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Development of Binary Deoxyribozyme sensors modified with LNA for the specific detection of methylated dsDNA

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Currently, the science and clinic are faced with the issue of rapid detection of epigenetic modifications, in particular, methylation. Methylation determines the nature of the availability of DNA sites, and therefore gene expression both in normal and pathological conditions. All the methods that are used to detect methylation sites today are limited in application due to their cost, complexity, or long sample preparation. Among examples of the current techniques are Oxford Nanopore sequencing, bisulfite sequencing, and various PCR-based methods. Protein-free enzyme technologies, in particular binary deoxyribozyme (BiDz) sensors, are under intensive development in the last decade. Such DNA constructs are capable of detecting specific DNA or RNA fragments with higher selectivity to complex mutations, including single nucleotide variations (SNV), than conventional hybridization approaches. After the recognition, the BiDzs perform signal propagation due to multiple cleavage turnover of a fluorescent substrate [1,2].

LNA (Locked Nucleic Acids) modifications, added to the analyte recognition strands, significantly increase the specificity of detection and reduce the limit of detection in comparison to unmodified versions. LNA are artificial nucleic acids that have a stabilized ribose in the 3' endo conformation via a methylene bridge between 2'-O and 4'-C [3]. LNA-modified BiDz sensors in this case will have more precise capabilities and we hope will be able to detect subtle changes in the affinity caused by the methylation sites. 5'-MethylCytosine (5-MeC) will be considered here as an SNV, and a strand with such a change will form a much stronger bond.

This project uses BRAF proto-oncogene as a model target gene. To test the hypothesis, we designed and tested 2 sets of BiDzs with LNA-modified deoxyribozymes. The designs showed a high background due to self-complementarity. In the second variant, that was compensated by the addition of the short single-stranded fragments designed to prevent self-assembly of the machine. The background level of several variants of the DNA machine with LNA insertion through one nucleotide and the signal level on DNA strands without methylation have been evaluated. Incubation was carried out at 37 °C for 1, 3, 6, and 24 hours.

The results indicate the inclusion of DNA into the strand and the activation of machines. The second compensating set of DNA machines demonstrates a better signal/background ratio when tested on unmethylated analytes, therefore being more promising and now subjecting to testing with methylated samples.

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References

1) Bengtson H. N. et al., https://doi.org/10.1016/j.bios.2017.02.051
2) Kolpashchikov D.M., https://doi.org/10.1021/acs.accounts.9b00098
3) Pabon-Martinez Y. V. et al., https://doi.org/10.1038/s41598-017-09147-8