

Product Information**RightZAP1.1**

Research use only

Catalog No: ZR-01**Lot No:** 0101**Contents:**

20 µg DNA, 0.5 µg/µl
in TE pH 7.5

Storage: -20°C**Features and Applications:**

RightZAP1.1 is a 32.5 kb *SfiI*-*PacI* DNA fragment that encompasses bp 3504-right end of the Ad5 genome. The E3 region is intact. The DNA was purified from a cosmid. It is used in combination with the shuttle vector pZAP1.1 to construct replication-deficient adenoviruses containing

transgenes in place of the E1 region of the Ad5 genome. The maximum transgene capacity of the resulting virus is 5.2 kb.

Quality Control Assays:**Ligation:**

A Left Arm containing a β -galactosidase expression cassette was purified as a *PacI*-*BstEII* fragment.

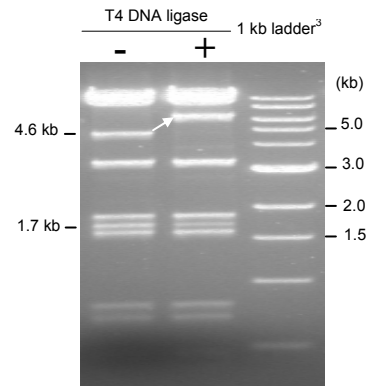
The following reaction was set up:

4 µl RightZAP1.1 (2 µg)
4 µl Left Arm (~ 300 ng)
8 µl H₂O
2 µl T4 DNA Ligase buffer
1 µl *PacI* (10 u/µl)¹
1 µl T4 DNA Ligase²

Total: 20 µl

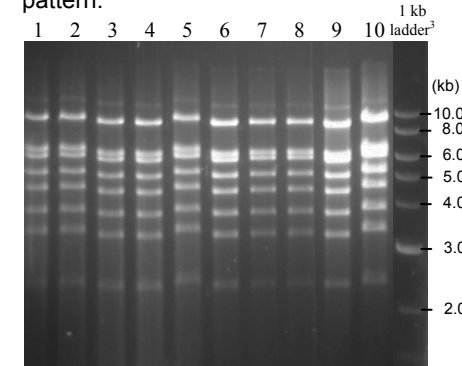
The reaction was performed for 1 hour at room temperature and stopped by inactivating the T4 Ligase for 10 min. at 65°C. The ligation product was digested with *BstEII*. In presence of T4 DNA ligase, the 4.6 kb fragment

containing the β Gal cassette is almost completely shifted to form a 6.3 kb DNA fragment with the 1.7 kb *BstEII* DNA fragment corresponding to the left end of RightZAP1.1.

**Virus Recovery:**

The ligation mixture (2 µg DNA) was transfected into 293 cells (passage 36, 6-cm dishes) using the calcium-phosphate method. The cell monolayer

was covered with agar 1 day after transfection and plaques were counted. The first plaque was visible 4 days after transfection. A total of 61 plaques were counted 9 days after transfection. Ten virus clones were amplified, their genomes were purified and analyzed by restriction with *HindIII*. All clones presented the expected restriction pattern.



¹ New England Biolabs #R0547

² New England Biolabs #M0202

³ New England Biolabs #323-2S