

## Publication:

A toolset of aequorin expression vectors for in planta studies of subcellular calcium concentrations in *Arabidopsis thaliana*.

Mehlmer N, Parvin N, Hurst CH, Knight MR, Teige M, Vothknecht UC.

J Exp Bot. 2012 Feb;63(4):1751-61. doi: 10.1093/jxb/err406. Epub 2012 Jan 2.

## Description

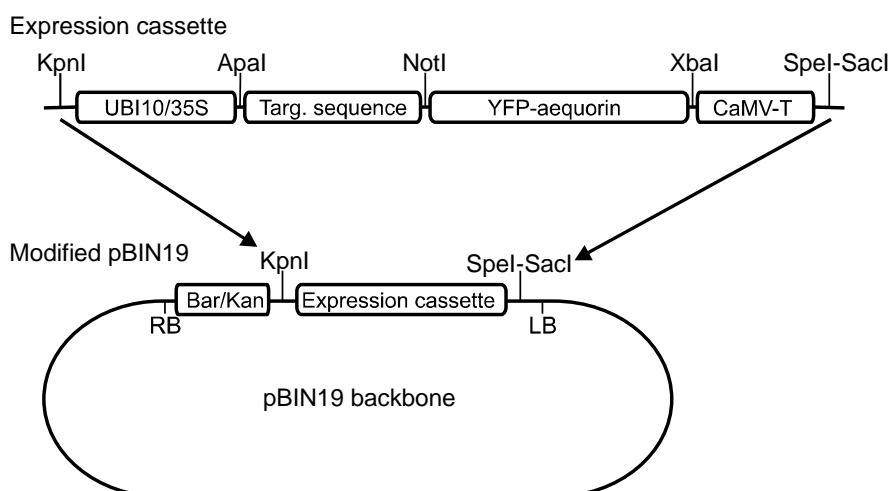
This collection contains a set of binary plasmids which targets the calcium sensor aequorin to different subcompartments of the plant cell. These constructs are aimed to generate transgenic *Arabidopsis* plants via agrobacterial transformation. The set includes constructs targeted to the cytosol, nucleus, inner surface of the plasma membrane, mitochondrial matrix, chloroplast stroma, as well as the inner and outer surface of the chloroplast envelope membranes.

## Detailed description

These sets of binary plasmids is based on the pBin19 binary plasmid (1). The coding sequence of aequorin was fused to different targeting peptides or organellar proteins as a means to localise it to various cell compartments. All constructs furthermore consist of a YFP-aequorin fusion that allows easy verification of the correct sub-cellular localisation. There are in total four sets of plasmids. The expression cassette is driven by either the 35S or the Ubiquitin-10 promoter, while at the same time plant selection resistances against Basta and kanamycin are available for each construct.

The modular set-up of the plasmids further allows the easy replacement of targeting sequences to include other compartments.

## The modified pBin19 plasmid



Localisation	Promoter	Plant selection	Paper (ID)
chloroplast	35S	Basta	pBIN-CHYA(B)
chloroplast	Ubiquitin	Basta	pBINU-CHYA(B)
chloroplast	35S	Kanamycin	pBIN-CHYA(K)
chloroplast	Ubiquitin	Kanamycin	pBINU-CHYA(K)
cytosol and nucleus	35S	Basta	pBIN-YA(B)
cytosol and nucleus	Ubiquitin	Basta	pBINU-YA(B)
cytosol and nucleus	35S	Kanamycin	pBIN-YA(K)
cytosol and nucleus	Ubiquitin	Kanamycin	pBINU-YA(K)
cytosol only	35S	Basta	pBIN-CYA(B)
cytosol only	Ubiquitin	Basta	pBINU-CYA(B)
cytosol only	35S	Kanamycin	pBIN-CYA(K)
cytosol only	Ubiquitin	Kanamycin	pBINU-CYA(K)
IE chloroplast	35S	Basta	pBIN-IEYA(B)
IE chloroplast	Ubiquitin	Basta	pBINU-IEYA(B)
IE chloroplast	35S	Kanamycin	pBIN-IEYA(K)
IE chloroplast	Ubiquitin	Kanamycin	pBINU-IEYA(K)
mitochondria	35S	Basta	pBIN-MYA(B)
mitochondria	Ubiquitin	Basta	pBINU-MYA(B)
mitochondria	35S	Kanamycin	pBIN-MYA(K)
mitochondria	Ubiquitin	Kanamycin	pBINU-MYA(K)
nucleus	35S	Basta	pBIN-NYA(B)
nucleus	Ubiquitin	Basta	pBINU-NYA(B)
nucleus	35S	Kanamycin	pBIN-NYA(K)
nucleus	Ubiquitin	Kanamycin	pBINU-NYA(K)
OE chloroplast	35S	Basta	pBIN-OEYA(B)
OE chloroplast	Ubiquitin	Basta	pBINU-OEYA(B)
OE chloroplast	35S	Kanamycin	pBIN-OEYA(K)
OE chloroplast	Ubiquitin	Kanamycin	pBINU-OEYA(K)
plasma membrane	35S	Basta	pBIN-PMYA(B)
plasma membrane	Ubiquitin	Basta	pBINU-PMYA(B)
plasma membrane	35S	Kanamycin	pBIN-PMYA(K)
plasma membrane	Ubiquitin	Kanamycin	pBINU-PMYA(K)

### Cited in

#### Genetically encoded Ca<sup>2+</sup> indicators: Properties and evaluation.

Pérez Koldenkova V, Nagai T.

Biochim Biophys Acta. 2013 Jan 22. pii: S0167-4889(13)00024-4. doi:

10.1016/j.bbamcr.2013.01.011.

#### The role of calcium in chloroplasts--an intriguing and unresolved puzzle.

Rocha AG, Vothknecht UC.

Protoplasma. 2012 Oct;249(4):957-66. Epub 2012 Jan 8.

### Reverences

#### 1) Binary Agrobacterium vectors for plant transformation.

Bevan, M.

Nucleic Acids Res. 1984.12(22): 8711-21.