Supporting Information

Dissection and reconstitution provide insights into electron transport in the membrane-bound aldehyde dehydrogenase complex of *Gluconacetobacter diazotrophicus*

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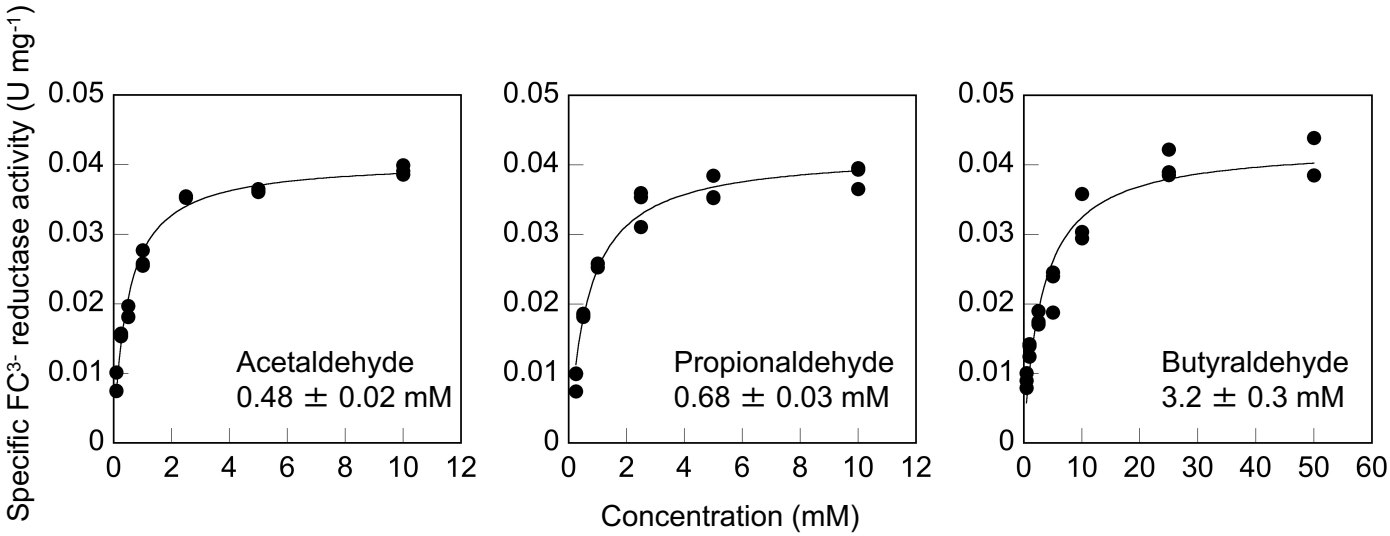

Fig. S1. Miah et al.

Fig. S1. K_M values of AldGH for short-chain aldehydes.
Aldehyde:ferricyanide (FC^{3-}) oxidoreductase activity at pH 6.0 of the crude soluble fraction of *Gluconacetobacter diazotrophicus* strain MR25 (PAL5 Δ*aldF* Δ*aldSLC* Δ*adhAB* ΔPQQ) assayed with deferent concentrations of acetaldehyde, propionaldehyde, or butyraldehyde. The V_{max} and K_M values were determined by fitting the data to the Michaelis–Menten equation using KaleidaGraph software v4.5 (Synergy Software, Reading, PA).

