User Manual

Version 1.1 – UM0020

# Gene Capture

For use with

GCE1001

GCE1002

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# Overview

## Background

Evercode split-pool combinatorial barcoding brings a simple workflow to large-scale single cell RNA-seq experiments. Gene Capture unlocks additional scalability by focusing on genes of interest to minimize sequencing costs. Gene Capture is a hybrid capture based enrichment technology (Figure 1) that is compatible with both the Immune1000 Panel and custom panels.

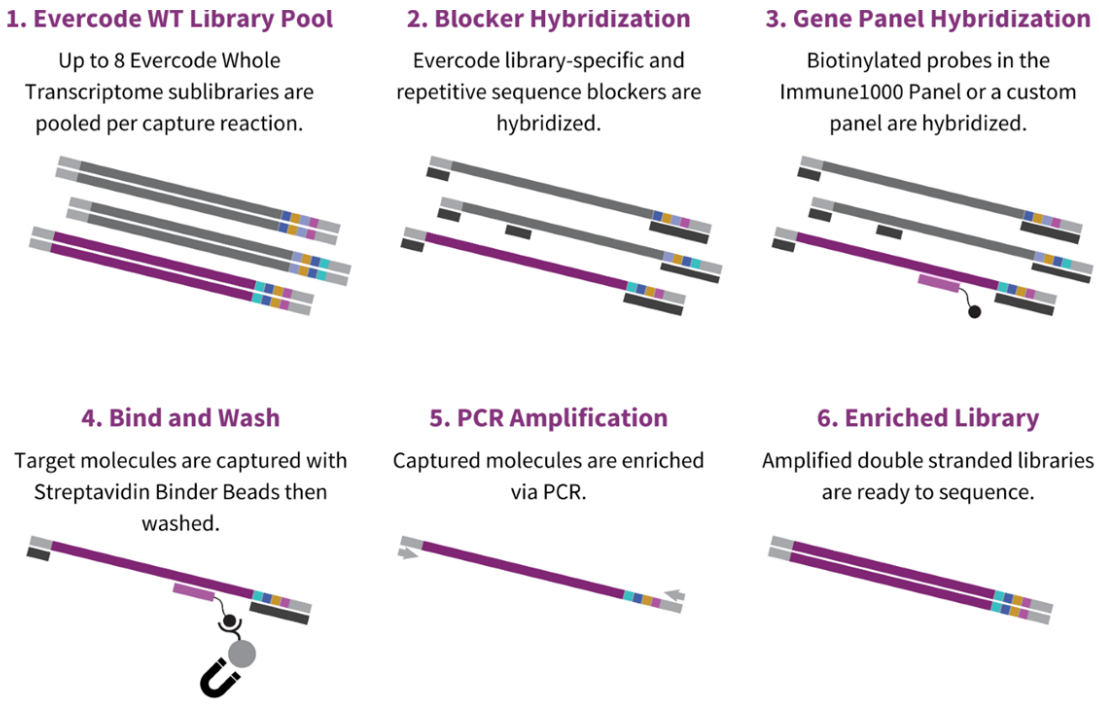
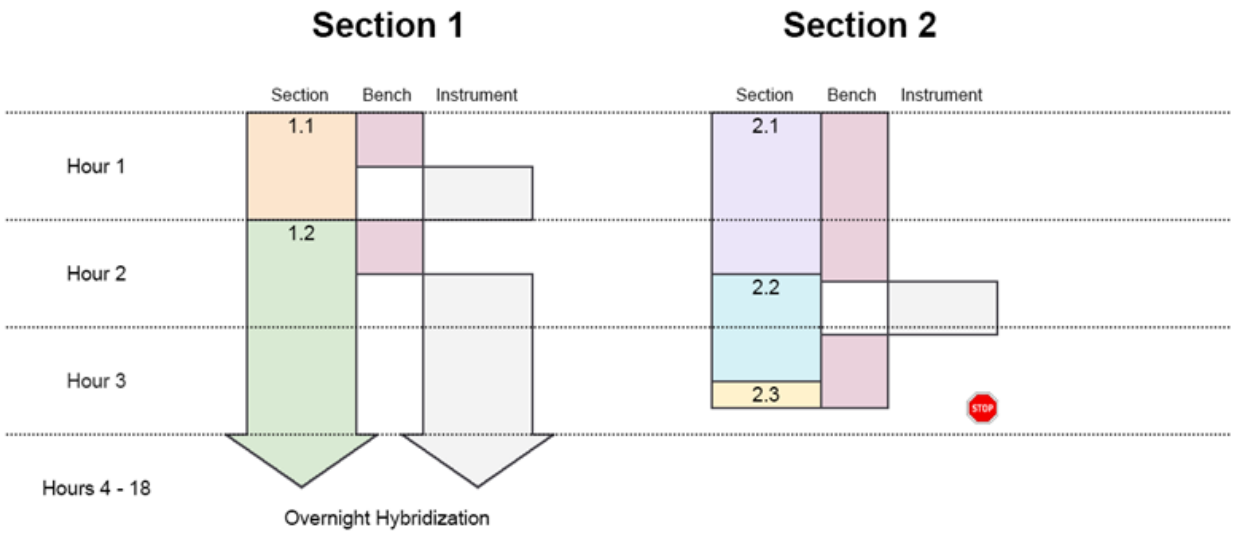



