GENOME EDITING OF HUMAN PLURIPOTENT STEM CELLS

Using the ArciTect™ CRISPR-Cas9 System

The ease-of-use and versatility of CRISPR-Cas9 has revolutionized human embryonic stem (ES) and induced pluripotent stem (iPS) cell (collectively referred to as human pluripotent stem cell; hPSC) research. This technological advance has enabled gene knockout and introduction or correction of specific mutations in hPSCs to further understanding of how individual genes and/or genetic variants impact biology and disease pathogenesis. ArciTect™ is designed to fully support genome editing in hPSCs, providing you with a rapid, flexible, and precise system to modify the genome as you see fit. From cell culture and single-cell survival (CloneR™; Catalog #05888) to experimental design, detection, and validation of editing efficiency, our continuously expanding toolkit contains qualified solutions for every step in the hPSC genome editing workflow. Our optimized and validated protocol (Document #27084) is specifically designed to work seamlessly with ArciTect™ offerings to minimize troubleshooting and maximize experimental success.

Why Use ArciTect™?

CUSTOMIZABLE. Design crRNA to target your sequence of interest.

FLEXIBLE. Meet your specific genome editing needs with multiple variations of Cas9.

RAPID. Immediate activity of CRISPR-Cas9 RNP.

REDUCED OFF-TARGET EFFECTS. Minimize potential off-target cutting with timely degradation of the RNP complex.

COMPLETE WORKFLOW. Obtain everything you need to culture, edit, and clone hPSCs.

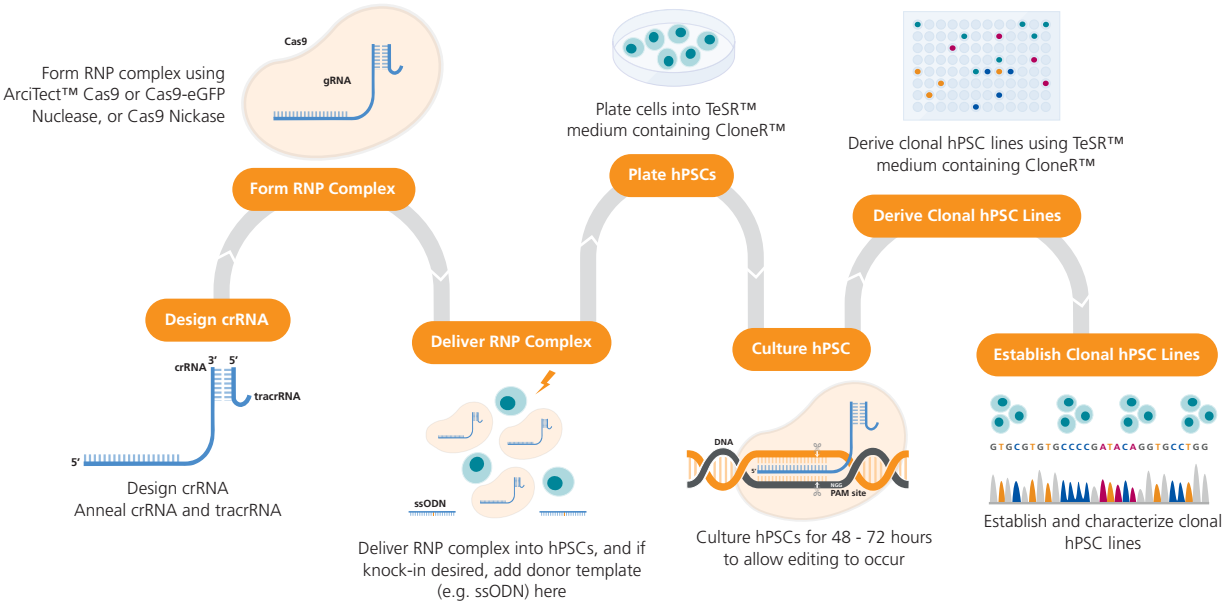

Figure 1. Experimental Workflow for Human Pluripotent Stem Cell (hPSC) Genome Editing

The guide RNA (gRNA) sequence is designed once a target locus for editing is identified. The ArciTect™ CRISPR-Cas9 ribonucleoprotein (RNP) complex is then prepared and delivered into hPSCs in single-cell suspension using electroporation with or without addition of a donor DNA template (e.g. single-stranded oligodeoxynucleotide, ssODN). Cells are then plated in hPSC maintenance medium (mTeSR™1 (Catalog #85850) or mTeSR™ Plus (Catalog #05825)) supplemented with CloneR™, to enhance survival of hPSCs plated as single cells. Editing efficiency can be analyzed after 48 - 72 hours using ArciTect™ T7 Endonuclease I Kit (Catalog #76021), via sequencing-based approaches, or by flow cytometry, if the experimental design permits. If generating clonal cell lines is desired, this can be accomplished by limited dilution cloning using mTeSR™1 or mTeSR™ Plus supplemented with CloneR™. Editing efficiency measurements can be used to inform the approximate number of clones for further characterization using sequencing-based approaches.

