JUMPCODE GENOMICS

DATASHEET

CRISPRclean™ Stranded Total RNA Prep with rRNA Depletion

(Human, Mouse, Rat)

CRISPR-based ribodepletion strategy improves sensitivity for the detection of lower expressing transcripts from mammalian tissues

Introduction

RNA sequencing has unleashed tremendous data generation to profile gene expression of diverse mammalian cell systems. The challenge is to efficiently eliminate abundant or uninformative sequences, such as ribosomal RNA (rRNA), in order to shift discovery to the previously, or lower expressing, and biologically interesting transcripts. CRISPRclean Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat) harnesses the power of CRISPR-based depletion with stranded RNA library preparation to specifically remove human, mouse, and rat rRNA sequences from adapter ligated cDNA libraries. CRISPRclean Stranded Total RNA Prep with rRNA Depletion reassigns sequencing reads from abundant molecules to higher value and lower expressing transcripts in an unbiased manner without disturbing the relative transcript abundance or complexity of the library. CRISPRclean Stranded Total RNA Prep with rRNA Depletion improves discovery of high value, biologically interesting transcripts, including low-expressing transcripts.

Highlights

- One-day workflow: 7.5 hours assay with 3 hours of hands-on time
- Effective single tube, multi-species depletion with CRISPR-Cas9 mediated technology
- Optimized library prep and depletion workflow generates high quality representation of transcripts
- Consistent full-length, uniform transcript coverage
- Increased sensitivity to detect lower expressing, biologically relevant transcripts

One-day workflow from RNA to sequencing ready library

Library preparation and rRNA depletion is completed through 7 steps with multiple safe stopping points (Figure 1). Greater than 98% of strand specificity is

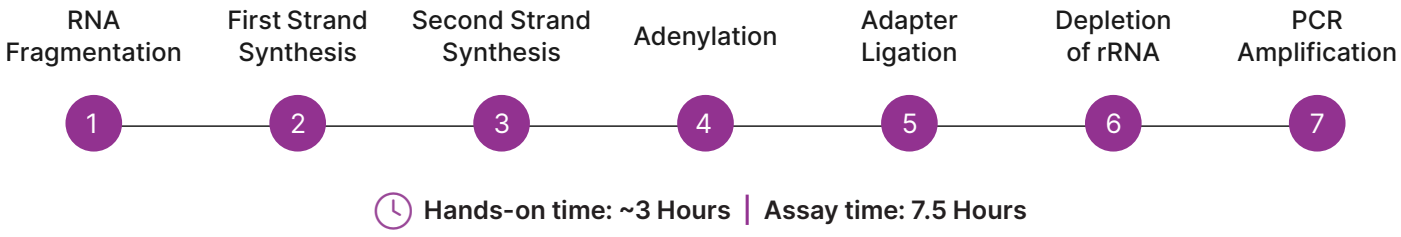

Figure 1: CRISPRclean Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat) is a simple and streamlined 1-day workflow from total RNA to sequencing-ready, strand-specific libraries in 7 steps with multiple safe stopping points.

achieved through incorporation of dUTP during second strand synthesis. The innovative step is the depletion of rRNA sequences in the adapter ligated libraries. CRISPR-powered depletion is performed in a single incubation to cleave rRNAs. Cas9 and guide RNAs are combined to form ribonucleoprotein complexes programmed to specifically remove rRNA sequences. Cleaved rRNA fragments cannot be amplified and are removed by size selection with magnetic beads. The final product is a directional and refined library ready for sequencing.

Methods

Libraries were prepared from universal reference total RNA for human, mouse, rat, chicken, cow, and dog at different input amounts ranging from 5 ng to 100 ng. Libraries were generated with CRISPRclean Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat) and sequenced on an Illumina® NextSeq™ 2000 instrument at 64M reads and 2×150 bp paired end reads. Data analysis was performed using a range of bioinformatic tools such as the RSeQC for transcript coverage and uniformity, STAR aligner for human genome and ERCC alignments, and custom pipelines were used to assess rRNA depletion levels and rates.