*Figure 1: Gene Capture Technology. Gene Capture enriches target genes (purple) by hybridization of biotinylated DNA probes (pink) and capture with streptavidin coated magnetic beads. Blockers (black) prevent non-specific binding during hybridization and capture. After PCR, enriched libraries are ready to sequence.*

## Protocol Timing




= Stopping Point

**Note:** Overnight hybridization should not exceed 17 hours.

# Important Guidelines

## 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 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:

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.

## Gene Panels

Gene Capture is compatible with the Immune1000 Panel and custom gene panels from Twist Bioscience®. 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 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 Capture kit includes the following boxes. Safety Data Sheets for these reagents can be provided upon request.

**Custom Gene Capture Hybridization Reagents,** store at -20°C, PN GC100

| 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 | GC104 | 1.5 mL tube | 1 |
|   | Enrichment Primer Mix | GC105 | 1.5 mL tube | 1 |
|   | Enrichment Amplification Mix | GC106 | 1.5 mL tube | 1 |

**Gene Capture Wash Reagents,** store at 4°C, PN GC300

| LABEL | ITEM | PN | FORMAT | QTY |
|---|---|---|---|---|
|   | Bead Wash Buffer A | GC301 | 1.5 mL tube | 1 |
|   | Bead Wash Buffer B | GC302 | 2 mL tube | 1 |
|   | Binding Buffer | GC303 | 5 mL tube | 1 |
|   | Streptavidin Binder Beads | GC304 | 1.5 mL tube | 1 |

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

**Immune1000 Gene Capture Hybridization Reagents,** store at -20°C, PN GC200

| 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 | GC104 | 1.5 mL tube | 1 |
|   | Enrichment Primer Mix | GC105 | 1.5 mL tube | 1 |
|   | Enrichment Amplification Mix | GC106 | 1.5 mL tube | 1 |
|   | Immune 1000 Panel | GC107 | 1.5 mL tube | 1 |

