QUICK PROTOCOL

# NxSeq® UltraLow DNA Library Kit, 12 Reactions with the NxSeq® Single Indexing Kits A and B

**(CAT. NO. 15012-1, 15100-1, 15200-1)**

Thaw all kit components on ice and set up reactions on ice. Equilibrate AMPure XP Beads to room temperature for 30 minutes before use and prepare fresh 70% ethanol before starting library prep. Refer to the full User Manual, MA166, when needed.

1. Add the following components in order to a 0.2 mL PCR tube and then mix gently by pipetting:

| Volume (µL) | Component |
|---|---|
| 17 - X | Nuclease-Free Water |
| 25 | 2X Buffer (2XB) |
| X | Sheared/fragmented DNA (50 pg – 75 ng) |
| 8 | Enzyme Mix (EM) |

2. Incubate the reactions in a programmed thermocylcer as follows:

| Step | Time (min) | Temperature |
|---|---|---|
| 1 | 20 | 25°C |
| 2 | 20 | 72°C |
| 3 | Hold | 4°C |

3. Add the following components to the tube in order at room temperature, and then mix gently by pipetting:

| Volume (µL) | Component | Important Notes |
|---|---|---|
| 3 | NxSeq Universal Adaptor | Dilute the NxSeq® Universal Adaptor in Adaptor Dilution Buffer based on the amount of input DNA (Step 1). 50 pg – 250pg: 1:60 dilution, >250 pg – 1 ng: 1:30 dilution, >1 ng: no dilution. |
| 4 | Ligase (LIG) | |

4. Incubate in thermocycler at 25°C for 30 min and then transfer the reaction contents to a 1.5 mL LoBind Tube.
5. Add 43 µL of Elution Buffer to each tube bringing the total to 100 µL before proceeding to the next step.
6. Add 80 µL (if mechanically sheared) or 100 µL (if enzymatically fragmented) of AMPure XP beads, mix, bind, and wash bound DNA per the User Manual, MA166. Elute DNA in 22 µL of Elution Buffer (EB) and transfer 20 µL of the eluate to a new 0.2 mL PCR tube.
7. Add the following components to the cleaned library DNA tube in the following order, and then mix gently by pipetting:

| Volume (µL) | Component |
|---|---|
| 25 | 2X PCR Master Mix (MM) |
| 5 | Single Indexed Primer Mix |

8. PCR amplify the adaptor-ligated fragments using the following program. Repeat Steps 2 - 3 a total of 4 to 16 times depending on starting DNA input amount (from Step1):

| Step | Time | Temperature | Cycle | Recommended Cycle Number by Input Amount | |
|---|---|---|---|---|---|
| 1 | 30 sec | 98°C | | 50 pg – 100 pg: 16 cycles | 2 ng – 10 ng: 8 cycles |
| **2** | **10 sec** | **98°C** | **5 – 16X** | 101 pg – 250 pg: 15 cycles | 11 ng – 25 ng: 7 cycles |
| **3** | **75 sec** | **72°C** | | 251 pg – 500 pg: 14 cycles | 26 ng – 50 ng: 6 cycles |
| 4 | 5 min | 65°C | | 501 pg – 750 pg: 13 cycles | 51 ng – 75 ng: 5 cycles |
| 5 | Hold | 4°C | | 751 pg – 1 ng: 12 cycles | |

9. Transfer the 50 µL PCR reaction to a new 1.5 mL LoBind Tube.
10. Add 50 µL of AMPure XP beads to bead clean the DNA as instructed in the User Manual, MA166. Elute the DNA in 102 µL of Elution Buffer (EB) and transfer 100 µL of eluate to a clean 1.5 mL LoBind tube.
11. Bead size select the DNA per the User Manual using 75 µL (if mechanically sheared) or 100 µL (if enzymatically sheared) of AMPure XP beads and elute in 22 µL of Elution Buffer (EB). Transfer 20 µL of the eluate (final library) to a new tube.
12. Quantify DNA by Qubit, check library size on Bioanalyzer and then calculate molarity. Sequence or store at -20°C.

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