QUICK REFERENCE

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Zero Blunt® TOPO® PCR Cloning Kit

Catalog Number K2800-20SC
Doc. Part no. 25-0649 **Pub. Part no.** MAN0003551 **Rev.** A.0

Introduction

Follow these instructions to TOPO® Clone your blunt-end PCR product into pCR™-Blunt II-TOPO® and transform the reaction into chemically competent *E. coli* cells. For transformation of electrocompetent cells, a diagram of the multiple cloning site, and a manual, see www.lifetechnologies.com/support or contact Technical Support.

Produce Blunt PCR Products

Use the appropriate protocol to produce blunt-end PCR products using a thermostable proofreading polymerase. Make sure that the final extension step is sufficient to fully extend your PCR products (7–30 minutes).

TOPO® Cloning Reaction

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

Reagent	Amount*
Fresh PCR Product	0.5–4 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.

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

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

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Transformation and Analysis

Follow the protocol in this section to transform chemically competent cells and to analyze positive clones. To transform electrocompetent cells, refer to the Zero Blunt® TOPO® PCR Cloning Kit manual.

One Shot® Chemical Transformation

1. Thaw 1 vial of One Shot® *E. coli* cells **on ice** for each transformation.
2. Add 2 µL of the TOPO® Cloning reaction to each vial of One Shot® cells to be transformed, and mix gently.
3. Incubate the vials 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 them at 37°C for 1 hour.
7. Spread 10–50 µL from each transformation on pre-warmed LB plates containing 50 µg/mL kanamycin or pre-warmed Low Salt LB plates containing 25 µg/mL Zeocin™ selective antibiotic. Refer to the Zero Blunt® TOPO® PCR Cloning Kit manual for a Low Salt LB medium recipe.
8. Incubate plates overnight at 37°C.

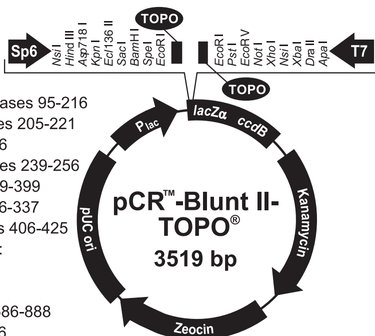
An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 colonies for analysis. **Proceed to Analyze Positive Clones**.

Transformation and Analysis, Continued

Analyze 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™ selective antibiotic.
2. Isolate plasmid DNA using your method of choice. For ultra-pure plasmid DNA, we recommend the PureLink® HQ Mini Plasmid Purification Kit (Cat. no. K2100-01).
3. Analyze the plasmid by restriction analysis or by sequencing to confirm the presence and 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

Limited Product Warranty

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