



Figure S1 Outgrowth of Polymerase-deficient Mutant Strains

Cell densities of the wild type and all combinations of mutant strains are plotted for 24 hours of growth in LB. Timepoints were taken every 45 minutes for the first 12 hours and every 90 minutes during the second 12 hours. All strains exhibited indistinguishable growth curves; representative data are shown. Black, wild type; light red, Pol II⁻; light green, Pol IV⁻; light blue, Pol V⁻; dark blue, Pol V⁺-only; dark green, Pol IV⁺-only; dark red, Pol II⁺-only; gray, triple mutant.

Table S1 Rif^R Single-nucleotide Polymorphisms Identified

All missense mutations in *rpoB* conferring rifampicin resistance (Rif^R) identified in this study are listed, along with the number observed within each strain background. Asterisks denote missense mutations conferring rifampicin resistance that to our knowledge have not previously been reported.

Missense Mutation						Observed in Strain							
Nucleotide	Previous Base	Mutation	Previous Codon	New Codon	AA Change	Wildtype	Pol II ⁻	Pol IV ⁻	Pol V ⁻	Pol V ⁺	Pol IV ⁺	Pol II ⁺	- / - / -
428	G	C*	CGT	CCT	R143P*	0	1	0	1	1	0	0	0
436	G	T	GTT	TTT	V146F	1	2	0	1	0	0	1	0
437	T	A*	GTT	GAT	V146D*	1	0	0	0	0	0	1	0
442	C	A	CAG	AAG	Q148K	0	0	1	0	0	0	0	0
443	A	C	CAG	CCG	Q148P	6	6	1	6	2	8	1	4
		G	CAG	CGG	Q148R	2	0	2	0	0	0	0	1
		T	CAG	CTG	Q148L	15	4	24	10	7	11	6	31
444	G	C	CAG	CAC	Q148H	0	0	1	0	0	0	0	0
		T	CAG	CAT	Q148H	0	0	0	1	1	0	1	1
446	T	C*	CTG	CCG	L149P*	0	0	0	2	0	0	0	0
448	C	G*	CAC	GAC	H150D*	0	0	0	0	1	0	0	0
1525	A	C	AGC	CGC	S509R	0	0	0	0	1	1	0	1
1526	G	T*	AGC	ATC	S509I*	1	0	0	0	0	0	0	0
1527	C	A	AGC	AGA	S509R	2	0	1	1	1	1	1	0
1532	T	A	CTG	CAG	L511Q	1	1	4	0	3	0	1	2
		C	CTG	CCG	L511P	1	2	0	2	1	0	0	1
		G	CTG	CGG	L511R	2	1	1	3	2	1	1	1
1534	T	C	TCT	CCT	S512P	3	4	6	5	7	5	1	4
1535	C	A	TCT	TAT	S512Y	0	4	1	3	5	1	2	3
		T	TCT	TTT	S512F	0	0	2	2	2	2	2	1
1537	C	A	CAG	AAG	Q513K	1	0	1	0	0	0	0	0
		G*	CAG	GAG	Q513E*	0	0	0	0	0	1	0	0
1538	A	C	CAG	CCG	Q513P	5	1	3	5	2	1	4	1
		G	CAG	CGG	Q513R	1	0	1	0	1	2	0	0
		T	CAG	CTG	Q513L	5	2	2	1	1	2	2	2
1539	G	T	CAG	CAT	Q513H	0	0	0	0	1	0	0	0
1546	G	A	GAC	AAC	D516N	0	1	0	1	0	2	1	0
		T	GAC	TAC	D516Y	1	0	1	1	1	0	0	0
1547	A	C	GAC	GCC	D516A	0	0	0	0	0	0	1	0
		G	GAC	GGC	D516G	11	9	7	16	11	7	17	8
		T	GAC	GTC	D516V	0	1	1	3	0	2	1	0
1552	A	G	AAC	GAC	N518D	1	0	0	1	0	0	1	0
1565	C	T	TCT	TTT	S522F	0	0	2	0	0	0	1	1
1574	C	G	ACG	AGG	T525R	0	0	0	0	0	2	0	0
1576	C	A	CAC	AAC	H526N	1	4	0	0	0	1	0	0
		G	CAC	GAC	H526D	0	0	1	2	1	1	1	0
		T	CAC	TAC	H526Y	6	7	11	8	12	9	6	10

