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We use this protocol and it's working

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cDNA Exome Capture v1.0.1

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ABSTRACT

This protocol describes a modified version of the Parse Biosciences Gene Capture protocol for full-length cDNA meant for both Illumina short read and Oxford Nanopore long read library prep and sequencing. We use Twist Biosciences exome panels to enrich for non-intronic reads for more meaningful long-read data. Each section matches the original protocol but with our modifications. Please see the attachment for the original Parse Biosciences protocol. The main deviations from the original Parse Biosciences protocol are 1. the use of cDNA amplification reagents instead of the amplification reagents included in the Gene Capture kit, and 2. modification of SPRI bead ratio from **1.8x** to **0.8x**. We perform this protocol on cDNA generated for a subset of **13,000 barcoded cells or nuclei** from the WT or WT Mega kits.

The product of this protocol is full-length, barcoded cDNA that excludes intron-only fragments. Each molecule should have a cell/nuclei barcode, UMI, and some fraction overlapping a region found in the Twist human or mouse exome panel. Given an **input of 500 ng** full-length, barcoded cDNA from the end of Section 2 (WT / WT Mega protocol), there should be plenty of captured cDNA (> 1 ug). In our experience, an input as low as 200 ng resulted in 700 ng captured cDNA.

The first part of the protocol, Section 1, describes setting up hybridization repetitive sequence blockers and of the actual panel, which runs overnight. Section 2 describes capture of the hybridized molecules to streptavidin beads and amplification of the captured product. **Section 2 section uses cDNA amplification reagents from the WT / WT Mega kits.**

ATTACHMENTS

SO_Gene+Capture+v1.0.1+User+Manual.pdf

MATERIALS

User Supplied Equipment and Consumables
The following materials and equipment are required to perform the protocol, but are not provided within the kit. Note that this list does not include standard laboratory equipment, such as freezers. Any questions regarding these items can be directed to support@parsebiosciences.com.

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Keywords: Parse Biosciences, Parse, Parse Bio, Evercode, snRNA-seq, scRNA-seq, Single cell, Split-seq, Gene capture, Exome capture, Exome, Mortazavi, Mouse, UCI, IGVF

	Item	Supplier	Part Number	Notes
	Vacuum Concentrator	Various Suppliers	Varies	Optional. Only needed if following Appendix A to concentrate sublibraries.
	Two Heat Blocks	Various Suppliers	Varies	Or equivalent water baths, bead baths, or thermomixers capable of holding temperatures from 48°C to 68°C and compatible with 1.5 mL, 2 mL, and 5 mL tubes.
	T100 Thermal Cycler	Bio-Rad Laboratories	1861096	Or an equivalent thermocycler compatible with 0.2 mL tubes and a heated lid capable of 105°C and 85°C.
	Microcentrifuge	Various Suppliers	Varies	Compatible with 1.5 mL and 0.2 mL tubes.
	Single Channel Pipettes: P20, P200, P1000	Various Suppliers	Varies	
	Vortex-Genie 2	Scientific Industries	SI-0236	Or an equivalent vortex mixer.
	6-Tube Magnetic Separation Rack	New England Biolabs	S1506S	Or an equivalent magnetic rack for 1.5 mL tubes.
	Parse Biosciences Magnetic Rack	Parse Biosciences	SB1004	Magnetic strength is critical. If 3rd party magnetic racks are used, the number of transcripts and genes detected per cell will be compromised. This magnetic rack is compatible with most 0.2 mL PCR tubes.
	Qubit Flex Fluorometer	Thermo Fisher Scientific	Q33327	Or an equivalent fluorometer.
	2100 Bioanalyzer	Agilent	G2939BA	Choose one.
	4200 TapeStation System	Agilent	G2991BA	

Equipment

	Item	Supplier	Part Number	Notes
	Pipette Tips TR LTS 20 µL, 200 µL, 1,000 µL	Rainin	17014961 17014963 17014967	Or appropriate sterile, DNA low-binding, and filtered pipette tips. We do not recommend using wide bore tips.
	Kapa® Pure Beads	Roche	KK8000 (5 mL) KK8001 (30 mL)	Choose one. We do not recommend substituting other magnetic beads.
	AMPure® XP Reagent	Beckman Coulter	A63880 (5 mL) A63881 (60 mL)	

