

VAHTS TGS DNA Library Prep Kit for ONT V2

TS202



Instruction for Use
Version 25.1

Contents

01/Product Description	02
02/Components	02
03/Storage	02
04/Applications	02
05/Self-prepared Materials	03
06/Notes	03
07/Mechanism & Workflow	04
08/Experiment Process	05
Protocol A: Single-Sample Sequencing	05
Protocol B: Barcoded-Sample Sequencing	08
09/FAQ & Troubleshooting	12

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

01/Product Description

VAHTS TGS DNA Library Prep Kit for ONT V2 is a ligation-based library preparation kit designed for the Nanopore sequencing platform. When used with compatible barcodes, it supports both single-sample and multiplex library preparation from genomic DNA (gDNA) and amplicons. This upgraded kit features a specifically optimized combination of high-activity mutant enzymes, paired with a finely tuned buffer system, delivering higher ligation efficiency and more robust library preparation performance. All reagents provided in the kit have undergone rigorous quality control and functional testing to ensure optimal stability and reproducibility of library preparation.

02/Components

Components		TS202-01 (24 rxns)
	End Prep Buffer	84 µl
	DNA Repair Buffer	84 µl
	End Prep Enzyme Mix	72 µl
	DNA Repair Mix	48 µl
	Barcode Rapid Ligase Mix	600 µl
	MP Adapter Ligation Buffer	240 µl
	MP Adapter Ligase	120 µl

▲ **DNA Damage Repair & End Preparation:** End Prep Buffer, DNA Repair Buffer, End Prep Enzyme Mix, and DNA Repair Mix.

▲ **Barcode Ligation:** Barcode Rapid Ligase Mix.

▲ **Motor Protein Ligation:** MP Adapter Ligation Buffer and MP Adapter Ligase.

03/Storage

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

04/Applications

It is applicable to library preparation for the Nanopore sequencing platform and is compatible with genomic DNA (gDNA) derived from animal, plant, and microbial sources. Recommended applications:

- ◇ Whole-genome sequencing
- ◇ Targeted sequencing

05/Self-prepared Materials

DNA Fragmentation: gDNA Shearing Tube (Vazyme #TDS01501);

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

Barcode Adapter:

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

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

▲ #TA20101 is supplied in tube format and contains 24 barcodes. #TAB20105 is supplied in plate format and contains 96 barcodes, and is compatible with high-throughput liquid handling workstation (Vazyme #VNL-96P).

3rd party materials (including motor protein adapters and nucleic acid purification buffers):

Ligation Sequencing Kit V14 (Nanopore #SQK-LSK114)

or Native Barcoding Auxiliary Kit V14 (Nanopore #EXP-NBA114);

▲ Motor protein adapters included in #SQK-LSK114 are designed for A/T ligation and single-sample library preparation, whereas those in #EXP-NBA114 are intended for sticky-end ligation and multiplexed library preparation, as barcode ligation generates sticky-end products.

▲ If #EXP-NBA114 is used, Elution Buffer (EB) is required for the elution step in purification of motor protein adapter-ligated products. The EB provided in Sequencing Auxiliary Vials V14 (Nanopore #EXP-AUX003) is recommended.

Clean Beads: VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880);

Other Materials: freshly prepared 80% ethanol, Nuclease-free ddH₂O, low-absorption EP tubes, PCR tubes, magnetic rack, PCR instrument, vortex mixer, etc.

06/Notes

1. It is recommended to verify the fragment length and integrity of input DNA prior to library preparation. Acceptable quantification methods include agarose gel electrophoresis, capillary electrophoresis, pulsed-field gel electrophoresis, and other equivalent techniques. For long-read sequencing applications, the starting material requires high-molecular-weight (HMW) DNA fragments; full-length genomic DNA (gDNA) is recommended for library preparation.
2. Input DNA amount, quality, and purity must meet the specified requirements. Insufficient or excessive DNA input, poor DNA quality (e.g., severe fragmentation or contamination with RNA or chemical impurities), or low purity may reduce library yield. Sample purity can be evaluated by measuring A260/280 and A260/230 ratio using a NanoDrop spectrophotometer. The recommended ratios are as follows:

$$A_{260}/A_{280} = 1.8$$

$$A_{260}/A_{230} = 2.0 - 2.2$$
3. Approximate Library DNA molar amount (fmol):

$$\text{Library DNA (fmol)} \approx (\text{Library DNA (ng)} \times 1,000) / (0.66 \times \text{Average library length (kb)})$$

