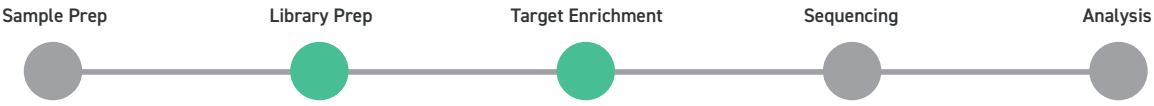
TWIST FOR ELEMENT

Exome 2.0 plus Comprehensive Exome Spike-in Workflow

For use with the Twist NGS Workflow

The Twist for Element, Exome 2.0 plus Comprehensive Exome Spike-in Workflow kit provides the reagents needed to prepare genomic DNA (gDNA) libraries using enzymatic gDNA fragmentation and the Twist Universal Adapter System – Element Compatible. The Twist Universal Adapter System – Element Compatible consists of Twist Universal Adapters – Element Compatible and Twist UDI Primers – Element Compatible. This manual details the steps for generating the amplified, indexed libraries and performing a target enrichment workflow. The resulting enriched DNA library can be sequenced on Element next-generation sequencing (NGS) systems.

This library preparation and target enrichment protocol is optimized for use with the Twist for Element, Exome 2.0 plus Comprehensive Exome Spike-in Workflow kit. This protocol should only be performed with 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, this protocol works in conjunction with the other component protocols.*

For Research Use Only. *Not intended for use in diagnostic procedures.*

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
107686: 96 rxn	Twist for Element, Exome 2.0 plus Comprehensive Exome Spike-in Workflow	Reagents for library construction and target enrichment	—
	Twist Library Preparation EF Kit 1, Element Compatible	· 5x Fragmentation Enzyme · 10x Fragmentation Buffer · DNA Ligation Mix · DNA Ligation Buffer	–20°C
	Twist Library Preparation Kit 2	DNA Purification Beads	2–8°C
	Twist for Element – Library Amplification Mix (2X)	Library Amplification Mix (2X)	–20°C
	Twist Universal Adapter System – Element Compatible	Twist Universal Adapters and Twist UDI Primers, provides unique dual-indexed combinations with 1 reaction per index pair	–20°C
	Twist Hybridization Reagents, Element Compatible	· Hybridization Mix · Hybridization Enhancer · Amplification Primers, Element Compatible	–20°C
	Twist Standard Wash Buffers v2	· Binding Buffer · Standard Wash Buffer 1+ · Wash Buffer 2	2–8°C
	Twist Exome 2.0 + Comp. Exome Spike-in	Fixed content enrichment panel for hybridization reactions. Designed to detect rare and inherited diseases, as well as germline cancers.	–20°C
	Twist Universal Blockers – Element Compatible	For the prevention of nonspecific capture: · Blocker Solution · Universal Blockers - Element Compatible	–20°C
	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	Twist Dry Down Beads** (sold separately)	For target enrichment and purification: · Streptavidin Binding Beads · DNA Purification Beads (contains additional volume for Alternate Pre-Hybridization DNA Concentration Protocol)	2–8°C

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

+ Buffer component in the new Standard Hyb kit v2.

LEGAL

This document may contain references to other third-party resources such as sources of information, hardware or software, products, or services and/or web sites owned or licensed by third parties. Twist Bioscience does not control or take responsibility for any third-party resources, including, without limitation, the accuracy, reliability, copyright compliance, compatibility, performance, legality, or any other aspect of third-party resources. The inclusion of such resources in this document does not imply endorsement by Twist Bioscience of any third-party resources.

Certain processes described in this document may be subject to patent rights or licenses in local jurisdictions, including those owned or licensed by parties other than Twist Bioscience. Purchase of this product does not include a license to perform any such processes. Users of this product may, therefore, be required to obtain a patent license depending upon the particular application and country in which the product is used before performing such processes.

Twist Bioscience, the various Twist logos, and the Twist NGS logo used herein are trademarks of Twist Bioscience Corporation. All other trademarks are the property of their respective owners.

INTENDED USE

This product is for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease or condition. Twist Bioscience assumes no liability regarding use of the product for applications in which it is not intended.

