Transcriptional control of the lateral-flagellar genes of *Bradyrhizobium diazoefficiens*

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Supplemental Material

Table S1. Strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics	Source
B. diazoefficiens		
USDA 110	Wild-type, Cm^r	ARS-USDA, Beltsville (MD)
LP 3004	USDA 110 spontaneous derivative, Cm^r, Sm^r	(1)
LP 3008	LP 3004 spontaneous derivative, higher motility, Cm^r, Sm^r	(2)
lafR::Km	USDA 110 derivative, *lafR* mutant by Km insertion	This study
Δ*flbT*$_L$	USDA 110 derivative, *flbT*$_L$ deletion mutant	This study
lafR ::Km pFAJ	*lafR*::Km mutant carrying the pFAJ1708 empty vector	This study
lafR ::Km pFAJ::*lafR*	*lafR*::Km mutant carrying the pFAJ::*lafR* plasmid	This study
lafR ::Km pFAJ::*flbT*$_L$	*lafR*::Km mutant carrying the pFAJ::*flbT*$_L$ plasmid	This study
*lafR*D50A	USDA 110 with the D50A point mutation in *lafR*	This study
*lafR*D50G	USDA 110 with the D50G point mutation in *lafR*	This study
*lafR*D50E	USDA 110 with the D50E point mutation in *lafR*	This study
Δ*flbT*$_L$ pFAJ	Δ*flbT*$_L$ mutant carrying the pFAJ1708 empty vector	This study
Δ*flbT*$_L$ pFAJ::*flbT*$_L$	Δ*flbT*$_L$ mutant carrying the pFAJ::*flbT*$_L$ plasmid	This study
USDA 110 pFAJ	USDA 110 carrying the pFAJ1708 vector	This study
USDA 110 pFAJ::*lafR*	USDA 110 carrying the pFAJ::*lafR* plasmid	This study
USDA 110 pFAJ::*flbT*$_L$	USDA 110 carrying the pFAJ::*flbT*$_L$ plasmid	This study
USDA 110 pCB::P*lafA1*	USDA 110 carrying the pCB::P*lafA1* plasmid	This study
USDA 110 pCB::P*lafA2*	USDA 110 carrying the pCB::P*lafA2* plasmid	This study
E. meliloti		
Rm2011	Wild-type, Sm^r	(3)
Rm2011mTn5STM.1.08.H02	Rm2011 *rem*::mTn*5* insertion	(4)
E. coli		
DH5α	F– *endA1 supE44 thi-1l-recA1 gyrA96 relA1 deoRD*(*lacZYA-argF*)U169	Bethesda Research Laboratory
S17-1	*E. coli* 294 Thi RP4-2-Tc::Mu-Km::Tn7 integrated into the chromosome	Bethesda Research Laboratory
Plasmids		
pG18mob2	*lacZ*α Gm^r	(5)
pK18mob*sacB*	*lacZ*α Km^r *sacB*	(6)
pFAJ1708	broad-host-range expression vector containing p*nptII* promoter, Tc^r	(7)
pUC4K	Plasmid with *nptI* gene (source for Km-resistance cassette) Ap^r, Km^r	(8)
pCB303	Promoterless β-galactosidase vector, replicative in *Bradyrhizobium*	(9)
pG::*lafR*	pG18*mob2* carrying the internal fragment of the *lafR* gene	This study
pG::*lafR*::Km	pG::*lafR* carrying the Km cassette in the middle of the *lafR* gene	This study
pG::*lafR*D50A	pG::*lafR* carrying the point mutation D50A	This study
pG::*lafR*D50G	pG::*lafR* carrying the point mutation D50G	This study
pG::*lafR*D50E	pG::*lafR* carrying the point mutation D50E	This study
pK*sacB*::*lafR*D50A	pK18mob*sacB* vector carrying the fragment subcloned from pG::*lafR*:D50A	This study
pK*sacB*::*lafR*D50G	pK18mob*sacB* vector carrying the fragment subcloned from pG::*lafR*:D50G	This study
pK*sacB*::*lafR*D50E	pK18mob*sacB* vector carrying the fragment subcloned from pG::*lafR*:D50E	This study
pK*sacB*::*flbT*$_L$	pK18mob*sacB* carrying the *flbT*$_L$ deletion construct	This study
pFAJ::*lafR*	pFAJ1708 carrying the complete coding sequence of *B. diazoefficiens lafR*	This study
pFAJ::*flbT*$_L$	pFAJ1708 carrying the complete coding sequence of *B. diazoefficiens flbT*$_L$	This study
pCB::P*lafA1*	pCB303 carrying the 657-bp fragment corresponding to the putative promoter region of *lafA1*	This study
pCB::P*lafA2*	pCB303 carrying the 591-bp fragment corresponding to the putative promoter region of *lafA2*	This study

Table S2. Primers used in this study.

