

Improved Gateway Binary Vector (ImpGWB)

1. All ImpGWB vectors confer resistance to spectinomycin in *E. coli* and *Agrobacterium tumefaciens*.
2. ImpGWB vectors confer resistance to kanamycin (pGWB4xx) or hygromycin (pGWB5xx) in plants.
3. Some of the vectors contain the 35S promoter upstream of the cloning site while others have none.
4. The N- or C-terminal tags are fused subsequent to the LR reaction.
5. Please read the Gateway instruction manual (Invitrogen) for procedures, etc.
6. See *Biosci. Biotechnol. Biochem.*, 71: 2095-2100 (2007) for the reference.

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 the licencing of Gateway™.

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Construction of ImpGWBs

The binary vector used as the starting plasmid for the construction of ImpGWBs was modified pPZP221 (Hajdukiewics, P. *et al. Plant Mol. Biol.*, 25, 989-994. 1994) which contained *aadA* for the selection of bacteria with spectinomycin. The pGWB4xx contained Pnos:NPTII:Tnos (Kmr) marker, and pGWB5xx contained Pnos:HPT:Tnos (Hygr) marker for plant selection.

The complete nucleotide sequence of ImpGWBs was registered under accession number AB294425 – AB294510.

Antibiotics for selection of ImpGWBs

Antibiotics can be used for selection in *E. coli* and *A. tumefaciens*.

All ImpGWBs : spectinomycin (100mg/l) **and** chloramphenicol (30mg/l)

All recombinant ImpGWB (after LR reaction) : spectinomycin (100mg/l)

Antibiotics can be used for selection in plant.

pGWB4xx : kanamycin

pGWB5xx : hygromycin

Amplification of ImpGWB vectors

- (1) Use competent cell of DB3.1 (Invitrogen 11782-018) or ccdB Survival T1 (Invitrogen C7510-03)
- (2) Spread on a plate containing spectinomycin (100 mg/l) **and** chloramphenicol (30 mg/l). We made and use 10 mg/ml (methanol) stock solution of chloramphenicol.
- (3) Pick up colony and culture in a liquid medium containing spectinomycin (100 mg/l) **and** chloramphenicol (30 mg/l)
- (4) Prepare plasmids from *E. coli* with plasmid purification kit. We usually use Mag Extractor plasmid (Toyobo NPK-301).

LR reaction and transformation

- (1) Reaction mixture can be scaled down. We usually use the following reaction:
- (2)

Entry clone	0.5 μ l	(about 150 ng)
BiFC vector	1.0 μ l	(about 150 ng)
LR clonase II	0.5 μ l	
H ₂ O	2.0 μ l	

