

MethylEasy™ Xceed

Rapid DNA Bisulphite Modification Kit

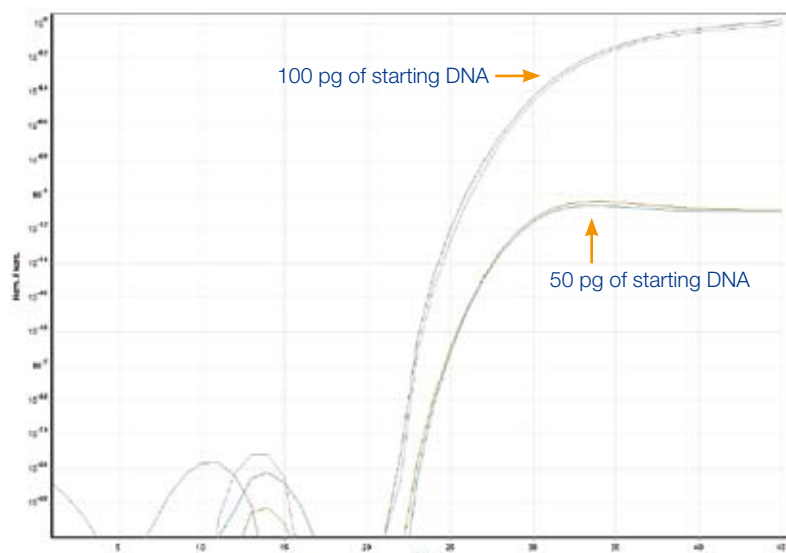
Take a Leap Forward in Methylation Detection



“Loss-less” conversion technology.

Lane 1: DNA (2µg) recovered and electrophoresed after using **MethylEasy™** conversion technology

Lane 2: (2µg) recovered and electrophoresed after using conventional bisulphite conversion.



Exquisite Sensitivity: Either 100 pg or 50 pg of DNA (16 or 8 mammalian cell equivalents respectively) was treated with **MethylEasy™ Xceed** as per the user manual and eluted in 12 µl. Nested PCR for the *Lim2* gene was performed on 4 µl of converted DNA. The second round was detected in real time on a Corbett Rotor-Gene 6000 using Syto-9 as the detection dye at a final concentration of 1 µM.

“The rapid bisulfite conversion time with **MethylEasy™ Xceed** greatly facilitates and accelerates our research on DNA methylation in stem cells”. Prof. Philippe Collas¹

- Easy to Use - No DNA pre-treatment necessary and easy column purification.
- Great new value per kit - Enough reagent for 40 conversions
- Markedly Faster Reactions - Processing time is 90 mins total from wild type to fully modified DNA
- More Sensitive - Start from as little as 50 picograms of DNA (approx. 8 mammalian cell equivalents)
- Excellent DNA Preservation - Reduce DNA degradation by more than 90% to obtain truly representative analysis of methylated cytosines
- Better Conversion - No evidence of DNA non-conversion found in sequencing analysis
- Internal controls - Included for absolute reaction confidence and product support
- From the original authors of the sodium bisulphite method*

*1992, Frommer et al., Proc. Nat. Acad. Sci. USA, 89, 1827-1831.

MethylEasy™ Xceed DNA Bisulphite Modification Kit

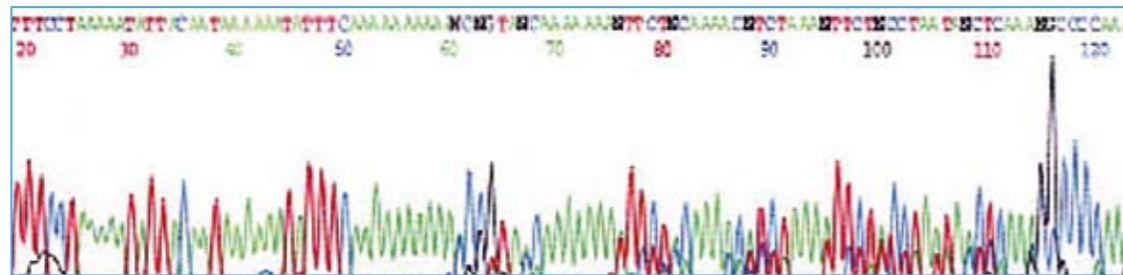
Conventional bisulphite treatments result in the loss of between 84% and 96% of the starting DNA², require pre-treatment with restriction endonuclease digests, the embedding of the DNA in agarose, or multiple tube changes and precipitations.

MethylEasy™ Xceed addresses all of these shortcomings and is a simple, sensitive and quick modification method that requires no DNA pre-treatment and as little as 50 pg of starting material. **MethylEasy™** "loss-less" sodium bisulphite technology results in virtually no loss of DNA, improved sensitivity, higher amplification efficiency, longer fragment generation and increased stability of the template DNA.

