



# CleanPlex<sup>®</sup> SARS-CoV-2 FLEX Research and Surveillance Panels

## User Guide

This user guide is for the following products for sequencing on Illumina<sup>®</sup> NGS platforms:

- CleanPlex<sup>®</sup> 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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## Revision History

Document	Date	Description of Change
UG4002-01	October 2020	<ul style="list-style-type: none"><li>Initial version</li></ul>
UG4002-02	November 2020	<ul style="list-style-type: none"><li>Updated the Applications section</li><li>Added the Terminology section</li><li>Updated data analysis guide suggestions</li><li>Updated the mPCR cycling conditions</li></ul>

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## Overview

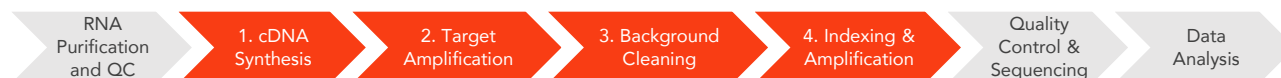
### Product Information

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 DNBSSEQ™ 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.



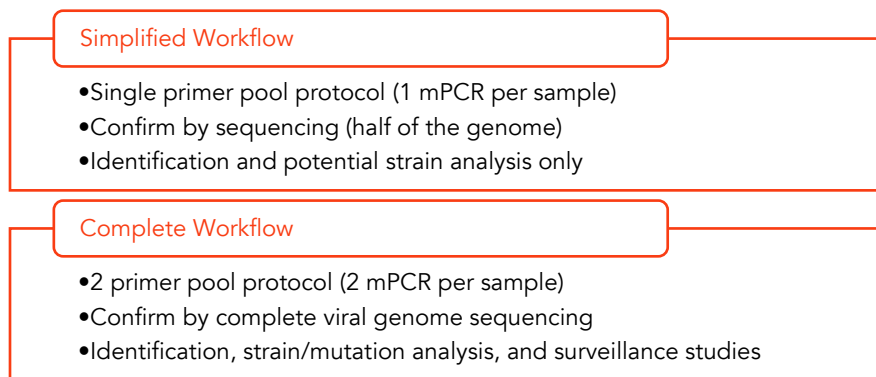
#### CleanPlex Target Enrichment and Library Preparation Workflow for Illumina Sequencing

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.



Organisms	Sample Types	Applications
<ul style="list-style-type: none"> <li>• Virus</li> </ul>	<ul style="list-style-type: none"> <li>• Purified RNA from bronchoalveolar lavage, tracheal aspirate, sputum, NP/OP swab, nasopharyngeal wash/aspirate or nasal aspirate</li> </ul>	<ul style="list-style-type: none"> <li>• Identify viral RNA from the <i>Coronaviridae</i> family</li> <li>• Confirm specific SARS-CoV-2 strain(s) present in samples through sequencing</li> <li>• Phylogenetic / Surveillance studies through in-depth sequence data analysis</li> <li>• High-sensitivity detection</li> </ul>

## FLEX Panel 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.

## Other Infectious Disease Applications

For additional SARS-CoV-2 research, we offer a variety of ready to use panels to support host susceptibility studies and co-Infections detection of other respiratory viruses.

Please visit the infectious diseases application page for additional information:

<https://www.paragongenomics.com/applications/infectious-disease/>

All panels can be found under Infectious Diseases Product Category:

<https://www.paragongenomics.com/product-category/products/ready-to-use-panels/infectious-disease/>

SARS-CoV-2 Related Ready to Use Panels		
Panel	SKU	Description
CleanPlex SARS-CoV-2 Research and Surveillance Panel	918011	Original SARS-CoV-2 full viral genome sequencing panel
CleanPlex SARS-CoV-2 FLEX Research and Surveillance Panel	918014	FLEX panel for frontline SARS-CoV-2 mutation research
CleanPlex Respiratory Research Panel	918301	Respiratory Panel contains additional viral content for concurrent Influenza and SARS-CoV-2 detection and flu subtyping
CleanPlex ACE2& TMPRSS2 Panel	916121	Host (human) germline panel for mutation analysis of ACE2 and TMPRSS2 and associated eQTL for expression level related research, and susceptibility investigations.

## Other Applications

For other targeted sequencing applications, Paragon Genomics also offer a while variety of both ready to use panel and custom panels for oncology, drug discovery, and more.

Visit our applications page for additional information: <https://www.paragongenomics.com/applications/>

Other Ready to Use Panels			
Panel	SKU	Application	Description
CleanPlex OncoZoom Cancer HotSpot Panel	916002	Oncology	Target 2,900+ hotspots in 65 genes with known cancer associations.
CleanPlex BRCA1 & BRCA2 Panel v3	916006	Oncology	Target the entire coding region including 20 bases of padding around all targeted coding exons in <i>BRCA1&amp;2</i>
CleanPlex TP53 Panel	916009	Oncology	Target the entire coding region and flanking intronic sequences of <i>TP53</i> for somatic mutations detection low as 1%
CleanPlex UMI Lung Cancer Panel	916066	Oncology	Interrogate 23 genes containing variants that are frequently mutated in lung cancer using a hotspot panel designed to work with cell-free DNA (cfDNA). Detect low-frequency variants below 0.5% MAF.
CleanPlex TMB 500 Panel	916074	Oncology	Interrogate 516 genes associated with the major solid tumor types to assess tumor mutational burden and characterize mutation profile.
OmniFusion™ RNA Lung Cancer Panel	917101	Oncology	Interrogate >530 known RNA fusion and additional novel RNA fusion using a single-primer method.
AccuFusion™ RNA Lung Cancer Panel	917104	Oncology	Interrogate ~280 known RNA fusions using a target specific dual-primer multiplex PCR method.
CleanPlex Mitochondrial Disease Panel	916063	Genetic Disease	Detect and investigate important variants with complete coverage of the entire human mitochondrial genome.
CleanPlex Hereditary Cancer Panel v2	916115	Genetic Disease	Interrogate 37 genes associated with cancers of the breast, ovary, uterus, skin, prostate, and gastrointestinal system, including rs12516 and rs8176318 in <i>BRCA1</i> and Boland inversion in <i>MSH2*</i> .
CleanPlex CFTR Panel	916117	Genetic Disease	Target the entire exon and include 20 bases of padding around all targeted coding exons for <i>CFTR</i> .
CleanPlex Custom NGS Panels	---	Variable	One-on-one service to design a custom panel specific to your applications and needs.



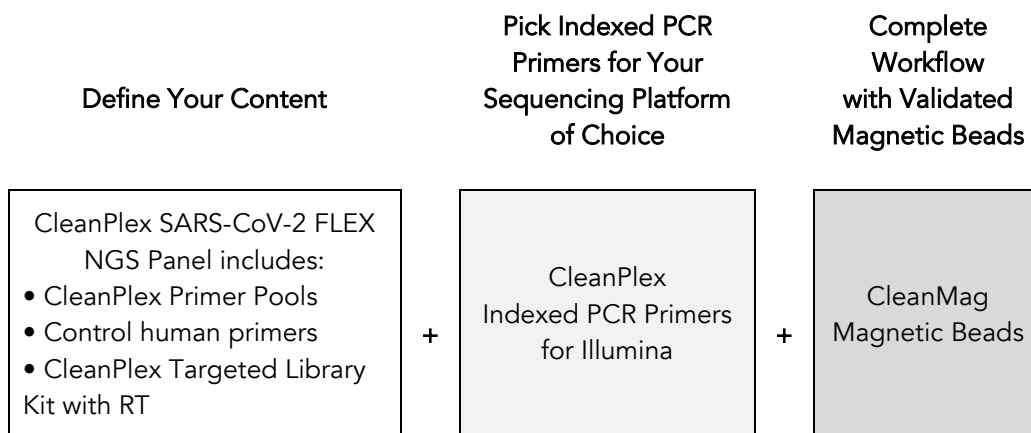
## Compatible Sequencing Instruments

CleanPlex Indexed PCR Primers for Illumina are used to generate CleanPlex target-enriched NGS libraries that are compatible with Illumina sequencing platforms, including NovaSeq<sup>®</sup> 5000/6000 Systems, HiSeq 3000/4000 Systems, HiSeq 2500 System, NextSeq<sup>®</sup> Series, MiSeq<sup>®</sup> System, MiniSeq<sup>®</sup> System, and iSeq<sup>®</sup> 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	25 µl	50 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](http://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.

