VAHTS TGS DNA Library Prep Kit for ONT

NB-54-0397

Contents

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#Cat: NB-54-0397 Size: 24rxns

1/ Product Description

VAHTS TGS DNA Library Prep Kit for ONT is based on ligation for library preparation. It is applicable for Nanopore sequencing, and single-sample or multiple-sample libraries can be prepared from 100 ng to 1.5 µg of gDNA and amplicons when combined with Native Barcode. This kit uses directed evolution mutant enzymes with high catalytic activity and carefully optimized buffer. It can directly perform Barcode Ligation without purification after DNA Damage Repair/End Preparation and the experiment process is streamlined. All the reagents provided in the kit have undergone rigorous quality control and functional testing to ensure the optimal stability and repeatability of library preparation.

2/Components

Components	NB-54-0397 (24 rxns)
End Prep & Repair Buffer	36 µl
End Prep & Repair Master Enzyme Mix	36 µl
Barcode Rapid Ligase Mix	96 µl
MP Adapter Fast Ligation Buffer	240 µl
MP Adapter Fast Ligase	120 µl

3/Storage

Store at -30 ~ -15°C and transport at ≤0°C.

4/Applications

It is applicable for preparing libraries for Nanopore sequencing, compatible with genomic DNA from various samples: animals, plants, microorganisms, etc. Recommended for:

- ◇ Whole genome sequencing
- ◇ tNGS targeted sequencing

5/Self-prepared Materials

DNA fragmentation: Shearing genomic DNA using the Covaris g-TUBE or other equivalent methods;
DNA quantification: Equalbit 1 × dsDNA HS Assay Kit (Neo Biotech # NB-54-0027);
3rd party materials: Native Barcoding Kit 24 V14);
Clean up magnetic beads: VAHTS DNA Clean Beads (Neo Biotech # NB-54-0060);
Other materials: Freshly prepared 80% ethanol, Nuclease-free ddH_2O; Low-adsorption EP tubes, PCR tubes; Magnetic rack, PCR instrument and Vortex Mixer, etc.

6/Notes

For research use only. Not for use in diagnostic procedures.

Due to various factors such as samples, protocols, equipments and operations, the parameters of the library preparation process may need to be adjusted according to the actual situation. In order to obtain high quality libraries, please make sure to carefully read the following notes.

6-1/DNA Quality

To ensure success, it is recommended to verify the fragment length and integrity of the Input DNA before library preparation (verification methods can include agarose gel electrophoresis or other equivalent methods). The amount, quality, and purity of the DNA must meet the requirements. Insufficient or excessive input, poor quality (e.g., highly fragmented, containing RNA or chemical contaminants) or low purity may all affect library yield.

6-2/Reaction System

This reaction system can be scaled up proportionally based on the actual needs.

7/Experiment Process

7.1/DNA Damage Repair and End Preparation

1. Thaw the End Prep & Repair Buffer and End Prep & Repair Master Enzyme Mix. Mix the reagents by inversion and place them on ice. Prepare the following solution in the PCR tube:

Components	Volume
Input DNA*	X µl
End Prep & Repair Buffer	1.5 µl
End Prep & Repair Master Enzyme Mix	1.5 µl
Nuclease-free ddH_2O	to 13.5 µl

*We recommend the amount of Input DNA range from 100 ng to 1.5 µg in this system.

2. Mix gently by pipetting and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
3. Perform the following program in a PCR instrument:

Components	Time
105°C (Preheat lid)	On
20°C	10 min*
65°C	10 min*
4°C	Hold

*If the reaction system is scaled up proportionally, the amount of Input DNA should be >1.5 µg, and the reaction time for each step can be extended to 15 min.

7.2/Barcode Ligation

1. Thaw the Native Barcode, mix by inversion and place them on ice.
2. Prepare the reaction solution as follows:

Components	Volume
Products from the previous step	13.5 µl
Native Barcode (NB01-24) *	2.5 µl
Barcode Rapid Ligase Mix	4 µl
Total	20 µl

* It is recommended to use the ONT product.

▲ Only use one Barcode per sample.

▲ Barcode Rapid Ligase Mix needs to be added last to prevent product or adapter self-ligation.

3. Mix gently by pipetting and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
4. Perform the following program in a PCR instrument:

Temperature	Time
105°C (Preheat lid)	On
20°C	20 min*
4°C	Hold

* If the reaction system is scaled up proportionally, the amount of Input DNA should be >1.5 µg, and the reaction time can be extended to 30 min.

7.3/Clean up

This step uses VAHTS DNA Clean Beads (Neo Biotech # NB-54-0060) to clean up the reaction product.

1. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.
2. Add 8 µl of VAHTS DNA Clean Beads (0.4 ×) to the 20 µl product after Barcode Ligation and slowly pipette 10 times to resuspend.
3. Incubate for 10 min at room temperature.
4. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully **discard the supernatant**.
5. Keep the tube on the magnetic rack and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec, carefully **discard the supernatant**.
6. Repeat step 5, wash twice in total.
7. Keep the tube always on the magnetic rack and air-dry the beads for 5 - 10 min.

▲ Do not over dry the magnetic beads, which may reduce the efficiency of DNA elution and affect the yield.

8. Remove the tube from the magnetic rack and add 35 µl of Nuclease-free ddH_2O or Elution Buffer (10 mM Tris-HCl, pH 8.0 - 8.5). Pipette gently to resuspend beads and incubate at 37°C for 10 min. If the beads are over-dry and cracked, please extend incubation time appropriately.
   ▲ Elution Buffer (10 mM Tris-HCl, pH 8.0 - 8.5) is beneficial for stability of the product. Incubation at 37°C is good for eluting completely. If pooling multiple samples for the Native Adapter Ligation, reducing the volume of Nuclease-free ddH_2O or Elution Buffer appropriately.
9. Place the tube on the magnetic rack until the supernatant is clear (~ 5 min).
10. Transfer 31 µl of supernatant into a new PCR tube and use 1 µl for Qubit quantification.
    ▲ This step can be stopped and products can be stored at -20°C for long-term preservation.

7-4/Native Adapter Ligation

1. Thaw the MP Adapter Fast Ligation Buffer and mix by inversion. Prepare the following solution in the PCR tube:

Components	Volume
Products from the previous step	30 µl
Native Adapter*	5 µl
MP Adapter Fast Ligation Buffer	10 µl
MP Adapter Fast Ligase	5 µl
Total	50 µl

* It is recommended to use the ONT product

▲MP Adapter Fast Ligase needs to be added last to prevent product or adapter self-ligation. Nanopore provide the amount of Native Adapter is sufficient. If the reaction system is scaled up proportionally, it is recommended that the amount of Native Adapter remain unchanged and increase the volume of other components in proportion.

2. Mix gently by pipetting and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
3. Perform the following program in a PCR instrument:

Temperature	Time
105°C (Preheat lid)	On
20°C	20 min*
4°C	Hold

* If the reaction system is scaled up proportionally, the amount of Input DNA should be >1.5 µg, and the reaction time can be extended to 30 min.

7-5/ Clean up

1. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.
2. Add 20 µl of VAHTS DNA Clean Beads (0.4 ×) to the 50 µl Native Adapter Ligation product and slowly pipette 10 times to resuspend.
3. Incubate for 10 min at room temperature.
4. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully **discard the supernatant**.

5. Remove the tube from the magnetic rack, add either 125 µl Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB). Pipetting 10 times to resuspent, then return the tube to the magnetic rack until the supernatant is clear. Carefully **discard the supernatant**.
   ▲ To enrich for DNA fragments of 3 kb or longer, LFB is recommended.
   ▲ To retain DNA fragments of all sizes, SFB is recommended.

6. Repeat step 5, wash twice in total.
7. Keep the tube always on the magnetic rack and air-dry the beads for 5 - 10 min.
   ▲ Do not over dry the magnetic beads, which may reduce the efficiency of DNA elution and affect the yield.

8. Remove the tube from the magnetic rack and add 17 µl of Nuclease-free ddH_2O or Elution Buffer (10 mM Tris-HCl, pH 8.0 - 8.5). Pipette gently to resuspend beads and incubate at 37°C for 10 min. If the beads are over-dry and cracked, please extend incubation time appropriately.
   ▲ Elution Buffer (10 mM Tris-HCl, pH 8.0 - 8.5) is beneficial for stability of the product. Incubation at 37°C is good for eluting completely.

9. Place the tube on the magnetic rack until the supernatant is clear (~ 5 min).
10. Transfer 15 µl of supernatant into a new PCR tube and continue to next step.

8/FAQ & Troubleshooting

◇ The elution efficiency is lower

1. There may be absorption errors due to the small volume of magnetic beads. After Barcode Ligation, it is recommended to add Nuclease-free ddH_2O to make up to 50 µl. Pipetting 20 µl of VAHTS DNA Clean Beads (0.4 ×) to clean up.
2. It may be caused by the aggregation of magnetic beads in the incubation process, and the products can be incubated on a vertical Rotating Mixer at 20 - 30 rpm.

◇ Amount of sequencing data is lower

1. DNA Damage Repair time may be insufficient. It is recommended to extend the time appropriately.
2. There may be the low ligation efficiency. It is recommended to extend the ligation time or increase the Native Barcodes concentration appropriately.

◇ N50 and N90 are shorter

1. Input DNA may contain the short fragments or fragmented DNA is too short. It is recommended to perform the size selection before library preparation.
2. Vortexing strongly may result in long fragments break and increasing short fragments during library preparation. It is recommended to mix by pipetting 10 times