Effect of Vector Density and Competence on Macromolecular Vector Transformation Efficiency

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Abstract To research the effects of vector quantity and competence on the positive cloning rate, with a known gene sequence but in the absence of DNA template, we artificially designed 26 primers to synthesize a target gene of 835 bp in vitro using overlapping PCR technique. The whole experiment design with two factors and six levels (36 combinations) was applied to study the effects of the vector density and competent cells on the macromolecular vector transformation efficiency. Based on the 1 500 ng target gene, the vector density grades were designed (50, 100, 150, 200, 250, 300 ng), and then the recombinant plasmids were transformed into Top10F', DH5, Stbl3, Epi400, JM108, SCSI. Results showed that the positive cloning rates of different vector amount from big to small were in the order of 200,250,300,150,100 and 50 ng. The maximum positive cloning rate of 200 ng reached 75%; and the average value was 28. 5%. The positive cloning rates of different competent cells from big to small were in the order of stbl3, Top10F', DH5, JM108, Epi400 and SCSI. Stbl3 was higher than other competent cells under any vector density, and its average positive cloning rate was 42.4%. Both the vector density and competent cells had significant effects on the macromolecular vector transformation efficiency. The optimal combination was C4 with 200 ng vector density and Stbl3, the positive cloning rate of which could reach 75%.

Key words Macromolecular vector, Vector density, Competence, Transformation efficiency

1 Introduction

The plasmid vector is the small DNA molecule having autonomous replication ability in cells, and the most common recombinant DNA carrier in genetic engineering. It can use the recombinant DNA technology to transport the purposefully strong DNA fragments into recipient cells for propagation and expression[1-7]. The DNA length of plasmid vector varies from a few thousand base pairs to hundreds of thousands of base pairs. Compared with the small molecular weight vector, the macromolecular vector has low extracellular recombination and connection efficiency, low transformation efficiency in competence, and low replication rate in the host cells, so it is easy to produce random mutation and deletion of target DNA and unstable expression in the passage[8-10]. Therefore, it needs an optimized recombination and connection system and an optimized competence transformation system, so as to improve the recombination and transformation efficiency of macromolecular vector[11-13]. In this study, the PCR (Polymerase Chain Reaction) technology was used to design a 835bp target gene synthesized from 26 primers, and this gene was connected with about 20 kb macromolecular vector pCMV5-Cla1-RyR1_WT to form the recombinant plasmid. On the basis of 1500 ng target gene substrate, the recombinant reaction of 6-gradient vector amount (50 ng, 100 ng, 150 ng, 200 ng, 250 ng and 300 ng) was carried out, and the recombinant reaction products obtained were transformed into 6 different kinds of competence (Top10F', DH5α, Stbl3, Epi400, JM108, SCSI) to form 36 different combinations. Then the rate of positive clones from the 36 different combinations was analyzed, respectively, in order to get the optimal vector amount and best competence in the recombinant cloning of macromolecular vector.

2 Materials and methods

2.1 Materials

2.1.1 Target gene. In this study, we selected one 835 bp target gene (Fig. 1). The overall GC content was 56.17%, it was not the gene with high GC content, and there was slight fluctuation in the GC content. Overall, it was easy to be amplified (Fig. 2). The target gene had no significant direct, inverted repeat, or autoreduplication, without difficulty in primer design.

GGGGCTGCTGACCTGGCTCATGTCCATCGATGTCAAGTACCAGATCTGGAAGTTCGGGGTCATCTTCACGGACAACT
CGTTCCTGTACCTGGGCTGGTACATGGTGATGTCCCTCCTGGGCCACTACAACAACTTCTTCTTTGCCGCCCACCTG
CTGGACATCGCCATGGGGGTCAAGACGCTGCGTACCATCCTCTCCTCTGTCACCCACAATGGGAAACAGCTGGTGAT
GACTGTGGGCCTCCTGGCCGTCGTGGTCTACCTGTACACTGTGGTGGCCTTCAACTTCTTCCGCAAGTTCTACAACA
AGAGCGAGGACGAGGACGAGCCGGACATGAAGTGCGATGACATGATGACGTGCTACCTGTTCCACATGTACGTGG
GCGTCCGGGCTGGCGGAGGCATCGGGGACGAGATCGAGGACCCAGCGGGCGATGAATACGAGCTCTACCGGGTGG
TCTTCGACATCACCTTCTTCTTCTTCGTCATTGTCATCCTGCTGGCCATCATCCAGGGTCTGATTATCGCCGCCTTCG
GCGAGCTCCGAGACCAGCAGGAGCAAGTGAAGGAAGATATGGAGACCAAATGCTTCATCTGCGGGATTGGCAGTG
ACTACTTCGATACCACGCCGCACGGCTTCGAGACCCACACGCTAGAGGAGCACAATCTGGCCAATTACATGTTCTTC
TTGATGTATCTGATAAACAAGGACGAGACGGAGCACACGGGCCAGGAGTCCTACGTCTGGAAGATGTATCAGGAGA
GGTGCTGGGACTTCTTCCCCGCCGGCGACTGCTTCCGCAAGCAGTACGAGGACCAGCTGAGCTGAGAAGCTTGCAT
GCCTGCAGGTCGACTCTAGAGGATCCCGGGTGGCATCCCTGTGAC

