

Detecting three-dimensional contacts of plasmid DNA with chromatin in HEK293T cells

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Motivation and Aim: Interphase chromosomes in cell nuclei are organized into functional domains that differ from each other with respect to their molecular composition. These include active and inactive chromatin domains, DNA repair compartments, nucleoli, Cajal bodies, transcription factories associated with active RNA polymerase II, etc. It was suggested that specific interactions of protein and/or RNA molecules with each other and with DNA sequences results in a biophysical process called liquid-liquid phase separation and explains formation of chromatin compartments [1]. However, the DNA motives determined compartmentalization pattern is still unknown due to the insufficient resolution of existing methods for analyzing the three-dimensional organization of the genome data. Here we propose a new method to uncover sequence determinants of chromatin compartmentalization.

Methods and Algorithms: We deliver the target DNA sequence as part of a plasmid to the studied cells, and using the 4C-experiment, we reveal preferences in three-dimensional contacts of the plasmid DNA sequence. We cloned the ancillary sequences required for the 4C-experiment into the pUC19 vector, transfected the resulting vector into HEK293T cells, and obtained and sequenced the 4C-libraries using NGS technologies.

Results: Bioinformatic analysis of the obtained data showed that using this approach, it is possible to detect spatial contacts of the plasmid with the genome with an efficiency of 7 %. Based on the analysis of the obtained data, modifications of plasmid vectors and the 4C-protocol were proposed, which allow increasing the amount of data obtained as a result of the 4C-experiment about 3 times.

Conclusion: as a result of our work, for the first time we have shown the possibility of using the 4C-method to study trans-contacts of plasmid DNA with chromatin in cell nucleus.

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References

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