Effective single tube, multi-species depletion with CRISPRclean

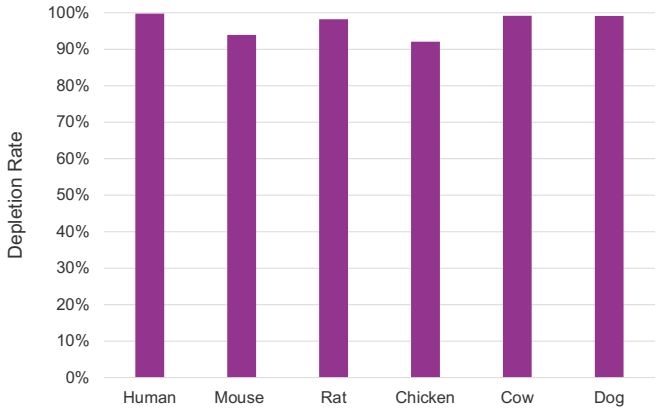

Figure 2: >90% rRNA depletion across mammalian species. Human, mouse, rat, chicken, cow and dog universal reference total RNAs were used to assess performance of the ribodepletion across multiple species. The rate of rRNA depletion was calculated based on the percentages of rRNA reads in depleted and undepleted libraries. CRISPRclean efficiently removes >90% of mammalian rRNA in a single tube depletion reaction.

Specific and unbiased

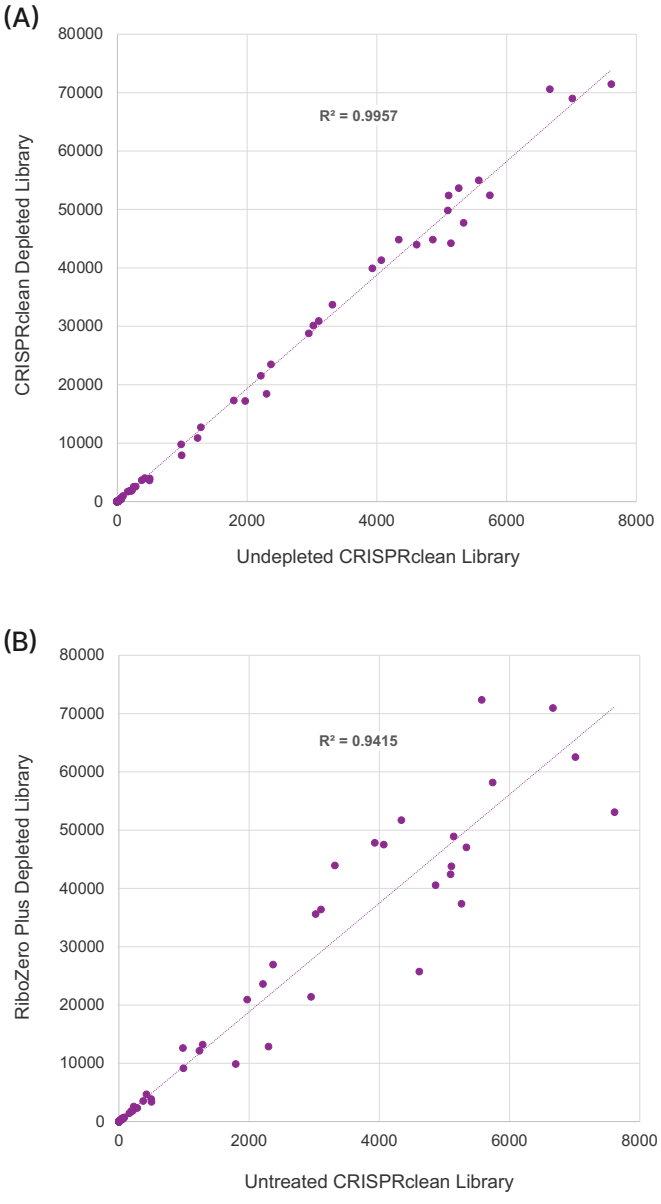

Figure 3: Specific and unbiased. 3A) CRISPRclean Stranded Total RNA Prep with rRNA Depletion produced extremely low library bias. ERCC reads counts were highly correlated, between depleted (y-axis) and undepleted libraries (x-axis), indicating the CRISPRclean rRNA depletion method was highly specific, unbiased, and accurate. This allowed for gene expression measurements to be more accurately represented than those that would be obtained from an undepleted sample. **3B)** Illumina® RiboZero™ Plus Depletion paired with Jumpcode stranded RNA prep shows lower ERCC read count correlation and higher bias.

