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The Effect of Size of Chromosomal DNA from *Escherichia coli* VC10 on Transformation of *Escherichia coli* HB101 by the Plasmid p328.5

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This study investigated the effects of the size of chromosomal DNA fragments on transformation efficacy of a plasmid. In our experiment, each of three chromosomal fragment sizes were co-transformed with varying amounts of plasmid DNA and results show that the conditions, plasmid alone, plasmid with 10kb, and plasmid with 20kb fragments gave no significant differences. On the other hand, plasmid with 30kb and plasmid with undigested chromosomal DNA exhibited a significantly decreased number of transformants compared to the above three conditions. Furthermore, when observing numbers of transformants with varying amounts of plasmid, we see that the plasmid alone and plasmid with 10kb conditions result in more transformants with increasing amounts of DNA and plasmid, whereas plasmid with 20kb and plasmid with 30kb increases with more DNA and plasmid but then decreases. Thus, in general, among the three DNA fragment conditions, there seems to be a decrease or at least a leveling off in the number of transformants as plasmid concentration increased — a marked difference from the consistent upward trend of the plasmid alone condition. Conversely, when contaminated with undigested DNA, transformation efficiency remained suppressed regardless of plasmid concentration. Based on these results, larger contaminating fragments exhibit lower transformation efficiencies then do smaller chromosomal fragments.

From experiment B6a (8), we observed that foreign chromosomal DNA inhibits plasmid transformation efficiency significantly in E. coli cells. One of the apparent differences between chromosomal DNA and plasmid is size. Therefore, in our experiment, we examined the effect of chromosomal DNA size on the efficiency of plasmid transformation in E. coli cells. In Y. Sheng's paper (9), it has been shown that transformation efficiency, measured in number of transformants per ug of DNA, drops with increasing size of the DNA. Molecules of 240kb chromosomal DNA transformed approximately 30 fold less well than molecules of 80kb. In other words, larger fragments of chromosomal DNA transform less effectively, and might be expected to compete more poorly than smaller fragments with plasmid to get into the host cell. As a result, we hypothesized that a smaller fragment of chromosomal DNA, when transformed with pure plasmid, will reduce plasmid transformation efficiently by competing for entry into the host cell. On the other hand, a larger fragment should not reduce plasmid transformation as much because it does not compete for entry into the host cell with plasmid as greatly as a small fragment does (8). If our hypothesis is correct, we would expect fewer E. coli transformants (ampicillin resistant cells) with a smaller fragment of chromosomal DNA and plasmid, and more transformants with a larger fragment of chromosomal DNA and plasmid.

MATERIALS AND METHODS

This experiment consisted of three parts: isolation of chromosomal DNA, isolation of plasmid DNA, and transformation of *E. coli* with the isolated DNA and plasmid.

Isolation of chromosomal DNA

Chromosomal DNA from *E. coli* VC10 was isolated (3) followed by purification with RNase (4). Concentration of purified chromosomal DNA was quantified by absorbance spectroscopy to determine the exact volume of DNA required for restriction endonuclease digestion using XbaI (1). Using an XbaI map of *E. coli* VC10 (7), we were able to identify fragments of 10, 20, and 30kb. It was then calculated that the 10kb fragments constituted 19% of the entire *E. coli* VC10 genome. Thus, in order to obtain 10ug of chromosomal DNA for each transformation, at least 5-6 times as much (50-60ug) DNA was digested. Digested fragments of DNA were run on a 0.5% agarose gel stained with ethidium bromide to isolate and resolve the different digestion products (1). Ethidium bromide was removed and DNA fragments were eluted from the gel as per the "BioRad Prep-A-Gene DNA Purification Matrix Instruction Manual", and concentration of DNA was again quantified by absorbance spectroscopy (1) to determine the exact volume of DNA needed to yield 10ug of DNA for transformation.

Isolation of plasmid

Plasmid DNA (p328.5) containing the ampicillin resistant gene was isolated from *E. coli* HB101 using the phenol extraction method (8) and also stored for later transformation.

