Chapter 1

Introduction

1.1 Background

In this modern day, genome editing has established itself as a fundamental tool for a wide range of applications, with a large portion of it being attributed to the CRISPR-Cas9 system and its development over the years, providing a simple yet reliable method of manipulating the genome (Javaid et al., 2022). Be that as it may, the success of the CRISPR-Cas9 system is entirely dependent on the successful delivery of the editing system into the cell's nucleus (Bloomer et al., 2022). As both the phospholipid bilayer found on the surface of all mammalian cells and nucleic acid inserts possess negative charges, the natural repulsion between the two creates a sort of defensive wall that must first be neutralized (Dutta et al., 2021; Ma et al., 2017; Pawłowski & Zielenkiewicz, 2024). In working around this, researchers have developed various approaches in delivering foreign material through the cell membrane, which are classified as one of three main categories: biological, physical, and chemical. While transfection may be carried out through biological means such as viral transduction, or physical techniques such as electroporation or microinjection, both approaches come with several limitations when compared to chemical-based transfection, involving cost, safety, and scalability (Chong et al., 2021; Fajrial et al., 2020; Merten et al., 2016). In chemical-based transfection, many have developed approaches of coating the DNA insert with a transfection agent capable of carrying it and fusing with, or penetrating the cellular membrane, effectively masking the insert's negative charge from the membrane during transfection (Chong et al., 2021; Dutta et al., 2021). Overall, chemical transfection approaches remain the most strategic choice, preventing excessive cell death, ensuring scalability with a considerably more efficient cost (Chong et al., 2021; Jamour et al., 2024).

Falling within the same chemical-based transfection category is lipid-based transfection, with the most notable one being the commercialized Lipofectamine platform (Li et al., 2019; Pavlov et al.,

2024). This platform has long been established as a sort of golden standard in delivering foreign DNA into cells, attributed to their typically high delivery rates across a wide range of cells (Cardarelli et al., 2016). However, the cost of this commercial reagent is a significant limitation, forming a major bottleneck that limits its availability for high-demand research (Chong et al., 2021; Ojha et al., 2025). On the other hand, other chemical transfection approaches, specifically polyethylenimine (PEI) and calcium phosphate-mediated transfections, are known to be more accessible, though with more optimization required to minimize cytotoxicity (Fahira et al., 2022; Roustazadeh et al., 2025).

Each cell type possesses distinct membrane compositions, endocytic pathway preferences, and metabolic activities, all of which can significantly impact transfection outcomes (Lin et al., 2020; National Human Genome Research Institute, 2024). Consequently, a highly efficient transfection method for a cell type may be ineffective, or even cytotoxic for another, highlighting the need for cell specific optimization prior to therapeutic applications. The A549 cell line, derived from human lung adenocarcinoma tissue, is an immortalized cell line often used as a model to study and develop respiratory gene therapy (Berg et al., 2025; Kohon et al., 2024; Korrodi-Gregório et al., 2016). Furthermore, these cells retain epithelial characteristics that are able to partially mimic the barrier tissues of the lung, a primary target for therapeutic interventions (Garcia-de-Alba, 2021; Wu et al., 2017). This lung epithelium cell represents a unique approach on assessing gene delivery systems due to their specialized phenotypes and cellular uptake mechanisms, specifically designed to protect against airborne pathogens and particles (Rothen-Rutishauser et al., 2023; Sasaki et al., 2021).

Given the cost constraints of commercial reagents and the cell-specific nature of transfection efficiency, a comprehensive comparison between the more cost-effective alternatives to the more established lipid-based standard is imperative. As A549 cells represent a critical model for respiratory research, and with transfection being a foundational technique in molecular biology, the findings of this study are expected to contribute towards a more practical framework for A549 implementation in genetic engineering studies, thereby enhancing experimental efficiency across different labs.

1.2 Objective

This study aims to develop and optimize a cost-effective CRISPR-Cas9 gene editing workflow in A549 cells by evaluating and comparing lipid-based transfection, calcium phosphate transfection, and polyethylenimine (PEI) chemical transfection methods for their efficiency in delivering a functional CRISPR-Cas9 construct to the AAVS1 locus. Validations will be carried out through genomic PCR amplification and the T7 Endonuclease I assay to assess on-target mutagenesis.

1.3 Hypothesis

It is hypothesized that PEI and calcium phosphate-mediated transfection methods will yield comparable efficiencies to the more costly lipid-based lipofectamine method in delivering a functional CRISPR-Cas9 construct into A549 cells, hoping to establish a more economically viable methodological foundation for future research.