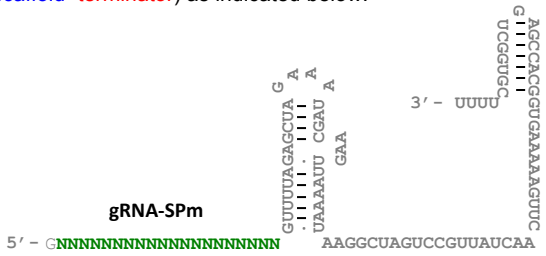
Choice of gRNAs for the Cas9 Orthologs

***S. pyogenes* Cas9 (SP)**: Find all 23bp genomic sites of the form 5'-NNNNNNNNNNNNNNNNNNNNGG-3' near your intended target site (ideally ±50bp). 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

```
TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCA
AGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACG
ATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATA
TTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTT
TAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTT
CGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGNNNNNNNNNNN
NNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTAT
CAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT
```



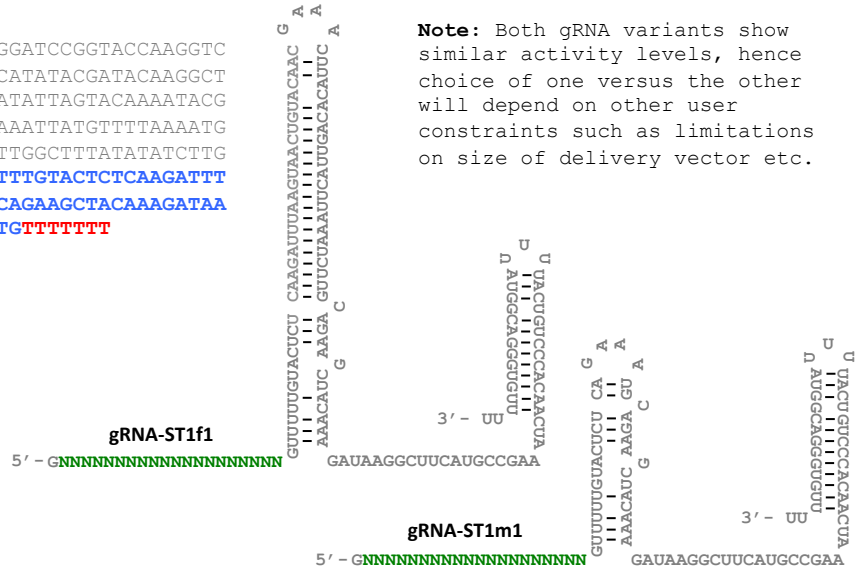
***S. thermophilus* CRISPR #1 Cas9 (ST1)**: Find all 27bp genomic sites of the form 5'-NNNNNNNNNNNNNNNNNNNNNNAGAAW-3' near your intended target site (ideally ±50bp). These may reside on the + or – strand. To create a gRNA expression fragment, incorporate 20bp of the protospacer sequence adjacent to the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

>gRNA_variant-ST1f1

```
TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTC
GGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCT
GTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACG
TGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATG
GACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTG
TGGAAAGGACGAAACACCGNNNNNNNNNNNNNNNNNNNNGTTTTTGTACTCTCAAGATTT
AAGTAACTGTACAACGAAACTTACACAGTTACTTAAATCTTGCAGAAGCTACAAAGATAA
GGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTTTT
```

>gRNA_variant-ST1m1

```
TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACT
GGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCC
ATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGA
GAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTA
GTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAG
TTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATG
CTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATAT
ATCTTGTGGAAAGGACGAAACACCGNNNNNNNNNNNNNNNNNNN
NNNGTTTTTGTACTCTCAGAAATGCAGAAGCTACAAAGATAA
GGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGG
TGTTTTTTT
```



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.

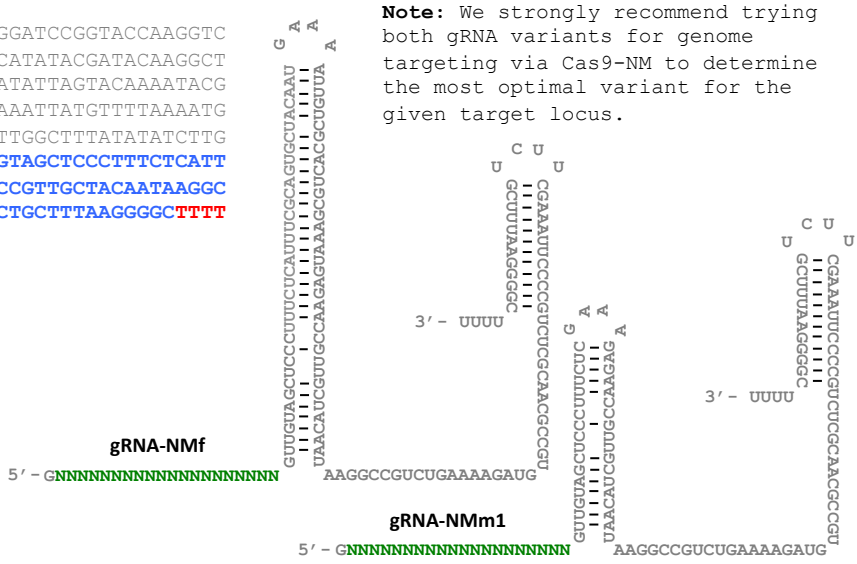
***N. meningitidis* Cas9 (NM)**: Find all 28bp genomic sites of the form 5'-NNNNNNNNNNNNNNNNNNNNNNNNGATT-3' near your intended target site (ideally ±50bp). These may reside on the + or – strand. To create a gRNA expression fragment, incorporate 20bp of the protospacer sequence adjacent to the PAM site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

>gRNA_variant-NMf

```
TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTC
GGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCT
GTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACG
TGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATG
GACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTG
TGGAAAGGACGAAACACCGNNNNNNNNNNNNNNNNNNNNGTTGTAGCTCCCTTTCTCATT
TCGCAGTGCTACAATGAAAATTGTCGCACTGCGAAATGAGAACCGTTGCTACAATAAGGC
CGTCTGAAAAGATGTGCCGCAACGCTCTGCCCCTTAAAGCTTCTGCTTTAAGGGGCTTTT
TTT
```

>gRNA_variant-NMm1

```
TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACT
GGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCC
ATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGA
GAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTA
GTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAG
TTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATG
CTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATAT
ATCTTGTGGAAAGGACGAAACACCGNNNNNNNNNNNNNNNNNNN
NNNGTTGTAGCTCCCTTTCTCGAAAGAGAACCGTTGCTACAA
TAAGGCCGTCTGAAAAGATGTGCCGCAACGCTCTGCCCCTTA
AAGCTTCTGCTTTAACGGGCTTTTTTT
```



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.

Prashant Mali & Kevin Esvelt (Church Lab), Version: 10-28-2013