diagenode

Innovating Epigenetics Solutions

MagMeDIP-seq Package

Magnetic Methylated DNA Immunopreciptation Package for NGS

Cat. No. C02010040 (10 rxns)

PACK CONTENT

Cat. No. C02010020 MagMeDIP qPCR Kit (x 10)
C05010020 iDeal Library Preparation kit (x 24)
C03010014 IPure kit v2 (x24)

USER GUIDE

Version 2 10_2020

Please read this manual carefully
before starting your experiment

Contents

Introduction

DNA methylation is a key epigenetic mechanism with important regulatory functions in biological processes such as genomic imprinting, control of transcription, embryonic development, X-chromosome inactivation, chromosome stability, and carcinogenesis.

DNA methylation occurs primarily as 5-methylcytosine (5-mC), and the Diagenode MagMeDIP kits take advantage of a specific antibody targeting this 5-mC to immunoprecipitate methylated DNA, which can be analyzed by qPCR or Next-Generation Sequencing (NGS). This methylation analysis is fast and highly specific as the IP has been optimized to specifically select and precipitate the methylated DNA by the use of our 5-mC monoclonal antibody 33D3 (Cat. No. C15200081), optimized buffers and protocol.

This package contains:

- MagMeDIP qPCR Kit (x10) including:
  - A XL GenDNA Extraction Module to prepare large amounts of DNA from cultured cells
  - Highly validated DNA shearing protocols on Diagenode Bioruptor®
  - All the reagents for the IP, including 5-mC antibody 33D3 and magnetic beads
  - Methylated and unmethylated DNA spike-in controls and their associated qPCR primers, allowing quality control of the IP
  - qPCR primers targeting positive and negative regions on human genome to monitor the success of the IP
- iPure Kit (x24) for highest quality purification before NGS
- iDeal Library Preparation Kit (x24) + SYBR to obtain a ready-to-sequence DNA on the Illumina platform

We recommend Diagenode's magnetic racks: DiaMag1.5 (Cat. No. B04000003) and DiaMag02 (Cat. No. B04000001) together with our MagMeDIP Kits. Diagenode's magnetic racks are designed to be used in IP experiments, keeping samples cool longer and allowing the use of small tubes to reduce the reaction volumes and use of reagents.

The kit ensures the use of a low amount of antibodies and buffers per reaction. The number of steps is reduced and handling is easier which makes this kit cost effective and simple to work with.

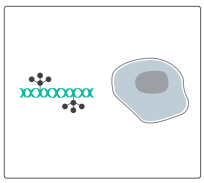
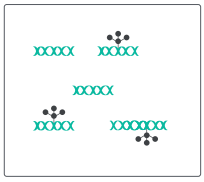
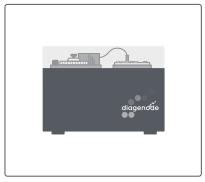
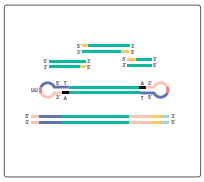
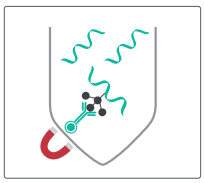
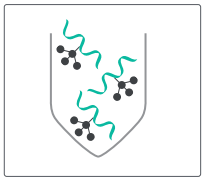
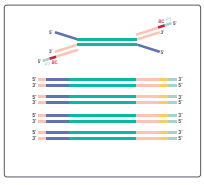
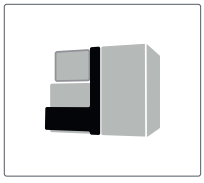
Diagenode's MagMeDIP-seq Package is perfectly designed for your high quality NGS analysis.

The MagMeDIP-seq Package can be used with DNA from every species.

Diagenode MagMeDIP Kits have been cited in many publications and chosen by leading laboratories worldwide thanks to their numerous benefits:

- Complete kits including DNA extraction module, antibody, spike-in controls and qPCR primer pairs for control regions
- Easy to use with user-friendly magnetic beads and racks
- Highly validated protocols compatible with qPCR (MagMeDIP qPCR Kit) and NGS downstream analyses (MagMeDIP-seq Package)
- MagMeDIP qPCR Kit is available as an automated version

Kit Method Overview & Timetable

Kit				Time needed	Day
MagMeDIP	STEP 1		Cell collection & lysis	30 min, overnight	1-2
	STEP 2		DNA extraction & purification	5 h	2
	STEP 3		DNA shearing	2 h	3
iDeal library prep	STEP 4		Library preparation	3 h	3
MagMeDIP	STEP 5		Methylated DNA immunoprecipitation	45 min, overnight	3-4
IPure	STEP 6		Methylated DNA isolation	90 min	4
iDeal library prep	STEP 7		Library amplification	45 min	4
	STEP 8		Quality control & Next-Generation Sequencing		

LEGEND

Methylated DNA (5-mC)

Unmethylated DNA

Magnetic bead

Sequencing adaptors

Antibody

Magnet

Kit materials

The MagMeDIP-seq Package is available in one format so that the kit content (Table below) is sufficient to perform 6 DNA extractions of 10 µg each and 10 methylated DNA Immunoprecipitations with 20 purifications and 20 library preparations.

***NOTE**: Upon receipt, store the components at the indicated temperatures.*

Table 1. Components of the MagMeDIP-seq Package

Component	Quantity	Storage
Magbeads (magnetic beads)	150 µl	4°C Do not freeze
Water	2x 2 ml	4°C
MagBuffer A (5x concentrated)	2 ml	4°C
MagBuffer B	100 µl	4°C
MagBuffer C	40 µl	-20°C
Antibody anti-5mC* (33D3 clone)	5 µl	-80°C
Methylated spike-in control	6 µl	-20°C
Unmethylated spike-in control	6 µl	-20°C
MagWash buffer-1	6 ml	4°C
MagWash buffer-2	4 ml	4°C
DNA Isolation Buffer (DIB)	4 ml	4°C
Proteinase K (100x)	20 µl	-20°C
Primer pair for Methylated spike-in ctrl (5 µM each)	50 µl	-20°C
Primer pair for Unmethylated spike-in ctrl (5 µM each)	50 µl	-20°C
Human TSH2B primer pair (5 µM each)	50 µl	-20°C
Human GAPDH primer pair (5 µM each)	50 µl	-20°C
200 µl tube strips	2	RT
Cap strips	2	RT
GenDNA Digestion Buffer	3 ml	4°C
GenDNA Proteinase K (200x)	300 µg/15 µl	-20°C
GenDNA precipitant	3 ml	4°C
GenDNA TE	3 ml	4°C
GenDNA RNAse (DNAse free)	5 µg/10 µl	-20°C

**Avoid freeze-thawing cycles of this very sensitive antibody! Make aliquots!*

iPure Kit v2 (x24)		
Component	**Quantity**	**Storage**
200 µl tube strips (8 tubes/strip) + cap strips	4 pc	RT
Buffer A	2.8 ml	4°C
Buffer B	115 µl	4°C
Wash buffer 1 w/o isopropanol	1.5 ml	4°C
Wash buffer 2 w/o isopropanol	1.5 ml	4°C
Buffer C	1.2 ml	4°C
iPure Beads v2	240 µl	4°C
Carrier**	48 µl	-20°C

