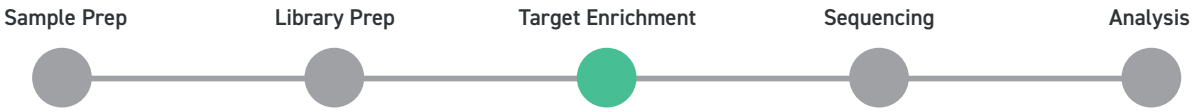
Twist Target Enrichment Fast Hybridization Protocol

For use with the Twist NGS Workflow

The Twist Fast Hybridization Target Enrichment protocol generates target-enriched DNA libraries for sequencing on Illumina next-generation sequencing (NGS) systems. This manual details the steps for a flexible hybridization that can require as little as 15 minutes but can be adjusted to suit your needs for optimum performance in the shortest amount of time possible.

A component of the Twist Target Enrichment for NGS workflow, this protocol is:

- Designed for single or multiplex hybridization reactions using either Twist fixed or custom panels; optional secondary panels (spike-ins) can also be added for additional content
- Optimized for use with Twist Library Preparation Kits
- Should only be performed with the reagents specified, or their equivalents



Twist NGS workflow. The complete NGS workflow takes you from sample preparation to NGS sequencing and data analysis. A component of this workflow, the Twist Fast Hybridization Target Enrichment Protocol works in conjunction with the other component protocols.

This product is for research use only.

PUBLISHED: May 2019

PROTOCOL COMPONENTS

Read the product packaging and storage recommendations carefully for each component, and store components as recommended below immediately upon arrival.

CATALOG #	NAME	DESCRIPTION	STORAGE
TWIST FAST HYBRIDIZATION AND WASH KIT WITH AMP MIX (For target enrichment with Twist Fast hybridization)			
101278: 2 rxn* 104180: 12 rxn 104181: 96 rxn	Twist Fast Hybridization Reagents (Box 1)	· Fast Hybridization Mix · Fast Hybridization Enhancer · Amplification Primers	–20°C
	Twist Fast Wash Buffers (Box 2)	· Fast Binding Buffer · Fast Wash Buffer 1 · Wash Buffer 2	2–8°C
	Equinox Library Amp Mix (Box 3)	· Equinox Library Amp Mix (2x)	–20°C
TWIST PROBE PANELS (ordered separately)			
Choice of panel type and reaction size	Twist Fixed Panel	Fixed content enrichment panel for hybridization reactions (for example, Twist Human Core Exome Panel)	–20°C
	Twist Custom Panel	Custom enrichment panel for hybridization reactions	–20°C
	(Optional) Secondary panel	Secondary enrichment panel for adding content to a fixed or custom panel	–20°C
TWIST BLOCKERS & BEADS FOR TARGET ENRICHMENT			
100856: 2 rxn 100578:12 rxn 100767: 96 rxn	Twist Universal Blockers	For the prevention of nonspecific capture: · Universal Blockers · Blocking Solution	–20°C
101262: 2 rxn 100983: 12 rxn 100984: 96 rxn	Twist Binding and Purification Beads**	For target enrichment and purification: · Streptavidin Binding Beads · DNA Purification Beads	2–8°C
104324: 2 rxn 104325: 12 rxn 104326: 96 rxn	Twist Dry Down Beads**	For target enrichment and purification: · Streptavidin Binding Beads · DNA Purification Beads (contains additional volume for Alternate Pre-Hybridization Solution Concentration Protocol)	2–8°C

**Catalog # 101278 does not contain box 3 for Equinox Library Amp Mix. The Amp mix required for 2 rxn enrichment workflow is included with Twist Library Prep Kits for 16 samples.*

*** Only one of these two bead kit products is required for execution of the entire protocol. When using vacuum concentration, utilize Twist Binding and Purification Beads. When following Alternate Pre-Hybridization Solution Concentration Protocol, utilize Twist Dry Down Beads (see Appendix B for more information).*

