Complete MHC Capture Kit

v.1.4

Introduction

Complete MHC Capture Kit, using CATCH-Seq, provides efficient capture of specific target regions from whole genome NGS libraries. The advantages of the CATCH-Seq technology are:

Low cost:
CATCH-Seq technology reduces the cost of NGS. We provide the lowest price for capture reagent with high quality.

Sample multiplexing:
CATCH-Seq technology allows you to multiplex samples in one NGS run to further reduce your cost.

Multiple target regions possible:
Single or multiple target regions can be captured.

Large target region:
CATCH-Seq can cover several hundred kilobases to several megabases of contiguous sequence. CATCH-Seq can cover exons, introns, 5' regulatory regions, 3' regulatory regions, and beyond.

Simple and fast protocol:
- Three steps for CATCH-Seq:
  - Hybridization of sample library with probe
  - Capture of hybridized library
  - PCR amplification of captured library
- Two day processing time.

Bisulfite conversion:
Bisulfite conversion is incorporated into the CATCH-Seq protocol for customers interested in DNA methylation.

Component

- Probe cocktail (Product No. UB-CM1)
- Hyb buffer
- W1 buffer
- W2 buffer
- Sera-Mag SpeedBeads dilution buffer
  - NaCl 2.5 M
  - PEG-8000 20%

Storage

Store kit at -20°C, stable up to 1 year

Reagent & Equipment Needed (not provided in this kit)

- MyOne streptavidin C1 dynabeads (Invitrogen #650.02)
- Sera-Mag SpeedBeads, carboxylate-modified (ThermoFisher, #65152105050250)
- 70% ethanol
- Library amplification primers
- Tris-HCl (10 mM)
- PCR reagent (recommended: Invitrogen Platinum Taq Cat# 10966-018)
- Betaine (5M, PCR grade)
- Mineral oil
- 96 well plate
- Magnetic particle concentrator (Invitrogen Dynal MPC -96S, #120.27)
- PCR thermal cycler
- 65°C water bath or 65°C heat block (compatible with 96-well plate)

Protocol

Attention:

- The amount of each capture reaction is for a 500 ng NGS DNA library (fluorometry recommended).
- Increase or decrease in the amount of library may result in unexpected low enrichment.
- Avoid many freeze-and-thaw cycles of probe cocktail.

Step 1: Hybridization: Hybridize NGS DNA sample library with specific probe.

1. Mix probe cocktail with NGS sample library in 96-well plate as following:

Probe cocktail (UB-CM1)	45 μl
Library (500 ng)	15 μl
Total	60 μl

2. Overlay a drop of mineral oil in wells with above mix
3. Incubate at 95°C for 2 min
4. Hold at 65°C for 16~24 hrs

Step 2: Capture: Capture DNA fragments hybridized with probe.

Reminder:

- Set water bath or heat block at 65°C
- Pre-heat W2 buffer at 65°C
- Place the following buffers at room temperature:
  - Hyb buffer
  - W1 buffer
  - Tris-HCl (10 mM)

1. Votex to 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 in 20 μl of hyb buffer with a pipette.
4. Transfer 60 μl of hybridized sample to 20 μl of Dynabeads solution, mix by pipette. Incubate at room temperature for 5 min
5. W1 washing: place plate on magnet for 1-2 min, aspirate supernatant, add 200 μl of W1 buffer gently, incubate at room temperature for 2 min, aspirate supernatant.
6. W2 washing: add 200 μl of pre-heated (65°C) W2 buffer gently, remove plate from magnet and incubate plate at 65°C (water bath or heat block) for 2 min, place plate on magnet for 10 sec, aspirate supernatant. Repeat W2 washing one more time.
7. Resuspend library:

- For standard sequencing:
  1) Add 200 μl of 70% ethanol gently without disturbing Dynabeads pellet, incubate at room temperature for 30 sec, aspirate supernatant COMPLETELY.

2) Air dry beads for 4 min.
3) Remove plate from magnet and resuspend Dynabeads in 30 μl of water with pipette
4) Seal the plate and heated at 95°C for 3 min, chill plate on ice.
5) Place plate on magnet and carefully transfer supernatant to a new tube.

- For bisulfite sequencing:
  1) Remove plate from magnet and resuspend Dynabeads in 40 μl of 10 mM Tris.
  2) Perform bisulfite conversion: We recommend Qiagen EpiTect Bisulfite Kit (Qiagen Cat# 59104). The above mixture (resuspended sample with Dynabeads) can be directly used for bisulfite conversion. Elute final library in 30 μl of water after bisulfite conversion.

Step 3: PCR enrichment: Amplify captured NGS sample library.

Reminder:
Sample multiplexing is possible if indexed primers are used for amplification of captured libraries.

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

Captured library	28
10X PCR buffer	5
dNTP (10 mM)	1
$MgCl_2$ (50 mM)	1.5
Forward primer (10 μM)	1
Reverse primer (10 μM)	1
Betaine (5 M)	12
Platinum Taq (Invitrogen)	0.5
Total	50 μl

2. Load sample on thermal cycler with the following reaction condition:
   95C 3 min, followed by 18-22 cycles of: 95C 30 sec
   62C 3 min
   Usually 18-22 PCR cycles are enough in most cases; fewer may be required.
3. Place PCR plate on ice. Load 5 μl PCR product on 2% agarose gel for confirmation of amplification.
4. Add more PCR cycles if needed.
5. Sera beads purification of PCR product (~45 μl)
   1)Add equal volume of 10X diluted Sera beads (Sera beads diluted with Sera beads dilution buffer) to PCR sample, mix by pipette, incubate for 4 min
   2)Load onto magnetic particle concentrator, incubate for 3 min, aspirate supernatant
   3)Add 180 μl of 70% ethanol, incubate for 2~3 min, aspirate supernatant. Repeat 70% ethanol washing one more time.
   4)Leave beads on magnet for 8 min to dry beads (DO NOT over dry beads)
   5)Remove plate from magnet, add 10-20 μl of water and mix by pipette
   6)Place plate on magnet, incubate for 1 min, transfer supernatant (containing final library) to a new tube
6. Quantification of final library: we recommended fluorometry or bioanalyzer for accurate quantification.

7. Sample multiplex (optional): sample multiplex is OK if index primers are used for amplification of captured libraries. Pool equal amount of each sample in one tube, quantify the concentration of mixed samples by fluorometry or bioanalyzer.
8. Library is ready for sequencing.

Related products

- **CATCH-Seq Target Enrichment Kit**
- **Complete MHC+LRC Capture Kit**
- **Total Human Depletion Kit**
- **Human Genome Enrichment Kit**

Ubiquity Genomics Inc.
601 Genome Way
Huntsville, AL 35806
Tel: 256-327-9833

For information and sales:
info@ubiquitygenomics.com

For technical support:
support@ubiquitygenomics.com