USER GUIDE

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# Zero Background™/Kan Cloning Kit

## Zero Background™ Cloning Kit with Selection on Kanamycin

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**For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.**

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# Contents

# Experienced Users Guide

## Introduction

The following procedure is designed to get experienced users quickly started with the Zero Background™/Kan Cloning Kit. The Introduction and Methods sections, along with appendices, provide detailed instructions for using the kit.

## Before starting

1. Prepare LB plates containing 25–50 µg/mL kanamycin (see page 15). Store the plates at 4°C.

   If you are using a cell line that contains the *lac*I$^q$ gene (i.e. TOP10F´, DH5αF´), include 1 mM IPTG in the plating medium to achieve complete induction.
2. Prepare LB or SOB liquid medium containing 25–50 µg/mL kanamycin for DNA minipreps.
3. Prepare or purchase chemically competent or electrocompetent TOP10 cells. See pages 18–21 for protocols to prepare competent cells. The minimal efficiencies required are $1 \times 10^8$ cfu/µg DNA for chemically competent cells and $1 \times 10^9$ cfu/µg DNA for electrocompetent cells.

   **Note**: For convenient high-efficiency transformation, we recommend One Shot® TOP10 Chemically Competent *E. coli* or One Shot® TOP10 Electrocompetent Cells, which are available separately (see page 24 for ordering information).
4. Determine a cloning strategy for ligating into pZErO®-2. See the detail of the multiple cloning site (page 4) for help.

## Ligate into pZErO®-2

Be sure to include a "no DNA", a "cells only", and linearized vector-only controls in your ligation experiments.

1. Digest 1 µg each of pZErO®-2 supercoiled vector (1 µL) and your DNA in total volumes of 10 µL using the buffer, temperature, and reaction conditions recommended by the manufacturer of the restriction enzyme. Optimal digestion time is 15–30 minutes. **DO NOT digest longer than 30 minutes.**
2. Inactivate the restriction enzyme(s) by heating the reaction at 65°C for 10–20 minutes or by phenol extraction. Dilute the cut vector to 10 ng/µL with TE buffer (provided). **If the enzyme is not heat inactivated** (i.e. *Eco*R V), phenol-extract the enzyme.
3. Ethanol precipitate the DNA and resuspend it in 90 µL of sterile water or TE buffer. We recommend that you use the linearized DNA immediately. You may store the DNA at −20°C for 1–2 weeks but the cloning efficiency may decrease.

*Procedure continued on the following page*

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### Ligate into pZErO®-2, Continued

*Procedure continued from previous page*

4. Prepare the ligation reaction. If ligating cohesive-ends, use a 2:1 insert: vector molar ratio; if ligating blunt-ends, use an insert: vector molar ratio between 3:1 and 10:1. See page 6 to determine how much insert you need. Set up the following 10 µL ligation reaction using the reagents supplied in the kit:

| | |
|---|---|
| Digested vector (~10 ng) | 1 µL |
| Digested DNA insert | x µL |
| Sterile water | to 8.5 µL |
| 10X Ligation Buffer (with ATP) | 1 µL |
| T4 DNA Ligase (4 U/µL) | 0.5 µL |
| Total Volume | 10 µL |

5. Incubate the reaction at 16°C for 30 minutes (cohesive-end ligations) or 60 minutes ( blunt-end ligations). **Do not** ligate at room temperature or exceed 1 hour for ligation and **do not** add PEG to the ligation reaction. Increased background levels have been observed under these conditions. Proceed to **Transformation**.

### Transformation

For more information on chemical transformation or electroporation, see pages 11–13.

1. Add 2 µL of each ligation reaction to a separate tube of competent cells (40–50 µL) and transform using your method of choice.
2. Plate 50–100 µL of each transformation mix on Low Salt LB-kanamycin plates. Let the liquid absorb, invert the plate, and incubate it at 37°C for 18–24 hours. Proceed to **Analyze Transformants**.

### Analyze transformants

1. Remove plates from the incubator. Pick at least 10 kanamycin resistant transformants and inoculate into 2 mL Low Salt SOB medium containing 25–50 µg/mL kanamycin. Grow 6–8 hours or overnight at 37°C.
2. Isolate the plasmid DNA by miniprep for restriction analysis or sequencing. Remember to purify the desired clone by streaking for single colonies and reconfirm the presence of insert before making a glycerol stock.
3. Prepare a glycerol stock of your desired clone for long-term storage by combining 0.85 mL of an overnight bacterial culture with 0.15 mL of sterile 100% glycerol. Mix by vortexing and transfer the stock to a labeled storage tube. Freeze the tube in liquid nitrogen or a dry ice/ethanol bath and store at –70°C.
4. After isolating the desired clone, proceed with further subcloning and/or analysis of your insert.

# Kit Contents and Storage

## Kit Contents and Storage

The Zero Background™/Kan Cloning Kit is shipped on dry ice, and it contains the following reagents:

| Item | Concentration | Storage |
|---|---|---|
| pZErO®-2 vector, supercoiled, 25 µg | 1 µg/µL in TE Buffer, pH 7.5 | –30°C to –10°C |
| Sterile water, 1 mL | Nuclease-free water | –30°C to –10°C |
| 10X Ligation Buffer, 100 µL | 60 mM Tris-HCl, pH 7.5<br>60 mM $MgCl_2$<br>50 mM NaCl<br>1 mg/mL bovine serum albumin<br>70 mM β-mercaptoethanol<br>1 mM ATP<br>20 mM dithiothreitol<br>10 mM spermidine | –30°C to –10°C |
| T4 DNA Ligase, 25 µL | 4.0 Weiss units/µL | –30°C to –10°C |
| TE Buffer, 2 mL | 10 mM Tris-HCl, pH 7.5<br>1 mM EDTA | –30°C to –10°C |
| Test Inserts, Blunt Ended ΦX174 *Hae* III DNA, 10 µL | 20 ng/µL in TE buffer, pH 7.5 | –30°C to –10°C |
| TOP10 cells, 1 stab | — | 2°C to 8°C |

## Genotype of TOP10

F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ*(ara-leu)*7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

## Intended Use

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

## Prepare TOP10 *E. coli* Glycerol Stocks

We recommend that you prepare a set of TOP10 *E. coli* glycerol master stocks within two weeks of receiving the kit. To prepare 5–10 glycerol master stocks for long-term storage:

1. Streak a small portion of the TOP10 cells that you have received as a stab on an LB plate.
2. Invert the plate and incubate it at 37°C overnight.
3. Isolate a single colony and inoculate into 5–10 mL of LB medium. Grow the culture to stationary phase ($OD_{600}$ = 1–2).
4. Mix 0.8 mL of the culture with 0.2 mL of sterile glycerol, and transfer to a cryovial.
5. Store the vials at –80°C. Use 1 master stock to create working stocks for regular use.

