Technical Note

Vector Compatibility with CRISPR Kit

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Document 1097102, Rev A, Jul 2024

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Introduction

The ScaleBio™ CRISPR Guide Enrichment Kit enables the capture of CRISPR guide sequences from CROP-seq style vectors through specific RT primers contained on the CRISPR RT Barcode Plate. An enrichment step after final cleanup of the RNA library uses a primer against the human U6 promoter to amplify the CRISPR guides. This Technical Note details the oligo sequences used in both the Cas9 CRISPR RT Barcode Plate and the CRISPR Enrichment PCR Module and outlines considerations taken to ensure vector compatibility.

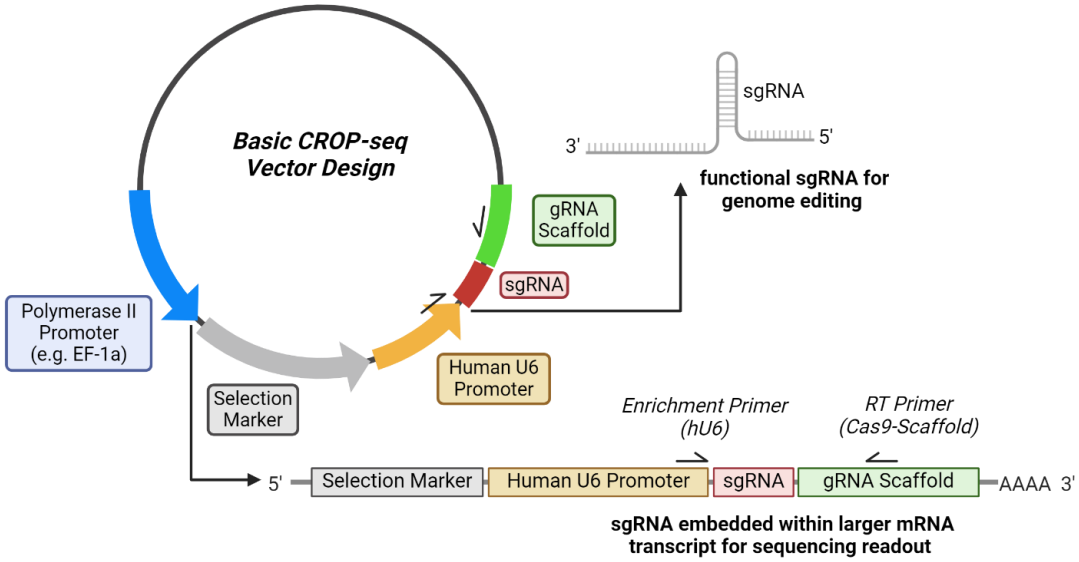
Vector Compatibility

CROP-seq integrates CRISPR/Cas9 genome editing with single cell RNA sequencing to enable high-throughput functional genomics screening at the single cell level (1). This method employs vectors that embed a copy of the CRISPR guide target sequence within a larger mRNA sequence (sgRNA) downstream of a known promoter. This allows for the capture of the guide sequence alongside that of the RNA library. To confirm compatibility of your plasmid with the ScaleBio CRISPR Guide Enrichment Kit please verify your vector contains the following:

1. Guide RNA must be embedded in a longer transcript (see “Example Vector”)
2. Guide RNA scaffold should contain the RT capture primer sequence* (see “CRISPR Kit Oligos – Cas9-specific RT primer”)
3. Guide RNA must be downstream of the human U6 promoter* (see “CRISPR Kit Oligos – CRISPR Enrichment Module”)

*Note that if the human U6 sequence or scaffold primer sequence is not found in the vector/target region, custom primers can be designed to enable capture of other sequences. Please contact support@scale.bio for more information.

Example Vector



Compatible Vector Examples:

Vector	Addgene Link
CROPseq-Guide-Puro	86708
CROPseq-puro-v2	127458
CROP-seq-opti	106280

CRISPR Kit Oligos

Cas9-specific CRISPR RT Primer:

RT Primer structure:
[5' Phosphate Overhang] – [UMI] – [RT Barcode] – [cas9 Scaffold Capture*]

Example sequence for ordering oligos:
/5Phos/CAGAGC NNNNNNNN RT Barcode ACTTTTTCAAGTTGATAACGGACTAGCCTTATTT

*Customization:
Note that the "cas9 Scaffold Capture" sequence can be replaced with any other sequence to prime other alternative Cas enzyme scaffolds, barcodes, or transcript regions. Please reach out to support@scale.bio for more information.

CRISPR Enrichment Module (downstream PCR primer):

U6-specific PCR Primer Sequence* :
P7 – i7 Index – Read2 – CTTGTGGAAAGGACGAAACACC

*Customization:
Note that this primer can be replaced with another sequence if U6 is not found in the plasmid or region of interest. Please reach out to support@scale.bio for more information.

References

1. Datlinger P, Rendeiro AF, Schmidl C, Krausgruber T, Traxler P, Klughammer J, Schuster LC, Kuchler A, Alpar D, Bock C. Pooled CRISPR screening with single-cell transcriptome readout. Nat Methods. 2017 Mar;14(3):297-301.

Document Revision History

Revision	Revision Date	Document ID	Changes
Rev A	Jul 2024	1097102	Initial release.