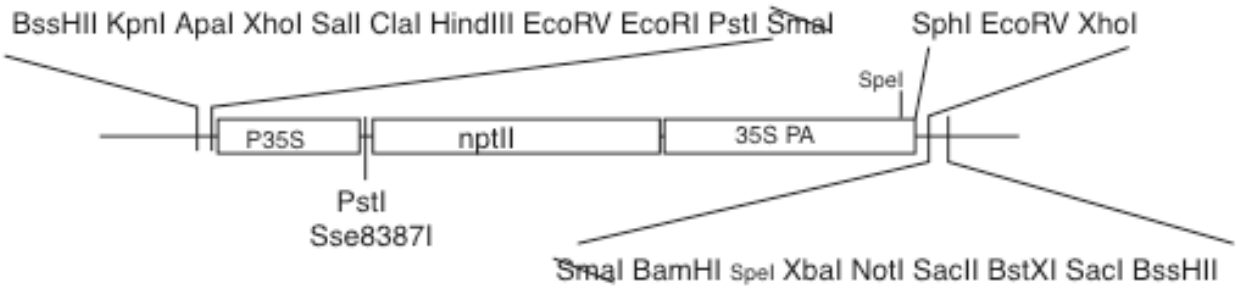
# 15 Available plasmids

1） gene disruption

| Name | marker | comment | ref |
|---|---|---|---|
| pTN3 | nptII | G418 resistant cassette | |
| pTN80 | nptII | Modified nptII cassette | |
| pTN81 | nptII | Modified nptII cassette | |
| pTN82 | nptII | Floxed modified nptII cassette | |
| pTN182 | NPTII | Floxed modified nptII cassette, MCS variant | |
| pTN86 | aph4 | Floxed modified aph4 cassette | |
| pTN186 | aph4 | Floxed modified aph4 cassette, MCS varinat | |
| pHIS14 | aph4 | Hygromycin resistant cassette | |
| p35S-Zeo | Ble | Zeocin resistant cassette | |

# pTN3

nptII cassette on pBluescript SKII(+)
A *Kpn*I-*Xho*I fragment from pMBL5 was inserted to the *Sma*I site of pBluescript SKII(+)




P35S: CaMV 35S promoter

Reference:
T. Nishiyama, Y. Hiwatashi, K. Sakakibara, M. Kato, and M. Hasebe. (2000)
Tagged Mutagenesis and Gene-trap in the Moss, *Physcomitrella patens* by Shuttle Mutagenesis. DNA Research 7: 9-17

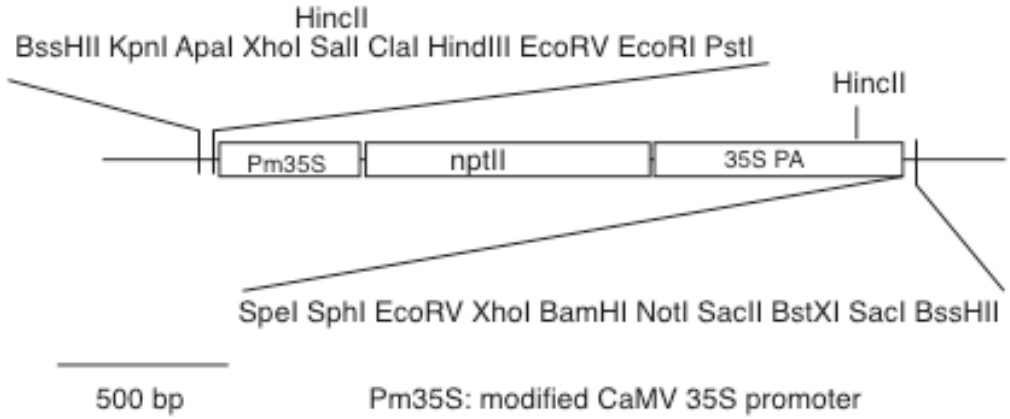
This document was written by Tomoaki Nishiyama
Any question may be sent to T. Nishiyama (tomoaki@nibb.ac.jp)

First edition 2001.1.16.

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

# pTN81

modified nptII cassette on pBluescript SKII+
The cauliflower mosaic virus 35S promoter of pTN3 was modified to confer kanamycin resistance in E.coli as well as G418 resistance in Physcomitrella. SpeI and XbaI sites was removed from pTN80 by digestion with BamHI and XbaI followed by blunting and self ligation.




EcoRV digest produces a 2.0 kb fragment (selection cassette) and a 3.0 kb fragment (vector).
EcoRV-HincII fragment is sufficient to confer G418 resistance in P. patens when targeted to Pphb7 locus.

