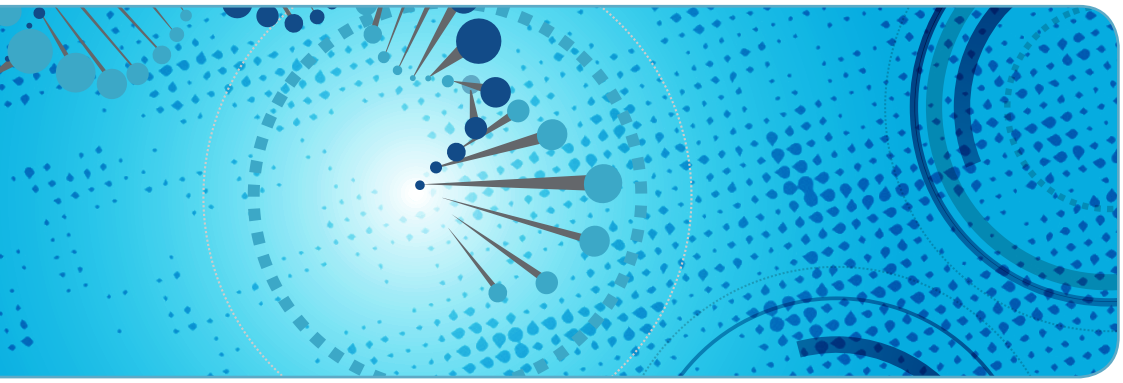
Lotus DNA Library Prep Kit



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IDT
INTEGRATED DNA TECHNOLOGIES

Revision history

Document version	Date released	Description of changes
2	June 2019	Emphasized use of fragmentation time specified in your Certificate of Analysis (COA)
1	April 2019	Original version

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Introduction

This guide provides instructions for the preparation of high-quality next generation sequencing (NGS) libraries from double-stranded DNA (dsDNA). The protocol describes the workflow for whole genome sequencing (WGS) and targeted sequencing applications, including enzymatic fragmentation and library construction.

The Lotus DNA Library Prep Kit is suitable for the following applications:

- Whole genome sequencing
- PCR-free sequencing
- Detection of germline-inherited single-nucleotide variations (SNVs) and insertions/deletions (indels)
- Hybridization capture of relevant genomic regions (e.g., the exome, or transcripts of interest)
- Low frequency somatic variation detection of SNVs and indels
- RNA-Seq starting with full-length, double-stranded cDNA input
- Metagenomic sequencing

The Lotus DNA Library Prep Kit can be used with a wide range of DNA inputs (1–250 ng) and may be used in conjunction with IDT adapters, including **Illumina TruSeq™–Compatible custom adapters** (stubby or full-length), **xGen Dual Index UMI Adapters—Tech Access**, and TA-ligation adapters.

Overview

This kit offers a versatile solution to streamline NGS sample preparation of dsDNA for sequencing on Illumina platforms. The technology provides rapid DNA fragmentation and library construction (**Figure 1**) to produce libraries for sequencing. This workflow suggests fragmentation times for high-quality DNA to obtain a mean of 350 bp inserts for WGS and 200 bp inserts for targeted sequencing, such as hybridization capture methods.

Tip: Although this kit can be used with damaged samples such as FFPE DNA, fragmentation time optimization may be necessary as FFPE samples generally require shorter fragmentation times.

The kit is used to generate libraries through an optional PCR workflow. PCR reagents are included in the kit for library amplification that should be used based on your library adapters and application. Adapters and index primers are *not* included in the kit; you must provide your own as needed.