Primer name (ref.)	Sequence	Use in the study
LafR_Fw	CGAAACAGGGGCGAAAGAC	Construction of the *lafR* insertional mutant
LafR_Rv	AAAAAGCTTGGTTGAGGAGGATGCCGAC	
LafRextFw	AAAATCTAGACACGCAGTAAGCGTTCAGT	Check the position of the crossing-over event in the *lafR* mutant and construction of pFAJ::*lafR*
LafRextRv	AAAAGGTACCGAGGCCGTTGTCGTTTTTCG	
6846D50A_Fw	TCGCCGCCTTCCTCCTC	Amplification of the *lafR* fragment with the desired point mutation
6846D50A_Rv	GAGGAGGAAGGCGGCGA	
6846D50G_Fw	TCGGCGCCTTCCTCCTC	
6846D50G_Rv	GAGGAGGAAGGCGCCGA	
6846D50E_Fw	TCGAAGCCTTCCTCCTC	
6846D50E_Rv	GAGGAGGAAGGCTTCGA	
FlbTUP_Fw	AAAAGAATTCCGACGGTCTCGGGCGTGCTT	Construction of the $flbT_L$ deletion mutant
FlbTUP_Rv	GCCGTCGACGGATCCGAGGCACTCGGCATCGTGACCACCG	
FlbTDW_Fw	TGCCTCGGATCCGTCGACGGCCATTGCCGGCCCCTATCCGAC	
FlbTDW_Rv	AAAAAAGCTTTTGCGCTGTTCGGCTCGCTG	
FlbTextFw	AAAATTCTAGAATCATCTCGATCGGCTTGTG	Construction of pFAJ::$flbT_L$
FlbTextRv	AAAAGGTACCCTGGTCGAACTGGACGACTT	
promA1_Fw	AATCTAGAGTCATGGCGGTCGAGTTC	Construction of pCB::P*lafA1*
promA1_Rv	AAACTGCAGATCCGTTCCGCGAGTTCATG	
promA2_Fw	AATCTAGATTGCAGACGAGTTGGTGAGG	Construction of pCB:: P*lafA2*
promA2_Rv	AAACTGCAGGGTTACATCGCGCAGGTCA	
LafR_Fw	CGAAACAGGGGCGAAAGAC	qRT-PCR studies
q6846int_Rv	GGTCCGATTCACTCGCAGA	
q6848_Fw	GCAACCGTCTCCTCCTCG	
q6848_Rv	TGACGGTTGAAGCGGCAT	
q6854_Fw	AATGGGATGTCGGTGGCG	
q6854_Rv	CCGTTACTTCGAGGCGCT	
q6861_Fw	AGGCCGCCGTCATATTCG	
q6861_Rv	ATGGTGCGTGGACGGATG	
q6864-2_Fw	CGAGACCCAGATACGGCG	
q6864-2_Rv	CGACAAGGTGACGGTGCT	
LafA2_Fw (10)	CCTCACCAACTCGTCTGCAA	
LafA2_Rv (10)	CCGTGTTCAGAGCGGTGTATT	
LafA1_Fw (10)	GGTTACATCGCGCAGGTCA	
LafA2_Rv (10)	GGGTGGACTCCTGGTTCATGT	
q6867_Fw	GAACAGCATCTTGAACGGCA	
q6867_Rv	CCGTTCCTCGTGATCGACAT	
q6868_Fw	CGGCGAGATTGGTCACGA	
q6868_Rv	CATCTGTTTGCAGCCGCC	
q6878_Fw	GTCCTCGTGCAGCGTCTT	
q6878_Rv	ATGAGCCAGTTGCCGTCC	

