

Oral Immunization of Rabbits with *S. enterica Typhimurium* Expressing *Neisseria gonorrhoeae*

Filamentous Phage Φ6 Induces Bactericidal Antibodies Against *N. gonorrhoeae*

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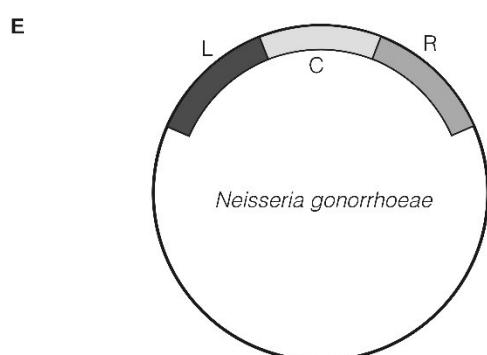
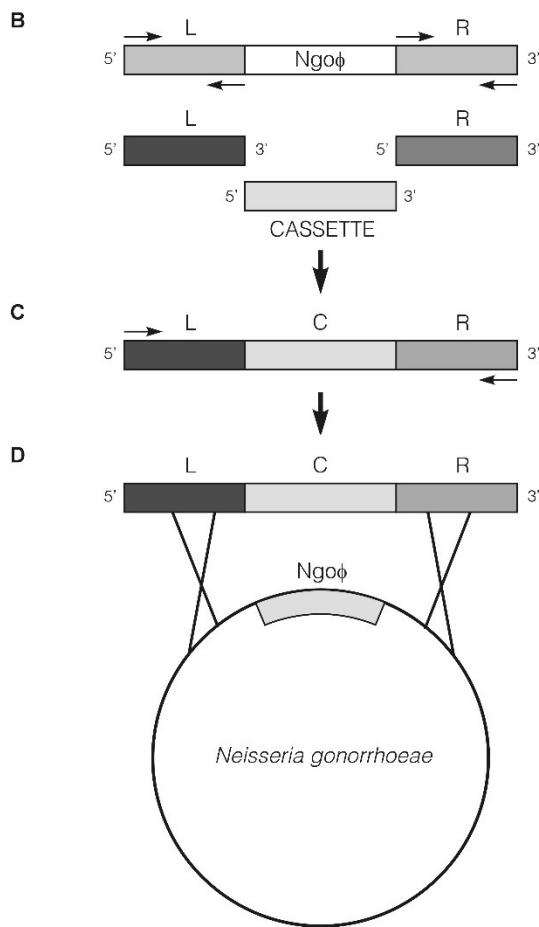
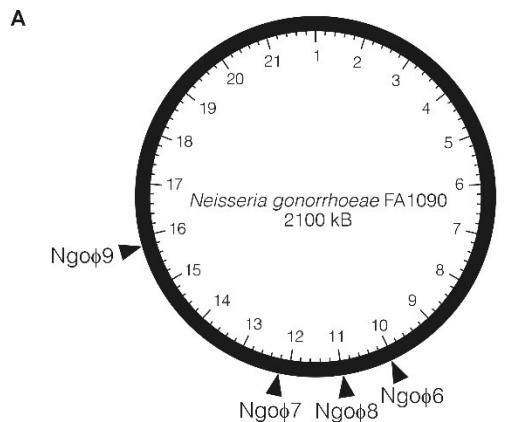
College Park, MD 20742

