Lotus DNA Library Prep Kit

For the targeted sequencing library construction workflow

Prepare reagents

1. Briefly vortex the Lotus Ligation Buffer and keep at room temperature.
2. Place all kit enzymes on ice for at least 20 minutes to allow enzymes to reach 4°C before pipetting.
3. Thaw other kit reagents, buffers, and primers on ice, then 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.

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 the Lotus Elution Buffer if necessary, then place tube on ice.

Reagent	Volume per sample (µL)
Lotus Elution Buffer	(19.5 – ×)
DNA	×
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 printed on the label of the kit box 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 (Beckman-Coulter) 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).
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 (min)
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 the magnet for 1 to 3 minutes.
12. Add 20 µL of the Lotus Elution Buffer to the sample tubes.
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 at room temperature.
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.

Perform PCR & cleanup

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

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 Table 1)	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 Table 1, but the exact number of cycles required must be empirically determined.

Table 1. 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 90 µL (1.8 ratio) of beads to each sample.
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.

The library is now ready for quantification, which can be performed using fluorometric methods (i.e., Qubit Fluorometer). A high-sensitivity DNA Agilent Bioanalyzer kit can be used to ensure desired library size.

See Lotus DNA Library Prep Kit protocol to view the comprehensive protocol.

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