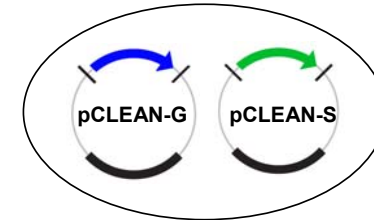


Novel dual binary vectors: pCLEAN series



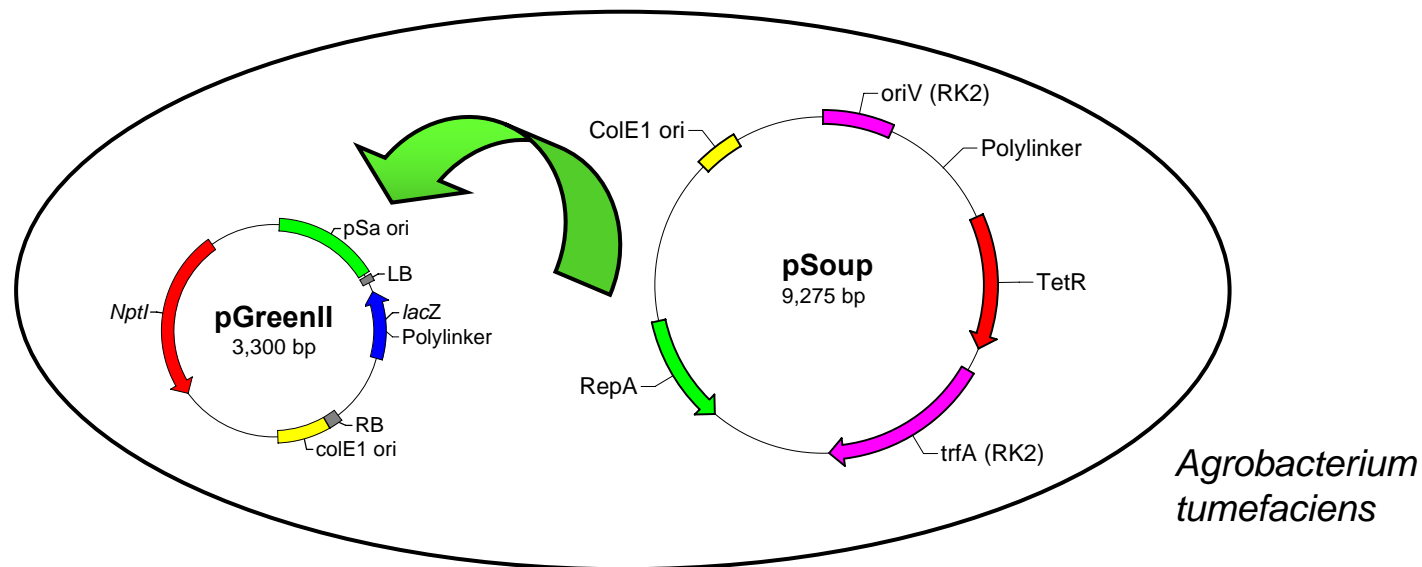
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pCLEAN vectors (Thole *et al.*, 2007) are compatible with the dual binary vector system pGreen and pSoup, where two binary vectors (*i.e.* pGreen and pSoup) are both present in the same *Agrobacterium*. In *Agrobacterium*, pSoup provides replication function *in trans* for pGreen (Hellens *et al.*, 2000). Both pGreen and pSoup can be maintained individually in *Escherichia coli*.

