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The Major Periplasmic Domain of YidC May Be Required for Polar Localization of a Green Fluorescence Protein Tagged YidC Variant Protein in *Escherichia coli*

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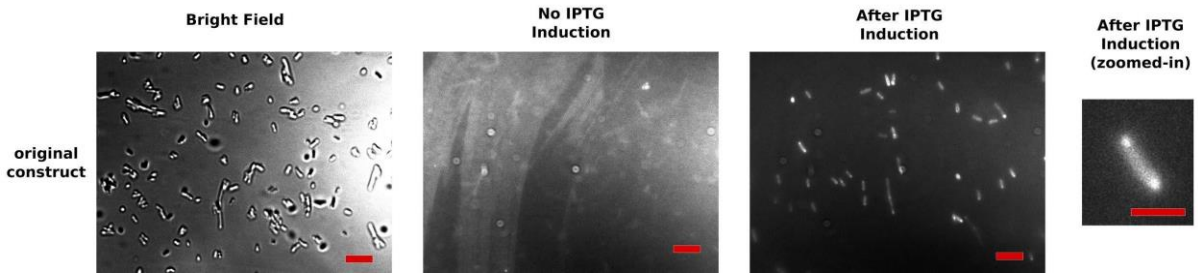
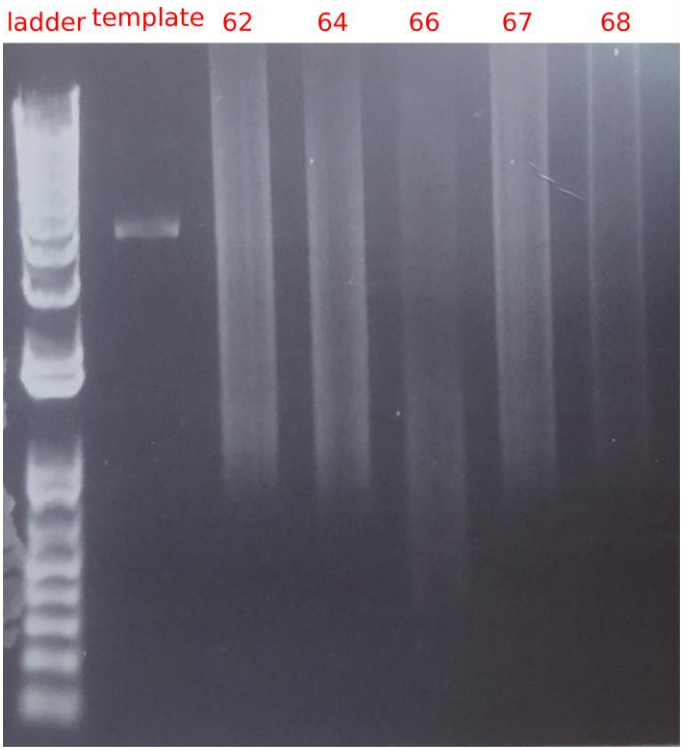
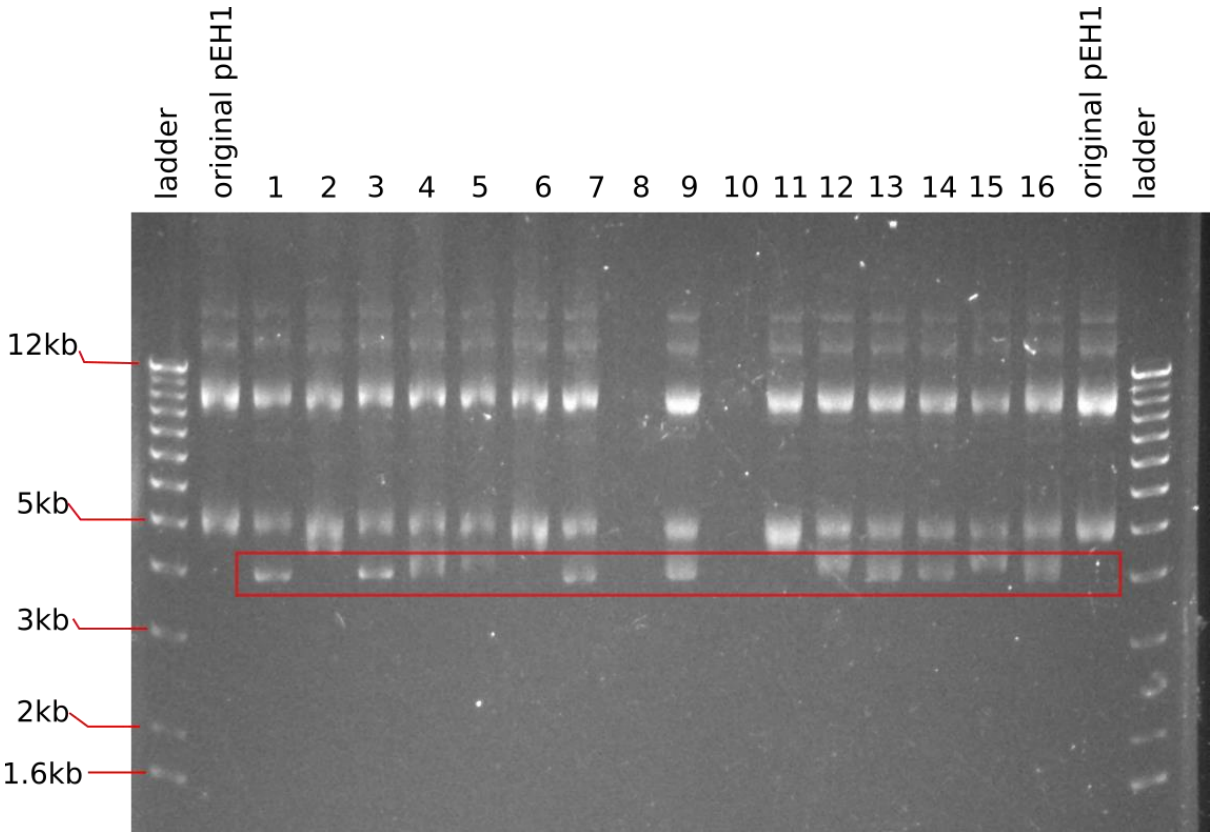
SUPPLEMENTAL MATERIAL



