDI149	TTACTTACCG	CGGTAAGTAA	GAGCTATCTA	GAGCTATCTA	TAGATAGCTC
UDI150	CCGGACGTTA	TAACGTCCGG	ACCTCGAGAG	ACCTCGAGAG	CTCTCGAGGT

UDI151	AACTGTGTTC	GAACACAGTT	CCGAATTCAC	CCGAATTCAC	GTGAATTCGG
UDI152	TATAGGACCT	AGGTCCTATA	AACGTCACGC	AACGTCACGC	GCGTGACGTT
UDI153	GCTAGGTCAA	TTGACCTAGC	TTGGTGTTCC	TTGGTGTTCC	GGAACACCAA
UDI154	TGATTGAAGC	GCTTCAATCA	CCAGGTGGAA	CCAGGTGGAA	TTCCACCTGG
UDI155	TTCGCACGCA	TGCGTGCGAA	TCATACCGAT	TCATACCGAT	ATCGGTATGA
UDI156	AGGTACCATT	AATGGTACCT	CGACGGTTGT	CGACGGTTGT	ACAACCGTCG
UDI157	TCGCGATACA	TGTATCGCGA	CACTCACACG	CACTCACACG	CGTGTGAGTG
UDI158	CCAATGTTAC	GTAACATTGG	TTGGCCACGA	TTGGCCACGA	TCGTGGCCAA
UDI159	CGAATTGTTG	CAACAATTCG	AATCGGTCGC	AATCGGTCGC	GCGACCGATT
UDI160	CATGACACGC	GCGTGTCATG	AGAACAATCG	AGAACAATCG	CGATTGTTCT
UDI161	TTCGGATCTA	TAGATCCGAA	CTATCGAAGT	CTATCGAAGT	ACTTCGATAG
UDI162	CCAGTTAAGA	TCTTAACTGG	TCGGCCTGAA	TCGGCCTGAA	TTCAGGCCGA
UDI163	CGGATGTGAC	GTCACATCCG	TCACTGTTCT	TCACTGTTCT	AGAACAGTGA
UDI164	AGATGCTTCA	TGAAGCATCT	GGTATCTAAC	GGTATCTAAC	GTTAGATACC
UDI165	AAGTAGTCCG	CGGACTACTT	CGTATTAAGG	CGTATTAAGG	CCTTAATACG
UDI166	GGACTCCGTT	AACGGAGTCC	TAGGAGTGTC	TAGGAGTGTC	GACACTCCTA
UDI167	GGTCACACCT	AGGTGTGACC	CTCCGAACTC	CTCCGAACTC	GAGTTCGGAG
UDI168	GGAACTCTGG	CCAGAGTTCC	ATGTCTCTCG	ATGTCTCTCG	CGAGAGACAT
UDI169	CAATCACTGG	CCAGTGATTG	AGGTGCACTT	AGGTGCACTT	AAGTGCACCT
UDI170	TATGTCAGTC	GACTGACATA	TTGGCCGCAT	TTGGCCGCAT	ATGCGGCCAA
UDI171	CAAGGATCGC	GCGATCCTTG	GGTGTCTGAG	GGTGTCTGAG	CTCAGACACC
UDI172	AAGTGGAACA	TGTTCCACTT	CCGTGCCATT	CCGTGCCATT	AATGGCACGG
UDI173	CCTATTGGAT	ATCCAATAGG	AAGATGACGA	AAGATGACGA	TCGTCATCTT
UDI174	TTAACGGTCT	AGACCGTTAA	TGTATTGCCA	TGTATTGCCA	TGGCAATACA
UDI175	GGTCAATAGT	ACTATTGACC	AACCATCGGC	AACCATCGGC	GCCGATGGTT
UDI176	GGAATTAGGC	GCCTAATTCC	CGTGCAACCT	CGTGCAACCT	AGGTTGCACG
UDI177	TTGTACCTAC	GTAGGTACAA	TTCTTGAGTG	TTCTTGAGTG	CACTCAAGAA
UDI178	CGAAGTCGCA	TGCGACTTCG	TCTGCAACAA	TCTGCAACAA	TTGTTGCAGA
UDI179	AACGTGACAA	TTGTCACGTT	CCGCTACACA	CCGCTACACA	TGTGTAGCGG
UDI180	TCAGTCGTTG	CAACGACTGA	CTCTGTCAGG	CTCTGTCAGG	CCTGACAGAG
UDI181	TAGCCGAATC	GATTCGGCTA	TTAACGGTCT	TTAACGGTCT	AGACCGTTAA
UDI182	CTAGCCACCA	TGGTGGCTAG	CGATGACCTT	CGATGACCTT	AAGGTCATCG

UDI183	ATCCTGGCCT	AGGCCAGGAT	AGGCAGGAGT	AGGCAGGAGT	ACTCCTGCCT
UDI184	CACAGGCGTT	AACGCCTGTG	AACGGACTCG	AACGGACTCG	CGAGTCCGTT
UDI185	CACTCACACG	CGTGTGAGTG	TTGGTTCGGC	TTGGTTCGGC	GCCGAACCAA
UDI186	TTCACATACG	CGTATGTGAA	CGCACTACCT	CGCACTACCT	AGGTAGTGCG
UDI187	TTGTGACGTA	TACGTCACAA	CCATACCACG	CCATACCACG	CGTGGTATGG
UDI188	CGGATCTTCC	GGAAGATCCG	GAATTCGGTA	GAATTCGGTA	TACCGAATTC
UDI189	AGCTGACATG	CATGTCAGCT	AGTCCTCCAC	AGTCCTCCAC	GTGGAGGACT
UDI190	GTGACGCTGT	ACAGCGTCAC	TAGTCATTCG	TAGTCATTCG	CGAATGACTA
UDI191	CCTTGTAACA	TGTTACAAGG	TTGAGGTCGC	TTGAGGTCGC	GCGACCTCAA
UDI192	CCTCTATAAG	CTTATAGAGG	CAACGTTATG	CAACGTTATG	CATAACGTTG

CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set E
96 reactions (SKU **716039)**

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	201	209	217	225	233	241	249	257	265	273	281
B	194	202	210	218	226	234	242	250	258	266	274	282
C	195	203	211	219	227	235	243	251	259	267	275	283
D	196	204	212	220	228	236	244	252	260	268	276	284
E	197	205	213	221	229	237	245	253	261	269	277	285
F	198	206	214	222	230	238	246	254	262	270	278	286
G	199	207	215	223	231	239	247	255	263	271	279	287
H	200	208	216	224	232	240	248	256	264	272	280	288

UDI	i7 Index Sequence	i7 Bases for Sample Sheet All Illumina Systems	i5 Index Sequence	i5 Bases for Sample Sheet	
				MiSeq, NovaSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
UDI193	TTGACTTCGA	TCGAAGTCAA	CGGAGGAATG	CGGAGGAATG	CATTCCTCCG
UDI194	ATAGGCCTCG	CGAGGCCTAT	GAGTCAGCCA	GAGTCAGCCA	TGGCTGACTC
UDI195	CACTTCAGGA	TCCTGAAGTG	GGAATTAGGC	GGAATTAGGC	GCCTAATTCC
UDI196	GGTAAGGATT	AATCCTTACC	TTCGCCACAC	TTCGCCACAC	GTGTGGCGAA
UDI197	TCTGCAACAA	TTGTTGCAGA	CCTCGCTTAC	CCTCGCTTAC	GTAAGCGAGG
UDI198	GGCCTAGATT	AATCTAGGCC	ACAGCGTGTG	ACAGCGTGTG	CACACGCTGT
UDI199	CAGTAGAGCC	GGCTCTACTG	TTCCGCTTCT	TTCCGCTTCT	AGAAGCGGAA
UDI200	CAACGTGGAC	GTCCACGTTG	CAGCGTCATT	CAGCGTCATT	AATGACGCTG
UDI201	AACTGCGGAG	CTCCGCAGTT	CCGTAGAACA	CCGTAGAACA	TGTTCTACGG
UDI202	TCACTGTTCT	AGAACAGTGA	CGGTTATCGT	CGGTTATCGT	ACGATAACCG
UDI203	CAATAAGAGC	GCTCTTATTG	TCTGGTATCA	TCTGGTATCA	TGATACCAGA
UDI204	TTCGTCTACA	TGTAGACGAA	AAGTATGCGT	AAGTATGCGT	ACGCATACTT
UDI205	CGACGACAAG	CTTGTCGTCG	TTCCTTCGAG	TTCCTTCGAG	CTCGAAGGAA
UDI206	TGTAAGACGA	TCGTCTTACA	GCTATGGATA	GCTATGGATA	TATCCATAGC
UDI207	TCCTCCTCTC	GAGAGGAGGA	AGGTACCATT	AGGTACCATT	AATGGTACCT
UDI208	GGTATCTAAC	GTTAGATACC	TTACGGAGTC	TTACGGAGTC	GACTCCGTAA
UDI209	TTCTTAAGCC	GGCTTAAGAA	TGAGGACTTA	TGAGGACTTA	TAAGTCCTCA
UDI210	TTGTACCAGA	TCTGGTACAA	TTGAGTTGCC	TTGAGTTGCC	GGCAACTCAA
UDI211	CCGAATTCAC	GTGAATTCGG	AGCTTCGCGA	AGCTTCGCGA	TCGCGAAGCT
UDI212	TTCTCCATTC	GAATGGAGAA	CATACGCCAG	CATACGCCAG	CTGGCGTATG
UDI213	CCAATTGACT	AGTCAATTGG	CAAGACCAGC	CAAGACCAGC	GCTGGTCTTG
UDI214	AGGTGATGCG	CGCATCACCT	GATAGACAGT	GATAGACAGT	ACTGTCTATC
UDI215	GGTGTCAATA	TATTGACACC	CGCTCGTGAA	CGCTCGTGAA	TTCACGAGCG

