**Adeosirus* virus mediated expression of first intron of *KLA* in CHO cells**

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**Abstract**

Through homologous recombination, a recombinant adenovirus was constructed with the *KLA* gene vector under the control of the *CMV* promoter, with and without an intron. The virus was used to infect *CHO* cells and the expression of the *KLA* gene was observed. It was found that the expression efficiency of the *KLA* gene vector with an intron was increased by 117% compared with the vector without an intron, indicating that the first intron of the *KLA* gene has a promoting function in gene expression in eukaryotic cells.

**Key words**

Pig growth hormone gene, first intron, adenovirus vector

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**Pig growth hormone (PGH)**

PGH, a growth hormone gene isolated from pig, is widely used in the study of animal growth hormone gene expression and regulation. This study was funded by the Natural Science Foundation of Hebei Province (A+/9602067) and the Science and Technology Commission of Shenzhen (A+/9648002). The first author is affiliated with the School of Biotechnology, Hebei Polytechnic Normal University, Cangli 066000, China. The article was received on 2002-02-01 and accepted on 2002-02-01.

**Materials and Methods**

**Materials**

**Plasmids**

- *KLA*:GV1 (recombinant adenovirus plasmid) was obtained from the plasmid collection of the Biotechnology Laboratory.
- *KHAL*:CM, *K7L*:CM plasmids were purchased from the Canadian Genentech Corporation.

**Bacteria and Cells**

- *X.41%* cells were a gift from Professor Liang Guodong, and *CHO* cells were stored in the laboratory.
- *E. coli DH5α* and *E. coli P3* were stored in the laboratory.

**Main Reagents**

- Restriction enzymes: *BamHI*, *SmaI*, *SphI*, *KpnI*, *T4* DNA Polymerase, *T4* DNA Ligase, *ClaI*, *SacI*, *EcoRI*, *BamHI*, *XhoI*, *KpnI*, *Klenow* DNA polymerase, *Lipo* (Gibco), *HPr*, *HRP*, *IgG* (Gibco), *pGH*, *pHeDNA-in*.

**Main Experimental Methods**

**Construction of *KLA*:CM Plasmid**

Using two-cut method, *pGH cDNA* was linearized at the 3' end of *KLA*:CM plasmid. After digestion with *SmaI* and *SphI*, and then with *BamHI* and *SfiI*, the linearized plasmid was used to transfect *CHO* cells. After successive selection of the antibiotic resistance, the positive clones were obtained. The recombinant plasmid was sequenced using the ABI3730XL automatic DNA sequencing system with the BigDye Terminator Cycle Sequencing Kit. The recombinant plasmid was identified using the plasmid克隆 strategy and sequenced using the ABI3730XL automatic DNA sequencing system with the BigDye Terminator Cycle Sequencing Kit.

**Construction of *KLA*:CM with First Intron Plasmid**

The *KLA*:CM plasmid was digested with *BamHI* and *SmaI* to isolate the *KLA*:CM plasmid fragment. The intron sequence was amplified using *KLA*:CM plasmid fragment and *pCMV/gfp* as template. The amplified intron fragment was digested with *BamHI* and *SfiI* and ligated to the *pCMV/gfp* plasmid. The recombinant plasmid was then used to transfect *CHO* cells. After successive selection of the antibiotic resistance, the positive clones were obtained. The recombinant plasmid was sequenced using the ABI3730XL automatic DNA sequencing system with the BigDye Terminator Cycle Sequencing Kit. The recombinant plasmid was identified using the plasmid克隆 strategy and sequenced using the ABI3730XL automatic DNA sequencing system with the BigDye Terminator Cycle Sequencing Kit.

**References**

- *HindIII*, *XbaI*, *SpeI*, *EcoRI*, *BamHI*, *XhoI*, *ClaI*, *SacI*, *KpnI*, *T4* DNA Polymerase, *T4* DNA Ligase, *ClaI*, *SacI*, *EcoRI*, *BamHI*, *XhoI*, *KpnI*, *Klenow* DNA polymerase, *Lipo* (Gibco), *HPr*, *HRP*, *IgG* (Gibco), *pGH*, *pHeDNA-in*.

**1. Introduction**

**1.1**

**1.1.1**

[pWH101]pZFCD-in[pCMV/gfp]

**1.2**

**1.2.1**

[pΔECM]pΔECMVCD]

**1.2.2**

[pΔECMVCD]pΔECMVCD-in[pCMV/gfp]
酶切结束后，在Klenow Buffer 2μL、BSA 10mg/mL、0.5μL Klenow Buffer 4 μL、1 μL 1mol/L dNTP，1μL]中，10min 75℃。10min Klenow Buffer 830bp 1040bp。酶切液中加入10μL。在室温下补平。处理使酶灭活。用酚、酚氯仿抽提，乙醇沉淀，将DNA片段溶于1/2μL中。后用EcoR I、Bgl II 酶切，用低熔点胶分别回收JK2L！和12D2L！的基因片段，电泳估计DNA片段含量。用单酶切EcoRI 质粒，以与上述相同的方法补平，后用EcoR I 酶切，用低熔点胶回收载体12，估计含量后分别与上述两个小片段进行连接，取连接液转化感受态菌。通过涂布含氨苄琼脂板，挑取单菌落，摇瓶培养细菌，提取质粒及酶切鉴定过程进行阳性克隆的筛选。