Transformation of E. coli

Fragmented chromosomal DNA (VC10) and plasmid DNA (p328.5) was co-transformed into $CaCl_2$ prepared competent HB101 host cells (8). Screening and analysis of transformants was carried out on LB-agar supplemented with ampicillin and regular LB-agar.

Transformation conditions

Our 15 transformation conditions were intended to be done as follows:

Tube #	Pure Plasmid (ug)	10kb Chromosomal DNA (ug)	20kb Chromosomal DNA (ug)	30kb Chromosomal DNA (ug)	Undigested Chromosomal DNA (ug)
1	**5.0**	0	0	0	0
2	**10.0**	0	0	0	0
3	**20.0**	0	0	0	0
4	**5.0**	**10.0**	0	0	0
5	**10.0**	**10.0**	0	0	0
6	**20.0**	**10.0**	0	0	0
7	**5.0**	0	**10.0**	0	0
8	**10.0**	0	**10.0**	0	0
9	**20.0**	0	**10.0**	0	0
10	**50**	0	0	**10.0**	0
11	**10.0**	0	0	**10.0**	0
12	**20.0**	10	0	**10.0**	0
13	**5.0**	0	0	0	**10.0**
14	**10.0**	0	0	0	**10.0**
15	**20.0**	0	0	0	**10.0**

However, due to complications in the elution method, we ended up with less chromosomal DNA than we expected. Thus, we altered the transformation conditions as follows:

Tube #	Pure Plasmid (ug)	10kb Chromosomal DNA (ug)	20kb Chromosomal DNA (ug)	30kb Chromosomal DNA (ug)	Undigested Chromosomal DNA (ug)
1	**4.0**	0	0	0	0
2	**5.0**	0	0	0	0
3	**8.0**	0	0	0	0
4	**10.0**	0	0	0	0
5	**4.0**	**2.0**	0	0	0
6	**8.0**	**4.0**	0	0	0
7	**5.0**	0	**2.5**	0	0
8	**10.0**	0	**5.0**	0	0
9	**5.0**	0	0	**2.5**	0
10	**10.0**	0	0	**5.0**	0
11	**5.0**	0	0	0	**2.5**
12	**10.0**	0	0	0	**5.0**

To ensure the success of transformation, the amount of chromosomal DNA used must be at least half of the amount of plasmid used because:

$$Ap = Ap - (Ap/(Ap+Ac))$$

(A = absorbance; p = plasmid; c = chromosomal DNA)

A few other modifications were made in the protocol along with the change of transformation conditions. The final volumes of all the 13 tubes were equalized to 110ul by adding additional Tris-EDTA buffer. Since the ratio of the volume of the tube (plasmid + chromosomal DNA + Tris-EDTA buffer) and the volume of competent cells should be 1:1 in order to ensure maximal transformation, we then added 100ul of competent cells into each tube.

RESULTS

The results of our experiment were contrary to what we hypothesized. Table 1 shows the number of transformants we obtained from the various conditions. The plasmid alone, plasmid with 10kb and plasmid with 20kb conditions did not make a difference in the number of transformations. Although the number of transformants vary, since the plate counts were gross estimates, it is clear that the small fragments did not decrease the plasmid transformation and might have enhanced the transformation at low amounts of plasmid. But the plasmid with 30kb condition caused a significant decrease in the number of transformants as compared to the control or the smaller fragments. The plasmid with undigested chromosomal DNA also showed a significant decrease in the number of transformants. Varying amounts of plasmid and DNA at constant ratios were used and were found to make a difference in all of the conditions. For the plasmid alone and plasmid with 10kb condition, as the amount of plasmid increased, the number of transformants increased as well. However, for the plasmid with 20kb and plasmid with 30kb DNA, the number of transformants increased with increasing amounts of plasmid up to a point and then decreased. Undigested chromosomal DNA exhibited low transformant numbers for both amounts of plasmid that were tested. Some of the plates counted resulted in TNTC (too numerous to count) in either both the duplicate plates or in only one, indicating some variation in the accuracy of the plating.

