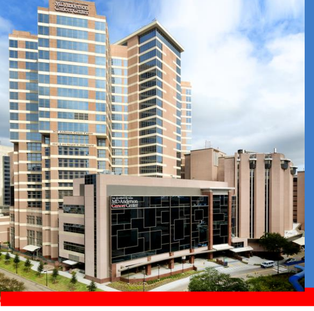

Determination of Optimal Cell and Plasmid Concentration for Transfection of I-SceI by DR-GFP Reporter

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Introduction

DNA damage is a common cause of cancer. One way it is caused is by interstrand crosslinks (ICLs); interstrand crosslinks are a result of two separate strands of DNA becoming connected by a covalent bond [1]. These ICLs are cytotoxic, causing several metabolic processes and DNA replication to be halted [2]. One of the cells main repair mechanisms for interstrand cross-links is homologous recombination (HR) [2].

While HR is preferred in healthy cells to eliminate ICLs, ICLs are commonly used as an avenue of chemotherapy in attempts to create more DNA damage, therefore promoting natural apoptosis. As a result, a cancer treatment that is currently being explored is a HR inhibitor drug. The HR inhibitor drug will be used to inhibit the HR mechanisms, therefore keeping the ICLs and allowing for quicker apoptosis of cancer cells. In the following study, direct repeat green fluorescent protein (DR-GFP) was transfected with the I-SceI plasmid to measure HR in cells. Specifically, the most effective cell and plasmid concentration was determined to achieve the highest transfection rate.

Materials and Methods

Plasmid Extraction

- The I-SceI plasmid was extracted using the standard protocol.

Cell Concentration

- The cells of concentrations $1*10^5$, $3*10^5$, $5*10^5$, and $9*10^5$ cells/mL were transfected with the I-SceI plasmid using the standard protocol.

Plasmid Concentration

- Once the ideal cell concentration was determined, the procedure was repeated with I-SceI plasmid amounts of 0.5ug,1.5ug, 1ug, & 2ug.

