VAHTS Universal Circularization Kit for MGI

NM202

Instruction for Use

Version 24.1

Vazyme

Contents

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

01/Product Description

VAHTS Universal Circularization Kit for MGI is a kit optimized for MGI high-throughput sequencing platforms. This kit is used to prepare a single-stranded circular DNA library for MGI sequencers from libraries with single barcode or dual barcode. All reagents included in the kit have undergone strict quality control and functional validation, ensuring the stability and reproducibility of library preparation.

02/Components

Components	NM202-01 (16 rxns)	NM202-02 (48 rxns)
Splint Oligo	128 µl	384 µl
Rapid Splint Buffer	96 µl	288 µl
Rapid DNA Ligase	96 µl	288 µl
Digestion Buffer	112 µl	336 µl
Digestion Enzyme	48 µl	144 µl

03/Storage

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

04/Applications

This kit is suitable for preparing a single-stranded circular DNA library for MGI sequencers from libraries with single barcode or dual barcode, compatible with various sample types: genomic DNA, cfDNA, FFPE DNA and Amplicons, etc. It can be compatible with various sample types: genomic DNA, cfDNA, FFPE DNA and Amplicons, etc.

05/Self-prepared Materials

◇ DNA purification magnetic beads: VAHTS DNA Clean Beads (Vazyme #N411);

◇ Library quantification: Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121), Qubit ssDNA Assay Kit (Invitrogen #Q10212);

◇ Other materials: Freshly prepared 80% ethanol, Nuclease-free ddH_2O; PCR tubes, Low-adsorption EP tubes; Magnetic rack, PCR instrument and Qubit Fluorometer, etc.

06/Notes

06-1/Quality Control of Circularization Library

1. It is recommended to use Qubit ssDNA Assay Kit (Invitrogen #Q10212) for quantifying the purified product after circularization.

2. For MGI high-throughput sequencing platform, the yield of the single-stranded circular DNA library after purification should not be less than 80 fmol. Refer to the following formula:

Conversion circular ssDNA fmol and ng:

Mass corresponding to 80 fmol circular ssDNA (ng) = 0.08 × Main fragment size of DNA (bp) × 0.33

06-2/Circularization Efficiency Calculation

1. The circularization efficiency of this kit for universal PCR library is calculated as follows:

   Circularization efficiency = Total amount of single-stranded circular DNA library/(total amount of input linear dsDNA library/2) × 100%

2. The circularization efficiency of this kit for PCR-Free library is calculated as follows:

   Circularization efficiency = Total amount of single-stranded circular DNA library/(total amount of input linear dsDNA library × 100%

06-3/Further Precautions

1. It is recommended to adjust and optimize the library preparation process according to the specific experimental design, sample characteristics, sequencing applications and instruments. The experimental procedures provided in the instructions are universal. The reaction parameters can be adjusted as needed to optimize the performance and efficiency.
2. Take out all of the components in advance. The Rapid DNA Ligase and Digestion Enzyme should be placed on ice for later use after mixing thoroughly. The other components should be thawed at room temperature. After thawing, mix thoroughly and centrifuge briefly, then put them on ice.
3. When preparing the reaction solution for each step, it is recommended to mix gently by pipetting more than 10 times. Vigorous oscillations may reduce the library yield.
4. It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
5. It is recommended that you use the PCR instruments with heated lids for reactions. Preheat the PCR instrument to reaction temperature before use.
6. Use designated equipment for each area and clean the area regularly to ensure a sterile working environment (use RNase, RNA and DNA Remover (Vazyme #R504)).

07/Sample Preparation

1. The recommended input DNA amount is 1 pmol. If the PCR product is not enough, the minimum input DNA amount should be no less than 0.5 pmol. If there are special requirements regarding the amount of input PCR product from the library prep kit, please obey the special requirements.
2. Refer to the formula below to calculate the mass in (ng) that corresponds to 1 pmol of dsDNA sample with varying fragment sizes.

Conversion between moles and mass of PCR products:

Corresponding mass of 1 pmol PCR products (ng) = Main fragment size of DNA (bp) × 0.66

3. Input DNA can be a single sample or multiplexed samples with different Barcodes.
4. If pool multiple samples, the pooling can be done according to each sample requires the same amount of sequencing data. The recommended total amount of multiplexed samples should be 1 pmol, and the main fragment size of the pooled libraried should be the same.
5. It is recommend the library with single barcode or dual barcode adapters for MGI to be pooled, cyclized and sequenced separately, the pooling rules of libraries with different barcodes refer to the corresponding manuals.

   Single barcode adapter for MGI:

   VAHTS DNA Adapters Set 8 for MGI (Vazyme #NM108);

   VAHTS PCR-Free DNA Adapters for MGI (Vazyme #NM109);

   Dual barcode adapter for MGI:

   VAHTS Maxi Unique Dual Barcode Primers Set 1 - Set 4 for MGI (Vazyme #NM34401 - NM34404);

   VAHTS Dual UMI UDB Adapters Set 1 - Set 8 for MGI (Vazyme #NM35101 - NM35108);

   TruePrep Dual Index Kit V1 for MGI (Vazyme #TDM201).

