pilQ Genomic Context in *Pseudomonas stutzeri* DBL332

P. stutzeri *pilQ* ORF

ATGAATACCACTCTCTCTCGTCTCGGCTTGTCTTTGCTGGCGGCGATTGTt
TTCGCCCGCGCTACTGGCCGCAAATCTTAATTCCCTCGATGTCGCATCGC
TACCCGGAGACCGCGTTGAGCTGAAGATGACGTTCGACGAGCCGGTGCCT
GCCCCGCGCGGATATACGCTCGACCAGCCAGCTCGCATCGCTCTAGATCT
GCCAGGTGTTTCCAGCAAGCTGACGTCCAAAAATCGCGAGCTCGGCGTTG
GTAATGCACGGAGTGTGACGGTAGTCGAGGCCCAGGGGCGCACGAGGCTT
ATCGTCAACCTCACGTCTCTGGTCCCTTATTCGACCCGAGTCGACGGGAG
TAATGTATTTGTCGTGCTTGGCGAAAGCGCTTCTGCTACAGAGCCCGTGT
CCGCAGCTTCGTCGGCGCCAGTCATTCCTATGACGAAGGAAAAGCTGGCC
CGGGCGAACGCTATCGACAACATCGACTTCAGGCGCGGCGAGGATGGCGC
AGGCAATATAGTCATAAGCCTGTCTGATCCATCCATCAGTCCGAGCATCG
AAGAGCAGGGTGGGAAGATTCGGGTCACGTTTGCCAAAACGCAGTTGCCC
GACTCCTTGCGTGTCCGTCTGGACGTTCAGGACTTCGCAACCCCAGTCAA
GTTCGTTGACGCAACCAGCAAGTCGGGCGATGCAAGTATCTCTATCGAGC
CGAGCGGGCGCTATGACTATCTAGCTTATCAGGCTGATGACAAGCTGACC
GTCAGCGTCAAGCCACTAAGCGAGGCTGATATCGAAAAGCGTAAAGCAGA
GCGCTTTGTTTATTCGGGTGAGAAGCTGTCACTGAACTTCCAGGACATCG
ACGTACGCTCCGTGCTGCAGCTAATTGCCGACTTCACTGATCTAAATCTT
GTTGCCAGTGATACTGTCCAAGGCAATATCACTCTGCGCTTACAAAACGT
ACCGTGGGATCAAGCGCTTGATCTGGTGCTGAAAACTAAGGGGCTCGATA
AGCGGCAAGTTGGAAACGTCTTGCTTGTTGCGCCAGCCGATGAGATCGCG
GCACGTGAAAGGCAAGAATTGGAGTCGAAGCGCCAAATTGCTGAACTTGC
GCCGCTACGTCGCGAGGTAGTTCAGGTGAACTATGCCAAGGCGTCCGACA
TTGCCAAGCTATTCC AAACGGTTACCAACAGTTCGGGGCAGGCAGATGAT
CGAGGCTCTATCGCTGTTGACGATCGCACAAATAATATAATTGCCTATCA
GACTCAAGAGCGCCTTGACGAGCTACGTCGCATCGTCACTCAATTGGATA
TTCCGGTCCGCCAAGTAATGATCGAGGCCCGGATTGTGGAGGCCAATGTC
GATTATGACAAAGCGTTAGGCGTGCGTTgGGGGGGGGAGTTTCACCTCAGG
CAACGCAGCGGTTTATGGTAAGGACGGCGCTACCACCTTTGATTCAGATA
CGGGTCGTGCTTATTTGCCAGGAACCGACACTATCGGGAACTTTACCGCT
AGTGACGGCGTTGCGCCGGTGCCCTTCGTTGACTTGGGTGTCGCGAACAG
CACCTCTGGCCTCGGCATTGGTTTCATCACTAATAATCTCATCTTAGATC
TGCAGCTTTCGGCAATGGAAAAGACTGGGAACGGGGAAAT CGTGTCGCAG
CCGAAAGTAA TGACTGCCGATAAAGAGACCGCCAAGATCCTTCGTGGCTC
AGAGATTCCATATCAAGAAGCCAGCTCCAGCGGTGCGACATCTACTTCGT
TCAAAGAGGCTGCTCTTTCGCTGGAAGTCACGCCGCTTATCACGCCAGAT
AATCGCGTGATTCTTGAGGTGAAGGTGACCAAGGACGCGGCGGATTTCAG
TCGTGCCGTAAACGGCGTGCCGCCAATCAACAAGAACGAGGTCAACGCCA
AGGTGTTGGTTGCTGATGGCGAGACCGTAGTTATTGGTGGTGTGTTCTCC
AATACGCAAACCCAGTCTGTCGACAAGGTTCCGTTCCTAGGTGACTTGCC
TTACGTAGGGCGGCTGTTCCGGCGCGACGTTGTGTCGGACGCCAAATCTG
AGCTGCTGATTTTCCTCACACCGCGTATCCTGAATCACCAGGCCATTGCA
GTGAATCGCTAA

Differences between wild-type and mutant *pilQ*

Mutant allele contains an extra “g” at position 1387 and is colored yellow above. This is a frameshift mutation that leads to a truncated protein. Wt PilQ is 704 amino acids long, mutant PilQ is 473

Amplified Construct for BP reaction

Color scheme matches sequence data above, and primer regions underlined in sequence above. No background color in primer sequence signifies nucleotides added to construct to allow recombination in Gateway BP reaction.