Comparison of Different CRISPR-Cas9 Methods[1]

Cas9	DNA	mRNA	Protein
Efficiency	+	++	+++
Specificity	+	++	+++
Degradation	> 72 hours	~ 72 hours	< 72 hours
Off-Target Cutting	High	Moderate	Low

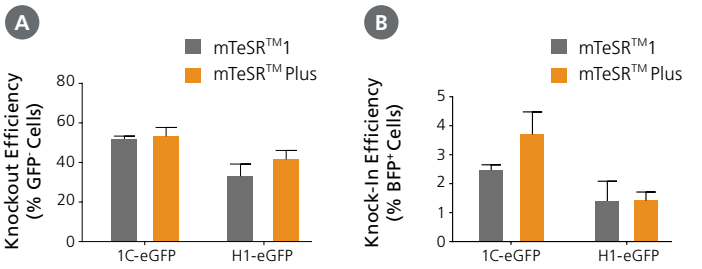

Figure 2. Efficient Genetic Knockout and Knock-In in hPSCs Using the ArciTect™ CRISPR-Cas9 System

H1-eGFP and 1C-eGFP hPSC lines were cultured in mTeSR™1 or adapted to mTeSR™ Plus hPSC maintenance medium for at least two passages prior to initial experiments. (A) Knockout (% GFP- cells) and (B) knock-in (% BFP+ cells) were measured by flow cytometry 72 hours after electroporation with ArciTect™ RNP complexes; n = 3. No significant differences were observed within cell lines.

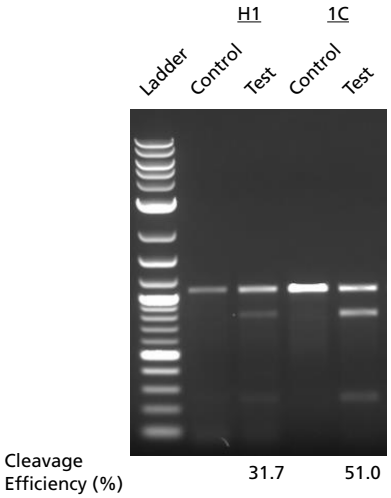

Figure 3. INDEL Detection by T7 Endonuclease I Assay

H1 ES cells or WLS-1C iPS cells were edited using ArciTect™ Human HPRT Positive Control Kit and INDEL formation (percent [%] cleavage efficiency) was assessed using ArciTect™ T7 Endonuclease I Kit. Control: Non-transfected cells; Test: HPRT-edited.

For complete instructions, refer to Technical Bulletin: Genome Editing of Human Pluripotent Stem Cells (Document #27084).

Reduce Off-Target Effects

The ArciTect™ product family is a ribonucleoprotein (RNP)-based Cas9 genome editing system. Unlike previous CRISPR technologies that utilize plasmid or mRNA-based systems, the ArciTect™ system results in timely degradation of the RNP complex, minimizing cleavage of off-target regions.

Product Information

Product	Size	Catalog #
ArciTect™ Cas9 Nuclease	100 µg 300 µg	76002 76004
ArciTect™ Cas9-eGFP Nuclease	100 µg	76006
ArciTect™ Cas9 Nickase	100 µg	76009
ArciTect™ crRNA	2 nmol 10 nmol 20 nmol	76010 76011 76012
ArciTect™ tracrRNA Kit	5 nmol Kit 10 nmol Kit 20 nmol Kit	76017 76018 76019
ArciTect™ Annealing Buffer (5X)	1 mL	76020
ArciTect™ Human HPRT Positive Control Kit	1 Kit	76013
ArciTect™ T7 Endonuclease I Kit	25 Reactions 125 Reactions	76021 76022
ArciTect™ High-Fidelity DNA Polymerase Kit	500 Reactions	76026

Reference

1. Liang X et al. (2015) Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection. J Biotechnol. 208: 44–53.