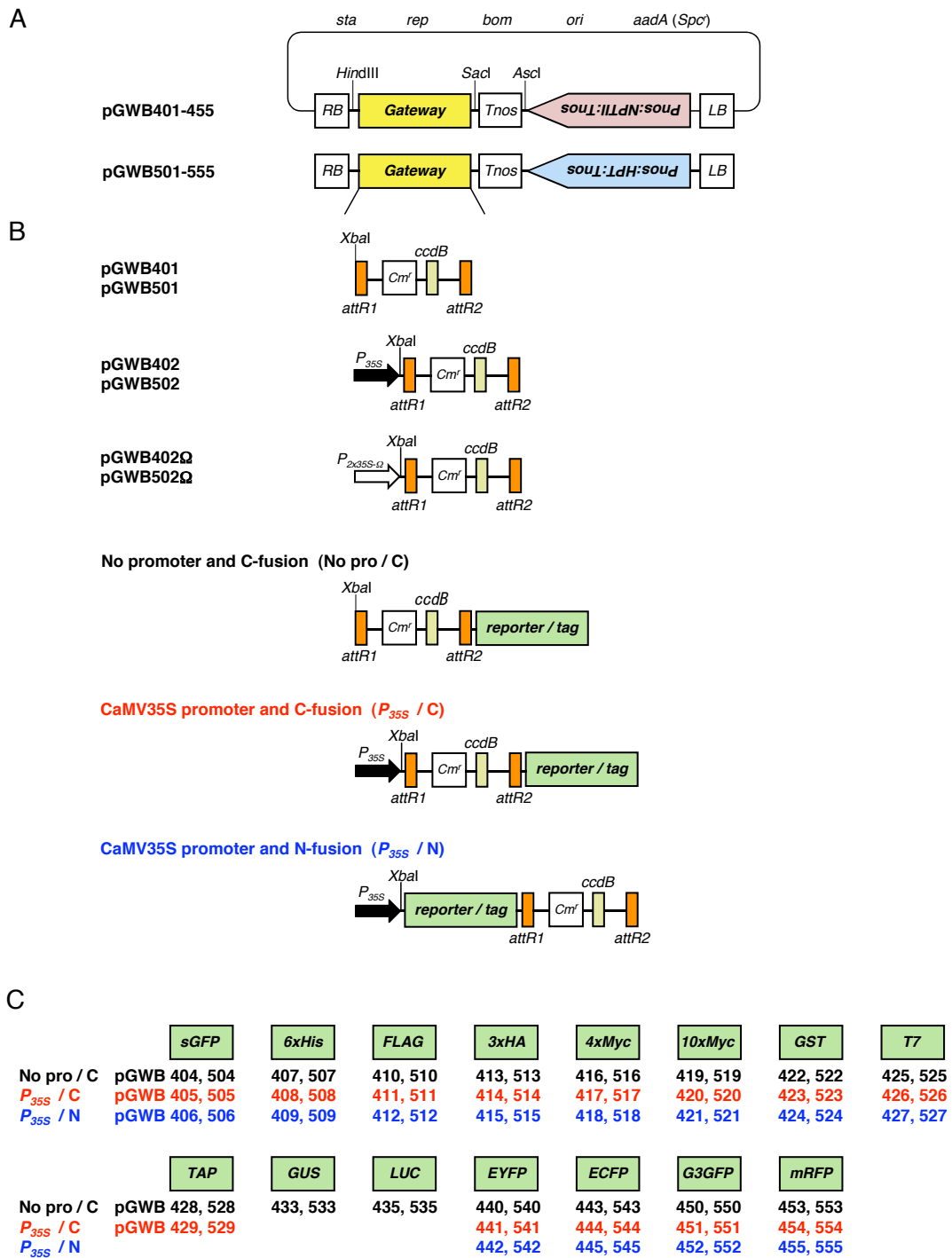
total 4.0 μ l

- (3) Incubate at 25 °C for more than 3hr.
- (4) 75 °C for 10 min. We do not use proteinase K solution to stop the reaction.
- (5) Add 2 μ l of reaction mixture to 5 μ l of DH5 α competent cell. We usually use Competent High DH5 α (Toyobo DNA-903). On ice for 30 min, 42 °C for 45 sec, on ice for 2 min, add 100 μ l of SOC, shake for 1hr.
- (6) Spread on a plate containing spectinomycin (50 mg/l).

Note

- (1) Linearization of ImpGWBs before performing the LR reaction is not necessary.
- (2) Use the *Escherichia coli* strain DH5alpha for cloning the product of the LR (BP) reactions, since negative selection with *ccdB* does not work in the *E.coli* which has F' episome (see the Invitrogen instruction manual).
- (3) Sometimes, *E.coli* with both the Entry Clone and recombinant ImpGWB are obtained subsequent to the cloning. We recommend that only the *E. coli* colonies with the recombinant ImpGWB be used.

Structure of ImpGWBs



A, Outline of ImpGWB. B, Structure of the region indicated *Gateway* in A. C, Reporters and tags. *Sta*, stability in *A. tumefaciens*; *rep*, broad host-range replication origin; *bom*, conjugational transfer; *ori*, ColE1 origin. *Cm^r*, chloramphenicol resistant marker; *Spc^r*, spectinomycin resistant marker.

List of the ImpGWBs

reporter/tag	selection marker	
	NosP-NPTII	NosP-HPT
R1-R2-Tnos	pGWB401	pGWB501
35S-R1-R2-Tnos	pGWB402	pGWB502
2x35S Ω -R1-R2-Tnos	pGWB402 Ω	pGWB502 Ω
R1-R2-sGFP-Tnos	pGWB404	pGWB504
35S-R1-R2-sGFP-Tnos	pGWB405	pGWB505
35S-sGFP-R1-R2-Tnos	pGWB406	pGWB506
R1-R2-6xHis-Tnos	pGWB407	pGWB507
35S-R1-R2-6xHis-Tnos	pGWB408	pGWB508
35S-6xHis-R1-R2-Tnos	pGWB409	pGWB509
R1-R2-FLAG-Tnos	pGWB410	pGWB510
35S-R1-R2-FLAG-Tnos	pGWB411	pGWB511
35S-FLAG-R1-R2-Tnos	pGWB412	pGWB512
R1-R2-3xHA-Tnos	pGWB413	pGWB513
35S-R1-R2-3xHA-Tnos	pGWB414	pGWB514
35S-3xHA-R1-R2-Tnos	pGWB415	pGWB515
R1-R2-4xMyc-Tnos	pGWB416	pGWB516
35S-R1-R2-4xMyc-Tnos	pGWB417	pGWB517
35S-4xMyc-R1-R2-Tnos	pGWB418	pGWB518
R1-R2-10xMyc-Tnos	pGWB419	pGWB519
35S-R1-R2-10xMyc-Tnos	pGWB420	pGWB520
35S-10xMyc-R1-R2-Tnos	pGWB421	pGWB521
R1-R2-GST-Tnos	pGWB422	pGWB522
35S-R1-R2-GST-Tnos	pGWB423	pGWB523
35S-GST-R1-R2-Tnos	pGWB424	pGWB524
R1-R2-T7-Tnos	pGWB425	pGWB525
35S-R1-R2-T7-Tnos	pGWB426	pGWB526
35S-T7-R1-R2-Tnos	pGWB427	pGWB527
R1-R2-TAP-Tnos	pGWB428	pGWB528
35S-R1-R2-TAP-Tnos	pGWB429	pGWB529
R1-R2-GUS-Tnos	pGWB433	pGWB533
R1-R2-LUC-Tnos	pGWB435	pGWB535
R1-R2-EYFP-Tnos	pGWB440	pGWB540
35S-R1-R2-EYFP-Tnos	pGWB441	pGWB541
35S-EYFP-R1-R2-Tnos	pGWB442	pGWB542
R1-R2-ECFP-Tnos	pGWB443	pGWB543
35S-R1-R2-ECFP-Tnos	pGWB444	pGWB544
35S-ECFP-R1-R2-Tnos	pGWB445	pGWB545
R1-R2-G3GFP-Tnos	pGWB450	pGWB550
35S-R1-R2-G3GFP-Tnos	pGWB451	pGWB551
35S-G3GFP-R1-R2-Tnos	pGWB452	pGWB552
R1-R2-mRFP-Tnos	pGWB453	pGWB553
35S-R1-R2-mRFP-Tnos	pGWB454	pGWB554
35S-mRFP-R1-R2-Tnos	pGWB455	pGWB555

Nucleotide sequences around *attR* sites of ImpGWBs

The *Hind*III (AAGCTT), *Xba*I (TCTAGA) and *Sac*I (GAGCTC) sites are shown.

