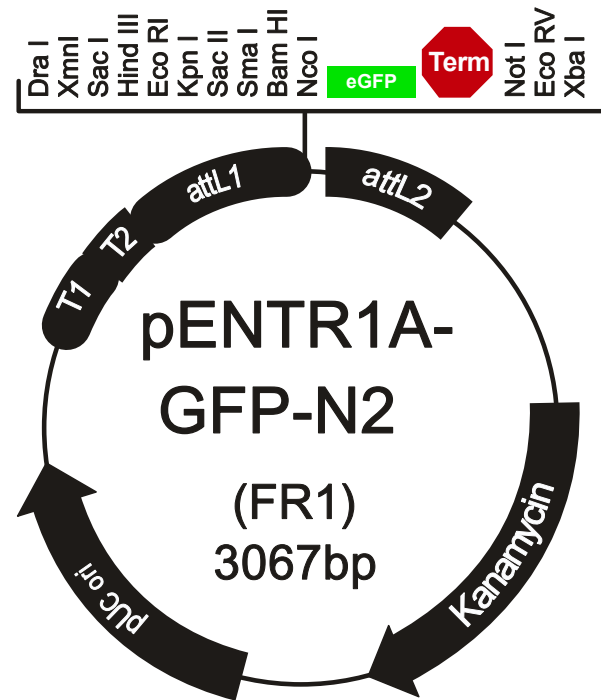Comments for pENTR1A-GFP-N2
3067 nucleotides

rrrB T1 transcription termination sequence: bases 106-149
rrrB T2 transcription termination sequence: bases 281-308
attL1: bases 358-457 (complementary strand)
enhanced green fluorescent protein (eGFP): bases 552-1271
attL2: bases 1296-1396

Kanamycin resistance gene: bases 1518-2327
pUC origin: bases 2392-3064

Top circular map labels (clockwise):
Dra I, Xmn I, Sac I, Hind III, Eco RI, Kpn I, Sac II, Sma I, Bam HI, Nco I, eGFP, Term, Not I, Eco RV, Xba I

attL1, attL2, T1, T2

pENTR1A-GFP-N2
(FR1)
3067bp

Kanamycin
pUC ori

Sequence map:

Dra I .... *Xmn I* .... *Sal I** .... *Xho I** .... *Sac I* *Hind III* ...... *Eco RI*
AAA GGA ACC AAT TCA GTC GAC TGG ATC TCG AGC TCA AGC TTC GAA TTC TGC
             Tyr Lys Ser Gly Leu Arg Ser Arg Ala Gln Ala Ser
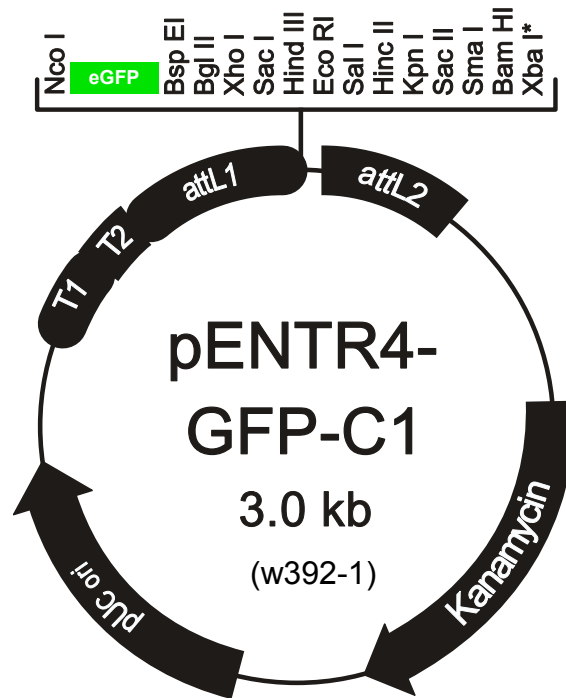
Pst I* *Sal I** .... *Kpn I* ... *Sac II* ... *Apa I* *Sma I* .... *Bam HI* .... *Nco I*
AGT CGA CGG TAC CGC GGG CCC GGG ATC CAC CGG CCG GTC GCC ACC **ATG GTG**
Asn Ser Ala Val Asp Gly Thr Ala Gly Pro Gly Ser Thr Gly Ser **Met**

...eGFP Term .... *Not I* .... *Xho I** *Eco RV* *Xba I* .... *att L2*
AGCGGCCGCACTCGAGATATCTAGACCAGCTTTCTTGTACAAA

Recommended *E.coli* strain for propagation: TOP10F'

The position of the genetic elements is approximative.

Made by Francis Rodier (Lawrence Berkeley National Laboratory)

Nco I

eGFP

Bsp EI
Bgl II
Xho I
Sac I
Hind III
Eco RI
Sal I
Hinc II
Kpn I
Sac II
Sma I
Bam HI
Xba I*

T1 T2

attL1

attL2

pENTR4-
GFP-C1

3.0 kb

(w392-1)

Kanamycin

pUC ori

Comments for pENTR4-GFP-C1
3028 nucleotides

rrrB T1 transcription termination sequence: bases 106-149
rrrB T2 transcription termination sequence: bases 281-308
attL1: bases 358-457 (complementary strand)
enhanced green fluorescent protein (eGFP): bases 463-1179
multiple cloning site (MCS): bases 1180-1256
att L2: bases
Kanamycin resistance gene: bases
pUC origin: bases

Recommended *E.coli* strain for propagation: TOP10F'

*Xba I site is methylated. Use methylation deficient strain in order to clone into this site.

