invitrogen

TOPO™ XL-2 Complete PCR Cloning Kit

USER GUIDE

Five-minute blunt-end cloning of extra long (up to 13 kb) PCR products

Catalog Number K805010 and K805020

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ThermoFisher SCIENTIFIC

The information in this guide is subject to change without notice.

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Revision	Date	Description
A.0	27 July 2016	User Guide to accompany new product release.

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Product information

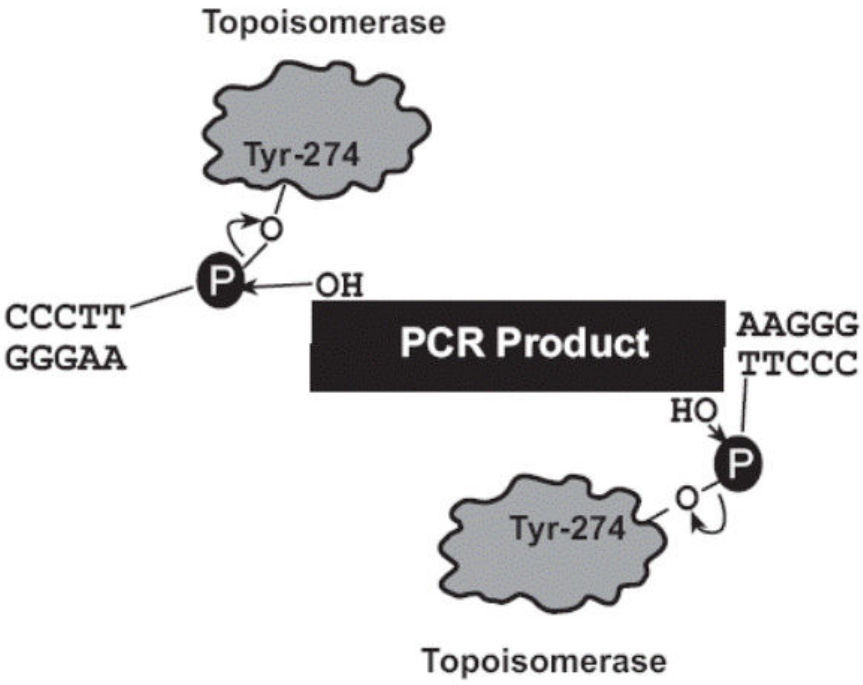
Product description

The TOPO™ XL-2 Complete PCR Cloning Kit provides a highly efficient one-step method ("TOPO™ Cloning") for the cloning of extra long PCR products generated by the Platinum™ SuperFi™ Green PCR Master Mix. No ligase is required for the TOPO™ Cloning reaction. Special gel purification reagents are provided to ensure efficient cloning of long, full-length PCR products.

TOPO™ Cloning reaction

The pCR-XL-2-TOPO™ Vector is supplied in linearized form with the *Vaccinia* virus DNA topoisomerase I covalently bound to the 3' end of each DNA strand ("TOPO™-activated" vector).

The *Vaccinia* topoisomerase I binds to duplex DNA at specific sites and cleaves the phosphodiester backbone after the 5'-CCCTT sequence in one strand. The energy from the broken phosphodiester backbone is conserved by formation of a covalent bond between the 3' phosphate of the cleaved strand and a tyrosyl residue (Tyr-274) of topoisomerase I. The phospho-tyrosyl bond between the DNA and enzyme can subsequently be attacked by the 5' hydroxyl of the original cleaved strand, reversing the reaction and releasing topoisomerase. TOPO™ Cloning exploits this reaction to efficiently clone PCR products. The TOPO™ Cloning reaction is subsequently transformed directly into competent cells.



ccdB gene

The pCR-XL-2-TOPO™ Vector allows direct selection of recombinant DNA by disrupting the lethal *E. coli* gene, *ccd*B. The vector contains the *ccd*B gene fused to the C-terminus of the LacZ*α* fragment. Ligation of a long PCR product disrupts expression of the *lac*Z*α* -*ccd*B gene fusion so that only positive recombinants grow of upon transformation. Cells containing the non-recombinant vector are killed upon plating, therefore, blue/white screening is not required.

Genotype of *E. coli* strain

Use One Shot™ OmniMAX™ 2 T1^R Chemically Competent *E. coli* for general cloning and blue/white screening. The strain is resistant to T1 bacteriophage.

