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Technical Note

A simple and inexpensive method for sending binary vector plasmid DNA by mail

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Abstract We describe a simple cost-effective technique for the transport of binary vector plasmid DNA by mail. Our results demonstrate that common multipurpose printing paper is a satisfactory substrate and superior to the more absorbent 3 MM chromatography paper for the transport of plasmid DNA through the mail. We show that depositing as little as 100 ng of purified plasmid onto multipurpose printing paper is sufficient to allow recovery of the mailed DNA. Also observed was an inverse correlation between vector size and recovery of intact plasmid DNA, thus larger binary vectors require the deposition of more plasmid to ensure successful recovery.

Key words: Binary vector, PCR, plasmid, transformation.

Plasmid binary vectors are useful plant molecular biology tools utilized in *Agrobacterium*-mediated transformation of plants (Komari et al. 2006; Hellens et al. 2000). The need to share these and other types plasmid vectors has become commonplace in the highly collaborative research environment. The lack of efficient distribution of useful plasmid vectors could significantly delay research progress by requiring the independent construction and verification of plasmids, thus duplicating the efforts already invested by other research groups. A common method of transport is to mail a few microliters of ethanol precipitated plasmid DNA in a microcentrifuge tube or in flame-sealed thin walled tubing (Mortimer and Kahn 2001). While functional, both methods entail the chance of DNA loss if the plastic becomes broken or the envelope is torn in transit. Commercial products are also available for the shipping of plasmid vectors (e.g. Whatman CloneSaver™ cards or the SampleMatrix™ materials from Biomatica), but these methods require the purchase of materials, are bulkier to send through the mail and are primarily designed for longer term storage of DNA samples.

In this report, we offer a simple solution that does not require use of any special equipment or materials and allows DNA shipment in a standard letter envelope. We present results from mailing various quantities of two *Agrobacterium* binary vectors and one smaller cloning vector and their subsequent recovery after transit.

To determine suitable conditions for sending plasmid DNA via the mail, we tested two common substrate materials and two elution solutions. We intentionally limited our examination to conditions and materials that are readily available in most if not all research labs. Three plasmid vectors, p409S-GUS, a pBINPLUS/ARS binary vector derivative (William Belknap, unpublished) which is 16.2 kilobase pair (kbp) in size; the pGPro1 binary vector which is 8.82 kbp in size (GenBank Accession DQ452049, Thilmony et al. 2006); and the cloning vector pCR2.1 which is 3.93 kbp in size (Invitrogen, Carlsbad, CA) were used for our analysis. Each of the plasmid vectors was purified from *E. coli* cultures using the GeneJet plasmid miniprep kit (Fermentas, Glen Burnie, MD) following the manufacturer's protocol. Common multipurpose printing/copying/typing acid-free white paper with 30% recycled content (216×279 mm, 20LB, 92 brightness, Aspen™ 30, Boise Cascade L.L.C., Boise, ID) and 3 MM chromatography paper (Whatman, Florham Park, NJ) were used as substrates for mailing the plasmid DNA samples. Although for our analyses, only the Aspen™ 30 multipurpose paper was used, we expect that any typical multipurpose printing paper will work equally well. We pipetted in duplicate 1000 ng, 500 ng, 100 ng, 10 ng, 1 ng or 0.1 ng of the plasmids onto the multipurpose printing paper and the 3 MM chromatography paper in volumes of 10 μl, marked the saturated areas with a pencil

Abbreviations: DNA, Deoxyribonucleic Acid; kbp, kilobase pair; PCR, Polymerase Chain Reaction; TE, 10 mM Tris pH 8.01 mM EDTA
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(typically spot sizes were 8–10 mm in diameter) and let them air dry. These samples were then placed in a standard letter envelope and sent (July, 2007) by first class mail via the U.S. postal service from Albany, CA to Wilmington, NJ and subsequently mailed back to Albany, CA traveling approximately 4,600 kilometers.

