**SUPPLEMENTARY MATERIALS**

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

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| **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. |

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

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| **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.

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| --- | --- | --- |
| 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.*

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| --- | --- | --- |
| Gene | Primer | Primer sequences |
| *gfp-aadA* | 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.

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

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

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| Gene | Primer | Primer sequences |
| *egfp* | Forward | 5′-TCCATGCCGTGAGTGATCCC-3′ |
| Reverse | 5′-GTCCGCCCTGAGCAAAGACC-3′ |
| Probe | 5′-FAM CAACGAGAAGCGCGATCACATG-BHQ1-3′ |
| *aadA* | Forward | 5′-GACATTGATCTGGCTATCTTGCTGA-3′ |
| Reverse | 5′-GAGTTCCATAGCGTTAAGGTTTCATT-3′ |
| Probe | 5′-R6G TAGCGCCTCAAATAGATCCTGTTCA-BHQ2-3′ |
| *ycf2* | Forward | 5′-CCCACACGAAGTTTGTGAATAAGTG-3′ |
| Reverse | 5′-GATTGAACAACCGGGAGCAA-3′ |
| Probe | 5′-ROX TGTCTGATAATGAGCAAGGAATATCCG-BHQ2-3′ |