



Optimizing Of The High-Throughput DNA Repair Assay For Immunotherapy

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Introduction

Fanconi anemia (FA), breast, and ovarian cancers are predisposed to by mutations in the BRCA2 (Breast Cancer gene 2) recombination gene. Homologous recombination repair (HR) is made up of a number of interconnected pathways that are involved in the repair of interstrand crosslinks (ICL) and double-stranded breaks in DNA [1]. Additionally, recombination contributes to DNA damage tolerance by helping DNA replication recover from stopped or broken replication forks. Following the creation of I-Sce1 induced double stranded breaks, green fluorescent protein (GFP) assay is used to evaluate HR by flow cytometry [2]. The goal of our project is to optimize this HR assay for high-throughput chemical screening to identify small compound agents that can inhibit HR repair and trigger DNA damage dependent immune response [4].

Materials and Methods

- **Transfection** - A process by which foreign nucleic acids are delivered into a eukaryotic cell to modify the host cell's genetic makeup [5].
 - I-sce1, GFP, and red fluorescent protein (RFP) plasmid was extracted using standard protocol.
 - Lipofectamine 3000 was used in all protocols.
- **Flow cytometry** - A technology that rapidly analyzes single cells or particles as they flow past single or multiple lasers while suspended in a buffered salt-based solution [6].

