CRISPR Guide Enrichment Extended Throughput Kit v1.1

Protocol

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Legal Notices

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Required Materials

ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1 (PN 945058) Contents:

Kit Module	Consumable	Part Number	Qty	Cap Color	Storage Temp
RNA Extended Throughput Tagment and i5 Index PCR Module (PN 936061)	Second Strand Buffer Conc.	202110009	1	Purple	-20°C
	Second Strand Enzyme Mix	202110010	1	Purple	-20°C
	Cleanup Enzyme v1.1	935999	1	Brown	-20°C
	Tagment Buffer Conc.	202100003	1	Red	-20°C
	Tagment Enzyme Mix	202100004	1	Red	-20°C
	Index PCR Enzyme Mix	202110012	2	Orange	-20°C
	Adaptor Primer i5 Barcode Plate	936013	1	-	-20°C
	Elution Buffer	202110014	1	Clear	-20°C
RNA Extended Throughput Workflow Consumables Module (PN 935985)	Tagment Stop Solution	202110017	1	Black	RT
	Index PCR Additive	202110016	1	Orange	RT
	Final Distribution Plate	202110018	1	-	RT
RNA Extended Throughput Adaptor Primer Module (PN 935986)	Adaptor Primer i7-2 Tube	936002	1	Orange	-20°C
	Adaptor Primer i7-3 Tube	936003	1	Orange	-20°C
	Adaptor Primer i7-4 Tube	936362	1	Orange	-20°C
RNA Extended Throughput CRISPR Guide Enrichment Module (PN 945060)	CRISPR PCR Enzyme Mix	202110021	3	Orange	-20°C
	CRISPR Amp Forward Primer	202110022	3	Blue	-20°C
	CRISPR Amp Reverse Primer 2	202110024	1	Blue	-20°C
	CRISPR Amp Reverse Primer 3	202110025	1	Blue	-20°C
	CRISPR Amp Reverse Primer 4	202110026	1	Blue	-20°C
	Elution Buffer	202110014	6	Clear	-20°C

Module	Part Number	Quantity
RNA Extended Throughput Tagment and i5 Index PCR Module	936061	3
RNA Extended Throughput Workflow Consumables Module	935985	3
RNA Extended Throughput Adaptor Primer Module	935986	1
RNA Extended Throughput CRISPR Guide Enrichment Module	945060	1

Required Cell Numbers

The 96-well Final Distribution Plate of the ScaleBio CRISPR Guide Enrichment Kit v1.1 is loaded with **1,600 cells per well** for a **total of 154,000 cells**. The unutilized remaining pooled cell suspension can be stored at -80°C until ready to process through the CRISPR Guide Enrichment Extended Throughput Kit v1.1 Protocol.

As the number of leftover cells may vary depending on the sample type processed, Table 1 helps to determine the amount of additional Final Distribution Plates that can be filled to capture the remaining pooled cells.

Note: The number of cells required to load the additional Final Distribution Plates are the absolute minimum cells that are needed. Users should incorporate an excess of cells for better ease of handling when planning their CRISPR Guide Enrichment Extended Throughput v1.1 experiment.

Table 1: Cell Number Requirements

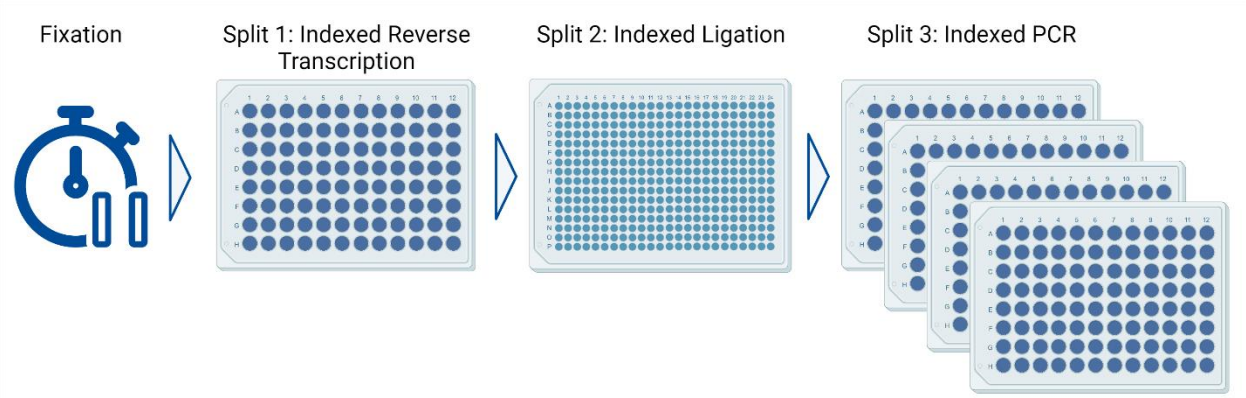
	CRISPR Guide Enrichment Kit v1.1	Additional Final Distribution Plates from the CRISPR Guide Enrichment Extended Throughput Kit v1.1		
		1	2	3
Minimum Total Cells Required to Load Final Distribution Plate(s)	154,000	308,000	462,000	616,000
Estimated Cells Recovered for Sequencing	125,000	250,000	375,000	500,000

