DEMONSTRATED PROTOCOL

SPRIselect Library Concentration for Targeted Gene Expression

Overview

The Targeted Gene Expression product is designed to enrich whole transcriptome analysis (WTA) libraries for relevant genes. Target enrichment is performed with gene-specific, biotinylated baits that hybridize to their complement DNA strand in the WTA library. Prior to library hybridization, the library is typically concentrated using a vacuum centrifuge (refer to Additional Guidance).

This protocol outlines an alternative method for concentrating libraries using SPRIselect. This protocol was demonstrated with Chromium Single Cell 3' and 5' Gene Expression libraries prepared from Peripheral Blood Mononuclear Cells, HOdgkins Lymphoma, and Mucose-associated Lymphoid tissue as well as Visium Spatial Gene Expression libraries prepared from human breast carcinoma, colorectal cancer, and spinal cord. The Targeted Gene Expression protocol was performed using the 10x Genomics Human Pan-Cancer Panel. Additional optimization may be required when working with libraries prepared from other cell or tissue types and when using custom panels.

Additional Guidance

Consult the Targeted Gene Expression - Single Cell (CG000293) or Spatial (CG000377) User Guide for the complete Targeted Gene Expression workflow.

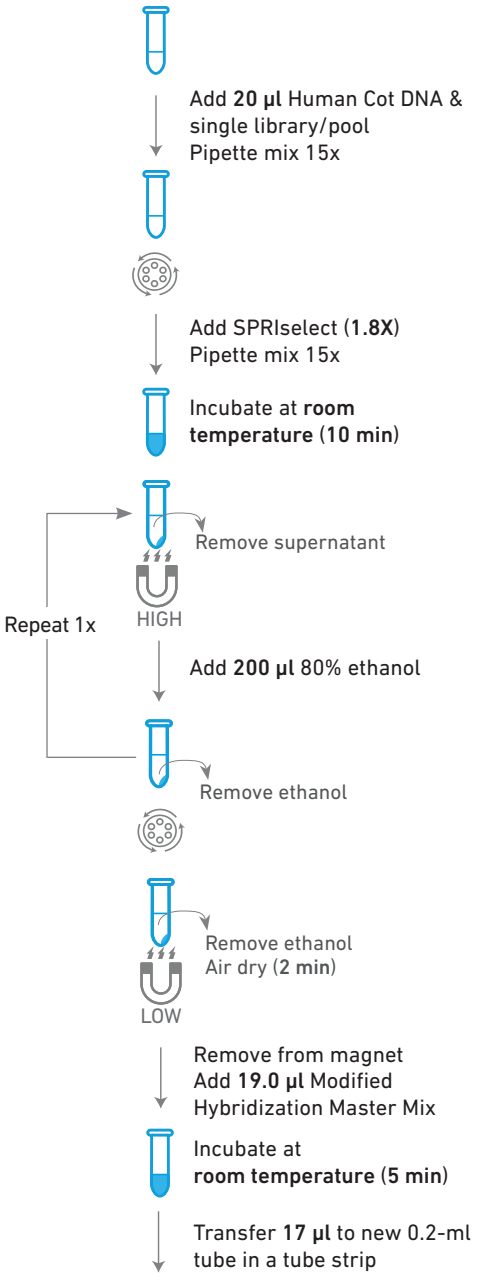
Specific Reagents & Consumables

Vendor	Item	Part Number
10x Genomics	Human Cot DNA*	3000478
	Hyb Buffer*	3000479
	Hyb Enhancer*	3000480
	Universal Blockers*	2000290
	Pre-designed Panel†	-
	10x Magnetic Separator	230003
Beckman Coulter	SPRIselect	B23318
Additional Buffers/Reagents		
80% Ethanol (prepare fresh)		

*Part of the 10x Genomics Target Hybridization Kit (PN-1000248)

† Select one pre-designed panel: Human Gene Signature Panel (PN-2000285/2000322), Human Immunology Panel (PN-2000286/2000323), Human Pan-Cancer Panel (PN-2000287/2000324), or Human Neuroscience Panel (PN-1000278/1000277)

Protocol Overview



Proceed to step 2.1e of Targeted Gene Expression - Single Cell or Spatial User Guide

SPRIselect Library Concentration Protocol

Equilibrate Gene Expression libraries and Human Cot DNA to **room temperature** and centrifuge briefly. For pooling information, consult the Targeted Gene Expression - Single Cell (CG000293) or Spatial (CG000377) User Guide and the Targeted Gene Expression Pooling Worksheet (CG000296).

1. Library Pooling

a. Add **20 µl** Human Cot DNA to one 0.2-ml tube in a tube strip for each sample being processed.

DO NOT add Universal Blockers

b. Add single library/pool to each tube containing Human Cot DNA. Pipette mix 15x and centrifuge briefly.

