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Optical pooled CRISPR screening coupling multiplexed gRNA detection and single-cell spatial multi-omics

Innovative Genomics Institute

BRUKER

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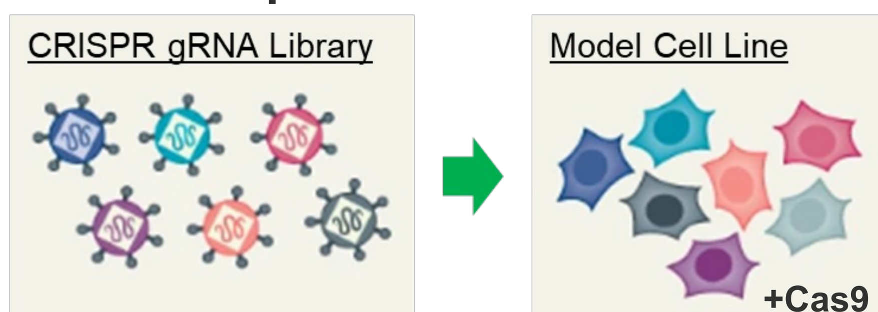
Introduction

CRISPR pooled screening is a powerful approach for studying gene function at scale, but conventional pooled assays and most optical pooled screening (OPS) methods are limited in the phenotypes they can measure. Sequencing-by-synthesis based OPS typically links perturbations to targeted reporter outputs or narrow imaging features, making it difficult to capture the full transcriptional and spatial consequences of genetic perturbation.

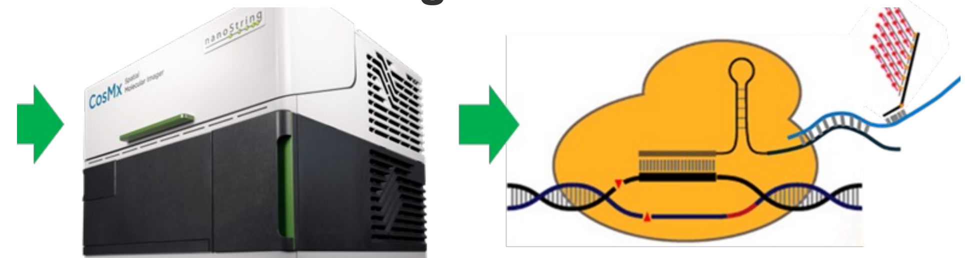
Spatial molecular imaging provides a complementary strategy by enabling direct *in situ* detection of perturbations together with rich molecular phenotypes in intact cells. The CosMx® Spatial Molecular Imager (SMI) is particularly well suited for this application because it supports single-cell, subcellular-resolution whole-transcriptome profiling within native spatial context. A key technical challenge, however, is the design of barcoded gRNA vectors that maintain CRISPR function while allowing sensitive and accurate *in situ* readout with minimal guide-barcode decoupling.

Technology and Methods

Pooled CRISPR perturbation



CosMx SMI for *in situ* guide detection



CosMx SMI for subcellular transcriptome profiling

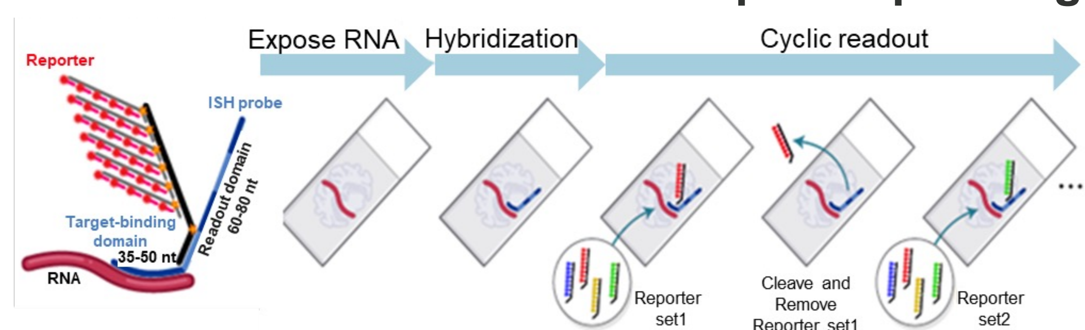


Fig. 1 Schematic of the spatial pooled CRISPR screening with CosMx SMI. The cyclic imaging chemistry enables readout of endogenous RNA and barcoded guide RNAs from the same cell.