Assay Introduction

The ScaleBio™ CRISPR Guide Enrichment Kit v1.1 allows the recovery of ~125,000 cells, which can be increased with the add-on protocol from the ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1.

After the addition of the first indexed barcode by reverse transcription, the cells are pooled and subsequently split onto the 384-well Ligation Barcode Plate where the second indexed barcode is added by ligation. The cells are then pooled again and counted to normalize the cell input to 1,600 cells per well going into the third split on the 96-well Final Distribution Plate (Figure 1).

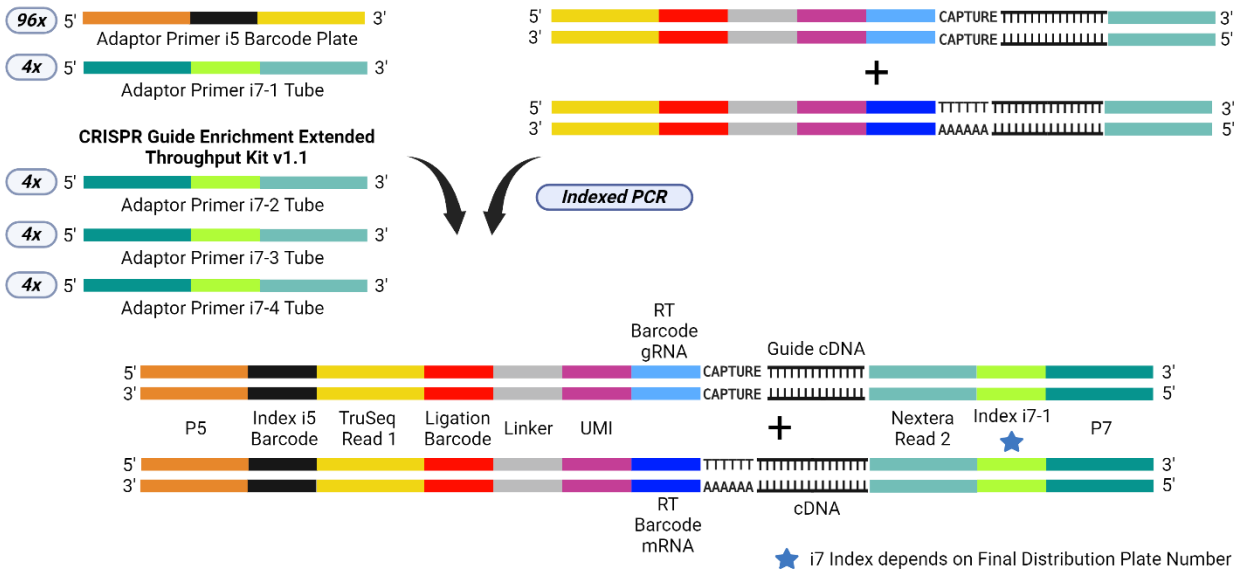
Figure 1: Procedure overview



This normalization step creates a leftover of pooled cells which may vary depending on the sample type processed.

