Supplementary Material

Depletion of MOM1 in non-dividing cells of *Arabidopsis* plants releases transcriptional gene silencing

[1]Muhammad Tariq, [2]Yoshiki Habu, Jerzy Paszkowski

Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland

[1]Corresponding author: Tel: 41 61 6975583; Fax: 41 61 6973976; E-mail: tariq@fmi.ch

[2]Present address: National Institute of Agrobiological Science, Tsukuba 305-8602, Ibaraki, Japan

Running title: Release of silencing in non-dividing cells of *Arabidopsis*

Plasmid construction and plant transformation

A fragment of 503 bp, which comprises the 3' end of the open reading frame and the 3'UTR, was amplified from a *MOM1* cDNA (Accession No. AF213627) clone using specific primers (At3'asF *Pst* I, 5'-GCTCCTCTGCAGCCTCAGGCATCTTC-3' and At3'asR *Xho* I, 5'-AGAGACTCGAGCCAACGAGTCGGTTC-3'), including recognition sites for different restriction endonucleases. Similarly, another set of primers (At3'sF *Xba* I, 5'-GGCTCCTCTAGAGCCTCAGGCATCT-3' and At3'sR *Sal* I, 5'-AGAGACCGTCGACAACGAGTCGGTTC-3') was used to amplify the same *MOM1* cDNA fragment.

For inverted repeat *MOM* (*IRMOM*) constructs driven either by the *PR-1* promoter of *Arabidopsis thaliana* or the CaMV*35S* promoter, a synthetic intron *syn7* (Goodal and Filipowicz, 1989) was inserted at *Xba* I and *Pst* I sites between the CaMV*35S*-promoter and terminator in the pDH51 (Pietrzak *et al*., 1986) plasmid. A PCR-amplified *MOM* fragment was digested with *Xho* I and *Pst* I and cloned in antisense orientation between the *syn7* intron and the CaMV*35S* terminator in pDH51, resulting in pDH*35SasMOM.* Similarly a second PCR-amplified, *Xba* I and *Sal* I-digested *MOM1* fragment was cloned in sense orientation between the CaMV*35S*-promoter and the *syn7* intron, resulting in pDH*35S-IRMOM*. Finally, the *35S-IRMOM* cassette was excised by *Eco* RI and inserted into pCambia1300 (Accession No. AF234296) binary vector, resulting in the pC*35S-IRMOM* used for plant transformation.

For the *PR1-IRMOM* construct, the pDH*35S-IRMOM* was digested with *Bam* HI and *Sac* I to release the *IRMOM* cassette, which was then cloned into *Bam* HI and *Sac* I sites

downstream of the 4258-bp *PR-1* promoter of *A. thaliana* (Lebel *et al*., 1998) to yield p*PR1-IRMOM*. The *PR1-IRMOM* cassette from p*PR1-IRMOM* was moved as an *Eco* RI fragment to pCambia1300 to generate pC*PR1-IRMOM*, which was used for plant transformation. pC*35S-IRMOM* and pC*PR1-IRMOM* were introduced into *A. thaliana* line 6b5 by *in planta* vacuum infiltration (Bechtold *et al*., 1993).

***Act2* (*Actin2*) primer sequence**

*Act2*F: CTAAGCTCTCAAGATCAAAGGC

*Act2*R: AACATTGCAAAGAGTTTCAAGG

References

Bechtold, N., Ellis, J. and Pelletier, G. (1993) In planta Agrobacterium mediated gene transfer by infiltration of adult Arabidopsis thaliana plants. *C. R. Acad. Sci. Paris, Life Sci.*, **316**, 1194-1199.

Goodall, G.J. and Filipowicz, W. (1989) The AU-rich sequences present in the introns of plant nuclear pre-mRNAs are required for splicing. *Cell*, **58**, 473-483.

Lebel, E., Heifetz, P., Thorne, L., Uknes, S., Ryals, J. and Ward, E. (1998) Functional analysis of regulatory sequences controlling PR-1 gene expression in Arabidopsis. *Plant J.*, **16**, 223-233.

Pietrzak, M., Shillito, R. D., Hohn, T. and Potrykus, I. (1986) Expression in plants of two bacterial antibiotic resistance genes after protoplast transformation with a new plant expression vector. *Nucleic Acids Res.*, **14**, 5857-5868.