A High-throughput Screening System for Barcoding Strategies

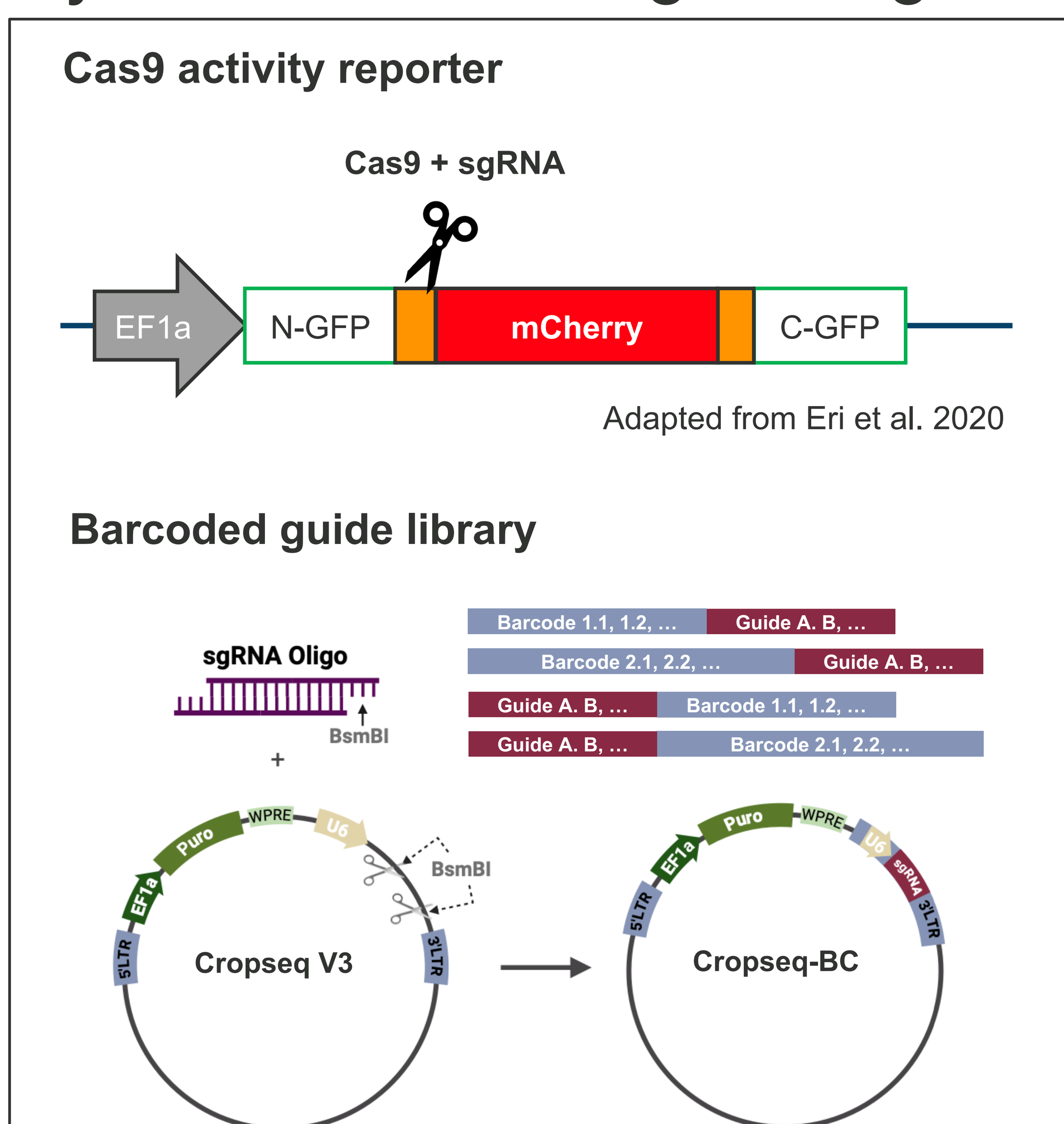
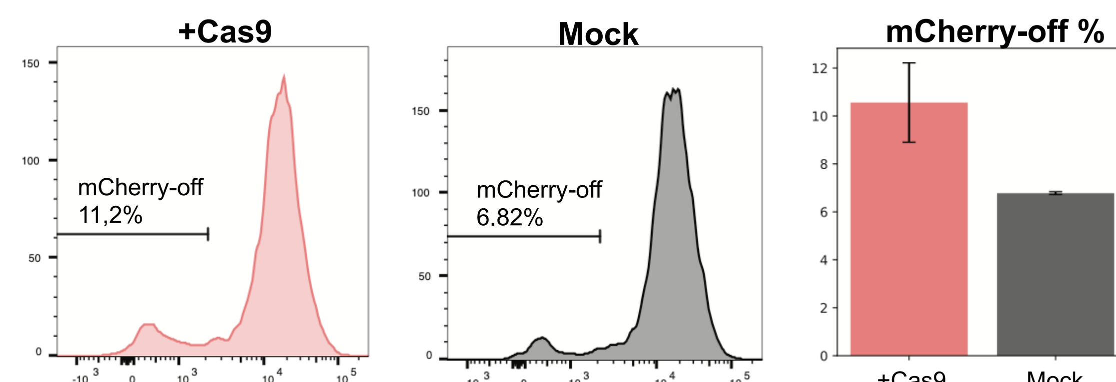


