

Supporting Information

Transcript Profiling of MRSA Biofilms Treated with a Halogenated Phenazine Eradicating Agent: A Platform for Defining Cellular Targets and Pathways Critical to Biofilm Survival

*Yasmeen Abouelhassan, Yanping Zhang, Shouguang Jin, and Robert W. Huigens III**

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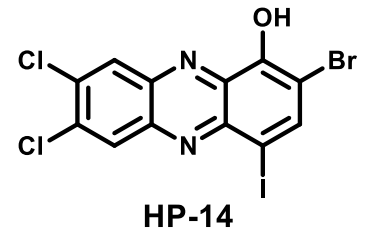
Supporting Information

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1.) MRSA Gene Symbols & Encoded Proteins.

Genes in this section are relevant to our transcript profiling investigations of established MRSA-1707 (MRSA BAA-1707, purchased from ATCC) biofilms treated with **HP-14**. The genes/symbols below were either used to validate RNA-seq findings (in qPCR experiments), or critical references to our mechanistic understandings of **HP-14**.



a) Genes Related to Iron Acquisition (with qPCR data reported):

isdB: iron-regulated surface determinant, mediates heme uptake (*J. Bacteriol.* **2006**, *188*, 8421-8429.)

sbnC: staphyloferrin B (siderophore) synthetase enzyme (*J. Biol. Chem.* **2016**, *291*, 29-40.)

sfaA: staphyloferrin A (siderophore) efflux transporter (*Infect. Immun.* **2017**, *85*, e00358-17.)

MW0695: hypothetical protein, similar to ferrichrome ABC transporters (ref. DOGAN, a genome database of microorganisms sequenced at NITE <http://www.bio.nite.go.jp/dogan/top>.)

fhuDI: ferric hydroxamate-binding lipoprotein (*J. Biol. Chem.* **2004**, *279*, 53152-53159.)

b) Gene Acronyms Related to Iron Acquisition (from WoPPER analysis; no qPCR data reported):

sir: staphylococcal iron regulated transporter (*Annu. Rev. Microbiol.* **2011**, *65*, 129-147.)

hts: heme transport system (*Annu. Rev. Microbiol.* **2011**, *65*, 129-147.)

c) Genes Not Related to Iron Acquisition (with qPCR data reported):

oppF: membrane-bound cytoplasmic ATP-binding protein; provides energy for peptide transport (*J. Bacteriol.* **2007**, *189*, 5119-5129.)

splB: serine protease; catalyzes the breakdown of proteins into peptides and amino acids (*J. Biol. Chem.* **2014**, *289*, 15544-15553.)

gap: glyceraldehyde-3-phosphate dehydrogenase; enzyme involved in glycolysis/ATP production (*J. Clin. Microbiol.* **2000**, *38*, 4351-4355.)

arcD: arginine/ornithine antiporter (*Nat. Comm.* **2017**, *8*, 14268.; *J. Bacteriol.* **2007**, *189*, 5976-5986.)

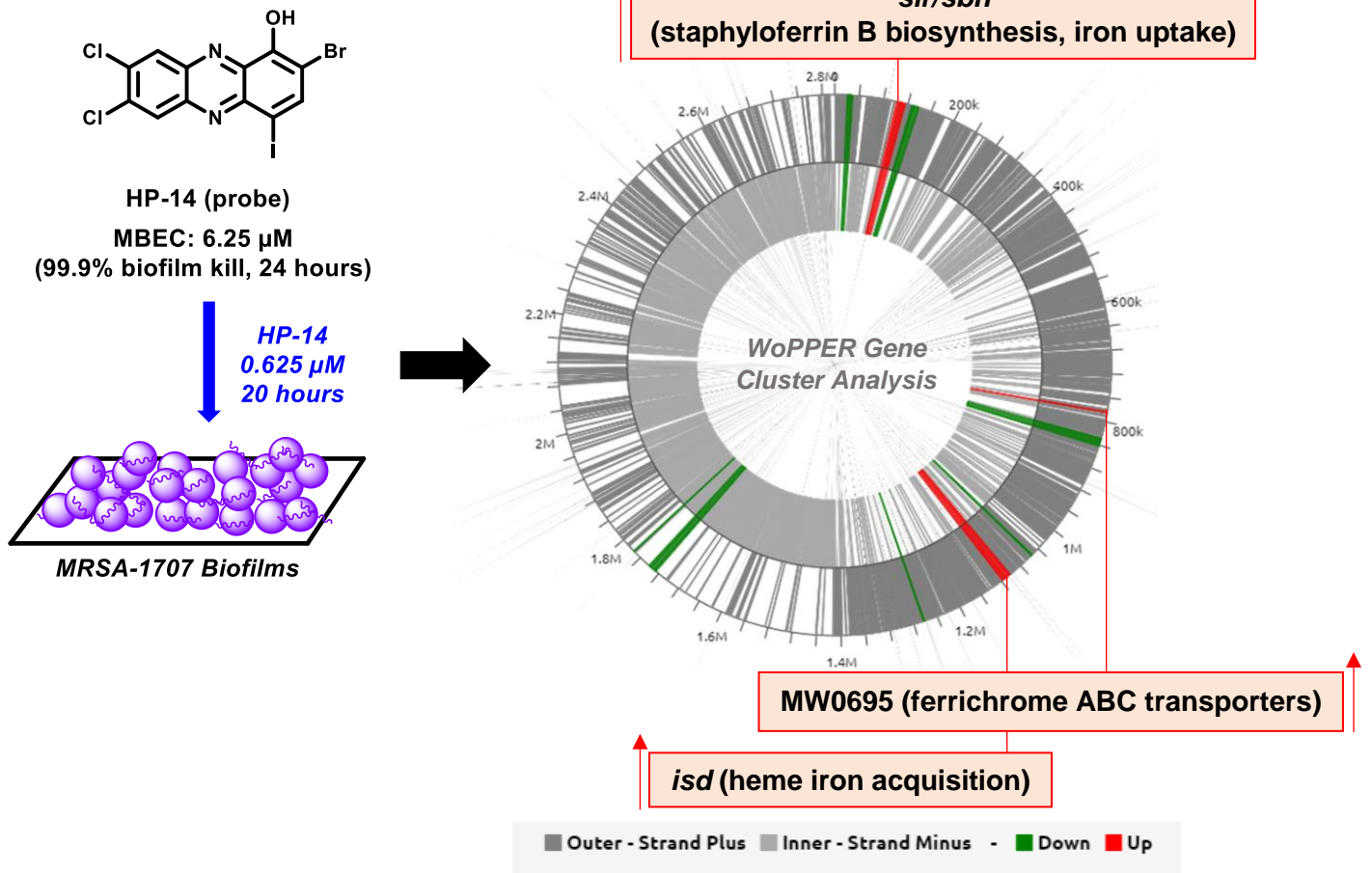
dnaC: DNA helicase required for elongation during DNA synthesis; involved in DNA repair (*FEMS Microbiol. Lett.* **2002**, *210*, 157-164.)

ureC: urease alpha subunit; virulence factor, essential in colonization of host organism & maintenance of bacterial populations in tissues (*Curr. Protein Pept. Sci.* **2012**, *13*, 789-806.)

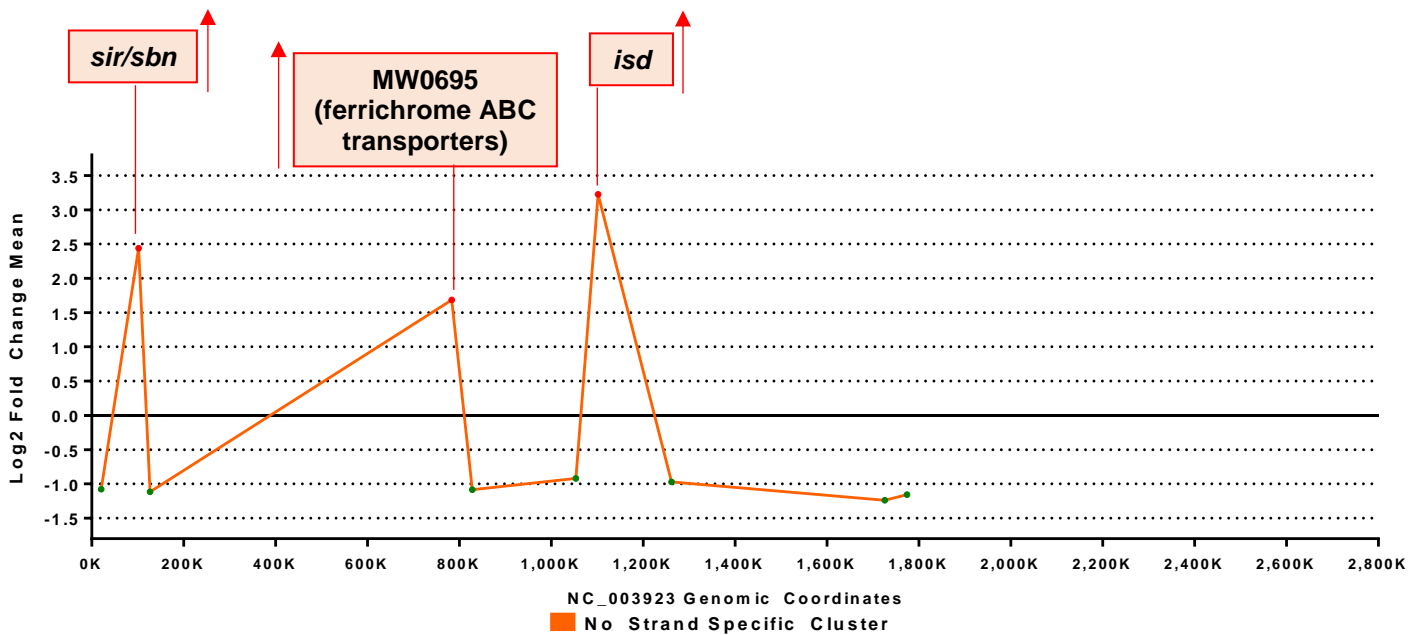
hemL: glutamate-1-semialdehyde 2,1-aminomutase; early gene of tetrapyrrole synthesis (heme biosynthesis; *Microbiol. Mol. Biol. Rev.* **2017**, *81*, e00048-16.; *Front. Microbiol.* **2017**, *8*, 1354.)

2.) Supporting Figures.

A)

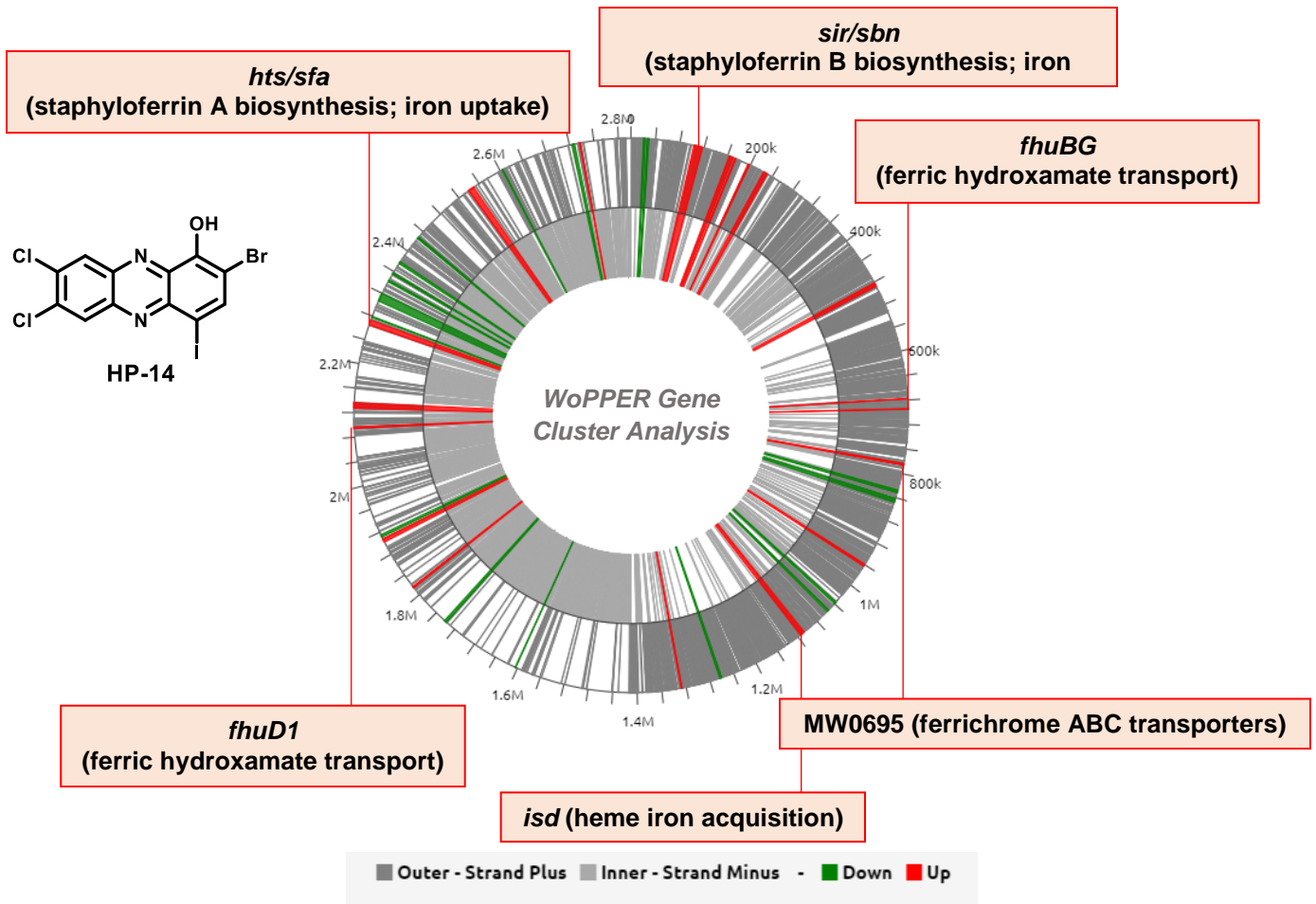


B)

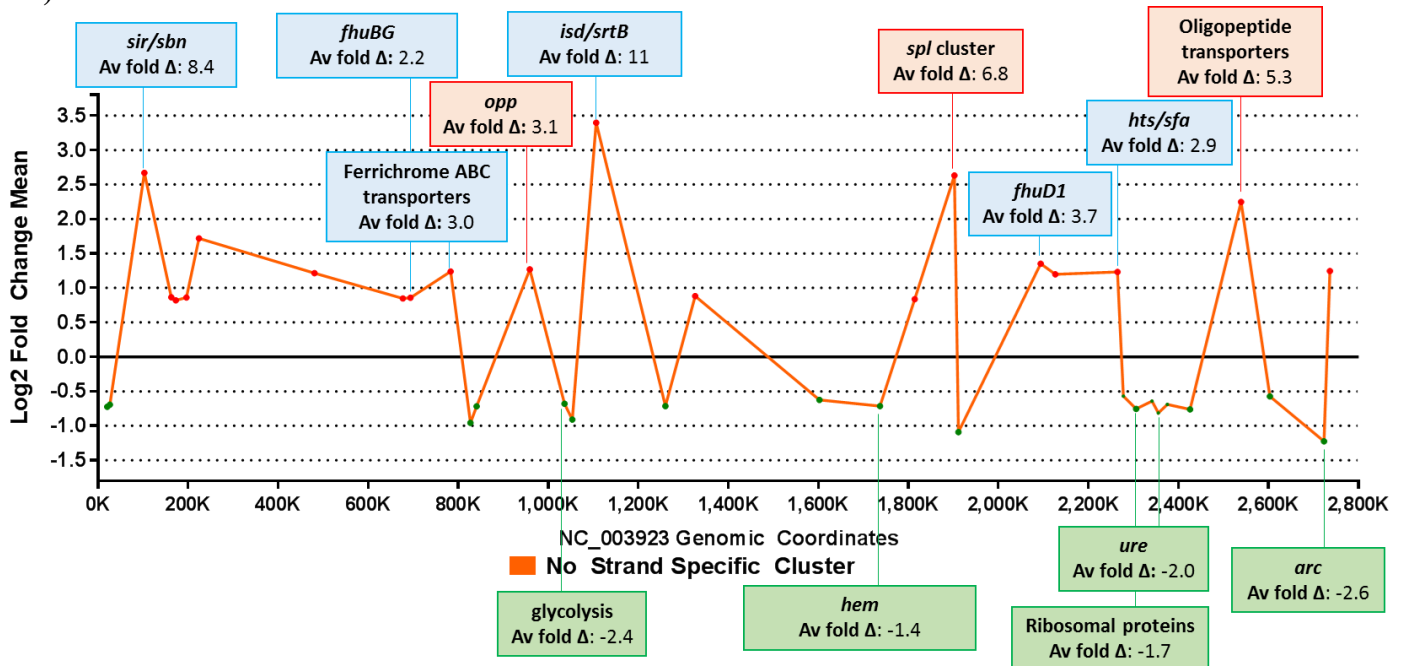


Supporting Figure 1: (A) Circular and (B) linear presentations of the 10 gene clusters identified from our initial WoPPER analysis that focused on the evaluation of the 646 genes that demonstrated ≥ 1.5 -fold change in gene expression upon **HP-14** (0.625 μ M, 1/10 MBEC for 20 hours) treatment of MRSA-1707 biofilms.

A)



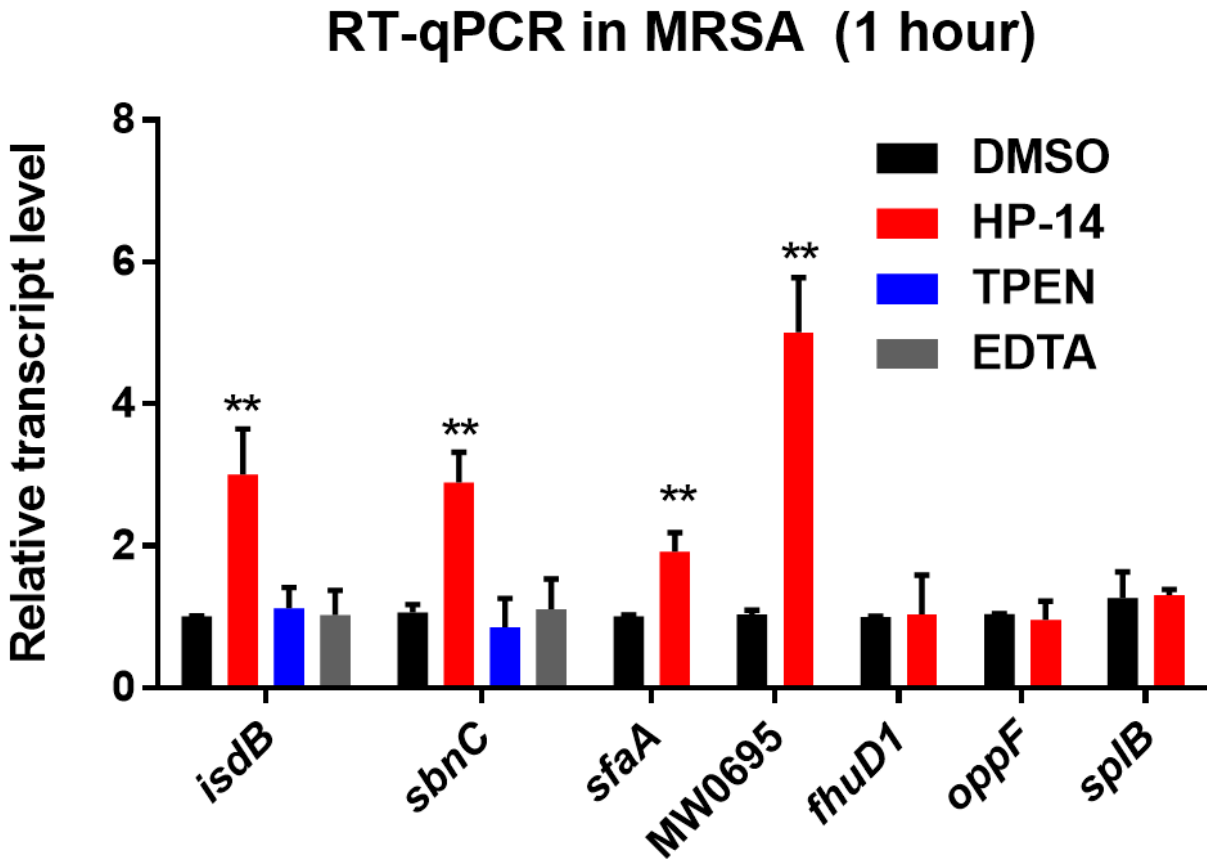
B)



Supporting Figure 2: (A) Circular and (B) linear presentations of 37 gene clusters identified from WoPPER analysis when analyzing all 2,738 MRSA-1707 biofilm genes following treatment with **HP-14** (0.625 μ M, 1/10 MBEC). This WoPPER analysis was performed to avoid missing any important gene cluster involvement (Supp. Fig. 1), which provided several key insights during these investigations.