Fig. 1 Complete sequence of target gene

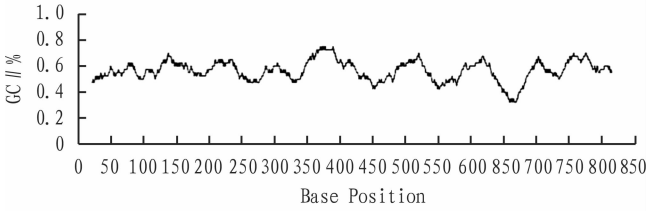

Fig. 2 GC content of target gene

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2.1.2 Target vector. Target vector was an artificially transformed Pcmv 5 carrier, and AMP resistance was added manually. The total length of vector was 19043 bp, and it was macromolecular plasmid vector (Fig. 3).

2.2 Test methods

2.2.1 Synthesis of target gene. According to gene sequences, the related companies were commissioned to design and synthesize 26 primers required by PCR amplification, and the primers were diluted to 20 pmol/uL. 2 uL of solution was taken from each primer and evenly mixed as mixed primer solution, and the target gene was synthesized through two rounds of PCR (Table 1).

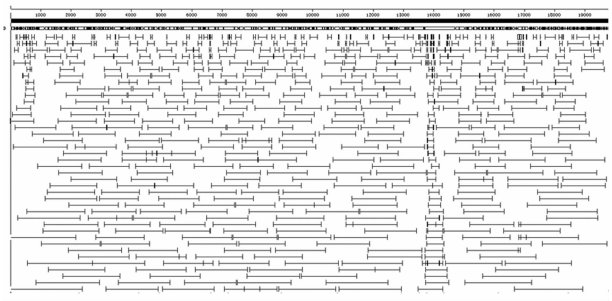


Fig. 3 Vector structure

Table 1 Primer sequence

Serial number	Sequence (5' to 3')	Base number
1	GGGGCTGCTGACCTGGCTCATGTCCATCGATGTCAAGTACCAGATCTGGAAG	52
2	GAACGAGTTGTCCGTGAAGATGACCCCGAACTTCCAGATCTGGTACTTGACATC	54
3	TCTTCACGGACAACTCGTTCCTGTACCTGGGCTGGTACATGGTGATGTCC	50
4	CGGCAAAGAAGAAGTTGTTGTAGTGGCCCAGGAGGGACATCACCATGTACCAGC	54
5	CAACAACTTCTTCTTTGCCGCCCACCTGCTGGACATCGCCATGGGGGTCAAGA	53
6	TTGTGGGTGACAGAGGAGAGGATGGTACGCAGCGTCTTGACCCCCATGGC	50
7	CTCTCCTCTGTCACCCACAATGGGAAACAGCTGGTGATGACTGTGGGCCTCCT	53
8	AAGGCCACCACAGTGTACAGGTAGACCACGACGGCCAGGAGGCCCACAGTCATC	54
9	CTGTACACTGTGGTGGCCTTCAACTTCTTCCGCAAGTTCTACAACAAGAGCGA	53
10	GCACTTCATGTCCGGCTCGTCCTCGTCCTCGCTCTTGTTGTAGAACTTGC	50
11	AGCCGGACATGAAGTGCGATGACATGATGACGTGCTACCTGTTCCACATGTACGT	55
12	TCGTCCCCGATGCCTCCGCCAGCCCGGACGCCCACGTACATGTGGAACAGGTAGC	55
13	GGCATCGGGGACGAGATCGAGGACCCAGCGGGCGATGAATACGAGCTCTAC	51
14	AAGAAGAAGGTGATGTCGAAGACCACCCGGTAGAGCTCGTATTCATCGCC	50
15	GTCTTCGACATCACCTTCTTCTTCTTCGTCATTGTCATCCTGCTGGCCATC	51
16	GGAGCTCGCCGAAGGCGGCGATAATCAGACCCTGGATGATGGCCAGCAGGATGA	54
17	CCTTCGGCGAGCTCCGAGACCAGCAGGAGCAAGTGAAGGAAGATATGGAG	50
18	CTGCCAATCCCGCAGATGAAGCATTTGGTCTCCATATCTTCCTTCACTTGCT	52
19	CTGCGGGATTGGCAGTGACTACTTCGATACCACGCCGCACGGCTTCGAGACCC	53
20	CATGTAATTGGCCAGATTGTGCTCCTCTAGCGTGTGGGTCTCGAAGCCGTG	51