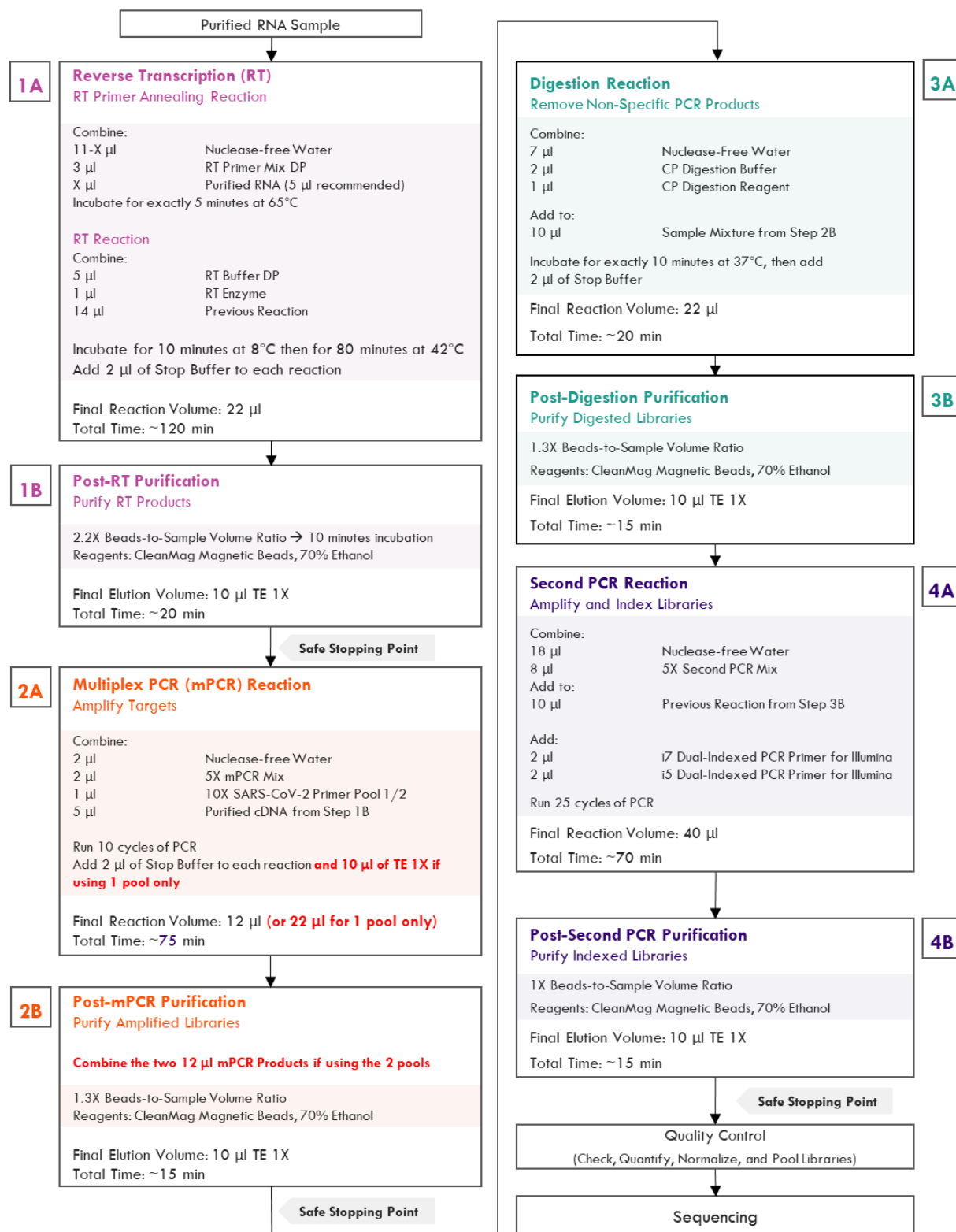
The components containing enzymes are viscous (10X SARS-CoV-2 pools, RT Enzyme Mix, 5X mPCR Mix, CP Digestion Reagent, 5X 2nd PCR Mix) and specially formulated for storage at -20°C without freezing to avoid freeze-thaw cycles to retain their full activity. Please avoid storing these components below -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/](http://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 µL 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 for 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, 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-2<sup>nd</sup> PCR Purification. After starting Digestion Step, samples must be carried through to the 2<sup>nd</sup> 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 $\mu$ l
RT Primer Mix DP	Purple Striped	3 $\mu$ l
Purified RNA	—	X $\mu$ l
<b>Total Volume per reaction</b>		<b>14 <math>\mu</math>l</b>

- 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 (with heated lid at 105°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
<b>Total Volume per reaction</b>		<b>20 µl</b>

- 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  $\mu$ l 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 $\mu$ l
Magnetic Beads to add	48 $\mu$ l



**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 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.

- 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  $\mu$ l of the purified RT reaction product. When working with a 1-pool workflow, the remaining 5  $\mu$ l 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  $\mu$ l of the purified RT reaction into two 5  $\mu$ l 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 5 $\mu$ L 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  $\mu$ l. **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  $\mu$ l 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  $\mu$ l 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 $\mu$ l (1 $\mu$ l with control)
10X diluted Control Human Primer (optional)	Lavender	(1 $\mu$ l)
5X mPCR Mix	Green	2 $\mu$ l
10X SARS-CoV-2 Primer Pool 1 or 10X SARS-CoV-2 Primer Pool 2	Varies	1 $\mu$ l
<b>Total Volume per reaction</b>		<b>5 <math>\mu</math>l</b>



**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 $\mu$ l
mPCR Master mix for Pool 1 or Pool 2	5 $\mu$ l
<b>Total Volume per reaction</b>	<b>10 <math>\mu</math>l</b>

- 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	$\infty$		

\* For thermal cyclers without adjustable ramp speed, the default setting can be used if max ramping speed is  $\leq 5^{\circ}\text{C}/\text{s}$ .

\*\* 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  $\mu$ l 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  $\mu\text{l}$  of TE Buffer to each sample for a total of 22  $\mu\text{l}$  volume.
  - When using 2-pool workflow, combine the 12  $\mu\text{l}$  multiplex PCR products for each sample for a total of 24  $\mu\text{l}$  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 $\mu\text{l}$ (12 $\mu\text{l}$ of sample + 10 $\mu\text{l}$ of TE)	24 $\mu\text{l}$
Magnetic Beads to add	29 $\mu\text{l}$	31 $\mu\text{l}$



**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  $\mu$ l 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
<b>Total Volume per reaction</b>		<b>10 µl</b>

Digestion Reaction Mixture	
Reagent	Volume per reaction
Digestion Reaction Master Mix	10 µl
Purified Sample from Step 2B	10 µl
<b>Total Volume per reaction</b>	<b>20 µl</b>

- 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  $\mu$ l** 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 $\mu$ l
Magnetic Beads	29 $\mu$ l



**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  $\mu$ l 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  $\mu$ l 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 open 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  $\mu$ l 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  $\mu$ l of master mix to each sample.

Second PCR Reaction Master Mix

Reagent	Cap Color	Volume per reaction
Nuclease-Free Water	—	18 $\mu$ l
5X 2nd PCR Mix	Blue	8 $\mu$ l
<b>Total Volume per reaction</b>		<b>26 <math>\mu</math>l</b>

Second PCR Reaction Mixture

Reagent	Volume per reaction
Second PCR Reaction Master Mix	26 $\mu$ l
Purified Sample from Step 3B	10 $\mu$ l
i5 Dual-Indexed PCR Primer for Illumina	2 $\mu$ l
i7 Dual-Indexed PCR Primer for Illumina	2 $\mu$ l
<b>Total Volume per reaction</b>	<b>40 <math>\mu</math>l</b>



**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 with a heated lid at 105°C, 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 FLEX 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.

\* For applications that specialize in very low viral count detection based on the Ct values we can make the following changes:

**For example:** to accommodate the increased 2 - 4 mPCR cycles to a total of 12 -14 cycles we can reduce the 2<sup>nd</sup> PCR cycle number by 2 - 4 cycles i.e. reduce from 24 cycles to 20 - 22 cycles.

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 2<sup>nd</sup> 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  $\mu$ l 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 open 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  $\mu$ l 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  $-20^{\circ}\text{C}$ .

**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  $-20^{\circ}\text{C}$  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 addition 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 FLEX 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 (30M PE reads/0.7M 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/](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' -AATGATACGGCGACCACCGAGATCTACACXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'

i7 Indexed Primer

5' -CAAGCAGAAGACGGCATAACGAGATXXXXXXXXGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-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

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#### CleanPlex Dual-Indexed PCR Primers for Illumina Set T

3 x 3 indexes, 9 reactions (SKU 716021)

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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.

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**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	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	GGAACCTA
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	i5 Index	Index Sequence	i5 Bases for Sample Sheet	
					MiSeq, NovaSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
Q7005	ATATTCAC	GTGAATAT	Q5001	AGCGCTAG	AGCGCTAG	CTAGCGCT
Q7006	GCGCCTGT	ACAGGCGC	Q5002	GATATCGA	GATATCGA	TCGATATC
Q7007	ACTCTATG	CATAGAGT	Q5007	ACATAGCG	ACATAGCG	CGCTATGT
Q7008	GTCTCGCA	TGCGAGAC	Q5008	GTGCGATA	GTGCGATA	TATCGCAC
Q7015	AGTAGAGA	TCTCTACT	Q5009	CCAACAGA	CCAACAGA	TCTGTTGG
Q7016	GACGAGAG	CTCTCGTC	Q5010	TTGGTGAG	TTGGTGAG	CTCACCAA
Q7017	AGACTTGG	CCAAGTCT	Q5013	AACCGCGG	AACCGCGG	CCGCGGTT
Q7018	GAGTCCAA	TTGGA CTC	Q5014	GGTTATAA	GGTTATAA	TTATAACC
Q7023	AATTCTGC	GCAGAATT				
Q7024	GGCCTCAT	ATGAGGCC				
Q7025	ATCTTAGT	ACTAAGAT				
Q7026	GCTCCGAC	GTCGGAGC				

## Plated Unique Dual-Indexed (UDI) PCR Primers for Illumina

A total of 384 unique dual indexes are offered on four 96 well PCR well plates (Sets 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	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	ATGTTACAGT	ATGTTACAGT	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

