xGen™ Exome Sequencing Kit Trinity for Element AVITI™ System

Element Biosciences

IDT INTEGRATED DNA TECHNOLOGIES

MA-00056 Rev. A

REVISION HISTORY

Version	Release date	Description of changes
1	October 2024	Initial release

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INTRODUCTION

xGen™ Hybridization Capture for Trinity on an Element Biosciences AVITI™ System simplifies exome sequencing unlike any previously available workflow. This workflow moves time consuming steps from the benchtop to your AVITI System, saving up to 4 hours of hands-on time, up to 28 hours in total time as compared to a competitive sequencing system, and eliminates the need for specialized equipment without compromising performance so you can focus on higher value projects

For your convenience, this protocol has been created in conjunction with Element Biosciences so users will not need to refer to multiple protocols for a single workflow and is a full set of instructions from DNA sample prep and hybridization, through to sequencing on the Element AVITI system.

OVERVIEW

This protocol describes the workflow for library preparation, followed by downstream 24-plex exome enrichment and sequencing using the Trinity workflow on the Element AVITI System. The xGen DNA Library Prep EZ UNI Kit includes enzymatic fragmentation to streamline the workflow for high-throughput research applications and automation, and is designed to produce next-generation sequencing (NGS) libraries from a broad range of double-stranded DNA inputs (100 pg to 1 µg).

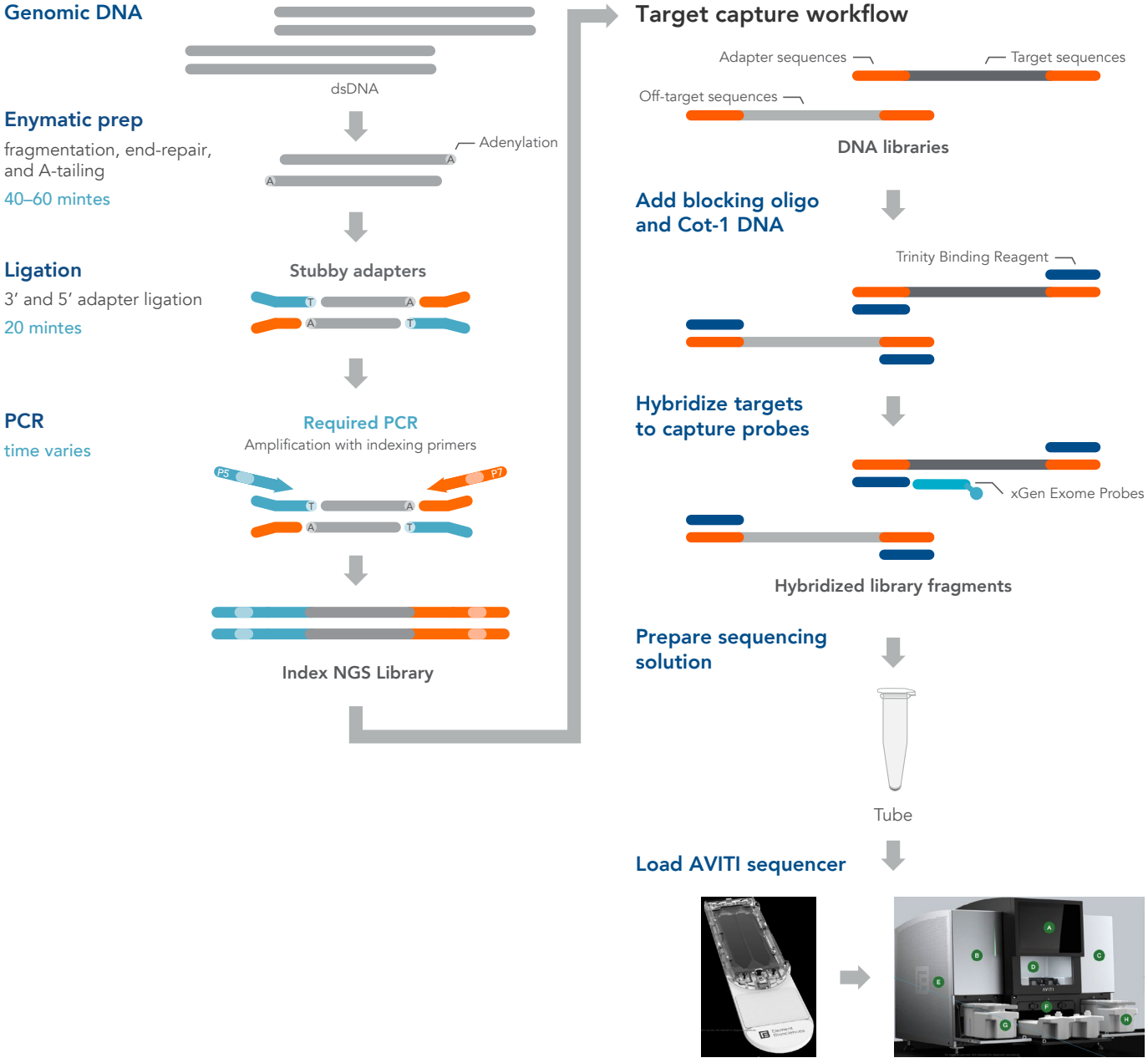

Figure 1. The xGen DNA Library Prep EZ UNI library preparation steps.