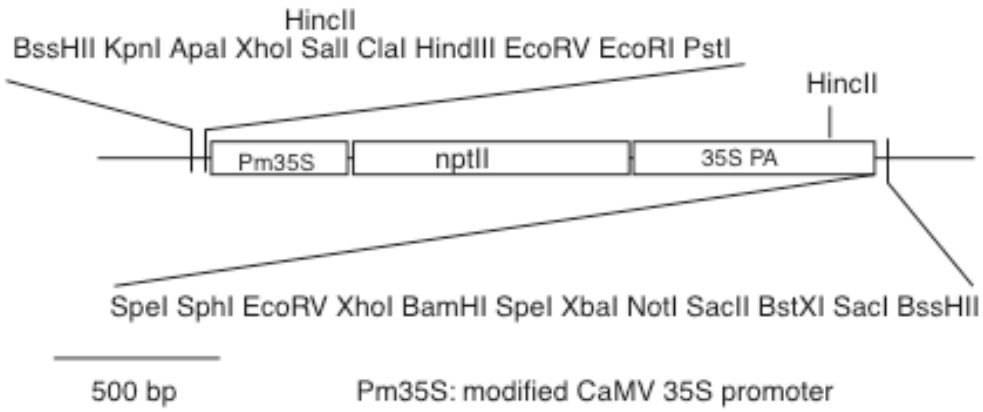
Any question may be sent to Tomoaki Nishiyama (tomoaki@nibb.ac.jp)

First edition 2001.1.24.

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

# pTN80

modified nptII cassette on pBluescript SKII+
The cauliflower mosaic virus 35S promoter of pTN3 was modified to confer kanamycin resistance in *E.coli* as well as G418 resistance in *Physcomitrella*.




*Eco*RV digest produces a 2.0 kb fragment (selection cassette) and a 3.0 kb fragment (vector).
*Eco*RV-*Hin*cII fragment is sufficient to confer G418 resistance in *P. patens* when targeted to *Pphb7* locus.

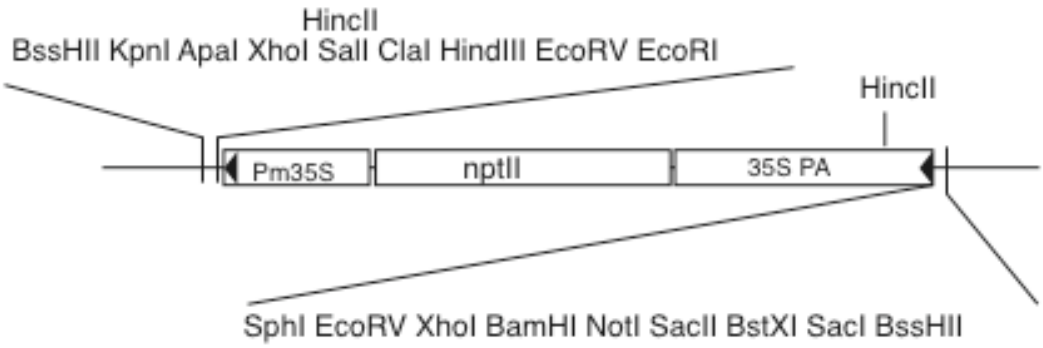
Any question may be sent to Tomoaki Nishiyama (tomoaki@nibb.ac.jp)

First edition 2001.1.16.

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

# pTN82

floxed modified nptII cassette on pBluescript SKII+
The cauliflower mosaic virus 35S promoter of pTN3 was modified to confer kanamycin resistance in E.coli as well as G418 resistance in Physcomitrella. Two loxP sites are inserted at the PstI and SpeI sites of pTN81 to make the whole cassette "floxed." The two loxP sites are in the same orientation, so that the marker cassette can be excised by the Cre site specific recombinase.




EcoRV digest produces a 2.1 kb fragment (the floxed selection cassette) and a 3.0 kb fragment (vector).

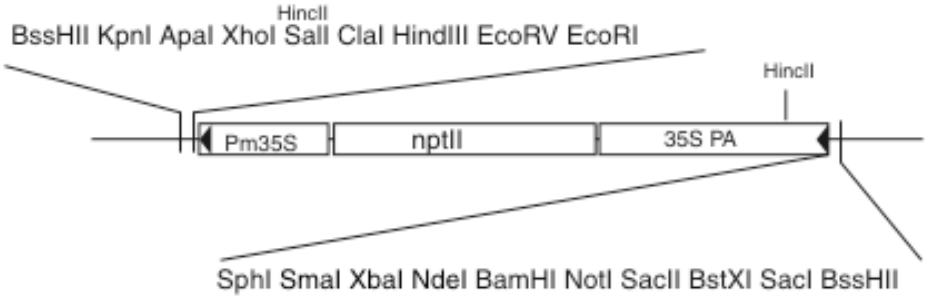
Any question may be sent to Tomoaki Nishiyama (tomoaki@nibb.ac.jp)

First edition 2001.3.16

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

# pTN182

floxed modified nptII cassette on pBluescript SKII+
The cauliflower mosaic virus 35S promoter of pTN3 was modified to confer kanamycin resistance in *E.coli* as well as G418 resistance in Physcomitrella. Two loxP sites are inserted at the *Pst*I and *Spe*I sites of pTN81 to make the cassette "floxed" (pTN82). The two loxP sites are in the same orientation, so that the marker cassette can be excised by the Cre site specific recombinase.