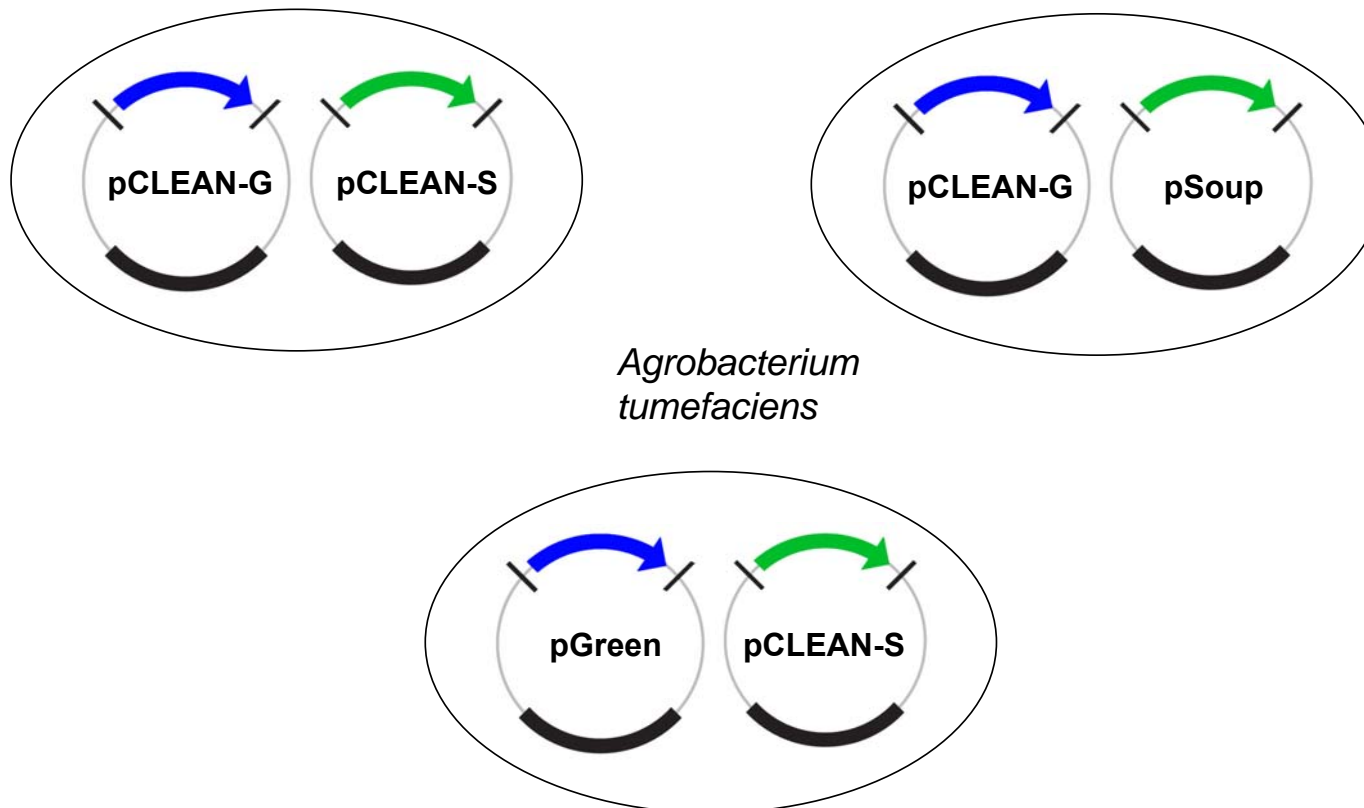


Information on basic pGreen and pSoup vectors can be found
http://www.pgreen.ac.uk/a_pls_fr.htm

There are two families of pCLEAN vectors:

- pCLEAN-G (which can be used as a pGreen equivalent)
- pCLEAN-S (which can be used as a pSoup equivalent)

pCLEAN, pGreen and pSoup vectors can be mixed and matched in *Agrobacterium*:



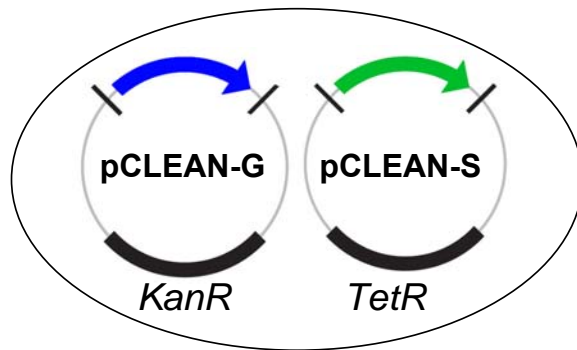
Added features in pCLEAN vectors:

(compared to other pGreen- and pSoup-based vectors)

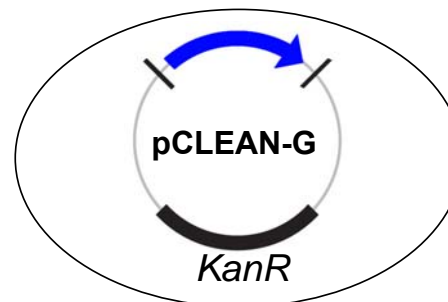
- **Counter selection of backbone transfer**
- **Modified left border (LB) of T-DNA**
- **Minimal T-DNA** (only 52 nt excluding the borders)
- **Reduced homology between pCLEAN and pSoup**
- **Multiple modified LBs**
- **Additional *vir* genes**
- **Exploiting backbone transfer for stable transformation**
- **Delivering multiple T-DNAs**

Using pCLEAN vectors:

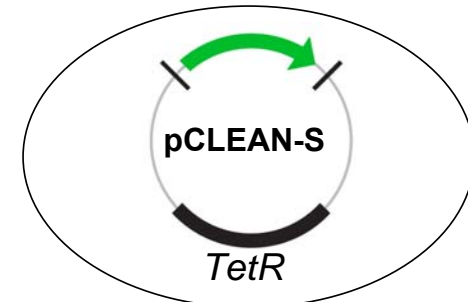
- pCLEAN-G vectors can be used as pGreen-like vectors as they share mostly the same backbone structure
- pCLEAN-S vectors can be used as pSoup-like vectors as they contain mostly the same backbone configuration



In Agrobacterium tumefaciens colonies grow after 48-72h @28°C on Kan 50 mg/l + Tet 7.5 mg/l or Kan 50 mg/l only



In Escherichia coli colonies grow after ~16h @37°C on Kan 50 mg/l



In Escherichia coli colonies grow after 24-48h @37°C on Tet 7.5 mg/l

Ordering information for pCLEAN binary vector system

1) Decide which/how many vectors in SET1 or SET2 of the pCLEAN vectors you wish to order:

SET1 (17 vectors) = **pCLEAN-G** basic vectors (pGreen-type)
+ **pCLEAN-S** basic vectors (pSoup-type)

