Supplementary Figure 1. *papX* linker-insertional mutants do not have impaired growth rate. Thirty 15-bp insertional mutants of *papX* in pDRM001, as well as WT *papX* in pDRM001, were electroporated into strain CFT073 Δ*papX*. Strains were diluted 1:100 in LB broth and OD_{600} was automatically recorded every 15 min by a bioanalyzer. No significant difference in growth rate was detected among the constructs. Name of transforming plasmid is shown on right.

Supplementary Figure 2. PapX is a dimer — (A) Recombinant purified PapX-His_6 and molecular-weight standards were run through a Sephacryl S-200 size-exclusion column at 1 ml/min and OD_{280} was measured in mAU over a 300 ml volume. Traces of each were superimposed by aligning the void-volumes. PapX-His_6 peak appears at approximately twice the predicted molecular weight of the monomer. **(B)** SDS-PAGE was performed on non-denatured recombinant PapX-His6. A major band appears at twice the molecular weight of the monomer (the minor band).

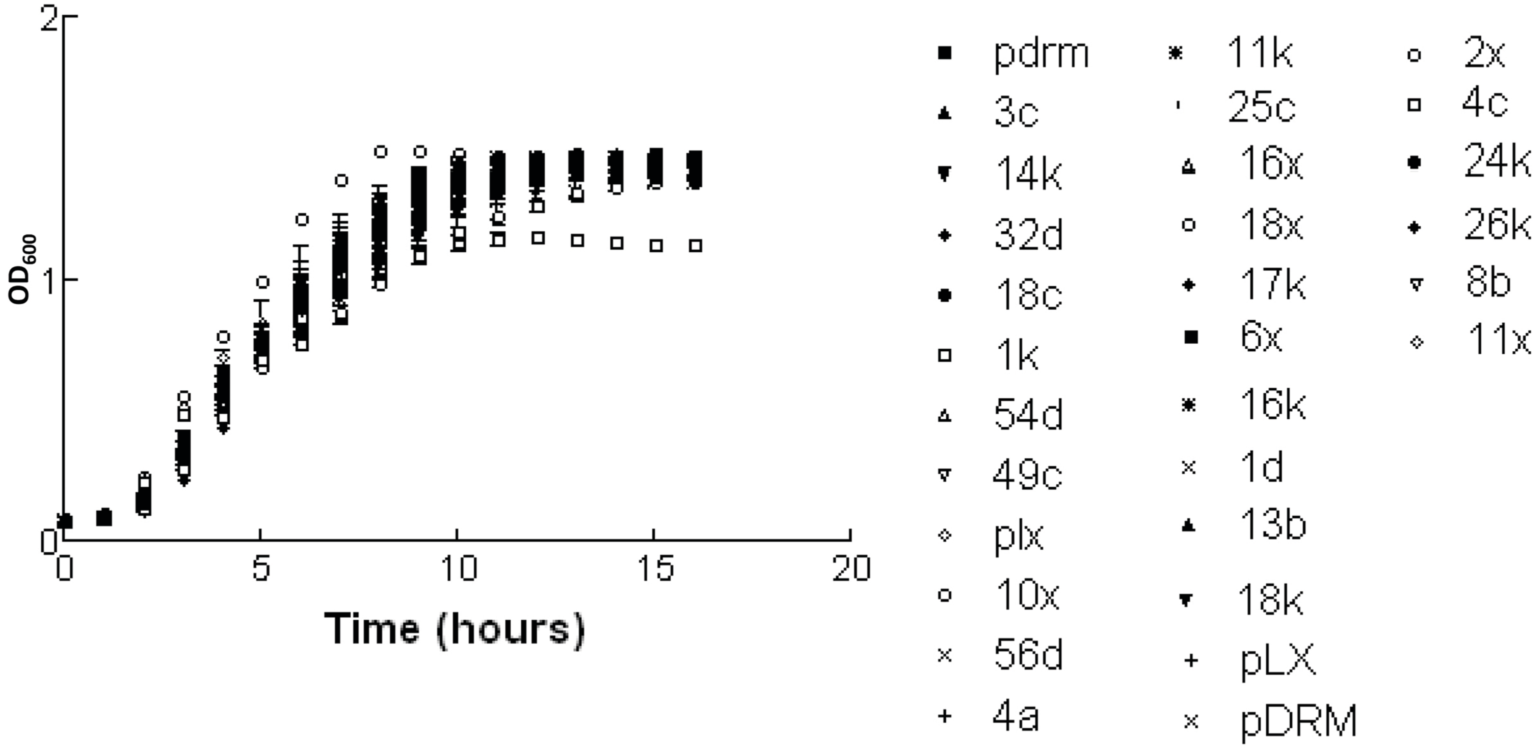
Supplementary Figure 3. Non-radiolabeled fluorescent DNase protect assay (A) 0.5 μg of recombinant purified PapX-His_6 was incubated with a 6-carboxyfluorescein end-labeled fragment derived from the first 360 bases of the *flhD* promoter and exposed to low-levels of DNase I for 1-20 minutes. Sheared fragments were processed by electrophoresis as for DNA sequencing to produce