DEMONSTRATED PROTOCOL

SPRIselect Library Concentration for Targeted Gene Expression

Overview

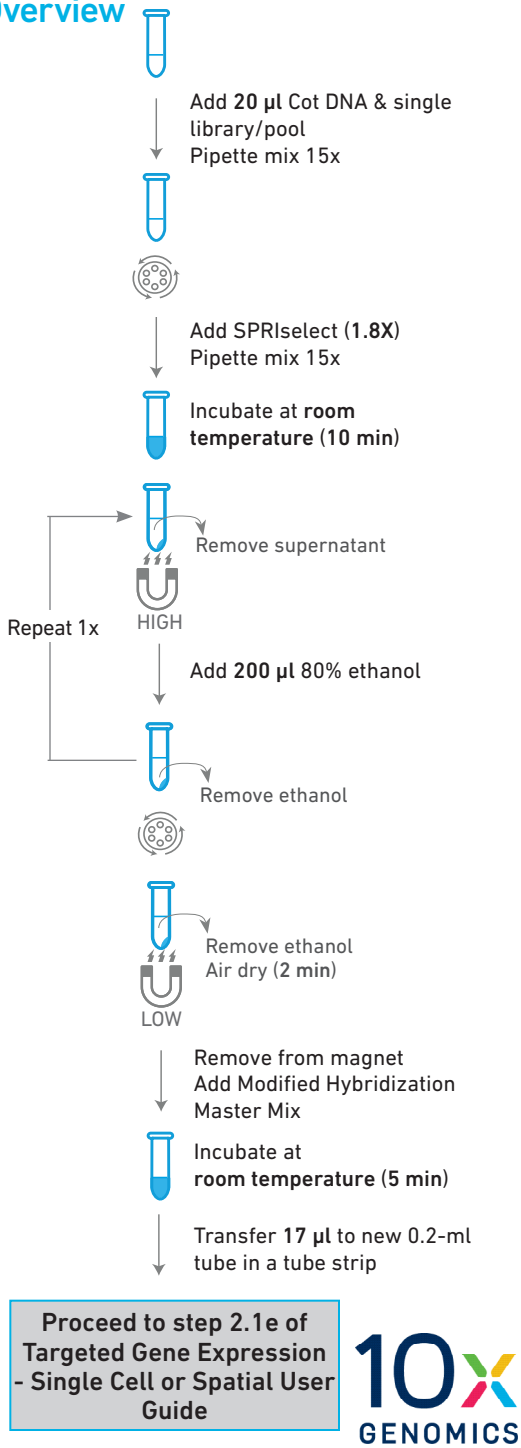
The Targeted Gene Expression product is designed to enrich whole transcriptome analysis (WTA) libraries for relevant genes. Target enrichment is performed with gene-specific, biotinylated baits that hybridize to their complement DNA strand in the WTA library. Prior to library hybridization, the library 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 xGen Custom Hyb Panels, xGen Lockdown Pools, or NGS Discovery Pools from IDT, and fully custom panels generated from Twist Oligo Pools.

Additional Guidance

Consult the Targeted Gene Expression - Single Cell (CG000293) or Spatial (CG000377) User Guide for the complete Targeted Gene Expression workflow. Consult the Double Stranded Biotinylated Bait Sets from Twist Oligo Pools (CG000412) Demonstrated Protocol for generating fully custom panels from Twist Oligo Pools.

Protocol Overview



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
Thermo Fisher Scientific	Nuclease-free Water	AM9937
IDT	NGS Discovery Pool	-
	Amplified Biotinylated Twist Oligo Pools *Generated by following Demonstrated Protocol CG000412*	-

*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)

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. The same amount of Cot DNA is used regardless of the number of libraries pooled per sample.

DO NOT add Universal Blockers

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

c. If using Amplified Biotinylated Twist Oligo Pools, add 300 ng baits to each tube containing libraries and Cot DNA. *DO NOT add baits if using Pre-Designed Panels of xGen Custom Hyb Panels, xGen Lockdown Pools, or NGS Discovery Pools from IDT. These panel types are added to the Modified Hybridization Master Mix in step 2.a.*

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, 2 , or 3 depending on panel type and vendor. Pipette mix and centrifuge briefly. Maintain at **room temperature**.

Table 1. Modified Hybridization Master Mix for Pre-designed Panels or Fully Custom Panels (xGen Custom Hyb Panels, xGen Lockdown Pools, or NGS Discovery Pools from IDT).

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 (xGen Custom Hyb Panels, xGen Lockdown Pools, or NGS Discovery Pools from IDT).

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.

Table 3. Modified Hybridization Master Mix for Fully Custom Panels (Amplified Biotinylated Twist Oligo Pools).