Supplemental TABLE 1

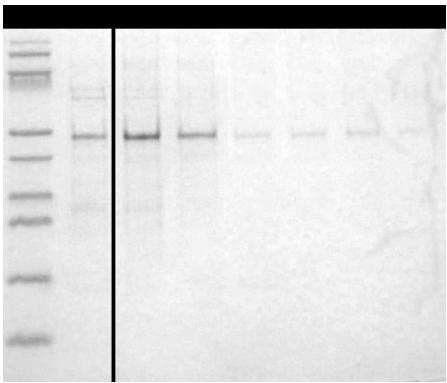
Primers used in this study

Primer	Sequence (5' → 3')	Use
ORF9F	AACTTTCGACGTGTCCGGGAACCTATGGAGACGGA	Cloning of ORF9 of NgoΦ6
ORF9R	AAGGC CGGGT GCTGATTGTTGGCGAACGCGACTA	into pET28a
PhageΦ9R	TTATTGATTCAATCGGTGTCTTCCC	Detection of the presence
PhageΦ9F	TTACGCCGATTGTAACCGCGATGGA	of phage NgoΦ9
PhageΦ8R	ATGGGCTTAAAGTGTCTGCCGAAA	Detection of the presence
PhageΦ8F	TTACGCCGATTGTAACCGCGATGGA	of phage NgoΦ8
PhageΦ7R	ATGATGACGATTCATACGCGCGC	Detection of the presence
PhageΦ7F	TTACGCCGATTGTAACCGCGATGGA	of phage NgoΦ7
PhageΦ6R	TTTCATTCCGGATTCCCACCTTCG	Detection of the presence
PhageΦ6F	ATGCGTAACGCCGTAGGATTGGATA	of phage NgoΦ6
Φ6LF	TCCGAACCTTGAAGCAA	Amplification of the
Φ6LR	CACTAAGCTTGGGATTACCCATTATGCGGATAT	left flank of NgoΦ6
Φ6RF	CACTCTGCAGCAGCGTTGCGAGGCTTAAGC	Amplification of the
Φ6RR	GCCGGCCGCCCGGACT	right flank of NgoΦ6
Φ7LF	GTCCTCGCCTCGCTCG	Amplification of the left
Φ7LR	GTGAAGCTTTGCCGGTGTATTGGTAGG	flank of NgoΦ7
Φ7RF	CACTCTGCAGAAATCGGTAAATTGCGT	Amplification of the right
Φ7RR	AGGGTTGGGGGGACCCATTCCCTTCTC	flank of NgoΦ7
Φ8LF	GACCCATTCCCCCTTCCTC	Amplification of the left
Φ8LR	GTGAAGCTTCCCGAAAGGAAAAAGG	flank of NgoΦ8
Φ8RF	CTCACTGCAGTATATCGATCCATCC	Amplification of the right
Φ8RR	GATGATGACGATTTCATAC	flank of NgoΦ8
Φ9LF	TATTAACACATAAAAACAAAGGA	Amplification of the left
Φ9LR	GTGAAGCTTCGATAATCCGAAAGG	flank of NgoΦ9
Φ9RF	CTCACTGCAATAAAATTCAACAAAATT	Amplification of the right
Φ9RR	GTTCGGCGGTGTTGCCCG	flank of NgoΦ9
Omega-PstFR	CAGTCTGCAGGAGTTAACGCCGC CGGAA	Amplification of the
Omega-HindIIIF	GACCTCGAGTTGCAACACCGAACGCTTG	spectinomycin cassette
OF16NheI	CTAGCTAGCATTATCTGTTACGGGAACAT	Cloning of Orf9 into
9FQER	GCAAGCTTTAGGACTTCCGCCATT	pET21A
ChlorF	ATCGTTAACCGTTGATCGGCACGTAAGAG	Amplification of the
ChlorR	ATCGTTAAATAGCACCAGGC GTTAAGGG	chloramphenicol cassette
KanF	CACTAAGCTTTATCTGGACAAGGGAAAACGC	Amplification of the
KanR	CACTCTGCAGGGT GGAATCGAAATCTCGTGA	Kanamycin cassette

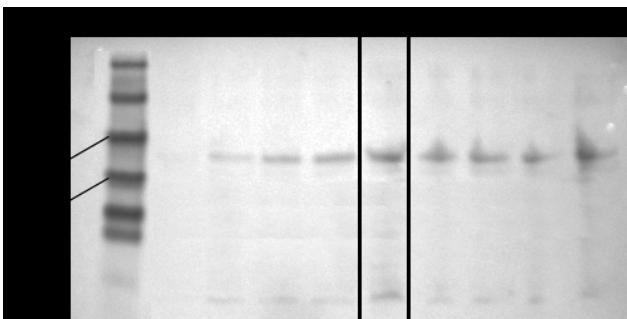
**Fig.S1. Schematic of method used to delete NgoΦ6, NgoΦ7, NgoΦ8 and NgoΦ9 from FA1090.** (A) Locations of the filamentous phage sequences on the FA1090 genome as determined by (28). (B) General scheme for removing phage sequences. Two amplicons (L-left and R-right) flanking each phage sequences were obtained by PCR and after digestion with HindIII (left flank DNA) and PstI (right flank DNA) ligated with the donor of antibiotic resistance cassette to produce a fragment (C). The ligation product containing antibiotic cassette integrated into left and right flanks of phages DNA was amplified by PCR using appropriate primers. Panel D indicates that the integration of the amplified DNA after *N. gonorrhoeae* transformation and selection of antibiotic resistant transformant (Panel E).



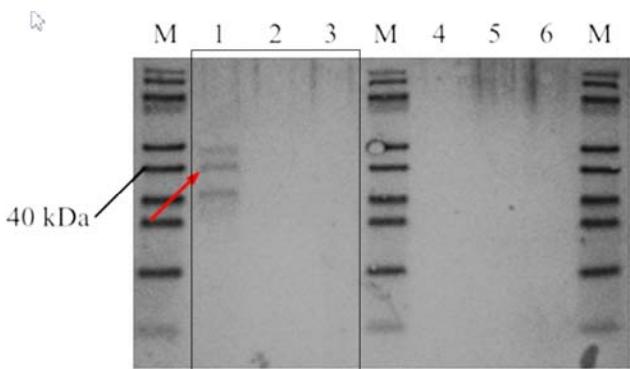
## Construction of Figure 1: Lanes M and 1



Lane 2



Lane 3, 4 and 5



Final image:

