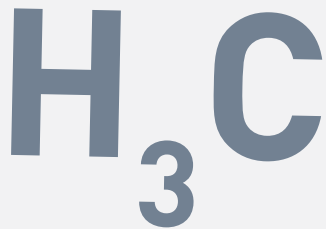




Innovating Epigenetics Solutions

DNA METHYLATION



- Solutions using bisulfite conversion and immunocapture
- Ideal for NGS, Sanger sequencing, Pyrosequencing, and qPCR

Understanding DNA Methylation

DNA methylation plays key roles in numerous biological processes such as genomic imprinting, control of transcription, embryonic development, X-chromosome inactivation, chromosome stability, and carcinogenesis.

In mammals, DNA methylation occurs primarily as 5-methylcytosine (5-mC), which is usually found at CpG dinucleotides. CpG-rich regions are called CpG islands and are generally near transcription start sites where they can play a role in gene regulation.

5-hydroxymethylcytosine (5-hmC) has been implicated with an alternative role as a regulator of gene expression and cellular differentiation during embryonic development, stem cell reprogramming, and carcinogenesis.

Studying 5-mC and 5-hmC

Sodium bisulfite converts unmethylated cytosine to uracil while methylated cytosines remain unchanged:

- Single nucleotide resolution
- Gene-specific and genome-wide analyses

Capture and quantification of methylated DNA

- Methylated/hydroxymethylated DNA immunoprecipitation (MeDIP/hMeDIP): Antibodies are used to immunoprecipitate methylated/ hydroxymethylated DNA. Enriched DNA is analyzed with locus-specific PCR or by NGS
- MethylCap: Methylated DNA-binding proteins (MBD protein) capture methylated DNA

Let Us Do Your DNA Methylation Assays for You

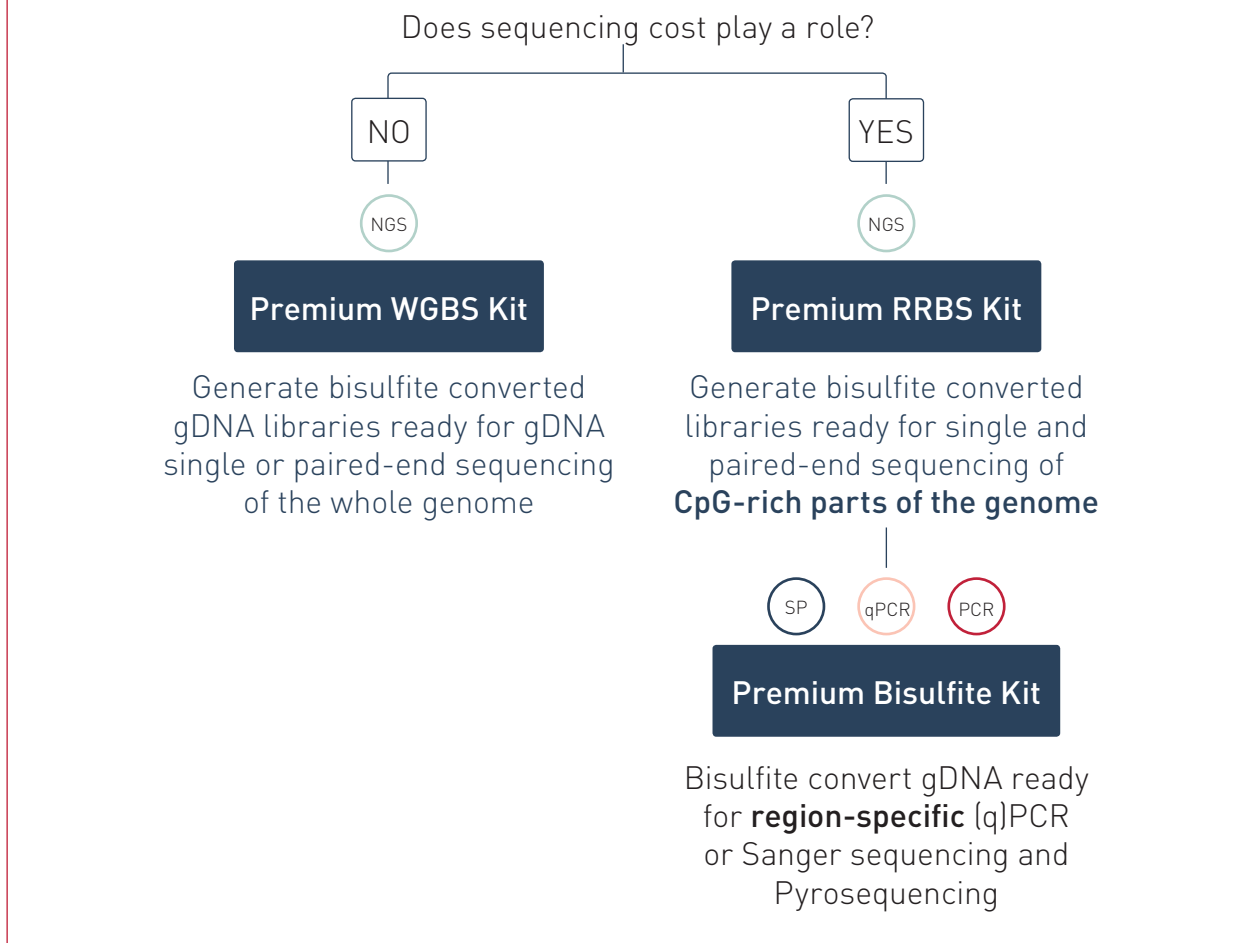
Diagenode offers DNA Methylation Profiling Services using Reduced Representation Bisulfite Sequencing (RRBS).

