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# T4 DNA Ligase

*Technical Bulletin 15244-2*

T4 DNA Ligase catalyzes the ATP-dependent formation of a phosphodiester bond between the 3´ hydroxyl end of a double-stranded DNA fragment and the 5´ phosphate end of the same or another DNA fragment (figure 1). It is widely used for generating recombinant DNA molecules in which DNA fragments of interest are covalently inserted into vector molecules. The enzyme also can be used to add adapters to the ends of DNA fragments in order to introduce the appropriate cohesive ends prior to cloning into a vector with cohesive ends. This bulletin describes conditions for using T4 DNA Ligase for direct cloning into plasmids, for cloning into λ DNA vectors, and for adding adapters to DNA fragments. See Table 1 (page 5) for a summary of the reaction parameters in the protocols.

When transforming bacteria with plasmid ligation products, the goal is to maximize the number of colonies containing recombinant plasmids with single inserts. This requires ligation reaction conditions that maximize the number of monomeric, circular molecules, since concatamers or linear molecules do not transform as efficiently. A number of ligation reaction parameters are important, including DNA concentration, the molar ratio of insert to vector, temperature, buffer composition, and enzyme concentration. Different protocols are recommended for cloning into circular vectors with cohesive ("sticky") ends and those with blunt ends. Because ligating blunt ends is less efficient, the process requires higher concentrations of enzyme and DNA, a longer incubation time, and a lower reaction temperature than are used for cohesive ends (1-3).

In contrast with plasmids, λ vector ligation products are packaged *in vitro* into bacteriophage, a procedure that works most efficiently with linear concatamers of DNA. Therefore, the protocol for cloning into λ DNA vectors with cohesive ends uses a relatively high vector DNA concentration that favors concatamer formation (4).

When ligation reaction products are used to transform bacteria, a high background of colonies containing nonrecombinant plasmids may result from the religation of the ends of the vector molecules. This background can be reduced by treating the linear vector DNA with bacterial alkaline phosphatase (BAP) (5) or calf intestinal alkaline phosphatase (CIAP) prior to insert ligation. The phosphatase removes the 5´ phosphate groups from each strand of the vector molecule, preventing T4 DNA Ligase from forming phosphodiester bonds between the two ends of the vector. Phosphatase treatment of the vector is not as important if the vector has been digested with two restriction endonucleases that generate incompatible ends. The use of BAP or CIAP may not be necessary if a method is employed to distinguish colonies containing vectors with inserts from those containing vectors without inserts (*e.g.*, "blue/white" screening by α-complementation), but phosphatase treatment will help maximize the number of recombinants. Phosphatase treatment is also used to reduce background in λ vector ligations.

In making recombinant DNA molecules, having the flexibility to change the restriction endonuclease sites at the ends of fragments is often helpful. Linkers and adapters are used for this purpose. Linkers are small double-stranded oligodeoxyribonucleotides that include the recognition sequence for a restriction endonuclease. Following ligation of the blunt-ended linkers to blunt-ended DNA fragments, digestion with the appropriate restriction endonuclease generates cohesive ends. Linker ligation is done with a large molar excess of linkers to increase the likelihood that every insert will have at least one linker ligated to each end and therefore generates DNA fragments with multiple linkers on each end. Restriction endonuclease digestion followed by gel-exclusion chromatography, ion-exchange chromatography, or gel electrophoresis removes the extra linkers from the insert. To protect the DNA insert's internal restriction endonuclease site, inserts that

# Introduction (cont.)

contain the same restriction endonuclease site as the linker are treated with the corresponding methylase before the linkers are added. Commercially available linkers often lack the 5´ phosphate groups required for ligation and must be phosphorylated using T4 polynucleotide kinase before ligation.

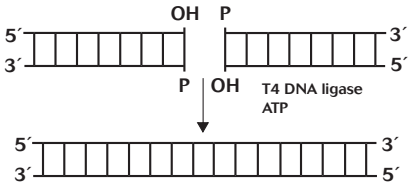
Adapters are short pieces of double-stranded DNA with one blunt end and one cohesive end which encodes for a restriction site. Adapters are formed by annealing two oligonucleotides together, forming a phosphorylated blunt end and a nonphosphorylated cohesive end. As with linkers, ligation is done with a large molar excess of adapters to increase the likelihood that every DNA will have an adapter ligated to its end. Protection of a DNA insert, by methylation, with an internal restriction endonuclease site identical to the adapter's cohesive site does not have to be done since restriction digestion is not necessary to generate the cohesive end. Once adapters are ligated to an insert, it is ligated to a phosphorylated vector.

