

Improved GATEWAY Binary Vector (ImpGWB)

1. ImpGWB vector consists of 4 kind of resistance marker series
pGWB4xx (Pnos:NPTII), kanamycin resistance
pGWB5xx (Pnos:HPT), hygromycin resistance
pGWB6xx (Pnos:bar), BASTA resistance
pGWB7xx (Pnos:GPT), tunicamycin resistance
2. Some of the vectors contain the 35S promoter upstream of the cloning site while others have none.
3. The N- or C-terminal tags are automatically fused subsequent to the LR reaction.
4. Please read the GATEWAY instruction manual (Invitrogen) for procedures, etc.

Please note that our ImpGWB series are available only for basic research. If you plan to use these ImpGWBs for commercial research, please contact Invitrogen about licencing of GATEWAY™.

Please do not distribute to other researchers without permission.

Tsuyoshi Nakagawa
Department of Molecular and Functional Genomics
Interdisciplinary Center for Research
Organization of Research
Shimane University
Matsue 690, Japan
tnakagaw@life.shimane-u.ac.jp
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The pGWB Series

“n” indicate selection markers, 4 (401 etc, kanamycin resistance), 5 (501 etc, hygromycin resistance), 6 (601 etc, BASTA resistance), 7 (701 etc, tunicamycin resistance).

- (1) pGWBn01 : [(no promoter, no tag) (--R1-CmR-*ccdB*-R2 --)]
- (2) pGWBn02 : [(35S promoter, no tag) (--35S promoter-R1-CmR-*ccdB*-R2--)]
- (3) pGWBn02- Ω : [(2x35S enhancer- Ω promoter, no tag) (--2x35S- Ω -R1- CmR-*ccdB*-R2-GUS--)]
- (4) pGWBn04 : [(no promoter, C-sGFP) (--R1- CmR-*ccdB*-R2-sGFP--)]
- (5) pGWBn05 : [(35S promoter, C-sGFP) (--35S promoter-R1- CmR-*ccdB*-R2-sGFP--)]
- (6) pGWBn06 : [(35S promoter, N-sGFP) (--35S promoter-sGFP-R1- CmR-*ccdB*-R2--)]
- (7) pGWBn07 : [(no promoter, C-6xHis) (--R1- CmR-*ccdB*-R2-6xHis--)]
- (8) pGWBn08 : [(35S promoter, C-6xHis) (--35S promoter-R1- CmR-*ccdB*-R2-6xHis--)]
- (9) pGWBn09 : [(35S promoter, N-6xHis) (--35S promoter-6xHis-R1- CmR-*ccdB*-R2--)]
- (10) pGWBn10 : [(no promoter, C-FLAG) (--R1- CmR-*ccdB*-R2-FLAG--)]
- (11) pGWBn11 : [(35S promoter, C-FLAG) (--35S promoter-R1- CmR-*ccdB*-R2-FLAG--)]
- (12) pGWBn12 : [(35S promoter, N-FLAG) (--35S promoter-FLAG-R1- CmR-*ccdB*-R2--)]
- (13) pGWBn13 : [(no promoter, C-3xHA) (--R1- CmR-*ccdB*-R2-3xHA--)]
- (14) pGWBn14 : [(35S promoter, C-3xHA) (--35S promoter-R1- CmR-*ccdB*-R2-3xHA--)]
- (15) pGWBn15 : [(35S promoter, N-3xHA) (--35S promoter-3xHA-R1- CmR-*ccdB*-R2--)]
- (16) pGWBn16 : [(no promoter, C-4xMyc) (--R1- CmR-*ccdB*-R2-4xMyc--)]
- (17) pGWBn17 : [(35S promoter, C-4xMyc) (--35S promoter-R1- CmR-*ccdB*-R2-4xMyc--)]
- (18) pGWBn18 : [(35S promoter, N-4xMyc) (--35S promoter-4xMyc-R1- CmR-*ccdB*-R2--)]
- (19) pGWBn19 : [(no promoter, C-10xMyc) (--R1- CmR-*ccdB*-R2-10xMyc--)]
- (20) pGWBn20 : [(35S promoter, C-10xMyc) (--35S promoter-R1- CmR-*ccdB*-R2-10xMyc--)]
- (21) pGWBn21 : [(35S promoter, N-10xMyc) (--35S promoter-10xMyc-R1- CmR-*ccdB*-R2--)]
- (22) pGWBn22 : [(no promoter, C-GST) (--R1- CmR-*ccdB*-R2-GST--)]
- (23) pGWBn23 : [(35S promoter, C-GST) (--35S promoter-R1- CmR-*ccdB*-R2-GST--)]
- (24) pGWBn24 : [(35S promoter, N-GST) (--35S promoter-GST-R1- CmR-*ccdB*-R2--)]
- (25) pGWBn25 : [(no promoter, C-T7) (--R1- CmR-*ccdB*-R2-T7--)]
- (26) pGWBn26 : [(35S promoter, C-T7) (--35S promoter-R1- CmR-*ccdB*-R2-T7--)]
- (27) pGWBn27 : [(35S promoter, N-T7) (--35S promoter-T7-R1- CmR-*ccdB*-R2--)]
- (28) pGWBn28 : [(no promoter, C-TAP) (--R1- CmR-*ccdB*-R2-TAP--)]
- (29) pGWBn29 : [(35S promoter, C-TAP) (--35S promoter-R1- CmR-*ccdB*-R2-TAP--)]

- (33) pGWBn33 : [(no promoter, C-GUS) (--R1- CmR-*ccdB*-R2-GUS--)]
- (35) pGWBn35 : [(no promoter, C-LUC) (--R1- CmR-*ccdB*-R2-LUC--)]

- (40) pGWBn40 : [(no promoter, C-EYFP) (--R1- CmR-*ccdB*-R2-EYFP--)]
- (41) pGWBn41 : [(35S promoter, C-EYFP) (--35S promoter-R1- CmR-*ccdB*-R2-EYFP--)]