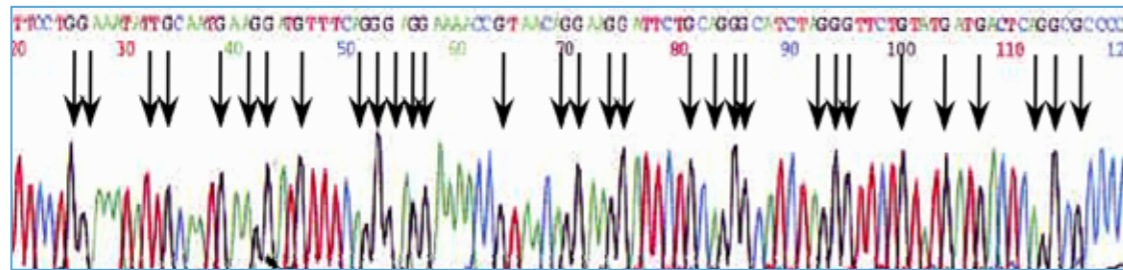
Better DNA Conversion

The sequencing chromatograms below show full conversion in the DNA treated with **MethylEasy™** technology, compared to many non-converted sites in the DNA converted with conventional methods (Arrows denote blocked sites.)

MethylEasy™ PCR product

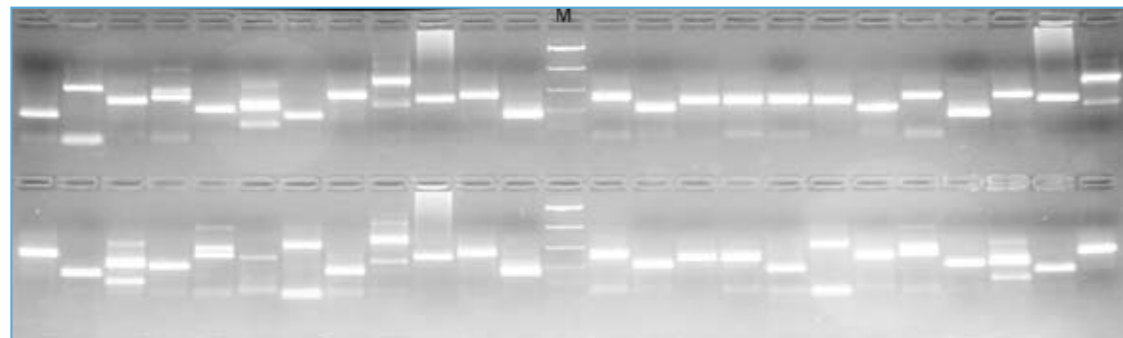


Conventional bisulphite PCR product



Excellent DNA Preservation

Typical PCR results after **MethylEasy™ Xceed** conversion of human genomic DNA is shown below. The results demonstrate whole genome conversion via the amplification of 48 different genomic loci from just 50 ng of starting DNA.

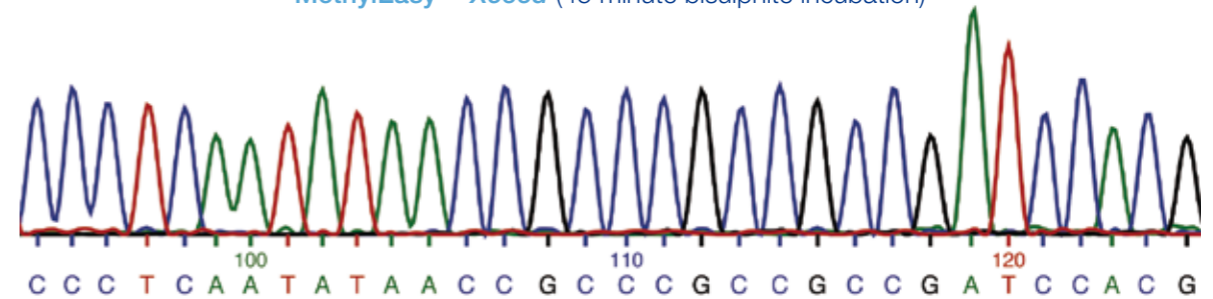


A total of 50 ng of human DNA was converted as per the **MethylEasy™ Xceed** product manual. The DNA was resuspended in 120 µL and a total of 2 µL was seeded into 48 different PCRs. All amplifications were two rounds, and 10 µL of the second round product was electrophoresed on a 2% agarose gel.

