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RMetSeq package for processing targeted MRE-seq

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Motivation and Aim: Methylation-sensitive restriction enzyme sequencing or MRE-seq is one of the methods for determining the level of DNA methylation. It usually involves comparing the results of sequencing samples after exposure to methyl sensitive and insensitive enzymes targeting the same restriction sites. This approach has some limitations, which leads to the fact that this method has not found wide application. We propose a new approach to the design of experiments and analysis of MRE-seq data, which allows integration of this analysis with standard NGS sequencing of exome and target panels with probe enrichment.
Methods and Algorithms: DNA isolated from 9 blood samples was sonicated in 300 and 500 bp modes. Thereafter, half of each sample was treated with the HpaII. All samples were enriched using SureSelect Custom DNA Target Enrichment Probes (which includes exomes of 37 genes of a total length of 168497 nucleotides) and sequenced using MiSeq. Genotyping analysis was performed using GATK4. The methylation level was assessed using the RMetSeq package published on github.com/alekseizarubin/RMetSeq.
Results: In the studied genomic regions, 159 restriction sites were found (with a coverage of more than 20). The methylation level ranged from 0.3 to 1. To assess the possibility of qualitative genotyping on MRE-seq data, modeling was performed on native DNA sequencing samples, DNA treated with restriction enzyme and a mixed sample. In the treated with restriction enzyme, ≈3 % of the target area is not available for analysis. The mixed sample showed the quality of the native sample, but required 5–10 % more than the average coverage for this. On average coverage of mixed samples, with an average coverage of 70X, 97.7 % of the target coverage of the region is more than 10.
Conclusion: Targeted MRE-seq and the RMetSeq package make it possible to simultaneously determine the level of DNA methylation without compromising the quality of genotyping in standard sequencing using probe enrichment.