JUMPCODE GENOMICS

DATASHEET

CRISPRclean™ Plus Stranded Total RNA Prep with rRNA Depletion

(Human, Mouse, Rat, Pan Bacteria)

CRISPR-based ribodepletion strategy optimized with stranded RNA prep increases coverage of lower-expressing transcripts from complex biological samples.

Introduction

The unbiased nature of shotgun metagenomic and metatranscriptomic sequencing has unlimited potential in the discovery and analysis of organisms co-existing in diverse biological systems (example: wastewater surveillance to profile viral genetic diversity across infected communities). In this datasheet nasopharyngeal swabs from clinically COVID positive patients were used to show how CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat, Pan Bacteria) can be applied to track viral spread during a pandemic. Much of the transcriptomic data captured is uninformative, due to the abundance of ribosomal RNA (rRNA) that interferes with efficient identification of biologically significant transcripts. CRISPRclean technology harnesses the power of the CRISPR system to deplete rRNA sequences from biological samples containing mixtures of mammalian, bacteria, and pathogenic agents. CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion is an optimized, workflow that simultaneously detects viral genomic data, microbiome composition, co-infections, and host gene expression. CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion reassigns sequencing reads from abundant molecules to higher value and lower expressing transcripts to understand gene expression of changing environmental conditions. In this datasheet we use SARS-CoV-2 (COVID) as a proxy for detection of RNA viruses in complex sample types containing eukaryotic, bacterial, and viral sequences. The adaptation of the revolutionary CRISPR technology to NGS library construction enables the process for discovery in shotgun metagenomic and metatranscriptomic sequencing.

Highlights

- Streamlined CRISPRclean workflow: stranded total RNA library prep with ribodepletion
- Ideal for analysis of samples containing complex mixtures of eukaryotic and bacterial rRNA
- 3 to 7-fold increase in coverage of SARS-CoV-2 and other genomes in nasopharyngeal (NSP) samples
- Single workflow to detect viral genomic data, microbiome composition, co-infections, and host gene expression

Workflow

Optimized for 9 hours assay time with 3.5 hours hands-on time from total RNA to sequencing ready libraries. >98% strand specificity is achieved through incorporation of dUTP during second strand synthesis. The innovative step is the CRISPR-powered depletion of bacterial, human, mouse, or rat rRNA sequences in adapter ligated libraries. CRISPR-powered depletion is performed in two successive incubations, the first to cleave bacterial rRNAs and the second to cleave eukaryotic rRNAs. Cas9 and guide RNAs are combined to form the ribonucleoprotein complex specifically programmed to cut specific rRNA sequences. Cleaved rRNA sequences cannot be amplified, and are removed by size selection with magnetic beads. The final product is a directional and refined library ready for short read next-generation sequencing (NGS).

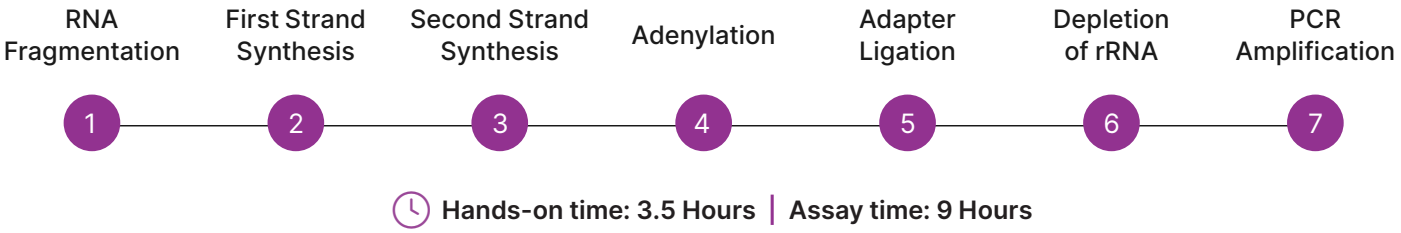

Figure 1: CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat, Pan Bacteria) is a streamlined workflow from total RNA to sequencing-ready, strand-specific libraries in 7 steps with multiple safe stopping points.

Methods

Total RNA was extracted from nasopharyngeal (NSP) swabs from clinically COVID positive patients with Ct values ranging from 16 to 38, using PerkinElmer® chemagic™ extraction method. A sub-set of this sample dataset is shown in this datasheet. Directional libraries were prepared using CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat, Pan Bacteria) from 10 ng of total RNA. Libraries were sequenced on Illumina® NextSeq™ 2000 instrument at 2×150 paired-end reads and down sampled to 80M reads for analysis. Analysis of data was performed using STAR aligner for COVID and human genomes, Kraken2 and IDseq for bacterial composition, and BWA for alignment to reads to SILVA database to calculate rRNA depletion metrics.

