



Innovating Epigenetics Solutions

# DNA METHYLATION

**SOLUTIONS TO STUDY DNA METHYLATION:  
TARGETED AND GENOME-WIDE**

Optimal for NGS, Sanger sequencing, Pyrosequencing & qPCR

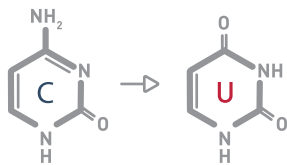


# Understanding DNA Methylation

DNA methylation plays key roles in numerous biological processes such as genomic imprinting, control of transcription, embryonic development, stem cell reprogramming and carcinogenesis. In mammals, DNA methylation occurs primarily as 5-methylcytosine (5-mC), which is usually found at CpG dinucleotides.

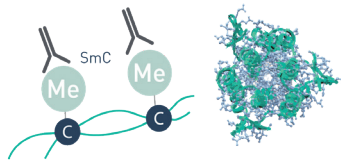
An additional mark, 5-hydroxymethylcytosine (5-hmC), has been implicated with an alternative role as a regulator of gene expression and cellular differentiation as well as an intermediary in the active demethylation process.

## Study 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 methylated/hydroxymethylated DNA** by choosing one of two techniques:

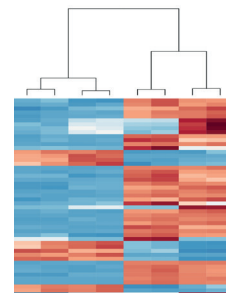
- Antibodies are used to capture methylated or hydroxymethylated DNA
- Methylated DNA-binding proteins (MBD) are used to capture methylated DNA

In both cases, enriched DNA is analyzed with locus-specific PCR or by NGS:

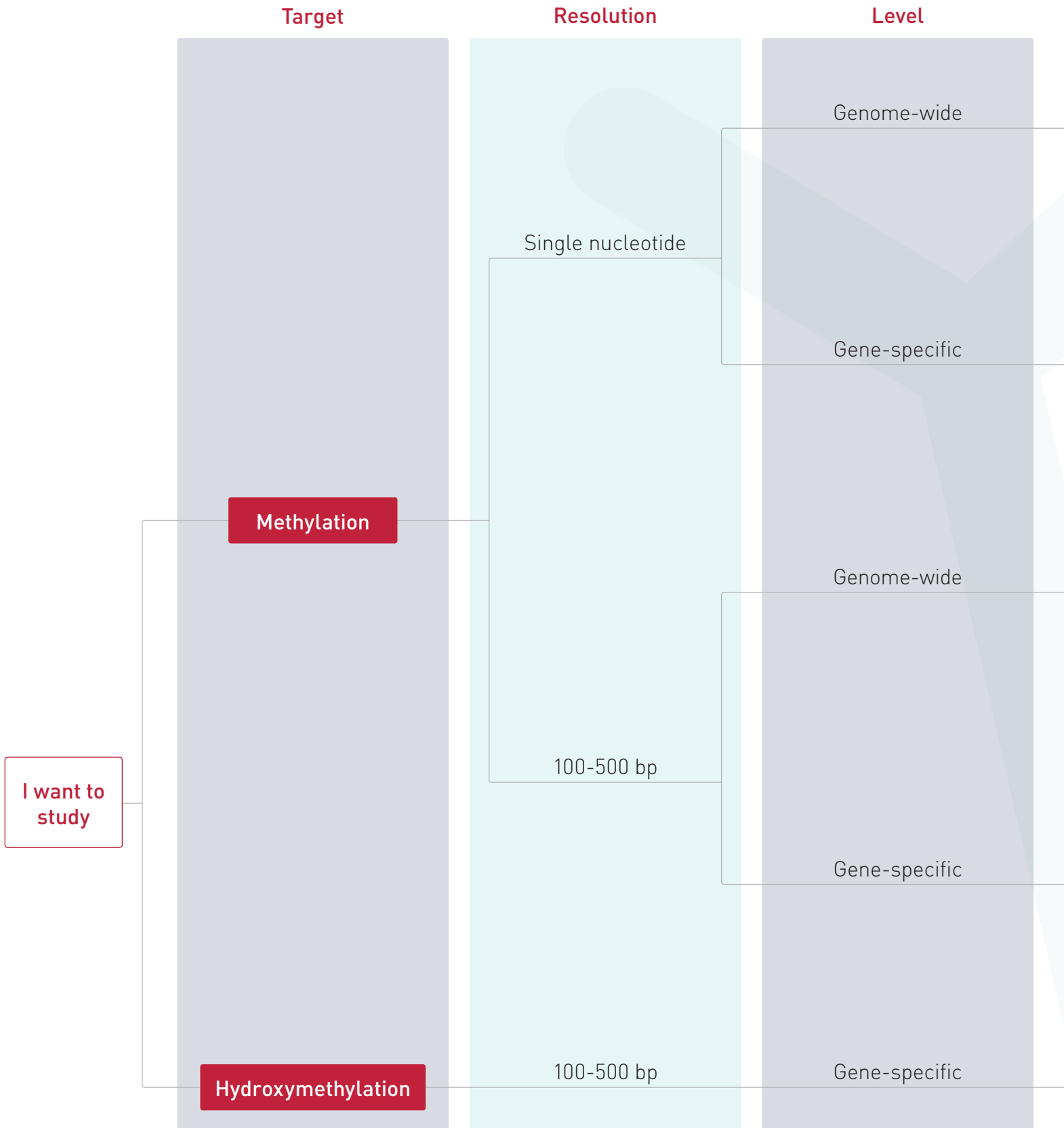
- Gene-specific and genome-wide analyses
- Compatible with degraded DNA or small fragments

