



Dovetail® Target Enrichment

USER GUIDE

VERSION 1.0

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Dovetail® Target Enrichment Kit Components and Storage

Each kit contains a sufficient supply of materials to perform 4 reactions. The Dovetail® Target Enrichment Kit comes as three boxes. Store the boxes as listed below immediately upon receipt.

Table 1. Dovetail® Target Enrichment Module 1 of 3 (shared across all target enrichment kits)

Component	Part Number	Storage
Streptavidin Binding Beads	CP1-EM-001	2°C to 8°C
DNA Purification Beads		
Binding Buffer		
Wash Buffer 1		
Wash Buffer 2		

Table 2. Dovetail® Hybridization Module 2 of 3 (shared across all target enrichment kits)

Component	Part Number	Storage
Universal Blockers	CP2-HM-001	-25°C to -15°C
Blocker Solution		
Hybridization Mix		
Hybridization Enhancer		
Amplification Primers		
Capture Amplification Mix		

Table 3. Dovetail® Enrichment Panel Module 3 of 3 (this module will consist of one of the panels below)

	Part Number	Storage
Dovetail® Human Pan Promoter Panel	CP3-PP-001	-25°C to -15°C
Dovetail® Mouse Pan Promoter Panel	CP3-PP-002	

User Supplied Reagents, Consumables and Equipment

Reagents

Table 4. Reagents

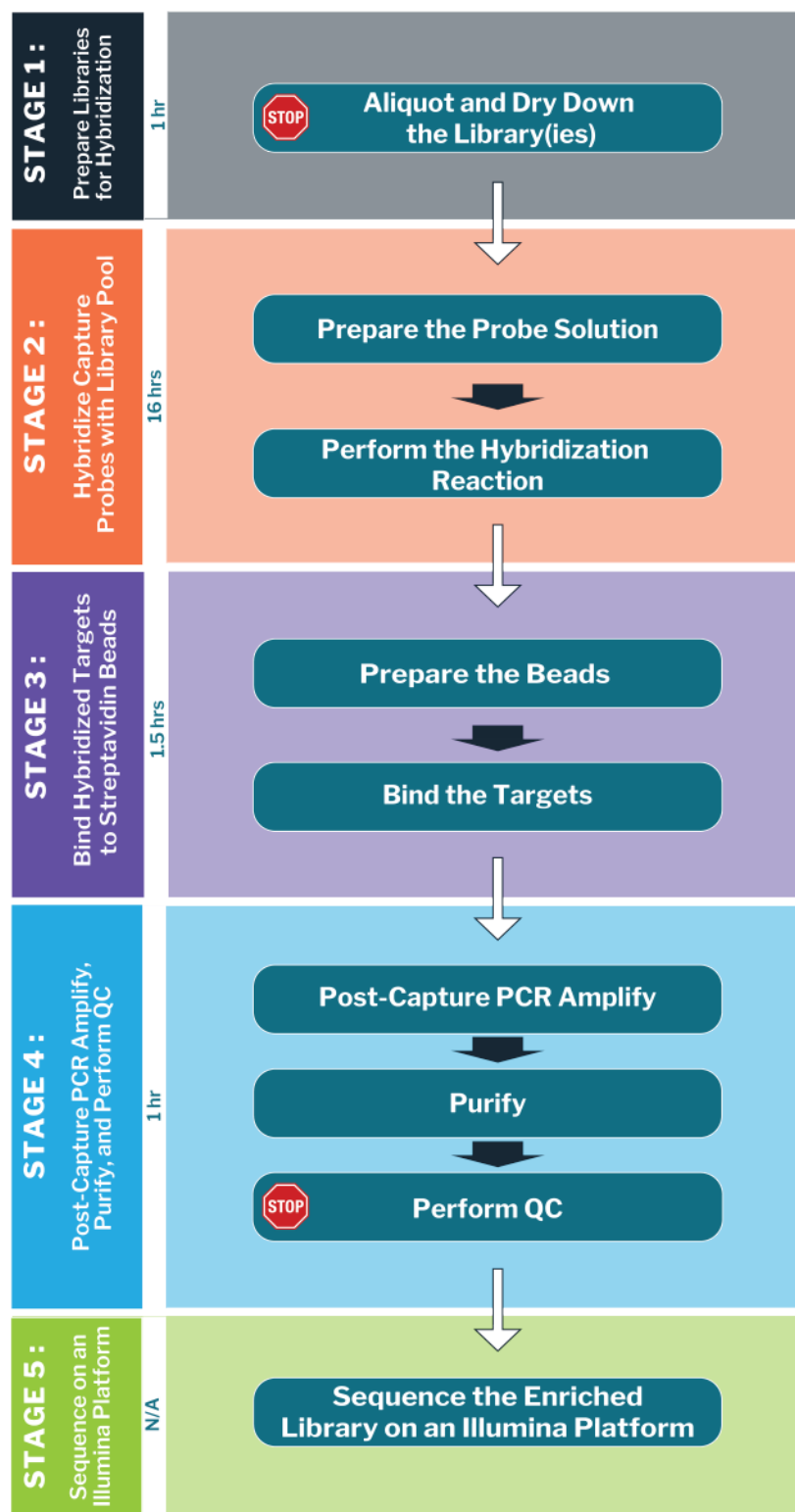
Reagent	Supplier
Ethanol (200 proof)	Generic
Molecular biology grade water	
TE buffer pH 8.0	

