



Alternative Sigma Factor RpoX Is a Part of the RpoE Regulon and Plays Distinct Roles in Stress Responses, Motility, Biofilm Formation, and Hemolytic Activities in the Marine Pathogen *Vibrio alginolyticus*

Dan Gu,^{a,c} Jun Zhang,^a Yuan Hao,^a Rongjing Xu,^d Yuanxing Zhang,^{a,b} Yue Ma,^{a,b} Qiyao Wang^{a,b}

^aState Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China

^bShanghai Engineering Research Center of Maricultured Animal Vaccines, Shanghai, China

^cJiangsu Key Laboratory of Zoonosis/Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, China

^dYantai Tianyuan Aquatic Co. Ltd., Yantai, Shandong, China

ABSTRACT Vibrio alginolyticus is one of the most abundant microorganisms in marine environments and is also an opportunistic pathogen mediating high-mortality vibriosis in marine animals. Alternative sigma factors play essential roles in bacterial pathogens in the adaptation to environmental changes during infection and the adaptation to various niches, but little is known about them for V. alginolyticus. Our previous investigation indicated that the transcript level of the gene rpoX significantly decreased in an RpoE mutant. Here, we found that rpoX was highly expressed in response to high temperature and low osmotic stress and was under the direct control of the alternative sigma factor RpoE and its own product RpoX. Moreover, transcriptome sequencing (RNA-seq) results showed that RpoE and RpoX had different regulons, although they coregulated 105 genes at high temperature (42°C), including genes associated with biofilm formation, motility, virulence, regulatory factors, and the stress response. RNA-seg and chromatin immunoprecipitation sequencing (ChIP-seq) analyses as well as electrophoretic mobility shift assays (EMSAs) revealed the distinct binding motifs of RpoE and RpoX proteins. Furthermore, quantitative real-time reverse transcription-PCR (qRT-PCR) analysis also confirmed that RpoX can upregulate genes associated with flagella, biofilm formation, and hemolytic activities at higher temperatures. rpoX abrogation does not appear to attenuate virulence toward model fish at normal temperature. Collectively, data from this study demonstrated the regulatory cascades of RpoE and an alternative sigma factor, RpoX, in response to heat and osmotic stresses and their distinct and overlapping roles in pathogenesis and stress responses in the marine bacterium V. alginolyticus.

IMPORTANCE The alternative sigma factor RpoE is essential for the virulence of *Vibrio alginolyticus* toward marine fish, coral, and other animals in response to sea surface temperature increases. In this study, we characterized another alternative sigma factor, RpoX, which is induced at high temperatures and under low-osmotic-stress conditions. The expression of *rpoX* is under the tight control of RpoE and RpoX. Although RpoE and RpoX coregulate 105 genes, they are programming different regulatory functions in stress responses and virulence in *V. alginolyticus*. These findings illuminated the RpoE-RpoX-centered regulatory cascades and their distinct and overlapping regulatory roles in *V. alginolyticus*, which facilitates unraveling of the mechanisms by which the bacterium causes diseases in various sea animals in response to temperature fluctuations as well as the development of appropriate strategies to tackle infections by this bacterium.

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Address correspondence to Yue Ma, mymarine@ecust.edu.cn, or Qiyao Wang, oaiwoivao@ecust.edu.cn.

D.G. and J.Z. contributed equally to this work.

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The Gram-negative bacterium *Vibrio alginolyticus* is a halophilic bacterium that is mainly found in marine and estuarine environments, causing high-mortality outbreaks of vibriosis in sea animals; this bacterium is also a notorious foodborne pathogen for humans (1, 2). Similar to other bacteria, the virulence of *V. alginolyticus* is strictly regulated by environmental factors such as cell density and temperature (3). In our previous study, we reported that the genes involved in the main virulence-associated characteristics of *V. alginolyticus*, such as biofilm formation, motility, extracellular proteases (Asp, Pep, and MviN), siderophore-dependent iron uptake systems, and type III and VI secretion systems (T3SS and T6SS), are tightly regulated by quorum sensing (QS) (4–10).

Alternative sigma (σ) factors are global regulators that enable the expression of genes associated with stress adaptation and virulence in response to diverse stimuli in both the environment and the host in vibrios (11-13). Our previous investigation demonstrated that the temperature-dependent binding of RpoE to distinct promoters appears to underlie a σ^{E} -controlled switch between the expression of virulence genes and adaptation to thermal stress (3). The RpoE protein is essential for the growth of V. alginolyticus, V. cholerae, and V. parahaemolyticus at high temperatures (3, 14–16). RpoS is another well-characterized alternative sigma factor, which controls virulence and cellular responses to reactive oxygen species (ROS), starvation, DNA damage, extreme temperatures, ethanol, and hyperosmolarity (17-19). In V. alginolyticus, RpoS is part of the regulatory networks of virulence and the LuxS quorum-sensing system and responds to high-temperature stress (20). In addition, the alternative sigma factor RpoH activates the transcription of genes involved in the heat shock response in various bacterial species, including vibrios (21). The RpoE protein can directly bind to the promoter of rpoH and activate its expression to respond and adapt to high-temperature conditions in vibrios (3, 22). In V. alginolyticus, the gene rpoX, annotated to encode an RpoS-like alternative sigma factor, was recently implicated as being involved in stress adaptation (23). The detailed regulatory roles associated with rpoX as well as the underlying mechanisms remain unclear.

Our recent transcriptomic analysis of *V. alginolyticus* identified that *rpoX* was significantly downregulated in the *rpoE* mutant (3). In this study, we characterized the roles of the *rpoX* gene in adaptation to heat stress and the regulation of virulence gene expression. The expression of *rpoX* was under the strict control of RpoE in response to high temperature and low osmotic stress. In addition, the regulons of RpoE and RpoX were defined with transcriptome sequencing (RNA-seq) and chromatin immunoprecipitation sequencing (ChIP-seq) analyses to illuminate their distinct and overlapping regulatory roles in the stress response, biofilm formation, motility, hemolytic activity, and virulence. These data enriched our understanding of the basis and regulatory networks of vibrio adaptation to osmosis, heat, and other stresses.

RESULTS

RpoX is induced by high temperature and low osmotic stress. A BLASTP analysis showed that the RpoX protein contains three conserved functional domains: sigma 70 region 1.2 (residues 27 to 59), sigma 70 region 2 (residues 65 to 125), and sigma 70 region 4 (residues 230 to 287) (see Fig. S1A in the supplemental material). In addition, an analysis of the conserved functional domains of the RpoE, RpoH, RpoS, and RpoD proteins from *V. alginolyticus* indicated that even though the RpoX protein was initially annotated as an RpoS-like sigma factor (23), this protein lacked the featured sigma region 3 of the RpoS protein. In addition, the three conserved domains of RpoX were highly similar (with 27% identity and 45% similarity) to the RpoH sigma factor, which could be directly modulated by the RpoE protein and was responsive to high-temperature stress (Fig. S1A). The BLASTP analysis also indicated that the *V. alginolyticus* protein shared 99%, 83%, and 78% identities with the homologous proteins



FIG 1 Stress-responsive *rpoX* expression in *V. alginolyticus*. (A) Western blot assay of RpoX levels in *V. alginolyticus* strains grown under various temperature or osmotic conditions. A Flag-tag-specific antibody was used to probe WT cells expressing RpoX-Flag driven by the native *rpoX* promoter. RpoB was used as a loading control. (B to D) Growth curves of WT, *ArpoX*, and *ArpoE* strains in LB medium containing 3% NaCl at 30°C, the normal growth conditions for this halophile (B); 3% NaCl at 42°C (C); and 0.5% NaCl at 30°C (D). Samples were taken and plate counted after serial dilutions with fresh LBS medium. (E and F) qRT-PCR analysis of the transcript levels of *rpoX*, *rpoE*, and *rpoH* in WT (E) and *ArpoX* (F) cells cultured under different stress conditions relative to WT and *ArpoX* cells grown in LBS medium at 30°C, respectively. Total RNA was isolated from the strains after 9 h of growth. The results are presented as the means \pm standard deviations (SD) (*n* = 3).

Vp1393 from *V. parahaemolyticus*, A1Q_0985 from *V. harveyi*, and ATB83_RS10190 from *V. splendidus*, respectively (Fig. S1B). These analyses indicated that RpoX is highly conserved in these bacteria and might be an important part of the RpoE regulon.

Our previous study indicated that the RpoE protein contributed to different stress responses in *V. alginolyticus*, such as the responses to high-temperature stress, osmotic stress, and H_2O_2 (3), and the *rpoS* mutant strain of *V. alginolyticus* was also defective in resistance to environmental stresses (20). Although RpoX has been suggested to be involved in stress adaptations, its exact biological roles remain undetermined (23). Therefore, we first examined the expression of RpoX under various stress conditions with a *V. alginolyticus* strain carrying an *rpoX* promoter reporter. RpoX protein could be expressed at a high temperature (42°C) and under low-osmotic-stress conditions (0.5% NaCl) (Fig. 1A). The growth of the wild-type (WT), $\Delta rpoE$, and $\Delta rpoX$ strains was monitored at 30°C (Fig. 1B) and 42°C (Fig. 1C) in Luria-Bertani (LB) broth containing 3% NaCl and at 30°C in LB broth containing 0.5% NaCl (Fig. 1D), respectively. The severely impaired growth of *V. alginolyticus* at 42°C in LB broth containing 0.5% NaCl excluded further experiments under these conditions. The $\Delta rpoE$ strain had a marked growth reduction under its optimal growth conditions (30°C with 3% NaCl) (Fig. 1B) and had a longer lag phase and reached a drastically lower stationary-phase growth rate than the WT strain at 42°C and in 0.5% NaCl (Fig. 1B to D). However, the $\Delta rpoX$ strain did not exhibit a significant difference in growth compared to the WT strain under these conditions (Fig. 1B to D). These investigations indicated that the RpoX protein can be induced at 42°C and in 0.5% NaCl but might not be required for the survival of *V. alginolyticus* under these conditions.

The RpoE sigma factor can directly regulate the expression of RpoH (3), which shares the same functional domains as the RpoX protein and responds to different types of stress. Therefore, we suspect that the $\Delta rpoX$ strain did not exhibit decreased survival at 42°C and in 0.5% NaCl due to induction of the expression of *rpoH* in the $\Delta rpoX$ strain, which might rescue bacterial growth under these stress conditions. After the failure of the trials to generate an *rpoH* deletion or null mutant to test this hypothesis, we resorted to detection of the expression of *rpoE*, *rpoX*, and *rpoH* in the WT and $\Delta rpoX$ strains at 42°C and in 0.5% NaCl. Indeed, the expression of rpoE, rpoX, and rpoH markedly increased in the WT strain cultured in 0.5% NaCl and at 42°C (Fig. 1E). In the rpoX mutant strain, the transcription level of rpoH in 0.5% NaCl and at 42°C was ~2-fold higher than that under culture conditions of 3% NaCl at 30°C, while the transcription of rpoE exhibited no significant difference between both culture conditions (Fig. 1F), which indicated that the increased expression of rpoH might compensate for the abrogation of RpoX protein to regulate the responses to these stress factors in the $\Delta rpoX$ strain. Moreover, these data also suggested that rpoX might be involved in the transcription of rpoE. Taken together, these findings suggested that RpoX is induced by and might be involved in the stress response to 42°C (high temperature) and 0.5% NaCl (low-osmotic-stress conditions).

RpoE and RpoX directly bind to the rpoX promoter and activate its transcription. Based on sequence analysis, the rpoX promoter region contains the conserved -35 motif "GCACTTT," the -10 motif "TGCTCA" for an RpoE-binding site (Fig. 2A), and the predicted transcriptional start site +1A. However, a 5' rapid amplification of cDNA ends (RACE) study found another transcriptional start site, +1G (Fig. 2A), which is located 104 bp downstream of the predicted RpoE-binding site, so we suggested that another promoter sequence might exist in the rpoX promoter. In addition, sequence analysis showed another putative sigma factor-binding sequence (TGTCTACA/ATA TAAA [-35/-10 motifs]), which was located directly upstream of the transcriptional start site +1G, and we speculate that this site might be the specific binding site of the RpoX protein (Fig. 2A). Overall, the sequence analysis showed that the *rpoX* promoter contains two conserved binding sites. An electrophoretic mobility shift assay (EMSA) was then used to determine whether the RpoE and RpoX proteins could directly bind to the rpoX promoter. As expected, the RpoE protein bound directly to the rpoX promoter region in a concentration-dependent manner in the presence of high concentrations (10-fold) of a nonspecific poly(dl-dC) competitor, and the RpoE protein could not bind to a negative-control DNA (Fig. 2B). In addition, the EMSA results also showed that the RpoX protein bound directly to its own promoter (Fig. 2C).

Given the direct interaction of the RpoE and RpoX proteins with the promoter of *rpoX*, we next determined whether the RpoE and RpoX proteins could regulate the expression of *rpoX in vivo*. We transformed the pBAD33::P_{*rpoX*}-Flag plasmid into WT, Δ *rpoE*, and Δ *rpoX* strains, and Western blotting confirmed that the expression of RpoX markedly decreased in the Δ *rpoX* mutant but was abolished in the Δ *rpoE* strain at 42°C (Fig. 2D). Taken together, these results show that the expression of *rpoX* was directly regulated by the sigma factors RpoE and RpoX.

Global analyses of the regulons of RpoE and RpoX in V. *alginolyticus.* Because RpoE and RpoX were found to be important in the response to high temperatures, we



FIG 2 RpoE and RpoX directly bind to the *rpoX* promoter. (A) Diagram showing the promoter region of the *rpoX* gene. The RpoE- and RpoX-binding sites and the ribosome-binding site (RBS) are underlined. The regions protected by RpoE/RpoX are shadow boxed. The transcription start sites are labeled as +1 and marked in red. The red letters "TGA" and "ATG" are the stop codon of N646_4611 and the start codon of *rpoX*, respectively. (B and C) EMSA of RpoE and RpoX specifically binding to the *rpoX* promoter. Various concentrations of the RpoE and RpoX proteins were added to mixtures of poly(dl-dC) and Cy5-labeled *rpoX* promoter DNA (a 282-bp fragment of the promoter adjoining the start codon "ATG," as indicated with the pair of arrows above the sequence). The same reactions were also carried out for a 300-bp fragment of the *gyrB* promoter region (negative control), which cannot be bound by RpoX and RpoE. (D) Western blotting of RpoX levels in WT, *ΔrpoE*, and *ΔrpoX* cells harboring pBAD33:P_{*rpoX*}-Flag and grown at normal (30°C) or high (42°C) temperatures. RpoB was used as a loading control for the blots.

identified genes that are regulated by RpoE and RpoX at high temperatures by comparing the transcriptomes of the WT, $\Delta rpoX$, and $\Delta rpoE$ strains at 42°C. The results showed that many genes were regulated by the RpoE and RpoX proteins at high temperatures (the 50 most upregulated and most downregulated genes are listed in Table 1). Comparison of the RNA-seq data for the WT and $\Delta rpoE$ strains at 42°C showed that 393 (8.4%) and 440 (9.4%) of the annotated genes were up- and downregulated (log₂ fold change [log₂FC] \geq 2 or log₂FC \leq -2; *P* < 0.001), respectively, in the $\Delta rpoE$ strains at 42°C showed that 240 (5.1%) and 408 (8.7%) genes were up- and downregulated (log₂FC \geq 2 or log₂FC \leq -2; *P* < 0.001), respectively, in the $\Delta rpoX$ strains at 42°C showed that 240 (5.1%) and 408 (8.7%) genes were up- and downregulated (log₂FC \geq 2 or log₂FC \leq -2; *P* < 0.001), respectively, in the $\Delta rpoX$ strain (Fig. 3B). To identify the coregulon of RpoE and RpoX, we compared the RNA-seq data and identified 105 overlapping genes between the regulons of RpoE and RpoX (Fig. 3C), including genes associated with biofilm formation, motility, and stress adaptation. These data suggested that both the RpoE and RpoX proteins are global regulators in *V. alginolyticus* in response to high-temperature stress.

The data presented in Fig. 3D and E describe the expression patterns of genes that are potentially associated with virulence (including genes associated with biofilm formation, motility, and virulence), regulatory factors, and stress responses. Our previous study showed that the RpoE protein can regulate motility and virulence (3), and the RNA-seq data confirmed that *rpoX* transcription could be positively regulated (fold change of -2.4) by RpoE. In addition, the RpoX protein controls the expression of various genes involved in biofilm formation and motility, virulence-associated genes, and regulatory factors. Finally, we also found that the RpoX protein is involved in the stress response (n = 15) via the regulation of heat shock and cold shock proteins, outer membrane proteins, and proteins associated with multidrug resistance. Taken together, our results demonstrated that RpoE and RpoX are important regulatory factors at high temperatures and are responsible for the regulation of virulence-associated genes.

RpoE- and **RpoX-binding motifs.** The binding motifs of RpoE and RpoX were generated by the MEME-suite tool (http://meme-suite.org) in search of the regulated genes' promoter regions in the identified regulon of RpoE and RpoX. As shown in Fig. 4A, the -10 box and -35 box were identified, and the conserved binding site of RpoE was found to be similar to the established RpoE-binding site identified by ChIP-seq in other bacteria (24); the results of our previous study also showed a similar binding site for RpoE in the promoter of *luxR* (TGACCTT for the -35 region and TCATCA for the -10 region) (3). In addition, based on the RNA-seq data for RpoX at high temperatures, the conserved -35 box and -10 box of the RpoX-binding motif were also revealed (Fig. 4B), and the -35 box and -10 box sequences were similar to the predicted binding sites in the promoter of *rpoX* (TGTCTACA/ATATAAA) (Fig. 2A). The binding motifs show marked differences between the binding sites of the RpoE and RpoX sigma factors.