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<b>UDI013</b>	CCGATTATTC	GAATAATCGG	TTCAGGAGAT	TTCAGGAGAT	ATCTCCTGAA
<b>UDI014</b>	ACACACTCCG	CGGAGTGTGT	AAGGCGTCTG	AAGGCGTCTG	CAGACGCCTT
<b>UDI015</b>	CGGTCGGTAA	TTACCGACCG	ACGCTTGACA	ACGCTTGACA	TGCAAGCGT
<b>UDI016</b>	GGCGAACACT	AGTGTTCGCC	CATGAAGTGA	CATGAAGTGA	TCACTTCATG
<b>UDI017</b>	AAGAACGTAG	CTACGTTCTT	TTACGACCTG	TTACGACCTG	CAGGTCGTAA
<b>UDI018</b>	TTCGTGTCGA	TCGACACGAA	ATGCAAGCCG	ATGCAAGCCG	CGGCTTGCAT
<b>UDI019</b>	AAGTTATCGG	CCGATAACTT	CTCCGTATAC	CTCCGTATAC	GTATACGGAG
<b>UDI020</b>	CGATGTCCAA	TTGGACATCG	GAATCTGGTC	GAATCTGGTC	GACCAGATTC
<b>UDI021</b>	TCTCAACGTT	AACGTTGAGA	CGGTCGGTAA	CGGTCGGTAA	TTACCGACCG
<b>UDI022</b>	TTCACTGGCC	GGCCAGTGAA	TCTGCTAATG	TCTGCTAATG	CATTAGCAGA
<b>UDI023</b>	CCGGAGACAT	ATGTCTCCGG	CTCTTATTCG	CTCTTATTCG	CGAATAAGAG
<b>UDI024</b>	GAAGCCCTTC	GAAGGCGTTC	CACCTCTAGC	CACCTCTAGC	GCTAGAGGTG
<b>UDI025</b>	TCTAGGAACA	TGTTCCTAGA	TTACTTACCG	TTACTTACCG	CGGTAAGTAA
<b>UDI026</b>	ACCTCGAGAG	CTCTCGAGGT	CTATGCCTTA	CTATGCCTTA	TAAGGCATAG
<b>UDI027</b>	TACCGTACAG	CTGTACGGTA	GGAAGGTACG	GGAAGGTACG	CGTACCTTCC
<b>UDI028</b>	TTGCCATAAG	CTTATGGCAA	GAGGAGACGT	GAGGAGACGT	ACGTCTCCTC
<b>UDI029</b>	GCTATGCGGA	TCCGCATAGC	ACGCAAGGCA	ACGCAAGGCA	TGCCTTGCCT
<b>UDI030</b>	AGGTGCTTGC	GCAAGCACCT	TATCCTGACG	TATCCTGACG	CGTCAGGATA
<b>UDI031</b>	TAGGACAGGC	GCCTGTCTTA	GAAGACCGCT	GAAGACCGCT	AGCGGTCTTC
<b>UDI032</b>	GATAGACAGT	ACTGTCTATC	CAACGTGGAC	CAACGTGGAC	GTCCACGTTG
<b>UDI033</b>	TACATGGACG	CGTCCATGTA	TAAGTGCTCG	TAAGTGCTCG	CGAGCACTTA
<b>UDI034</b>	TTGCAGTTAG	CTAACTGCAA	CACATCGTAG	CACATCGTAG	CTACGATGTG
<b>UDI035</b>	ACCACAAGCA	TGCTTGTGGT	ACTACCGAGG	ACTACCGAGG	CCTCGGTAGT
<b>UDI036</b>	TGTGCTTACA	TGTAAGCACA	GATGTGTCTT	GATGTGTCTT	AGAACACATC
<b>UDI037</b>	ACGCAACGAG	CTCGTTGCGT	AAGTGCCTGA	AAGTGCCTGA	TACGACACTT
<b>UDI038</b>	CACCTCTAGC	GCTAGAGGTG	GGAGAACCAC	GGAGAACCAC	GTGGTTCTCC
<b>UDI039</b>	TTCTCCGCTT	AAGCGGAGAA	TGTACGAACT	TGTACGAACT	AGTTCGTACA
<b>UDI040</b>	CAGCGTCATT	AATGACGCTG	GGATGAGTGC	GGATGAGTGC	GCACCTATCC
<b>UDI041</b>	CGGCTACCAA	TTGGTACGCG	TAGTAGGACA	TAGTAGGACA	TGTCTACTA
<b>UDI042</b>	TTCACCTTCA	TGAAGGTGAA	ACGCCTCGTT	ACGCCTCGTT	AACGAGGCGT
<b>UDI043</b>	AAGCCACTAC	GTAGTGGCTT	CACCGCTGTT	CACCGCTGTT	AACAGCGGTG
<b>UDI044</b>	TTCTGTTACG	CGTAACAGAA	TCTATAGCGG	TCTATAGCGG	CCGCTATAGA
<b>UDI045</b>	TTATGGCCTT	AAGGCCATAA	CCGATGGACA	CCGATGGACA	TGTCCATCGG
<b>UDI046</b>	GGTCTATGAA	TTCATAGACC	TTCAACATGC	TTCAACATGC	GCATGTTGAA
<b>UDI047</b>	TCGGAGTTGG	CCAACTCCGA	GGAGTAACGC	GGAGTAACGC	CGGTTACTCC
<b>UDI048</b>	CATACTCGTG	CACGAGTATG	AGCCTTAGCG	AGCCTTAGCG	CGCTAAGGCT
<b>UDI049</b>	TTGGTAGCGG	CCGCTACCAA	TTACCTCAGT	TTACCTCAGT	ACTGAGGTAA

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<b>UDI050</b>	GGAGGTTTCAG	CTGAACCTCC	CAGGCATTGT	CAGGCATTGT	ACAATGCCTG
<b>UDI051</b>	TAACAAGGCC	GGCCTTGTTA	GTGTTCCACG	GTGTTCCACG	CGTGGAACAC
<b>UDI052</b>	TCTGCGTTAA	TTAACGCAGA	TTGATCCGCC	TTGATCCGCC	GGCGGATCAA
<b>UDI053</b>	CGCACTACCT	AGGTAGTGCG	GGAGGCTGAT	GGAGGCTGAT	ATCAGCCTCC
<b>UDI054</b>	AAGTTACACG	CGTGTAACCT	AACGTGACAA	AACGTGACAA	TTGTCACGTT
<b>UDI055</b>	CGTCACAAGT	ACTTGTGACG	CACAAGCTCC	CACAAGCTCC	GGAGCTTGTG
<b>UDI056</b>	CAACGCATGG	CCATGCGTTG	CCGTGTTGTC	CCGTGTTGTC	GACAACACGG
<b>UDI057</b>	CGTACAAGG	CCTGTAGCG	TTGAGCCAGC	TTGAGCCAGC	GCTGGCTCAA
<b>UDI058</b>	TCACGTATGT	ACATACGTGA	GCGTTACAGA	GCGTTACAGA	TCTGTAACGC
<b>UDI059</b>	GGATATCAAG	CTTGATATCC	TCCAGACATT	TCCAGACATT	AATGTCTGGA
<b>UDI060</b>	ACATCGGCTG	CAGCCGATGT	TCGAACTCTT	TCGAACTCTT	AAGAGTTCGA
<b>UDI061</b>	TAGCGCATGA	TCATGCGCTA	ACCTTCTCGG	ACCTTCTCGG	CCGAGAAGGT
<b>UDI062</b>	TGGACGGAGT	ACTCCGTCCA	AGACGCCAAC	AGACGCCAAC	GTTGGCGTCT
<b>UDI063</b>	CAAGGCTGTC	GACAGCCTTG	CAACCGTAAT	CAACCGTAAT	ATTACGGTTG
<b>UDI064</b>	CAGATAACCG	CGGTTATCTG	TTATGCGTTG	TTATGCGTTG	CAACGCATAA
<b>UDI065</b>	CCGTGGAGTA	TACTCCACGG	CTATGAGAAC	CTATGAGAAC	GTTCTCATAG
<b>UDI066</b>	TGCCGGAAGT	ACTTCCGGCA	AAGTTACACG	AAGTTACACG	CGTGTAACCT
<b>UDI067</b>	GCAGCTTCAC	GTGAAGCTGC	GCAATGTGAG	GCAATGTGAG	CTCACATTGC
<b>UDI068</b>	AGAAGAGCAA	TTGCTCTTCT	CGAAGTCGCA	CGAAGTCGCA	TGCGACTTCG
<b>UDI069</b>	TACGTGCGTT	AACGCACGTA	CCTGATTCAA	CCTGATTCAA	TTGAATCAGG
<b>UDI070</b>	CCTGCAGTAA	TTACTGCAGG	TAGAACGTGC	TAGAACGTGC	GCACGTTCTA
<b>UDI071</b>	CCTCAACTGG	CCAGTTGAGG	TTCGCAAGGT	TTCGCAAGGT	ACCTTGCGAA
<b>UDI072</b>	TTAACGCACA	TGTGCGTTAA	TTAATGCCGA	TTAATGCCGA	TCGGCATTAA
<b>UDI073</b>	AAGCACTAGT	ACTAGTGCTT	AGAACAGAGT	AGAACAGAGT	ACTCTGTTCT
<b>UDI074</b>	GTGTTCCACG	CGTGGAACAC	CCATCTGTTC	CCATCTGTTC	GAACAGATGG
<b>UDI075</b>	CCACTTCCAT	ATGGAAGTGG	TTCGTAGGTG	TTCGTAGGTG	CACCTACGAA
<b>UDI076</b>	TGTGATCTCA	TGAGATCACA	GCACGGTACA	GCACGGTACA	TGTACCGTGC
<b>UDI077</b>	CACCAAGGAC	GTCCTTGGTG	TGTCAAGAGG	TGTCAAGAGG	CCTCTTGACA
<b>UDI078</b>	TTCCACGCTC	GAGCGTGGAA	TCTAAGGTAC	TCTAAGGTAC	GTACCTTAGA
<b>UDI079</b>	ACAGCGTGTG	CACACGCTGT	GAACGGAGAC	GAACGGAGAC	GTCTCCGTTC
<b>UDI080</b>	TGTACAACCA	TGGTTGTACA	CGTACCATC	CGTACCATC	GATGGTAGCG
<b>UDI081</b>	TGTGAGTGAT	ATCACTCACA	TTACGGTAAC	TTACGGTAAC	GTTACCGTAA
<b>UDI082</b>	TCTACCTCCG	CGGAGGTAGA	TTCAGATGGA	TTCAGATGGA	TCCATCTGAA
<b>UDI083</b>	TTGTCAACTC	GAGTTGACAA	TAGCATCTGT	TAGCATCTGT	ACAGATGCTA
<b>UDI084</b>	CAAGTTCGGC	GCCGAACTTG	GGACGAGATC	GGACGAGATC	GATCTCGTCC
<b>UDI085</b>	TGTGAGGCCT	AGGCCTCACA	AGGTTCTGTT	AGGTTCTGTT	AACAGAACCT
<b>UDI086</b>	CTAACAGAGA	TCTCTGTTAG	CATACTCGTG	CATACTCGTG	CACGAGTATG

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<b>UDI087</b>	AATCGTCGGA	TCCGACGATT	CCGGATACCA	CCGGATACCA	TGGTATCCGG
<b>UDI088</b>	AACATAGCCT	AGGCTATGTT	ATGTCCACCG	ATGTCCACCG	CGGTGGACAT
<b>UDI089</b>	CAAGAGAACG	CGTTCTCTTG	CACCAAGTGG	CACCAAGTGG	CCACTTGGTG
<b>UDI090</b>	CCATAGACAA	TTGTCTATGG	TTGAGTACAC	TTGAGTACAC	GTGTACTION
<b>UDI091</b>	TGTATCCATC	GATGGATACA	CGGTCCGTA	CGGTCCGTA	TACGGAACCG
<b>UDI092</b>	CGCCTAAGTG	CACTTAGGCG	GGAGGTCCTA	GGAGGTCCTA	TAGGACCTCC
<b>UDI093</b>	TAGCCAGTGT	ACACTGGCTA	CCTGCTTGGA	CCTGCTTGGA	TCCAAGCAGG
<b>UDI094</b>	CAGTGGCGAT	ATCGCCACTG	TTCACGTCAG	TTCACGTCAG	CTGACGTGAA
<b>UDI095</b>	TTCACGTCAG	CTGACGTGAA	AACATAGCCT	AACATAGCCT	AGGCTATGTT
<b>UDI096</b>	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
<b>A</b>	97	105	113	121	129	137	145	153	161	169	177	185
<b>B</b>	98	106	114	122	130	138	146	154	162	170	178	186
<b>C</b>	99	107	115	123	131	139	147	155	163	171	179	187
<b>D</b>	100	108	116	124	132	140	148	156	164	172	180	188
<b>E</b>	101	109	117	125	133	141	149	157	165	173	181	189
<b>F</b>	102	110	118	126	134	142	150	158	166	174	182	190
<b>G</b>	103	111	119	127	135	143	151	159	167	175	183	191
<b>H</b>	104	112	120	128	136	144	152	160	168	176	184	192