TABLE OF CONTENTS

MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate libraries using the Twist for Element, Exome 2.0 plus Comprehensive Exome Spike-in Workflow.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Ethanol (200 proof)	—
Molecular biology grade water	—
10 mM Tris-HCl pH 8 (optional)	—
Buffer EB (optional)	Qiagen
1.5-ml microcentrifuge tubes	VWR
Thin-walled PCR 0.2-ml strip-tubes	Eppendorf
96-well thermal cycling plates	VWR
Qubit dsDNA Broad Range Quantitation Assay	Thermo Fisher Scientific
Qubit dsDNA High Sensitivity Quantitation Assay	Thermo Fisher Scientific
Agilent DNA 7500 Kit	Agilent Technologies
Agilent High Sensitivity DNA Kit	Agilent Technologies
EQUIPMENT	
Pipettes and tips	—
Vortex mixer	—
1.5-ml compatible magnetic stand	Beckman Coulter
96-well compatible magnetic plate	Alpaqua, Permagen Labware
Benchtop mini centrifuge for 0.2-ml tubes	—
Thermomixer (preferred) or heat block for 1.5-ml tubes x2	Eppendorf
Thermal cycler (96-well) with heated lid	—
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 instructions provided. Twist cannot guarantee the performance of the Twist for Element, Exome 2.0 plus Comprehensive Exome Spike-in Workflow if modifications are made to the protocol.

This library preparation method may yield more material than needed for target enrichment. Excess product can be stored at –20°C for later use.

Do NOT mix or combine the same reagents from different lots.

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.

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

GUIDELINES FOR SAMPLES

gDNA SAMPLES

- Input DNA should be suspended in molecular biology grade water, 10 mM Tris-HCl pH 8.0, or Buffer EB.
- It is important to remove all cations and chelators from the starting gDNA sample. The presence of cations and chelators may affect the initial fragmentation reaction.
- For genomic DNA (gDNA) samples, correct input quantity is critical for achieving optimal yield and library fragment length.
- The recommended DNA input is 50 ng of purified gDNA.
- Using higher or lower mass input may require optimization of the following steps in library preparation to achieve optimal performance.
  - Incubation time for fragmentation at 32°C (Step 1.1, page 9)
  - Amount of Twist Universal Adapters - Element Compatible (Step 2.1, page 11)
  - Incubation time for ligation reaction (Step 2.4, page 12)
- Use the Thermo Fisher Scientific Qubit dsDNA Broad Range Quantitation Assay to accurately quantify input purified gDNA.
- Measuring DNA concentration by absorbance at 260 nm is not recommended.

FOR TECHNICAL SUPPORT, CONTACT CUSTOMERSUPPORT@TWISTBIOSCIENCE.COM.

PROTOCOL OVERVIEW

This protocol begins with genomic DNA (gDNA) and generates target-enriched DNA libraries for subsequent sequencing on Element NGS systems. It features enzymatic fragmentation and Twist Universal Adapters - Element Compatible with Twist UDI Primers - Element Compatible. This protocol allows you to perform gDNA library preparation in 3 hours. Then, amplified, indexed gDNA libraries are used to generate target-enriched DNA libraries for sequencing on Element NGS systems.

LIBRARY PREPARATION

	ENZYMATIC FRAGMENTATION WITH UNIVERSAL ADAPTERS AND UDI PRIMERS (GENOMIC DNA, 50 NG STARTING DNA MATERIAL)	TIME
STEP 1	**Perform DNA Fragmentation, End Repair, and dA-tailing** dA-tailed DNA fragments	**1 hour**
STEP 2	**Ligate Twist Universal Adapters and Purify** gDNA libraries ready for indexing	**1 hour**
STEP 3	**PCR Amplify Using Twist UDI Primers, Purify, and Perform QC** Amplified, indexed libraries	**1 hour**

TARGET ENRICHMENT

	HYBRIDIZATION TARGET ENRICHMENT WORKFLOW (AMPLIFIED, INDEXED LIBRARIES)	TIME
STEP 1	**Prepare Libraries for Hybridization** Indexed library pool	**1 hour**
STEP 2	**Hybridize Capture Probes With Pools** Hybridized targets in solution	**16 hours**
STEP 3	**Bind Hybridized Targets to Streptavidin Beads** Captured targets on beads	**1.5 hours**
STEP 4	**Post-Capture PCR Amplify, Purify, and Perform QC** Enriched libraries	**1 hour**
STEP 5	**Sequencing on an Element AVITI™ System** Libraries ready for sequencing on Element AVITI system	—

STEP 1 LIBRARY PREP: PERFORM DNA FRAGMENTATION, END REPAIR, AND DA-TAILING

Perform enzymatic fragmentation of input gDNA and subsequent end repair and dA-tailing to generate dA-tailed DNA fragments.

