flgK Genomic Context in *Pseudomonas stutzeri* DBL332

5' upstream of *flgK* in *P. stutzeri* DBL332

TTCATCGCAACCATGCTGCCCATGGCGGAAAAAGCGGCCAAGCGCCTTGGCGTCG
AGCCACGTTTCCTCGTCGCACAAGCCGCGCTGGAAACCGGTTGGG
GCAAATCGATCATCAAGCAGAAGGACGGCACAAACAGCCACAACCTGTTTGGCATC
AAGTCCACTGGTTGGGAAGGTGAAGCCGCGAAGGTCACTACCAC
CGAGTACGTGAATGGCAAGGCGACCAAGCAGGTCGCAGGTTTCCGAGCGTATGAC
TCGTTCGAGCACAGCTTCAATGACTACGTCCGGTTGCTGGAGAAC
AACGATCGTTACAAGCCAGCCATTCAGGTGGCCAGTGCGTCCGGCGACTCCGAAC
GCTTCGTCAAGGAATTGCAGCGAGCCGGTTACGCGACCGACCCGC
AATACGCGAGCAAAATCAACCAGATCGCCCGCAAAGTGCAGACATATCAGACAATC
GCCGACGCGAGTTCGGCGCCTGCGGTACGGACTAGAGGCTAATC

P. stutzeri *flgK* ORF

ATGGCTGACCTACTGAACATTGGCCTGTCGGGGCTGAGCGCGAGCAAAAGCCAGC
TGTCGATCACCGGCCACAATATCAGCAACGTCAATACTCCCGGCT
TCAGCCGGCAGGACGCATCCCAGGCTACCCGCGTTCCTCAGTTCAGTGGGGCGG
GTTACGTTGGCTCGGGTACGACGCTGGTGGAGATTCGCCGGAGCTA
CAGTGAGTTTCTGACCAGCCAGCTGCGCAGCAGCACCTCGTTAAGTAGCGATGTC
GAAGCTTATAAAAGCCAGATCGACCAGCTCGATTCGCTTCTGGCC
GGCAGCACGACGGGTATTACGCCTTCGTTGCAGCAGTTTTTTTCGGCCTTCCAAAC
GGCAGCGGAGGATCCGTCCAACATCCCTGCGCGTCAGTTGGTAC
TGGCGGAGGCGGAAGGCCTGGCGCGTCGATTCAATACGGTCTCTGATCGATTGAC
CGAGCAGAACGACTTTACCAACAAGCAAATGGCGGCGGTTACCGA
TCAGGTCAACCGTCTTGCAGGCAGCATCGGCAGCTTGAACAATGCGATCGCGATC
GCTTCGGCTAATGGCCAGCAGCCCAACGACCTGCTCGATGCACGG
GACGAAGCGGTGCGTCAGCTATCGACCTACATCGGCGTCACCGTAACGCCGCAGG
ACGACAACAGCTTCAATATCTCCGTTGGTACAGGTCAGCCGCTGG
TCGTGGGAAGCACGGTGAGCCGTTTGGAAGTCGTGCCGGGGCAGAGCGATCCCA
ATCGCTACGAAATCCAGTTTGTCAGCGGCAACTCGCGGCAGGGCAT
TACGTCGCAGATCACCGGCGGCGAGCTGGGCGGATTGATCCGTTATCGCGAGGAA
GTGCTGGACCCGACCATGAACTCGCTCGGCAGACTGGCGCTTGCG
GTAAGTGATCAGATCAACTCGCAACAGACGCAGGGGCTCGACCTGAAGGGGCAAG
TGGGCGAAGCCCTGTTTGGTGACTACAACGCACCCTCGCTGATGG
GCCTGCGTGCCACCCCTTTCAATGGCAATACGTCAGCGGCCGACGCGGCATTGGA
GATTACAGACACCGGTAATCTGACGATCAGCGACTATCGTTTGGA
ATTCGACGGAACGAATTACTCGGCAAGGCGGCTGAGTGACAACGCCAGCATCAGC
GTTACCCAAAATGCCGGGCCGCCCGCGTACTTGAGTTTTGCTGAC
AGCGCAGGGAAGGATCAGGGCTTCCAGGTCTCTATCAGCGGCACCCCTAGCGCAG
GCGACAAATTCTCGTTGCAACCGACTCGACGCGGTGCCACGGACA
TCAAGACAGTACTGGATCAGGCGGATCAGCTAGCTTTCGCGGCGCCTGTAAGGGC
GCAAAGCAATTTACAGAATGCCGGTACAGGTTTAATCAGTCAGCC
TGAGACGTCCGATACGGTGGACATGGACGCGCTGAAAGCTGCGCTTCCGGCCGAC