WORKFLOW

This workflow contains minimal hands-on steps, reducing the time it takes to prepare linear libraries for sequencing on the AVITI™ system.

Library preparation has minimal enzymatic incubations and bead-based cleanups, thereby reducing the sample handling and overall library preparation time to under 2 hours before library amplification. There are three major activities outlined in this protocol for library preparation:

- **Enzymatic preparation**. Performs fragmentation, end-repair, and dA-tailing of dsDNA in a single reaction. The final fragmentation profile is dependent on both incubation temperature and time.
- **Adapter ligation**. Performs ligation using a stubby Y adapter. Ligation with the Stubby Adapter for Element Sequencing requires amplification with indexing primers to incorporate sample indexing sequences and to add the flow cell attachment sequences.
- **PCR amplification**. Amplify libraries based on the adapter and DNA input used.

There are three major activities outlined in this protocol for Hybridization capture:

- **Pooling and dry-down**. Combine up to twenty-four (24) individually indexed linear libraries for on-sequencer capture, and perform hybridization using the xGen Exome v2 Panel.
- **Hybridization and sequencing preparation**. Perform the appropriate dilution and preparation steps of the hybridized library pool for capture and sequencing on the AVITI™ sequencing platform.
- **Capture and sequencing**. Capture and sequence the prepared hybridization pool directly on the AVITI™ platform.

Library preparation

1	Prepare reagents	• Thaw reagents on ice • Prepare fresh 80% ethanol	Total time: Varies (~20 min)
2	Perform enzymatic preparation	• Set up dsDNA fragmentation, end-repair, and A-tailing • Run Enzymatic Prep program	Total time: 40–60 min
3	Perform ligation	• Add stubby adapters	Total time: 20 min
4	Clean up ligation reaction	• Purify ligation product	Total time: 20 min
	Safe stopping point (store @ –20°C)		
5	Perform PCR & cleanup*	• Add index sequences • Increase available library for sequencing	Total time: PCR, 10–30 min Cleanup, 20 min
	Safe stopping point (store @ –20°C)		

Hybridization capture & sequencing

6	Library Pooling and dry-down	• Pool up to twenty-four (24) linear libraries • Dry-down the pooled library reaction	Total time: Varies (< 90 min)
7	Hybridization	• Set up a hybridization reaction, using xGen Exome v2 Panel • Run Hybridization program	Total time: 16 hours
8	Prepare for sequencing	• Dilute and add key sequencing reagents	Total time: < 10 min
9	Start sequencing run	• Load and begin sequencing on the AVITI platform	Total time: 30 min

CONSUMABLES AND EQUIPMENT

Consumables—IDT

Workflow component	Product name	Reaction size	Catalog number
xGen Exome Sequencing Kit Trinity for Element – Catalog number 10022463	xGen DNA Library Prep EZ UNI Kit	96 rxn	10009822
	xGen Stubby Adapter UDI Primers for Element	96 rxn	10017037
	xGen Hyb Capture Kit Trinity for Element	16 rxn	10022462
	xGen Exome Hyb Panel v2	4 rxn	10005151

Kit contents—P/N 10009822

Kits contain sufficient reagents for the preparation of 96 libraries (10% excess volume provided).

	Components	Volume	Storage
End prep	• Buffer K1	317 µL	–20°C
	• Reagent K2	476 µL	
	• Enzyme K3	634 µL	
Adapter ligation	• Buffer W1	1218 µL	
	• Enzyme W3	424 µL	
PCR amplification	• PCR Master Mix	2640 µL	
	• Reagent R1*	—	
Additional reagents	Low EDTA TE	20 mL	Room temperature

* Reagent R1 are amplification primers compatible with other sequencing systems and is not used for Element Biosciences sequencing. They will not be used for Element Biosciences sequencing.

Kit contents—P/N 10017037

Kits contain sufficient reagents for the preparation of 96 libraries (10% excess volume provided).

	Components	Volume	Storage
xGen Stubby Adapter -UDI Primers for Element	xGen Stubby Adapter for Element	15 uM Tube	–20°C
	xGen Indexing Primers for Element	10 uM single use plate	

Kit contents—P/N 1022462

Kits contain sufficient reagents for the preparation of 16 libraries (10% excess volume provided).