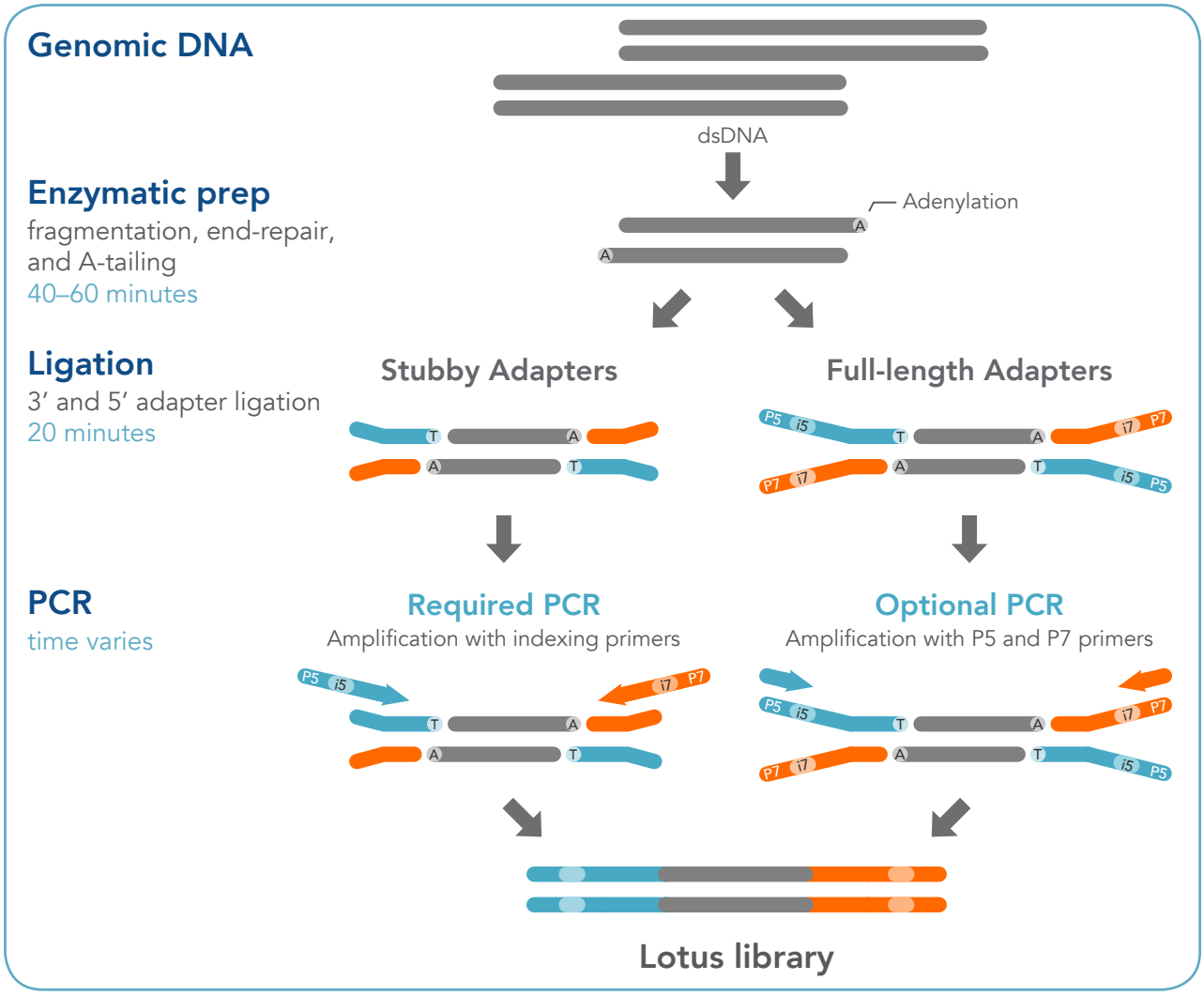

Figure 1. Lotus DNA library preparation illustrated.

Protocol options

Adapter recommendations (adapters not included in kit)

One of the following types of IDT adapters are strongly recommended for use with the Lotus DNA Library Prep Kit:

- TruSeq™–Compatible Stubby Adapters and Indexing Primers
- TruSeq™–Compatible Full-length Adapters
- xGen Dual Index UMI Adapters—Tech Access

Notes:

- xGen Dual Index UMI Adapters are full-length adapters.
- Other adapters compatible with TA-ligation/ligation to A-tailed fragments may be used.

