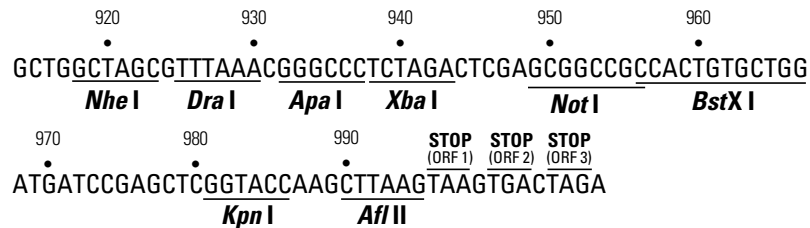
**pShuttle2 MCS**

Restriction Map and Multiple Cloning Site (MCS) of pShuttle2 Vector. Unique restriction sites are in bold.

Description

pShuttle2 is a cloning vector designed for use with the Adeno-X™ Expression System (Cat. No. 631513; 631022). In the Adeno-X System, standard ligation techniques are used to transfer a gene-specific expression cassette from pShuttle2 to a replication-deficient, Ad5 genome (2). The pShuttle2 expression cassette consists of the human cytomegalovirus immediate early promoter ($P_{CMV IE}$), a multiple cloning site (MCS), and the SV40 polyadenylation signal (SV40 poly A). The entire cassette is flanked by unique *I-Ceu I* and *PI-Sce I* restriction sites so that it can be excised and directly ligated to Adeno-X Viral DNA—the adenoviral genome. The vector backbone also contains the pUC origin (pUC ori) and a kanamycin resistance gene (Kan^r) for propagation and selection in *E. coli*.

Use

Insert your full-length cDNA into the MCS of pShuttle2 using any of the unique restriction sites shown. Your cDNA insert must contain a start codon for proper promoter driven expression in mammalian cells. After the gene of interest has been cloned into pShuttle2, excise the expression cassette by digesting the plasmid with *I-Ceu I* and *PI-Sce I* according to the one-step double digestion procedure in the Adeno-X Expression System User Manual (PT3414-1). Because the termini of the excised expression cassette fragment are compatible with the cloning site in Adeno-X Viral DNA, the cassette can be directly ligated to Adeno-X DNA to form a recombinant adenoviral vector.



Clontech

United States/Canada
800.662.2566

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR31250; published 14 January 2003)

Location of features

- Human immediate early cytomegalovirus promoter (P_{CMVIE})
Enhancer region: 336–832
TATA Box: 827–832
Transcription start point: 853
- Multiple Cloning Site (MCS): 918–995
- SV40 polyadenylation signal: 1038–1043
- pUC origin of replication: 1393–2036
- Kanamycin resistance (aminoglycoside phosphotransferase) gene: 2710–3525
- I-*Ceu* I Recognition sequence: 3–28

```

      10      20
      •      •
5'..TAAC TATAACGGTCTAAGGTAGCGA..3'
3'..ATTGATATTGCCAGGATTCATCGCT..5'
      ▲

```

- PI-*Sce* I Recognition sequence: 1133–1171

```

      1140      1150      1160      1170
      •      •      •      •
5'..ATCTATGTCGGGTGCGGAGAAAGAGGTAATGAAATGGCA..3'
3'..TAGATACAGCCACGCCTCTTTCTCCATTACTTTACCGT..5'
      ▲

```

Propagation in *E. coli*

- Suitable host strains: DH5 α and other general purpose strains.
- Selectable marker: plasmid confers resistance to kanamycin (50 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

References

1. Adeno-X Expression System (January 2000) *Clontechniques* **XV** (1):8–10.
2. Mizuguchi, H. & Kay, M. A. (1999) *Hum. Gene Ther.* **10**:2013–2017.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc.. This vector has not been completely sequenced.

Notice to Purchaser

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes, nor is it intended for human use. Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Clontech Laboratories, Inc.

Clontech, Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc.
Clontech is a Takara Bio Company. ©2005