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

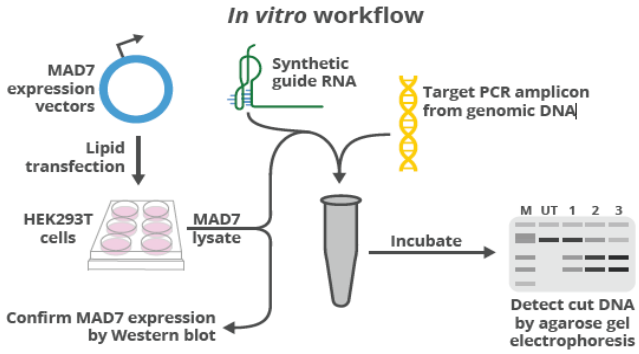

Figure 1. To determine if MAD7 expressed in mammalian cells might perform comparable to observations in bacterial and yeast systems, a MAD7 expression vector was transfected into HEK293T cells using DharmaFECT Duo transfection reagent (Dharmacon cat #T-2010-01). After 48 hours, cell lysates were harvested 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.

MAD7 shows high-efficiency cutting in mammalian cells

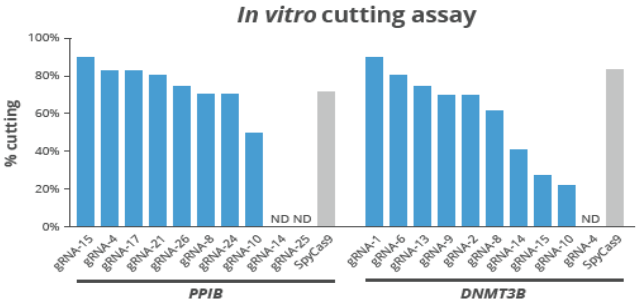

Figure 2. Following the workflow outlined in Figure 1, efficient *in vitro* cutting was observed with 8/10 and 9/10 gRNAs for *PPIB* and *DNMT3B*, respectively. The data demonstrate that MAD7 is expressed and folds correctly to form a functioning nuclease that cleaves DNA in a reconstituted system. SpyCas9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

Workflow for MAD7 *in vivo* gene editing activity in mammalian cells

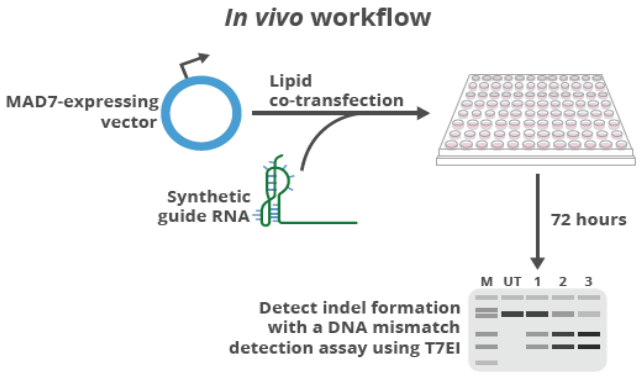

Figure 3. To confirm that MAD7 performs effectively in a mammalian system, a MAD7 expression vector (200 ng) was co-transfected with synthetic gRNA (25 nM) using DharmaFECT Duo transfection reagent (Dharmacon cat #T-2010-01) in a 96-well format. Cell lysate was harvested 72 hours post-transfection, and 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.

MAD7 successfully forms indels in mammalian cells

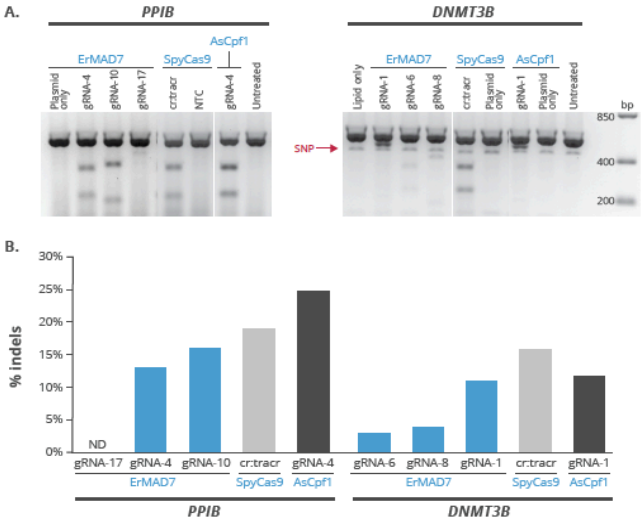

Figure 4. TThe T7EI mismatch detection assay workflow detailed in Figure 3 was followed to test *in vivo* performance of MAD7 in mammalian cells. Panel A shows the gels used to estimate the percentage of indels formed. Two and three gRNAs targeting *PPIB* and *DNMT3B*, respectively, showed efficient 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

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