T4 DNA Ligase is the product of gene 30 of the *Escherichia coli* bacteriophage T4. It is a monomer with a molecular weight of 68 ± 6.8 kDa (6). T4 DNA Ligase is purified from *E. coli* λ lysogen NM989 (7). It is supplied in 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM dithiothreitol, 50% (v/v) glycerol at a concentration of 1 unit/µl. T4 DNA Ligase High Concentration is supplied at a concentration of 5 units/µl.

### Unit definition

One Weiss unit of T4 ligase catalyzes the exchange of 1 nmol $^{32}$P-labeled pyrophosphate into ATP in 20 min at 37°C (8). T4 DNA Ligase is measured in Weiss units. Other suppliers may use cohesive-end ligation units. One Weiss unit is equal to ~300 cohesive-end units.

**Figure 1 - Joining of two double-stranded DNA molecules by T4 DNA Ligase.**




# Materials

In addition to T4 DNA ligase and the insert and vector DNAs, the following reagents and equipment are required for the protocols described below:

**All protocols:**

- 5X DNA ligase buffer [250 mM Tris-HCl (pH 7.6), 50 mM $MgCl_2$, 5 mM ATP, 5 mM dithiothreitol, 25% (w/v) polyethylene glycol 8000]. Store at -20°C. This buffer is included with T4 DNA Ligase.
- Autoclaved, 1.5-ml microcentrifuge tubes
- Autoclaved, distilled water
- Microcentrifuge (15,000 X *g*)
- 70°C water bath

**Ligations for plasmid cloning:**

- 14°C water bath (required for ligation of blunt-ended fragments only)
- 0.5 M EDTA

**Ligation of DNA adapters to blunt-ended DNA:**

- DNA adapters
- T4 polynucleotide kinase
- 0.1 M Dithiothreitol (DTT)
- 5X Adapter buffer [330 mM Tris (pH 7.6), 50 mM $MgCl_2$, 5 mM ATP]
- 16°C water bath
- 37°C water bath

## Ligation protocol for plasmid cloning of DNA fragments with cohesive ends

The following reaction conditions are for ligation of DNA inserts with cohesive ends to DNA vectors with complementary cohesive ends to produce circular recombinant molecules. A molar ratio of 3:1 insert:vector is recommended. See "Additional Information" for a discussion of how to calculate the required quantities of DNA. See "Analysis of Ligations for Transformation" for information on control reactions.

1. In an autoclaved, 1.5-ml microcentrifuge tube, dilute an aliquot of T4 DNA Ligase to 0.1 unit/µl in 1X Ligase Reaction Buffer. Mix gently. Centrifuge briefly to bring the contents to the bottom of the tube.
   **Note:** The ligase must be used immediately after dilution.
2. To an autoclaved, 1.5-ml microcentrifuge tube, add the following:

| Component | Amount |
|---|---|
| 5X ligase buffer | 4 µl |
| vector DNA | 3-30 fmol |
| insert DNA | 9-90 fmol |
| autoclaved distilled water | to 19 µl |

3. Add 1.0 µl (0.1 units) of diluted T4 DNA Ligase. Mix gently. Centrifuge briefly to bring the contents to the bottom of the tube.
4. Incubate at 23°C to 26°C for at least 1 h.
5. Add 1 µl of 0.5 M EDTA to inactivate the enzyme. Store the reaction at 4°C.
6. Dilute an aliquot of the ligation reaction five-fold in autoclaved, distilled water and use it to transform competent cells.

