Supporting Information

Table S1. P5 sequence in predicted extracellular loops 1 to 5 for representative NTHi isolates.

		NTHi isolate														
		Patient 1			Patient 2		Patient 3			Patient 4		Patient 5				Patient 6
P5	**Loop sequence**	**1a**	**1b**	**1c**	**2a**	**2b**	**3a**	**3b**	**3c**	**4a**	**4b**	**5a**	**5b**	**5c**	**5d**	**6a**
Loop1	GQASFHDGVHALHDGVHALARQNKAGYHRNS							X	X							
	GQGSFHDGINNNGAIKQDLEL--GYGHRRNT	X	X	X	X	X										
	GQGSFHDGINNNGAIKENLTS--GYGYRRNT						X									
	GQGSFHDGINNNGAIQKDLEL--GYGYRRNT												X	X		
	GQGSFHDGINRNRVIIDGLPGDYG---RRNT											X				
	GQGSFHDGINNNGAIKNNLPGY--YGYRRNT									X						
	GQASFHDGINNNGAIKKDLLGLASYGYRRNT										X					
	GQASFHDGLRALARENKV-------GYHRNS														X	
	GQGSFHDGINNNGAIKQNLSSAN-YGYRRNT															X
Loop2	DNFGRAKLRSNGQALAKHTN						X									
	DDFGRAKFRVAGKPKVKHTN							X	X							
	DNFGRAKLREAGKPRAKHTN	X	X	X	X	X										
	DDFGRAKGREKGRTVAKHTN												X	X		
	DNFGRVKARQERKTLAKHTN									X		X				
	DDFGRVKLRLAGKPKVKHTN										X					
	DDFGRAKGREKGKTVAKHTN														X	
	DNFGRVKLREAGKPKAKHTN															X
Loop3	VRSDYKFY----GTRDHKKGR							X	X							
	VRSDYKFYEVANGTRERKKGR									X		X				
	VRSDYKVYEVADGTRDHKKGR						X									
	VRSDYKFYADANGTRDHKKGR	X	X	X	X	X										
	VRSDYKLYNKNSSTLKDLGEH													X		
	VRSDYKFYDKNGSTFKNPGEH												X			
	VRSDYKFYEDANGTRDHKKGR										X					
	VRSDYKLYNKNSSSNSTLKNL														X	
	VRSDYK-YEVANGTRDHKKGR															X
Loop4	LTRVGKYRAQDK						X									
	LTRVGKYRPQDK	X	X	X	X	X		X	X		X		X	X		X
	LTRVGKYRTQKT														X	
	LTRVGKFRTQDK									X		X				
Loop5	RFGQGAAP-VVAAPEVVSKT	X	X	X	X	X	X	X	X	X	X	X	X			
	RFGQGEAP-VVAAPEMVSKT															X
	RFGQ-AAP-VVAAPEMVSKT													X		
	RFGQGAAP-VVAAPEMVSKT														X	

Table S2. Self-aggregation of NTHi strains (t=h).

Patient	Strain	0	1	1.5	2	3	4	5	6	7
	E. coli C600	1,03±0,04	0,99±0,05	0,97±0,04	0,97±0,03	0,99±0,03	0,98±0,02	0,98±0,03	1±0,04	0,990±05
1	NTHi1a	1,02±0,05	0,50±0,06[b]	0,25±0,06[b]	0,14±0,06	0,07±0,03	0,06±0,01	0,04±0,01	0,03	0,03
	NTHi1b	1,06±0,03	0,54±0,10[b]	0,31±0,12[b]	0,17±0,09	0,08±0,02	0,06±0,01	0,04	0,04	0,04
	NTHi1c	1,01± 0,08	0,45±0,09[b]	0,21±0,02[b]	0,1±0,04	0,05± 0,02	0,04	0,04	0,04±0,01	0,02
2	NTHi2a	1,02±0,03	0,30±0,07	0,14±0,04	0,06±0,03	0,04±0,01	0,03±0,01	0,03±0,02	0,03±0,02	0,02±0,01
	NTHi2b	1,04±0,02	0,27±0,15	0,1±0,05	0,06±0,01	0,04±0,02	0,03±0,02	0,02±0,02	0,03±0,02	0,02±0,01
3	NTHi3a	1,03±0,04	1,01±0,05[a]	1,04±0,02[a]	0,98±0,05[a]	0,94±0,07[a]	0,98±0,06[a]	0,92±0,1[a]	0,88±0,08[a]	0,89±0,07[a]
	NTHi3b	1,04±0,04	1,02±0,05[a]	1±0,06[a]	1,02±0,07[a]	0,92±0,08[a]	0,89±0,18[a]	0,86±0,23[a]	0,84±0,18[a]	0,85±0,22[a]
	NTHi3c	1,02±0,06	1,03±0,03[a]	1,04±0,06[a]	1,02±0,04[a]	0,96±0,02[a]	0,96±0,04[a]	0,94±0,03[a]	0,92±0,03[a]	0,93±0,03[a]
5	NTHi5b	1,05±0,01	0,41±0,08	0,20±0,02	0,11±0,02	0,07±0,01	0,05±0,02	0,03±0,02	0,02±0,02	0,02±0,02
6	NTHi6a	1,03±0,04	0,83±0,11[c]	0,58±0,17[c]	0,33±0,14	0,13±0,04	0,08±0,02	0,05±0,01	0,04±0,02	0,03±0,02

[a] OD_{600nm} values for series isolated from patient 3 were significantly higher ($p<0.0001$ from 1 h to the end of the assay) than those obtained for the other NTHi strains tested.

[b] OD_{600nm} values for NTHi1a, 1b and 1c were significantly higher than those obtained for NTHi2a and 2b at 1 ($p<0.0001$) and 1,5 h ($p<0.001$).

[c] NTHi6a presented OD_{600nm} values higher than those obtained for strains from patients 1 and 2 at 1-1.5 h ($p<0.0001$).

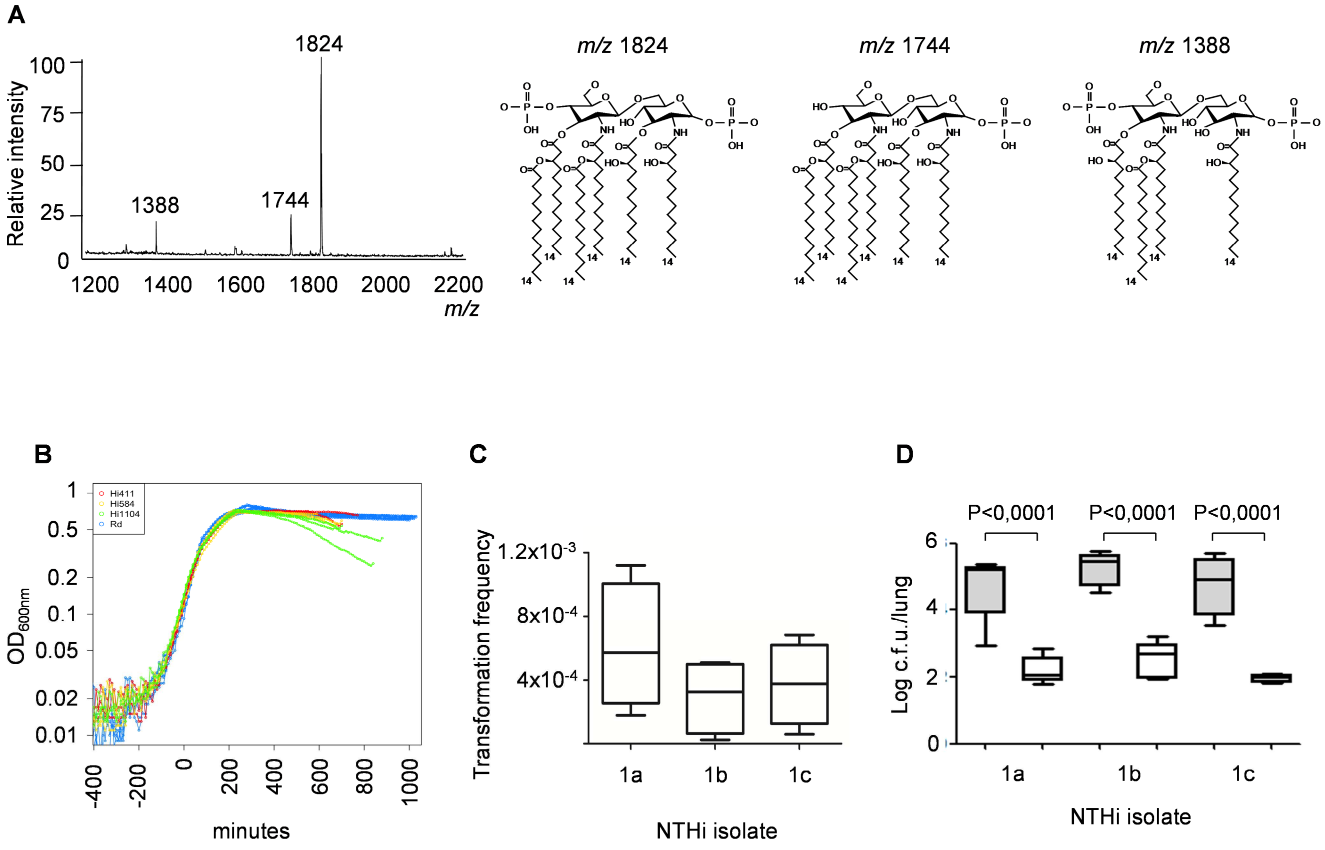
Table S3. Lipid A structures identified in the NTHi isolates analyzed.

$[M-H]^-$	Fatty acid substitutions			
	C3’	C2’	C3	C2
1824	14:0[3-O(14:0)]	14:0[3-O(14:0)]	14:0(3-OH)	14:0(3-OH)
1744	14:0[3-O(14:0)]	14:0[3-O(14:0)]	14:0(3-OH)	14:0(3-OH)
1388	OH	14:0[3-O(14:0)]	14:0(3-OH)	14:0(3-OH)

Table S4. Summary statistics for draft genome assemblies.

Features	**RR3131**	**NTHi1a**	**NTHi1b**	**NTHi1c**
# of reads	2,349,021	13,668,344	3,420,997	6,535,653
% overlapped	19.7%	40.1%	39.9%	29.4%
# contigs	21	23	17	16
total length (bp)	1,891,683	1,787,944	1,794,570	1,803,206
N50 (bp)	198,461	224,231	246,701	247,076
median (bp)	36,044	24,844	38,138	51,313
largest contig (bp)	414,414	507,594	426,945	508,640

Figure S1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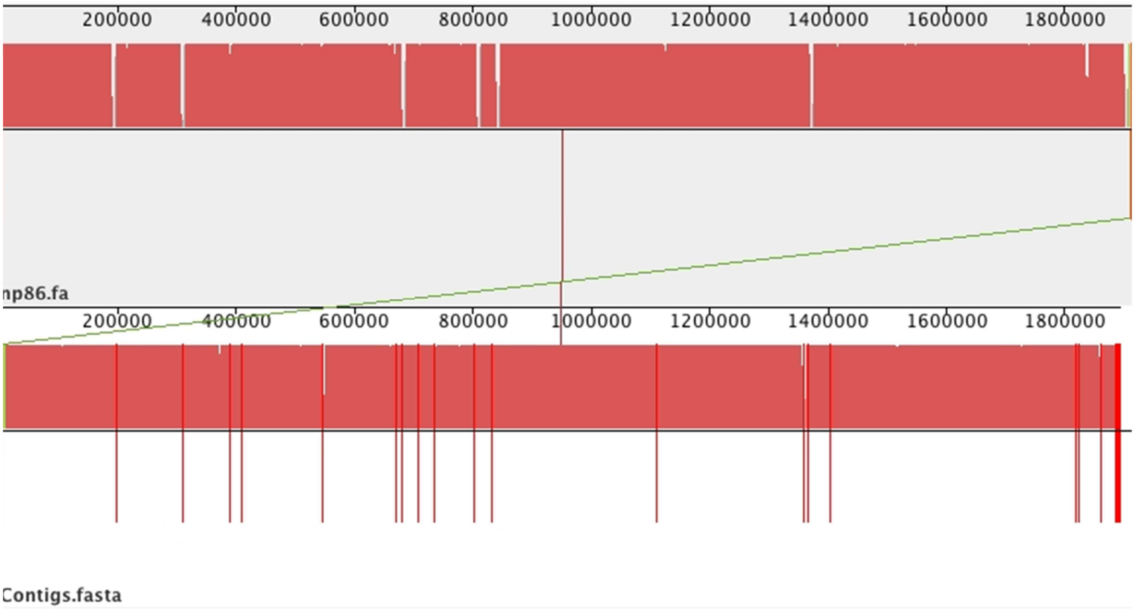
1388
1744
Relative intensity
100
75
50
25
0
1200
1400
1600
1800
2000
2200
m/z
m/z 1824
m/z 1744
m/z 1388
B
Hi411
Hi584
Hi1104
Rd
OD600nm
1
0.5
0.2
0.1
0.05
0.02
0.01
-400
-200
0
200
400
600
800
1000
minutes
C
Transformation frequency
1.2x10-3
8x10-4
4x10-4
1a
1b
1c
NTHi isolate
D
P<0,0001
P<0,0001
P<0,0001
Log c.f.u./lung
6
4
2
0
1a
1b
1c
NTHi isolate

Figure S2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1000000
1200000
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1600000
1800000
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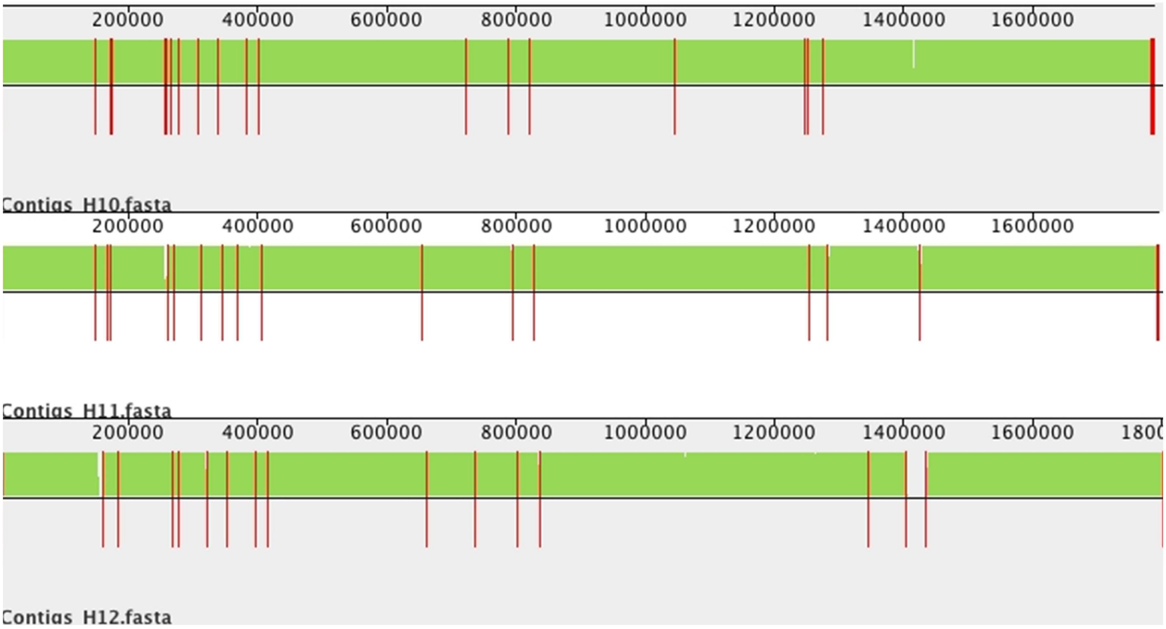
400000
600000
800000
1000000
1200000
1400000
1600000
1800000
Contigs.fasta

