xGEN PRISM DNA LIBRARY PREP KIT

Perform end repair

Before starting the protocol, make sure the AMPure and PEG/NaCl reagents are at room temperature (20–25°C). Also, prepare a fresh 80% ethanol solution.

1. Add 50 µL of each sample into a low, nucleic acid binding PCR plate.

   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.

End Repair Master Mix	
Component	Volume—per reaction (µL)
End Repair Buffer	6
End Repair Enzyme	3
Total volume (µL):	9

   Note: If there is precipitate in the End Repair Buffer, vortex until the precipitate becomes clear in solution.

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

End Repair program		
Step	Temperature*	Time
1	20°C	30 min
2	4°C	hold

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

7. In preparation for Post-end repair cleanup, make the Ligation 1 Master Mix.

Ligation 1 Master Mix	
Component	Volume—per reaction (µL)
Ligation 1 Buffer	25
Ligation 1 Adapter	2
Ligation 1 Enzyme	3
Total volume (µL):	30

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

8. After the End repair program reaches 4°C, proceed immediately to Post-end repair cleanup.

Perform post-end repair cleanup

Note: Before starting cleanup, make sure you have prepared the Ligation 1 Master Mix.

1. 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, up to 5 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. Remove and discard the cleared supernatant; make sure 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; overdry beads will decrease final library yield.

9. Proceed immediately to Ligation 1.

Perform ligation 1

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

   Important! Make sure that the beads are fully resuspended.

3. Run the following thermal cycler program:

Ligation 1 program		
Step	Temperature*	Time
1	20°C	15 min
2	65°C	15 min
3	4°C	hold

* If possible, set lid temperature to 70°C. If lid cannot be programmed, set 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.

Perform ligation 2

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

Ligation 2 Master Mix	
Component	Volume—per reaction (µL)
Ligation 2 Buffer	4.5
Ligation 2 Adapter	4
Ligation 2 Enzyme A	0.5
Ligation 2 Enzyme B	1
Total volume (µL):	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! Make sure that the beads are fully resuspended

   Note: If you notice droplets on the sides of the well, gently centrifuge.

5. Run the following thermal cycler program:

Ligation 2 program		
Step	Temperature*	Time
1	65°C	30 min
2	4°C	hold

* If possible, set lid temperature to 70°C. If lid cannot be programmed, set to 105°C.

6. After program completes, proceed immediately to Post-ligation 2 cleanup.

Perform post-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, up to 5 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.
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; overdry 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 liquid to clear completely for 1–2 minutes.

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

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

    Safe Stop: Store the plate at –20°C overnight.

Run PCR amplification

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

1. Add 5 µL of xGen UDI Primer Pairs to each well.

   Note: If using alternative indexing primers, contact IDT application support to ensure that they are compatible with this protocol.

2. Add 25 µL of HiFi HotStart ReadyMix to each well, then pipette 10 times to mix.
3. Seal the plate, then briefly centrifuge.
4. Run the following thermal cycler program:

PCR program			
Step	Temperature*	Time	
1	98°C	45 sec	
2	98°C	15 sec	Based on sample input (see Table 1)
	60°C	30 sec	
	72°C	30 sec	
3	72°C	1 min	
4	4°C	∞	

* If possible, set lid temperature to 70°C. If lid cannot be programmed, set to 105°C.

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

	Number of cycles	
Input mass (ng)	gDNA or cfDNA	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 Post-PCR cleanup.

Perform post-PCR Cleanup

1. Add 65 µL of AMPure beads (1.3X volume) 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, up to 5 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.
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.
8. Dry the beads at room temperature for 1–3 minutes.

   Important! Never allow the beads to dry for >5 minutes; overdry 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.
12. 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.

13. Carefully transfer 30 uL of eluted DNA into a new well.

The library is now ready for quantification, which can be performed using fluorometric methods (i.e., Qubit Fluorometer) or qPCR. Digital electrophoresis [i.e., Agilent TapeStation® system or Agilent Bioanalyzer system] can also be used to assess the library size.

For more information, use the complete xGen Prism DNA Library Prep Kit protocol (download also available at www.idtdna.com/protocols).

Technical support: applicationsupport@idtdna.com

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