

SUPPLEMENTAL MATERIALS AND METHODS

Agroinjection in tomato fruits

The agroinjection method is adapted from Orzaez et al. (2006). Purified plasmid DNA was transformed into C58 *A. tumefaciens* competent cells. For each construct, a single colony was inoculated into 5 mL YEB media, supplemented with 50 mg/L spectinomycin and 37 mg/L rifampicin, and grown at 28°C overnight. Cultures were transferred to 250 mL flask containing 50 mL induction media supplemented with the same antibiotics and incubated overnight at 28°C. Cells were pelleted by centrifugation at 3200 x g for 20 min and resuspended in infiltration media (Orzaez et al., 2006) to an OD₆₀₀=0.2, followed by incubation at 28°C for 160 min prior to infiltration. Tomato fruits (cv MicroTom) were injected through the base (stylar end) with a 1 mL syringe and 0.6 x 30 needles. Infiltration media containing *Agrobacterium* cells was injected until the air spaces beneath the epidermis were filled, requiring up to 1 mL in larger fruits. Approximately 20 to 30 fruits were injected per construct, across a range of developmental stages and results for each constructs were entirely consistent. Representative samples are shown in Figure 2. Infiltration medium containing untransformed *Agrobacterium* cells was used as a negative control. Different plants were used for each treatment to prevent possible *Agrobacterium* cross-contamination between fruits. Following infiltration, fruits were left attached to the plants for 7 days before harvesting, because such an incubation period yielded higher transformation efficiency (unpublished results). Two to three vertical sections (approximately 2 mm thickness) were cut from each fruit samples and incubated with GUS staining solution (Jefferson et al., 1987) into 30 mL tubes overnight in the dark at 28°C. Fruit sections were cleared in 100% methanol for 6 to 8 h, blotted dry, and photographed.

Gateway constructs for amiRNA expression

We tested whether an amiRNA precursor sequence captured and subcloned in the Gateway format would remain an efficient inducer of gene silencing. For this purpose, we selected with WMD an amiRNA targeting the transcript coding for the Arabidopsis phytoene desaturase *PDS*, necessary for β -carotene biosynthesis. *pds* mutants fail to accumulate photo-protective carotenoids and exhibit an easily scorable albino phenotype caused by chlorophyll degradation (Wang et al., 2005).

According to Schwab et al. (2006), the plasmid pRS300 was used as template to introduce an amiRNA sequence specific to the *Arabidopsis thaliana* PDS gene (5'-TCTACTGGCATACAAAGCGTT-3') into the *miR319a* precursor by site-directed mutagenesis (also see the detailed online protocol for more information; http://wmd2.weigelworld.org/themes/amiRNA/pics/Cloning_of_artificial_microRNAs.pdf). The four oligonucleotides provided by the designer for the mutagenesis are (from 5' to 3'):

I miR-s gaTCTACTGGCATACAAAGCGTTtctctctttgtattcc;
II miR-a gaAACGCTTTGTATGCCAGTAGAtcaaagagaatcaatga;
III miR*s gaAAAGCTTTGTATGGCAGTAGTtcacaggtcgtgatatg;
IV miR*a gaACTACTGCCATACAAAGCTTTtctacatatattct.

Overlapping PCR amplification steps were performed as described in the original cloning protocol except that, in the final PCR reaction, the oligonucleotides A and B (based on the pBSK backbone) were replaced by two alternative oligonucleotides, attB1-amiRNA-fw and attB2-amiRNA-rev, carrying the *attB1* and *attB2* sites, respectively. These two primers can be used for the amplification of any amiRNA derived from the *miR319a* precursor backbone.

attB1-amiRNA-fw:

5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCCCCAAACACACGCTCGGA-3'

attB2-amiRNA-rev:

5'-GGGACCACTTTGTACAAGAAAGCTGGGTCCCATGGCGATGCCTTAA-3'.

The resulting PCR product containing the *AtamiR-pds* precursor was cloned into pDONR221 by BP clonase reaction, then subcloned into the pK7WG2 destination vector by LR clonase reaction, to yield the pK7-B1-*AtamiR-pds*-B2 expression (CaMV 35S promoter) vector.