## Let Us Do Your DNA Methylation Assays for You

Diagenode offers **DNA Methylation Profiling Services** using our different technologies, and we provide you with ready-to-publish data.



# Find your DNA methylation analysis solution



# according to your needs

25x10<sup>6</sup> CpGs coverage

NGS

## Premium WGBS Kit

Generate bisulfite-converted libraries ready for single or paired-end sequencing of the **whole genome**

C02030034

4x10<sup>6</sup> CpGs coverage

NGS

## Premium RRBS Kit

Generate bisulfite-converted libraries ready for single and paired-end sequencing of **CpG-rich parts of the genome**

C02030032

SP qPCR PCR

## Premium Bisulfite Kit

Bisulfite convert gDNA ready for **region-specific analysis** like (q)PCR, Sanger/ pyrosequencing (SP)

C02030030

Antibody

NGS

## MagMeDIP-seq Package

From gDNA extraction to library preparation - prepare ready-to-sequence libraries of **immunoprecipitated methylated DNA**

C02010040

MBD domain

NGS

## MethylCap Kit + MicroPlex

Prepare libraries of captured methylated DNA combining **MBD technology** with the **MicroPlex Library Prep Kit**

C02020010

C05010014

Antibody

qPCR

## MagMeDIP qPCR

Precipitate methylated DNA with a **specific antibody**

C02010020

CITED IN  
nature

MBD domain

qPCR

## MethylCap Kit

Capture methylated DNA via methyl-binding domain (MBD) of human **MeCP2 protein**

