# Epigenetic dynamics during Intestinal maturation

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## Research significance

Genetic and environmental factors affect gut development and, in some cases, could be identified as risk factors for the emergence of IBD and colon cancer. While the genetic basis for those is largely studied, the epigenetic changes which happen as a result of the environmental conditions are still unclear.

In order to examine this effect, Prof. Nahum Shpigel developed a xenograft system to follow human gut development for several month. Small-intestine segments are taken from fetuses (derived from early terminated pregnancies) and implanted the back on SCID mice. The xenograft can be maintained for ~ 6 month and shows normal morphology and histology markers of pediatric matured human intestine.

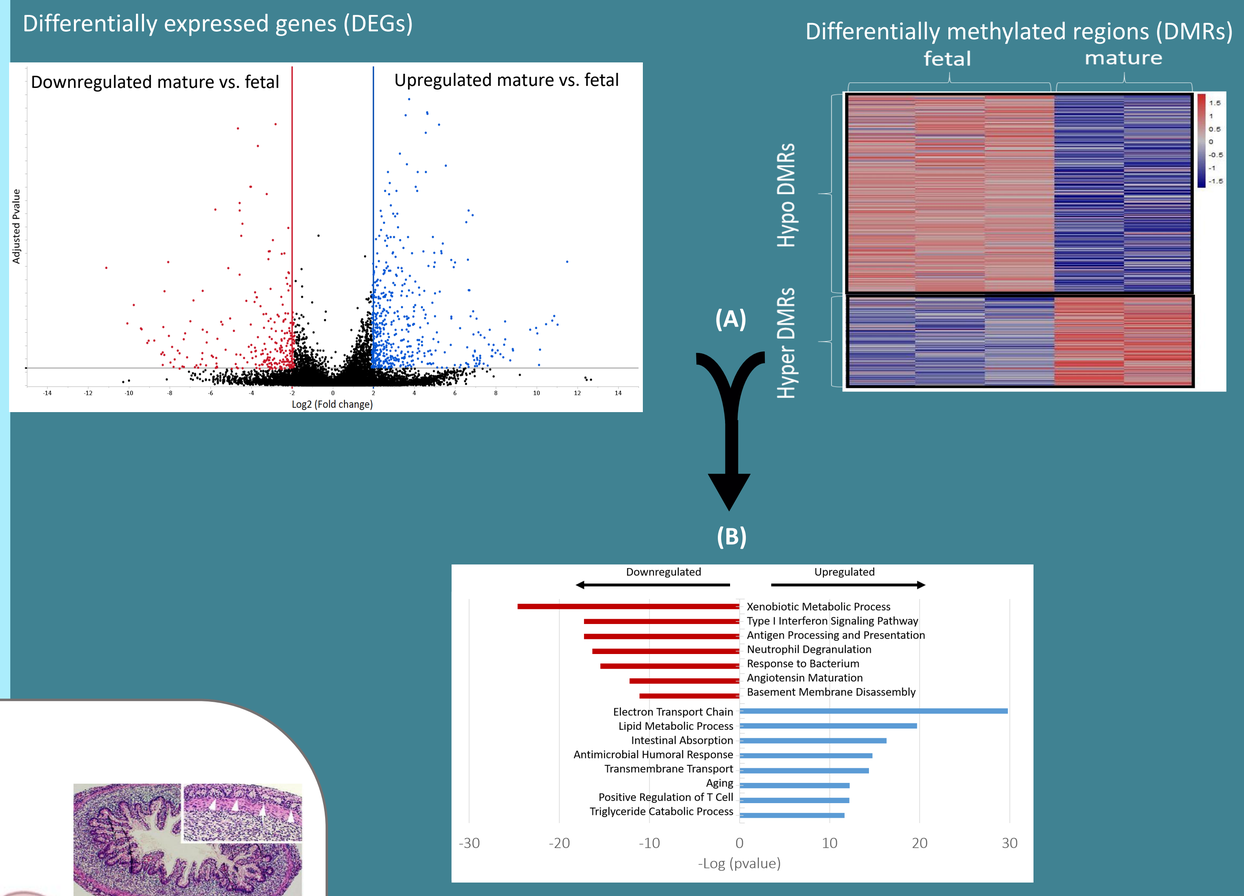
## Hypothesis:

Changes in methylation pattern during intestinal maturation regulate transcriptional changes.

