**SUPPLEMENTARY MATERIAL**

# The CRISPR/Cas system in *Neisseria meningitidis* affects adhesion to human nasopharyngeal epithelial cells

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**This supplement contains:**

**Supplementary Material & Methods**

*Bacterial Strains and Growth Conditions*

All *Escherichia coli* and *Neisseria meningitidis* strains used in this study are listed in Table S1. *E. coli* were cultivated overnight at 37 °C on *Luria-Bertani* (LB) agar plates in an incubator or in LB media (peptone 10 g, yeast extract 5 g, NaCl 10 g, ddH2O ad 1000 ml) at 200 rpm in an incubator shaker. To select requested recombinant clones, the medium was supplemented with the appropriate antibiotic before usage. The final antibiotic concentrations used for *E. coli* were 100 µg/ml for ampicillin; 30 µg/ml for kanamycin; 250 µg/ml for erythromycin and 30 µg/ml for chloramphenicol. *N. meningitidis* were cultivated overnight at 37 °C with 5% $CO_2$ on Columbia blood agar plates with 5% sheep blood (COS) (*bioMérieux*, Nürtingen) or on Difco™ GC Medium Base (GCB) (*BD*, Heidelberg) plates with Kellogg's supplements I and II (22.2 mM glucose, 0.68 mM glutamine, 0.45 mM co-carboxylase, 1.23 mM Fe(NO3)3; *Sigma-Aldrich*, Schnelldorf) ($GCB^{++}$). The selection of recombinant clones occurred similar to *E. coli* by adding antibiotics to the $GCB^{++}$ agar plates. The final antibiotic concentrations used for *N. meningitidis* were 100 µg/ml for kanamycin; 7 µg/ml for erythromycin and 7 µg/ml for chloramphenicol. For cultivation in liquid culture, the bacteria were inoculated into proteose peptone medium (PPM) supplemented with 500 µl 8.4 % $NaHCO_3$, 500 µl 2M $MgCl_2$ and 1 ml PolyViteX (*bioMérieux*, Nürtingen) in a total volume of 100 ml and incubated at 37°C at 200 rpm in an incubator shaker.

*Construction of CRISPR mutant and rnc and tracrRNA complemented strains*

The 8013 ΔCRISPR strain was constructed by replacing the CRISPR-array (nucleotide position 1921685 to 1923370 in FM999788.1) with a kanamycin resistance cassette. Strain 8013 was transformed with the plasmid pBJ106 followed by $Kan^R$ selection as described above. For complementation of the Δ*rnc* and

ΔtracrRNA mutants, wildtype copies of *rnc* (NMV-1713) or tracrRNA (nucleotide position 1916964 to1916858 in FM999788.1) were cloned between the *Nsi*I and the *Bam*HI sites of pCompInd[1]. The resulting plasmids were transformed into the respective mutant strain and the transformants were selected using chloramphenicol.All mutants were confirmed by PCR as shown in Table S4. PCRs for verifying strains or transformants were performed in a T3 Thermocycler (*Biometra*, Göttingen) with 0.05 U/µl *Taq* DNA Polymerase (*New England Biolabs*, Frankfurt) using either 10 ng of genomic DNA or 1 µl bacterial lysate as templates. Chromosomal DNAs were isolated using QIAamp DNA Mini Kit (*Qiagen*, Hilden) according to the manufacturers' instructions.

*Southern Blot for the confirmation of cas9 genotype*

One microgram of genomic DNA of each genotype was digested with *Hinc*II overnight at 37°C. Digested chromosomal DNA was separated in a 0.8% agarose gel and stained for monitoring the DNA digestion. The gel was depurinated by incubation in 0.25M HCl for 20 min and denaturated in 5M NaOH/1.5M NaCl for 30 min before neutralization in 0.5M TRIS, pH 7.5/1.5M NaCl for 30min. Afterwards, the fractionated DNA was transferred from the gel onto a nylon membrane by capillary transfer overnight. After UV cross linking (Thermo Fisher, Frankfurt) and prehybridization, the membrane was hybridized overnight at 48°C with a DIG-labeled DNA probe in high SDS hybridization buffer (5x SSC; 0,1% (w/v) Lauroylsarcosine; 0,02% (w/v) SDS; 1% Blocking reagent). The DNA probe for *cas9* detection was constructed using primers 1227/1228 while the probe for *blp* detection was constructed using primers 1229/1230. The membrane was washed in wash buffer 1 (2xSSC, 0.1% SDS) for 10 min at room temperature and for 30 min in wash buffer 2 (0.1xSSC, 1%SDS) at 68°C. After blocking the membrane for 30 min at room temperature in buffer 2 (1%

