Degradation of the Phycobilisomes in *Synechocystis* sp. PCC6803: Evidence for an essential NblA1/NblA2 heterodimer formation

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TABLE S1. Strains, plasmids and oligonucleotides used in this study.

FIGURE S1. Plasmid construction. *A,* Constructs for the interaction analysis of $NblA1_{6803}$ and $NblA2_{6803}$ via FRET *in vivo*. The DNA sequences encoding $nblA1_{6803}$ and $nblA2_{6803}$ and its promoter and terminator regions, respectively, were amplified by PCR. The marked restriction sites were generated by mutagenesis and the encoding regions for *yfp* and *cer* were cloned in the restriction sites, respectively. *B,* Map of the *cpcA* region of plasmid pGEX/cpcA6803. Construct for the heterologous expression of the apoprotein of Phycocyanin (cpcA) with N-terminally tagged GST. *C,* Map of the $clpC_{6803}$ region of plasmid pET11/ClpC. Construct for the heterologous expression of $ClpC_{6803}$. *D,* Map of the $nblA1_{6803}$ region of plasmid pACYC/A1_GST. Construct for the heterologous expression of $NblA1_{6803}$ with C-terminally tagged GST. *E,* Map of the *nblA2* region of plasmid pACYC/A2_GST.Construct for the heterologous expression of NblA2 with C-terminally tagged GST. *F,* Map of the $nblA1_{6803}$ and $nblA2_{6803}$ region of plasmid pACYC/His_A1, A2_GST. Construct for the heterologous co-expression of $NblA1_{6803}$ with N-terminally tagged His and NblA2 with C-terminally tagged GST. *G,* Map of the $nblA1_{6803}$ and $nblA2_{6803}$ region of plasmid pACYC/His_A2, A1_GST. Construct for the heterologous co-expression of NblA2 with N-terminally tagged His and $NblA1_{6803}$ with C-terminally tagged GST. *H,* Map of the $nblA1_{6803}$ and $nblA2_{6803}$ region of plasmid pACYC/A2, A1_GST. Construct for the heterologous co-expression of $NblA2_{6803}$ and $NblA1_{6803}$ with C-terminally tagged GST. *I,* Map of the *clpP1* and *clpR* region of plasmid pACYC/ClpP1_His, ClpR. Construct for the heterologous co-expression of ClpP1 with C-terminally tagged His and ClpR. *J,* Construct for complementation analysis of the non-bleaching phenotype of the Synechocystis 6803 Δ*nblA1*/*nblA2* double mutant. The DNA sequences encoding $nblA1_{6803}$ and $nblA2_{6803}$ and its promoter and terminator regions, respectively, were amplified by PCR. *K,* Construct for complementation analysis of the non-bleaching phenotype of the *Synechocystis* 6803 Δ*nblA1*/*nblA2* double mutant. The DNA sequences encoding *nblA* of *Nostoc* 7120 and the promoter and terminator regions of $nblA1_{6803}$ and $nblA2_{6803}$, respectively, were amplified by PCR.

TABLE S1.

Strain, plasmid or oligonucleotides	Description	Source or reference
Synechocystis strains		
Synechocystis sp. PCC6803	wild-type	This reference
Synechocystis 6803 Δ*nblA2* *Synechocystis* 6803 Δ*nblA1/nblA2* mutant		This reference
Plasmids		
pGEX-2TK	Expression vector carrying *gst*	GE Healthcare, Waukesha, WI
pGEX/cpcA6803	Expression vector carrying *gst-cpcA*	This reference
pGEX-2TK/NdeI	Expression vector carrying additional *Nde*I site	Karradt *et al*., 2008
pACYCDuet-1	Vector for coexpression of two genes, containing two multiple cloning sites (MCS)	Novagen, Darmstadt
pACYC/GST	Vector for coexpression of two genes, carrying additional *gst* gene (MCS2)	
pACYC/A1_GST	Vector for expression of *nblA1-gst* (MCS2)	This reference
pACYC/A2_GST	Vector for expression of *nblA2-gst* (MCS2)	This reference
pACYC/His_A2, A1_GST	Vector for coexpression of *his-nblA2* (MCS1) and *nblA1-gst* (MCS2)	This reference
pACYC/His_A1, A2_GST	Vector for coexpression of *his-nblA1* (MCS1) and *nblA2-gst* (MCS2)	This reference
pACYC/A2, A1_GST	Vector for coexpression of *nblA2* (MCS1) and *nblA1-gst* (MCS2)	This reference
pACYC/ClpP1-His	Vector for expression of *clpP1-his* (MCS1)	
pACYC/ClpP1-His, ClpR	Vector for coexpression of *clpP1-his* (MCS1) and *clpR* (MCS2)	This reference
pEmYFP-N1	Vector containing a monomeric variant of YFP [Zacharias *et al*., 2002]	provided by Silvia Scolari [Scolari *et al*., 2009; Engel *et al*, 2010]
pEmCFP-N1	Vector containing a monomeric variant of CFP [Zacharias *et al*., 2002]	provided by Silvia Scolari [Scolari *et al*., 2009]
pEmCer-N1	Vector containing a monomeric variant of pCerulean [Zacharias *et al*., 2002; Rizzo *et al*., 2004]	provided by Silvia Scolari [Dissertation]
pET11a	Expression vector	Novagen, Darmstadt
pET11/ClpC	Vector for expression of *clpC*	This reference
pET15b	Expression vector	Novagen, Darmstadt
pET15b/P6803NblA7942	Cloning vector carrying P_{6803}-$nblA_{7942}$	This reference