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
Nuclease-free Water*	4.5	19.8	39.6
Total	**19**	**83.6**	**167.2**

*Amplified Biotinylated Twist Oligo Pools are added to libraries prior to SPRIselect concentration, due to volume limitations in the Modified Hybridization Master Mix.

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.
*If using Amplified Biotinylated Twist Oligo Pools, include the volume of the baits in the sample volume.

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).

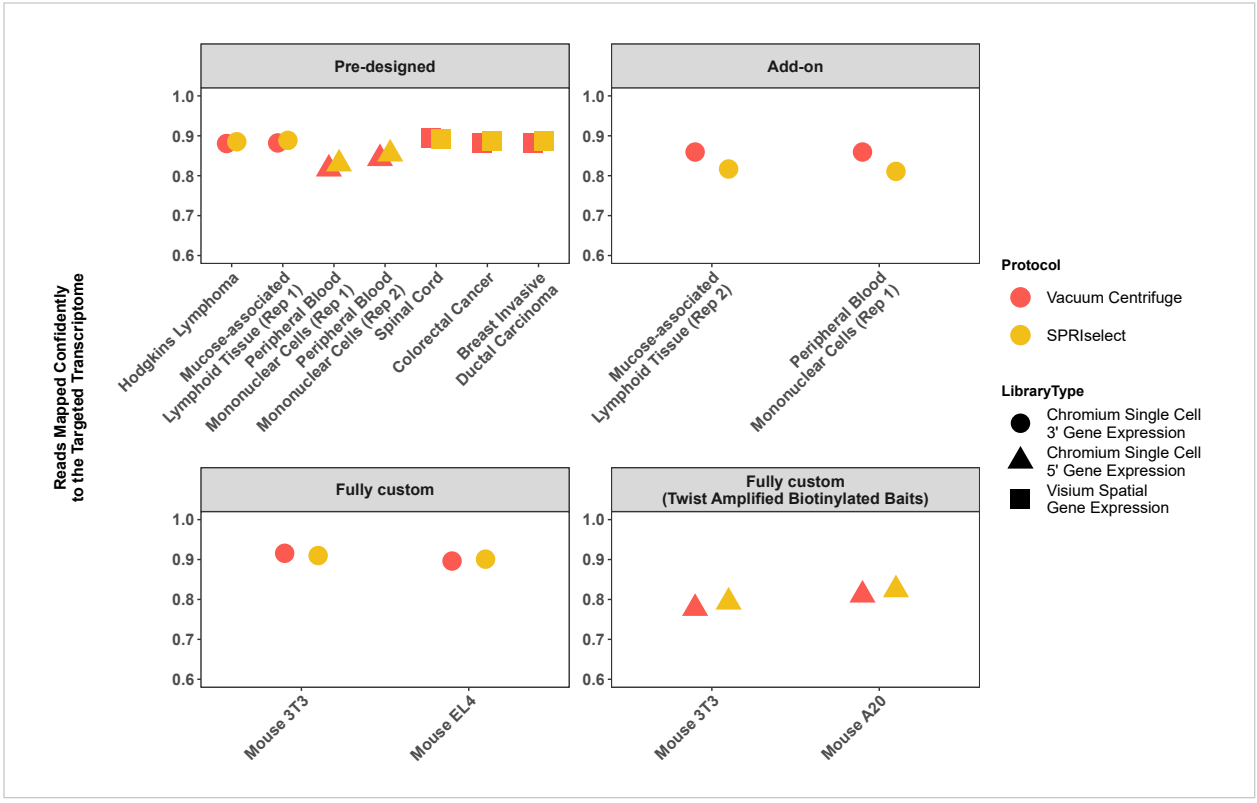


Figure 1. Reads mapped confidently to the targeted transcriptome. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

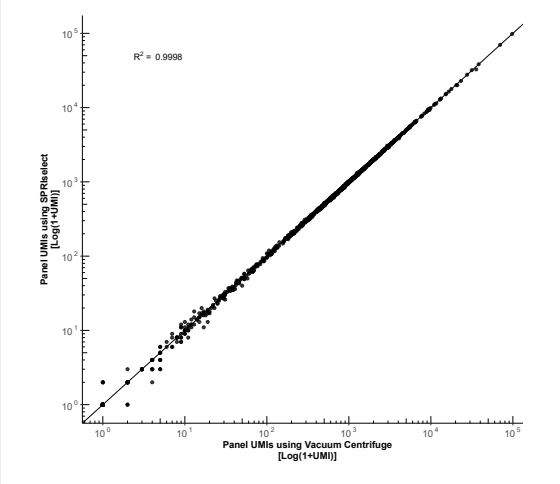


Figure 2. UMI recovery. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

References

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

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