firefly® technical note

Twist Target Enrichment Standard Hybridization v1 Workflow

Overview

- **Twist Target Enrichment Standard Hybridization v1 Workflow** (Twist Hybridization and Target Capture Protocol, Manual DOC-001240 REV 3.0)
- The Twist Target Enrichment workflow enables high-specificity capture of genomic regions of interest through probe-based hybridisation, streptavidin bead binding, and post-capture amplification. The workflow supports both fixed and custom probe panels, delivering robust enrichment performance with high on-target rates, low off-target capture, and uniform coverage profiles.
- This automated method was developed for **12 pooled hybridisation reactions** using Eppendorf twin.tec PCR plates and the **Alpaqua Magnum FLX magnet**. The use of alternative labware may require optimisation. The protocol is validated for firefly software version **1.9.x**.
- **Instrument configuration:** firefly, 6-head Genomics configuration
- **Development software version:** v1.9.x
- **Deviation(s) from the published Twist method**:
  - Reservoir volumes have been adjusted to accommodate firefly reservoir dead volume. Updated reservoir calculations are provided in **Table C**.
  - Ethanol volumes during post-capture cleanup have been optimised to allow **single-transfer removal** using **125 µL tips**, improving wash efficiency and reducing tip consumption.
  - Hybridisation, wash, and binding temperatures are executed through firefly thermal modules and the heated shaker, resulting in minor timing adjustments to maintain correct thermal conditions.

firefly protocols

Protocol number	Protocol name	firefly run time (minutes)	Thermocycler run time (minutes)
1 of 4	Protocol 4: Hybridise Capture Probes with Pools	~7 minutes	16 hours at 70 °C (twist_robot_hyb)
2 of 4	Protocol 5: Prepare Streptavidin Beads	~38 minutes	None
3 of 4	Protocol 6: Bind the Targets	~120 minutes	None
4 of 4	Protocol 7: Post-capture PCR & Purification	~46 minutes	PCR: 9–12 minutes + plate drying time

Table 1. Overview of protocols to process 12 hybridisation pools using the Twist Target Enrichment workflow on SPT Labtech's firefly. Actual run times may vary depending on user and laboratory conditions.

Input variables

This workflow does not require any user-defined input variables.

All parameters used in the automated hybridisation, bead preparation, target binding, and post-capture PCR workflows are fixed within the supplied firefly protocols.

If you require a different input configuration, please contact **SPT Labtech fireflysupport**.

sptlabtech

Master Mix Calculations – Reservoir Volumes

Protocol	Reagent	Total Required Volume (ul)
5 – Bead Prep	Binding Buffer	10,080*
5 – Bead Prep	Streptavidin Binding Beads	1,440
6 – Bind Targets	Wash Buffer 2	8,400*
6 – Bind Targets	Wash Buffer 1	2,880*
6 – Bind Targets	Nuclease-Free Water	1,000
7 – Post-Capture PCR	PCR Mix	405
7 – Post-Capture PCR	Water	700
7 – Post-Capture PCR	SPRI Beads	850
7 – Post-Capture PCR	80% Ethanol	6,080*

Table 2. Master mix volumes required to run the Twist Target Enrichment Standard Hybridization v1 workflow on the SPT Labtech firefly liquid handler. Values marked with * indicate reagents that are distributed across multiple reservoirs. For alternative input requirements, please contact SPT Labtech fireflysupport.

Consumables and Accessories

Pipette Head Consumable Type	Product Number(s)	Protocol 4	Protocol 5	Protocol 6	Protocol 7
100 µL Tips (Filtered), EZ-Load Strip Tips	125-008-EZ-FS	6×*	8×*	14×*	14×*

Table 3. Pipette Head consumables required for each protocol of the Twist Target Enrichment Standard Hybridization v1 workflow on the SPT Labtech firefly liquid handler.
*Each count refers to individual EZ-Load strips (8 tips per strip). One box of 125-008-EZ-FS contains 12 strips; therefore, the total number of strips used across this workflow equals the equivalent of approximately 3 full boxes.

Dispense Head Consumable Type	Product Number(s)	Protocol 4	Protocol 5	Protocol 6	Protocol 7
Syringes (Ultra-low Retention)	4150-07208	—	—	—	—
Syringes (Standard)	4150-07200	—	3×	—	5×
Reservoirs (Standard)	4150-07103	—	3×	4×	2×
Reservoirs (Low Dead Volume)	4150-07202	—	—	1×	3×

Table 4. Dispense Head consumables required for each protocol, based on reservoir usage extracted from the automated firefly workflows for the Hybridisation method.

