

SUPPLEMENTARY MATERIALS

PECULIARITIES OF *eGFP* GENE EXPRESSION IN TRANSPLASTOMIC TOBACCO PLANTS *NICOTIANA TABACUM* L. CV. PETIT HAVANA

Yu. V. Sidorchuk^{a,*}, P. A. Belavin^a, A. A. Zagorskaya^a, T. V. Marenkova^a, V. V. Kuznetsov^a,
E. S. Khajrulina^a, E. V. Deineko^a

^aInstitute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk,
Russia

Corresponding author: Yu. V. Sidorchuk

*e-mail: sidorch@bionet.nsc.ru

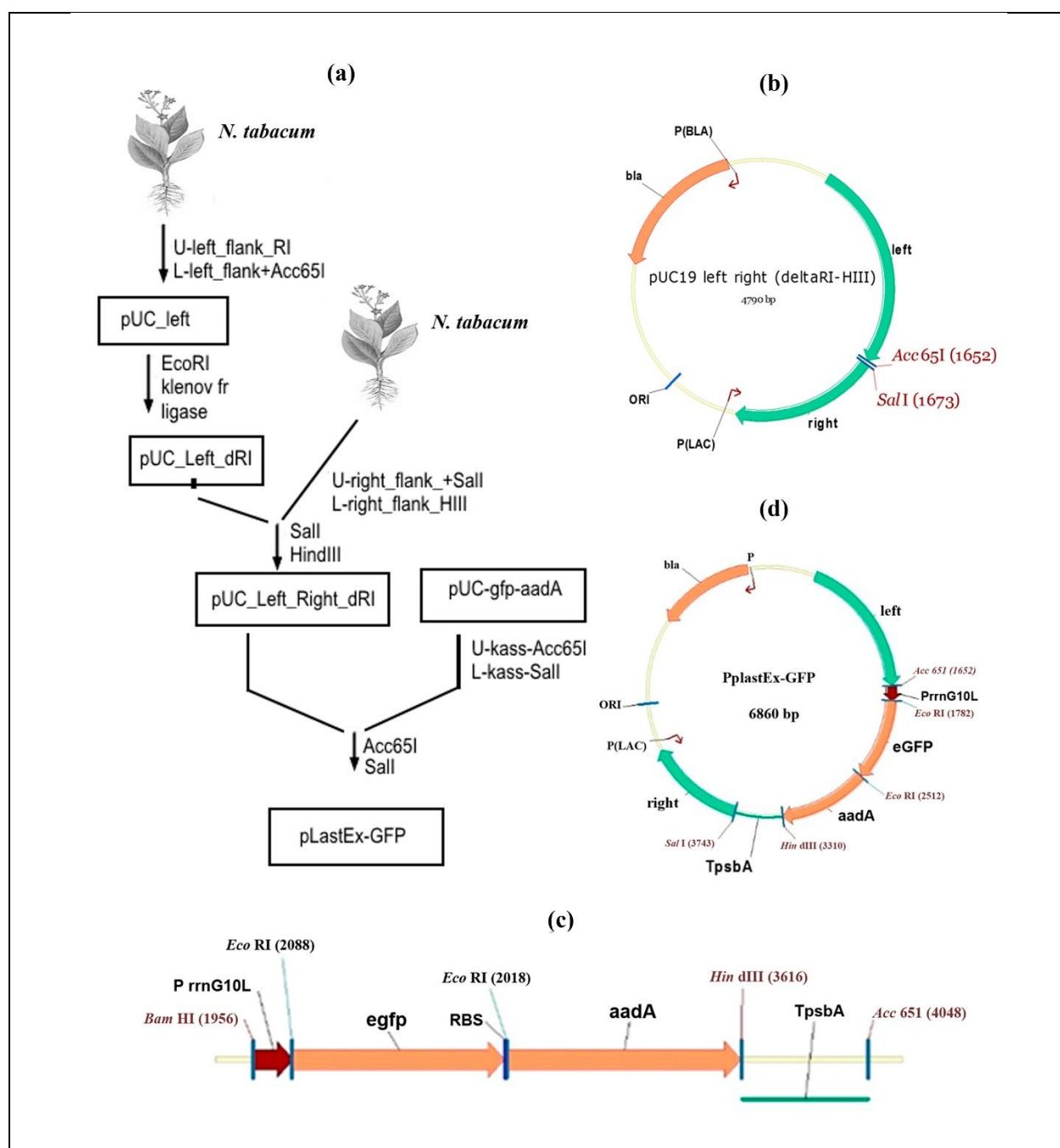


Fig. S1. Design of the expression vector pPlastEx-GFP.

a – general scheme of construction; b – intermediate plasmid pUC19_left_right-(deltaRI-HIII); c – expression cassette, where *PrrnG10L* is the promoter of the rRNA operon (*Prrn*), fused with the leader sequence of gene 10 of phage T7 (*G10L*), *egfp* is the coding sequence of the green fluorescent protein gene, RBS is the ribosome binding site, *aadA* is the coding sequence of the spectinomycin resistance gene, *TpsbA* is the terminator of the *psbA* gene encoding the D1 protein of photosystem II; d – final plasmid (vector) pPlastEx-GFP, used for transformation of the plastid genome.