***This product is shipped at 4°C. Store it at -20°C upon arrival. This is an optimized buffer (NOT CARRIER DNA).*

iDeal Library Preparation Kit (x24) + SYBR	
Component	**Storage**
iDeal Library End Repair/dA-Tailing Enzyme (green)	-20°C
iDeal Library End Repair/dA-Tailing Buffer (green)	-20°C
iDeal Library Ligation Master Mix (red)	-20°C
iDeal Library Ligation Enhancer (red)	-20°C
iDeal Library PCR Master Mix (blue)	-20°C
iDeal Library Adaptor for Illumina (red)	-20°C
iDeal Library Uracil Excision Reagent (red)	-20°C
iDeal Library Universal PCR Primer for Illumina (blue)	-20°C
100 x SYBR (brown tube)	-20°C

iDeal Library Preparation Kit (x24) + SYBR				
Component	**Index Primer Sequence**	**Expected index Primer Sequence Read**	**Quantity**	**Storage**
iDeal Library Index 1 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATCGTGATGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ATCACG	10 µl	-20°C
iDeal Library Index 2 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATACATCGGTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CGATGT	10 µl	-20°C
iDeal Library Index 3 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATGCCTAAGTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	TTAGGC	10 µl	-20°C
iDeal Library Index 4 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATTGGTCAGTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	TGACCA	10 µl	-20°C
iDeal Library Index 5 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATCACTGTGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ACAGTG	10 µl	-20°C
iDeal Library Index 6 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATATTGGCGTG ACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GCCAAT	10 µl	-20°C
iDeal Library Index 7 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATGATCTGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CAGATC	10 µl	-20°C
iDeal Library Index 8 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATTCAAGTGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	ACTTGA	10 µl	-20°C
iDeal Library Index 9 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATCTGATCGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GATCAG	10 µl	-20°C
iDeal Library Index 10 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATAAGCTAGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	TAGCTT	10 µl	-20°C
iDeal Library Index 11 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATGTAGCCGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	GGCTAC	10 µl	-20°C
iDeal Library Index 12 Primer for Illumina	5'-CAAGCAGAAGACGGCATACGAGATTACAAGGTGA CTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3'	CTTGTA	10 µl	-20°C

Where -s- indicates phosphorothioate bond.

***NOTE**: If fewer than 12 indexes are used in a lane for sequencing, it is recommended to use the following indexes:*

- *Pool of 2 samples: Index #6 and 12*
- *Pool of 3 samples: Index #4, 6 and 12*
- *Pool of 6 samples: Index #2, 4, 5, 6, 7 and 12*

Required materials not provided

Materials and Reagents

- Gloves to wear at all steps
- RNAse/DNase-free 1.5ml, 2ml and 50ml conical tubes
- Ice-cold PBS buffer
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol
- Ethanol
- Nuclease-free Water
- 10 mM Tris-HCl, pH 8.0 or 0.1X TE
- AMPure® XP Beads (Beckman Coulter, Inc. #A63881)
- Isopropanol

Equipment

- Centrifuges for 1.5 ml tubes and 50 ml conical tubes (4°C)
- Microcentrifuge for 0.2 ml tubes
- Thermomixer (95°C)
- Thermocycler
- Cell counter
- Magnetic rack – DiaMag 1.5ml (Cat. No. B04000003)
- Magnetic rack – DiaMag 0.2ml (Cat. No. B04000001)
- Rotating wheel such as DiaMag Rotator (Cat. No. B05000001)
- Bioruptor sonication device from Diagenode and the associated tubes:
  - **Bioruptor Pico (Diagenode, Cat. No. B01060010)**, 0.5/0.65 ml tube holder (Cat. No. B01200043) or the holder (Cat. No. B01200051) with the 0.65 ml adaptor (Cat. No. B01200054), and 0.65 ml Bioruptor Microtubes (Cat. No. C30010011) or

- **Bioruptor Plus (Diagenode, Cat. No. B01020003)**, 0.5/0.65 ml tube holder (Cat. No. B01200043), and 0.5 ml TPX Microtubes (Cat. No. C30010013)

- Reagents and equipment for quantitative PCR
- Reagents and equipment for DNA analysis such as electrophoresis on agarose gel, BioAnalyzer (Agilent) or Fragment Analyzer (Agilent)
- Reagents and equipment for DNA quantification such as Qubit® Fluorometer (ThermoFisher Scientific)

Optional supplies available seperately

The MagMeDIP-seq Package contains all reagents you need from your DNA extraction to your MeDIP assay and your library preparation. However four of the products mentioned in the protocol can be purchased additionally if needed:

Additionnal supplies (included and available separately)

Component	Cat. No.	Format
Human meDNA primer pair (TSH2B)	C17011041-500	500 µl
Human unDNA primer pair (GAPDH)	C17011047-500	500 µl
XL GenDNA Extraction Module	C03030020	500 µl
Antibody anti-5-mC (33D3 clone)	C15200081	10/100/500 µg

Optional supplies (not included and available separately)

Component	Cat. No.	Format
DNA Methylation control package	C02040012	40 rxns
Mouse meDNA primer pair (TSH2B)	C17021042-500	500 µl
Mouse unDNA primer pair (GAPDH)	C17021045-500	500 µl
Rat meDNA primer pair (TSH2B)	C17031043-500	500 µl
Rat unDNA primer pair (GAPDH)	C17031046-500	500 µl
Index Primer Set 2 for iDeal Library Prep	C05010021	12 indexes

Remarks before starting

DNA extraction

The quality of the DNA to be used in MagMeDIP is important. Thus, we recommend using Diagenode XL GenDNA Extraction Module (included in this kit) for the DNA extraction. It was optimized for the preparation of genomic DNA (gDNA) from cultured cells.

The XL GenDNA Extraction Module for gDNA isolation provides sufficient volume of reagents for the preparation of 6 x 10 µg gDNA batches, each obtained from 1 to 1.5 million cultured cells.

Starting amount

Concentrations of all the reagents in the MagMeDIP-seq Package are optimized for a starting amount of 1 µg of sheared DNA per reaction.

> ***NOTE**: If starting material is limiting, it is possible to decrease the starting amount of sheared DNA down to 100 ng per reaction, but this may result in an increase of the background.*

Guidelines regarding the required amount of cells:

- From 1.5 million cells, 8 to 12 µg of gDNA can be expected
- Some of the isolated gDNA will be used as a control for DNA preparation efficiency
- 1 µg of sheared DNA is needed per IP
- Some of the sheared DNA will be used as a control checking for: (a) shearing efficiency and (b) the IP experiment efficiency (see section Input below)

DNA shearing

For an efficient and best resolution IP experiment, the gDNA has to be sheared into fragments around 200 bp on the Bioruptor Pico (Cat. No. B01060010) or on the Bioruptor Plus (Cat. No. B01020001).

Before using the sheared DNA, we recommend analyzing the size of the fragments after shearing using a dedicated device such as the BioAnalyzer (Agilent) or the Fragment Analyzer (Agilent).

Magnetic beads

This kit includes magnetic beads (Magbeads). Please make sure the beads do not dry out during the procedure as this may result in reduced performance. Keep the Magbeads homogenous in suspension at all times when pipetting. Variation in the amount of beads will decrease reproducibility. Do not freeze the Magbeads.

Input

"Input sample" corresponds to the sheared DNA that undergoes the full MeDIP-seq procedure without incubation with the antibody and magnetic beads (Magbeads). The input sample is used as a reference to calculate the recovery by qPCR. It is also used by most bioinformatics tools for analysis of MeDIP-seq data. It serves to determine the bias which may result from experimental condition. We recommend including one input sample for each sheared DNA sample that undergoes MeDIP-seq.

Methylated and unmethylated spike-in controls

The MagMeDIP-seq Package contains one methylated and one unmethylated spike-in control that can be added directly to DNA samples before the IP. Those two spike-in controls are not homologous to any model species and will not interfere with the sample of interest.

To check the efficiency of the MeDIP experiment, this kit also includes primer pairs (targeting methylated and unmethylated spike-in controls' regions) to calculate their recovery by qPCR.