blocking reagent in maleine acid buffer), the membrane was incubated for further 30 min in anti-Dig_Ap (1:10000 in buffer 2, Roche, Basel). After incubation in detection buffer (0,1M Tris-HCl, 0,1M NaCl, pH 9.5) and 1% CSPD (25mM, Roche) for each 5 min at room temperature, the membrane was developed with ChemiDoc MP System according to the manufacturer's instructions.

*RT-PCR for the confirmation of blp expression*

For RT-PCR, 2 µg of DNase-treated total RNA isolated with the Hot-Phenol method was transcribed into cDNA. The RNA samples were incubated together with each 2 microliter Nona Random Primer (Sigma-Aldrich, Steinheim) for 5min at 70°C prior to 5min on ice. The samples were incubated with the prepared mastermix (20mM dATP, 20mM dGTP, 20mM dTTP, 20mM dCTP, 0.04M DTT, 40U RNase Out, 100U Reverse Transcriptase Superscript II, 5xFS buffer (Invitrogen)) for 2 hours at 42°C prior to 15min at 70°C. After RNA digestion using RNaseA (Roche, Basel) for 45min at 37°C, the cDNA got purified using the QIAquick PCR purification kit (QIAGEN, Hilden) according to the manufacter`s instructions. RT-PCRs were performed in a T3 Thermocycler (*Biometra*, Göttingen) with 0.05 U/µl *Taq* DNA Polymerase (*New England Biolabs*, Frankfurt) using 100 ng of cDNA as a template. The sequences of the oligonucleotides are given in Table S3 and the PCR conditions in Table S4.

*Construction of a* N. meningitidis cas9::*3xFLAG strain*

To construct a *cas9*::3xFLAG-tagged strain, a plasmid (pNHBJ03) containing the 3xFLAG and the aphA-1 cassette flanked by 300 nt up- and downstream of the cas9 stop codon was cloned into *E. coli*. First, 300 nt upstream of the *cas9* stop codon were amplified by PCR from chromosomal DNA of 8013 with primers JVO_10487 and JVO_10488. The primer JVO_1088 contains the 3xFLAG sequence at the 3`end, leading to the addition of the 3xFLAG tag directly downstream of the stop codon of

*cas9*. The resulting PCR product was digested with *Bam*HI and *Eco*RI. Next the downstream region of the *cas9* gene was amplified by PCR from chromosomal DNA of 8013 with primers 10489 and 10490 and the resulting PCR fragment was digested with *Eco*RI and *Hind*III. These fragments were cloned into the pBluescript II SK (+) vector along with a *Eco*RI digested fragment of pUC4K containing the apha-1 kanamycin cassette, generating p*cas9*-3xFLAG::aphA-1 plasmid. The plasmid was checked by colony PCR with primer pair kb9 and 329. This plasmid was then linearized and used for transformation of the 8013 strain. Transformants were first isolated by selection for kanamycin resistance. The functionality of the FLAG-tagging was verified by performing Western Blot analysis on protein samples corresponding to an $OD_{600}$ of 0.5 of the kanamycin resistant clones using an anti-FLAG primary antibody (Monoclonal ANTI-FLAG M2, Sigma, #F1804).