C02020010

qPCR

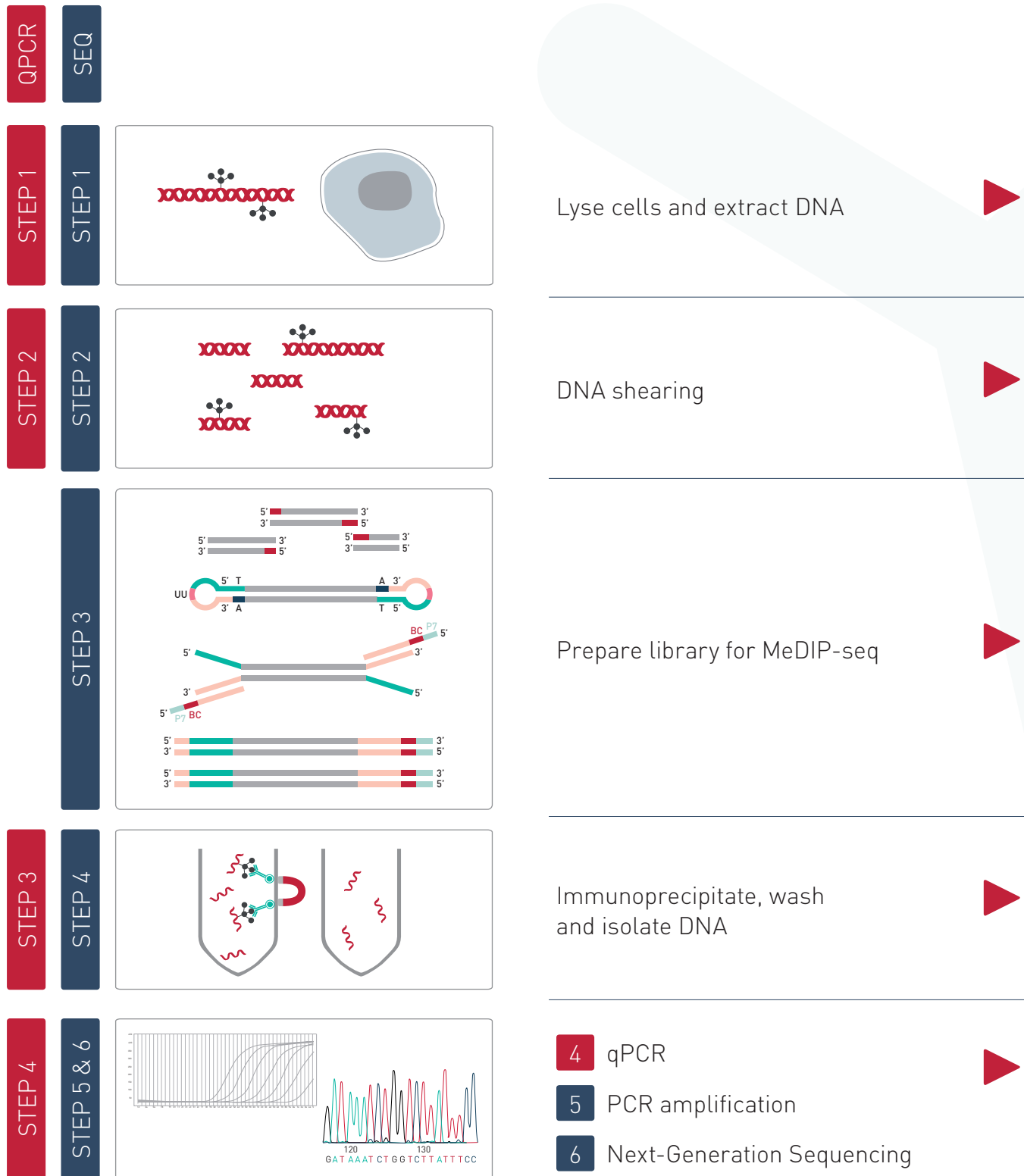
## hMeDIP Kit

Precipitate hydroxymethylated DNA with a **specific antibody**

C02010031

# MagMeDIP qPCR/hMeDIP or MagMeDIP-seq

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



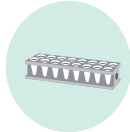
AUTO EQUIPMENT NEEDS WHICH KIT FOR WHICH STEP?