Reagents Required

- Genomic DNA (gDNA): 50 ng per sample
- Molecular biology grade water (chilled)
- Optional 10 mM Tris-HCl pH 8 or Buffer EB
- Qubit dsDNA Broad Range Quantitation Assay (or equivalent)
- From the Twist Library Preparation EF Kit 1, Element Compatible:
  - 5x Fragmentation Enzyme
  - 10x Fragmentation Buffer

Before You Begin

- Thaw 5x Fragmentation Enzyme and gDNA samples on ice, then mix by flicking the tube with a finger.
- Thaw 10x Fragmentation Buffer on ice, then mix by pulse vortexing for 2 seconds.

PREPARE THE THERMAL CYCLER, SAMPLES, AND REAGENTS

1.1

Program the thermal cycler with the following conditions. Set the temperature of the heated lid to 70°C. Start the program to pre-chill the thermal cycler.

STEP	TEMPERATURE	TIME
1	4°C	HOLD
2	32°C	22 min*
3	65°C	30 min
4	4°C	HOLD

**Adjustments may be required based on DNA mass input and/or quality*

1.2

Use the Qubit dsDNA Broad Range Quantitation Assay to determine the concentration of your gDNA samples.

1.3 Dilute the gDNA samples to 5 ng/µl with water, 10 mM Tris-HCl pH 8, or Buffer EB. Mix well with gentle pipetting.

1.4 Add 10 µl of each diluted gDNA sample (50 ng total gDNA) into a thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate and place on ice.

1.5 Pulse-spin to ensure all of the solution is at the bottom of the tube.

PERFORM FRAGMENTATION, END REPAIR, AND DA-TAILING

1.6 Prepare an enzymatic fragmentation master mix in a 1.5-ml microcentrifuge tube on ice. Use the volumes listed below. Mix thoroughly by gentle pipetting.

REAGENT	VOLUME PER REACTION*
Water (chilled)	25 µl
10x Fragmentation Buffer	5 µl
5x Fragmentation Enzyme	10 µl
Total	**40 µl**

**Prepare a master mix for multiple reactions.*

1.7 Add 40 µl enzymatic fragmentation master mix (from Step 1.6) to each 10 µl gDNA sample well or tube and mix well by gentle pipetting. Cap the tube and keep the reaction on ice.

1.8 Pulse-spin the sample plate or tubes and immediately transfer to the pre-chilled thermal cycler.

1.9 Proceed to steps 2–4 of the thermal cycler program (32°C step of the thermocycler program in Step 1.1 above).

NOTE: While the thermal cycler program is running, prepare the reagents for Step 2: Ligate Twist Universal Adapters and Purify (see Before You Begin).

1.10 When the thermal cycler program is complete and the sample block has returned to 4°C, remove the samples from the block and place on ice.

PROCEED IMMEDIATELY TO STEP 2: LIGATE TWIST UNIVERSAL ADAPTERS AND PURIFY

STEP 2 LIBRARY PREP: LIGATE TWIST UNIVERSAL ADAPTERS AND PURIFY

Ligate Twist Universal Adapters - Element Compatible to the dA-tailed DNA fragments from Step 1 and purify to generate gDNA libraries ready for index introduction through amplification in Step 3.

Reagents Required

- dA-tailed DNA fragments (from Step 1.10)
- Ethanol
- Molecular biology grade water (chilled)
- Optional 10 mM Tris-HCl pH 8 or Buffer EB
- From the Twist Library Preparation EF Kit 1, Element Compatible:
  - DNA Ligation Mix
  - DNA Ligation Buffer
- From the Twist Universal Adapter System - Element Compatible:
  - Twist Universal Adapters - Element Compatible
- From the Twist Library Preparation Kit 2:
  - DNA Purification Beads

Before You Begin

- Thaw on ice:
  - Twist Universal Adapters - Element Compatible (tube; utilized for all samples)
  - DNA Ligation Mix
  - DNA Ligation Buffer
- Prepare 1 ml 80% ethanol for each sample (for use in Steps 2 and 3 of library preparation).
- Equilibrate DNA Purification Beads to room temperature for at least 30 minutes (for use in Steps 2 and 3 of library preparation).
- Program a thermal cycler to incubate the samples at 20°C with the heated lid set to minimum temperature or turned off. Start the program so that the cycler is at 20°C when the samples are prepared.

