# Publications "EZ DNA Methylation Kits"



## EZ DNA Methylation-Lightning Kit

Researchers studying circulating unmethylated and methylated preproinsulin (INS) DNA in type 1 diabetes (T1D) sought to test the differences between subjects with new-onset T1D and controls. DNA was extracted from serum using the ZR Serum DNA Kit™ and bisulfite converted with the EZ DNA Methylation Kit™ or the EZ DNA Methylation-Lightning Kit™. Droplet digital PCR was used to measure unmethylated and methylated serum levels. The data demonstrated that elevations in both unmethylated and methylated INS DNA occurs in new-onset T1D and that levels of these DNA species change during T1D evolution. The use of methylation levels as potential biomarkers for diabetes may prove to be a novel tool in the near future.

Fisher MM, et al. (2015) Elevations in Circulating Methylated and Unmethylated Preproinsulin DNA in New-Onset Type 1 Diabetes. Diabetes. 2015 Nov;64(11):3867-72. doi: 10.2337/db15-0430. Epub 2015 Jul 27.

The EZ DNA Methylation-Lightning™ Kit was used to bisulfite convert DNA from prefrontal cortex samples of patients with psychiatric diseases. The researchers then used a recently developed bisulfite padlock probe-based approach for ultra-deep mapping of modified cytosines in patients affected or unaffected by major psychosis. Their findings revealed significant epigenetic differences between patients and controls and also identified interesting epigenetic age effects.

Pal M et al. (2015) High Precision DNA Modification Analysis of HCG9 in Major Psychosis. Schizophr Bull. 2015 Jun 15. pii: sbv079.

In order to investigate why endogenes are less susceptible to RNA silencing than transgenes, researchers from Germany used the EZ DNA Methylation-Lightning™ Kit to bisulfite convert DNA from locally and systemically silenced transgenic plant leaves. After amplifying the bisulfite-converted DNA using ZymoTaq™ polymerase and bisulfite sequencing, the researchers were able to conclude that cDNA transgenes are prone to post-transcriptional gene silencing and RNA-directed DNA methylation while endogene-resembling transgenes are resistant to systemic silencing and DNA methylation.

Dadami E. et al. (2014) An endogene-resembling transgene is resistant to DNA methylation and systemic silencing. RNA Biology. 11:7, 1-8

DNA from FACS-purified prospermatogonia (PSG) was bisulfite converted using the EZ DNA Methylation-Lightning™ Kit. Genome-wide single-base-resolution DNA methylome analysis revealed epigenetic deficiencies in mutant germ cells specifically cell types expressing Dnmt3L. The data also implies that genomes of germ cells may have the highest overall levels of DNA methylation which is proposed to be a consequence of Dnmt3L.

Vlachogiannis G et al. The Dnmt3L ADD Domain Controls Cytosine Methylation Establishment during Spermatogenesis. Cell Reports 10, 1–13 February 17, 2015

Cerebellar cortex DNA from both Autism spectrum disorder (ASD) and healthy individuals were bisulfite converted using the EZ DNA Methylation-Lightning Kit in parallel to immunoprecipitation (with either 5-hmC or 5-mC antibodies). Next, 5-hydroxymethylcytosine (5-hmC) and 5-methylcytosine (5-mC) were measured with pyrosequencing. The researchers reported that an increase of MeCP2 binding at GAD1 and RELN promoters is associated with increased levels of 5-hmC in ASD cerebellum.

Zhubi A et al. (2014) Increased binding of MeCP2 to the GAD1 and RELN promoters may be mediated by an enrichment of 5-hmC in autism spectrum disorder (ASD) cerebellum. Transl Psychiatry. 4:e349.

The EZ DNA Methylation-Lightning Kit to bisulfite convert DNA extracted from cheek cells or preserved kidney tissues from dogs with clinical cases of moderate to severe renal dysplasia. It was found that wild-type alleles of the Cox-2 promoter are not methylated, however, allelic variants of the Cox-2 promoter that result in clinical manifestation of renal dysplasia have aberrant methylation.

