1. CRISPR

   This is the streamlined protocol we ended up using from NEB once we had our sgRNA (20 base pairs) made by IDT and our DNA (ZZ) synthesized by IDT

   FROM New England Biolabs

   Cas9 nuclease, *S. pyogenes*, complexed with an sgRNA

   .Cas9 Nuclease, *S. pyogenes*, is an RNA-guided endonuclease that catalyzes site-specific cleavage of double stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif) (1). The PAM sequence, NGG, must follow the targeted region on the opposite strand of the DNA with respect to the region complementary sgRNA sequence.

1. Assemble the reaction at room temperature in the following order:
   a. 20ul of Nuclease Free Water
   b. 3ul of 10x Cas9 Nuclease Reaction buffer
   c. 3ul of 300nM sgRNA
   d. 1ul of 1uM Cas 9 Nuclease, *S. pyogenes*
2. Incubate at 25C for 10min, then add
   a. 3ul of 30nM substrate DNA (with the zz mutation)
3. Mix thoroughly and pulse-spin in a microfuge.
4. Incubate at 37°C for 15 minutes.
5. Add 1 µl of Proteinase K to each sample.
6. Mix thoroughly andpulse-spin in a microfuge.
7. Incubate at room temperature for 10 minutes.
8. Proceed with fragment analysis.

References:

1. Jinek et al. (2012) *Science* 337 (6096) 816-821.
2. Larson et al. (2013) *Nature Protocol* 8 (2180-2196).
3. Mali et al. (2013) *Science* 339 (6121): 823-826.