The *attR*1-CmR-*ccdB*-*attR*2 cassette is underlined.

pGWB401, 501 : [(no promoter, no tag) (--35S promoter-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)

pGWB402, 502: [(35S promoter, no tag) (--35S promoter-R1-*ccdB*-R2 --)]

(AAGCTT) -- 35S 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)

pGWB402Ω, 502Ω: [(2x35S omega, no tag) (--35S promoter-R1-*ccdB*-R2 --)]

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

pGWB404, 504 : [(no promoter, C-sGFP) (--R1-*ccdB*-R2–sGFP--)]

(AAGCTT)GCATGCCTGCAGGC (TCTA A)TC ACA AGT TTG TAC AAA AAA --(Cm-R, *ccdB*)--
TTC TTG TAC AAA GTG GTT ATC ATG --(*sGFP* sequence) –AAG TAA AGCGGCC (GAGCTC)

(1) As a result of a mistake during the fill-in reaction while constructing pGWB4, the *Xba*I site TCTAGA was changed to TCTAA. This should not, however, affect the results of your experiments.

(2) The initiation codon ATG (double-underlined) is from the sGFP coding sequence. The termination codon TAA of sGFP is indicated by bold-underline.

pGWB405, 505 : [(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 --(*sGFP* sequence) -- AAG TAA AGCGGCC (GAGCTC)

- (1) As a result of a mistake during the fill-in reaction while constructing pGWB5, the *Xba*I site TCTAGA was changed to TCTAA. This should not, however, affect the results of your experiments.
- (2) The initiation codon ATG (double-underlined) is from the sGFP coding sequence. The termination codon TAA of sGFP is indicated by bold-underline.

pGWB406, 506: [(35S promoter, N-sGFP) (--35S promoter-sGFP-R1-ccdB-R2--)]
 (AAGCTT) -- 35S promoter //--(TCTAGA)GGATCC ATG --(sGFP 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 initiation codon ATG (double-underlined) is from the sGFP coding sequence. The termination codon TAA (bold-underlined) is derived from *GFP*.

pGWB407, 408, 410, 411, 413, 414, 416, 417, 419, 420, 422, 423, 425, 426, 428, 429, 433, 435, 440, 441, 443, 444, 450, 451, 453, 454, 507, 508, 510, 511, 513, 514, 516, 517, 519, 520, 522, 523, 525, 526, 528, 529, 533, 535, 540, 541, 543, 544, 550, 551, 553, 554 (no promoter or 35S promoter, C-tag)

No promoter --- 07, 10, 13, 16, 19, 22, 25, 28, 33, 35, 40, 43, 50, 53

35S promoter --- 08, 11, 14, 17, 20, 23, 26, 29, 41, 44, 51, 54

(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 sequence of each tag is indicated below.

pGWB409, 412, 415, 418, 421, 424, 427, 442, 445, 552, 554 (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 sequence of each **tags** is indicated below.

Nucleotide and amino acid sequences around *attB* sites after LR reaction

pGWB401 (no promoter; no fusion)
pGWB501

AAGCTTGCATGCCTGCAGGTCGACTCTAGAGTTATCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GIT GAT AAC AGC GCT TAG AGCTC

attB1

attB2

pGWB402 (CaMV35S promoter; no fusion)
pGWB502

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GIT GAT AAC AGC GCT TAG AGCTC

attB1

attB2

X P A F L Y K V V D N S A *

pGWB402Ω (2xCaMV35S-Ω promoter; no fusion)
pGWB502Ω

AAGCTTGCATGGCTAGAG-(2xCaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGGACCGCTGAAATCACCAAG

TGTATTTTACAACAATTACCAACAACAACAACAACAACAACAACAATTACTATTACAATTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GIT GAT AAC AGC GCT TAG AGCTC

attB1

attB2

X P A F L Y K V V D N S A *

pGWB404 (no promoter, C-fusion to sGFP)
pGWB504

AAGCTTGCATGCCTGCAGGCTAATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATC ATG-(sGFP)-AAG TAA AGCGGCCCGAGCTC

X P A F L Y K V V I M K *

attB1

attB2

pGWB405 (CaMV35S promoter; C-fusion to sGFP)
pGWB505

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GIT CGA TCT AGA GGA TCC ATG-(sGFP)-AAG TAA AGCGGCCCGAGCTC

X P A F L Y K V V R S R G S M K *

attB1

attB2

pGWB406 (CaMV35S promoter; N-fusion to sGFP)
pGWB506

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAGACACGGGGGACTCTAGAGGATCC

ATG-(sGFP)-CTG TAC ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATG TAC AAG TAA AGCGGCCCGAGCTC

M L Y I T S L Y K K A G X X P A F L Y K V V M Y K *

attB1

attB2

pGWB407 (no promoter; C-fusion to 6xHis)
pGWB507

AAGCTTGCATGCCTGCAGGTCGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GIT GAT AAC AGC CAT CAT CAT CAT CAT CAT GCT TAG AGCTC

X P A F L Y K V V D N S H H H H H H A *

attB1

attB2

pGWB408 (CaMV35S promoter; C-fusion to 6xHis)
pGWB508

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GIT GAT AAC AGC CAT CAT CAT CAT CAT CAT GCT TAG AGCTC

X P A F L Y K V V D N S H H H H H H A *

attB1

attB2

pGWB409 (CaMV35S promoter; N-fusion to 6xHis)
pGWB509

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAGACACGGGGGACTCTAGA

ATG AGC CAT CAT CAT CAT CAT CAT GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC

M S H H H H H H A V I T S L Y K K A G X X P A F L Y K V V I T *

attB1

attB2

pGWB410 (no promoter; C-fusion to FLAG)
pGWB510

AAGCTTGATGCCTGCAGTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GAC TAC AAG GAT GAC GAT GAC AAG GCT TAG AGCTC
X P A F L Y K V V D N S D Y K D D D D D K A *
attB1 attB2

pGWB411 (CaMV35S promoter; C-fusion to FLAG)
pGWB511

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GAC TAC AAG GAT GAC GAT GAC AAG GCT TAG AGCTC
X P A F L Y K V V D N S D Y K D D D D D K A *
attB1 attB2

pGWB412 (CaMV35S promoter; N-fusion to FLAG)
pGWB512

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGA

ATG AGC GAC TAC AAG GAT GAC GAT GAC AAG GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
M S D Y K D D D D K A V I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB413 (no promoter; C-fusion to 3xHA)
pGWB513

AAGCTTGATGCCTGCAGTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GGG TTA ATT AAC ATC TTT
X P A F L Y K V V D N S G L I N I F
attB1 attB2

TAC CCA TAC GAT GTT CCT GAC TAT GCG GGC (HA) GGA TCC (HA) GCT CAG TGC AGC GCT TAG AGCTC
Y P Y D V P D Y A G G S A Q C S A *

pGWB414 (CaMV35S promoter; C-fusion to 3xHA)
pGWB514

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GGG TTA ATT AAC ATC TTT
X P A F L Y K V V D N S G L I N I F
attB1 attB2

TAC CCA TAC GAT GTT CCT GAC TAT GCG GGC (HA) GGA TCC (HA) GCT CAG TGC AGC GCT TAG AGCTC
Y P Y D V P D Y A G G S A Q C S A *

pGWB415 (CaMV35S promoter; N-fusion to 3xHA)
pGWB515

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGA

ATG AGC GGG TTA ATT AAC ATC TTT TAC CCA TAC GAT GTT CCT GAC TAT GCG GGC (HA) GGA TCC (HA) GCT CAG TGC AGC GCT GTT
M S G L I N I F Y P Y D V P D Y A G G S A Q C S A V

ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB416 (no promoter; C-fusion to 4xMyc)
pGWB516

AAGCTTGGGTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GGG TTA ATT AAC GGT
X P A F L Y K V V D N S G L I N G
attB1 attB2

GAA CAA AAG CTA ATC TCC GAG GAA GAC TTG AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT AGC GCT TAG AGCTC
E Q K L I S E E D L N G N G L D G N G N G S A *

pGWB417 (CaMV35S promoter; C-fusion to 4xMyc)
pGWB517

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTGAGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GGG TTA ATT AAC GGT
X P A F L Y K V V D N S G L I N G
attB1 attB2

GAA CAA AAG CTA ATC TCC GAG GAA GAC TTG AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT AGC GCT TAG AGCTC
E Q K L I S E E D L N G N G L D G N G N G S A *

pGWB418 (CaMV35S promoter; N-fusion to 4xMyc)
pGWB518

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTGAGAGAGACACGGGGGACTCTAGA

ATG AGC GGG TTA ATT AAC GGT GAA CAA AAG CTA ATC TCC GAG GAA GAC TTG AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT AGC GCT GTT
M S G L I N G E Q K L I S E E D L N G N G L D G N G N G S A V

ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB419 (no promoter; C-fusion to 10xMyc)
pGWB519

AAGCTTGGGTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GGG TTA ATT AAC GGT
X P A F L Y K V V D N S G L I N G
attB1 attB2

GAA CAA AAG CTA ATC TCC GAG GAA GAC TTG AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT (Myc) AAC GGA CTC GAC GGT
E Q K L I S E E D L N G N G L D G N G N G N G L D G

(Myc) AAC GGT (Myc) AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT GCT TAG AGCTC
N G N G N G L D G N G N G A *

pGWB420 (CaMV35S promoter; C-fusion to 10xMyc)
pGWB520

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTGAGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC GGG TTA ATT AAC GGT
X P A F L Y K V V D N S G L I N G
attB1 attB2

GAA CAA AAG CTA ATC TCC GAG GAA GAC TTG AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT (Myc) AAC GGA CTC GAC GGT
E Q K L I S E E D L N G N G L D G N G N G N G L D G

(Myc) AAC GGT (Myc) AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT GCT TAG AGCTC
N G N G N G L D G N G N G A *

pGWB421 (CaMV35S promoter; N-fusion to 10xMyc)
pGWB521

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTGAGAGAGACACGGGGGACTCTAGA

ATG AGC GGG TTA ATT AAC GGT GAA CAA AAG CTA ATC TCC GAG GAA GAC TTG AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT
M S G L I N G E Q K L I S E E D L N G N G L D G N G N G

(Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT (Myc) AAC GGA CTC GAC GGT (Myc) AAC GGT (Myc) AAC GGT AGC GCT GTT
N G L D G N G N G N G L D G N G N G S A V

ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB422 (no promoter; C-fusion to GST)
pGWB522

AAGCTTGGTCTAGAGTTATCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(GST)-TCC GCT TAG AGCTC
X P A F L Y K V V D N S M S A *
attB1 attB2

pGWB423 (CaMV35S promoter; C-fusion to GST)
pGWB523

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAACACGGGGGACTCTAGAGTTA
TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(GST)-TCC GCT TAG AGCTC
X P A F L Y K V V D N S M S A *
attB1 attB2

pGWB424 (CaMV35S promoter; N-fusion to GST)
pGWB524

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAACACGGGGGACTCTAGA
ATG AGC ATG-(GST)-GGA TCC GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
M S M G S A V I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB425 (no promoter; C-fusion to T7)
pGWB525

AAGCTTGGTCTAGAGTTA
TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT GCT TAG AGCTC
X P A F L Y K V V D N S M A S M T G G Q Q M G A *
attB1 attB2

pGWB426 (CaMV35S promoter; C-fusion to T7)
pGWB526

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAACACGGGGGACTCTAGAGTTA
TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT GCT TAG AGCTC
X P A F L Y K V V D N S M A S M T G G Q Q M G A *
attB1 attB2

pGWB427 (CaMV35S promoter; N-fusion to T7)
pGWB527

AAGCTTGCATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTCAATTTGGAGAGAACACGGGGGACTCTAGA ATG AGC
M S
ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
M A S M T G G Q Q M C A V I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB428 (no promoter; C-fusion to TAP)
pGWB528

AAGCTTGGGCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC
X P A F L Y K V V D N S
attB1 attB2

AAG AGA AGA TGG AAA AAG AAT TTC ATA GCC GTC TCA GCA GCC AAC CGC TTT AAG AAA ATC TCA TCC TCC GGG GCA CTT GAT TAT GAT ATT CCA ACT ACT GCT AGC
K R R W K K N F I A V S A A N R F K K I S S S G A L D Y D I P T T A S
CBP

GAG AAT TTG TAT TTT CAG GGT GAG CTC AAA ACC GCG GCT CTT GCG CAA CAC GAT GAA GCC GTG GAC AAC AAA TTC AAC AAA GAA CAA CAA AAC GCG TTC TAT GAG
E N L Y F Q G E L K T A A L A Q H D E A V D N K F N K E Q Q N A F Y E
TEV Protein A

ATC TTA CAT TTA CCT AAC TTA AAC GAA GAA CAA CGA AAC GCC TTC ATC CAA AGT TTA AAA GAT GAC CCA AGC CAA AGC GCT AAC CTT TTA GCA GAA GCT AAA AAG
I L H L P N L N E E Q R N A F I Q S L K D D P S Q S A N L L A E A K K

CTA AAT GAT GCT CAG GCG CCG AAA GTA GAC AAC AAA TTC AAC AAA GAA CAA CAA AAC GCG TTC TAT GAG ATC TTA CAT TTA CCT AAC TTA AAC GAA GAA CAA CGA
L N D A Q A P K V D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R

AAC GCC TTC ATC CAA AGT TTA AAA GAT GAC CCA AGC CAA AGC GCT AAC CTT TTA GCA GAA GCT AAA AAG CTA AAT GGT GCT CAG GCG CCG AAA GTA GAC GCG AAT
N A F I Q S L K D D P S Q S A N L L A E A K K L N G A Q A P K V D A N

TCC GCG GGG AAG TCA ACC TGA GCT TAG AGCTC
S A G K S T *

pGWB429 (CaMV35S promoter; C-fusion to TAP)
pGWB529

AAGCTTGCATGCCTCAGGT-(CaMV35S)-CGCAAGACCCCTCTATATAAGGAAGTTCATTTGAGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC
X P A F L Y K V V D N S
attB1 attB2

AAG AGA AGA TGG AAA AAG AAT TTC ATA GCC GTC TCA GCA GCC AAC CGC TTT AAG AAA ATC TCA TCC TCC GGG GCA CTT GAT TAT GAT ATT CCA ACT ACT GCT AGC
K R R W K K N F I A V S A A N R F K K I S S S G A L D Y D I P T T A S
CBP

GAG AAT TTG TAT TTT CAG GGT GAG CTC AAA ACC GCG GCT CTT GCG CAA CAC GAT GAA GCC GTG GAC AAC AAA TTC AAC AAA GAA CAA CAA AAC GCG TTC TAT GAG
E N L Y F Q G E L K T A A L A Q H D E A V D N K F N K E Q Q N A F Y E
TEV Protein A

ATC TTA CAT TTA CCT AAC TTA AAC GAA GAA CAA CGA AAC GCC TTC ATC CAA AGT TTA AAA GAT GAC CCA AGC CAA AGC GCT AAC CTT TTA GCA GAA GCT AAA AAG
I L H L P N L N E E Q R N A F I Q S L K D D P S Q S A N L L A E A K K

CTA AAT GAT GCT CAG GCG CCG AAA GTA GAC AAC AAA TTC AAC AAA GAA CAA CAA AAC GCG TTC TAT GAG ATC TTA CAT TTA CCT AAC TTA AAC GAA GAA CAA CGA
L N D A Q A P K V D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R

AAC GCC TTC ATC CAA AGT TTA AAA GAT GAC CCA AGC CAA AGC GCT AAC CTT TTA GCA GAA GCT AAA AAG CTA AAT GGT GCT CAG GCG CCG AAA GTA GAC GCG AAT
N A F I Q S L K D D P S Q S A N L L A E A K K L N G A Q A P K V D A N

TCC GCG GGG AAG TCA ACC TGA GCT TAG AGCTC
S A G K S T *

pGWB433 (no promoter; C-fusion to GUS)
pGWB533

AAGCTTGCATGCCTCAGGTGACTGACTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(GUS)-CAA TGA GCT TAG AGCTC
X P A F L Y K V V D N S M Q *
attB1 attB2

pGWB435 (no promoter; C-fusion to LUC)
pGWB535

AAGCTTGCATGCCTCAGGTGACTGACTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(LUC)-GTG-TAA GCT TAG AGCTC
X P A F L Y K V V D N S M V *
attB1 attB2

pGWB440 (no promoter; C-fusion to EYFP)
pGWB540

AAGCTTGATGCCTGCAGGTCGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(EYFP)-AAG GCT TAG AGCTC
X P A F L Y K V V D N S M K A *
attB1 attB2

pGWB441 (CaMV35S promoter; C-fusion to EYFP)
pGWB541

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(EYFP)-AAG GCT TAG AGCTC
X P A F L Y K V V D N S M K A *
attB1 attB2

pGWB442 (CaMV35S promoter; N-fusion to EYFP)
pGWB542

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGA

ATG AGC ATG-(EYFP)-AAG GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT ATA ACC TAG AGCTC
M S M K A V I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB443 (no promoter; C-fusion to ECFP)
pGWB543

AAGCTTGATGCCTGCAGGTCGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(ECFP)-AAG GCT TAG AGCTC
X P A F L Y K V V D N S M K A *
attB1 attB2

pGWB444 (CaMV35S promoter; C-fusion to ECFP)
pGWB544

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(ECFP)-AAG GCT TAG AGCTC
X P A F L Y K V V D N S M K A *
attB1 attB2

pGWB445 (CaMV35S promoter; N-fusion to ECFP)
pGWB545

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGA

ATG AGC ATG-(ECFP)-AAG GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT ATA ACC TAG AGCTC
M S M K A V I T S L Y K K A G X X P A F L Y K V V I T *
attB1 attB2

pGWB450 (no promoter; C-fusion to G3GFP)
pGWB550

AAGCTTGATGCCTGCAGGTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(G3GFP)-AAA TAA GCT TAG AGCTC
X P A F L Y K V V D N S M K *

pGWB451 (CaMV35S promoter; C-fusion to G3GFP)
pGWB551

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(G3GFP)-AAA TAA GCT TAG AGCTC
X P A F L Y K V V D N S M K *

pGWB452 (CaMV35S promoter; N-fusion to G3GFP)
pGWB552

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGA

ATG AGC ATG-(G3GFP)-AAA GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
M S M K A V I T S L Y K K A G X X P A F L Y K V V I T *

pGWB453 (no promoter; C-fusion to mRFP)
pGWB553

AAGCTTGATGCCTGCAGGTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(mRFP)-GCC TAA GCT TAG AGCTC
X P A F L Y K V V D N S M A *

pGWB454 (CaMV35S promoter; C-fusion to mRFP)
pGWB554

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGAGTTA

TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(mRFP)-GCC TAA GCT TAG AGCTC
X P A F L Y K V V D N S M A *

pGWB455 (CaMV35S promoter; N-fusion to mRFP)
pGWB555

AAGCTTGATGCCTGCAGGT-(CaMV35S)-CGCAAGACCTTCTCTATATAAGGAAGTTCATTTTCATTGGAGAGACACGGGGGACTCTAGA

ATG AGC ATG-(mRFP)-AAA GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (c1one) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
M S M K A V I T S L Y K K A G X X P A F L Y K V V I T *

The sequences between *Hind*III and *Sac* I sites (see “Structure of ImpGWB”) are shown for all vectors. To clearly show the relation of functional elements, linefeeds are irregularly inserted in the sequences. The *att*B1 and *att*B2 sites are underlined. N indicates a nucleotide derived from an entry clone (green) and X indicates an amino acid residue corresponding to the N-containing codon if the entry clone encodes a protein. The CaMV35S promoter is shown in blue, where the region between -46 and +8 is represented by nucleotide sequence with the predicted transcription start site (underlined larger letter). The Ω translation enhancer equipped in pGWB402 Ω and 502 Ω is indicated in orange. Reporters and tags are indicated in red. Only partial sequences are shown for long reporters/tags, whose whole sequences are indicated in a later section, and can also be referred to the corresponding vector sequences registered in the public databases. The region of calmodulin binding peptide (CBP), TEV protease cleavage site (TEV), and protein A in TAP are underlined for pGWB428, 429, 528, and 529. In C-fusion vectors, the peptide sequence derived from *att*B2 region becomes the linker between the entry clone and reporter/tag. If the entry clone is a promoter fragment lacking translational initiation codon, the first downstream ATG around *att*B2 site (i.e. the native initiation codon of the reporter, indicated by red M) will be used for translational initiation. In N-fusion vectors, the peptide sequence derived from *att*B1 region becomes the linker between reporter/tag and the entry clone, and the peptide sequence derived from *att*B2 region (gray italic) is added at the C-terminus of the fusion protein only if the entry clone lacks the termination codon in it.

Reporters and tags

The full sequences of long reporter and tag are shown.

pGWB404, 405, 406, 504, 505, 506 (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 pGWB406 and 506 (35S promoter, N-GFP).

