

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

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Colibri™ 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 bead-based post-ligation purification of adaptor-ligated DNA libraries for whole-exosome sequencing (WES) samples. It replaces the *Post-ligation cleanup* section in the workflow provided in the *Colibri™ PS DNA Library Prep Kit for Whole-Exome Sequencing User Guide*.

Required materials

Components from the Colibri™ PS DNA Library Prep Kits for Illumina®
DNA Cleanup Beads
Wash Buffer (diluted with 96% ethanol)
Elution Buffer
Other consumables
Dual index adaptor-ligated DNA sample
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 adaptor ligated library

Perform all the cleanup steps at room temperature.

Bind the library

1. Mix the Dual Index Adaptor-ligated DNA library (70 µL) with 56 µL of DNA Cleanup Beads by vortexing until you have obtained a homogeneous suspension.
2. Briefly centrifuge the tube 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 a greater amount of adaptor and adaptor dimers in the final library.

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.

Bind the library a second time

1. Remove the tube from the magnetic rack and add 70 µL of Elution Buffer, then vortex to mix thoroughly.
2. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 1 minute at room temperature.
3. Add 70 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.
4. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 5 minutes at room temperature.
5. (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.
6. 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.
7. Keep the reaction tube in the magnetic rack and add 200 µL of Wash Buffer (pre-mixed with ethanol), then incubate for 30 seconds at room temperature.

IMPORTANT! Do not resuspend the magnetic beads in Wash Buffer.

8. Carefully remove and discard the supernatant using a pipette.
9. Repeat steps 7–8.
10. Briefly centrifuge the reaction tube and place it in a magnetic rack. Without disturbing the pellet, carefully remove any remaining supernatant and residual ethanol.
11. 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 vortex to mix thoroughly.
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, proceed to library amplification, or store the eluted DNA library at 4°C for up to 1–2 weeks. For longer term, store the library at –20°C.

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Revision history: Pub. No. MAN0025491

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

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