> ***CAUTION:*** *If you use the controls provided with the separate* ***DNA Methylation control package (C02040012)*** *as spike-in controls, please note that they can interfere with plant species. Please check FAQ section p.43*

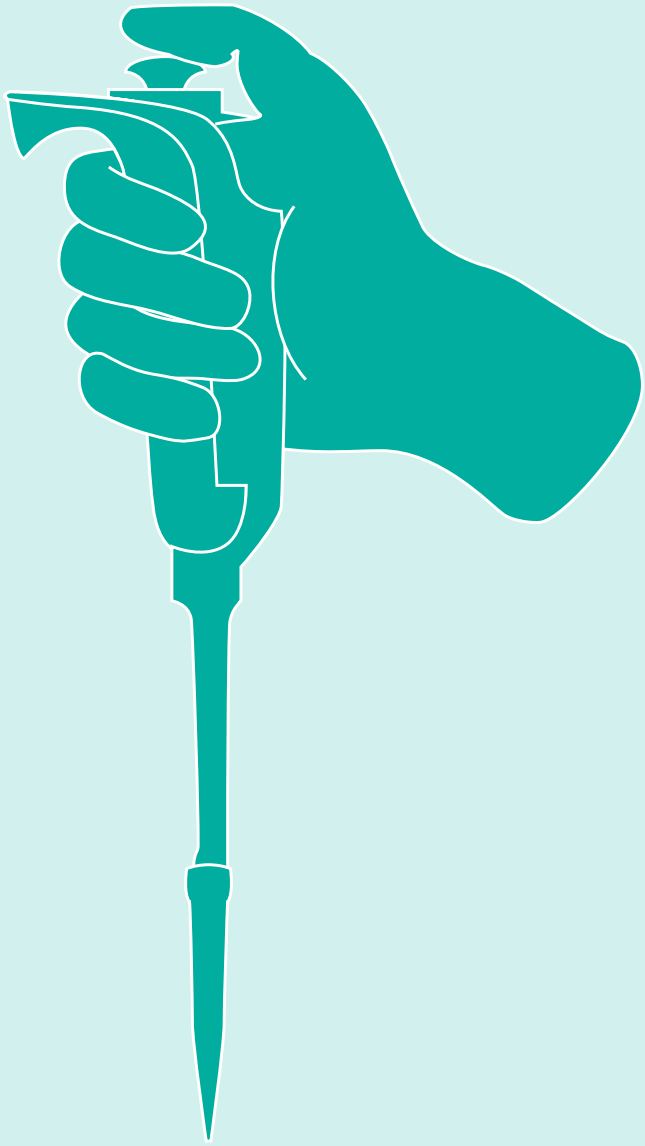
Technique for DNA isolation

The MagMeDIP qPCR Kit includes a DNA Isolation Buffer (DIB) providing DNA suitable for qPCR analysis. However DNA of higher purity is needed for Next-Generation Sequencing (NGS), therefore we recommend using the IPure kit v2 (Cat. No. C03010015) instead. The IPure kit v2 is the only DNA purification kit that is specifically optimized for extracting very low amounts of DNA after MeDIP.

Quantitative PCR analysis

This kit contains two primer pairs targeting methylated (TSH2B) and unmethylated (GAPDH) human regions. For each primer pair, run the input sample alongside the IP samples. As downstream analysis, qPCR reactions are recommended at least in duplicates (although triplicates are recommended as for potential outliers' identification).

PROTOCOL



STEP 1

Cell collection & lysis

1 *Day 1* *30 minutes, overnight*

MAGMEDIP QPCR KIT CAT. NO. C02010020

Starting material: cultured cells

***NOTE**: For recommendations about starting amounts of material, please refer to section "Remarks before starting"*

1.1 Pellet suspension culture out of its serum containing medium or trypsinize adherent cells and collect cells from the flask. Centrifuge at 300g for 5 minutes at 4°C.

1.2 Discard the supernatant. Resuspend cells in **5 to 10 ml ice-cold PBS**. Count cells. Centrifuge at 300g for 5 minutes. Discard the supernatant. Repeat this resuspension and centrifugation step once more. This step is to wash the cells.

- Meanwhile, place the GenDNA Digestion Buffer at room temperature and the GenDNA Proteinase K on ice (to be used at Point 1.3 below).

***NOTE**: If needed, cell pellets can be fresh frozen at this step and stored at -80°C for several months.*

1.3 Prepare the complete Digestion Buffer by adding **5 µl GenDNA Proteinase K** to **1 ml GenDNA Digestion Buffer**.

1.4 Resuspend cells in complete Digestion Buffer.

- For 1 to 1.5 million cells, use up to **500 µl complete Digestion Buffer**.
- It might be necessary to use more buffer to avoid viscosity when performing the extractions.

1.5 Incubate the samples with shaking (500 rpm) at 50°C for 12 to 18 hours in tightly capped tubes.

STEP 2

Nucleic acid extraction & purification

2 *Day 2* *5 hours*

MAGMEDIP QPCR KIT CAT. NO. C02010020

2.1 Thoroughly extract the samples with an equal volume of phenol/chloroform/isoamyl alcohol (work under a fume-hood).

- Add **1 volume** (500 µl according to step 1.4) **of phenol/chloroform/isoamyl alcohol (25:24:1)**.
- Incubate the samples at room temperature for 10 minutes on a rotating wheel. Use gentle rotation, do not vortex.

2.2 Centrifuge at 1700g for 10 minutes.

- If the phases do not resolve properly, add another volume of GenDNA Digestion Buffer omitting Proteinase K, and repeat the centrifugation.
- If there is a thick layer of white material at the interface between the phases, repeat the extraction.

2.3 Transfer the aqueous (top) layer to a new tube.Increase volume to avoid viscosity if necessary and pipette slowly.

2.4 Thoroughly extract the samples with **500 µl of chloroform/isoamyl alcohol (one volume)**.

2.5 Incubate for 10 minutes at room temperature.

2.6 Centrifuge at 1700g for 10 minutes.

2.7 Transfer aqueous layer to a new 2 ml tube.

2.8 Add **250 µl of GenDNA precipitant** (which is ½ volume) and **1 ml of 100% ethanol** (2 volumes).

2.9 Recover gDNA by centrifugation at 1700g for 5 minutes.

- Do not use higher speed to avoid genomic DNA fragmentation
- This brief precipitation in the presence of an optimized high salt precipitant (GenDNA precipitant) reduces the amount of RNA in the DNA sample. For long-term storage, it is convenient to leave the DNA in the presence of ethanol.

2.10 Rinse the pellet with **70% ethanol**. Decant ethanol and air-dry the pellet.

- It is important to rinse extensively to remove any residual salt or phenol

2.11 Resuspend the pellet of gDNA at **~1 mg/ml in GenDNA TE** until dissolved. Shake gently at room temperature or at 65°C for several hours to facilitate solubilization. Store at 4°C.

- From 1-1.5 million cells, ~8 to 12 µg of gDNA can be expected (in a volume of 8 to 12 µl)
- From 3 million cells, ~20 to 30 µg of gDNA can be expected (in a volume of 20 to 30 µl)

NOTE: *At this step the DNA quality can be analyzed by electrophoresis on 0.8% agarose gel or with a Fragment Analyzer (Agilent) and Standard Sensitivity Genomic DNA Analysis Kit (DNF-487, Agilent) (please refer to manufacturer's instructions).*

2.12 If present, residual RNA has to be removed at this step by adding **2 µl of GenDNA RNAse (DNAse-free)** per ml of DNA sample and incubating 1 hour at 37°C, followed by phenol/chloroform extraction and ethanol precipitation (same protocol as above, starting from step 2.1).

2.13 Store the gDNA at 4°C until the shearing.

NOTE*: For long term storage the gDNA can be placed at -20°C.*

STEP 3

DNA shearing

3 *Day 3* *2 hours*

MAGMEDIP QPCR KIT CAT. NO. C02010020

Genomic DNA must be randomly sheared by sonication on Diagenode's Bioruptor to generate fragments around 400 bp (see Figure 1 in"Example of results" section). To perform the MagMeDIP, 1.2 µg of sheared DNA is needed in a volume smaller than 55 µl.

3.1 Dilute the required amount of DNA sample in GenDNA TE and transfer DNA sample into appropriate sonication tubes (see below).