>Primer DBL3 (BP tailing primer F)

GGGGACAAGTTTGTACAAAAAAGCAGGCTCC

>Primer DBL4 (BP tailing primer R)

GGGGACCACTTTGTACAAGAAAGCTGGGTG

>Primer DBL353 (*pilQ* allele F)
CAAAAAAGCAGGCTCCTTACTTTCGGCTGCGACACG

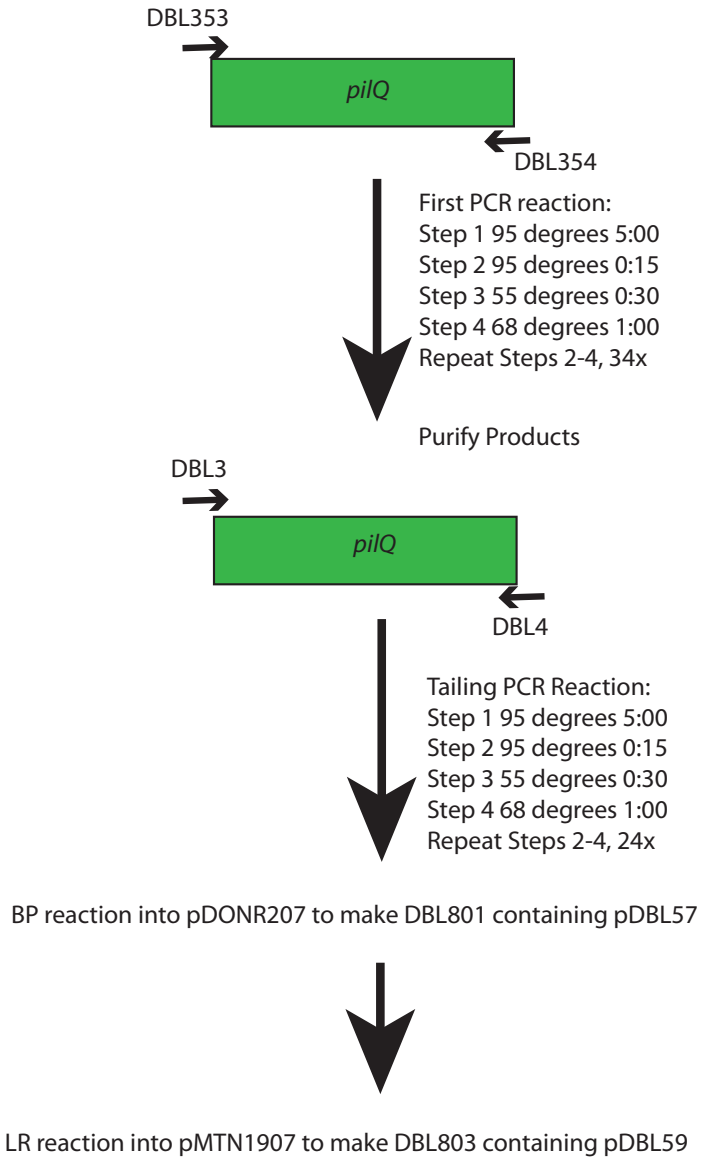
>Primer DBL354 (*pilQ* allele R)
GAAAGCTGGGTGAAACGGTTACCAACAGTTCGGGGC

Creation of Plasmids for *pilQ* Allele Swap Strain in *P. stutzeri* DBL332

General overview of using Gateway reactions and pMTN1907 for creating deletions within *Pseudomonas* can be found here:

https://dx.doi.org/10.6084/m9.figshare.3204175.v1

For this particular mutant, schematic of deletion construction is represented below.



Creation *pilQ* allele swap strains in *P. stutzeri* DBL332

Mate DBL803 with either DBL332
-Spin down 1mL of overnight culture of each
-Rinse all strains 2x 1mL 10Mm MgCl2, resuspend in 1mL 10Mm MgCl2.
-Mix recipient strain with DBL829 and DAB42 (*E. coli* containing plasmid pRK2013) in total volume of 100uL of 10Mm MgCl2.

-Ratio of 1:1:1
-Plate on Plain LB for 2 days at 27 degrees
-Resuspend in 1mL 10Mm MgCl2
-Plate 100uL of resuspension and 100uL of 1:10 dilution of resuspension on LB+ tetracycline 10, rifampicin 50, nitrofurantoin 40
-Pick single *Pseudomonas colonies* to overnight liquid culture in LB
-Plate 100uL of overnight culture onto 5% sucrose KB plates
-Screen for *pilQ* deletions (will not be able to carry out natural transformation of DNA, and colonies will not spread on KB media)