## Ligation protocol for plasmid cloning of DNA fragments with blunt ends

The following reaction conditions are for ligation of DNA inserts with blunt ends to DNA vectors with blunt ends to produce circular recombinant molecules. A molar ratio of 3:1 insert:vector is recommended. See "Additional Information" for a discussion of how to calculate the required quantities of DNA. See "Analysis of Ligations for Transformation" for information on control reactions. For maximum yield of colonies containing recombinant plasmids, incubation of the ligation reaction at 14°C for 24 h is recommended (step 4); however, in situations where a lower yield is acceptable, incubation at 23°C to 26°C for 4 h will permit ligation and transformation to be performed in the same day.

1. In an autoclaved, 1.5-ml microcentrifuge tube, dilute an aliquot of T4 DNA Ligase to 0.5 units/µl with 1X Ligase Reaction Buffer.
   **Note:** The ligase must be used immediately after dilution.
2. To an autoclaved, 1.5-ml microcentrifuge tube add the following:

| Component | Amount |
|---|---|
| 5X ligase buffer | 4 µl |
| vector DNA | 15-60 fmol |
| insert DNA | 45-180 fmol |
| autoclaved distilled water | to 18 µl |

3. Add 2 µl (1 unit) of the diluted T4 DNA Ligase. Mix gently. Centrifuge briefly to bring the contents to the bottom of the tube.
4. Incubate at 14°C for 24 h.
5. Add 1 µl of 0.5 M EDTA to inactivate the enzyme. Store the reaction at 4°C.
6. Dilute an aliquot of the ligation reaction five-fold in autoclaved, distilled water and use it to transform competent cells.

# Analysis of ligations for plasmid cloning

Ligation reactions for transformation are best analyzed by actual transformation of bacteria. Ligation reagents can be tested by gel analysis of ligation reaction products.

## Transformation

For most applications, transformation of competent cells is the ultimate test of a ligation reaction because not all of the high molecular weight forms created in a reaction will transform cells efficiently. Several ligation controls necessary to identify the source of any ligation problems are described below.

1. **Supercoiled vector, or control DNA provided with competent cells.** Perform a transformation reaction and plate the number of cells that is expected to generate 50 to 100 colonies per plate, based on the anticipated transformation efficiency of the competent cells. The expected number of colonies should be seen, indicating that the competent cells are transforming with high efficiency. The control DNA provided with competent cells is supercoiled monomer; vector DNA preparations that contain other forms will not transform as efficiently. Transformation efficiencies will be ~10-fold lower for ligation of inserts to vectors than for intact control DNA.
2. **Restriction endonuclease-digested vector.** Perform a transformation with an amount of vector DNA equivalent to that contained in the fraction of the ligation reaction being used for the experimental transformations. Few or no colonies should be seen, indicating complete restriction endonuclease digestion of the vector. The presence of colonies indicates incomplete digestion of the vector that will cause a background of colonies containing nonrecombinant vector in the experimental transformations.
3. **Restriction endonuclease-digested, religated vector**. Set up a ligation reaction using the same amount of vector DNA that is used in the experimental ligations and use it to transform competent cells. Religation of vectors with cohesive ends should result in ≥50% of the number of colonies obtained with supercoiled vector DNA, indicating that the components of the ligation reaction are working; religation of vectors with blunt ends should yield 10% to 20% of the number of colonies obtained with supercoiled vector DNA. This is an appropriate control only with vectors that have been digested with a single restriction endonuclease; double-digested vectors may not religate because the ends are incompatible and the small DNA fragment that is released from between the two sites is sometimes lost during ethanol precipitation of the DNA.
4. **Restriction endonuclease-digested, dephosphorylated, religated vector.** Set up a ligation reaction using the same amount of vector DNA that is used in the experimental ligations and use it to transform competent cells. Few or no colonies should be observed, indicating complete dephosphorylation of the vector. The dephosphorylated vector is not ligated by T4 DNA Ligase.
5. **No vector.** Perform a mock transformation of competent cells, to which no DNA is added. No colonies should be seen, indicating that the selecting antibiotic on the agar plates is potent and that the competent cells are pure.

