

DNA Ligation Kit (Cat.# 6021)

Application: Comparison of linker efficiency using the DNA Ligation Kit vs. T4 DNA Ligase

Takara's DNA Ligation Kit (Cat. # 6021) is a two-component system consisting of an optimized buffer system and T4 DNA Ligase. This system allows for rapid ligation reactions and high transformation efficiency.

Methods:

Vector: 100 ng (50 fmol) pUC118 *Hinc* II/BAP (Cat. # 3322)

Linker: 2.6–130 ng (0.5–25 pmol) *Bgl*/II linkers (5'-CAGATCTG-3') (Cat. # 4621P, discontinued)

The dephosphorylated vector and phosphorylated linkers were combined in a total volume of 5 μ l. Ligation was performed with either the DNA Ligation Kit (Cat. # 6021) or T4 DNA Ligase. For the DNA Ligation Kit, Solution A (25 μ l) and Solution B (5 μ l) were added, and the mixture was incubated at 16°C for 30 minutes. For the T4 DNA ligase reaction, 350 U (2.8 Weiss units) of T4 DNA ligase in standard ligation buffer was added, and the mixture was incubated at 16°C for 16 hours. After ligation, a portion of the reaction mixture was transformed into competent JM109* cells. Transformants were isolated on LB-Amp plates containing X-Gal and IPTG.

*The titer of JM109 cells used was at least 1.5×10^8 colony forming units (cfu) per μ g of supercoiled pUC118 vector DNA.

Results:

Transformation efficiency was calculated after counting the number of white colonies.

Table 1. Transformation Efficiency (colonies per μ g pUC118 DNA)

Linker/Vector (Molar Ratio)	DNA Ligation Kit	T4 DNA Ligase
10	2.0×10^6	1.2×10^6
50	8.0×10^6	3.2×10^6
100	3.0×10^7	2.3×10^6
500	2.5×10^7	2.4×10^6

Conclusion:

More transformants were obtained from ligations performed with Takara's DNA Ligation Kit than T4 DNA ligase-mediated reactions for each of the linker/vector ratios (molar ratios of 10, 50, 100, and 500). Additionally, the use of the DNA Ligation Kit reduced the ligation reaction time to 30 minutes versus 16 hours required for T4 DNA ligase.