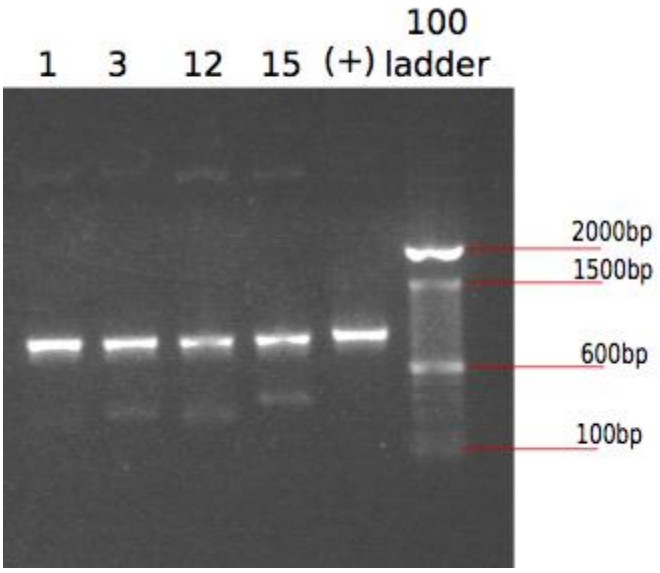
S.FIG 1. Localization of YidC-GFP in BL21(DE3). pEH1 construct containing the original YidC-GFP fusion protein, expressed under lac-T7 promoter, was transformed into BL21(DE3). YidC-GFP was expressed by incubating the overnight culture of transformed BL21(DE3) in 1 mM IPTG for 3 hours. Bright field and fluorescent images (FITC) were taken at 1000x magnification. Samples were not fixed; mounted by placing the sample on a microscope slide and covered by glass cover slip. Scale bars = 2 um.