LIGATE TWIST UNIVERSAL ADAPTERS

2.1 Add 5 µl Twist Universal Adapters - Element Compatible into each sample well or tube containing the dA-tailed DNA fragments from Step 1. Mix gently by pipetting and keep on ice.

2.2 Prepare the ligation master mix in a 1.5-ml microcentrifuge tube on ice as indicated below. Mix well by gentle pipetting.

REAGENT	VOLUME PER REACTION*
Water (chilled)	15 µl
DNA Ligation Buffer	20 µl
DNA Ligation Mix	10 µl
Total	**45 µl**

**Prepare a master mix for multiple reactions.*

2.3 Add 45 µl of the ligation master mix to the sample from Step 2.1 and mix well by gentle pipetting. Seal or cap the tubes and pulse-spin to ensure all solution is at the bottom of the tube.

2.4 Incubate the ligation reaction at 20°C for 15 minutes in the thermal cycler, then move the samples to the bench top. Proceed to the Purify step.

⚠ IMPORTANT: Turn off the heated lid or set to minimum temperature.

NOTE: While the thermal cycler program is running, prepare the reagents for Step 3: PCR Amplify Using Twist UDI Primers, Purify, and Perform QC (see Before You Begin).

PURIFY

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

2.6 Add 80 µl (0.8x) of homogenized DNA Purification Beads to each ligation sample from Step 2.4. Mix well by vortexing.

2.7 Incubate the samples for 5 minutes at room temperature.

2.8 Place the samples on a magnetic plate for 1 minute or until the supernatant is clear.

2.9 The DNA Purification Beads form a pellet, leaving a clear supernatant. Without removing the plate or tubes from the magnetic plate, remove and discard the supernatant.

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

2.11 Repeat the wash once, for a total of two washes, while keeping the samples on the magnetic plate.

2.12 Carefully remove all remaining ethanol with a 10-µl pipette, making sure not to 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.

2.13 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.

2.14 Remove the plate or tubes from the magnetic plate and add 17 µl water, 10 mM Tris-HCl pH 8, or Buffer EB to each sample. Mix by pipetting until homogenized.

2.15 Incubate at room temperature for 2 minutes.

2.16 Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.

2.17 Transfer 15 µl of the clear supernatant containing the ligated and indexed libraries to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate, making sure not to disturb the bead pellet.

PROCEED TO STEP 3: PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY, AND PERFORM QC

STEP 3 LIBRARY PREP: PCR AMPLIFY USING TWIST UDI PRIMERS, PURIFY, AND PERFORM QC

Amplify the adapted gDNA libraries with Twist UDI Primers - Element Compatible, purify them, and perform quality control (QC) analysis to complete library preparation.

Reagents Required

- Ligated, adapted libraries (from Step 2.17)
- 80% Ethanol (from Step 2)
- Equilibrated DNA Purification Beads (from Step 2)
- Molecular biology grade water
- Optional 10 mM Tris-HCl pH 8 or Buffer EB
- From the Twist for Element - Library Amplification Mix (2X):
  - Library Amplification Mix (2X)
- From the Twist Universal Adapter System - Element Compatible:
  - Twist UDI Primers - Element Compatible

Before You Begin

- Thaw on ice:
  - Twist UDI Primers - Element Compatible (plate with single-use primers)
  - Library Amplification Mix (2X)

PREPARE THE THERMAL CYCLER

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

	STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
1	Initialization	98°C	45 seconds	1
2	Denaturation	98°C	45 seconds	6–8*
	Annealing	60°C	30 seconds	
	Extension	72°C	30 seconds	
3	Final Extension	72°C	1 minute	1
4	Final Hold	4°C	HOLD	–

**6–8 cycles is recommended when starting with 50–100 ng of high-quality gDNA.*

PERFORM PCR

3.2 Add 10 µl of Twist UDI Primers - Element Compatible from the provided 96-well plate to each of the gDNA libraries from Step 2.17 and mix well by gentle pipetting.

NOTE: For index selection and multiplexing, refer to the pooling guidelines in Appendix A.

