JUMPCODE GENOMICS

APP NOTE

CRISPRclean® Plus for SARS-CoV-2 Shotgun Metatranscriptomic Sequencing

CRISPR-based ribodepletion removes RNA "noise" from assays, allowing key insights into variants, as well as co-infections and host responses.

Like other viruses, SARS-CoV-2 mutates frequently, producing new strains that continue to pose a public health threat. As each new wave appears, epidemiological surveillance is the first line of defense, offering crucial insights into genomic variations, as well as offering other research and potential clinical insights.

Shotgun metatranscriptomic sequencing is an invaluable tool to understand this (or any) constantly evolving virus, offering unbiased data on SARS-CoV-2 variants, potential co-infections and host responses.

Unfortunately, most of the transcriptomic data can be uninformative, as noise from overabundant human and bacterial ribosomal RNA (rRNA) sequences drowns out the signal from important transcripts. In some samples, rRNA accounts for 90% of total RNA.

Traditionally, these rRNA sequences are removed before library preparation. CRISPRclean offers a novel way to remove rRNA after library preparation, immediately before sequencing.

In addition, these rRNA sequences obscure low-expressing transcripts. Removing these less informative molecules offers tremendous opportunity to provide higher-coverage of reads on the more interesting RNA molecules.

CRISPRclean Plus Stranded Total RNA Prep with rRNA depletion can provide more insights into complex samples. In this app note, CRISPRclean Plus is used with COVID positive and negative nasopharyngeal (NSP) samples which contain a mixture of human and bacterial cells.

CRISPRclean Plus: The Value of Eliminating Noise

Next-generation sequencing has been a powerful tool for discovery. Unfortunately, much of that sequencing power can be misspent decoding biologically uninformative sequences.

Complex samples often contain a mixture of bacterial and human cells, which are constantly expressing rRNA, muddying the transcriptomic picture. Jumpcode Genomics has adapted CRISPR to build more precise NGS libraries, using its molecular scissors to cleave rRNA, which are then excluded from PCR amplification. This cleaning process makes shotgun metatranscriptomic sequencing more effective, delivering the essential information labs need to fully understand a sample.

CRISPRclean Plus depletes rRNA sequences from human, and more than 200 bacterial species, to help researchers identify rare, biologically relevant sequences.

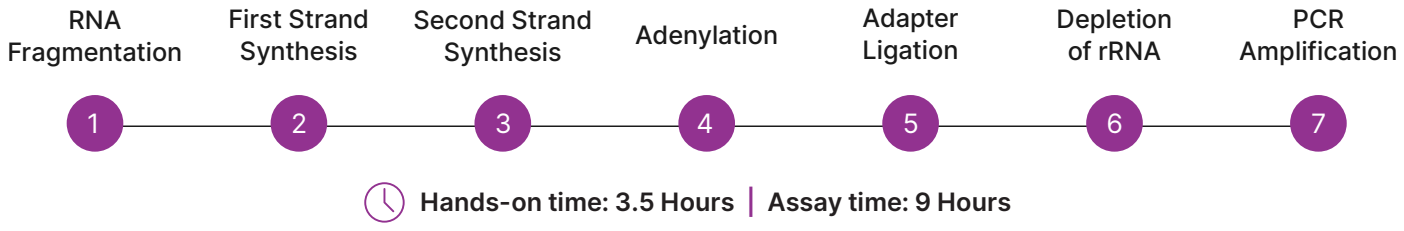

Figure 1: CRISPRclean Plus produces a directional and refined library that is ready for short read next-generation sequencing.

Using CRISPRclean Plus, labs can detect SARS-CoV-2 with the same sensitivity as PCR and obtain greater genomic coverage. This increased coverage means scientists can identify SARS-CoV-2 transcripts, as well as hard-to-detect potential co-infections and host responses.

Workflow

CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion prepares strand- specific RNA libraries, depleting human, mouse, rat and over 200 bacterial species' rRNA.

- Complete workflow takes just 9 hours, with 3.5 hours of hands-on time;
- The final product is a directional, depleted library compatible with short read NGS instruments;
- The kits contain necessary reagents to process purified total RNA sample through library preparation for sequencing.