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TABLE OF CONTENTS

MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate enriched libraries using the Twist Fast Hybridization Target Enrichment workflow.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Ethanol (200 proof)	—
Molecular biology grade water	—
1.5-ml microcentrifuge tubes	VWR
Thin-walled PCR 0.2-ml strip-tubes	Eppendorf
96-well thermal cycling plates	VWR
1.5-ml compatible magnetic stand	Beckman Coulter, Thermo Fisher Scientific
96-well compatible magnetic plate	Alpaqua, Permagen Labware
Qubit dsDNA High Sensitivity Quantitation Assay	Thermo Fisher Scientific
Agilent High Sensitivity DNA Kit	Agilent Technologies
EQUIPMENT	
Pipettes and tips	—
Vortex mixer	—
Thermomixer (preferred) or heat block for 1.5-ml tubes x2	—
Thermomixer for 1.5-ml tubes	Eppendorf
Thermal cycler (96 well) with heated lid	—
Lab shaker, rocker, rotator	—
Fluorometer (Qubit 3.0)	Thermo Fisher Scientific
2100 Bioanalyzer	Agilent Technologies
Vacuum concentrator (If unavailable, see Appendix B)	—

GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol, and follow the provided instructions. Twist cannot guarantee the performance of the Twist Fast Hybridization Target Enrichment Workflow if modifications are made to the protocol.

Test the compatibility of your thermal cycler and PCR tubes by incubating them at 95°C for up to 5 minutes to ensure the PCR tubes do not crack under heat and pressure. Adjust the tightness of the thermal cycler lid and/or use a spacer specific to the thermal cycler model.

If using a non-human capture panel, replace the Blocker Solution with species-specific blocking solution (not provided).

This protocol details different methods for mixing reagents (gentle pipetting, flicking or tapping, vortexing), depending on the volume, vessel, and reagents involved.

The Fast Hybridization Mix is a viscous reagent. Pipette slowly to ensure accuracy.
For technical support, contact customersupport@twistbioscience.com

PROTOCOL OVERVIEW

This protocol begins with amplified, indexed genomic DNA (gDNA) libraries and generates target-enriched DNA libraries for sequencing on Illumina next-generation sequencing (NGS) systems. It features a flexible hybridization time of 15 minutes to 4 hours, which allows the entire target enrichment workflow to be completed in less than one day. This flexibility allows you to adjust the hybridization time as needed to balance your workflow and performance needs:

- Longer (1–4 hour) hybridizations in the Twist Fast Hybridization Solution can improve performance
- Can also accommodate workflows that require an overnight hybridization
- Refer to Appendix A for further information on how hybridization time impacts performance

	FAST HYBRIDIZATION TARGET ENRICHMENT WORKFLOW (AMPLIFIED INDEXED LIBRARIES)	TIME
STEP 1	**Prepare libraries for hybridization** Indexed library pool STOPPING POINT	**1 hour**
STEP 2	**Hybridize capture probes with pools** Hybridized targets in solution	**0.5 hour** **Plus flexible** **15 minutes to 4 hours**
STEP 3	**Bind hybridized targets to streptavidin beads** Captured targets on beads	**1.5 hour**
STEP 4	**Post-capture PCR amplify, purify, and perform QC** Enriched libraries STOPPING POINT	**1 hour**
STEP 5	**Sequence on an Illumina platform** Libraries ready for sequencing on Illumina platform	—

STEP 1 PREPARE LIBRARIES FOR HYBRIDIZATION

This step involves aliquoting the appropriate amount of amplified, indexed libraries (generated previously in library preparation) and preparing the hybridization reaction solution. For a list of Twist Library Preparation Kit options, see twistbioscience.com/products/ngs.

- When multiplexing, follow the pooling guidelines included as Appendix A in the Twist Library Preparation Protocol used.
- If using another library preparation method, use the pooling guidelines specific to that method.
- If vacuum concentrator is unavailable, see Appendix B.

Reagents Required

- Amplified, indexed library
- Twist fixed or custom panel
- Twist custom secondary (spike-in) panel(s) (optional)
- Twist Universal Blockers:
  - Universal Blockers
  - Blocker Solution (If using a non-human capture panel, replace with species-specific blocking solution, not provided)

Before You Begin

- Thaw all required reagents on ice, then pulse-vortex for 2 seconds to mix and pulse-spin.
- In preparation for Step 2 (Hybridize Capture Probes with Pools), also thaw at room temperature:
  - From the Twist Fast Hybridization Reagents:
    - Fast Hybridization Mix
    - Hybridization Enhancer

ALIQUOT THE LIBRARIES

This protocol supports a single-plex or multi-plex (up to 8-plex) hybridization capture. The amount of indexed library to use depends on the number of indexed samples per pool.