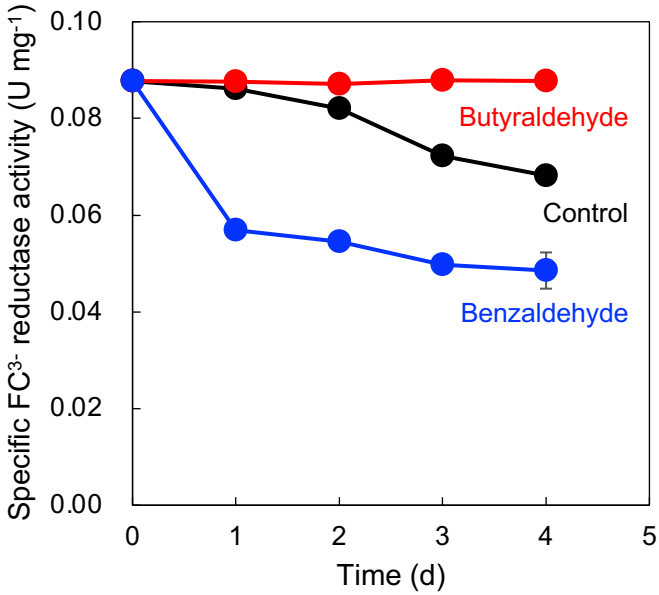

Fig. S2. Miah et al.

Fig. S2. Effect of butyraldehyde on stability of AldGH. The crude soluble fraction of *Gluconacetobacter diazotrophicus* strain MR25 was kept at 4° C for 4 days in the presence of 2 mM butyraldehyde (red line) or 50 mM benzaldehyde (blue line; Adachi *et al*., 1980), or in the absence of aldehyde (black line). Aldehyde:ferricyanide (FC^{3-}) oxidoreductase activity at pH 6.0 was assayed once a day. Mean values and standard deviations from triplicate assays are shown.

Reference

Adachi O, Tayama K, Shinagawa E, Matsushita K, Ameyama M. 1980. Purification and characterization of membrane-bound aldehyde dehydrogenase from Gluconobacter suboxydans. Agric Biol Chem 44:503-515.

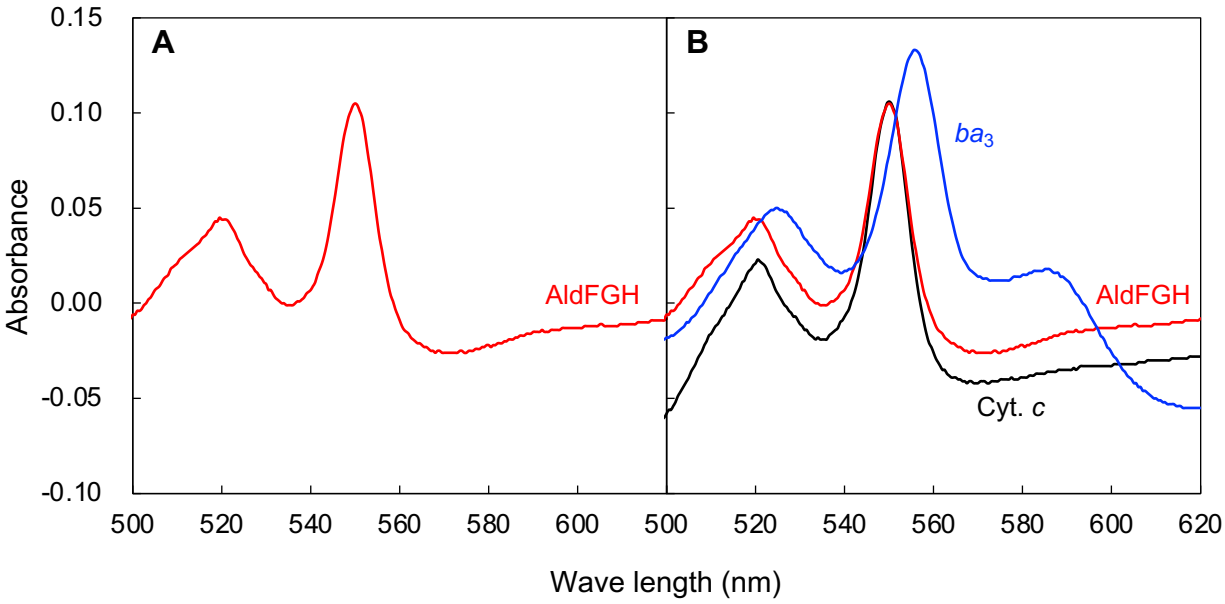

Fig. S3. Miah et al.