The workflow begins with RNA fragmentation through high-temperature incubation and continues to first and second strand synthesis, converting RNA fragments into cDNA libraries. The assay achieves 98% strand specificity by incorporating dUTP during second strand synthesis. Adenylation modifies the 3' ends of the double-stranded cDNA and dATP prepares the library for adapter ligation. After ligating the unique dual index adapters, the library is ready for depletion.

CRISPR depletion happens in two successive incubations. The first cleaves bacterial rRNAs and the second cleaves eukaryotic rRNAs. Cas9 and guide RNAs are combined to form the ribonucleoprotein complex that cuts the targeted rRNA sequences. Cleaved rRNA cannot be amplified and is removed through size selection with magnetic beads.

CRISPRclean Plus in Action

Study design

To assess CRISPRclean Plus for COVID-19 surveillance, Jumpcode studied 60 NSP samples with Ct values ranging from 16 to 39: 45 were COVID-positive and 15 COVID-negative samples. Each sample was split into three, with one control and two replicates for depletion. Each subsample went through library construction, depletion, sequencing, and analysis.

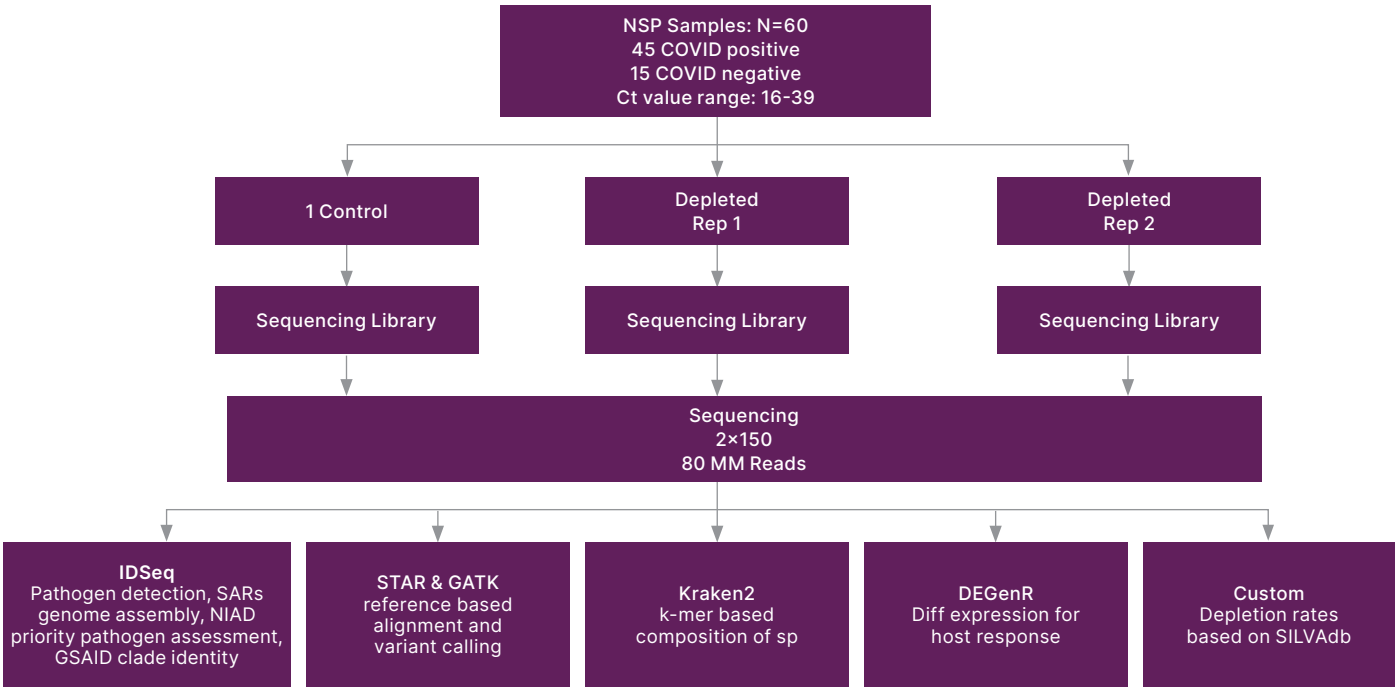

Figure 2: The experimental setup from NSP sample through library preparation, CRISPRclean Plus depletion, sequencing, and analysis.