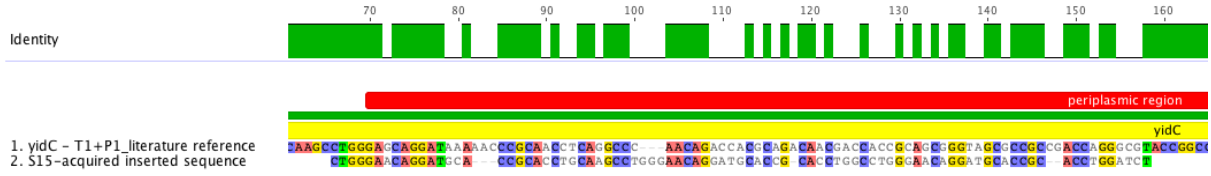
S.FIG 2. DNA gel of gradient PCR with homologous-end primer design. PCR was performed using homologous-end deletion primers, Pfu polymerase, and annealing gradient of 60 to 70C. The original pEH-1 plasmid (template) and PCR products were analyzed by gel electrophoresis in 0.8% agarose gel (TAE). 180V (6V/cm) for 1 hour in 1x TAE buffer.



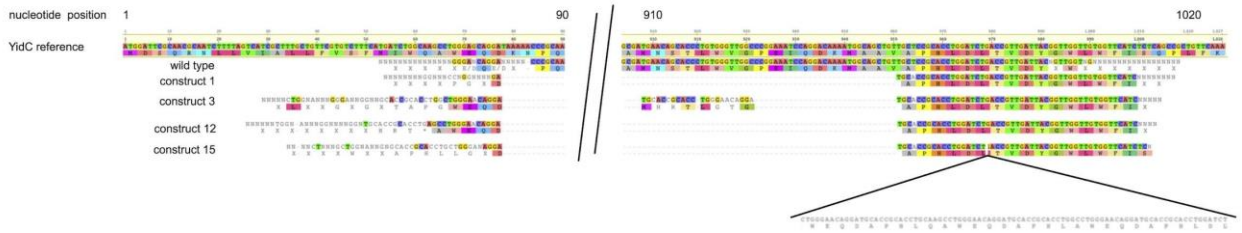
S.FIG 3. Size analysis of putative YidC-ΔP1-GFP containing pEH-1 constructs. pEH-1 plasmids that were circularized and maintained from the linear PCR product were harvested from DH5α, and analyzed for size based on migration speed using gel electrophoresis. Sample plasmids were mixed 1:1 ratio with original pEH-1, and then loaded into the gel. As control, the original unmodified pEH-1 was also loaded. DNA samples ran in 0.8% agarose gel (TAE). 180V (6V/cm) for 120min in 1x TAE buffer. Red rectangle box highlights the region where the YidC ΔP1-GFP containing pEH-1 constructs are expected to appear if they are present in the sample. DNA bands around 5kb were thought to be unmodified pEH-1 plasmid in supercoiled form.



S.FIG 4. DNA gel of Hot-start PCR performed using exterior forward and interior primers targeting the P1 deletion region. PCR was performed with Platinum Taq. Construct #1, 3, 12, and 15 are used as templates. PCR was also performed with the original pEH-1 plasmid was used as template--positive control. PCR products were analyzed by gel electrophoresis in 1% agarose gel (TAE). 180V (6V/cm) for 30 min in 1x TAE buffer. Control was expected to result in a PCR product around 900 nucleotides. Constructs 1, 3, 12, and 15 were expected to result in no PCR products.



S.FIG 5. Alignment of acquired inserted in construct 15 to YidC reference. ClustalW pairwise alignment was used to align the acquired insertion against the reference. Identical nucleotides sequences are coloured. Yellow area represents YidC. Red area represents P1 domain of wild type YidC. Percent similarity between the two sequences was 57%.