Fig.2 A guide barcoding strategy screening platform based on a mCherry-off, Cas9 activity reporter and a modified Cropseq construct. Thousands of barcoding strategies can be screened in one experiment.



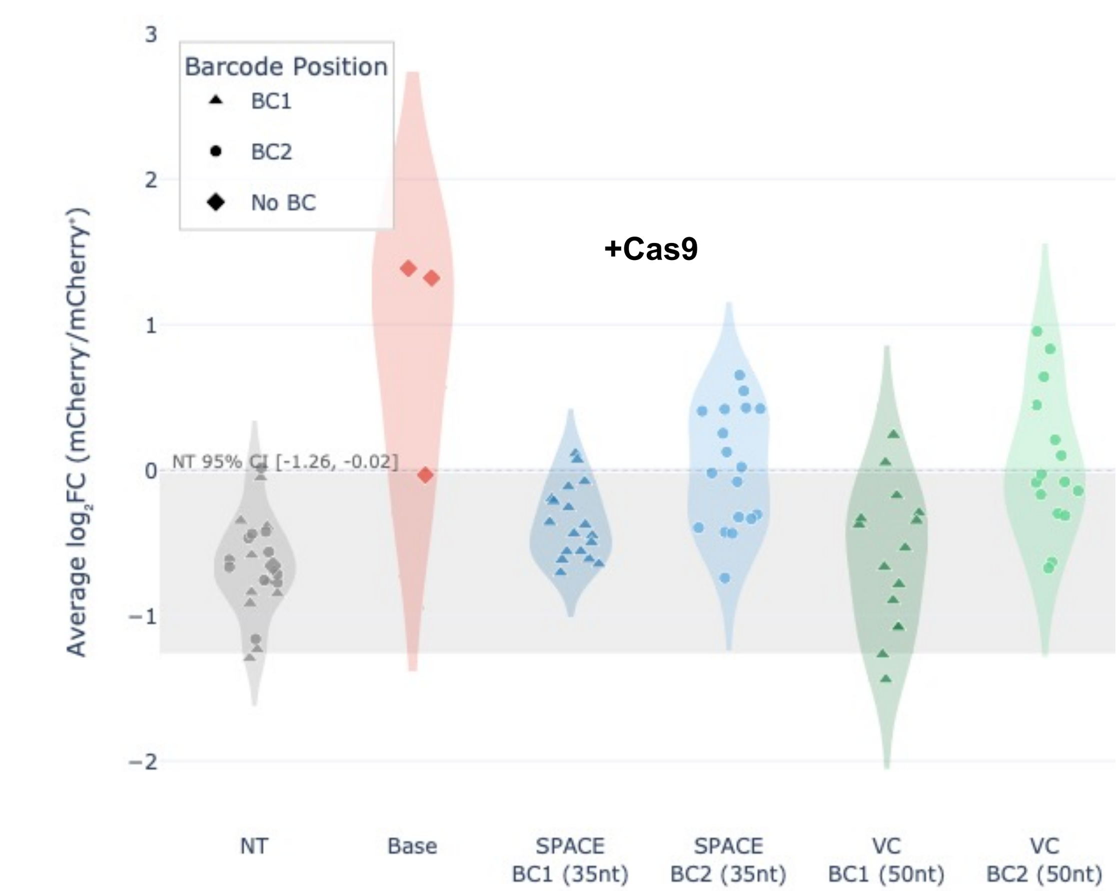


Fig.3 Significant increase of mCherry-off population in library infected-reporter cells upon transient Cas9 delivery. Guide barcoding strategies that preserve Cas9 activity were identified.