UDI216	CCGACAGTCT	AGACTGTCGG	TCTCTAACAG	TCTCTAACAG	CTGTTAGAGA
UDI217	GAGTCCAGAT	ATCTGGACTC	ACCTAGGAGG	ACCTAGGAGG	CCTCCTAGGT
UDI218	CCTTATTCTC	GAGAATAAGG	TCTGTACCTT	TCTGTACCTT	AAGGTACAGA
UDI219	GGCGACAACA	TGTTGTCGCC	CTCAGGCCAT	CTCAGGCCAT	ATGGCCTGAG
UDI220	AACACCGCAG	CTGCGGTGTT	TTGTGCAGCC	TTGTGCAGCC	GGCTGCACAA
UDI221	CGGAGTTATC	GATAACTCCG	TAGCCGAATC	TAGCCGAATC	GATTCGGCTA
UDI222	GTACAAGGAT	ATCCTTGTAC	AAGCCTGTTA	AAGCCTGTTA	TAACAGGCTT
UDI223	TATACGCGTA	TACGCGTATA	TGTACAGTAG	TGTACAGTAG	CTACTGTACA
UDI224	CAATTGGTGG	CCACCAATTG	CGATTCTGCC	CGATTCTGCC	GGCAGAATCG
UDI225	GGCCTTCACA	TGTGAAGGCC	TTGCTAAGGA	TTGCTAAGGA	TCCTTAGCAA
UDI226	GCAGTCAAGG	CCTTGACTGC	ACTCCTTGGC	ACTCCTTGGC	GCCAAGGAGT
UDI227	CCGACGCATT	AATGCGTCGG	GAAGGCGAAC	GAAGGCGAAC	GTTCGCCTTC
UDI228	GTGTAGTCTT	AAGACTACAC	CAATACCTTG	CAATACCTTG	CAAGGTATTG
UDI229	CTGCACTGAC	GTCAGTGCAG	CGACGACAAG	CGACGACAAG	CTTGTCGTCG
UDI230	TTCTGGTGAG	CTCACCAGAA	GAACCTGACC	GAACCTGACC	GGTCAGGTTC
UDI231	AAGTACGAGA	TCTCGTACTT	TTGCCTCGCA	TTGCCTCGCA	TGCGAGGCAA
UDI232	CCTAATCTGA	TCAGATTAGG	TTCGTGTCGA	TTCGTGTCGA	TCGACACGAA
UDI233	TTCTTGAGTG	CACTCAAGAA	TGGATGGCAA	TGGATGGCAA	TTGCCATCCA
UDI234	GAATGGCTCT	AGAGCCATTC	TTCACCAGCT	TTCACCAGCT	AGCTGGTGAA
UDI235	CGGAATCGTG	CACGATTCCG	CCTGAGTAGC	CCTGAGTAGC	GCTACTCAGG
UDI236	CAGGCTCCAA	TTGGAGCCTG	AGGTGTCCGT	AGGTGTCCGT	ACGGACACCT
UDI237	CAAGTCGTAA	TTACGACTTG	GTCTGGTTGC	GTCTGGTTGC	GCAACCAGAC
UDI238	CCGACCTTAA	TTAAGGTCGG	CTCTTAGATG	CTCTTAGATG	CATCTAAGAG
UDI239	TGACAGAACC	GGTTCTGTCA	TATCACCTGC	TATCACCTGC	GCAGGTGATA
UDI240	GGTGCGTATC	GATACGCACC	CAGAGGCAAG	CAGAGGCAAG	CTTGCCTCTG
UDI241	AGCTTCGCGA	TCGCGAAGCT	CCGGTCAACA	CCGGTCAACA	TGTTGACCGG
UDI242	CCGTCTTAAC	GTTAAGACGG	TCACGAGGTG	TCACGAGGTG	CACCTCGTGA
UDI243	GTATGACCGG	CCGGTCATAC	CCATAGACAA	CCATAGACAA	TTGTCTATGG
UDI244	TTAAGCTGAC	GTCAGCTTAA	GAGCTTGGAC	GAGCTTGGAC	GTCCAAGCTC
UDI245	TATCCGCGGT	ACCGCGGATA	TACGGTGTTG	TACGGTGTTG	CAACACCGTA
UDI246	TTGATGCAAC	GTTGCATCAA	TTCAACTCGA	TTCAACTCGA	TCGAGTTGAA
UDI247	TTGGTGCACA	TGTGCACCAA	AAGGCAGGTA	AAGGCAGGTA	TACCTGCCTT

UDI248	GACCACAGAT	ATCTGTGGTC	CGGCCAATTC	CGGCCAATTC	GAATTGGCCG
UDI249	GGATCTTGTG	CACAAGATCC	CAACCGGACA	CAACCGGACA	TGTCCGGTTG
UDI250	CAGCTAGCAG	CTGCTAGCTG	AACTTGGCCG	AACTTGGCCG	CGGCCAAGTT
UDI251	TTCGACCGGT	ACCGGTCGAA	TGGAACATAG	TGGAACATAG	CTATGTTCCA
UDI252	TAGAACGTGC	GCACGTTCTA	TTCGGATCTA	TTCGGATCTA	TAGATCCGAA
UDI253	TTCCTTCCTT	AAGGAAGGAA	CGGAATCGTG	CGGAATCGTG	CACGATTCCG
UDI254	CTATCTCTCT	AGAGAGATAG	TCTAATCGGT	TCTAATCGGT	ACCGATTAGA
UDI255	AATAGGAACC	GGTTCCTATT	GCTGGAATTA	GCTGGAATTA	TAATTCCAGC
UDI256	CGCTCGTGAA	TTCACGAGCG	CGCTTCTCAC	CGCTTCTCAC	GTGAGAAGCG
UDI257	AGGTTGTGCC	GGCACAACCT	TAGACTCCTG	TAGACTCCTG	CAGGAGTCTA
UDI258	TTCTGAGTCA	TGACTCAGAA	CCGTTGATTG	CCGTTGATTG	CAATCAACGG
UDI259	CCTGAGATCG	CGATCTCAGG	CGAACCTCCA	CGAACCTCCA	TGGAGGTTCG
UDI260	TTCCAGCAGG	CCTGCTGGAA	TTGGAAGTTG	TTGGAAGTTG	CAACTTCCAA
UDI261	TATACAGCTC	GAGCTGTATA	CCAGGAGTAC	CCAGGAGTAC	GTACTCCTGG
UDI262	CCGTCAGGTT	AACCTGACGG	AGGTTCGTCG	AGGTTCGTCG	CGACGAACCT
UDI263	CCACGAGCTT	AAGCTCGTGG	GACCTGAAGA	GACCTGAAGA	TCTTCAGGTC
UDI264	GAGCTTGGAC	GTCCAAGCTC	TTAACGCACA	TTAACGCACA	TGTGCGTTAA
UDI265	CGAAGTCTAG	CTAGACTTCG	TCGGAGTTGG	TCGGAGTTGG	CCAACTCCGA
UDI266	TTACCTTGGA	TCCAAGGTAA	CGATGACTCC	CGATGACTCC	GGAGTCATCG
UDI267	TGCTACCAAG	CTTGGTAGCA	TATAGGTTGG	TATAGGTTGG	CCAACCTATA
UDI268	ACGCCTCGTT	AACGAGGCGT	GACAAGTGTT	GACAAGTGTT	AACACTTGTC
UDI269	CCATCTTCTG	CAGAAGATGG	TTCTCCGGAA	TTCTCCGGAA	TTCCGGAGAA
UDI270	GGATGTATCA	TGATACATCC	ACACACTCCG	ACACACTCCG	CGGAGTGTGT
UDI271	AACTACGCGC	GCGCGTAGTT	CTGGTCACTA	CTGGTCACTA	TAGTGACCAG
UDI272	CGCAGACAAC	GTTGTCTGCG	TTCGTGCCAC	TTCGTGCCAC	GTGGCACGAA
UDI273	CAGCGCTAAG	CTTAGCGCTG	AGATCATGGA	AGATCATGGA	TCCATGATCT
UDI274	GGAGGCTGAT	ATCAGCCTCC	GAGTATGTAC	GAGTATGTAC	GTACATACTC
UDI275	GAGCACTGCA	TGCAGTGCTC	TAGAACACCT	TAGAACACCT	AGGTGTTCTA
UDI276	GTCTGAGCTC	GAGCTCAGAC	CCAGTTAAGA	CCAGTTAAGA	TCTTAACTGG
UDI277	TTGTCCAGGT	ACCTGGACAA	CGCTTATCTG	CGCTTATCTG	CAGATAAGCG
UDI278	TTGGAAGTTG	CAACTTCCAA	GAGCTCTTAC	GAGCTCTTAC	GTAAGAGCTC
UDI279	CACAGGATGG	CCATCCTGTG	TCTCAAGGCG	TCTCAAGGCG	CGCCTTGAGA

