# Whole-exosome sequencing - Purification of amplified post capture libraries for Collibri™ DNA Library Prep Kits for Illumina® systems

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**Note:** For safety and biohazard guidelines, see the "Safety" appendix in the Collibri™ DNA Library Prep Kit for Illumina® User Guides (Pub. Nos. MAN0025533, MAN0025534). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## About the protocol

This section describes bead-based purification of amplified post-capture DNA libraries for whole-exosome sequencing (WES) samples.

## Required materials

| **Components from the Collibri™ ES DNA Library Prep Kits for Illumina®** |
|---|
| DNA Cleanup Beads |
| Wash Buffer (diluted with 96% ethanol) |
| Elution Buffer |
| **Other consumables** |
| Amplified post-capture DNA libraries |
| 96% ethanol, molecular biology grade (used for diluting the Wash Buffer before first use) |
| Eppendorf™ 1.5-mL DNA LoBind microcentrifuge tubes |
| 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 post-capture DNA library

Perform all the cleanup steps at room temperature.

### Bind the library

1. Mix amplified post-capture library (50 µL) with 50 µL of DNA Cleanup Beads by vortexing until you have obtained a homogeneous suspension.

   **Note:** It is **not** necessary to recover the supernatant or remove the Target Enrichment and Amplification Beads from the amplified PCR product.

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.

### 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 and add 32 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 a magnetic rack for 2 minutes or until the beads have formed a tight pellet. Wait for the solution to clear before proceeding to the next step..

4. Keep the tube in the magnetic rack, and transfer 30 µL of the supernatant (i.e., the eluate) to a new tube for storage.

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

   **Note:** Use Nuclease-free 1.5-mL tubes, such as Eppendorf™ 1.5-mL DNA LoBind microcentrifuge tubes (or equivalent) for long term storage.

5. 14. Proceed to the assessment of the DNA library size and yield.

6. After purification, proceed immediately to sequencing, or store the enriched libraries at –20°C.

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| Revision | Date | Description |
|---|---|---|
| A.0 | 14 January 2022 | Replacement post-capture purification protocol for Collibri DNA Library Prep Kits for Whole-Exome Sequencing. |

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