Overexpression vectors

No	Name	Promoter	marker	Target site	Comment & references
1	pPpMAD2 7133	E7133	nptII	PpMAD2	
2	pPpMAD2 7133 rev	E7133	nptII	PpMAD2	
3	pPphb7 7133	E7133	nptII	Pphb7	
4	pPphb7 7133 rev	E7133	nptII	Pphb7	
5	pTFH9119	Actin	nptII	PpMAD2	
6	pPpMAD2 Actin rev	Actin	nptII	PpMAD2	
7	pTFH15.3	Actin	nptII	Pphb7	
8	pZAG1	Actin	zeocin	213 locus	GFP-fusion
9	pMAK1	7133	zeocin	213 locus	
10	pCMAK1	7133	zeocin	213 locus	
11	pTFH38.9N	CaMV35S	zeocin	213 locus	mRFP-fusion
12	pTFH38.9C	CaMV35S	zeocin	213 locus	mRFP-fusion
13	pKS2	Actin	NptII		GFP-fusion
14	pActN-GFP	Actin	NptII		GFP-fusion
15	pTFH22.4	Actin	NptII GFP		
16	pTFH38.6N	CaMV35S	None		mRFP-fusion, Transient expression
17	pTFH38.6C	CaMV35S	None		mRFP-fusion, Transient expression
18	pTKM1	Actin	None		Transient expression

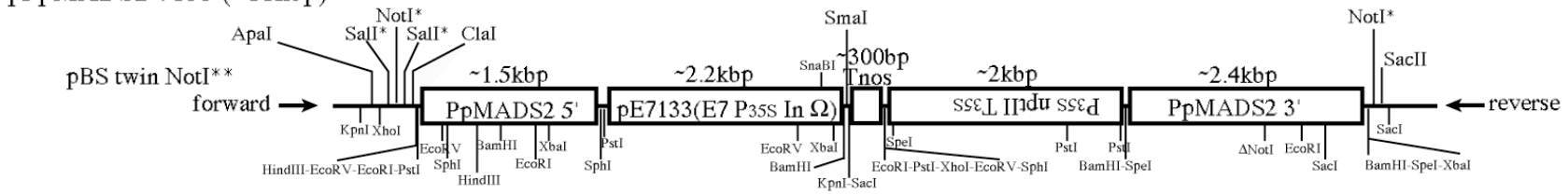
No. 1, 2, 3, and 4. pPpMAD2 7133, pPpMAD2 7133 rev, pPphb7 7133, and pPphb7 7133 rev.

Restriction sites indicated above the construct have a unique restriction site, indicated under the contruct have multiple sites.

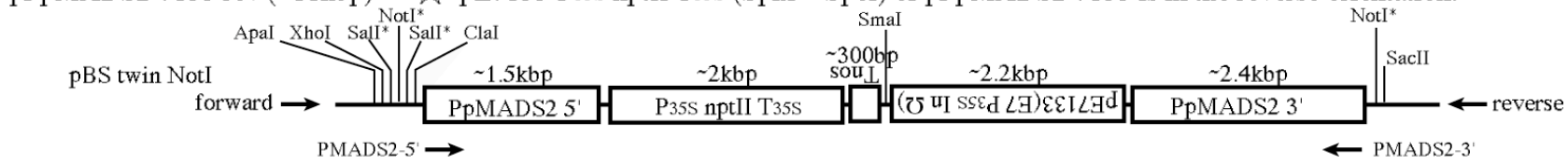
* Not unique but does not cut the insert.

(Revised by T.Fujita 12/1/01)

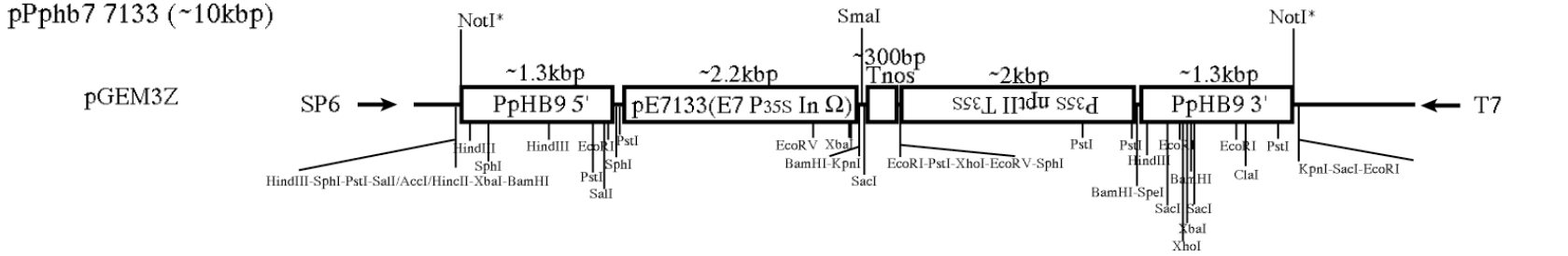
pPpMADS2 7133 (~11kbp)



