

Supplemental file 1 – recombinant DNA manipulations

Isolation and Characterization of Arabidopsis Fip1 cDNAs - cDNAs derived from Fip1-encoding mRNAs were isolated from a number of sources by PCR and RT/PCR. Potential Fip1-encoding genes were identified in the *Arabidopsis* genome (<http://www.Arabidopsis.org/home.html>), with TBLASTN and BLASTP using the human and yeast Fip1 amino acids sequences as search queries. Based on the results, primers were designed to amplify the cDNA coding regions of AtFip1[V] as listed in Table 1. Primer sets for AtFip1[V] (5'FL/3'INT, 5'INT/3'INT1, 5'INT1/3'FL) were used in PCR reactions with first strand cDNA or with a 3-6 kb cDNA expression library for *Arabidopsis* (CD4-16; ABRC-DNA Stock Center; (1) as templates. PCR reactions contained a final concentration of 1X PCR buffer (Invitrogen), 5mM MgCl₂, 125µM dNTPs, and 1U of Taq DNA polymerase (Invitrogen), with 100 ng of each primer and template. PCR reactions were run at 95°C for 2 min, then 35 cycles of 95°C for 30 s, 55°C for 60 s, 72°C for 90s, and one step at 72°C for 7 min. PCR products of expected size (1478 bp, 1460 bp, and 899 bp) were subcloned into pGEM-T Easy vector and sequenced as described above. These three pGEM clones represent the 5'-end (N; bases 1-1478), middle region (M; bases 1220-2692) and 3'-end (C; bases 2672-3588) of the full-length coding region, respectively. All three clones are in the 5' to 3' direction from the T7 promoter in the vector. The pGEM-N and pGEM-M clones were digested with *Ava*I and *Nsi*I (American Allied Biochemical; AAB). All further digests were performed with AAB restriction enzymes. The resulting fragments M fragment (approx. 1200 bp) was ligated into pGEM-N with T4 DNA ligase (Invitrogen). This clone pGEM-N+M (bases 1-2692) and the pGEM-C clone (bases 2672-3588) were transformed into a dam-/dcm- strain of *E. coli* (Strain ER2925-New England Biolabs; NEB) to eliminate DNA methylation of a *Cla*I restriction site in both clones. Plasmid DNA was purified and digested with *Nco*I and *Cla*I. The resulting N+M fragment (2672 bp) was ligated into pGEM-C. The resulting clone yields a full-length protein coding region of 3588 bp, termed pGEM-AtFip1-V(FL). One clone was isolated for the very 5'-end of AtFip1-V (N) that contained a premature stop codon. This recombinant can only encode amino acids 1-137. This recombinant interacted with other subunits and was used for subsequent characterizations.

Cloning of cDNAs encoding Arabidopsis polyadenylation factor subunits - Database searches of the *Arabidopsis* genome with the yeast Pfs2 and human CstF 50, 64 and 77 subunits as search queries using TBLASTN and BLASTP identified potential homologs to each subunit. Based on the sequence information, primers were designed to amplify the cDNA coding regions of these genes using primers listed in Table 1. All of the following clones were generated by PCR under the same reaction conditions used for AtFip1-V.

For the cloning of the *Arabidopsis* Pfs2p homolog, primer sets of 5'FL/3'INT and 5'INT/3'FL were used in a PCR reaction with first strand cDNA as template. PCR products of approx. sizes (1212bp and 897bp) were subcloned into pGEM-T Easy vector and sequenced as mentioned above. Both clones were in the 5' to 3' direction from the T7 promoter in the vector. Both clones were then digested with *Eco*RV. The pGEM-1212 and pGEM-897 clones were further digested with *Sal*I and *Eco*RI, respectively, generating a fragment from pGEM-897 of approx. 900bp. The fragments were then treated with DNA Polymerase I, Large (Klenow) Fragment (Invitrogen) and purified. The 900bp fragment was ligated into pGEM-1212 and digested again with *Sal*I. The resulting clone represents a protein-coding region that is 1945bp.