pET22b	Expression vector	Novagen, Darmstadt
pET22b/His-ClpC	Expression vector carrying *his-clpC*	This reference
pET22/A1_YFP, A2	Cloning vector carrying P_{6803}-*nblA1yfp-nblA2*-T_{6803}	This reference
pET22/A1_YFP, A2_Cer	Cloning vector carrying P_{6803}-*nblA1yfp-nblA2Cer*-T_{6803}	This reference
pET22/A1, A2_Cer	Cloning vector carrying P_{6803}-*nblA1-nblA2Cer*-T_{6803}	This reference
pET22/A2_YFP, A2_Cer	Cloning vector carrying P_{6803}-*nblA2yfp-nblA2Cer*-T_{6803}	This reference
pET22/YFP_A1, A2	Cloning vector carrying P_{6803}-yfp*nblA1-nblA2*-T_{6803}	This reference
pET22/ A1, Cer_A2	Cloning vector carrying P_{6803}-*nblA1*-cer*nblA2*-T_{6803}	This reference
pET22/YFP_A1, Cer_A2	Cloning vector carrying P_{6803}-yfp*nblA1*-cer*nblA2*-T_{6803}	This reference
pET22/P6803-NblA7120-T6803	Cloning vector carrying P_{6803}-*nblA*$_{7120}$-T_{6803}	This reference
pVZ321	Shuttle vector containing Cm^r and Km^r resistance cassette	Zinchenko *et al*., 1999
pVZ/A1_YFP, A2	Shuttle vector containing P_{6803}-*nblA1yfp-nblA2*-T_{6803}	This reference
pVZ/A1_YFP, A2_Cer	Shuttle vector containing P_{6803}-*nblA1yfp-nblA2Cer*-T_{6803}	This reference
pVZ/A1, A2_Cer	Shuttle vector containing P_{6803}-*nblA1-nblA2Cer*-T_{6803}	This reference
pVZ/A2_YFP, A2_Cer	Shuttle vector containing P_{6803}-*nblA2yfp-nblA2Cer*-T_{6803}	This reference
pVZ/YFP_A1, A2	Shuttle vector containing P_{6803}-yfp*nblA1-nblA2*-T_{6803}	This reference
pVZ/ A1, Cer_A2	Shuttle vector containing P_{6803}-*nblA1*-cer*nblA2*-T_{6803}	This reference
		This reference
pVZ/YFP_A1, Cer_A2	Shuttle vector containing P_{6803}-yfp*nblA1*-cer*nblA2*-T_{6803}	This reference
pVZ/P6803-NblA7120-T6803	Shuttle vector carrying P_{6803}-*nblA*$_{7120}$-T_{6803}	This reference

