



Structural insights into the iron nitrogenase complex

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Supplementary Information

Table S1: Primers used in this study.

Primer	Target	Sequence (5'-3')	Purpose
oMM0021	B10S genomic DNA	GCAGCGTGAAGCAGCCCGTTTCGGAATTCC G	Construction of pMM0064
oMM0023	pRhon5Hi-2	CTGCTTCACGCTGCCGCAAG	Construction of pMM0064
oMM0027	pBS85	GTTTTTGGTCTCAGCCAATCCCTGGG	Construction of pMM0021
oMM0028	pBS85	GGCTGAGACCAAAAACATATTCTCAATAAA CCC	Construction of pMM0021
oMM0033	pOGG024	CACCACAGGTCTCGGGGTTATGCAGCGGA AAAGG	Construction of pMM0002
oMM0034	pOGG024	TCAGTAGGTCTCGAGCATGGTGAGAATCC AGGGGTCC	Construction of pMM0002
oMM0035	B10S genomic DNA	TACAACAGGTCTCGTGCTCCCGAGGCGAC	Construction of pMM0002
oMM0036	B10S genomic DNA	CATCATGGTCTCGACCGGGTAAGGAGTTC CTGTC	Construction of pMM0002
oMM0037	B10S genomic DNA	CACCACAGGTCTCGACCCATTCCAAGGGC CG	Construction of pMM0002
oMM0038	B10S genomic DNA	CAATCAGGTCTCGTGGCTGCAGATCCAGT CCGTA	Construction of pMM0002
oMM0054	B10S genomic DNA	CGTAGTCTGACGATGCGCACTTC	Construction of pMM0057
oMM0055	B10S genomic DNA	GTCAAAGGAGGCAAGCCCCATCACGAG	Construction of pMM0057
oMM0056	B10S genomic DNA	GGGCTTGCCCTCTTTGACTTGCTCGGGTT	Construction of pMM0057
oMM0057	B10S genomic DNA	GGATCCTCTAGTGCAGAACCGAATCCGAA AGC	Construction of pMM0057
oMM0145	pRhon5Hi-2	ATCGCCGCGCAACCTGGATCCGAATTCGA GCTCCGTCGACAAGCTTG	Construction of pMM0064
oMM0146	B10S genomic DNA	CTCGAATTCGGATCCAGGTTGCGCGGCGA TG	Construction of pMM0064

oMM0161	pMM0064	AAAGCGGTGTCCGAGATGACCGGCCAGC	Construction of pMM0073
oMM0162	pMM0064	CCGAGATCTTCGGGAGCGCCTGAAGC	Construction of pMM0073
oMM0163	pMM0064	CATCTCGGACACCGCTTTTCAGGAAGGC	Construction of pMM0073
oMM0164	pMM0064	CCGAAGATCTCGGGCCGTCTCTTGGGC	Construction of pMM0073
oMM0223	pMM0119	CGTTCAACCTGCCGCCGAATGGAGCCACC CGCAGTTCGAAAAAT	Construction of pMM0190
oMM0224	pMM0119	TTTCGCTGATATCGGTCATCTGTCCCTTATT TTTCGAACTGCGGGTGGCT	Construction of pMM0190
oMM0227	pK18mobSacB	GATTTAGGTCTCTGTAAAACGACGGCCAGT GC	pK18mobSacB backbone amplification for Gibson assembly
oMM0228	pK18mobSacB	GATTTAGGTCTCTCGTAATAGCGAAGAGGC CCG	pK18mobSacB backbone amplification for Gibson assembly
oMM0284	B10S genomic DNA	GATTTAGGTCTCTTTACGCGGACATCATC TTCGG	Construction of pMM0105
oMM0286	B10S genomic DNA	GATTTAGGTCTCAGAGATCCGCGCTCAGG TGC	Construction of pMM0105
oMM0287	B10S genomic DNA	GATTTAGGTCTCTTACGAACGAATATCTGG CGGCGG	Construction of pMM0105
oMM0288	B10S genomic DNA	GATTTAGGTCTCTTCTCCAGATTGGTGGAA TGGCCAAGG	Construction of pMM0105
oMM0323	B10S genomic DNA	ATGCAAGCTTGGCACTGGCCGTCGTTTTAC GGTCTCGCAGCGGTC	Construction of pMM0133
oMM0324	B10S genomic DNA	CCCTAGCCCCCCTGGAGACGAACACGCG CGA	Construction of pMM0133
oMM0325	B10S genomic DNA	GCGCGTGTTTCGTCTCCAGGGGGGGCTAG GGTG	Construction of pMM0133

