









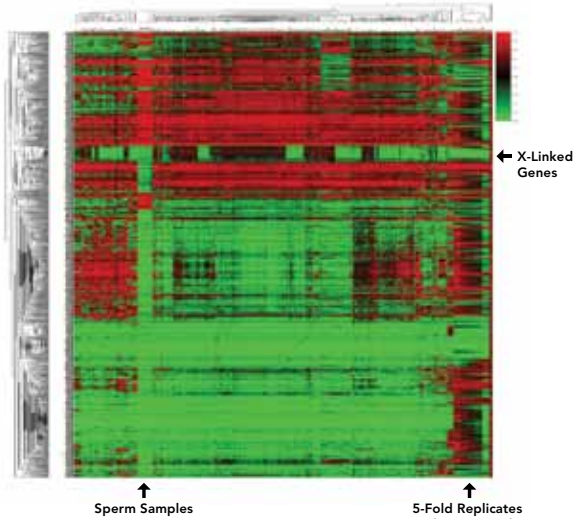




methylated DNA reference samples were selected for the final assay.

The unmethylated reference samples were generated by genome-wide amplification of human genomic DNA. After amplification, DNA methylation was diluted > 1000-fold, effectively rendering the amplified genomic DNA unmethylated. The methylated templates were generated by in vitro methylation using a CpG-methylase, SssI.

Figure 6: Cluster Analysis Of DNA Methylation Data For 1,505 CPG LOCI On 288 DNA Samples



### 4. Conclusion

The GoldenGate Assay for Methylation is a highly reproducible and multiplexed method for the high-throughput quantitative measurement of DNA methylation. This method provides not only a discrete measure of positive versus negative DNA methylation, but also a continuous measure of levels of DNA methylation. For a 17% difference in absolute methylation level (e.g., 10% vs. 27%), signals are expected to have largely non-overlapping distributions. Importantly, the assay can detect as little as 2.5% methylation status for some CpG sites. Unlike restriction enzyme-based methods, assay probes can be designed specifically for many of the CpG sites in the genome, and assay oligos can be designed to interrogate the Watson strand, the Crick strand, or both strands at each CpG site. Assay end products are processed using a Sentrix® Universal Array Matrix (SAM). As a result, gene or CpG sets can be refined iteratively, because no custom pre-defined arrays need to be developed. This method can detect changes in methylation status at up to 1,536 different CpG sites simultaneously, using only 500 ng of genomic DNA for duplicate assays. Coupled with integrated genomic analysis tools, the Illumina GoldenGate Methylation platform will provide powerful insight into epigenetic mechanisms of gene regulation.

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