Results

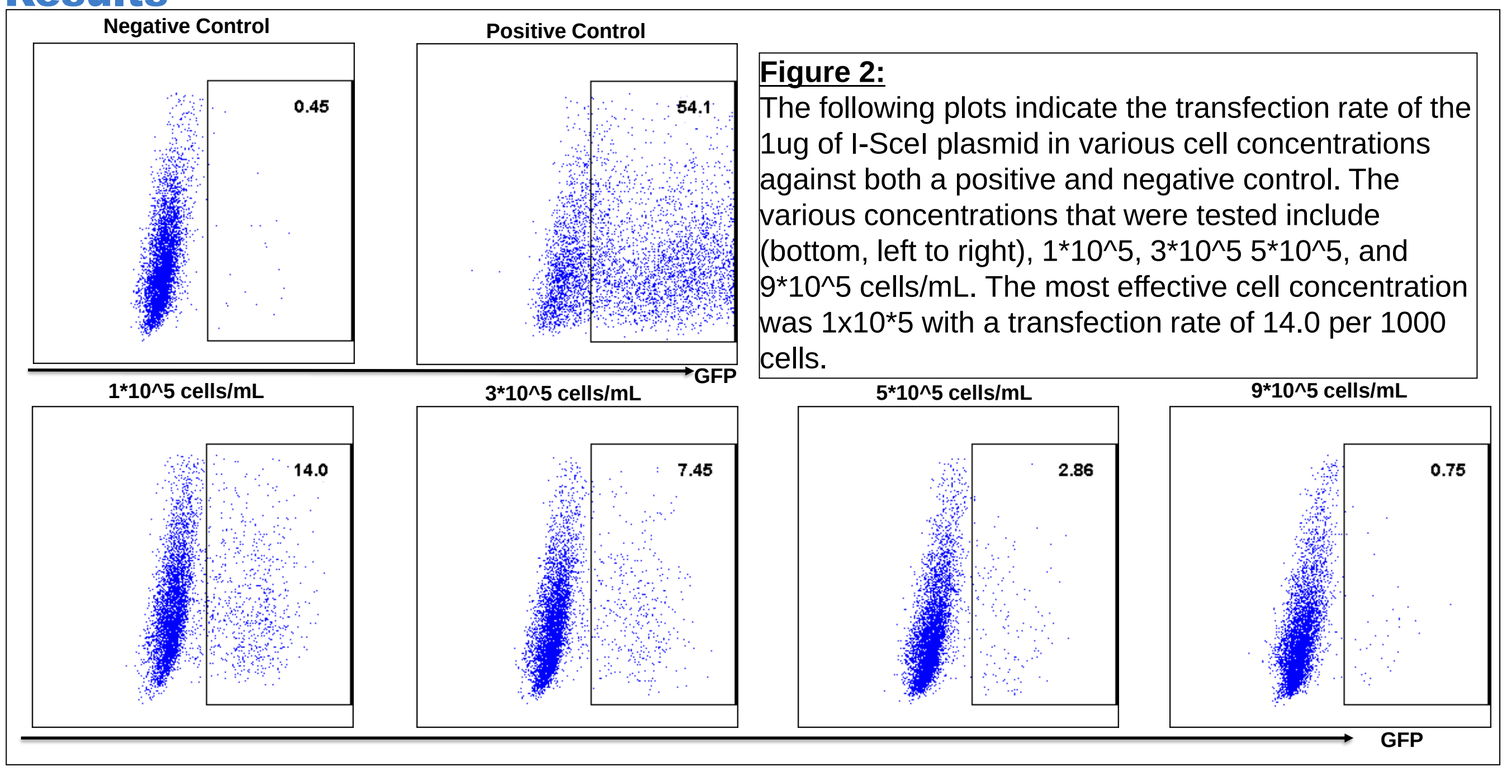

Figure 2:
The following plots indicate the transfection rate of the 1ug of I-SceI plasmid in various cell concentrations against both a positive and negative control. The various concentrations that were tested include (bottom, left to right), 1*10^5, 3*10^5 5*10^5, and 9*10^5 cells/mL. The most effective cell concentration was 1x10*5 with a transfection rate of 14.0 per 1000 cells.

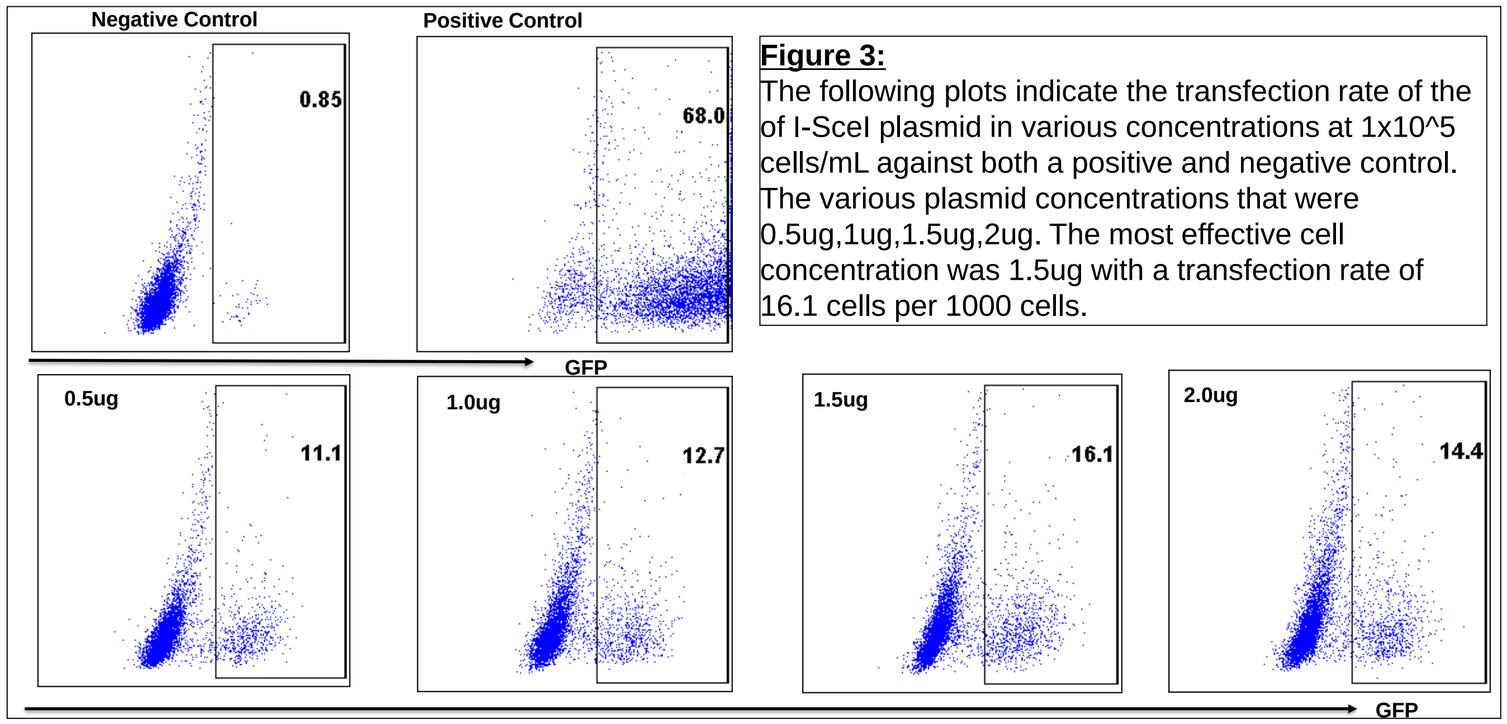

Figure 3:
The following plots indicate the transfection rate of the of I-SceI plasmid in various concentrations at 1x10^5 cells/mL against both a positive and negative control. The various plasmid concentrations that were 0.5ug,1ug,1.5ug,2ug. The most effective cell concentration was 1.5ug with a transfection rate of 16.1 cells per 1000 cells.

