Quick Ligation™ Kit

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M2200S

30 Reactions   **Lot: 1011202**   **Exp: 2/14**
**Store at –20°C**

Description: The Quick Ligation™ Kit enables ligation of cohesive end or blunt end DNA fragments in 5 minutes at room temperature (25°C).

Applications:

- Cloning into vectors
- Library construction
- TA cloning
- Linker ligation
- Recircularization of linear DNA

Kit Components:

Quick T4 DNA Ligase (recombinant)	0.03 ml
2X Quick Ligation Reaction Buffer	1.00 ml

Ligation Time Course (0–15 minutes)
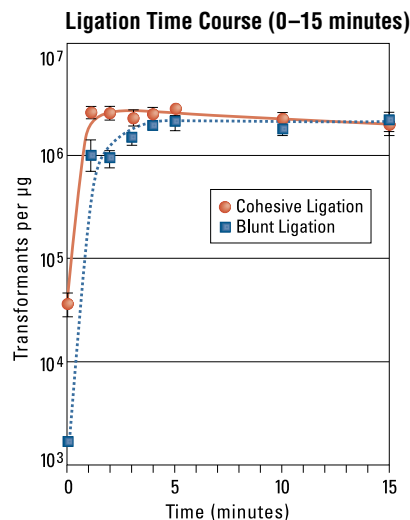
Ligation Time Course: LITMUS 28 vector (NEB #N3628) was cut with either EcoRV (blunt) or HindIII (cohesive), treated with calf intestinal phosphatase and gel purified. Blunt inserts from a HaeIII digest of φX174 DNA and cohesive inserts from a HindIII digest of λ DNA were ligated into the respective vectors at a 3:1 insert:vector ratio using the Quick Ligation Kit. Ligation products were transformed into chemically competent E. coli DH-5α cells and grown overnight on LB-amp plates at 37°C.

Quick T4 DNA Ligase supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

2X Quick Ligation Reaction Buffer:
132 mM Tris-HCl
20 mM $MgCl_2$
2 mM dithiothreitol
2 mM ATP
15% Polyethylene glycol (PEG 6000)
pH 7.6 @ 25°C

Note: Mix thoroughly before use. Avoid repeated freeze/thaw cycles.

Quality Controls

The Quick Ligation Kit is tested for transformation efficiency using the following protocol.

LITMUS 28 vector is cut with either EcoRV (blunt) or HindIII (cohesive), treated with calf intestinal phosphatase and gel purified. Blunt inserts from a HaeIII digest of φX174 DNA and cohesive inserts from a HindIII of digest of λ DNA are ligated into the respective vectors at a 3:1 insert:vector ratio using the Quick Ligation Protocol. Ligation products are transformed as described.

Each lot exceeds the following standards:

	Efficiency (transformants/µg)	
	Recircularization	Insertion
Cohesive ends	$> 5 \times 10^6$	$> 1 \times 10^6$
Blunt ends	$> 2 \times 10^6$	$> 1 \times 10^6$
Uncut vector	2×10^7	

Quick Ligation Protocol

1. Combine 50 ng of vector with a 3-fold molar excess of insert. Adjust volume to 10 µl with dH_2O.

2. Add 10 µl of 2X Quick Ligation Reaction Buffer and mix.

3. Add 1 µl of Quick T4 DNA Ligase and mix thoroughly.

4. Centrifuge briefly and incubate at room temperature (25°C) for 5 minutes.

5. Chill on ice, then transform or store at –20°C.

6. **Do not heat inactivate.**
   Heat inactivation dramatically reduces transformation efficiency.

Transformation Protocol:

Quick Ligation products may be transformed by many different methods. The following protocol is recommended by New England Biolabs.

1. Thaw competent cells on ice.

2. Chill approximately 5 ng (2 µl) of the ligation mixture in a 1.5 ml microcentrifuge tube.

3. Add 50 µl of competent cells to the DNA. Mix gently by pipetting up and down or flicking the tube 4–5 times to mix the cells and DNA. Do not vortex.

4. Place the mixture on ice for 30 minutes. Do not mix.

5. Heat shock at 42°C for 30 seconds*. Do not mix.

6. Add 950 µl of room temperature media* to the tube.

7. Place tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.

8. Warm selection plates to 37°C.

9. Spread 50–100 µl of the cells and ligation mixture onto the plates.

10. Incubate overnight at 37°C

* Please note: For the duration and temperature of the heat shock step as well as for the media to be used during the recovery period, please follow the recommendations provided by the competent cells' manufacturer.

Plasmid Recircularization

Use the Quick Ligation Protocol with 50 ng of linearized vector without insert.

Example: LITMUS 28 cloning vector was cut with HindIII. The reaction mixture was heated at 65°C for 20 minutes to inactivate the endonuclease. The DNA was recircularized using the Quick Ligation Protocol and transformed.

Vector:	(50 ng) 2.5 µl
Insert:	None
dH_2O:	7.5 µl
2X Quick Ligation Reaction Buffer:	10 µl
Quick T4 DNA Ligase	1 µl

Results (transformants/µg):

Control plasmid (uncut)	1.3×10^7
Linearized plasmid	1.1×10^4
Recircularized plasmid	2.1×10^7

Cohesive End Ligation

Use the Quick Ligation Protocol with 50 ng of linearized dephosphorylated vector and a 3:1 molar excess of insert.

