The *Neisseria meningitidis* CRISPR-Cas9 System Enables Specific Genome Editing in Mammalian Cells

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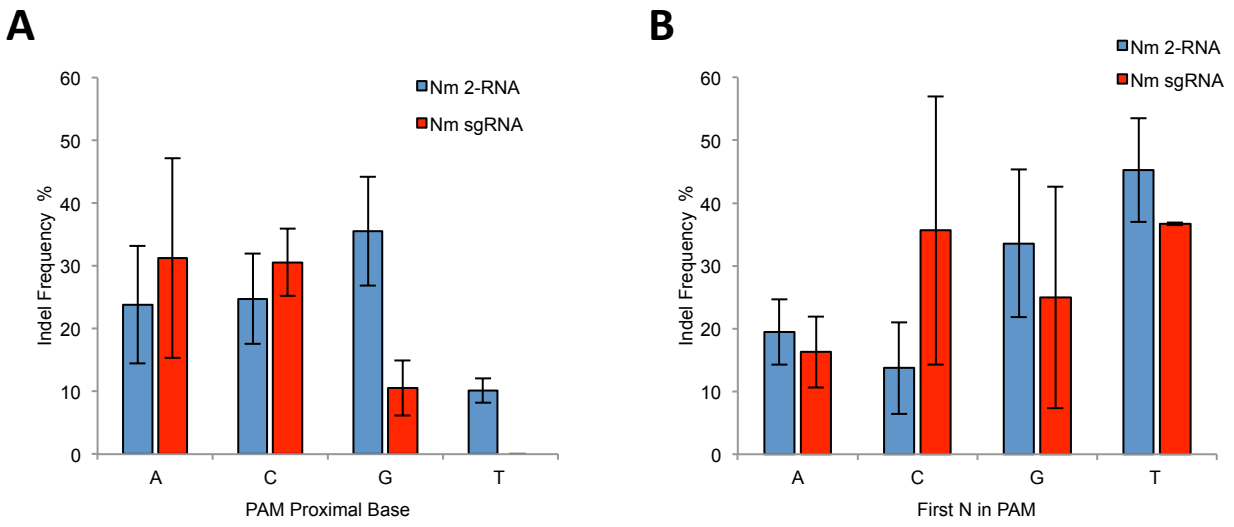
Supplementary Information

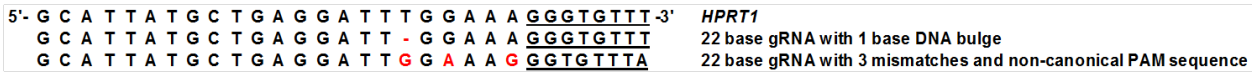
Supplementary Figures

Supplementary Tables



Supplementary Figure S1: Effect of sequence on *Nm* Cas9 activity. (a) The effect of the PAM proximal base on *Nm* Cas9 activity. Data represents the average activity level observed at 4, 6, 2, and 2 endogenous sites for targets containing A, C, G, or T at the PAM proximal position. **(b)** The effect of the first N in the PAM on *Nm* Cas9 activity. Data represents the average activity level observed at 7, 3, 2, and 2 endogenous sites for targets containing A, C, G, or T at the first position of the PAM. All experiments were carried out in HEK293T cells and all data derived from T7EI assays.