Supporting Figure 3: Time course RT-qPCR validation of MRSA-1707 biofilm transcripts up-regulated & down-regulated upon treatment with **HP-14** (0.625 μ M) at (A) 1 hour, (B) 4 hours, (C) 8 hours and (D) 20 hours (next few pages). EDTA and TPEN were tested at 5 μ M. * P-value \leq 0.05, ** P-value \leq 0.01

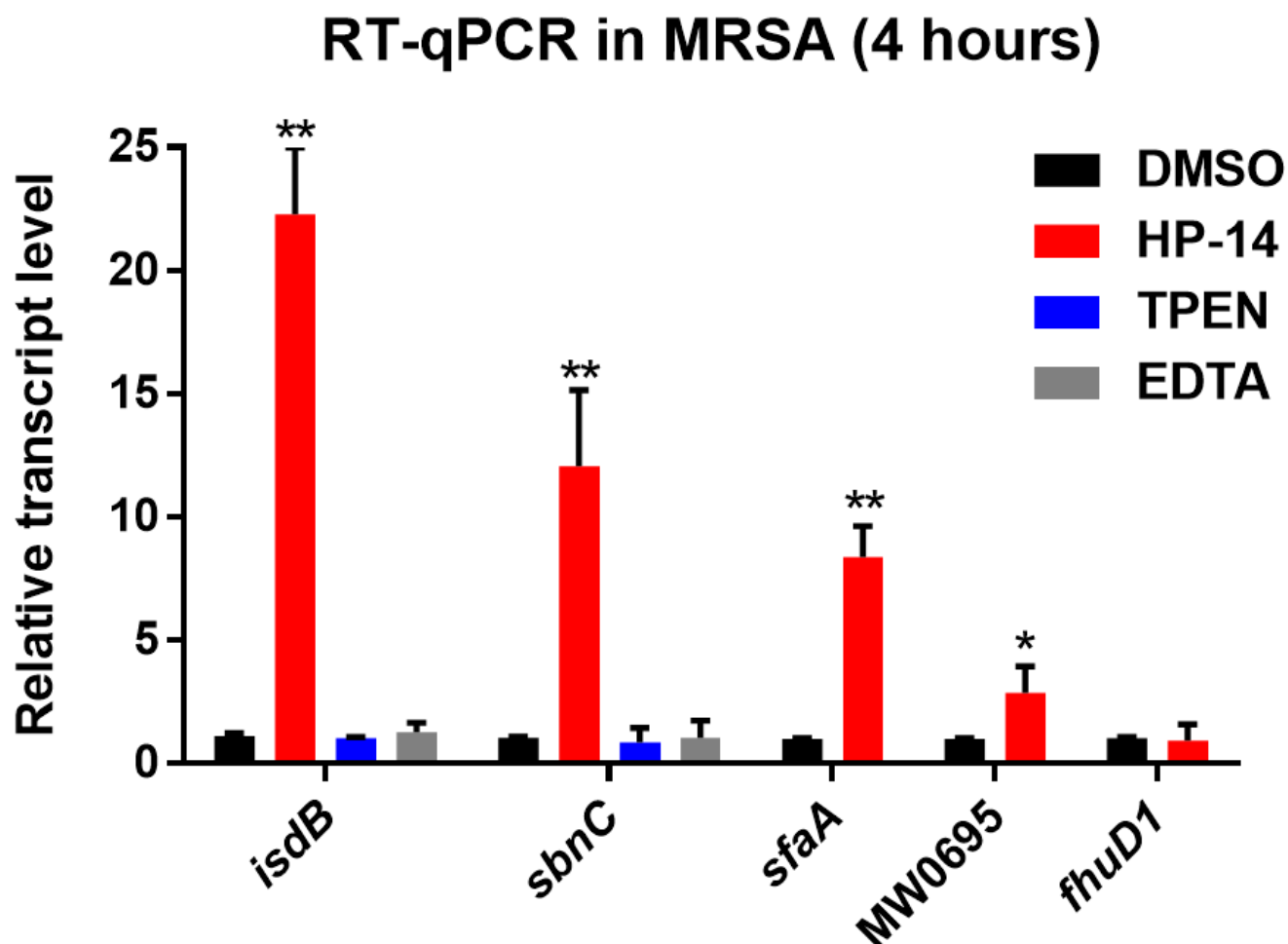
A) 1 hour time point (note the rapid activation of *isdB*, *sbnC*, *sfaA* & MW0695 in MRSA-1707 biofilms upon **HP-14** treatment)



Gene	DMSO	HP-14	TPEN	EDTA	Gene Information / Function
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	
<i>isdB</i>	1.0 \pm 0.004	3.0 \pm 0.640	1.1 \pm 0.298	1.0 \pm 0.346	hemoglobin receptor required for heme iron utilization
<i>sbnC</i>	1.1 \pm 0.110	2.9 \pm 0.423	0.9 \pm 0.406	1.1 \pm 0.429	staphyloferrin B (siderophore) biosynthesis; iron acquisition
<i>sfaA</i>	1.0 \pm 0.017	1.9 \pm 0.271	--	--	staphyloferrin A (siderophore) efflux; iron acquisition
MW0695	1.0 \pm 0.055	5.0 \pm 0.774	--	--	hypo. protein, similar to ferrichrome ABC transporters
<i>fhuD1</i>	1.0 \pm 0.006	1.0 \pm 0.550	--	--	utilize ferric hydroxamates as a source of iron
<i>oppF</i>	1.0 \pm 0.006	1.0 \pm 0.260	--	--	provides energy for peptide transport; bacterial nutrition
<i>splB</i>	1.3 \pm 0.369	1.3 \pm 0.079	--	--	serine protease; proteolysis (breakdown of proteins)

Supporting Figure 3 (continued)

B) 4 hour time point



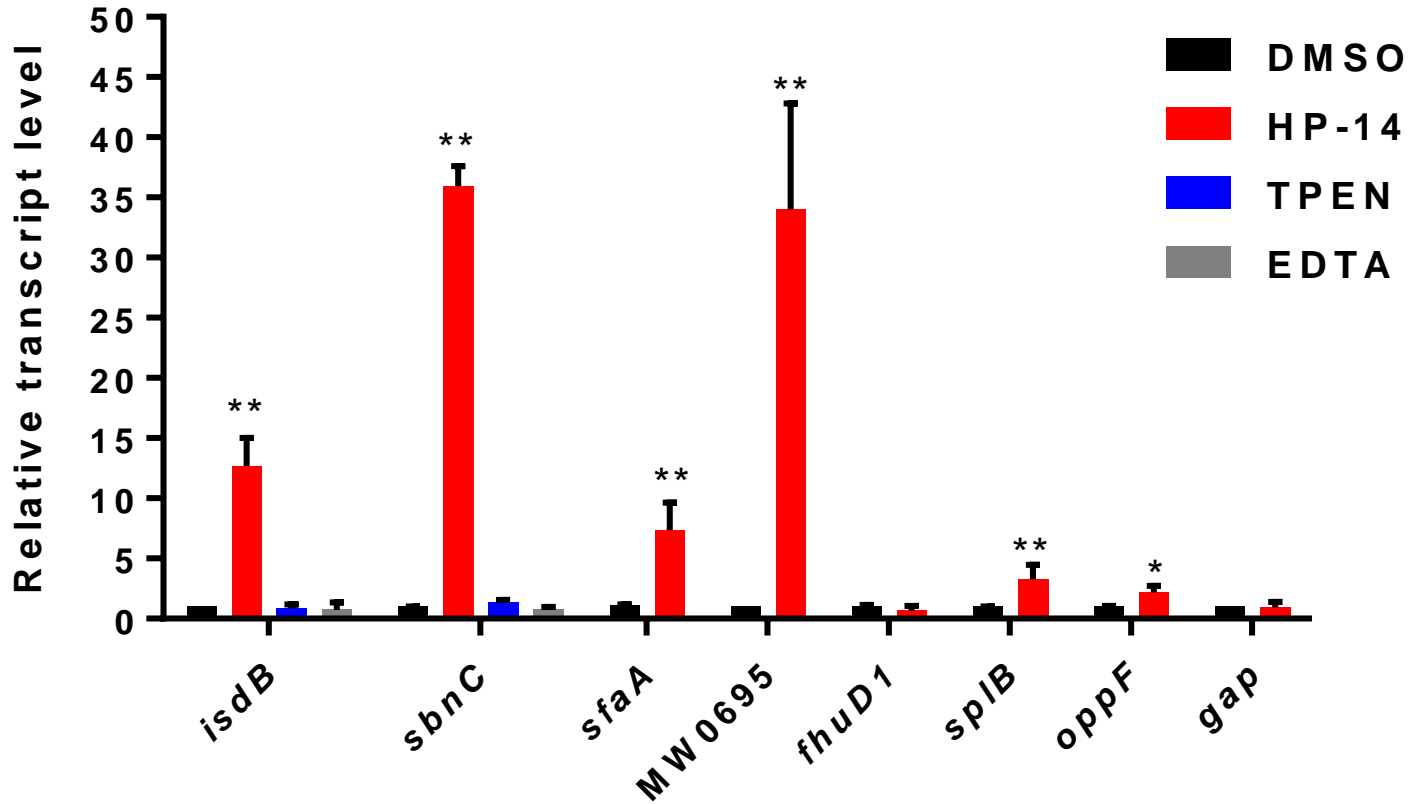
Gene	DMSO	HP-14	TPEN	EDTA	Gene Information / Function
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
<i>isdB</i>	1.1 ± 0.137	22.3 ± 2.70	1.0 ± 0.050	1.3 ± 0.382	hemoglobin receptor required for heme iron utilization
<i>sbnC</i>	1.0 ± 0.052	12.1 ± 3.07	0.9 ± 0.585	1.1 ± 0.681	staphyloferrin B (siderophore) biosynthesis; iron acquisition
<i>sfaA</i>	1.0 ± 0.021	8.4 ± 1.25	--	--	staphyloferrin A (siderophore) efflux; iron acquisition
<i>MW0695</i>	1.0 ± 0.012	2.9 ± 1.09	--	--	hypo. protein, similar to ferrichrome ABC transporters
<i>fhuD1</i>	1.0 ± 0.031	0.9 ± 0.68	--	--	utilize ferric hydroxamates as a source of iron

* P-value ≤ 0.05, ** P-value ≤ 0.01

Supporting Figure 3 (continued)

C) 8 hour time point

RT-qPCR (MRSA Biofilms; 8 hours)



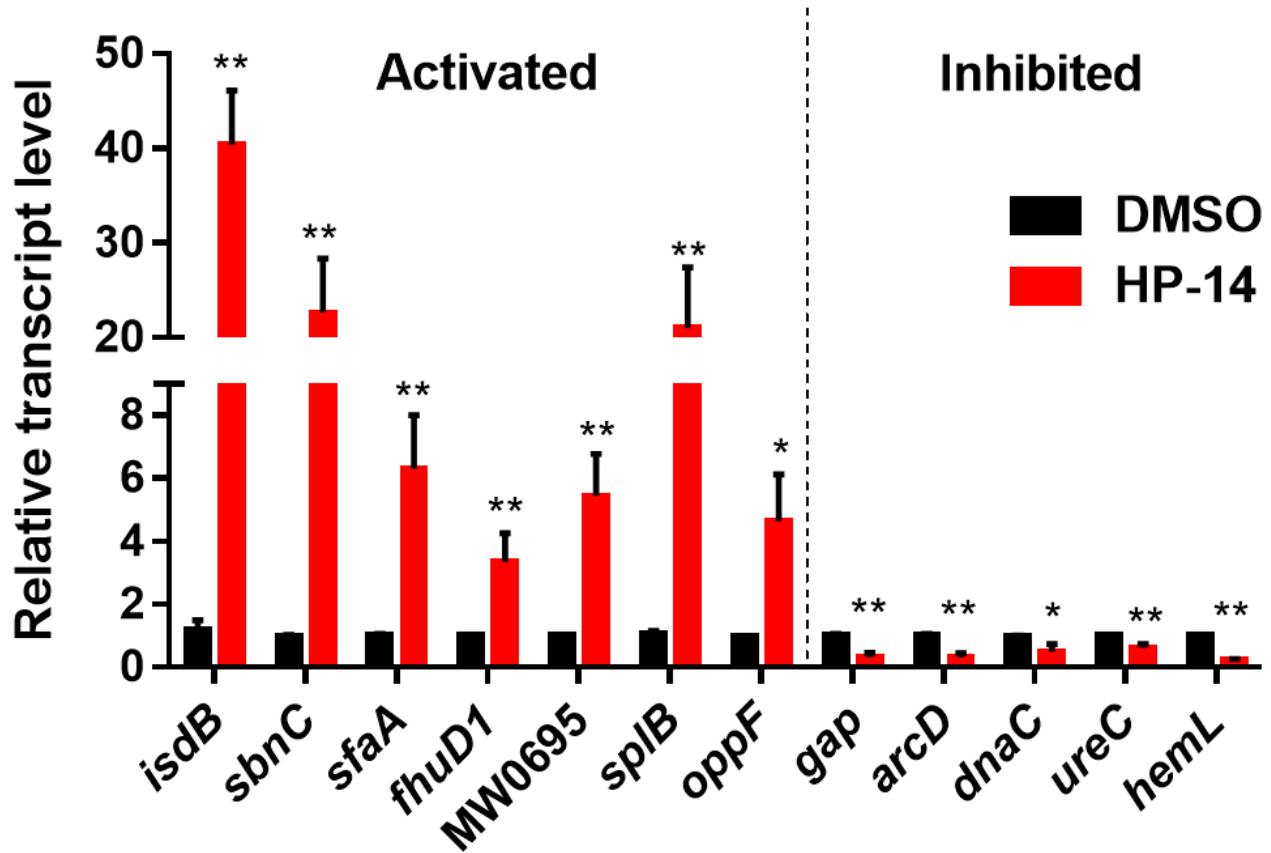
Gene	DMSO	HP-14	TPEN	EDTA	Gene Information / Function
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
<i>isdB</i>	1.0 ± 0.010	12.7 ± 2.35	0.9 ± 0.311	0.8 ± 0.606	hemoglobin receptor required for heme iron utilization
<i>sbnC</i>	1.0 ± 0.012	36.0 ± 1.68	1.3 ± 0.269	0.8 ± 0.219	staphyloferrin B (siderophore) biosynthesis; iron acquisition
<i>sfaA</i>	1.1 ± 0.079	7.4 ± 2.30	--	--	staphyloferrin A (siderophore) efflux; iron acquisition
<i>MW0695</i>	1.0 ± 0.015	34.0 ± 8.78	--	--	hypo. protein, similar to ferrichrome ABC transporters
<i>fhuD1</i>	1.1 ± 0.084	0.6 ± 0.41	--	--	utilize ferric hydroxamates as a source of iron
<i>splB</i>	1.0 ± 0.018	3.3 ± 1.20	--	--	serine protease; proteolysis (breakdown of proteins)
<i>oppF</i>	1.0 ± 0.031	2.2 ± 0.57	--	--	provides energy for peptide transport; bacterial nutrition
<i>gap</i>	1.0 ± 0.012	0.9 ± 0.47	--	--	glyceraldehyde-3-phosphate dehydrogenase; glycolysis

* P-value ≤ 0.05, ** P-value ≤ 0.01

Supporting Figure 3 (continued)

D) 20 hour time point

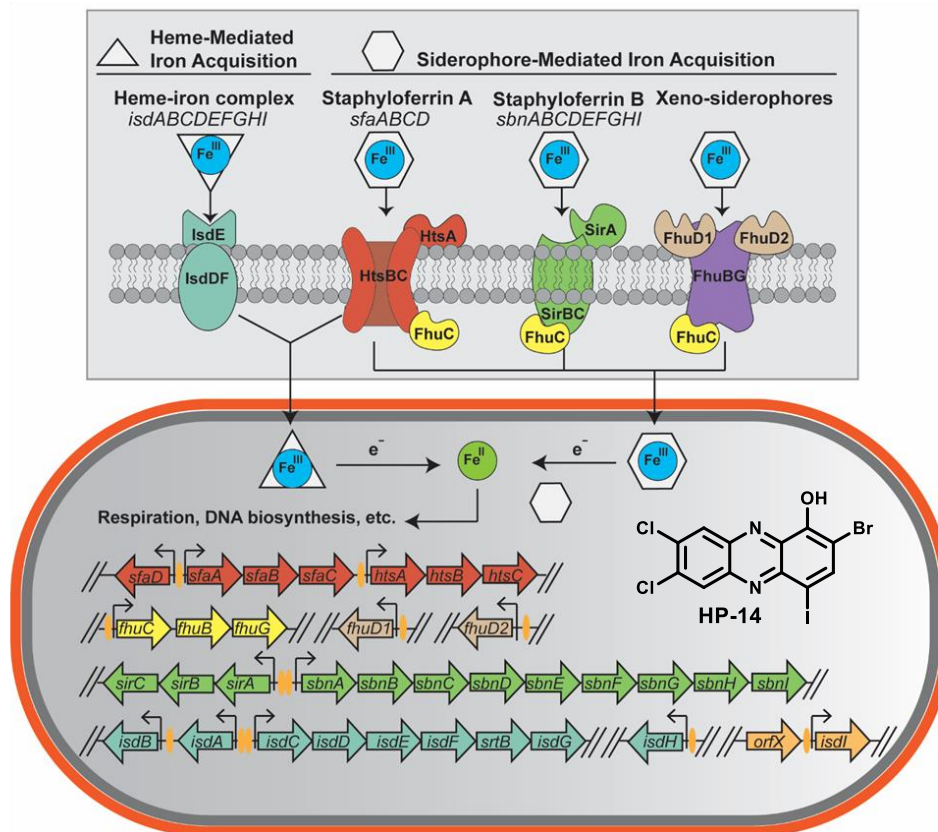
RT-qPCR in MRSA (20 hour)



Gene	DMSO	HP-14	Gene Information / Function
	Mean ± SD	Mean ± SD	
<i>isdB</i>	1.2 ± 0.298	40.4 ± 5.70	hemoglobin receptor required for heme iron utilization
<i>sbnC</i>	1.0 ± 0.010	22.7 ± 5.71	staphyloferrin B (siderophore) biosynthesis; iron acquisition
<i>sfaA</i>	1.0 ± 0.035	6.3 ± 1.70	staphyloferrin A (siderophore) efflux; iron acquisition
<i>MW0695</i>	1.0 ± 0.025	5.4 ± 1.34	hypothetical protein, similar to ferrichrome ABC transporters
<i>fhuD1</i>	1.0 ± 0.023	3.3 ± 0.92	utilize ferric hydroxamates as a source of iron
<i>spIB</i>	1.1 ± 0.077	21.1 ± 6.34	serine protease; proteolysis (breakdown of proteins into polypeptides & amino acids)
<i>oppF</i>	1.0 ± 0.006	4.6 ± 1.49	provides energy for peptide transport; bacterial nutrition
<i>gap</i>	1.0 ± 0.040	0.32 ± 0.14	glyceraldehyde-3-phosphate dehydrogenase; enzyme involved in glycolysis
<i>arcD</i>	1.0 ± 0.031	0.31 ± 0.14	arginine/ornithine antiporter
<i>dnaC</i>	1.0 ± 0.012	0.49 ± 0.25	DNA helicase required for elongation during DNA synthesis; involved in DNA repair
<i>ureC</i>	1.0 ± 0.016	0.63 ± 0.12	urease alpha subunit; virulence factor
<i>hemL</i>	1.0 ± 0.012	0.25 ± 0.02	glutamate-1-semialdehyde 2,1-aminomutase; heme biosynthesis

* P-value ≤ 0.05, ** P-value ≤ 0.01

Gene	Fold Δ
<i>isdB</i>	10.73
<i>isdA</i>	10.37
<i>isdC</i>	14.09
<i>isdD</i>	14.13
<i>isdE</i>	12.08
<i>isdF</i>	10.45
<i>srtB</i>	17.39
<i>isdG</i>	10.41
<i>isdH</i>	3.78
<i>orfX</i>	1.94
<i>isdI</i>	1.14
<i>sirB</i>	4.30
<i>sirA</i>	8.63
<i>sirC</i>	1.31
<i>sbnA</i>	12.43
<i>sbnB</i>	15.13
<i>sbnC</i>	16.02



Gene	Fold Δ
<i>sbnD</i>	14.11
<i>sbnE</i>	9.15
<i>sbnF</i>	8.37
<i>sbnG</i>	6.38
<i>sbnH</i>	6.16
<i>sbnI</i>	5.55
<i>fhuC</i>	2.61
<i>fhuB</i>	2.32
<i>fhuG</i>	1.99
<i>fhuD1</i>	5.03
<i>fhuD2</i>	2.52
<i>sfaD</i>	1.60
<i>sfaA</i>	4.58
<i>sfaB</i>	1.55
<i>sfaC</i>	1.61
<i>htsA</i>	3.85
<i>htsB</i>	3.84

Supporting Figure 4: Iron acquisition systems in *S. aureus* with corresponding fold changes of MRSA-1707 biofilm gene transcripts (activated) following treatment with **HP-14** (0.625 μ M, 1/10 MBEC) for 20 hours.

