ZYMO RESEARCH

The Beauty of Science is to Make Things Simple®

High-throughput, magnetic bead-based procedure for methylation analysis using the EZ-96 DNA Methylation™ MagPrep Kits.

Introduction

The need to detect and quantify DNA methylation efficiently and accurately has become even more crucial in many areas of biology. The well proven bisulfite conversion chemically modifies non-methylated cytosine into uracil, while methylated cytosine remains unchanged. The methylation profile can then be analyzed using applications including pyrosequencing, reduced representation bisulfite sequencing (RRBS), etc. Magnetic bead-based bisulfite converted DNA clean-up on the Hamilton Microlab STAR platform enables high-throughput automated solutions for methylation analysis.
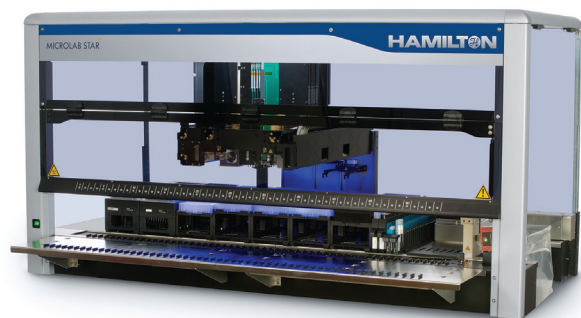
Materials and Methods

Genomic DNA (gDNA) was first extracted from healthy human blood with the *Quick*-DNA™ Miniprep Plus Kit (Cat. No. D4068). Forty-eight replicate samples of isolated gDNA (600 ng) were used as input for bisulfite treatment using the EZ-96 DNA Methylation-Lightning™ MagPrep Kit (Cat. No. D5040). Twenty-four of the extracted gDNA samples were processed manually and the other 24 samples were processed using the Hamilton Microlab STAR.

The Hamilton Microlab STAR used in this test has 8 channels, Autoload (optional), CO-RE MPH-96, CO-RE Grip, Hamilton Heater shaker, ZR-96 MagStand (Cat. No. P1005), and all required tips and reagent carriers.

The DNA concentration was analyzed using Thermo Scientific NanoDrop™ 2000 UV-Vis Spectrophotometer.

Multiplex amplification was performed using locus-specific primer pairs and the Fluidigm® Access Array™ System. The resulting amplicons were pooled for harvesting and subsequent barcoding according to Fluidigm guidelines. After barcoding, samples were cleaned up using the ZR-96 DNA Clean and Concentrator™ (Cat. No. D4023) and then prepared for massively parallel sequencing using an Illumina MiSeq paired-end sequencing run. The protocol workflow is shown in Figure 1 (page 2).

Results and Discussion

Consistent Yields and High Quality

DNA concentration, recovered volume, and yields from replicate DNA samples were compared between 24 manually processed samples and 24 samples processed on the Microlab STAR. The results indicate that automation is comparable with the manual process (Figure 2). The manual samples have a CV of 7.1% and the automated samples have a CV of 5.4%.
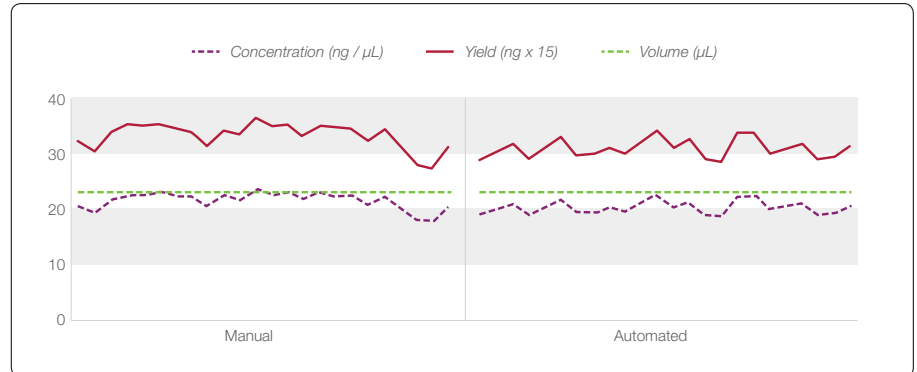

Figure 2: Comparison between manual and automated (Microlab STAR) sample processing using EZ DNA Methylation Lightning.