- Single base resolution at the CpG level
- High throughput: ideal for processing large numbers of patient samples
- Compatible with all vertebrate species
- Identification of differentially methylated regions (DMRs)
- Validated for low inputs and different sample types
- Ready-to-publish data

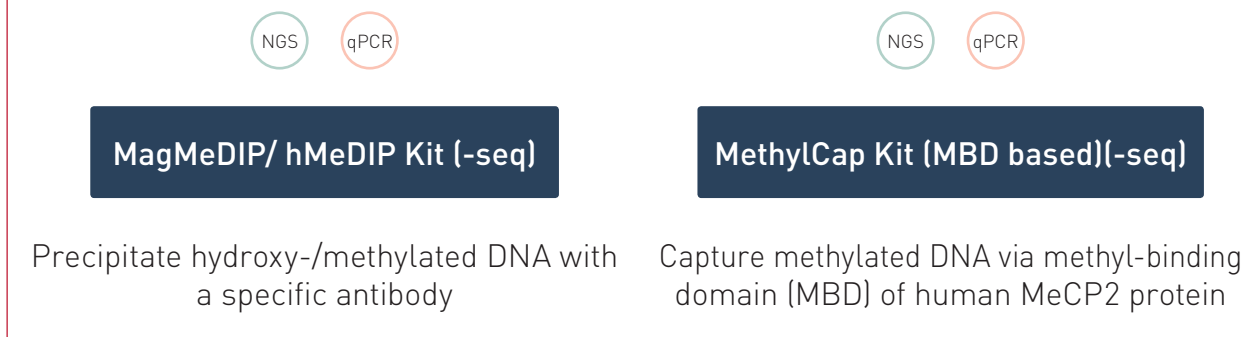
Choose your DNA methylation analysis solution by resolution requirements

