xGen™ Exome Sequencing Kit Trinity™ for Element™ Using a 1-Hour Hybridization Reaction

The method presented here is provided by IDT. This method uses the xGen™ Exome Sequencing Kit Trinity™ for Element™ protocol with minor modifications for compatibility with a 1-hour hybridization reaction time. This method can be used in similar experiments but may not be fully optimized for your application. IDT does not guarantee this method and application scientists at IDT can only provide general guidance with limited troubleshooting support. For more information and support for Element Biosciences products and sequencing on the AVITI™ platform, please see the Element Biosciences website.

OVERVIEW

This protocol describes the workflow for library preparation and end-polishing, followed by downstream 24-plex exome enrichment and sequencing using the Trinity workflow on the Element AVITI sequencing system with a 1-hour hybridization reaction time.

The workflow contains minimal hands-on steps, thus reducing the time it takes to prepare linear libraries for sequencing on the AVITI system. An xGen DNA EZ UNI Library Preparation Kit uses minimal enzymatic incubations and bead-based cleanups to reduce sample handling and overall library preparation time to under 2-hours prior to library amplification. Following the creation of libraries, an end-polishing step is performed to ensure sample compatibility with the use of a 1-hour hybridization step. There are four 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 reagent requires amplification with indexing primers to incorporate sample indexing sequences and to add the flow cell attachment sequences.
- **PCR amplification.** Amplifies libraries based on the adapter and DNA input used.
- **End-polishing.** Uses a Library Amplification Primer Mix for Element Sequencing to ensure libraries are compatible with sequencing on the AVITI instrument when using a 1-hour hybridization reaction.

There are three major activities outlined in this protocol for hybridization and sequencing on the AVITI system.

- **Pooling and dry-down.** Combines up to 24 unique indexed end-polished linear libraries for on-sequencer capture and sequencing on the AVITI system.
- **Hybridization and sequencing preparation.** Performs the appropriate dilution and preparation steps of the hybridized library pool for capture and sequencing.
- **Sequencing.** Perform capture and sequencing of the prepared hybridized library pool on the AVITI instrument.

Genomic DNA

Enymatic prep

fragmentation, end-repair, and A-tailing

40–60 mintes

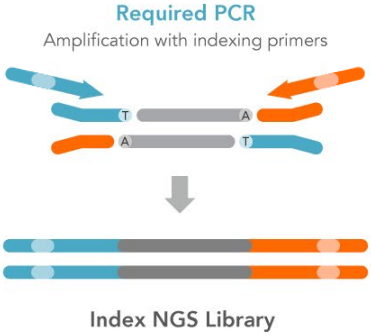
Ligation

3′ and 5′ adapter ligation

20 mintes

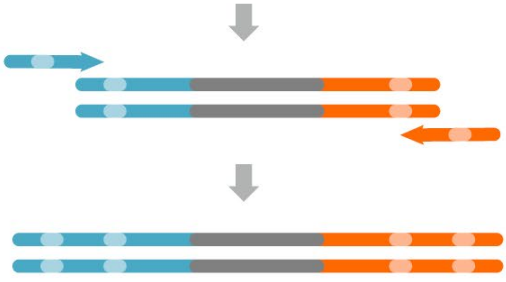
PCR

time varies



End polishing

time varies



Target capture workflow

Hybridize targets to capture probes

Hybridized library fragments

Prepare sequencing solution

Tube

Load AVITI sequencer

Library preparation and End-Polishing

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 Safe stopping point (store @ -20 °C)	• Purify ligation product	Total time: 20 min
5	Perform PCR & cleanup* Safe stopping point (store @ -20 °C)	• Add index sequences • Increase available library for sequencing	Total time: PCR 10 - 30 min Cleanup 20 min
6	Perform Library End-Polishing Safe stopping point (store @ -20 °C)	• Amplify with PCR Amplification Primers • Cleanup end-polished libraries	Total time: PCR 10 - 20 min Cleanup 20 min