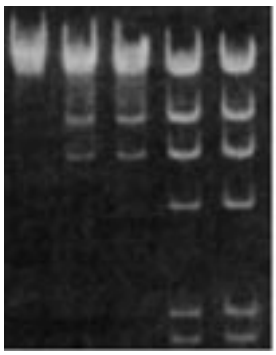
## Gel analysis

The ligation reagents can be tested by performing a ligation reaction with a molecular size marker such as the 1 Kb DNA Ladder or λ DNA/*Hind* III Fragments (9,10). Compare the ligation reaction products to unligated DNA on an agarose gel (figure 2). The ligation reaction should contain a high molecular weight smear and few low molecular weight bands. To test for the presence of ligation inhibitors, perform a ligation reaction in which some of the insert or vector DNA is included along with some marker DNA. If ligation of the DNA marker fragments occurs alone but is not observed when other DNA is added, then an inhibitor is present in the vector or insert DNA.

Gel analysis is not a good method of evaluating ligation reactions for transformation due to the low concentration of DNA. Although gel electrophoresis of a ligation reaction should reveal the disappearance of the vector and insert fragments and the appearance of higher molecular weight forms, not all high molecular weight forms will transform efficiently.

**Figure 2 - Ligation of λ DNA/ *Hind* III fragments analyzed by agarose gel electrophoresis.**

Using 1 µg of l λ DNA/ *Hind* III fragments, a series of 20-µl reaction mixtures containing 0, 0.001, 0.01, 0.1, or 0.5 units (right to left) of T4 DNA Ligase were incubated at 22°C for 1 h.



# Protocol for ligation of adapters to DNA fragments with blunt ends

An adapter adds cohesive ends directly to any blunt-ended DNA fragment. An adapter has a phosphorylated blunt end and a nonphosphorylated cohesive end. A 20:1 molar ratio (adapter:insert) is recommended. The adapted DNA, after excess adapter removal, can be ligated directly into a phosphorylated vector with compatible cohesive ends. However, if a dephosphorylated vector is used, the adapted DNA must be phosphorylated. Phosphorylation of the adapted DNA and ligation to the vector can be done conveniently in a single buffer as described in the protocol below. This one buffer reaction allows ligation and phosphorylation to be performed without any organic extraction or ethanol precipitation, which minimizes DNA losses. When using a vector that does not need phosphorylation, ligation can be done using the 5X DNA Ligase Buffer provided with the enzyme. See "Additional Information" for a discussion of how to calculate the required quantities of DNA.

## Adapter addition to DNA

1. To an autoclaved 1.5-ml microcentrifuge tube on ice, add the following:

| Component | Amount |
|---|---|
| blunt-ended DNA | 0.5 pmol |
| 5X Adapter buffer | 10 µl |
| adapter | 10 pmol |
| 0.1 M DTT | 7 µl |
| autoclaved distilled water | up to 45 µl |

2. Add 5µl of T4 DNA Ligase (1 unit/µl) and mix gently.
3. Incubate at 16°C for a minimum of 16 h.
4. Heat the reaction at 70°C for 10 min to inactivate the T4 DNA Ligase.
5. Place on ice.

**Table 1 - Reaction parameters for ligation**

| Type of Ligation | | | | | |
|---|---|---|---|---|---|
| | Plasmid | | | | |
| Parameter | Cohesive Ends | Blunt Ends | Adapters | Linkers | λ |
| T4 DNA Ligase (units) | 0.1 | 1 | 5 | 2 | 1 |
| Amount of DNA | | | | | |
| Vector (fmol) | 3-30 | 15-60 | - | - | - |
| Insert (fmol) | 9-90 | 45-180 | 500 | 1400 | - |
| Linker (pmol) | - | - | - | 150 | - |
| Adapter (pmol) | - | - | 10 | - | - |
| Vector (ng) | - | - | - | - | 1000 |
| Insert (ng) | - | - | - | - | 50 |
| Molar ratio | | | | | |
| Insert:vector | 3:1 | 3:1 | - | - | - |
| Linker:insert | - | - | - | 100:1 | - |
| Adapter:insert | - | - | 20:1 | - | - |
| Temperature (°C) | 23-26 | 14 | 16 | 14 | 4 |
| Time (h) | ≥1 | 24 | 16 | 16 | ≥16 |
| Reaction volume (µl) | 20 | 20 | 50 | 50 | 10 |

## Phosphorylation of adapted DNA

6. Add 3 µl (30 units) of T4 polynucloetide kinase and mix gently.
7. Incubate 30 min at 37°C.
8. Heat the reaction at 70°C for 10 min to inactivate the T4 polynucleotide kinase.
9. Place on ice.
10. Remove excess adapters by column chromatography (*i.e.* cDNA Size Fractionation Columns) or by gel electrophoresis and then ligate into an appropriate vector.