3.2 Shear DNA by sonication using the Bioruptor. Choose the protocol and consumables which are adapted to your device.

***CAUTION**: Only use the recommended tubes for high quality results.*

- When using the **Bioruptor Pico** (Cat. No. B01060010) use 0.65 ml Bioruptor Microtubes (Cat. No. C30010011) and the holder (Cat. No. B01200051) with the 0.65 ml adaptor (Cat. No. B01200054) and shear your 100 µl samples for 13 cycles (sonication parameters: 30'' ON and 30'' OFF).

***NOTE:** For a volume of sample below 100 µl, use 0.2 ml Microtubes for Bioruptor Pico (Cat. No. C30010020) and the holder (Cat. No. B01200051) with the 0.2 ml adaptor (Cat. No. B01200053) for 16 samples, then follow the protocol https://www.diagenode.com/files/protocols/protocol-dna-shearing-tubes-02ml.pdf*

- When using the **Bioruptor Plus** (Cat. No. B01020001) use Diagenode 0.5 ml Microtubes (Cat. No. C30010013) with the 0.5 ml tube holder (Cat. No. B01200043) and shear your 100 µl samples for 30 cycles (sonication parameters: 30'' ON and 30''

STEP 4

Library preparation

3 *Day 3* *3 hours*

IDEAL LIBRARY PREPARATION KIT CAT. NO. C05010020

END PREPARATION

4.1 Mix the following components in a nuclease-free tube:

iDeal LibraryEnd Repair/dA-Tailing Enzyme Mix (green)	3 µl
iDeal Library End Repair/dA-Tailing Buffer (green)	6.5 µl
Sheared DNA (100 ng/µl)	12 µl*
Nuclease-free water	43.5 µl*
TOTAL volume	65 µl

**__NOTE__: If the DNA sample is not at a concentration of 100 ng/µl, adjust the volume of water and DNA: 1.2 µg of DNA is required and the total volume of incubation mix must remain 65 µl.*

4.2 Mix by pipetting followed by a quick spin to collect all liquid from the sides of the tube.

4.3 Place in a thermocycler, with the heated lid on, and run the following program:

Temperature	Time
20°C	30 minutes
65°C	30 minutes
4°C	Hold

ADAPTOR LIGATION

4.4 Add the following components and mix well:

iDeal Library Ligation Master Mix (red)	15 µl
iDeal Library Adaptor for Illumina (red)	2.5 µl
iDeal Library Ligation Enhancer (red)	1 µl
TOTAL volume	83.5 µl

4.5 Incubate at 20°C for 15 minutes in a thermal cycler. Add **3 µl of iDeal Library Uracil Excision Reagent (red)**. Mix well and incubate at 37°C for 15 minutes.

SIZE SELECTION

CAUTION: *The following size selection protocol is for libraries with 200 bp inserts only. For libraries with different size of inserts, refer to Table 2 for the appropriate volume of beads to be added. The size selection protocol is based on a starting volume of 100 µl.*

4.6 Add **13.5 µl of nuclease-free water** to each sample to have a final volume of 100 µl.

4.7 Carefully resuspend the room temperature AMPure XP beads by shaking and light vortexing until no pellet is visible at the bottom of the container.

4.8 Add **55 µl of AMPure XP beads**. Mix by pipetting 8 – 10 times until the mixture is homogeneous. Incubate at room temperature for 10 minutes.

4.9 Quickly spin the tube, place it on the DiaMag02 and wait until the beads are completely bound to the magnet (~ 2 minutes).

4.10 Without disturbing the pellet, carefully aspirate and transfer the supernatant to a new tube. Discard the beads that contain the unwanted large fragments.

4.11 Add **25 µl of AMPure XP beads**. Mix by pipetting 8 – 10 times until the mixture is homogeneous. Incubate at room temperature for 10 minutes.

4.12 Quickly spin the tube, place it on the DiaMag02 and wait until the beads are completely bound to the magnet (~ 2 minutes).

4.13 Carefully aspirate by pipette and discard the supernatant without disturbing the beads that contain the desired DNA targets. Wash the beads pellet 2 times as follows:

- With the tubes on the magnet, add **100 µl** of freshly prepared **80% ethanol** without disturbing the bead pellet and wait for 5 seconds.
- Carefully aspirate by pipette and discard the supernatants without disturbing the pellet.

4.14 Leaving the tube open, let dry the beads on the DiaMag02 for 3 minutes.

4.15 Remove tubes from DiaMag02 and elute DNA by resuspending the beads in **25 µl of 10 mM Tris-HCl** or 0.1 X TE, pH 8.0. Incubate for 10 minutes at room temperature.

4.16 Quickly spin the tube, place it on the DiaMag02 and wait until the beads are completely bound to the magnet (~ 2 minutes).

4.17 Without disturbing the pellet, carefully aspirate and transfer 23 µl of supernatant to a new tube.

4.18 The library is now ready for selection of methylated DNA by immunoprecipitation. Proceed to step 5 by using the MagMeDIP kit.

Table 2. Recommended conditions for bead based size selection.

	Approximate insert size	150 bp	200 bp	250 bp	300-400 bp	400-500 bp	500-700 bp
Library parameters	Total Library size (insert + adaptor)	270 bp	320 bp	400 bp	400-500 bp	500-600 bp	600-800 bp
Volume to be added	1st Bead Selection	65 µl	55 µl	45 µl	40 µl	35 µl	30 µl
	2nd Bead Selection	25 µl	25 µl	25 µl	20 µl	15 µl	15 µl

STEP 5

Methylated DNA immunoprecipitation

3-4 *Day 3-4* *90 minutes, overnight*

MAGMEDIP QPCR KIT CAT. NO. C02010020

BEADS PREPARATION

5.1 Determine the number of IP reactions to be run. It is recommended to perform two IPs per DNA sample. Input will not undergo immunoprecipitation and is therefore not considered as an IP.

5.2 Prepare **MagBuffer A 1x** (bead wash buffer) as described in the table below. The volumes contain a sufficient excess.

	1 IP	2 IPs	3 IPs	4 IPs	5 IPs	6 IPs	7 IPs	8 IPs	9 IPs	10 IPs
MagBuffer A (5x)	20 µl	40 µl	60 µl	80 µl	100 µl	120 µl	140 µl	160 µl	180 µl	200 µl
Water	80 µl	160 µl	240 µl	320 µl	400 µl	480 µl	560 µl	640 µl	720 µl	800 µl

5.3 Take the required amount of **Magnetic beads (Magbeads)** and transfer it to a clean 1.5 ml tube. **11 µl of beads** are needed **per IP**.

***NOTE**: Keep beads in liquid suspension at all handling steps, as drying will result in reduced performance.*

5.4 Place the tube on the DiaMag1.5 to discard the supernatant. Keep the beads.

5.5 Wash the magnetic beads (Magbeads) twice with ice-cold MagBuffer A 1x (bead wash buffer). To wash the beads, add the required volume of MagBuffer A 1x directly to the beads: **27.5 µl of MagBuffer A 1x** are needed **per IP**. Resuspend the beads by pipetting up and down

several times, spin the tubes and place them in the DiaMag1.5. Wait for 1 minute to allow the beads to be captured by the magnet and remove the supernatant.

5.6 After washing, resuspend the beads in **22 µl of MagBuffer A 1x per IP**. Keep on ice.

***CAUTION**: Do not freeze the beads.*

DNA IMMUNOPRECIPITATION

5.7 In a new 1.5 ml tube, prepare the Mag master mix as described in the following table. The volumes also include all inputs needed (1 input/IP) and contain a sufficient excess.

