

LigaFast™ Rapid DNA Ligation System

5–15 minutes from DNA to Transformation

The LigaFast Rapid DNA Ligation System is designed for efficient ligation of sticky-end DNA inserts into plasmid vectors in just 5 minutes (blunt-end inserts in as little as 15 minutes). Rapid ligation is based on the combination of T4 DNA Ligase with a unique 2X Rapid Ligation Buffer. The LigaFast System is designed to eliminate any further purification prior to transformation of ligated DNA. The specially formulated 2X Rapid Ligation Buffer requires no additional ATP or Mg²⁺ prior to use.

Benefits:

- **Flexible:** Use with 5', 3' or blunt-ended DNA inserts.
- **Fast:** Ligation of sticky-end DNA inserts in 5 minutes, blunt-ended inserts in 15 minutes.
- **Convenient:** No requirement to purify ligated DNA prior to transformation in *E. coli*. Ligations performed at room temperature.
- **Ready-to-use:** No additional buffer modifications required prior to use.
- **Efficient:** Results comparable with standard overnight ligations.

Standard Protocol for DNA ligations using LigaFast Rapid DNA Ligation System

The following ligation reaction of a 3kb vector and a 0.5kb insert DNA uses a 1:2 vector:insert ratio.

1. Assemble the following reaction in a sterile microcentrifuge tube:

Vector DNA	100ng
Insert DNA	33ng
2X Rapid Ligation Buffer	5µl
T4 DNA Ligase	3u
Nuclease-Free water to a final volume of	10µl

2. Incubate the reaction at room temperature for 5 minutes for sticky-ended ligations, or 15 minutes for blunt-ended ligations.
3. Remove aliquot of ligated DNA for direct transformation into competent cells of choice.

5 Minute Sticky End Ligations

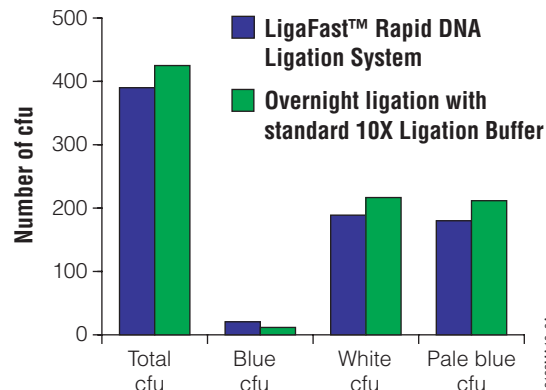


Figure 1. Comparison of overnight ligations and the LigaFast Rapid DNA Ligation System using a DNA insert with 5' overhangs. A DNA insert with 5' overhangs was ligated into a *Sa*I digested, dephosphorylated pGEM® vector. Ligations were conducted either at 4°C overnight using 3 units of T4 DNA Ligase with the provided 10X buffer, or at room temperature for 15 minutes using the LigaFast Rapid DNA Ligation System (3 units of T4 DNA Ligase with 2X Rapid Ligation Buffer). Ligated DNA was transformed into competent JM109 cells and plated on indicator media. White and pale blue colonies were confirmed to contain recombinant vector by restriction enzyme analysis.

15 Minute Blunt-Ended Ligations

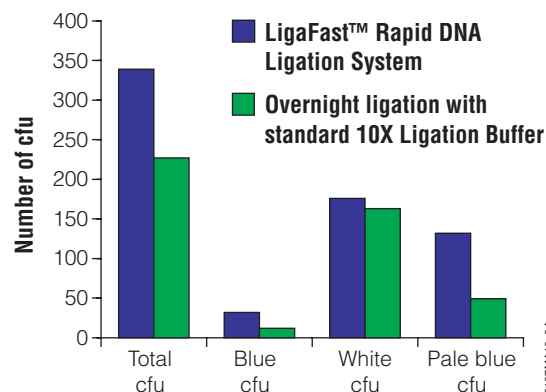


Figure 2. Comparison of overnight ligations and the LigaFast Rapid DNA Ligation System using blunt-ended DNA inserts. Blunt-ended DNA fragments were ligated into a *Eco*R V digested, dephosphorylated pGEM® vector. Ligations were conducted either at 4°C overnight using 3 units of T4 DNA ligase with the provided 10X buffer, or at room temperature for 15 minutes using the LigaFast Rapid DNA Ligation System (3 units of T4 DNA Ligase with 2X Rapid Ligation Buffer). Ligated DNA was transformed into competent JM109 cells and plated on indicator media. White and pale blue colonies were confirmed to contain recombinant vector by restriction enzyme analysis.