The expected sequence after excision is shown below.

```
GTGAGCGCGCGTAATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCATAACTT
    /                                       /     /     /     //        /     /       /    /
    BssHII                                KpnI    ApaI     XhoI  AccI     ClaI  HindIII     EcoRI
                                                        SalI                EcoRV

CGTATAGCATACATTATACGAAGTTATCCCTCACACCGGTGACGGGGATCGCATGC
                                /                    /
                              SgrAI              SphI
```

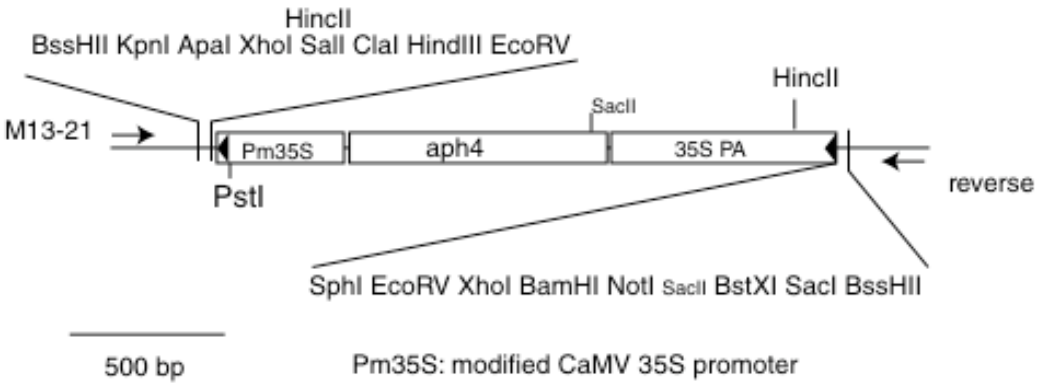
Any question may be sent to Tomoaki Nishiyama (tomoaki@nibb.ac.jp)

First edition 2004.1.22

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

# pTN86

Floxed hygromycin resistance cassette on pBluescript SKII+. *Eco*RI, *Pst*I, and *Eco*T14I sites were removed from aph4 gene (gb:V01499) on pHTS 14 with PCR. Two loxP sites are inserted at *Eco*RI and *Spe*I sites of pTN81 and nptII coding sequence was replaced with this modified coding siquence. This plasmid confers resistance to hygromycin at 30mg/l in *E. coli*.



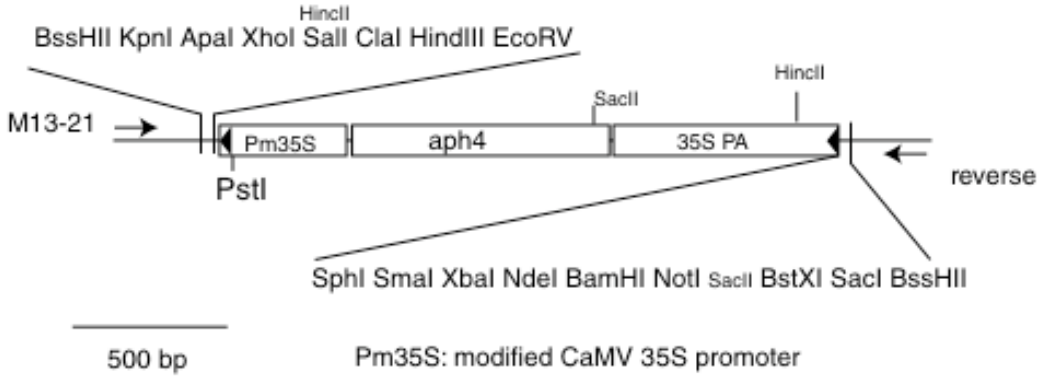

Any question may be sent to Tomoaki Nishiyama (tomoaki@nibb.ac.jp)

First edition 2003.7.31
revised on 2003.8.7
revised on 2003.10.5

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

# pTN186

Floxed hygromycin resistance cassette on pBluescript SKII+. *Eco*RI, *Pst*I, and *Eco*T14I sites were removed from aph4 gene (gb:V01499) on pHTS14 with PCR. Two loxP sites are inserted at *Eco*RI and *Spe*I sites of pTN81 and nptII coding sequence was replaced with this modified coding siquence (pTN86). EcoRV and XhoI sites in the 3' multiple cloning site were replaced with *Sma*I, *Xba*I, and *Nde*I sites. Construction of a disruption vector can be done by blunt end cloning of 5' and 3' flanking regions into *Eco*RV and *Sma*I sites.



