Appendix 1

Construction of binary vectors for mEosFP-tagging and plant transformation

From the pcDNA3 EosFP T158H/V123T vector [1], we amplified the 681 bp *monomeric EosFP* (*mEosFP*) fragment by proofreading PCR with the two primers PDS1224 (5'-ATAGGATCCAGTACTATGAGTGCGATTAAGCCAG-3') and PDS1225 (5'-ATAGGATCC TTATCGTCTGGCATTGTCAG-3'), which introduced a N-terminal *Bam*HI and *Sca*I restriction sites and also a C-terminal *Bam*HI restriction site. Subsequently this fragment was introduced into the pGD120 vector [2] by *Bam*HI restriction and ligation, creating the vector CZN319. A Gateway box (GW) with reading frame B (Invitrogen) was introduced upstream of the *mEosFP* fragment by *Sca*I restriction and ligation, creating the vector CZN320. The expression cassette containing the *CaMV35S promoter_GW_mEosFP_NOS terminator* from CZN320 was then introduced into the binary vector pGD121 [3] by double digestion with *Asc*I/*Pac*I and ligation, creating the destination vector CZN321. Subsequently, the *CaMV35S* promoter element was cut out from CZN321 by double digestion with *Asc*I/*Xba*I followed by self-ligation, creating the destination vector CZN351. This destination vector was used to make the final expression clones by LR reaction with the previously made entry vector containing the genomic clone of the MADS box gene *APETALA1* [4]. The expression clone *gAP1:mEosFP* (CZN389) was then transformed to wild type *Arabidopsis thaliana* Columbia-0 plants via *Agrobacterium tumefaciens* strain GV3101 using the floral dip method [5].

Appendix 2

Confocal Laser Scanning Microscopy of mEosFP-tagged proteins

To observe the localisation of mEosFP-tagged proteins in living plant material, the inflorescence was dissected and embedded as previously described [6]. Confocal Laser

Scanning Microscopy of the tissue was performed with a Leica SPE DM5500 upright microscope with a 63x ACS APO (NA 1.15 CORR) lens, using the LAS AF 1.8.2 software. The green version of the photoconvertible mEosFP tag was excited with the 488 nm solid state laser and scanned at a bandwidth of 505-550 nm, while the photoconverted red version of the mEosFP tag was excited with the 532 nm solid state laser and scanned at a bandwidth of 575-620 nm. The obtained confocal z-stacks were median filtered, converted to 3-D maximum projections and adjusted with Adobe Photoshop version 5.0. Photoconversion of the mEosFP fluorophore was performed on selected areas by using the maximal square zoom function (approximately 5 to 7 meristematic cells) and repeatedly scanning this zoomed-in area in one z-section with 405 nm laser light. Several laser intensities and photoconversion times were tested in order to obtain optimal photoconversion of the mEosFP-tagged MADS domain proteins.

References

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