

Hieff™ LongSeq Amplicon End Repair and Ligation Module

13306ES

INSTRUCTIONS FOR USE

Ver. EN20260302

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Product description

Hieff™ LongSeq Amplicon End Repair and Ligation Module is a library preparation kit specifically developed for the third-generation Nanopore sequencing platform. When used in combination with 13304ES, it enables library construction for PCR products of various lengths.

This kit is designed for amplicon library preparation. It features a streamlined workflow where the product undergoes end repair and A-tailing without the need for intermediate purification, allowing for direct barcode ligation. This makes the experimental procedure more convenient.

- Compatible with input amounts ranging from 50 ng to 1.5 µg.
- Library preparation time is approximately 2 hours.
- Stringent quality control for batch-to-batch performance and stability.

Specifications

Product Name	Cat.NO.	Specification
Hieff™ LongSeq Amplicon End Repair and Ligation Module Amplicon Library Preparation Module	13306ES08	8 T
	13306ES24	24 T
	13306ES96	96 T

Components

Components No.		Name	13306ES08	13306ES24	13306ES96
			8 T	24 T	96 T
13306-A	●	Endprep Buffer	56 µL	168 µL	672 µL
13306-B	●	Endprep Enzyme	24 µL	72 µL	288 µL
13306-C	○	Rapid Ligase Master Mix	280 µL	840 µL	4×840 µL

Storage

This product should be stored at -25~-15°C for 1 years.

Instructions

1. Materials to be Prepared by User

- 1) **DNA Quantification:** 1× dsDNA HS Assay Kit (Cat# 12642ES); Qubit Assay Tubes (Cat# 83509ES); or equivalent products from other vendors.
- 2) **DNA Quality Control:** TapeStation DNA ScreenTape or other equivalent instruments.
- 3) **Barcode Adapter:**
 - Hieff™ Native Barcode Kit for ONT, Set 1 (Cat# 13317)
 - Hieff™ Native Barcode Kit for ONT, Set 2 (Cat# 13318)
 - Hieff™ Native Barcode Kit for ONT, Set 3 (Cat# 13319)
 - Hieff™ Native Barcode Kit for ONT, Set 4 (Cat# 13320)
 - Native Barcoding Expansion 1-12 (Nanopore #EXP-NBD104)
 - Native Barcoding Expansion 13-24 (Nanopore #EXP-NBD114)

Native Barcoding Expansion 1-96 (Nanopore #EXP-NBD196)

4) Motor Protein Adapter:

Adapter Mix II Expansion (Nanopore #EXP-AMII001)

Native Adapter (Nanopore #EXP-NBA114)

5) Motor Protein Ligation Module and Purification Beads: Hieff™ Adapter Ligation Module for ONT (Cat# 13304), Hieff NGS™ DNA Selection Beads V2 (Cat# 12418), AMPure XP Beads (Beckman), or equivalent products from other vendors.

6) Nucleic Acid Purification Buffers:

Additional Long Fragment Buffer (LFB) (Nanopore #EXP-LFB001)

Additional Short Fragment Buffer (SFB) (Nanopore #EXP-SFB001)

7) Other Materials: Freshly prepared 80% ethanol, Nuclease-free ddH₂O, 0.5 M EDTA, TE Buffer (10 mM Tris-HCl, pH 8.0~8.5 + 1 mM EDTA), low-binding EP tubes, PCR tubes, wide-bore pipette tips, magnetic rack, thermal cycler, and vortex mixer, etc.

2. Workflow

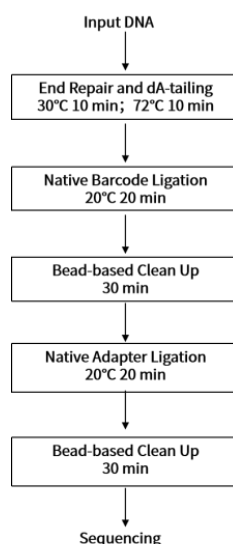


Figure 1. Library Preparation Workflow for PCR Products

3. Protocol

3.1 End Repair / dA-Tailing

This step is used for DNA end blunting, 5' phosphorylation, and 3' dA-tailing.

1) Thaw Endprep Buffer and Endprep Enzyme on ice. Mix by inversion, briefly centrifuge, and keep on ice. Prepare the reaction mixture in a PCR tube according to the volumes specified in Table 1.

