Cat. # 3262
3263

For Research Use

TaKaRa

pRI 101 DNA series

(High-expression vectors for plant cell transformation)

Product Manual

v201807Da

Table of Contents

I. Description

pRI 101 DNA series are binary vectors for expressing foreign gene in plant cells, which has the 35S promoter of cauliflower mosaic virus (CaMV) and a 5'non-coding region (5'-UTR) of alcohol dehydrogenase (ADH) gene. The 5'-UTR of ADH functions as a translational enhancer in plants[1]. There are two types of vectors, pRI 101-AN DNA having a 5'-UTR of *Arabidopsis* ADH (AtADH 5'-UTR) and pRI 101 ON DNA having a 5'-UTR of rice ADH (OsADH 5'-UTR). The pRI 101-AN is for dicotyledonous plants such as tobacco or *Arabidopsis* and the pRI 101-ON is for monocotyledonous plants such as rice.
The pRI 101 DNA are shuttle vectors, and replicates autonomously in *E. coli* and *Rhizobium* (*Agrobacterium*). In *E. coli*, these vectors are high copy number plasmid because these have replication origin same as that of pUC type plasmid (ColE1 ori), and these are maintained stably in *Rhizobium* (*Agrobacterium*) also with mutant type replication origin of Ri plasmid (Ri-ori)[2]. The pRI 101 DNA are possible to integrate target gene in plant chromosome stably because the cloning site of these products are located at the position closer to Right Border (RB) of T-DNA than the selection marker for plant, so the target gene is not deleted.

＊ These products were developed by Takara Bio Inc. to which Nara Institute of Science and Technology provided supports and samples.

II. Components

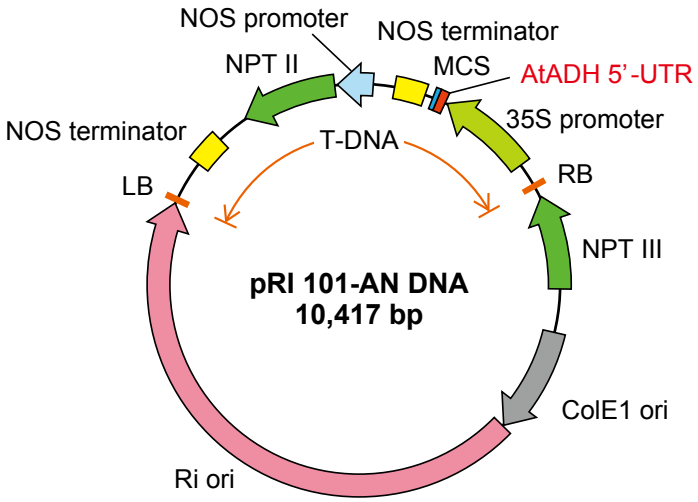
pRI 101-AN DNA (Cat.#3262)	10 μg
pRI 101-ON DNA (Cat.#3263)	10 μg

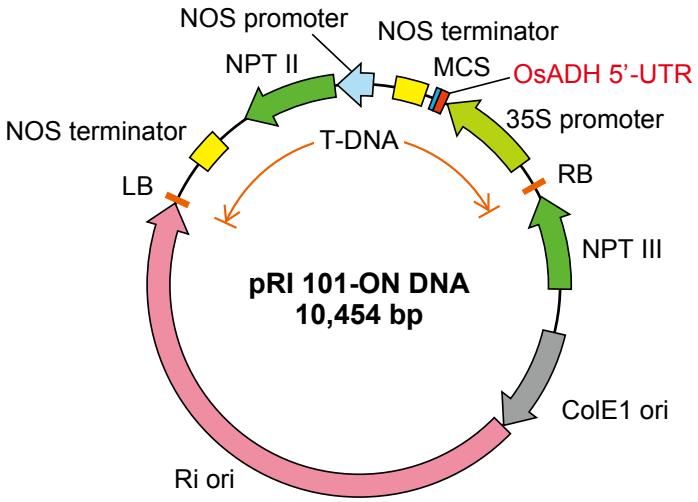
- Concentration 0.5 μg/μl
- Form 10 mM Tris-HCl, pH 8.0
  1 mM EDTA

