JUMPCODE GENOMICS

APP NOTE

CRISPRclean® Single Cell RNA Boost Kit for identification of rare and additional cell types in PBMCs

Increasing the number of identified cell types, unique UMIs, and genes in single cell RNA-seq data

Identifying the individual immune cell types within peripheral blood mononuclear cells (PBMCs) can provide insight into disease origins and how the immune system changes in response to infection, disease, and therapeutic interventions.

PBMCs are commonly studied because they represent a broad spectrum of cell types and are highly heterogeneous, enabling discovery into a broad range of immune responses.

Single cell RNA sequencing (scRNA-seq) offers a powerful way to identify and profile different cell types within PBMC samples, including identifying biological significant rare cell types such as circulating tumor cells, antigen-specific T-cells, and invariant natural killer T cells.

Unfortunately, most of the data from scRNA-seq can be uninformative, with as much as 95% attributable to noise, including noise from uninformative reads.[1]

Uninformative reads not only take up sequencing real estate, but they are also not used for downstream analysis. CRISPRclean® Single Cell RNA Boost Kit is a molecular technique that leverages CRISPR to remove these superfluous uninformative reads in scRNA-seq libraries. Through an in silico depletion across single cell data from 14 tissue types, we specifically designed CRISPRclean to remove unwanted reads mapping to ribosomal, mitochondrial, non-variable (housekeeping) genes, and un-annotated genomic intervals. Click here to access the complete list of contents that CRISPRclean Single Cell RNA Boost Kit depletes.

These uninformative reads can obscure low-expressing transcripts, restricting the identification of rare cell types and limiting the number of distinct cell populations identified. Removing these uninformative reads allows redistribution of reads to informative transcriptional reads, increasing the data available for biological interpretation.

The CRISPRclean Workflow

CRISPRclean utilizes specially designed single-guide RNAs to deplete uninformative reads from scRNA-seq libraries in a simple, integrated 3-step process. This process requires less than 2 hours of total assay time and inserts directly into your existing Chromium single cell 3' library construction workflow (Figure 1).

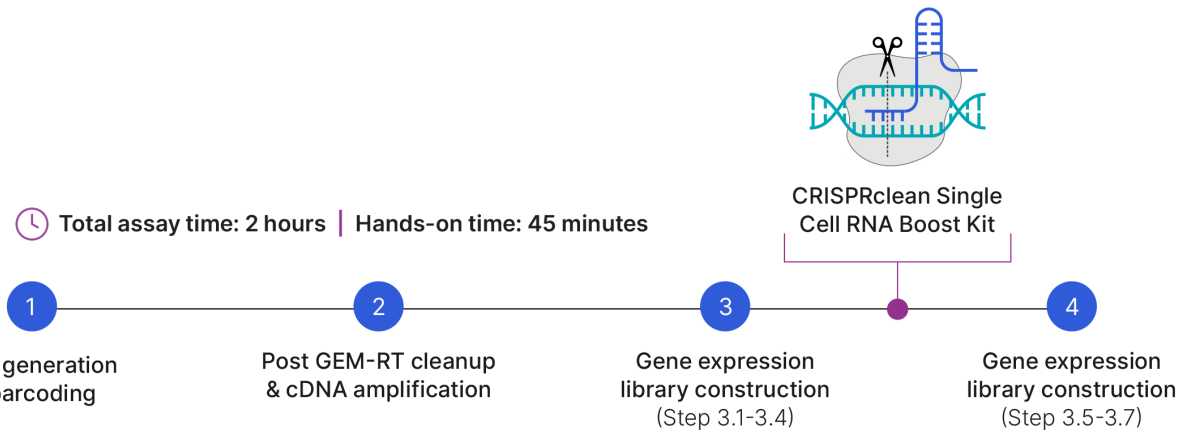

Figure 1: CRISPRclean workflow for single cell RNA-seq libraries.

CRISPRclean in Action

To assess the power of boosting signal-to-noise using CRISPRclean for identifying cell clusters, CRISPRclean depletion was compared with an undepleted control using a PBMC scRNA-seq library. At 25,800 reads per cell, depletion increased the number of unique molecular identifiers (UMIs) by ~1.5-fold compared with control (Figure 2). In total, 280 more genes and 456 more UMIs were recovered per cell.

Cell annotations for PBMCs were performed automatically using predefined legacy markers from published repositories containing greater than 1 million PBMCs.[2] Using this automated tool means no human interpretation is required, allowing unbiased comparison between control and depleted samples.

Depletion resulted in the identification of one additional cell type: CD16+ Monocyte, which has a frequency of 2% (Figure 3). Subclusters for these cell types are present in the control samples, but they do not meet the unique transcriptional signatures and confidence thresholds to be categorized as different cell states.

