Element Biosciences

TWIST BIOSCIENCE

Workflow Protocol

Twist for Element Exome 2.0 + Comprehensive Exome Spike-in Library Preparation and Standard Hybridization With Trinity™ Sequencing Workflow

For use with the Twist NGS Workflow

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Chapter 1: Overview

The Twist for Element Trinity, Exome 2.0 + Comprehensive Spike, Standard Hyb 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 using the Trinity sequencing workflow.

This library preparation and target enrichment protocol is optimized for use with the Twist for Element Trinity, Exome 2.0 + Comprehensive Spike-In, Standard Hyb Workflow kit. This protocol should only be performed with reagents specified or their equivalents.

Protocol Components

Read the product packaging and storage recommendations carefully for each component. Store components as recommended below immediately upon arrival. Note that this protocol requires reagents sold from both Twist Bioscience and Element Biosciences.

Catalog #	Name	Description	Storage
Twist for Element Trinity Reagents And Kits			
109326*	Twist for Element Trinity, Exome 2.0 + Comp Spike, Standard Hyb Workflow	Reagents for constructing libraries and performing standard hybridization. The resulting enriched libraries are compatible with Element Trinity workflows.	
107681	Twist Library Preparation EF Kit 1, Element Compatible	• 5x Fragmentation Enzyme • 10x Fragmentation Buffer • DNA Ligation Mix • DNA Ligation Buffer	–20°C
100573	Twist Library Preparation Kit 2	DNA Purification Beads	2–8°C
107779	Twist for Element – Library Amplification Mix (2X)	Library Amplification Mix (2X)	–20°C
107682	Twist Universal Adapter System – Element Compatible	• Twist Universal Adapters - Element Compatible • Twist UDI Primers - Element Compatible	–20°C
109297	Twist Standard Hybridization Reagents, Element Trinity Compatible	• Hybridization Mix • Hybridization Enhancer • Blocker Solution	–20°C
110506	Twist Exome 2.0 + Comp Spike, Trinity Compatible	Fixed content enrichment panel for hybridization reactions. Designed to detect rare and inherited diseases, as well as germline cancers.	–20°C
Element Trinity Reagents And Kits**			
830-00029	Trinity Binding Reagent	Reagent for Trinity hybridization	-20°C
860-00019: 2 x 75 860-00020: 2 x 150	Trinity Sequencing Kit	Trinity Flow Cell AVITI™ Buffer Bottle Trinity Sequencing Reagent Trinity Sequencing Cartridge Library Loading Buffer Pouch	2-8°C Room temperature -20°C -20°C -20°C

* Includes all the library preparation and target enrichment reagents listed below. These items must be purchased from Twist Bioscience (twistbioscience.com).
** These reagents must be purchased from Element Biosciences (www.elementbiosciences.com).

User-Supplied Materials

The following materials or their equivalent are required to generate libraries using the Twist for Element, Exome 2.0 + 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
5 ml Eppendorf tubes	Eppendorf
Thin-walled 0.2 ml PCR strip-tubes	Eppendorf
96-well thermal cycling plates	VWR
Qubit dsDNA Broad Range Quantitation Assay	Thermo Fisher Scientific
Agilent DNA 7500 Kit	Agilent Technologies
Equipment	
Pipettes and tips	—
Vortex mixer	—
96-well compatible magnetic plate	Alpaqua, Permagen Labware
1.5 ml compatible magnetic stand	Beckman Coulter
Benchtop mini centrifuge for 0.2 ml tubes	—
Thermomixer (preferred) or heat block	Eppendorf
Thermal cycler (96-well) with heated lid	—
Fluorometer (Qubit 3.0)	Thermo Fisher Scientific
2100 Bioanalyzer	Agilent Technologies
Vacuum concentrator	—