chromatograms representing all sheared fragments. Fluorescence of the 6-carbosyfluorescein is shown on the y-axis. Position of the fragment is shown on the x-asis. **(A)** Representative chromatogram of a fragment exposed to DNase I for 1 minute in the absence of PapX protein **(B)** Representative chromatogram of a fragment exposed to DNase I for 1 minute in the presence of 0.5 μg of PapX. **(C)** Representative chromatogram of a fragment exposed to DNase I for 20 minutes in the presence of 0.5 μg of PapX. Ratios of peak heights remained the same between conditions.

Supplementary Table 1. Bacterial strains and plasmids used in this study.

Plasmid or strain name	Function
pMCSG7	Recombinant PapX-His $_6$
pMCSG7_R127G	PapX-His $_6$ including an Arg^{127}Gly point mutant
pRS551	Promoterless *lacZ* containing vector
pRS551$_A$	*lacZ* fused to the first 130 bases of *flhD* promoter
pRS551$_B$	*lacZ* fused to the first 215 bases of *flhD* promoter
pRS551$_C$	*lacZ* fused to the first 360 bases of *flhD* promoter
pRS551$_D$	*lacZ* fused to the first 501 bases of *flhD* promoter
pRS551$_E$	*lacZ* fused to the first 597 bases of *flhD* promoter
pRS551$_F$	*lacZ* fused to the first 718 bases of *flhD* promoter
pLX3607 or pVector	Empty IPTG-inducible vector
pDRM001 or pPxWT	Wildtype *papX* made in pLX3607
pDRM002 or pR127G	*papX*_Arg^{127}Gly constructed in pDRM001
pDRM003 or pK54N	*papX*_Lys^{54}Asn constructed in pDRM001