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ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTG
M V S K G E E L F T G V V P I L V E L D G D V
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
N G H K F S V S G E G E G D A T Y G K L T L K
TTCATCTGCACCACCGGCAAGCTGCCCGTGCCTGGCCACCCTCGTGACCACCTCACCTACGGCGTG
F I C T T G K L P V P W P T L V T T F T Y G V
CAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCGAAGGC
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
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
CAGAACACCCCATCGGCGACGGCCCGTGCTGCTGCCGACAACCACTACCTGAGCACCCAGTCCGCC
Q N T P I G D G P V L L P D N H Y L S T Q S A
CTGAGCAAAGACCCCAACGAGAAGCGGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCGGGATC
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 *
```

pGWB413, 414, 415, 513, 514, 515 (3xHA) 120bp

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

GGGTTAATTAACATCTTTTACCCATACGATGTTCTGACTATGCGGGCTATCCCTATGACGTCCGGAC
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)].

pGWB416, 417, 418, 516, 517, 518 (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.

pGWB419, 420, 421, 519, 520, 521 (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
GAAGAAGACTTGAACGGACTCGACGGTGAACAAAAGTTGATTTCTGAAGAAGATTGAACGGTGAACAA
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
GACGGTGAACAAAAGTTGATTTCTGAAGAAGATTGAACGGTGAACAAAAGCTAATCTCCGAGGAAGAC
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
TCTGAAGAAGATTGAACGGTGAACAAAAGCTAATCTCCGAGGAAGACTTGAACGGTAGC
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 pGWB419, 420, 519 and 520.

pGWB422, 423, 424, 522, 523, 524 (GST) 678bp

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

ATGTCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGAATAT
M S P I L G Y W K I K G L V Q P T R L L L E Y
CTTGAAGAAAAATATGAAGAGCATTGTATGAGCGGATGAAGGTGATAAATGGCGAAACAAAAAGTTT
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
GCCATCATACGTTATATAGCTGACAAGCACAAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATT
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
GAAACTCTCAAAGTTGATTTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGT
E T L K V D F L S K L P E M L K M F E D R L C
CATAAACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTT
H K T Y L N G D H V T H P D F M L Y D A L D V
GTTTTATACATGGACCAATGTGCCTGGATGCGTTCCTCCAAAATTAGTTTGTAAAAAACGTATTGAA
V L Y M D P M C L D A F P K L V C F K K R I E
GCTATCCACAAATTGATAAGTACTTGAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAA
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

pGWB433, 533 (GUS) 1,653bp

ATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAACTCGACGGCCTGTGGCATTCAAGTCTG
M L R P V E T P T R E I K K L D G L W A F S L
GATCGCGAAAACGTGGAATTGATCAGCGTTGGTGGAAAGCGCGTTACAAGAAAGCCGGGCAATTGCT
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
CAGCGGAAGTCTTTATACCGAAAGGTTGGCAGGCCAGCGTATCGTGCTGCGTTTCGATGCGGTCACT
Q R E V F I P K G W A G Q R I V L R F D A V T
CATTACGGCAAAGTGTGGGTCAATAATCAGGAAGTGTGGAGCATCAGGGCGGCTATACGCCATTTGAA
H Y G K V W V N N Q E V M E H Q G G Y T P F E
GCCGATGTCACGCCGTATGTTATTGCCGGGAAAAGTGTACGTATCACCGTTTGTGTGAACAACGAACTG
A D V T P Y V I A G K S V R I T V C V N N E L
AACTGGCAGACTATCCCGCCGGAATGGTGATTACCGACGAAAACGGCAAGAAAAAGCAGTCTTACTTC
N W Q T I P P G M V I T D E N G K K K Q S Y F
CATGATTTCTTAACTATGCCGGAATCCATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGGGTG
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
GTCACAGCCAAAAGCCAGACAGAGTGTGATATCTACCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTG
V T A K S Q T E C D I Y P L R V G I R S V A V
AAGGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTGGTTCATGAAGATGGG
K G E Q F L I N H K P F Y F T G F G R H E D A
GACTTGGTGGCAAAGGATTTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGGATTGGG
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
ATCGTGGTGATTGATGAAACTGCTGCTGTGGCTTTAACCTCTCTTAGGCATTGGTTTCGAAGCGGGC

I V V I D E T A A V G F N L S L G I G F E A G
AACAAGCCGAAAGAAGTGTACAGCGAAGAGGCAGTCAACGGGGAAACTCAGCAAGCGCACTTACAGGCG
N K P K E L Y S E E A V N G E T Q Q A H L Q A
ATTAAGAGCTGATAGCGCGTGACAAAAACCACCCAAGCGTGGTGATGTGGAGTATTGCCAACGAACCG
I K E L I A R D K N H P S V V M W S I A N E P
GATACCGTCCGCAAGGTGCACGGGAATATTTGCGGCCACTGGCGGAAGCAACCGGTAAACTCGACCGG
D T R P Q G A R E Y F A P L A E A T R K L D P
ACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGCGAGCTCACACCGATACCATCAGCGATCTCTTT
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
GTACTGGAAAAGAAGTCTGGCCTGGCAGGAGAAACTGCATCAGCCGATTATCATCACCGAATACGGC
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
CTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAACAGGTATGGAATTTGCC
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
AAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAACCGCAG
K P K S A A F L L Q K R W T G M N F G E K P Q
CAGGGAGGCAAACAATGA
Q G G K Q *

pGWB435, 535 (LUC) 1,653bp

ATGGAAGACGCCAAAAACATAAAGAAAGGCCGGCCATTCTATCCGCTGGAAGATGGAACCGCTGGA
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
ATCGAGGTGGACATCACTTACGCTGAGTACTTCGAAATGTCCGTTCCGTTGGCAGAAGCTATGAAACGA
I E V D I T Y A E Y F E M S V R L A E A M K R
TATGGGCTGAATACAAATCACAGAATCGTCGTATGCAGTGAAAACCTCTCTTCAATTCTTTATGCCGGTG
Y G L N T N H R I V V C S E N S L Q F F M P V
TTGGGCGGTTATTTATCGGAGTTCAGTTGCGCCCGGAACGACATTTATAATGAACGTGAATTGCTC
L G A L F I G V A V A P A N D I Y N E R E L L
AACAGTATGGGCATTTTCGAGCCTACCGTGGTGTTCGTTTCCAAAAGGGGTTGCAAAAATTTTGAAC
N S M G I S Q P T V V F V S K K G L Q K I L N
GTGCAAAAAAGCTCCCAATCATCAAAAATTATTATCATGGATTCTAAAACGGATTACCAGGGATTT