F′ {*pro*AB *lac*I ^{q} *lac*ZΔM15 *Tn*10(Tet ^{R}) Δ(*ccdA*B)} *mcr*A Δ(*mrr hsd*RMS-*mcr*BC) Φ 80(*lac*Z)ΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A *pan*D

Primer sequences

The sequences of the T3 and T7 sequencing primers are provided in the following table.

Primer	Sequence	Concentration
T3	ATTAACCCTCACTAAAGGGA	385 pmol
T7	TAATACGACTCACTATAGGG	407 pmol

Kit contents and storage

The TOPO™ XL-2 Complete PCR Cloning Kit consists of three boxes. Box 1 and Box 3 are shipped on dry ice, while Box 2 is shipped at room temperature. Store items as directed in the following table upon receipt.

Box	Contents	Storage
1	TOPO™ XL-2 PCR Cloning Reagents (includes Platinum™ SuperFi™ Green PCR Master Mix reagents)	–30°C to –10°C
2	PureLink™ Quick Gel Extraction and PCR Purification Combo Kit	Room temperature (15–30°C)
3	One Shot™ OmniMAX™ 2 T1^{R} Chemically Competent *E. coli* [1]	–85°C to –68°C

[1] See page 6 for genotype information.

The contents of each box are listed in the following tables.

Component	Cat. No. K805010 (10 reactions)	Cat. No. K805020 (20 reactions)
TOPO™ XL-2 PCR Cloning Reagents (Box 1)		
Platinum™ SuperFi™ Green PCR Master Mix (2X)	1.25 mL	2 × 1.25 mL
SuperFi™ GC Enhancer (5X)	1.25 mL	1.25 mL
Nuclease-free Water	1.25 mL	2 × 1.25 mL
pCR-XL-2-TOPO™ Vector (10 ng/µL)	10 µL	20 µL
Salt Solution	50 µL	50 µL
T3 Primer (0.1 µg/µL)	25 µL	25 µL
T7 Primer (0.1 µg/µL)	25 µL	25 µL
XL Control PCR Template (25 ng/µL)	10 µL	10 µL
XL Control PCR Primers (0.2 µg/µL)	10 µL	10 µL
PureLink™ Quick Gel Extraction and PCR Purification Combo Kit Reagents (Box 2)		
Gel Solubilization Buffer (L3)	2 × 90 mL	2 × 90 mL
Binding Buffer (B2)	15 mL	15 mL
Wash Buffer (W1)	16 mL	16 mL
Elution Buffer (E1)	15 mL	15 mL
PureLink™ Clean-up Spin Columns (in Wash Tubes)	15 each	30 each
PureLink™ Elution Tubes	15 tubes	30 tubes

Component	Cat. No. K805010 (10 reactions)	Cat. No. K805020 (20 reactions)
One Shot™ OmniMAX™ 2 T1R Chemically Competent *E. coli* (Box 3)		
Competent cells	11 × 50 µL	21 × 50 µL
S.O.C. Medium (Store at 4°C or room temperature)	6 mL	6 mL
pUC19 Control DNA (10 pg/µL)	50 µL	50 µL

Workflow

Produce blunt-end PCR product using the Platinum™ SuperFi™ Green PCR Master Mix

▼

Purify the blunt-end PCR product with the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit

▼

Set up the TOPO™ Cloning reaction by mixing together the purified PCR product with the pCR-XL-2-TOPO™ Vector

▼

Incubate for 5 minutes at room temperature

▼

Transform the TOPO™ Cloning reaction into One Shot™ OmniMAX™ 2 T1R Chemically Competent *E. coli*

▼

Select and analyze colonies for insert

▼

Isolate plasmid DNA and perform DNA sequencing

Produce blunt-end PCR products

Materials required but not provided

- Thermocycler
- DNA template and primers for PCR product
- 1X TAE buffer (40 mM Tris-acetate, pH 8, 1 mM EDTA).
- DNA gel stain (e.g. SYBR™ Safe DNA Gel Stain or ethidium bromide)

PCR guidelines

- Refer to the **Platinum™ SuperFi™ Green PCR Master Mix user guide** for additional information on thermocycling conditions and performing PCR.
- Optimize PCR conditions to produce a single, discrete PCR product.
- PCR products can also be stored at –20°C until ready for purification.