pDRM004 or pR127K	*papX*_Arg^{127}Lys constructed in pDRM001
pDRM005 or pT21A	*papX*_Thr^{21}Ala constructed in pDRM001
pDRM006 or pR51A	*papX*_Arg^{51}Ala constructed in pDRM001
pDRM008 or pR55G	*papX*_Arg^{55}Gly constructed in pDRM001
pDRM009 or pH71A	*papX*_His^{71}Ala constructed in pDRM001
pDRM010 or pR101A	*papX*_Arg^{101}Ala constructed in pDRM001
pDRM011 or pR106A	*papX*_Arg^{106}Ala constructed in pDRM001
pDRM012 or pK113N	*papX*_Lys^{113}Asn constructed in pDRM001
pDRM013 or pK118A	*papX*_ Lys^{118}Ala constructed in pDRM001
pDRM014 or pR119A	*papX*_Arg^{119}Ala constructed in pDRM001
pDRM015 or pR123A	*papX*_Arg^{123}Ala constructed in pDRM001
pDRM016 or pR126A	*papX*_Arg^{126}Ala constructed in pDRM001
pDRM017 or pK129A	*papX*_ Lys^{129}Ala constructed in pDRM001
pDRM018 or pK130A	*papX*_ Lys^{130}Ala constructed in pDRM001
pKD3	λ red encoding chloramphenicol resistance cassette
pKD4	λ red encoding kanamycin resistance cassette
pCP20	λ red encoding recombinase to remove cassettes
pKD46	λ red encoding recombinase to insert cassettes
CFT073	Prototypical strain of pyelonephritogenic UPEC
CFT073 Δ*papX*	Deletion of *papX* in strain CFT073
CFT073 Δ*papX*Δ*lacZ*	Deletion of *papX* and *lacZ* in strain CFT073
CFT073 Δ*lacZ*	Deletion of *lacZ* in strain CFT073
K12 MG1655	Fecal/commensal strain K12 MG1655

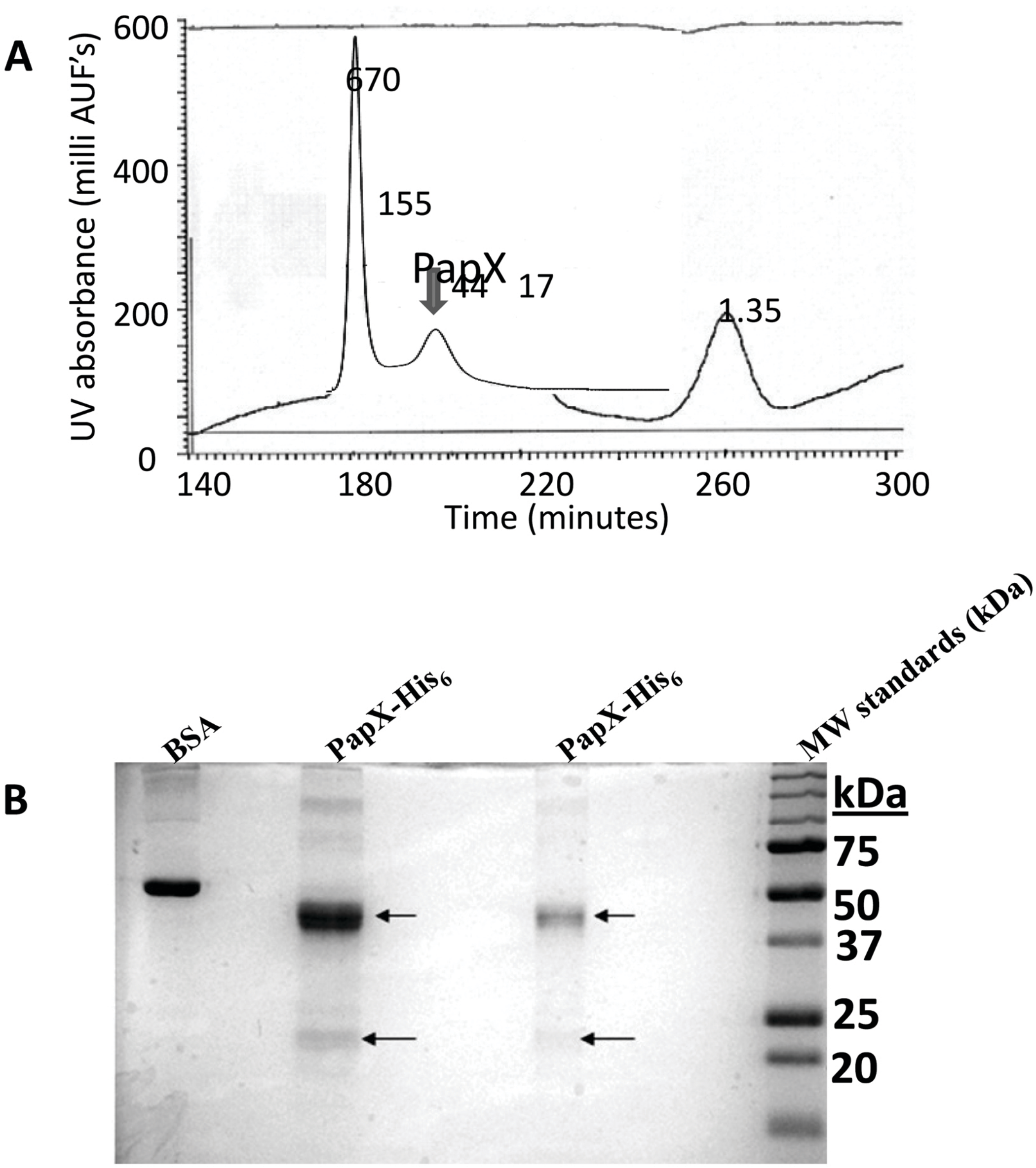
Supplementary Table 2. Primers used in this study.