V Q K K L P I I Q K I I I M D S K T D Y Q G F
CAGTCGATGTACAGTTTCGTCACATCTCATCTACCTCCGGTTTTAATGAATACGATTTTGTGCCAGAG
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
ATCATTCCGGATACTGCGATTTTAAAGTGTGTTCCATTCCATCACGGTTTTGGAATGTTTACTACTC
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
CTTCAGGATTACAAGATTCAAAGTGCCTGCTGGTGCCAACCCTATTCTCCTTCTCGCCAAAAGCACT
L Q D Y K I Q S A L L V P T L F S F F A K S T
CTGATTGACAAATACGATTTATCTAATTTACACGAAATTGCTTCTGGTGGCGCTCCCTCTCTAAGGAA
L I D K Y D L S N L H E I A S G G A P L S K E
GTCGGGGAAGCGGTTGCCAAGAGTTCCATCTGCCAGGTATCAGGCAAGGATATGGGCTCACTGAGACT
V G E A V A K R F H L P G I R Q G Y G L T E T

ACATCAGCTATTCTGATTACCCGAGGGGGATGATAAACCGGGCGGGTGGTAAAGTTGTTCCATTT

T S A I L I T P E G D D K P G A V G K V V P F
TTTGAAGCGAAGGTTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAATCAAAGAGGCGAACTGTGT
F E A K V V D L D T G K T L G V N Q R G E L C
GTGAGAGGTCCTATGATTATGTCCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGATTGACAAG
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
CTGAAGTCTCTGATTAAGTACAAAGGCTATCAGGTGGCTCCCGCTGAATTGGAATCCATCTTGCTCCAA
L K S L I K Y K G Y Q V A P A E L E S I L L Q
CACCCCAACATCTTCGACGCAGGTGTCGCAGGTCTTCCGACGATGACGCGGTGAACTTCCGCGCGCC
H P N I F D A G V A G L P D D D A G E L P A A
GTTGTTGTTTTGGAGCACGGAAGACGATGACGGAAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTA
V V V L E H G K T M T E K E I V D Y V A S Q V
ACAACCGCGAAAAAGTTGCGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGGAAAA
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 *

pGWB440, 441, 442, 540, 541, 542 (EYFP) 717bp

ATGGTGAGCAAGGGCGAGGAGCTGTTACCCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
M V S K G E E L F T G V V P I L V E L D G D V
AACGGCCACAAGTTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAAG
N G H K F S V S G E G E G D A T Y G K L T L K
TTCATCTGCACCACCGGAAGCTGCCCGTGCCCTGGCCACCCTCGTGACCACCTTCGGCTACGGCCTG
F I C T T G K L P V P W P T L V T T F G Y G L
CAGTGCTTCGCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGGC
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
CAGAACACCCCATCGGGCAGGGCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCC
Q N T P I G D G P V L L P D N H Y L S Y Q S A
CTGAGCAAAGACCCCAACGAGAAGCGGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATC
L S K D P N E K R D H M V L L E F V T A A G I
ACTCTGGCATGGACGAGCTGTACAAG
T L G M D E L Y K

pGWB443, 444, 445, 543, 544, 545 (ECFP) 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
AACGGCCACAAGTTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCGTAAG
N G H K F S V S G E G E G D A T Y G K L T L K
TTCATCTGCACCACCGGAAGCTGCCCGTGCCTGGCCACCCTCGTGACCACCCTGACCTGGGGCGTG
F I C T T G K L P V P W P T L V T T L T W G V
CAGTGCTTCAGCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGGC
Q C F S R Y P D H M K Q H D F F K S A M P E G
TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCGCGCGGAGGTGAAGTTC
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
CAGAACACCCCATCGGGCAGGGCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCAGTCCGCC
Q N T P I G D G P V L L P D N H Y L S T Q S A
CTGAGCAAAGACCCCAACGAGAAGCGGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATC
L S K D P N E K R D H M V L L E F V T A A G I
ACTCTGGCATGGACGAGCTGTACAAG
T L G M D E L Y K

pGWB450, 451, 452, 550, 551, 552 (G3GFP) 717bp

ATGAGTAAAGGAGAAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTGAATTAGATGGTGATGTTAAT
M S K G E E L F T G V V P I L V E L D G D V N
GGGCACAAATTTTCTGTCAAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACCTACCCTTAAATTT
G H K F S V S G E G E G D A T Y G K L T L K F
ATTTGCACTACTGGAAAACCTGTTCCATGGCCAACACTTGTACTACTTTTCGCTTACGGTGTGCAG
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
GTGCAGGAGAGGACCATCTTCTTCAAGGACGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAG
V Q E R T I F F K D D G N Y K T R A E V K F E
GGAGACACCCTCGTCAACAGGATCGAGCTTAAGGGAATCGATTTCAGGAGGACGGAAAACATCCTCGGC
G D T L V N R I E L K G I D F K E D G N I L G
CACAAGTTGGAATACAACCTTCAACTCCCACAACGTGTACATCATGGCAGACAAAACAAAAGAATGGAATC
H K L E Y N F N S H N V Y I M A D K Q K N G I
AAAGTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAA
K V N F K I R H N I E D G S V Q L A D H Y Q Q
AATACTCCAATTGGCGATGGCCCTGTCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTT
N T P I G D G P V L L P D N H Y L S T Q S A L
TCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCTTCTTGAGTTTGTAAACAGCTGCTGGGATTACA
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 *

The termination codon TAA is not included in pGWB452 and 552.

pGWB453, 454, 455, 553, 554, 555 (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
AAGGTGACCAAGGGCGGCCCTGCCCTTCGCTGGGACATCCTGTCCCCTCAGTTCAGTACGGCTCC
K V T K G G P L P F A W D I L S P Q F Q Y G S
AAGGCCTACGTGAAGCACCCCGCGACATCCCGACTACTTGAAGCTGTCCTTCCCGAGGGCTTCAAG
K A Y V K H P A D I P D Y L K L S F P E G F K
TGGGAGCGCGTGATGAACCTTCGAGGACGGCGGGCTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGAC
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
AAGACCATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCGAGGACGGCGCCCTGAAGGGCGAGATC
K T M G W E A S T E R M Y P E D G A L K G E I
AAGATGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAG
K M R L K L K D G G H Y D A E V K T T Y M A K
AAGCCCGTGACGCTGCCCGGCCCTACAAGACCGACATCAAGCTGGACATCACCTCCACAACGAGGAC
K P V Q L P G A Y K T D I K L D I T S H N E D
TACACCATCGTGGAACAGTACGAGCGCGCGAGGGCCGCCACTCCACCGCGCCTAA
Y T I V E Q Y E R A E G R H S T G A *

The termination codon TAA is not included in pGWB455 and 555.