NEBNext FFPE DNA Repair Mix (M6630) -Protocol for use with Other User-supplied Library Construction Reagents (M6630)

Symbols

⚠	*This caution sign signifies a step in the protocol that has multiple paths leading to the same end point but is dependent on a user variable, like the amount of input DNA.*
●	*Colored bullets indicate the cap color of the reagent to be added to a reaction.*

3.1 NEBNext FFPE Repair

Input amount should be determined based on recommendations by end user supplied library preparation kits.

1. Mix the following components in a sterile nuclease-free tube:
   FFPE DNA 53.5 µl
   ● (green) FFPE DNA Repair Buffer 6.5 µl
   ▫ (green) NEBNext FFPE DNA Repair Mix 2 µl

   Total volume 62 µl
2. Mix by pipetting followed by a quick spin to collect all liquid from the sides of the tube.
3. Incubate at 20°C for 15 minutes.

3.2 Cleanup Using AMPure XP Beads

1. Vortex AMPure XP Beads to resuspend.
2. Add 186 µl (3X) of resuspended AMPure XP Beads to the repair reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
3. Incubate for 5 minutes at room temperature.
4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.
5. Add 200 µl of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
6. Repeat Step 5 once.
7. Air dry beads for 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.
   Caution: Do not overdry the beads. This may result in lower recovery of DNA target.
8. Remove the tube/plate from the magnet. Elute DNA target by adding 40 µl 0.1X TE to the beads. Mix well on a vortex mixer or by pipetting up and down, and incubate for 2 minutes at room temperature. Put the tube/PCR plate in the magnetic stand until the solution is clear.
9. Without disturbing the bead pellet, carefully transfer 32 µl of the supernatant to a fresh, sterile microfuge tube.
10. Proceed to library construction using end-user supplied reagents.