

BiFC System info:

This Bimolecular Fluorescence Complementation system is based on the 172 N-terminal amino acids of **EYFP**, and the 85 C-terminal aa.s of **ECFP** (aa.s 154-239).

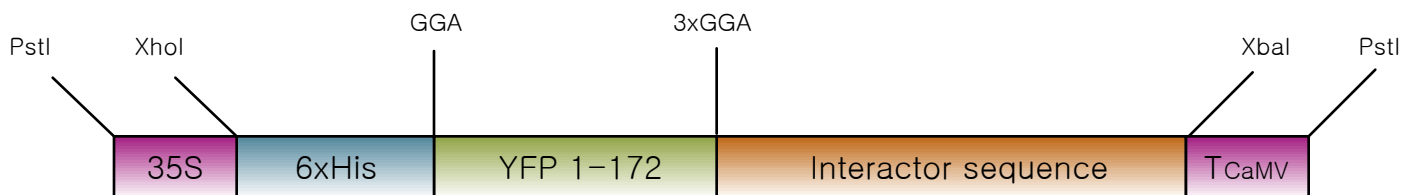
Use of the 4 different vectors yields C- and N-terminal fusions of a putative interactor protein to both of the of the fluorescent protein halves.

All 4 vectors are binary with a backbone derived from pZP312, a single **35S** promoter and a CaMV terminator (derived from pRT100).

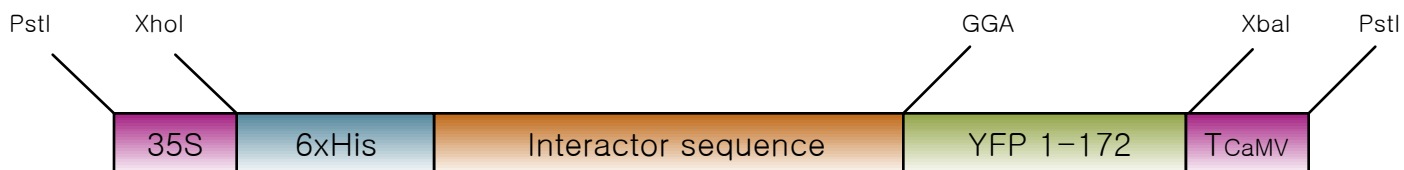
The vectors confer **Spectinomycin** resistance in *E. coli* and *Agrobacterium*, and **Basta** resistance in plants.

All vectors contain a **6xHis-tag** allowing for simple affinity-based purification of the expressed proteins.

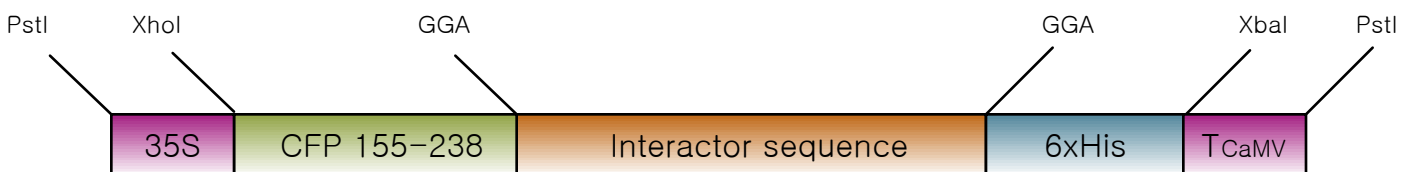
pNXGW



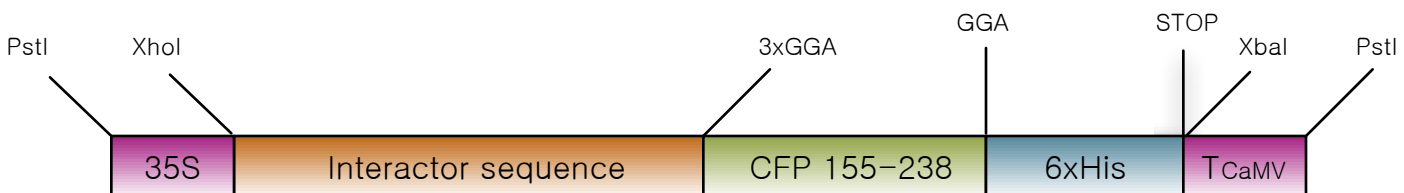
pXNGW



pCXGW



pXCGW



pXCGW

```

                Bg/II      attR1                                attR2
----421 ggacctcgag aga tct ACA AGT TTG TAC AAA AAA ---CmR---ccdB---TTC TTG TAC AAA GTG GTG
aag ctt ggt gga gct ggt gga gct ggt gga gct gcc ga c aag cag aag aac ggc atc aag gccaaactt--
HindIII      3XGGA
```