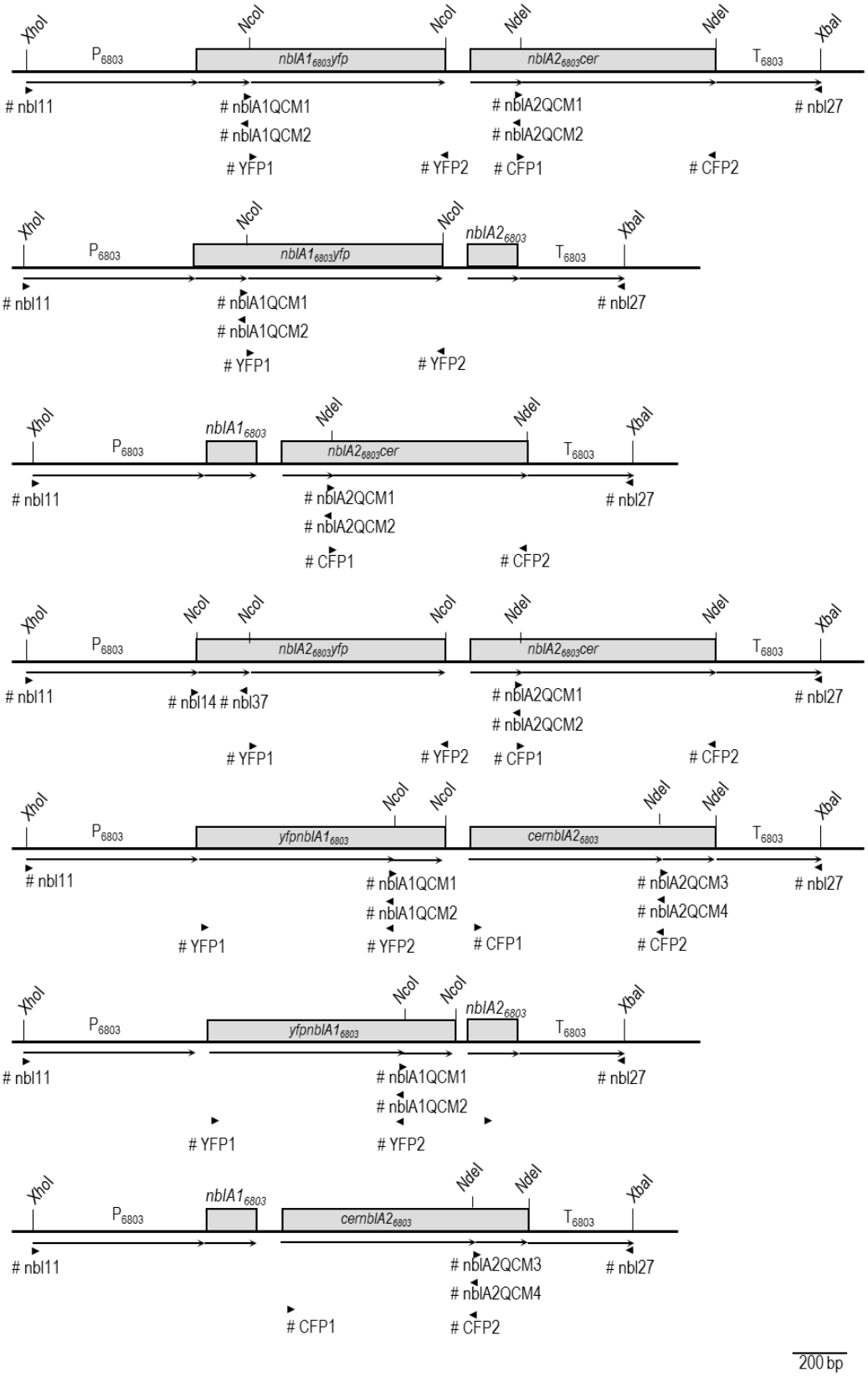
Oligonucleotides[a]	
# cpcA_1	GATACCGGATCCATGAAAACCCC
# cpcA_2	GAATTCAAAACTGACTAGCTCAG
# nbl7	GCAACACATATGAAACC
# nbl11	CCAAACAACATTGGGCTCGAGAGTTAG
# nbl13	CGAAGGATTCAGGTTCCATGGCTGTTG
# nbl14	GGAGTGCGATCGCCATGGTCAACAACG
# nbl21	GCCTAAACCTAGAATTCTGCTAG
# nbl24	GGAGTGCGATCCATATGATCAACAACG
# nbl26	CCTAGAGTTGTGCATATGGAGGAGTG
# nbl27	GCAACAACAAGAGTTTCTAGACCGGG
# nbl28	CCATATGGGGCCCCTGGAACAGAACTTCCAGACCTAGGGGCTCCAG
# nbl31	ACATATGGGGCCCCTGGAACACAACTTCCAGGGGGAGGAGTGAATTTTTC
# nbl32	CTTGGAGGAATTCAGCTATG
# nbl33	CTAAACCTAAGCTTGTGCTAG
# nbl34	ATTAGAATTCCGCCATGATCAACAACG
# nbl37	GAGTTGTGCTACCATGGGAGTGAAT
# nblA-4	GACAGCAAGCTTATTTTTTTGCC