III. Storage -20℃

IV. Vector map and cloning site

IV-1. Vector map

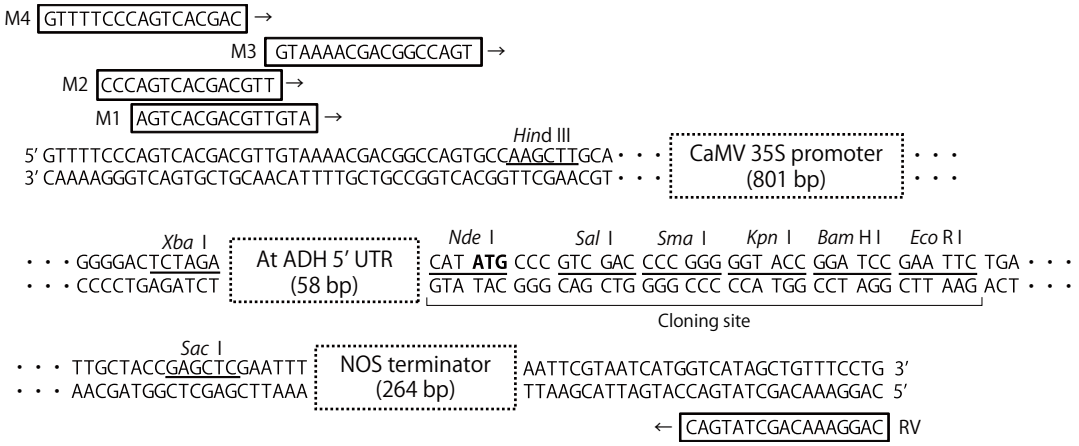




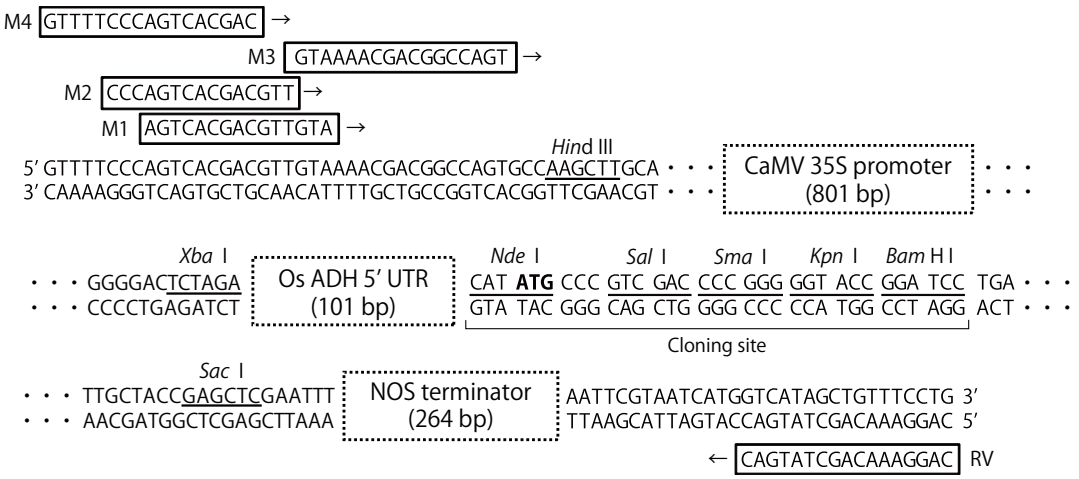
ColEi ori	:	Replication origin in *E. coli*
Ri ori	:	Replication origin in *Rhizobium* (*Agrobacterium*)
RB, LB	:	T-DNA border sequences
NOS-promoter, NOS-terminator	:	Promoter or terminator for expression of selectable marker gene in plant
NPTII	:	Selectable marker gene in plant
AtADH 5'-UTR, OsADH 5'-UTR	:	5' non-coding region of Arabidopsis or Rice Alcohol dehydrogenase gene (translational enhancer)
35S promoter	:	Cauliflower mosaic virus (CaMV) promoter for expression of target gene in plant
NPTIII	:	Selectable marker gene in *E. coli* or *Rhizobium* (*Agrobacterium*)