Reagent (µl)	1 IPs	2 IPs	3 IPs	4 IPs	5 IPs	6 IPs	7 IPs	8 IPs	9 IPs	10 IPs
MagBuffer A 5x*	24	60	84	108	132	168	192	216	240	264
MagBuffer B	6	15	21	27	33	42	48	54	60	66
meDNA spike-in control	0.3	0.75	1.05	1.35	1.65	2.1	2.4	2.7	3	3.3
unDNA spike-in control	0.3	0.75	1.05	1.35	1.65	2.1	2.4	2.7	3	3.3
Nuclease-free Water	2.4	6	8.4	10.8	13.2	16.8	19.2	21.6	24	26.4

** Contains detergent; if its appearance is cloudy and crystallized please warm gently prior to use.*

5.8 In 1.5 ml tubes, prepare the incubation mix as described in the table below.

	Volume per 1 IP + 1 input
Mag master mix	33 µl
Adaptor-ligated DNA	22 µl
Water	35 µl
TOTAL volume	90 µl

5.9 Incubate the incubation mix at 95°C for 3 minutes.

5.10 Quickly chill the incubation mix on ice. Perform a pulse spin to consolidate your sample.

5.11 Take out **7.5 µl (that will be your 10% input)** from each tube and transfer to a new labeled 0.2 ml tube (or 8tube-strip). Keep the input sample at 4°C: it is to be used as a control of starting material and it is therefore not to be used in IP.

5.12 Then, transfer **75 µl of incubation mix** for each IP into one 200 µl tube (using the provided 200 µl tube strips or individual 200 µl tubes that fit in the DiaMag02). Keep at 4°C.

5.13 In a new tube, dilute the antibody 1:2 with water (e.g. mix 1 µl of antibody with 1 µl of water).

***NOTES**:*

- *Discard remaining master mixes not used on the day.*
- *Do not omit the dilution step as the amount of antibody to be used is critical.*

5.14 Prepare the diluted antibody mix as described in the following table. The volumes contain a sufficient excess. **Mix the diluted antibody 1:2, MagBuffer A 5x and water first and add MagBuffer C at the end**.

Reagents (µl)	1 IP	2 IPs	3 IPs	4 IPs	5 IPs	6 IPs	7 IPs	8 IPs	9 IPs	10 IPs
Antibody 1:2	0.30	0,75	1.05	1.50	1.80	2.10	2.40	3.00	3.30	3.60
MagBuffer A 5x	0.60	1,50	2.10	3.00	3.60	4.20	4.80	6.00	6.60	7.20
Water	2.10	5,25	7.35	10.50	12.60	14.70	16.80	21.00	23.10	25.20
MagBuffer C	2.00	5,00	7.00	10.00	12.00	14.00	16.00	20.00	22.00	24.00
TOTAL volume	5.00	12,50	17.50	25.00	30.00	35.00	40.00	50.00	55.00	60.00

5.15 Add **5 µl of diluted antibody mix** to each 200 µl IP tube containing the incubation mix (from point 5.12)

5.16 Mix and add **20 µl of washed Magbeads** to each 200 µl IP tube (from point 5.6). The final volume is 100 µl.

***NOTE**: Keep the beads homogenously in suspension at all times when pipetting. Variation in the amount of beads will lead to lower reproducibility.*

5.17 Place on a rotating wheel at 4°C overnight.

DNA WASHES

5.18 Place the MagWash buffers and the DiaMag02 on ice.

5.19 Spin down and place the IP tubes in the ice-cold the DiaMag02, wait 1 minute and discard the buffer.

5.20 Wash the DNA IP Samples 3 times as follows. Add per tube, **100 µl ice-cold MagWash Buffer-1**, close the tube caps, invert the 8-tube strip to resuspend the beads, incubate for 5 minutes at 4°C on a rotating wheel (40 rpm), spin and place in the DiaMag02. Wait 1 minute and discard the buffer. Keep the captured beads.

***NOTE**:*

- *Do not disturb the captured beads attached to the tube wall.*
- *Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning in the DiaMag02.*

5.21 Wash the beads once with **100 µl ice-cold MagWash Buffer-2**.

5.22 After the last wash, discard the last traces of Wash Buffer and keep the bead pellets on ice. The bound DNA can be now purified from Magbeads.

STEP 6

Methylated DNA isolation

Day 4  *90 minutes*

IPURE KIT V2 CAT. NO. C03010014

DNA ELUTION

6.1 Take the input samples, centrifuge briefly and from now onwards treat the input DNA samples and IP samples in parallel.

6.2 Prepare the Elution Buffer by mixing Buffer A and B as follows:

Elution Buffer	1 rxn
Buffer A	115.4 µl
Buffer B	4.6 µl
TOTAL volume	120 µl

- Place Buffer A at 25°C during 30 minutes before use.
- **100 µl of Elution buffer** are needed per IPure reaction (20 µl excess).
- 1 IPure reaction corresponds to the purification 1 MeDIP or 1 input sample.

***CAUTION**: Make sure when working with Buffer A, that there are no crystals left in solution. Otherwise heat up gently and mix until complete disappearance of such crystals.*

6.3 Add **50 µl of Elution Buffer** to the bead pellets (tube strip) from step 5.22.

6.4 Add **92.5 µl of Elution Buffer** to your input sample in the 200 µl tube (or 8-tube strip) from step 5.11.

6.5 Incubate IP samples and input DNA for 15 minutes at room temperature on a rotating wheel (40 rpm).

6.6 Spin the 8-tube strip and place it into the DiaMag02. After 1 minute, transfer the supernatants to a new labelled 8-tube strip.

6.7 For the IP samples only, repeat the incubation of the bead pellets for 15 minutes at room temperature on a rotating wheel (40 rpm) in **50 µl Elution Buffer**.

- For input DNA samples: 1 elution in 100 µl.
- For IP samples: 2 elutions in 50 µl (total volume 100 µl).

6.8 Spin the 8-tube strip and place it into the DiaMag02. wait 1 minute and transfer the supernatants to a new 8-tube strip.

Elutions of IP and input samples are now completed in 100 µl.

DNA BINDING

6.9 Add **2 µl of carrier** to each IP and input sample. Vortex briefly and perform a short spin.

6.10 Add **100 µl of 100% isopropanol** to each IP and input sample. Vortex briefly and perform a short spin.

- Following the addition of isopropanol the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.

6.11 Resuspend the provided **magnetic beads** and transfer **10 µl** to each IP and input sample.

- Keep magnetic beads in liquid suspension during storage at

4°C and at all handling steps, as drying will result in reduced performance.

- The final volume is now 212 µl per IPure reaction.

6.12 Incubate IP and input samples for 10 minutes at room temperature on a rotating wheel (40 rpm).

DNA WASHING

6.13 When it is the first use of the kit prepare the Wash Buffer 1 containing 50% isopropanol as follows:

	24 rxns
Wash Buffer 1 w/o isopropanol	1.5 ml
Isopropanol (100%)	1.5 ml
Wash Buffer 1 TOTAL volume	3 ml

CAUTION: *Never leave the bottle open to avoid evaporation.*

6.14 Briefly spin the tubes, place in the DiaMag02, wait 1 minute and discard the supernatant.

6.15 Add **100 µl Wash Buffer** 1 per tube, close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).

- Do not disturb the captured beads attached to the tube wall.
- Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning into the DiaMag02. Rack.

6.16 When it is the first use of the kit prepare the Wash buffer 2 containing 50% isopropanol as follows:

	24 rxns
Wash Buffer 1 w/o isopropanol	1.5 ml
Isopropanol (100%)	1.5 ml
Wash Buffer 1 TOTAL volume	3 ml

6.17 Briefly spin the tubes, place into the DiaMag02, wait 1 minute and discard the buffer.

6.18 Add **100 µl Wash buffer 2** per tube, close the tubes, invert the 8 tube strip to resuspend the beads and incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).

- Do not disturb the captured beads attached to the tube wall.
- Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning in the DiaMag02.

DNA ELUTION

6.19 Briefly spin the tubes and place them into the DiaMag02, wait 1 minute and discard the buffer.

6.20 Add **25 µl Buffer C** per tube, close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 15 minutes at room temperature on a rotating wheel (40 rpm). Resuspend the pelleted beads using the pipet and make sure that you drop them on the bottom of the tube.

6.21 Spin the 8-tube strip and place it into the DiaMag02, wait 1 minute and transfer the supernatant into a new 1.5 ml tube.

6.22 Place the DNA on ice and check the IP success by qPCR (**Step 7**), proceed to library amplification using the iDeal Library Preparation Kit (**Step 8**) or store the DNA at -20°C or -80°C until further use.