After transit through the mail, the encircled DNA sample areas were excised with a clean scissors and placed into individual 1.5 ml microcentrifuge tubes. The plasmid DNA was eluted using 100 μl of either sterile distilled water or TE (10 mM Tris, pH 8.0, 1 mM EDTA). Each sample was incubated at room temperature for 10 min and then centrifuged at 20,800×g (Eppendorf 5417C microcentrifuge) for 2 min. The eluates, comprising 90 μl from the multipurpose paper and approximately 50 μl from the 3 MM paper, were transferred to new microcentrifuge tubes and stored at −20°C.

To examine both the integrity and concentration of the plasmid DNA in the eluates, we loaded 10 μl of each on a 0.8% agarose gel, resolved by electrophoresis, and visualized with ethidium bromide staining and ultraviolet light. The image of the pGPro1 samples eluted with water and TE from multipurpose paper is shown in Figure 1. Plasmid DNA was visible only in the TE eluates from the 1000 ng and 500 ng spots for the 8.82 kbp pGPro1 vector. Most of the visible plasmid DNA in the TE eluate appeared to be either linearized or nicked open circles. Substantially less DNA was visible in the samples eluted from the 3 MM chromatography paper (data not shown). The 1000 and 500 ng samples eluted with water from the printing paper (Figure 1) and 3 MM paper (data not shown) exhibited a weak smear in the gel below the size of the supercoiled plasmid, suggesting the presence of partially degraded DNA. Similar results were observed for the 3.93 kbp vector pCR2.1 eluted with TE, but the water eluted 1000 and 500 ng pCR2.1 samples contained weak visible bands of linearized plasmid DNA (data not shown). The 16.2 kbp p409S-GUS vector samples did not exhibit any visible bands on the gel for either the TE or water eluted samples. Overall, these results support the conclusion that TE is superior to sterile water as an elutant because it releases more intact plasmid DNA from the paper substrates.

To further test the eluted DNA, we performed PCR with primers designed to amplify a fragment of the kanamycin resistance gene present within each plasmid. The purified plasmids used for spotting served as positive controls. The PCR amplification conditions used for 25 μl reactions were: 95°C, 3 min for 1 cycle; 94°C, 30 s, 58°C, 30 s, 72°C, 30 s for 20 cycles; and 72°C, 5 min. The primers nptIF56 5′-ttccaacatggatgctgatttatatgg-3′ and nptIR56 5′-catcgccatgtgtcacgac-3′ were used to amplify a 637 bp fragment of the pGPro1 *nptI* gene. The primers nptIIF57 5′-gattgaacaagatggattgcacgc-3′ and nptIIR58 5′-ccacagtcgatgaatccagaaaagc-3′) were used amplify a 626bp fragment from the pCR2.1 and p409s-GUS *nptII* genes.

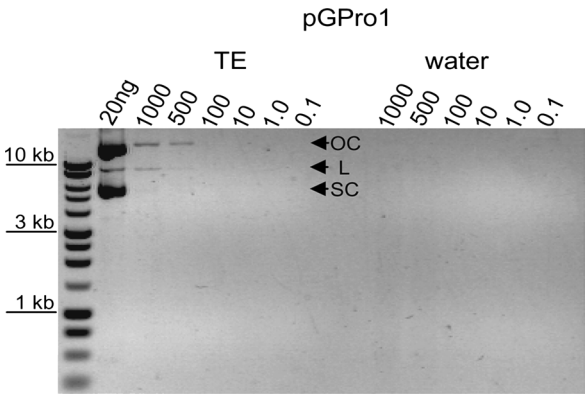

Figure 1. Gel electrophoresis of eluted pGPro1 DNA. Lane 2 contains 20 ng of purified pGPro1 binary vector plasmid. The eluates from multipurpose printing paper spotted with 1000, 500, 100, 10, 1.0 and 0.1 ng of pGPro1 DNA were loaded as indicated in the figure. The samples eluted with TE are shown on the left; those eluted with water are shown on the right. The relaxed open circular (OC), linear (L) and supercoiled circular (SC) plasmid DNA bands are identified with arrows. Labels for the size standard are shown on the left.