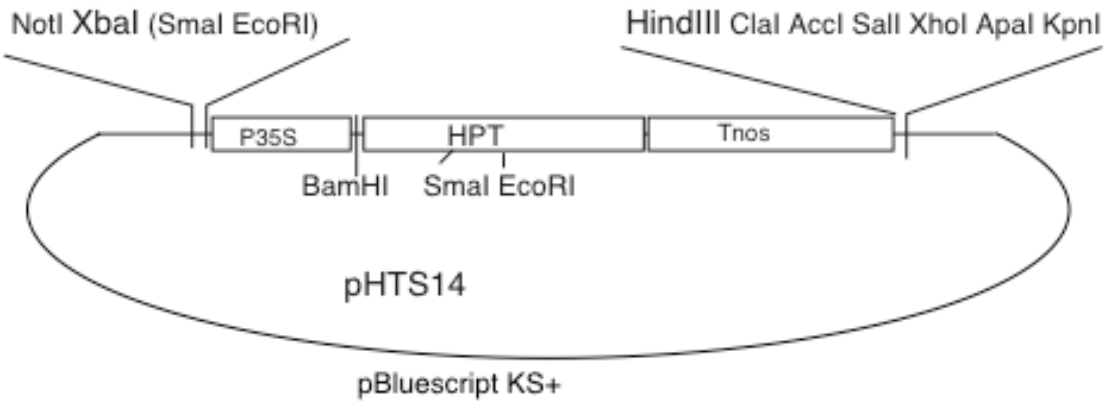

Any question may be sent to Tomoaki Nishiyama (tomoaki@nibb.ac.jp)

First edition 2004.1.22

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

# pHTS14

hygromycin resistance cassette on pBluescript KS(+)
Detailed information is not available.

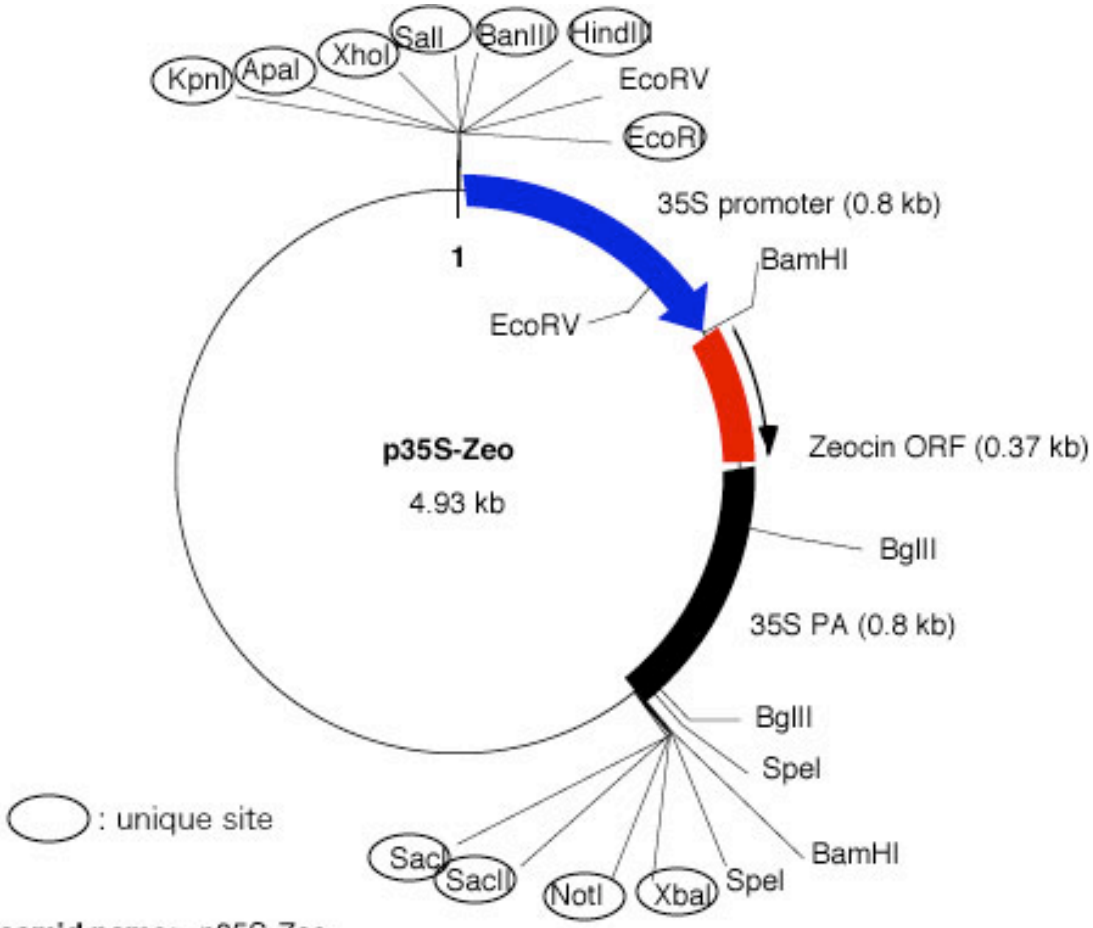



This plasmid was a gift from Dr. Hirokazu Tsukaya.
This map was drawn after his hand-written map. The metric may not be accurate.
Only *Xba*I and *Hin*dIII are tested to have a unique recognition site.

