Species-specific residues calibrate SoxR sensitivity to redox-active molecules

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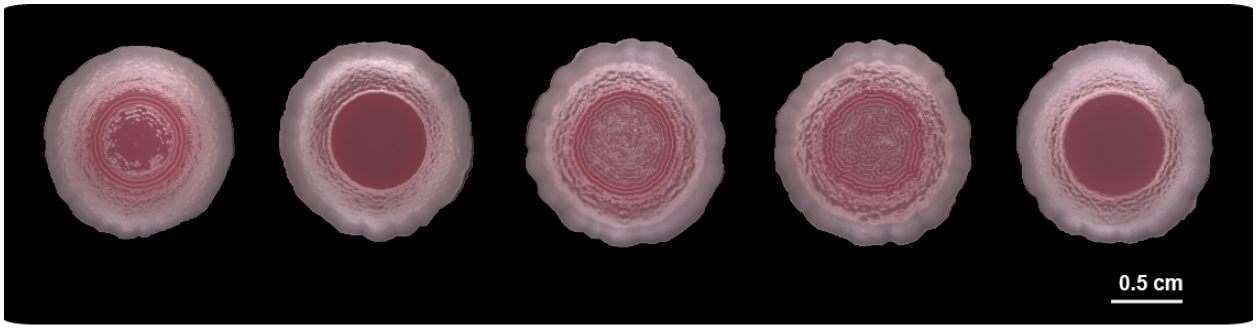
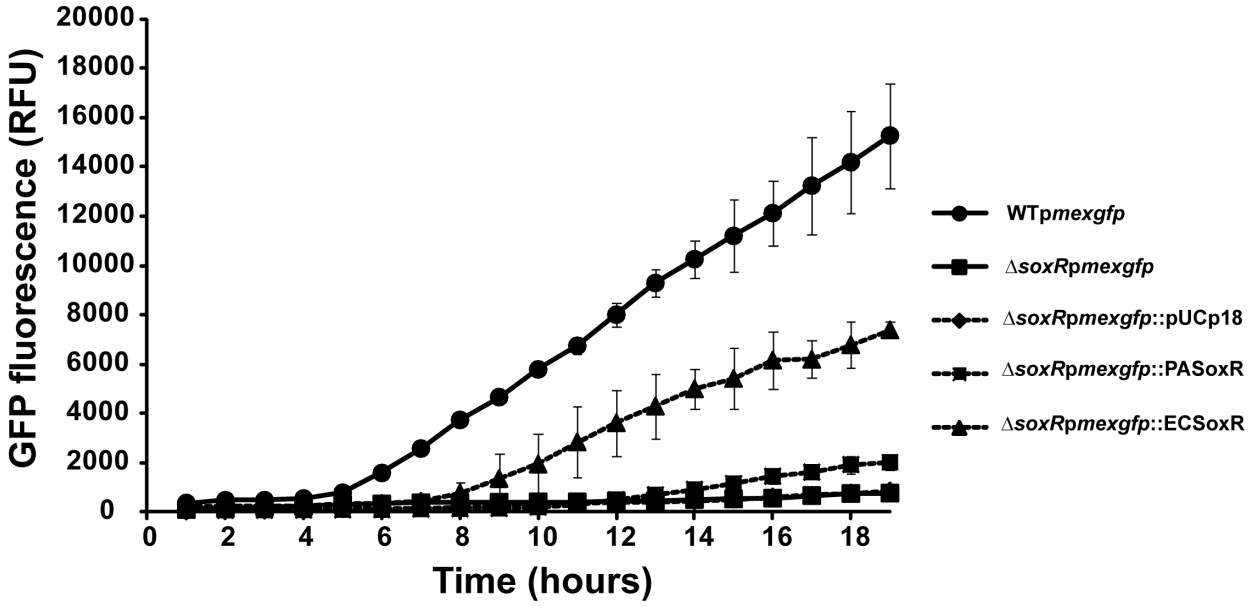
Supplementary Figure S1.

