

**Supplementary material for Benoit *et al.*, 2018 (MMI-16779). Iron-sulfur protein maturation in *Helicobacter pylori*: identifying a Nfu-type cluster carrier protein and its iron-sulfur protein targets.**

**Tables S1, S2 and S3.**

**Table S1.** Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
<b><u>Strain</u></b>		
<b><u>E. coli</u></b>		
TOP10	Cloning strain	Invitrogen
Rosetta (DE3) RIL	BL21 derivative. Host for protein overproduction; Cm <sup>r</sup>	Novagen
BTH101	<i>cya-99</i> mutant. Host for bacterial adenylate cyclase-based two hybrid	(Karimova <i>et al.</i> , 1998)
<b><u>H. pylori</u></b>		
26695	Parental strain	(Tomb <i>et al.</i> , 1997)
26695 <i>Anfu</i>	26695 with <i>Δhp1492::cat</i> ; Cm <sup>r</sup>	This study
43504	Parental strain	ATCC
43504 <i>Anfu</i>	43504 with <i>Δhp1492::cat</i> ; Cm <sup>r</sup>	This study
X-47	Parental, mouse colonizing strain	(Veyrier <i>et al.</i> , 2013)
X47 <i>Anfu</i>	X47 with <i>Δhp1492::cat</i> ; Cm <sup>r</sup>	This study
SLB1355	43504 with pILL- <i>nifU</i> ; Cm <sup>r</sup>	This study
SLB1372	SLB1355 with <i>ΔnifU::aphA3</i> ; conditional <i>nifU</i> mutant; Cm <sup>r</sup> Kan <sup>r</sup>	This study
<b><u>Plasmid</u></b>		
pET21b	Cloning and expression vector; Amp <sup>r</sup>	Invitrogen
pET-Nfu	pET21b with <i>hp1492 (nfu)</i> ORF cloned at <i>NdeI-XhoI</i> ; Amp <sup>r</sup>	This study
pET-NifS	pET21b with <i>hp0220 (nifS)</i> ORF cloned at <i>NdeI-BamHI</i> ; Amp <sup>r</sup>	(Olson <i>et al.</i> , 2000)
pET-NifU	pET21b with <i>hp0221 (nifU)</i> ORF cloned at <i>NdeI-EcoRI</i> ; Amp <sup>r</sup>	(Olson <i>et al.</i> , 2000)
pUC20-cat	Source of <i>cat</i> cassette; Cm <sup>r</sup>	(Wang and Taylor, 1990)
pBC-Kan	Source of <i>aphA3</i> cassette; Kan <sup>r</sup>	(Taylor <i>et al.</i> , 1988)
pILL2150	<i>E. coli-H. pylori</i> shuttle plasmid; Cm <sup>r</sup>	(Boneca <i>et al.</i> , 2008)
pILL- <i>nifU</i>	pILL2150 with <i>hp0221 (nifU)</i> ORF cloned at <i>NdeI-BamHI</i> ; Cm <sup>r</sup>	This study
pKT25	BACTH; allows for C-terminal fusions to T25 fragment of CyaA; Kan <sup>r</sup>	(Karimova <i>et al.</i> , 1998)
pKNT25	BACTH; allows for N-terminal fusions to T25 fragment of CyaA; Kan <sup>r</sup>	(Karimova <i>et al.</i> , 1998)
pUT18	BACTH; allows for N-terminal fusions to T18 fragment of CyaA; Amp <sup>r</sup>	(Karimova <i>et al.</i> , 1998)
pUT18C	BACTH; allows for C-terminal fusions to T18 fragment of CyaA; Amp <sup>r</sup>	(Karimova <i>et al.</i> , 1998)
pKT25- <i>zip</i>	pKT25 with <i>zip</i> gene; positive control for BACTH; Kan <sup>r</sup>	(Karimova <i>et al.</i> , 1998)
pUT18C- <i>zip</i>	pUT18C with <i>zip</i> gene; positive control for BACTH; Amp <sup>r</sup>	(Karimova <i>et al.</i> , 1998)
pKT25- <i>nifS</i>	pKT25 with <i>hp0220 (nifS)</i> ORF cloned at <i>XbaI-KpnI</i> ; Kan <sup>r</sup>	This study
pKT25- <i>nifU</i>	pKT25 with <i>hp0221 (nifU)</i> , ORF cloned at <i>XbaI-KpnI</i> ; Kan <sup>r</sup>	This study