Table 1: Effects of VC10 Chromosomal DNA on the Transformation of *E. coli* HB101 with p328.5 Plasmid

Transformation Condition	Observed Colony Numbers at Different Amounts of Plasmid (ug)*				
	0	4	5	8	10
Plasmid alone	0	TNTC	573	1372	1980
Plasmid and 10kb DNA	0	1076	NT	1512	NT
Plasmid and 20kb DNA	0	NT	1604	NT	1140
Plasmid and 30kb DNA	0	NT	780	NT	344
Plasmid and undigested DNA	0	NT	31	NT	24

* all numbers are averages of estimated duplicate plate counts

TNTC: too numerous to count

NT: not tested

Table 2 shows the number of transformants per viable cell (viable cell count number not shown). Consistent with data in table 1, the plasmid alone, plasmid with 10kb and plasmid with 20kb conditions showed similar transformation efficiencies. The plasmid with 30kb and plasmid with undigested DNA both caused significant decreases in efficiency relative to the other three conditions

Table 2: Number of Transformants per Viable Cell

Transformation Condition	Transformants per Viable Cell at Different Amounts of Plasmid (ug)				
	0	4	5	8	10
Plasmid alone	0	*	1.44E-04	3.44E-04	4.96E-04
Plasmid and 10kb DNA	0	2.70E-04	NT	3.79E-04	NT
Plasmid and 20kb DNA	0	NT	4.02E-04	NT	2.86E-04
Plasmid and 30kb DNA	0	NT	1.95E-04	NT	8.61E-05
Plasmid and undigested DNA	0	NT	7.64E-04	NT	5.89E-06

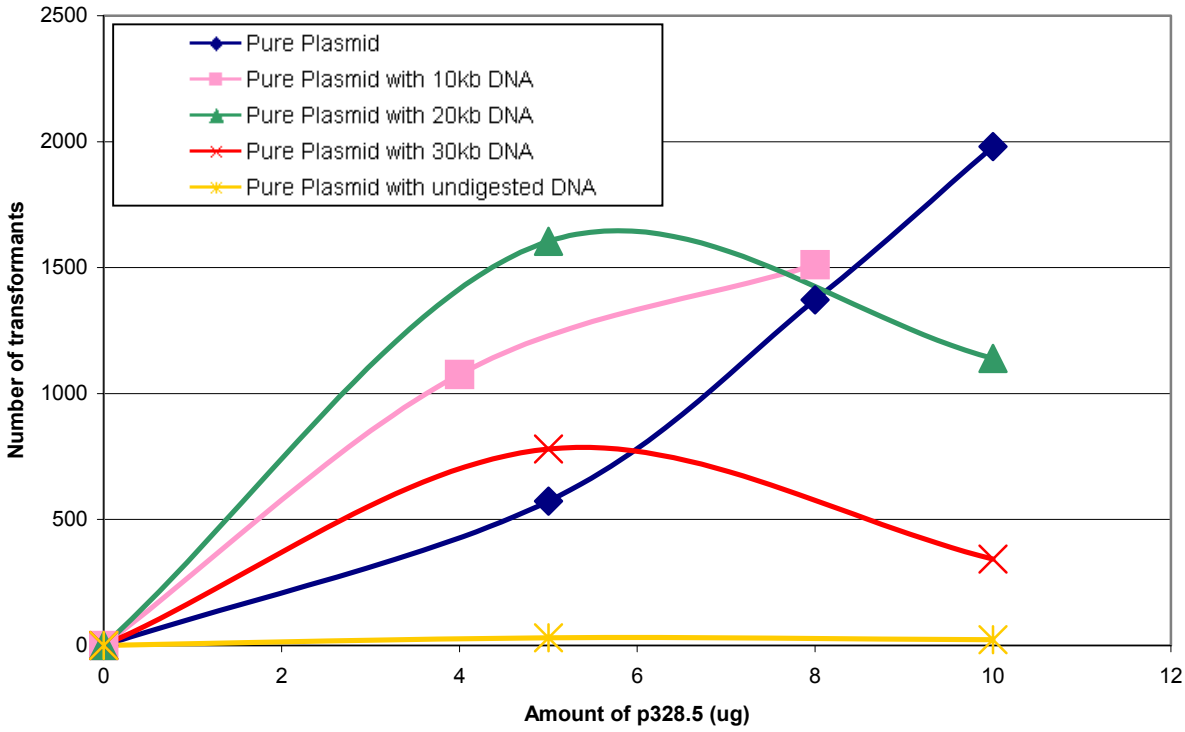
* colony count is TNTC therefore not calculated

NT: not tested

Figure 1 is a graph of the data represented in table 1. The graph shows the plasmid alone and plasmid with 10kb showed similar trends and the plasmid with 20kb and plasmid with 30kb showed similar trends. The plasmid alone and plasmid with 10kb conditions exhibited increasing numbers of transformants with increasing amounts of plasmid and DNA. The plasmid with 20kb and plasmid with 30kb conditions exhibited increasing numbers of transformants with increasing amounts of plasmid and DNA but then peaked and decreased. However, the amount of plasmid at which the peak occurred cannot be determined because the 0 to 5ug range and the 5 to 10ug range of

plasmid were not tested. Since the number of transformants obtained in all condtions are estimates and may be erroneous due to plating errors, the increases shown in the graph for the plasmid alone, plasmid with 10kb and plasmid with 20kb condtions should be ignored. For the plasmid with undigested DNA condition, the number of transformants remained low and did not change significantly when varying amounts of plasmid and DNA were used.

Figure 1: Number of *E. coli* HB101 Transformants with Plasmid p328.5 in varying amounts of VC10 Chromosomal DNA



DISCUSSION

Figure 1 shows that when E. coli HB101 is transformed with plasmid p328.5 alone, the number of transformants increased as the amount of plasmid increased. However, in Table 1 the TNTC of the 4ug plasmid alone condition was assumed to be an error. This upward trend is followed by the plasmid and 10kb DNA condition. This implies that contaminating DNA of 10kb length does not change the fact that a higher concentration of plasmid yields a higher number of transformants. However, looking closely, the plasmid and 10kb condition does not parallel that of the plasmid alone condition. Plasmid alone exhibits a steeper trend at >5ug of plasmid; in contrast, plasmid and 10kb becomes less steep at >4ug as compared to lower amount of plasmid. The trends, if extrapolated, would intersect at around 8.5ug of plasmid. This may signify that contaminating DNA of 10kb was able to effect a decrease in transformants starting at 8.5ug of plasmid. When transforming with an amount of plasmid lower than 8.5ug, contaminating DNA not only does not decrease but seems to enhance the transformation efficiency.

Two conditions, plasmid with 20kb and with 30kb, form distinctly different trends. Again, in Figure 1, we see that contaminating DNA only seemed to decrease transformation efficiency at higher amounts of plasmid. The length of the contaminating DNA played a role. At 20kb, we again see a enhanced transformation efficiency at lower amounts of plasmid. The trend intersects with that of plasmid alone at around 8ug of plasmid. This observation confirms the above postulate that contaminating DNA was capable of decreasing transformation efficiency only at amounts of plasmid that are higher than 8ug. Below 8ug, the transformation efficiency seemed to be dependent on the length of the contaminating DNA. 10kb fragments increased transformation to a lesser extent than 20kb fragments. This enhancement may be due to presence of other cofactors in the solutions of DNA fragments. The effect depends on the amount of plasmid (<8ug) because a higher concentration of DNA was transformed with larger amounts of plasmid, and more DNA can offset the effect of these cofactors. Another reason for enhancement

may lie in the fact that these 2 fragments (10kb and 20kb) are not themselves much larger than than the transforming 4.4kb plasmid p328.5, at least not large when compared to undigested chromosome. It may be that the presence of small contaminating fragments can increase transformation efficiency, although the mechanism by which this occurs and the amount of DNA at which the effect levels off is unknown.

