DOI 10.18699/SBB-2022-600

# Assessment of the nucleotide excision repair system activity *ex vivo*

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**Key words:** nucleotide excision repair, ex vivo methods, DNA damages

*Motivation and Aim*: Nucleotide excision repair (NER) is one of the main repair systems in cells of living organisms, which is responsible for removal of a wide range of bulky DNA lesions. The use of approaches focused on exploring the structure and functions of proteins involved in NER would enable elucidation of the process mechanism and identification of the main stages affecting its efficiency as well as the composition and structure of multiprotein complexes that arise and function at different NER stages [1, 2]. Nevertheless, the development of approaches to investigating and comparing the efficiency of bulky lesion repair in living cells (*ex vivo*) remains topical for both fundamental and applied research.
*Methods and Algorithms*: Recombinant plasmids containing bulky nFlu and nAnt lesions (hereinafter referred to as nFlu and nAnt DNA respectively) were synthesized. Transfection of cells with the plasmid was performed using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's protocol. To analyze the efficiency of NER of nFlu- and nAnt-DNA in HEK 293T human embryonic kidney cells we assessed the time of emergence of cells whose fluorescence indicated recovery of TagRFP protein expression using the Cell-IQ MLF system (Chip-Man Technologies, Finland) for long-term intravital monitoring of cells at the Common Use Center of the Institute of Cytology and Genetics, SB RAS. Cells were pictured at 10 min intervals in phase contrast and fluorescence modes using a Nikon CFI Plan Fluorescence DL ×10 objective. The resulting images were analyzed using the ImageJ and Cell-IQ Analyzer software.
*Results*: Our constructed plasmids containing bulky nFlu or nAnt lesions near the *tagrfp* gene promoter were shown to undergo repair in eukaryotic cells (HEK 293T) and that they can be used to analyze the efficiency of NER *ex vivo*. A comparative analysis of the time dependence of fluorescent cells accumulation after transfection with nFlu- and nAnt-DNA revealed that there are differences in how efficient their repair by the NER system of HEK 293T cells can be.
*Conclusion*: The proposed method enables assessing the efficiency of removal of bulky nAnt and nFlu lesions from model plasmids by the NER system of HEK 293T cells. The method is a promising tool for studying NER; it enables comparing both the repair status of various cells and the efficiency of repair of various structural lesions.
*Acknowledgements*: This study was supported by the Russian Science Foundation (project No. 19-74-10056).

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