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Antisense RNA Targeting the First Periplasmic Domain of YidC in *Escherichia coli* Appears to Induce Filamentation but Does Not Affect Cell Viability

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SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Antisense periplasmic *yid*C cloning into pHN678 and pBAD24. We ligated both the 50 bp and the 248 bp fragments into pBAD24 and pHN678. Since pBAD24 and pHN678 respectively contains the ampicillin and chloramphenicol resistance genes, the transformed colonies were plated on ampicillin and chloramphenicol plates to select for transformants with the correct plasmid. From our experiment, we found that pBAD24 exhibited lower transformation efficiency than pHN678 as exhibited by the lower number of colonies (data not shown).

Gel electrophoresis for the confirmation of successful restriction digestion. In order to confirm successful restriction digestion of pHN678 and pBAD24 with EcoRI and HindIII, gel electrophoresis was performed to confirm plasmid size and check for star activity. Both the pBAD24 and pHN678 bands appeared between the 4 kb and 5 kb marker on the GeneRuler DNA Ladder. pHN678 migrated slightly further than pBAD24 (Data not shown). This is expected since pBAD24 is 4.5kb and pHN678 is 4.0kb. No star activity was observed through the lack of non-specific bands.This experiment also serves as a confirmation that the plasmids we received are indeed pBAD24 and pHN678.

Colony counts for competent cells transformed with pHN678 containing the 50 bp insert. As shown in Table S1, pZ worked as a control for transformation and grew to numbers which were TMTD. The positive control, the uncut plasmid, is expected to have many colonies indicative of transformation success. With the addition of ligase, cells transformed with digested vector resulted in many colonies, which confirmed that the ligase is functional. As expected, cells transformed with the cut vector alone showed no colonies. This result also confirmed proper digestion of vectors. However, both our positive and negative controls exhibited few colonies. Transformation was successful with ligated vectors and the highest efficiency is at 1:1 vector to insert ratio, with decreasing efficiency at higher insert to vector concentrations.

Confirmation of successful transformation of antisense constructs via single restriction endonuclease digest and gel electrophoresis. Since plasmids with the 50 bp insert would have the *Eco*RI and *Hin*dIII sites destroyed, 10 transformed colonies from each plate was selected and restriction digested with *Eco*RI. The control would have an intact *Eco*RI site which can be cut and linearized. The transformants with ligated insert cannot be cut with *Eco*RI and would remain supercoiled. As shown in FIG S3, the positive control was cut by *Eco*RI and linearized, which corresponds to the ~4000 bp band on the linear ladder.

As shown in FIG S4, Colony 9 and 10 (lane 11 and 12) of pBAD24 and colony 1, 3, 4, 5, 6, and 8 (lane 3, 5, 6, 7, 8, 10) of pHN678 (FIG S3) showed bands at ~4000 bp on the supercoiled ladder which indicates their *Eco*RI site was destroyed during ligation with the 50 bp insert. Colony 2 and 7 (lane 4 and 9) of pHN678 (FIG S3) have a band at ~4000 bp on the linear ladder, which is due to the cut plasmid re-ligating without the insert.

Colony PCR of the transformants was performed on pBAD24 and pHN678 with the putative 248 bp insert. Colonies with the insert should show a 248bp PCR product. In FIG S5, lane 8 is the positive control with the 248bp PCR product, lane 9 is a negative control with pHN678 without insert, and lane 10 contains no template DNA which should not have amplification. We determined that the pHN678 vector we used for colony 2 (lane 9) may have been a mis-labelled ligated product, as we meant to use pHN678 without an insert. As shown in FIG S5, colony 3 of pBAD24 (lane 4) and colony 1, 2, and 3 of pHN678 (lane 5, 6, and 7) showed a band between 200 bp and 300 bp. These colonies appear to contain the desired 248 bp insert.

pBAD Sequencing. For pNEARB509 (pBAD24-a*yid*C with 50bp insert), the primer used was one strand of the oligonucleotide sequence. The presence of the sequencing product indicated that the fragment was successfully inserted (FIG S9). For pBAD24, the insert were present but we did not analyze whether the inserts were in the correct direction.

LISVKTDVLDLTINTRGGDVEQALLPAYPKELNSTQPFQLLETSPQFIYQAQSGLTG
RDGPDNPANGPRPLYNVEKDAYVLAEGQNELQVPMTYTDAAGNTFTKTFVLKRG
DYAVNVNYNVQNAGEKPLEISSFGQLKQSITLPPHLDTGSSNFALHTFRGAAYSTPD
EKYEKYKFDTIADNENLNISSKGGWVAMLQQYFATAWIPHNDGTNNFYTANLGNG
IAAIGYKSQPVLVQPGQTGAMNSTLWVGPEIQDKMAAVAPHLDLTVDYGWLWFIS
QPLFKL

FIG S1 Region(s) of the *yid*C first periplasmic domain targeted by primers for antisense periplasmic *yid*C construct. Dark grey region was used to generate the 50bp oligonucleotide while the light grey region was used to generate the 248 bp insert.

