Cat #. 3264_3267

For Research Use

TaKaRa

pRI 201 DNA Series

(High-Expression Vectors for Plant Transformation)

Product Manual

v202007Da

Table of Contents

I. Description

pRI 201 DNA vectors are designed for expressing target genes in transformed plant cells. These vectors retain the backbone of pRI 101 vectors[1] (Cat. #3262/3263), which includes an alcohol dehydrogenase (ADH) gene-derived 5′ untranslated region (5′-UTR) (translational enhancer region) downstream of the 35S promoter from cauliflower mosaic virus (CaMV). In addition, they have a heat shock protein (HSP) gene-derived terminator in place of the nopaline synthase (NOS) gene-derived terminator, allowing higher target gene expression compared with the pRI 101 vectors[2]. Further, multigene transformation with a single vector is possible by cloning of an expression cassette (promoter + enhancer + gene of interest + terminator) into the cloning site (MCS2) downstream of the HSP terminator.

The pRI 201 vectors has two types of vectors: pRI 201-AN DNA and pRI 201-ON DNA. Carrying an Arabidopsis ADH-derived 5′ -UTR (AtADH 5′ -UTR), pRI 201-AN DNA is suitable for dicotyledonous plants. With a rice ADH-derived 5′ -UTR (OsADH 5′ -UTR), pRI 201-ON DNA is compatible with monocotyledonous plants. Positive control vectors (pRI 201-AN-GUS DNA and pRI 201-ON-GUS DNA) containing the β-glucuronidase (GUS) gene are also available.

pRI 201-AN DNA and pRI 201-ON DNA are binary vectors for plant transformation and have a mutant-type replication origin (Ri ori) from the *Rhizobium rhizogenes* Ri plasmid[3]. These vectors also have both a replication origin (ColE1 ori) derived from pUC plasmids, which allows maintenance at a high-copy-number in *E. coli*, and a multicloning site located near the right border (RB) of T-DNA relative to the plant selection marker (NPT II), which allows stable integration of the target gene into a plant chromosome.

＊ These vectors were developed by Takara Bio Inc. with the support of the Nara Institute of Science and Technology, which provided technical assistance and samples.

II. Components

pRI 201-AN DNA	(Cat. #3264)	10 μg
pRI 201-ON DNA	(Cat. #3265)	10 μg
pRI 201-AN-GUS DNA＊	(Cat. #3266)	10 μg
pRI 201-ON-GUS DNA＊	(Cat. #3267)	10 μg

(Concentration: 0.5 μg/μl)
＊ pRI 201-AN-GUS DNA and pRI 201-ON-GUS are not available in the U.S.

III. Storage

−20°C

Note: The products are stable for 2 years from date of receipt under proper storage conditions.