ATTACCCTGACTTACAACGCCGGTAGTCCTCCAACCATCTCTGGT
TCAGGCGTGACTCAGGTGAGCCCGGCCGTCTTCACCCCGGGACAGACCAACGAG
CTGGCTGTCACCATCGATGGCCACAGCTTTACATTCACGGTTAGCG
GCCGCCCTCAGGCAGGTGACACCTTTACGATTGGCTTCAACCAGAACGGTGTGTC
GGACAACCGAAACGCCTTGAAAATGGTGGATCTTCAGACAAAGCA
GACAATCGGAGTTGATCCGAGCGTGTCAGGTTCAGGGAAGAGTTTCACCGACGGT
TATGGCGAACTCGTCGAGCGTGTCGGAACGCTGACGGCTCAGGCG
CGAATGGACAGCGATGCGACCACCGCGATTCTCAAACAAGCCACCGATAATCGAGA
CTCGCTATCCGCGGTCAACCTGGACGAAGAGGCGGCCAACCTGA
TCAAGTTCGAGCAGTACTACAACGCTTCTGCTCAGATCATTCAAGTTGCGCGCACC
ATGTTCGATACATTGATCAGCACCTTCAGGTAA

3' downstream of *flgK* in *P. stutzeri* DBL332

TAGGCCCGCCCATGCGTATCTCGACCTTGCAAGCCTTCAATAATGGCGTAACCGGC
ATCCAGCGAAACTATTCCAATGCGGCCCGTACCCAAGAGCAGAT
CAGTACTGGCAACCGCATTCTTACGCCTGCCGACGATCCGGTTGCATCGGTGCGC
CTGCTTCAGCTCGAACAGCAGCAGAGCGTGCTGGGTCAGTACAAC
ACGAACTTGACGGCGGCGCAGAACAGCCTGACTCAGGAAGAAGTGACCTTGAACT
CGGTCAATACCGTACTGCAGCGCGTCCGAGAGCTGGCGGTGCAGG
CGGGCAACGGTTCGCTCGATCCCCAGGATCGTAAGGCCATCGCTGCCGAACTGGG
TGAACGGGAGGATGAGTTGATGTCCTTGATGAATACGCGCAACGC
GCGCGGTGAGTATTTATTCTCTGGCTTCCAGGGCAAAACCCAACCTTTCGTGCGTG
CTGCTGACGGAACCTATAGCTACGCGGGTGATGAAGGGCAGCGA

Synthesized 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.

GGGGACAAGTTTGTACAAAAAAGCAGGCTCC

GCCAAGCGCCTTGGCGTCGAGCCACGTTTCCTCGTCGCACAAGCCGCGCTGGAA
A C C G G T T G G G
GCAAATCGATCATCAAGCAGAAGGACGGCACAAACAGCCACAACCTGTTTGGCATC
AAGTCCACTGGTTGGGAAGGTGAAGCCGCGAAGGTCACTACCAC
CGAGTACGTGAATGGCAAGGCGACCAAGCAGGTCGCAGGTTTCCGAGCGTATGAC
TCGTTCGAGCACAGCTTCAATGACTACGTCCGGTTGCTGGAGAAC
AACGATCGTTACAAGCCAGCCATTCAGGTGGCCAGTGCGTCCGGCGACTCCGAAC
GCTTCGTCAAGGAATTGCAGCGAGCCGGTTACGCGACCGACCCGC
AATACGCGAGCAAAATCAACCAGATCGCCCGCAAAGTGCAGACATATCAGACAATC
GCCGACGCGAGTTCGGCGCCTGCGGTACGGACTAGAGGCTAATC
TAGGCCCGCCCATGCGTATCTCGACCTTGCAAGCCTTCAATAATGGCGTAACCGGC
ATCCAGCGAAACTATTCCAATGCGGCCCGTACCCAAGAGCAGAT
CAGTACTGGCAACCGCATTCTTACGCCTGCCGACGATCCGGTTGCATCGGTGCGC

CTGCTTCAGCTCGAACAGCAGCAGAGCGTGCTGGGTCAGTACAAC
ACGAACTTGACGGCGGCGCAGAACAGCCTGACTCAGGAAGAAGTGACCTTGAACT
CGGTCAATACCGTACTGCAGCGCGTCCGAGAGCTGGCGGTGCAGG
CGGGCAACGGTTCGCTCGATCCCCAGGATCGTAAGGCCATCGCTGCCGAACTGGG
TGAACGGGAGGATGAGTTGATGTCCTTGATGAATACGCGCAACGC
GCGCGGTGAGTATTTATTCTCTGGCTTCCAGGGCAAAACCCAACCTTTCGTGCGTG
CTGCTGACGGAACC