- (42) pGWBn42 : [(35S promoter, N-EYFP) (--35S promoter-EYFP-R1- CmR-*ccdB*-R2--)]
- (43) pGWBn43 : [(no promoter, C-ECFP) (--R1- CmR-*ccdB*-R2-ECFP--)]
- (44) pGWBn44 : [(35S promoter, C-ECFP) (--35S promoter-R1- CmR-*ccdB*-R2-ECFP--)]
- (45) pGWBn45 : [(35S promoter, N-ECFP) (--35S promoter-ECFP-R1- CmR-*ccdB*-R2--)]
-
- (50) pGWBn50 : [(no promoter, C-G3GFP) (--R1- CmR-*ccdB*-R2-G3GFP--)]
- (51) pGWBn51 : [(35S promoter, C-G3GFP) (--35S promoter-R1- CmR-*ccdB*-R2-G3GFP--)]
- (52) pGWBn52 : [(35S promoter, N-G3GFP) (--35S promoter-G3GFP-R1- CmR-*ccdB*-R2--)]
- (53) pGWBn53 : [(no promoter, C-mRFP) (--R1- CmR-*ccdB*-R2-mRFP--)]
- (54) pGWBn54 : [(35S promoter, C-mRFP) (--35S promoter-R1- CmR-*ccdB*-R2-mRFP)]
- (55) pGWBn55 : [(35S promoter, N-mRFP) (--35S promoter-mRFP-R1- CmR-*ccdB*-R2)]
-
- (59) pGWBn59 : [(no promoter, C-tagRFP) (--R1- CmR-*ccdB*-R2-tagRFP--)]
- (60) pGWBn60 : [(35S promoter, C-tagRFP) (--35S promoter-R1- CmR-*ccdB*-R2-tagRFP)]
- (61) pGWBn61 : [(35S promoter, N-tagRFP) (--35S promoter-tagRFP-R1- CmR-*ccdB*-R2)]

Notes:

1. pGWBn01 is used for simple cloning, pGWBn02 and pGWBn02- Ω are for expression driven by the 35S promoter or 2x35S- Ω promoter, pGWBn33 for promoter-GUS analysis, pGWBn35 for promoter-luciferase analysis, and others are for fusion of reporters and tags.
2. Tags could be added to either the N- or C- terminus of *Your Clone*. For example, in pGWBn10 “C-FLAG” means that after the LR reaction with the vector, FLAG will be fused at the C-terminal of *Your Clone*.
3. DNA sequences enclosed in [] are introduced into the *HindIII-SacI* site of the binary vector pPZP221 [Hajdukiewicz et al. (1994) *Plant Mol. Biol.* **25**: 989-994; see next page]. R = the *attR* site.

Construction of pGWBs

The binary vector used as the starting plasmid for the construction of ImpGWBs was pPZP221 [Hajdukiewicz et al. (1994) *Plant Mol. Biol.* **25**: 989-994]. The *aacC1* selection marker of pPZP221 was replaced with Pnos:NPTII:Tnos (pGWB4xx, kanamycin resistance), Pnos:HPT:Tnos (pGWB5xx, hygromycin resistance), Pnos:bar:Tnos (pGWB6xx, BASTA resistance), or Pnos:GPT:Tnos (pGWB7xx, tunicamycin resistance). The *HindIII-SacI* region in pPZP221 was replaced by the GATEWAY cassette and tag in the ImpGWB as shown below.

RB-(HindIII)-GATEWAY-(SacI)-Tnos-[Tnos-marker-Pnos]-LB

The DNA fragment diagrammed above is present between LB and RB of binary vector. Note that the marker unit is in reverse orientation.

Antibiotics can be used for selection in bacteria are as below.

ImpGWBs: spectinomycin, chloramphenicol
recombinant ImpGWB (after LR reaction) : spectinomycin.

The principle behind the GATEWAY technology and the pGWB vector series could be generalized as follow.

- (1) Subsequent to the LR reaction with an Entry Clone and a ImpGWB vector, the *attR1*-(CmR, *ccdB*)-*attR2* cassette in the pGWB will be replaced by the *attB1*-(*Your Clone*)-*attB2*.
- (2) The tag will be fused automatically in-frame to *Your Clone*, if the primer was designed according to the GATEWAY system (see instruction manual by Invitrogen). Note that the peptide encoded by the *attB* and linker region will be included in your construct, linking the tag to *Your Clone*.
- (3) N indicates the nucleotide (and X the amino acid) that should be included in *Your Clone*.

Important Note

- (1) Linearization of ImpGWBs by digestion is not necessary. (Digestion with *XhoI* before performing the LR reaction cause high efficiency in LR reaction.) The efficiency of the LR reaction depends on the topology of the plasmids in the following order (see instruction manual of Invitrogen).

Either or both plasmids linear > both plasmids relaxed >> both plasmids supercoiled

The *Xho*I linker was introduced into *Not*I site (of GATEWAY cassette just downstream of *att*R1) in all ImpGWBs. This *Xho*I site is unique.

*Xho*I

RB-(HindIII)-GATEWAY-(SacI)-Tnos-[Tnos-marker-Pnos]

- (2) Select with spectinomycin (100 ug/ml) after the LR reaction.
- (3) Use the *Escherichia coli* strain DH5alpha for cloning the product of the LR (BP) reactions, since negative selection with *ccd*B does not work in the *E. coli* which has F' episome (see instruction manual of Invitrogen).
- (4) Sometimes, *E. coli* with both the Entry Clone and recombinant ImpGWB are obtained subsequent to the cloning. I recommend that only the *E. coli* colonies with only the recombinant ImpGWB be used.

pGWBn01 : [(no promoter, no tag) (--R1-ccdB-R2 --)]

(AAGCTT) GCATGCCTGCAGGTCGAC
(TCTAGA)GTTA TCA ACA AGT TTG TAC AAA AAA --(CmR, ccdB)--TTC TTG TAC AAA GTG
GTT GAT AAC AGC GCT TA(G AGCTC)

- (1) The *Hind*III (AAGCTT), *Xba*I (TCTAGA) and *Sac*I (GAGCTC) sites are shown
- (2) The *att*R1-CmR-*ccd*B-*att*R2 cassette is underlined.