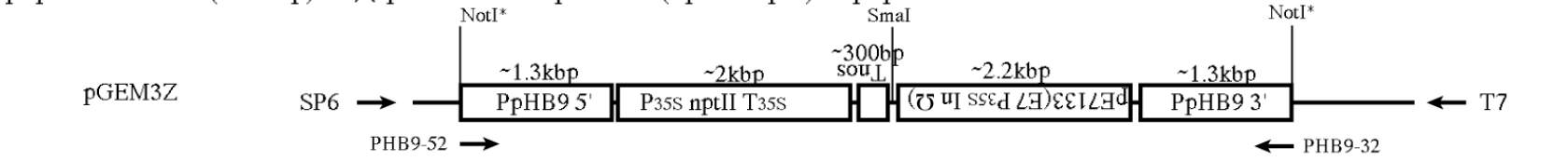
pPpMADS2 7133 rev (~11kbp) ☆ pE7133-P35S nptII T35S (SphI～SpeI) of pPpMADS2 7133 is in the reverse orientation.



pPphb7 7133 (~10kbp)



pPphb7 7133 rev (~10kbp) ☆ pE7133-P35S nptII T35S (SphI～SpeI) of pPpHB9 7133 is in the reverse orientation.

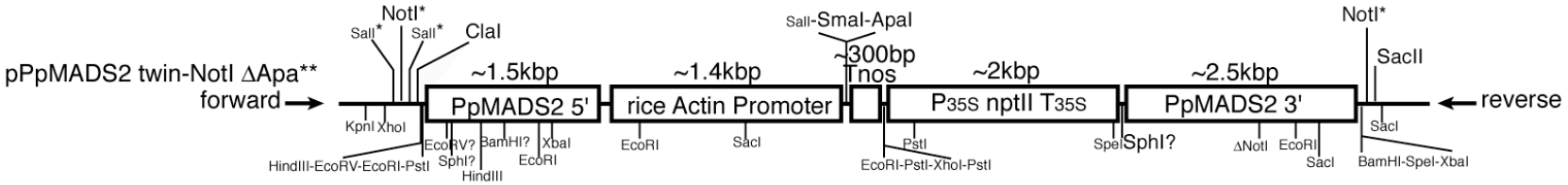


No. 5 and 6. pTFH9119 and pPpMAD2 Actin rev

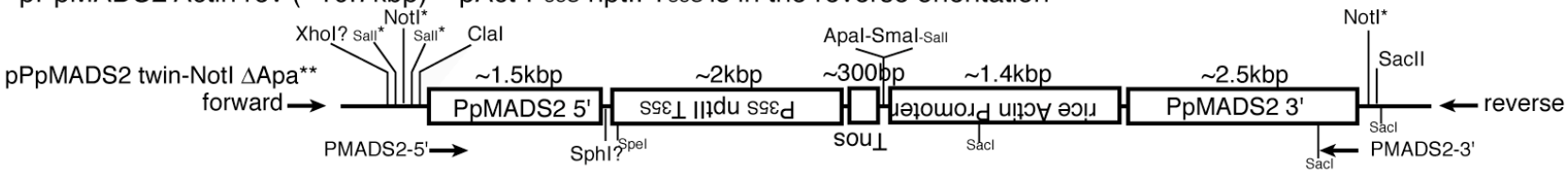
Restriction sites with a single recognition site are written with a large font, while restriction sites with multiple recognition sites are written small.

* Not unique but does not the insert

pTFH9119 (#6)
= pPpMADS2 Actin (~10.7kbp)