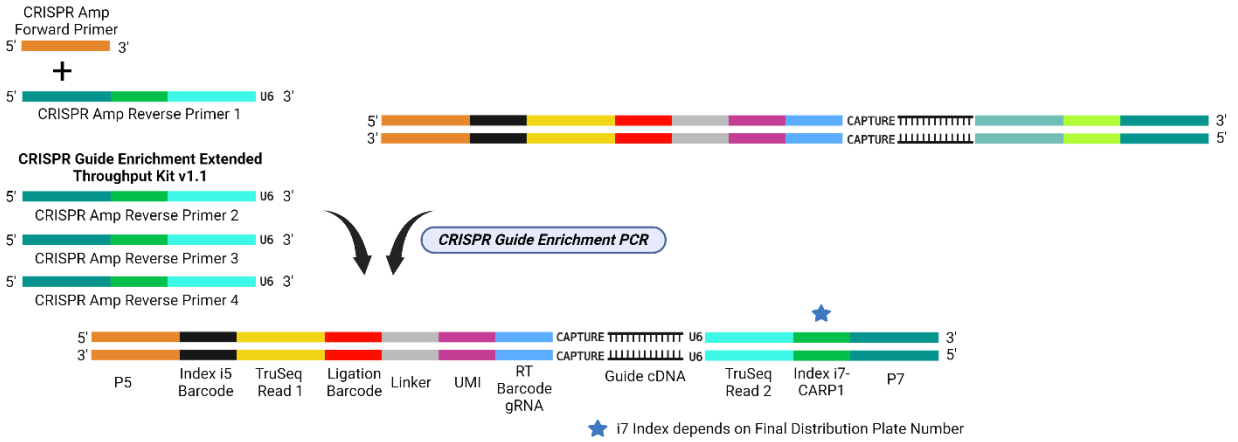
With the ScaleBio CRISPR Guide Enrichment Extended Throughput v1.1 Kit, these leftover pooled cells may be used to fill additional Final Distribution Plates on Split 3 to increase the cell recovery of the assay overall. This is achieved by providing additional unique i7 indices that are added to the libraries during the Indexed PCR step (Figure 2).

Figure 2: Primer Combinations during Indexed PCR for Extended Throughput



Furthermore, during the CRISPR Guide Enrichment PCR reaction, the CRISPR Libraries are enriched with a new set of unique i7 indices to differentiate them from the RNA Libraries (Figure 3).

Figure 3: Primer Combinations during CRISPR Guide Enrichment PCR for Extended Throughput



For correct downstream sample demultiplexing during data analysis, it is important to note which Adaptor Primer i7 is used with which CRISPR Amp Reverse Primer for each Final Distribution Plate that is being processed.

The throughput of the recovered cells can be increased incrementally up to ~500,000 cells by purchasing the CRISPR Guide Enrichment Extended Throughput Kit v1.1. Please review chapter *Required Cell Numbers* for considerations on the input as well as output cell number requirements for the add-on protocol.

The CRISPR Guide Enrichment Extended Throughput Kit v1.1 Protocol provides instructions on processing the additional Extended Throughput Kit. This protocol should be used in conjunction with the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol as the initial steps of reverse transcription and ligation remain the same.

Please contact support@scale.bio or your local Field Application Scientist for assistance with this workflow.

Step 1: Initial Distribution and Reverse Transcription

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- **At Step 4**: Load **10,000 cells per well** on the initial Cas9 CRISPR RT Barcode Plate (**5 µL** of cell suspension with a concentration of **2000 cells per µL**) to ensure high cell numbers after ligation for loading Extended Throughput Kits.

Step 2: Ligation

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.

Step 3: Final Distribution

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	CRISPR Guide Enrichment Extended Throughput Kit v1.1			
RNA Extended Throughput Workflow Consumables Module	Final Distribution Plate	1	2	3

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- Use the contents of the ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1 modules listed above.
- **At Step 3**: repeat the process from step 3 onwards to load the required amount of additional Final Distribution Plates with the leftover cell suspension. The cell suspension is already diluted in Wash Buffer at a final concentration of **400 cells per µL**.

Step 4: Second Strand Synthesis and Cleanup Enzyme Digestion

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	CRISPR Guide Enrichment Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput Tagment and i5 Index PCR Module	Second Strand Buffer Conc.	1	2	3
	Second Strand Enzyme Mix	1	2	3
	Cleanup Enzyme v1.1	1	2	3

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- Use the contents of the ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1 modules listed above.