pKT25- <i>nfu</i>	pKT25 with <i>hp1492 (nfu)</i> ORF cloned at <i>XbaI- KpnI</i> ; Kan <sup>r</sup>	This study
pKT25- <i>hp0779</i>	pKT25 with <i>hp0779 (acnB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pKNT25- <i>nifU</i>	pKNT25 with <i>hp1492 (nrfU)</i> ORF cloned at <i>XbaI- KpnI</i> ; Kan <sup>r</sup>	This study
pKNT25- <i>nfu</i>	pKNT25 with <i>hp1492 (nfu)</i> ORF cloned at <i>XbaI- KpnI</i> ; Kan <sup>r</sup>	This study
pKNT25- <i>hp0779</i>	pKNT25 with <i>hp0779 (acnB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18- <i>nfu</i>	pUT18 with <i>hp1492 (nfu)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>nifS</i>	pUT18C with <i>hp0220 (nifS)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>nifU</i>	pUT18C with <i>hp0221 (nifU)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>nfu</i>	pUT18C with <i>hp1492 (nfu)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0117</i>	pUT18C with <i>hp0117</i> (hypothetical) ORF cloned at <i>PstI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0132</i>	pUT18C with <i>hp0132 (sdaA)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0138</i>	pUT18C with <i>hp0138</i> (hypothetical) ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0142</i>	pUT18C with <i>hp0142 (mutY)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0191</i>	pUT18C with <i>hp0191 (frdA)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0207</i>	pUT18C with <i>hp0207 (apbC, mrp)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0269</i>	pUT18C with <i>hp0269 (miaB-like)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0277</i>	pUT18C with <i>hp0277 (ferredoxin)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0285</i>	pUT18C with <i>hp0285 (miaB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0400</i>	pUT18C with <i>hp0400 (ispH)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0468</i>	pUT18C with <i>hp0468</i> (hypothetical) ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0568</i>	pUT18C with <i>hp0568</i> (hypothetical) ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0585</i>	pUT18C with <i>hp0585 (nth)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0588</i>	pUT18C with <i>hp0588 (oorD)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0625</i>	pUT18C with <i>hp0625(ispG)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0631</i>	pUT18C with <i>hp0631 (hydA)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0654</i>	pUT18C with <i>hp0654 (mqnD)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0656</i>	pUT18C with <i>hp0656 (mqnC)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0665</i>	pUT18C with <i>hp0665 (hemN)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0666</i>	pUT18C with <i>hp0666 (glpC)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0734</i>	pUT18C with <i>hp0734 (rimO)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0768</i>	pUT18C with <i>hp0768 (moaA)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0779</i>	pUT18C with <i>hp0779 (acnB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp0934</i>	pUT18C with <i>hp0934 (queE)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1089</i>	pUT18C with <i>hp1089 (addB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1109</i>	pUT18C with <i>hp1109 (porD)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1222</i>	pUT18C with <i>hp1222 (dld)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1226</i>	pUT18C with <i>hp1226 (hemN)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1261</i>	pUT18C with <i>hp1261 (nuoB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1266</i>	pUT18C with <i>hp1266 (nqoB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1268</i>	pUT18C with <i>hp1268 (nuoI)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1356</i>	pUT18C with <i>hp1356 (nadA)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study
pUT18C- <i>hp1406</i>	pUT18C with <i>hp1406 (bioB)</i> ORF cloned at <i>XbaI- KpnI</i> ; Amp <sup>r</sup>	This study

pUT18C- <i>hp1428</i>	pUT18C with <i>hp1428</i> ( <i>rlmN</i> ) ORF cloned at XbaI- KpnI; Amp <sup>r</sup>	This study
pUT18C- <i>hp1508</i>	pUT18C with <i>hp1508</i> ( <i>ferredoxin</i> ) ORF cloned at XbaI- KpnI; Amp <sup>r</sup>	This study
pUT18C- <i>hp1540</i>	pUT18C with <i>hp1540</i> ( <i>fbcF</i> ) ORF cloned at XbaI- KpnI; Amp <sup>r</sup>	This study

