VAHTS Circularization Kit for MGI

NM201

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
Follow Us

Instruction for Use

Version 22.1

Contents

01/Product Description

The VAHTS Circularization Kit for MGI is a kit optimized for the high-throughput sequencing platform of MGI. This kit can convert PCR products with adapters to single-stranded circularization DNA libraries dedicated to MGI high-throughput sequencer. All reagents provided in the kit have undergone rigorous quality control and functional validation to ensure the optimal stability and repeatability of library preparation.

02/Components

Components	NM201-01 (16 rxns)	NM201-02 (48 rxns)
Splint Oligo	160 μl	480 μl
Rapid Splint Buffer	240 μl	720 μl
Rapid DNA Ligase	80 μl	240 μl
Digestion Buffer	128 μl	384 μl
Digestion Enzyme	32 μl	96 μl

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

This kit is applicable to all PCR amplification libraries prepared by MGI high-throughput sequencing platform library preparation kits.

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: Qubit Fluorometer, absolute ethanol, Nuclease-free ddH_2O, Nuclease-free PCR tube, low-adsorption EP tube, magnetic stand, PCR instrument, etc.

06/Sample Preparation

◇ The amount of Input DNA recommended for this kit is 1 pmol, and the minimum amount is 0.5 pmol. If the library preparation kit has special requirements for circularization input, the amount of DNA should be used as required.

◇ For DNA with different fragment sizes, 1 pmol molecules correspond to different masses. Please refer to the following formula or table to calculate the Input DNA amount:

Formula 1 Conversion between moles and mass of PCR products:

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

Table 1. Corresponding mass of 1 pmol PCR products with different fragment sizes

Main band size of inserted fragments (bp)	Main band size of PCR products (bp)	Corresponding mass of 1 pmol (ng)
150	234	155
200	284	190
250	334	220
300	384	255
350	434	290
400	484	320
450	534	355
500	584	390

◇ Input DNA can be a single sample or a mixture of multiple samples with different Barcodes. When being mixed, they should meet the Barcodes mixing requirements. Please refer to the instructions of the VAHTS DNA Adapters Set 8 for MGI (Vazyme #NM108) to select appropriate Barcodes for mixing.

◇ The recommended total amount of mixed samples is 1 pmol; If the amount of data required for each sample is the same, balanced mix should be adopted. The mass required for each sample should be calculated according to the following formula:

Formula 2 The mass required for a single sample in mixed samples should be calculated as follows:

The mass required for a single sample (ng) = the mass corresponding to 1 pmol Input DNA (ng)/the number of samples mixed N

07/Notes

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

07-1/About magnetic beads

1. Magnetic beads should be balanced to room temperature before use. Otherwise, it will affect the capture efficiency.
2. The beads should be vortexed vigorously and fully mixed before using.
3. When the sample and beads are fully mixed and placed on the magnetic stand for separation, the supernatant should be pipetted after the solution is completely clear, and 2 - 3 μl should be left when the supernatant is pipetted. If the beads are accidentally pipetted, low yield and poor sorting effect will be caused. And the subsequent enzyme reaction may even be affected. In this case, the beads can be mixed and placed on the magnetic stand again for re-separation. Due to different suction forces of magnetic stands or other reasons, the default separation time may need to be extended to completely separate the beads and the liquid.
4. Freshly prepared 80% ethanol should be used to rinse the beads.
5. Keep the PCR tube on the magnetic stand during the whole rinsing process, and the beads should not be disturbed. After the second rinsing of beads with 80% ethanol, the supernatant should be fully removed.
6. The beads should be dried at room temperature before elution. Insufficient drying is easy to cause residue of ethanol and affect the following reaction; Excessive drying will cause the crack of beads and reduce the purification yield. The beads can be fully dried after drying at room temperature for 5 - 10 min under normal conditions. Please do not dry them by heating (such as drying in an oven at 37℃).
7. The purified products can be stored temporarily at -20℃.

07-2/About quality control of circularization library

1. After the purification of single-stranded circularized products, Qubit ssDNA Assay Kit is recommended to quantify the purified products.
2. For the high-throughput sequencing platform of MGI, the yield of purified single-stranded circularized products should be more than 80 fmol to be enough for sequencing twice. Calculate according to the following formula or refer to Table 2.

Formula 3 Conversion between moles and mass of single-stranded circularization products:

The mass corresponding to 80 fmol single-stranded circularization products (ng) =

0.08 × main band size of single-stranded library (nt) × 0.33

Table 2. Corresponding yield of 80 fmol single-stranded circularization products

Main band size of inserted fragments (nt)	Main band size of single-stranded circularization library (nt)	Corresponding yield of 80 fmol (ng)
150	234	6.2
200	284	7.5
250	334	8.8
300	384	10.1
350	434	11.5
400	484	12.8
450	534	14.1
500	584	15.4

07-3/Other Notes

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 Enzyme should be placed on ice for later use after instantaneous centrifugation. The other components should be thawed at room temperature. After thawing, mix thoroughly and centrifuge briefly before putting them on ice.
3. When preparing the reaction solution for each step, it is recommended to gently pipette for more than 10 times. Violent oscillations may result in a decrease in library yields.
4. To avoid cross-contamination of the samples, it is recommended to use pipette tips with filter. Please change the pipette tips when pipetting different samples.
5. The experimental area should be cleaned regularly with 0.5% sodium hypochlorite, 10% bleach or RNase, RNA and DNA Remover (Vazyme #R504) to ensure the cleanliness of the experimental environment.