## Analysis of adapter ligation

Adapter addition is assayed most readily by nondenaturing polyacrylamide gel electrophoresis. A 10-µl aliquot of the adapter ligation reaction is electrophoresed on a 12% (w/v) polyacrylamide gel and the DNA is visualized by ethidium bromide staining. The ligation of two adapters to each other at their blunt ends will be evidenced by a fragment twice the size as the original adapter. Although this does not verify that the adapters have ligated to the DNA, it does show that the ligation reaction is functionally sound.

## Ligation protocol for λ cloning of DNA fragments with cohesive ends

The following reaction conditions are for ligation of DNA inserts to dephosphorylated λ arms that have been previously ligated at their *cos* ends.

1. In an autoclaved, 1.5-ml microcentrifuge tube, dilute an aliquot of T4 DNA Ligase to 0.5 units/µl with 1X T4 Ligase Reaction Buffer.
   **Note:** The ligase must be used immediately after dilution.
2. To an autoclaved, 1.5-ml microcentrifuge tube, add the following:

| Component | Amount |
|---|---|
| 5X ligase buffer | 2 µl |
| λ vector DNA | 1 µg |
| insert DNA | 50 ng |
| autoclaved distilled water | up to 8 µl |

3. Add 2 µl (1 unit) of the diluted T4 DNA Ligase. Mix gently. Centrifuge briefly to bring the contents to the bottom of the tube.
4. Incubate at 4°C for at least 16 h.
5. Heat the reaction at 70°C for 10 min to inactivate the T4 DNA Ligase.
6. Package the reaction products into bacteriophage.
   **Note:** Packaging may be inhibited by polyethylene glycol concentrations >1%. Because the ligation reaction contains 5% polyethylene glycol, it is necessary to dilute the ligation reaction if it contributes more than 20% of the final volume of the packaging reaction.

## Troubleshooting

Because ligation is assayed by transformation of bacteria, it is important to be sure that the transformation procedure itself is working. The controls described in "Analysis of Ligations for Plasmid Cloning" will help to distinguish between problems with the ligation reaction and problems with the competent cells, the selection medium, the restriction endonuclease digestion of the vector, and the phosphatase treatment of the vector. Because transformation of some competent cells is inhibited by components of the ligation reaction, the reaction should be diluted five-fold before being used for transformation (13). Some possible causes of unsuccessful ligation are listed below along with suggested solutions.

| Possible Causes | Suggested Solutions |
|---|---|
| Inhibitors of DNA ligase are present in the insert or vector DNA (See "Analysis of Ligations for Plasmid Cloning" for more information.) | Extract the DNA with buffer-saturated phenol (14), then extract with chloroform:isoamyl alcohol, then precipitate with ammonium acetate and ethanol (15).<br><br>Be sure that the DNA is free of phenol and that the phosphate concentration is < 25 mM and the NaCl concentration is < 50 mM.<br><br>Be sure that the DNA is free of contaminating DNA that might compete for ligation to the insert or vector (*e.g.*, linker fragments, DNA fragments from which the insert was incompletely purified). |
| DNA ligase is inactive (See "Analysis of Ligations for Plasmid Cloning" for procedures to check ligase activity.) | Use fresh ligase. |
| ATP or DTT in the reaction buffer has degraded | Use 5X ligase buffer that is < 24 months old. Store the buffer at -20°C. |
| The insert and vector DNA (or adapters) have incompatible ends | Confirm that the vector and insert have been digested with the same restriction endonuclease or with different restriction endonucleases that generate compatible ends.<br><br>If the vector has been digested with two restriction endonucleases, be sure that both digestions are complete (16).<br><br>If ligating blunt-ended molecules, treat the vector and/or insert with T4 DNA polymerase to be sure that the ends are blunt (17). |
| DNA is degraded by nonspecific endodeoxyribonucleases contaminating the reaction mixture. | Use fresh buffer, fresh ligase, and autoclaved, distilled water. |
| Restriction endonucleases are present, causing redigestion of ligated products | After digestion of the vector and insert DNA, remove restriction endonucleases by extraction with buffer-saturated phenol (14), extraction with chloroform:isoamyl alcohol, and ethanol precipitation (15). |
| Concentrations of DNAs are incorrect | Check concentration of DNAs by gel electrophoresis. |