A. Colony morphology assay

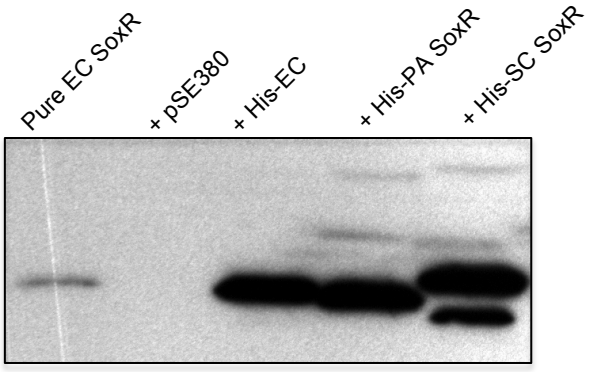


B. Phenazine-dependent activation of *P. aeruginosa* SoxR regulon

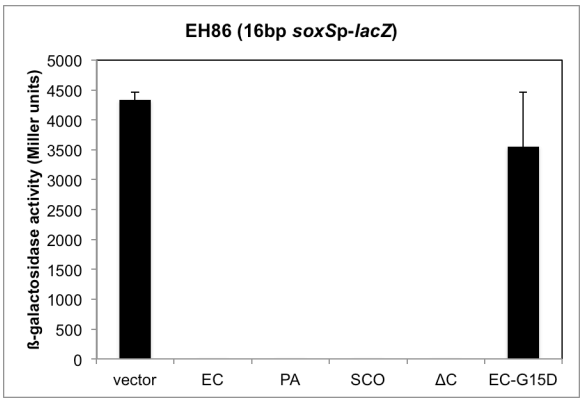


Supplementary Figure S2.

