Enabling high-throughput CRISPR screening with the BD Rhapsody™ System

Introduction

Single-cell CRISPR screening enables high-resolution functional genomics by linking thousands of targeted gene perturbations to transcriptomics and phenotypic responses within individual cells. This approach facilitates systemic interrogation of gene regulatory networks and identification of functional elements and elucidates mechanisms underlying drug sensitivity and resistance. The BD Rhapsody™ System provides an integrated workflow for pooled CRISPR screens combined with whole transcriptome or targeted scRNA-seq, enabling cost-efficient and scalable analysis of perturbation effects. Furthermore, incorporation of BD® OMICS-One Protein Panels allows simultaneous quantification of cell surface proteins, delivering a multiomics perspective on gene perturbation responses. For large-scale studies, the BD Rhapsody™ HT Xpress System supports high-throughput single-cell CRISPR screening, ensuring robust and reproducible data generation.

Highlights

- Seamless integration with existing workflows on the BD Rhapsody™ Single-Cell Analysis System
- Flexibility to scale experimental design using BD whole transcriptome analysis or targeted mRNA assays
- Supported by custom targeted panels
- Compatible with common CRISPR vectors with poly-A capture (CROP-seq, Perturb-seq)
- Supports CRISPRko, CRISPRi and CRISPRa
- Multiomics compatible using CITE-seq

Workflow diagram

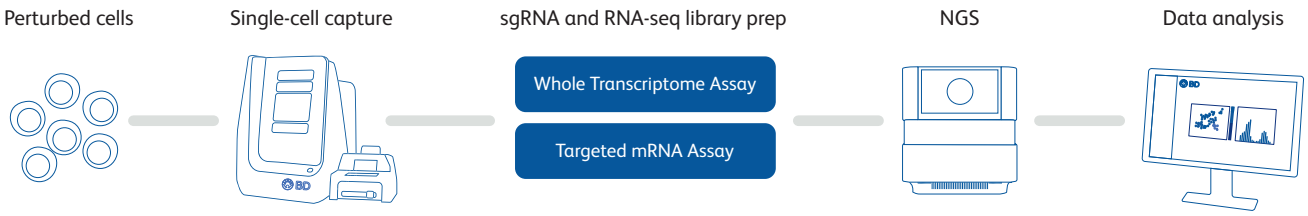


Figure 1. Workflow for single-cell CRISPR screening using the BD Rhapsody™ System. Cells that are subjected to CRISPR perturbations and carry single guide RNAs (sgRNAs) are loaded onto the BD Rhapsody™ HT Xpress System, where individual cells are captured in microwells. Both mRNA and sgRNA molecules are hybridized to barcoded beads via poly(A) capture, enabling single-cell resolution. sgRNAs are selectively amplified in parallel with either the BD whole transcriptome analysis or targeted mRNA assays. Indexed libraries are then prepared for next generation sequencing. Downstream analysis, including sgRNA assignment per cell and transcriptome profiling, can be performed using the BD Rhapsody™ Sequence Analysis Pipeline and BD Cellismo™ Data Visualization Tool.

Seamless integration of library preparation steps

A.

Integration with the Whole Transcriptome Analysis Assay

sgRNA Amplification

After the last step, proceed with a brief bead wash before continuing with WTA library preparation.

B.

Integration with the Targeted mRNA Assay

Targeted Panel with sgRNA Amplification

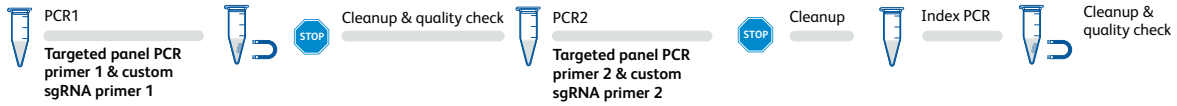


Figure 2. Integration of sgRNA library preparation with the BD whole transcriptome analysis assay (A) and BD targeted mRNA assay (B).