Workflow component	Product name	Volume	Catalog number
xGen Hyb Capture Kit Trinity for Element – Catalog number 1022462	xGen Human Cot DNA, 150 µL	150 µL	1080769
	xGen 2x Hybridization Buffer	16 rxn kit	1072277
	xGen Hyb Buffer Enhancer	16 rxn kit	1080587
	xGen Exome Hyb Panel v2	96 rxn	10005153

Consumables—Element

Item	Supplier	Catalog number
Trinity 2 x 75 Sequencing Kit Includes: Trinity Flow Cell Trinity 2 x 75 Cartridge Trinity Sequencing Reagent Library Loading Buffer Buffer Bottle	Element Biosciences	860-00019
Trinity 2 x 150 Sequencing Kit Includes: Trinity Flow Cell Trinity 2 x 150 Cartridge Trinity Sequencing Reagent Library Loading Buffer Buffer Bottle	Element Biosciences	860-00020
Trinity Binding Reagent	Element Biosciences	830-00029

Consumables—Other suppliers

Item	Supplier	Catalog number
Absolute ethanol (200 proof)	Various suppliers	Varies
Purification beads		
SPRIselect™ purification beads, or equivalent		B23317/B23318/B2331
Agencourt® AMPure® XP-PCR purification beads, or equivalent		A63880 or A63881
Experion™ DNA 1K Analysis Kit, or equivalent	Bio-Rad	700-7107
High Sensitivity DNA Kit	Agilent	5067-4626
High Sensitivity D1000 ScreenTape®, or equivalent	Agilent	5067-5584
Fluorometric DNA quantification assay kit		
Qubit™ dsDNA HS Assay Kit, or equivalent	Thermo Fisher Scientific	Q32851 or Q32854
Qubit dsDNA BR Assay Kit, or equivalent	Thermo Fisher Scientific	Q32850 or Q32853
KAPA Biosystems® Library Quantification Kit	Roche	KK4824
Nuclease-Free Water	Various suppliers	Varies
PCR tubes, 0.2 mL		
96-well, low-bind PCR plates		
Low-bind DNA Tubes, 1.5 mL		
Aerosol-resistant tips and pipettes ranging from 2–1000 µL		

Equipment

Item	Supplier	Catalog #
Digital electrophoresis		
Experion Electrophoresis Station, or equivalent 2100 Electrophoresis Bioanalyzer, or equivalent 2200 TapeStation System/4200 TapeStation System, or equivalent	Bio-Rad Agilent Agilent	700-7010 G2939BA G2965AA or G2991AA
Qubit 4 Fluorometer, or equivalent	Thermo Fisher Scientific	Q33226
qPCR system	Various Suppliers	Varies
Magnet options (choose one):		
Magnetic Separator Plate Magnetic PCR Strip Magnetic Separator Rack	Permagen Permagen	MSP750 MSR812
Microcentrifuge	Various Suppliers	Varies
Vortex		
Thermal Cycler		
Vacuum concentrator	Thermo Fisher Scientific SpeedVac® System, or equivalent	Varies
Microcentrifuge	Various Suppliers	Varies
Vortex		
Thermal Cycler		

Reagent handling

Important: Always store kit reagents at –20°C, except for the xGen Low EDTA TE Buffer which can be stored at room temperature.

Note: The enzymes provided in this kit are temperature sensitive. Appropriate care should be taken during storage and handling. To maximize use of enzyme reagents, remove enzyme tubes from –20°C storage and place on ice for at least 10 minutes prior to pipetting. Attempting to pipette enzymes at –20°C may result in reagent loss.

Except for Buffer W1 and enzymes, briefly vortex the reagents after thawing them on ice. Spin all tubes in a microcentrifuge to collect contents before opening.

Thaw Buffer W1 (for Ligation Master Mix) at room temperature. Buffer W1 is viscous and requires special handling during pipetting. When ready for use, pipette slowly to draw the accurate quantity.

To create master mixes, scale reagent volumes as appropriate, using 5% excess volume to compensate for pipetting loss. Add reagents to the master mix in the specified order, as stated throughout the protocol. Once prepared, master mixes should be stored on ice until used.

Avoid cross-contamination

Important: To reduce the risk of DNA and library contamination, physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed. We recommend taking these steps to avoid cross-contamination:

- Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
- Use barrier pipette tips to avoid exposure to potential contaminants.
- Always change pipette tips between each sample.

Size selection during cleanup steps

This protocol has been optimized with SPRISelect® beads (Beckman Coulter) but can also be used with Agencourt® AMPure® XP beads (Beckman Coulter) or equivalent bead-based nucleotide purification products. If other beads are used, solutions and conditions for DNA binding may differ. Consider these points before performing dsDNA size selection:

- Post-enzymatic fragmentation, analyze the size distribution by electrophoretic methods to determine the fragment size of your dsDNA samples.
- Left side size selection is recommended for this protocol.
- To customize size selection, use Beckman Coulter's SPRISelect User Guide for conditions not discussed here.

