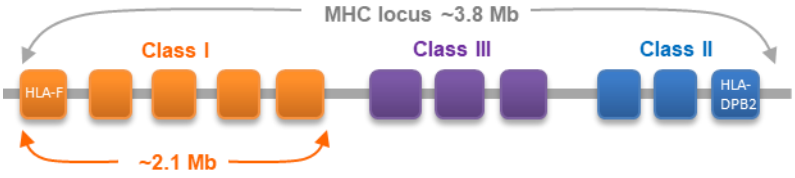
MHC Class I Capture Kit

Catalog No.	32013S	32013L
Reactions	24 reactions	96 reactions

Description

The **MHC Class I Capture Kit** was developed to capture the entire MHC Class I locus from whole genome NGS libraries based on the CATCH-Seq technology. Comprehensive sequencing of the entire Class I locus with our kit make it possible to detect SNPs, indels, and structural variants that are not covered by other MHC targeted sequencing reagents.



Our MHC Class I kit enables the most cost-effective targeted sequencing of 2.1 Mb of the human MHC Class I locus, including all coding and non-coding regions.

Captured sample multiplexing:

- **Single Index** (Cat. # 30072 Multiplexing Index Primers): For kits with 24 reactions..
- **Unique dual index** (Cat. # 30075 Multiplexing Unique Dual Index Primers): For kits with 96 reactions.

Features

- **Full MHC Class I region**
  - Covers 2.1 MB of MHC Class I 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.	32013S	32013L
MHC-I-B Probe	1440 ul	5760 ul
RS Buffer	672 ul	2688 ul
W1 Buffer	14.4 ml	57.6 ml
W2 Buffer	9.6 ml	38.4 ml

Storage Condition

- Store MHC-I-B Probe 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)
- PCR reagents
- Primers
- SPRI Beads (BioDynami Cat.# 40051) or equivalent
- Mineral oil

Protocol

Step 1. 1st Hybridization

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

MHC-I-B Probe	30
NGS Library (500 ng)	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. 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 2. 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 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.
    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 3. PCR: Amplify captured NGS library. Use below reaction as a reference.

1. Mix the following in 96-well PCR plate:

Captured library (from step 2)	23
Primers*	2
2X PCR mix*	25
Total	50 μl

* Not included in the kit

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 4. 2nd Hybridization

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

MHC-I-B Probe	30
PCR mixture (from step 3)	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 5. 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 26 μl of water. Seal the plate and heated at 95°C for 3 min, chill plate on ice.
10. Place plate on magnet, transfer 23 μl of supernatant to a new PCR plate.

Step 6. Final PCR: Amplify captured libraries. Use below reaction as a reference.

1. Mix the following in 96-well PCR plate:

Captured library	23
Primers*	2
2X PCR mix*	25
Total	50 μl

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	10-20 cycles**
Annealing/extension	65°C	70 seconds	
Final Extension	65°C	2 minutes	1
Hold	4°C		

* Not included in the kit
** As a reference:
10-15 cycles for 4 MB target region;
11-16 cycles for 2 MB target region;
12-17 cycles for 1 MB target region;
13-18 cycles for 500 KB target region;
14-19 cycles for 250 KB target region;

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 7. 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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