3.) Supporting Tables.

Supporting Table 1: Top 30 upregulated gene transcripts from RNA-seq of MRSA-1707 biofilms upon treatment with **HP-14** (20 hours at 0.625 μ M).

	Gene	Symbol	Product	Fold Δ	P-value
1	MW1017	<i>srtB</i>	NPQTN specific sortase B	17.4	2.9×10^{-137}
2	MW0091	<i>sbnC</i>	Staphyloferrin B (siderophore) biosynthesis protein SbnC	16.0	4.8×10^{-171}
3	MW0090	<i>sbnB</i>	Putative ornithine cyclodeaminase (staphyloferrin B)	15.1	2.3×10^{-116}
4	MW1014	<i>isdD</i>	Conserved hypothetical protein (iron-regulated surface determinant)	14.1	9.9×10^{-122}
5	MW0092	<i>sbnD</i>	Hypothetical efflux protein (staphyloferrin B)	14.1	6.0×10^{-151}
6	MW1013	<i>isdC</i>	Conserved hypothetical protein (iron-regulated surface determinant)	14.1	4.4×10^{-131}
7	MW1754	<i>splB</i>	Serine protease SplB [Genomic island nu Sa beta2]	13.7	2.9×10^{-107}
8	MW0089	<i>sbnA</i>	Hypothetical protein; similar to cysteine synthase (staphyloferrin B)	12.4	5.7×10^{-119}
9	MW1015	<i>isdE</i>	Hypothetical protein; similar to ferrichrome ABC transporter	12.1	2.0×10^{-123}
10	MW1755	<i>splA</i>	Serine protease SplA [Genomic island nu Sa beta2]	10.9	1.6×10^{-111}
11	MW1011	<i>isdB</i>	Conserved hypothetical protein (iron-regulated surface determinant)	10.7	4.5×10^{-170}
12	MW1016	<i>isdF</i>	Hypothetical protein; similar to ferrichrome ABC transporter	10.5	1.4×10^{-120}
13	MW1018	<i>isdG</i>	Conserved hypothetical protein (iron-regulated surface determinant)	10.4	4.0×10^{-64}
14	MW1012	<i>isdA</i>	Cell surface protein (iron-regulated surface determinant)	10.4	2.3×10^{-121}
15	MW1753	<i>splC</i>	Serine protease SplC [Genomic island nu Sa beta2]	9.6	1.3×10^{-129}
16	MW0093	<i>sbnE</i>	Staphyloferrin B (siderophore) biosynthesis protein SbnE	9.2	3.3×10^{-130}
17	MW0088	<i>sirA</i>	Iron-regulated ABC transporter siderophore-binding protein SirA	8.6	6.4×10^{-156}
18	MW0094	<i>sbnF</i>	Staphyloferrin B (siderophore) biosynthesis protein SbnF	8.4	3.1×10^{-125}
19	MW2389	n.a.	Oligopeptide transporter putative membrane permease domain	7.8	8.1×10^{-126}
20	MW2388	n.a.	Oligopeptide transporter putative ATPase domain	7.5	3.6×10^{-119}
21	MW1752	<i>splF</i>	Serine protease SplF [Genomic island nu Sa beta2]	7.4	3.4×10^{-131}
22	MW2393	n.a.	Conserved hypothetical protein	6.8	3.0×10^{-78}
23	MW2392	n.a.	Conserved hypothetical protein	6.7	1.7×10^{-73}
24	MW0095	<i>sbnG</i>	Putative 2-dehydro-3-deoxyglucarate aldolase (staphyloferrin B)	6.4	8.0×10^{-96}
25	MW0096	<i>sbnH</i>	Putative diaminopimelate decarboxylase (staphyloferrin B)	6.2	4.1×10^{-102}
26	MW0194	n.a.	Hypothetical protein; similar to NADH-dependent dehydrogenase	5.7	1.9×10^{-89}
27	MW0195	n.a.	Conserved hypothetical protein	5.6	2.0×10^{-84}
28	MW0097	<i>sbnI</i>	Hypothetical protein (staphyloferrin B)	5.6	1.4×10^{-86}
29	MW2387	n.a.	Oligopeptide transporter putative ATPase domain	5.5	3.9×10^{-101}
30	MW2394	n.a.	Hypothetical protein; similar to diaminopimelate epimerase	5.3	1.4×10^{-62}

Note: All corresponding gene names and symbols were retrieved from DOGAN, a genome database of microorganisms sequenced at NITE (<http://www.bio.nite.go.jp/dogan/top>).

Supporting Table 2: Top 30 down-regulated gene transcripts from RNA-seq of MRSA-1707 biofilms upon treatment with **HP-14** (20 hours at 0.625 μ M).

	Gene	Symbol	Product	Fold Δ	P-value
1	MW2281	n.a.	Conserved hypothetical protein	-4.5	1.4 x 10 ⁻⁸⁴
2	MW2280	n.a.	Hypothetical protein; similar to ABC transporter and ATP-binding protein	-4.3	6.9 x 10 ⁻⁹⁹
3	MW_RS08680	n.a.	Hypothetical protein ^a	-3.8	1.9 x 10 ⁻²⁴
4	MW0734	<i>gap</i>	Glyceraldehyde-3-phosphate dehydrogenase	-3.7	8.4 x 10 ⁻⁶⁴
5	MW1834	n.a.	Hypothetical protein; similar to ferritin	-3.7	3.0 x 10 ⁻⁶²
6	MW2554	<i>arcD</i>	Arginine/ornithine antiporter	-3.6	1.6 x 10 ⁻²⁵
7	MW1762	<i>basD</i> ^b	Hypothetical protein; similar to lantibiotic epidermin biosynthesis protein EpiD	-3.3	2.4 x 10 ⁻²⁹
8	MW0100	<i>butA</i>	Acetoin(diacetyl)reductase	-3.0	1.5 x 10 ⁻³⁰
9	MW1625	<i>lysP</i>	Lysine-specific permease	-3.0	5.6 x 10 ⁻²⁹
10	MW2552	n.a.	Hypothetical protein; similar to transcription regulator Crp/Fnr family protein	-3.0	2.0 x 10 ⁻¹⁸
11	MW0958	n.a.	Conserved hypothetical protein	-2.9	1.4 x 10 ⁻³⁸
12	MW2578	n.a.	Hypothetical protein	-2.9	1.7 x 10 ⁻³²
13	MW2553	<i>arc</i>	Carbamate kinase	-2.9	1.5 x 10 ⁻¹⁷
14	MW0108	n.a.	Hypothetical protein	-2.8	2.6 x 10 ⁻⁴³
15	MW1226	<i>lexA</i>	SOS regulatory LexA protein	-2.8	2.5 x 10 ⁻²³
16	MW1152	<i>infB</i>	Translation initiation factor IF-2	-2.8	1.2 x 10 ⁻³²
17	MW1641	<i>pykA</i>	Pyruvate kinase	-2.8	6.3 x 10 ⁻⁴³
18	MW1763	<i>basC</i> ^b	Hypothetical protein; similar to lantibiotic epidermin biosynthesis protein EpiC2	-2.7	1.2 x 10 ⁻²⁶
19	MW2622	n.a.	Hypothetical protein	-2.6	1.3 x 10 ⁻²
20	MW1611	<i>hemL</i>	Glutamate-1-semialdehyde 2,1-aminomutase	-2.6	7.1 x 10 ⁻²³
21	MW0738	<i>eno</i>	Enolase (2-phosphoglycerate dehydrogenase)	-2.6	5.1 x 10 ⁻³⁴
22	MW1435	n.a.	Hypothetical protein; similar to Cro-like protein (<i>Strep. pyogenes</i> M1 GAS)	-2.5	1.6 x 10 ⁻²³
23	MW1612	<i>hemB</i>	Delta-aminolevulinic acid dehydratase	-2.5	2.5 x 10 ⁻²²
24	MW1468	<i>bmfBB</i>	Branched-chain alpha-keto acid dehydrogenase E2	-2.5	1.8 x 10 ⁻²¹
25	MW0774	n.a.	Hypothetical protein	-2.5	1.7 x 10 ⁻²⁸
26	MW1680	n.a.	Chorismate mutase homologue	-2.4	7.9 x 10 ⁻²³
27	MW0016	<i>dnaC</i>	Replicative DNA helicase	-2.4	5.2 x 10 ⁻²⁰
28	MW1642	<i>pfk</i>	6-Phosphofructokinase	-2.4	3.7 x 10 ⁻³⁰
29	MW2330	n.a.	Conserved hypothetical protein	-2.4	2.1 x 10 ⁻³⁰
30	MW1250	n.a.	4-Oxalocrotonate tautomerase	-2.4	3.3 x 10 ⁻²¹

Notes: All corresponding gene names and symbols were retrieved from DOGAN, a genome database of microorganisms sequenced at NITE (<http://www.bio.nite.go.jp/dogan/top>) unless otherwise noted. ^a Information retrieved from ATGC, Alignable Tight Genomic Cluster: ATGC052 (ftp://dmk-brain.ecn.uiowa.edu/ATGC/data/ATGC052/genomes/Staphylococcus_aureus_aureus_MW2.GCF_000011265.1.html). ^b Information retrieved from KEGG GENOME Database (http://www.genome.jp/kegg-bin/show_organism).

Supporting Table 3: Genes involved in responses to reactive oxygen species (ROS) from MRSA-1707 biofilms treated with **HP-14** (20 hours at 0.625 μ M).

Gene	Symbol	Function	Fold Δ	P-value
MW0356	<i>ahpF</i>	Alkyl hydroperoxide reductase subunit F	- 1.4	9.3×10^{-6}
MW0357	<i>ahpC</i>	Alkyl hydroperoxide reductase subunit C	- 1.3	5.1×10^{-3}
MW0726	<i>trxB</i>	Thioredoxine reductase	1.0	7.8×10^{-1}
MW0107	<i>sodM</i>	Superoxide dismutase	- 1.2	2.8×10^{-2}
MW1505	<i>sodA</i>	Superoxide dismutase SodA	- 1.8	2.7×10^{-11}

Note: Our interest with ROS-related genes is due to the known redox activity of phenazine antibiotics.

Conclusion: Based on the data in Supporting Table 3, **HP-14** does not operate through a ROS-based mechanism of action. Minimal or non-significant reductions in gene transcripts observed upon **HP-14** treatment of MRSA biofilms.

Supporting Table 4: Genes related to quorum sensing, virulence, biofilm attachment/formation and toxin/antitoxin systems from MRSA-1707 biofilms treated with **HP-14** (20 hours at 0.625 μ M).

Gene	Symbol	Function	Category	Fold Δ	P-value
MW1960	<i>agrB</i>	accessory gene regulator B	Quorum Sensing	- 1.1	1.7×10^{-1}
MW1961	<i>agrD</i>	AgrD	Quorum Sensing	- 1.1	4.9×10^{-1}
MW1962	<i>agrC</i>	AgrC	Quorum Sensing	- 1.4	4.2×10^{-5}
MW1963	<i>agrA</i>	accessory gene regulator A	Quorum Sensing	- 2.1	7.3×10^{-13}
MW2585	<i>icaR</i>	ica operon transcriptional regulator IcaR	Biofilm Attachment	1.8	3.6×10^{-12}
MW2586	<i>icaA</i>	intercellular adhesion protein A	Biofilm Attachment	1.1	4.0×10^{-1}
MW2587	<i>icaD</i>	intercellular adhesion protein D	Biofilm Attachment	- 1.3	3.3×10^{-1}
MW2588	<i>icaB</i>	intercellular adhesion protein B	Biofilm Attachment	1.5	1.8×10^{-3}
MW2589	<i>icaC</i>	intercellular adhesion protein C	Biofilm Attachment	1.2	4.5×10^{-2}
MW1992	n.a.	mRNA interferase MazF ^a	TX / AT System	1.3	8.5×10^{-3}
MW1993	n.a.	Antitoxin MazE ^a	TX / AT System	1.0	7.4×10^{-1}
MW1991	<i>rsbU</i>	Sigma regulation protein RsbU	TX / AT System	1.2	5.7×10^{-3}
MW1990	<i>rsbV</i>	Anti-sigmaB factor antagonist	TX / AT System	1.2	2.4×10^{-2}
MW1989	<i>rsbW</i>	Anti-sigmaB factor	TX / AT System	1.0	8.5×10^{-1}
MW1988	<i>sigB</i>	Sigma factor B	TX / AT System	1.1	4.2×10^{-1}
MW2330	n.a.	Antitoxin YefM ^b	TX / AT System	-2.4	2.1×10^{-30}
MW2329	n.a.	Toxin YoeB ^b	TX / AT System	-1.7	9.9×10^{-12}
MW0125	<i>cap8B</i>	Capsular polysaccharide synthesis enzyme Cap8B	Virulence / Biofilm	-1.1	1.5×10^{-1}
MW0126	<i>cap8C</i>	Capsular polysaccharide synthesis enzyme Cap8C	Virulence / Biofilm	-1.2	2.7×10^{-2}
MW0127	<i>cap8D</i>	Capsular polysaccharide synthesis enzyme Cap8D	Virulence / Biofilm	-1.3	1.4×10^{-3}
MW0128	<i>cap8E</i>	Capsular polysaccharide synthesis enzyme Cap8E	Virulence / Biofilm	-1.4	3.4×10^{-5}
MW0129	<i>cap8F</i>	Capsular polysaccharide synthesis enzyme Cap8F	Virulence / Biofilm	-1.5	1.3×10^{-7}
MW0130	<i>cap8G</i>	Capsular polysaccharide synthesis enzyme Cap8G	Virulence / Biofilm	-1.4	1.4×10^{-4}
MW0131	<i>cap8H</i>	Capsular polysaccharide synthesis enzyme Cap8H	Virulence / Biofilm	-1.3	3.0×10^{-4}
MW0132	<i>cap8I</i>	Capsular polysaccharide synthesis enzyme Cap8I	Virulence / Biofilm	-1.3	2.1×10^{-3}
MW0133	<i>cap8J</i>	Capsular polysaccharide synthesis enzyme Cap8J	Virulence / Biofilm	-1.4	1.9×10^{-3}
MW0134	<i>cap8K</i>	Capsular polysaccharide synthesis enzyme Cap8K	Virulence / Biofilm	-1.3	7.6×10^{-4}
MW0135	<i>cap8L</i>	Capsular polysaccharide synthesis enzyme Cap8L	Virulence / Biofilm	-1.5	2.3×10^{-7}
MW0136	<i>cap8M</i>	Capsular polysaccharide synthesis enzyme Cap8M	Virulence / Biofilm	-1.3	5.0×10^{-3}
MW0137	<i>cap8N</i>	Capsular polysaccharide synthesis enzyme Cap8N	Virulence / Biofilm	-1.4	5.2×10^{-5}
MW0138	<i>cap8O</i>	Capsular polysaccharide synthesis enzyme Cap8O	Virulence / Biofilm	-1.2	7.0×10^{-2}
MW0139	<i>cap8P</i>	Capsular polysaccharide synthesis enzyme Cap8P	Virulence / Biofilm	-1.3	5.3×10^{-4}

Note: TX / AT System = Toxin / Antitoxin system

Conclusion: Based on the data in Supporting Table 4, **HP-14** demonstrates minimal impacts on quorum sensing, biofilm attachment, toxin-antitoxin systems and capsular polysaccharide synthesis. Each of these genes are known to have a role in biofilm or persister cell formation.

Supporting Table 5: Primers used for qPCR validation experiments.

Gene	Symbol	Forward primer	Reverse primer
MW0091	<i>sbnC</i>	TCCGGTACATCCTTGGCAAT	GACATGGTTATACGGTGCGC
MW1011	<i>isdB</i>	CCAGCAGCAAAAGCCACTAA	CGAGAGTTTGGTGCCTATG
MW1754	<i>splB</i>	TCAAGTTGAAGAGCGTGCAA	GGTGATCCAGAGTTTCCGCT
MW0734	<i>gap</i>	GTGGTTTCCGCGTAAATGGT	CGCCTGCTTCAATATGAGCT
MW0695	n.a.	CTGGAACGATGGAATGGGCT	CGTGCGCAATGAGATGAAGG
MW1945	<i>fhuD1</i>	TGGATTGAAGAGTGGGACGA	CCAAACCACGACCCCATGTA
MW0871	<i>oppF</i>	TGAATCAGAACGCAGTCGCA	TCGTAGTTGTGCCGCTTCTT
MW2106	<i>sfaA</i>	GACGATGTGTGCCATTGGTG	TTGTCGTACGTGCCCATGTA
MW2554	<i>arcD</i>	TCGGTATGACGTCAGAGGGT	TACTGCACCCTCGATACCGA
MW0016	<i>dnaC</i>	ACAGCAGGGTTCAACCGAAA	CAGCACCCATCTCTAGCGAG
MW1611	<i>hemL</i>	CCAGATTCTCCTGGTGTGCC	CAATGCGCCGTATTCAGTCG
MW2208	<i>ureC</i>	ATACTGAAGGTGCTGGTGGC	CTTCACCTACACGGCCCAT
MW1668	<i>ptaA</i>	TCCTAGCGAGTTCAGTTGCA	CCAATGGAATGTAGCTGCGA

Notes: Primers were designed using OligoPerfect Primer Designer (Thermofisher). During these studies, *ptaA* was used as the housekeeping gene for MRSA-1707 (*S. aureus* MW2) biofilms.

Supporting Table 6: WoPPER analysis of 10 gene clusters identified from **HP-14** treatment of MRSA-1707 biofilms (646 genes analyzed based on ≥ 1.5 -fold change in transcript levels).