**Gene Capture Wash Reagents,** store at 4°C, PN GC300

| LABEL | ITEM | PN | FORMAT | QTY |
|---|---|---|---|---|
|   | Bead Wash Buffer A | GC301 | 1.5 mL tube | 1 |
|   | Bead Wash Buffer B | GC302 | 2 mL tube | 1 |
|   | Binding Buffer | GC303 | 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 |
|---|---|---|---|
| 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 | Or 8-channel pipettes can be substituted for 12-channel pipettes. |
| 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 |

| ITEM | SUPPLIER | PN | NOTES |
|---|---|---|---|
| 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 |
|---|---|---|---|
| AMPure® XP Reagent | Beckman Coulter® | A63880 (5 mL)<br>A63881 (60mL) | Choose one. We do not recommend substituting other magnetic beads. |
| KAPA® Pure Beads | Roche® | KK8000 (5 mL)<br>KK8001 (30mL) | |
| 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<br>17014963<br>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. |

| ITEM | SUPPLIER | PN | NOTES |
|---|---|---|---|
| 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 (Ampure XP or KAPA Pure Beads) 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 reaction | Thaw on ice. |
| ● Hybridization Mix | Gene Capture Hybridization Reagents (-20°C) | 1 | Heat at 65°C for 10 minutes, then keep at room temp. |
| ● Blocker Solution | Gene Capture Hybridization Reagents (-20°C) | 1 | Thaw on ice. Briefly centrifuge before use. |
| ● Evercode Blocker Solution | Gene Capture Hybridization Reagents (-20°C) | 1 | |
| ● Immune1000 Panel or Twist Bioscience Custom Panel | Gene Capture Hybridization Reagents (-20°C) | 1 | |

| ITEM | SOURCE | QTY | HANDLING AND STORAGE |
|---|---|---|---|
| Hybridization Enhancer | Gene Capture Hybridization Reagents (-20°C) | 1 | Thaw on ice. Briefly centrifuge before use. |

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 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 Capture reaction for a maximum of 800 ng of input.

**Note:** See Notes Before Starting 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.
12. Vortex the SPRI beads until fully mixed. Add 1.8x of SPRI beads to each library pool.

**Note:** For example, if a Library Pool is 100 µL, add (1.8 x 100 µL) = 180 µL of SPRI beads.

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<br>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. 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.

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

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.

# 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 (Ampure XP or KAPA Pure Beads) to room temperature for at least **30 minutes**.
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 |
|---|---|---|---|
| ● Streptavidin Binder Beads | Gene Capture Wash Reagents (-20°C) | 1 | Equilibrate at room temperature for at least 30 minutes. |
| ● Binding Buffer | Gene Capture Wash Reagents (-20°C) | 1 | Heat tube at 48°C for 5 minutes. Then keep at room temperature. |
| ● Bead Wash Buffer A | Gene Capture Wash Reagents (-20°C) | 1 | Heat tube at 68°C. |
| ● Bead Wash Buffer B | Gene Capture Wash Reagents (-20°C) | 1 | Heat tube at 48°C. |
| ● Enrichment Amplification Mix | Gene Capture Hybridization Reagents (-20°C) | 1 | Thaw on ice.<br>Briefly centrifuge before use. |
| ● Enrichment Primer Mix | Gene Capture Hybridization Reagents (-20°C) | 1 | Thaw at room temperature, then place on ice. |

6. Check if precipitate is dissolved, then equilibrate the Binding Buffer to room temperature. Keep the 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 Binding Buffer to the tube 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 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 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.

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 capture product.

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

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

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.

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

| AMPLIFICATION PROTOCOL | | | |
|---|---|---|---|
| Run Time | Lid Temperature | Sample Volume | |
| ~30 min | 105°C | 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.

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

8. 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.

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

10. 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**.

**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.

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).

15. Remove the tube(s) from the magnetic rack. Fully resuspend each bead pellet with **32 µL** of nuclease-free water.
16. Incubate the tube(s) at room temperature for **5 minutes**.
17. 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).

18. 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.

19. Proceed immediately to Section 2.3.

## 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 according to the manufacturer's instructions. See Figure 2 for the expected size distribution.

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

4. 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.

