

# Preparing NGS libraries using the xGen™ DNA Library Prep Kit EZ and Adept™ Library Compatibility Kit for sequencing on the Element AVITI™ System

The method presented here is provided by IDT. This demonstrated protocol uses the xGen DNA Library Prep Kit EZ with the Element Adept Library Compatibility Kit and can be used as a starting point for creating next generation sequencing (NGS) libraries intended for downstream sequencing by the Element AVITI System. This method can be used in similar experiments but may not be fully optimized for your application. IDT does not guarantee these methods, 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 System, see [Element website](#).

## Overview

xGen DNA Library Prep Kit EZ from IDT is a fast and flexible solution designed to produce NGS libraries from a broad range of double-stranded DNA inputs. This demonstrated protocol describes the workflow for whole genome sequencing (WGS) on the AVITI platform using the xGen DNA Library Prep Kit EZ with the Adept Library Compatibility Kit, and an optional downstream workflow for target enrichment with xGen Hybridization Capture Panels. Sequencing with the Element AVITI System requires circularized libraries containing AVITI-specific surface primer sequences which can be achieved by using the Element Adept Library Compatibility Kit. To ensure compatibility for sequencing on the AVITI System, the Element Adept Library Compatibility Kit must be used with libraries prepared using the xGen DNA Library Prep Kit EZ or hybridization captured libraries.

There are four major activities outlined in this protocol:

- **Enzymatic preparation**—performs fragmentation, end-repair, and dA-tailing of dsDNA.
- **Adapter ligation**—performs ligation of adapters.
- **PCR amplification**—amplify and index libraries.
- **Adept circularization**—prepare for AVITI sequencing with annealing, circularization, and digestion steps.

|   |                                                                                                                                                                                                                                               |                                                                                                                                                     |                                               |
|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| 1 | Prepare reagents                                                                                                                                                                                                                              | <ul style="list-style-type: none"> <li>Thaw reagents on ice</li> <li>Prepare fresh 80% ethanol</li> </ul>                                           | Total time: Varies (~20 min)                  |
| 2 | Perform enzymatic preparation                                                                                                                                                                                                                 | <ul style="list-style-type: none"> <li>Set up dsDNA fragmentation, end-repair, and A-tailing</li> <li>Run Enzymatic Prep program</li> </ul>         | Total time: 40–60 min                         |
| 3 | Perform ligation                                                                                                                                                                                                                              | <ul style="list-style-type: none"> <li>Add stubby adapters</li> </ul>                                                                               | Total time: 20 min                            |
| 4 | Clean up ligation reaction                                                                                                                                                                                                                    | <ul style="list-style-type: none"> <li>Purify ligation product</li> </ul>                                                                           | Total time: 20 min                            |
|   |  <b>Safe stopping point</b> (store @ –20°C)                                                                                                                  |                                                                                                                                                     |                                               |
| 5 | Perform PCR & cleanup*                                                                                                                                                                                                                        | <ul style="list-style-type: none"> <li>Add index sequences (for stubby adapters only)</li> <li>Increase available library for sequencing</li> </ul> | Total time: PCR, 10–30 min<br>Cleanup, 20 min |
|   |  <b>Safe stopping point</b> (store @ –20°C)  <b>Optional hybrid capture</b> |                                                                                                                                                     |                                               |
| 6 | Anneal splint oligo                                                                                                                                                                                                                           | <ul style="list-style-type: none"> <li>Denature library and add splint</li> </ul>                                                                   | Total time: 15 min                            |
| 7 | Circularization                                                                                                                                                                                                                               | <ul style="list-style-type: none"> <li>Phosphorylate the 5' end of linear library</li> </ul>                                                        | Total time: 25 min                            |
| 8 | Digestion                                                                                                                                                                                                                                     | <ul style="list-style-type: none"> <li>Remove carryover linear DNA</li> </ul>                                                                       | Total time: 17 min                            |
| 9 | Perform cleanup                                                                                                                                                                                                                               | <ul style="list-style-type: none"> <li>Purify circularization reaction</li> </ul>                                                                   | Total time: 20 min                            |

Figure 1. Workflow of the demonstrated protocol.