*Plasmids*

Al plasmids used in this study are listed in Table S2. *E. coli* DH5α cells (*Thermo Fisher*, Frankfurt) were used for CRISPR knock-out and *E. coli* XL1-Blue cells (*Stratagene*, La Jolla) were used for *rnc* and tracrRNA complementation. All plasmid constructions were verified by Sanger sequencing. For cloning purposes PCR reactions were performed with Q5® High-Fidelity DNA polymerase (*New England Biolabs,* Frankfurt). Restriction enzymes and T4 DNA ligase were purchased from *New England Biolabs* (Frankfurt). A complete list of the oligonucleotides used for cloning and PCR verification is provided in Table S3. For CRISPR knock-outs in 8013 the plasmid pBJ106 was used. Approximately 600 bp upstream and downstream of the CRISPR array were amplified with primer pair 11443/11115 and 11116/11444 using 8013 genomic DNA as template. The oligonucleotides were modified to introduce *Bam*HI/*Eco*RI sites at the 5´ and 3´ ends of the upstream fragment, and *Eco*RI/*Hind*III sites at the 5´ and 3´ ends of the downstream fragment. The delivery

plasmid pUC4K (*GE Healthcare*, Freiburg) was digested with *Eco*RI restriction enzyme and purified using the Invisorb Spin DNA Extraction Kit (*STRATEC Biomedical AG*, Birkenfeld) to gain *Eco*RI flanked kanamycin resistance cassettes. These fragments were cloned into the *Hind*III and *BamHI* double digested pBluescript SK vector (*Stratagene*, La Jolla) yielding the knock-out plasmid pBJ106. For genetic complementation the gene of interest was amplified by PCR using genomic DNA of 8013 as template with oligonucleotides introducing *Nsi*I and *Bam*HI restriction sites at the 5´ and 3´ ends of the fragment. The primer pair 1043/1044 was used for *rnc* complementation and 1047/1048 used for tracrRNA complementation. The resulting fragments were cloned between the *Nsi*I and *Bam*HI sites of pCompInd replacing the *lac* operon to yield plasmids pAH-1 and pAH-2 respectively.

*Cell culture and cell adhesion experiments*

The human nasopharyngeal epithelial cell line Detroit562 (ATCC® number CCL-138™) was used for all subsequent cell culture experiments and cultivated in Eagle's minimum essential medium (EMEM) (Lonza, Basel, Switzerland) with 10% feta calf serum (Thermo Fisher, Frankfurt/Main, Germany), 1% nonessential amino acids (Lonza) and 1% sodium pyruvate (Lonza) (EMM+++) at 37°C and 5% $CO_2$. Cell adhesion assays were performed as described in ref. [2]. Briefly, Detroit562 cells were seeded onto 24-well tissue culture plates (Corning Costar) at a density of $5\times10^4$ cells per well and were grown to $\approx1\times10^5$ prior to infection. Cells were infected with bacteria at a multiplicity of infection (MOI) of 20 either in presence of EMM+++ medium at 37°C and 5% $CO_2$. After 4 hours of infection, the number of adherent cell-bacteria in the supernatant was determined by lysis with 1% saponin for 15 min and subsequent determination of colony-forming units (CFU) by plating appropriate dilutions of the lysates of blood agar. For each strain, the adhesion rate was calculated as the number of CFU recovered after 4 h of infection divided by the seeded CFU

determined in parallel. The relative adhesion rate is the adhesion rate of the mutant divided by the adhesion rate of the wild-type strain. All strains were tested in triplicate, and experiments were repeated at least three times.

*RIP-Seq of* N. meningitidis Cas9-*3xFLAG.*

*N. meningitidis* mutant strain expressing 3×FLAG-tagged Cas9 protein and wild-type strain (control) were grown in 100ml GCBL++ medium (50ml x 2 flasks) at 37°C in the presence of kanamycin until $OD_{600nm}$ of 0.5 and 1.5. For each strain cells equivalent to an $OD6_{00nm}$ of 50 were collected in parallel and subjected to Cas9 coIP and control coIP as described in ref. 3.

Cells were harvested by centrifugation at 6,000g for 20 min at 4 °C. Afterwards, cell pellets were resuspended in 1ml Lysis Buffer (20mM Tris-HCl, pH 8.0, 150mM KCl, 1mM MgCl2, 1mM dithiothreitol (DTT)) and subsequently centrifuged (5 min, 11,000g,4 °C). The pellets were shock-frozen in liquid nitrogen and stored at -80 °C. Frozen pellets were thawed on ice and resuspended in 0.8 ml Lysis Buffer. An equal volume of glass beads was then added to the cell suspension. Cells were then lysed using a Retsch MM40 ball mill (30 s-1, 10min) in pre-cooled blocks (4 °C) and centrifuged for 2min at 15,200g, 4 °C. The supernatant was transferred to a new tube, and an additional 0.4 ml of Lysis Buffer was added to the remaining un-lysed cells with beads. Lysis of the remaining cells was achieved by a second round of lysis at 30 s-1 for 5min. Centrifugation was repeated and this second supernatant was combined with the first one. The combined supernatant was centrifuged again for 30 min at 15,200g, 4 °C for clarification and the resulting supernatant (lysate fraction) was transferred to a new tube. The lysate was incubated with 35 ml anti-FLAG antibody (Monoclonal ANTI-FLAG M2, Sigma, #F1804) for 30 min at 4 °C on a rocker. Next, 75 ml of Protein A-Sepharose (Sigma, #P6649), prewashed with Buffer