This document was written by Tomoaki Nishiyama (tomaoki@nibb.ac.jp)

First edition 2001.1.16.

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan




**Plasmid name:** p35S-Zeo

**Plasmid size:** 4.93 kb

**Constructed by:** Yuji Hiwatashi

**Construction date:** 24 Oct, 2002

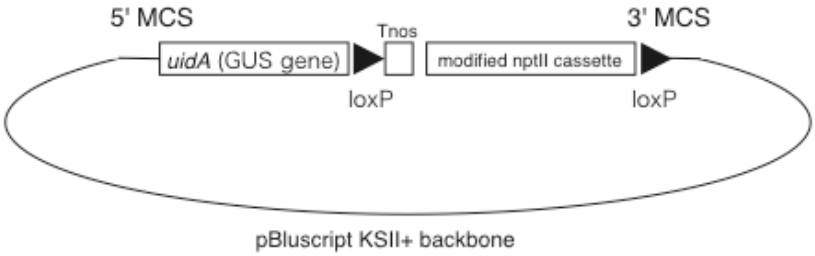
**Comment&Reference:** Zeocin expression cassette, pBluescript KSII(+)

2）GUS（GFP）expression vectors

| Name | reporter | marker | comment | ref |
|---|---|---|---|---|
| pGUSmutNPTII | uidA | nptII | GUS (M1L): to fuse GUS to a C terminal | |
| pTN83 | uidA | nptII | Modified nptII cassette: to fuse GUS to a C terminal | |
| pTN84 | uidA | nptII | Floxed modified nptII cassette: to fuse GUS to a C terminal | |
| pTN85 | uidA | nptII | GUS (M1L), Floxed modified nptII cassette: to fuse GUS to a C terminal | |
| pGFPmutNPTII | gfp | nptII | GFP(M1L): to fuse GFP to a C terminal | |
| pYHG2 | gfp | aph4 | GFP(M1L): to fuse GFP to a C terminal | |
| plinkerYHG2 | gfp | aph4 | GFP(M1L), linker sequence just before GFP;  to fuse GFP to a C terminal | |
| pHIZ2 | gfp | Ble | GFP(M1L): to fuse GFP to a C terminal | |
| pHIZ3 | gfp | ble | to fuse GFP to a N terminal | |

# pTN83

This plasmid is intended for replacing the coding sequence or inserting a reporter gene before the stop codon. This plasmid contains *uidA* reporter gene, nopaline synthase polyadenylation signal and the modified nptII cassette (see pTN80). The nopaline synthase polyadenylation signal and modified nptII cassette is flanked by loxP sites, so that the polyadenylation signal and selection marker can be, potentially, removed with Cre enzyme, thereby effect of foreign sequence will be kept minimal.




5' MCS

```
        10        20        30        40        50        60
GAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGTGGTCAGT
      / /     ///     /       /     /     /
     EcoICRI HspAlI  NotI     XbaI  SpeI  BamHI
     SacI BstXI       Eco52I
          SacII       BstZI
                      EagI

        70        80        90        100       110       120
CCCTTATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAACTCGACGGCCTGT
```

3' MCS

```
      4270      4280      4290      4300      4310      4320
TCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCG
        / /           /       /       /     /         /   / /
    PstI  EcoRI       HindIII         SalI  XhoI      ApaI  Acc65I
                      BanIII          PaeR7I    PspOMIKpnI
                      Bsp106I         BsoBI
                      BspDI
                      ClaI
```

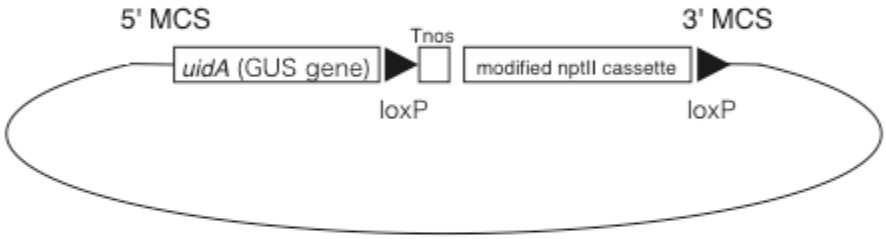
Any question may be sent to Tomoaki Nishiyama (tomoaki@nibb.ac.jp)

