
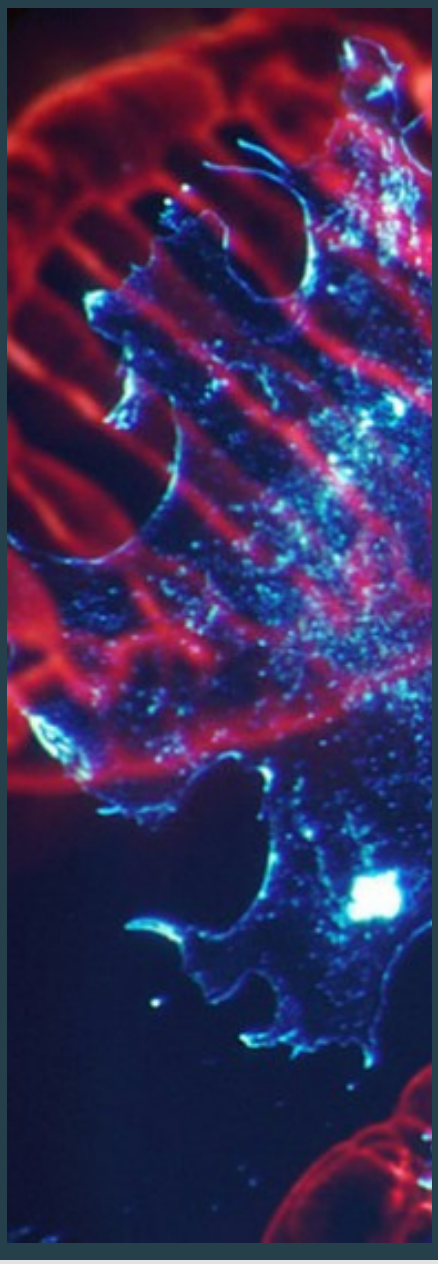
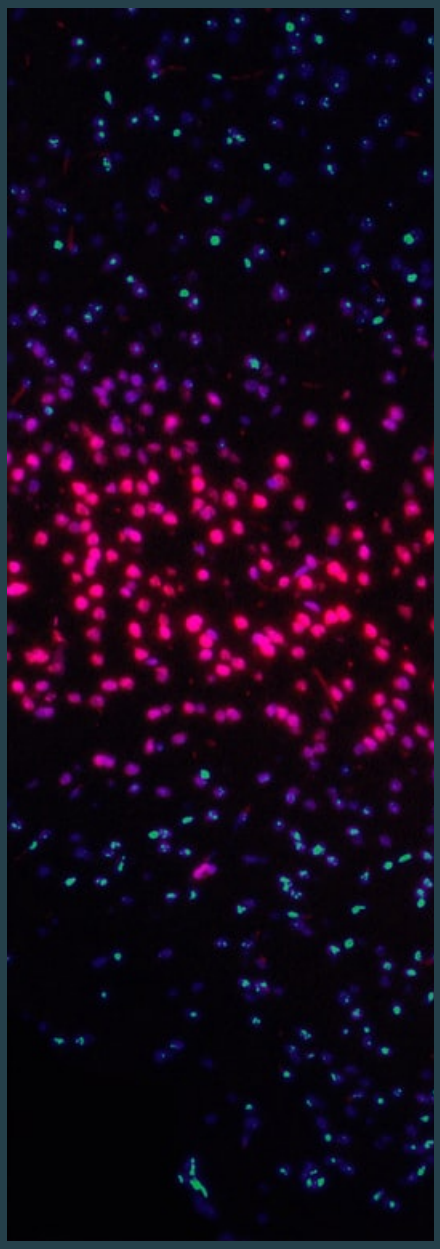

# Epigenetic Analysis:
## Bioinformatic Applications for Research

fios GENOMICS

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# 1.What is epigenetics analysis

## 1.1 What is epigenetics?

**Epigenetics is the study of physical modifications of DNA which do not alter the underlying genetic sequence.** This encapsulates heritable changes which induce phenotypic variation without corresponding genotypic variation, and includes DNA methylation and histone modifications. These modifications affect how cells interpret gene sequences, which in turn change the expression pattern of genes. Epigenetic modifications essentially underpin the cellular diversity within an organism.