Identification of RpoX-binding regions by ChIP-seq. We further used ChIP-seq experiments to investigate the possible RpoX-binding loci on the chromosome of V. alginolyticus cultured at a high temperature (42°C) and under low-osmotic-stress conditions (0.5% NaCl). We identified 9 enriched loci (fold change of >2.0; P < 0.01) harboring RpoX-binding peaks at a high temperature (42°C) but only 2 enriched loci exhibiting peaks under low-osmotic-stress conditions (0.5% NaCl), and these 2 peaks were also included in the high-temperature peaks (Table 1). Only 4 of these 9 peaks were located in intergenic regions. We thus chose the 4 related regions for EMSAs. Among these enriched loci, the rpoX promoter was first identified as a binding substrate of RpoX with a fold enrichment of 10.9, and EMSAs also confirmed that the RpoX protein could directly bind to its own promoter (Fig. 2C). In addition, the additional 3 peaks upstream of N646_4603 (7.6-fold), N646_4601 (6.1-fold), and N646_1623 (4.8-fold) were found to be located in distinct promoter regions (Fig. 5A to C). As expected, EMSAs validated that the RpoX protein bound directly to these promoters in the presence of high concentrations (10-fold) of a nonspecific poly(dl-dC) competitor (Fig. 2C and Fig. 5A to C). The gyrB promoter region was used as the

