Experiment	Plasmid	Primer	Sequence	Restriction site	Comments
NepR Start codon experiment	pPR9TT	NepRstarts-UP	ATAT**GGTACC**TTGGCGTCCAGAACAAGCTC	KpnI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*.
		Start1-LO	ATAT**CTGCAG**TCCTCGACGCCGAAGTTCAT	PstI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*. First start codon.
		Start2-LO	ATAT**CTGCAG**GGTACGTGTTCGATCATGTC	PstI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*. Second start codon.
		Start3-LO	ATAT**CTGCAG**CCCTTGCGTTTGTCTTCCAT	PstI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*. Third start codon.
		Start(1)23-LO	ATAT**CTGCAG**TCTTCCGCCGGTACGTGTTCGATCGCGTCCTCGACGCCGAAGTTCATCGG	PstI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*. Mutation of second and third start codons.
		Start1(2)3-LO	ATAT**CTGCAG**TCTTCCGCCGGTACGTGTTCGATCATGTCCTCGACGCCGAAGTTCGCCGG	PstI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*. Mutation of first and third start codons.
		Start12(3)-LO	ATAT**CTGCAG**TCTTCCATCGGTACGTGTTCGATCGCGTCCTCGACGCCGAAGTTCGCCGG	PstI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*. Mutation of first and second start codons.
		Start123-LO	ATAT**CTGCAG**TCTTCCGCCGGTACGTGTTCGATCGCGTCCTCGACGCCGAAGTTCGCCGG	PstI	Primer use to clone the promoter region and the different initiation starts of *nepR* in pPR9TT for translational fusion with *lacZ*. Mutation of first, second and third start codons.
Bacterial Two-Hybrid experiment	pKT25	T25-PhyR-UP	ATAT**TCTAGA**GATGAGTCTTCTTGCTCGCTT	XbaI	Primers used to clone *phyR* into pKT25 for BTH experiment. To make pKT25-*phyRD192A*, we used these primers and strain FC1211 as a template for PCR.
		T25-PhyR-LO	ATAT**GGTACC**TCAGGCCGCCTTAGCGG	KpnI	
		T25-PhyRSL-LO	ATAT**GGTACC**TCAGGTCGCCAGCTCGGCG	KpnI	Primer used to clone *phyR* sigma-like domain into pKT25 for BTH experiment.
		T25-SigT-UP	ATAT**TCTAGA**GATGGTCGCGGAACAGGTC	XbaI	Primers used to clone *sigT* into pKT25 for BTH experiment.
		T25-SigT-LO	ATAT**GGTACC**TCACCGCAAGGCGCTCAAT	KpnI	
		T25-NepRFL-UP	ATAT**TCTAGA**GATGAACTTCGGCGTCGAGG	XbaI	Primers used to clone *nepRFL* into pKT25 for BTH experiment.
		T25-NepRFL-LO	ATAT**GGTACC**CTACTCGCCCCCCGCC	KpnI	
		T25-NepRSV-UP	ATAT**TCTAGA**GCAGCAGGCGATCGGCGT	XbaI	Primers used to clone *nepRSV* into pKT25 for BTH experiment.
		T25-NepRSV-LO	ATAT**GGTACC**CTACTCGGCCTTGCGGAGGAT	KpnI	
	pUT18c	18c-NepRFL-UP	ATAT**GGATCC**GATGAACTTCGGCGTCGAGG	BamHI	Primers used to clone *nepRFL* into pUT18c for BTH experiment.
		18c-NepRFL-LO	ATAT**GAATTC**CTACTCGCCCCCCGCC	EcoRI	
		18c-NepRSC2-UP	ATAT**GGATCC**GATGATCGAACACGTACCGATG	BamHI	Primer used to clone *nepRSC2* into pUT18c for BTH experiment.
		18c-NepRSC3-UP	ATAT**GGATCC**GATGGAAGACAAACGCAAGGG	BamHI	Primer used to clone *nepRSC3* into pUT18c for BTH experiment.
		18c-NepRSV-UP	ATAT**GGATCC**GCAGCAGGCGATCGGCGT	BamHI	Primer used to clone *nepRSV* into pUT18c for BTH experiment.
		18c-NepRSV-LO	ATAT**GAATTC**CTACTCGGCCTTGCGGAGGAT	EcoRI	
		18c-NepRpolyA-UP	GTCAACGCAGCAGCAGCAGACGAATTTCTCGCCA	-	Primer used to mutate NepR linker into poly-alanine.
		18c-NepRpolyA-LO	TTCGTCTGCTGCTGCTGCGTTGACGACCTCGTCG	-	
		18c-NepRSV+linker	TATT**GGATCC**GACTAATGGTGTTTCGGTTGGTAACGGTATGGCAAACGGTGCTAGTGGAAA CGGTCTTCTCGGGCCGGGTGCGGCACACGGCGTAGGAGGCCAGCAGGCGATCGGCGTC AAGCTGCGGCAGATGTTCGACGAGGTCGTCAACGAGCCGGTGCCCGACGAATTTCTCGC CATCCTCCGCAAGGCCGAGTAG**GAATTC**TATA	BamHI-EcoRI	Gblocks Gene fragment from IDT. Used to insert a 30 aa linker between T18 adenylate cyclase subunit and NepRsv.
