PCR-free NGS DNA Library Prep Kit (illumina platform)

Catalog No.	30040S	30040L	30041S	30041L
Index type	non-index	non-index	index	index
Reactions	24 reactions	48 reactions	48 reactions	96 reactions

Description

The **PCR-free NGS DNA Library Prep Kit** was developed for construction of high quality libraries for next generation sequencing (illumina platform) without PCR. The PCR-free library can reduce library prep bias, improve coverage of difficult areas, and enhance the detection of sequence variants. The kit is compatible with DNA fragments generated from both enzymatic methods and mechanical methods. Library multiplexing is possible with dual indexes.

	End polishing	Adaptor addition	Beads purification
Hands-on time	1 min	1 min	3 min
Walk-away time	20 min	20 min	7 min

Two index types are available for the kit:

Non-index (Cat.# 30040S and 30040L): Libraries do not have index.
Index (Cat.# 30041S and 30041L): Each library contains one i5 index and one i7 index. Library multiplexing up to 96 samples is possible.

Features

- Total time: 1 hr
- Hands-on time: 5 min
- Easy procedure: Ready-to-use master mix & Less reaction components
- Input DNA amount from 100 ng to 1 ug

Component

Catalog No.	30040S	30040L	30041S	30041L
DL1 Buffer	96 ul	192 ul	192 ul	384 ul
DL1 Enzyme	96 ul	192 ul	192 ul	384 ul
DL2A Buffer	576 ul	1152 ul	1152 ul	2304 ul
DL2 Enzyme	48 ul	96 ul	96 ul	192 ul
Sodium Chloride (2 M)***	960 ul	1920 ul	1920 ul	3840 ul
Adaptor	96 ul*	192 ul*	4 ul** X48	4 ul** X96

*non-indexed adaptor
**indexed adaptor
***Sometimes the tube of Sodium Chloride (2 M) may crack during dry ice shipping. Customers need to prepare Sodium Chloride (2 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 (2 M)***
- Magnetic Beads (BioDynami Cat.# 40051) or equivalent

Library and Index Information

Non-Indexed library (Cat.# 30040S and 30040L)
Library adaptor:

5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'
5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG-3'

Indexed library (Cat.# 30041S and 30041L)
Index locations of the indexed libraries:

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5′AATGATACGGCGACCACCGAGATCTACACNNNNNNNNACACTCTTTCCCTACACGACGCTCTTCCGATCT-insert-
3′TTACTATGCCGCTGGTGGCTCTAGATGTGNNNNNNNNTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-insert-

     -insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG 3′
     -insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGNNNNNNNNNTAGAGCATACGGCAGAAGACGAAC 5′
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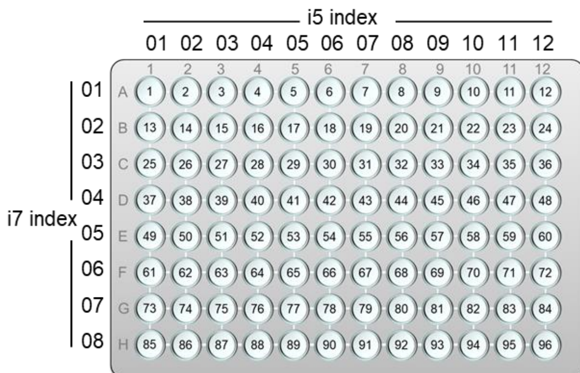
Note: i5 index: NNNNNNN (in yellow) is the index sequence, 5' to 3' direction.
I7 index: NNNNNNN (in red) is the index sequence, 5' to 3' direction.

List of indexes can be downloaded from:
https://www.biodynami.com/documents/BioDynami-PCR-free-Index.xls

For Cat.# 30041S and 30041L, the indexed adaptors have been aliquot into 96-well plates by column (i5) and row (i7). Each well contains one i5 index and one i7 index as shown in figure. Example: library #71 has i5-11 index and i7-06 index.



Protocol

Step 1: End polishing

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

Sheared DNA	42 ul (100 ng~1 ug)
DL1 Buffer	4 ul
DL1 Enzyme	4 ul
Total	50 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 DL2A Buffer is needed for precise aliquot.

DL2A Buffer	24 ul
Adaptor	4 ul
DL2 Enzyme	2 ul
Total	30 ul

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

Step 3: Beads 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 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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Oct. 2022