UDI280	TCTAACTGCC	GGCAGTTAGA	CTAAGTACCA	CTAAGTACCA	TGGTACTTAG
UDI281	TCTCATGTGA	TCACATGAGA	TCGACAAGCC	TCGACAAGCC	GGCTTGTCGA
UDI282	CCAACGAATA	TATTCGTTGG	TTCGACATCA	TTCGACATCA	TGATGTCGAA
UDI283	GAAGACCGCT	AGCGGTCTTC	AGTGGTACTT	AGTGGTACTT	AAGTACCACT
UDI284	AATCGGTCGC	GCGACCGATT	TTGCACTTGT	TTGCACTTGT	ACAAGTGCAA
UDI285	GGACGAGATC	GATCTCGTCC	GTCTTCGCAG	GTCTTCGCAG	CTGCGAAGAC
UDI286	CCTATAATGG	CCATTATAGG	CAGGCTCCAA	CAGGCTCCAA	TTGGAGCCTG
UDI287	CGTGGTCTGT	ACAGACCACG	CCAGGTTACG	CCAGGTTACG	CGTAACCTGG
UDI288	TGTGTGGAAT	ATTCCACACA	CAATCGCCTA	CAATCGCCTA	TAGGCGATTG

CleanPlex Unique Dual-Indexed PCR Primers for Illumina Set F
96 reactions (SKU **716040)**

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	297	305	313	321	329	337	345	353	361	369	377
B	290	298	306	314	322	330	338	346	354	362	370	378
C	291	299	307	315	323	331	339	347	355	363	371	379
D	292	300	308	316	324	332	340	348	356	364	372	380
E	293	301	309	317	325	333	341	349	357	365	373	381
F	294	302	310	318	326	334	342	350	358	366	374	382
G	295	303	311	319	327	335	343	351	359	367	375	383
H	296	304	312	320	328	336	344	352	360	368	376	384

UDI	i7 Index Sequence	i7 Bases for Sample Sheet All Illumina Systems	i5 Index Sequence	i5 Bases for Sample Sheet	
				MiSeq, NovaSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
UDI289	TTCGTTCCGG	CCGGAACGAA	CTACACTATG	CTACACTATG	CATAGTGTAG
UDI290	CTGTCTTGCT	AGCAAGACAG	AAGAAGATCC	AAGAAGATCC	GGATCTTCTT
UDI291	AGATTCAACC	GGTTGAATCT	CACGGTGTAT	CACGGTGTAT	ATACACCGTG
UDI292	AGAGTGTTGA	TCAACACTCT	CGGTAAGTGG	CGGTAAGTGG	CCACTTACCG
UDI293	TTGGCTCATA	TATGAGCCAA	CCAGACTGAG	CCAGACTGAG	CTCAGTCTGG
UDI294	GCTCCAATAT	ATATTGGAGC	GCTAGGTCAA	GCTAGGTCAA	TTGACCTAGC
UDI295	CTAACGAGTT	AACTCGTTAG	GGTGCCTCAT	GGTGCCTCAT	ATGAGGCACC
UDI296	GCTATGGATA	TATCCATAGC	TAACAACCTC	TAACAACCTC	GAGGTTGTTA
UDI297	GGTATGGTGA	TCACCATACC	TTGGCAAGAA	TTGGCAAGAA	TTCTTGCCAA
UDI298	GTCTTCGCAG	CTGCGAAGAC	GGAAGTGGTT	GGAAGTGGTT	AACCACTTCC
UDI299	TTCGCCACAC	GTGTGGCGAA	TCTAACCTTC	TCTAACCTTC	GAAGGTTAGA
UDI300	CAACCACCTT	AAGGTGGTTG	GTATGACCGG	GTATGACCGG	CCGGTCATAC
UDI301	CAATCGAGCG	CGCTCGATTG	CCTGTGTATT	CCTGTGTATT	AATACACAGG
UDI302	AATCCGACAC	GTGTCGGATT	TTCTCCGCTT	TTCTCCGCTT	AAGCGGAGAA
UDI303	CATGTTGTGG	CCACAACATG	CCACTGGTAA	CCACTGGTAA	TTACCAGTGG
UDI304	TTCCATCGGA	TCCGATGGAA	AATCCGACAC	AATCCGACAC	GTGTCGGATT
UDI305	CGCTTATCTG	CAGATAAGCG	TTCACCTTCA	TTCACCTTCA	TGAAGGTGAA
UDI306	TTCGACATCA	TGATGTCGAA	TATAGGACCT	TATAGGACCT	AGGTCCTATA
UDI307	TCTAGAAGGT	ACCTTCTAGA	TTACCGTGAT	TTACCGTGAT	ATCACGGTAA
UDI308	GAGCGGAACT	AGTTCCGCTC	ACGTTAGAGT	ACGTTAGAGT	ACTCTAACGT
UDI309	AGCAGACTAA	TTAGTCTGCT	CAGCGCTAAG	CAGCGCTAAG	CTTAGCGCTG
UDI310	ATCGCCGACA	TGTCGGCGAT	ACAGGTACTC	ACAGGTACTC	GAGTACCTGT
UDI311	GGTGTTAAGT	ACTTAACACC	CTAGATAGCA	CTAGATAGCA	TGCTATCTAG
UDI312	ACTCCTTGGC	GCCAAGGAGT	TTGGACCTTA	TTGGACCTTA	TAAGGTCCAA

