diagenode

A Hologic Company

Auto MethylCap kit

Methylated DNA Capture kit

Cat. No. C02020011 (48 rxns)

USER GUIDE

Version 6 10_2020

Contents

Introduction

Overview

Methylation of CpG dinucleotides is generally associated with epigenetic silencing of transcription and is maintained through cellular division. Multiple CpG sequences are rare in mammalian genomes, but frequently occur at the transcriptional start site of active genes, with most clusters of promoter CpGs being hypomethylated (1).

The binding specificity of the H6-GST-MBD fusion protein to un-, hemi- and fully methylated DNA was evaluated using synthetic DNA that either contained three methylated CpGs (GAM3), three hemimethylated CpG's or no methylated CpGs (GAM). Hemimethylated DNA does not stably interact with the MBD of MeCP2. A single fully methylated CpG is sufficient for the interaction between the H6-GST-MBD fusion protein and methylated DNA, whereas there is little binding to a hemimethylated target sequence (1).

Reference:
1. Kangaspeska S, Stride B, Métivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, Benes V, Gannon F, Reid G. 2008 Transient cyclical methylation of promoter DNA. Nature 452(7183):112-5.

Product description

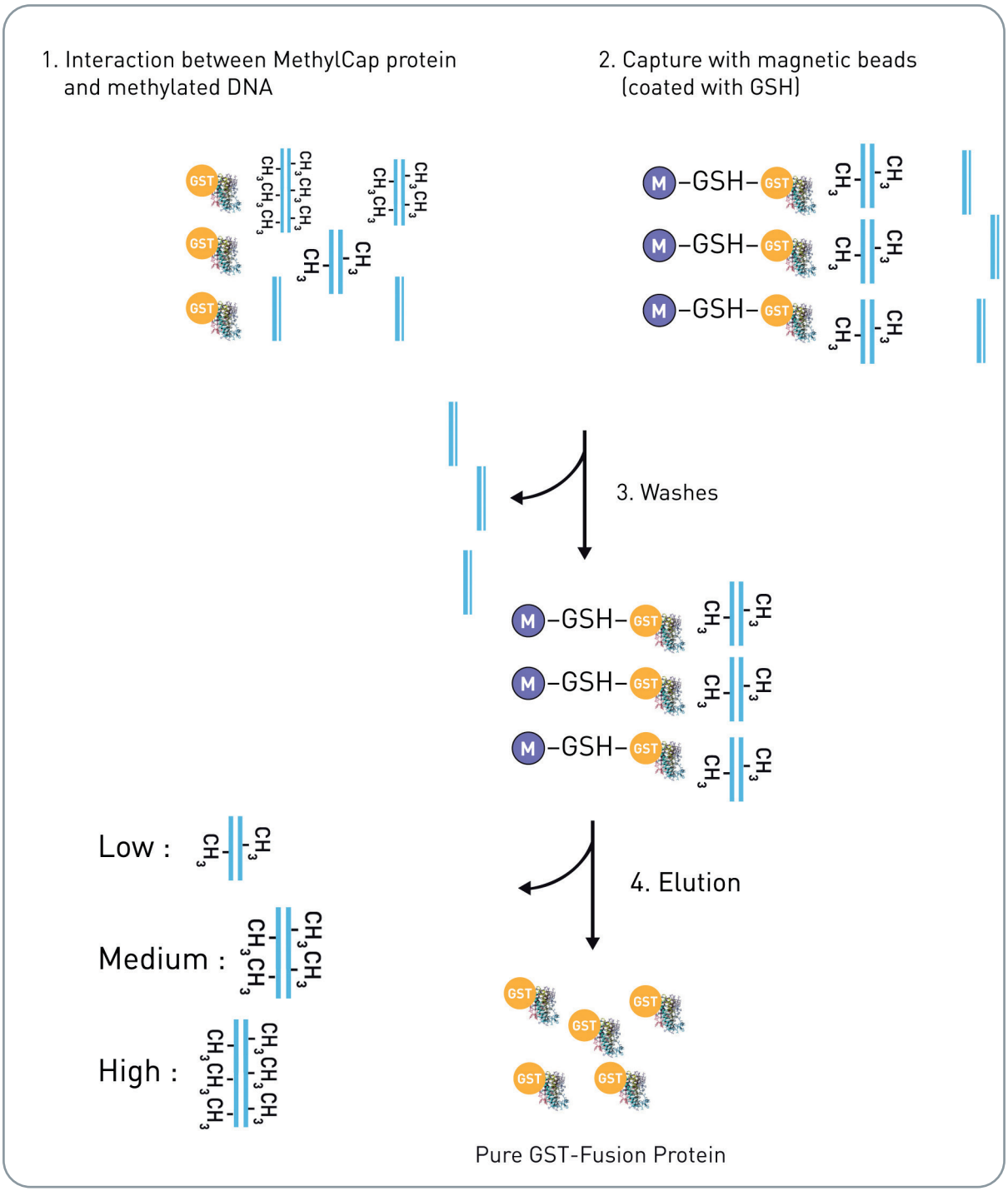
The Auto MethylCap kit allows to specifically capture DNA fragments containing methylated CpGs. The assay is based on the affinity purification of methylated DNA using the MethylCap protein (Cat. No. C02020012) which has been extensively validated. The latter consists of the methyl-CpG-binding domain (MBD) of human MeCP2, as a C-terminal fusion with Glutathione-S-transferase (GST) containing an N-terminal His6-tag.

