xGen™ DNA Library Prep EZ UNI Kit

Element Biosciences™ compatibility

REVISION HISTORY

Version	Release date	Description of changes
1	September 2024	Initial release

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INTRODUCTION

The xGen DNA Library Prep EZ Kit are designed to produce next-generation sequencing (NGS) libraries from a broad range of double-stranded DNA inputs (100 pg to 1 µg). This protocol describes the workflow for whole genome sequencing (WGS) and xGen Hybridization Capture for targeted sequencing. It also includes enzymatic fragmentation to streamline the workflow for high-throughput research applications and automation.

- The xGen DNA Library Prep EZ UNI Kit is compatible with the xGen Stubby Adapter - UDI Primers for Element, to create libraries that are native to the Element AVITI sequencer.

The kit produces libraries of equivalent complexity and quality. Also, the kit includes the PCR amplification reagents.

The xGen DNA Library Prep EZ Kits support the following research applications:

- Whole genome sequencing (WGS)
- Hybridization capture of targeted genomic regions (e.g., exome)
- Metagenomic sequencing
- PCR-free sequencing
- Detection of germline inherited SNVs and indels
- Low-frequency somatic variation detection of SNVs and indels
- Copy number variation detection

OVERVIEW

The xGen DNA Library Prep EZ Kit streamline NGS sample preparation of dsDNA for sequencing on AVITI™ platforms. The kit provide rapid DNA fragmentation and library construction to generate libraries for sequencing (Figure 1). Detailed instructions are provided for obtaining a mean aligned insert of 350bp or 200bp for direct or targeted sequencing. A protocol amendment is available for larger insert sizes (up to 550 bp) to guide you in using the xGen Deceleration Module.