08/Experiment Process

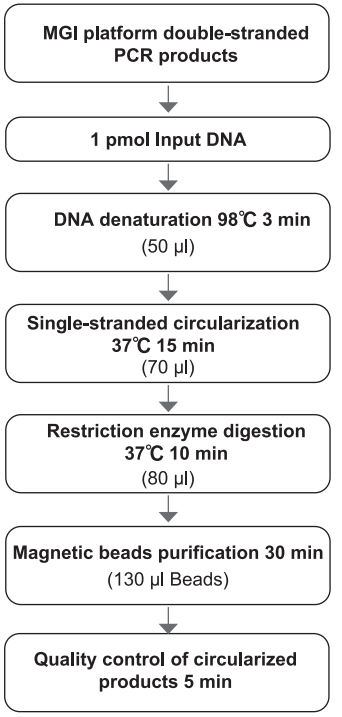

Fig 1. Workflow of Single-stranded Circularization Library Construction

08-1/Denaturation

1. According to the fragment length of Input DNA, 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	40 μl
Splint Oligo	10 μl
Total	50 μl

3. Mix gently and centrifuge briefly. Place the PCR tube on the PCR instrument and run the following program:

Temperature	Time
Hot lid	On
98℃	3 min

4. Chill it on ice immediately. After 2 min ice bath, centrifuge briefly and carry out single-stranded circularization immediately.

08-2/Single-stranded DNA circularization

1. Mix and briefly centrifuge Rapid Splint Buffer after thawing and keep it on ice.
2. Prepare the following single-strand circularization reaction solution on ice:

Components	Volume
Reaction product of the previous step	50 μl
Rapid Splint Buffer	15 μl
Rapid DNA Ligase	5 μl
Total	70 μl

3. Mix gently and centrifuge briefly.
4. Put the above PCR tube in the PCR instrument, and carry out the circularization reaction according to the following conditions:

Temperature	Time
Hot lid	Off
37℃	15 min
4℃	Hold

▲ High hot lid temperature may affect the actual reaction temperature. It is recommended to turn off the hot lid of the PCR instrument or set hot lid temperature at 40℃.

5. After the reaction, carry out enzymatic digestion immediately after instantaneous centrifugation.

08-3/Restriction enzyme digestion

1. Mix and briefly centrifuge Digestion Buffer after thawing and keep it on ice.
2. Prepare the digestion reaction solution on ice according to the following system:

Components	Volume
Reaction product of the previous step	70 μl
Digestion Buffer	8 μl
Digestion Enzyme	2 μl
Total	80 μl

3. Mix gently and centrifuge briefly. Place the PCR tube in the PCR instrument, and carry out the digestion reaction according to the following conditions:

Temperature	Time
Hot lid	Off
37℃	10 min
4℃	Hold

▲ High hot lid temperature may affect the actual reaction temperature. It is recommended to turn off the hot lid of the PCR instrument or set hot lid temperature at 40℃.

4. After the reaction, carry out purification immediately after instantaneous centrifugation.

08-4/Purification of circularized products

1. Balance VAHTS DNA Clean Beads to room temperature and vortex thoroughly before use.
2. Pipette 130 μl VAHTS DNA Clean Beads into the digestion products. Mix thoroughly by pipetting at least 10 times. Incubate at room temperature for 10 min.
3. Centrifuge briefly and put the PCR tube on the magnetic stand. Carefully remove the supernatant after the solution is clear (about 5 min).
4. Keep the PCR tube on the magnetic stand, and then add 200 μl freshly prepared 80% ethanol to rinse the magnetic beads. Carefully discard the supernatant.
5. Repeat Step 4 and make sure 80% ethanol was completely removed.
6. Keep the PCR tube on the magnetic stand. Open the lid to air dry the beads for 5 - 10 min until there is no ethanol residue.
7. Remove the PCR tube from the magnetic stand, and add 22 μl ddH_2O for DNA elution. Mix thoroughly by pipetting at least 10 times. Incubate at room temperature for 10 min.
8. After instantaneous centrifugation, place the PCR tube on the magnetic stand until the liquid is clear (about 3 min). Transfer 20 μl supernatant to a new PCR tube.

▲ The purified circularized products can be temporarily stored at -20℃. Repeated freezing and thawing should be avoided.

08-5/Quality control of circularized products

Use Qubit ssDNA Assay Kit to quantify the library. Refer to 07/Notes for details.

Vazyme

www.vazyme.com