pPpMADS2 Actin rev (~10.7kbp) pAct-P35S nptII T35S is in the reverse orientation



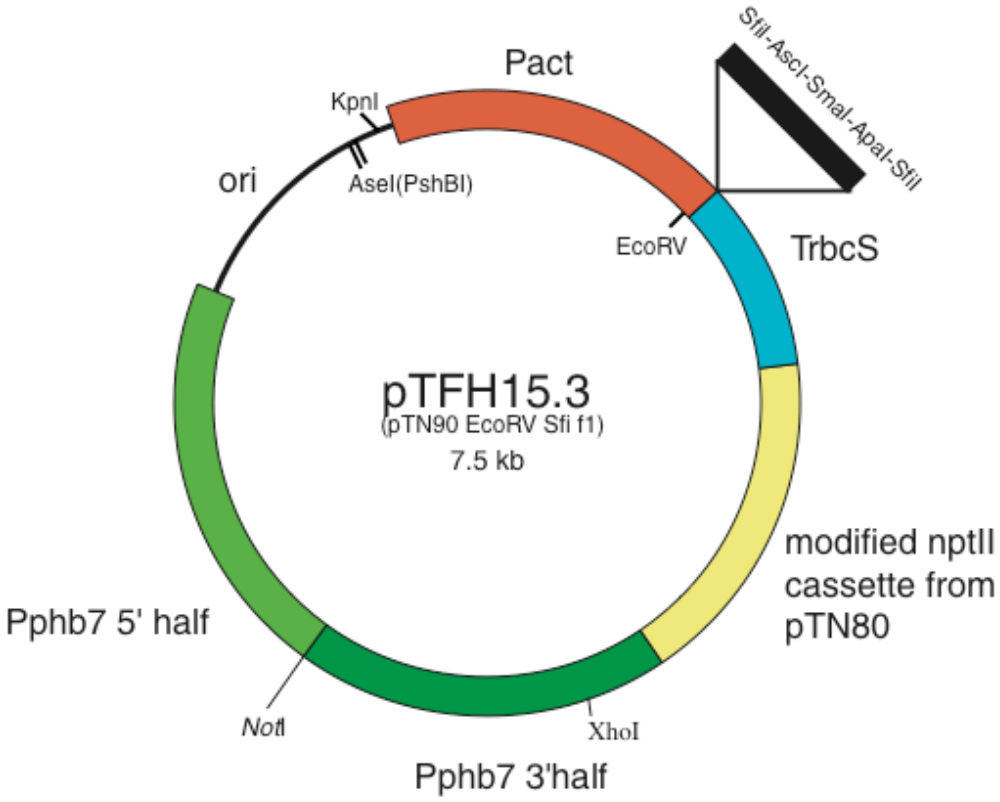
TFH#9-1(16), #9-1(19)

last modified on 1/22/01
translated 10/2/03

No. 7. pTFH15.3

TFH#15-3(8)

5/30/01



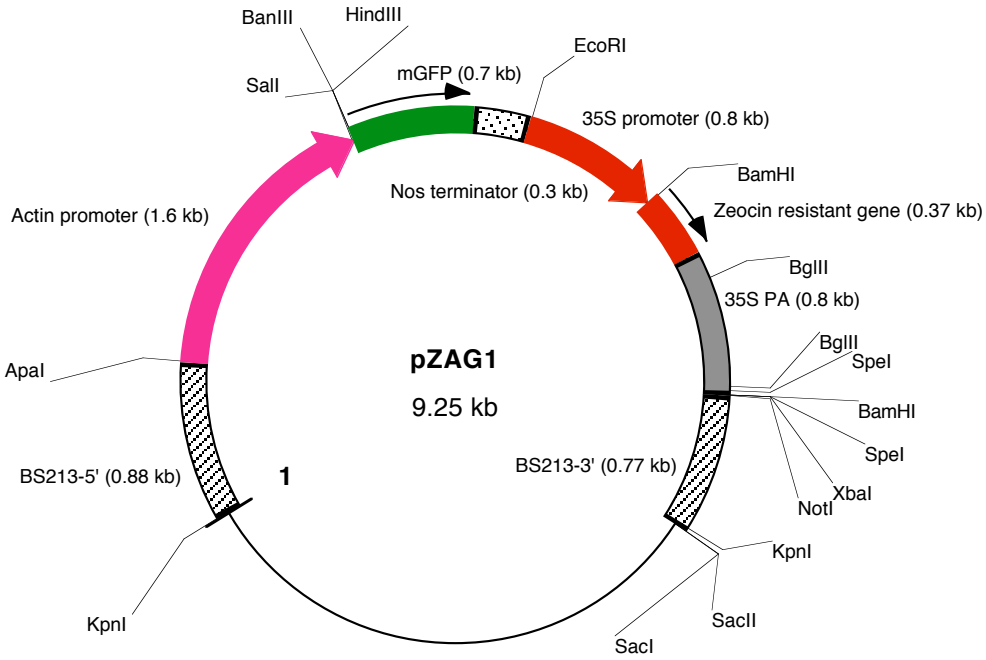
* pTFH15.3r = pTN90 EcoRV Sfi r1, opposit direction of the linker

```
  EcoRV SfiI          AscI      SmaI    ApaI   SfiI
aaGATATCGGCCATTCAGGCCGGCGCGCCACCCGGGAGGGCCCGGCCGATTTGGCCatca
```

No cut-- SpeI, BsmI, MluI, PmaCI, PmeI,
1 cut -- SacII, Sse8387I, RsrII, NruI, BssII, BstEII, KpnI, SmaI, AscI, ApaI, XhoI
more than 2 cut -- SgrAI

TFH#15-4(1), #15-4(5), #15-3(2), #22-1(1)