STEP 7

qPCR on control regions

4 *Day 4* *› 2 hours*

MAGMEDIP QPCR KIT CAT. NO. C02010020

At this step it is possible to check the MeDIP efficiency by performing qPCR on a few microliters of each sample.

The MagMeDIP qPCR Kit includes four validated primer pairs:

1. Primer pair for Methylated spike-in ctrl (5µM each)
2. Primer pair for Unmethylated spike-in ctrl (5µM each)
3. Human TSH2B (also known as HIST1H2BA) primer pair (5µM each) (methylated region)
4. Human GAPDH primer pair (5µM each) (unmethylated region)

***NOTE**: Primer pairs for mouse and rat are available! For more information please visit www.diagenode.com*

7.1 Take an aliquote of the eluted DNA and dilute it 10 times (e.g. take **4.4 µl of eluted DNA** and add **39.6 µl of water**). **5 µl of the diluted DNA** are needed per reaction.

7.2 Prepare your qPCR mix using a SYBR Green PCR master mix and start qPCR

***NOTES**: We recommend performing qPCR in duplicates or triplicates.*

7.3 Example of qPCR mix:

Reagents	Volume for 1 reaction
Primer pair (Stock: 5 µM each)	1 µl
2x SYBR Green PCR master mix	12.5 µl
IP'ed diluted DNA	5 µl
Nuclease-free water	6.5 µl
TOTAL volume	25 µl

7.4 Use the following qPCR program:

Step	Temperature	Time & Cycles	
1. Denaturation*	95°C	7 minutes	
2. PCR Amplification	95°C	15 seconds	X 40 cycles
	60°C	60 seconds	
	95°C	1 minute	
3. Melting curve**	Follow qPCR instrument manufacturer recommendations		

**Please check carefully supplier's recommendations about Taq polymerase activation time and temperature.*
***Include and inspect the melting curves to ensure that primer pairs amplify only a single specific product.*

7.5 Data analysis. Some major advices how to analyse qPCR results are given below.

Data interpretation

The efficiency of methylated DNA immunoprecipitation of a particular genomic locus can be calculated from qPCR data and reported as a recovery of starting material using the following formula:

% recovery= 2^[Ct(10% input) - 3.32 - Ct(IP sample)] x 100

In this example, 2 is the amplification efficiency, Ct (IP sample) and Ct (10% input) are threshold values obtained from exponential phase of qPCR for the IP sample and input sample respectively; the compensatory factor (3.32) is used to take into account the dilution 1:10 of the input.

***NOTE**: This equation assumes that the qPCR is 100% efficient (amplification efficiency = 2). For accurate results, the amplification efficiency of the primer pairs has to be close to 100% meaning that for each cycle the amount of product doubles (E=2).The real amplification efficiency, if known, should be used.*

Background determination

The final goal of IP is to calculate the enrichment in the same IP sample of:

- The specific DNA fragments (corresponding to the methylated DNA) in comparison with background (corresponding to unmethylated DNA).
- This enrichment can be calculated as a ratio of specific signal over background.

enrichment= % recovery (specific locus) / % recovery (background locus)

STEP 8

Library amplification

4 *Day 4*  *› 2 hours*

IDEAL LIBRARY PREPARATION KIT CAT. NO. C05010020

OPTIMAL CYCLE NUMBER

Before the amplification, you will have to determine the optimal number of PCR cycles by doing a qPCR.

***NOTE**: For this step only 1 µl of each library will be used.*

8.1 Prepare the Quantification Mix as described in the table below for the number of desired reactions. Mix by pipetting and keep on ice until use.

Component	Volume per reaction
iDeal Library PCR Master Mix (blue)	5 µl
iDeal Library Index Primer 1 (blue)	0.1 µl*
iDeal Universal PCR Primer (blue)	0.1 µl*
100X SYBR (brown tube)	0.1 µl*
Nuclease-free water	3.7 µl
TOTAL Quantification Mix	9 µl

**If the number of reactions is small, an intermediate dilution can be used if the needed volume is too low for pipetting. The volume of nuclease-free water should be adapted to keep a final volume of 9 µl per reaction.*

8.2 Dispense **9 µl of the Quantification Mix** into 0.2 ml tubes or strips according to the number of libraries.

8.3 Add **1 µl of IP or input sample** in each tube and mix by pipetting.

8.4 Briefly spin the tubes and run the qPCR program described below.

Cycle step	Temperature	Time	Cycles
Initial denaturation	95°C	5 minutes	1
Denaturation	95°C	10 seconds	X 20
Annealing/extension	60°C	75 seconds	
Melting curve	Follow qPCR instrument manufacturer recommendations		

***CAUTION** : Keep IP and input samples on ice during qPCR.*

8.5 Analyse the Ct values. The optimal cycle number for the amplification of the rest of the MeDIP samples is typically Ct (rounded up)+2 (e.g. for a Ct of 6.82 use 9 amplification cycles).

***NOTE**: The Ct value is highly dependent on the thermocycler you use, as well as the way you analyse the qPCR results. Thus when using the kit for the first time you may need to verify that the Ct+2 rule applies well in your conditions. As general guidelines, for a MeDIP-seq experiment starting from 1 µg of untreated DNA, the number of cycles to apply is typically around 9 for IP samples and around 7 for input samples.*

PCR AMPLIFICATION

8.6 Mix the following components in sterile strip tubes:

Immunoprecipitated DNA	17 µl
iDeal Library PCR Master Mix 2X (blue)	25 µl
iDeal Library Index Primer* (blue)	0.5 µl
iDeal Universal PCR Primer* (blue)	0.5 µl
Nuclease-free water	7 µl
TOTAL volume	50 µl

**If fewer than 4 indexes are used in a lane for sequencing, it is recommended to use the following indexes:*

- *Pool of 2 samples: Index #6 and 12*
- *Pool of 3 samples: Index #4, 6 and 12*

8.7 Transfer tubes to a pre-programmed thermal cycler and incubate as follows.

Cycle step	Temperature	Time	Cycles
Initial denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	Ct+2
Annealing/extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

CLEAN-UP

8.8 Carefully resuspend the room temperature AMPure XP beads by shaking and light vortexing until no pellet is visible at the bottom of the container.

8.9 Add **55 µl of AMPure XP beads**. Mix by pippeting 8-10 times until the mixture is homogenous.

8.10 Incubate at room temperature for 10 minutes.

8.11 Quickly spin the tube, place it on the DiaMag02 and wait until the beads are completely bound to the magnet (~ 2 minutes).

8.12 Carefully aspirate by pipette and discard the supernatant without disturbing the beads that contain the desired DNA targets.

8.13 Wash the beads pellet 2 times as follows:

- With the tube on the magnet, add **100 µl** of freshly prepared **80% ethanol** without disturbing the beads pellet and wait for 5 seconds.
- Carefully aspirate by pipette and discard the supernatant without disturbing the pellet.

8.14 Leaving the tube open, let dry the beads on the DiaMag02 for 3 minutes.

Remove tubes from DiaMag02 and elute DNA by resuspending the beads in **33 µl of 10mM Tris-HCl** or **0.1XTE, pH 8**.

***NOTE**: If the DNA concentration is critical, it is possible to reduce the elution volume, down to 15 µl.*

8.15 Incubate for 10 minutes at room temperature.

8.16 Quickly spin the tube, place it on the DiaMag02 and wait until the beads are completely bound to the magnet (~ 2 minutes).

8.17 Without disturbing the pellet, carefully aspirate and transfer 30 µl of supernatant to a new tube.

STEP 9

Quality control and Next-Generation Sequencing

9.1 Determine the concentrations of your samples by the use of a fluorescence-based assay such as the Qubit High Sensitivity assay (ThermoFischer Scientific).

9.2 Run a part of each library on a High Sensitivity chip for BioAnalyzer (Agilent) or on Fragment Analyzer (Agilent) according to the manufacturer's instructions.