q6881_Fw	TCAGCAGCACCGCATCAA	
q6881_Rv	TGGCTCGTCTCGAAAGCA	
phaR_Fw	GGTCAAGGATGGCGAAGA	
phaR_Rv	GATCTGCTTGCGGAACTT	
relA_Fw	TACAATCCCAACACCAACGA	
relA_Rv	CAATCGCCAGCAACAATTT	
6849_Fw	ATCGGCGGCGGATGATTT	
6849ext_Rv	CCAGGTTCGTGCGGAGAG	
int6849.5FW	CTCTCCGCACGAACCTGG	
int6849.5RV	GTCGGATGGTCTCGCTCG	
6850ext_Fw	CGAGCGAGACCATCCGAC	
6850_Rv	ATCGCCAGCCCGTTCATC	
q6858Fw2	ATTGCCCGAACCGCTCTT	
q6858Rv2	TCGCGAACGTCAACACCA	
q6859Fw	TCCCTCGCGCTGTTTCAG	
q6859Rv	AGTTCACTTCGGTGCGGG	
int58-59_Fw	TTGTTGGATTGCGCGGACA	
int58-59_Rv	GCCGAAAACGACCTGACAAC	
6860_Fw	CGACTGTGACGAGGGCTG	
6860_Rv	GACTCGATCCGCGAGCTG	
int60-61_Fw	ACGTCCCGCGCATATTGA	
int60-61_Rv	GCTCGGCGAACTCGAACT	
fliCIFw (10)	CCTCACCAACTCGTCTGCAA	RT-PCR to determine the operon structure of the regulon
fliCIRv	CCGTGTTCAGAGCGGTGTATT	
fliCIIFw (10)	GGTTACATCGCGCAGGTCA	
fliCIIRv	GGGTGGACTCCTGGTTCATGT	
int65-66 Fw	GACAGGTTGGGTCCTTCACA	
int65-66 Rv	GGCTTGTGGCTCTGTGACTC	
6876	GCATTCCGTCGAGCACCT	
6876	TCGACCATCGCCACCAAC	
6877	CCTGCTTGTGCTCGTCCT	
6877	CGTCTCGGCGATCTGCAT	
int76-77	GCTTGCGACGATGCGAGT	
int76-77	AGTCGAAGCAGACGACCC	
6879_Fw	CGTGAGCGAGACACCGAA	
6879_Rv	CCTGATGAAGCTCGGGCG	
q6881_Fw	TCAGCAGCACCGCATCAA	
q6881_Rv	TGGCTCGTCTCGAAAGCA	
int79-80_Fw	ACGTCTCGGTCTCTTCCG	
int79-80_Rv	AAGATCGACCAGGCCCAC	

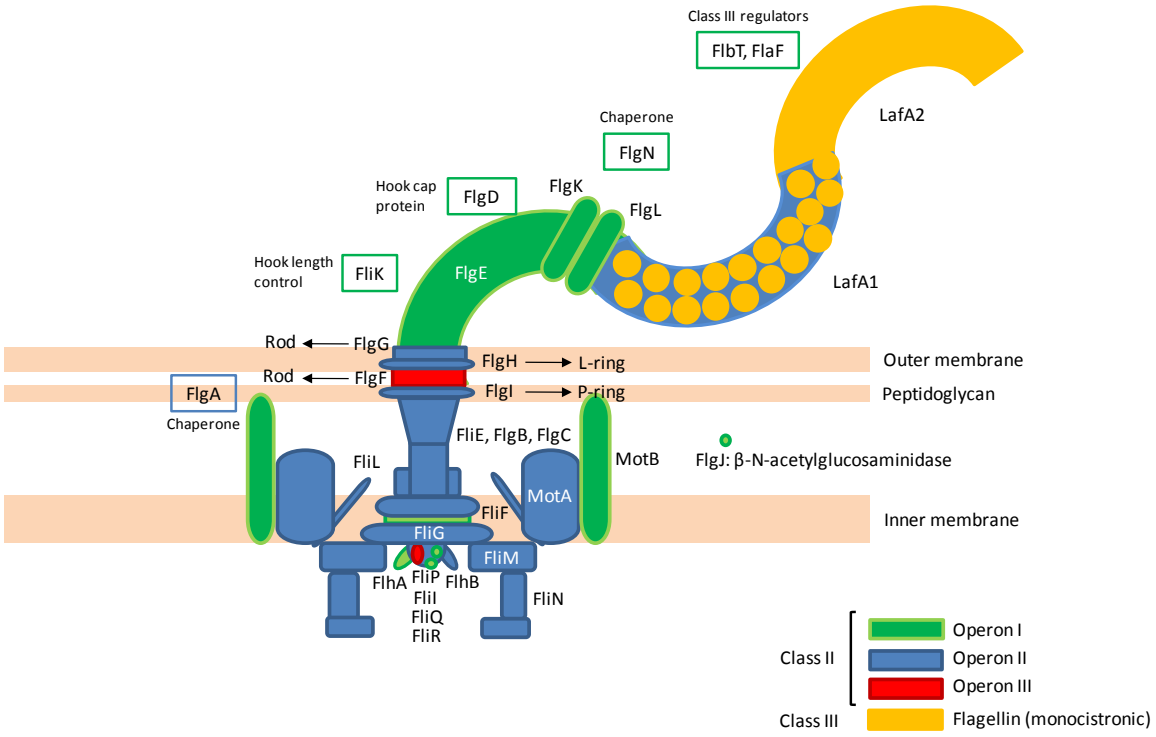

Fig. S1. General structure of the lateral flagellum of *B. diazoefficiens* depicting the gene products that we could identify, with a color code indicating which operon encodes each one. For the flagellins, the *lafA1* gene seemed to be expressed as part of Operon II, but also as an independent monocistronic unit.