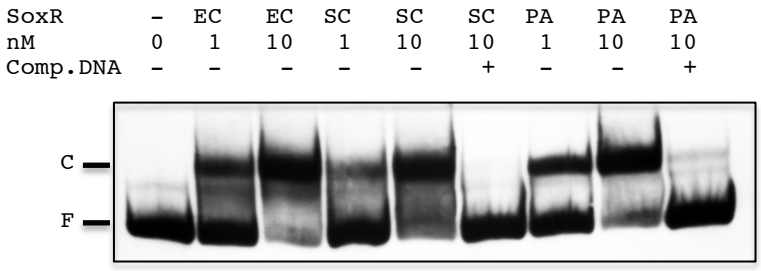
A. His-SoxR expression in *E. coli*



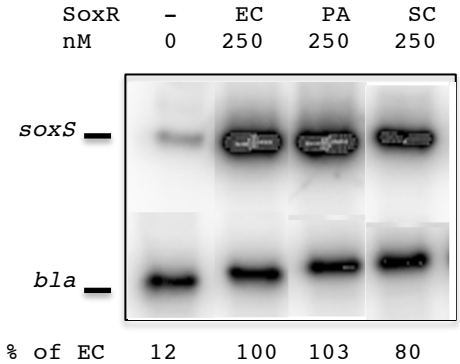
B. *soxS* promoter binding *in vivo*



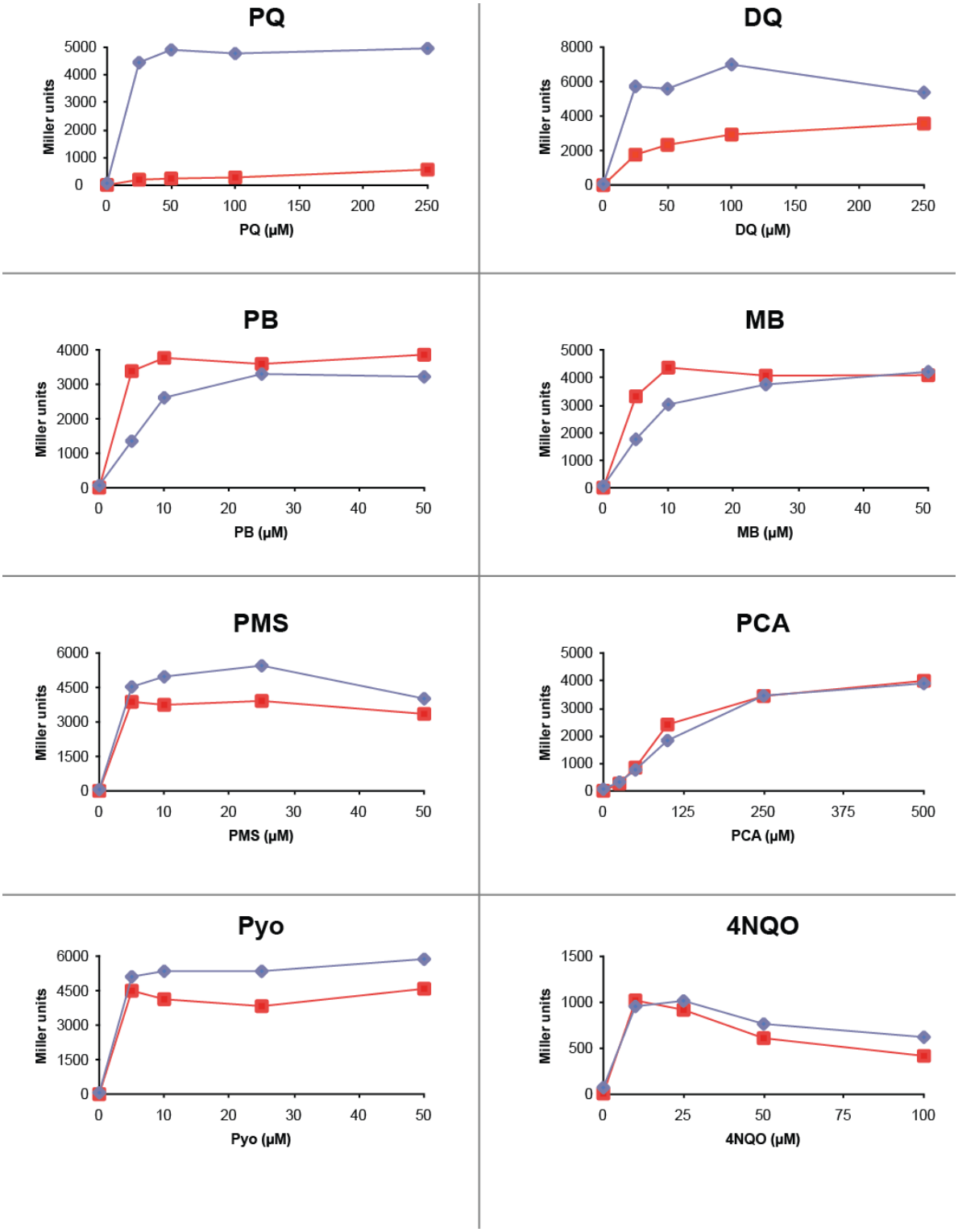
C. *soxS* promoter binding *in vitro*



D. *In vitro* transcription of *soxS* gene

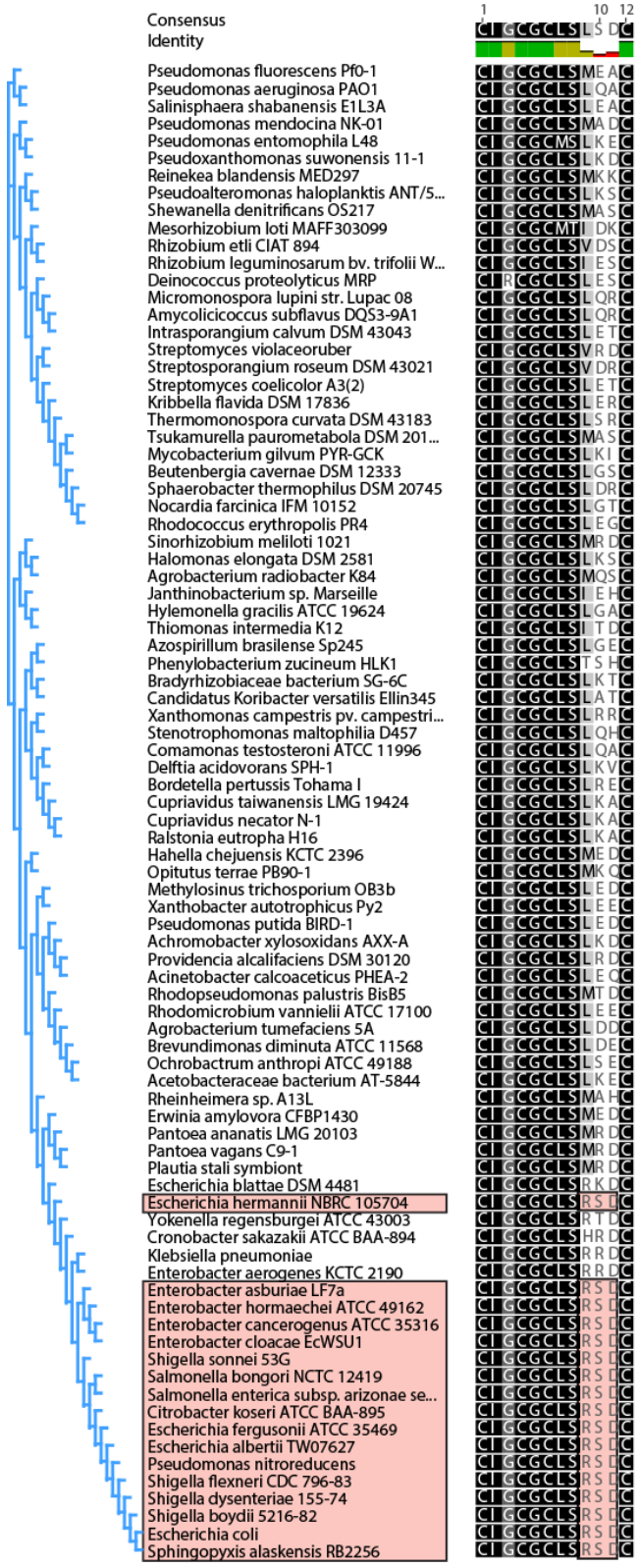


Supplementary Figure S3

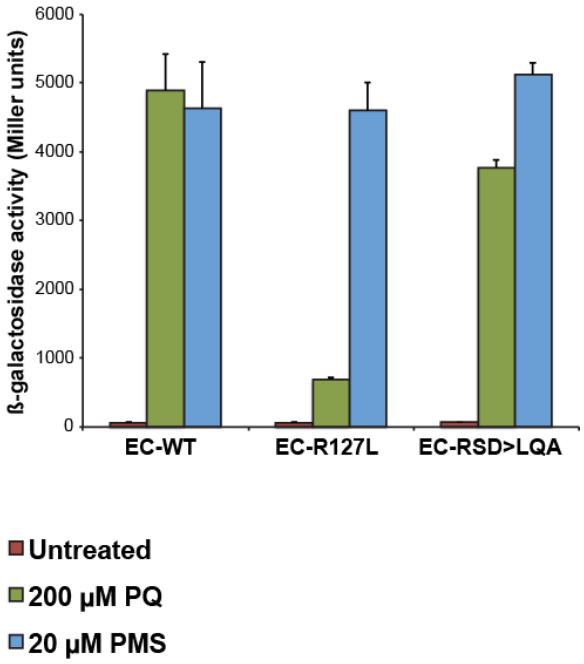


Supplementary Figure S4

A. Allignment of SoxR [2Fe-2S] cluster domain



B. Activity of *E. coli* SoxR mutants



Supplementary information figure legends

Figure S1. Complementation of a *P. aeruginosa* Δ*soxR* mutant by *E. coli* or *P. aeruginosa* SoxR.

A. Colony morphology assay. Cultures were grown for 16 h in LB medium supplemented with 300 μg mL^{-1} carbenicillin. Ten microliters of each culture was spotted on agar plates (1% tryptone, 1% agar, supplemented with 20 μg mL^{-1} Coomassie Blue and 40 μg mL^{-1} Congo Red) for two days before imaging (Keyence VHX1000 microscope). As reported previously (Dietrich *et al.*, 2008), wild type PA14 started to wrinkle after two days of growth while the Δ*soxR* mutant remained smooth. Complementation of the Δ*soxR* mutant with *P. aeruginosa* SoxR (Δ*soxR:PAsoxR*) or *E. coli* SoxR (Δ*soxR:ECsoxR*) restored the wrinkled colony morphotype of wild type.