Fig. S3. Reduced *minus* oxidized difference spectrum of pyridine hemochromogen of the AldFGH complex. Purified AldFGH complex (0.30 mg protein mL^{-1}) was treated with pyridine and NaOH. The reduced *minus* oxidized difference spectrum of the treated sample was recorded (red trace in panels A and B). The heme *c* content in the AldFGH complex was calculated at 2.1 mol heme *c* (mol AldFGH complex)$^{-1}$, using a molecular mass for the protein complex of 142 kDa. For comparison, horse heart cytochrome *c* (60 µg protein mL^{-1}) was used for determination of heme *c* (black trace in panel B). The heme *c* content in horse heart cytochrome *c* was calculated as 1.0 mol heme *c* (mol cytochrome *c*)$^{-1}$, using a molecular mass for the protein of 12 kDa. For another comparison, hemes *b* and *a* were extracted from partially purified cytochrome ba_3 ubiquinol oxidase by acid–acetone treatment, followed by evaporation of the acetone (Puustinen & Wikström, 1991). A reduced *minus* oxidized difference spectrum was recorded for the pyridine hemochromogen of the extracted hemes (blue trace in panel B).

Reference

Puustinen A, Wikström M. 1991. The heme groups of cytochrome *o* from *Escherichia coli*. Proc Natl Acad Sci U S A 88:6122-6.

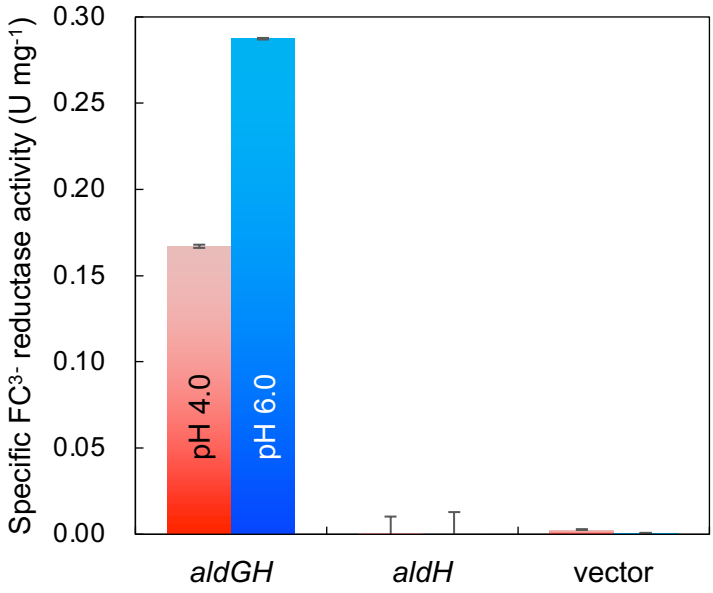

Fig. S4. Miah et al.

Fig. S4. The *aldG* gene is essential for ALDH activity. *Acetobacter pasteurianus* strain mNS4 (Δ*adhAB* Δ*aldFGH* Δ*aldSLC*) harboring pTM8 (*aldGH*$^{+}$), pTM10 (*aldH*$^{+}$), or pCM62 (vector control) was cultivated overnight in YPGD medium containing 50 μg mL^{-1} tetracycline at 30° C. Acetaldehyde:ferricyanide (FC^{3-}) oxidoreductase activity of crude soluble fractions was measured at pH 4.0 and 6.0.