A



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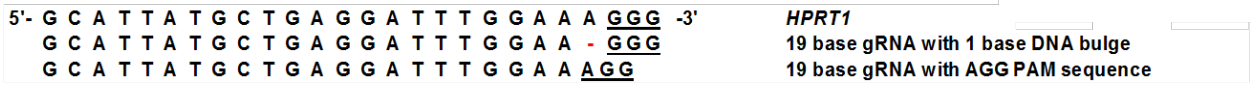
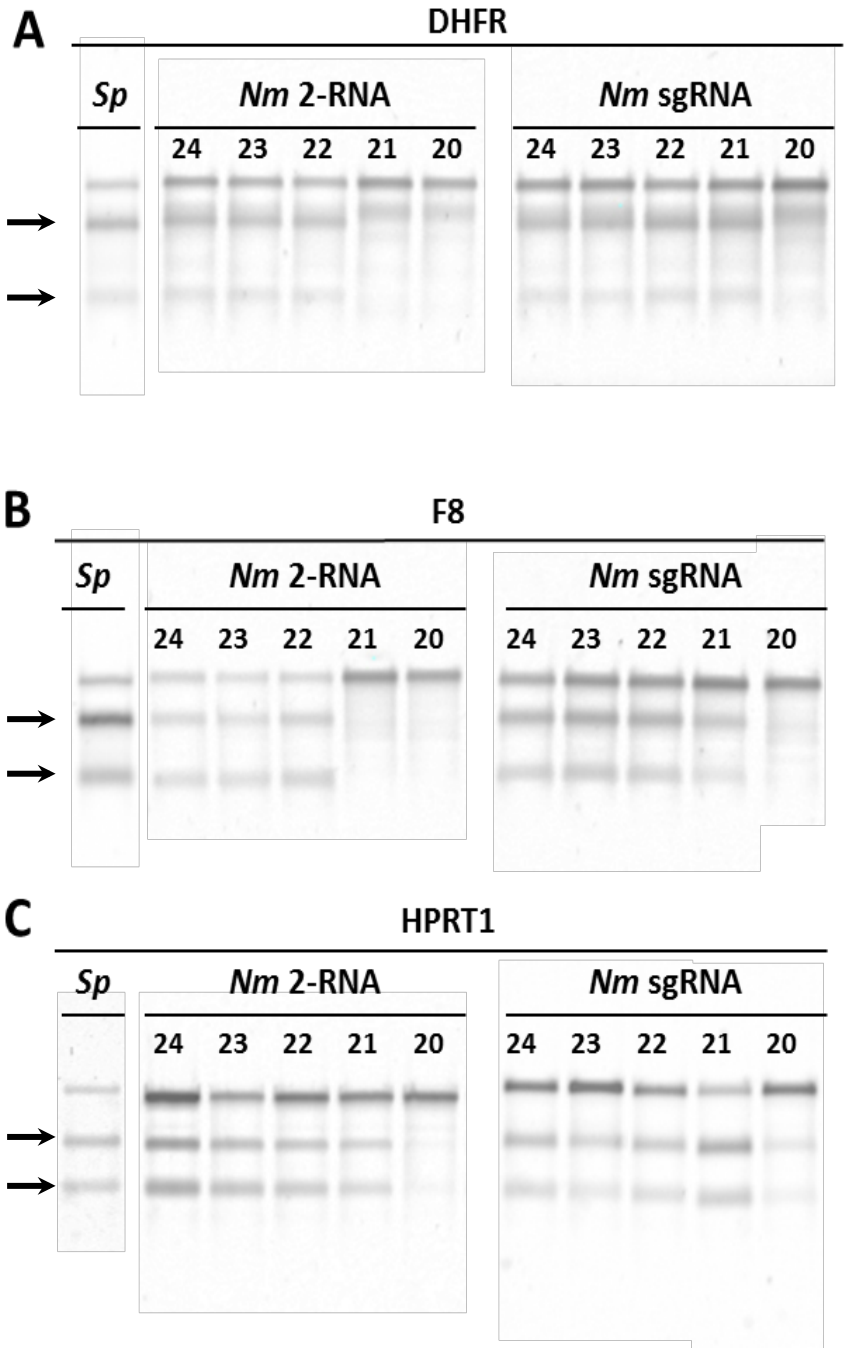
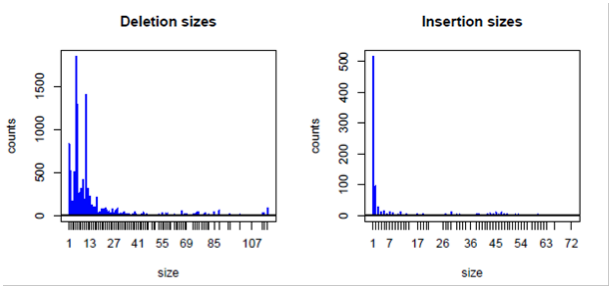