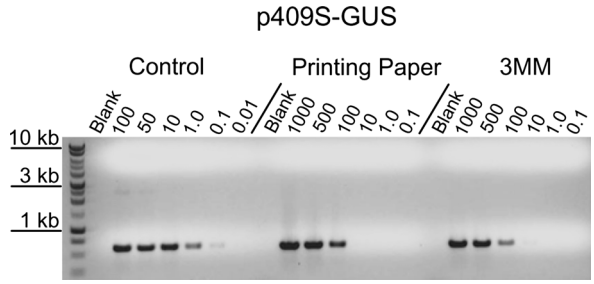

Figure 2. Gel electrophoresis of PCR-amplified fragments using p409S-GUS DNA samples eluted with TE from multipurpose and 3 MM chromatography paper. Control reactions with 100, 50, 10, 1.0, 0.1 and 0.01 ng of p409S-GUS DNA are shown. The samples eluted with TE from multipurpose paper (center) or 3 MM chromatography paper (right) spotted with 1000, 500, 100, 10, 1.0 and 0.1 ng of p409S-GUS DNA were used as templates in each PCR reaction as indicated. Labels for the size standard are shown on left.

A 0.5 μl aliquot of the TE-eluted samples or control plasmids was used as template for PCR. Following 20 amplification cycles, ten microliters of each reaction were visualized on an ethidium bromide stained gel. The results observed for the p409S-GUS samples are shown in Figure 2. The kanamycin resistance gene fragment of the expected size was visibly amplified from the 1000 ng, 500 ng and 100 ng eluates. Somewhat surprisingly, very similar results were observed for the all the samples regardless of paper type, plasmid vector or elutant. We did observe, using densitometry analysis (Thomson and Ow 2006), that the PCR bands present from multipurpose paper samples exhibited on average, 20% stronger bands than those eluted from the 3 MM paper.

This suggests that despite the lack of visible DNA bands in some of the eluted samples, they all contained amplifiable amounts of plasmid DNA (albeit fragmented).

Finally we used the eluates from each of the 3 highest DNA concentrations to transform *E. coli* competent cells. Standard techniques were used for bacterial growth, DNA manipulation and *E. coli* transformation as previously described (Sambrook et al. 1989). *E. coli* XL2 blue (Stratagene, La Jolla, CA) competent cells were transformed using 5 μl of eluate. The transformation efficiency of the competent cells used was 7.65×10^6 colony forming units/μg of plasmid DNA. Following transformation and recovery, the bacterial cultures were serially diluted and plated on LB solid medium containing 50 mg L^{-1} kanamycin as previously described (Katagiri et al. 2002). After an overnight incubation at 37°C, the resulting colonies were counted and used to calculate the total number of transformed bacteria produced from each transformation. The results for the TE-eluted samples are graphed in Figure 3. The numbers of transformed colonies produced by samples eluted from the multipurpose printing paper were dramatically higher than those observed for the 3 MM samples. This was particularly obvious when comparing the results for pGPro1 and p409S-GUS (Figure 3). Also clear from the results is that the number of kanamycin resistant colonies produced is inversely proportional to the size of the plasmid vector (Figure 3A), and that the elution efficiencies of intact plasmid DNA of the two larger binary vectors from the chromatography paper is less than those observed for the small vector pCR2.1 (Figure 3B).

Although considerably fewer colonies were observed for the 100 ng TE-eluate samples, even this modest amount of original plasmid DNA was sufficient to allow successful recovery of transformants after mailing when the multipurpose printing paper was used as the substrate. For the water-eluted samples, only the smallest vector pCR2.1 generated a substantial number of colonies from both the multipurpose and 3 MM paper (data not shown). The 1000 ng pGPro1 printing paper water eluate was able to generate some kanamycin resistant colonies, but none were recovered from comparable 3 MM water eluate. We were also unable to generate any kanamycin resistant colonies with transformation of the p409s-GUS water-eluted samples.

