Purchaser Notification

Catalog no. K2800-20SC

Limited Use Label License No. 54: *ccd*B-Fusion Vectors

This product is the subject of one or more of U.S. Patent Numbers 5,910,438 and 6,180,407 and corresponding foreign patents and is sold under license from the Université Libre de Bruxelles for research purposes only. 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, contact: Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, CA 92008. Tel: 760-603-7200; Fax: 760-602-6500

Limited Use Label License No. 118: TOPO® Cloning Products

The TOPO® Cloning Technology products and their use are the subject of one or more of U.S. Patent Nos. 5,766,891, 6,548,277 and/or other pending U.S. and foreign patent applications licensed to Invitrogen Corporation.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). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

Part no. 25-0649C Rev. Date: 17 Jan 2005

Corporate Headquarters
Tel: 800 955 6288
Fax: 760 602 6500
tech_service@invitrogen.com

invitrogen™

For contact information for other countries, visit our Web site at www.invitrogen.com.

1

Zero Blunt® TOPO® PCR Cloning Kit

Catalog no. K2800-20SC

QUICK REFERENCE CARD

Instructions are provided to TOPO® Clone your PCR product into pCR®-Blunt II-TOPO® and transform the reaction into chemically competent *E. coli* cells. For transformation of electrocompetent *E. coli* cells, a diagram of the multiple cloning site, and detailed instructions, refer to the Zero Blunt® TOPO® PCR Cloning Kit manual available from www.invitrogen.com or Technical Service.

Producing Blunt PCR Products

Produce blunt-end PCR products using a thermostable proofreading polymerase and your own protocol. End the PCR reaction with a final 7 to 30 minutes extension step.

TOPO® Cloning Reaction

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

Reagent	Amount*
Fresh PCR Product	0.5 to 4 µl
Salt Solution	1 µl
Sterile Water	add to a total volume of 5 µl
pCR®-Blunt II-TOPO®	1 µl
Final Volume	**6 µl**

*For transformation of chemically competent *E. coli* only.

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

Corporate Headquarters
Tel: 800 955 6288
Fax: 760 602 6500
tech_service@invitrogen.com

invitrogen™

For contact information for other countries, visit our Web site at www.invitrogen.com.

2

Transformation and Analysis

Protocols to transform chemically competent cells and to analyze positive clones are provided below. If you wish to transform electrocompetent cells, refer to the Zero Blunt® TOPO® PCR 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 µl of the TOPO® Cloning reaction to a vial of One Shot® *E. coli* and mix gently.
3. Incubate on ice for 5-30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Add 250 µl 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 µl from each transformation on prewarmed LB plates containing 50 µg/ml kanamycin or prewarmed Low Salt LB plates containing 25 µg/ml Zeocin™. Refer to the Zero Blunt® TOPO® PCR Cloning Kit manual for a recipe for Low Salt LB medium.
8. Incubate plates overnight at 37°C.
9. An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 colonies for analysis. Proceed to **Analyzing Positive Clones**.

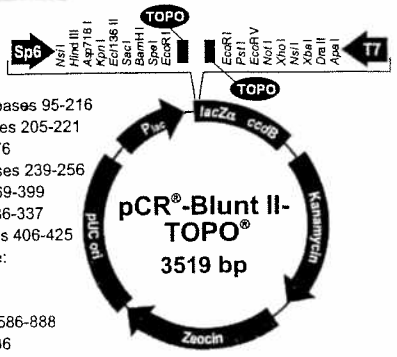
3

Transformation and Analysis, cont'd

Analyzing Positive Clones

1. Culture the 10 colonies overnight in LB medium containing 50 µg/ml kanamycin or Low Salt LB medium containing 25 µg/ml Zeocin™.
2. Isolate plasmid DNA using your method of choice. For ultra-pure plasmid DNA, we recommend the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01).
3. Analyze the plasmid by restriction analysis or by sequencing to confirm the presence and correct orientation of the insert.

Map of pCR®-Blunt II-TOPO®



lac promoter/operator region: bases 95-216
M13 Reverse priming site: bases 205-221
*lac*Z-alpha ORF: bases 217-576
SP6 promoter priming site: bases 239-256
Multiple Cloning Site: bases 269-399
TOPO®-Cloning Site: bases 336-337
T7 promoter priming site: bases 406-425
M13 (-20) Forward priming site: bases 433-448
Fusion joint: bases 577-585
ccdB lethal gene ORF: bases 586-888
kan promoter: bases 1099-1236
Kanamycin resistance ORF: bases 1237-2031
Zeocin resistance ORF: bases 2238-2612
pUC origin: bases 2724-3397