E2 cDNA

PCR GBV-C/HCV E2 cDNA 559bp pGEX-E2 1324bp
DNA pPIC9K pPIC9K-GST-E2 Pichia pastoris GS115 His+ Mut' 0.5% 5% 50% GST-E2 10% 95%

Q78 A 1000-3064 2002-02-0187-06

RNA GBV-C/HCV 9.1 9.4kbp
HCV 2900 E2 NS1-NS5
E2 11-10

GBV-C/HCV HGV E2 cDNA HIV
HGV E2 four 45
HIV 50 E2 HIV E2

HGV E2 cDNA pThioHisA-E2 pGEX-5X-1-E2 E2cDNA pThioHisA-E2 Bgl II-Sma I pGEX-5X-1

1 Pichia pastoris

1.1 DH5α supE44 ΔlacU169 F80 lacZ Δ M15 hsdr17 recA1 endA1 gyrA96 thi-1 relA1ompC LB21 DE3 hsdS37 λc1ts1537
Sam7 nin5 lacV5-17 11580 confiscating
HGV E2 cDNA pThioHisA-E2 E2cDNA pThioHisA-E2 Bgl II-Sma I pGEX-5X-1

Pichia pastoris GS115 his4 Invitrogen

庚型肝炎病毒区在毕赤酵母中的表达及抗原性鉴定

王卓华 叶 凯 徐 洪 马辉文

利用ABC技术，从含有庚型肝炎病毒(DE&0BFGD&9#)包膜蛋白(JJ8KL)的质粒(LD9M09#)中，扩增得
到能够编码日本血吸虫谷胱甘肽硫转移酶(DN2)和DE&0BFGD&9#包膜蛋白(JJ8KL)的融合基因片段。将此片段插入到酵母表达载体LAOB8P中，使之位于α因子信号肽下游，且与之同框。通过电激转化将构建的重组表达质粒LAOB8P0DN209#插入到DN!!J菌株染色体中。筛选G;Q表型的转化子，震荡培养，用$TJU甲醇诱导表达J?后，在培养液中得到表达的DN209#融合蛋白。经过表达条件的优化，DN209#蛋白可占培养液中总蛋白的J$U。通过谷胱甘肽亲和层析柱纯化，DN209#融合蛋白的纯度可达8JU左右。以庚型肝炎病人血清为探针，进行免疫印迹及9VONI实验，结果表明该融合蛋白具有能被庚型肝炎病人血清特异性识别的抗原性。
酵母菌培养基的固体培养基:甲醇液体培养基。置

1.7

1.8

1.9

1.10

1.11

2

2.1
**Fig. 1** Construction of recombinant plasmid pPIC9K-GST-E2

**Fig. 2** Restriction map of plasmids pPIC9K-E2

1. λ/HindIII molecular marker
2. pPIC9K-GST-E2 digested with PstI [6960bp + 2949bp + 6931bp]
3. pPIC9K digested with PstI [2949bp + 6327bp]
4. pPIC9K-GST-E2 digested with BglII [8199bp + 2403bp]
5. pPIC9K digested with BglII [2403bp + 6873bp]
6. pPIC9K-GST-E2 digested with SalI [10602bp]
7. pPIC9K digested with SalI [9271bp]
8. GST-E2 gene fragment amplified with PCR [1324bp]

**2.2 GST-E2**

*P. pastoris* GS115

![GST-E2](image)

**P. pastoris** GS115/pPIC9K-GST-E2

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<td>14.4</td>
<td>20.1</td>
<td>31.0</td>
<td>43.0</td>
<td>66.2</td>
<td>97.4</td>
<td>123</td>
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**Fig. 3** SDS-PAGE analysis of the expression products

1. Molecular weight standard
2. Induced culture supernatant of *Pichia pastoris* GS115/pPIC9K-GST-E2
3. - 9. Induced supernatant of *Pichia pastoris* GS115/pPIC9K-GST-E2 induced for 4 days

**Fig. 4** GST-E2 fusion proteins purified with GST-E2

|  |  |  |  |  |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| 97.4 | 66.2 | 31.0 | 14.4 |

