4-hydroxy-2-nonenal antimicrobial toxicity is neutralized by an intracellular pathogen.

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Abstract

Pathogens encounter numerous antimicrobial responses, including the reactive oxygen species (ROS) burst. ROS-mediated oxidation of host membrane poly-unsaturated fatty acids (PUFAs) generates the toxic alpha-beta carbonyl 4-hydroxy-2-nonenal (4-HNE). Though studied extensively in the context of sterile inflammation, 4-HNE's role during infection remains limited. Here we found that 4-HNE is generated during bacterial infection and that the intracellular pathogen *Listeria monocytogenes* induces a specific set of genes in response to 4-HNE exposure. A component of the *L. monocytogenes* 4-HNE response is the expression of the genes *rha1* and *rha2* which code for two NADPH-dependent oxidoreductases that collectively counter 4-HNE toxicity. Heterologous expression of *rha1/2* in *Bacillus subtilis* significantly increased bacterial resistance to 4-HNE both *in vitro* and following phagocytosis by murine macrophages. Our work demonstrates that 4-HNE is a previously unappreciated component of ROS-mediated toxicity and that *L. monocytogenes* has evolved specific countermeasures to survive within its presence.

Introduction

Innate immune detection of bacterial infection initiates a complex inflammatory response characterized by production of cytokines and small molecule mediators involved in driving antimicrobial immunity. A key aspect of intrinsic cellular immunity is the production of highly reactive molecules, including reactive oxygen (ROS) and nitrogen (RNS) species (Nathan and Cunningham-Bussel 2013). Unlike the highly specific targeting of infectious agents by adaptive immune responses, ROS and RNS exhibit indiscriminate toxicity toward biological systems through their capacity to react with lipid, amino acid, and nucleic acid moieties that are conserved among both eukaryotic host and invading microbe (Patel et al.

1999, Jacobson 1996). While such indiscriminate noxious metabolite production provides protection against infection, many bacterial pathogens have evolved a diverse array of mechanisms to directly detoxify or repair damaged cellular components following ROS and RNS encounters (Fang 2004, Staerck, et al. 2017).

ROS and RNS encompass a broad group of distinct molecules. While nitric oxide, hydrogen peroxide, hypochlorite, and superoxide are well characterized molecular components of the innate immune response, these molecules give rise to numerous secondary metabolites that may also contribute to host defense against infection. An initial characteristic of the inflammatory response is the mobilization of arachidonic acid from cellular membranes. While typically involved in generation of eicosanoids, arachidonic acid exposure to oxygen radicals derived from the ROS burst leads to peroxide-mediated structural rearrangement and the generation of breakdown products, the best studied of which is 4-hydroxy-2-nonenal (4-HNE), a highly reactive membrane-permeable molecule (Hanna and Hafez, 2018). Over the last 40 years, the production of 4-HNE has been well documented at sites of sterile inflammation and has been associated with many disease pathologies, including atherosclerosis (Uchida et al., 1994), Alzheimers' (Sayre et al., 2002), diabetes (Pillon et al., 2012), obstructive pulmonary disease (Rahman et al., 2002) and chronic liver disease (Paradis. et al., 1997). Although arachidonic acid mobilization and reactive oxygen species generation are well-known and well-studied components of innate immune responses, there are a dearth of studies characterizing the role of 4-HNE during infectious disease.

In this study, we demonstrate that 4-HNE is generated during bacterial infection both in cell culture and *in vivo*, is able to penetrate the bacterial cell envelope and access the cytoplasm, and that 4-HNE generation leads to bacterial growth delay or death. We observed that the intracellular bacterial pathogen *Listeria monocytogenes* is highly resistant to the bactericidal effects of 4-HNE and that several specific transcriptional responses are induced in response to toxic 4-HNE exposure, including two enzymes, *rha1* and *rha2*, whose loss sensitizes *L. monocytogenes* to 4-HNE toxicity. When *rha1/2* are expressed in the 4-HNE sensitive and avirulent organism *B. subtilis*, they significantly increase bacterial survival in the presence of 4-HNE both *in vitro* and following phagocytosis by murine macrophages. Our findings are consistent with the premise that 4-HNE is a heretofore unrecognized component of ROS-mediated antimicrobial defense and that pathogens have specific and multifaceted detoxification systems used to evade 4-HNE-mediated cytotoxicity in order to promote infection.

57

58 **Results**

59 **4-HNE accumulates during *L. monocytogenes* infection.**

60 4-HNE is a highly reactive electrophilic $\alpha\beta$ -unsaturated aldehyde that undergoes Michael addition
 61 with nucleophilic amino acids, resulting in stable conjugates that correlate with cellular levels of free 4-HNE.
 62 Monoclonal antibodies to these adducts are routinely used to monitor 4-HNE levels in cells (Majima,
 63 Nakanishi-Ueda, and Ozawa. 2002). To investigate 4-HNE production during bacterial infection, we infected
 64 murine hepatocytes with *L. monocytogenes* and quantified 4-HNE conjugates using dot blots of whole cell
 65 lysates at various times post-infection. During the course of a 24-hour infection, a continuous increase in 4-
 66 HNE protein conjugates relative to β -actin control was observed (Fig 1A). To interrogate the impact of
 67 bacterial infection on host production of 4-HNE *in vivo*, mice were infected intravenously with *L.*
 68 *monocytogenes* constitutively expressing GFP. At 48 hours post infection, tissues were harvested, fixed,
 69 and analyzed by immunohistochemistry. Clear foci of infection were visible in the liver with no change in the
 70 abundance of 4-HNE protein conjugates (Supp Fig 1A-D). In the spleen, however, the bacteria were
 71 diffusely distributed throughout the organ and the entire spleen of the infected mouse exhibited increased
 72 staining for 4-HNE protein conjugates (Figs 1B-E).

73 The grossly different observations between the liver and spleen were somewhat surprising given our
 74 previous observation that infection of murine hepatocytes with *L. monocytogenes* *in vitro* induced
 75 accumulation of 4-HNE conjugates. These observations are likely a consequence of several factors. The
 76 liver is a major site of small molecule detoxification and hepatocytes are known to produce high levels of
 77 many of the 4-HNE metabolizing proteins, including aldo-keto reductases, alcohol dehydrogenase, and the
 78 4-HNE glutathione transferase, GSTA4 (Zheng et al, 2014). Additionally, the immune driven mobilization of
 79 arachidonic acid and ROS precursors that lead to 4-HNE generation may result in elevated accumulation of
 80 this host aldehyde in the spleen.

81 At higher levels of magnification, we observed that 4-HNE conjugates were not evenly distributed
 82 among all cells. The majority of cells exhibited diffuse and constant staining and a subset showed very dark
 83 and robust staining for 4-HNE conjugates. At 100x magnification, the most pronounced signal for 4-HNE
 84 conjugates had punctate staining resembling the staining of the bacteria with anti-GFP antibody (Fig 1F-G),

85 consistent with 4-HNE conjugate formation on the bacteria. While these observations do not provide
 86 quantitative measures of 4-HNE levels, they establish that 4-HNE was indeed elevated following bacterial
 87 infection and suggest that bacteria encounter and may be directly chemically modified by this metabolite
 88 during infection.

89

Fig 1. 4-HNE accumulates during intracellular bacterial infection by *L. monocytogenes*.

(A) 4-HNE accumulation in TIB73 murine hepatocytes during intracellular *L. monocytogenes* infection. 4-HNE adduct accumulation is assessed by dot blot whole cell Western and normalized to actin levels. Data are normalized 4-HNE levels as percent of 4-HNE at hour 0 of infection. Western image below is representative. (B) 4-HNE accumulation during 48-hour murine infection by GFP+ *L. monocytogenes* assessed by immunohistochemistry analysis. Spleen (infected) anti-4-HNE. (C) spleen (uninfected) anti-4-HNE. (D) spleen (infected) anti-GFP 25x magnification. (E) spleen (infected) anti-4-HNE 25x magnification (F) spleen (infected) anti-GFP 100x magnification. (G) spleen (infected) anti-4-HNE 100x magnification. Antigens detected with 3,3-diaminobenzidine staining by horseradish peroxidase and cellular nuclei imaged with Hematoxylin counterstain. Data in (A) are pooled from two independent experiments. Statistics in (A) are an ordinary one-way ANOVA with a Dunnett's multiple comparison test against hour 0. Error bars are mean +/- SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

90

91 **4-HNE causes damage through the targeting of nucleophilic protein moieties and *L. monocytogenes***
 92 **is uniquely resistant to 4-HNE-mediated death.**

93 Electrophilic stress due to 4-HNE conjugation to proteins causes eukaryotic cells to undergo
 94 apoptosis following intermediate 4-HNE exposure (5-40 μ M) and necrosis at higher concentrations (40-
 95 100 μ M) (Dalleau et al., 2013). However, due to 4-HNE's lipophilicity it is believed to accumulate to
 96 significantly higher levels (0.3-5mM) near and within membranes than what is typically considered cytotoxic
 97 (Zimniak, 2011; Esterbauer, Schaur and Zollner, 1991; Uchida, 2003).

98 To characterize bacterial sensitivity to 4-HNE toxicity, we exposed bacteria within the Firmicutes
 99 phylum to a wide range of 4-HNE concentrations and assessed viability. We observed variability in survival,
 100 ranging from a 4-log reduction in CFU for *B. subtilis*, a 2-log reduction for *Staphylococcus aureus*, a half-log
 101 reduction for *Enterococcus faecalis* to less than a 40% reduction for *L. monocytogenes* (Fig 2A). We
 102 observed a dose dependent decrease in growth of *L. monocytogenes* with increased exposure to 4-HNE
 103 (Fig 2B). The significant resistance of *L. monocytogenes* to 4-HNE exposure was unexpected. Due to the
 104 conserved nature of 4-HNE targets, we hypothesized that 4-HNE would exert similar damaging effects on
 105 bacteria as on eukaryotic cells. Thus, we first interrogated the ability of 4-HNE to generate protein adducts
 106 in *L. monocytogenes*. Dot blots of *L. monocytogenes* bacterial cell lysates indicated an increase in 4-HNE-
 107 protein adducts that correlated with increased 4-HNE exposure (Fig 2C), establishing that this aldehyde
 108 penetrates the bacterial cell envelope and impacts cytosolic proteins.

109 4-HNE adduct accumulation can result in protein misfolding and crosslink-induced aggregation.
 110 Eukaryotic cells clear 4-HNE damaged proteins through proteasome and autophagy-mediated pathways
 111 (Zhang and Forman, 2017). Bacteria target damaged proteins for degradation through the proteases that
 112 comprise the heat shock response (Parsell and Lindquist, 1993). This pathway is primarily transcriptionally
 113 regulated (Yura, Nagai and Mori, 1993), so RT-qPCR was performed on a subset of heat shock genes
 114 representing two major groups of heat shock genes in *L. monocytogenes*: HrcA-regulated chaperones and
 115 CtsR-regulated proteases (Roncarati and Scarlato, 2017). When *L. monocytogenes* was exposed to 640 μ M
 116 4-HNE for 20 minutes, the four genes tested (*clpC*, *clpE*, *dnaK*, *groES*) were significantly induced compared
 117 to vehicle controls (Fig 2D). These data, combined with the dot blot results, support the hypothesis that 4-
 118 HNE causes protein damage to which *L. monocytogenes* mounts a heat shock response. The elevated

Fig 2. 4-HNE impacts bacterial viability, replication and damage to cellular proteins.

(A) Survival of mid-log (0.4-0.8OD) *Listeria monocytogenes* (Lm), *Enterococcus faecalis* (Ef), *Staphylococcus aureus* (Sa) and *Bacillus subtilis* (Bs) following exposure to various concentrations of 4-HNE or mock vehicle (ethanol) in PBS at 37 °C for 1 hour. Data are reported as recovered CFU normalized to mock treated controls. (B) Growth of *L. monocytogenes* in TSB at 37°C with various concentrations of 4-HNE added at time zero. (C) Anti-4-HNE dot blot of soluble bacterial lysates from mid-log *L. monocytogenes* suspended in PBS and treated with increasing concentrations of 4-HNE for 30 minutes. Protein levels were normalized (3μg total protein) and signal quantified by densitometry on a Licor Odyssey Fc. (D) qRT-PCR measurement of expression of indicated genes in midlog *L. monocytogenes* in TSB treated with 640 μM 4-HNE for 20 minutes. Expression normalized to 16S rRNA levels. Data in figures (A) and (B) are in biological triplicate. Data in (C) and (D) are biological duplicate. Statistics for (D) are unpaired t-tests of $\Delta\Delta Ct$ values of treated samples versus untreated samples for each gene. Error bars are mean $\pm$ SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

***L. monocytogenes* expresses potential 4-HNE detoxification enzymes that contribute to its survival in the presence of 4-HNE.**

Our data suggest that *L. monocytogenes* is exposed to 4-HNE during infection and that only high concentrations of this aldehyde impact its growth. We hypothesized that *L. monocytogenes* may express genes involved in countering the cytotoxic effects of 4-HNE. To probe further, we performed global transcriptome analysis during 4-HNE exposure using RNA sequencing. Over one hundred genes were induced greater than 10-fold in response to 4-HNE exposure, including several of the heat shock genes previously identified by qRT-PCR analysis (Fig 3A, Suppl Fig 2A, Suppl Table 1).

Eukaryotic cells utilize several reductases to detoxify 4-HNE (Mol et al., 2017). Two reductases were highly induced in our global transcriptome, *lmo0103* and *lmo0613*, which we refer to as *rha1* and *rha2* (resistance to host alkenals 1 and 2), respectively. Phyre2 analysis of Rha1 predicted high structural homology to CLA-ER, a flavin-dependent enone reductase from *Lactococcus plantarum* (PDB: 4QLY)(Hou et al, 2015). A Phyre2 analysis of Rha2 revealed structural similarity to a few crotonyl-CoA carboxylase/reductases (PDBs: 3KRT, 4Y0K, and 5A3J) and a plant chloroplast oxoene reductase (PDB: 5A3V), two enzymes with the capacity to reduce enone-containing lipophilic substrates. Given the predicted reductase activity of Rha1 and Rha2 and their structural similarity to proteins that metabolize enone-containing compounds, we further investigated their role in 4-HNE resistance.

Induction of *rha1* and *rha2* in response to 4-HNE exposure was found to be 34 and 90-fold, respectively, by RT-qPCR. To assess the specificity of their induction, we exposed *L. monocytogenes* to a sublethal concentration of a panel of aldehydes (Suppl Fig 2B,C), including 4-HNE; 4-HHE (4-hydroxy-2-hexenal), a similar but shorter chain $\alpha\beta$ -unsaturated aldehyde produced from the oxidation of ω -3 fatty acids (Awada et al., 2012); methylglyoxal, a reactive byproduct of glycolysis; propionaldehyde, an alpha hydrogen aldehyde; and malondialdehyde, another product of lipid peroxidation (Esterbauer, Schaur, and Zollner, 1991). Both *rha1* and *rha2* were most strongly induced by 4-HNE exposure, with much less induction by 4-HHE and negligible induction with the other tested compounds (Fig 3B). We next assessed if these genes are induced by *L. monocytogenes* during intracellular infection. At 6 hours post-infection of macrophages we found that there was significant induction of both genes compared to growth in BHI broth (Fig 3C). Together these transcriptional studies suggested that the *rha1/2* genes are not components of a general aldehyde response but rather specific responses to 4-HNE encountered during infection.

These intriguing transcriptional results suggested that *rha1* and *rha2* may function in 4-HNE resistance. Individual and double mutants of these genes were generated and assessed by competition experiments for survival relative to WT *L. monocytogenes* following 4-HNE exposure. Control mixtures left untreated in PBS exhibited no significant difference between mutant and control strains (Suppl Fig 2D).

Fig 3. 4-HNE exposure induces resistance genes in *L. monocytogenes*.