Hybridization capture & sequencing

7	Library Pooling and dry-down	• Pool up to twenty-four (24) linear libraries • Dry-down the pooled library reaction	Total time: Varies (< 90 min)
8	Hybridization	• Set up a hybridization reaction, using xGen Exome v2 Panel • Run Hybridization program	Total time: 60 min
9	Prepare for sequencing	• Dilute and add key sequencing reagents	Total time: < 10 min
10	Start sequencing run	• Load and begin sequencing on the AVITI platform	Total time: 30 min

CONSUMABLES AND EQUIPMENT

Consumables – IDT

Product Name	Reaction size	Catalog number
xGen DNA Library Prep EZ UNI Kit	96 rxn	10009822
xGen Stubby Adapter UDI Primers for Element	96 rxn	10017037
xGen Library Amplification Primer Mix for Element	96 rxn	10016959
xGen Human Cot DNA, 150 µL	150 µL	1080769
xGen 2X Hybridization Buffer	16 rxn	1072277
xGen Exome Hyb Panel v2	4 rxn	10005151

Kit Contents – IDT

IDT Kit	Component	Volume	Storage
xGen DNA Library Prep EZ UNI Kit	Buffer K1	317 µL	–20°C
	Reagent K2	476 µL	
	Enzyme K3	634 µL	
	Buffer W1	1,218 µL	
	Enzyme W3	424 µL	
	PCR Master mix	2,640 µL	
	Reagent R1*	-	
	Low EDTA TE	20 mL	Room temperature
xGen Stubby Adapter UDI Primers for Element	xGen Stubby Adapter for Element	15 µM tube	–20°C
	xGen Indexing Primers for Element	10 µM single use plate	

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

Consumables – Element Biosciences

Product Name	Catalog number
Trinity 2 x 150 Sequencing Kit:	860-00020
• Trinity Flow Cell	
• Trinity Sequencing Buffer Bottle	
• Trinity 2 x 150 Sequencing Cartridge	
• Trinity Sequencing Reagent	
• Library Loading Buffer	
Trinity Binding Reagent	830-00029

Consumables – Other suppliers

Item	Supplier	Catalog number
Agencourt® AMPure® XP-PCR purification beads	Beckman Coulter	A63880 / A63881
High Sensitivity D1000 Reagent Kit	Agilent	5067-4626
High Sensitivity D1000 Screen Tape®		5067-5584
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851
Purified Nuclease-Free Water	Various suppliers	Varies
Absolute ethanol (200 proof)		
PCR tubes, 0.2 mL		
Low-bind PCR plates, 0.2 mL		
Low-bind DNA Tubes, 1.5 mL		
0.2 mL Plate Seals		
Aerosol-resistant tips and pipettes ranging from 2 – 1000 µL		

Equipment

Item	Supplier	Catalog number
Agilent TapeStation 4200 System	Agilent	G2991AA
Magnetic Separator Plate	Permagen	MSP750
Qubit 4 Fluorometer	Thermo Fisher Scientific	Q33226
DNA vacuum concentration	Various suppliers	Varies
Microcentrifuge		
Vortex		
Thermal Cycler		

GUIDELINES

Reagent storage and 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.

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

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:

Components	Volume per sample (µL)
Buffer K1	3
Reagent K2	1.5
Enzyme K3	6
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.

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

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)

*xGen Stubby Adapters for Element are offered at a 15 µM stock concentration.

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

Components	Volume per sample (µL)
Buffer W1	12
Enzyme W3	4
Low EDTA TE	9
xGen Stubby Adapter for Element	5
Total Master Mix volume	30
Fragmented DNA 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 the 30 µL of pre-mixed ligation master mix to the tubes containing fragmented DNA.

Note: If you do not add the xGen Stubby Adapter for Element into the ligation master mix, ensure you add 5 µL of this reagent to each well containing fragmented DNA before continuing.