Spatial Multiomic Profiling for CRISPR Pool Transduced Cells

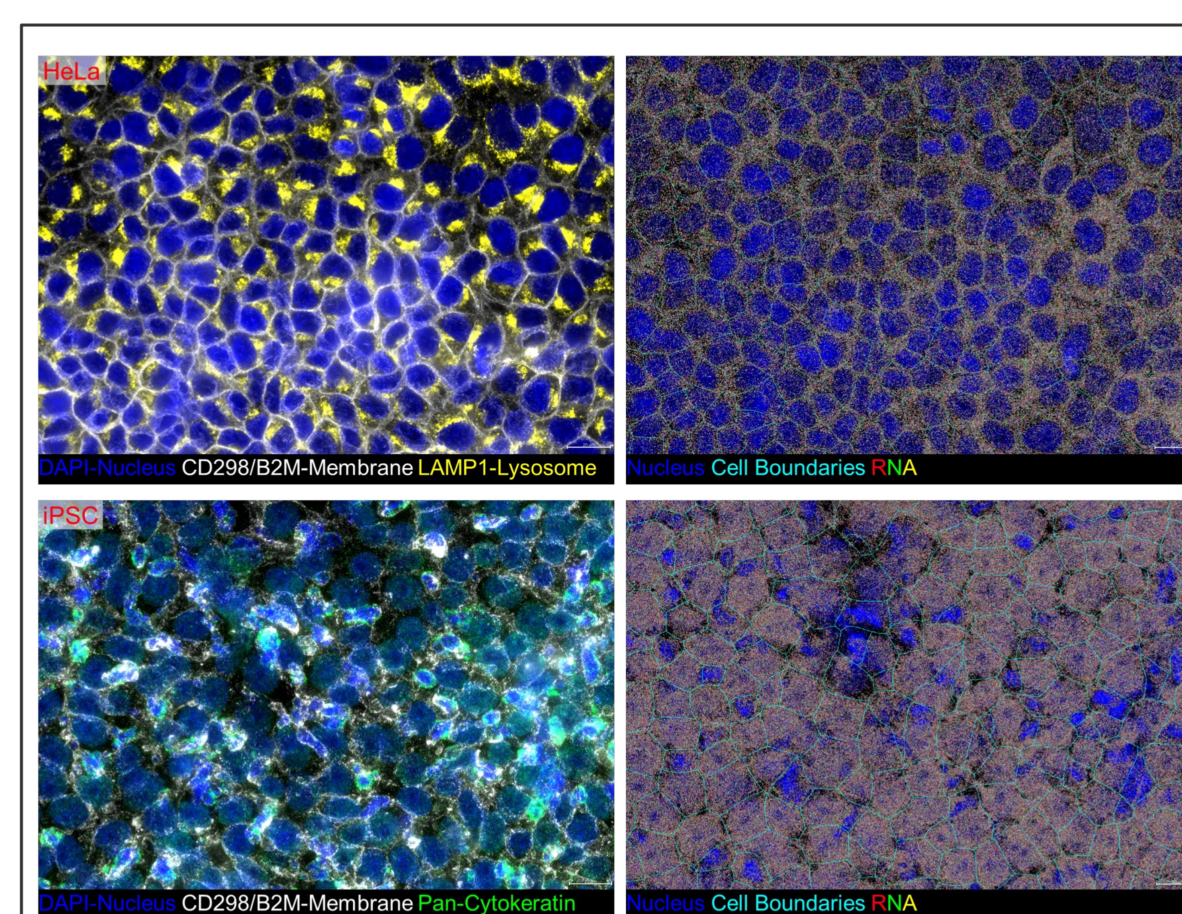


Fig. 4 CosMx SMI provides same-cell multiomic information covering protein, whole-transcriptome RNA, and CRISPR