Ideal for analysis of samples containing complex mixtures of organisms

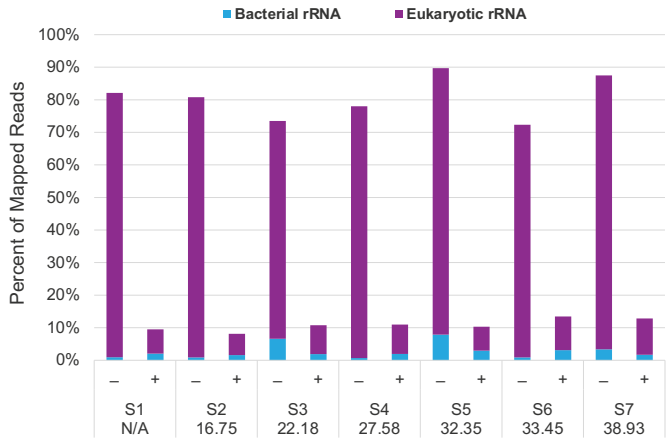

Figure 2: CRISPRclean removed >80% abundant rRNA sequences from clinical NSP samples. Comparison of clinical NSP samples before and after depletion showed efficient (> 80%) depletion of bacterial and eukaryotic rRNA using clinical NSP samples across different Ct values from negative to 38.93, which has very low viral load.

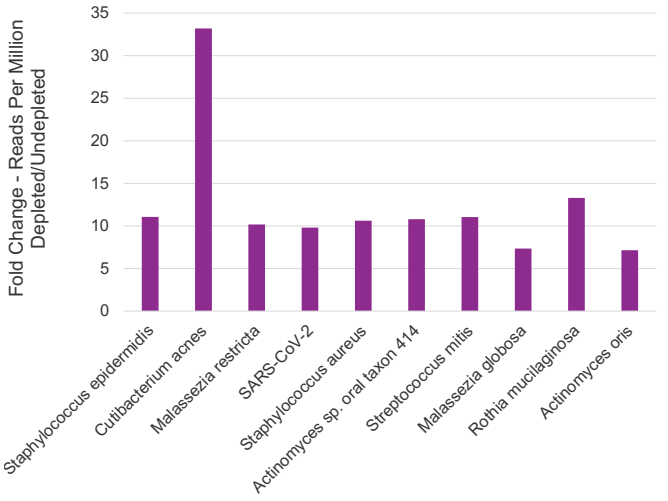

Figure 3: Increased detection sensitivity. Top ten high abundance organisms in depleted sample S8 (Ct value 26.94). High abundant organisms on average increased 10-fold reads per million compared to undepleted samples.

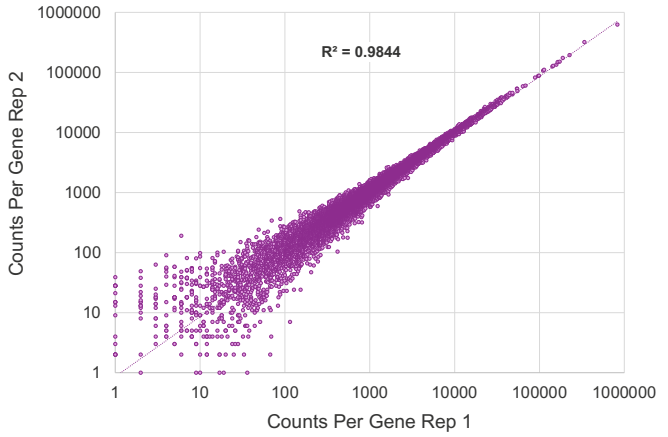

Figure 4: Highly reproducible. Gene expression signals from replicates were highly correlated with linearity R^2 at 0.9844. R^2 close to 1.0, showed that human gene expression was highly conserved across replicated depletions.

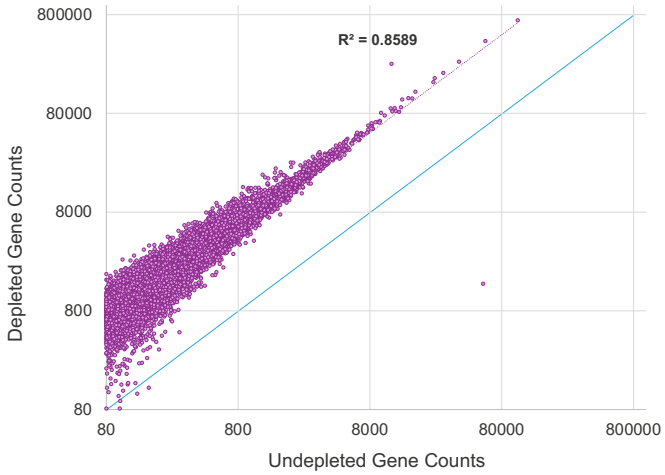

Figure 5: More genes are counted in human gene expression for depleted compared to undepleted sample S7. Gene expression signals before and after depletion are highly correlated with linearity R2 at 0.8589. R2 close to 1.0 showed that human gene expression was conserved between undepleted and depleted sample S7. Gene counts were higher for depleted sample S7. Blue line at y=x showed how the signals would line up if the gene counts between the depleted and the undepleted were balanced. A shift closer to the left showed that gene counts were higher for depleted sample S7.