07/Mechanism & Workflow

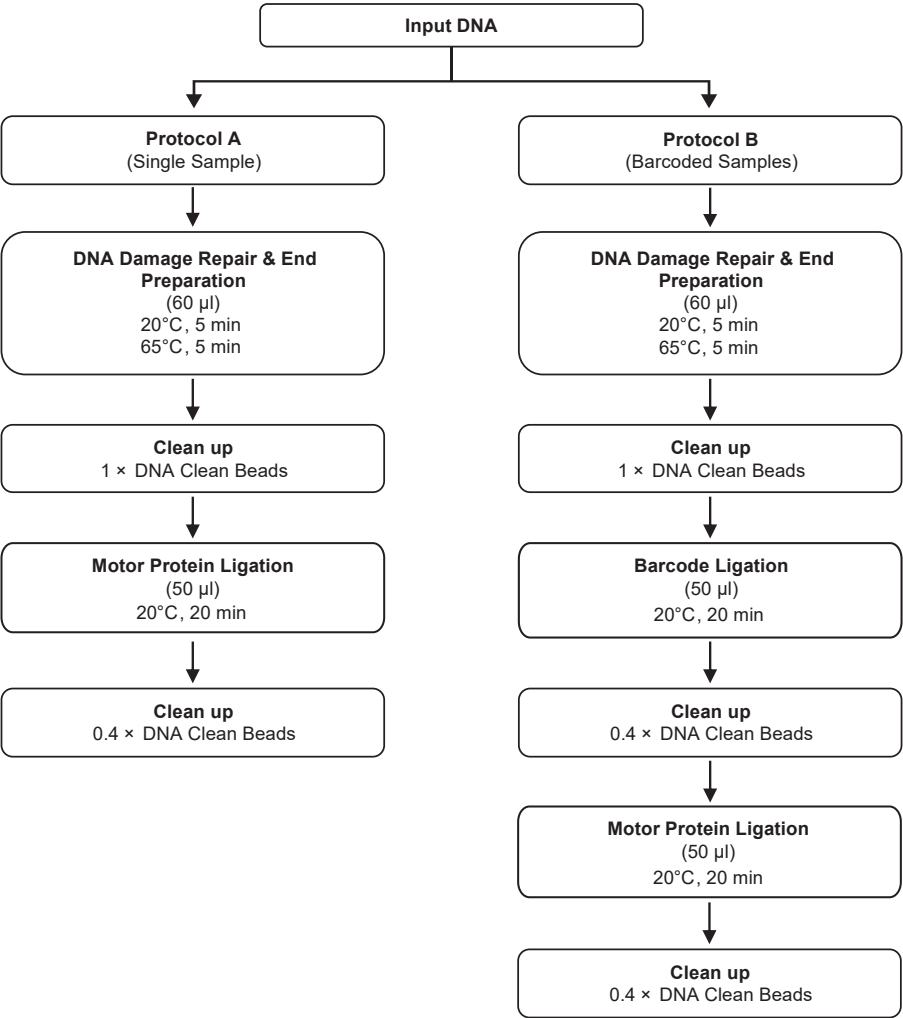


Fig 1. Workflow of VAHTS TGS DNA Library Prep Kit for ONT V2

08/Experiment Process

Protocol A: Single-Sample Sequencing

For single-sample sequencing on a single flow cell, use the Ligation Sequencing Kit V14 (Nanopore #SQK-LSK114) and prepare the library according to Protocol A.

08-1/DNA Damage Repair & End Preparation

This step is performed to repair DNA damage, generate blunt ends, phosphorylate the 5' ends, and add a dA tail to the 3' ends of the DNA fragments.

