NGS Dual Barcode DNA Circularization Kit for MGI

Cat. No.: HYD221

Product Description

NGS Dual Barcode DNA Circularization Kit for MGI is a single-stranded DNA circularization kit specifically developed for MGI high-throughput sequencing platforms. Featuring high-quality enzymes and an optimized buffer formulation, the kit significantly improves reaction efficiency, enabling the entire circularization and digestion workflow to be completed within 30 minutes. The kit is compatible with amplified library products generated using PCR adapters with dual barcodes conforming to BGI standards. Except for limitations imposed by the library preparation kit itself, this circularization kit is not restricted to specific MGI sequencing platforms.

Components

Components	24T	96T
DB Splint Oligo	96 μL	576 μL
Splint Buffer	240 μL	2×720 μL
Ligase	80 μL	480 μL
Digestion Buffer	128 μL	768 μL
Digestion Enzyme	32 μL	192 μL

Storage

Store at -25 ~ -15°C, valid for 1 year.

Notes

1. About the operation

1) For your safety and health, always wear a laboratory coat and disposable gloves during operation.
2) Before use, thaw all kit components at room temperature. After thawing, mix thoroughly by inversion several times, briefly centrifuge, and place on ice until use.
3) When preparing reaction mixtures, mix by pipetting up and down or gentle vortexing. Avoid vigorous mixing, as it may reduce library yield.

4) To prevent cross-contamination between samples, use filter pipette tips and change tips whenever handling different samples.
5) Perform all reactions in a thermal cycler equipped with a heated lid. Preheat the thermal cycler to approximately the required reaction temperature before use.
6) Clean all laboratory work areas regularly by wiping with 0.5% sodium hypochlorite or 10% bleach solution to maintain a clean working environment.
7) For Research Use Only.

2. Sample Requirements and Preparation

2.1 DNA Input Requirements

1) The recommended input amount for this kit is 1 pmol of DNA. If the library yield is insufficient, the input amount can be reduced to a minimum of 0.5 pmol.
2) For specific circularization input requirements, the DNA input amount may be adjusted accordingly. However, it is generally recommended not to exceed 2 pmol.
3) The mass corresponding to 1 pmol of DNA varies depending on fragment size. Calculate the required DNA input amount using Equation 1 or refer to Table 1.

Equation1 Conversion between mole number and mass of PCR products

The mass(ng) of 1 pmol PCR product(ng) =DNA main fragment size(bp)× 0.66

Table 1. PCR products of different fragment sizes correspond to the yield of 1 pmol

Insert size(bp)	PCR products main size(bp)	1 pmol correspond product(ng)
150	300	198
200	350	231
250	400	264
300	450	29T

2.2 Sample Pooling Requirements

1) The input DNA can consist of a single sample or a pool of multiple samples carrying different barcodes.
2) The recommended total DNA input for pooled samples is 1 pmol. If equal sequencing depth is required for each sample, pool the samples in equal amounts. Calculate the required DNA mass for each sample according to Equation 2.

Equation2 Calculation of the required mass of a single sample in a mixed sample

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

3) The total volume of the DNA input, whether from a single sample or a pooled sample, should be adjusted to 34 μL for circularization. If the sample volume is less than 34 μL, add ddH_2O to bring the final volume to 34 μL.

3. Beads-based CleanUp

1) Allow the magnetic beads to equilibrate to room temperature before use. Failure to do so may result in reduced purification yield.
2) Thoroughly resuspend the beads before each use by vortexing or pipetting up and down.
3) Prepare fresh 80% ethanol immediately before use for bead washing, as improper ethanol preparation or storage may affect recovery efficiency.
4) Before product elution, air-dry the beads at room temperature. Insufficient drying may result in residual ethanol, which can interfere with subsequent reactions. Over-drying may cause the beads to crack and reduce purification yield. Under normal conditions, approximately 5 minutes of air-drying at room temperature is recommended.
5) For purification and storage of single-stranded circular DNA products, elute with TE Buffer. The purified products can be stored at -20°C for up to 1 month.

4. Library Quality Analysis

1) After purification, it is recommended to quantify the purified single-stranded circular DNA products using the ssDNA Assay Kit.
2) The recommended yield of purified single-stranded circular DNA should be ≥80 fmol, which is sufficient for two sequencing runs.
3) The required amount can be calculated using Equation 3 or determined by referring to Table 2.