Primer	Sequence (5'→ 3')	Function
*papX*H1P1	...TGCCTGAAGCTATCCGGCATACTCAGGCATTTCACGCTTTGTGTAGGCTGGAGCTGCTTC	λ red mutants
*papX*H2P2	..AGAAATCAGGCAGGAATAAATTGTAGTGGAAAGTCGATGTATGGGAATTAGCCATGGTCC	λ red mutants
*lacZ*H1P1	...TTACGCGAAATACGGGCAGACATAGCCTGCCCGGTTATTA	λ red mutants
*lacZ*H2P2	...ATGACTATGATTACGGATTCTCTGGCCGTCGTATTACAAC	λ red mutants
gapA 29bp fwd	...ACTCCACTCACGGCCGTTTCGACGGTACCG	Gel shift
gapA 29bp rev	...CGGTACCGTCGAAACGGCCGTGAGTGGAGT	Gel shift
papX 29bp fwd	...TTACGGTGAGTTATTTTAACTGTGCGCAAC	Gel shift
papX 29bp rev	...GTTGCGCACAGTTAAAATAACTCACCGTAA	Gel shift
EMSA154_F	...TCGCTACACTGGCAAGCAAA	Gel shift
EMSA154_R	...GTTGTGCGGTAAGTGTTTGT	Gel shift
*papX*RTfwd	...CGCATATCCCTTTCGGGAGCATTT	qPCR
*papX*RTrev	...TCCGCTGCGAATAATGTGGTGAGA	qPCR
*gapA*RTfwd	...CGTTAAAGGCGCTAACTTCG	qPCR
*gapA*RTrev	...ACGGTGGTCATCAGACCTTC	qPCR
*flhD*RTfwd	...TCCGCTATGTTTCGTCTCGGCATA	qPCR
*flhD*RTrev	...ACCAGTTGATTGGTTTCTGCCAGC	qPCR
*flhC*RTfwd	...AAACTGGCTTGTGTAATGGCGTCG	qPCR
*flhC*RTrev	...TCAACAAACCGCACCAATGTCCAG	qPCR
T^{21}Afwd	...GTAATATGGAGAATAATGAAATGAATAACGCAGACACATTAGAAAAAATAATCAGACAC	Site-directed mutant
T^{21}Arev	......CATTATACCTCTTATTACTTTACTTATTGCGTCTGTGTAATCTTTTTTATTAGTCTGTG	Site-directed mutant
R^{51}Afwd	...GGAGCATTTGTTGATGCAACTCTGTATTGCCACAAATAAAAGAATGCAG	Site-directed mutant
R^{51}Arev	...CTGCATTCTTTTATTTGTGGCAATACAGAGTTGCATCAACAAATGCTCC	Site-directed mutant
K^{54}Nfwd	...AGATATATTATCCTGCATTCTGTTATTTGTGCGAATACAGAGTTGCA	Site-directed mutant
K^{54}Nrev	...TGCAACTCTGTATTCGCACAAATAACAGAATGCAGGATAATATATCT	Site-directed mutant
R^{55}Gfwd	...AGATATATTATCCTGCATTCCTTTATTTGTGCGAATACAGAGTTGC	Site-directed mutant