# Additional information

## Reaction conditions

Temperature affects the rate and extent of ligation. Blunt end ligation at 26°C produces 90% as many transformants in 4 h as are obtained in 23 h and 25-fold more transformants than are obtained at 4°C in 4 h (1); however, overnight incubation at 14°C produces 4-fold more transformants than a 4 h incubation at room temperature (2).

T4 DNA Ligase requires $Mg^{2+}$. Using the unit assay conditions, the optimal concentration is 10 mM; at 3 mM $Mg^{2+}$, 35% of maximal activity is observed; at 30 mM $Mg^{2+}$, 80% of maximal activity is observed. $Mn^{2+}$ can substitute for $Mg^{2+}$; however, at the optimal $Mn^{2+}$ concentration of 10 mM, the activity is 25% of that observed with 10 mM $Mg^{2+}$ (8).

The optimal pH range for T4 DNA Ligase is 7.2 to 7.8. At pH 6.9 the enzyme is 46% as active as it is at pH 7.6; at pH 8.0, it is 65% as active as it is at pH 7.6 (6).

High molecular weight polymers such as polyethylene glycol (PEG), Ficoll, bovine serum albumin, and glycogen have been shown to enhance both blunt end and cohesive end ligation by T4 DNA Ligase (18). When ligation is assayed by the number of transformants obtained, a sharp optimum is seen at a PEG 8000 concentration of 5% (1).

Hexamine cobalt chloride has been shown to enhance ligation of blunt-ended fragments when ligation is assayed by gel electrophoresis (19), but it does not increase the number of transformants obtained when ligations are performed according to the protocols described above (1).

Although T4 RNA ligase was reported to enhance the ligation of blunt-ended DNA fragments by T4 DNA Ligase (20), subsequent studies were unable to confirm this finding (18).

## Inhibitors

dATP is a competitive inhibitor of T4 DNA Ligase, resulting in a 60% decrease in activity at a concentration of 66 µM. The following compounds do not inhibit ligase at a concentration of 66 µM: GTP, CTP, UTP, dGTP, dCTP, dTTP, ADP, AMP, $NAD^+$ (8). Transfer RNA does not inhibit T4 DNA Ligase (21).

The following ions inhibit T4 DNA Ligase almost completely at concentrations of 0.2 M: $K^+$, $Cs^+$, $Li^+$, $NH_4^+$ (6); however, $NH_4^+$ is not inhibitory at a concentration of 10 mM (22). NaCl at a concentration of 50 mM causes negligible inhibition of blunt end ligation and 15% inhibition of cohesive end ligation; at a concentration of 150 mM, NaCl inhibits blunt end ligation by 50% and cohesive end ligation by 40%. Potassium phosphate at a concentration of 50 mM inhibits blunt end ligation by 50% and cohesive end ligation by 5% (23). EDTA inhibits T4 DNA Ligase by chelating $Mg^{2+}$ ions.

## Ligation of linkers to DNA fragments with blunt ends

Ligation of linkers to blunt-ended DNA is performed at a 100:1 molar ratio of linker to insert. If the linkers are not phosphorylated they must be phosphorylated with T4 polynucleotide kinase prior to ligation. Linker addition is typically done in a 50 µl volume with 2 units of T4 DNA Ligase, 1.4 pmol blunt-ended DNA, 150 pmol of phosphorylated linkers in 1X T4 DNA Ligase Buffer for 16 h at 14°C. Digestion with the appropriate restriction endonuclease is necessary to remove excess linkers. Phenol extraction, chloroform:isoamyl alcohol extraction, and ethanol precipitation of the DNA are recommended before restriction endonuclease digestion to ensure complete digestion. If you omit these steps, it will be necessary to inactivate the ligase by heating to 70°C for 10 min and to decrease the polyethylene glycol concentration by diluting the ligation reaction at least 2.5-fold before performing the restriction endonuclease digestion.