Whiteley MH. (2014) Allelic variation in the canine Cox-2 promoter causes hypermethylation of the canine Coz-2 promoter in clinical cases of renal dysplasia. Clin Epigenetics. 6(1):7.

Researchers from New York evaluated the DNA methylation patterns between individuals with Autism Spectrum Disorders (ASD) and typical developing (TD) controls. Using the EZ DNA Methylation-Lightning Kit, DNA extracted from the homogeneous ectodermal cells type was bisulfite converted and analyzed using a quantitative genome-wide DNA methylation assay. DNA methylation patterns in individuals with ASD were shown to be dysregulated compared to the typical developing individuals.

Esther RB et al. (2014) Mosaic Epigenetic Dysregulation of Ectodermal Cells in Autism Spectrum Disorder. PLoS One.

To determine the role Tet1 may have in activating meiotic genes, whole-genome bisulfite sequencing libraries were generated to compare the methylation levels of wild-type and Tet1-mutated mouse primordial germ cells (PGCs). The authors found that libraries bisulfite converted using the EZ DNA Methylation-Lightning™ Kit from Zymo Research were fully converted and post-mapping filtering was not required, whereas libraries processed with the Sigma Imprint Kit had some poorly converted reads, which required post-mapping filtering. The results of the findings showed Tet1 is not responsible for global demethylation in PGCs but is involved in specific meiotic gene activation via DNA demethylation.

Yamaguchi, S., et al. (2012) Tet1 controls meiosis by regulating meiotic gene expression. Nature 492: 443-447.

**EZ DNA Methylation-Gold Kit**

Researchers from the National Institute of Plant Genome Research in New Delhi used the EZ DNA Methylation-Gold™ Kit to identify the level of cytosine methylation in genomic DNA from Tomato leaf curl New Delhi virus. Through DNA methylation-specific RNA silencing, the researchers aimed to target the viral genomic regions of this virus to potentially determine an alternate defense mechanism for generating transgenic plants to prevent yield loss.

Sahu P et al. Post-transcriptional and Epigenetic Arms of RNA Silencing: A Defense Machinery of Naturally Tolerant Tomato Plant Against Tomato Leaf Curl New Delhi Virus. Plant Mol Biol Rep (2014) 32:1015-1029

The EZ DNA Methylation-Gold™ Kit was used to bisulfite convert DNA extracted from Thy-1 (+) and Thy-1 (-) lung fibroblasts and tissue. Data from methylation-specific PCR and bisulfite sequencing enabled researchers to show that epigenetic regulation of Thy-1 could be a novel and potentially reversible mechanism in fibrosis that may offer the possibility of new therapeutic options.

Sanders Y et al. Thy-1 Promoter Hypermethylation A Novel Epigenetic Pathogenic Mechanism in Pulmonary Fibrosis. Am J Respir Cell Mol Biol. 2008 Nov; 39(5):610-8

Researchers from China analyzed the role of DNA methylation in the life cycle of a parasitic nematode, Trichinella spiralis and characterized the methylome of three life-cycles states in this organism. Genomic DNA of T. spiralis muscle larvae, adults, and new-born larvae was isolated and methylated cytosine levels were determined by MethylC-seq.

Gao F et al. (2012) Differential DNA methylation in discrete developmental stages of the parasitic nematode Trichinella spiralis. Genome Biol. 13(10):R100.

Genomic DNA was extracted from paraffin-embedded (FFPE) renal cancer tissue blocks and was bisulfite converted using EZ DNA Methylation-Gold Kit. The authors found CST6 and LAD1 methylation to strongly associate with progression-free and overall survival of patients undergoing VEGF-targeted therapy for metastasized renal cell cancer, suggesting these sites as candidate biomarkers for predicting disease progression and response to therapy.

Peters I et al. (2014) DNA methylation biomarkers predict progression-free and overall survival of metastatic renal cell cancer (mRCC) treated with antiangiogenic therapies. PLoS One. 9(3):e91440.

European scientists used the EZ DNA Methylation-Gold Kit and pyrosequencing to verify results from a DNA methylation array. They found that anaplastic Pleomorphic xanthoastrocytoma (PXA) has increasing DNA promoter hypermethylation compared with grade II samples.

