A simple treatment of DNA in a ligation mixture prior to electroporation improves transformation frequency

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A high frequency of transformation of bacteria of about 1-5×10^10 cells per µg of supercoiled plasmid DNA (1, 2) is now routinely obtained when a dense suspension of Escherichia coli (MC1061/P3; Invitrogen) cells (1×10^10 cells/ml) are exposed to a brief (3-5 msec) high voltage electrical pulse. However, if the DNA sample is dissolved in a ligation mixture with a high salt concentration, the transformation frequency is decreased substantially. To circumvent this difficulty, dilution (3), dialysis (4), precipitation (5) or heat inactivation (6) of the ligation mixtures have been reported. However, none of them are entirely satisfactory. They hardly exceed the frequency of the competent cells prepared by a chemical treatment we developed recently (7), in which the DNA in a ligation mixture can be directly introduced without any treatments, giving transformation frequency of 1-3×10^9 cfu per 1 µg of pBR322 DNA. Here we report a new protocol which retains about 42% of the original high transformation frequency in a ligation mixture after a simple treatment.

MC1061/P3 cells were prepared for electroporation as described previously (1, 2). pBR322 DNA (10 pg/µl) was dissolved either in TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) or in a ligation mixture (20 mM Tris-HCl [pH 7.5], 0.1 mM EDTA, 10 mM NaCl, 4 mM MgCl₂, 10 mM (NH₄)SO₄, 100 mM KCl, 0.15 mM βNAD, 0.04 mM dNTP and 50 µg/ml Bovine Serum Albumin) supplemented with E. coli DNA ligase, DNA Polymerase and RNase H as described (8). The ligation mixture is from the Okayama-Yberg method for the preparation of cDNA library of high quality (8). Heat inactivation of the ligation mixture (6) gave only a little improvement, resulting in 0.36% of the original frequency as shown in Table 1a and 1c. Hence we additionally conducted combinations of either phenol—chloroform (1:1) extraction (Table 1f) or denaturation by filtering through a Millipore microfilter (UFCP3TK50) (Table 1d, e, f), which separately improved the frequency (from 1.9% to 19.3%). Performing these treatments altogether, that is, filtration after a heat treatment and a phenol—chloroform extraction, it resulted in 6.3×10^8 cfu per 1 µg of pBR322 DNA as shown in Table 1g. This is almost a 1000-fold improvement as compared with the case without treatment (Table 1b), and is about 42% of the original frequency.

We summarize our new procedure as follows; (1) Incubate a DNA sample in ligation mixture at 70°C for 30 min. (2) Add an equal volume of phenol—chloroform, vortex and spin in a microfuge for 2 min. (3) To the supernatant, add an equal volume of chloroform, vortex and spin again. (4) Apply the supernatant onto a Millipore microfilter (UFCP3TK50), spin down the sample at 3000×g for 20 min. (5) Add 300 µl of TE and drain the filter by spinning again at 3000×g for 20 min. Repeat this step three times. (6) Add 30 µl TE onto the filter and vortex. (7) Remove the upper part of the filter, insert it into a microtube whose lid is cut off, then spin down for 5 seconds to recover the DNA. (8) Add 3 µl of this solution to a dense suspension of 50 µl cells (MC1061) for electroporation.

We found that up to 4.0 µl of fully treated solutions could be added to the prewashed dense suspension of cells (50 µl) without lowering the transformation frequency. The addition of tRNA carrier to aid ethanol precipitation of DNA during the process of cDNA library preparation also showed no effect.

REFERENCES


Table 1

<table>
<thead>
<tr>
<th>DNA sample</th>
<th>[A]</th>
<th>Treatment*</th>
<th>[B]</th>
<th>Frequency # (cfu/µg pBR322)</th>
<th>% of total</th>
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</thead>
<tbody>
<tr>
<td>In TE (control)</td>
<td>(a)</td>
<td>– – –</td>
<td>(1.5 ± 0.3)×10^10</td>
<td>100</td>
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<tr>
<td>In ligaton mixture</td>
<td>(b)</td>
<td>– – –</td>
<td>(0.6 ± 0.3)×10^7</td>
<td>0.94</td>
<td></td>
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<tr>
<td></td>
<td>(c)</td>
<td>– + –</td>
<td>(5.4 ± 1.0)×10^6</td>
<td>0.36</td>
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<tr>
<td></td>
<td>(d)</td>
<td>– – +</td>
<td>(2.8 ± 1.2)×10^6</td>
<td>1.9</td>
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</tr>
<tr>
<td></td>
<td>(e)</td>
<td>+ + +</td>
<td>(2.8 ± 1.0)×10^9</td>
<td>18.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(f)</td>
<td>+ + +</td>
<td>(2.9 ± 0.8)×10^9</td>
<td>19.3</td>
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</tr>
<tr>
<td></td>
<td>(g)</td>
<td>+ + +</td>
<td>(6.3 ± 0.7)×10^9</td>
<td>42.0</td>
<td></td>
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</table>

*Treatment (+ means that the treatment is performed): [A] Heat the sample at 70°C for 30 min. [B] Extract the sample by phenol/chloroform (1:1), then by chloroform. [C] Samples are filtered through a Millipore microfilter (UFCP3TK50) and subsequently washed by 300 µl of TE solutions three times.

*Standard deviation of five separate experiments using the same lot of dense suspension of E. coli cells.

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