Step 5: Tagmentation and Index PCR

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	CRISPR Guide Enrichment Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput Workflow Consumables Module	Tagment Stop Solution	1	2	3
	Index PCR Additive	1	2	3
RNA Extended Throughput Tagment and i5 Index PCR Module	Tagment Buffer Conc.	1	2	3
	Tagment Enzyme Mix	1	2	3
	Index PCR Enzyme Mix	2	4	6
	Adaptor Primer i5 Barcode Plate	1	2	3
RNA Extended Throughput Adaptor Primer Module	Adaptor Primer **i7-X** Tube	AP-i7-2	AP-i7-3	AP-i7-4
		936002	936003	936362

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- Use the contents of the ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1 modules listed above.
- **At Step 18**: prepare one Index PCR Master Mix for each additional Final Distribution Plate that is being processed by the Extended Throughput Kit. Substitute *Adaptor Primer i7-X* in Table 2 with one of the following reagent tubes:
    - Adaptor Primer i7-2
    - Adaptor Primer i7-3
    - Adaptor Primer i7-4

Table 2: Index PCR Master Mix

Reagent	Volume (µL)
Index PCR Additive	240
Adaptor Primer i7-X	48
Index PCR Enzyme Mix	2400
Total Volume	**2688**

Caution: Write down each Adaptor Primer i7 used for each Final Distribution Plate.

- **At Step 22**: use one Adaptor Primer i5 Barcode Plate for each additional Final Distribution Plate that is being processed by the Extended Throughput Kit. The plate contains 96 unique barcodes that are identical on each Adaptor Primer i5 Barcode Plate.

Step 6: Index PCR Purification

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	CRISPR Guide Enrichment Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput Tagment and i5 Index PCR Module	Elution Buffer	1	2	3

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- Use the contents of the ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1 modules listed above.

Step 8: CRISPR Guide Enrichment PCR

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	CRISPR Guide Enrichment Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput CRISPR Guide Enrichment Module	CRISPR PCR Enzyme Mix	1	2	3
	CRISPR Amp Forward Primer	1	2	3
	Elution Buffer	1	2	3
	CRISPR Amp Reverse Primer X	CARP2	CARP3	CARP4
		202110024	202110025	202110026

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- Use the contents of the ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1 modules listed above.
- **At Step 2**: prepare one CRISPR Enrichment PCR Master Mix for each additional Final Distribution Plate that is being processed by the Extended Throughput Kit. Substitute *CRISPR Amp Reverse Primer X* in Table 3 with one of the following reagent tubes, depending on the Adaptor Primer i7 used in *Step 5: Tagmentation and Index PCR*:
    - CRISPR Amp Reverse Primer 2 used with AP-i7-2
    - CRISPR Amp Reverse Primer 3 used with AP-i7-3
    - CRISPR Amp Reverse Primer 4 used with AP-i7-4

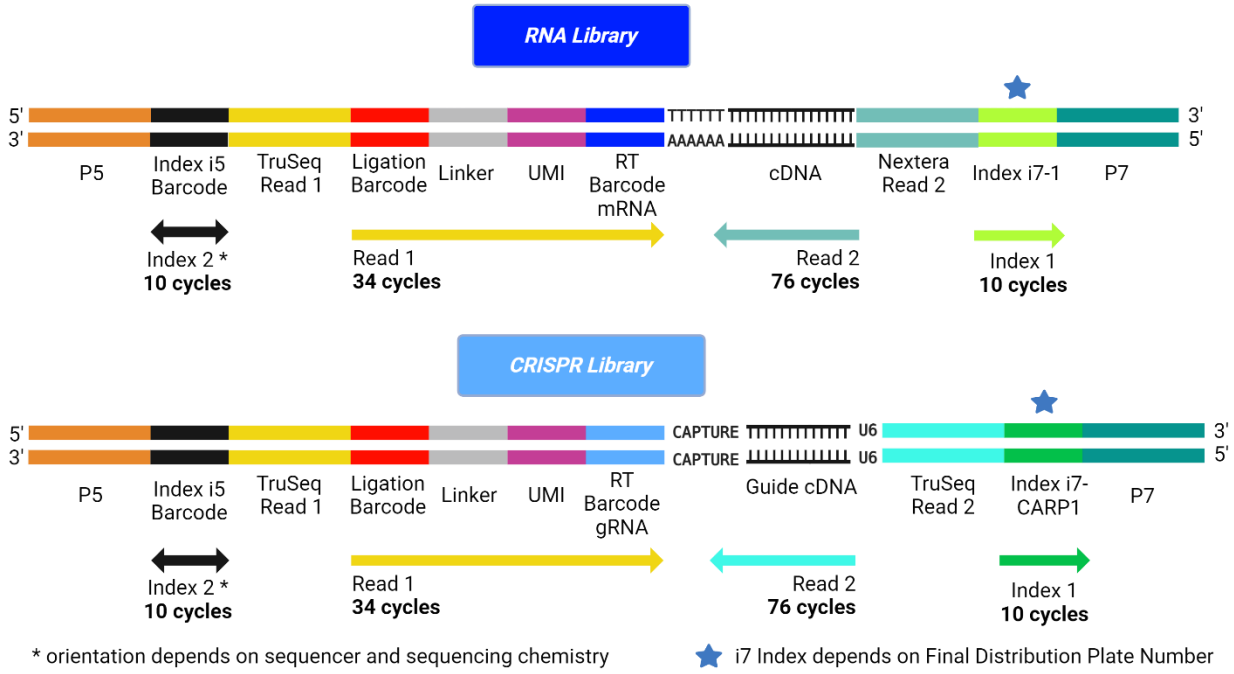
Table 3: CRISPR Enrichment PCR Master Mix