IV-2. Cloning site

pRI 101-AN DNA



pRI 101-ON DNA



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

V. Protocol

1) Generate a plasmid by cloning your gene of interest in the cloning site downstream of AtADH 5′-UTR or OsADH 5′-UTR. Select a digestion site of restriction enzyme, considering the reading frame of the pRI 101 DNA vector. A position of the translational enhancer (AtADH 5′-UTR and OsADH 5′-UTR) and a start codon of the gene of interest may affect translational activity of the gene[1, 3)].
2) Transform the constructed plasmid into *Agrobacterium*, then produce a transformed *Agrobacterium*.
3) Transform a target plant using the transformed *Agrobacterium*.

VI. Experimental Example

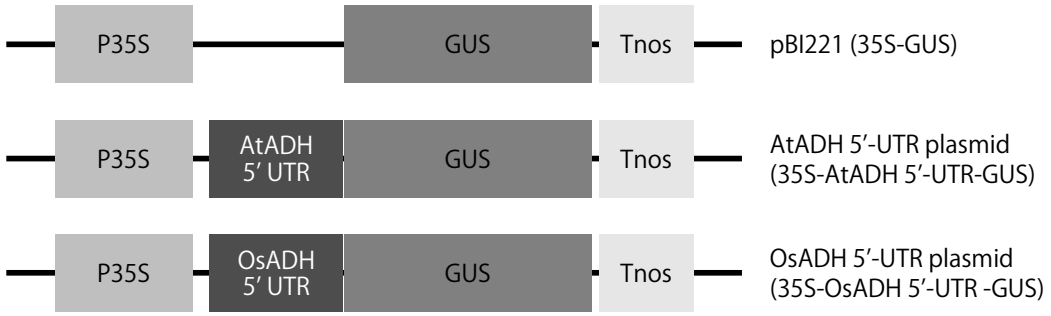
VI-1. Confirmation of enhancer function (transient expression using pUC vector) :

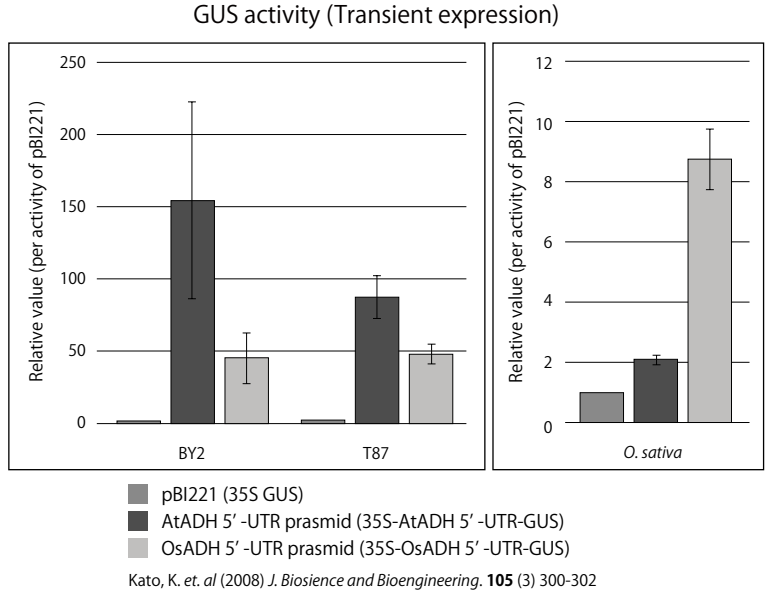
Method :

