鸡传染性喉气管炎病毒
gB

gB

1
2
3
4

ILTV

PCR

pY-α-gB

hsp70

pR83

prR-α-gB

M. smegmatis mc²

ELISA

M. smegmatis mc²

pR-α-gB

Western blot

1

EID₉₀

ILTV

SPF

1

Bacillus calmette-guerrini

BCG

HeBO₁

Escherichia coli

JMI109

pY-α-gB

M. smegmatis mc²

155

ILTV

IL-16

ILTV

Plasmid P2

Nhe I

EcoRI

PI

5′-CGGCTAGCATGGCTAGCCTTGAGAAATGC-3′

5′-TGATCTTTGACGAAAGTACGCTTGC-3′

P1

1.2mmol/L

1μL

4nmol/L

10×Buffer

5μL

dNTP

25mmol/L

LA

3U

1μL

ddH₂O

0.37μL

min

1 min

94℃

2 min

30℃

72℃

10 min

pR-α-gB

ILTV

gB

pY-α-gB

pY-α-gB

pY-α-gB

pY-α-gB

pY-α-gB

pY-α-gB

pY-α-gB

pY-α-gB

pY-α-gB

pY-α-gB

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pY-α-gB

pY-α-gB

pY-α-gB
2.3 pY-α-gB pY-α-gB gB Xba 1 Hinc II pY-α α-gB EcoR I gB 1.5 kbp
2.4 hsp-α-gB hsp-α-gB gB pY-α-gB pY-α-gB gB pY-α-gB gB

Fig. 1 Electrophoresis of PCR products of hsp-α-gB gene
1. hsp-α-gB gene 2.9 kb 2. λ-Eco T14 I digest marker.

2.5 ELISA M. smegmatis mc² 15S pR-α-gB pR-α-gB pR-α-gB pR-α-gB

Fig. 2 Analysis of expressed products with Western blot
1. Low molecular weight protein markers 2. Control of pR3 with SDS-PAGE
3. gB harboring pR-α-gB with SDS-PAGE
4. gB harboring pR-α-gB with Western blot
5. gB harboring pR-α-gB with Western blot
6. Western blot with gB
7. Western blot with pR3
8. Western blot with pR-α-gB

2.1 pY-a pY-a EcoR I Nco I 150 bp
2.2 ILTV gB PCR

Western blot

1. Hsp-α-gB 2.9 kb 2. λ-Eco T14 I digest marker.

1.1 ELISA M. smegmatis mc² 15S pR-α-gB pR-α-gB

Western blot

1. Hsp-α-gB 2.9 kb 2. λ-Eco T14 I digest marker.

2.5 ELISA M. smegmatis mc² 15S pR-α-gB

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Western blot

1. Hsp-α-gB 2.9 kb 2. λ-Eco T14 I digest marker.
2.11 分枝杆菌穿梭表达 M. smegmatis mc^2 155 pR-α-gB

Table 1  The neutralization of SPF chick embryo assay for the recombinant bacteria strain

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of chick embryos</th>
<th>Observational time /d</th>
<th>Attacked embryos number/ survived embryos number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera of vaccination with active vaccine</td>
<td>10</td>
<td>10</td>
<td>0/10</td>
</tr>
<tr>
<td>Sera of vaccination with normal saline</td>
<td>10</td>
<td>10</td>
<td>10/10</td>
</tr>
<tr>
<td>Sera of vaccination with recombinant bacteria strain</td>
<td>10</td>
<td>10</td>
<td>1/10</td>
</tr>
</tbody>
</table>

3


Cloning and Expression of gB Gene of Infectious Laryngotraceatitis Virus in *M. Smegmatis*

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Abstract Firstly, the complete gB gene of a domestic isolation strain were amplified by PCR and the 2.6 kb gene fragment was obtained. Then the recombinant plasmid pY-α-gB was constructed by cloning PCR product into pY-α vector and the shuttle expression plasmid pR-α-gB was constructed by cloning the hsp-α-gB gene into the downstream sequences of pR3 vector. The recombinant plasmid was identified by restriction enzyme digestion and the sequence analysis which was electrophoreted into *M. smegmatis mc² 155*. At last, the expressed gB proteins were successfully detected by ELISA and Western blot which seems to be immunogenic crucially. The recombinant bacterial strain *M. smegmatis mc² 155* pR-α-gB could protect SPF chick embryo from one lethal dose of ILTV.

Key words Polymerase chain reaction Cloning Expression The shuttle expression vector

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