Eric Campeau September 2007

pENTR4 -eGFP C1 MCS

*att*L1

```
GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
CCCGGGGTTT ATTACTAAAA TAAAACTGAC TATCACTGGA CAAGCAACGT TGTTTAACTA
```

```
AAG CAA TGC TTT TTT ATA ATG CCA ACT TTG TAC AAA AAA GCA GGC TCC
TTC GTT ACG AAA AAA TAT TAC GGT TGA AAC ATG TTT TTT CGT CCG AGG
```

Nco I   **Bsp EI**   **Bgl II** **Xho I**   **Sac I** **Hind III**

ACC ATG G ...eGFP... TAC AAG TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG
    Met   Tyr Lys Ser Gly Leu Arg Ser Arg Ala Gln Ala Ser

Eco RI   **Sal I**   **Kpn I** **Sac II** **Apa I, Sma I** **Bam HI**   **Xba I**

AAT TCT GCA GTC GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA **TCT AGA**
Asn Ser Ala Val Asp Gly Thr Ala Gly Pro Gly Ser Thr Gly Ser Arg
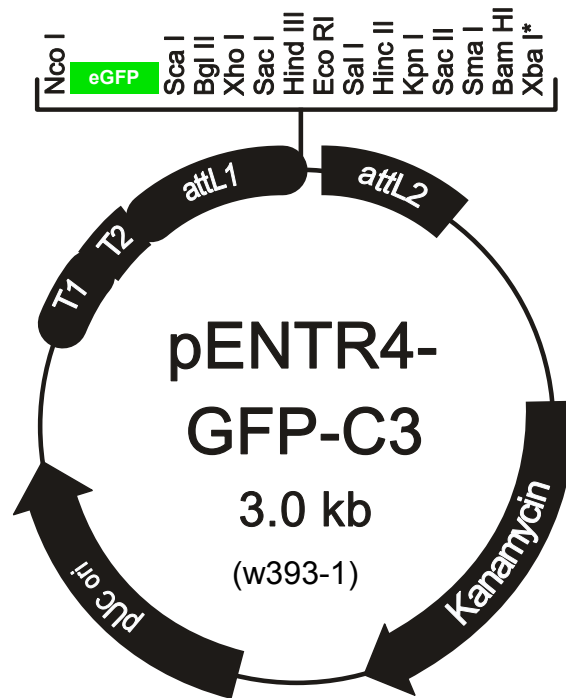
```
A CCA GCT TTC TTG TAC AAA
```

*att*L2

```
GTTGGCATTA TAAGAAAGCA TTGCTTATCA ATTTGTTGCA ACGAACAGGT CACTATCAGT
CAACCGTAAT ATTCTTTCGT AACGAATAGT TAAACAACGT TGCTTGTCCA GTGATAGTCA
```

reverse primer binding site

```
CAAAATAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG CCCGTGTCTC AAAATCTCTG
GTTTTATTTT AGTAATAAAC GGTAGGTCGA CGTCGAGACC GGGCACAGAG TTTTAGAGAC
```

```
ATGTTACATT
TACAATGTAA
```

** Please note that there are no stop codons after the Xba I site.

Eric Campeau September 2007

NcoI
eGFP
ScaI
BglII
XhoI
SacI
HindIII
EcoRI
SalI
HincII
KpnI
SacII
SmaI
BamHI
XbaI*

attL1
attL2
T1 T2
pENTR4-GFP-C3
3.0 kb
(w393-1)
Kanamycin
pUC ori

Comments for pENTR4-GFP-C3
3024 nucleotides

rrnB T1 transcription termination sequence: bases 106-149
rrnB T2 transcription termination sequence: bases 281-308
attL1: bases 358-457 (complementary strand)
enhanced green fluorescent protein (eGFP): bases 463-1179
multiple cloning site (MCS): bases 1180-1256
attL2: bases
Kanamycin resistance gene: bases
pUC origin: bases

Recommended *E.coli* strain for propagation: TOP10F'

*Xba I site is methylated. Use methylation deficient strain in order to clone into this site.

Eric Campeau September 2007

pENTR4 -eGFP-C3 MCS

*att*L1

```
GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
CCCGGGGTTT ATTACTAAAA TAAAACTGAC TATCACTGGA CAAGCAACGT TGTTTAACTA
```

```
AAG CAA TGC TTT TTT ATA ATG CCA ACT TTG TAC AAA AAA GCA GGC TCC
TTC GTT ACG AAA AAA TAT TAC GGT TGA AAC ATG TTT TTT CGT CCG AGG
```

Nco I   *Sca* I   *Bgl* II   *Xho* I   *Sac* I   *Hind* III   *Eco* RI

```
ACC ATG G ...eGFP... TAC AAG TAC TCA GAT CTC GAG CTC AAG CTT CGA
    Met               Tyr Lys Tyr Ser Asp Leu Glu Leu Lys Leu Arg
```

        Sal I   *Kpn* I   *Sac* II *Apa* I, *Sma* I *Bam* HI   *Xba* I

```
ATT CTG CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GAT CTA GA
Ile Leu Gln Ser Thr Val Pro Arg Ala Arg Asp Pro Pro Asp Leu
```

```
A CCA GCT TTC TTG TAC AAA
```

*att*L2

```
GTTGGCATTA TAAGAAAGCA TTGCTTATCA ATTTGTTGCA ACGAACAGGT CACTATCAGT
CAACCGTAAT ATTCTTTCGT AACGAATAGT TAAACAACGT TGCTTGTCCA GTGATAGTCA
```

reverse primer binding site

```
CAAAATAAAA TCATTATTTG CCATCCAGCT GCAGCTCTGG CCCGTGTCTC AAAATCTCTG
GTTTTATTTT AGTAATAAAC GGTAGGTCGA CGTCGAGACC GGGCACAGAG TTTTAGAGAC
```

```
ATGTTACATT
TACAATGTAA
```

*** Please note that there are no stop codons after the Xba I site.

Eric Campeau September 2007