Arabidopsis transformation and PDS silencing

The pXK7AtamiRpds expression vector was transferred into *Agrobacterium tumefaciens* C58C1 (pMP90) by electroporation and transformed into Arabidopsis plants by floral dip (Clough and Bent, 1998). T1 seeds were germinated on media containing 2.2 g/L of Murashige-Skoog medium (Sigma), 1.5% sucrose, 1.2 % plant agar and 50 mg/L kanamycin. Seed germination was performed as described (Harrison et al., 2006).

Out of approximately 700 seedlings, 10 and 4 T1 plants transformed with the *35Spro:amiR-pds* transgene displayed a complete and variegated albino phenotype, respectively, presumably resulting from *PDS* silencing, and were markedly different

from kanamycin-sensitive and kanamycin-resistant positive control T1 seedlings (Supplemental Fig. S3). The observed albino phenotype was comparable to that previously reported for mutations in the same target gene, indicating that the *attB* sites added to the amiRNA precursor did not affect silencing efficiency.

To conclude, it is advantageous to adopt the Gateway cloning format to capture amiRNA precursor sequences because any such entry clone pEN-L1-amiRNA-L2 can be recombined at will with any promoter (i.e. tissue-specific or inducible promoters) in a single Multisite LR clonase reaction. Tissue-restricted silencing is particularly attractive considering that amiRNA-induced gene silencing is not likely to spread systemically (Alvarez et al., 2006; Schwab et al., 2006).

Creation of new hpRNA destination and donor vectors

We adapted the original Gateway hairpin cloning strategy (Wesley et al., 2001) and created bipartite destination vectors in which, from 5' to 3', the *ccdB* gene was flanked by the *attR4* and *attR2* sites in the first Gateway MultiSite cassette, and by *attR2* and *attR1* in the second. Both cassettes were separated by the pHELLSGATE12-derived intron spacer. This construct was transferred into the T-DNA region of the pPZP200 binary vector together with a plant selectable marker coding for either kanamycin, hygromycin or phosphinotricin resistance, resulting in the pK8GWIm24GW, pH8GWIm24GW and pB8GWIm24GW intro-spacer destination vectors (collectively named p*8GWim24GW; Supplemental Fig. 3C). hpRNA expression clones were assembled into these destination vectors in two successive steps: (1) an intermediate entry clone was created by LR recombination between a promoter (pEN-L4-promoter-R1) and a GST (pEN-L1-GST-L2) entry clone (Supplemental Fig. 3A); (2) the resulting co-integrate plasmid (pEN-L4-promoter-B1-GST-L2) and the same GST entry clone (pEN-L1-GST-L2) were recombined simultaneously into the pK8GWIm24GW destination vector via a double LR clonase reaction (Supplemental Fig. 3B and C) resulting in the desired specific hpRNA expression clone (Supplemental Fig. 3D). This flexible cloning scheme can be used to silence genes through the expression of hpRNAs in a constitutive, inducible or tissue-specific manner provided that the necessary promoters are available as entry clones.

Some experiments require transcription of different hpRNAs under the control of the same promoter. To bypass the two-step cloning procedure described above, we converted existing hpRNA expression clones into novel hpRNA destination vectors via a particular reverse BP reaction. It is based on an intro-spacer donor

vector, pDONR P1-R2-I-R2-P1 (Supplemental Fig. 3E), designed for reverse BP recombination with any hpRNA expression clone that contains a specific promoter (*attB4*-promoter-*attB1*) and a pair of inverted GSTs separated by a spacer (*attB1*-GST-*attB2*-spacer-*attB2*-TSG-*attB1*). In such a BP clonase reaction, only the two *attB1* sites from the expression clone recombined with the two *attP1* sites from the donor vector. As a result, the original GST hpRNA expression cassette downstream of the promoter of interest was entirely replaced by the cassette originating from the donor vector containing the *ccdB* gene in direct repeats (Supplemental Fig. 3F). We applied this scheme to create the specific hpRNA destination vectors corresponding to all five promoters available as entry clones (Supplemental Table III). Additional cloning procedure details are provided here below.