Reagent	Volume (µL)
Nuclease-free water	90
CRISPR Amp Forward Primer	15
CRISPR Amp Reverse Primer X	15
CRISPR PCR Enzyme Mix	150
Total volume	**270**

Caution: Write down each CRISPR Amp Reverse Primer used for each Final Distribution Plate.

Step 11: Sequencing Parameters

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- The combination of i7 Indices used for the RNA and CRISPR Libraries depends on the number of processed Final Distribution Plates utilized in the Extended Throughput Protocol (Table 4).



Caution: To ensure correct sample demultiplexing, libraries processed by multiple ScaleBio CRISPR Guide Enrichment Extended Throughput Kits require both the i5 and the i7 indices to be sequenced.

Table 4: Index i7 Combinations for the CRISPR Guide Enrichment Extended Throughput Kit v1.1

Final Distribution	RNA Library		CRISPR Library	
	Reagent	PN	Reagent	PN
Plate 2	Adaptor Primer i7-2	936002	CRISPR Amp Reverse Primer 2	202110024
Plate 3	Adaptor Primer i7-3	936003	CRISPR Amp Reverse Primer 3	202110025
Plate 4	Adaptor Primer i7-4	936362	CRISPR Amp Reverse Primer 4	202110026

Appendix B: CRISPR Guide Enrichment PCR from a Single Column

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	CRISPR Guide Enrichment Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput CRISPR Guide Enrichment Module	CRISPR PCR Enzyme Mix	1	2	3
	CRISPR Amp Forward Primer	1	2	3
	Elution Buffer	1	2	3
	CRISPR Amp Reverse Primer X	CARP2	CARP3	CARP4
		202110024	202110025	202110026

- Follow the instructions as described in the ScaleBio CRISPR Guide Enrichment Kit v1.1 Protocol.
- Use the contents of the ScaleBio CRISPR Guide Enrichment Extended Throughput Kit v1.1 modules listed above.
- **At Step 2**: prepare one CRISPR Enrichment PCR Master Mix for each additional Final Distribution Plate that is being processed by the Extended Throughput Kit. Substitute *CRISPR Amp Reverse Primer X* in Table 3 with one of the following reagent tubes, depending on the Adaptor Primer i7 used in *Step 5: Tagmentation and Index PCR*:
  - CRISPR Amp Reverse Primer 2 used with AP-i7-2
  - CRISPR Amp Reverse Primer 3 used with AP-i7-3
  - CRISPR Amp Reverse Primer 4 used with AP-i7-4

Table 5: CRISPR Enrichment PCR Master Mix for a Single Column

Reagent	Volume (µL)
Nuclease-free water	7.5
CRISPR Amp Forward Primer	1.25
CRISPR Amp Reverse Primer X	1.25
CRISPR PCR Enzyme Mix	12.5
Total Volume	**22.5**

Caution: Write down each CRISPR Amp Reverse Primer used for each Final Distribution Plate.

Document Revision History

Revision	Revision Date	Document ID	Changes
Rev A	Dec 2023	1020770	Initial release.
Rev B	Jul 2024	1020770	Update consumables storage temperature.