## Storage and stability

Store T4 DNA Ligase and 5X ligase buffer at -20°C.

## Inactivation

T4 DNA Ligase can be inactivated by heating to 70°C for 10 min, or by adding EDTA to 25 mM to chelate the $Mg^{2+}$ ions in the reaction mixture.

# Additional information (cont.)

### Calculation of molar ratios

One microgram of a 1000-bp DNA fragment is equivalent to 3000 fmol of double-stranded DNA ends. The amount of double-stranded DNA corresponding to a given number of ends can be calculated as follows:

$$\mu g\ DNA = fmoles\ DNA \times \frac{1\ \mu g}{3000\ fmol} \times \frac{size\ of\ DNA\ in\ bp}{1000\ bp}$$

**Example:** A 1600-bp insert with cohesive ends is to be cloned into a 2700 bp vector. Ten fmol of vector will be used. An insert:vector molar ratio of 3:1 is desired. The number of micrograms of DNA corresponding to 10 fmol of ends of vector can be calculated:

$$\text{amount of vector} = 10\ fmol \times \frac{1\ \mu g}{3000\ fmol} \times \frac{2700\ bp}{1000\ bp}$$

$$= 0.009\ \mu g$$

$$= 9\ ng$$

The number of femtomoles of insert ends required is 3 times the number of fmoles of vector ends, or 30 fmol. The amount of double-stranded insert DNA required is:

$$\text{amount of insert} = 30\ fmol \times \frac{1\ \mu g}{3000\ fmol} \times \frac{1600\ bp}{1000\ bp}$$

$$= 0.016\ \mu g$$

$$= 16\ ng$$

### Other activities of T4 DNA ligase

T4 DNA Ligase will ligate DNA/RNA hybrid molecules with low efficiency, joining either DNA to DNA or DNA to RNA. It will ligate double-stranded RNA molecules with extremely low efficiency (6). The enzyme can be used to repair nicks in double-stranded DNA (6,8). Single-stranded nucleic acids are not substrates for T4 DNA Ligase.

# Ordering information

| Description | Concentration | Quantity | Cat. No. |
|---|---|---|---|
| T4 DNA Ligase | 1 unit/µl | 100 units | 15224-017 |
| | 1 unit/µl | 500 units | 15224-025 |
| | 5 units/µl | 250 units | 15224-041 |
| T4 DNA Ligase Buffer | | 2 X 1 ml | 46302-018 |
| Bacterial Alkaline Phosphatase (BAP) | 150 units/µl | 2,500 units | 18011-015 |
| Calf Intestinal Alkaline Phosphatase (CIAP) | 20 units/µl | 1,000 units | 18009-019 |
| | 1 unit/µl | 1,000 units | 18009-027 |
| DNA Polymerase I, Large (Klenow) Fragment | 3-9 units/µl | 100 units | 18012-021 |
| | 3-9 units/µl | 500 units | 18012-039 |
| | 3-9 units/µl | 2 X 500 units | 18012-096 |
| T4 Polynucleotide Kinase | 10 units/µl | 200 units | 18004-010 |
| | 10 units/µl | 1,000 units | 18004-028 |
| 1 Kb DNA Ladder | | 250 µg | 15615-016 |
| | | 1,000 mg | 15615-024 |
| λ DNA/*Hind* III Fragments | | 500 µg | 15612-013 |
| Dithiothreitol | | 5 g | 15508-013 |
| Phenol | | 500 g | 15509-037 |
| Buffer-Saturated Phenol | | 100 ml | 15513-039 |
| | | 400 ml | 15513-047 |
| Phenol:Chloroform:Isoamyl Alcohol | | 100 ml | 15593-031 |
| | | 400 ml | 15593-049 |
| Tris | | 1 kg | 15504-020 |
| S.N.A.P.™ Gel Purification Kit | | 25 rxns | K1999-25 |
| S.N.A.P.™ UV-Free Gel Purification Kit | | 25 rxns | K2000-25 |

For research use only. Not for diagnostic or therapeutic use in humans or animals.

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