The CaMV 35S promoter was removed from the pHELLSGATE12 hpRNA expression cassette and its 5' *attR1* site replaced by an *attR4* site. The resulting *SacI*-*SpeI* fragment (from 5' to 3': *attR4*-*ccdB*-*attR2*-intron_spacer-*attR2*-*ccdB*-*attR1*-OC*ster*) was cloned into the T-DNA region of one of three *SacI*-*SpeI*-linearized modified pPZP200 binary vectors, that also carried a plant selectable marker coding for either kanamycin (K), hygromycin (H) or phosphinotricin (B) resistance, resulting in a set of three intron-spacer destination vectors named p*8GWIm24GW (where * represents the K, H or B marker).

Novel hpRNA vectors can be created by recombination between appropriate plasmids in alternating LR and BP clonase reactions. Specific hpRNA expression clones were generated carrying each of the five fruit promoters following the two-step LR recombination illustrated in Supplemental Fig. 3 (A-D). Note that in this scheme, the pEN-L4-promoter-R1 and pEN-L1-GST-L2 recombined to generate the intermediate entry clone (Supplemental Fig. 3A) should carry different bacterial selectable markers, for example coding for kanamycin and gentamycin resistance, respectively, in this case. The resulting LR clonase reaction products were transformed into DH5 α cells selected on medium containing both antibiotics.

To build the intron spacer donor vector (pDONR P1-R2-I-R2-P1; Supplemental Fig. 3E), the *attL2* site in pENTR1A (Invitrogen) was replaced by the *attL1* to create pEN-L1L1. This plasmid was recombined with pHELLSGATE12 in an LR clonase reaction with the aim to transfer the entire *attR1*-*attR1* portion of pHELLSGATE12, excluding the CaMV 35S promoter, into the new pDONRP1-R2-I-R2-P1 donor clone carrying the *attP1*-*ccdB*-*attR2*-intron_spacer-*attR2*-*ccdB*-*attP1* assembly. Following transformation in DB3.1 competent cells, clones were selected on kanamycin LB plates, that shared the same backbone as the pENTR-L1-*ccdB*-L1 entry clone (Supplemental Fig. 4).

The novel specific hpRNA destination vectors carrying the *PPC2*, *IMA* and *TPRP* promoters were created as shown (Supplemental Figure 3D-F), following *Bam*HI linearization of the initial expression clone to enable the selective recovery of the desired backbone, including the spectinomycin resistance marker. However, the same procedure could not be implemented for constructs carrying the large *PG* and *CRC* promoters because no appropriate unique restriction site was available for linearization of the expression clones. Instead, a *PGpro* and *CRCpro* hpRNA expression clones were recombined in a double reverse BP clonase reaction with the *Eco*RV-linearized pDONR221 that contains an *att*L1-*ccdB-Cm-att*L2 Gateway cassette (Supplemental Fig. 5). Because the subsequent selection of chloramphenicol-resistant DB3.1 cells could not distinguish between single and double recombination products, sufficient clones were screened via restriction analysis to identify the latter. Cloning efficiency with specific hpRNA destination vectors was verified by creating derivative-specific hpRNA expression in LR recombination with pEN-L1-GST-L2 clones. Most clones recovered after DH5 α cell transformation had the expected structure characterized by GST inverted repeats separated by the intron spacer and downstream of the corresponding tissue-specific promoter.