Table 1. Reaction System for Enzymatic Digestion / End Repair / dA-Tailing

Name	Volume (μL)
Input DNA	x
Endprep Buffer	7
Endprep Enzyme	3
ddH ₂ O	Up to 60

2) Mix gently by pipetting (do not vortex) and briefly centrifuge to collect the reaction mixture at the bottom of the tube.

3) Place the PCR tube in a thermal cycler and run the program set in Table 2:

Table 2. PCR Program for Enzymation / End Repair / dA-Tailing

Temperature	Time
Heat Lid 105°C	On
30°C	10 min
72°C	10 min
4°C	Hold

3.2 Native Barcode Ligation

This step is used to ligate Native Barcodes to both ends of the product from Step 3.1.

1) Thaw Native Barcode and Rapid Ligase Master Mix on ice. Mix by flicking, briefly centrifuge, and keep on ice. Prepare the reaction mixture in a PCR tube according to the volumes specified in Table 3.

Table 3. Reaction System for Native Barcode Ligation

Name	Volume (μL)
Product from Step 3.1	60
Native Barcode*	2.5
Rapid Ligase Master Mix	35
ddH ₂ O	Up to 100

[Note]: We recommend using the Yesea Associated products (Cat# 13317~13320) or ONT designated products (Nanopore #EXP-NBD104/EXP-NBD114/EXP-NBD196) for Native Barcode.

2) Mix gently by pipetting (do not vortex) and briefly centrifuge to collect the reaction mixture at the bottom of the tube.

3) Place the PCR tube in a thermal cycler and run the program set in Table 4:

Table 4. PCR Program for Native Barcode Ligation

Temperature	Time
Heat Lid 105°C	Off
20°C	20 min
4°C	Hold

3.3 Termination of Native Barcode Ligation (Optional)

This step uses EDTA to terminate the ligation reaction from Step 3.2. Termination is required before pooling samples; failure to terminate before pooling may result in cross-ligation between different groups of Barcodes. Termination is optional for single-tube purification.

1) Preparation: Prepare 0.5 M EDTA in advance.

2) Add 4 μL of 0.5 M EDTA to the Native Barcode ligation product, mix thoroughly, and proceed to the purification step.

3.4 Purification of Native Barcode Ligation Product

This step purifies the product from Step 3.2 or 3.3 using Hieff NGS™ DNA Selection Beads V2 (Cat# 12418).

- 1) Preparation: Remove the Hieff NGS™ DNA Selection Beads V2 and allow them to equilibrate at room temperature for 30 minutes. Prepare 80% ethanol.
 - 2) Vortex or invert the magnetic beads thoroughly to ensure homogeneity before use.
 - 3) For libraries >1 Kb, add 40 µL of Hieff NGS™ DNA Selection Beads V2 (0.4×, Beads:DNA = 0.4:1) to the product from Step 3.2 or 3.3. Mix by pipetting 10 times and incubate at room temperature for 10 minutes. For libraries <1 Kb, add 60 µL of beads (0.6×, Beads:DNA = 0.6:1).
 - 4) Briefly centrifuge the PCR tube and place it on a magnetic rack. After the solution clears (approx. 5 min), carefully remove the supernatant.
 - 5) Keep the PCR tube on the magnetic rack. Add 200 µL of freshly prepared 80% ethanol to wash the beads, incubate at room temperature for 30 sec, and carefully remove the supernatant.
 - 6) Repeat Step 5 for a total of two washes.
 - 7) Keep the PCR tube on the magnetic rack, open the lid, and air-dry the beads until all ethanol has evaporated (do not exceed 5 min).
 - 8) Remove the PCR tube from the magnetic rack. Add 33 µL of Nuclease-free ddH₂O or elution buffer (10 mM Tris-HCl, pH 8.0-8.5) to cover the beads. Pipette to mix and incubate at 37°C for 10 minutes. If the beads are dry or cracked, extend the incubation time appropriately.
- Note: If multiple samples will be pooled for Native Adapter ligation, you may reduce the volume of elution buffer appropriately.
- 9) Place the PCR tube on the magnetic rack and wait until the solution clears (approx. 5 min).
 - 10) Transfer 31 µL of the supernatant to a new PCR tube and take 1 µL for Qubit quantification.

3.5 Native Adapter Ligation

This step ligates the Native Adapter to the purified product from Step 3.4.

- 1) Thaw Rapid Ligation Reaction Buffer (5×), Rapid T4 DNA Ligase, and Native Adapter on ice. Mix by inversion, briefly centrifuge, and keep on ice. Prepare the reaction mixture according to the volumes specified in Table 5.

