

# Choice of gRNAs for the Cas9 Orthologs

**S. pyogenes Cas9 (SP):** Find all 23bp genomic sites of the form 5'-NNNNNNNNNNNNNNNNNNNG-3' near your intended target site (ideally  $\pm 50$ bp). These may reside on the + or - strand. To create a gRNA expression fragment, incorporate 20bp of the protospacer sequence adjacent to the PAM site into the DNA fragment (**U6 promoter\_gRNA spacer+scaffold+terminator**) as indicated below:

>gRNA\_variant-SPm

TGTACAAAAAGCAGGCTTAAAGGAACCAATTAGTCGACTGGATCCGGTACCA  
AGGTCGGGCAGGAAGAGGGCTTATTTCCATGATTCTCATATTGCAATACGG  
ATACAAGGGTGTAGAGGAGATAATTAGAATAATTGACTGTAAACACAAAAGATA  
TTAGTACAAAATACGTGACGTAGAAAAGTAATAATTCTTGGTAGTTGCAGTT  
TAAAATTATGTTTTAAATGGATATCATATGCTTACCGTAACTGTAAAAGTATT  
CGATTTCTGGCTTATATATCTTGGAAGGACGAAACACCCNNNNNNNNNNNN  
**NNNNNNNNNGTTTAGAGCTAGAAATAGCAAGTAAATAAGGCTAGTCGGTTAT**  
**CAACTTGGCAACCGAGTCGGTGT**TTTTTT

**S. thermophilus CRISPR #1 Cas9 (ST1):** Find all 27bp genomic sites of the form 5'-NNNNNNNNNNNNNNNNNNNNNNNNNNNAGAAW-3' near your intended target site (ideally  $\pm 50$ bp). These may reside on the + or - strand. To create a gRNA expression fragment, incorporate 20bp of the protospacer sequence adjacent to the PAM site into either of the two DNA fragments as indicated below (**U6 promoter\_gRNA spacer+scaffold +terminator**):

>gRNA\_variant-ST1f1

TGTACAAAAAAGCAGGTTAAAGGAACCAATTCACTGACTGGATCCGGTACCAAGGTC  
GGCAGGAAGAGGGCTATTCCTCATGATTCTCATATTCGCATATACTGATACAAGGCT  
GTTAGAGAGATAATTAGAATTAAATTGACTGTAAACACAAAGATATTGATACAAAATAC  
TGACGTAGAAAGTAATAATTCTGGTAGTTGCAGTTAAATTATGTTTAAATC  
GACTATCATATGCTAACCGTAACCTGGAAAGATTTCGATTCTGGCTTATATATCTTC  
TGGAAAGGAGCAAACACCGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  
**AAGTAACGTACAACGAAACTTACACAGTTACTTAAATCTGCAGAACGCTACAAAGATA**  
**GGCTTCATGCCGAATCACACCCCTGTCAATTATGGCAGGGTGTTTTTT**

**Note:** Both gRNA variants show similar activity levels, hence choice of one versus the other will depend on other user constraints such as limitations on size of delivery vector etc.

>gRNA\_variant-ST1m1

TGTACAAAAAAGCAGGTTAAAGGAACCAATTCACTGACT  
GGATCCGGTACCAAGTCGGCAGGAAGAGGGCTATTCTCC  
ATGATTCCTCATATTGCATATACAGATAACAGCTGTTAGA  
GAGATAATTAGAATTATTCAGTGTAAACACAAGATTATA  
GTACAAAATACGTGACCTGAGAAAGTAATAATTCTGGTAG  
TTTCAGTTTAAAATTATGTTTAAATGGACTATCATATG  
CTTACCGTAACCTGAAAGTATTCGATTTCTGGCTTATAT  
ATCTTGAAAGGAGCAAACACC**GNNNNNNNNNNNNNNNN**  
**NNNGTTTTGTACTCTCAGAAATCAGAACGCC**  
**GCCTTCATGCCGAAATCACACCCCTGTCATTTATGGCAGGG**

**N. meningitidis Cas9 (NM):** Find all 28bp genomic sites of the form 5'-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNGATT-3' near your intended target site (ideally  $\pm 50$ bp). These may reside on the + or - strand. To create a gRNA expression fragment, incorporate 20bp of the protospacer sequence adjacent to the PAM site into either of the two DNA fragments as indicated below (U6 promoter-gRNA spacer+scaffold+terminator):

>gRNA variant=NMF

>gca\_variant\_RNA  
TGTACAAAAAAAGCAGGGCTTAAAGGAACCAATTCACTGCACTGGATCCGGTACCAAGGTC  
GGGCAGGAAGAGGGCCTATTTCCATGATTCTTCATATTCGATATACTGATACAAGGCT  
GTTAGAGAGATAATTAGAATTAAATTGACTGAAACACAAAGATATTAGTACAAAATAC  
TGACGTAGAAAGTAATAATTCTGGGTAGTTGCAGTTTAAATTATGTTAAATAC  
GACTATCATATGCTTACCGTACTGAAAGTATTGCACTTCTGGCTTATATATCTT  
TGGAAAGGACGAAACACGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  
TCTGGTAGCTCCCCCTTCATT  
TCGCAGTGCATCACAAATGAAAGATGTGCCACTGCGAACATGGAACGGCTGTCATAATAAGGG  
CGCTGAAAGATGTGCCGCAACGCTCTGCCCTAAAGCTCTGCTTAAAGGGCCTT  
TTT

**Note:** We strongly recommend trying both gRNA variants for genome targeting via Cas9-NM to determine the most optimal variant for the given target locus.

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>**gRNA\_variant-NMm1**  
TGTACAAAAAAGCAGGCTTAAAGGAACCAATTCACTGACT  
GGATCCGGTACCAAGTCGGGCAGGAAGAGGGCTATTCCCC  
ATGATTCCTCATATTTCGATACAGATAACAGCTGTTAGA  
GAGATAATTAGAATTATTTGACTGTAAACACAAGATATTAA  
GTACAAAATACGTGACCTGAGAAAGTAATAATTCTTGGTAG  
TTTCAGTTTAAATTATGTTTAAATGGACTATCATATG  
CTTACCGTAACTTGAAAGTATTCGATTCTGGCTTATAT  
ATCTTGTGAAAGGACCAAACACC**GNNNNNNNNNNNNNNNNNN**  
**NNN****GTTGTAGCTCCCTTCTCGAAAGAGAACCGTTGCTACAA**  
**TAAGGCCGTCTGAAAGATGTGCCGCAACGCTCTGCCCTTA**

**Note:** We strongly recommend trying both gRNA variants for genome targeting via Cas9-NM to determine the most optimal variant for the given target locus.