
# Dovetail® Pan Promoter Kit for LinkPrep™ Kit

## USER GUIDE

VERSION 1.0

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# Kit Components and Storage

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

**Table 1. Dovetail® Target Enrichment Module (shared across all pan promoter 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 for LinkPrep™ Kit (shared across all pan promoter kits)**

| Component | Part Number | Storage |
|---|---|---|
| Nextera Blocker | CP2-HM-002 | -25°C to -15°C |
| Blocker Solution | | |
| Hybridization Mix | | |
| Hybridization Enhancer | | |
| Amplification Primers | | |
| Capture Amplification Mix | | |

**Table 3. Dovetail® Promoter Enrichment Panel (this module will consist of one of the panels below depending on whether the human or mouse pan promoter kit is used)**

| Component | 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**

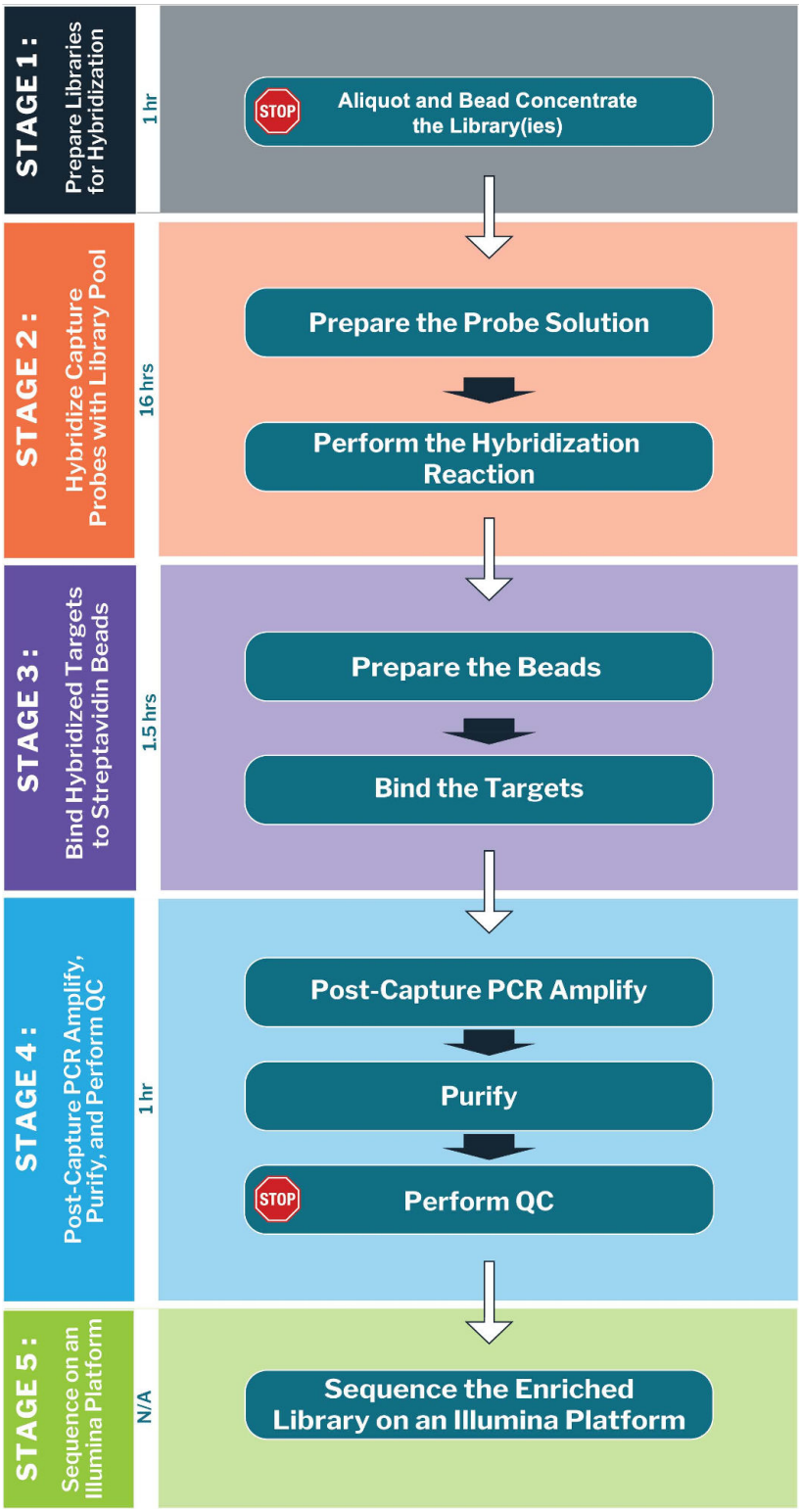
| Reagent | Supplier |
|---|---|
| Ethanol (200 proof) | Generic |
| Molecular biology grade water | |
| TE buffer pH 8.0 | |

## 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 | |
| Centrifuge for 0.2 mL tubes | |
| Water bath | |
| Heat block | |
| SPRIselect Beads | Beckman Coulter (B23317) |
| 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 |

# 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 libraries (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 indicated in the Dual Index Primers Appendix of the Dovetail® LinkPrep™ Kit User Guide.
- This stage involves aliquoting the appropriate library volume for hybridization (the library(ies) generated previously using Dovetail® LinkPrep™ Kit).