Table 5. Reaction System for Native Adapter Ligation

Name	Volume (µL)
Purified Product from Step 3.4	30
Rapid Ligation Reaction Buffer (5×)	20
Native Adapter (NA)*	5
Rapid T4 DNA Ligase	5
ddH ₂ O	Up to 100

[Note]: We recommend using the ONT designated product for Native Adapter (NA) (Nanopore #EXP-NBA114).

- 2) Mix gently by pipetting (do not vortex) and briefly centrifuge to collect the reaction mixture at the bottom of the tube.
- 3) Place the PCR tube in a thermal cycler and run the program set in Table 6:

Table 6. PCR Program for Native Adapter Ligation

Temperature	Time
Heat Lid 105°C	Off
20°C	20 min

3.6 Purification of Native Adapter Ligation Product

This step purifies the reaction product using Hieff NGS™ DNA Selection Beads V2 (Cat# 12418).

- 1) Preparation: Remove the Hieff NGS™ DNA Selection Beads V2 and allow them to equilibrate at room temperature for 30 minutes. Retrieve the Nanopore Associated nucleic acid wash buffer.
- 2) Vortex or invert the magnetic beads thoroughly to ensure homogeneity.
- 3) For libraries >1 Kb, add 40 µL of Hieff NGS™ DNA Selection Beads V2 (0.4×, Beads:DNA = 0.4:1) to the product from Step 3.5. Mix by pipetting 10 times and incubate at room temperature for 10 minutes. For libraries <1 Kb, add 60 µL of beads (0.6×, Beads:DNA = 0.6:1).
- 4) Briefly centrifuge the PCR tube and place it on a magnetic rack to separate the beads from the liquid. After the solution clears (approx. 5 min), carefully remove the supernatant.
- 5) Add 200 µL of Long Fragment Buffer (LFB)* or Short Fragment Buffer (SFB)** to resuspend the beads (Do not use 80% ethanol!!!). Mix by pipetting 10 times. Return the PCR tube to the magnetic rack, wait for the solution to clear, and remove the supernatant. (The volume of LFB or SFB can be adjusted based on your previous experimental conditions!)

***If enriching for DNA fragments of 3 kb or longer, we recommend using LFB (Nanopore #EXP-NBA114);**

****If aiming to retain a broader range of DNA fragment lengths, we recommend using SFB (Nanopore #EXP-NBA114).**

- 6) Repeat Step 5 for a total of two washes.
- 7) Place the PCR tube on the magnetic rack and remove the residual supernatant.
- 8) Remove the PCR tube from the magnetic rack. Add 18 µL of EB buffer or elution buffer (10 mM Tris-HCl, pH 8.0-8.5) to cover the beads. Pipette to mix and incubate at 37°C for 10 minutes. If the beads are dry or cracked, extend the incubation time appropriately.
- 9) Place the PCR tube on the magnetic rack to separate the beads from the liquid until the solution clears (approx. 5 min).
- 10) Transfer 16 µL of the supernatant to a new PCR tube, take 1 µL for Qubit quantification, and store the library at 4°C.

Common Issues and Troubleshooting

1. How to proceed with low-quality samples (DIN < 5)?

- ❖ Minimize sample degradation during extraction by reducing grinding time or mechanical shearing time.
- ❖ Extend the repair time or increase the input amount (Note: Input DNA must not exceed the upper limit recommended in the manual).

2. Low library yield?

- ❖ Inaccurate volume or inhomogeneous mixing of beads during the Native Barcode purification step. We recommend mixing the beads thoroughly before pipetting and ensuring accurate aspiration (avoid touching beads with the tip or creating air bubbles).
- ❖ Over-drying of beads during the purification step. We recommend proceeding to the next step when the bead surface appears matte.

- ❖ For long-fragment libraries, use wide-bore pipette tips for mixing to avoid DNA fragmentation.

3. Low effective data output after sequencing?

- ❖ Incomplete ligation or insufficient amount of Native Barcode or Native Adapter. We recommend extending the ligation time or increasing the amount used.
- ❖ Failure to use the Nanopore 配套 nucleic acid wash buffer during the purification of the Native Adapter ligation product, or incorrect selection of wash buffer. We recommend selecting the appropriate wash buffer based on the fragment size.
- ❖ Do not aspirate the magnetic beads when collecting the supernatant during the final purification step!

Note:

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[illegible]



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Together to make a healthier and brighter world