Preparing NGS libraries using xGen™ cfDNA & FFPE DNA Library Prep MC and Adept™ Library Compatibility Kit for sequencing with the Element AVITI™ system

The method presented here is provided by IDT. This method combines the xGen cfDNA & FFPE DNA Library Prep MC Kit with Adept circularization and can be used as a starting point for creating next generation sequencing (NGS) libraries intended for downstream AVITI sequencing. 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 cfDNA & FFPE DNA library kits are optimized for use with 1–250 ng of degraded sample input, such as cell-free DNA (cfDNA) or DNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples. The technology utilized in library construction enables high conversion of degraded or damaged samples into sequencer-ready libraries without the need for adapter titration. Unique chemistry and sequence modifications allow for independent tagging of the top and bottom strands of the library, using our proprietary ligation strategy with fixed UMI sequences. This demonstrated protocol describes the workflow for preparing xGen cfDNA & FFPE libraries for whole genome sequencing (WGS) on the AVITI platform using the Adept Library Compatibility Kit, and an optional downstream workflow for target enrichment using xGen Hybridization Capture. 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. The Element Adept Library Compatibility Kit must be used with prepared xGen cfDNA & FFPE libraries (or hybridization captured libraries) in this workflow to ensure library compatibility for sequencing on the AVITI System.

The workflow includes five major steps:

- **End repair.** Converts cfDNA, or sheared input DNA, into blunt-ended DNA ready for ligation.
- **Ligation 1.** Ligation 1 Enzyme catalyzes the single-stranded addition of the Ligation 1 Adapter to the 3′ ends of the insert DNA.
- **Ligation 2.** Ligation 2 Adapter acts as a primer to gap-fill the bases complementary to the UMI, followed by ligation to the 5′ end of the DNA insert to create a fully double-stranded product.
- **PCR amplification.** HiFi PCR Mix is added to perform indexing PCR for sequencing.
- **Adept circularization.** Prepare for AVITI sequencing with annealing, circularization, and digestion steps.