# Introduction

## Product Overview

### System Overview

This kit is designed to clone cohesive or blunt-ended DNA fragments with a low background of non-recombinants. The technology described here is based on vectors containing the lethal *E. coli* gene, *ccd*B (Bernard, *et al.*, 1994). The pZErO®-2 vector allows direct selection of inserts via disruption of a lethal gene. Very high cloning efficiencies (~95%) are often achieved without the need for exotic strains, X-Gal, calf intestinal phosphatase (CIP), or other components. *ccd*B, which inhibits growth of transformed cells containing non-disrupted *ccd*B, is constitutively expressed in cells that do not carry the *lac*I$^q$ gene.

### pZErO®-2 vector

The cloning vector, pZErO®-2, contains the *ccd*B gene fused to the C-terminus of LacZα. Insertion of a DNA fragment disrupts expression of the *lacZα-ccd*B gene fusion permitting growth of only positive recombinants. Cells that contain non-recombinant vector are killed. The vector also contains:

- The kanamycin resistance gene for selection in *E. coli*
- The f1 origin of replication for single-strand rescue
- A versatile multiple cloning site with 17 unique sites
- Flanking Sp6 and T7 promoter/priming sites for *in vitro* transcription and sequencing
- All of the M13 universal primer sites for sequencing.

### Recommended *E. coli* host

*E. coli* TOP10 is the recommended host strain for pZErO®-2. Because this strain does not contain a *lac*I$^q$ gene, the *ccd*B gene will be constitutively expressed without the need for IPTG induction.

Strains that contain an F plasmid are not recommended for transformation and selection of recombinant clones. The F plasmid encodes the CcdA protein, which acts as an inhibitor of the CcdB protein (see page 2 for a more detailed explanation).

Any *E. coli* strain that contains the complete Tn*5* transposable element (i.e. DH5αF´IQ™, SURE, SURE2) encodes the kanamycin resistance gene. For the most efficient selection, we highly recommend that you choose an *E. coli* strain that does not contain the Tn*5* gene (e.g. TOP10).

Do not use INVαF´ cells. The transformation efficiency of INVαF´ is very low using pZErO®-2 and selection on kanamycin.

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### Mechanism of action of the CcdB protein

The CcdB protein acts by poisoning bacterial DNA-gyrase (topoisomerase II), an essential enzyme that catalyzes the ATP-dependent negative supercoiling of DNA. DNA gyrase acts by creating a transient double-strand nick in the DNA substrate, passing the DNA helix through the break to decrease the linking number, and then resealing the nick. During the breaking-resealing reaction, the 5´ phosphate termini are covalently linked to a tyrosine residue in the A subunit of DNA gyrase (*gyr*A). This gyrase-DNA intermediate is called the cleavable complex. The CcdB protein has been shown both *in vivo* (Bernard and Couturier, 1992) and *in vitro* (Bernard, *et al.*, 1993) to poison the cleavable complex by inhibiting the resealing of the double-strand nick in the DNA. This causes DNA breakage, activation of the SOS response, and cell death.

### The *ccd*B gene

The *ccd*B gene is found in the *ccd* (control of cell death) locus on the F plasmid. This locus contains two genes, *ccd*A and *ccd*B, which encode proteins of 72 and 101 amino acids respectively (Karoui, *et al.*, 1983; Ogura and Hiraga, 1983; and Miki, *et al.*, 1984). The *ccd* locus participates in stable maintenance of F plasmid by post-segregational killing of cells that do not contain the F plasmid (Jaffé, *et al.*, 1985). The CcdB protein is a potent cell-killing protein when its action is not inhibited by the CcdA protein. The half-life of the CcdA protein is shorter than that of the CcdB protein. Persistence of the CcdB protein leads to death of bacterial segregants that do not contain the F plasmid. Overexpression of the CcdB protein causes cell death when its action is not prevented by sufficient CcdA protein (Van Melderen, *et al.*, 1994).

# Methods

## Clone into pZErO®-2

### Introduction

Selecting cloned inserts using the Zero Background™ technique is extremely powerful. However, because of the nature of selection, do not propagate the vector in common laboratory strains. We have developed ligation and transformation procedures to optimize the use of the pZErO®-2 vector provided in this kit. The kit contains enough vector for ~25 restriction digestions and ~2000 ligations.

### Before starting

1. Prepare LB plates containing 25–50 µg/mL kanamycin (see page 15). Store the plates at 4°C.

   If you are using cells that carry the *lac*I$^q$ gene, include 1 mM IPTG in the plating medium to achieve complete induction.
2. Prepare LB or SOB medium containing 25–50 µg/mL kanamycin liquid medium for DNA minipreps.
3. Prepare or purchase chemically competent or electrocompetent TOP10 cells. See pages 18–21 for protocols to prepare competent cells; see page 24 for ordering information. The minimal efficiencies required are $1 \times 10^8$ cfu/µg DNA for chemically competent cells and $1 \times 10^9$ cfu/µg DNA for electrocompetent cells.
4. Determine a cloning strategy for ligation into pZErO®-2. See the detail of the multiple cloning site (page 4) for help.

### Important

The LacZ/CcdB fusion protein can be made nonlethal by minor changes in the peptide sequence within the multiple cloning site. Disruption of the LacZ/CcdB fusion protein can occur by frameshift, addition of stop codons, or by alterations in the fusion peptide sequence. We recommend the following precautions to avoid isolating non-recombinants:

- Dephosphorylation of pZErO®-2 is not required or recommended.
- Do not overdigest (more than 30 minutes) with restriction enzymes and take precautions to prevent nuclease contamination. Any problems with high background (self-ligation of the vector that produces colonies) are generally caused by low levels of exonuclease contamination in your restriction enzyme digest, ligation reaction, or the solution containing your insert. Use the buffers and water provided in the kit. If you need to use your own materials, all buffers and water used should be autoclaved extensively or boiled for 5–10 minutes in a microwave oven.
- Exonuclease digestion of vector ends may cause a frameshift mutation resulting in disruption of the *lacZα-ccd*B gene if the vector self-ligates. This results in a high back-ground of non-recombinants. We recommend using the highest quality restriction enzymes for digesting pZErO®-2.