IV. Vector Map and Cloning Sites

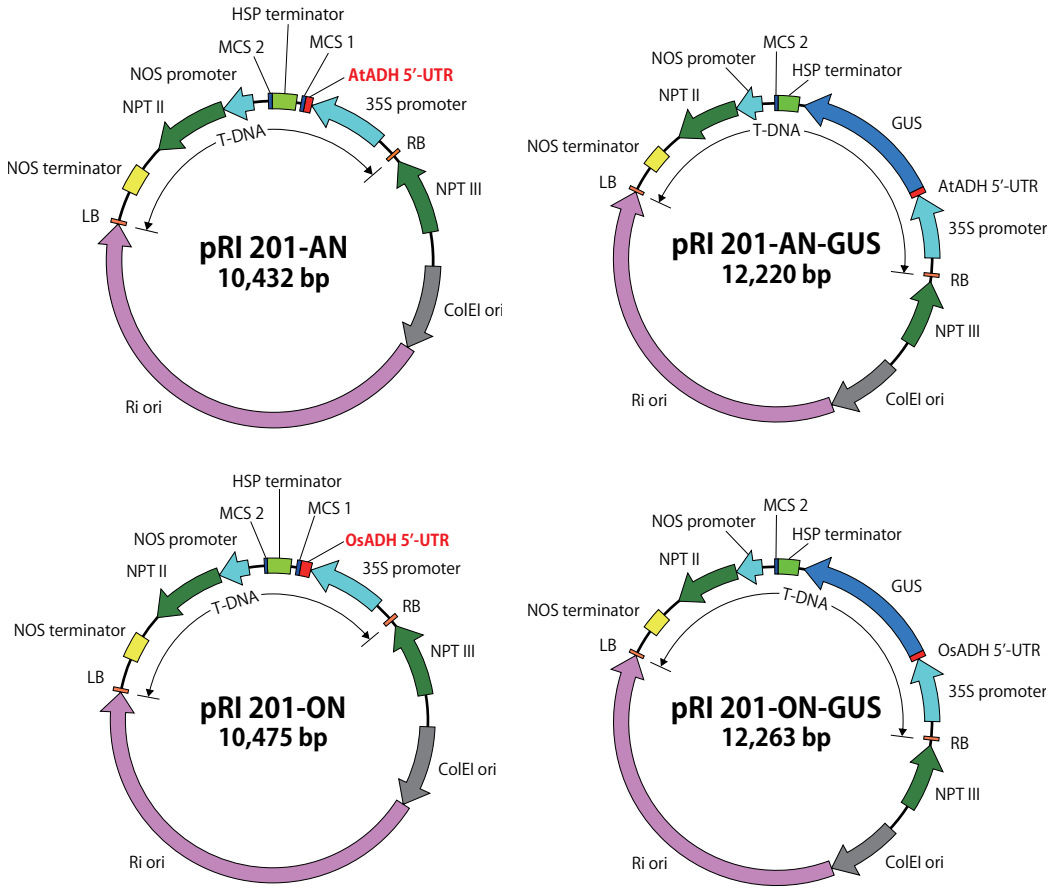
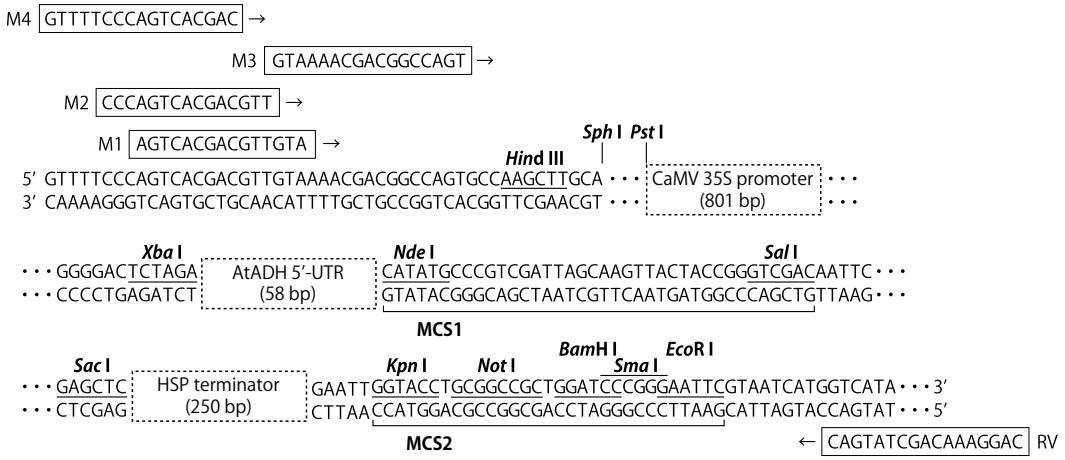


Figure 1. Vector Map.

