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Product Information

pACD4 TargeTron®Vector Set
No selectable marker

Catalog Number **TV0010**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The pACD4 vector set (no selectable marker) is a set of four linearized vectors intended for use with the TargeTron Gene Knockout System when the use of a selectable marker is not desired. Each vector in the pACD4 vector set is similar to the pACD4K-C TargeTron vector, but without a kanamycin RAM marker to select for chromosomal insertion. When these non-RAM TargeTron vectors express the ribonucleoprotein complex (RNP) and intron RNA is inserted into the specific sequence of interest, there is no selectable marker present in the group II intron insertion sequence. The pACD4 vector set also differs from the pACD4K-C TargeTron vector in that each vector in this set contains either an A, C, G, or T base in the δ+1 position.[1] This base variation facilitates proper base pairing of group II intron precursor RNA for different target site designs and results in more efficient precursor RNA splicing to form active TargeTron RNPs.[1]

Reagents

Set contains 2 µg of each vector supplied at 20 ng/µl in 10 mM Tris-HCl, pH 8.0, with 1 mM EDTA. The vectors have been linearized with *Hin*d III and *Bsr*G I.

Precautions and Disclaimer

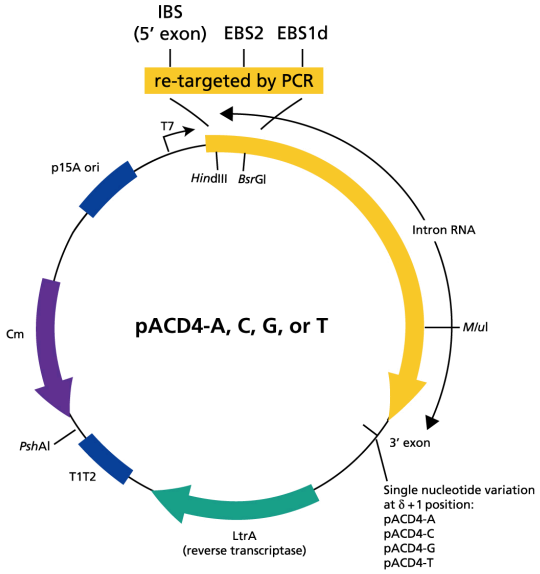
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store at –20 °C. Shipped on wet ice.

Vector Features

Location (bp)	Feature
585-1497	p15A ori
1741-1762	T7 promoter
1802-1826	5' exon (IBS)
1827-2741	intron RNA
2049-2053	EBS2 (exon binding sequence 2)
2102-2111	EBS1d
2742	δ+1 position
2742-2751	3' exon
2988-4787	LtrA ORF
4920-5190	T1/T2 transcriptional terminator
5861-219	chloramphenicol (Cm) resistance



Procedure

A. Vector selection

In general, the pACD4-C vector can be used. In some cases, upon performing colony PCR to locate a mutant, the efficiency of insertion may be just below what is reasonable to screen (for example, >100 colonies must be screened). If a mutant cannot be found, cloning into an alternative vector (pACD4-A, T, or G) may increase the insertion efficiency so that <100 colonies could be screened by colony PCR.

To choose the appropriate vector, match the vector name with the nucleotide at the δ+1 position in the target site. For example, if the algorithm suggests the target site shown below, vector pACD4-A would be the appropriate vector to use.

CCATCTTGCGATAGACCGGATCTTTAGCGC – intron – **A**GTTTTTACGTCCAC

↑

δ +1 position

The δ+1 position is the nucleotide immediately after the intron insertion in the 3′ exon.

B. Additional plasmid information

Vectors are provided pre-linearized with *Hin*d III and *Bsr*G I. Chloramphenicol (25 μg/ml) is used to propagate each pACD4 vector. These vectors require the use of T7 RNA polymerase for intron expression. When using DE3 strains or a T7 RNA polymerase under the control of the *lac* UV5 promoter, glucose is typically included to provide additional suppression of the *lac* UV5 promoter prior to IPTG-induction. T7 RNA polymerase may also be supplied by co-transforming plasmid pAR1219 that expresses T7 RNA polymerase, Catalog Number T2076.

C. Sub-cloning into host specific shuttle vectors

Prior to sub-cloning, the pACD4 vectors need to be circularized. This may be accomplished using the *lacZ* control reaction in the TargeTron Kit (Product No. TA0100). For adaptation to another host, it is advised to sub-clone intron components first from pACD4-C and target an easily screenable gene. Sub-cloning the region bound by *Hin*d *III* and *Psh*A I into an alternative host shuttle vector downstream of a host specific promoter should result in a functional TargeTron expression vector. The *Hin*d III-*Psh*A I region should contain the intron RNA and LtrA reverse transcriptase coding regions, as well as the transcriptional terminator. In the final TargeTron shuttle vector, the *Hin*d III and *Bsr*G I sites should be unique since these are used to routinely re-target the intron to knockout specific genes.

D. Delivery of heterologous DNA

Since these vectors do not contain the kan-RAM marker, heterologous DNA may be cloned into the *Mlu* I site in the intron for delivery to specific DNA sites. The *Mlu* I site has been used to successfully deliver a trimethoprim-RAM, a kanamycin-RAM (plasmid pACD4K-C in the TA0100 kit), and a *lacZα* gene.[3] The efficiency of the intron maybe affected by insertions at the *Mlu* I site. A good starting point is to attempt to insert an intron containing heterologous DNA into an easily screenable or selectable gene, such as *lacZ*.

For detailed TargeTron gene disruption protocols, see the latest User Guide for the TargeTron Gene Knockout System, Catalog Number TA0100, at www.sigma-aldrich.com

References

1. Perutka, J., *et al.*, Use of computer-designed group II introns to disrupt *Escherichia coli* DExH/D-box protein and DNA helicase genes. *J. Mol. Biol.*, **336** (2), 421-39 (2004)
2. Zhong, J., *et al.*, Targeted and random bacterial gene disruption using a group II intron (targetron) vector containing a retrotransposition-activated selectable marker. *Nucleic Acids Res.*, **31**(6), 1656-64 (2003).
3. Jones, J.P., *et al.*, Retargeting mobile group II introns to repair mutant genes. *Mol. Ther.*, **11**(5), 687-94 (2005).

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