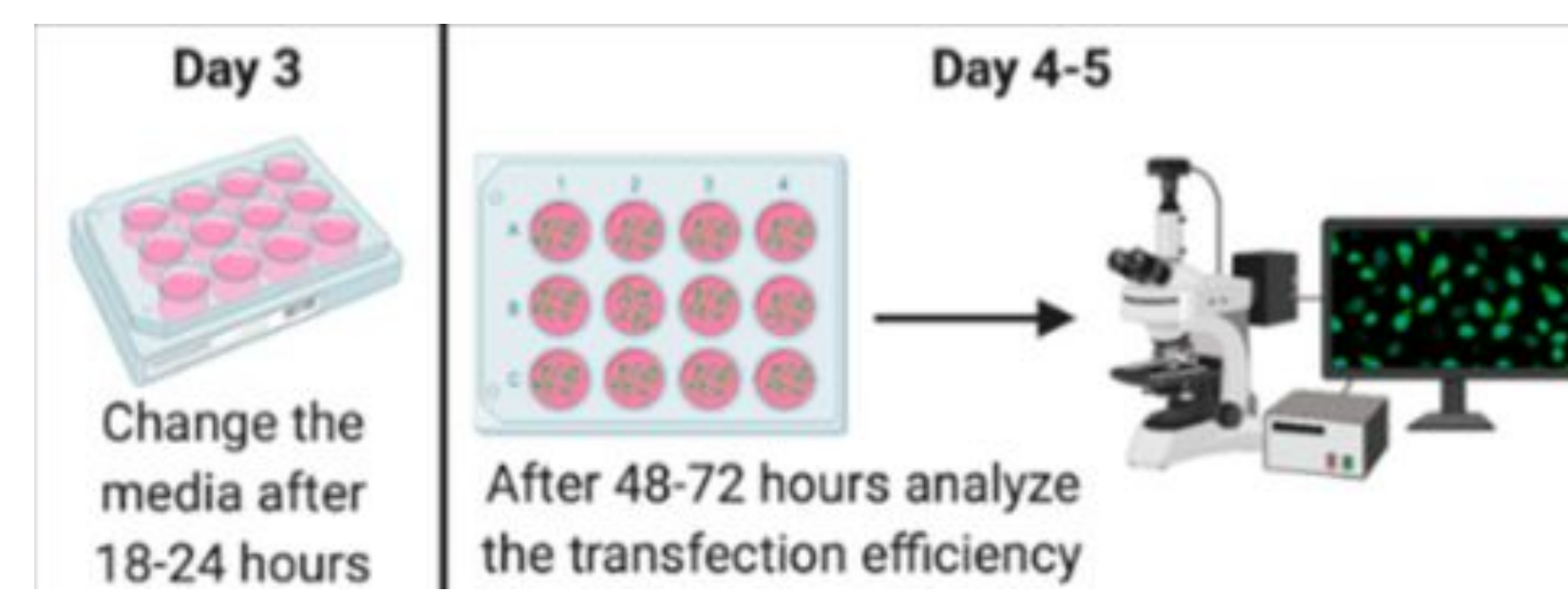
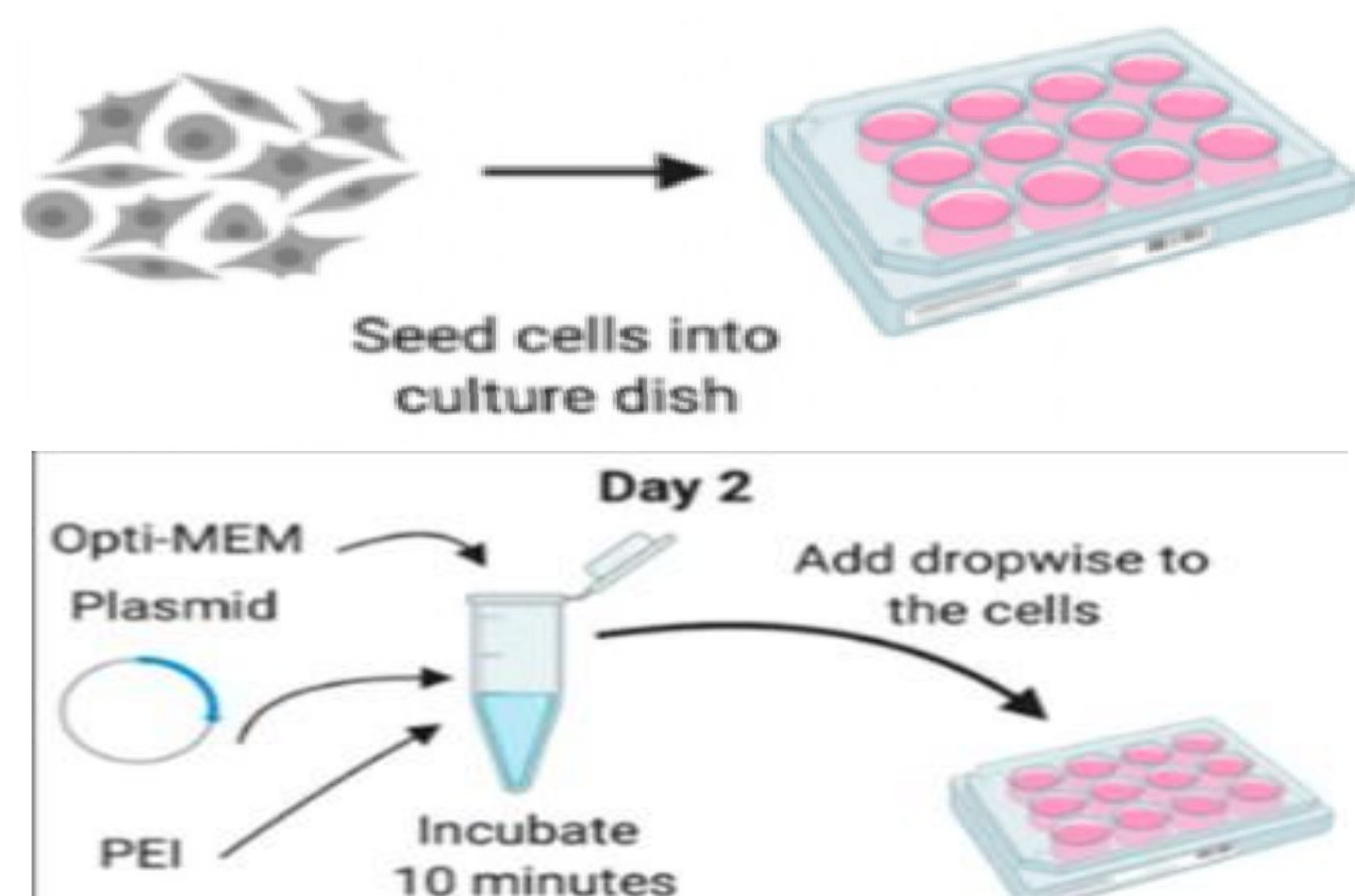


Figure 1. Experimental Design of Transfection.

Above is the experimental design of transfection used [3].

Figure 2. To the right is the four quadrant scatter plot showing what cells are expressed.