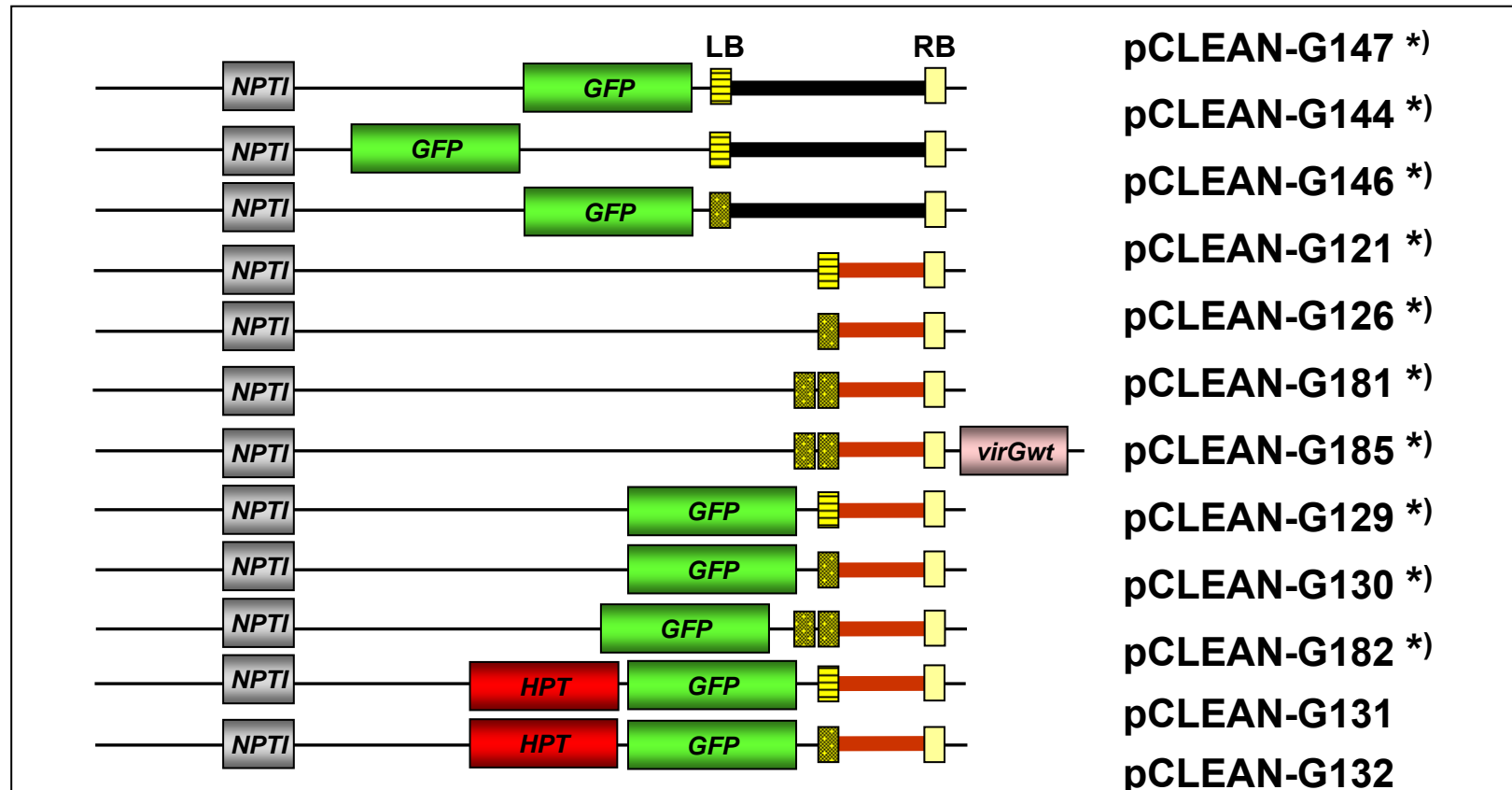
SET2 (10 vectors) = **pCLEAN-G** and **pCLEAN-S** vectors containing transgenes in T-DNA

2) The cost per order for SET1 is £40 and for SET1 + SET2 is £60 (payable by credit card). A Material Transfer Agreement form and Credit Card Order form can be obtained from Vera Thole or Philippe Vain.