Amplify PCR product

1. Set up a PCR reaction according to the following table.

Component	50-µL reaction
Water, nuclease-free	to 50 µL
Platinum™ SuperFi™ Green PCR Master Mix (2X) [1]	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
(*Optional*) SuperFi™ GC Enhancer (5X) [2]	10 µL

[1] Provides $MgCl_2$ at a final concentration of 1.5 mM.
[2] Recommended for target sequences with >65% GC content.

2. 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[1]	varies	10 seconds	
Extend	72°C	15–30 seconds/kb	
Final Extension	72°C	5 minutes	1X
	4°C	Hold	

[1] For the recommended annealing temperature, go to **thermofisher.com/tmcalculator**

Amplify the 7-kb control PCR product

1. Set up a PCR reaction according to the following table to produce the 7-kb control PCR product.

Component	50-µL reaction
Water, nuclease-free	23 µL
Platinum™ SuperFi™ Green PCR Master Mix (2X)	25 µL
XL Control PCR Primers (0.2 µg/µL)	1 µL
XL Control PCR Template (25 ng/µL)	1 µL

2. Amplify the control PCR product using the following cycling parameters.

Step	Temperature	Time	Cycles
Initial Denaturation	98°C	30 seconds	1X
Denaturation	98°C	10 seconds	30X
Anneal	56°C	10 seconds	
Extend	72°C	2 minutes	
Final Extension	72°C	5 minutes	1X
	4°C	Hold	

Analyze PCR product

After producing the blunt-end PCR product, analyze 5–10 µL by agarose gel electrophoresis to verify the size, quality, and quantity of the PCR product.

- If you have a single discrete band (e.g. the 7-kb control PCR product) prepare the PCR product by column purification (see "Column purify the PCR product" on page 11)
- If you **do not** have a single, discrete band, isolate the desired PCR product by gel purification (see "Gel purify the PCR product" on page 12)

Before first use of the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit

Materials required but not provided

- 96-100% ethanol
- 100% isopropanol

Prepare Wash Buffer (W1)

1. Add 64 mL 96–100% ethanol to the bottle containing Wash Buffer (W1).
2. Check the box on the Wash Buffer (W1) label to indicate that ethanol was added.
3. Store the Wash Buffer (W1) with ethanol at room temperature.

Prepare Binding Buffer (B2)

1. Add 10 mL of 100% isopropanol to the bottle containing Binding Buffer (B2).
2. Check the box on the Binding Buffer (B2) label to indicate that isopropanol was added.
3. Store the Binding Buffer (B2) with isopropanol at room temperature.

Column purify the PCR product

Materials required but not provided

- Microcentrifuge capable of centrifuging at ≥10,000 × *g*
- DNase-free pipettes and tips

Guidelines for PCR purification

- Maintain PCR volume of 50 µL.
- Each PureLink™ Clean-up Spin Column can purify up to **40 µg of DNA**. To purify larger amounts of DNA, use additional PureLink™ Clean-up Spin Columns.
- Perform all centrifugation steps at room temperature.
- Always use sterile water with pH 7–8.5, if performing elution with water.

Dilute and bind DNA

1. Add 4 volumes of Binding Buffer (B2) with isopropanol to 1 volume of PCR reaction (50 µL) to dilute the sample. Mix well.
2. Add the diluted sample to a PureLink™ Clean-up Spin Column in a Wash Tube.
3. Centrifuge the PureLink™ Spin Column at room temperature at 10,000 × *g* for 1 minute.
4. Discard the flow through and replace the PureLink™ Spin Column into the Wash Tube.
5. Proceed to "Wash DNA" on page 12.

Wash DNA

1. Add 650 µL Wash Buffer (W1) with ethanol to the PureLink™ Spin Column.
2. Centrifuge the PureLink™ Spin Column at 10,000 × *g* for 1 minute at room temperature. Discard the flow-through and replace the PureLink™ Spin Column into the Wash Tube .
3. Centrifuge the PureLink™ Spin Column at maximum speed for 2–3 minutes at room temperature to remove any residual Wash Buffer. Discard the Wash Tube.
4. Place the PureLink™ Spin Column in a clean 1.7-mL PureLink™ Elution Tube (supplied with the kit).
5. Proceed to "Elute DNA".