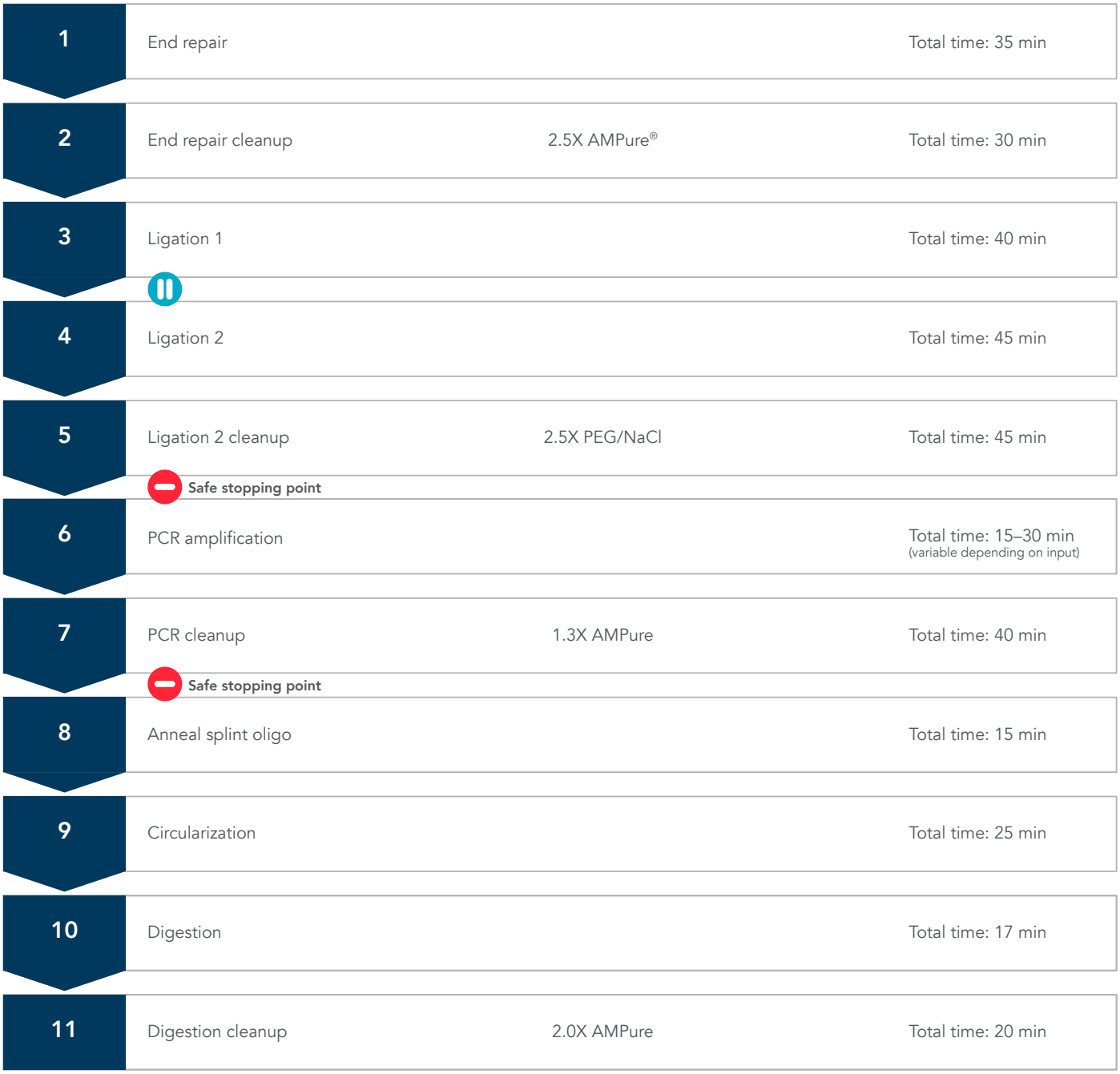

Figure 1. Workflow of the demonstrated protocol.

Consumables and equipment

Consumables—IDT

Item	Catalog #
xGen cfDNA & FFPE DNA Library Prep v2 MC Kit or xGen cfDNA & FFPE DNA Library Prep MC Kit (Note—polymerase mix not included)	10010207 10006203
IDTE pH 8.0 (1X TE Solution), 300 mL	11-05-01-13
xGen UDI Primer Pairs, Plate 1, 8 nt	10005922

Consumables—Other suppliers

Item	Supplier	Catalog number
Element Adept Library Compatibility Kit	Element Biosciences	830-00003
Polymerase Mix (Note—this is only required if not using v2 kit)	General laboratory supplier	Varies
Buffer EB (10 mM Tris-HCl, pH 8.5), 250 mL	Qiagen	19086
Absolute ethanol (200 proof)	General laboratory supplier	Varies
Purification beads		
Agencourt® AMPure XP 5 mL (22 rxn) or 60 mL (270 rxn)	Beckman Coulter	A63880 or A63881
Digital electrophoresis chips and associated reagents (choose one):		
Experion® DNA 1K Analysis Kit	Bio-Rad	700-7107
High Sensitivity DNA Kit	Agilent	5067-4626
High Sensitivity D1000 ScreenTape®	Agilent	5067-5584
twin.tec® 96 Well LoBind® PCR Plates	Eppendorf	0030129504
DNA LoBind® Tubes, 1.5 mL	Eppendorf	022431021
Filtered low bind pipette tips	General laboratory supplier	Varies
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	032851 or 032854

Equipment

Item	Supplier	Catalog #
Microcentrifuge	General laboratory supplier	Varies
Thermal cycler		
Qubit 4 Fluorometer, or similar DNA quality analyzer	Thermo Fisher Scientific	033226
Magnet options (choose one):		
Permagen 96-well side pull magnet	Thermo Fisher Scientific	NC1568572
Magnum® EX Universal Magnet Plate	Alpaqua	A000380
Magnetic Stand-96, or similar magnetic stand	Thermo Fisher Scientific	AM10027
Magnet for 1.5mL tubes or deep-well plate	General laboratory supplier	Varies

Guidelines

Reagent storage and handling

Always store the xGen cfDNA & FFPE DNA Library Prep MC reagents at –20°C, except for PEG/NaCl, which can be stored either at room temperature or at –20°C.

Note: The enzymes provided in this kit are temperature-sensitive and appropriate care should be taken during storage and handling.

Note: The xGen 2x HiFi PCR Mix may be in a liquid state after storage in –20°C condition; this is expected and will not impact product integrity. This is only supplied in the v2 kit.