3.3 Add 25 µl of Library Amplification Mix (2X) to the gDNA libraries from Step 3.2 and mix well by gentle pipetting.

3.4 Pulse-spin sample plate or tube and immediately transfer to the thermal cycler. Start the program (programmed in Step 3.1).

3.5 Remove the sample(s) from the block when the thermal cycler program is complete. Proceed to the Purify step.

PURIFY

3.6 Vortex the pre-equilibrated DNA Purification Beads until mixed.

3.7 Add 50 µl (1x) of homogenized DNA Purification Beads to each ligation sample from Step 3.5. Mix well by vortexing.

3.8 Incubate the samples for 5 minutes at room temperature.

3.9 Place the samples on a magnetic plate for 1 minute.

3.10 The DNA Purification Beads form a pellet, leaving a clear supernatant. Without removing the plate or tubes from the magnetic plate, remove and discard the supernatant.

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

3.12 Repeat this wash once, for a total of two washes, while keeping the samples on the magnetic plate.

3.13 Carefully remove all remaining ethanol with a 10-µl pipette, making sure not to 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.

3.14 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.

3.15 Remove the plate or tubes from the magnetic plate and add 22 µl water, 10 mM Tris-HCl pH 8, or Buffer EB to each sample. Mix by pipetting until homogenized.

3.16 Incubate at room temperature for 2 minutes.

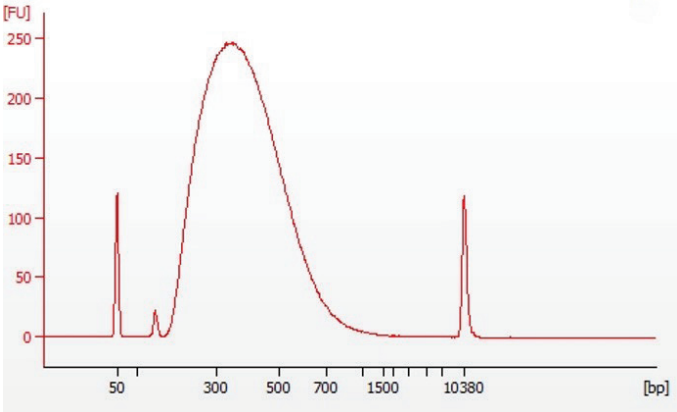
3.17 Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.

3.18 Transfer 20 µl of the clear supernatant containing the amplified, indexed libraries to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate, making sure not to disturb the bead pellet.

PERFORM QC

3.19 Quantify and validate the size range of each library using the Qubit dsDNA Broad Range Quantification Assay Kit and Agilent DNA 7500 Kit.

50 ng of high-quality gDNA and 8 cycles of PCR should result in a final concentration ≥180 ng/µl (note: for fewer PCR cycles, this concentration value will be lower) and the average fragment length should be 350–400 bp using a range setting of 150–1000 bp.



Representative electropherogram of a purified library generated with input of 50 ng of high-quality gDNA and 8 cycles of PCR. There may be a small peak at ~150 bp which should not impact library performance in target enrichment.

NOTES: If the average fragment length is not in the range of 350–400 bp:

- Input DNA concentration may be inaccurate. Typically, using too much DNA leads to shorter fragments, while not using enough DNA leads to larger fragments.
- The presence of cations and chelators may also affect the average fragment length.
- If neither of the above factors applies, optimize the 32°C fragmentation in Step 1.1 by changing the time in 3-minute increments—increase time to produce shorter fragments and decrease time to produce longer fragments.

You can proceed with concentrations <180 ng/µl (note: a concentration ≥180 ng/µl is expected for 8 PCR cycles), but low concentrations may reflect inefficient sample preparation and can result in low library diversity after hybridization.

STOPPING POINT: If not proceeding immediately to target enrichment, store the amplified, indexed libraries at –20°C.

STEP 1 TARGET ENRICHMENT: 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. When multiplexing, follow the pooling guidelines included in Appendix A.

If a vacuum concentrator is unavailable, see Appendix B.

Reagents Required

- Amplified, indexed library

THIS PROTOCOL SUPPORTS AN 8-PLEX HYBRIDIZATION CAPTURE.

ALIQUOT AND DRY DOWN THE LIBRARY

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

- The amount of each library will be 187.5 ng and the total mass of the pool will be 1,500 ng.
- Divide the amount of each indexed library per pool (187.5 ng) by the concentrations measured in ng/µl from the library preparation QC.