1.1

Use the concentration of each amplified, indexed library to calculate the volume (in µl) of each library needed for hybridization:

- Determine the amount of each indexed library per pool from the table below.
- Divide the amount of each indexed library per pool by the concentrations measured in ng/µl from the library preparation QC.

  For example; If multiplexing eight libraries per hybridization reaction, the amount of each library will be 187.5 ng while the total mass of the pool will be 1,500 ng.

1.1 (continued)

NUMBER OF INDEXED SAMPLES PER POOL	AMOUNT OF EACH INDEXED LIBRARY PER POOL	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 use a smaller amount; using less, however, may result in decreased library complexity.
- More than 1,500 ng (1.5 µg) total DNA can be used; do not, however, use more than 4 µg total DNA, as this might lead to incomplete enrichment.
- If using Alternate Pre-Hybridization Solution Concentration Protocol, total volume of library pool cannot exceed 16 µl. Not recommended for use with panels < 3 Mb.

1.2

Transfer the calculated volumes from each amplified indexed library to an indexed library pool reaction tube for each hybridization being performed. Clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate are recommended to avoid unnceccesary transfers in downstream steps.

PREPARE THE PRE-HYBRIDIZATION SOLUTION

1.3

Add the following volumes of reagents to each amplified indexed library to create a pre-hybridization solution. Mix by flicking the tube(s).

REAGENT	VOLUME
Twist Probe Panel	4 µl
Optional: Secondary Panel (If a secondary panel is not used, do not add water as the entire solution will be dried down)	4 µl
Universal Blockers	8 µl
Blocker Solution	5 µl

1.4

Pulse-spin the tube(s) and ensure there are minimal bubbles present.

1.5

Dry the pre-hybridization solution (library, probes, blockers) in the tube(s) used for the hybridization reaction using a SpeedVac system (or a similar evaporator device) using low or no-heat.

NOTE: If alternate method to drydown is desired, proceed to Appendix B: Alternate Pre-Hybridization Solution Concentration Protocol.

⚠ IMPORTANT: Step 3.1 to 3.7 should be performed concurrently to the pre-hybridization solution dry down and Step 2 for hybridizations under 30 minutes.

SAFE STOPPING POINT: The dried pre-hybridization solution may be stored at -20°C for up to 24 hours before proceeding to Step 2.

PROCEED TO STEP 2: HYBRIDIZE CAPTURE PROBES WITH POOLS

STEP 2 HYBRIDIZE CAPTURE PROBES WITH POOLS

Use the aliquoted libraries and hybridization reaction solution from Step 1, as well as the Fast Hybridization Mix and Hybridization Enhancer you thawed in Step 1.

⚠ IMPORTANT: Before proceeding with this step, test the compatibility of your thermal cycler and PCR tubes or plates by incubating them at 95°C for up to 5 minutes to ensure they do not crack under heat and pressure. Adjust the tightness of the thermal cycler lid and/or use a spacer specific to the thermal cycler model.

Reagents Required

- Dried hybridization reaction (from Step 1.5)
- Reagents thawed in Step 1:
  - Fast Hybridization Mix
  - Hybridization Enhancer

Before You Begin

Program a 96-well thermal cycler with the following conditions and set the heated lid to 85°C:

	TEMPERATURE	TIME
STEP 1	95°C	HOLD
STEP 2	95°C	5 minutes
STEP 3	60°C	15 min to 4 hours[1]

[1]*Set this hybridization time as needed for your application (anywhere from 15 minutes to 4 hours).*

RESUSPEND THE PRE-HYBRIDIZATION SOLUTION

2.1 Heat the Fast Hybridization Mix at 65°C for 10 minutes, or until all precipitate is dissolved. Vortex and use immediately.

⚠ IMPORTANT: Do not allow the Fast Hybridization Mix to cool to room temperature.