Example: Two 2 kb fragments from a HindIII digest of λ DNA were copurified from an agarose gel and inserted into HindIII cleaved, dephosphorylated pUC19 vector. The DNA was ligated using the Quick Ligation Protocol and transformed.

Vector:	(50 ng) 3.3 µl
Insert (3:1):	(122.5 ng) 4.9 µl
dH_2O:	1.8 µl
2X Quick Ligation Reaction Buffer:	10 µl
Quick T4 DNA Ligase	1 µl

Results (transformants/µg):

Control plasmid (uncut)	2.0×10^7
Vector without insert	1.1×10^5
Vector + insert	2.1×10^6

Blunt End Ligation

Use the Quick Ligation Protocol with 50 ng of linearized, dephosphorylated vector and a 3:1 molar excess of insert.

Example: A 1.9 kb EcoR V fragment was generated from λ DNA by PCR, cleaved to create EcoRV ends and inserted into EcoR V cleaved, dephosphorylated LITMUS 28 vector. The DNA was ligated using the Quick Ligation Protocol and transformed.

Vector:	(50 ng) 2.0 µl
Insert (3:1):	(130 ng) 1.7 µl
dH_2O:	6.3 µl
2X Quick Ligation Reaction Buffer:	10 µl
Quick T4 DNA Ligase	1 µl

Results (transformants/µg):

Control plasmid (uncut)	1.1×10^7
Vector without insert	5.0×10^3
Vector + insert	1.4×10^5

Linker Ligation

Use the Quick Ligation Protocol with 50–250 ng of blunt-ended DNA and a 20-fold excess of linker. If necessary, the protocol can be directly scaled up for larger amounts of DNA.

(See other side)

CERTIFICATE OF ANALYSIS

Example: Dephosphorylated, EcoRV-digested LITMUS 28 vector was ligated to HindIII linkers (8 mer) using the Quick Ligation Protocol. Ligation products were purified, digested with HindIII, repurified and recircularized using the Quick Ligation Protocol.

Vector:	(1 µg) 6.7 µl
Linkers (20:1):	(20 pmols) 3.5 µl
dH$_2$O:	34.8 µl
2X Quick Ligation Reaction Buffer:	50 µl
Quick T4 DNA Ligase	5 µl

Results (transformants/µg):

Control plasmid (uncut)	6.2×10^6
Vector without linkers	3.0×10^3
Hind III cut vector	3.9×10^6
Vector + linkers	2.0×10^5

Usage Notes

Some of the most critical parameters which should be controlled to ensure successful ligation and transformation are addressed below.

Cells: Competent cells can vary by several logs in their competence. Perceived ligation efficiency directly correlates to the competence of the cells used for transformation. Always transform uncut vector as a control for comparison purposes.

Electroporation: Electroporation can increase transformation efficiency by several logs. Before using the products of a Quick Ligation reaction for electrotransformation, it is necessary to reduce the PEG concentration. We recommend a spin column purification.

DNA: Purified DNA for ligations can be dissolved in dH$_2$O (Milli-Q water or equivalent is preferable); TE or other dilute buffers also work well. For optimum ligation, the volume of DNA and insert should be 10 µl before adding 2X Quick Ligation Reaction Buffer. For DNA volumes greater than 10 µl, increase the volume of 2X Quick Ligation Reaction Buffer such that it remains 50% of the reaction and correspondingly increase the volume of ligase.

The overall concentration of vector + insert should be between 1–10 µg/ml for efficient ligation. Insert:vector ratios between 2 and 6 are optimal for single insertions. Ratios below 2:1 result in lower ligation efficiency. Ratios above 6:1 promote multiple inserts. If you are unsure of your DNA concentrations, perform multiple ligations with varying ratios.

Time: Most ligations performed using the Quick Ligation Kit reach an end point at 5 minutes or less at 25°C. Incubation beyond this time provides no additional benefit. In fact, transformation efficiency starts to decrease after 2 hours and is reduced by up to 75% if the reaction is allowed to go overnight at 25°C.

Biology: Some DNA structures, including inverted and tandem repeats, are selected against by *E. coli*. Some recombinant proteins are not well tolerated by *E. coli* and can result in poor transformation or small colonies.

Calculation of Molarity of Ends

Molarity = [(µg/µl) ÷ (base pairs x 650 daltons)] x 2 ends

Example:

• Calculate the molarity of ends for a linearized 5 kb vector that has a concentration of 250 ng/µl:

[(0.25 µg/µl) ÷ (5000 x 650 daltons)] x 2 = 154 nM

• Calculate the molarity of ends if you put 50 ng of this vector in a 20 µl ligation reaction:

50 ng ÷ 20 µl = 0.0025 µg/µl
[(0.0025 µg/µl) ÷ (5000 x 650)] x 2 = 1.54 nM

• Determine the amount of a 1 kb insert needed to achieve a 3:1 insert:vector ratio:

3 X 1.54 nM = 4.62 nM
[(? µg/µl) ÷ (1000 x 650)] x 2 = 4.62 nM
0.0015 µg/µl x 20 µl = 0.03 µg = 30 ng

• Does this reaction fall within the optimal range of 1–10 µg/ml?

(50 ng vector + 30 ng insert) ÷ 20 µl = 4 µg/ml