<p><b>IP-Star® Compact Automated System</b> B03020002</p>	<p><b>Bioruptor®</b> B01020001 B01060010</p>	<p>Magnetic rack <b>DiaMag02</b> B04000001</p>	<p><b>XL GenDNA Extraction Module</b> C03030020</p>	<p><b>iDeal Library Preparation Kit</b> C05010020</p>	<p>DNA isolation with <b>IPure Kit v2</b> C03010014</p>	<p><b>hMeDIP Kit</b> C02010031</p>	<p><b>MagMeDIP qPCR Kit</b> C02010020</p>	<p><b>MagMeDIP-seq Package</b> C02010040</p>
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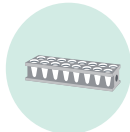
✓ ✓ ✓ ✓ ✓



✓ ✓ ✓



✓ ✓ ✓

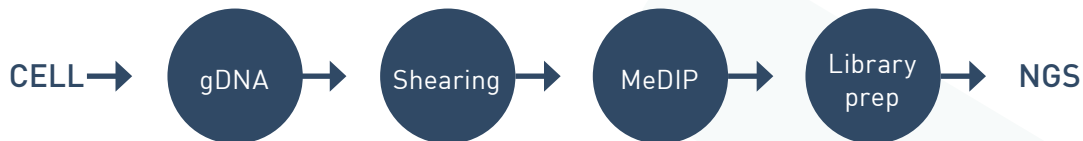


✓ ✓ ✓ ✓

5-6 4 4 5-6


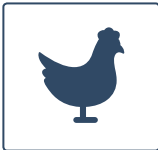

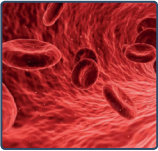
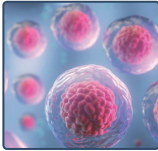
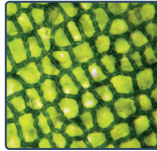
# Your Full MeDIP-seq Solution

The **MagMeDIP-seq Package**, our complete solution, includes MagMeDIP qPCR, IPure, iDeal Library prep and an **optimized MeDIP-seq protocol** to offer the highest **accuracy, reliability** and **performance**.

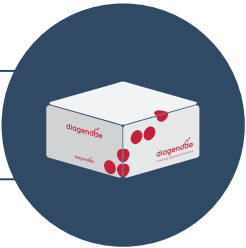


- **Start-to-finish** : Complete kits include DNA extraction module, highly validated antibody 33D3, spike-in controls and qPCR primer pairs for control regions
- **Easy to use** with user-friendly magnetic beads and racks
- **Validated protocols** provide reproducible and **consistent data** - using as low as 100 ng input DNA
- **Highest quality purification** with IPure
- Validated **shearing protocol** with the Bioruptor
- Suitable for **all species**

## Species and sample possibilities - no limit

<b>SPECIES</b>	 <i>Human</i>	 <i>Animals</i>	 <i>Plants</i>	<b>The possibilities are limitless</b>
	 <i>Blood</i>	 <i>Stem cells</i>	 <i>Tissue</i>	
	<b>SAMPLES</b>			