***NOTE**: In some cases, for example if adaptor dimers (around 125 bp) are still present, a new clean-up can be performed. In this case, the volume of AMPure beads to use should be 30 µl instead of 50 µl.*

9.3 Your libraries are now ready for pooling and sequencing.

***NOTE**: Individual libraries, quantified and purified according to the above protocol, can be pooled at desired molar ratios to allow multiplex sequencing. Libraries that are being pooled must have been prepared with different indexes (for multiplexing and index pooling guidelines refer to Indexes, page 9). The minimal molar concentration needed to sequence the pool depends on the requirements of the sequencing platform. The total molarity is the sum of all the individual libraries' molarities in the final volume, e.g. if you add 5 µl of a 10 nM library to 5 µl of a 20 nM library, you have 10 µl of a 15 nM pool. If libraries are prepared from similar input amounts, they can be pooled by combining equal volume aliquots of each library.*

Example of Results

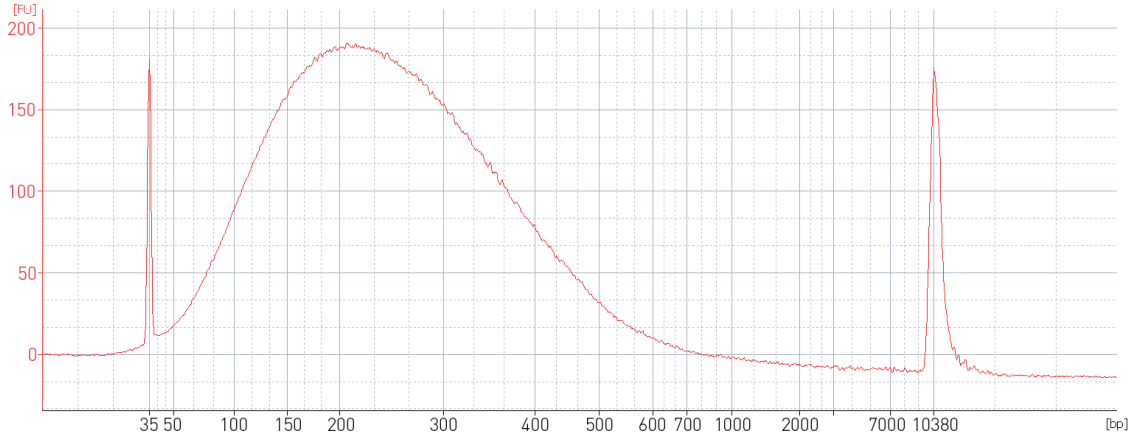


Figure 1. BioAnalyzer (Agilent) High Sensitivity DNA chip profile of sheared genomic DNA: smear around 200 bp. The genomic DNA was diluted in GenDNA TE to reach a concentration of 100 ng/µl and 100 µl were sheared in a 0.65 ml Bioruptor Microtube (Cat. No. C30010011). The following program was used: 13 Cycles [30 seconds "ON" & 30 seconds "OFF"].

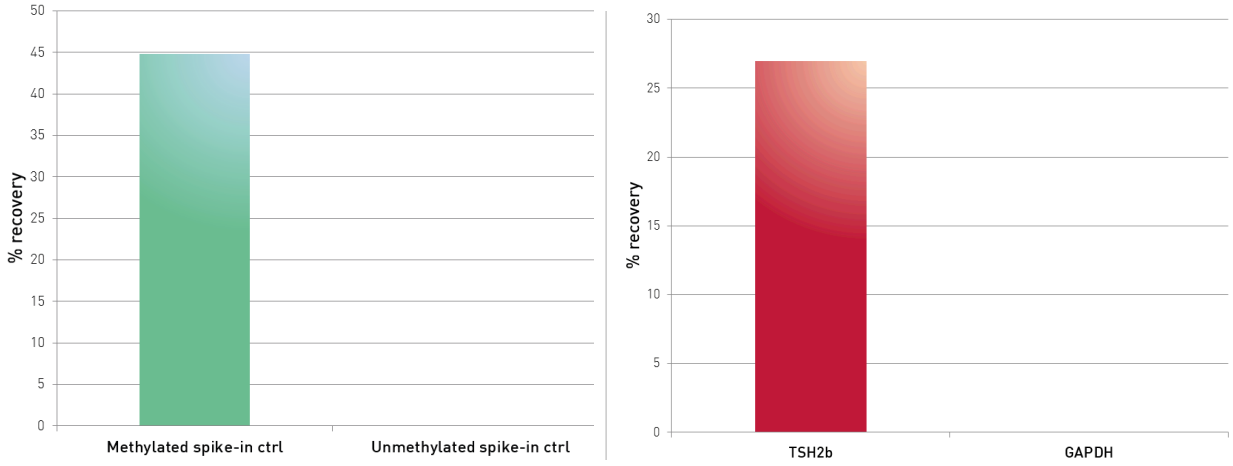


Figure 2. qPCR analysis after IP. MeDIP-seq assays were performed manually using 100 ng cfDNA (left) and 1 µg DNA from blood (right), both with the MagMeDIP-seq Package (Diagenode). The IP was performed including the methylated and unmethylated spike-in controls from MagMeDIP qPCR kit, together with the human DNA samples. The DNAs were isolated/purified using IPure kit V2. Afterwards, qPCR were performed using the primer pairs included in the MagMeDIP-qPCR kit.

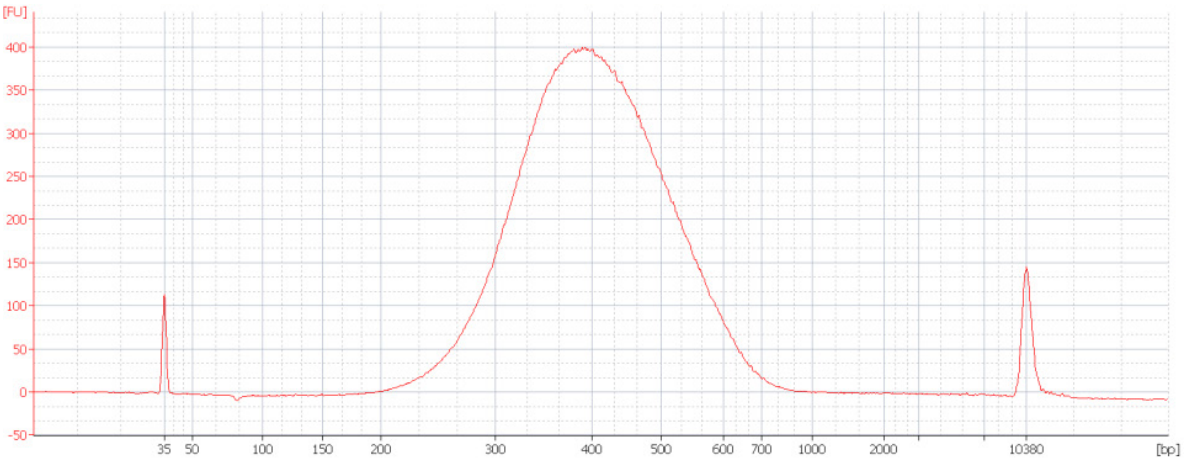


Figure 3. Library quality control. At the end of the MeDIP-seq experiment, the distribution of the fragments sizes was assessed by loading 7 ng of library on BioAnalyzer (Agilent).