Results

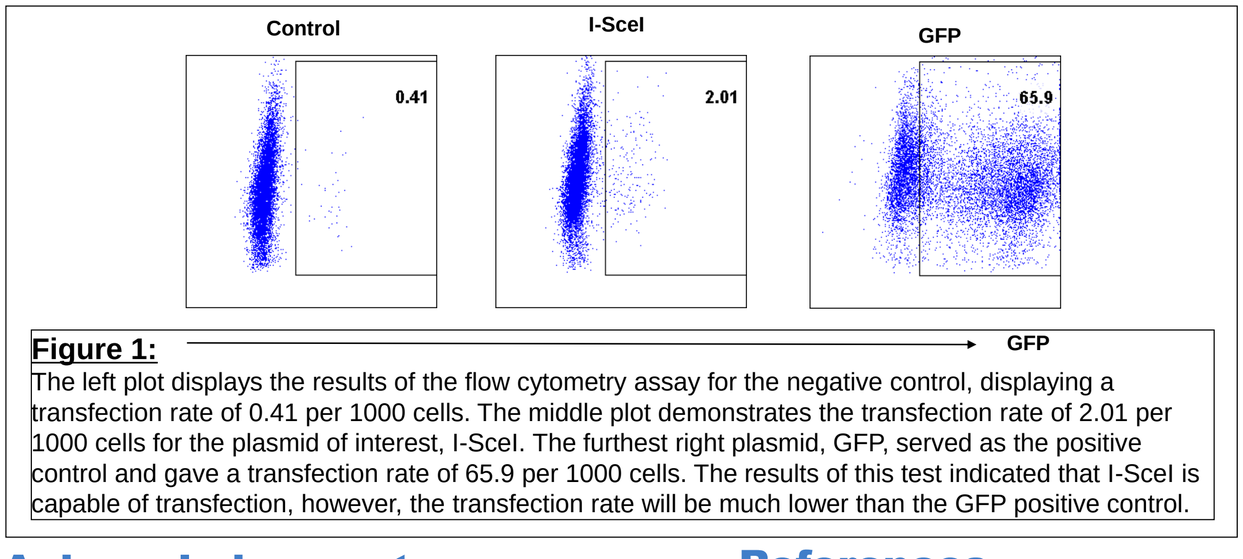

Figure 1:
The left plot displays the results of the flow cytometry assay for the negative control, displaying a transfection rate of 0.41 per 1000 cells. The middle plot demonstrates the transfection rate of 2.01 per 1000 cells for the plasmid of interest, I-SceI. The furthest right plasmid, GFP, served as the positive control and gave a transfection rate of 65.9 per 1000 cells. The results of this test indicated that I-SceI is capable of transfection, however, the transfection rate will be much lower than the GFP positive control.

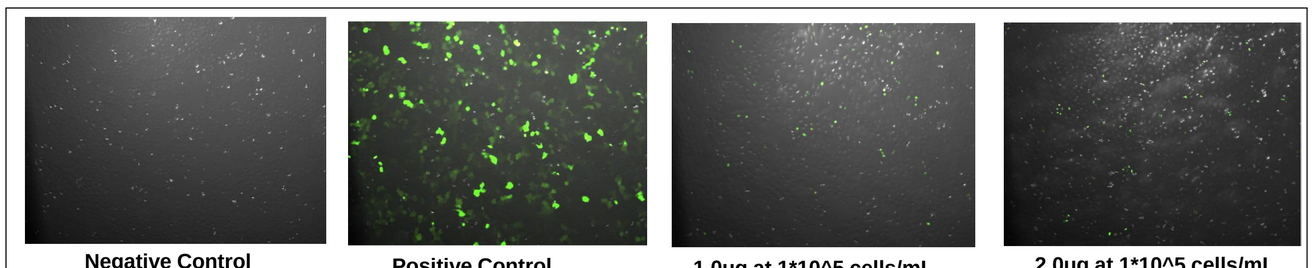

Figure 4:
The following images illustrate the success of the transfection in the cell. The negative control indicates that there are no transfected cells due to absence of a green fluorescent color. The positive control indicates that there was a successful transection. The plate with 1.0ug of plasmid in 1*10^5 cells/mL and 2.0ug of plasmid in 1*10^5 cells/mL indicates a successful transfection similar to the rate of transfection displayed in Figures 2 and 3.

Acknowledgements

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References

1) Nakanishi et al. “Homologous Recombination Assay for Interstrand Cross-Link Repair.” *Methods Molecular Biology.* 1998;745:283-291.

2) Cleary et al. “Biomarker-Guided Development of DNA Repair Inhibitors.” *Cell Press*. 2020; 78: 1070-1085.

Conclusions

The determined cell concentration, plasmid concentration, and produced transfection rate can be used in the future to test the effectiveness of an HR inhibit drug by determining how much the transfection rate was reduced. Additionally, further studies can be done to determine the correct concentration and ideal time of drug introduction to ensure optimal HR inhibition.