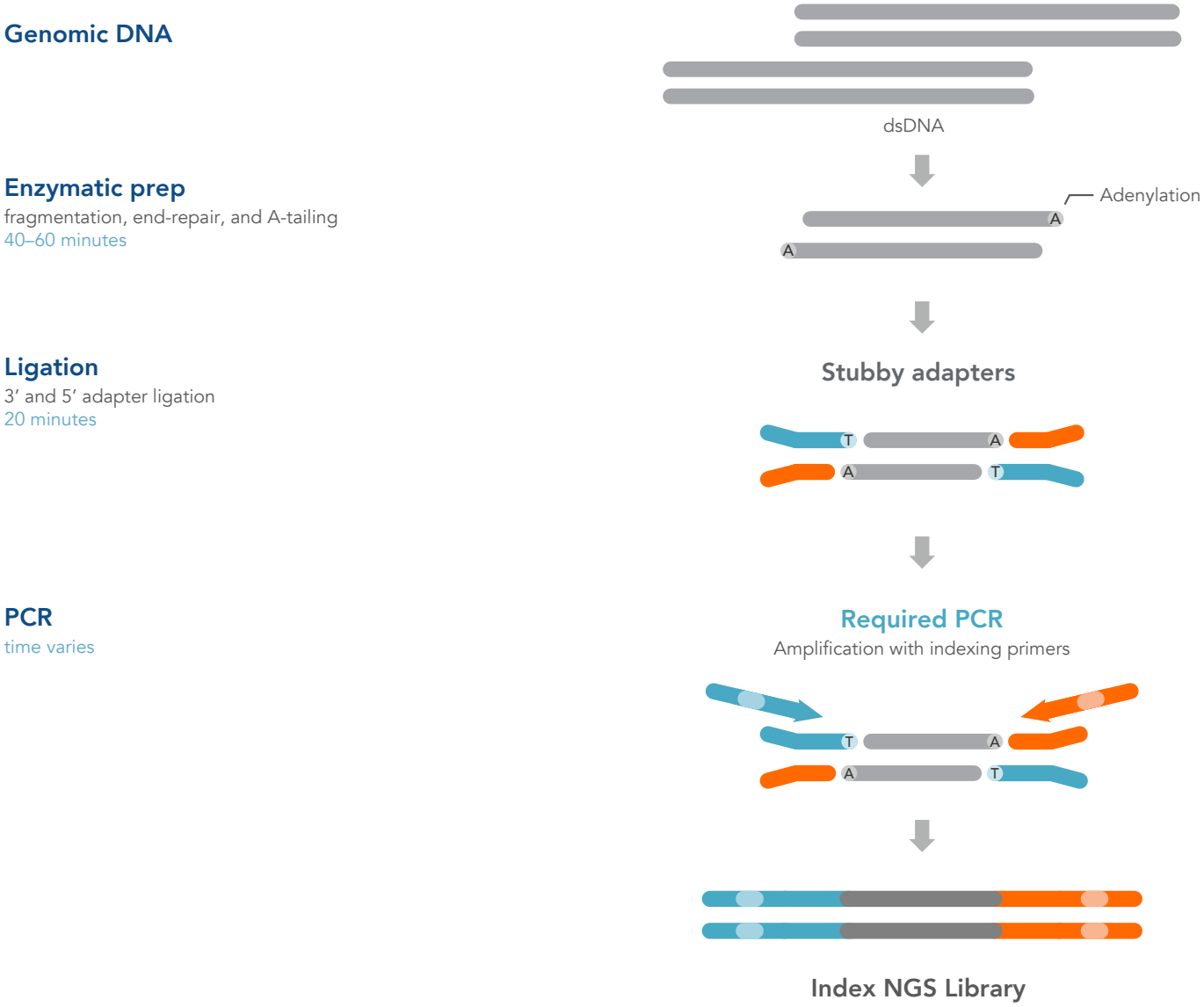

Figure 1.The xGen DNA Library Prep EZ UNI library preparation steps.

WORKFLOW

This workflow contains 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:

- **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 stubby Y adapters. Ligation with the Stubby Y Adapter requires amplification with indexing primers to incorporate sample indexing sequences and to add the flow cell attachment sequences, Stubby Adapter for Element sequencing.
- **PCR amplification.** Amplify libraries based on the adapter and DNA input used.

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 (for stubby adapters only) • Increase available library for sequencing	Total time: PCR, 10–30 min Cleanup, 20 min

* Use of stubby adapter requires PCR to add index sequences to the library.

† Required for PCR-free.

CONSUMABLES AND EQUIPMENT

Kit contents—P/N 10009864, 10009822

Kits contain sufficient reagents for the preparation of 16, or 96 libraries (10% excess volume provided).

	Components	16 rxn	96 rxn	Storage
End prep	• Buffer K1	53 µL	317 µL	–20°C
	• Reagent K2	80 µL	476 µL	
	• Enzyme K3	106 µL	634 µL	
Adapter ligation	○ Buffer W1	201 µL	1218 µL	
	○ Enzyme W3	67 µL	424 µL	
PCR amplification	• PCR Master Mix	436 µL	2640 µL	
	• Reagent R1**	87 µL	528 µL	
Additional reagents	Low EDTA TE	20 mL	20 mL	Room temperature

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

Consumables—IDT

Workflow component	Product name	Index number	Reaction size	Catalog number
xGen core reagents	xGen DNA Library Prep EZ UNI Kit	N/A	16 rxn	10009864
	xGen DNA Library Prep EZ UNI Kit	N/A	96 rxn	10009822
	xGen Stubby Adapter-UDI Primers Element	001-096	96 rxn	10017037
	xGen Stubby Adapter-UDI Primers Element	001-016	16 rxn	10017036

*For index sequences, see the Index Sequence Master List. For adapter sequences, see Appendix B. For custom indexing options, contact us.

Consumables—Other suppliers

Item	Supplier	Catalog number
Absolute ethanol (200 proof)	Various suppliers	Varies
Purification beads		
SPRIselect™ purification beads, or equivalent	Beckman Coulter	B23317/B23318/B2331
Agencourt® AMPure® XP-PCR purification beads, or equivalent	Beckman Coulter	A63880 or A63881
Digital electrophoresis chips and associated reagents (choose one):		
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
Library Quantification Kit	Various Suppliers	Varies
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	Bio-Rad	700-7010
2100 Electrophoresis Bioanalyzer, or equivalent	Agilent	G2939BA
2200 TapeStation System/4200 TapeStation System, or equivalent	Agilent	G2965AA or G2991AA
Qubit 4 Fluorometer, or equivalent	Thermo Fisher Scientific	Q33226
qPCR system	Various Suppliers	Varies
Magnet options (choose one):		
Magnetic Separator Plate	Permagen	MSP750
Magnetic PCR Strip Magnetic Separator Rack	Permagen	MSR812
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–1 µg. 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

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 for xGen DNA Library Prep EZ UNI), as shown:

Important: Adapter dilution is necessary to achieve low levels of adapter dimer. For certain applications, adapter dilution may be adjusted to achieve 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, 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. 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 to not 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. Quick 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 Low EDTA TE to the sample tubes
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	Minimum recommended cycles for >4 nM* with 350 bp insert		Minimum recommended cycles for >500 ng* with 200 bp insert
	xGen DNA Library Prep EZ UNI		xGen DNA Library Prep EZ UNI
1 µg	0–3	3**	1–2
100 ng	0–3	5	5
10 ng	6–7	9	9
1 ng	9–10	12	12
100 pg	11–12	15	15

**When indexing by PCR, a minimum of 3 cycles is required to attach adapter sequences, irrespective of whether a sufficient library amount is available following ligation.