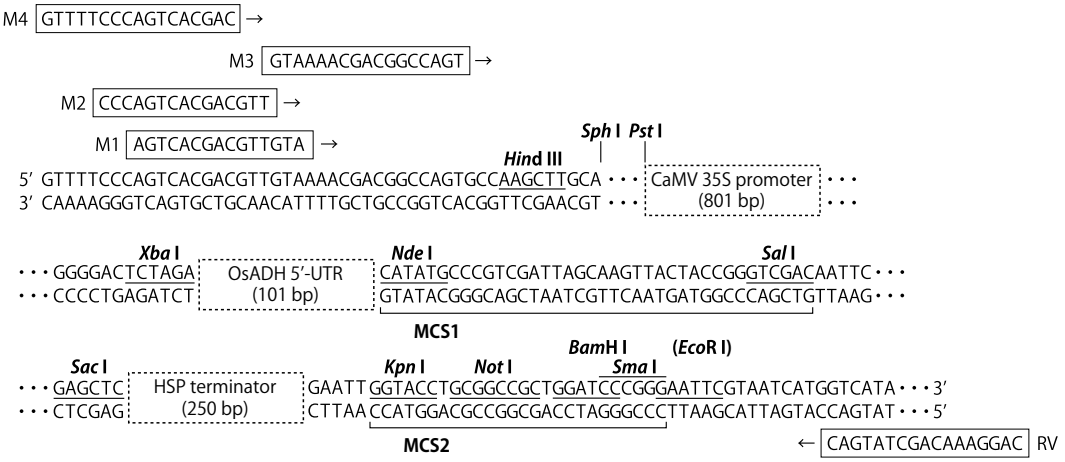
Note: pRI 201-AN-GUS DNA and pRI 201-ON-GUS are not available in the U.S.

ColE1 ori	: Replication origin in *E. coli* (*E. coli*)
Ri ori	: Replication origin in *Rhizobium* (*Rhizobium rhizogenes (Agrobacterium rhizogenes)*)
RB, LB	: T-DNA border sequences for integration in plants (*Rhizobium radiobacter (Agrobacterium tumefaciens)*)
NOS promoter, NOS terminator	: Promoter or terminator for gene expression in plants (*Rhizobium radiobacter (Agrobacterium tumefaciens)*)
HSP terminator:	: Terminator for gene expression in plants (*Arabidopsis thaliana*)
GUS	: β-glucuronidase gene (*E. coli*)
NPT II	: Selection marker gene in plants (*E. coli*)
AtADH 5′-UTR, OsADH 5′-UTR	: Translational enhancer region (*Arabidopsis thaliana* or *Oryza sativa*)
35S promoter	: Promoter for gene expression in plants (CaMV)
NPT III	: Selection marker gene in *E. coli* and *Rhizobium* (*Agrobacterium*) (kanamycin-resistance) (*Streptococcus faecalis*)