UDI	i7 Index Sequence	i7 Bases for Sample Sheet for All Illumina Systems	i5 Index Sequence	i5 Bases for Sample Sheet	
				MiSeq, NovaSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
<b>UDI097</b>	TATAGGCGAT	ATCGCCTATA	TTGGCTCATA	TTGGCTCATA	TATGAGCCAA
<b>UDI098</b>	CAGGAATCCG	CGGATTCCTG	CAGAATACGG	CAGAATACGG	CCGTATTCTG
<b>UDI099</b>	CCACGTGTGA	TCACACGTGG	TGTATAGGTC	TGTATAGGTC	GACCTATACA
<b>UDI100</b>	GGAAATGCTGC	GCAGCATTCC	GTATACCACA	GTATACCACA	TGTGGTATAC
<b>UDI101</b>	TTCACCACGG	CCGTGGTGAA	AACTGGACGG	AACTGGACGG	CCGTCCAGTT
<b>UDI102</b>	CCGTTCTGTG	CACAGAACGG	TGTGAGTGAT	TGTGAGTGAT	ATCACTCACA
<b>UDI103</b>	TTCGATCCAT	ATGGATCGAA	AACTCAGCAA	AACTCAGCAA	TTGCTGAGTT
<b>UDI104</b>	AGGTGAGACC	GGTCTCACCT	AGACGATTGA	AGACGATTGA	TCAATCGTCT
<b>UDI105</b>	TACGGTGTG	CAACACCGTA	CGGCTTGTTT	CGGCTTGTTT	GAACAAGCCG
<b>UDI106</b>	CCAATATTCG	CGAATATTGG	TTCGGTGCTG	TTCGGTGCTG	CAGCACGGAA
<b>UDI107</b>	GCTGGAATTA	TAATTCAGC	CGAATACGAT	CGAATACGAT	ATCGTATTCG
<b>UDI108</b>	TAACCGCGAC	GTCGCGGTTA	ACCTCACCAG	ACCTCACCAG	CTGGTGAGGT
<b>UDI109</b>	CGACGCAGAA	TTCTGCGTCG	TTCGTACACC	TTCGTACACC	GGTGTACGAA
<b>UDI110</b>	AAGTATGCGT	ACGCATACTT	AAGTACGAGA	AAGTACGAGA	TCTCGTACTT
<b>UDI111</b>	TTGTGCAGCC	GGCTGCACAA	TCGGACCTCT	TCGGACCTCT	AGAGGTCCGA
<b>UDI112</b>	TTGGCTTGGT	ACCAAGCCAA	CCGCCTTGTA	CCGCCTTGTA	TACAAGCGCG
<b>UDI113</b>	GGACAATTGG	CCAATGTCC	GCGTATGAGC	GCGTATGAGC	GCTCATACGC
<b>UDI114</b>	AAGGCGTCTG	CAGACGCCTT	TTGAGCTCTG	TTGAGCTCTG	CAGAGCTCAA
<b>UDI115</b>	TCTGGCAATT	AATTGCCAGA	AACGTACCGT	AACGTACCGT	ACGGTACGTT
<b>UDI116</b>	TCTGGTATCA	TGATACCAGA	GGCCTTCACA	GGCCTTCACA	TGTGAAGGCC
<b>UDI117</b>	TAACAACCTC	GAGGTGTTA	TGTGCACTGG	TGTGCACTGG	CCAGTGCACA
<b>UDI118</b>	CTGATACCT	AGAGTATCAG	GGATACAGGT	GGATACAGGT	ACCTGTATCC
<b>UDI119</b>	CATACGCCAG	CTGGCGTATG	CCAATGTTAC	CCAATGTTAC	GTAACATTGG
<b>UDI120</b>	CGAGATGACC	GGTCATCTCG	GCTATGCGGA	GCTATGCGGA	TCCGCATAGC

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<b>UDI121</b>	TTGTTTCGACA	TGTCGAACAA	CCAGAATCTA	CCAGAATCTA	TAGATTCTGG
<b>UDI122</b>	TTCGTGCCAC	GTGGCACGAA	CCAATTAGCA	CCAATTAGCA	TGCTAATTGG
<b>UDI123</b>	TCTAAGGCTT	AAGCCTTAGA	CGTGTATGA	CGTGTATGA	TCATAACACG
<b>UDI124</b>	AGCCTTAGCG	CGCTAAGGCT	TGTGCCGGTT	TGTGCCGGTT	AACCGGCACA
<b>UDI125</b>	GGTCGTGATT	AATCACGACC	CACCAGAAGT	CACCAGAAGT	ACTTCTGGTG
<b>UDI126</b>	CGACAGCTAC	GTAGCTGTCT	TCTGCGTTAA	TCTGCGTTAA	TTAACGCAGA
<b>UDI127</b>	ACCTTACGTG	CACGTAAGGT	AGCTTAGAGG	AGCTTAGAGG	CCTCTAAGCT
<b>UDI128</b>	CATGAAGTGA	TCACTTCATG	TTGCGACCAC	TTGCGACCAC	GTGGTCGCAA
<b>UDI129</b>	AGACGCCAAC	GTTGGCGTCT	CGAAGTCTAG	CGAAGTCTAG	CTAGACTTCG
<b>UDI130</b>	TTGGCGTGTG	CACACGCCAA	GCTGAAGATA	GCTGAAGATA	TATCTTCAGC
<b>UDI131</b>	TTCACAGTGT	ACACTGTGAA	TCTGTTAGAC	TCTGTTAGAC	GTCTAACAGA
<b>UDI132</b>	AGAACAATCG	CGATTGTTCT	TGTACAACCA	TGTACAACCA	TGGTTGTACA
<b>UDI133</b>	CAGTAGCCGA	TCGGCTACTG	CTATTGTGTG	CTATTGTGTG	CACACAATAG
<b>UDI134</b>	CCTCTTACAA	TTGTAAGAGG	GAAGCAGCTG	GAAGCAGCTG	CAGCTGCTTC
<b>UDI135</b>	AACGGACTCG	CGAGTCCGTT	CCGCAGTAGT	CCGCAGTAGT	ACTACTGCGG
<b>UDI136</b>	TTGAGTACAC	GTGTACTCAA	AAGGTTGCTT	AAGGTTGCTT	AAGCAACCTT
<b>UDI137</b>	AACGTCACGC	GCGTGACGTT	CTCTCTTCTA	CTCTCTTCTA	TAGAAGAGAG
<b>UDI138</b>	TCAGACGCCT	AGGCGTCTGA	GGATCTTGTG	GGATCTTGTG	CACAAGATCC
<b>UDI139</b>	CCTCGTAAGT	ACTTACGAGG	AGCGATTAAC	AGCGATTAAC	GTTAATCGCT
<b>UDI140</b>	TTACGACCTG	CAGGTCGTAA	GAAGGCATAA	GAAGGCATAA	TTATGCCTTC
<b>UDI141</b>	AACTAGCGTA	TACGCTAGTT	AGCAGACTAA	AGCAGACTAA	TTAGTCTGCT
<b>UDI142</b>	GCACGACAGA	TCTGTCTGTC	AAGCACTAGT	AAGCACTAGT	ACTAGTGCTT
<b>UDI143</b>	TGCCAAGATC	GATCTTGGCA	TTAGACAGCG	TTAGACAGCG	CGTGTCTFAA
<b>UDI144</b>	TGGCTCTCCA	TGGAGAGCCA	TTAGGCACAA	TTAGGCACAA	TTGTGCCTAA
<b>UDI145</b>	CGAGATTGGT	ACCAATCTCG	TTCCGGCACT	TTCCGGCACT	AGTGCCGGAA
<b>UDI146</b>	GTGTCACGAC	GTCGTGACAC	TTGTATGGCT	TTGTATGGCT	AGCCATACAA
<b>UDI147</b>	CGACTAGAGA	TCTCTAGTCG	TGGATCGATT	TGGATCGATT	AATCGATCCA
<b>UDI148</b>	CAACCGTAAT	ATTACGGTTG	CGGAATCACC	CGGAATCACC	GGTGATTCCG
<b>UDI149</b>	TTACTTACCG	CGGTAAGTAA	GAGCTATCTA	GAGCTATCTA	TAGATAGCTC
<b>UDI150</b>	CCGGACGTTA	TAACGTCCGG	ACCTCGAGAG	ACCTCGAGAG	CTCTCGAGGT
<b>UDI151</b>	AACTGTGTTT	GAACACAGTT	CCGAATTCAC	CCGAATTCAC	GTGAATTCGG
<b>UDI152</b>	TATAGGACCT	AGGTCTTATA	AACGTCACGC	AACGTCACGC	GCGTGACGTT
<b>UDI153</b>	GCTAGGTCAA	TTGACCTAGC	TTGGTGTTC	TTGGTGTTC	GGAACACCAA
<b>UDI154</b>	TGATTGAAGC	GCTTCAATCA	CCAGGTGGAA	CCAGGTGGAA	TTCCACCTGG
<b>UDI155</b>	TTCGCACGCA	TGCGTGCAG	TCATACCGAT	TCATACCGAT	ATCGGTATGA
<b>UDI156</b>	AGGTACCATT	AATGGTACCT	CGACGGTTGT	CGACGGTTGT	ACAACCGTCG
<b>UDI157</b>	TCGCGATACA	TGTATCGCGA	CACTCACACG	CACTCACACG	CGTGTGAGTG

