

Gateway Vectors for BiFC

1. The enhanced YFP (EYFP) are used (Split EYFP).
2. The Fusion fusion gene is expressed by CaMV35S promoter.
3. The N- or C-terminal fragments of EYFP are fused subsequent to the LR reaction.
4. Please read the Gateway instruction manual (Invitrogen) for procedures, etc.

Please note that our BiFC vectors are available for basic research only. If you plan to use them for commercial research, please contact Invitrogen about the licencing of Gateway™.

Please do not distribute to other researchers without permission.

Tsuyoshi Nakagawa
Department of Molecular and Functional Genomics
Center for Integrated Research in Science
Shimane University
Matsue 690-8504, Japan
tnakagaw@life.shimane-u.ac.jp

Construction of BiFC vectors

The vector used as the starting plasmid was pUGW2 and pUGW0 (Nakagawa, T. *et al.*, *J. Biosci. Bioeng.*, 104, 34-41. 2007) which were constructed from pUC119. The EYFP (239 amino acids) were separated into nEYFP (1-174) and cEYFP (175-239), then introduced into pUGW2 and pUGW0, respectively, to make four types of vectors.

- (1) nEYFP/pUGW2 (35S pro, C-nEYFP)
- (2) cEYFP/pUGW2 (35S pro, C-cEYFP)
- (3) nEYFP/pUGW0 (35S pro, N-nEYFP)
- (4) cEYFP/pUGW0 (35S pro, N-cEYFP)

Antibiotics

All BiFC vectors : ampicillin (50 mg/l) **and** chloramphenicol (30 mg/l)

All recombinant BiFC vectors (after LR reaction) : ampicillin (50 mg/l)

Amplification of BiFC vectors

- (1) Use competent cell of DB3.1 (Invitrogen 11782-018) or ccdB Survival T1 (Invitrogen C7510-03)
- (2) Spread on a plate containing ampicillin (50 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 ampicillin (50 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

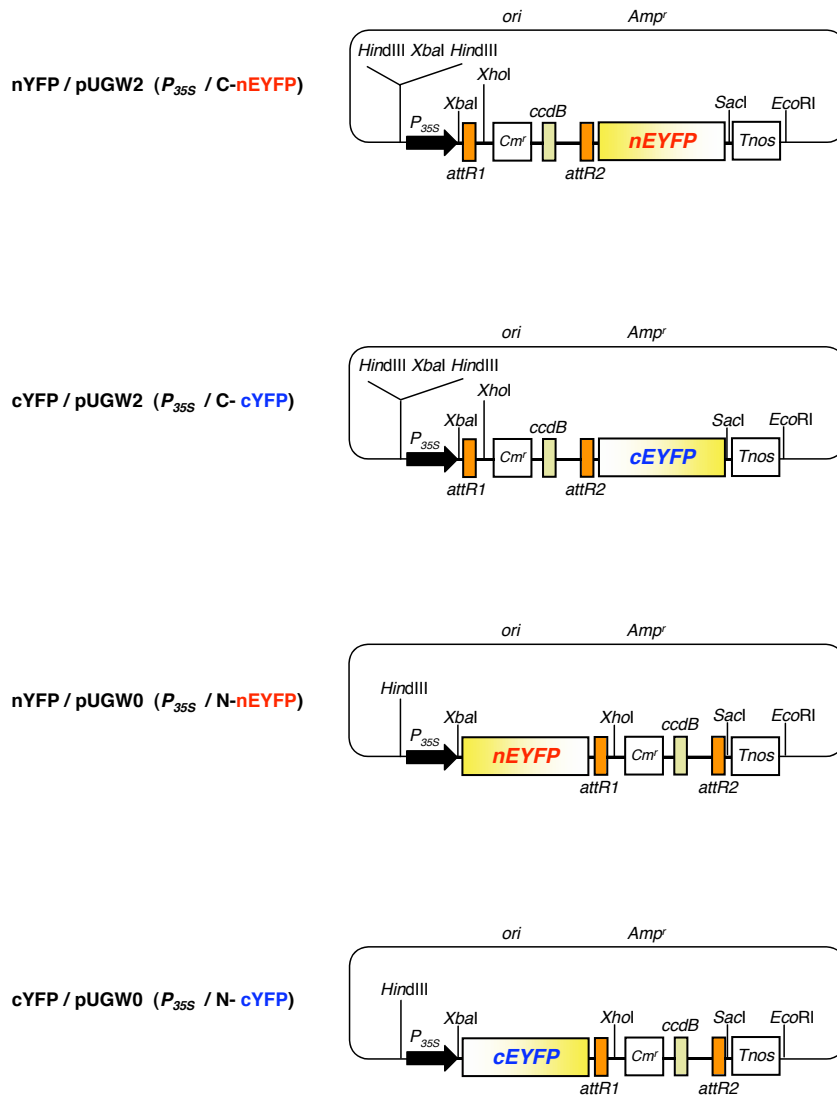
- (3) Incubate at 25 °C for more than 3hr.