## References

- Boneca, I.G., Ecobichon, C., Chaput, C., Mathieu, A., Guadagnini, S., Prevost, M.C., *et al.* (2008). Development of inducible systems to engineer conditional mutants of essential genes of *Helicobacter pylori*. *Appl. Environ. Microbiol.* **74**(7): 2095-2102.
- Karimova, G., Pidoux, J., Ullmann, A. and Ladant, D. (1998). A bacterial two-hybrid system based on a reconstituted signal transduction pathway. *Proc Natl Acad Sci U S A* **95**(10): 5752-5756.
- Olson, J.W., Agar, J.N., Johnson, M.K. and Maier, R.J. (2000). Characterization of the NifU and NifS Fe-S cluster formation proteins essential for viability in *Helicobacter pylori*. *Biochemistry* **39**(51): 16213-16219.
- Taylor, D.E., Yan, W., Ng, L.K., Manavathu, E.K. and Courvalin, P. (1988). Genetic characterization of kanamycin resistance in *Campylobacter coli*. *Ann Inst Pasteur Microbiol* **139**(6): 665-676.
- Tomb, J.F., White, O., Kerlavage, A.R., Clayton, R.A., Sutton, G.G., Fleischmann, R.D., *et al.* (1997). The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388**(6642): 539-547.
- Veyrier, F.J., Ecobichon, C. and Boneca, I.G. (2013). Draft Genome Sequence of Strain X47-2AL, a Feline *Helicobacter pylori* Isolate. *Genome Announc* **1**(6).
- Wang, Y. and Taylor, D.E. (1990). Chloramphenicol resistance in *Campylobacter coli*: nucleotide sequence, expression, and cloning vector construction. *Gene* **94**(1): 23-28.

**Table S2.** Primers used in this study

Name	Sequence (5'-3')*	Usage
NifU-NdeI	aggaccatATGGCAAACATGATTTA	Expression of <i>nifU</i> in pILL2150
NifU-BamHI	gtcctggatccTCCTCAAATCGGTAA	Expression of <i>nifU</i> in pILL2150
Nfu-NdeI	aggaccatATGATAGAATTTAGCGATG	Expression of <i>nfu</i> in pET21b
Nfu-XhoI	cgaatctcgagATGCCTATAATCCTTAAAGC	Expression of <i>nfu</i> in pET21b
$\Delta$ nifU-1	AATGGCAGGCGCAGCGGGAC	Construction of <i>AnifU::aphA3</i> conditional mutant
$\Delta$ nifU-2	gtataacatagtagcgaCTTTGCCATTTTGATTCC	Construction of <i>AnifU::aphA3</i> conditional mutant
$\Delta$ nifU-3	gaattgttttagtagcgaGGTGTACCGATTTGAA	Construction of <i>AnifU::aphA3</i> conditional mutant
$\Delta$ nifU-4	ACGGGGTTTTGGTTTTGTCC	Construction of <i>AnifU::aphA3</i> conditional mutant
$\Delta$ nfu-1	CAGGCGAAACGATACGGCCC	Construction of <i>Anfu::cat</i> mutant
$\Delta$ nfu-2	atccactttcaatctatcATAATGAGCCATTTTTATC	Construction of <i>Anfu::cat</i> mutant
$\Delta$ nfu-3	cccagtttgcgactgataaTAAGCTTTAAGGATTATAG	Construction of <i>Anfu::cat</i> mutant
$\Delta$ nfu-4	ATCACCTAGAGCTGAACATC	Construction of <i>Anfu::cat</i> mutant

