

GENOME EDITING OF HUMAN PLURIPOTENT STEM CELLS (hPSCs)

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.** Multiple variations of Cas9 to suit your specific genome editing needs.
- RAPID.** No need for transcription and translation.
- REDUCED OFF-TARGET EFFECTS.** Timely degradation of the RNP complex to minimize potential off-target cutting.

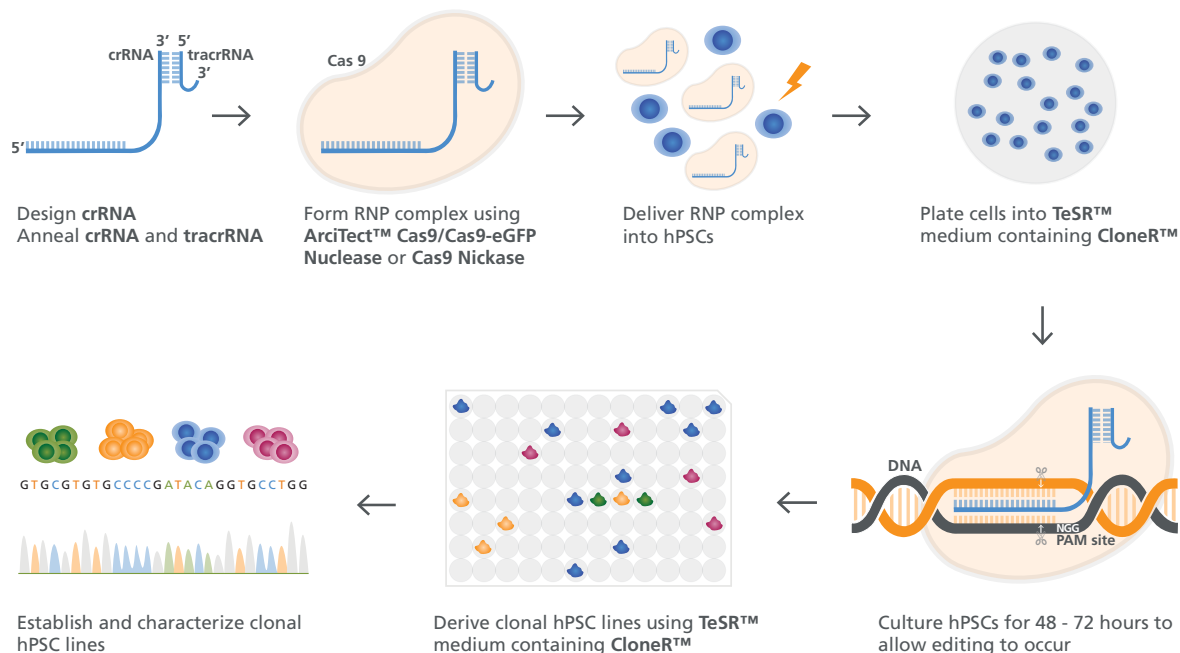


Figure 1. Genome Editing Workflow for hPSCs

The guide RNA (gRNA) complex, consisting of a crRNA and tracrRNA, can be customized to target the desired genomic region of interest. Next, the ribonucleoprotein (RNP) complex, consisting of the gRNA complex and Cas9 protein (Cas9/Cas9-eGFP nuclease or Cas9 nickase), is formed and delivered into hPSCs by electroporation or chemical-based transfection. The hPSCs are then cultured in TeSR™ medium (e.g. mTeSR™1, Catalog #85850) containing CloneR™ (Catalog #05888), to increase cloning efficiency and single-cell survival, for 48 - 72 hours to allow editing to occur. Following editing, clonal hPSC lines can be established and characterized for downstream applications.

GENOME EDITING OF HUMAN PLURIPOTENT STEM CELLS (hPSCs)

Table 1. Comparison of Different CRISPR-Cas9 Methods¹

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

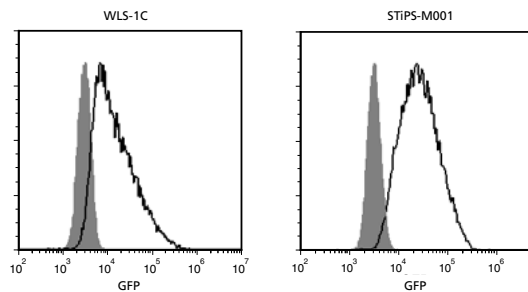


Figure 2. Cas9-eGFP Detection by Flow Cytometry

WLS-1C (left) or MTIPS-M001 (right) iPS cells were transfected with Cas9-eGFP RNP complex; eGFP was detected by flow cytometry 24 hours after transfection. Filled histogram: Non-transfected control; Solid line histogram: Cas9-eGFP-transfected cells.

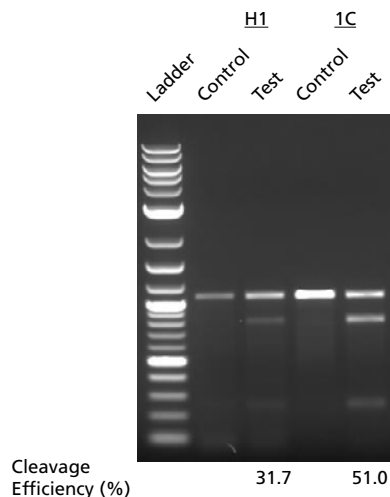


Figure 3. INDEL Detection by T7 Endonuclease I Assay

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.

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	50 µg	76001
	100 µg	76002
	300 µg	76004
ArciTect™ Cas9-eGFP Nuclease	50 µg	76005
	100 µg	76006
ArciTect™ Cas9 Nickase	10 µg	76007
	50 µg	76008
	100 µg	76009
ArciTect™ crRNA	2 nmol	76010
	10 nmol	76011
	20 nmol	76012
ArciTect™ tracrRNA Kit	5 nmol Kit	76017
	10 nmol Kit	76018
	20 nmol Kit	76019
ArciTect™ Annealing Buffer (5X)	1 mL	76020
ArciTect™ Human HPRT Positive Control Kit	1 Kit	76013
ArciTect™ T7 Endonuclease I Kit	25 Reactions	76021
	125 Reactions	76022

Reference

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

Copyright © 2018 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, CloneR, and ArciTect are trademarks of STEMCELL Technologies Canada Inc. TeSR is a trademark of WARF. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.