For the cloning of the *Arabidopsis* CstF50 homolog, AtCstF50 primers 5'FL/3'INT were used in a PCR reaction with first strand cDNA as template. A PCR product of expected size (1190 bp) was subcloned into pGEM-T easy vector and sequenced as mentioned above. This clone is base 1 to 1190 of the predicted 1290 bp coding region. The 3'-end was amplified in a continuous (1-step) reverse transcriptase RCR (RT-PCR) reaction as mentioned above using primers 5'INT/3'FL. A RT-PCR product of expected size (685 bp) was digested with *Bgl*II and *Eco*RI and then subcloned into *Bam*HI and *Eco*RI digested pBLUESCRIPT-(KS) (Stratagene) using T4 DNA ligase (Invitrogen) and resulting clone sequenced. The pGEM clone was then digested with *Spe*I and *Not*I restriction enzymes and the resulting fragment (approx. 1200 bp) from the digest was ligated into the pBLUESCRIPT clone that had also been digested with *Spe*I and *Not*I. The resulting clone was then digested with *Bam*HI and then religated together to yield a full-length protein coding region of 1290 bp.

For the cloning of the *Arabidopsis* CstF64 homolog, AtCstF64 primers 5'FL/3'FL were used in a PCR reaction with a 2-3 kb cDNA expression library for *Arabidopsis* (CD4-15; ABRC-DNA Stock Center; (1)) as template. The resulting PCR product of expected size (1386 bp) was subcloned into pGEM-T Easy vector and sequenced as previously mentioned above. This represents the full-length protein coding region of the cDNA for AtCstF64.

For the cloning of the *Arabidopsis* CstF77 homolog, AtCstF77 primers 5'FL/3'FL were used in a PCR reaction with first strand cDNA as template. PCR product of expected size (2320 bp) was subcloned into pGEM-T Easy vector and sequenced as previously mentioned above. This represents the full-length protein coding region of the cDNA for AtCstF77.

For the cloning of the *Arabidopsis* CFI-25 homolog, AtCFI-25 primers 5'FL/3'FL were used in a PCR reaction with first strand cDNA as template. PCR product of expected size (669bp) was subcloned into pGEM-T easy vector and sequenced as previously mentioned above. This represents the full-length protein coding region of the cDNA for AtCFI-25.

For the cloning of the *Arabidopsis* PABN1 homolog, a full length cDNA was obtained from ABRC-DNA Stock Center ((2); clone #U10366). AtPABN1 primers 5'FL/3'FL were used in a PCR reaction with clone #U10366 as template and PCR product of expected size (684 bp) was subcloned into pGEM-T easy vector and sequenced as previously mentioned above. The resulting pGEM recombinants were digested with BglII and the resulting fragment (approx. 675bp) was subcloned into BamHI digested pGAD-C(1), pGBD-C(1), and pMAL-C2x for yeast two-hybrid and pulldown analysis.

A cDNA for an *Arabidopsis* PAP from chromosome IV was also generated. The protein coding region was amplified and cloned into three different fragments, the NTD which spans bases 1 to 910, MR that spans bases 390 to 1500, and CTD which spans bases 1440 to 2403. Clones of MR and CTD fragments were gifts in the form of pGEM-T Easy vector clones from Lisa Meeks. The NTD fragment was amplified from first strand cDNA using AtPAPIV primers 5'FL/3'INT in a PCR reaction. PCR product of expected size (910 bp) was subcloned into SmaI digested pBLUESCRIPT-(KS) and sequenced. pBLUE-NTD (pB-N) and pGEM-MR clones were then digested with NheI and NotI restriction enzymes. A fragment of approximately 1 kbp from pGEM-MR was ligated into the pB-N clone. The resulting pB-N-MR clone and pGEM-CTD were then digested with StuI and NotI restriction enzymes. A fragment of approx. 900 bp from the pGEM-CTD digest was then ligated into pB-N-MR yielding a full-length clone of 2403 bp. Full length cDNA was used as template in a PCR reaction with AtPAPIV primers 5'MR/3'MR and the resulting product (approx. 1110bp) was digested with BglII and then subcloned into BamHI digested pGEX-2T (Pharmacia Biotech).

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