**Immunoprecipitation kit**  
C02010040 - MagMeDIP-seq Package - 10 rxns



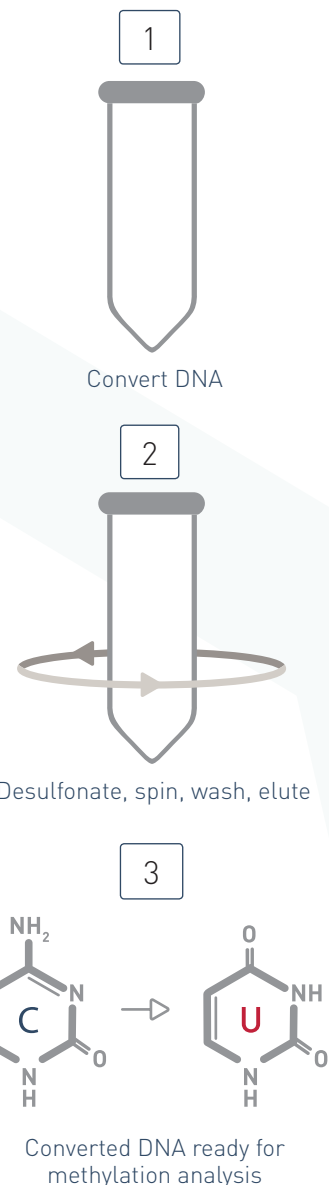
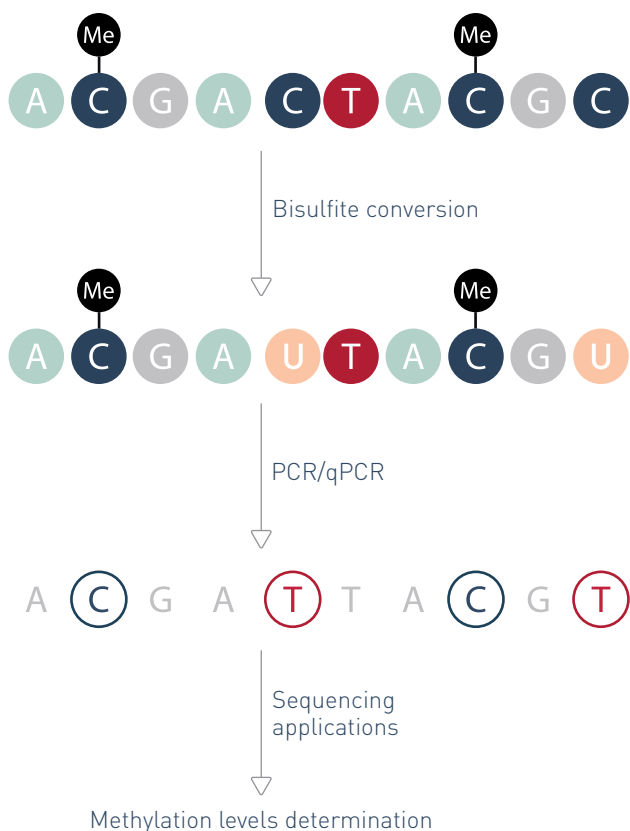




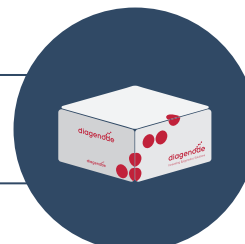
# Bisulfite conversion

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.

