FAQs for Cas9 Nuclease, *S. pyogenes*

1. Why do I observe incomplete digestion?
   - Incomplete digestion may be caused by an incorrect ratio of Cas9 Nuclease, sgRNA, and target site. For complete digestion we recommend a 10:10:1 molar ratio of Cas9 Nuclease:sgRNA:target site.
   - Incomplete digestion may be caused by a suboptimal sequence of the sgRNA. Verify the sequence and design of the sgRNA transcription template.
   - Incomplete digestion may be caused by poor quality sgRNA. Verify the integrity of the sgRNA by gel electrophoresis.
   - Incomplete digestion may be caused by using suboptimal buffer. Please use the 10X Cas9 Nuclease Buffer included with the enzyme.

2. Why does digestion efficiency differ between two SgRNAs?
   - Digestion efficiency may be influenced by sgRNA design. Verify the sequence and design of the sgRNA transcription template.
   - Digestion efficiency may be influenced by sgRNA quality. Verify the integrity of the sgRNA by gel electrophoresis.

3. Does NEB provide plasmids for gRNA cloning?
   We do not distribute plasmids for sgRNA cloning, but we recommend that you visit Addgene if you wish to obtain sgRNA plasmids. Alternatively, oligonucleotide templates encoding sgRNA transcribed by a T7 promoter can be used in conjunction with an in vitro transcription kit such as the NEB T7 Quick High Yield RNA Synthesis Kit.