**Epigenetic modifications are dynamic and can occur naturally.** However, several factors such as environment and lifestyle, disease stage, and ageing, all affect the epigenome in context-specific ways. Epigenetic modification is a tightly regulated process and in normal, healthy cells it is essential to regulate various processes; inappropriate epigenetic modification can have long-lasting effects as well as deleterious results in a cell or tissue.

## 1.2. Why analyse epigenetic modification?

Disease Mechanisms

Development of Therapies

The understanding of epigenetic processes has an important role in the research of cancers, metabolic syndromes, brain disorders and development amongst other areas. By analysing epigenetic changes, investigations into disease mechanisms and the development of therapies which target the epigenome are facilitated.

Epigenetic modifications are influenced by the environment and lifestyle, which makes them important to consider as part of clinical research studies to further understand how a drug may specifically benefit certain sub-populations. Additionally, an understanding of where or how epigenetic changes could be altered or reversed may lead to the development and marketing of novel therapies.

# 1.3. Importance in research

Epigenetic analyses are relevant to many areas of research.
Epigenetic studies can achieve:

- Greater insight into protein–DNA interactions
- Resolution of cell type- or tissue-specific methylation patterns
- Characterisation of the response of cells or tissues to epigenome-modifying agents
- An enhanced understanding of the mechanisms regulating gene expression in response to environmental stresses or during developmental processes.

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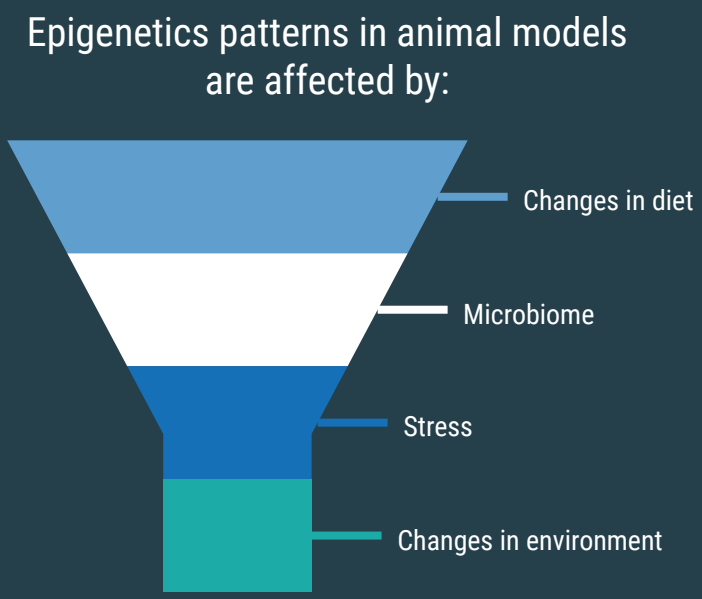
The effect of the environment and environmental stressors on epigenetic modifications are not limited to human subjects in research. Animal models can also be subject to epigenetic modifications; understanding how changes in diet, environment, microbiome, or stress affect epigenetic patterns can give a deeper understanding into results from preclinical phases of trials and why results perhaps differed between study sites or from the expected outcome.



# 2. How to quantify epigenetic modifications

There are many ways to measure epigenetic modifications. Some of the most common modifications and their study are detailed below.

## 2.1. DNA methylation

DNA methylation (and hydroxymethylation) is the most well characterised epigenetic modification. It is a process whereby methyl groups are covalently added to one of two types of DNA nucleotides: cytosine or adenine. Cytosine methylation is far more common across different species of eukaryotes and prokaryotes; in mammals, DNA methylation is found almost exclusively in cytosine-guanine (CpG) dinucleotide pairings. Adenine methylation has been observed in bacteria, plants, and mammals but is less well characterised.

CpG sites, where a cystosine nucleotide is followed by a guanine nucleotide in the 5'-3' direction, occur throughout the genome in a non-random distribution within CpG islands, shelves, shores or seas. The mean length of CpG islands is typically between 500-1500 base pairs long, and islands are generally found near promoter regions of genes. This is one method by which DNA methylation can impact upon gene expression.

The functional effect of DNA methylation is highly context-dependent. It is a mechanism by which cells can tightly and dynamically regulate gene expression in response to developmental or environmental factors, as this can be achieved in the absence of modification of the DNA sequence. DNA methylation can be measured directly at targeted sites by array (such as Illumina EPIC arrays) or with greater resolution up to the genome level through bisulphite sequencing.

