

Wizard[®] Plus Minipreps for the Isolation of Binary Plasmids from *Agrobacterium tumefaciens*



By Ross A. Rupert, M.S., and Paul J. Bottino, Ph.D.
Department of Cell Biology and Molecular Genetics, University of Maryland at College Park

Corresponding author: e-mail to ross.a.rupert@usa.dupont.com

Minor modification of the Wizard[®] Plus Minipreps DNA Purification System^(a) procedure allows for the efficient isolation of binary vector DNA from *Agrobacterium tumefaciens*. Following purification, plasmid DNA can be digested with restriction endonucleases and analyzed by gel electrophoresis. The modified procedure serves as an effective method for screening *A. tumefaciens* cells for the presence of introduced binary vectors.

INTRODUCTION

As a powerful tool for the study of gene function and expression, *Agrobacterium*-mediated transformation has become central to plant biology (1,2). Prior to transformation, binary vector constructs are generated and introduced into *A. tumefaciens* cells. A critical step involves the screening of cells for the presence of the introduced plasmids. A number of methods are currently in use for such analysis (39). However, these procedures are expensive and labor-intensive. We present here a modification to the standard Wizard[®] Plus Minipreps procedure (10) for rapid, economical and efficient isolation of binary plasmid DNA from *A. tumefaciens*.

MODIFIED WIZARD[®] PLUS MINIPREP PROCEDURE

To begin, AB plates prepared with appropriate antibiotic and streaked with *A. tumefaciens* cells are incubated at 28°C for 4 days. AB medium (11,12) contains 3g/L K₂HPO₄, 1g/L NaH₂PO₄, 1g/L NH₄Cl, 300mg/L MgSO₄, 150mg/L KCl, 1mg/L CaCl₂, 2.5mg/L FeSO₄ and 5.5g/L glucose (pH 7.2). Next, 3ml of AB medium, containing appropriate antibiotic, are inoculated using a loopful of cells and incubated overnight at 28°C with shaking (250rpm). Prior to harvesting the cells, a fresh solution of lysozyme (10mg/ml) in 10mM Tris (pH 8.0) is prepared, and an aliquot of TE buffer is preheated to 70°C. Cells are harvested by centrifugation in a microcentrifuge at 8,000 x g for two minutes; then the protocol is followed as outlined in Figure 1. Following purification, plasmid DNA can be analyzed by restriction endonuclease digestion and gel electrophoresis. Figure 2 demonstrates typical results of such an analysis.



Figure 1. Modified Wizard[®] Plus Minipreps procedure.

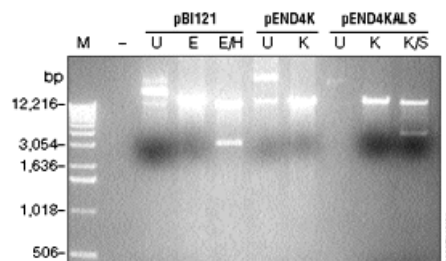


Figure 2. Restriction endonuclease digestion and gel electrophoresis of binary vectors pBI121, pEND4K and pEND4KALS. *A. tumefaciens* strain LBA 4404 does not harbor a binary vector and served as a negative control (lane). Plasmids were linearized by single enzyme digestions, and the inserts of pBI121 and pEND4KALS were released by double enzyme digestions. Abbreviations: U, uncut; E, *EcoR* I; H, *Hind* III; K, *Kpn* I; S, *Sal* I.

CONCLUSION

Minor modifications of the Wizard[®] Plus Minipreps procedure allow efficient isolation of binary plasmid DNA from *A. tumefaciens*. The plasmid DNA is sufficiently pure for restriction analysis to confirm the presence of insert. Most methods used for such analysis involve costly and labor-intensive steps such as protease digestion, phenol/chloroform extraction, ether extraction, ethanol precipitation and RNase digestion. Although required for the isolation of total DNA from *A. tumefaciens*, these methods are not necessary for the exclusive purification of binary plasmid DNA. The modified Wizard[®] Plus Minipreps procedure presented here serves as a more efficient alternative.

Since binary plasmids are larger than 10kb and are present in low copy number in *A. tumefaciens*, minipreps typically give a low DNA yield. Most procedures have been optimized to overcome this problem. Accordingly, the Wizard[®] Plus Minipreps procedure was optimized by the addition of a lysozyme digestion step and the use of preheated TE buffer. The cell walls of *A. tumefaciens* are partially resistant to lysis. The addition of the lysozyme incubation improves lysis and increases plasmid yield several-fold. (We found that increasing the amount of lysozyme added, up to 100µl from a 10mg/ml stock, improved plasmid yield.) Resuspending plasmids of 10kb and larger in 70°C TE buffer is recommended by Promega and increases yield several-fold.

High copy number plasmid yield can often be improved by increasing the culture time prior to harvesting. Growing *E. coli* for as long as 24 hours allows them to reach stationary phase where plasmid copy number is the highest. *A. tumefaciens* harboring binary plasmids were grown for 12, 24 or 48 hours prior to using the modified Wizard[®] Plus Minipreps procedure. No further increase in plasmid yield was seen after 12 hours.

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Ordering Information

Product	Size	Cat.#
Wizard [®] Plus Minipreps DNA Purification Systems	50 preps	A7100
	100 preps	A7500

	250 preps	A7510
Wizard [®] Minipreps DNA Purification Resin ^(a)	250ml	A7141
Wizard [®] Minicolumns	250	A7211

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Product	Size	Cat.#
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Wizard [®] Plus SV Minipreps DNA Purification Systems + Vacuum Adapters ^(b)	50 preps	A1340
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Wizard [®] Plus SV Minipreps DNA Purification Systems + Vacuum Adapters ^(b)	250 preps	A1470

Editor's Notes: *The Wizard[®] Plus SV Minipreps Systems feature the flexible 'spin' and 'vacuum' (i.e., 'SV') format allowing the user the option of how to purify DNA.*

Also, we recommend that E. coli harboring high-copy number plasmid be grown for 16 hours prior to plasmid purification.

^(a)U.S. Pat. Nos. 5,658,548 and 5,808,041, and Australian Pat. No. 689815 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures.

^(b)Patent Pending.

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