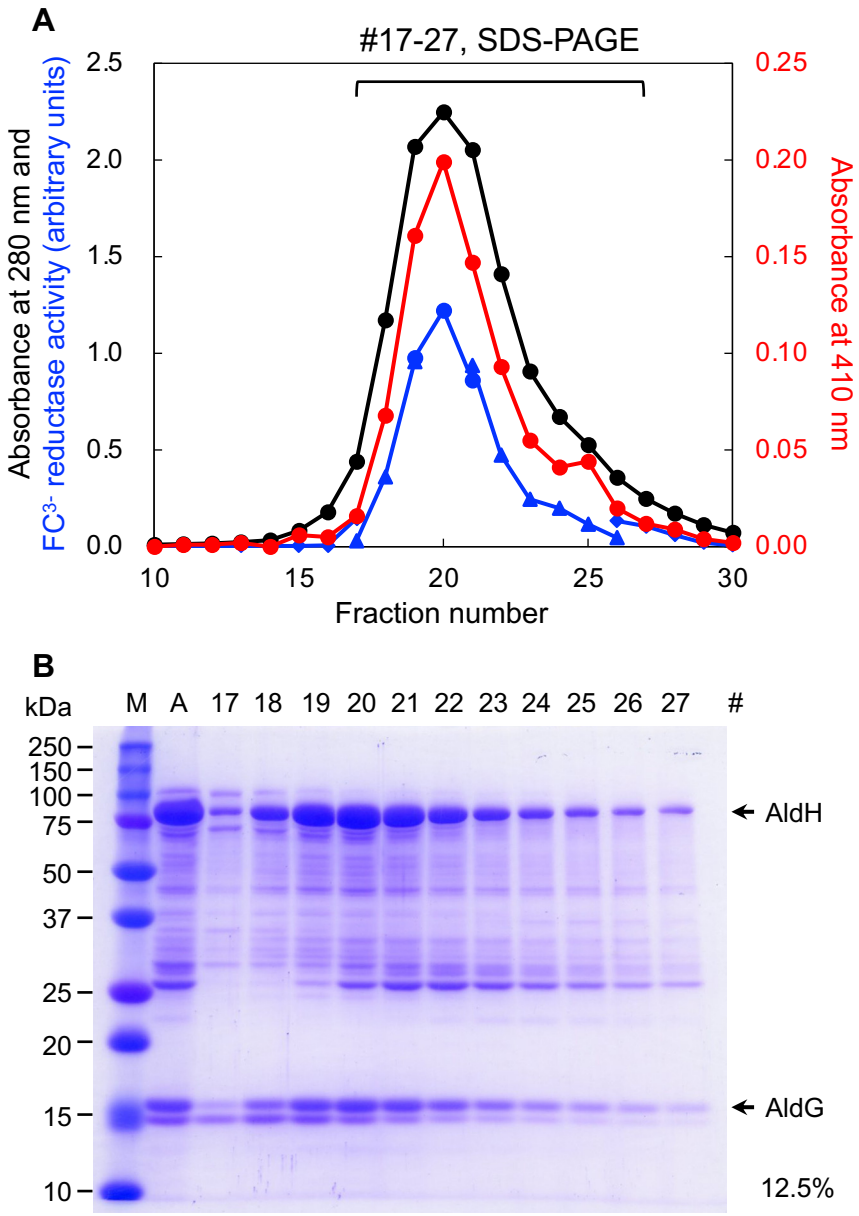

Fig. S5. Miah et al.

Fig. S5. Superdex 200 gel filtration column chromatography of AldGH. The AldGH subcomplex (0.19 mg mL^{-1}) purified by DEAE-Toyopearl, Phenyl-Toyopearl, and hydroxyapatite chromatographies was concentrated using an Amicon Ultra (50 k). Two hundred microliters of the concentrated AldGH (8.9 mg protein mL^{-1}) were applied to a Superdex 200 column (10/300) and eluted with 50 mM K^+-phosphate (pH 6.0) containing 2 mM butyraldehyde. A. Acetaldehyde:ferricyanide (FC^{3-}) oxidoreductase activity at pH 5.0 (blue), and absorbances at 280 nm (black) and 410 nm (red). B. Six microliters of each of fractions 17 to 27 were applied to SDS-PAGE (12.5% acrylamide) and the gels were stained with Coomassie Brilliant Blue R-250. Molecular mass standards and the concentrated, purified AldGH subcomplex before gel filtration (11 µg protein) were loaded in lanes M and A, respectively.