Cloning Site of pRI 201-AN DNA

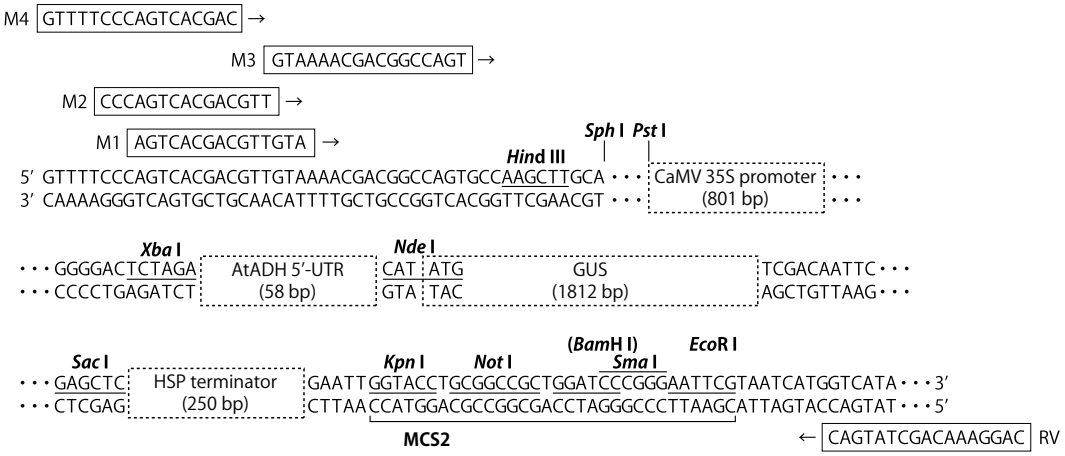


Cloning Site of pRI 201-ON DNA

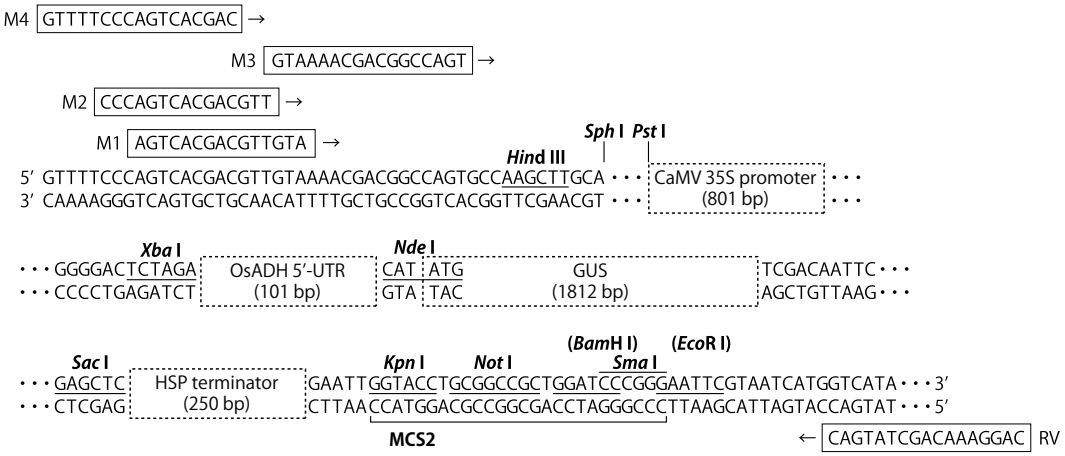


Note: There is an *Eco*R I site in OsADH 5'-UTR.

Cloning Site of pRI 201-AN-GUS DNA



Cloning Site of pRI 201-ON-GUS DNA



Note: There is an *Eco*RI site in OsADH 5′-UTR, while the GUS gene has a *Bam*H I site.

Figure 2. Multicloning site and expression cassette structure.

V. Protocol

1. Plasmid Construction

A. Cloning a gene into pRI 201-AN or pRI 201-ON

1) Clone DNA encoding a target protein into the MCS1 site (*Nde* I/*Sal* I) of pRI 201-AN or pRI 201-ON DNA between the translational enhancer region (AtADH 5′-UTR or OsADH 5′-UTR) and the HSP terminator.
2) The *Nde* I site of MCS1 is recommended for cloning the target gene because the locations of enhancer and start codon (ATG) may affect translational activity [1, 4, 5] and the ATG sequence in the *Nde* I site may be used as a translational start codon.
3) Transform *E. coli* competent cells with the resulting vector and select transformants on LB plates with kanamycin (50 μg/ml).

<Alternative protocol using the In-Fusion HD Cloning Kit>

In-Fusion HD Cloning Kit w/ Cloning Enhancer (Cat. #639633) allows you to join two or more fragments, e.g. vector and insert, as long as they share 15 bases of homology at each end.

1) Digest pRI 201-AN or pRI 201-ON DNA with *Nde* I and *Sal* I and then purify the linearized vector.
2) Amplify the target gene's open reading frame (ORF) by PCR.
   - PCR primers must be designed in such a way that they generate PCR products containing ends that are homologous to those of the vector
   - The 5′-primer has 15 bases immediately upstream of the *Nde* I site on the vector at its 5′-end , and the 3′-primer has 15 bases immediately downstream of the *Sal* I site on the vector at its 5′-end.