A, was added and the mixture was rocked for another 30 min at 4 °C. After centrifugation at 15,200g for 1min, the supernatant was removed. Pelleted beads were washed five times with 1 ml Lysis Buffer. Finally, 500 ml Lysis Buffer was added to the beads and RNA and proteins were separated by phenolchloroform-isoamyl alcohol (ROTH 985.1) extraction and precipitated as described previously[3].

From each coIP, 400–1,000 ng of RNA was recovered. 80 ul of 1x protein loading buffer (62.5mM Tris-HCl, pH 6.8, 100mM DTT, 10% (v/v) glycerol, 2% (w/v) SDS, 0.01% (w/v) bromophenol blue) was added to the final protein sample precipitated along with beads. This sample was termed the coIP sample. For verification of a successful coIP, protein samples equivalent to 5.0 $OD_{600nm}$ of cells were obtained during different stages of the coIP (culture, lysate and supernatant) for further western blot analysis. Two hundred fifty microlitres of 1x protein loading buffer was added to those protein samples and boiled for 8 min. Protein sample corresponding to an OD600 of 0.1 (lysate and supernatant fraction) and 1 (coIP sample (proteins precipitated from beads) were used for western blot analysis.

*cDNA library construction and sequencing*.

cDNA libraries of RNAseq samples were constructed by vertis Biotechnology AG, Germany (http://www.vertis-biotech.com/), as described previously[3].

*Read mapping of RNA-seq data*.

Raw data is available at GEO under accession GSE85252. Illumina reads in FASTQ format were trimmed with a cut-off phred score of 20 using the program fastq_quality_trimmer from FASTX toolkit version 0.10.1(http://hannonlab.cshl.edu/fastx_toolkit/). The following steps were performed using subcommand "create", "align" and "coverage" in the tool READemption version 0.3.0[4]: The poly(A)-tail sequences were computationally removed, and a size filtering

step was applied to eliminate the reads shorter than 12 nt. The remaining reads were mapped to the reference genome sequence of *N. meningitidis* 8013 (NC_017501.1) using segemehl version 0.1[5]. Each coverage files were normalized by the total number of mapping reads and then multiple by one million. Integrated Genome Viewer[6] was used to visualize the coverage plots. An overview over the read mapping statistics is given in Table S5.

*Analysis of Cas9 RIP-seq data*

Raw reads were trimmed for a Phred quality of 20 and sequencing adaptors and using the FASTX toolkit version 0.0.13 and Cutadapt version 1.3[5] then mapped using the READemption pipeline[4] , segemehl and the lack remapper[7]. Read counts were summed for all genomic features contained in the NCBI annotation and imported into R for further analysis. Enrichment analysis was conducted using the edgeR package[8]. Normalization factors were calculated using the trimmed means of M values method between FLAG-tagged Cas9 RIP-seq libraries and non-flag-tagged mock pull-down control libraries, using only those features with greater than five reads in the control library. For enrichment analysis, all features containing less than five reads in the flag-tagged library were excluded. The respective read mapping statistical data are summarized in Table S6.