Gene	Symbol	Product	Fold Δ	P-value
Cluster 1 Iron-regulated Surface Determinant (Heme Iron Acquisition); Average Fold Δ: 9.96 \pm 5.79				
MW1004	n.a.	conserved hypothetical protein	1.7	1.0 x 10 ⁻⁴
MW1011	<i>isdB</i>	conserved hypothetical protein	10.7	4.5 x 10 ⁻¹⁷⁰
MW1012	<i>isdA</i>	cell surface protein	10.4	2.3 x 10 ⁻¹²¹
MW1013	<i>isdC</i>	conserved hypothetical protein	14.1	4.4 x 10 ⁻¹³¹
MW1014	<i>isdD</i>	conserved hypothetical protein	14.1	9.9 x 10 ⁻¹²²
MW1015	<i>isdE</i>	hypothetical protein; similar to ferrichrome ABC transporter	12.1	2.0 x 10 ⁻¹²³
MW1016	<i>isdF</i>	hypothetical protein; similar to ferrichrome ABC transporter	10.5	1.4 x 10 ⁻¹²⁰
MW1017	<i>srtB</i>	NPQTN specific sortase B	17.4	2.9 x 10 ⁻¹³⁷
MW1018	<i>isdG</i>	conserved hypothetical protein	10.4	4.0 x 10 ⁻⁶⁴
MW1022	<i>pheT</i>	Phe-tRNA synthetase beta chain	- 1.7	3.4 x 10 ⁻¹⁰
Cluster 2 Staphyloferrin B Biosynthesis (Siderophore; Iron Acquisition); Average Fold Δ: 9.0 \pm 5.3				
MW0085	<i>sarH1</i>	staphylococcal accessory regulator A homologue	- 2.0	2.9 x 10 ⁻²¹
MW0087	<i>sirB</i>	iron-regulated ABC transporter siderophore permease protein SirB	4.3	1.8 x 10 ⁻⁴³
MW0088	<i>sirA</i>	iron-regulated ABC transporter siderophore-binding protein SirA	8.6	6.4 x 10 ⁻¹⁵⁶
MW0089	<i>sbnA</i>	hypothetical protein; similar to cysteine synthase	12.4	5.7 x 10 ⁻¹¹⁹
MW0090	<i>sbnB</i>	putative ornithine cyclodeaminase	15.1	2.3 x 10 ⁻¹¹⁶
MW0091	<i>sbnC</i>	siderophore biosynthesis protein SbnC	16.0	4.8 x 10 ⁻¹⁷¹
MW0092	<i>sbnD</i>	hypothetical efflux protein	14.1	6.0 x 10 ⁻¹⁵¹
MW0093	<i>sbnE</i>	siderophore biosynthesis protein SbnE	9.2	3.3 x 10 ⁻¹³⁰
MW0094	<i>sbnF</i>	siderophore biosynthesis protein SbnF	8.4	3.1 x 10 ⁻¹²⁵
MW0095	<i>sbnG</i>	putative 2-dehydro-3-deoxyglucarate aldolase	6.4	8.0 x 10 ⁻⁹⁶
MW0096	<i>sbnH</i>	putative diaminopimelate decarboxylase	6.2	4.1 x 10 ⁻¹⁰²
Cluster 3 Ferrichrome ABC Transporter Permease (Iron Acquisition); Average Fold Δ: 3.55 \pm 0.21				
MW0695	n.a.	hypothetical protein; similar to ferrichrome ABC transporter permease	3.7	8.5 x 10 ⁻⁴⁵
MW0696	n.a.	hypothetical protein; similar to ferrichrome ABC transporter permease	3.4	8.3 x 10 ⁻³³
Cluster 4 Glycolysis (Metabolism of Carbohydrates); Average Fold Δ: -2.57 \pm 0.29				
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰
Cluster 5 Glycolysis (Metabolism of Carbohydrates); Average Fold Δ: -2.56 \pm 0.79				
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4 x 10 ⁻⁶⁴
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0 x 10 ⁻²⁵
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6 x 10 ⁻²⁴
MW0747	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 2.1	2.1 x 10 ⁻¹²
Cluster 6 Function Unknown; Average Fold Δ: -2.33 \pm 0.48				
MW0108	n.a.	hypothetical protein	- 2.8	2.6 x 10 ⁻⁴³
MW0113	<i>drm</i>	phosphopentomutase	- 2.2	3.3 x 10 ⁻²⁰
MW0119	n.a.	hypothetical protein; similar to lactococcal phosphatase homologue	- 1.9	2.0 x 10 ⁻¹⁹
Cluster 7 Uroporphyrinogen III Biosynthesis (Heme Biosynthesis); Average Fold Δ: -2.25 \pm 0.41				
MW1595	<i>rpmA</i>	50S ribosomal protein L27 (BL30) (BL24)	- 1.7	9.1 x 10 ⁻¹¹
MW1611	<i>hemL</i>	glutamate-1-semialdehyde 2,1-aminomutase	- 2.6	7.1 x 10 ⁻²³
MW1612	<i>hemB</i>	delta-aminolevulinic acid dehydratase	- 2.5	2.5 x 10 ⁻²²
MW1613	<i>hemD</i>	uroporphyrinogen III synthase	- 2.3	5.2 x 10 ⁻²²
Cluster 8 Function Unknown; Average Fold Δ: -2.22 \pm 0.66				
MW0957	<i>purD</i>	phosphoribosylamine-glycine ligase PurD	- 1.6	2.8 x 10 ⁻⁸
MW0958	n.a.	conserved hypothetical protein	- 2.9	1.4 x 10 ⁻³⁸
MW0959	n.a.	hypothetical protein; similar to cation ABC transporter	- 2.1	5.0 x 10 ⁻¹⁹
Cluster 9 Function Unknown; Average Fold Δ: -2.20 \pm 0.83				
MW1152	<i>infB</i>	translation initiation factor IF-2	- 2.8	1.2 x 10 ⁻³²
MW1153	<i>rbfA</i>	ribosome-binding factor A	- 1.6	5.0 x 10 ⁻⁹
Cluster 10 Function Unknown; Average Fold Δ: -2.1 \pm 0.32				

MW0015	<i>rplI</i>	50S ribosomal protein L9	- 2.4	9.4×10^{-27}
MW0016	<i>dnaC</i>	replicative DNA helicase	- 2.4	5.2×10^{-20}
MW0019	<i>vicK</i>	two-component sensor histidine kinase	- 1.6	2.5×10^{-9}
MW0020	n.a.	conserved hypothetical protein	- 2.0	1.3×10^{-15}
MW0021	n.a.	conserved hypothetical protein	- 2.1	8.2×10^{-17}

Notes: Genes were inserted into WoPPER analysis web tool (<https://wopper.ba.itb.cnr.it/Articles>) to identify gene clusters that had altered transcript profiles upon treatment of MRSA-1707 biofilms with **HP-14**. All gene symbols and names in this table were retrieved from DOGAN which is a genome database of microorganisms sequenced at NITE (<http://www.bio.nite.go.jp/dogan/top>).

Supporting Table 7: WoPPER analysis of 37 gene clusters identified from **HP-14** treatment of MRSA-1707 biofilms (all 2,738 genes examined).

Cluster 1: <i>isd/srtB</i> (Heme Iron Acquisition); Average Fold Δ: 11.0 \pm 5.1				
Genes	Symbol	Function	Fold Δ	P-value
MW1011	<i>isdB</i>	conserved hypothetical protein	10.7	4.5 x 10 ⁻¹⁷⁰
MW1012	<i>isdA</i>	cell surface protein	10.4	2.3 x 10 ⁻¹²¹
MW1013	<i>isdC</i>	conserved hypothetical protein	14.1	4.4 x 10 ⁻¹³¹
MW1014	<i>isdD</i>	conserved hypothetical protein	14.1	9.9 x 10 ⁻¹²²
MW1015	<i>isdE</i>	hypothetical protein; similar to ferrichrome ABC transporter	12.1	2.0 x 10 ⁻¹²³
MW1016	<i>isdF</i>	hypothetical protein; similar to ferrichrome ABC transporter	10.5	1.4 x 10 ⁻¹²⁰
MW1017	<i>srtB</i>	NPQTN specific sortase B	17.4	2.9 x 10 ⁻¹³⁷
MW1018	<i>isdG</i>	conserved hypothetical protein	10.4	4.0 x 10 ⁻⁶⁴
MW1020	n.a.	hypothetical protein; similar to rRNA methylase	- 1.1	3.9 x 10 ⁻¹
Cluster 2: <i>sir/sbn</i> (Staphyloferrin B Biosynthesis & Transport; Iron Acquisition); Average Fold Δ: 8.4 \pm 4.9				
Genes	Symbol	Function	Fold Δ	P-value
MW0086	<i>sirC</i>	iron-regulated ABC transporter siderophore permease protein SirC	1.3	2.7 x 10 ⁻⁵
MW0087	<i>sirB</i>	iron-regulated ABC transporter siderophore permease protein SirB	4.3	1.8 x 10 ⁻⁴³
MW0088	<i>sirA</i>	iron-regulated ABC transporter siderophore-binding protein SirA	8.6	6.4 x 10 ⁻¹⁵⁶
MW0089	<i>sbnA</i>	hypothetical protein; similar to cysteine synthase	12.4	5.7 x 10 ⁻¹¹⁹
MW0090	<i>sbnB</i>	putative ornithine cyclodeaminase	15.1	2.3 x 10 ⁻¹¹⁶
MW0091	<i>sbnC</i>	siderophore biosynthesis protein SbnC	16.0	4.8 x 10 ⁻¹⁷¹
MW0092	<i>sbnD</i>	hypothetical efflux protein	14.1	6.0 x 10 ⁻¹⁵¹
MW0093	<i>sbnE</i>	siderophore biosynthesis protein SbnE	9.2	3.3 x 10 ⁻¹³⁰
MW0094	<i>sbnF</i>	siderophore biosynthesis protein SbnF	8.4	3.1 x 10 ⁻¹²⁵
MW0095	<i>sbnG</i>	putative 2-dehydro-3-deoxyglucarate aldolase	6.4	8.0 x 10 ⁻⁹⁶
MW0096	<i>sbnH</i>	putative diaminopimelate decarboxylase	6.2	4.1 x 10 ⁻¹⁰²
MW0097	<i>sbnI</i>	hypothetical protein	5.6	1.4 x 10 ⁻⁸⁶
MW0098	n.a.	hypothetical protein	1.6	1.7 x 10 ⁻⁴
Cluster 3: <i>spl</i> (Serine Proteases); Average Fold Δ: 6.8 \pm 4.9				
Genes	Symbol	Function	Fold Δ	P-value
MW1751	<i>hsdM</i>	type I restriction enzyme EcoR124II M protein homologue	1.1	1.5 x 10 ⁻¹
MW1752	<i>splF</i>	serine protease SplF [Genomic island nu Sa beta2]	7.4	3.4 x 10 ⁻¹³¹
MW1753	<i>splC</i>	serine protease SplC [Genomic island nu Sa beta2]	9.6	1.3 x 10 ⁻¹²⁹
MW1754	<i>splB</i>	serine protease SplB [Genomic island nu Sa beta2]	13.7	2.9 x 10 ⁻¹⁰⁷
MW1755	<i>splA</i>	serine protease SplA [Genomic island nu Sa beta2]	10.9	1.6 x 10 ⁻¹¹¹
MW1756	n.a.	hypothetical protein [Genomic island nu Sa beta2]	3.5	6.2 x 10 ⁻²
MW1757	n.a.	hypothetical protein; similar to Ear protein [Genomic island nu Sa beta2]	1.6	2.7 x 10 ⁻⁴
Cluster 4: Oligopeptide Transporter System (Bacterial Nutrition, Signaling & Virulence); Average Fold Δ: 5.3 \pm 2.0				
Genes	Symbol	Function	Fold Δ	P-value
MW2384	n.a.	hypothetical protein	1.4	1.3 x 10 ⁻⁴
MW2386	n.a.	hypothetical protein; similar to antibiotic resistance protein	4.0	3.8 x 10 ⁻⁷²
MW2387	n.a.	oligopeptide transporter putative ATPase domain	5.5	3.9 x 10 ⁻¹⁰¹
MW2388	n.a.	oligopeptide transporter putative ATPase domain	7.5	3.6 x 10 ⁻¹¹⁹
MW2389	n.a.	oligopeptide transporter putative membrane permease domain	7.8	8.1 x 10 ⁻¹²⁶
MW2390	n.a.	oligopeptide transporter putative membrane permease domain	3.8	8.0 x 10 ⁻⁷⁸
MW2391	n.a.	oligopeptide transporter putative substrate binding domain	4.6	9.7 x 10 ⁻⁸⁶
MW2392	n.a.	conserved hypothetical protein	6.7	1.7 x 10 ⁻⁷³
MW2393	n.a.	conserved hypothetical protein	6.8	3.0 x 10 ⁻⁷⁸
MW2394	n.a.	hypothetical protein; similar to diaminopimelate epimerase	5.3	1.4 x 10 ⁻⁶²
Cluster 5: <i>fhuD1</i> (Ferric Hydroxamate Transport; Iron Acquisition); Average Fold Δ: 3.7 \pm 1.4				
Genes	Symbol	Function	Fold Δ	P-value
MW1944	n.a.	hypothetical protein	3.6	6.8 x 10 ⁻³⁴
MW1945	<i>fhuD1</i>	hydroxamate siderophore binding lipoprotein	5.0	2.6 x 10 ⁻⁵⁸
MW1946	n.a.	hypothetical protein; similar to Na ⁺ -transporting ATP synthase	2.3	6.4 x 10 ⁻²³

Cluster 6: Function Unknown; Average Fold Δ: 3.6 \pm 2.5				
Genes	Symbol	Function	Fold Δ	P-value
MW0190	n.a.	hypothetical protein; similar to maltose/maltodextrin-binding protein	1.7	6.2 x 10 ⁻¹¹
MW0191	n.a.	maltose/maltodextrin transport permease homologue	4.8	1.2 x 10 ⁻⁷⁶
MW0192	n.a.	maltose/maltodextrin transport permease homologue	3.4	1.5 x 10 ⁻⁵²
MW0193	n.a.	hypothetical protein; similar to NADH-dependent dehydrogenase	5.3	9.0 x 10 ⁻⁸⁴
MW0194	n.a.	hypothetical protein; similar to NADH-dependent dehydrogenase	5.7	1.9 x 10 ⁻⁸⁹
MW0195	n.a.	conserved hypothetical protein	5.6	2.0 x 10 ⁻⁸⁴
MW0196	n.a.	conserved hypothetical protein	- 1.0	8.1 x 10 ⁻¹
Cluster 7: Oligopeptide Transport Systems (Bacterial Nutrition, Signaling & Virulence); Average Fold Δ: 3.1 \pm 0.6				
Genes	Symbol	Function	Fold Δ	P-value
MW0869	n.a.	hypothetical protein; similar, oligopeptide transport syst. permease protein OppC	2.3	4.9 x 10 ⁻¹³
MW0870	<i>oppD</i>	oligopeptide transport system ATP-binding protein OppD homologue	3.3	4.9 x 10 ⁻³⁶
MW0871	<i>oppF</i>	oligopeptide transport system ATP-binding protein OppF homologue	3.6	3.1 x 10 ⁻⁴⁵
MW0872	n.a.	hypothetical protein; similar to peptide binding protein OppA	3.0	5.2 x 10 ⁻³⁷
Cluster 8: Ferrichrome ABC Transporters (Iron Acquisition); Average Fold Δ: 3.0 \pm 0.6				
Genes	Symbol	Function	Fold Δ	P-value
MW0695	n.a.	hypothetical protein; similar to ferrichrome ABC transporter permease	3.7	8.5 x 10 ⁻⁴⁵
MW0696	n.a.	hypothetical protein; similar to ferrichrome ABC transporter permease	3.4	8.3 x 10 ⁻³³
MW0697	n.a.	hypothetical protein; similar to ferrichrome ABC transport, ATP-binding protein	2.8	2.5 x 10 ⁻²⁷
MW0698	n.a.	lipoprotein, similar to ferrichrome ABC transporter	2.3	9.1 x 10 ⁻²⁰
Cluster 9: Fructose & Mannose Metabolism; Average Fold Δ: 2.9 \pm 1.2				
Genes	Symbol	Function	Fold Δ	P-value
MW2562	n.a.	fructose phosphotransferase system enzyme fruA homologue	3.7	6.3 x 10 ⁻⁵³
MW2563	<i>pmi</i>	mannose-6-phosphate isomerase	2.0	6.0 x 10 ⁻¹⁸
Cluster 10: Function Unknown; Average Fold Δ: 2.9 \pm 1.3				
Genes	Symbol	Function	Fold Δ	P-value
MW0594	n.a.	conserved hypothetical protein	2.0	1.7 x 10 ⁻¹⁹
MW0595	n.a.	hypothetical protein; similar to ABC transporter ATP-binding protein	3.8	6.3 x 10 ⁻⁴⁸
Cluster 11: Staphyloferrin A Biosynthesis; Average Fold Δ: 2.9 \pm 1.3				
Genes	Symbol	Function	Fold Δ	P-value
MW2100	n.a.	conserved hypothetical protein	2.0	6.8 x 10 ⁻²¹
MW2102	<i>htsB</i>	hypothetical protein; similar to ferrichrome ABC transporter (permease)	3.8	9.3 x 10 ⁻⁵⁴
MW2103	<i>htsA</i>	hypothetical protein; similar to ferrichrome ABC transporter	3.9	1.0 x 10 ⁻⁶⁷
MW2104	<i>sfaC</i>	conserved hypothetical protein	1.6	7.4 x 10 ⁻¹³
MW2105	<i>sfaB</i>	conserved hypothetical protein	1.6	4.4 x 10 ⁻⁹
MW2106	<i>sfaA</i>	hypothetical protein; similar to transporter	4.6	6.4 x 10 ⁻⁷²
Cluster 12: Arginine Deaminase (Utilization of Arginine for Energy, Anaerobic Conditions); Average Fold Δ: -2.6 \pm 0.8				
Genes	Symbol	Function	Fold Δ	P-value
MW2552	n.a.	hypothetical protein; similar to transcription regulator Crp/Fnr family protein	- 3.0	2.0 x 10 ⁻¹⁸
MW2553	<i>arcC</i>	carbamate kinase	- 2.9	1.5 x 10 ⁻¹⁷
MW2554	<i>arcD</i>	arginine/ornithine antiporter	- 3.6	1.6 x 10 ⁻²⁵
MW2555	<i>arcB</i>	ornithine transcarbamoylase	- 2.1	7.5 x 10 ⁻¹³
MW2556	<i>arcA</i>	arginine deiminase	- 1.6	3.6 x 10 ⁻⁹
Cluster 13: Function Unknown; Average Fold Δ: 2.5 \pm 1.8				
Genes	Symbol	Function	Fold Δ	P-value
MW1674	n.a.	hypothetical protein	3.8	5.5 x 10 ⁻⁷⁵
MW1675	<i>fhs</i>	formyltetrahydrofolate synthetase	1.2	5.3 x 10 ⁻²
Cluster 14: Function Unknown; Average Fold Δ: 2.4 \pm 0.7				
Genes	Symbol	Function	Fold Δ	P-value
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0 x 10 ⁻³⁶
MW0428	<i>treP</i>	PTS enzyme II, phosphoenolpyruvate-dependent, trehalose-specific	2.1	1.1 x 10 ⁻²¹
MW0429	n.a.	alpha-glucosidase	2.7	5.2 x 10 ⁻³⁴
MW0430	n.a.	hypothetical protein; similar to trehalose operon transcriptional repressor	3.3	1.9 x 10 ⁻⁴⁸