Protoplasts of tobacco cultured cell BY-2, *Arabidopsis* cultured cell T87, or rice (*Oryza sativa*) was prepared, and transformed with the plasmid containing B-glucuronidase (GUS) gene described below by electroporation. At 16 - 18 hours after transformation, the cell extracts of the protoplasts were obtained. GUS activity in the extracts were examined. The GUS activity is based on a fluorescence intensity of 4-methylumbelliferone (4MU) produced by digestion of 4-methylumbelliferyl-β-D-glucuronide (4-MUG) as a substrate.

Result :

The GUS activity of CaMV 35S promoter in the plasmid having 5′-UTR was significantly higher than that of the promoter without 5′-UTR enhancer. The Arabidopsis ADH 5′-UTR showed high enhance activity in BY-2 and T87 cells, but hardly in rice. On the other hand, the OsADH 5′-UTR had high activity not only in BY-2 and T87, but in rice protoplast.





Kato, K. *et. al* (2008) *J. Biosience and Bioengineering*. **105** (3) 300-302

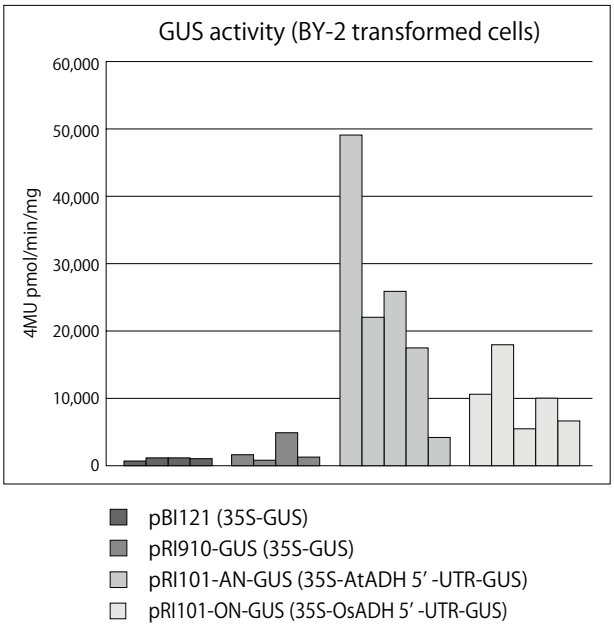
VI-2. Expression of a gene of interest in transformant (using a binary vector) :

Method :

The GUS gene containing pRI 101-AN or pRI 101-ON plasmid was constructed as described in VI-1 section, and transformed into tobacco cultured cell BY-2 by *Agrobacterium* method. The GUS activity of the obtained transformants was measured by identical method as described in VI-1 section.

Result :

Many transformants with the pRI 101-AN-GUS and pRI 101-ON-GUS having ADH 5′-UTR express higher GUS activity than transformants with pBI 121 or pRI 910-GUS, whose structure is same as pBI 221 plasmid shown in VI-1.



VII. References

1) T Sugio, J Satoh, H Matsuura, A Shinmyo, and K Kato.
J Bioscience and Bioengineering. (2008) **105** (3): 300-302.
2) R Nishiguchi, M Takanami, and A Oka. *Molecular and General Genetics.* (1987) **206**: 1-8.
3) J Satoh, K Kato, and A Shinmyo. *J Bioscience and Bioengineering*. (2004) **98** (1): 1-8.

VIII. Related Products

pRI 909 DNA (Cat. #3260)
pRI 910 DNA (Cat. #3261)
pRI 201-AN DNA (Cat. #3264)
pRI 201-ON DNA (Cat. #3265)
pRI 201-AN-GUS DNA (Cat. #3266) *
pRI 201-ON-GUS DNA (Cat. #3267) *
Agrobacterium tumefaciens LBA4404 Electro-Cells (Cat. #9115)

* Not available in all geographic locations. Check for availability in your area.