Data analysis

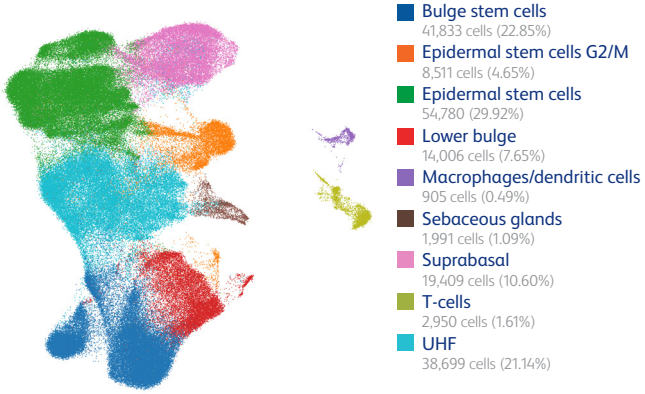
CRISPR screening using the BD Rhapsody™ System allows streamlined data analysis. FASTA files containing identifier names and guide RNA sequences (for CROP-seq) or barcode sequences (for Perturb-seq) can be easily integrated into the BD Rhapsody™ Sequence Analysis Pipeline by adding them to the Supplemental Reference input (for whole transcriptome assays) or the Targeted Reference input (for the targeted mRNA panels). After pipeline execution, UMI counts for each guide per cell are included in the main Cell x Gene count matrix, with the identifier names from the FASTA file appearing as guide 'genes'. Results can also be visualized in the BD Cellismo™ Data Visualization Tool using the .CELLISMO output file generated by the pipeline.

CRISPR screening with the BD whole transcriptome analysis assay

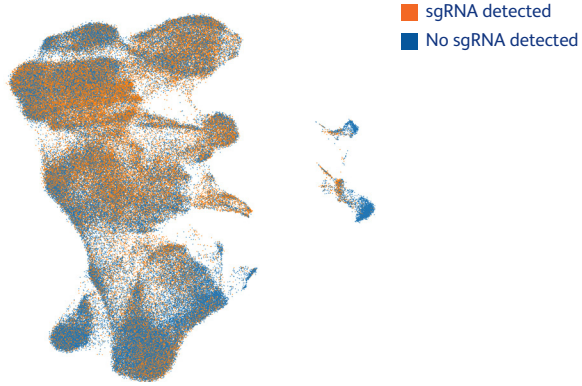
The peer-reviewed publication "In Vivo Single-Cell CRISPR Uncovers Distinct TNF Programmes in Tumour Evolution" (Renz PF, et al. *Nature*, 2024) highlights how CRISPR screening can be used with the BD whole transcriptome analysis assay. In this study, a modified CROP-seq vector (CROPseq-Guide-Puro, Addgene #86708) was employed in an in vivo CRISPR application that uncovered important tumor necrosis factor (TNF) signaling modules. Approximately 150 sgRNAs were used, with 3 sgRNAs per gene. Using an elaborate experimental design, sgRNAs were delivered into Cas9-expressing mouse embryos through lentiviral infection that enabled the identification of at least nine distinct sgRNA-containing cell types. Approximately 500,000 cells were captured in the BD Rhapsody™ System and further analyzed.

We re-analyzed the available published data to showcase some examples of downstream analysis (Figures 3 and 4).

A.



B.