MW0432	n.a.	conserved hypothetical protein	1.4	2.1×10^{-5}
Cluster 15: Branched Chain Amino Acid Synthesis; Average Fold Δ: 2.4 ± 0.3				
Genes	Symbol	Function	Fold Δ	P-value
MW1977	<i>ilvD</i>	dihydroxy-acid dehydratase	2.1	7.5×10^{-12}
MW1978	<i>ilvB</i>	acetolactate synthase large subunit	2.0	2.8×10^{-14}
MW1979	n.a.	hypothetical protein, similar to acetolactate synthase small subunit	2.7	5.7×10^{-4}
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2×10^{-18}
MW1981	<i>leuA</i>	2-isopropylmalate synthase	2.5	3.5×10^{-22}
MW1982	<i>leuB</i>	3-isopropylmalate dehydrogenase	2.2	1.7×10^{-20}
MW1983	<i>leuC</i>	3-isopropylmalate dehydratase large subunit	2.6	1.2×10^{-28}
MW1985	<i>ilvA</i>	threonine dehydratase	3.0	4.9×10^{-45}
Cluster 16: Function Unknown; Average Fold Δ: -2.4 ± 0.7				
Genes	Symbol	Function	Fold Δ	P-value
MW1761	n.a.	hypothe. pr.; epidermin leader peptide processing serine protease epip precursor	- 1.6	3.0×10^{-8}
MW1762	n.a.	hypothe. protein; similar to lantibiotic epidermin biosynthesis protein EpiD	- 3.3	2.4×10^{-29}
MW1763	n.a.	hypothe. protein; similar to lantibiotic epidermin biosynthesis protein EpiC	- 2.7	1.2×10^{-26}
MW1764	n.a.	hypothe. protein; similar to lantibiotic epidermin biosynthesis protein EpiB	- 2.0	5.7×10^{-23}
Cluster 17: Glycolysis (Metabolism of Carbohydrates); Average Fold Δ: -2.4 ± 1.0				
Genes	Symbol	Function	Fold Δ	P-value
MW0733	<i>gapR</i>	glycolytic operon regulator	- 1.4	3.6×10^{-4}
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4×10^{-64}
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0×10^{-25}
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6×10^{-24}
Cluster 18: Function Unknown; Average Fold Δ: 2.3 ± 0.7				
Genes	Symbol	Function	Fold Δ	P-value
MW1214	n.a.	aspartate kinase homologue	2.8	6.0×10^{-48}
MW1215	<i>dhoM</i>	homoserine dehydrogenase	1.8	1.2×10^{-15}
Cluster 19: Function Unknown; Average Fold Δ: -2.2 ± 0.6				
Genes	Symbol	Function	Fold Δ	P-value
MW0957	<i>purD</i>	phosphoribosylamine--glycine ligase PurD	- 1.6	2.8×10^{-8}
MW0958	n.a.	conserved hypothetical protein	- 2.9	1.4×10^{-38}
MW0959	n.a.	hypothetical protein; similar to cation ABC transporter	- 2.1	5.0×10^{-19}
MW0960	n.a.	conserved hypothetical protein	- 1.9	7.1×10^{-16}
Cluster 20: <i>fhu</i> (Ferric Hydroxamate Transporters; Iron Acquisition); Average Fold Δ: 2.2 ± 0.2				
Genes	Symbol	Function	Fold Δ	P-value
MW0610	<i>fhuB</i>	ferric hydroxamate transport permease	2.3	7.0×10^{-36}
MW0611	<i>fhuG</i>	ferric hydroxamate transport permease	2.0	9.1×10^{-23}
Cluster 21: Function Unknown; Average Fold Δ: -2.2 ± 2.7				
Genes	Symbol	Function	Fold Δ	P-value
MW2279	n.a.	hypothetical protein; similar to membrane protein	- 1.0	9.4×10^{-1}
MW2280	n.a.	hypothetical protein; similar to ABC transporter, ATP-binding protein	- 4.3	6.9×10^{-99}
MW2281	n.a.	conserved hypothetical protein	- 4.5	1.4×10^{-84}
MW2282	n.a.	hypothetical protein; similar to two component response regulator	1.0	8.3×10^{-1}
Cluster 22: Function Unknown; Average Fold Δ: 2.1 ± 0.8				
Genes	Symbol	Function	Fold Δ	P-value
MW0141	n.a.	conserved hypothetical protein	3.9	7.2×10^{-40}
MW0142	<i>aldA</i>	aldehyde dehydrogenase homologue	1.9	3.9×10^{-9}
MW0143	n.a.	hypothetical protein; similar to cation-efflux system membrane protein CzcD	1.6	7.6×10^{-9}
MW0144	n.a.	hypothetical protein	1.8	7.3×10^{-6}
MW0145	n.a.	hypothetical protein; similar to alpha-helical coiled-coil protein SrpF	1.7	1.4×10^{-11}
MW0146	n.a.	hypothetical protein; similar to nitrate transporter	1.9	3.5×10^{-13}
MW0147	n.a.	hypothetical protein; similar to membrane lipoprotein SrpL	1.8	2.9×10^{-13}
Cluster 23: Function Unknown; Average Fold Δ: 2.1 ± 0.1				
Genes	Symbol	Function	Fold Δ	P-value

MW0164	n.a.	conserved hypothetical protein	2.2	2.5×10^{-25}
MW0166	n.a.	hypothetical protein; similar to sucrose phosphotransferase enzyme II	2.0	1.3×10^{-16}
Cluster 24: Function Unknown; Average Fold Δ: 2.1 ± 0.6				
Genes	Symbol	Function	Fold Δ	P-value
MW0151	<i>fdh</i>	NAD-dependent formate dehydrogenase	2.5	1.4×10^{-22}
MW0152	n.a.	hypothetical protein, similar to integral membrane protein LmrP	1.6	3.0×10^{-10}
Cluster 25: <i>ure</i> Operon (Encodes for Urease); Average Fold Δ: -2.0 ± 0.1				
Genes	Symbol	Function	Fold Δ	P-value
MW2208	<i>ureC</i>	urease alpha subunit	- 2.2	2.1×10^{-32}
MW2210	<i>ureF</i>	urease accessory protein UreF	- 2.0	1.8×10^{-18}
MW2211	<i>ureG</i>	urease accessory protein UreG	- 1.9	1.7×10^{-18}
Cluster 26: Function Unknown; Average Fold Δ: -2.0 ± 0.8				
Genes	Symbol	Function	Fold Δ	P-value
MW0015	<i>rplI</i>	50S ribosomal protein L9	- 2.3	9.4×10^{-27}
MW0016	<i>dnaC</i>	replicative DNA helicase	- 2.4	5.2×10^{-20}
MW0017	<i>purA</i>	adenylosuccinate synthase	- 1.1	3.9×10^{-1}
Cluster 27: Function Unknown; Average Fold Δ: -1.9 ± 0.3				
Genes	Symbol	Function	Fold Δ	P-value
MW0019	<i>vicK</i>	two-component sensor histidine kinase	- 1.6	2.5×10^{-09}
MW0020	n.a.	conserved hypothetical protein	- 2.0	1.3×10^{-15}
MW0021	n.a.	conserved hypothetical protein	- 2.1	8.2×10^{-17}
Cluster 28: Function Unknown; Average Fold Δ: -1.9 ± 0.3				
Genes	Symbol	Function	Fold Δ	P-value
MW2229	n.a.	formate dehydrogenase homologue	- 1.5	7.3×10^{-7}
MW2230	n.a.	hypothetical protein; similar to <i>lyt</i> divergon expression attenuator LytR	- 2.2	2.7×10^{-18}
MW2231	n.a.	hypothetical protein; similar to suppressor protein <i>subB</i>	- 1.9	1.7×10^{-13}
Cluster 29: Quinol Oxidase Polypeptides (Oxidative Phosphorylation); Average Fold Δ: -1.9 ± 0.3				
Genes	Symbol	Function	Fold Δ	P-value
MW0941	n.a.	hypothetical protein; similar to quinol oxidase polypeptide IV QoxD	- 1.8	1.9×10^{-10}
MW0942	<i>qoxC</i>	Quinol oxidase polypeptide III QoxC	- 1.5	7.4×10^{-06}
MW0943	<i>qoxB</i>	Quinol oxidase polypeptide I QoxB	- 2.1	3.9×10^{-16}
MW0944	n.a.	hypothetical protein; similar to quinol oxidase polypeptide II QoxA	- 2.2	4.4×10^{-23}
Cluster 30: Function Unknown; Average Fold Δ: -1.8 ± 0.7				
Genes	Symbol	Function	Fold Δ	P-value
MW1150	n.a.	conserved hypothetical protein	- 1.4	2.2×10^{-5}
MW1151	n.a.	hypothetical protein; similar to ribosomal protein L7AE family	- 1.4	5.7×10^{-7}
MW1152	<i>infB</i>	translation initiation factor IF-2	- 2.8	1.2×10^{-32}
MW1153	<i>rbfA</i>	ribosome-binding factor A (P15B protein)	- 1.6	5.0×10^{-9}
Cluster 31: <i>moe</i> (Molybdopterin Biosynthesis); Average Fold Δ: -1.8 ± 0.4				
Genes	Symbol	Function	Fold Δ	P-value
MW2191	<i>moeA</i>	molybdopterin biosynthesis protein <i>moeA</i>	- 2.1	9.1×10^{-21}
MW2192	<i>moaC</i>	molybdenum cofactor biosynthesis protein C	- 1.3	7.5×10^{-5}
MW2193	<i>moaB</i>	molybdopterin precursor biosynthesis <i>moaB</i>	- 1.8	5.6×10^{-19}
MW2194	<i>moeB</i>	molybdopterin biosynthesis protein <i>moeB</i>	- 1.4	3.3×10^{-6}
MW2195	<i>modC</i>	molybdenum transport ATP-binding protein ModC	- 2.2	2.5×10^{-15}
Cluster 32: Ribosomal Proteins; Average Fold Δ: -1.7 ± 0.2				
Genes	Symbol	Function	Fold Δ	P-value
MW2142	<i>rplQ</i>	50S ribosomal protein L17 (BL15) (BL21)	- 1.8	5.4×10^{-15}
MW2143	<i>rpoA</i>	DNA-directed RNA polymerase alpha chain	- 1.8	2.4×10^{-15}
MW2144	<i>rpsK</i>	30S ribosomal protein S11 (BS11)	- 1.9	2.2×10^{-17}
MW2145	<i>rpsM</i>	30S ribosomal protein S13	- 1.9	3.6×10^{-17}
MW2148	<i>adk</i>	adenylate kinase	- 1.6	3.7×10^{-9}
MW2149	<i>secY</i>	preprotein translocase SecY subunit	- 1.7	1.9×10^{-13}
MW2150	<i>rplO</i>	50S ribosomal protein L15	- 1.5	1.3×10^{-7}

MW2151	<i>rpmD</i>	50S ribosomal protein L30	- 1.3	3.2 x 10 ⁻⁴
MW2152	<i>rpsE</i>	30S ribosomal protein S5 (BS5)	- 1.6	1.4 x 10 ⁻⁸
MW2153	<i>rplR</i>	50S ribosomal protein L18	- 1.7	5.9 x 10 ⁻¹³
MW2154	<i>rplF</i>	50S ribosomal protein L6	- 1.9	4.8 x 10 ⁻¹⁶
MW2155	<i>rpsH</i>	30S ribosomal protein S8 (BS8)	- 1.8	2.4 x 10 ⁻¹⁴
MW2157	<i>rplE</i>	50S ribosomal protein L5 (BL6)	- 2.0	1.1 x 10 ⁻¹⁹
MW2158	<i>rplX</i>	50S ribosomal protein L24	- 1.8	1.1 x 10 ⁻¹⁶
MW2159	<i>rplN</i>	50S ribosomal protein L14	- 2.0	4.3 x 10 ⁻¹⁹
MW2160	<i>rpsQ</i>	30S ribosomal protein S17	- 1.9	9.1 x 10 ⁻¹⁹
MW2161	<i>rpmC</i>	50S ribosomal protein L29	- 1.7	2.4 x 10 ⁻¹⁴
MW2162	<i>rplP</i>	50S ribosomal protein L16	- 1.8	4.7 x 10 ⁻¹⁴
MW2163	<i>rpsC</i>	30S ribosomal protein S3	- 1.7	2.0 x 10 ⁻¹²
MW2164	<i>rplV</i>	50S ribosomal protein L22	- 1.8	3.8 x 10 ⁻¹⁵
MW2165	<i>rpsS</i>	30S ribosomal protein S19	- 1.8	4.9 x 10 ⁻¹³
MW2167	<i>rplW</i>	50S ribosomal protein L23	- 1.9	7.8 x 10 ⁻¹⁶
MW2168	<i>rplD</i>	50S ribosomal protein L4	- 1.7	7.2 x 10 ⁻¹³
MW2169	<i>rplC</i>	50S ribosomal protein L3 (BL3)	- 1.5	8.4 x 10 ⁻⁹
MW2170	<i>rpsJ</i>	30S ribosomal protein S10	- 1.3	2.0 x 10 ⁻³

Cluster 33: Function Unknown; Average Fold Δ: -1.7 ± 0.3

Genes	Symbol	Function	Fold Δ	P-value
MW0746	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 1.2	1.4 x 10 ⁻¹
MW0747	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 2.1	2.1 x 10 ⁻¹²
MW0749	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 2.3	9.1 x 10 ⁻¹²
MW0750	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 1.9	1.7 x 10 ⁻¹⁰
MW0751	n.a.	putative primase [Genomic island nu Sa alpha3mw]	- 1.5	1.1 x 10 ⁻⁵
MW0752	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 1.4	6.9 x 10 ⁻⁴
MW0753	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 1.7	1.8 x 10 ⁻⁵
MW0754	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 1.8	3.5 x 10 ⁻¹⁰
MW0755	n.a.	hypothetical protein [Genomic island nu Sa alpha3mw]	- 1.8	2.1 x 10 ⁻⁸

Cluster 34: *bfm* (Catalyzes Branched Chain Fatty Acid Production); Average Fold Δ: -1.7 ± 0.1

Genes	Symbol	Function	Fold Δ	P-value
MW1469	<i>bfmBAB</i>	branched-chain alpha-keto acid dehydrogenase E1	- 1.8	9.5 x 10 ⁻¹¹
MW1470	<i>bfmBAA</i>	branched-chain alpha-keto acid dehydrogenase E1	- 1.6	8.9 x 10 ⁻⁸

Cluster 35: Function Unknown; Average Fold Δ: -1.7 ± 0.2

Genes	Symbol	Function	Fold Δ	P-value
MW2111	n.a.	glycine betaine transporter opuD homologue	- 1.5	9.2 x 10 ⁻⁷
MW2112	n.a.	hypothetical protein; similar to alginate lyase	- 1.9	1.8 x 10 ⁻¹⁸

Cluster 36: Uroporphyrinogen III Biosynthesis (Early Heme Biosynthesis); Average Fold Δ: -1.4 ± 1.6

Genes	Symbol	Function	Fold Δ	P-value
MW1609	n.a.	conserved hypothetical protein	1.2	8.1 x 10 ⁻³
MW1611	<i>hemL</i>	glutamate-1-semialdehyde 2,1-aminomutase	- 2.6	7.1 x 10 ⁻²³
MW1612	<i>hemB</i>	delta-aminolevulinic acid dehydratase	- 2.5	2.5 x 10 ⁻²²
MW1613	<i>hemD</i>	uroporphyrinogen III synthase	- 2.1	5.2 x 10 ⁻²²
MW1614	<i>hemC</i>	porphobilinogen deaminase	- 1.1	4.2 x 10 ⁻¹

Cluster 37: Function Unknown; Average Fold Δ: -1.3 ± 0.2

Genes	Symbol	Function	Fold Δ	P-value
MW2440	n.a.	hypothetical protein; similar to transcription regulator MarR family	- 1.5	3.8 x 10 ⁻⁷
MW2441	n.a.	conserved hypothetical protein	- 1.2	4.5 x 10 ⁻²
MW2442	n.a.	conserved hypothetical protein	- 1.2	2.0 x 10 ⁻²

Notes: Significant genes were inserted into WoPPER analysis web tool (<https://wopper.ba.itb.cnr.it/Articles>) with the corresponding log-2 fold change to outcome the gene clusters. All corresponding gene symbols and gene names are retrieved from DOGAN which is a genome database of microorganisms sequenced at NITE (<http://www.bio.nite.go.jp/dogan/top>).

Supporting Table 8: KEGG pathway analysis from **HP-14** (20 hours at 0.625 μ M) treatment of MRSA-1707 biofilms. Gene transcripts that are activated or inhibited (≥ 2 -fold change in transcription of gene products) in MRSA-1707 biofilms by **HP-14** in various pathways are indicated in this analysis.

sam01100			Metabolic Pathways	
Gene	Symbol	Function	Fold Δ	P-value
MW0096	<i>sbnH</i>	putative diaminopimelate decarboxylase	6.2	4.1×10^{-102}
MW0151	<i>fdh</i>	NAD-dependent formate dehydrogenase	2.5	1.4×10^{-22}
MW0306	n.a.	hypothetical protein; similar to transport protein SgaT	2.4	9.8×10^{-32}
MW0308	n.a.	hypothetical protein; similar to PTS fructose-specific enzyme IIBC component	2.1	4.6×10^{-11}
MW0426	<i>gltB</i>	glutamate synthase large subunit	2.3	3.3×10^{-23}
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0×10^{-36}
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4×10^{-64}
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0×10^{-25}
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6×10^{-24}
MW0943	<i>qoxB</i>	Quinol oxidase polypeptide I QoxB	- 2.1	3.9×10^{-16}
MW0944	n.a.	hypothetical protein; similar to quinol oxidase polypeptide II QoxA	- 2.2	4.4×10^{-23}
MW1051	n.a.	hypothetical protein; similar to carbamate kinase	2.0	1.2×10^{-16}
MW1214	n.a.	aspartate kinase homologue	2.8	6.0×10^{-48}
MW1217	<i>thrB</i>	homoserine kinase homologue	2.1	7.6×10^{-27}
MW1250	n.a.	4-oxalocrotonate tautomerase	- 2.4	3.3×10^{-21}
MW1468	<i>bmfBB</i>	branched-chain alpha-keto acid dehydrogenase E2	- 2.5	1.8×10^{-21}
MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase, accB homologue	2.1	5.9×10^{-8}
MW1611	<i>hemL</i>	glutamate-1-semialdehyde 2,1-aminomutase	- 2.6	7.1×10^{-23}
MW1612	<i>hemB</i>	delta-aminolevulinic acid dehydratase	- 2.5	2.5×10^{-22}
MW1613	<i>hemD</i>	uroporphyrinogen III synthase	- 2.3	5.2×10^{-22}
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3×10^{-43}
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7×10^{-30}
MW1680	n.a.	chorismate mutase homologue	- 2.4	7.9×10^{-23}
MW1707	n.a.	proline dehydrogenase homologue	2.2	6.0×10^{-25}
MW1977	<i>ilvD</i>	dihydroxy-acid dehydratase	2.1	7.5×10^{-12}
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7×10^{-4}
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2×10^{-18}
MW1981	<i>leuA</i>	2-isopropylmalate synthase	2.5	3.5×10^{-22}
MW1982	<i>leuB</i>	3-isopropylmalate dehydrogenase	2.2	1.7×10^{-20}
MW1983	<i>leuC</i>	3-isopropylmalate dehydratase large subunit	2.6	1.2×10^{-28}
MW1985	<i>ilvA</i>	threonine dehydratase	3.0	4.9×10^{-45}
MW2191	<i>moeA</i>	molybdopterin biosynthesis protein moeA	- 2.1	9.1×10^{-21}
MW2208	<i>ureC</i>	urease alpha subunit	- 2.2	2.1×10^{-32}
MW2475	<i>rocA</i>	1-pyrroline-5-carboxylate dehydrogenase	2.1	5.0×10^{-19}
MW2553	<i>arcC</i>	carbamate kinase	- 2.9	1.5×10^{-17}
MW2555	<i>arcB</i>	ornithine transcarbamoylase	- 2.1	7.5×10^{-13}
MW2562	n.a.	fructose phosphotransferase system enzyme fruA homologue	3.7	6.3×10^{-53}
MW2563	<i>pmi</i>	mannose-6-phosphate isomerase	2.0	6.0×10^{-18}
sam02010			ABC Transporter	
Gene	Symbol	Function	Fold Δ	P-value
MW0087	<i>sirB</i>	iron-regulated ABC transporter siderophore permease protein SirB	4.3	1.8×10^{-43}
MW0088	<i>sirA</i>	iron-regulated ABC transporter siderophore-binding protein SirA	8.6	6.3×10^{-156}
MW0191	n.a.	maltose/maltodextrin transport permease homologue	4.8	1.2×10^{-76}
MW0192	n.a.	maltose/maltodextrin transport permease homologue	3.4	1.5×10^{-52}
MW0594	n.a.	conserved hypothetical protein	2.0	1.7×10^{-19}
MW0595	n.a.	hypothetical protein; similar to ABC transporter ATP-binding protein	3.8	6.3×10^{-48}
MW0609	<i>fhuC</i>	ferric hydroxamate transport ATP-binding protein	2.6	3.9×10^{-37}
MW0610	<i>fhuB</i>	ferric hydroxamate transport permease	2.3	7.0×10^{-36}
MW0695	n.a.	hypothetical protein; similar to ferrichrome ABC transporter permease	3.7	8.5×10^{-45}
MW0696	n.a.	hypothetical protein; similar to ferrichrome ABC transporter permease	3.4	8.3×10^{-33}
MW0697	n.a.	hypothetical pr.; similar to ferrichrome ABC transporter ATP-binding protein	2.8	2.5×10^{-27}
MW0698	n.a.	lipoprotein, similar to ferrichrome ABC transporter	2.3	9.1×10^{-20}
MW0869	n.a.	hypothetical pr.; similar, oligopeptide transport syst. permease protein OppC	2.3	4.9×10^{-13}
MW0870	<i>oppD</i>	oligopeptide transport system ATP-binding protein OppD homologue	3.3	4.9×10^{-36}
MW0871	<i>oppF</i>	oligopeptide transport system ATP-binding protein OppF homologue	3.6	3.1×10^{-45}

