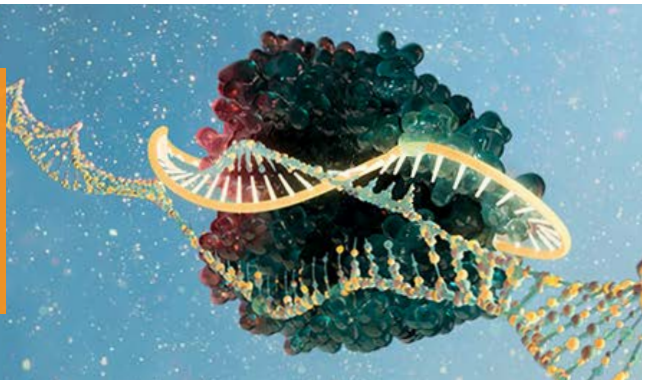
CUT UNCERTAINTY OUT OF GENOME EDITING

Using the ArciTect™ CRISPR-Cas9 System



CRISPR-Cas9, an RNA-guided genome editing technology, is revolutionizing cell biology due to the ease and efficiency by which it enables genetic manipulation of mammalian cells.

The ArciTect™ product family is a ribonucleoprotein (RNP)-based CRISPR-Cas9 genome editing system. With validated reagents and protocols, the ArciTect™ system enables you to perform high-efficiency genome editing and generate functional gene-edited cells. The RNP complex is composed of the purified Cas9 protein and custom synthetic guide RNA (gRNA) that can be efficiently delivered into cells using chemical transfection or electroporation. Once inside the cell, RNP complexes will not induce the cellular immune response and experience timely degradation to limit off-target editing (Table 1).

To learn more, visit **www.stemcell.com/ArciTect**.

Why Use ArciTect™?

EFFICIENT. Maximize delivery and expression in difficult-to-manipulate cell types by using RNP complexes.

EASY TO USE. Simplify genome editing with an integrated guide RNA design tool and cell-type-specific protocols.

RAPID. Get to your results faster with ready-to-use purified Cas9 proteins and synthetic guide RNAs.

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

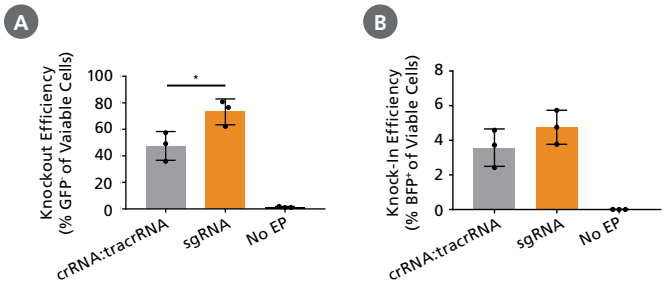


Figure 1. Efficient Genetic Knockout and Knock-In in Human Pluripotent Stem Cells Using the ArciTect™ CRISPR-Cas9 System

1C-eGFP hPSC lines were cultured in mTeSR™1 (Catalog #85850) supplemented with CloneR™ (#05888) for 24 hours after electroporation with CRISPR-Cas9 RNP complexes containing ArciTect™ Cas9 Nuclease and either ArciTect™ crRNA:tracrRNA duplexes or sgRNA targeting GFP, co-delivered with ssODN encoding nucleotides to convert GFP to BFP. (A) Knockout (% GFP^- cells) and (B) knock-in (% BFP^+ cells) efficiency were measured by flow cytometry 3 days after electroporation; n = 3. Control samples were not electroporated (no EP). Error bars represent standard deviation.

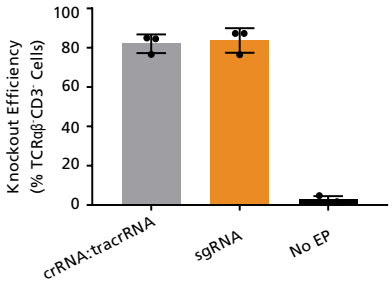


Figure 2. Efficient TRAC Knockout in Human Primary T Cells Using the ArciTect™ CRISPR-Cas9 System

Human T cells were activated with ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) for 3 days and the cells were electroporated with ArciTect™ RNP-complexes containing either ArciTect™ crRNA:tracrRNA duplexes or sgRNA targeting the T cell receptor (TCR) alpha constant (TRAC) locus. Knockout efficiency was assessed 3 days after electroporation by flow cytometry analysis of TCRαβ and CD3 expression; n = 3 donors. Control samples were not electroporated (no EP). Error bars represent standard deviation.

Table 1. 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

Build Your ArciTect™ CRISPR-Cas9 System

Purified Cas9 Proteins

Choose from a variety of purified Cas9 proteins. All versions of Cas9 contain a nuclear localization sequence for rapid translocation into the nucleus. Cas9 can be complexed with guide RNA to form an RNP complex.

Product	Description	Size	Catalog #
ArciTect™ Cas9 Nuclease	Cas9 nuclease for the generation of double-strand breaks	100 µg 300 µg	76002 76004

T7 Endonuclease I Assay Reagents

Estimate CRISPR-Cas9 editing efficiency in your experiments.

Product	Description	Size	Catalog #
ArciTect™ T7 Endonuclease I Kit	Rapidly determine CRISPR-Cas9 genome editing efficiency	25 Reactions 50 Reactions	76021 76022
ArciTect™ High-Fidelity DNA Polymerase Kit	High-fidelity polymerase for ultra-low error rates in PCR amplification	1 Kit	76026

Guide RNA (gRNA)

Custom design the gRNA to target your genomic sequence of interest. The gRNA is composed of a CRISPR RNA (crRNA) and tracrRNA annealed as a crRNA:tracrRNA duplex, or a single guide RNA (sgRNA). The first three 5′ and 3′ terminal residues of either ArciTect™ gRNA format contain 2′-O-methyl and phosphorothioate modifications to improve stability and increase editing efficiency.

Product	Description	Size	Catalog #
ArciTect™ sgRNA	Custom-designed sgRNA containing the crRNA and tracrRNA regions within a single molecule	4 nmol	200-0013
ArciTect™ crRNA*	Custom-designed crRNA for gRNA generation by complexing with tracrRNA	2 nmol 10 nmol 20 nmol	76010 76011 76012
ArciTect™ tracrRNA Kit*	Trans-activating crRNA for gRNA generation	5 nmol 10 nmol 20 nmol	76017 76018 76019

**ArciTect™ crRNA and ArciTect™ tracrRNA Kit are required together.*

Positive Control Kit

Optimize transfection protocols using our positive control kit, complete with HPRT-targeting gRNA and primers for PCR amplification prior to the T7 Endonuclease I Assay.

Product	Description	Size	Catalog #
ArciTect™ Human HPRT Positive Control Kit	Positive control for CRISPR-Cas9 genome editing	1 Kit	76013

References

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