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## Genome wide hydroxymethylation tested using the HELP-GT assay shows redistribution in cancer

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5-hydroxymethylcytosine (5-hmC) is a recently discovered epigenetic modification that is altered in cancers. Genome-wide assays for 5-hmC determination are needed as many of techniques for 5-methylcytosine (5-mC) determination, including methyl-sensitive restriction digestion and bisulfite sequencing cannot distinguish between 5-mC and 5-hmC. Glycosylation of 5-hmC residues by beta-Glucosyl Transferase ( $\beta$ -GT) can make CCGG residues insensitive to digestion by MspI. Restriction digestion by HpaII, MspI or MspI after  $\beta$ -GT conversion, followed by adapter ligation, massive parallel sequencing and custom bioinformatic analysis allowed us determine distribution of 5-mC and 5-hmC at single base pair resolution at MspI restriction sites. The resulting HELP-GT assay identified 5-hmC loci that were validated at global level by LC-MS and the locus specific level by qRT-PCR of 5-hmC pulldown DNA. Hydroxymethylation at both promoter and intragenic locations correlated positively with gene expression. Analysis of pancreatic cancer samples revealed striking redistribution of 5-hmC sites in cancer cells and demonstrated enrichment of this modification at many oncogenic promoters such as GATA6. The HELP-GT assay allowed global determination of 5-hmC and 5-mC from low amounts of DNA and with the use of modest sequencing resources. Redistribution of 5-hmC seen in cancer highlights the importance of determination of this modification in conjugation with conventional methylome analysis.

## Biography

Sanchari Bhattacharyya has completed her Ph.D. from Albert Einstein College of Medicine, Department of Pathology and pursuing postdoctoral studies from the department of Developmental and Molecular Biology, Cancer Center of the same institution. She has published two first author papers and several co-author papers in reputed journals and is continuing with different projects on cancer epigenomics.

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