	Item	Supplier	Part Number	Notes
	Nuclease-Free Water	Sigma-Aldrich	W4502	Or equivalent nuclease-free water.
	Ethyl Alcohol, Pure	Sigma-Aldrich	459844	Or equivalent 100% non-denatured ethanol.
	DNA LoBind® Tubes, 1.5 mL, Snap Cap	Eppendorf	022431021	Or equivalent DNA low-binding, nuclease-free 1.5 mL tubes.
	TempAssure® PCR 8-Tube Strips, 0.2 mL	USA Scientific	1402-4700	Or equivalent nuclease-free 0.2 mL PCR tubes.
	Qubit dsDNA HS (High Sensitivity) Assay Kit	Thermo Fisher Scientific	Q33230 (100 assays) Q33231 (500 assays)	Or equivalent fluorescent DNA dye based quantification kit.
	High Sensitivity DNA Kit	Agilent	5067-4626	Choose one that corresponds to the chosen Bioanalyzer or TapeStation.
	High Sensitivity D1000 ScreenTape and Reagents	Agilent	5067-5592 (screen tape) 5067-5593 (sample buffer and ladder)	

Consumables

BEFORE START INSTRUCTIONS

User Supplied Equipment and Consumables: Before starting an experiment, check the “User Supplied Equipment and Consumables” section and confirm that your lab has all of the supplies that are not provided by the kit. Avoid substituting custom materials for those that are provided in the kit. Each item has been deliberately chosen to attain optimal results.

Input: This protocol begins with indexed sublibraries generated with an Evercode Whole Transcriptome kit. Up to 8 sublibraries can be pooled into each Gene Capture reaction. Thus, all 8 sublibraries from an Evercode WT kit can be pooled into 1 reaction, and all 16 sublibraries from an Evercode WT Mega would require 2 reactions. We recommend adding 100 ng of each sublibrary to a Gene Capture reaction for a maximum of 800 ng of input. To ensure even coverage, sublibraries should be mixed in equal proportion. However, if any sublibraries have less than 100 ng of input, sublibraries can be pooled to:
(A) Maintain the desired proportion between samples with lower final library complexity. or (B) Add the maximum amount of lower concentration indexed sublibraries, which will result in the highest overall complexity but impact the proportion of reads between samples.

Nonhuman Samples: If using a non-human samples, the Blocker Solution can be replaced with a species-specific blocking solution (not provided). The provided Blocker Solution may still reduce off target binding in other vertebrate species but less effectively.

Concentration Sublibraries: Sublibraries can be concentrated with vacuum centrifugation rather than SPRI bead concentration. See Appendix for a protocol.

Gene Panels: Gene Capture is compatible with the Immune1000 Panel and custom gene panels from Twist Biosciences. The Immune1000 Panel is included with Immune1000 Gene Capture (GCE1002). For Custom Gene Capture (GCE1001), a panel is not provided and should be purchased separately from Twist Biosciences. Custom panel design can vary based on experimental goals, but we recommend that probes span the MANE transcript of each gene with non-overlapping probes, including the 3’ UTR. For additional details about panel design, contact us at support@parsebiosciences.com.

For additional questions not discussed above, please contact us at support@parsebiosciences.com. We also have a library of additional resources and videos on our support site at https://support.parsebiosciences.com/.

Section 1: Hybridization

1 **1.1 Prepare cDNA**

1.1 Prepare for hybridization with the following checklist:

- Fill an ice bucket
- Set a heat block to **65C**
- Program a thermocycler to **95C** and set the heated lid to 105C
- Equilibrate SPRI beads (Ampure XP or KAPA Pure Beads) to room temperature for at least **30 minutes**
- Prepare at least 600 µL of 85% ethanol per reaction
- Take out magnetic rack for 1.5 mL tubes

1.2 Gather the following items and handle as indicated below:

	Item	Location	Quantity	Format	Handling and Storage
	Evercode WT or WT Mega cDNA	User Stored Location (-20°C)	8 per hybridization reaction	0.2 mL PCR tube	Thaw on ice.
	Hybridization Mix	Gene Capture Hybridization Reagents (-20°C)	1	1.5 mL tube	Heat at 65°C for 10 min, then keep at room temp.
	Blocker Solution	Gene Capture Hybridization Reagents (-20°C)	1	1.5 mL tube	Thaw on ice. Briefly centrifuge before use.
	Evercode Blocker Solution	Gene Capture Hybridization Reagents (-20°C)	1	1.5 mL tube	Thaw on ice. Briefly centrifuge before use.
	Mouse Exome Panel or Human Comprehensive Exome (Twist Biosciences cat. 102036 or 102032)	-20C	1	1.5 mL tube	Thaw on ice. Briefly centrifuge before use.
	Hybridization Enhancer	Gene Capture Hybridization Reagents	1	1.5 mL tube	Thaw on ice. Briefly centrifuge before use.

1.3 Incubate the **Hybridization Mix** in the heat block at **65°C** for **10 minutes,** or until no precipitate is present before proceeding.

1.4 Incubate the **Hybridization Mix** at room temperature for **5 minutes** to equilibrate the solution.

1.5 Vortex **cDNA** (2-3 sec). Briefly centrifuge (~2 sec).

1.6 For each hybridization reaction, we recommend adding **500 ng** of each **cDNA** sample to a Gene Capture reaction. However, we've performed gene capture and long read library prep with as low as 200 ng of cDNA input. **Do NOT pool cDNA samples, they do not have the fourth subpool barcode (Illumina index)!**

1.7 If the total volume of any cDNA sample is less than 100 µL, bring the volume up to 100 µL with nuclease-free water.

1.8 Vortex the SPRI beads until fully mixed. Add **0.8x** of SPRI beads to each library pool.

Note: For example, if a cDNA sample is 100 µL, add (0.8 x 100 µL) = **80 µL** of SPRI beads.

1.9 Vortex the tube(s) for 5 sec. Briefly centrifuge (~2 sec).

1.10 Incubate tube(s) at room temperature for **5 minutes**.

1.11 Place the tube(s) in an 1.5 mL magnetic rack and wait for all the beads to bind to the magnet (~3 min: liquid should be clear).

Critical! Ensure the supernatant is completely clear before proceeding. Discarding any beads in the supernatant will result in a reduction of transcripts and genes detected per cell.

1.12 With tube(s) still on the magnetic rack, slowly remove and discard the clear supernatant.

1.13 Without resuspending beads, add **300 µL** of 85% ethanol to each tube using a P1000 and wait **1 minute**.

1.14 Using a pipette, aspirate and discard the ethanol from each tube.

1.15 Without resuspending beads, add another **300 µL** of 85% ethanol to each tube using a P1000 and wait **1 minute**.

1.16 Using a pipette, aspirate and discard all of the ethanol from each tube (it may be necessary to remove the final few drops with a P20 pipette). With the tube still on the rack, air dry the beads (~2 min).

Critical! Do NOT over-dry the beads. Over-drying of beads can lead to substantial losses in yield. “Cracking” of the beads is a sign of over-drying

1.17 Remove tube(s) from the magnetic rack. Proceed directly to Section 1.2.

2 1.2 Hybridization

2.1 Add the following reagents individually to each **cDNA sample** from step 1.1.17 . Mix by flicking the 1.5 mL tube(s). If making a master mix for multiple samples, scale up volumes by 10%.

Item	Volume (µL)
Evercode Blocker Solution	7
Blocker Solution	5
Total	12

Add to Each cDNA Sample

2.2 For each **cDNA sample**, spin down and carefully transfer the entire volume (12 µL) into new 0.2 mL tube(s) using a P20 set to 15 µL.

2.3 In a new 0.2 mL tube(s) for each **cDNA sample**, prepare a **Probe Solution** as follows. Mix by flicking the tube(s). If making a master mix for multiple samples, scale up volumes by 10%.