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



**Bisulfite conversion based kit**  
 C02030030 - Premium Bisulfite Kit - 50 rxns

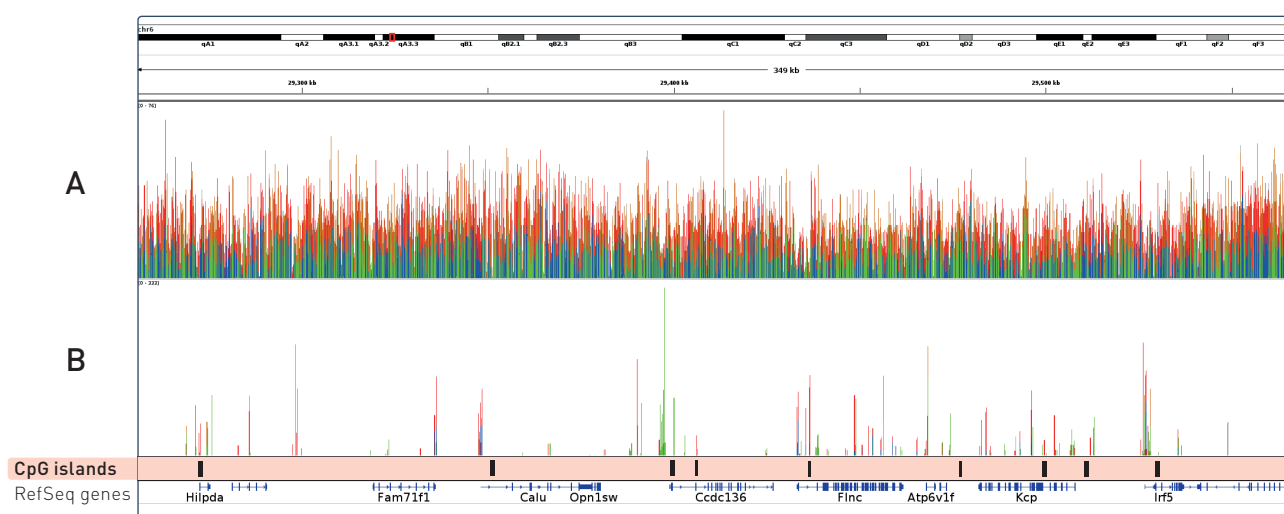


# Whole Genome Bisulfite Sequencing (WGBS)

Whole Genome Bisulfite Sequencing (WGBS) is a **single nucleotide resolution** technique that allows the user to study DNA methylation sites and their role in **gene regulation**. Diagenode's Premium WGBS Kit is designed to prepare **single and paired-end** bisulfite converted DNA libraries for sequencing using Illumina® platforms.

Diagenode's Premium WGBS Kit contains specially designed enzymes and buffers needed for genome-wide bisulfite sequencing.

- Wide range of DNA inputs: **5 ng - 1 µg**.
- Adaptor sets (6-12-24) available separately for multiplexing
- ChIP-bisulfite-sequencing compatible



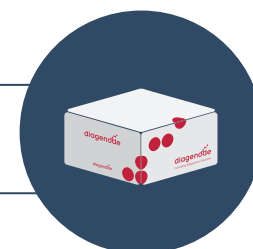
**Visualization of the alignment bam files on Integrative Genome Viewer (IGV) shows excellent coverage (A) of the whole genome using WGBS and (B) of the CpGs areas using RRBS.**

**(A)** WGBS was performed using the Premium Whole Genome Bisulfite Sequencing (WGBS) kit.

**(B)** RRBS was performed using the Premium Reduced Representation Bisulfite Sequencing (RRBS) kit. For both, after sequencing, reads were aligned on the mm10 reference genome.

**Bisulfite conversion based kit**

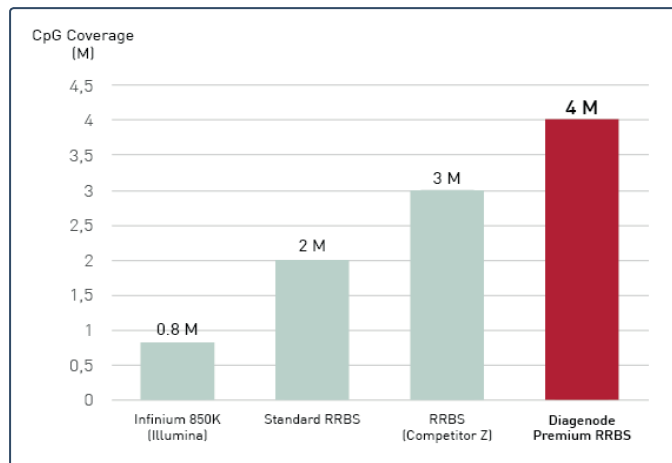
C02030034 - Premium WGBS Kit - 8 rxns



## 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 8 human/mouse samples per sequencing lane on HiSeq 3000
- **High efficiency and minimal bias** – 99.5% bisulfite conversion rate and reduced amplification



### Superior coverage.

Comparison of CpG coverage between competing technologies.

### EXPERTS RECOMMEND IT

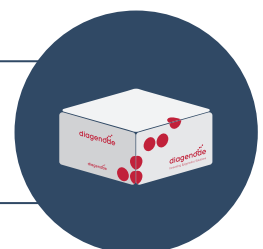
“Our lab has used Diagenode’s Premium RRBS kit on rat brain samples. The protocol is understandable, logical, well-written and is easy to follow. I found it fantastic that I could ask questions from the company, and their answers were really useful. We were able to construct a library, which we ran on BioAnalyzer, and the results looked very nice and ready to be sequenced. I would definitely recommend my colleagues to use the Premium RRBS kit from Diagenode.”

