pCMV-Script XR Predigested Vector

Instruction Manual

Catalog #212224
Revision C.0

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212224-12
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pCMV-Script XR Predigested Vector

**MATERIALS PROVIDED**

<table>
<thead>
<tr>
<th>Materials provided</th>
<th>Concentration</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCMV-Script XR predigested vector&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>30 ng/μl</td>
<td>55 μl (1650 ng)</td>
</tr>
<tr>
<td>XR LacZ test insert (600 bp)</td>
<td>10 ng/μl</td>
<td>3 μl (30 ng)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The pCMV-Script XR predigested vector is digested with EcoRI/XhoI and CIAP-treated.

<sup>b</sup> The complete sequence and restriction sites for the pCMV-Script vector can be found at www.genomics.agilent.com.

**STORAGE CONDITIONS**

- pCMV-Script XR Predigested Vector: –20°C
- XR LacZ Test Insert: –20°C

**ADDITIONAL MATERIALS REQUIRED**

- T4 DNA ligase
- TE buffer<sup>§</sup>
- High efficiency competent cells (≥5 × 10⁹ cfu/μg DNA)
- LB-kanamycin agar plates<sup>§</sup>
- X-gal (5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside)
- IPTG (isopropyl-β-D-thio-galactopyranoside)
- Taq DNA polymerase
- Taq DNA polymerase buffer

**NOTICE TO PURCHASER**

The use of the CMV Promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation and licensed FOR RESEARCH USE ONLY. For further information, please contact UIRF at 319-335-4546.

<sup>§</sup> See Preparation of Media and Reagents.
INTRODUCTION

The pCMV-Script XR predigested vector is derived from a high-copy-number pUC-based plasmid and is designed to allow protein expression in mammalian systems. Mammalian expression is driven by the human cytomegalovirus (CMV) immediate early promoter to promote constitutive expression of cloned inserts in a wide variety of cell lines. Selection is made possible in bacteria by the kanamycin-resistance gene under control of the prokaryotic β-lactamase promoter. The neomycin-resistance gene is driven by the SV40 early promoter, which provides stable selection with G418 in mammalian cells.1

The pCMV-Script XR vector does not contain an ATG initiation codon. A translation initiation sequence must be incorporated if the DNA fragment to be cloned does not have an initiating ATG codon or an optimal sequence for initiating translation, such as the Kozak sequence [GCC(A/G)CCATGG].2

The pCMV-Script XR vector is predigested with EcoRI and XhoI restriction enzymes. The multiple cloning site (MCS) contains unique restriction enzyme recognition sites organized with alternating 5′ and 3′ overhangs to allow serial exonuclease III/mung bean nuclease deletions. T3 and T7 RNA polymerase promoters flank the polylinker for in vitro RNA synthesis. The choice of promoter used to initiate transcription determines which strand of the DNA insert will be transcribed.

The pCMV-Script XR vector can be rescued as single-stranded (ss) DNA. The plasmid contains a 454-nucleotide filamentous f1 phage intergenic region (M13-related) that includes the 307 bp origin of replication. The orientation of the f1 origin in pCMV-Script allows the rescue of antisense ssDNA by a helper phage. This ssDNA can be used for dideoxynucleotide sequencing (Sanger method) or site-directed mutagenesis.
Figure 1  Circular map and polylinker sequence of the pCMV-Script vector. The complete vector sequence is available at www.genomics.agilent.com. The vector supplied in this kit has been digested with EcoRI and XhoI restriction enzymes, and does not contain the sequence between EcoRI and XhoI.
**CLONING PROTOCOL FOR THE pCMV-SCRIPT XR VECTOR**

The pCMV-Script XR vector is designed for the convenient insertion of DNA inserts compatible with the *Eco*RI/*Xho*I cloning site. This vector features an MCS with eleven unique, conveniently arranged restriction enzyme sites for subcloning the DNA sequence of interest. Expression is driven by the human CMV promoter, a strong promoter that allows high-level constitutive expression in a variety of mammalian cell lines. The vector has a neomycin-resistance gene for selection of stable cell lines (see Figure 1).

