ZYMO RESEARCH
The Beauty of Science is to Make Things Simple®

EZ-96 DNA Methylation™ MagPrep Series

High-throughput, automated, magnetic bead-based bisulfite conversion of DNA with the Tecan Freedom EVO® for methylation analysis.

Introduction

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, and many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis and methylation-sensitive arbitrarily primed PCR. However, the most common techniques used today still rely on bisulfite conversion.

Treating DNA with bisulfite chemically modifies non-methylated cytosine into uracil, methylated cytosine remains unchanged. Once converted, the methylation profile of the DNA can be determined using the desired downstream application. For single locus analysis, the region of interest is generally amplified following bisulfite conversion (i.e., bisulfite PCR) and then sequenced. However, recent advances in methylation detection allow the investigation of genome-wide methylation patterns, technologies include array-based methods, Pyrosequencing®, reduced representation bisulfite sequencing (RRBS), and whole genome bisulfite sequencing.

To this point all bisulfite conversion products have been dependent on manual manipulation of spin plates and columns or been of limited throughput. By adapting the clean-up of bisulfite converted DNA to a magnetic bead based procedure and coupling it to the Freedom EVO® platform, Zymo Research has opened the door to high-throughput bisulfite conversion of samples for methylation analysis.

Automation Equipment

- Tecan Freedom EVO®
- Freedom EVOware®
- 8 channel Liquid Handling Arm (LiHa), configured for Disposable Tips (DiTis)
- Robotic Manipulation Arm (RoMa)
- Te-Shake™ Shaker
- 96-well Magnetic Stand

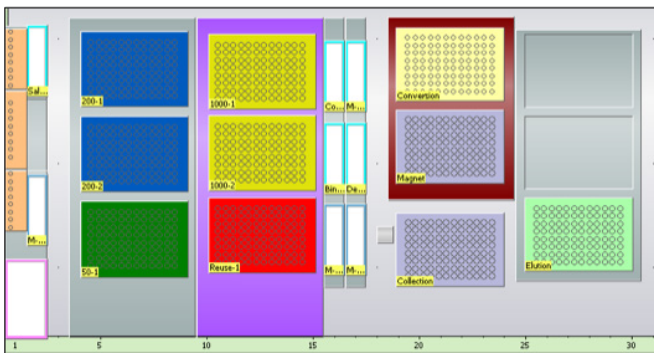

Figure 1. Example Deck Layout

	Compatible with Illumina Infinium® Arrays	High Speed	Fastest, Most Convenient	Input Cells and Tissues Directly!
	EZ DNA Methylation™	EZ DNA Methylation-Gold™	EZ DNA Methylation-Lightning™	EZ DNA Methylation-Direct™
Conversion Time	12-16 hr.	2.5 hr.	1 hr.	3.5 hr.
Conversion Efficiency	> 99%	> 99%	> 99.5%	> 99.5%
Input (volume)	500 pg - 2 µg DNA (≤ 45 µl)	500 pg - 2 µg DNA (≤ 50 µl)	100 pg - 2 µg DNA (≤ 20 µl)	50 pg - 2 µg DNA, 10 - 105 cells (≤ 20 µl)

Figure 2. Bisulfite conversion kit selection.

Overview of Procedure

Genomic DNA samples of 50 pg to 2 µg are used as input for the bisulfite conversion reaction. The reaction is incubated, the samples are desulphonated and cleaned, then eluted from the binding beads. Recovered DNA can be used for various downstream applications including bisulfite PCR.