(A) Global gene expression of midlog *L. monocytogenes* in TSB treated with 640 μM 4-HNE or ethanol control for 20 minutes. RPKM: reads per kilobase million. Genes of interest *rha1*, *rha2* and heat shock class members are indicated in blue, red and orange, respectively. (B) qRT-PCR of expression of *rha1* and *rha2* genes after 20-minute treatment of mid-log bacteria in TSB media with 500μM of selected aldehydes: 4-HNE (4-hydroxy-2-nonenal), 4-HHE (4-hydroxy-2-hexenal), MG (methylglyoxal), PA (propionaldehyde) and MDA (malondialdehyde). (C) qRT-PCR analysis of expression of *rha1* and *rha2* at 6 hours post infection in J774 macrophages (mφ). (D) Competitive index of midlog WT and mutant *L. monocytogenes* in PBS treated with 640 μM 4-HNE at 37°C for one hour. Data in figures (A) and (B) are in biological duplicate. (C) is in biological triplicate. (D) is in technical triplicate, representative of at least two independent experiments. Statistics in (B) is an ordinary one-way ANOVA with Dunnett's multiple comparison test of $\Delta\Delta Ct$ values of the treated samples against untreated samples. Statistics in (C) are unpaired t-test between broth and macrophage samples. Statistics in (D) are unpaired t-test between WT and mutant *L. monocytogenes* competition pairs. Error bars are mean $\pm$ SD. *, $p < 0.05$; **, $p < 0.01$.

Among mixtures exposed to 640 μM 4-HNE, unmarked WT and marked WT showed no significant difference in 4-HNE survival. Loss of *rha2* had no effect on 4-HNE survival while $\Delta rha1$ had a modest 5-fold reduction relative to WT. However, the $\Delta rha1\Delta rha2$ mutant exhibited a 50-fold competitive defect compared to WT *L. monocytogenes* that was rescued by either *rha1* or *rha2* expression in *trans*, demonstrating that both genes must be absent for the toxic effect to manifest (Fig 3D). The $\Delta rha1\Delta rha2$ *L. monocytogenes* was used to infect macrophages and mice. During cellular infection, we observed a ~50% reduction in CFU at 2 hours post-infection (Suppl fig 2E), which rebounded to WT levels at later time points. In mice infected via intravenous injection no significant phenotype was observed at 48 hours post infection in either the spleen or the liver (Suppl fig 2F). These findings provide evidence that Rha1 and Rha2 are specific and important

for 4-HNE resistance, though other factors likely contribute to *L. monocytogenes* capacity to counteract this metabolite *in vivo*.

Recombinant Rha1 and Rha2 metabolize 4-HNE and ectopic expression confers 4-HNE resistance to sensitive bacteria.

In order to determine if these putative enone reductases can directly utilize 4-HNE as a substrate, we generated recombinant Rha1 and Rha2 proteins. As controls for these studies, we generated catalytically dead variants of the two proteins by mutating amino acids predicted to be involved in flavin or NADPH binding (Suppl Fig 3A). All proteins were expressed and characterized for NADPH oxidation in the presence and absence of 4-HNE. Only the WT variants of Rha1 and Rha2 exhibited NADPH oxidation upon addition of 4-HNE (Fig 4A), consistent with their capacity to mediate NADPH dependent reduction of the enone.

We hypothesized that Rha1 and Rha2 could confer 4-HNE resistance to a sensitive organism. To this end, each gene and corresponding catalytically dead variant were expressed individually and in combination in *B. subtilis*, which exhibited 300-fold more sensitivity than *L. monocytogenes* upon 4-HNE exposure (Fig 2A, suppl fig 3B). Consistent with our observations of the *L. monocytogenes rha* deletion mutants, expression of *rha2* in *B. subtilis* had no effect on growth in the presence of 4-HNE, *rha1* gave modest protection against 640μM 4-HNE, and expression of both *rha1* and *rha2* had the largest growth rescue, reducing lag time by up to 3 hours (Suppl Fig 3C, Fig 4B). We then focused on *B. subtilis* expressing both *rha1* and *rha2* genes, as this strain had the most robust phenotype. When assessed for bacterial survival following 4-HNE treatment, *B. subtilis* expressing the functional enzymes exhibited nearly a 2-log survival advantage relative to the control strain expressing enzymatically dead *rha1/2* (Fig 4C). Additionally, soluble cellular fractions from *B. subtilis* exposed to 4-HNE and probed for 4-HNE protein adducts by dot blot revealed a ~70% reduction in 4-HNE conjugates in the *B. subtilis* strain expressing both of the active *rha1* and *rha2* genes versus their catalytically dead counterparts (Suppl Fig 3D, Fig 4D).

To determine if 4-HNE resistance conferred by *rha1/2* could contribute to bacterial survival within mammalian cells, *B. subtilis rha1/2* strains were assessed for viability following phagocytosis by primary bone marrow-derived macrophages. We found that *B. subtilis* expressing the active forms of Rha1 and

Rha2 maintained a significantly higher CFU over the course of eight hours than the *B. subtilis* expressing the catalytically dead form (Fig 4E). To determine whether this survival advantage was due to 4-HNE resistance, we measured *B. subtilis* survival within bone marrow-derived macrophages from *gp91^{phox}* mice deficient in oxidase cytochrome b-245, which are unable to produce the reactive oxygen burst and therefore 4-HNE (Esterbauer, Schaur, and Zollner, 1991). Consistent with the role of *rha1* and *rha2* in resistance to a ROS-derived factor, the protective effect of WT *rha1/2* expression was eliminated in the absence of *gp91^{phox}* (Fig 4E). Together, these observations revealed that expression of *rha1* and *rha2* in *B. subtilis* imparts resistance to 4-HNE toxicity and impacts bacterial survival in the host cell's ROS burst.

Fig 4. Recombinant Rha1 and Rha2 metabolize 4-HNE and ectopic expression confers 4-HNE resistance to sensitive bacteria. (A) Rates of NADPH oxidation (200µM) by WT and mutant variants of Rha1 and Rha2 in the presence of 4-HNE. (B) Growth of *B. subtilis* expressing either the WT or MUT (catalytically dead mutant) versions of *rha1* and *rha2* in TSB at 37 °C in the presence of the indicated concentrations of 4-HNE added at time zero. Dotted line represents OD₆₀₀ 0.5. (C) Survival of *B. subtilis* WT and MUT in PBS at 37 °C with 160µM 4-HNE for one hour. (D) 4-HNE conjugates from *B. subtilis* WT and MUT soluble cell lysates (3 µg total protein) three hours after 4-HNE treatment as assessed by dot blot and quantified by densitometry on a Licor Odyssey Fc. (E) *B. subtilis* WT and MUT survival following phagocytosis by Interferon gamma-activated primary WT or phagosomal oxidase-deficient bone marrow derived macrophages (WT or *gp91^{phox}* pBMMs). All experiments in figure performed in biological triplicate. Statistics in (A) are unpaired t-tests comparing the hours to OD 0.5 between WT and MUT *B. subtilis* *pHT01::rha1/2*. Statistics in (B), (C) and (D) are unpaired t-tests comparing WT and MUT *B. subtilis* *pHT01::rha1/2*. Error bars are mean +/- SD. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001.

Discussion

In this study, we provide evidence that ROS-derived metabolite 4-HNE accumulates during *L. monocytogenes* infection both in tissue culture and in mice. We also show that 4-HNE exhibits antimicrobial effects on several bacterial species and that in the highly resistant intracellular pathogen *L. monocytogenes*, exposure to this aldehyde induces a specific and robust transcriptional profile. Among the highest induced genes are components of the heat shock response, consistent with aldehyde induced protein damage, a known effect of 4-HNE exposure. In addition, two genes, *rha1* and *rha2* are highly and specifically induced by 4-HNE exposure, and these two enzymes turnover 4-HNE through NADPH-dependent reduction *in vitro*. Disruption of *rha1* and *rha2* in *L.*

monocytogenes results in a decrease in viability in the presence of 4-HNE, and heterologous expression of *rha1* and *rha2* in *B. subtilis* conferred increased tolerance to 4-HNE toxicity in this non-pathogenic and 4-HNE-sensitive organism. Rha1 and Rha2 expression in *B. subtilis* also allowed for greater survival following phagocytosis by bone marrow derived macrophages in a manner entirely dependent upon phagocyte ROS generation. Together this work supports

the conclusion that 4-HNE represents one of the individual molecular components of ROS-mediated host defense through its direct antimicrobial effects on bacteria and that pathogens have likely evolved complex mechanisms of surviving its encounter within eukaryotic hosts (Fig 5).

There are many parallels between the chemical and biological functions of 4-HNE and other toxic metabolites that function in antimicrobial defense. The freely diffusible and highly reactive diatomic gas nitric oxide (NO) is produced during infection (Iyengar, Stuehr and Marletta, 1987; Stuehr and Marletta, 1985) and has a direct role in preventing bacterial growth (Nathan and Hibbs, 1991). However, due to the conservation of its reactive targets, elevated levels of NO also exert pathological effects during both sterile

Figure 5. Model of *rha1/2*-mediated 4-HNE resistance in *L. monocytogenes*.

inflammation and acute infections (Nagafuji et al., 1995, Galley and Webster, 1998). 4-HNE is membrane diffusible, highly reactive, and contributes to disease pathology due to its cytotoxic activity toward eukaryotic cells. These parallels, together with our findings that bacterial infection induces 4-HNE production are consistent with the premise that 4-HNE represents a component of ROS-mediated host defense, among such other toxic metabolites as superoxide, hydrogen peroxide, and hypochlorite.

While a role for 4-HNE in host antimicrobial defense has yet to be appreciated in mammals, plants utilize a variety of lipophilic molecules generated by the oxidation of polyunsaturated fatty acids (PUFAs), collectively referred to as oxylipins. While generally considered to be involved in signal transduction, many oxylipins can directly inhibit bacterial growth (Prost et al., 2005) and 4-HNE itself is a component of the oxylipin burst in soybean where it serves an anti-fungal function (Vaughn and Gardner, 1993). Because 4-HNE is one of several distinct metabolites produced following oxidation of PUFAs in mammals, it is conceivable that other reactive byproducts of this process also contribute to microbial defense in a similar manner.

To survive within the sterile tissues of eukaryotic hosts, bacterial pathogens often counteract the toxic effects of the immune response. Our discovery of two genes that confer synergistic resistance to 4-HNE in *L. monocytogenes* begin to provide insight into the mechanisms by which 4-HNE toxicity might be overcome. *In vitro* studies suggest that Rha1 and Rha2 both metabolize 4-HNE in an NADPH-dependent manner, suggesting redundant functions. Redundancy in bacterial resistance to ROS is a relatively common phenomenon, including the need to eliminate five individual enzymes in *Salmonella enterica* Serovar Typhimurium to exhibit a phenotype in the presence of hydrogen peroxide (Hébrard et al., 2009) and simultaneous disruption of four enzymes in *Bacillus anthracis* to observe a phenotype in the presence of superoxide (Cybulski et al., 2009). Such redundancy in bacterial detoxification programs likely decreases the chances that genetic drift or other genomic damage would render an organism defenseless against oxidative stress.

While Rha1 and Rha2 both contribute to 4-HNE resistance, the $\Delta rha1\Delta rha2$ *L. monocytogenes* strain still exhibits several logs of survival benefit relative to the related organism *B. subtilis*, suggesting that other mechanisms of 4-HNE resistance remain to be identified. Among the many uncharacterized genes induced during 4-HNE exposure, *Imo0796* shows homology to *bcnA*, a secreted lipocalin in *Burkholderia*

cenocepacia which sequesters long-chain lipophilic antibiotics (El-Halfawy et al., 2017). 4-HNE, with its long hydrophobic tail, could conceivably be neutralized in an analogous manner. It is possible many intrinsic resistance properties of *L. monocytogenes* are not reflected through transcriptional responses. For instance, addition of amine containing constituents on the cell's surface through lysinylation of teichoic acids and/or lipids, as well as deacetylation of peptidoglycan, may provide a nucleophile reactivity barrier that prevents 4-HNE entry into the bacterial cell. Additionally, $\alpha\beta$ -unsaturated aldehydes have preferential reactivity toward sulfhydryl groups, including cysteine and glutathione, and it is expected that thiolate depletion would be the major mechanism of 4-HNE toxicity (Lopachin and Gavin, 2014). Indeed, the thiol responsive transcription factor *spxA1* was induced >2-fold in response to 4-HNE and the magnitude of heat shock gene induction mirrored results reported for *B. subtilis* following diamide treatment, a potent inducer of disulfide stress (Ole Leichert, Scharf, and Hecker, 2003). While our findings provide initial molecular insight into one pathogen's resistance to 4-HNE, it is clear that many details are yet to be revealed.

Taken together, our findings extend the range of antimicrobial molecules generated through the reactive oxygen burst to include the byproducts of lipid peroxidation. Additionally, bacteria whose infection cycles involve intimate exposure to these molecules, such as *L. monocytogenes*, have specific and finely regulated detoxification and protection programs against this toxicity. Future investigation of the impacts of 4-HNE on a diverse array of organisms with varied infection models will highlight the importance of this metabolite on host defense and the varied mechanisms by which pathogens counteract its toxicity to promote infection.

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Author Contributions

HT, APM and JJW conceptualized the studies, HT, APM, AJP and RCG performed the experiments, HT and JJW wrote the original draft of the manuscript, APM, AJP and RCG edited and reviewed the manuscript, and JJW, HT, AJP and APM acquired funding. JJW supervised and administered the project.

Competing Interests

The authors declare no competing interests.

Methods

Strains and routine growth conditions

Unless otherwise specified, *L. monocytogenes* was grown at 37°C degrees in tryptic soy broth (TSB) media and *E. coli* at 37°C degrees in Luria-Bertani (LB) media with appropriate antibiotic selection. Unless otherwise noted, *B. subtilis* was struck on LB plates with appropriate antibiotics and induction agent overnight at 30°C, after which the biomass was scraped off the plates, resuspended in LB media, passed 6 to 10 times through a 27-gauge needle to break up clumps and chains, then normalized to an OD₆₀₀ of 1. When required for selection, antibiotic concentrations used in this study were as follows – *L. monocytogenes* selections: streptomycin 200µg/mL, chloramphenicol 5µg/mL; *E. coli* selections: ampicillin 50µg/mL; *B. subtilis* selections: chloramphenicol 10µg/mL; tissue culture: gentamicin 50µg/mL.

DNA manipulation and plasmid construction

All DNA manipulation procedures followed standard molecular biology protocols. Primers were synthesized and purified by Integrated DNA Technologies (IDT). HiFi polymerase (Kapa Biosystems, #KK2102), FastDigest restriction enzymes (Thermo Fisher Scientific #FD0274) and T4 DNA ligase (Thermo Scientific #K1423) were used for plasmid construction, with the exception of *pHT01::rha1/2_WT* and

pHT01::rha1/2_MUT which were generated using Gibson Assembly MasterMix (NEB, #E2611S). DNA sequencing was performed by Genewiz Incorporated.

Bacterial infection dot blot

TIB73 cells were lysed in whole cell lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% Triton X-100) with EDTA (1 μ M) and Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, #78442). Protein concentration was determined using the Pierce BCA protein assay kit (Fisher Scientific, #PI23227). Lysates were resuspended in 1X PBS to achieve 2 μ g of protein per 3 μ l, which was the volume spotted out onto nitrocellulose membrane (Bio-Rad, #1620115). Four to six technical replicates were spotted per biological replicate. Membranes were blocked in 1X TBS-T (Tris-buffered saline with 0.1% Triton X-100) with 5% BSA (sterile filtered) for 1 hour at room temperature, incubated for 1 hour with anti-4-hydroxynonenal (HNE) antibody (Abcam #ab46545, diluted 1:1000) and anti-actin antibody (Santa Cruz Biotechnology #sc-8432, diluted 1:500), washed and then incubated with the appropriate IRDye secondary antibodies (LI-COR). Membrane fluorescence was visualized and densitometric analyses were performed using an Odyssey CLx imager and Image Studio software (LI-COR).

Mouse infections

L. monocytogenes was grown overnight statically at 30°C degrees in Brain Heart Infusion (BHI) broth, then back-diluted using 1.2 mL of overnight culture to 4.8 mL of fresh BHI and grown for 1 hour at 37°C degrees shaking. OD₆₀₀ of these cultures were taken and, using the conversion of 1 OD = 1.7x10⁹ CFU, diluted to 5x10⁵ CFU/mL with PBS. 200 μ l were then injected into female mice between 6-8 weeks of age retro-orbitally (1x10⁵ CFU/mouse) and livers and spleens were harvested at 48 hours post infection. Livers were homogenized in 10mL of cold 0.1% IGEPAL and spleens were homogenized in 5mL using a Tissue Tearor Model 985370 (Biospec Products) at 10,000 RPM for 5 seconds/organ. Homogenates were diluted in PBS and plated on LB plates to enumerate CFU. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Washington.

