DNA Ligation Kit, Mighty Mix (Cat.# 6023)

Application: Using DNA Ligation Kit, Mighty Mix for Fast Ligation

With the DNA Ligation Kit, Mighty Mix (Cat. # 6023) reagent, 5 minute ligations are possible. In this experiment, the efficiency of cohesive and blunt ended ligations were compared for standard and fast ligation protocols.

Methods

Cohesive end (*Hin*dIII-digested vector and insert) and blunt end (*Hin*cII-digested vector and insert) ligations were performed using two protocols: (1) standard protocol: 16°C for 30 minutes, and (2) fast protocol: 25°C for 5 minutes. The ligation efficiencies of the two protocols were compared using either the DNA Ligation kit, Mighty Mix or a fast ligation kit from Company D (for which a 25°C, 5 minute ligation is recommended by the manufacturer).

Results

After ligation, transformants were selected on LB-Amp plates containing X-Gal and IPTG. The numbers of blue and white colonies were counted and are are shown in Figures 1 and 2.

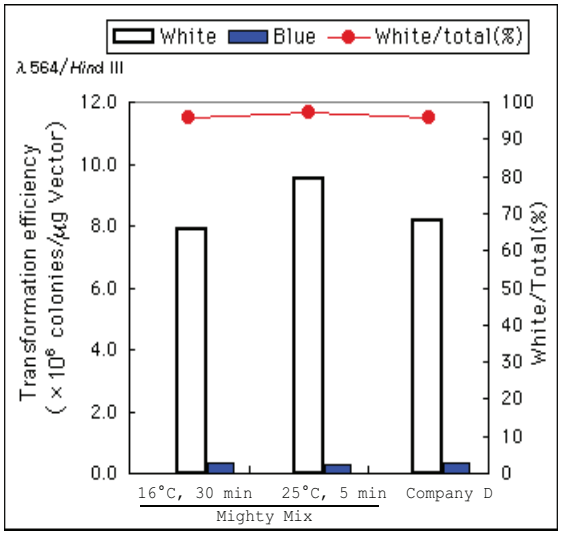

Figure 1. Cohesive end ligation. *Hin*dIII-digested λ DNA fragment (564 bp) and vector were ligated using either the standard or fast protocol with the DNA Ligation Kit, Mighty Mix or Company D's fast ligation kit. The bars show the number of white and blue colonies per µg vector (transformation efficiency) and the red line indicates the number of white colonies/total number of colonies (%).

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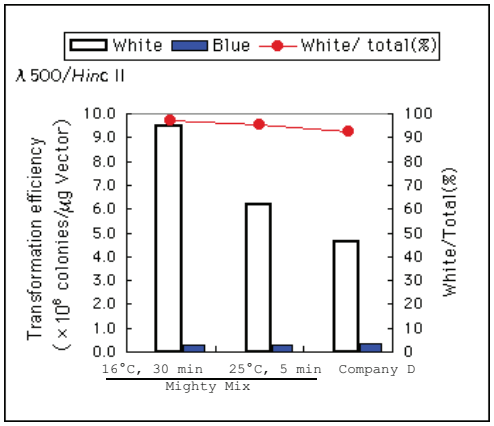

Figure 2. Blunt end ligation. *Hin*cII-digested λ DNA fragment (500 bp) and vector were ligated using either the standard or fast protocol with the DNA Ligation Kit, Mighty Mix or Company D's fast ligation kit. The bars show the number of white and blue colonies per µg vector (transformation efficiency) and the red line indicates the number of white colonies/total number of colonies (%).

Conclusions

The ligation efficiency for cohesive end ligations was the same for both the standard and fast protocols using the Mighty Mix reagent. For blunt end DNA ligation, higher efficiency was obtained using the standard protocol (16°C, 30 minutes). However, when the rapid protocol (25°C, 5 minutes) was used, the ligation efficiency was higher with Takara's kit as compared to Company D's kit. Overall, the Mighty Mix reagent enables efficient ligation of DNA inserts into vectors in just 5 minutes.