2.2 Resuspend the dried pre-hybridization solution from Step 1.5 in 20 µl Fast Hybridization Mix.

NOTES:

- If this resuspended solution requires transfer into a secondary vessel for hybridization, mix by flicking and please wait an addition 5 minutes for resuspension.
- Fast Hybridization Mix is viscous. Pipette slowly to ensure accuracy.
- Small white particles may be present in the capture probes. They do not affect the final capture product.

2.3 Pulse-spin the tube(s) and ensure there are no bubbles present.

PERFORM THE HYBRIDIZATION REACTION

2.4 Add 30 µl Hybridization Enhancer to the top of the pre-hybridization solution.

2.5 Pulse-spin the tube(s) to ensure all solution is at the bottom of the tube(s).

NOTE: Hybridization Enhancer settles on top of the hybridization reaction after the pulse-spin. This does not affect the final capture product.

2.6 Transfer the tube(s) to the preheated thermal cycler and move to Steps 2 and 3 of the thermocycler program.

⚠ IMPORTANT: Make sure the tube is sealed tightly to prevent evaporation over the incubation. Confirm the 95°C step on the thermocycler is started before proceeding to other actions.

PROCEED TO STEP 3: BIND HYBRIDIZED TARGETS TO STREPTAVIDIN BEADS

STEP 3 BIND HYBRIDIZED TARGETS TO STREPTAVIDIN BEADS

Reagents Required

- Hybridization reactions (from Step 2.7)
- From the Twist Fast Wash Buffers:
  - Fast Binding Buffer
  - Fast Wash Buffer 1
  - Wash Buffer 2
- From Twist Binding and Purification Beads or Twist Dry Down Beads:
  - Streptavidin Binding Beads

Before You Begin

Inspect the following for precipitate, and if a precipitate is observed, heat at 48°C until it is dissolved:

- Fast Binding Buffer
- Fast Wash Buffer 1
- Wash Buffer 2

For each hybridization reaction:

- Equilibrate 800 µl Binding Buffer to room temperature
- Preheat 450 µl Fast Wash Buffer 1 to 70°C
  - The temperature of this Fast Wash Buffer 1 can be altered to tune off-target and uniformity in a use-case specific manner. Please see Appendix A for more information.
- Preheat 700 µl Wash Buffer 2 to 48°C

Equilibrate the Streptavidin Binding Beads to room temperature for at least 30 minutes

In preparation for Step 4 (Post-Capture PCR Amplify, Purify, and Perform QC):

- DNA Purification Beads (from the Twist Binding and Purification Beads or Twist Dry Down Beads) — equilibrate to room temperature for at least 30 minutes
- Equinox Amp Mix (2x)*—thaw on ice
- Amplification Primers (from the Twist Fast Hybridization and Wash Kit) — thaw on ice

**Catalog # 101278 does not contain box 3 for Equinox Library Amp Mix. The Amp Mix required for 2 rxn enrichment is included with Twist Library Prep Kits for 16 samples.*

PREPARE THE BEADS

3.1 Vortex the pre-equilibrated Streptavidin Binding Beads until mixed.

3.2 Add 100 µl Streptavidin Binding Beads to a 1.5-ml microcentrifuge tube. Prepare one tube for each hybridization reaction.

3.3 Add 200 µl Fast Binding Buffer to the tube(s) and mix by pipetting.

3.4 Place the tube(s) on a magnetic stand for 1 minute, then remove and discard the clear supernatant. Make sure to not disturb the bead pellet. Remove the tube from the magnetic stand.

3.5 Repeat the wash (Steps 3.3 and 3.4) two more times for a total of three washes.

3.6 After removing the clear supernatant from the third wash, add a final 200 µl Fast Binding Buffer and resuspend the beads by vortexing until homogenized.

BIND THE TARGETS

3.7 After the hybridization (Step 2.7) is complete, open the thermal cycler lid and quickly transfer the volume of each hybridization reaction including Hybridization Enhancer into a corresponding tube of washed Streptavidin Binding Beads from Step 3.6. Mix by pipetting and flicking.

NOTE: Rapid transfer directly from the thermal cycler at 60°C is a critical step for minimizing off-target binding. Do not remove the tube(s) of hybridization reaction from the thermal cycler or otherwise allow it to cool to less than 60°C before transferring the solution to the washed Streptavidin Binding Beads.