No. 8. pZAG1



Plasmid name:pZAG1

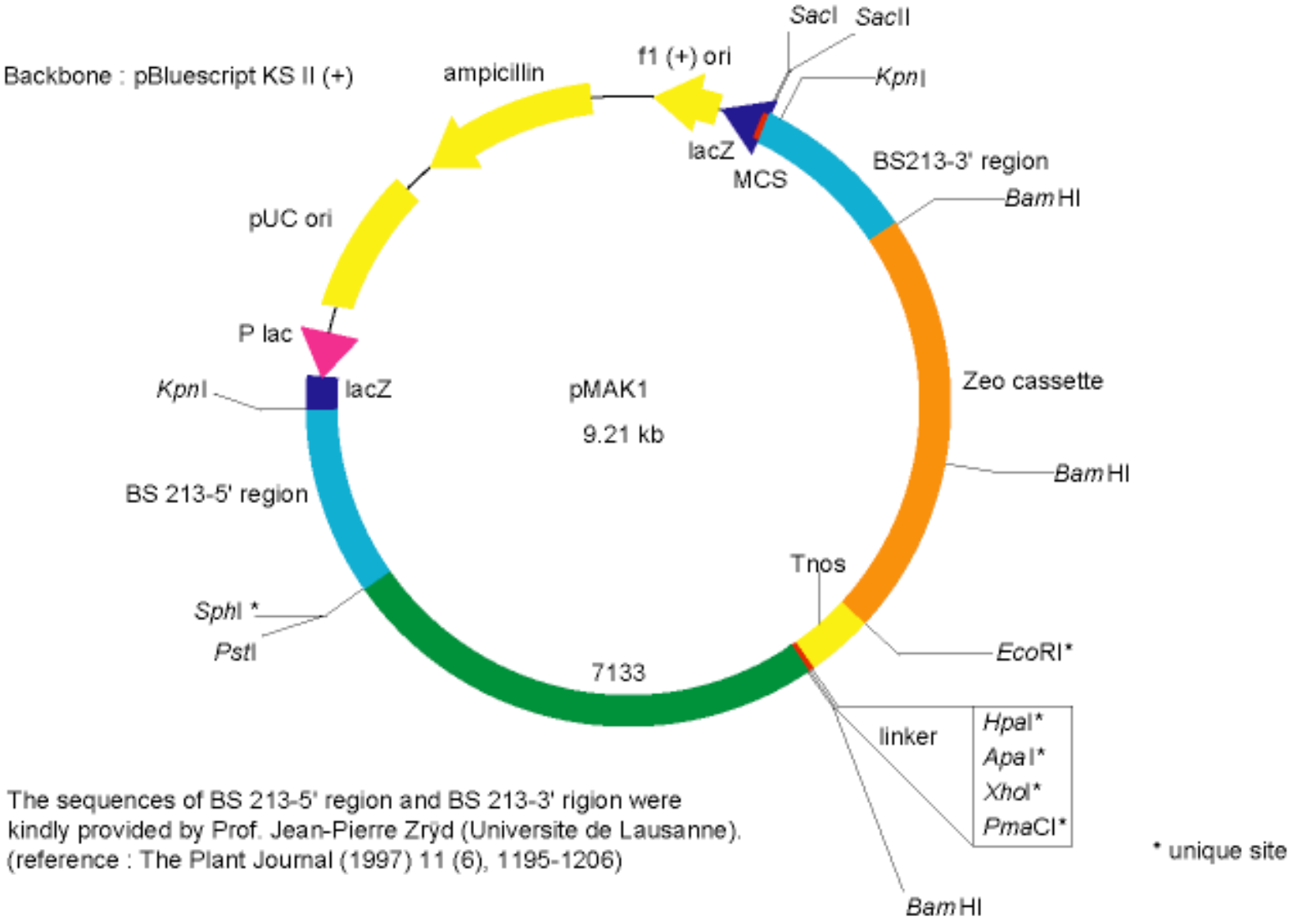
Plasmid size:9.25 kb

Constructed by: Yuji Hiwatashi

Construction date:12 Feb., 2003

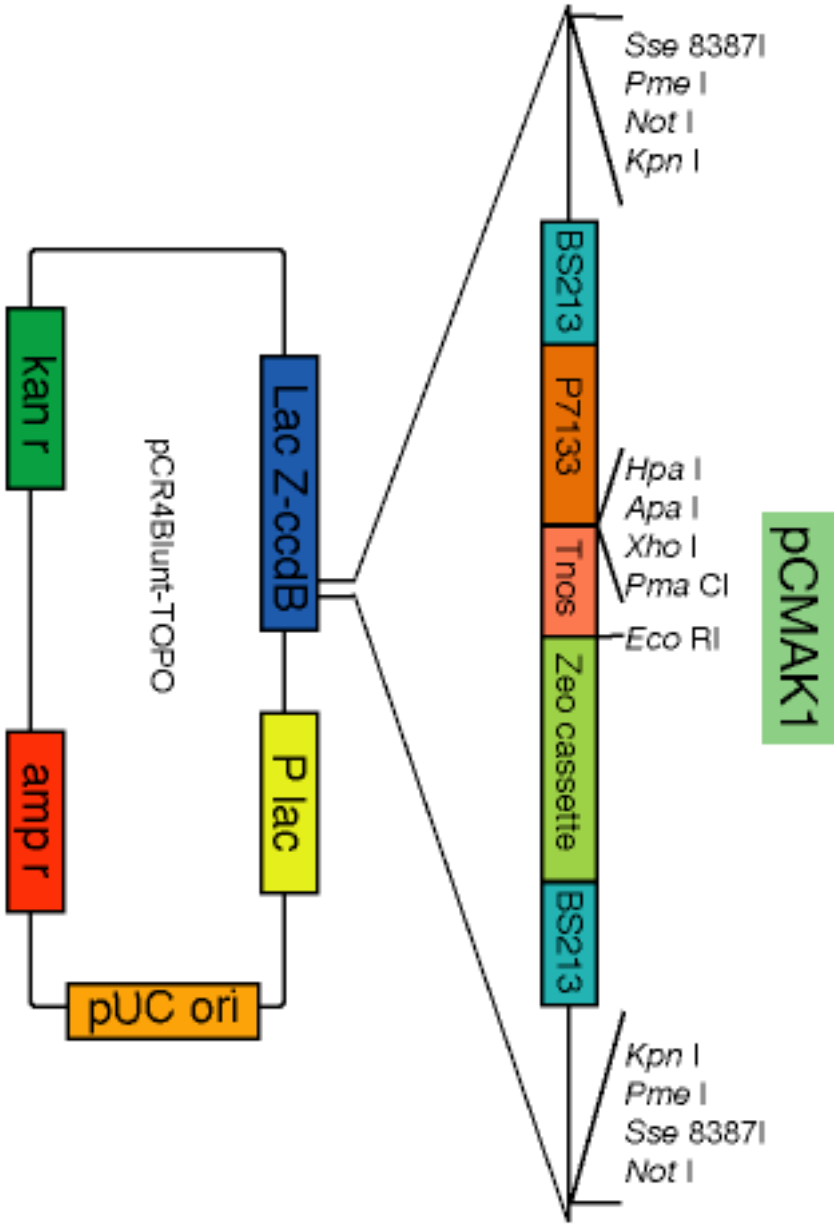
Comment&Reference:mGFP (start codon: ATG->TTG) expression vector
under control of rice actin promoter,
zeocin resistant, pBluescriptKSII.
BS213 fragments are used as a platform for integration.

No.9. pMAK1

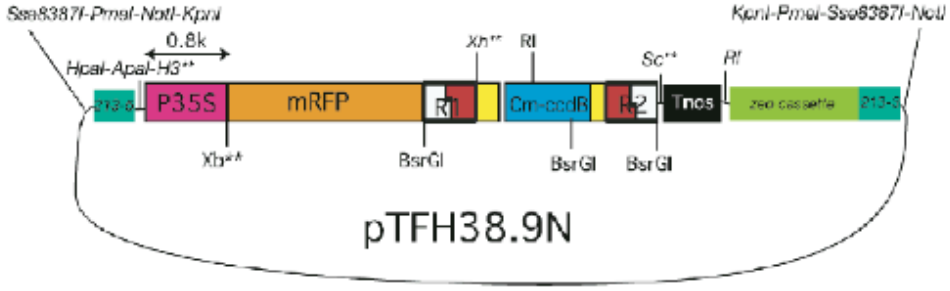