R^{55}Grev	...GCAACTCTGTATTCGCACAAATAAAGGAATGCAGGATAATATATCT	Site-directed mutant
H^{71}Afwd	...CTGAATTTCTGGGGGTGTATGGAATAAATGCCTCAGTATATATGGTTCT	Site-directed mutant
H^{71}Arev	...AGAACCATATATACTGAGGCATTTATTCCATACACCCCCAGAAATTCAG	Site-directed mutant
R^{101}Afwd	...TAAGCCAGAAACTTCAGTTTACCGCAACTAATATTACCCGCATTACAG	Site-directed mutant
R^{101}Arev	...CTGTAATGCGGGTAATATTAGTTGCGGTAAACTGAAGTTTCTGGCTTA	Site-directed mutant
R^{106}Afwd	...TCAGTTTACCAGAACTAATATTACCGCCATTACAGATTTTTTAGAAAAAGCC	Site-directed mutant
R^{106}Arev	...GGCTTTTTCTAAAAAATCTGTAATGGCGGTAATATTAGTTCTGGTAAACTGA	Site-directed mutant
K^{113}Nfwd	...TAATATTACCCGCATTACAGATTTTTTAGAAAACGCCGGATATGTAAAAAG	Site-directed mutant
K^{113}Nrev	...CTTTTTACATATCCGGCGTTTTCTAAAAAATCTGTAATGCGGGTAATATTA	Site-directed mutant
K^{118}Afwd	...TTACAGATTTTTTAGAAAAAGCCGGATATGTAGCAAGGATGGATAGCAGGG	Site-directed mutant
K^{118}Arev	...CCCTGCTATCCATCCTTGCTACATATCCGGCTTTTTCTAAAAAATCTGTAA	Site-directed mutant
R^{119}Afwd	...AGATTTTTTAGAAAAAGCCGGATATGTAAAAGCGATGGATAGCAGGGAGG	Site-directed mutant
R^{119}Arev	...CCTCCCTGCTATCCATCGCTTTTACATATCCGGCTTTTTCTAAAAAATCT	Site-directed mutant
R^{123}Afwd	...GTAAAAAGGATGGATAGCGCGGAGGATCGCCGTGCTAA	Site-directed mutant
R^{123}Afwd	...TTAGCACGGCGATCCTCCGCGCTATCCATCCTTTTTAC	Site-directed mutant
R^{126}Afwd	...AAAAAGGATGGATAGCAGGGAGGATGCCCGTGCTAAAAAA	Site-directed mutant
R^{126}Arev	...TTTTTTAGCACGGGCATCCTCCCTGCTATCCATCCTTTTT	Site-directed mutant
R^{127}Gfwd	...TAGCAGGGAGGATCGCGGTGCTAAAAAAATCAGT	Site-directed mutant
R^{127}Grev	...ATCGTCCCTCCTAGCGCCACGATTTTTTTAGTCA	Site-directed mutant