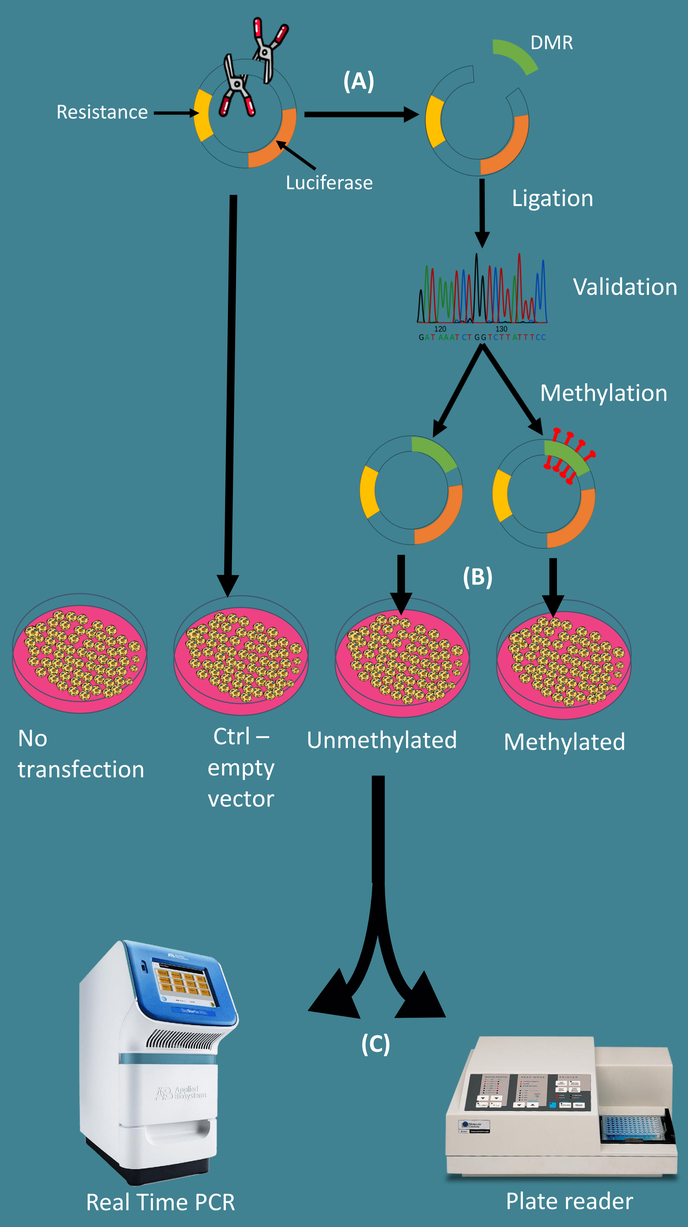
## Objectives:

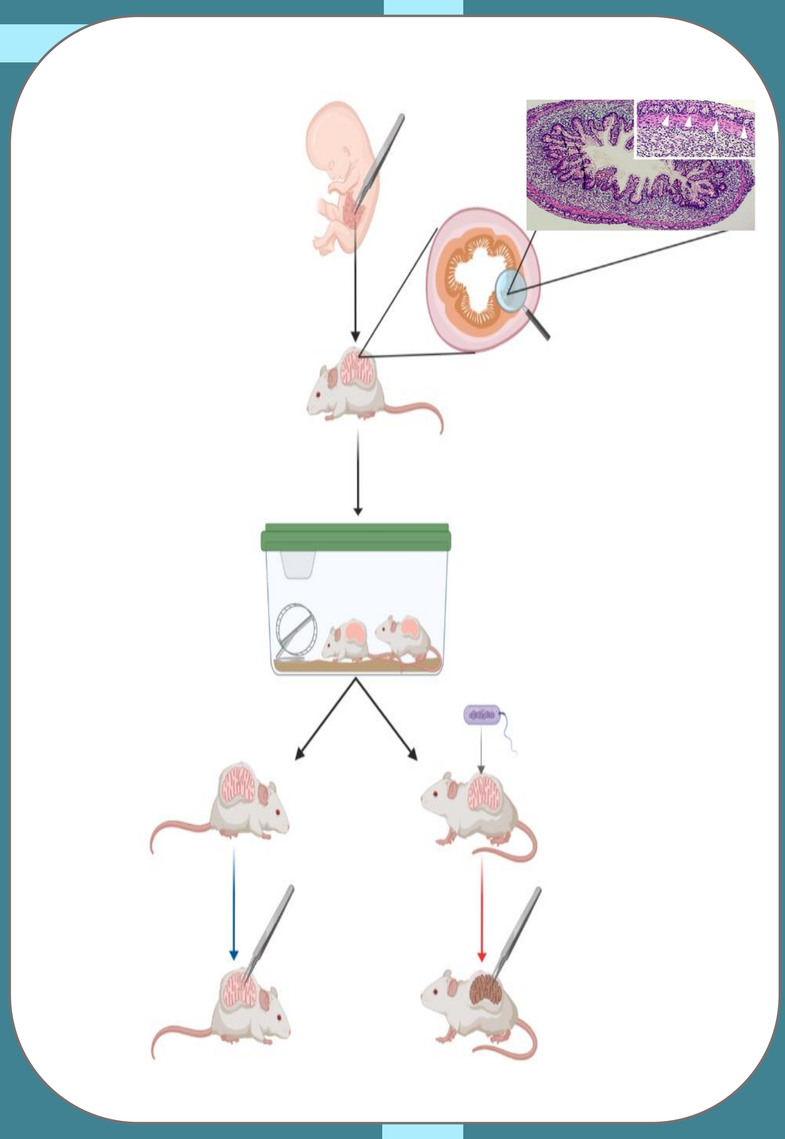
1. Uncover the gene expression profile of the developing intestine.
2. Intersect between change in methylation pattern of DMRs and gene expression.
3. Validate the relation between specific DMRs to gene regulation.