barcodes in HeLa cells and induced pluripotent stem cells (iPSC).

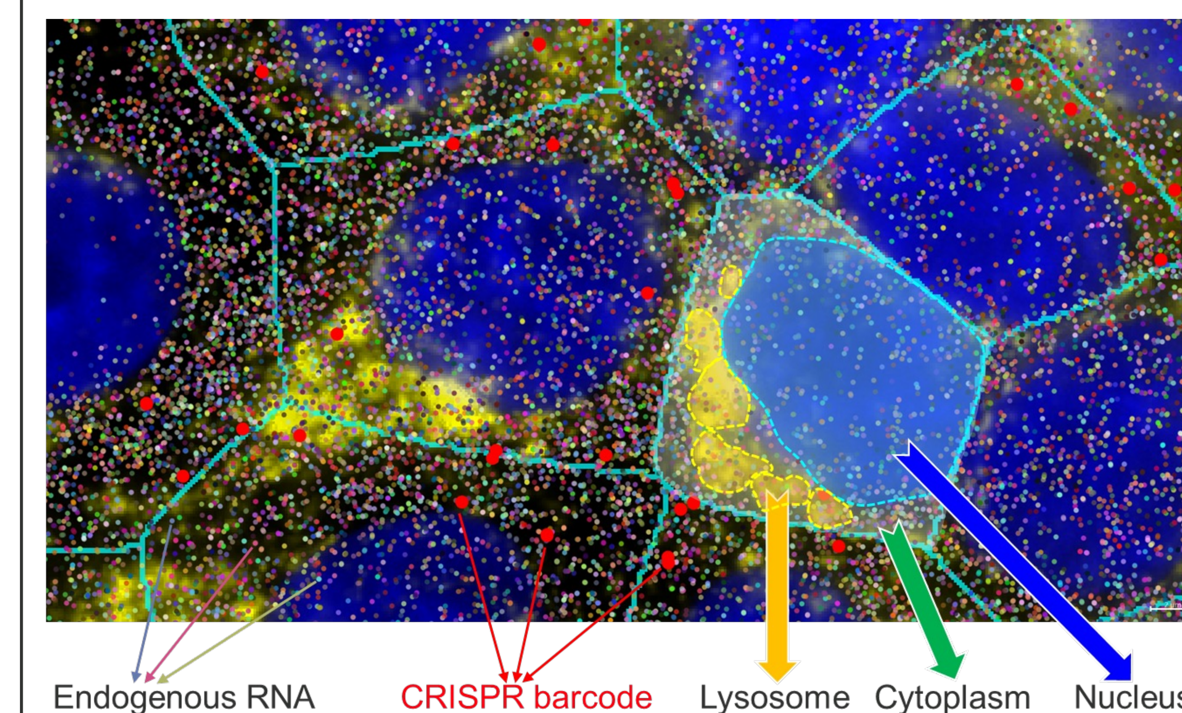


Fig. 5 CosMx SMI enables subcellular localization of endogenous RNAs and CRISPR associated barcodes.