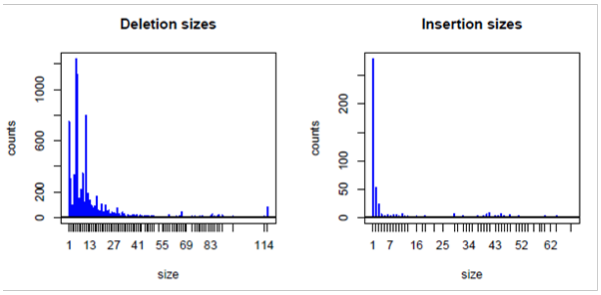
Supplemental Figure S2: Alternative modeling of DNA bulges as shorter gRNAs with alternate PAMs. A. A *Nm* gRNA with a deletion at position 6 models a DNA bulge at this position. The alternative is a scenario with 3 mismatches close to the PAM and a PAM sequence not recognized by *Nm* (NNNNTTTA). **B.** A *Sp* gRNA with a deletion at position 1 was designed to model a DNA bulge at this position. However, due to the next base being a G, it creates a new PAM sequence for the 19 nt *Sp* gRNA.



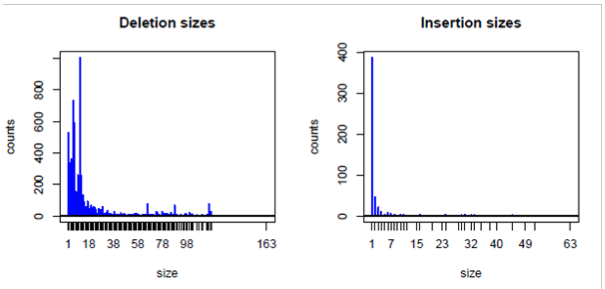
Supplementary Figure S3: RepresentativeT7EI assay gel of CRISPR-Cas9 activity with gRNA lengths of 20 to 24 nt. **A**. Activity of CRISPR-Cas systems targeting *DHFR*. **B**. Activity of CRISPR-Cas systems targeting *F8*_Site 2. **C**. Activity of CRISPR-Cas systems targeting *HPRT1*. Expected cleavage bands highlighted by arrows.



