A Quick Start Guide to using MAD7

STEP		DESCRIPTION
1	**Get the MAD7 sequence**	Download the DNA sequence at www.inscripta.com/madzymes.
2	**Optimize the sequence**	The MAD7 sequence is codon-optimized for expression in *E. coli.* Tools for codon and/or sequence optimization are widely available (e.g., Thermo Fisher Scientific or IDT®).
3	**Synthesize the MAD7 gene**	Use your favorite vendor - we have successfully used Invitrogen™ GeneArt™ (Thermo Fisher Scientific) or IDT® in the past.
4	**Clone MAD7 into an expression vector**	We recommend starting with inducible and/or low-level constitutive promoters depending on your host organism, although it is possible that a range of expression levels may need to be explored to identify the right conditions for a particular application.
5	**Design and synthesize gRNA**	Each crRNA contains a 5′-GGAATTTCTACTCTTGTAGAT sequence and should be designed to target 5′-YTTN-3′ PAMs using the first 21 nucleotides directly adjacent to the 3′ side of the PAM. The overall gRNA design is as follows: 5′-GGAATTTCTACTCTTGTAGAT NNNNNNNNNNNNNNNNNNNN-3′ The following crRNA repeat sequences are also compatible with MAD7 editing: 1. GGAATTTCTACTaTTGTAGAT 2. GGAATTTCTACTgTTGTAGAT 3. GGAATTTCTACTtTTGTAGAT 4. GGAATTTCTACTagTGTAGAT
6	**Clone gRNA into expression vector**	Consider expressing gRNAs under a strong promoter and in medium- to high-copy vectors such as pBR322 or puc19 for optimal expression and nuclease activity. Make sure all vectors (from step 4 and 6) are compatible. It is also possible to use a single vector to express both MAD7 and the gRNA.
7	**Transform MAD7 and gRNA vectors into species of interest**	If MAD7 and gRNA are contained on different vectors, consider first transforming with the MAD7 vector and confirming uptake of the plasmid. Next, transform the gRNA vector into the MAD7 competent cells and begin experiments. If MAD7 and gRNA are on the same vector, simply transform the vector into your cells of interest and perform cutting/editing experiments as desired.

Summary Note: Expression of proteins and gRNA is often species dependent. Best practices for your particular organism should be used 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 specific vectors, origins, codon usages, and/or promoters, among other features.