DNA input considerations

This kit works with a broad range of DNA inputs, ranging from 100 pg to 100 ng. Our kit can use:

- **High Quality gDNA**: Quantify with Qubit or similar fluorometric method.
- **Formalin-fixed paraffin-embedded (FFPE) DNA**: Quantify using xGen Input DNA Quantification Primers (e.g., Human Alu primers). See the Input DNA Quantification Assay for more details.
- **Amplicons**: Quantify with Qubit or similar fluorometric method.

For high quality samples, dsDNA concentration can be determined using Qubit (Thermo Fisher Scientific), or a similar fluorometric method, to measure the size and concentration of your DNA samples.

To quantify the concentration of low-quality human DNA samples, qPCR can be performed using the xGen Input DNA Quantification Primers (Cat. No. 10009856). Our primers will help you accurately assess the usable amount of genomic DNA in the samples and its integrity. The xGen Input DNA Quantification Primers are for research use only (RUO).

Important: For specific input quantities recommended in this protocol, refer to the total DNA quantified after fragmentation.

Fragmentation parameters

When utilizing a new lot of the fragmentation enzyme, you may experience variation in the required fragmentation times. Refer to your certificate of analysis (CoA) for specific fragmentation time recommendations for the lot number that you received.

Tip: To find the CoA, enter the lot number of your kit (found on the bottom of the packaging) at idtdna.com/COA. Alternatively, read the CoA provided with your kit on the label inside the product box for lot-specific fragmentation parameters.

Fragmentation times provided in this protocol are for high quality samples. You may need to determine shorter fragmentation time for samples of compromised quality (e.g., FFPE).

Additionally, the xGen Deceleration Module can be used with this kit to achieve larger aligned insert sizes of 550 bp if needed.

EDTA in elution buffers

The enzymatic preparation reaction is sensitive to high concentrations of EDTA, which is usually introduced by elution buffers in the final steps of the DNA extraction or purification process. A high concentration of EDTA, such as 1 mM in standard TE buffer, will slow the reaction, resulting in larger insert sizes. Alternatively, no EDTA (if eluted in Tris buffer only) will result in faster fragmentation and smaller insert sizes.

Our standard enzymatic prep conditions are determined using 0.1 mM EDTA TE (as provided in this kit) and requires 1.5 µL of Reagent K2 for fragmentation.

If DNA is eluted in standard TE with 1 mM EDTA, perform a buffer exchange using a column, or bead-based purification protocol (3x SPRIselect® from Beckman Coulter is recommended for minimum loss of sample gDNA). Alternatively, you can adjust the amount of Reagent K2 used in the Enzymatic Prep step to no more than 3x to achieve the desired fragment length (up to 4.5 µL of Reagent K2 per reaction).

If DNA is resuspended in 10 mM Tris or water (e.g., Buffer EB from Qiagen, 10 mM Tris-HCl, pH 8.5) without EDTA, Reagent K2 is not needed during Enzymatic Prep.

Automation

This protocol is amenable to automation. A 10% overage volume of reagents is supplied in the xGen DNA Library EZ kits to accommodate automation. IDT does not supply automated liquid handling instruments or consumables but collaborates with automation solution providers and customers to develop optimized scripts for use of our kits with liquid handling platforms. Contact your instrument vendor or contact us if you plan to use this kit with your automated liquid handling system.

Note: The IDT xGen Deceleration Module can be used to enable room temperature reaction setup and fragmentation times suitable for high-throughput use.

PROTOCOL—LIBRARY PREPARATION

Enzymatic prep

Important: Keep the Enzymatic Prep Master Mix and the DNA samples on ice until they are loaded in the thermal cycler to safeguard against fragmentation. Enzymes are active at room temperature and may fragment DNA to undesired sizes.

1. Transfer the DNA sample to a sterile, 0.2 mL PCR tube. Adjust sample volume to a total of 19.5 µL using Low EDTA TE, then place the tube on ice.

Components	Volume per sample (µL)
Low EDTA TE	(19.5 -x)
DNA	x
Total volume	**19.5**

2. Set up the thermal cycler with the Enzymatic Prep program, as described below, with the lid set to 70°C (heated lid required).

Step	Temperature * (°C)	Time
Hold	4	∞
Fragmentation	32	Variable (see note)
Inactivation	65	30 minutes
Hold	4	Less than 1 hour

* Lid temperature needs to be set to 70°C

Important: Fragmented samples can be kept at 4°C for no longer than 1 hour.

Note: See your Certificate of Analysis (CoA) for fragmentation time recommendations for the individual lot number that you received. Reaction times may be optimized for individual samples. Specifically, for sample inputs <25 ng, longer fragmentation times may be required.