Elute DNA

1. Add 50 µL Elution Buffer (E1) or sterile, distilled water (pH >7.0) to the center of the PureLink™ Spin Column.

   Note: Elution can be performed with 30 µL Elution Buffer (E1) if smaller volumes are required, particularly for larger sized amplicons.
2. Incubate the PureLink™ Spin Column at room temperature for 1 minute.
3. Centrifuge the PureLink™ Spin Column at maximum speed for 1 minute.
4. Remove and discard the PureLink™ Spin Column. **The elution tube contains the purified PCR product** in an elution volume of ~48 µL.
5. Proceed to "Perform TOPO™ Cloning reaction" on page 15.

Store the purified DNA

Keep the purified DNA at 4°C for immediate use, or make aliquots of the DNA and store at –20°C for long-term storage. Avoid repeated freezing and thawing of the DNA.

Gel purify the PCR product

Materials required but not provided

- Agarose gel containing the DNA fragment
- Weighing paper or weigh trays
- Digital scale sensitive to 0.001 g
- 50°C water bath or heat block
- 1.7-mL polypropylene microcentrifuge tubes
- Clean, sharp razor blade
- Microcentrifuge capable of centrifuging at ≥10,000 × *g*
- DNase-free pipettes and tips

Guidelines for gel purification

- DNA fragments can be purified from TAE and TBE agarose gels with different melting points without modifying the protocol. **Follow these specific directions for gels containing ~1% agarose.**
- The PureLink™ Quick Gel Extraction and PCR Purification Combo Kit has been tested for use with the TOPO™ XL-2 Complete PCR Cloning Kit using PCR products of up to 13 kb in size.
- Ensure that the PCR product of interest is completely separated from other DNA fragments on the agarose gel.
- Each PureLink™ Clean-up Spin Column can purify up to **40 µg of DNA**. To purify larger amounts of DNA, use additional PureLink™ Clean-up Spin Columns. For best results, use 1 PureLink™ Clean-up Spin Column per 10 µg of PCR product loaded onto the gel.

Excise and weigh agarose gel slice

1. Use a clean, sharp razor blade to cut out the section of the ~1% agarose gel containing your desired DNA fragment.

   Note: Be sure to excise the 7 kb control fragment if the control PCR product is run on the same agarose gel.

2. Trim the gel slice by removing excess agarose surrounding the DNA fragment.

3. Weigh the gel slice containing the DNA fragment, and place the gel slice into a 1.7-mL microcentrifuge tube.

   Note: The maximum amount of starting material is 400 mg of agarose per tube. If the gel slice exceeds 400 mg, cut the gel into smaller slices of ≤400 mg. Place additional gel slices into separate microcentrifuge tubes. During the purification procedure (page 14), an additional PureLink™ Spin Column is required for each extra gel slice.

4. Proceed to "Solubilize the gel slice".

Solubilize the gel slice

1. Add 3 volumes of Gel Solubilization Buffer (L3) for every 1 volume of gel (e.g., add 1.2 mL Gel Solubilization Buffer for a 400-mg gel slice).

2. Incubate the tube containing the gel slice for at least 10 minutes in a 50°C water bath or heat block. Invert the tube every 3 minutes to ensure complete gel dissolution.

3. Incubate the tube for an additional 5 minutes at 50°C after the gel slice appears dissolved.

4. (*Optional*) For optimal DNA yields, add 1 gel volume isopropanol to the dissolved gel slice (e.g., add 400 µL isopropanol for a 400-mg gel slice). Mix well.

5. Proceed to "Bind DNA" on page 14.

Bind DNA

1. Pipet the dissolved gel slice into the center of a PureLink™ Clean-up Spin Column inside a Wash Tube.

   Note: Do not load >400 mg agarose per PureLink™ Spin Column.

2. Centrifuge the tube at 10,000 × *g* for 1 minute. Discard the flow through and replace the PureLink™ Spin Column into the Wash Tube.

3. Proceed to "Wash DNA".

Wash DNA

1. Add 500–700 µL Wash Buffer (W1) with ethanol, to the PureLink™ Spin Column.

2. Centrifuge the PureLink™ Spin Column at 10,000 × *g* for 1 minute. Discard the flow-through and replace the PureLink™ Spin Column into the Wash Tube.