First edition 2003.5.9
updated on 2003.7.30

Tomoaki Nishiyama
National Institute for Basic Biology
Myoudaiji-cho, Okazaki-shi,
444-8585 Japan

pTN84

This plasmid is intended for replacing the coding sequence or inserting a reporter gene before the stop codon. This plasmid contains *uidA* reporter gene, nopaline synthase polyadenylation signal and the modified nptII cassette (see pTN80). The nopaline synthase polyadenylation signal and modified nptII cassette is flanked by loxP sites, so that the polyadenylation signal and selection marker can be, potentially, removed with Cre enzyme, thereby effect of foreign sequence will be kept minimal.




5' MCS

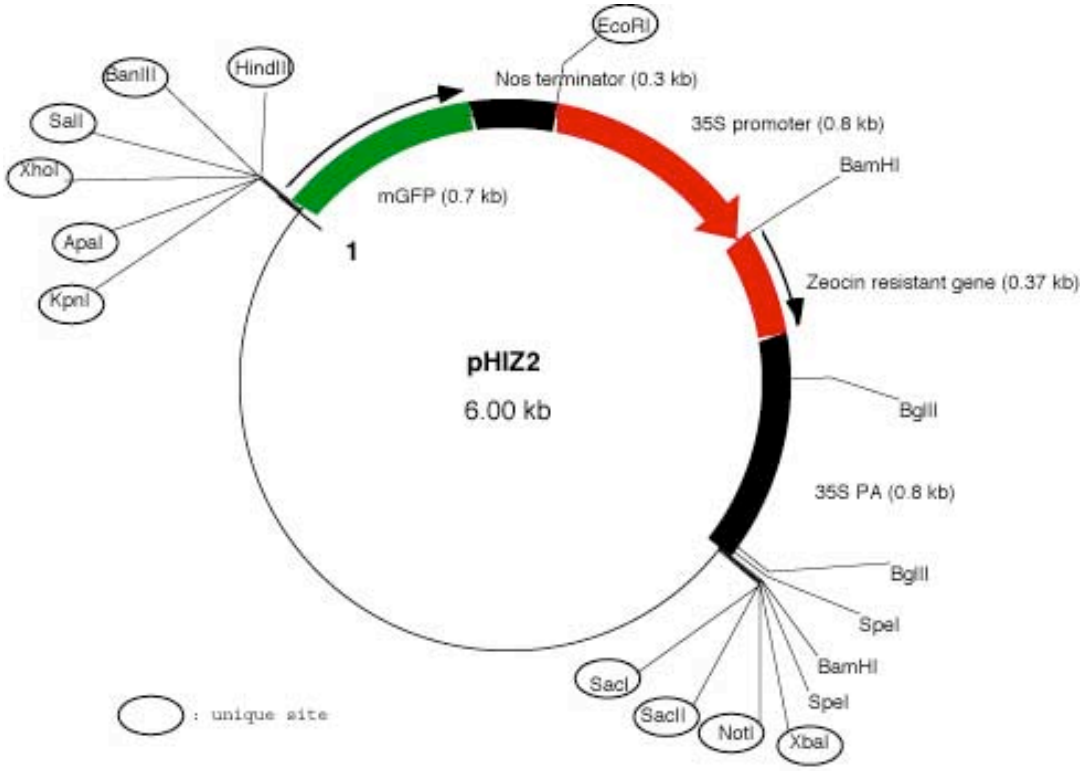
```
        10         20         30         40         50         60
AGCGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCCCTCGAGG
                                    /   / /   /    /     /
                                    Acc65IApaI     XhoI  SalI
                                    KpnI

        70         80         90        100        110        120
TCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCCTCAGTCCCTTATGTTACGT
         /    /          /          /
        ClaI  HindIII     EcoRI PstI
```

3' MCS

```
      4270       4280       4290       4300       4310       4320
ATAGCATACATTATACGAAGTTATCCCTCACACCGGTGACGGGGATCGGGGGGATCCACT
                                 /                     /     /
                                 AgeI                  BamHI SpeI

      4330       4340       4350       4360       4370
AGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAATTCGCCC
   /      /          //     / /
   XbaI  NotI   BstXI      SacI
                SacII
```




```
                                                          ATG (a start codon of GUS)
                                                                  ↓
5'MCS     TTG GGT ACC GGG CCC CCC CTC GAG GTC GAC GGT ATC GAT AAG CTT TTG GTG AG
              Kpn I   Apa I       Xho I   Sal I     Cla I  HindIII

3'MCS      - BamHI* - SpeI* - Xba I - Not I - Sac II - Sac I

              *: multiple site
```