# Consumables and equipment

## Consumables—IDT

| Item                          | Catalog # |
|-------------------------------|-----------|
| xGen DNA Library Prep Kit EZ  | 10009821  |
| xGen UDI Primers Plate 1, 8nt | 10005922  |

\* For index sequences, see the Index Sequence Master List. For adapter sequences, see [Appendix B](#). For custom indexing options, [contact us](#).

 **Note:** xGen Normalase™ CDI and UDI primers are compatible with this workflow. However, the xGen Normalase Module Kit cannot be used. See the [IDT Index webpage](#) for more information on these xGen indexes.

## Consumables—Other suppliers

| Item                                                                | Supplier                 | Catalog number      |
|---------------------------------------------------------------------|--------------------------|---------------------|
| Element Adept Library Compatibility Kit                             | Element Biosciences      | 830-00003           |
| Absolute ethanol (200 proof)                                        | Various suppliers        | Varies              |
| Magnetic purification beads (choose one):                           |                          |                     |
| SPRIselect™ purification beads, or equivalent                       | Beckman Coulter          | B23317/B23318/B2331 |
| 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    |
| PCR tubes, 0.2 mL                                                   |                          |                     |
| 96-well, low-bind PCR plates                                        |                          |                     |
| Low-bind DNA Tubes, 1.5 mL                                          | Various suppliers        | Varies              |
| Aerosol-resistant tips and pipettes ranging from 2–1000 µL          |                          |                     |

## Equipment

| Item                                                           | Supplier                 | Catalog #          |
|----------------------------------------------------------------|--------------------------|--------------------|
| Digital electrophoresis (choose one):                          |                          |                    |
| 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             |
| Magnet options (choose one):                                   |                          |                    |
| Magnetic Separator Plate                                       | Permagen                 | MSP750             |
| Magnetic PCR Strip Magnetic Separator Rack                     | Permagen                 | MSR812             |
| Magnet for 1.5 mL tubes or deep-well plate                     |                          |                    |
| Microcentrifuge                                                | Various Suppliers        | Varies             |
| Vortex                                                         |                          |                    |
| Thermal Cycler                                                 |                          |                    |

## Reagent handling

**!** **Important:** Always store kit reagents at  $-20^{\circ}\text{C}$ , except for the Low EDTA TE which can be stored at room temperature.

**☰** **Note:** The enzymes provided are temperature sensitive. Appropriate care should be taken during storage and handling. To maximize use of enzyme reagents, remove enzyme tubes from  $-20^{\circ}\text{C}$  storage and place on ice for at least 10 minutes prior to pipetting. Attempting to pipette enzymes at  $-20^{\circ}\text{C}$  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.

## DNA input considerations

The xGen DNA Library Prep Kit EZ works with a broad range of DNA inputs, ranging from 100 pg to 1  $\mu\text{g}$ . This kit can use high quality gDNA, amplicons, and formalin-fixed paraffin-embedded (FFPE) DNA. To quantify the concentration of low-quality human DNA samples, qPCR can be performed using xGen Input DNA Quantification Primers (Cat. No. 10009856).

**!** **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. 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).

## EDTA in elution buffers

The enzymatic preparation reaction is sensitive to high concentrations of EDTA. A high concentration of EDTA, such as 1 mM in standard 1X TE buffer, will slow the reaction, resulting in larger insert sizes. Alternatively, no EDTA (i.e., 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 (Low EDTA TE)].

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 (Beckman Coulter) is recommended for minimum loss of sample gDNA, although you may need to optimize your buffer exchange method depending on sample source. 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  $\mu$ L of Reagent K2 per reaction).

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



**Note:** For more information on automation, size-selection, and avoiding contamination see the full [xGen DNA Library Prep Kit EZ protocol](#).