1577	A	T	CAC	CTC	H526L	0	2	1	0	1	0	1	0
1578	C	A	CAC	CAA	H526Q	0	0	0	0	0	0	0	1
		G	CAC	CAG	H526Q	0	0	0	0	1	0	0	0
1585	C	T	CGT	TGT	R529C	1	1	1	1	0	0	0	0
1586	G	A	CGT	CAT	R529H	0	4	1	3	3	1	0	0
		T	CGT	CTT	R529L	0	2	0	0	0	0	0	1
1592	C	A	TCC	TAC	S531Y	1	1	0	0	1	0	0	0
		G*	TCC	TGC	S531C*	0	0	0	0	1	0	0	0
		T	TCC	TTC	S531F	1	2	0	2	2	6	6	3
1594	G	C*	GCA	CCA	A532P*	0	1	0	0	0	0	1	0
1595	C	A	GCA	GAA	A532E	1	0	0	0	2	2	0	1
1597	C	G*	CTC	GTC	L533V*	1	0	0	0	0	0	0	0
1598	T	A	CTC	CAC	L533H	0	0	1	1	0	1	0	1
		C	CTC	CCC	L533P	1	6	2	4	1	2	1	0
		G	CTC	CGC	L533R	2	0	0	0	0	0	0	1
1600	G	A	GGC	AGC	G534S	0	1	0	0	3	1	0	3
		T	GGC	TGC	G534C	2	2	4	1	0	2	0	1
1601	G	A	GGC	GAC	G534D	0	1	0	0	1	0	0	1
		C	GGC	GCC	G534A	4	1	2	2	3	0	0	1
		T	GGC	GTC	G534V	2	0	2	0	2	0	0	0
1607	G	T*	GGC	GTC	G536V*	3	0	1	1	0	0	0	0
1610	G	A	GGT	GAT	G537D	0	1	1	0	2	0	0	0
1687	A	C	ACC	CCC	T563P	13	31	21	25	18	28	33	18
1691	C	A*	CCT	CAT	P564H*	1	0	0	0	0	0	0	0
		T	CCT	CTT	P564L	1	3	1	2	2	0	4	2
1702	A	G*	AAC	GAC	N568D*	0	1	0	0	0	0	0	0
1708	G	T	GGT	TGT	G570C	3	2	0	1	0	1	2	1
1709	G	C	GGT	GCT	G570A	2	1	0	0	4	0	0	0
1712	T	A*	CTG	CAG	L571Q*	0	1	1	0	1	1	0	0
1714	A	C	ATC	CTC	I572L	3	2	2	3	4	0	1	4
		T	ATC	TTC	I572F	2	0	1	0	2	1	1	1
1715	T	A	ATC	AAC	I572N	3	0	3	0	2	0	0	2
		C	ATC	ACC	I572T	1	0	0	0	0	1	0	0
		G	ATC	AGC	I572S	3	5	6	3	6	9	5	6
1716	C	G	ATC	ATG	I572M	1	0	0	0	0	0	0	0
1721	C	A	TCT	TAT	S574Y	2	0	0	0	1	5	2	2
	C	T	TCT	TTT	S574F	2	0	2	0	2	4	3	4
2060	G	A	CGT	CAT	R687H	0	0	1	0	0	0	0	0

Table S2 Rif^R Deletions and Amplifications Identified

All unique deletions and amplifications in *rpoB* conferring rifampicin resistance (Rif^R) identified in this study are listed. Some deletions were identified more than once within a given strain. The codons affected are provided.