For all non-enzyme reagents, thaw on ice, then briefly vortex to mix well. Remove enzyme tubes from –20°C storage and place on ice just before use. Briefly centrifuge the tubes in a microcentrifuge to collect contents before opening.

To create master mixes, scale reagent volumes as appropriate, using 10% excess volume to compensate for pipetting loss. Add reagents in the order listed when preparing master mixes, then pulse-vortex to mix and briefly centrifuge.

Note: You may observe precipitation in the End Repair Buffer. Continue vortexing until no precipitate can be observed.

Important: Use extra caution when handling Ligation 1 and Ligation 2 adapter tubes. Never handle Ligation 2 adapter prior to, or during Ligation 1 Master Mix setup and handling. Trace contamination of Ligation 2 adapter into Ligation 1 adapter has been shown to induce adapter-dimer formation.

DNA input considerations

This kit works with a wide range of DNA inputs, ranging from 1–250 ng. Input DNA should be stored in IDTE buffer (10 mM Tris-Cl, pH 8.0; 0.1 mM EDTA) or Buffer EB (Qiagen, 10 mM Tris HCl, pH 8.5).

Important: Input quantities recommended in this protocol refer to the total DNA quantified after fragmentation.

DNA should be an appropriate size before library construction. For genomic DNA, or DNA derived from FFPE samples, using a Covaris® or similar DNA shearing instrument can create fragments with an average insert size of 150–300 base pairs. As cfDNA typically has an average size of 160 base pairs, no further fragmentation is required.

For FFPE samples, use standard quality control methods, such as Q-ratio with qPCR or the DNA Integrity Number (DIN) using size distribution [i.e., Bioanalyzer instrument (Agilent) or similar DNA quality analyzer].

These methods can help you choose the appropriate number of PCR cycles for your DNA sample. For cfDNA, we suggest assessing the size distribution with electrophoresis.

Note: If large molecular weight DNA is present, an additional cleanup may be necessary to remove genomic DNA contamination. However, this additional cleanup can reduce sample complexity and mass.

Note: For more information on avoiding cross contamination, and size selection during clean-ups see the complete xGen cfDNA & FFPE DNA Library Prep v2 MC protocol.

Protocol

End repair

Before starting the protocol, ensure that the AMPure and PEG/NaCl reagents are at room temperature (20–25°C). Also, prepare a fresh 80% ethanol solution in nuclease-free water.

1. Add 50 µL of each sample into a low-bind PCR plate that resists nucleic acid adsorption.

   Note: If sample volume <50 µL, use IDTE buffer (10 mM Tris-Cl, pH 8.0; 0.1 mM EDTA) or Buffer EB (10 mM Tris-HCl, pH 8.5) to bring the volume up to 50 µL.

2. For each sample, make the following End Repair Master Mix:

Components	Volume per sample (µL)
End Repair Buffer	6
End Repair Enzyme	3
Total volume:	9

   Tip: If there is precipitate in the End Repair Buffer, vortex until the precipitate becomes clear in solution. The resulting Master Mix is viscous and requires careful pipetting.

3. Pulse-vortex the master mix for 10 sec, then briefly centrifuge. Keep the master mix on ice.
4. Add 9 µL of End Repair Master Mix to each well. Using a pipette set to 40 µL, pipette 10 times to mix, then seal the plate. Alternatively, seal the plate and vortex mix for minimum of 10 seconds.
5. Run the following thermal cycler program:

End Repair program		
Step	Temperature* (°C)	Time
End repair	20	30 min
Hold	4	Hold

   * Set the lid temperature to OFF, or to 40°C.

   While the end repair program runs, make the Ligation 1 Master Mix in preparation for end repair cleanup.

Ligation 1 Master Mix	
Components	Volume per sample (µL)
Ligation 1 Buffer	25
Ligation 1 Adapter	2
Ligation 1 Enzyme	3
Total volume:	30

6. Pulse vortex the master mix for 10 seconds, then briefly centrifuge. Keep the master mix on ice until ready to use.

   Important: Use extra caution when handling Ligation 1 and Ligation 2 adapter tubes: never handle Ligation 2 adapter before or during Ligation 1 Master Mix setup and handling. Trace contamination of Ligation 2 adapter into Ligation 1 adapter has been shown to induce adapter-dimer formation.