Application	Best choice	Alternatives
Whole genome sequencing, metagenomics, PCR-free sequencing (≥100 ng input)	TruSeq™-Compatible Full-length Adapters	xGen Dual Index UMI Adapters—Tech Access
Whole genome sequencing, metagenomics with PCR (1–250 ng input), exome sequencing, and targeted germline sequencing (SNVs, indels)	TruSeq™-Compatible Stubby Adapters and Indexing Primers	TruSeq™-Compatible Full-length Adapters or xGen Dual Index UMI Adapters—Tech Access
Low-level mutation detection, down to ~1% frequency	xGen Dual Index UMI Adapters—Tech Access	—
RNA-seq starting with full-length, double-stranded cDNA input	xGen Dual Index UMI Adapters—Tech Access	TruSeq™-Compatible Full-length Adapters or TruSeq™-Compatible Stubby Adapters and Indexing Primerss

Additional reagents and kits for hybridization capture from IDT

The Lotus DNA Library Prep Kit is compatible with a suite of IDT hybridization capture products including:

- xGen Hybridization and Wash Kit
- xGen Universal Blockers—TS Mix and 10 bp TS Mix
- xGen Lockdown Panels and Probe Pools
- xGen Gene Capture Pools
- xGen Library Amplification Primers

Workflow

This protocol contains minimal enzymatic incubations and bead-based purification steps, thereby reducing sample handling and overall library preparation time to under 2 hours before library amplification. There are 3 major activities to perform in this protocol:

- **Enzymatic preparation—fragmentation, end-repair, and dA-tailing of dsDNA**. These reactions are all performed in a single step. The fragmentation profile achieved is dependent on both incubation temperature and time.
- **Ligation of either full-length or stubby P5 and P7 adapters**. When using full-length adapters, the final PCR step with Lotus PCR primers is optional and can be used to increase library yield. Stubby adapters (sometimes called truncated adapters) require amplification with indexing primers to incorporate sample indexing sequences and to add the flow cell attachment sequences, P5 and P7, for Illumina sequencing.
- **Optional PCR amplification**. Choose to amplify your libraries based on adapter and DNA input used.

Important! To perform a PCR-free WGS workflow, full-length adapters are required.

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 full-length or stubby adapters	Total time: 20 min
4	Clean up ligation reaction	• Purify ligation product	Total time: 20 min
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
6	Perform purification†	• Remove unincorporated primers (PCR-amplified) or adapters (PCR-free)	Total time: 20 min

PCR-free
library construction workflow
steps 1–4 & 6

1. Prepare reagents
2. Perform enzymatic preparation
3. Perform ligation
4. Clean up ligation reaction
6. Perform purification†

Targeted sequencing
library construction workflow
steps 1–5

1. Prepare reagents
2. Perform enzymatic preparation
3. Perform ligation
4. Clean up ligation reaction
5. Perform PCR & cleanup*

PCR-amplified
library construction workflow
steps 1–6

1. Prepare reagents
2. Perform enzymatic preparation
3. Perform ligation
4. Clean up ligation reaction
5. Perform PCR & cleanup*
6. Perform purification†

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

† Required for all WGS workflows (PCR-amplified/PCR-free).

Consumables and equipment

The Lotus DNA Library Prep Kits are available in 2 sizes with reagents (10% excess volume) for the preparation of either 16 or 96 libraries.

Tips:

- PCR reagents are also supplied in the kit for amplified WGS applications and pre-capture PCR. However, these reagents are not compatible with post-hybridization capture PCR.
- Primers are included for both WGS sequencing and pre-hybridization capture amplification of libraries generated with full-length adapters.
- IDT stubby adapters (not included in kit) come with indexing primers. PCR is used to add index sequences and amplify DNA.

Consumables from IDT

Item	Size	Catalog #	Storage
Lotus DNA Library Prep Kit	16 rxn	10001073	See Kit contents
	96 rxn	10001074	
Adapters Recommendations (choose one type): TruSeq™–Compatible Stubby Adapters and Indexing Primers TruSeq™–Compatible Full-length Adapters xGen Dual Index UMI Adapters—Tech Access "TA-ligation" adapters for dsDNA	Varies	www.idtdna.com/NGS-adapters	–20°C
Duplex Buffer	10 x 2 mL	11-01-03-01	–20°C
Nuclease-Free Water	10 x 2 mL	11-04-02-01	Room temperature

Kit contents

Item	Storage
Lotus Enzymatic Prep Buffer	–20°C
Lotus Enzymatic Prep Reagent	–20°C
Lotus Enzymatic Prep Enzyme	–20°C
Lotus Ligation Buffer	–20°C
Lotus Ligation Enzyme	–20°C
Lotus PCR Reagent	–20°C
Lotus PCR Buffer	–20°C
Lotus PCR Enzyme	–20°C
Lotus PCR Primers (P5 and P7 library amplification primers)	–20°C
Lotus Elution Buffer	Room temperature (20–25°C)