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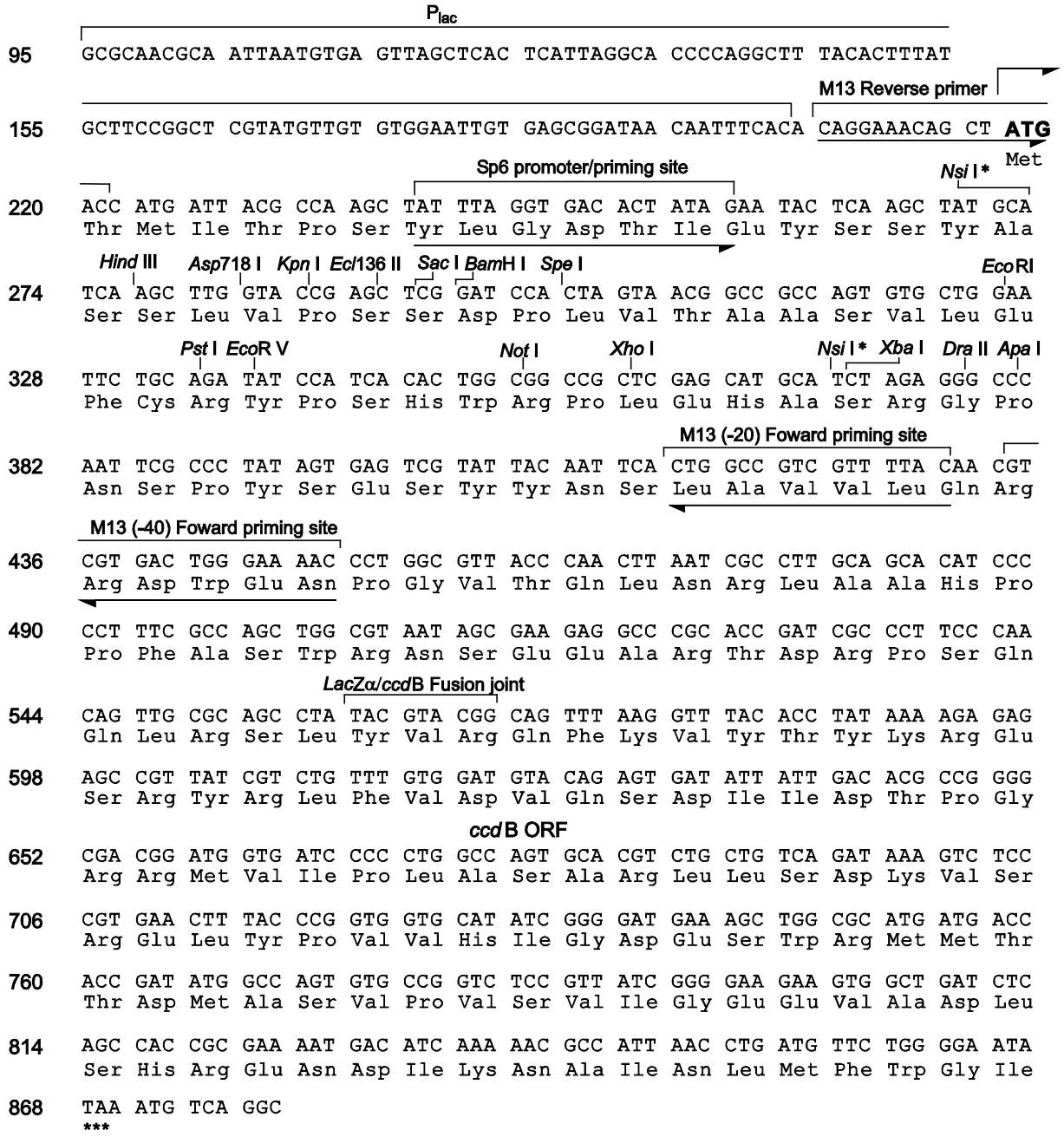
# Clone into pZErO®-2, Continued

## Detail of the Multiple Cloning Site

The following diagram shows the multiple cloning site and surrounding sequences. Restriction sites are marked to indicate the cleavage site. Cloning into the multiple cloning site disrupts expression of the *ccd*B gene.




*The two *Nsi* I sites in the polylinker are the only *Nsi* I sites in the vector.

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# Clone into pZErO®-2, Continued

## Isolate and Purify inserts

For best results, phenol-extract PCR reactions and precipitate the DNA before ligating PCR products into pZErO®-2.

If you gel-purify your insert before ligating into pZErO®-2, you may see a higher background of colonies without insert because of nucleases contamination. To reduce nuclease contamination, **do not** use communal ethidium bromide baths. Use solutions that are free of nucleases, and use high quality agarose.

## Ligate into pZErO®-2

Be sure to include a "no cells", a "cells only", and linearized vector controls.

1. Digest 1 µg each of pZErO®-2 supercoiled vector (1 µL) and your DNA in total volumes of 10 µL for 15–30 minutes using the buffer, temperature, and reaction conditions recommended by the manufacturer of the restriction enzyme. **DO NOT OVERDIGEST.**

   To ligate blunt fragments, digest pZErO®-2 with 10–20 units of *Eco*R V for 15–30 minutes. Adjusting insert: vector quantities to a molar ratio of 2:1 for a cohesive-end ligation and between 3:1 to 10:1 for a blunt-end ligation increases ligation efficiency. Adjust digestion quantities accordingly.

2. **For enzymes that are inactivated by heat**, add TE buffer to a final volume of 100 µL and heat reaction to 70°C for 10 minutes, cool the reaction to room temperature, then place the reaction on ice. The final concentration of the reaction will be 10 ng/µL.

   You may use the enzyme digestion directly in the ligation reaction (step 5 of this procedure) unless the enzyme is not fully inactivated by heat.

   **If the enzyme(s) are not fully inactivated by heat** (e.g. *Bam*H I, *Eco*R V, *Kpn* I, *Pst* I), extract the digest with 10 µL phenol/chloroform; precipitate the DNA with 1/10 volume 3 M sodium acetate, pH 5.6, and 2 volumes 100% ethanol; centrifuge; and carefully wash the pellet with 80% ethanol. Air dry the pellet and resuspend in 90 µL of TE buffer. Assuming 90% recovery, the DNA concentration will be 10 ng/µL.

3. To verify complete digestion and recovery, electrophorese a 20 µL aliquot of the digestion on a 1% agarose gel.

4. If you are ligating cohesive-ends, use 2:1 insert: vector molar ratio. If you are ligating blunt-ends, increase the insert: vector molar ratio to between 3:1 to 10:1. See **Calculate molar ratios** on page 6 to determine how much insert you need. Set up the following 10 µL ligation reaction using the supplied reagents:

| | | |
|---|---|---|
| Digested vector (~10 ng) | | 1 µL |
| Digested DNA insert | | x µL |
| Sterile water | to a final volume of | 8.5 µL |
| 10X Ligation Buffer (with ATP) | | 1 µL |
| T4 DNA Ligase (4 U/µL) | | 0.5 µL |
| Total Volume | | 10 µL |

5. Incubate at 16°C for 30 minutes (cohesive-end ligations) or 60 minutes (blunt-end ligations). **Do not ligate at room temperature.** Place vials on ice. Proceed to **Transformation**, page 8.

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# Clone into pZErO®-2, Continued

## Calculate molar ratios

To clone your insert into pZErO®-2, you need to know the concentration of your insert DNA solution. Determine the concentration by $OD_{260}$, agarose gel electrophoresis, fluorescence, or with the Qubit® dsDNA Assay Kit (see page 24 for ordering information). Use the concentration to calculate the volume required to achieve a particular molar ratio of vector to insert.