Performance

Using COVID samples from NSP, CRISPRclean Plus effectively removed around 90% of human and bacterial rRNA (figure 3).

After depletion, researchers were able to confidently call the strains down to a cycle threshold (Ct) approaching 30. The assay showed 100% COVID genome coverage to Ct of 27. This approach detected

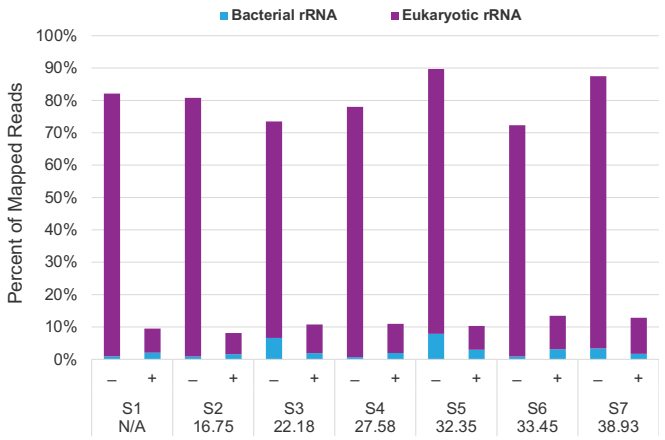

Figure 3: CRISPRclean Plus removes more than 90% of both bacterial and eukaryotic rRNA.

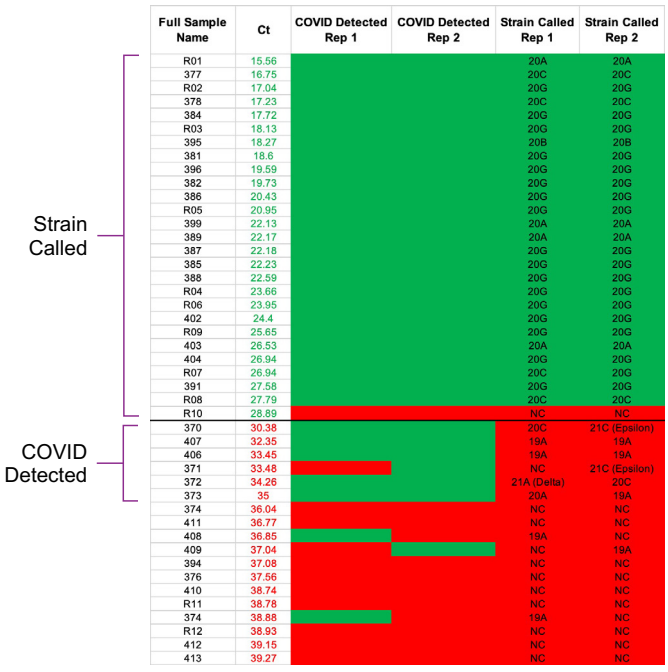

Figure 4: CRISPRclean Plus enhanced detection sensitivity with complete COVID genome coverage for samples with Ct of 27 and below. For low viral load samples with Ct values greater than 27, CRISPRclean Plus detected COVID transcripts, without identifying specific strains.

COVID transcripts, without identifying specific strains, to Ct of 36 The higher Ct numbers correlate with lower viral particle concentrations. This enhanced sensitivity allows researchers to design more efficient and cost-effective assays to maintain surveillance. Statistical relevance at lower sequencing depth also streamlines data analysis.

Removing rRNA from samples reallocates sequencing reads to drive additional coverage and find data that was previous hidden by rRNA noise. Depletion can increase the number of bacterial species detected from 500 to 1,000 species (figure 6).

CRISPRclean Plus also helps rule out COVID and identify other pathogens. In some cases, a sample may be COVID-negative while being positive for another other pathogen that might cause similar symptoms.

