Circularization Kit For MGI

Cyclization kit (MGI platform)

Catalog number: C666001 (16 rxns)

Storage conditions:

Box 1: Store at -20°C and transport on dry ice.

Box 2: Store at 2°C~8°C and transport in ice packs.

Products content

Box 1: Cyclization reagent

Component	c666001 (16 rxns)
Splint Oligo	20 μl
5×Splint Buffer T4	250 μ
DNA Ligase	50 μl
Digestion Buffer	20 μl
Digestion Enzyme I	70 μl
Digestion Enzyme III	25 μl

Box 2: DNA Purification Recovery Beads

Component	
CMPure	1.5 ml/tube×4 tubes

Products Introduction

The Cyclization Kit is a modular kit tailored for the MGI high-throughput sequencing platform. With this kit, PCR products after junction ligation can be prepared into single-stranded circular DNA libraries suitable for MGI sequencers. All reagents provided in the kit have been subjected to stringent quality control and functional validation to maximize the stability and reproducibility of library construction.

Provide your own instruments, reagents and consumables

1. Magnetic frame: DynaMagTM-2 (Cat. No. 12321D) is recommended.
2. "Qubit" 3.0 Fluorescence Quantimeter (ThermoFisher, Cat. No. Q33216)
3. Qubit" ssDNA Assay Kit (Invitrogen, Cat. No. Q10212)
4. Anhydrous ethanol, EB (10 mM Tris-HCl, pH 8.0), NF Water (pH between 7.0 and 8.0).
5. reaction tubes: low adsorption PCR tubes with 1.5 mlEP tubes are recommended: 5.

Tip: It is recommended to use a high quality filter tip to prevent contamination of kits and libraries.

Pre-experiment Preparation and Important Notes

1. Sample preparation.

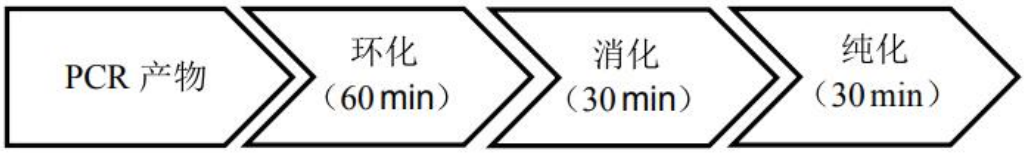
PCR product: 2330 ng total (total amount when multiple PCR products are mixed) in a volume of 49 pL (if the volume of PCR product is insufficient, add NF Water to bring the total volume to 49 pl). -PCR product: Fragment size: The fragment peak is between 200-500 bp. -PCR product fragment size: Fragment peaks between 200-500 bp. -PCR product modification: Fixed sequences (with Index) for MGISEQ-2000, MGISEQ-200 and BGISEQ-500 sequencing platforms were added.

2. Reagent preparation

-Remove the corresponding reagents from the kit, centrifuge briefly, and place the enzyme mixture on ice until ready to use: buffers need to be dissolved at room temperature before use, then centrifuged with shaking and placed on ice until ready to use, and NF Water and EB are placed at room temperature until ready to use: "Please make up the mixture on ice:

Precipitation may appear after the buffer in the kit is dissolved, the precipitation does not affect the function of the reagent, please shake and mix well until the precipitation disappears and then use.

Schematic diagram of the cyclization process



procedure

cyclize

1. 1 wl of Splint Oligo was added to the 49JI PCR product. The product was denatured and incubated on a PCR instrument at 95°C for 3 min, then immediately transferred to an ice bath and allowed to stand for 2 min.

individual parts making up a compound	Volume (μl)
PCR product	49
Splint Oligo	1
total volume	50

2. The reaction mixture was prepared on ice according to the following system.

individual parts making up a compound	CW volume (μl)
5×Splint Buffer	49
T4 DNA Ligase	1
total volume	50

3. Add 15ul of the above reaction mixture to 50μl of denatured DNA.

4. Place the above PCR tubes on the PCR instrument under the following conditions Reaction.

temp	timing
Hot cover 38°C	on
37°C	60 min
4°C	Hold

digest

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

individual parts making up a compound	volumetric
Digestion Buffer	0.8
Digestion Enzyme I	3.9
Digestion Enzyme III	1.3
NF Water	2
total volume	8

2. After the cyclization reaction, add 8l of digestion reaction solution directly to the cyclization system, mix well, centrifuge briefly and then place the PCR tube on the PCR instrument and react under the following conditions.

individual parts making up a compound	timing
Hot cover 38°C	on
37°C	60 min
4°C	Hold

3. Purification was carried out immediately after the reaction.

Purification of digestive products

1. Remove CMPure at room temperature 30 minutes prior to use and mix well with shaking.

2. Transfer the digested product to a 1.5 mIEP tube, pipette 340 pICMPure into the digested product, mix well by gently blowing 10 times with a pipette and incubate for 10 minutes at room temperature.

3. Instantaneous centrifugation, place the EP tube on a magnetic rack and let stand for 5 minutes until the liquid is clear, pipette and discard the supernatant.

4. Keep the EP tube fixed on a magnetic rack, add 250ul of freshly prepared 80% ethanol, let it stand at room temperature for 1 minute, then carefully discard the supernatant.

5. Repeat step 4 once, try to suck up the liquid at the bottom of the tube: Note: Do not suck up the magnetic beads, so as not to affect the yield.

6. Keep the EP tube fixed on the magnetic rack, open the cap and dry it at room temperature for 5-10 minutes.

7. Remove the EP tube from the magnetic rack, add 35ul of EB or NF Water for DNA elution, pipette blow to mix and dissolve at room temperature for 10 min.

8. Centrifuge instantaneously, place the EP tube on a magnetic rack and let stand for 2 minutes until the liquid is clarified, transfer the supernatant to a new EP tube. -Store at 20C and leave to prepare DNB.