CACCCAGCTTTCTTGTACAAAGTGGTCCCC

>Primer DBL575 (*flgK* deletion test F)
CACAACCTGTTTGGCATCAAGTC

>Primer DBL576 (*flgK* deletion test R)
ATCAACTCATCCTCCCGTTCACCC

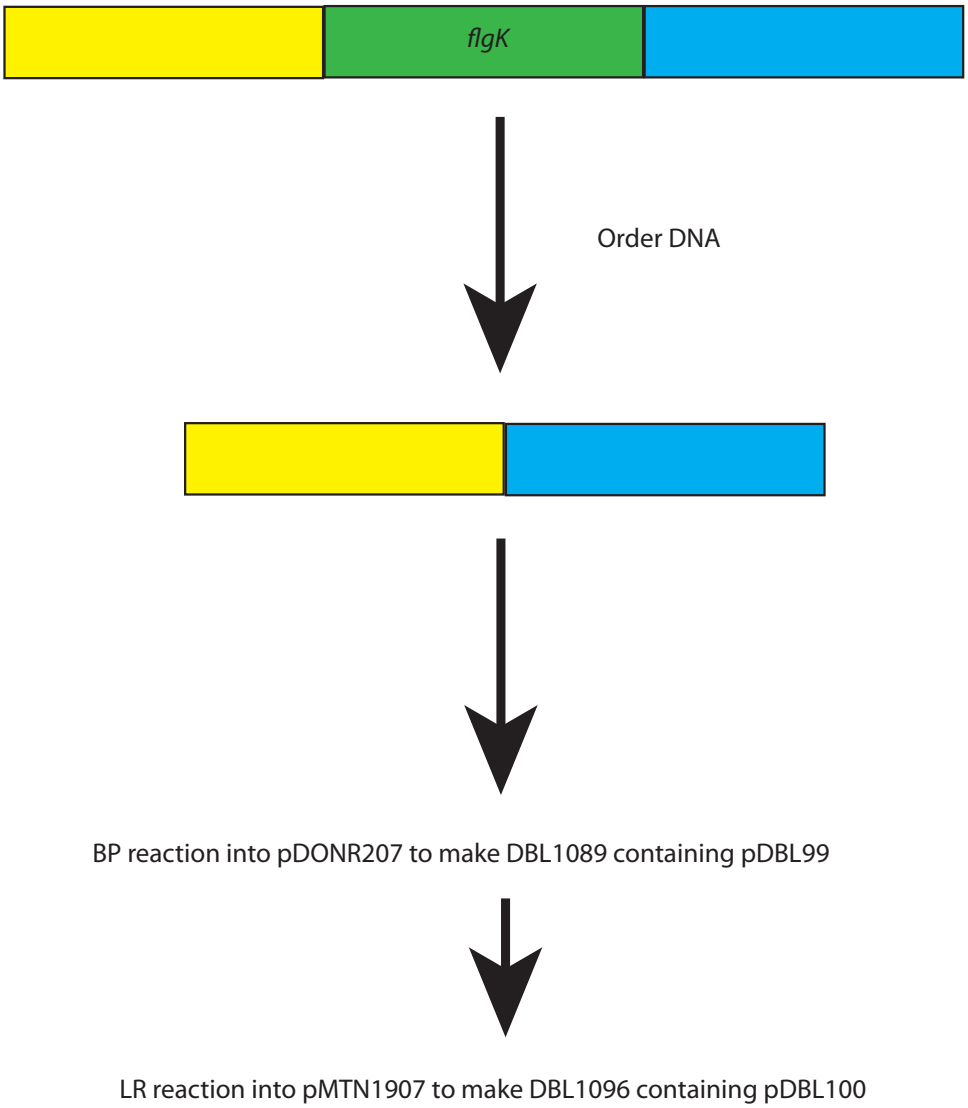
738bp product if deletion

Creation of Plasmids for *flgK* Deletion 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 *flgK* Deletion Strains in *P. stutzeri* DBL332

Naturally transform DBL332 with DBL1096

-Grow DBL332 overnight at 27 degrees in Saltwater LB
-Add ~10uL of DBL1096 plasmid to 50-100uL of DBL332
-Plate on SWLB agar plate

-Grow plate for two days at 27 degrees
-Resuspend in 1mL 10Mm MgCl2
-Plate 100uL of resuspension and 100uL of 1:10 dilution of resuspension on KB+ tetracycline 10, rifampicin 50, nitrofurantoin 40
-Pick single *Pseudomonas colonies* to overnight liquid culture in KB
-Plate 100uL of overnight culture onto 5% sucrose KB plates
-Screen for *flgK* deletions (immotile in 0.25% LB agar)