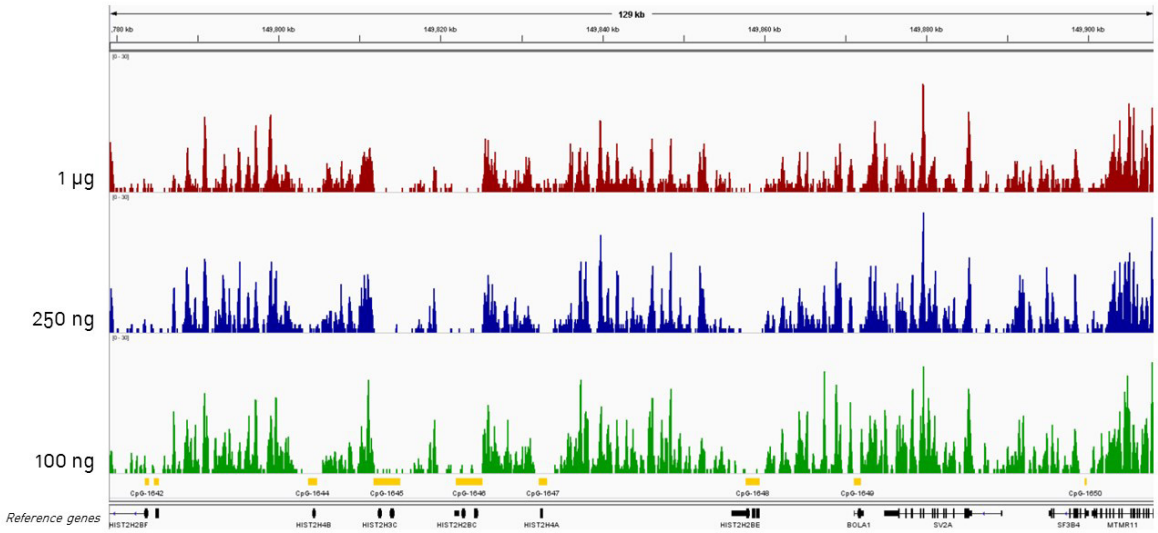


Figure 4. Sequencing profiles of MeDIP-seq libraries prepared from different starting amounts of DNA. The Diagenode MagMeDIP-seq Package was used to prepare samples containing decreasing starting amounts of DNA (from the top down: 1000 ng (red), 250 ng (blue), 100 ng (green)) originating from human blood, and they were sequenced an on Illumina HiSeq 4000 with 2x75 PE reads. The reads were mapped to the human genome (hg19) with bwa and the alignments were loaded into IGV (the tracks use an identical scale). The CpG islands (CGIs) are marked by yellow boxes in the bottom track.

FAQs

What is the resolution for MagMeDIP?

The resolution is related to the size of the fragments. Usually the sheared DNA is a smear of 100-600 bp which means that the resolution is also 100-600 bp.

Would it be possible to obtain genomic DNA by other means and start using the kit at the DNA shearing step? Can we clean up the DNA by column, to minimize the contact with phenol?

The XL GenDNA extraction module allows the extraction of high molecular weight DNA, but you can definitely use columns instead of the double phenol/chloroform extraction, as long as you are confident with the quality of the genomic DNA you will get. The most important is to obtain pure and high molecular weight DNA. You can choose which DNA extraction protocol you want to use to reach this goal and then start using the MagMeDIP qPCR kit from the immunoprecipitation step.

I will shear the DNA using the Bioruptor Pico. How do I choose the right tubes?

For the shearing, the size of the tubes is not important as long as you use the recommended volume according to each format and the recommended tubes. To help you with DNA shearing we have developed a tool available at https://www.diagenode.com/en/dna-shearing-guide that contains all the information needed to choose the best shearing protocol for DNA.

Can I shear DNA using an enzyme for MagMeDIP DNA preparation?

As long as you obtain DNA fragments from 100-600 bp, you can use the fragmentation technique of your choice. We usually do not recommend using restriction enzymes because the fragmentation is less random compared to sonication.

Can I use more than 1 µg DNA for the MeDIP? Will it increase the overall yield?

It is possible to use more DNA, but the amount of antibody may need to be adjusted, requiring further optimization without a guarantee of improved output. Therefore, we recommend following the standard and optimized protocol. To increase the output we suggest to process several samples with 1 µg and to pool them at the end.

What is the lowest DNA amount that we can investigate with your MagMeDIP qPCR kit?

We recommend using at least 100 ng of sheared DNA per IP.

Can I use another antibody with the kit?

This kit has been validated with the provided antibody only - Cat. No. C15200081, therefore we do not recommend changing the antibody. Moreover not all antibodies are compatible with the beads which are included in the kit.

What is the reference of the antibody used in the MagMeDIP qPCR kit? Which amount of antibody per IP should I use?

The antibody used is the Cat. No. C15200081. To ensure efficient IP it is important to use the diluted antibody as described in the protocol. A lack of antibody can result in low IP efficiency whereas a large excess of antibody might lead to lower specificity.

What are the internal controls that can be added to the incubation mix during MeDIP? Is this a required step?

The internal spike-in controls allow you to check the immunoprecipitation efficiency of a positive and a negative template in the same tube as your DNA of interest. The purpose of these controls is to confirm antibody specificity. It is better to add them to every sample, but you can also choose to add a spike-in control to only one sample per experimental condition.

Is it possible to buy other spike-in control tubes or primers? If possible, are they the same as in the initial MagMeDIP-seq Package?

The MagMeDIP-qPCR kit contains enough spike-in controls for all the reactions. If you need more spike-in controls, you can buy the **DNA Methylation Control Package (Cat. No. C02040012)** which provides other methylated and unmethylated controls and their associated primers. Those controls are different since they are produced from the genome of Arabidopsis thaliana and may therefore interfere with plant samples. Nevertheless they were validated with MagMeDIP-seq Package and are compatible with every other sample species.

Please check the product datasheet on the website for more information: https://www.diagenode.com/en/p/dna-methylation-control-package-40-rxns.

What are the lengths of the PCR products?

Here are the lengths of the PCR products obtained with the different primer pairs:

- TSH2B: 170 pb
- unDNA: 92 pb
- GAPDH: 64 pb
- meDNA1 : 81 pb

I did not obtain a Ct value for the negative control GAPDH. Which value should I use for the calculation?

The absence of a qPCR signal for GAPDH after the MeDIP, means that the IP was highly specific since GAPDH signal is a measurement of the background. Therefore it already shows that the background is very low. If you want to put a Ct value you can apply the maximal number of qPCR cycles performed (usually 40).

Why do we need to add the adaptors before doing the immunoprecipitation? Would it be possible to do the IP first and then make the libraries from the resulting DNA ?

Before MeDIP, the DNA has to be denaturated in order to allow the antibody to target the 5-mC. As the standard Illumina adaptors are doublestranded, for the ligation to work, the DNA template has to be doublestranded as well. This is why adaptor ligation has to be performed before DNA denaturation and thus before MeDIP for MeDIP-seq protocols.

Is the Microplex Kit compatible with the MagMeDIP kit?

With MeDIP-seq, the immunoprecipitated DNA is single-stranded and thus is not compatible with our MicroPlex Library Preparation Kit. Library preparation must be performed before MeDIP. All steps using the MicroPlex Kit are performed in the same tube which makes difficult to stop in the middle to perform the MeDIP. We recommend using the iDeal Library Preparation Kit instead.

Do I have to add the spike-in controls to all samples or only to control samples that will not be sequenced?

The spike-in controls are designed for qPCR validation that you may want to do before sequencing. If you do not perform qPCR validation there is no need to add them because they will not be sequenced.

I would like to use the methylated internal control provided in the MagMeDIP-seq Package as positive spike-in control for sequencing. Has this methylated control been used as internal sequencing control before?

In the Diagenode MeDIP-seq protocol, the spike-in controls are added after the library preparation step, therefore they do not have the adaptors that are required for sequencing. For a use in sequencing, the spike-in controls should be added before the library preparation step, but it has not been tested so far.

Related products

Product	Cat. No.
MagMeDIP-seq x48	C01020021
iDeal Library Preparation Kit x24	C05010020
Index Primer Set 2 (iDeal Library Preparation Kit x24)	C05010021
IPure kit v2 x100	C03010015
XL GenDNA Extraction Module	C03030020
DNA Methylation control package	C02040012
5-methylcytosine (5-mC) monoclonal antibody 33D3	C15200081
DiaMag 0.2ml - magnetic rack	B04000001
DiaMag 1.5ml - magnetic rack	B04000003
DiaMag rotator	B05000001
Bioruptor® Pico sonication device	B01060010

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