**Supplementary Methods—NEBNext Ultra DNA Library Prep for Illumina**

Note: All steps performed on ice unless specified.

*End Prep.*

For each sample, 55.5 µL fragmented gDNA (initial concentration 3 ng/µL) was combined with 3.0 µL End Prep Enzyme Mix and 6.5 µL End Repair Reaction Buffer (10X). The reagents were thoroughly pipette-mixed and incubated on a thermocycler at 20°C for 30 minutes, followed by 65°C for 30 minutes.

*Adaptor Ligation.*

To the End Prep reaction mixture, 15 µL Blunt/TA Ligase Master Mix, 1.0 µL Ligation Enhancer, and 2.5 µL NEBNext Adaptor for Illumina were added and pipette-mixed well. The mixture was incubated on a thermocycler at 20°C for 15 minutes. 3.0 µL USER Enzyme was added, pipette-mixed, and returned to a thermocycler at 37°C for 15 minutes. The reaction mixture was cleaned using AMPure XP beads at a 1:1 ratio by volume, with a final elution volume of 15 µL. DNA concentration was measured by Qubit.

*PCR Enrichment.*

25 µL NEBNext Q5 HotStart HiFi PCR Master Mix, 15 µL Adaptor Ligated DNA fragments, 5 µL Index Primer (unique per sample), and 5 µL Universal Primer were combined and thoroughly pipette-mixed. PCR was performed as follows:

|  |  |  |  |
| --- | --- | --- | --- |
| **Cycle Step** | **Temperature (°C)** | **Time** | **Cycles** |
| Initial Denaturation | 98 | 30 seconds | 1 |
| Denaturation | 98 | 10 seconds | 8\* |
| Annealing/Extension | 65 | 75 seconds |
| Final Extension | 65 | 5 minutes | 1 |
| Hold | 4 | ∞ |  |
| \*Cycle number determined by input DNA amount of ca. 50 ng | | | |

PCR products were cleaned using AMPure XP beads at a 1:1 ratio by volume, with a final elution volume of 33 µL.