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 reduced performance of the enrichment.

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 unnecessary transfers in downstream steps.

NOTE: Check for a proper seal on the tube(s) as evaporation may occur leading to decreased performance.

1.3 Pulse-spin the indexed library pool tube(s) to minimize the amount of bubbles present.

1.4

Dry the indexed library pool(s) using a vacuum concentrator using low or no heat.

NOTE: If an alternate method to dry down is desired, proceed to Appendix B: Alternate Pre-Hybridization DNA Concentration Protocol.

STOPPING POINT: If not proceeding immediately to Step 2, store the dried indexed library pool at –20°C for up to 24 hours.

PROCEED TO STEP 2: HYBRIDIZE CAPTURE PROBES WITH POOLS

STEP 2 TARGET ENRICHMENT: HYBRIDIZE CAPTURE PROBES WITH POOLS

Use the dried indexed library pool(s) from Step 1.4 for performing the hybridization reaction.

⚠ 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

- Indexed library pool(s) from Step 1.4
- Twist Exome 2.0 + Comp. Exome Spike-in
- From Twist Hybridization Reagents, Element Compatible:
  - Hybridization Mix
  - Hybridization Enhancer
- From Twist Universal Blockers - Element Compatible:
  - Universal Blockers - Element Compatible
  - Blocker Solution

Before You Begin

- Thaw all required reagents on ice, then pulse-vortex for 2 seconds to mix and then pulse-spin.
- Set a heat block to 65°C.
- Program a 96-well thermal cycler to 95°C and set the heated lid to 105°C.

PREPARE THE PROBE SOLUTION

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

2.2 Prepare a probe solution in a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate as indicated in the table below. Mix by flicking the tube(s).

REAGENT	VOLUME
Hybridization Mix	20 µl
Twist Exome 2.0 + Comp. Exome Spike-in	2 µl
Water	6 µl
Total	**28 µl**

NOTES: Hybridization Mix is very viscous. Pipette slowly to ensure accurate pipetting. Small white particles may be present in the Twist Exome 2.0 + Comp. Exome Spike-in. This will not affect the final capture product.

2.3 Resuspend the dried indexed library pool (from Step 1.4) by adding the reagents described below. Mix by flicking the tube(s).

REAGENT	VOLUME
Dried Indexed Library Pool	–
Blocker Solution	5 µl
Universal Blockers - Element Compatible	7 µl
Total	**12 µl**

PERFORM THE HYBRIDIZATION REACTION

2.4 Heat the probe solution to 95°C for 2 minutes in a thermal cycler with the lid at 105°C, then immediately cool on ice for 5 minutes.

2.5 While the probe solution is cooling on ice, heat the tube containing the resuspended indexed library pool at 95°C for 5 minutes in a thermal cycler with the lid at 105°C, then equilibrate both the probe solution and resuspended indexed library pool to room temperature on the benchtop for 5 minutes.

2.6 Vortex and spin down the probe solution, then transfer the entire volume to the resuspended indexed library pool. Mix well by vortexing.

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

2.8 Add 30 µl Hybridization Enhancer to the top of the entire capture reaction.

2.9 Pulse-spin the tube(s) to ensure there are no bubbles present.

⚠ IMPORTANT: Seal the tube(s) tightly to prevent excess evaporation over the 16-hour incubation.

2.10 Incubate the hybridization reaction at 70°C for 16 hours in a thermal cycler with the lid at 85°C.

NOTE: Halting hybridization between 15–17 hours will not affect downstream capture quality.

PROCEED TO STEP 3: BIND HYBRIDIZED TARGETS TO STREPTAVIDIN BEADS

STEP 3 TARGET ENRICHMENT: BIND HYBRIDIZED TARGETS TO STREPTAVIDIN BEADS

Reagents Required

- Hybridization reactions (from Step 2.10)
- Molecular biology grade water
- From the Twist for Element - Library Amplification Mix (2X):
  - Library Amplification Mix (2X)
- From the Twist Hybridization Reagents, Element Compatible:
  - Amplification Primers, Element Compatible
- From the Twist Standard Wash Buffers v2:
  - Binding Buffer
  - Standard Wash Buffer 1+
  - Wash Buffer 2
- From Twist Binding and Purification Beads or Twist Dry Down Beads:
  - Streptavidin Binding Beads
  - DNA Purification Beads