Strains	Mutation	Nucleotide	Previous Codon	AA Position	Previous AA	New Codon	New AA
Pol II ⁺	Deletion	1516	TTC GGT TCC	506/8	FGS	-	-
Pol II ⁺	Deletion	1519	GGT TCC	507/8	GS	-	-
Pol II ⁺	Deletion	1587	-	530	I	-	-
Pol II ⁺	Deletion	1594	GCA	532	A	-	-
Pol II ⁺ , Pol V ⁺	Deletion	1594	GCA CTC	532/3	AL	-	-
Pol IV ⁺	Deletion	1594	GCA CTC GGC	532/4	ALG	-	-
Pol II ⁺	Deletion	1594	GCA CTC GGC CCA	532/5	ALGP	-	-
Pol V ⁺ , Pol IV ⁺	Deletion	1604	CCA GGC GGT	535/7	PGG	-	-
WT	Amplification	1528-1557	-	-	-	CAG CTG TCT CAG TTT ATG GAC CAG AAC AAC	QLSQFMDQNN
- / - / -	Amplification	1585-1590	-	-	-	CGT ATC	RI

Table S3 Strain-specific Mutation Spectrum

The absolute number of mutations identified in each class of mutation, as well as the percent of overall mutations they represent, within each strain is provided.

	WT		Pol II ⁻		Pol IV ⁻		Pol V ⁻		Pol V ⁺		Pol IV ⁺		Pol II ⁺		- / - / -	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
GC → AT	11	9%	21	17%	22	17%	19	15%	29	22%	25	20%	23	18%	25	20%
GC → TA	22	18%	17	14%	12	9%	10	8%	15	12%	13	10%	9	7%	12	9%
GC → CG	9	7%	4	3%	4	3%	5	4%	12	9%	5	4%	2	2%	1	1%
AT → GC	21	17%	22	18%	18	14%	30	24%	21	16%	17	13%	20	16%	14	11%
AT → TA	27	22%	11	9%	38	30%	15	12%	17	13%	18	14%	13	10%	39	30%
AT → CG	34	27%	46	38%	34	27%	45	36%	35	27%	48	38%	46	36%	36	28%
Deletions	0	0%	0	0%	0	0%	1	1%	1	1%	2	2%	14	11%	0	0%
Insertions	1	1%	0	0%	0	0%	0	1%	0	0%	0	0%	0	0%	1	1%
Total Identified	125		121		128		125		130		128		127		128	

Extended Experimental Procedures:

Table S4 qRT-PCR Primers used in this study, related to Figure 6

Primer Name	Sequence (5'-3')
<i>polB</i> (internal) Forward	agttcgaaatcaagcgagga
<i>polB</i> (internal) Reverse	tgcacaatggcactatcgtt
<i>dinB</i> (internal) Forward	gacctgcggtgatgtacaaa
<i>dinB</i> (internal) Reverse	tcgcttcacattcagaccag
<i>umuC</i> (internal) Forward	ttatctgttcccgcctcgttt
<i>umuC</i> (internal) Reverse	cagttttaccgacgcgctat
<i>umuD</i> (internal) Forward	tcgtcaaagcaagtggatgat
<i>umuD</i> (internal) Reverse	gatggtaatggcgagtagc
<i>polA</i> (internal) Forward	ctttttccagcttcgcaatc
<i>polA</i> (internal) Reverse	agttcaaacgctggactgct
<i>dnaE</i> (internal) Forward	taccatttccacgtcaacga
<i>dnaE</i> (internal) Reverse	cccggacatgatcagttttt
<i>recA</i> (internal) Forward	cagcatcgataaacgcacag
<i>recA</i> (internal) Reverse	gaagaccgttccatggatgt
<i>sulA</i> (internal) Forward	tgcggtgttaaccagagttg
<i>sulA</i> (internal) Reverse	tcaggctatgcacatcgttc
<i>gapA</i> (internal) Forward	gtgatccggctaacctgaaa
<i>gapA</i> (internal) Reverse	gtcctggccagcatatattgt
<i>sbmC</i> (internal) Forward	tcgggaagcgtaaagtaacc
<i>sbmC</i> (internal) Reverse	gttgacaggtttccatctcgt
<i>fis</i> (internal) Forward	cgaacaacgcgtaaattctg
<i>fis</i> (internal) Reverse	attgcatcaccatgtccaac
<i>lexA</i> (internal) Forward	ttgcaggaagaggaagaagg
<i>lexA</i> (internal) Reverse	ttatgcactgccagcaagtc
<i>dps</i> (internal) Forward	ctgccatggtatccagatga
<i>dps</i> (internal) Reverse	tgctttataccgcaacgat