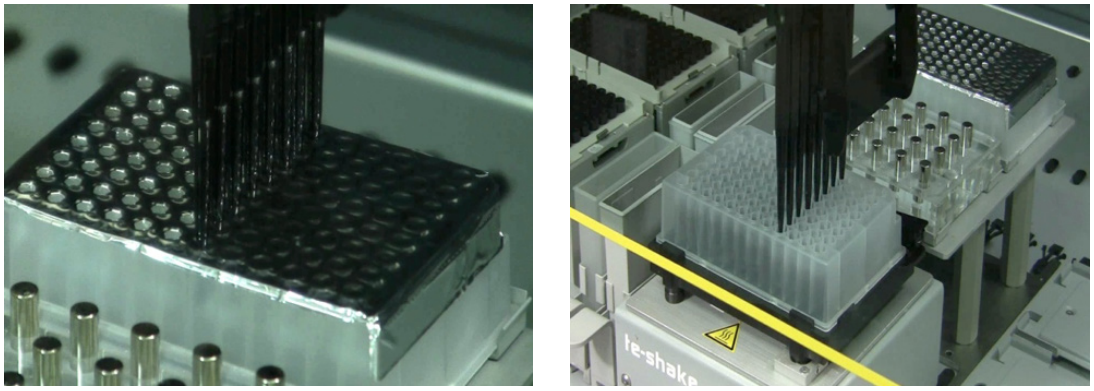

Figure 3. Transfer from conversion plate to binding plate. Conversion reactions in a foil-sealed PCR plate are transferred to a separate plate containing binding buffer and magnetic beads (right). Close-up of samples are being aspirated from the conversion plate (left).

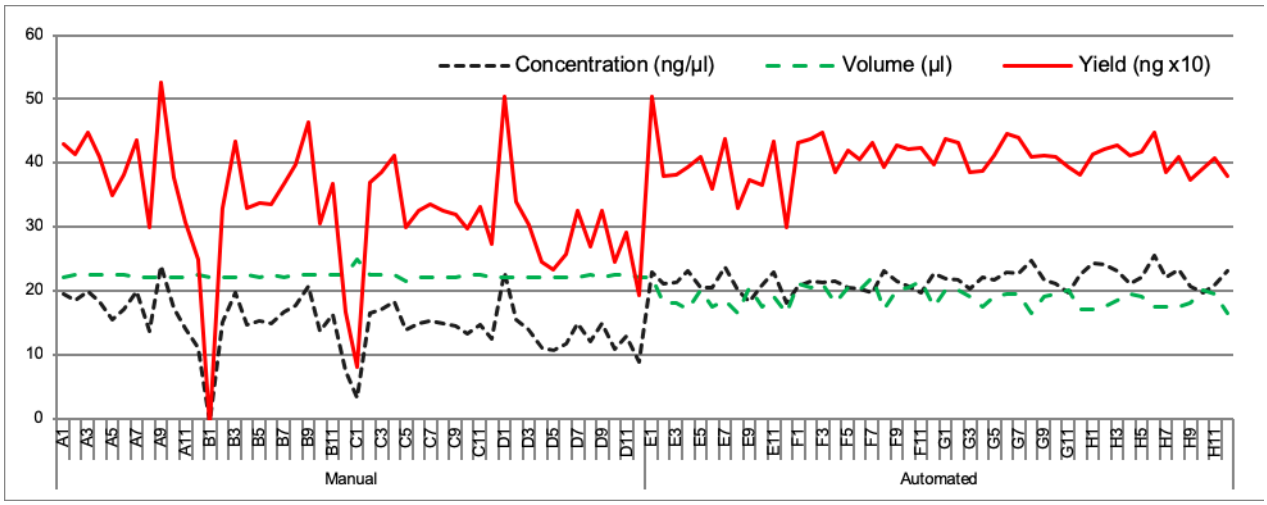

Figure 4. Consistent recoveries with automated processing. Graph shows concentration, recovered volume and total yield for replicate bisulfite converted DNA samples across a 96-well plate. Half of the samples (rows A-D) were processed manually. The other half of the samples (rows E-H) were processed using the Freedom EVO® platform and a dedicated script.

