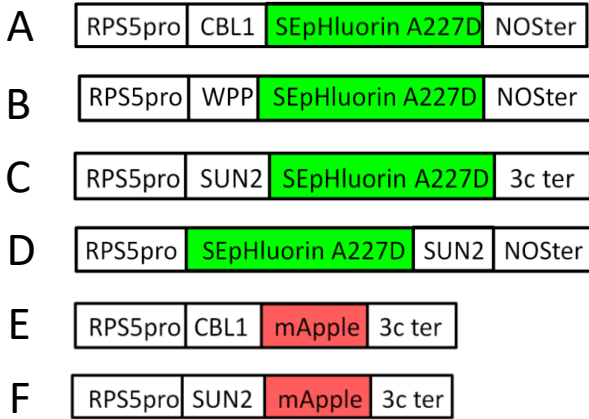
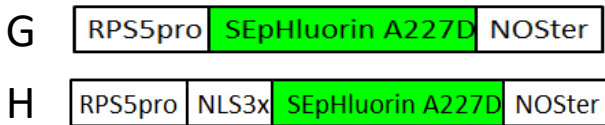


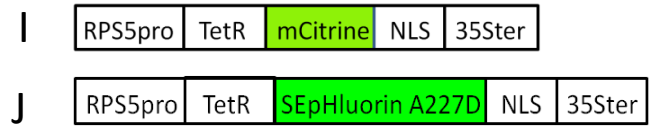
Membrane localized constructs:



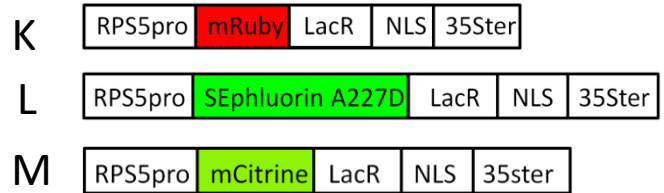
Cyto- and nucleoplasm constructs:



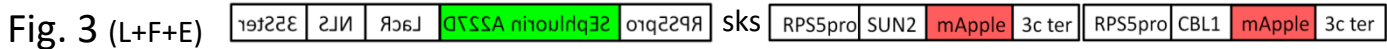
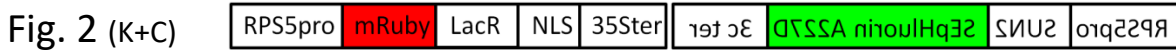
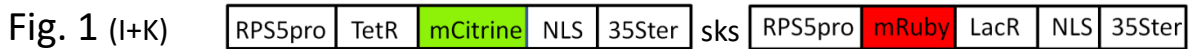
Constructs: with tet repressor



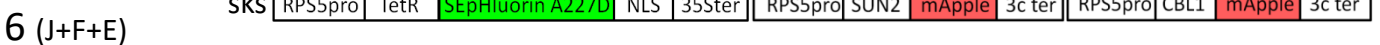
Constructs: with lac repressor



Combinations used in indicated figures:



Data Sheet



pH sensitivity of fluorescent proteins

mCitrine – pH-insensitive (Griesbeck et al., 2001)

SEpHluorinA227D – pH-sensitive (Matzke and Matzke, 2015)

mRuby – pH-insensitive (Kredel et al., 2009)

mApple – pH-sensitive, but fast bleaching (Shaner et al., 2008)

Data Sheet 1: Constructs and pH sensitivity of fluorescent proteins used in this study

Constructs were produced avoiding *SalI*, *XhoI* and *SacII* sites – if necessary having DNA synthesis carried out by Genscript (www.genscript.com) using standard molecular biology techniques. Building block constructs A-L were assembled on modified pBC plasmids (Stratagene, Cat. Nr. 21215) between *SalI* and *XhoI* sites. *SalI/XhoI* digestion releases the construct from the pBC plasmids. The released construct was ligated into the *SalI* site of binary vector pPZP221 with a 35Spro-driven gentamicin selection marker (Hajdukiewicz et al., 1994). The above binary vector constructs can be cut again with *SalI* and for construct combinations, more *SalI/XhoI* assembled constructs can be added. Tagging the *SalI/XhoI* assembled construct with *SalI SacII KAN SacII XhoI* (abbreviated as ‘sks’ in the combination constructs) allows direct selection for the combination constructs used for **Figs. 1-5**. The KAN selection marker can be deleted with *SacII* (combination construct **Fig. 2**), and a third *SalI/XhoI* assembled construct can be added (combination constructs **Figs 3 and 4**).

The respective binary vectors containing the desired transgene construct were introduced via *Agrobacterium*-mediated transformation into *Arabidopsis thaliana* using the floral dip method (Clough and Bent 1998). Seeds harvested from the dipped plants were surface sterilized and dispersed on solid Murashige and Skoog medium containing cefotaxime (200µg/µl) to eliminate agrobacteria and gentamicin (100µg/µl) for selection of transgenic plants (primary transformants).

Abbreviations: CBL1, a plasma membrane targeting motif (Batistic et al., 1998); SUN2, targeting motif for inner nuclear membrane (INM) Graumann et al., 2010, 2014); WPP, an outer nuclear membrane (ONM) targeting sequence (Deal and Henikoff, 2010); NOSTer, nopaline synthase transcriptional terminator; 3Cter, transcriptional terminator from the *rbcS3C* gene (Benfey et al., 1989); 35Ster, transcriptional from cauliflower mosaic virus; *RPS5A* pro, promoter from the *RIBOSOMAL PROTEIN S5* (At3G11940) (Weijers et al., 2001); TetR, tetracycline repressor protein; LacR, Lac repressor protein; NLS, nuclear localization signal from SV40.

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