After the LR reaction, the following sequence will be obtained.

(AAGCTT) GCATGCCTGCAGGTCGAC
(TCTAGA)GTTA TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (Your Clone) NAC CCA
GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GCT TA(G AGCTC)

- (1) The *att*B1 and *att*B2 sequences are underlined.
- (2) The in-frame termination codon TAG (double-underlined) is present downstream of *att*B2.

pGWBn02 : [(35S promoter, no tag) (--35S promoter-R1-ccdB-R2 --)]

pGWBn02-Ω: [(2x35S-Ω promoter, no tag) (--2x35S-Ω promoter-R1-ccdB-R2 --)]

(AAGCTT) – (35S or 2x35S-Ω promoter) --//
(TCTAGA)GTTA TCA ACA AGT TTG TAC AAA AAA --(CmR, ccdB)--TTC TTG TAC AAA GTG
GTT GAT AAC AGC GCT TA(G AGCTC)

- (1) The *HindIII* (AAGCTT), *XbaI* (TCTAGA) and *SacI* (GAGCTC) sites are shown
- (2) The *attR1-CmR-ccdB-attR2* cassette is underlined.
- (3) The sequence upstream of the *XbaI* site is the same as in pBI121

After LR reaction, the following sequence will be obtained

(AAGCTT) -- (35S or 2x35S-Ω promoter) --//
(TCTAGA)GTTA TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (*Your Clone*) NAC CCA
M ----- (X) (P)
GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GCT TA(G AGCTC)
(A) (F) (L) (Y) (K) (V) (V) (D) (N) (S) (A) *

- (1) Include the initiation codon ATG in *Your Clone* as pGWBn02 and pGWBn02-Ω do not have one.
- (2) The inclusion of a termination codon in *Your Clone* is not necessary since one is present downstream of *attB2*. The amino acid residues in () are encoded by the *attB2* and the linker region, and will be included if the termination codon TAG (double-underlined) of the vector is used.
- (3) N indicates the nucleotide (and X the amino acid) that should be included in *Your Clone*.

pGWBn04 : [(no promoter, C-sGFP) (--R1-ccdB-R2-sGFP--)]

(AAGCTT) --//
(TCTA A)TC ACA AGT TTG TAC AAA AAA --(Cm-R, ccdB)-- TTC TTG TAC AAA GTG
GTG ATC ATG --(*GFP* sequence) --(GAGCTC)

- (1) As a result of a mistake during the fill-in reaction while constructing pGWBn04, the *Xba*I site TCTAGA was changed to TCTAA. However, I think it would not affect the results of your experiments.
- (2) The initiation codon ATG (double-underlined) is from the GFP coding sequence.

After the LR reaction, the following sequence will be obtained.

(AAGCTT) --//
(TCTA A)TC ACA AGT TTG TAC AAA AAA GCA GGC TNN (*Your Clone*) NAC CCA GCT
(X) (P) (A)
TTC TTG TAC AAA GTG GTG ATC ATG --(*GFP* sequence) --(GAGCTC)
(F) (L) (Y) (K) (V) (V) (I) M

- (1) If *Your Clone* has only a 5' region/promoter without an initiation codon, translation will start at the ATG (double-underlined) of *GFP*.
- (2) If *Your Clone* contains at least a part of an ORF, C-terminal GFP fusion will be made. In that case, the peptide sequence made up of the amino acid in () will link *Your Clone* and GFP.
- (3) N indicates the nucleotide (and X the amino acid) that should be included in *Your Clone*, if C-terminal GUS fusion is to be made.
- (4) The sequences from the termination codon of *GFP* to *Sac*I site (see below) was generated by the following steps: (i) *Not*I digest, (ii) fill-in, and (iii) *Sac*I linker (8-mer) ligation.

TAAAGCGGCC(GAGCTC)

pGWBn05 : [(35S promoter, C-sGFP) (--35Spromoter-R1-ccdB-R2-sGFP--)]

```
(AAGCTT) -- 35S promoter --//  
(TCT AA)T CAA ACA AGT TTG TAC AAA AAA --(CmR, ccdB)--TTC TTG TAC AAA GTG  
GTT CGA TCT AGA GGA TCC ATG --(GFP sequence) --(GAGCTC)
```

- (1) As a result of a mistake during the fill-in reaction while constructing pGWBn05, the XbaI site TCTAGA was changed to TCTAA. However, I think it would not affect the result of your experiments.
- (2) The initiation codon ATG (double-underlined) is from the *GFP* coding sequence.

After LR reaction, the following sequence will be obtained.

```
35S promoter -(TCT AA)T CAA ACA AGT TTG TAC AAA AAA GCA GGC TNN (Your Clone)  
NAC CCA GCT TTC TTG TAC AAA GTG GTT CGA TCT AGA GGA TCC ATG --  
-X- -P- -A- -F- -L- -Y- -K- -V- -V- -R- -S- -R- -G- -S- -M-  
(GFP sequence) -- (GAGCTC)
```

- (1) Include an initiation codon ATG in *Your Clone*. Protein fusion with the C-terminal GFP will be linked by the peptide sequence derived from the *attB2* and linker region as shown in the above sequence.
- (2) N indicates the nucleotide (and X the amino acid) that is needed to be included in *Your Clone*.
- (3) The DNA sequence downstream of *GFP* is the same as in pGWBn04.
- (4) Hyphenations flanking the amino acid residues are used for proper spacing.

pGWBn06 : [(35S promoter, N-sGFP) (--35S promoter-sGFP-R1-ccdB-R2--)]

```
(AAGCTT) -- 35S promoter --(GFP sequence)--//  
--- C(TG TAC A)TC ACA AGT TTG TAC AAA AAA --(CmR, ccdB)--TTC TTG TAC AAA  
GTG GTG ATG TAC AAG TAA AGCGGCCC(GAGCTC)
```

- (1) The GATEWAY conversion cassette was introduced into the *Bsr*GI (TGTACA) site (in the 3' end of *GFP*).
- (2) The termination codon TAA (double-underlined) is derived from *GFP*.

