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METHYLATION PROFILE ANALYSIS OF DNA-HALO STRUCTURE BY SYNCHROTRON-BASED FTIR SPECTROSCOPY

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Introduction: DNA methylation is a major regulator of transcriptional activity and alongside other epigenetic modifications it introduces specific level of chromatin complexity. Fourier transform infrared (FTIR) spectroscopy is a rapid, non-destructive, and label-free technique for identifying subtle changes in all bio-macromolecules, and it has been used as a method of choice for studying DNA conformation. The present study was designed to explore the use of synchrotron-based FTIR spectroscopy to monitor the subtle changes on molecular level regarding the DNA methylation status of cytosine in the whole genome.

Methods: For FTIR-based DNA methylation analysis *in situ*, DNA-HALO samples were prepared using slightly modified methodology for nuclear HALO preparations where DNA-HALOs are liberated of any protein residues but preserve higher order chromatin structure.

Results: Using FTIR spectroscopy we analysed and compared methylation profiles of isolated genomic DNA and DNA-HALO samples. DNA-HALO structure shows more distinct peaks in fingerprint region of spectra. DNA-HALO structure is more accurate for detecting bonds in unmemthylated cytosine as specific infrared peaks are defined as vibrations of bonds in unmethylated cytosine at 1151 cm^{-1} and 1357 cm^{-1}. The ratio of integrated area under the peak 1151 cm^{-1} over integrated area under the peak that represents PO_2^- backbone vibrations can be used to assess level of unmethylated cytosine and thus methylation rate in the DNA-HALO samples.

Conclusion: This study demonstrates potential of FTIR spectroscopy to detect DNA methylation in DNA-HALO samples more precisely compared to classical DNA extraction procedure that yield unstructured whole genomic DNA.

Key words: HALO structure; FTIR spectroscopy; DNA methylation; Epigenetic marks; Chromatin

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