MW0872	n.a.	hypothetical protein; similar to peptide binding protein OppA	3.0	5.2×10^{-37}
MW0958	n.a.	conserved hypothetical protein	- 2.9	1.4×10^{-38}
MW0959	n.a.	hypothetical protein; similar to cation ABC transporter	- 2.1	5.0×10^{-19}
MW1015	<i>isdE</i>	hypothetical protein; similar to ferrichrome ABC transporter	12.1	2.0×10^{-123}
MW1016	<i>isdF</i>	hypothetical protein; similar to ferrichrome ABC transporter	10.5	1.4×10^{-120}
MW1945	<i>fhuD1</i>	ferric hydroxamate siderophore binding lipoprotein	5.0	2.6×10^{-58}
MW2102	n.a.	hypothetical protein; similar to ferrichrome ABC transporter (permease)	3.8	9.3×10^{-54}
MW2103	n.a.	hypothetical protein; similar to ferrichrome ABC transporter (binding protein)	3.9	1.0×10^{-67}
MW2195	<i>modC</i>	molybdenum transport ATP-binding protein ModC	- 2.2	2.5×10^{-15}
MW2196	<i>modB</i>	probable molybdenum transport permease	- 2.3	7.2×10^{-19}
MW2202	<i>fhuD2</i>	ferric hydroxamate siderophore binding lipoprotein	2.5	5.3×10^{-31}
MW2280	n.a.	hypothetical protein; similar to ABC transporter, ATP-binding protein	- 4.3	6.9×10^{-99}
MW2281	n.a.	conserved hypothetical protein	- 4.5	1.4×10^{-84}
MW2387	<i>opp-1F</i>	oligopeptide transporter putative ATPase domain	5.5	3.9×10^{-101}
MW2388	<i>opp-1D</i>	oligopeptide transporter putative ATPase domain	7.5	3.6×10^{-119}
MW2389	<i>opp-1C</i>	oligopeptide transporter putative membrane permease domain	7.8	8.1×10^{-126}
MW2390	<i>opp-1B</i>	oligopeptide transporter putative membrane permease domain	3.8	8.0×10^{-78}
MW2391	<i>opp-1A</i>	oligopeptide transporter putative substrate binding domain	4.6	9.7×10^{-86}

sam01110 Biosynthesis of Secondary Metabolites

Gene	Symbol	Function	Fold Δ	P-value
MW0096	<i>sbnH</i>	putative diaminopimelate decarboxylase	6.2	4.1×10^{-102}
MW0426	<i>gltB</i>	glutamate synthase large subunit	2.3	3.3×10^{-23}
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0×10^{-36}
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4×10^{-64}
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0×10^{-25}
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6×10^{-24}
MW1018	<i>isdG</i>	conserved hypothetical protein	10.4	4.0×10^{-64}
MW1214	n.a.	aspartate kinase homologue	2.8	6.0×10^{-48}
MW1217	<i>thrB</i>	homoserine kinase homologue	2.1	7.6×10^{-27}
MW1468	<i>bmfBB</i>	branched-chain alpha-keto acid dehydrogenase E2	- 2.5	1.8×10^{-21}
MW1557	n.a.	hypothetical protein, similar to acetyl-CoA carboxylase	2.1	5.9×10^{-8}
MW1611	<i>hemL</i>	glutamate-1-semialdehyde 2,1-aminomutase	- 2.6	7.1×10^{-23}
MW1612	<i>hemB</i>	delta-aminolevulinic acid hydratase	- 2.5	2.5×10^{-22}
MW1613	<i>hemD</i>	uroporphyrinogen III synthase	- 2.3	5.2×10^{-22}
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3×10^{-43}
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7×10^{-30}
MW1680	n.a.	chorismate mutase homologue	- 2.4	7.9×10^{-23}
MW1707	n.a.	proline dehydrogenase homologue	2.2	6.0×10^{-25}
MW1977	<i>ilvD</i>	dihydroxy-acid hydratase	2.1	7.5×10^{-12}
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7×10^{-4}
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2×10^{-18}
MW1981	<i>leuA</i>	2-isopropylmalate synthase	2.5	3.5×10^{-22}
MW1982	<i>leuB</i>	3-isopropylmalate dehydrogenase	2.2	1.7×10^{-20}
MW1983	<i>leuC</i>	3-isopropylmalate hydratase large subunit	2.6	1.2×10^{-28}
MW1985	<i>ilvA</i>	threonine hydratase	3.0	4.9×10^{-45}
MW2555	<i>arcB</i>	ornithine transcarbamoylase	- 2.0	7.5×10^{-13}
MW2563	<i>pmi</i>	mannose-6-phosphate isomerase	2.0	6.0×10^{-18}

sam01120 Microbial Metabolism in Diverse Environments

Gene	Symbol	Function	Fold Δ	P-value
MW0096	<i>sbnH</i>	putative diaminopimelate decarboxylase	6.2	4.1×10^{-102}
MW0151	<i>fdh</i>	NAD-dependent formate dehydrogenase	2.5	1.4×10^{-22}
MW0306	n.a.	hypothetical protein; similar to transport protein SgaT	2.4	9.8×10^{-32}
MW0308	n.a.	hypothetical protein; similar to PTS fructose-specific enzyme IIBC component	2.1	4.6×10^{-11}
MW0426	<i>gltB</i>	glutamate synthase large subunit	2.3	3.3×10^{-23}
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0×10^{-36}
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4×10^{-64}
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0×10^{-25}
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6×10^{-24}
MW1051	n.a.	hypothetical protein, similar to carbamate kinase	2.0	1.2×10^{-16}
MW1214	n.a.	aspartate kinase homologue	2.8	6.0×10^{-48}
MW1217	<i>thrB</i>	homoserine kinase homologue	2.1	7.6×10^{-27}
MW1250	n.a.	4-oxalocrotonate tautomerase	- 2.4	3.3×10^{-21}

MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase	2.1	5.9 x 10 ⁻⁸
MW1611	<i>hemL</i>	glutamate-1-semialdehyde 2,1-aminomutase	- 2.6	7.1 x 10 ⁻²³
MW1612	<i>hemB</i>	delta-aminolevulinic acid dehydratase	- 2.5	2.5 x 10 ⁻²²
MW1613	<i>hemD</i>	uroporphyrinogen III synthase	- 2.3	5.2 x 10 ⁻²²
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰
MW2208	<i>ureC</i>	urease alpha subunit	- 2.2	2.1 x 10 ⁻³²
MW2444	<i>ddh</i>	D-specific D-2-hydroxyacid dehydrogenase	- 2.3	1.3 x 10 ⁻²⁷
MW2553	<i>arcC</i>	carbamate kinase	- 2.9	1.5 x 10 ⁻¹⁷
MW2562	n.a.	fructose phosphotransferase system enzyme fruA homologue	3.7	6.3 x 10 ⁻⁵³
sam01130 Biosynthesis of Antibiotics				
Gene	Symbol	Function	Fold Δ	P-value
MW0096	<i>sbnH</i>	putative diaminopimelate decarboxylase	6.2	4.1 x 10 ⁻¹⁰²
MW0426	<i>gltB</i>	glutamate synthase large subunit	2.3	3.3 x 10 ⁻²³
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0 x 10 ⁻³⁶
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4 x 10 ⁻⁶⁴
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0 x 10 ⁻²⁵
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6 x 10 ⁻²⁴
MW1214	n.a.	aspartate kinase homologue	2.8	6.0 x 10 ⁻⁴⁸
MW1468	<i>bmfBB</i>	branched-chain alpha-keto acid dehydrogenase E2	- 2.5	1.8 x 10 ⁻²¹
MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase	2.1	5.9 x 10 ⁻⁸
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰
MW1680	n.a.	chorismate mutase homologue	- 2.4	7.9 x 10 ⁻²³
MW1707	n.a.	proline dehydrogenase homologue	2.2	6.0 x 10 ⁻²⁵
MW1977	<i>ilvD</i>	dihydroxy-acid dehydratase	2.1	7.5 x 10 ⁻¹²
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7 x 10 ⁻⁴
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2 x 10 ⁻¹⁸
MW1985	<i>ilvA</i>	threonine dehydratase	3.0	4.9 x 10 ⁻⁴⁵
MW2555	<i>arcB</i>	ornithine transcarbamoylase	- 2.1	7.5 x 10 ⁻¹³
MW2563	<i>pmi</i>	mannose-6-phosphate isomerase	2.0	6.0 x 10 ⁻¹⁸
sam01230 Biosynthesis of Amino Acids				
Gene	Symbol	Function	Fold Δ	P-value
MW0096	<i>sbnH</i>	putative diaminopimelate decarboxylase	6.2	4.1 x 10 ⁻¹⁰²
MW0426	<i>gltB</i>	glutamate synthase large subunit	2.3	3.3 x 10 ⁻²³
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0 x 10 ⁻³⁶
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4 x 10 ⁻⁶⁴
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0 x 10 ⁻²⁵
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6 x 10 ⁻²⁴
MW1214	n.a.	aspartate kinase homologue	2.8	6.0 x 10 ⁻⁴⁸
MW1217	<i>thrB</i>	homoserine kinase homologue	2.1	7.6 x 10 ⁻²⁷
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰
MW1680	n.a.	chorismate mutase homologue	- 2.4	7.9 x 10 ⁻²³
MW1977	<i>ilvD</i>	dihydroxy-acid dehydratase	2.1	7.5 x 10 ⁻¹²
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7 x 10 ⁻⁴
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2 x 10 ⁻¹⁸
MW1981	<i>leuA</i>	2-isopropylmalate synthase	2.5	3.5 x 10 ⁻²²
MW1982	<i>leuB</i>	3-isopropylmalate dehydrogenase	2.2	1.7 x 10 ⁻²⁰
MW1983	<i>leuC</i>	3-isopropylmalate dehydratase large subunit	2.6	1.2 x 10 ⁻²⁸
MW1985	<i>ilvA</i>	threonine dehydratase	3.0	4.9 x 10 ⁻⁴⁵
MW2555	<i>arcB</i>	ornithine transcarbamoylase	- 2.1	7.5 x 10 ⁻¹³
sam01200 Carbon Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0151	<i>fdh</i>	NAD-dependent formate dehydrogenase	2.5	1.4 x 10 ⁻²²
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4 x 10 ⁻⁶⁴
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0 x 10 ⁻²⁵
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6 x 10 ⁻²⁴
MW1051	n.a.	hypothetical protein; similar to carbamate kinase	2.0	1.2 x 10 ⁻¹⁶
MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase	2.1	5.9 x 10 ⁻⁸
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³

MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰
MW1985	<i>ilvA</i>	threonine dehydratase	3.0	4.9 x 10 ⁻⁴⁵
MW2553	<i>arcC</i>	carbamate kinase	-2.9	1.5 x 10 ⁻¹⁷
sam02024 Quorum Sensing				
Gene	Symbol	Function	Fold Δ	P-value
MW0869	n.a.	hypothetical pr.; similar, oligopeptide transport syst. permease protein OppC	2.3	4.9 x 10 ⁻¹³
MW0870	<i>oppD</i>	oligopeptide transport system ATP-binding protein OppD homologue	3.3	4.9 x 10 ⁻³⁶
MW0871	<i>oppF</i>	oligopeptide transport system ATP-binding protein OppF homologue	3.6	3.1 x 10 ⁻⁴⁵
MW0872	n.a.	hypothetical protein; similar to peptide binding protein OppA	3.0	5.2 x 10 ⁻³⁷
MW1752	<i>splF</i>	serine protease SplF	7.4	3.4 x 10 ⁻¹³¹
MW1763	<i>bsaC</i>	hypothetical protein; similar to lantibiotic epidermin biosynthesis protein EpiC	- 2.7	1.2 x 10 ⁻²⁶
MW1764	<i>bsaB</i>	hypothetical protein; similar to lantibiotic epidermin biosynthesis protein EpiB	- 2.0	5.7 x 10 ⁻²³
MW1963	<i>agrA</i>	accessory gene regulator A	- 2.1	7.3 x 10 ⁻¹³
sam01210 2-Oxocarboxylic Acid Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1214	n.a.	aspartate kinase homologue	2.8	6.0 x 10 ⁻⁴⁸
MW1977	<i>ilvD</i>	dihydroxy-acid dehydratase	2.1	7.5 x 10 ⁻¹²
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7 x 10 ⁻⁴
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2 x 10 ⁻¹⁸
MW1981	<i>leuA</i>	2-isopropylmalate synthase	2.5	3.5 x 10 ⁻²²
MW1982	<i>leuB</i>	3-isopropylmalate dehydrogenase	2.2	1.7 x 10 ⁻²⁰
MW1983	<i>leuC</i>	3-isopropylmalate dehydratase large subunit	2.6	1.2 x 10 ⁻²⁸
sam00290 Valine, Leucine & Isoleucine Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW1977	<i>ilvD</i>	dihydroxy-acid dehydratase	2.1	7.5 x 10 ⁻¹²
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7 x 10 ⁻⁴
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2 x 10 ⁻¹⁸
MW1981	<i>leuA</i>	2-isopropylmalate synthase	2.5	3.5 x 10 ⁻²²
MW1982	<i>leuB</i>	3-isopropylmalate dehydrogenase	2.2	1.7 x 10 ⁻²⁰
MW1983	<i>leuC</i>	3-isopropylmalate dehydratase large subunit	2.6	1.2 x 10 ⁻²⁸
MW1985	<i>ilvA</i>	threonine dehydratase	3.0	4.9 x 10 ⁻⁴⁵
sam02060 Phosphotransferase System (PTS)				
Gene	Symbol	Function	Fold Δ	P-value
MW0166	n.a.	hypothetical protein; similar to sucrose phosphotransferase enzyme II	2.0	1.3 x 10 ⁻¹⁶
MW0306	n.a.	hypothetical protein; similar to transport protein SgaT	2.4	9.8 x 10 ⁻³²
MW0308	n.a.	hypothetical protein; similar to PTS fructose-specific enzyme IIBC component	2.1	4.6 x 10 ⁻¹¹
MW0428	<i>treP</i>	PTS enzyme II	2.1	1.1 x 10 ⁻²¹
MW2562	n.a.	fructose phosphotransferase system enzyme fruA homologue	3.7	6.3 x 10 ⁻⁵³
sam00010 Glycolysis / Gluconeogenesis				
Gene	Symbol	Function	Fold Δ	P-value
MW0734	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase	- 3.7	8.4 x 10 ⁻⁶⁴
MW0735	<i>pgk</i>	phosphoglycerate kinase	- 2.2	5.0 x 10 ⁻²⁵
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6 x 10 ⁻²⁴
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰
sam00230 Purine Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0113	<i>drm</i>	phosphopentomutase	- 2.2	3.3 x 10 ⁻²⁰
MW1051	n.a.	hypothetical protein; similar to carbamate kinase	2.0	1.2 x 10 ⁻¹⁶
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³
MW2208	<i>ureC</i>	urease alpha subunit	- 2.2	2.1 x 10 ⁻³²
MW2553	<i>arcC</i>	carbamate kinase	- 2.9	1.5 x 10 ⁻¹⁷
sam00860 Porphyrin & Chlorophyll Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1018	<i>isdG</i>	conserved hypothetical protein	10.4	4.0 x 10 ⁻⁶⁴
MW1611	<i>hemL</i>	glutamate-1-semialdehyde 2,1-aminomutase	- 2.6	7.1 x 10 ⁻²³
MW1612	<i>hemB</i>	delta-aminolevulinic acid dehydratase	- 2.5	2.5 x 10 ⁻²²
MW1613	<i>hemD</i>	uroporphyrinogen III synthase	- 2.3	5.2 x 10 ⁻²²
sam02020 Two-Component System				
Gene	Symbol	Function	Fold Δ	P-value
MW0198	n.a.	hypothetical protein; similar to two-component response regulator	- 2.2	1.6 x 10 ⁻²⁰