NifS-Xba	agtactctagagTTACAACGAATTTATTTAG	NifS fusion for BACTH
NifS-Kpn	agcacggtaccgaATAAGAGCTTGAAATATTTTC	NifS fusion for BACTH
NifU-Xba	agtactctagagGCAAAACATGATTTAGTG	NifU fusion for BACTH
NifU-Kpn	agcacggtaccgaAATCGGTAACACCCTG	NifU fusion for BACTH
Nfu-Xba	agtactctagagATAGAATTTAGCGATGAAG	Nfu fusion for BACTH
Nfu-Kpn	agcacggtaccgaAAGCTTATCAAACCTCTTAG	Nfu fusion for BACTH
HP0117-PstI	agtactctgagGCTAAAGAAAATCCGCC	HP0117 fusion for BACTH
HP0117-KpnI	agcacggtaccgaAAACGCGCAATAAAACTCC	HP0117 fusion for BACTH
HP0132-Xba	agtactctagagGCTAGTTTTTCTATTTTATC	HP0132 fusion for BACTH
HP0132-Kpn	agcacggtaccgaGCATTTTAAGGTTTTGGC	HP0132 fusion for BACTH
HP0138-Xba	agtactctagagGAAAAATACCATAGCGACC	HP0138 fusion for BACTH
HP0138-Kpn	agcacggtaccgaCTCATAAATCACCCCTTCTAAG	HP0138 fusion for BACTH
HP0142-Xba	agtactctagagGAAACTTTACACAACGCC	HP0142 fusion for BACTH
HP0142-Kpn	agcacggtaccgaACCCCAAATAAATTTTTTTG	HP0142 fusion for BACTH
Hp0191-Xba	agtactctagagAGTGATAATGAACGAACG	HP0191 fusion for BACTH
Hp0191-Kpn	agcacggtaccgaGCGGCTTTTACCCACTTTC	HP0191 fusion for BACTH
Hp0207-Xba	agtactctagagCTCACCCAAGAAGATGTC	HP0207 fusion for BACTH
Hp0207-Kpn	agcacggtaccgaATGCGAGCAAGCATGGGT	HP0207 fusion for BACTH
Hp0269-Xba	agtactctagagAAAGTTTATATTGAAACCATG	HP0269 fusion for BACTH
Hp0269-Kpn	agcacggtaccgaGTTGCCTTTAGTGGCCGCAT	HP0269 fusion for BACTH
Hp0277-Xba	agtactctagagTCATTATTGGTGAATGATG	HP0277 fusion for BACTH
Hp0277-Kpn	agcacggtaccgaATCTTGCTCTTTTAAGC	HP0277 fusion for BACTH
HP0285-Xba	agtactctagagAAAAAAGTCTATTTCAAAC	HP0285 fusion for BACTH
HP0285-Kpn	agcacggtaccgaGAAAACGGCATGATTTTCCC	HP0285 fusion for BACTH
HP0400-Xba	agtactctagagGAAATTAAAATGGCTAAGG	HP0400 fusion for BACTH
HP0400-Kpn	agcacggtaccgaAATCGTGCTGATTTTTTGC	HP0400 fusion for BACTH
HP0468-Xba	agtactctagagAGCGTTTTGAAATTGCATG	HP0468 fusion for BACTH
HP0468-Kpn	agcacggtaccgaAATGAAAGTAACCTTATG	HP0468 fusion for BACTH
HP0568-Xba	agtactctagagCCTTTGGAATTATTTGAAAAAG	HP0568 fusion for BACTH
HP0568-Kpn	agcacggtaccgaTTTTTTATAACGAATGAGAG	HP0568 fusion for BACTH
Hp0585-Xba	agtactctagagAAAATGGGTTTAAAACG	HP0585 fusion for BACTH
Hp0585-Kpn	agcacggtaccgaCGCTTTAAAGCTAGCTTTAG	HP0585 fusion for BACTH
HP0588-Xba	agtactctagagGCTAAAATGAGCGCTCCAG	HP0588 fusion for BACTH
HP0588-Kpn	agcacggtaccgaTTTGTCTCTCCCTTCTAAA	HP0588 fusion for BACTH
HP0625-Xba	agtactctagagCTAGAAAATAGAGTTAAGAC	HP0625 fusion for BACTH
HP0625-Kpn	agcacggtaccgaATCCTTTAAACTTTTTTCTC	HP0625 fusion for BACTH
Hp0631-Xba	agtactctagagTTCTACGATGAAAAAAG	HP0631 fusion for BACTH
Hp0631-Kpn	agcacggtaccgaCTCTTTGTTTTTGATCG	HP0631 fusion for BACTH
HP0654-Xba	agtactctagagGACTTTTTAGAAAAAGTATT	HP0654 fusion for BACTH
HP0654-Kpn	agcacggtaccGAAAATTTCTGTATAAAATTAT	HP0654 fusion for BACTH
HP0656-Xba	agtactctagagGCAAGAAATGTAAAGGGCTA	HP0656 fusion for BACTH
HP0656-Kpn	agcacggtaccgaTACCTTTGCTTTAGCCGGAT	HP0656 fusion for BACTH