5. Thoroughly mix samples by moderate vortexing for 5 seconds and briefly centrifuge.

6. Place samples in the pre-programmed thermal cycler and run the Ligation program from step 1 of this section.
7. 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, using a heated lid set to 105°C. Adjust the number of cycles based on the initial DNA input amount.

PCR amplification program			
Step	Temperature (°C)	Time	Cycles
Polymerase activation	98	45 seconds	1
Denaturation	98	15 seconds	Varies (see "Cycling recommendations for PCR amplified workflow" table below)
Annealing	60	30 seconds	
Extension	72	30 seconds	
Final Extension	72	60 seconds	1
Hold	4	∞	1

*Lid Temperature should be OFF.

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. Add 25 µL of the PCR master mix component to each well containing eluted samples.

3. Add 5 µL of a unique dual index primer using the xGen UDI Primers for Element single use plate in each well containing sample.

Components	Volume per sample (µL)
PCR Master Mix	25
xGen UDI Primers for Element	5
Total Master Mix volume	30
Eluted sample	20
Total volume	50

4. Mix thoroughly by pipetting several times.

5. Place the samples onto a pre-programmed thermal cycler and run the PCR amplification program from Step 1.

6. When the PCR program is complete, vortex the room temperature SPRIselect beads until the solution is homogenous before continuing to Post-PCR cleanup.

Post-PCR cleanup

1. Add 90 µL bead volume (for 1.8x ratio) to each sample.

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.

Library end-polishing

1. Prepare 50 – 100 ng of each linear library into 20.0 µL of nuclease-free water or TE buffer (10 mM Tris, pH 8.0 and 0.1 mM EDTA).

2. Prepare an end-polishing master mix by adding the components shown below:

Components	Volume per sample (µL)
xGen Library Amplification Primer Mix for Element*	5
KAPA HiFi HotStart Ready Mix	25
Total volume	30

*If necessary, dilute the amplification primers to ensure a final primer concentration of 1 µM each.

3. Add 30 µL of the prepared end-polishing master mix to each sample to create a reaction volume of 50 µL.

4. Set up a thermal cycler with the PCR amplification program shown below using a heated lid set at 105°C. Adjust the number of cycles based on the input amount used.

PCR amplification program			
Step	Temperature (°C)	Time	Cycles
Polymerase activation	98	45 seconds	1
Denaturation	98	45 seconds	100 ng input: 5 cycles 50 ng input: 6 cycles
Annealing	60	30 seconds	
Extension	72	30 seconds	
Final Extension	72	60 seconds	1
Hold	4	∞	1

*Lid temperature should be set to 105°C.

Post-end-polishing cleanup

1. Add 50 µL bead volume (for 1.0x ratio) to each sample.
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 and sizing, which can be performed using fluorometric methods (i.e., Qubit™ Fluorometer) and Agilent TapeStation, or equivalent.

Preparation of Trinity Sequencing Cartridge

Note: Thaw the Trinity Sequencing Cartridge at 2–8°C overnight out of direct light. Alternatively, the cartridge may be thawed in a room temperature water bath out of direct light and then placed at 2–8°C. Allow approximately 2.5 hours to thaw a 2 x 150 sequencing cartridge.

Hybridization

1. In an appropriately sized vessel, combine up to twenty-four (24) uniquely indexed libraries to create a 12 µg multiplexed library pool.

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 a hybridization master mix in a 1.5 mL tube by combining the components shown below. It is recommended to multiply by the number of reactions performed and add a 10% overfill.

Components	Volume per sample (µL)
xGen 2X Hybridization Buffer	8.5
xGen Exome Hyb Panel v2	4
Nuclease-Free Water	4.5
Total volume	17

8. Vortex or pipette mix the master mix thoroughly.
9. Add 17 µL of the master mix to each tube or plate well containing dried DNA.
10. Pipette mix, then incubate the sample at room temperature for 5 – 10 minutes.