After LR reaction, the following sequence will be obtained.

```
(AAGCTT) -- 35S promoter --(GFP sequence)--//  
--- C(TG TAC A)TC ACA AGT TTG TAC AAA AAA GCA GGC TNN (Your Clone) NAC  
--- -L- -Y- -I- -T- -S- -L- -Y- -K- -K- -A- -G- -X- ---- -X- (X)  
CCA GCT TTC TTG TAC AAA GTG GTG ATG TAC AAG TAA AGCGGCCC(GAGCTC)  
(P) (A) (F) (L) (Y) (K) (V) (V) (M) (Y) (K) *
```

- (1) The inclusion of a termination codon in *Your Clone* is not necessary since one is present downstream of *attB2*. The amino acid residues in () is encoded by the *attB2* and the linker region, and will be included if the termination codon TAG of the vector is used.
- (2) The C-terminus of the original GFP is LYK*.
- (3) The 5' end sequence of *GFP* is as shown below.

```
TCTAGAGGATCC ATG --(GFP sequence)--  
XbaI BamHI M
```

pGWBn07, n08, n10, n11, n13, n14, n16, n17, n19, n20, n22, n23, n25, n26, n28, n29, n33, n35, n40, n41, n43, n44, n50, n51, n53, n54, n59, n60 (no promoter or 35S promoter, C-tag)

No promoter --- n07, n10, n13, n16, n19, n22, n25, n28, n33, n35, n40, n43, n50, n53, n59

35S promoter --- n08, n11, n14, n17, n20, n23, n26, n29, n41, n44, n51, n54, n60

(AAGCTT) -- (no promoter or 35S promoter) --//

(TCTAGA)GTTA TCA ACA AGT TTG TAC AAA AAA --(CmR,ccdB)--TTC TTG TAC AAA GTG
GTT GAT AAC AGC tag GCT TA(G AGCTC)

- (1) The *Hind*III (AAGCTT), *Xba*I (TCTAGA) and *Sac*I (GAGCTC) sites are shown.
- (2) The *att*R1-CmR-*ccdB*-*att*R2 cassette is underlined.
- (3) The sequence of each tag is indicated in the “Tags” section.

After LR reaction, the following sequence will be obtained.

(AAGCTT) -- (no promoter or 35S promoter) --//

(TCTAGA)GTTA TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (Your Clone) NAC CCA
M ----- -X- -P-
GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC tag GCT TA(G AGCTC)
-A- -F- -L- -Y- -K- -V- -V- -D- -N- -S- tag -A- *

- (1) Include an initiation codon ATG in *Your Clone*. Protein fusion with the C-terminal tag will be linked by the peptide sequence derived from the *att*B2 and linker region as shown in the above sequence.
- (2) Translation will stop at the termination codon TAG (double-underlined) after the tag.

pGWBn09, n12, n15, n18, n21, n24, n27, n42, n45, n52, n55, n61 (35S promoter, N-tag)

```
(AAGCTT) -- 35S promoter --//  
(TCTAGA) ATG AGC tag GCT GTT ATC ACA AGT TTG TAC AAA AAA --(CmR,ccdB)--TTC  
TTG TAC AAA GTG GTG ATA ACC TA(G AGCTC)
```

- (1) The *Hind*III (AAGCTT), *Xba*I (TCTAGA) and *Sac*I (GAGCTC) sites are shown.
- (2) The *att*R1-CmR-*ccdB-att*R2 cassette is underlined.
- (3) The sequence of each tag is indicated in the “Tags” section.

After the LR reaction, the following sequence will be obtained.

```
(AAGCTT) -- 35S promoter --//  
(TCTAGA) ATG AGC tag GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN  
M- -S- tag -A- -V- -I- -T- -S- -L- -Y- -K- -K- -A- -G- -X-  
(Your Clone) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TA(G AGCTC)  
--- --- (X) (P) (A) (F) (L) (Y) (K) (V) (V) (I) (T) *
```

- (1) The inclusion of a termination codon in *Your Clone* is not necessary since one is present downstream of *att*B2. The amino acid residues in () is encoded by the *att*B2 and the linker region, and will be included if the termination codon TAG (double-underlined) of the vector is used.

Tags

Hyphenations in the amino acid sequences are used for proper spacing.

pGWBn04, n05, n06 (sGFP) 720bp

The sequence of sGFP (ATG to TAA) is as described by Chiu et al., (1996) Current Biol., 6: 325-330 .

Note that the C-terminal K is converted to I in pGWB6 (35S promoter, N-GFP). (see the section on pGWB6)

```
ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
M- V- S- K- G- E- E- L- F- T- G- V- V- P- I- L- V- E- L- D- G- D- V-
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
N- G- H- K- F- S- V- S- G- E- G- E- G- D- A- T- Y- G- K- L- T- L- K-
TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCACCTACGGCGTG
F- I- C- T- T- G- K- L- P- V- P- W- P- T- L- V- T- T- F- T- Y- G- V-
CAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGGC
Q- C- F- S- R- Y- P- D- H- M- K- Q- H- D- F- F- K- S- A- M- P- E- G-
TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC
Y- V- Q- E- R- T- I- F- F- K- D- D- G- N- Y- K- T- R- A- E- V- K- F-
GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG
E- G- D- T- L- V- N- R- I- E- L- K- G- I- D- F- K- E- D- G- N- I- L-
GGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCATATATCATGGCCGACAAGCAGAAGAACGGC
G- H- K- L- E- Y- N- Y- N- S- H- N- V- Y- I- M- A- D- K- Q- K- N- G-
ATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
I- K- V- N- F- K- I- R- H- N- I- E- D- G- S- V- Q- L- A- D- H- Y- Q-
CAGAACACCCCCATCGGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCC
Q- N- T- P- I- G- D- G- P- V- L- L- P- D- N- H- Y- L- S- T- Q- S- A-
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATC
L- S- K- D- P- N- E- K- R- D- H- M- V- L- L- E- F- V- T- A- A- G- I
ACTCACGGCATGGACGAGCTGTACAAGTAA
T- H- G- M- D- E- L- Y- K- *
```