Consumables and Equipment

Table 5. Consumables and Equipment

Consumable/Equipment	Supplier
1.5 mL microcentrifuge tubes	Generic
Thin-walled 0.2 mL PCR tubes	
Magnetic separation rack for 1.5 mL tubes	
Pipettes and tips	
Agitating thermal mixer for 1.5 mL tubes	
Thermal cycler	
Vortex mixer	
Vacuum concentrator	
Centrifuge for 0.2 mL tubes	
Water bath	
Heat block	
Qubit Fluorometer	Thermo Fisher Scientific
Qubit dsDNA High Sensitivity Assay Kit	Thermo Fisher Scientific
TapeStation System (or Bioanalyzer)	Agilent Technologies
Agilent High Sensitivity D1000 Kit (or Agilent High Sensitivity DNA Kit)	Agilent Technologies

Dovetail® Target Enrichment Protocol Overview



Stage 1: Prepare Libraries for Hybridization

As you prepare for Stage 1, keep the following in mind:

- » This protocol supports a single library or pooled library (up to 8-plex) approach to the hybridization capture.
- » If you wish to multiplex libraries for hybridization capture, ensure the recommended pooling guidelines are followed as suggested in the Index Primers Appendix of the Dovetail® Omni-C™ Kit User Guide or the Dovetail® Micro-C Kit User Guide.
- » This stage involves aliquoting the appropriate library volume for hybridization (the library(ies) generated previously using Dovetail® Omni-C™ Kit or Dovetail® Micro-C Kit).

Before You Begin

- » Thaw Dovetail® Omni-C™ or Micro-C library(ies) at room temperature and store on ice.

Aliquot and Dry Down the Library

1. In the last step of Dovetail Omni-C or Micro-C assay, you quantified the concentration (ng/μL) of the library. Use this concentration and the table below to calculate the volume (μL) needed for each library that will be pooled prior to the hybridization reaction.

For example, if multiplexing eight libraries in a single hybridization reaction, the amount of each library will be 187.5 ng and the total mass of the pool will be 1,500 ng.

Number of Libraries Pooled Per Hybridization Reaction	Amount of Each Library	Total Mass Per Pool
1	500 ng	500 ng
2	500 ng	1,000 ng
3	500 ng	1,500 ng
4	375 ng	1,500 ng
8	187.5 ng	1,500 ng



NOTES

- » If the amount of library you have is insufficient, you can proceed with a smaller amount, however, this may result in decreased library complexity.
2. For each hybridization reaction, pool the libraries by transferring the above-calculated volumes for each library into a clean thin-walled 0.2 mL PCR tube.
 3. Quick-spin the 0.2 mL tube containing the pooled libraries to minimize the amount of bubbles present.

STAGE 1: PREPARE LIBRARIES FOR HYBRIDIZATION

4. Dry the library pool using a vacuum concentrator using low or no heat.



SAFE STOP If not proceeding immediately to Stage 2, store the dried library pool at -20°C for up to 24 hours.

5. Proceed to Stage 2: Hybridize Capture Probes with Library Pool.

Stage 2: Hybridize Capture Probes with Library Pool

As you prepare for Stage 2, keep the following in mind:

- » This stage involves performing the hybridization reaction of the promoter panel with the pooled libraries from Stage 1.

