

User Manual

Version 1.4 – UMGSE1001



Gene Select

For use with

GSE1001

GSE1002



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Overview

Background

Evercode™ split-pool combinatorial barcoding brings a simple workflow to large-scale single cell RNA-seq experiments. Gene Select unlocks additional scalability by focusing on genes of interest to minimize sequencing costs. Gene Select is a hybrid capture based enrichment technology that is compatible with both the Immune1000 Panel and custom panels.

Following Evercode barcoding, hybridization probes selectively enrich cDNA libraries for transcripts of interest prior to sequencing, allowing researchers to profile hundreds to thousands of curated genes across large cell numbers at a fraction of the sequencing depth required for whole-transcriptome analysis.

The table below provides a high-level overview of the workflow.

GENE SELECT TECHNOLOGY	
1	 Evercode Whole Transcriptome sublibraries are pooled for the gene selection reaction.
2	 After hybridization of blockers to the Evercode library specific and repetitive sequences, biotinylated probes in the Immune1000 Panel or a custom panel are hybridized.
3	 Target molecules are captured with Streptavidin Binder beads, then washed.

GENE SELECT TECHNOLOGY	
4	 Captured molecules are enriched with PCR. Amplified double stranded libraries are ready to sequence.

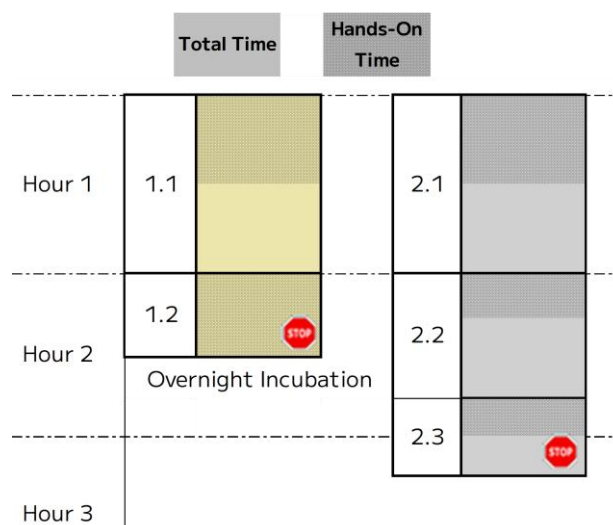
Protocol Timing

The table below provides details of the total and hands-on time required for the whole transcriptome workflow. A visual representation of the workflow is shown below the table.

DESCRIPTION	TIME	HANDS-ON-TIME	SAFE STOPPING POINTS
Section 1: Hybridization			
1.1 Pool libraries	60 min	30 min	
1.2 Hybridization	15-17 hrs	30 min	≤17 hrs in the thermocycler
Section 2: Capture and Amplification			
2.1 Bind and wash	60 min	30 min	
2.2 PCR and SPRI Cleanup	40 min	10 min	
2.3 Enriched Library Quantification	20 min	10 min	-20°C ≤ 3 months



Note: Overnight hybridization should not exceed 17 hours.



Important Guidelines

The following guidelines provide additional information to obtain optimal performance. For additional questions not discussed below, 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/>.

User Supplied Equipment and Consumables

- Before starting an experiment, check the “User Supplied Material and Equipment” section and confirm that your lab has all of the supplies that are not provided by the kit. Avoid substituting custom materials for those 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. Each Gene Select reaction can accommodate up to 8 pooled sublibraries, and each Gene Select kit supports 2 reactions, allowing up to 16 sublibraries to be processed per kit.
- We recommend adding 100 ng of each sublibrary to a Gene Select 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:
 - i. Maintain the desired proportion between samples with lower final library complexity.
 - ii. 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.

Non-Human Samples

- If using 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.