TABLE 1 Genes coregulated by RpoE and RpoX at 42°C

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Note: Appl: -9.17 -3.9.7 -3.9.7 Note: 330 Protectical protein of ExoQ family, involved -2.49 -42.09 -42.09 Note: 303 Protectical protein of ExoQ family, involved -2.49 -2.29 -7 Note: 1010 Hypothetical protein in prox -3.20 -71.18 Y Note: 1010 Hypothetical protein -3.20 -71.18 Y Note: 3788 Hypothetical protein -6.66 -71.91 - Note: 4.660 AEC transporter outer membrane component -2.25 -17.99 Y Note: 4.600 AEC transporter outer membrane component -2.25 -16.89 Y Note: 4.600 Hypothetical protein -2.35 -17.99 Y Note: 4.600 Hypothetical protein -2.25 -17.33 Y Note: 4.601 Hypothetical protein -2.15 -11.33 Y Note: 4.602 Hypothetical protein -3.17 Y Y Note: 4.604 Hypothetical protein -3.13 -6.13 -6.13 N	N646_4684	Hypothetical protein	-4./4	- /9.41		
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Node_0.232 Pututive method and protein of Exod jammy, movined -2.49 -2.43 T Node_0.1030 In exopolyasecharide production -33.13 Y Y Node_0.1030 Hypothetical protein -44.43 -33.13 Y Y Node_0.401 Hypothetical protein -44.33 -2.20 Y Y Node_0.401 Hypothetical protein -6.05 -1.139 Y Node_0.402 Att transporter outer membrane component -2.55 -1.699 Y Node_0.402 Hypothetical protein -2.51 -1.189 Y Node_0.402 Hypothetical protein -2.52 -1.699 Y Node_0.402 Hypothetical protein -3.17.4 -1.195 Y Node_0.402 Hypothetical protein -2.23 -10.07 Y Node_0.202 Hypothetical protein -2.23 -10.07 Y Node_0.203 Hypothetical protein -2.23 -8.63 - Node_0.204 Hypothetical protein -3.24 -5.7 -<	N646_3940	Hypothetical protein	-5.11	-47.04		V
Nede_461 Pypothetical protein, RpoX -4.44 -39.73 Y Y Nede_1050 Hypothetical protein, RpoX -44.33 -32.0 -31.18 Nede_3050 Hypothetical protein -46.33 -29.20 Y Nede_3780 Hypothetical protein -60.6 -71.91 - Nede_4600 AGC transporter outer membrane component -25.5 -16.69 Y Nede_4601 Hypothetical protein -29.71 -16.78 Y Nede_4602 Hypothetical protein -21.5 -11.33 Y Nede_46210 Hypothetical protein -21.5 -11.33 Y Nede_2010 Hypothetical protein -23.2 -10.05 Y Nede_2021 Hypothetical protein -3.33 -10.07 Y Nede_1319 Hypothetical protein -3.33 -10.07 Y Nede_46010 Hypothetical protein -3.33 -10.07 Y Nede_462116 Hypothetical protein -3.08 -7.61 Y Nede_462116 </td <td>N646_0523</td> <td>Putative membrane protein of ExoQ family, involved</td> <td>-2.49</td> <td>-42.23</td> <td></td> <td>Ŷ</td>	N646_0523	Putative membrane protein of ExoQ family, involved	-2.49	-42.23		Ŷ
Notel_abil Prypotetical protein -34.4 -39.7.3 Y Y Notel_ability -31.18 -31.18 Y Notel_ability -32.00 -31.18 Y Notel_ability -4.33 -32.00 Y Notel_ability -4.64 -21.91 Y Notel_ability -25.6 -17.69 Y Notel_ability -25.7 -17.69 Y Notel_ability -25.8 -16.78 Y Notel_ability -25.8 -16.74 Y Notel_ability Notel_ability -25.8 -16.74 Y Notel_ability Notel_ability -25.7 -11.95 Y Notel_ability Pyonbetical protein -31.7 -11.95 Y Notel_ability Pyonbetical protein -22.15 -11.33 Y Notel_ability Pyonbetical protein -32.3 -8.51 Y Notel_ability Pyonbetical protein -32.4 -7.18 Y Notel_ability <td>NGAG AGIO</td> <td>in exopolysaccharide production</td> <td></td> <td>20.72</td> <td>X.</td> <td>V</td>	NGAG AGIO	in exopolysaccharide production		20.72	X.	V
NoteL. (10-30) Pryponetical protein -3.10 -31.18 No664601 Hyponetical protein -6.06 -21.91 No664604 Hyponetical protein -6.06 -21.91 No664605 ABC transporter outer membrane component -2.35 -11.89 Y No664606 ABC transporter outer membrane component -2.35 -16.89 Y No666602 Hyponetical protein -2.97.1 -16.78 Y No666602 Hyponetical protein -31.74 -11.35 Y No666602 Hyponetical protein -2.15 -11.33 Y No666203 Hyponetical protein -2.16 -11.35 Y No666204 Hyponetical protein -2.12 -11.33 Y No666012 Hyponetical protein -2.23 -10.67 Y No666024 Hyponetical protein -3.12 -9.17 Y No666034 Hyponetical protein -2.23 -6.33 -8.51 Y No666034 Hyponetical protei	N646_4610	Hypothetical protein, Rpox	-4.44	-39.73	Ŷ	Ŷ
Note:	N646_1050	Hypothetical protein	-3.20	-31.18	X	
nvaba nybonickal protein -0.05 -11.91 Ne64_0262 ABC transporter outer membrane component -225 -17.99 Ne64_066 ABC transporter outer membrane component -225 -16.89 Y Ne64_062 Hypothetical protein -29.71 -16.78 Y Ne64_0618 FMM-dependent NADH-accreductase -26.8 -16.44 Ne64_0722 Hypothetical protein -21.5 -11.33 Ne64_08292 Polar flagellar protein -21.6 -10.69 Ne64_0818 Hypothetical protein -22.2 -10.37 Y Ne64_0818 Hypothetical protein -23.3 -10.07 Y Ne64_0180 Hypothetical protein -23.3 -8.55 - Ne64_0391 Hypothetical protein -23.4 -7.16 - Ne64_0322 Hypothetical protein -32.4 -7.16 - Ne64_0332 Hypothetical protein -4.83 -8.55 Y Ne64_0332 Hypothetical protein -4.948 -6.43 - <td>N646_4601</td> <td>Hypothetical protein</td> <td>-44.33</td> <td>-29.20</td> <td>Y</td> <td></td>	N646_4601	Hypothetical protein	-44.33	-29.20	Y	
Node, 2020 Putative guida tosylitalisatizate - 3.2.8 - 19.04 Node, 1186 Sodium-type flagellar protein MotY - 2.35 - 17.99 Node, 1186 Sodium-type flagellar protein MotY - 2.35 - 16.89 Y Node, 1186 Sodium-type flagellar protein - 2.35 - 16.78 Y Node, 012 FMM-dependent NADF-azoreductase - 2.68 - 16.44 Y Node, 022 Hypothetical protein - 31.74 - 11.95 Y Node, 2020 Hypothetical protein - 20.26 - 10.69 Y Node, 2020 Polar flagellar protein - 3.33 - 10.07 Y Node, 2020 Polar flagellar protein - 3.32 - 8.63 - 10.07 Node, 1511 Hypothetical protein - 3.32 - 8.63 - 10.07 Node, 3039 Hypothetical protein - 4.32 - 6.43 - 7.18 Node, 3039 Hypothetical protein - 4.52 - 6.73 - 7.18 Node, 3049 Hypothetical protein - 2.27 - 6.38 4.98	N040_3708	Hypothetical protein	-0.06	-21.91		
Nebs Abc. Litarbjörder Outer methodale Component -2.33 -17.99 Ne64_1165 Sodium-type Hagellar protein -2.33 -16.89 Y Ne64_602 Hypothetical protein -2.9.71 -16.78 Y Ne64_6105 FMN-dependent NADH-azoreductase -2.66 -16.44 Y Ne64_6100 Hypothetical protein -3.17 -11.95 Y Ne64_6201 Hypothetical protein -2.15 -11.33 Y Ne64_5216 Hypothetical protein -2.12 -10.69 Y Ne64_6139 Hypothetical protein -3.03 -8.55 Y Ne64_608 Hypothetical protein -3.03 -8.55 Y Ne64_6039 Hypothetical protein -3.08 -7.66 Y Ne64_6039 Hypothetical protein -4.83 -8.51 Y Ne64_6039 Hypothetical protein -4.71 -6.43 Y Ne64_6039 Hypothetical protein -4.71 -6.43 Y Ne64_60390 Hypothetical protein	N040_0520		-5.20	- 19.04		
Index Job Science 1	N040_4000	ABC transporter outer membrane component	-2.55	-17.99		V
Nobel, 2002 PhyDinetical protein -2.97.1 -10.78 Nobel, 5198 Hypothetical protein -6.38 -14.22 Nobel, 5198 Hypothetical protein -3.74 -11.95 Y Nobel, 5292 Hypothetical protein -2.15 -11.33 Y Nobel, 5292 Polar flagellar FlgF -2.52 -10.69 Y Nobel, 5292 Polar flagellar Jortein -3.12 -9.17 Y Nobel, 1591 Hypothetical protein -2.33 -8.63 -1.069 Nobel, 1591 Hypothetical protein -3.08 -7.66 -7.18 Nobel, 1591 Hypothetical protein -3.08 -7.66 -7.18 Nobel, 3939 Hypothetical protein -4.52 -6.73 -6.44 Nobel, 3939 Hypothetical protein -4.52 -6.73 -6.44 Nobel, 3939 Hypothetical protein -2.57 -6.38 -7.18 Nobel, 3949 Hypothetical protein -2.57 -6.38 -7.18 Nobel, 3040 GTP cyclohydrolase II	N040_1180	Sodium-type hagenar protein Moti	-2.35	-16.89		ř
Index depth depth field in value depth dept	N646_4602	Appointerical protein	-29.71	-16.78		
Nede_ds38 Phypothetical protein -0.38 -14.22 Nede_dc00 Hypothetical protein -2.15 -11.35 Y Nede_2016 Hypothetical protein -2.15 -11.35 Y Nede_2016 Hypothetical protein -2.15 -11.35 Y Nede_2292 Polar flagellar Flgf -2.52 -10.69 Y Nede_4008 Hypothetical protein -3.93 -10.07 Y Nede_4008 Hypothetical protein -3.12 -9.17 Y Nede_15191 Hypothetical protein -2.23 -8.63 Y Nede_0339 Hypothetical protein -3.08 -7.66 Y Nede_0339 Hypothetical protein -4.52 -6.73 Y Nede_0389 Hypothetical protein -4.71 -6.44 Y Nede_0562 Hypothetical protein -2.75 -6.38 Y Nede_6.370 GTP cyclohydrolase II -2.03 -5.99 Y Nede_6.036 Cytochrome c xidase, subunit II -2.05	N646_0512	FMIN-dependent NADH-azoreductase	-2.68	- 16.44		
Nade_acou Hypothetical protein -21.7.4 -11.33 Ne66_072 Hypothetical protein -20.26 -10.69 Ne66_1399 Hypothetical protein -20.26 -10.37 Y Ne66_1399 Hypothetical protein -3.93 -10.07 Y Ne66_1399 Hypothetical protein -3.12 -9.17 Ne66_151 Hypothetical protein -3.12 -9.17 Ne66_039 Putative acetytransferase -5.03 -8.55 - - Ne66_039 Hypothetical protein -3.08 -7.66 - - Ne66_0391 Hypothetical protein -4.83 -6.41 - - Ne66_0394 Hypothetical protein -4.98 -6.73 - - Ne66_0394 Hypothetical protein -2.470 -6.38 - - Ne66_0394 Putative dioxygenase -2.40 -6.38 - - Ne66_0314 Transcriptional regulator, GntR family protein -2.75 -6.28 - - - -	N646_4598	Hypothetical protein	-6.38	-14.22	X	
No46_2/12 Hypothetical protein -2.15 -11.33 No46_219 Polar flagellar FigF -2.52 -10.69 No46_22929 Polar flagellar FigF -2.52 -10.16 No46_40139 Hypothetical protein -3.93 -10.07 No46_15191 Hypothetical protein -3.12 -9.17 No46_0708 Putative acetyltransferase -5.03 -8.55 No46_0708 Putative acetyltransferase -5.03 -8.51 Y No46_0708 Hypothetical protein -3.08 -7.66 -7.63 No46_0389 Hypothetical protein -4.52 -6.73 -6.44 No46_0552 Hypothetical protein -4.52 -6.73 -6.38 No46_0562 Hypothetical protein -2.27 -6.38 -6.44 No46_0562 Hypothetical protein -2.28 -5.39 -5.99 No46_0714 Transcriptional regulator, GntR family protein -2.28 -5.39 -5.99 No46_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 <t< td=""><td>N646_4600</td><td>Hypothetical protein</td><td>-31./4</td><td>-11.95</td><td>Ŷ</td><td></td></t<>	N646_4600	Hypothetical protein	-31./4	-11.95	Ŷ	
Nede_2916 Proponentical protein -2.2.20 -10.39 Y Nede_1399 Hypothetical protein -1.41 -10.16 Y Nede_1399 Hypothetical protein -3.12 -9.17 Y Nede_0708 Putative aceptransferase -5.03 -8.53 Y Nede_0708 Putative aceptransferase -5.03 -8.55 Y Nede_0399 Hypothetical protein -3.248 -7.18 Y Nede_0399 Hypothetical protein -4.433 -6.44 Y Nede_0390 Hypothetical protein -4.52 -6.73 Y Nede_0390 Hypothetical protein -4.98 -6.44 Y Nede_0390 Hypothetical protein -2.57 -6.38 Y Nede_0384 Putative dioxgenase -2.40 -5.38 Y Nede_0362 Cytochrome coxidase, subunit II -2.03 -5.99 Y Nede_0464 505 ribosomal protein 131 -0.46 -5.88 Y Nede_0.176 Hypothetical protein	N646_0722	Hypothetical protein	-2.15	-11.33		
Nede_139 Piair Tagletial right -2.22 -10.3 T Nede_139 Hypothetical protein -3.93 -10.07 Nede_1161 Hypothetical protein -3.12 -9.17 Nede_15191 Hypothetical protein -2.23 -8.63 Nede_0709 Hypothetical protein -2.33 -8.55 Nede_0539 Hypothetical protein -3.08 -7.66 Nede_0539 Hypothetical protein -4.83 -6.44 Nede_0539 Hypothetical protein -4.98 -6.44 Nede_0552 Hypothetical protein -4.71 -6.38 Nede_0562 Hypothetical protein -2.75 -6.38 Nede_0714 Transcriptional regulator, GntR family protein -2.75 -6.38 Nede_0352 Cytochrome coxidase, subunit II -2.03 -5.99 Nede_0352 Cytochrome coxidase, subunit II -2.28 -5.39 Nede_0352 Cytochrome coxidase, subunit II -2.28 -5.39 Nede_0352 Print-related protein -111.19 -4.81	N646_2916	Hypothetical protein	-20.26	-10.69		V
No46_ 1399 Hypothetical protein -1.41 -10.16 N646_ 6108 Hypothetical protein -3.93 -10.07 N646_ 51161 Hypothetical protein -3.12 -9.17 N646_ 610708 Putative acety/transferase -5.03 -8.55 N646_ 609 Hypothetical protein -4.83 -8.51 Y N646_ 0532 Hypothetical protein -3.248 -7.18 N646_ 0390 Hypothetical protein -4.98 -6.44 N646_ 0384 Putative dioxygenase -2.40 -6.38 N646_ 0384 Putative dioxygenase -2.40 -6.38 N646_ 0462 Cytochrome c oxidase, subunit II -2.03 -5.99 N646_ 0363 Cytochrome c oxidase, subunit II -2.28 -5.39 N646_ 0363 Putative dioxygenase -4.40 -4.81 N646_ 0363 Putative dioxidase/2-oxohepta-3- -2.42 -4.98 N646_ 0363 Putative agular polysaccharide biosynthesi -4.11 -4.49 N646_ 0363 Putative agular polysaccharide biosynthesis -4.10	N646_2929	Polar flagellar FIGF	-2.52	-10.37		Ŷ
No46, 2400B Hypothetical protein -3.93 -10.07 N646_15151 Hypothetical protein -2.23 -8.63 N646_0708 Putative aceplytransferase -5.03 -8.55 N646_0708 Hypothetical protein -4.83 -8.51 Y N646_0339 Hypothetical protein -3.08 -7.66 - N646_0328 Hypothetical protein -4.52 -6.73 - N646_0328 Hypothetical protein -4.498 - - N646_0326 Hypothetical protein -4.71 -6.43 - N646_0370 GTP cyclohydrolase II -2.27 -6.38 - N646_0429 Cytochrome c oxidase, subunit II -2.03 -5.99 - N646_038 Putative divotein -2.28 -5.39 - N646_038 Z-keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 - N646_038 Putative diprotein -2.269 -4.81 - N646_0303 Hypothetical protein -3.03 -4.81	N646_1399	Hypothetical protein	-1.41	-10.16		
Nede_1161 Hypothetical protein -3.12 -9.17 Nede_5708 Putative acetyltransferase -5.03 -8.55 Nede_60339 Hypothetical protein -3.08 -7.66 Nede_0532 Hypothetical protein -3.248 -7.66 Nede_0339 Hypothetical protein -4.98 -6.44 Nede_0330 Hypothetical protein -4.71 -6.43 Nede_0334 Putative dioxygenase -2.40 -6.38 Nede_046 OGP cyclohydrolase II -2.23 -5.99 Nede_042 Oythetical protein -2.42 -6.38 Nede_044 S05 ribosomal protein IC31 -0.46 -5.88 Nede_044 S05 ribosomal protein IC31 -0.46 -5.88 Nede_0352 2.4ket -4pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 Nede_0363 Hypothetical protein -2.69 -4.98 Nede_037 Hypothetical protein -3.03 -4.81 Nede_0384 Prin-related protein -3.03 -4.81 Nede_0300 Putative	N646_4608	Hypothetical protein	-3.93	-10.07		
Ne46_0708 Putative acetyltransferase -5.03 -8.55 N646_0708 Hypothetical protein -4.83 -8.51 Y N646_0703 Hypothetical protein -3.08 -7.66 -7.66 N646_0329 Hypothetical protein -32.48 -7.18 -7.18 N646_0389 Hypothetical protein -4.92 -6.43 -6.43 N646_0384 Putative dioxygenase -2.40 -6.38 -6.44 N646_0347 GTP cyclohydrolase II -2.57 -6.38	N646_1161	Hypothetical protein	-3.12	-9.17		
No46_00708 Putative actety/transferase -5.03 -8.55 No46_009 Hypothetical protein -4.83 -8.51 Y No46_3393 Hypothetical protein -3.08 -7.66 -7.66 No46_0532 Hypothetical protein -4.52 -6.73 -6.44 No46_0384 Putative dioxygenase -2.40 -6.38 -6.38 No46_0384 Putative dioxygenase -2.40 -6.38	N646_1591	Hypothetical protein	-2.23	-8.63		
No46_4009 Hypothetical protein -4.8.3 -8.51 T N646_3939 Hypothetical protein -32.48 -7.16 N646_0390 Hypothetical protein -4.52 -6.73 N646_0390 Hypothetical protein -4.71 -6.43 N646_0390 Hypothetical protein -4.71 -6.38 N646_03470 GTP cyclohydrolase II -2.57 -6.38 N646_03470 GTP cyclohydrolase II -2.03 -5.99 N646_1446 505 ribosomal protein L31 -0.46 -5.38 N646_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- -2.28 -5.39 N646_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 men-1,7-dioic acid hydratase - - -4.81 N646_0301 Hypothetical protein -111.19 -4.81 N646_5300 Putative capsular polysaccharide biosynthesis -4.10 -4.59 Y N646_013 Hypothetical protein -3.03 -4.81 - Y N646_0230 Putative capsular pol	N646_0708	Putative acetyltransferase	-5.03	-8.55	X.	
Ne46_033 Hypothetical protein -3.08 -7.66 N646_0332 Hypothetical protein -4.52 -6.73 N646_0339 Hypothetical protein -4.52 -6.43 N646_0330 Hypothetical protein -4.71 -6.63 N646_0384 Putative dioxygenase -2.40 -6.38 N646_0370 GTP cyclohydrolase II -2.57 -6.38 N646_0384 Putative dioxygenase -2.40 -5.99 N646_0385 Cytochrome c oxidase, subunit II -0.46 -5.88 N646_0385 2-Keto-4-penetonate hydratase/2-oxohepta-3- -2.28 -5.39 N646_0385 2-Keto-4-penetonate hydratase/2-oxohepta-3- -2.42 -4.98 ene-1.7-dioic acid hydratase - - - N646_0385 2-Keto-4-penetonate hydratase/2-oxohepta-3- -2.69 - - N646_0301 Hypothetical protein -111.19 -4.81 - N646_030 Putative capsular polysaccharide biosynthesis -4.10 -4.59 Y N646_013 Hypothetical protein	N646_4609	Hypothetical protein	-4.83	-8.51	Ŷ	
No46_0332 Hypothetical protein -32.48 -7.18 No46_0390 Hypothetical protein -4.52 -6.73 No46_0300 Hypothetical protein -4.71 -6.43 No46_0304 Putative dioxygenase -2.40 -6.38 No46_0317 Transcriptional regulator, GnR family protein -2.57 -6.38 No46_0201 Transcriptional protein L31 -0.46 -5.89 No46_0176 Hypothetical protein -2.28 -5.39 No46_0176 Hypothetical protein -2.69 -4.98 ene-1,7-dioic acid hydratase -2.69 -4.98 No46_013 Hypothetical protein -3.03 -4.81 No46_2320 Hypothetical protein -3.03 -4.81 No46_2402 Hypothetical protein -3.03 -4.81 No46_2548 Glycerol dehydrogenase -4.40 -4.31 No46_2548 Glycerol dehydrogenase -2.70 -3.74 Y No46_0131 Hypothetical protein FIM -2.05 -3.74 Y No46_0213	N646_3939	Hypothetical protein	-3.08	-7.66		
No46_0389 Hypothetical protein -4.52 -6.73 No46_0390 Hypothetical protein -4.71 -6.44 No46_0384 Putative dioxygenase -2.57 -6.38 No46_0374 GTP cyclohydrolase II -2.57 -6.38 No46_0714 Transcriptional regulator, GnR family protein -2.75 -6.24 No46_0714 Transcriptional regulator, GnR family protein -2.75 -6.24 No46_0176 Hypothetical protein -2.28 -5.39 No46_0176 Hypothetical protein -2.24 -4.98 ene-1,7-dioic acid hydratase -ene-1,7-dioic acid hydratase -6.44 No46_0302 Hypothetical protein -111.19 -4.81 No46_0303 Putative capsular polysaccharide biosynthesis -4.00 -4.31 No46_0304 Hypothetical protein FIFF -5.32 -3.76 Y No46_0313 Caboxythesphonoenolpyruste phosphonomutase -2.70 -3.71 Y No46_045 Hypothetical protein -7.04 -3.52 Y Y No46_0456	N646_0532	Hypothetical protein	-32.48	-7.18		
No46_0330Hypothetical protein -4.98 -6.44 N646_052Hypothetical protein -4.71 -6.43 N646_0384Putative dioxygenase -2.40 -6.38 N646_0714Transcriptional regulator, GntR family protein -2.75 -6.24 N646_0714Transcriptional regulator, GntR family protein -2.75 -6.24 N646_0714Transcriptional regulator, GntR family protein -2.28 -5.99 N646_0716Hypothetical protein -2.28 -5.39 N646_0385 2 -Keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 ene-1,7-dioic acid hydratase -111.19 -4.81 N646_0303Hypothetical protein -2.69 -4.98 N646_0303Hypothetical protein -3.03 -4.81 N646_2548Glycerol dehydrogenase -4.40 -4.31 N646_1338Flagellar polysaccharide biosynthesis -4.10 -4.59 N646_0713Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.71 N646_0713Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.61 N646_031Hypothetical protein -7.04 -3.52 N646_032Duidoreutase -6.58 -3.26 YN646_032Divothetical protein -7.04 -3.52 N646_033Hypothetical protein -7.04 -3.52 N646_0431Hypothetical protein -7.04 -3.52 N646_0432Divothetical protein -2.207 -3.16 N646_032Divo	N646_0389	Hypothetical protein	-4.52	-6./3		
No46_0362 Hypothetical protein -4.7.1 -6.43 No46_0344 Putative dioxygenase -2.40 -6.38 No46_03470 GTP cyclohydrolase II -2.57 -6.38 No46_014 Transcriptional regulator, GntR family protein -2.75 -6.24 No46_0176 Hypothetical protein -2.03 -5.99 No46_0176 Hypothetical protein -0.46 -5.88 No46_0176 Hypothetical protein -2.28 -5.39 No46_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98	N646_0390	Hypothetical protein	-4.98	-6.44		
No46, 2034 Putative dioxygenase -2.40 -6.38 No46, 3470 GTP cyclohydrolase II -2.57 -6.38 No46, 4629 Cytochrome c oxidase, subunit II -2.03 -5.99 No46, 4629 Cytochrome c oxidase, subunit II -2.03 -5.99 No46, 140 505 ribosomal protein L31 -0.46 -5.88 No46, 0176 Hypothetical protein -2.28 -5.39 No46, 0136 Pytothetical protein -2.42 -4.98 ene-1.7-dioic acid hydratase/2-oxohepta-3- -2.42 -4.98 weithetical protein -111.19 -4.81 No466,0013 Hypothetical protein -13.03 -4.81 No464,2548 Glycerol dehydrogenase -4.40 -4.31 No464,2544 Putative fimbrial assembly protein FIHF -5.32 -3.76 Y No464,0713 Carboxyphosphoneonlopyruvate phosphonomutase -2.70 -3.71 Y No464,0713 Carboxyphosphoneonlopyruvate phosphonomutase -2.70 -3.71 Y No464,0713 Carboxyphosphoneonlopyruvate phosphono	N646_0562	Hypothetical protein	-4./1	-6.43		
Ne46_34/0GIP cyclohydrolase II -2.57 -6.38 Ne46_0714Transcriptional regulator, GntR family protein -2.75 -6.24 Ne46_144650S ribosomal protein L31 -0.46 -5.88 Ne46_03852-Keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 ene-1.7-dioic acid hydratase -111.19 -4.81 Ne46_0330Hypothetical protein -2.69 -4.98 Ne46_0302Hypothetical protein -3.03 -4.81 Ne46_0303Hypothetical protein -3.03 -4.81 Ne46_04302Hypothetical protein -3.03 -4.81 Ne46_04302Hypothetical protein -3.03 -4.81 Ne46_0530Putative capsular polysaccharide biosynthesis -4.10 -4.59 Ne46_1338Flagellar biosynthesis protein FIhF -5.32 -3.76 YNe46_1338Flagellar biosynthesis protein FIhF -5.32 -3.61 YNe46_0456Hypothetical protein -2.36 -3.61 YNe46_0431Hypothetical protein -2.36 -3.61 YNe46_0432Oxidoreductase -6.58 -3.26 YNe46_0432Oxidoreductase -6.58 -3.26 YNe46_0432Oxidoreductase -2.04 -2.99 YNe46_0432Oxidoreductase -2.64 -2.99 YNe46_0432Oxidoreductase -2.64 -2.99 YNe46_0456Enoyl-CoA hydratase -2.04 -2.99 YNe46_045	N646_0384	Putative dioxygenase	-2.40	-6.38		
N646_00/14 Iranscriptional regulator, GntR family protein -2.75 -6.24 N646_4629 Cytochrome c oxidase, subunit II -2.03 -5.99 N646_1446 505 ribosomal protein L31 -0.46 -5.88 N646_0176 Hypothetical protein -2.28 -5.39 N646_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 ene-1,7-dioic acid hydratase -111.19 -4.81 N646_0313 Hypothetical protein -111.19 -4.81 N646_530 Putative capsular polysaccharide biosynthesis -4.10 -4.59 Y N646_0713 Glycerol dehydrogenase -4.40 -4.31 Y N646_0454 Putative fimbrial assembly protein PIM -2.05 -3.74 Y N646_0455 Hypothetical protein -2.36 -3.51 Y N646_0456 Hypothetical protein -2.36 -3.51 Y N646_0430 Putative finbrial assembly protein PIM -2.35 -3.61 Y N646_0456 Hypothetical protein -3.33 -3.26 Y N646_0430 Putative phenylacetate-CoA ligase <t< td=""><td>N646_3470</td><td>GIP cyclohydrolase II</td><td>-2.57</td><td>-6.38</td><td></td><td></td></t<>	N646_3470	GIP cyclohydrolase II	-2.57	-6.38		
No46_24629 Cytochrome c oxidase, subunt II -2.03 -5.99 No46_1446 505 ribosomal protein L31 -0.46 -5.39 No46_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- ene-1,7-dioic acid hydratase -2.42 -4.98 No46_0303 Hypothetical protein -111.19 -4.81 No46_3044 Pirin-related protein -3.03 -4.81 No46_2508 Putative capsular polysaccharide biosynthesis -4.10 -4.59 No46_2548 Glycerol dehydrogenase -4.40 -4.31 No46_1338 Flagellar biosynthesis protein FlhF -5.32 -3.76 Y No46_0456 Hypothetical protein -2.36 -3.71 Y No46_0456 Hypothetical protein -2.36 -3.61 Y No46_0456 Hypothetical protein -2.36 -3.61 Y No46_0456 Hypothetical protein -3.52 Y Y No46_0456 Hypothetical protein -2.36 -3.61 Y No46_0456 Hypothetical protein -2.33 -3.26 <t< td=""><td>N646_0/14</td><td>Transcriptional regulator, GntR family protein</td><td>-2.75</td><td>-6.24</td><td></td><td></td></t<>	N646_0/14	Transcriptional regulator, GntR family protein	-2.75	-6.24		
N646_1446 505 ribosomal protein LS1 -0.46 -5.88 N646_0176 Hypothetical protein -2.28 -5.39 N646_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- -2.42 -4.98 N646_03844 Prin-related protein -111.19 -4.81 N646_0530 Putative capsular polysaccharide biosynthesis -4.10 -4.59 Y N646_1384 Flagellar biosynthesis protein FIhF -5.32 -3.76 Y N646_0713 Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.71 Y N646_0431 Hypothetical protein -7.04 -3.52 Y Y N646_2724 DNA-binding response regulator Pho8 -2.33 -3.26 Y N646_0320 Putative phenylacetate-CoA ligase -6.58 -3.26 Y N646_2270 Hypothetical protein	N646_4629	Cytochrome c oxidase, subunit II	-2.03	-5.99		
N646_0176 Hypothetical protein -2.28 -5.39 N646_0385 2-Keto-4-pentenoate hydratase/2-oxhepta-3- ene-1,7-dioic acid hydratase -2.42 -4.98 N646_0013 Hypothetical protein -111.19 -4.81 N646_0013 Hypothetical protein -3.03 -4.81 N646_0530 Putative capsular polysaccharide biosynthesis -4.10 -4.59 Y N646_0530 Putative capsular polysaccharide biosynthesis -4.40 -4.31 -5.32 -3.76 Y N646_013 Flagellar biosynthesis protein FIhF -5.32 -3.76 Y Y N646_0456 Hypothetical protein -2.05 -3.71 Y Y N646_0456 Hypothetical protein -2.36 -3.61 Y Y N646_0456 Hypothetical protein -2.36 -3.61 Y Y N646_04030 Putative phenylacetate-CoA ligase -6.58 -3.26 Y N646_0422 Oxidoreductase -6.58 -3.26 Y N646_0422 Hypothetical protein -3.55 -3.16 Y N646_0422 Hypothetical pro	N646_1446	50S ribosomal protein L31	-0.46	-5.88		
N646_0385 2-Keto-4-pentenoate hydratase/2-oxohepta-3- ene-1,7-dioic acid hydratase -2.42 -4.98 N646_3844 Pirin-related protein -2.69 -4.98 N646_0013 Hypothetical protein -111.19 -4.81 N646_4302 Hypothetical protein -3.03 -4.81 N646_2530 Putative capsular polysaccharide biosynthesis -4.10 -4.59 Y N646_2548 Glycerol dehydrogenase -4.40 -4.31 Y N646_1338 Flagellar biosynthesis protein FIHF -5.32 -3.76 Y N646_0713 Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.71 Y N646_0456 Hypothetical protein -2.36 -3.51 Y N646_4030 Putative phenylacetate-CoA ligase -8.34 -3.41 N646_2720 DNA-binding response regulator PhoB -2.33 -3.26 Y N646_2270 Hypothetical protein -2.07 -3.16 Y N646_3227 CsuA -2.207 -3.06 Y N646_6453 Amino acid ABC transporter, periplasmic amino-acid-binding protein -2.64 -2.89	N646_0176	Hypothetical protein	-2.28	-5.39		
Neffect Base of the second sec	N646_0385	2-Keto-4-pentenoate hydratase/2-oxohepta-3-	-2.42	-4.98		
N646_3844Print-related protein -2.69 -4.98 N646_0013Hypothetical protein -111.19 -4.81 N646_4302Hypothetical protein -3.03 -4.81 N646_0530Putative capsular polysaccharide biosynthesis -4.10 -4.59 YN646_2548Glycerol dehydrogenase -4.40 -4.31 YN646_1338Flagellar biosynthesis protein FlhF -5.32 -3.76 YN646_0713Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.71 YN646_0456Hypothetical protein -7.04 -3.52 -3.61 N646_4030Putative phenylacetate-CoA ligase -8.34 -3.41 YN646_4032Oxidoreductase -6.58 -3.26 YN646_42270Hypothetical protein -3.55 -3.16 YN646_456Hopothetical protein -2.07 -3.36 YN646_457Csula -2.207 -3.26 YN646_4032Oxidoreductase -6.58 -3.26 YN646_457Hypothetical protein -2.04 -2.99 YN646_455Amino acid ABC transporter, periplasmic amino-acid- binding protein -2.64 -2.89 YN646_675Hypothetical protein -2.06 -2.71 YN646_2932Flagellar P-ring protein Flgl -3.54 -2.67 YN646_2931Hypothetical protein -2.66 -2.67 Y		ene-1,7-dioic acid hydratase				
N646_0013Hypothetical protein -111.19 -4.81 N646_4302Hypothetical protein -3.03 -4.81 N646_0530Putative capsular polysaccharide biosynthesis -4.10 -4.59 YN646_2548Glycerol dehydrogenase -4.40 -4.31 1N646_1338Flagellar biosynthesis protein FIhF -5.32 -3.76 YN646_0713Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.71 YN646_0456Hypothetical protein -7.04 -3.52 -3.61 N646_4031Hypothetical protein -7.04 -3.52 YN646_2724DNA-binding response regulator PhoB -2.33 -3.26 YN646_2270Hypothetical protein -3.55 -3.16 YN646_4532CsuA -22.07 -3.06 YN646_4645Enoyl-CoA hydratase -2.04 -2.99 YN646_4675Hypothetical protein -2.64 -2.89 YN646_2932Flagellar P-ring protein FIgl -3.54 -2.67 Y	N646_3844	Pirin-related protein	-2.69	-4.98		
N646_4302Hypothetical protein-3.03-4.81N646_0530Putative capsular polysaccharide biosynthesis-4.10-4.59YN646_2548Glycerol dehydrogenase-4.40-4.31YN646_1338Flagellar biosynthesis protein FIFF-5.32-3.76YN646_0713Carboxyphosphonoenolpyruvate phosphonomutase-2.70-3.71YN646_0456Hypothetical protein-2.36-3.61YN646_4031Hypothetical protein-2.36-3.61YN646_4030Putative phenylacetate-CoA ligase-8.34-3.41YN646_2724DNA-binding response regulator PhoB-2.33-3.26YN646_4032Oxidoreductase-6.58-3.26YN646_4272DNA-binding response regulator PhoB-2.07-3.16YN646_4508Enoyl-CoA hydratase-2.04-2.99YN646_6455Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.64-2.89-2.67N646_2932Flagellar P-ring protein FIgI-3.54-2.67YN646_2932Flagellar P-ring protein FIgI-3.54-2.67Y	N646_0013	Hypothetical protein	-111.19	-4.81		
N646_0530Putative capsular polysaccharide biosynthesis-4.10-4.59YN646_2548Glycerol dehydrogenase-4.40-4.31N646_1338Flagellar biosynthesis protein FlhF-5.32-3.76YN646_1844Putative fimbrial assembly protein PilM-2.05-3.74YN646_0713Carboxyphosphonoenolpyruxte phosphonomutase-2.70-3.71YN646_0456Hypothetical protein-7.04-3.52-3.61N646_4031Hypothetical protein-7.04-3.52YN646_4030Putative phenylacetate-CoA ligase-8.34-3.41YN646_2724DNA-binding response regulator PhoB-2.33-3.26YN646_4032Oxidoreductase-6.58-3.26YN646_2270Hypothetical protein-3.55-3.16YN646_4508Enoyl-CoA hydratase-2.04-2.99YN646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.06-2.71YN646_2932Flagellar P-ring protein Flgl-3.54-2.67Y	N646_4302	Hypothetical protein	-3.03	-4.81		
N646_2548Glycerol dehydrogenase -4.40 -4.31 N646_1338Flagellar biosynthesis protein FlhF -5.32 -3.76 YN646_1844Putative fimbrial assembly protein PilM -2.05 -3.74 YN646_0713Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.71 YN646_0456Hypothetical protein -2.36 -3.61 YN646_4031Hypothetical protein -7.04 -3.52 YN646_4030Putative phenylacetate-CoA ligase -8.34 -3.41 YN646_4032Oxidoreductase -6.58 -3.26 YN646_2270Hypothetical protein -3.55 -3.16 YN646_432Oxidoreductase -6.58 -3.26 YN646_45272CsuA -22.07 -3.06 YN646_4508Enoyl-CoA hydratase -2.04 -2.99 YN646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein -2.64 -2.89 YN646_2292Flagellar P-ring protein Flgl -3.54 -2.67 Y	N646_0530	Putative capsular polysaccharide biosynthesis	-4.10	-4.59		Y
N646_1338Flagellar biosynthesis protein FlhF-5.32-3.76YN646_1844Putative fimbrial assembly protein PilM-2.05-3.74YN646_0713Carboxyphosphonoenolpyruvate phosphonomutase-2.70-3.71YN646_0456Hypothetical protein-2.36-3.61YN646_4031Hypothetical protein-7.04-3.52YN646_4030Putative phenylacetate-CoA ligase-8.34-3.41YN646_4032Oxidoreductase-6.58-3.26YN646_2270Hypothetical protein-3.55-3.16YN646_3227CsuA-22.07-3.06YN646_6453Enoyl-CoA hydratase-2.04-2.99YN646_6645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.06-2.71YN646_2932Flagellar P-ring protein FlgI-3.54-2.67YN646_0301Hypothetical protein-2.06-2.71Y	N646_2548	Glycerol dehydrogenase	-4.40	-4.31		
N646_1844Putative fimbrial assembly protein PilM-2.05-3.74YN646_0713Carboxyphosphonoenolpyruvate phosphonomutase-2.70-3.71N646_0456Hypothetical protein-2.36-3.61N646_4031Hypothetical protein-7.04-3.52N646_4030Putative phenylacetate-CoA ligase-8.34-3.41N646_2724DNA-binding response regulator PhoB-2.33-3.26N646_4032Oxidoreductase-6.58-3.26N646_2270Hypothetical protein-3.55-3.16N646_2270Hypothetical protein-2.07-3.06N646_3227CsuA-22.07-3.06YN646_6455Enoyl-CoA hydratase-2.04-2.99N646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.06-2.71N646_4675Hypothetical protein-2.06-2.71N646_2932Flagellar P-ring protein Flgl-3.54-2.67Y	N646_1338	Flagellar biosynthesis protein FlhF	-5.32	-3.76		Y
N646_0713Carboxyphosphonoenolpyruvate phosphonomutase -2.70 -3.71 N646_0456Hypothetical protein -2.36 -3.61 N646_4031Hypothetical protein -7.04 -3.52 N646_4030Putative phenylacetate-CoA ligase -8.34 -3.41 N646_2724DNA-binding response regulator PhoB -2.33 -3.26 N646_4032Oxidoreductase -6.58 -3.26 N646_2270Hypothetical protein -3.55 -3.16 N646_3227CsuA -22.07 -3.06 N646_4508Enoyl-CoA hydratase -2.04 -2.99 N646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein -2.66 -2.71 N646_2932Flagellar P-ring protein Flgl -3.54 -2.67 YN646_0301Hypothetical protein -5.28 -2.67 Y	N646_1844	Putative fimbrial assembly protein PilM	-2.05	-3.74		Y
N646_0456Hypothetical protein -2.36 -3.61 N646_4031Hypothetical protein -7.04 -3.52 N646_4030Putative phenylacetate-CoA ligase -8.34 -3.41 N646_2724DNA-binding response regulator PhoB -2.33 -3.26 N646_4032Oxidoreductase -6.58 -3.26 N646_2270Hypothetical protein -3.55 -3.16 N646_3227CsuA -22.07 -3.06 N646_4508Enoyl-CoA hydratase -2.04 -2.99 N646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein -2.64 -2.89 N646_2932Flagellar P-ring protein Flgl -3.54 -2.67 YN646_0301Hypothetical protein -5.28 -2.67 Y	N646_0713	Carboxyphosphonoenolpyruvate phosphonomutase	-2.70	-3.71		
N646_4031Hypothetical protein-7.04-3.52N646_4030Putative phenylacetate-CoA ligase-8.34-3.41N646_2724DNA-binding response regulator PhoB-2.33-3.26N646_4032Oxidoreductase-6.58-3.26N646_2270Hypothetical protein-3.55-3.16N646_3227CsuA-22.07-3.06YN646_4508Enoyl-CoA hydratase-2.04-2.99N646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.66-2.71N646_2932Flagellar P-ring protein Flgl-3.54-2.67YN646_0301Hypothetical protein-5.28-2.67Y	N646_0456	Hypothetical protein	-2.36	-3.61		
N646_4030Putative phenylacetate-CoA ligase-8.34-3.41N646_2724DNA-binding response regulator PhoB-2.33-3.26YN646_4032Oxidoreductase-6.58-3.26YN646_2270Hypothetical protein-3.55-3.16YN646_3227CsuA-22.07-3.06YN646_4508Enoyl-CoA hydratase-2.04-2.99YN646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.64-2.89YN646_2932Flagellar P-ring protein Flgl-3.54-2.67YN646_0301Hypothetical protein-5.28-2.67Y	N646_4031	Hypothetical protein	-7.04	-3.52		
N646_2724DNA-binding response regulator PhoB-2.33-3.26YN646_4032Oxidoreductase-6.58-3.26YN646_2270Hypothetical protein-3.55-3.16YN646_3227CsuA-22.07-3.06YN646_4508Enoyl-CoA hydratase-2.04-2.99YN646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.64-2.89YN646_2932Flagellar P-ring protein Flgl-3.54-2.67YN646_0301Hypothetical protein-5.28-2.67Y	N646_4030	Putative phenylacetate-CoA ligase	-8.34	-3.41		
N646_4032 Oxidoreductase -6.58 -3.26 N646_2270 Hypothetical protein -3.55 -3.16 N646_3227 CsuA -22.07 -3.06 Y N646_4508 Enoyl-CoA hydratase -2.04 -2.99 -2.09 N646_0645 Amino acid ABC transporter, periplasmic amino-acid- -2.64 -2.89 -2.04 N646_4675 Hypothetical protein -2.06 -2.71 -2.67 Y N646_2932 Flagellar P-ring protein Flgl -3.54 -2.67 Y N646_0301 Hypothetical protein -5.28 -2.67 Y	N646_2724	DNA-binding response regulator PhoB	-2.33	-3.26		Y
N646_2270 Hypothetical protein -3.55 -3.16 N646_3227 CsuA -22.07 -3.06 Y N646_4508 Enoyl-CoA hydratase -2.04 -2.99 -2.09 N646_0645 Amino acid ABC transporter, periplasmic amino-acid- binding protein -2.64 -2.89 N646_4675 Hypothetical protein -2.06 -2.71 N646_2932 Flagellar P-ring protein Flgl -3.54 -2.67 Y N646_0301 Hypothetical protein -5.28 -2.67 Y	N646_4032	Oxidoreductase	-6.58	-3.26		
N646_3227 CsuA -22.07 -3.06 Y N646_4508 Enoyl-CoA hydratase -2.04 -2.99 -2.04 -2.99 N646_0645 Amino acid ABC transporter, periplasmic amino-acid- binding protein -2.64 -2.89 -2.71 N646_2932 Flagellar P-ring protein Flgl -3.54 -2.67 Y N646_0301 Hypothetical protein -5.28 -2.67 Y	N646_2270	Hypothetical protein	-3.55	-3.16		
N646_4508Enoyl-CoA hydratase-2.04-2.99N646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.64-2.89N646_4675Hypothetical protein-2.06-2.71N646_2932Flagellar P-ring protein Flgl-3.54-2.67YN646_0301Hypothetical protein-5.28-2.67	N646_3227	CsuA	-22.07	-3.06		Y
N646_0645Amino acid ABC transporter, periplasmic amino-acid- binding protein-2.64-2.89N646_4675Hypothetical protein-2.06-2.71N646_2932Flagellar P-ring protein Flgl-3.54-2.67YN646_0301Hypothetical protein-5.28-2.67	N646_4508	Enoyl-CoA hydratase	-2.04	-2.99		
N646_4675Hypothetical protein-2.06-2.71N646_2932Flagellar P-ring protein Flgl-3.54-2.67YN6460301Hypothetical protein-5.28-2.67Y	N646_0645	Amino acid ABC transporter, periplasmic amino-acid-	-2.64	-2.89		
No40_4073 Hypothetical protein -2.06 -2.71 N646_2932 Flagellar P-ring protein Flgl -3.54 -2.67 Y N646_0301 Hypothetical protein -5.28 -2.67 Y	N646 4675	Upothetical protein	-2.06	_ 2 71		
No40_2732 Flagelidi P-fling protein Figi -3.34 -2.07 Y N646_0301 Hypothetical protein -5.28 -2.67	N646 2022	Hypothetical protein Elagollar Diring protoin Elal	-2.00	-2.71		V
	N646 0301	Hypothetical protein	-5.28	-2.67		