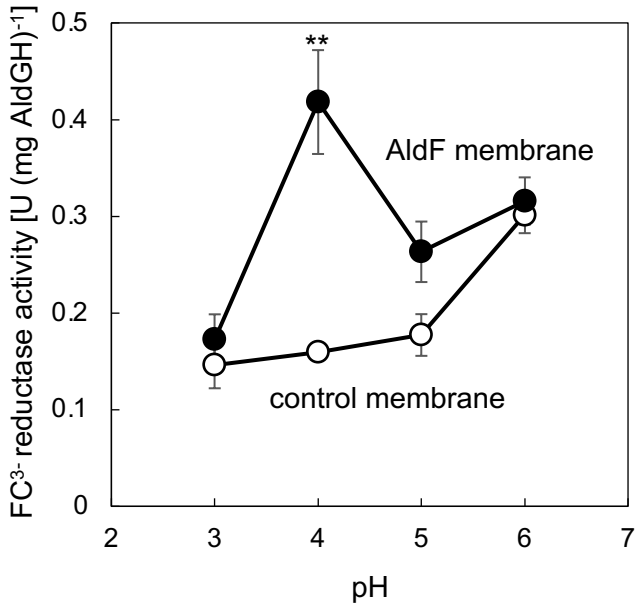

Fig. S6. Miah et al.

Fig. S6. Effect of AldF on ferricyanide reductase activity of AldGH. The crude soluble fraction of *A. pasteurianus* mNS4 harboring pTM8 (*aldGH*$^+$) was incubated with the membrane fraction of strain mNS4 harboring either pCM62 (vector control) or pTM14 (*aldF*$^+$). One volume of the soluble fraction of AldGH (7.2 mg protein mL^{-1}) and one volume of the membrane suspension (13 mg protein mL^{-1} for control membranes, 16 mg protein mL^{-1} for AldF membranes) were mixed and incubated at 30° C for 10 min. Acetaldehyde:ferricyanide (FC^{3-}) oxidoreductase activity was assayed at pH 3.0 to 6.0. The specific activity was calculated with reference to the soluble fraction containing AldGH. Mean values and standard deviations from triplicate enzyme assays are shown. **P < 0.01 (between the AldF membrane and the control membrane, using Student's t-test).

