

## Chapter 6.5

### VECTORS FOR REVERSE GENETICS AND EXPRESSION ANALYSIS

Stig Uggerhøj Andersen, Cristina Cvitanich, Mette Grønlund, Hanne Busk, Dorthe Bødker Jensen, and Erik Østergaard Jensen\*

*Laboratory of Gene Expression, Department of Molecular Biology, University of Aarhus, Gustav Wieds Vej 10, DK-8000 Aarhus C., DENMARK.; \*Corresponding author.*

Email: [ej@mb.au.dk](mailto:ej@mb.au.dk)

Phone: +45 8942 5014 Fax: +45 8942 5012

Keywords: Vectors, transformation, reverse genetics, expression analysis, protein localization

*The binary Agrobacterium pPZP211 vector is stable and fully sequenced. We have produced derivatives of this vector that can be used for general over-expression, nodule-specific expression, expression pattern analysis, and protein localization studies.*

#### INTRODUCTION

The pPZP211 (Hajdukiewicz et al., 1994) derivatives are divided into two groups, pZ and pZN. The pZ vector series is based on the original pPZP211 vector, in which various constructs have been inserted into the pUC18 multiple cloning site (MCS). The presence of the strong cauliflower mosaic virus 35S (CaMV35S) enhancer-promoter (ep35S) (Guilley et al., 1982) in the pZ vector series could cause problems by overruling the specificity of a promoter under investigation, as observed with a similar vector (Hansen et al., 1999). To avoid this effect, ep35S was replaced with the *Agrobacterium tumefaciens* nopaline synthase promoter (pNos) in the pZN vector series.

#### pZ-35S

The pZ-35S vector is designed for over-expression of a gene of interest (GOI) from the CaMV35S promoter (p35S) (Figure 1A). The MCS allows cloning of a GOI between p35S and the *A. tumefaciens* nopaline synthase terminator (pAnos). The pZ-35S vector has successfully been used to over-express intrinsic factor in

A.J. Márquez (Editorial Director). 2005. *Lotus japonicus* handbook. pp. 289-292.  
<http://www.springer.com/life+sci/plant+sciences/book/978-1-4020-3734-4>

*Arabidopsis thaliana* (Fedosov et al., 2003).

### **pZ-35S-GFP**

The pZ-35S-GFP vector (Figure 1B) is designed for protein localization studies. An Xba I restriction site allows in frame fusion to Green Fluorescent Protein (GFP). p35S drives expression of the fusion protein. The pZ-35S-GFP vector has been used to determine the localization of CPP1 and NDX proteins by transient expression in onion epidermal cells and *Cantharantus roseus* cells, respectively (Cvitanich et al., 2000; Grønlund et al., 2003).

### **pZ-LB**

The pZ-LB vector (Figure 1C) is designed for nodule specific expression from the *Glycine max* leghemoglobin *lbc3* promoter (plbc3), which is active only in the central infected tissue of the root nodule (Stougaard et al., 1987). The MCS allows cloning of a GOI between plbc3 and pAnos. The pZ-LB vector has been used to express *gus* in nodules (Cvitanich et al., 2000).

### **pZ-Enod12**

The pZ-Enod12 vector (Figure 1D) is designed for nodule specific expression from the *Pisum sativum enod12* promoter (penod12). This promoter is active in nodule primordia and young nodules. Expression from the promoter has also been observed in pollen (Grønlund et al., 2003). The MCS allows cloning of a GOI between penod12 and pAnos.

### **pZN**

This vector (Figure 1E) is designed to avoid ep35S influence on promoters used for tissue specific expression or expression pattern analysis. To this end, ep35S, used to control NptII expression in pPZP211, was replaced by pNos.

### **pZN-LB**

This vector (figure 1F) is the same as pZ-LB but ep35S was replaced by pNos.

### **pZN-Enod12**

This vector (Figure 1G) is the same as pZ-Enod12 but ep35S was replaced by pNos.

### **pZN-GUS**

The pZN-GUS vector (Figure 1H) was designed for expression pattern analysis using an intron-containing  $\beta$ -glucuronidase reporter gene (GUSint). The MCS allows cloning of a promoter of choice in front of the promoterless GUSint. The

GUSint gene is interrupted by the second intron (IV2) of the potato ST-LSI gene (Vancanneyt et al., 1990) to avoid expression in bacteria.

