NGS DNA Fragmentation & Library Prep Kit (illumina platform)

Catalog No.	30026S	30026L	30028S	30028L	30030S	30030L
Index type	non-index	non-index	index	index	unique dual index	unique dual index
Reactions	24 reactions	48 reactions	24 reactions	48 reactions	96 reactions	96 reactions X2

Description

The **NGS DNA Fragmentation & Library Prep Kit** (illumina 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.

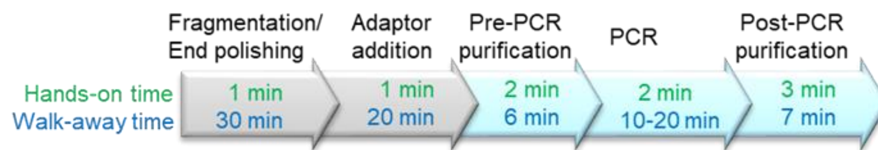


Fig. 1 kit workflow

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).

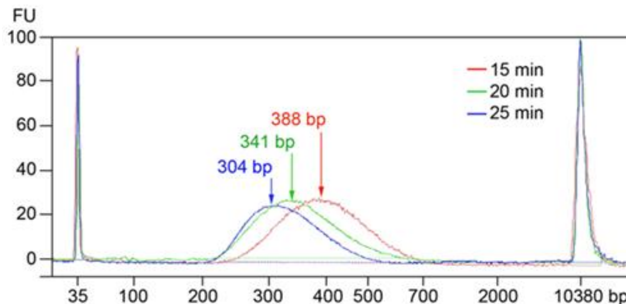


Fig. 2 Incubation time of step 1 at 20°C is inversely correlated with library size.

Three index types are available for the kit:

Non-index (Cat.# 30026S and 30026L): Libraries do not have index.

Index (Cat.# 30028S and 30028L): Each of our index primers contains a unique barcode sequence with 6 bases that can be used to identify libraries. Library multiplexing up to 48 samples is possible.

Unique dual index (Cat.# 30030S and 30030L): Library multiplexing up to 96 samples is possible with unique dual indexes. We have developed a **4-Base Difference Index System**. The system allows us to make indexes that have at least 4 bases different from each other in the 8 bases index length. Our unique dual indexing primers remove sequencing errors such as index hopping, index cross-contamination, mis-assignment of reads, amplification errors, and de-multiplexing errors. The primer set includes 96 pre-mixed unique pairs of i5 and i7 index primers in a 96-well plate.

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.	30026S	30026L	30028S	30028L	30030S	30030L
AD1 Enzyme	120 ul	240 ul	120 ul	240 ul	480 ul	960 ul
AD2 Buffer	456 ul	912 ul	456 ul	912 ul	1824 ul	3648 ul
AD2 Enzyme	24 ul	48 ul	24 ul	48 ul	96 ul	192 ul
EF Buffer	48 ul	96 ul	48 ul	96 ul	192 ul	384 ul
Primers	120 ul	240 ul	5 ul* X24	5 ul* X48	5 ul** X96	10 ul** X96
Sodium Chloride (1.25 M)***	960 ul	1920 ul	960 ul	1920 ul	3840 ul	7680 ul
PCR mix	600 ul	1200 ul	600 ul	1200 ul	2400 ul	4800 ul

*Index primers
**Unique dual index primers

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 (1.25 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

Non-Index (Cat.# 30026S and 30026L)
Primer sequences:

Primer-1 5'-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3'
Primer-2 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'

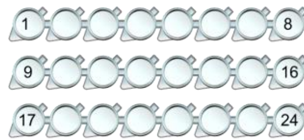
Index (Cat.# 30028S and 30028L)
Sequence of the final library with index location:

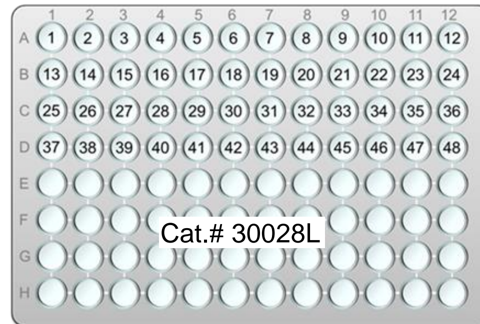
```
5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-insert-
3'TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-insert-

          -insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNATCTCGTATGCCGTCTTCTGCTTG 3'
          -insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGNNNNNNNTAGAGCATACGGCAGAAGACGAAC 5'
```

Note: NNNNNNN (in red) is the index sequence, 5' to 3' direction.

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

For Cat.# 30028S, primers will be shipped in 8-stripe PCR tubes with index labels at both ends as shown below. For Cat.# 30028L, primers will be shipped in 96-well plates. Below is the index layout.


Cat.# 30028S

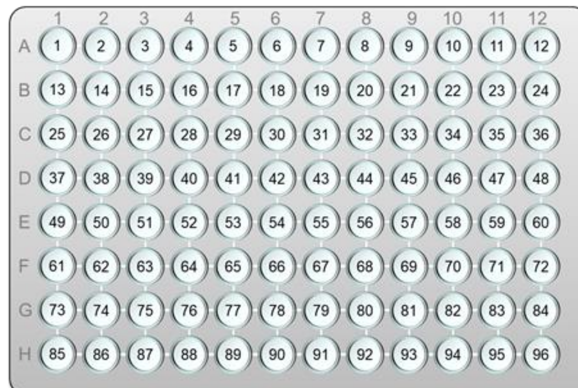


Unique Dual Index (Cat.# 30030S and 30030L)
Sequence of the final library with index locations:

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

    -insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG 3′
    -insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGNNNNNNNNNTAGAGCATACGGCAGAAGACGAAC 5′
```

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-Unique-Dual-Index.xls



The 96 unique dual index primers have been aliquot in the 96-well plate as shown on left.

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 AD2 Buffer is needed for precise aliquot. The total volume is 40 ul.

AD2 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.

Limited Label License

The product is developed and sold exclusively for research purposes and *in vitro* use only. The product or its any individual component has not been tested for use in diagnostics or drug development, and is not suitable for administration to human or animal.
The purchaser of this product is granted a limited, non-transferable right to use the purchased amount of the product only for internal, research purposes for the sole benefit of the purchaser. The buyer cannot sell or otherwise transfer (i) this product (ii) its components or (iii) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for commercial purposes. This product is for internal research purposes only and is not for use in commercial purposes of any kind. "Commercial purposes" includes any activity for which a party receives consideration and may include, but is not limited to, (1) use of the product or its components or derivatives in manufacturing, (2) transfer or sale of vectors made with the product or components or derivatives of the product, (3) use of this product or components or derivatives of the product made therefrom to provide a service, information, or data to a third party in return for a fee or other consideration, or (4) resale of the product or its components or derivatives, whether or not such product or its components or derivatives are resold for use in research. If the purchaser is not willing to accept the limitations of this limited use statement, BioDynami is willing to accept return of the products with a full refund. For information on obtaining additional rights, please contact support@BioDynami.com

About PCR master mix:
This product is licensed from Bio-Rad Laboratories, Inc. under U.S. Pat. Nos. 6,627,424,7,541,170, 7,560,260, 7,670,808, 7,666,645, 7,919,296, 8,232,078, 8,367,376, 8,415,129, 8,445,249, 8,470,573, 8,476,045, 8,895,283, and 8,900,846 and corresponding patents in other countries for use only in: (a) standard (non-real time) PCR in the research field only, but not digital PCR; (b) real-time PCR for internal product research and development purposes only, and not for sales to end-users within the research field; (c) any in-vitro diagnostic application, including applications using real-time PCR, but not digital PCR; and (d) any non-PCR applications in DNA sequencing, isothermal amplification, and the production of synthetic DNA.

BioDynami

Oct. 2022