Sublibraries Concentration

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

- When using the SPRI beads to concentrate the libraries during section 1.1, the SPRI beads will stay in the solution throughout the workflow. SPRI beads will be removed during the post-PCR library clean-up in section 2.2. However, during section 2.1, streptavidin beads are also used to capture target molecules. Therefore during section 2 (through PCR amplification) both SPRI and Streptavidin beads will be together with the sample.

Gene Panels

- Gene Select is compatible with the Immune1000 Panel and custom gene panels from Twist Bioscience®. The Immune1000 Panel is included with Immune1000 Gene Select (GSE1002). For Custom Gene Select (GSE1001), a panel is not provided and should be purchased separately from Twist Bioscience. 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.

Part List

The Custom Gene Select kit includes the following boxes. Safety Data Sheets for these reagents can be provided upon request.

Custom Gene Select Hybridization Reagents, store at -20°C, PN GS100

LABEL	ITEM	PN	FORMAT	QTY
	Hybridization Mix	GC101	1.5 mL tube	1
	Hybridization Enhancer	GC102	1.5 mL tube	1
	Blocker Solution	GC103	1.5 mL tube	1
	Evercode Blocker Solution	GC108	0.5 mL tube	1
	Enrichment Primer Mix	GC105	0.5 mL tube	1
	Enrichment Amplification Mix	GC106	0.5 mL tube	1

Gene Select Wash Reagents, store at 4°C, PN GS300

LABEL	ITEM	PN	FORMAT	QTY
	Bead Wash Buffer A	GC301	1.5 mL tube	1
	Bead Wash Buffer B	GC302	2 mL tube	1
	Streptavidin Binding Buffer	GC305	8 mL tube	1
	Streptavidin Binder Beads	GC304	1.5 mL tube	1

The Immune1000 Gene Select kit includes the following boxes. Safety Data Sheets for these reagents can be provided upon request.

Immune1000 Gene Select Hybridization Reagents, store at -20°C, PN GS200

LABEL	ITEM	PN	FORMAT	QTY
	Hybridization Mix	GC101	1.5 mL tube	1
	Hybridization Enhancer	GC102	1.5 mL tube	1
	Blocker Solution	GC103	1.5 mL tube	1
	Evercode Blocker Solution	GC108	0.5 mL tube	1
	Enrichment Primer Mix	GC105	0.5 mL tube	1
	Enrichment Amplification Mix	GC106	0.5 mL tube	1
	Immune 1000 Panel	GC107	0.5 mL tube	1

Gene Select Wash Reagents, store at 4°C, PN GS300

LABEL	ITEM	PN	FORMAT	QTY
	Bead Wash Buffer A	GC301	1.5 mL tube	1
	Bead Wash Buffer B	GC302	2 mL tube	1
	Streptavidin Binding Buffer	GC305	5 mL tube	1
	Streptavidin Binding Beads	GC304	1.5 mL tube	1

User Supplied Material and Equipment

The following materials and equipment are required to perform the protocol but are not provided with the kit. This list does not include standard laboratory equipment, such as freezers.

Equipment

ITEM	SUPPLIER	PN	NOTES	CHECK LIST
Parse Biosciences Magnetic Rack	Parse Biosciences	SB1004	We do not recommend alternative 0.2 mL PCR tube racks, as they may result in lower transcript and gene detection.	☐
Microcentrifuge	Various Suppliers	Varies	Compatible with 1.5 mL and 0.2 mL tubes.	☐
Two Heat Blocks	Various Suppliers	Varies	Or equivalent water bath, bead bath, or thermomixer capable of holding temperatures from 48°C to 68°C and compatible with 1.5 mL, 2 mL, and 5 mL tubes.	☐
Hemocytometer	Sigma-Aldrich®	Z359629	Or other cell counting device. We recommend validating alternatives relative to a hemocytometer.	☐
Single Channel Pipettes: P20, P200, P1000	Various Suppliers	Varies		☐
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.	☐
6-Tube Magnetic Separation Rack	New England Biolabs®	S1506S	Or an equivalent magnetic rack for 1.5 mL tubes.	☐
Vortex-Genie 2®	Scientific Industries®	SI-0236	Or alternative vortex or mixer	☐
Qubit™ Flex Fluorometer	Thermo Fisher Scientific®	Q33327	Or an equivalent fluorometer.	☐
2100 Bioanalyzer	Agilent®	G2939BA	Choose one.	☐
4200 TapeStation System	Agilent	G2991BA		

