Plant Molecular Biology **15**: 173–174, 1990.

Plant Molecular Biology Update

Nopaline synthase gene is expressed in *Escherichia coli* and in cucumber cells under a hybrid promoter

Yedidya Gafni
Department of Plant Genetics and Breeding, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

Recevied 22 March 1990; accepted 29 March 1990

Key words: Agrobacterium tumefaciens, *trp* promoter, *Cucumis sativus*, plasmids, binary vectors, nopaline

Abstract

Fusion of the nopaline synthase gene (*nos*) to the *Escherichia coli trp* promoter gave rise to a hybrid promoter (*tros*). Under control of this hybrid element, synthesis of nopaline was observed in *E. coli* as well as in cucumber cells transformed with the described vector.

A hybrid promoter that is functional in *Escherichia coli* and in cucumber cells has been constructed by fusion of the nopaline synthase gene (*nos*) to the *E. coli trp* promoter. This chimeric *trp-nos(tros)* control unit contained the *nos* 'TATA box' but not the 'CAAT box' and upstream sequences of the original promoter, and it was shown to be expressed in *E. coli* [3]. The *tros* promoter being attached to the *nos* structural gene was placed on a wide host range replicon [1] between the right and the left border sequences of the T-DNA of pTi T37 (Fig. 1). Under control of this *tros* hybrid element, synthesis of nopaline was observed in *E. coli* as well as in cucumber (*Cucumis sativus*) cells transformed with the described pVOL2 vector. Cucumber hypocotyls were inoculated with *A. tumefaciens* strain A519 bearing the pVOL2 as well as its own Ti plasmid [4]. The *A. tumefaciens* strain A519 is able to incite crown gall tumors but the tumors do not produce nopaline [2]. Nopaline assays were performed on the transformed *E. coli* and on the plant tumor extracts according to Gafni & Chilton [3]. Levels of expression of the nopaline synthase gene under the *tros* promoter are reasonable cmpared with amounts produced by C58-induced tumor cells (Fig. 2). The pVOL2 vector with a slight modification can be used as a potential vector for 'one step' cloning of a gene to achieve its expression first in *E. coli* and then in transformed plant cells. Shortening of the *nos* promoter to − 32 and attaching it to the *E. coli trp* promoter, does not affect *nos* expression in cucumber cells.

This paper is contribution no. 2698-E, 1989 series from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

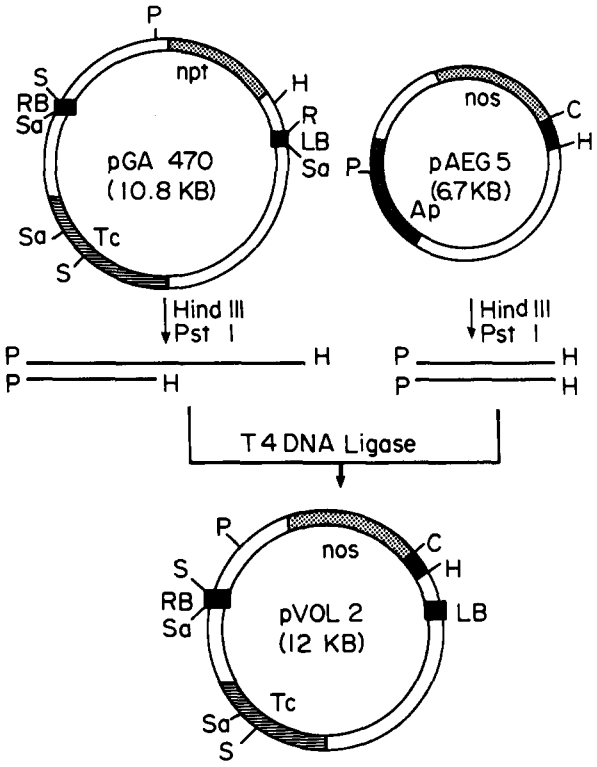

Fig. 1. Construction of pVOL2 containing *nos* structural gene under control of a hybrid *tros* promoter. pAEG5 and pGA470 were digested with *Hind* III and *Pst* I. The fragment with the *nos* gene was ligated between the right and the left border sequences of the T-DNA of pGA470, replacing the NPT II gene. Symbols: H, *Hind* III; P, *Pst* I; LB, left border; RB, right border; Tc; tetracyclin.

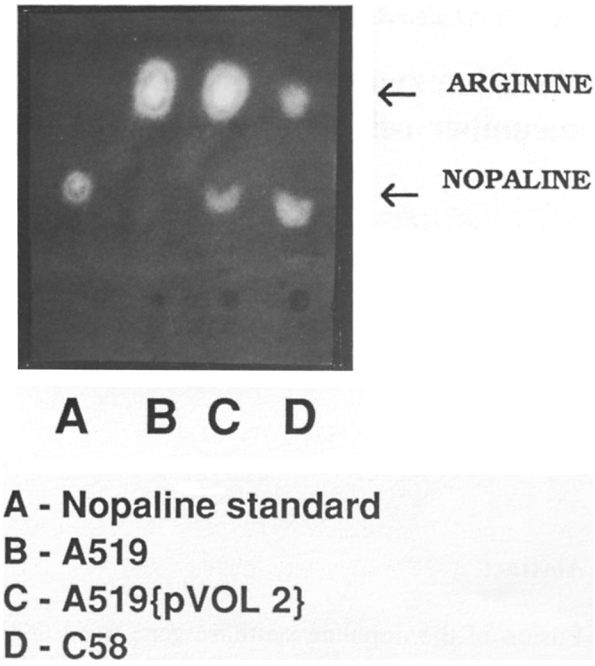

Fig. 2. Nopaline production in cucumber cells transfromed with *nos* gene under *tros* promoter. Nopaline assays were performed according to Gafni *et al.* [3].

References

1. An G, Watson BD, Stachel S, Gordon MP, Nester EW: New cloning vehicles for transformation. EMBO J 4: 277–284 (1985).
2. Chilton WS, Rinehart KL, Chilton M-D: Structure and stereochemistry of succinamopine. Biochemistry 23: 3290–3297 (1984).
3. Gafni Y, Chilton M-D: Expression of the nopaline synthase gene in *Escherichia coli.* Gene 39: 141–146 (1985).
4. Matsumoro S, Machida Y, Takebe I: A rapid method for assaying tumorigenicity of *Agrobacterium tumefaciens.* Plant Mol Biol Rep 4: 42–47 (1986).