3. Centrifuge the PureLink™ Spin Column at maximum speed for 2–3 minutes to remove any residual Wash Buffer and ethanol.

4. Discard the Wash Tube and place the PureLink™ Spin Column in a clean 1.7-mL PureLink™ Elution Tube (supplied with the kit).

5. Proceed to "Elute DNA".

Elute DNA

1. Add 50 µL Elution Buffer (E1) to the center of the PureLink™ Spin Column.

   Note: Elution can be performed with 30 µL Elution Buffer (E1) if smaller volumes are required, particularly for larger sized amplicons.

2. Incubate the PureLink™ Spin Column for 1 minute at room temperature.

3. Centrifuge the PureLink™ Spin Column at maximum speed for 1 minute.

4. Remove and discard the PureLink™ Spin Column. **The elution tube contains the purified PCR product** in an elution volume of ~48 µL.

5. Proceed to "Perform TOPO™ Cloning reaction" on page 15.

Store the purified DNA

Keep the purified DNA at 4°C for immediate use, or make aliquots of the DNA and store at –20°C for long-term storage. Avoid repeated freezing and thawing of the DNA.

Perform TOPO™ Cloning

Guidelines for TOPO™ Cloning

After purification of your PCR product, you are ready to perform TOPO™ Cloning of the insert into the pCR-XL-2-TOPO™ Vector. It is important to have everything you need set up and ready to use to ensure the best possible results.

Note: The blue color of the pCR-XL-2-TOPO™ Vector solution is normal and is used to assist visualization of the solution.

For best results, a 1:1 molar ratio of insert to vector is recommended.

$$\frac{(length\ of\ insert\ (bp))}{(length\ of\ vector\ (3956bp))} \times 10\ ng\ of\ vector = ng\ of\ insert\ needed\ for\ 1{:}1$$

For example, if the insert is 7 kb in length:

$$\frac{(7000bp\ insert)}{(3956bp\ vector)} \times 10\ ng\ vector = 17.69\ ng\ of\ insert\ needed\ for\ 1{:}1$$

Perform TOPO™ Cloning reaction

1. Set up the TOPO™ Cloning reaction in a sterile microcentrifuge tube using the volumes in the following table.

Reagent	6-µL reaction
Column or gel purified PCR product[1]	up to 4 µL
pCR-XL-2-TOPO™ Vector (10 ng/µL)	1 µL
Salt Solution	1 µL

[1] Use the 7-kb control PCR product for control reactions.

2. Mix gently, then briefly centrifuge the tube.
3. Incubate the tube for at least 5 minutes at room temperature (~25°C).

   Note: Extending the incubation time up to 30 minutes may increase cloning efficiency for long PCR products.
4. Place the tube on ice and Proceed immediately to "Perform transformation reaction" on page 16.

   Note: The TOPO™ Cloning reaction can be stored on ice or frozen at –20°C for up to 24 hours. A decrease in the transformation efficiency can occur, but the cloning efficiency should remain high.

Perform transformation reaction

Materials required but not provided

- LB plates containing 50 µg/mL kanamycin and 1 mM IPTG or LB plates containing 100 µg/mL ampicillin and 1 mM IPTG (see Appendix A, "Recipes").

  Note: At least one plate is required for each transformation reaction. Additional plates may be required if transformation reactions are plated using different dilutions of the transformed competent cells.
- LB plate containing 50 µg/mL kanamycin (for 7-kb PCR product control reaction)
- LB plate containing 100 µg/mL ampicillin (for pUC19 control reaction)
- 42°C water bath
- 37°C shaking and non-shaking incubator
- General microbiological supplies (e.g. plates, spreaders)

Before you begin

- Thaw the vial of S.O.C. medium and allow it to warm to room temperature.
- Pre-warm the required number of selective plates at 37°C for 30 minutes.
- Place 1 vial of One Shot™ OmniMAX™ 2 T1^R Chemically Competent *E. coli* for each transformation on ice, and allow the cells to fully thaw (2–5 minutes).

Transform competent cells

1. Add 2 µL of the TOPO™ Cloning reaction into a vial of One Shot™ OmniMAX™ 2 T1^R Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down.**
2. (*Optional*) Add 1 µL of the pUC19 control plasmid into a vial of One Shot™ OmniMAX™ 2 T1^R Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down.**
3. Incubate for 30 minutes on ice.
4. Heat-shock the cells for 30 seconds in a 42°C water bath.
5. Immediately place the tubes on ice and incubate for 2 minutes.
6. Add 250 µL of room temperature S.O.C. medium.
7. Cap the tube tightly and shake the tube horizontally at 225 rpm for 1 hour at 37°C.
8. Proceed to "Plate transformed cells" on page 17.