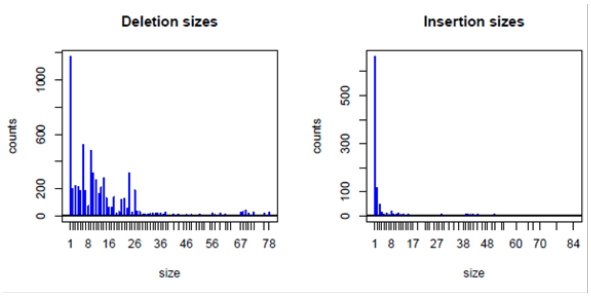
Nm 2-RNA *HPRT1*



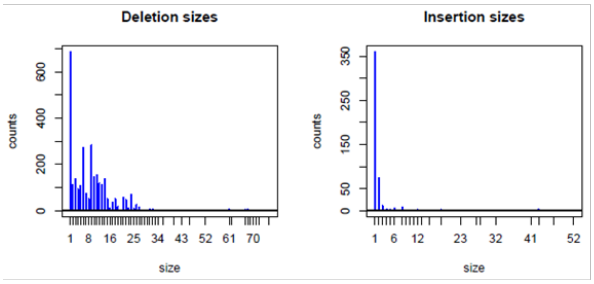
Nm sgRNA *HPRT1*



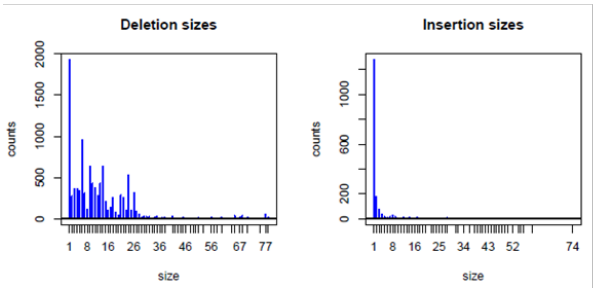
Sp *HPRT1*



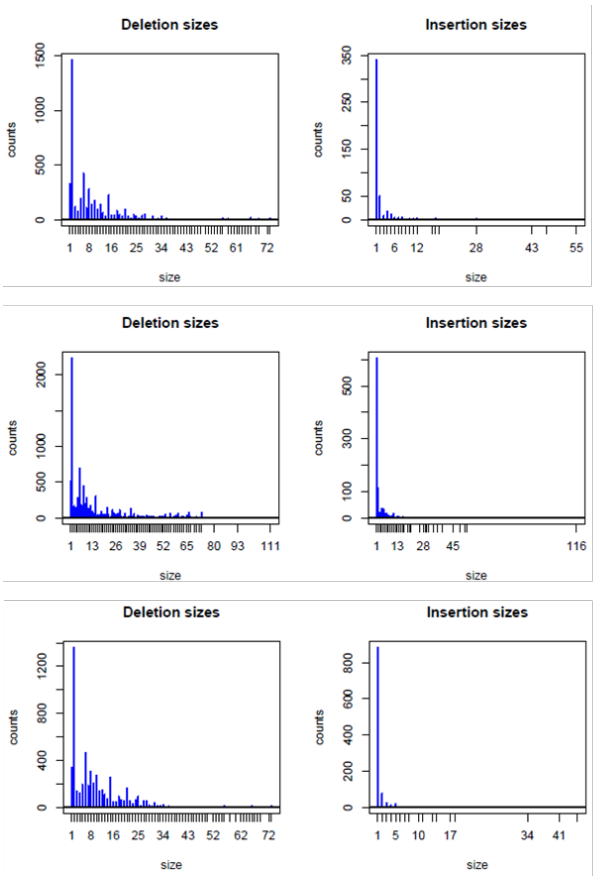
Nm 2-RNA *DHFR*



Nm sgRNA *DHFR*



Sp *DHFR*



Nm 2-RNA *F8*

Nm sgRNA *F8*

Sp *F8*

Supplementary Figure S4: Indel profiles of different CRISPR-Cas9 systems. Indel profiles generated by *Sp* and *Nm* CRISPR-Cas9 systems at three distinct genomic loci. All data derived from processed illumina MiSeq reads and analyzed using the CRISPR Genome Analyzer webtool[1].

Supplementary Table S2: Luficerase SSA assay data.