Consumables

ITEM	SUPPLIER	PN	NOTES	CHECKLIST
AMPure® XP Reagent	Beckman Coulter®	A63880 (5 mL) A63881 (60mL)	Choose one. We do not recommend substituting other magnetic beads.	☐
KAPA® Pure Beads	Roche®	KK8000 (5 mL) KK8001 (30mL)		
SPRIselect Reagent	Beckman Coulter®	B23317 (5mL) B23318 (60mL)		
MAGFLO® NGS magnetic beads for NGS size selection and PCR purification	INTEGRA	7000 (1 mL) 7002 (50 mL)		
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.	☐
Pipette Tips TR LTS 20 µL, 200 µL, 1000 µL	Rainin®	17014961 17014963 17014967	Or appropriate DNA low-binding, DNase/RNase-free, and filtered pipette tips. Do not use wide bore tips.	☐
Ethyl alcohol, Pure	Sigma-Aldrich	459844	Or equivalent 100% non-denatured ethanol.	☐
Nuclease-Free Water	Sigma-Aldrich	W4502	Or equivalent nuclease-free water.	☐
High Sensitivity DNA Kit	Agilent	5067-4626	Choose one that corresponds to the chosen Bioanalyzer or TapeStation.	☐
High Sensitivity D5000 ScreenTape and Reagents	Agilent	5067-5592 (screen tape) 5067-5593 (sample buffer and ladder)		

Section 1: Hybridization

1.1. Pool Libraries

Libraries are pooled and prepared for hybridization reaction.

To pool libraries:

1. Fill a bucket with ice.
2. Set a heat block to 65°C.
3. Program a thermocycler to 95°C and set the heated lid to 105°C.
4. Equilibrate SPRI beads (SPRISelect, Ampure XP, KAPA Pure Beads, or MAGFLO) to room temperature for at least **30 minutes**.
5. Prepare at least **700 µL** of 85% ethanol per reaction and the magnetic rack for 1.5 mL tubes.
6. Gather the following items and handle as indicated below.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
Evercode WT Indexed Sublibraries	User Stored Location (-20°C)	8 per hybridization reaction	Thaw on ice.
● Hybridization Mix	Gene Select Hybridization Reagents (-20°C)	1	Heat at 65°C for 10 minutes, then keep at room temp.
● Hybridization Enhancer	Gene Select Hybridization Reagents (-20°C)	1	Thaw on ice. Briefly centrifuge before use.
● Blocker Solution	Gene Select Hybridization Reagents (-20°C)	1	
● Evercode Blocker Solution	Gene Select Hybridization Reagents (-20°C)	1	
● Immune1000 Panel or Twist Bioscience Custom Panel	Gene Select Hybridization Reagents (-20°C)	1	

7. Incubate the ● Hybridization Mix in the heat block at 65°C for **10 minutes** or until no precipitate is present before proceeding.
8. Incubate the ● Hybridization Mix at room temperature for **5 minutes** to equilibrate the solution.
9. Vortex sublibraries (**2-3 seconds**). Briefly centrifuge (~2 seconds).
10. For each hybridization reaction, pool up to 8 indexed sublibraries into a 1.5 mL tube to make a Library Pool. We recommend adding **100 ng** of each sublibrary to a Gene Select reaction for a maximum of **800 ng** of input.



Note: See Important Guidelines if your sublibraries contain less than 100 ng of total mass.

