



## Size Selection of NEXTflex™ Small RNA Sequencing Kit v3 Libraries Using the Sage Pippin Prep System

This protocol describes use of the Sage Pippin Prep system for automated size selection of small RNA libraries created with the NEXTflex™ Small RNA Sequencing Kit v3. The protocol is designed to recover final library products of ~150 bp, which represent miRNAs and siRNAs.

Follow the NEXTflex Small RNA Sequencing Kit v3 protocol through completion of Step G: PCR Amplification. Instead of proceeding with Step H2: PAGE Size Selection & Cleanup, complete these steps:

### Create a Pippin Prep size selection program:

1. In the Pippin Prep software, create a protocol for use with the “3% DF Marker F.” Select the “Range” collection mode and enter 115 for “BP start” and 170 for “BP end.”
2. Click the button marked “Use Internal Standards.”
3. Ensure the lane numbers match the “Ref Lane” values.
4. Save the protocol with an appropriate name.

### Purify PCR products:

1. To each sample, add 40 µL of NEXTflex™ Cleanup Beads and 65 µL of isopropanol and mix well by pipette.
2. Incubate for 5 minutes.
3. Magnetize sample until solution is clear.
4. Remove and discard supernatant.
5. 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.
6. Incubate sample for 3 minutes. After one minute, remove any residual liquid that may have collected at the bottom of the well.
7. Remove plate from magnetic stand and resuspend bead pellet in 32 µL of Resuspension Buffer by pipetting. Ensure that beads are completely resuspended.
8. Incubate for 2 minutes.
9. Magnetize until solution is clear.
10. Transfer 30 µL of supernatant to a new well or microcentrifuge tube.

### Run purified PCR products on Pippin Prep according to manufacturer's instructions.

Collect size selected fraction (40 µL) and transfer each sample to a clean well of a 96-well plate.

### Purify size-selected product:

1. To each 40 µL sample, add 40 µL of NEXTflex™ Cleanup Beads and 80 µL of isopropanol and mix well by pipette.
2. Incubate for 5 minutes.
3. Magnetize sample until solution is clear.
4. Remove and discard supernatant.
5. 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.
6. Incubate sample for 3 minutes. After one minute, remove any residual liquid that may have collected at the bottom of the well.
7. Remove plate from magnetic stand and resuspend bead pellet in 13 µL of Resuspension Buffer by pipetting. Ensure that beads are completely resuspended.
8. Incubate for 2 minutes.
9. Magnetize until solution is clear.
10. Transfer 12 µL of supernatant to a clean well or microcentrifuge tube. This is your sequencing library.
11. Check the size distribution and concentration of the final library by Bioanalyzer High Sensitivity DNA Assay (Agilent).