## 1. Identifying regulatory motifs in the developing intestine:

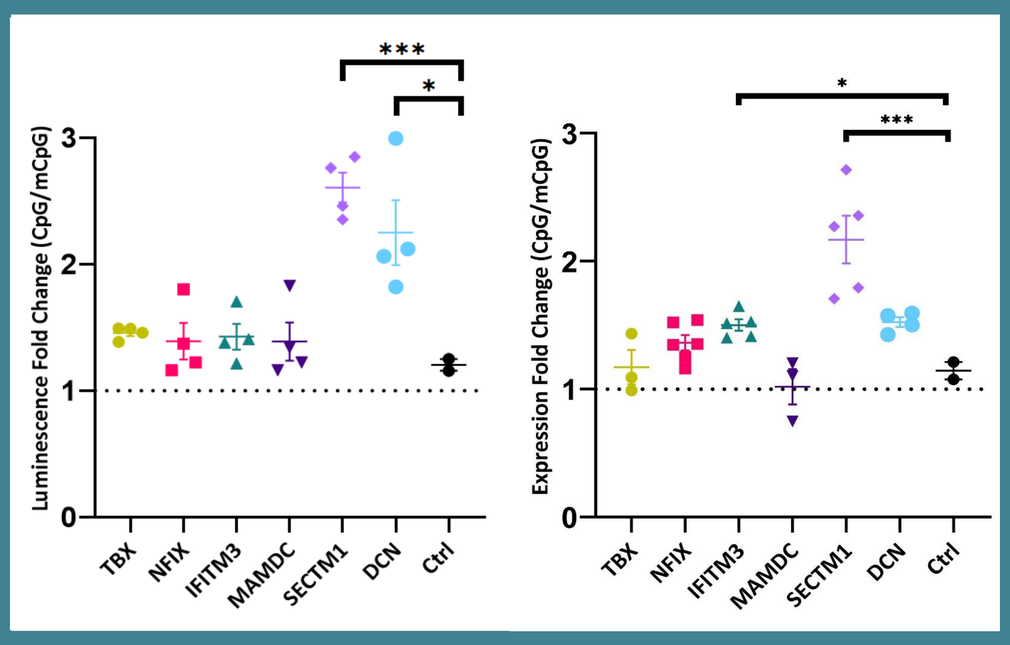



## 2. Testing DNA motif regulatory ability:

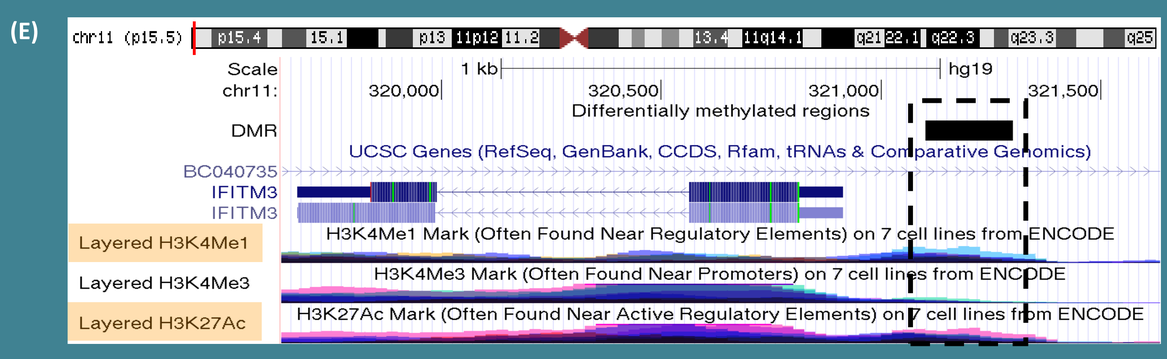


## 3. DNA methylation affects putative enhancers




**(A)** To examine the regulatory aspect of the DMR on DEG, selected DMRs were inserted to plasmids containing no CpGs and methylated. Both methylated and unmethylated plasmids were transfected to HEK293T cells **(B)** DMRs were cloned, methylated and transfected to HEK293T cell. **(C)** Difference between methylated and unmethylated plasmids was measured trough Luciferase gene expression using luciferase assay and RT-qPCR. **(D)** Overall, 3 genes have showed significant change in expression after methylation in comparison to control.




**(A)** RRBS analysis for differentially methylated regions (DMRs) and RNA sequencing analysis for differentially expressed genes (DEGs). **(B)** Upregulated and Downregulated biological processes trough GO analysis **(C)** 153 downregulated DEGs were in the vicinity (+/-50Kb) of hypermethylated DMRs (pv <<0.05), and 227 upregulated genes are found next to hypomethylated sites (pv <<0.05). **(D)** 6 DMRs were chosen for functional verification **(E)** **UCSC** Genome browser's map showing the location of one DMR (next to IFITM3 gene) along with enhancer specific histone modification (H3K4me1 & H3K27Ac)

## Conclusions

In this project, we examined for the first-time gene expression and DNA methylation profiles of human maturing intestine in the xenograft model. We uncovered hundreds of DEGs and thousands of DMRs and **verified the function of 3 DMRs as gene enhancers, up-regulating gene expression levels when unmethylated**.

The long-term goal of the study is to draw a line between early life events (like inflammation) and their effect on the epigenome to the future prospective for diseases like IBD and cancer.