Automation of the EZ-96 DNA Methylation™ MagPrep Kits

High-throughput, magnetic bead-based procedure for bisulfite conversion of DNA for methylation analysis on the Microlab® STAR™.

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 Microlab STAR platform enables high-throughput automated solutions for methylation analysis.

Materials and Methods

Genomic DNA (gDNA) was extracted from healthy human blood with Zymo Research Quick-DNA™ Miniprep Plus Kit (Cat. #D4068). Forty-eight samples of isolated gDNA (600 ng) were used as input for bisulfite treatment using Zymo EZ-96 DNA Methylation-Lightning™ MagPrep Kit (Cat. #D5040). Twenty-four of the extracted gDNA samples were processed manually and the other 24 samples were processed using the Microlab STAR.

The Microlab STAR used was configured with 8 Independent Pipetting Channels, Autoload (optional), CO-RE® 96 Multi-Probe Head (MPH), CO-RE Grippers, Hamilton Heater Shaker (HHS), Zymo magnetic rack, 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. #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).

130 µL Lighting Conversion Reagent + 20 µL DNA Sample, Mixing

Thermal Cycler (98 °C for 8 min, 54 °C for 60 min, 4 °C)

600 µL M-Binding Buffer + 10 µL MagBinding Beads, Mixing

RT for 5 min, Transfer to Magnetic Rack, Settling for 5 min, Remove Supernatant

400 µL M-Wash Buffer Washing

200 µL L-Desulphonation Buffer at RT for 15 – 20 min, Remove Supernatant

400 µL M-Wash Buffer Washing

Dry Bead at 55 °C for 20 – 30 min

25 µL M-Elution Buffer, 55 °C for 4 min

Transfer Eluate to Elution Plate

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

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%.

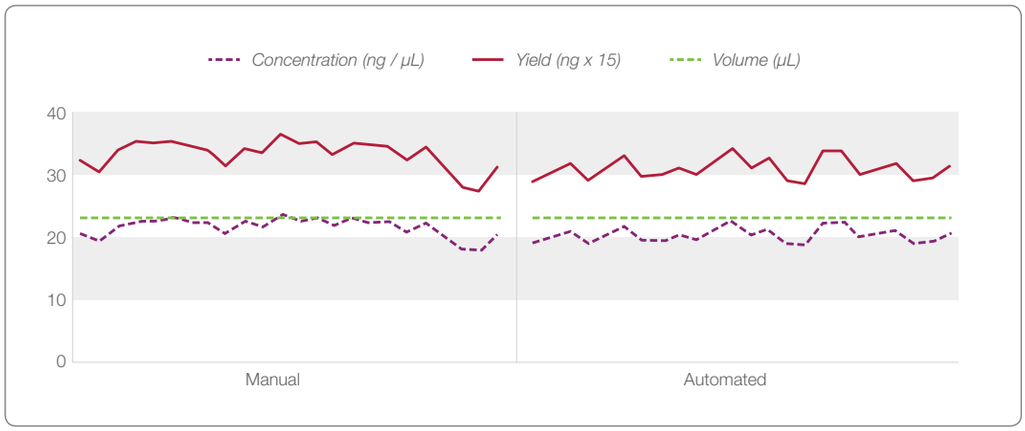

Figure 2: Comparison between manual and automated (Microlab STAR) sample processing.

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.

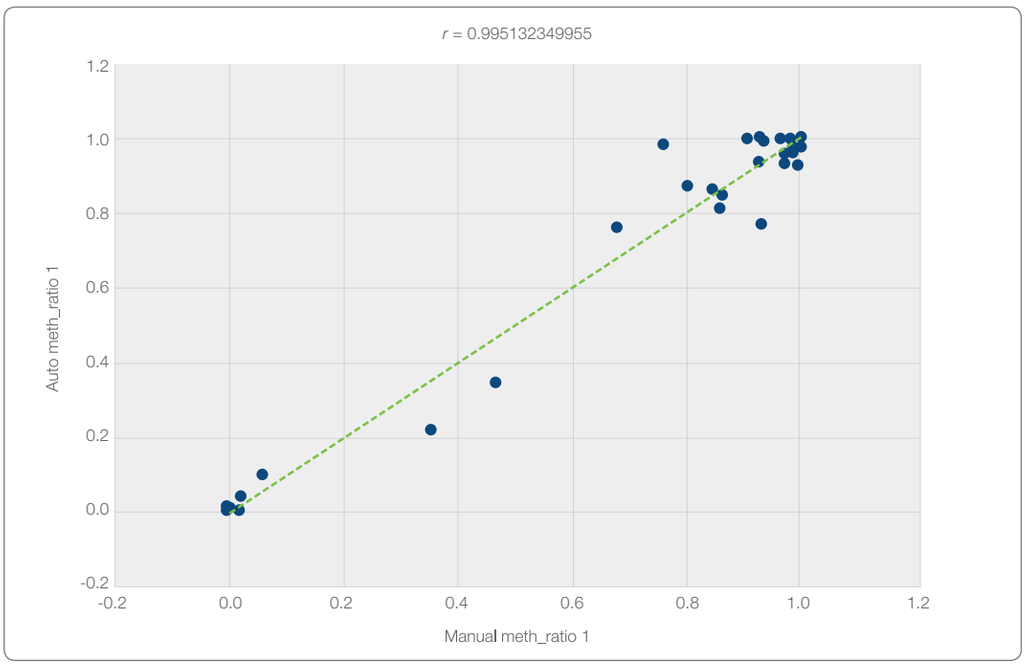

Figure 3: High correlation of methylation ratio values with scatterplot showing the correlation of several gene loci comprising 131 intersecting unique CpG sites between a single representative automated and manual comparison. The mean of the correlation scores (r-value) for all sample comparisons is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

Conclusions

Samples processed using the EZ-96 DNA Methylation-Lightning MagPrep procedures with the 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.

Products

Description	Zymo Research Catalog Number	Kit 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	*High Speed*	*Input Cells and Tissues Directly*	*Fastest, Most Convenient*
	EZ DNA Methylation	**EZ DNA Methylation-Gold**	**EZ DNA Methylation-Direct**	**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)

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