4-HNE histology

Two female mice were infected as outlined above, in addition to one uninfected control mouse. The livers and spleens were harvested at 48 hours post infection and placed in 10% neutral buffered formalin for 24 hours, after which the organs were removed from formalin and placed in PBS for 24 hours. Paraffin embedded tissues were sliced and prepared as slides. Slides were then deparaffinized for 30 minutes at 60°C. All subsequent manipulations were performed on a Leica Bond Automated Immunostainer. Antigen retrieval for GFP was performed by HIER 2 (EDTA) treatment for 20 minutes at 100°C. Antigen retrieval for 4-HNE was performed by citrate treatment for 20 minutes at 100°C. Then a Leica Bond peroxide block was performed for 5 minutes at room temperature, and normal goat serum (10% in TBS) was added for 20 minutes at room temperature. Primary antibody was added (GFP 1:500), (Rab IgG 1:1000), (4-HNE 1:200) in Leica Primary antibody diluent, for 30 minutes at room temperature. Leica Bond Polymer was added for 8 min at room temperature, after which the samples were washed with Leica Bond Mixed Refine (DAB) detection solution twice for 10 minutes at room temperature. Hematoxylin Counterstain was added for 4 minutes and the samples were cleared to xylene. Finally, samples were mounted with synthetic resin mounting medium on a 1.5cm coverslip and imaged with a Hamamatsu Nanozoomer Whole Slide Scanner and a Keyence BZ-X710 Microscope.

L. monocytogenes 4-HNE dot blots

L. monocytogenes were sub-cultured from overnight stationary cultures 1:100 into fresh media and grown to mid-log (0.4-0.8 OD₆₀₀). The bacteria were normalized to OD 1, washed twice with sterile PBS and resuspended in sterile PBS. A range of 4-HNE concentrations were added to the bacteria and the samples were placed at 37°C for 30 minutes. Upon completion, the bacteria were washed twice with PBS and spun at 10,000g for 5 minutes, then resuspended in fresh PBS. The bacteria were then sonicated using a narrow tip sonicator at 20% power, 1 second on 1 second off for 10 seconds and placed on ice. The bacteria were then spun at 4°C at 10,000g for 30 minutes. The subsequent lysate was transferred to fresh Eppendorf tubes containing Halt Proteinase and Phosphatase Inhibitor (Thermo Fisher Scientific, #78442) and stored at -80°C until use. For the dot blots, the protein concentration was normalized using BCA (Fisher Scientific, #PI23227) and 3µg in 3µL was spotted onto nitrocellulose membrane (Bio-Rad, #1620115). The nitrocellulose was then dried, blocked for 45 minutes in 5% dry milk, washed 3 times with TBS-T and

primary 4-HNE antibody was added at 1:200 dilution (Abcam, #ab46545). The antibody was left on overnight at 4°C rocking. The primary antibody was then washed off with TBS-T 3 times and secondary antibody (Licor, #926-32211) was added at 1:8000 for 45 minutes at RT. The secondary antibody was then washed off with TBS-T twice, TBS once and the blot was imaged on a Licor Odyssey Fc. Relative densitometric analysis was performed using Licor Image Studio software.

RNA extraction from broth cultures of *L. monocytogenes*

L. monocytogenes stationary phase overnights were subcultured 1:100 into fresh TSB media and grown shaking at 37°C to mid-log (0.4-0.8 OD₆₀₀). Then a final concentration of 640µM 4-HNE (Cayman #32100) or vehicle (100% ethanol) was added to the bacteria, which continued to grow at 37°C shaking for 20 minutes. After 20 minutes, ice cold 100% methanol was added in equal volume to the culture flask and placed at -20C overnight. The next day the bacteria were spun down and resuspended in 400µL AE buffer (50mM NaOAc pH 5.2, 10mM EDTA in molecular grade water). The resuspended bacteria were then mixed with 400µL acidified 1:1 phenol:chloroform pH 5.2 (Fisher Scientific, # BP1753I) and 40µL 10% sodium dodecyl sulfate (SDS) and was vortexed for 10 minutes in a multi-tube vortexer. The tubes were then transferred to a 65C heat block for ten minutes, after which the mixture was transferred to a Heavy Phase-lock tube (VWR #10847-802) and spun down for 5 minutes at 17,000g. Then the aqueous layer was transferred into tubes containing 1mL 100% ethanol and 40µL 3M NaOAc and placed at -20C for 6 hours. Then tubes were spun at 17,000g for 30 minutes at 4C, the ethanol was aspirated and 500µL 70% ethanol was added. The tubes were then centrifuged at 17,000g for 10 minutes at room temperature and the supernatant was aspirated. The RNA pellet was then dried in a speed vacuum concentrator for 5 minutes and resuspended in RNA-free molecular grade water. The extracted RNA was then treated with DNase (Ambion Life Technologies #AM1907) for an hour at 37°C and used for downstream processing.

RNA-sequencing

RNA was processed using the Ovation Complete Prokaryotic RNA-Seq Library System (NuGEN, #0363-32, 0326-32, 0327-32) according to the manufacturer's instructions to a final pooled library concentration of 3nM. Libraries were sequenced on an Illumina HiSeq 2500 (SR50) at The Genomics Resource at the Fred

Hutchinson Cancer Research Center. Image analysis and base calling were performed using Illumina's Real Time Analysis v1.18.66.3 software, followed by 'demultiplexing' of indexed reads and generation of FASTQ files using Illumina's bcl2fastq Conversion Software v1.8.4. Reads determined by the RTA software to pass Illumina's default quality filters were concatenated for further analysis. The FASTQ files were aligned and analyzed using Rockhopper software (McClure et al., 2013). These data have been deposited to the GEO and are accessible using accession number GSE150188.

qRT-PCR

Bacteria were grown in the same manner as for RNA-seq except 500µM of each aldehyde tested was added to the culture at mid-log (0.4-0.8 OD₆₀₀). The RNA was extracted by the acidified phenol method as listed above and DNase treated and reverse-transcribed using the iScript Reverse Transcription Supermix (Bio-Rad, #1708840). SYBR Green (Thermo Fisher Scientific # K0223) was then used to amplify genes of interest and CT values and relative expression were normalized using CFX Maestro Software (Bio-Rad #12004110).

Intracellular RNA extraction

RNA extraction from macrophages was performed as previously described (Sigal, Pasechnek and Herskovits, 2016). J774 macrophages were seeded at a density of 2.0x10⁷ cells/dish in three 150 mm dishes in 30mL media and incubated overnight. The next day, overnight *L. monocytogenes* culture grown at 30°C was washed twice with PBS and added to the cells at a MOI of 50. After one hour, the cells were washed twice with PBS and media containing gentamicin was added. Eight hours post-infection, cells were washed once with PBS and lysed by addition of cold nuclease-free water. Lysate was collected by scraping and centrifugation at 800g for 3 min at 4°C. Supernatants were passed through 0.45 µm filters in a vacuum apparatus, and filters were collected in conical tubes. Filters were vortexed with 650µL sterile AE buffer for one minute and centrifuged briefly. Bacteria-containing AE buffer was collected and used for immediate RNA extraction as described above.

Survival assays Firmicute panel

Bacteria were inoculated overnight in TSB and grown at 37°C. The next day the bacteria were subcultured 1:1000 into fresh TSB and allowed to reach mid-log (0.4-0.8 OD₆₀₀). At mid-log the ODs of the bacteria were normalized to OD₁, washed twice in sterile PBS and resuspended in sterile PBS. Then the bacteria were diluted 1:100 into sterile PBS in Eppendorf tubes and various concentrations of 4-HNE were added. The bacteria were then placed at 37°C for an hour. After an hour, the bacteria were plated on LB plates to enumerate CFU.

L. monocytogenes competition experiments

Colonies of *L. monocytogenes* were picked off BHI plates and inoculated into 2mL TSB which was then grown shaking at 37°C to mid-log (0.5-0.8 OD₆₀₀). At mid-log the bacteria were normalized to OD₆₀₀ 1, washed twice in sterile PBS and resuspended in sterile PBS. Then the bacteria were diluted 1:100 into sterile PBS and appropriate strains were mixed together in a 1:1 ratio, after which 640μM of 4-HNE or vehicle (ethanol) was added. The bacteria were then placed at 37°C for one hour. 2x concentrated TSB media was added to the bacterial-PBS solution and the cells recovered for an hour at 37°C. The bacteria were then plated on BHI-streptomycin and BHI-chloramphenicol plates for competitive strain differentiation. CFUs were enumerated after 24-48 hours of growth.

Purified protein expression and enzyme kinetics

rha1 and *rha2* ORFs were cloned into pET20B vectors and transformed into BL21 *E. coli* and grown overnight in LB-ampicillin. The overnights were subcultured 1:100 in 2L baffled flasks until mid-log (0.5-0.8 OD₆₀₀) after which 0.5mM IPTG was added. The *pET20b::rha2* *E. coli* were induced for 4 hours at 37°C shaking. 0.1% w/v riboflavin was added to *E. coli pET20B::rha1* and the flask was moved to grow at 17°C for 18 hours shaking. Upon completion, the bacteria were spun down, resuspended in buffer A (30mM K₂HPO₄, 300mM NaCl, pH 8) and sonicated on ice with a large sonicator tip at 80% power 1 sec on 1 sec off for 30 seconds total. They were then spun down at 15,000 g for 45 minutes at 4°C and the supernatant was passed over a nickel resin column (Thermo Fisher Scientific, # PI88222) and eluted using buffer B (30mM K₂HPO₄, 300mM NaCl, 500mM Imidazole, pH 8). The final protein was then transferred into PBS using a

desalting column (Bio-Rad #7322010). For purification of Rha1 10μM FMN (Sigma #F2253) was added at every step of purification.

Enzyme turnover assays were performed at 37°C in 96 well clear bottom plates (Genesee Scientific, #25-104) in a Synergy HTX plate reader in 200μL PBS using 200μM NADPH, 0.2μM enzyme and a range of 4-HNE concentrations from 0 to 0.4mM. Rha1 turnover was performed in the presence of 10μM FMN.

B. subtilis growth curves

B. subtilis expressing genes of interest on the pHT01 plasmid (Nguyen, Phan, and Schumann, 2007) were struck on LB-chloramphenicol plates on day 1 and grown overnight at 30C. On day 2, colonies were re-struck on LB chloramphenicol IPTG 1mM LB plates overnight at 30C. On day 3, biomass was scraped and processed as described in the “bacterial culturing” section above. The bacteria were then normalized to an OD₆₀₀ of 1 and inoculated 1:100 into a 96-well plate containing TSB chloramphenicol and 0.5mM IPTG. 4-HNE was then added to the bacteria at various concentrations and the bacteria were allowed to grow at 37°C in Synergy HTX plate reader for 12 hours with shaking.

B. subtilis 4-HNE dot blot

B. subtilis were grown and processed in the same manner as for the growth curves above. Upon OD₆₀₀ 1 normalization the bacteria were resuspended in TSB-chloramphenicol with 0.5mM IPTG and 250μL of this mix was transferred to a sterile Eppendorf tube into which 640μM 4-HNE was added. The tubes were then incubated at 37°C for 3 hours. At 3 hours, the bacteria were spun down at 17,000g for 1 minute. The supernatant was then aspirated and the pellet flash frozen in liquid nitrogen. At this point, the bacteria were stored at -80C until further processing. Once removed from the -80C, the bacteria were thawed at room temperature and resuspended in 250μL PBS. The bacteria were then sonicated using a narrow tip sonicator at 20% power, 1sec on 1sec off for 10 seconds and placed on ice. The bacteria were then spun at 4°C at 5,000g for 10 minutes. The subsequent lysate was transferred to fresh Eppendorf tubes containing Halt Proteinase and Phosphatase Inhibitor (Thermo Fisher Scientific, #78442) and stored at -80C until use. For the dot blots, the protein concentration was normalized using BCA (Fisher Scientific, #PI23227) and 3μg in 3μL was spotted onto nitrocellulose membrane (Bio-Rad, #1620115). The nitrocellulose was then dried,

blocked for 45 minutes in 5% dry milk, washed three times with TBS-T and primary 4-HNE antibody was added at 1:200 dilution (Abcam, #ab46545). The antibody was left on overnight at 4°C rocking. The primary antibody was then washed off with TBS-T three times and secondary antibody (Licor, #926-32211) was added at 1:8000 for 45 minutes at RT. The secondary was then washed off with TBST twice, TBS once, and then the blot was imaged on a Licor Odyssey Fc. Relative densitometric analysis was performed using Licor Image Studio software.

L. monocytogenes macrophage infection

0.5 x 10⁶ primary murine macrophages from WT C57BL/6 mice were plated in BMM media (DMEM with 10% heat inactivated fetal bovine serum, 1mM L-glutamine, 2mM sodium pyruvate and 10% L929-conditioned medium) in a tissue culture treated 24-well dish (Greiner Bio, #662165) with the addition of 100ng recombinant murine IFN-γ (Peprotech, #315-05) for 18 hours. Inoculants of *L. monocytogenes* were statically grown at 30°C overnight, washed twice with sterile PBS and resuspended in PBS before an MOI of 0.1 was added to each macrophage well. The cells were left to sit for an hour after which all wells were washed twice with PBS and gentamicin was added to all but one well, which was lysed in 500μL water and plated for CFU on LB plates. The remainder of the wells were washed twice with PBS and then lysed and plated for CFU at hours 2, 6 and 9 post-infection.

B. subtilis macrophage survival assay

B. subtilis were grown on LB-chloramphenicol 1mM IPTG plates and processed into LB chloramphenicol as described above. Once the bacteria were normalized to OD₆₀₀ 1 in LB chloramphenicol, an MOI of 100 was added to 0.5 x 10⁶ primary murine macrophages from WT C57BL/6 or C57BL/6 deficient for phox (*gp91^{phox-/-}*) mice (The Jackson Laboratory, stock # 002365) that have been activated using 100ng/well recombinant murine IFN-γ (Peprotech, #315-05) for 18 hours. The cells were spininfected at 200g for 5 minutes. At 1.5 hours, cells were washed 2x with sterile PBS and gentamicin was added to the cells. pBMMs were lysed in 500μL cold water at hours 2, 6, 7, and 8 and plated for CFU. Colonies were enumerated after overnight growth at 30°C on LB plates.

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Supplemental Fig 1.

4-HNE accumulation in the liver during 48-hour murine infection by GFP+ *L. monocytogenes* assessed by immunohistochemistry analysis.

- A. Liver (infected) anti-4-HNE
- B. Liver (uninfected) anti-4-HNE
- C. Liver (infected) anti-GFP 10X magnification
- D. Liver (infected) anti-HNE 10X magnification

Supplemental Fig 2.

- Global gene expression of midlog *L. monocytogenes* in TSB treated with ethanol mock control (panel 1) or 640 μ M 4-HNE (panel 1) for 20 minutes. RPKM: reads per kilobase million.
- Aldehyde structures used in the experiment. 4-HNE: 4-hydroxy-2-nonenal, 4-HHE: 4-hydroxy-2-hexenal, MDA: malondialdehyde, MG: methylglyoxal, PA: propionaldehyde
- CFU post treatment with sublethal concentrations of listed aldehydes. Performed in technical duplicate.
- Competitive index of 1 hour mock treated (ethanol) *L. monocytogenes* in PBS.
- CFU/well of WT and $\Delta rha1\Delta rha2$ *L. monocytogenes* in 100ng recombinant murine IFN- γ activated WT primary murine macrophages. Performed in biological triplicate.
- CFU/organ of WT and $\Delta rha1\Delta rha2$ ($\Delta\Delta$) *L. monocytogenes* at 48 hours intravenous murine infection.

Statistics in (E) are unpaired t-tests comparing WT and $\Delta\Delta$ *L. monocytogenes* CFU at hour 2 post infection. Statistics in (F) are unpaired t-tests comparing WT and $\Delta\Delta$ *L. monocytogenes* CFU within each organ.

Error bars are mean $\pm$ SD. ns > 0.05; *, p < 0.05. In figure (F), the line is drawn at the median of data.

Supplemental Fig 3.

