EFFECT OF PLASMID SIZE ON THE TRANSFORMATION OF

*E.COLI* HB101

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ABSTRACT

Plasmids are one of the most common vectors used in cloning. Many factors have been reported to affect the bacterial transformation by plasmids. This paper attempts to find the effect of plasmid size on the transformation efficiency of *E.coli* HB101. Our results showed that the small-sized plasmids are more effective in transforming the bacteria *E.coli* HBCOL than the large ones (5.7-6kb) compared to (4.1-4.8kb). The transformation efficiency with the small-sized plasmids reaches between $6 \times 10^{-7}$ to $6 \times 10^{-6}$ comparing with $4.1 \times 10^5$ to $4.6 \times 10^5$ of the large-sized plasmid.

We conclude that the small-sized plasmids are more suitable to use in cloning than large-sized plasmids.

Keywords: Plasmid, *E.coli* HB 101, cloning vectors.
تأثر حجم البلازيميد على تحول بكتريا القولون E. coli HB101

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الخلاصة

تعتبر البلازيميدات من المواد الوراثية الشائعة الاستخدام في الهندسة الوراثية وتختلف هذه في حجمها من بلازيميد إلى نوع آخر. ومن خلال الإحاثات العلمية التي أجريت سابقا فانه لوحظ وجود عوامل كثيرة تؤثر على فعالية هذه البلازيميدات كنواقل في الهندسة الوراثية ومن هذه العوامل حجم البلازيميدات وتأثير ذلك على كفاءة التحول في الخلايا المضيفة.

في هذا البحث تم استخدام أحماض مختلفة من البلازيميدات (pSp 6-4) من بكتريا القولون E. coli HB101 كمضيف. أوضح النتائج المتصاعدة انتباذة كبيرة في تحديد كفاءة تحول بكتريا القولون حيث كانت كفاءة التحول عالية في البكتريا عند استخدام بلازيميدات صغيرة الحجم (6 x 10^6 - 6 x 10^7) مقارنة بكفاءة تحول تبلغ 4.6 x 10^5 - 4.1 x 10^6 في البلازيميدات كبيرة الحجم (6.0 - 7.0 x 10^7) كليلول قاعدة) وهو ماوضح بان البلازيميدات صغيرة الحجم أفضل كنواقل في الهندسة الوراثية من تلك الكبيرة الحجم لأنها توفر كفاءة تحول أعلى.
INTRODUCTION

Many genera of bacteria are naturally competent. These include *Bacillus*, *Streptococcus*, *Neisseria*, *Haemophilis* and *Moraxella* species. Others such as *E. coli* are not naturally competent. Similarly, naturally competent bacteria are either inefficiently or not transfected by phage and plasmid DNA (1). In 1970, techniques have been developed which enable some non competent bacteria to be transformed or transfected (2, 3, 4, 5). These technological advances have enabled genetics exchange to take place between completely unrelated organisms (6,7,8,9). Several studies have been reported that transformation efficiency can be affected by many factors such as kind of vector (10), DNA fragment (9,11), bacterial strain (12), salt ions (13), growth conditions (14,15,16) and others (17,18,19,20). This paper discussed the effect of plasmid size on the transformation of *E. coli* HB101.

MATERIALS & METHODS

Materials:
Bacterial strain: The bacteria *E. coli* HB101 was purchased from Bio-Rad company-USA.
Plasmids: 3 Kb PsP plasmids (cloned with different sized human fragments ranged between 1.1 to 3.0 Kb to give total size between 4.1 to 6.0 Kb) were purchased from Amersham international plc-UK.
LB medium:
10g/L Bacto-Tryptone, 5g/L Bacto-yeast Extract, 5g/L NaCl, adjust the pH to 7.3 with NaOH autoclave to sterilize. Allow the autoclaved medium to cool to 55°C and add ampicillin (final concentration 100 ug/ml). For LB plates, 1.5% bacto-agar(15ug/L) was added prior to autoclaving.
Solutions:
0.1M MgCl2 and 0.1M CaCl2 solutions
Ampicillin stock: Stock solution of Ampicillin (Sigma) to final concentration of 25mg/ml was prepared and added to media at concentration of 100 ug/ml.

Methods:
Preparation of competent bacteria: *E. coli* was transformed by a modification of the procedure of Dagert and Ehrlich 1979(17). *E. coli* HB101 was inoculated in 10 ml of LB medium and incubated at 37°C overnight with shaking (150 rpm). 1 ml of an overnight culture was added to 250 ml flasks containing 50 ml LB medium and shaken (150 rpm) at 37°C.
After the optical density of the culture reach approximately 0.5 at 550nm A, flask removed, placed in an ice bath for 15 mines and the culture centrifugated at 2500 rpm for 5 mines at 4C. The medium was removed and the pellet resuspended in 20 ml of ice cold 0.1 M MgCl2. The bacterial suspension was transferred to sterilized universal tubes, placed on ice for 5 mines , centrifugated as before and the buffer was removed .The pellet was resuspended in 4 ml of ice cold 0.1M CaCl2 and chilled for overnight in an ice bath .Serial dilutions were made from the competent bacteria and the viability of bacteria was counted (40X10^7).

