

Figure S1. *ispD* library clones do not confer FSM resistance.

FSM sensitivity of *E. coli* expressing pDX-*ispD* plasmid-borne *ispD* alleles (*ispD1-6*). Data are normalized to growth in the absence of treatment. Data points and error bars represent mean \pm S.E.M.

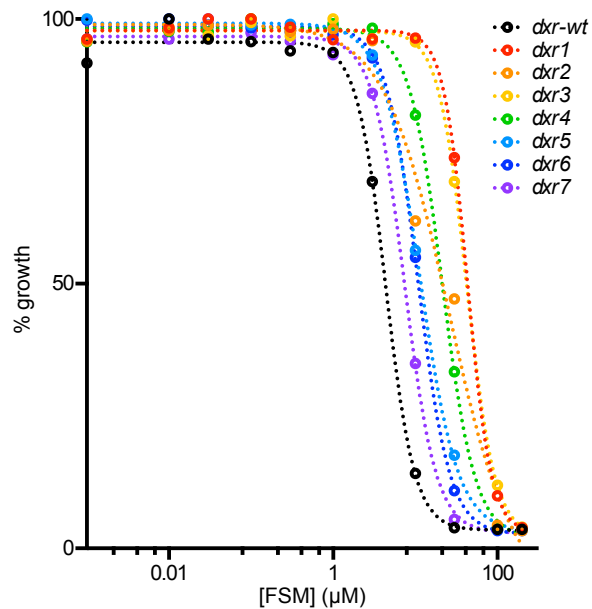


Figure S2. *dxr* library clones confer FSM resistance.

FSM sensitivity of *E. coli* expressing plasmid-borne pDX-*dxr* *dxr* alleles (*dxr1-7*).

Data are representative of at least three independent biological experiments and are normalized to growth in the absence of treatment. Data points and error bars represent mean \pm S.E.M.

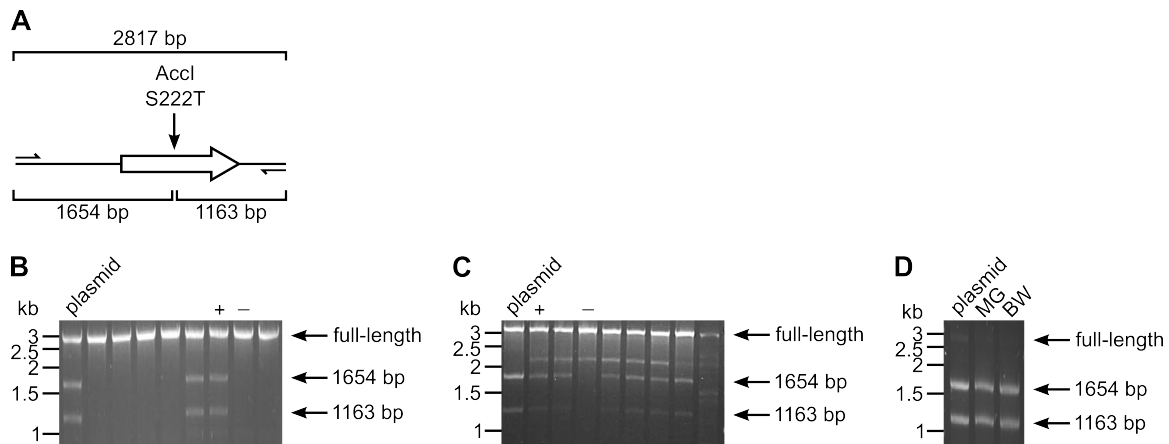


Figure S3. Allelic replacement of *dxr*.

Site-directed mutagenesis of the *dxr* gene was used to create a novel *Accl* site and *dxr*-S222T mutation. Clones were screened by PCR amplification of the region surrounding the *dxr* locus, followed by *Accl* digestion of the PCR product.

(A) Genomic context of the *dxr*-S222T mutation. Coding sequence of *dxr* gene shown as a thick white arrow. S222T mutation and *Accl* restriction site indicated. PCR primer annealing sites shown as half-arrows. Resulting PCR product is 2817 bp; *Accl* digestion results in 1654 and 1163 bp fragments.