UDI313	GGAACCTTGC	GCAAGGTTCC	TGTAGCACAG	TGTAGCACAG	CTGTGCTACA
UDI314	TGTGTCCTAT	ATAGGACACA	TTGACGTCTT	TTGACGTCTT	AAGACGTCAA
UDI315	CAACACATTC	GAATGTGTTG	CCATCTCCGT	CCATCTCCGT	ACGGAGATGG
UDI316	GTAACATGCA	TGCATGTTAC	CTAACAGAGA	CTAACAGAGA	TCTCTGTTAG
UDI317	ACGCAAGGCA	TGCCTTGCGT	ACCTTACGTG	ACCTTACGTG	CACGTAAGGT
UDI318	CCGTGTTACT	AGTAACACGG	AGGTGCTTGC	AGGTGCTTGC	GCAAGCACCT
UDI319	TTCGGTTGTG	CACAACCGAA	GACTAGGTCC	GACTAGGTCC	GGACCTAGTC
UDI320	AGGTGTCCGT	ACGGACACCT	TCGAAGTTCT	TCGAAGTTCT	AGAACTTCGA
UDI321	CGCTGTAACG	CGTTACAGCG	CTATCTCTCT	CTATCTCTCT	AGAGAGATAG
UDI322	AACCAATGGT	ACCATTGGTT	CCGTTCTCCT	CCGTTCTCCT	AGGAGAACGG
UDI323	TCGAGGCAGT	ACTGCCTCGA	ATGCCGTAAC	ATGCCGTAAC	GTTACGGCAT
UDI324	GAATCTGGTC	GACCAGATTC	CAAGCTATTC	CAAGCTATTC	GAATAGCTTG
UDI325	CAAGAAGGCG	CGCCTTCTTG	GGTCACACCT	GGTCACACCT	AGGTGTGACC
UDI326	TTGGACCTTA	TAAGGTCCAA	TCTGTGAAGA	TCTGTGAAGA	TCTTCACAGA
UDI327	TGTATTACGC	GCGTAATACA	TTCGATCTTG	TTCGATCTTG	CAAGATCGAA
UDI328	AGGCGTTACC	GGTAACGCCT	TACCGCGGAA	TACCGCGGAA	TTCCGCGGTA
UDI329	TCTATGCTGT	ACAGCATAGA	TGGACGGAGT	TGGACGGAGT	ACTCCGTCCA
UDI330	CCATCCATCT	AGATGGATGG	CGTTGACTAT	CGTTGACTAT	ATAGTCAACG
UDI331	TTGCATGTTC	GAACATGCAA	GAAGGTCAGA	GAAGGTCAGA	TCTGACCTTC
UDI332	TCGTGGATGG	CCATCCACGA	ATCGCCGACA	ATCGCCGACA	TGTCGGCGAT
UDI333	GACAGGAGCA	TGCTCCTGTC	AAGACGACTT	AAGACGACTT	AAGTCGTCTT
UDI334	CCTGCTGAAG	CTTCAGCAGG	CGGTTACGAG	CGGTTACGAG	CTCGTAACCG
UDI335	AACTCAGCAA	TTGCTGAGTT	TGACCAGCAC	TGACCAGCAC	GTGCTGGTCA
UDI336	AGGTTCTGTT	AACAGAACCT	CATGTTGTGG	CATGTTGTGG	CCACAACATG
UDI337	CGGAGGAATG	CATTCCTCCG	TGCAGAAGCT	TGCAGAAGCT	AGCTTCTGCA
UDI338	TTCGTCGCTA	TAGCGACGAA	GTGCGATCGA	GTGCGATCGA	TCGATCGCAC
UDI339	CCAGGATAGA	TCTATCCTGG	GCTCATTCAT	GCTCATTCAT	ATGAATGAGC
UDI340	GAGACAAGCT	AGCTTGTCTC	TTCGAGTGTA	TTCGAGTGTA	TACACTCGAA
UDI341	ACTGCTGTCG	CGACAGCAGT	CCACTTCCAT	CCACTTCCAT	ATGGAAGTGG
UDI342	TTCCACTTAC	GTAAGTGGAA	CAGTGGCTTA	CAGTGGCTTA	TAAGCCACTG
UDI343	ACGTATACTC	GAGTATACGT	TCCTCCAGTG	TCCTCCAGTG	CACTGGAGGA
UDI344	CAAGATTCCA	TGGAATCTTG	AATGGCACAC	AATGGCACAC	GTGTGCCATT
UDI345	TTCGTTGTAC	GTACAACGAA	GGAGTGAGCT	GGAGTGAGCT	AGCTCACTCC
UDI346	AAGATGACGA	TCGTCATCTT	CCTCTTACAA	CCTCTTACAA	TTGTAAGAGG

