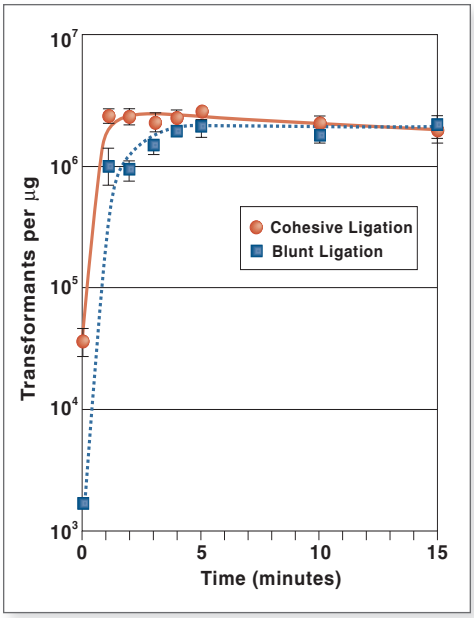
Quick Ligation™ Kit

The Quick Ligation™ Kit enables ligation of cohesive end or blunt end DNA fragments in 5 minutes at room temperature.

Ligation Time Course



LITMUS 28 vector (NEB #N3628S) 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 Protocol. Ligation products were transformed into chemically competent E. coli DH-5α cells and grown overnight on LB-amp plates at 37°C.

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 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 and mix gently by pipetting up and down.
4. Incubate on ice for 30 minutes.
5. Heat shock for 2 minutes at 37°C, chill on ice for 5 minutes.
6. Add 950 µl of room temperature media and incubate at 37°C for 1 hour.
7. Spread 100 µl onto the appropriate solid medium.
8. Incubate overnight at 37°C.

(see other side)

Applications

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

Kit Includes

- Quick T4 DNA Ligase (recombinant)
- 2X Quick Ligation Buffer

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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:	2.5 µl (50 ng)
Insert:	None
dH_2O:	7.5 µl
2X Quick Ligation 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 Hind III 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:	3.3 µl (50 ng)
Insert (3:1):	4.9 µl (122.5 ng)
dH_2O:	1.8 µl
2X Quick Ligation 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 EcoR V ends and inserted into EcoR V cleaved, dephosphorylated LITMUS 28 vector.
The DNA was ligated using the Quick Ligation Protocol and transformed.

Vector:	2 µl (50 ng)
Insert (3:1):	1.7 µl (103 ng)
dH_2O:	6.3 µl
2X Quick Ligation 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

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_2O (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 Buffer. For DNA volumes greater than 10 µl, increase the volume of 2X Quick Ligation 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.

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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.
Example: Dephosphorylated, EcoR V-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:	6.7 µl (1 µg)
Linkers (20:1):	3.5 µl (20 pmols)
dH_2O:	34.8 µl
2X Quick Ligation 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

Calculation of Molarity of Ends

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

Example:

1. 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
2. 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
3. Determine the amount of a 1 kb insert should be added 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
4. Does this reaction fall within the optimal range of 1–10 µg/ml?
   (50 ng vector + 30 ng insert) ÷ 20 µl = 4 µg/ml

Ordering Information

PRODUCT	NEB #	SIZE	PRICE
Quick Ligation Kit	M2200 S/L	30/150 reactions	$95/$380

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