Streamlined Restriction Digestion, Dephosphorylation and Ligation Procedure

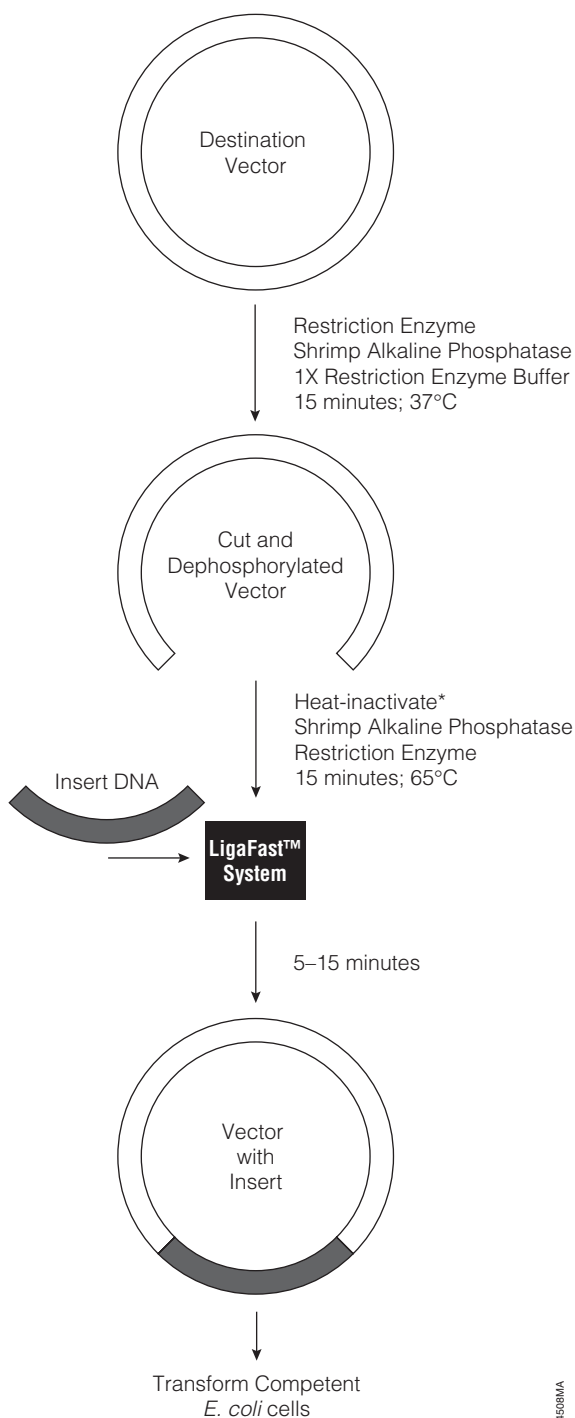
1. Combine restriction digestion and dephosphorylation of DNA vector in 1X restriction enzyme buffer. Use 15 units of restriction enzyme and 10 units Shrimp Alkaline Phosphatase (SAP) per microgram vector in a final volume of 30–50µl. Incubate at 37°C for 15 minutes. This is a sufficient amount of SAP to completely dephosphorylate the vector regardless of overhang type (5', 3', or blunt) in any Promega restriction enzyme buffer.
2. Heat-inactivate both restriction enzyme and SAP for 15 minutes at 65°C.
Note: Not all restriction enzymes can be heat inactivated. If the enzyme cannot be heat inactivated, purify cut/dephosphorylated vector with the Wizard® SV Gel and PCR Clean-Up System. Full purification is achieved in just 15 minutes and vector DNA can be eluted in as little as 15µl of water.
3. Centrifuge briefly then remove 1–2µl of vector for ligation with appropriate DNA insert using T4 DNA Ligase and 2X Rapid Ligation Buffer from the LigaFast™ Rapid DNA Ligation System. Incubate at 15°C for 5 minutes (3' or 5' ends), or 15 minutes for blunt ends, in a final reaction volume of 10–50µl. We recommend starting with a 1:2 molar ratio of vector:insert DNA.
4. Transform the ligated material directly into competent *E. coli* cells.

Ordering Information

Product	Size	Cat. #
LigaFast™ Rapid DNA Ligation System	30 reactions	M8221
	150 reactions	M8225
Shrimp Alkaline Phosphatase	500u	M8201
Wizard® SV Gel and PCR Clean-Up System	50 preps	A9281

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* Not all restriction enzymes can be heat-inactivated.

Figure 3. Streamlined subcloning procedure.

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