pGWBn07, n08, n09 (6xHis) 18bp

CATCATCATCATCATCAT

H- H- H- H- H- H-

pGWBn10, n11, n12 (FLAG) 24bp

GACTACAAGGATGACGATGACAAG

D- Y- K- D- D- D- D- K-

pGWBn13, n14, n15 (3xHA) 120bp

Please consult <http://pingu.salk.edu/users/forsburg/> for further information

GGGTTAATTAACATCTTTTACCCATACGATGTTCCCTGACTATGCGGGCTATCCCTATGACGTCCCGGAC

G- L- I- N- I- F- Y- P- Y- D- V- P- D- Y- A- G- Y- P- Y- D- V- P- D-

TATGCAGGATCCTATCCATATGACGTTCCAGATTACGCTGCTCAGTGCAGC

Y- A- G- S- Y- P- Y- D- V- P- D- Y- A- A- Q- C- S-

(1) The three HA units are underlined.

(2) The 54th nucleotide is a G in the reported sequence, but it is a C in our vectors. This does not affect the identity of the amino acid, however [CCG (P) to CCC (P)].

pGWBn16, n17, n18 (4xMyc) 171bp

Please consult <http://pingu.salk.edu/users/forsburg/> for further information

GGGTTAATTAACGGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTGAACAAAATTAATCTCA

G- L- I- N- G- E- Q- K- L- I- S- E- E- D- L- N- G- E- Q- K- L- I- S-

GAAGAAGACTTGAACGGACTCGACGGTGAACAAAAGTTGATTTCTGAAGAAGATTTGAACGGTGAACAA

E- E- D- L- N- G- L- D- G- E- Q- K- L- I- S- E- E- D- L- N- G- E- Q-

AAGCTAATCTCCGAGGAAGACTTGAACGGTAGC

K- L- I- S- E- E- D- L- N- G- S

The four Myc units are underlined.

pGWBn19, n20, n21 (10xMyc) 402bp

GGGTTAATTAACGGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTGAACAAAATTAATCTCA
G- L- I- N- G- E- Q- K- L- I- S- E- E- D- L- N- G- E- Q- K- L- I- S-
GAAGAAGACTTGAACGGACTCGACGGTGAACAAAAGTTGATTTCTGAAGAAGATTTGAACGGTGAACAA
E- E- D- L- N- G- L- D- G- E- Q- K- L- I- S- E- E- D- L- N- G- E- Q-
AAGCTAATCTCCGAGGAAGACTTGAACGGTGAACAAAATTAATCTCAGAAGAAGACTTGAACGGACTC
K- L- I- S- E- E- D- L- N- G- E- Q- K- L- I- S- E- E- D- L- N- G- L-
GACGGTGAACAAAAGTTGATTTCTGAAGAAGATTTGAACGGTGAACAAAAGCTAATCTCCGAGGAAGAC
D- G- E- Q- K- L- I- S- E- E- D- L- N- G- E- Q- K- L- I- S- E- E- D-
TTGAACGGTGAACAAAATTAATCTCAGAAGAAGACTTGAACGGACTCGACGGTGAACAAAAGTTGATT
L- N- G- E- Q- K- L- I- S- E- E- D- L- N- G- L- D- G- E- Q- K- L- I-
TCTGAAGAAGATTTGAACGGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTAGC
S- E- E- D- L- N- G- E- Q- K- L- I- S- E- E- D- L- N- G- S-

- (1) The ten Myc units are underlined.
- (2) The last codon AGC (Ser) is not included in pGWBn19 and n20.

pGWBn22, n23, n24 (GST) 678bp

The full-length *GST* sequence was derived from pGEX2T

ATGTCCCCTATACTAGGTTATTTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGGGAATAT
M- S- P- I- L- G- Y- W- K- I- K- G- L- V- Q- P- T- R- L- L- L- E- Y-
CTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTT
L- E- E- K- Y- E- E- H- L- Y- E- R- D- E- G- D- K- W- R- N- K- K- F-
GAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATG
E- L- G- L- E- F- P- N- L- P- Y- Y- I- D- G- D- V- K- L- T- Q- S- M-
GCCATCATACGTTTATATAGCTGACAAGCACAAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATT
A- I- I- R- Y- I- A- D- K- H- N- M- L- G- G- C- P- K- E- R- A- E- I-
TCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACTTT
S- M- L- E- G- A- V- L- D- I- R- Y- G- V- S- R- I- A- Y- S- K- D- F-
GAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTTGAAGATCGTTTATGT
E- T- L- K- V- D- F- L- S- K- L- P- E- M- L- K- M- F- E- D- R- L- C-
CATAAAACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTT
H- K- T- Y- L- N- G- D- H- V- T- H- P- D- F- M- L- Y- D- A- L- D- V-
GTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAA
V- L- Y- M- D- P- M- C- L- D- A- F- P- K- L- V- C- F- K- K- R- I- E-
GCTATCCCACAAATTGATAAGTACTTGAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAA
A- I- P- Q- I- D- K- Y- L- K- S- S- K- Y- I- A- W- P- L- Q- G- W- Q-
GCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGTTCGCGTGGATCC
A- T- F- G- G- G- D- H- P- P- K- S- D- L- V- P- R- G- S-