Martinez R et al. (2014) DNA methylation alterations in grade II- and anaplastic pleomorphic xanthoastrocytoma. BMC Cancer. 14:213.

Genomic DNA was extracted from T cells from blood and DNA methylation was analyzed by Methylated DNA immunoprecipitation (MeDIP) and Microarray Hybridization. Next, the researchers validated the MeDIP data by Illumina Infinium HumanMethylation450 arrays and by pyrosequencing. This study presents preliminary evidence for genome-wide variation in promoter DNA methylation associated with chronic physical aggression.

Guillemin C et al. (2014) DNA methylation signature of childhood chronic physical aggression in T cells of both men and women. PLoS One. 9(1):e86822.

DNA was extracted from cervical cytology samples and the presence of HPV16 was confirmed by PCR. DNA was then bisulfite converted using EZ DNA Methylation-Gold kit, amplified, and sequenced. The authors noted an increase in HPV16 biomarker methylation with increasing histologic severity.

Brandsma JL et al. (2014) Methylation of Twelve CpGs in Human Papillomavirus Type 16 (HPV16) as an Informative Biomarker for the Triage of Women Positive for HPV16 Infection. Cancer Prev Res (Phila). 7(5):526-33.

Researchers from Siberian Branch of the Russian Academy of Sciences and Charite-Universitatsmedizin Berlin evaluated three commercially available bisulfite modification kits using cell-free circulating DNAs (cfcDNA) in blood. They showed that the conversion efficiency of EZ DNA Methylation-Gold™ Kit is close to 99% and this kit also provided the highest recovery (> 86%) regardless of the initial DNA input. This paper suggests that the EZ DNA Methylation-Gold™ Kit is the most appropriate tool for studying bisulfite modification of low concentration and fragmented cfcDNA.

Bryzgunova O, et al. (2013) Efficacy of Bisulfite Modification and Recovery of Human of Genomic and Circulating DNA Using Commercial Kits, European Journal of Molecular Biology. Vol. 1, No. 1, 2013, pp. 1-8. doi: 10.11648/j.ejmb.20130101.11

Researchers used the EZ DNA Methylation-Gold kit from Zymo Research to investigate the role of the antiviral protein Daxx on the methylation of retroviral DNA. They found that Daxx was responsible for promoting methylation of the viral genome, and it may play a direct role in the epigenetic silencing of viral gene expression because Daxx was observed to associate with both DNA methyltransferase enzymes and viral DNA.

Shalginskikh N, Poleshko A, Skalka AM, Katz RA. (2013) Retroviral DNA methylation and epigenetic repression are mediated by the antiviral host protein Daxx. J Virol. 87(4):2137-50.

To understand the basis of inheritance of DNA methylation patterns from gametes to offspring in zebrafish, Next-Gen sequencing libraries were constructed from genomic DNA of sperm, oocytes, and embryos from different stages. The libraries were bisulfite converted using the EZ DNA Methylation-Gold kit from Zymo Research. Sequencing results showed early embryos inherit DNA methylomes from sperm rather than oocytes.

Jiang, L. et al. (2013) Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. Cell 153:773-784.

The EZ DNA Methylation-Gold™ Kit was used to investigate RNA-dependent RNA polymerase 6 (RDR6)-mediated RNA-directed DNA methylation of transposable elements (TEs) in Arabidopsis thaliana. The authors identified RDR6-specific small interfering RNAs (siRNAs) that were expressed from transcriptionally active TEs and demonstrated that these siRNAs were responsible for initiating the silencing of active transposable elements by promoting DNA methylation through an RNA-dependent mechanism.

Nuthikattu S, McCue AD, Panda K, Fultz D, Defraia C, Thomas EN, & Slotkin RK. (2013) The Initiation of Epigenetic Silencing of Active Transposable Elements Is Triggered by RDR6 and 21-22 Nucleotide Small Interfering RNAs. Plant physiology, 162(1), 116-31.