LITERATURE CITED

- Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y** (2006) Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. *Plant Cell* **18**: 1134-1151
- Bartel DP** (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**: 281-297
- Clough SJ, Bent AF** (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* **16**: 735-743
- Harrison SJ, Mott EK, Parsley K, Aspinall S, Gray JC, Cottage A** (2006) A rapid and robust method of identifying transformed *Arabidopsis thaliana* seedlings following floral dip transformation. *Plant Methods* **2**: 19
- Jefferson RA, Kavanagh TA, Bevan MW** (1987) GUS fusions: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J* **6**: 3901-3907

- Orzaez D, Mirabel S, Wieland WH, Granell A** (2006) Agroinjection of tomato fruits. A tool for rapid functional analysis of transgenes directly in fruit. *Plant Physiol* **140**: 3-11
- Schwab R, Ossowski S, Riestter M, Warthmann N, Weigel D** (2006) Highly specific gene silencing by artificial microRNAs in Arabidopsis. *Plant Cell* **18**: 1121-1133
- Wang T, Iyer LM, Pancholy R, Shi X, Hall TC** (2005) Assessment of penetrance and expressivity of RNAi-mediated silencing of the Arabidopsis phytoene desaturase gene. *New Phytol* **167**: 751-760
- Wesley SV, Helliwell CA, Smith NA, Wang M, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP, Gleave AP, Green AG, Waterhouse PM** (2001) Construct design for efficient, effective and high-throughput gene silencing in plants. *Plant J* **27**: 581-590

SUPPLEMENTAL FIGURES

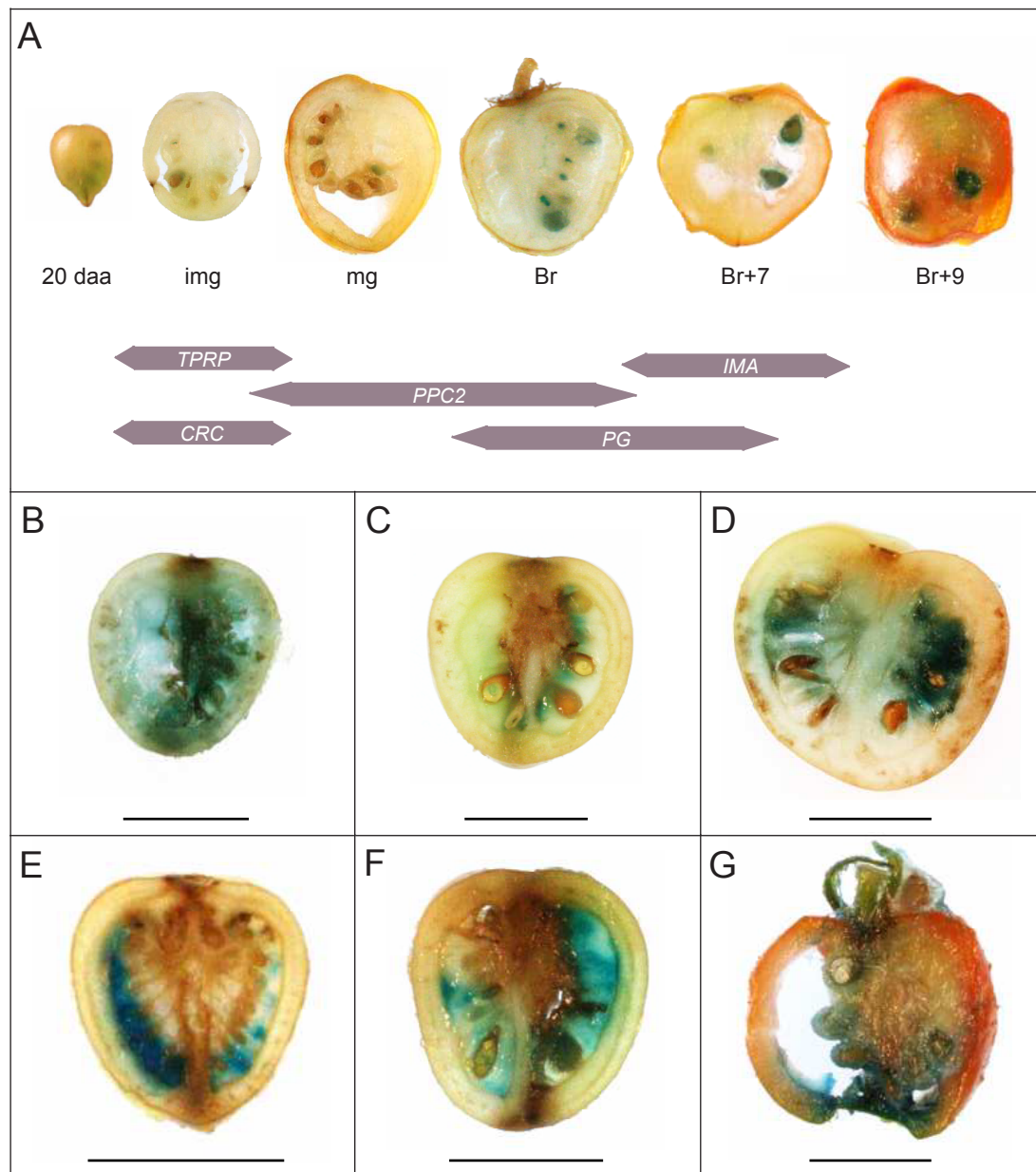
Supplemental Fig. S1. *GUS* activity in tomato fruits (cv. Micro Tom) agroinjected with *promoter:GUS* transgenes generated via MultiSite Gateway cloning. A, Uninjected control fruits stained for endogenous *GUS* activity at the stages shown. Arrows mark the developmental stages at which promoter activity was observed in the fruit for the indicated genes. B-G, representative images of fruits agroinjected with each of the five *promoter:GUS* constructs. B, *TPRPpro:GUS* (img). C, *CRCpro:GUS* (img). D, *PPC2pro:GUS* (mg). E, *PPC2pro:GUS* (b). F, *PGpro:GUS* (b). G, *IMApr:GUS* (b+7d). Letters refer to the stage at which fruits were collected: daa, day after anthesis; img, immature green; mg, mature green; b, breaker; b+7d, breaker plus 7 days. Size bar = 10 mm.