**Fig. 5** SDS-PAGE analysis of the expression products

1. Molecular weight standard
2. Culture supernatant of methanol induced *Pichia pastoris* GS115/pPIC9K-GST-E2
3. GST-E2 fusion protein purified from the culture supernatant of methanol induced *Pichia pastoris* GS115/pPIC9K-GST-E2
4. GST-E2 fusion protein purified from the cell lysate of lactose induced *E. coli* BL2 DE3/pGEX-5X-1-E2

**Table 1**

|  |  |  |  |  |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| 97.4 | 66.2 | 31.0 | 14.4 |

2.3 GST-E2**

*P. pastoris* His+
2.4 E2

Western blot analysis of the expression products

2.5

Table 1 ELISA of HGV E2 antigens probed with GBV-C/HGV-RNA positive and healthy human sera

<table>
<thead>
<tr>
<th>GST-E2a</th>
<th>Nb</th>
<th>P</th>
<th>P/Nb</th>
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<tbody>
<tr>
<td>Expressed in E. coli</td>
<td>0.018</td>
<td>0.022</td>
<td>1.222</td>
</tr>
<tr>
<td>Expressed in P. pastoris</td>
<td>0.020</td>
<td>0.123</td>
<td>6.150</td>
</tr>
</tbody>
</table>

a GST-E2 fusion protein expressed either by E. coli Bl2[D2]pGEX-5X-1-E2 or by Pichia pastoris/pPIC9K-GST-E2
b OD490 of negative control well probed with healthy human sera. OD490 of positive well probed with sera from the patients infected by GBV-C/HGV

2.6 E2

HGV E2 ELISA

3

HGV
菌株在没有任何选择压力的条件下生长速度较快,而且外源基因的表达由强启动子启动,所以目的蛋白具有天然活性。与其它真核表达系统相比,生长速度较快,而且外源基因的表达由强启动子启动,所以目的蛋白具有天然活性。与其它真核表达系统相比,生长速度较快,而且外源基因的表达由强启动子启动,所以目的蛋白具有天然活性。与其它真核表达系统相比,生长速度较快,而且外源基因的表达由强启动子启动,所以目的蛋白具有天然活性。与其它真核表达系统相比,生长速度较快,而且外源基因的表达由强启动子启动,所以目的蛋白具有天然活性。与其它真核表达系统相比,生长速度较快,而且外源基因的表达由强启动子启动,所以目的蛋白具有天然活性。与其它真核表达系统相比,生长速度较快,而且外源基因的表达由强启动子启动,所以目的蛋白具有天然活性。
Expression and Characterization of Envelope Protein 2 Gene of Hepatitis G Virus in *Pichia pastoris*

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Abstract  A cDNA fragment locating at the putative envelop protein E2 region of GBV-C/HGV fused with *Schistosoma japonicum* glutathione S-transferase GST was amplified with PCR from plasmid pGEX-E2. The amplified DNA fragment was inserted into plasmid pGEX-5X-1 at the downstream of the coding sequences of GST in the same reading frame with the gene of GST. The fusion gene fragment of GST-E2 was amplified with PCR using the recombinant plasmid pGEX-5X-1-E2 as the template. The amplified 1324 bp DNA fragment of GST-E2 was inserted into *Pichia pastoris* expression vector pPIC9K in reading frame with α-secretion signal peptide. The plasmid pPIC9K-GST-E2 was transformed into *Pichia pastoris* GS115 with electroporation. The transformants were selected and induced to express the 54kD GST-E2 fusion protein which could be specifically recognized by both the antiserum directed against E2 and against GST. The GST-E2 fusion protein was purified with Sepharose 4B glutathione affinity chromatography to a purity of 95%. The expression was optimized to achieve the highest expression level of GST-E2 fusion protein which was accumulated up to 50% of total proteins in the culture supernatant. The GST-E2 protein derived from the recombinant *Pichia pastoris* was proved possessing antigenicity and high specificity by ELISA probed with sera from the patients infected by GBV-C/HGV.

Key words  GBV-C/HGV E2 protein  *Pichia pastoris* expression  antigenicity

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