

## TA Cloning® Kits

**Catalog Number** K2000-01, K2000-40, K2020-20, K2020-40, K2030-01, K2030-40, K2040-01, K2040-40

**Doc. Part no.** 25-0069 **Pub. No.** MAN0006844 **Rev.** 2.00

### Produce PCR Products

1. Produce PCR products using Taq polymerase and your own protocol. End the PCR reaction with a final 7–10 minute extension step.
2. Analyze 10 µL of each PCR sample by agarose gel electrophoresis.
3. Quantify the amount of DNA for each PCR product.

### Clone into pCR® 2.1

1. Ligation Reaction (10 µL):

Sterile Water	up to 5 µL
5X ExpressLink™T4 DNA Ligase Buffer	2 µL
pCR®2.1vector (25 ng/µL)	2 µL
Fresh PCR product (~10 ng)	x µL
ExpressLink™T4 DNA Ligase (5 units)	1 µL
Total volume	10 µL

2. Incubate the ligation reaction at room temperature for a minimum of 15 minutes. Longer incubation times increase the cloning efficiency.
3. Centrifuge the ligation reactions briefly and place on ice.
4. Proceed to One Shot® Chemical Transformation.

### One Shot® Chemical Transformation

1. Thaw One Shot® cells on ice.  
**Note:** SOC medium, and positive control are provided in kits with the One Shot® chemically competent cells.
2. Pipet 1–2 µL of each ligation reaction into the cells and gently stir the mixture with a pipette tip to mix.
3. Incubate the vials on ice for 30 minutes.
4. Heat shock the cells for 30 seconds at 42°C without shaking. Transfer the vials to ice.
5. Add 250 µL of S.O.C. medium to each vial.
6. Shake the vials at 37°C for 1 hour at 225 rpm.

7. Plate 10–200 µL from each transformation vial on an LB plate containing X-gal and 50 µg/mL kanamycin or 100 µg/mL ampicillin. Add IPTG if using TOP10F' cells.

8. Incubate the plates overnight at 37°C.

### Analyze Positive Clones

1. Pick at least 10 transformants for analysis.
2. Analyze the plasmid DNA by restriction analysis or sequencing to verify the correct insert and orientation. We recommend using the Pure Link™ HQ Mini Plasmid Purification Kit for purifying your plasmid DNA.
3. Purify the colony and make a glycerol stock for long-term storage. We recommend that you store a stock of plasmid DNA at –20°C.

### Obtaining Support

A detailed protocol, "Original TA Cloning® Kit" is available online, go to:

**[www.lifetechnologies.com/support](http://www.lifetechnologies.com/support)**

At the website, you can also :

- Access additional products used with the Original TA Cloning® Kit
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**[techsupport@lifetech.com](mailto:techsupport@lifetech.com)**)
- Obtain information about customer training
- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search for other user documents including SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Download detailed protocols and manuals

### Limited product warranty

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