Technical Note

RNA v1.1 Custom RT Capture

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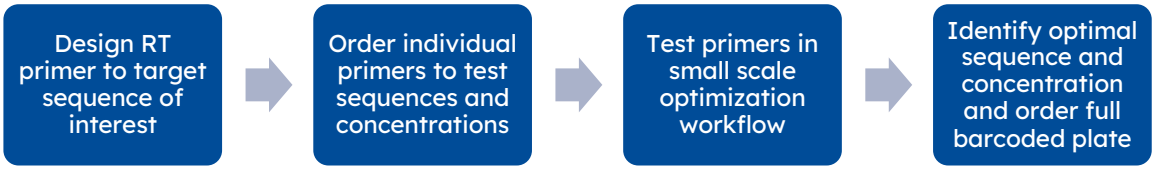
Table of Contents

Introduction

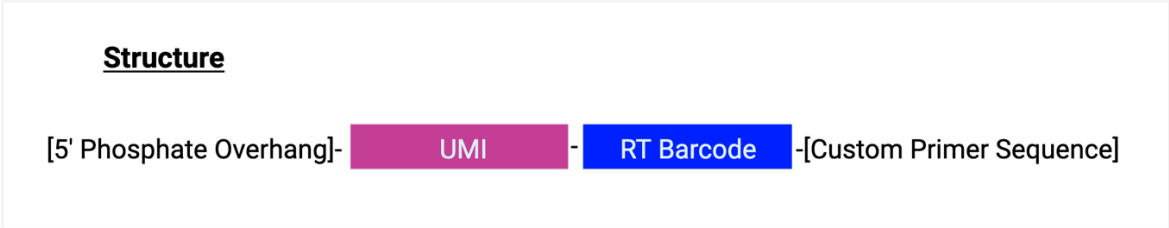
This protocol provides guidelines, suggestions, and protocol adjustments that should be used when designing and testing a custom reverse transcription (RT) primer, which can be spiked into the ScaleBio Single Cell RNA Sequencing Kit v1.1 (950884) workflow. This can be helpful to amplify signal from targets that are of particular interest, are not poly-adenylated, or are too far from the 3' end of the mRNA to be captured by traditional 3' single-cell RNA seq library preparation. This protocol should be used in conjunction with the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol (1020796) and references specific steps and tables in these documents.

Figure 1: Recommended Primer Design and Testing Workflow



Primer Design Considerations

Figure 2: RT Primer Structure



Ordering:
/5Phos/CAGAGC- [NNNNNNNN] - [RT Barcode] - [Custom 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: RT barcodes that should be appended per well can be found at: https://github.com/ScaleBio/ScaleRna/blob/master/references/3lvlRNA_rt.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 3), 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 3: 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 primer in the Single Cell RNA Sequencing 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

- Continue with Appendix A: Purification of a Single Column from Final Distribution Plate for the optimization study.

Reach out to support@scale.bio with any questions.

Document Revision History

Revision	Revision Date	Document ID	Changes
A	Jan 2025	1219666	Initial release.