3. Begin the Enzymatic Prep program by chilling the thermal cycler to 4°C.
4. Prepare the Enzymatic Prep Master Mix by adding the components in the order shown:

Enzymatic Prep Master Mix	
Components	**Volume per reaction (µL)**
• Buffer K1	3.0
• Reagent K2	1.5
• Enzyme K3	6.0
Total volume	**10.5**

5. Vortex the Enzymatic Prep Master Mix for 5 seconds, then briefly centrifuge. Keep mix on ice until ready to use.

Important: Ensure that the Enzymatic Prep Master Mix is mixed thoroughly before and after the addition of DNA samples to prevent incomplete fragmentation.

6. Add 10.5 µL of the premixed Enzymatic Prep Master Mix to each tube containing DNA samples and low EDTA TE to reach a final volume of 30 µL.
7. Thoroughly vortex the sample tubes for 5 seconds.
8. Briefly centrifuge the sample tubes, then immediately place in the chilled thermal cycler and advance the Enzymatic Prep program to the 32°C fragmentation step.
9. While the enzymatic prep program runs, prepare the Ligation Master Mix.

Adapter ligation

1. Before starting adapter ligation, preset a thermal cycler according to the program listed below with lid heating **OFF**.

Ligation program		
Step	Temperature* (°C)	Time
Ligation	20	20 minutes
Hold	4	Hold

* Lid temperature should be OFF

2. For DNA input <25 ng, dilute adapters (e.g., xGen Stubby Adapter for Element), as shown:

Important: Adapter dilution is necessary to achieve low levels of adapter dimer. For certain applications, adapter dilution may be adjusted to achieve the best results.

DNA input	Adapter*
≥25 ng	No dilution
10 ng	10-fold (1:10)
1 ng	20-fold (1:20)
100 pg	30-fold (1:30)

* For xGen DNA Library Prep EZ UNI xGen Stubby Adapters for Element are used at a 15 µM stock concentration.

3. Prepare the Ligation Master Mix by referring to the table below. Add components in the order shown.

xGen DNA Library Prep EZ UNI Master Mix	
Components	Volume per reaction (µL)
• Buffer W1	12
• Enzyme W3	4
Low EDTA TE	9
xGen Stubby Adapter for Element	5
Total Master Mix	**30**
Fragmented sample	30
Total volume	**60**

* If preparing the ligation master mix ahead of time, add the adapter to the Master Mix just prior to use.

Important: Slowly pipette the viscous Buffer W1 to avoid bubbles and to ensure accuracy.

4. When the Enzymatic Prep program is complete, add pre-mixed Ligation Master Mix to the tubes containing fragmented DNA:
   - 30 µL for xGen DNA Library Prep EZ UNI

5. For xGen DNA Library Prep EZ UNI samples ONLY, add the full-length Indexed Y Adapters individually to each sample.
6. Thoroughly mix samples by moderate vortexing for 5 seconds and briefly centrifuge.
7. Place samples in the pre-programmed thermal cycler and run the Ligation program from step 1 of this section.
8. After the ligation program is complete, proceed immediately to Post-ligation cleanup.

Post-ligation cleanup

Important: Make sure magnetic beads are equilibrated to room temperature before starting this section.

1. Prepare fresh 80% ethanol solution.
2. Vortex the beads until the solution is homogenous.
3. Add 48 µL of beads to each sample at room temperature (ratio of bead to sample is 0.8).
4. Thoroughly mix samples by moderate vortexing for 5 seconds, then briefly centrifuge.
5. Incubate the samples for 5 minutes at room temperature.
6. Place the samples on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
7. Remove and discard the supernatant without disturbing the pellet (less than 5 µL may be left behind).
8. Add 180 µL of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Be careful not to disturb the pellet.
9. Incubate for 30 seconds, then carefully remove the ethanol solution using a pipette.
10. Repeat steps 8 and 9 for a second ethanol wash.
11. Quickly spin the samples in a microcentrifuge, then place them back on the magnetic rack. Remove any residual ethanol solution from the bottom of the tube with a clean pipette tip.
12. Remove the samples from the magnetic rack.
13. Add 20 µL Low EDTA TE to the sample tubes based on your application as shown.
14. Incubate the samples at room temperature for 2 minutes.
15. Place the samples on a magnetic rack until the solution clears and a pellet is formed (~2 minutes).
16. Carefully transfer the clear solution into a clean tube, being careful to avoid any bead carryover.

Safe Stop: DNA libraries can be stored overnight at –20°C.

PCR amplification

1. Set up the thermal cycler with the PCR program as shown below, with a heated lid set to 105°C. Adjust the number of cycles based on input amount and workflow.