S.FIG 6. Alignment of four constructs with P1 in-frame deletion to reference YidC sequence. Nucleotide sequences of wild type control and the four constructs were aligned to the sequence in Geneious, and then also translated according to the +1 frame of the reference sequence. Nucleotide and amino acid sequences that agree with that of YidC reference are highlighted with colour, otherwise they are colorless.

Table S1. Nucleotide sequence of deletion-confirmation PCR products

PCR Product	Nucleotide Sequence
S1-extP-YidC	5’ NNNNNNNNGGNNNCCNGGNNNNGATGCACCGCACCTGGATCTGACCGTTGATTACG GTTGGTTGTGGTTCATCNNNNNNNN 3’
S3-extP-YidC	5’ NNNNNCTGGNANNNGGGANNGGNNGCACCGCACCTGGCTGGGAACAGGATGCACC GCACCTGGGAACAGGATGCACCGCACCTGGATCTGACCGTTGATTACGGTTGGTTGT GGTTCATCNNNNN 3’
S12-extP-YidC	5’ NNNNNNTGGNANNNGGNNNNGGNTGCACCGCACCTGAGCCTGGGAACAGGATGCA CCGCACCTGGATCTGACCGTTGATTACGGTTGGTTGTGGTTCATCNNNN 3’
S15-extP-YidC	5’ NNNNCTNNNGCTGGNANNGNGCACCGCACCTGCTGGGANAGGATGCACCGCACCTG GATCTCTGGGAACAGGATGCACCGCACCTGCAAGCCTGGGAACAGGATGCACCGCA CCTGGCCTGGGAACAGGATGCACCGCACCTGGATCTGACCGTTGATTACGGTTGGTT GTGGTTCATCTCN 3’
Positive control_extP-YidC	5’ NNNNNNNNNNNNNNNGGGANCAGGANNNNNCCCGCAACCTCAGGCCCAACAGACC ACGCAGACAACGATCACCGCAGCGGGTAGCGCCGCCGACCAGGGCGTACCGGCCAG TGGCCAGGGGAAACTGATCTCGGTTAAGACCGACGTGCTTGATCTGACCATCAACAC CCGTGGTGGTGATGTTGAGCAAGCTCTGCTGCCTGCTTACCCGAAAGAGCTGAACTC TACCCAGCCGTTCCAGCTGTTGGAAACTTCACCGCAGTTTATTTATCAGGCACAGAG CGGTCTGACCGGTCGTGATGGCCCGGATAACCCGGCTAACGGCCCGCGTCCGCTGTA TAACGTTGAAAAAGACGCTTATGTGCTGGCTGAAGGTCAAAACGAACTGCAGGTGCC GATGACGTATACCGACGCGGCAGGCAACACGTTTACCAAAACGTTTGTCCTGAAACG TGGTGATTACGCTGTCAACGTCAACTACAACGTGCAGAACGCTGGCGAGAAACCGCT GGAAATCTCCTCGTTTGGTCAGTTGAAGCAATCCATCACTCTGCCACCGCATCTCGAT ACCGGAAGCAGCAACTTCGCACTGCACACCTTCCGTGGCGCGGCGTACTCCACGCCT GACGAGAAGTATGAGAAATACAAGTTCGATACCATTGCCGATAACGAAAACCTGAA CATCTCTTCGAAAGGTGGTTGGGTGGCGATGCTGCAACAGTATTTCGCGACGGCGTG GATCCCGCATAACGACGGTACCAACAACTTCTATACCGCTAATCTGGGTAACGGCAT CGCCGCTATCGGCTATAAATCTCAGCCGGTACTGGTTCAGCCTGGTCAGACTGGCGC GATGAACAGCACCCTGTGGGTTGGCCCGGAAATCCAGGACAAAATGGCAGCTGTTG CTCCGCACCTGGATCTGACCGTTGATTACNGTTGGTNGNNNNNNNNNNNNNNNNN 3’