Consumables from other suppliers

Item	Supplier	Catalog #
Absolute ethanol (200 proof)	General laboratory supplier	Varies
Purification bead options (choose one):		
SPRIselect® Reagent, 5 mL	Beckman-Coulter	B23317
Agencourt® AMPure® XP–PCR purification beads, 5 mL	Beckman-Coulter	A63880
Digital electrophoresis		
Bio-Rad Experion™ DNA 1K Analysis Kit,or equivalent	Bio-Rad	700-7107
High Sensitivity DNA Kit, or equivalent	Agilent	5067-4626
High Sensitivity D1000 ScreenTape, or equivalent	Agilent	5067-5584
twin.tec® PCR plate 96 LoBind, semi-skirted	Eppendorf	0030129504
Library Quantification Kit – Illumina/Universal	Kapa Biosystems	KK4824
MAXYMum Recovery® Microtubes, 1.7 mL	VWR	22234-046
MAXYMum Recovery PCR Tubes, 0.2 mL flat cap	VWR	22234-056
Microseal® 'B' PCR Plate Sealing Film	Bio-Rad	MSB1001
Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851 or Q32854

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
Microcentrifuge	General laboratory supplier	Varies
Thermal cycler	General laboratory supplier	Varies
Magnet options:		
Magnum™ EX Universal Magnet Plate	Alpaqua	A000380
Magnetic Stand-96	Thermo Fisher Scientific	AM10027
qPCR system	General laboratory supplier	Varies
Qubit® 4 Fluorometer, or equivalent	Thermo Fisher Scientific	Q33226

Guidelines

Reagent handling

Always store the Lotus DNA Library Prep Kit reagents at –20˚C with the exception of Lotus Elution Buffer, which is stored at room temperature.

The enzymes provided in this kit are temperature sensitive, and appropriate care should be taken during storage and handling. To maximize use of enzyme reagents, when ready to use, remove enzyme tubes from –20˚C storage and place on ice (not in a cryocooler) for at least 20 minutes to allow enzymes to fully thaw and reach 4˚C prior to pipetting.

Caution: Attempting to pipette enzymes at –20˚C may result in reagent loss.

After thawing on ice (4˚C), briefly vortex all reagents, except the enzymes, to mix well. Spin all tubes in a microcentrifuge to collect contents before opening.

Keep Lotus Ligation Buffer at room temperature during handling and library preparation. Lotus Ligation Buffer is very viscous and requires special attention during pipetting. Once ready for use, pipette it very slowly so that you accurately draw the desired quantity.

To create master mixes, scale reagent volumes as appropriate, using 10% excess volume to compensate for pipetting loss. Add reagents in order listed when preparing master mixes. Once prepared, master mixes should be stored ON ICE until used.

Important!

- Except for the Lotus Ligation Buffer, keep all kit reagents ON ICE during handling and preparation.
- Only proceed when all kit reagents, including the Lotus Elution Buffer, have equilibrated to an ice-cold temperature (i.e., place on ice for at least 20 minutes to allow reagents to fully thaw and reach 4˚C).
- Assemble all master mixes ON ICE and scale volumes as appropriate, using 10% excess volume to compensate for pipetting loss.
- Always add reagents to the master mix in the order specified in this protocol.

Avoid cross-contamination

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.

Follow the instructions below 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 tips between each sample.

Size selection during clean up steps

This protocol has been optimized with AMPure XP beads (Beckman Coulter) for WGS and targeted sequencing applications but can also be used with SPRIselect beads (Beckman Coulter). If other beads are used, solutions and conditions for DNA binding may differ.

Consider the following for performing efficient size selection:

- After the enzymatic preparation, analyze the samples' size distribution by electrophoretic methods to determine the median fragment size of your fragmented dsDNA samples.
- The size selections used in this protocol perform left side size selection (also called single-size selection), and are designed to produce an average insert size of 350 bp for WGS workflows, or an average insert size of 200 bp for targeted sequencing workflows.

Tip: For customizing size selection, see Beckman Coulter's SPRIselect User Guide on guidance with optimizing SPRI conditions not included in this protocol.

DNA input considerations

This kit works with a wide range of DNA inputs: 1–250 ng. For PCR-free WGS, use a minimum of 100 ng of DNA input and full-length adapters.