- Modeling of Rha1 and Rha2 binding pockets with FMN (Rha1) and NADPH (Rha2) with corresponding predicted coordinating amino acid labeled. Coordinating amino acids are labeled. Blue indicates carbon, red indicates oxygen, orange indicates phosphorous. Rha1 is a predicted flavin containing reductase. In comparison between the structure of CLA-ER and the homology model of Rha1 predicted by Phyre2 analysis, several conserved residues lining the flavin binding pocket were identified and the N47A mutant was generated. Rha2 is a predicted NADPH reductase. Similar structural models were generated by Phyre2 analysis and a Y195A mutation in a conserved tyrosine residue predicted to be involved in NADPH co-substrate binding was generated. Figure generated in PyMol.
- L. monocytogenes* and *B. subtilis* growth curves in TSB after exposure to various concentrations of 4-HNE. (green circles: 0 μ M, blue triangles: 320 μ M, red triangles: 640 μ M, light blue squares: 1280 μ M). Data performed in technical duplicate and representative of at least three independent experiments.
- Growth curves of *B. subtilis*::*pHT01* expressing various *rha1* and *rha2* constructs in TSB after exposure to various concentrations of 4-HNE. Dotted line represents OD₆₀₀ 0.5. Performed in biological triplicate.
- 4-HNE dot blots of *B. subtilis*::*pHT01* expressing various *rha1* and *rha2* constructs after 3 hours of exposure to 640 μ M 4-HNE in TSB. Performed in biological triplicate. Statistics in (C) is an unpaired t-test comparing the hours to OD 0.5 between *B. subtilis* *pHT01::rha1*-WT and *pHT01::rha1*-N47A. Error bars are mean $\pm$ SD. *, $p < 0.05$.

Supplemental Table 1

Data presented here are sorted by q-value (statistical significance) < 0.05						
Synonym	Product	Expression mock	Expression HNE	qValue wt vs HNE	hne fold over mock	lmo numbers
LMRG_00296	oxidoreductase	3	716	0	238.6666667	lmo0613
LMRG_00295	hypothetical protein	3	542	0	180.6666667	lmo0612
predicted RNA	antisense: LMRG_00484	15	2673	0	178.2	#N/A
LMRG_00999	mercuric ion binding protein	2	332	0	166	lmo1852
LMRG_01869	hypothetical protein	41	6735	0	164.2682927	lmo2829
LMRG_02304	peptidoglycan bound protein	1	137	0	137	lmo0880
predicted RNA	-	19	2296	0	120.8421053	#N/A
LMRG_00177	hypothetical protein	63	6244	0	99.11111111	lmo0496
LMRG_02097	ATP-dependent Clp protease ATP-binding subunit ClpE	47	4494	0	95.61702128	lmo0997
LMRG_01000	Cu2+-exporting ATPase	33	2999	0	90.87878788	lmo1853
LMRG_01001	hypothetical protein	18	1628	0	90.44444444	lmo1854
LMRG_02351	hypothetical protein	3	260	0	86.66666667	lmo0102
LMRG_00484	Ycel like family protein	75	5695	0	75.93333333	lmo0796
LMRG_01602	hypothetical protein	30	1977	0	65.9	lmo2230
predicted RNA	antisense: LMRG_02097	8	527	0	65.875	#N/A
LMRG_02352	hypothetical protein	2	123	0	61.5	lmo0103
LMRG_00333	hypothetical protein	5	306	0	61.2	lmo0646
predicted RNA	antisense: LMRG_01626	3	183	0	61	#N/A
LMRG_01633	OsmC/Ohr family protein	12	668	0	55.66666667	lmo2199
LMRG_02052	hypothetical protein	2	83	0	41.5	lmo0953
LMRG_01632	MarR family transcriptional regulator	69	2700	0	39.13043478	lmo2200
predicted RNA	antisense: LMRG_00177	6	226	0	37.66666667	#N/A
LMRG_00482	hypothetical protein	19	714	0	37.57894737	lmo0794
predicted RNA	-	14	476	0	34	#N/A
LMRG_02677	CtsR family transcriptional regulator	6	188	0	31.33333333	lmo0229
predicted RNA	-	8	244	0	30.5	#N/A
LMRG_00129	hypothetical protein	8	239	0	29.875	lmo0437
LMRG_01948	general stress protein 26	33	973	0	29.48484848	lmo2748
LMRG_00446	hypothetical protein	13	378	0	29.07692308	lmo0758
LMRG_02675	ATP:guanido phosphotransferase	12	344	0	28.66666667	lmo0231
LMRG_01674	hypothetical protein	59	1650	0	27.96610169	lmo2158
LMRG_02676	hypothetical protein	12	319	0	26.58333333	lmo0230
LMRG_00581	Clp protease	51	1311	0	25.70588235	lmo1138
LMRG_00448	hypothetical protein	6	154	0	25.66666667	lmo0760
LMRG_00137	hypothetical protein	15	374	0	24.93333333	lmo0445
LMRG_02646	internalin C2	6	140	1.17E-285	23.33333333	lmo0263
LMRG_00447	glyoxylase	4	92	1.91E-252	23	lmo0759
predicted RNA	-	7	159	7.63E-193	22.71428571	#N/A
LMRG_00583	hypothetical protein	16	344	1.04E-243	21.5	lmo1140
LMRG_01626	ATP-dependent chaperone ClpB	80	1707	2.00E-230	21.3375	lmo2206

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LMRG_01029	30S ribosomal protein S14	40	852	4.55E-255	21.3	lmo1882
LMRG_01627	phosphoglycerate mutase	154	3240	4.37E-233	21.03896104	lmo2205
LMRG_00334	hypothetical protein	37	771	4.93E-241	20.83783784	lmo0647
LMRG_02768	hypothetical protein	4	83	9.40E-207	20.75	lmo1694
LMRG_01754	phosphate transport system regulatory protein PhoU	5	102	6.31E-187	20.4	lmo2494
LMRG_00489	hypothetical protein	14	276	4.94E-214	19.71428571	lmo0800
LMRG_02611	succinyl-diaminopimelate desuccinylase	6	117	2.33E-182	19.5	lmo0265
LMRG_01764	membrane protein	72	1388	3.23E-219	19.27777778	lmo2484
LMRG_02736	hypothetical protein	9	171	3.09E-199	19	lmo2391
predicted RNA	-	43	815	1.23E-167	18.95348837	#N/A
predicted RNA	antisense: LMRG_00335	22	414	7.52E-178	18.81818182	#N/A
LMRG_01219	chaperonin GroS	81	1484	4.17E-188	18.32098765	lmo2069
LMRG_02000	dihydroxyacetone kinase	4	72	1.49E-144	18	lmo2697
predicted RNA	antisense: LMRG_01296	13	233	1.77E-235	17.92307692	#N/A
LMRG_00285	hypothetical protein	6	107	2.64E-116	17.83333333	lmo0602
LMRG_00449	hypothetical protein	4	71	3.23E-110	17.75	lmo0761
LMRG_02693	CDF family cation efflux system protein	22	389	1.54E-161	17.68181818	lmo2575
LMRG_02002	dihydroxyacetone kinase DhaK subunit	23	405	5.57E-165	17.60869565	lmo2695
predicted RNA	-	14	241	9.22E-157	17.21428571	#N/A
LMRG_02674	ATP-dependent Clp protease ATP-binding subunit ClpC	37	626	9.91E-140	16.91891892	lmo0232
LMRG_02808	hypothetical protein	7	118	2.54E-130	16.85714286	lmo2132
LMRG_02146	Mg2+ transporter-C family protein	16	269	5.53E-139	16.8125	lmo2602
LMRG_02218	universal stress protein	12	201	1.25E-136	16.75	lmo2673
LMRG_00666	hypothetical protein	14	234	4.12E-137	16.71428571	lmo1220
LMRG_00293	internalin	6	100	2.29E-133	16.66666667	lmo0610
LMRG_00411	pyruvate oxidase	42	697	3.15E-148	16.5952381	lmo0722
LMRG_02697	nicotinamidase	24	398	8.74E-139	16.58333333	lmo2571
LMRG_02701	hypothetical protein	22	359	3.17E-133	16.31818182	lmo2567
LMRG_01972	DNA binding 3-demethylubiquinone-9 3-methyltransferase domain-containing protein	51	784	3.22E-127	15.37254902	lmo2724
predicted RNA	antisense: LMRG_02046	14	213	3.84E-177	15.21428571	#N/A
LMRG_01675	hypothetical protein	9	134	8.77E-110	14.88888889	lmo2157
LMRG_00221	tagatose 1,6-diphosphate aldolase	183	2721	1.34E-99	14.86885246	lmo0539
LMRG_01814	glutamate decarboxylase	33	484	1.49E-147	14.66666667	lmo2434
LMRG_02001	dihydroxyacetone kinase L subunit	16	233	1.17E-98	14.5625	lmo2696
predicted RNA	antisense: LMRG_02042	16	232	1.45E-174	14.5	#N/A
predicted RNA	antisense: LMRG_00221	15	214	9.08E-169	14.26666667	#N/A
LMRG_02003	OsmC/Ohr family protein	35	499	1.57E-103	14.25714286	lmo0903
predicted RNA	antisense: LMRG_00364	14	198	4.14E-134	14.14285714	#N/A
LMRG_01780	Clp protease	241	3361	2.53E-101	13.94605809	lmo2468
predicted RNA	antisense: LMRG_02779	31	431	8.30E-100	13.90322581	#N/A
LMRG_02382	hypothetical protein	5	67	1.75E-124	13.4	lmo0133
predicted RNA	-	35	469	6.70E-92	13.4	#N/A

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predicted RNA	antisense: LMRG_00073	15	199	1.12E-122	13.26666667	#N/A
predicted RNA	antisense: LMRG_00704	24	317	5.17E-154	13.20833333	#N/A
LMRG_00131	hypothetical protein	6	79	3.36E-70	13.16666667	lmo0439
LMRG_01236	peptidoglycan binding protein	16	207	4.29E-84	12.9375	lmo2085
LMRG_02633	50S ribosomal protein L25 Ctc-form	13	167	3.18E-71	12.84615385	lmo0211
predicted RNA	antisense: LMRG_01869	23	295	1.89E-146	12.82608696	#N/A
predicted RNA	-	37	465	4.87E-132	12.56756757	#N/A
LMRG_02472	arginine deiminase	42	527	1.70E-94	12.54761905	lmo0043
LMRG_00273	hypothetical protein	5	62	1.74E-56	12.4	lmo0591
LMRG_01761	hypothetical protein	20	241	1.17E-69	12.05	lmo2487
LMRG_02147	amidase	10	120	3.25E-63	12	lmo2603
LMRG_01619	hypothetical protein	41	486	2.61E-69	11.85365854	lmo2213
LMRG_02290	hypothetical protein	46	514	4.62E-52	11.17391304	#N/A
LMRG_02036	hypothetical protein	7	77	1.99E-69	11	lmo0937
LMRG_02295	hypothetical protein	11	121	8.24E-51	11	lmo0871
LMRG_01753	phosphate ABC transporter ATP-binding protein	13	142	2.10E-50	10.92307692	lmo2495
predicted RNA	-	36	390	1.30E-88	10.83333333	#N/A
predicted RNA	-	23	247	1.02E-92	10.73913043	#N/A
LMRG_01763	PspC domain-containing protein	46	485	9.81E-47	10.54347826	lmo2485
predicted RNA	antisense: LMRG_00365	79	826	2.26E-45	10.4556962	#N/A
LMRG_02013	succinate-semialdehyde dehydrogenase	31	322	1.48E-65	10.38709677	lmo0913
LMRG_00341	hypothetical protein	81	831	1.21E-48	10.25925926	lmo0654
LMRG_02696	riboflavin biosynthesis protein RibD domain-containing protein	35	355	2.82E-47	10.14285714	lmo2572
LMRG_02695	NADPH2:quinone reductase	14	142	9.70E-42	10.14285714	lmo2573
LMRG_02247	hypothetical protein	113	1146	3.80E-61	10.14159292	lmo0822
LMRG_00272	hypothetical protein	16	158	4.99E-44	9.875	lmo0590
LMRG_02732	hypothetical protein	7	69	3.37E-37	9.857142857	lmo2387
LMRG_02398	zinc transport system substrate-binding protein	9	86	1.56E-31	9.555555556	lmo0153
predicted RNA	-	30	285	1.62E-51	9.5	#N/A
LMRG_02094	hypothetical protein	8	76	1.20E-33	9.5	lmo0994
predicted RNA	antisense: LMRG_00288	29	266	1.50E-55	9.172413793	#N/A
LMRG_02622	listeriolysin regulatory protein	55	490	1.14E-42	8.909090909	lmo0200
predicted RNA	-	128	1134	3.82E-31	8.859375	#N/A
LMRG_02011	hypothetical protein	24	211	4.50E-33	8.791666667	lmo0911
predicted RNA	-	36	315	5.00E-29	8.75	#N/A
LMRG_02042	hypothetical protein	574	4865	2.49E-28	8.475609756	lmo0944
predicted RNA	-	24	201	3.30E-25	8.375	#N/A
LMRG_01757	hydrolase	58	475	2.44E-33	8.189655172	lmo2491
LMRG_01153	aryl-alcohol dehydrogenase	11	90	2.59E-25	8.181818182	lmo2005
LMRG_01816	hypothetical protein	26	212	1.29E-25	8.153846154	lmo2432
LMRG_01353	hypothetical protein	15	120	9.60E-25	8	lmo1613
predicted RNA	-	26	207	6.40E-37	7.961538462	#N/A

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predicted RNA	antisense: LMRG_01149	29	229	1.18E-33	7.896551724	#N/A
LMRG_02734	hypothetical protein	58	456	1.24E-26	7.862068966	lmo2389
predicted RNA	-	36	277	1.50E-21	7.694444444	#N/A
LMRG_00897	transcriptional regulator ZurR	91	699	3.06E-30	7.681318681	lmo1445
LMRG_02476	hypothetical protein	17	130	3.36E-23	7.647058824	lmo0047
predicted RNA	-	82	622	1.82E-21	7.585365854	#N/A
LMRG_02738	hypothetical protein	14	106	9.18E-22	7.571428571	lmo2393
LMRG_01680	thioredoxin	99	742	6.82E-26	7.494949495	lmo2152
predicted RNA	-	104	778	4.76E-23	7.480769231	#N/A
LMRG_01444	hypothetical protein	111	823	2.31E-27	7.414414414	lmo1526
LMRG_01755	HTH-type transcriptional repressor czrA	25	185	2.83E-19	7.4	lmo2493
LMRG_02579	hypothetical protein	284	2051	5.97E-09	7.221830986	lmo0292
predicted RNA	antisense: LMRG_00364	33	235	8.76E-27	7.121212121	#N/A
LMRG_00357	hypothetical protein	10	71	8.56E-18	7.1	lmo0669
LMRG_02415	hypothetical protein	174	1233	2.19E-17	7.086206897	lmo0170
predicted RNA	antisense: LMRG_02779	31	216	4.02E-31	6.967741935	#N/A
LMRG_01296	zinc transport system substrate-binding protein	339	2339	7.35E-14	6.899705015	lmo1671
LMRG_00928	heat-inducible transcription repressor HrcA	139	954	1.44E-19	6.863309353	lmo1475
LMRG_00292	hypothetical protein	38	259	2.51E-19	6.815789474	lmo0609
LMRG_01737	sigma-54 modulation protein	92	619	9.23E-23	6.72826087	lmo2511
LMRG_02884	secreted protein	18	121	4.42E-17	6.722222222	lmo0479
LMRG_00926	chaperone DnaK	276	1849	6.39E-07	6.699275362	lmo1473
LMRG_01028	hypothetical protein	81	537	1.75E-17	6.62962963	lmo1881
LMRG_02679	hypothetical protein	10	66	3.04E-17	6.6	lmo2588
predicted RNA	-	31	202	2.61E-26	6.516129032	#N/A
LMRG_02215	hypothetical protein	14	91	4.97E-16	6.5	lmo2670
LMRG_02239	hypothetical protein	269	1736	1.84E-15	6.453531599	lmo2692
LMRG_02731	hypothetical protein	13	83	5.22E-14	6.384615385	lmo2386
LMRG_01140	alpha-acetolactate decarboxylase	347	2173	1.58E-12	6.262247839	lmo1992
LMRG_00521	hypothetical protein	17	106	2.08E-13	6.235294118	lmo1059
predicted RNA	-	133	829	5.00E-14	6.233082707	#N/A
LMRG_00743	glycerol-3-phosphate dehydrogenase	32	198	5.21E-18	6.1875	lmo1293
LMRG_01431	glycerol uptake facilitator protein	12	73	6.40E-12	6.083333333	lmo1539
predicted RNA	-	56	340	7.37E-17	6.071428571	#N/A
LMRG_01762	hypothetical protein	31	188	2.63E-16	6.064516129	lmo2486
LMRG_01218	chaperonin GroL	281	1684	5.23E-06	5.992882562	lmo2068
LMRG_02694	hypothetical protein	241	1407	4.30E-14	5.838174274	lmo2574
LMRG_01756	hypothetical protein	338	1964	2.88E-13	5.810650888	lmo2492
predicted RNA	-	168	971	2.98E-13	5.779761905	#N/A
LMRG_02700	hypothetical protein	14	80	2.05E-11	5.714285714	lmo2568
LMRG_01573	hypothetical protein	14	79	1.95E-09	5.642857143	lmo2258
LMRG_02331	hypothetical protein	24	133	3.16E-10	5.541666667	lmo0083

