Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Circularization Kit For MGI

Product Number:PCK13

Shipping and Storage

Box 1： Store at -20°C; transportation conditions: dried ice.

Box 2： Store at 2°C~8°C; transportation conditions: ice bag.

Components

Box 1: Cyclization reagents

Component	PCK13
Splint Oligo	20μL
T4 DNA Ligase	250μL
5×Splint Buffer	50μL
Digestion Buffer	20μL
Digestion Enzyme I	70μL
Digestion Enzyme III	25μL

Box 2: DNA Purification Beads

Component	PCK13
Magnetic Bead	1.5mL/tube ×4

Description

Cyclation kit is a modular kit tailored for MGI's high-throughput sequencing platform. This kit can be used to prepare PCR products from the spliced joint into single-stranded circular DNA libraries suitable for MGI sequencers. All reagents provided in the kit have undergone strict quality control and functional verification to ensure maximum stability and repeatability of library construction.

Consumables and Equipment

1. Magnetic rack: DynaMagTM-2 is recommended.
2. Qubit®3.0 ThermoFisher.
3. Qubit®3.0 ssDNA Assay Kit.
4. Anhydrous ethanol, EB (10mM Tris-HCl, pH 8.0), NF Water (pH 7.0 to 8.0) .
5. Reaction tube: It is recommended to use low adsorption PCR tube and EP tube.

Tips: It is recommended to use high quality filter gun head placement kit, library sample contamination

Preparation

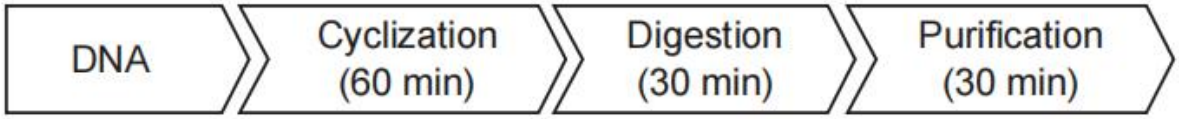
1. Sample preparation
   - 1.1. PCR products: a total of 330 or more ng (multiple PCR product mix, refers to the total) after mixing, the volume of 49μL (if the PCR product itself insufficient volume, supplementary NF Water to total volume 49μL).
   - 1.2. PCR products fragment size: fragment peaks between 200-500bp.
   - 1.3. Modification: PCR products has joined for MGISEQ-2000, and BGISEQ MGISEQ -200-500 such as sequencing platform (Index) of fixed sequence.
2. Preparation of reagents
   - 2.1. Take out corresponding reagent kit, centrifugation, enzyme mixture on the ice to use: Before use, the buffer should be dissolved at room temperature, centrifuged by shock, placed on ice for use, NF Water and EB at room temperature for use.

2.2. Please compound mixture on the ice.

2.3. Please make reaction system on the ice.

2.4. Kit buffer after dissolving in possible precipitation, precipitation does not affect the reagent function, please fully vibration blending to precipitation disappear after use.

Workflow



Protocol

Cyclization

1. 1μL was added to the 49μL PCR product, denatured and incubated at 95℃ on the PCR instrument for 3 min, and then immediately transferred to an ice bath and left for 2 min.

Component	Volume (μL)
PCR product	49
Splint Oligo	1
Total volume	50

2. Prepare reaction mixture on the ice according to the following system:

Component	volume (μL)
5×Splint Buffer	13
T4 DNA ligase	2
Total volume	15

3. Add the above 15μL reaction mixture to the 50μL denatured DNA;

4. Place the PCR tube above on the PCR instrument, and the reaction conditions are as follows:

Temperature	Time
Heated lid 38°C	On
37°C	60 min
4°C	Hold

Digestion by enzyme cutting

1. Prepare the digestive reaction solution on the ice according to the following system:

Component	Volume (μL)
Digestion Buffer	0.8
Digestion Enzyme I	3.9
Digestion Enzyme III	1.3
NF Water	2
Total	8

2. After the cyclization reaction, add 8μL digestive reaction solution directly into the cyclization system, mix it well, and then place the PCR tube on the PCR instrument after a short centrifugation. The reaction conditions are as follows:

Temperature	Time
Heated lid 38°C	On
37°C	30 min
4°C	Hold

3. Purification was carried out immediately after the reaction.

Purification of digestion products

1. Take Magnetic Bead out at room temperature 30 minutes in advance and mix thoroughly before use;
2. The digestive products are transferred to 1.5mL EP tube, 340μL Magnetic Bead is absorbed into the digestive products, gently blown 10 times with pipette and thoroughly mixed, incubated at room temperature for 10 minutes;
3. Instantaneous centrifugation, put the EP tube on the magnetic rack, and let it stand for 5 minutes until the liquid is clarified. The pipette absorbs and discards the supernatant;
4. Keep the EP tube fixed on the magnetic rack, add 250μL freshly prepared 80% ethanol, stand for 1 minute, and carefully discard the supernatant;
5. Repeat step 4 once, try to dry the bottom of the tube liquid;

   Note: Do not absorb magnetic beads, so as not to affect the yield.
6. Keep the EP tube fixed on the magnetic rack, open the tube cover, and dry at room temperature for 5-10 minutes;
7. Remove the EP tube from the magnetic rack, add 35μL EB or NF Water for DNA elution, blow and mix with pipette and dissolve at room temperature for 10 minutes;
8. Place the EP tube on the magnetic rack for instantaneous centrifugation, let it stand for 2 minutes until the liquid clears, and transfer the supernatant to the new EP tube. Store at -20℃ for DNB preparation.

Library quality control

Use Qubit™ ssDNA Assay Kit or Quant-iT™ OliGreen® figure SSDNA Reagent single strand DNA quantification kit quantifies the library.