# nblana2.23	GGAGTCTGCCATGGACCAACCAATC
# nblana2.24	GCGACACATATGAACTCTATGCCGG
# clpc_3	GGATTTAGCATATGTTTGAACGC
# clpc_5	GCTGGGGATCCTAATTATTCAAC
# CFP1	CGGTCGCCCATATGGTGAGCAAGGG
# CFP2	CCGCTTTACATATGCAGCTCGTCCATG
# YFP1	GGTCGCCACCATGGTGAGCAAG
# YFP2	CGCTTTCCATGGACAGCTCGTCCATG
# nblA1_QCM1	GCGCCAGGCTCCCCTGGAGCCCATGGGTTAAGCACAGGCAGGC
# nblA1_QCM2	GCCTGCCTGTGCTTAACCCATGGGCTCCAGGGGAGCCTGGCGC
# nblA1_QCM3	CGCCTTGGAGGGCAACAGCCATGGAACCTGAATCCTTCGATCTCA
# nblA1_QCM4	TGAGATCGAAGGATTCAGGTTCCATGGCTGTTGCCCTCCAAGGCG
# nblA2_QCM1	GATTTGATGAAAAATTCACTCCATATGTAGCACAACTCTAGGTTTAGG
# nblA2_QCM2	CCTAAACCTAGAGTTGTGCTACATATGGAGTGAATTTTTCATCAAATC
# nblA2_QCM3	CATTTTTTCAGGAGTGCGATCCATATGATCAACAACGAAGCCTTTAACC
# nblA2_QCM4	GGTTAAAGGCTTCGTTGTTGATCATATGGATCGCACTCCTGAAAAAATG
# clpR_1	CAACCTGACCATATGGAAATAAC
# clpR_2	GAATGGAAACCTCGAGAATCACTG
# clpP1_1	GTAACAACACCCCATGGCCATGATTCC
# clpP1_2	GAGCTAAATACACCATGGAAATAGGGTC

[a] Oligonucleotides read in 5´ to -3´ direction. Restriction sites in oligonucleotides are underlined.

FIGURE S1.

A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NcoI
NdeI
NdeI
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nblA1 6803yfp
nblA2 6803cer
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nblA1QCM1
nblA1QCM2
YFP1
YFP2
nblA2QCM1
nblA2QCM2
CFP1
CFP2
nbl27
nblA1 6803
nblA2 6803
nbl14 # nbl37
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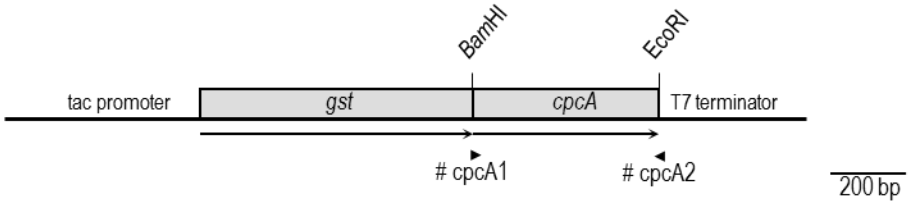
200 bp

B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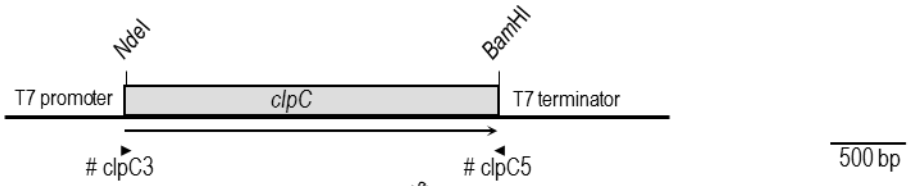
EcoRI
tac promoter
gst
cpcA
T7 terminator
cpcA1
cpcA2
200 bp

