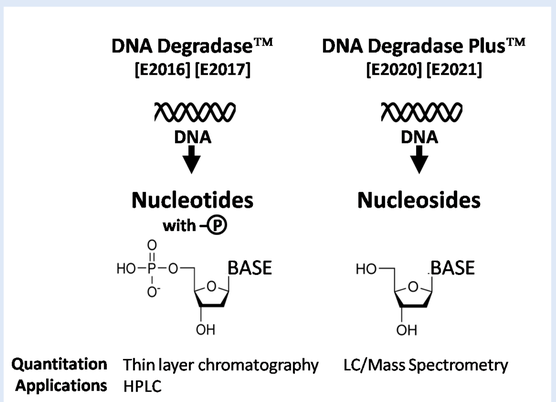
Rapid Enzymatic DNA Degradation for Quantitation of 5-Methylcytosine and 5-Hydroxymethylcytosine

James L. Yen & Xi Yu Jia
Zymo Research Corp., Irvine, CA

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

Abstract

Modification of DNA, namely 5-methylcytosine, has been recognized to be one of the dominant phenomenon in the field of epigenetics. Fluctuations in global DNA methylation levels have implications in development, cancer, and aging. Therefore methods for precise quantification of global DNA methylation (i.e. - HPLC and LC/MS) are powerful tools to gain a better understanding in these areas. To address the need for a rapid and convenient sample preparation method for global methylation quantitation, we have developed a one hour, one-step enzymatic procedure for DNA degradation with DNA Degradase™ and DNA Degradase Plus™. DNA Degradase™ and DNA Degradase Plus™ degrade DNA to single nucleotides and nucleosides, respectively. Nucleotides are easily quantitated by thin layer chromatography (TLC) or high performance liquid chromatography (HPLC), while nucleosides (lacking a charged phosphate) are ideal for quantitation by mass spectrometry (LC/MS).



Furthermore, we have validated DNA Degradase™ and DNA Degradase Plus™ by HPLC and LC/MS, respectively. Sampling a range of biological sources, DNA Degradase Plus™ coupled with LC/MS has proven to be a powerful method for detection and quantification of 5-methylcytosine as well as 5-hydroxymethylcytosine.

Aims

1. Evaluate quality of sample preparation using conventional digestion methods versus DNA Degradase™ with HPLC.
2. Evaluate quality of LC/MS results from samples treated with DNA Degradase Plus™.
3. Evaluate LC/MS as a method for 5-methylcytosine and 5-hydroxymethylcytosine identification.

Results

AIM1

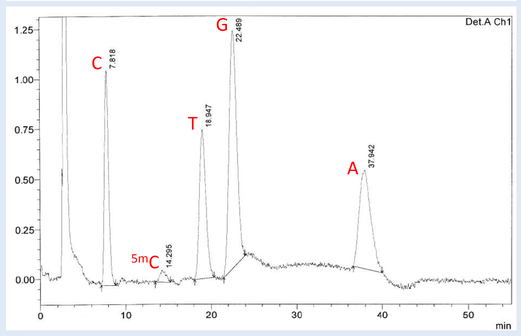
Method:
DNA Degradase™ and HPLC

Purpose:
Compare quality of HPLC results for global methylation quantitation using "conventional" sample preparation methods versus DNA Degradase™.

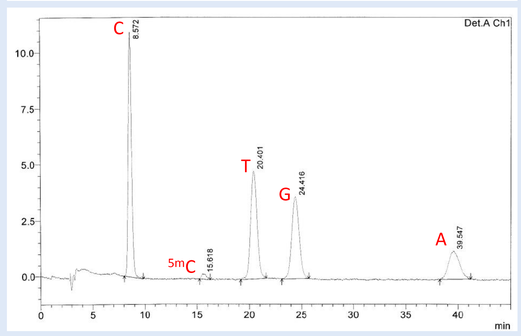
Procedure:
Genomic DNA samples from biological replicate animals. DNA was extracted from tissue source from animals of the same genetic background and fed the same diet.

Results
DNA Degradase™ gives high quality HPLC results.

Conventional Method



DNA Degradase™



	3µg	10µg	28.5µg	Mean	SD
C	18.36	16.89	17.87	17.71	0.75
^{5m}C	1.58	1.31	1.12	1.34	0.23
T	19.77	18.35	17.82	18.65	1.01
G	37.17	40.17	38.10	38.48	1.54
A	23.12	23.27	25.09	23.83	1.10

	3µg	5µg	7µg	Mean	SD
C	33.5	32.8	33.2	33.32	0.37
^{5m}C	1	1.3	1.1	1.12	0.12
T	28.3	28.3	27.9	27.89	0.64
G	24.5	24.4	24.7	24.78	0.55
A	12.7	13.2	13.1	13.17	0.44