When 30kb fragments are taken into consideration, the picture is much harder to analyze. At 5ug, 30kb contaminating DNA resulted in 780 transformants, which was 136% that of plasmid alone, but resulted in only 63% and 49% of the total number of transformants in the 10kb and 20kb conditions, respectively. In addition, the 30kb trend intersects with the plasmid alone trend at around 6ug of plasmid — 2 units lower than was seen before. It now appears that the effect of a longer contaminating fragment causing a larger number of transformants (the effect noticed earlier) peaked somewhere between 20kb and 30kb. In other words, contaminating DNA longer than 20kb made transformation less efficient.

Undigested DNA resulted in far fewer transformants than any of the other conditions, which can probably be best explained by its obvious much larger size than the other contaminating fragments. Undigested VC10 chromosome is 4.6Mb; the 10kb to 30kb fragments range from 0.2 to 0.65% of the total chromosome size (2). Table 1 also shows that using a larger amount of plasmid did not seem to have a significant effect (decrease) on number of transformants. It can be assumed that, when contaminated with undigested DNA, transformation efficiency did not depend on the amount of plasmid at all; it remained suppressed at any plasmid concentration.

A consensus among the 3 DNA fragment conditions is that there was a decrease or at least a leveling off in the number of transformants as plasmid concentration increased — a marked difference from the consistent upward trend of the plasmid alone condition. The consensus seems to be that contaminating DNA decreased transformation efficiency to a higher extent at larger plasmid concentrations. At larger amounts of plasmid, the amount of contaminating DNA introduced into the transformation reaction also increased proportionately in a ratio of 2:1. In other words, at higher concentration of plasmid, there was an accompanying higher concentration of DNA. Perhaps, when this contaminating DNA reaches a certain concentration level, transformation efficiency gradually decreases. Competition usually comes about when contaminating DNA occupies the pores that are induced by heat shock (5). A higher concentration of DNA would occupy a larger number of pores, and hence create a decrease in number of transformants. It has been found that when transforming with contaminating DNA, competition becomes pronounced at 0.5ug of total DNA (contaminating or otherwise) per transformation of 2 to 5 x 10^8 cells (5). When contaminating DNA was in excess, competition becomes negligible when total DNA mass is restricted to less than 0.5 ug. Figure 1 shows that for 20kb and 30kb, both trends peaked and began leveling off at around 5 to 6 ug of plasmid, when DNA was at 2.5 to 3ug. Maybe 2.5 to 3ug was this “excess” amount of DNA above which competition became pronounced.

With undigested DNA, however, the length of the chromosome was so pronounced that its competition against plasmid transformation was no longer dependent on its concentration. This phenomenon tentatively proves that the larger the size of contaminating DNA, the lower the transformation efficiency, although it is contrary to our initial hypothesis. In fact, Table 1 shows very clearly that transformant numbers decrease down the 5ug and 10ug columns from 20kb to undigested. We may see a more convincing trend if different plasmid amounts and larger DNA fragments was tested.

