Highly efficient genome editing and cell engineering in stem cells using CRISPR/Cas9

Shantanu Kumar, Xiqian Liang, Xin Yu, Hillary Barbour, Yanfei Zou, Jason Potter, Jon Chesnut and Namratha Ravinder
Department of Synthetic Biology, Department of Cell Biology, Life Sciences Solutions, Thermo Fisher Scientific, 5791 Van Allen Way, Carlsbad, CA 92008

ABSTRACT

Genome editing in induced pluripotent stem cells (iPSCs) has been demonstrated to be highly effective for generating disease models for both monogenic and complex genetic disorders. For successful genome editing of iPSCs many factors need to be considered such as choice of growth media, genome editing tools, and nucleic acid delivery methods. Currently many genome editing tools require large quantities of RNA, involve tedious cloning steps and are relatively more toxic to iPSCs. To overcome these challenges we developed a highly purified transfection grade CRISPR/Cas9 ribonucleaseprotein (RNP) complex, which is highly efficient in genome editing with minimal toxicity. We also optimized a transfection condition to maximize delivery and genome editing in stem cells using CRISPR/Cas9 RNP complex. Discussed here are the results from different CRISPR/Cas9 formats tested across wide variety of cell types including stem cells. Using these formats we have edited mouse embryonic stem cells (ESCs) and human iPSCs with 80% to 60% genome cleavage efficiencies, respectively. The methods described here facilitate efficient disease model generation thereby accelerating research in the field of gene therapy and regenerative medicine.

INTRODUCTION

Genome editing in induced pluripotent stem cells (iPSCs) has been demonstrated to be highly effective for generating disease models for both monogenic and complex genetic disorders. For successful genome editing and downstream application of iPSCs, many factors need to be considered, such as choice of growth media, extracellular matrix, genome editing tools, and nucleic acid (NA) delivery methods. Here we have described feeder-free culture of stem cells and a genome editing protocol that can facilitate efficient disease model generation.

The clustered regularly interspaced short palindromic repeat (CRISPR) system from Streptococcus pyogenes has become a powerful technology for genome editing, and it can be used to rapidly generate engineered cell lines and model organisms. It is a simple system comprising a catalytic unit, Cas9, and a short noncoding guide RNA (gRNA) that targets specific sequence. It is an attractive route for genome engineering in a wide variety of hosts (1-3). We have developed various CRISPR/Cas9 formats that can be used to edit genomes in a wide variety of cell types, including stem cells (4). Using these formats we achieved greater than 50% target-specific DNA cleavage in mouse embryonic stem cells (ESCs) and human iPSCs, and ESCs (4). The methods described here show great potential as highly efficient gene editing tools in stem cells.

MATERIALS AND METHODS

1. Mouse ESCs culture and transfection

Mouse E14TG2a x 4 ESCs were cultured mouse inactivated embryonic fibroblasts (MEFs; strain ICR) in the presence of recombinant human interleukin-6 (IL-6) in mouse ESC medium. Before transfection, cells were adapted to feeder-free conditions and maintained on plates coated with attachment factor protein in mouse ESC-conditioned medium.

2. Transfection

Lipofectamine™ and RNAiMAX transfection complexes have been used to mediate delivery of CRISPR components into these cells. Transfection efficiency was determined using different protocols such as electroporation and transfection.

3. Human iPSCs and ESCs transfection

Option 1: Feeder-dependent adaptation of CRISPR/Cas9 for transfection

Feeder-dependent human embryonic stem cells (iPSCs) are cultured on MEF feeder cells (strain ICR) in human embryonic stem cell medium containing 20% KnockOut™-serum Replacement, 10 Mm MEM Non Essential Amino Acids Solution, 55 Mm L-glutamine and 4 Mm human IFG-2 recombinant protein in DMEM/F12. Cells were adapted to feeder-free conditions before electroporation and transfection.

Option 2: Culturing cells in feeder-free conditions in Essential Medium Cells harvested using TrypLE™ Express Enzyme by incubating 2–3 minutes in a 37 °C humidified incubator. Single-cell suspensions were prepared. For lipid-based transfection, a 24-well tissue culture plate coated with Gelat® matrix was used. Each well was seeded with approximately 5 x 10⁴ cells in 500µL of Essential Medium containing 10 Mm ROX antibiotic and allowed to recover overnight.

3. GeneArt® CRISPR/Cas9 Formats

A. All-in-one Plasmid

- Bacterial-based expression system
- No need for specific promoter
- Relatively low cost
- Easy to use
- Antibody against Cas9
- Complementary design
- Stable RNP complex

B. CRISPR/Cas9 (mRNA/mRNA + RNP)

- No need for specific promoter
- Requires specific sequence
- No antibody against Cas9
- Complementary design
- Stable RNP complex

C. CRISPR/Cas9 (mRNA + RNP)

- No need for specific promoter
- Requires specific sequence
- No antibody against Cas9
- Complementary design
- Stable RNP complex

D. CRISPR/Cas9 (mRNA)

- No need for specific promoter
- Requires specific sequence
- No antibody against Cas9
- Complementary design
- Stable RNP complex

E. CRISPR/Cas9 (RNP)

- No need for specific promoter
- Requires specific sequence
- No antibody against Cas9
- Complementary design
- Stable RNP complex

REFERENCES


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PRESENTERS CONTACT INFORMATION
shantanu.kumar@thermofisher.com