NGS NGS
 SP Sanger, Pyrosequencing
 qPCR qPCR
 PCR PCR

Study methylation with single base resolution

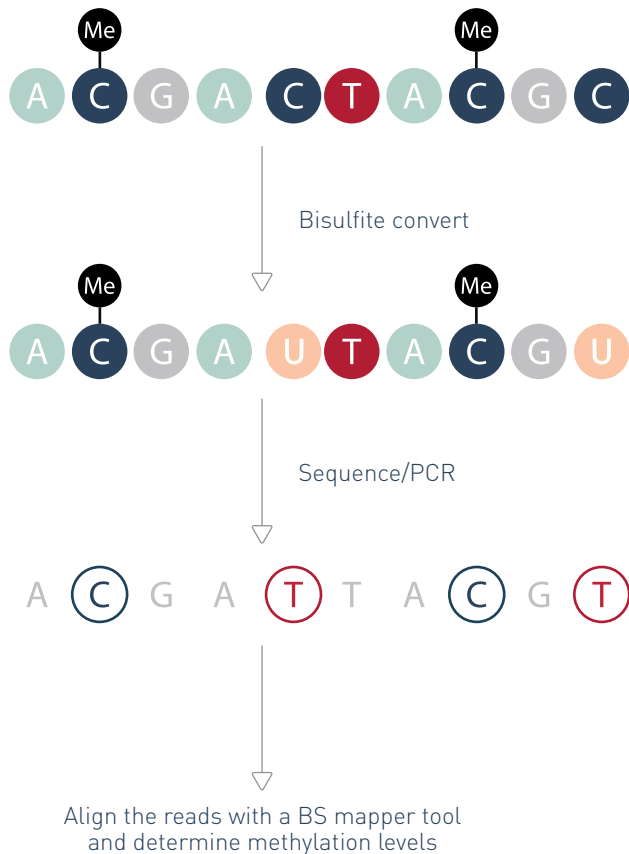


Study methylation 100-500 bp resolution



Bisulfite conversion

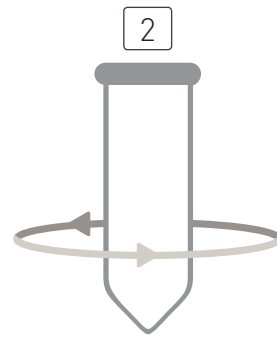
Bisulfite conversion reagent chemically modifies non-methylated cytosines (C) into uracil (U) while methylated cytosines (5-mC) remain unchanged. For single locus analysis, the region of interest is amplified with PCR which can be followed by Sanger sequencing or Pyrosequencing.



- **Premium Bisulfite Kit** 50 rxns C02030030
- **MethylEasy™ kit Xceed** 40rxns C02030021

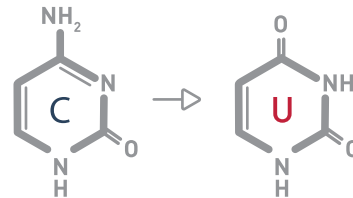


Convert DNA



Desulfonate, spin, wash, elute

3



Converted DNA ready for methylation analysis

- **Rapid** bisulfite conversion of DNA - only 1 hour reaction time
- **Simple** workflow, 3 steps
- **High-yields** of converted DNA for methylation analysis



Reduced Representation Bisulfite Sequencing (RRBS)

Methylation typically targets cytosine in a CpG context in vertebrates.

In RRBS, by using the restriction enzyme MspI (CCGG target sites) followed by size selection, DNA is enriched to represent CpG-rich regions (including CpG islands). Thus, RRBS is a **powerful** and **cost-effective** method to efficiently analyze DNA methylation at the **single nucleotide level** at approximately 20x lower cost compared to whole genome bisulfite sequencing (WGBS).

- **Excellent coverage** – 4 million CpGs
- **Confidence in results** – Positive and negative spike-in controls to check conversion efficiency
- **Cost-efficient** – Multiplex up to 6 or 8 human/mouse samples per sequencing lane on HiSeq2500 or HiSeq3000
- **High efficiency and minimal bias** – 99.5% bisulfite conversion rate and reduced amplification



- **Premium RRBS Kit**
24 rxns C02030032, 96 rxns C02030033
- **Premium WGBS Kit**
8 rxns C02030034