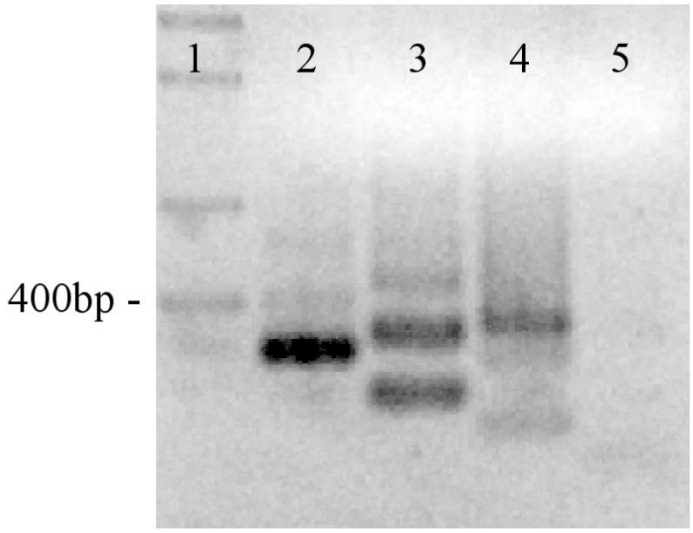

FIG S2 PCR amplification of YidC periplasmic region. YidC periplasmic region was amplified with 3 forward primers and 1 reverse primer giving rise to amplicons of 248bp (lane 2), 147bp (lane 3), and 80bp (lane 4) (Table S2). Only the 248bp amplicon had a specific product at ~250 bp.

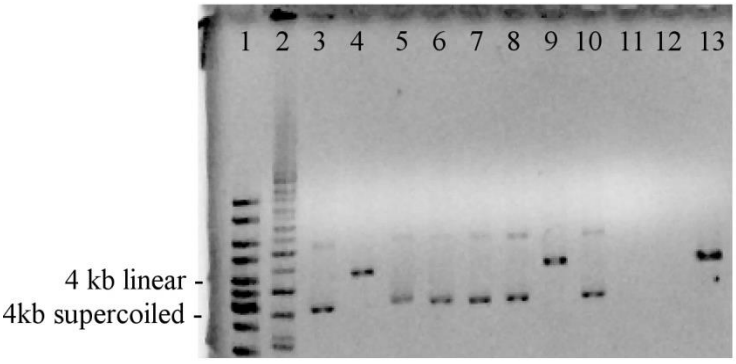

FIG S3. Screening pHN678 containing the insert by testing for presence of *Eco*RI site. The positive control (Lane 13) had an intact *Eco*RI site and could be linearized corresponding to a 4kb linear sequence. Colony 9 and 10 (lane 11 and lane 12) showed bands at ~4000bp on the supercoiled ladder, indicating successful ligation with insert.

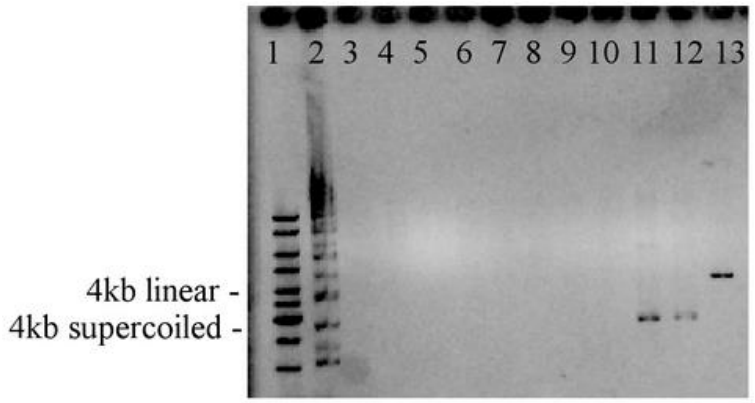

FIG S4 Screening pBAD24 containing the insert by testing for presence of *Eco*RI site. The positive control (Lane 13) had an intact *Eco*RI site and could be linearized corresponding to a 4kb linear sequence. Colony 9 and 10 (lane 11 and lane 12) showed bands at ~4000 bp on the supercoiled ladder, indicating successful ligation with insert.

