SARS-CoV-2 post-ligation cleanup protocol for Collibri™ ES DNA Library Prep Kits for Illumina® systems

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Collibri™ ES DNA Library Prep Kit for Illumina® User Guide* (Pub. No. MAN0018545). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Purify the adaptor-ligated library

This protocol describes bead-based post-ligation purification of adaptor-ligated DNA library for SARS-CoV-2 samples. It replace the section for *Post-ligation double-sided size selection* in the workflow provided in the Collibri™ ES DNA Library Prep Kit for Illumina® User Guide.

Required materials

Components from the Collibri™ ES DNA Library Prep Kit 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 tube, 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.

Bind the adaptor-ligated 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 allow the sample to bind for more than 5 minutes.Over-incubation can result in greater amount of adaptor and adaptor dimers in the final library.

3. After incubation, briefly centrifuge to collect all the droplets at the bottom of the tube, then place the tube in the magnetic rack for 2 minutes or until the beads have formed a tight pellet.

   Note: The time required for the 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. To remove residual ethanol, briefly centrifuge the reaction tube and place it in the magnetic rack, then carefully remove any remaining supernatant with a pipette without disturbing the pellet.

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! Leave the plate in contact with the magnet as beads should not be resuspended during this washing step.

Elute the adaptor-ligated library

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. After incubation, briefly centrifuge the tube to collect all the droplets at the bottom, then place it in the magnetic rack for 2 minutes or until the beads have formed a tight pellet.

6. 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.

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. To remove residual ethanol, briefly centrifuge the reaction tube and place it in the magnetic rack, then carefully remove any remaining supernatant with a pipette without disturbing the pellet

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** over-dry by prolonged incubation for more than 5 minutes. Over-drying significantly decreases the elution efficiency.

12. Remove the tube from the magnetic rack, add 25 µL of Elution Buffer, then vortex to mix thoroughly.

13. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 1 minute at room temperature.

14. Place the tube in the 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.

15. 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 pellet of magnetic beads was disturbed, mix the sample and let the beads settle to the side of the tube on the magnet again.

16. Store the eluted DNA library at 4°C for up to 1–2 weeks or at –20°C for long-term storage, or immediately proceed to the next step (Select the concentration normalization method: Qubit-based normalization as described on page 25 or bead-based normalization as described on page 31).

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