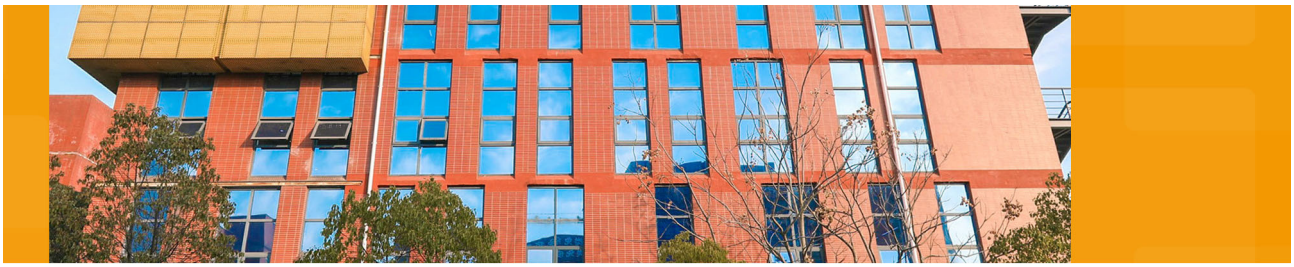
HB220412

Hieff NGS™ Fast-Pace DNA Cyclization Kit for MGI®

Cat# 13341



INSTRUCTION FOR USE

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Product Information

Product Name	Cat#	Specification
Hieff NGS™ Fast-Pace DNA Cyclization Kit for MGI®	13341ES16	16 T
	13341ES96	96 T

Product Description

Hieff NGS™ Fast-Pace DNA Cyclization Kit for MGI® is a single-strand cyclization kit specifically designed for MGI® high-throughput sequencing platforms. The use of high-quality enzymes and optimized Buffer significantly improves reaction efficiency, enabling the entire cyclization and digestion process to be completed in less than 30 minutes. This kit is suitable for all standard dual-label PCR adapter library connected to MGI® platforms, and is not limited to different MGI sequencing platforms except for the limitations of the library-prep reagents.

Product Components

Components			13341ES16	13341ES96
13341-A	◌	Splint Oligo	96 μL	576 μL
13341-B	◌	Splint Buffer	240 μL	2×720 μL
13341-C	◌	Ligase	80 μL	480 μL
13341-D	◯	Digestion Buffer	128 μL	768 μL
13341-E	◯	Digestion Enzyme	32 μL	192 μL

Shipping and Storage

All the components are shipped with ice packs and can be stored at -20°C for one year.

Cautions

I About the operation

1. For your safety and health, please wear lab coats and disposable gloves for operation.
2. Please thaw each component of the kit at room temperature before use. Please invert the thawed reagents several times, briefly spin down, and put them on ice until use.
3. It is highly recommended to mix the reagents by pipetting up-and-down or by gentle vortexing when setting up the reactions. Vigorous vortexing may impact the library yield.
4. It is highly recommended to use filtered pipet tips to avoid cross-contamination. Be sure to change pipet tips when processing different samples.
5. It is highly recommended to pre-heat the lid of the thermocycler for each reaction step.
6. Improper operations may very likely cause carry-over contaminations through aerosols, impacting the experiment accuracy. It is highly recommended to divide the experiment environment into the pre-PCR and post-PCR regions, with separate sets of devices and disposables in each area. Perform routine cleaning for each area by wiping the surfaces with 0.5% sodium hypochlorite or 10% bleach.
7. For research use only!

