Technical Note

CRISPR Custom Guide Capture

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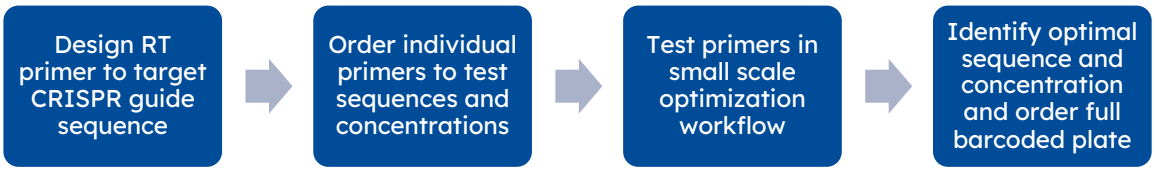
Table of Contents

Introduction

This protocol provides guidelines, suggestions, and protocol adjustments that should be used when designing and testing a custom CRISPR Guide RT primer, which can be spiked into the RT Plate while preparing a CRISPR library using the CRISPR Guide Enrichment Kit [955100]. This protocol should be used in conjunction with the CRISPR Guide Enrichment Protocol [1020765] and references specific steps and tables in these documents.

Figure 1: Recommended Primer Design and Testing Workflow



Primer Design Considerations

Figure 2: General Primer Structure

[5' Phosphate overhang]- [UMI] - [CRISPR RT Barcode] - [Custom Guide Primer Sequence]

For Ordering:
/5Phos/CAGAGC- [NNNNNNNN] - [CRISPR RT Barcode] - [Custom Guide Primer Sequence]

Custom primer design and ordering:

Metric	Recommendation
Design location	Primers 20-60 bases downstream of the target sequence have yielded good recovery with internal testing.
Melting Temperature (Tm)	Primers in the 60-70°C range tested successfully internally.
Purification	Standard desalting
Format	In solution, resuspended in Low TE Buffer (10mM Tris-HCl pH 8.0 and 0.1 mM EDTA solution)

Note: CRISPR RT barcodes that should be appended per well can be found at: https://github.com/ScaleBio/ScaleCRISPR/blob/master/references/guideRt.txt

Conditions to test during small-scale optimization:

The 96 wells of the RT Barcode Plate can be used to test multiple primers, concentrations, etc. in a single experiment. In addition to the location and sequence of the primer (addressed above), other recommended conditions to test can be found below:

Condition	Notes
Primer Concentration*	The recommended starting concentration is 1 µL of 20µM primer added to each RT well. Users may find performance is better titrating this concentration lower (for example, 1 µL of 10µM primer) or, in some occasions, higher. Suggested to test 3-4 concentrations.
Primer Design	See recommendations above. Suggested to test 3-4 primer designs.

*Note that unique oligos will need to be ordered for each RT well in which a sequence/condition is being tested. Careful experimental planning should be performed to ensure that enough cells are recovered per condition to judge primer performance.

Example Experimental Design

Table 1. RT wells to test with number of cells recovered per well

Number of RT wells used to test the condition	Cells recovered per column of the Final Distribution Plate	Cells recovered per Final Distribution Plate
1	100	1,300
2	215	2,600
4	430	5,200
8	860	10,400

An example experimental layout is below (Figure 2), with expected cell yields if two columns of the Final Distribution Plate (~20,000 cells) were taken through sequencing (Table 2).

Note: All wells in the RT plate must be used. Any wells that are not used to test primers may be filled with control cells.

Figure 2: RT Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Primer A Conc 1	Primer C Conc 1										
B	Primer A Conc 1	Primer C Conc 1										
C	Primer A Conc 2	Primer C Conc 2										
D	Primer A Conc 2	Primer C Conc 2										
E	Primer B Conc 1	Primer D Conc 1										
F	Primer B Conc 1	Primer D Conc 1										
G	Primer B Conc 2	Primer D Conc 2										
H	Primer B Conc 2	Primer D Conc 2										

Table 2: Expected Cell Yields

Condition	Oligo(s) ordered	Number of Cells
Primer A, Concentration 1	Primer A, Barcode 1A Primer A, Barcode 1B	430
Primer A, Concentration 2	Primer A, Barcode 1C Primer A, Barcode 1D	430
Primer B, Concentration 1	Primer B, Barcode 1E Primer B, Barcode 1F	430
Primer B, Concentration 2	Primer B, Barcode 1G Primer B, Barcode 1H	430
Primer C, Concentration 1	Primer C, Barcode 2A Primer C, Barcode 2B	430
Primer C, Concentration 2	Primer C, Barcode 2C Primer C, Barcode 2D	430
Primer D, Concentration 1	Primer D, Barcode 2E Primer D, Barcode 2F	430
Primer D, Concentration 2	Primer D, Barcode 2G Primer D, Barcode 2H	430

Changes to Main Protocol

The below modifications are applicable when optimizing a custom RT guide capture primer in the CRISPR Guide Enrichment Kit workflow.

RT Barcode Plate: Initial Distribution and Reverse Transcription

- After Step 1 (thawing of RT Barcode Plate) add 1 µL of each selected primer concentration to the appropriate well of the RT Barcode Plate.

Index PCR Purification and CRISPR Guide Enrichment PCR

- Continue with Appendix A: Purification of a Single Column from Final Distribution Plate, followed by Appendix B: CRISPR Guide Enrichment PCR from a Single Column, and finally, Appendix C: CRISPR Guide Enrichment PCR Purification from a Single Column and Quality Control.

Note: The vector must have the hU6 promotor to be compatible with the CRISR Guide Enrichment Module. Refer to the CRISPR Vector Compatibility Tech Note (1097102).

The following are examples of vectors that would require additional customization and may not be supported by Scale Bio.

- Non-hU6 promotors
- Multiple guides in one vector
- Short or non-polyadenylated guide transcripts

Before beginning optimization, please confirm vector compatibility. Reach out to support@scale.bio with any questions.

Document Revision History

Revision	Revision Date	Document ID	Changes
A	Jan 2025	1219660	Initial release.