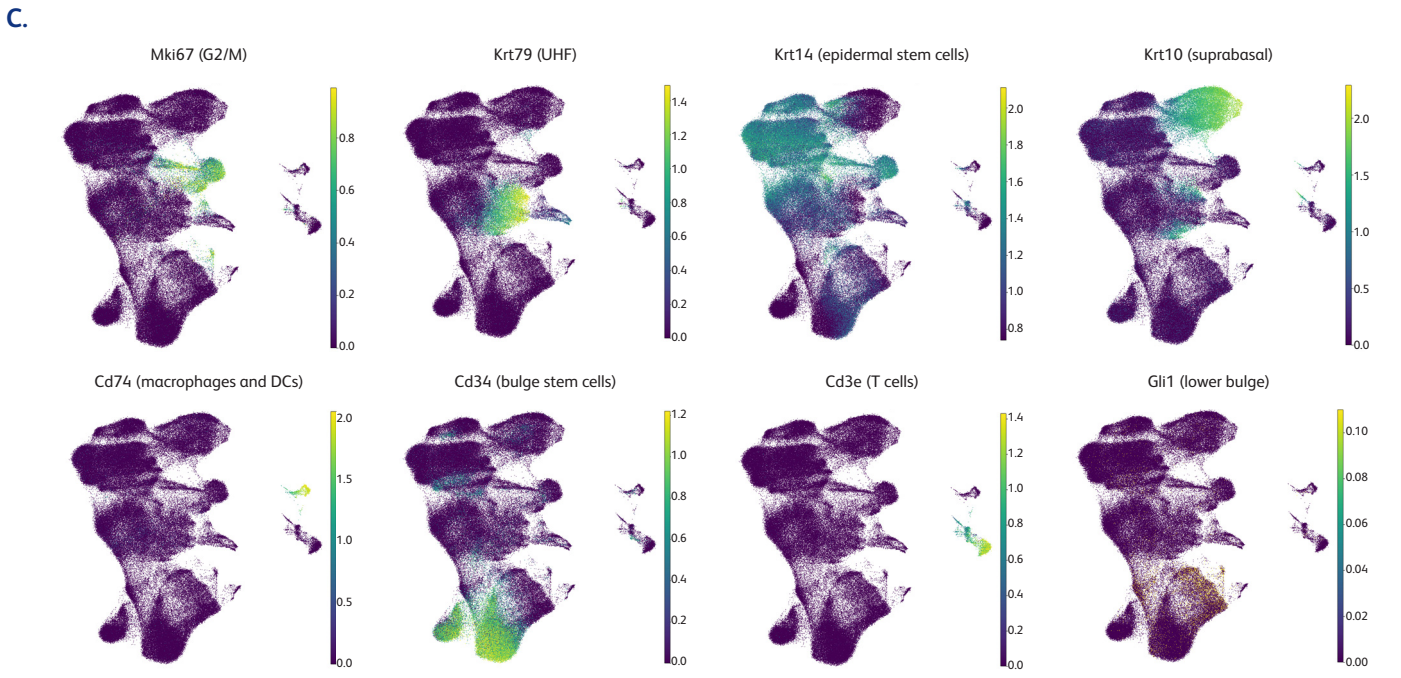


Figure 3. Data generated using the BD Cellismo™ Data Visualization tool showing a UMAP-generated plot with cell type assignments (A) and that sgRNAs are uniformly represented in all major cell populations, suggesting unbiased detection of sgRNAs (B). Cell-type specific gene-expression markers are shown in (C).