7. After the End Repair program reaches 4°C, proceed immediately to end repair cleanup.

End repair cleanup

Note: Before starting cleanup, make sure the Ligation 1 Master Mix has already been prepared.

1. Thoroughly resuspend AMPure XP beads before use, then add 147.5 µL of AMPure beads (2.5X volume) to each well and pipette 10 times to thoroughly mix.
2. Incubate the plate at room temperature for 10 minutes.
3. Place the plate on a magnet and wait for the liquid to clear completely or at least for 2 minutes.

   Important: If the solution is not clear after 2 minutes, keep the plate on the magnet until solution clears. If beads are discarded, sequencing library yield and quality may suffer.

4. With a clean P20 pipette tip, remove and discard any trace amount of supernatant that remains.

   Important: Be careful not to remove any beads.

5. Keeping the plate on the magnet, add 160 µL of 80% ethanol and incubate for 30 seconds.
6. Remove and discard the supernatant.
7. Use a P20 pipette tip to remove any residual ethanol.
8. Dry the beads at room temperature for 1–3 minutes.

   Important: Never allow the beads to dry for >5 minutes; over-dry beads will decrease final library yield.

9. Proceed immediately to Ligation 1.

Ligation 1

1. Remove the plate from the magnet, then add 30 µL Ligation 1 Master Mix.
2. Pipette mix a minimum of 10 times, then seal the plate.

   Important: Make sure the samples are thoroughly mixed and that the beads are fully resuspended before proceeding.

3. Run the following thermal cycler program:

Ligation 1 program		
Step	Temperature* (°C)	Time
Ligation	20	15 min
Inactivation	65	15 min
Hold	4	Hold

* If possible, set lid temperature to 70°C. If lid cannot be programmed, set the thermal cycler heat block to 105°C.

Safe Stop: The plate can temporarily remain at 4°C (no more than 2 hours). It is normal for beads to settle during this reaction.

4. Proceed to Ligation 2.

Ligation 2

1. For each sample, prepare the Ligation 2 Master Mix.

Ligation 2 Master Mix	
Components	Volume per sample (µL)
Ligation 2 Buffer	4.5
Ligation 2 Adapter	4
Ligation 2 Enzyme A	0.5
Ligation 2 Enzyme B	1
Total volume:	10

2. Pulse-vortex the master mix for 10 seconds, then briefly centrifuge. Keep the master mix on ice until ready to use.
3. Add 10 µL of the Ligation 2 Master Mix to each well.
4. Using a pipette set to 35 µL, pipette 10 times to mix, then seal the plate.

   Important: Ensure the samples are thoroughly mixed, and make sure the beads are fully resuspended before proceeding.

   Note: If necessary, briefly centrifuge to collect contents to the bottom of the wells.

5. Run the following thermal cycler program:

Ligation 2 program		
Step	Temperature* (°C)	Time
Ligation	65	30 min
Hold	4	Hold

* If possible, set lid temperature to 70°C. If lid cannot be programmed, set the thermal cycler heat block to 105°C.

6. After thermal cycler program is completed, proceed immediately to ligation 2 cleanup.

Ligation 2 cleanup

1. Add 100 µL of PEG/NaCl (2.5X volume) to each well, then pipette 10 times to mix.
2. Incubate the plate at room temperature for 10 minutes.
3. Place the plate on a magnet and wait for the liquid to clear completely or at least for 2 minutes.

   Important: If solution is not clear after 2 minutes, keep the plate on the magnet until solution clears. If beads are discarded, sequencing library yield and quality may suffer.

4. Use a clean P20 pipette tip to remove and discard any trace amount of supernatant that remains.

   Important: Be careful not to remove any beads.

5. Keeping the plate on the magnet, add 160 µL of 80% ethanol and incubate for 30 seconds.
6. Remove and discard the supernatant.
7. Use a P20 pipette tip to remove any residual ethanol.

   Important: Make sure all the ethanol has been removed before proceeding.

8. Dry the beads at room temperature for 1–3 minutes.

   Important: Never allow the beads to dry for >5 minutes; over-dry beads will decrease final library yield.