HP0665-Xba	agtact <b>tctagag</b> CAAACCATTGATTTTAAAA	HP0665 fusion for BACTH
HP0665-kpn	agcac <b>ggtaccga</b> TAGCGTTTTACTGAAATGTT	HP0665 fusion for BACTH
HP0666-Xba	agtact <b>tctagag</b> AATGAAAATATTAATGAAAA	HP0666 fusion for BACTH
HP0666-Kpn	agcac <b>ggtaccga</b> TTCAGCGCTTTTAAAGGC	HP0666 fusion for BACTH
HP0734-Xba	agtact <b>tctagag</b> CAAGTTAAAGAAAACAAAC	HP0734 fusion for BACTH
HP0734-Kpn	agcac <b>ggtaccga</b> AAAAGGGCTTAAACCTTAGC	HP0734 fusion for BACTH
HP0768-Xba	agtact <b>tctagag</b> TTAGTAGATAGTTTTAATAG	HP0768 fusion for BACTH
HP0768-Kpn	agcac <b>ggtaccga</b> CCCCCTGTGTAGTAAACG	HP0768 fusion for BACTH
HP0779-Xba	agtact <b>tctagag</b> ATGAAAGATTTTTTAGAAG	HP0779 fusion for BACTH
HP0779-Kpn	agcac <b>ggtaccga</b> GAGCCTGAAATCTCCATTAAG	HP0779 fusion for BACTH
HP0934-Xba	agtact <b>tctagag</b> AAACTCCCGGTCGTTGAGAG	HP0934 fusion for BACTH
HP0934-Kpn	agcac <b>ggtaccga</b> AAACCCTTTTGATTATCCC	HP0934 fusion for BACTH
HP1089-Xba	agtact <b>tctagag</b> AACTTAGAAAACTTTTTTTAG	HP1089 fusion for BACTH
HP1089-Kpn	agcac <b>ggtaccga</b> TCGGTTGCACATGTCTTTAA	HP1089 fusion for BACTH
HP1109-Xba	agtact <b>tctagag</b> AAAGATTGGAACGAATTTG	HP1109 fusion for BACTH
HP1109-Kpn	agcac <b>ggtaccga</b> TGATTTTTTCTTTTCTTG	HP1109 fusion for BACTH
HP1222-Xba	agtact <b>tctagag</b> CGTGTGGAAGAAAATTATC	HP1222 fusion for BACTH
HP1222-Kpn	agcac <b>ggtaccga</b> AAGCGTGCAAGCATCCACTA	HP1222 fusion for BACTH
HP1226-Xba	agtact <b>tctagag</b> AAAATGAGAGAAATAAATATG	HP1226 fusion for BACTH
HP1226-Kpn	agcac <b>ggtaccga</b> TAACAGCCACAAAGCC	HP1226 fusion for BACTH
HP1261-Xba	agtact <b>tctagag</b> CAACAAGCACCGGTTGTTC	HP1261 fusion for BACTH
HP1261-Kpn	agcac <b>ggtaccga</b> CACTAACCTTTGGGAGCGTC	HP1261 fusion for BACTH
HP1266-Xba	agtact <b>tctagag</b> ATCACAATGAATATCAATGG	HP1266 fusion for BACTH
HP1266-Kpn	agcac <b>ggtaccga</b> TGCTTGCTCCTTTAAATCCAC	HP1266 fusion for BACTH
HP1268-Xba	agtact <b>tctagag</b> GCCAAACAAGAATACAAGC	HP1268 fusion for BACTH
HP1268-Kpn	agcac <b>ggtaccga</b> AACATTTTCATCTCCCTTATTG	HP1268 fusion for BACTH
HP1356-Xba	agtact <b>tctagag</b> CCAAGTATAACGATTTAAAAG	HP1356 fusion for BACTH
HP1356-Kpn	agcac <b>ggtaccga</b> AGATAACTCCATCATTTTAG	HP1356 fusion for BACTH
HP1406-Xba	agtact <b>tctagag</b> CAAGAGATTTTTTTATGTTC	HP1406 fusion for BACTH
HP1406-Kpn	agcac <b>ggtaccga</b> ATGACAGCTTGTCGCCATTGTC	HP1406 fusion for BACTH
HP1428-Xba	agtact <b>tctagag</b> AAAGCTAGTATTTATGATTTTC	HP1428 fusion for BACTH
HP1428-Kpn	agcac <b>ggtaccga</b> AATTTGCTGAGAGAGTTTTTTC	HP1428 fusion for BACTH
HP1508-Xba	agtact <b>tctagag</b> CTTGAAACTTCTAGCCATTT	HP1508 fusion for BACTH
HP1508-Kpn	agcac <b>ggtaccga</b> ATCCTCACTTGGTGCAATAAAC	HP1508 fusion for BACTH
HP1540-Xba	agtact <b>tctagag</b> GCAGATATCAAAGGCGTG	HP1540 fusion for BACTH
HP1540-Kpn	agcac <b>ggtaccga</b> CGCTTTAGCCATCATTTTC	HP1540 fusion for BACTH