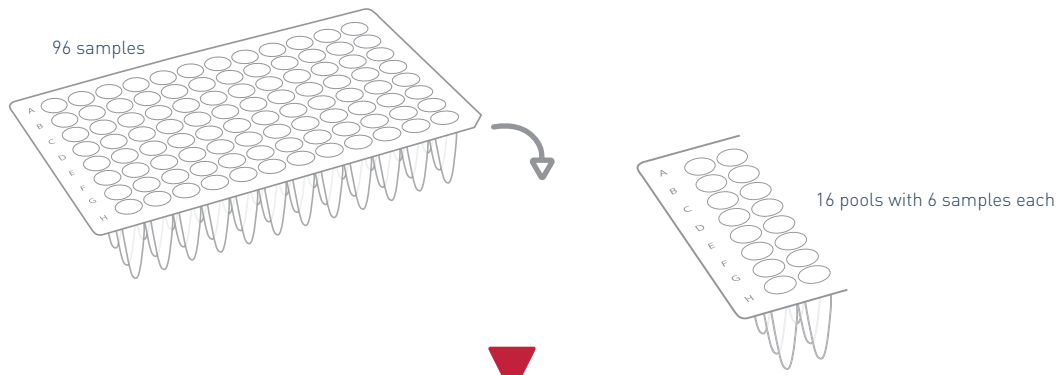
1 Msp I digestion of isolated gDNA



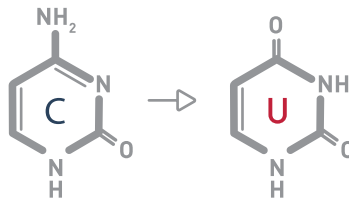
2 Ends preparation

3 Adaptor ligation and size selection with AMPure[®] XP beads

4 Sample pooling allows sequencing of 6 samples on 1 Illumina[®] HiSeq2500 lane



5 Bisulfite conversion

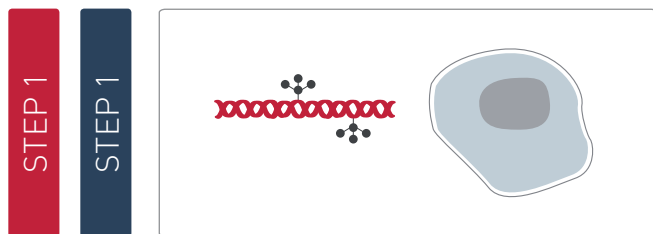


6 PCR amplification and clean up

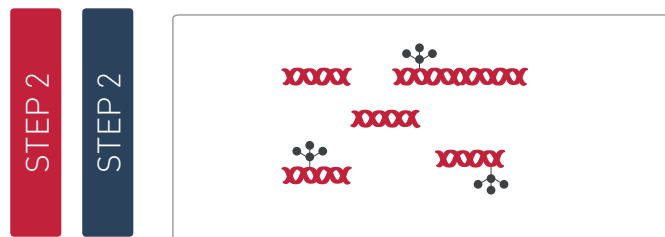
7 Next Generation Sequencing

MagMeDIP/ hMeDIP or MagMeDIP-seq/hMeDIP-seq

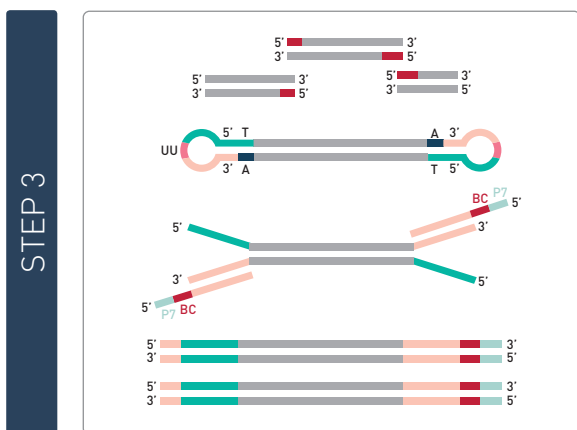
Perform DNA immunoprecipitation (-sequencing) to estimate the DNA modification status of your sample using the 5-methylcytosine antibody (5-mC Ab) or 5-hydroxymethylcytosine antibody (5-hmC Ab).



