BioDynami

NGS DNA Fragmentation & Library Prep Kit (MGI platform)

Catalog No.	34028S	34028L
Index type	24 indexes	96 indexes
Reactions	24 reactions	96 reactions

Description

The **NGS DNA Fragmentation & Library Prep Kit** (MGI platform) was developed for construction of high-quality libraries for next generation sequencing. The kit uses intact genomic DNA as input DNA without an additional DNA fragmentation step. Our technology provides a fast and simple workflow (Fig. 1). DNA libraries can be generated around 1.5 hours.

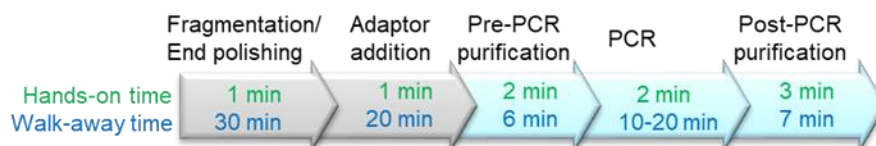


Fig. 1 kit workflow

The incorporation of DNA fragmentation in the kit makes it possible to directly use intact genomic DNA as input DNA without the need of mechanical DNA shearing or enzymatic DNA fragmentation. The library size is inversely correlated with the incubation time of step 1 at 20°C (Fig. 2).

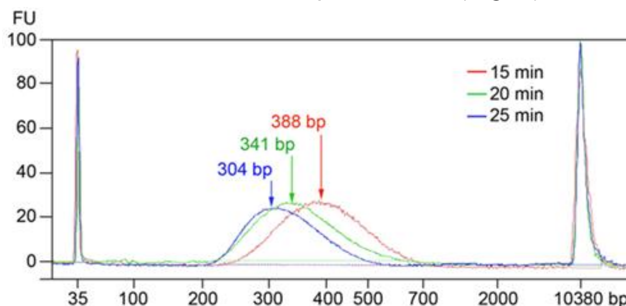


Fig. 2 Incubation time of step 1 at 20°C is inversely correlated with library size.

Features

- 1.5-hour protocol from intact genomic DNA to NGS library
- Intact genomic DNA as input, DNA fragmentation is not needed.
- Works with both EDTA-free DNA and DNA resuspended in TE buffer
- Simple workflow: Less steps
- Guaranteed quality: Higher library conversion efficiency

Component

Catalog No.	34028S	34028L
AD1 Enzyme	120 ul	480 ul
AD2M Buffer	456 ul	1824 ul
AD2 Enzyme	24 ul	96 ul
EF Buffer	48 ul	192 ul
Sodium Chloride (1.25 M)	960 ul	3840 ul
Primers	5 ul X24	5 ul X96
PCR mix	600 ul	2400 ul

Storage Condition

- Store kit at -20°C, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- PCR thermal cycler
- 96-well PCR plate
- 80% ethanol (prepare before use)
- Sodium Chloride (1.25 M)*
- Magnetic Beads (BioDynami Cat.# 40051) or equivalent

*Sometimes the tube of Sodium Chloride (1.25 M) may crack during dry ice shipping. Customers need to prepare Sodium Chloride (1.25 M) in this case.

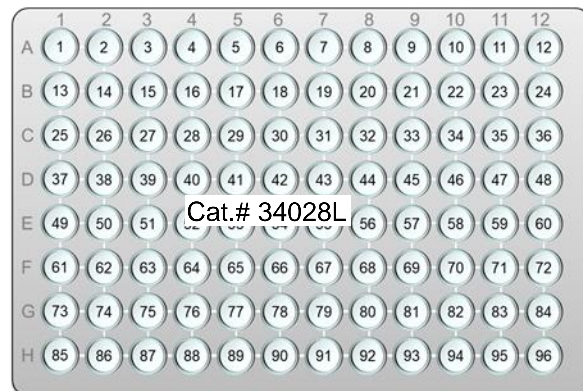
Library and Index Information

Index (Cat.# 34028S and 34028L)

