

Lotus DNA Library Prep Kit

For the PCR-free library construction workflow

For PCR-free WGS, use a minimum of 100 ng of DNA input and full-length adapters.

Prepare reagents

- Briefly vortex the Lotus Ligation Buffer and keep at room temperature.
- Place all kit enzymes on ice for at least 20 minutes to allow enzymes to reach 4°C before pipetting.
- Thaw other kit reagents, buffers, and primers on ice, then briefly vortex to mix well.
- Spin all tubes in a microcentrifuge to collect contents before opening.
- 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.

- Transfer the DNA sample (100–250 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 – x)
DNA	x
Total volume	19.5

- 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.

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

- 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.
- 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.
- Thoroughly vortex to mix for 5 seconds.
- 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.
- 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. Dilute your adapters (e.g., TruSeq™-Compatible Full-length Adapters) in Duplex Buffer to 15 µM if supplied at a different concentration.
- 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 µL 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 50 µL of 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.
- 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 purification

! **Important!** For WGS applications, we recommend performing this purification procedure to remove excess adapter, minimizing adapter dimers and index hopping.

1. Vortex the AMPure XP beads until the solution is homogeneous.
2. Add 32.5 μL (ratio 0.65) of beads to each sample.
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 qPCR. Fluorometric methods (i.e., Qubit Fluorometer) cannot be used for quantification because they cannot distinguish fully from partially ligated molecules. 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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