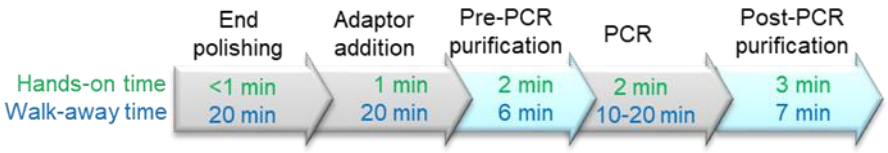
ChIP-Seq Library Prep Kit (illumina platform)

Catalog No.	30032S	30032L	30034S	30034L	30036S	30036L
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 **ChIP-Seq Library Prep Kit** (illumina platform) was developed for the construction of high quality libraries using 5 ng to 400 ng of ChIP DNA as input. The kit is compatible with ChIP DNA fragments generated from both enzymatic methods and mechanical methods (sonication, nebulization etc.).



Three index types are available for the kit:

Non-index (Cat.# 30032S and 30032L): Libraries do not have index.

Index (Cat.# 30034S and 30034L): 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.# 30036S and 30036L): 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

- 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
- Input ChIP DNA: From 5 ng to 400 ng

Component

Catalog No.	30032S	30032L	30034S	30034L	30036S	30036L
CS1 Buffer	72 ul	144 ul	72 ul	144 ul	288 ul	576 ul
CS1 Enzyme	48 ul	96 ul	48 ul	96 ul	192 ul	384 ul
CS2 Buffer	336 ul	672 ul	336 ul	672 ul	1344 ul	2688 ul
CS2 Enzyme	24 ul	48 ul	24 ul	48 ul	96 ul	192 ul
Primers	120 ul	240 ul	5 ul* X24	5 ul* X48	5 ul** X96	10 ul** X96
Sodium Chloride (1.67 M)***	432 ul	864 ul	432 ul	864 ul	1728 ul	3456 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 (1.67 M)***
- Magnetic Beads (BioDynami Cat.# 40051) or equivalent

***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.

Library and Index Information

Non-Index (Cat.# 30032S and 30032L)
Primer sequences:

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

Index (Cat.# 30034S and 30034L)
Sequence of the final library with index location:

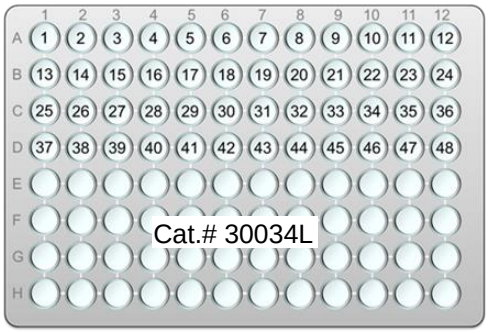
```
5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-insert-
3'TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-insert-

          -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.# 30034S, primers will be shipped in 8-stripe PCR tubes with index labels at both ends as shown below. For Cat.# 30034L, primers will be shipped in 96-well plates. Below is the index layout.

Cat.# 30034S



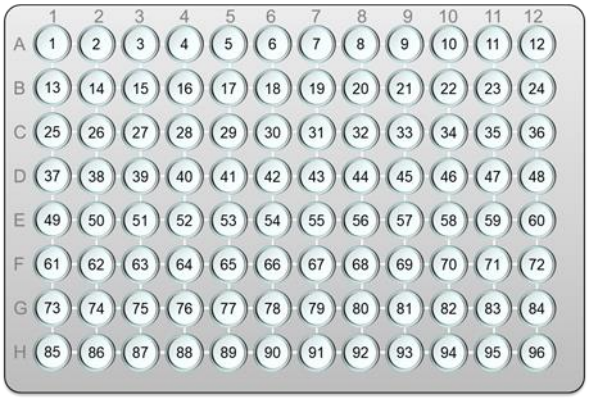
Cat.# 30034L

Unique Dual Index (Cat.# 30036S and 30036L)
Sequence of the final library with index locations:

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

-insert-AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG 3′
-insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGNNNNNNNNTAGAGCATACGGCAGAAGACGAAC 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: End polishing

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

ChIP DNA	10 ul (5 ng~400 ng)
CS1 Buffer	3 ul
CS1 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 CS2 Buffer is needed for precise aliquot.

CS2 Buffer	14 ul
CS2 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	6-13 cycles***
Annealing/extension	65°C	70 seconds	
Final Extension	65°C	2 minutes	1
Hold	4°C		

*** As a reference:
10-13 cycles for 5-10 ng input;
8-11 cycles for 10-20 ng input;
7-10 cycles for 20-50 ng input;
6-9 cycles for 50-400 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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