Dr. Vera Thole
tel: +44 (0) 1603-450617
e-mail: vera.thole@bbsrc.ac.uk

Dr. Philippe Vain
tel: +44 (0) 1603-450612
e-mail: philippe.vain@bbsrc.ac.uk

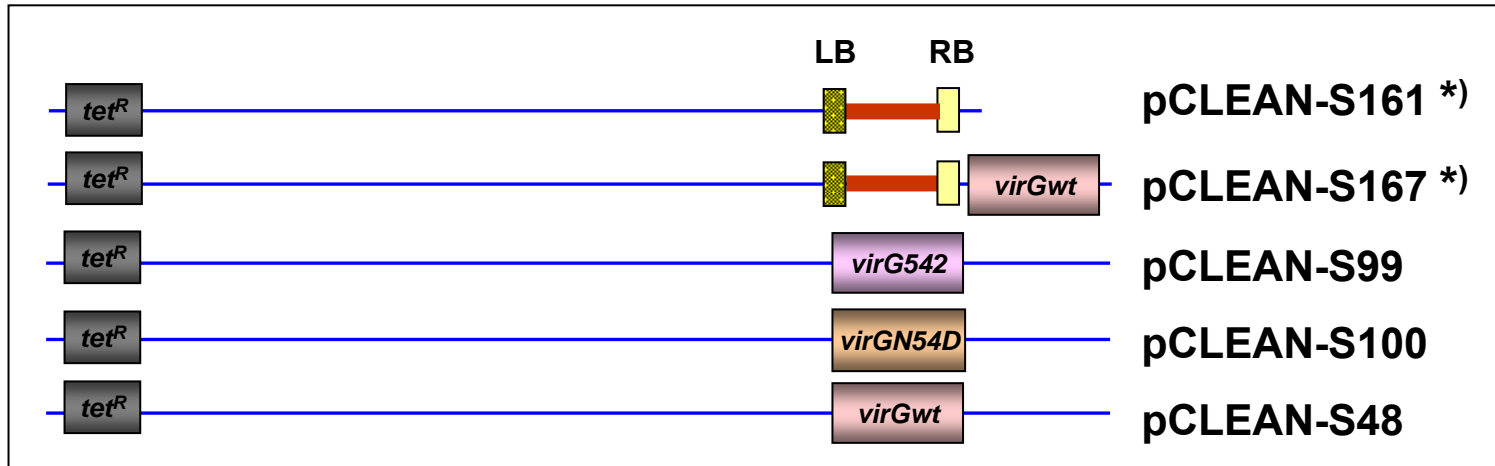
SET1: Basic pCLEAN-G vectors (pGreen-based)



*) complete vector sequence available in public databases

see legend on page 9

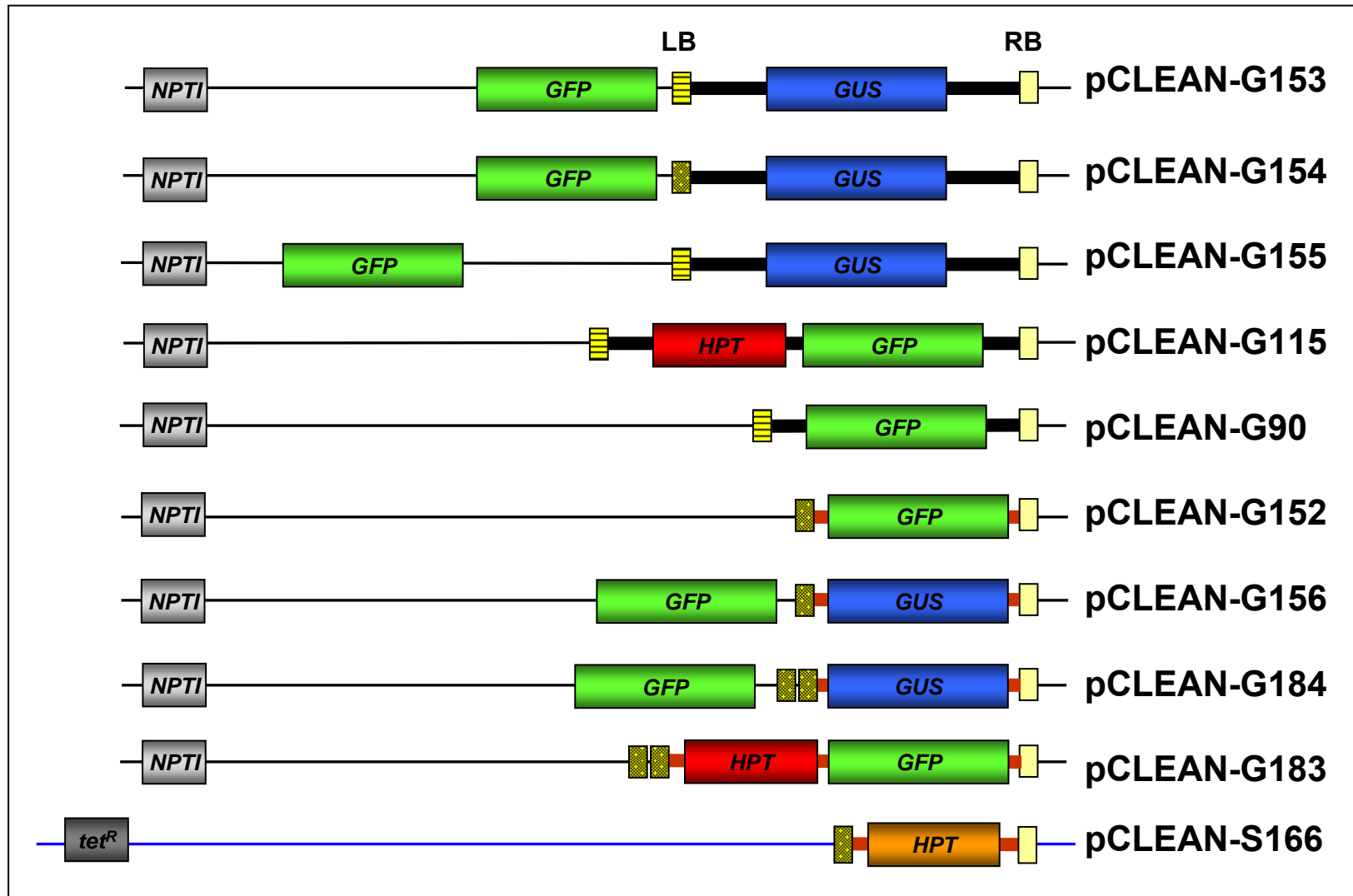
SET1: pCLEAN-S vectors (pSoup-based)