The sequences of BS 213-5' region and BS 213-3' rigion were kindly provided by Prof. Jean-Pierre Zrÿd (Universite de Lausanne).
(reference : The Plant Journal (1997) 11 (6), 1195-1206)

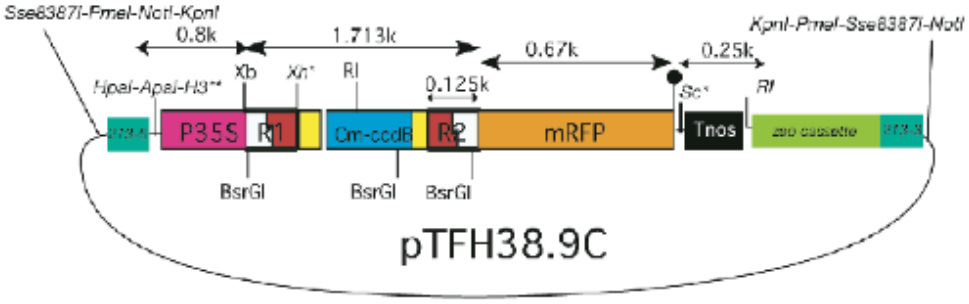
No. 10. pCMAK1


Sse 8387I
Pme I
Not I
Kpn I
BS213
P7133
Tnos
Zeo cassette
BS213
Hpa I
Apa I
Xho I
Pma CI
Eco RI
pCMAK1
Kpn I
Pme I
Sse 8387I
Not I
kan r
pCR4Blunt-TOPO
Lac Z-ccdB
P lac
amp r
pUC ori