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<b>UDI158</b>	CCAATGTTAC	GTAACATTGG	TTGGCCACGA	TTGGCCACGA	TCGTGGCCAA
<b>UDI159</b>	CGAATTGTTG	CAACAATTCG	AATCGGTTCGC	AATCGGTTCGC	GCGACCGATT
<b>UDI160</b>	CATGACACGC	GCGTGTTCATG	AGAACAATCG	AGAACAATCG	CGATTGTTCT
<b>UDI161</b>	TTCGGATCTA	TAGATCCGAA	CTATCGAAGT	CTATCGAAGT	ACTTCGATAG
<b>UDI162</b>	CCAGTTAAGA	TCTTAACTGG	TCGGCCTGAA	TCGGCCTGAA	TTCAGGCCGA
<b>UDI163</b>	CGGATGTGAC	GTCACATCCG	TCACTGTTCT	TCACTGTTCT	AGAACAGTGA
<b>UDI164</b>	AGATGCTTCA	TGAAGCATCT	GGTATCTAAC	GGTATCTAAC	GTTAGATACC
<b>UDI165</b>	AAGTAGTCCG	CGGACTACTT	CGTATTAAGG	CGTATTAAGG	CCTTAATACG
<b>UDI166</b>	GGACTCCGTT	AACGGAGTCC	TAGGAGTGTC	TAGGAGTGTC	GACACTCCTA
<b>UDI167</b>	GGTCACACCT	AGGTGTGACC	CTCCGAACTC	CTCCGAACTC	GAGTTCGGAG
<b>UDI168</b>	GGAACTCTGG	CCAGAGTTCC	ATGTCTCTCG	ATGTCTCTCG	CGAGAGACAT
<b>UDI169</b>	CAATCACTGG	CCAGTGATTG	AGGTGCACTT	AGGTGCACTT	AAGTGCACCT
<b>UDI170</b>	TATGTCAGTC	GACTGACATA	TTGGCCGCAT	TTGGCCGCAT	ATGCGGCCAA
<b>UDI171</b>	CAAGGATCGC	GCGATCCTTG	GGTGTCTGAG	GGTGTCTGAG	CTCAGACACC
<b>UDI172</b>	AAGTGGAACA	TGTTCCACTT	CCGTGCCATT	CCGTGCCATT	AATGGCACGG
<b>UDI173</b>	CCTATTGGAT	ATCCAATAGG	AAGATGACGA	AAGATGACGA	TCGTCACTTT
<b>UDI174</b>	TTAACGGTCT	AGACCGTTAA	TGTATTGCCA	TGTATTGCCA	TGGCAATACA
<b>UDI175</b>	GGTCAATAGT	ACTATTGACC	AACCATCGGC	AACCATCGGC	GCCGATGGTT
<b>UDI176</b>	GGAAATTAGGC	GCCTAATTCC	CGTGCAACCT	CGTGCAACCT	AGGTTGCACG
<b>UDI177</b>	TTGTACCTAC	GTAGGTACAA	TTCTTGAGTG	TTCTTGAGTG	CACTCAAGAA
<b>UDI178</b>	CGAAGTCGCA	TGCGACTTCG	TCTGCAACAA	TCTGCAACAA	TTGTTGCAGA
<b>UDI179</b>	AACGTGACAA	TTGTCACGTT	CCGCTACACA	CCGCTACACA	TGTGTAGCGG
<b>UDI180</b>	TCAGTCGTTG	CAACGACTGA	CTCTGTCAGG	CTCTGTCAGG	CCTGACAGAG
<b>UDI181</b>	TAGCCGAATC	GATTCGGCTA	TTAACGGTCT	TTAACGGTCT	AGACCGTTAA
<b>UDI182</b>	CTAGCCACCA	TGGTGGCTAG	CGATGACCTT	CGATGACCTT	AAGGTCATCG
<b>UDI183</b>	ATCCTGGCCT	AGGCCAGGAT	AGGCAGGAGT	AGGCAGGAGT	ACTCCTGCCT
<b>UDI184</b>	CACAGGCGTT	AACGCCTGTG	AACGGACTCG	AACGGACTCG	CGAGTCCGTT
<b>UDI185</b>	CACTCACACG	CGTGTGAGTG	TTGGTTCGGC	TTGGTTCGGC	GCCGAACCAA
<b>UDI186</b>	TTCACATACG	CGTATGTGAA	CGCACTACCT	CGCACTACCT	AGGTAGTGCG
<b>UDI187</b>	TTGTGACGTA	TACGTCACAA	CCATACCACG	CCATACCACG	CGTGGTATGG
<b>UDI188</b>	CGGATCTTCC	GGAAGATCCG	GAATTCGGTA	GAATTCGGTA	TACCGAATTC
<b>UDI189</b>	AGCTGACATG	CATGTCAGCT	AGTCCTCCAC	AGTCCTCCAC	GTGGAGGACT
<b>UDI190</b>	GTGACGCTGT	ACAGCGTCAC	TAGTCATTCG	TAGTCATTCG	CGAATGACTA
<b>UDI191</b>	CCTTGTAACA	TGTTACAAGG	TTGAGGTCGC	TTGAGGTCGC	GCGACCTCAA
<b>UDI192</b>	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
<b>A</b>	193	201	209	217	225	233	241	249	257	265	273	281
<b>B</b>	194	202	210	218	226	234	242	250	258	266	274	282
<b>C</b>	195	203	211	219	227	235	243	251	259	267	275	283
<b>D</b>	196	204	212	220	228	236	244	252	260	268	276	284
<b>E</b>	197	205	213	221	229	237	245	253	261	269	277	285
<b>F</b>	198	206	214	222	230	238	246	254	262	270	278	286
<b>G</b>	199	207	215	223	231	239	247	255	263	271	279	287
<b>H</b>	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
<b>UDI193</b>	TTGACTTCGA	TCGAAGTCAA	CGGAGGAATG	CGGAGGAATG	CATTCCTCCG
<b>UDI194</b>	ATAGGCCTCG	CGAGGCCTAT	GAGTCAGCCA	GAGTCAGCCA	TGGCTGACTC
<b>UDI195</b>	CACCTCAGGA	TCCTGAAGTG	GGAATTAGGC	GGAATTAGGC	GCCTAATTC
<b>UDI196</b>	GGTAAGGATT	AATCCTTACC	TTCGCCACAC	TTCGCCACAC	GTGTGGCGAA
<b>UDI197</b>	TCTGCAACAA	TTGTTGCAGA	CCTCGCTTAC	CCTCGCTTAC	GTAAGCGAGG
<b>UDI198</b>	GGCCTAGATT	AATCTAGGCC	ACAGCGTGTG	ACAGCGTGTG	CACACGCTGT
<b>UDI199</b>	CAGTAGAGCC	GGCTCTACTG	TTCCGCTTCT	TTCCGCTTCT	AGAAGCGGAA
<b>UDI200</b>	CAACGTGGAC	GTCCACGTTG	CAGCGTCATT	CAGCGTCATT	AATGACGCTG
<b>UDI201</b>	AACTGCGGAG	CTCCGCAGTT	CCGTAGAACA	CCGTAGAACA	TGTCTACGG
<b>UDI202</b>	TCACTGTTCT	AGAACAGTGA	CGGTTATCGT	CGGTTATCGT	ACGATAACCG
<b>UDI203</b>	CAATAAGAGC	GCTCTTATTG	TCTGGTATCA	TCTGGTATCA	TGATACCAGA
<b>UDI204</b>	TTCGTCTACA	TGTAGACGAA	AAGTATGCGT	AAGTATGCGT	ACGCATACTT
<b>UDI205</b>	CGACGACAAG	CTTGTCGTCG	TTCCTTCGAG	TTCCTTCGAG	CTCGAAGGAA
<b>UDI206</b>	TGTAAGACGA	TCGTCTTACA	GCTATGGATA	GCTATGGATA	TATCCATAGC
<b>UDI207</b>	TCCTCCTCTC	GAGAGGAGGA	AGGTACCATT	AGGTACCATT	AATGGTACCT
<b>UDI208</b>	GGTATCTAAC	GTTAGATACC	TTACGGAGTC	TTACGGAGTC	GACTCCGTAA
<b>UDI209</b>	TTCTTAAGCC	GGCTTAAGAA	TGAGGACTTA	TGAGGACTTA	TAAGTCTCA
<b>UDI210</b>	TTGTACCAGA	TCTGGTACAA	TTGAGTTGCC	TTGAGTTGCC	GGCAACTCAA
<b>UDI211</b>	CCGAATTCAC	GTGAATTCGG	AGCTTCGCGA	AGCTTCGCGA	TCGCGAAGCT
<b>UDI212</b>	TTCTCCATTC	GAATGGAGAA	CATACGCCAG	CATACGCCAG	CTGGCGTATG
<b>UDI213</b>	CCAATTGACT	AGTCAATTGG	CAAGACCAGC	CAAGACCAGC	GCTGGTCTTG
<b>UDI214</b>	AGGTGATGCG	CGCATCACCT	GATAGACAGT	GATAGACAGT	ACTGTCTATC
<b>UDI215</b>	GGTGCAATA	TATTGACACC	CGCTCGTGAA	CGCTCGTGAA	TTCACGAGCG
<b>UDI216</b>	CCGACAGTCT	AGACTGTCGG	TCTCTAACAG	TCTCTAACAG	CTGTTAGAGA
<b>UDI217</b>	GAGTCCAGAT	ATCTGGACTC	ACCTAGGAGG	ACCTAGGAGG	CCTCTAGGT