     5′-primer: **CACTGTTGATACATA**TGNNNNNNNNNNNNNNN (for pRI 201-AN)

     5′-primer: **GAGGGGGATTACATA**TGNNNNNNNNNNNNNNN (for pRI 201-ON)

     3′-primer: **ATTCAGAATTGTCGA**NNNNNNNNNNNNNNNN

   (The bold represents vector sequence and the underlined ATG's are the start of an ORF. N's represent the sequence corresponding to the gene of interest.)
3) Perform In-Fusion reaction using the PCR product and the linearized vector by following the protocol that comes with the In-Fusion HD Cloning Kit and then transform *E. coli* competent cells (Stellar Competent Cells (Cat. #636763) are ideal for use with the In-Fusion HD Cloning Kit). Select transformants on LB plates with kanamycin (50 μg/ml).

B. Cloning two genes into pRI 201-AN or pRI 201-ON

1) Clone the ORF of target gene A into the MCS1 site (*Nde* I/*Sal* I) of pRI 201-AN or pRI 201-ON DNA.
2) Clone an expression cassette (promoter + enhancer + target gene B + terminator) into the MCS2 site downstream of the HSP terminator.
3) Transform *E. coli* competent cells with the resulting vector and select transformants on LB plates with kanamycin (50 μg/ml).

<Alternative protocol using the In-Fusion HD Cloning Kit>

In-Fusion HD Cloning Kit allows you to join two or more fragments, e.g. vector and insert, as long as they share 15 bases of homology at each end.

1) Clone target gene A into the MCS1 site (*Nde* I/*Sal* I) of pRI 201-AN DNA or pRI 201-ON DNA following the protocol outlined in Step A.
2) Digest the resulting vector with two types of MCS2 restriction enzymes that have no recognition sequence in the target gene A, and then purify. (For example, cut with *Not* I and *Sma* I.)
3) Amplify the expression cassette region (CaMV 35S promoter + AtADH (or OsADH) + gene B + HSP terminator) on other pRI 201-AN or pRI 201-ON DNA by PCR. Use a 5′-primer that has the 15 bases immediately upstream of the *Not* I site on the vector at its 5′-end and a 3′-primer that has the 15 bases immediately downstream of the *Sma* I site on the vector at its 5′-end.
4) Perform the In-Fusion reaction using the amplified expression cassette (containing target gene B) and the linearized plasmid (carrying target gene A) and then transform *E. coli* competent cells (Stellar Competent Cells are ideal for use with the In-Fusion HD Cloning Kit). Select transformants on LB plates with kanamycin (50 μg/ml).

2. Transformation of the constructed plasmid into Agrobacterium competent cells.

A. Transform the constructed plasmid (carrying target gene A or genes A and B) into Agrobacterium competent cells (*Agrobacterium tumefaciens* LBA4404 Electro-Cells, Cat. #9115) to produce Agrobacterium transformants.

B. Select transformants on LB plates with kanamycin (50 μg/ml) and streptomycin (100 μg/ml). (For transient expression in plant protoplasts, introduce the constructed plasmid by electroporation or other.)

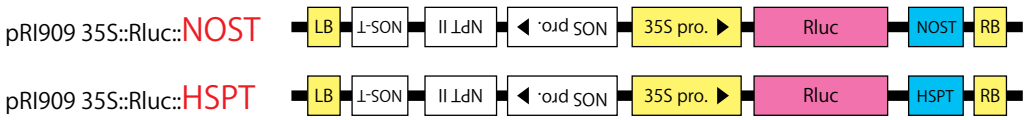
3. Transform a plant using the Agrobacterium transformants.