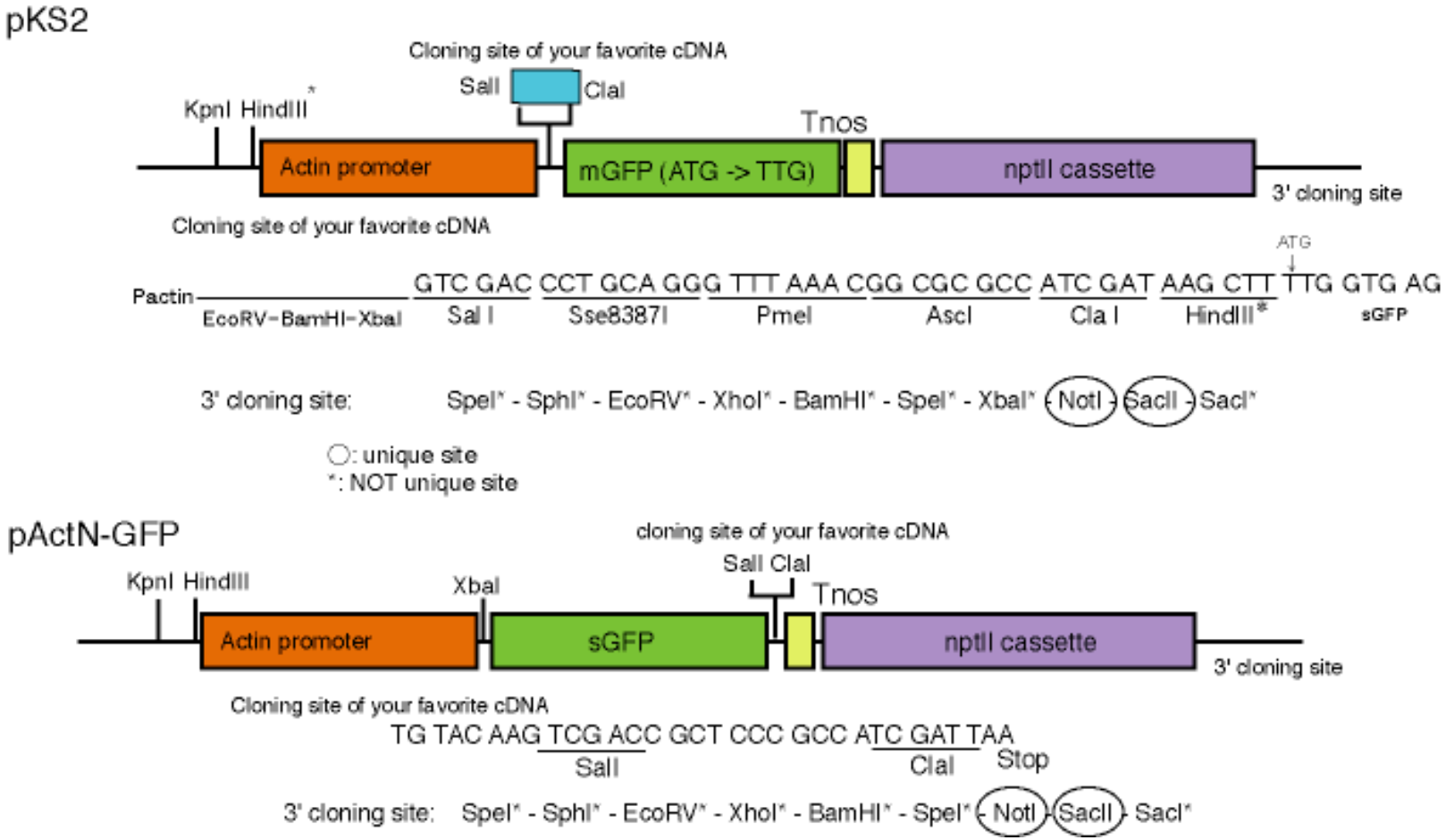
No. 11 and 12. pTFH38.9N and pTFH38.9C.



P35S-mRFP-gateway cassette-Tnos in pTFH38.6N was partially cut by H3-RI, then inserted into H3-RI of pCMAK2 (Km and Amp, and Cm res). Gateway A cassette is used.



P35S-gateway cassette-mRFP-Tnos in pTFH38.6C was partially cut by H3-RI, then inserted into H3-RI of pCMAK2 (Km and Amp, and Cm res). Gateway B cassette is used.