A

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OmpR      MQENYKILVVDDDMRLRALLERYLTEQGFQVRSVANAEQMDRLLTRESFHLMVLDLMLPG--EDGLSICRRLRSQS
LafR      -----MYIIVDDRESVTNSYVGGLVREGVSSIGFSSGEFWDWLQSASESDLAAVDAFLLGDCDARGSLPRAMRKRS
Rem       -----MIVVVDDRAILVKDGYASLFGREGIPSTGFDDPREFGEWVSSAADSDIDAVEAFLLGQGESTLTLPRAIRDRS
FtcR      -----MIVVVDDRDMVTEGYSSWFGREGITTTGFTPTDFDEWVESVPEQDIMAIEAFLLGECADQHRLPARIRERC

OmpR      NPMPIIMVTAKGEEVDRIVGLEIGADDYIPKPFNPRELLARIRAVLRRQANELPGAPSQEEAVIAFGKFKLNLGTR
LafR      -SAPIIAMSGQKMLKNTLELFESGVDDVVYVPIHLREILARTAAIARRRVGELP----RP----CETRIQVFFNGR
Rem       -RAPVIAMSDTPSLENTLALFDCGVDDVVRKPVHPREILARVAAIRRRLTAI----ANFT----DIGPIRVFADGR
FtcR      -KAPVIAVNDRPSLEHTLELFQSGVDDVVRKPVHVREILARINAIRRRAGASATSGADGT----QLGPIRVFSDGR

OmpR      EMFREDEPMPLTSGEFAVLKALVSHPREPLSRDKLMNLARGREYSAM-ERSIDVQISRLRRMVEEDPAHPRYIQTV
LafR      DPEIAGHALTLPRRELRILEYMVSNHGKWITKTQIFNAVYGIFESTFDESVIESHVSKLRKKLRDRLGFDA-IVAR
Rem       DPEINGEVFALPRRERRILEYLVANRGRRVSKSQIFNAIYGIFDEDVEENVVESHISKLRKKLRKKLGFDP-IDSK
FtcR      DPQINGIDFPLPRRERRILEYLIANRGRRLNKVQIFSAIYGIFDSEVEENVVESHISKLRKKLRGQLGFDP-IDSK

OmpR      WGLGYVFVPDGSKA----------------------------
LafR      RYVGYRLNIPAGETVDVPMQELDEVGILLNRAHIAPAAYPAGN
Rem       RFLGYCIDWN-------------------------------
FtcR      RFLGYCINIE-------------------------------
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B

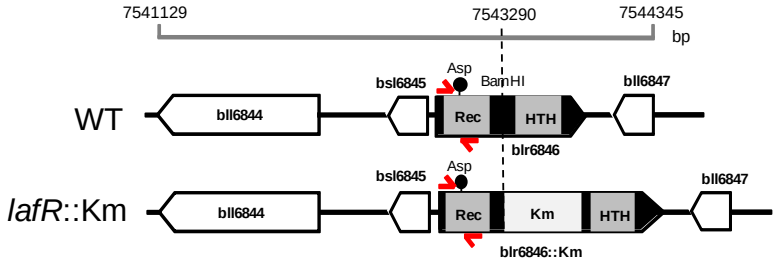

Fig. S2. *Structure of blr6846 (*lafR*)*

A. Deduced amino-acid sequence in comparison with the OmpR from *E. coli*, the Rem from *E. meliloti*, and the FtcR from *B. melitensis*. The conservative positions are shadowed in black. The position of the phosphorylable Asp50, which amino acid is present in OmpR but not in Rem and FtcR, is shown shadowed in red. Underlined are the receiver (upstream) and helix-turn-helix (downstream) domains.

B. Gene structure of the blr6846 wild-type (WT) and *lafR*::Km mutant. The positions of the receiver (Rec) and helix-turn-helix (HTH) domains (dark-gray shading), the *Bam*HI restriction site (vertical dashed line) between the two, and the Km-cassette insertion (light-gray shading in the mutant) are indicated. The arrows (red) mark the position of the primers used for RT-PCR. Note that both the wild-type and the mutant mRNAs can be amplified with these primers. The dot indicates the position of the phosphorylable Asp50.