pGWBn25, n26, n27 (T7) 33bp

ATGGCTAGCATGACTGGTGGACAGCAAATGGGT
M- A- S- M- T- G- G- Q- Q- M- G

pGWBn28, n29 (TAP) 546bp

AAGAGAAGATGGAAAAAGAATTTTCATAGCCGTCTCAGCAGCCAACCGCTTTAAGAAAATCTCATCCTCC
K- R- R- W- K- K- N- F- I- A- V- S- A- A- N- R- F- K- K- I- S- S- S-
GGGGCACTTGATTATGATATTCCTCAACTACTGCTAGCGAGAATTTGTATTTTCAGGGTGAGCTCAAACC
G- A- L- D- Y- D- I- P- T- T- A- S- E- N- L- Y- F- Q- G- E- L- K- T-
GCGGCTCTTGCGCAACACGATGAAGCCGTGGACAACAAATTC AACAAAGAACAACAAAACGCGTTCTAT
A- A- L- A- Q- H- D- E- A- V- D- N- K- F- N- K- E- Q- Q- N- A- F- Y
GAGATCTTACATTTACCTAACTTAAACGAAGAACAACGAAACGCCTTCATCCAAAGTTTAAAAGATGAC
E- I- L- H- L- P- N- L- N- E- E- Q- R- N- A- F- I- Q- S- L- K- D- D-
C CAAGCCAAAGCGCTAACCTTTTAGCAGAAGCTAAAAAGCTAAATGATGCTCAGGCGCCGAAAGTAGAC
P- S- Q- S- A- N- L- L- A- E- A- K- K- L- N- D- A- Q- A- P- K- V- D-
AACAAATTC AACAAAGAACAACAAAACGCGTTCTATGAGATCTTACATTTACCTAACTTAAACGAAGAA
N- K- F- N- K- E- Q- Q- N- A- F- Y- E- I- L- H- L- P- N- L- N- E- E-
CAACGAAACGCCTTCATCCAAAGTTTAAAAGATGACCCAAGCCAAAGCGCTAACCTTTTAGCAGAAGCT
Q- R- N- A- F- I- Q- S- L- K- D- D- P- S- Q- S- A- N- L- L- A- E- A-
AAAAAGCTAAATGGTGCTCAGGCGCCGAAAGTAGACGCGAATTCGCGGGGAAGTCAACCTGA
K- K- L- N- G- A- Q- A- P- K- V- D- A- N- S- A- G- K- S- T- *

- (1) The CBP sequence is underlined.
- (2) TEV site is underlined in bold.
- (3) The protein A sequence is double-underlined.

pGWBn33 (GUS) 1812bp

ATGTTACGTCCTGTAGAAAACCCCAACCCGTGAAATCAAAAACTCGACGGCCTGTGGGCATTCACTCTG
M L R P V E T P T R E I K K L D G L W A F S L
GATCGCGAAAACCTGTGGAATTGATCAGCGTTGGTGGGAAAGCGCGTTACAAGAAAGCCGGGCAATTGCT
D R E N C G I D Q R W W E S A L Q E S R A I A
GTGCCAGGCAGTTTTAACGATCAGTTCGCCGATGCAGATATTCGTAATTATGCGGGCAACGTCTGGTAT
V P G S F N D Q F A D A D I R N Y A G N V W Y
CAGCGCGAAGTCTTTATACCGAAAAGTTGGGCAGGCCAGCGTATCGTGCTGCGTTTTGATGCGGTCACT
Q R E V F I P K G W A G Q R I V L R F D A V T
CATTACGGCAAAGTGTGGGTCAATAATCAGGAAGTATGAGCATCAGGGCGGCTATACGCCATTTGAA
H Y G K V W V N N Q E V M E H Q G G Y T P F E
GCCGATGTCACGCCGATGTTATTGCCGGAAAAGTGTACGTATCACCGTTTGTGTGAACAACGAACCTG
A D V T P Y V I A G K S V R I T V C V N N E L
AACTGGCAGACTATCCCGCCGGGAATGGTGATTACCGACGAAAACGGCAAGAAAAGCAGTCTTACTTC
N W Q T I P P G M V I T D E N G K K K Q S Y F
CATGATTTCTTTAACTATGCCGGAATCCATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGGGTG
H D F F N Y A G I H R S V M L Y T T P N T W V
GACGATATCACCGTGGTGACGCATGTCGCGCAAGACTGTAACCACGCGTCTGTTGACTGGCAGGTGGTG
D D I T V V T H V A Q D C N H A S V D W Q V V
GCCAATGGTGATGTCAGCGTTGAACTGCGTGATGCGGATCAACAGGTGGTTGCAACTGGACAAGGCACT
A N G D V S V E L R D A D Q Q V V A T G Q G T
AGCGGGACTTTGCAAGTGGTGAATCCGCACCTCTGGCAACCGGGTGAAGGTTATCTCTATGAACTGTGC
S G T L Q V V N P H L W Q P G E G Y L Y E L C
GTCACAGCCAAAAGCCAGACAGAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCACTGGCAGTG
V T A K S Q T E C D I Y P L R V G I R S V A V
AAGGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGCG
K G E Q F L I N H K P F Y F T G F G R H E D A
GACTTGGCTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGGATTGGG
D L R G K G F D N V L M V H D H A L M D W I G
GCCAACTCCTACCGTACCTCGCATTACCCTTACGCTGAAGAGATGCTCGACTGGGCAGATGAACATGGC
A N S Y R T S H Y P Y A E E M L D W A D E H G
ATCGTGGTGATTGATGAAACTGCTGCTGCTCGGCTTTAACCTCTCTTTAGGCATTGGTTTTCGAAGCGGGC
I V V I D E T A A V G F N L S L G I G F E A G
AACAAGCCGAAAAGAACTGTACAGCGAAGAGGCAGTCAACGGGGAAACTCAGCAAGCGCACTTACAGGCG
N K P K E L Y S E E A V N G E T Q Q A H L Q A
ATTAAGAGCTGATAGCGCGTGACAAAACCCCAAGCGTGGTGATGTGGAGTATTGCCAACGAACCG
I K E L I A R D K N H P S V V M W S I A N E P
GATACCCGTCGCAAGGTGCACGGGAATATTTGCGCCACTGGCGGAAGCAACGCGTAAACTCGACCCG
D T R P Q G A R E Y F A P L A E A T R K L D P
ACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGCGACGCTCACACCGATACCATCAGCGATCTCTTT