1. Thaw End Prep Buffer and DNA Repair Buffer and mix well by inversion. Mix End Prep Enzyme Mix and DNA Repair Mix well by inversion. Keep all reagents on ice. Prepare the reaction solution in a sterile PCR tube on ice as follows:

Components	Volume	
Input DNA*	x μ l	
End Prep Buffer	3.5 μ l	
DNA Repair Buffer	3.5 μ l	
End Prep Enzyme Mix	3 μ l	
DNA Repair Mix	2 μ l	
ddH ₂ O	To 60 μ l	

* Recommended Input gDNA amount under the current setup: 1 - 2 μ g.

* Components for this step can be purchased separately using VAHTS Repair Module for ONT V2 (Vazyme #TN2025).

2. Mix by flicking the tube and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
3. Place the tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid105°C	On
20°C	5 min
65°C	5 min
4°C	Hold

08-2/Clean up

VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880) can be used for this step.

1. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.
2. Add 60 μ l of the resuspended beads (1 $\times$) to 60 μ l of the product from the previous step, and mix by flicking the tube.
3. Incubate at room temperature for 5 min.
4. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min). Keep the tube on the magnet and carefully pipette off 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 after the solution is clear (~ 5 min).
6. Repeat step 5 (wash twice in total).
7. Keep the PCR tube on the magnetic rack, and air-dry the beads until there is no residual ethanol.
 - ▲ Avoid over-drying as it can reduce the DNA recovery efficiency.
 - ▲ For beads drying time, please refer to the FAQ.
8. Remove the tube from the magnetic rack and add 32 µl of Nuclease-free ddH₂O. Gently tap the tube to mix, and incubate for 5 min at room temperature. Extend the incubation time as needed when the beads are dry or cracked.
9. Place the tube on the magnetic rack until the supernatant is clear (~ 5 min).
10. Transfer 30 µl of the supernatant to a new PCR tube.
 - ▲ The product can be temporarily stored at 4°C; however, long-term storage is not recommended and should not exceed 3 h to ensure optimal library preparation performance.

08-3/Motor Protein Ligation

This step is performed to ligate the Motor Protein Adapter to the repaired products.

1. Thaw MP Adapter Ligation Buffer and mix by inversion. Mix MP Adapter Ligase well by inversion. Keep them on ice for later use. Prepare the reaction solution in a PCR tube on ice as follows:

Components	Volume	
Purified end-repaired product	30 µl	
Ligation Adapter (LA)*	5 µl	
MP Adapter Ligation Buffer	10 µl	■
MP Adapter Ligase	5 µl	■
Total	50 µl	

* Nanopore #SQK-LSK114 is recommended as the Ligation Adapter (LA).

* Components for this step can be purchased separately using VAHTS MP Ligation Module for ONT V2 (Vazyme #TN2027).

▲ To prevent self-ligation, add the reagents in the following order: Ligation Adapter (LA) first, followed by MP Adapter Ligation Buffer, and finally MP Adapter Ligase.

2. Mix by flicking the tube and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
3. Place the tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
20°C	20 min
4°C	Hold

08-4/Clean up

VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880) can be used for this step.

1. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.
2. Add 20 μ l of VAHTS DNA Clean Beads (0.4 $\times$) to the 50 μ l Motor Protein Ligation product and mix by flicking the tube.
3. Incubate at room temperature for 10 min.
4. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully.
5. Remove the tube from the magnetic rack, add either 125 μ l Long Fragment Buffer (LFB) or 125 μ l Short Fragment Buffer (SFB). **Do not use 80% ethanol.** Mix by flicking the tube, place the tube on the magnetic rack, then carefully discard the supernatant after the solution is clear (~ 5 min).
 - ▲ 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.
 - ▲ Do not over dry the magnetic beads, which may reduce the efficiency of DNA elution and affect the yield.
 - ▲ For bead drying time, please refer to the FAQ.
8. Remove the tube from the magnetic rack and add 15 μ l 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.
 - ▲ The EB provided in Nanopore #SQK-LSK114 is recommended.
 - ▲ Incubation at 37°C is recommended to ensure thorough elution.
 - ▲ Gently tap the tube every 2 min to facilitate elution during incubation.
9. Place the tube on the magnetic rack until the solution is clear (~ 5 min).
10. Transfer 13 μ l of the supernatant to a new PCR tube for the subsequent reaction.
11. Refer to the table below to prepare the appropriate library amount based on library length. Library loading volumes may vary between different sequencing platforms and can be adjusted accordingly.

