TECH NOTE

Enable novel discovery by reallocating reads onto low and medium expression targets

Combined CRISPRclean® and Evercode™ technology boosts sequencing saturation and reads mapping to the transcriptome

Introduction

Parse Bioscience Evercode™ combinatorial barcoding technology has eliminated the need for expensive hardware such as microfluidic instruments for single-cell sequencing. With a throughput of 10,000 to 1 million cells in a single experiment, combinatorial barcoding allows low multiplet rates without restrictions in cell sizes and numbers. CRISPRclean Single Cell RNA Boost Kit removes uninformative genes such as ribosomal, mitochondrial, and non-variable genes from single cell libraries prior to sequencing, allowing redistribution of these reads onto informative transcripts. Combining CRISPRclean technology with Evercode™ technology, researchers can produce more data with less sequencing saving researchers' valuable costs and enabling discovery on lower expression transcripts.

Methods

Primary bone marrow cells (PBMCs) were split into two conditions: control and CRISPRclean treated condition. They were sequenced on a NextSeq550 with the following read structure: Read 1: 74, Index 1:6, Read 2: 86. All samples were normalized to 65M reads for analysis.

Results

The boost in the percentage of reads mapping to the transcriptome can be seen in Figure 1. The control sample has 64% of reads mapping to the transcriptome while the CRISPRclean condition has 80% of reads mapping to the transcriptome. Additionally, the fraction of reads mapping to ribosomal RNA drops from 8% to 0.3%. Importantly, the sequencing saturation for the control sample is 50% while in the depleted sample that metric increases to 62%. This metric shows that more of the transcript is captured with the same amount of sequencing using CRISPRclean depletion.

Statistic	Control	CRISPRclean
Sequencing Saturation	50.8%	61.9%
Fraction of reads mapping to transcriptome	64.3%	80.6%
Fraction of reads mapping to rRNA	8.4%	0.3%

Figure 1: Key metrics comparing results of sequencing the control sample compared to the CRISPRclean depleted sample.

In Figure 2, violin plots are used to compare the control to the depleted sample in terms of their percent mitochondrial and ribosomal reads. The CRISPRclean treatment has reduced both the mitochondrial and ribosomal reads significantly. Reads are then remapped onto medium and low-expression transcripts that remain in the population.

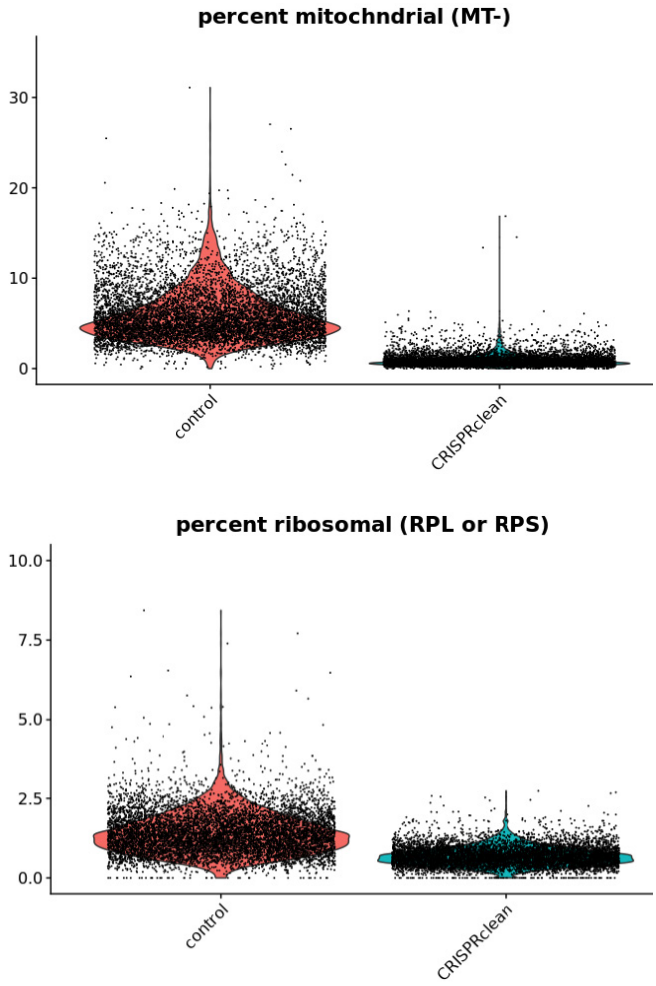

Figure 2: Violin plots showing reduction in both mitochondrial content (2A) and ribosomal content (2B) between the control sample and the CRISPRclean depleted sample.

Similarly, in Figure 3, we have a visual representation of the reduction in reads associated with both mitochondrial (2A) and ribosomal content (2B) as well as the non-variable gene content that is also targeted by CRISPRclean depletion.

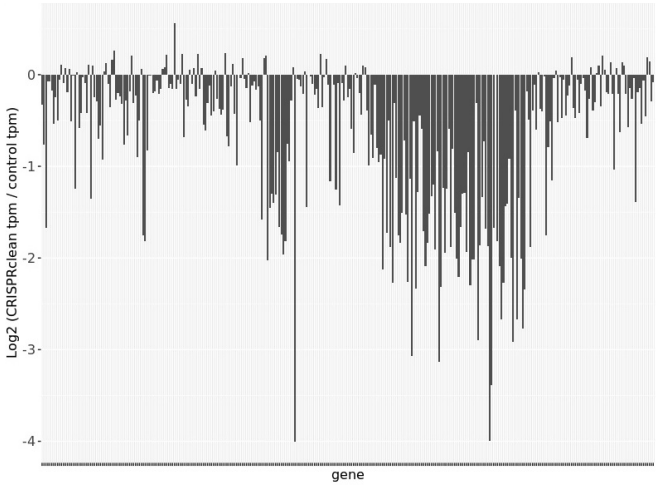

Figure 3: Depletion shows significantly depleted genes for 316 targets. Log2 of the ratio of the CRISPRclean TPM (transcripts per million) over the control TPM gives significant fold change differences.

Conclusion

Combining Evercode™ combinatorial barcoding technology with CRIPRclean technology gives users a boost in sequencing saturation and reads mapping to the transcriptome, reducing cost for researchers and enabling novel discovery by reallocating reads onto low and medium expression targets.

To learn more, visit jumpcodegenomics.com

Ordering information

Catalog	Product name	Samples
KIT1018	CRISPRclean Single Cell RNA Boost Kit	24