2 重组腺病毒的构建

2.1 pAEvCVD pAEvCVD-in 穿梭质粒的构建

在穿梭质粒的EcoRI 和EcoRI’位点上插入由Hind III、Bgl II双酶切得到的启动子，构建成EcoRI质粒；然后在EcoRI质粒的EcoRI启动子下分别插入EV-AM、EV-AM’，分别构建出EcoRI和EcoRI’穿梭质粒，见图1。构建的质粒经酶切鉴定证明结构正确。图1 ! EcoRI和EcoRI’穿梭质粒的构建策略

结果

2.2 pAEvCVD pAEvCVD-in穿梭质粒分别与腺病毒重组质粒I$MN$% &’(共转染(SK细胞，通过二者在SK细胞中的同源基因重组(构建策略见图？)，分别构建出含EV-AM基因的重组腺病毒$MN$%&>8-E和含EV-AM’基因的重组腺病毒$MN$%&’(&>8-E。

图K为共转染1OE后?SK细胞部分区域出现的病变现象。分别用两种重组腺病毒感染满度为J/Q以上的?SK细胞，DE后就可见到类似于图K典型的$GI现象。通过负染方法在电镜下观察两

DS1

生物工程学报

1J卷
重组腺病毒构建策略

图5 电镜下重组腺病毒颗粒的形态（左图6>？，右图??）

为鉴定是否为含@FG-CHE或含@FG-CHED重组腺病毒，又通过方法对其做进一步鉴定（从含@FG-CHE重组腺病毒CHE中应该扩增出J!?1@的片段；从含@FG-CHED重组腺病毒CHE中应该扩增出KJ?1@的片段）。首先采用G#+*方法提取两种重组腺病毒CHE，以此CHE作模板，通过设计的引物进行8&I扩增，结果在&AB&CD+/E3的CHE中扩增出J!?1@的CHE片段；在&AB&CD#(D+/E3的CHE中扩增出KJ?1@的CHE片段（见图6>）。说明本文已得到了相应的两种重组腺病毒。经过多次传代，&AB&CD+/E3重组腺病毒的滴度(L&MC)>?达N?O 7%P>，&AB&CD#(D+/E3重组腺病毒的滴度达N?O N?。

图6 从重组腺病毒CHE中扩增的特异片段

由图可见，感染后5K内，生长激素的表达量很低，从5K开始表达量急剧上升，特别是在5K X 期
期间，上升的幅度较大，到表达量达到最高。随后趋于下降。下降的原因可能是由于病毒对细胞的损伤作用及细胞后期发生裂解，使表达出的基因发生降解所致。这一结果说明由腺病毒介导基因可以在真核细胞中进行分泌性表达。

第一内含子对基因表达的影响：用\(+\)和\(-\)重组腺病毒分别感染满度为\(!56\)的\(\)孔板\(\)细胞，每种重组腺病毒同时感染\%孔。感染后换细胞专用无血清培养基，取感染后\$75\)的细胞培养液，分别测定培养液中\&'\)的含量。测定结果见表1。

<table>
<thead>
<tr>
<th>No.</th>
<th>CMVCD&amp;Ad</th>
<th>CMVCD&amp;Ad</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1166.7</td>
<td>618.0</td>
</tr>
<tr>
<td>2</td>
<td>1143.0</td>
<td>480.0</td>
</tr>
<tr>
<td>3</td>
<td>1348.2</td>
<td>512.4</td>
</tr>
<tr>
<td>4</td>
<td>968.1</td>
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<tr>
<td>5</td>
<td>1078.2</td>
<td>704.4</td>
</tr>
<tr>
<td>6</td>
<td>1209.9</td>
<td>391.2</td>
</tr>
<tr>
<td>7</td>
<td>987.6</td>
<td>523.2</td>
</tr>
<tr>
<td>8</td>
<td>1400.0</td>
<td>561.9</td>
</tr>
</tbody>
</table>

Means 1162.7 ± 155.39 536.6 ± 93.93

1 Test  \(P < 0.01\)

第1栏：\(\) CHO \(\) pGH \(\) cDNA

第2栏：\(\) CMVCD-reAd CMVCD-in-reAd CMVCD-reAd CMVCD-in-reAd

第3栏：\(\) pGH \(\) CHO \(\) pGH \(\) cDNA 536.6 ± 93.93ng/mL

1162.7 ± 155.39ng/mL  \(P < 0.01\)

171%

第4栏：\(\) pGH \(\) CHO \(\) pGH \(\) cDNA 117%

3

REFERENCES

Expression of Adenovirus-mediated pGH cDNA with First Intron in CHO Cells

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Abstract The recombinant adenoviruses containing pGH cDNA and pGH cDNA with the first intron under the control of CMV promoter were constructed respectively by homogenous recombination method. The results showed that the recombinant adenoviruses could mediate pGH cDNA expression in CHO cells infected with the recombinant adenoviruses. The expression level of pGH cDNA with the first intron increased by 117% compared with pGH cDNA without intron. This indicates that the first intron of pGH gene have the function of improving the expression of the pGH gene.

Key words porcine growth hormone, first intron, adenovirus vector

Received 18-20-2001
This work was supported by a grant from Hebei Natural Science Fund and Shenzhen Scientific Committee No. 98023.

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