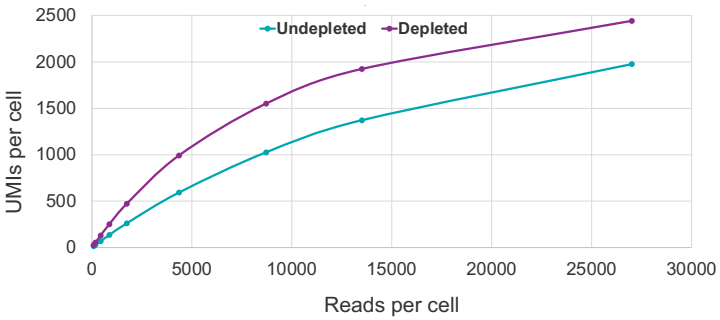

Figure 2: CRISPRclean depletion increases the number of recovered UMIs per cell.

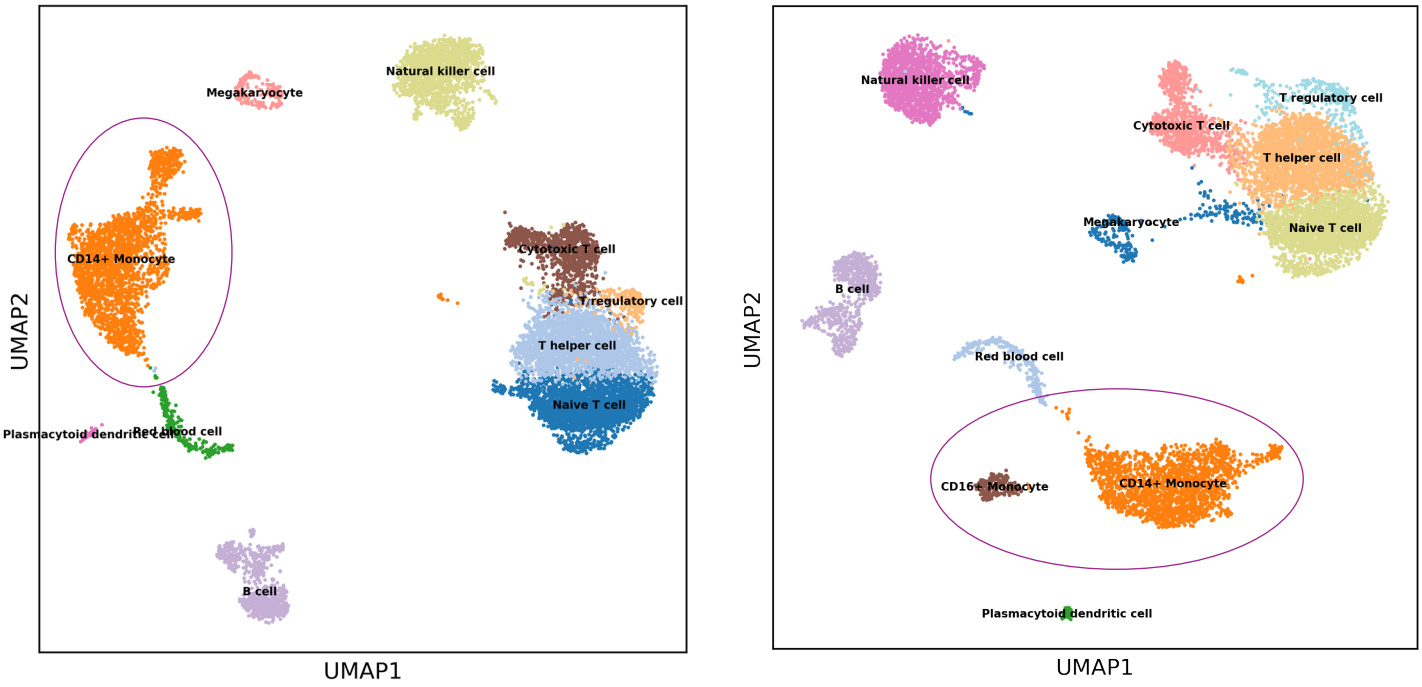

Figure 3: Depletion using CRISPRclean recovers one additional cell type.

In addition to the recovery of an additional cell type, depletion enhances the amount of information associated with how cells communicate with each other. Focusing on receptor-ligand expression at cellular resolution is essential for understanding complex biological processes and is particularly prescient for immune samples. Using CellPhoneDB to perform such analysis illustrates the value of performing depletion, considering there is a 1.5-2.2 fold enhancement in the amount of statistical information retained. Furthermore, it highlights the importance of the role of CD16+ Monocytes on cellular communication, a cell type that could not have been distinguished without depletion. [3]

