*Trans*NGS® Universal Circularization Kit For MGI®

Please read the datasheet carefully prior to use.

Cat. No. KC401

Storage: at -20°C for one year

Description

*Trans*NGS® Universal Circularization Kit For MGI® is designed for MGI high-throughput sequencers and suitable for efficient and rapid preparation of single-stranded circular DNA library that can be sequenced on MGI high-throughput sequencers from dsDNA of MGI dual or single barcode library or non-MGI library converted by libraries. All reagents provided in this kit have passed stringent quality control and functional verification procedures to ensure stability and reproducibility of library preparation to the maximum extent possible.

Features

- High library conversion rate
- Short operation time

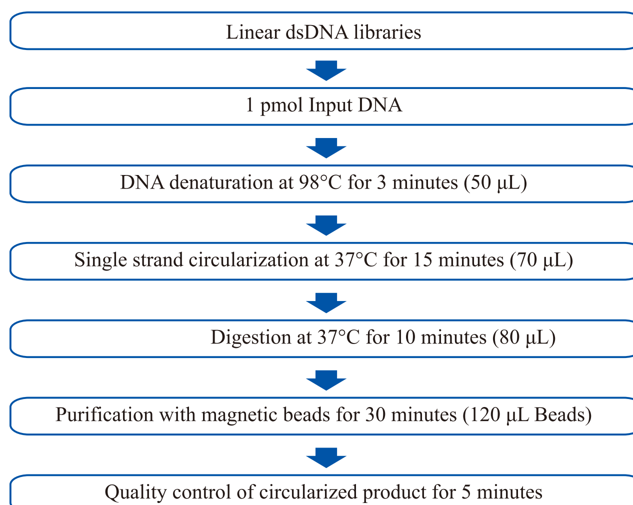
Applications

- dsDNA of MGI dual barcode library
- dsDNA of MGI single barcode library
- dsDNA of non-MGI library converted by libraries

Kit Contents

Component	KC401-01 (12 rxns)	KC401-02 (96 rxns)
Splint Oligo IV	120 µl	960 µl
Rapid DNA Ligase	12 µl	96 µl
Rapid Splint Buffer	228 µl	2×912 µl
Digestion Enzyme Mix	24 µl	192 µl
Digestion Buffer	96 µl	768 µl

Workflow



Sample Requirements

1. Sample requirement

Sample: purified dsDNA library (MGI dual barcode library, MGI single barcode library or non-MGI library converted by libraries). It is recommended to dilute sample with Nuclease-free Water.

Note: It is recommended to use *Trans*NGS® Transformation Kit For MGI®(KC301) to convert non-MGI library.

2. Input requirement

The recommended input amount is 1 pmol. The minimum input DNA amount should be no less than 0.5 pmol and not higher than 2 pmol to ensure high circularization efficiency and meet the requirements of MGI sequencer. If the library prep kit has a special circularization input requirement, the required input amount is added according to the requirements of the library prep kit.

3. Sample pooling requirement

Input DNA can be a single sample or multiple samples with different barcodes. Multiple samples must satisfy specific barcodes combination requirements. The recommended total amount of multiple samples should be 1 pmol, and the mixing ratio is adjusted according to the amount of sequencing data, sample concentration, and fragment length.

4. Sample mass calculation

1 pmol of DNA with different fragment sizes corresponds to different masses, and the sample amount can be calculated according to formula 1 or selected according to table 1.

Formula 1:

$$\text{The mass (ng) corresponding to 1 pmol PCR products} = \frac{\text{DNA main fragment size (bp)}}{\text{1000 bp}} \times 660 \text{ ng}$$

Table 1 The yield corresponding to 1 pmol PCR products

Main fragment size of amplified product (bp)	Yield (ng)
281	185
331	220
381	250
431	285
481	320
531	350
581	385
631	420

Reagents not included in the kit

DNA purification: *MagicPure*® Size Selection DNA Beads (Cat. No. EC401); freshly prepared 80% ethanol; Nuclease-free Water.

DNA quantification: 1 × dsDNA HS Assay Kit (Cat. No. GS401); Qubit™ ssDNA Assay Kit (ThermoFisher).

Instruments and consumables: Qubit fluorometer; thermal cycler; Nuclease-free PCR tube; magnetic stand, etc.

