USER GUIDE

invitrogen™
by life technologies™

Zero Background™ Cloning Kit

(pZErO®-1 Vector)

Catalog number K2500-01

Revision date 29 March 2012

Publication part number 25-0152

MAN0000036

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Contents

Experienced Users Guide

Introduction

The following procedure is designed to get experienced users quickly started with the Zero Background™ Kit. Information is provided elsewhere in the manual if you need help with any of the steps.

Before starting

1. Prepare Low Salt LB plates containing 50 µg/mL Zeocin™ (Low Salt LB-Zeocin™, see page 17). Store the plates at 4°C. Plan on using **2** plates per transformation.

   If using a cell line carrying the *lac*I^q gene, include IPTG in the plating medium to achieve complete induction.
2. Prepare Low Salt LB or SOB liquid medium containing 50 µg/mL Zeocin™ for DNA minipreps.
3. Prepare or purchase chemically competent or electrocompetent TOP10 cells. See page 22 for protocols to prepare competent cells. The minimal efficiencies required are 1×10^8 cfu/µg DNA for chemically competent cells and 1×10^9 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 28 for ordering information).
4. Determine a cloning strategy for ligation into pZErO®-1. Refer to the details of the multiple cloning site (page 5) for help.

Ligate into pZErO®-1

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

1. Digest 1 µg each of pZErO®-1 supercoiled vector (1 µL) and your DNA in total volumes of 10 µL using the recommended buffer, temperature, and reaction conditions described by the manufacturer of the restriction enzyme. Optimal digestion time is 15–30 minutes. **Do not digest for 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 (e.g. *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 next page

Continued on next page

Ligate into pZErO®-1, 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 3:1–10:1. See page 7 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** in the following section.

Transformation

For more information on transformation methods, see pages 12–14.

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-Zeocin™ plates. Let the liquid absorb, invert the plate, and incubate at 37°C for 18–24 hours. Proceed to **Analyzing Transformants** in the following section.

Analyze transformants

1. Remove the plates from the incubator. Pick at least 10 Zeocin™ resistant transformants, and inoculate into 2 mL Low Salt SOB medium containing 50 µg/mL Zeocin™. 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 to a labeled storage tube. Freeze the tube in liquid nitrogen or a dry ice/ethanol bath and store at –70°C.
4. After you isolate the desired clone, proceed with further subcloning and/or analyzing your insert.

Kit Contents and Storage

Kit Contents

The Zero Background™ Cloning Kit contains the following reagents:

Item	Concentration	Storage
pZErO®-1 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 60 mM $MgCl_2$ 50 mM NaCl 1 mg/mL bovine serum albumin 70 mM β-mercaptoethanol 1 mM ATP 20 mM dithiothreitol 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 1 mM EDTA	–30°C to –10°C
TOP10 cells, 1 stab	—	2°C to 8°C
Zeocin™ antibiotic, 125 mg	100 mg/mL in water	–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

***TE buffer, pH 7.5**: 10 mM Tris-HCl, 1 mM EDTA, pH 7.5

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

Prepare TOP10 *E. coli* glycerol stocks

We recommend that you prepare a set of 5–10 TOP10 *E. coli* glycerol master stocks within 2 weeks of receiving the kit:

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.
4. Grow the culture to stationary phase (OD_{600} = 1–2).
5. Mix 0.8 mL of the culture with 0.2 mL of sterile glycerol, and transfer to a cryovial.
6. Store the vials at –80°C. Use one master stock to create working stocks for regular use.

Product Use

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

Introduction

Description of the System

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 cloning vector, pZErO®-1, contains the *ccd*B gene fused to the C-terminus of LacZα. Insertion of a DNA fragment disrupts the expression of the *lacZα-ccd*B gene fusion, permitting growth of only positive recombinants. Cells that contain non-recombinant vector are killed. Using the pZErO®-1 vector, very high cloning efficiencies (~95%) are often achieved without the need for exotic strains, X-Gal, calf intestinal phosphatase (CIP), or other components. The vector also contains:

- the Zeocin™ resistance gene for selection in *E. coli*
- the f1 origin of replication for single strand rescue
- a versatile multiple cloning site with 17 sites
- flanking Sp6 and T7 RNA promoter/priming sites for *in vitro* transcription
- all M13 universal primer sites for sequencing

Recommended *E. coli* host

TOP10 *E. coli* is the recommended strain for general use of pZErO®-1, but other host strains may also be used. Because the TOP10 *E. coli* strain does not carry the *lac*I^q gene, the *ccd*B gene is constitutively expressed without the need for IPTG induction. If you use a strain that contains the *lac*I^q gene, you must induce the expression of the *ccd*B gene with 1 mM IPTG.