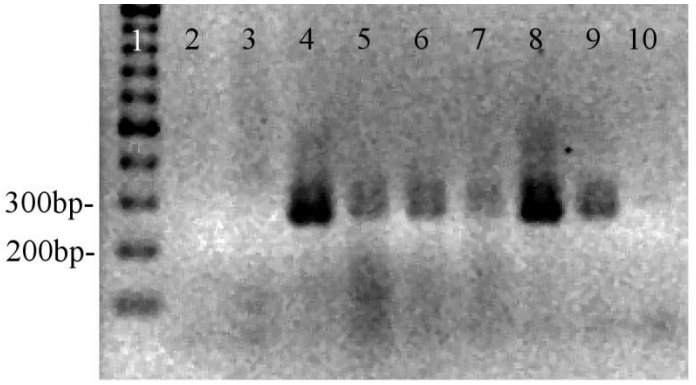

FIG S5 Screening pBAD24 and pHN678 with the putative 248 bp insert via colony PCR. The positive control (Lane 8) had the 248 bp PCR product and the negative control (Lane 9) should contain the empty pHN678 vector. Lane 2-4 contain PCR products from colonies transformed with pBAD24 and the 248 bp insert. Lane 5-7 contain PCR products from colonies transformed with pHN678 and the 248 bp insert. Lane 10 contains no template DNA.

ACCCCCTCNACCCCTTGAGGATTTTCANCNAGGGGNCAGGCCCTGAACGTGANGGGGGTCGCANGTGTC
CTTGGTGGGGGTATTGAGACCCTGTTAAAGGCCATCTTGCCCCTTGCCCCTCCGGCGCTCACCTTTCNNCC
CAATCTCACTGTAGGCCTCGGCCNTCTTATCTTTTTGCAGTTCATTGTACAGGCCTTCATGAGGGTTCTTCC
TTCNCGGNTTTCCCCCNNTCTCTGGTCCCGGCCNNNTCTCTTGTCNAACACATCGTACTCCTCNTNNCNTC
NTANATNAANCTCGTTATAAAGNTGGTTCTGCTCCTCCTTGTACTCGGGGGNNTCGGCGCTCCTGGTGAA
CTTCACTCTCANTTCATTTCCTCCTTCTTCTTCTTTTGGAAANCGGCAGCTAGATCCATCTTCCTCTTGAGT
ACTTTGTANNGGCCTCGGAAATGGTGGTGTGAATATATACTGGATTTTGTTTCTGCCCCGTTTGCANTAAA
GGGTGATAACCATTGCCAGGANAANGACCCNNAAAGTCCCGGCCAGGGNCGCCGANATGTTNATATCAC
ACGNAAGANCCAGCCCCNNCNTGNANGCTGAGCACCCCGGCNCGGGCCGGCACGCCTCTAGGCTCATGA
ACATGTGCTGAGACGCNTTGNNGNGCGACTGTGTAGGTGGGCACGGAGCTNNACATCANGGTTGCNACA
GCTNAGGACCCNCGNGACTGAGGANCCGNNGANNCCANCACCCCATAACANAGNCAACCTCGTAGNAG
ANNAGTGTGGCNNNNNANTNNNAGGCTGTCTCATCNCTTTGCAGACTGATCNTTTTNATAAGACTTGNCT
CTAGTANTTNTCNTAGATGATGNGCATCNTGGAANTGACANCTANATTANCAGANGGNGGNTCANNANN
NNTCNNTCANNNCNGNCNGNTCAAANNCTNNNTCNGGGAANNNNNGCGAATGCCGTCTANNNCNNGGN
CGNGGGAGGGGANNNNCCNN

FIG S6 Sequencing result for pNEARH2481.

GAGAAGTATGAGAAATACAAGTTCGATACCATTGCCGATAACGAAAACCTGAACATCTCTTCGAAAGGT
GGTTGGGTGGCGATGCTGCAACAGTATTTCGCGACGGCGTGGATCCCGCATAACGACGGTACCAACAAC
TTCTATACCGCTAATCTGGGTAACGGCATCGCCGCTATCGGCTATAAATCTCAGCCGGTACTGGGAATTC
CCATGGCACCACCACCACCACCACTGCATGGTTAATTCCTCCTGAAATTGTTATCCGCTCACAATTCCACA
CATTATACGAGCCGGATGATTAATTGTCAACAGCTCATTTCAGAATATTTGCCAGAACCGTTATGATGTC
GGCGCAAAAAACATTATCCAGAACGGGAGTGCGCCTTGAGCGACACGAATTATGCAGTGATTTACGACC
TGCAGGCATGCATTTACGTTGACACCATCGAATGGTGCAAAACCTTTCGCGGTATGGCATGATAGCGCCC
GGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGG
TGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAA
GTGGAAGCGGCGATGGCGGANCTGAATTACATTCCCAACCGCGTGGCNCANCNACTGGCGGGCAAACAG
TCGTTGCTGANNNNNGTTGCCNCCTCCAGTCTGGCCCTGCACGCGCNGTCGCAAATTGTCNNGNCNATTA
NATCTCNCGCCGATNAATTGGGNNNCNNGNGAGNNNNTGTNAANGGNNNANCACNNNNNNNNNNNAAN
CCTNTTTTTCNNNCNAGNGNN

FIG S7 Sequencing result for pNEARH2482.