Plate Type	Product Number(s)	Protocol 4	Protocol 5	Protocol 6	Protocol 7
Eppendorf twin.tec 96-well PCR Plate	0030128648	3×*	—	3×*	3×*
Abgene 1.2 mL Deepwell Plate	AB-0661	—	1×	1×	—
Abgene 0.8 mL Deepwell Plate	AB-0765	—	1×	1×	1×
PCR Plate (96-well)	N/A	—	—	—	1×

Table 5. Table detailing the types and quantities of plates required for each protocol.
*Plate counts reflect the number of plates used within each protocol. Some plates may be counted more than once, as they serve as the final plate for one protocol and the starting plate for the subsequent protocol.

Accessory Type	Product Number(s)	Protocol 4	Protocol 5	Protocol 6	Protocol 7
Tip Stand	3276-08075	3×	4×	7×	7×
Tip Loading Cassette	FFY-A-01-EZL-SL-5	3×	4×	7×	7×
Strip Tip Insert – 8-Channel	FFY-A-01-EZL-096-SC-8	3×	4×	7×	7×
Thermal Adapter for PCR Plate (96-well)	3276-01065	1×	—	—	—
Alpaqua Magnum FLX Magnet	A000400	—	1×	1×	1×

Table 6. This table lists the accessories needed for each protocol, with quantities based on column usage.

Workflow overview

Step numbers in protocol descriptions refer to steps in the Twist Target Enrichment Standard Hybridization v1 Protocol User Guide.

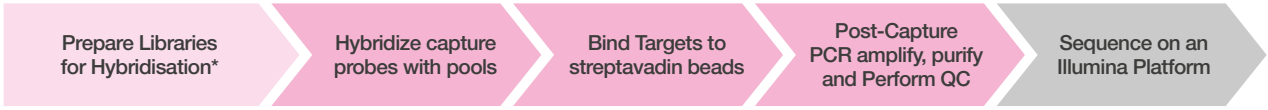

Figure 1. Workflow overview for automating the Twist Target Enrichment Standard Hybridization v1 workflow on firefly. Steps shown in bright pink indicate processes automated by firefly. The pale pink step (*) represents library preparation performed prior to this workflow (see Twist Library Preparation EF 2.0 technote). The grey step represents downstream sequencing performed off instrument.

Workflow details

Step numbers in protocol descriptions refer to steps in the Twist Protocol User Guide.

Protocol 1 of 4 – Step 1: Hybridise Capture Probes With Pools

This protocol performs probe hybridisation by combining pooled libraries with the Twist Probe Mix, Blocker Mix, and Hybridisation Enhancer, followed by sealed incubation to allow probe–target hybrid formation according to the Twist protocol.

Prior to executing this protocol:

- **1.1** – Program the thermal cycler with the hybridisation conditions specified in the manual (e.g., 95°C denaturation followed by hybridisation at 70°C for the required duration).
- **1.2** – Prepare the Probe Mix and Blocker Mix according to the Twist protocol.
- **1.3** – Ensure pooled libraries are dried down and resuspended per Twist recommendations before combining with Probe Mix.

Protocol 2 of 4 – Step 2: Prepare Streptavidin Beads

This protocol prepares streptavidin-coated magnetic beads for capture by washing them in Binding Buffer and equilibrating them according to the Twist workflow, ensuring bead performance and specificity for the subsequent target-binding step.

Prior to executing this protocol:

- **2.1** – Remove streptavidin beads from cold storage and allow them to equilibrate to room temperature for at least 30 minutes.
- **2.2** – Prepare the appropriate volume of Binding Buffer following Twist specifications.
- **2.3** – Ensure magnets and deepwell plates are correctly positioned on the deck.

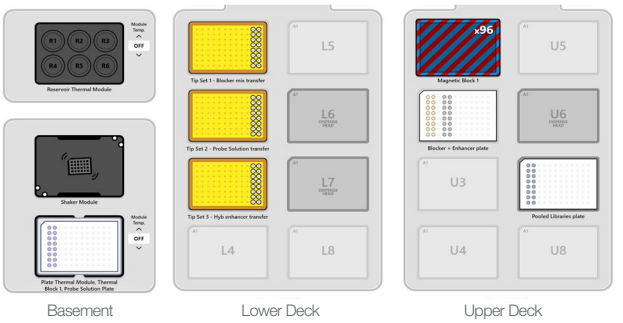

Figure 2. Protocol 1 of 4 – Step 1: Hybridise Capture Probes with Pools initial deck layout.

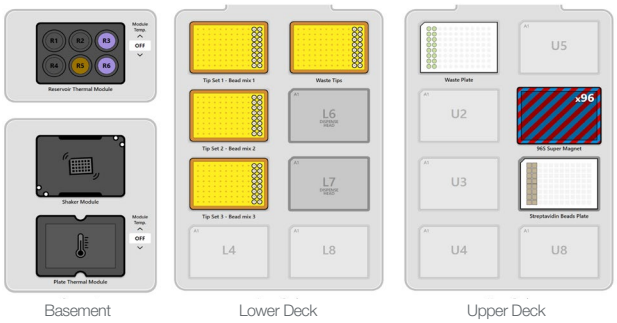

Figure 3. Protocol 2 of 4 – Step 2: Prepare Streptavidin Beads initial deck layout.