UDI347	CCGCACTCAA	TTGAGTGCGG	CTAGCAGACG	CTAGCAGACG	CGTCTGCTAG
UDI348	TCTAGCCTAG	CTAGGCTAGA	CACTATGGCG	CACTATGGCG	CGCCATAGTG
UDI349	CTCTGTCAGG	CCTGACAGAG	TAGATACTGG	TAGATACTGG	CCAGTATCTA
UDI350	GTATACCACA	TGTGGTATAC	TAGGACAGGC	TAGGACAGGC	GCCTGTCCTA
UDI351	GTCACTTAAG	CTTAAGTGAC	AACTAGCGTA	AACTAGCGTA	TACGCTAGTT
UDI352	AGAGATTGTG	CACAATCTCT	GGTACTGCTG	GGTACTGCTG	CAGCAGTACC
UDI353	TTGGTTCGGC	GCCGAACCAA	CCAGAATGGC	CCAGAATGGC	GCCATTCTGG
UDI354	ACGCTTGACA	TGTCAAGCGT	TTCTGAGTCA	TTCTGAGTCA	TGACTCAGAA
UDI355	CACTGCCAAG	CTTGGCAGTG	GGCCTAGATT	GGCCTAGATT	AATCTAGGCC
UDI356	CCGACATTGC	GCAATGTCGG	CGAACCACAG	CGAACCACAG	CTGTGGTTCG
UDI357	AGGCCGATGT	ACATCGGCCT	GCGTAATGTT	GCGTAATGTT	AACATTACGC
UDI358	TCTGAGGACC	GGTCCTCAGA	GAAGTGACTC	GAAGTGACTC	GAGTCACTTC
UDI359	CAGATCCGCA	TGCGGATCTG	AGACCTCCGT	AGACCTCCGT	ACGGAGGTCT
UDI360	GAAGCAGCTG	CAGCTGCTTC	CGGATCGGAT	CGGATCGGAT	ATCCGATCCG
UDI361	GTGCGATCGA	TCGATCGCAC	TTGAATCTCC	TTGAATCTCC	GGAGATTCAA
UDI362	GAGTATGTAC	GTACATACTC	GCAACCTATA	GCAACCTATA	TATAGGTTGC
UDI363	TTCCTTCGAG	CTCGAAGGAA	ATAGGCCTCG	ATAGGCCTCG	CGAGGCCTAT
UDI364	CCACGGATTC	GAATCCGTGG	TCTCATGTGA	TCTCATGTGA	TCACATGAGA
UDI365	ATGTTCACGT	ACGTGAACAT	CACGGCTAGT	CACGGCTAGT	ACTAGCCGTG
UDI366	TTCGTGGACT	AGTCCACGAA	CCTCCTTATT	CCTCCTTATT	AATAAGGAGG
UDI367	CGCAATAGAA	TTCTATTGCG	GTGTCACGAC	GTGTCACGAC	GTCGTGACAC
UDI368	CCGTCATATG	CATATGACGG	TCTGTGACAT	TCTGTGACAT	ATGTCACAGA
UDI369	ACTACCGAGG	CCTCGGTAGT	GTAACATGCA	GTAACATGCA	TGCATGTTAC
UDI370	AGGTTCACCA	TGGTGAACCT	CACTGCCAAG	CACTGCCAAG	CTTGGCAGTG
UDI371	TTCGAAGACG	CGTCTTCGAA	TAGCTGAGGT	TAGCTGAGGT	ACCTCAGCTA
UDI372	TTCAGATGGA	TCCATCTGAA	CCAGGATAGA	CCAGGATAGA	TCTATCCTGG
UDI373	CCGTTCGTAT	ATACGAACGG	TTGCATGACA	TTGCATGACA	TGTCATGCAA
UDI374	TCTCAAGGCG	CGCCTTGAGA	ACGCAACGAG	ACGCAACGAG	CTCGTTGCGT
UDI375	CGACTGATGC	GCATCAGTCG	TTGTGTGATG	TTGTGTGATG	CATCACACAA
UDI376	GAAGATTAGC	GCTAATCTTC	CAAGAGCTGG	CAAGAGCTGG	CCAGCTCTTG
UDI377	TCGAACGTAA	TTACGTTCGA	TTGCGTCCGA	TTGCGTCCGA	TCGGACGCAA
UDI378	GCAAGATACT	AGTATCTTGC	CCACCTTGAG	CCACCTTGAG	CTCAAGGTGG
UDI379	TCTGTTAGAC	GTCTAACAGA	CGAGCGATAG	CGAGCGATAG	CTATCGCTCG
UDI380	TATCACCTGC	GCAGGTGATA	CTCGGCTTCA	CTCGGCTTCA	TGAAGCCGAG

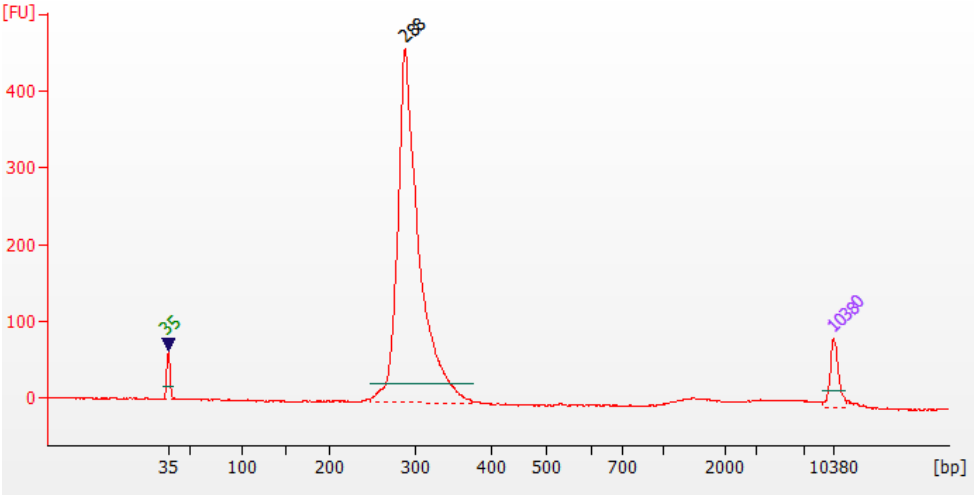
UDI381	TGGTAAGCCT	AGGCTTACCA	GTGACGCTGT	GTGACGCTGT	ACAGCGTCAC
UDI382	CGTGTTATGA	TCATAACACG	TCTGGCAATT	TCTGGCAATT	AATTGCCAGA
UDI383	AGAAGTTGGA	TCCAACTTCT	TCTAGCTAAC	TCTAGCTAAC	GTTAGCTAGA
UDI384	TTAGACAGCG	CGCTGTCTAA	AGATGGTTAG	AGATGGTTAG	CTAACCATCT