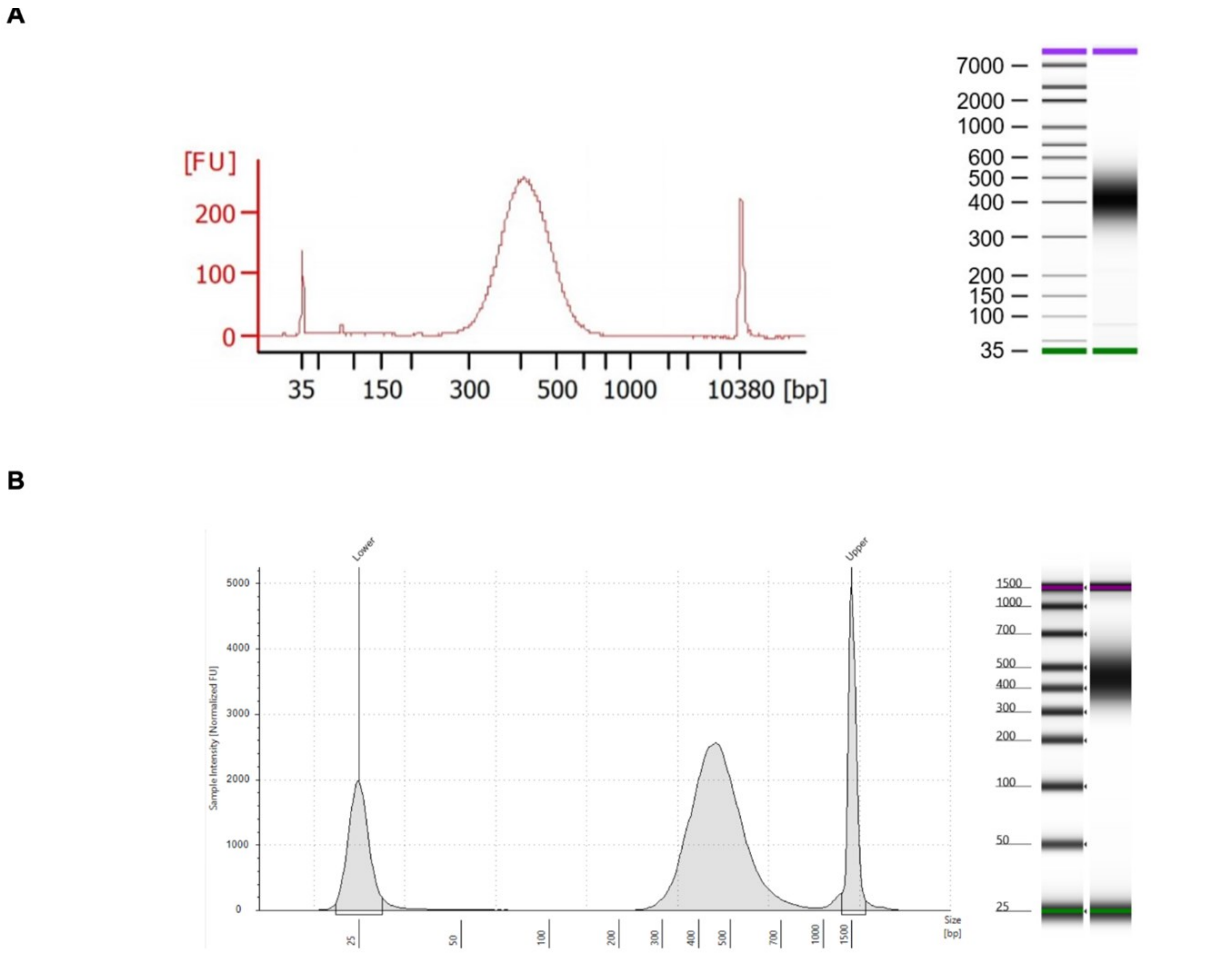



*Figure 2: Expected Size Distribution before Illumina Sequencing (A) Example trace of DNA from enriched sublibraries run on a Bioanalyzer. (B) Example trace of DNA from enriched sublibraries run on a TapeStation.*

**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.

# Appendix

## 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.

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