Multiplex Targeted Amplification and Sequencing

Sequence reads were aligned according to the reference genome using Bismark, an aligner optimized for bisulfite sequence data and methylation calling. The methylation level of each accessed cytosine was estimated as the number of reads reporting a C, divided by the total number of reads reporting a C or T. Sequence data from sample replicates were graphed and assessed for correlation of DNA methylation values using linear regression analysis. Results show high correlation, indicating no significant difference between manual and automation processing.
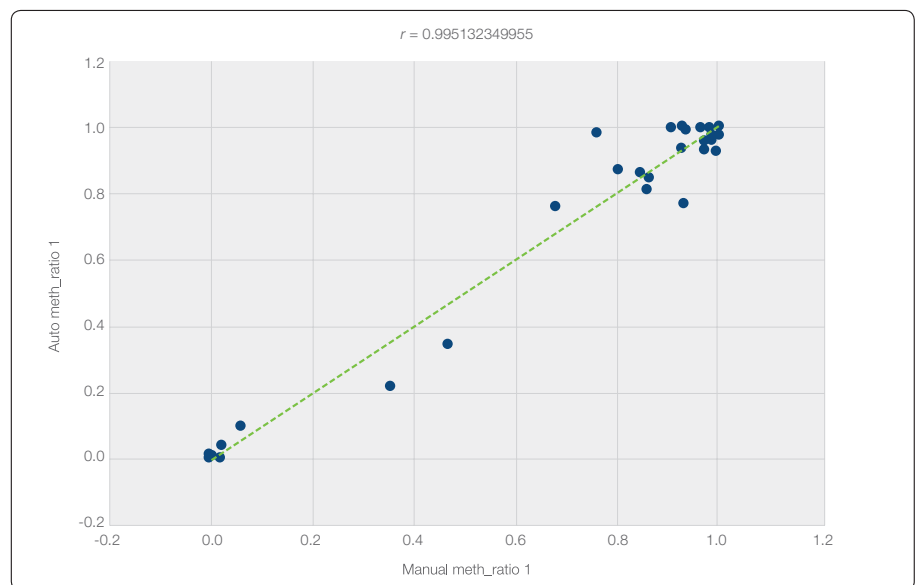

Figure 3: High correlation of methylation ratio values. Scatterplot shows the correlation ("meth_ratio") of several gene loci comprising 131 intersecting unique CpG sites between a single representative automated and manual comparison. The mean of the correlation scores (r-value) for all sample comparisons is r = 0.989199971 ± 0.006039335.

Figure 1 workflow steps:

- 130 µl Lighting Conversion Reagent
- Thermal Cycler (98 °C for 8 min, 54 °C for 60 min, 4 °C)
- 600 µl M-Binding, Mixing
- 20 µl MagBinding Beads, Shaking 10 minutes
- Magnetic Rack Separation
- 400 µl M-Wash Buffer Washing
- 200 µl L-Desulphonation Buffer at RT, 15 minutes
- 400 µL M-Wash Buffer Washing x2
- Dry Beads at 55°C
- 25 µl M-Elution Buffer, Shaking 5 minutes
- Transfer Eluate

Figure 1: EZ-96 DNA Methylation-Lightning MagPrep workflow.

Conclusions

Samples processed using the EZ-96 DNA Methylation-Lightning™ MagPrep procedures with the Hamilton Microlab STAR perform comparably with manual pipetting techniques and methods. This is shown by the successful recovery, amplification, and sequencing of both automated and manually processed samples, enabling an efficient solution for reliable high-throughput bisulfite conversion.

Automation advantage includes reducing processing time of 96 samples from 8 hours to 1.5 hours, reduced manual tracking, and increased regulatory compliance.

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

Bisulfite Conversion Kit Selection

	Compatible with Illumina Infinium® Arrays **EZ DNA Methylation**	*High Speed* **EZ DNA Methylation-Gold**	*Input Cells and Tissues Directly* **EZ DNA Methylation-Direct**	*Fastest, Most Convenient* **EZ DNA Methylation-Lightning**
Conversion Time	12 – 16 hr	2.5 hr	3.5 hr	1 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)	50 pg – 2 µg DNA, $10 – 10^5$ cells (≤ 20 µl)	100 pg – 2 µg DNA (≤ 20 µl)