Epigenetic profiling of plant LTR retrotransposon copies using Nanopore sequencing

Omarov M.[*1, 2], Merkulov P.[1], Gvaramiya S.[1], Kirov I.[1]*

muradok98@gmail.com
@Murad__Omarov

[1]All-Russia Research Institute of Agricultural Biotechnology, Moscow, Russia;
[2] National Research University Higher School of Economics, Moscow, Russia

Summary. DNA methylation is a major barrier that inhibits transposable elements (TEs) activity playing an important role in the control of genome integrity. Unfortunately, due the repetitive nature of TEs, the methylation profile of individual recently originated TE copies could not be established using short-read bisulfite sequencing. But Nanopore sequencing provides great opportunities for DNA methylation profiling. DNA methylation can be directly detected from native DNA reads by tracking changes in electrical potential without any chemical conversion. Here we obtained epigenetic profiling and comparison of LTR retrotransposon copies from ATCOPIA93 family in wild type and mutant plant with defective methylation system (*ddm*). We at the first time for plants carried out Cas9-targeted Nanopore sequencing (CANS)[3] followed by detection of DNA methylation in three different contexts (CG, CHG and CHH) by recently published tool based on using BLSTM neural networks [1]. We found up to 50% decrease of methylation level in mutant plants corroborating with bisulfite sequencing[2] results. In conclusion, we showed that Nanopore-based DNA methylation detection in combination with Cas9-targeted sequencing are useful approaches for illuminating the epigenetic regulation of TEs.

To investigate methylation profile of individual copies we applied recently developed protocol of targeted Nanopore sequencing with Cas9-based enrichment[3] (CANS) of *EVD* retrotransposon copies (Fig.1) in *ddm* mutants, which are characterized by increasing of TEs mobility[2], and wild type plants.

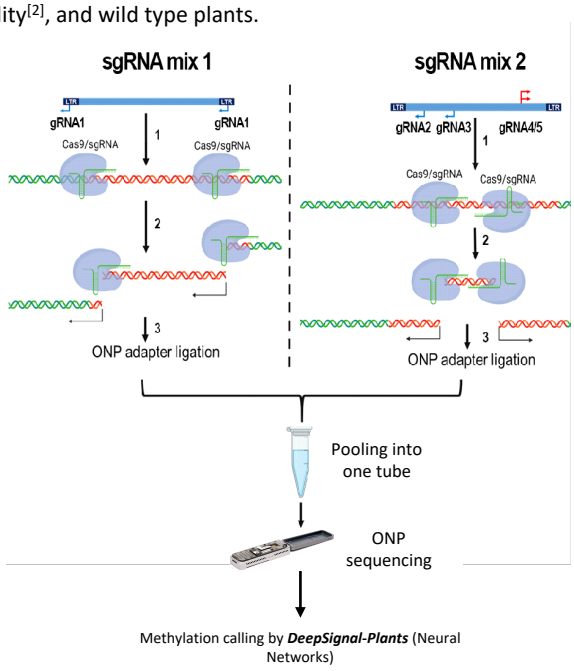

Figure 1. Scheme of the pipeline for obtaining abundance of TE-related long reads and methylation calling

In plants with defective methylation system (*ddm*), a decrease in methylation level is expected. This is consistent with bisulfite sequencing (BS) results (Fig. 2).

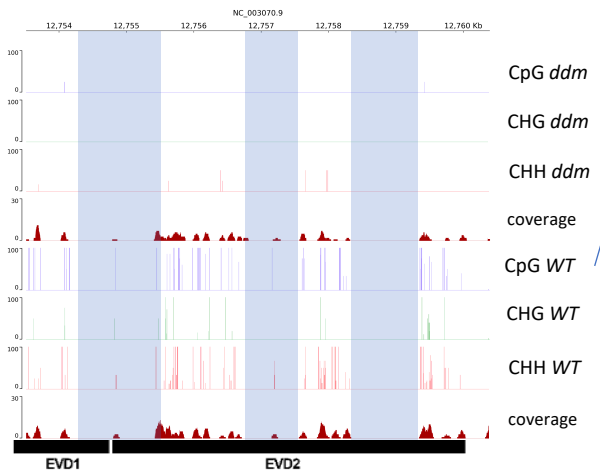

Figure 2. Plot of the methylation profiles from bisulfite sequencing in CpG, CHG and CHH context in genome browser. Blue squares represent indeterminable regions.

But compared to bisulfite sequencing, CANS does a much better job in highly repetitive and low complexity regions (Fig. 3), which is extremely important in TEs copies profiling.

For methylation calling from long reads we used recently published algorithm[2] based on using neural networks, which were trained on plants genome methylation data.

Conclusion. We showed that Nanopore-based DNA methylation detection in combination with Cas9-targeted sequencing are useful approaches for illuminating the epigenetic regulation of TEs.

References:

[1] Peng Ni et al., «Genome-wide Detection of Cytosine Methylations in Plant from Nanopore sequencing data using Deep Learning»., Biorxiv, 2021

[2] Zemach A. et al., «The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin»., Cell, 2013

[3] Gabrieli T. et al., «Selective nanopore sequencing of human BRCA1 by Cas9-assisted targeting of chromosome segments (CATCH)»., Nucleic Acids Research, 2018

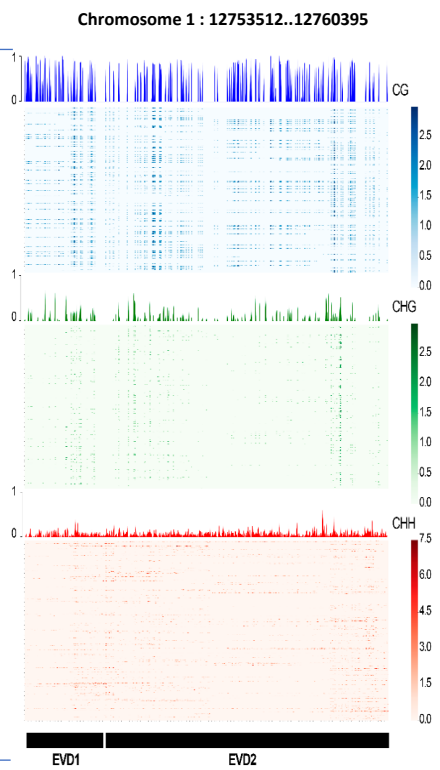

Figure 3. Methylation profiles from CANS in CpG, CHG and CHH context. Each row in matrix corresponds to pattern of methylated cytosines in single read