

SUPPLEMENTARY MATERIALS

**Histidine-Triad Hydrolases Provide Resistance to Peptide-Nucleotide Antibiotics**

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**Table S1. Primers used in the study.**

Primer	Sequence 5'-3'	Procedure
HmiA1_up	<u>CATGA</u> ACGATAAAGCCACCATCGAAATCAAGA AAGATGAAAAGAAAGCGGAGCCGAAGAAAGT TGTGGTGGTTAAAACCAGCATCAAGGCCGGTC CGGCGGCGTTCAACT <u>A</u>	Cloning into pRSFDuet-1
HmiA1_down	<u>AGCTTAGTTGA</u> ACGCCCGCCGGACCGGCCTTGAT GCTGGTTTTAACCACCACAACCTTCTTCGGCTC CGCTTCTTTTCATCTTTCTTGATTTCGATGGTG GCTTTATCGT <u>T</u>	
HmiA2_up	<u>CATGA</u> ACGAGAAAACCGCCCAGGAGAGCCAGA AGACCGAATCGCCGAAAGCCGAAACCCCGGCC AAAAAGGCCGTGATTGTGAAAACCCGCATTAA AGCGGGTCCGGGCGGTGGTGGCCTTGTGCATCC GATCGCCAACT <u>A</u>	
HmiA2_down	<u>AGCTTAGTTGG</u> CGATCGGATGCACAAGGCCAC CACCGCCCGGACCCGCTTTAATGCGGGTTTTCA CAATCACGGCCTTTTTGGCCGGGGTTTTCGGCTT	

	TCGGCGATT <u>CGGTCTTCTGGCTCTCCTGGGCGG</u> TTTTCTCGTT	
HmiB1_F_N deI	ATTTAT <u>CATATGAAAGACCACCTCTGGC</u>	
HmiB1_R_ KpnI	TTATAT <u>GGTACCTTATGCCTTGGGGACCTC</u>	
HmiB2_F_N deI	TATTAT <u>CATATGAACGACTATCTCTGGC</u>	
HmiB2_R_ KpnI	TTATAT <u>GGTACCTTAGGGCTGTGGCGTGGAGGG</u>	
HmiP1P2P3 _F_EcoRI	TTATAT <u>GAATTCCATGGCCATCCCCTTCTCCC</u>	Cloning into pACYCduet-1
HmiP1P2P3 _R_KpnI	TATATT <u>GGTACCTTACGGGTGGGCGATGACG</u>	
HmiB1_F_B amHI	ATAATT <u>GGATCCGATGAAAGACCACCTCTGGCT</u> GCTGC	Cloning into pET22_MBP
HmiB1_R_S all	ATAATT <u>GTCGACTTATGCCTTGGGGACCTCTTG</u> ACGG	
HmiB2_F_B amHI	ATAATT <u>GGATCCGAACGACTATCTCTGGCTGCT</u> CCCC	
HmiB2_R_S all	ATAATT <u>GTCGACCACTCAGCCGGACCCTGGACT</u> CAGG	
pBAD30_Sa IRBS_R_Sal I	TATATT <u>GTCGACTTCCTCCTGTTAGCCCAAAA</u> AAACGGGTATGGAGAAACAG	Construction of pBAD30_SalRB S vector

pBAD30_F_ HindIII	TATTATA <u>AAGCTT</u> GGCTGTTTTGGCGG	
MccH_F_Sa II	TTATAG <u>TCGACAT</u> GAGCGCCACCGACACC	Cloning into pBAD30_SalRB S
MccH_R_Hi ndIII	TTATA <u>AAGCTT</u> TTAAATCTCTGCGGCGGC	
HmiHinT_F _SalI	ATATA <u>AAGTCGACAT</u> GTCCAAGTGCCTCTTCTG	
HmiHinT_R HindIII	TTATATA <u>AAGCTT</u> TACCCCGGCGGCCACCCCAT G	
EcoHinT_F_ SalI	ATTATAG <u>TCGACAT</u> GGCAGAAGAACTATATTC AGCAAAAT	
EcoHinT_R _HindIII	ATTATA <u>AAGCTT</u> TACAGACCTTTATGCGCCAG CATT	
MccH_F_N deI	TTATAC <u>ATATGAGCGCCACCGACACC</u>	Cloning into pET22(b)
MccH_R_X hoI	ATTATA <u>CTCGAGAATCTCTGCGGCGGCCTTG</u>	
HmiHinT_F _NdeI	ATATA <u>ACATATGTCCAAGTGCCTCTTCTG</u>	
HmiHinT_R XhoI	ATATAT <u>CTCGAGCCCCGGCGGCCACCCCATG</u>	
EcoHinT_F_ NdeI	ATTATAC <u>ATATGGCAGAAGAACTATATTCAGC</u> AAAAT	

EcoHinT_R _XhoI	ATTATACTCGAGCAGACCTTTATGCGCCAGCAT TGGT	
MccH_F44 H_F	CCCAACGCGGCCG <b><u>CCAC</u></b> ACCGTGGTGGCGCC CAAG	Mutagenesis
MccH_F44 H_R	GCCACCACGGT <b><u>GTG</u></b> GCCGCGCGTTGGGGAA CGGCG	
MccH_H99 N_F	GAGGGCATGGGCATTGATA <b><u>AAC</u></b> GCCCACGTGAA GCTCACGCCGCTGC	
MccH_H99 N_R	GAGCTTCACGTGGG <b><u>GTT</u></b> TATCAATGCCCATGCC CTCGGCGAACAG	
MccH_H101 N_F	CATGGGCATTGATCACGCC <b><u>AAC</u></b> GTGAAGCTCA CGCCGCTGCACGG	
MccH_H101 N_R	GCGGCGTGAGCTTCAC <b><u>GTT</u></b> GGCGTGATCAATG CCCATGCCCTCGG	
MccH_K103 H_F	ATTGATCACGCC <b><u>CAC</u></b> GTGCACCTCACGCCGCTG CACGGCCTCCCC	
MccH_K103 H_R	GCAGCGGCGTGAG <b><u>GTG</u></b> CACGTGGGCGTGATCA ATGCCCATGCC	

Sequences of restriction endonucleases recognition sites are shown in plain typeface and are underlined; Sequences corresponding to ribosomal binding sites are highlighted in bold; Codons corresponding to mutated amino acids are underlined and highlighted in bold.