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STM Reagent Improves Robustness of Infinium® Methylation Assay

Introduction

The Infinium Methylation Assay detects cytosine methylation (an important factor in many human diseases) based on highly multiplexed genotyping of bisulfite-converted genomic DNA (gDNA). Upon treatment with bisulfite, unmethylated cytosines are deaminated to uracil, while methylated cytosines remain unaffected. The assay interrogates these chemically-differentiated loci using two site-specific probes, one designed for the methylated locus and another for the unmethylated. Single-base extension of the probes incorporates a DNP-or biotin-labeled ddNTP, which is subsequently stained with a fluorescence reagent in order to determine the signal intensity ratio of methylated vs unmethlylated residues[1].

The X-Stain protocol used for fluorescence staining has been updated with an improved reagent that delivers highly robust performance across HumanMethylation27 BeadChips. According to the conventional X-Stain protocol, the hybridized probes are single-base extended, then stained using Labeling Two-Color Master Mix (LTM)[2]. In the updated protocol, LTM is replaced by Superior Two-Color Master Mix (STM), the standard reagent for all Infinium HD BeadChips.

Analysis of Data Quality and Consistency

To evaluate the consistency of the data produced by each reagent, replication experiments were performed with six samples that were independently assayed using either STM or LTM. The correlation coefficients for all probe signals indicated a very strong linear relationship between replicates. Evaluation of the individual samples showed r2 > 0.99 in all cases tested (Table 1). Overall, this analysis demonstrated that STM and LTM produce highly consistent data, meaning that samples processed with either stain can be analyzed within the same GenomeStudio® project.

A comparison study was performed over a larger range of samples to evaluate how the reagents performed across a more diverse sample set. Forty-eight reference gDNA samples were assayed using both STM and LTM to compare the CpG detection accuracy. As illustrated in Figure 1, STM showed reduced variability and an increased percentage of known CpG sites detected from the reference samples. These results suggest that STM provides more robust data quality and better reproducibility over a broader range of samples and BeadChips for the Infinium Methylation Assay.

Updated Staining Procedure

All Infinium Assays based on HD BeadChips use STM as the standard staining reagent. Although the HumanMethylation27 BeadChip is not HD-formatted, STM can be incorporated into the assay protocol with only minor procedural modifications.

For the automated assay protocol, an update to the Tecan robot setup is recommended. In the updated procedure, once the BeadChips have been placed in the Chamber Rack and slid into the robot bed, the XStain HD BeadChip task should be selected on the robot PC (Figure 2). After selecting this task, the STM reagent tubes can be placed on the robot in the same location that the LTM tubes would have occupied. After this point, the assay can be completed as normal by following the conventional protocol.

For the manual assay protocol, the only required procedural change is to replace the LTM reagent tubes with STM tubes before starting an experiment.

Samples		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Sample5_LTM_rep1	1		0.997	0.995	0.997	0.998	0.998	0.998	0.998	0.998	0.993	0.994	0.992	0.994	0.995	0.991	0.995	0.992	0.993
Sample5_LTM_rep2	2			0.996	0.998	0.997	0.998	0.996	0.996	0.997	0.996	0.996	0.995	0.996	0.996	0.994	0.996	0.995	0.995
Sample5_LTM_rep3	3				0.996	0.995	0.995	0.996	0.997	0.995	0.993	0.995	0.991	0.992	0.993	0.994	0.993	0.994	0.995
Sample5_LTM_rep4	4					0.997	0.998	0.997	0.996	0.997	0.995	0.995	0.994	0.995	0.996	0.993	0.996	0.994	0.995
Sample5_LTM_rep5	5						0.999	0.997	0.997	0.998	0.994	0.994	0.994	0.995	0.996	0.991	0.995	0.992	0.993
Sample5_LTM_rep6	6							0.997	0.997	0.998	0.995	0.994	0.994	0.995	0.996	0.992	0.996	0.992	0.994
Sample5_LTM_rep7	7								0.999	0.998	0.993	0.995	0.992	0.993	0.994	0.992	0.994	0.992	0.995
Sample5_LTM_rep8	8									0.998	0.993	0.995	0.991	0.993	0.994	0.992	0.994	0.993	0.995
Sample5_LTM_rep9	9										0.993	0.994	0.992	0.993	0.995	0.991	0.995	0.992	0.993
Sample5_STM_rep1	1											0.998	0.998	0.997	0.998	0.997	0.998	0.998	0.997
Sample5_STM_rep2	2												0.997	0.996	0.998	0.997	0.998	0.998	0.998
Sample5_STM_rep3	3													0.997	0.998	0.995	0.998	0.997	0.996
Sample5_STM_rep4	4														0.998	0.994	0.997	0.996	0.995
Sample5_STM_rep5	5															0.996	0.999	0.997	0.997
Sample5_STM_rep6	6																0.996	0.998	0.997
Sample5_STM_rep7	7																	0.997	0.997
Sample5_STM_rep8	8																		0.997
Sample5_STM_rep9	9																		

Tha above table shows correlations between beta values, which represent methylation levels of the CpG sites. Analysis of replicates treated with STM and LTM show a strong correlation, with r2 > 0.99 across all samples tested.

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
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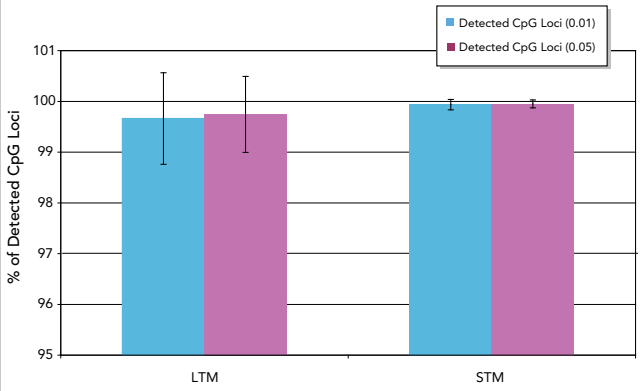
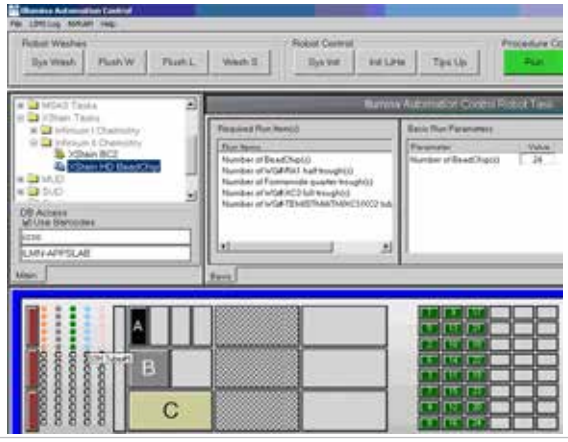
Figure 1: CPG Sites Detected from Reference Sample



In a 48-sample CpG detection analysis, STM produced less variability and an overall improvement in signal detection accuracy compared to LTM

Figure 2: Tecan Robot Setup Modification for the Assat Automated Protocol



To prepare the Tecan robot for STM, select
XStain Tasks | Infinium II Chemistry | X-Stain HD BeadChip

Conclusions

Replacing LTM with STM in the Infinium Methylation Assay produces results that are highly consistent under either reagent condition. However, when evaluated over a broader range of samples and BeadChips, the STM reagent proves to be more robust, and can modestly improve the signal detection accuracy of the Infinium Methylation Assay.

Additional Information

Visit our website or contact us at the address below to learn more about Infinium products and protocols.

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