## Before You Begin

- Thaw Dovetail® LinkPrep™ library(ies) at room temperature and store on ice.

## Aliquot and Bead Concentrate the Library

1. In the last step of Dovetail LinkPrep 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 1.5 mL tube.

**NOTES**

- If the total volume of the library pool is 5 µL, transfer the pool from the 1.5 mL tube to a 0.2 mL tube and proceed to Stage 2 (i.e. skip steps 3-16).
- If the total volume of the library pool is < 5 µL, bring the volume up to 5 µL using molecular biology grade water then transfer the pool from the 1.5 mL tube to a 0.2 mL tube and proceed to Stage 2 (i.e. skip steps 3-16).
- If the total volume of the library pool is > 5 µL, continue to steps 3-16 to bead concentrate the pool then proceed to Stage 2.

3. Quick spin the 1.5 mL tube containing the pooled libraries to minimize the amount of bubbles present.
4. Vortex the SPRIselect beads thoroughly (>30 seconds) to resuspend.
5. Add 1.8x resuspended SPRIselect beads to the 1.5 mL tube containing the DNA library(ies).
6. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
7. Incubate the tube at room temperature, off the magnetic rack, for 5 minutes.
8. Quick spin the tube and place it in the magnetic rack for 5 minutes or until the solution is clear. Discard the supernatant.
9. Leave the tube in the magnetic rack and wash the beads **twice** with 200 µL fresh 80% ethanol. Do not resuspend the the beads for these washes. Add the ethanol, wait for 1 minute then discard the supernatant.
10. After the last wash, quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10 µL pipet tip to remove all traces of ethanol.
11. Air dry the beads for 3 minutes in the magnetic rack until no residual ethanol remains. **Do not over dry the beads.**
12. Off the magnetic rack, resuspend the beads in 6 µL of molecular biology grade water.
13. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the the beads start to settle).
14. Incubate at room temperature, off the magnetic rack, for 2 minutes.
15. Quick spin the tube and place it in the magnetic rack for 1 minute.
16. Transfer 5 µL of the supernatant (concentrated library(ies)) to a new 0.2 mL PCR tube.
17. Proceed to Stage 2.

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

# 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 µL of $H_2O$ 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.
  - **Library pool from Stage 1 (if stored at -20°C)**
  - Pan Promoter Panel
  - Hybridization Mix
  - Hybridization Enhancer
  - Nextera Blocker
  - 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 µL) 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 µL |
| Pan Promoter Panel | 4 µL |
| Water | 4 µL |
| **Total** | **28 µL** |



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

4. Add to the library pool (from Stage 1) the reagents described below. Mix by pipetting up and down. Set aside at room temperature to be used later in 2.2.

| Reagent | Volume |
|---|---|
| Library Pool | 5 µL |
| Blocker Solution* | 5 µL |
| Nextera Blocker | 2 µL |
| **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 µL 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 µL library pool/blocker mixture at 95°C for 5 minutes in a thermal cycler with the lid at 105°C.
4. Equilibrate both the probe solution and the 12 µL library pool/blocker mixture to room temperature on the benchtop for 5 minutes.
5. Quick-spin both tubes.
6. Carefully add the probe solution to the library pool/blocker mixture. 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 µL 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 µL 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 µL) 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 µL of 48°C Wash Buffer 2. Pipet up and down to fully mix.

> **NOTE** Remember to place the remaining Wash Buffer 2 (~ 500 µL) 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 µL 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 µL 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)<br>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 µL 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 µL 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 µL 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 µL TE Buffer pH 8.0. 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 µL of the **SUPERNATANT** (the enriched library) to a clean 1.5 mL tube. Discard the beads.

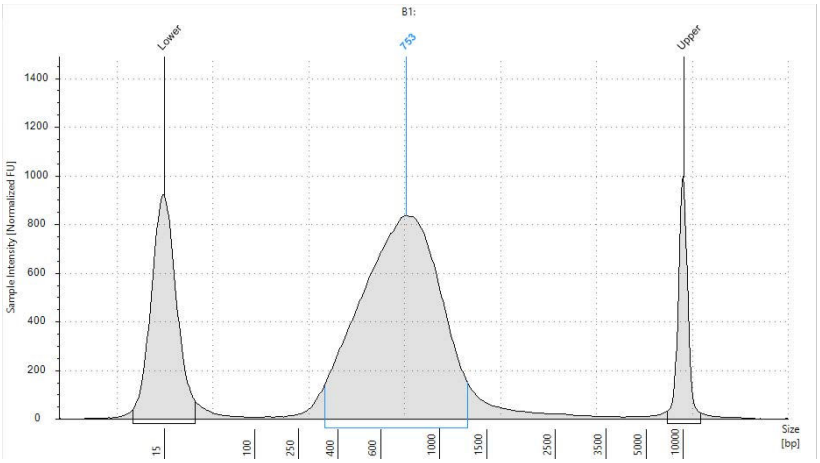
> **SAFE STOP** The library can be stored at -20°C for up to 6 months.

## 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/µL 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/µL to avoid overloading the tape. When using the Agilent Bioanalyzer HS DNA kit, load 0.5 µL of the final sample.

| System | Recommended Kit |
|---|---|
| TapeStation | High Sensitivity D1000 |
| Bioanalyzer | High Sensitivity DNA |

**Figure 1. Electropherogram of the enriched library.**




# 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).