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Generation of Stable Universal HEK293 Cell Line with Integrated FRT-sites for Subsequent Generating Protein Producing Cell Lines

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Introduction. In recent years, the global pharmaceutical market has experienced significant growth. The Russian market alone reached 2.5 billion rubles in 2022, with 18.3% attributed to biotechnological drugs. Thanks to the adopted state strategy for the development of the pharmaceutical industry, market growth is expected to continue until 2030. [1] In 2023-2024, there was a slight decrease due to stabilization after a sharp increase during the COVID-19 pandemic, while positive trends continued, such as the inclusion of the biotechnology sector in the list of priority areas of scientific and technological development in 2024. [2]
The main platform for producing biotechnological drugs is cell lines that produce therapeutic recombinant proteins. Developing and improving such cell lines is a complex technological process, making its optimization an important task.

Main Body. This study describes the development of an HEK293 cell line with FRT sites integrated into the genome, enabling site-specific insertion of a target expression cassette carrying a therapeutic protein gene.
The study was divided into two stages: integration of FRT sites into the HEK293 genome was the first stage and verification of the efficiency of targeted insertion of an expression cassette with a fluorescent protein into these sites was the second one.
The integration of FRT sites into the genome was carried out using SpCas9 through homologous recombination. For the integration a donor plasmid was constructed using restriction-ligation methods. This plasmid contained FRT sites, a gene providing resistance to a selective antibiotic and homology arms for targeted genome integration. The integration process involved SpCas9 endonuclease, encoded by a separate plasmid. Using lipofectamine-mediated co-transfection, the donor plasmid and SpCas9-expressing plasmid were introduced into an adherent HEK293 cell culture.
Following transient plasmid expression, cells were monoclonally selected in 96-well plates and transferred to a medium containing the selective antibiotic corresponding to the resistance gene in the donor plasmid. Successful integration was confirmed through microscopy by observing the growth and proliferation of transfected cells in the selective medium, while wild-type HEK293 control cells died.
On the second stage, to test the efficiency of insertion via integrated FRT sites, a second plasmid was constructed containing an expression cassette with a blue fluorescent protein gene flanked by FRT sites identical to those in the donor plasmid. Monoclones selected in the first stage underwent a second round of co-transfection with: a plasmid carrying the fluorescent protein gene flanked by FRT sites and a plasmid encoding the Flp recombinase, which recognizes FRT sites and mediates recombination. The resulting population of transfected monoclones was enriched using a cell sorter based on fluorescence intensity in the blue spectrum. Further research plans include selecting monoclones with the highest fluorescence intensity and conducting additional analyses of insertion copy number using real-time PCR.

Conclusion. Experiments on targeted FRT site integration demonstrated sufficient efficiency in HEK293 cells, resulting in monoclones with FRT sites integrated into the genome. Additional experiments to verify targeted cassette insertion efficiency and characterize the obtained monoclones are in their final stages.

References:

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