R^{127}Kfwd	...GATGGATAGCAGGGAGGATCGCAAGGCTAAAAAAATCAGTCTGACAT	Site-directed mutant
R^{127}Krev	...ATGTCAGACTGATTTTTTTAGCCTTGCGATCCTCCCTGCTATCCATC	Site-directed mutant
K^{129}Afwd	...CAGGGAGGATCGCCGTGCTGCAAAAATCAGTCTGACATCTG	Site-directed mutant
K^{129}Arev	...CAGATGTCAGACTGATTTTTGCAGCACGGCGATCCTCCCTG	Site-directed mutant
K^{130}Afwd	...GGGAGGATCGCCGTGCTAAAGCAATCAGTCTGACATCTGAA	Site-directed mutant
K^{130}Arev	...TTCAGATGTCAGACTGATTGCTTTAGCACGGCGATCCTCCC	Site-directed mutant
*papX*MltplxRev	...TCCGCTGCGAATAATGTGGTGAGA	Multiplex PCR
XMltPlxfwd	...GCGCGCTTGTACACAGACAGTGTG	Multiplex PCR
*focX*MltPlxfwd	...CAGGCGTGTGGAAGCTGATG	Multiplex PCR
*papX*MltPlxfwd	...CCTGACCATCGGCAGTCGCCTC	Multiplex PCR
DNase*flhD*rev	...TCAGCAACTCGGAGGTATGC	DNase protect assay
DNase*flhD*Afwd	...ACCAAAAAGGTGGGTCTGCT	DNase protect assay
DNase*flhD*Bfwd	...AAAATCGCAGCCCCCCTCCG	DNase protect assay
DNase*flhD*Cfwd	...TTTGCTTGCCAGTGTAGCGA	DNase protect assay
DNase*flhD*Dfwd	...GTTGTGCGGTAAGTGTTTGT	DNase protect assay
DNase*gapA*fwd	...ACGTGACTGATTCTA	DNase protect assay
DNase*gapA*rev	...CAGCTATTTGTTAGTG	DNase protect assay
*flhD*EHrev	...TCAGCAACTCGGAGGTATGC	*lacZ*-fusions
*flhD*Afwd	...ACCAAAAAGGTGGGTCTGCT	*lacZ*-fusions
*flhD*Bfwd	...AAAATCGCAGCCCCCCTCCG	*lacZ*-fusions
*flhD*Cfwd	...TTTGCTTGCCAGTGTAGCGA	*lacZ*-fusions
*flhD*Dfwd	...GTTGTGCGGTAAGTGTTTGT	*lacZ*-fusions
*flhD*Efwd	...ATGTACTGATTTCCCATATT	*lacZ*-fusions
*flhD*Ffwd	...TGGAGAAACGACGCAATCCC	*lacZ*-fusions

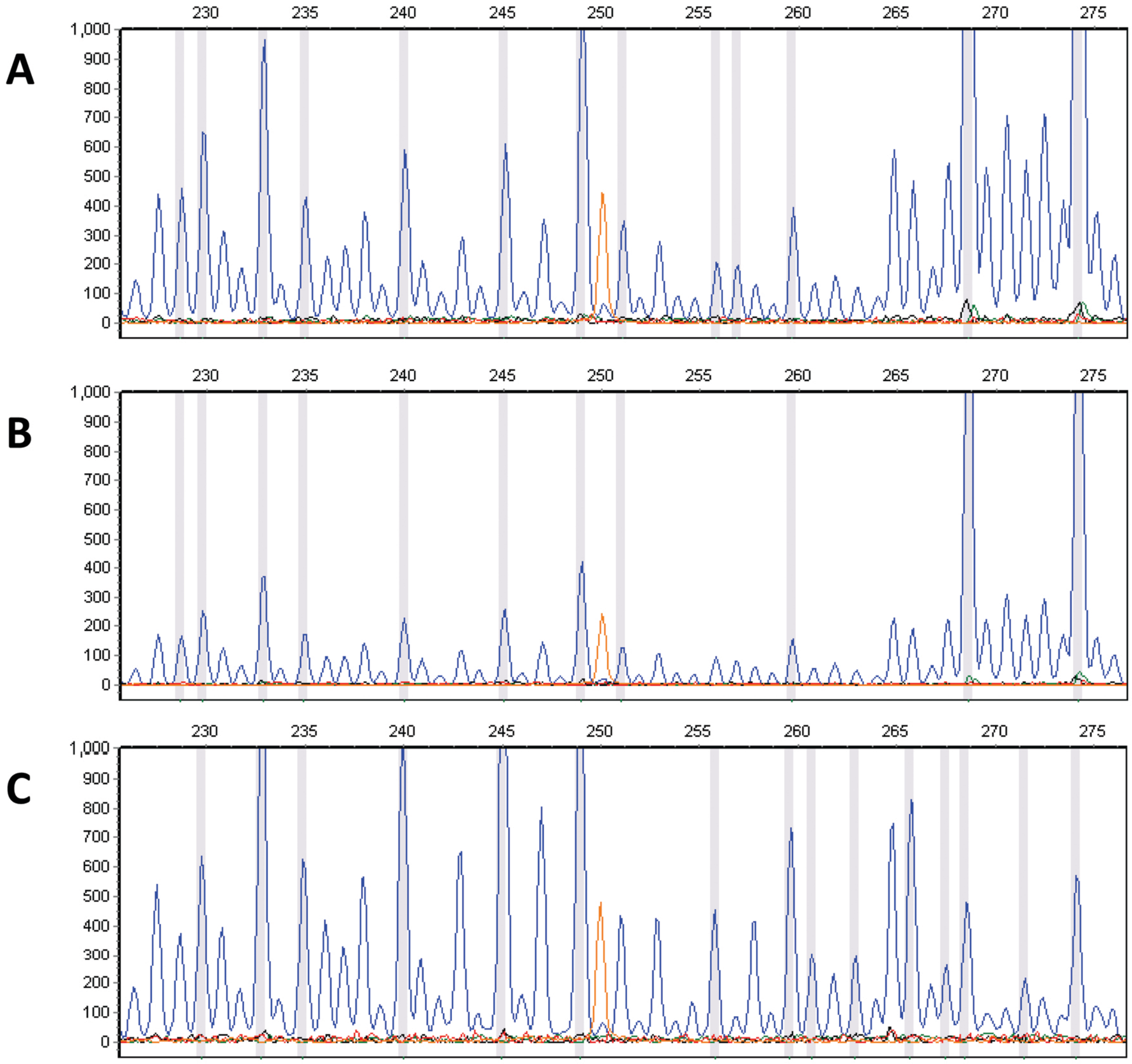


Growth kinetics of CFT073 Δ*papX* linker-insertional transformants

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3