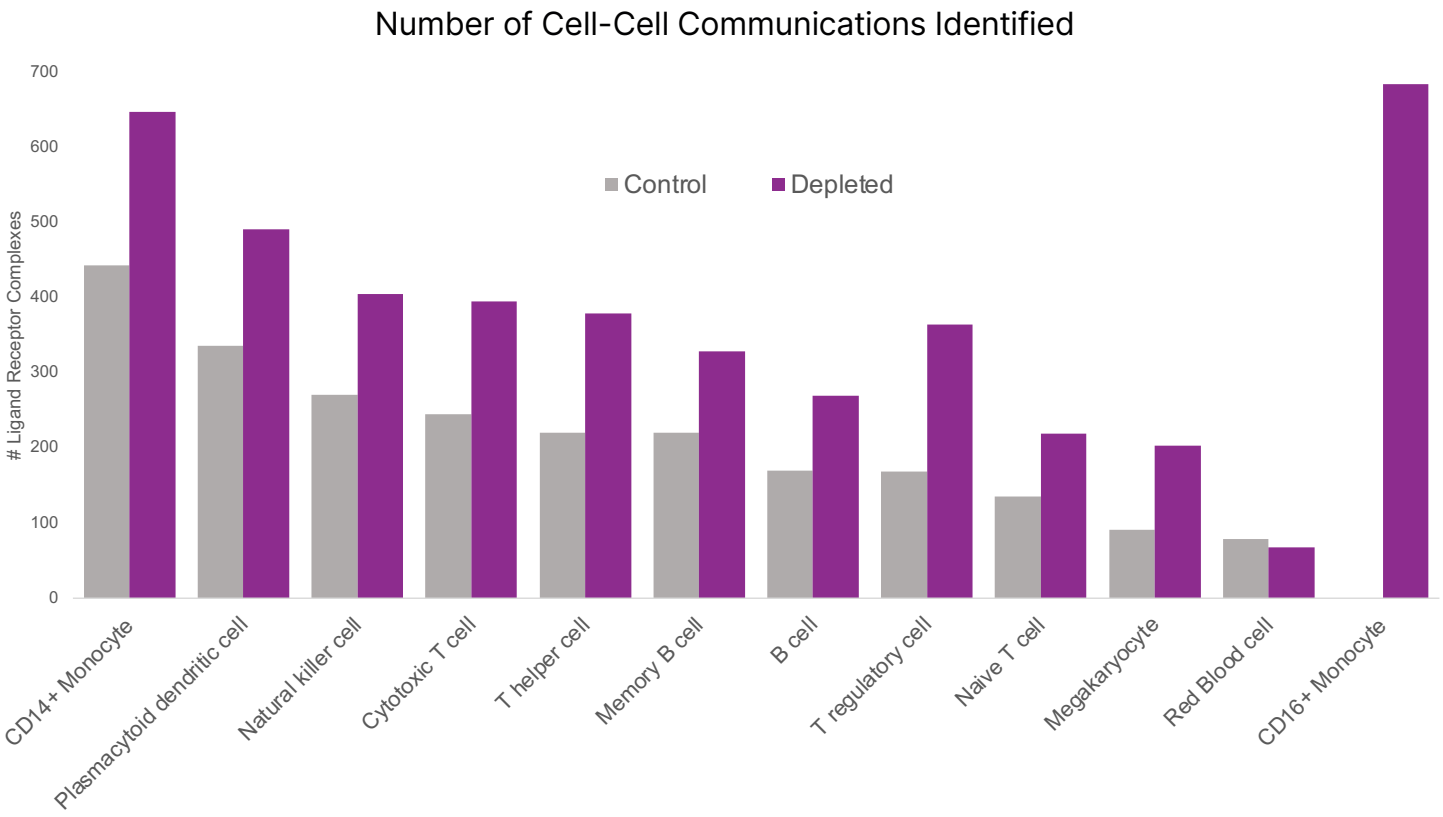

Figure 4: Depletion improves the resolution of cell-to-cell communication by a factor of 1.5-2.2 fold enhancement. The number of ligand receptor complexes found after depletion demonstrates the importance of the role of CD16+ Monocytes on cellular communication, a cell type that could not have been distinguished without depletion.

Conclusion

Depleting uninformative reads using CRISPRclean in PBMC samples boosts usable data by 50% and increases the number of identified cell types in downstream analysis, including rare cell types, providing greater biological insight.

To learn more, visit jumpcodegenomics.com

References

1. Aparicio, L., Bordyuh, M., Blumberg, AJ. et al. A Random Matrix Theory Approach to Denoise Single-Cell Data. Patterns 1(3), 100035 (2020).
2. Li, B., Gould, J., Yang, Y. et al. Cumulus provides cloud-based data analysis for large-scale single-cell and single-nucleus RNA-seq. Nat Methods 17, 793–8 (2020).
3. Efremova, M., Vento-Tormo, M., Teichmann, S.A. et al. CellPhoneDB: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes. Nat Protoc 15, 1484–1506 (2020).