**Borbála Veto, Institute of Enzymology,  
Budapest, Hungary**

### Bisulfite conversion based kits

C02030032 - Premium RRBS Kit - 24 rxns

C02030033 - Premium RRBS Kit - 96 rxns





## Diagenode RRBS SIP – Software for Intelligent Pooling

Pooling techniques allow you to manipulate **fewer tubes**, providing an **easier-to-handle** and **cost-effective** protocol.



### POOLING

**Pool Size**

4 24

4 8 12 16 20 24

**Choose CSV File**

Browse... test\_009.csv

Upload complete

**Decimal Separator**

Comma

Dot

Choose the right decimal separator that is used for your Ct values in your CSV file

Diagenode's new online **intelligent pooling aid** provides the **optimal pool design** for RRBS to meet your **specific sample** and analysis needs:

- **Time-saving** – Avoid complex calculations
- **Highest pooling efficiency** based on qPCR quantification – Bring the power of pooling to the highest
- **Powerful** – Incorporates advanced aspects such as number of samples per pool required, the separation between projects, and more
- **Accurate** – Identify outliers



# A quick glance: DNA methylation kit features

		Bisulfite conversion		
		Premium Bisulfite	RRBS	WGBS
FEATURES	Resolution	Single nt	Single nt	Single nt
	Downstream application	Sanger, Pyroseq, (q)PCR	NGS	NGS
	Initial fragment size (bp)	gDNA	gDNA	100-400
	Input DNA (ng)	0.1-2000	100	5-1000
	Turnaround time	1.5-2h	4-5 days	2-3 days
	BS conv. reagent	✓	✓	✓
	Restriction enzyme	na	✓	na
	All buffers	✓	✓	✓
	Controls		✓	
	All library prep reagents	na	✓	✓
KIT CONTENT	Indexes	na	24	24 included (C05010032 for more)
	Purification	✓	✓	✓
	Beads for IP	na	na	na
	Automated Kits	C02030030 (40 rxns)	na	na
	Manual Kits	C02030031 (50 rxns)	C02030032 (24 rxns) C02030033 (96 rxns)	C02030034 (8 rxns)
ORDERING	SERVICES	✓	✓	✓

## KITS

Hydroxy-/Methylated DNA Immunoprecipitation		MBD based	
MagMeDIP qPCR	MagMeDIP-seq Package	hMeDIP	MethylCap
100-500 bp	100-500 bp	100-500 bp	100-500 bp
qPCR, NGS	NGS	qPCR, NGS	qPCR, NGS
100-600 (200)	200	100-600 (200)	100-600 (200)
100-1000	100-1000	>1000	1000
2-3 days	4	2-3 days	1-2 days
na	na	na	na
na	na	na	na
✓	✓	✓	✓
✓	✓	✓	
Separately in iDeal Library prep C05010020	✓ 24 included (C05010032 for more)	Separately in iDeal Library prep C05010020	Separately in MicroPlex Library prep C05010013, C05010014
✓	✓	✓	Separately in IPure v2 C03010015 or MicroChIP Diapure C03040001
✓	✓	✓	✓
C02010013 (10 rxns) C02010014 (48 rxns)	na	(16 rxns) mAb rat: C02010033 mAb mouse: C02010034 polyAb rabbit: C02010035	C02020011 (48 rxns)
C02010020 (10 rxns) C02010021 (48 rxns)	C02010040 (10 rxns)	(16 rxns) mAb rat: C02010030 mAb mouse: C02010031 polyAb rabbit: C02010032	C02020010 (48 rxns)
MagMeDIP-seq	✓	hMeDIP-seq	

na: not applicable

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[www.diagenode.com](http://www.diagenode.com)

