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 and Visium Spatial Gene Expression libraries prepared from a variety of cell and tissue types. The Targeted Gene Expression protocol was performed using 10x Genomics pre-designed panels, as well as add-on and fully custom panels purchased as NGS Discovery Pools from IDT.

Additional Guidance

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

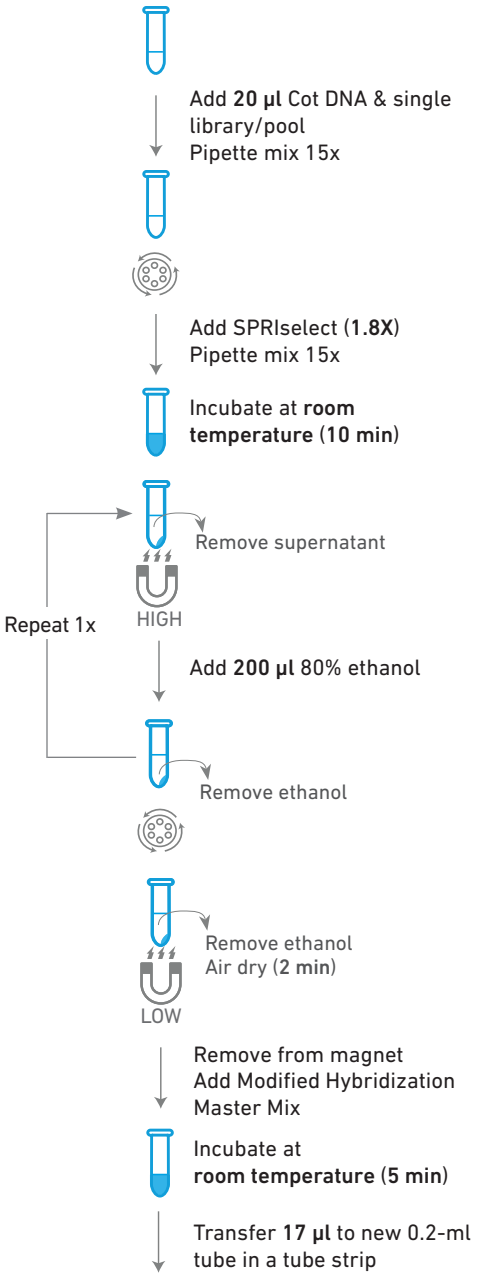
Specific Reagents & Consumables

Vendor	Item	Part Number
10x Genomics	Cot DNA* Formerly Human Cot DNA	3000478
	Hyb Buffer*	3000479
	Hyb Enhancer*	3000480
	Universal Blockers*	2000290
	Pre-designed Panel†	-
	10x Magnetic Separator	230003
Beckman Coulter	SPRIselect	B23318
IDT	NGS Discovery Pool	-
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 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** 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 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 according to Table 1 or 2 depending on panel type. Pipette mix and centrifuge briefly. Maintain at **room temperature**.

Table 1. Modified Hybridization Master Mix for Pre-designed or Fully Custom Panels.

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 or Fully Custom Panel Working Dilution*	4.5	19.8	39.6
Total	**19**	**83.6**	**167.2**

*Consult User Guide for Fully Custom Panel dilutions.

Table 2. Modified Hybridization Master Mix for Add-on panels.

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
Add-on Panel Working Dilution*	2.2	9.7	19.4
Pre-designed Panel	4.5	19.8	39.6
Total	**21.2**	**93.3**	**186.6**

*Consult User Guide for Add-on Panel dilutions.

b. Vortex to resuspend SPRIselect reagent. Based on the total volume of the sample (Cot DNA + libraries), add SPRIselect (**1.8X**) to each sample and pipette mix 15x. For example, add **90 µl** of SPRIselect to **50 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 for Pre-designed/Fully Custom Panels, or **21.2 µl** Modified Hybridization Master Mix for Add-on panels.

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, Chromium Single Cell 3' and 5' Gene Expression libraries and 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 30-50% reduced targeted library yield. All samples, regardless of concentration method, had a final library yield between 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).

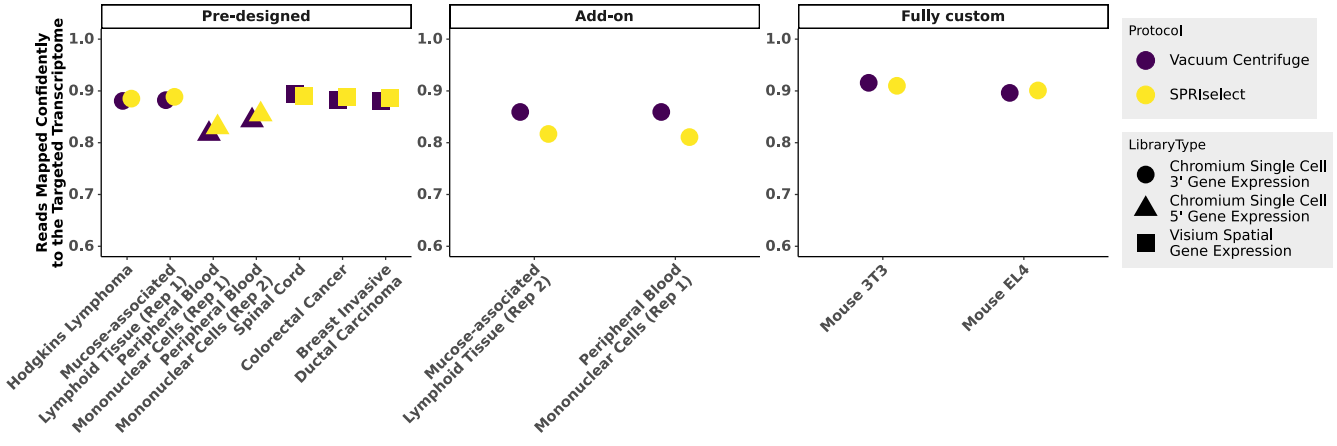


Figure 1. Reads mapped confidently to the targeted transcriptome. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

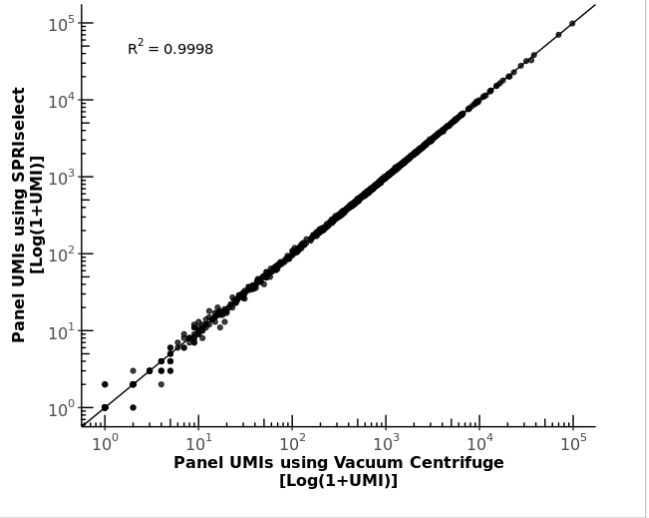


Figure 2. UMI recovery. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

References

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

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA