

Fur-Mediated Activation of Gene Transcription in the Human Pathogen
Neisseria gonorrhoeae

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Running title: Fur functions as a transcriptional activator

Keywords: Fur, transcriptional activation, *Neisseria gonorrhoeae*

Supplementary Table 1. The primers used in RT-PCR of the putative Fur activated genes.

Primers	Sequence	Replicon size
rmp_Fw	GTTACGATGCTGCGGATTTT	407 bp
rmp_Rv	GAAACCATTTCCCTGTCTGC	
N37_368_Fw	TGTCGCAAGTCGATATTTCC	223 bp
N37_590_Rv	TTGATTTCTTTACGCCTTG	
N43_RT_Fw	AAATGGCTGGATACGCAACT	176 bp
N43_RT_Rv	GCATCGACGTTGTTCTGTTC	
76_RT_FW	GCTCACAACCGGAGTATGG	186 bp
76_RT_Rv	TGGATTTGATTTFCGGTTTCA	
N199_22_Fw	ACCCTGCACATTTCCAAACT	235 bp
N199_256_Rv	TTTGAGTGGGCGAGACATAG	
N233_21_Fw	ACTGATTGCCCTCGCACT	235 bp
N233_255_Rv	GCCGATGCTGTAAAGTTTGA	
N376_41_Fw	AACTCGATTTTCGACAAAGCC	216 bp
N376_256_Rv	CCATGGCGATGGTGTATTTA	
432_RT_FW	TGGAAACTGGAAACTTCCCT	179 bp
432_RT_Rv	GTCAGTCCGTAGCGCAAAC	
N575_19_Fw	AACCAAACACCAAACGACCT	209 bp
N575_227_Rv	CCCATACCGAAGCCTATTTTC	
711_RT_FW	TCGTATCCTCGGTCATGAAG	190 bp
711_RT_Rv	CGTGCCGTCAATCATATAGC	
N775_1908_Fw	GGGTAAAGGCATGATCCAGT	212 bp
N775_2119_Rv	ACACCATCGCCAAAGTCATA	
N794_RT_Fw	GGAAGAAGAAACCACACACG	195 bp
N794_RT_Rv	TAGTCTTGAGCCTCTTCGCA	
N904_RT_Fw	GGTCAGCCTGCCTATTCATC	175 bp
N904_RT_Rv	CCGCTTTGGATTTCGTATTCT	
1205_RT_FW	TTCGGCAACTACATTTACGC	196bp
1205_RT_Rv	TCAGACGGCCTCGTACATAG	
N1207_RT_Fw	TATGTCGAGAGCCTGTCTGC	166 bp
N1207_RT_Rv	GGTAGTCGTGGATTTCCGTT	
1275_RT_Fw	ATTGGGCAAATACGTCTTCC	177 bp
1275_RT_Rv	GATTGGATGTGCCAAGTACG	
N1276_RT_Fw	TGGAATACCGCTACTGGACA	208 bp
N1276_RT_Rv	GCTTTGAAGCTGAATGTGGA	
1282_RT_Fw	TGGAGGTGGTAAGATTGTGC	195bp
1282_RT_Rv	AACGTCCGGCAGACATAAA	
1290_RT_Fw	GCATCCTGTTTCAGCAAGGTA	200bp
1290_RT_Rv	AGAAGGTCAGACACATGGCA	
1317_RT_Fw	GACGGCGTAAAGGCAGAC	175 bp
1317_RT_Rv	GGGCTATCGGCATAAACAAT	
1430_RT_Fw	CCACATCTCCAAACTCAACG	183 bp
1430_RT_Rv	TCGTGTTTGAGCGAGAAATC	
N1685_306_Fw	CCAATTCAACACCTTTGTCTG	207 bp
N1685_512_Rv	ATAAATTCATCGCTGCTTGC	
N1751_RT_Fw	CAAGGATGAAGTTCGACGTG	153 bp
N1751_RT_Rv	TCAGGACGACGATAAACACC	
N1788_902_Fw	GTCTGACCACGCACGAATAC	239 bp
N1788_1140_Rv	GGCTTCTTCATAGCCTGTCTG	
N1789_206_Fw	AAGAAATCGCAAGCCTCAAC	224 bp
N1789_429_Rv	TGCGTACATTTCCGCATC	
N1842_RT_Fw	AATCTTCGAACTGGCTACCG	185 bp
N1842_RT_Rv	CTTTCAGACCGACGATTTCA	

1850_RT_Fw	GCGAACATCCGATTATGTTG	164 bp
1850_RT_Rv	TGGAACGTGTACTGCCATTT	
N1851_3956_Fw	GTTTCGGTGAGATGGAGGTT	208 bp
N1851_4163_Rv	TCGATATCCAAACCCAGTGA	
1859_RT_Fw	CTTCGTCAATCAGGATGCAG	178 bp
1859_RT_Rv	ATTACCGACGAGTGCATCAA	
N1865_RT_Fw	TGCTTCAGGATGAAGATTTCC	199 bp
N1865_RT_Rv	GACACGGTAGGAATGCCTTT	
1913_RT_Fw	CCGCACAACAAGCAAATAT	244 bp
1913_RT_Rv	AAGATGCCTTTACGCTTTGC	
N1937_99_Fw	TCGGTGGGTATCACTGATGT	248 bp
N1937_346_Rv	CAATCCAACCTGCAAATCCTG	
1957_RT_Fw	ATCGTAATCGGTTTCGGTTTC	185 Bp
1957_RT_Rv	CCGATGAGGTAAAGGACGAT	
N1960_101_Fw	GCGAGGATGATGACAAACAG	158 bp
N1960_258_Rv	AGCGGGACCGTGTTTAAG	
N2011_RT_Fw	AAGTGTCCGCTATGTCGAA	180 bp
N2011_RT_Rv	GGAACAAGGCTTCATTGGTT	
2068_RT_Fw	AAACCTGATTATCCGCCCTA	173 bp
2068_RT_Rv	ACCAGATATGTTTCGGGCAGT	
2116_RT_Fw	TTGATTGAAGGACGGGATTT	181 bp
2116_RT_Rv	AAACCAAATCGCGAATAACC	
2137_RT_Fw	AAATTCAAGACGGCGAAATC	187 bp
2137_RT_Rv	TGAAACACCATGCCGATATT	
N2162_181_Fw	CAACAAACATTCCTCGATGC	212 bp
N2162_392_Rv	GTCGGATCGATGAAATACCA	

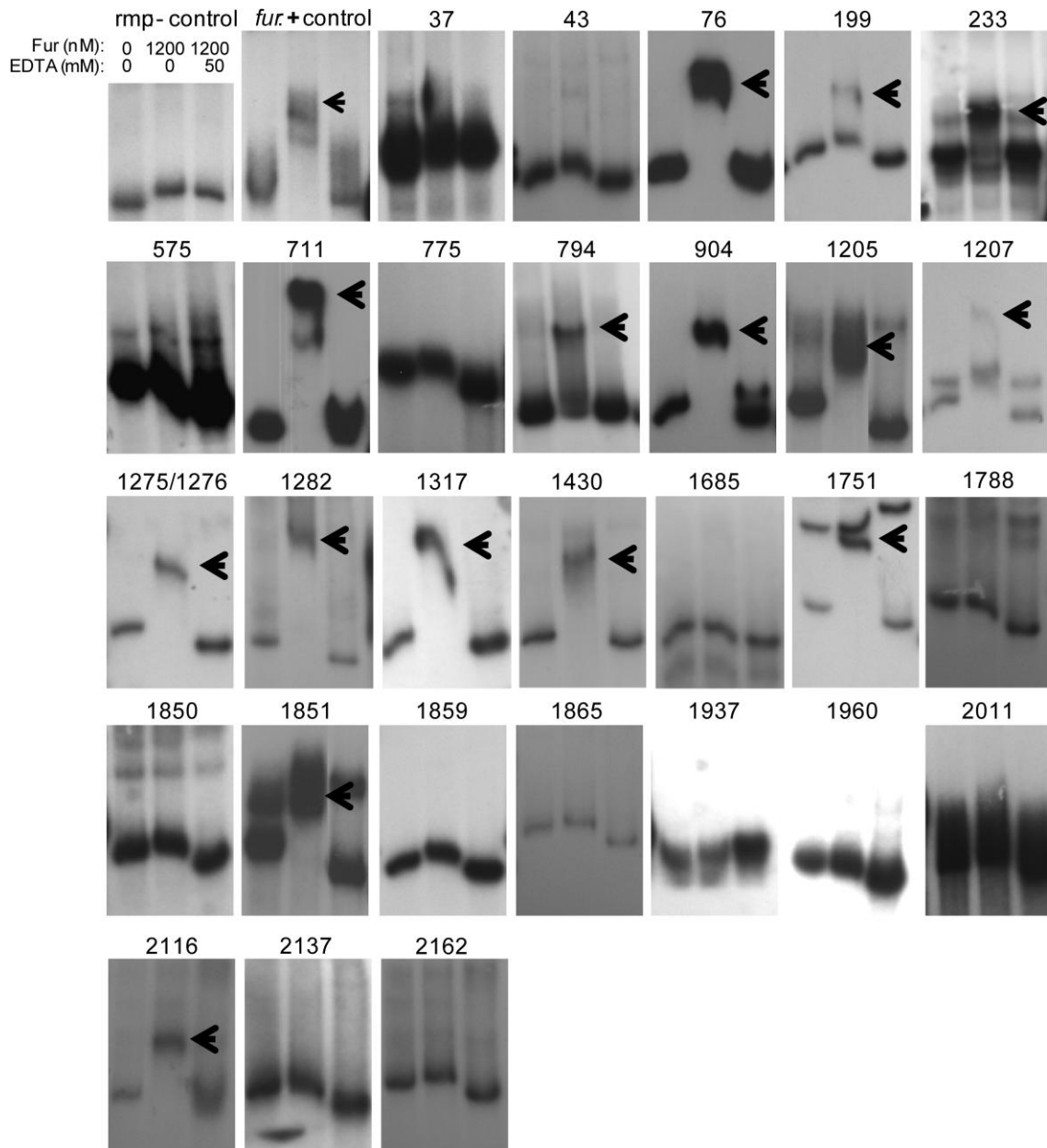
Supplementary Table 2. The primers used for amplifying the probes containing the putative promoter region of each gene for EMSA.

Primers	Sequences	Length of probe	Positions*
fur_1300_BamHI_Fw	GGTCAGGATCCTGCGTTCTTTGAT	505 bp	-305~+200
fur_HindIII_R3	TTTAAGCTTTCATAATATTCAA		
rmp-BamHI_Fw	CGGGATCCGAATTCCTATCCGATTTGCCG	100 bp	-95~-1
rmp_pHindIII_Rv	CCAAGCTTTTATTCCCTCATTAGATTTGTA		
37_pBamHI_Fw	TTTGGATCCGCATAACAGCCGA	504 bp	-491~+3
37_pHindIII_Rv	GGGAAGCTTCATGGTTTGTGTC		
43_pBamHI_Fw	GGGGGATCCATACAAACAGAAAG	527 bp	-500~+17
43_pHindIII_Rv	GGGAAGCTTATTTGTTGGTAGGG		
76_pBamHI_Fw	TTTGGATCCAAACCGCCGCAATC	436 bp	403~+23
76_pHindIII_Rv	TTTAAGCTTCTGCCGGTTCGGTCTG		
199_pBamHI_Fw	TTTGGATCCCTCGAACGACATGA	527 bp	-500~+17
199_pHindIII_Rv	TTTAAGCTTAATTCGGAGACGTG		
233_pBamHI_F4	TTTGGATCCAAACCCGCCCGAAC	452 bp	-413~+29
233_pHindIII_R2	GGGAAGCTTGCAATCAGTGC GGCAAG		
575_pBamHI_Fw	TTTGGATCCAGGCAGAGCAGCTT	527 bp	-500~+17
575_pHindIII_Rv	TTTAAGCTTTCGGGGGTGTCTGT		
711_pBamHI_Fw	TTTGGATCCGCCACAACGACA	467 bp	-397~+60
711_pHindIII_Rv	TTTAAGCTTCGGGCGGGGTTTTTC		
775_pBamHI_Fw	TGTGGATCCGGGCGGCGATATC	503 bp	-492~-1
775_pHindIII_Rv	GGGAAGCTTCATGGATACTCTC		
794_pBamHI_F	AGATGGATCCTTAGTTTTAGCAGC	233 bp	-210~+13
794_pHindIII_R	CAAAAGCTTGATTGCCTTGCAT		
869_pBamHI_Fw	TTTGGATCCGCGAAGCCTTCTAT	527 bp	-500~+17
869_pHindIII_Rv	GGGAAGCTTATCACAGAAGCAAG		
904_pBamHI_Fw	GGGGGATCCGCCAAATAACTGCT	527 bp	-500~+17
904_pBamHI_Fw	GGGAAGCTTGGAATGGTTGCGCT		
1189_pBamHI_Fw	TTTGGATCCGGGCGGGGGCTC	525 bp	-500~+15
1189_pHindIII_Rv	GGGAAGCTTGGCGGTTTGATT		
1205_pBamHI_Fw	TTTGGATCCGCACGGCGACTTCCTG	503 bp	-477~+16
1205_pHindIII_Rv	TTTAAGCTTGGCTTCTCGTCGATT		
1207_pBamHI_F2	TTTGGATCCGGCAGGCGATTTTG	484 bp	-472~-2
1207_pHindIII_R2	TTTAAGCTTGGATGCCGCTGAA		
1275_pBamHI_Fw	TTTGGATCCAGAAAAGTAGGGGG	527 bp	-500~+17
1275_pBamHI_Fw	GGGAAGCTTATATCGTCTTTCGT		
1282_pBamHI_Fw	TTTGGATCCGTTACGGTGGTGA	527 bp	-500~+17
1282_pHindIII_Rv	GGGAAGCTTGATGCCGTCTGAA		
1317_pBamHI_Fw	TTTGGATCCCACGCCGCGACA	526 bp	-500~+16
1317_pHindIII_Rv	GGGAAGCTTAGTGTGTTATCTT		
1430_pBamHI_Fw	GGGGGATCCGCCAAAGCCGAAGCAC	457 bp	-393~+54
1430_pHindIII_Rv	GGGAAGCTTGGCGCAACGGAAAA		
1685_pBamHI_F2	AAAGGATCCGCGCCTGAAATT	476 bp	-473~-8
1685_pHindIII_R	AAAAAGCTTTCGGAAAGGC		
1751_pBamHI_Fw	TTTGGATCCGGATTTAACCGTC	527 bp	-500~+17
1751_pHindIII_Rv	GGGAAGCTTAAATAAGCGGACA		
1788_pBamHI_Fw	TTTGGATCCTATCGGCAACTAT	526 bp	-500~+16
1788_pHindIII_Rv	GGGAAGCTTTTGGGGTAAAT		
1823_pBamHI_Fw	TTTGGATCCGATCAAGCACGA	540 bp	-500~+30
1823_pHindIII_R2	GGGAAGCTTAACAGCAGGTTGAA		
1842_pBamHI_F4	CCCGGATCCTTCCAATTGGCATTGAA	507 bp	-442~+55
1842_pHindIII_R2	TTTAAGCTTCGATGGTGCCAACGTTA		

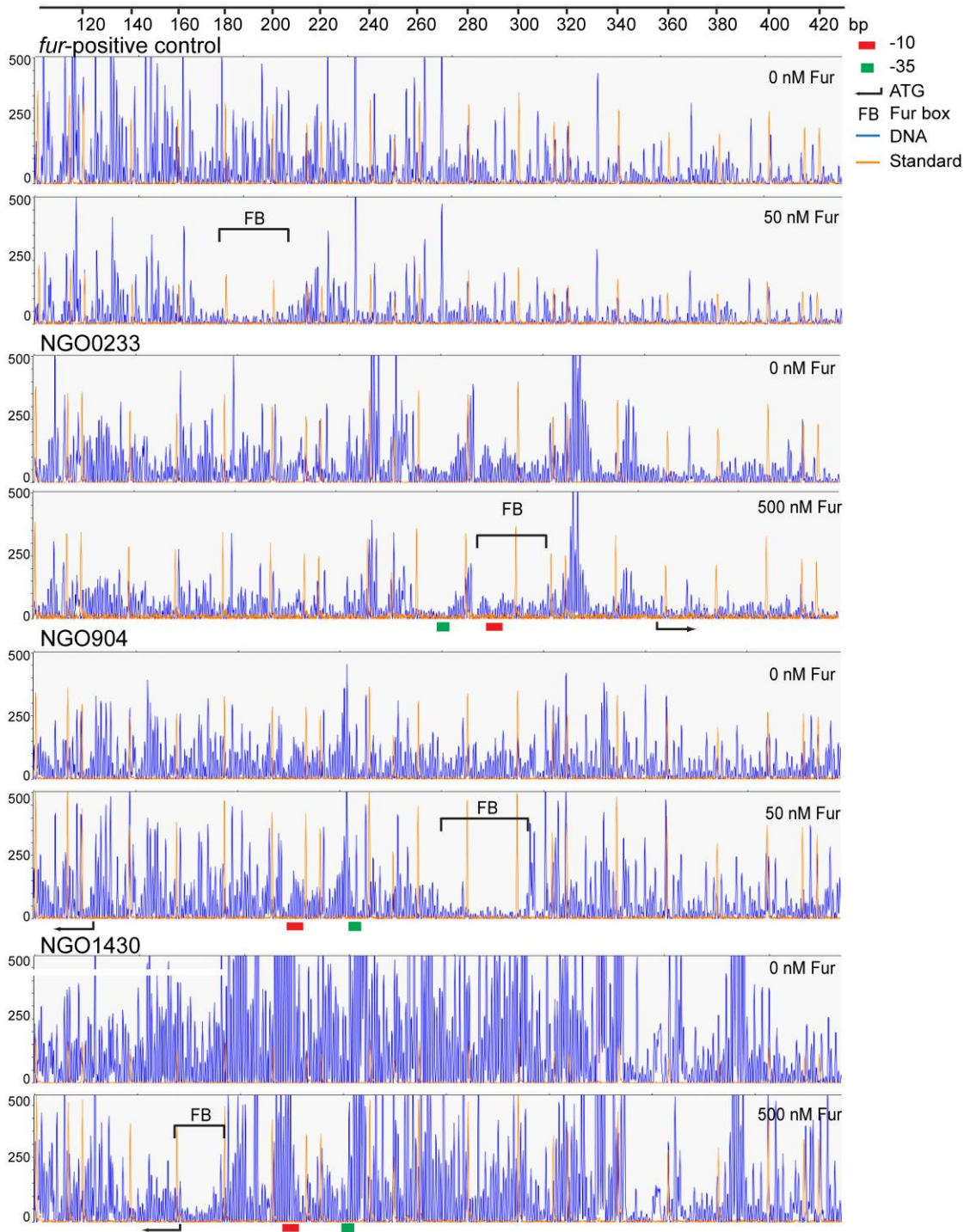
1850_pBamHI_F3	<u>TTTGGATCC</u> GCACGCGGTTCTACC	504 bp	-443~+41
1850_pHindIII_R2	TTT <u>AAGCTT</u> CCTGCGGTTTGCAACG		
1851_pBamHI_F3	<u>TTTGGATCC</u> GCTGGTGCCGGTGCT	495 bp	-430~+55
1851_pHindIII_R2	TTT <u>AAGCTT</u> CCCGCTTTGCAAACTCT		
1859_pBamHI_F2	<u>TTTGGATCC</u> TTAGCCATGGCAATATCC	493 bp	-426~+57
1859_pHindIII_R2	TTT <u>AAGCTT</u> CCCCGAAACCCATGACG		
1865_pBamHI_Fw	GGGG <u>GATCC</u> GAAAGCCAA	523 bp	-500~+15
1865_pHindIII_Rv	GGGA <u>AAGCTT</u> CGTCCTCTGT		
1913_pBamHI_F2	<u>TTTGGATCC</u> GACGGCATCTGTTTGACG	419 bp	-356~+53
1913_pHindIII_R2	TTT <u>AAGCTT</u> TTGGAACCTGTGGGGATGA		
1937_pBamHI_F2	<u>TTTGGATCC</u> GAAACTTTCAGGCGACTTTG	247 bp	-213~+26
1937_pHindIII_R2	TTT <u>AAGCTT</u> CCCGTTCACACCTAAACGA		
1960_pBamHI_F2	<u>TTTGGATCC</u> GGGATTGATGTTGATGC	504 bp	-480~+14
1960_pHindIII_Rv	TTT <u>AAGCTT</u> CGCGCCCCAAAC		
2011_pHindIII_R	GGGA <u>AAGCTT</u> AGTTCATCAGAAATC	504 bp	-486~+8
2011_pBamHI_F2	<u>TTTGGATCC</u> CGGCAGCTACGAG		
2068_pBamHI_Fw	<u>TTTGGATCC</u> GAAACGCTCGC	525 bp	-500~+15
2068_pHindIII_Rv	GGGA <u>AAGCTT</u> AATCAGGGAGGT		
2116_pBamHI_Fw	GGGG <u>GATCC</u> ACGCGCGTATTC	525bp	-500~+15
2116_pHindIII_Rv	GGGA <u>AAGCTT</u> CGGGAAGACGGG		
2137_pBamHI_Fw	<u>TTTGGATCC</u> GGTTCGGCAGTTT	526 bp	-500~+16
2137_pHindIII_Rv	GGGA <u>AAGCTT</u> GTCCAAAATAAT		
2162_pBamHI_Fw	GGGG <u>GATCC</u> AGAAGAGTGGGA	525 bp	-500~+15
2162_pHindIII_Rv	GGGA <u>AAGCTT</u> GTGGTGAGTGTT		

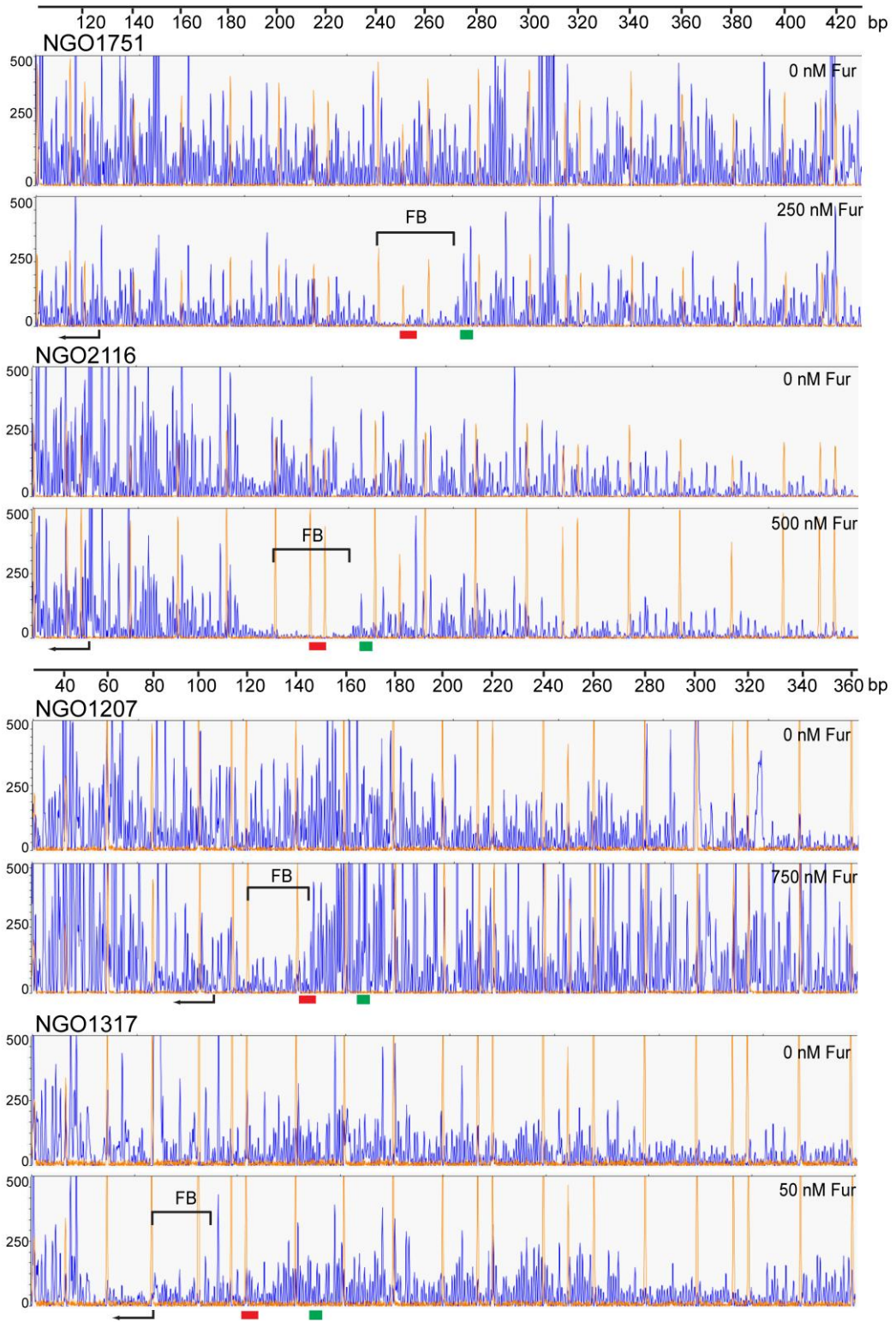
***Positions:** The Positions of each PCR product are relative positions to the translational start codon ATG of the gene, which is defined as +1 in this case.

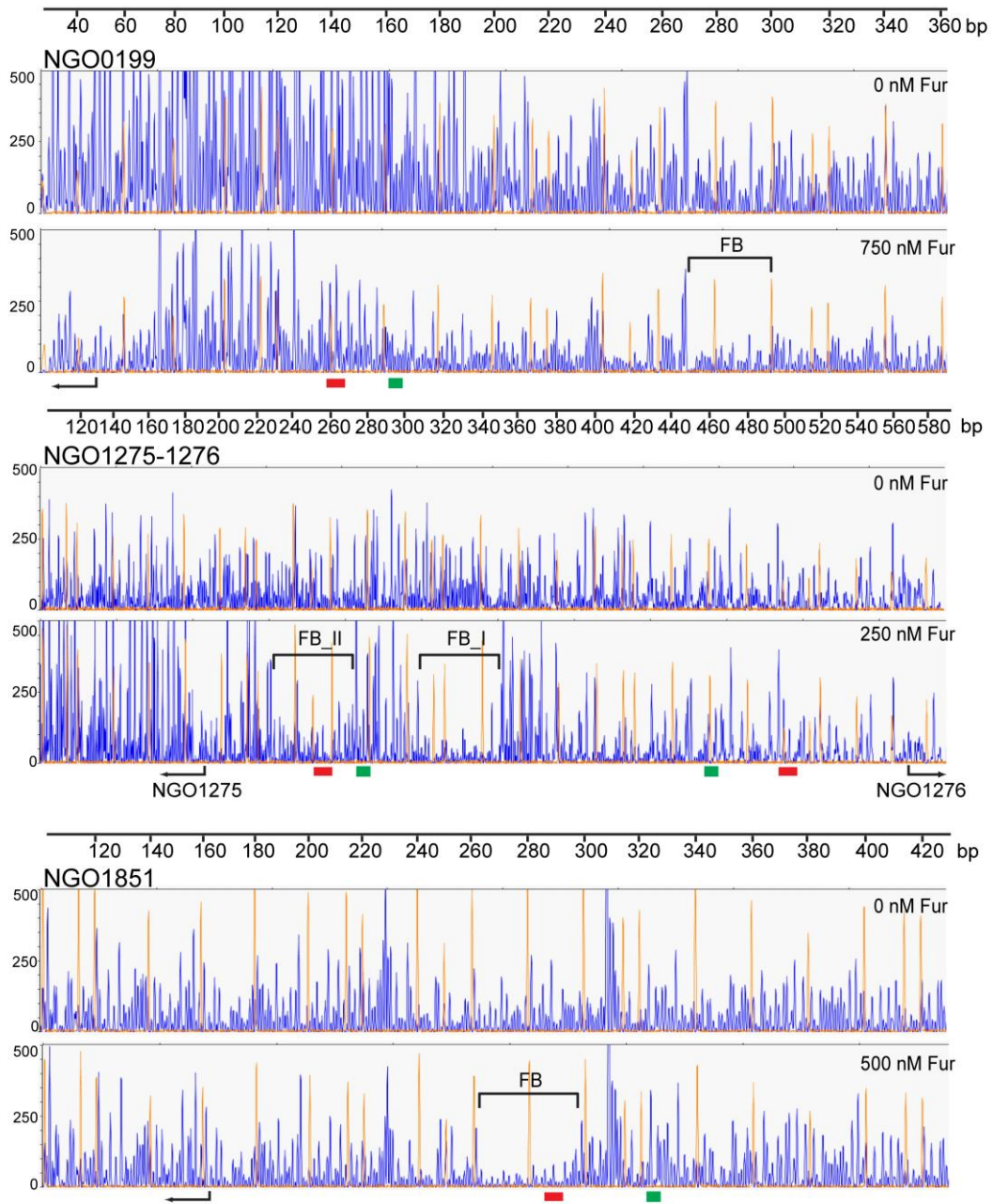
****BamHI and HindIII sites are underlined in each primer.**



Supplementary Figure 1. Binding of Fur to the putative promoter regions of the iron-activated genes of strain F62 *in vitro*. Electrophoretic mobility shift assay (EMSA) of ³²P-labeled putative promoter DNA after the incubation with Fur. The shift of the Fur-bound promoter is indicated by an arrow. On each panel, Lane 1, free DNA without the addition of Fur; Lane 2, 1200 nM Fur; and Lane 3, 1200 nM Fur + 50 mM EDTA. The gene designations of *N. gonorrhoeae* F62 were assigned according to their homologue in *N. gonorrhoeae* FA1090.







Supplementary Figure 2. DNase I foot printing of the promoter regions of the Fur activated genes to Fur. Fur protected regions are indicated by black brackets. The promoter motifs, -10 and -35, predicted by BPR0M were labeled as a red and green box, respectively. The translational start site (ATG) is indicated by an arrow. Since NGO1275 and NGO1276 share the same promoter regions, we used one probe for both genes to demonstrate Fur binding. The gene designations of *N. gonorrhoeae* F62 were assigned according to their homologue in *N. gonorrhoeae* FA1090.