Before You Begin

- Preheat a thermomixer (preferred) or heat block to 68°C
- Preheat a thermomixer (preferred) or heat block to 48°C
- Preheat the following tubes at 48°C until any precipitate is dissolved:
  - Binding Buffer
  - Standard Wash Buffer 1+
  - Wash Buffer 2
- For each hybridization reaction:
  - Equilibrate 800 µl Binding Buffer to room temperature
  - Equilibrate 225 µl Standard Wash Buffer 1 to 68°C
  - Leave 700 µl Wash Buffer 2 at 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):

- Thaw on ice:
  - Library Amplification Mix (2X)
  - Amplification Primers, Element Compatible
- Equilibrate DNA Purification Beads (from the Twist Binding and Purification Beads or Twist Dry Down Beads) to room temperature for at least 30 minutes

+ Buffer component in the new Standard Hyb kit v2

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 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 Binding Buffer and resuspend the beads by vortexing until homogenized.

3.7 Heat the resuspended beads at 68°C for at least 10 minutes before continuing to Step 3.8.

BIND THE TARGETS

3.8 After the hybridization (Step 2.10) is complete, open the thermal cycler lid and directly transfer the volume of each hybridization reaction into a corresponding tube of preheated Streptavidin Binding Beads from Step 3.7. Mix by pipetting and flicking.

⚠ IMPORTANT: Rapid transfer directly from the thermal cycler at 70°C is a critical step for minimizing off-target binding. Do not remove the tube(s) of the hybridization reaction from the thermal cycler or otherwise allow it to cool to less than 70°C before transferring the solution to the washed Streptavidin Binding Beads. Allowing to cool to room temperature for less than 5 minutes will result in as much as 10–20% increase in off-target binding.

3.9 Incubate the tube(s) of the hybridization reaction with the Streptavidin Binding Beads for 5 minutes at 68°C, agitation is not required.

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

3.10 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).

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

3.12 Remove and discard the clear supernatant including the Hybridization Enhancer. 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.

3.13 Remove the tube(s) from the magnetic stand and add 200 µl 68°C Standard Wash Buffer 1+. Mix by pipetting.

3.14 Incubate the tube(s) for 5 minutes at 68°C.

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

3.16 Transfer the entire volume from Step 3.15 (~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: This step reduces background from non-specific binding to the surface of the tube.

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

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

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

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

3.21 Repeat the wash (Steps 3.17–3.20) two more times, for a total of three washes.

3.22 After the final wash, use 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 the supernatant, the bead pellet may be briefly spun to collect the supernatant at the bottom of the tube or plate and returned to the magnetic plate.

3.23 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

+ Buffer component in the new Standard Hyb kit v2

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

Reagents Required

- Streptavidin Binding Bead slurry (from Step 3.23)
- Ethanol
- Molecular biology grade water
- Reagents thawed and equilibrated in Step 3:
  - DNA Purification Beads
  - Library Amplification Mix (2X)
  - Amplification Primers, Element Compatible
- 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 OF CYCLES
1	Initialization	98°C	45 seconds	1
2	Denaturation	98°C	45 seconds	8
	Annealing	60°C	30 seconds	
	Extension	72°C	30 seconds	
3	Final Extension	72°C	1 minute	1
4	Final Hold	4°C	HOLD	–

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.

REAGENT	VOLUME
Streptavidin Binding Bead Slurry	22.5 µl
Amplification Primers, Element Compatible	2.5 µl
Library Amplification 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 (programmed in Step 4.1).

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

PURIFY

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

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

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

4.13 Repeat this wash once, for a total of two washes, while keeping the tube on the magnetic plate.

4.14 Carefully remove all remaining ethanol using a 10-µl pipette, 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.

4.15 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.

4.16 Remove the tube(s) from the magnetic plate and add 32 µl water, 10 mM Tris-HCl pH 8, or Buffer EB to each capture reaction. Mix by pipetting until homogenized.