	Item	Volume (µL)
	Hybridization Mix	20
	Twist Biosciences Panel	4
	Nuclease-free Water	4
	Total	28

Probe Solution

2.4 Heat the **Probe Solution(s)** for **2 minutes** at **95°C** in a thermocycler with the lid heated to 105°C, then immediately cool for **5 minutes** on ice.

2.5 Heat the **cDNA sample(s)** for **5 minutes** at **95°C** in a thermocycler with the lid heated to 105°C, then immediately place at room temperature.

2.6 Incubate the **Probe Solution(s)** and **cDNA sample(s)** at room temperature for 5 minutes.

2.7 Set a thermocycler to **70°C** with the lid at **85°C** for infinite time.

2.8 Vortex the tube(s) for 5 sec. Briefly centrifuge (~2 sec).

2.9 Transfer the entire volume (28 µL) of the **Probe Solution** into each **cDNA sample** to create the **Hybridization Reaction**.

2.10 Vortex the tube(s) for 5 sec. Briefly centrifuge (~2 sec).

2.11 Without mixing, add **30 µL** of **Hybridization Enhancer** to the top of the meniscus of the **Hybridization Reaction** from step 9.

Note: Do not mix after adding the Hybridization Enhancer. It should form a distinct layer above the hybridization reaction.

2.12 Briefly centrifuge tube(s) to ensure there are no bubbles present.

2.13 Ensure tubes are tightly capped to prevent excess evaporation.

2.14 Incubate each **Hybridization Reaction** at **70°C** for **15-17 hours** in a thermocycler with the lid at 85°C.

Critical! Rapid transfer directly from the thermocycler at 70°C is a critical step for minimizing off-target binding. Do not remove the Hybridization Reaction tube(s) from the thermocycler or otherwise allow it to cool to less than 70°C until Step 2.1.12.

Section 2: Capture and Amplification

3 **2.1 Bind and Wash**

3.1 Prepare the following:

- Gather two heat blocks, set one to **68°C** (for 1.5 mL tube) and the other to **48°C** (for 2 mL and 5 mL tubes).
- Program a thermocycler to **95°C** and set the heated lid to 105°C.
- Equilibrate **Streptavidin Binder Beads** to room temperature for at least **30 minutes**.
- Equilibrate SPRI beads (Ampure XP or KAPA Pure Beads) to room temperature for at least **30 minutes**.
- Prepare at least 400 µL of 85% ethanol per reaction.
- Take out magnetic rack for 1.5 mL tubes.

3.2 Note: For performing gene capture on cDNA, you need to use reagents from the **WT** or **WT Mega cDNA Amplification box: Amplification Reaction Solution** and **Amplification Master Buffer.**

Gather the following items and handle as indicated below:

	Item	Location	Quantity	Format	After taking out
	Streptavidin Binder Beads	Gene Capture Wash Reagents (4°C)	1	1.5 mL tube	Equilibrate at room temperature for at least 30 minutes.
	Binding Buffer	Gene Capture Wash Reagents (4°C)	1	1.5 mL tube	Heat tube at 48°C for 5 minutes.
	Bead Wash Buffer A	Gene Capture Wash Reagents (4°C)	1	1.5 mL tube	Heat tube at 68°C.
	Bead Wash Buffer B	Gene Capture Wash Reagents (4°C)	1	1.5 mL tube	Heat tube at 48°C.
	Amplification Reaction Solution	cDNA Amplification (-20°C)	1	1.5 mL tube	Thaw on ice. Briefly centrifuge before use.
	Amplification Master Buffer	cDNA Amplification (-20°C)	1	1.5 mL tube	Thaw on ice. Briefly centrifuge before use.

3.3 Check if precipitate is dissolved, then equilibrate the **Binding Buffer** to room temperature. Keep **Bead Wash Buffer A** and **Bead Wash Buffer B** in their respective heat blocks.

3.4 Ensure the **Streptavidin Binder Beads** have been equilibrated to room temperature for at least 30 minutes. Vortex the **Streptavidin Binder Beads** until mixed.