MW1763	<i>bsaC</i>	hypothetical protein; similar to lantibiotic epidermin biosynthesis protein EpiC	- 2.7	1.2 x 10 ⁻²⁶
MW1764	<i>bsaB</i>	hypothetical protein; similar to lantibiotic epidermin biosynthesis protein EpiB	- 2.0	5.7 x 10 ⁻²³
MW1963	<i>agrA</i>	accessory gene regulator A	- 2.1	7.3 x 10 ⁻¹³
sam00620 Pyruvate Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase	2.1	5.9 x 10 ⁻⁸
MW1641	<i>pykA</i>	pyruvate kinase	- 2.8	6.3 x 10 ⁻⁴³
MW1981	<i>leuA</i>	2-isopropylmalate synthase	2.5	3.5 x 10 ⁻²²
MW2444	<i>ddh</i>	D-specific D-2-hydroxyacid dehydrogenase	- 2.3	1.3 x 10 ⁻²⁷
sam00910 Nitrogen Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0426	<i>gltB</i>	glutamate synthase large subunit	2.3	3.3 x 10 ⁻²³
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0 x 10 ⁻³⁶
MW1051	n.a.	hypothetical protein; similar to carbamate kinase	2.0	1.2 x 10 ⁻¹⁶
MW2553	<i>arcC</i>	carbamate kinase	-2.9	1.5 x 10 ⁻¹⁷
sam00220 Arginine Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW1051	n.a.	hypothetical protein; similar to carbamate kinase	2.0	1.2 x 10 ⁻¹⁶
MW2208	<i>ureC</i>	urease alpha subunit	- 2.2	2.1 x 10 ⁻³²
MW2553	<i>arcC</i>	carbamate kinase	- 2.9	1.5 x 10 ⁻¹⁷
MW2555	<i>arcB</i>	ornithine transcarbamoylase	- 2.1	7.5 x 10 ⁻¹³
sam01501 Beta-Lactam Resistance				
Gene	Symbol	Function	Fold Δ	P-value
MW0869	n.a.	hypothetical pr.; similar, oligopeptide transport syst. permease protein OppC	2.3	4.9 x 10 ⁻¹³
MW0870	<i>oppD</i>	oligopeptide transport system ATP-binding protein OppD homologue	3.3	4.9 x 10 ⁻³⁶
MW0871	<i>oppF</i>	oligopeptide transport system ATP-binding protein OppF homologue	3.6	3.1 x 10 ⁻⁴⁵
MW0872	n.a.	hypothetical protein; similar to peptide binding protein OppA	3.0	5.2 x 10 ⁻³⁷
sam00260 Glycine, Serine & Threonine Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6 x 10 ⁻²⁴
MW1214	n.a.	aspartate kinase homologue	2.8	6.0 x 10 ⁻⁴⁸
MW1217	<i>thrB</i>	homoserine kinase homologue	2.1	7.6 x 10 ⁻²⁷
MW1985	<i>ilvA</i>	threonine dehydratase	3.0	4.9 x 10 ⁻⁴⁵
sam00660 C5-Branched Dibasic Acid Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7 x 10 ⁻⁴
MW1982	<i>leuB</i>	3-isopropylmalate dehydrogenase	2.2	1.7 x 10 ⁻²⁰
MW1983	<i>leuC</i>	3-isopropylmalate dehydratase large subunit	2.6	1.2 x 10 ⁻²⁸
sam00680 Methane Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0151	<i>fdh</i>	NAD-dependent formate dehydrogenase	2.5	1.4 x 10 ⁻²²
MW0737	<i>pgm</i>	2,3-diphosphoglycerate-independent phosphoglycerate mutase	- 2.2	3.6 x 10 ⁻²⁴
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰
sam00250 Alanine, Aspartate & Glutamate Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0426	<i>gltB</i>	glutamate synthase large subunit	2.3	3.3 x 10 ⁻²³
MW0427	<i>gltD</i>	NADH-glutamate synthase small subunit	2.6	4.0 x 10 ⁻³⁶
MW2475	<i>rocA</i>	1-pyrroline-5-carboxylate dehydrogenase	2.1	5.0 x 10 ⁻¹⁹
sam05150 Staphylococcus aureus Infection				
Gene	Symbol	Function	Fold Δ	P-value
MW0759	<i>sec4</i>	enterotoxin type C precursor	2.0	1.3 x 10 ⁻¹⁶
MW1012	<i>isdA</i>	cell surface protein	10.4	2.3 x 10 ⁻¹²¹
MW1768	<i>lukE</i>	leukotoxin Luke	2.2	4.9 x 10 ⁻¹⁰
sam00770 Pantothenate & CoA Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW1977	<i>ilvD</i>	dihydroxy-acid dehydratase	2.1	7.5 x 10 ⁻¹²
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7 x 10 ⁻⁴
MW1980	<i>ilvC</i>	alpha-keto-beta-hydroxyacyl reductoisomerase	2.4	1.2 x 10 ⁻¹⁸
sam00051 Fructose & Mannose Metabolism				

Gene	Symbol	Function	Fold Δ	P-value
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7×10^{-30}
MW2562	n.a.	fructose phosphotransferase system enzyme fruA homologue	3.7	6.3×10^{-53}
MW2563	<i>pmi</i>	mannose-6-phosphate isomerase	2.0	6.0×10^{-18}
sam00500 Starch & Sucrose Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0166	n.a.	hypothetical protein; similar to sucrose phosphotransferase enzyme II	2.0	1.3×10^{-16}
MW0428	<i>treP</i>	PTS enzyme II	2.1	1.1×10^{-21}
MW0429	n.a.	alpha-glucosidase	2.7	5.2×10^{-34}
sam00520 Amino Sugar & Nucleotide Sugar Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0292	<i>nanA</i>	N-acetylneuraminase lyase subunit	2.7	3.4×10^{-29}
MW2563	<i>pmi</i>	mannose-6-phosphate isomerase	2.0	6.0×10^{-18}
sam00030 Pentose Phosphate Pathway				
Gene	Symbol	Function	Fold Δ	P-value
MW0113	<i>drm</i>	phosphopentomutase	- 2.2	3.3×10^{-20}
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7×10^{-30}
sam03030 DNA Replication				
Gene	Symbol	Function	Fold Δ	P-value
MW0016	<i>dnaC</i>	replicative DNA helicase	- 2.4	5.2×10^{-20}
MW1323	n.a.	hypothetical protein; similar to cell wall enzyme EbsB	2.3	7.3×10^{-10}
sam00650 Butanoate Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0100	<i>butA</i>	acetoin reductase	- 3.0	1.5×10^{-30}
MW1979	n.a.	hypothetical protein; similar to acetolactate synthase small subunit	2.7	5.7×10^{-4}
sam00640 Propanoate Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1468	<i>bmfBB</i>	branched-chain alpha-keto acid dehydrogenase E2	- 2.5	1.8×10^{-21}
MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase, accB homologue	2.1	5.9×10^{-8}
sam00190 Oxidative Phosphorylation				
Gene	Symbol	Function	Fold Δ	P-value
MW0943	<i>qoxB</i>	Quinol oxidase polypeptide I QoxB	- 2.1	3.9×10^{-16}
MW0944	n.a.	hypothetical protein; similar to quinol oxidase polypeptide II QoxA	- 2.2	4.4×10^{-23}
sam00300 Lysine Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW0096	<i>sbmH</i>	putative diaminopimelate decarboxylase	6.2	4.1×10^{-102}
MW1214	n.a.	aspartate kinase homologue	2.8	6.0×10^{-48}
sam00330 Arginine & Proline Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1707	n.a.	proline dehydrogenase homologue	2.2	6.0×10^{-25}
MW2475	<i>rocA</i>	1-pyrroline-5-carboxylate dehydrogenase	2.1	5.0×10^{-19}
sam00053 Ascorbate & Aldarate Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0306	n.a.	hypothetical protein; similar to transport protein SgaT	2.4	9.8×10^{-32}
MW0308	n.a.	hypothetical protein; similar to PTS fructose-specific enzyme IIBC component	2.1	4.6×10^{-11}
sam03010 Ribosome				
Gene	Symbol	Function	Fold Δ	P-value
MW0015	<i>rplI</i>	50S ribosomal protein L9	- 2.3	9.4×10^{-27}
MW1107	<i>rpmB</i>	50S ribosomal protein L28	- 2.0	8.4×10^{-17}
sam00630 Glyoxylate & Dicarboxylate Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW0151	<i>fdh</i>	NAD-dependent formate dehydrogenase	2.5	1.4×10^{-22}
sam00790 Folate Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW2191	<i>moeA</i>	molybdopterin biosynthesis protein moeA	- 2.1	9.1×10^{-21}
sam00052 Galactose Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7×10^{-30}
sam00970 Aminoacyl-tRNA Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value

MW1626	<i>thrS</i>	threonyl-tRNA synthetase 1	- 2.2	1.1 x 10 ⁻²⁰
sam00622 Xylene Degradation				
Gene	Symbol	Function	Fold Δ	P-value
MW1250	n.a.	4-oxalocrotonate tautomerase	- 2.4	3.3 x 10 ⁻²¹
sam00261 Monobactam Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW1214	n.a.	aspartate kinase homologue	2.8	6.0 x 10 ⁻⁴⁸
sam01220 Degradation of Aromatic Compounds				
Gene	Symbol	Function	Fold Δ	P-value
MW1250	n.a.	4-oxalocrotonate tautomerase	- 2.4	3.3 x 10 ⁻²¹
sam00280 Valine, Leucine & Isoleucine Degradation				
Gene	Symbol	Function	Fold Δ	P-value
MW1468	<i>bmfBB</i>	branched-chain alpha-keto acid dehydrogenase E2	- 2.5	1.8 x 10 ⁻²¹
sam00362 Benzoate Degradation				
Gene	Symbol	Function	Fold Δ	P-value
MW1250	n.a.	4-oxalocrotonate tautomerase	- 2.4	3.3 x 10 ⁻²¹
sam00270 Cysteine & Methionine Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1214	n.a.	aspartate kinase homologue	2.8	6.0 x 10 ⁻⁴⁸
sam00061 Fatty Acid Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase, accB homologue	2.1	5.9 x 10 ⁻⁸
sam01212 Fatty Acid Metabolism				
Gene	Symbol	Function	Fold Δ	P-value
MW1557	n.a.	hypothetical protein; similar to acetyl-CoA carboxylase, accB homologue	2.1	5.9 x 10 ⁻⁸
sam00400 Phenylalanine, Tyrosine & Tryptophan Biosynthesis				
Gene	Symbol	Function	Fold Δ	P-value
MW1680	n.a.	chorismate mutase homologue	- 2.4	7.9 x 10 ⁻²³
sam03018 RNA Degradation				
Gene	Symbol	Function	Fold Δ	P-value
MW1642	<i>pfk</i>	6-phosphofructokinase	- 2.4	3.7 x 10 ⁻³⁰

Note: KEGG Mapper-Search Pathway Data obtained from the following link: https://www.genome.jp/kegg/tool/map_pathway1.html.

4.) Biological Methods.

a.) Total RNA Extraction of Treated and Untreated MRSA-1707 (*S. aureus* MW2) Biofilms:

Biofilm Formation: MRSA BAA-1707 (MRSA-1707, or *S. aureus* MW2) was grown in fresh tryptic soy broth with 0.5 % glucose (TSBG) to an optical density (OD₆₀₀ ~ 0.8 - 1.0). Then 1 mL of this culture was added to 24-well plate coated with 0.1% gelatin. The plate was then incubated for 20 hours at 37 °C under static conditions to form biofilms. Following biofilm formation, the contents of the well was discarded leaving only the biofilm at the bottom.

Treating Established Biofilms with HP-14: HP-14 was added to the established MRSA-1707 biofilm in TSBG at the desired concentration (0.625 µM, 1/10 MBEC value). In addition, the same volume of DMSO (vehicle) was added as a negative control. The plate was then incubated under static conditions for 20 hours at 37 °C. After the incubation period, the liquid culture was discarded leaving only the surface-attached biofilm.

Extraction of Total RNA from MRSA-1707 Biofilms: 0.5 mL of RNAprotect Bacteria Reagent (Qiagen) was added for 5 minutes to the plate then the biofilm suspension was scraped and transferred into 2 mL tubes. The bacterial cells were then centrifuged for 1 minute at 15,000 xg, then the supernatant was removed. Total RNA was extracted using the RiboPure RNA Purification Kit, Bacteria (Invitrogen, cat# AM1925) according to the manufacturer's protocols. Genomic DNA was digested using the materials supplied by the kit. Each experiment was performed in three replicates. Note: TPEN and EDTA treated biofilms followed the same protocol.

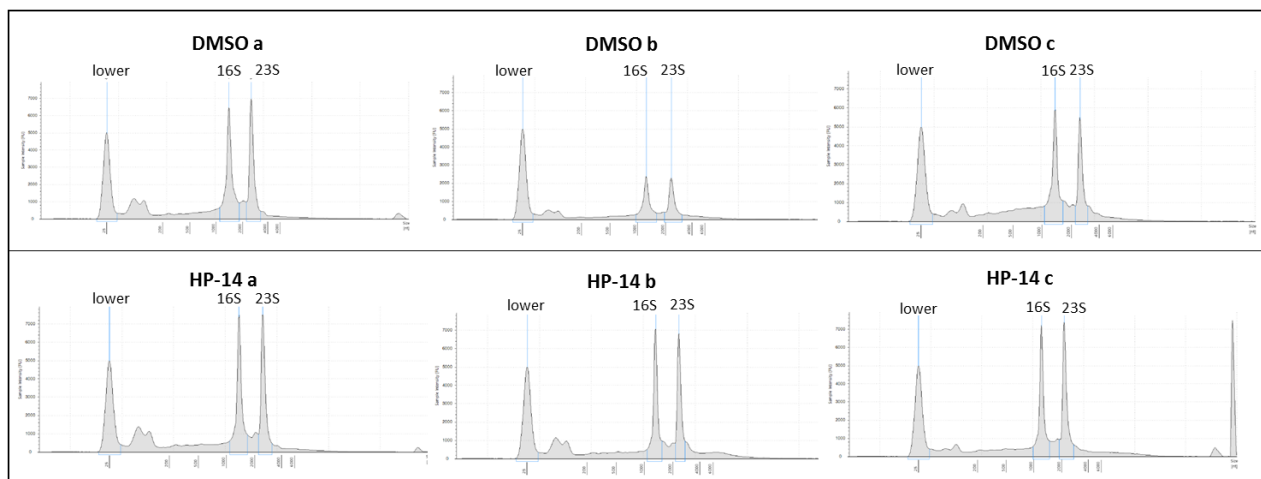
RiboPure RNA Purification kit, Bacteria protocol as provided by the manufacturer (<https://www.thermofisher.com/order/catalog/product/AM1925>):

1. For each sample, pour ~ 250 µL of ice-cold Zirconia Beads into a 0.5 mL screw cap tube.
2. Add 350 µL RNAwiz to the cell pellet and re-suspend by vortexing vigorously for 10 to 15 seconds.
3. Transfer the cells in RNAwiz to a tube containing 250 µL Zirconia Beads and securely fasten the lid.
4. Vortex the tubes for 10 minutes then centrifuge for 5 min at 4 °C.
5. Transfer the bacterial lysate to a fresh 1.5 mL tube and discard the Zirconia Beads. Estimate the lysate volume while transferring the lysate (note: typically 200 to 250 µL of lysate is recovered at this step).
6. Add 0.2 volumes chloroform to the lysate.
7. Shake vigorously for 30 seconds, then incubate 10 minutes at room temperature. Adding chloroform and incubating at room temperature will allow the aqueous and organic phases to be separated by centrifugation.
8. Centrifuge for 5 minutes at 4 °C.
9. Transfer the aqueous phase (top), containing the partially purified RNA, to a fresh 1.5 mL Tube. Estimate the lysate volume while transferring the lysate (note: typically 200 to 250 µL of aqueous phase is recovered at this step).
10. Add 0.5 volumes of 100% ethanol to the aqueous phase recovered and mix thoroughly.
11. Transfer the sample to the Filter Cartridge, close the lid, and centrifuge for ~1 minute, or until all the liquid is through the filter.
12. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.
13. Wash the filter by adding 700 µL Wash Solution 1 to the Filter Cartridge, and centrifuge for ~ 1 minute or until all of the liquid is through the filter.
14. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.
15. Wash the filter by adding 500 µL Wash Solution 2/3 to the Filter Cartridge and centrifuge for ~1 minute, or until all of the liquid is through the filter.
16. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.
17. Repeat with a second 500 µL aliquot of Wash Solution 2/3.

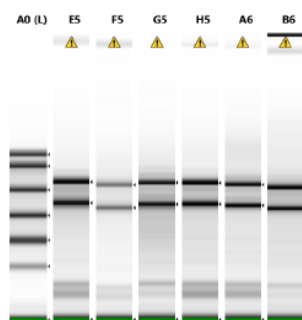
18. Spin the Filter Cartridge for 1 minute to remove excess wash.
19. Transfer the Filter Cartridge to a fresh 2 mL Collection Tube.
20. Elute RNA by applying 25 to 50 μ L Elution Solution, preheated to 95 to 100 $^{\circ}$ C, to the center of the filter.
21. Centrifuge for 1 minute.
22. Repeating the elution step with a second 25 to 50 μ L aliquot of preheated Elution Solution will maximize total RNA yields.
23. DNase treatment: Add 10X DNase buffer equal to 1/9 volume of the RNA and add 4 μ L of DNase 1 (2 U/ μ L)
24. Incubate 30 minutes at 37 $^{\circ}$ C so that the DNase 1 can digest the genomic DNA.
25. Add a volume of DNase Inactivation Reagent equal to 20% of the volume of RNA treated to each sample. For example, if 100 μ L of RNA is treated with DNase, then add 20 μ L of DNase Inactivation Reagent.
26. Vortex the tube of RNA after adding the DNase Inactivation Reagent to mix well.
27. Store at room temperature for 2 minutes flicking the tube once or twice during this period to re-suspend the DNase Inactivation Reagent.
28. Centrifuge the sample for \sim 1 minute at maximum speed to pellet the DNase Inactivation Reagent, then transfer the RNA solution to a new RNase-free tube (not supplied with the kit).

b.) RNA Quality Control Information:

RNA concentration was determined on Qubit[®] 2.0 Fluorometer (ThermoFisher/Invitrogen, Grand Island, NY), RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Total RNA with RNA integrity numbers (RIN) \geq 7 were used for RNA-seq library construction.



Gel Image



RIN ^e	RIN ^e	RIN ^e	RIN ^e	RIN ^e	RIN ^e
8.6	8.3	7.8	8.9	9.0	8.9

Sample	23S/16S	[ng / μ L]	RIN
DMSO (a)	0.8	77.5	8.6
DMSO (b)	0.9	37.1	8.3
DMSO (c)	0.7	70.9	7.8
HP-14 (a)	0.9	76.3	8.9
HP-14 (b)	0.9	70.3	9.0
HP-14 (c)	1.0	83.0	8.9

c.) RNA-seq Library Preparation:

rRNAs were removed by starting with 2000 ng of total RNA (Illumina Ribo-Zero Magnetic Kit for bacteria following the manufacturer's protocol). The depleted RNA were then used for library construction with NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, catalog # E7530) according to the manufacturer's user guide. Briefly, 8 μ l of rRNA removal solution was used for 2000 ng of total RNA for ribosomal removal. 5 μ L of purified ribosomal removal product was fragmented using the first strand synthesis reaction buffer mix by heating at 94 °C for 8 minutes. This was then followed by first strand cDNA synthesis using reverse transcriptase and random primers. Synthesis of ds cDNA was done using the second strand master mix provided in the kit. The resulting double-stranded cDNA was end-repaired, dA-tailing and ligated with NEBNext adaptors. Finally, the library was enriched by PCR amplification, and purified by Agencourt AMPure beads (Beckman Coulter, catalog # A63881).

d.) Library QC and Pooling:

Barcoded libraries were sized on the bioanalyzer, quantitated by QUBIT. Typically, a 200-1000 broad library peak is observed. Quantitative PCR is used to validate the library's functionality, using the KAPA library quantification kit (Kapa Biosystems, catalog number: KK4824). Six individual samples were pooled equimolarly for one lane of HiSeq 3000 2X100 cycles run. RNA library construction was performed at the Interdisciplinary Center for Biotechnology Research (ICBR), Gene Expression & Genotyping Core, University of Florida (UF).

e.) Illumina HiSeq 3000 Sequencing Run:

Sequencing was performed on the Illumina HiSeq3000 instrument using the clustering and sequencing reagents provided by Illumina. Paired-end, 2 x 100 cycles runs required the adding together of reagents from the 150 cycles and the 50 cycles kits (Cat# FC-410-1002, FC-410-1001, and PE-410-1001). Sequencing reactions were set up using 5 μ L of library (2.5 nM). Libraries were first denatured with 5 μ l 0.1 N sodium hydroxide for 8 minutes at room temperature. This was followed by neutralization with 5 μ l of 200 mM Tris (pH 7.5), and mixing with 35 μ L of the ExAmp reagents (contained in the PE-410-1001 clustering kit) according to the manufacturer's protocol. Samples were clustered in the cBot clustering station using the "HiSeq 3000/4000 HD Exclusion Amp v1.0" protocol. Runs were set by choosing the 'Generate FASTQ only' workflow in the HiSeq Control Software v 3.3.76 in the computer station that runs the HiSeq3000 sequencing machine (Illumina, USA). Under these run conditions, the cluster pass-filter was 70-75%, with a yield of 300-325 million pass-filter reads per lane. The % \geq Q30 score was typically above 95%. RNA-seq projects that consisted of large number of samples were done by adding unique barcodes (single- or dual-indexed) to each sample, followed by generating a single pool for sequencing in as many lanes as necessary for a yield of ~ 50 million reads per sample. The reads that passed Illumina quality control filtering were used as raw data for further bioinformatics analysis.

f.) RNA-Seq Data Analysis:

The quality of the RNA-Seq sequence data was first evaluated using FastQC¹ prior to further downstream analysis. Low quality sequences were removed and poor quality part of the reads were trimmed using Trimmomatic². The Star Aligner³ was used to map high quality paired-end reads to NC_003923.1⁴ genome. Gene expression was obtained using RSEM⁵. The expected read counts and Fragments Per Kilobase of transcript per Million mapped reads (FPKM), were extracted for further analysis. The estimated read counts were taken as input for edgeR⁶ to perform DE analysis using exact test. The thresholds for calling significantly differential expressed genes were set at FDR 0.01, the fold change of greater than 2, and the average FPKM for at least one of each comparison group is higher than 0.

