

pCR™ 8/GW/TOPO® TA Cloning® Kits

Catalog numbers K2500-20SC and K2520-20SC

Publication part number 25-0806 MAN0005691 Revision Date 20 April 2012

Description

Instructions are provided to TOPO® Clone your PCR product into the TOPO® vector and transform the reaction into chemically-competent *E. coli* cells. For transformation of electrocompetent *E. coli* cells, a map of the TOPO® vector, a diagram of the multiple cloning site, and a manual, refer to www.lifetechnologies.com or contact Technical Support.

Produce PCR Products

Produce PCR products using *Taq* polymerase and your own protocol. End the PCR reaction with a final 7–30 minute extension step.

TOPO® Cloning Reaction

1. Set up the following 6 μ L TOPO® Cloning reaction:

Reagent	Amount
Fresh PCR Product	0.5–4 μ L
Salt Solution	1 μ L
Sterile Water	add to a total volume of 5 μ L
TOPO® Vector	1 μ L
Final Volume	6 μ L

2. Mix gently and incubate for 5 minutes at room temperature.
3. Place tube on ice. Proceed to **Transformation and Analysis**.

Transformation and Analysis

Protocols to transform chemically-competent cells and to analyze positive clones are provided below. Use the **Rapid One Shot[®] Chemical Transformation** protocol if you wish to obtain transformants as quickly as possible. To transform electrocompetent cells, refer to the pCR[®]8/GW/TOPO[®] TA Cloning[®] Kit manual for instructions.

One Shot[®] Chemical Transformation

1. Thaw on ice 1 vial of One Shot[®] *E. coli* cells for each transformation.
2. Add 2 μ L of the TOPO[®] Cloning reaction from step 3 on page 1 to a vial of One Shot[®] *E. coli* and mix gently.
3. Incubate the tube on ice for 5–30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Add 250 μ L of room temperature S.O.C. Medium to the cells.
6. Cap the tubes and shake at 37°C for 1 hour.
7. Spread 10–50 μ L from each transformation on prewarmed LB plates containing 100 μ g/mL spectinomycin.
8. Incubate plates overnight at 37°C.
9. An efficient TOPO[®] Cloning reaction should produce several hundred colonies, with cloning efficiency > 95%. Pick ~10 colonies and proceed to **Analyze Positive Clones**.

Transformation and Analysis, Continued

Rapid One Shot[®] Chemical Transformation

1. Thaw **on ice** 1 vial of One Shot[®] *E. coli* for each transformation.
2. Add 4 μL of the TOPO[®] Cloning reaction from step 3 on page 1 to a vial of One Shot[®] *E. coli* and mix gently.
3. Incubate on ice for 5 minutes.
4. Spread 50 μL from each transformation on **prewarmed** LB plates containing 100 $\mu\text{g}/\text{mL}$ spectinomycin. Incubate plates overnight at 37°C.
5. An efficient TOPO[®] Cloning reaction should produce several hundred colonies, with cloning efficiency > 95%. Pick ~10 colonies and proceed to **Analyze Positive Clones**.

Analyze Positive Clones

1. Culture the 10 colonies overnight in LB medium containing 100 $\mu\text{g}/\text{mL}$ spectinomycin.
2. Isolate plasmid DNA using your method of choice.
To prepare plasmid DNA for sequencing, use the PureLink[®] HQ Mini Plasmid Purification Kit (Cat. no. K2100-01).
3. Analyze the plasmid by restriction digest or sequencing to confirm the presence and orientation of the insert. Use the GW1 and GW2 primers included in the kit for sequencing.
4. *Optional*: Transfer your gene into a destination vector of choice using Gateway[®] Technology. Refer to the pCR[®]8/GW/TOPO[®] TA Cloning[®] Kit manual for guidelines.

Purchaser Notification

Limited Use Label License: ULB ccdB Selection Technology

ccdB selection technology is described in Bernard et al., "Positive Selection Vectors Using the F Plasmid ccdB Killer Gene" Gene 148 (1994) 71-74. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). For licensing information for use in other than research, please contact: outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

©2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation 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 www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

