TOPO™ XL-2 Complete PCR Cloning Kit

invitrogen

QUICK REFERENCE Pub. No. MAN0016193 Rev. A.0

Print options

Package contents

Catalog No.	Size
K8050-10	10 reaction
K8050-20	20 reaction

Kit contents

Storage conditions

Store Box 1 at –30°C to –10°C
Store Box 2 at room temperature
Store Box 3 at –85°C to –68°C

Required materials

- Template: DNA
- PCR thermocycler
- Microcentrifuge
- Agarose gel electrophoresis equipment
- 42°C water bath
- 37°C incubator

Timing

- Perform PCR: 30 minutes to 3 hours
- Purify PCR product: 15–20 minutes
- TOPO™ Cloning reaction: 5 minutes
- Transformation reaction: 2 hours

Product description

- The TOPO™ XL-2 Complete PCR Cloning Kit provides all the components needed for long fragment PCR cloning in a single kit format.
- Platinum™ SuperFi™ Green PCR Master Mix is used to generate long PCR products with high specificity and fidelity.
- The PureLink™ Quick Gel Extraction and PCR Purification Kit is used to obtain high yields of purified PCR product despite large fragment sizes.
- One Shot™ OmniMAX™ 2 T1^{R} Chemically Competent *E. coli* have superior transformation efficiency to ensure sufficient transformants for evaluation.

Online resources

Visit our product page for additional information and protocols. For support, visit **thermofisher.com/support**.

Protocol outline

A. Produce blunt-ended PCR product
B. Purify PCR product
C. Perform TOPO™ Cloning reaction
D. Transform TOPO™ Cloning reaction
E. Select and analyze colonies

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PCR protocol for TOPO™ XL-2 Complete PCR Cloning Kit

The following procedure is used to produce blunt-ended PCR products for TOPO™ Cloning. See the **Platinum™ SuperFi™ Green PCR Master Mix User Guide** for additional information on thermocycling conditions and performing PCR. For details on purification of PCR products, see the **TOPO™ XL-2 Complete PCR Cloning Kit User Guide**.

Step 1 — Prepare PCR mix

Combine the following components in a sterile PCR tube.

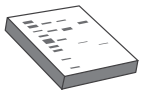
Component	50-µL reaction
Water, nuclease-free	to 50 µL
Platinum™ SuperFi™ Green PCR Master Mix (2X)	25 µL
10 µM forward primer (0.5 µM final concentration)	2.5 µL
10 µM reverse primer (0.5 µM final concentration)	2.5 µL
Template DNA (5–50 ng of gDNA or 1 pg to 10 ng of plasmid DNA)	varies
(*Optional*) SuperFi™ GC Enhancer (5X)	10 µL

Step 2 — Amplify PCR product

Use the following cycling parameters for amplicons <10 kb.

Step	Temperature	Time	Cycles
Initial Denaturation	98°C	30 seconds	1X
Denaturation	98°C	5–10 seconds	25–35X
Anneal	varies	10 seconds	
Extend	72°C	15–30 seconds/kb	
Final Extension	72°C	5 minutes	1X
	4°C	Hold	

Step 3 — Analyze PCR product



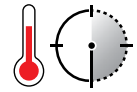
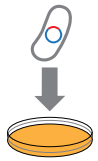
Analyze 5–10 µL by agarose gel electrophoresis to verify the size, quality, and quantity of the PCR product.

Step 4 — Purify PCR product

- If you have a single discrete band, prepare the PCR product by column purification or gel purification.
  Note: Gel purification results in optimal cloning efficiency.
- If you **do not** have a single, discrete band, isolate the desired PCR product by gel purification.

Cloning and transformation protocol for TOPO™ XL-2 Complete PCR Cloning Kit

The following procedure is used to perform TOPO™ Cloning and transformation into One Shot™ OmniMAX™ 2 T1^{R} Chemically Competent *E. coli*. For details on analyzing transformants, see the **TOPO™ XL-2 Complete PCR Cloning Kit User Guide**.

<table>
<tr><th>Step</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1</td><td> 
Prepare TOPO™ reaction mix</td><td>Combine the following components in a sterile PCR tube. Use a 1:1 molar ratio of insert to vector.
<table><tr><th>Component</th><th>6-µL reaction</th></tr><tr><td>Column or gel purified PCR product</td><td>up to 4 µL</td></tr><tr><td>pCR-XL-2-TOPO™ Vector (10 ng/µL)</td><td>1 µL</td></tr><tr><td>Salt Solution</td><td>1 µL</td></tr></table></td></tr>
<tr><td>2</td><td> 
5 min
Incubate TOPO™ reaction mix</td><td>a. Gently mix, then briefly centrifuge.
b. Incubate at room temperature for 5 minutes.
c. Place the tube on ice.</td></tr>
<tr><td>3</td><td> 
42˚C 30 s
Transform competent cells</td><td>a. Add 2 µL of the TOPO™ Cloning reaction into a vial of competent cells.
b. Incubate for 30 minutes on ice.
c. Heat-shock the cells for 30 seconds in a 42°C water bath.
d. Immediately place the tubes on ice and incubate for 2 minutes.
e. Add 250 µL of room temperature S.O.C. medium.
f. Cap the tube tightly and shake the tube horizontally at 225 rpm for 1 hour at 37°C.</td></tr>
<tr><td>4</td><td> 
Plate transformed cells</td><td>a. Spread 50–150 µL from each TOPO™ Cloning transformation reaction on a pre-warmed LB plate containing kanamycin or ampicillin, and 1 mM IPTG.
b. Incubate plates overnight at 37°C.</td></tr>
<tr><td>5</td><td> 
Analyze transformants</td><td>▪ Analyze transformants by colony PCR.
▪ Analyze transformants by restriction enzyme digestion.</td></tr>
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