Index ID	Index Sequence	Index ID	Index Sequence	Index ID	Index Sequence
Index #1	GCTTGTTCAG	Index #9	CCGATGACGT	Index #17	GACGCGGTAT
Index #2	AACAAGCACT	Index #10	TTATCTCGAG	Index #18	ACGAGACGTC
Index #3	TTGCCAGTGA	Index #11	AGCCGATACC	Index #19	CTACTCAAGA
Index #4	CGAGTCAGTC	Index #12	GATGACGTTA	Index #20	TGTTATTCCG
Index #5	GATAGTAACG	Index #13	TCGCGATGTC	Index #21	CCGTCACTGA
Index #6	TGAGTGGCTA	Index #14	AGTGACACCA	Index #22	TGACGCAACT
Index #7	CCGTCATTAC	Index #15	GACATTCAAG	Index #23	GTTGTTGCTC
Index #8	ATCCACCGGT	Index #16	CTATCGGTGT	Index #24	AACAAGTGAG

For Cat.# 34028S, primers will be shipped in 8-stripe PCR tubes with index labels at both ends as shown below. For Cat.# 34028L, primers will be shipped in 96-well plates. Below is the index layout.

Cat.# 34028S



Cat.# 34028L

The 96 index primers have been aliquot in the 96-well plate as shown above.

Protocol

Step 1: Fragmentation/End polishing

1) The following reaction **MUST** be assembled on ice. Add the following to a 96-well PCR plate:

For EDTA-free DNA samples:

Genomic DNA	13 ul (100~500 ng)
EF Buffer	2 ul
AD1 Enzyme	5 ul
Total	20 ul

For DNA samples in TE buffer:

Genomic DNA	15 ul (100~500 ng)
AD1 Enzyme	5 ul
Total	20 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 20 min*, 75°C for 10 min. Proceed immediately to step 2.
   * Incubation time affects library size. Longer time will result in shorter library size (use Fig. 2 as a reference).

Step 2: Adaptor addition

1) Add the following to the above reaction mixture. Slow pipetting of the viscous AD2M Buffer is needed for precise aliquot. The total volume is 40 ul.

AD2M Buffer	19 ul
AD2 Enzyme	1 ul
Total	20 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 20 min.
4) Add Sodium Chloride (1.25 M) 40 ul to the reaction mixture. Proceed immediately to step 3.

Step 3: Pre-PCR purification

1) Resuspend Magnetic Beads and transfer 40 ul to the above reaction mixture, mix by pipetting and incubate for 3 min.
2) Load the plate on a magnet, incubate for 2 min, and discard the supernatant carefully. Remove all residual supernatant without disturbing the beads.
3) Add 180 ul of 80% ethanol, incubate for 30 sec, and discard the supernatant carefully. Remove all residual ethanol without disturbing the beads.
4) Remove the plate from the magnet, resuspend the beads in 22 ul of water.
5) Load the plate on the magnet, incubate for 1 min, and transfer 20 ul supernatant (containing library) to a new tube without disturbing the beads.

Step 4: PCR

1) Mix the following in a PCR plate:

Library	20 ul
Primers	5 ul
PCR mix	25 ul
Total	50 ul

2) Put PCR plate on a thermal cycler, start PCR with the following condition:

Step	Temperature	Time	Cycles
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	6-9 cycles
Annealing/extension	65°C	70 seconds	
Final Extension	65°C	2 minutes	1
Hold	4°C		

Step 5: Post-PCR purification

1) Resuspend Magnetic Beads and transfer 40 ul to the above reaction mixture, mix by pipetting and incubate for 3 min.
2) Load the plate on a magnet, incubate for 2 min, and discard the supernatant carefully.
3) Add 180 ul of 80% ethanol, incubate for 30 sec, and discard the supernatant carefully. Remove all residual ethanol without disturbing the beads.
4) Remove the plate from the magnet, resuspend the beads in 22 ul of water or Tris-HCl (10 mM).
5) Load the plate on the magnet, incubate for 1 min, and transfer 20 ul supernatant (containing library) to a new tube without disturbing the beads.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to BioDynami.com for Material Safety Data Sheet of the product.

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