*) complete vector sequence available in public databases

see legend on page 9

SET2: pCLEAN-G and pCLEAN-S vectors with transgenes in T-DNA



see legend on page 9

Description of constituents of pCLEAN vectors:

Bacterial elements:

NPTI

NPTI (Hellens et al., 2000)

tet^R

tet^R from pAlter (Hellens et al., 2000)

virGwt

Wild-type **virG** (Vain et al., 2004)

virG542

virG from pTiBo542 (Vain et al., 2004)

virGN54D

virG mutant from pAD1289 (Vain et al., 2004)



pGreenII original suboptimal LB (Hellens et al., 2000)



pCLEAN consensus LB



RB



pGreenII original large T-DNA (Hellens et al., 2000; GenBank Accession No. EU048862)



pCLEAN-type small T-DNA



pGreenII-based vector backbone (GenBank Accession No. EF590266)



pSoup-based vector backbone (GenBank Accession No. EU048870)

Tet- tetracycline resistance
NPTI- kanamycin resistance
HPT- hygromycin resistance
GFP- green fluorescent protein
GUS- β-glucuronidase
NOS- nopaline synthase
CaMV- cauliflower mosaic virus
SPA- soybean-derived poly(A) signal

Plant expression units:

HPT

CaMV35S_{promoter}::maize shrunken1-intron1::HPT:: NOS_{terminator} (Vain et al., 1996)

HPT

NOS_{promoter}::HPT:: NOS_{terminator} (derived from pSLJ261; Jones et al., 1992)