Transformation of competent \textit{E.coli} HB101:

Five sizes (4.10, 4.50, 5.50, 5.70, 6.00, Kb) of the plasmid pSp (with Amp R gene) were used in the transformation. 20ng of chilled plasmid DNA was added to the competent bacteria in each tube, tubes were placed on ice for 30mins and heat shocked by placing the tubes at 42C for 2 mins. Then the tubes were chilled for 10mins.1ml from LB medium was then added to each tube and incubated for 1 hr at 37C.200ul from a serial dilutions from each transformation mixture were mixed with 3ml LB top agar containing 50ug /ml ampicillin and poured on top of LB agar plates containing 50ug / ml ampicillin. Three plates from each dilution were used. The plates were incubated at 37C for 24- 48 hrs and the transformation efficiency determined.

Calculation of transformation efficiency (Colony forming units-cfu):

The transformation efficiency was calculated according to Zhiming et al, 2005 (12) and Kushner, 1978 (21).

Transformation efficiency is defined as the number of cfu produced by 1 ug of plasmid DNA, and is measured by performing a control transformation reaction using a known quantity of DNA then calculating the number of cfu formed per microgram DNA.

Transformants-cfu-: No. of bacteria colonies X dilution ratio X original transformation volume / plated volume.

Transformation Efficiency: Transformants cfu / plasmid DNA (ug)

Transformation Frequency: Transformation efficiency/viable cell.

\textbf{RESULTS & DISCUSSION}

The data (Table 1) shows that size plays an important role in transformation. The result indicate that the small sized plasmids showed the highest transformation efficiencies (3X10^6, 3.5X10^5) and also the transformation frequency was 2-11 times greater than large sized plasmids. Other factors such as total transforments and
percentage of cells transformed were also indicate the seem conclusion. These results indicate that the small sized plasmids are suitable in cloning with E.coli HB101 more than largest.

Table-1: Effect of plasmid size on different factors related to E.coli HB101 transformation

<table>
<thead>
<tr>
<th>Plasmid size Kb</th>
<th>Transformation efficiency</th>
<th>Total transforments</th>
<th>Transformation frequency</th>
<th>% of cell transformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.10</td>
<td>3.5X10^6</td>
<td>7X10^4</td>
<td>0.0875</td>
<td>0.1750</td>
</tr>
<tr>
<td>4.50</td>
<td>3 X10^6</td>
<td>6X10^4</td>
<td>0.0750</td>
<td>0.1500</td>
</tr>
<tr>
<td>5.50</td>
<td>2.5X10^6</td>
<td>5X10^4</td>
<td>0.0625</td>
<td>0.1250</td>
</tr>
<tr>
<td>5.70</td>
<td>4.1X10^5</td>
<td>7.5X10^3</td>
<td>0.0102</td>
<td>0.0007</td>
</tr>
<tr>
<td>6.00</td>
<td>4.6X10^5</td>
<td>8X10^3</td>
<td>0.0115</td>
<td>0.0105</td>
</tr>
</tbody>
</table>

Many factors have been shown to influence the transformation of E. coli with plasmids. Some of these have been shown to stimulate the efficiency of the transformation such as salt ions (21); growth conditions (11, 19, 22); nature of DNA (18, 23) and the concentration of the DNA which is used in the transformation (24,25 ).The current results represented in the table indicated that the size of plasmids which are used in the transformation have an important rule in the process. The transformation efficiency was increased to 10 times with the small sized plasmids (2.5-3.5X10^6), while transformation with large sized plasmids have low efficiencies (4.1-4.6X10^5). These results mean that the transformation efficiency was increased in opposite proportion to the plasmid size. The effect of the plasmid size which has been demonstrated in this paper could be due to the number of plasmid copies in the DNA concentration which used in the experiments. Because the small sized plasmids have a large number of molecules for a given DNA concentration (20ng) compared with the large sized plasmid, the chance of the small sized plasmids to transformed E.coli HB101 is great compared with the large sized plasmids and that might explain why the small size plasmids have a high transformation efficiency and frequency.

This mean that the transformation efficiency increase in opposite proportion to the size and linear with number of plasmid in a given DNA concentration.
This in agreement with results reported by Cosoly and Oishi (1973) (26) and Atkins et al (1987) (27) who reported that the number of transformants was increased in proportion to the DNA transforming concentration. Also they found that the relationship between transformation efficiency and DNA concentration was linear in the range of limiting DNA concentration. The high transformation efficiency of E.coli was also reported by others (12, 28, 29, 30, 31). In bacteria other than E.coli, the size of the DNA also found to influence the transformation efficiency in Bacillus subtilis (27), Bacillus brevis (32), Actinobacter (9), Salmonella (33), Corynebacteria (34), Agrobacter (35), Streptococcus (36) and xanthomonas camestris campestis (27,37).
REFERENCES