After LR reaction:

```

----421 ggacctcgag aga tct ACA AGT TTG TAC AAA AAA GCA GGC TNN (your clone) NAC CCA GCT TTC
TTG TAC AAA GTG GTG aag ctt ggt gga gct ggt gga gct ggt gga gct gcc ga c aag cag aag aac ggc
atc aag gccaaactt ---
```

If your entry clone is in pENTR/TOPO

```

----421 ggacctcgag aga tct ACA AGT TTG TAC AAA AAA GCA GGC TCC GCG GCC GCC  CCC TTC ACC (your
clone) AAG GGT GGG CGC GCC GAC CCA GCT TTC TTG TAC AAA GTG GTG aag ctt ggt gga gct ggt gga
gct ggt gga gct gcc gac aag cag aag aac ggc atc aag gccaaactt ---
```

pCX GW

```

                1XGGA      HindIII      attR1
--241 atggacgagc tgtacaag ggt gga gct aag ctt ACA AGT TTG TAC AAA AAA ---CmR---ccdB---TTC
TTG TAC AAA GTG GTG aga tct ggt gga gct cat cat cat cat cat cat taa tctag agtccgcaaa a----
attR2                Bg/II      1XGGA                                6XHis
```

After LR reaction:

```

----421 ggacctcgag aag ctt ACA AGT TTG TAC AAA AAA GCA GGC TNN (your clone) NAC CCA GCT TTC
TTG TAC AAA GTG GTG aga tct ggt gga gct cat cat cat cat cat cat taa tctag agtccgcaaa a ----
```

If your entry clone is in pENTR/TOPO

```

----421 ggacctcgag aag ctt ACA AGT TTG TAC AAA AAA GCA GGC TCC GCG GCC GCC CCC TTC ACC (your
clone) AAG GGT GGG CGC GCC GAC CCA GCT TTC TTG TAC AAA GTG GTG aga tct ggt gga gct cat cat
cat cat cat cat taa tctag agtccgcaaa a ----
```

pXNGW

-----421 ggacctcgag atgaatcatc atcatcat cat *Bg/II* *attR1*
TTG TAC AAA GTG GTG *aag ctt ggt gga gct atg gtgagcaag ggcgaggagc tgttcaccgg ggtggtgcc atcctg*---
attR2 *HindIII*
After LR reaction;

-----421 ggacctcgag atgaatcatc atcatcat cat *aga tct* ACA AGT TTG TAC AAA AAA GCA GGC TNN (your
clone) NAC CCA GCT TTC TTG TAC AAA GTG GTG *aag ctt ggt gga gct atg gtgagcaag ggcgaggagc*
tgttcaccgg ggtggtgcc atcctg---

If your entry clone is in pENTR/TOPO:

-----421 ggacctcgag atgaatcatc atcatcat cat *aga tct* ACA AGT TTG TAC AAA AAA GCA GGC TCC GCG
GCC GCC CCC TTC ACC (your clone) AAG GGT GGG CGC GCC GAC CCA GCT TTC TTG TAC AAA GTG GTG *aag*
ctt ggt gga gct atg gtgagcaag ggcgaggagc tgttcaccgg ggtggtgcc atcctg---

pNXGW

-----971 *aacatc**ggt gga gct ggt gga gct ggt gga gct* *aag ctt* *HindIII* *attR1*
---CmR---ccdB---TTC TTG TAC AAA GTG GTG *aga tct taa tctaga* gtccgcaaaa atcaccagtc---
attR2 *Bg/II* *XbaI*

After LR reaction;

-----971 *aacatc**ggt gga gct ggt gga gct ggt gga gct* *aag ctt* ACA AGT TTG TAC AAA AAA GCA GGC TNN
(your clone) NAC CCA GCT TTC TTG TAC AAA GTG GTG *aga tct taa tctaga* gtccgcaaaa atcaccagtc ---
Bg/II *XbaI*

If your entry clone is in pENTR/TOPO:

-----971 *aacatc**ggt gga gct ggt gga gct ggt gga gct* *aag ctt* ACA AGT TTG TAC AAA AAA GCA GGC TCC
GCG GCC GCC CCC TTC ACC (your clone) AAG GGT GGG CGC GCC GAC CCA GCT TTC TTG TAC AAA GTG GTG
aga tct taa tctaga gtccgcaaaa atcaccagtc ---
Bg/II *XbaI*