The EZ DNA Methylation – Gold™ kit was used for the bisulfite treatment of DNA from tumor tissues of Dutch and Chinese patients with Lynch syndrome. Data from methylation-specific PCR and pyrosequencing of the bisulfite-converted DNA suggested that the absence of polyadenylation in an expressed gene, TACSTD1, may result in epigenetic inactivation of its downstream neighbor, MSH2, possibly contributing to disease.

Ligtenberg, MJ, et al. (2009) Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. Nature Genetics 41(1): 1

Genomic DNA from HeLa cells was treated with sodium bisulfite and desulfonated using the EZ DNA Methylation-Gold Kit. Bisulfite-converted DNA was used for library preparation and sequencing to analyze the DNA methylation status of the intergenic spacer region of the ribosomal DNA locus.

Rothbart et al. (2012) Association of UHRF1 with methylated H3K9 directs the maintenance of DNA methylation. Nat Struct Mol Biol. 19(11):1155-60.

Honeybee genomic DNA was bisulfite converted with the EZ DNA Methylation-Gold™ kit. The converted DNA was used for CHARM analysis and whole genome bisulfite sequencing to compare DNA methylation levels between subclasses of worker bees.

Herb, B.R., et al. (2012). Reversible switching between epigenetic states in honeybee behavioral subcastes. Nature Neurosci, 15(10): 1371-1373.

To study the effect of curcumin on DNA methylation in colorectal cancer (CRC) cells, genomic DNA from three CRC cell lines was bisulfite converted using the EZ DNA Methylation – Gold™ kit. Data gathered using various assays, such as microarrays, pyrosequencing, and real-time PCR, suggest that treatment with curcumin induces gene- and cell-line-specific methylation changes and that these changes have a direct impact on the expression of genes involved in major biological processes.

Link A, et al. (2013) Curcumin Modulates DNA Methylation in Colorectal Cancer Cells. PLoS ONE 8(2):e57709.

**EZ DNA Methylation Kit**

Researchers from Germany used the EZ DNA Methylation Kit™ to bisulfite convert genomic DNA from ex vivo isolated T cell subsets to study the changes in DNA methylation during differentiation of naïve T cells into Th cell subsets. Further methylome analysis through methylation sensitive high-resolution melting (MS-HRM) and pyrosequencing demonstrated that CD4+ T cells share more demethylated regions with Th17 cells when compared to Th1 cells, and that overall Th17 cells display the highest number of demethylated regions. Through this analysis, the researchers were able to identify a unique Th17-specific epigenetic signature and reinforce the finding that Th cell differentiation is associated with major epigenetic changes.

Yang BH, et. al. (2015) Development of a unique epigenetic signature during in vivo Th17 differentiation. Nucleic Acids Res. Feb 18;43(3):1537-48. doi: 10.1093/nar/gkv014. Epub 2015 Jan 15.

Researchers from Brazil studying bipolar disorder (BD), aimed to identify epigenetic mechanisms associated with the development and progression of BD. The EZ DNA Methylation Kit™ was used to bisulfite treat DNA samples from patients with BD, first degree relatives, and healthy controls, prior to DNA methylation analysis by pyro sequencing. Based on previous experiments of epigenetic mechanisms modulating the FKBP51 feed-back loop and evidence suggesting the FKBP5 gene plays a role in altered DNA methylation patterns, the researchers focused on regions of the FKBP5 gene during the DNA methylation analysis. Patients with BD presented with increased intronic methylation levels at the FKBP5 gene region which may be responsible for reduced mRNA expression that was previously demonstrated by the group. This is one of the first studies to look at epigenetic mechanisms of the FKBP5 gene in patients with BD and their first-degree relatives.

Fries GR, et. al. (2014) Hypothalamic-Pituitary-Adrenal Axis Dysfunction and Illness Progression in Bipolar Disorder. Int J Neuropsychopharmacol Oct 31; 18(1). pii: pyu043. doi: 10.1093/ijnp/pyu043

Researchers studying circulating unmethylated and methylated preproinsulin (INS) DNA in type 1 diabetes (T1D) sought to test the differences between subjects with new-onset T1D and controls. DNA was extracted from serum using the ZR Serum DNA Kit™ and bisulfite converted with the EZ DNA Methylation Kit™ or the EZ DNA Methylation-Lightning Kit™. Droplet digital PCR was used to measure unmethylated and methylated serum levels. The data demonstrated that elevations in both unmethylated and methylated INS DNA occurs in new-onset T1D and that levels of these DNA species change during T1D evolution. The use of methylation levels as potential biomarkers for diabetes may prove to be a novel tool in the near future.