While our results indicate that 100 ng of DNA is sufficient to successfully transfer plasmid binary vectors via the mail, we recommend that at least 500 ng of plasmid be deposited onto multipurpose paper to be mailed as a standard letter. Elution with TE is preferable to water and use of multipurpose printing paper will allow the use of smaller elution volumes so as to concentrate the eluted DNA. Although heat shock competent cells were successfully used for transformation, the use of electro-competent cells will further increase the yield of transformed bacteria thus reducing the requirement for depositing larger amounts of DNA.

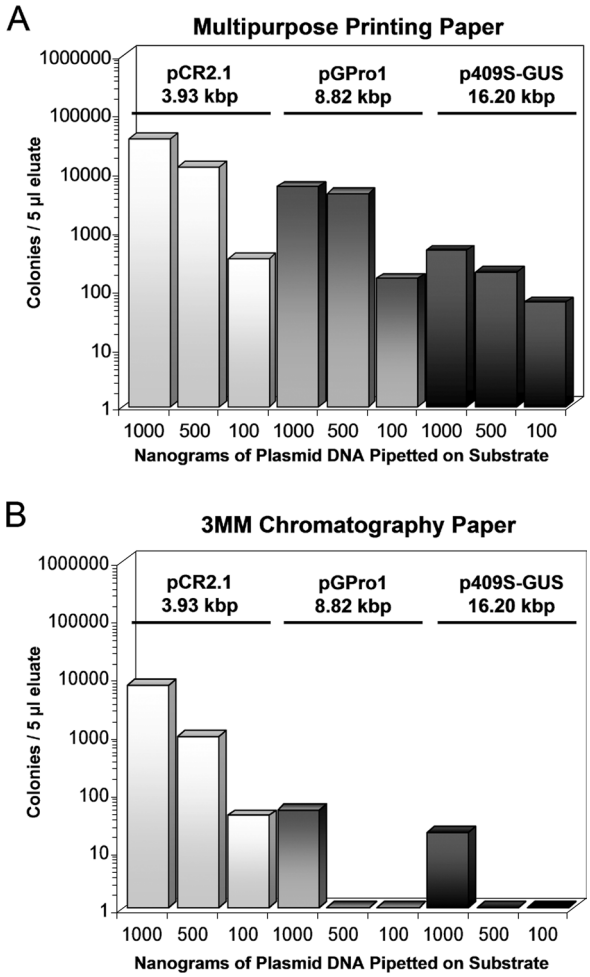

Figure 3. Transformation efficiencies of plasmid samples eluted with TE from multipurpose (A) and 3 MM chromatography (B) paper. The total number of colonies from transformation with 5 μl aliquots of the 1000, 500 and 100 ng eluates for plasmids pCR2.1 (light gray), pGPro1 (dark gray) and p409S-GUS (black) are shown on a log scale.

It is clear from the results that multipurpose printing paper is a superior substrate when compared to 3 MM paper. This conclusion is supported by both gel electrophoresis and *E. coli* transformation results that demonstrated that the multipurpose printing paper eluates contained more intact plasmid than the 3 MM eluates. The precise explanation of why the multipurpose paper performed better is not obvious, but it is evident that the more absorbent nature of the 3 MM paper contributes to inefficient elution yielding a smaller volume of total eluate that contains more dilute plasmid DNA.

The authors have successfully used this method for the transfer of plasmid vectors within the continental United States as well as internationally to several labs in Europe

and Asia. For mailing overseas, we suggest that the spotted DNA samples be wrapped in plastic wrap or parafilm to protect them from moisture. We believe this simple, inexpensive method of mailing plasmid DNA will be a useful technique for globally distributing and receiving research materials among colleagues.

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