2. SPRIselect Cleanup

Before preparing Hybridization Master Mix, thaw Hyb Buffer for **10 min** at maximum speed in a thermomixer set to **65°C**. Verify no precipitate. Cool to room temperature. Thaw remaining reagents at **room temperature**, centrifuge briefly.

a. Prepare Modified Hybridization Master Mix. Pipette mix and centrifuge briefly. Maintain at **room temperature**.

Table 1. Modified Hybridization Master Mix

Modified Hybridization Master Mix Add reagents in order listed	1X (µl)	4X + 10% (µl)	8X + 10% (µl)
Universal Blockers	2.0	8.8	17.6
Hyb Buffer	9.5	41.8	83.6
Hyb Enhancer	3.0	13.2	26.4
Pre-designed Panel	4.5	19.8	39.6
Total	**19**	**83.6**	**167.2**

b. Vortex to resuspend SPRIselect reagent. Based on the volume of the sample, add SPRIselect (**1.8X**) to each sample and pipette mix 15x. For example, add **90 µl** of SPRIselect to **40 µl** sample.

c. Incubate for **10 min** at **room temperature**.

d. Place on a 10x Magnetic Separator•**High** position (magnet•**High**) until the solution clears.

e. Remove the supernatant.

f. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

g. Remove the ethanol.

h. Repeat steps f and g for a total of 2 washes.

i. Centrifuge briefly and place on the magnet•**Low.**

j. Remove any remaining ethanol. Air dry for **2 min**. DO NOT exceed **2 min** as this will decrease elution efficiency.

k. Remove from the magnet and add **19 µl** Modified Hybridization Master Mix.

l. Incubate **5 min** at **room temperature**.

m. Place on the magnet•**Low** until the solution clears.

n. Transfer **17 µl** into a new 0.2-ml tube.

o. Proceed **immediately** to step 2.1e (Library Hybridization) of the Targeted Gene Expression -Single Cell or Spatial User Guide.

Results

To demonstrate the efficiency of this protocol, two Chromium Single Cell 3' Gene Expression libraries, two Chromium Single Cell 5' Gene Expression libraries, and three Visium Spatial Gene Expression libraries were concentrated using both methods. Targeting and complexity metrics for these two methods are similar, but with up to a 30% reduced targeted library yield for single cell and spatial libraries prepared from long cDNA. For Visium Spatial Gene Expression libraries prepared from short cDNA, this reduction was up to 45%. All samples, regardless of concentration method, had a final library yield of 10 nM and 300 nM. The fraction of reads mapping to the targeted transcriptome across all sample types was not altered in the SPRIselect method (Figure 1). The UMI recovery of targeted genes between the vacuum centrifuge method and SPRIselect method was highly correlated (R^2=0.998) and similar in magnitude (Figure 2).

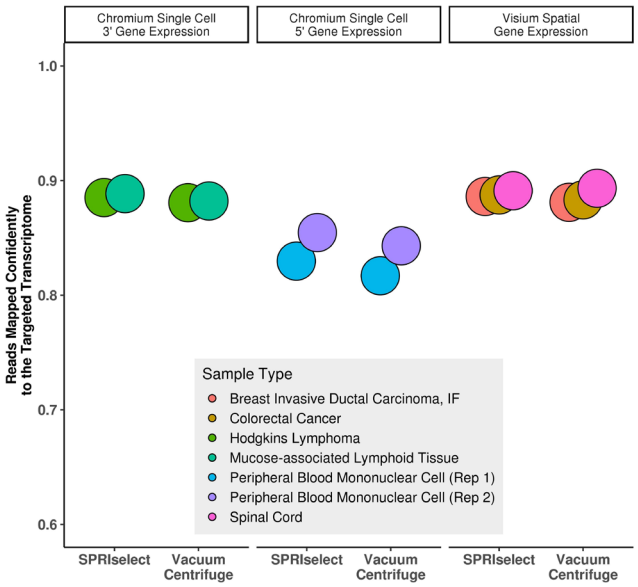


Figure 1. Reads mapped confidently to the targeted transcriptome. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

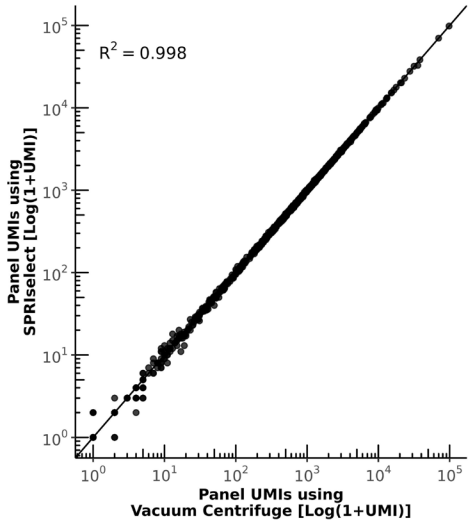


Figure 2. UMI recovery. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

References

- Targeted Gene Expression - Single Cell User Guide (CG000293)
- Targeted Gene Expression - Spatial User Guide (CG000377)
- Targeted Gene Expression Library Pooling Worksheet (CG000296)

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