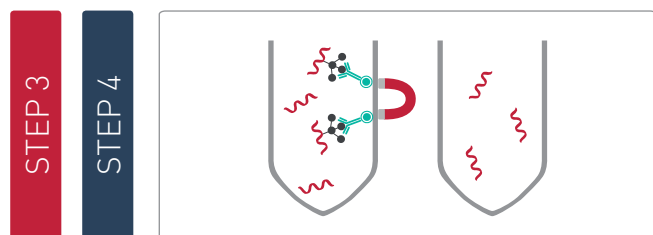
Collect and lyse cells



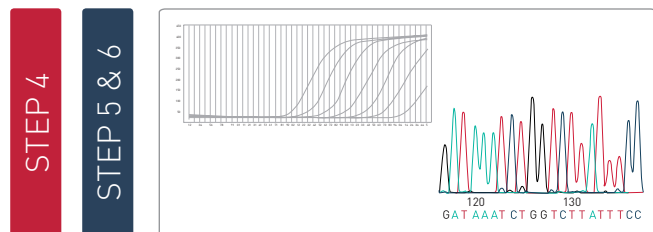
Extract and shear DNA



Prepare library for MeDIP-seq



Immunoprecipitate, wash and isolate DNA



4 qPCR

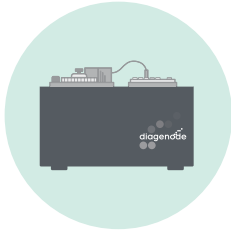
5 PCR amplification

6 Next-Generation Sequencing

Optimal solutions from Diagenode



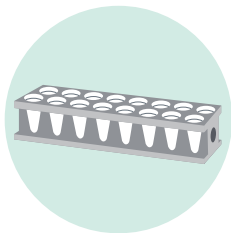
- **XL GenDNA Extraction Module** C03030020



- **XL GenDNA Extraction Module** C03030020
- **Bioruptor® Pico** B01060001



- **iDeal Library Preparation Kit** C05010020



- **MagMeDIP Kit** C02010020
- **hMeDIP Kit** C02010030-35
- **DNA isolation with IPure Kit v2** C03010014
- **Magnetic rack DiaMag02** B04000001



- **iDeal Library Preparation Kit** C05010020



A quick glance: DNA methylation kit features

	Bisulfite conversion			
	Premium Bisulfite	MethylEasy Xceed	RRBS	
Resolution	Single nt	Single nt	Single nt	
Downstream application	Sanger, Pyroseq, (q)PCR	Sanger, Pyroseq, (q)PCR	NGS	
Initial fragment size (bp)	gDNA	gDNA	gDNA	
Input DNA (ng)	0.1-2000	0.05-5000	100	
Turnaround time	1.5-2h	1.5-2h	4-5 days	
BS conv. reagent	✓	✓	✓	
Restriction enzyme	na	na	✓	
All buffers	✓	✓	✓	
Controls		✓	✓	
All library prep reagents	na	na	✓	
Indexes	na	na	24	
Purification (columns/beads)	✓	✓	✓	
Beads for IP	na	na	na	
Format (rxn)	40 50 (Auto only)	40	24; 96	
Automation	✓	na	Automatable	
ORDERING	Kits	C02030030 C02030031	C02030021	C02030032 C02030033

na: not applicable

KIT

	Hydroxy-/Methylated DNA Immunoprecipitation		MBD based
WGBS	MagMeDIP (seq)	hMeDIP (seq)	MethylCap (seq)
Single nt	100-500 bp	100-500 bp	100-500 bp
NGS	NGS, qPCR	NGS, qPCR	NGS, qPCR
100-400	100-600 [200]	100-600 [200]	100-600 [200]
5-1000	1000	>1000	1000
2-3 days	2-3 days	2-3 days	1-2 days
✓	na	na	na
na	na	na	na
✓	✓	✓	✓
	✓	✓	
✓			
24 Separately C05010032	Separately in iDeal Library prep C05010020	Separately in iDeal Library prep C05010020	Separately in MicroPlex Library prep C05010012, C05010014
✓	Separately in IPure v2 C03010015	Separately in IPure v2 C03010015	Separately in IPure v2 C03010015
na	✓	✓	✓
8	10; 48	16	48
Automatable	✓	✓	✓
C02030034	C02010020 C02010021	(mAb rat) C02010030, C02010033 (mAb mouse) C02010031, C02010034 (polyAb rabbit) C02010032, C02010035	C02020010 C02020011

Shop online in our EpiStore at
www.diagenode.com