Important! Input quantities recommended in this protocol refer to the total DNA quantified *before* DNA fragmentation.

EDTA in elution buffers

The enzymatic preparation reaction is very sensitive to high concentrations of EDTA. EDTA is usually introduced via elution buffers in the final steps of the DNA extraction, or purification process. If DNA has been stored or eluted in standard TE with 1 mM EDTA, perform a buffer exchange using a column, or bead-based, purification. Alternatively, you can adjust the amount of Lotus Enzymatic Prep Reagent in the Enzymatic Prep step to no more than 3X volume to achieve the desired fragment length. TE with 0.1 mM EDTA is most suitable for DNA elution and requires only 1.5 µL of Lotus Enzymatic Prep Reagent for fragmentation.

Tip: Store any input DNA in Lotus Elution Buffer (provided in this kit) to maximize fragmentation efficiency. Or, elute the purified DNA in:

- DNA suspension buffer (Teknova: 1 mM Tris-HCl; 0.1 mM EDTA, pH 8.0)

or

- Buffer EB (Qiagen, 10 mM Tris-HCl, pH 8.5)

Quantification

For high quality samples, we recommended determining dsDNA concentration using a Qubit Fluorometer, or a similar fluorometric method.

Fragmentation parameters

Refer to your certificate of analysis (CoA) for specific fragmentation time recommendations for the lot number you receive. When utilizing a new lot, you may experience variation in required fragmentation time. To find the CoA, enter the lot number of your kit (found on the bottom of the packaging) at www.idtdna.com/COA.

Recommended PCR cycles

For applications that require amplification, this protocol provides minimum PCR cycle recommendations for sequencing of libraries prepared from high-quality genomic DNA (see Tables 1–2). For samples of compromised quality, additional cycles may be required. Yields are approximate and will vary by sample type.

Protocol

Prepare reagents

1. Briefly vortex Lotus Ligation Buffer and keep at room temperature.
2. Place all kit enzymes on ice (not in a cryocooler) for at least 20 minutes to allow enzymes to reach 4°C before pipetting.
3. Thaw other kit reagents, buffers, and primers on ice (4°C), briefly vortex to mix well.
4. Spin all tubes in a microcentrifuge to collect contents before opening.
5. Prepare a fresh 80% ethanol solution using 200-proof/absolute ethanol and Nuclease-Free Water. At least 1.5 mL of 80% ethanol solution will be used per sample.

Perform enzymatic preparation

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 (1–250 ng) to a 0.2 mL PCR tube, adjust the volume to a total of 19.5 µL using Lotus Elution Buffer, if necessary, and place tube on ice.

Reagent	Volume per sample (µL)
Lotus Elution Buffer	(19.5 – x)
DNA	x
Total volume	**19.5**

2. Set up the thermal cycler with the Enzymatic Prep program as described, with the heated lid **set to 70°C**:

Step	Temperature (°C)	Duration
Hold	4	∞
Enzymatic prep	32	Varies (see Note below)
	65	30 minutes
Hold	4	∞

Important! Fragmented samples can be kept at 4°C no more than 1 hour.

Note: See your Certificate of Analysis (CoA) for fragmentation time recommendations for the lot number you receive. Reaction times may need to be optimized for individual samples. Specifically, for sample inputs <25 ng, longer fragmentation may be required. To find the CoA, enter the lot number, which can be found on a label on the bottom of the kit, at www.idtdna.com/COA.

3. Begin Enzymatic Prep program to chill thermal cycler to 4°C.

4. Prepare the Enzymatic Prep Master Mix by adding the components in the order shown:

Reagent	Volume per sample (µL)
Lotus Enzymatic Prep Buffer	3
Lotus Enzymatic Prep Reagent*	1.5
Lotus Enzymatic Prep Enzyme	6
Total volume: Enzymatic Prep Master Mix	**10.5**

* If samples are in 1 mM EDTA, using 2X–3X volume of Lotus Enzymatic Prep Reagent will reduce EDTA-induced under-fragmentation.

Note: Keep reagents and master mix on ice as much as possible.

5. Gently vortex the Enzymatic Prep Master Mix for 5 seconds.

Important! Thoroughly mix the Enzymatic Prep Master Mix before *and after* adding to your DNA samples. Because this master mix is viscous, failure to mix thoroughly could result in incomplete fragmentation.

