

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*	3000478
	Formerly Human Cot DNA	3000479
	Hyb Buffer*	3000480
	Hyb Enhancer*	2000290
	Universal Blockers*	-
	Pre-designed Panel†	230003
10x Magnetic Separator		
Beckman Coulter	SPRIselect	B23318
IDT	NGS Discovery Pool	-

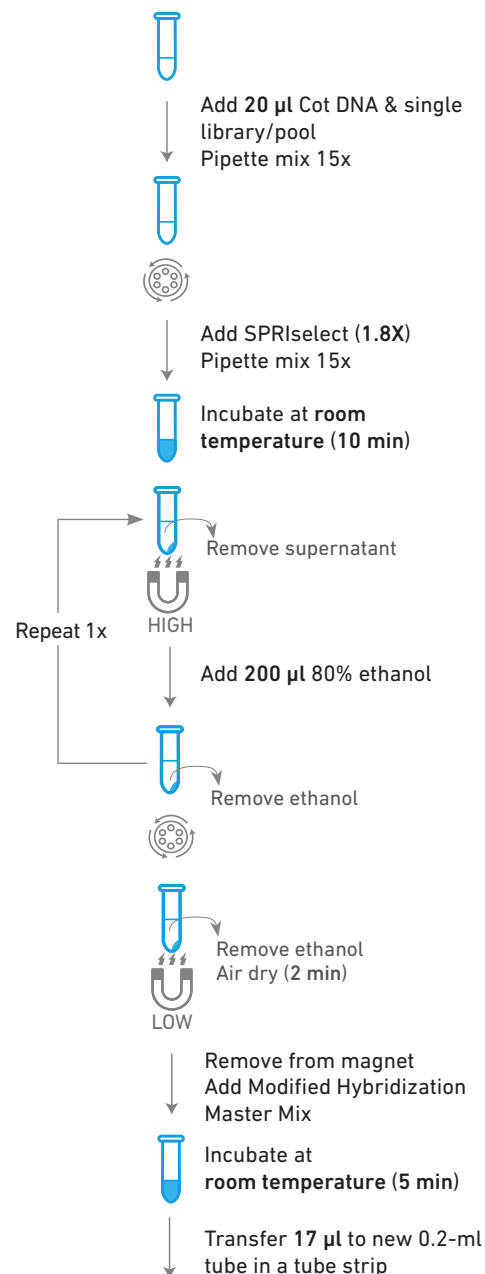
Additional Buffers/Reagents

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

10x
GENOMICS

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).

