CRISPR-MAD7 activity in mammalian cells

Introduction

MAD7™ is a Class 2 Type V CRISPR-Cas system utilizing a T-rich PAM sequence (YTTN). Gene editing using MAD7 has been demonstrated in both E. coli and yeast organisms, and now, in mammalian cells. Here we confirm that MAD7 expressed in human HEK293T cells and combined with a chemically synthesized guide RNA (gRNA; ten per gene) targeting PPIB or DNMT3B genes results in high-efficiency cleavage in an in vitro cutting assay. When MAD7 expression vectors are co-transfected with synthetic gRNA in the same cell line, indel formation is detected using a DNA mismatch detection assay demonstrating that this novel nuclease is fully functional in mammalian gene editing experiments.

Workflow for MAD7 in vitro cutting activity in mammalian cells

Figure 1. To determine if MAD7 expressed in mammalian cells might perform comparable to observations in bacterial and yeast systems, a MAD7 expression vector was transfected into HEK293T cells using DharmaFECT Duo transfection reagent (Dharmacon cat #T-2010-01). After 48 hours, cell lysates were harvested and used in an in vitro cutting assay testing ten (per gene target) chemically synthesized guide RNAs (gRNAs; Dharmacon) targeting amplicons prepared from genomic DNA for the mammalian PPIB and DNMT3B genes.

Figure 2. Following the workflow outlined in Figure 1, efficient in vitro cutting was observed with 8/10 and 9/10 gRNAs for PPIB and DNMT3B, respectively. The data demonstrate that MAD7 is expressed and folds correctly to form a functioning nuclease that cleaves DNA in a reconstituted system. SpyCas9 = Streptococcus pyogenes Cas9; ND = not detected.
Workflow for MAD7 in vivo gene editing activity in mammalian cells

**Figure 3.** To confirm that MAD7 performs effectively in a mammalian system, a MAD7 expression vector (200 ng) was co-transfected with synthetic gRNA (25 nM) using DharmaFECT Duo transfection reagent (Dharmacon cat #T-2010-01) in a 96-well format. Cell lysate was harvested 72 hours post-transfection, and indel detection was performed using primers from the Edit-R PPIB crRNA Control Kit (Dharmacon cat #UK-007050-01-05) and Edit-R DNMT3B crRNA Control Kit (Dharmacon cat #UK-007060-01) in a T7EI mismatch detection assay. M = marker; UT = untreated.

**Figure 4.** The T7EI mismatch detection assay workflow detailed in Figure 3 was followed to test in vivo performance of MAD7 in mammalian cells. Panel A shows the gels used to estimate the percentage of indels formed. Two and three gRNAs targeting PPIB and DNMT3B, respectively, showed efficient editing, confirming activity of the MAD7 nuclease in mammalian cells. The data is graphed in Panel B. ErMAD7 = Eubacterium rectale MAD7; SpyCas9 = Streptococcus pyogenes Cas9; AsCpf1 = Acidaminococcus sp. Cas12a; SNP = this band occurs in the negative control and is not related to CRISPR-mediated activity, likely representing a SNP; NTC = non-targeting control; cr:tracr = synthetic crRNA and tracrRNA for SpyCas9; ND = not detected.

**Conclusion**
- MAD7 can be expressed in mammalian cells; high-efficiency cleavage of DNA in a reconstituted system strongly suggests that the nuclease folds correctly in the mammalian cell milieu to enable proper enzyme activity
- Multiple crRNAs can be designed for efficient cleavage both in vitro and in cells
- MAD7 effectively causes targeted indel formation in mammalian cells

Download the MADzyme™ DNA sequence

at INSCRIPTA.COM/MAD7