General Notes and Precautions

- This protocol supports 24-plex pooled hybridization reaction. One Trinity sequencing run on an AVITI System supports a single hybridization reaction for up to a 24-plex library pool.
- 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 Exome 2.0 + Comprehensive Exome Spike-in Library Preparation and Standard Hybridization With Trinity Sequencing 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 6)
    - Amount of Twist Universal Adapters - Element Compatible (Step 2.1, page 8)
    - Incubation time for ligation reaction (Step 2.4, page 9)
- 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

Step	Enzymatic Fragmentation with Universal Adapters and UDI Primers (Genomic DNA, 50 ng starting DNA material)	Time
1	**Perform DNA Fragmentation, End Repair, and dA-tailing** dA-tailed DNA fragments	1 Hour
2	**Ligate Twist Universal Adapters and Purify** gDNA libraries ready for indexing	1 Hour
3	**PCR Amplify Using Twist UDI Primers, Purify, and Perform QC** Amplified, indexed libraries	1 Hour

Hybridization

Step	Hybridization and Trinity Sequencing Workflow (Amplified, indexed libraries)	Time
1	**Prepare Libraries for Hybridization** Indexed library pool	1 hour
	Stopping point	
2	**Hybridize Capture Probes With Pools** Hybridize targets in the solution	16 hours
3	**Trinity Sequencing Run Setup** Libraries ready for Trinity sequencing on an Element platform	45 minutes

Chapter 2: Library Preparation

STEP 1

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. When multiplexing, follow the pooling guidelines included in Appendix A.

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

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

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 the 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.

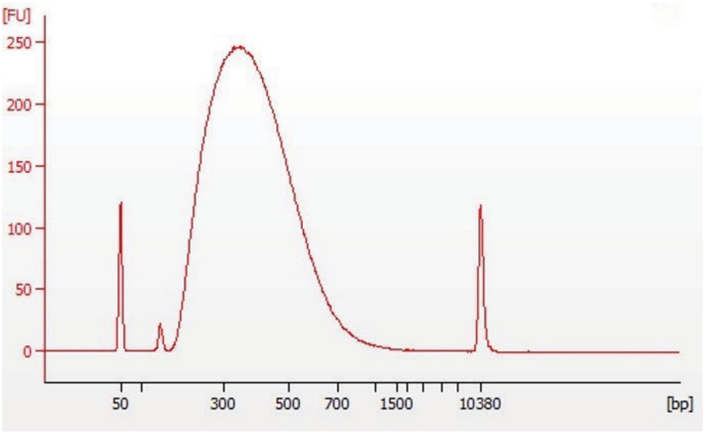
3.18 Transfer 20 µl of the clear supernatant containing the amplified, indexed libraries to a clean thin-walled PCR 0.2 ml strip-tube or well of a 96-well thermal cycling plate, making sure not to disturb the bead pellet.

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.

Expect 50 ng of high-quality gDNA and 8 cycles of PCR to result in a final concentration ≥ 180 ng/µl (for fewer PCR cycles, this concentration value will be lower). 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). 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.

Chapter 3: Hybridization

STEP 1

Target Enrichment: Prepare Libraries for Hybridization

This step involves aliquoting the appropriate amount of amplified, indexed libraries (generated previously in library preparation). When multiplexing, follow the pooling guidelines included in Appendix A.

Reagents Required

- Amplified and indexed linear library (generated in Chapter 2: Library Preparation)

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:

- Expect up to 24 indexed samples in one hybridization reaction to be 4000 ng.
- Divide the amount of each indexed library per pool by the concentrations measured in ng/µl from the library preparation QC.
- This protocol supports a single or multiplex (up to 24-plex) hybridization capture.
- Each Trinity flow cell can support up to 24 exome samples.
- For libraries prepared with UDI 47 or UDI 67, double the input amount. These unique dual indexes typically require higher input amounts to ensure a more balanced representation.