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 the sample.
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 thermal cycling program		
Step	Temperature (°C)	Time
Initialization	95	5 minutes
Hybridization	80	1 hour
Hold	80	∞

Initiate a sequencing run

1. On the Home screen of the Element AVITI™ instrument, select **New Run**.
2. If the 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, Side B,** or **Both**).

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 as shown below and then select Next.

Kit Type	Index 1	Index 2	Read 1	Read 2
2 x 150	12	9	151	151

Inspect and mix reagents

1. Gently invert the Trinity Sequencing Cartridge 10 times to mix.
2. Tape the base of the cartridge on the benchtop to remove any large droplets from the tube tops.
3. Inspect the small tubes to ensure reagents are settled on the bottom.
4. Place the cartridge into a clean sequencing basket and lock the clips.

Prepare sequencing solution

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. Remove the hybridization reaction from the thermal cycler and briefly centrifuge. Place the reaction on the bench.
2. 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

3. Combine the following reagents on ice to prepare the sequencing solution in a 5 mL tube. Pipette gently to mix thoroughly.

Sequencing Reaction Components	Volume per sample (µL)
Trinity Sequencing Reagent	72
Library Loading Buffer	2,028
Diluted Hybridization Reaction	100
Total volume	2,200

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 the full 2,200 µL sequencing solution to the library well of the cartridge.
3. Twist to remove each shipping lock from the cartridge lid.

Confirm reagent preparation

1. On the AVITI touchscreen, 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 a sequencing 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 previously used consumables and dispose of them properly.
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 water 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** to automatically open the nest door.

Load flow cell

1. Remove the previously used flow cell.
2. Unpackage the new Trinity flow cell and load it onto the nest.
3. Select **Close Nest** to close the nest door.
4. Select **Next**.

Review and start the run

1. Review the run parameters to ensure they are set properly for your run.

2. Select **Run** to begin sequencing.

Library performance

Example libraries were prepared using an initial 100 ng input of Coriell NA12878 genomic DNA (gDNA) and annealed with xGen Stubby Adapters and UDI Primers for Element using an xGen DNA EZ UNI Library Prep Kit. The libraries underwent an end-polishing process using xGen Library Amplification Primer Mix for Element. After end-polishing two (2) independent preparations of the same libraries underwent dry-down, and hybridization before being sequencing on the Element Biosciences AVITI instrument.

The use of a 1-hour hybridization reaction was shown to produce acceptably performing results based on NGS functional metrics. The flanked on-target percentage (86.54 – 87.24%) and fold-80 base penalty (1.42 – 1.50) showed the material was able to achieve acceptable uniform target coverage depth Figure 1. The Picard metrics detailing the percent target coverage at both 20x (95.85 – 96.99%) and 30x (87.33 – 90.88%) emphasize the ability of this protocol to produce acceptable sequencing coverage Figure 2.