The Auto MethylCap kit has been optimized to perform automated immunoprecipitation of chromatin using the IP-Star® Compact Automated System (Cat. No. B03000002) enabling highly reproducible results and allowing for high throughput.

Librairies of captured methylated DNA can be prepared for next-generation sequencing (NGS) by combining MBD technology with the MicroPlex Library Preparation kit (Cat. No. C05010001 and C05010002).

Kit Method Overview

Prior to MethylCap, DNA is extracted and sheared using the Bioruptor® Sonicator (see our online DNA shearing Guide: https://www.diagenode.com/en/dna-shearing-guide).



Kit Materials

Kit content

The kit content is sufficient to perform 48 capture reactions (meDNA captures).
The kit content is described in Table 1. Upon receipt, store the components at the temperatures indicated in Table 1.

Table 1. Kit content

Component	Description	Format	Storage
Buffer B (Capture)		40 ml	4°C
Wash Buffer 1		16 ml	4°C
Wash Buffer 2		32 ml	4°C
MethylCap Beads	Do not freeze	1700 µl	4°C
MethylCap Protein		55 µl	-20°C/-80°C
Low Elution Buffer		16 ml	4°C
Medium Elution Buffer		16 ml	4°C
High Elution Buffer		32 ml	4°C
hum meDNA primer pair (TSH2B)		500 µl	-20°C
hum unDNA primer pair (GAPDH)		500 µl	-20°C

Table 2. Components available separately

Component	Cat. No.	Format	Storage
hum meDNA primer pair (TSH2B)	C17011041-500	500 µl	-20°C
hum unDNA primer pair (GAPDH)	C17011047-500	500 µl	-20°C
mouse meDNA primer pair (TSH2B)	C17021042-500	500 µl	-20°C
mouse unDNA primer pair (GAPDH)	C17021045-500	500 µl	-20°C
rat meDNA primer pair (TSH2B)	C17031043-500	500 µl	-20°C
rat unDNA primer pair (GAPDH)	C17031046-500	500 µl	-20°C
MethylCap protein	C02020012	50 rxns	-20°C/-80°C
200 µl tube strips (12 tubes/strip) + cap strips	C30020001	80	RT
200 µl tube strips (8 tubes/strip) + cap strips	C30020002	120	RT
Tips (bulk)	C30040020	1000	RT
Tips (box)	C30040021	10x96	RT

Table 3. Modules available separately

Description	Comments	Reference	Quantity
XL GenDNA Extraction Module	For easy and fast DNA extraction	C03030020	60 rxns
IPure kit v2	For high-efficiency DNA purification	C03010014 & C03010015	24 rxns & 100 rxns
MicroPlex Library Preparation Kit v3	For DNA library preparation	C05010001 & C05010002	48 rxns & 96 rxns

Auto Protocol

STEP 1. DNA shearing

Genomic DNA must be randomly sheared by sonication to generate fragments around 400 bp (see example below). To perform the MethylCap, at least 1 µg of sheared DNA is needed in a volume smaller than 20 µl.

To choose the best protocol for the sonication with Bioruptor, use our online DNA Shearing Guide: https://www.diagenode.com/en/dna-shearing-guide

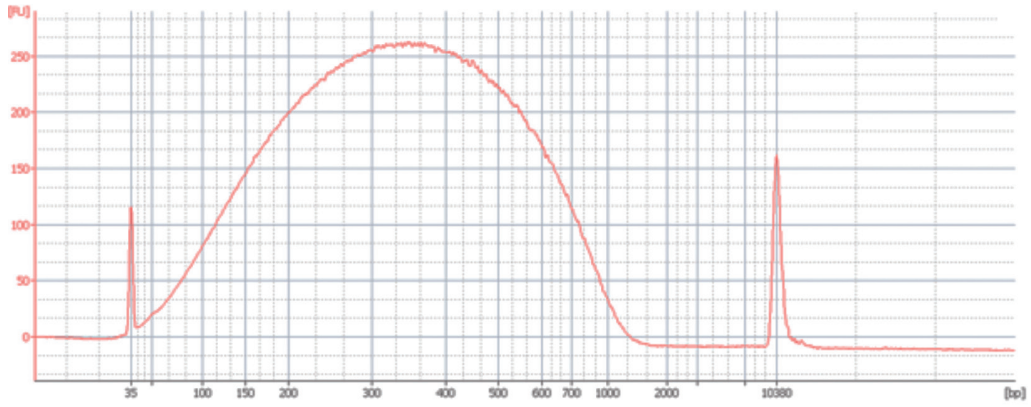
Only use the recommended tubes!

