One-Pot Hybridization Reaction Protocol

For use with the Twist NGS Workflow

This document outlines an alternative hybridization reaction protocol that replaces the “Hybridize Capture Probes with Pools” step for Twist Standard Hyb v1, Standard Hyb v2, and FlexPrep Target Enrichment workflows. It is designed to limit the number of transfer steps required for the setup of capture reactions while maintaining high performance. This protocol can be used in conjunction with the alternative bead-based concentration dry down method or the SpeedVac dry down method. On automation, we recommend using both the bead-based concentration dry down method and this one-pot dry down method.

The user is encouraged to compare samples generated with this one-pot protocol directly to the standard hybridization reaction setup to ensure that the desired sequencing metrics are unaffected for the given sample input.

Please refer to the following links for updated versions of the standard protocols:

- **Target Enrichment Standard Hyb v1:** twistbioscience.com/resources/protocol/twist-target-enrichment-standard-hybridization-v1-protocol
- **Target Enrichment Standard Hyb v2:** twistbioscience.com/resources/protocol/twist-target-enrichment-standard-hybridization-v2-protocol
- **FlexPrep Target Enrichment:** twistbioscience.com/resources/protocol/twist-flexprep-target-enrichment-protocol

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PREPARE THE PRE-HYBRIDIZATION SOLUTION

Reagents Required

- Indexed library pool(s) from Step titled “Hybridize Capture Probes with Pools”
- Twist fixed or custom panel
- Twist custom secondary (spike-in) panel(s) (optional)
- From Twist Hybridization Reagents (or similarly named box):
  - Hybridization Mix
  - Hybridization Enhancer
- Twist Universal Blockers:
  - Universal Blockers
  - Blocker Solution (If using a non-human capture panel, replace with species-specific blocking solution, not provided)
  - ⚠ IMPORTANT: If using the FlexPrep target enrichment protocol or a bead-based concentration method, these reagents were already added to the library pool solution in a previous step. Omit the addition of these reagents in Step 3 below.

Before You Begin

- Thaw all required reagents on ice, then pulse-vortex for 2 seconds to mix and pulse-spin.
- Set a heat block to 65°C.

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Program a thermal cycler with the following conditions. Set the temperature of the heated lid to 85°C.

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
1	95°C	5 min	1
2	70°C	HOLD	—

2

Heat the Hybridization Mix at 65°C for 10 minutes, or until all precipitate is dissolved, then cool to room temperature on the benchtop for 5 minutes

3

Resuspend the Dried Indexed Library Pool by adding the reagents described below. Mix the entire Hybridization Reaction thoroughly by pipetting, making sure to not generate bubbles. Pulse-spin to ensure all solution is at the bottom of the tube.

NOTES: For users adding optional secondary panel (spike-in) content, add 4 µl of probes in place of water. The Hybridization Mix is very viscous. Pipette slowly to ensure accurate pipetting volume.

⚠ IMPORTANT: If using a bead-based concentration protocol, Blocker Solution and Universal Blockers have been added in a previous Step. In this case, do not add additional amounts of these reagents.

REAGENT	VOLUME PER REACTION*
Dried Indexed Library Pool	—
Blocker Solution*	5 µl
Universal Blockers	7 µl
Hybridization Mix	20 µl
Twist Fixed or Custom Panel	4 µl
Optional: Secondary Panel (in place of water)	4 µl
Water (up to total volume)	(0–4) µl
Total	**40 µl**

* ⚠ IMPORTANT: If using a non-human capture panel, replace with species-specific blocking solution, not provided.

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Add 30 µl Hybridization Enhancer to the top of the entire capture reaction. Pulse-spin the tube(s) to ensure there are no bubbles present.

⚠ IMPORTANT: Make sure the sample is tightly sealed to prevent excess evaporation over the 16-hour incubation.

5

Transfer the Hybridization Reaction into the thermal cycler and start the thermal cycler program. Incubate for 16 hours at the 70°C HOLD step.

PROCEED DIRECTLY TO THE STEP ENTITLED "BIND HYBRIDIZED TARGETS TO STREPTAVIDIN BEADS" IN THE TARGET ENRICHMENT PROTOCOL BEING USED.