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<b>UDI218</b>	CCTTATTCTC	GAGAATAAGG	TCTGTACCTT	TCTGTACCTT	AAGGTACAGA
<b>UDI219</b>	GGCGACAACA	TGTTGTCGCC	CTCAGGCCAT	CTCAGGCCAT	ATGGCCTGAG
<b>UDI220</b>	AACACCGCAG	CTGCCGTGTT	TTGTGCAGCC	TTGTGCAGCC	GGTGCACAA
<b>UDI221</b>	CGGAGTTATC	GATAACTCCG	TAGCCGAATC	TAGCCGAATC	GATTCCGGCTA
<b>UDI222</b>	GTACAAGGAT	ATCCTTGATC	AAGCCTGTTA	AAGCCTGTTA	TAACAGGCTT
<b>UDI223</b>	TATACGCGTA	TACGCGTATA	TGTACAGTAG	TGTACAGTAG	CTACTGTACA
<b>UDI224</b>	CAATTGGTGG	CCACCAATTG	CGATTCTGCC	CGATTCTGCC	GGCAGAATCG
<b>UDI225</b>	GGCCTTCACA	TGTGAAGGCC	TTGCTAAGGA	TTGCTAAGGA	TCCTTAGCAA
<b>UDI226</b>	GCAGTCAAGG	CCTTGACTGC	ACTCCTTGGC	ACTCCTTGGC	GCCAAGGAGT
<b>UDI227</b>	CCGACGCATT	AATGCGTCGG	GAAGGCGAAC	GAAGGCGAAC	GTTCCGCTTC
<b>UDI228</b>	GTGTAGTCTT	AAGACTACAC	CAATACCTTG	CAATACCTTG	CAAGGTATTG
<b>UDI229</b>	CTGCACTGAC	GTCAGTGCAG	CGACGACAAG	CGACGACAAG	CTTGTCGTCG
<b>UDI230</b>	TTCTGGTGAG	CTCACCAGAA	GAACCTGACC	GAACCTGACC	GGTCAGGTTC
<b>UDI231</b>	AAGTACGAGA	TCTCGTACTT	TTGCCTCGCA	TTGCCTCGCA	TGCAGGCAA
<b>UDI232</b>	CCTAATCTGA	TCAGATTAGG	TTCGTGTCGA	TTCGTGTCGA	TCGACACGAA
<b>UDI233</b>	TTCTTGAGTG	CACTCAAGAA	TGGATGGCAA	TGGATGGCAA	TTGCCATCCA
<b>UDI234</b>	GAATGGCTCT	AGAGCCATTC	TTCACCAGCT	TTCACCAGCT	AGCTGGTGAA
<b>UDI235</b>	CGGAATCGTG	CACGATTCCG	CCTGAGTAGC	CCTGAGTAGC	GCTACTCAGG
<b>UDI236</b>	CAGGCTCCAA	TTGGAGCCTG	AGGTGTCCGT	AGGTGTCCGT	ACGGACACCT
<b>UDI237</b>	CAAGTCGTAA	TTACGACTTG	GTCTGGTTGC	GTCTGGTTGC	GCAACCAGAC
<b>UDI238</b>	CCGACCTTAA	TTAAGGTCGG	CTCTTAGATG	CTCTTAGATG	CATCTAAGAG
<b>UDI239</b>	TGACAGAACC	GGTTCTGTCA	TATCACCTGC	TATCACCTGC	GCAGGTGATA
<b>UDI240</b>	GGTGCATATC	GATACGCACC	CAGAGGCAAG	CAGAGGCAAG	CTTGCCCTCTG
<b>UDI241</b>	AGCTTCGCGA	TCGCGAAGCT	CCGGTCAACA	CCGGTCAACA	TGTTGACCGG
<b>UDI242</b>	CCGTCTTAAC	GTTAAGACGG	TCACGAGGTG	TCACGAGGTG	CACCTCGTGA
<b>UDI243</b>	GTATGACCGG	CCGGTCATAC	CCATAGACAA	CCATAGACAA	TTGTCTATGG
<b>UDI244</b>	TTAAGCTGAC	GTCAGCTTAA	GAGCTTGGAC	GAGCTTGGAC	GTCCAAGCTC
<b>UDI245</b>	TATCCGCGGT	ACCGCGGATA	TACGGTGTG	TACGGTGTG	CAACACCGTA
<b>UDI246</b>	TTGATGCAAC	GTTGCATCAA	TTCAACTCGA	TTCAACTCGA	TCGAGTTGAA
<b>UDI247</b>	TTGGTGACAA	TGTGCACCAA	AAGGCAGGTA	AAGGCAGGTA	TACCTGCCTT
<b>UDI248</b>	GACCACAGAT	ATCTGTGGTC	CGGCCAATTC	CGGCCAATTC	GAATTGGCCG
<b>UDI249</b>	GGATCTTGTG	CACAAGATCC	CAACCGGACA	CAACCGGACA	TGTCCGGTTG
<b>UDI250</b>	CAGCTAGCAG	CTGCTAGCTG	AACTTGCCCG	AACTTGCCCG	CGGCCAAGTT
<b>UDI251</b>	TTCGACCGGT	ACCGGTCGAA	TGGAACATAG	TGGAACATAG	CTATGTTCCA
<b>UDI252</b>	TAGAACGTGC	GCACGTTCTA	TTCGGATCTA	TTCGGATCTA	TAGATCCGAA
<b>UDI253</b>	TTCCTTCCTT	AAGGAAGGAA	CGGAATCGTG	CGGAATCGTG	CACGATTCCG
<b>UDI254</b>	CTATCTCTCT	AGAGAGATAG	TCTAATCGGT	TCTAATCGGT	ACCGATTAGA

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<b>UDI255</b>	AATAGGAACC	GGTTCCTATT	GCTGGAATTA	GCTGGAATTA	TAATTCCAGC
<b>UDI256</b>	CGCTCGTGAA	TTCACGAGCG	CGCTTCTCAC	CGCTTCTCAC	GTGAGAAGCG
<b>UDI257</b>	AGGTTGTGCC	GGCACAACCT	TAGACTCCTG	TAGACTCCTG	CAGGAGTCTA
<b>UDI258</b>	TTCTGAGTCA	TGACTCAGAA	CCGTTGATTG	CCGTTGATTG	CAATCAACGG
<b>UDI259</b>	CCTGAGATCG	CGATCTCAGG	CGAACCTCCA	CGAACCTCCA	TGGAGGTTTCG
<b>UDI260</b>	TTCCAGCAGG	CCTGCTGGAA	TTGGAAGTTG	TTGGAAGTTG	CAACTTCCAA
<b>UDI261</b>	TATACAGCTC	GAGCTGTATA	CCAGGAGTAC	CCAGGAGTAC	GTACTCCTGG
<b>UDI262</b>	CCGTCAGGTT	AACCTGACGG	AGGTTCTGTCG	AGGTTCTGTCG	CGACGAACCT
<b>UDI263</b>	CCACGAGCTT	AAGCTCGTGG	GACCTGAAGA	GACCTGAAGA	TCTTCAGGTC
<b>UDI264</b>	GAGCTTGGAC	GTCCAAGCTC	TTAACGCACA	TTAACGCACA	TGTGCGTTAA
<b>UDI265</b>	CGAAGTCTAG	CTAGACTTCG	TCGGAGTTGG	TCGGAGTTGG	CCAACTCCGA
<b>UDI266</b>	TTACCTTGGA	TCCAAGGTAA	CGATGACTCC	CGATGACTCC	GGAGTCATCG
<b>UDI267</b>	TGCTACCAAG	CTTGGTAGCA	TATAGGTTGG	TATAGGTTGG	CCAACTTATA
<b>UDI268</b>	ACGCCTCGTT	AACGAGGCGT	GACAAGTGTT	GACAAGTGTT	AACACTTGTC
<b>UDI269</b>	CCATCTTCTG	CAGAAGATGG	TTCTCCGGAA	TTCTCCGGAA	TTCCGGAGAA
<b>UDI270</b>	GGATGTATCA	TGATACATCC	ACACACTCCG	ACACACTCCG	CGGAGTGTGT
<b>UDI271</b>	AACTACGCGC	GCGCGTAGTT	CTGGTCACTA	CTGGTCACTA	TAGTGACCAG
<b>UDI272</b>	CGCAGACAAC	GTTGTCTGCG	TTCGTGCCAC	TTCGTGCCAC	GTGGCACGAA
<b>UDI273</b>	CAGCGCTAAG	CTTAGCGCTG	AGATCATGGA	AGATCATGGA	TCCATGATCT
<b>UDI274</b>	GGAGGCTGAT	ATCAGCCTCC	GAGTATGTAC	GAGTATGTAC	GTACATACTC
<b>UDI275</b>	GAGCACTGCA	TGCAGTGCTC	TAGAACACCT	TAGAACACCT	AGGTGTTCTA
<b>UDI276</b>	GTCTGAGCTC	GAGCTCAGAC	CCAGTTAAGA	CCAGTTAAGA	TCTTAACTGG
<b>UDI277</b>	TTGTCCAGGT	ACCTGGACAA	CGCTTATCTG	CGCTTATCTG	CAGATAAGCG
<b>UDI278</b>	TTGGAAGTTG	CAACTTCCAA	GAGCTCTTAC	GAGCTCTTAC	GTAAGAGCTC
<b>UDI279</b>	CACAGGATGG	CCATCCTGTG	TCTCAAGGCG	TCTCAAGGCG	CGCCTTGAGA
<b>UDI280</b>	TCTAACTGCC	GGCAGTTAGA	CTAAGTACCA	CTAAGTACCA	TGGTACTTAG
<b>UDI281</b>	TCTCATGTGA	TCACATGAGA	TCGACAAGCC	TCGACAAGCC	GGCTTGTCGA
<b>UDI282</b>	CCAACGAATA	TATTCGTTGG	TTCGACATCA	TTCGACATCA	TGATGTCGAA
<b>UDI283</b>	GAAGACCGCT	AGCGGTCTTC	AGTGGTACTT	AGTGGTACTT	AAGTACCACT
<b>UDI284</b>	AATCGGTGCG	GCGACCGATT	TTGCACTTGT	TTGCACTTGT	ACAAGTGCAA
<b>UDI285</b>	GGACGAGATC	GATCTCGTCC	GTCTTCGCAG	GTCTTCGCAG	CTCGAAGAC
<b>UDI286</b>	CCTATAATGG	CCATTATAGG	CAGGCTCCAA	CAGGCTCCAA	TTGAGCCTG
<b>UDI287</b>	CGTGGTCTGT	ACAGACCACG	CCAGGTTACG	CCAGGTTACG	CGTAACTGG
<b>UDI288</b>	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
<b>A</b>	289	297	305	313	321	329	337	345	353	361	369	377
<b>B</b>	290	298	306	314	322	330	338	346	354	362	370	378
<b>C</b>	291	299	307	315	323	331	339	347	355	363	371	379
<b>D</b>	292	300	308	316	324	332	340	348	356	364	372	380
<b>E</b>	293	301	309	317	325	333	341	349	357	365	373	381
<b>F</b>	294	302	310	318	326	334	342	350	358	366	374	382
<b>G</b>	295	303	311	319	327	335	343	351	359	367	375	383
<b>H</b>	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
<b>UDI289</b>	TTCGTTCCGG	CCGGAACGAA	CTACACTATG	CTACACTATG	CATAGTGTAG
<b>UDI290</b>	CTGTCTTGCT	AGCAAGACAG	AAGAAGATCC	AAGAAGATCC	GGATCTTCTT
<b>UDI291</b>	AGATTCAACC	GGTTGAATCT	CACGGTGTAT	CACGGTGTAT	ATACACCGTG
<b>UDI292</b>	AGAGTGGTGA	TCAACACTCT	CGGTAAGTGG	CGGTAAGTGG	CCACTTACCG
<b>UDI293</b>	TTGGCTCATA	TATGAGCCAA	CCAGACTGAG	CCAGACTGAG	CTCAGTCTGG
<b>UDI294</b>	GCTCCAATAT	ATATTGGAGC	GCTAGGTCAA	GCTAGGTCAA	TTGACCTAGC
<b>UDI295</b>	CTAACGAGTT	AACTCGTTAG	GGTGCCTCAT	GGTGCCTCAT	ATGAGGCACC
<b>UDI296</b>	GCTATGGATA	TATCCATAGC	TAACAACCTC	TAACAACCTC	GAGGTTGTTA
<b>UDI297</b>	GGTATGGTGA	TCACCATACC	TTGGCAAGAA	TTGGCAAGAA	TTCTTGCCAA
<b>UDI298</b>	GTCTTCGCAG	CTGCGAAGAC	GGAAGTGGTT	GGAAGTGGTT	AACCCTTCC
<b>UDI299</b>	TTCGCCACAC	GTGTGGCGAA	TCTAACCTTC	TCTAACCTTC	GAAGGTTAGA
<b>UDI300</b>	CAACCACCTT	AAGGTGGTTG	GTATGACCGG	GTATGACCGG	CCGGTCATAC
<b>UDI301</b>	CAATCGAGCG	CGCTCGATTG	CCTGTGTATT	CCTGTGTATT	AATACACAGG
<b>UDI302</b>	AATCCGACAC	GTGTGGGATT	TTCTCCGCTT	TTCTCCGCTT	AAGCGGAGAA
<b>UDI303</b>	CATGTTGTGG	CCACAACATG	CCACTGGTAA	CCACTGGTAA	TTACCAGTGG
<b>UDI304</b>	TTCCATCGGA	TCCGATGGAA	AATCCGACAC	AATCCGACAC	GTGTGGGATT
<b>UDI305</b>	CGTTATCTG	CAGATAAGCG	TTCACCTTCA	TTCACCTTCA	TGAAGGTGAA
<b>UDI306</b>	TTCGACATCA	TGATGTGCGAA	TATAGGACCT	TATAGGACCT	AGGTCCCTATA
<b>UDI307</b>	TCTAGAAGGT	ACCTTCTAGA	TTACCGTGAT	TTACCGTGAT	ATCACGGTAA
<b>UDI308</b>	GAGCGGAACT	AGTTCGCTC	ACGTTAGAGT	ACGTTAGAGT	ACTCTAACGT
<b>UDI309</b>	AGCAGACTAA	TTAGTCTGCT	CAGCGCTAAG	CAGCGCTAAG	CTTAGCGCTG
<b>UDI310</b>	ATCGCCGACA	TGTCGGCGAT	ACAGGTACTC	ACAGGTACTC	GAGTACCTGT
<b>UDI311</b>	GGTGTTAAGT	ACTTAACACC	CTAGATAGCA	CTAGATAGCA	TGCTATCTAG
<b>UDI312</b>	ACTCCTTGGC	GCCAAGGAGT	TTGGACCTTA	TTGGACCTTA	TAAGGTCCAA
<b>UDI313</b>	GGAACCTTGC	GCAAGTTCC	TGTAGCACAG	TGTAGCACAG	CTGTGCTACA
<b>UDI314</b>	TGTGTCCTAT	ATAGGACACA	TTGACGTCTT	TTGACGTCTT	AAGACGTCAA