3.5 Add **100 µL** of **Streptavidin Binder Beads** to a new 1.5 mL tube for each hybridization reaction.

3.6 Add **200 µL** of **Binding Buffer** to the tube with **Streptavidin Binder Beads**. Mix thoroughly by pipetting up and down 10x with a P200 set to 200 µL.

3.7 Place the tube(s) in an 1.5 mL magnetic rack and wait for all the beads to bind to the magnet (~2 min: liquid should be clear).

3.8 Using a pipette, aspirate and discard the supernatant from each tube. Remove tube(s) from the magnetic rack.

3.9 Repeat steps 6-8 twice for a total of three washes

3.10 Add **200 µL** of **Binding Buffer** and resuspend the beads by vortexing until fully homogenized.

3.11 Heat the resuspended beads for **68°C** for **10 minutes** before proceeding to the next step.

3.12 Set a P200 pipette to 80 µL. After the hybridization reaction from Section 1.2 is complete, open the thermocycler lid and immediately transfer the entire volume of each **Hybridization Reaction** into a corresponding tube of preheated **Streptavidin Binder Beads** from step 11. Mix by pipetting and flicking.

Critical! Rapid transfer directly from the thermocycler at 70°C is a critical step for minimizing off-target binding. Do not remove the Hybridization Reaction tube(s) from the thermocycler or otherwise allow it to cool to less than 70°C before transferring the solution to the washed Streptavidin Binding Beads. Allowing the Hybridization Reaction to cool to room temperature for more than 5 minutes will result in as much as 10–20% increase in off-target binding.

3.13 Incubate the tube(s) containing the **Hybridization Reaction** and **Streptavidin Binder Beads** mixture at **68°C** for **5 minutes.**
Critical! Do not vortex. Aggressive mixing is not required.

3.14 Remove the tube(s) from the heat block. Briefly centrifuge (~2 sec).

3.15 Place the tube(s) in a magnetic rack and wait for all the beads to bind to the magnet (~2 min: liquid should be clear).

3.16 Using a pipette, aspirate and discard the clear supernatant from each tube.
*Note: Some **Hybridization Enhancer** reagent residue may be visible after supernatant removal and throughout each wash step. This will not affect the final capture product.*

3.17 Remove the tube(s) from the magnetic rack and add **200 µL** of **68°C Bead Wash Buffer A**. Mix by pipetting.

3.18 Incubate the tube(s) at **68°C** for **5 minutes** and place **Bead Wash Buffer A** back into the 68°C heat block.

3.19 Briefly centrifuge the tube(s).

3.20 
For each hybridization reaction, transfer the entire volume from step 19 (~200 µL) into new 1.5 mL tube(s).
Critical! This step reduces background from non-specific binding to the surface of the tube.

3.21 Place the tube(s) in an 1.5 mL magnetic rack and wait for all the beads to bind to the magnet (~1 min: liquid should be clear).

3.22 Using a pipette, aspirate and discard the clear supernatant from each tube.

3.23 Remove the tube(s) from the magnetic rack and add **200 µL** of **48°C Wash Buffer B**. Mix by pipetting and briefly centrifuge.

3.24 Incubate the tube(s) at **48°C** for **5 minutes** and place **Bead Wash Buffer B** back into the 48°C heat block.

3.25 Place the tube(s) in a magnetic rack and wait for all the beads to bind to the magnet (~1 min: liquid should be clear).

3.26 Using a pipette, aspirate and discard the clear supernatant from each tube.

3.27 Repeat steps 23-26 twice for a total of three washes.

3.28 While incubating the tubes at 48°C during the wash steps, in a new 1.5 mL tube, make the **Amplification Reaction Solution** by adding **Amplification Master Buffer** and **Amplification Primer Mix**. Mix well and store on ice.