1. Determine the concentration of the insert in µg/mL.
2. Use the following formula to calculate the amount of insert needed to give a molar ratio of 2:1 between insert and linearized pZErO®-2. Note that the amount of pZErO®-2 is 10 ng.

$$\text{x ng insert} = \frac{(2)\ (\text{bp insert})\ (10\text{ ng linearized pZErO®-2})}{(3297\text{ bp pZErO®-2})}$$

**Note:** If you are performing a blunt-ended ligation, calculate the insert: vector molar ratio to be between 3:1 and 10:1 by replacing the 2 with the appropriate molar ratio in the preceding equation.

3. Based on the calculation above, calculate the volumes needed for the ligation reaction.

## General guidelines for control reactions

The following table gives some suggestions for possible control reactions for the experiments presented in this manual.

| Experiment | Control | Reason |
|---|---|---|
| Ligation and Transformation | No DNA | Checks for contamination of ligation reagents. |
| | Linearized vector only | Checks for nuclease contamination in your reactions. A frameshift mutation will disrupt *ccdB* function, resulting in a high background of colonies that will not contain insert. |
| | Cells only | Checks for the presence of antibiotic in the plates and contamination of competent cells and SOC medium. |
| | Supercoiled vector (e.g. pUC19) | Checks the efficiency of the competent cells. See **Transformation**, page 8. |
| | Test Inserts | Checks general ligation conditions.<br>Confirms disruption of *ccd*B expression. See page 7 for more information. |

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# Clone into pZErO®-2, Continued

## Use the test inserts

Use the test inserts to check the general ligation conditions and to confirm the lack of *ccd*B function when its expression is disrupted. To use the test inserts, digest the pZErO®-2 vector with *Eco*R V, and ligate the test inserts (*Hae* III digested ΦX174 DNA) into the vector. Tranform the ligation mixture into competent TOP10 cells, and plate the transformation mix onto LB-kanamycin plates.

1. Digest 1 µg of pZErO®-2 with *Eco*R V (10 units) for 15–30 minutes.
2. **IMPORTANT! *Eco*R V is not heat inactivated**.

   Extract digested vector with phenol/chloroform and precipitate the DNA in the aqueous layer with 1/10 volume 3 M sodium acetate pH 5.6 and 2 volumes of ethanol. Carefully wash the pellet with 80% ethanol and air dry.
3. Resuspend the DNA pellet in 90 µL TE buffer.
4. Set up the following 10 µL ligation reaction:

| | |
|---|---|
| Digested vector (~10 ng) | 1 µL |
| Test inserts, blunt-ended ΦX174 DNA (20 ng/µL) | 1 µL |
| Sterile water | 6.5 µL |
| 10X ligation buffer | 1 µL |
| T4 DNA Ligase (4 U/µL) | 0.5 µL |
| Total Volume | 10 µL |

5. Set up a "vector only" ligation reaction:

| | |
|---|---|
| Digested vector (~10 ng) | 1 µL |
| Sterile water | 7.5 µL |
| 10X ligation buffer | 1 µL |
| T4 DNA Ligase (4 U/µL) | 0.5 µL |
| Total Volume | 10 µL |

6. Incubate the ligation reactions at 16°C for 1 hour. Proceed to **Transformation**, pages 8–13. Use 2 µL of the ligation reaction to transform TOP10 cells and plate 10 µL of each transformation reaction.

The number of transformants per plate varies from 50–2000 colonies per 10 µL using competent TOP10 cells. The cloning efficiency should be ~95%. Since neither the *Hae* III nor the *Eco*R V sites are regenerated, inserts may be released with *Nsi* I or a combination of other enzymes. Digestion of a recombinant plasmid will release one of 11 possible ΦX174 DNA fragments (bp): 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, or 1353. Note that tandem inserts may occur. This will not affect disruption of *ccd*B expression. Φ174 DNA has no sites for *Apa* I, *Bam*H I, *Eco*R I, *Hin*d III, *Kpn* I, *Not* I, *Nsi* I, *Spe* I, or *Xba* I.

## Determine cloning efficiency

To determine the cloning efficiency, compare the number of colonies produced in the test insert ligation (10 µL plate) to the total number of colonies seen on the 10 µL test insert ligation plate plus the number of colonies on the 10 µL vector only plate.

$$\text{cloning efficiency} = \frac{\text{(colonies on test insert plate)}}{\text{(colonies on test insert plate) + (colonies on vector only plate)}} \times 100\%$$

# Transformation

## Introduction

After performing the cloning reaction, chemically transform or electroporate your pZErO®-2 construct into competent TOP10 *E. coli*, and plate the transformed cells onto LB-kanamycin plates (see **Recipes**, page 15). After incubating the plates for 24 hours, analyze kanamycin-resistant colonies by DNA miniprep and restriction mapping to find the desired clones. This section includes protocols to transform chemically competent or electrocompetent *E. coli*.

For a high-efficiency and convenient transformation, we recommend One Shot® TOP10 Chemically Competent *E. coli* or One Shot® TOP10 Electrocompetent Cells, which are available separately (see page 24 for ordering information). For instructions on preparing chemically competent or electrocompetent cells, see pages 18 and 20, respectively.

## Controls

See the section on **Control Reactions** (page 6) to determine which controls to include.

## General handling

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transform the cells immediately after you thaw them on ice. Mix by stirring with a pipette tip, not by pipetting up and down.

**Important**

If you are using cells that carry a *lac*I$^q$ gene, IPTG is required to induce expression from the *lac* promoter. Be sure to include IPTG in the agar medium at a final concentration of 1 mM. **Do not spread IPTG on the plate.**

*Continued on next page*

# **Transformation,** Continued

**Before starting**

- If transforming by electroporation, you need:
  - an electroporation device
  - sterile, glass transfer pipettes or pipette tips
  - electroporation cuvettes (contact Technical Support for ordering information, page 25)
  - sterile, 15-mL polypropylene snap-cap tubes
  - SOC medium
- Equilibrate a water bath to 42°C.
- Warm one vial of SOC medium to room temperature.
- Determine the total number of transformations, including controls. You need two LB-kanamycin plates per ligation/transformation. Place an appropriate number of 10-cm diameter LB-kanamycin agar plates in a 37°C incubator to remove excess moisture.
- To test the transformation efficiencies of your cells, prepare a stock solution (10 pg/µL) of any supercoiled plasmid (e.g. pUC19, pBR322) to use as a control for transformation. Be sure to use appropriate antibiotic plates to test the transformation efficiency of your competent cells.
- Obtain a test-tube rack (float) that will hold all transformation tubes to allow simultaneous heat shock in the water bath at 42°C.