08/Workflow

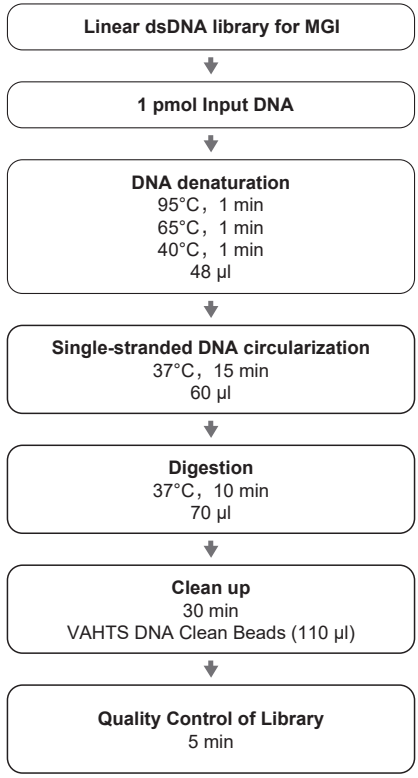

Fig 1. Workflow of VAHTS Universal Circularization Kit for MGI

09/Experiment Process

09-1/DNA Denaturation

1. According to the fragment length of Input DNA (linear dsDNA library for MGI), add 1 pmol Input DNA into a 0.2 ml PCR tube. Fill up to 40 μl with Nuclease-free ddH_2O.
2. Mix and briefly centrifuge Splint Oligo after thawing and keep it on ice. Add the following reagents into an Nuclease-free tube:

Components	Volume
Input DNA	X μl
Splint Oligo	8 μl
Nuclease-free ddH_2O	To 48 μl

3. Mix gently by pipetting and centrifuge briefly.

4. Place the PCR tube into the PCR instrument and run the following program:

Temperature	Time
Preheat lid	On
95°C	1 min
65°C	1 min
40°C	1 min
4°C	Hold

5. When the program is completed, centrifuge briefly to collect the solution at the bottom of the tube, and place on ice.

09-2/Single-stranded DNA Circularization

1. Mix and briefly centrifuge Rapid Splint Buffer after thawing and keep it on ice.

2. Prepare the following circularization reaction solution on ice:

Components	Volume	
Products from previous step	48 µl	
Rapid Splint Buffer	6 µl	■
Rapid DNA Ligase	6 µl	■
Total	60 µl	

3. Mix gently by pipetting and centrifuge briefly.

4. Place the PCR tube into the PCR instrument and run the following program:

Temperature	Time
Preheat lid	Off
37°C	15 min
4°C	Hold

5. When the program is completed, centrifuge briefly to collect the solution at the bottom of the tube, and place on ice.

09-3/Digestion

1. Mix and briefly centrifuge Digestion Buffer after thawing and keep it on ice.

2. Prepare the following reaction solution on ice:

Components	Volume	
Products from previous step	60 µl	
Digestion Buffer	7 µl	■
Digestion Enzyme	3 µl	■
Total	70 µl	

3. Mix gently by pipetting and centrifuge briefly to collect the solution at the bottom of the tube.

4. Place the PCR tube into the PCR instrument and run the following program:

Temperature	Time
Preheat lid	Off
37°C	10 min
4°C	Hold

5. When the program is completed, centrifuge briefly to collect the solution at the bottom of the tube, and place on ice.

09-4/Clean up

1. Keep the VAHTS DNA Clean Beads at room temperature for 30 min. Resuspend the beads by vortexing.
2. Add 110 μl of VAHTS DNA Clean Beads to the Digestion product and slowly pipette 10 times to resuspend. Incubate at room temperature for 5 min.

   ▲ Magnetic beads are relatively viscous, please ensure to aspirate a sufficient volume and dispense slowly when using a pipette.
3. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~ 5 min), carefully **discard the supernatant**.
4. 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**.
5. Repeat step 4, wash twice in total.
6. Keep the tube always on the magnetic rack and air-dry the beads for 3 - 5 min.

   ▲ Do not over dry the magnetic beads, which may reduce the efficiency of DNA elution and affect the yield.
7. Remove the tube from the magnetic rack and add 22 μl of Nuclease-free ddH_2O. Pipette gently to resuspend beads and incubate at room temperature for 5 min. If the beads are overdry and cracked, please extend incubation time appropriately.
8. Place the tube on the magnetic rack until the supernatant is clear (~ 3 min). Transfer 20 μl of supernatant into a new PCR tube and perform the quality control of library.

   After clean up, the purification product can be stored at -20°C for one month.

09-5/Quality Control of Library

Use Qubit ssDNA Assay Kit (Invitrogen #Q10212) to quantify the library.

10/FAQ & Troubleshooting

◇ The cyclized product yield is abnormal

1. Input DNA (linear dsDNA Library for MGI) is not quantitatively accurate, resulting in the amount of Input is too little or too many. It is recommended that the initial input be limited within 0.5 - 2 pmol, and the optimal input is 1 pmol.
2. The main size of fragmented DNA is inaccurate (due to adapter dimers, over-amplification of large fragments, broad peaks, etc.), resulting in the amount of Input is too little or too many. It is recommended to control the library size within the appropriate range.
3. The enzymatic activity is affected due to improper storage. It is recommended to use a new kit for circularization.
4. The cyclized product is inaccurately quantified. It is recommended to recalibrate the instrument before each quantification. Take 2 μl of the cyclized product and 198 μl of Qubit (Invitrogen #Q10212), mix thoroughly by vortexing and then quantify.
5. Improper experimental procedures. For example, the reagents and reaction system are not mixed thoroughly. It is recommended to vortex the buffer and oligo until they are clear and homogeneous, and mix the enzymes by flicking or vortexing gently. After adding samples at each step, make sure to pipette or vortex gently before running the PCR. The reaction system in the PCR tubes should appear clear and homogeneous to avoid bubbles.

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