3.8 Mix the tube(s) of the hybridization reaction with the Streptavidin Binding Beads for 30 minutes at room temperature on a shaker, rocker, or rotator at a speed sufficient to keep the solution mixed.

NOTE: **Do not vortex.** Aggressive mixing is not required.

3.9 Remove the tube(s) containing the hybridization reaction with Streptavidin Binding Beads from the mixer and pulse-spin to ensure all solution is at the bottom of the tube(s).

3.10 Place the tube(s) on a magnetic stand for 1 minute.

3.11 Remove and discard the clear supernatant including the Hybridization Enhancer. Do not disturb the bead pellet.

NOTE: A trace amount of Hybridization Enhancer may be visible after supernatant removal and throughout each wash step. It will not affect the final capture product.

3.12 Remove the tube(s) from the magnetic stand and add 200 µl preheated Fast Wash Buffer 1. Mix by pipetting.

3.13 Incubate the tube(s) for 5 minutes at the same temperature selected for Fast Wash Buffer 1 equilibration (65–70°C).

NOTE: The temperature of Wash Buffer 1 can be altered to tune off-target and uniformity in a use-case specific manner. Please see Appendix A for more information.

3.14 Place the tube(s) on a magnetic stand for 1 minute.

3.15 Remove and discard the clear supernatant. Make sure to not disturb bead pellet.

3.16 Remove the tube(s) from the magnetic stand and add an additional 200 µl of preheated Fast Wash Buffer 1. Mix by pipetting.

3.17 Incubate the tube(s) for 5 minutes at the same temperature selected for Step 3.13.

3.18 Pulse-spin to ensure all solution is at the bottom of the tube(s).

3.19 Transfer the entire volume from Step 3.18 (~200 µl) into a new 1.5-ml microcentrifuge tube, one per hybridization reaction. Place the tube(s) on a magnetic stand for 1 minute.

⚠ IMPORTANT: A tube transfer is required at this step as it reduces background due to non-targeted library that may stick to the surface of the tube.

3.20 Remove and discard the clear supernatant. Make sure to not disturb the bead pellet.

3.21 Remove the tube(s) from the magnetic stand and add 200 µl of 48°C Wash Buffer 2. Mix by pipetting, then pulse-spin to ensure all solution is at the bottom of the tube(s).

3.22 Incubate the tube(s) for 5 minutes at 48°C.

3.23 Place the tube(s) on a magnetic stand for 1 minute.

3.24 Remove and discard the clear supernatant. Make sure to not disturb the bead pellet.

3.25 Repeat the wash (Steps 3.21–3.24) two more times, for a total of three washes.

3.26 After the final wash, using a 10 µl pipette to remove all traces of supernatant. Proceed immediately to the next step. Do not allow the beads to dry.

NOTE: Before removing supernant, the bead pellet may be briefly spun to collect supernatant at the bottom of the tube or plate and returned to the magnetic plate.

3.27 Remove the tube(s) from the magnetic stand and add 45 µl water. Mix by pipetting until homogenized, then incubate this solution, hereafter referred to as the Streptavidin Binding Bead slurry, on ice.

PROCEED TO STEP 4: POST-CAPTURE PCR AMPLIFY, PURIFY, AND PERFORM QC

STEP 4 POST-CAPTURE PCR AMPLIFY, PURIFY, AND PERFORM QC

Reagents Required

- Streptavidin Binding Bead slurry (from Step 3.27)
- Ethanol
- Molecular biology grade water
- Reagents thawed and equilibrated in Step 3:
  - DNA Purification Beads
  - Equinox Library Amp Mix (2x)
  - Amplification Primers
- Agilent Bioanalyzer High Sensitivity DNA Kit (or equivalent)
- Thermo Fisher Scientific Qubit dsDNA High Sensitivity Quantitation Assay.

Before You Begin

- Prepare 500 µl 80% ethanol for each Streptavidin Binding Bead slurry to be processed.