Example of shearing for MethylCap using the Bioruptor® Pico

The genomic DNA was diluted in TE buffer 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:

- Cycles: [15 seconds "ON" & 90 seconds "OFF"]
- 8 cycles



Agilent High Sensitivity DNA chip profile of sheared genomic DNA: smear around 400 bp

STEP 2. Auto capture of methylated DNA

Prepare reagents

1. Prepare aliquots of the MethylCap protein

   a. Thaw the MethylCap protein on ice.

   b. Make 5 aliquots of 11 µl (10 capture reactions per aliquot) to avoid multiple freeze-thaw cycles.

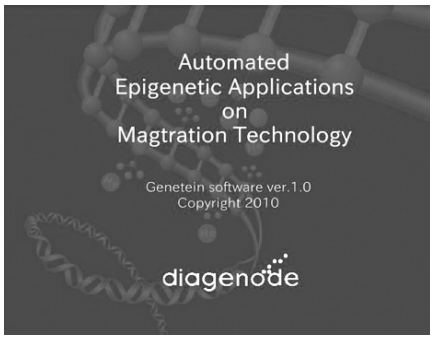
   c. Store at -80°C.

2. Prepare DNA mix tube without MethylCap protein

   a. In a new 1.5 ml tube, prepare the capture reaction mix without MethylCap protein. For one reaction, see volume needed below. Vortex for 5 seconds, at medium power and keep on ice.

Reagent	Volume per capture reaction and INPUT sample (1 µg of DNA)
Sheared DNA (0.1 µg/µl)*	12 µl
Buffer B	129.8 µl
TOTAL VOLUME	**141.8 µl**

Running a protocol



Diagenode Splash Screen – A0

After the software start-up screen disappears, the Diagenode splash screen is displayed for several seconds, and then disappears.

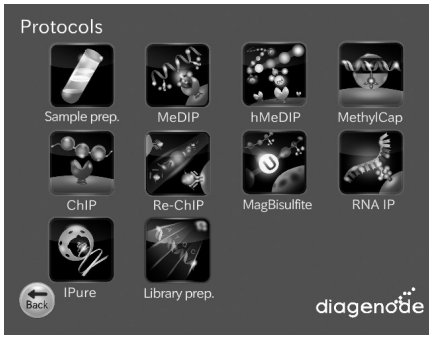
Start Screen – Top menu

After the Digenode splash screen disappears, the start screen is displayed. This is the first active window; it allows the user to enter into three different parts of the software.

USER ACTIONS:

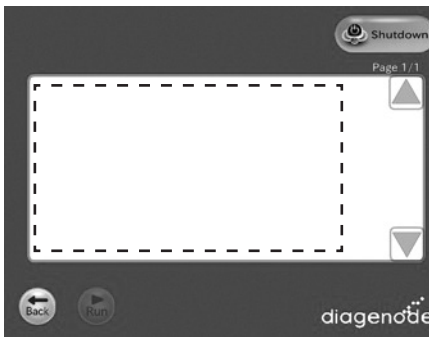
Buttons:

- Protocols
- Maintenance (for technical service)
- Information (Diagenode contact details)



Protocols screen

All available protocols are displayed on this screen.

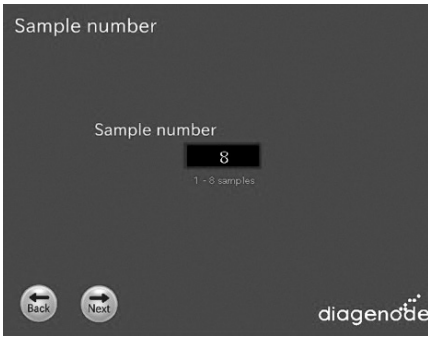


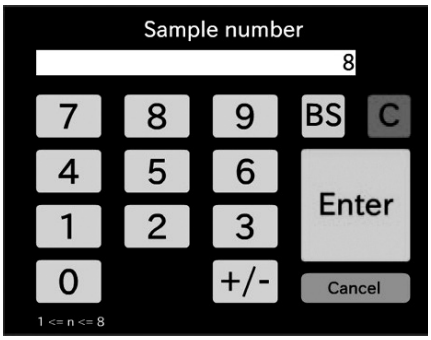
Screen – [Categories Name] Protocol List

After the user presses the "[Categories Name]" button, the "[Categories Name]" appears. When selected the protocol on the protocol list, the "Run" button shall turn executable.

Buttons:

- The user presses the "Back" button. The user returns to the "Protocols" screen.
- The user presses the "Shutdown" button. The screen shall be changed to "Power Off".
- The user presses the "Run" button. The screen shall be changed to "Sample number".
- ▲ Page up the list box.
- ▼ Page down the list box





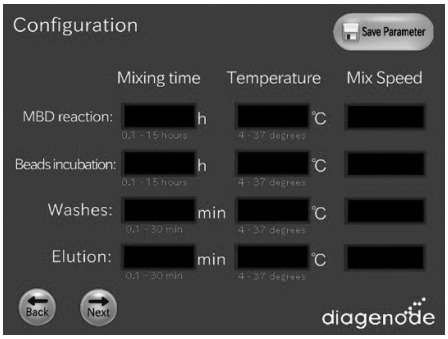
Keyboard

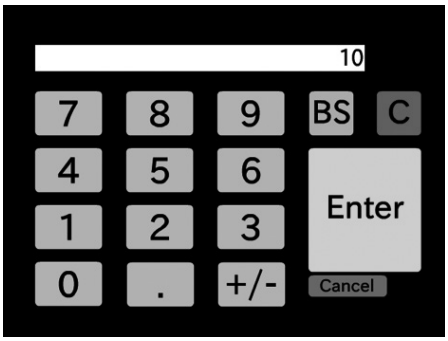
Screen – Sample number

After the user presses the "Run" button, the "Sample number" appears.

