Small fragment DNA library cleanup protocol for Collibri™ PS DNA Library Prep Kits for Illumina® systems

Modified protocol for short (100–150 bp) physically sheared DNA or intact cfDNA for use with Illumina® next-generation sequencing (NGS) platforms

Catalog Numbers A38612024, A38614096, A38612024W, A38614096W, A38613024, A43611024, A43612024 A43613024, A38614196

Pub. No. MAN0026159 Rev. A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Collibri™ PS DNA Library Prep Kit for Illumina® User Guide* (Pub. No. MAN0018546). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

About the protocol

This protocol is used to generate small fragment DNA libraries with a target insert size of 100–150 bp. Construction is performed as described in the user guide for Collibri™ PS DNA Library Prep Kits for Illumina® (Pub. No. MAN0018546), except the modified protocols for small fragment DNA library cleanup replace the following purification steps:

- Post-ligation purification of the adaptor-ligated DNA library (user guide, page 21; see page 1 for replacement procedure)
- Purification of the amplified DNA library (user guide, page 31; see page 3 for replacement procedure)

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 (One-sided size selection)

This section replaces the post-ligation purification of adaptor-ligated DNA library procedure described on page 21 of the Collibri™ PS DNA Library Prep Kits for Illumina® 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
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, or 96-well plate
Microcentrifuge
Magnetic rack

Bind the library

1. Mix the Dual Index Adaptor-ligated DNA library (70 µL) with 40 µL of DNA Cleanup Beads and 20 µL of 96% ethanol 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 10 minutes at room temperature.

   IMPORTANT! Do not extend the binding step for more than 10 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.

5. Remove the tube from the magnetic rack, then add 70 µL of Elution Buffer. Vortex to mix thoroughly.

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

7. Add an additional 80 µL of DNA Cleanup Beads and 40 µL of 96% ethanol directly to the bead suspension in Elution Buffer. Mix by vortexing until you have obtained a homogeneous suspension.

8. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 10 minutes at room temperature.

9. Briefly centrifuge the tube to collect all the droplets at the bottom, then place the tube in a magnetic rack for 2 minutes.

10. 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, 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 have formed 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, you can proceed to PCR amplify the library, or store the DNA library at 4°C for 1–2 weeks. For longer term, store the library at –20°C.

Purify the amplified DNA library (small fragment)

This section replaces the purification of the amplified DNA library procedure described on page 31 of the Collibri™ PS DNA Library Prep Kits for Illumina® User Guide.

Perform all cleanup steps at room temperature.

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
Eppendorf™ 1.5-mL DNA LoBind microcentrifuge tubes, or 96-well plate
Microcentrifuge
Magnetic rack

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.

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 a greater amount of primers and primer-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.

5. Remove the tube from the magnetic rack, then add 50 µL of Elution Buffer, then vortex to mix thoroughly.

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

7. Add an additional 50 µL of DNA Cleanup Beads directly to the bead suspension in Elution Buffer, then mix by vortexing until you have obtained a homogeneous suspension.

8. Briefly centrifuge the tube to collect all the droplets at the bottom, then incubate for 5 minutes at room temperature.

9. (*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.

10. 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 have formed 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. Proceed to assessment of the DNA library size and yield (see “Verification of size distribution and quality of prepared DNA libraries” on page 4).

6. After purification, you can store the amplified DNA library 4°C for 1–2 weeks. For longer term, store the library at –20°C until ready for sequencing.

Verification of size distribution and quality of prepared DNA libraries

Verify the size distribution and quality of prepared DNA library by performing capillary electrophoresis analysis on the Agilent™ 2100 Bioanalyzer™ Instrument (or any similar instrument) using the Agilent™ High Sensitivity DNA Kit.

Required materials

- Agilent™ 2100 Bioanalyzer™ Instrument (Agilent G2938A)
- Agilent™ High Sensitivity DNA Kit (Agilent 5067-4626)
- Nuclease-free water

Analyze the size distribution of the amplified library

1. Remove 1 µL from each prepared DNA library (i.e., purified and amplified DNA library) and dilute it 3–5-fold in nuclease-free water.

2. Analyze 1 µL of the diluted DNA library using the appropriate chip on the Agilent™ 2100 Bioanalyzer™ Instrument with the Agilent™ High Sensitivity DNA Kit.

3. Using the 2100 Expert software, perform a smear analysis to determine the average library length using a size range of 150–1000 bp. Check for the expected size distribution of library fragments and for the absence of residual adaptor or adaptor dimers peaks near 140 bp.

   Note: For instructions on how to perform the smear analysis, refer to the *Agilent™ 2100 Bioanalyzer™ 2100 Expert User's Guide* (Agilent, Pub. No. G2946-90004).

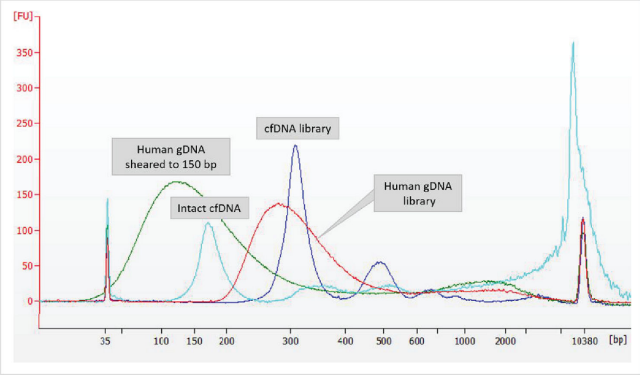

Figure 1

Typical Agilent™ 2100 Bioanalyzer trace of small DNA fragment libraries generated using the Collibri™ PS DNA Library Prep Kit

and modified cleanup protocols. Small fragment DNA libraries were prepared from 10 ng of physically sheared (Covaris™ E220) human gDNA and intact cfDNA using the Collibri™ PS DNA Library Prep Kit. cfDNA and human gDNA libraries were purified following post-ligation cleanup and amplified. Post amplification cleanup was applied to all libraries. Aliquots of the sample were collected, then electrophoregrams were generated on an Agilent™ 2100 Bioanalyzer™ Instrument.

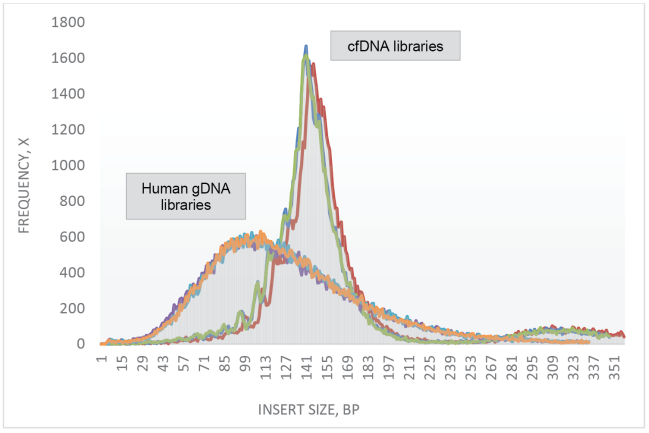

Figure 2

Insert size distribution. Small fragment human gDNA and cf DNA libraries were sequenced on MiSeq system (2x150 bp). Insert sizes were determined by using QualiMap v.2.2.1.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.