**Cloning Considerations**

- A translation initiation sequence must be incorporated in the insert DNA if the DNA fragment to be cloned does not have an initiating ATG codon. For optimal translation, include a Kozak sequence. A complete Kozak sequence includes **CCATGG**, although **CCATGG**, or the core **ATG**, is sufficient.

- The insert should have ends compatible with the *Eco*RI/*Xho*I ends of the pCMV-Script XR vector.

- The insert DNA should be suspended in a volume of TE buffer§ that will allow the concentration of the insert DNA to be the same as the concentration of the vector DNA (0.03 μg/μl).

**Ligating the Insert**

For ligation, the ideal insert-to-vector molar ratio of DNA is variable; however, a reasonable starting point is a 1:1 insert-to-vector ratio. The ratio is calculated using the following equation:

\[
X \mu g \text{ of insert} = \frac{(\text{Number of base pairs in insert}) \times (0.03 \mu g \text{ of pCMV-Script vector})}{4278 \text{ bp of pCMV-Script vector}}
\]

where \(X\) is the quantity of insert (in micrograms) required for a 1:1 insert-to-vector molar ratio. Multiply \(X\) by 2 to get the quantity of insert required for a 2:1 ratio.
1. Prepare one control and two experimental 5-μl ligation reactions by adding the following components to separate sterile 1.5-ml microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Ligation reaction components</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepared pCMV-Script XR vector (0.03 μg/μl)</td>
<td>1.0 μl</td>
<td>1.0 μl</td>
</tr>
<tr>
<td>Prepared insert (0.03 μg/μl)</td>
<td>0.0 μl</td>
<td>X μl</td>
</tr>
<tr>
<td>XR LacZ test insert (0.01 μg/μl)</td>
<td>1.0 μl</td>
<td>0 μl</td>
</tr>
<tr>
<td>rATP [10 mM (pH 7.0)]</td>
<td>0.5 μl</td>
<td>0.5 μl</td>
</tr>
<tr>
<td>Ligase buffer (10×)§</td>
<td>0.5 μl</td>
<td>0.5 μl</td>
</tr>
<tr>
<td>T4 DNA ligase (4 U/μl)</td>
<td>0.5 μl</td>
<td>0.5 μl</td>
</tr>
<tr>
<td>Double-distilled H₂O (ddH₂O) to 10 μl</td>
<td>1.5 μl</td>
<td>Y μl</td>
</tr>
</tbody>
</table>

* This control verifies the ability of the vector to ligate the test insert.

b These experimental ligation reactions vary the insert-to-vector ratio. We recommend preparing reactions with 1:1 and 2:1 ratios. Expect a majority of the transformant colonies to represent recombinants.

2. Incubate the reactions overnight at 4°C.

**TRANSFORMATION**

1. Transform competent bacteria with 1–5 μl of the ligation reactions. Refer to Hanahan (1983) for a protocol for producing competent cells. When performing the control with the XR LacZ test insert, transform the ligation reaction into cells suitable for blue/white screening, such as the Agilent XL1-Blue strain.

   **Note** Use competent cells with transformation efficiencies ≥5 × 10⁹ cfu/μg for preparing libraries. (Competent cells with transformation efficiencies of ≥5 × 10⁹ cfu/μg are also available from Agilent.)

2. Plate the experimental transformants on LB-kanamycin agar plates. Plate the control transformants on LB-kanamycin agar plates containing X-gal and IPTG. Blue colonies on the control plates contain the test insert.

   § See Preparation of Media and Reagents
VERIFICATION OF INSERT PERCENTAGE, SIZE, AND ORIENTATION

Individual colonies can be examined by PCR directly from the colony or by restriction analysis to identify the vectors with inserts and determine the insert size and orientation. T3 and T7 primers are recommended for use in PCR amplification and sequencing from the pCMV-Script XR vector.

Polymerase Chain Reaction Amplification of DNA from Individual Colonies

The presence and size of a DNA insert in a pCMV-Script XR vector may be determined by PCR amplification of DNA from individual colonies.