B. Expression of *E. coli* SoxR in the *P. aeruginosa* PA14 Δ*soxR* mutant mediates phenazine-dependent activation of the SoxR regulon. The *mexGHI-ompD* operon encodes an efflux pump that is part of *P. aeruginosa's* SoxR regulon. It is expressed in a SoxR- and phenazine-dependent manner in the stationary phase of planktonic cultures. To test if *E. coli* SoxR could complement a PA14 Δ*soxR* mutant, we made transcriptional *gfp* reporter constructs under the control of the *mexG* promoter. Strains were diluted to an optical density of 0.05 at 500 nm and cell density and GFP fluorescence were monitored for 19 hours. Data represent the mean and standard deviation of three experiments. Complementation with *P. aeruginosa* and *E. coli* SoxR both restored *mexG* expression in the Δ*soxR* mutant.

Figure S2. SoxR protein expression, *soxS* promoter binding, and *in vitro* transcription of the *soxS* gene.

A. SoxR protein levels in *E. coli*. EH46 cells expressing empty vector (pSE380) or histidine-tagged SoxR proteins from *E. coli* (His-EC SoxR), *P. aeruginosa* (His-PA SoxR), or *S. coelicolor* (His-SC SoxR) were grown at 37°C for 2.5 h. Total cell extract (50 µg per lane) was resolved on a 15% SDS-polyacrylamide gel and subjected to immunoblot analysis using anti-histidine antibody (GE Healthcare). Purified histidine-tagged *E. coli* SoxR (10 ng) was loaded as a control. His-SC SoxR migrates as a higher molecular weight species than His-EC and His-PA SoxR.

B. SoxR binding to *soxS* promoter *in vivo*. *E. coli* strain EH86 (Δ*soxRS* lysogenized with λ[16bp *soxS* promoter-*lacZ* reporter]) was transformed with vector control, histidine-tagged *E. coli, P. aeruginosa* or *S. coelicolor soxR* genes, C-terminal truncated *S. coelicolor soxR,* or the *E. coli* DNA binding variant G15D. The wild type *soxS* promoter has a 19-bp spacer separating the -10 and -35 elements. The shortened (16-bp) *soxS* promoter renders *soxS* transcription constitutive and promoter occupation by SoxR prevents access to RNA polymerase (Hidalgo and Demple, 1997). Low ß-galactosidase activity in this background thus indicates specific promoter binding by SoxR, while high ß-galactosidase activity indicates defective promoter binding as demonstrated by cells expressing the *E. coli* SoxR DNA-binding mutant G15D (Fig. 2D, Chander *et al.* 2003). The values shown represent the means and standard errors of three independent experiments.

C. SoxR protein binding to *soxS* promoter *in vitro*. A DIG-end-labeled fragment (180 bp) containing the *soxS* promoter was incubated with 1 nM or 10 nM purified histidine-tagged SoxR proteins from *E. coli* (EC), *S. coelicolor* (SC), or *P. aeruginosa* (PA). Protein-bound complexes (C) were separated from free DNA (F) on a 5% native polyacrylamide gel. SoxR binding

specificity was demonstrated by the addition of a 500-fold molar excess of unlabeled probe (Comp. DNA).

D. Transcription of the *soxS* gene *in vitro*. Purified histidine-tagged SoxR proteins (250 nM) from *E. coli* (EC), *P. aeruginosa* (PA), or *S. coelicolor* (SC) were incubated with a plasmid containing the *soxS* and *bla* genes, *E. coli* σ^{70}-RNA polymerase, and four ribonucleotide triphosphates for 15 min at 37°C. The *soxS* and *bla* transcripts were quantified by primer extension analysis as described (Chander and Demple, 2004). Reactions were electrophoresced on 8% polyacrylamide, 6 M urea gels and quantified on a Storm phosphorimager. The *bla* gene is a SoxR-independent transcript and serves as a loading control. The amount of *soxS* mRNA is reported as a percent of the amount obtained with *E. coli* SoxR.

Figure S3. Comparison of the transcriptional response of *E. coli* and *P. aeruginosa* SoxR to varying doses of redox-active molecules.

E. coli strain EH46 (Δ*soxRS* lysogenized with λ[*soxS* promoter-*lacZ* promoter] expressing histidine-tagged *E. coli* SoxR (blue lines) or *P. aeruginosa* SoxR (red lines) were treated with the indicated concentrations of various redox-active drugs for 1 h before the assay for ß-galactosidase activity (Miller units).