Optimized library prep and depletion workflow generates high quality representation of transcripts

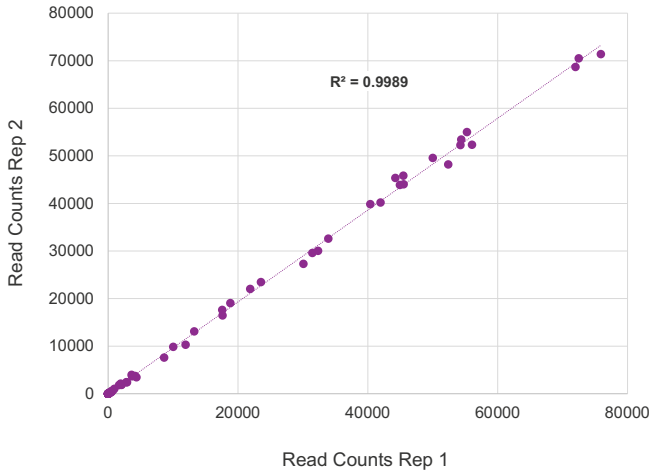

Figure 4: Robust and reproducible. High reproducibility of read counts in replicate depleted CRISPRclean libraries. Sequence read counts of ERCC control spiked into 5 ng of UHR RNA input displayed high correlation (R^2 values for linear fit) between depleted library replicates using CRISPRclean.

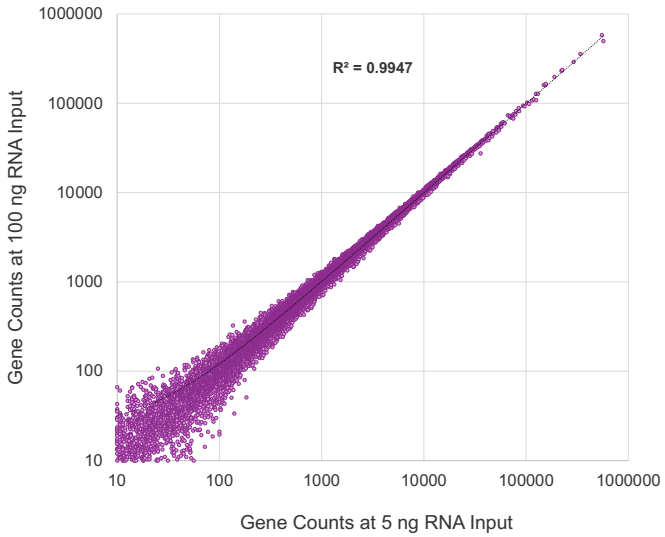

Figure 5: Highly correlated human gene expression across different inputs. CRISPRclean showed highly correlated gene expression between 5 ng and 100 ng UHR RNA inputs. Highly correlated expression meant the CRISPRclean workflow was very specific for ribosomal RNA removal and robust regardless of input amount.