Fisher MM, et al. (2015) Elevations in Circulating Methylated and Unmethylated Preproinsulin DNA in New-Onset Type 1 Diabetes. Diabetes. 2015 Nov;64(11):3867-72. doi: 10.2337/db15-0430. Epub 2015 Jul 27.

Chromatin from clear cell renal cell carcinoma patient tissues were treated with M.SssI to methylate CpGs in open chromatin regions. DNA was then isolated and bisulfite-converted using the EZ DNA Methylation Kit and submitted for HM450 analysis. M.SssI-treated/untreated DNA methylation values were then compared to simultaneously measure endogenous DNA methylation and chromatin accessibility in renal carcinoma and adjacent normal samples.

Becket E., et. al. (2015) Identification of DNA methylation-independent epigenetic events underlying clear cell renal cell carcinoma (Cancer Research). Jan 12, 2016. doi: 10.1158/0008-5472

The EZ DNA Methylation™ Kit was used to bisulfite convert DNA to study the methylation levels of various genes related to the JAK-STAT signaling pathway in T-cell lymphoblastic lymphoma (T-LBL). The researchers used methylation specific PCR and sequencing to further elucidate the role of JAK2 and more specifically SOCS3, a suppressor of cytokine signaling in this disease. The data supports current literature demonstrating that SOCS3 is often hypermethylated in T-LBL patients and that these patients may benefit from treatment with DNA methylation inhibitors.

Roncero AM et al. (2015) Contribution of JAK2 mutations to T-cell lymphoblastic lymphoma development. Leukemia. Jul 28. doi: 10.1038/leu.2015.202

Researchers from New York investigated the role of changes in epigenome structure in age-related changes of gene expression and ovarian function. The EZ DNA Methylation™ Kit was used to bisulfite convert DNA purified from human ovarian granulosa cells. Next generation sequencing technologies such as RRBS further allowed the researchers to link epigenome structure to age-related physiology and pathology in female fertility.

Yu B et al. DNA methylome and transcriptome sequencing in human ovarian granulosa cells links age-related changes in gene expression to gene body methylation and 3'-end GC density. Oncotarget. 2015 Feb 28;6(6):3627-43

This study investigated the effects of high-fat overfeeding in healthy low birth weight and normal birth weight individuals. Genomic DNA from both groups was extracted from blood samples and bisulfite treated using EZ DNA Methylation Kit. Bisulfite converted DNA was assessed using Illumina's Infinium 27k BeadArray. Lower DNA methylation plasticity in skeletal muscle was found in the low birth weight group and this could potentially contribute to the understanding the link between low birth weight and increase risk of type 2 diabetes.

Jacobsen SC et al. (2014) Young men with low birthweight exhibit decreased plasticity of genome-wide muscle DNA methylation by high-fat overfeeding. Diabetologia. 57(6):1154-8.

Researchers established and systematically evaluated a statistical method to predict methylation patterns in a target tissue using more easily accessed surrogate tissues. DNA was bisulfite converted using EZ DNA Methylation Kit and profiled using Illumina Infinium HumanMethylation27 and HumanMethylation450 arrays. The novel statistical model showed improved accuracy in predicting the methylation in the target tissue.

Ma B et al. (2014) Predicting DNA methylation level across human tissues. Nucleic Acids Res. 42(6):3515-28.

Researchers identified disease-associated DNA methylation differences for atopic dermatitis. DNA methylations patterns were investigated in whole blood, T cells, B cells, lesional, and non-lesional epidermis from both healthy and atopic dermatitis patients. Striking methylation differences were found between lesional epidermis and healthy control epidermis, opening the door to future investigations of epigenetic mechanisms in atopic dermatitis.

Rodriguez E et al. (2014) An Integrated Epigenetic and Transcriptomic Analysis Reveals Distinct Tissue-Specific Patterns of DNA Methylation Associated with Atopic Dermatitis. J Invest Dermatol.

