O.D.260 Inc.

Product Information

RightZAP1.2

Research use only

Catalog No: ZR-02
Lot No: 0411

Contents:

20 μg DNA, 0.5 μg/μl
in TE pH 7.5

Storage: -20°C

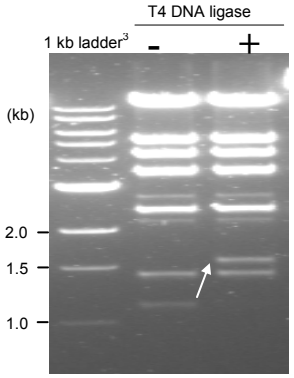
Features and Applications:
RightZAP1.2 DNA is a 29.8 kb linear DNA fragment that encompasses bp 3504-right end of the Ad5 genome. The E3 region is deleted (2.7 kb). The DNA was purified from a cosmid. It is used in combination with the shuttle vector pZAP1.1 to construct replication-deficient adenoviruses containing transgenes in place of the E1 region of the Ad5 genome. The maximum transgene capacity of the resulting virus is 7.9 kb.

Quality Control Assays:
Ligation:
The following reaction was set up:

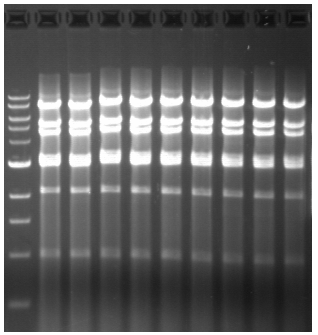
2 μl RightZAP1.2
2 μl Control Left Arm (ΔE1)
4 μl H_2O
1 μl T4 DNA Ligase buffer
0.5 μl *Pac*I (10 u/μl)[1]
0.5 μl T4 DNA Ligase[2]

Total: **10 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 *Bst*XI. This enzyme generates a 1108-bp DNA fragment corresponding to the left end of RightZAP1.2. In presence of T4 DNA ligase, this fragment is almost completely shifted to form a 1530-bp DNA fragment with the 430-bp long control left arm.



Virus Recovery:
The ligation mixture (2 μg DNA) was transfected into 293 cells (passage 38, 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 plaques were visible 4 days after transfection. A total of 87 plaques were counted 9 days after transfection. Nine virus clones were amplified, their genomes were purified and analyzed by restriction with *Hin*dIII. All clones presented the expected restriction pattern.



[1] New England Biolabs #R0547
[2] New England Biolabs #M0202
[3] New England Biolabs #323-2S