AIM2

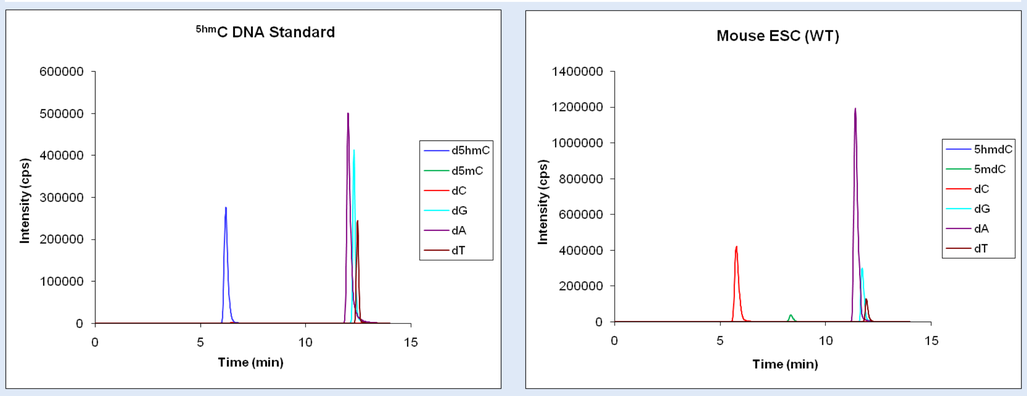
Method:
DNA Degradase Plus™ and LC/MS

Purpose:
Evaluate quality of LC/MS results using DNA Degradase Plus™.

Procedure:
A ^{5hm}C DNA standard (Zymo Research Corp.) and mouse ESC genomic DNA were digested with DNA Degradase Plus™ (1 hour) and nucleosides were analyzed via LC/MS.

Results
DNA Degradase Plus™ treatment of DNA gave high resolution chromatograms as detected by LC/MS. The method efficiently identifies all four canonical bases as well as 5-methylcytosine and 5-hydroxymethylcytosine. Resolution is comparable to "conventional" methods (data not shown).

DNA Degradase Plus™



AIM3

Method:
DNA Degradase Plus™ and LC/MS

Purpose:
Evaluate potential of high throughput sample processing using LC/MS to identify ^{5hm}C in various biological sources.

Procedure:
DNA was extracted from various sample sources and digested with DNA Degradase Plus™ (1 hour) and nucleosides were analyzed via LC/MS.

Results
Zebrafish embryonic DNA showed detectable levels of ^{5hm}C whereas none was detected in Arabidopsis and Chlamydomonas DNA.

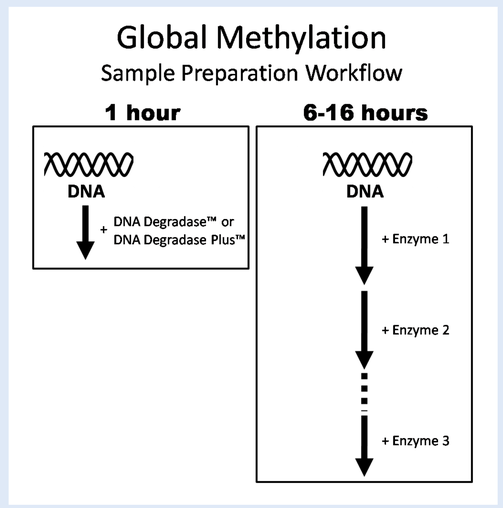
DNA Degradase Plus™

DNA Source	^{5m}C	^{5hm}C
Arabidopsis	11.38%	0.00%
Zebrafish Embryo	12.93%	0.39%
Chlamydomonas	1.03%	0.00%

Conclusions

The increased recognition that global DNA methylation profiles change in diseased states as well as in normal development has led to the necessity for innovative technologies that can facilitate reliable and efficient workflow for global DNA methylation studies.

Results show that DNA Degradase™ and DNA Degradase Plus™ coupled to HPLC and LC/MS (respectively), are valuable tools for global methylation analysis. The ease and efficiency of this method as compared to tedious conventional methods allows researchers greater ability for processing of large sample numbers.



Recently, the discovery of 5-hydroxymethylcytosine (^{5hm}C) in the mammalian genome (ESC and Purkinje cells) has spurred interest in identifying this novel epigenetic modification in other biological contexts (1, 2). To date, the most sensitive and reliable tool for looking for 5-hydroxymethylcytosine in DNA is mass spectrometry (3). Therefore to evaluate this method, we treated DNA samples from various organisms with DNA Degradase Plus™ and analyzed them via LC/MS. Interestingly, we were able to identify 5-hydroxymethylcytosine in zebrafish (*Danio rerio*) embryo genomic DNA. This result implicates 5-hydroxymethylcytosine as being a conserved epigenetic mark in development, not only in mammals, but in simple vertebrates as well.

Acknowledgements

Special thanks to our collaborators:
HPLC experiments and results were kindly provided by K. Armstrong of AgResearch Limited (New Zealand).
LC/MS experiments and results were kindly provided by T. Le (Dr. Fan Lab UCLA).

References

1. Tahiliani *et al. Science* 2009 Vol. 324.
2. Kriaucionis *et al. Science* 2009 Vol. 324.
3. Song *et al. Analytical Chemistry* 2005 Vol. 77.

Copyright © 2010 Zymo Research Corp.