2. Prepare the PCR Master Mix
   a. For Indexing PCR, Prepare PCR Master Mix by following these guidelines. Add 25uL 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. Afterward, 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	50

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 the specified bead volume to each sample as shown:

Application	Average insert size (bp)	Sample volume (µL)	Bead volume (µL)
Direct sequencing	350	50	32.5 (ratio 0.65)*
Hybridization capture	200	50	90 (ratio: 1.8)

* Although 350 bp insert size is suggested for direct sequencing and 200 bp for hybridization capture, both insert sizes are compatible with either application, depending on the desired read length and sample processing.

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 to not 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. Quick 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. Add 21 µL of Low EDTA TE to the sample tubes and mix well until homogenous.
11. Incubate sample tubes at room temperature for 2 minutes.
12. Place the samples on a magnetic rack until the solution clears and a pellet is formed (~2 minutes).
13. 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.

14. 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 Library Prep Kit EZ. The sample was fragmented enzymatically to create 200 bp inserts. The prepared inserts were ligated to xGen Stubby Y adapters that add ~135 bp of sequence onto each ~200 bp library fragment. The final expected size of the library fragments is ~330 bp. In Figure 2, Agilent High-Sensitivity DNA traces show the expected trace of the final library fragment (blue line).

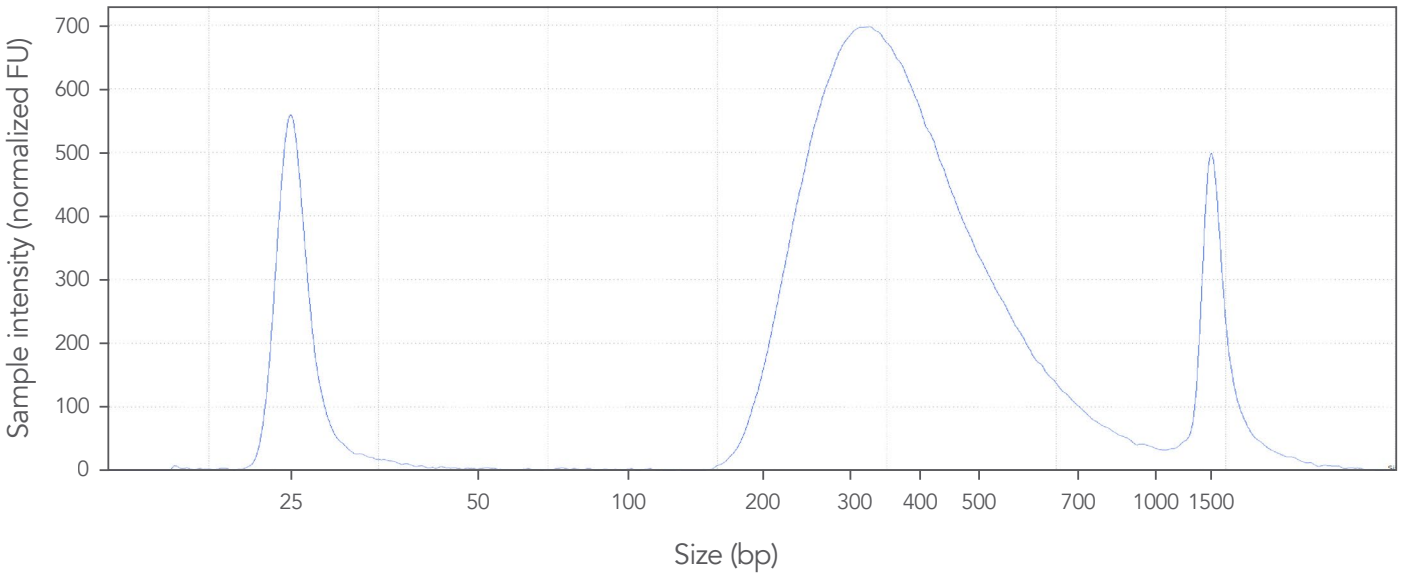

Figure 2. Representative TapeStation traces of a final library preparation (blue). The resulting library (blue) showed a peak height of ~ 330 bp; this is the expected library size after adding the adapters (~ 135 bp) to 200 bp enzymatically sheared genomic DNA (gDNA).

APPENDIX A: RECOMMENDED PCR CYCLES FOR HYBRIDIZATION CAPTURE

The recommendations below are based on using 200 bp of fragmented, Qubit-quantified, high-quality NA12878 Coriell DNA with the xGen DNA Library Prep EZ Kit.

Note: If you are using reduced quality DNA samples, additional PCR cycles may be necessary.

DNA input into xGen DNA Library Prep EZ UNI (ng)	Minimum recommended PCR cycles to reach >500 ng yield with 200 bp insert
100	8
25	10
10	11
1	14

APPENDIX B: INDEXED ADAPTER SEQUENCES

For the master list of sequences, see Element UDI sequences Master List found on the xGen Indexing page. The sequences will help in preparing your AVITI™ sequencing sample sheet on the instrument of your choice.

APPENDIX C: 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 DNA Library Prep EZ UNI Kit

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

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