Evaluation of DNA Plasmid Storage Conditions

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Abstract: Several pBR DNA plasmid storage conditions were evaluated in this study, including the storage form (liquid or dried on 3M paper), the buffer used to dissolve the plasmid, and temperature. Storage in the liquid form resulted in increased colony formation compared to DNA dried on paper, which is a more convenient and often preferred method for shipping plasmids. The TE buffer was superior to autoclaved H2O for DNA storage. The most important factor for long-term storage was temperature, and storage at -20°C in TE buffer showed good results (ca. 107 cfu/µg plasmid DNA) for up to 270 days. In contrast, stored DNA plasmid in H2O or dried on Whatman 3M paper at 20°C, preferred for ordinary DNA shipment, resulted in deteriorated yields after 90 days. In conclusion, my results indicate that 20°C is acceptable for short-term storage (up to 18 days), but TE buffer and -20°C should be used for longer-term storage.

Keywords: Plasmid, DNA, storage, temperature, transform, shipment.

INTRODUCTION

DNA storage is one of the most important factors in DNA recombinant experiments. Molecular biology depends on several techniques, including DNA recombination, transformation, amplification, and storage. Therefore, DNA storage is important for many scientific investigations. In addition, plasmids are shipped worldwide. Therefore, identification of simple and stable DNA storage methods is critical. In the present study, DNA storage conditions, in terms of temperature, buffer type, and storage form, were evaluated for up to 270 days.

MATERIAL AND METHODS

Standard protocols were used for the molecular biological experiments [1]. Plasmid DNA was prepared with a Qiagen Midi-prep kit (Qiagen Inc., Valencia, CA, USA). Competent E. coli cells (XL-1blue; Stratagene Corp., La Jolla, CA, USA) were prepared using Inoue’s protocol [2], which yields 10–100 million cfu/µg and is one of the best protocols for preparation of competent cells. Inoue’s protocol is an improved one of the widely used Hanahan’s transformation protocol [3].

Three factors were evaluated, including the liquid or dried form of the DNA plasmid, TE buffer or autoclaved H2O, and three temperatures (20, 4, and -20°C). Briefly, the pBR322 DNA plasmid (final concentration, 1 ng/µl) was prepared in TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA) or autoclaved H2O. Plasmid DNA (10 ng) was stored in a liquid or dried form, the latter on Whatman 3M paper (WhatmannInc., Clifton, NJ, USA) (~2 mm²) in a 0.5-ml tube. After each storage period (0, 2, 7, 18, 90, 150, 270 days) at each temperature (20, 4, and -20°C), plasmid samples in paper form were soaked in 50-µl H2O at 4°C for 12 h to recover the plasmid. The same volume (50 µl) of H2O was added to the liquid samples.

Approximately 1 ng of each plasmid DNA sample was transformed on the same day. As transformation is often dependent on culture condition, all plasmid DNA was transformed on the same day using the same competent cell preparation. After 1 h incubation at 37°C, one tenth of each sample was plated on a 90-mm dish (55 cm²) containing ampicillin (50 µg/ml), and the colonies were enumerated the following day. With this protocol, colony number on one plate yields number x 10⁴ cfu/µg plasmid DNA, as it reflects colonies from 100 pg of transformed plasmid DNA. The transformation efficiency of the control plasmid DNA was 1.34 × 10⁷ cfu.

STATISTICS

Data are presented as means ± standard error. Significance (p < 0.05) was determined by one-way or two-way analyses of variance. Student’s t-tests were used when the means of two groups were compared. A p-value < 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

DNA quality was evaluated initially by electrophoresis (Fig. 1a). On day 18 at a storage temperature of 20°C, electrophoresis clearly showed a significant decrease in DNA quantity, indicating time-dependent changes. Fig. (1b) shows typical results of plasmid transformation on day 90. The DNA plasmid in TE buffer (Fig.1b-i) yielded the greatest number of colonies, whereas DNA plasmid in H2O or dried on What man 3M paper (Fig. 1b-iv) resulted in relatively few colonies.

The difference between the liquid and dried DNA was evaluated statistically (Fig. 1c). Storage as the liquid form
resulted in more colonies than the dried form on days 90, 150, and 270 (*p<0.05, n = 6). Interestingly, no significant difference was observed between the two conditions on day 18.

Next, buffer conditions were evaluated. TE buffer is thought to be better for DNA storage, as it is buffered to pH 7.5 and contains EDTA to chelate divalent ions; thus, TE buffer should be superior to autoclaved H2O for long-term storage. Indeed, TE buffer showed better results on days 90, 150, and 270 (Fig. 2). No significant difference was observed on day 18.

Temperature dependency was evaluated using the plasmid samples in TE buffer (Fig. 3). Use of -20°C was beneficial for long-term storage. No significant decrease in colony number was observed on day 270 compared to that on day 0 (open blue circles). Storage at 4°C resulted in a time-dependent decrease in colony number (open black circles). Storage at 20°C resulted in significantly decreased numbers of colonies on days 7, 18, 90, 150, and 270 (open red circles). However, > 200 colonies (x10⁴ cfu/µg) were formed on day 270 under this storage condition. Nevertheless, the data revealed that -20°C is the optimum temperature for long-term DNA plasmid storage.

The performance of the dried form of plasmid stored in TE buffer was also evaluated (Fig. 4). Colony number tended to decrease at -20°C with this DNA plasmid storage form (open blue squares), although the difference was not significant. Storage as the dried form at 4 and 20°C resulted in decreased colony formation in a temperature-dependent manner; thus, these conditions are not suitable for long-term storage.
storage (particularly 20°C). In addition, either few or no colonies were occasionally formed after plasmid recovery from the dried form. Taken together, these data indicate that any of the aforementioned conditions (TE or H₂O, liquid or dried, three temperatures: 20, 4 or -20°C) can be used to store plasmid DNA for 2 weeks, but the liquid form at 20°C should be utilized for long-term storage. Considering the significant decrease in the number of colonies formed on day 90, DNA plasmids in the dried form should be recovered by day 18 (Fig. 4). This finding is important, if one considers plasmid shipment methods. My results indicate that plasmid DNA can be shipped in either the liquid or dried form at room temperature, but that it should be recovered within 2–3 weeks, transformed, and amplified. The amplified plasmid DNA should be maintained at -20°C for long-term storage. In many laboratories, researchers send plasmid samples with Rosman’s protocol, with TE buffer dried form at room temperature [4]. Our data indicate possible difficulties with the protocol, as I got only 169 ± 18 colonies (x10⁴ cfu/µg) with modified Rosman’s protocol (plasmid in TE buffer, dried form, at 20°C, Fig. (4) red open square) on Day 150. Furthermore, I got mere 31 ± 2 colonies (x10⁴ cfu/µg) on Day 270 (Fig. 4 red open square). Considering relatively unstable preparation of competent cells (sometimes less than 1 × 10⁵ cfu/µg with Hanahan’s protocol), it seems not advisable to stock plasmid DNA in the Rosman’s condition for a long-term storage.

In conclusion, DNA plasmids in a liquid or dried form can be used for shipment, but should be maintained in liquid form at -20°C for longer term storage of more than several weeks’ duration.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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