Figure S3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800000
1000000
1200000
1400000
1600000
Contigs H10.fasta
200000
400000
600000
800000
1000000
1200000
1400000
1600000
Contigs H11.fasta
200000
400000
600000
800000
1000000
1200000
1400000
1600000
180
Contigs H12.fasta

Figure S4

```
NTHi2a SapC          MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
NTHi2b               MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
NTHi1c               MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
NTHi1b               MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
NTHi1a               MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
NTHi3a               MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
NTHi3b               MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
NTHi3c               MQNKEPDEFRESTSIFQIWLRFRQNTIALFSFYLLIALIFTALFASYLAPYADNRQFIGQ 60
                     ************************************************************

NTHi2a               ELMPPSWVDRGKIAFFFGADDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
NTHi2b               ELMPPSWVDRGKIAFFFGADDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
NTHi1c               ELMPPSWVDRGKIAFFFGADDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
NTHi1b               ELMPPSWVDRGKIAFFFGADDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
NTHi1a               ELMPPSWVDRGKIAFFFGTDDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
NTHi3a               ELMPPSWVDRGKIAFFFGTDDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
NTHi3b               ELMPPSWVDRGKIAFFFGTDDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
NTHi3c               ELMPPSWVDRGKIAFFFGTDDLGRDILSRLIMGTRYTLGSALLVVFSVAIIGGALGIIAG 120
                     ******************:*****************************************

NTHi2a               LLKGIKARFVGHIFDAFLSLPILLIAVVISTLMEPSLWNAMFATLLAILPYFIHTIYRAI 180
NTHi2b               LLKGIKARFVGHIFDAFLSLPILLIAVVISTLMEPSLWNAMFATLLAILPYFIHTIYRAI 180
NTHi1c               LLKGIKARFVGHIFDAFLSLPILLIAVVISTLMEPSLWNAMFATLLAILPYFIHTIYRAI 180
NTHi1b               LLKGIKARFVGHIFDAFLSLPILLIAVVISTLMEPSLWNAMFATLLAILPYFIHTIYRAI 180
NTHi1a               LLKGIKARFVGHIFDAFLSLPILLIAVVISTLMEPSLWNAMFATLLAILPYFIHTIYRAI 180
NTHi3a               LLKGIKARFVGHIFDAFLSLPILLIAIVISTLMEPSLWNAMFATLLAILPYFIHAIYQAI 180
NTHi3b               LLKGIKARFVGHIFDAFLSLPILLIAIVISTLMEPSLWNAMFATLLAILPYFIHAIYQAI 180
NTHi3c               LLKGIKARFVGHIFDAFLSLPILLIAIVISTLMEPSLWNAMFATLLAILPYFIHAIYQAI 180
                     **************************:***************************:**:**

NTHi2a               QKELEKDYVVMLKLEGISNQALLKSTILPNITVIYIQEVAHAFVIAVLDISALSFISLGA 240
NTHi2b               QKELEKDYVVMLKLEGISNQALLKSTILPNITVIYIQEVAHAFVIAVLDISALSFISLGA 240
NTHi1c               QKELEKDYVVMLKLEGISNQALLKSTILPNITVIYIQEVAHAFVIAVLDISALSFISLGA 240
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NTHi3a               QQELKKDYVLMLKLEGISNWELLKSTILPNITVIYIQEVARAFVIAVLDISALSFISLGA 240