II Sample input requirement

1. The recommended amount of Input DNA for this kit is 1 pmol; If the PCR product is insufficient, the minimum input can be reduced to 0.5 pmol.
2. If there is a special requirement for the amount of cyclization input, the required amount of input will according to the requirements of the library preparation kit.
3. Different fragment sizes of DNA 1 pmol molecules correspond to different masses, the required amount of input DNA can be

calculated according to Formula 1 or selected by referring to Table 1:

Equation 1 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 1pmol

Insert size(bp)	PCR products main size(bp)	1 pmol correspond product(ng)
150	300	198
200	350	231
250	400	264
300	450	297

Samples mixing requirements

1. Input DNA can be a single sample or a mixture of multiple samples with different barcodes.
2. When mixing samples, you need to meet the requirements of barcodes mixing, you can refer to the manual of the Double Label PCR Connector Kit to select the appropriate Barcodes for mixing.
3. The recommended total sample size for mixing is 1 pmol, if the amount of data required for each sample is the same, the amount is mixed equally, and the quality required for each sample is calculated according to Equation 2:

Equation 2 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

4. Single or mixed samples should be 34 μL in volume when used for cyclization, and supplemented to 34 μL with ddH2O if insufficient.

III Bead-based Clean Up

1. The beads should be equilibrated to room temperature before use, otherwise it will lead to a decrease in the purification yield.
2. The beads should be fully mixed before each use by pipetting or vortexing.
3. The 80% ethanol used for bead rinsing should be ready to use, otherwise the recovery efficiency will be affected.
4. The beads should be dried at room temperature before the product is eluted. Insufficient drying can easily cause absolute ethanol residues to affect subsequent reactions; over-drying will lead to bead cracking and thus reduce the purification yield. Normally, it dries at room temperature for about 5 min.
5. The single-strand circles product is purified and stored, which can be eluted using TE Buffer, and the product can be stored at -20 °C for 1 month.

IV Library Quality Analysis

1. It is recommended to quantify the purified product using the Qubit® ssDNA Assay Kit single-stranded DNA fluorescent dye reagent after purification.
2. For MGI high-throughput sequencing platform, the yield should be ≥ 80 fmol after purification of the single-chain ring product (enough for 2 times on-the-machine sequencing).
3. Library Quality can be calculated according to Equation 3 or with reference to Table 2.

Equation 3 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)×0.33

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

Insert size(bp)	PCR product main size(bp)	80 fmol corresponding output(ng)
150	230	6.07
200	280	7.39
250	330	8.71
300	380	10.03

Instructions

I Required Materials Not Included

1. Purified magnetic beads: Cat #12601, Hieff NGS™ DNA Selection Beads or Cat#A63880, AMPure XP Beads or other equivalent products.
2. Library QC: Cat # Q10212, Thermo fisher Qubit® ssDNA Assay Kit.
3. Other materials: absolute ethanol, sterilized ultrapure water, TE Buffer (10 mM Tris-HCl, pH 8.0-8.5+0.1 mM EDTA), low adsorption EP tube, PCR tube, magnetic rack, thermal instrument, etc.