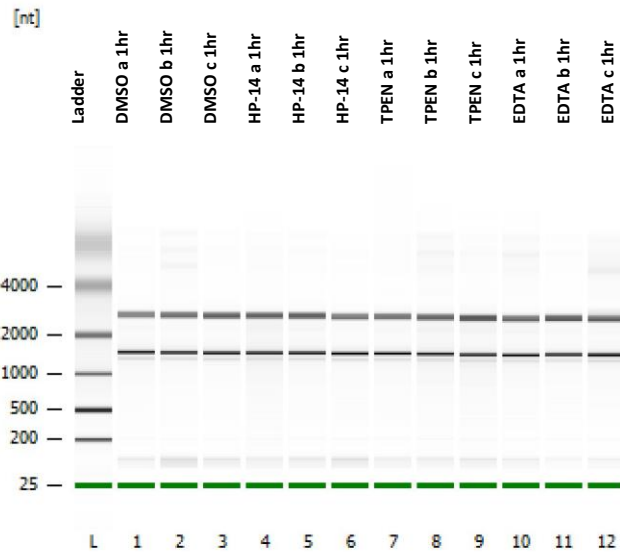
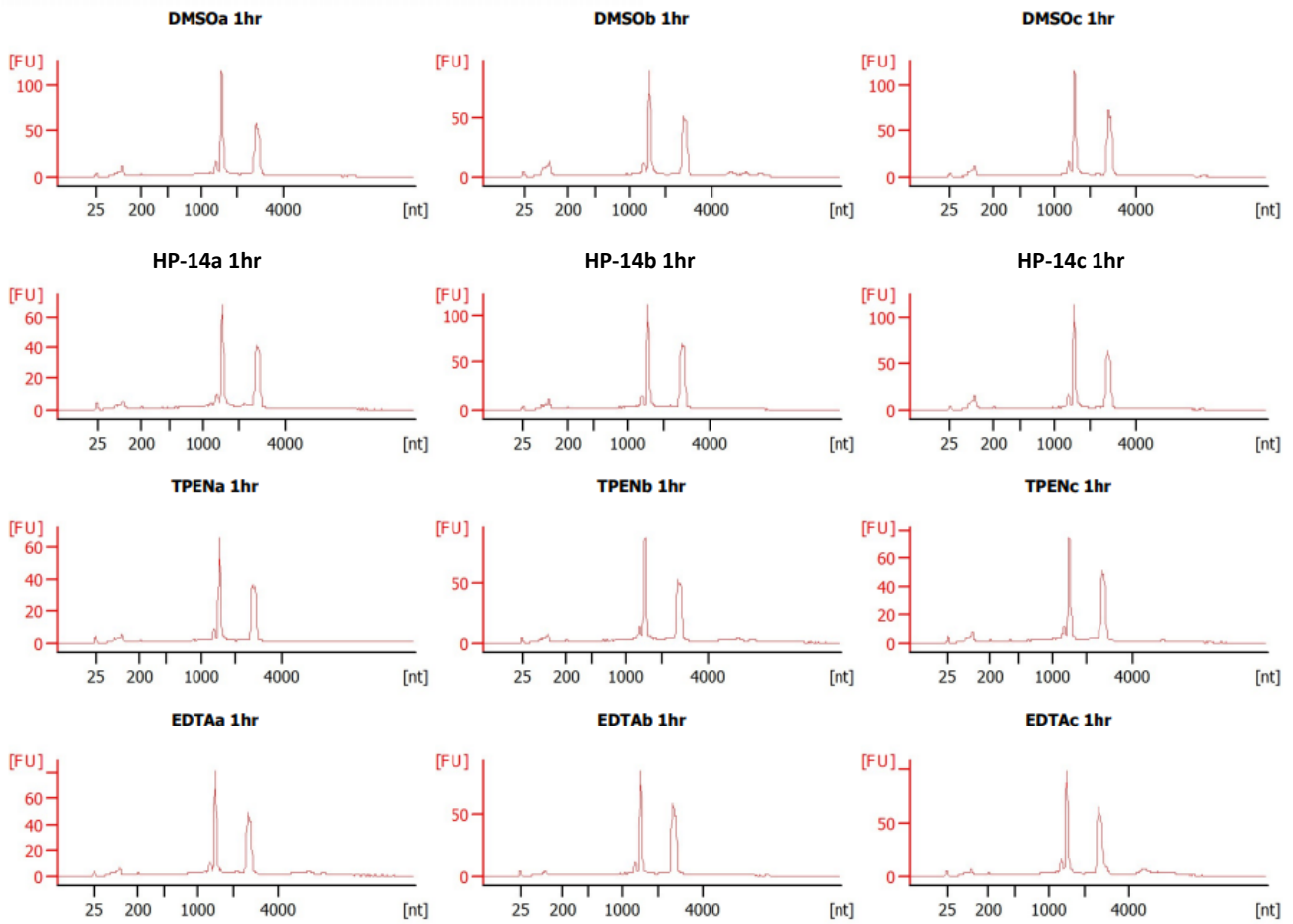
g.) Quantitative Real-Time PCR (qPCR) for Select Gene Transcripts (Validation):

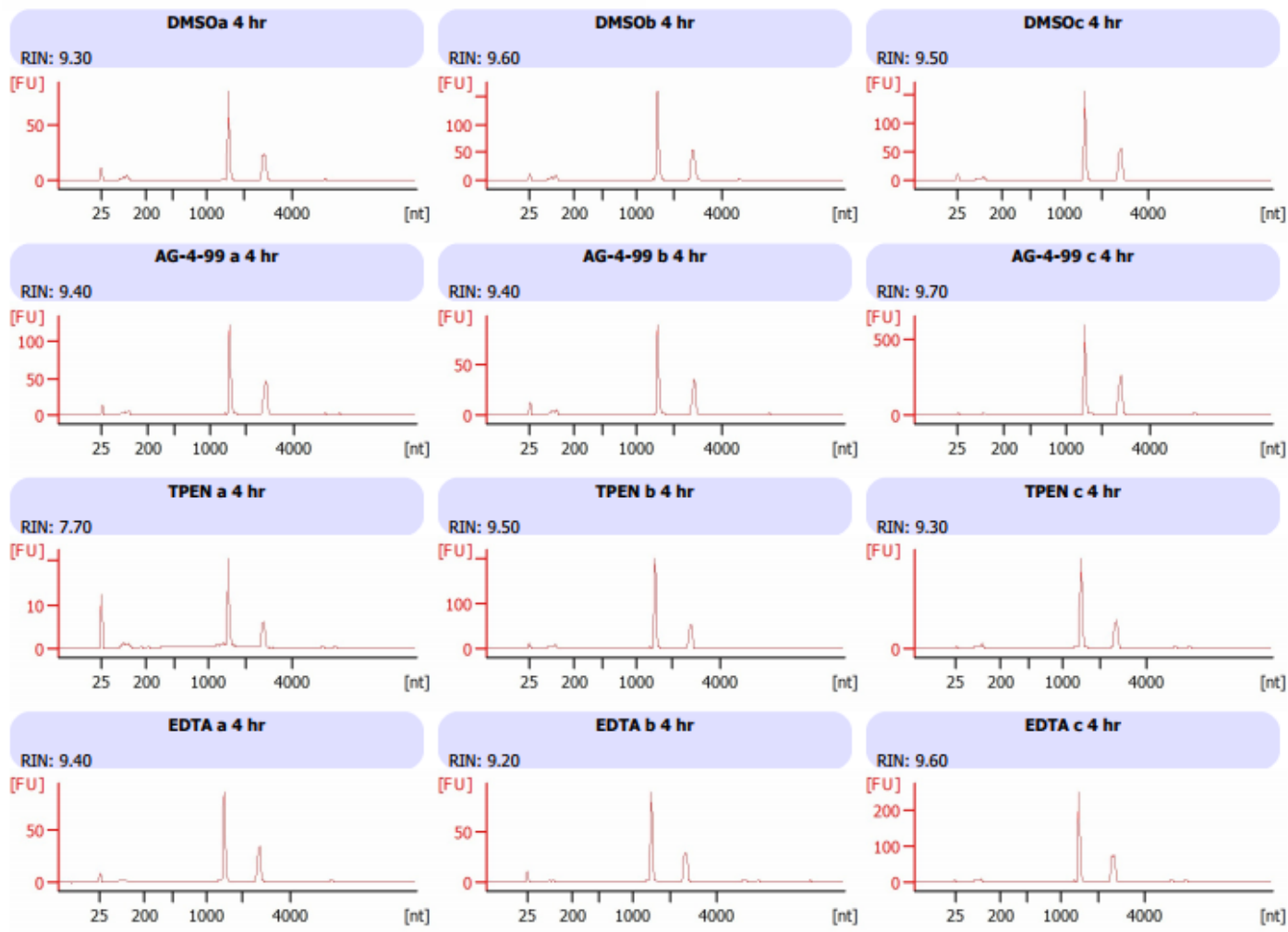
Total RNA was isolated from MRSA-1707 biofilms treated and untreated with **HP-14** (see RNA extraction methods for details). Real-time PCR reactions were performed using the Power SYBR Green RNA-to-C_T 1-Step Kit (Applied Biosystems cat# 4389986) using the manufacturer's guidelines. The materials in the table below were added to a 1.5 mL Eppendorf tube on ice.

Material	Amount / Reaction (20 μ L)	Triplicate + Excess (70 μ L)
SYBR	10 μ L	35 μ L
Primer (5 nM)_forward	1.5 μ L	5.25 μ L
Primer (5 nM)_reverse	1.5 μ L	5.25 μ L
Rt enzyme	0.16 μ L	0.56 μ L
RNA	30 ng	105 ng
Water	20 μ L	70 μ L

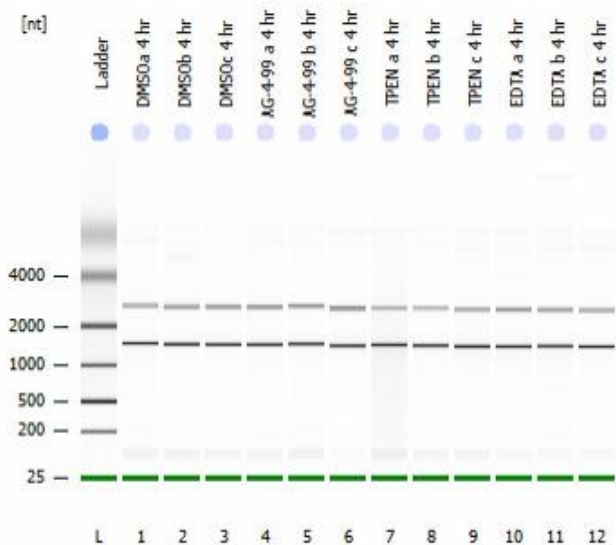
After all contents were added to Eppendorf tubes, they were mixed by centrifugation for 1 minute at 10,000 $\times g$. 20 μ L were then removed from the reaction tubes and were added to each well of a MicroAmp Optical 96-Well Reaction Plate with Barcode (Applied Biosystems 4306737) on ice. The plate was then sealed with MicoAmp Optical Adhesive Film (Applied Biosystems 4311971). The plate was centrifuged for 2 minutes at 1200 $\times g$. qPCR was carried out on an ABI 7300 sequence detection system using the thermocycler program: 30 minutes at 50 $^{\circ}$ C, 10 minutes at 95 $^{\circ}$ C, 15 seconds at 95 $^{\circ}$ C (40 cycles) and 1 minute at 60 $^{\circ}$ C. Relative gene expression changes were calculated using the $\Delta\Delta$ CT method. For each experiment, the CT values of each gene tested were normalized to the CT values of the housekeeping gene *ptaA*. Graphs and data analysis were performed using the GraphPad Prism 6. All primers are listed in Supporting Table 5. All qPCR data were generated from three independent experiments.

i.) Time Course Quality Control Data for 1, 4 and 8 Hour MRSA Biofilm Experiments:

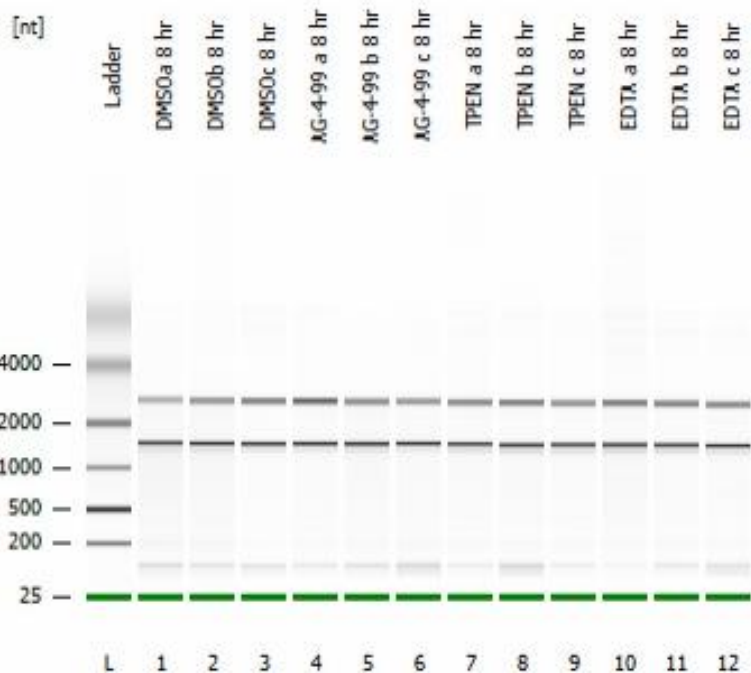
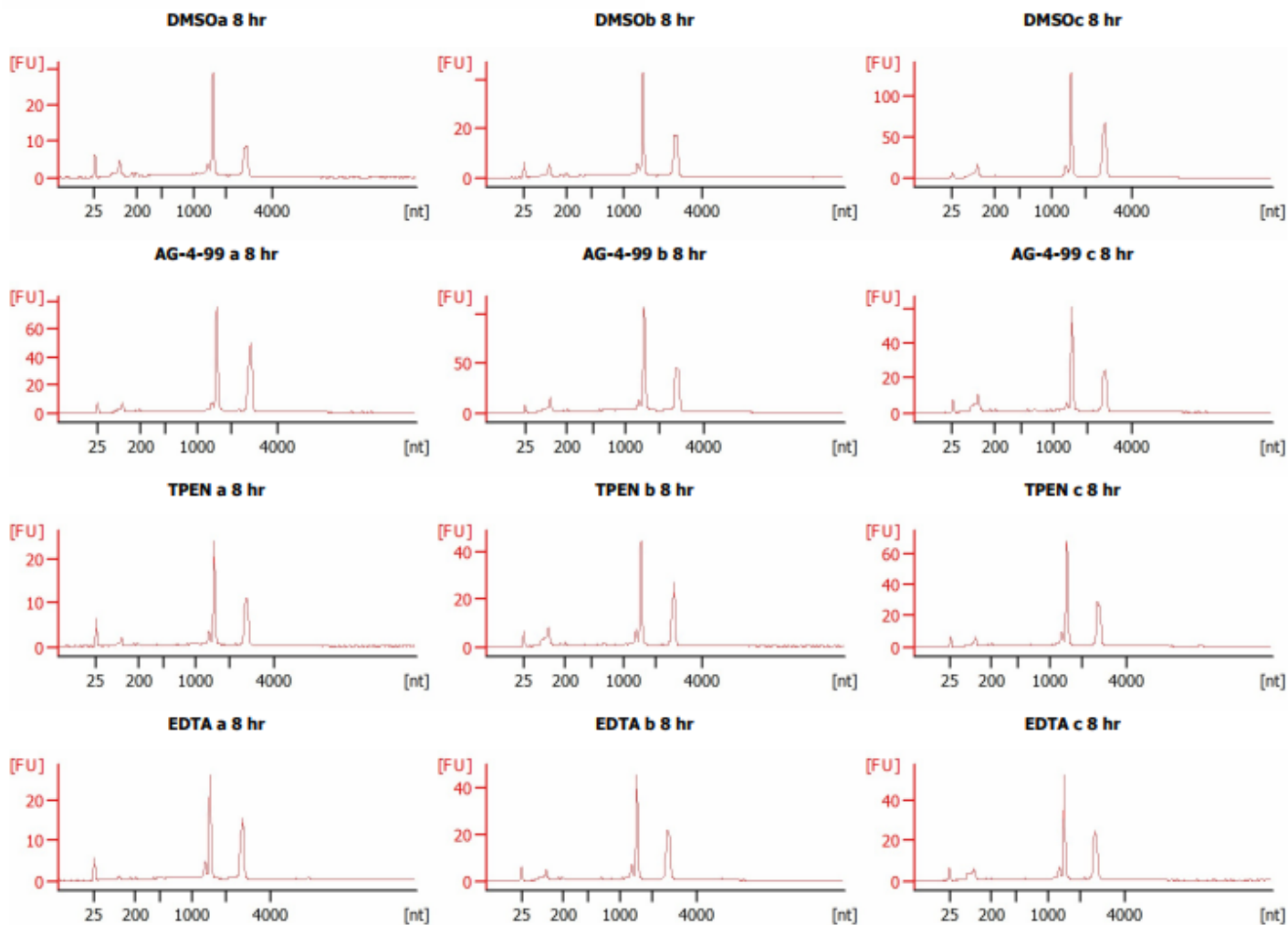




Electrophoresis File Run Summary



Note: AG-4-99 in the above image is the notebook code for is compound **HP-14**.



Note: AG-4-99 in the above image is the notebook code for is compound **HP-14**.

Summary of the RNA concentrations and the RIN (RNA Integrity Number) numbers obtained from the RNA samples (independent experiments) isolated from MRSA-1707 biofilms treated with **HP-14** at 0.625 μ M for 1, 4 and 8 hours.

Sample	1-hr treatment		4-hr treatment		8-hr treatment	
	[ng / μ L]	RIN	[ng / μ L]	RIN	[ng / μ L]	RIN
DMSO (a)	512	8.6	111	9.3	97.1	7.3
DMSO (b)	445	8.9	229	9.6	94	7.1
DMSO (c)	530	9.1	213	9.5	241	9.1
HP-14 (a)	366	8.2	179	9.4	155	9.0
HP-14 (b)	534	8.9	124	9.4	232	8.2
HP-14 (c)	518	8.9	813	9.7	132	8.4
TPEN (a)	286	8.8	39	7.7	46	8.5
TPEN (b)	465	8.3	243	9.5	101	8.4
TPEN (c)	403	8.4	526	9.3	125	8.4
EDTA (a)	397	8.7	119	9.4	54	8.1
EDTA (b)	374	9.2	125	9.2	92	8.4
EDTA (c)	536	8.9	317	9.6	102	8.6

5.) References.

- 1.) Babraham Bioinformatics. FASQC. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- 2.) Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics (Oxford, England)* **30**, 2114-2120 (2014).
- 3.) Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics (Oxford, England)* **29**, 15-21 (2013).
- 4.) *Staphylococcus aureus* subsp. *aureus* MW2 DNA, complete genome. https://www.ncbi.nlm.nih.gov/nuccore/NC_003923.
- 5.) Li, B. & Dewey, C. N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**, 323 (2011).
- 6.) Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics (Oxford, England)* **26**, 139-140 (2010).