NEXTflex™ Small RNA Sequencing Kit v3 No Size Selection Protocol

The NEXTflex Small RNA-Seq v3 kit is designed to enrich final library products of ~150-160 bp, corresponding the RNAs of ~20-30 nt., by depleting larger products during the cleanups following reverse transcription and PCR. To retain larger products, please use the following modifications to the protocol.

Follow the NEXTflex Small RNA Sequencing Kit v3 protocol through completion of Step E: Reverse Transcription-First Strand Synthesis. Instead of proceeding with Step F: Bead Cleanup in the manual, complete these steps:

Step F: Bead Cleanup

1. Add 10 µL of Adapter Depletion Solution to each sample and mix well by pipette.
2. Add 40 µL of NEXTflex Cleanup Beads and 90 µL of isopropanol and mix well by pipette.
3. Incubate for 5 minutes.
4. Magnetize sample until solution is clear.
5. Remove and discard supernatant.
6. Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove with a P200 or P300 set to 200 µL. Repeat this step for a total of 2 ethanol washes. IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.
7. Incubate sample for 3 minutes. After one minute, remove any residual liquid that may have collected at the bottom of the well.
8. Remove plate from magnetic stand and resuspend bead pellet in 20 µL of Nuclease-free Water by pipetting. Ensure that beads are completely resuspended.
9. Incubate for 2 minutes.
10. Magnetize until solution is clear.
11. Transfer 18 µL of supernatant to a new well. Proceed to STEP G: PCR Amplification in the manual. For gel-free library preparation, after STEP G, instead of proceeding with STEP H1 from the manual, complete STEP H1 below. For PAGE-based size selection, follow Step H2: PAGE Size Selection and Cleanup from the manual, but cut out the band or bands corresponding the size of the product of interest (20 nt RNA= 146 bp library product).

Step H1: Gel-Free Size Selection and Cleanup

1. Add 62.5 µL of NEXTflex Cleanup Beads to each sample and mix well by pipette.
2. Incubate for 5 minutes.
3. Magnetize sample for 5 minutes, or until solution appears clear.
4. Remove and discard supernatant.
5. Add 180 µL of freshly prepared 80% ethanol, incubate for 30 seconds, and remove all of the supernatant. Repeat this step for a total of 2 ethanol washes. IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.
6. Incubate sample for 3 minutes. After one minute, remove all residual liquid that may have collected at the bottom of the well.
7. Remove plate from magnetic stand and resuspend bead pellet in 13.5 µL of Resuspension Buffer by pipetting volume up and down. Ensure that beads are completely resuspended.
8. Incubate for 2 minutes.
9. Magnetize sample for 3 minutes or until solution appears clear.
10. Transfer 12 µL of supernatant to a new well or clean microcentrifuge tube. This is your sequencing library.
11. Check the size distribution of the final library by Bioanalyzer High Sensitivity DNA Assay (Agilent) and the concentration by Qubit dsDNA HS Assay (Life Technologies). See Figure 4A in Appendix A for sample Bioanalyzer trace.

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