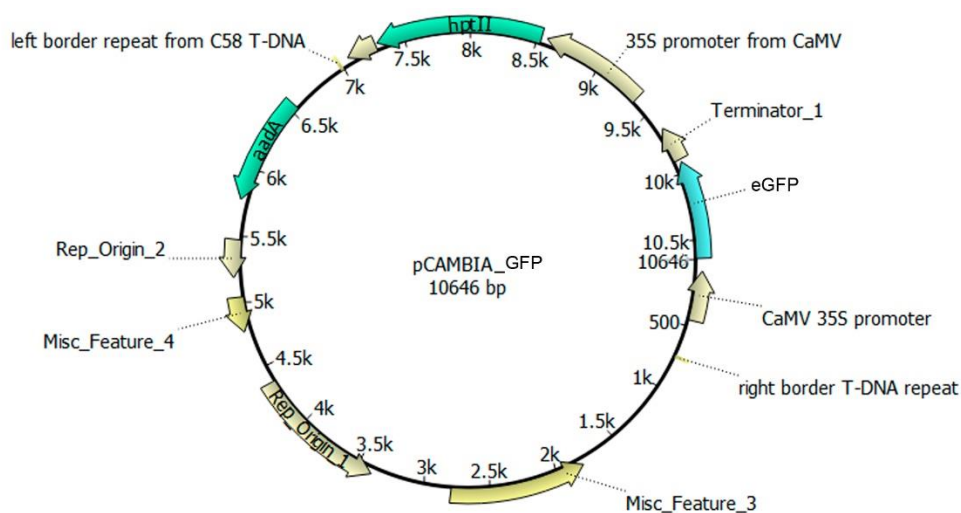


Fig. S2. Vector for obtaining nuclear transformants expressing a reporter gene *egfp*.

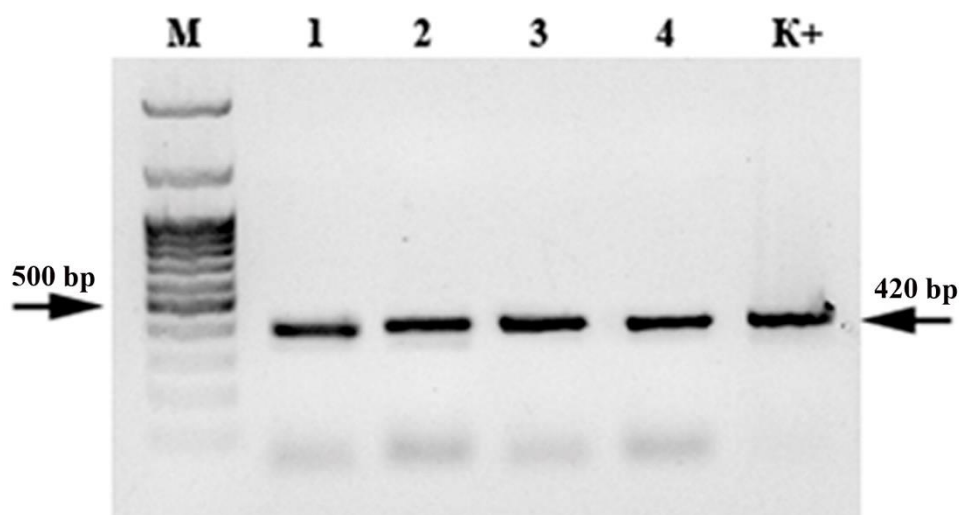


Fig. S3. Gel electrophoresis of amplification products in 1% agarose gel (using 4 samples as an example) confirming the presence of the *egfp* gene in the genome of nuclear tobacco transformants T₀.

Table S1. Primers structure for amplification of homology flanks to the insertion site.

Gene	Primer	Primer sequences
Homology flank left	Forward	5' GGTGAATTCGTTCCCGGGC 3'
	Reverse	5' GCCGGTACCGCTGGGCCATCCTGGACTTG 3'
Homology flank right	Forward	5' CCCGTCGACAGCTGCGCCAGGGAAAAGAA 3'
	Reverse	5' CATAAAGCTTTGTATCGGCTAAGTTCA 3'

Table S2. Primers structure for amplification of expression cassette *gfp-aadA*.

Gene	Primer	Primer sequences
<i>gfp-aadA</i>	Forward	5' AGCGGTACCCGCCGTCGTTCAATGAGAAT 3'
	Reverse	5' GCTGTCGACCCAAGATCCAAGATAAAGTA 3'

Table S3. Primers structure for *gfp* and *aadA* genes detection in genome of nuclear transformants and plastid genome of transplastomic plants by PCR.

Gene	Primer	Primer sequences	Fragment length, b.p.
Nuclear transformants			
<i>egfp</i>	Forward	5'-TTG TGC CCC AGG ATG TTG CC-3'	420
	Reverse	5'-GGT GAG CAA GGG CGA GGA GC-3'	
Transplastomic plants			
<i>egfp</i>	Forward	5'-GAGGAGCTGTTCACCGGG-3'	702
	Reverse	5'-CTTGTACAGCTCGTCCATGC-3'	
<i>aadA</i>	Forward	5'-ATGGCAGAAGCGGTGATCG-3'	290
	Reverse	5'-GCTCGAAGATACCTGCAAGAATGTC-3'	

Table S4. Primers and probes for multiplex qRT-PCR.

Gene	Primer	Primer sequences
<i>egfp</i>	Forward	5'-TCCATGCCGTGAGTGATCCC-3'
	Reverse	5'-GTCCGCCCTGAGCAAAGACC-3'
	Probe	5'-FAM CAACGAGAAGCGCGATCACATG-BHQ1-3'
<i>aadA</i>	Forward	5'-GACATTGATCTGGCTATCTTGCTGA-3'
	Reverse	5'-GAGTTCCATAGCGTTAAGGTTTCATT-3'
	Probe	5'-R6G TAGCGCCTCAAATAGATCCTGTTCA-BHQ2-3'
<i>ycf2</i>	Forward	5'-CCCACACGAAGTTTGTGAATAAGTG-3'
	Reverse	5'-GATTGAACAACCGGGAGCAA-3'
	Probe	5'-ROX TGTCTGATAATGAGCAAGGAATATCCG-BHQ2-3'