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Product Information

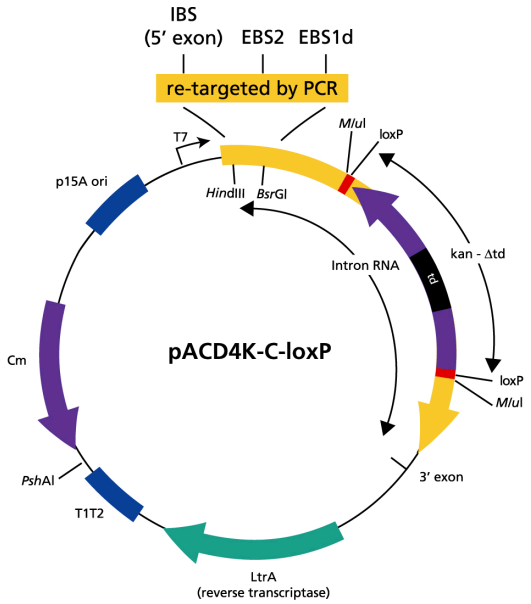
TargeTron® Vector pACD4K-C-loxP

Catalog Number **T2826**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The TargeTron vector, pACD4K-C-loxP, is a 7,745 bp *Escherichia coli* expression vector to be used in conjunction with the TargeTron Gene Knockout System, Catalog Number TA0100. This vector differs from the original pACD4K-C vector in that it has loxP sites flanking each end of the kanamycin ORF. The addition of the loxP sites on this vector allows for multiple site-specific knockouts in the same bacterial chromosome. After an initial "targetron" chromosomal insertion, the kanamycin resistance marker used to select for insertions can be removed via Cre-loxP mediated recombination.[1,2] After removal of the kanamycin resistance marker, sequential "targetron" insertions can be generated.[3]



Map Features of TargeTron pACD4K-C-loxP vector

Feature	Map Position
p15A ori	585-1497
T7 promoter	1741-1762
5′ exon (IBS)	1802-1826
Intron RNA	1827-4182
EBS2 (exon binding sequence 2)	2049-2053
EBS1d	2102-2111
5′ loxP site	2523-2555
Kanamycin-RAM	2556-3923
td group I intron	2853-3245
3′ loxP site	3924-3957
3′ exon	4183-4192
LtrA ORF	4429-6228
T1/T2 transcriptional terminator	6365-6644
Chloramphenicol (Cm) resistance	7305-219

loxP sites that flank the kanamycin-RAM:

5′ loxP sequence
5′ - CTACTTCGTATAGCATACATTATACGAAGTTAT - 3′

Note: The mutated 5′ loxP site shown above was found to be more efficient than the wild-type loxP site in conjunction with targeted group II intron insertions.

3′ loxP sequence:
5′ – ATAACTTCGTATAGCATACATTATACGAAGTTAT – 3′

Reagent
Supplied at 25 ng/µl in 10 mM Tris-HCl, pH 8.0, with 1 mM EDTA. The provided pACD4K-C-loxP vector is linearized at the *Hin*d III and *Bsr*G I sites.

Precautions/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.

Procedures

A. Removal of kanamycin by Cre-loxP mediated recombination

1. After the initial "targetron" insertional knockout has been confirmed, make the knockout strain competent using the RapidTransit™ Transformation Kit , Catalog Number R2653, or an alternative method for making competent cells.
2. Obtain a plasmid expressing Cre-recombinase that has a selectable marker other than kanamycin (e.g., chloramphenicol, tetracycline, etc.).
3. Transform the knockout strain with the Cre plasmid according to the supplier's recommendations.
4. Grow and select for transformants at the appropriate temperature with the appropriate antibiotic.[4]
5. Once transformants are obtained, express the Cre recombinase according to supplier recommendations (e.g., shifting temperature from 30 °C to 37 °C for plasmids with thermosensitive promoters).
6. Check for removal of kanamycin by replica plating isolated colonies on LB and LB+Kan (25 µg/ml).
7. Further verification for removal of kanamycin (~1.0 kb) can be done by colony PCR using gene specific or intron specific primers spanning the kanamycin ORF.
8. The PCR amplicon can then be submitted for sequencing. There will be a single loxP scar remaining within the "targetron" once the kanamycin has been removed.

B. Sub-cloning into host specific shuttle vectors

The TargeTron system has previously been adapted to other bacterial hosts by sub-cloning the essential intron components into shuttle/expression vectors. Prior to sub-cloning, the pACD4K-C-loxP vector needs to be circularized. This can be done by running the *lacZ* control reaction in the TargeTron kit (Product Number TA0100). For adaptation to another host, it is advised to 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.

C. Delivery of heterologous DNA

The lox-P kanamycin RAM is bound by two *Mlu* I sites. The loxP-kan-RAM can be removed by *Mlu* I digestion and replaced with other DNA such as promoters (for mitigating polar effects, if needed), reporter genes, other antibiotic RAM-type markers, etc. The *Mlu* I site has been used to successfully deliver a trimethoprim–RAM,[3] a kanamycin-RAM (plasmid pACD4K-C in the TA0100 kit and the pACD4K-C-loxP plasmid provided here), and a *lacZ*α gene.[5] The efficiency of the intron may be 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*.

References:

1. Abremski, K. *et al*., J. Biol. Chem., **261** (1), 391-396 (1986).
2. Hartung, M. *et al*., J. Biol. Chem., **273** (36), 22884-22891 (1998).
3. Zhong, J. *et al*., Nucleic Acids Res., **31** (6), 1656-1664 (2003).
4. Buchholz, F. *et al*., Nucleic Acids Res., **24** (15), 3119-3119 (1996).
5. Jones, J.P. *et al*., Mol. Ther., **11** (5), 687-94 (2005).

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