Supplemental Fig. S2. Albino phenotype resulting from *PDS* silencing in Arabidopsis. Two 5-day-old T1 albino seedlings transformed with a *35Spro:amiR-At-pds* Gateway transgene (center and right) compared to a seedling transformed with a *35S:GFP* control (left).

Supplemental Fig. S3. Creation of novel hpRNA destination vectors. Promoter and GST entry clones (A) fused to generate an intermediate clone (B) in a first LR reaction. The resulting pEN-L4-promoter-B1-GST-L2 together with the GST entry clone were recombined in a second LR clonase reaction with the intron-spacer destination vectors (C) to produce an expression clone (D). Novel specific hpRNA destination vectors (F) were produced by reverse BP recombination of *Bam*HI-linearized expression clones with pDONR P1-R2-I-R2-P1 (E). Open arrows represent recombination between compatible *att* sites and arrowheads indicate the direction of transfer into the selected backbone.

Supplemental Fig. S4. Construction of the intron-spacer donor vector pDONR P1-R2-I-R2-P1. Open arrows represent recombination between compatible *att* sites and arrowheads indicate the direction of transfer into the selected backbone.

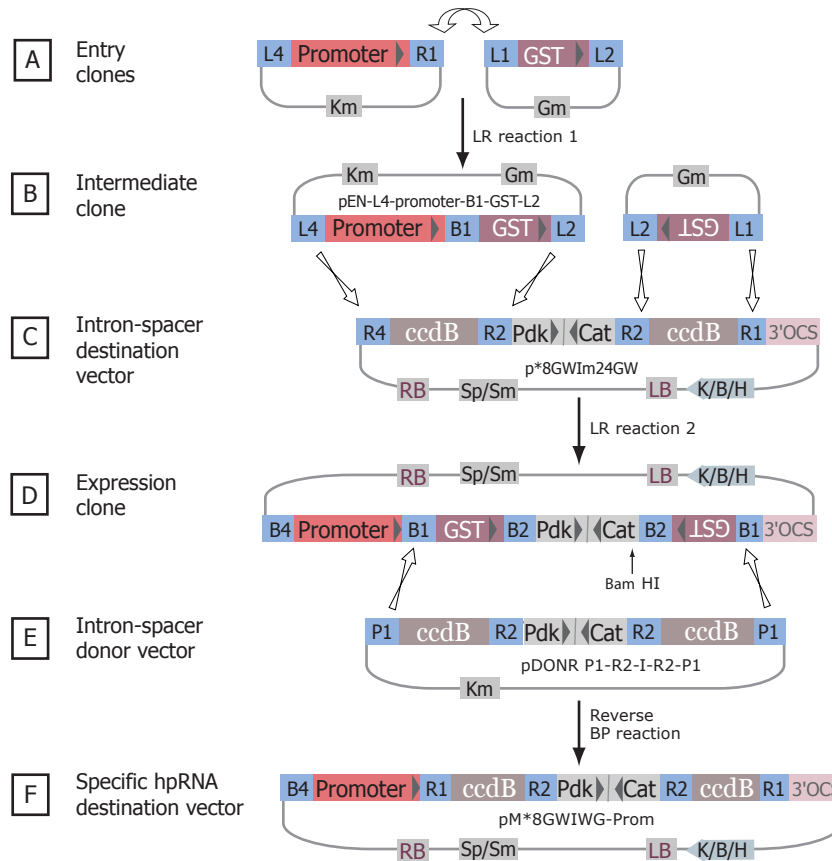
Supplemental Fig. S5. Alternative scheme to generate specific hpRNA destination vectors. Open arrows represent recombination between compatible *att* sites and arrowheads indicate the direction of transfer into the selected backbone.



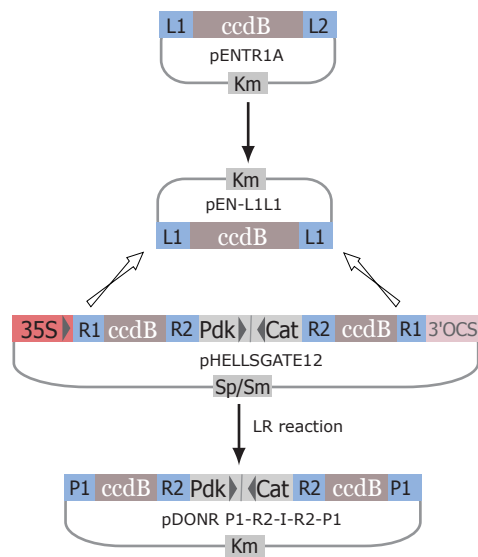
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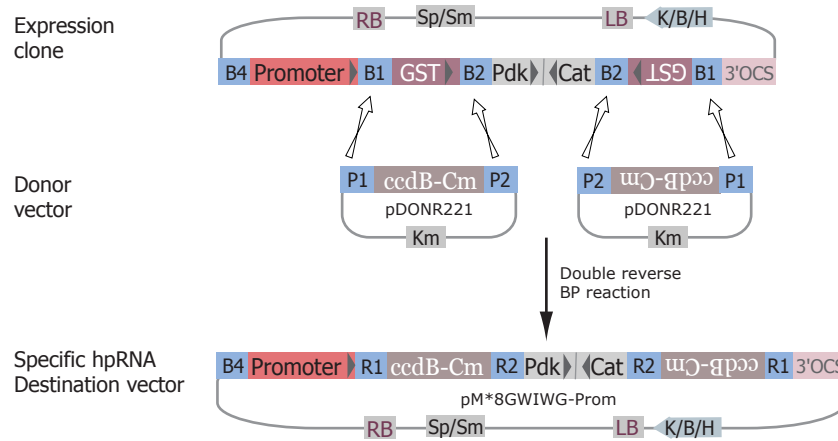
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Supplemental Table S1. Theoretical promoter sequences

>PPC2 1967 bp

ATACATTCTACTTTGAAGTTGTTTAATGAGGTAATAGGACACCTGCAAAGTTAAAATATC
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>TPRP 2645 bp

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>MATRIOSKA 534 bp

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>PG 4859 bp

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CTCTCTCTTTCTTTCAAGAGCGGTTTTCA

Supplemental Table S2. Primers designed for PCR amplification of genetic elements and for validation of recombined plasmids.