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LMRG_00472	mannose-specific PTS system IIA component	21	116	1.38E-10	5.523809524	lmo0784
LMRG_01815	tributylin esterase	120	658	1.48E-10	5.483333333	lmo2433
LMRG_00679	thioredoxin	104	569	2.18E-12	5.471153846	lmo1233
LMRG_02248	aldo/keto reductase	111	604	2.23E-10	5.441441441	lmo0823
LMRG_02813	hypothetical protein	13	70	3.75E-09	5.384615385	lmo1789
predicted RNA	antisense: LMRG_00288	144	768	7.93E-11	5.333333333	#N/A
LMRG_02609	hypothetical protein	25	131	1.77E-10	5.24	lmo0267
LMRG_02055	N-acetylglucosamine-6-phosphate deacetylase	168	880	5.95E-09	5.238095238	lmo0956
LMRG_01656	hypothetical protein	17	89	1.72E-09	5.235294118	lmo2176
LMRG_01794	hypothetical protein	13	68	9.42E-10	5.230769231	lmo2454
LMRG_02812	MarR family transcriptional regulator	39	202	6.79E-10	5.179487179	lmo1788
LMRG_01384	thiol peroxidase	43	222	4.11E-10	5.162790698	lmo1583
LMRG_00345	endonuclease III	13	66	1.24E-08	5.076923077	lmo0658
LMRG_00879	osmoprotectant transport system permease	43	218	2.60E-10	5.069767442	lmo1427
LMRG_02056	glucosamine-6-phosphate isomerase	59	299	8.90E-12	5.06779661	lmo0957
predicted RNA	antisense: LMRG_02397	80	403	8.77E-09	5.0375	#N/A
LMRG_05029	Met tRNA	81	403	5.84E-09	4.975308642	#N/A
LMRG_00878	osmoprotectant transport system substrate-binding protein	79	391	4.62E-09	4.949367089	lmo1426
LMRG_01641	arsenate reductase	24	118	1.05E-07	4.916666667	lmo2191
LMRG_02054	hypothetical protein	50	243	8.38E-11	4.86	lmo0955
LMRG_01491	HTH-type transcriptional regulator ytlI	27	131	1.45E-08	4.851851852	lmo2352
LMRG_01795	hypothetical protein	161	779	2.32E-08	4.838509317	lmo2453
predicted RNA	antisense: LMRG_02397	53	256	1.65E-08	4.830188679	#N/A
LMRG_01822	arsenate reductase	72	346	9.16E-09	4.805555556	lmo2426
predicted RNA	antisense: LMRG_01678	93	446	3.44E-08	4.795698925	#N/A
LMRG_00469	mannose-specific PTS system IID component	13	62	1.15E-08	4.769230769	lmo0781
LMRG_02397	peptide/nickel transport system substrate-binding protein	1002	4775	0.005247719	4.765469062	lmo0152
LMRG_00488	hypothetical protein	107	509	3.27E-08	4.757009346	lmo0799
LMRG_00826	peptidase T-like protein	62	294	2.14E-08	4.741935484	lmo1375
LMRG_00877	osmoprotectant transport system permease	43	203	7.10E-09	4.720930233	lmo1425
predicted RNA	antisense: LMRG_01840 LMRG_01839	272	1280	5.55E-09	4.705882353	#N/A
LMRG_01066	hypothetical protein	73	340	3.46E-10	4.657534247	lmo1919
LMRG_01679	ribonucleotide reductase-associated flavodoxin	204	949	1.84E-07	4.651960784	lmo2153
LMRG_00470	mannose-specific PTS system IIC component	43	200	7.17E-09	4.651162791	lmo0782
LMRG_00294	FMN-dependent NADH-azoreductase 1	24	111	2.83E-07	4.625	lmo0611
LMRG_01469	hypothetical protein	95	436	2.53E-08	4.589473684	lmo1501
LMRG_00132	hypothetical protein	50	227	1.15E-07	4.54	lmo0440
LMRG_00925	chaperone DnaJ	105	471	3.15E-07	4.485714286	lmo1472
LMRG_02764	hypothetical protein	228	1021	2.47E-06	4.478070175	lmo1690
LMRG_01207	protoheme IX farnesyltransferase	15	67	1.93E-06	4.466666667	lmo2057
LMRG_01068	hypothetical protein	24	107	3.84E-07	4.458333333	lmo1921
predicted RNA	-	47	209	2.45E-11	4.446808511	#N/A

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LMRG_00885	glutathione reductase	18	80	1.03E-06	4.444444444	lmo1433
LMRG_02076	hypothetical protein	18	80	3.98E-06	4.444444444	lmo0976
LMRG_00290	ABC transporter	70	311	4.35E-07	4.442857143	lmo0607
predicted RNA	antisense: LMRG_01556	60	266	2.64E-06	4.433333333	#N/A
LMRG_01154	acetolactate synthase catabolic	85	376	2.83E-07	4.423529412	lmo2006
LMRG_00927	co-chaperone GrpE	74	326	9.01E-09	4.405405405	lmo1474
LMRG_02350	hypothetical protein	164	704	9.58E-09	4.292682927	lmo0101
LMRG_02320	sigma-B negative regulator	24	103	2.85E-06	4.291666667	lmo0896
LMRG_00836	hypothetical protein	207	885	8.45E-06	4.275362319	lmo1384
LMRG_00880	osmoprotectant transport system ATP-binding protein	62	264	2.14E-06	4.258064516	lmo1428
predicted RNA	antisense: LMRG_02384	72	306	6.76E-06	4.25	#N/A
predicted RNA	-	42	177	2.49E-09	4.214285714	#N/A
LMRG_02053	hypothetical protein	32	133	8.57E-06	4.15625	lmo0954
LMRG_02666	ribonuclease III family protein	25	103	1.17E-05	4.12	lmo0240
predicted RNA	antisense: LMRG_02397	54	221	7.03E-06	4.092592593	#N/A
LMRG_00891	superoxide dismutase	110	450	2.97E-06	4.090909091	lmo1439
LMRG_00289	transcription regulator MarR family protein	183	748	8.08E-06	4.087431694	lmo0606
LMRG_02216	hypothetical protein	16	65	2.31E-05	4.0625	lmo2671
LMRG_01690	hypothetical protein	34	138	2.03E-06	4.058823529	lmo2557
LMRG_01850	phosphatidylglycerophosphatase A	76	307	9.10E-07	4.039473684	lmo2398
LMRG_02758	hypothetical protein	26	105	9.71E-06	4.038461538	lmo1684
LMRG_02845	GntR family transcriptional regulator	34	137	6.75E-06	4.029411765	lmo0902
LMRG_00291	ABC transporter	152	610	1.44E-04	4.013157895	lmo0608
LMRG_02414	sugar uptake protein	28	112	1.18E-05	4	lmo0169
predicted RNA	antisense: LMRG_05509	42	167	3.33E-06	3.976190476	#N/A
LMRG_01770	thioredoxin-disulfide reductase	90	356	2.26E-05	3.955555556	lmo2478
LMRG_02869	mannose-specific PTS system IIB component	17	67	8.85E-05	3.941176471	lmo0783
predicted RNA	antisense: LMRG_05515	42	165	5.69E-06	3.928571429	#N/A
LMRG_00261	hypothetical protein	56	220	1.95E-05	3.928571429	lmo0579
LMRG_01851	thioredoxin-like protein	23	89	1.88E-05	3.869565217	lmo2397
LMRG_01678	ribonucleoside-diphosphate reductase	617	2371	0.039824699	3.842787682	lmo2154
LMRG_02667	cysteinyl-tRNA synthetase	54	207	3.60E-05	3.833333333	lmo0239
LMRG_02665	TrmH family RNA methyltransferase group 3	63	241	1.51E-06	3.825396825	lmo0241
LMRG_00884	hypothetical protein	337	1276	2.19E-04	3.786350148	lmo1432
LMRG_02082	deblocking aminopeptidase	22	83	5.76E-05	3.772727273	lmo0982
LMRG_00266	hypothetical protein	135	508	5.74E-05	3.762962963	lmo0584
LMRG_00707	hypothetical protein	54	203	2.40E-06	3.759259259	lmo1258
predicted RNA	antisense: LMRG_02771	49	184	7.32E-08	3.755102041	#N/A
LMRG_00032	hypothetical protein	27	101	1.99E-04	3.740740741	lmo0341
LMRG_05008	Arg tRNA	50	187	1.45E-04	3.74	#N/A
LMRG_00898	zinc transport system permease	61	228	1.63E-05	3.737704918	lmo1446
LMRG_02663	RNA polymerase sporulation-specific sigma factor	181	673	1.27E-04	3.718232044	lmo0243

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LMRG_01937	hypothetical protein	21	78	9.21E-05	3.714285714	lmo2759
LMRG_00278	hypothetical protein	78	289	7.49E-06	3.705128205	lmo0596
LMRG_02786	carbonic anhydrase	33	122	8.27E-05	3.696969697	lmo0811
LMRG_01114	hypothetical protein	153	558	3.50E-04	3.647058824	lmo1967
LMRG_01297	hypothetical protein	117	425	5.59E-05	3.632478632	lmo1670
predicted RNA	antisense: LMRG_00515 LMRG_00514	119	430	1.75E-04	3.613445378	#N/A
LMRG_01870	hypothetical protein	53	190	2.12E-04	3.58490566	lmo2828
LMRG_00337	membrane protein	31	111	2.04E-04	3.580645161	lmo0650
LMRG_02349	hypothetical protein	169	605	6.05E-05	3.579881657	lmo0100
LMRG_00753	cell division suppressor protein yneA	19	68	5.66E-04	3.578947368	lmo1303
LMRG_00745	host factor-I protein	243	867	5.76E-05	3.567901235	lmo1295
LMRG_02959	multidrug resistance protein	37	131	1.00E-05	3.540540541	lmo1712
predicted RNA	antisense: LMRG_01609	75	262	5.43E-04	3.493333333	#N/A
LMRG_00835	isopentenyl-diphosphate delta-isomerase type 2	292	1019	0.002406516	3.489726027	lmo1383
LMRG_02063	hypothetical protein	70	244	1.20E-04	3.485714286	lmo0964
LMRG_02317	anti-sigma-B factor antagonist	77	267	1.85E-04	3.467532468	lmo0893
LMRG_00354	antibiotic transport system ATP-binding protein	82	282	4.34E-04	3.43902439	lmo0667
LMRG_00899	zinc uptake system ATP-binding protein zurA	55	189	3.94E-05	3.436363636	lmo1447
LMRG_01823	glycine cleavage system H protein	37	127	4.03E-04	3.432432432	lmo2425
LMRG_02050	hypothetical protein	38	130	2.17E-04	3.421052632	lmo0951
LMRG_01811	hypothetical protein	56	191	4.20E-04	3.410714286	lmo2437
LMRG_00258	hypothetical protein	18	61	3.96E-04	3.388888889	lmo0576
LMRG_02643	type III pantothenate kinase	149	504	5.73E-04	3.382550336	lmo0221
LMRG_02644	chaperonin HslO	41	138	1.68E-04	3.365853659	lmo0222
LMRG_01556	gp27	114	383	4.07E-04	3.359649123	#N/A
LMRG_02714	general stress protein 13	143	480	6.94E-05	3.356643357	lmo2369
LMRG_01615	hypothetical protein	87	292	3.01E-04	3.356321839	lmo2217
LMRG_02735	hypothetical protein	159	527	7.10E-04	3.314465409	lmo2390
LMRG_02445	cytochrome aa3 quinol oxidase subunit IV	31	102	0.00194844	3.290322581	lmo0016
LMRG_00520	hypothetical protein	58	190	0.001066446	3.275862069	lmo1058
LMRG_01365	hypothetical protein	81	265	2.92E-04	3.271604938	lmo1602
predicted RNA	antisense: LMRG_02224	73	238	0.001730138	3.260273973	#N/A
LMRG_01796	carboxylesterase	47	153	4.39E-04	3.255319149	lmo2452
predicted RNA	antisense: LMRG_05511	75	244	0.001100033	3.253333333	#N/A
LMRG_01357	thioredoxin-like protein ytpP	29	93	0.003804507	3.206896552	lmo1609
LMRG_02718	cellobiose-specific PTS system IIB component	31	99	0.002545682	3.193548387	lmo2373
LMRG_00235	hypothetical protein	410	1307	0.006174283	3.187804878	lmo0553
LMRG_02293	hypothetical protein	22	70	0.001841892	3.181818182	lmo0869
predicted RNA	antisense: LMRG_02834	111	353	0.001722101	3.18018018	#N/A
LMRG_02245	hypothetical protein	29	92	0.001924661	3.172413793	lmo0820
LMRG_05032	Arg tRNA	58	184	0.002078209	3.172413793	#N/A
predicted RNA	antisense: LMRG_01571	112	355	0.001936706	3.169642857	#N/A

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LMRG_00103	pyruvate,water dikinase	25	79	0.001765837	3.16	lmo0410
LMRG_02041	starvation-inducible DNA-binding protein	846	2651	0.029780284	3.13356974	lmo0943
LMRG_01113	hypothetical protein	94	293	0.001053115	3.117021277	lmo1966
LMRG_02007	hypothetical protein	83	258	0.001066446	3.108433735	lmo0907
LMRG_02057	GntR family transcriptional regulator	48	149	0.001066276	3.104166667	lmo0958
LMRG_02610	hypothetical protein	31	96	0.002731204	3.096774194	lmo0266
LMRG_01575	protease I	32	99	0.002100988	3.09375	lmo2256
LMRG_02263	MarR family transcriptional regulator	32	99	0.002865296	3.09375	lmo0840
LMRG_01833	FeS assembly ATPase SufC	278	857	0.006208014	3.082733813	lmo2415
LMRG_02437	diamine N-acetyltransferase	33	101	0.002572121	3.060606061	lmo0009
LMRG_02792	hypothetical protein	76	231	0.001558446	3.039473684	#N/A
predicted RNA	-	73	220	0.00451781	3.01369863	#N/A
LMRG_02375	hypothetical protein	33	99	0.004438201	3	lmo0126
LMRG_00417	hypothetical protein	45	133	0.004725323	2.955555556	lmo0729
LMRG_01568	hypothetical protein	42	124	0.002673939	2.952380952	lmo2263
predicted RNA	-	88	256	0.005176832	2.909090909	#N/A
LMRG_02343	hypothetical protein	21	61	0.008216044	2.904761905	lmo0094
predicted RNA	-	82	236	0.003455037	2.87804878	#N/A
LMRG_02077	hypothetical protein	37	106	0.00581421	2.864864865	lmo0977
LMRG_02938	serine O-acetyltransferase	22	63	0.013315824	2.863636364	lmo0238
predicted RNA	antisense: LMRG_00233	117	333	0.008711523	2.846153846	#N/A
LMRG_01576	hypothetical protein	37	105	0.017136024	2.837837838	lmo2255
LMRG_01402	DNA polymerase I	55	156	0.005065534	2.836363636	lmo1565
LMRG_02117	PTS system glucose-specific transporter subunit IIA	82	230	0.0032698	2.804878049	lmo1017
LMRG_00080	hypothetical protein	209	584	0.010165448	2.794258373	lmo0387
LMRG_02274	hypothetical protein	99	275	0.010550408	2.777777778	lmo0851
LMRG_01777	NADPH dehydrogenase	31	86	0.006728628	2.774193548	lmo2471
predicted RNA	antisense: LMRG_05504	63	174	0.018568862	2.761904762	#N/A
LMRG_02089	hypothetical protein	57	157	0.013197896	2.754385965	lmo0989
LMRG_00336	hypothetical protein	23	63	0.025737754	2.739130435	lmo0649
predicted RNA	antisense: LMRG_05508	87	237	0.012951781	2.724137931	#N/A
LMRG_00236	hypothetical protein	89	242	0.018535735	2.719101124	lmo0554
LMRG_02259	hypothetical protein	109	295	0.003559122	2.706422018	lmo0836
LMRG_00512	polypeptide deformylase	243	649	0.008885988	2.670781893	lmo1051
LMRG_00081	hypothetical protein	48	128	0.018674629	2.666666667	lmo0388
LMRG_00173	transcription regulator LysR	29	77	0.018961544	2.655172414	lmo0492
LMRG_01007	peptide-methionine (S)-S-oxide reductase	482	1278	0.041761156	2.651452282	lmo1860
predicted RNA	antisense: LMRG_05517	97	257	0.014391515	2.649484536	#N/A
predicted RNA	antisense: LMRG_00479	94	248	0.023529652	2.638297872	#N/A
LMRG_02673	DNA repair protein RadA	41	108	0.010760569	2.634146341	lmo0233
LMRG_01624	hypothetical protein	63	165	0.005014105	2.619047619	lmo2208
LMRG_00519	L-lactate dehydrogenase	79	206	0.021438437	2.607594937	lmo1057