	iPSC	HeLa
Number of Segmented Cells	34610	59466
Mean Cell Area (μm²)	358.1	320.5
Mean Transcripts Per Cell	14199	2953
Median Transcripts Per Cell	14066	2796
90-Percentile Transcripts Per Cell	24131	4557
Mean Unique Genes Per Cell	5400	1586
Median Unique Genes Per Cell	5755	1551
Mean Transcripts Per μm²	39.7	9.2
NegProbe Per Plex Per Cell	0.056	0.021
FalseCode Per Plex Per Cell	0.056	0.006

Fig. 6 (left) Performance metrics table for CosMx detection efficiency in CRISPR barcode transduced iPSC and HeLa cells. (right) Expression profile correlation between CRISPR barcode detected and non-detected cells. This control experiment demonstrated that the introduced barcode alone did not induce significant transcriptomic changes.

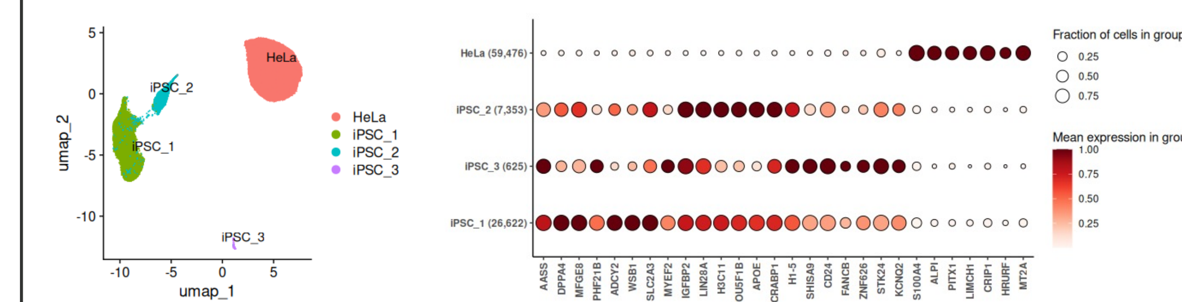


Fig. 7 The CosMx WTX panel enables robust separation of different cell lines and intra-population cell states. (left) UMAP; (right) DEG heatmap.