(B) Screening of eight clones from MG1655 parent strain. Plasmid control (pKOV-*dxr*S222T) and example positive (+) and negative (-) clones indicated. Right, expected sizes of full-length PCR product and fragments following *Accl* digestion.

(C) Screening of eight clones from BW25113 $\Delta bamB \Delta tolC$ parent strain. Plasmid control (pKOV-*dxr*S222T) and example positive (+) and negative (-) clones indicated. Right, expected sizes of full-length PCR product and fragments following *Accl* digestion.

(D) Extended *AccI* digest of PCR products. Length of digest was extended to 16 hours to confirm loss of the uncut band in pKOV-dxrS222T plasmid control and presumptive positive clones from both MG1655 (MG) and BW25113 $\Delta bamB \Delta tolC$ (BW) parent strains. Right, expected sizes of full-length PCR product and fragments following *AccI* digestion.

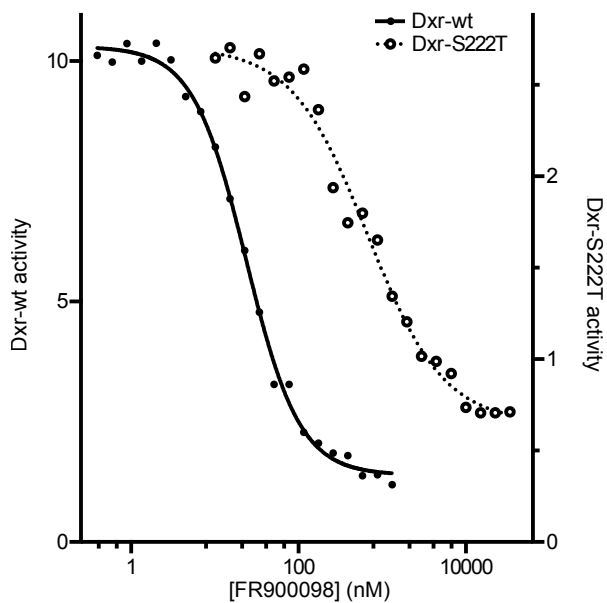


Figure S4. Purified recombinant Dxr-S222T is resistant to FR900098 in vitro.

FR900098 sensitivity of purified, recombinant wild-type (●) and S222T (○) Dxr enzymes. Data shown are representative of at least three independent experiments. Activity is measured in $\mu\text{mol}/\text{min}/\text{mg}$ of enzyme. Data points and error bars represent mean \pm S.E.M.

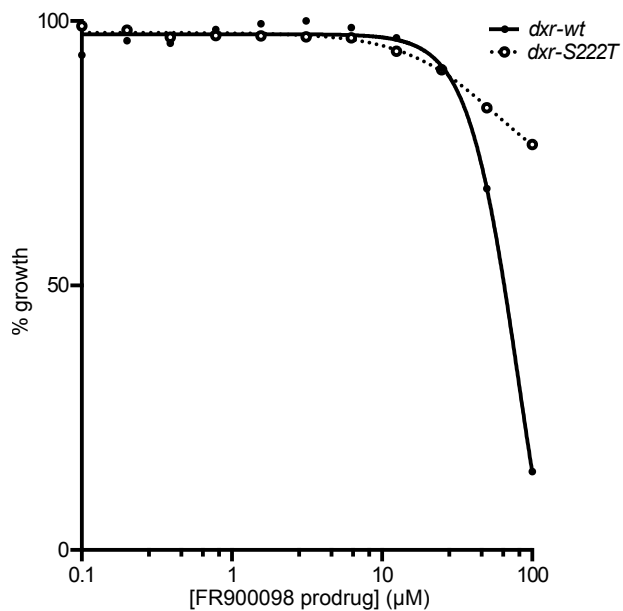


Figure S5. *E. coli* strain MG1655 is insensitive to an FR900098 prodrug.

FR900098 prodrug sensitivity of *E. coli* strain MG1655, in which the native wild-type *dxr* allele is present (●) or has been replaced with an S222T (○) allele. Data are normalized to growth in the absence of treatment. Data points and error bars represent mean \pm S.E.M.