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Quick Start Guide: Gene editing with MAD7 in yeast

STEP		DESCRIPTION
1	**Get the MAD7 sequence**	Download the DNA sequence at inscripta.com/madzymes.
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-terminus.
3	**Synthesize the MAD7 gene**	Use your favorite vendor; GenScript, IDT and Thermo Fisher Scientific have been successfuly used.
4	**Clone MAD7 into an expression vector**	We recommend using a vector and Pol II promoter system that has optimal activity in your specific strain. Matching a 2-µM vector with a low-expression promoter for the CRISPR nucleases commonly results in greater gene editing efficiency. Antibiotic resistance (e.g., KanMx), Auxotrophic markers (e.g., Ura3), or other indicators can be used for selection.
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′-GTCTGGCCCCAAATTCTAATTTCTACTGTTG-TAGAT-3′) to the 5′ end of the spacer region • The overall gRNA design is as follows: 5′-GTCTGGCCCCAAATTCTAATTTCTACTGTTGTAGATNNNNNNNNNNNNNNNNNNNN-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., SNR52 or pRPR1); this can be in the same vector that is expressing MAD7 or in a separate vector.
7	**Deliver MAD7 and gRNA into yeast**	If MAD7 and gRNA are encoded by different vectors, they can be sequentially (MAD7 and then gRNA) or simultaneously transformed into cells. If MAD7 and gRNA are in the same vector, simply transform the vector into your cells. Perform gene editing experiments as desired. *Note: A DNA donor template is required if performing precise editing. DNA donors can be chemically synthesized single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) or cloned into the vector expressing the gRNA. The length of the 5′ and 3′ homology arms will depend on the length of the desired precise edit and will need to be optimized for your system.*

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.