(Continued on next page)

TABLE 1 (Continued)

Gene identification ^a	Annotation ⁶	Fold change (∆rpoE/WT)	Fold change (∆ <i>rpoX/</i> WT)	Promoter region bound by RpoX determined by ChIP-seq ^c	Virulence- associated gene ^d
N646 4059	Hypothetical protein	-2.10	-2.66	· ·	
N646_2799	Small protein A	-2.16	-2.60		
N646_2936	Flagellin	-2.28	-2.57		Y
N646_3225	CsuC	-15.77	-2.49		Ŷ
N646 4417	Hypothetical protein	-10.82	-2.40		•
N646_3072	Hypothetical protein	-2.52	-2.36		
N646_4028	Hypothetical protein	-14 67	-2.36		
N646_0455	Hypothetical protein	-2.10	-2.30		
N646_0799	Hypothetical protein	-3.72	-2.27		
N646_0531	Periplasmic protein involved in polysaccharide	-16.16	-2.27		Y
	export				
N646_0807	Hypothetical protein	-2.45	-2.24		
N646_3224	CsuD	-5.06	-2.22		Y
N646_4029	Putative high-affinity branched-chain-amino-acid transport ATP-binding protein	-11.73	-2.21		
N646 0145	Tryptophanyl-tRNA synthetase	-2.99	-2.19		
N646 3223	CsuE	-2.97	-2.17		Y
N646_0685	Hypothetical protein	-3.58	-2.04		
N646 4419	Hypothetical protein	-2.18	-2.03		
N646_0561	Formate dehydrogenase accessory protein	-3.67	-2.00		
N646_0626	Hypothetical protein	5.81	2.05		
N646_2558	Hypothetical protein	2.20	2.05		
N646_0313	Imidazolonepropionase	2.47	2.07		
N646 2232	Hypothetical protein	3.12	2 10		
N646_3280	Pyruvate formate-lyase	2.88	2.10		
N646 1747	Aspartate carbamovitransferase regulatory subunit	2.60	2.15		
N646 3214	Hypothetical protein	3 47	2.15		
N646_0304	Outer membrane protein	2.02	2.19		
N646_0641	Hypothetical protein	3.82	2.28		
N646_3434	Putative ribosomal protein N-acetyltransferase	2 58	2 39		
N646 2428	LITP-qlucose-1-phosphate uridylyltransferase	2.36	2.35		
N646 4424	Hypothetical protein	2.55	2.17		
N646 4184	Hypothetical protein	2.06	2.56		
N646_3925	Putative acriflavine resistance protein	2.00	2.50		
N646_3664	Thermolabile hemolysin	2.75	2.65		Y
N646_4456	Putative KHG/KDPG aldolase	2.73	2.03		•
N646_3202	Putative ABC transporter membrane-spanning	2.03	2.96		
N646 3782	Hypothetical protein	3 47	415		
N646_3806	Alcohol dehydrogenase, zinc-binding domain	3.66	4.21		
N646 3556	Putative hydrolase	3.26	5 04		
N646_2057	Hypothetical protein	2.95	5.26		
N646_2057	Argining ABC transporter periplasmic	11 25	5.20		
	arginine-binding protein	11.25	5.50		
N646_3755	Putative muconate cycloisomerase I	2.24	6.51		
N646_3685	Hypothetical protein	5.6/	7.51		
N646_3856	Hypothetical protein	3.03	7.62		
N646_3885	Glyceraldehyde-3-phosphate dehydrogenase	2.77	/.82	Y.	
N646_4611	Hypothetical protein	1.02	-1.27	Y	
N646_1623	505 ribosomal protein L19	1.09	2.37	Y	
N646 1624	tkina (quanine-/V')-methyltransferase	1.03	2.6/	Y	

^{*a*}All the genes with differential expression with a *P* value of <0.001.

^bFMN, flavin mononucleotide.

cY indicates that the promoter region of the gene was also bound by RpoX, as identified by ChIP-seq analysis.

 $^{d}\mathrm{Y}$ indicates that the gene was annotated as a virulence-associated genes.

negative control, and no peak was found in the promoter region of *gyrB* during ChIP analysis (Fig. 5D). Overall, four genes containing *rpoX*-binding sites were identified by EMSAs and ChIP-seq analysis. Among these four ChIP-identified promoter regions, *rpoX*, N646_4604 (N646_4603 and N646_4604 are in the same operon), and N646_4601 were positively regulated and N646_1623 was negatively regulated by RpoX, as revealed by



FIG 3 Comparative analyses of the transcriptional responses of *V. alginolyticus* to *rpoE* and *rpoX* abrogation at 42°C. (A and B) Pie charts representing genes differentially transcribed in $\Delta rpoE$ (A) and $\Delta rpoX$ (B) cells compared to WT cells grown in LBS medium at 42°C. (C) Venn diagrams showing overlapping genes with significantly increased or decreased transcript abundances (FC ≥ 2 or FC ≤ -2 ; adjusted *P* value [P_{adj}] of $<1 \times 10^{-2}$) in response to different culture conditions. (D and E) MA plots depicting changes in gene expression between $\Delta rpoE$ and WT strains (E) in LBS medium at 42°C. The log₂ value of the ratios of the abundances of each transcript between the two conditions (M) (*y* axis) is plotted against the average log₂ value of the abundance of that transcript under both conditions (A) (*x* axis).

RNA-seq analysis. Interestingly, the gene N646_4604 encodes RTX-type hemolysin D (HlyD), and HlyD has been reported to be a hemolysin in other bacteria (25), further suggesting a role for RpoX in the pathogenesis of *V. alginolyticus*.

RpoX modulates biofilm formation, motility, and hemolytic activities in V. *alginolyticus.* In our RNA-seq analysis, we found that several flagellum-related genes were regulated by the RpoE and RpoX proteins. We thus further investigated the roles of *rpoX* in the motility of this bacterium. The swimming ability was significantly reduced in the $\Delta rpoX$ strain compared with the WT strain, and *rpoX* complementation restored the swimming ability at high temperatures (Fig. 6A); however, there was no significant difference in swarming abilities between the WT and $\Delta rpoX$ strains (Fig. 6A).

Furthermore, biofilm formation was significantly decreased in the $\Delta rpoX$ strain compared with the WT strain, and biofilm formation was restored when the rpoX gene was

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FIG 4 Conserved binding site of RpoE and RpoX generated by RNA-seq data. The most significant RpoE-binding motif (A) or RpoX-binding motif (B) was derived from an RNA-seq binding sequence generated by the MEME-suite tool. The height of each letter represents the relative frequency of each base at different positions in the consensus sequence.

complemented (Fig. 6B). As shown in Fig. 2A, N646_4611 encodes a GGDEF family protein and is located upstream of the rpoX gene; GGDEF family proteins have been identified as regulators of biofilm formation and motility (24). Therefore, we constructed a Δ N646_4611 mutant strain and an N646_4611-overexpressing strain driven by the pBAD promoter in the ΔN646_4611 mutant strain (N646_4611^{OE}/ΔN646_4611) with the induction of L-arabinose, which showed no apparent influence on V. alginolyticus biofilm formation. Biofilm formation was significantly reduced in the $\Delta N646_4611$ mutant strain and enhanced in the N646 4611^{OE}/ Δ N646 4611 strain (Fig. 6B). To determine whether RpoX regulates biofilm formation via N646_4611, we overexpressed the N646_4611 protein in the $\Delta rpoX$ strain, N646_4611^{OE}/*ΔrpoX*, and the level of biofilm formation by the strain was significantly lower than that by the N646_4611^{OE}/ Δ N646_4611 strain but higher than that by the N646_4611^{OE}/ $\Delta rpoX$ strain (Fig. 6B), which suggested that RpoX might not regulate biofilm formation via the N646_4611 protein. RNA-seq data showed that N646_4604, encoding RTX-type hemolysin D, was downregulated in the $\Delta rpoX$ strain. $\Delta rpoX$ cells showed weaker hemolytic activity than WT cells, and complementation of *rpoX* restored the activity to the WT level at 30°C and 42°C. The Δ *rpoE* mutant strain showed drastically reduced hemolytic activity similar to that of the $\Delta hlyD$ strain (Fig. 6C).

Quantitative real-time reverse transcription-PCR (qRT-PCR) was then further used to verify the roles of *rpoX* in the above-mentioned genes' expression. The results showed that flagellum-related genes (*flgD*, *flgC*, and *flgB*) were downregulated in the $\Delta rpoX$ strain compared with the WT strain, and the expression of these genes was restored to WT levels in the *rpoX*-complemented strain (Fig. 6D). Moreover, the expression of the exopolysaccharide biosynthesis- and biofilm formation-associated gene *exoQ* and the hemolysin-related gene *hlyD* was significantly downregulated in the $\Delta rpoX$ strain compared to the WT strain, and complementation restored the expression of these



FIG 5 ChIP-seq analysis of genes directly bound and regulated by RpoX. (A to C) N646_4603 (A), N646_4601 (B), and N646_1623 (C) were used for peak comparison of ChIP-seq (left) and EMSA (right) results. The fold enrichment of each of the typical promoters bound by RpoX is shown. (D) A 300-bp fragment of the *gyrB* promoter region is shown as the negative control, which cannot be bound by RpoX. B, bound DNA; F, free DNA. The numbers above each of the peaks indicate the enrichment fold change relative to the control.

genes (Fig. 6D). Taken together, these results demonstrated that the RpoX protein could modulate the expression of flagellum-, biofilm-, and hemolysis-related genes in *V. alginolyticus*.

Zebrafish were used as a model system to test the impact of RpoE and RpoX on the virulence of *V. alginolyticus*. The 50% lethal dose (LD₅₀) values for the WT and $\Delta rpoE$ strains were 2.5 × 10⁵ and 6.6 × 10⁶ CFU/fish at 30°C, respectively, demonstrating an essential role of *rpoE* in *V. alginolyticus* virulence. However, the $\Delta rpoX$ strain exhibited an LD₅₀ value of 1.8 × 10⁵ CFU/fish (Fig. 6E), indicating that the deletion of *rpoX* did not significantly impair virulence toward fish. Collectively, these data illuminated the RpoE-RpoX-centered regulatory cascades and their distinct and overlapping regulatory functions in pathogenesis and in stress responses in *V. alginolyticus*.

DISCUSSION

Sigma factors can interact with the RNA polymerase (RNAP) core enzyme to generate an RNAP holoenzyme and initiate the transcription of a specific set of genes responsible for the stress response and virulence (26). Here, we identified the *rpoX* gene, included as part of the regulon of RpoE, and genetic analysis showed that RpoX lacked the region 3 domain that is present in the RpoS protein. We speculate that the RpoX protein might be a paralog of RpoH because they share the same functional domains (see Fig. S1A in the supplemental material) and 45% overall similarity, and both proteins are alternative sigma factors under subhierarchical control by RpoE and are involved in high-temperature and low-osmotic-stress responses (Fig. 1 and 2) (3). Moreover, the high expression level of RpoH seems to be able to rescue the growth defects of the $\Delta rpoX$ strain under high-temperature and low-osmotic-stress conditions (Fig. 1F). Further experiments with the *rpoH* null mutant to compare the regulons of RpoH and RpoX as well as their recognized promoters will validate their homology and functional redundancy in response to stresses.

The dozens of established alternative sigma factors, i.e., *rpoH*, *rpoN*, *rpoE*, and *rpoS*, etc., are all subject to tight regulation under various specific physiological conditions (12). Interestingly, our data indicated that the expression of *rpoX* was induced under both low-osmotic-stress conditions and high temperatures (Fig. 1). Although how these



FIG 6 RpoX positively regulates motility, biofilm formation, and hemolytic activities. (A) Motility assays of WT, $\Delta rpoX$, and $rpoX^+$ strains. Diluted cultures were spotted onto swimming and swarming plates (containing 0.3% and 1.5% agar, respectively) and incubated for 48 h or 12 h at 42°C. Three independent cultures were used for each strain, and a representative result is displayed. (B) Assays of biofilm formation by different strains. For WT, $\Delta N646_4611$, $\Delta rpoX$, and $rpoX^+$ strains, biofilm formation in glass tubes containing LBS medium after 48 h of culturing was assayed. N646_4611^OE/\DeltaN646_4611 and N646_4611^OE/\Delta rpoX strains were cultured in LBS medium with 0.04% L-arabinose for 48 h. The results are presented as the means \pm SD (n = 3). **, P < 0.01; ***, P < 0.001; NS, not significant (by t test). (C) Hemolytic activities of WT, $\Delta rpoX$, $\Delta rpoE$, $rpoX^+$, and $\Delta luxR$ strains grown on sheep blood agar plates at 30°C (top) and at 42°C (bottom). (D) qRT-PCR analysis of the transcripts of the selected genes. Total RNA was isolated from the $\Delta rpoX$, and $rpoX^+$ strains after 12 h of growth in liquid culture. The results are presented as the means \pm SD (n = 3). (E) Median lethal dose (LD₅₀) of WT, $\Delta rpoX$, and $\Delta rpoE$ strains in zebrafish. Series of dilutions of WT, $\Delta rpoX$, and $\Delta rpoE$ strains were intramuscularly inoculated into fish that were acclimated at 30°C for 4 weeks. A total of 30 fish were used for each of the dilutions. The infected fish were cultivated at 30°C and monitored for 7 days. The results are presented as the means \pm SD (n = 3). *, P < 0.05 (by t test).

conditions exert influences on *rpoX* expression warrants further investigation, these observations suggested that this alternative sigma factor may be induced *in vivo* in marine animals or at extremely high sea surface temperatures, orchestrate gene expression in response to these stresses, and thus facilitate *Vibrio* adaptation under both *in vivo* and *in vitro* conditions of the hosts. Indeed, our further transcriptomic and phenotypic investigations indicated that RpoX was involved in the expression of various genes (Table 1 and Fig. 6). We thus unraveled a novel RpoX-involved

signal transduction pathway in vibrios to respond to low-osmotic-stress and high-temperature stimuli.

As an alternative sigma factor, RpoE is released via regulated intramembrane proteolysis of the anti-sigma factor RseA triggered by membrane stresses (13) and has been found to be essential for stress adaptation and virulence in response to environmental stimuli in various bacterial pathogens, such as V. parahaemolyticus, V. harveyi, V. cholerae, and Salmonella (15, 16, 27-29). The RpoE protein can directly regulate the expression of the RpoH protein and is responsible for the high-temperature stress response of V. alginolyticus (3). In this study, we found that the RpoE protein can also bind directly to the promoter and control the expression of the RpoX protein (Fig. 2). RNA-seq was used to identify the regulons of the RpoX and RpoE proteins at a high temperature (42°C). The results showed that the regulon of RpoE contains more genes than that of RpoX (Fig. 3A and B), which, in addition to the result that the RpoE protein can directly bind to the promoter of rpoX and trigger the expression of rpoX (Fig. 2B to D), further confirmed that RpoX is at the subhierarchical level in the RpoE regulatory cascade; i.e., RpoE may act upstream of the regulatory cascade of RpoX. ChIP-seq and EMSA results also showed that the *rpoX* gene can be directly regulated by RpoX (Fig. 2C).

Sigma factors have been reported to be essential regulators of virulence, and we found that many virulence-associated genes, i.e., the genes encoding hemolysin (N646_4604 and N646_3664), exopolysaccharides (N646_0523 and N646_0530), flagella (N646_1186, N646_2929, N646_1338, N646_1844, N646_2932, and N646_2936), and type I pili (N646_3227, N646_3225, N646_3224, and N646_3223) (Fig. 7), were regulated by RpoX or RpoE at high temperatures (Table 1). Accordingly, our study has shown that RpoX was involved in the pathogenesis-related regulation of motility, biofilm formation, and hemolytic activity (Fig. 6). Although the $\Delta rpoX$ mutant strain did not show apparent attenuation of lethality to zebrafish (Fig. 6E), it may be related to the pathogenesis process and virulence factor production in an undiscerned manner in response to stresses such as reactive oxygen species (ROS), osmotic changes, and temperatures (23). In V. alginolyticus, swimming ability was increased in the $\Delta rpoE$ strain at high temperatures (3), while this ability was significantly decreased in the $\Delta rpoX$ strain, suggesting that the regulation of motility by RpoX is independent of RpoE. Taken together, these analyses indicate that RpoX might modulate stress adaptation in an RpoE-dependent manner but regulate motility in an RpoE-independent manner. RNAseq analyses also support the idea that RpoX might be involved in some RpoEindependent processes and signaling in V. alginolyticus (Table 1).

We thus present a putative scenario where RpoE and RpoX are involved in the heat stress response in *V. alginolyticus* (Fig. 7). The release of RpoE protein tethered to the inner membrane into the cytoplasm could be a response to high temperatures, triggering the degradation of the anti-sigma factor RseA (3, 16, 27, 28). The RpoE protein can directly bind to the promoter of *rpoX* and control the expression of this gene. The RpoE sigma factor can directly regulate *rpoH* and *rpoX* to mediate the high-temperature response. However, our RNA-seq analysis indicated that, in addition to the high-temperature response, the RpoX protein may be able to regulate many other pathways, such as those associated with virulence processes, ABC transport, flagellar assembly, F-type ATPases, carbon metabolism, type I/II secretion systems, and two-component systems (Fig. 7 and Table 1). In addition, RpoX regulates its own expression, a feature exhibited by many other alternative sigma factors (21, 30). The question remains regarding how RpoE responds to heat or other stresses to orchestrate the expression of *rpoX*, *rpoH*, and the gene encoding itself, *rpoE*, as well as other alternative sigma factors.

