A CRISPR-Powered Universal Infectious Disease Assay

Keith Brown,[3] Azeem Siddique,[1,3] Dhanya Ramachandran,[1,3] Gaia Suckow,[1,3] Nils Homer,[2] Jay Carey,[2] Phillip Ordoukhanian,[1,3] Steve Head,[1,3] Stan Nelson[3]
1 The Scripps Research Institute, La Jolla, CA; 2 Fulcrum Genomics, Somerville, MA; 3 Jumpcode Genomics, San Diego, CA

JUMPCODE GENOMICS

To learn more visit:
JumpcodeGenomics.com

Highlights

- A CRISPR-based method of depletion of uninformative NGS sequences
- Allows detection of all viral, bacterial and fungal pathogens in a single test
- High detection sensitivity

Background

The COVID-19 pandemic has brought awareness to the dangers of emerging pathogens to global human health and welfare. Sensitivity and flexibility are important features for methods used to detect emerging pathogens. While PCR testing is rapid and sensitive, a significant advantage next generation sequencing (NGS) approaches have over PCR-based analyses is the ability to detect previously undiscovered pathogens while also providing genomic information that can detect SARS-CoV-2 genome sequence, identify source of co-infection, and the host transcriptional response in a single workflow. The critical component enabling this approach is Jumpcode CRISPRclean technology which removes abundant human and bacterial ribosomal RNA sequences from NGS libraries.

Methods

CRISPRclean was applied to contrived infected tissue samples including human lung RNA spiked with serially diluted amounts of SARS-CoV-2 RNA and bacterial RNA. NEB RNA libraries were prepared and treated with CRISPRclean protocol, then sequenced on NovaSeq6000 and NextSeq500 instruments. Data analysis was performed using Jumpcode proprietary software to measure alignment and depletion rates, the Silva database for rRNA read alignment, and Kraken2 and CosmosID pipelines for k-mer based metagenomic investigation.

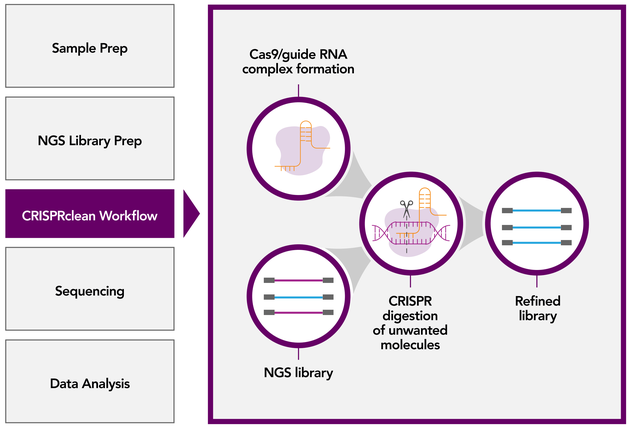

Figure 1: CRISPRclean workflow seamlessly fits into NGS workflows.

Results

CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome. For the 60 viral copies of SARS-CoV-2 sample, the number of reads mapping to the SARSCoV-2 genome increases from ~10,000 reads to ~70,000 reads. A similar increase in reads occurs for S. aureus. The percentage of SARS-CoV-2 genome covered at 1X and 10X also increases. Data were evaluated using the CosmosID shotgun metagenomic pipeline. Similar results were achieved even after downsampling the datasets to 5M reads. There is a ~4-fold increase in bacterial species detection in these stool samples after CRISPRclean treatment. SARS-CoV-2 was detectable by sequencing at only 60 copies (0.000001%) in the sample.

Conclusions

Metatranscriptomics powered by CRISPR-mediated depletion of rRNA offers a robust methodology to acquire viral genomic data, microbiome composition, co-infection information, and the transcriptional status of the host immune response in a single workflow. This sequencing-based approach can be available on the first day of the next viral outbreak and should be considered as a first-line test for novel zoonotic virus detection.

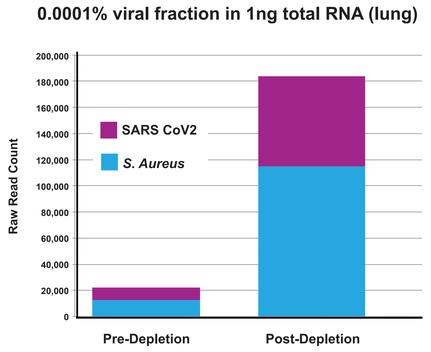

Figure 2: Number of reads aligning to the *S. aureus* and SARS-CoV-2 genomes before and after CRISPRclean depletion.

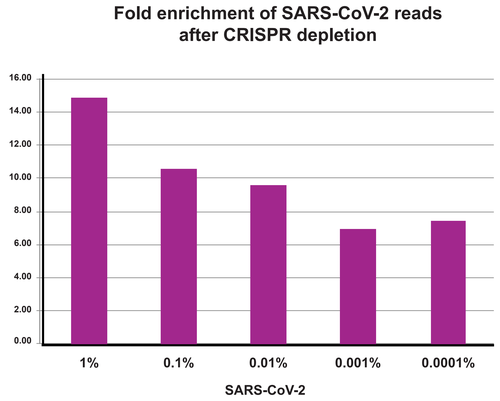

Figure 3: Fold enrichment of SARS-CoV-2 reads after CRISPRclean depletion of libraries prepared from contrived samples.

SARs COV-2 fraction of total RNA	% of genome covered at 1x	% genome covered at 10x
1.0000%	100%	100%
0.1000%	100%	100%
0.0100%	100%	100%
0.0010%	80%	48%
0.0001%	30%	7%

Table 1: Coverage of the SARS-CoV-2 genome at 50 million reads.

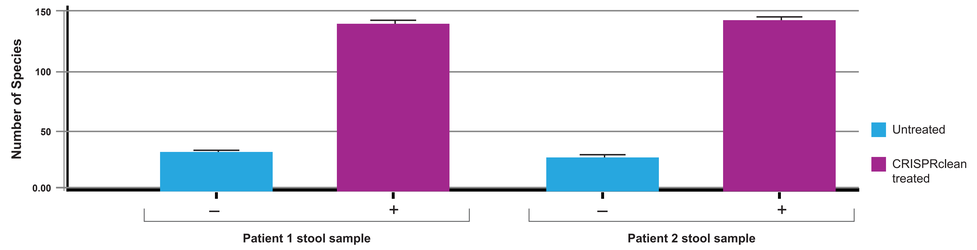

Figure 4: Bacterial species composition of patient stool samples before and after CRISPRclean depletion. Sequencing data downsampled to 20 million reads.