Before You Begin

- » Test the compatibility of your thermal cycler and PCR tube by incubating a test PCR tube with 50 μ L of H₂O at 95°C for up to 5 minutes to ensure it does not crack under heat and pressure.
- » Thaw the following reagents on ice. Vortex to mix for 2 seconds and quick-spin prior to use.
 - » Dried pooled libraries from Stage 1, step 4 (if stored at -20°C)
 - » Pan Promoter Panel
 - » Hybridization Mix
 - » Hybridization Enhancer
 - » Universal Blockers
 - » Blocker Solution
- » Set a heat block to 65°C.
- » Program a thermal cycler to 95°C and set the heated lid to 105°C.

2.1 Prepare the Probe Solution

1. Heat the Hybridization Mix at 65°C in the heat block for 10 minutes, or until no precipitate is visible.
2. Cool to room temperature on the benchtop for 5 minutes.
3. For each hybridization reaction, prepare a probe solution (28 μ L) in a clean thin-walled 0.2 mL PCR tube as indicated in the table below. Mix by pipetting up and down. Set aside at room temperature to be used later in 2.2.

Reagent	Volume
Hybridization Mix	20 μ L
Pan Promoter Panel	4 μ L
Water	4 μ L
Total	28 μL



NOTE Hybridization Mix is very viscous. Pipette slowly to ensure accurate pipetting.

4. Resuspend the dried library pool (from Step 4 in Stage 1) by adding the reagents (12 μ L) described below. Mix by pipetting up and down. Set aside at room temperature to be used later in 2.2.

Reagent	Volume
Dried Library Pool	N/A
Blocker Solution*	5 μ L
Universal Blockers	7 μ L
Total	12 μL

***If using mouse promoter panel, replace with Mouse Blocker Solution.**

2.2 Perform the Hybridization Reaction

1. Heat the tube containing the 28 μ L probe solution ([step 3, in 2.1 Prepare The Probe Solution](#)) to 95°C for 2 minutes in a thermal cycler with the lid at 105°C.
2. Immediately cool on ice for 5 minutes.
3. While the probe solution is cooling on ice, heat the tube containing the 12 μ L resuspended library pool at 95°C for 5 minutes in a thermal cycler with the lid at 105°C.
4. Equilibrate both the probe solution and resuspended library pool to room temperature on the benchtop for 5 minutes.
5. Quick-spin both tubes.
6. Carefully add the probe solution to the resuspended library pool. Vortex to mix. This tube now contains your hybridization reaction.



NOTE If the hybridization reaction tube is warped, transfer the mixture to a new 0.2 mL tube.

7. Quick-spin the tube to ensure all solution is at the bottom.
8. Add 30 μ L of Hybridization Enhancer to the tube.
9. Quick-spin the tube to ensure there are no bubbles present.



NOTE Check the seal on the tube and seal the tube tightly to prevent excess evaporation over the 16-hour incubation to prevent decreased performance.

10. Incubate the tube at 70°C for 16 hours in a thermal cycler with the lid at 85°C.
11. Proceed to Stage 3: Bind Hybridized Targets to Streptavidin Beads.

Stage 3: Bind Hybridized Targets to Streptavidin Beads

As you prepare for Stage 3, keep the following in mind:

- » This stage involves pulling down the biotinylated hybridization products with paramagnetic streptavidin beads.

Before You Begin

- » Preheat the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 at 48°C until no precipitate is visible.
- » When no precipitate is visible, equilibrate the Binding Buffer and Wash Buffer 1 to room temperature. Transfer 700 µL of Wash Buffer 2 into a new 1.5 mL tube and keep it heated at 48°C.
- » Equilibrate the Streptavidin Binding Beads to room temperature for at least 30 minutes.

3.1 Prepare the Beads

1. Vortex the Streptavidin Binding Beads vial thoroughly to resuspend the beads.
2. Transfer 100 µL of the resuspended Streptavidin Binding Beads to a new 1.5 mL tube.
3. Place the 1.5 mL tube containing the beads in the magnetic rack for 5 minutes (or until the solution looks clear). Discard the supernatant.
4. Remove the tube from the magnetic rack and wash the beads with 200 µL of Binding Buffer pipetting up and down to resuspend the beads. Place the tube in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant. Make sure to not disturb the bead pellet.
5. Repeat step 4 twice, for a total of three washes.
6. After removing the supernatant from the third wash, remove the tube from the magnetic rack and resuspend the beads in 200 µL of Binding Buffer. Pipet up and down to fully mix.