PCR amplification program			
Step	Cycles	Temperature (°C)	Time
Initial denaturation	1	98	45 seconds
Denaturation	Varies (see table "Cycling recommendation for PCR-amplified workflows)	98	15 seconds
Annealing		60	30 seconds
Extension		72	30 seconds
Final extension	1	72	1 minute
Hold	1	4	∞

* Lid should be heated to 105°C

Cycling recommendations for PCR-amplified workflows	
DNA input	Recommended cycles
100 ng	5–7
10 ng	9–12
1 ng	12–15
100 pg	15–18

2. Prepare the PCR Master Mix by following these guidelines. Add 25 µL of the prepared PCR master mix to the eluted samples, followed by 5 µL from the appropriate well of the xGen UDI Primers for Element plate. Afterwards, mix by pipetting several times.

xGen DNA Library Prep EZ UNI Master Mix	
Components	Volume per reaction (µL)
• PCR Master Mix	25
• xGen UDI Primers for Element	5
Total Master Mix volume	30
Eluted sample	20
Total volume	

3. Place samples into preprogrammed thermal cycler and run the PCR Amplification Program.
4. When the PCR program is complete, vortex the room temperature SPRIselect beads until the solution is homogenous.
5. Proceed to Post-PCR cleanup.

PCR cleanup

1. Add 90 µL bead volume (for 1.8x ratio) to each sample: Average insert size 200 bp, Sample volume 50 µL, and bead volume 90 µL (ratio: 1:8).
2. Vortex sample tubes, then briefly centrifuge.
3. Incubate the samples for 5 minutes at room temperature.
4. Place the samples on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
5. Remove and discard the supernatant, using a clean pipette tip, without disturbing the pellet (less than 5 µL may be left behind).
6. Add 180 µL of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Be careful not to disturb the pellet.
7. Incubate for 30 seconds, then carefully remove the ethanol solution.
8. Repeat steps 4 and 5 for a second ethanol wash.
9. Quickly spin the samples in a microcentrifuge, then place them back on the magnetic rack. Remove any residual ethanol solution from the bottom of the tube with a clean pipette tip.
10. Remove the samples from the magnetic rack.
11. Add 21 µL of Low EDTA TE to the sample tubes and mix well until homogenous.
12. Incubate sample tubes at room temperature for 2 minutes.
13. Place the samples on a magnetic rack until the solution clears and a pellet is formed (~2 minutes).
14. Carefully transfer 20 µL clear solution (eluted DNA) into a clean tube, being careful to avoid any bead carryover.

    Safe Stop: Libraries can be stored overnight at –20°C.
15. The library is now ready for quantification, which can be performed using fluorometric methods (i.e., Qubit™ Fluorometer) or qPCR.

Example of data output

Example libraries were prepared from NA12878 Coriell DNA (Coriell Institute) using the xGen DNA EZ UNI Library Prep Kit. The sample was fragmented enzymatically to create 200 bp inserts. The prepared inserts were ligated to xGen Stubby Adapters for Element. The final expected size of the library fragment is ~ 314 bp. In Figure 2, Agilent High Sensitivity DNA traces show final library fragments.

