

## Product Information

### TargeTron® Vector pJIR750ai

Catalog Number **T7701**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

TargeTron vector pJIR750ai is a 10,262 bp expression vector intended for use with the TargeTron Gene Knockout System, Catalog Number TA0100. This circularized vector can be used for targeted gene knockouts in gram-positive bacteria such as *Clostridium perfringens* type A.<sup>1</sup> Expression of the group II intron RNA is under the control of the  $\beta$ -2 toxin gene promoter, *cpb2*.<sup>1</sup> As supplied, the pJIR750ai vector is re-targeted to the  $\alpha$ -toxin (*plc*) gene.  $\alpha$ -toxin, a phospholipase C, is an extracellular toxin produced by all *C. perfringens* isolates.<sup>1</sup> For validation in a specific *C. perfringens* strain, this vector can be used directly to knockout *plc* without any further modifications using the enclosed protocol. In order to re-target this vector to knockout other genes, the *plc* specific IBS-EBS fragment (350 bp) between the *Hind* III and *Bsr*G I sites can be cut out and replaced with another gene specific fragment. For re-targeting protocols, refer to the TargeTron Gene Knockout System User Guide, Catalog Number TA0100, at sigma-aldrich.com.

Key Features for the TargeTron Vector pJIR750ai
pIP404 ori
Chloramphenicol resistance ( <i>E. coli</i> and <i>C. perfringens</i> )
pMB1 ori
<i>cpb2</i> ( $\beta$ -2-P) promoter
Intron RNA
LtrA ORF

### Reagent

Supplied at a concentration of 100 ng/ $\mu$ l in 10 mM Tris-HCL, pH 8.0, 1 mM EDTA

### 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/Stability

Store at  $-20\text{ }^{\circ}\text{C}$ .

### Cloning and propagation in *E. coli*

Plasmid pJIR750ai is maintained in *E. coli* using 15  $\mu$ g/ml chloramphenicol.

### Procedure for $\alpha$ -toxin (*plc*) knockout in *C. perfringens*

1. Electroporate pJIR750ai into *C. perfringens* ATCC 3624 type A.
2. Grow cells at 37  $^{\circ}\text{C}$  in TGY (tryptone glucose yeast) medium for 3 hours without shaking.
3. Plate on BHI (brain heart infusion) agar containing chloramphenicol (15  $\mu$ g/ml).
4. Incubate under anaerobic conditions at 37  $^{\circ}\text{C}$  overnight.
5. Perform colony PCR to confirm TargeTron integration using either of the primer combinations listed in the table below.

**Note:** Depending on the colony PCR reagents and procedure, using the *plc*-F and Intron Specific primers may give more sensitive colony PCR results for detecting targetron insertions.

### $\alpha$ -toxin (*plc*) forward primer

5'-GTGAGGTTATGTTAATTATATGGTATAATTTCAATGC

### $\alpha$ -toxin (*plc*) reverse primer

5'-AGTTACAATCATAGCATGAGTTCCTGTTCC

### Intron Specific primer

5'-GTGTTTACTGAACGCAAGTTTCTAATTTTCGGT

## Results: Colony PCR

Primers	Wild-type <i>C. perfringens</i>	Mutant <i>plc targetron</i>
<i>plc</i> -F <i>plc</i> -R	200 bp	1.1 kb
<i>plc</i> -F Intron Specific	No product	876 bp

### *plc*-50/51a target sequence

TAGACTTTAGTTGATGCCCCAGCCCATAGG -  
intron – CTAGTTGCTAGCGTA

In addition to ATCC 3624 type A, the *plc*-50/51a target sequence is present exactly as listed above in *C. perfringens* str. 13 (per NCBI microbial BLAST).

### Procedure for testing $\alpha$ -toxin activity

$\alpha$ -toxin activity can be monitored by the presence of a white halo around colonies on BHI agar containing egg yolk (4% [vol/vol] egg yolk).<sup>1</sup> *plc*-50/51a mutants should not produce a white halo on BHI agar containing egg yolk when compared to wild type *C. perfringens*.<sup>1</sup>

### Procedure for curing donor plasmid (pJIR750ai) after targetron insertion

The re-targeted donor plasmid is crucial for making intron insertional mutants, however, it is not necessary for maintaining the intron insertion on the chromosome. For intron insertions in the sense orientation, it is possible for the intron to splice out of mRNA with the assistance of the LtrA protein encoded on pJIR750ai. Although the intron cannot splice out when inserted in the antisense ORF orientation, donor plasmid curing is still recommended. Therefore, for accurately observing phenotypic effects of a given disruption, the donor plasmid should be cured from the insertional mutant strain. In order to cure pJIR750ai after a knockout has been confirmed, subculture the knockout strain for several days in FTG (fluid thioglycolate) medium (without antibiotic) and subsequently screen for chloramphenicol sensitive colonies on BHI agar plates.<sup>1</sup>

**Note:** If the targeted gene is essential or partially essential for cell survival, curing of the donor plasmid could be more difficult or unattainable.<sup>2</sup>

### Procedure for delivery of heterologous DNA

pJIR750ai has an *Mlu* I restriction site located within group II intron RNA coding sequence. Digestion at the *Mlu* I site allows for insertion of other DNA such as promoters (for mitigating polar effects), reporter genes, *loxP* recombination sequences, other antibiotic RAM-type markers, etc. The *Mlu* I site has been used to successfully deliver *tetM* and *abiD* genes<sup>3</sup>, a trimethoprim –RAM<sup>4</sup>, a kanamycin-RAM (plasmid pACD4K-C, TA0100 kit), a removable kanamycin-RAM flanked with *loxP* sites (plasmid pACD4K-C-*loxP*, Cat. No. T2826), and a *lacZ $\alpha$*  gene<sup>5</sup>. 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. For instance, the  $\alpha$ -toxin 50/51a targeting sequence in pJIR750ai is very good control to test the effects of heterologous DNA inserted at the *Mlu* I site on intron mobility.

### References:

1. Chen, Y., et.al. *Appl Environ Microbiol.* **71**(11):7542-7547 (2005).
2. Yao, J., et.al. *RNA.* **12**(7):1271-1281 (2006).
3. Frazier, C., et al., *Appl Environ Microbiol.* **69**(2):1121-1128 (2003).
4. Zhong, J., et al., *Nucleic Acids Res.* **31**(6):1656-1664 (2003).
5. Jones, J.P., et al., *Mol. Ther.* **11**(5):687-94 (2005).

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