PREPARE THE BEADS, THERMAL CYCLER, AND PCR MIX

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

STEP	TEMPERATURE	TIME	NUMBER CYCLES
1 Initialization	98°C	45 seconds	1
2 Denaturation	98°C	15 seconds	Varies
Annealing	60°C	30 seconds	
Extension	72°C	30 seconds	
3 Final Extension	72°C	1 minute	1
4 Final Hold	4°C	HOLD	–

CUSTOM PANEL SIZE	NUMBER CYCLES
>100 Mb	5
50–100 Mb	7
10–500 Mb	8
1–10 Mb	9
500–1,000 kb	11
100–500 kb	13
50–100 kb	14
<50 kb	15

NOTE: Number of amplification cycles may vary depending on hybridization reaction size.

4.2 If the Streptavidin Binding Bead slurry has settled, mix by pipetting.

4.3 Transfer 22.5 µl of the Streptavidin Binding Bead slurry to a 0.2-ml thin-walled PCR strip-tube(s). Keep on ice until ready to use in the next step.

NOTE: Store the remaining 22.5 µl water/Streptavidin Binding Bead slurry at –20°C for future use.

4.4 Prepare a PCR mixture by adding the following reagents to the tube(s) containing the Streptavidin Binding Bead slurry. Mix by pipetting.

4.4 (continued)

REAGENT	VOLUME PER REACTION
Streptavidin Binding Bead Slurry	22.5 µl
Amplification Primers, ILMN	2.5 µl
Equinox Library Amp Mix (2x)	25 µl
Total	**50 µl**

PCR AMPLIFY

4.5 Pulse-spin the tubes, transfer them to the thermal cycler and start the cycling program.

4.6 When the thermal cycler program is complete, remove the tube(s) from the block and immediately proceed to purification.

PURIFY

4.7 Vortex the DNA Purification Beads to mix.

4.8 Add 90 µl (1.8x) homogenized DNA Purification Beads to the tube(s) from Step 4.6. Mix well by vortexing.

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

4.9 Incubate for 5 minutes at room temperature.

4.10 Place the tube(s) on a magnetic plate for 1 minute or until the supernatant is clear.

4.11 Without removing the tube(s) from the magnetic plate, remove and discard the clear supernatant.

4.12 Wash the DNA Purification Bead pellet with 200 µl freshly prepared 80% ethanol for 1 minute, then remove and discard the ethanol. Repeat this wash once, for a total of two washes, while keeping the tube on the magnetic plate.

4.13 Using a 10 µl pipet, remove all residual ethanol, making sure to not disturb the bead pellet.

NOTE: Before pipetting, the bead pellet may be briefly spun to collect ethanol at the bottom of the plate or tube and returned to the magnetic plate.

4.14 Air-dry the bead pellet on the magnetic plate for 5 minutes or until the bead pellet is dry. Do not overdry the bead pellet.

4.15 Remove the tube(s) from the magnetic plate and add 32 µl water. Mix by pipetting until homogenized and incubate at room temperature for 2 minutes.

4.16 Place the tube(s) on a magnetic plate and let stand for 3 minutes or until the beads fully pellet.

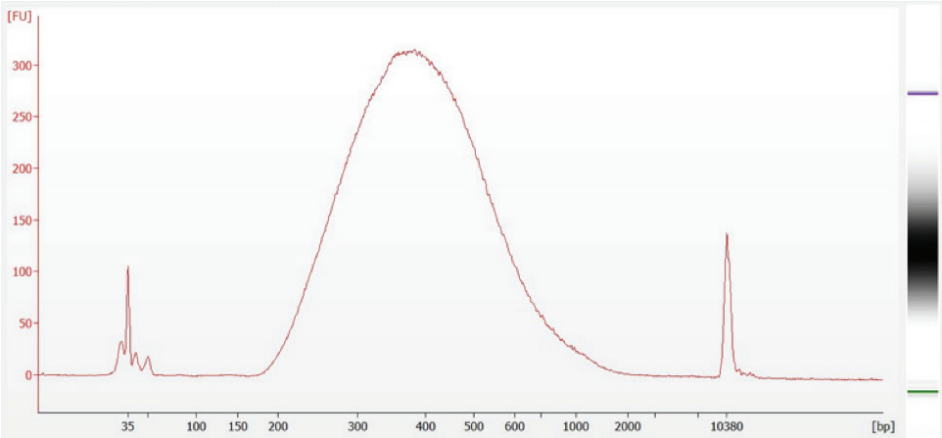
4.17 Transfer 30 µl of the clear supernatant containing the enriched library to a clean thin-walled PCR 0.2-ml strip-tube, making sure not to disturb the bead pellet.