Fragment library length	Flow cell loading amount
Short fragments (<1 kb)	100 fmol
Medium fragments (1 - 10 kb)	35 - 50 fmol
Long fragments (>10 kb)	300 ng

▲ The product can be temporarily stored at 4°C; however, long-term storage is not recommended and should not exceed 5 days to ensure optimal sequencing results.

▲ Approximate input DNA molar amount (fmol):

$$\text{Library DNA (fmol)} \approx (\text{Library DNA (ng)} \times 1,000) / (0.66 \times \text{Average library length (kb)})$$

Protocol B: Barcoded-Sample Sequencing

For multiplexed sequencing of multiple samples on a single flow cell, use the Native Barcoding Auxiliary Kit V14 (Nanopore #EXP-NBA114) and Sequencing Auxiliary Vials V14 (Nanopore #EXP-AUX003), in combination with Vazyme #TA20101 (tube format, 24 Barcodes) or Vazyme #TAB20105 (plate format, 96 Barcodes), and prepare the library according to Protocol B.

▲ For multiplexed library preparation, it is recommended to use samples derived from the same species or samples with similar DNA fragment length distributions to ensure a more uniform sequencing output across all barcoded samples.

08-1/DNA Damage Repair & End Preparation

This step is performed to repair DNA damage, generate blunt ends, phosphorylate the 5' ends, and add a dA tail to the 3' ends of the DNA fragments.

- 1. Thaw End Prep Buffer and DNA Repair Buffer and mix well by inversion. Mix End Prep Enzyme Mix and DNA Repair Mix well by inversion. Keep the reagents on ice. Prepare the reaction solution in a sterile PCR tube on ice as follows:

Components	Volume
Input DNA*	x µl
End Prep Buffer	3.5 µl
DNA Repair Buffer	3.5 µl
End Prep Enzyme Mix	3 µl
DNA Repair Mix	2 µl
ddH ₂ O	To 60 µl

- * Recommended Input DNA amount under the current setup:
Genomic DNA (gDNA): For >4 barcodes: 400 ng of input DNA per sample;
For ≤4 barcodes: 1 µg per sample.
PCR-amplified products: ≥200 fmol per sample.
- * Components for this step can be purchased separately using VAHTS Repair Module for ONT V2 (Vazyme #TN2025).

- 2. Mix by flicking the tube and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
- 3. Place the tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
20°C	5 min
65°C	5 min
4°C	Hold

08-2/Clean up

VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880) can be used for this step.

1. Equilibrate the beads to room temperature and mix well by vortexing.
2. Add 60 µl of the resuspended beads (1 ×) to 60 µl of the product from the previous step, and gently tap the tube to mix.
3. Incubate at room temperature for 5 min.
4. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min). Keep the tube on the magnet and carefully pipette off 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. Keep the tube on the magnet and carefully pipette off the supernatant.
6. Repeat step 5 (wash twice in total).
7. Keep the PCR tube on the magnetic rack, and air-dry the beads until there is no residual ethanol.
 ▲ Avoid over-drying as it can reduce the DNA recovery efficiency.
 ▲ For beads drying time, please refer to the FAQ.
8. Remove the tube from the magnetic rack and add 25 µl of Nuclease-free ddH₂O to resuspend the beads. Gently tap the tube to mix, and incubate for 5 min at room temperature. Extend the incubation time as needed when the beads are dry or cracked.
9. Place the tube on the magnetic rack until the solution is clear (~ 5 min).
10. Transfer 22.5 µl of the supernatant to a new PCR tube.
 ▲ The product can be temporarily stored at 4°C; however, long-term storage is not recommended and should not exceed 3 h to ensure optimal library preparation performance.