In summary, this investigation presented an RpoE-RpoX-centered heat stress response regulatory cascade. These data facilitate an improved understanding of the regulatory networks of various alternative sigma factors contributing to their distinct and overlapping regulatory functions in stress responses and virulence in the pathogen *V. alginolyticus*.



FIG 7 Schematic of the regulation network of RpoX in *V. alginolyticus*. Pathway analysis was performed with the Kobas 3.0 algorithm. The various pathways and their respective cellular locations, as well as the regulatory roles of RpoX, are illustrated with arrows (activation) or bar-ended lines (repression) and are discussed in the text. 3P, 3-phosphate; TCA, tricarboxylic acid.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The strains and plasmids used in this study are listed in Table 2. The *V. alginolyticus* strains were grown in Luria-Bertani (LB) broth containing 3% sodium chloride (LBS broth) at 30°C as the normal growth conditions. *Escherichia coli* DH5 α (λpir), *E. coli* SM10 (λpir), and *E. coli* BL21(DE3) were grown in LB broth at 37°C. When appropriate, the medium was supplemented with carbenicillin (100 μ g ml⁻¹), chloramphenicol (25 μ g ml⁻¹), kanamycin (50 μ g ml⁻¹), or L-arabinose (0.2 mg ml⁻¹).

Deletion mutant and complemented strain construction. In-frame deletion mutants were generated as described in a previous study (10). The fragment was cloned into the Xbal sites of the suicide vector pDM4 (31), and the resulting plasmid was transformed into *E. coli* DH5 α λpir . After sequencing, pDM4 derivatives were transformed into *E. coli* SM10 λpir . This plasmid was introduced into *V. alginolyticus* by conjugation. The double-crossover recombinant was selected on LBS agar containing 15% sucrose. The mutation was confirmed by PCR and sequencing. A fragment containing the intact *rpoX* gene (a 282-bp fragment of the promoter adjoining the start codon "ATG" and the open reading frame [ORF]) and the Flag sequence was cloned into the plasmid pBAD33 to construct a complementation strain (3).

Immunoblot analysis. For the immunoblot assay, supernatants and bacterial cell pellets were harvested at the same optical density measured at 600 nm (OD₆₀₀). Next, 15 μ l of each sample was loaded onto a 12% denaturing polyacrylamide gel, and proteins were resolved by electrophoresis and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). The membranes were blocked with a 10% skim milk powder solution, incubated with a 1:2,000 dilution of Flag-specific (Sigma-Aldrich, St. Louis, MO) mouse antiserum, and incubated with a 1:2,000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology, CA). Finally, the blots were visualized with an enhanced chemiluminescence reagent (Thermo Fisher Scientific Inc., Waltham, MA).

Growth curves. Bacteria were incubated overnight and then diluted 1:100 in 50 ml of fresh LBS medium. The bacteria were then grown in LBS medium at 30° C or 42° C or in LB broth containing 0.5%

TABLE 2 Bacterial strain	ns and plasmid	s used in	this study
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Strain or plasmid	Description ^a	Source or reference
Strains		
Escherichia coli		
DH5 α λpir	Host for π -requiring plasmids	Laboratory collection
SM10 λpir	Host for π -requiring plasmids; conjugal donor; Km ^r	40
BL21(DE3)	Host strain for protein expression	Novagen
BL21/pET22b::rpoE	BL21; expression of RpoE; Km ^r	This study
BL21/pET22b::rpoX	BL21; expression of RpoX; Km ^r	This study
Vibrio alginolyticus		
EPGS	Wild type; fish isolate; CCTCC strain AB 209306; Carb ^r	Laboratory collection
ΔrpoE	EPGS; in-frame deletion in <i>rpoE</i> ; Carb ^r	3
ΔrpoX	EPGS; in-frame deletion in <i>rpoX</i> ; Carb ^r	This study
ΔluxR	EPGS: in-frame deletion in <i>luxR</i> : Carb ^r	3
ΔhlyD	EPGS; hlyD disrupted; Carbr Cmr	This study
ΔΝ646 4611	EPGS; GGDEF domain deletion in EPGS 03411; Carb	This study
Δasp	EPGS; disrupted in <i>asp</i> ; Carb ^r Cm ^r	6
rpoX ⁺	$\Delta r p \delta X$; pBAD33 carrying the intact <i>r p \delta X</i> gene	This study
N646_4611 ^{OE} /ΔN646_4611	ΔN646_4611; pBAD33 carrying the ORF of N646_4611	This study
N646 4611 ^{OE} /Δ <i>rpoX</i>	$\Delta r poX$; pBAD33 carrying the ORF of EPGS 03411; Carb ^r Cm ^r	This study
WT/pBAD33::Flag	EPGS; pBAD33 carrying the Flag gene; Carb ^r Cm ^r	This study
WT/pBAD33::Prov-Flag	EPGS; pBAD33 carrying the intact rpoX-Flag gene; Carbr Cmr	This study
Δ <i>rpoE</i> /pBAD33::P _{max} -Flag	$\Delta r poE$; pBAD33 carrying the intact <i>rpoX</i> -Flag gene; Carb ^r Cm ^r	This study
ΔrpoX/pBAD33::P _{mox} -Flag	$\Delta r poX$; pBAD33 carrying the intact <i>rpoX</i> -Flag gene; Carb ^r Cm ^r	This study
WT/pDM8::Proof	EPGS; pDM8 carrying the promoter region of <i>rpoX</i> ; Carb ^r Cm ^r	This study
$\Delta / uxO/pDM8::P_{rnox}$	$\Delta luxO$; pDM8 carrying the promoter region of <i>rpoX</i> ; Carb ^r Cm ^r	This study
ΔluxR/pDM8::P _{rpoX}	$\Delta luxR$; pDM8 carrying the promoter region of <i>rpoX</i> ; Carb ^r Cm ^r	This study
Plasmids		
pDM4	Suicide vector; pir dependent; R6K; SacBR; Cm ^r	41
pDM8	pSup202 derivative containing promoterless <i>lacZ</i> ; Cm ^r Tc ^r	42
pBAD33	Carrying a <i>mob</i> gene in pBAD33; Cm ^r	3
pET28a	Expression vector; Km ^r	Novagen
pDM4::rpoX	pDM4 with <i>rpoX</i> fragment deleted from nt 4–576; Cm ^r	This study
pDM4::N646 4611	pDM4 with GGDEF fragment deleted from nt 27–234; Cm ^r	This study
pBAD33::P _{max} -Flag	Plasmid expressing <i>rpoX</i> -Flag driven by P _{rnox} ; Cm ^r	This study
pBAD33::Flag	pBAD33 derivative Flag expression plasmid; Cm ^r	This study
pBAD33::N646_4611	pBAD33 derivative EPGS_03411 expression plasmid; Cm ^r	This study
pDM8::P _{rnox}	pDM8 carrying the promoter region of <i>rpoX</i> ; Cm ^r	This study
pET22b::rpoE	pET22b carrying the <i>rpoE</i> ORF; Km ^r	This study
pET22b::rpoX	pET22b carrying the <i>rpoX</i> ORF; Km ^r	This study

^ant, nucleotides.

NaCl at 30°C, and live bacterial counts were determined at 2, 4, 6, 8, 10, 12, and 24 h. At each time point, 100 μ l of fresh culture was serially diluted with phosphate-buffered saline (PBS), and the dilutions were spread onto plates containing solidified LBS medium. The live bacterial count for each plate was obtained after cultivation for 12 h at 30°C.

Total RNA extraction. Bacteria were incubated overnight and then diluted 1:100 in LBS medium. The bacteria were then grown at 30°C or 42°C and harvested after 9 h. Total RNA was isolated using an RNA extraction kit (Tiangen, Beijing, China). The RNA samples were digested with DNase I (Promega, Madison, WI, USA) to eliminate genomic DNA contamination. Before reverse transcription, regular PCR was routinely performed using the isolated RNA sample as a template to confirm that there was no DNA contamination.

5' RACE. We performed 5' RACE (rapid amplification of cDNA ends) experiments as previously described (3). Six micrograms of total RNA was subjected to dephosphorylation using tobacco acid pyrophosphatase (TAP) (Epicentre) for 60 min at 37°C. The RNA oligonucleotide linker was ligated to total RNA using T4 RNA ligase (New England Biolabs, Beverly, MA, USA) according to the manufacturer's instructions. cDNA was synthesized using avian myeloblastosis virus (AMV) reverse transcriptase (RT) (TaKaRa) according to the manufacturer's instructions, using a random primer. First-round PCR amplification was performed using RACE-adapter and the primer *rpoX*-RACE (Table 3), and second-round PCR amplification was performed using RACE-adapter-nested primers and *rpoX*-RACE-nested primers (Table 3). The single resulting band was extracted, subcloned, and sequenced.

Quantitative real-time reverse transcription-PCR. Equal amounts of RNA (1 μ g) were used to generate cDNA (Toyobo, Tsuruga, Japan) using 6-mer random primers. Three independent qRT-PCR experiments were performed, and each experiment was run in triplicate. The primers for qRT-PCR (Table 3) were designed using the NCBI primer selection tool with predicted product sizes ranging from 100 to 200 bp. The reactions were run on an Applied Biosystems 7500 real-time system (Applied Biosystems), and the transcript levels were normalized to the 16S rRNA levels in each sample by using the $\Delta\Delta C_{\tau}$ method.