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### One Shot® TOP10 chemical transformation

1. Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.
2. Thaw, on ice, one 50 µL vial of One Shot® cells for each ligation/transformation.
3. Pipet 2 µL of each ligation reaction directly into the competent cells, and mix by tapping gently. Use 1 µL of diluted control plasmid for the transformation control. You can store the remaining ligation mixture(s) at –20°C.
4. Incubate the vial(s) on ice for 15 minutes.
5. Incubate the vial(s) for 30–45 seconds in the 42°C water bath (i.e. heat shock). Do not mix or shake.
6. Remove the vial(s) from the 42°C bath and quickly place them on ice for 1 minute.
7. Add 250 µL of pre-warmed SOC medium to each vial. (SOC is a rich medium; you must practice good sterile technique to avoid contamination.)
8. Place the vial(s) in a microcentrifuge rack on its side, and secure them with tape to avoid loss of the vial(s). Shake the vial(s) at 37°C for exactly 1 hour at 225 rpm in a shaking incubator.
9. Place the vial(s) with the transformed cells on ice.
10. Spread 10–50 µL from each transformation vial on separate, labeled LB-kanamycin agar plates. We recommend that you plate 2 different volumes to ensure well-spaced colonies. For plating smaller volumes, add 20 µL SOC to ensure even spreading.

    **Note**: Plate the pUC19 control transformation on LB plates containing 50 µg/mL ampicillin.
11. Invert the plate(s) and incubate at 37°C overnight. Proceed to **Analyze Transformants**, page 13.

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### One Shot® electroporation

**Use ONLY electrocompetent cells for electroporation to avoid arcing. Do not use the One Shot® TOP10 chemically competent cells for electroporation.**

1. Add 2 µL of the ligation reaction into a sterile microcentrifuge tube containing 50 µL of electrocompetent *E. coli* and mix gently. **Do not mix by pipetting up and down. Avoid formation of bubbles.** Transfer the cells to a 0.1-cm cuvette.
2. Electroporate your samples using your own protocol and your electroporator.
3. Immediately add 250 µL of room-temperature SOC Medium.
4. Transfer the solution to a 15-mL snap-cap tube (e.g. Falcon®) and shake the tube at 37°C for 1 hour.
5. Spread 10–50 µL from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µL of SOC Medium. We recommend that you plate 2 different volumes to ensure that at least 1 plate will have well-spaced colonies.
6. Invert the plate(s) and incubate at 37°C overnight. Proceed to **Analyze Transformants**, page 13.

### Chemical transformation

Use the following chemical transformation procedure if you have generated your own competent cells from the TOP10 *E. coli* provided as a stab with the kit. You may not have the same degree of transformation efficiency regularly attained when using One Shot® chemically competent cells; prepare additional SOC for this procedure (see **Recipes**, page 16).

1. Equilibrate a water bath or heat block to 42°C. Remove the appropriate number of tubes of frozen TOP10 chemically competent cells (50 µL each) and thaw on ice.
2. Add 2–5 µL of each ligation reaction to a separate tube of competent cells. Mix gently with the pipette tip. **Do not pipet up and down**. Repeat for all ligations.

   **Note**: If the cell competency is less than $1 \times 10^8$ cfu/µg, you may need to use more of the ligation mixture.
3. For control reactions, add 1 µL (10 ng) of each supercoiled plasmid (e.g. pUC19) to a separate tube of cells.
4. Incubate all tubes on ice 20 minutes.
5. Transfer all tubes to 42°C heat block or water bath and incubate for 1 minute, then place on ice for 1 minute.
6. Add 450 µL of room temperature SOC medium to each tube and shake at 225 rpm for 60 minutes at 37°C. Place on ice. Place the tubes horizontally in the shaker to maximize aeration, and secure them with tape.
7. Plate 25 and 100 µL of each transformation mix on LB-kanamycin plates. Let all the liquid absorb into agar, invert the plate(s), and incubate at 37°C for 18–24 hours. Proceed to **Analyze Transformants**, page 13.

   **Note**: If the cell competency is less than $1 \times 10^8$ cfu/µg, you may need to plate more of each transformation mix.

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### Electroporation transformation

**Note:** Prepare additional SOC for this procedure (see **Recipes**, page 16).

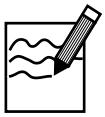
1. Remove the appropriate number of microcentrifuge tubes of TOP10 electrocompetent cells from the –80°C freezer, and thaw the cells on ice. Chill the electroporation cuvettes on ice.
2. Set up your electroporation device for electroporation of bacteria using the manufacturer's instructions.
3. Dilute the ligation reaction with 10 µL of sterile water and place at 65°C for 5 minutes.

   **Note**: Ligation reactions are diluted to reduce the salt concentration. Excess salt may cause arcing during electroporation. Heating to 65°C inactivates the ligase.
4. Add 2 µL of the ligation reaction to each tube containing 80 µL competent cells. Repeat for all ligation reactions.
5. For the control reactions, add 1 µL (10 ng) of each supercoiled plasmid (e.g. pUC19) to a separate tube of 40 µL competent cells.
6. Incubate all tubes on ice for 1 minute.
7. Take one sample at a time and transfer the cell/DNA mix to an electroporation cuvette. Be sure not to trap air bubbles in the sample. Place the cuvette in the chamber, and discharge the electrical pulse.
8. Remove the cuvette, and **immediately** add 450 µL of room temperature SOC medium into the cuvette. Transfer the contents of the cuvette to a 15-mL snap-cap polypropylene tube (Falcon 2059 or similar), and place the tube on ice.
9. Repeat Steps 7–8 until all samples have been transferred to 15-mL tubes.
10. Incubate the tubes with shaking (200–225 rpm) at 37°C for 60 minutes. Place the tubes horizontally in the shaker to maximize aeration, and secure them with tape.
11. Plate 25 µL and 100 µL of the transformation mix on LB-kanamycin agar plates. After the liquid is absorbed into the agar, invert the plates, and incubate them at 37°C for 18–24 hours. Proceed to **Analyze Transformants**, page 13.

*Continued on next page*

### Analyze transformants

1. Remove the plates from the incubator. Pick at least 10 kanamycin-resistant transformants and inoculate into 2 mL LB or SOB medium containing 25–50 µg/mL kanamycin. Grow 6–8 hours or overnight at 37°C.
2. Isolate plasmid DNA by miniprep for restriction analysis. You may need to phenol-extract miniprep DNA to prevent smearing on agarose gels. Remember to isolate the desired clone by streaking for single colonies, and reconfirm the presence of insert before making a glycerol stock.
3. Prepare a glycerol stock of your desired clone for long-term storage by combining 0.85 mL of the mid-log bacterial culture with 0.15 mL of sterile 100% glycerol in a labeled cryovial. Mix the contents of the cryovial by vortexing, and freeze the tube in liquid nitrogen or a dry ice/ethanol bath. Store the glycerol stocks at –80°C.
4. After isolating the desired clone, you may proceed with further subcloning and/or analysis of your insert.



**Note**

If you need ultra-pure plasmid DNA for automated or manual sequencing, we recommend using the PureLink® HQ Mini Plasmid Purification or PureLink® HiPure Plasmid Miniprep kits (see page 24 for ordering information). Refer to www.lifetechnologies.com/support or contact Technical Support (page 25) for more information on a large selection of plasmid purification columns.