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LMRG_00458	transcriptional regulator	25	65	0.018961544	2.6	lmo0770
LMRG_01623	hypothetical protein	25	65	0.021265447	2.6	lmo2209
LMRG_01403	formamidopyrimidine-DNA glycosylase	34	88	0.021453834	2.588235294	lmo1564
predicted RNA	antisense: LMRG_05505	123	317	0.025246604	2.577235772	#N/A
LMRG_00701	hypothetical protein	178	457	0.02523123	2.56741573	#N/A
LMRG_02615	HlyD family secretion protein	39	100	0.021634833	2.564102564	lmo0193
predicted RNA	antisense: LMRG_05514	96	245	0.03036087	2.552083333	#N/A
LMRG_01055	hypothetical protein	42	107	0.043428349	2.547619048	lmo1908
predicted RNA	antisense: LMRG_00554	67	170	8.34E-04	2.537313433	#N/A
LMRG_02244	hypothetical protein	89	225	0.030493736	2.528089888	lmo0819
LMRG_01069	hypothetical protein	42	106	0.01320132	2.523809524	lmo1922
LMRG_00205	hypothetical protein	48	121	0.033431119	2.520833333	lmo0524
LMRG_00090	hypothetical protein	52	131	0.032359701	2.519230769	lmo0397
LMRG_00442	hypothetical protein	54	136	0.019786837	2.518518519	lmo0754
LMRG_02037	protein-tyrosine phosphatase	157	392	0.033431119	2.496815287	lmo0938
predicted RNA	-	238	591	0.026337976	2.483193277	#N/A
LMRG_02386	peptide/nickel transport system permease	146	362	0.020624817	2.479452055	lmo0137
LMRG_02635	peptidyl-tRNA hydrolase	54	131	0.039822953	2.425925926	lmo0213
LMRG_00280	transcription regulator CRP/FNR family protein	26	63	0.044916888	2.423076923	lmo0597
LMRG_01847	hydrolase	43	104	0.032798066	2.418604651	lmo2401
LMRG_01856	transcriptional regulator	46	111	0.016880131	2.413043478	lmo2842
LMRG_01036	hypothetical protein	107	255	0.033198142	2.38317757	lmo1889
LMRG_01051	hypothetical protein	47	112	0.020005519	2.382978723	lmo1904
LMRG_02049	hypothetical protein	134	319	0.044879257	2.380597015	lmo0950
LMRG_02946	hypothetical protein	252	595	0.021633399	2.361111111	#N/A
LMRG_02108	hypothetical protein	252	586	0.041761156	2.325396825	lmo1008
LMRG_00452	lipoate-protein ligase A	37	86	0.038929387	2.324324324	lmo0764
LMRG_01834	FeS assembly protein SufD	118	273	0.042967549	2.313559322	lmo2414
LMRG_01047	aspartate 1-decarboxylase	341	784	0.044283805	2.299120235	lmo1900
LMRG_01054	dihydrodipicolinate reductase	64	147	0.02980491	2.296875	lmo1907
LMRG_02444	cytochrome aa3 quinol oxidase subunit III	64	145	0.038929387	2.265625	lmo0015
predicted RNA	-	353	799	0.04803035	2.263456091	#N/A
LMRG_01210	hypothetical protein	76	169	0.035520736	2.223684211	lmo2060
LMRG_02727	multicomponent Na ⁺ :H ⁺ antiporter subunit E	124	63	0.032125345	0.508064516	lmo2382
LMRG_02008	hypothetical protein	65	33	0.020005519	0.507692308	lmo0908
LMRG_01700	homoserine dehydrogenase	201	101	0.047175323	0.502487562	lmo2547
LMRG_02800	sodium transport system permease	48	24	0.035658352	0.5	lmo2140
LMRG_02585	aminotransferase	52	26	0.038929387	0.5	lmo0286
LMRG_02250	hydroxymethylglutaryl-CoA reductase	48	24	0.03981199	0.5	lmo0825
LMRG_00681	aspartate kinase	182	91	0.040562131	0.5	lmo1235
LMRG_02620	UDP-N-acetylglucosamine diphosphorylase/glucosamine-1-phosphate N- acetyltransferase	130	65	0.04364084	0.5	lmo0198
LMRG_01394	acetyl-CoA carboxylase carboxyl transferase subunit beta	48	24	0.044283805	0.5	lmo1573

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LMRG_02124	hypothetical protein	44	22	0.047789583	0.5	lmo1024
LMRG_02312	hypothetical protein	283	141	0.04803035	0.498233216	lmo0888
LMRG_00784	secreted protein	257	128	0.04215241	0.498054475	lmo1334
LMRG_00413	hypothetical protein	350	173	0.039495623	0.494285714	lmo0724
LMRG_01002	D-alanyl-D-alanine carboxypeptidase	81	40	0.023124458	0.49382716	lmo1855
predicted RNA	-	1606	790	0.033748261	0.491905355	#N/A
LMRG_02641	tRNA(Ile)-lysine synthetase	147	72	0.03871668	0.489795918	lmo0219
LMRG_01347	D-amino acid aminotransferase	333	163	0.03990536	0.489489489	lmo1619
LMRG_00908	hypothetical protein	190	93	0.002408231	0.489473684	lmo1456
LMRG_00995	manganese transport system membrane protein mntC	178	87	0.029152211	0.488764045	lmo1848
LMRG_00537	teichoic acids export ATP-binding protein tagH	84	41	0.020653577	0.488095238	lmo1075
LMRG_02621	ribose-phosphate pyrophosphokinase 1	355	173	0.038289935	0.487323944	lmo0199
LMRG_01143	deoxyribose-phosphate aldolase	142	69	0.037493838	0.485915493	lmo1995
LMRG_00883	hypothetical protein	266	129	0.041730146	0.484962406	lmo1431
LMRG_02502	phosphoribosylformylglycinamide synthase II	31	15	0.021382699	0.483870968	lmo1769
LMRG_02167	30S ribosomal protein S17	461	223	0.034746604	0.48373102	lmo2623
LMRG_01773	phosphoglucosyltransferase/phosphomannomutase	54	26	0.03885516	0.481481481	lmo2475
LMRG_01892	hypothetical protein	52	25	0.016954793	0.480769231	lmo2804
LMRG_00993	multidrug resistance protein norM	50	24	0.015638073	0.48	lmo1846
LMRG_00685	rdgB/HAM1 family non-canonical purine NTP pyrophosphatase	221	106	0.026237749	0.479638009	lmo1239
predicted RNA	-	1672	800	0.013365301	0.4784689	#N/A
LMRG_01697	hypothetical protein	46	22	0.034356578	0.47826087	lmo2550
LMRG_02180	1,4-dihydroxy-2-naphthoate octaprenyltransferase	67	32	0.018674629	0.47761194	lmo2635
LMRG_02684	response regulator	74	35	0.023677106	0.472972973	lmo2583
LMRG_02879	hypothetical protein	55	26	0.009199536	0.472727273	lmo0528
LMRG_01168	isoleucyl-tRNA synthetase	70	33	5.73E-04	0.471428571	lmo2019
LMRG_00536	teichoic acid transport system permease	51	24	0.029891732	0.470588235	lmo1074
LMRG_00965	ribulose-phosphate 3-epimerase	149	70	0.026728806	0.469798658	lmo1818
LMRG_02252	pyruvate:ferredoxin oxidoreductase	66	31	0.026347347	0.46969697	lmo0829
LMRG_01406	replication initiation and membrane attachment protein	330	155	0.035630534	0.46969697	lmo1561
LMRG_01374	NifS/icsS protein	49	23	0.013354675	0.469387755	lmo1593
predicted RNA	-	868	406	0.020408525	0.467741935	#N/A
LMRG_00211	N-acetylglucosaminyltransferase	30	14	0.044813253	0.466666667	lmo0529
LMRG_00281	biotin biosynthesis protein BioY	133	62	0.010993339	0.466165414	#N/A
LMRG_01793	phosphopyruvate hydratase	1243	579	0.02779667	0.465808528	lmo2455
LMRG_00301	hypothetical protein	41	19	0.021453834	0.463414634	lmo0618
LMRG_01330	ABC transporter ATP-binding protein	237	109	0.021382699	0.459915612	lmo1636
LMRG_01284	hypothetical protein	192	88	0.023052624	0.458333333	lmo2130
LMRG_01373	septation ring formation regulator EzrA	313	143	0.030958788	0.45686901	lmo1594
LMRG_00905	hypothetical protein	125	57	0.007034615	0.456	lmo1453
LMRG_01063	beta-lactamase	145	66	0.015455111	0.455172414	lmo1916
LMRG_01685	hypothetical protein	33	15	0.047803696	0.454545455	lmo2147

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LMRG_00758	hypothetical protein	97	44	0.008216044	0.453608247	lmo1308
LMRG_00703	alpha-phosphotrehalase	181	82	0.02165941	0.453038674	lmo1254
LMRG_02699	peptide/nickel transport system substrate-binding protein	371	168	0.032359701	0.452830189	lmo2569
LMRG_02168	50S ribosomal protein L29	349	158	0.009456532	0.452722063	lmo2624
LMRG_02150	DNA-directed RNA polymerase subunit alpha	685	310	0.045972061	0.452554745	lmo2606
LMRG_00952	ribonuclease III	250	113	0.011835797	0.452	lmo1805
LMRG_01062	malate dehydrogenase	113	51	0.014960466	0.451327434	lmo1915
LMRG_02185	heptaprenyl diphosphate synthase component I	246	111	0.018334167	0.451219512	lmo2640
LMRG_00534	pyruvate carboxylase	404	182	0.038260918	0.45049505	lmo1072
LMRG_00961	hypothetical protein	60	27	0.014081458	0.45	lmo1814
LMRG_02820	hypothetical protein	145	65	0.0383802	0.448275862	lmo1796
predicted RNA	antisense: LMRG_02657	654	293	0.03871668	0.448012232	#N/A
LMRG_01034	N6-adenine-specific DNA methylase	76	34	0.005810536	0.447368421	lmo1887
LMRG_02656	large subunit ribosomal protein L10	1275	569	0.032743346	0.44627451	lmo0250
LMRG_01253	glutamine amidotransferase subunit pdxT	299	133	0.009044345	0.444816054	lmo2102
LMRG_00941	nicotinate nucleotide adenyltransferase	135	60	0.006294116	0.444444444	lmo1488
LMRG_00765	di-trans,poly-cis-decaprenylcistransferase	187	83	0.013134209	0.443850267	lmo1315
LMRG_02051	hypothetical protein	210	93	0.006922704	0.442857143	lmo0952
LMRG_02360	hypothetical protein	113	50	0.004666342	0.442477876	lmo0111
LMRG_02181	thiamine biosynthesis lipoprotein	118	52	0.016805534	0.440677966	lmo2636
LMRG_02599	hypothetical protein	66	29	0.007640077	0.439393939	lmo0276
LMRG_02177	30S ribosomal protein S10	746	327	0.013197896	0.438337802	lmo2633
LMRG_02712	glucose-6-phosphate isomerase	963	422	0.037643237	0.438213915	lmo2367
LMRG_02088	peptide chain release factor 3	176	77	0.013046027	0.4375	lmo0988
LMRG_01408	threonyl-tRNA synthetase	112	49	0.015305858	0.4375	lmo1559
LMRG_02432	hypothetical protein	117	51	0.031589071	0.435897436	lmo0004
LMRG_00802	hypothetical protein	590	254	0.006832118	0.430508475	lmo1352
LMRG_00550	hypothetical protein	135	58	0.007061656	0.42962963	lmo1088
LMRG_00147	hypothetical protein	35	15	0.004337716	0.428571429	lmo0455
LMRG_02348	hypothetical protein	138	59	0.004955323	0.427536232	lmo0099
LMRG_00771	hypothetical protein	134	57	0.004312974	0.425373134	lmo1321
LMRG_02278	D-alanine--D-alanine ligase	146	62	0.005807016	0.424657534	lmo0855
LMRG_01638	peptide/nickel transport system permease	721	305	0.04919203	0.423023578	lmo2194
LMRG_02501	phosphoribosylformylglycinamide synthase I	31	13	0.049232295	0.419354839	lmo1770
LMRG_05006	Gly tRNA	5433	2278	0.010459305	0.419289527	#N/A
LMRG_01772	aldose 1-epimerase	456	191	0.010762338	0.418859649	lmo2476
LMRG_00744	tRNA delta(2)-isopentenylpyrophosphate transferase	203	85	0.004477661	0.418719212	lmo1294
LMRG_00944	ribosome biogenesis GTPase YqeH	696	291	0.049164568	0.418103448	lmo1491
LMRG_01303	methionine adenosyltransferase	137	57	0.004283812	0.416058394	lmo1664
LMRG_01473	uridine kinase	41	17	0.04301591	0.414634146	lmo1497
LMRG_01360	DNA segregation ATPase FtsK/SpoIIIE	152	63	0.010095766	0.414473684	lmo1606
LMRG_00704	trehalose-specific PTS system IIBC component	162	67	0.0063181	0.413580247	lmo1255