21	CACAATCTGGCCAATTACATGTTCTTCTTGATGTATCTGATAAACAAGGAC	51
22	ACTCCTGGCCCGTGTGCTCCGTCTCGTCCTTGTTTATCAGATACATCAAGAA	52
23	CACACGGGCCAGGAGTCCTACGTCTGGAAGATGTATCAGGAGAGGTGCTGGG	52
24	ACTGCTTGCGGAAGCAGTCGCCGGCGGGGAAGAAGTCCCAGCACCTCTCCTGATA	55
25	CTGCTTCCGCAAGCAGTACGAGGACCAGCTGAGCTGAGAAGCTTGCATGCCTGCA	55
26	GTCACAGGGATGCCACCCGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTT	55

2.2.2 Setting of different amount of vector. 10 μL of recombinase was put into 36 PCR tubes, respectively, and these tubes were divided into 6 groups (A, B, C, D, E, F). 6 tubes in each group were numbered A1-A6, B1-B6, C1-C6, D1-D6, E1-E6 and F1-F6, respectively, and different amount of vector was added according to Table 2 (with group A as an example). Using PCR instrument, they reacted at 50℃ and were preserved at −20℃.

Table 2 Ligation system

No.	1	2	3	4	5	6
Recombinase	10	10	10	10	10	10
DNA001(1500ng/)	1	1	1	1	1	1
Vector(50ng/)	1	2	3	4	5	6
ddH_2O	8	7	6	5	4	3

2.2.3 Setting of different competence. As shown in Table 3, different competence was set.

2.2.4 Bacteria detection result judgment. There were 36 plates, and 24 plaques were picked from each plate for electrophoresis. There were too many electrophoresis samples, and the result did not directly display electrophoretogram. And the number of positive clones from every 24 clones was counted and divided by 24 to get the rate of positive clones. The positive clones of bacteria de-

tection product were judged as follows: electrophoretic bands were matched with ladder; 1149 bp meant that the positive clones were denoted as 1; 412 bp meant that the empty vector was denoted as 0; the primer dimer meant that the variegated bacteria sample was denoted as 0.

Table 3 Transformation combination

Top10F'	DH5α	Stbl3	Epi400	JM108	SCSI
A1	B1	C1	D1	E1	F1
A2	B2	C2	D2	E2	F2
A3	B3	C3	D3	E3	F3
A4	B4	C4	D4	E4	F4
A5	B5	C5	D5	E5	F5
A6	B6	C6	D6	E6	F6

3 Results

3.1 Synthesis of target gene As shown in Fig. 4, PCR products of the target gene were 750 – 1000 bp, consistent with the theoretical value of 855 bp.

