QUICK REFERENCE

TOPO® TA Cloning® Kits

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Introduction

Follow these instructions to use the TOPO® TA Cloning® Kit to clone your PCR product into the TOPO® vector and transform the reaction into chemically competent *E. coli* cells. For transformation of electrocompetent cells, a map of the TOPO® vector, a diagram of the multiple cloning site, and additional detailed instructions, refer to the TOPO® TA Cloning® Kit manual available from **www.lifetechnologies.com/manuals.**

Produce PCR products

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

TOPO® kit cloning reaction

1. Set up the following 6 µL reaction:

Reagent	Amount*
Fresh PCR Product	0.5–4 µL
Salt Solution	1 µL
Sterile Water	add to a total volume of 5 µL
TOPO® Vector	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.**

Transformation and Analysis

Follow the protocol in this section to transform chemically competent cells and to analyze positive clones. Use the **Rapid One Shot® chemical transformation** protocol if you are using *ampicillin selection only* and if you wish to obtain transformants as quickly as possible. For instructions to transform electrocompetent cells, refer to the TOPO® TA Cloning® 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 X-gal and 50–100 µg/mL ampicillin or 50 µg/mL kanamycin. For TOP10F' *E. coli*, add both IPTG and X-gal to LB plates.
8. Incubate plates overnight at 37°C.
9. An efficient TOPO® cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis. Do not pick dark blue colonies. Proceed to **Analyze positive clones.**

Transformation and Analysis, Continued

Rapid One Shot® chemical transformation (for ampicillin selection only)

1. Thaw 1 vial of One Shot® *E. coli* cells *on ice* for each transformation.
2. Add 4 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 minutes.
4. Spread 50 µL from each transformation on pre-warmed LB plates containing X-gal and 50–100 µg/mL ampicillin. For TOP10F' *E. coli*, add both IPTG and X-gal to LB plates.
5. Incubate plates overnight at 37°C.
6. An efficient TOPO® cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis. Do not pick dark blue colonies. Proceed to **Analyze positive clones**.

Analyze positive clones

1. Culture the 10 colonies overnight in LB medium containing 50–100 µg/mL ampicillin or 50 µg/mL kanamycin.
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.

Limited Product Warranty

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