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Toward analyzing interactions between plasmid DNA and chromatin in HEK293T cells

Yan A.P.[1, 2]*, Salnikov P.A.[1, 2], Gridina M.M.[1, 2], Belokopytova P.S.[1, 2], Fishman V.S.[1, 2]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Novosibirsk State University, Novosibirsk, Russia*

* *a.yan@g.nsu.ru*

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Motivation and Aim: With the advent of methods for capturing chromosome conformation, studies of interphase nucleus chromatin organization have received a new impetus for development. Using these approaches, elementary units of native chromatin stacking – topologically associated domains (TADs) formed by different mechanisms in vertebrates and invertebrates – were discovered for different animal species [1]. However, how nucleus chromatin interacts with extracellular DNA such as viral and plasmid DNA in space remains poorly understood. This work focuses on developing a method to detect spatial contacts between plasmid DNA and chromatin of eukaryotic cells based on 4C technology.

Methods and Algorithms: We transfect HEK293T cells with a plasmid vector adapted for the 4C experiment. Presumably, in the nucleus, chromatin proteins bind to plasmid DNA and condition its distribution within the nucleus. Treatment of cells with formaldehyde ensures fixation of spatial contacts of genomic and plasmid DNA. We perform the 4C procedure on this material. The resulting 4C library contains predominantly DNA fragments that include the plasmid sequence and the nearby genomic DNA sequence. In this way, the sequences of genome loci that were in contact with the plasmid are revealed.

Results: Optimization of the standard conditions of the 4C method and the design of a special plasmid vector made it possible to efficiently detect plasmid-DNA contacts: over 90 % of 4C library reads contain genomic DNA sequence. The remaining share consists of reads formed as a result of incomplete hydrolysis of plasmid DNA or recovery of the full-length plasmid molecule after ligation. Such products are defined as intramolecular cis contacts and are a characteristic fraction in libraries obtained by chromosome conformation capture methods. We also showed that at least part of the contacts between plasmid and chromatin are not an artifact of the experiment, but are formed within the nucleus. For this purpose, we performed a 4C experiment on a mixture of transfected human cells, mouse cells, and solubilized plasmid. The sequences of the transfected plasmid and the one added to the mixture of cells differed from each other, which allowed us to distinguish the chromatin contacts formed with their participation from each other. The plasmid transfected into cells formed more contacts with human DNA than with mouse DNA, while ligations of the plasmid added to the mixture of cells with DNA of both species occurred with approximately the same frequency (all calculations were performed taking into account the ratio of concentrations of different chromatins in the reaction). Statistically significant differences between the contact patterns of plasmids with DNA from the two species ($p < 0.001$) suggest that about 30 % of the

contacts formed between plasmid transfected into cells and human chromatin are formed through biological patterns within the nucleus rather than by random diffusion in solution.

Conclusion: The proposed 4C-plasmid experiment allows us to detect spatial contacts of plasmid DNA with eukaryotic chromatin. In the future, this approach opens prospects for testing various short DNA sequences cloned into the vector as possible genetic determinants of nucleus compartmentalization and search for methylated loci of the genome.

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References

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