Membrane localized constructs:

A RPS5pro | CBL1 | SEpHluorin A227D | NOSter

B RPS5pro | WPP | SEpHluorin A227D | NOSter

C RPS5pro | SUN2 | SEpHluorin A227D | 3c ter

D RPS5pro | SEpHluorin A227D | SUN2 | NOSter

E RPS5pro | CBL1 | mApple | 3c ter

F RPS5pro | SUN2 | mApple | 3c ter

Cyto- and nucleoplasm constructs:

G RPS5pro | SEpHluorin A227D | NOSter

H RPS5pro | NLS3x | SEpHluorin A227D | NOSter

Constructs: with tet repressor

I RPS5pro | TetR | mCitrine | NLS | 35Ster

J RPS5pro | TetR | SEpHluorin A227D | NLS | 35Ster

Constructs: with lac repressor

K RPS5pro | mRuby | LacR | NLS | 35Ster

L RPS5pro | SEphluorin A227D | LacR | NLS | 35Ster

M RPS5pro | mCitrine | LacR | NLS | 35ster

Combinations used in indicated figures:

Fig. 1 (I+K) RPS5pro | TetR | mCitrine | NLS | 35Ster sks RPS5pro | mRuby | LacR | NLS | 35Ster

Fig. 2 (K+C) RPS5pro | mRuby | LacR | NLS | 35Ster | 3c ter | SEpHluorin A227D | SUN2 | RPS5pro

Fig. 3 (L+F+E) 35Ster | NLS | LacR | SEphluorin A227D | RPS5pro sks RPS5pro | SUN2 | mApple | 3c ter RPS5pro | CBL1 | mApple | 3c ter

Fig. 4 (M+F+E) 35Ster | NLS | LacR | mCitrine | RPS5pro sks RPS5pro | SUN2 | mApple | 3c ter RPS5pro | CBL1 | mApple | 3c ter

Fig. 5 (K+L) RPS5pro | mRuby | LacR | NLS | 35Ster sks RPS5pro | SEpHluorin A227D | LacR | NLS | 35Ster

Data Sheet 6 (J+F+E) sks RPS5pro | TetR | SEpHluorin A227D | NLS | 35Ster RPS5pro | SUN2 | mApple | 3c ter RPS5pro | CBL1 | mApple | 3c ter

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 *Sal*I, *Xho*I and *Sac*II 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 *Sal*I and *Xho*I sites. *Sal*I/*Xho*I digestion releases the construct from the pBC plasmids. The released construct was ligated into the *Sal*I 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 *Sal*I and for construct combinations, more *Sal*I/*Xho*I assembled constructs can be added. Tagging the *Sal*I/*Xho*I assembled construct with *Sal*I *Sac*II KAN *Sac*II *Xho*I (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 *Sac*II (combination construct **Fig. 2**), and a third *Sal*I/*Xho*I 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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