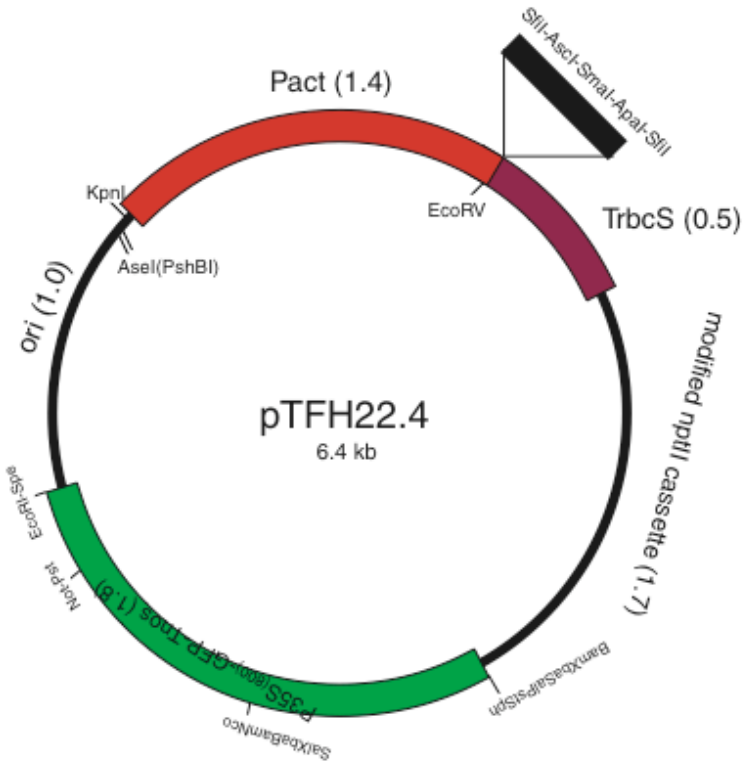


No. 13 and 14. pKS2 and pACtN-GFP.

No. 15. pTFH22.4.

TFH#22-4(1)

5/31/01



MCS EcoRV SfiI AscI SmaI ApaI SfiI

aaGATATCGGCCATTCAGGCCGGCGCGCCACCCGGGAGGGCCCGGCCGATTTGGCCatca

P35S(800)-Sph/Sma from pBI221 was inserted into pTFH15.3::dProGFP-Sph/PmaCI.

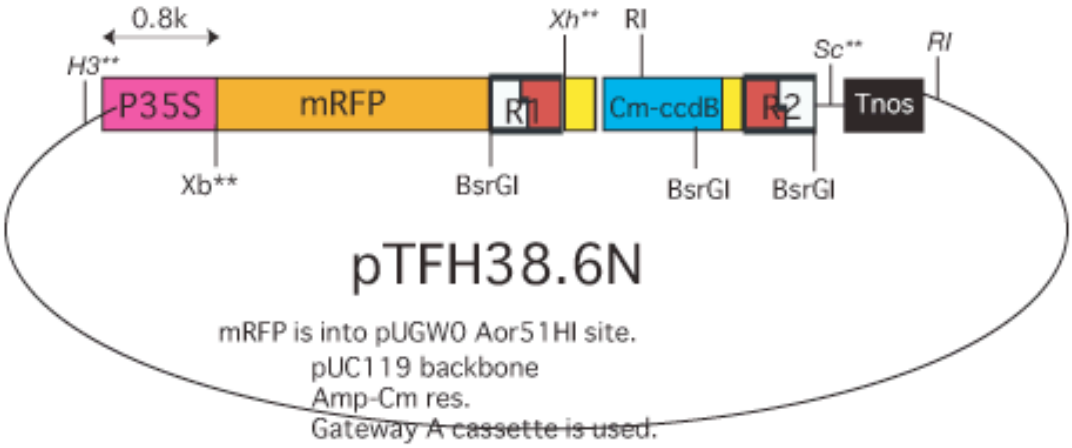
No cut-- BsmI, MluI, PmeI,

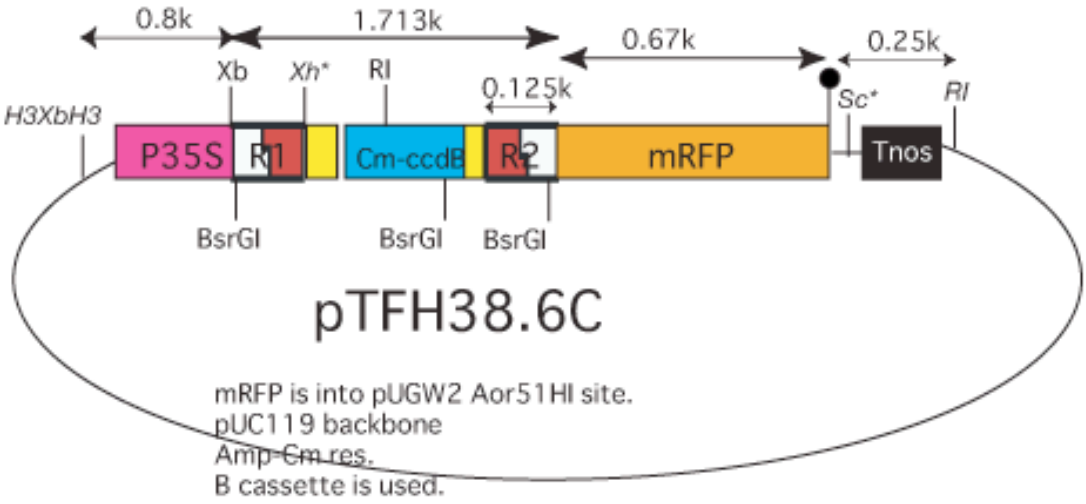
1 cut --SphI, AscI, ApaI, SmaI (TFH#22-4(1, 6))
SacII, Sse8387I, RsrII, NruI, BssII, BstEII, KpnI, SpeI, PmaCI, EcoRV