## Supplemental References


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## Supplemental tables

**Table S1** Strains used in this study

| Species | Strain | Relevant genotypes | Source |
|---|---|---|---|
| *Escherichia coli* | XL1-Blue MRF' | *Δ((mcrA)183Δ(mcrCB-hsdSMR-mrr)173 recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac F´ proAB lacIqZΔM15 Tn10 Tetr* | *Stratagene*, La Jolla, USA |
| *E. coli* | DH5α | *fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17* | *Thermo Fisher*, Frankfurt DE |
| *N. meningitidis* | 8013 | wild-type | |
| *N. meningitidis* | 8013 | Δ*cas9*::Km$^r$ | Zhang *et al.*[9] |
| *N. meningitidis* | 8013 | ΔtracrRNA::Km$^r$ | Zhang *et al.*[9] |
| *N. meningitidis* | 8013 | Δ*rnc*::Km$^r$ | Zhang *et al.*[9] |
| *N. meningitidis* | 8013 | ΔCRISPR::Km$^r$ | This study |
| *N. meningitidis* | 8013 | Δ*cas9*::Km$^r$ C*cas9* Ery$^r$ | Zhang *et al.*[9] |
| *N. meningitidis* | 8013 | ΔtracrRNA::Km$^r$ *CtracrRNA* Cm$^r$ | This study |
| *N. meningitidis* | 8013 | Δ*rnc*::Km$^r$ C*rnc* Cm$^r$ | This study |
| *N. meningitidis* | 8013 | | |

**Table S2** Plasmids

| Plasmid | Relevant characteristics | Resistance | Reference |
|---|---|---|---|
| pBluescript-SK | Standard cloning vector | $Amp^r$ | *Stratagene,* La Jolla US |
| pCompInd | Standard vector for complementation | $Cm^r$ | Ieva *et al.*[1] |
| pUC4K | Delivery plasmid harbouring kanamycin resistance cassette | $Km^r$ | *GE Healthcare*, Freiburg DE |
| pBJ106 | ΔCRISPR | $Amp^r/Km^r$ | this study |
| pAH-1 | pCompInd -*lac* +*rnc* | $Amp^r/Cm^r$ | this study |
| pAH-2 | pCompInd -*lac* +tracrRNA | $Amp^r/Cm^r$ | this study |
| pNHBJ03 | cas9 FLAG tagging | $Amp^r/Km^r$ | this study |

**Table S3** Oligonucleotides

| ID[a] | Sequence (5'-3')[b] | Target site |
|---|---|---|
| 242 | AATACGACTCACTATAGGGC | pBluescript-SK |
| 243 | ACCATGATTACGCCAAGC | pBluescript-SK |
| 328 | GGTATTGATAATCCTGATATGAA | Tn*903* Kanamycin resistance cassette |
| 329 | GATGGTCGGAAGAGGCATAAATT | Tn*903* Kanamycin resistance cassette |
| 970 | TTCGCCCAAATCGCAGGAAATGA | 8013 *rnc* |
| 971 | TGTTTTGAAACAGCAGGCACACG | 8013 *rnc* |
| 988 | CCGAACCGCTGGCGCGTTAA | 8013 NMV0959 locus |
| 989 | ATTAATGCCGCCTCCGTCGG | 8013 *porA* locus |
| 1031 | GAAGCGAACTCAATCCATTCA | 8013 CRISPR locus |
| 1032 | CAATGGAAAGCTAATGTATTA | 8013 CRISPR locus |
| 1043 | gttttATGCAT$_{NsiI}$GGAATACGTTGGGGGAAAAC | 8013 *rnc* |
| 1044 | gttttGGATCC$_{BamHI}$AAAGGCTCCAAAAGGAGCC TTTTCATTTCTTTTTCCTCTTCAGC | 8013 *rnc* |
| 1047 | gttttATGCAT$_{NsiI}$CCGTAAACAACGTTGCAAATAA | 8013 tracrRNA |
| 1048 | gttttGGATCC$_{BamHI}$AAAGGCTCCAAAAGGAGCC TTTTAAACGATGCCCCTTAAAGCAG | 8013 tracrRNA |
| 1049 | CACTTTCCCTGTTTCTATG | *cat* Chloramphenicol resistance cassette |
| 1227 | CGCCAAGAAGAAAACCGCAA | Cas9-probe forward |
| 1228 | TTTCGCTGCCCAATACCAGT | Cas9-probe reverse |
| 1229 | ATTGGCGCGAAAAATCGGAC | blp-probe forward |
| 1230 | CCGCACTTTTTCTGATGGGC | blp-probe reverse |
| 11115 | gcgcgcGAATTC$_{EcoRI}$ACTTCGACGGGAAATCCTTAT | 8013 downstream CRISPR |
| 11116 | gcgcgcGAATTC$_{EcoRI}$CAGCCGTTGCGATAAGCGAAC | 8013 upstream CRISPR |
| 11443 | GGATCC$_{BamHI}$CGTGATACATTTGTACGAACA | 8013 downstream CRISPR |
| 11444 | AAGCTT$_{HindIII}$CAGTTTGAGAGTAAAGCAGGG | 8013 upstream CRISPR |
| 10487 | gttttGGATCC$_{BamHI}$AAAGATGAAGAAGATTGGCAA | 8013 Cas9 locus |
| 10488 | gttttGAATTC$_{EcoRI}$TTACTATTTATCGTCGTCATCTT TGTAGTCGATATCATGATCTTTATAATCACCGTC ATGGTCTTTGTAGTCACGGACAGGCGGCGTTTT TT | 8013 Cas9 locus |
| 10489 | gttttGAATTC$_{EcoRI}$ATTAATCTATCCCTGTTTCAG<br>gttttAAGCTT$_{HindIII}$GCAGCCATTGTCCGCAGGGCA | 8013 cas9 locus<br>8013 Cas9 locus |