Guide	Sequence	Sp sgRNA	Nm sgRNA	Nm 2-RNA
HPRT1	GCAUUAUGCUGAGGAUUUGGAAA	73.31 ± 3.89	52.61 ± 3.07	41.26 ± 0.92
mm_22	G**A**AUUAUGCUGAGGAUUUGGAAA	n.t.	n.t.	33.02 ± 1.88
mm_21	GC**G**UUAUGCUGAGGAUUUGGAAA	n.t.	n.t.	10.3 ± 0.2
mm_20	GCA**A**UAUGCUGAGGAUUUGGAAA	n.t.	33.95 ± 1.93	12.43 ± 0.45
mm_19	GCAU**A**AUGCUGAGGAUUUGGAAA	62.6 ± 2.13	15.61 ± 1.42	6.69 ± 0.27
mm_18	GCAUU**G**UGCUGAGGAUUUGGAAA	10.11 ± 0.77	31.3 ± 6.52	8.69 ± 0.22
mm_17	GCAUUA**A**GCUGAGGAUUUGGAAA	8.73 ± 0.47	11.76 ± 2.14	1.84 ± 0.07
mm_16	GCAUUAU**A**CUGAGGAUUUGGAAA	37.86 ± 3.13	18.15 ± 4.01	3.1 ± 0.07
mm_15	GCAUUAUG**A**UGAGGAUUUGGAAA	4.45 ± 0.44	15.23 ± 3.46	4.14 ± 0.19
mm_14	GCAUUAUGC**A**GAGGAUUUGGAAA	5.52 ± 0.44	9.51 ± 0.54	2.07 ± 0.07
mm_13	GCAUUAUGCU**A**AGGAUUUGGAAA	29.73 ± 2.2	13.47 ± 1.17	7.74 ± 0.13
mm_12	GCAUUAUGCUG**G**GGAUUUGGAAA	23.05 ± 0.71	35.99 ± 5.16	13.09 ± 0.47
mm_11	GCAUUAUGCUGA**A**GAUUUGGAAA	7.71 ± 0.54	10.8 ± 1.55	2.63 ± 0.04
mm_10	GCAUUAUGCUGAG**A**AUUUGGAAA	24.28 ± 0.17	11.35 ± 1.56	2.87 ± 0.07
mm_9	GCAUUAUGCUGAGG**G**UUUGGAAA	54.23 ± 3.02	14.29 ± 0.74	2.94 ± 0.06
mm_8	GCAUUAUGCUGAGGA**A**UUGGAAA	5.42 ± 0.08	6.45 ± 0.36	0.96 ± 0.06
mm_7	GCAUUAUGCUGAGGAU**A**UGGAAA	43.81 ± 1.85	12.22 ± 1.42	15.55 ± 0.23
mm_6	GCAUUAUGCUGAGGAUU**A**GGAAA	15.82 ± 1.48	5.94 ± 0.05	2.97 ± 0.12
mm_5	GCAUUAUGCUGAGGAUUU**A**GAAA	9.56 ± 0.21	6.8 ± 0.53	1.97 ± 0.03
mm_4	GCAUUAUGCUGAGGAUUUG**A**AAA	8.64 ± 0.06	6.63 ± 0.66	41.19 ± 2.93
mm_3	GCAUUAUGCUGAGGAUUUGG**G**AA	30.66 ± 0.65	10.38 ± 1.04	5.48 ± 0.21
mm_2	GCAUUAUGCUGAGGAUUUGGA**G**A	65.65 ± 3.27	10.84 ± 1.33	6.2 ± 0.2
mm_1	GCAUUAUGCUGAGGAUUUGGAA**G**	64.46 ± 6.06	7.59 ± 0.47	16.14 ± 0.42
Ins_22	G**A**CAUUAUGCUGAGGAUUUGGAAA	n.t.	n.t.	40.55 ± 0.86
Ins_21	GC**U**AUUAUGCUGAGGAUUUGGAAA	n.t.	n.t.	22.3 ± 0.35
Ins_20	GCA**G**UUAUGCUGAGGAUUUGGAAA	n.t.	41.4 ± 4.3	18.06 ± 0.35
Ins_19	GCAU**A**UAUGCUGAGGAUUUGGAAA	50.49 ± 2.59	36.04 ± 3.51	40.15 ± 1.76
Ins_18	GCAUU**G**AUGCUGAGGAUUUGGAAA	29.57 ± 1.23	10.82 ± 1.13	12.12 ± 0.46
Ins_17	GCAUUA**G**UGCUGAGGAUUUGGAAA	11.39 ± 0.48	1.3 ± 0.15	0.99 ± 0.09
Ins_16	GCAUUAU**A**GCUGAGGAUUUGGAAA	1.39 ± 0.07	1.33 ± 0.14	1.13 ± 0.09
Ins_15	GCAUUAUG**A**CUGAGGAUUUGGAAA	0.97 ± 0.05	1.17 ± 0.16	2.96 ± 0.36
Ins_14	GCAUUAUGC**A**UGAGGAUUUGGAAA	1.14 ± 0.05	2.21 ± 0.09	37.99 ± 3.47
Ins_13	GCAUUAUGCU**A**GAGGAUUUGGAAA	0.87 ± 0.05	1.03 ± 0.04	2.76 ± 0.22