PERFORM QC

4.18

Validate and quantify each enriched library using an Agilent Bioanalyzer High Sensitivity DNA Kit and a Thermo Fisher Scientific Qubit dsDNA High Sensitivity Quantitation Assay.

NOTE: When using the Agilent Bioanalyzer High Sensitivity DNA Kit, load 0.5 µl of the final sample. Average fragment length should be 375–425 bp using a range setting of 150–1,000 bp. Final concentration may vary and is dependent on panel size, library input, hybridization reaction size, and the number of PCR cycles.



Electropherogram generated by an Agilent High Sensitivity DNA analysis of the enriched gDNA library samples that were prepared as described. Note the single prominent peak.

STOPPING POINT: If not proceeding immediately, store the Enriched Library sample at –20°C for up to 24 hours.

STEP 5 SEQUENCING ON AN ILLUMINA PLATFORM

Sequence the Enriched Libraries on an Illumina platform. Sequencing protocols and settings will depend on the application and instrumentation used. Please contact customersupport@twistbioscience.com for recommendations.

END OF WORKFLOW

APPENDIX A: TEMPERATURE ADJUSTMENT OF FAST WASH BUFFER 1

Process stringency is controlled in the Fast Wash Buffer 1 step. Adjusting the temperature of the Fast Wash Buffer 1 will change the underlying Picard performance metrics in a predictable manner. Representative values of important Picard metrics are shown in Figure A1. Please refer to this figure and the Fast Wash Buffer 1 temperatures on the x-axis when optimizing panel performance around these metrics. Results shown below are with the Twist Human Core Exome at a 4 hour hybridization time.

Performance metrics improve with increased hybridization time. Performance expectations are diagramed below in Figure A2. Results shown below are with the Twist Human Core Exome with a 70°C fast wash buffer 1 temperature.

Small panels (under 0.1 Mb) will suffer greater off-target than larger panels. Off target ranges expected for different panel sizes are diagramed below in Figure A3. Metrics are plotted based on a 70°C fast wash buffer 1 temperature.

Adjust the temperature of the Fast Wash Buffer 1 to change the stringency of your wash step. Relative values for important Picard metrics are shown in the figure below. Please refer to this figure and the Fast Wash Buffer 1 temperatures on the x-axis when troubleshooting any issues surrounding these metrics. Results shown below are with the Twist Human Core Exome.

FIGURE A1
Expected increase in panel off target with respect to panel size with a 70°C Fast Wash Buffer 1 temperature.

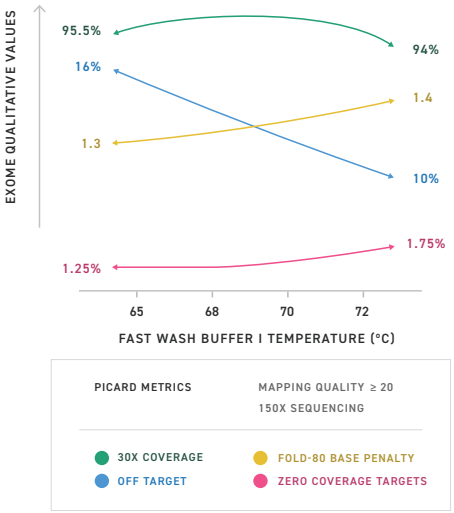


FIGURE A2
Representative target enrichment performance versus hybridization time with a 70°C Fast Wash Buffer 1 temperature.