# Troubleshooting

| Observation | Reason | Solution |
|---|---|---|
| Very few or no transformants arise | DNA is lost during precipitation | Use more DNA. Be careful not to lose the DNA pellet during the precipitation and wash steps. |
| | Insert not ligating properly | Check the subcloning strategy. |
| | Molar ratio of insert to vector is incorrect | Determine the concentration of insert and calculate the correct molar ratio. |
| | Transformation efficiency of *E. coli* strain is too low | Chemically competent cells should yield ~$1 \times 10^9$ transformants/µg DNA. Electrocompetent cells should yield >$1 \times 10^9$ transformants/µg DNA. Check transformation efficiency with a control vector. |
| High background of transformants that do not contain inserts | The vector is overdigested with restriction enzymes | Use the minimum amount of enzyme necessary to digest the vector. Limit digests to 15–30 minutes. |
| | If using cells that carry the *lac*$I^q$ gene, insufficient amount of IPTG in plate | IPTG must be in excess to achieve proper induction and cell death. |
| | Reagents are contaminated with nucleases | Use the reagents supplied with the kit or autoclave all reagents used for cloning (especially water). |
| Thin "lawn" of cells on plate | If using cells that carry the *lac*$I^q$ gene, plate contained insufficient amount of IPTG | Be sure to add the correct amount of IPTG to the plate medium. Let the medium cool sufficiently before adding IPTG. **Do not** spread IPTG onto plates. |
| | Insufficient amount of antibiotic is used in medium | Be sure to add the correct amount of antibiotic to the medium. |
| DNA migrates anomalously on agarose gels (bands run at a larger molecular weight than expected and seem slightly smeared) | Protein is bound to DNA | Extract the DNA with phenol/chloroform during plasmid preparation or use the PureLink® HQ Mini Plasmid Purification Kit (see page 24 for ordering information). |

# Appendix

## Recipes

### Low Salt LB Agar Plates with Kanamycin

Low Salt LB Medium (per liter)
1% Tryptone
0.5% Yeast Extract
0.5% NaCl
1.5% Agar
pH 7.5

**Note**: As the salt concentration of the medium decreases, the activity of aminoglycoside antibiotics (e.g. streptomycin, kanamycin) increases. You may find that 25 μg/mL kanamycin is sufficient to select transformants. Test your host strain for sensitivity to 25 μg/mL kanamycin before selecting transformants.

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 5 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.5 with 5 M NaOH, add 15 g agar, and bring the volume to 1 liter.
3. Autoclave for 20 minutes on liquid cycle.
4. Let agar cool to ~55°C. Add kanamycin to a final concentration of 25–50 μg/mL.

   If using a cell line that carries the *lac*$I^q$ gene, add IPTG to a final concentration of 1 mM (1 mL/liter).
5. Pour into 10-cm petri plates. Let the plates harden, then invert and store at 4°C. Plates containing kanamycin and IPTG are stable for 1–2 weeks.

### Low Salt LB-Kanamycin Medium

Low Salt LB Medium (per liter)
1% Tryptone
0.5% Yeast Extract
0.5% NaCl
pH 7.5

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 5 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.5 with 5 M NaOH and bring the volume to 1 liter.
3. Autoclave for 20 minutes on liquid cycle.
4. Let solution cool to ~55°C. Add kanamycin to a final concentration of 25–50 μg/mL. Store the medium at 4°C.

*Continued on next page*

# **Recipes,** Continued

## SOB Medium

SOB (per liter)
2% Tryptone
0.5% Yeast Extract
0.05% NaCl
2.5 mM KCl
10 mM $MgCl_2$

1. Dissolve 20 g tryptone, 5 g yeast extract, and 0.5 g NaCl in 950 mL deionized water.
2. Make a 250 mM KCl solution by dissolving 1.86 g of KCl in 100 mL of deionized water. Add 10 mL of this stock KCl solution to the solution in step 1 of this procedure.
3. Adjust pH to 7.5 with 5 M NaOH and add deionized water to 1 liter.
4. Autoclave this solution, cool to ~55°C, and add 10 mL of sterile 1 M $MgCl_2$ and kanamycin, if desired.
5. Store at room temperature or 4°C. **Do not add IPTG.**

## SOC Medium

SOB Medium
20 mM glucose

1. Prepare and autoclave the SOB medium as described above.
2. After autoclaving, cool the solution to ~55°C, and add 10 mL of sterile 1 M $MgCl_2$ and 7.2 mL of 50% glucose.
3. Store at room temperature or 4°C.

*Continued on next page*

### FSB Transformation Solution

10 mM potassium acetate, pH 7.5
45 mM $MnCl_2$-$4H_2O$
10 mM $CaCl_2$-$2H_2O$
100 mM KCl
3 mM hexaamminecobalt chloride (Contact Aldrich at 1-800-558-9160 to order)
10% glycerol

1. Prepare 100 mL of 1 M potassium acetate by dissolving 9.82 g in 90 mL deionized water. Adjust pH to 7.5 with 2 M acetic acid. Bring the volume up to 100 mL.
2. For 100 mL of FSB transformation solution combine the following ingredients:

| | |
|---|---|
| 1 mL | 1 M potassium acetate, pH 7.5 |
| 890 mg | $MnCl_2$-$4H_2O$ |
| 150 mg | $CaCl_2$-$2H_2O$ |
| 750 mg | KCl |
| 80 mg | hexaamminecobalt chloride |
| 10 mL | 100% glycerol |
| 80 mL | deionized water |

3. Carefully adjust pH to 6.4 with 0.1 N HCl. If you go past the correct pH, remake solution. Do not readjust pH with base.
4. Adjust the final volume to 100 mL with deionized water and filter sterilize. Store at 4°C.

### DMSO

It is very important to use fresh, analytical grade DMSO. If you routinely transform cells by chemical means using the method of Hanahan, 1983, you probably have frozen aliquots of DMSO in your laboratory; if not, then follow this procedure:

1. Order the smallest amount of analytical grade DMSO.
2. When the DMSO arrives, take 5–10 mL and aliquot 200–500 µL per microcentrifuge tube. You may use the rest of the DMSO for other applications or you may aliquot the remainder for competent cells. It depends on whether you plan to use the method described in this manual on a routine basis.
3. Freeze these tubes at –20°C and use one tube per preparation of competent cells. Discard any remaining DMSO in the tube. **Use a fresh tube for every preparation of competent cells.**

# Protocol for Chemically Competent Cells

## Introduction

This following protocol describes how to make chemically competent cells for transformation with plasmid DNA (Hanahan, 1983). These cells are not a substitute for electrocompetent cells used for electroporation. To make chemically competent cells, grow the cells to mid-log phase, wash them with FSB solution, and treat them with DMSO. Freeze the competent cells in a dry ice/ethanol bath and stored at –80°C.

## Yield

This protocol will yield enough cells for about 60 transformations. The expected efficiency of chemically competent TOP10 cells is $1 \times 10^8$ cfu/µg supercoiled DNA. This is also the minimum efficiency needed to obtain 100–200 colonies per 100 µL transformation mix.

**Important**

**Sterile technique is absolutely essential to avoid contamination of the competent cells.** Remember to use **sterile** solutions, medium, and supplies.