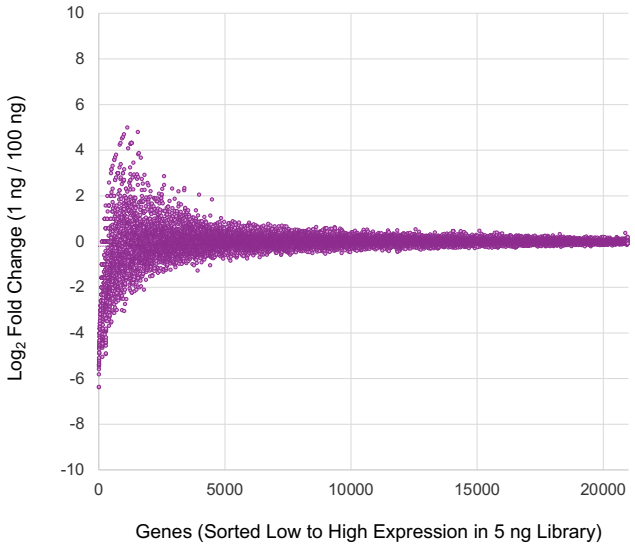

Figure 6: CRISPRclean comparison between 5 ng and 100 ng RNA input. CRISPRclean showed minimal difference in human gene expression levels at 5 ng and 100 ng UHR RNA inputs. Gene level counts were determined for 5 ng and 100 ng inputs, plotted on a Log2 scale (y-axis) and sorted from low to high expression in the 5 ng library. Minimal deviation was seen from a Log2 fold change of 0 which represented highly correlation gene expression measurements across a range of RNA inputs.

Consistent full-length, uniform transcript coverage

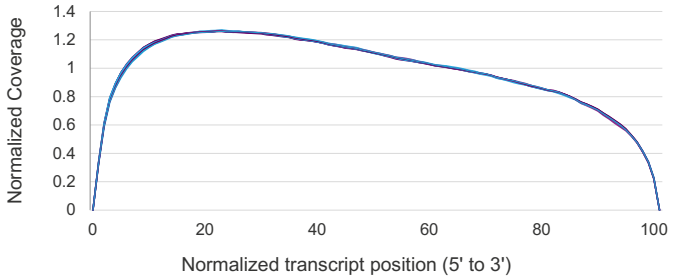

Figure 7: Uniform transcript coverage from 5’ to 3’ across different inputs. CRISPRclean libraries provide uniform coverage across the length of transcripts. Transcript position was analyzed across genes detected with >10 reads in CRISPRclean libraries prepared from 3 replicates of each 5 ng, 25 ng and 100 ng of UHR RNA input.

Increased sensitivity to detect lower-expressing, biologically relevant transcripts

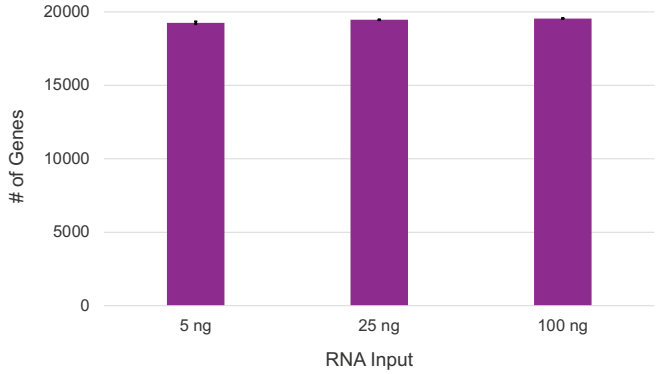

Figure 8: Consistent gene detection at 10x coverage in depleted libraries. The number of genes is assessed at 10x coverage in libraries prepared with 5 ng, 25 ng, and 100 ng UHR RNA. Gene detection is measured by the number of genes detected at 64M (2×150) paired-end reads pass filter.

Summary

CRISPRclean Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat) is a simple and streamlined workflow from total RNA to sequencing-ready, strand-specific libraries in 7.5 hours assay time and 3 hours hands-on time. It is highly effective at removing interfering mammalian ribosomal RNA. CRISPRclean Stranded Total RNA Prep with rRNA Depletion (Human, Mouse, Rat) is a high quality, directional RNA library preparation kit with a novel CRISPR-mediated depletion strategy that increases detection sensitivity of lower expressing and biologically relevant transcripts while generating consistent full-length, uniform transcript coverage with minimal off-target.

Specifications

Application	Whole-transcriptome
Assay time	7.5 hrs
Hands-on time	3 hrs
Sample types	Total RNA
Input quantity	5 to 100 ng
Depletion mechanism	CRISPR-Cas9 mediated
Strand specificity	> 98% directional and strand specific
Compatible species	Human, mouse, rat
Designed to deplete	Human 5S, 5.8S, 18S and 28S rRNA genes, 45S rRNA precursor, mitochondrial 12S and 16S rRNA genes.
Multiplex	Up to 96 unique dual index adapter barcodes

To learn more, visit jumpcodegenomics.com

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