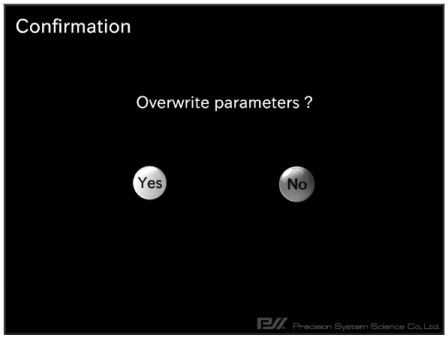
Buttons:

- The user presses the "Sample number" Text box. The screen will be changed to keyboard.
- The user presses the "Back" button. The user returns to the "Protocol List" screen.
- The user presses the "Next" button. The screen shall be changed to "Configuration" or "Layout information".





Keyboard



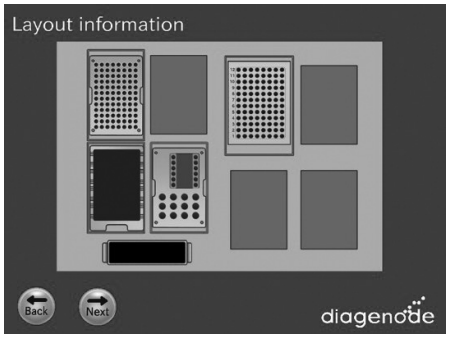
Screen – Configuration

After the user presses the next button from the "Sample number" screen, the "Configuration" screen appears.

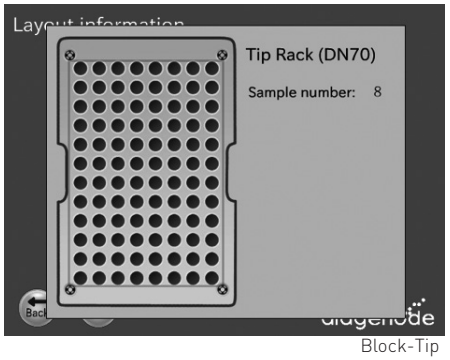
Buttons:

- The user presses the "Back" button. The user returns to the "Protocol List" screen.
- The user presses the "Next" button. The screen shall be changed to "Layout information".
- The user presses the "Save Parameter" button. The screen will be changed to "Save Parameter - Confirmation".
  - OK – Current parameters shown in the Display View will be stored to the [Protocol].ptd. And, returns the user to the display of the "Configuration" screen.
  - No – Returns the user to the display of the "Configuration" screen.
- The user presses the Text box. The screen will be changed to Keyboard or Speed list menu.