## Required solutions

For each preparation, prepare the following solutions (see **Recipes**, pages 15–17):

- 5 mL SOB medium in a sterile culture tube
- 250 mL SOB in a sterile 500-mL or 1-liter culture flask
- FSB solution (~25 mL)
- Fresh, reagent grade DMSO

## Protocol for Chemically Competent Cells

**Day 1:**

1. Streak TOP10 *E. coli* on an LB plate, invert the plate, and incubate at 37°C overnight.

**Day 2:**

2. Inoculate 5 mL of SOB medium in a sterile culture tube with one colony from the LB plate.
3. Grow the cells overnight (12–16 hours) in a shaking incubator (200–225 rpm) at 37°C.

**Day 3:**

4. For each preparation, place the following items on ice or at 4°C.
   - Two 250-mL sterile centrifuge bottles
   - Two 50-mL sterile centrifuge tubes
   - Two 5-mL sterile pipettes
5. Inoculate 250 mL of fresh SOB medium in a 500-mL or 1-liter culture flask with 2.5 mL of the overnight culture.
6. Grow the culture at 37°C at 200–225 rpm in a shaking incubator until the $OD_{550}$ reaches between 0.55–0.65 (2–3 hours).

*Procedure continued on next page*

*Continued on next page*

# Protocol for Chemically Competent Cells, Continued

**Protocol for Chemically Competent Cells,** Continued

**Day 3**, Continued:

7. Divide the culture between the two cold (0–4°C), sterile 250-mL centrifuge bottles, and place on ice for 30 minutes.
8. Centrifuge the 250-mL bottles at 2,000 × g for 10–15 minutes at 0–4°C.
9. Decant the medium, and resuspend each pellet in 10 mL of cold (0–4°C) FSB solution. Transfer the resuspended cells to two cold, sterile, 50-mL centrifuge tubes. Incubate the tubes on ice for 15 minutes.
10. Centrifuge the tubes at 2,000 × g for 10–15 minutes at 0–4°C.
11. Decant the buffer, and resuspend each pellet in 1.8 mL cold FSB solution using a sterile 5-mL pipette.
12. While gently swirling the tubes, slowly add 65 µL of DMSO drop by drop to each tube. Incubate the tubes on ice for 15 minutes.
13. While gently swirling the tubes, slowly add an additional 65 µL of DMSO drop by drop to each tube.
14. Combine the cell suspensions from both tubes into one, and incubate the tube on ice for 15 minutes. Keep on ice.
15. Prepare a dry ice/ethanol bath.
16. For each preparation, place approximately sixty 1.5-mL microcentrifuge tubes on ice. Keep cell suspension on ice.
17. Pipette 50 µL of cell suspension into each tube.
18. As soon as all of the cell suspension is aliquoted, quick-freeze the tubes in the dry ice/ethanol bath and store at –80°C.

# Protocol for Electrocompetent Cells

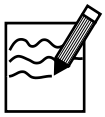
## Introduction

This procedure describes how to prepare cells for transformation with plasmid DNA by electroporation. The washing step in the protocol ensures that salts are removed from the cell solution to reduce the conductivity. High conductivity may result in arcing during electroporation.

Use electrocompetent cells only for electroporation. **Do not** use them for any other transformation procedure.

## Yield

The following procedure will yield enough electrocompetent cells for about 30 transformations.



**Note**

The expected efficiency of the electrocompetent TOP10 cells is $1 \times 10^9$ cfu/µg supercoiled DNA. This is the minimum efficiency needed to obtain 100–200 colonies per 100 µL of the transformation reaction.

**Important**

**Sterile technique is absolutely essential to avoid contamination of the electrocompetent cells.**

## Protocol for electrocompetent cells

**Day 1:**

1. Streak TOP10 *E. coli* on an LB plate, invert the plate, and incubate at 37°C overnight.
2. Prepare the following solutions. Store the LB media at room temperature, and the glycerol solution and water at 4°C.
   - 50 mL LB medium in a 250-mL sterile culture flask
   - 1 liter of LB medium in a 2-liter or 4-liter sterile culture flask
   - 50 mL of sterile 10% glycerol
   - 1 liter of sterile water

**Day 2:**

3. Inoculate the 50 mL of LB medium in a 250-mL culture flask with a single colony from the LB plate and incubate at 37°C with shaking (200–225 rpm) for 12–16 hours (overnight).

**Day 3:**

4. For each preparation, pre-chill on ice or at 4°C:
   - Two sterile 500-mL centrifuge bottles
   - Two sterile 50-mL centrifuge tubes
   - Two sterile 25-mL pipettes
   - One sterile 5-mL pipette

*Procedure continued on next page*

*Continued on next page*

# Protocol for Electrocompetent Cells, Continued

**Protocol for electrocompetent cells,** Continued

**Day 3**, Continued:

5. Inoculate 1 liter of LB medium in a 2-liter or 4-liter flask with the 50 mL of overnight culture. Grow the 1 liter culture in a shaking incubator (200–225 rpm) at 37°C until the $OD_{550}$ is between 0.5–0.6 (approximately 2–3 hours).
6. Transfer the 1-liter culture to the two chilled, sterile 500-mL centrifuge bottles, and incubate on ice for 30 minutes.
7. Centrifuge the cultures at 2,000 × g for 15 minutes at 0–4°C. Keep the cell pellet, and decant the broth. Place the bottles back on ice.
8. Resuspend the cell pellet in each bottle in approximately 500 mL of ice cold sterile water.
9. Centrifuge cells at 2,000 × g for 15 minutes at 0–4°C. Keep the pellet, and decant the water. Place the bottles back on ice.
10. Resuspend the cells in each bottle in approximately 250 mL of ice cold sterile water.
11. Centrifuge cells at 2,000 × g for 15 minutes at 0–4°C. Decant the water and place the bottles back on ice.
12. Using a pre-chilled sterile 25-mL pipette, resuspend the cells in each bottle in 20 mL of ice cold sterile 10% glycerol. Transfer each cell suspension to a chilled, sterile 50-mL centrifuge tube.
13. Centrifuge the cells at 4,000 × g for 15 minutes at 0–4°C. Decant the 10% glycerol, and place the tubes on ice.
14. Resuspend each cell pellet in 1 mL of ice cold sterile 10% glycerol. Using a pre-chilled 5-mL pipette, pool the cells into one of the 50-mL tubes. Keep the tubes containing the cells on ice.
15. Prepare a dry ice/ethanol bath.
16. For each preparation, place thirty-five to forty 1.5-mL microcentrifuge tubes on ice, and pipet 40 µL of the cell suspension into each tube. Keep the cell suspension and tubes on ice until you have aliquoted all of the cell solution.
17. After you have aliquoted all of the cell solution, quick-freeze the tubes in the dry ice/ethanol bath. Store the electrocompetent cells at –80°C until ready for use.