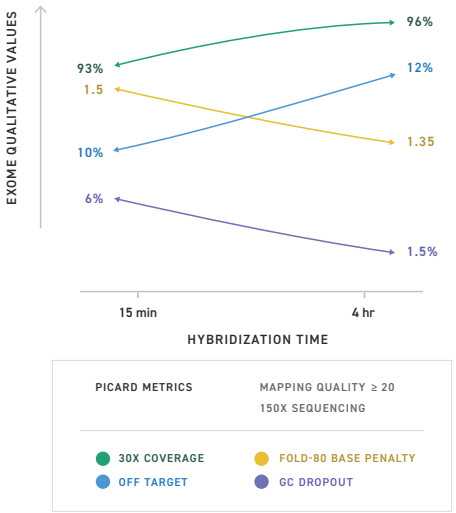
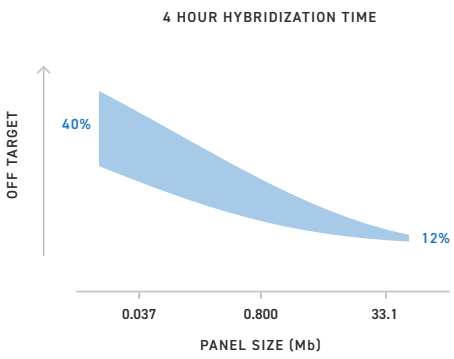


FIGURE A3
Representative target enrichment performance versus Fast Wash Buffer 1 temperature at a 4 hour hybridization time.



END OF APPENDIX A

APPENDIX B: ALTERNATE PRE-HYBRIDIZATION SOLUTION CONCENTRATION PROTOCOL

Reagents Required

- Pre-hybridization solution from Step 1.3
- Ethanol
- Molecular biology grade water
- From Twist Dry Down Beads:
  - DNA Purification Beads
- From the Twist Fast Hybridization Reagents:
  - Fast Hybridization Mix

Before you begin:

- Equilibrate DNA Purification Beads to room temperature for at least 30 minutes
- Thaw at room temperature:
  - Fast Hybridization Mix
- Prepare 500ul of fresh 80% ethanol per hybridization
- Heat Fast Hybridization Mix at 65°C for 10 minutes

  NOTE: This protocol cannot be used if the total volume for each pre-hybridization solution is greater than 33 µl. Decreased post-capture amplification yield and increased AT dropout occur with DNA Purification Beads volume of greater than 100 µl. Not recommended for use with panels < 3 Mb.
- Program a 96-well thermal cycler with the following conditions and set the heated lid to 85°C:

TEMPERATURE	TIME
95°C	HOLD
95°C	5 minutes
60°C	1 hour[1]

[1]Set this hybridization time as needed for your application (anywhere from 1 to 4 hours).

CONCENTRATE THE PRE-HYBRIDIZATION SOLUTION

1 Vortex the pre-equilibrated DNA Purification Beads until well mixed.

2 Add 3.0X DNA Purification Beads based on volume of pre-hybridzation solution from Step 1.3 and mix well by pipetting or vortexing.

3 Incubate for 5min at room temperature.

4 Place the tube(s) on a magnetic plate or rack for 1 minute or until solution is clear.

5 The DNA Purification Beads form a pellet, leaving a clear supernatant. Without removing the plate or tube(s) from the magnetic plate or rack, remove and discard the clear supernatant.

6 Wash the bead pellet by gently adding 200µl freshly prepared 80% ethanol (do not disturb the pellet). Incubate for 1 minute, then remove and discard the ethanol.

7 Repeat this wash once, for a total of two washes, while keeping the tube(s) on the magnetic plate or rack.

8 Briefly spin the tube(s) to pellet the beads on the bottom of the tube(s).

9 Carefully remove all remaining ethanol using a 10 µl pipette, making sure to not disturb the bead pellet.

10 Air dry the bead pellet for 1-5 min until mostly dry. Do not overdry the pellet.

11 Resuspend the bead pellet in 20ul of heated Fast Hybridization Mix by vortexing or pipetting up and down.

NOTE: Vortex Fast Hybridization Mix immediately before use. Do not allow the Fast Hybridization Mix to cool to room temperature.

12 Pulse-spin the tube(s) and ensure there are no bubbles present.

13 Proceed to Step 2.4.

END OF APPENDIX B

LAST REVISED: February 2, 2022

REVISION	DATE	DESCRIPTION
4.0	Feb 2, 2022	• Minor language update to clarify steps
3.0	Nov 12, 2021	• Addition of appendix providing optional bead based alternative to the SpeedVac system for pre-hybridization solution
2.0	May 14, 2021	• Library Amplification Mix is now included with the 12 reactions and 96 reactions enrichment kits. • Kit catalog numbers, kit component list, and workflow steps are updated to include Library Amplification Mix. • Minor workflow steps are updated for more clarity