Spatially Resolved Decoding of CRISPR Perturbation Identities

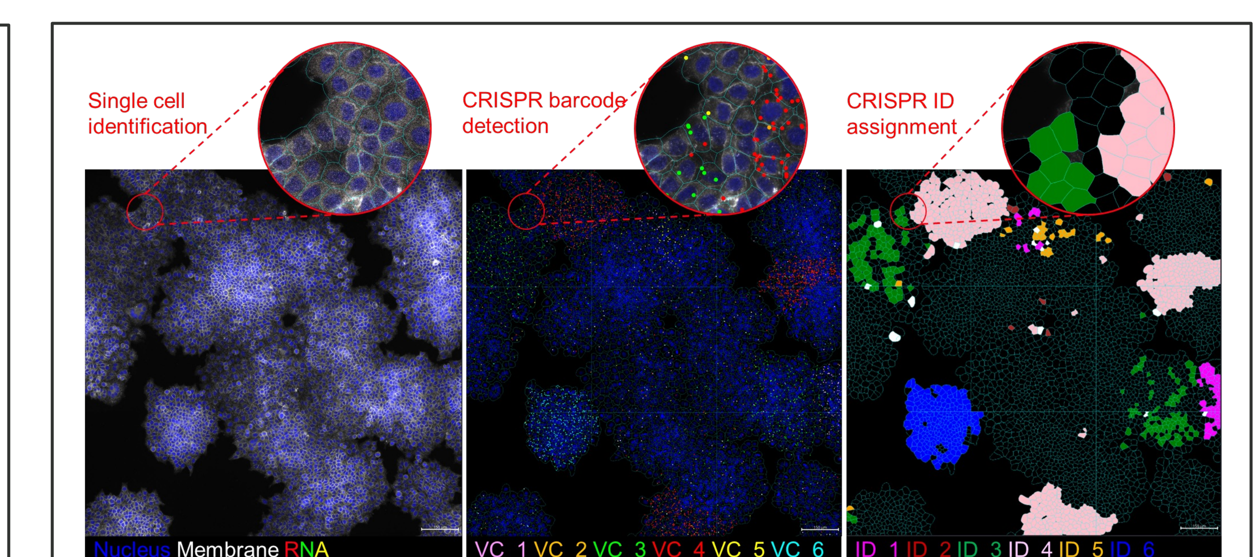


Fig. 8 Workflow of spatial CRISPR perturbation identification. Single cells are identified and segmented with multimodal morphology staining, and then the detected CRISPR barcode molecules are assigned to each cell. The CRISPR ID per cell is determined upon a set of filtering criteria and thresholds.

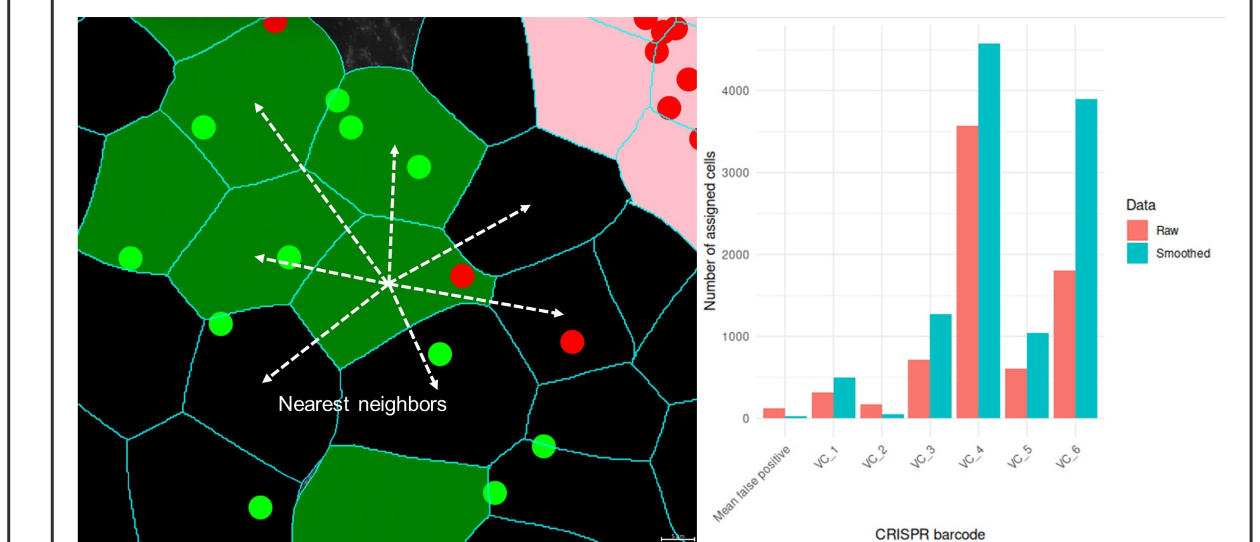


Fig. 9 Owing to the unique gRNA-barcode vector design and the nature of spatial single-cell data matrix, a nearest-neighbor based smoothing was implemented for CRISPR ID calling, for which the cellular CRISPR barcode counts are weight-calculated with the nearby CRISPR barcodes (left). Such processing not only enhances the assigning rate for low-count cells but also suppresses the false positive barcode calls (right).

Conclusions

Here, we establish a practical framework for large-scale pooled CRISPR screening on CosMx SMI. We constructed and validated a unique barcoded gRNA library based on a modified CROP-seq design and identified an optimized vector architecture that supports spatial barcode detection while preserving CRISPR functionality. As a proof of concept, we show that barcoded gRNAs can be robustly detected within the standard CosMx whole-transcriptome assay workflow, enabling pooled perturbation assignment alongside unbiased, spatially resolved molecular profiling. Together, these results provide a foundation for multimodal spatial CRISPR screening in complex biological systems.

References

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