

Supplemental Data

Crystal Structure of a Functional Dimer of the PhoQ Sensor Domain

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Constructions and Cloning for Mutant Variants

Variants of PhoQ in which amino acids at residues 50, 54, or 179 were substituted were made using pLPQ3 (*HindIII-NcoI*) and pLPQ3 λ as follows. pLPQ3 (*HindIII-NcoI*) [1] is a pBR322-derived plasmid in which the *phoP-phoQ* operon is driven by the *lacUV5* promoter and *HindIII* and *NcoI* restriction enzyme sites have been introduced at codons 42-44 and 189-191 of *phoQ*, respectively. The mutations that create the *HindIII* site are silent and the mutations that create the *NcoI* site result in a serine in place of the wild type tyrosine at codon 189. pLPQ3 λ (1) is a derivative of pLPQ3 (*HindIII-NcoI*) in which a ~1.2 kb *HindIII-NcoI* stuffer fragment corresponding to bp 23901-25157 of λ has been cloned into the *HindIII-NcoI* backbone of pLPQ3 (*HindIII-NcoI*). The following primers were used for the mutagenesis:

P1: 5'-GATCGGTTATAGCGTAAGCTTCGATAAAACTACGTTTCGGC-3'

P2: 5'-CCAGCTCCAGAACATGTAGGAACCTTTTGTAGCTCCACCGG-3'

P3: 5'-GATCGGTTATAGCGTAAGCTTCGATAAAACTACGTTTCGGCTGTTACGTGACGAGAGC-3'

P4: 5'-GATCGGTTATAGCGTAAGCTTCGATAAAACTACGTTTGACCTGTTACGTGGC-3'

P5: 5'-CCAGCTCCAGAACATGTAGGAACCTTTTGTAGCTCCACCGGAATGGTGCGCACCACC-3'

Restriction enzyme recognition sites are italicized in each primer (*HindIII* in P1, P3, and P4; *AflIII* in P2 and P4). Mutagenized codons are underlined. PCR products were generated using pLPQ3 (*HindIII-NcoI*) as a template and the following primer pairs for each listed mutation:

G54D	P3/P2
R50D	P4/P2
D179R	P1/P5
R50D/D179R	P4/P5

The resulting DNA fragments were then digested with *HindIII* and *AflIII* and cloned into the *HindIII-NcoI* backbone of pLPQ3 λ . Note that this procedure results in restoration of the wild type tyrosine codon in the *phoQ* gene at position 189. All mutations were confirmed by double stranded DNA sequencing of the resulting plasmid. The mutations were then moved into a lower copy number vector by sub-cloning the *EcoRI-SalI* *P_{lacUV5-phoP-phoQ}* fragment (2) into the *EcoRI-SalI* backbone of pGB2 (3).

References

1. Regelman, A. G, Lesley, J. A., Mott, C., Stokes, L., and Waldburger, C. D. (2002) *J. Bacteriol.* **184**, 5468-5478.
2. Waldburger, C. D., and Sauer, R. T. (1996) *J. Biol. Chem.* **271**, 26630-26636.
3. Churchward, G., Belin, D., and Nagamine, Y. (1984) *Gene* **31**, 165-171.