6. Add 10.5 µL of premixed Enzymatic Prep Master Mix to each tube containing DNA samples and Lotus Elution Buffer to reach a final volume of 30 µL.

7. Thoroughly vortex to mix for 5 seconds.

8. Briefly centrifuge the sample in a microcentrifuge, then *immediately* place in the chilled thermocycler and advance the Enzymatic Prep program to the 32°C fragmentation step.

9. Continue the Enzymatic Prep program to completion.

Important! Fragmented samples can be kept at 4°C no more than 1 hour.

Perform ligation

Tip: To prepare for the Clean up ligation reaction procedure (following this section), begin equilibrating the AMPure XP beads for at least 30 minutes.

1. If you are using DNA inputs <25 ng, dilute your adapters (e.g., TruSeq™–Compatible Adapter) in Duplex Buffer (IDT), as described:

DNA input (ng)	Adapter dilution	Stock concentration (µM)
≥25	No dilution	15
10	10-fold (1:10)	1.5
1	20-fold (1:20)	0.75

2. Prepare the Ligation Master Mix, adding the components in the order shown:

Reagent	Volume per sample (µL)
Lotus Elution Buffer	10
Lotus Ligation Buffer (room temperature)	12
Lotus Ligation Enzyme	4
Total volume: Ligation Master Mix	**26**

Important! Slowly pipette the viscous Lotus Ligation Buffer to avoid bubbles and ensure accuracy. Keep reagents and master mix on ice except for the Lotus Ligation buffer.

3. Gently vortex Ligation Master Mix for 5 seconds.
4. When the Enzymatic Prep program is complete, add 26 µL of premixed Ligation Master Mix to the tubes containing your fragmented DNA samples (total volume is 56 µL).
5. Add 4 uL of adapter to the mixture and place tube on ice (total volume is 60 µL).

Note: For WGS sequencing applications with DNA inputs <25 ng, use diluted adapters from step 1 (above).

6. Thoroughly vortex to mix for 5 seconds.
7. Set up the thermal cycler with the Ligation program as described, with lid heating **OFF**, or **set at 40°C**.

Step	Temperature (°C)	Duration (minute)
Ligation	20	20
Hold	4	∞

8. Run the samples in the thermal cycler with the Ligation program.
9. Immediately proceed to Clean up ligation reaction.

Clean up ligation reaction

Note: Make sure the AMPure XP beads are equilibrated to room temperature before you begin.

1. Vortex the beads until the solution is homogeneous.
2. Add 48 µL of beads to each sample at room temperature (ratio of beads to sample is 0.8).
3. Vortex mix, then briefly centrifuge the samples in a tabletop microcentrifuge.
4. Incubate the samples for 5 minutes at room temperature.
5. Place the sample on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
6. Remove and discard the supernatant without disturbing the pellet (less than 5 µL may be left behind).
7. Add 180 µL of freshly prepared 80% ethanol solution to the sample while it is still on the magnetic rack. Do not disturb the pellet.
8. Incubate for 30 seconds, then carefully remove the ethanol solution.
9. Repeat steps 7 and 8 for a second ethanol wash.
10. Using a new pipette tip, remove any residual ethanol solution from the bottom of the tube.
11. Allow beads to dry on magnet for 1 to 3 minutes.
12. Add Lotus Elution Buffer to the sample tubes based on your application as shown:

Application	Lotus Elution Buffer (µL)
PCR-free	50
Targeted sequencing	20
PCR-amplified	20

13. Pipet mix until homogeneous.
14. Incubate at room temperature for 5 minutes.
15. Place the sample tubes on a magnetic rack for 2 minutes.
16. Transfer the supernatant containing your library to a clean tube, being careful to avoid any bead carryover.

    Safe stopping point: Libraries can be stored overnight at –20°C.

17. For PCR-free libraries, go directly to Perform purification. For targeted sequencing and PCR-amplified libraries, proceed to Perform PCR & cleanup (optional).

Perform PCR & cleanup (optional)

Complete this section if you are generating **PCR-amplified** and **targeted sequencing libraries**.

Notes:

- Make sure the AMPure XP beads are equilibrated to room temperature.
- Review Guidelines for tips on avoiding cross-contamination.

