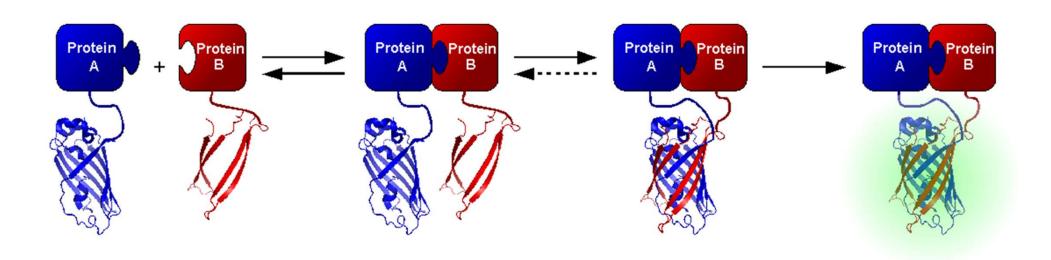
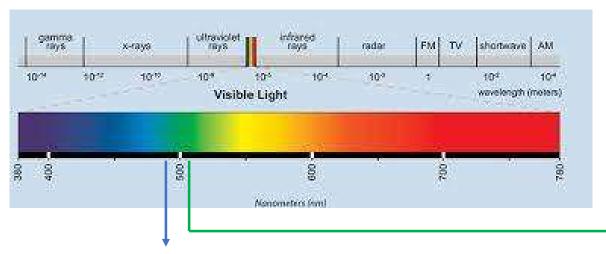
GFP can be expressed as two separate segments that combine within cells to yield a functional fluorescent protein:

Ex. BiFC (Bimolecular fluorescence complementation)



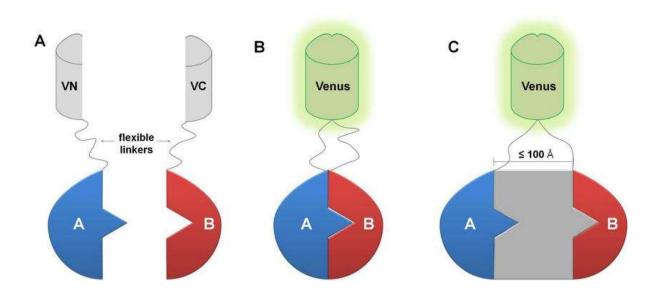
GFP - Protein that exhibits bright green fluorescence when exposed to light in the blue to UV range



Emission peak at 509 nm

488 nm – excitation peak for EGFP

Bimolecular Fluorescence Complementation (BiFC)



Yellow fluorescent protein (YFP)

-"Venus"- Genetic mutant of green fluorescent protein (GFP).

The requirement for fluorophore reconstitution is spatial proximity of the two proteins of interest, not necessarily direct interaction between them.

All commonly used BiFC vectors encode linker sequences (of varying lengths, but typically at least 5 amino acids long) between the FP fragment and the gene of interest, which may be important to provide sufficient structural flexibility of the fusion proteins to facilitate FP fragment reconstitution after interaction between the proteins of interest has occurred.

CLC (clathrin light chain)



CHC (clathrin heavy chain)