| ID[a] | Sequence (5'-3')[b] | Target site |
|---|---|---|
| 10490 | | |

[a]ID-number 0-9999 according to the AG SCHOEN oligonucleotide collection, 10000-99999 according to the common IMIB oligonucleotide collection

[b]Restriction sites are underlined, small letters indicate nonsense-nucleotides

**Table S4** PCRs

| Primer 1[a] | Primer 2[a] | Description and Usage | ET[b] (s) | AT[c] | PM[d] |
|---|---|---|---|---|---|
| 11443 | 11115 | 8013 CRISPR knock-out | 10 | 63 °C | Q5 |
| 11444 | 11116 | 8013 CRISPR knock-out | 10 | 63 °C | Q5 |
| 1031 | 1032 | 8013 CRISPR knock-out verification | 210 | 60 °C | *Taq* |
| 328 | 1031 | 8013 CRISPR knock-out verification | 40 | 55 °C | *Taq* |
| 329 | 1032 | 8013 CRISPR knock-out verification | 40 | 55 °C | *Taq* |
| 329 | 11444 | 8013 CRISPR knock-out verification/ knock-out vector check | 40 | 50 °C | *Taq* |
| 328 | 11443 | 8013 CRISPR knock-out verification/ knock-out vector check | 40 | 50 °C | *Taq* |
| 1043 | 1044 | 8013 *rnc* complementation | 20 | 53 °C | Q5 |
| 971 | 1049 | 8013 *rnc* complementation verification | 180 | 65 °C | *Taq* |
| 971 | 988 | 8013 *rnc* complementation verification | 180 | 65 °C | *Taq* |
| 970 | 989 | 8013 *rnc* complementation verification | 180 | 65 °C | *Taq* |
| 1047 | 1048 | 8013 tracrRNA complementation | 20 | 53 °C | Q5 |
| 1049 | 989 | 8013 tracrRNA complementation verification | 180 | 65 °C | *Taq* |
| 1048 | 988 | 8013 tracrRNA complementation verification | 180 | 65 °C | *Taq* |
| 1047 | 989 | 8013 tracrRNA complementation verification | 180 | 65 °C | *Taq* |
| 970 | 971 | 8013 *rnc* knock-in verification/ knock-out exclusion | 40 | 64 °C | *Taq* |
| 243 | 1049 | pCompInd integration control | 150 | 50 °C | *Taq* |
| 243 | 242 | pBluescript –SK integration control | 180 | 58 °C | *Taq* |
| 1227 | 1228 | RT-PCR for *cas9* expression and construction of probe for Northern blot | 25 | 52 °C | *Taq* |
| 1229 | 1230 | RT-PCR for *blp* expression and construction of probe for Northern blot | 25 | 52 °C | *Taq* |
| 10487 | 10488 | 8013 cas9 FLAG tagging | 20 | 58°C | *Q5* |
| 10489 | 10490 | 8013 cas9 FLAG tagging | 20 | 58°C | *Q5* |