Name	Sequences (from 5' to 3') ^a	Application
<i>PPC2-attB4</i> forward	GGGGACA ACTTTGTATAGAAAAGTTGATACATTCTACTTTGAAGTTGTT	Amplification of <i>PPC2pro</i> (5')
<i>PPC2-attB1</i> reverse	GGGGACTG CTTTTTTGTACAAACTTGAACCCCTTTTTACTCAA	Amplification of <i>PPC2pro</i> (3')
<i>TPRP-attB4</i> forward	GGGGACA ACTTTGTATAGAAAAGTTGAATAGGCGAGTGAGATGGAA	Amplification of <i>TPRPpro</i> (5')
<i>TPRP-attB1</i> reverse	GGGGACTG CTTTTTTGTACAAACTTGGGCAATGAACAAAGTTCCAAG	Amplification of <i>TPRPpro</i> (3')
<i>IMA-attB4</i> forward	GGGGACA ACTTTGTATAGAAAAGTTGCATCAGTGAGGGCCACACA	Amplification of <i>IMApr</i> (5')
<i>IMA-attB1</i> reverse	GGGGACTG CTTTTTTGTACAAACTTGCTCTTCTAATGATTCAGATAAAT	Amplification of <i>IMApr</i> (3')
GWM13 forward	GTAAAACGACGGCCAGTCTTA	Validation of promoter entry clones (5') located in vector backbone, outside the <i>attL1</i> site
GWM13 reverse	CCAGGAAACAGCTATGACCAT	Validation of promoter entry clones (3') located in vector backbone, outside the <i>attL2</i> site
INDU 5F reverse	TCGCGCTGATACCAGAC	Validation of pK7B4-promoter-B1-GUSintron-B2 expression clones (Table 2) located in 5' end of <i>GUS</i>
LHGROATG4-145-reverse	CAACGCGTCAGTGGGCTGAT	Validation of pK7B4-promoter-B1-LHGATO4-B2 expression clones (Table 2) located in 5' end of <i>LhG4</i>

^a The *attB* sites are in bold.

Supplemental Table S3. Composition of MicroTom transformation media (per liter)

Components	Liquid KCMS	Solid KCMS	2Z	Rooting medium	0.5x MS
MS (basal salt; Duchefa MO221) (g)				2.2	
MS (Including vitamins; Duchefa , MO222) (g)	4.4	4.4	4.4		2.2
Sucrose (g)	20	20	30	10	15
KH ₂ PO ₄ (mg)	200	200			
pH	5.8	5.7	5.8	5.8	5.9
Agar (g)	8		8	7	8
Thiamine (mg L ⁻¹)	0.9	0.9			
Acetophenone (μM)	200	200			
2,4D (μg L ⁻¹)	200				
Kinetin (μg L ⁻¹)	100				
R3 vitamins (μL)					500
Nitsch vitamins x1000 (mL)			1	1	
Zeatin riboside (mg L ⁻¹)			2l		
Timentin ((mg L ⁻¹)			250, then 150	75	

Supplemental Table S4. Vitamins for MicroTom transformation media

Vitamins	Compound	Concentration
R3	Thiamine	1 g L ⁻¹
	Nicotinic acid	0.5 g L ⁻¹
	Pyridoxine	0.5 g L ⁻¹
Nitsch	Biotin	0.05 mg L ⁻¹
	Folic acid	0.5 mg L ⁻¹
	Glycine	2 mg L ⁻¹
	Myo-inositol	100 mg L ⁻¹
	Nicotinic acid	5 mg L ⁻¹
	Pyridoxine HCl	0.5 mg L ⁻¹
	Thiamine	0.5 mg L ⁻¹

Supplemental Table S5. Specific hpRNA modular destination vectors

Promoter	Corresponding modular hp vector
<i>PPC2</i>	pMK8GWIWGPPC2
<i>PG</i>	pMK8GWIWGPG
<i>TPRP</i>	pMK8GWIWGTPRP
<i>IMA</i>	pMK8GWIWGIMA
<i>CRC</i>	pMK8GWIWGCRC