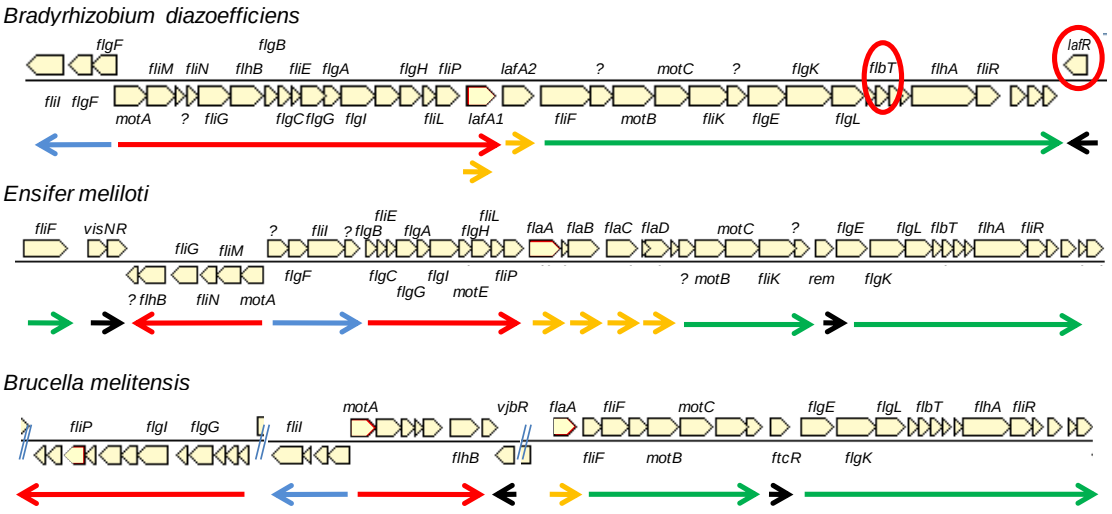

Fig. S3. Comparison of the flagellar-gene clusters of *B. diazoefficiens* (lateral flagellum), *E. meliloti*, and *B. melitensis*. The operon organizations are indicated (in colors) below each cluster. Encircled (in red) are *lafR* and *flbT*$_L$, which loci were studied in this work. Note that the orientation of the gene cluster of *B. diazoefficiens* has been rotated with respect to the orientation given in Rhizobase in order to visualize the synteny of the three clusters.

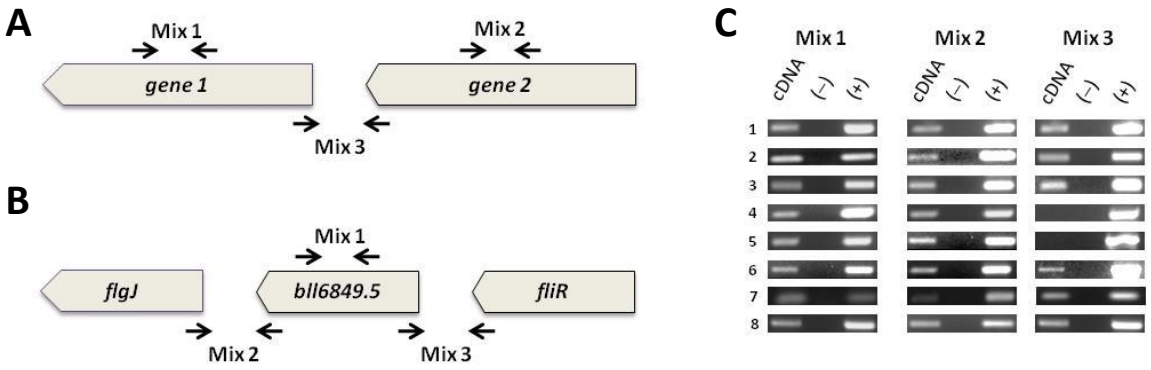

Fig. S4. *Strategy to find whether two contiguous genes are cotranscribed in the same polycistronic mRNA*.

A. Scheme of the general strategy where RT-PCR amplifications are done from internal gene sequences (mix 1 and 2) and intergenic sequences (mix 3).
B. The particular strategy followed with $flgJ_L$, bll6849.5, and $fliR_L$.
C. RT-PCR results obtained with the strategy outlined in panels **A** and **B** for the regions numbered 1-8 in Figure 2A (cDNA). Amplification from the genomic DNA was the positive control (+), and amplification without template was the negative control (–).

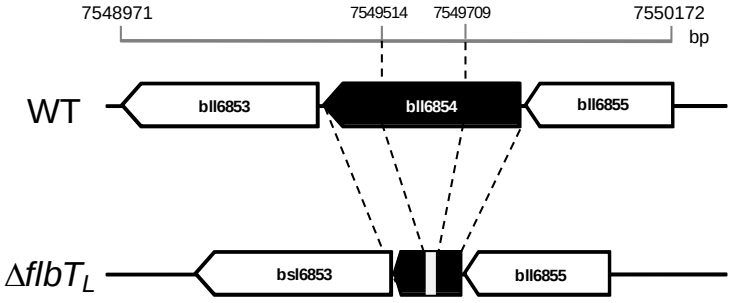

Fig. S5. Scheme of the deletion produced in bll6854 ($flbT_L$) without alteration of the reading frame

A

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LafA1  1    ACGGCGGGTTGGACCC--CACCCACAT-----CAGTCCAAAGGCATGATGCCGCCCCTCC  53
LafA2  1     CGACAGGTTGGGTCCTTCACACGCATTTCCGCGGCCCAAAGGCATGATGCCGCCCTGTC  59