Speed list menu

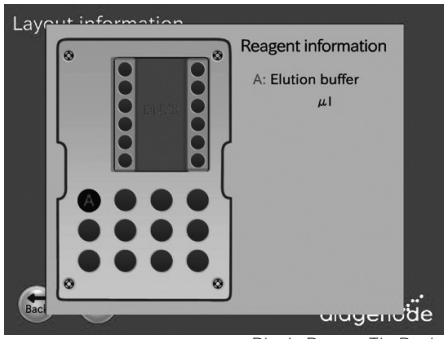


Layout information

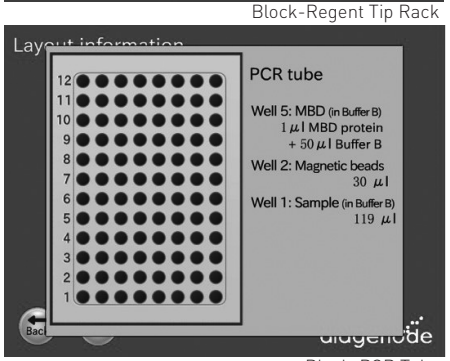


Block-Tip

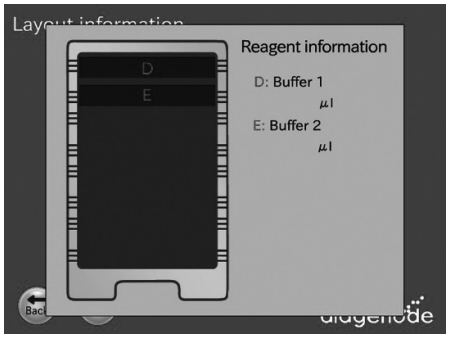
ONE ELUTION



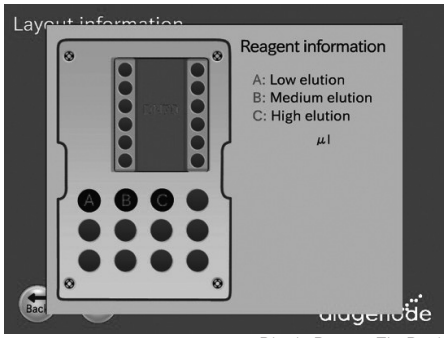
Block-Regent Tip Rack



Block-PCR Tube



FRACTIONATED ELUTION



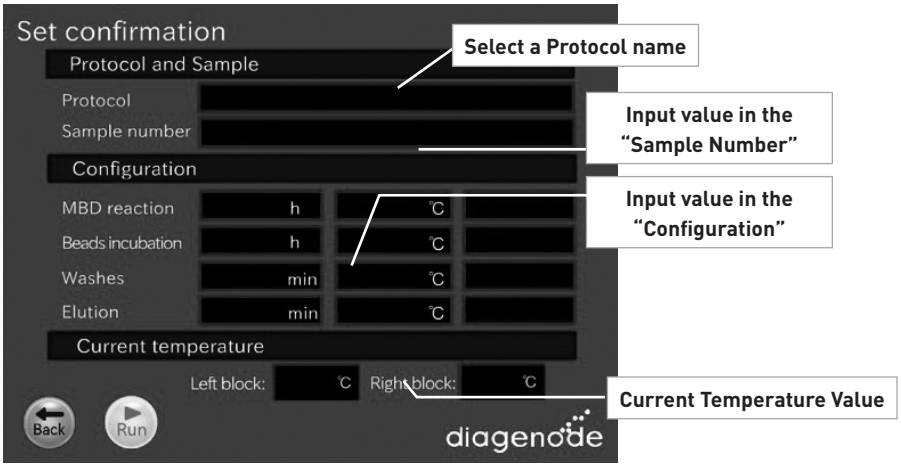
Block-Regent Tip Rack

Screen – Layout Information

After the user presses the "next" button from "Sample number" screen or "Configuration" screen, the "Layout Information" screen appears.

Buttons:

- The user presses the "Back" button. The user returns to the previous screen.
- The user presses the "Next" button. The screen changed to "Set confirmation".
- When the user presses a block, that block is magnifies on the work surface layout background. The magnified view provides a better display of the correct method setup for that block on the work surface.
- Based on the selected protocols, the user follows the indications provided in the screens to set up correctly the different reagents and samples.



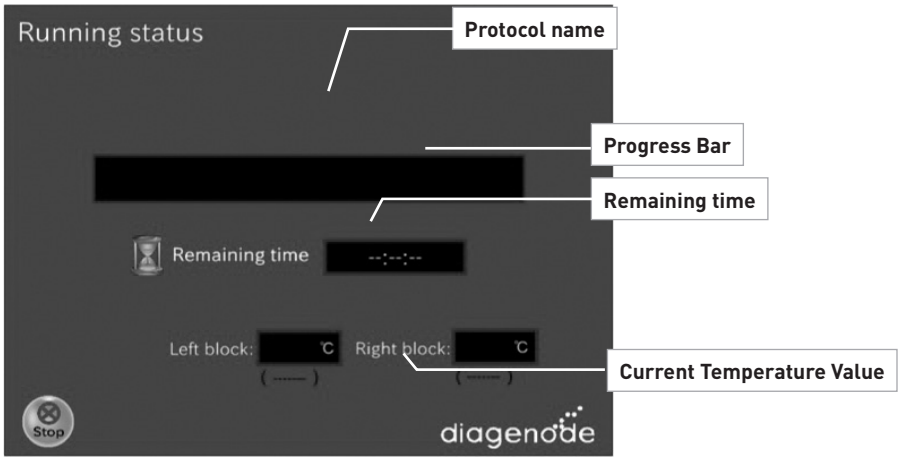
Screen – Set confirmation

After the user presses the "next" button in the "Layout information" screen, the "Set confirmation" screen appears.

At this point, user is expected to be ready to press RUN.

Buttons:

- The user presses the "Back" button. The user returns to the Layout information screen.
- The user presses the "Run" button. This is the expected action when user gets to this display after reviewing blocks. Runs the protocol.



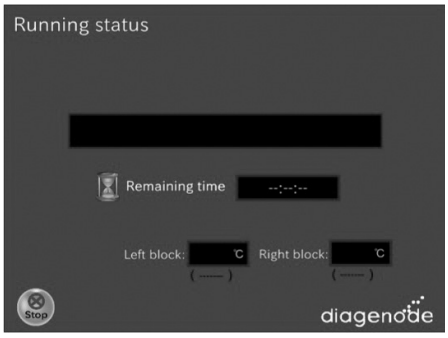
Screen – Running

After the user presses the "Run" button in the "Set confirmation" screen, the "Running" screen appears.

Buttons:

- The user presses the "Stop" button. The screen changes to "Stop Dialog".