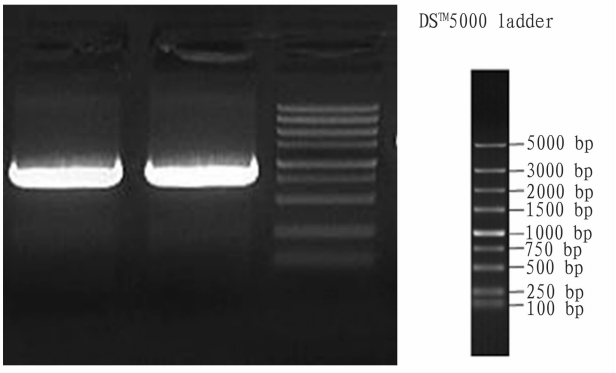
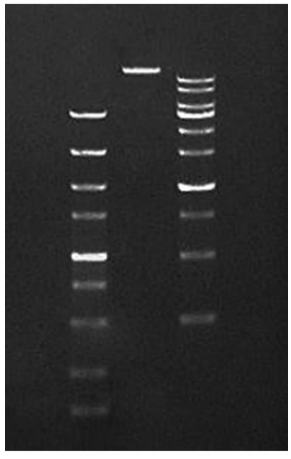


Fig. 4 Electrophoretogram of PCR products

3.2 Linearization of vector As shown in Fig. 5, the theoretical size after enzyme digestion was 19043 bp and 98 bp, and the minimum value of 5000 Ladder was 100 bp, so 98 bp could not be displayed in the figure. The enzyme digestion result was consistent with the theoretical value.



Note: Band 1, 2, 3 represented 5000 bp Ladder, enzyme digestion sample and 10 kb Ladder, respectively.

Fig. 5 Electrophoretogram of vector enzyme digestion

3.3 Growth of transformed colonies As shown in Fig. 6, in 6 kinds of competence, there were a total of 36 plates (A_1-F_6), and the colonies with vector amount of 50, 100, 150, 200, 250 and 300 ng were added, respectively. It was clearly found that the colonies on the 36 plates grew well.

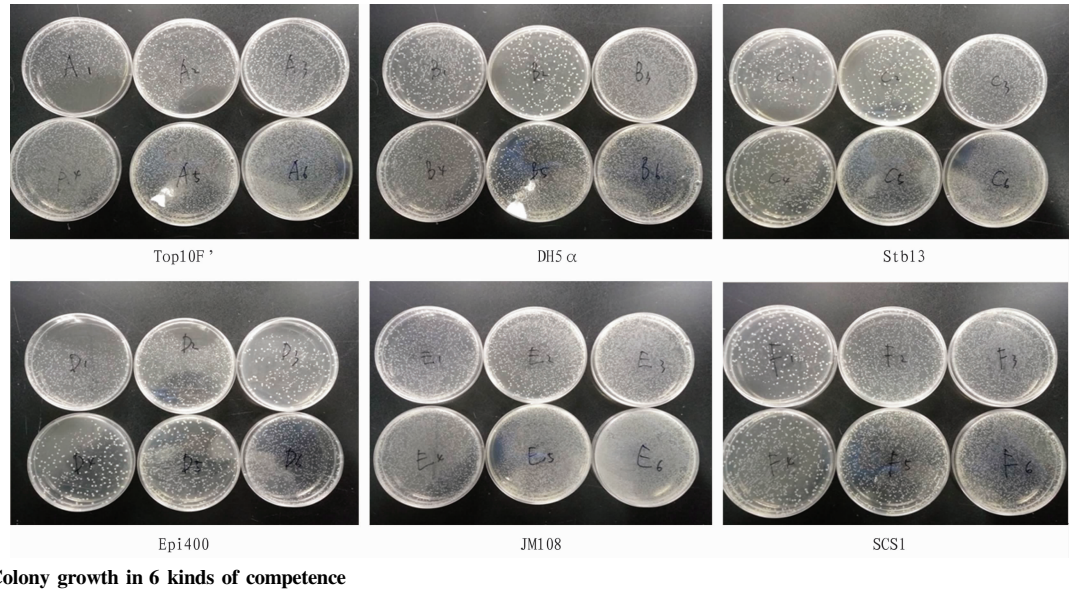


Fig. 6 Colony growth in 6 kinds of competence

3.4 Rate of positive clones From Table 4 and Fig. 7, it was found that different vector density had a significant impact on the rate of positive clones of macromolecular vectors, and in terms of the average rate of positive clones, 200 ng > 250 ng > 300 ng > 150 ng > 100 ng > 50 ng. In all kinds of competence, the rate of positive clones was highest at 200 ng, the highest rate was up to 75%, and the average rate reached 28.5%. If the vector density was less than 100 ng, the transformation efficiency was less than 10%. Different competence had a significant effect on the rate of positive clones of macromolecular vector, and the average rate of positive clones was in the order of stbl3 > Top10F' > DH5α > Jm108 > Epi400 > Scsl. The best competence was stbl3, and its rate of positive clones was higher than that of other kinds of competence under any vector density, and the average rate of positive clones was 42.4%. The average rate of positive clones was below 10% for Jm108, Epi400 and Scsl.