LafA1  54   GTACCGGTGAAAGCCCGGTATGTCCCCTCGTGAAAATGTAACGCGTACAT-AAAGGGACA  112
LafA2  60   GTACCGGTGCAAGTCCGGTATGTCCCC-----AAACTC-AATTCAATCGTCAAAGGGACA  113

LafA1  113  TTCGCAATG---TCAAGCCTGCTTACGAACTCGACCGCCATGACCGCGCTCCAGACCCTG  169
LafA2  114  T-CAAAATGGGTTCTAGCCTCCTCACCAACTCGTCTGCAATGACCGCGCTGCAGACC    169
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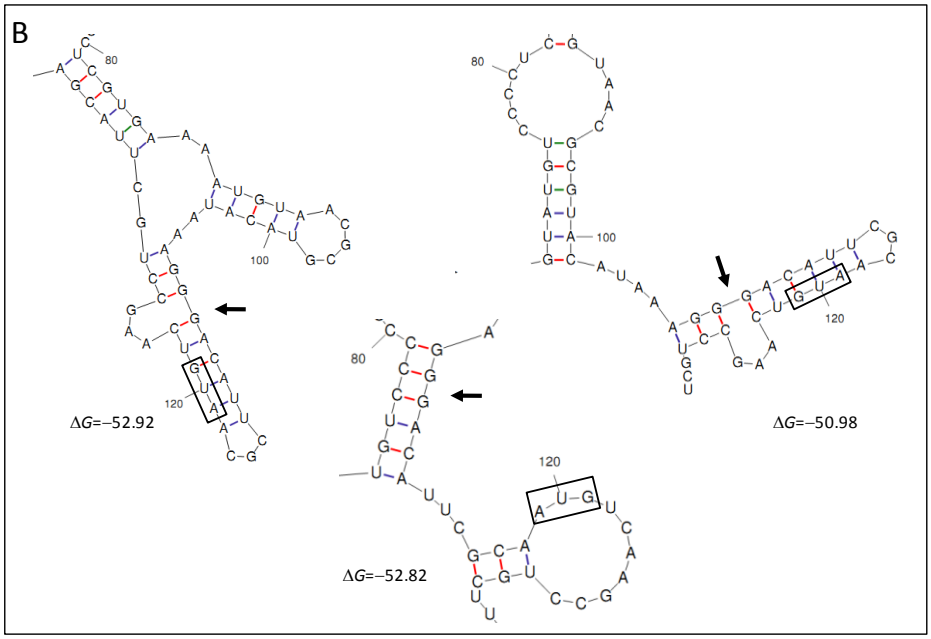


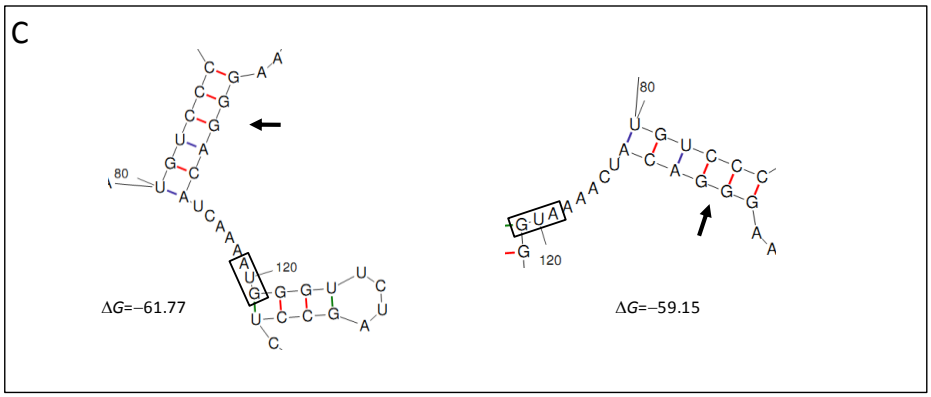
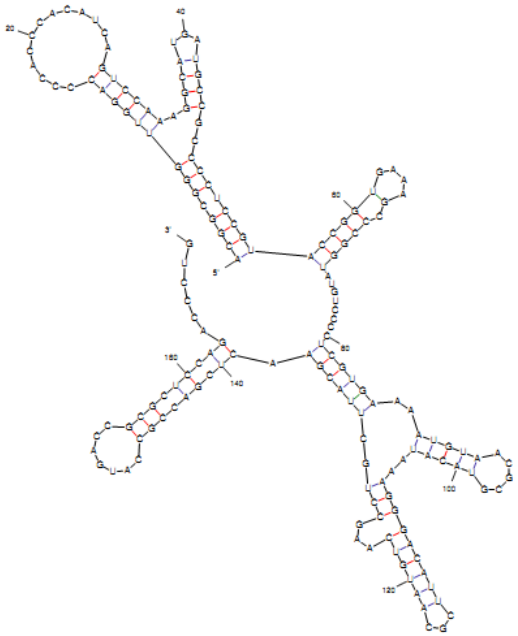
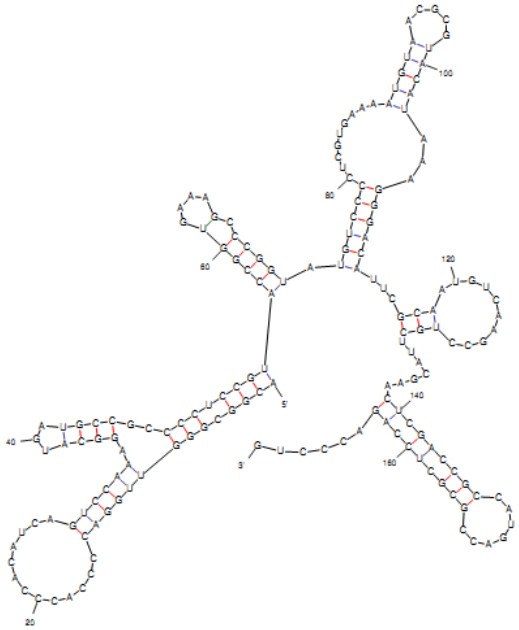