Increased sensitivity to detect lower-expressing, biologically relevant transcripts

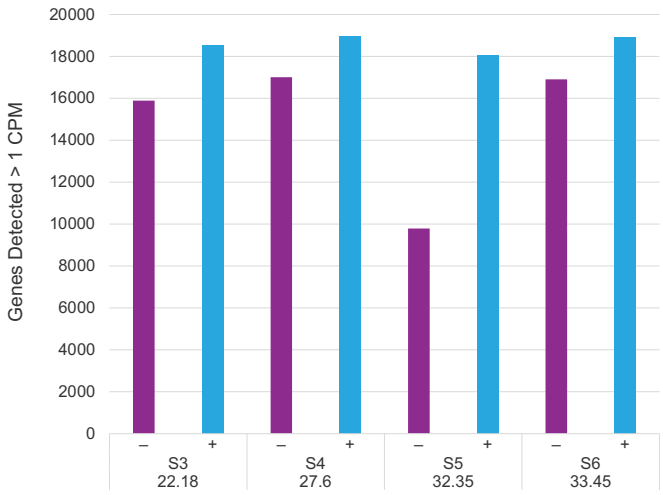

Figure 6: More human genes were detected in NSP samples after depletion. Number of human genes detected at greater than 1 CPM (Read count cutoff of 80 was used as CPM of 1.)

Single workflow to detect viral genomic data, microbiome composition, co-infections, and host gene expression

Sample	Ct	Depletion	COVID Detected	Strain Called
S1	neg	-	Not detected	No call
		+	Not detected	No call
S2	16.75	-	Detected	20C
		+	Detected	20C
S3	22.18	-	Detected	20G
		+	Detected	20G
S4	27.60	-	Detected	No call
		+	Detected	20G
S5	32.35	-	Not detected	No call
		+	Detected	No call
S6	33.45	-	Not detected	No call
		+	Detected	No call
S7	38.93	-	Not detected	No call
		+	Detected	No call

Table 1: Better RNA virus detection and strain calling results with CRISPRclean. COVID strain 20G was called only in the depleted sample S4 (Ct of 27.60). COVID presence was detected only in depleted samples, S5 and S6 samples at 32.25 and 33.45 Ct, respectively.

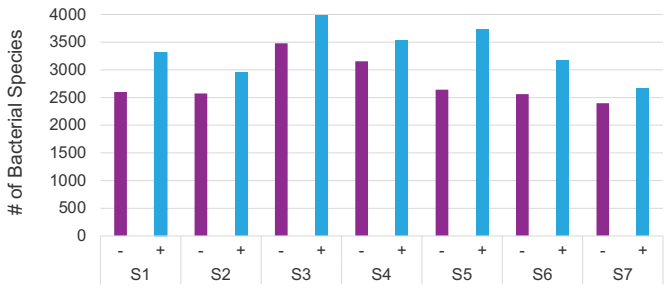

Figure 7: Increased bacterial detection sensitivity with clinical NSP samples.

Summary

CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion is an optimized workflow that simultaneously detects viral genomic data, microbiome composition, co-infections, and host gene expression. CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion reassigns sequencing data from abundant molecules to higher value and lower expressing transcripts in order to understand gene expression of changing environmental conditions or treatments. In this datasheet we have presented data using nasopharyngeal swabs collected from clinical patients with SARS-CoV-2 as a proxy RNA virus. Data has showed that CRISPR-powered depletion strategy significantly improves virus strain typing calling, increases the number of human genes detected above noise, and increases the number of bacterial species detected compared to undepleted samples. CRISPRclean Plus Stranded Total RNA Prep with rRNA Depletion streamlines the process for routine assay development and discovery in biological systems made up of diverse organisms and pathogens using shotgun metagenomic and metatranscriptomic sequencing.

To learn more, visit jumpcodegenomics.com

Specifications

Application	Metatranscriptomics, infectious disease surveillance
Assay time	9 hrs
Hands-on time	3.5 hrs
Sample types	Complex samples with mixtures of eukaryotes and prokaryotes such as nasopharyngeal, saliva, gut, fecal, and more.
Input quantity	5 - 100 ng
Method	RNA-Sequencing
Depletion Mechanism	CRISPR-based rRNA depletion
Strand specificity	>98% directional and strand specific
Compatible species	Human, mouse, rat, bacteria
Designed to deplete	Human 5S, 5.8S, 18S and 28S, 45S rRNA precursor, mitochondrial 12S and 16S rRNA genes. 212 bacteria representing all phyla: 5S, 16S, and 23S rRNA gene.
Multiplex	Up to 96 unique dual index adapter barcodes

Ordering information

Catalog	Product name	Reactions
KIT1014	CRISPRclean™ Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat)	24
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