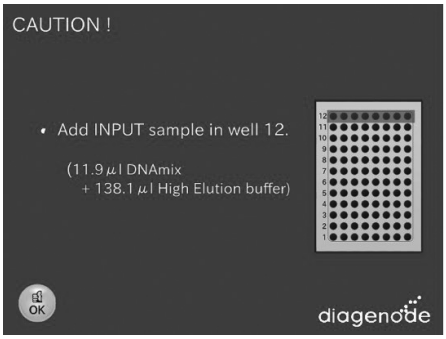
Status screen is preferred as a progress bar that moves across the screen as the step progresses

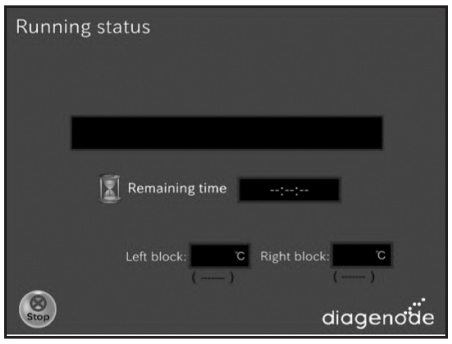


Screen – Running status

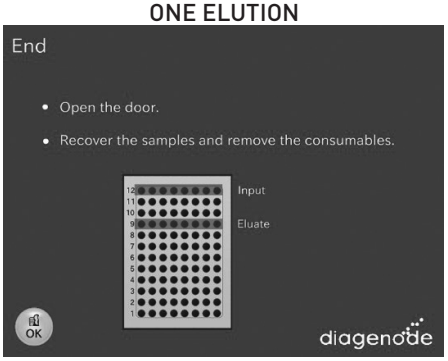
This screen gives informations about the current running step of the protocol.

The user can check through this screen the passed and remaining time of the experiment.

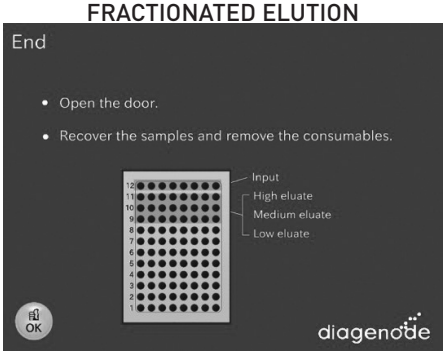




ONE ELUTION



FRACTIONATED ELUTION



Screen – Finish/End

When the protocol is complete, a window appears telling user the run is over. The screen behind this window should be the Startup screen. When OK is pressed, then the Startup screen appears and the user can immediately begin to remove their sample and prepare the next run.

At this point, user is expected to be ready to press RUN.

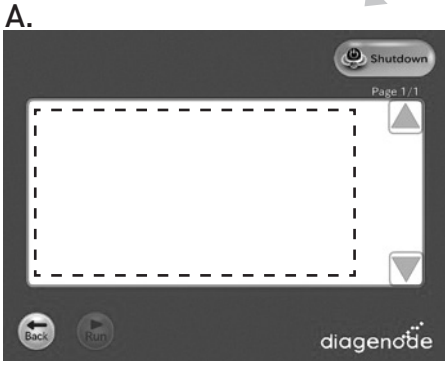
Buttons:

- The user presses the "OK" button. Then screen shall be changed to "[Categories Name] Protocol List".

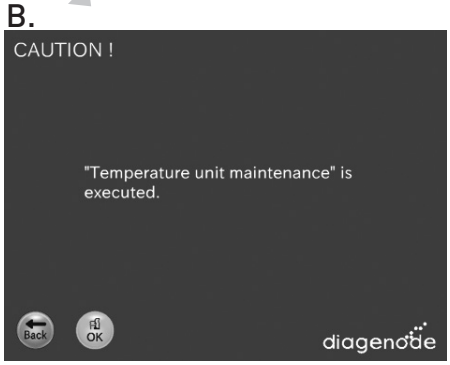
Screen – Caution !

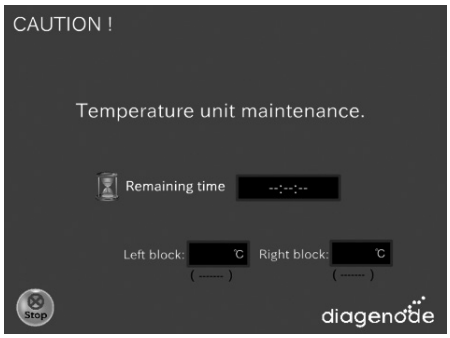
When the protocol finishes the user can return to the protocol list (screen **A.**) or warm the peltier block (screen **B.**) to eliminate possible condensation in the block.

A.



B.





STEP 3. DNA purification

Purification of all fractions and INPUT can be performed by using the IPure Kit v2 (Cat. No. C03010014 & CC03010015).

NOTE: We recommend to follow the IPure v2 protocol directly at the DNA binding step by adding 150 µL of isopropanol, 2 µL of carrier and 10 µL of IPure beads to each sample. You can then follow the standard IPure v2 protocol starting from Step 2 to Step 4.