- (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 ampicillin (50 mg/l).

Note

- (1) Linearization of BiFC vectors before performing the LR reaction is not necessary. If you want to linealize BiFC vectors, use *Xho*I.
- (2) Use the *Escherichia coli* strain DH5 α for cloning the product of the LR reactions, since negative selection with *ccd*B does not work in the *E.coli* which has F' episome (see the Invitrogen instruction manual).
- (3) The pUGW2 has the structure of *Hind*III-*Xba*I-*Hind*III-CaMV35S pro-*Xba*I around the CaMV35S promoter. The CaMV35S promoter is removed from pUGW2 by digestion of *Xba*I and following self ligation.
- (4) BiFC vectors are used for transient expression experiments. See protocol of particle gun, protoplast transformation with PEG, etc., on the website.

Structure of BiFC vectors



Structure of the BiFC gateway vectors. *ori*, ColE1 origin; *Amp^r*, ampicillin resistant marker; *Cm^r*, chloramphenicol resistant marker.

Sequence of BiFC vectors

The *HindIII* (AAGCTT), *XbaI* (TCTAGA) and *SacI* (GAGCTC) sites are shown. The *attR1-CmR-ccdB-attR2* cassette is underlined. The termination codon in the vector are bold-underlined.

n/cEYFP/pUGW2 (35S promoter, C-n/cEYFP)

(AAGCTT)GGG(TCTAGA)CCC(AAGCTT) -- (35S promoter) --//

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

nEYFP (M1-D174) + TAG

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTA
M V S K G E E L F T G V V P I L V E L D G D V
AACGGCCACAAGTTGAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
N G H K F S V S G E G E G D A T Y G K L T L K
TTCATCTGCACCACCGGCAAGCTGCCCGTGGCCACCTCGTGACCACCTTCGGCTACGGCCTG
F I C T T G K L P V P W P T L V T T F G Y G L
CAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGGC
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
GGGCACAAGCTGGAGTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC
G H K L E Y N Y N S H N V Y I M A D K Q K N G
ATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACTAG
I K V N F K I R H N I E D *

cEYFP ATG + (G175-TAA)

ATGGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGGGACGGCCCGTGTCTGCTG
M G S V Q L A D H Y Q Q N T P I G D G P V L L
CCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATG
P D N H Y L S Y Q S A L S K D P N E K R D H M
GTCCTGCTGGAGTTCGTGACCGCCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
V L L E F V T A A G I T L G M D E L Y K *

n/cEYFP / pUGE0 (35S promoter, N-n/cEYFP)