11. If the total volume of any Library Pool is less than 100 µL, bring the volume up to **100 µL** with nuclease-free water. If the volume is more than 100 µL continue to the next step
12. Vortex the SPRI beads until fully mixed. Add 1.8x volume of SPRI beads to each library pool.



Note: For example, if a Library Pool is 100 µL, add $(1.8 \times 100 \text{ µL}) = 180 \text{ µL}$ of SPRI beads. If the volume is more than 100 µL adjust the SPRI beads volume accordingly.

13. Vortex the tube(s) for **5 seconds**. Briefly centrifuge (~2 seconds).
14. Incubate tube(s) at room temperature for **5 minutes**.
15. Place the tube(s) in a 1.5 mL magnetic rack and wait for all the beads to bind to the magnet (~3 minutes: ensure the liquid is clear).
16. With tube(s) still on the magnetic rack, slowly remove and discard the clear supernatant.
17. Without resuspending beads, add **300 µL** of 85% ethanol to each tube using a P1000 and wait **1 minute**.
18. Using a pipette, aspirate and discard the ethanol from each tube.
19. Without resuspending beads, add another **300 µL** of 85% ethanol to each tube using a P1000 and wait **1 minute**.

20. 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 minutes).



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.

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

1.2. Hybridization

Biotinylated probes in the Immune1000 or the Custom Panel are hybridized to the pooled libraries.

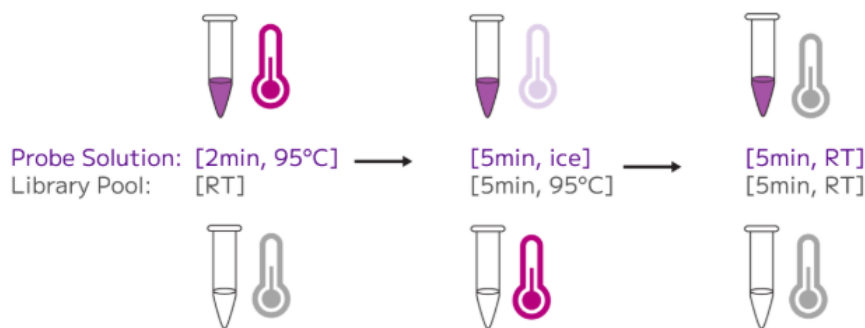
1. Add the following reagents individually to each Library Pool from step 1.1.21. Mix each reagent to the solution by gentle pipetting. Mix the solution by flicking the 1.5 mL tube(s).

ADD TO EACH LIBRARY POOL	
ITEM	VOLUME (μL)
● Evercode Blocker Solution	7
● Blocker Solution	5
TOTAL	12

2. For each Library Pool, spin down and carefully transfer the entire volume (**12 μL**) into **new 0.2 mL** tube(s) using a P20 set to 15 μL. Place tube(s) on ice.
3. In a **new 0.2 mL** tube(s) for each Library Pool, prepare a Probe Solution as follows. Mix by flicking the tube(s).

PROBE SOLUTION	
ITEM	VOLUME (μL)
● Hybridization Mix	20
● Immune1000 Panel OR Twist Bioscience Custom Panel	4
Nuclease-free Water	4
TOTAL	28

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.
5. While the Probe Solution(s) cools on ice, heat the Library Pool(s) for **5 minutes** at 95°C in a thermocycler with the lid heated to 105°C, then immediately place at room temperature.



6. Separately, incubate both the Probe Solution(s) and Library Pool(s) at room temperature for **5 minutes**.
7. Set a thermocycler to 70°C with the lid at 85°C for infinite time.
8. Vortex the Probe Solution(s) for **5 seconds**. Briefly centrifuge (~2 seconds).
9. Transfer the entire volume (**28 µL**) of the Probe Solution into each Library Pool to create the Hybridization Reaction.
10. Vortex the tube(s) for **5 seconds**. Briefly centrifuge (~2 seconds).
11. Without mixing, add **30 µL** of ● Hybridization Enhancer to the top of the meniscus of the Hybridization Reaction from step 9.
12. Briefly centrifuge tube(s) to ensure there are no bubbles present.
13. Ensure tubes are tightly capped to prevent excess evaporation.
14. Incubate each Hybridization Reaction at 70°C for **15-17 hours** in a thermocycler with the lid at 85°C.