# 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                      |
| <b>Total volume</b> | <b>19.5</b>            |

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

| Enzymatic Prep program |                    |                     |
|------------------------|--------------------|---------------------|
| 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 sample (µL) |
| • Buffer K1               | 3.0                    |
| • Reagent K2              | 1.5                    |
| • Enzyme K3               | 6.0                    |
| <b>Total volume</b>       | <b>10.5</b>            |

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 enough 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 (Reagent W5) as shown:

 **Important:** Adapter dilution is necessary to help 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) |

3. Prepare the Ligation Master Mix. Add components in the order shown.

| xGen DNA Library Prep Kit EZ Master Mix |                        |
|-----------------------------------------|------------------------|
| Components                              | Volume per sample (µL) |
| • Buffer W1                             | 12                     |
| • Enzyme W3                             | 4                      |
| • Reagent W5<br>(stubby adapter)*       | 5                      |
| Low EDTA TE                             | 9                      |
| <b>Total Master Mix</b>                 | <b>30</b>              |
| Fragmented sample                       | 30                     |
| <b>Total volume</b>                     | <b>60</b>              |

\* 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 30  $\mu$ L pre-mixed Ligation Master Mix to the tubes containing fragmented DNA.
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 purification 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  $\mu$ L 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  $\mu$ L may be left behind).
8. Add 180  $\mu$ L 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 at room temperature, 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 20  $\mu$ L 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  $-20^{\circ}\text{C}$ .

## PCR amplification

**Note:** Use the IDT PCR Master Mix supplied in xGen DNA Library Prep Kit EZs for both direct sequencing and pre-hybridization capture PCR workflows. If performing direct sequencing, >17 nM yield is needed. If performing hybridization capture, see [Appendix A](#) for PCR cycling recommendations.

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<br>(see table below) | 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* |                                       |
|--------------------------|---------------------------------------|
| DNA input                | Minimum recommended cycles for >17 nM |
| 1 µg                     | 3**                                   |
| 100 ng                   | 4–5                                   |
| 10 ng                    | 7–8                                   |
| 1 ng                     | 11–12                                 |
| 100 pg                   | 13–14                                 |

\*Optimization may be needed to determine correct cycling conditions for different sample types

\*\*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. Add the following components to the eluted library sample:

| Components           | Volume per reaction (µL) |
|----------------------|--------------------------|
| • PCR Master Mix     | 25                       |
| xGen UDI primer pair | 5                        |
| Sample               | 20                       |
| <b>Total volume</b>  | <b>50</b>                |

3. Gently vortex for 5 seconds and briefly centrifuge.
4. Place samples into pre-programmed thermal cycler and run the PCR Amplification program.
5. When the PCR program is complete, vortex the room temperature beads until the solution is homogenous.
6. Proceed to [Post-PCR cleanup](#).

## Post-PCR cleanup

1. Add the specified bead volume to each sample as shown:

| Average insert size (bp) | Sample volume (μL) | Bead volume (μL)   |
|--------------------------|--------------------|--------------------|
| 350                      | 50                 | 32.5 (ratio: 0.65) |
| 200                      | 50                 | 90 (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 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.

 **Note:** If performing direct sequencing, continue with the Adept library circularization protocol for AVITI sequencing below, using the Element Adept Library Compatibility Kit. If performing hybridization capture, stop here and complete hybridization capture following the [xGen hybridization capture of DNA libraries protocol](#). Note that post-capture PCR cycles may need to be adjusted. See [Appendix A](#) for more information. After the hybridization capture protocol is complete, follow the Adept library circularization protocol for AVITI sequencing listed below.

# Adept library circularization protocol for AVITI sequencing

All reagents for the remainder of the protocol are provided in the Element Adept Library Compatibility Kit. The Adept library circularization protocol accepts inputs of 0.5 pmol in 30  $\mu$ L (equivalent to 16.67 nM in 30  $\mu$ L). Input amounts as low as 0.2 pmol can be used if needed; however, this may result in material loss. After quantification of linear libraries, normalize libraries to  $\geq 16.67$  nM and pool based on the sequencing throughput requirements needed for each sample. Up to 384 uniquely indexed libraries can be pooled for a single circularization reaction (remember to not pool libraries with same index pair). If the final pool contains  $>30$   $\mu$ L volume, a speed vac or bead-based method can be used to concentrate library to 30  $\mu$ L. If the final pool contains  $<30$   $\mu$ L, add Elution Buffer to bring to 30  $\mu$ L volume.