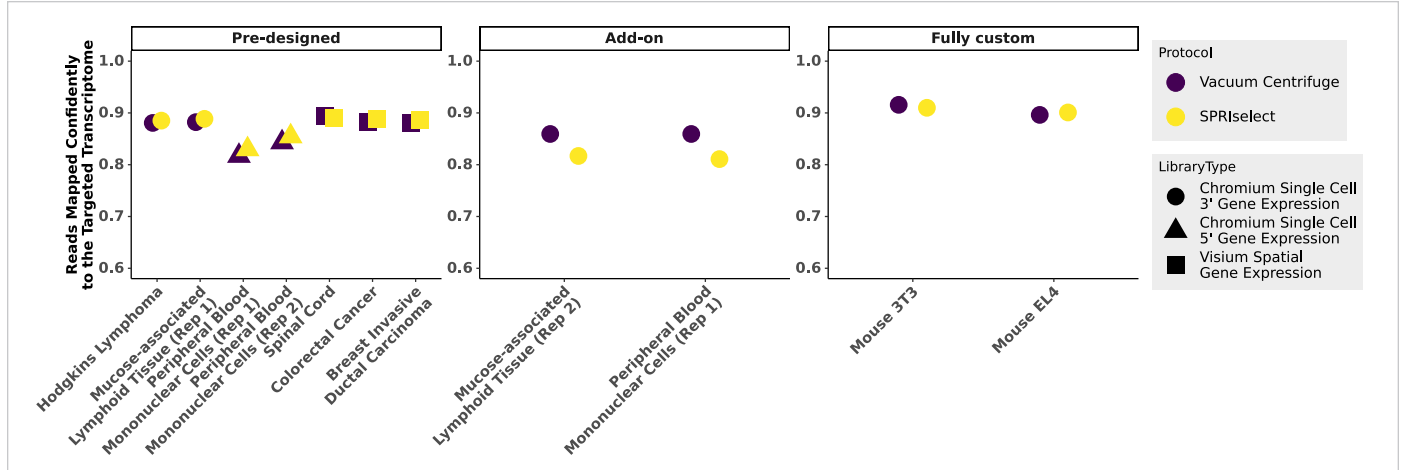


Figure 1. Reads mapped confidently to the targeted transcriptome. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

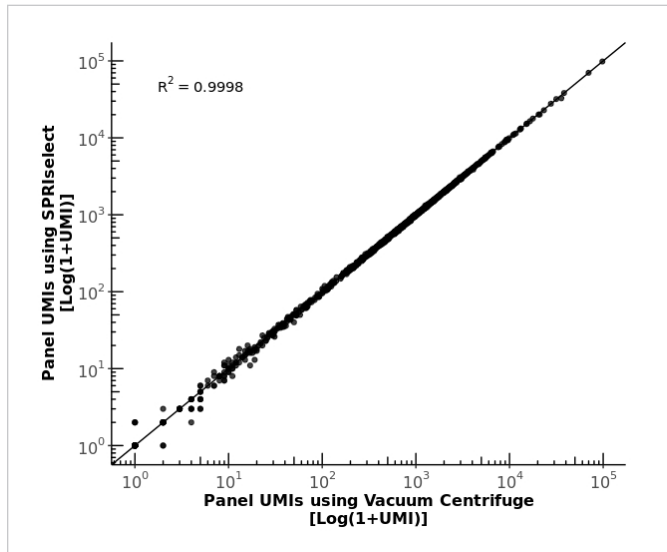


Figure 2. UMI recovery. Assay performance using SPRIselect is similar to using a vacuum centrifuge.

References

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

© 2021 10x Genomics, Inc. (10x Genomics). All rights reserved. Duplication and/or reproduction of all or any portion of this document without the express written consent of 10x Genomics, is strictly forbidden. Nothing contained herein shall constitute any warranty, express or implied, as to the performance of any products described herein. Any and all warranties applicable to any products are set forth in the applicable terms and conditions of sale accompanying the purchase of such product. 10x Genomics provides no warranty and hereby disclaims any and all warranties as to the use of any third-party products or protocols described herein. The use of products described herein is subject to certain restrictions as set forth in the applicable terms and conditions of sale accompanying the purchase of such product. A non-exhaustive list of 10x Genomics' marks, many of which are registered in the United States and other countries can be viewed at: www.10xgenomics.com/trademarks. 10x Genomics may refer to the products or services offered by other companies by their brand name or company name solely for clarity, and does not claim any rights in those third-party marks or names. 10x Genomics products may be covered by one or more of the patents as indicated at www.10xgenomics.com/patents. The use of products described herein is subject to 10x Genomics Terms and Conditions of Sale, available at <http://www.10xgenomics.com/legal-notices>, or such other terms that have been agreed to in writing between 10x Genomics and user. All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

The use of 10x Genomics products in practicing the methods set forth herein has not been validated by 10x Genomics, and such non-validated use is NOT COVERED BY 10x GENOMICS STANDARD WARRANTY, AND 10x GENOMICS HEREBY DISCLAIMS ANY AND ALL WARRANTIES FOR SUCH USE. Nothing in this document should be construed as altering, waiving or amending in any manner 10x Genomics terms and conditions of sale for the Chromium Controller or the Chromium Single Cell Controller, consumables or software, including without limitation such terms and conditions relating to certain use restrictions, limited license, warranty and limitation of liability, and nothing in this document shall be deemed to be Documentation, as that term is set forth in such terms and conditions of sale. Nothing in this document shall be construed as any representation by 10x Genomics that it currently or will at any time in the future offer or in any way support any application set forth herein.

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