Researchers used the EZ DNA Methylation kit for bisulfite conversion of DNA extracted from pancreatic ductal adenocarcinoma tissues. Genome-wide DNA methylation patterns were analyzed to show that aberrant DNA methylation patterns are widespread in pancreatic cancer, particularly in cancer-signaling pathways known to be important in pancreatic carcinogenesis.

Nones K et al. (2014) Genome-wide DNA methylation patterns in pancreatic ductal adenocarcinoma reveal epigenetic deregulation of SLIT-ROBO, ITGA2 and MET signaling. Int J Cancer.

The authors investigated the association between human newborn neurobehaviour and placental leptin DNA methylation. Bisulfite pyrosequencing was employed using the bisulfite-treated DNA for leptin promoter methylation detection. Infant neurobehaviour was evaluated with the NICU Network Neurobehavioral Scales. Increased methylation of the placental leptin promotor was associated with a statistically significant neurobehavioral profile in male infants, suggesting a role in early neurodevelopement.

Lesseur C et al. (2014) Sex-specific associations between placental leptin promoter DNA methylation and infant nerubeharior. Psychoneuroendocrinology. 40:1-9.

The EZ DNA Methylation™ Kit from Zymo Research was used by researchers to investigate DNA methylation changes in the development of osteoclast cells. Osteoclasts are derived from monocytes in the hematopoietic system, and these cells can degrade bones, which is an important step in the healing process of broken bones. The researchers observed that certain genes

exhibited DNA hypermethylation, and other genes showed a decrease in DNA methylation during differentiation of monocytes to osteoclasts, leading them to conclude that DNA methylation is a key epigenetic mechanism that regulates this process.

de la Rica L, et al. (2013) PU.1 target genes undergo Tet2-coupled demethylation and DNMT3b-mediated methylation in monocyte-to-osteoclast differentiation. Genome Biol. 12;14(9):R99.<

In this publication, the authors provide a roadmap of modifications, strategies, and troubleshooting approaches to optimize sequencing of multiplexed libraries for Reduced Representation Bisulfite Sequencing (RRBS). Furthermore, the researchers found that several other published methods that recommended two rounds of bisulfite conversion with the Qiagen EpiTect kit for complete bisulfite conversion of human RRBS libraries resulted in significant loss of the template. The scientists achieved more consistent bisulfite conversion of size-selected libraries using the EZ DNA Methylation kit from Zymo Research. The use of the kit resulted in consistent conversion of human genomic DNA and minimal loss during the process.

Chatterjee A. et al. (2012) Technical Considerations for Reduced Representation Bisulfite Sequencing with Multiplexed Libraries. Journal of Biomedicine and Biotechnology Volume

In a study to determine how global DNA methylation patterns change with age, researchers used the EZ DNA Methylation Kit to bisulfite convert genomic DNA blood samples taken from newborn babies and 100+ year old patients. The authors subsequently subjected the bisulfite-converted DNA to whole genome bisulfite sequencing, 450 K CpG site microarray analysis, and bisulfite genomic sequencing and pyrosequencing, and they found that genome wide DNA methylation content significantly decreased with age.

Heyn H, Li N, Ferreira HJ, et al. (2012) Distinct DNA methylomes of newborns and centenarians. Proc Natl Acad Sci U S A. 109(26):10522-7.

In a genome-wide study investigating DNA methylation levels in acute myeloid leukemia cells, researchers used the EZ DNA MethylationTM Kit to bisulfite convert genomic DNA obtained from clinical samples. PCR amplification followed by bisulfite sequencing of the converted DNA helped to show that patients diagnosed with acute myeloid leukemia have distinct methylomes in their cancer cells compared to normal cells.

Saied et al. (2012) Genome wide analysis of acute myeloid leukemia reveal leukemia specific methylome and subtype specific hypomethylation of repeats. PLoS One. 7(3):e33213.