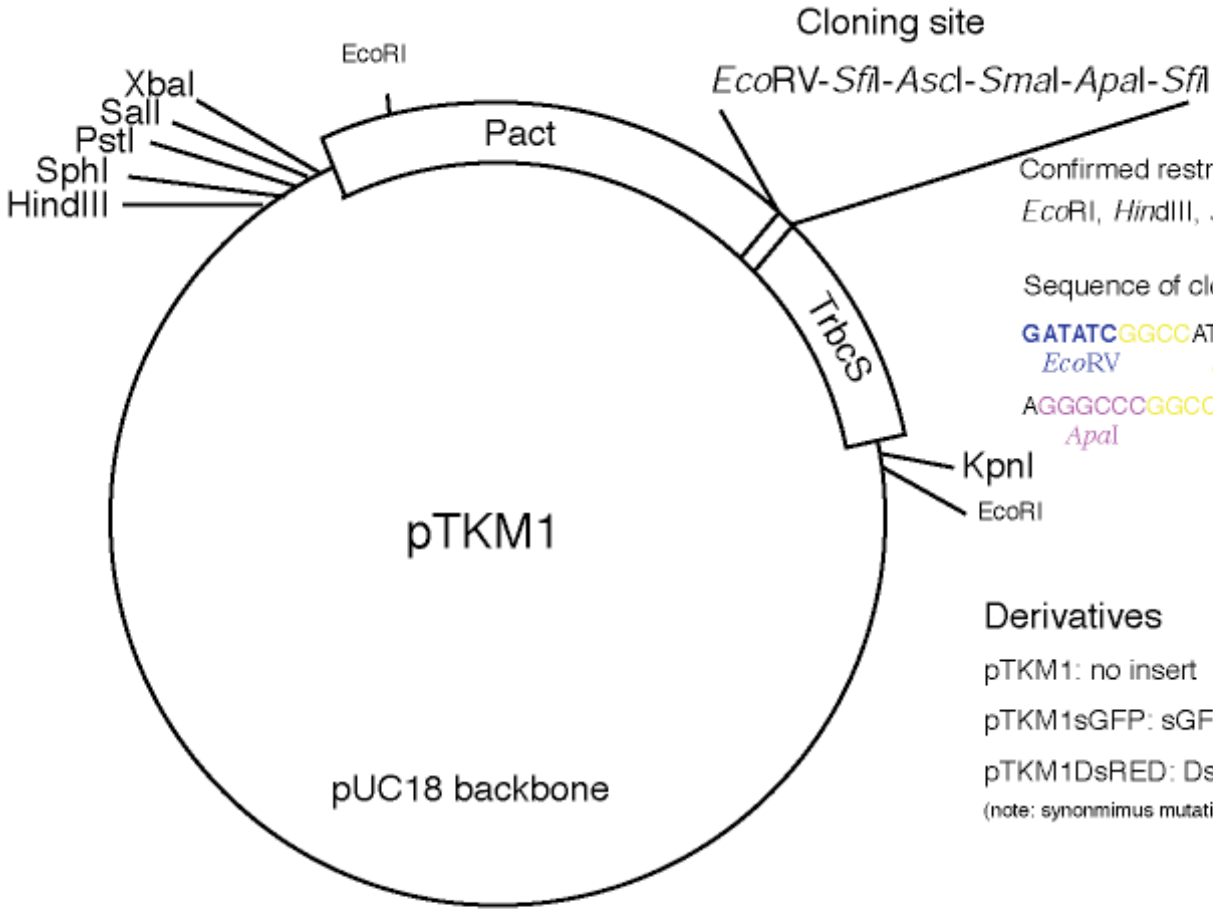
more than 2 cut -- SgrAI, SalI(2sites)

TFH#15-4(1), #15-4(5), #15-3(2), #22-1(1)(7)

No. 16 and 17. pTFH38.6N and pTFH38.6C.







Confirmed restriction enzyme site by digestion;
*Eco*RI, *Hin*dIII, *Sma*I, *Apa*I, *Kpn*I.

Sequence of cloning site (confirmed by sequencing)

GATATCGGCCATTCAGGCCGGCGCGCCACCCGGG
*Eco*RV *Sfi*I *Asc*I *Sma*I

AGGGCCCGGCCGATTTGGCC
*Apa*I *Sfi*I

Derivatives

pTKM1: no insert

pTKM1sGFP: sGFP insert between *Sma*I-*Apa*I

pTKM1DsRED: DsRED insert between *Asc*I-*Apa*I

(note: synonmimus mutation may occur in DsRED, A at 338 may be G)

Actin promoter, cloning site and rbcS terminater are derived from pTFH15.3. The fragment (approx. 2kb) was inserted in SmaI site of pUC18.

April 10, 2003 by Takashi Murata

No. 18. pTKM1.