Strains that contain an F plasmid are not recommended for transformation and selection of recombinant clones. The F plasmid encodes the CcdA protein that 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 *ble* (bleomycin) resistance genes. These strains will confer resistance to Zeocin™. For the most efficient selection, choose an *E. coli* strain that does not contain the Tn*5* gene (e.g. TOP10, DH5, DH10).

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

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Description of the System, Continued

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 and cell death.

*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 the CcdA protein does not inhibit its action. 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).

Mechanism of action of Zeocin™

Zeocin™ is a member of the bleomycin family of cytotoxins that kills a wide range of prokaryotic and eukaryotic cells by binding chromosomal DNA and causing random double-stranded breaks. A Zeocin™ resistance gene, *ble*, has been isolated from *Streptoalloteichus hindustanus*. The *ble* gene product inactivates Zeocin™ by binding directly to it in a stoichiometric fashion and preventing chromosomal DNA from being bound and cleaved. The Zeocin™ resistance gene is included in this cloning vector as an alternative to β-lactamase. Since the Zeocin™ resistance protein is overproduced in the cytoplasm and not secreted like β-lactamase, there is no danger of Zeo^S satellite colonies arising. For more information on Zeocin™, see page 20.

Methods

Clone into pZErO®-1

Introduction

Selecting cloned inserts using the Zero Background™ technique is extremely powerful; however, because of the nature of selection, we do **not** recommend propagating the vector in common laboratory strains. We have developed ligation and transformation procedures to optimize the use of the pZErO®-1 vector provided in this kit. The kit contains enough vector for ~25 restriction digestions and ~2,000 ligations.

Important

Zeocin™ is inactivated by >110 mM salt concentrations. The salt concentration must either be reduced or the concentration of Zeocin™ increased to compensate. It is also important to maintain the pH between 7.0–7.5 to prevent inactivation of the drug.

Failure to lower the salt content and maintain the pH of your medium between 7.0–7.5 will result in non-selection due to inactivation of Zeocin™.

To prevent inactivation of Zeocin™, use Low Salt LB-Zeocin™ medium when plating transformation reactions. We recommend growing positive recombinants in Low Salt SOB-Zeocin™ liquid medium for better growth and higher plasmid yields. Using other liquid media such as Low Salt LB, YT, or TB may produce unsatisfactory results. The table below summarizes the media you can use.

Technique	**Media** (see **Recipes**, page 17)
Plating transformation reaction	Low Salt LB-Zeocin™ (50 µg/mL Zeocin™)
	YT-Zeocin™ (50 µg/mL Zeocin™)
Growing positive transformants in liquid culture	Low Salt SOB-Zeocin™ (50 µg/mL Zeocin™)

Continued on next page

Clone into pZErO®-1, Continued

Before starting

1. Prepare Low Salt LB-Zeocin™ plates (50 µg/mL) (see page 17). Store the plates at 4°C.

   If using cells carrying the *lac*I^q gene, include 1 mM IPTG in the plating medium to achieve complete induction.
2. Prepare Low Salt SOB-Zeocin™ (50 µg/mL) liquid medium for DNA minipreps.
3. Prepare or purchase chemically competent or electrocompetent TOP10 cells. See pages 22–25 for protocols to prepare competent cells. The minimal efficiencies required are 1×10^8 cfu/µg DNA for chemically competent and 1×10^9 cfu/µg DNA for electrocompetent cells.

   Note: For 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 28 for ordering information).
4. Determine a strategy for cloning into pZErO®-1. See the details of the multiple cloning site (page 5) for help.