# pZErO®-2 Vector

## Description of pZErO®-2

pZErO®-2 is a 3297 bp cloning vector that allows direct selection of positive recombinants via disruption of the lethal gene, *ccd*B. Expression of *ccd*B results in the death of cells containing non-recombinant vector. The product of the *ccd*B gene is expressed as a fusion protein to the LacZα peptide fragment to exploit the proven disruption technology of LacZα, and to include the M13 forward and reverse priming sites in the vector.

## Features of pZErO®-2

The important elements of pZErO®-2 are described in the following table. The pZErO®-2 vector has been completely sequenced and all features have been functionally tested.

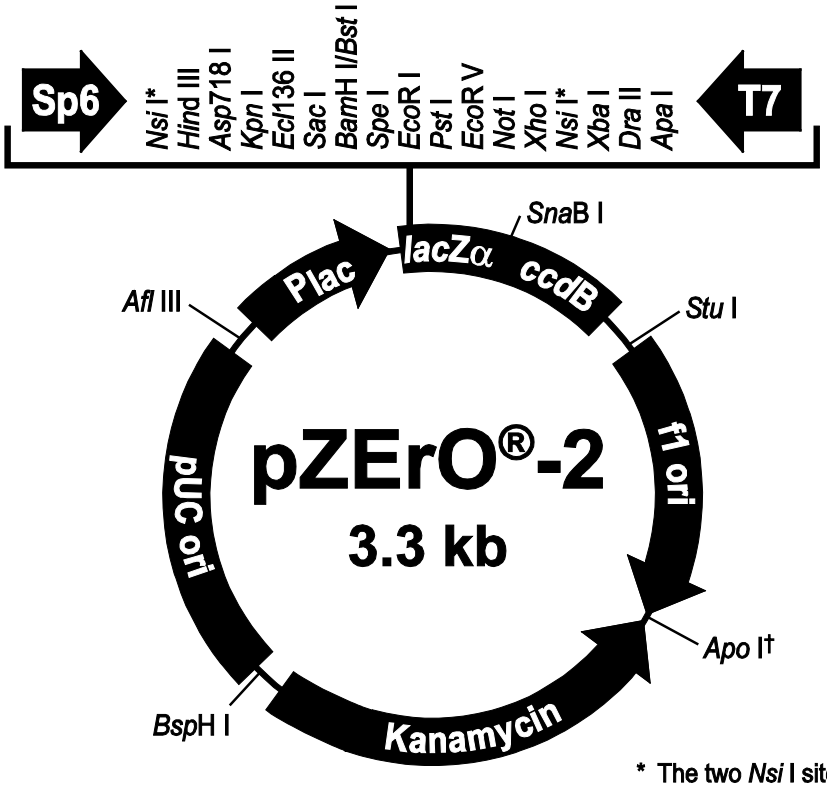
| Features | Function |
|---|---|
| Promoter ($P_{lac}$) | Allows inducible expression of *lac*Zα-*ccd*B gene fusion. |
| M13 Reverse priming site | Permits sequencing of your insert. |
| Sp6 promoter/priming site | Allows *in vitro* transcription of the sense strand and sequencing of your insert. |
| Multiple Cloning Site (17 unique sites) | Permits insertion of desired gene to disrupt expression of the *ccd*B gene. |
| *lac*Zα-*ccd*B fusion gene | • Provides lethal selection against non-recombinant vector.<br>• Uses the LacZα disruption technology to clone inserts.<br>• Includes all the universal M13 forward and reverse priming sites for sequencing. |
| T7 promoter/priming site | Allows *in vitro* transcription of the anti-sense strand and sequencing of your insert. |
| M13 Forward (–20,–40) priming sites | Permit sequencing of your insert. |
| f1 origin | Allows isolation of single-stranded DNA from *E. coli* strains containing the F´. |
| Kanamycin resistance gene | Provides resistance to the antibiotic kanamycin. Derived from the bacterial transposon Tn*5.* |
| pUC origin | Allows high copy replication and maintenance of the plasmid in *E. coli*. |

*Continued on next page*

# pZErO®-2 Vector, Continued

## Map of pZErO®-2

The following figure summarizes the features of the pZErO®-2 vector. The sequence is available from www.lifetechnologies.com/support or Technical Support (page 25). Details of the multiple cloning site are shown on page 4.




**Comments for pZErO®-2**
**3297 nucleotides**

Lac Promoter/Operator Region: bases 95-216
M13 Reverse Priming Site: bases 205-221
*Lac*Zα ORF: bases 217-558
Sp6 Promoter/Priming Site: bases 239-256
Multiple Cloning Site: bases 269-381
T7 Promoter/Priming Site: bases 388-407
M13 (-20) Forward Priming Site: bases 415-430
M13 (-40) Forward Priming Site: bases 434-450
Fusion Joint: bases 559-567
*ccd*B Lethal Gene ORF: bases 568-870
f1 origin: bases 895-1307
Kanamycin Resistance ORF: bases 2116-1322 (C)
pUC origin: bases 2502-3175

* The two *Nsi* I sites in the MCS are the only sites in the vector.

† There are two tandem *Apo* I sites at this location. *Apo* I also recognizes the *Eco*R I site.

# Accessory Products

## Additional Products

The following table lists additional products that may be used with the Zero Background™/Kan Cloning Kit. For more information, visit www.lifetechnologies.com/support or contact **Technical Support,** page 25.

| Product | Quantity | Catalog no. |
|---|---|---|
| Platinum® *Taq* DNA Polymerase | 100 reactions | 10966-018 |
| | 250 reactions | 10966-026 |
| | 500 reactions | 10966-034 |
| *Taq* DNA Polymerase, Recombinant | 100 units | 10342-053 |
| | 500 units | 10342-020 |
| PCR OpTimizer™ Kit | 100 reactions | K1220-01 |
| PCR SuperMix High Fidelity | 100 reactions | 10790-020 |
| One Shot® TOP10 Chemically Competent Cells | 10 reactions | C4040-10 |
| | 20 reactions | C4040-03 |
| One Shot® TOP10 Electrocompetent Cells | 10 reactions | C4040-50 |
| Electroporation Cuvettes, 0.1-cm | 50 each | P410-50 |
| Electroporation Cuvettes, 0.2-cm | 50 each | P450-50 |
| PureLink® HQ Mini Plasmid Purification Kit | 100 preps | K2100-01 |
| PureLink® HiPure Plasmid Miniprep Kit | 25 preps | K2100-02 |
| | 100 preps | K2100-03 |
| Kanamycin Sulfate | 5 g | 11815-024 |
| | 25 g | 11815-032 |
| Kanamycin Sulfate (100X), liquid | 100 mL | 15160-054 |
| Qubit® dsDNA Assay Kit, High Sensitivity | 500 assays | Q32854 |
| Qubit® dsDNA Assay Kit, Broad-Range | 500 assays | Q32853 |
| Qubit® 2.0 Fluorometer | 1 each | Q32866 |

# Technical Support

## Obtaining support

For the latest services and support information for all locations, go to www.lifetechnologies.com/support.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## Safety Data Sheets (SDSs)

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

## Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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# Notes