3.2 Bind the Hybridized Targets

1. After the hybridization step ([step 10 of Stage 2.2 Perform the Hybridization Reaction](#)) is complete, open the thermal cycler lid and directly transfer the hybridization reaction to the 1.5 mL tube containing the Streptavidin Binding Beads resuspended in 200 µL of Binding Buffer. Mix by pipetting up and down.



NOTE Rapid transfer directly from the thermal cycler at 70°C is a critical step for minimizing off-target binding. Do not remove the hybridization reaction tube from the thermal cycler or otherwise allow it to cool to less than 70°C before transferring the solution to the Streptavidin Binding Beads suspended in 200 µL of Binding Buffer.

2. Incubate the tube at 25°C for 30 minutes in an agitating thermal mixer set at 1,250 rpm.



NOTE Do not vortex. Aggressive mixing is not required.

3. Quick-spin the tube to ensure all solution is at the bottom.
4. Place the tube in the magnetic rack for 1 minute (or until the solution looks clear).
5. Remove and discard the supernatant. Do not disturb the bead pellet.



NOTE Some Hybridization Enhancer may be visible after supernatant removal and throughout each wash step. It will not affect the final capture product.

6. Remove the tube from the magnetic rack and resuspend the beads in 200 μ L of Wash Buffer 1. Pipet up and down to fully mix.
7. Quick-spin the tube to ensure all solution is at the bottom.
8. Transfer the entire volume (~ 200 μ L) into a new 1.5 mL tube.



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

9. Place the tube in the magnetic rack for 1 minute (or until the solution looks clear).
10. Remove and discard the supernatant. Make sure to not disturb the bead pellet.
11. Remove the tube from the magnetic rack and resuspend the beads in 200 μ L of 48°C Wash Buffer 2. Pipet up and down to fully mix.



NOTE Remember to place the remaining Wash Buffer 2 (~ 500 μ L) back at 48°C.

12. Quick-spin the tube to ensure all solution is at the bottom.
13. Incubate the tube for 5 minutes at 48°C.
14. Place the tube in the magnetic rack for 1 minute (or until the solution looks clear).
15. Remove and discard the supernatant. Make sure to not disturb the bead pellet.
16. Repeat steps 11 to 15 twice, for a total of three washes.
17. After the final wash, quick-spin the tube and place it in the magnetic rack for 1 minute. Use a 10 μ L pipette tip to remove all traces of Wash Buffer 2. Proceed immediately to the next step. **Do not allow the beads to dry.**
18. Remove the tube from the magnetic rack and resuspend the beads in 45 μ L of molecular biology grade water. Pipet up and down to fully mix.
19. Incubate this solution on ice. Hereafter, this solution is referred to as the Streptavidin Binding Bead Slurry.
20. Proceed to Stage 4: Post-Capture PCR Amplify, Purify and Perform QC.

Stage 4: Post-Capture PCR Amplify, Purify, and Perform QC

As you prepare for Stage 4, keep the following in mind:

- » This Stage involves PCR amplification of the biotinylated hybridized targets followed by DNA purification and QC.

Before You Begin

- » Equilibrate DNA Purification Beads to room temperature for at least 30 minutes.
- » Thaw on ice the Capture Amplification Mix and Amplification Primers. Vortex for 2 seconds to mix and quick-spin prior to use.
- » Prepare 500 µL of 80% ethanol.

4.1 Post-Capture PCR Amplify

1. Program a thermal cycler with the following conditions. Set the heated lid to 105°C.

Step	Temperature	Time	Cycles
Enzyme Activation	98°C	45 seconds	1
Denature	98°C	15 seconds	8 (if multiplex) 9 (if singleplex)
Anneal	60°C	30 seconds	
Extend	72°C	30 seconds	
Extend	72°C	1 minute	1
Hold	4°C	Hold	

2. If the Streptavidin Binding Bead Slurry has settled, pipet up and down to fully mix.
3. Transfer 22.5 µL of the Streptavidin Binding Bead Slurry to a thin-walled 0.2 mL PCR tube. Keep on ice until ready to use in the next step. Store the remaining 22.5 µL of Streptavidin Binding Bead Slurry at –20°C for future use.
4. Prepare a PCR mixture by adding the following reagents to the tube containing the Streptavidin Binding Bead slurry. Pipet up and down to fully mix.

Temperature	Volume Per Reaction
Streptavidin Binding Bead Slurry	22.5 µL
Amplification Primers	2.5 µL
Capture Amplification Mix	25 µL
Total	50 µL

5. Quick-spin the tube, place in the thermal cycler and start the cycling program ([set in step 1 of 4.1](#)).
6. When the thermal cycler program is complete, remove the tube from the block and immediately proceed to 4.2: Purify step.