C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clpC5
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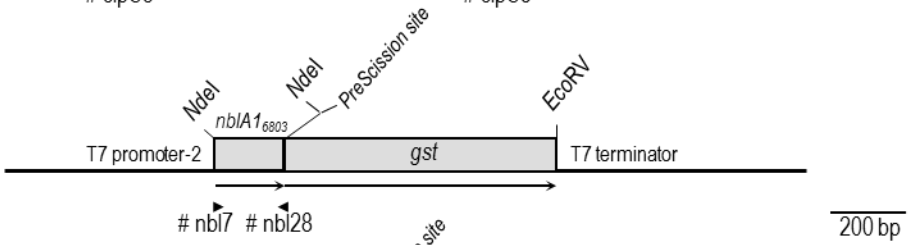
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gst
T7 terminator
nbl7
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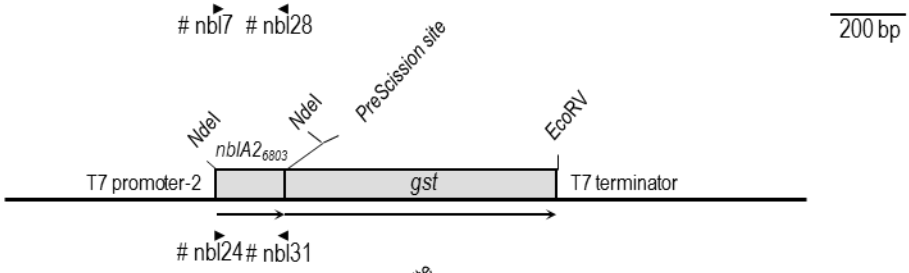
E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T7 promoter-2
gst
T7 terminator
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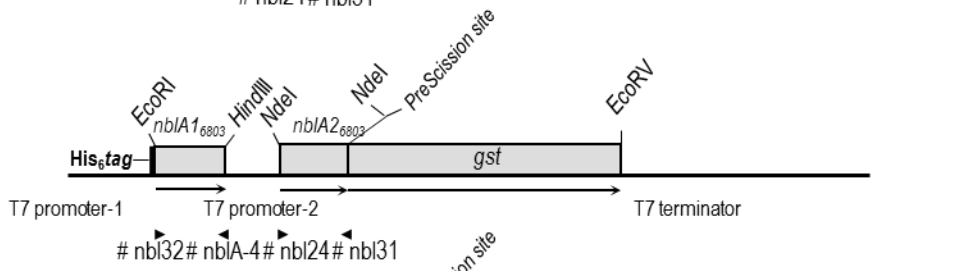
nbl31

F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HindIII
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NdeI
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gst
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T7 promoter-2
T7 terminator
nbl32
nblA-4
nbl24
nbl31

G


EcoRI
nblA2 6803
HindIII
NdeI
NdeI
nblA1 6803
PreScission site
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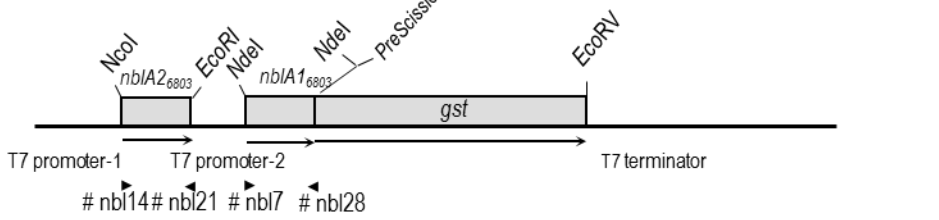
H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T7 terminator
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nbl7
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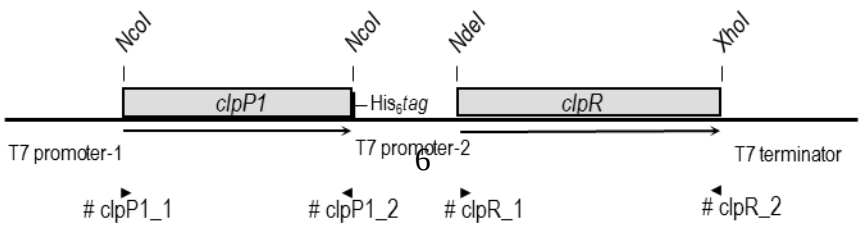
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T7 terminator
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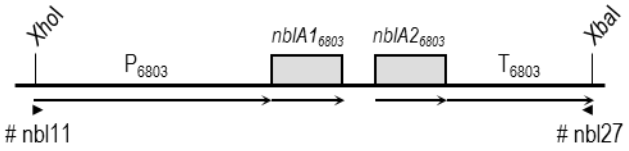
clpP1_2
clpR_1
clpR_2

J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P 6803
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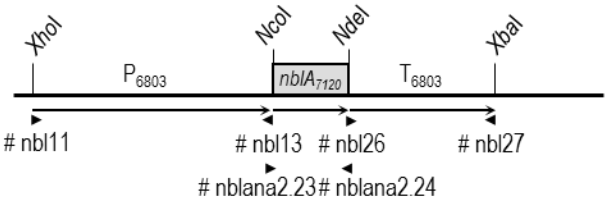
K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nblana2.24