1. Prepare the PCR Master Mix, adding the components in the order shown:

Reagent	Volume per sample (µL)
Lotus Elution Buffer	10
Lotus PCR Reagent	4
Lotus PCR Buffer	10
Lotus PCR Enzyme	1
Total volume: PCR Master Mix	**25**

Note: Keep reagents and master mix on ice.

2. Gently vortex PCR Master Mix for 5 seconds.
3. Add 25 µL of the premixed PCR Master Mix to the entire eluted library sample (20 µL), then vortex mix (total volume is 45 µL).
4. Add 5 µL of PCR primers to the PCR Master Mix based on your adapter type, as shown:

Adapter type	Primer	Stock concentration	Final concentration
Full-length	Lotus PCR Primers	6 µM (each primer)	600 nM
Stubby	Sample Indexing Primer mix*	10 µM (each primer)	1 µM

* Sample Indexing Primers must be purchased separately.

5. Briefly centrifuge the sample tube in a microcentrifuge, then put on ice.
6. Set up the thermal cycler with the PCR program as described, with a heated lid **set to 105°C**.

Step	Cycles	Temperature (°C)	Duration (seconds)
Activate enzyme	1	98	30
Amplify	Varies (see Tables 1–2)	98	10
		60	30
		68	60
Hold	—	4	∞

Important! The number of cycles required to produce sufficient library for sequencing will depend on sample input quantity and quality. In the case of low-quality samples including FFPE DNA, the number of cycles required may vary based on the quality of the sample and amount of usable DNA present. Approximate guidelines for high quality DNA are as indicated in Tables 1–2, but the exact number of cycles required must be empirically determined.

Table 1. Cycling recommendations for PCR-amplified workflows—yields ~4 nM final library.

Input material (ng)	Minimum PCR cycles
≥25	4
10	6
1	9

Table 2. Cycling recommendations for targeted sequencing workflows—yields ≥500 ng of material for capture.

Input material (ng)	Minimum PCR cycles
250	4–5
100	6–7
25	8–9
10	9–10
1	13–14

7. When the PCR program is complete, vortex the room-temperature AMPure XP beads until the solution is homogeneous.
8. Add the specified bead volume to each sample as shown:

Application	Average insert size (bp)	Sample volume (µL)	Magnetic bead volume (µL)
PCR-amplified	350	50	32.5 (ratio: 0.65)
Targeted sequencing	200	50	90 (ratio: 1.8)

9. Vortex mix then briefly centrifuge the samples in a tabletop microcentrifuge.
10. Incubate the samples for 5 minutes at room temperature.
11. Place the sample on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
12. Remove and discard the supernatant without disturbing the pellet (less than 5 µL may be left behind).
13. Keeping the sample on the magnetic rack, add 180 µL of freshly prepared 80% ethanol solution without disturbing the pellet.
14. Incubate for 30 seconds, then carefully remove the ethanol solution.
15. Repeat steps 13 and 14 for a second ethanol wash.
16. With a new pipette tip, remove any residual ethanol solution from the bottom of the tube.
17. Allow beads to dry on the magnet for 1 to 3 minutes.
18. Add 22 µL of Lotus Elution Buffer to the sample tubes, and mix well by pipetting up and down until homogeneous.
19. Incubate at room temperature for 5 minutes.
20. Place the sample tubes on a magnetic rack for 2 minutes.

21. Transfer the supernatant (20 µL) containing the final library to a clean tube. Be careful to avoid any bead carryover.

    Safe stopping point: Libraries can be stored overnight at -20°C.

22. For PCR-amplified libraries proceed to Perform purification.

23. For targeted sequencing, the library is now ready for quantification, which can be performed using fluorometric methods (i.e., Qubit Fluorometer).

    Note: See the xGen hybridization capture of DNA libraries protocol.

Perform purification

Complete this section if you are generating **PCR-free** and **PCR-amplified libraries**.

Important! For WGS applications, we recommend performing this purification procedure to remove excess adapter (PCR-free libraries) or primers (PCR-amplified libraries) minimizing adapter dimers and index hopping.