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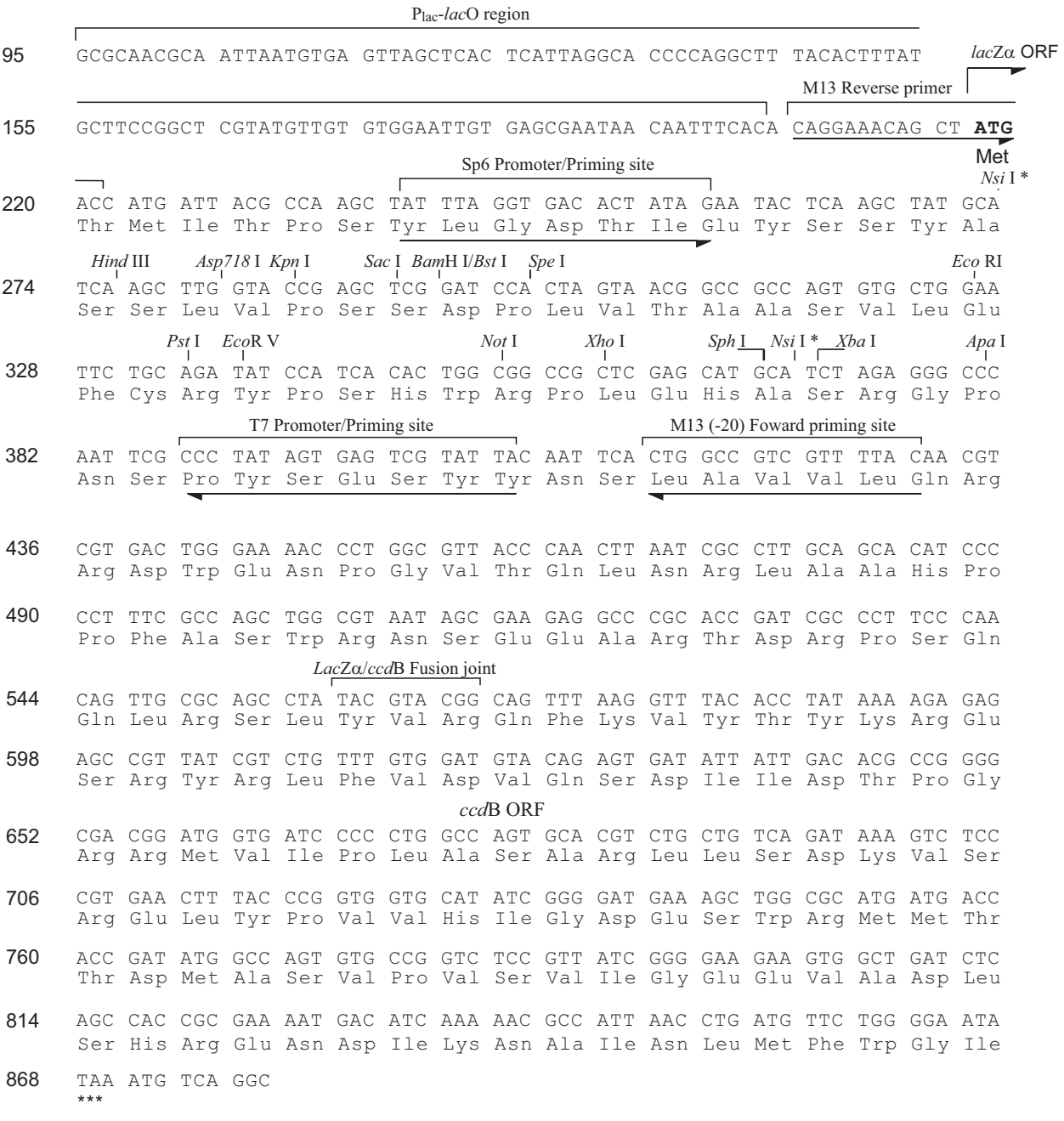
Detail of the multiple cloning site

The following diagram shows the pZero®-1 multiple cloning site and surrounding sequences. Restriction sites indicate where the enzyme cuts. Cloning into the multiple cloning site disrupts expression of the *ccd*B gene. The pZErO®-1 vector is a cloning vector; it is not designed to be an expression or fusion vector.



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Clone into pZErO®-1, Continued

Isolate and Purify inserts

For best results, phenol-extract PCR products and precipitate the DNA before ligating PCR products into pZErO®-1.

If you gel-purify your insert before ligating into pZErO®-1, you may see a higher background of colonies without insert because of nuclease contamination. To reduce nuclease contamination, do **not** use communal ethidium bromide baths. Use solutions that are free of nucleases, and high quality agarose.

Important considerations

- **Do not dephosphorylate pZErO®-1.**
- **Do not overdigest pZErO®-1 or your insert with restriction enzymes, and take precautions to prevent nuclease contamination.**
- Autoclave or extensively boil for 5–10 minutes in a microwave oven all water and buffers used in the experiments to ensure that they are nuclease-free. We recommend that you use the sterile, nuclease-free water and buffers provided with the kit.
- 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 background of non-recombinants.