TABLE 3 Primers used in this study

AppoSupPE CTAGIGGGGCCTICTAGATIGCARTACASTICG AppoSupPE CGANAATICGARCAGTICAGGTICAGATICAGTIGG AppoSupPE TGAACTICAGATITTIAGCCICCAGACCAGACGG AppoSupPE TGAACTICAGATITTIAGCCICAGACCAGCG AppoSupPE TGAACGACCAGACGAGCAAGTICCCCAGACCAGCAGACAGCAGCAGACGAGACG	Primer	Sequence (5'-3')
Appot-Jown-F CCTAAAAATCTGACAGTTCACCCGG Appot-Jown-F TGAAATCTGACATCCGGTACCCGGG Appot-Jown-F TGGGGAAGCTAGGTACCCGCGGACAATGTTCGCGCAGCAGCGGCG Appot-Jown-R CGGGGAAGCTAGGTACCCCGGACGAGCGCG Appot-Jown-R ACGGCCCATTTAATCGGCGGAGCG Appot-Jown-R ACGGCCATTTAATCGGCGGAGCG Appot-Jown-F ATGGGCCATTTAATCGGCGGAGCG Appot-Jown-F ATGGGCCACCTTAGCGGAGCGACATTTAGTGGGGGGACCTTGGCGGAGCTTGGGGGGGG	Δ <i>rpoX</i> -up-F	CTAGTGGGGCCCTTCTAGATGCAATTCATTTGAGTATCAGTTTGG
Appol-down-F TGAACTGCAGATTTTTACCCCCCTATCCIGG Appol-down-R CGGGAAGCCCAGGCTAGCCCGTACTCCACACACCGG Appol-dowl-R ACGGAAGCCCAGGCGACCGACGCA Appol-dowl-R ACGAACGGACCGACGGACGCA Appol-dowl-R ACGAACGGACCGACGCACCGACGCA Appol-dowl-R ACGAACGCACGACGCACCGACGCACACCACCGCGCGCACGCCACG	Δ <i>rpoX</i> -up-R	GCTAAAAATCTGACAGTTCATCCGTGACAGCTCGG
AppoX-down=R CGGGAAGCTCAGGTACCCCTGACTTTAACCTTCAACCACCACCAGA AppoX-out-F TCGGGAAGCCAGGCAATGTTGCC AppoX-out-F ACGGACCATTTATGGTCAGGAGCA AppoX-out-F ATGGACCATTTATGGTCAGGAGCA AppoX-out-F ATGGACCACCATCGATCGGACGGAC AppoX-in-F ATGGACCACCATCCGATCGGACGGACACCATTGTGGGGAC AppoX-in-F ATGGACGCACGTCCACCCGCCGCGCCGACCACACTTGTGGGCCGGACCTGACGCGCGGACGGA	Δ <i>rpoX</i> -down-F	TGAACTGTCAGATTTTTAGCCCCTTATCCTAGCCG
App8-out-F TGCTGGGAGACCGAGCAHAGTIGCC App8-out-R ACGAGCGATTATAGTIGCAGACGAC App8-in-F ATGAAGCATTAGTIGGGA App8-in-R TTAAACCGACACTGAGTIGGAGA Ab465 401-up-R ATGAAGCATCATGGATGGGAGA Ab465 401-up-R ATGAAGGACCTATGCCAGGACATTGCTGATTG Ab465 401-uborn-F TGGGAGTGCCATGCGAGGTCACATGCTGACAGCTGGACACTGGCAAGCATGCGAAGCTAGCCAAGGACTAGCAAGCA	Δ <i>rpoX</i> -down-R	CGGGAGAGCTCAGGTTACCCCTGACTTTAACCTTCAACACATCGA
AppSoluteR ACGAGCCATTANTGGTCCAGAGCA AppSvinF ATGAMCGATTCGTGTCACTGGGA AppSvinF TTAAACCCAACCATCGGAATTCGTGGA AppSvinF ATGAMCGATTCGTGTCACTTGGGACATTCGTGGACGCCAGTCATTAGGACTTGGGCCCAGTCATTAGGACTTGGGCCAGTCATTGGGACGCAATTGCGTAGTGATTG AbM64_611-up-F GGGAGCTAGCGTCCCAGTTGTCGAGTCGATTGGCGACAGCCGGAACCCAGTGGGAAAGCCAGTGGGAAAGCCATAGGCGGAACGCCGAATTCGGGACAGCCGGAACGCCGAATGGGGCGAACGCCGAATGGGCGAACGCGGGAACGCCGAATGGGGAACGCCGAATGGGGCGAAGCCGAATGGGGCGACGACCGAAGGCGAATGGGCGAACGCGGAAGCGACAAGCGAATGGGCGAAGCGGAGGGCGAAGCGGAGGGCGAAGGCGGAGGGCGGACGGCGG	Δ <i>rpoX</i> -out-F	TGCTGGAGACCGAGCAATAGTTGCC
ArpoXinFi ATGAAGAATCATCGTTGTCAGTTGGGA Anbels_4011-up-F GAGCTCAGGTTACCCGCATGCAAGATTGGACTTGGCCCAGTCATAATG Abbels_4011-up-R ATAACTGGACAGCTATGCCAGGACATTGGCTGATTG Abbels_4011-down F TGGAAGTGACGTTCCCCAGTGCAAGTCCTGACTGCCGAAGCTAGTGAAGGAT Abbels_4011-down F CCCTCGGATGCAAGCGCGCCCCTGACAGCTGGGACCTGGAAGCATGGCAAGCAT Abbels_4011-down F CCCTCGGATGCAAGCGGCCCCCACACCGCCGCACAGCAGCCGGACCAAGCATGGCAAGCATGGCAAGCATGGAAGCAT Abbels_4011-ub-F GAGTACCGGGCCCCACCACCCACC Abbels_4011-ub-F CCCTCGGCTGCGTGCGTGTGTGGGCCCTGCACGCGGCCCGGACCAAATGCGGACGAAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	Δ <i>rpoX</i> -out-R	ACGAGCCATTTAATGGTGCAGAGCA
AppoXin R TTANACCCATECATCGAATCGCAGA Abries, 461 up-F GAGCTAGGTACTCCCGATCGAATCGTAGGACTTTATGGCACTTGCAATGCATATG Abries, 461 up-F TGGAGTGCACGTCACATTGTAACGACTGTCAGC Abries, 461 up-F TGGAGTGCACGTCACATTGTGCACGACTCACGACGCCACACGCCGAACACCACGACGCACAACCCAGCGCAAATCGACGCACACCCACGACGCAAATCGA Abries, 461 up-F CCCTCGGTGCGCGAACAACCAATGCGC Abries, 461 up-F CGAGTACGCGCGAACACCAATGCGC Abries, 461 up-F CCCTGGCGCGAACACCAATGCGCCGACGCCAATGCGGACCCCACATGCGGAACGCAAATGCAGCCGACGCAATGCGGCCGAATGCGGCCGAATGCGGCCGACGCCACGCCGACGCGACGCACGACGCAATGCGGCGCCGACGCGCGCCGCCGCCCGC	Δ <i>rpoX</i> -in-F	ATGAAAGAATCGTTGTCAGTTGGGA
Abide 4o11up-F GAGCTCAGGTTACCCGCATTATATGGACTTTGGCGCCGCCGCATATATG Abide 4o11up-R ATACTTGGACGCCACATCGCAGCATTGTGGCCCGCACATCGCAGCTGGAACCTAGCAAGCA	Δ <i>rpoX</i> -in-R	TTAAACCCAACCATCGAATCGGAGA
Ab645.401-up-R ATAACTIGGAACGTCACTCACCAGCATTG Ab645.401-down-F TGGAGATGACGCTUCATATTAACAGACTGCAGC Ab645.401-down-R CCCTCGAGTACGCGCTACATAGTGGGGCCCTGGACAGCTGGCAAACGC Ab645.401-down-R CCCTCGAGTACGCGCCAAATCG Ab645.401-down-R CCCTCGAGTACGCCTACTAGTGGGGCCCCGACAACCGC Ab645.401-lour-F CGAGACAACAAATCGATGCGC Ab645.401-lour-F CCATCCGCTGTTTTTGGGCCAATCGCCAATTCGGCCAATTCGGCCAATTCGGCCAATTCGGCCCAATTCGGCCCAATTCGGCCCAATTCGGCCCAATTCGGCCCAATTGCGTCCCTCTTAATTCGTTCG	ΔN646_4611-up-F	GAGCTCAGGTTACCCGCATGCAAGATCTATTATGGACTTTGGCGCAGTCATAATG
Abide 4611 down F TGGAGATGACGTICCAAGTTATAACGAGACGTGGAAGCTAGCAAGCGAGAGCAGCAAGCA	ΔN646_4611-up-R	ATAACTTGGAACGTCATCTCCAGCAATTGCTGATTG
Abids 461 I-down-R CCCTCGAGTACGCGTCACTAGTGGGGCCTGGAACCTAGCAAAGCAT Abids 461 Jour-F CAGTACGCGCCCAAATCG Abids 461 Jour-F CGAACAACAAATCAATGCCAA Abids 461 Jour-F CCATCCCGTGCTGCTGCTGCGC Abids 461 Jour-F CCATCCCGCTTTTTTGGCTAGCAATTGCGC Abids 461 Jour-F CCATCCCGTTTTTTGGCTAGCAATTGCGCCAATTGCGTCAATTGCGTCCAATTGCGTCCCAATTGCGTCCGTC	ΔN646_4611-down-F	TGGAGATGACGTTCCAAGTTATAACAGACTGTCAGC
Abiese, 4611-out-F GAGTACCCGACCAACCAATCGG Abiese, 4611-in-F CACCTGATTGACCAATTGGC Abiese, 4611-in-F CACATCATTGACCAATTGGC Abiese, 4611-in-R GTAAACTCAGGATAGTAGCG mpoX-HagRI CLTGTCGGCGTCGTCGTGTGGTCGTCGTGGCACATTGGGCAAATTGAGACGGI mpoX-HagRI CLTGTCGGCGTCGTCGTGTGGTGGTCGTGGCGCGCGCTGTGAGGGAAATGAGAGCGI pBapRa CGTACCGGTGGTCGTTGTGTGACGAACGGACGGI Mede, 4611-Hag-R CCTGTCGTCGTCGTCGTGGTGGTCGTGGGCGCGGAGGGGGG pDabPapRa CGTGGTCGTCGTGTGTGGTGGTCGTGGGCGCGGGAGGGGG pDabPapRa CTGGGTGGTCGTGTGGTGGTGGTGGTGGGGAGGGGGGGGG	ΔN646_4611-down-R	CCCTCGAGTACGCGTCACTAGTGGGGGCCCTGACAGCTCGGAACCTAGCAAAGCAT
Abida, 4611-out-R CGAGACAAACAATCAATCACCCAC Mode, 4611-In-F CACCTGACTAGCAC Abida, 4611-In-R GTAAACTCAGGATAGTAAGC Mode, 4611-In-R GTAAACTCAGGATAGTAAGC Mode, 4611-Flag-F CCATATCCCGTTTTTTGGCCTACCAATTCAGGCCAATTGGGTACAAATACTAT Ipox Flag-R GGTCAGCATGGGTACCTTTCTCCTCTTTAATACTGGTGGTCGTCGTCGTCGTCGTGTGTGCTGTGGTGCTCTTGTAGTCAATTGGTGCGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCG	ΔN646_4611-out-F	GAGTACGCGGCGAACAACCAAATCG
Abide, 4611-in-F CACCTGATTGAGCAATTGGC Morde, 4611-in-R GTAAACTCAGGATAGAGC mpok-Flag F CCATACCCGGTTTTTTGGCTACGAATCAGCCAATTGGCTACAATACTAT mpok-Flag RI CTGTGCGTGCTCTGTGAGTCAACCCGAACTGGAACGACGT Flag R2 GGTCACCATGGGCTACCTTTTGATCAACCCAACCGCGACGGTCGAGGGTGCAGGTGAGGT N684_4611-Flag -F CCATACCCGTTTTTGGCGTCAGGCGTACGCGTGAGGCGTGAGGCGTGAGGGTGAGG N684_4611-Flag -F CCATACCCGGTTGCTGGCGTGACGATGCTTCACGCGAAGCGCGCGGGGGGGG	ΔN646_4611-out-R	CGAGACAACAAATCAATGCCCAC
Abida 4011-In-R GTAAACTCAGGATAGTAAGC PoxFlag-F CCATACCGATTTIGGGCTAGCCAATTCAGCCAAATTCGGTACAAATACTAT moxFlag-R1 CTIGTCGTCGTCGTCCTTGTAGTCAACCCAACCATCGGACACGT Hag-R2 GGTCACCATGGTACCTTGTAGTCTACTGCAACCAACCAAC	ΔN646_4611-in-F	CACCTGATTGAGCAATTGGC
poxFlag-F CCATACCCGTITITITGGGCTASCGAATICAGGCCAATTGCGGTACAATACTAT poxFlag-R1 CTIGGTGGTGCTCTIGTAGTCAACCCAGGAATTGGGGAACGT Flag-R2 GGTCACCATGGGGTACCTITITAGTGGCTAGGCGGAGCGT Nel4_611-Flag-F CCCATACCCGTITITTGGGCTAGGCAATCGTGACGGCGAAGCGT Nel4_611-Flag-R2 CTIGTGGTGGTGCTGTGTAGTCACTGACGGACGATCGA pooF_pT22b-F ATGGGATCGAAGGAGCGACGACGACGACGACGACGACGACGACGACG	ΔN646_4611-in-R	GTAAACTCAGGATAGTAAGC
moxFiba_R1 CTGTCGTCGTCGTCGTCGTCGTCACCAACCCAACCAATCGAATCGGAACGGT Hag-R2 GGTCACCATGGGTCACTTGTCGTCGTCGTGTCGTGTGTGT	rpoX-Flag-F	CCATACCCGTTTTTTTGGGCTAGCGAATTCAGGCCAATTTGCGTACAAATACTAT
Flaghz GGTCAGCATGGGTACCTTTCTCTCTTTAATTACTTGTCGTGTCGTTGTAGAG N666_4611-Flag-R CCTGTCGTCGTCGTCGTGTGTGTAATGCAACGAACGAACG	rpoX-Flag-R1	CTTGTCGTCGTCGTCCTTGTAGTCAACCCAACCATCGAATCGGAGACGT
Neão. 4611 - Flag-F CCATACCCGATTITITIGGCTAGCAACCATAGAGC Nedo. A611 - Flag-R CTGTCGCTCGTCCTCTGTAGTCATTCAGCCAACTCGAAGCCAGGTGA Words PET22b-F ATCGGATCCGATGAAGCAGCTGACCGATC mpobr.pET22b-R ATATGTCGACACCCCACTGTGGAAAAGAGTCGTTGGAAGCGAGTGTGGA MPONPET22b-R ATCGGATCCATGAAGAGATCGTTGGCAAAGAGTCGTGGGA MPONPET22b-R ATCGCGACCACCCCCCAATTGCGTACAAATCATG PDM8-PpoXF ATCCCGGGGGCCACTGGACAATCCATGGG PDM8-PpoXF CCCGGGAGCCCGTACAATCCAATGCGGG PDM8-PpoXF CCCGGGAGCCCGTACAATCCAATGCACGGG PDM8-PpoXF CCCGGGAATTCCTGTAGA PDM8-PpoXF CCCGGAATTCCTGTAGA PDM8-PpoXF CCCGGAATTCCTGTAGA RACE-adapter CGCGGAATTCCTGTAGA RACE-adapter2 CCGCGAATTCCTGTAGA RACE-adapter2 CCCCCACGGCACCCGGCCCCAATTGCGTACAATACTTG Ppox95-F TGCCTCCACGGCCGCCCCGGCCCAATTGCGCAAATACTTG Ppox95-F TGCCTCCACGGCCGCGCCCCCGGCCCCCCGGCCCCCGGCCCCCGGCCCC	Flag-R2	GGTCAGCATGGGTACCTTTCTCCTCTTTAATTACTTGTCGTCGTCGTCCTTGTAGTC
Ned4_a611-Haor R CTIGICGTCGTCGTCGTCGTCATCACTTACCTGACGAAGGCAGCGGTGA ppde-pE122b-F ATCGGATCCCATGAAGGAAGCGAGCTGACCGATC ppob-pE122b-R ATCGGATCCCATGAAAGAATCGATGGAGGAGCGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	N646 4611-Flag-F	CCATACCCGTTTTTTTGGGCTAGCGAATTCTTCAGCCGTAAAATGAAGCATAGAG
ppoEpET22bF ATCGGATCCGATGACCGATC ppoEpET22b-R ATATGTCGACGCGTTGCAAAGAGCACCTGACCGATC ppoXpET22b-R ATATGTCGACACCTACACCAACCACACCAGACAGCAGAC ppoXpET22b-R ATATGTCGACACCTACCTACACCAGACAGCAGCAGCAGCAGAC pDM8-PpoXF ATCCCGGGGGCCAATTGCGTGACAGCTCGG pDM8-PpoXF ATCCCGGGGGCCAATTGCGTGACAGCTCGG pDM8-PpoXF CCCGGGATCCCTGTAGAAGCAACCAATCGTGACAGCTCGG pDM8-PpoXF CCCGGGATTCCTGTAGAA pDM8-PpoXF CCCGGGATTCCTGTAGAAGCAAC pDM8-PpoXF CCCGGGATTCCTGTAGAAGCAAC pACE-adapter2 CCGCGAATTCCTGTAGAACGAAC RACE-adapter2 CCCGCGAATTCCTGTAGAACGAAC PpocyC9FF TGCCTGCACGTCGACCATTGCGTACAATAGCAAGAAAAA PpocyC9FF TGCCTCCACGTCGACGCACGCGGC PpocyC9FF TGCCTCCACGTCGACGCATCGCGCACATTGCGTACAAAGAAGAAC PpocyC9FF TGCCTCCACGTCGCACATTGCGTACAAAGCAAGAC PpocyC9FF TGCCTCCACGTCGCACCATTGCGCAAAGACAAGCAAGC PpocyC9FF TGCCTCCACGTCGCACATTCCAGCACAAAGATTAAACGAAGC PpocoC9FF TGCCTCCACGTCGCACATTCCAGCACAAAGATTAAACGAAGC PpocoC9FF TGCCTCCACGTCGCACATTCCAGCACACAAGAC PpocoC9FF TGCCTCCACGTCGCCACATCACACACAAGACCTGGCACATCCAC	N646 4611-Flag-R	CTTGTCGTCGTCGTCCTTGTAGTCATTCACTTGACGAAGCGCAGGTTGA
pp6_pT22b-R ATATGTCGACGCGTGTCAAAAGAAGGTCTGATT px0x-pET22b-F ATACGGATCCATGAAAGAATCGTTGGGAA pDMB-Pp0XF ATACGGATCACAAACACCAACGATCGAAGAGA pDMB-Pp0XR ATCCCGGGGACACCAATTCGCGCGAATGCGGAG pDMB-Pp0XR ATCCCGGGGACACAGTTCATCGCGGACAGAGCCAATTGCGG pDMB-Pp0XR CCCGGGACACCAGTTCATCGCGGCACAGCGGC pDMB-Pp0XR CCCGGGAATTCCCTGTAGAACAGCGCGCGC pDMR-Pp0XR CCCGGGAATTCCCTGTAGAACAGCGGCACC RACE-adapter CCCGGGAATTCCCTGTAGAACGACCGAC RACE-adapter2 CCCGCGAATTCCCGGGCCAATTGCGGCAACTCTGG Pp0x057F TGCCTCCAGGTCGACGATCCGGCCAATTGCGGACAATCACTGG Pp0x057F TGCCTCCAGGTCGACGATCCGGCCAATTGCGGGC Pp0x057F TGCCTCCAGGTCGACGATCCGGCCAATTGCGGGC Pp0x057F TGCCTCCAGGTCGACGATCAATCGGACACAAGAACAAGAACCAAGAC Pp0x057F TGCCTCCAGGTCGACGATCAATCGGACATACAAGCAAGACCAAGACCAAGACAAGACACAAGACACAAGACACAAGACACAAGAGAGAAGA	rpoE-pET22b-F	ATCGGATCCGATGAACGAGCAGCTGACCGATC
ipoX-pET22b-F ATCGGATCCATGAAACGAATICGTGTCAGTTGGGA ipoX-pET22b-R ATAGTGCAACCAACCCAACCATGGAAA ipoX-pET22b-R ATCCCCGGACAACCCAACCAATAGCGACAA ipoX-pDX-F ATCCCCGGAGAGCCCAATTTGCGTACAAATACTTG ipoX-PoX-F ATCCCGGGGGCACAATTGCGTACAAATACATGCCGA ipoX-RACE-nested TCACGAGACAAAACAAATCAATGCCGCA ipoX-RACE-nested CCACGATTTCCTGTAGAACGAAC RACE-adapter GCGGGAATTCCTGTAGAACGAAC RACE-adapter2 GCGGGAATTCCCTGTAGAACGAAC RNA-Linker AUAUGCGCGAAUUCCUUGAGAACGAACACUAGAAGAAA P _{puoCV5} -F TGCCTGCAGGTCGACGATTGCGACACATTGGTACAAATACTTG P _{modv0} -SF TGCCTGCAGGTCGACGATTGCGACACATTGCGTACAAATACTTG P _{modv0} -SF TGCCTGCAGGTCGACGATTGCGACGACGTGGTGACAATACGAAGAC P _{modv0} -SF CTCTASTGCTGACGATCACACCGACACAAGAAAGTTACAAACCAAGAC P _{modv0} -SF CTCCTGCAGGTCGACGATTCCGGTCAACATTACGACACAAGGCTTG P _{modv0} -SF CTCCTGCAGGTCGACGATCACAACGACAAAGGAAAAGTGACACAGGC P _{modv0} -SF TGCCTGCAGGTCGACGATCACACACCACAGAAAGGCTTG P _{modv0} -SF TGCCTGCAGGTCGACGATCACACACCACAGACACACAGAACGCTGGC P _{modv0} -SF TGCCTGCAGGTCGACACATCAGACCACTTTGCGACAAAGGCTTG P _{modv0} -SF TGCCTGCACGTCGCACACACA	rpoE-pET22b-R	ATATGTCGACGCGTTGCAAAAGAGGTCTGATT
pvX-pET22b-R ATATGTCGACAACCCARACCATCGAATGCGACA pDM8-PrpoX-F ATCCCGGGAGCAATTTGCGTACAAATACTTG pDM8-PrpoX-R ATCCCCGGGTGACAATTTCCTGGACAACTAATACTTG pDM8-PrpoX-R ATCCCGGGGTGACAACTAATCATGCTGG poX-RACE-nested TCACGGACAACAAATCAATCCATCCATGCGGG poX-RACE CGCGGAATTCCTGTAGAG RACE-adapter GCGCGAATTCCTGTAGAGCAAC RACE-adapter2 GCGCGAATTCCTGTAGAGCAACCAACAAGAAATACTTG ProxC95-F TGCCTGCAGGTCGACCGAUCCCUAGAAGGAAA ProxC95-F TGCCTGCAGGTCGACCGATCGCCAATTGCGACAAGAAA ProxC95-F TGCCTGCAGGTCGACGATCGCCAATTGCGACAACAAAGAAAAACCAAAGGACGAGCGGG ProxC95-F TGCCTGCAGGTCGACGATCGCCACTGCTGGTTTTCCTTCT ProxC95-F TGCCTGCAGGTCGACGGACCAATTGCAACAAAGCAAAGC	rpoX-pET22b-F	ATCGGATCCATGAAAGAATCGTTGTCAGTTGGGA
pDM8-PrpoX-F ATCCCGGGAGGCCANTTGCGTGACAGCTGG pDM8-PrpoX-R ATCCCGGGTGACAGTTGCCGGGACAGCTGG pDM8-PrpoX-R ATCCCGGGTGACAGTTGCCGGACGTGG pDM8-PrpoX-RE CACGAACAAATCAATGCCCG mpoX-RACE GCGGGAATTCCTGTGAGA RACE-adapter GCGGGAATTCCTGTGAGAGGAAC RACE-adapter GCGGGAATTCCTGTGAGAGGAAC RACE-adapter2 GCGGGAATTCCTGTGAGAGGAACACAUGAAGAAAA RNA-Linker AUAUGGCGAAUUCCUGUAGAACGAACACAUGAAGAAAA PrpoX-95-F TGCCTGCAGGTCGACGATCGGCCAATTTGCGTACAAATACTTG PrpoX-95-F TGCCTGCAGGTCGACGATCCGGCGACGATCAACTACAAATACTTG Prox0-95-F TGCCTGCAGGTCGACGATCAGTGCTTTTTCTTCT Prox0-05-F TGCCTGCAGGTCGACGATCAACCAAAGTATACAAACCAAGAC Prox0-05-F TGCCTGCAGGTCGACGATCAGCACTCTTTTGCAAATGGCTTG Prox0-05-F TGCCTGCAGGTCGACGATCAGCACAT Prox0-05-F TGCCTGCAGGTCGACGATCAGACCACAATAGAT gyrB cy5-F TGCCTGCAGGTCGACGATCAGCACTTTGCAAATGGCTG gyrB cy5-F TGCCTGCAGGTCGACGATGACACACAAAGAGTGAGCA gyrB cy5-F TGCCTGCAGGCGACGGCGACCACT gyrB cy5-F TGCCTGCAGGCGACGCACACT gyrB cy5-F TGCCTGCAGGTGGACGACACACCACC	rpoX-pET22b-R	ATATGTCGACAACCCAACCATCGAATCGGAGA
pDM8-Pp0x-R ATCCCGGGTGACAGTTCATCCGTGACAGCTCGG pDV8-Pp0x-R TCACGAGACAACAAGATCATGCCCCA pox-RACE CAACATTICGGCGCCTTTCATCA RACE-adapter GCGCGAATTCCTGTAGA RACE-adapter2 GCGCGAATTCCTGTAGA RACE-adapter2 GCGCGAATTCCTGTAGAACGAAC RNA-Linker AUAUGCGCGCAAUUCCUGUAGAACGAACAAATACTTG Pp0xQ5-F TGCCCTCCAGGTCGACGACCGCCGCCAATTTGCGTACAAATACTTG Pp0xQ5-F TGCCCTCCAGGTCGACGACTCCGCCGCAATTTGCTCTCT Pw646_4003C95-F TGCCTGCAGGTCGACGATCCGGCGACTTCCTCT Pw646_4003C95-F TGCCTGCAGGTCGACGATCCCGGCACAT Pw646_4003C95-F TGCCTGCAGGTCGACGATCAATCGACAATGCAAATGAAGACC Pw646_4003C95-F TGCCTGCAGGTCGACGATCCGGCACAT Pw646_4003C95-F TGCCTGCAGGTCGACGATCAATCGACAATGCAAATGAAGCCTGG Pw646_4003C95-F TGCCTGCAGGTCGACGATCAATCGACAAAGTAATACAAACCAAAGAC Pw646_4003C95-F TGCCTGCAGGTCGACGATCAATCGACAAAGTAATACAAACCAAAGAC Pw646_4003C95-F TGCCTGCAGGTCGACGACCAATCGACAAAGTGACCAATGCAATGACCAAGACGATGCACTGACAATGCAACAAAGTGACCACC Pw646_4003C95-F TGCCTGCCTAGGTCGACGACGACCACTGACAATGCAAAGTGACACTGACAATGACAATGAAGACGATGACAATGAAGACGATGACAATGAAGAAGTTGACAA yyf8 cy5-F TGCCTGCCTGCAGGTCGACGACGACGACGACGACGACAAGAAGTTGAGAC Pw646_407FF TAATGAACAATGGCCGTAGAAC Pw646_407FF GCTCTGCCTGCAGTGTGAAAACGCGGTGAA N646_30997RTF GCCTGCCGGTGTAACCTAGC </td <td>pDM8-PrpoX-F</td> <td>ATCCCGGGAGGCCAATTTGCGTACAAATACTTG</td>	pDM8-PrpoX-F	ATCCCGGGAGGCCAATTTGCGTACAAATACTTG
TpDX-RACE TCACGAGACAACAAATGATGCCCA rpDX-RACE CAACATTTCGCGCGCTTCTTCATCA RACE-adapter GCGCGAATTCCTGTAGA RACE-adapter2 GCGCGAATTCCTGTAGAC RNA-Linker AUAUGCGCGACGATCGGCCAACGUGAAGAACAAAGAAA Ppox/SP-F TGCCTGCAGGTCGACGATCGGCCAATTGCGTGCAACACUAGAAGAAA Ppox(SP-F TGCCTGCAGGTCGACGATCGGCCAATTGCGTGCAACACUAGAAGAAA Ppox(SP-F TGCCTGCAGGTCGACGATCGGCCAATTGCGTCACAATACTTG Phote.doi/SP-F TGCCTGCAGGTCGACGATCGGCCAATTGCGACAAAGAAAAAGCAAAGACAAAGCAAGAGACAAAGAGATAACCAAGGCT Phote.doi/SP-F TGCCTGCAGGTCGACGACTCATCGACAACAAAGTAATACAAACCAAGAC Phote.doi/SP-F TGCCTGCAGGTCGACGACTCATTGCACACAAAGTAATACAAACCAAGAC Phote.doi/SP-F TGCCTGCAGGTCGACGATCAATGCACAAAGTAATACAAACCAAGAC Phote.doi/SP-F TGCCTGCAGGTCGACGACTCATTTGCAAAGGCATGA Phote.doi/SP-F TGCCTGCAGGTCGACGATCAGACTGTTTTGCAATAGGCTTG Phote.doi/SP-F TGCCTGCAGGCGACGACTGACAAATACAAAGGCCTGGCAGA Phote.doi/SP-F TGCCTGCAGGTCGACGATCAGACTGAGCAGA Phote.doi/SP-F TGCCTGCAGGCGACGACACCACCAC Phote.doi/SP-F TGCCTGCAGGCGACGACACTGGCACAAT Phote.doi/SP-F TGCCTGCAGTGAGACGACCACAC Phote.doi/SP-F TGCCTGCAGGACGACACACT Phote.doi/SP-F	pDM8-PrpoX-R	ATCCCGGGTGACAGTTCATCCGTGACAGCTCGG
TpoX-RACE CAACATTICGCGCGCTTCTTCATCA RACE-adapter GCGCGAATTCCTGTAGA RACE-adapter2 GCGCGAATTCCTGTAGA RACE-adapter2 GCCGCGAATTCCTGTAGAACGAAC RNA-Linker AUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA PpostQ5-F TGCCTGCAGGTCGACGACTCGCGCCAGTTGCGACAAAATACTTG PpostQ5-F TGCCTGCAGGTCGACGACTCGCGCAGTTTGCGTACAAATACTTG PpostQ5-F TGCCTGCAGGTCGACGATCTCCGACACACAAAAGTATACAAAACCAAGAC Phote_doing/5-F TGCCTGCAGGTCGACGATCTCGGACAACAAAAGTATACAAACCAAGAC Phote_doing/5-F TGCCTGCAGGTCGACGATCCAGCACAAAAGGTATACAAAACCAAGAC Phote_doing/5-F TGCCTGCAGGTCGACGATCCAGCACACAAAAGTATACAAAACCAAGAC Phote_doing/5-F TGCCTGCAGGTCGACGATCCAGCACTCAAAAGGATAACAAGACCAAGC Phote_doing/5-F TGCCTGCAGGTCGACGATCCAGACTCTTTGCAAATGGACCAGACAGA	rpoX-RACE-nested	TCACGAGACAACAAATCAATGCCCA
RACE-adapter GCGCGAATTCCTGTAGA RACE-adapter2 GCCGCGAATTCCTGTAGACGAAC RNA-Linker AUAUGCGCGGAAUUCCUGUAGAAGAACACUAGAAGAAA P _{pox} Cy5-F TGCCTGCAGGCGACGATCGGCCAATTGCGTACAAATACTTG P _{pox} Cy5-F TGCCTGCAGGTCGACGATCGGCCAATTGCGTACAAATACTTG P _{pox} Cy5-F TGCCTGCAGGTCGACGATCTGGCCAGTTCTTCTCT P _{Ne64, 4603} Cy5-F TGCCTGCAGGTCGACGATCCGACCAATACAAAGACAAAGACAAGACCAAGACGATCGACGATCGACAATACAAACAA	rpoX-RACE	CAACATTTCGCGCGCTTCTTCATCA
RACE-adapter2 GCGCGAATTCCTGTAGAACGAAC RNA-Linker AUAUGCGCGAAAUUCCUGUAGAACGAAC RNA-Linker AUAUGCGCGAAAUUCCUGUAGAACGAACACUAGAAGAAA P _{pox} Cy5-F TGCCTGCAGGTCGACGATCGGCGACCAATTGCGTACAATTGCGT P _{pox} Cy5-F TGCCTGCAGGTCGACCGATCGCGCGTCATTTCTCT P _{Nota6,4003} Cy5-F TGCCTGCAGGTCGACCGATCGACCACCGGC P _{Nota6,4003} Cy5-F TGCCTGCAGGTCGACGATCGACCACCGGCACAAAGATACAAACCAAAGCC P _{Nota6,4003} Cy5-F TGCCTGCAGGTCGACGATCGACCACTTTTTGCAAATGGCTTG P _{Nota6,4003} Cy5-F TGCCTGCAGGTCGACGATCAACTCTTTTGCAAATGGCTTG P _{Nota6,4003} Cy5-F TGCCTGCAGGTCGACGATCAACTCTTTTGCAAATGGCTG P _{Nota6,4003} Cy5-F TGCCTGCAGGTCGACGATCAACTCTTTTGCAAATGGCTG P _{Nota6,1023} Cy5-F TGCCTGCAGGTCGACGATCAACTCTTTTGCAAATGACC P _{Nota6,1023} Cy5-F TGCCTGCAGGTCGACGATCAACTCATAAATAAAT gy78 Cy5-F TGCCTGCAGGTCGACCACTCAATCAAAAGGCGTGAGC gy78 Cy5-F TGTTGCTCAAGGCGTAGAC gy78 Cy5-R CCACCTTCATACATGAAACTGACCACC gy78 Cy5-R CCACCTTCAAAAGTGACCGACG gy78 Cy5-R CCACCTTCAAAGTGACCCACC gy78 Cy5-R CCACCTTCAAAGTGACCCACC gy78 Cy5-R CCACCTGCAGGTGTGAACC gy78 Cy5-R CCACCTGCAAGTGCGACGAC gy78 Cy5-R CCACCTGCAAGTGCGCACG gy78 Cy5-R CCACCTGCAAGTGCGCACCC gy78 Cy5-R CCACCTGCAAG	RACE-adapter	GCGCGAATTCCTGTAGA