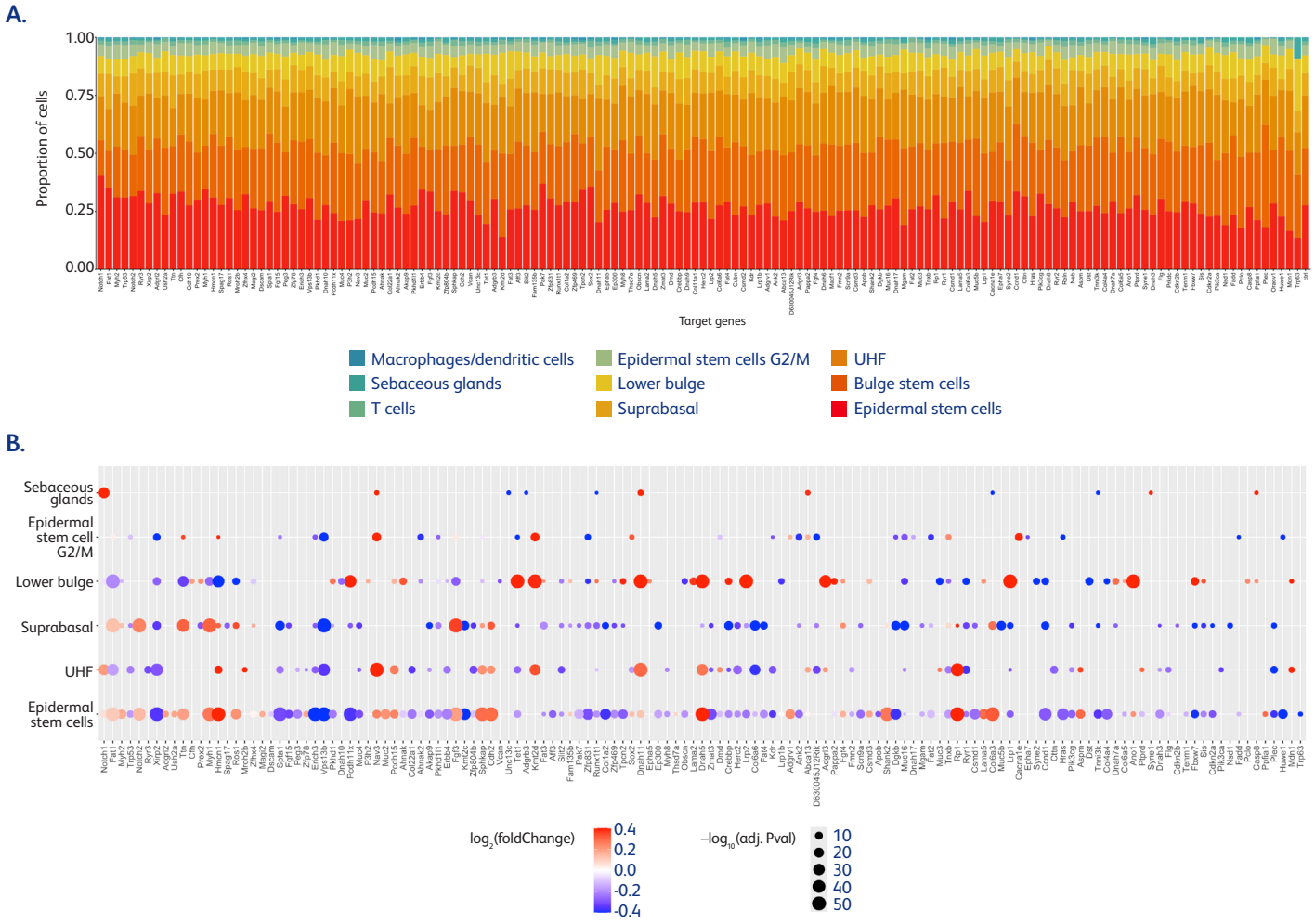


Figure 4. Further analysis with sgRNAs, similarly shown in the publication. (A) Proportion of the 150 sgRNAs found in various cell types shown. **(B)** Effect of perturbatuions on the TNF signaling gene module. In both figures, sgRNA perturbations are shown on the x-axis ranked from the most (left) to least (right) abundant.

CRISPR screening with the BD targeted mRNA assay

An example of a CRISPR activation (CRISPRa) screen using a modified CROP-seq vector (CROPseq-Guide-Puro, Addgene #86708) on the BD Rhapsody™ platform using a targeted mRNA approach is demonstrated in the publication "Sensitive Dissection of a Genomic Regulatory Landscape Using Bulk and Targeted Single-Cell Activation" (Vučićević D, et al. *Cell Genom*, 2025). In this publication, sgRNA was amplified concurrently alongside a custom targeted gene panel to determine CRISPRa-responsive elements. The authors in this study developed TESLA-seq (targeted single-cell activation), which used approximately 1,000 sgRNAs and had an mRNA targeted panel of approximately 150 genes. The authors reported sgRNA detection in 99% of cells with a minimum of two guide RNA molecules/cell.

Conclusion

Single-cell CRISPR screening is a powerful tool with which to deeply characterize thousands of genomic perturbations in millions of cells within a single experiment. The BD Rhapsody™ System provides a seamless end-to-end solution for CRISPR studies with flexible options for multiomic approaches at varying scales of experimental designs that generate high-quality data, while still remaining cost efficient.

Ordering information

Description	Size	Cat. No.
BD OMICS-One™ WTA Next Amplification Kit	8 reactions	572620
BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit	4 reactions	633774
BD Rhapsody™ HT Xpress Package		666625

Contact your local BD field application specialist to design custom and supplemental targeted panels.

References

1 Vučićević D, Hsu CW, Lopez Zepeda LS, et al. Sensitive dissection of a genomic regulatory landscape using bulk and targeted single-cell activation. *Cell Genom.* 2025;5(10):100984. doi:10.1016/j.xgen.2025.100984

2 Renz PF, Ghoshdastider U, Baghai Sain S, et al. In vivo single-cell CRISPR uncovers distinct TNF programmes in tumour evolution. *Nature.* 2024;632(8024):419-428. doi:10.1038/s41586-024-07663-y

3 BD Rhapsody™ System BD OMICS-One™ WTA Next Library Preparation Protocol, 23-24991

4 BD Rhapsody™ System mRNA Targeted Library Preparation Protocol, 23-24121

Additional Resources

Other research using the BD Rhapsody™ System technology for CRISPR screening experiments:

1 Bexte T, Albinger N, Al Ajami A, et al. CRISPR/Cas9 editing of NKG2A improves the efficacy of primary CD33-directed chimeric antigen receptor natural killer cells. *Nat Commun.* 2024;15(1):8439. doi:10.1038/s41467-024-52388-1

2 Borrelli C, Gurtner A, Arnold IC, Moor AE. Stress-free single-cell transcriptomic profiling and functional genomics of murine eosinophils. *Nat Protoc.* 2024;19(6):1679-1709. doi:10.1038/s41596-024-00967-3

3 Ling S, Zhang X, Dai Y, et al. Customizable virus-like particles deliver CRISPR-Cas9 ribonucleoprotein for effective ocular neovascular and Huntington's disease gene therapy. *Nat Nanotechnol*. 2025;20(4):543-553. doi:10.1038/s41565-024-01851-7

BD Life Sciences, Milpitas, CA 95035, U.S.

bdbiosciences.com