Upper lane 2-9: Linearized plasmid DNA cleavage assay using Cas9$_{HNH1}$ variants with enhanced (eSpCas9(1.1)) specificity, with a time range from 0 to 120min, resolved by agarose gel.

Upper lane 10-17: Linearized plasmid DNA cleavage assay containing 1 bp PAM-distal end mismatches between DNA and sgRNA using Cas9$_{HNH1}$ variants with enhanced (eSpCas9(1.1)) specificity, with a time range from 0 to 120min, resolved by agarose gel.

Lower lane 2-9: Linearized plasmid DNA cleavage assay containing 2 bp PAM-distal end mismatches between DNA and sgRNA using Cas9$_{HNH1}$ variants with enhanced (eSpCas9(1.1)) specificity, with a time range from 0 to 120min, resolved by agarose gel.

Lane 2-9: Linearized plasmid DNA cleavage assay containing 3 bp PAM-distal end mismatches between DNA and sgRNA using Cas9$_{HNH1}$ variants with enhanced (eSpCas9(1.1)) specificity, with a time range from 0 to 120min, resolved by agarose gel.

Lane 10-17: Linearized plasmid DNA cleavage assay containing 4 bp PAM-distal end mismatches between DNA and sgRNA using Cas9$_{HNH1}$ variants with enhanced (eSpCas9(1.1)) specificity, with a time range from 0 to 120min, resolved by agarose gel.
Lane 2-9: Linearized plasmid DNA cleavage assay using Cas9\textsubscript{HNH1} variants with reduced (Cas9(L847R)) specificity, with a time range from 0 to 32min, resolved by agarose gel.
Lane 10-17: Linearized plasmid DNA cleavage assay containing 1 bp PAM-distal end mismatches between DNA and sgRNA using Cas9\textsubscript{HNH1} variants with reduced (Cas9(L847R)) specificity, with a time range from 0 to 32min, resolved by agarose gel.

Lane 2-9: Linearized plasmid DNA cleavage assay containing 2 bp PAM-distal end mismatches between DNA and sgRNA using Cas9\textsubscript{HNH1} variants with reduced (Cas9(L847R)) specificity, with a time range from 0 to 60min, resolved by agarose gel.
Lane 10-17: Linearized plasmid DNA cleavage assay containing 3 bp PAM-distal end mismatches between DNA and sgRNA using Cas9\textsubscript{HNH1} variants with reduced (Cas9(L847R)) specificity, with a time range from 0 to 90min, resolved by agarose gel.
Lane 2-9: Linearized plasmid DNA cleavage assay containing 4 bp PAM-distal end mismatches between DNA and sgRNA using Cas9\textsubscript{\textsc{Hnh1}} variants with reduced (Cas9(L847R)) specificity, with a time range from 0 to 180 min, resolved by agarose gel.