NTHi3b               QQELKKDYVLMLKLEGISNWELLKSTILPNITVIYIQEVARAFVIAVLDISALSFISLGA 240
NTHi3c               QQELKKDYVLMLKLEGISNWELLKSTILPNITVIYIQEVARAFVIAVLDISALSFISLGA 240
                     *:**:****:*********  *******************:*******************

NTHi2a               QRPTPEWGAMIKDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKAINQHQE 295
NTHi2b               QRPTPEWGAMIKDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKAINQHQE 295
NTHi1c               QRPTPEWGAMIKDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKAINQHQE 295
NTHi1b               QRPTPEWGAMIKDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKAINQHQE 295
NTHi1a               QRPTPEWGAMIKDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKAINQHQE 295
NTHi3a               QRPTPEWGAMIRDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKTINQHQE 295
NTHi3b               QRPTPEWGAMIRDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKTINQHQE 295
NTHi3c               QRPTPEWGAMIRDSLELLYLAPWTVLLPGFAIIFTILLSIIFSNGLTKTINQHQE 295
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```

Figure S5

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NTHi1b KdkA MHQFQQDNQYFIFNFDRTFEQATEFFQAEFWQKQERVIGSAKGRGTTYFLQTEDWFGVNC 60
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NTHi1a      MHQFQQDNQYFIFNFDRTFEQATEFFQAEFWQKQERVIGSAKGRGTTYFLQTEDWFGVNC 60
NTHi2a      MHQFQQDNQYFIFNFDRTFEQATEFFQAEFWQKQERVIGSAKGRGTTYFLQTEDWFGVNC 60
NTHi2b      MHQFQQDNQYFIFNFDRTFEQATEFFQAEFWQKQERVIGSAKGRGTTYFLQTEDWFGVNC 60
NTHi3b      MHQFQQDNQYFIFNFDRTFDQATEFFQAEFWQKQERLIGSAKGRGITYFLQTEDWFGVNC 60
NTHi3c      MHQFQQDNQYFIFNFDRTFDQATEFFQAEFWQKQERLIGSAKGRGITYFLQTEDWFGVNC 60
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NTHi1b      ALRHYYRGGLWGKLNKDRYRFSALDTTRSFAEFHLLQRLYEAGLPVPKPIAARIQKGKLG 120
NTHi1c      ALRHYYRGGLWGKLNKDRYRFSALDTTRSFAEFHLLQRLYEAGLPVPKPIAARIQKGKLG 120
NTHi1a      ALRHYYRGGLWGKLNKDRYRFSALDTTRSFAEFHLLQRLYEAGLPVPKPIAARIQKGKLG 120
NTHi2a      ALRHYYRGGLWGKLNKDRYRFSALDTTRSFAEFHLLQRLYEAGLPVPKPIAARIQKGKLG 120
NTHi2b      ALRHYYRGGLWGKLNKDRYRFSALDTTRSFAEFHLLQRLYEAGLPVPKPIAARIQKGKLG 120
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NTHi3c      ALRHYYRGGLWGKLNKDRYRFSALETTRSFAEFHLLQRLYEAGLPVPKPIAARIQKGKLG 120
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NTHi1c      ICYQADILTEKIENAQDLTALLQTQTLPKETWRQIGRLIRKLHDLQICHTDLNAHNILLQ 180
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NTHi2a      ICYQADILTEKIENAQDLTALLQTQTLPKETWRQIGRLIRKLHDLQICHTDLNAHNILLQ 180
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NTHi3b      ICYQADILTEKIENAQDLTALLQTQTLPKETWTQIGRLIRKLHDLQICHTDLNAHNILLQ 180
NTHi3c      ICYQADILTEKIENAQDLTALLQTQTLPKETWTQIGRLIRKLHDLQICHTDLNAHNILLQ 180
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NTHi2b      QAEQEQKCWLLDFDKCGEKSGDFWKAQNLNRLKRSFEKEVGRMNIQFTEQNWADLTSAYHQ 241
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