08-3/Barcode Ligation

This step is performed to ligate the Barcode to both ends of the repaired products.

1. Mix Barcode Rapid Ligase Mix well by inversion and keep it on ice for later use. Prepare the reaction solution in a sterile PCR tube on ice as follows:

Components	Volume
Purified end-repaired product	22.5 µl
Barcode Adapter*	2.5 µl
Barcode Rapid Ligase Mix	25 µl
Total	50 µl

* Vazyme #TA20101 (tube format, 24 barcodes) or Vazyme #TAB20105 (plate format, 96 barcodes) is recommended as the Barcode Adapter.

* Barcode Rapid Ligase Mix for this step can be purchased separately using VAHTS BD Ligation Module for ONT V2 (Vazyme #TN2026).

▲ Add the Barcode Adapter first, followed by the Barcode Rapid Ligase Mix, to prevent self-ligation.

- Mix by flicking the tube and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
- Place the tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
20°C	20 min
4°C	Hold

08-4/Clean up

VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880) can be used for this step.

- Equilibrate the beads to room temperature and mix well by vortexing.
- Add 20 µl of the resuspended beads (0.4 ×) to 50 µl of the product from the previous step, and gently tap the tube to mix.
- Incubate at room temperature for 10 min.
- Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min). Keep the tube on the magnet and carefully pipette off the supernatant.
- 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. Keep the tube on the magnet and carefully pipette off the supernatant.
- Repeat step 5 (wash twice in total).
- Keep the PCR tube on the magnetic rack, and air-dry the beads until there is no residual ethanol.
 - ▲ Avoid over-drying as it can reduce the DNA recovery efficiency.
 - ▲ For beads drying time, please refer to the FAQ.
- Remove the tube from the magnetic rack and add 33 µl of Nuclease-free ddH₂O to resuspend the beads. Gently tap the tube to mix, and incubate at 37°C for 10 min.
 - ▲ For motor protein adapter ligation of pooled samples, where the input volume of barcode ligation products is limited, the elution volume of Nuclease-free ddH₂O per sample may be appropriately reduced (≥12 µl to ensure elution efficiency) to maintain nucleic acid utilization.
 - ▲ Gently tap the tube every 2 min to facilitate elution during incubation.
- Place the tube on the magnetic rack until the solution is clear (~ 5 min).
- Transfer 31 µl of the supernatant to a new PCR tube and use 1 µl for Qubit quantification.
 - ▲ When pooling multiple samples with similar DNA fragment sizes, equimolar pooling is recommended. Adjust the total amount of barcode-ligated products to 500 ng - 1.2 µg for subsequent motor protein adapter ligation.
 - ▲ The purified product can be used directly for downstream motor protein adapter ligation or stored at 4°C or -20°C overnight.

08-5/Motor Protein Ligation

This step is performed to ligate the motor protein adapter to the barcoded DNA.

1. Thaw MP Adapter Ligation Buffer and mix well by inversion. Mix MP Adapter Ligase well by inversion. Keep them on ice for later use. Prepare the reaction solution in a PCR tube on ice as follows:

Components	Volume
Purified, barcode adapter-ligated DNA	30 μ l
Native Adapter (NA)*	5 μ l
MP Adapter Ligation Buffer	10 μ l 
MP Adapter Ligase	5 μ l 
Total	50 μ l

* Nanopore #EXP-NBA114 is recommended as the Native Adapter (NA).

* Components for this step can be purchased separately using VAHTS MP Ligation Module for ONT V2 (Vazyme #TN2027).

- ▲ Add the Native Adapter (NA) first, followed by the MP Adapter Ligation Buffer, and finally add the MP Adapter Ligase, to prevent self-ligation. For scaled-up reactions, keep the Native Adapter (NA) amount unchanged and scale the remaining components accordingly.
2. Mix by flicking the tube and avoid vortexing, briefly centrifuge to collect the solution at the bottom of the tube.
3. Place the tube into the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
20°C	20 min
4°C	Hold

08-6/Clean up

VAHTS DNA Clean Beads (Vazyme #N411) or AMPure XP Beads (Beckman #A63880) can be used for this step.