VI. Experimental Examples

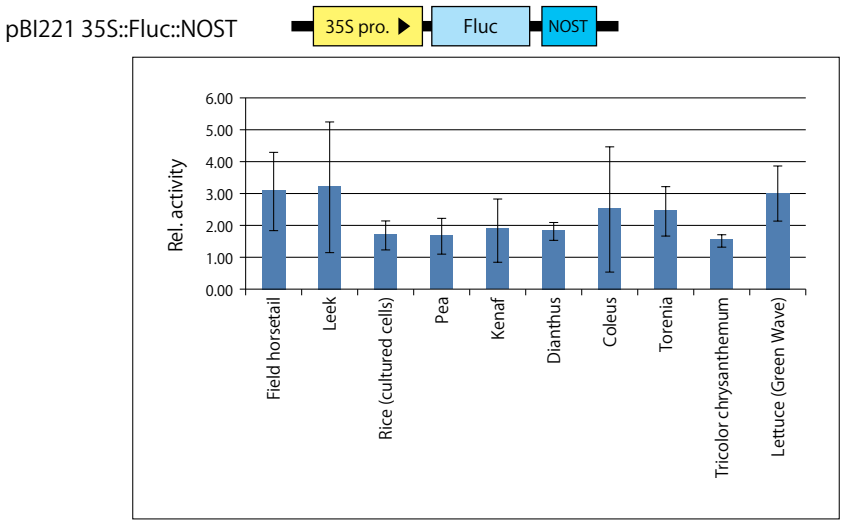
1. Comparison of HSP and NOS terminator using transient gene expression

- Protoplasts were prepared from various plants and transformed by PEG method with pRI909 plasmid carrying either HSP terminator or NOS terminator downstream of the *Renilla* Luciferase (Rluc) gene. pBI221 35S::Fluc::NOST was co-transfected as a control.
- Proteins were recovered from protoplasts 6 to 7 hours after plasmid transfection to assay the activities of Rluc and Fluc. The relative Rluc/Fluc activity obtained using a NOS terminator-containing plasmid was assigned a value of 1.
- Relative Rluc/Fluc activity using an HSP terminator-containing plasmid was measured and compared to that obtained with the NOS terminator.

Rluc plasmids



Fluc for normalizing transfection efficiency

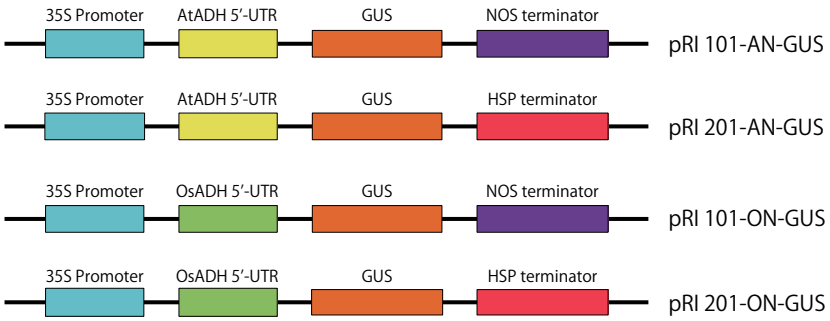


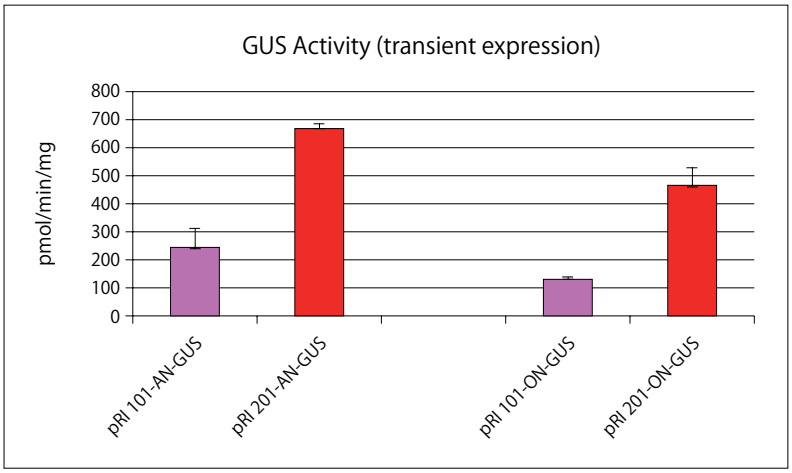
Result: An HSP terminator-containing plasmid showed higher expression compared with a NOS terminator-containing plasmid across all plants tested.