oMM0326	B10S genomic DNA	GCGATCGGTGCGGGCCTCTTCGCTATTAC GCTCGGGTTTTTCGGTGGTGG	Construction of pMM0133
oMM0384	pRhon5Hi-2	GCCGTGGGTCGATGTTTGATGTTAGTCTTA TCTGAAAGTTGTGC	Construction of pMM0119
oMM0385	pRhon5Hi-2	CGATCTCGGCTTGAACGAATTGACCTTTTC TCCGACGAATAGAGT	Construction of pMM0119
oMM0386	pOGG024	AATTCGTTCAAGCCGAGATCG	Construction of pMM0119
oMM0387	pOGG024	TAACATCAAACATCGACCCACG	Construction of pMM0119
oMM0389	pMM0073	GATTTAGGTCTCAGGAGCAGCCCGTTCCGG AATTCC	Construction of pMM0119
oMM0390	pMM0073	GATTTAGGTCTCTAGCGGGTCACCACACGT TGAGG	Construction of pMM0119
oMM0394	B10S genomic DNA	GATTTAGGTCTCTCTGGTCAAGATCCTCGA CGC	Construction of pMM0131
oMM0395	B10S genomic DNA	GATTTAGGTCTCTCCAGCGACTTGCCGATA CCACCTTTG	Construction of pMM0131
oMM0396	B10S genomic DNA	GATTTAGGTCTCAGGAGATCCCGATCCCGT TCGAATG	Construction of pMM0131
oMM0397	B10S genomic DNA	CAAATCGACGACCACCATGGCCAACACGC TTTTTCG	Construction of pMM0131
oMM0510	pMM0119	CCTTCCAAGGAGCGACCCATGCACCACC ATCATCACCATA	Construction of pMM0190
oMM0511	pMM0119	CCGTAAATGGCGATCTTGCGGGTATGGTG ATGATGGTGGTGC	Construction of pMM0190

Table S2: Plasmids used in this study.

Plasmid	Code	Relevant Features	Source or Reference
pK18mobSacB	pMM0056	Suicide vector, <i>oriT</i> (mobilizable), <i>sacB</i> , Kan ^R	[1]
pK18mobSacB- <i>draTG</i>	pMM0105	Suicide vector, <i>oriT</i> (mobilizable), <i>sacB</i> , Kan ^R , homologous recombination sites for <i>draTG</i> locus	This study
pK18mobSacB- <i>gtal</i>	pMM0133	Suicide vector, <i>oriT</i> (mobilizable), <i>sacB</i> , Kan ^R , homologous recombination sites for <i>gtal</i> locus	This study
pK18mobSacB- <i>nifHDK</i>	pMM0131	Suicide vector, <i>oriT</i> (mobilizable), <i>sacB</i> , Kan ^R , homologous recombination sites for <i>nifHDK</i> locus	This study
pK18mobSacB- <i>modABC</i>	pMM0057	Suicide vector, <i>oriT</i> (mobilizable), <i>sacB</i> , Kan ^R , homologous recombination sites for <i>modABC</i> locus	This study
pBS85	pMM0017	Suicide vector, <i>oriT</i> (mobilizable), Tet ^R	[2]
pBS85-Bsal	pMM0021	Suicide vector, <i>oriT</i> (mobilizable), Tet ^R , Bsal cutting site	This study
pBS85-Bsal- <i>genR</i>	pMM0002	Suicide vector, <i>oriT</i> (mobilizable), Tet ^R , Bsal cutting site, Gen ^R , homologous recombination sites for <i>anfHDGK</i> locus	This study
pRhon5Hi-2	pMM0031	Broad host range plasmid, Kan ^R	[3]
pRhon5Hi-2- <i>anfHDGK</i>	pMM0064	Broad host range plasmid, <i>anfHDGK</i> operon, Kan ^R	This study
pRhon5Hi-2- <i>anfHDGK</i> -Golden Gate	pMM0073	Broad host range plasmid, <i>anfHDGK</i> operon with silent mutation in Bsal cutting site, Kan ^R	This study
pOGG024	pMM0114	Broad host range plasmid, <i>oriT</i> (mobilizable), <i>lacZα</i> cassette for golden gate cloning (Bsal), Gen ^R	Addgene plasmid #113991
pOGG024- <i>kanR</i>	pMM0119	Broad host range plasmid, <i>oriT</i> (mobilizable), <i>lacZα</i> cassette for golden gate cloning (Bsal), Kan ^R	This study
pOGG024- <i>kanR</i> - <i>anfHDGK</i>	pMM0119	Broad host range plasmid, <i>oriT</i> (mobilizable), <i>anfHDGK</i> operon, Kan ^R	This study
pOGG024- <i>kanR</i> - <i>anfHDGK</i> -Strep/His	pMM0190	Broad host range plasmid, <i>oriT</i> (mobilizable), <i>anfHDGK</i> operon with N-terminal His ₆ -tag and C-terminal Strep tag II, Kan ^R	This study

Table S3: Strains used in this study.

Strain	Genotype	Source or Reference
<i>Rhodobacter capsulatus</i> B10S	Wildtype	[4]
<i>Rhodobacter capsulatus</i> MM0425	$\DeltaanfHDGK::genR \Delta modABC \Delta draTG \Delta gtaI \Delta nifHDK$	This study
<i>Rhodobacter capsulatus</i> MM0436 (expression strain)	$\DeltaanfHDGK::genR \Delta modABC \Delta draTG \Delta gtaI \Delta nifHDK$	This study
<i>Escherichia coli</i> DH5 α	F ⁻ $\Phi80lacZ\Delta M15 \Delta(lacZYA-argF)$ U169 <i>recA1 endA1 hsdR17(r_k⁻, m_k⁺) phoA supE44 thi-1 gyrA96 relA1 λ⁻</i>	Thermo Scientific (Waltham, USA) Fisher Inc. (USA) catalogue #18265017
<i>Escherichia coli</i> ST18	RP4-2 <i>Tc::Mu Km::Tn7 $\Delta hemA$</i> mutant	[5]

SI References

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