1. Equilibrate the beads to room temperature and mix well by vortexing.
2. Add 20 μ l of the resuspended beads ($0.4 \times$) to 50 μ l of the product from the previous step, and gently tap the tube to mix.
 - ▲ For scaled-up reactions, adjust the amount of $0.4 \times$ beads accordingly.
3. Incubate at room temperature for 10 min.
4. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min). Keep the tube on the magnet and carefully pipette off the supernatant.
5. Remove the tube from the magnetic rack and add 125 μ l of Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB) to resuspend the beads. **Do not use 80% ethanol.** Place the tube on a magnetic rack until the solution is clear (~ 5 min). Keep the tube on the magnet and carefully pipette off 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 PCR tube on the magnetic rack, and air-dry the beads.
 - ▲ For beads drying time, please refer to the FAQ.
8. Remove the tube from the magnetic rack and add 15 µl of Elution Buffer (EB) to resuspend the beads. Gently tap the tube to mix, and incubate at 37°C for 10 min.
 - ▲ The EB provided in Nanopore #SQK-LSK114 or #EXP-AUX003 is recommended.
 - ▲ Gently tap the tube every 2 min to facilitate elution during incubation.
9. Place the tube on the magnetic rack until the solution is clear (~ 5 min).
10. Transfer 13 µl of the supernatant to a new PCR tube for the subsequent reaction.
11. Refer to the table below to prepare the appropriate library amount based on library size. Library loading volumes may vary between different sequencing platforms and can be adjusted accordingly.

Library size	Library loading amount
Short fragments (<1 kb)	100 fmol
Medium fragments (1 - 10 kb)	35 - 50 fmol
Long fragments (>10 kb)	300 ng

▲ The product can be temporarily stored at 4°C; however, long-term storage is not recommended and should not exceed 5 days to ensure optimal sequencing results.

▲ Approximate Library DNA molar amount (fmol):

$$\text{Library DNA (fmol)} \approx (\text{Library DNA (ng)} \times 1,000) / (0.66 \times \text{Average library length (kb)})$$

09/FAQ & Troubleshooting

◇ Low elution yield

Possible causes and solutions:

1. Beads aggregation during incubation. It is recommended to incubate on a vertical mixer at 20 - 30 rpm. **Do not use a vortex mixer.**
2. Over-dried beads during purification. It is recommended to extend the incubation time as needed.

◇ Short N50 and N90

Possible causes and solutions:

1. Excessive small fragments in the input template or insufficient fragment length during shearing. It is recommended to perform size selection prior to library preparation.
2. Excessive agitation during library preparation. It is recommended to mix by gently by tapping the tube instead of vortexing during library preparation.

◇ **Smaller reaction volume for sample pooling**

When preparing libraries from pooled samples using a smaller reaction volume:

1. If each sample has a relatively high nucleic acid concentration and the required sequencing output is low, the reaction can be scaled down to a 1/4-scale. Refer to the [Small-Volume Protocol](http://www.vazymeglobal.com) available on www.vazymeglobal.com.
2. If each sample has a low nucleic acid concentration, see [04-1/DNA Damage Repair & End Preparation](#) in the [Small-Volume Protocol](#) for the recommended input volume. However, if the resulting input mass does not meet the required total input mass, Protocol B is recommended.
3. For multiplexed library preparation, Protocol B is recommended if higher data output per sample is required.

◇ **Beads drying time**

1. AMPure XP Beads: Air-dry for 30 sec at room temperature.
2. VAHTS DNA Clean Beads: Air-dry for 2 - 5 min at room temperature
3. The beads are sufficiently dry once the pellet loses its shine. Avoid over-drying, as this will negatively impact DNA recovery.
4. Drying time may vary depending on the volume of beads used, as well as ambient temperature and humidity conditions.



Vazyme Biotech Co.,Ltd.

www.vazyme.com

400-600-9335 (China) +86 400-168-5000 (Global)

support@vazyme.com