(This data was supplied by Dr. Ko Kato of the Laboratory for Metabolic Regulation of Plant Cells, Graduate School of Biological Sciences, Nara Institute of Science and Technology)

2. Expression in cultured plant cells (transient expression)

- Protoplast was prepared from cultured tobacco cells (BY-2) and transformed by electroporation with one of the plasmids (pRI 101 and pRI 201 vectors) containing the β-glucuronidase (GUS) gene.
- After 2 days of incubation, protoplasts transformants were homogenized. GUS activity was assayed based on fluorescence intensity of 4-methylumbelliferone (4 MU) generated in the degradation of the substrate, 4-methylumbelliferyl-β-D-glucuronide (MUG).



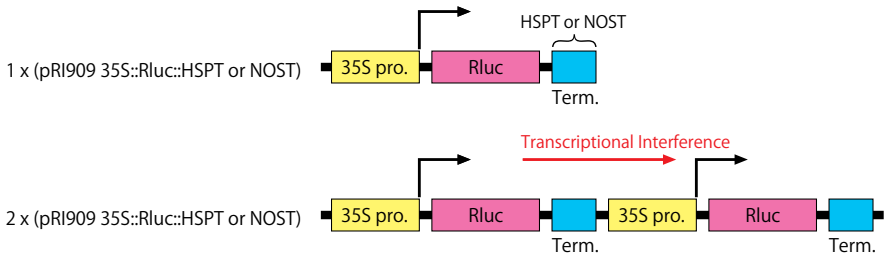


Result: GUS activity of HSP terminator-containing pRI 201 plasmids was 2- to 3-fold higher than that of NOS terminator-containing pRI 101 plasmids. And the AtADH enhancer is same activity as the OsADH enhancer in BY-2 cells (derived from dicotyledonous tobacco plants).

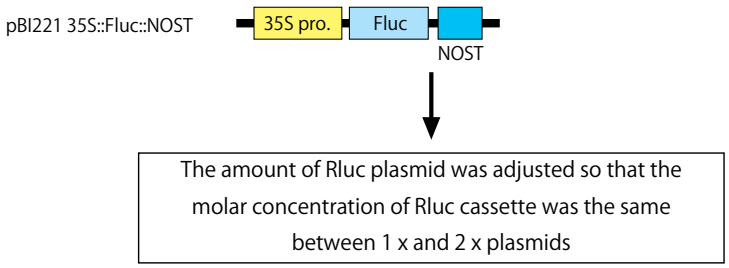
3. Activity of HSP terminator in vectors with two cloned genes (transient expression)

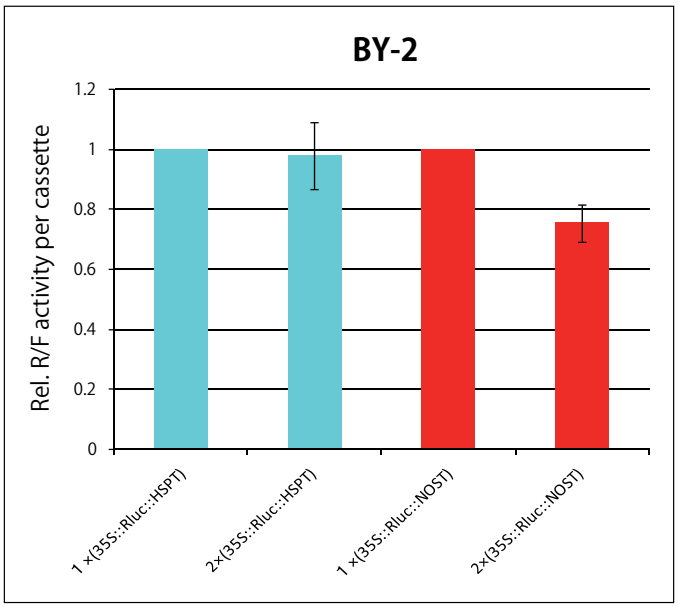
- Cultured tobacco cells (BY-2) and lettuce protoplasts were transformed with an expression plasmid containing one (1X) or two (2X) tandemly repeated expression cassettes comprised of 35S promoter - Rluc - NOS terminator or 35S promoter - Rluc - HSP terminator. the effect of NOS and HSP terminators were compared in copy number on Rluc.
- The amount of plasmid was adjusted so that both 1X and 2X had the same number of expression cassettes. Based on the relative expression level of Rluc versus Fluc control, the relative ratios of expression between 1X and 2X were determined.