CRITICAL! Do not mix after adding the ● Hybridization Enhancer. It should form a distinct layer above the hybridization reaction.



Note: Hybridizing for less than 15 hours or more than 17 hours will impact hybridization efficiency.

Section 2: Capture and Amplification

2.1. Bind and Wash

Target molecules are captured.

1. 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).
2. Equilibrate ● Streptavidin Binder Beads and the SPRI beads (SPRISelect, Ampure XP, KAPA Pure Beads, or MAGFLO) to room temperature for at least **30 minutes**.



Note: See Important Guidelines, "Sublibraries Concentration" for clarifications about the Streptavidin Beads and the SPRI beads in the sample.

3. Prepare at least 400 µL of 85% ethanol per reaction.
4. Prepare the magnetic rack for 1.5 mL tubes.
5. Gather the following items and handle as indicated below.

ITEM	SOURCE	QTY	HANDLING AND STORAGE
● Bead Wash Buffer A	Gene Select Wash Reagents (4°C)	1	Heat tube at 68°C and keep at 68°C.
● Bead Wash Buffer B	Gene Select Wash Reagents (4°C)	1	Heat tube at 48°C and keep at 48°C.
○ Streptavidin Binding Buffer	Gene Select Wash Reagents (4°C)		Heat tube at 48°C for 5 minutes. Then keep at room temperature.
● Streptavidin Binder Beads	Gene Select Wash Reagents (4°C)	1	Equilibrate at room temperature for at least 30 minutes.
● Enrichment Primer Mix	Gene Select Hybridization Reagents (-20°C)	1	Thaw at room temperature, then place on ice.
● Enrichment Amplification Mix	Gene Select Hybridization Reagents (-20°C)	1	Thaw on ice. Briefly centrifuge before use.

6. Check the ○ Streptavidin Binding Buffer for precipitate. If precipitate exist, warm up the buffer for **5 minutes** at 37°C and pipette mix to equilibrate buffer. Keep ● Bead Wash Buffer A and ● Bead Wash Buffer B in their respective heat blocks.
7. Ensure the ● Streptavidin Binder Beads have been equilibrated to room temperature for at least **30 minutes**. Vortex the ● Streptavidin Binder Beads until mixed.
8. Add **100 µL** of ● Streptavidin Binder Beads to a new 1.5 mL tube for each hybridization reaction.
9. Add **200 µL** of ○ Streptavidin Binding Buffer to the tube(s) with ● Streptavidin Binder Beads. Mix thoroughly by pipetting 10x with a P200 set to 200 µL.
10. Place the tube(s) in a 1.5 mL magnetic rack and wait for all the beads to bind to the magnet (~2 minutes: liquid should be clear).
11. Using a pipette, aspirate and discard the supernatant from each tube. Remove tube(s) from the magnetic rack.
12. Repeat steps 9-11 twice for a total of three washes.
13. Add **200 µL** of ○ Streptavidin Binding Buffer and resuspend the beads by vortexing until fully homogenized.
14. Heat the resuspended beads at 68°C for **10 minutes** before proceeding to the next step.
15. 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 2.1.14. Mix by pipetting.



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.

16. Incubate the tube(s) containing the Hybridization Reaction and ● Streptavidin Binder Beads mixture at 68°C for **5 minutes**.
17. Remove the tube(s) from the heat block. Do not vortex, briefly centrifuge (~2 seconds).
18. Place the tube(s) in a magnetic rack and wait for all the beads to bind to the magnet (~2 minutes: liquid should be clear).

19. 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 captured product.