Protocol

1. DNA denaturation

(1) The concentration of input DNA library is detected with 1 × dsDNA HS Assay Kit (Cat. No. GS401). Add 1.0 pmol DNA to PCR tube according to main fragment size. Add Nuclease-free Water to make a total volume of 40 μl.

(2) Thaw Splint Oligo IV, turn the tube upside down to mix, and place on ice for later use.

(3) Prepare the following system in PCR tube.

Component	Volume
Input DNA	40 μl
Splint Oligo IV	10 μl
Total	50 μl

(4) After mixing, denaturation at 98 °C for 3 minutes in a thermal cycler (with the heated lid set at 105°C). When the program is completed, immediately place the PCR tube on ice for 2 minutes.

(5) Centrifuge briefly and immediately proceed to single strand circularization.

2. Single strand circularization

(1) Thaw Rapid Splint Buffer, turn the tube upside down to mix, and place on ice for later use.

(2) Prepare the following system on ice.

Component	Volume
DNA denaturation product	50 μl
Rapid Splint Buffer	19 μl
Rapid DNA Ligase	1 μl
Total	70 μl

(3) Pipet 10 times gently, do not shake to mix, and centrifuge briefly to collect the reaction solution to the bottom of the tube.

(4) Place the PCR tube into the thermal cycler. Run the program with the following conditions.

Component	Volume
Heated lid	Off
37℃	15 min
4℃	Hold

(5) When the program is completed, centrifuge briefly, and immediately proceed to digestion.

3. Digestion

(1) Thaw Digestion Buffer, turn the tube upside down to mix, and place on ice for later use.

(2) Prepare the following system on ice.

Component	Volume
Single strand circularization product	70 μl
Digestion Buffer	8 μl
Digestion Enzyme Mix	2 μl
Total	80 μl

(3) Pipet 10 times gently, do not shake to mix, and centrifuge briefly to collect the reaction solution to the bottom of the tube.

(4) Place the PCR tube into the thermal cycler. Run the program with the following conditions.

Temperature	Volume
Heated lid	Off
37℃	10 min
4℃	Hold

(5) When the program is completed, centrifuge briefly, and immediately proceed to purification.

4. Purification of circularization product

(1) Take out the *MagicPure*® Size Selection DNA Beads from 2-8°C, incubate for at least 30 minutes and vortex well to mix.

(2) Pipet 120 µl of the beads into the digested product, pipet gently or low-speed votex to mix, and incubate at room temperature for 5 minutes.

(3) Centrifuge briefly, place the tube on the magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), discard the supernatant.

(4) Add 200 µl of freshly prepared 80% ethanol to the tube on the magnetic stand, do not pipet the beads. Incubate at room temperature for 30 seconds. Discard the supernatant.

(5) Repeat step (4) once.

(6) Air dry the beads at room temperature until the beads just begin to crack (about 5 minutes) while the tube is on the magnetic stand.

(7)Remove the tube from the magnetic stand. Add 22 µl Nuclease-free Water to elute DNA. Mix the beads thoroughly by pipetting gently or low-speed vortexing. Incubate at room temperature for 2 minutes.

(8) Place the tube on the magnetic stand. Incubate at room temperature until the solution is clear (about 2 minutes). Make sure the beads settle to the magnet completely.

(9) Carefully transfer 20 µl of the eluate to a clean 1.5 ml centrifuge tube. The product can be stored at -20°C.

5. Quality control of circularized product

Quantify the digestion product with the Qubit™ ssDNA Assay Kit (ThermoFisher).

Note

- Thaw the Buffers in the kit at room temperature, mix thoroughly, centrifuge briefly Buffers and Enzymes, and place on ice for later use;
- The samples after DNA denaturation should be immediately placed on ice for incubation;
- Avoid vigorously shaking when mixing the reaction solution to prevent the enzyme activity from decreasing, which will lead to a decrease in circularization efficiency;
- For the MGI high-throughput sequencing platform, the yield of the purified single-stranded circularization product should reach more than 80 fmol to be sufficient for two sequencing runs;
- Clean each experimental area regularly (wipe with 0.5% sodium hypochlorite or 10% bleach) to ensure the cleanliness of the experimental environment.

For research use only, not for clinical diagnosis.

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