T R P I T C V N V M F C D A H T D T I S D L F
GATGTGCTGTGCCTGAACCGTTATTACGGATGGTATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAG
D V L C L N R Y Y G W Y V Q S G D L E T A E K
GTACTGGAAAAAGAACTTCTGGCCTGGCAGGAGAACTGCATCAGCCGATTATCATCACCGAATACGGC
V L E K E L L A W Q E K L H Q P I I I T E Y G
GTGGATACGTTAGCCGGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGAGTATCAGTGTGCATGG
V D T L A G L H S M Y T D M W S E E Y Q C A W
CTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAACAGGTATGGAATTCGCC
L D M Y H R V F D R V S A V V G E Q V W N F A
GATTTTGCACCTCGCAAGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCGC
D F A T S Q G I L R V G G N K K G I F T R D R
AAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTAAAAACCGCAG
K P K S A A F L L Q K R W T G M N F G E K P Q
CAGGGAGGCAACAATGA
Q G G K Q *

pGWBn35 (LUC) 1653bp

ATGGAAGACGCCAAAAACATAAAGAAAGGCCCGGCCATTCTATCCGCTGGAAGATGGAACCGCTGGA
M E D A K N I K K G P A P F Y P L E D G T A G
GAGCAACTGCATAAGGCTATGAAGAGATACGCCCTGGTTCCTGGAACAATTGCTTTTACAGATGCACAT
E Q L H K A M K R Y A L V P G T I A F T D A H
ATCGAGGTGGACATCACTTACGCTGAGTACTTCGAAATGTCCGTTTCGGTTGGCAGAAGCTATGAAACGA
I E V D I T Y A E Y F E M S V R L A E A M K R
TATGGGCTGAATACAAATCACAGAATCGTCGTATGCAGTGAAAACCTCTTCAATTCTTTATGCCGGTG
Y G L N T N H R I V V C S E N S L Q F F M P V
TTGGGCGCGTTATTTATCGGAGTTGCAGTTGCGCCCGCAACGACATTTATAATGAACGTGAATTGCTC
L G A L F I G V A V A P A N D I Y N E R E L L
AACAGTATGGGCATTTTCGAGCCTACCGTGGTTCGTTTCCAAAAAGGGTTGCAAAAAATTTTGAAC
N S M G I S Q P T V V F V S K K G L Q K I L N
GTGCAAAAAAAGCTCCCAATCATCCAAAAATTATTATCATGGATTCTAAAACGGATTACCAGGGATTT
V Q K K L P I I Q K I I I M D S K T D Y Q G F
CAGTCGATGTACACGTTTCGTCACATCTCATCTACCTCCCGGTTTTAATGAATACGATTTTGTGCCAGAG
Q S M Y T F V T S H L P P G F N E Y D F V P E
TCCTTCGATAGGGACAAGACAATTGCACTGATCATGAACTCCTCTGGATCTACTGGTCTGCCTAAAGGT
S F D R D K T I A L I M N S S G S T G L P K G
GTCGCTCTGCCTCATAGAACTGCCTGCGTGAGATTCTCGCATGCCAGAGATCCTATTTTTGGCAATCAA
V A L P H R T A C V R F S H A R D P I F G N Q
ATCATTCCGATACTGCGATTTTAAGTGTGTTCCATTCCATCACGGTTTTTGAATGTTTACTACTCT
I I P D T A I L S V V P F H H G F G M F T T L
GGATATTTGATATGTGGATTTTCGAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTCTGAGGAGC
G Y L I C G F R V V L M Y R F E E E L F L R S
CTTCAGGATTACAAGATTCAAAGTGCCTGCTGGTGGCAACCCTATTCTCCTTCTTCGCCAAAAGCACT
L Q D Y K I Q S A L L V P T L F S F F A K S T
CTGATTGACAAATACGATTTATCTAATTTACACGAAATTGCTTCTGGTGGCGCTCCCCTCTCTAAGGAA
L I D K Y D L S N L H E I A S G G A P L S K E
GTCGGGGAAGCGGTTGCCAAGAGGTTCCATCTGCCAGGTATCAGGCAAGGATATGGGCTCACTGAGACT
V G E A V A K R F H L P G I R Q G Y G L T E T
ACATCAGCTATTCTGATTACACCCGAGGGGATGATAAACCGGGCGCGGTTCGGTAAAGTTGTTCCATTT
T S A I L I T P E G D D K P G A V G K V V P F
TTTGAAGCGAAGGTTGTGGATCTGGATACCGGAAAACGCTGGGCGTTAATCAAAGAGGCGAACTGTGT
F E A K V V D L D T G K T L G V N Q R G E L C
GTGAGAGGTCCTATGATTATGTCCGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGATTGACAAG
V R G P M I M S G Y V N N P E A T N A L I D K
GATGGATGGCTACATTCTGGAGACATAGCTTACTGGGACGAAGACGAACACTTCTTCATCGTTGACCGC
D G W L H S G D I A Y W D E D E H F F I V D R
CTGAAGTCTCTGATTAAGTACAAAGGCTATCAGGTGGCTCCCGCTGAATTGGAATCCATCTTGCTCAA

L K S L I K Y K G Y Q V A P A E L E S I L L Q
CACCCCAACATCTTCGACGCAGGTGTCGCAGGTCTTCCCGACGATGACGCCGGTGAACCTCCCGCCGCC
H P N I F D A G V A G L P D D D A G E L P A A
GTTGTTGTTTTGGAGCACGAAAGACGATGACGGAAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTA
V V V L E H G K T M T E K E I V D Y V A S Q V
ACAACCGCAAAAAAGTTGCGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGAAAA
T T A K K L R G G V V F V D E V P K G L T G K
CTCGACGCAAGAAAAATCAGAGAGATCCTCATAAAGCCAAGAAGGGCGAAAGATCGCCGTGTAA
L D A R K I R E I L I K A K K G G K I A V *