## 2.2. ChIP-seq analysis

Chromatin immunoprecipitation sequencing (ChIP-seq) analysis can be used to analyse DNA-protein interactions. It can also be used for both transcription factor determination and histone modification assessment.

ChIP allows for greater resolution of investigation at specific DNA sites that interact directly with transcription factors or other proteins. It can produce a list of target DNA sites which are bound by a protein of interest, allowing for analysis of both the interaction patterns of a specific protein with DNA and the patterns of chromatin modifications.

# 2.3. ATAC-seq analysis

Assay for transposase-accessible chromatin using sequencing (ATAC-seq) allows for investigation into genome-wide chromatin changes and chromatin-accessibility signatures. It is a fast and sensitive analysis method to characterise chromatin conformation; preparation can be completed within three hours, and unlike ChIP-seq, no antibodies are required.

ATAC-seq identifies accessible regions of DNA by probing open sections of chromatin with a mutated version of a Tn5 transposase. Transposases are enzymes which bind to the end of transposons and catalyse their transposition to another section of the genome. The use of a mutated transposase allows for the cleaving and tagging of accessible areas of double-stranded DNA. Tagged DNA fragments can then be purified and sequenced, to find regions of increased accessibility.

# 2.4. CLIP-seq analysis

Cross-linking immunoprecipitation sequencing (CLIP-seq) can be used to analyse protein-RNA interaction and binding sites or locate RNA modification sites on a transcriptome-wide scale.

CLIP cross-links RNA-protein complexes using ultraviolet (UV) light. The UV light causes covalent bonds to form between a protein and nucleotide in close proximity, with the proteins of interest then isolated via immunoprecipitation. Once the cross-linked section of RNA has been identified, interaction sites can be identified through read mapping.

# 3. Challenges and resolutions for epigenetic research

**There are a number of challenges associated with epigenetic research.** The assays used to detect epigenetic modifications are diverse and the approach employed is dependent upon the type of epigenetic modification under study, while a lack of comparative validation studies have been conducted to fully corroborate these approaches. The diversity of assays and epigenetic markers makes it difficult to perform validation studies at the clinical research stage.

While DNA methylation (Section 2.1) is the most well characterised epigenetic modification, only a limited number of methylation markers have been clinically validated for routine use. Assays that are developed for use in research laboratories and preclinical studies often are not reproduced during clinical-stage validation studies. Contrasting reports in published literature can mean that candidate genes are used in research when levels of methylation are misstated. Lorincz (2012) found that in prostate and breast cancer samples, methylation levels within candidate genes were important for both diagnostic and prognostic aspects, however a number of these candidates again were not reproduced or validated in clinical studies as biomarkers. There is also the need to return actionable results; large-scale epigenomic projects are still in their infancy and there are no consensus criteria to assess clinical validity or actionability from epigenetic studies.

Moving forward, large-scale epigenome projects are needed to better understand and produce evidence for the epigenetic causes of diseases. Future clinical studies should aim to reproduce and validate candidates and assays developed for use in research laboratories and preclinical studies.

Further comparative studies into specific diseases or families of disease and their associated epigenetic modifications will assist with the validation of the epigenetic markers that are currently associated with those diseases.

# 4. Future of epigenetic research

**Epigenetic research is an evolving field, with new techniques being developed to delve deeper into the epigenome, epigenetic regulation and links to health and disease.** The use of high-throughput analysis approaches will continue to provide, at greater resolution, new evidence suggesting associations and causal mechanistic insight into specific DNA modifications and their impact on disease.

Future research will likely continue to assess the impact of epigenetics on drug discovery and other preclinical and clinical research. The importance of how epigenetic modifications can affect response to drugs, as well as how factors such as lifestyle and disease stage can affect the prevalence of these modifications, needs further research as well as embedding in all areas of therapeutic discovery.

The application of epigenetics in research areas such as oncology and gastrointestinal disease will help to fully understand these diseases' epigenetic components and assist with future therapeutic interventions.