Equation3 Conversion between molar number and mass of single-strand circles

The mass(ng) of 80 fmol single-strand circle = 0.08× DNA main size(bp)×0.3

Table 2. Different sizes PCR product of 80 fmol correspond to single-strand circle yields

Insert size(bp)	PCR product main size(bp)	80 fmol corresponding output(ng)
150	300	7.92
200	350	9.24
250	400	10.56
300	450	11.88

Instructions

1. Required Materials and Equipment (User-Supplied)

1) DNA Purification Beads: NGS DNA Selection Beads (Cat. # HYD511), AMPure XP Beads (Cat. # A63880), or equivalent products.
2) Library Quantification: ssDNA Assay Kit (Cat. # HYD412) or Qubit® ssDNA Assay Kit (Thermo Fisher Scientific, Cat. # Q10212).
3) Other Materials and Equipment: 80% ethanol, low-retention microcentrifuge tubes, magnetic racks, PCR thermal cycler, and other standard laboratory supplies.

2. Procedure

2.1 Denaturation

1) Based on the library fragment size, transfer 1 pmol of DNA library into a 0.2 mL PCR tube and adjust the volume to 34 μL with TE Buffer.
2) Thaw the reagents listed in Table 3, mix thoroughly by inversion, and place on ice until use.
3) Prepare the reaction mixture on ice according to Table 3.

Table 3. DNA denaturation system

Components	Volume
The Single or mixed double-stranded libraries	34 μL
DB Splint Oligo	6 μL

4) Mix thoroughly and denature at 98°C for 3 minutes in a thermal cycler. Immediately transfer the sample to ice, incubate on ice for 2 minutes, and briefly centrifuge.

   Note: Some thermal cyclers rapidly cool down after the program ends. Failure to remove the sample promptly may result in DNA reannealing and reduced circularization efficiency. To prevent this, set the thermal cycler to 98°C (Hold). Once the sample is placed in the thermal cycler, start a timer for 3 minutes, then immediately remove the sample and place it on ice.

2.2 DNA cyclization

1) Thaw the reagents listed in Table 4, mix thoroughly by inversion, and place on ice until use.
2) Prepare the reaction mixture on ice according to Table 4.

Table 4. Single-strand cyclization system

Components	Volume
Products from above	40 μL
Splint Buffer	15 μL
Ligase	5 μL

3) Gently mix by pipetting up and down or low-speed vortexing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.
4) Place the PCR tube in a thermal cycler and run the program specified in Table 5 for single-stranded DNA circularization.

Table 5. Single-strand cyclization system

Temperature	Time
Hot lid 105℃	OFF
37 ℃	15 min
4 ℃	Hold

2.3 Enzyme digestion

1) Thaw the reagents listed in Table 6, mix thoroughly by inversion, and place on ice until use.
2) Prepare the reaction mixture on ice according to Table 6.

Table 6. Enzyme digestion Mix

Components	Volume
Products from above	60 μL
Digestion Buffer	8 μL
Digestion Enzyme	2 μL

3) Gently mix by pipetting up and down or low-speed vortexing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.
4) Place the PCR tube in a thermal cycler and run the reaction program specified in Table 7.

Table 7. Digestion reaction procedure

Temperature	Time
Hot lid 105℃	OFF
37 ℃	10 min
4 ℃	Hold

5) Upon completion of the reaction, briefly centrifuge the tube and proceed immediately to purification.

2.4 Purification of digestion products

1) Preparation: Remove NGS DNA Selection Beads or Beckman AMPure XP Beads from storage and allow them to equilibrate to room temperature for at least 30 minutes. Prepare fresh 80% ethanol.
2) Thoroughly resuspend the beads by vortexing or repeated inversion.
3) Add 120 μL of NGS DNA Selection Beads to the digestion product and incubate at room temperature for 10 minutes.
4) Briefly centrifuge the PCR tube and place it on a magnetic rack to separate the beads from the solution. Once the solution has cleared (approximately 2 minutes), carefully remove the supernatant.
5) Keep the PCR tube on the magnetic rack. Add 200 μL of freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 seconds, then carefully remove the supernatant.
6) Repeat Step 5 once more, for a total of two washes.
7) Keep the PCR tube on the magnetic rack, open the tube lid, and air-dry the beads until cracks just begin to appear.
8) Remove the PCR tube from the magnetic rack. Add 22 μL of TE Buffer and thoroughly resuspend the beads by vortexing or gently pipetting up and down. Incubate at room temperature for 10 minutes.
9) Briefly centrifuge the tube and place it on the magnetic rack. Once the solution has cleared (approximately 2 minutes), carefully transfer the supernatant to a new PCR tube.

***Stopping point: Cyclized purification product, can be stored at-20"C for one month**

2.5 The Digestion production QC

Quantify digestion products using ssDNA Assay Kit, more details see Note 4.