Ligate into pZErO®-1

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

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

   To ligate blunt fragments, digest pZErO®-1 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 3:1–0:1 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 incubate the reaction mix at 70°C for 10 minutes. Cool 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 4 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 and precipitate the DNA with 1/10 volume 3 M sodium acetate, pH 5.6, and 2 volumes 100% ethanol. Centrifuge the tube containing precipitated DNA, 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.

   Procedure continued on next page

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Ligate into pZErO®-1, Continued

3. To verify complete digestion and estimate recovery, run an aliquot of the digestion mixture 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 3:1–10:1. See the following section, **Calculate molar ratios**, 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 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 the vials on ice. Proceed to **Transformation**, page 11.

Calculate molar ratios

To clone your insert into pZErO™-1, you need to know the concentration of your insert DNA solution. You may determine the concentration by OD_{260}, agarose gel electrophoresis, or fluorescence. 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®-1 (see the following **Note)**. Note that the amount of pZErO®-1 is 10 ng.

$$\text{x ng insert} = \frac{(2)\ (\text{bp insert})\ (10\text{ ng linearized pZErO®-1})}{(2{,}808\text{ bp pZErO®-1})}$$

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

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

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General guidelines for control reactions

The following table gives some suggestions for possible control reactions for the experiments presented in this manual. It is useful to have control data to evaluate your experiments or if you need to contact Technical Support (page 29) for assistance.

Experiment	Control	Reason
Ligation and Transformation	No DNA	Checks for contamination of ligation reagents.
	Linearized vector only	Checks for uncut or self-ligated vector. If the vector religates, it will probably be lethal and very few transformants should arise.
	Cells only	Checks for the presence of antibiotic in the plates and for contamination of competent cells and S.O.C. medium.
	Supercoiled vector (e.g. pUC19)	Checks the efficiency of the competent cells. See **Transformation**, page 11.
	Test Inserts	Checks general ligation conditions, and confirms disruption of *ccd*B expression. See **Use the test inserts**, page 9.

Continued on next page

Using 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®-1 vector with *Eco*R V, and ligate the test inserts (*Hae* III digested ΦX174 DNA) into the vector. Transform the ligation mixture into competent TOP10 cells, and plate the transformation mix onto Low Salt LB-Zeocin™ plates.

1. Digest 1 µg of pZErO®-1 with *Eco*R V (10 units) for 15–30 minutes.
2. ***Eco*R V is not heat inactivated**. Extract the digested vector with phenol/chloroform, and precipitate the DNA in the aqueous layer with 1/10 volume of 3 M sodium acetate pH 5.6 and 2 volumes of ethanol. Carefully wash the pellet with 80% ethanol and let the pellet 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. Longer incubation times may increase the yield of ligated products. Proceed to **Transformation**, page 10. Use 2 µL of the ligation reaction to transform TOP10 cells.

To determine the cloning efficiency, pick 10–20 colonies and isolate plasmid DNA. To release the test inserts, digest with *Nsi* I or a combination of other enzymes. Digesting 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. ΦX174 DNA has no sites for *Apa* I, *Bam*H I, *Eco*R I, *Hin*d III, *Kpn* I, *Not* I, *Spe* I, *Sph* I, or *Xba* I. The cloning efficiency should be ~95%.

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Analyze clones

To determine the cloning efficiency, pick 10–20 colonies and isolate plasmid DNA. To release the test inserts, digest with *Nsi* I or a combination of other enzymes. Digestion of a recombinant plasmid containing the test insert 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. ΦX174 DNA has no sites for *Apa* I, *Bam*H I, *Eco*R I, *Hin*d III, *Kpn* I, *Not* I, *Spe* I, *Sph* I, or *Xba* I. The cloning efficiency should be ~95%.

Determine cloning efficiency

To determine the cloning efficiency, compare the number of colonies produced in the test insert ligation (10–50 µL per plate) to the total number of colonies seen on the test insert ligation plate plus the number of colonies on the vector only plate. Be sure to correct for the volume plated.

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

Transformation

Introduction

After performing the cloning reaction, chemically transform or electroporate your pZErO®-1 construct into competent TOP10 *E. coli*, and plate the transformed cells onto Low Salt LB-Zeocin™ plates (see **Recipes**, page 17). After incubating the plates for 24 hours, analyze Zeocin™ 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 28 for ordering information). For instructions on preparing chemically competent or electrocompetent cells, see pages 22 and 24, respectively.