**Plasmid name:** pHIZ2

**Plasmid size:** 6.00 kb

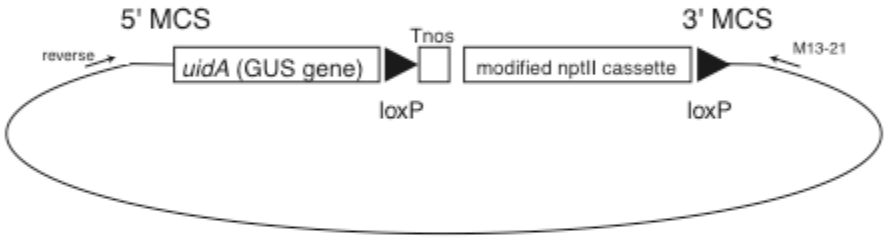
**Constructed by:** Yuji Hiwatashi

**Construction date:** 2 Nov., 2002

**Comment&Reference:** mGFP (start codon: ATG->TTG) expression vector, zeocin resistant, pBluescriptKSII

pTN85

This plasmid is intended to generate C-terminus GUS fusion construct, that is, inserting *uidA* (GUS coding sequence) without its start codon before the stop codon. This plasmid contains *uidA* reporter gene (M1L), nopaline synthase polyadenylation signal and the modified nptII cassette (see pTN80). The nopaline synthase polyadenylation signal and modified nptII cassette is flanked by loxP sites, so that the polyadenylation signal and selection marker can be, potentially, removed with Cre enzyme, thereby effect of foreign sequence will be kept minimal.




pBluscript KSII+ backbone

5' MCS

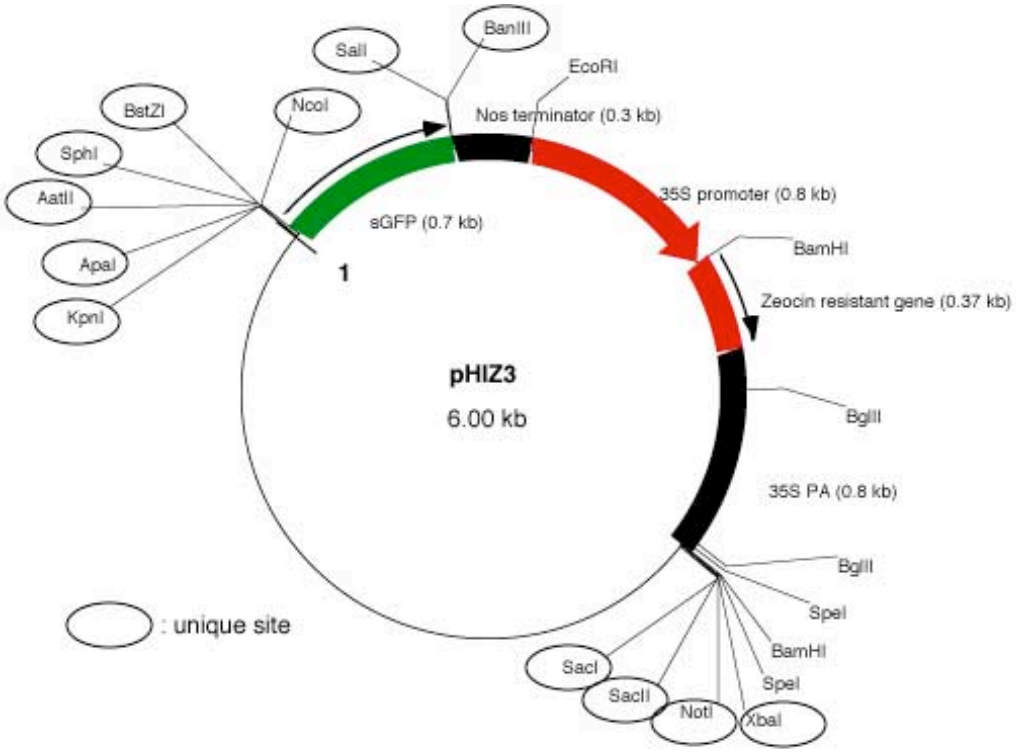
```
        10         20         30         40         50         60
CGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCCCCCCTCGAGGTC
                                  /    /     /     /     /
                                 Acc65IApaI      XhoI  SalI
                                 KpnI

        70         80         90        100        110        120
GACGGTATCGATAAGCTTTTGTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAA
       /    /
     ClaI  HindIII
```

3' MCS

```
      4270       4280       4290       4300       4310       4320
CCGGTGACGGGGATCGGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCT
                  /     /     /      /            //        /
                 BamHI SpeI  XbaI   NotI    BstXI       SacI
                                             SacII

      4330
CCAAT
```




