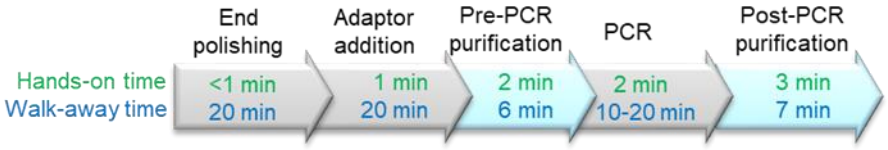
NGS Cell-free DNA Library Prep Kit (MGI Platform)

Catalog No.	34031S	34031L
Index type	index	index
Reactions	24 reactions	96 reactions

Description

The **NGS Cell-free DNA Library Prep Kit** (MGI Platform) was developed for the construction of high-quality libraries using 1 ng to 50 ng of cell-free DNA (cfDNA) as input. The kit has a simple work flow and a fast procedure.



Features

- Fast
  - Total time: 1.5 hrs
  - Hands-on time: 10 min
- Easy procedure
  - Ready-to-use master mix
  - Less reaction components
- Less magnetic beads required: Reduced more than 50%
- Guaranteed quality: Higher library conversion efficiency
- Low input DNA: From 1 ng to 50 ng

Component

Catalog No.	34031S	34031L
KH1 Buffer	72 ul	288 ul
KH1 Enzyme	48 ul	192 ul
KH2M Buffer	336 ul	1344 ul
KH2 Enzyme	24 ul	96 ul
Sodium Chloride (1.67 M)*	432 ul	1728 ul
Index Primers	5 ul X24	5 ul X96
PCR mix	600 ul	2400 ul

*Sometimes the tube of Sodium Chloride (1.67 M) may crack during dry ice shipping. Customers need to prepare Sodium Chloride (1.67 M) in this case.

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 Solution (1.67 M)*
- Magnetic Beads (BioDynami Cat.# 40051) or equivalent

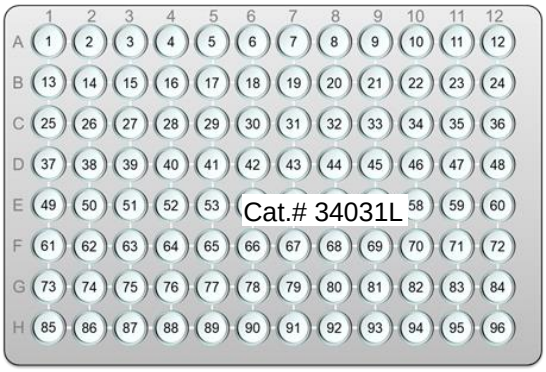
Library and Index Information

Index (Cat.# 34031S and 34031L)

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.# 34031S, primers will be shipped in 8-stripe PCR tubes with index labels at both ends as shown below. For Cat.# 34031L, primers will be shipped in 96-well plates. Below is the index layout.

Cat.# 34031S



Cat.# 34031L

Protocol

Step 1: End polishing

1) Add the following to one well of a 96-well PCR plate:

cfDNA	10 ul (1 ng~50 ng)
KH1 Buffer	3 ul
KH1 Enzyme	2 ul
Total	15 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 15 min, 70°C for 5 min. Proceed immediately to step 2.

Step 2: Adaptor addition

1) Add the following to Step 1 reaction mixture. Slow pipetting of the viscous KH2M Buffer is needed for precise aliquot.

KH2M Buffer	14 ul
KH2 Enzyme	1 ul
Total	15 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 20 min.
4) Add Sodium Chloride (1.67 M) 18 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	7-14 cycles***
Annealing/extension	65°C	70 seconds	
Final Extension	65°C	2 minutes	1
Hold	4°C		

*** As a reference:
12-14 cycles for 1-5 ng input;
10-12 cycles for 5-10 ng input;
8-11 cycles for 10-20 ng input;
7-10 cycles for 20-50 ng input;

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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About PCR master mix:
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