Q2 RFP+	Q1 GFP+ or RFP+
Q3 No GFP+ or RFP+	Q4 GFP+

Results

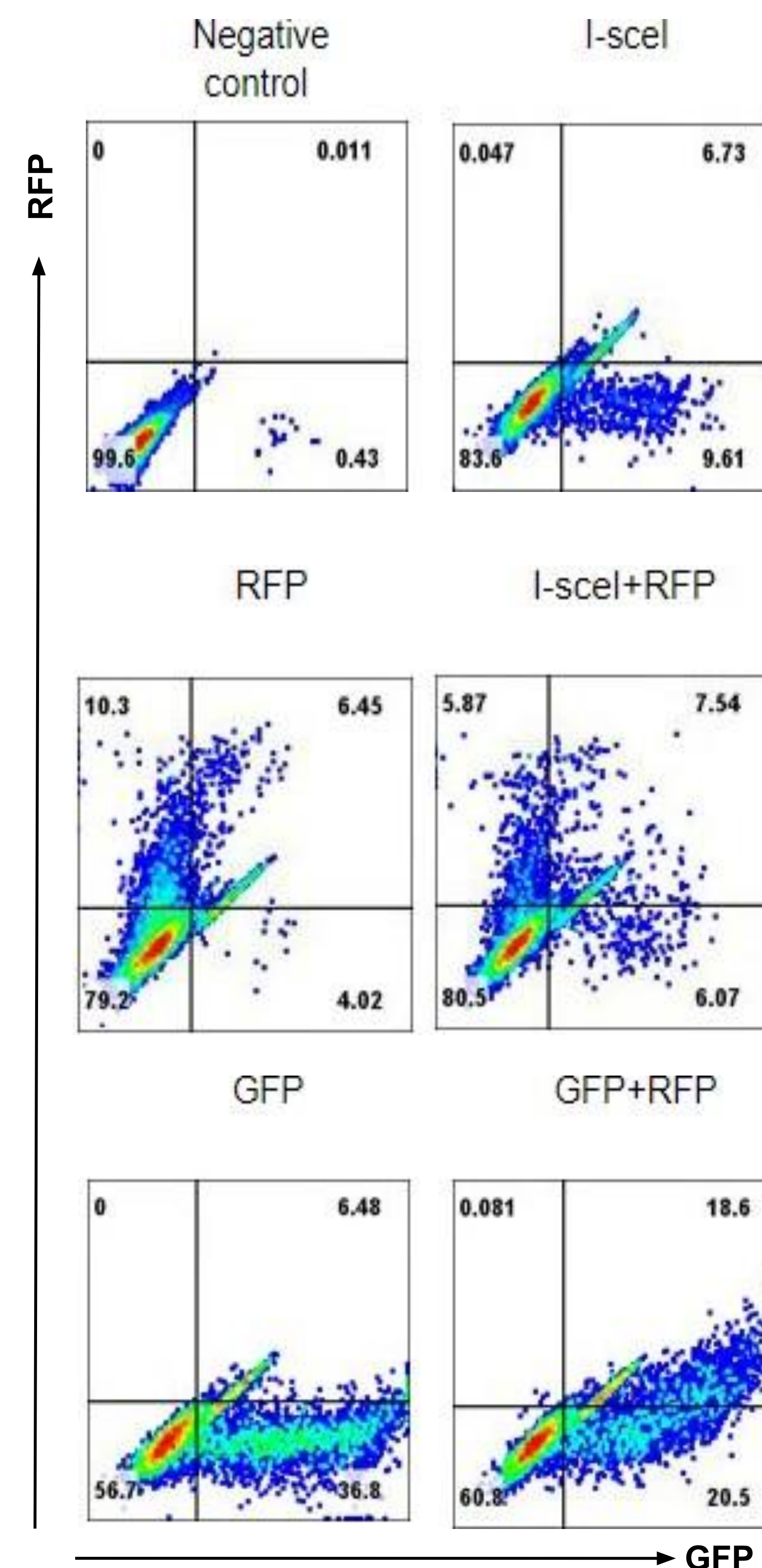


Figure 3. Results of Flow Cytometry DR-GFP Transfection From 6 Samples Using a 6-Well Plate.

The plots above indicate the transfection rate of the I-Sce1, RFP and GFP plasmids against both a positive and negative control. Each graph represents 3 micrograms of plasmid with the exception of negative control. The GFP+RFP is a 1:1 ratio.