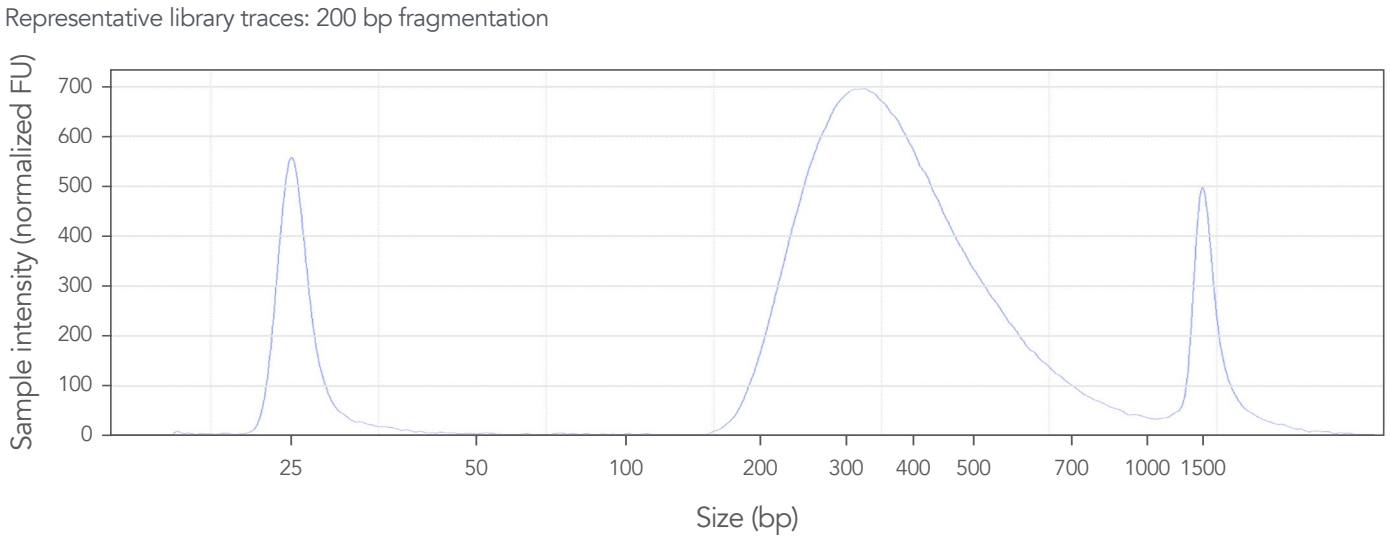

Figure 2. Representative TapeStation traces for 200 bp xGen DNA EZ UNI Libraries. The resulting library (blue) showed a peak height of ~314 bp; this is the expected library size after adding the xGen Stubby Adapters for Element and amplified by using the xGen UDI Primers for Element.

Perform hybridization reaction

1. In an appropriately sized vessel, combine up to twenty-four (24) uniquely indexed libraries to create a 5–6 µg multiplexed library pool. Use 2x input for sample with UDI-67

   Note: It is recommended to perform this step in a low-bind plate format to reduce the risk of sample loss. However, a tube format may be used if necessary.

   Tip: When using a plate format mark the wells that contain DNA before drying down the plate since they will not be distinguishable from empty wells after dry-down.

2. Add 5 µL of xGen Human Cot DNA.
3. Add 5 µL of Trinity Binding Reagent.
4. Vortex to mix and spin down.
5. Dry down the mixture in a SpeedVac system, or equivalent, low (less than 40°C) or no heat

   Safe Stop: Dried down samples may be stored at –20°C for no more than one (1) week.

6. Thaw all contents of the xGen Hybridization reagents to room temperature.

   Note: Inspect the tube of 2X Hybridization Buffer for crystallization of salts. If crystals are present, heat the tube at 65°C, shaking intermittently, until the buffer is completely solubilized.

7. Create the Hybridization master mix in a 1.5 mL tube. Multiply by the number of hybridization reactions and add a 10% overfill.

Hybridization Master Mix components	Volume per reaction (µL)
xGen 2X Hybridization Buffer	8.5
xGen Hybridization Buffer Enhancer	2.7
xGen Exome Hyb Panel	4
Nuclease-Free Water	1.8

8. Vortex or pipette mix the Hybridization Master Mix to mix well
9. Add 17 µL of the Hybridization Master Mix to each tube or plate well containing dried DNA
10. Pipette mix, then incubate at room temperature for 5–10 min.

    Note: If you have resuspended your dried down sample using a tube format, you must transfer the resuspended 17 µL reaction to a low-bind 0.2 mL plate before continuing.

11. Securely seal the plate, vortex, and then briefly centrifuge.
12. Place the plate in a thermal cycler and run the HYB program as shown below, with a heated lid set to 100°C.

HYB program (lid set at 100°C)	
95°C	30 sec
65°C	16 h
65°C	Hold

TRINITY SEQUENCING

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

Prepare consumables

1. Thaw the Trinity Sequencing Cartridge at 2–8°C overnight, out of direct light.
2. Alternatively, the Trinity Sequencing Cartridge may be thawed in a 20°C water bath out of direct light, and then placed at 2–8°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.

Initiate a sequencing run

1. On the Home screen of the Element AVITI™ instrument, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a used flow cell.
3. Select **Sequencing**.
4. Select the side for sequencing (**Side A**, **Both**, or **Side B**).
5. For "Chemistry Type", select **Trinity**.

Define run parameters

1. In the "Run Name" field, enter a unique run name.
2. If applicable, select **Browse** and import the run manifest.
3. In the "Storage" drop-down menu, select an appropriate storage location for the run.
4. In the "Sequencing Kit" drop-down menu, select the appropriate Trinity Sequencing Kit.
5. In the "Panel" drop-down menu, select **xGen Exome Kit for Trinity**.
6. In the "Cycles" field, enter the number of cycles for each read, and then select **Next**.

Default Default Cycle Values				
Kit type	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. Gently invert the Trinity Sequencing Cartridge 10 times to mix.
2. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.
3. Inspect the small tubes to make sure reagents are settled at the bottom.
4. Place the cartridge into a clean cartridge basket and lock the clips.

Prepare Sequencing Solutions

Important: Ensure the Trinity Sequencing Cartridge is completely thawed. The cartridge may be thawed in a 20°C water bath outside of direct light prior to use. However, it should be placed at 2–8°C once fully thawed.

1. About 5 minutes before completion of the overnight hybridization, combine the following reagents on ice to prepare the sequencing solution in a 5 mL tube or 15 mL Falcon tube.

