JUMPCODE GENOMICS

DATASHEET

CRISPRclean® Single Cell RNA Boost Kit

Double your transcriptomic reads for 10x Genomics® Chromium™ Next GEM Single Cell 3’ libraries

Introduction

There are vast opportunities to apply single cell genomics to the unanswered in biology. With its unique ability to study the individuality of cells, single cell genomics has become an increasingly common, widely adopted approach. This research will not only have important implications for disease but can revolutionize biology. In order to profile large amounts of individual cells, transcriptional profiling with single cell RNA Seq provides the most thorough analysis for large amounts of individual cells. This empowers researchers with the knowledge of what genes are expressed, in what quantity, and how they differ across the cells within a sample at the single cell level.

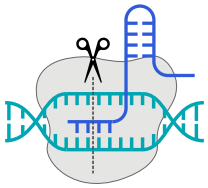
Single cell studies require a significant amount of sequencing to understand transcript levels within individual cells. It can take up anywhere from 50,000 to 150,000 reads per cell in order to get the informative, low abundant genes you want for calling differences between cells—with reads for a single sample often exceeding 150 million reads.

Traditionally, single cell data processing incorporates certain filtering and normalization steps prior to cell clustering and downstream interpretation. Instead of removing those reads in-silico, CRISPRclean removes those reads in-vitro ahead of sequencing, redistributing 50% sequencing reads to unique biologically relevant transcripts —allowing you to maximize gene and UMI sensitivity.

Highlights

- Gain a deeper view of expression profiles of individual cells
- 1.5x improvement in gene and UMI detection sensitivity
- Boost usable data by cutting wasted sequencing by ~50%
- Depletes sequences not used for secondary analysis including: Unaligned reads, ribosomal, mitochondrial, non-variable genes
- Simple 3-step protocol integrated into 10x Genomics® Chromium™ Next GEM Single Cell 3’ workflow

CRISPRclean leverages Cas9 and a specifically designed guide set to remove reads filtered by secondary analysis. CRISPRclean Single Cell RNA Boost Kit gives you the ability to cut through the noise with minimal impact in your workflow, and maximum confidence on your results.



CRISPRclean Single Cell RNA Boost Kit

Total assay time: 2 hours | Hands-on time: 45 minutes

1. GEM generation & barcoding
2. Post GEM-RT cleanup & cDNA amplification
3. Gene expression library construction (Step 3.1-3.4)
4. Gene expression library construction (Step 3.5-3.7)

Figure 1: CRISPRclean is a simple 3-step protocol integrated into the 10x Genomics® Chromium™ Next GEM Single Cell 3’ workflow prior to the final PCR amplification.

Content for depletion was designed by analyzing a cohort of publicly available single cell 10x Genomics® data, roughly 30-50% of reads aligned to the genome but not the transcriptome, and thus, were conventionally ignored. By tailoring guides to deplete these genomic intervals in addition to the highest expressed protein coding ribosomal and mitochondrial genes, we exhibited the ability to redistribute ~50% of reads through in-silico depletion across samples representing 14 sample types (Table 1).

Designed to deplete	Description
Ribosomal, mitochondrial	Poly A ribosomal, mitochondrial genes. Abundant ribosomal and mitochondrial rRNA
Unaligned reads	Reads that do not align to the transcriptome
Non-variable genes	155 genes commonly expressed across 14 different sample types

Table 1: CRISPRclean Single Cell RNA Boost Kit redistributed ~50% of reads through in-silico depletion by depleting unaligned intervals and abundant protein coding ribosomal and mitochondrial genes.

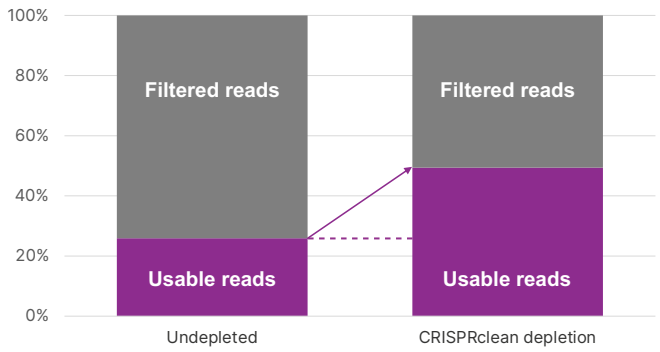


Figure 2: 100% increase in reads mapped to the transcriptome with CRISPRclean. Filtered reads were not used for secondary analysis.