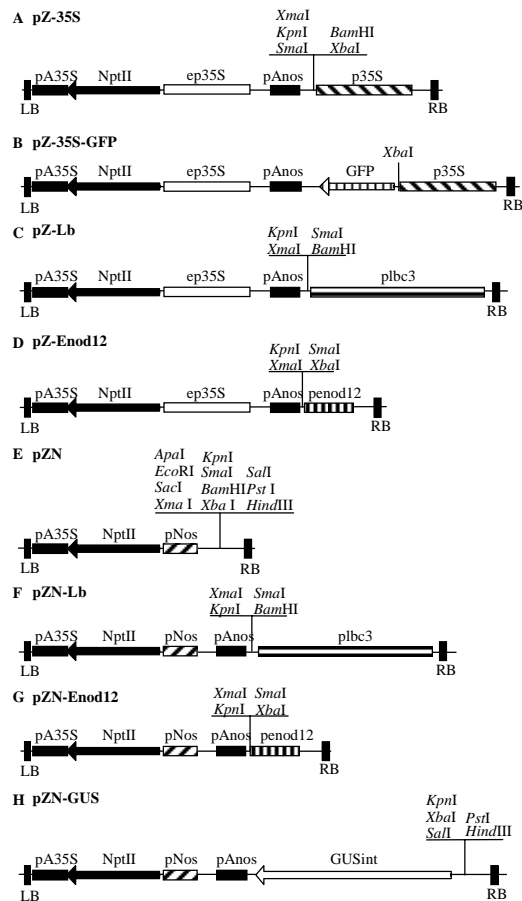


Figure 1. Vectors (pPZP211 derivatives) for reverse genetics and expression analysis. Only the region between left border (LB) and right border (RB) is shown. Unique restriction sites are indicated by restriction enzyme names. All vectors confer bacterial resistance to spectinomycin. pA35S: CaMV35S terminator. NptII: neomycin phosphotransferase II gene. ep35S: CaMV35S enhancer-promoter. p35S: CaMV35S promoter. pAnos: A. tumefaciens nopaline synthase terminator. pNos: A. tumefaciens nopaline synthase promoter. GFP: Green Fluorescent Protein. plbc3: Glycine max leghemoglobin lbc3 promoter. penod12: Pisum sativum enod12 promoter. GUSint: intron-containing  $\beta$ -glucuronidase reporter gene.

A.J. Márquez (Editorial Director). 2005. *Lotus japonicus* handbook. pp. 289-292.  
<http://www.springer.com/life+sci/plant+sciences/book/978-1-4020-3734-4>

## REFERENCES

- Cvitanich C, Pallisgaard N, Nielsen KA, Hansen AC, Larsen K, Pihakaski-Maunsbach K, Marcker KA, and Jensen EO. (2000) **CPP1, a DNA-binding protein involved in the expression of a soybean leghemoglobin c3 gene.** *Proceedings of the National Academy of Sciences USA* 97, 8163-8168.
- Fedosov SN, Laursen NB, Nexø E, Moestrup SK, Petersen TE, Jensen EO, and Berglund L. (2003) **Human intrinsic factor expressed in the plant *Arabidopsis thaliana*.** *European Journal of Biochemistry* 270, 3362-3367.
- Grønlund M, Gustafsen C, Roussis A, Jensen D, Nielsen LP, Marcker KA, and Jensen EO. (2003) **The *Lotus japonicus ndx* gene family is involved in nodule function and maintenance.** *Plant Molecular Biology* 52, 303-316.
- Guilley H, Dudley RK, Jonard G, Balazs E, and Richards KE. (1982) **Transcription of Cauliflower mosaic virus DNA: detection of promoter sequences, and characterization of transcripts.** *Cell* 30, 763-773.
- Hajdukiewicz P, Svab Z, and Maliga P. (1994) **The small, versatile pPZP family of Agrobacterium binary vectors for plant transformation.** *Plant Molecular Biology* 25, 989-994.
- Hansen AC, Busk H, Marcker A, Marcker KA, and Jensen EO. (1999) **VsENBP1 regulates the expression of the early nodulin PsENOD12B.** *Plant Molecular Biology* 40, 495-506.
- Stougaard J, Sandal N, Grøn A, Kühle A, and Marcker KA. (1987) **5' Analysis of the soybean leghaemoglobin *lbc3* gene: regulatory elements required for promoter activity and organ specificity.** *EMBO Journal* 6, 3565-3569.
- Vancanneyt G, Schmidt R, O'Connor-Sanchez A, Willmitzer L, and Rocha-Sosa M. (1990) **Construction of an intron-containing marker gene: splicing of the intron in transgenic plants and its use in monitoring early events in Agrobacterium-mediated plant transformation.** *Molecular General Genetics* 220, 245-250.