pGWBn40, n41, n42 (EYFP) 717bp

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
M V S K G E E L F T G V V P I L V E L D G D V
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
N G H K F S V S G E G E G D A T Y G K L T L K
TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTG
F I C T T G K L P V P W P T L V T T F G Y G L
CAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGC
Q C F A R Y P D H M K Q H D F F K S A M P E G
TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC
Y V Q E R T I F F K D D G N Y K T R A E V K F
GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG
E G D T L V N R I E L K G I D F K E D G N I L
GGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC
G H K L E Y N Y N S H N V Y I M A D K Q K N G
ATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
I K V N F K I R H N I E D G S V Q L A D H Y Q
CAGAACACCCCATCGGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCC
Q N T P I G D G P V L L P D N H Y L S Y Q S A
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGGATC
L S K D P N E K R D H M V L L E F V T A A G I
ACTCTCGGCATGGACGAGCTGTACAAG
T L G M D E L Y K

pGWBn43, n44, n45 (EGFP) 717bp

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
M V S K G E E L F T G V V P I L V E L D G D V
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
N G H K F S V S G E G E G D A T Y G K L T L K
TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTGGGGCGTG
F I C T T G K L P V P W P T L V T T L T W G V
CAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCCAAGGC
Q C F S R Y P D H M K Q H D F F K S A M P E G
TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC
Y V Q E R T I F F K D D G N Y K T R A E V K F
GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG
E G D T L V N R I E L K G I D F K E D G N I L
GGGCACAAGCTGGAGTACAACACTACATCAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGC
G H K L E Y N Y I S H N V Y I T A D K Q K N G
ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
I K A N F K I R H N I E D G S V Q L A D H Y Q
CAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCCACAACCACTACCTGAGCACCCAGTCCGCC
Q N T P I G D G P V L L P D N H Y L S T Q S A
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGGATC
L S K D P N E K R D H M V L L E F V T A A G I
ACTCTCGGCATGGACGAGCTGTACAAG
T L G M D E L Y K

pGWBn50, n51, n52 (G3GFP) 717bp

ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAAT
M S K G E E L F T G V V P I L V E L D G D V N
GGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACCTTACCCTTAAATTT
G H K F S V S G E G E G D A T Y G K L T L K F
ATTTGCACTACTGGAAAACCTTACCTGTTCCATGGCCAACACTTGTTACTACTTTTCGCTTACGGTGTGCAG
I C T T G K L P V P W P T L V T T F A Y G V Q
TGCTTCTCAAGATACCCAGATCATATGAAGCGGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGATAC
C F S R Y P D H M K R H D F F K S A M P E G Y
GTGCAGGAGAGGACCATCTTCTTCAAGGACGACGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAG
V Q E R T I F F K D D G N Y K T R A E V K F E
GGAGACACCCTCGTCAACAGGATCGAGCTTAAGGGAATCGATTTCAAGGAGGACGGAAACATCCTCGGC
G D T L V N R I E L K G I D F K E D G N I L G
CACAAGTTGGAATACAACCTTCAACTCCCACAACGTGTACATCATGGCAGACAAACAAAAGAATGGAATC
H K L E Y N F N S H N V Y I M A D K Q K N G I
AAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAA
K V N F K I R H N I E D G S V Q L A D H Y Q Q
AATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTT
N T P I G D G P V L L P D N H Y L S T Q S A L
TCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACA
S K D P N E K R D H M V L L E F V T A A G I T
CATGGCATGGATGAACTATACAAATAA
H G M D E L Y K *

Termination codon (TAA) is included in pGWBn50 and pGWBn51 (C-G3GFP). Termination codon is not included in pGWBn52 (N-G3GFP).

pGWBn53, n54, n55 (mRFP) 678bp

ATGGCCTCCTCCGAGGACGTCATCAAGGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCGTGAAC
M A S S E D V I K E F M R F K V R M E G S V N
GGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCAGACCGCCAAGCTG
G H E F E I E G E G E G R P Y E G T Q T A K L
AAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCAGTACGGCTCC
K V T K G G P L P F A W D I L S P Q F Q Y G S
AAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGCCTTCCCCGAGGGCTTCAAG
K A Y V K H P A D I P D Y L K L S F P E G F K
TGGGAGCGCGTGATGAACTTCGAGGACGGCGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGAC
W E R V M N F E D G G V V T V T Q D S S L Q D
GGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAG
G E F I Y K V K L R G T N F P S D G P V M Q K
AAGACCATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATC
K T M G W E A S T E R M Y P E D G A L K G E I
AAGATGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACCCGAGGTCAAGACCACCTACATGGCCAAG
K M R L K L K D G G H Y D A E V K T T Y M A K
AAGCCCGTGACGCTGCCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGAC
K P V Q L P G A Y K T D I K L D I T S H N E D
TACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCACTCCACCGGCGCCTAA
Y T I V E Q Y E R A E G R H S T G A *

Termination codon (TAA) is included in pGWBn53 and pGWBn54 (C-mRFP). Termination codon is not included in pGWBn55 (N-mRFP).

pGWBn59, n60, n61 (tagRFP) 714bp

ATGGTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTG
M V S K G E E L I K E N M H M K L Y M E G T V
AACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGA
N N H H F K C T S E G E G K P Y E G T Q T M R
ATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGC
I K V V E G G P L P F A F D I L A T S F M Y G
AGCAGAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTC
S R T F I N H T Q G I P D F F K Q S F P E G F
ACATGGGAGAGAGTCAACACATACGAAGACGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAG
T W E R V T T Y E D G G V L T A T Q D T S L Q
GACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAG
D G C L I Y N V K I R G V N F P S N G P V M Q
AAGAAAACACTCGGCTGGGAGGCCAACACCGAGATGCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGA
K K T L G W E A N T E M L Y P A D G G L E G R
AGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTCAAGACCACATACAGATCC
S D M A L K L V G G G H L I C N F K T T Y R S
AAGAAACCCGCTAAGAACCTCAAGATGCCCCGGCGTCTACTATGTGGACCACAGACTGGAAAGAATCAAG
K K P A K N L K M P G V Y Y V D H R L E R I K
GAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGC
E A D K E T Y V E Q H E V A V A R Y C D L P S
AAACTGGGGCACAACTTAATTGA
K L G H K L N *

Termination codon (TGA) is included in pGWBn59 and pGWBn60 (C-tagRFP). Termination codon is not included in pGWBn61 (N-tagRFP).

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