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# **ANALYSIS OF GENOMEWIDE PATTERNS OF DNA METHYLATION IN RESPONSE TO AN EARLY TEMPERATURE STRESS IN RAINBOW TROUT**

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## **Introduction**

We aim to investigate the potential role of epigenetic marks in the expression of phenotypes and their variability in fish, in particular to study whether the epigenetic marks established in response to an environmental stress depend on the genetic background. The environmental stress chosen here is temperature, a known induction factor of epigenetic marks in fish. In this context, rainbow trout isogenic lines are the material of choice. Within each line, all fish have the same genome i.e. there is no genetic variability. This allows the comparison of epigenetic marks among several individuals with the same genotype. The objective of this study was to test whether temperature regime experienced during early development leads to epigenetic modifications within and between lines.

## **Material and methods**

Six rainbow trout isogenic lines were chosen. For each line, half of the eggs were incubated at standard temperature (12°C) and the other half at high temperature (16°C), from eyed-stage to hatching. At eyed-stage just before hatching, analysis of HSP47 gene expression was performed by qPCR on 3 pools of 5 eggs per line and per incubation temperature. Also, genomewide patterns of DNA methylation were analysed by EpiRADseq on the same biological material. EpiRADseq is a reduced-representation library-based approach that has been recently developed and tested on a single clone of water fleas. The protocol was here modified to account for genetic variability and allow both within and between-lines comparisons.

## **Results**

An overexpression of HSP47 gene in the 16°C batches confirmed that the early temperature stress was successful. In total, 284 825 EpiRAD loci were defined, among which 102 354 were present in only one sample. In order to compare the lines, preliminary analysis was restricted to 57 129 loci that were common to the 6 lines. Globally, 325 loci spread across the genome (3 to 18 loci per chromosome) were differentially methylated between the two incubation temperatures, 169 loci being less methylated at 16°C compared to 12°C and 156 loci more methylated. This number differed between lines, ranging from 14 to 143 depending on the line (Fig. 1).

## **Discussion**

Rainbow trout isogenic lines are a unique biological model to study the interactions between genetics, epigenetics and environment. This study contributes to the understanding of the ability of organisms to cope with changing environmental conditions. Overall, the great majority of observed changes in methylation in response to an early temperature stress seem to be dependent on the genetic background. However, further studies are required. Preliminary analyses should be deepened by investigating the function of the genes located near differentially methylated loci. Analysis of expression of DNMT genes (DNA methyltransferase, involved in DNA methylation) could help to understand the establishment of differential methylation profiles during an early temperature stress. In the future, the impact of a longer exposure to high temperatures during early development could also be tested.

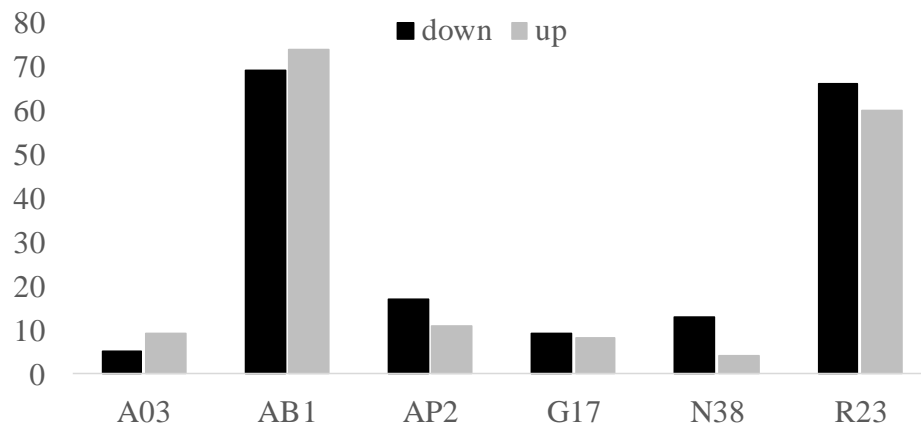


Figure 1. Number of differentially methylated loci between the two incubation temperatures (12°C vs. 16°C) for 6 rainbow trout isogenic lines. Down: less methylated at 16°C compared to 12°C; up: more methylated at 16°C compared to 12°C.