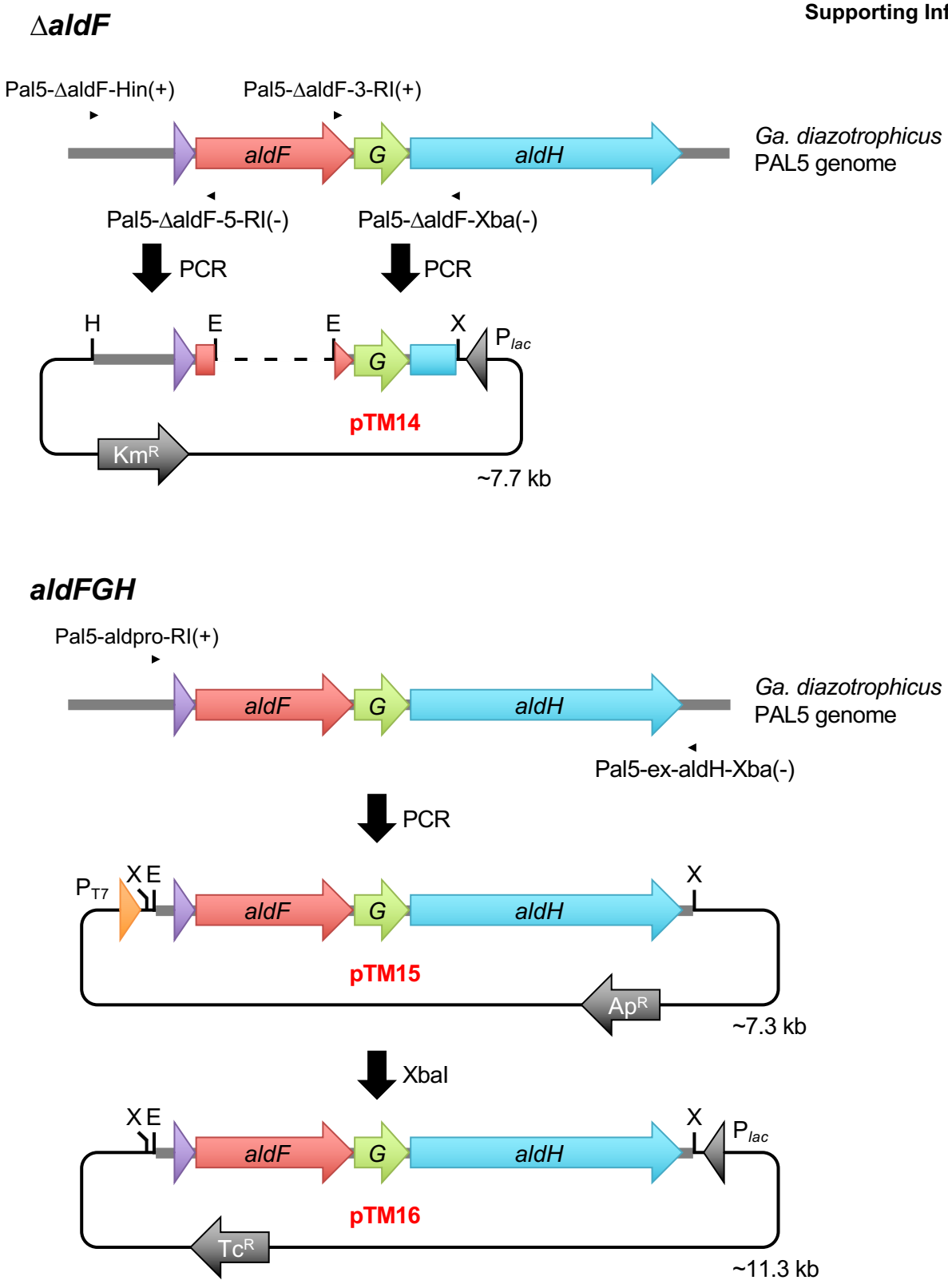

Fig. S7. Miah et al.