Table 4 The rate of positive clones Unit: %

Concen tration // ng	A(Top10F')	B(DH5α)	C(Stbl3)	D(Epi400)	E(JM108)	F(SCSI)	Average
50	0.0	0.0	4.2	0.0	0.0	0.0	0.7
100	4.2	4.2	12.5	0.0	4.2	0.0	4.2
150	8.3	12.5	41.7	4.2	8.3	4.2	13.2
200	33.3	29.2	75.0	8.3	16.7	8.3	28.5
250	29.2	20.8	66.7	4.2	8.3	4.2	22.2
300	20.8	12.5	58.3	0.0	4.2	4.2	16.7
Average	16.0	13.2	42.4	2.8	6.9	3.5	–

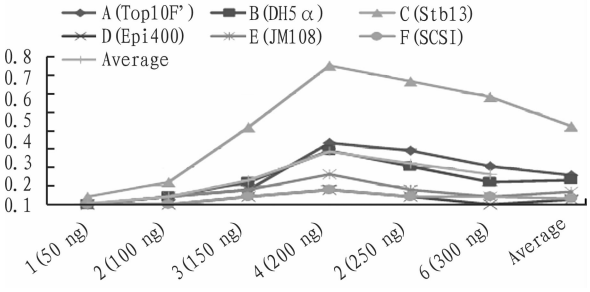


Fig. 7 The rate of positive clones

4 Conclusions and discussions

In this study, for the 20 kb macromolecular vector, on the basis of 1500 ng target gene substrate, we set the vector amount with six gradients (50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 300 ng), and the recombinant reaction products obtained were transformed into six different kinds of competence (Top10F', DH5α, Stbl3, Epi400, JM108, SCSI) to form 36 test combinations. The results showed that both the vector amount and competence significantly affected the rate of positive clones. When the vector amount in the reaction system was too low, the recombinant reaction could not get enough positive clones, and the screening efficiency was very low. When the vector amount in the reaction system was too high, many empty vectors might be selected, affecting the rate of positive clones. For the 20 kb vector, when the vector molecular weight was about 200 ng, the rate of positive clones was highest. Compared with other kinds of competence, Stbl3 had a greater ability to copy large vectors. The optimal combination was 200 ng vector density and Stbl3, and the rate of positive clones could reach 75%. For the common recombinant test, the vector sample amount was far less than 200 ng, and taking the common puc57 series vector for example, the vector sample amount for recombination reaction was only about 30 ng[1, 3, 4]. When there was a large vector in the laboratory, the rate of positive clones might be low and even 0. Due to different genotypes for different competence, there were large differences in copying rate of different sizes and different types of plasmid[14-18]. In this study, the vector used was close to 20 kb, and it was difficult to clone compared with the commonly used 3 – 4 kb vector. The macromolecular vector had features of low recombination efficiency, low transformation efficiency, low copying rate, unstable expression, difficult extraction, separation and purification[8-10], so many domestic DNA companies were not willing to accept cloning of macromolecular vector, and even if there are corresponding services, the service price was high due to high failure rate and experimentation cost. In this study, with 20kb vector as example, we selected the vector amount with six gradients and six kinds of competence, indicating that the vector amount and competence indeed had a significant impact on the rate of positive clones. We obtained the optimal combination which can be used as reference for improving the cloning efficiency of macromolecular vector.

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cause behind the big data, there is the privacy of the group information. The cloud computing concerns the network security, and the standard for implementation of the platform economy is the high degree of customer information security. Mobile Internet also imposes higher requirements on the network carrier, of which some parts need establishing support system of intellectual property right, to guarantee the security of operating environment. On the other hand, as maker groups, their innovative and creative inspiration has characteristic of originality. At present, there is serious problem of plagiarism at current domestic market, which poses a great challenge for protection of intellectual property rights. Therefore, at the time of building "Maker" urban culture in Shanghai, it is required to firstly make innovation on the original systems, in other words, the institution should go first.

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