4.2 Purify

1. Vortex the DNA Purification Beads vial thoroughly to resuspend the beads.
2. Add 50 μ L of the DNA Purification Beads to the tube from [step 6 of 4.1](#). Vortex to mix.



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

3. Incubate for 5 minutes at room temperature.
4. Place the tube in the magnetic rack for 1 minute (or until the solution looks clear).
5. **Leave the tube in the magnetic rack** and aspirate and discard the supernatant.
6. **Leave the tube in the magnetic rack** and wash the beads with 200 μ L of freshly prepared 80% ethanol. Do not resuspend the beads for this wash. Simply add the ethanol, wait for 1 minute then discard the ethanol supernatant.
7. Repeat step 6 once, for a total of two washes.
8. Quick-spin the tube and place it in the magnetic rack for 1 minute. Use a 10 μ L pipette tip to remove any traces of ethanol.
9. Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains. **Do not over dry the beads.**
10. Remove the tube from the magnetic rack and resuspend the beads in 32 μ L TE Buffer pH 8.0 (you can use the TE Buffer supplied in the Dovetail® Omni-C™ or Micro-C kit). Pipet up and down to fully mix.
11. Incubate at room temperature for 2 minutes.
12. Place the tube in the magnetic rack for 3 minutes (or until the solution looks clear).
13. Transfer 30 μ L of the **SUPERNATANT** (the enriched library) to a clean 1.5 mL tube. Discard the beads.



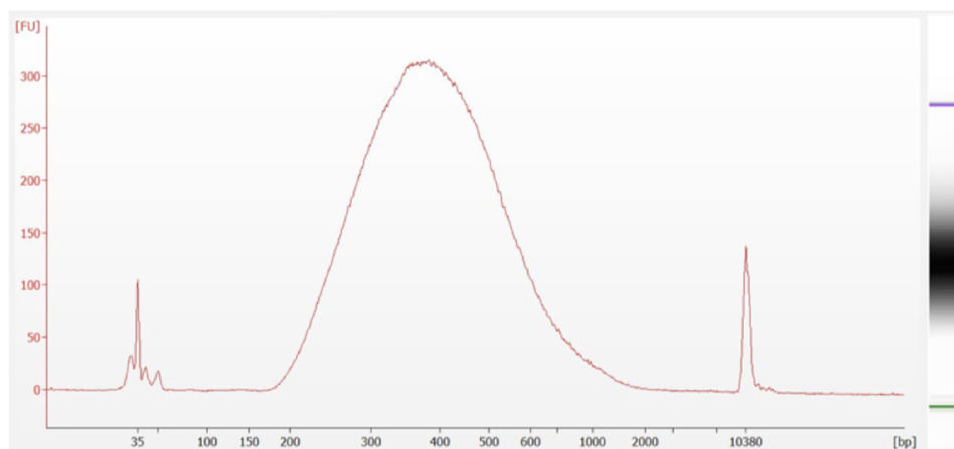
SAFE STOP The library can be stored at -20°C for up to 6 months.

4.3 Perform QC

1. Quantify your enriched library using a Qubit Fluorometer and Qubit dsDNA HS Kit. The final concentration is typically ≥ 15 ng/ μ L but depends on the number of pooled libraries per hybridization reaction.
2. Use a TapeStation or Bioanalyzer to verify the size distribution of your enriched library (see below for the recommended kit for each system). The size range is expected to be between 150 bp and 1,000 bp (Figure 1) with an average fragment length of 375 – 425 bp.
When using the Agilent TapeStation HS D1000 ScreenTape, make sure your sample is diluted to 1 ng/ μ L to avoid overloading the tape. When using the Agilent Bioanalyzer HS DNA kit, load 0.5 μ L of the final sample.

System	Recommended Kit
TapeStation	High Sensitivity D1000
Bioanalyzer	High Sensitivity DNA

Figure 1. Electropherogram generated by an Agilent High Sensitivity DNA analysis of the enriched library. Note the single prominent peak.



Stage 5: Sequence on an Illumina Platform

Sequence the enriched library on an Illumina platform in paired-end mode. We recommend 150 M read pairs (2 x 150 bp) per sample. Cantata Bio provides all kit users with access to QC analysis pipeline available on readthedocs (<https://dovetail-capture.readthedocs.io/en/latest/index.html>).