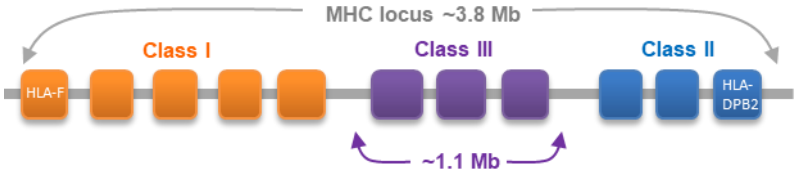
MHC Class III Capture & Library Prep Kit

Catalog No.	32018S	32018L
Reactions	24	96

Description

The **MHC Class III Capture & Library Prep Kit** was developed to combine NGS library prep and MHC locus capture. The integration of library prep and MHC capture not only simplifies the procedure, but also enhances the capture efficiency. Comprehensive sequencing of the entire Class III locus with our kit makes it possible to detect SNPs, indels, and structural variants that are not covered by other MHC targeted sequencing reagents.



Our MHC Class III kit enables the most cost-effective targeted sequencing of 1.1 Mb of the human MHC Class I locus, including all coding and non-coding regions.

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

- **Integration of NGS library prep and MHC capture**
  - Simplifies the procedure
  - Enhances the capture efficiency
- **Sequencing of MHC Class III region**
  - Covers 1.1 MB of MHC Class III region
  - Covers exons, introns, 5' regulatory regions, 3' regulatory regions, and beyond.
- **Easy detection of SNPs, indels, and structural variants**
  - The only reagent provides intact sequence information
- **Low cost**
- **Sample multiplexing**: Further reduces the cost

Component

Catalog No.	32018S	32018L
DL1 Buffer	96 ul	384 ul
DL1 Enzyme	96 ul	384 ul
DL2 Buffer	672 ul	2688 ul
DL2 Enzyme	48 ul	192 ul
MHC-III-A Probe	960 ul	3840 ul
MHC-III-B Probe	720 ul	2880 ul
RS Buffer	840 ul	3360 ul
W1 Buffer	14.4 ml	57.6 ml
W2 Buffer	9.6 ml	38.4 ml

Non-index Primers	48 ul	192 ul
UDI Primers	5 ul X24	5 ul X96
PCR mix	1200 ul	4800 ul

Storage Condition

- Store components (except W1, W2 and RS Buffer) at -20°C, stable up to 12 months.
- Store W1, W2 and RS Buffer at room temperature, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- PCR thermal cycler
- Hybridization oven
- Heat block (compatible with 96-well plate)
- 96-well PCR plate
- 80% ethanol (prepare before use)
- MyOne streptavidin C1 dynabeads (ThermoFisher)
- SPRI Beads (BioDynami Cat.# 40051) or equivalent
- Mineral oil

Library and Index Information

Sequence of the final library with index locations:

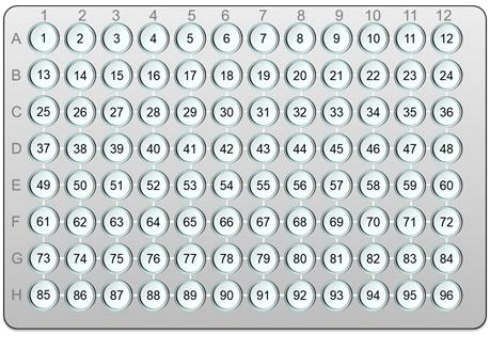
```
5'AATGATACGGCGACCACCGAGATCTACACNNNNNNNNACACTCTTTCCCTACACGACGCTCTTCCGATCT-insert-
3'TTACTATGCCGCTGGTGGCTCTAGATGTGNNNNNNNNTGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-insert-

        -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

For Cat.# 32018S, UDI primers will be shipped in 8-stripe PCR tubes with index labels at both ends as shown below. For Cat.# 32018L, primers will be shipped in 96-well plates. Below is the index layout.

Cat.# 32018S



Cat.# 32018L

Protocol

Step 1. NGS Library Prep: End polishing

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

Sheared DNA	42 (500-700 ng)
DL1 Buffer	4
DL1 Enzyme	4
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. NGS Library Prep: Adaptor addition

1. Add the following to Step 1 reaction mixture. Slow pipetting of the viscous DL2 Buffer is needed for precise aliquot.

DL2 Buffer	28
DL2 Enzyme	2
Total	30 ul

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

Step 3. 1st Hybridization

1. Warm probe at 37°C, invert the tube several times to dissolve. Mix the following in a 96-well plate:

MHC-III-A Probe	40
NGS Library (from step 2)	80
Total	120 μl

2. Overlay a drop of mineral oil in wells with above mix. Seal the wells with cap or sealing tape.
3. Heat at 95°C for 5 min
4. Transfer plate to a hybridization oven, hybridize with the following condition:
    1) 75°C for 6-8 hrs
    2) 70°C overnight
    3) 65°C for 6-8 hrs
    4) 60°C overnight

Step 4. 1st Capture

Note: Warm the W1 and W2 buffer at 37°C until the buffer is clear. Invert the bottle several times to mix.

1. Resuspend MyOne Streptavidin C1 Dynabeads, transfer 20 μl of Dynabeads to 96-well plate
2. Place plate on magnet for 1-2 min, aspirate supernatant.
3. Remove plate from magnet and resuspend Dynabeads with 21 μl of RS Buffer.
4. Transfer 120 μl of hybridized sample to 21 μl of Dynabeads solution, mix by pipette. Incubate at room temperature for 5 min.
5. W1 washing:
    a. Place plate on magnet for 1-2 min, aspirate supernatant.
    b. Remove plate from magnet, add 200 μl of W1 Buffer, resuspend Dynabeads by pipetting, and incubate at room temperature for 2 min.
    c. Place plate on magnet for 1-2 min, and aspirate supernatant.
    d. Repeat W1 washing one more time.
6. Add 200 μl of 80% ethanol gently without disturbing Dynabeads, incubate at room temperature for 30 sec, aspirate supernatant COMPLETELY.
7. Air dry beads for 4 min.
8. Remove plate from magnet and resuspend Dynabeads in 26 μl of water. Seal the plate and heated at 95°C for 3 min, chill plate on ice.
9. Place plate on magnet, transfer 23 μl of supernatant to a new PCR plate.

Step 5. PCR: Amplify captured NGS library

1. Mix the following in the new PCR plate:

Captured library (from step 4)	23
Non-index Primers	2
PCR mix	25
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	10 cycles
Annealing/extension	65°C	70 seconds	
Final Extension	65°C	2 minutes	1
Hold	4°C		

Step 6. 2nd Hybridization

1. Warm probe at 37°C, invert the tube several times to dissolve. Mix the following in a 96-well plate:

MHC-III-B Probe	30
PCR mixture (from step 5)	50
Total	80 μl

2. Overlay a drop of mineral oil in wells with above mix. Seal the wells with cap or sealing tape.
3. Incubate at 95°C for 5 min
4. Put plate in the hybridization oven, hybridize with the following condition:
    1) 75°C for 6-8 hrs
    2) 70°C overnight
    3) 65°C for 6-8 hrs
    4) 60°C overnight

Step 7. 2nd Capture

Note: Warm the W1 and W2 buffer at 37°C until the buffer is clear. Invert the bottle several times to mix.

1. Resuspend MyOne Streptavidin C1 Dynabeads, transfer 20 μl of Dynabeads to 96-well plate.
2. Place plate on magnet for 1-2 min, aspirate supernatant.
3. Remove plate from magnet and resuspend Dynabeads with 14 μl of RS Buffer.
4. Transfer 80 μl of hybridized sample to 14 μl of Dynabeads solution, mix by pipette. Incubate at room temperature for 5 min.
5. W1 washing:
    a. Place plate on magnet for 1-2 min, aspirate supernatant.
    b. Remove plate from magnet, add 200 μl of W1 Buffer, resuspend Dynabeads by pipetting, and incubate at room temperature for 2 min.
    c. Place plate on magnet for 1-2 min, and aspirate supernatant.
6. W2 washing:
    a. Remove plate from magnet, add 200 μl of W2 Buffer,
    b. Resuspend Dynabeads by pipetting, incubate plate at 65°C (water bath, heat block or thermal cycler) for 5 min,
    c. Place plate on magnet for 10 sec, aspirate supernatant.
    d. Repeat W2 washing one more time.
7. Add 200 μl of 80% ethanol gently without disturbing Dynabeads, incubate at room temperature for 30 sec, aspirate supernatant COMPLETELY.
8. Air dry beads for 4 min.
9. Remove plate from magnet and resuspend Dynabeads in 23 μl of water. Seal the plate and heated at 95°C for 3 min, chill plate on ice.
10. Place plate on magnet, transfer 20 μl of supernatant to a new PCR plate.

Step 8. Final PCR: Amplify captured NGS library

1. Mix the following in the new PCR plate:

Captured library (from step 7)	20
UDI Primers	5
PCR mix	25
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	12-17 cycles
Annealing/extension	65°C	70 seconds	
Final Extension	65°C	2 minutes	1
Hold	4°C		

3. Place PCR plate on ice. Load 5 μl PCR product on 2% agarose gel for confirmation of amplification. Add more PCR cycles if needed.

Step 9. Beads Purification

1. Resuspend SPRI 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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