II Workflow

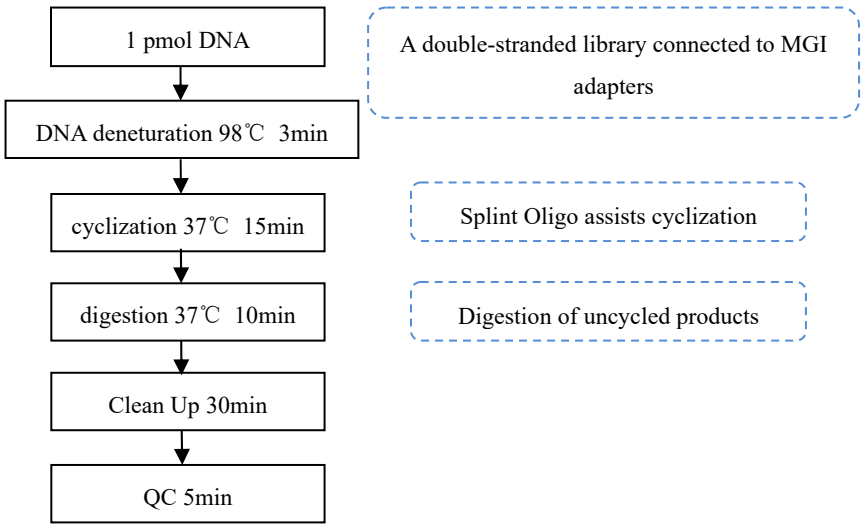

Figure 1 single-strand cyclization Library Prep workflow

III Operation steps

3.1 Denaturation

1. According to the size of the library, take 1 pmol library to 0.2 ml PCR tube and supplement to 34 μL with ddH2O.
2. After thawing the reagents in Table 3, mix well upside down and place on ice for later.
3. Prepare the Table 3 reaction system **on ice**.

Table 3 DNA denaturation system

Name	Volume(μL)
The Single or mixed double-stranded libraries	34
Splint Oligo	6
Total	40

4. After mixing, denaturate at 98 °C on a thermal cycler for 3 min, then immediately place on ice, and centrifuge instantaneously after 2 min of ice bath.

3.2 Single-strand DNA cyclization

1. After thawing the reagents in Table 4, mix well upside down and place on ice for later.

2. Prepare the Table 4 reaction system on ice.

Table 4 Single-strand cyclization system

Name	Volume(μL)
Products from above	40
Splint Buffer	15
Ligase	5
Total	60

3. Mix well by gently pipetting up and down or shaking at low speed, and centrifuge the reaction solution briefly to the bottom of the tube..

4. Place the PCR tube on the thermal cycler and perform a single-strand cyclization reaction according to the reaction procedure shown in Table 5.

Table 5 Single- stranded cyclization reaction procedures

Temperature	Duration
Hot lid 105°C	OFF
37°C	15min
4°C	Hold

3.3 Enzyme digestion

1. After thawing the reagents in Table 6, mix well upside down and place on ice for later.

2. Prepare the reaction system shown in Table 6 **on ice.**

Table 6 Enzyme digestion Mix

Name	Volume(μL)
Products from above	60
Digestion Buffer	8
Digestion Enzyme	2
Total	70

3. Mix wellby gently pipetting up and down or shaking at low speed, and centrifuge briefly to centrifuge the reaction solution to the bottom of the tube.

4. Place the PCR tube on the thermal cycler and react according to the conditions of Table 7：

Table 7 Digestion reaction procedure

Temperature	Duration
Hot lid 105°C	OFF
37°C	10 min
4°C	Hold

5. After the reaction is completed, centrifuge instantaneously and process purification immediately.

3.4 Purification of digestion products

This step uses magnetic beads to purify the product of step 3.3

1. Preparation: Remove the Hieff NGS™ DNA Selection Beads or Beckman AMPure XP Beads from the refrigerator and equilibrate at room temperature for at least 30 min. Prepare 80% ethanol.

2.Vortex or invert the beads sufficiently to ensure adequate mixing.

3.Add 120 μL of Hieff NGS™ DNA Selection Beads into the digestion product, mix well by vortexing or pipetting, and incubate at room temperature for 10 min.

4.Centrifuge the PCR tube briefly and place in a magnetic stand to separate the beads and liquid, and after the solution is clear (about 2 min), carefully remove the supernatant.

5.Keep the PCR tube in the magnetic stand, add 200 μL of freshly formulated 80% ethanol to rinse the beads, and after incubating at room temperature for 30 sec, carefully remove the supernatant.

6.Repeat step 5 for a total of two rinses.

7.Keep the PCR tube in the magnetic stand at all times, and air dry the beads until they have just cracked.

8.Remove the PCR tube from the magnetic stand, add 22 μL TE Buffer, mix well by vortexing or blow pipetting, allowing to stand at room temperature for 10 min.

9.Centrifuge briefly, keep the PCR tube in a magnetic stand and carefully move the supernatant to a new PCR tube after the solution is clear (about 2min).

★Stopping point: Cyclized purification product, can be stored at -20 ° C for one month.

3.5 The Digestion production QC

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

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