QUICK REFERENCE

invitrogen
by life technologies™

TOPO® TA Cloning® Kits

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Produce PCR products

1. Produce PCR products using *Taq* polymerase. Include a final extension step of 7–10 minutes to ensure 3′ adenylation.
2. Verify there is only one PCR product of the proper size in each reaction by agarose gel electrophoresis.

Perform TOPO® cloning

1. Set up a 6 µL TOPO® Cloning reaction for each PCR product:

Reagent	Volume
Fresh PCR product	0.5–4 µL
Salt solution	1 µL
Water	add to a final volume of 5 µL
TOPO® Vector	1 µL

2. Mix gently and incubate for 5 minutes at room temperature.
3. Place tubes on ice. Proceed to **Transform One Shot® cells** or **Electroporate One Shot® cells**.

Transform One Shot® cells

1. Thaw One Shot® *E. coli* or equivalent competent cells on ice.
2. Add 2 µL of the TOPO® Cloning reaction to a vial of One Shot® *E. coli* or equivalent competent cells and mix gently.
3. Incubate on ice for 5–30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking. Proceed to **Recover and plate cells**.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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Electroporate One Shot® cells

1. Thaw competent cells on ice.
2. Dilute the TOPO® Cloning reaction 4-fold with sterile water.
3. Add 2 µL of the diluted TOPO® Cloning reaction to a vial of One Shot® Electrocomp™ *E. coli* or equivalent competent cells and mix gently.
4. Electroporate your samples. Refer to the manual if arcing occurs.
5. Proceed to **Recover and plate cells**.

Recover and plate cells

1. Add 250 µL of room temperature S.O.C. Medium to the cells. For electroporated cells, transfer the sample to a 15-mL culture tube.
2. Cap the tubes and shake at 37°C for 1 hour.
3. Spread 10–50 µL from each transformation on prewarmed LB plates containing X-gal, and either 50–100 µg/mL ampicillin or 50 µg/mL kanamycin. For TOP10F′ *E. coli*, add both IPTG and X-gal to the LB plates.
4. Incubate plates overnight at 37°C.
5. 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 10 colonies in LB medium containing 50–100 µg/mL ampicillin or 50 µg/mL kanamycin.
2. Isolate plasmid DNA using your method of choice.
3. Analyze the plasmid by restriction analysis or by sequencing to confirm the presence and correct orientation of the insert.

For more information, refer to www.lifetechnologies.com/manuals

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