1. For each colony to be examined, prepare a PCR amplification reaction containing the following components:

   4.0 μl of 10× Taq DNA polymerase buffer  
   0.4 μl of dNTP mix (25 mM each dNTP)  
   40.0 ng of T3 primer  
   40.0 ng of T7 primer  
   0.4 μl of 10% (v/v) Tween® 20  
   1.0 U of Taq DNA polymerase  
   dH₂O to a final volume of 40 μl

<table>
<thead>
<tr>
<th>Vector</th>
<th>Primer</th>
<th>Nucleotide sequence (5´ to 3´)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCMV-Script vector</td>
<td>T3</td>
<td>AATTAACCCTCACTAAAGGG</td>
</tr>
<tr>
<td></td>
<td>T7</td>
<td>GTAATACGACTCACTATAGGGGCC</td>
</tr>
</tbody>
</table>

2. Stab a transformed colony with a sterile toothpick and swirl cells from the colony into the amplification reaction mixture. Immediately following inoculation into the reaction mixture, remove the toothpick and streak onto antibiotic-containing patch plates for future reference.

3. Gently mix each reaction, then overlay the reactions with 30 μl of mineral oil and perform PCR using the following cycling parameters:

<table>
<thead>
<tr>
<th>Number of cycles</th>
<th>Temperature</th>
<th>Length of time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cycle</td>
<td>94°C</td>
<td>4 minutes</td>
</tr>
<tr>
<td></td>
<td>50°C</td>
<td>2 minutes</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>2 minutes</td>
</tr>
<tr>
<td>30 cycles</td>
<td>94°C</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td>56°C</td>
<td>2 minutes</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>1 minute</td>
</tr>
<tr>
<td>1 cycle</td>
<td>72°C</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

4. Analyze the PCR products to determine insert sizes using standard 1% (w/v) agarose gel electrophoresis. Because the forward and reverse PCR/sequencing primers are located on both sides of the MCS, the expected size of the PCR product should be 150 bp plus the size of the insert. Additional information can be obtained by restriction analysis of the PCR products.
## Transfection into Mammalian Cells

For protocols for transfection into mammalian cell lines please see Sambrook, *et al.* (1989).^4^

## Preparation of Media and Reagents

### LB Agar (per Liter)
- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar
- Add deionized H₂O to a final volume of 1 liter
- Adjust pH to 7.0 with 5 N NaOH
- Autoclave
- Pour into petri dishes (~25 ml/100-mm plate)

### LB Broth (per Liter)
- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- Add deionized H₂O to a final volume of 1 liter
- Adjust to pH 7.0 with 5 N NaOH
- Autoclave

### LB–Kanamycin Agar (per Liter)
- Prepare 1 liter of LB agar
- Autoclave
- Cool to 55°C
- Add 5 ml of 10-mg/ml, filter-sterilized kanamycin
- Pour into petri dishes (~25 ml/100-mm plate)

### LB–Kanamycin Agar Plates Containing X-gal and IPTG (per Liter)
- Prepare 1 liter of LB agar
- Autoclave
- Cool to 55°C
- Add 5 ml of 10-mg/ml-filter-sterilized kanamycin
- Add X-gal [prepared in dimethylformamide (DMF)] to a final concentration of 80 μg/ml
- Add IPTG (prepared in sterile distilled water) to a final concentration of 20 mM
- Pour into petri dishes (~25 ml/100-mm plate)

### TE Buffer
- 10 mM Tris-HCl (pH 7.5)
- 1 mM EDTA

### 10× Ligase Buffer
- 500 mM Tris-HCl (pH 7.5)
- 70 mM MgCl₂
- 10 mM dithiothreitol (DTT)

**Note** rATP is added separately in the ligation reaction

### Alternative Method
- Spread 100 μl of 10 mM IPTG and 100 μl of 2% X-gal on LB-kanamycin agar plates 30 minutes prior to plating the transformations
REFERENCES


ENDNOTES

Tween® is a registered trademark of ICI Americas, Inc.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at http://www.genomics.agilent.com. MSDS documents are not included with product shipments.