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| **About the Measure** | |
| **Domain** | Smoking Cessation, Harm Reduction, and Biomarkers |
| **Measure** | DNA Methylation Profile: Epigenetics of Cigarette Smoking Exposure |
| **Definition** | A genome-wide DNA methylation analysis which provides an epigenetic signature of cigarette smoking exposure for individual study participants. |

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| **About the Protocol** | |
| **Description of Protocol** | This is a laboratory protocol to determine sites of DNA methylation. The DNA was extracted from biospecimens taken from smokers. Genome-wide association study (GWAS) analysis is conducted to identify associations between methylated sites and smoking-related phenotypes, also known as epigenome-wide association studies (EWAS). |
| **Protocol Text** | The genomic DNA can be subjected to bisulfite-conversion (as described here), methyl-CpG binding domain (MBD) enrichment, antibody enrichment, or ten eleven translocation (TET) oxidation before analyzing by microarray or next-generation sequencing platform (Yong, 2016). Choosing whether to use a microarray-based assay versus a sequencing assay depends on whether coverage or throughput is most important.  Studies of DNA methylation associations with cigarette smoking phenotypes have typically used DNA extracted from whole blood samples. However, DNA may also be extracted from other cell types (e.g., CD4+ T-cells, monocytes), or tissue or saliva samples. When analyzing samples such as saliva, whose DNA content is contributed by different cell types (buccal and blood), additional adjustments must be made to control for cellular heterogeneity (Philibert, 2020).  After obtaining methylation data, a variety of data processing and statistical analysis methods may be employed to test for associations between DNA methylation and the phenotype of interest.  **Methylation assay**  Extracted genomic DNA is bisulfite converted using a bisulfite conversion kit such as EZ DNA Methylation kit (Zymo Research, Orange County, CA) or EpiTect Fast DNA kit (Qiagen, Germany) according to the manufacturer’s instructions. There are a variety of options for obtaining DNA methylation profiles, microarray-based and sequencing-based methodologies are prevalent.  *Microarray-based assay*  Bisulfite-converted DNA samples may be assayed for genome-wide DNA methylation using a contemporary microarray, such as the Infinium Human Methylation 850K array (Illumina, San Diego, CA), which contains more than 850,000 CpGs (Illumina, 2016).  *Sequencing-based assay*  Sequencing-based DNA methylation assays include methylated DNA immunoprecipitation (MeDIP) sequencing (Xing, 2018), whole-genome (WGBS) or reduced representation (RRBS) bisulfite sequencing. Assay kits such as the [TruSeq DNA Methylation Kit](http://owl.fish.washington.edu/halfshell/bu-git-repos/LabDocs/protocols/Commercial_Protocols/Illumina_truseq-dna-methylation-library-prep-guide-15066014-a.pdf) (Illumina) and [MethylDetect](https://www.methyldetect.com/wp-content/uploads/2017/10/MethylDetect-DNA-Methylation-Assay-Protocol-V1.pdf) are commercially available. |
| **Participant** | Adults ≥18 years of age |
| **Source** | Joehanes, R., Just, A. C., Marioni, R. E., Pilling, L. C., Reynolds, L. M., Mandaviya, P. R., Guan, W., Xu, T., Elks, C. E., Aslibekyan, S., Moreno-Macias, H., Smith, J. A., Brody, J. A., Dhingra, R., Yousefi, P., Pankow, J. S., Kunze, S., Shah, S. H., McRae, A. F., Lohman, K., … London, S. J. (2016). Epigenetic Signatures of Cigarette Smoking. *Circulation. Cardiovascular genetics*, *9*(5), 436–447.  Xing, X., Zhang, B., Li, D., Wang T. Comprehensive Whole DNA Methylome Analysis by Integrating MeDIP-seq and MRE-seq. Methods Mol Biol. 2018;1708:209-246. |
| **Language of Source** | English |
| **Personnel and Training Required** | Laboratory training in DNA sample isolation methods and in the use of microarray or sequencing assays is required. All analysts must be CLIA-certified and demonstrate proficiency in the analysis before handling samples. However, it is noted that there are several commercial entities that offer DNA methylation analysis as a service. |
| **Equipment Needs** | This method requires use of microarray-based and/or sequencing-based assays. |
| **Protocol Type** | Bioassay |
| **General References** | Illumina, Inc. (2016, June 10). *Field Guide to Methylation Methods.* Pub No. Pub. No. 270-2013-001.  Murat, K., Grüning, B., Poterlowicz, P. W., Westgate, G., Tobin, D. J., & Poterlowicz, K. (2020). Ewastools: Infinium Human Methylation BeadChip pipeline for population epigenetics integrated into Galaxy. *GigaScience*, *9*(5), giaa049.  Niu, L., Xu, Z., & Taylor, J. A. (2016). RCP: a novel probe design bias correction method for Illumina Methylation BeadChip. *Bioinformatics (Oxford, England)*, *32*(17), 2659–2663.  Yong, WS., Hsu, FM. & Chen, PY. Profiling genome-wide DNA methylation. *Epigenetics & Chromatin* **9,**26 (2016). |
| **Process and Review** | Not Applicable |