Supplemental Figure 1. Engineering a 7His-tag sequence at the 5' or the 3' end of *ndhB* in the tobacco plastid genome. (A) Nucleotide and amino acid sequences in the vicinity of the introduced His-tag. Addition of a 7His-encoding sequence was performed with a PCR-based site-mutagenesis approach (ExSite PCR-based site directed mutagenesis kit, Stratagene). The mutagenic primers (O1 and O2) were designed with a complementary region and a tail of 7 His codons (CAT codon). B5'-tag, construct carrying the His-tag at the 5' end of *ndhB*. Addition of the tag-sequence downstream the ATG codon creates a new *Nsi*I restriction site. B3'-tag, construct carrying the His-tag at the 3' end of *ndhB* and a point mutation (T to G transversion) creating a *Fsp*I site. Added or modified nucleotides appear in bold. (B) Construction of plastid transformation vectors. Plasmid pCTM9 (Rumeau *et al*, 2004) contains a *Bam*HI-*Pst*I ptDNA fragment encompassing a mutated version of *16srDNA* conferring spectinomycin resistance. Plasmid pPS8 is a *Pst*I subclone of pTB12 (Shinozaki *et al* 1986) containing a ptDNA fragment contiguous to pCTM9 and carrying *ndhB*. His-tag additions to *ndhB* were performed from pPS8. Plastid transformation vector pB5'-tag was obtained by ligating the *Pst*I-*Hind*III fragment of pPS8 containing the 5' end His-tagged *ndhB* gene to pCTM9. The tagged 3'-end of *ndhB* was excised as a *Nde*I-*Stu*I restriction fragment, the 488 bp insert was used to replace the same restriction fragment excised from pPS8. From the modified pPS8 the *Pst*I fragment was excised and cloned into the *Pst*I site of pCTM9 to create the transformation vector pB3'-tag. Resulting constructs pB5'-tag and pB3'-tag were used to transform tobacco protoplasts (0.25×10^6) by polyethylene glycol (PEG) treatment (O'Neill *et al*, 1993). DNA-treated protoplasts were cultured in liquid medium, colonies were then grown in spectinomycin-containing solid medium ($1 mg.mL^{-1}$) and plants were regenerated from resistant calli as described (Medgyesy, 1994). Vertical arrows represent recombination endpoints. Dotted lines represent the vector portion of pBSII. BHI, BamHI. (C) PCR analysis. DNA from wild type (WT) or from spectinomycin resistant tobacco plants (B5'-tag and B3'-tag) was extracted and used as template for PCR with O3, O4 or O5, O6. Following amplification, PCR product obtained with B5'-tag DNA and O3, O4 (980bp) was digested with *Nsi*I; the PCR product obtained with B3'-tag DNA and O5, O6 (1050bp) was digested with FspI.). Following digestion, no trace of the full size wild type (WT) PCR fragment was observed, demonstrating homoplasmy of B5'- and B3'-tag transformants.

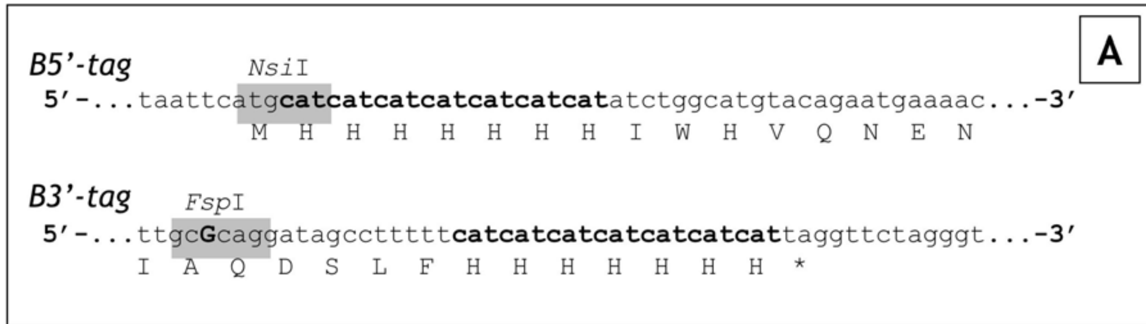
O1: 5'-ATGATGATGATGATGATGATGAAAAAGGCTATCCTGCGCAATTGCAAT -3'
O2: 5'-CATCATCATCATCATCATCATGCTATAACAGAGTTTCTCTTATTCGTA -3'
O3: 5'-AGAATCGGAATTGATCGATAGATT-3'

O4: 5'-AGGTCAGGAGTGCATTGATGA-3'

O5: 5'-CTCCCACTCCAGTCGTTGCTT-3'

O6: 5'-TCCGATTACGCGTATTCCTAATCCTA-3'

Supplemental Figure 1



B
ptDNA
155939 bp
rbcL
IRB
IRA
16S
16S

C
WT B5'-tag
μg: 0.1 0.25 1.25 2.5
980
750
230
WT B3'-tag
0.1 0.25 1.25 2.5
1050
750
300

BHI trnI Spec 16S rDNA trnV 3'-rps 12 PstI pCTM9
pPS8 PstI rps7 5' Hind ndhB StuI 3 NdeI trnL PstI
O3 O4 O5 O6
BHI trnI Spec 16S rDNA trnV 3'-rps 12 PstI rps7 NsiI Hind pB5'-tag
His-tag
BHI trnI Spec 16S rDNA trnV 3'-rps 12 PstI rps7 5' Hind ndhB StuI 3' FspI NdeI trnL PstI pB3'-tag
His-tag