4.17 Incubate at room temperature for 2 minutes.

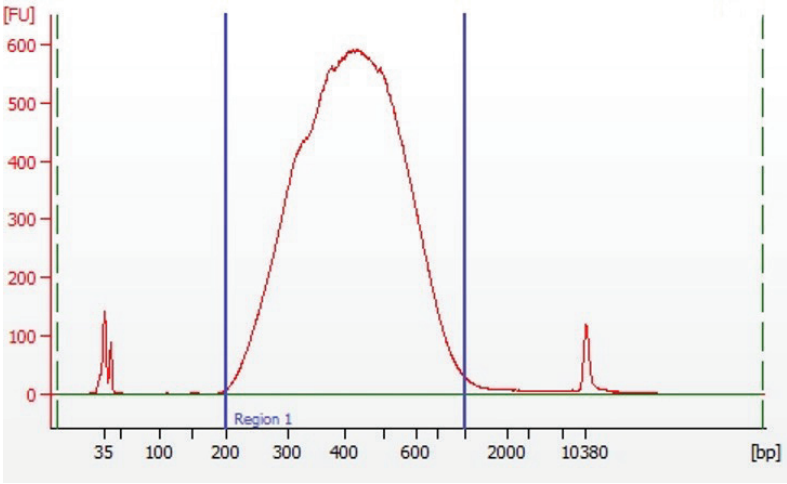
4.18 Place the plate or tube(s) on a magnetic plate and let stand for 3 minutes or until the beads fully pellet.

4.19 Transfer 30 µl of the clear supernatant containing the enriched library to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate, making sure to not disturb the bead pellet.

PERFORM QC

4.20 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 375–450 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 to sequencing, store the enriched library sample at –20°C for up to 24 hours.

STEP 5 TARGET ENRICHMENT: SEQUENCING ON AN ELEMENT AVITI SYSTEM

Sequence the enriched libraries on an Element AVITI System. Sequencing protocols and settings depend on the application. Please contact customersupport@twistbioscience.com for recommendations.

END OF WORKFLOW

APPENDIX A: ELEMENT-COMPATIBLE TWIST UNIVERSAL ADAPTER SYSTEM - SEQUENCES AND POOLING GUIDELINES

UDI SEQUENCES

For a complete guide of the Twist UDI Primers - Element Compatible used in the Twist Universal Adapter System - Element Compatible, please refer to the Twist UDI Primers - Element Compatible Reference Spreadsheet and the Sample Sheet Template.

POOLING GUIDELINES

Twist UDI Primers - Element Compatible are base balanced for next-generation sequencing on a column basis. When pooling unique dual-indexed libraries for 8-plex hybridization, it is recommended that libraries be selected from a single column except column 6. Column 6 is optimized for >16-plex pools. Multiple columns may be selected in any desired combination across a single plate for sequencing.

Twist UDI Primers — Element Compatible plate layouts and pooling guidelines.
Twist Universal Adapter System - Element Compatible, 96 Samples (107682)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

APPENDIX B: PRE-HYBRIDIZATION DNA CONCENTRATION PROTOCOL

Reagents Required

- Amplified, indexed library pool(s) from Step 1.2
- Ethanol
- Molecular biology grade water
- From Twist Dry Down Beads:
  - DNA Purification Beads
- From Twist Universal Blockers - Element Compatible:
  - Universal Blockers - Element Compatible
  - Blocker Solution

Before You Begin

- Equilibrate DNA Purification Beads to room temperature for at least 30 minutes.
- Vortex the pre-equilibrated DNA Purification Beads until well mixed.
- Prepare 500 µl fresh 80% ethanol for each sample to be processed.

CONCENTRATE THE DNA LIBRARIES

1 Add 1.8x homogenized DNA Purification Beads to the tube(s) containing the DNA libraries from Step 1.2. Mix well by vortexing.

NOTE: For amplified, indexed library pool(s) with a volume of less than 10 µl, bring the volume up to 10 µl with water.

2 Incubate for 5 minutes at room temperature.

3 Pulse-spin to ensure all the solution is at the bottom of the tube(s) and place the tube(s) on a magnetic plate or rack for 3 minutes or until the solution is clear.

4 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.

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

6 Repeat this wash once, for a total of two washes, while keeping the tube on the magnetic plate.

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

NOTE: Pulse-spin if necessary to ensure complete removal of ethanol.

8 Air-dry the bead pellet on a magnetic plate for 1–5 minutes or until the bead pellet is dry. Do not overdry the bead pellet.

9 Remove the tube(s) from the magnetic plate or rack and add 7 µl Universal Blockers - Element Compatible and 5 µl Blocker Solution. Mix by pipetting until homogenized.

10 Proceed to Step 2.1 and continue the protocol omitting Step 2.3.

END OF APPENDIX