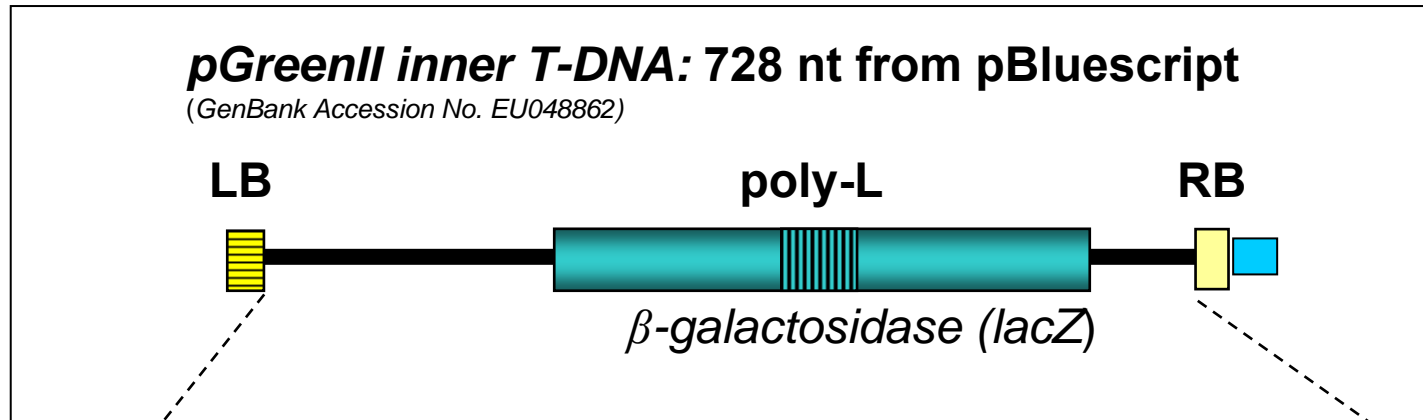
GFP


CaMV35S_{promoter}:: mgfp5-ER::SPA_{terminator} (derived from pGVT1; Thole and Rawsthorne, 2003)

GUS

CaMV35S_{promoter}:: gusA-int::SPA_{terminator} (derived from pGVT5; Thole and Rawsthorne, 2003)

Comparison of pGreenII- and pCLEAN-type T-DNAs



 RB overdrive sequence
 (Peralta et al. 1986)

Consensus of 25-bp terminal sequences of octopine and nopaline-type T-DNA regions:

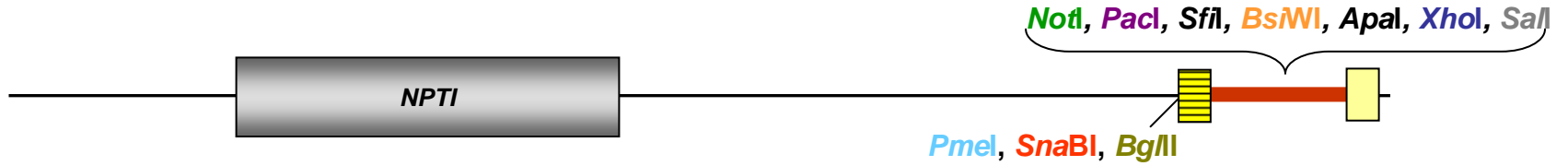
C GT T
 TGGCAGGATATATT X TGTAAC
G AG C

(Slightom et al., 1985)

Consensus RB	TGACAGGATATATTGGCGGGTAAAC	
pGreenII vectors RB	TGACAGGATATATTGGCGGGTAAAC	 RB

Consensus LB	TGGCAGGATATATTGTGGTGTAAC	
pGreenII vectors LB	TGGCAGGATATATTGTGGTGTAAC	 LB
New pCLEAN consensus LB	TGGCAGGATATATTGTGGTGTAAC	 LB

- Original pGreenII LB lacks an “A” compared to the consensus sequence
- **New pCLEAN LB sequence is identical with the consensus sequence**