9. Remove the plate from the magnet, then add 20 µL of Buffer EB.
10. Seal the plate, then gently vortex (use 70% vortex capacity) to resuspend beads.
11. Allow the plate to incubate at room temperature for 5 minutes to elute DNA off the beads.
12. Place the plate on a magnet and wait for the beads to be cleared from the liquid (approximately 1–2 minutes).

    Note: Depending on the strength of your magnet, you may need to wait longer.

13. Carefully transfer 20 µL of the cleared liquid containing the eluted DNA into a new well. Proceed to PCR amplification or pause here.

    Safe Stop: The plate can be stored at –20°C overnight.

PCR amplification

Note: Sample index barcodes are introduced during PCR; double check that a unique primer pair is used for each sample.

Note: If using the v2 kit, use the provided xGen 2x HiFi PCR Mix, if not using the v2 kit use a comparable polymerase mix from an alternate vendor.

1. Add 5 µL of xGen UDI Primer Pairs to each well.
2. Add 25 µL of xGen 2x HiFi PCR Mix (or polymerase mix from alternate vendor) to each well, then pipette 10 times to mix.
3. Seal the plate, then briefly centrifuge.
4. Run the following thermal cycler program:

   Note: Only use standard cycling conditions for the PCR program. Fast cycling has been shown to negatively impact library yields.

PCR amplification program			
Step	Cycles	Temperature* (°C)	Time
Polymerase activation	1	98	45 seconds
Denaturation	Varies based on sample input (see Table 1 below)	98	15 seconds
Annealing		60	30 seconds
Extension		72	30 seconds
Final extension	1	72	1 minute
Hold	—	4	∞

* Set lid temperature to 105°C. If the lid cannot be programmed, set the thermal cycler block to 105°C.

Table 1. Recommended PCR cycling parameters to yield >500 ng.

Input mass (ng)	Number of cycles	
	gDNA or dsDNA	FFPE*
1	11–13	14–16
10	9–11	11–13
25	7–9	9–11
100	5–7	7–9
250	4–6	6–8

* For lower-quality FFPE samples, we recommend using the higher number of recommended PCR cycles from Table 1. For very low quality FFPE (DIN 1–2), you may not obtain 500 ng, but we do not recommend increasing the number of PCR cycles.

5. After the program completes, proceed to PCR cleanup.

PCR cleanup

1. Add 65 µL of AMPure beads (1.3X volume), or similar bead-based DNA purification product, to each well, then pipette 10 times to thoroughly mix.
2. Incubate the plate at room temperature for 5 minutes.
3. Place the plate on a magnet and wait for the liquid to clear completely or at least for 2 minutes.

   Important: If solution is not clear after 2 minutes, keep the plate on the magnet until solution clears. If beads are discarded, sequencing library yield and quality may suffer.
4. Remove and discard the cleared supernatant; make sure not to remove any beads.

   Important: Be careful not to remove any beads.
5. Keeping the plate on the magnet, add 160 µL of 80% ethanol, then incubate for 30 seconds.
6. Remove and discard the supernatant.
7. Use a P20 pipette tip to remove any residual ethanol.

   Important: Make sure all the ethanol has been removed before proceeding.
8. Dry the beads at room temperature for 1–3 minutes.

   Important: Never allow the beads to dry for >5 minutes; over-dry beads will decrease final library yield.
9. Remove the plate from magnet, then add 31 µL of Buffer EB.
10. Seal the plate and gently vortex (use 70% vortex capacity) to resuspend beads.
11. Allow the plate to incubate at room temperature for 5 minutes to elute DNA off beads. Then place the plate on a magnet and wait for the liquid to clear completely for 1–2 minutes.

    Note: Depending on the strength of your magnet, you may need to wait longer.
12. Carefully transfer 30 µL of eluted DNA into a new well.
13. 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. Read the paragraph below for yield information required post- capture. After the hybridization capture protocol is complete, follow the Adept library circularization protocol for AVITI sequencing listed below.

Adept circularization protocol for AVITI sequencing platform

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 µL (equivalent to 16.67 nM in 30 µL). 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 ≥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 µL volume, a speed vac or bead-based method can be used to concentrate library to 30 µL.

Note: More information and troubleshooting can be found on the Element website.