DNA from human colorectal tumors and normal adjacent tissues was bisulfite converted using Zymo's EZ DNA Methylation Kit and converted DNA was analyzed by Next-Gen sequencing. Whole genome bisulfite sequencing revealed focal hypermethylation at CpG islands within regions of long-range hypomethylation and possible silencing programs directed by the three-dimensional structure of chromatin within the nucleus of cancer cells.

Berman et al. (2011) Regions of focal DNA hypermethylation and long-range hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains. Nat Genet. 44(1): 40-6.

**EZ DNA Methylation-Direct Kit**

Bisulfite treatment of DNA from fibroblast cells of Aicardi-Goutières syndrome (AGS) patients was performed using the EZ DNA Methylation-Direct™ Kit prior to MethylC-seq. Results showed a global loss of DNA methylation from patients with TREX1, RNASEH2, and SAMHD1 mutations, indicating epigenetic abnormalities in AGS.

Lim YW, et. al. (2015). Genome-wide DNA hypomethylation and RNA:DNA hybrid accumulation in Aicardi-Goutières syndrome. Elife.

In this publication, researchers investigated the regulatory effects of BRCA and glucocorticoid receptor (GR). Genomic DNA extracted from both ovarian cancer and normal ovarian tissue was bisulfite converted using EZ DNA Methylation-Direct Kit and BRCA1 promoter methylation was analyzed. Immunochemistry, real-time PCR, regression analysis, knockdown, and overexpression experiments were also performed to examine the relationship between BRCA1 and GR expression levels, which were found to positively correlate in cancer tissues.

Fang YY et al. (2014) Glucocorticoid receptor repression mediated by BRCA1 inactivation in ovarian cancer. BMC Cancer. 14:188.

Epigenetic changes were shown to be induced in the midbrain of adult mice by social isolation from 3-6 months of age. Genomic DNA was isolated from the midbrain of the "lonely" mice and was bisulfite converted using the EZ DNA Methylation-Direct Kit. Bisulfite pyrosequencing was performed and the researchers found that social isolation of adult male C57BL/6 mice led to global DNA methylation changes in the midbrain.

Siuda D et al. (2014) Social isolation-induced epigenetic changes in midbrain of adult mice. J Physiol Pharmacol. 65(2):247-55.

The EZ DNA Methylation-Direct Kit was used by researchers to bisulfite convert DNA from breast cancer cell lines and patient tissues prior to performing methylation-specific PCR (MSP) and pyrosequencing. They found that DNA methylation is involved in the regulation of heparanase during breast cancer progression and significantly correlated with clinical stage.

Jiao F et al. (2014) DNA methylation of heparanase promoter influences its expression and associated with the progression of human breast cancer. PLoS One. 9(3):e92190.

Researchers extracted DNA from in vitro differentiated mouse and human embryonic stem cells, the DNA was used for both mDIP microarray analysis and bisulfite deep sequencing. They show that ES cell lines and cells derived from them are subject to significant aberrant CpG island de novo methylation which is exacerbated by differentiation in vitro and may inhibit normal differentiation.

Ludwig G et al. (2014) Aberrant DNA Methylation in ES Cells. PLoS One. 9(5):e96090.

Researchers showed that DNA methylation is involved in the regulation of TMPRSS2, a downstream androgen receptor signaling gene important in prostate cancer. Prostate cancer cells were subjected to a bisulfite conversion using EZ DNA Methylation-Direct Kit and methylation levels were analyzed. The data suggest that high levels of DNMT1 lead to the transcriptional repression of TMPRSS2 in AR-negative prostate cancer cells.

Chu M et al. (2014) Hypermethylation-mediated transcriptional repression of TMPRSS2 in androgen receptor-negative prostate cancer cells.

Researchers at Harvard Medical School used the EZ DNA Methylation-Direct™ Kit to bisulfite convert DNA in breast cancer studies prior to performing methylation-specific PCR (MSP) assays. They found that overexpression of miR-22 epigenetically silenced the expression of another miRNA, miR-200, by targeting TET enzymes – leading to increased DNA methylation at the miR-200 promoter.

Song SJ, et al. (2013) MicroRNA-Antagonism Regulates Breast Cancer Stemness and Metastasis via TET-Family-Dependent Chromatin Remodeling. Cell, 154 (2), 311-24