STEP 4. qPCR

qPCR analysis of the following fractions: Flow-through, wash 1, wash 2, Low, Medium and High.

Control primers available at Diagenode

Primer pairs	Specificity	Input DNA sample (which includes Ctrls) amplification :	meDNA capture (which includes Ctrls) amplification :
Human meDNA primer pair (TSH2B)	Human DNA	Yes (if sample is human DNA)	Yes
Human unDNA primer pair (GAPDH)			No
Mouse meDNA primer pair (TSH2B)	Mouse DNA	Yes (if sample is mouse DNA)	Yes
Mouse unDNA primer pair (GAPDH)			No
Rat meDNA primer pair (TSH2B)	Rat DNA	Yes (if sample is Rat DNA)	Yes
Rat unDNA primer pair (GAPDH)			No

1. Prepare your qPCR mix using SYBR Green PCR master mix and start out qPCR.

   qPCR mix (total volume of 25 µl/reaction):
   - 1.00 µl of provided primer pair (stock: 10 µM each: reverse and forward)
   - 12.50 µl of master mix (e.g.: iQ SYBR Green supermix)
   - 5.00 µl of isolated DNA or diluted purified DNA sample (see above for DNA dilutions)
   - 6.50 µl of water

qPCR cycles:

	Temperature	Time	Cycles
PCR Amplification	95°C	7 minutes	x1
	95°C	15 seconds	x40
	60°C	60 seconds	
	95°C	1 minute	x1
Melting curve	65°C and increment of 0.5°C per cycle	1 minute	x60

2. When the PCR is done, analyse the results.

Data interpretation

The efficiency of Methyl DNA capture (meDNA-CAP) of particular genomic locus can be calculated from qPCR data and reported as a recovery of starting material: % (meDNA-CAP/ Total input).

% (meDNA-CAP/ Total input)= 2^[(Ct(10%input) - 3.32) - Ct(meDNA-CAP)]x 100%

Here 2 is the AE (amplification efficiency), Ct (meDNA-CAP) and Ct (10%input) are threshold values obtained from exponential phase of qPCR for the methyl DNA sample and input sample respectively; the compensatory factor (3.32) is used to take into account the dilution 1:10 of the input. The recovery is the % (meDNA-CAP/ Total input).

STEP 5. Sequencing

The samples collected in the fractions of interest and the input samples can also be analyzed by Next-Generation Sequencing (NGS).

1. Prepare DNA libraries for Illumina® NGS platforms with the MicroPlex Library Preparation Kit v3 (Cat. No. C05010001) by following Diagenode's instruction for kit handling and protocol.

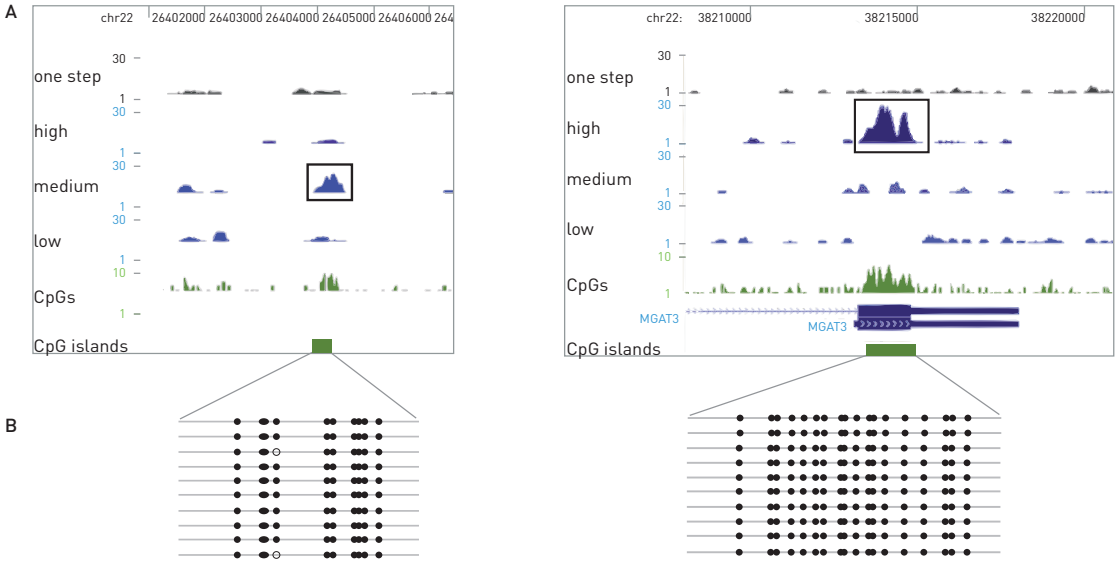
2. Quantify the libraries and check their profiles on a BioAnalyzer or Fragment Analyzer (Agilent) as explained in the corresponding manuals.

3. Prepare equimolar pools of the samples that will be sequenced in the same lane. We recommend to target 50 million of raw reads per sample. The requirements for a final library concentration depend on a sequencer and may vary between different sequencing service providers. The usual range is between 5-20 nM in a final volume 10-15 µl but we recommend inquiring with your sequencing platform.