Number of Indexed Samples Per Pool	Amount of Each Indexed Library per Pool
24	166.67 ng

1.2 Transfer the calculated volumes from each amplified, indexed library to a tube or plate well for each hybridization being performed. A clean thin-walled PCR 0.2 ml strip-tube or well of a 96-well thermal cycling plate is recommended to avoid unnecessary transfers in downstream steps.

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

1.4 Dry the indexed pool with a SpeedVac using low or no heat.

STOPPING POINT: If not proceeding immediately to Step 2: Hybridize Capture Probes With Pools, 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 from Step 1 to perform 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 from Step 1
- Twist Exome 2.0 + Comp Spike, Trinity Compatible
- From Twist Standard Hybridization Reagents, Element Trinity Compatible:
  - Hybridization Mix
  - Hybridization Enhancer
  - Blocker Solution
- Trinity Binding Reagent (Element, catalog # 830-00029)

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.
- Program a 96-well thermal cycler to 95°C and set the heated lid to 105°C.

Prepare 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 the 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 Spike, Trinity Compatible	2 µl
Water	6 µl
Total	**28 µl**

NOTE: Hybridization Mix is viscous. Pipette slowly to ensure accurate pipetting. Small white particles may be present in the capture probes, which does not affect the final capture product.

2.3 Resuspend the dried indexed library from Step 1 by adding the reagents described below. Mix by flicking the tubes.

Reagent	Volume
Dried Indexed Library	-
Blocker Solution	5 µl
Trinity Binding Reagent	5 µl
Water	2 µl
Total	**12 µl**

Perform the Hybridization Reaction

2.4 Heat the probe solution tube at 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 library/binding solution at 95°C for 5 minutes in a thermal cycler with the lid at 105°C, then equilibrate both the probe solution and the resuspended indexed library pool to room temperature for 5 minutes.

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

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

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

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

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

2.10 Run the following thermal cycler program to incubate the hybridization reaction.

Temperature	Time
Lid set to 85°C	
72°C	16 hours
72°C	Hold

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

Chapter 4: Sequencing with the Trinity Sequencing Workflow

Pool hybridization reactions and sequence on an Element AVITI System.

The following instructions are intended for an experienced operator of an AVITI System. For comprehensive instructions, see the user guide for your instrument on the Element website.

Prepare Consumables

1.1 Gather the following consumables from the Trinity Sequencing Kit:

- Hybridization reaction (from Step 2.10 of Hybridization)
- Trinity Sequencing Reagent
- Library Loading Buffer
- Trinity Sequencing Cartridge
- Trinity Flow Cell
- AVITI Buffer Bottle

1.2 Thaw the sequencing cartridge in a water bath set to 20°C.

- Allow approximately 1.5 hours to thaw a 2 x 75 cartridge.
- Allow approximately 2.5 hours to thaw a 2 x 150 cartridge.
- Alternatively, thaw the cartridge overnight at 2°C to 8°C.

Initiate a Sequencing Run

1.3 On the Home screen, select **New Run**.

1.4 If prompted that the flow cell is missing, load a used flow cell.

1.5 Select **Sequencing**.

1.6 Select the side for sequencing: **Side A**, **Both**, or **Side B**.

1.7 For chemistry type, select **Trinity**.

1.8 For a Manual Run, proceed to *Define Run Parameters*. For a Planned Run, select the run and storage connection, and then select **Next**. Proceed to *Inspect and Mix Reagents*.