Methods

PBMC samples were isolated from a donor and prepared using 10x Genomics® Chromium™ Next GEM Single Cell 3' Reagent Kit (v3.1) protocol. 150 ng of cDNA product collected at the end of Step 3.4 in the Next GEM Single Cell 3' Reagent Kit protocol was used as starting material for CRISPRclean Single Cell RNA Boost Kit protocol.

The 10x protocol was resumed at step 3.5 with depleted libraries. Four libraries were loaded onto a P3-100 cycle flow cell and sequenced on a NextSeq™ 2000. We recovered ~11,000 cells per sample, each with >25,000 reads per cell.

Raw sequencing reads were processed through Cell Ranger and then used the filtered feature barcode matrix and performed secondary analysis using Pegasus, the python based single cell toolbox. This included all cell filtering and normalization as well as the generation of UMAP plots and single cell trajectory plots.

1.5x improvement in detection sensitivity with depletion

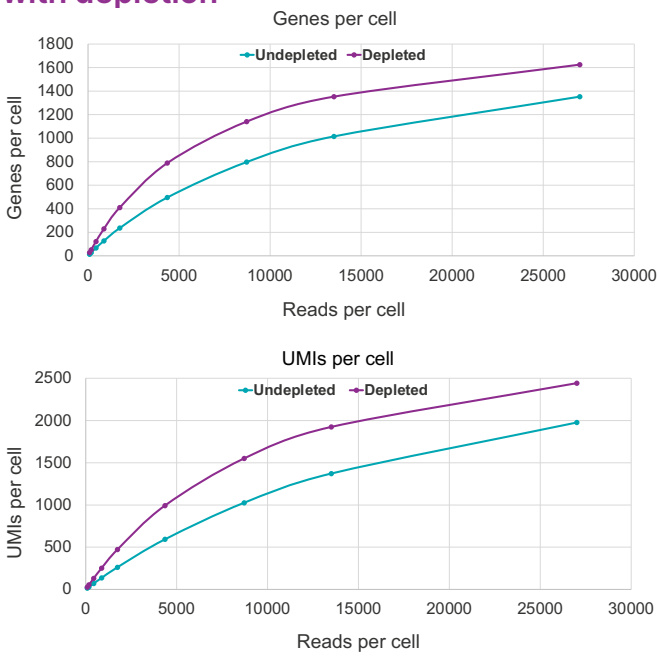


Figure 3: 1.5x improvement in detection sensitivity of genes and UMIs per cell for depleted PBMC samples compared to undepleted sample.

Equivalent performance with ~50% less reads

	Undepleted	CRISPRclean Depleted
Reads per cell	27,000	13,500
Number of cells	11,479	11,479
Genes per cell	1,353	1,340
UMIs per cell	1,976	1,905

Table 2: Detect the same number of genes and UMIs with reduced sequencing.