Ins_12	GCAUUAUGCUG**U**AGGAUUUGGAAA	1.57 ± 0.1	1.07 ± 0.06	10.9 ± 1.1
Ins_11	GCAUUAUGCUGA**U**GGAUUUGGAAA	1.52 ± 0.06	1.13 ± 0.06	6.64 ± 0.43
Ins_10	GCAUUAUGCUGAG**A**GAUUUGGAAA	0.79 ± 0.02	1.07 ± 0.04	4.17 ± 0.2
Ins_9	GCAUUAUGCUGAGG**U**AUUUGGAAA	0.83 ± 0.03	1 ± 0.05	1.14 ± 0.05
Ins_8	GCAUUAUGCUGAGGA**G**UUUGGAAA	0.8 ± 0.03	0.94 ± 0.09	1.56 ± 0.37
Ins_7	GCAUUAUGCUGAGGAU**A**UUGGAAA	0.82 ± 0.04	1.12 ± 0.11	1.46 ± 0.07
Ins_6	GCAUUAUGCUGAGGAUU**A**UGGAAA	0.95 ± 0.05	1.38 ± 0.13	1.13 ± 0.05
Ins_5	GCAUUAUGCUGAGGAUUU**A**GGAAA	0.81 ± 0.04	1.77 ± 0.52	1.86 ± 0.09
Ins_4	GCAUUAUGCUGAGGAUUUG**A**GAAA	0.89 ± 0.02	1.38 ± 0.17	1.07 ± 0.04
Ins_3	GCAUUAUGCUGAGGAUUUGG**U**AAA	0.8 ± 0.02	1.35 ± 0.07	1.12 ± 0.06
Ins_2	GCAUUAUGCUGAGGAUUUGGA**U**AA	0.96 ± 0.02	1.43 ± 0.11	1.18 ± 0.08
Ins_1	GCAUUAUGCUGAGGAUUUGGAA**U**A	0.95 ± 0.01	1.44 ± 0.08	1.11 ± 0.1
Ins_0	GCAUUAUGCUGAGGAUUUGGAAA**U**	1.22 ± 0.02	1.45 ± 0.15	0.76 ± 0.06
Δ_22	G-AUUAUGCUGAGGAUUUGGAAA	n.t.	n.t.	34.56 ± 0.82
Δ_21	GC-UUAUGCUGAGGAUUUGGAAA	n.t.	n.t.	4.72 ± 0.14
Δ_19/20	GCA-UAUGCUGAGGAUUUGGAAA	66.24 ± 5.44	27.76 ± 1.85	4.37 ± 0.16
Δ_18	GCAUU-UGCUGAGGAUUUGGAAA	45.19 ± 4.07	3.19 ± 0.08	1.09 ± 0.06
Δ_17	GCAUUA-GCUGAGGAUUUGGAAA	8.26 ± 0.7	1.08 ± 0.02	5.52 ± 0.04
Δ_16	GCAUUAU-CUGAGGAUUUGGAAA	2.37 ± 0.17	0.71 ± 0.02	1.09 ± 0.02
Δ_15	GCAUUAUG-UGAGGAUUUGGAAA	1.09 ± 0.01	1.02 ± 0.02	1.19 ± 0.11
Δ_14	GCAUUAUGC-GAGGAUUUGGAAA	13.99 ± 0.66	0.86 ± 0.03	2.89 ± 0.15
Δ_13	GCAUUAUGCU-AGGAUUUGGAAA	5.39 ± 0.06	0.89 ± 0.03	1.16 ± 0.04
Δ_12	GCAUUAUGCUG-GGAUUUGGAAA	1.89 ± 0.03	0.91 ± 0.02	1.07 ± 0.03
Δ_10/11	GCAUUAUGCUGA-GAUUUGGAAA	3.54 ± 0.04	0.92 ± 0.02	1.18 ± 0.03
Δ_9	GCAUUAUGCUGAGG-UUUGGAAA	1.17 ± 0.06	0.9 ± 0.05	1.09 ± 0.06
Δ_6/7/8	GCAUUAUGCUGAGGA-UUGGAAA	1.25 ± 0.04	26.13 ± 0.98	1.91 ± 0.07
Δ_4/5	GCAUUAUGCUGAGGAUUU-GAAA	3.58 ± 0.06	1.06 ± 0.05	1.06 ± 0.04
Δ_1/2/3	GCAUUAUGCUGAGGAUUUGG-AA	53.94 ± 0.72	4.24 ± 0.27	6.19 ± 0.36

Supplementary Table S2: Relative light unit measurements from luciferase assays. Data represents the average values from biological triplicates ± the standard error of the mean. n.t. = not determined.

Supplementary References

1. Guell, M., Yang, L. & Church, G.M. Genome editing assessment using CRISPR Genome Analyzer (CRISPR-GA). *Bioinformatics* **30**, 2968-2970 (2014).