

Supplementary Information for: Mechanism and Diversity of the Erythromycin Esterase Family of Enzymes. By Kate Pengelly, Mariya Morar, Kalinka Koteva, Gerard D. Wright

Supplemental Table 1. Primers used for *ere* gene amplification or mutagenesis.

Enzyme	Mutation	Primer Pair (5' → 3')
EreA	WT	Forward GGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGA CATATGACATGGAGAACGACCAGAAC* Reverse GGGGACCACTTTGTACAAGAAAGCTGGGTCGGATCCCTATAGGGC AACCAGGCTGTCC
EreB	WT	Forward GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGA CATATGAGGTTTCGAAGAATGGGTC Reverse GGGGACCACTTTGTACAAGAAAGCTGGGTCGGATCCCTATAGGGC AACCAGGCTGTCC
	E43A	Forward GGAGATACCCGAGTTGTAGCATTAGGTGCAAATTCTCATTTC Reverse Complement GAAATGAGAATTTGCACCTAATGCTACAACCTCGGGTATCTCC
	E74A	Forward CGTTTGCTTTTGCATTTGGTTTTGCTG Reverse Complement CAGCAAAACCAAATGCAAAAGCAAACG
	H285A	Forward GAGCAGAAAGTGATAGTAGCAGCTAATGCACATATTCAAAAAACA CC Reverse Complement GGTGTTTTTTGAATATGTGCATTAGCTGCTACTACTATCACTTTCTG CTC
	H46A	Forward CGAGTTGTAGCATTAGGTGAAAATTCTGCTTTCATAAAAGAATTCT TTTTGTTACG Reverse Complement CGTAACAAAAAGAATTCTTTTATGAAAGCAGAATTTTCACCTAATG CTACAACTCG
	H288A	Forward GTGATAGTAGTAGCACATAATGCAGCTATTCAAAAAACACCC Reverse Complement CCATCATACAGAATGGGTGTTTTTTGAATAGCTGCATTATGTGCTAC TACTATCAC

* Represented in bold font are either gene specific regions – for wild type genes or mutagenesis sites – for mutant genes.

Supplemental Table 2. pGENThreader fold predictions for EreA and EreB.

Alignment	Confidence	Net Score	p- value	Aln Score	Aln. Length	Str. Length	Seq. Length
EreA							
3B55	CERT	104.0	2e-9	392	371	408	406
2QGM	CERT	103.7	2e-9	412	371	392	406
1O57	LOW	33.4	0.02	36	234	270	406
EreB							
3B55	CERT	168.9	4e-16	892	389	408	419
2QGM	CERT	148.4	5e-14	824	378	392	419
3G79	MEDIUM	39.4	0.005	51	329	475	419

Supplemental Table 3. Macrolide inactivation products generated by Ere enzymes.

Reaction	Molecular Mass	Observed m/z (amu)		Retention Time (min)
		+EMS	-EMS	
erythromycin alone	733.5	733.9	-	16.1
erythromycin + EreB	751.5 hydrolysis product or hemiketal	751.6	750.2	15.1
	733.5 final dehydration product	733.8	732.0	17.1
erythromycin + EreA	751.5 hydrolysis product or hemiketal	751.4	750.2	15.1
	733.5 final dehydration product	734.0	732.2	17.1
clarithromycin alone	747.5	748.8	-	16.2
clarithromycin + EreB	765.5	766.9	-	14.5
roxithromycin alone	836.5	838.0	-	16.8
roxithromycin + EreB	854.5	856.0	-	15.1

Supplemental Figure 1. EreA pH profiles. **A.** Dependence of $\log(V_{max})$ on pH with pKa value of 6.2 shown as a dotted line. **B.** Dependence of $\log(V_{max}/K_M)$ on pH with pKa values of 5.6 and pKb value of 9.1 shown as dotted lines.