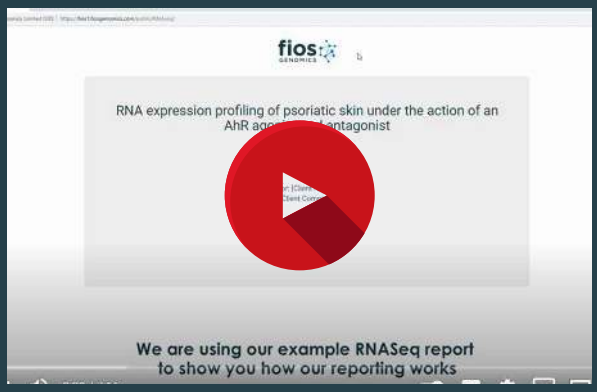
## Publications in epigenetics

**DNA Methylation Associated With Diabetic Kidney Disease in Blood-Derived DNA**
in collaboration with Queen's University Belfast.

# 5. Our expertise

## Get the most out of your research

A walkthrough of our data analysis reports




**Fios Genomics has experience with epigenetic analysis related to different therapeutic areas and disease indications.** We can offer bioinformatic analysis services to interrogate genetic variation and association with clinical outcomes or phenotype including at the genome-wide level, and can handle all types of epigenetic modification data. We also have established in-house pipelines for the analysis of epigenetic data.

## Request a sample of our analysis reports

sample

**Examples of projects where we have successfully helped our clients include:**

- Standard methylation analysis using Illumina 450k and 850k arrays and bisulfite sequencing
- Identification of DNA-protein interaction sites through ChIP-seq analysis
- Evaluation of methylation levels at baseline against age, adjusting for disease status of patients
- Metanalysis of disease vs healthy samples using methylation data generated from multiple studies
- Assessment of sites of interest in human fibroblast cells following UV exposure and application of treatments

# Fios Genomics

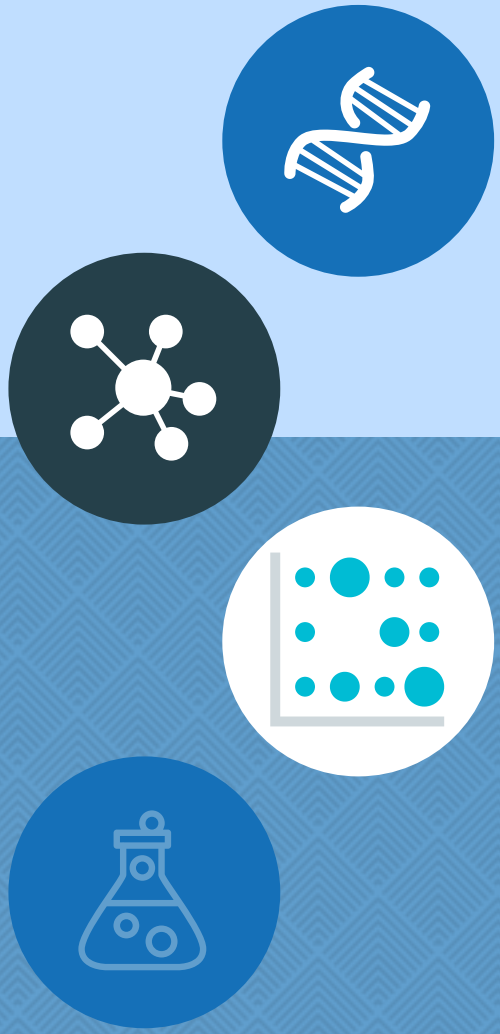
**Fios Genomics is a bioinformatic analysis provider** helping our clients to gain more insight from their research data.

## OVERVIEW

With over 10 years of experience in supporting scientists, researchers and bioinformaticians in data analysis, Fios have extensive experience in handling all types of datasets for drug discovery & development, diagnostics, agricultural research, veterinary medicine and applied research across all species.

Our specialised team of bioinformaticians, statisticians, and biologists are able to analyse and interpret any genomic, transcriptomic, proteomic & metabolomic data, independent of the platform used.

find more



**"We have utilized the Bioinformatics team at Fios Genomics for many of our drug discovery projects, as they provide expertise in the analysis of complex bioinformatic datasets. We have been consistently impressed with the rigor of FIOS' work, their communication throughout the projects, and the rapid speed at which they complete their analyses."**

- Dr Scott Ribich, Vice President of Biology at Accent Therapeutics

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