\* Upper case letters indicate *H. pylori*-derived sequences; bold letters indicate newly generated restriction sites; *aphA3*-or *cat*-specific sequences are underlined. All primers were purchased from Integrated DNA Technology (IDT), Coralville, IA

**Table S3- Growth of co-transformed *E. coli* BTH101 cells in M63-maltose minimal medium: additional results.**

FUSION PROTEIN 1 (in pUT18C or pUT18)		Growth (OD <sub>595</sub> ) in M63 after 72h at 30°C (Mean ± SD, n=3-5)		
		FUSION PROTEIN 2 (in pKT25 or pKNT25)		
Fusion protein 1	<i>H. pylori</i> Protein ID	T25 only (control)	T25-HP0779 (AcnB)	HP0779-T25 (AcnB)
T18 only	None (neg. cont.)	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
T18-HP0220	NifS	0.03 ± 0.02	0.25 ± 0.01	0.15 ± 0.03
T18-HP0221	NifU	0.02 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
T18-HP1492	Nfu	0.02 ± 0.01	0.09 ± 0.03	0.04 ± 0.01
HP1492-T18	Nfu	0.05 ± 0.01	0.14 ± 0.04	0.04 ± 0.01

FUSION PROTEIN 1 (in pUT18)		FUSION PROTEIN 2 (in pKT25 or pKNT25)			
Fusion protein 1	<i>H. pylori</i> Protein ID	T25-HP0220 (NifS)	T25-HP0221 (NifU)	HP0221-T25 (NifU)	T25-HP1492 (NfU)
HP1492-T18	Nfu	0.06 ± 0.01	0.15 ± 0.07	0.07 ± 0.01	0.20 ± 0.05
T18-Zip	T25-Zip	Interaction & Color key	Not detectable	Moderate	
Positive control	0.31 ± 0.05		Weak	Strong	