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

21. Incubate the tube(s) at 68°C for **5 minutes**. Briefly centrifuge.

22. For each hybridization reaction, transfer the entire volume from step 21 (~200 µL) into new 1.5 mL tube(s).



CRITICAL! This step reduces background from non-specific binding to the surface of the tube.

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

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

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

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

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

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

29. Repeat steps 25-28 twice for a total of three washes.

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

31. Remove the tube(s) from the magnetic rack and resuspend in **45 µL** of nuclease-free water. Mix by pipetting until homogenized, then incubate on ice. This solution will be referred to as Streptavidin Binding Bead Slurry.

32. Proceed immediately to Section 2.2.

2.2. PCR and SPRI Clean Up

Captured targets are amplified.

1. Prepare **400 µL** of 85% ethanol per reaction.
2. Prepare the magnetic rack for 1.5 mL tubes.
3. Mix the Streptavidin Binding Bead Slurry by pipetting.
4. For each hybridization reaction, prepare a PCR mixture in a new 0.2 mL PCR strip as follows. Then, mix by pipetting, centrifuge briefly, and store on ice.

PCR MIXTURE	
Reagents	Volume
Streptavidin Binding Bead Slurry	22.5 µL *
● Enrichment Primer Mix	2.5 µL
● Enrichment Amplification mix	25 µL
Total	50 µL



Note: *Only half of the streptavidin binding bead slurry will be used at this step. The remaining slurry can be stored at -20C.

5. Determine the number of PCR cycles required based on the table below. Although these recommendations are generally appropriate, the number of cycles may need to be optimized.

No of Gene Target in Panel	Total PCR Cycles
0-25	16
26-50	15
51-100	14
101-200	13
201-400	12
401-800	11
801-1600 (Immune1000 Panel)	10
1601-3200	9



Note: If using less than 800 ng of total input, 1-2 additional PCR cycles may need to be added.

- Place the tube(s) into a thermocycler and run the following program.

AMPLIFICATION PROGRAM			
Run Time		~30 minutes	
Lid Temperature		105°C	
Sample Volume		50 µL	
Step	Time	Temperature	Cycles
1	45 sec	98°C	1
2	15 sec	98°C	Variable, see above
3	30 sec	60°C	
4	30 sec	72°C	
5	1 min	72°C*	1
6	Hold	4°C	1



CRITICAL! If using more than 1 panel with different cycling recommendations, libraries should be amplified in separate thermocyclers according to the recommendations in step 5.

- As soon as the program reaches 4°C, place the tube(s) at room temperature and proceed immediately to the next step.
- Ensure the SPRI beads have been equilibrated to room temperature for at least **30 minutes**. Vortex the SPRI beads until fully mixed. Add **90 µL** of SPRI beads (1.8x) to each tube. Vortex for **5 seconds**.



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

- Incubate tube(s) at room temperature for **5 minutes**.
- 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 minutes: liquid should be clear).

11. With tubes still on the magnetic rack, remove and discard the clear supernatant.
12. With tubes still on the magnetic rack, add **180 µL** of 85% ethanol to each tube.
13. Incubate tube(s) at room temperature for **1 minute**.
14. With tubes still on the magnetic rack, remove and discard the supernatant.
15. Repeat steps 12-14 once more for a total of 2 washes. Remove any residual ethanol with a P20 pipette.
16. With tubes still on the magnetic rack, air dry the beads (~30 seconds).



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.

17. Remove the tube(s) from the magnetic rack. Fully resuspend each bead pellet with **32 µL** of nuclease-free water.
18. Incubate the tube(s) at room temperature for **5 minutes**.
19. 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 minutes: liquid should be clear).
20. With tubes still on the magnetic rack, transfer **30 µL** of the supernatant containing the purified enriched sublibraries into new 0.2 mL tube(s). Store on ice.
21. Proceed immediately to Section 2.3.