Anneal splint oligo

1. Ensure all reagents are thawed completely and remain on ice during remainder of protocol.
2. Briefly vortex Adept Annealing mix and briefly centrifuge.
3. Add 13 µL of Adept Annealing Mix to each well of a 96-well plate containing library:

Annealing Mix	
Components	Volume per sample (µL)
• Pooled linear library (at 16.67 nM)	30
• Adept Annealing Mix	13
Total volume:	43

4. Set a pipette to 33 µL and pipette mix each reaction 10 times.
5. Seal the plate and briefly centrifuge.
6. Run the following thermal cycler program:

Step	Temperature* (°C)	Time
1	95	5 minutes
2	37	5 minutes
Hold	37	∞

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

7. Once completed, remove the plate from the thermocycler, centrifuge briefly, and immediately proceed to the next step.

Circularize library

1. Gently flick Ligation Enzyme 1 and Ligation Enzyme 2 to mix, briefly centrifuge and store on ice.
2. Vortex Ligation Buffer to mix and briefly centrifuge.
3. Prepare the Circularization Master Mix by adding the components in the order shown:

Circularization Mix	
Components	Volume per sample (µL)
• Ligation Buffer	5
• Ligation Enzyme 1	1
• Ligation Enzyme 2	1
Total volume:	7

4. Set pipette to 75% of master mix volume and pipettete mix 10 times on ice.
5. Add 7 µl of Circularization Mix to each reaction, set pipette to 38 µL and pipette mix each reaction 10 times.
6. Seal the plate and briefly centrifuge.
7. Run the following thermal cycler program:

Step	Temperature* (°C)	Time
1	37	10 minutes
2	65	10 minutes
Hold	4	∞

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

8. Once completed, remove the plate from the thermocycler, 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. Prepare the Digestion Master Mix by adding the components in the order shown to each reaction:

Circularization Mix	
Components	Volume per sample (µL)
• Digestion Enzyme 1	2
• Digestion Enzyme 2	2
Total volume:	4

3. Set pipette to 75% of the master mix volume and pipette mix 10x on ice.
4. Add 4 µL of Circularization Mix to each reaction, set pipette to 41 µL and pipette mix each reaction 10 times.
5. Seal the plate and briefly centrifuge.
6. Run the following thermal cycler program:

Step	Temperature* (°C)	Time
1	37	10 minutes
2	80	2 minutes
Hold	4	∞

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

7. Once completed, remove the plate from the thermocycler, centrifuge briefly, and immediately proceed to the next step.

Digestion cleanup

1. Prepare fresh 80% ethanol solution.
2. Transfer each reaction (54 µL) to a new deep well plate or larger tube.
3. Vortex the room temperature beads until the solution is homogenous.
4. Add 108 µL of beads to each sample at room temperature (ratio of bead to sample is 2).
5. Mix samples vigorously (recommend 2 minutes of vortexing at 1500–1800 rpm for plates or pipetting 120 µL vigorously 10x times 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 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.
10. Incubate for 30 seconds, then carefully remove the ethanol solution.
11. Repeat steps 9 and 10 for a second ethanol wash.
12. Using a 10 µL or 20 µL 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 µL 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 µL and pipette mix > 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 is formed (~2 minutes).
18. Carefully transfer 30 µL clear solution (eluted circularized library) into a clean tube, being careful to avoid any bead carryover.

    Safe Stop: Libraries can be stored at –20°C for ≤ 15 days.

19. The circularized library is now ready for quantification, which must be performed using qPCR with the 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 10 ng of cell-free DNA from a human donor (BioChain) using the xGen cfDNA & FFPE DNA Library Prep MC Kit. A polymerase mix from an alternate vendor was used for PCR amplification. Prepared libraries contain fragments that represent both mono- and di- nucleosomal fractions in the cfDNA samples. Size profiles for a whole genome library containing both cfDNA nucleosomal fractions (blue), and a hybridization-captured library (orange) are shown in Figure 2 and show comparable traces. After the hybridization capture, libraries were circularized using the Adept Circularization kit in preparation for sequencing on the AVITI platform. Sequencing quality metrics for 69 Adept-treated libraries sequenced using the Element AVITI System (2 x 150 paired-end reads) are shown in Table 2.