**Note:** more information and troubleshooting can be found on the [Element website](#).

1. Ensure all reagents are thawed completely and remain on ice during the remainder of the protocol.
2. Briefly vortex Adept Annealing Mix reagent and briefly centrifuge.
3. Set up the thermal cycler with the Adept Annealing program, as described below, with the lid set to 105°C.

| Adept Annealing program |                   |           |
|-------------------------|-------------------|-----------|
| Step                    | Temperature* (°C) | Time      |
| 1                       | 95                | 5 minutes |
| 2                       | 37                | 5 minutes |
| Hold                    | 37                | Hold      |

\* Lid temperature needs to be set to 105°C

4. Prepare the Annealing Mix by adding the components in the order shown to each well (containing library) of a 96-well plate:

| Annealing Mix                            |                              |
|------------------------------------------|------------------------------|
| Components                               | Volume per sample ( $\mu$ L) |
| • Pooled linear library<br>(at 16.67 nM) | 30                           |
| • Adept Annealing Mix                    | 13                           |
| <b>Total volume</b>                      | <b>43</b>                    |

5. Set a pipette to 33  $\mu$ L and mix each reaction by pipetting 10x.
6. Seal the plate and briefly centrifuge.
7. Place samples into pre-programmed thermal cycler and run the Adept Annealing program.
8. Once completed, remove the plate from the thermal cycler, centrifuge briefly and immediately proceed to the next step.

## Circularize library

1. Gently flick Ligation Enzyme 1 and Ligation Enzyme 2 to mix, then briefly centrifuge and store on ice.
2. Vortex Ligation Buffer to mix and briefly centrifuge.
3. Set up the thermal cycler with the Adept Circularization program, as described below, with the lid set to 75°C.

| Adept Circularization program |                  |            |
|-------------------------------|------------------|------------|
| Step                          | Temperature (°C) | Time       |
| 1                             | 37               | 10 minutes |
| 2                             | 65               | 10 minutes |
| Hold                          | 4                | Hold       |

\* Lid temperature needs to be set to 75°C

4. Prepare the Circularization Master Mix by adding the components in the order shown.

| Circularization Master Mix |                        |
|----------------------------|------------------------|
| Components                 | Volume per sample (µL) |
| • Ligation Buffer          | 5                      |
| • Ligation Enzyme 1        | 1                      |
| • Ligation Enzyme 2        | 1                      |
| <b>Total volume</b>        | <b>7</b>               |

5. Set pipette to 75% of Circularization Master Mix volume and mix by pipetting 10x while stored on ice.
6. Add 7 µL of Circularization Master Mix to each reaction, set pipette to 38 µL and mix each reaction 10x by pipetting.
7. Seal the plate and briefly centrifuge.
8. Place samples into pre-programmed thermal cycler and run the Adept Circularization Program.
9. Once completed, remove the plate from the thermal cycler, centrifuge briefly and immediately proceed to the next step.

## Digest Linear DNA

1. Gently flick Digestion Enzyme 1 and Digestion Enzyme 2 to mix, briefly centrifuge, and store on ice.
2. Set up the thermal cycler with the Adept Digestion program, as described below, with the lid set to 105°C.

| Adept Digestion program |                  |            |
|-------------------------|------------------|------------|
| Step                    | Temperature (°C) | Time       |
| 1                       | 37               | 10 minutes |
| 2                       | 80               | 2 minutes  |
| Hold                    | 4                | Hold       |

\* Lid temperature needs to be set to 105°C

3. 4. Prepare the Circularization Master Mix by adding the components in the order shown.

| Digestion Master Mix |                        |
|----------------------|------------------------|
| Components           | Volume per sample (µL) |
| • Digestion Enzyme 1 | 2                      |
| • Digestion Enzyme 2 | 2                      |
| <b>Total volume</b>  | <b>4</b>               |

