

TA Cloning® Kit Dual Promoter (pCR® II)

Catalog Number K2050-01, K2050-40, K2060-01, K2060-40, K2070 20, and K2070-40

Pub. No. MAN0007771 **Rev.** 1.00

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® II

Set up the following 10 µL ligation reaction:

Fresh PCR product (~10 ng)	X µL
5X ExpressLink™T4 DNA Ligase Ligase (5 units)	2 µL
pCR®II vector (25 ng/µL)	2 µL
Water to a total volume of	9 µL
ExpressLink™T4 DNA Ligase Ligase (5 units)	1 µL
Final volume	10 µL

Incubate the ligation reaction at room temperature for a minimum of 15 minutes. Longer incubation times increase the cloning efficiency.

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 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.

Analyse Positive Clones

1. Pick at least 10 white transformants for plasmid isolation.
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, "TA Cloning® Kit Dual Promoter (pCR® II) is available online, go to:

www.lifetechnologies.com/support

At the website, you can also :

- Access additional products used with the TA Cloning® Kit Dual Promoter pCR® II
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at

www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

DISCLAIMER: LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

© 2013 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners.

Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit lifetechnologies.com/support or email techsupport@lifetech.com

lifetechnologies.com

