Full Spectrum of In Vitro Disease Models with Optimized CRISPR/Cas9 Gene Editing in Difficult-to-Transfect Blood-Lineage Cells

Huanyu Jin, Zhongsheng Yu, Vladimir Pak, Yin Zhang, Padmaja Tummala, Pavithra Rajeswaran, Diana Nguyen, Monika Maleszewska, Jinling Li, Charles Cao, Ruby Yanru Chen-Tsai
AppliedStemCell, Inc., Milpitas, CA

INTRODUCTION

- CRISPR gene editing in cell lines, the workhorse of preclinical and biomedical research, enables the generation of unlimited in vitro models with precise gene modifications and advanced gene expression design that are physiologically relevant.
- CRISPR genome editing in certain types of cells, such as suspension cells and several blood lineage cell lines is very inefficient and problematic.
- Contributing factors include low transfection efficiency of CRISPR reagents, cytotoxicity, and low or undesired gRNA and Cas9 activities in the cell lines.
- In this poster, we describe techniques optimized for CRISPR genome editing in some difficult-to-transfect blood-lineage cell lines, the factors influencing efficiency such as cytotoxicity, and the types of modifications achieved (double gene knockouts, large fragment knock-in, point mutation).
- These results demonstrate that different cell lines may require different approaches or modified protocols to deliver CRISPR components for efficient and successful modifications of the targeted gene.

CRISPR/Cas9 Cell Line Engineering

Using a DNA-dependent protein kinase inhibitor to improve efficiency of HDR mediated sequence insertion:

A

- B

Using Cas9-RNP:

A

- B

Gene Knockout in Leukemia Cell Lines, KG-1 Cells

Using Cas9-plasmid:

A

- B

Double Gene Knockout in Jurkat Cell Lines

Using Cas9-plasmid:

A

- B

Large Fragment Deletion in Jurkat Cell Lines

Using Cas9-plasmid:

A

- B

Knock-in & Knockout in T2 Lymphoblastic Cell Line

Using DNA-based CRISPR reagents:

A

- B

CONCLUSION

- CRISPR/Cas9 is a powerful tool for engineering many different cell lines from several species.
- CRISPR/Cas9 enables the generation of knockout, knock-in and point mutation lines with precise genetic modifications.
- CRISPR efficiency can be limited by the type or cell line being engineered, especially difficult-to-transfect cell lines such as blood lineage cells.
- A variety of factors such as cell growth parameters, cell line sensitivity and cytotoxicity to transfected CRISPR reagents, can impact efficiency and reliability in genome editing.
- Optimizing protocols and transfection conditions for these blood lineage cells can contribute to higher cell survival, efficiency and success rate.
- Our data demonstrate that different cell lines may require different approaches or modified protocols to deliver CRISPR/Cas9 components for efficiency and success modifications of targeted genes.

www.appliedstemcell.com | 1-408-773-8007 | info@appliedstemcell.com

2018 AAACB