(AAGCTT) -- 35S promoter --//

(TCTAGA) ATG AGC **n/cEYFP** GCT GTT ATC ACA AGT TTG TAC AAA AAA -- (CmR, ccdB) --TTC

TTG TAC AAA GTG GTG ATA ACC TA(G AGCTC)

nEYFP M1-D174

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA

M V S K G E E L F T G V V P I L V E L D G D V

AACGGCCACAAGTTCAAGCTGTCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG

N G H K F S V S G E G E G D A T Y G K L T L K

TTCATCTGCACCACCGGCAAGCTGCCCGTGGCCACCTCGTGACCACCTTCGGCTACGGCCTG

F I C T T G K L P V P W P T L V T T F G Y G L

CAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCGAAGGC

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

GGGCACAAGCTGGAGTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC

G H K L E Y N Y N S H N V Y I M A D K Q K N G

ATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGAC

I K V N F K I R H N I E D

cEYFP ATG + (G175-K239) K239 is last amino acid of EYFP

ATGGGCAGCGTGCAGCTCGCCGACCCTACCAGCAGAACACCCCATCGGGGACGGCCCGTGTGCTG

M G S V Q L A D H Y Q Q N T P I G D G P V L L

CCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGGATCACATG

P D N H Y L S Y Q S A L S K D P N E K R D H M

GTCCTGCTGGAGTTCGTGACCGCCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG

V L L E F V T A A G I T L G M D E L Y K

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

nEYFP / pUGW2 (CaMV35S promoter; C-fusion to nEYFP)

AAGCTTGGGTCTAGACCAAGCTTGCATGCTGCAGGT-(CaMV35S)-CGCAAGACCCCTCCTCTATATAAGGAAGTTCATTTTCATTTGGAGAGAACACGGGGGACTCTAGAGTTA
 TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (clone) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-(nEYFP)- GAC TAG GCT TAG AGCTC
 X P A F L Y K V V D N S M D *

attB1 *attB2*

cEYFP / pUGW2 (CaMV35S promoter; C-fusion to cEYFP)

AAGCTTGGGTCTAGACCAAGCTTGCATGCTGCAGGT-(CaMV35S)-CGCAAGACCCCTCCTCTATATAAGGAAGTTCATTTTCATTTGGAGAGAACACGGGGGACTCTAGAGTTA
 TCA ACA AGT TTG TAC AAA AAA GCA GGC TNN (clone) NAC CCA GCT TTC TTG TAC AAA GTG GTT GAT AAC AGC ATG-GGC-(cEYFP)- AAG-TAA GCT TAG AGCTC
 X P A F L Y K V V D N S M G K *

attB1 *attB2*

nEFP / pUGW0 (CaMV35S promoter; N-fusion to nEYFP)

AAGCTTGCATGCTGCAGGT-(CaMV35S)-CGCAAGACCCCTCCTCTATATAAGGAAGTTCATTTTCATTTGGAGAGAACACGGGGGACTCTAGA
 ATG AGC ATG-(nEYFP)-GAC GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (clone) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
 M S M D 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*

nEFP / pUGW0 (CaMV35S promoter; N-fusion to cEYFP)

AAGCTTGCATGCTGCAGGT-(CaMV35S)-CGCAAGACCCCTCCTCTATATAAGGAAGTTCATTTTCATTTGGAGAGAACACGGGGGACTCTAGA
 ATG AGC ATG-GGC-(cEYFP)-AAG GCT GTT ATC ACA AGT TTG TAC AAA AAA GCA GGC TNN (clone) NAC CCA GCT TTC TTG TAC AAA GTG GTG ATA ACC TAG AGCTC
 M S M G 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*

The sequences between *Hind*III and *Sac* I sites (see “Structure of BiFC vectors”) 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. The CaMV35S promoter is shown in orange, where the region between -46 and +8 is represented by a nucleotide sequence with the predicted transcription start site (underlined larger letter). The nEYFP is indicated in red and cYFP is indicated in blue. Only partial sequences are shown for n/cEYFP, whose whole sequences are indicated in the former section. In C-fusion vectors, the peptide sequence derived from *att*B2 region becomes the linker between the entry clone and n/cEYFP. In N-fusion vectors, the peptide sequence derived from *att*B1 region becomes the linker between n/cEYFP 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.