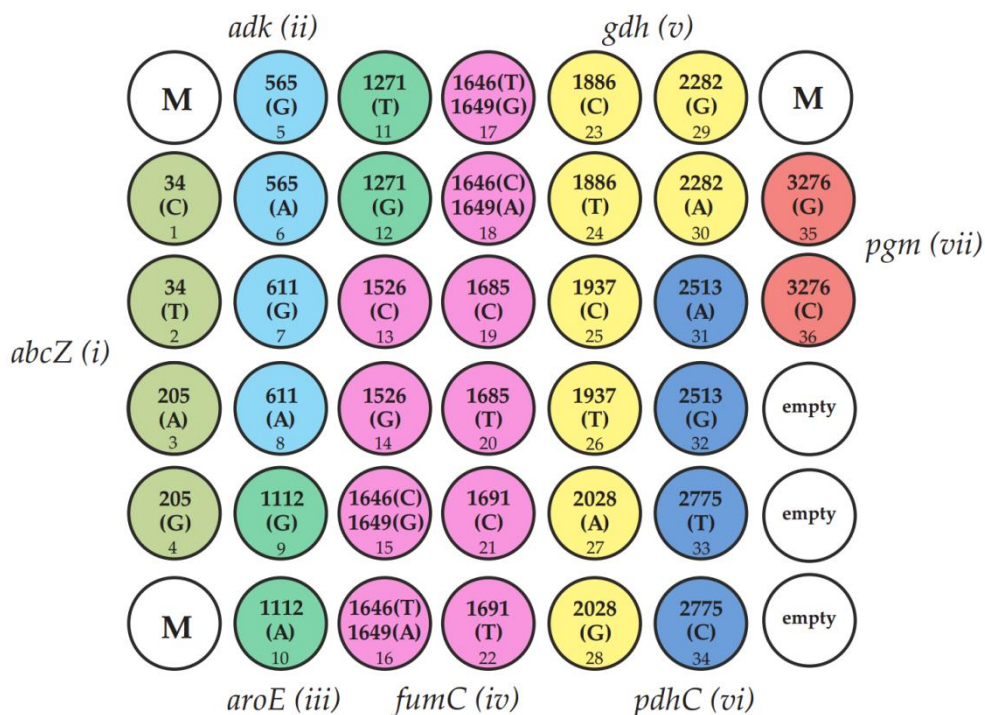


Supplementary File S1. Identifying informative polymorphisms in *Neisseria gonorrhoeae* MLST-related genes using a microarray-based assay

Microarray layout



List of oligonucleotides immobilized on the microarray elements

	Gene	Set	Position of analyzed nucleotide	Probe sequence, 5'-3'	Length, b.p.
1	<i>abcZ</i>	i	34(C)	GGCGAAATTCGCGATTTATT	20
2	<i>abcZ</i>		34(T)	GGCGAAATTTGCGATTTATT	20
3	<i>abcZ</i>		205(A)	GCCGGAACAAAAAATCG	20
4	<i>abcZ</i>		205(G)	GCCGGAACGAAAAAATCG	20
5	<i>adk</i>	ii	565(G)	GTACATTGGCGCAAGCCGAA	20
6	<i>adk</i>		565(A)	GTACATTGGCACAAGCCGAA	20
7	<i>adk</i>		611(G)	GACGCGGTCGTTGAAATCGA	20
8	<i>adk</i>		611(A)	GACGCGGTCATTGAAATCGA	20
9	<i>aroE</i>	iii	1112(G)	GGCGGATGTGAACGGCGGTT	20
10	<i>aroE</i>		1112(A)	GGCGGATGTAAACGGCGGTT	20
11	<i>aroE</i>		1271(T)	CGACAATCGGGTGCGAAAAAA	21
12	<i>aroE</i>		1271(G)	CGACAATCGGGGCGAAAAAA	21
13	<i>fumC</i>	iv	1526(C)	AATCAAAATCCCCGAAAACG	20
14	<i>fumC</i>		1526(G)	AATCAAAATGCCCGAAAACG	20
15	<i>fumC</i>		1646(GC)	TATGGCGGGCGCGTCGGGCAA	21
16	<i>fumC</i>		1646(TA)	TATGGCGGGTGCATCGGGCAA	21
17	<i>fumC</i>		1649(TG)	TATGGCGGGTGCATCGGGCAA	21

18	<i>fumC</i>		1649(CA)	TATGGCGGGCGCATCGGGCAA	21
19	<i>fumC</i>		1685(C)	TATGCCCCGTCATCGCCTACA	20
20	<i>fumC</i>		1685(T)	TATGCCCCGTTATCGCCTACA	20
21	<i>fumC</i>		1691(C)	CCGTTATCGCCTACAACCTC	20
22	<i>fumC</i>		1691(T)	CCGTTATCGCTTACAACCTC	20
23	<i>gdh</i>	v	1886(C)	TAAATACATCGAAAGCGTGC	20
24	<i>gdh</i>		1886(T)	TAAATACATTGAAAGCGTGC	20
25	<i>gdh</i>		1937(C)	AGAGCGCGGCGAGTTTTACG	20
26	<i>gdh</i>		1937(T)	AGAGCGCGGTGAGTTTTACG	20
27	<i>gdh</i>		2028(A)	GCCCCCGCCAGCTTGGATG	19
28	<i>gdh</i>		2028(G)	GCCCCCGCCGGCTTGGATG	19
29	<i>gdh</i>		2282(G)	ACGCATGGCGGGCAAAGTGG	20
30	<i>gdh</i>		2282(A)	ACGCATGGCAGGCAAAGTGG	20
31	<i>pdhC</i>	vi	2513(A)	CGTGGAAGCAGACATCTGGT	20
32	<i>pdhC</i>		2513(G)	CGTGGAAGCGGACATCTGGT	20
33	<i>pdhC</i>		2775(T)	CTGCGCCGCTTCTTTGAAGT	20
34	<i>pdhC</i>		2775(C)	CTGCGCCGCCTCTTTGAAGT	20
35	<i>pgm</i>	vii	3276(G)	GCCAAAGCCGAATTTGAAGG	20
36	<i>pgm</i>		3276(C)	GCCAAAGCCCAATTTGAAGG	20
empty	Empty gel	-	-	-	-
M	Marker elements	-	-	-	-

Multiplex PCR

Two rounds of multiplex PCR were performed to amplify the *abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC*, and *pgm* loci. The reaction mixture consisted of DNA polymerase (SibEnzyme, Russia), dNTPs (Evrogen, Russia), fluorescently labeled Cy5-dUTP (Lumiprobe, Russia), and the corresponding forward and reverse primers (refer to the Table below). The template used for the first round of amplification was *N. gonorrhoeae* genomic DNA (1 µl per mixture). PCR was performed using an S1000 thermal cycler (Bio-Rad, USA) with the following conditions: initial denaturation at 95°C for 5 minutes, followed by 15 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 30 seconds.

For the second round of amplification, PCR fragments obtained during the first amplification round (1 µl each) were used as a template. PCR was performed with the following conditions: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 30 seconds.

The mixtures were supplemented with forward and reverse primers for the second round of amplification (Table below). To obtain predominantly single-stranded DNA fragments, excess reverse primers were added. The mixtures of PCR products, which contained mainly single-stranded fluorescently labeled fragments after the second round of amplification, were combined and utilized for hybridization on microarrays.

Primers for the first amplification round

	Direction	Gene	Sequence, 5'-3'	Concentration in the reaction mixture (mM)
1	for	<i>abcZ</i>	AATCGTTTATGTACCGCAGG	2
2	rev	<i>abcZ</i>	GAGAACGAGCCGGGATAGGA	2
3	for	<i>adk</i>	AGGCAGGCACGCCCTTGG	2
4	rev	<i>adk</i>	CAATACTTCGGCTTTCACGG	2
5	for	<i>aroE</i>	GCGGTCAATACGCTGGTG	2
6	rev	<i>aroE</i>	ATGATGTTGCCGTACACATA	2
7	for	<i>fumC</i>	TCCGGCTTGCCGTTTGTGTCAG	2
8	rev	<i>fumC</i>	TTGTAGGCGGTTTTGGCGAC	2
9	for	<i>gdh</i>	CCTTGGCAAAGAAAGCCTGC	2
10	rev	<i>gdh</i>	GCGCACGGATTTCATTCGG	2
11	for	<i>pdhC</i>	TCTACTACATCACCCCTGATG	2
12	rev	<i>pdhC</i>	ATCGGCTTTGATGCCGTATTT	2
13	for	<i>pgm</i>	CGGCGATGCCGACCGCTTGG	2
14	rev	<i>pgm</i>	GGTGATGATTTCGGTTGCGCC	2

Primers for the second amplification round

	Direction	Gene	Sequence, 5'-3'	Concentration in the reaction mixture (mM)
1	for	<i>abcZ</i>	AATCGTTTATGTACCGCAGG	1
2	rev	<i>abcZ</i>	CCAGTAACAATACATCGGGC	15
3	for	<i>adk</i>	GGTCAAAGAACGCATCGCG	1
4	rev	<i>adk</i>	GTAGGTAACGTGGTAAGTACGG	10
5	for	<i>aroE</i>	ATTGCCAACCGTACCCG	1
6	rev	<i>aroE</i>	AGAGGGCGTAGGAAGCC	10
7	for	<i>fumC</i>	CCTGAACAAAATCGCCAACG	1
8	rev	<i>fumC</i>	AGTTTTTCGTTGAAGCTGTTGC	10
9	for	<i>gdh</i>	CCTTGGCAAAGAAAGCCTGC	1
10	rev	<i>gdh</i>	GACAGATTTCGATGGTCAGCG	10
11	for	<i>pdhC</i>	TATGCCGACCGTATCCGC	1
12	rev	<i>pdhC</i>	ATCGGCTTTGATGCCGTATTT	10
13	for	<i>pgm</i>	AAGTGTTGGACAACCTGCC	1
14	rev	<i>pgm</i>	GGAAGCACGCATCAGACC	20

Microarray hybridization

The Multiplex PCR product mixture (20 μ L) was added to 10 μ L of hybridization buffer (0.3 M HEPES (pH 7.5), 3.0 M guanidine thiocyanate, 30 mM EDTA). The microarray hybridization chambers were filled with the mixtures (30 μ L), and the microarrays were incubated at 37°C for 6-12 hours. After incubation, the hybridization chambers were removed, and the microarray surfaces were washed three times with distilled water and air-dried.