VAHTS TGS DNA Library Prep Kit for ONT

TS201

Vazyme

Instruction for Use

Version 24.2

Vazyme

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

VAHTS TGS DNA Library Prep Kit for ONT is based on ligation for library preparation. It is compatible with Nanopore sequencing and supports library preparation from either genomic DNA (gDNA) or amplicon DNA, accommodating both single-sample and multiplexed workflows. 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.

02/Components

Components	TS201-01 (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

03/Storage

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

04/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

05/Self-prepared Materials

DNA fragmentation:

gDNA Shearing Tube (Vazyme #TDS01501);

DNA quantification:

Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121);

Barcode for ONT:

VAHTS TGS DNA BD Adapters Set 1 for ONT (Vazyme #TA20101);

VAHTS TGS DNA BD Adapters Set 1 - Set 4 for ONT (Plate) (Vazyme #TAB20105);

3rd party materials:

Ligation Sequencing Kit V14 (Nanopore #SQK-LSK114);

Native Barcoding Kit 24 V14 (Nanopore #SQK-NBD114.24);

Native Barcoding Kit 96 V14 (Nanopore #SQK-NBD114.96);

Clean up magnetic beads:
VAHTS DNA Clean Beads (Vazyme #N411);
Other materials:
Freshly prepared 80% ethanol, Nuclease-free ddH_2O, Low-adsorption
EP tubes, PCR tubes; Magnetic rack, PCR instrument and Vortex Mixer, etc.

06/Notes

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.

06-1/QC Input DNA

To ensure experimental success, it is recommended to check the fragmented length and integrity of Input DNA before library preparation (verification methods include agarose gel electrophoresis or other equivalent methods). It is important that the Input DNA meets the quantify and quality requirements. Using too little or too much DNA, or DNA of poor quality (e.g., highly fragmented or containing RNA or chemical contaminants) or low purity can affect library yield.

06-2/Reaction System

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

07/Workflow

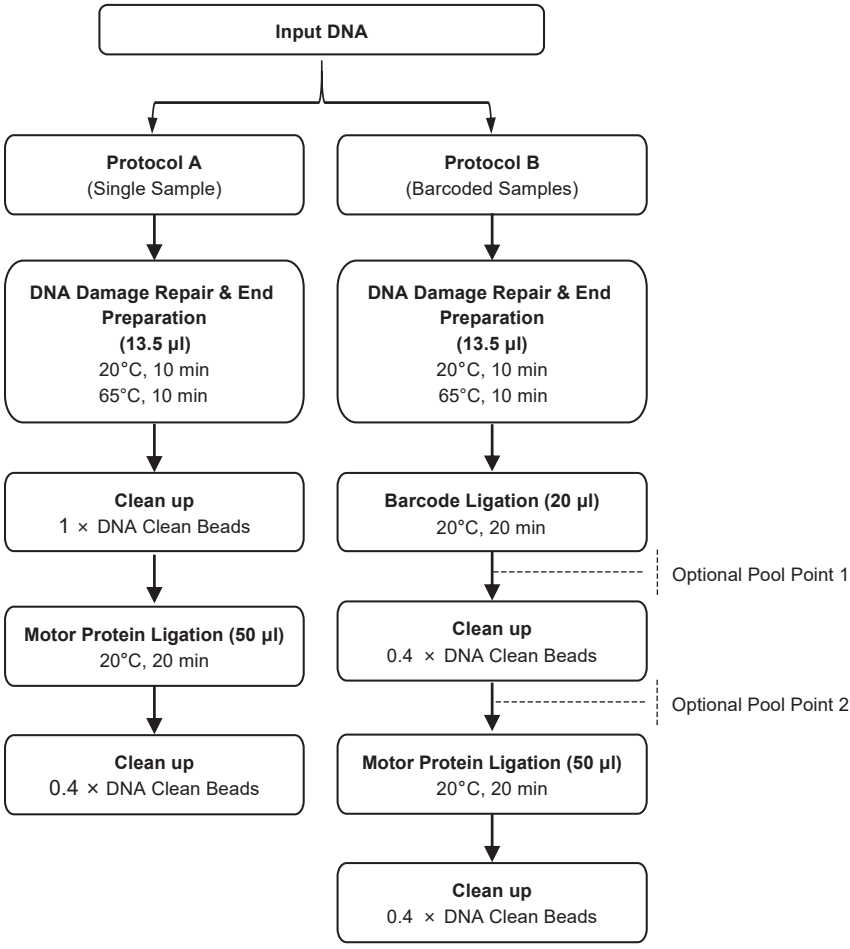

Fig 1. Workflow of VAHTS TGS DNA Library Prep Kit for ONT

08/Experiment Process

◇ Protocol A: Single Sample for Sequencing

This protocol describes how to carry out sequencing of single DNA sample using Ligation Sequencing Kit V14 (Nanopore #SQK-LSK114).

08-1/DNA Damage Repair & 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

* For optimal performance, 1 μg of high molecular weight gDNA is recommended per reaction.

2. Mix by flicking the tube 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	10 min*
65°C	10 min*
4°C	Hold

* If the reaction 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.