4. Sequence the libraries on Illumina® NGS platforms and analyze the results.

Results

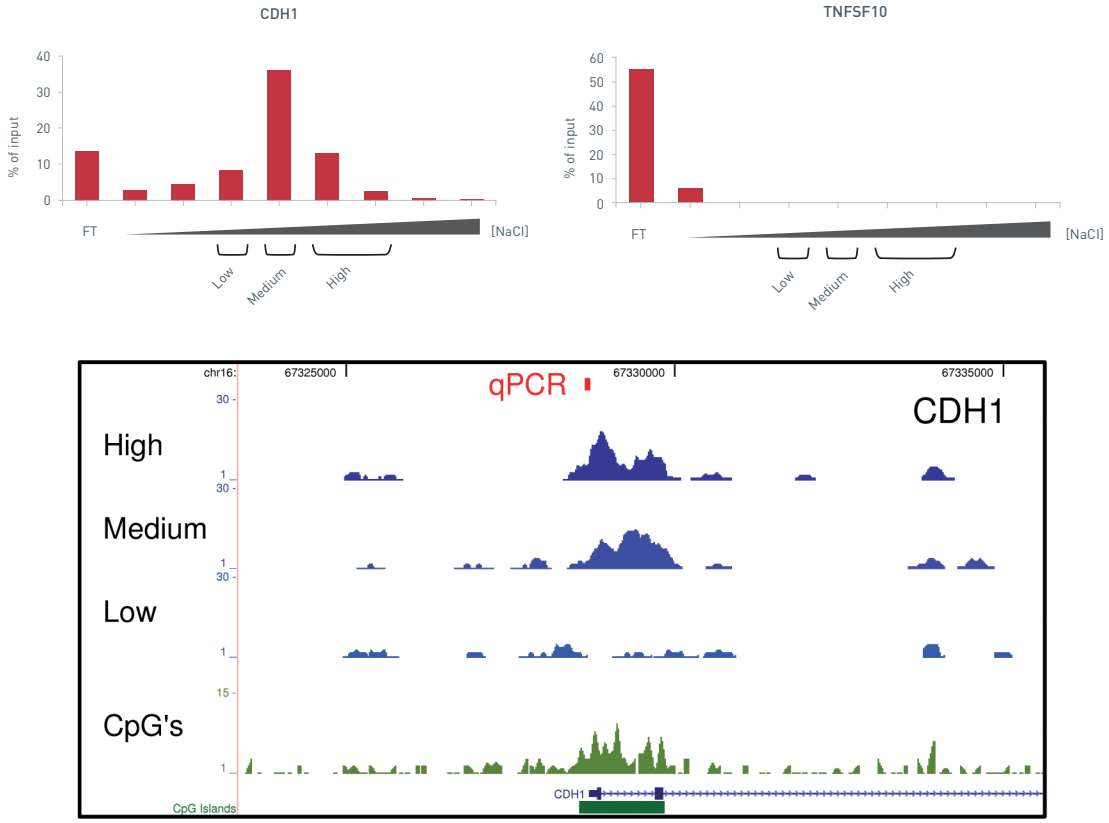
MBD-seq (Methylbinding domain - sequencing) allows for detection of genomic regions with different CpG density



Data provided by Henk Stunnenberg (Nijmegen Center for Molecular Life Sciences - The Netherlands)

Figure 1.
Using MBD-seq, two methylated regions were detected in different elution fractions according to their methylated CpG density (A). Low, Medium and High refer to the sequenced DNA from different elution fractions with increasing salt concentration. Methylated patterns of these two different methylated regions were validated by bisulfite conversion assay (B).

MethylCap results



Data provided by Henk Stunnenberg (Nijmegen Center for Molecular Life Sciences - The Netherlands).

Figure 2.

MethylCap assays were performed using DNA from NB4 cells and the MethylCap kit (Diagenode). Differential fractionation of double stranded DNA based on CpG methylation density was performed using increasing salt concentration during the elution steps. (A) qPCR results in a methylated (CDH1-CpG) and a unmethylated (TNFSF10) region show the % of recovery of captured DNA compared to the input in the different fractions. (B) Results have been confirmed by sequencing the captured DNA in the different elution fractions.

Troubleshooting Guide

Error Cause	Remedy
IP-Star cannot be switched on	IP-Star is not receiving power. Check that the power cord is connected to the workstation and to the wall power outlet.
Computer cannot be switched on	Computer is not receiving power. Check that the power cord is connected to the computer and to the wall power outlet.
IP-Star shows no movement when a protocol is started	IP-Star is not switched on. Check that the IP-Star is switched on.
IP-Star shows abnormal movement when a protocol is started	The pipettor head may have lost its home position. In the Software, select "Manual Operation/Home". After confirming that the pipettor head moves to the home position, run the protocol again.
Aspirated liquid drips from the disposable tips	Dripping is acceptable when ethanol is being handled. For other liquids: air is leaking from the syringe pumps. Grease or replace the O-rings. If the problem persists, contact DIAGENODE Technical Services.

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