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LMRG_01038	recombination protein U	179	74	0.005263869	0.413407821	lmo1891
LMRG_00275	formate/nitrite transporter	119	49	0.001306163	0.411764706	lmo0593
LMRG_02176	50S ribosomal protein L3	506	207	0.006832118	0.409090909	lmo2632
LMRG_00870	nucleotide pyrophosphatase	93	38	0.008061528	0.408602151	lmo1418
LMRG_00966	ribosome small subunit-dependent GTPase A	142	58	0.008512739	0.408450704	lmo1819
LMRG_00901	endonuclease 4	130	53	0.006954465	0.407692308	lmo1449
LMRG_01124	ribonuclease Z	32	13	0.030716609	0.40625	lmo1977
predicted RNA	antisense: LMRG_01720	160	65	0.04102053	0.40625	#N/A
LMRG_00674	ribonuclease HIII	32	13	0.043051938	0.40625	lmo1228
LMRG_02280	alpha/beta fold family hydrolase	106	43	0.001883725	0.405660377	lmo0857
LMRG_05034	Gly tRNA	4718	1889	0.005916195	0.400381518	#N/A
LMRG_01084	ribosome-associated GTPase EngA	200	80	0.004192147	0.4	lmo1937
LMRG_00954	3-oxoacyl-ACP reductase	45	18	0.014038157	0.4	lmo1807
LMRG_01714	F-type H ⁺ -transporting ATPase subunit C	484	193	0.001306163	0.398760331	lmo2534
LMRG_00529	GTP-binding protein TypA/BipA	224	89	0.006238853	0.397321429	lmo1067
LMRG_01169	cell division initiation protein	68	27	0.014408909	0.397058824	lmo2020
LMRG_01582	PIT family inorganic phosphate transporter	754	298	0.017102841	0.395225464	lmo2249
LMRG_00773	hypothetical protein	947	373	0.003406903	0.393875396	lmo1323
LMRG_02271	polar amino acid transport system ATP-binding protein	112	44	0.001070677	0.392857143	lmo0848
LMRG_00832	hypothetical protein	396	155	0.001640079	0.391414141	lmo1380
LMRG_00318	2-haloalkanoic acid dehalogenase	296	114	0.002014978	0.385135135	lmo0635
LMRG_02770	hypothetical protein	65	25	0.007034615	0.384615385	lmo1696
LMRG_01926	antibiotic transport system ATP-binding protein	39	15	0.012232232	0.384615385	lmo2769
LMRG_01340	hypothetical protein	136	52	0.001194099	0.382352941	lmo1626
LMRG_05045	Lys tRNA	1810	690	0.00345001	0.38121547	#N/A
LMRG_01099	diaminopimelate decarboxylase	108	41	0.002777291	0.37962963	lmo1952
LMRG_01079	trans-hexaprenyltranstransferase	467	177	0.002933682	0.379014989	lmo1932
LMRG_01090	hypothetical protein	45	17	0.005014008	0.377777778	lmo1943
LMRG_02586	D-methionine transport system substrate-binding protein	398	150	0.002200451	0.376884422	lmo0285
LMRG_02315	serine/threonine-protein kinase rsbT	146	55	7.89E-04	0.376712329	lmo0891
LMRG_02119	hypothetical protein	77	29	6.28E-04	0.376623377	lmo1019
LMRG_00657	integral membrane protein	40	15	0.036269745	0.375	lmo1211
LMRG_02175	50S ribosomal protein L4/L1	884	331	0.002853719	0.374434389	lmo2631
LMRG_01243	glycine betaine transporter	59	22	3.96E-04	0.372881356	lmo2092
LMRG_00419	hypothetical protein	43	16	0.039856044	0.372093023	lmo0731
LMRG_01363	peroxiredoxin	250	93	0.002708912	0.372	lmo1604
LMRG_01792	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	1390	517	0.002493673	0.371942446	lmo2456
LMRG_00812	exodeoxyribonuclease VII small subunit	119	44	0.012379762	0.369747899	lmo1362
LMRG_00612	threonine-phosphate decarboxylase	38	14	0.00563131	0.368421053	lmo1169
LMRG_05036	Lys tRNA	3699	1354	0.001375696	0.366044877	#N/A
LMRG_01414	delta-aminolevulinic acid dehydratase	41	15	0.004441472	0.365853659	lmo1554
predicted RNA	antisense: LMRG_01081	707	257	0.006238853	0.363507779	#N/A

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LMRG_00392	hypothetical protein	295	107	0.0016435	0.362711864	lmo0703
LMRG_02759	glutamate-1-semialdehyde 2,1-aminomutase 2	91	33	5.05E-04	0.362637363	lmo1685
LMRG_01412	porphobilinogen deaminase	50	18	0.002877714	0.36	lmo1556
predicted RNA	-	225	81	0.011560716	0.36	#N/A
LMRG_02744	multiple sugar transport system permease	53	19	0.002233874	0.358490566	lmo0179
LMRG_02784	fructokinase	90	32	1.69E-04	0.355555556	lmo0813
LMRG_01443	bifunctional preprotein translocase subunit SecD/SecF	249	88	0.001306163	0.353413655	lmo1527
predicted RNA	-	2015	710	3.40E-04	0.35235732	#N/A
predicted RNA	-	375	132	4.83E-05	0.352	#N/A
LMRG_01141	pyrimidine-nucleoside phosphorylase	37	13	0.002721735	0.351351351	lmo1993
LMRG_01033	carboxypeptidase Taq	180	63	8.59E-04	0.35	lmo1886
LMRG_01717	ATP synthase F1 alpha subunit	277	96	9.11E-04	0.346570397	lmo2531
LMRG_00171	shikimate 5-dehydrogenase	52	18	0.002151164	0.346153846	lmo0490
LMRG_01691	fructose-16-bisphosphate aldolase class II	2798	968	0.001156859	0.345961401	lmo2556
LMRG_01630	3-oxoacyl-ACP synthase	67	23	2.92E-04	0.343283582	lmo2202
LMRG_01696	transcription termination factor Rho	35	12	0.001999326	0.342857143	lmo2551
LMRG_01097	segregation and condensation protein B	794	272	7.23E-04	0.34256927	lmo1950
LMRG_01425	septum site-determining protein minC	38	13	0.006680086	0.342105263	lmo1545
LMRG_00852	acetyltransferase	328	112	3.96E-04	0.341463415	lmo1400
LMRG_01385	site-specific DNA-methyltransferase	484	163	5.43E-04	0.33677686	lmo1582
LMRG_02473	30S ribosomal protein S6	520	175	4.20E-04	0.336538462	lmo0044
predicted RNA	-	4219	1411	0.003535268	0.334439441	#N/A
predicted RNA	-	560	187	0.001804117	0.333928571	#N/A
LMRG_02187	serine/threonine protein phosphatase	105	35	3.64E-04	0.333333333	lmo2642
LMRG_01230	hypothetical protein	42	14	9.19E-04	0.333333333	lmo2079
LMRG_01382	protease IV	36	12	0.002942662	0.333333333	lmo1585
LMRG_01195	2-dehydropantoate 2-reductase	30	10	0.004772802	0.333333333	lmo2046
LMRG_01790	phosphoglycerate kinase	686	228	5.42E-04	0.332361516	lmo2458
LMRG_01098	ScpA/B protein	103	34	2.76E-05	0.330097087	lmo1951
LMRG_01059	hypothetical protein	73	24	3.33E-05	0.328767123	lmo1912
predicted RNA	-	229	75	0.039007579	0.327510917	#N/A
LMRG_00736	DNA topoisomerase IV B subunit	513	168	0.005301755	0.32748538	lmo1286
LMRG_01058	hypothetical protein	43	14	8.40E-04	0.325581395	lmo1911
LMRG_01997	aldo/keto reductase family oxidoreductase	83	27	4.52E-05	0.325301205	lmo2700
LMRG_02122	response regulator LiaR	163	53	1.52E-05	0.325153374	lmo1022
LMRG_00084	hypothetical protein	114	37	0.00194844	0.324561404	lmo0391
LMRG_00762	hypothetical protein	37	12	0.027913956	0.324324324	lmo1312
LMRG_00085	hypothetical protein	130	42	5.72E-06	0.323076923	lmo0392
LMRG_01441	hypothetical protein	78	25	0.003860523	0.320512821	lmo1529
LMRG_02900	beta-glucoside operon transcriptional antiterminator	94	30	8.10E-06	0.319148936	lmo2773
LMRG_01713	ATP synthase F0 A subunit	421	134	1.23E-04	0.318289786	lmo2535
LMRG_02658	50S ribosomal protein L11	2141	680	5.50E-04	0.317608594	lmo0248

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LMRG_00287	hypothetical protein	41	13	0.019268586	0.317073171	lmo0604
LMRG_00871	hypothetical protein	121	38	5.23E-06	0.314049587	lmo1419
LMRG_00475	amino acid transporter AAT family protein	287	90	9.08E-05	0.31358885	lmo0787
predicted RNA	-	354	111	0.003199764	0.313559322	#N/A
LMRG_02425	membrane protein OxaA	428	134	6.66E-05	0.313084112	lmo2854
LMRG_00759	hypothetical protein	48	15	0.004644073	0.3125	lmo1309
LMRG_02516	glutamyl-tRNA(Gln) amidotransferase subunit A	308	96	1.24E-04	0.311688312	lmo1755
LMRG_00163	cfr family radical SAM enzyme	289	90	7.62E-05	0.311418685	lmo0482
LMRG_00749	glutamine synthetase type I	621	191	1.08E-04	0.307568438	lmo1299
LMRG_02485	adenylosuccinate synthetase	154	47	2.18E-05	0.305194805	lmo0055
LMRG_00942	hypothetical protein	59	18	0.009270389	0.305084746	lmo1489
LMRG_00216	hypothetical protein	69	21	5.69E-06	0.304347826	lmo0534
LMRG_00774	hypothetical protein	793	241	8.03E-06	0.303909206	lmo1324
LMRG_02868	AAT family amino acid transporter	33	10	4.88E-04	0.303030303	lmo0798
LMRG_00377	hypothetical protein	33	10	0.001095668	0.303030303	lmo0689
LMRG_01981	ABC transporter CydDC cysteine exporter CydC	308	93	5.50E-05	0.301948052	lmo2715
LMRG_02279	UDP-N-acetylmuramoylalanyl-D-glutamyl-2	170	51	7.76E-06	0.3	lmo0856
LMRG_01891	hypothetical protein	47	14	0.004544726	0.29787234	lmo2805
LMRG_01637	peptide/nickel transport system permease	1132	336	7.25E-04	0.296819788	lmo2195
LMRG_02751	hypothetical protein	118	35	7.56E-06	0.296610169	lmo0186
LMRG_00955	malonyl CoA-acyl carrier protein transacylase	98	29	2.02E-06	0.295918367	lmo1808
LMRG_00223	iron complex transport system substrate-binding protein	122	36	1.29E-06	0.295081967	lmo0541
LMRG_00288	hypothetical protein	75	22	3.80E-06	0.293333333	lmo0605
LMRG_01979	cytochrome d ubiquinol oxidase subunit II	580	170	2.49E-05	0.293103448	lmo2717
LMRG_02153	50S ribosomal protein L36	3824	1111	1.42E-05	0.290533473	lmo2609
LMRG_01252	pyridoxine biosynthesis protein	341	99	1.11E-05	0.290322581	lmo2101
LMRG_01182	cell division protein FtsA	428	124	1.93E-05	0.289719626	lmo2033
LMRG_02671	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	2304	665	2.72E-04	0.288628472	lmo0235
predicted RNA	antisense: LMRG_01430 LMRG_01429	427	123	0.001841892	0.288056206	#N/A
LMRG_02114	glycine betaine/proline transport system ATP-binding protein	293	84	9.67E-06	0.28668942	lmo1014
LMRG_00263	SAM-dependent methyltransferase	155	44	1.07E-05	0.283870968	lmo0581
LMRG_05004	Lys tRNA	2329	660	7.03E-06	0.283383426	#N/A
LMRG_01489	hypothetical protein	120	34	1.09E-05	0.283333333	lmo2354
LMRG_01771	UDP-glucose 4-epimerase	110	31	3.42E-07	0.281818182	lmo2477
LMRG_02510	neurotransmitter:Na ⁺ symporter	64	18	3.12E-07	0.28125	lmo1761
LMRG_01242	argininosuccinate lyase	25	7	3.35E-04	0.28	lmo2091
LMRG_01331	DNA binding 3-demethylubiquinone-9 3-methyltransferase domain-containing protein	193	54	3.19E-07	0.279792746	lmo1635
LMRG_02081	hypothetical protein	175	48	6.10E-07	0.274285714	lmo0981
LMRG_01866	glycerate kinase	77	21	1.60E-07	0.272727273	lmo2832
predicted RNA	-	837	228	2.67E-05	0.272401434	#N/A
LMRG_01718	ATP synthase F1 gamma subunit	788	213	2.31E-06	0.270304569	lmo2530
LMRG_01018	phosphomannomutase	37	10	1.37E-05	0.27027027	lmo1871

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LMRG_02183	NADH dehydrogenase	592	160	5.06E-05	0.27027027	lmo2638
LMRG_01013	phosphotransferase	26	7	0.00230137	0.269230769	lmo1866
LMRG_02798	hypothetical protein	272	73	2.06E-06	0.268382353	lmo2142
predicted RNA	-	358	96	0.011052388	0.268156425	#N/A
LMRG_02864	hypothetical protein	64	17	0.001034586	0.265625	#N/A
LMRG_00810	hypothetical protein	57	15	2.40E-05	0.263157895	lmo1360
LMRG_02626	actin-assembly inducing protein ActA	153	40	1.42E-07	0.261437908	lmo0204
LMRG_01364	aminopeptidase	153	40	2.03E-07	0.261437908	lmo1603
LMRG_01003	purine nucleoside phosphorylase	81	21	2.60E-06	0.259259259	lmo1856
LMRG_00381	flagellar motor switch protein FliN/FliY	722	185	2.34E-07	0.256232687	lmo0693
LMRG_02710	CTP synthase	594	152	6.70E-07	0.255892256	lmo2559
LMRG_01341	hypothetical protein	43	11	7.57E-07	0.255813953	lmo1625
LMRG_05050	His tRNA	232	59	6.68E-04	0.254310345	#N/A
predicted RNA	-	284	72	0.005807016	0.253521127	#N/A
predicted RNA	-	222	56	0.005060871	0.252252252	#N/A
LMRG_01488	hypothetical protein	282	71	1.21E-07	0.25177305	lmo2355
LMRG_00553	hypothetical protein	64	16	7.57E-07	0.25	lmo1091
LMRG_00393	hypothetical protein	128	32	2.24E-06	0.25	lmo0704
LMRG_02496	AIR carboxylase	40	10	3.98E-04	0.25	lmo1775
LMRG_02070	D-alanine transfer protein	1034	255	2.17E-05	0.246615087	lmo0971
LMRG_02872	hypothetical protein	1602	393	8.01E-08	0.245318352	lmo0694
predicted RNA	-	213	52	0.001184674	0.244131455	#N/A
LMRG_02827	translation initiation factor IF-3	4106	997	2.11E-06	0.242815392	lmo1785
predicted RNA	-	5311	1286	3.68E-08	0.242138957	#N/A
LMRG_00384	hypothetical protein	2429	588	3.52E-07	0.242074928	lmo0695
LMRG_00843	simple sugar transport system permease	87	21	4.89E-09	0.24137931	lmo1391
LMRG_01659	hypothetical protein	29	7	1.10E-05	0.24137931	lmo2173
LMRG_00772	transcription termination factor NusA	287	69	2.34E-09	0.240418118	lmo1322
predicted RNA	-	887	213	1.16E-07	0.240135287	#N/A
LMRG_01712	ATP synthase I	150	36	2.41E-07	0.24	lmo2536
LMRG_00394	flagellar hook-associated protein FlgK	573	137	3.41E-08	0.239092496	lmo0705
predicted RNA	antisense: LMRG_01792	176	42	8.59E-04	0.238636364	#N/A
LMRG_02307	membrane protein	202	48	1.26E-09	0.237623762	lmo0883
LMRG_00819	phosphate butyryltransferase	131	31	7.29E-11	0.236641221	lmo1369
LMRG_00811	exodeoxyribonuclease VII large subunit	89	21	4.10E-10	0.235955056	lmo1361
LMRG_01867	beta-phosphoglucomutase	51	12	1.08E-05	0.235294118	lmo2831
predicted RNA	-	290	68	0.001100033	0.234482759	#N/A
LMRG_02321	SulP family sulfate permease	64	15	1.99E-10	0.234375	lmo0897
LMRG_02978	hypothetical protein	64	15	0.001125382	0.234375	#N/A
LMRG_01636	peptide/nickel transport system substrate-binding protein	2391	560	1.75E-07	0.234211627	lmo2196
LMRG_00987	pyrimidine operon attenuation protein/uracil phosphoribosyltransferase	47	11	6.92E-05	0.234042553	lmo1840
LMRG_01978	cytochrome bd-I oxidase subunit I	159	37	1.15E-08	0.232704403	lmo2718