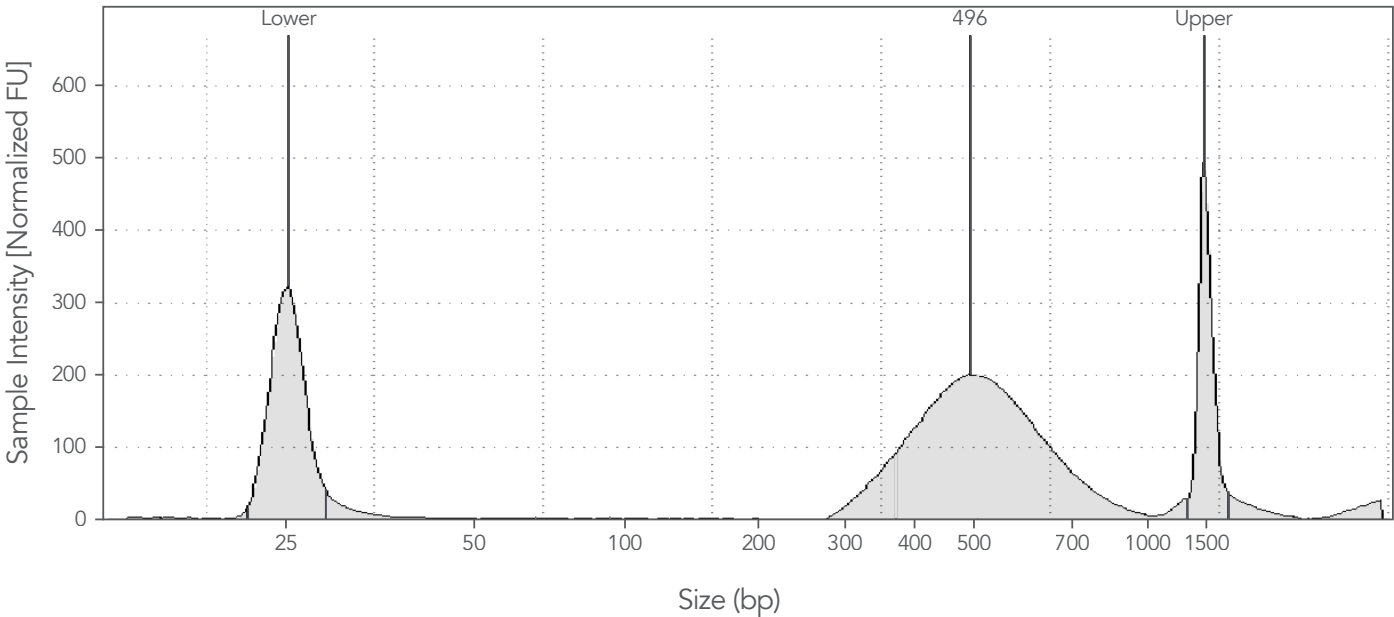
4. Set pipette to 75% of the Digestion Master Mix volume and mix by pipetting 10x while stored on ice.
5. Add 4  $\mu\text{L}$  of Digestion Master Mix to each reaction, set pipette to 41  $\mu\text{L}$  and mix each reaction by pipetting 10x.
6. Seal the plate and briefly centrifuge.
7. Place samples into pre-programmed thermal cycler and run the Adept Digestion program.
8. Once completed, remove the plate from the thermal cycler, centrifuge briefly, and immediately proceed to the next step.

## Cleanup

1. Prepare fresh 80% ethanol solution.
  2. Transfer each reaction (54  $\mu\text{L}$ ) to a new deep-well plate or larger tube.
  3. Vortex room-temperature beads until the solution is homogenous.
  4. Add 108  $\mu\text{L}$  of beads to each sample at room temperature (ratio of bead to sample is 2:1).
  5. Mix samples vigorously (recommend 2 minutes of vortexing at 1500–1800 rpm for plates or pipetting 120  $\mu\text{L}$  vigorously >10x for tubes).
  6. Incubate the samples for 5 minutes at room temperature.
  7. Place the samples on a magnetic rack until the solution clears and a pellet has formed (~3–5 minutes).
  8. Remove and discard the supernatant using a clean pipette tip, without disturbing the pellet.
  9. Add 200  $\mu\text{L}$  of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Be careful to not disturb the pellet.
  10. Incubate for 30 seconds at room temperature, then carefully remove the ethanol solution.
  11. Repeat steps 9 and 10 for a second ethanol wash.
  12. Using a 10  $\mu\text{L}$  or 20  $\mu\text{L}$  pipette, remove residual ethanol.
  13. Air-dry the beads unsealed or uncapped for 3–5 minutes until the pellet loses shine. Do not over dry beads.
  14. Once dry, remove the samples from the magnet.
  15. Add 32  $\mu\text{L}$  of Elution Buffer to the sample tubes and mix well until homogenous (recommend vortexing plates at 1500–1800 rpm for 2 minutes, or for tubes set a pipette to 24  $\mu\text{L}$  and mix by pipetting >10x until fully resuspended).
  16. Incubate sample tubes at room temperature for 2 minutes.
  17. Place the samples on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
  18. Carefully transfer 30  $\mu\text{L}$  clear solution (eluted circularized library) into a clean tube, being careful to avoid any bead carryover.
-  **Safe Stop:** Libraries can be stored at  $-20^{\circ}\text{C}$  for  $\leq 15$  days.
19. The circularized library is now ready for quantification, which must be performed using qPCR with Element provided standards and primers. Protocol for quantification and sequencing can be found on [Element website](#).

