Oligo	Sequence $5' \rightarrow 3'$	Use
pOD1-F	GATCCAAATACTGCAGTTTAACTTTAAGAAGGAGATATACC	pOD1
pOD1-R	CATGGGTATATCTCCTTCTTAAAGTTAAACTGCAGTATTTG	
pOD2-F	GATCCAAATACTGCAGCTTAGAATATTGAAGGAGATATACCCATCTATTATAAATAGTGCA	- pOD2 from pOD1
pOD2-R	CTATTTATAATAGATGGGTATATCTCCTTCAATATTCTAAGCTGCAGTATTTG	
pOD3-F	GATCCAAATACTGCAGAGATACAATAAGAAGGAGATATACCCATCTGTATTAAATAGTGCA	pOD3 from pOD2
pOD3-R	CTATTTAATACAGATGGGTATATCTCCTTCTTATTGTATCTCTGCAGTATTTG	
pOD4-F	GTTTAACTTTAAGAAGGAGATATACATATGTAATAAGAAGGAGATATACC	- pOD4 from pOD1
pOD4-R	CATGGGTATATCTCCTTCTTATATATATGTATATCTCCTTCTTAAAGTTAAACTGCA	
pOD5-F	GTTTAACTTTAAGAAGGAGATATACATATGTAGTAAGAAGGAGATATACATATGTAATAAGAAGGAGATATACC	pOD5 from pOD1
pOD5-R	CATGGGTATATCTCCTTCTTATTACATATGTATATCTCCTTCTTACTACATATGTATATCTCCTTCTTAAAGTTAAA CTGCA	
pOD6-F	GTTTAACTTTAAGAAGGAGTAAGAAGGAGATATACC	pOD6 from pOD1
pOD6-R	CATGGGTATATCTCCTTCTTACTCCTTCTTAAAGTTAAACTGCA	
pOD7-F	GTTTAACTTTAAGAAGGAGTAAGAAGGAGTAAGAAGGAGATATACC	pOD7 from pOD1
pOD7-R	CATGGGTATATCTCCTTCTTACTCCTTCTTAAAGTTAAACTGCA	
pOD8-F	GTTTAACTTTAAGAAGGAGATATACATATGGGTTAATAAGAAGGAGATATACC	pOD8 from pOD1
pOD8-R	CATGGGTATATCTCCTTCTTATTAACCCATATGTATATCTCCTTCTTAAAGTTAAACTGCA	
pOD9-F	GTTTAACTTTAAGAAGGAGATATACATATGGGATAGTAAGAAGGAGATATACATATGGGTTAATAAGAAGGAGA TATACC	- pOD9 from pOD1
pOD9-R	CATGGGTATATCTCCTTCTTATTAACCCATATGTATATCTCCTTCTTACTATCCCATATGTATATCTCCTTCTTAAA GTTAAACTGCA	
pOD10-F	CATGGGTGAAAATTTATATTTTCAAAG	TEV in pOD1

pOD10-R	CATGCTTTGAAAATATAAATTTTCACC	
pOD11-F	GTTTAACTTTAAGAAGGAGATATACATATGGGAGAAGGAGATATACC	pOD11 from pOD10 pOD17 from pOD1
pOD11-R	CATGGGTATATCTCCTTCTCCCATATGTATATCTCCTTCTTAAAGTTAAACTGCA	
pOD12-F	GTTTAACTTTAAGAAGGAGATATACATATGGGAGAAGGAGATATACATATGGGAGAAGGAGATATACC	pOD12 from pOD10 pOD18 from pOD1
pOD12-R	CATGGGTATATCTCCTTCTCCCATATGTATATCTCCTTCTCCCATATGTATATCTCCTTCTTAAAGTTAAACTGCA	
pOD13-F	GTTTATCTTTATGAAGGAGATATACC	pOD13 from pOD10 pOD19 from pOD1
pOD13-R	CATGGGTATATCTCCTTCATAAAGATAAACTGCA	
pOD14-F	GATCCAAATACTGCAGCTTACAATATTGAAGGAGATATACATATGGGTATTATAAATAGTGCA	pOD14 from pOD13 pOD15 from pOD14 pOD22 from pOD15 pOD20 from pOD19 pOD21 from pOD20
pOD14-R	CTATTTATAATACCCATATGTATATCTCCTTCAATATTGTAAGCTGCAGTATTTG	
P16Srrn-F	CAAGCGGTGGAGCATGTGG	generation of hybridization probes
P16Srrn-R	GGCGGTGTGTACAAGGCCC	
P7247	CCCAGAAAGAGGCTGGCCC	
P7244	CCCAAGGGGGGGGAACTGC	
PZF9	TTTTCATATGAGTAAAGGAGAAGAACTT	
PZF10	TTTTATTAATGATTAGTTCATCCATGCC	



Supplemental Figure 1. Isolation of homoplasmic transplastomic tobacco lines obtained with pOD transformation vectors. Total cellular DNA samples were digested with the restriction enzyme BgIII, separated in 1% agarose gels and hybridized to a *psaB*-specific probe (cp. Figure 1). Absence of the 3.5 kb hybridization signal specific to the wild-type plastid genome indicates homoplasmy of the transplastomic lines. Instead, all transplastomic lines show a hybridization signal of 5.9 kb. The size difference of 2.4 kb corresponds to the added sizes of the *gfp* and *aadA* cassettes.