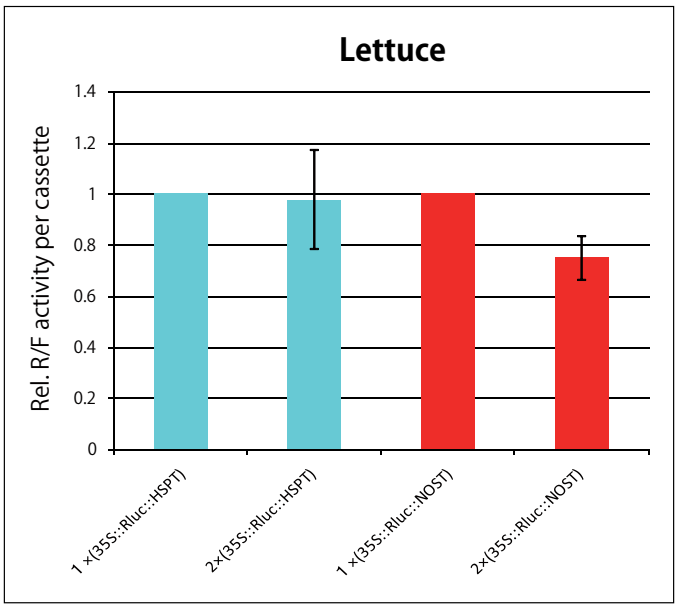
Rluc plasmids



Fluc plasmid for normalizing transfection efficiency







Result: In the HSP terminator-containing cassette, the expression were nearly identical between two (2X) cassettes and one (1X) cassette, indicating that it is effective for the concurrent expression of two genes when integrated into tandem expression cassettes. In contrast, the expression was lower for two (2X) cassettes compared with one (1X) cassette in the NOS terminator-containing cassette, suggesting the NOS terminator interfered to some extent with transcription.

(This data was supplied by Dr. Ko Kato of Laboratory for Metabolic Regulation of Plant Cells, Graduate School of Biological Sciences, Nara Institute of Science and Technology)

VII. References

1) T Sugio, J Satoh, H Matsuura, A Shinmyo and K Kato. *J Bioscience and Bioengineering.* (2008) **105** (3): 300-302.
2) S Nagaya, K Kawamura, A Shinmyo and K Kato. *Plant and Cell Physiology*. (2010) **51** (2): 328-332.
3) R Nishiguchi, M Takanami, and A Oka. *Molecular and General Genetics*. (1987) **206**: 1-8.
4) J Satoh, K Kato and A Shinmyo. *J Bioscience and Bioengineering*. (2004) **98** (1): 1-8.
5) T Sugio, H Matsuura, T Matsui, M Matsunaga, T Nosho, S Kanaya, A Shinmyo and K Kato. *J Bioscience and Bioengineering*. (2010) **109** (2): 170-173.

VIII. Related Products

Agrobacterium tumefaciens LBA4404 Electro-Cells (Cat. #9115)
pRI 909 DNA (Cat. #3260)
pRI 910 DNA (Cat. #3261)
pRI 101-AN DNA (Cat. #3262)
pRI 101-ON DNA (Cat. #3263)
In-Fusion® HD Cloning Kit w/Cloning Enhancer（Cat. #639633 - 639635）
Stellar Competent Cells (Cat. #636763)