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| --- | --- |
| sgRNA | UUGCACCUACCUUCUGGAUGUACGUCAUAGUUCCCCUGAGAAAUCAGGGUUACUAUGAUAAGGGCUUUCUGCCUAAGGCAGACUGACCCGCGGCGUUGGGGAUCGCCUGUCGCCCGCUUUUGGCGGGCAUUCCCCAUCCUU |
| On-target DNA | CAAAGAGCTCCTCGTCCAGTGGGAAGAGAGCTGATCTCATTTGTAAGGAATACCCTCTTCATCCCCCACCCTTGCCATTGATCTATTCATTCCATCTCCATGACAACAGGAAGAGAGGGCCCGGCGTGAGGAGGAGGAGAACAGGAGGAAGGCTGAGGATGAGGCCCGGAAGAAGAAGGCTCTGTCCAACATGATGCACTTTGGAGGGTACATCCAGAAGGTAGGTGCAAAGCAGCATCGGGCACCAGGACACCCCAGTGTATCCTCAAGGCCGCCTTTGCTTGGATCCATGAAGAAATTCCCAACTGCTGGTGGCTGAAGTCTAAGGTCTGCTCATGTCTAGCCCCTGAGCTGTCTATCAGCCTGACCATGGTTCAGTAGGAGGGCTCTGCTGTGTGTGACAGTTAGAACACTAATATGTCTCCAAATTCTGGCTCCCCAAAGGGACAACTGGGAGAATCTTGGGTCCTGGAGTCCAT |
| Off-target DNAPAM proximal mismatch (5-6 bp AT 🡪 CA) | CAAAGAGCTCCTCGTCCAGTGGGAAGAGAGCTGATCTCATTTGTAAGGAATACCCTCTTCATCCCCCACCCTTGCCATTGATCTATTCATTCCATCTCCATGACAACAGGAAGAGAGGGCCCGGCGTGAGGAGGAGGAGAACAGGAGGAAGGCTGAGGATGAGGCCCGGAAGAAGAAGGCTCTGTCCAACATGATGCACTTTGGAGGGTACcaCCAGAAGGTAGGTGCAAAGCAGCATCGGGCACCAGGACACCCCAGTGTATCCTCAAGGCCGCCTTTGCTTGGATCCATGAAGAAATTCCCAACTGCTGGTGGCTGAAGTCTAAGGTCTGCTCATGTCTAGCCCCTGAGCTGTCTATCAGCCTGACCATGGTTCAGTAGGAGGGCTCTGCTGTGTGTGACAGTTAGAACACTAATATGTCTCCAAATTCTGGCTCCCCAAAGGGACAACTGGGAGAATCTTGGGTCCTGGAGTCCAT |
| Off-target DNAPAM distal mismatch (19-20 bp TG 🡪 CA) | CAAAGAGCTCCTCGTCCAGTGGGAAGAGAGCTGATCTCATTTGTAAGGAATACCCTCTTCATCCCCCACCCTTGCCATTGATCTATTCATTCCATCTCCATGACAACAGGAAGAGAGGGCCCGGCGTGAGGAGGAGGAGAACAGGAGGAAGGCTGAGGATGAGGCCCGGAAGAAGAAGGCTCTGTCCAACATGATGCACTTTGGAGGGTACATCCAGAAGGTAGGcaCAAAGCAGCATCGGGCACCAGGACACCCCAGTGTATCCTCAAGGCCGCCTTTGCTTGGATCCATGAAGAAATTCCCAACTGCTGGTGGCTGAAGTCTAAGGTCTGCTCATGTCTAGCCCCTGAGCTGTCTATCAGCCTGACCATGGTTCAGTAGGAGGGCTCTGCTGTGTGTGACAGTTAGAACACTAATATGTCTCCAAATTCTGGCTCCCCAAAGGGACAACTGGGAGAATCTTGGGTCCTGGAGTCCAT |

**Supplementary File 3.** Nucleic acidsequences used in the *Geo*Cas9 *in vitro* off-target assay. The23 base pair spacer sequence of gRNA is underlined. The spacer sequence within the DNA sequences is highlighted yellow, and the PAM are highlighted in blue.