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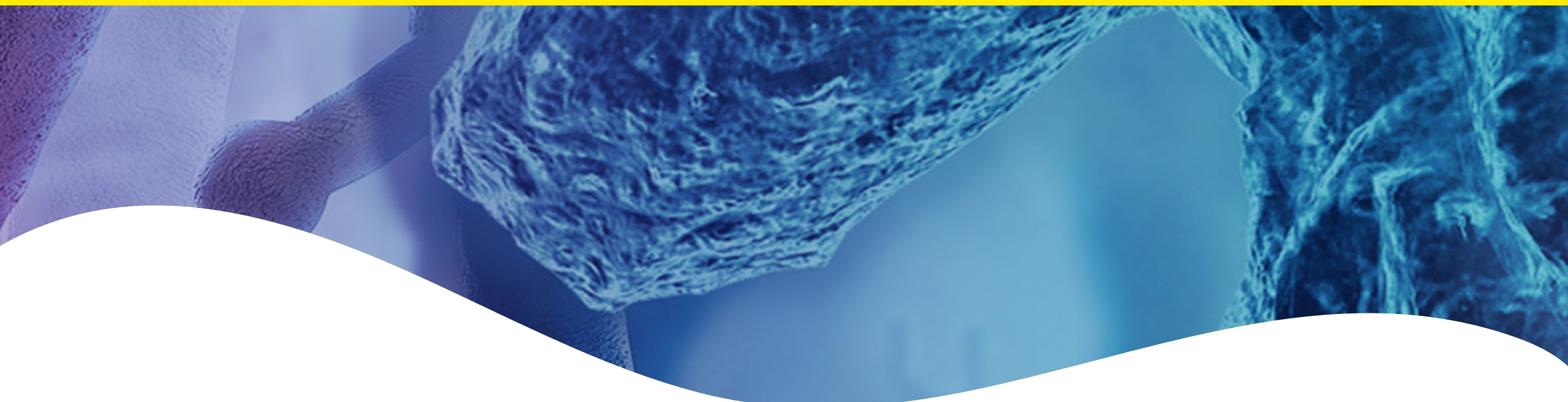
No size selection protocol



Size selection & cleanup

Materials

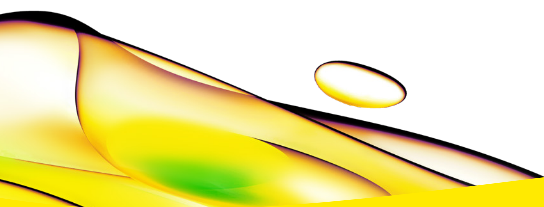
- White Cap - Nuclease-Free Water
- Yellow Cap - Resuspension Buffer
- Clear Cap Bottle - NEXTFLEX® Cleanup Beads

User supplied

- 80% Ethanol, freshly prepared
- Magnetic Stand
- **26 µL of PCR Product (from Step F)**

1. Ensure the volume of all samples is 26 µL. If less, add Nuclease-free Water to bring the entire volume up to 26 µL.
2. Add 47 µL of NEXTFLEX® Cleanup Beads and mix well by pipetting.
3. Incubate for 5 minutes.
4. Remove and discard supernatant.
5. Add 200 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 17 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 15 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 LabChip® or equivalent and the concentration by Qubit dsDNA HS Assay (Life Technologies).



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