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<b>UDI315</b>	CAACACATTC	GAATGTGTTG	CCATCTCCGT	CCATCTCCGT	ACGGAGATGG
<b>UDI316</b>	GTAACATGCA	TGCATGTTAC	CTAACAGAGA	CTAACAGAGA	TCTCTGTTAG
<b>UDI317</b>	ACGCAAGGCA	TGCCTTGCGT	ACCTTACGTG	ACCTTACGTG	CACGTAAGGT
<b>UDI318</b>	CCGTGTTACT	AGTAACACGG	AGGTGCTTGC	AGGTGCTTGC	GCAAGCACCT
<b>UDI319</b>	TTCGGTTGTG	CACAACCGAA	GACTAGGTCC	GACTAGGTCC	GGACCTAGTC
<b>UDI320</b>	AGGTGTCCGT	ACGGACACCT	TCGAAGTTCT	TCGAAGTTCT	AGAACTTCGA
<b>UDI321</b>	CGCTGTAACG	CGTTACAGCG	CTATCTCTCT	CTATCTCTCT	AGAGAGATAG
<b>UDI322</b>	AACCAATGGT	ACCATGGGTT	CCGTTCTCCT	CCGTTCTCCT	AGGAGAACGG
<b>UDI323</b>	TCGAGGCAGT	ACTGCCTCGA	ATGCCGTAAC	ATGCCGTAAC	GTTACGGCAT
<b>UDI324</b>	GAATCTGGTC	GACCAGATTC	CAAGCTATTC	CAAGCTATTC	GAATAGCTTG
<b>UDI325</b>	CAAGAAGGCG	CGCCTTCTTG	GGTCACACCT	GGTCACACCT	AGGTGTGACC
<b>UDI326</b>	TTGGACCTTA	TAAGGTCCAA	TCTGTGAAGA	TCTGTGAAGA	TCTTCACAGA
<b>UDI327</b>	TGTATTACGC	GCGTAATACA	TTCGATCTTG	TTCGATCTTG	CAAGATCGAA
<b>UDI328</b>	AGGCGTTACC	GGTAACGCCT	TACCGCGGAA	TACCGCGGAA	TTCCGCGGTA
<b>UDI329</b>	TCTATGCTGT	ACAGCATAGA	TGGACGGAGT	TGGACGGAGT	ACTCCGTCCA
<b>UDI330</b>	CCATCCATCT	AGATGGATGG	CGTTGACTAT	CGTTGACTAT	ATAGTCAACG
<b>UDI331</b>	TTGCATGTTC	GAACATGCAA	GAAGGTCAGA	GAAGGTCAGA	TCTGACCTTC
<b>UDI332</b>	TCGTGGATGG	CCATCCACGA	ATCGCCGACA	ATCGCCGACA	TGTCGGCGAT
<b>UDI333</b>	GACAGGAGCA	TGCTCCTGTC	AAGACGACTT	AAGACGACTT	AAGTCGTCTT
<b>UDI334</b>	CCTGCTGAAG	CTTCAGCAGG	CGGTTACGAG	CGGTTACGAG	CTCGTAACCG
<b>UDI335</b>	AACTCAGCAA	TTGCTGAGTT	TGACCAGCAC	TGACCAGCAC	GTGCTGGTCA
<b>UDI336</b>	AGGTTCTGTT	AACAGAACCT	CATGTTGTGG	CATGTTGTGG	CCACAACATG
<b>UDI337</b>	CGGAGGAATG	CATTCCTCCG	TGCAGAAGCT	TGCAGAAGCT	AGCTTCTGCA
<b>UDI338</b>	TTCGTCGCTA	TAGCGACGAA	GTGCGATCGA	GTGCGATCGA	TCGATCGCAC
<b>UDI339</b>	CCAGGATAGA	TCTATCCTGG	GCTCATTTCAT	GCTCATTTCAT	ATGAATGAGC
<b>UDI340</b>	GAGACAAGCT	AGCTTGCTCTC	TTCGAGTGTA	TTCGAGTGTA	TACACTCGAA
<b>UDI341</b>	ACTGCTGTCTG	CGACAGCAGT	CCACTTCCAT	CCACTTCCAT	ATGGAAGTGG
<b>UDI342</b>	TTCCACTTAC	GTAAGTGGAA	CAGTGGCTTA	CAGTGGCTTA	TAAGCCACTG
<b>UDI343</b>	ACGTATACTC	GAGTATACGT	TCCTCCAGTG	TCCTCCAGTG	CACTGGAGGA
<b>UDI344</b>	CAAGATTCCA	TGGAATCTTG	AATGGCACAC	AATGGCACAC	GTGTGCCATT
<b>UDI345</b>	TTCGTTGTAC	GTACAACGAA	GGAGTGAGCT	GGAGTGAGCT	AGCTCACTCC
<b>UDI346</b>	AAGATGACGA	TCGTCATCTT	CCTCTTACAA	CCTCTTACAA	TTGTAAGAGG
<b>UDI347</b>	CCGCACTCAA	TTGAGTGC GG	CTAGCAGACG	CTAGCAGACG	CGTCTGCTAG
<b>UDI348</b>	TCTAGCCTAG	CTAGGCTAGA	CACTATGGCG	CACTATGGCG	CGCCATAGTG
<b>UDI349</b>	CTCTGTCAAG	CCTGACAGAG	TAGATACTGG	TAGATACTGG	CCAGTATCTA
<b>UDI350</b>	GTATACCACA	TGTGGTATAC	TAGGACAGGC	TAGGACAGGC	GCCTGTCCTA
<b>UDI351</b>	GTCACTTAAG	CTTAAGTGAC	AACTAGCGTA	AACTAGCGTA	TACGCTAGTT
<b>UDI352</b>	AGAGATTGTG	CACAATCTCT	GGTACTGCTG	GGTACTGCTG	CAGCAGTACC



