Chapter 1

Introduction

1.1 Background

The recently introduced endonuclease-based gene editing, CRISPR-Cas9, in 2012 by Doudna and Charpentier, becomes a breakthrough that enables a reliable, flexible, high specificity and effectiveness genome editing (Strzyz, 2020). CRISPR-Cas9 is considered as a transformative tool in many fields related to genome editing, such as; biomedical research for gene function and disease mechanisms study to explore potential therapeutic strategy, bioinformatics for the discovery of novel patterns and relationships through genomic datasets, and biotechnology for precise DNA modification in wide array of organisms which includes bacteria, yeast, plants (i.e., GMO crops), and animals to improve traits or create novel function. Hence, a staple tool for molecular biology applications (Ansori et al., 2023; Gostimskaya, 2022). Development of and efficient and cost-effective protocols are particularly important to create reliable, reproducible and feasible data within limited resources.

Transfection is one of the most critical determinants of successful gene editing, as it directly influences the delivery of CRISPR-Cas9 components into target cells and, consequently, the overall editing efficiency (Setia et al., 2026). Various transfection platforms have been developed to facilitate nucleic acid delivery into mammalian cells, asides from physical transfection of electrophoration and biological transfection through viruses that may be cost-inefficient and toxic to the cells, complex and tedious, respectively (Luft and Ketteler, 2015). Such chemical transfections are widely commercialized and adopted in many research laboratories due to their cost-effectiveness and ease to use. Chemical transfection platforms include lipid-based reagents such as Lipofectamine™ 3000, inorganic methods such as calcium phosphate (CaP) precipitation, and cationic polymer-based carriers such as polyethylenimine (PEI). Previous papers show that efficiency was comparable, however there are no

papers that compares all of the three transfection methods of Lipofectamine™ 3000, CaP and PEI toward CRIPSR-Cas9 research targeting AAVS1 locus (Chong et al., 2021; Hasan et al., 2021; Jahanafrooz et al., 2022). Here, HEK293T is a cell line of choice due to its easiness to transfect (Tan et al., 2021) and the existence of a safe harbor for gene editing in the locus adeno-associated virus integration site 1 (AAVS1), located in the nonessential region of PPP1R12C gene of human chromosome 19 in HEK293T allows for genetic editing without disrupting any vital cellular functions (Hayashi et al., 2020; Oceguera-Yanez et al., 2016). Not only that, this cell has been utilized by Hermantara et al. (2024) team for CRISPR-Cas9 research targeting AAVS1, and this cell able to generate CRISPR-Cas9 proteins upon transfection with plasmid containing human U6 and CMV promoter (Falah et al., 2024). Plasmid 1400 and GuideA78 is a promising Cas9 vector and guide, which upon synergized, it allows the production of Cas9 enzyme linked with GFP and sgRNA targeting AAVS1 to induce double-strand break (DSB) that leads to either deletions or insertions caused by NHEJ.

By comparatively evaluating Lipofectamine™ 3000 transfection efficiency in HEK293T cells with different transfection platforms; CaP and PEI through Tracking of Indels by DEcomposition (TIDE) assay, will enable the identification of the most effective and reproducible transfection platform. Hence this research may provide valuable methodological insights, improve experimental success rates in future cohorts, and contribute to the further field of genome editing by clarifying practical considerations in CRISPR-Cas9 nucleic acid delivery.

1.2 Objective

To evaluate three transfection method of Lipofectamine™ 3000, CaP and PEI nucleic acid's CRISPR-Cas9 genome editing efficiency toward HEK293T cells' AAVS1 locus safe harbour site by indels detection using T7EI mutagenesis assay

The scope of the project is to perform three transfection methods Lipofectamine™ 3000, CaP and 2:1 and 4:1 PEI ratio using purified CRISPR-Cas9 plasmid of p1400 & pGuideA78, and performing T7EI mutagenesis assay

1.3 Hypothesis

1. H_0 : The TIDE assay analysis of the three transfection methods does not result in significant differences

   H_1 : At least one of the TIDE assay analysis of the three transfection methods does result in significant differences