CACCACCACGNCCACTGCATGGTTAATTCCTCCTACNAGTTTTGGCGGATGAGAGAAGATTTTCAGCCTG
ATACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACAGAATTTGCCTGGCGGCAGTAGCGCGGTG
GTCCCACCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTANTGCCGATGGTAGTGTGGGGTCTCCCC
ATGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGT
TTTATCTGAGGGGGAGGCAGAATAAATGATCATATCGTCAATTATTACCTCCACGGGGAGAGCCTGAGCA
AACTGGCCTCAGGCATTTGAGAAGCACACGGTCACACTGCTTCCGGTAGTCAATAAACCGGTAAACCAG
CAATAGACATAAGCGGCTATTTAACGACCCTGCCCTGAACCGACGACCGGGTCGAATTTGCTTTCGAATT
TCTGCCATTCATCCGCTTATTATCACTTATTCAGGCGTAGCAACCAGGCGTTTAAGGGCACCAATAACTGC
CTTAAAAAAAATTACGNNNCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGAC
ATGGAAGCCATCACAAANGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGT
ATAAATATTTGCCCATCGTGAAAACGGGGNGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAAAC
TGGGTGAAACTCNCCCAGGGATTGNNNGANACNAAANCATATTCTCNATAAACCCTTTAGGGNAANTNN

FIG S8 Sequencing result for pNEARH50.

NNNNNNNNNNNNNNNNNNNNNNNCCNANNNANNNNNNNNTANCGGNNAANNCANGNGAGGANNTA
TNGNATATNGACATCNTGNNANANAANNNGNGTTGCGGCNNGGGNGNGCGNGCCNNCATNNNCGTCCC
GCACGAACGNNACNCCCGGGACCCTGANNGANNGGAGTTGGGGTTNNNTGGNGGGTATTGCGCNGCAAT
TTCTCCNCGATAATGACGTNATGNNATGGGTCGGCCGGGGAGTCTACCCTCCTNNCNACACTNNTCCTCG
GNNACTTCGCCACNCNCNNGANNNTTTTCGCTGATGCNTATACACTTTTCCNCGNNCTGNTNNCGNNACN
CCNAAGGTNNNNGGTAAANNCGTCTCNTGAGNTGTATNCTATTTGTCATTGGTTTTATTNATGTTNTGCCA
AGATNCTTGGNAANGCACNAATAATANTTNCCANCNTGAANNGTGNNNTCNTNGNGTGGCANNCCTTAC
NNGANGNANGCNNTTGCCTANNANNNTTNCCNACNANNNGATGACNATCTCTAGGCTAANTGGNGNNGT
AACTGATACCTTGATTGAGTCTGCANTGANNNNANTATTNNATNATTTGNNNATTGAAACTCTCNGTAGA
TGGCTCCTGNGACGACTCAGTGTTCATCATCGATNNGTGTTAATCGATCGCNTTTAGAAAAGAAAGAAAA
TGATAGNAATACCTCCNNCCTNNAG

FIG S9 Sequencing result for pNEARB509.

Table S1 Colony counts for competent cells transformed with vector containing the 50 bp insert

pBAD24		1:1	1:2	1:3	1:4
Experimental	Plasmid + Insert	43	39	28	15
Control	Uncut plasmid	7			
Control	Cut plasmid	18			
Control	Cut plasmid + Ligase	8			

pHN678		1:1	1:2	1:3	1:4
Experimental	Plasmid + Insert	38	34	20	15
Control	Uncut plasmid	0			
Control	Cut plasmid	0			
Control	Cut plasmid + Ligase	2			

Table S2 Primer sequences (and features) used in the construction of the 80bp and 147 bp antisense sequences.

Primer	Restriction overhang	Primer sequence (5'to 3')*	Product size (base pair)
yidC-f-80 (forward *yidC*)	*Hin*dIII	*gat*aagcttCGACGGTACCAACAACTTCTATAC	80 (with yidC-back)
yidC-f-147 (forward *yidC*)	*Hin*dIII	*gat*aagcttTCTCTTCGAAAGGTGGTTGG	147 (with yidC-back)

*Upper case nucleotides are complementary to sequence; lower case nucleotides are restriction enzyme sequence; italicized nucleotides are additional nucleotides added to complete restriction enzyme site to PCR products.