[a]ID-number 0-9999 according to the AG SCHOEN oligonucleotide collection, 10000-99999 according to the common IMIB oligonucleotide collection

[b] elongation time

[c] annealing temperature ($T_a$)

[d] utilized polymerase: “Q5” for Q5® High-Fidelity DNA polymerase, “*Taq*” for *Taq* polymerase

**Table S5: Mapping statistics for *N. meningitidis* RNA-seq.**

| **Libraries** | **$OD_{600nm}$=0.5 wild-type** | **$OD_{600nm}$=1.5 wild-type** | **$OD_{600nm}$=0.5 Δ*cas9*** | **$OD_{600nm}$=1.5 Δ*cas9*** |
|---|---|---|---|---|
| No. of input reads | 2069152 | 1888269 | 2266894 | 2091807 |
| No. of reads - PolyA detected and removed | 1485504 | 1503893 | 1519607 | 1603801 |
| No. of reads - Single 3' A removed | 134308 | 81813 | 165981 | 111967 |
| No. of reads – Unmodified | 449340 | 302563 | 581306 | 376039 |
| No. of reads - Removed as too short | 113141 | 91638 | 92928 | 122777 |
| No. of reads - Long enough and used for alignement | 1956011 | 1796631 | 2173966 | 1969030 |
| Total no. of aligned reads | 1708913 | 1705711 | 2039178 | 1842931 |
| Total no. of unaligned reads | 247098 | 90920 | 134788 | 126099 |
| Total no. of uniquely aligned reads | 1193216 | 1372567 | 1435239 | 1394628 |
| Total no. of alignments | 3806753 | 3099632 | 4500362 | 3776717 |
| Total no. of split alignments | 0 | 0 | 0 | 0 |
| Percentage of aligned reads (compared to total input reads) | 82,59 | 90,33 | 89,95 | 88,1 |
| Percentage of uniquely aligned reads (in relation to all aligned reads) | 69,82 | 80,47 | 70,38 | 75,67 |

**Table S6: Mapping statistics for *N. meningitidis* cas9 RIP-seq.**

| **Libraries** | **CoIP_0.5 NoFLAG** | **CoIP_0.5 FLAG** | **CoIP_1.5 NoFLAG** | **CoIP_1.5 FLAG** |
|---|---|---|---|---|
| No. of input reads | 1017732 | 444271 | 1335868 | 518063 |
| No. of reads - PolyA detected and removed | 708174 | 414040 | 959516 | 498074 |
| No. of reads - Single 3' A removed | 60375 | 5668 | 69488 | 4006 |
| No. of reads – Unmodified | 249183 | 24563 | 306864 | 15983 |
| No. of reads - Removed as too short | 33075 | 6146 | 35007 | 6568 |
| No. of reads - Long enough and used for alignement | 984657 | 438125 | 1300861 | 511495 |
| Total no. of aligned reads | 956151 | 426319 | 1270362 | 499294 |
| Total no. of unaligned reads | 28506 | 11806 | 30499 | 12201 |
| Total no. of uniquely aligned reads | 447486 | 323761 | 830569 | 413740 |
| Total no. of alignments | 2522861 | 650158 | 2605816 | 647044 |
| Total no. of split alignments | 0 | 0 | 0 | 0 |
| Percentage of aligned reads (compared to total input reads) | 93,95 | 95,96 | 95,1 | 96,38 |
| Percentage of uniquely aligned reads (in relation to all aligned reads) | 46,8 | 75,94 | 65,38 | 82,87 |

**Supplemental Figure Captions**

**Supplemental Figure 1** Mutant construction in *N. meningitidis* strain 8013

Depicted are the CRISPR/Cas loci in 8013 (A) and the pCompInd integration site (B) as well as the linearized plasmids aligned to their point of integration. There is a scale in bp above the genes denoting the place in the genome according to the published genome sequences. Bold white arrows indicate genes and their reading direction, gene fragments are indicated by white boxes. Promotors were predicted by BPROM and are depicted as small angled arrows. Terminators were predicted by FindTerm and are depicted as loops. Loci of non-coding RNAs are indicated by white boxes with angled arrows. Black boxes indicate non-neisserial DNA in particular resistance cassettes. Vertical lines indicate CRISPR spacer. Drawn to scale.