Troubleshooting Guide

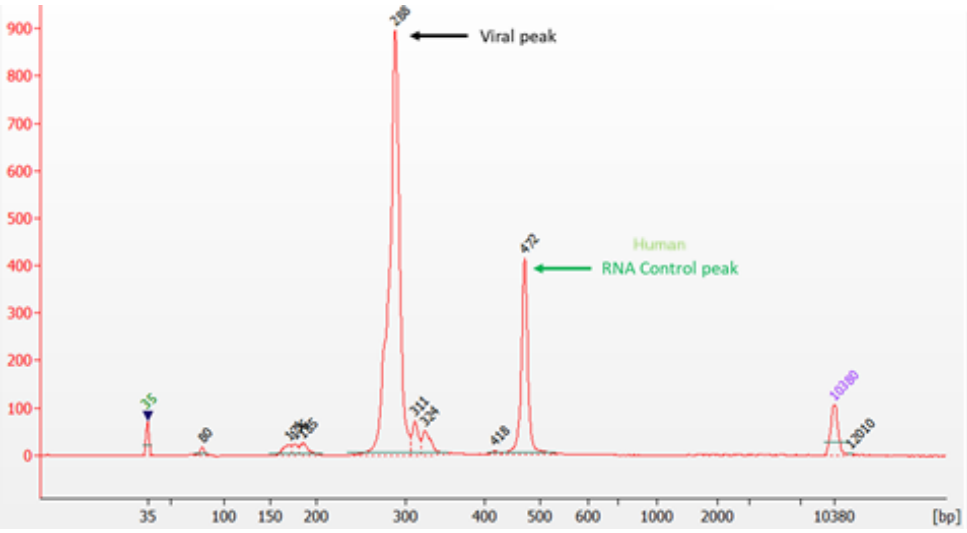
Examples of Libraries Prepared with CleanPlex SARS-CoV-2 FLEX Panel

Depending on the workflow and fragment analyzer used, the CleanPlex SARS-CoV-2 FLEX Panel library peak should be between 250 and 350 bp. Below is a representative Agilent® 2100 Bioanalyzer® trace generated using SARS-CoV-2 FLEX Panel.

CleanPlex SARS-CoV-2 FLEX Panel



CleanPlex SARS-CoV-2 Panel with control human housekeeping primer



Potential Causes for Extra Peaks and Suggested Solutions

Peaks around 70–90 bp are index primer dimers from the Second PCR Reaction and result from incomplete removal of low molecular weight DNA fragments during the final magnetic bead purification (Post-Second PCR Purification). When these peaks are high, it usually indicates one or more of the following:

- Inaccurate pipetting of magnetic beads when making a large number of libraries in a short period of time.
- Insufficient removal of supernatant and/or ethanol washes during the last purification.
- Adding more than specified amount of indexed primers to Second PCR Reaction Primer.

Peaks around 150–190 bp are residues of digested nonspecific amplification products and adapter dimers. They come from incomplete removal of low molecular weight DNA fragments during the Post-Digestion Purification or nonspecific products formed during or after the Multiplex PCR (mPCR) Reaction due to deviation from protocol or high concentration of panel. The digestion reagent degrades nonspecific amplification products into small pieces, which are then removed during magnetic bead purification. These peaks are usually caused by one or more of the following:

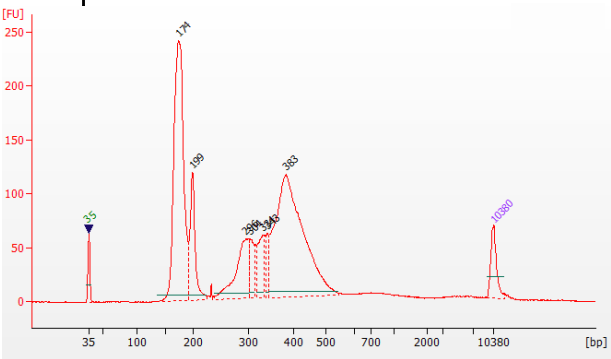
- Inaccurate pipetting of magnetic bead volume.
- Insufficient mixing of reaction solutions or bead and sample mixture.
- Insufficient removal of supernatant and ethanol washes during purification.
- Allowing mPCR products to sit for too long (and form nonspecific products) before purification.
- CleanPlex Custom NGS Panels may require panel titration for optimal performance. Often dimers will decrease by reducing the panel concentration in the mPCR Reaction Mixture.
- Incorrect annealing time, specifically unnecessarily longer annealing time.
- Cross contamination of Pool 1 and Pool 2 primers in mPCR reactions.

Broad peaks spread across 600 – 10,000 bp range are nonspecific products due to overamplification. Double check that the correct mPCR and Second PCR cycle numbers were used. PCR cycles are determined based on the amplicon count per pool of your panel, RNA input amount, and RNA quality. Try reducing the Second PCR cycles by 2-3 cycles if issue persists. If you're working with a custom panel for organisms with a much smaller genome than human's, consider reducing the RNA input and Second PCR cycles accordingly.