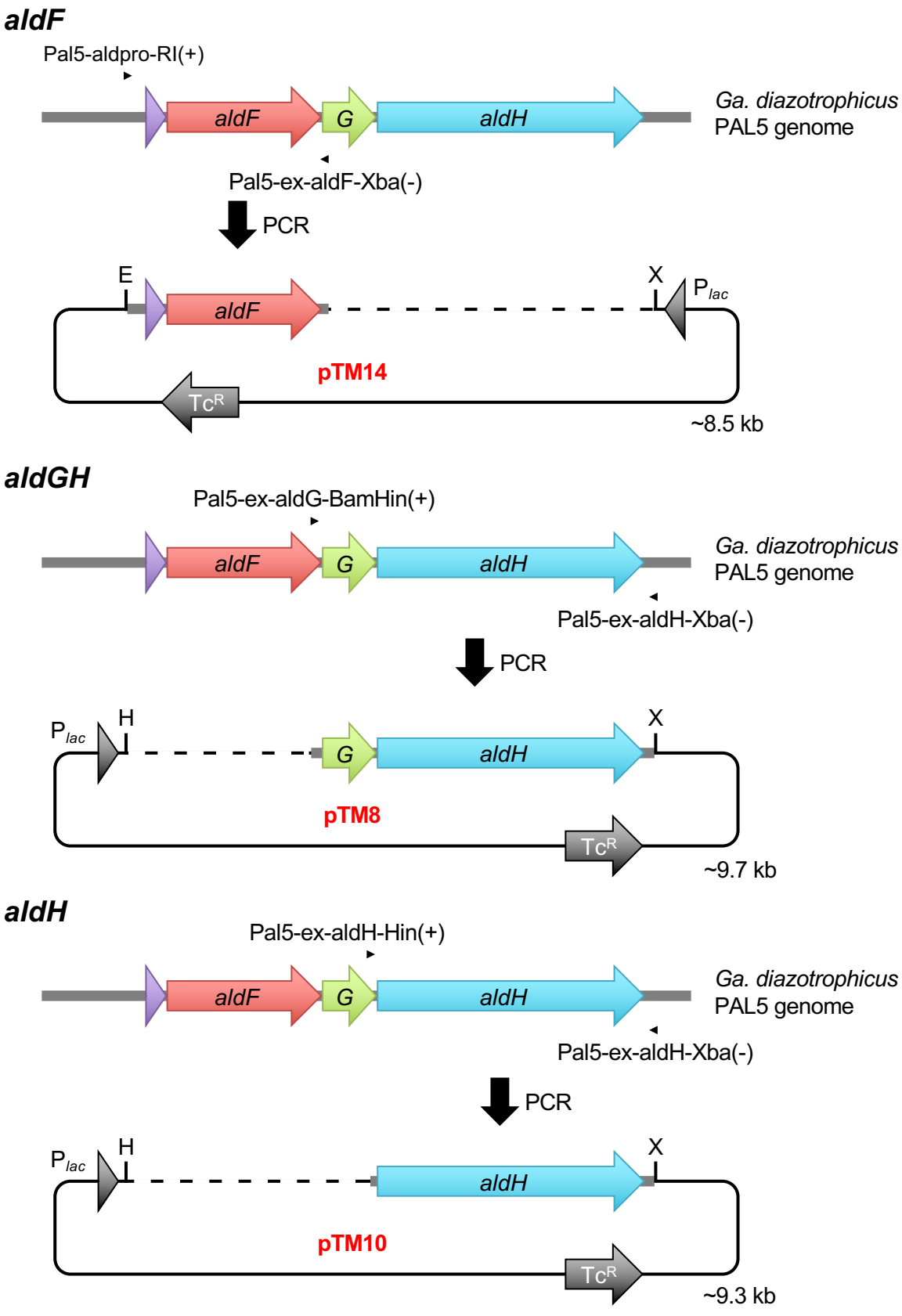

Fig. S7. Miah et al.

Fig. S7. Miah et al. (caption)

Fig. S7. Construction of plasmids used in this study. All the DNA fragments used in this study were prepared by PCR using genomic DNA from *Ga. diazotrophicus* strain PAL5. The positions where each DNA primer binds to the genomic DNA are shown by black arrow heads with the primer name. H, *Hin*dIII; X, *Xba*I; E, *Eco*RI; purple arrowhead, putative promoter region for *aldFGH*; red arrow, *aldF*; green arrow, *aldG*; blue arrow, *aldH*; gray arrowheads, *lac* promoter; orange arrowhead, T7 promoter; gray arrow, antibiotic resistance genes (Ap^R, ampicillin resistance; Km^R, kanamycin resistance; Tc^R, tetracycline resistance); dashed line, no DNA.

Table S1. Q_2 reductase activity of the reconstituted AldFGH complex.

Sample	Protein amount in the assay mix (ng)		Q_2 reductase activity in the assay mix (mU)
	AldGH	AldF	
Purified AldGH subcomplex	96	-	n.d.[a]
Partially purified AldF	-	88×10^3	n.d.
Reconstituted	96	88×10^3	2.6 ± 0.4

[a]n.d., not detected.

Table S2. Oligonucleotides used in this study.

Name	Sequence (5' to 3')	Objective
Pal5-ex-aldG-BamHin(+)	ggatccaagcttgagatgacagacggagcc	Expression of *aldGH*
Pal5-ex-aldH-Hin(+)	aagcttcgcgacgccatcaagaag	Expression of *aldH*
Pal5-ex-aldH-Xba(-)	tctagattcaggttgatgcggtgccg	Expression of *aldFGH*, *aldGH*, and *aldH*
Pal5-aldpro-RI(+)	gaattcatcgtgacggccgatgg	Expression of *aldFGH* and *aldF*
Pal5-ex-aldF-Xba(-)	tctagatgtcggggctccgtctg	Expression of *aldF*
Pal5-D-aldF-Hin(+)	aagcttcgctatgacgggc	Construction of Δ*aldF*
Pal5-D-aldF-5-RI(-)	gaattcgatgctggcggtgtcg	Construction of Δ*aldF*
Pal5-D-aldF-3-RI(+)	gaattctaccattccctgaccg	Construction of Δ*aldF*
Pal5-D-aldF-Xba(-)	catctagaccgaccagcttc	Construction of Δ*aldF*