Plate transformed cells

1. Spread 50–150 µL from each TOPO™ Cloning transformation reaction on a pre-warmed LB plate containing 50 µg/mL kanamycin and 1 mM IPTG, or 100 µg/mL ampicillin and 1 mM IPTG.
   - If performing a pUC19 control, dilute 10 µL of the pUC19 control transformation reaction in 20 µL of S.O.C. medium and spread on a LB plate containing 100 µg/mL ampicillin.
   - If performing a 7-kb PCR product control, spread 50 µL of the 7-kb PCR product control transformation reaction on a LB plate containing 50 µg/mL kanamycin and 1 mM IPTG.

   Note: Larger amplicons will produce fewer colonies, so plating cells at a higher concentration may be necessary.
2. Incubate plates overnight at 37°C.
3. Proceed to "Analyze transformants".

A successful TOPO™ Cloning reaction produces several hundred colonies, while the pUC19 control plasmid (used to check transformation efficiency) has an expected efficiency is 1×10^9 cfu/µg DNA.

Analyze transformants

Analyze transformants by colony PCR

The following protocol is a general PCR protocol to directly analyze positive transformants with the T3 and T7 primers. If you are using this technique for the first time, we recommend performing restriction enzyme analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template.

1. Prepare a PCR master mix in a 1-mL microcentrifuge tube. Scale volumes according to the number of colonies to be tested. For a single colony, add:
   - 25 µL of Platinum™ SuperFi™ Green PCR Master Mix
   - 1 µL each of the forward and reverse PCR primer
   - 23 µL of nuclease-free water
2. Prepare one 50-µL aliquot of PCR master mix in a 0.2-mL PCR tube for each colony to be tested.
3. Pick an individual colony from the transformation plate and resuspend it into a PCR tube.

   Note: Be sure to preserve each colony on a patch plate for further analysis.
4. Incubate the reaction for 10 minutes at 94°C to lyse the cells and inactivate nucleases.
5. Amplify for 20–30 cycles.
6. For the final extension, incubate at 72°C for 10 minutes. Store at 4°C.
7. Visualize by agarose gel electrophoresis.

Analyze transformants by restriction enzyme digestion

1. Pick individual colonies from the plate.

   Note: Be sure to preserve each colony on a patch plate for further analysis.

2. Culture each colony overnight in 3 mL of LB medium containing 50 µg/mL kanamycin or 100 µg/mL ampicillin.

3. Isolate plasmid DNA from each culture sample using your method of choice.

   Note: If ultra-pure plasmid DNA for subsequent automated or manual sequencing is required, the PureLink™ HQ Mini Plasmid DNA Purification Kit (K210001) is recommended.

4. Analyze the plasmids by restriction analysis to confirm the presence and correct orientation of the insert.

Verification of positive clones

Sequence your construct using the T3 and T7 sequencing primers included in the kit to confirm the sequence of your gene of interest. See Appendix B, "Map of pCR-XL-2-TOPO™ Vector" for the sequence surrounding the TOPO™ Cloning site. For the full sequence of the pCR-XL-2-TOPO™ Vector go to **thermofisher.com/support**.

Store bacterial colonies

After identifying the clone containing the desired amplicon, purify the colony and make a glycerol stock for long-term storage.

1. Streak the original colony out on a LB plate containing 50 µg/mL kanamycin or 100 µg/mL ampicillin to produce single colonies.

2. Isolate a single colony and inoculate into 1–2 mL of LB containing 50 µg/mL kanamycin and/or 100 µg/mL ampicillin.

3. Grow until culture is at mid-log phase (between 0.6–1.0 OD_{600}).

4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.

5. Store at –80°C.

Recipes

LB media

Composition

- 1.0% Tryptone
- 0.5% Yeast Extract
- 1.0% NaCl
- pH 7.0

Prepare LB media

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi.
4. Allow solution to cool, and store at room temperature or at 4°C.
5. Add antibiotic(s) (50 µg/mL of kanamycin and/or 100 µg/mL of ampicillin) prior to use.

