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## Technologies for Methylome Studies

<b>Status</b>	Current
<b>Competition</b>	Development of New Technologies Competition
<b>Sector</b>	Development of New Technologies
<b>Genome Centre</b>	Ontario Genomics Institute
<b>Project Leader</b>	Art Petronis

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### Project Description

The ultimate goal of the Human Genome Project was to determine the sequence of the 3 billion "genetic elements", or nucleotides, that make up the human genome. The human sequence of the human genome is of enormous value and promises to revolutionize biological research and clinical medicine. It also comes with the realization that the complete genome sequence is only a beginning in our understanding of human biology. One of the greatest mysteries is the regulation of genes present within each cell. Despite being genetically identical, cells from different tissues look differently and perform very different functions. For example, although dopamine system genes are present in the cells of brain, muscle, and skin, such genes are active in neurons but not in muscle or skin cells. It is now believed that tissue-specific expression is achieved by the epigenetic regulation of genes via processes such as DNA methylation. More specifically, one of the nucleotides, namely cytosine, can be present in two functional states – methylated or unmethylated. Methylated cytosines are sometimes referred as the 5<sup>th</sup> base of human DNA.

DNA methylation profiles are highly variable across different cells, even in the same organism, and such variation depends on tissue, age, sex, diet, and numerous other factors. There is increasing evidence that epigenetic factors can cause various human diseases: cancer, schizophrenia, diabetes, asthma, among numerous others. Over the last decade a series of new methods have been developed to investigate DNA methylation profiles across large DNA regions - chromosomes and even entire genomes. Unfortunately, all these methods exhibit significant limitations as they require large amounts of DNA, interrogate only a small fraction of methylatable nucleotides or are able to scan only short DNA fragments.

This Genome Canada application is dedicated to development of a set of new technologies for genome-wide DNA methylation, or methylome, analysis. The first project will attempt to combine two powerful technologies: targeted deposition of extended groups of biopolymers on DNA, and the application of microarrays. Engineered DNA methyltransferases, enzymes that methylate DNA, will be used to attach fluorescent labels on unmethylated cytosines, and these labeled DNA fragments will be interrogated on tiling microarrays. Microarrays are small pieces of glass containing millions of short DNA sequences that will hybridize to and highlight the unmethylated DNA fragments. This new approach exhibits numerous advantages over the existing methods for DNA methylation profiling in terms of simplicity, sensitivity, informativeness, and robustness. Another effort will be dedicated to mapping methylated cytosines in the entire genome of a single cell. The main challenge consists of dealing with a very small amount of DNA extracted from a single cell, which is less than 1/100,000 of 1/1,000,000 of a gram.

Through a number of manipulations, DNA fragments that contain no methylated cytosines will be separated from the ones that contain a high density of methylated cytosines. The unmethylated fraction of a single cell genome will be amplified using polymerase chain reaction and analyzed using microarrays. This procedure will be individually performed on numerous cells, and cell specific genome-wide methylation profiles will be identified. Finally, we will attempt to adapt the so-called deep sequencing for fine methylomic studies of complex genomes that should generate very detailed DNA methylation maps across a number of cells in one experiment. This technology is based on simultaneous sequencing of millions of chemically modified DNA fragments.

The new technologies may significantly contribute to our understanding of development, tissue differentiation, aging, among numerous other important questions of the life sciences. Even more importantly, the genome wide DNA methylation studies may allow for the identification of unique and informative signatures of common human diseases, such as cancer, diabetes, schizophrenia, multiple sclerosis, among numerous others. This effort may be of critical importance for the developing of new early diagnostic tests and individualized treatments.