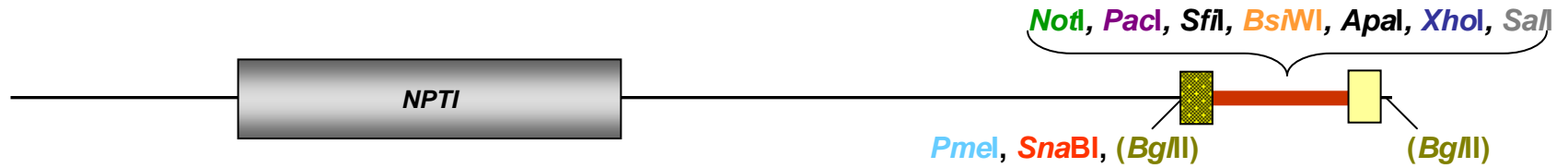
Sequence of pCLEAN-G121 (2638 nt) (*GenBank Accession no. EU186081*):

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LB
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RB RB overdrive

```

(Note: Lower case letter indicates pGreenII0000-based backbone sequence.)



Sequence of pCLEAN-G126 (2645 nt) (GenBank Accession no. EU186082):

```

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LB

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GCCGCTTAATTAAGCCGTACCGGCCCTCGAGTCGACGTTCTCTTGACAGGATATATTGGCGGTAACTAAGTCGCTGTATGTGTTTGTGGATCT

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RB

RB overdrive

(Note: Lower case letter indicates pGreenII0000-based backbone sequence.)



Sequence of pCLEAN-G181 (2718 nt) (*GenBank Accession no. EU186083*):

```

catgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcttgctggcggttttccataggctccgccccctgacgagcatcacaaaaatcgacgctcaagtcaaggtgg
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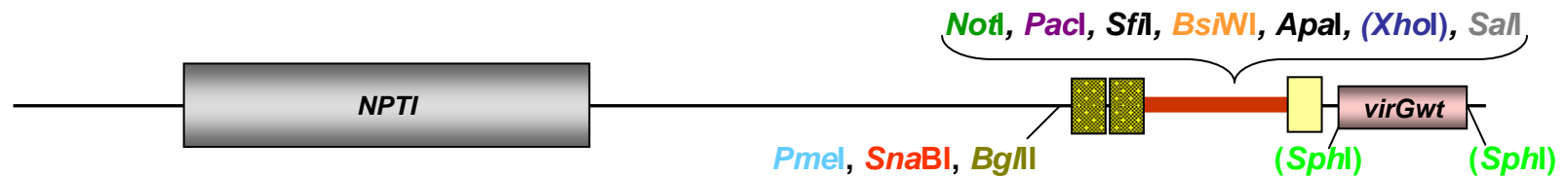
LB

GCGGTCGAGATGGATCT**TGGCAGGATATATTGTGGTGTAAAC**GTTTCCT**GCGGCCGCTTAATTAAAGGC**CGTACGGGCCCT**CGAGTCGACG**TTCCCT**TGACAGGATATATTGGCGGGTAAACTA**
spacer **LB** **RB**

AGTCGCTGTATGTGTTTGTGTTGAGATCCTCTAGGGCATGCAAGCTGATCTGGATCT

RB overdrive

(Note: Lower case letter indicates pGreenII0000-based backbone sequence.)



Sequence of pCLEAN-G185 (3793 nt) (GenBank Accession no. EU186084):

```

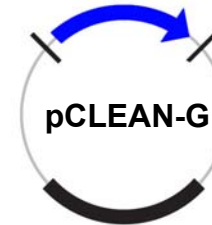
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CGATGTGCCCATCACACTGCGCATGCAAGCTGATCTGGATCT
    
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(Note: Lower case letter indicates pGreenII0000-based backbone sequence.)

Summary of GenBank accession numbers

pCLEAN-G vectors:

EU186081	pCLEAN-G121
EU186082	pCLEAN-G126
EU186090	pCLEAN-G129
EU186091	pCLEAN-G130
EU186089	pCLEAN-G144
EU186088	pCLEAN-G146
EU186087	pCLEAN-G147
EU186083	pCLEAN-G181
EU186092	pCLEAN-G182
EU186084	pCLEAN-G185



pCLEAN-S vectors:

EU186085	pCLEAN-S161
EU186086	pCLEAN-S167



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