Gene Editing with MAD7™ in mammalian cells Quick Start Guide

STEP		DESCRIPTION
1	Get the MAD7 sequence	Download the DNA sequence at inscripta.com/MAD7
2	Optimize the sequence	The MAD7 nucleotide sequence provided is codon-optimized for expression in *E. coli*. Tools for codon and/or sequence optimization are widely available (e.g., GenScript, IDT, or Thermo Fisher Scientific). Nuclear localization signal(s) and/or epitope tag(s) can also be added to the vector in-frame with MAD7 at the N- or C-terminus.
3	Synthesize the MAD7 gene	Use your favorite vendor; GenScript, IDT and Thermo Fisher Scientific have been successfully used.
4	Clone MAD7 into an expression vector	We recommend using a vector containing a Pol II promoter (e.g., CMV, Ef1α) that has optimal activity in your specific cell type or cell line. Higher CRISPR nuclease expression commonly results in greater gene editing efficiency. A selectable marker such as antibioticresistance or fluorescent reporter gene can also be added to the vector.
5	Design and synthesize guide RNA (gRNA)	• Obtain the genomic DNA sequence surrounding the desired edit(s) • Identify PAM sequences (5′-YTTN-3′) near the desired edit site(s) • Choose the first 21 nucleotides directly adjacent to the 3′ end of the PAM; this is the gene-targeting spacer region (5′-NNNNNNNNNNNNNNNNNNNN-3′) • Append the constant repeat region (5′-GTCAAAAGACCTTTGGAATTTCTACTCTTG-TAGAT-3′) to the 5′ end of the spacer region Note: A shorter repeat region (5′-GGAATTTCTACTCTTGTAGAT-3′) is also functional for indel formation using chemically synthesized gRNA • gRNAs can be synthesized as DNA for cloning or as RNA for direct delivery into cells • The overall gRNA design is as follows: 5′- GTCAAAAGACCTTTGGAATTTCTACTCTTGTAGATNNNNNNNNNNNNNNNNNNNN-3′
6	Clone gRNA into an expression vector	The gRNAs can be cloned into an expression vector and expressed using a Pol III promoter (e.g., U6, H1); this can be in the same vector that is expressing MAD7 or in a separate vector.
7	Deliver MAD7 and gRNA into mammalian cells	If MAD7 and gRNA are encoded by different vectors, they can be co-transfected or co-electroporated into cells. If MAD7 and gRNA are in the same vector, simply transfect or electroporate the vector into your cells. Additionally, a MAD7-expressing vector can also be co-delivered with synthetic gRNA. Perform gene editing experiments as desired.

Summary Note:
Protein and gRNA expression are often species dependent. Use best practices for your particular organism to clone and express MAD7 and associated gRNA under conditions expected to produce a functional nuclease system. Such practices typically require design and/or evaluation of features including specific vectors, origins, codon usages, and/or promoters.