LB agar plates

Composition

- 1.0% Tryptone
- 0.5% Yeast Extract
- 1.0% NaCl
- 15 g/L agar
- pH 7.0

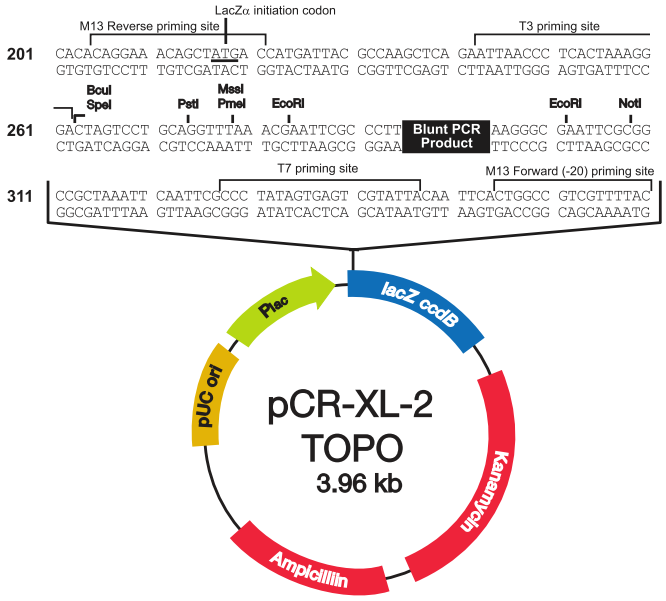
Prepare LB agar plates

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH.
3. Add 15 g agar, and bring the volume up to 1 liter.
4. Autoclave on liquid cycle for 20 minutes at 15 psi.
5. After autoclaving, allow solution to cool to ~55°C, then add IPTG (1 mM final concentration) and antibiotic(s) (50 µg/mL of kanamycin and/or 100 µg/mL of ampicillin).

   Note: Addition of 1 mM IPTG improves cloning efficiency, particularly with large sized inserts.
6. Immediately pour solution into 10 cm plates and allow the agar to solidify.
7. Store plates inverted at 4°C in the dark.

B

Map of pCR-XL-2-TOPO™ Vector



Element	Position
lac promoter region	bases 2–216
Transcription start site	base 179
M13 Reverse priming site	bases 205–221
LacZα-*ccdB* gene fusion	bases 217–810
T3 priming site	bases 243–262
TOPO™ Cloning site	bases 294–295
T7 priming site	bases 328–347
M13 Forward (–20) priming site	bases 355–370
Kanamycin resistance gene	bases 1159–1953
Ampicillin resistance gene	bases 2203–3063
pUC origin	bases 3161–3834

C

Analyze 7-kb control transformants

Analyze 7-kb control transformants by restriction enzyme digestion

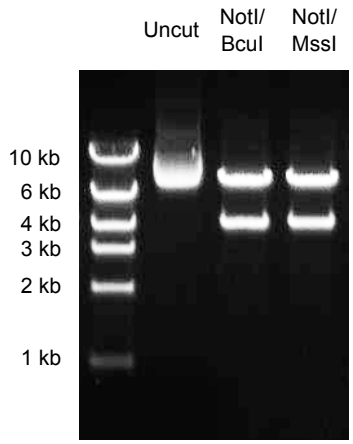
1. Pick 5 individual colonies from the 7-kb control plate.
2. Culture each colony overnight in 3 mL of LB medium containing 50 µg/mL kanamycin.
3. Isolate plasmid DNA from each culture sample using your method of choice.
4. Digest 500 ng of each plasmid with the following Anza™ restriction enzymes:

Component	20-µL reaction
Anza™ 10X Red Buffer	2 µL
Anza™ 1 NotI restriction enzyme	1 µL
Anza™ 3 BcuI or Anza™ 24 MssI restriction enzyme	1 µL
Plasmid DNA (500 ng)	varies
Water, nuclease-free	up to 20 µL

5. Incubate tubes at 37°C for 15 minutes.
6. Analyze 20 µL of each restriction digestion reaction on a 1% agarose gel.

Example of restriction enzyme digestion for the 7-kb control

Double digestion of the 7-kb control results in a banding pattern with a 7-kb fragment (insert) and a 3.96-kb fragment (vector) when analyzed by gel electrophoresis.



D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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