Figure 1 shows a puzzling inexplicable observation, namely the 3 contaminating fragments all seemed to enhance transformation efficiency at low concentrations of plasmid. Even though the concentration of contaminating DNA in these transformation reactions may have been too low to compete with the plasmid, it still cannot fully explain why DNA had the ability to increase transformation. The 3 fragment conditions share several commonalities that distinguished them from the other 2 conditions (plasmid alone and with undigested DNA). They were all generated by a restriction digest, followed by electrophoresis, and eluted using a relatively inexact technique (by eye-balling the ladder only) that resulted in approximate size fragments. They were all of different genetic sequences and all relatively unpure DNA (as evidenced by their low A_{280} to A_{260} absorbance ratios). Thus, the effect of transformation enhancement was confounded by these variables — heterologous sizes, non-identical sequence, alcohol and ion contaminants — that may have been the reason behind this increased efficiency. It has been proven that pores have no sequence specificity for DNA of different sources, therefore the different sequences of DNA was unlikely to have affected plasmid transformation (6). However, it remains to be seen if other chemicals within the transformation reaction could have increased its efficiency. For example, combinations of Mn^{2+}, Ca^{2+}, Rb^{2+} and other ions have been found to be beneficial to plasmid transformation into E. coli MM294 strain; the strain transforms more readily with these ion supplements than in the standard Ca^{2+} condition (6). Perhaps such similar cofactors were present in our 3 DNA fragment transformation conditions. The effect of these cofactors leveled off at higher plasmid and DNA concentrations (around 5ug of plasmid for the 10kb and 20kb conditions), indicating that the beneficial effect could be competed by adding more contaminating DNA.

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REFERENCES

1. **Ausubel, F. M.** 1999. Short Protocols in molecular biology: a compendium of methods from current protocols in molecular biology (4th Ed.). New York: Wiley.
2. **Blattner, F. R., et al.** 1997. The complete gene sequence of *Escherichia coli* K-12. Science. **277:**1453-1474.
3. **Dill, B.** 2000. Microbiology 201 & 202 Laboratory Manual. Pages 51 – 54. University of British Columbia, Vancouver, BC.
4. **Dill, B.** 1998. Microbiology 322: Microbiological Techniques I Laboratory Manual. Pages 83 – 84. University of British Columbia, Vancouver, BC.
5. **Hanahan, D., J. Jessee, F. R. Bloom.** 1991. Plasmid transformation of *Escherichia coli* and other bacteria. Methods Enzymol. **204:**63-113.
6. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557-580.
7. **Miller, J. H.** 1992. A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for *Escherichia coli* and Related Bacteria. Page 2.49. Cold Spring Harbor Laboratory Press.
8. **Ramey, W. D.** 2002. Microbiology 421: Laboratory of Experimental Microbiology (B6a). University of British Columbia, Vancouver, BC.
9. **Sheng, Y.** 1995. Transformation of *Escherichia coli* with large DNA molecules by electroporation. Nucleic Acids Research. **23:**1990-1996

APPENDIX

1. Sample calculation of DNA concentration from spectroscopy measurements (for the 20kb chromosomal DNA fragment):

Absorbance (at wavelength)	1 in 25 dilution	1 in 50 dilution
260	0.098	0.040
270	0.095	0.041
280	0.065	0.036

Since the ratio of absorbance at 260nm to absorbance at 280nm is less than 2, the following empirical formula was used:

$$C \text{ (in ug/ml)} = 63 \times A_{260} - 36 \times A_{280}$$

1/25 dilution: $63 \times 0.098 - 36 \times 0.065 =$ **3.834**
$3.834 \times 25 = 95.85$
1/50 dilution: $63 \times 0.040 - 36 \times 0.036 =$ **1.224**
$1.224 \times 50 = 61.20$

Therefore, the concentration 20kb chromosomal fragment is: $\frac{(95.85 + 61.20)}{2}$ = **78.525 ug/ml**

2. Sample calculation of transformation efficiency (number of transformants per viable cell) using viable cell count (for the 5ug plasmid alone condition):

Dilution	Number of colonies	Dilution factor	Viable cell count
10^{-4}	206	10^{4}	2.06E06
10^{-5}	19	10^{5}	1.90E06
10^{-6}	2	10^{6}	2.00E06
10^{-7}	1	10^{7}	1.00E07

Viable cell count (average of all 4 plates) in 0.1ml of culture is:

$$\frac{2.06E06 + 1.90E06 + 2.00E06 + 1.00E07}{4} = \textbf{3.99E06}$$

Therefore, the transformation efficiency of the 5ug plasmid alone condition is:

$$\frac{575 \text{ colonies}}{3.99E06} = \textbf{1.44E-04} \text{ transformants per viable cell}$$