Results

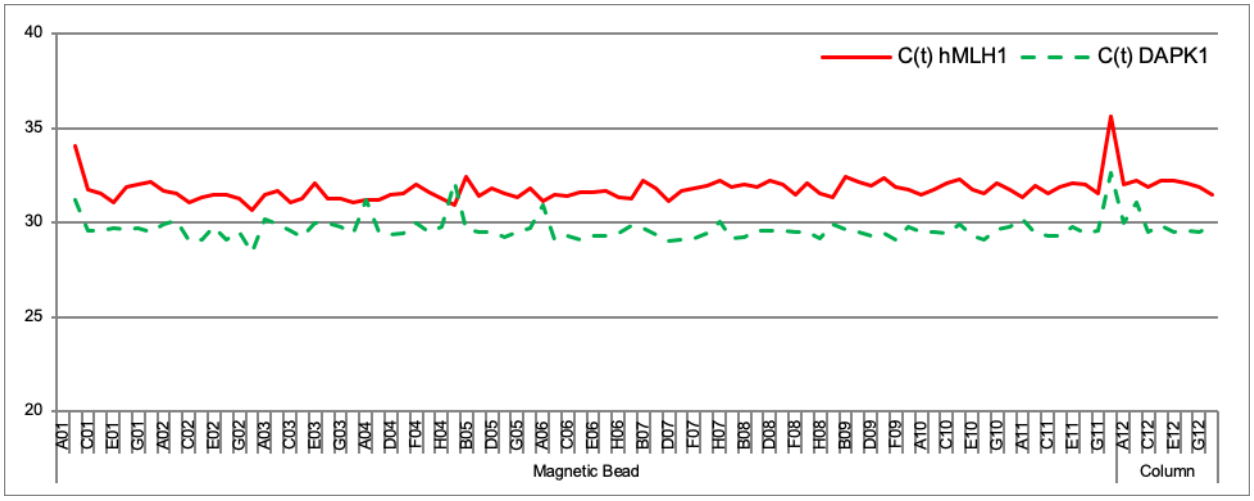

Figure 5. Consistent amplification of bisulfite converted genomic DNA. An entire plate of replicate samples was bisulfite converted and then cleaned-up using either the automated magnetic bead based system ("Magnetic Bead", A1-H11) or by spin-column based methods ("Column", A12-H12). Real-time PCR was performed using 1 µl of each eluted DNA for each of two primer sets. Threshold cycle (C(t)) values for the two primer sets are shown above.

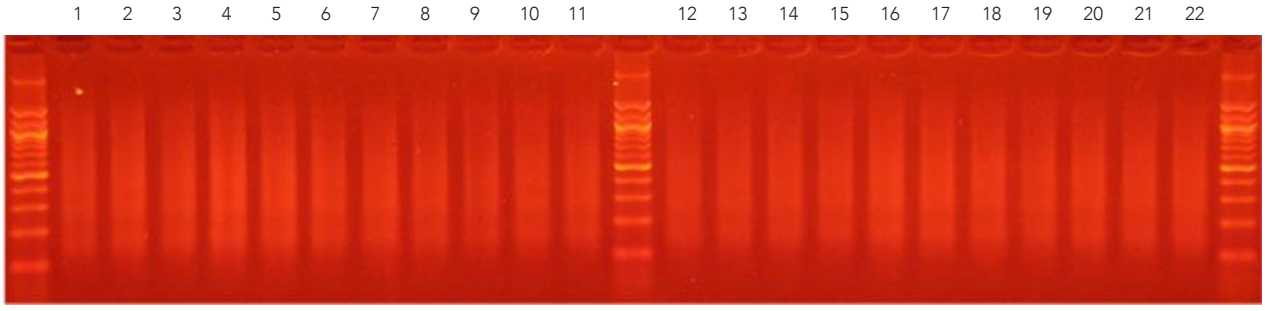

Figure 6. Consistent DNA profiles following bisulfite conversion. Comparison of DNA following clean-up by column based (lanes 1, 2) or automated magnetic bead-based (lanes 3-22) procedures. Samples were resolved in 2% agarose gel with 100 bp ladder for scale.

Conclusions

Samples processed using the EZ-96 DNA Methylation™ MagPrep procedures with the Freedom EVO® platform perform comparably with established spin-column based procedures. This is shown by recovery, amplification and profile of the converted DNA, demonstrating the power of these protocols for processing large numbers of samples with the same reliability that has come to be expected from Zymo Research's bisulfite conversion products.

Specifications

- DNA Input: Samples containing between 50 pg and 2 µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.
- Conversion Efficiency: > 99.0 - 99.5% of non-methylated cytosine residues are converted to uracil; > 99.0 - 99.5% protection of methylated cytosines.

Product	Cat. No.	Kit Size
EZ-96 DNA Methylation™ MagPrep	D5040 D5041	4 x 96 preps 8 x 96 preps
EZ-96 DNA Methylation-Gold™ MagPrep	D5042 D5043	4 x 96 preps 8 x 96 preps
EZ-96 DNA Methylation-Direct™ MagPrep	D5044 D5045	4 x 96 preps 8 x 96 preps
EZ-96 DNA Methylation-Lightning™ MagPrep	D5046 D5047	4 x 96 preps 8 x 96 preps