Example of data output

Example libraries were prepared using 100 ng of NA12878 DNA (Coriell Institute) using the xGen DNA Library Prep Kit EZ. The sample was enzymatically fragmented to ~350 bp, and an additional ~135 bp of sequence was added to each library fragment via the ligation and indexing steps for an average expected fragment size of ~485 bp. A TapeStation High-Sensitivity DNA trace (Agilent) of a pre-Adept-treated library shows a final linear library fragment size peak at ~496 bp (Figure 1). Sequencing quality metrics for 69 Adept-treated libraries sequenced using the Element AVITI System (2 x 150 paired-end reads) are shown in Table 1.



**Figure 1. Representative TapeStation trace for 350 bp DNA fragments subjected to library preparation.** The resulting library showed a peak fragment size of ~496 bp; this is the expected library size after adding the adapters and index sequences (~135 bp).

**Table 1. Sequencing metrics obtained on the Element AVITI System.**

| Polonies      | Q30 (%) | Index Assignment (%) | Total Yield (Gb) |
|---------------|---------|----------------------|------------------|
| 1,042,412,054 | 93.6    | 95.7                 | 299.305          |

Table 1 shows the sequencing metrics obtained on the Element AVITI System. The sequencing run included 69 xGen libraries that had been circularized using the Adept Library Compatibility Kit.

## Appendix A: Recommended PCR cycles for pre-hybridization capture PCR and post-hybridization capture information

For hybridization capture, >500 ng yield is recommended per library; therefore, more PCR cycles are needed during library preparation than if performing direct sequencing. The cycling recommendations below are for PCR amplification during library preparation. These values are based on high-quality gDNA (NA12878, Coriell Institute) used with the xGen DNA Library Prep Kit EZ.

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

| DNA input into<br>xGen DNA Library Prep Kit EZ (ng) | Minimum recommended<br>xGen DNA Library Prep Kit EZ<br>PCR cycles to reach >500 ng |
|-----------------------------------------------------|------------------------------------------------------------------------------------|
| 100                                                 | 8                                                                                  |
| 25                                                  | 10                                                                                 |
| 10                                                  | 11                                                                                 |
| 1                                                   | 14                                                                                 |

If performing hybridization capture after library preparation, proceed following the [xGen hybridization capture of DNA libraries protocol](#) with potential modifications needed to the post-capture PCR cycling in order to generate enough captured library for the Adept Library Compatibility Kit. The need to add additional PCR cycles will be dependent on panel size, multiplex status, and number of libraries being sequenced. Optimization will be needed. After hybridization capture is complete, perform the Adept Circularization protocol as described above.

## Appendix B: Indexed adapter sequences

The full-length adapter sequences are listed below. The underlined text indicates the location of the index sequences, which is 8 for UDI. These sequences represent the adapter sequences following completion of the indexing PCR step.

### Index 1 (i7) Adapters

5' – GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG – 3'

### Index 2 (i5) Adapters

5' – AATGATACGGCGACCACCGAGATCTACACYYYYYYYACACTCTTTCCCTACACGACGCTCTTCCGATCT – 3'

For the master list of sequences, see [Index Sequences Master List](#). The sequences will help in preparing your sequencing sample sheet on the instrument of your choice.

## xGen DNA Library Prep Kit EZ with Adept Compatibility Kit for AVITI sequencing

For more information, go to [www.idtdna.com/ContactUs](http://www.idtdna.com/ContactUs)

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