Controls

See the section on **Control Reactions** (page 8) to determine which controls you wish to include.

We recommend that you test the efficiency of the competent cells contained in the One Shot® Kit using the pUC19 control plasmid:

1. Prepare LB agar plates containing 50 µg/µL ampicillin.
2. Dilute the 10 ng/µL pUC19 stock solution (provided with the One Shot® Kit) 1:10 in sterile water to prepare a 1 ng/µL solution.
3. Prepare a 10 pg/µL pUC10 working solution by transferring 1 µL of the 1 ng/µL stock into 99 µL of sterile water.
4. Transform 1 µL (10 pg) of the working vector solution into 50 µL of competent cells according to the transformation protocol next page.
5. Calculate the transformation efficiency as transformants per 1 µg of plasmid. The cells should have an efficiency of 10^9 transformants/µg of the control plasmid.

Handling competent cells

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical stress caused by pipetting. Start the transformation procedure immediately following the thawing of the cells on ice, and perform all mixing by stirring with a pipette tip, **not** by pipetting up and down.

Important

If using cells that carry a *lac*I^q gene (i.e. TOP10F´, DH5αF´), 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

Before starting

- Equilibrate a water bath to 42°C.
- Warm 1 vial of S.O.C. medium to room temperature.
- Place an appropriate number of 10-cm diameter Low Salt LB-Zeocin™ agar plates in a 37°C incubator to remove excess moisture (use 1 plate for each transformation).
- Obtain a test tube rack (float) that will hold all transformation tubes to allow simultaneous heat shock in the water bath at 42°C.

One Shot® TOP10 chemical transformation protocol

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 vial(s) from the 42°C bath and quickly place them on ice for 1 minute.
7. Add 250 µL of pre-warmed S.O.C. medium to each vial. (S.O.C. 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 Low Salt LB-Zeocin™ agar plates. We recommend that you plate 2 different volumes to ensure well-spaced colonies. For plating smaller volumes, add 20 µL S.O.C. 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 15.

Continued on next page

One Shot® electroporation protocol

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.

   Note: If you have problems with arcing, see page 14.
3. Immediately add 250 µL of room temperature S.O.C. Medium.
4. Transfer the solution to a 15-mL snap-cap tube (e.g. Falcon) and shake 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 S.O.C. 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 15.

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 high degree of transformation efficiency regularly attained when using One Shot® chemically competent cells.

Note: You need to prepare additional S.O.C. for this procedure (see **Recipes**, page 17).

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 pipette up and down**. Repeat for all ligations.

   Note: If the cell competency is $<1 \times 10^8$ 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 S.O.C. 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 Low Salt LB-Zeocin™ 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 15.

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

Continued on next page

Electroporation transformation

You need to prepare additional S.O.C. for this procedure (see **Recipes**, page 17).

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 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 S.O.C. 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. Repeat Steps 7–8 until all samples have been transferred to 15-mL tubes.
9. 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.
10. Plate 25 µL and 100 µL of the transformation mix on Low Salt LB-Zeocin™ agar plates. After the liquid is absorbed into the agar, invert the plates, and incubate them at 37°C for 18–24 hours.

Continued on next page

Analyze Transformants

1. Remove plates from the incubator. Pick 10 Zeocin™ resistant transformants and inoculate into 2 mL SOB-Zeocin™ medium with 50 µg/mL Zeocin™. Grow 6–8 hours or overnight at 37°C.
   Using SOB-Zeocin™ medium is crucial for optimal growth and plasmid yield.
2. Purify plasmid DNA by miniprep for restriction analysis. 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 an overnight 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 analyzing your insert.

Note

If you need ultra-pure plasmid DNA for automated or manual sequencing, we recommend using the PureLink® HQ Mini Plasmid Purification Kit or the PureLink® HiPure Plasmid Miniprep Kit (see page 28 for ordering information). Refer to **www.lifetechnologies.com/support** or contact Technical Support (page 29) for more information on a large selection of plasmid purification columns.

Troubleshooting

Troubleshooting

The table below provides solutions to possible problems you might encounter.

