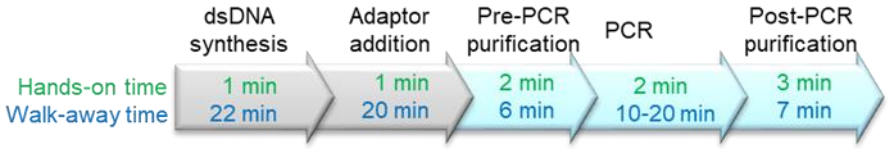
Bisulfite Sequencing Library Prep Kit (illumina platform)

Catalog No.	30093A	30093F	30093S	30093L
Index type	unique dual index	unique dual index	unique dual index	unique dual index
Reactions	24	48	96	96X2

Description

The **Bisulfite Sequencing Library Prep Kit** (illumina platform) was developed for construction of high-quality libraries using bisulfite treated DNA (50 ng - 500 ng) as input. It is known that bisulfite treatment of completed NGS libraries causes tremendous damage to the libraries. By using bisulfite treated DNA as input, the kit overcomes the significant library loss due to the bisulfite conversion. The kit contains a mixture of PCR polymerases that have high-fidelity amplification and uracil tolerance which is ideal for bisulfite treated DNA. The final library is strand specific.



Library multiplexing up to 96 samples is possible with Unique Dual Indexes (UDI). 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-base 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

- Fast and simple
  - Total time: 1.5 hrs
  - Hands-on time: 10 min
- Directional library
- Less magnetic beads required: Save more than 50%
- Bisulfite treated DNA input: From 50 ng to 500 ng

Component

Catalog No.	30093A	30093F	30093S	30093L
BS1 Buffer	72 ul	144 ul	288 ul	576 ul
BS1 Enzyme	48 ul	96 ul	192 ul	384 ul
BS2 Buffer	336 ul	672 ul	1344 ul	2688 ul
BS2 Enzyme	24 ul	48 ul	96 ul	192 ul
UDI Primers	5 ul X24	5 ul X48	5 ul X96	10 ul X96
Sodium Chloride (2.12 M)*	720 ul	1440 ul	2880 ul	5760 ul
PCR mix	600 ul	1200 ul	2400 ul	4800 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 Solution (2.12 M)*
- Magnetic Beads (BioDynami Cat.# 40051) or equivalent

*Sometimes the tube of Sodium Chloride (2.12 M) may crack during dry ice shipping. Customers need to prepare Sodium Chloride (2.12 M) in this case.

Library and Index Information

Sequence of the final library with index locations:

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

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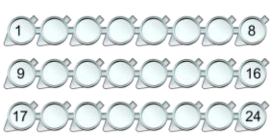
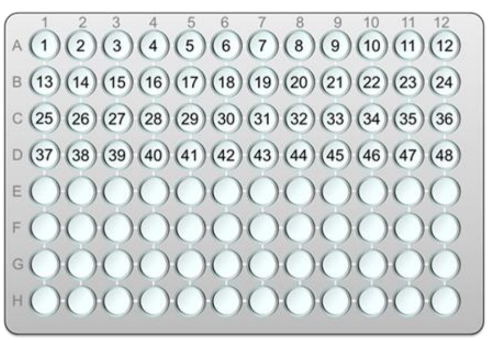
Note: i5 index: NNNNNNN (in yellow) is the index sequence, 5’ to 3’ direction.
I7 index: NNNNNNN (in red) is the index sequence, 5’ to 3’ direction.

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

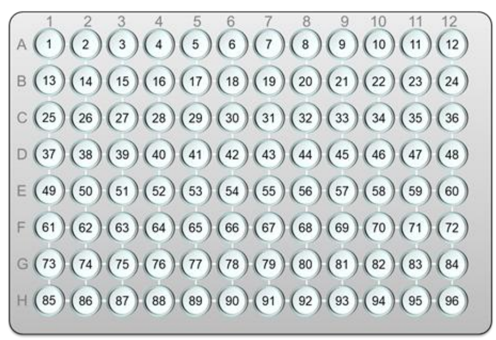
For Cat.# 30093A, UDI primers will be shipped in 8-stripe PCR tubes with index labels at both ends as shown below. For Cat.# 30093F, 30093S, and 30093L, primers will be shipped in 96-well plates. Below is the index layout.



Cat.# 30093A



Cat.# 30093F



Cat.# 30093S & 30093L

Strand Specificity

Read 1: Reverse complementary strand
Read 2: Original strand

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                                   i5 index                    Reverse complementary strand                     i7 index
5’AATGATACGGCGACCACCGAGATCTACACNNNNACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNAGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNATCTCGTATGCCGTCTTCTGCTTG
3’TTACTATGCCGCTGGTGGCTCTAGATGTGNNNNTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGANNNNNNNNNTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGNNNNTAGAGCATACGGCAGAAGACGAAC
                                                                   Original strand
```

Protocol

Step 1: dsDNA synthesis

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

Bisulfite treated DNA	25 ul (50 ng ~ 500 ng)
BS1 Buffer	3 ul
BS1 Enzyme	2 ul
Total	30 ul

2) Mix by pipetting ten times.
3) Incubate at 50°C for 2 min, 30°C for 20 min. Proceed immediately to step 2.

Step 2: Adaptor addition

1) Add the following to Step 1 reaction mixture. Slow pipetting of the viscous BS2 Buffer is needed for precise aliquot.

BS2 Buffer	14 ul
BS2 Enzyme	1 ul
Total	15 ul

2) Mix by pipetting ten times.
3) Incubate at 20°C for 20 min.
4) Add Sodium Chloride (2.12 M) 30 ul to the reaction mixture. Proceed immediately to 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
UDI 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	95°C	2 minutes	1
Denaturation	95°C	30 seconds	7-10 cycles**
Annealing	60°C	30 seconds	
Extension	68°C	45 seconds	
Final Extension	68°C	2 minutes	1
Hold	4°C		

** As a reference:
9-10 cycles for 50-100 ng input;
8-9 cycles for 100-200 ng input;
7-8 cycles for 200-500 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:
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.

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