## CleanPlex SARS-CoV-2 FLEX Research and Surveillance NGS Panel User Guide

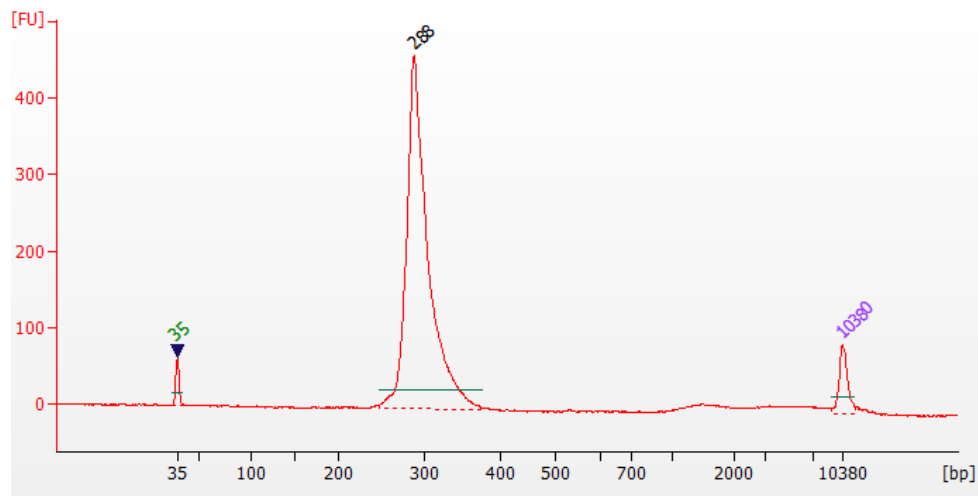
<b>UDI353</b>	TTGGTTCGGC	GCCGAACCAA	CCAGAATGGC	CCAGAATGGC	GCCATTCTGG
<b>UDI354</b>	ACGCTTGACA	TGTCAAGCGT	TTCTGAGTCA	TTCTGAGTCA	TGACTCAGAA
<b>UDI355</b>	CACTGCCAAG	CTTGGCAGTG	GGCCTAGATT	GGCCTAGATT	AATCTAGGCC
<b>UDI356</b>	CCGACATTGC	GCAATGTCGG	CGAACCACAG	CGAACCACAG	CTGTGGTTTCG
<b>UDI357</b>	AGGCCGATGT	ACATCGGCCT	GCGTAATGTT	GCGTAATGTT	AACATTACGC
<b>UDI358</b>	TCTGAGGACC	GGTCCTCAGA	GAAGTGACTC	GAAGTGACTC	GAGTCACTTC
<b>UDI359</b>	CAGATCCGCA	TGCGGATCTG	AGACCTCCGT	AGACCTCCGT	ACGGAGGTCT
<b>UDI360</b>	GAAGCAGCTG	CAGCTGCTTC	CGGATCGGAT	CGGATCGGAT	ATCCGATCCG
<b>UDI361</b>	GTGCGATCGA	TCGATCGCAC	TTGAATCTCC	TTGAATCTCC	GGAGATTCAA
<b>UDI362</b>	GAGTATGTAC	GTACATACTC	GCAACCTATA	GCAACCTATA	TATAGGTTGC
<b>UDI363</b>	TTCCTTCGAG	CTCGAAGGAA	ATAGGCCTCG	ATAGGCCTCG	CGAGGCCAT
<b>UDI364</b>	CCACGGATTC	GAATCCGTGG	TCTCATGTGA	TCTCATGTGA	TCACATGAGA
<b>UDI365</b>	ATGTTACAGT	ACGTGAACAT	CACGGCTAGT	CACGGCTAGT	ACTAGCCGTG
<b>UDI366</b>	TTCGTGGACT	AGTCCACGAA	CCTCCTTATT	CCTCCTTATT	AATAAGGAGG
<b>UDI367</b>	CGCAATAGAA	TTCTATTGCG	GTGTCACGAC	GTGTCACGAC	GTCGTGACAC
<b>UDI368</b>	CCGTCATATG	CATATGACGG	TCTGTGACAT	TCTGTGACAT	ATGTCACAGA
<b>UDI369</b>	ACTACCGAGG	CCTCGGTAGT	GTAACATGCA	GTAACATGCA	TGCATGTTAC
<b>UDI370</b>	AGGTTACCA	TGGTGAACCT	CACTGCCAAG	CACTGCCAAG	CTTGGCAGTG
<b>UDI371</b>	TTCGAAGACG	CGTCTCGAA	TAGCTGAGGT	TAGCTGAGGT	ACCTCAGCTA
<b>UDI372</b>	TTCAGATGGA	TCCATCTGAA	CCAGGATAGA	CCAGGATAGA	TCTATCCTGG
<b>UDI373</b>	CCGTTTCGTAT	ATACGAACGG	TTGCATGACA	TTGCATGACA	TGTCATGCAA
<b>UDI374</b>	TCTCAAGGCG	CGCCTTGAGA	ACGCAACGAG	ACGCAACGAG	CTCGTTGCGT
<b>UDI375</b>	CGACTGATGC	GCATCAGTCG	TTGTGTGATG	TTGTGTGATG	CATCACACAA
<b>UDI376</b>	GAAGATTAGC	GCTAATCTTC	CAAGAGCTGG	CAAGAGCTGG	CCAGCTCTTG
<b>UDI377</b>	TCGAACGTAA	TTACGTTCGA	TTGCGTCCGA	TTGCGTCCGA	TCGGACGCAA
<b>UDI378</b>	GCAAGATACT	AGTATCTTGC	CCACCTTGAG	CCACCTTGAG	CTCAAGGTGG
<b>UDI379</b>	TCTGTTAGAC	GTCTAACAGA	CGAGCGATAG	CGAGCGATAG	CTATCGCTCG
<b>UDI380</b>	TATCACCTGC	GCAGGTGATA	CTCGGCTTCA	CTCGGCTTCA	TGAAGCCGAG
<b>UDI381</b>	TGTAAGCCT	AGGCTTACCA	GTGACGCTGT	GTGACGCTGT	ACAGCGTCAC
<b>UDI382</b>	CGTGTTATGA	TCATAACACG	TCTGGCAATT	TCTGGCAATT	AATTGCCAGA
<b>UDI383</b>	AGAAGTTGGA	TCCAACCTCT	TCTAGCTAAC	TCTAGCTAAC	GTTAGCTAGA
<b>UDI384</b>	TTAGACAGCG	CGCTGTCTAA	AGATGGTTAG	AGATGGTTAG	CTAACCATCT

## Troubleshooting Guide

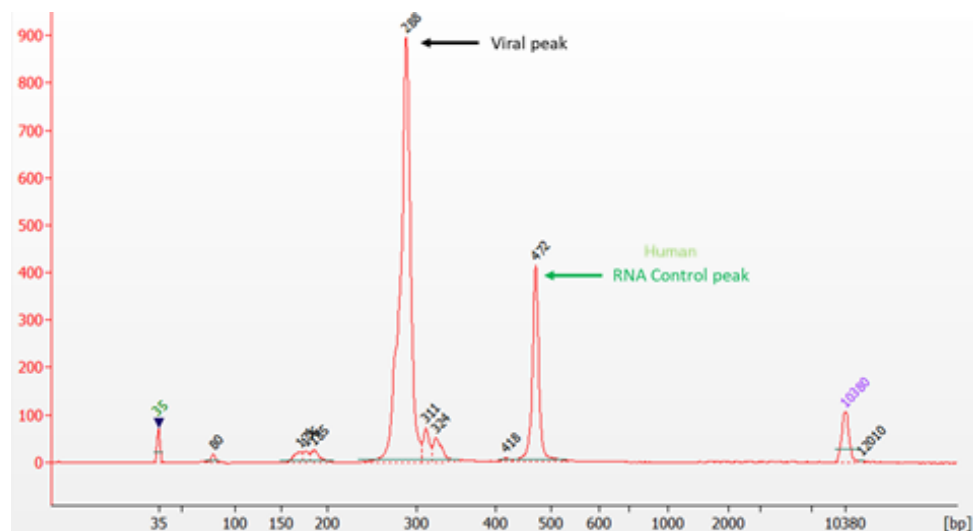
### Examples of Libraries Prepared with CleanPlex SARS-CoV-2 FLEX Panel

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:

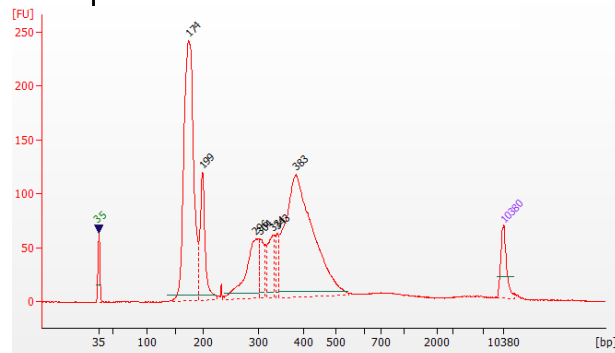
- Inaccurate pipetting of magnetic bead volume.
- Insufficient mixing of reaction solutions or bead and sample mixture.
- Insufficient removal of supernatant and ethanol washes during purification.
- Allowing mPCR products to sit for too long (and form nonspecific products) before purification.
- CleanPlex 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.

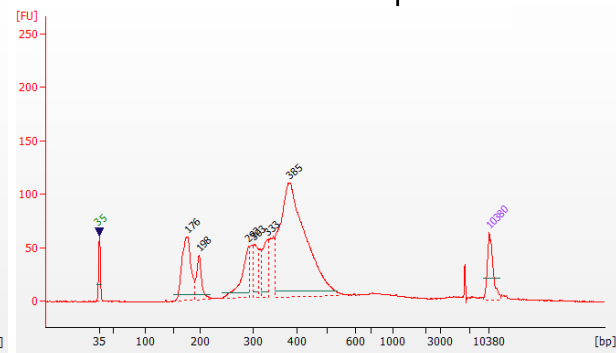
## Removing nonspecific products from final libraries

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  $\mu$ L**. 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 <https://www.paragongenomics.com/faq-items/> 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/> using the associated order account after the purchase has been shipped.

#### 5. Downstream analysis.

Paragon Genomics could provide any additional design files to be used in virus detection or strain identification. SOPHiA Genomic also provides [analysis support](#) for the CleanPlex SARS-CoV-2 Panel. For suggestions or more information on analysis solutions, please contact [sales@paragongenomics.com](mailto:sales@paragongenomics.com) or [techsupport@paragongenomics.com](mailto:techsupport@paragongenomics.com).

## Technical Support

For technical assistance, please contact Paragon Genomics Technical Support.

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**Email:** [techsupport@paragongenomics.com](mailto:techsupport@paragongenomics.com)

**Website:** [www.paragongenomics.com](http://www.paragongenomics.com)

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