

| PhoQ allele | Fold activation | PhoQ allele | Fold activation |
|-------------|-----------------|-------------|-----------------|
| R219C | 2.27 | R236S | 15.63 |
| P220H | 3.16 | L239F | 7.09 |
| I221N | 1.29 | L239P | 5.60 |
| R226W | 3.02 | P241L | 1.84 |
| E227V | 2.74 | P241T | 1.46 |
| E227K | 15.10 | E242K | 1.37 |
| V228I | 3.64 | T244K | 9.68 |
| R229L | 4.97 | R245C | 2.35 |
| E230A | 13.29 | R245L | 9.63 |
| E230G | 19.33 | E246D | 2.02 |
| E230K | 12.48 | L247Q | 4.35 |
| L231F | 5.27 | L247P | 7.74 |
| L231R | 3.24 | L250H | 2.09 |
| L231I | 12.12 | R252C | 2.08 |
| L231V | 9.05 | L254I | 8.79 |
| L231P | 11.63 | L254F | 26.59 |
| E232G | 17.52 | L254V | 3.73 |
| E232K | 36.16 | N255T | 21.78 |
| E232V | 16.41 | N255I | 24.46 |
| D233G | 1.14 | L257Q | 0.84 |
| D233Y | 2.26 | L258F | 6.39 |
| D233V | 3.99 | E261K | 13.28 |
| H234Q | 5.69 | Y265C | 14.78 |
| R236H | 19.33 | N266S | 4.68 |

Table S1. Fold activation of the different PhoQ alleles when compared to wild-type grown under PhoQ repressive conditions. The mean activity of alkaline phosphatase was determined for each allele in at least three independent experiments and then compared to the mean wild-type activity. All strains were grown in N minimal media in the presence of 10 mM MgCl₂. Amino acid substitutions resulting in 10 fold increase in activity above the wild-type are shown over a grey background.