Figure S4. The [2Fe-2S] cluster domain of SoxR has a hypervariable stretch of three amino acids.

A. Shown is a sequence allignment of the [2Fe-2S] cluster domain of the SoxR proteins represented in the phylogenetic tree in Fig. 3B. The blue tree on the left of the allignment is a different representation of the SoxR tree from Fig. 3B, and was generated accordingly. [2Fe-2S] clusters containing the RSD motif are highlighted in red.

B. ß-galactosidase activity was measured in EH46 cells (*soxS* promoter-*lacZ* reporter) expressing wild type or mutant *E. coli soxR* alleles. The mutations are within the [2Fe-2S] cluster domain. Activity was measured in untreated cells (red columns) or cells treated for 1 h with 200 µM PQ (green columns) or 20 µM PMS (blue columns).

Table S1. Primers used in this study

Cloning	**Sequence (5' → 3')**		
pET-F	CGC GTC GAC TCA CTA TAG GGG AAT TGT G		
pET-R	GCT TTG TTA GCA GCC G		
380F-Bam	CCG CCG GAT CCG ACA TCA TAA CGG TTC TGG C		
380R-Bam	GCA GAT CTG TCA TGA TG		
pmexG-F	TAC CAA GCT TCT CGT GGC CAA CCA GAA TAG		
pmexG-R	TTG CGA ATT CGT CGT TCC TTG TGC TGG TC		
PA and EC Mutagenic*	**Sequence (5' → 3')**		
PA-V64I	AAG GTC GCC CAG CGG ATC GGC ATT CCC CTC G		
PA-R82H	CCC TGC CGG CCG GGC ACA GCC CTA GCG CGG C		
PA-P84L	CGG CCG GGC GCA GCC TTA GCG CGG CGG ACT G		
PA-A94S	TGG GCG CGC CTG TCG TCG CAG TGG AAG GAG G		
PA-L125R	GCG GCT GCC TGT CGC GCC AGG CCT GCC CGT TG		
PA-Q113E	CTG CTG TTG CGC GAC GAA CTG GAC GGC TGC A		
PA-Q126S	GGC TGC CTG TCG CTC TCG GCC TGC CCG TTG CG		
PA-A127D	GCC TGT CGC TCC AGG ACT GCC CGT TGC GCA AC		
PA-RSD	GCG GCT GCC TGT CGC GCT CGG ACT GCC CGT TGC GCA AC		
PA-AAA	TGC GGC TGC CTG TCG GCC GCG GCC TGC CCG TTG CGC		
PA-L125A	TGC GGC TGC CTG TCG GCC CAG GCC TGC CCG TTG		
PA-ASD	TGC GGC TGC CTG TCG GCC TCG GAC TGC CCG TTG		
PA-C118A	GAC CAA CTG GAC GGC GCC ATC GGT TGC GGC TG		
EC-LQA	TGT GGC TGC CTT TCG CTC CAA GCT TGC CCG TTG CGT AAC		
SCO Mutagenic*	**Sequence (5' → 3')**		
SCO-S154stop	GGA GCG CCG CGG CTG AAC CGC CAG GGG C		
qRT-PCR	**Sequence (5' → 3')**	**Amplicon**	**Size (bp)**
hrdB-F	CAT GCG CTT CGG ACT CA	*hrdB*	95
hrdB-R	ACT CGA TCT GGC GGA TG		
1178-F	TCA AGG TCC GGC AGG TCT A	*SCO1178*	82
1178-R	CCG TCC TCC TGC TTG GT		
2478-F	GAG ATC ACC CCG AAA CTG G	*SCO2478*	104
2478-R	AAG TGC CAG TCG ATG ACG TT		
4266-F	GAT GGG CAT CCT CCA GTT C	*SCO4266*	104
4266-R	CGT TCT TCG CGT ACT GCA C		

* Sequence of forward primers used to mutagenize *P. aeruginosa, E. coli* and *S. coelicolor soxR* genes cloned in plasmid pSE380 using either Invitrogen's GENEART site-directed mutagenesis kit (for *P. aeruginosa* and *E. coli*) or Stratagene QuikChange site-directed mutagenesis kit (for *S. coelicolor*). Underlined sequence indicates change from original nucleotide. Reverse primers are complementary to forward primers.