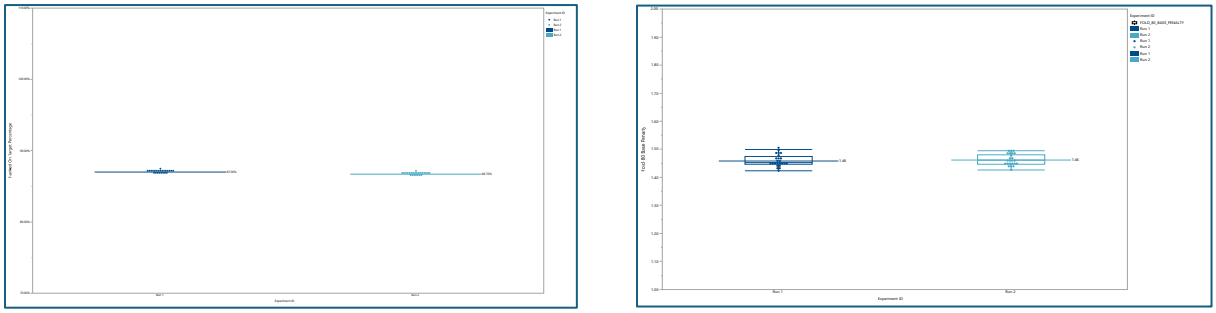


Figure 1, both the flanked on-target percentage and fold-80 base penalty functional NGS metrics evaluated suggest that the use of a 1-hour hybridization reaction is still able to produce uniform and sufficient target coverage depth both between and among sequencing events. Independent sequencing events of a 12 µg, 24-plex hybridized reaction were subsampled to 40 million reads per sample and analyzed using Picard. Error bars shown in the figure represent the standard deviation of the measurement.

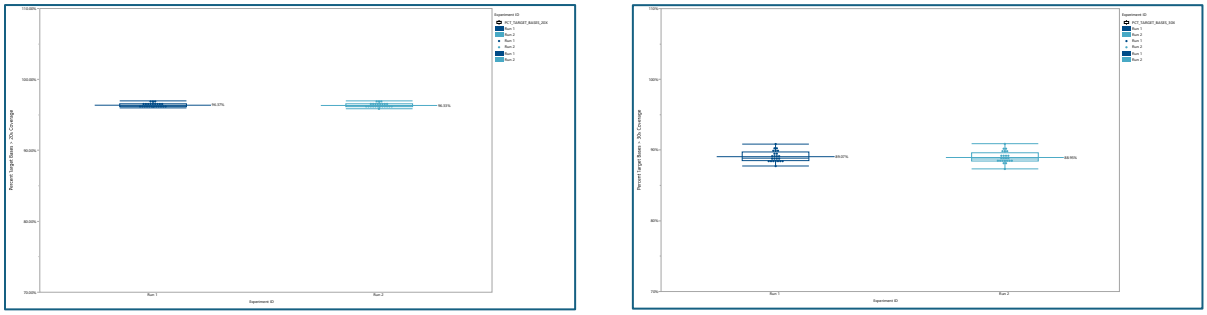


Figure 2, the Picard percent target bases coverage at both 20x and 30x demonstrate acceptable sequencing depth and coverage between and among samples using a 1-hour hybridization reaction. Independent sequencing events of a 12 µg, 24-plex hybridized reaction were subsampled to 40 million reads per sample and analyzed using Picard. Error bars shown in the figure represent the standard deviation of the measurement.

Table 1, a summary of the sequencing metric variability observed among both independent sequencing events. Both flow cells passed all instrument quality specifications.

NGS sequencing metric	Run number	Minimum observed	Average observed	Maximum observed	%CV
Flanked on-target percentage	1	86.84%	87.00%	87.24%	0.13%
	2	86.54%	86.70%	86.97%	0.13%
% target base coverage at 20x	1	96.00%	96.37%	96.967%	0.23%
	2	95.85%	96.33%	96.99%	0.27%
% target base coverage at 30x	1	87.74%	89.07%	90.86%	0.83%
	2	87.33%	88.95%	90.88%	0.91%
Fold-80 base penalty	1	1.42	1.46	1.50	1.41%
	2	1.43	1.46	1.49	1.30%

Table 2, a summary of the sequencing metric variability observed between each independent sequencing event. Both flow cells passed all instrument quality specifications.

NGS sequencing metric	Minimum observed	Average observed	Maximum observed	%CV
Flanked on-target percentage	86.54%	86.85%	87.24%	0.22%
% target base coverage at 20x	95.85%	96.35%	96.99%	0.25%
% target base coverage at 30x	87.33%	89.01%	90.88%	0.87%
Fold-80 base penalty	1.42	1.46	1.50	1.36%

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.

Revision history

Version	Release date	Description of changes
1	December 2024	Initial release

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