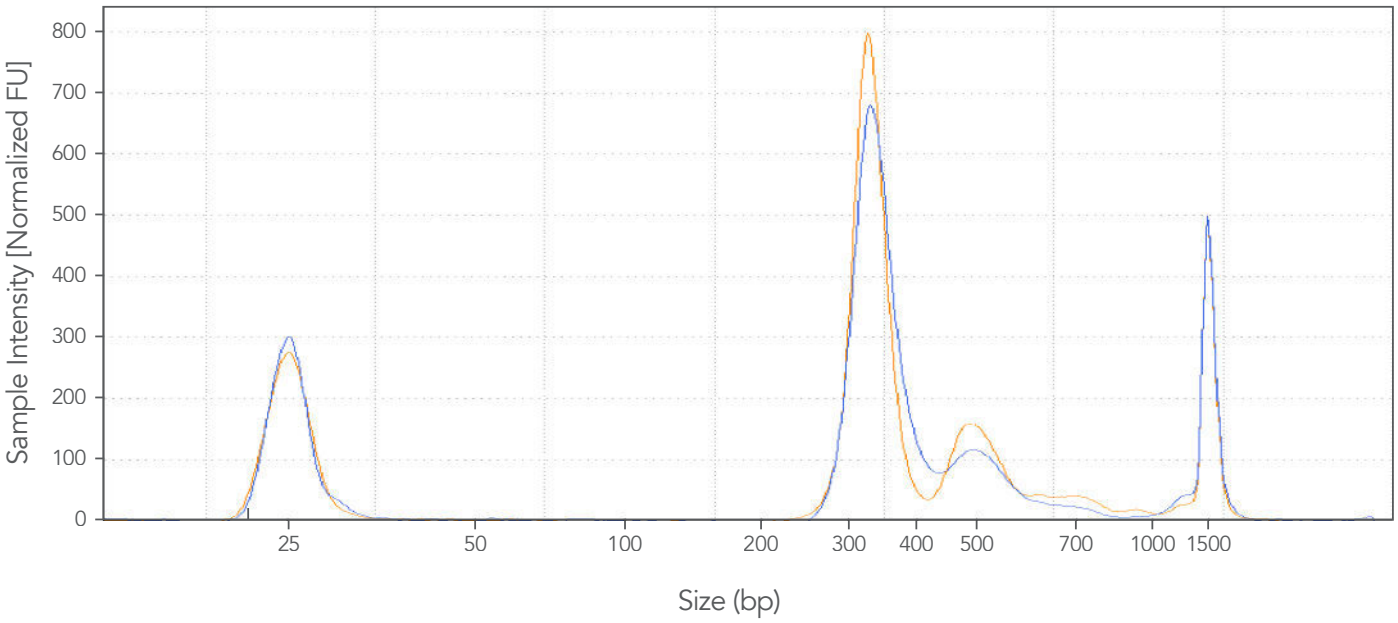

Figure 2. Representative TapeStation (Agilent) trace for cfDNA fragments subjected to library preparation (blue) and hybridization capture (orange). The resulting library showed a peak height of ~339 bp and ~502 bp; this is the expected library size after adding the adapters and index sequences.

Table 2. 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.

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

Appendix A: Fixed UMI sequences

The Ligation 1 Adapter contains 32 optimized, fixed UMI sequences that are 8 base pairs in length.

Adapter name	UMI sequence	Adapter name	UMI sequence
UMI_1	GAGACGAT	UMI_17	GCACAACT
UMI_2	TTCCAAGG	UMI_18	GCGTCATT
UMI_3	CGCATGAT	UMI_19	GAAGGAAG
UMI_4	ACGGAACA	UMI_20	ACTGAGGT
UMI_5	CGGCTAAT	UMI_21	TGAAGACG
UMI_6	GCTATCCT	UMI_22	GTTACGCA
UMI_7	TGGACTCT	UMI_23	AGCGTGTT
UMI_8	ATCCAGAG	UMI_24	GATCGAGT
UMI_9	CTTAGGAC	UMI_25	TTGCGAAG
UMI_10	GTGCCATA	UMI_26	CTGTTGAC
UMI_ 11	TCGCTGTT	UMI_27	GATGTGTG
UMI_12	TTCGTTGG	UMI_28	ACGTTCAG
UMI_13	AAGCACTG	UMI_29	TTGCAGAC
UMI_14	GTCGAAGA	UMI_30	CAATGTGG
UMI_ 15	ACCACGAT	UMI_31	ACGACTTG
UMI_16	GATTACCG	UMI_32	ACTAGGAG

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