08-2/Clean up

This step uses VAHTS DNA Clean Beads (Vazyme #N411) 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 13.5 μl of VAHTS DNA Clean Beads (1 ×) to the 13.5 μl product after DNA Damage Repair & End Preparation and mix by flicking the tube.
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. Mix by flicking the tube to resuspend beads and incubate for 10 min at 37°C. Every 2 min, agitate the sample by gently flicking for 10 sec to encourage DNA elution. If the beads are over-dry and cracked, please extend incubation time appropriately.
   ▲ 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 31 µl of supernatant into a new PCR tube and use 1 µl for Qubit quantification.
    ▲ Take forward the repaired and end-prepped DNA into the adapter ligation step. However, at this point it is also possible to store the sample at 4°C overnight.

08-3/Motor Protein 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	
Ligation 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 Nanopore #SQK -LSK114 for Protocol A.

▲ MP Adapter Fast Ligase needs to be added last to prevent product or adapter self-ligation. Nanopore provide the amount of Ligation Adapter (Nanopore #SQK-LSK114) is sufficient. If the reaction system is scaled up proportionally, it is recommended that the amount of Ligation Adapter remain unchanged and increase the volume of other components in proportion.

2. Mix by flicking the tube 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

08-4/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 Motor Protein Ligation product and mix by flicking the tube.
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 125 μl Short Fragment Buffer (SFB). Mix by flicking the tube, 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 Elution Buffer (Nanopore #SQK-LSK114). Mix by flicking the tube and incubate at 37°C for 10 min. If the beads are over-dry and cracked, please extend incubation time appropriately.
   ▲ 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 sequence.

◇ Protocol B: Barcoded Samples for Sequencing

This protocol describes how to carry out sequencing of barcoded samples using Native Barcoding Kit (Nanopore #SQK-NBD114.24, Nanopore #SQK-NBD114.96).

08-1/DNA Damage Repair & 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 to input 200 fmol (130 ng for 1 kb amplicons) DNA per sample to be barcoded. If using >4 barcodes, recommended to input 400 ng gDNA per sample; if using ≤4 barcodes, recommended to input 1 μg gDNA per sample.

2. Mix by flicking the tube 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	10 min
65°C	10 min
4°C	Hold

08-2/Barcode Ligation

1. Thaw the TGS DNA Adapters for ONT, 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
TGS DNA Adapters for ONT*	2.5 μl
Barcode Rapid Ligase Mix	4 μl
Total	20 μl

* It is recommended to use the Vazyme #TA20101 or Vazyme #TAB20105-01. But you can also use the ONT product (Nanopore #SQK-NBD114.24, Nanopore #SQK-NBD114.96).

▲ Only use one Barcode per sample.

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

3. Mix by flicking the tube 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

▲ You can choose to perform the **08-3/Clean up** directly or pool the samples.

Optional Pool Point 1: This protocol is for pooling the barcoded samples before clean up.

1. Add the following volume of EDTA to each well and mix thoroughly by pipetting and centrifuge briefly.

Components	Volume
Products from the previous step	20 µl
EDTA*	4 µl

* It is recommended to use the EDTA (blue cap) from Nanopore #SQK -NBD114.24.

2. Pool all the barcodes samples in the 1.5 ml tube.

	Volume per sample	For 6 samples	For 12 samples	For 24 samples
Total volume for preps using blue cap EDTA	24 µl	144 µl	288 µl	576 µl

08-3/Clean up

This step uses VAHTS DNA Clean Beads (Vazyme #N411) 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 **X µl** of VAHTS DNA Clean Beads (0.4 ×) to the product after Barcode Ligation or Optional Pooling Point 1. Mix by flicking the tube.
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.Mix by flicking the tube and incubate at 37°C for 10 min. If the beads are over-dry and cracked, please extend incubation time appropriately.
   ▲ 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 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.
    ▲ Take forward the barcoded DNA library to the adapter ligation and clean up step. However, you may store the sample at 4°C overnight.

Optional Pool Point 2：This point can be for pooling the barcoded samples after clean up.

▲ It is recommended to pool samples using equal Input DNA (ng) to ensure consistency. If the total volume of pooled sample exceeds 30 µl, the total volume of Native Adapter Ligation reaction solution can be proportionally scaled up. However, the volume of the Native Adapter should remain constant at 5 µl.

08-4/Motor Protein 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 Nanopore #SQK-NBD114.24 or Nanopore #SQK-NBD114.96 for Protocol B.

▲ MP Adapter Fast Ligase needs to be added last to prevent product or adapter self-ligation. Nanopore provide the amount of Native Adapter (Nanopore #SQK-NBD114.24, Nanopore #SQK-NBD114.96) 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 by flicking the tube 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

08-5/Clean up

1. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.
2. Add **X μl** of VAHTS DNA Clean Beads (0.4 ×) to the product after Motor Protein Ligation. Mix by flicking the tube.
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 125 μl 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 Elution Buffer (Nanopore #SQK-NBD114.24, Nanopore #SQK-NBD114.96). Mix by flicking the tube and incubate at 37°C for 10 min. If the beads are over-dry and cracked, please extend incubation time appropriately.
   ▲ 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 sequence.

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