RNA-Linker AUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA P _{pob} (y5-F TGCCTGCAGGTCGACGATCGGCCAATTTGCGTACAAATACTTG P _{pob} (y5-R TGCCTGCAGGTCGACGATCGCCGCGACAATACTTG P _{pob} (y5-R TGCCTGCAGGTCGACGATCGCCGCGG P _{Neda,4003} (y5-F TGCCTGCAGGTCGACGATCGACAGCTCGAC P _{Neda,4001} (y5-F TGCCTGCAGGTCGACGATCGACAACAAAGATAACAAAGAAC P _{Neda,4001} (y5-F TGCCTGCAGGTCGACGATCGACAATGGACTTG P _{Neda,4001} (y5-F TGCCTGCAGGTCGACGATCAGACTGAACAAGATACAAACCAAGAC P _{Neda,4001} (y5-F TGCCTGCAGGTCGACCGATCAGACTCTTGCAATGGCTTG P _{Neda,4001} (y5-F TGCCTGCAGGTCGACCGATCAGACTTTGCAATGGCTTG P _{Neda,1023} (y5-F TGCCTGCAGGTCGACCGATCAGACTTACAAGAGAAAGTTGAGC gyrB (y5-F TGCCTGCAGGTCGACGCATCGCACTATCAGAAAAGTTGAGC gyrB (y5-F TGCTGCAGGCTGAGGCTAGAC ppoF-qRT-R TGTTGCTCAAGGCGTAGAC ppoF-qRT-R GCGAGTTGACCATGACCACC rpoA-qRT-R GCCTGCTTGCAGGCGCACGA rpoH-qRT-R GCCAGTTGGATGAACCACC rpoH-qRT-F GCGAGTTAGGCTGTGTAA N646_3697qRTF GCCAGTTGGAACCTAGC N646_3697qRTF GCCAGTTGGCAGCGTGTGTAA N646_3697qRTR ATCGTTTCGAGCTGCAGCAGCGGCG N646_3695qRTF GCCAGCCCCTCTAGCC N646_3695qRTF GCCCCCCCTGGCAGCGCGCGG N646_3695qRTR ATCGTTTCCACCAGACCACC <td< td=""><td>RACE-adapter2</td><td>GCGCGAATTCCTGTAGAACGAAC</td></td<>	RACE-adapter2	GCGCGAATTCCTGTAGAACGAAC
PpppXY5-FTGCCTGCAGGTCGACGATCGGCCAATTTGCGTACAAATACTTGPpppXY5-RTGACAGTTCATCCGTGACAGCTCGGPhot64.6035Y5-FTGCCTGCAGGTCGACGATCTCGACTGTTTTTCTTCTPhot64.6035Y5-RATTTATCATTATCCCACCGCACPhot64.6001Y5-FTGCCTGCAGGTCGACGATCAATCGACAACAAAGTATACAAACCAAGACPhot64.6001Y5-FTGCCTGCAGGTCGACGATCAGCACAATAGGCTTGPhot64.6001Y5-FTGCCTGCAGGTCGACGATCGAGCATCAATGGACAACAAAGTATACAAACCAAGACPhot64.6001Y5-FTGCCTGCAGGTCGACGATCGAGCATCAGACAACAAAGTGATGCPhot64.6001Y5-FTGCCTGCAGGTCGACGATCGAGCTTTTGCAAATGGCTTGPhot64.6001Y5-FTGCCTGCAGGTCGACGATCGAGCACTATCAGAGAAAGTGAGCgyf8 cy5-FTGCCTGCAGGTCGACGATCGACATCAAGGAGAAAGTTGAGCgyf8 cy5-FTGCCTGCAGGTCGACGATCGACACAGAGAAAGTTGAGCgyf8 cy5-RCCACCTTCATACATGAAGTGATCApp0-qRT-FTGTTGCTCAAAGGCCGTGGAACpp0-qRT-FTGTTGCTCAAGGCCGCACCpp0-qRT-FGCCAGTTGCATGACACCACCpp0-qRT-FGCCAGTTGCACTGAACACCACCpp0+qRT-FGCCAGTTGCGCGCGTGTGAAN646_3697qRTFACAAGGCCCGCTGTGTAAN646_3695qRTFAGCAAGCGTTTGACCCGAACAN646_3695qRTRATCCTTCGCCAGTACCAN646_3695qRTRCACCTGCCGCCAGTAGCN646_3695qRTRCCCCGCCCTTCAACCAAGACN646_3695qRTFCGCGTCCCTTCAACCAAATCN646_3695qRTRCCCCGCCCTTCAACCAAATCN646_3695qRTRCCCCGCCCTCAACCAAATCN646_3695qRTRCCCCGCCCTTCAACCAAATCN646_3695qRTRCCCCGCCCTTCAACCAAATCN646_4604qRTRGTAAACCACGAGGCGATCCAN646_4604qRTRGTAAACCACGAGGCGATCCAN646_4604qRTRGTAA	RNA-Linker	AUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA
PhysicTGACAGTTCATCCGTGACAGCTCGGPhysicTGCCTGCAGGTCGACGATCTCGACTGCTGTTTTTCTTCTPhysicATTTATCATTATCCCACCGCACPhysicATTTATCATTATCCCACCGCACPhysicAGCCTGCAGGTCGACGATCAATCGACAACAAAGTATACAAACCAAGACPhysicTGCCTGCAGGTCGACGATCAATCGACAACAAAGTATACAAACCAAGACPhysicTGCCTGCAGGTCGACGATCAATCGACAACAAAGTATACAAACCAAGACPhysicTGCCTGCAGGTCGACGATCAAACTGATACAAACGAATAAACTGATACTAAATAAA	Pvcv5-F	TGCCTGCAGGTCGACGATCGGCCAATTTGCGTACAAATACTTG
PhotoTGCCTGCAGGTCGACGATCTCGACTGCTTTTCTTCTPhota6_46325y5-FTGCCTGCAGGTCGACGATCATCGACGACGATCAACAAAGTATACAAACCAAGACPhota6_46325y5-FTGCCTGCAGGTCGACGATCAACCAACAAAGTATACAAACCAAGACPhota6_4601cy5-RCTCTAGTTCTGACTCCGGCACATPhota6_1622cy5-FTGCCTGCAGGTCGACGATCAGACTAAATGAATAAATgyr8 cy5-FTGCCTGCAGGTCGACGATCGACTATCAGAGAAGTTGAGCgyr8 cy5-RCCACCTTCATACATGAAGTGATCArpoE-qRT-FTGTTGCTCAGAGTCGACGATCGACATATCAGAGAAGTTGAGCrpoZ-qRT-FTGTTGCTCAGGGCGTGAGACrpoX-qRT-FTAATGAACATGGCCGCACGrpoX-qRT-FGCGAGTTGGACGTGGACGACCACGrpoX-qRT-RGCCTGCTTTCACCCCTGAGk646_3697qRTRAACAAGGCCCGCTGTTGAAN646_3697qRTRAACAAGGCCCGCTGTTGATAN646_3695qRTRAGCAGGGTTGGACGTGGCGAGACN646_3695qRTRGTCCTGCCCGCAGTGGTGAAN646_3695qRTRCACCGGTTTGCACCGACN646_3695qRTRCCCCGCTTTCACCCCAAATCCN646_3695qRTRCCCCGCTTTCACCCCAAATCN646_3695qRTRCCCCGGTTGGTGAAAN646_3695qRTRCCCCGCTCTTCACCCACACN646_3695qRTRCCCCGCCCATGTCTCGAAAATCN646_3695qRTRCCCCGGTCCTCAAAATCCAAATCN646_3695qRTRCACCGGTTGGTGAAAN646_3695qRTRCACCGGTTGGTGAAAN646_3695qRTRCACCGGTCCAAATCCAAAATCN646_3695qRTRCACCGGCACAAATCCAAAACCAAATCN646_3695qRTRCACGCGCCACAAATCN646_3695qRTRCACGCGCCACAGATGGTGAAAACCAAAACCAAATCN646_4604qRTRGCACGTACCGACAGAGTGGGAAAACCAAAATCN646_4604qRTRGTAAACCCGGACACTGGTGGAAAAACCAAAAACCAAAAACCAAAAACAAAAACCAAAAACCAAAA	P _{max} cv5-R	TGACAGTTCATCCGTGACAGCTCGG
Non-Non-SystemATTTATCATTATCCCACCGCACPNotad4001CyS-FTGCCTGCAGGTCGACGATCAATCGACAAAAGTATACAAACCAAGACPNotad4001CyS-RCTCTAGTTCTGACTCCGGCACATPNotad4001CyS-RTGCCTGCAGGTCGACGATCAGACTCTTTTGCAAATGGCTTGPNotad1022CyS-FTGCCTGCAGGTCGACGATCAGACTCTAATCAAAACCGAATGACTgyrB cyS-FTGCCTGCAGGTCGACGATCAGACTCATAAAAAATgyrB cyS-RCCACCTTCATAGAAGGATCAGACCgyrB cyS-RCCACCTTCATAGAAGGACCATCGACACTAGAAGTGAGCgyrB cyS-RCCACCTTCATGAAGGCGAGACrpoE-qRT-FTGTTGCTCAAGGGCGTAGACrpoZ-qRT-FTAATGAACAATGGCCGCACGrpoX-qRT-FGCCTGCTTTCACCCCGAGrpoH-qRT-RGCCGAGTTAGGTGTGAGCCTrpoH-qRT-RGCCCAGTTGGACAANo46_3697qRTFAGCAAGCGCTTTGCAACAAGCGCGAAANo46_3697qRTFAGCAAGCGCTTTGGAACAACAAGGCCGAAGAANo46_3697qRTFAGCAAGCGCTTTGGAACAACAAGCGCGAAAAAGTATACAAAGACAACAAAGACAAAGAAGCGTTTTGAGCCGAAAAAGAAGAGAAGAAGAAGAAGAAGAAGAAGAA	PNAG AGO2CV5-F	TGCCTGCAGGTCGACGATCTCGACTGCTGTTTTCTTCT
No.dd. 400 (75 F)TGCCTGCAGGTCGACGATCAATCGACAAAGTATACAAACCAAGACNo.dd. 400 (75 F)TGCCTGCAGGTCGACGATCAATCGACAAAGTATAACCAAAGCAAAGTATAACCAAAGCANo.dd. 1023 (75 F)TGCCTGCAGGTCGACGATCAGACTCTTTGCAAATGGCTTGgy/B (75 F)TGCCTGCAGGTCGACGATCGCACTATCAGAGAAAGTTGAGCgy/B (75 F)TGCCTGCAGGTCGACGATCGCACTATCAGAGAAAGTTGAGCgy/B (75 F)TGCCTGCAGGTCGACGATCGCACTATCAGAGAAAGTTGAGCgy/B (75 F)TGCTGCAGGGCGAGACgy/B (75 F)TGCTGCAGGGCGAGACgy/B (75 F)CCACCTTCATACATGAACGGCCACAAAGTTGAGCgy/B (75 F)TGTTGCTAAGGGCGAGACgy/B (75 F)TGTTGCCAAGGGCGAGACgy/B (75 F)TGTTGCCAAGGGCGAGACgy/B (75 F)TGTTGCCAAGGGCGAGGACgy/B (75 F)TGTTGCCAAGGGCGAGGACgy/B (75 F)TGTTGCCAAGGGCGAGACgy/B (75 F)TGTTGCCAAGGGCGACGACgy/B (75 F)TGTTGCCAAGGGCGAGACgy/B (75 F)TGCCTGCCAGGTAGACgy/B (75 F)TGCTGCCAGGTGGAACgy/B (75 F)TGTTGCCAGGGCGCACGgy/B (75 F)TGTGCCCGCAGGACCgy/B (75 F)TGCTGCCAGTAGGCGCCgy/B (75 F)GCCTGCCTGCAAGACCACpol-qRT-FGCCGTGTGTGAAgo/H-qRT-FGCCGGTTGTGACgo/H-qRT-FGCCGGTTGTGACgo/H-qRT-FGCCGGTTGTGCGGGGgo/H-qRT-FGCCGGTTGTCACCCAGCGCGACAgo/H-qRT-FGCCGGTCCTTCACCAAATCNo46_3095qRTRGCCCTGCCCGCAGAACCCACNo46_3095qRTRCACCGGCCCTGCACAAATCNo46_3053qRTFCGCGTTGCACCAAATCNo46_60523qRTFGCCGCTACCCAGGGGGACANo46_4004	PNEAE AEO2CV5-R	ATTTATCATTATCCCACCGCAC
Posted_actionCTCTAGTTCTGACTCCGGCACATPosted_actionTGCCTGCAGGTCGACGATCCACATTGCAAATGGCTTGPosted_actionTTTTAAATTCCTAGAATAAACTGATACTAAATAAATPosted_actionTGCCTGCAGGTCGACGATCCACATACTAAATAAATgyrB cy5-FTGCCTGCAGGTCGACGATCGCACTATACAGAAAGTTGAGCgyrB cy5-RCCACCTTCATACATGAAGTGATCArpoE-qRT-RCGATTGCACTGAACACCACCrpoX-qRT-RCGATTGCACTGACACACCCrpoX-qRT-RGCCTGCGTTTCACCCCTGAGrpoH-qRT-RGCCTGGCTTTCACCCCTGAGrpoH-qRT-RGCCCAGTTAGATGAACACCACCrpoH-qRT-RGCCCAGTTACGTGAGACN646_3697qRTFGCTCAGTTTCGCCAGGTGAAAN646_3697qRTFGCTCAGTTTCGCAGGTGGTGAAAN646_3697qRTFGGTCCAGTTTGCGCAGCTGTGAAN646_3695qRTFGTCCCTGCTTTGACCCGAN646_3695qRTFGTCCCTGCTTCACCACCACN646_3695qRTFGTCCTGCCTCCAGCAATCCN646_3695qRTFGTCCCTGCCAGTAACCTAACCN646_3695qRTFGTCCTGCCCAGTAACCAAACCN646_3695qRTFGTCCTGCCCAGTAACCAACCN646_60523qRTFCGCCGCTTCTCACCACACN646_60523qRTFGCCCGCCTCCAGCAAATCCN646_604qRTFGCAGTACCGAGAGCTGCGAN646_604qRTFGCAGTACCGAGAGCTGGGGN646_4604qRTFGCAGTACCGAGAGCTGGGAAAN646_4604qRTFGCAGTACCGAGAGCGATCCAN646_4604qRTFGCAGTACCGAGAGCTGGGAAAN646_4604qRTFGAGACCTTTCAGCCCCAGTAATN646_4604qRTFGAGACACTTGCGGAGAAATCN646_4604qRTFGAGAACCTAGCGGGGAAAATCN646_4604qRTFGAGACACTTGCGCAGGAGAAATCN646_4604qRTFGAGACACTGCGCAGGAGCAAATC <td>PN646_4601CV5-F</td> <td>TGCCTGCAGGTCGACGATCAATCGACAACAAAGTATACAAACCAAGAC</td>	PN646_4601CV5-F	TGCCTGCAGGTCGACGATCAATCGACAACAAAGTATACAAACCAAGAC
Posde_1c32Cy5-FTGCCTGCAGGTCGACGATCAGACTCTTTTGCAAATGGCTTGPosde_1c32Cy5-RTTTTTAAATTCCTAGAATAAACTGATACTAAATAAATgyf8 cy5-FTGCCTGCAGGTCGCACGATCGCACTATCAGAGAAAGTTGAGCgyr8 cy5-RCCACCTTCATACATGAAGTGATCArp0E-qRT-FTGTTGCTCAAGGCGTAGACrp0E-qRT-RCGATTGCACGACACCrp0A-qRT-FTAATGAACAATGGCCGCACGrp0H-qRT-RGCCGAGTTACGCCCTGAGrp0H-qRT-RGCCAGTTGCTGTGAACrp0H-qRT-RGCCAGTTCGCCCGAGACAN646_3697qRTRACAAGGCCCGCTGTGAAAN646_3695qRTFAGAGAAGCGTTTTGAGCCGGAN646_3695qRTFGTCCTCGCCTAGGACACCN646_3695qRTRCCCCGGTGTGTCAACCACACACACACACACACACACACAC	PNEAE AGOICV5-R	CTCTAGTTCTGACTCCGGCACAT
PowerTTTTTAAATTCCTAGAATAAACTGATACTAAATAAATgyr8 cy5-FTGCCTGCAGGTCGACGATCGCACTATCAGAGAAAGTTGAGCgyr8 cy5-FCCACCTTCATACATGAAGTGATCAgyr8 cy5-RCCACCTTCATACATGAAGTGATCArpoE-qRT-FTGTTGCTCAAGGGCGTAAGACrpoZ-qRT-FCGATTGCACTGAACACCACCrpoX-qRT-RGCTCTGCTTTCACCCTGAGrpoH-qRT-RGCGAGTTAGGGCGTGGGAAstpoH-qRT-RGCGAGTTAGGGCGTGGGAAstpoH-qRT-RGCGAGTTCCGCAGGGACACstpoH-qRT-RGCTCAGTTCTCGCAGGTACAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3695qRTFAGAGAAGCCGCTTTGAGCCGAN646_3695qRTFGCTCCGCAGTACCTAGCN646_3695qRTFGCTCCGCCAGTACCTAGCN646_3695qRTFGCCCCGGTTGCCAGGTACCN646_3695qRTFGCCCCGGTTGCCAGACCTAGCN646_3695qRTFGCCCCGGTTGCCAGACCTAGCN646_3695qRTFGCCCCGGTTGCCAACCTAGCN646_3695qRTFGCCCCGGTTGCCCAGTAACCTAGCN646_3695qRTFGCCCGCCCTCCAACAAATCN646_0523qRTFGCCGCCCCCGCAGAACCCACN646_6523qRTFGCAGTACCGAGAGCTGCGAGAATCN646_4604qRTFGCAGTACCGAGACTTGGTGGN646_4604qRTFGCAGTACCGAGACTGGGAGAA165 RNA-qRT-FAAAGCACTTTCAGTCGGAGGAA165 RNA-qRT-FTGCCCCCCCGCCAGTAAT	PN646_1622CV5-F	TGCCTGCAGGTCGACGATCAGACTCTTTTGCAAATGGCTTG
NobeTGCCTGCAGGTCGACGATCGCACTATCAGAGAAAGTTGAGCgyrB cy5-FTGCCTGCAGGTCGACGATCGCACTATCAGAGAAAGTTGAGCgyrB cy5-RCCACCTTCATACATGAAGTGATCArpoE-qRT-FTGTTGCTCAAGGGCGTAGACrpoX-qRT-FTAATGAACAATGGCCGCACGrpoX-qRT-RGCCTCTGCTTTCACCCCTGAGrpoH-qRT-RGCGAGTTAGGTGTGAGCCTrpoH-qRT-RGCGAGTTAGGTGTGAGCCTrpoH-qRT-RGCGAGTTAGGTGTGAGCCTrpoH-qRT-RGCGAGTTAGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTGCCAGGTACAN646_3697qRTFAACAAGGCCCGCTGTGATAN646_3696qRTFAGAGAAGCGTTTTGAGCCGAN646_3695qRTFGTCCTCGCCGCAGTAGCCGCGTGN646_3695qRTFGCCCCGTTGTCACACCACN646_3695qRTFGCCCCGCTGTGATAN646_3695qRTFGCCCCGCCGTAGCGCGGTGN646_3695qRTFCGCCGTCCTTCACCCAAATCN646_0523qRTFCGCGTCCCTTCACCAAATCN646_0523qRTRACAGCTCGCACAGAGTGTCAAN646_0523qRTRACAGCTCGCACAGAGTGTCAAN646_4604qRTFGCAGTACCGAGAGTGCGAN646_4604qRTFGCAGTACCGAGAGTGGAA165 RNA-qRT-FAAAGCACTTCAGCCCAGTAAT165 RNA-qRT-FAAAGCACTTCACCCCAGTAAT	Parce reactive-R	ТТТТАААТТССТАGААТАААСТGАТАСТАААТАААТ
gyr B cy5-RCCACCTTCATACATGAAGTGATCArpoE-qRT-FTGTTGCTCAAGGGCGTAGACrpoE-qRT-RCGATTGCACTGAACACCCACCrpoX-qRT-RCGATTGCACTGAACATGGCCGCACGrpoX-qRT-RGCTCTGCTTTCACCCCTGAGrpoH-qRT-FGCGAGTTAGGTGTGAGCCTrpoH-qRT-RATAGCATCGGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3696qRTRAACAAGGCCCGCTGTTGATAN646_3695qRTFGTCCTGGCTGGTGAGCTGGN646_3695qRTFGTCCTCGCCAGTACCTAGCN646_3695qRTFGTCCTCGCCAGTACCTAGCN646_0523qRTFCACCGGTTTGTCAACCACAN646_0523qRTFCGCGCTCCTTCAACCAAATCN646_0523qRTRACAGCTCGCACAGATGTCAAN646_04qRTFGCAGTACCGAGACTTGGTGGN646_04qRTFGCAGTACCGAGAGCGTGGGGN646_4604qRTFGTAAACCACGAGGCGATCAN646_4604qRTFAACAGCACGAGGCATTCAACAAN646_157AAAGCACTTCAGCAGAGGCGAAN646_165 AGFTFGTAACCCAGAAGTGCAAN646_165 AGFTFGCAGTACCGAGAGCGATCCAN646_165 AGFTFGCAGTACCGAGAGTGCAAN646_165 AGFTFGCAGTACCGAGAGTGCAAN646_165 AGFTFGCAGTACCGAGAGTGCAAN646_165 AGFTFGCAGTACCGAGAGCGATCCAN646_165 AGFTFGCAGTACCGAGAGTGCAAN646_165 AGFTFGCAGTACCGAGAGCGATCCAN646_165 AGFTFGCAGTACCGAGAGCGATCCAN646_165 AGFTFGCAGTACCGAGAGCGATCCAN646_165 AGFTFGCAGTACCAGAGGCGATCCAN646_165 AGFTFGCAGTACCAGAGGCGATCCAN646_165 AGFTFGCAGTACCGAGAGCGAGCATCCAN646_1	avrB cv5-F	TGCCTGCAGGTCGACGATCGCACTATCAGAGAAAGTTGAGC
pol-qRT-FTGTTGCTCAAGGGCGTAGACrpoE-qRT-FTAATGAACAATGGCCGCACGrpoX-qRT-FTAATGAACAATGGCCGCACGrpoX-qRT-RGCTCTGCTTTCACCCCTGAGrpoH-qRT-FGCGAGTTAGGTGTTGAGCCTrpoH-qRT-RATAGCATCGGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTRAACAAGGCCGCGTGTGATAN646_3696qRTRAAGAAAGCGCTTTTGAACCTGGN646_3695qRTFGTCCTCGCCAGTAACN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTRATTCGTTTCGAAGCTGCGTGN646_3695qRTRCACCGGTTTGTTCACACCACN646_0523qRTFCGCGTCCCTTCAACCAAATCN646_0523qRTRCACCGGTTGTTCACACCACN646_04qRTFGCAGTACCGAGACTTGGTGGN646_4604qRTFGCAGTACCGAGACCTGGGGN646_4604qRTFGCAGTACCGAGACCTAGCN646_4604qRTFGCAGTACCGAGACCTAGTGAAN646_4604qRTFAAAGCACTTGCGGGAACCA165 RNA-qRT-FAAAGCACTTTCCAGCCACTAATCN646_461TGCAGTACCGAGAACTTGGTGGAAA165 RNA-qRT-RTGCGCTTTACGCCCAGTAAT	avrB cv5-R	CCACCTTCATACATGAAGTGATCA
rpoE-qRT-RCGATTGCACTGAACACCACCrpoX-qRT-FTAATGAACAATGGCCGCACGrpoX-qRT-RGCTCTGCTTTCACCCCTGAGrpoH-qRT-FGCGAGTTAGGTGTTGAGCCTrpoH-qRT-RATAGCATCGGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTRAACAAGGCCCGCTGTTGATAN646_3696qRTFAGAGAAGCGTTTTGAGCCGAN646_3695qRTRATTCGTTTCGAAGCTGGCGGN646_3695qRTRGTCCTCGCCAGTAACCTAGCN646_3695qRTRCACCGGTTTGTTCAACCAACN646_3695qRTRCACCGGTTTGTTCAACCAACN646_0523qRTFCGCGTCCCTTCAACCAAATCN646_0523qRTFCGCGTCCCTTCAACCAAATCN646_4604qRTFGCAGTACCGAGACTTGGTGGN646_4604qRTRGTAAACCACGAGGCGATCCA165 RNA-qRT-FAAAGCACTTTCAGCCCAGTAAT	rpoE-gRT-F	TGTTGCTCAAGGGCGTAGAC
rpxX-qRT-FTAATGAACAATGGCCGCACGrpxX-qRT-RGCTCTGCTTTCACCCCTGAGrpoH-qRT-FGCGAGTTAGGTGTTGAGCCTrpoH-qRT-RATAGCATCGGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTRAACAAGGCCCGCTGTTGATAN646_3696qRTFAGAGAAGCGTTTTGAGCCGAN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFCACCGGTTTGTTCAACCAACN646_0523qRTFCACCGGTTCCTCAACCAAATCN646_0523qRTFGCGCTCCCTCAACCAAATCN646_4604qRTFGCAGTACCGAGAATGTCAAN646_4604qRTRGTAAACCACGAGGCGATCCA165 RNA-qRT-FAAAGAT-165 RNA-qRT-RTGCGCTTTACGCCCAGTAAT	rpoE-gRT-R	CGATTGCACTGAACACCACC
rpX-qRT-RGCTCTGCTTTCACCCCTGAGrpOH-qRT-FGCGAGTTAGGTGTTGAGCCTrpOH-qRT-RATAGCATCGGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTRAACAAGGCCCGCTGTTGATAN646_3696qRTFAGAGAAGCGTTTTGAGCCGAN646_3696qRTRATTCGTTTCGAAGCTGCGTGN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFCACCGGTTTGTTCACACCACN646_0523qRTFCGCGTCCCTTCAACCAAATCN646_0523qRTFGCAGTACCGAGAGTGTCAAN646_4604qRTFGCAGTACCGAGAGTGCGGN646_4604qRTRGTAAACCACGAGGGGATCCA16S RNA-qRT-FAAAGCACTTTCAGCCCAGTAAT	rpoX-qRT-F	TAATGAACAATGGCCGCACG
rpoH-qRT-FGCGAGTTAGGTGTTGAGCCTrpoH-qRT-RATAGCATCGGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTRAACAAGGCCCGCTGTTGATAN646_3696qRTFAGAGAAGCGTTTTGAGCCGAN646_3696qRTRATTCGTTTCGAAGCTGCGTGN646_3695qRTRGTCCTCGCCAGTAACCTAGCN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTRCACCGGTTTGTTCACACCACN646_3695qRTRCACCGGTCCTTCAACCACACN646_0523qRTFCGCGTCCCTTCAACCAAATCN646_0523qRTFGCAGTACCCACGAGTGTCAAN646_4604qRTFGCAGTACCGAGAGTGTGAGN646_4604qRTRGTAAACCACGAGGGAACTCAA16S RNA-qRT-FAAAGCACTTTCAGTCGTGAGGAA16S RNA-qRT-RTGCGCTTTACGCCCAGTAAT	rpoX-qRT-R	GCTCTGCTTTCACCCCTGAG
rpoH-qRT-RATAGCATCGGCGCTGTGTAAN646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTRAACAAGGCCCGCTGTTGATAN646_3696qRTFAGAGAAGCGTTTTGAGCCGAN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFCACCGGTTTGTTCACACCACN646_3695qRTRCACCGGTTTGTTCACACCACN646_3695qRTRCACCGGTTTGTTCACACCACN646_3695qRTRCACCGGTTCGTCACCACCACN646_0523qRTFCGCGTCCCTTCAACCAAATCN646_0523qRTFGCAGTACCGACAGATGTCAAN646_4604qRTFGCAGTACCGAGACTTGGTGGN646_4604qRTRGTAAACCACGAGGCGATCCA16S RNA-qRT-FAAAGCACTTTCAGTCGTGAGGAA16S RNA-qRT-RTGCGCTTTACGCCCAGTAAT	rpoH-aRT-F	GCGAGTTAGGTGTTGAGCCT
N646_3697qRTFGCTCAGTTCTCGCAGGTACAN646_3697qRTRAACAAGGCCCGCTGTTGATAN646_3696qRTFAGAGAAGCGTTTTGAGCCGAN646_3696qRTRATTCGTTTCGAAGCTGCGTGN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFGTCCTCGCCAGTAACCTAGCN646_3695qRTFCACCGGTTTGTTCACACCACN646_3695qRTFCGCGTCCCTTCAACCAAATCN646_0523qRTFCGCGTCCCTTCAACCAAATCN646_0523qRTFGCAGTACCGAGAGTGTCAAN646_4604qRTFGCAGTACCGAGAGTTGGGGN646_4604qRTFGTAAACCACGAGGCGATCCA165 RNA-qRT-FAAAGCACTTTCAGTCGTGAGGAA165 RNA-qRT-RTGCGCTTTACGCCCAGTAAT	rpoH-aRT-R	ATAGCATCGGCGCTGTGTAA
N646_3697qRTR AACAAGGCCGCTGTTGATA N646_3696qRTF AGGAAGCGTTTTGAGCCGA N646_3696qRTR ATTCGTTTCGAAGCTGCGTG N646_3695qRTF GTCCTCGCCAGTAACCTAGC N646_3695qRTF GTCCTCGCCAGTAACCTAGC N646_3695qRTR CACCGGTTTGTTCACACCAC N646_3695qRTR CACCGGTTTGTTCACACCAC N646_3695qRTR CACCGGTTCTTCAACCAAATC N646_0523qRTF CGCGTCCCTTCAACCAAATC N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_4604qRTF GCAGTACCGAGACTTGGTGG N646_4604qRTF GTAAACCACGAGGCGATCCA 165 RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 165 RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_3697aRTE	GCTCAGTTCTCGCAGGTACA
N646_3696qRTF AGAGAAGCGTTTTGAGCCGA N646_3696qRTR ATTCGTTTCGAAGCTGCGTG N646_3695qRTF GTCCTCGCCAGTAACCTAGC N646_3695qRTR CACCGGTTTGTTCACACCAC N646_3695qRTR CACCGGTTTGTTCACACCAC N646_0523qRTF CGCGTCCCTTCAACCAAATC N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_4604qRTF GCAGTACCGAGACTTGGTGG N646_4604qRTR GTAAACCACGAGGCGATCCA 165 RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 165 RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_3697aRTR	AACAAGGCCCGCTGTTGATA
N646_3696qRTR ATTCGTTTCGAAGCTGCGTG N646_3695qRTF GTCCTCGCCAGTAACCTAGC N646_3695qRTR CACCGGTTTGTTCACACCAC N646_0523qRTF CGCGTCCCTTCAACCAAATC N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_0523qRTR GCAGTACCGAGACTTGGTGG N646_0523qRTR GCAGTACCGACGACATGTCAA N646_4604qRTF GCAGTACCGAGACTTGGTGG N646_4604qRTR GTAAACCACGAGGCGATCCA 165 RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 165 RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_3696gRTE	AGAGAAGCGTTTTGAGCCGA
N646_3695qRTF GTCCTCGCCAGTAACCTAGC N646_3695qRTR CACCGGTTTGTTCACACCAC N646_0523qRTF CGCGTCCCTTCAACCAAATC N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_0523qRTR GCAGTACCGACAGATGTCAA N646_4604qRTF GCAGTACCGAGGCGATCCA N646_4604qRTR GTAAACCACGAGGCGATCCA 165 RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 165 RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_3696gRTR	ATTCGTTTCGAAGCTGCGTG
N646_3695qRTR CACCGGTTTGTTCACACCAC N646_0523qRTF CGCGTCCCTTCAACCAAATC N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_4604qRTF GCAGTACCGAGACTTGGTGG N646_4604qRTR GTAAACCACGAGGCGATCCA 165 RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 165 RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_3695aRTE	GTCCTCGCCAGTAACCTAGC
N646_0523qRTF CGCGTCCCTTCAACCAAATC N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_4604qRTF GCAGTACCGAGACTTGGTGG N646_4604qRTR GTAAACCACGAGGCGATCCA 165 RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 165 RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_3695aRTR	CACCGGTTTGTTCACACCAC
N646_0523qRTR ACAGCTCGCACAGATGTCAA N646_4604qRTF GCAGTACCGAGACTTGGTGG N646_4604qRTR GTAAACCACGAGGCGATCCA 16S RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 16S RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_0523gRTE	CGCGTCCCTTCAACCAAATC
N646_4604qRTF GCAGTACCGAGACTTGGTGG N646_4604qRTR GTAAACCACGAGGCGATCCA 16S RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 16S RNA-qRT-R TGCGCTTTACGCCCAGTAAT	N646_0523gRTR	ACAGCTCGCACAGATGTCAA
N646_4604qRTR GTAAACCACGAGGGGATCCA 16S RNA-qRT-F AAAGCACTTTCAGTCGTGAGGAA 16S RNA-gRT-R TGCGCTTTACGCCCAGTAAT	N646_4604gRTF	GCAGTACCGAGACTTGGTGG
16S RNA-gRT-F AAAGCACTTTCAGTCGTGAGGAA 16S RNA-gRT-R TGCGCTTTACGCCCAGTAAT	N646_4604gRTR	GTAAACCACGAGGCGATCCA
16S RNA-aRT-R TGCGCTTTACGCCCAGTAAT	16S RNA-gRT-F	AAAGCACTTTCAGTCGTGAGGAA
	16S RNA-gRT-R	TGCGCTTTACGCCCAGTAAT