Define Run Parameters

1.9 In the Run Name field, enter a unique name.

1.10 To import the run manifest, select **Browse** and navigate to the run manifest.

1.11 In the Storage drop-down menu, select a storage location.

1.12 In the Sequencing Kit drop-down menu, select the appropriate **Trinity Sequencing Kit**.

1.13 In the Panel drop-down menu, select **Twist for Element, Trinity Exome Workflow**.

1.14 In the Cycles fields, enter the number of cycles for each read, and then select **Next**.

Kit Size	Default Cycle Values for Trinity			
	Index 1	Index 2	Read 1	Read 2
2 x 75	12	9	76	76
2 x 150	12	9	151	151

Inspect and Mix Reagents

1.15 Gently invert the Trinity Sequencing Cartridge 10 times to mix reagents.

1.16 Tap the Trinity Sequencing Cartridge base on the benchtop to remove any large droplets from the tube tops.

1.17 Inspect the small tubes to make sure reagents are settled at the bottom.

1.18 Insert the Trinity Sequencing Cartridge into a clean cartridge basket and lock the clips.

Prepare the Sequencing Solution

1.19 When the 16-hour incubation at 72°C is complete, remove the hybridization reaction from the thermal cycler and briefly centrifuge. Place the reaction on the benchtop.

1.20 Immediately dilute the 70 µl hybridization reaction with 130 µl of library loading buffer. Mix thoroughly by gentle pipetting.

NOTE: The remaining hybridization reaction volume can be stored at -20°C for up to a week.

1.21 Prepare the sequencing solution on ice in a 5 ml tube as follows. Mix thoroughly by gentle pipetting.

Component	Volume
Library Loading Buffer	2053 µl
Trinity Sequencing Reagent	72 µl
Diluted hybridization reaction	75 µl
Total	**2200 µl**

Add Sequencing Solution to the Cartridge

1.22 Using a new 1 ml pipette tip, pierce the Library well of the sequencing cartridge. Push the foil to the edges.

1.23 Transfer the 2200 µl of prepared sequencing solution to the Library well in the sequencing cartridge.

1.24 Twist to remove each shipping lock from the cartridge lid.

Confirm Reagent Preparation

1.25 Select the **Invert cartridge** checkbox to confirm that the reagents are mixed.

1.26 Select the **Insert into basket** checkbox to confirm that the Trinity Sequencing Cartridge is in the cartridge basket.

1.27 Select the **Load hybed reaction** checkbox to confirm that the Trinity Sequencing Cartridge contains the hybridized reaction.

1.28 Select **Next**.

Load Reagents and Buffer

1.29 Open the reagent bay door and remove any used consumables.

1.30 Slide the basket containing the thawed Trinity Sequencing Cartridge into the reagent bay until it stops.

1.31 Slide the AVITI Buffer Bottle into the reagent bay until it stops.

1.32 Close the reagent bay door, and then select **Next**.

Empty Waste and Prime Reagents

1.33 Open the waste bay door, remove the waste bottle, and close the transport cap.

1.34 Open the transport cap and the vent cap and empty the waste bottle.

1.35 Close the vent cap and reload the empty waste bottle.

1.36 Select **Next** to automatically start priming.

1.37 During priming, bring a new Trinity Flow Cell to room temperature for ≥ 5 minutes. Do not open the pouch.

1.38 When priming is complete, select **Next**. The nest door opens automatically.

Load the Flow Cell

1.39 Remove the used flow cell from the nest.

1.40 Unpackage the new Trinity Flow Cell and load it onto the nest.

1.41 Select **Close Nest**, and then select **Next**.

Review and Start the Run

1.42 Review the run parameters to ensure proper setup.

1.43 Select **Run** to start sequencing.

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, it is recommended that libraries be selected from a single column. Up to three columns may be selected in any desired combination across a single plate for sequencing.

Twist Universal Adapter System - Element Compatible (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

Document History

Date of Release	Document Number	Description
December 2024	DOC-001514 2.0 / MA-00054 B	Updated general notes to state that one Trinity sequencing run supports a single hybridization for up to a 24-plex library pool. Updated kit configuration to support a 24-plex hybridization reaction. Changed the order in which steps are performed for Preparing Sequencing Solution. Updated name of sequencing basket to cartridge basket. Added statement to double volumes for libraries prepared with UDI 47 and UDI 67. Added option to select a planned run.
October 2024	DOC-001514 1.0 / MA-00054 A	Initial release.

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