Gain a deeper view of expression profiles

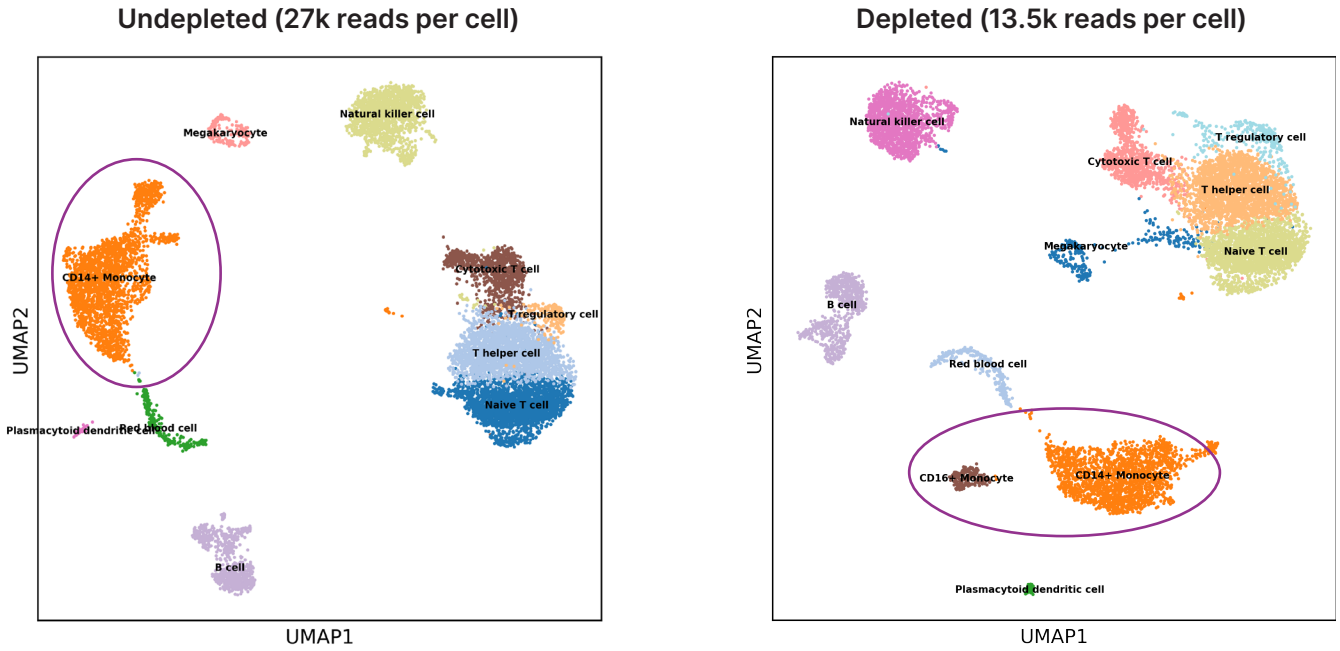


Figure 4: UMAP plots of cell clusters with and without depletion using PBMC samples showed that depletion did not perturb cell type calls. 1 additional cell types were identified in depleted samples.

	Undepleted (27k reads /cell)		Depleted (13.5k reads/cell)	
Cell Type	**Cell Number**	**Cell Frequency**	**Cell Number**	**Cell Frequency**
Naïve T Cell	2,414	22%	2,216	21%
CD14+ Monocyte	2,260	21%	1,949	18%
T Helper Cell	2,254	21%	2,372	22%
Natural Killer Cell	1,468	14%	1,444	13%
Cytotoxic T Cell	994	9%	982	9%
B Cell	693	6%	683	6%
Red Blood Cell	246	2%	272	3%
T Regulatory Cell	203	2%	295	3%
Megakaryocyte	165	2%	288	3%
Plasmacytoid Dendritic Cell	67	1%	61	1%
CD16+ Monocyte	**0**	**0%**	**209**	**2%**
	10,764		10,771	

Table 3: Improvement in cell frequency for CRISPRclean depleted PBMC samples compared to undepleted samples using Pegasus, a secondary analysis single cell toolbox.

Summary

CRISPRclean Single Cell RNA Boost for 10x Genomics® focuses on the signal, not the noise—to specifically remove uninformative sequences and achieve a nearly 50% reduction in raw sequencing reads. Gain a deeper view of the expression profile of cells while still detecting the same number of genes with the CRISPRclean Single Cell RNA Boost Kit.

To learn more, visit jumpcodegenomics.com

Specifications

Assay time	2 hours
Hands-on time	45 min
Input	Uses one of four Chromium™ cDNA aliquots per prep
Method	Single cell 3’ gene expression libraries for 10x Genomics®
Designed to deplete	• Unaligned reads • Ribosomal • Mitochondrial • Non-variable genes
Jumpcode validated	10x Genomics® Chromium™ Next GEM Single Cell 3’ Reagent Kits v3.1

Ordering information

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