Observation	Reason	Solution
Very few or no transformants arise	Loss of DNA during precipitation	Be careful not to lose the DNA pellet when precipitating and washing.
	Insert not ligating properly	Check your subcloning strategy.
	Incorrect insert to vector molar ratio	Determine the concentration of insert and calculate the correct molar ratio.
	Low transformation efficiency of *E. coli* strain	Check transformation efficiency with the pUC19 control vector (page 10). Chemically competent cells should yield $> 1 \times 10^8$ transformants/µg DNA. Electrocompetent cells should yield $>1 \times 10^9$ transformants/µg DNA.
High background of transformants which do not contain inserts	Overdigestion of vector with restriction enzymes	Limit digests to 15–30 minutes.
	Nuclease contamination in reagents	Use the reagents supplied with the kit or autoclave all reagents used for cloning (especially water).
	If using a cell line carrying the *lac*I^q gene, there may be an insufficient amount of IPTG in plate medium	IPTG must be in excess to achieve proper induction and killing of background colonies without inserts.
Thin "lawn" of cells on plate	Insufficient amount of Zeocin™ in plate medium	Be sure to add the correct amount of Zeocin™ to the plate medium and let medium cool sufficiently before adding.
	High salt in LB medium	Make sure you make LB medium with 5 g/liter NaCl.
DNA migrates anomalously on agarose gels (bands run at a larger molecular weight than expected and seem slightly smeared)	Protein bound to DNA	Extract the DNA with phenol/chloroform during preparation of plasmid or after restriction digest.

Appendix

Recipes

Low Salt LB Agar Plates with Zeocin™

Low Salt LB Medium (per liter)

1% Tryptone
0.5% Yeast Extract
0.5% NaCl
1.5% Agar
pH 7.5

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and **5 g NaCl** in 950 mL of 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. Thaw the 100 mg/mL Zeocin™ stock solution and add to a final concentration of 50 µg/mL (500 µL/liter of medium).

   Sufficient Zeocin™ is provided to make 2 liters of 50 µg/mL Zeocin™ medium.

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

YT-Zeocin™ Plates

YT medium (per liter)

0.8% Tryptone
0.5% Yeast extract
0.25% NaCl
1.5% agar

1. For 1 liter, dissolve 8 g tryptone, 5 g yeast extract, and 2.5 g NaCl in 900 mL of deionized water.
2. Adjust the pH to 7.5 with 5 M NaOH, add 15 g agar, and bring up the volume to 1 liter.
3. Autoclave for 20 minutes on liquid cycle.
4. Cool to the medium to ~55°C. Thaw the 100 mg/mL Zeocin™ stock solution.
5. Add Zeocin™ to 50 µg/mL final concentration (500 µL).

Continued on next page

SOB Medium with Zeocin™

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 of 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.
3. Adjust the pH to 7.5 with 5 M NaOH, and bring up the volume to 1 liter with deionized water.
4. Autoclave this solution, cool to ~55°C, and add 10 mL of sterile 1 M $MgCl_2$.
5. If preparing medium with Zeocin™, add Zeocin™ to a final concentration of 50 µg/mL.
6. Store the medium at room temperature or 4°C.

S.O.C. Medium

S.O.C. (per liter)

SOB
20 mM glucose

1. After making SOB medium (see above), add 7.2 mL of 50% glucose after step 4.
2. Store the medium at room temperature or 4°C. **Do not add Zeocin™.**

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 (Aldrich #20309-2; 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 the solution. Do **not** readjust pH with base.
4. Adjust the final volume to 100 mL with deionized water, and filter sterilize the solution. 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 you can.
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.**