1. Vortex the AMPure XP beads until the solution is homogeneous.
2. Add beads to each sample as shown:

Application	Sample volume (µL)	Bead volume (µL)
PCR-free	50	32.5 (ratio 0.65)
PCR-amplified	20	24 (ratio 1.2)

3. Vortex mix, then briefly centrifuge the samples in a tabletop microcentrifuge.
4. Incubate the samples for 5 minutes at room temperature.
5. Place the sample on a magnetic rack until the solution clears and a pellet has formed (~2 minutes).
6. Remove and discard the supernatant without disturbing the pellet (less than 5 µL may be left behind).
7. Keeping the sample on the magnetic rack, add 180 µL of freshly prepared 80% ethanol solution.
8. Incubate for 30 seconds, then carefully remove the ethanol solution.
9. Repeat steps 7 and 8 for a second ethanol wash.
10. With a new pipette tip, remove any residual ethanol solution from the bottom of the tube.
11. Allow beads to dry on the magnet for 1 to 3 minutes.
12. Add 20 µL of Lotus Elution Buffer to the sample tubes, then mix well by pipetting up and down until homogeneous.
13. Incubate at room temperature for 5 minutes.
14. Place the sample tubes on a magnetic rack for 2 minutes at room temperature.

15. Transfer the supernatant containing the final library to a clean tube. Be careful to avoid any bead carryover.

16. Store freshly prepared libraries at 4˚C (or long term at –20˚C).

The library is now ready for quantification, which can be performed using fluorometric methods (i.e., Qubit Fluorometer) or qPCR. For PCR-fee libraries, qPCR is required as fluorometric methods cannot distinguish fully from partially ligated molecules. A high-sensitivity DNA Agilent Bioanalyzer kit can be used to ensure desired library size.

Appendix: Troubleshooting

Problem	Possible cause	Action/explanation
Library migrates unexpectedly during Bioanalyzer analysis	Broad library size distribution for 350 bp insert size	Agilent High Sensitivity DNA traces for a library insert of 350 bp fragmentation run larger than expected at a ~560 bp mode. This is due to the broad size distribution of the library; however, smaller inserts will preferentially cluster. When sequenced, an aligned insert size of 350 bp will be obtained.
	Over-amplification of library leads to the formation of heteroduplex structures that migrate abnormally	• Quantify library by qPCR, as other quantification methods will not accurately detect heteroduplex library molecules. • Perform the minimum number of PCR cycles necessary to avoid over-amplification.
	Migration behavior over-estimates the library size of PCR-free libraries due to partially single-stranded adapters	PCR-free libraries containing 350 bp inserts should migrate to a ~800 bp peak on the Agilent High Sensitivity Chip.
DNA does not fragment properly—under digestion (High molecular weight profile of fragmented DNA is observed)	Input DNA was in a buffer with greater than 0.1 mM EDTA	Use a buffer exchange column or bead-based method to clean up your input DNA before fragmentation. Alternatively, use 2–3X volume of Lotus Enzymatic Prep Reagent in your fragmentation reaction.
	Improper mixing of reagents	Make sure the fragmentation mix is adequately mixed before and after adding to sample DNA.
DNA does not fragment properly—over digestion	Reaction left at room temperature	Make sure the Enzymatic Prep Master Mix and the DNA sample are kept on ice until placed onto the pre-chilled thermal cycler.
	Sample integrity compromised	Fragmentation time must be optimized for DNA samples that are not high quality (e.g., FFPE). We have observed more rapid digestion for FFPE samples.
Incomplete resuspension of beads after ethanol wash during cleanup or 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 with Lotus Elution Buffer within 3 minutes of removal of any residual ethanol.
Shortage of ligation enzyme (Lotus Ligation Enzyme)	Pipetting enzymes at –20°C, instead of 4°C	Allow ligation enzyme reagents (especially the Lotus Ligation Enzyme) to equilibrate to 4°C on ice for 20 minutes before pipetting.
Retention of liquid in pipette tip	Viscous reagents (i.e., Lotus Ligation Buffer) may stick to pipette tips, especially for non-low retention tips.	Pipette up and down repeatedly to release all liquid from the pipette tip.

For additional help with your library prep, please contact Applications Support.

Integrated DNA Technologies, Inc. (IDT) is your Advocate for the Genomics Age. For more than 30 years, IDT's innovative tools and solutions for genomics applications have been driving advances that inspire scientists to dream big and achieve their next breakthroughs. IDT develops, manufactures, and markets nucleic acid products that support the life sciences industry in the areas of academic and commercial research, agriculture, medical diagnostics, and pharmaceutical development. We have a global reach with personalized customer service. See what more we can do for **you** at www.idtdna.com.

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applicationsupport@idtdna.com

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