Fig. S6. Structure of the 5'UTRs of *lafA1* and *lafA2*.
A. BLAST nucleotide sequence alignment of the 5'UTR regions considering the transcription start site at 118 nt upstream of the ATG initiation codon (ref. 34 in the main article) and including the first 17 codons (ref. 39 in the main article). Squared is the gap found at the 3' end of a sequence complementary to the ribosome-binding site (RBS). Underlined are the RBS and the ATG initiation codon.
B, C. Predicted secondary structures of the region containing the RBS (arrow) and ATG (squared) sequences in three representative structures of *lafA1* (**B**) and two of *lafA2* (**C**).
D, E. Predicted stable secondary structures of the 5'UTRs of *lafA1* (**D**) and *lafA2* (**E**).

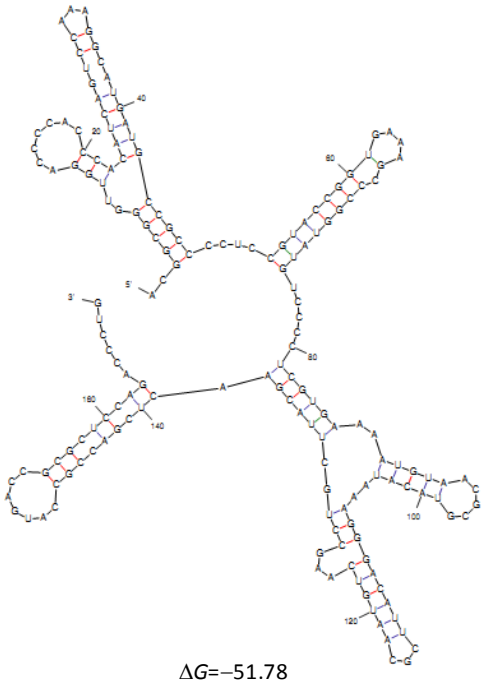
D



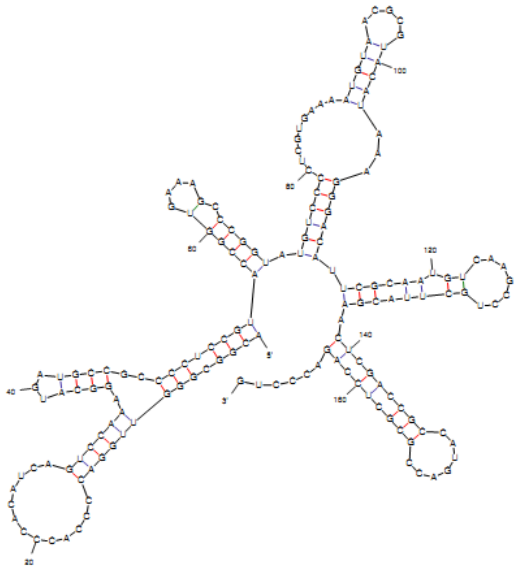
ΔG=−52.92



ΔG=−52.82



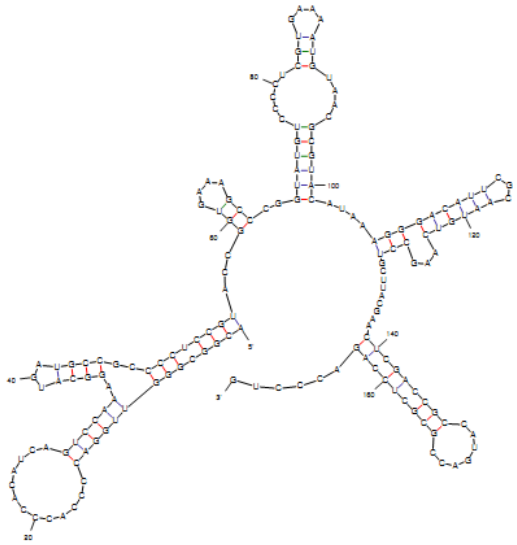
ΔG=−51.78



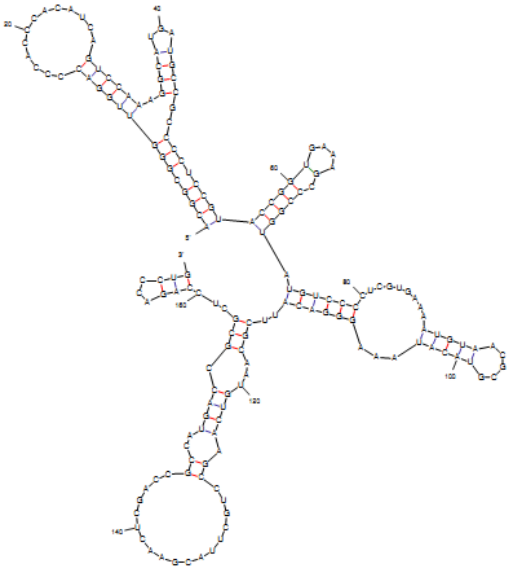
ΔG=−51.11

Fig. S6. (continued)

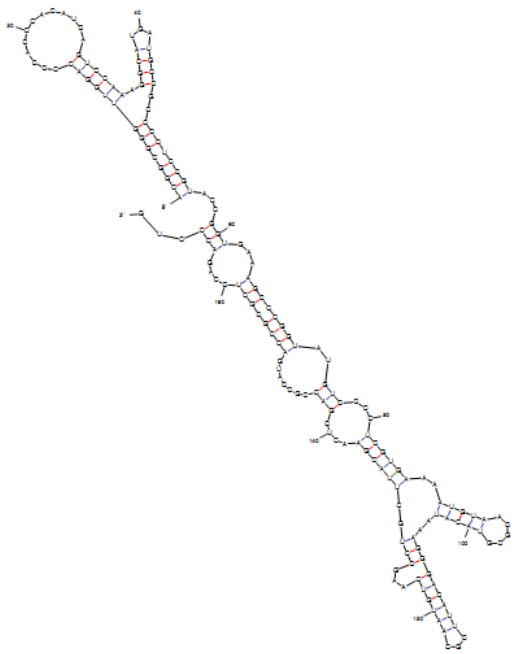
D



ΔG=−50.98



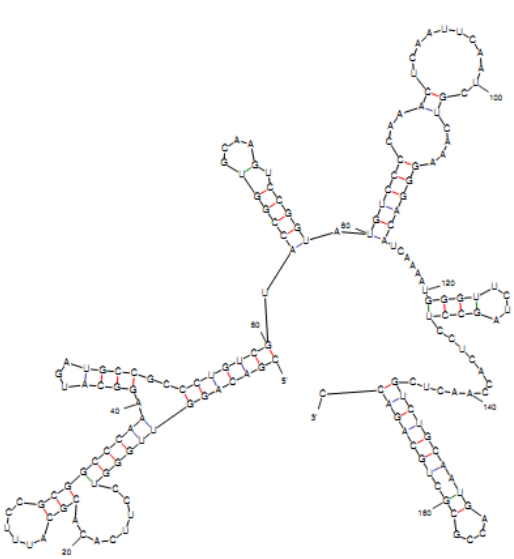
ΔG=−50.55

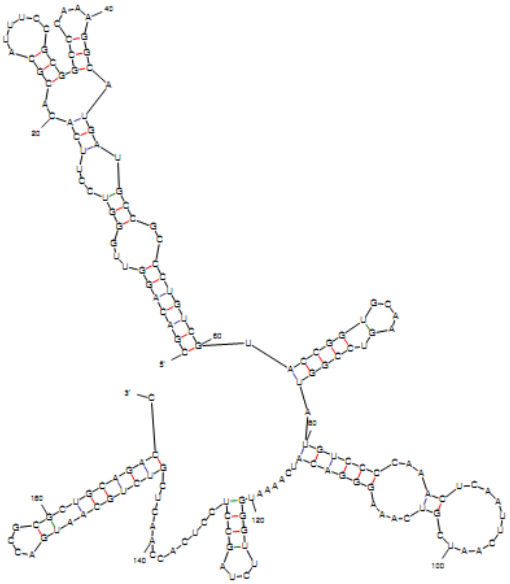


ΔG=−50.40

Fig. S6. (continued)

E





ΔG=–61.77

ΔG=–59.15

Fig. S6. (continued)

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