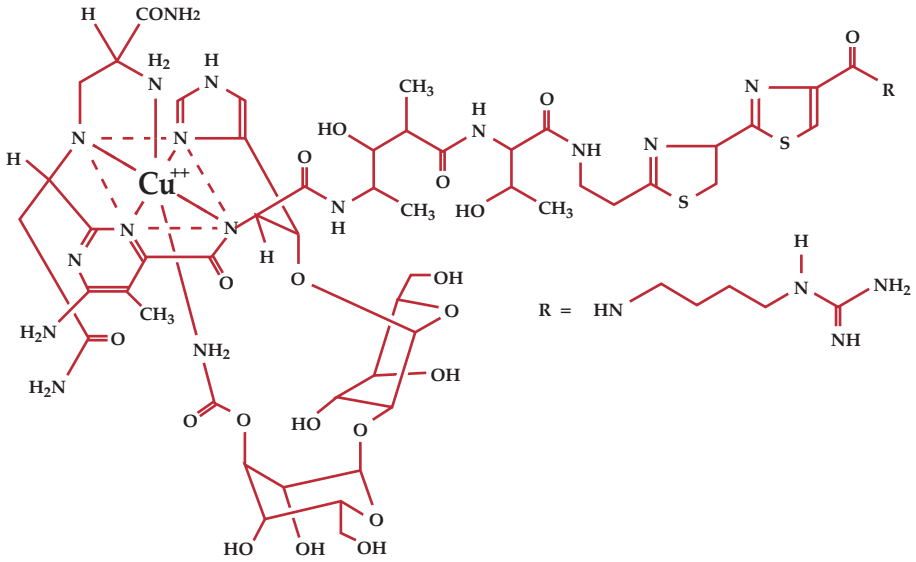
Zeocin™ Selective Antibiotic

Description

Zeocin™ selective antibiotic belongs to a family of structurally related bleomycin/phleomycin-type antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong antibacterial and antitumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells. Zeocin™ selective antibiotic is not as toxic as bleomycin on fungi. As a broad-spectrum antibiotic Zeocin™ selective antibiotic is particularly useful, allowing selection in a number of cell types containing vectors with a Zeocin™ resistance gene.

Chemical properties

Zeocin™ selective antibiotic is a basic, water-soluble compound isolated from *Streptomyces verticillus* as a copper-chelated glycopeptide. The presence of copper gives the solution its blue color. The chemical formula for Zeocin™ selective antibiotic is $C_{55}H_{86}O_{21}N_{20}S_2Cu$-HCl and the molecular weight is 1527.5. It contains several unique amino acids, sugars, and aliphatic amines. For general information about the family of bleomycin antibiotics, see (Berdy, 1980) (**Reference** section). The general structure of Zeocin™ selective antibiotic is shown below.



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Mechanism of action

The exact mechanism of action of Zeocin™ selective antibiotic is not known; however, it is thought to be the same as bleomycin and phleomycin due to its similarity to these drugs and its inhibition by the *Sh ble* resistance protein (see the following section). The copper/glycopeptide complex is selective and involves chelation of copper (Cu^{2+}) by the amino group of the α-carboxamide, single nitrogen atoms of both the pyrimidine chromophore and the imidazole moiety, and the carbamoyl group of mannose. The copper-chelated form is inactive. When the antibiotic enters the cell, the copper cation is reduced from Cu^{2+} to Cu^{+} and removed by sulfhydryl compounds in the cell. Upon removal of the copper, Zeocin™ selective antibiotic is activated to bind DNA and cleave it causing cell death (Berdy, 1980). **Salt concentrations greater than 110 mM and acidity or basicity inactivate Zeocin™ selective antibiotic; therefore, it is necessary to reduce the salt in bacterial medium and adjust the pH to 7.5 to make sure the drug remains active.**

Resistance to Zeocin™ Selective Antibiotic

A Zeocin™ resistance protein has been isolated and characterized (Gatignol, *et al.*, 1988; Drocourt, *et al.*, 1990). This protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), is a 13,665 D protein that binds Zeocin™ selective antibiotic in a stoichiometric manner. The binding of Zeocin™ selective antibiotic inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to the Zeocin™ selective antibiotic.

Note

The *Sh ble* gene can be excised from pZErO®-1 without the promoter using *PmL* I or with the *bla* (β-lactamase) promoter using *Ssp* I and *Dra* I.

Protocol for Chemically Competent Cells

Introduction

The 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 yields enough cells for approximately 60 transformations. The expected efficiency of chemically competent TOP10 cells is 1×10^8 cfu/µg of 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 contaminating the competent cells. Remember to use **sterile** solutions, medium, and supplies.

Solutions required

For each batch of chemically competent cells, prepare the following solutions (see **Recipes**, page 17):

5 mL SOB medium in a sterile culture tube

250 mL SOB medium 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

The following 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 yields enough electrocompetent cells for approximately 30 transformations.

Note

The expected efficiency of the electrocompetent TOP10 cells is 1×10^9 cfu/µg of 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 contaminating 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
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).

