Abstract

Despite its wide range of applications, the success of CRISPR-Cas9 genome editing is still reliant on the efficient delivery of the CRISPR components across the cellular membrane. A549 cells are a primary model for respiratory gene therapy with there being no study that has systematically compared transfection efficiencies in this cell line. This study aimed to develop and optimize a cost-effective CRISPR-Cas9 gene editing workflow by assessing and comparing lipid-based, calcium phosphate, and polyethylenimine (PEI)-based chemical transfection methods in delivering a functional CRISPR-Cas9 construct targeting the AAVS1 locus in A549 cells. However, inconclusive findings were obtained from the experiment, not being able to prove the hypothesis that PEI and calcium phosphate are able to yield efficiencies comparable to Lipofectamine. This finding is largely attributed to persistent nonspecific AAVS1 primer amplification that likely compromised true mutation signals in the T7 Endonuclease I assay. Consequently, the study pivoted towards the well characterized EMX1 locus, where two highly specific sgRNA candidates were designed with superior off-target profiles compared to published benchmarks. Future studies are therefore recommended to prioritize proper quality control, include transfection efficiency controls, and utilize Sanger sequencing with TIDE analysis for quantitative validation.

Keywords: CRISPR-Cas9, Chemical Transfection, A549 Cells, T7 Endonuclease I Assay, sgRNA Design