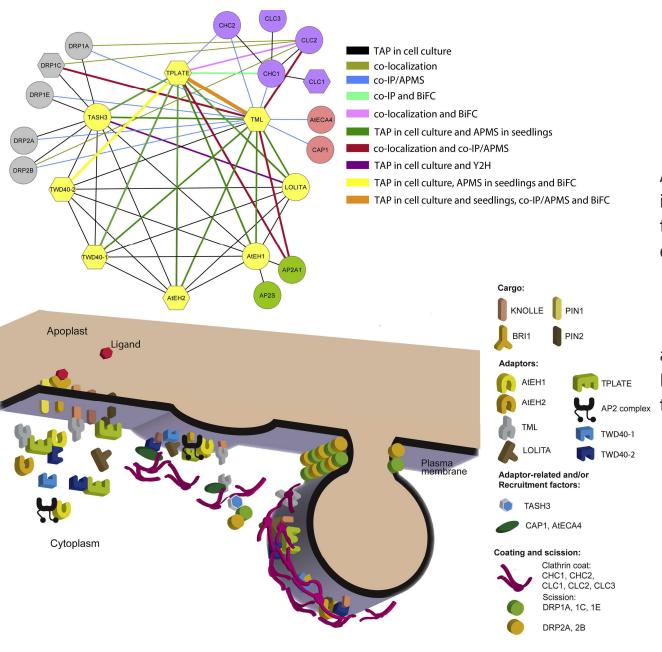
Created with SnapGene®

(3872) BfuAl - BspMI (3667) SbfI (3867) HindIII SbfI (3883) BseRI (47) (3661) SphI (3846 .. 3863) M13 Forward PshAI (183) (3651) HindIII PshAI (183) (3832 .. 3854) M13/pUC Forward BmgBI (246) EcoRV (274) (3630 .. 3647) M13 Forward BmgBI (246) (3707) PluTI 35S promoter (310 .. 330) (3616 .. 3638) M13/pUC Forward EcoRV (274) (3705) Sfol (3704) Narl (3491) PluTI 355 promoter (310 .. 330) (3703) Kasl Accl (445) (3489) Sfol (3653) **BstAPI** Sall (444) Hincll (446) (3488) Nari (3652) Ndel Btgl - Ncol - Styl (460) Accl (445) (3487) Kasl (3619 .. 3638) pRS-marker Hincll (446) EGFP-N (507 .. 528) (3437) BstAPI (3497 .. 3519) pGEX 31 Btgl - Ncol - Styl (460) (3436) Ndel (3457) EcoO1091 Bsql (552) (3403 .. 3422) pRS-marker (3441 .. 3459) pBRforEco M13 fwd CaM. CaMV 355 promo EGFP-C (656 .. 677) (3281 .. 3303) pGEX 3' (3403) Aatii BsrGI (712) (3401) Zrai (3298) Pfol EXFP-R (768 .. 787) Eagl - Noti (725) (3285) Sspl (3241) EcoO1091 Afiii (770) (3225 .. 3243) pBRforEco BsaBI (804) (3031 .. 3050) Amp-R BtgZI (813) (3187) Aatii BsrGI (928) Nsil (861) (3185) Zral (2961) Scal pBCC155 Eagl - Noti (941) pBCN155 3685 bp Afiii (986) (3069) Sspl MauBI (965) BsaBI (1020) (2880) Tsol (2815 .. 2834) Amp-R Apol - EcoRi (1017) Nsil (1077) (2745) Scal M13 Reverse (1031 .. 1047) M13/pUC Reverse (1044 .. 1066) (2664) Tsol MauBI (1181) Apol - EcoRI (1233) M13 Reverse (1248 .. 1264) BspQI - SapI (1256) L4440 (1262 .. 1279) (2542) Bsal (2326) **Bsal** (2481) AhdI 2000 BspQI - SapI (1472) (2265) AhdI L4440 (1478 .. 1495) pBR322ori-F (1513 .. 1532) pBR322ori-F (1729 .. 1748) AlwNI (1788) (2004) AlwNI

BiFC vector for transient expression of EGFP (1–155)

BiFC vector for transient expression of EGFP (155–239)

There is no gene for selection on plants



The TPLATE Adaptor Complex Drives Clathrin-Mediated Endocytosis in Plants

A large network of accessory proteins also function in linking the cargo as well as membrane lipids to the AP-2/clathrin assembly, inducing membrane curvature for invagination of the PM into vesicles.

adaptor proteins with domains such epsin N-terminal homology (ENTH), AP180 N-AP2 complex terminal homology (ANTH):

ENTH – pollen specific

ECA4 – ANTH domain adaptor protein CAP1 - ANTH domain adaptor protein

TPLATE is a plant-specific adaptin-like protein