For example, if processing one cDNA sample, add 60.5 µL of **Amplification Primer Mix** to 60.5 µL of **Amplification Master Buffer.**

# Subpools	1	2	3	4	5	6	7	8
Amplification Master Buffer	60.5	121	181.5	242	302.5	363	423.5	484
Amplification Primer Mix	60.5	121	181.5	242	302.5	363	423.5	484
Total	121	242	363	484	605	726	847	968

3.29 Remove any residual supernatant with a P20 pipette. Proceed immediately to the next step.

3.30 **New Step 30:** Remove the tube(s) from the magnetic rack and resuspend in **100 µL** of the **Amplification Reaction Solution**. Mix by pipetting until homogenized, **move to PCR strip tubes,** then incubate on ice. This solution will be referred to as **Streptavidin Binding Bead Slurry**.
Note: Move slurry to PCR tubes for the following amplification reaction.

4 2.2 PCR and SPRI Clean Up

4.1 **Start cDNA amplification.**

Note: For primer annealing, steps 3 and 6 below (*) have different time and temperature settings. Double check the settings you input into the thermocycler before starting the amplification protocol.

	Run Time	Lid Temperature	Subpool Volume
	50-70 min	105C	100 µL

Amplification Overview

	Step	Time	Temperature
	1	3 min	95C
	2	20 sec	98C
	3	*45 sec	*65C
	4	3 min, then go to step 2, repeat 4 times (5 cycles total)	72C
	5	20 sec	98C
	6	*20 sec	*67C
	7	3 min	72C
	8	5 min	72C
	9	Hold	4C

Amplification Protocol

Note: No 2nd cycling!

4.2 As soon as the program reaches 4°C, place the tube(s) at room temperature and proceed immediately to the next step.

4.3 Ensure the SPRI beads have been equilibrated to room temperature for at least 30 minutes. Vortex the SPRI beads until fully mixed. Add **80 µL** of SPRI beads (0.8x) to each tube. Mix by vortexing for 5 sec.

*Note: It is not necessary to recover supernatant or remove **Streptavidin Binding Beads** from the amplified PCR product.*

4.4 Incubate tube(s) at room temperature for **5 minutes**

4.5 Place the tube(s) on the high position of the magnetic rack for 0.2 mL tubes and wait for all the beads to bind to the magnet (~2 min: liquid should be clear).

4.6 With tubes still on the magnetic rack, remove and discard the clear supernatant.

4.7 With tubes still on the magnetic rack, add **180 µL** of 85% ethanol to each tube.

4.8 Incubate tube(s) at room temperature for **1 minute.**

4.9 With tubes still on the magnetic rack, remove and discard the supernatant.

4.10 Repeat steps 10-12 once more for a total of 2 washes. Remove any residual ethanol with a P20 pipette.

4.11 With tubes still on the magnetic rack, air dry the beads (~30 sec).

Critical! Do NOT over-dry the beads. Over-drying of beads can lead to substantial losses in yield. “Cracking” of the beads is a sign of over-drying.

4.12 Remove the tube(s) from the magnetic rack. Fully resuspend each bead pellet with **32 µL** of nuclease-free water.

4.13 Incubate the tube(s) at room temperature for **5 minutes**.

4.14 Place the tube(s) on the low magnet position of the magnetic rack for 0.2 mL tubes and wait for all the beads to bind to the magnet (~2 min: liquid should be clear).

4.15 With tubes still on the magnetic rack, transfer 30 µL of the supernatant containing the purified enriched cDNA into new 0.2 mL tube(s). Store on ice.

5 2.3 Enriched cDNA Quantification

5.1 Measure the concentration of the cDNA using the Qubit dsDNA HS protocol.
Note: Be sure to record sample concentrations as they will be needed for further downstream steps.

5.2 Run 1 µL of the cDNA on a Bioanalyzer or TapeStation. Use the concentration obtained from the Qubit to determine the appropriate dilution necessary (check manufacturer specifications, 1:10 dilution is generally appropriate).

5.3 Captured cDNA can be stored at this point at 4C for up to 2 days or at -20C for up to 3 months. If you wish to continue, proceed directly to

1. Short-read protocol: **Section 3: Preparing Libraries for Sequencing** in either the **Evercode WT** or **Evercode WT Mega** protocol.

2. Long-read protocol: **ONT Library Prep for Split-seq cDNA**.

[STOPPING POINT].