Direct Transformation of *Neisseria gonorrhoeae* by Gel-Isolated DNA

Kristen Hoikka Pritchard¹,² and H. Steven Seifer²

**Abstract**

The naturally competent organism, *Neisseria gonorrhoeae*, can efficiently transform a marker carried on DNA purified in low-melting-temperature agarose without prior purification or dilution. Neither the agarose or buffer components inhibit transformation frequencies, but exposure to UV irradiation completely abrogates transformation.

**Index Entries:** *Neisseria gonorrhoeae*; DNA transformation; low-melting agarose; UV irradiation; ethidium bromide.

While almost every species can be induced to take up plasmid DNA by chemical or physical means, only a limited number of bacterial species are naturally competent for DNA transformation. Naturally competent bacteria have specific DNA uptake mechanisms and also allow recombination with homologous sequences within the bacterial chromosome. It is often desirable to transform a size-selected fragment of digested DNA into an organism; however, the process of isolating the DNA fragment of interest on an agarose gel, excising the band, and extracting the DNA from the agarose is time-consuming and often results in loss of DNA sample. Here we show that the transformation efficiency of DNA in agarose by *Neisseria gonorrhoeae* is as efficient as that of purified DNA, provided the DNA is not exposed to UV irradiation.

Plasmid pNG1721CAT(1), containing the *N. gonorrhoeae pilE2* gene fused to a promoterless chloramphenicol acetyltransferase gene (encoding chloramphenicol resistance) was linearized with *PstI*. This plasmid transforms *N. gonorrhoeae* with high efficiency. In our first attempt at isolation, the digested DNA was run on a low-melting-temperature agarose gel in the presence of ethidium bromide. The DNA was visualized with UV light, and the fragment of interest was excised. The gel slice was melted at 65°C and added to piliated gonococci, but this DNA did not produce detectable transformation. In contrast, a similar amount of the unfractionated DNA produced transformation frequencies of about $10^{-2}$ transformants/CFU. We assumed that the ethidium bromide complexed with the DNA was inhibiting transformation. The gel was then run without ethidium bromide, and only the marker lane was stained. The desired fragment was located and isolated by lining up the stained and unstained halves of the gel and visualizing with UV light. This DNA still could not be transformed, which prompted us to investigate whether the agarose or UV-irradiation was inhibiting transformation. Table 1 shows that with 500 ng/mL of *PstI* digested pNG1721CAT, high transformation frequencies were obtained with purified DNA, purified DNA mixed with agarose, and DNA electrophoresed in agarose and melted. The presence of agarose did not affect the transformation frequency. However, when the DNA was exposed to

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312 nm UV irradiation, even for a few seconds, the transformation frequency decreased by more than $10^4$ fold (Table 1).

To obtain efficient transformation with gel-isolated DNA, the digested DNA and a molecular weight marker were electrophoresed on a 1% low-melting-temperature agarose gel (NuSieve GTG, FMC Bioproducts, Rockland, ME) with 1X TBE buffer without ethidium bromide. After electrophoresis, the molecular weight marker lane was cut off and stained with ethidium bromide. The marker lane was visualized with UV light (312 nm), and a reference cut was made corresponding to the migration distance of the DNA fragment to be excised. In the absence of UV light the marker lane was aligned with the rest of the gel. Using the reference cut as a guide, a gel slice containing the DNA fragment of interest was excised and stored at 4°C in a microcentrifuge tube. To determine the concentration of the DNA in the agarose, the agarose-DNA was remelted, and dilutions were run on an agarose gel along with dilutions of DNA of known concentrations. By visual comparisons, the amount of DNA in the agarose was estimated. The desired amount of remelted agarose-DNA was used directly in gonococcal transformation following a standard protocol (2).

This protocol is applicable to any cloned DNA fragment providing the DNA fragment can efficiently transform gonococci. Two factors will influence transformation efficiency when using cloned fragments. For efficient transformation, the DNA fragment should contain at least one copy of the gonococcal uptake sequence (4). However, if the marker encoded by the fragment provides for positive selection of a phenotype, fragments without an uptake sequence can be transformed at reduced frequency. Transformation without an uptake sequence requires relatively large concentrations of DNA (>40 μg/mL) (5). Additionally, the size of the homologous region relative to the heterologous insertion on the DNA is also an important factor (5). While both of these factors greatly influence the frequency of transformation, we have used a 0.9-kb fragment with no uptake sequences to introduce a 1.0-kb heterologous insertion at low frequency (C. Wright and H. S. Seifert, unpublished). Our general experience with over 40 different fragments is that any cloned fragment greater than 2 kb in size will transform as long as the heterologous insertion is less than 2 kb.

Other researchers have shown that E. coli can be transformed efficiently with DNA in remelted agarose provided the agarose concentration is diluted to less than 0.02% and the UV exposure is kept to a minimum (6). However, this also dilutes the DNA concentration. Here we show that when transforming N. gonorrhoeae the agarose-DNA does not have to be diluted, and the fragment of interest can be excised without exposure to damaging UV irradiation. This agarose-DNA transforms as well as purified DNA and does not require time-consuming purification steps. It is surprising that short exposure to 312 nm UV-light inactivates the transforming potential of this DNA. The extreme sensitivity of DNA transformation to UV-light suggests that specific mechanisms may prevent transformation of damaged DNA.

### Table 1

<table>
<thead>
<tr>
<th>Transforming DNA</th>
<th>Transformants/CFUa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified DNA</td>
<td>$3.5 \times 10^{-3}$</td>
</tr>
<tr>
<td>Purified DNA + agaroseb</td>
<td>$3.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>Purified DNA + UV</td>
<td>$&lt;2.8 \times 10^{-7}$</td>
</tr>
<tr>
<td>Purified DNA + agarose + UV</td>
<td>$&lt;3.6 \times 10^{-8}$</td>
</tr>
<tr>
<td>Agarose-DNAc</td>
<td>$2.4 \times 10^{-3}$</td>
</tr>
<tr>
<td>Agarose-DNA + UV</td>
<td>$&lt;1.1 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

*aCFU = colony forming units. < indicates that transformation was below that level of detection.

*bRefers to purified DNA that was mixed with melted low-melting temperature agarose.

*cRefers to a DNA fragment excised from a low-melting temperature agarose gel, and the melted agarose DNA mixture was used in transformation.