Procedure continued on next page

Continued on next page

Protocol for Electrocompetent Cells, Continued

Protocol for electrocompetent cells, Continued

Day 3, Continued:

6. Transfer the 1 liter culture to the 2 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.

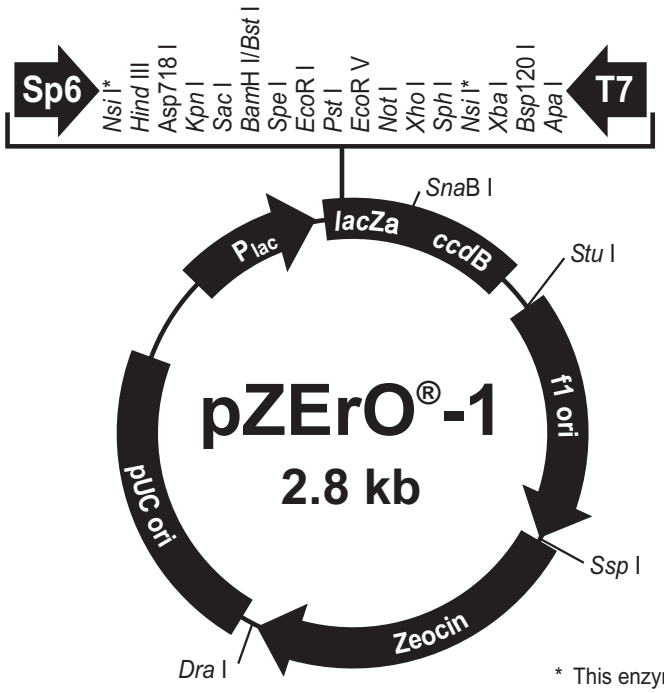
pZErO®-1 Vector

Description of pZErO®-1

pZErO®-1 is a 2808 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. The Zeocin™ resistance gene reduces vector size and prevents formation of satellite colonies, which is common when selecting vectors on ampicillin. The Zeocin™ antibiotic also selects against contaminating plasmids that may be present with the insert DNA during ligation. Gel purification of inserts is not necessary.

Map of pZErO®-1

The figure below summarizes the features of the pZErO®-1 vector.



* This enzyme has two recognition sites both of which are found in the multiple cloning site.

Comments for pZErO®-1
2808 nucleotides

Lac Promoter/Operator Region: bases 95-216
M13 Reverse Priming Site: bases 205 - 221
LacZa 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
Fusion Joint: bases 559-567
*ccd*B Lethal Gene ORF: bases 568-870
f1 Ori: bases 895-1310
Zeocin™ Resistance ORF: bases 1527-1901
pUC Ori: bases 2058-2640

Continued on next page

Features of pZErO®-1

The following table describes the important elements of pZErO®-1. All features have been functionally tested. The sequence of pZErO®-1 has been compiled from information in sequence databases, published sequences, and other sources.

Features	Function
*lac*Zα–*ccd*B fusion gene	• Provides lethal selection against non-recombinant vector. • Utilizes the LacZα disruption technology to clone inserts. • Includes the M13 forward and reverse priming sites for sequencing
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, and disrupts the expression of the *ccd*B gene
T7 promoter/priming site	Allows *in vitro* transcription of the anti-sense strand and sequencing of your insert
M13 Forward (–20) priming site	Permits sequencing of your insert
Sh ble	Provides resistance to the Zeocin™ antibiotic. Expression of *Sh ble* is driven using the *bla* (β-lactamase) promoter.
f1 origin	Allows isolation of single-stranded DNA from *E. coli* strains containing the F´.
pUC-derived origin of replication	Allows replication and maintenance of the plasmid in *E. coli.*

Accessory Products

Additional Products

Many of the reagents supplied with the Zero Background™ Cloning Kit and other reagents suitable for use with the kit are available separately. Ordering information for these reagents is provided in the following table. For details, visit **www.lifetechnologies.com/support** or contact **Technical Support,** page 29.

Product	Amount	Cat. 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
PureLink® HQ Mini Plasmid Purification Kit	100 preps	K2100-01
PureLink® HiPure Plasmid Miniprep Kit	25 preps	K2100-02
	100 preps	K2100-03
Zeocin™ Selection Reagent	1 g	R250-01
	5 g	R250-05

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:

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- 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 (SDS)

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

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Limited Use Label License No. 54: *ccd*B-Fusion Vectors

ccdB selection technology is described in Bernard et al., "Positive Selection Vectors Using the F Plasmid ccdB Killer Gene" Gene 148 (1994) 71-74. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). For licensing information for use in other than research, please contact: Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008; Phone (760) 603-7200 or e-mail **outlicensing@lifetech.com**.

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Notes