		18c-PhyR-UP	TATT**GGATCC**GATGAGTCTTCTTGCTCGCTT	BamHI	Primers used to clone *phyR* into pUT18c for BTH experiment.
		18c-PhyR-LO	TATA**GAATTC**CTCAGGCCGCCTTAGCGG	EcoRI	
		18c-PhyRSL-LO	TATA**GAATTC**CTCAGGTCGCCAGCTCGGCG	EcoRI	Primer used to clone *phyR* sigma-like domain into pUT18c for BTH experiment.
		18c-SigT-UP	TATT**GGTACC**GATGGTCGCGGAACAGGTC	KpnI	Primers used to clone *sigT* into pUT18c for BTH experiment.
		18c-SigT-LO	TATA**GAATTC**CTCACCGCAAGGCGCTCAAT	EcoRI	
SPR and phosphorylation experiments	pMalc2g	MBP-NepRSV-UP	TATA**GAATTC**CAGCAGGCGATCGGCGT	EcoRI	Primers used to clone *nepRSV* in pMalc2g (MBP tag).
		MBP-NeprSV-LO	TATA**AAGCTT**CTACTCGGCCTTGCGGAGGAT	HindIII	
	pET28c	PhyRD192A-UP	TTTT**CATATG**AGTCTTCTTGCTCGCTTGGC	NdeI	Primers used to clone *phyRD192A* into pET28c. pKT25-*phyRD192A* was used as a template for PCR.
		PhyRD192A-LO	TATA**GAATTC**TCAGGCCGCCTTAGCGGT	EcoRI	
SigT/NepR interaction assay	pETDuet	PDSigT1-UP	GGCC**GAATTC**GATGGTCGCGGAACAGGTCG	EcoRI	Primers used to clone *sigT* into pETDuet first cloning site.
		PDSigT1-LO	ATAT**GCGGCCGC**TCACCGCAAGGCGCTCAAT	NotI	
		PDNepRFL2-UP	ATTT**CATATG**AACTTCGGCGTCGAGG	NdeI	Primers used to clone *nepRFL* into pETDuet second cloning site.
		PDNepRFL2-LO	ATAT**GGTACC**CTACTCGCCCCCCGCC	KpnI	
		PDNepRSV2-UP	ATTT**CATATG**CAGCAGGCGATCGGCGT	NdeI	Primers used to clone *nepRSV* into pETDuet second cloning site.
		PDNepRSV2-LO	ATAT**GGTACC**CTACTCGGCCTTGCGGAGGAT	KpnI	
		PDMBP-UP	TTGT**CATATG**AAAACTGAAGAAGGTAAAC	NdeI	Primer used (with PDNepRFL2-LO, PDNepRSV2-LO, PDMBP-LO) to amplify *MBP-nepRFL*, *MBP-nepRSV* or *MBP* from the different pMalc2g constructs and to insert PCR fragment into pETDuet second cloning site.
		PDMBP-LO	ATAT**GGTACC**CTAGAATTCTACGTAGTGTGCCG	KpnI	Primer used with PDMBP-UP to amplify *MBP* from pMalc2g and to insert PCR fragment into pETDuet second cloning site.
CB15 deletion strains	pNPTS138 Δ*nepRsigT*	ΔnepRsigT-UP	ATAT**GAATTC**AGCCAGATGGCGTGGAA	EcoRI	Primers used to clone Δ*nepRsigT* fragment into pNPTS138 to make a CB15 Δ*nepRsigT* strain.
		ΔnepRsigT1-MID	GCGCGGCCGGTAAAGACGGCGGCGCCCGCCCCCTTTGGCC	-	
		ΔnepRsigT2-MID	GGCCAAAGGGGCGGGCGCCGCCGTCTTTACCGGCCGCGC	-	
		ΔnepRsigT-LO	ATAT**GTCGAC**AGCGCCTGGAACCAGGG	SalI	
NepR vanillate inducible expression in CB15	pMT806	806NepRFL-UP	TATA**CATATG**AACTTCGGCGTCGAGGA	NdeI	Primers used to clone *nepRFL* in pMT806 plasmid.
		806NepRFL-LO	TATA**CTCGAG**CTACTCGCCCCCCGCC	XhoI	
		806NepRSV-UP	TATA**CATATG**CAGCAGGCGATCGGCGT	NdeI	Primers used to clone *nepRSV* in pMT806 plasmids.
		806NepRSV-LO	TATA**CTCGAG**CTACTCGGCCTTGCGGAGGAT	XhoI	
		HA-NepR-UP	ATAT**CATATG**TACCCATACGATGTTCCAGATTACGCTATGAACTTCGGCGT	NdeI	Primers used to fuse an HA tag at the C- and N-terminus of NepR.
		NepR-HA-LO	ATAT**CTCGAG**CTAAGCGTAATCTGGAACATCGTATGGGTACTCGCCCCCCGCCG	XhoI	
		HA-NepRSV-UP	ATAT**CATATG**TACCCATACGATGTTCCAGATTACGCTCAGCAGGCGATCGG	NdeI	
		NepRSV-HA-LO	ATAT**CTCGAG**CTAAGCGTAATCTGGAACATCGTATGGGTAACGCTCGGCCTTGC	XhoI	