Sequencing Reaction Components	Volume (µL)
Trinity Sequencing Reagent	72
Trinity Library Loading Buffer	2,038
Total Volume	2,110

2. Remove the hybridization reaction from the thermal cycler and place on the bench
3. Immediately add 183 µL of the Library Loading Buffer to dilute the Hybridization Reaction. This is now the diluted hybridization reaction.

   Safe Stop: The diluted hybridization reaction volume can be stored at –20°C for up to one (1) week

4. Pipette gently to mix thoroughly.
5. Immediately after pipette mixing, transfer 90 µL of the diluted hybridization reaction to the sequencing solution prepared in Step 1 above.
6. Pipette gently to mix thoroughly.

Add Sequencing Solution to the Trinity Sequencing Cartridge

1. Using a new 1 mL pipette tip, pierce the center of the Library well of the sequencing cartridge.
2. Transfer 2200 µL sequencing solution to the Library well.
3. Twist to remove each shipping lock from the cartridge lid.

Confirm reagent preparation

1. Select the **Invert cartridge** checkbox to confirm that the cartridge was mixed.
2. Select the **Insert into basket** checkbox to confirm that the sequencing cartridge is in the cartridge basket.
3. Select the **Load hybed reaction** checkbox to confirm that the sequencing cartridge contains the hybridized reaction.
4. Select **Next**.

Load reagents and buffer bottle

1. Open the reagent bay door and remove any used consumables.
2. Slide the basket containing the thawed Trinity Sequencing Cartridge into the reagent bay until it stops.
3. Slide the AVITI Buffer bottle into the reagent bay until it stops.
4. Close the reagent bay door, and then select **Next**.

Empty waste and prime reagents

1. Open the waste bay door, remove the waste bottle, and close the transport cap.
2. Open the transport cap and the vent cap to empty the waste bottle.
3. Close the vent cap and reload the empty waste bottle.
4. Select **Next** to automatically start priming the reagents.
5. During priming, bring a new flow cell to room temperature for ≥ 5 minutes. **Do not** open the pouch.
6. When priming is complete, select **Next**. The nest door opens automatically.

Load Flow Cell

1. Remove the used flow cell.
2. Unpackage the new Trinity Flow Cell and load it onto the nest.
3. Select **Close Nest**, and then select **Next**.

Review and start the run

1. Review the run parameters to ensure proper setup.
2. Select **Run** to begin sequencing.

APPENDIX A: INDEXED ADAPTER SEQUENCES

For the master list of sequences, see Index Sequences Master List found on the xGen Indexing page.

APPENDIX B: TROUBLESHOOTING

Issue	Possible cause	Suggested solution
Library migrates unexpectedly on Bioanalyzer.	Over-amplification of library leads to the formation of heteroduplex structures that migrate slowly.	• Quantify library by qPCR, as other quantification methods will not accurately quantify heteroduplex library molecules. • Perform a lower number of PCR cycles than necessary to avoid over-amplification.
DNA is under fragmented (larger than expected molecular weight).	Input DNA was in a buffer with greater than 0.1 mM EDTA. Improper mixing of reagents.	• Use a buffer exchange column or bead-based clean-up before fragmentation or use up to 3x volume of Reagent K2 in your fragmentation reaction. • Ensure fragmentation mixture is adequately mixed prior to and after adding to DNA samples.
DNA is over fragmented (smaller than expected molecular weight).	Reaction left at room temperature. Sample integrity compromised.	• Ensure the Enzymatic Prep master mix and the DNA sample are kept on ice until placed onto the pre-chilled thermal cycler. • Fragmentation time must be optimized for DNA samples that are not high quality (e.g., FFPE). For more information, refer to the xGen Deceleration Module.
Incomplete resuspension of beads after ethanol wash during purification steps.	Over-drying of beads.	• Continue pipetting the liquid over the beads to break up clumps for complete resuspension. To avoid over-drying, resuspend beads immediately after the removal of residual ethanol.
Shortage of enzyme reagents.	Pipetting enzymes at –20°C	• Place enzyme reagents on ice for 10 minutes prior to pipetting.
Retention of liquid in pipette tip.	Viscous reagents (i.e., Buffer W1) may stick to pipette tip, especially for non-low retention tips.	• Pipette up and down several times to ensure all liquid is released from the pipette tip.
Unexpected increase in adapter dimers.	Improper adapter dilution. Improper bead purification. Stubby Adapter not added to the ligation master mix just before use.	• Use the specified dilution for your input DNA quantity. • Use the specified bead volume, particularly during post-PCR purification. • Add xGen Stubby Adapter for Element to the Ligation Master Mix just before use.

xGen™ Exome Sequencing Kit Trinity for Element AVITI™ System

For more information, go to: **www.idtdna.com/ContactUs**

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