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LMRG_00803	hypothetical protein	86	20	5.80E-09	0.23255814	lmo1353
LMRG_02136	CRISPR-associated protein cas2	56	13	3.17E-04	0.232142857	#N/A
predicted RNA	-	358	83	0.001804117	0.231843575	#N/A
LMRG_01583	hypothetical protein	986	228	8.19E-09	0.231237323	lmo2248
LMRG_01213	hypothetical protein	39	9	7.00E-04	0.230769231	lmo2063
LMRG_01676	hypothetical protein	39	9	7.11E-04	0.230769231	lmo2156
LMRG_02071	D-alanine--poly(phosphoribitol) ligase subunit 2	1124	259	7.20E-10	0.230427046	lmo0972
LMRG_02426	ribonuclease P	211	48	4.46E-09	0.227488152	lmo2855
LMRG_01741	cell division ATP-binding protein FtsE	721	163	6.66E-10	0.226074896	lmo2507
LMRG_00981	dihydroorotate dehydrogenase electron transfer subunit	49	11	3.31E-06	0.224489796	lmo1834
LMRG_01743	D-glutamyl-L-m-Dpm peptidase P45	1944	436	9.47E-08	0.224279835	lmo2505
LMRG_00956	fatty acid/phospholipid synthesis protein PlsX	90	20	1.29E-10	0.222222222	lmo1809
predicted RNA	-	389	86	3.72E-05	0.221079692	#N/A
predicted RNA	-	242	53	2.11E-04	0.219008264	#N/A
predicted RNA	-	256	56	2.67E-04	0.21875	#N/A
LMRG_01788	transcriptional regulator	719	156	3.22E-10	0.216968011	lmo2460
LMRG_00957	fatty acid biosynthesis transcriptional regulator	598	129	4.58E-12	0.215719064	lmo1810
LMRG_01577	AGZA family MFS transporter xanthine/uracil permease	182	39	2.34E-10	0.214285714	lmo2254
LMRG_01453	nitrogen regulatory protein P-II	33	7	5.43E-04	0.212121212	lmo1517
LMRG_00391	hypothetical protein	496	105	3.72E-11	0.211693548	lmo0702
LMRG_02060	protease	177	37	7.11E-12	0.209039548	lmo0961
LMRG_02270	glutamine ABC transporter	144	30	2.32E-13	0.208333333	lmo0847
predicted RNA	-	221	46	7.94E-06	0.208144796	#N/A
LMRG_00087	extracellular P60 protein	135	28	2.80E-11	0.207407407	lmo0394
LMRG_00151	hypothetical protein	92	19	6.63E-08	0.206521739	#N/A
LMRG_01742	cell division transport system permease	762	157	2.04E-11	0.206036745	lmo2506
LMRG_00535	iron complex transport system substrate-binding protein	68	14	2.93E-09	0.205882353	lmo1073
predicted RNA	-	787	162	4.55E-09	0.205844981	#N/A
predicted RNA	-	254	52	7.39E-06	0.204724409	#N/A
LMRG_00386	flagellar hook protein FlgE	362	74	8.09E-12	0.20441989	lmo0697
LMRG_02607	oligopeptide transport system permease oppC	54	11	2.17E-12	0.203703704	lmo0269
LMRG_00370	flagellar basal-body rod protein FlgG	54	11	2.43E-07	0.203703704	lmo0682
LMRG_01980	ABC transporter CydDC cysteine exporter CydD	430	87	1.19E-11	0.202325581	lmo2716
LMRG_01073	chorismate mutase	195	39	4.57E-11	0.2	lmo1926
LMRG_01428	50S ribosomal protein L21	6089	1215	3.38E-08	0.199540154	lmo1542
LMRG_00395	flagellar hook-associated protein 3	517	101	2.40E-13	0.195357834	lmo0706
LMRG_02773	hypothetical protein	385	75	4.01E-15	0.194805195	lmo1699
LMRG_02134	hypothetical protein	423	82	1.41E-16	0.193853428	lmo2594
predicted RNA	-	301	58	9.22E-06	0.19269103	#N/A
LMRG_02073	D-alanine--poly(phosphoribitol) ligase subunit 1	270	52	2.81E-14	0.192592593	lmo0974
predicted RNA	antisense: LMRG_01691	203	39	1.42E-05	0.192118227	#N/A
LMRG_01472	O-methyltransferase	73	14	1.24E-09	0.191780822	lmo1498

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predicted RNA	-	335	64	1.11E-05	0.191044776	#N/A
LMRG_02624	listeriolysin O	168	32	1.15E-13	0.19047619	lmo0202
LMRG_00398	hypothetical protein	2607	494	7.01E-14	0.189489835	lmo0709
predicted RNA	antisense: LMRG_02182	431	81	9.73E-15	0.187935035	#N/A
LMRG_02783	enoyl-(acyl carrier protein) reductase	256	48	5.67E-15	0.1875	lmo0814
LMRG_01791	triosephosphate isomerase	659	123	9.75E-15	0.186646434	lmo2457
LMRG_00761	hypothetical protein	70	13	2.10E-12	0.185714286	lmo1311
LMRG_00389	flagellar motor switch protein FliN/FliY	890	165	2.56E-14	0.185393258	lmo0700
LMRG_01744	hypothetical protein	65	12	5.56E-12	0.184615385	lmo2504
predicted RNA	-	378	68	2.00E-06	0.17989418	#N/A
LMRG_00388	flagellar motor switch protein FliM	1078	191	2.38E-16	0.177179963	lmo0699
LMRG_00953	acyl carrier protein	362	64	1.58E-14	0.17679558	lmo1806
predicted RNA	-	665	117	1.11E-10	0.17593985	#N/A
LMRG_00104	hypothetical protein	74	13	2.12E-11	0.175675676	lmo0412
LMRG_00215	ACT domain-containing protein	74	13	2.26E-07	0.175675676	lmo0533
LMRG_00390	hypothetical protein	274	48	5.67E-19	0.175182482	lmo0701
LMRG_00662	bax protein	190	33	7.49E-21	0.173684211	lmo1216
LMRG_01508	hypothetical protein	1747	301	1.15E-14	0.172295363	lmo2335
LMRG_00404	flagellar assembly protein H	2207	379	3.22E-17	0.171726325	lmo0715
LMRG_02138	CRISPR-associated protein	47	8	1.46E-21	0.170212766	#N/A
predicted RNA	-	259	44	7.88E-07	0.16988417	#N/A
predicted RNA	-	282	47	2.32E-07	0.166666667	#N/A
LMRG_00365	flagellar biosynthetic protein FliQ	571	95	4.06E-24	0.166374781	lmo0677
LMRG_00407	hypothetical protein	319	53	5.84E-24	0.166144201	lmo0718
LMRG_02746	multiple sugar transport system substrate-binding protein	103	17	3.42E-21	0.165048544	lmo0181
LMRG_00385	flagellar hook capping protein	1003	165	2.51E-22	0.164506481	lmo0696
LMRG_01715	ATP synthase F0 subunit B	1436	236	6.64E-20	0.164345404	lmo2533
LMRG_02238	autolysin	1956	321	1.78E-16	0.164110429	lmo2691
predicted RNA	-	476	78	1.71E-10	0.163865546	#N/A
LMRG_00476	hypothetical protein	55	9	3.69E-21	0.163636364	lmo0788
LMRG_02379	hypothetical protein	110	18	9.32E-20	0.163636364	lmo0130
predicted RNA	-	385	63	6.88E-08	0.163636364	#N/A
LMRG_01487	hypothetical protein	44	7	2.13E-06	0.159090909	#N/A
predicted RNA	-	1512	236	6.50E-18	0.156084656	#N/A
LMRG_02059	hypothetical protein	154	24	2.94E-25	0.155844156	lmo0960
predicted RNA	-	2547	383	7.86E-27	0.150372988	#N/A
LMRG_00840	ABC transporter	293	44	1.28E-24	0.150170648	lmo1388
predicted RNA	-	181	27	1.95E-09	0.149171271	#N/A
predicted RNA	-	222	33	1.25E-09	0.148648649	#N/A
LMRG_02072	membrane protein	622	92	6.60E-26	0.147909968	lmo0973
predicted RNA	-	325	48	1.43E-10	0.147692308	#N/A
LMRG_02623	1-phosphatidylinositol phosphodiesterase	299	44	3.76E-31	0.147157191	lmo0201

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LMRG_02306	hypothetical protein	367	54	2.22E-31	0.147138965	lmo0882
LMRG_00859	pyruvate formate-lyase activating enzyme	48	7	1.99E-11	0.145833333	lmo1407
LMRG_02137	CRISPR-associated protein cas1	69	10	4.98E-17	0.144927536	#N/A
LMRG_00045	long-chain fatty acid CoA ligase (AMP-binding)	152	22	2.06E-33	0.144736842	lmo0354
LMRG_00402	flagellar M-ring protein FliF	1309	189	4.43E-19	0.144385027	lmo0713
LMRG_01507	1-phosphofructokinase	791	111	3.77E-29	0.140328698	lmo2336
LMRG_01506	hypothetical protein	679	95	9.72E-33	0.139911635	lmo2337
LMRG_05005	Leu tRNA	1696	237	1.78E-30	0.139740566	#N/A
predicted RNA	-	266	37	8.02E-10	0.139097744	#N/A
LMRG_01779	APA family basic amino acid/polyamine antiporter	72	10	3.67E-23	0.138888889	lmo2469
LMRG_00760	hypothetical protein	36	5	1.02E-13	0.138888889	lmo1310
LMRG_01376	N-acetyl-gamma-glutamyl-phosphate reductase	58	8	2.16E-18	0.137931034	lmo1591
LMRG_00980	dihydroorotate oxidase	51	7	5.29E-14	0.137254902	lmo1833
LMRG_00400	flagellar basal-body rod protein FlgC	633	85	8.02E-41	0.134281201	lmo0711
LMRG_05035	Leu tRNA	1337	178	1.08E-27	0.133133882	#N/A
predicted RNA	-	370	49	3.61E-11	0.132432432	#N/A
LMRG_00778	tRNA pseudouridine synthase B	152	20	1.08E-32	0.131578947	lmo1328
LMRG_00399	flagellar basal-body rod protein FlgB	604	79	1.27E-41	0.130794702	lmo0710
predicted RNA	antisense: LMRG_01743	177	23	1.17E-10	0.129943503	#N/A
predicted RNA	-	524	68	5.21E-20	0.129770992	#N/A
LMRG_02012	nitrite transporter NirC	509	66	3.81E-33	0.129666012	lmo0912
predicted RNA	-	324	42	2.16E-18	0.12962963	#N/A
LMRG_01584	hypothetical protein	124	16	2.06E-16	0.129032258	#N/A
LMRG_00397	flagellar biosynthesis protein fliS	1993	257	5.01E-35	0.12895133	lmo0708
LMRG_00406	hypothetical protein	1247	160	1.60E-36	0.128307939	lmo0717
predicted RNA	-	219	28	1.83E-13	0.127853881	#N/A
LMRG_01241	argininosuccinate synthase	90	11	5.43E-32	0.122222222	lmo2090
LMRG_00405	flagellar protein export ATPase FliI	550	66	1.79E-41	0.12	lmo0716
LMRG_00985	aspartate carbamoyltransferase	34	4	3.73E-18	0.117647059	lmo1838
predicted RNA	-	1199	134	3.87E-22	0.1117598	#N/A
LMRG_00841	simple sugar transport system ATP-binding protein	127	14	1.26E-55	0.11023622	lmo1389
LMRG_00978	orotate phosphoribosyltransferase	37	4	3.01E-15	0.108108108	lmo1831
predicted RNA	-	192	20	8.11E-19	0.104166667	#N/A
predicted RNA	-	279	29	5.63E-31	0.103942652	#N/A
LMRG_00842	simple sugar transport system permease	165	17	5.55E-56	0.103030303	lmo1390
LMRG_01579	aspartate aminotransferase	235	24	1.18E-75	0.10212766	lmo2252
LMRG_00403	flagellar motor switch protein FliG	592	60	8.87E-68	0.101351351	lmo0714
LMRG_01976	acetyl-CoA synthetase	773	78	8.55E-63	0.100905563	lmo2720
LMRG_00858	formate acetyltransferase	355	35	9.14E-72	0.098591549	lmo1406
LMRG_00984	dihydroorotase	64	6	1.11E-35	0.09375	lmo1837
LMRG_00376	hypothetical protein	270	25	2.17E-74	0.092592593	lmo0688
LMRG_00366	flagellar biosynthesis protein FliR	119	11	1.32E-41	0.092436975	lmo0678

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LMRG_00387	hypothetical protein	338	31	2.43E-45	0.091715976	lmo0698
predicted RNA	-	1163	105	1.17E-62	0.090283749	#N/A
LMRG_00706	hypothetical protein	1530	137	4.97E-90	0.089542484	lmo1257
LMRG_00396	flagellar hook-associated protein 2	1259	111	3.48E-82	0.08816521	lmo0707
LMRG_00401	flagellar hook-basal body complex protein FliE	337	29	6.46E-57	0.086053412	lmo0712
LMRG_00368	flagellar biosynthesis protein FlhA	95	8	1.26E-74	0.084210526	lmo0680
LMRG_02347	mannose-specific PTS system IID component	216	18	2.87E-83	0.083333333	lmo0098
LMRG_00364	flagellar biosynthetic protein FliP	410	34	6.85E-123	0.082926829	lmo0676
LMRG_05037	Thr tRNA	401	33	1.30E-37	0.082294264	#N/A
predicted RNA	-	379	30	2.01E-36	0.079155673	#N/A
LMRG_00982	carbamoyl-phosphate synthase large subunit	89	7	2.07E-117	0.078651685	lmo1835
LMRG_02908	hypothetical protein	140	11	1.14E-38	0.078571429	#N/A
LMRG_00367	flagellar biosynthetic protein FlhB	272	21	5.76E-133	0.077205882	lmo0679
LMRG_01064	formate acetyltransferase	689	53	4.57E-119	0.076923077	lmo1917
predicted RNA	-	281	21	3.94E-48	0.074733096	#N/A
LMRG_01580	polar amino acid transport system ATP-binding protein	436	32	5.02E-157	0.073394495	lmo2251
LMRG_01838	hypothetical protein	151	11	1.30E-66	0.072847682	lmo2410
LMRG_00979	orotidine 5'-phosphate decarboxylase	127	9	3.19E-70	0.070866142	lmo1832
LMRG_01269	hypothetical protein	466	33	3.13E-154	0.070815451	lmo2115
predicted RNA	-	505	35	3.98E-56	0.069306931	#N/A
LMRG_00983	carbamoyl-phosphate synthase small subunit	130	9	8.52E-85	0.069230769	lmo1836
predicted RNA	-	188	13	2.11E-45	0.069148936	#N/A
predicted RNA	-	772	52	5.12E-116	0.067357513	#N/A
LMRG_02874	flagellar motor switch protein FliN	611	40	2.69E-133	0.065466448	lmo0675
LMRG_00374	chemotaxis protein MotB	872	54	7.09E-193	0.061926606	lmo0686
predicted RNA	-	326	20	3.99E-66	0.061349693	#N/A
LMRG_00748	HTH-type transcriptional regulator glnR	87	5	1.99E-60	0.057471264	lmo1298
LMRG_01268	hypothetical protein	301	17	1.10E-188	0.056478405	lmo2114
predicted RNA	-	341	19	2.61E-95	0.055718475	#N/A
LMRG_00369	flagellar biosynthesis regulator FlhF	222	12	2.50E-219	0.054054054	lmo0681
predicted RNA	-	674	36	5.75E-87	0.053412463	#N/A
LMRG_00375	hypothetical protein	512	26	5.22E-242	0.05078125	lmo0687
predicted RNA	antisense: LMRG_01332	355	17	1.73E-116	0.047887324	#N/A
predicted RNA	-	315	15	1.95E-118	0.047619048	#N/A
LMRG_02345	mannose-specific PTS system IIAB component manL	939	42	0	0.044728435	lmo0096
predicted RNA	-	249	11	1.37E-135	0.044176707	#N/A
LMRG_00046	fumarate reductase flavoprotein subunit	570	25	0	0.043859649	lmo0355
LMRG_05003	Thr tRNA	412	18	1.15E-143	0.04368932	#N/A
predicted RNA	-	208	9	1.68E-121	0.043269231	#N/A
LMRG_00373	chemotaxis protein MotA	849	36	0	0.042402827	lmo0685
predicted RNA	-	386	15	7.22E-180	0.038860104	#N/A
predicted RNA	antisense: LMRG_01332	284	11	5.53E-170	0.038732394	#N/A

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LMRG_02346	mannose-specific PTS system IIC component	549	20	0	0.036429872	lmo0097
LMRG_00371	chemotaxis protein methyltransferase	191	6	0	0.031413613	lmo0683
LMRG_00986	uracil permease	535	16	0	0.029906542	lmo1839
predicted RNA	-	373	11	0	0.029490617	#N/A
predicted RNA	-	606	13	0	0.021452145	#N/A
LMRG_01332	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	4772	98	0	0.020536463	lmo1634
predicted RNA	-	3702	61	0	0.01647758	#N/A
LMRG_00372	hypothetical protein	1276	21	0	0.01645768	lmo0684
LMRG_01479	glutamate decarboxylase	809	12	0	0.014833127	lmo2363
predicted RNA	-	237	3	0	0.012658228	#N/A
predicted RNA	-	660	8	0	0.012121212	#N/A
LMRG_01480	glutamate/gamma-aminobutyrate antiporter	1094	12	0	0.010968921	lmo2362