Whole-exosome sequencing - Post-amplification cleanup protocol for Collibri™ PS DNA Library Prep Kits for Illumina® systems

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Collibri™ PS DNA Library Prep Kit for Whole-Exome Sequencing User Guide* (Pub. No. MAN0025533). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

About the protocol

This protocol describes post-amplification cleanup of the DNA libraries for whole-exosome sequencing (WES) samples. It replaces the *Purification of the amplified DNA libraries* section in the workflow provided in the *Collibri™ PS DNA Library Prep Kit for Whole-Exome Sequencing User Guide*.

Required materials

Components from the Collibri™ PS DNA Library Prep Kits for Illumina®
DNA Cleanup Beads
Wash Buffer (diluted with 96% ethanol)
Elution Buffer
Other consumables
PCR-amplified DNA library
96% ethanol, molecular biology grade (used for diluting the Wash Buffer before first use)
Eppendorf™ 1.5-mL DNA LoBind microcentrifuge tubes, 0.2-mL PCR 8-tube strips with attached caps, or 96-well plate
Microcentrifuge
Magnetic rack

Before starting

- Ensure that the appropriate volume of 96% ethanol (as noted on the bottle) was added to the Wash Buffer before first use.
- Ensure that the DNA Cleanup Beads, Wash Buffer, and Elution Buffer are at room temperature.
- Gently vortex the DNA Cleanup Beads to completely resuspend the magnetic beads in the solution.

Purify the amplified DNA library

Perform all the cleanup steps at room temperature.

Bind the library

1. Mix amplified post-capture library (50 µL) with 55 µL of DNA Cleanup Beads by vortexing until you have obtained a homogeneous suspension.
2. Briefly centrifuge the tube containing the amplified DNA library and bead mixture to collect all the droplets at the bottom, then incubate for 5 minutes at room temperature.

   IMPORTANT! Do not extend the binding step for more than 5 minutes.Over-incubation can result in lower DNA yields.
3. Briefly centrifuge the tube to collect all the droplets at the bottom, then place the tube in a magnetic rack for 2 minutes or until the beads have formed a tight pellet.

   Note: The time required for complete capture of the cleanup beads can vary depending on the reaction vessel and the magnet used. Optimize the capture time accordingly.
4. Keep the reaction tube in the magnetic rack, then carefully remove and discard the supernatant using a pipette. Ensure that all the supernatant is removed.

   Note: If the bead pellet is disturbed, mix the sample and allow the beads to form a tight pellet on the magnet again.
5. Remove the tube from the magnetic rack, then add 50 µL of Elution Buffer, then vortex to mix thoroughly.
6. Add 65 µL of fresh DNA Cleanup Beads directly to the bead suspension in Elution Buffer, then mix by vortexing until you have obtained a homogeneous suspension.
7. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 5 minutes at room temperature.
8. (*Optional*) If the bead pellet is disturbed, briefly centrifuge the tube to collect all the droplets at the bottom, then place the tube back in the magnetic rack for 2 minutes.
9. Keep the tube in the magnetic rack, then carefully remove and discard the supernatant using a pipette. Ensure that all the supernatant is removed.

   Note: If the bead pellet is disturbed, mix the sample and allow the beads to form a tight pellet on the magnet again.

Wash the magnetic bead pellet

1. Keep the reaction tube in the magnetic rack and add 200 µL of Wash Buffer (pre-mixed with ethanol).

   IMPORTANT! Do not resuspend the magnetic beads in Wash Buffer.
2. Incubate for 30 seconds at room temperature, then carefully remove and discard the supernatant using a pipette.
3. Repeat steps 1–2.
4. Briefly centrifuge the reaction tube and place it in a magnetic rack. Without disturbing the pellet, carefully remove any remaining supernatant and residual ethanol.
5. Keep the reaction tube in the magnetic rack and air dry the magnetic beads for 1 minute at room temperature, or until there are no droplets of ethanol left on the walls of the tube.

   IMPORTANT! Do not dry the magnetic beads for more than 5 minutes. Over-drying significantly decreases the elution efficiency.

Elute the library

1. Remove the tube from the magnetic rack, add 25 µL of Elution Buffer, then mix the suspension thoroughly by pipetting up and down or vortexing.
2. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 1 minute at room temperature.
3. Place the tube in the magnetic rack for 2 minutes or until the beads form a tight pellet. Wait for the solution to clear before proceeding to the next step.
4. Without removing the tube from the magnetic rack, transfer 22–23 µL of the supernatant to a new sterile tube for storage.

   Note: If the bead pellet is disturbed, mix the sample and repeat steps 2–4.
5. After purification, the amplified DNA library can be stored at 4°C for 1–2 weeks. For longer term, store the library at –20°C until ready for exome enrichment.

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Revision	Date	Description
A.0	14 January 2022	Replacement post-amplification cleanup protocol for Collibri PS DNA Library Prep Kit for Whole-Exome Sequencing.