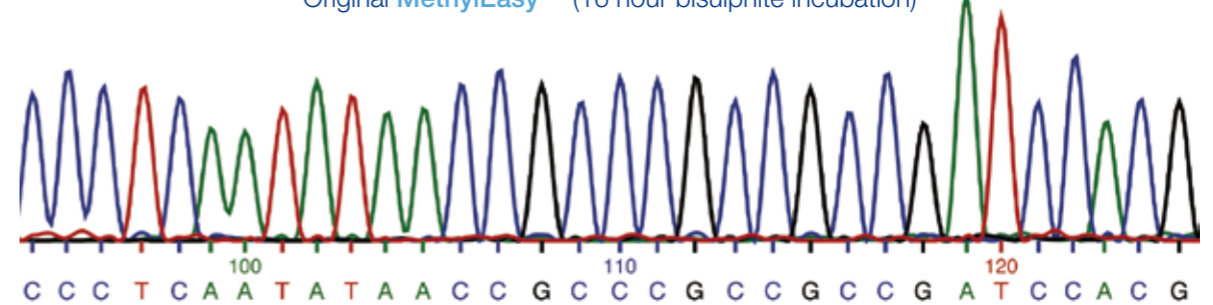
Consistent Results as Compared to Original MethylEasy™

The new **MethylEasy™ Xceed** method achieves the same results in 90 minutes that took 6-18 hours with the original **MethylEasy™** method. The figure below compares the sequence data of PCR fragments amplified from DNA that had been treated with either the **MethylEasy™ Xceed** or Original **MethylEasy™** protocols.

MethylEasy™ Xceed (45 minute bisulphite incubation)



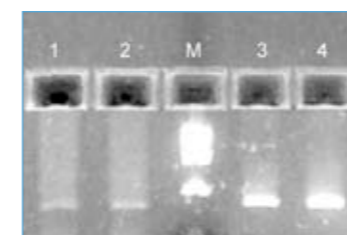
Original MethylEasy™ (16 hour bisulphite incubation)



The above sequence shows a portion of the Myog gene that was assayed for CpG methylation using either the **MethylEasy™ Xceed** kit (top panel) or the original **MethylEasy™** kit. The sequence was generated using a reverse primer and hence any methylated cytosines are represented as guanines. The above data was generated during beta-testing in the laboratory of Prof Philippe Collas¹.

More Sensitive

Unsurpassed sensitivity of **MethylEasy™ Xceed** is seen below in the amplification of 2 separate PCRs when starting with only 100 pg or 50 pg of mammalian DNA.

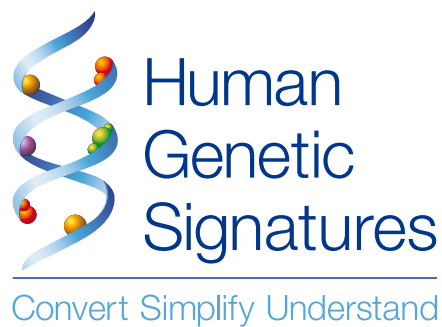


Either 100 pg or 50 pg of DNA (16 or 8 mammalian cell equivalents respectively) was treated with **MethylEasy™ Xceed** as per the user manual and eluted in 12 µL. Nested PCR for the Lim2 gene was performed on 4 µL of converted DNA. The second round amplification was electrophoresed on a 2% agarose gel. Lanes 1 and 2: 50 pg starting DNA; Lanes 3 and 4: 100 pg starting DNA; M: DNA marker.

Easy to Use

MethylEasy™ technology does not require any DNA pre-treatment (i.e restriction digestion) and the DNA is now purified via a simple column. The vastly reduced processing time is demonstrated below:

Original MethylEasy™	MethylEasy™ Xceed
Denature DNA with NaOH and incubate for 15 minutes	Denature DNA with NaOH and incubate for 15 minutes
Add conversion reagents and incubate for 4-16 hours	Add conversion reagents and incubate for 45 mins
Precipitate and wash the DNA, 45 to 75 minutes	Purify the DNA via column for 10 mins
Resuspend the DNA and desulphonate for 30 mins to 1 hour.	Desulphonate for 20 mins
The DNA sample is now fully converted – 6 to 18 hours total time	The DNA sample is now fully converted – 90 minutes total time



For further information on **MethylEasy™** technology
visit our website at www.geneticsignatures.com
or email info@geneticsignatures.com

US and International Patents Pending

¹Philippe Collas is Professor at the Faculty of Medicine of the University of Oslo, Norway. He obtained his Ph.D. in 1991 from the University of Massachusetts and since has worked as Researcher in the private and academic sector. His research activities focus on nuclear reprogramming and stem cell epigenetics. Collas has also been a consultant for various Biotech Companies worldwide.

²Grunau C, Clark SJ, Rosenthal A. Bisulfite genomic sequencing: systematic investigation of critical experimental parameters. *Nucleic Acids Res.* 2001 Jul 1;29(13):E65-5

Human Genetic Signatures Pty Ltd

22 Delhi Road, Macquarie Park, NSW 2113, Australia • Phone: +61 2 9870 7580 • Fax: +61 2 9889 4034