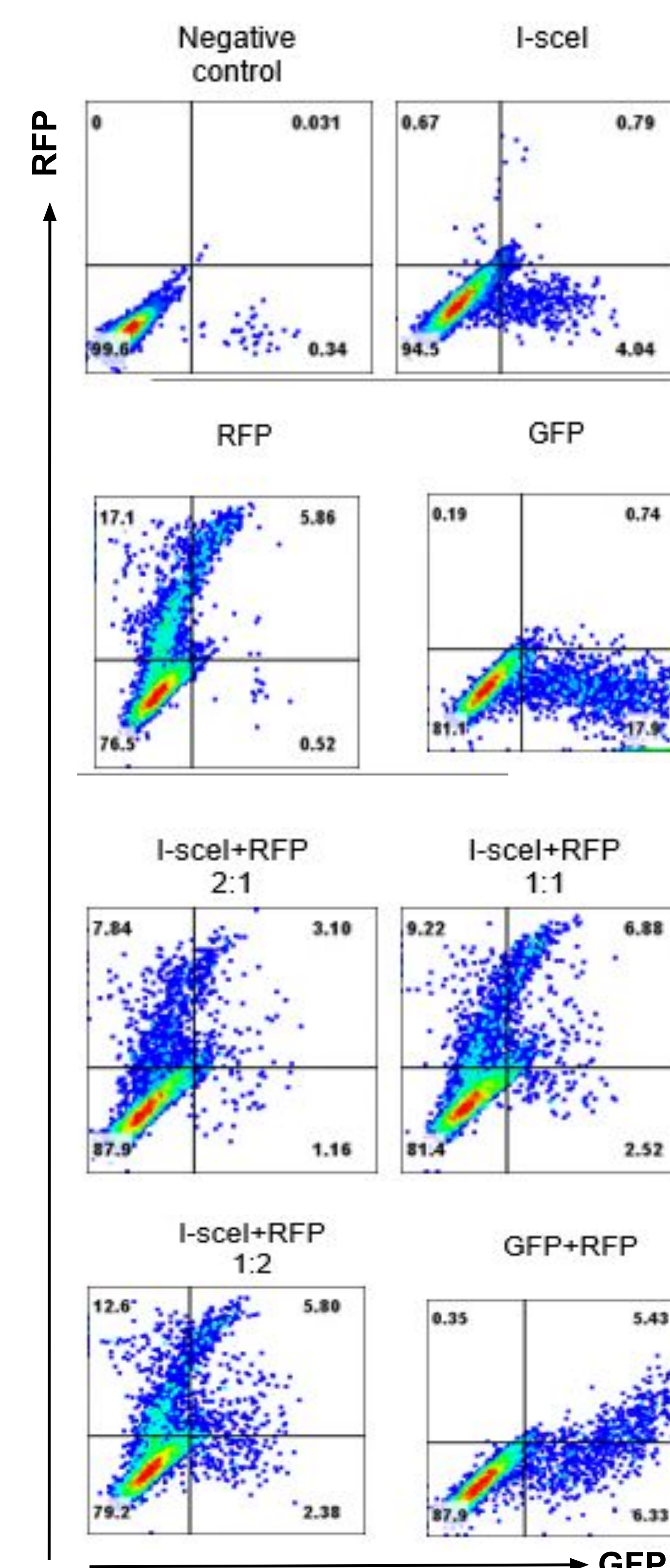


Figure 4. Results of Flow Cytometry DR-GFP Transfection From 8 Samples Using (8) 60 mm Dishes.

The plots above indicate the transfection rate of the I-Sce1, RFP and GFP plasmids against both a positive and negative control.

Each graph represents 3 micrograms of plasmid with the exception of negative control. The GFP+RFP is a 1:1 ratio. The different I-sce1+RFP plasmid concentration ratios were: 2:1, 1:1, & 1:2, respectively. The most effective cell concentration was I-sce1+RFP 1:2 with 12.6% of RFP cells.

Conclusions

Through the experiments, we optimize transfection conditions to achieve the best efficiency of HR repair assay in a high-throughput format.

Changing the calculated cell concentration, plasmid concentration, and produced GFP intensity, we can achieve better GFP signaling to measure HR repair efficiency. Future tests of the efficacy of an HR inhibitor drug can be done by estimating how much the GFP intensity is reduced. Additionally, more research can be done to establish the proper drug introduction time and the concentration for achieving the best HR inhibition. We expect the new HR inhibitors have a potential to modulate immune responses through regulating DNA damage accumulation for immunotherapy.

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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. Li, X., Heyer, WD. Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res* 18, 99-113 (2008). <https://doi.org/10.1038/cr.2008.1>
3. Kachkin, D. V., Khorolskaya, J. I., Ivanova, J. S., & Rubel, A. A. (2020). An Efficient Method for Isolation of Plasmid DNA for Transfection of Mammalian Cell Cultures. *Methods and Protocols*, 3(4), 69. MDPI AG. Retrieved from <http://dx.doi.org/10.3390/mps3040069>
4. Zhang, L., Peng, Y., Uray, I. P., Shen, J., Wang, L., Peng, X., Brown, P. H., Tu, W., & Peng, G. (2017). Natural product β -thujaplicin inhibits homologous recombination repair and sensitizes cancer cells to radiation therapy. *DNA repair*, 60, 89-101. <https://doi.org/10.1016/j.dnarep.2017.10.009>
5. Chong, Z. X., Yeap, S. K., & Ho, W. Y. (2021). Transfection types, methods and strategies: a technical review. *PeerJ*, 9, e11165. <https://doi.org/10.7717/peerj.11165>
6. McKinnon K. M. (2018). Flow Cytometry: An Overview. *Current protocols in immunology*, 120, 5.1.1-5.1.11. <https://doi.org/10.1002/cpim.40>