Abstract

CRISPR-Cas9 genome editing has become a revolutionary technology in fields of biomedical, bioinformatic and biotechnology, which enables more precise and adaptable genome editing in mammalian cells in comparison to conventional genome editing methods. A critical determinant of successful genome editing is the optimization of nucleic acids transfection method. Amongst the traditional transfection methods, chemical transfection is widely sought due to its highly commercialized and ease to use. Hence, this study aims to evaluate three chemical transfection methods of Lipofectamine™ 3000, CaP and PEI genome editing efficiency in HEK293T using p1400 and pGuideA78 plasmid through T7EI mutagenesis assay. However, the T7EI assay result of this experiment could not be determined due to the unspecific binding of AAVS1 primers during PCR, producing multiple bands that leads to the unresolved T7EI digestion products, preventing reliable assessment of genome editing activity. For future studies, the HPRT1 locus is recommended as an alternative target due to the well-known genomic instability in HEK293T causing the production of multiple AAVS1 bands. Thus, the use of the third sgRNA design targeting exon 2 of HPRT1 for 6-thioguanine metabolite selection knockout studies. It is suggested to use inverted fluorescence microscopy to visualize fluorescent cells for determining transfection efficiency in well-plates. It is suggested to perform viable cell counting post-transfection during cell harvesting to determine transfection reagent toxicity.

Keywords: p1400, pGuideA78, HEK293T, Lipofectamine™ 3000, CaP, PEI, T7EI, editing efficiency