Electrophoretic mobility shift assay. The purification of 6×His-tagged RpoE and RpoX from *E. coli* BL21(DE3) with nickel affinity chromatography was performed as previously described (3). For electrophoretic mobility shift assays (EMSAs), purified 6×His-tagged RpoE or RpoX was incubated with different Cy5-labeled DNA probes (Table 2) in 20 μ l of loading buffer (10 mM NaCl, 0.1 mM dithiothreitol [DTT], 0.1 mM EDTA, 10 mM Tris [pH 7.4]). After the mixture was incubated at 25°C for 30 min, the samples were

resolved using 6% polyacrylamide gel electrophoresis in $0.5 \times$ TBE (Tris-boric acid-EDTA) buffer on ice at 100 V for 120 min. Next, the gels were scanned using a Typhoon FLA 9500 instrument (GE Healthcare, Uppsala, Sweden).

RNA-seq analysis. For RNA-seq analysis of the $\Delta rpoX$ or $\Delta rpoE$ strain, bacteria were incubated overnight and then diluted 1:100 in LBS medium. The bacteria were then grown at 42°C and harvested after 9 h. The subsequent procedures and statistical analysis were performed as previously described (32).

ChIP-seq analysis. For the ChIP-seq analysis of RpoX, the pBAD33::Proox-Flag and pBAD33::Flag plasmids, encoding RpoX-Flag and the Flag tag alone (control), respectively, were transferred to the $\Delta rpoX$ strain. Cultures of each strain grown overnight in LBS medium at 42°C or in LB medium containing 0.5% NaCl at 30°C were diluted (1:100) in 50 ml of fresh LBS medium with 0.04% L-arabinose. After 9 h of growth with shaking, the protein-DNA complexes in the bacterial cells were fixed in vivo with rifampin at a final concentration of 150 μ g/ml under the corresponding conditions for 20 min (33) and then cross-linked in vivo with 1% formaldehyde at room temperature for 10 min. Cross-linking was stopped by the addition of 125 mM glycine. The following procedures and statistical analysis were performed as previously described (34). Briefly, bacterial cells were sonicated in SDS lysis buffer, and the DNA was fragmented to 100 to 500 bp and immunoprecipitated (IP) with Flag-labeled beads. IP DNA was collected in elution buffer, followed by reversion of the DNA-protein cross-links and purification of the DNA by phenol-chloroform. DNA fragments were used for library construction with the VAHTS Turbo DNA library prep kit and then sequenced with a MiSeq sequencer (Illumina, San Diego, CA). ChIP-seq reads were mapped to the V. alginolyticus EPGS genome. The enriched peaks were identified using MACS software (35), followed by MEME analysis to generate the RpoX-binding motif (36). KEGG pathway analysis was performed with Kobas 3.0 to illustrate the enriched gene function (37).

Motility, biofilm, and hemolytic activity assays. The motility assay was performed as previously described (10). Cultures grown overnight were diluted to an OD_{600} of 1.0 and then spotted onto LBS medium containing 0.3% (swimming) and 1.5% (swarming) agar. After incubation at 30°C for 12 h and 24 h, respectively, bacterial motility was observed. The experiments were performed at least three times, and one representative result is shown.

The biofilm assay was performed as previously described (10). Cultures grown overnight (50 μ l) were diluted to 5 ml in LBS medium in glass tubes and incubated at 30°C without shaking for 48 h. A total of 0.04% L-arabinose, which exerts no apparent influence on biofilm formation of the WT, was added to LBS medium to induce the pBAD promoter. The total biofilm was measured by 2% crystal violet staining. The experiments were performed at least three times, and one representative result is shown.

Hemolytic activity assays were performed as previously described (4, 28, 38). *V. alginolyticus* strains were grown to mid-log phase in LBS medium at 30°C. The bacterial cells were centrifuged at $500 \times g$, washed three times with PBS, and then resuspended with PBS to a final concentration of $0.5 \times 10^{\circ}$ CFU/ml. For the blood agar assay, a suspension of 5% defibrinated sheep blood erythrocytes was added to LBS agar medium (45°C to 50°C), mixed gently, and poured into plates. Pellets of 5-µl bacterial suspensions were dropped onto the blood agar plates. The plates were incubated at 30°C or 42°C for 12 h. The experiments were performed at least three times, and one representative result is shown.

LD_{so} determination. Median lethal dose (LD_{so}) determination for the WT, $\Delta rpoX$, or $\Delta rpoE$ strain in the zebrafish infection model was performed as previously described (39). Healthy fish, each weighing approximately 0.25 g, were obtained from a commercial farm and acclimatized to the laboratory conditions for at least 15 days. Zebrafish were anesthetized with tricaine methanesulfonate (catalog no. MS-222; Sigma-Aldrich) at a concentration of 80 mg/liter. Groups of 10 fish each were injected intramuscularly with bacterial cells adjusted to the required concentrations. Fish mortality was monitored over a period of 7 days postinfection. Fish injected with PBS only served as negative controls. The LD_{so} values were calculated as described previously (39). The animal work presented here was approved by the Animal Care Committee, East China University of Science and Technology (approval no. 2006272).

Statistical analysis. GraphPad Prism (version 6) was used to perform the statistical analyses. To compare gene expression or CFU between the groups, a two-tailed Student's unpaired *t* test was used. A *P* value of <0.05 was considered significant.

Data availability. The sequence reads were deposited in the SRA database under accession no. SRP152034.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM .00234-19.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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