Safe stopping point: The sequencing-ready libraries can be stored at -20°C for up to 3 months.

2.3. Enriched Library Quantification

1. Measure the concentration of each enriched library from Section 2.2 with the Qubit dsDNA HS (High Sensitivity) Assay Kit according to the manufacturer's instructions.
2. Assess the size distribution of each purified DNA with a High Sensitivity DNA Kit on the Agilent Bioanalyzer System or Tapestation HSD1000 according to the manufacturer's instructions. See Figure 1 for the expected size distribution.
3. For sequencing, refer to run configuration details in the appropriate user manual used to generate the Evercode Whole Transcriptome Libraries pooled in Step 1.1.10.

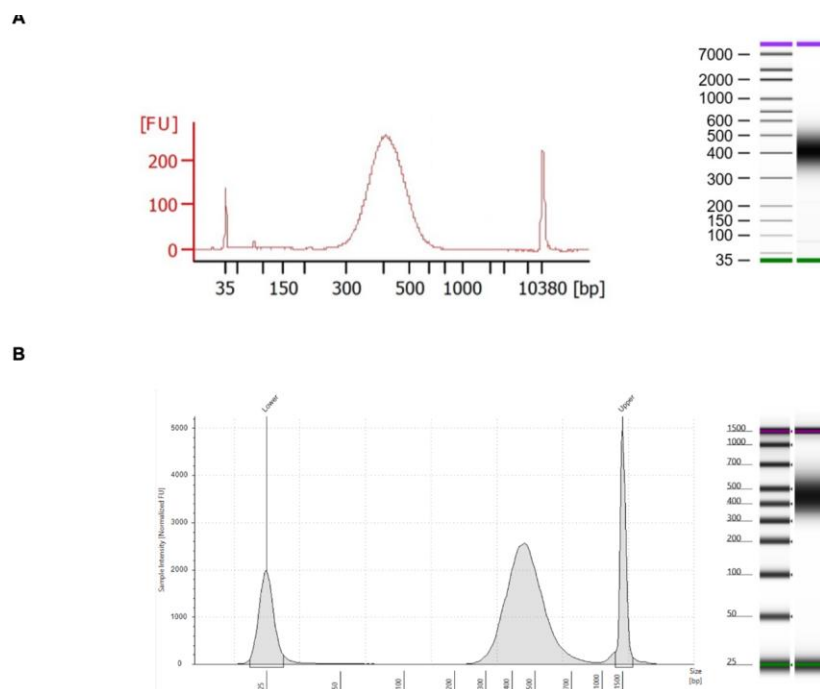


Figure 1: Expected Size Distribution before Illumina Sequencing (A) Example trace of DNA from enriched sublibraries run on a Bioanalyzer. (B) Example trace of DNA from enriched sublibraries run on a TapeStation.



Note: The traces above are representative of typical Bioanalyzer and TapeStation of DNA from enriched sublibraries. There should be a peak between 400-500 bp. The prominence of the trace is dependent on amount of DNA loaded into the Bioanalyzer or TapeStation. Samples with minor deviations can still produce high quality data.

Note: There may be an additional peak present on the Bioanalyzer. If there is a 400-500 bp peak, it should not impact sequencing or data quality, but care should be taken when calculating the library concentration before sequencing.

Appendices

Appendix A: Vacuum Concentration

1. Pool 100 ng of each indexed library into a 0.2 mL PCR tube. Briefly centrifuge.
2. Dry the pool of sublibraries using a vacuum concentrator without heat or on the lowest heat setting.
3. The dried Library Pool can be stored at -20°C for 1 week. Otherwise, proceed directly to section 1.2 Hybridization, Step 1.2.2 should be skipped.

Appendix B: Revision History

Version	Description	Date
1.1	Initial release	February 2024
1.2	Updated product name	August 2024
1.3	Section 2.2.4: added a note	July 2026
1.4	Updated for Evercode MAX	September 2026



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