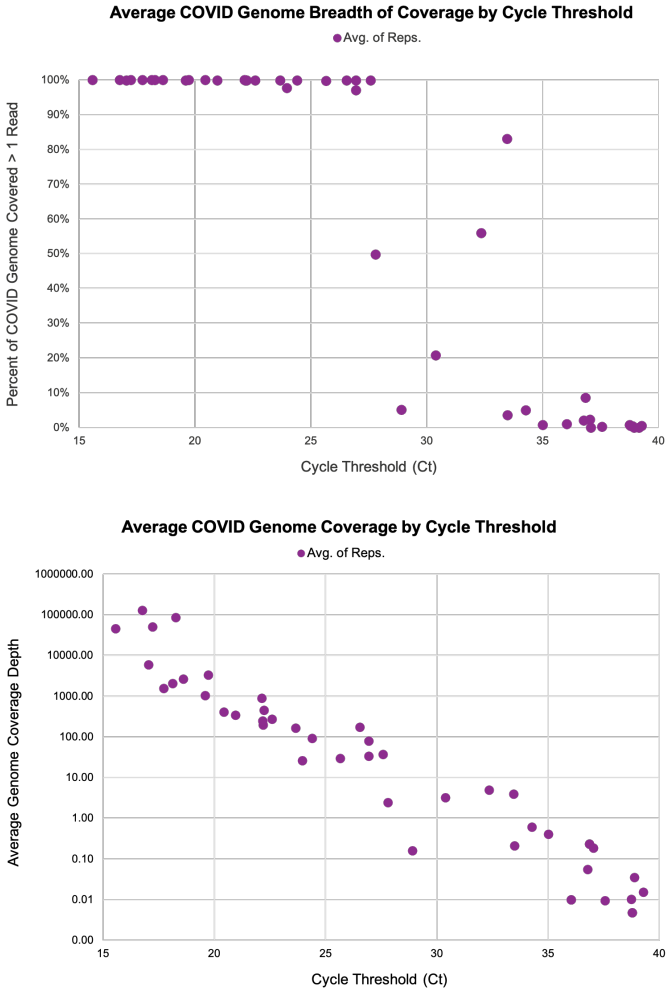

Figure 5: Precise results at high cycle thresholds helps labs find more information while reducing sequencing costs.

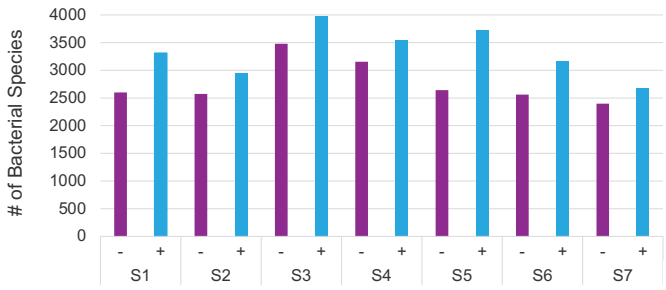

Figure 6: Ribosomal RNA depletion reveals hidden bacterial species, boosting discovery.

Sequencing done following rRNA depletion detected rhinovirus, which is often implicated in the common cold. In one case, the rhinovirus was likely a coinfection; in another, it was the primary infection. By reducing the background noise, researchers could tease out more information from these complex samples.

This research has also shown more sensitivity and coverage when assessing host responses, showing upregulated transcripts for T-cell chemotaxis, natural killer cell regulation, macrophage migration and other critical immune responses (figure 7).

Understanding these host response signatures could give researchers, clinicians and public health officials new weapons understand the current pandemic and fight future pandemics In some cases, findings like this can inform efforts to mitigate a hyperactive immune response.

Conclusion

CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion is an optimized workflow that detects bacterial and viral transcripts as well as host gene expression.

This simple workflow reassigns sequencing data from abundant molecules to higher value and lower-expressing transcripts to provide better insights into gene expression. CRISPR-powered rRNA depletion significantly improves virus strain calling, boosts the number of human genes detected above noise and increases detection of bacterial species, compared to

Figure 7: More sensitive sequencing allows researchers to develop a more precise picture of host responses to COVID and other pathogens.

undepleted samples.

To learn more, visit jumpcodegenomics.com

Overall CRISPRclean Plus:

- Depletes more than 90% of eukaryotic and bacterial rRNA in clinical NSP samples;
- Provides three to seven-fold increase in SARS-CoV-2 coverage;
- Detects viral data, microbiome composition, co-infections and host gene expression in a single workflow;
- Increases human gene detection sensitivity.

Ordering information

Catalog	Product name	Reactions
KIT1016	CRISPRclean™ Plus Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat, Pan Bacteria)	24
KIT1017	CRISPRclean™ Unique Dual Index Adapter Plate for RNA Prep (Set A)	96 UDI