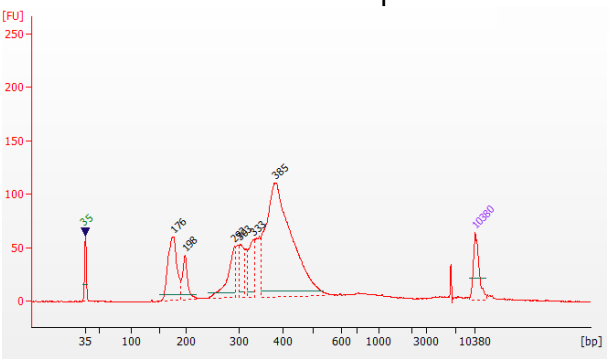
Removing nonspecific products from final libraries

If short nonspecific products described above are present in significant amounts, they can be reduced by pooling indexed libraries (that will be sequenced in the same lane) and performing one additional round of 1X magnetic bead-based purification if the pooled library **concentration is >3,000 pM** and the **total volume is >20 µL**. Low volume and low concentration libraries should not be bead-purified again as it will lead to significant loss of the library of interest. In the figures below, the Agilent® 2100 Bioanalyzer® trace on the left shows a CleanPlex Hereditary Cancer NGS library that contains significant adapter dimers due to poor library preparation. The Bioanalyzer trace on the right shows the same library after an additional 1X magnetic bead-based purification. The bead purification can be repeated once more if volume and concentration still meet the criteria above. We recommend keeping short adapter dimer peaks to less than 5% for best results. When possible it's always better to repeat the library preparation with the correct steps so nonspecific products do not form in the first place.

Before purification



After additional 1X bead-based purification



Potential Reasons for No Library Peaks and No In-Sample Positive Control Peak

- 30% ethanol instead of 70% ethanol was used in DNA or cDNA purification with magnetic beads.
- Magnetic beads were not added for one or more of the purification steps.
- Stop Buffer was not added or was added too late after Digestion Reaction, resulting in over-digestion of the samples. This may happen when handling a large number of samples.
- RNA input was too low due to incorrect quantification, try using more input RNA.
- RNA quality is extremely degraded. Try using more input RNA.
- Incompatible indexed PCR primers were using in the Second PCR Reaction. Only use CleanPlex Indexed PCR Primers for Illumina with CleanPlex NGS Panels for Illumina.
- A weak or incompatible magnetic rack was used to perform magnetic bead purification, resulting in significant bead loss. Do **not** use magnetic racks designed for 1.5 ml tubes.

Additional Resources

Please visit www.paragongenomics.com/product/faq/ for additional troubleshooting resources.

Data Analysis Recommendations for Illumina

Paragon Genomics offers this panel of 343 amplicons covering 99.7% of the SARS-CoV-2 genome (MN908947/NC_045512.2) with 92 bases uncovered at the ends of the genome and 1 control amplicon covering the TATA Binding protein (TBP) housekeeping gene of the human (host) control. The 343 amplicons covering the SARS-CoV-2 genome are distributed into two pools with amplicon size (without index and adapter) range from 116-196bp (median 149bp), the panel can be used for Illumina 2 X 150. Below are some notes on data processing.

We recommend the Broad Institute's GATK Best Practice (https://software.broadinstitute.org/gatk/best-practices/) as general guiding principles for sequencing data analysis.

1. Adapter Trimming.

With amplicon size ranges from 116 to 196bp, it is recommended to trim leftover adapter sequences for 2X150 sequencing before read mapping.

Following is an example command with open source software cutadapt (https://cutadapt.readthedocs.io/en/stable/).

```
cutadapt -g CCTACACGACGCTCTTCCGATCT \
-a AGATCGGAAGAGCACACGTCTGAA \
-A AGATCGGAAGAGCGTCGTGTAGG \
-G TTCAGACGTGTGCTCTTCCGATCT \
-e 0.1 -O 9 -m 20 -n 2 \
-o R1_out.fq.gz -p R2_out.fq.gz R1_in.fq.gz R2_in.fq.gz \
> cutadapt_report.output.txt
```

2. Map reads to reference genome.

The design was based on MN908947/NC_045512.2 and it is recommended to perform read mapping against the reference sequence of NC_045512.2. Bwa mem is recommended for read mapping and de-duplication procedure shall be skipped.

3. Trim primer sequences.

Before construction of a consensus genome sequence, it is recommended to remove primer sequences. Software package *fgbio* is recommended. It requires primer genomic coordinates in a tab delimited file which will be provided by Paragon Genomics to customers upon request.

Following is an example command.

```
java -jar fgbio-1.2.0-e7ac607-SNAPSHOT.jar TrimPrimers -i input.bam -o
output.primerTrim.bam -p primer_info.tab -H true
```

4. Calculate QC metrics.

In order to assess the quality of the sequencing results, it is recommended to assign mapped reads to amplicons based on mapping position. Subsequently, the following metrics can be used to measure general performance of the panel.

- **Mapping Rate:** Percentage of reads mapped to reference genome. It assesses primer-dimers and other PCR artifacts.
- **On-Target Rate:** Percentage of mapped reads that aligned to the targeted regions. It assesses binding/amplification specificity of designed primers.
- **Coverage Uniformity:** Percentage of amplicons with read depth equal to or greater than 20% of mean read depth of all amplicons in the panel. It measures performance uniformity of amplicons in the panel.

To accommodate the calculation, a file in BED format listing amplicon start and end coordinates will be provided to customers. The BED file can be downloaded from
https://www.paragongenomics.com/my-account/downloads/
from the associated order account after the purchase has been shipped.

5. Downstream analysis.

Paragon Genomics could provide required design files to be used in virus detection or strain identification. The suggestions on analysis solutions, please contact sales@paragongenomics or techsupport@paragongenomics.com

Technical Support

For technical assistance, please contact Paragon Genomics Technical Support.

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