Protocol 3 of 4 – Step 3: Bind the Targets

This protocol performs the capture of hybridised DNA fragments by binding them to streptavidin-coated beads, followed by a series of washes at Twist-recommended temperatures to remove nonspecific molecules and enrich for the desired targets.

Prior to executing this protocol:

- **3.1** – Ensure hybridisation plates from Protocol 1 are held at the correct temperature prior to transfer.
- **3.2** – Preheat Wash Buffer 2 to 48°C as required by the Twist protocol.
- **3.3** – Confirm all wash buffers have been loaded into the correct reservoirs on the firefly deck.

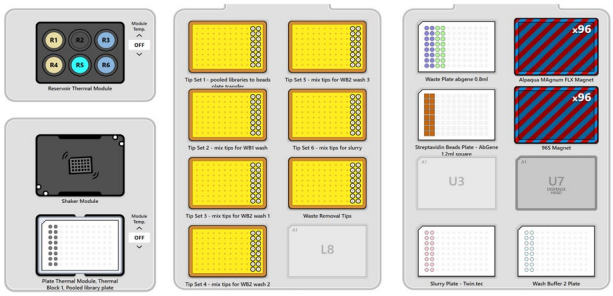

Figure 4. Protocol 3 of 4 – Step 3: Bind the Targets initial deck layout.

Protocol 4 of 4 – Step 4: Post-capture PCR Amplification and Purification

This protocol amplifies the enriched libraries recovered from the bead capture step using Twist-recommended PCR conditions, followed by SPRI cleanup to generate purified post-capture libraries suitable for QC and sequencing.

Prior to executing this protocol:

- **4.1** – Program the thermal cycler using the PCR conditions specified within the Twist user manual.
- **4.2** – Prepare PCR Master Mix and ensure correct loading of SPRI beads, ethanol, and elution volumes into reservoirs.
- **4.3** – Confirm that the PCR plate and thermal adapter are properly seated.

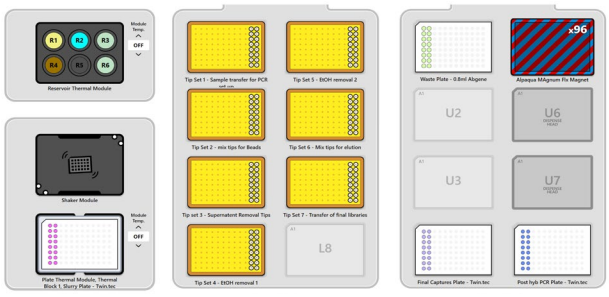

Figure 5. Protocol 4 of 4 – Step 4: Post-capture PCR Amplification and Purification initial deck layout.

For questions regarding SPT Labtech's firefly, contact our support team at fireflysupport@sptlabtech.com

Appendix: Full Consumables List

Supplier	Part Name	Part Number	Hybridisation Workflow (12 Pools)
SPT Labtech	firefly® EZ-Load Strip Tips, 100 µL, Filtered, Sterile (8 tips per strip)	125-008-EZ-FS	42 strips*
SPT Labtech	Universal Tip Stand	3276-08075	21×
SPT Labtech	Universal Tip Loading Cassette	FFY-A-01-EZL-SL-5	21×
SPT Labtech	Strip Tip Insert – 8 Channel Offset	FFY-A-01-EZL-096-SC-8	21×
SPT Labtech	Thermal Adapter for PCR Plate (96 well)	3276-01065	1×
SPT Labtech	firefly® Low Dead Volume Reservoirs	4150-07202	3×
SPT Labtech	firefly® Standard Reservoirs	4150-07103	8×
SPT Labtech	Ultra-Low Retention Syringes	4150-07208	1×
SPT Labtech	Standard Syringes	4150-07200	1×
Eppendorf	twin.tec PCR Skirted 96-well Plate	0030128648	9×
Thermo Fisher Scientific	Abgene 1.2 mL Deepwell Plate	AB-0661	2×
Thermo Fisher Scientific	Abgene 0.8 mL Deepwell Plate	AB-0765	3×

Table 7. Complete consumables list for running the Twist Target Enrichment Standard Hybridization v1 workflow on the SPT Labtech firefly.
*Strip counts refer to individual EZ-Load strips. One box of 125-008-EZ-FS contains 12 strips; therefore, the workflow uses the equivalent of ~3.5 boxes.