目的cDNAのクローニング部位

TG TAC AAG TCG ACC GCT CCC GCC ATC GAT TAA
SalI ClaI Stop
GFP CDS

**Plasmid name:** pHIZ3

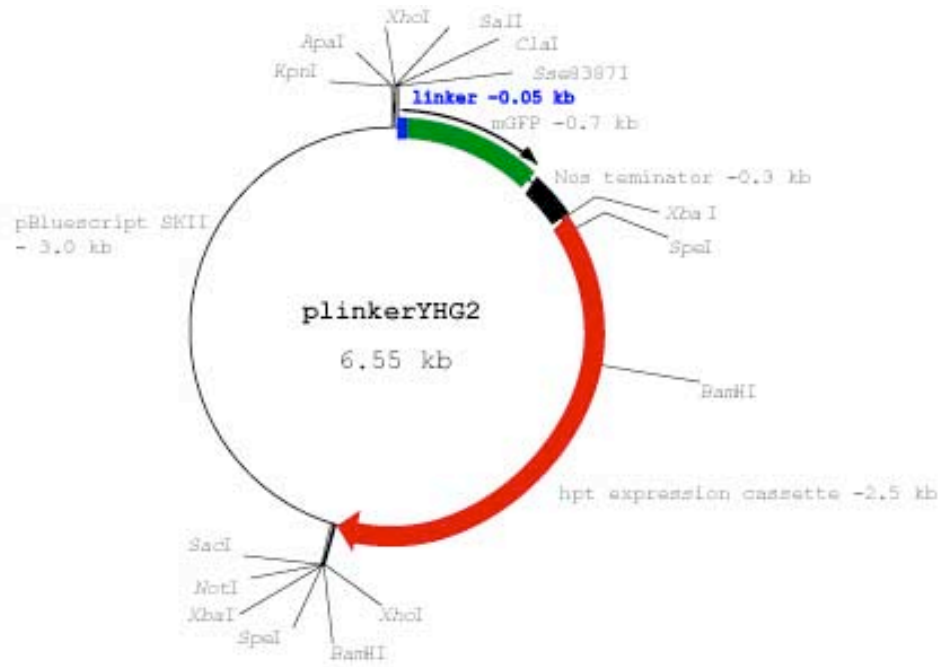
**Plasmid size:** 6.00 kb

**Constructed by:** Yuji Hiwatashi

**Construction date:** 2 Nov., 2002

**Comment&Reference:** sGFP (stop codon: TAA->TCG in a SalI site) expression vector,

zeocin resistant, pBluescriptKSII




5'MCS TTG GGT ACC GGG CCC CCC CTC GAG GTC GAC GGT ATC GAT TCC CTG CAG GCA
Kpn I Apa I Xho I* Sal I Cla I Sse8387 I

ATG (a start codon of GFP)

GGA GCA GGA GCA GGA GCA GGA GCA GGA GCA GGT CAG CTT TTG GTG AG

Hind III(AAGCTT)

3'MCS XhoI* - BamHI* - SpeI* - XbaI* - NotI - SacII? - SacI

*: multiple site

**Plasmid name:**plinkerYHG2

**Plasmid size:**6.55 kb

**Constructed by:**Junko Kawai

**Construction date:**20, Mar. 2003

**Comment&Reference:**Linker fragment (TAC GAT TCC CTG CAG GCA GGA GCA GGA GCA GGA GCA GGA GCA GGA GCA GGT CAG CTT) was inserted between ClaI and HindIII sites into pYHG2 plasmid (just upstream of the GFP gene).
HindIII site of 5'MCS of pYHG2 plasmid was eliminated to avoid putative protease recognition site (Lys-Leu).
Junction sequences is

TAC GAT TCC CTG CAG GCA **GGA GCA GGA GCA GGA GCA GGA GCA GGA GCA GGT CAG CTT** TTG

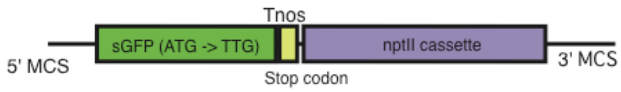
Cla I Sse8387 I

GFP

Hind III(AAGCTT)

Ile-Asp-Ser-Leu-Gln-Ala-**Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Gln-Leu**

pGFPmutNPTII




5' MCS

TTG GGT ACC GGG CCC CCC CTC GAG GTC GAC GGT ATC GAT AAG CTT TTG GTG AG
Kpn I * Apa I * Xho I Sal I * Cla I * HindIII *

ATG ↓ sGFP

3' MCS SpeI - SphI - EcoRV* - XhoI - BamHI* - SpeI - XbaI - NotI* - SacII* - SacI

* : unique site

Comment:
The GFP coding region was PCR-amplified and cloned into pTN3. A start codon of the GFP was modified into TTG in this plasmid.
This plasmid contains *GFP* (M1L), nopaline polyadenilation signal and a nptII cassette.

Bachbone:
pBluescriptII SKII (+)