One Shot® PIR1 and PIR2 Competent *E. coli*

Catalog no. C1010-10, C1111-10

Version E
13 April 2004
25-0334

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

Overview

Introduction

The PIR1 and PIR2 One Shot® Chemically Competent *E. coli* are for use with vectors that contain the R6Kγ origin of replication (i.e. pUni/V5-His-TOPO®). The *pir* gene encodes the replication protein π, which is required to replicate and maintain plasmids containing the R6Kγ origin. The information in this manual covers the following kits:

Product	Reactions	Catalog no.
PIR1 One Shot® Chemically Competent *E. coli*	10	C1010-10
PIR2 One Shot® Chemically Competent *E. coli*	10	C1111-10

Contents

The PIR1 and PIR2 One Shot® Chemically Competent *E. coli* Kits contain the following:

- 11 tubes each containing 50 µl of chemically competent *E. coli*
- 5 ng supercoiled pUC19 plasmid (10 pg/µl in 5 mM Tris-HCl, .5 mM EDTA, pH 8.0) for testing transformation efficiency
- SOC medium (6 ml) for plating

Genotypes

PIR1: You may use this strain for cloning and maintenance of your donor vector (i.e. pUniV5/His-TOPO®) construct (or other vector containing the R6Kγ origin). This strain contains a mutant allele of the *pir* gene that maintains the donor vector construct at ~250 copies per cell.

F- *Δlac169 rpoS(Am) robA1 creC510 hsdR514 endA recA1 uidA(ΔMluI)::pir-116*

PIR2: This strain is recommended for maintaining constructs that express toxic genes or for libraries. Use this strain for cloning and maintenance of your donor vector construct (or other vector containing the R6Kγ origin). This strain contains the wild-type *pir* gene for maintenance of the vector at ~15 copies per cell.

F- *Δlac169 rpoS(Am) robA1 creC510 hsdR514 endA recA1 uidA(ΔMluI)::pir*

General Handling

Be extremely gentle when working with competent *E. coli*. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transformation should be started immediately following the thawing of the cells on ice, and the reaction mixed by swirling or tapping the tube gently, not by pipetting.

Product Qualification

To qualify PIR1 and PIR2 cells:

1. 50 µl of competent cells are transformed with 10 pg of supercoiled pUC19 plasmid. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be:
   - PIR1: > 1 x 10^9 cfu/µg DNA
   - PIR2: > 1 x 10^8 cfu/µg DNA
2. Transformation efficiency is also confirmed with supercoiled pUni/V5-His (10 pg). Transformed cultures are plated on LB plates containing 50 µg/ml kanamycin and the transformation efficiency calculated.
3. Untransformed cells are plated on:LB plates containing 100 µg/ml ampicillin, 25 µg/ml streptomycin, 50 µg/ml kanamycin, 15 µg/ml tetracycline, or 15 µg/ml chloramphenicol to verify the absence of antibiotic- resistant contamination.
4. To verify the absence of phage contamination, 0.5-1 ml of competent cells are added to LB top agar and poured onto LB plates. After overnight incubation, no plaques should be detected.

Transformation of Chemically Competent Cells

Materials Supplied by the User

You will need the following items for transformation:

- 37°C shaking and non-shaking incubator
- 10 cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice
- 42°C water bath

Before Starting

- Equilibrate a water bath to 42°C.
- Warm the vial of SOC medium to room temperature.
- Warm plates in a 37°C incubator for 30 minutes (we recommend using two plates for each transformation).
- Obtain a test tube rack that will hold all transformation tubes so that they can all be put into a 42°C water bath at once.

Transformation Procedure

The instructions provided below are for general use. Specific instructions for particular applications such as Echo™ Cloning are provided in the donor vector manual.

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 1 to 5 µl of each ligation reaction directly into the competent cells and mix by tapping gently. **Do not mix by pipetting up and down.** The remaining ligation mixture(s) can be stored at -20°C.
4. Incubate the vial(s) on ice for 30 minutes.
5. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
6. Remove vial(s) from the 42°C bath and place them on ice.
7. Add **250 µl** of pre-warmed SOC medium to each vial. (SOC is a rich medium; sterile technique must be practiced to avoid contamination.)
8. Place the vial(s) in a microcentrifuge rack on its side and secure 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. Spread 20 µl to 200 µl from each transformation vial on separate, labeled LB agar plates containing the appropriate antibiotic. **Note**: We recommend that you plate two volumes to ensure that one plate will have well-spaced colonies. For plating small volumes, add 20 µl SOC to allow even spreading.
10. Invert the plate(s) and incubate at 37°C overnight.
11. Select colonies and isolate plasmid DNA. Analyze by restriction digest, PCR, or sequencing.

Transformation Control

We recommend that you test the transformation efficiency of the competent cells contained in the One Shot® Kit. This can be accomplished by using the supercoiled pUC19 plasmid supplied with the kit as described below.

- Prepare LB agar plates containing 100 µg/ml ampicillin
- Transform 1 µl (10 pg) into 50 µl of competent cells according to the transformation protocol on the previous page
- Plate the control transformation as follows:

Strain	pUC19 (pg)	Volume to Plate
PIR1	10	10 µl + 20 µl SOC
PIR2	10	50 µl

- Incubate overnight at 37°C and count colonies. Calculate transformation efficiency using the formula below.

Calculation

Calculate the transformation efficiency as transformants per 1 µg of plasmid DNA.

$$\frac{\text{\# of colonies}}{\text{10 pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µl total transformation volume}}{\text{X µl plated}} = \frac{\text{\# transformants}}{\text{µg plasmid DNA}}$$

Expected transformation efficiency:

Strain	Transformation Efficiency
PIR1	$\geq 1 \times 10^9$ cfu/µg supercoiled plasmid
PIR2	$\geq 1 \times 10^8$ cfu/µg supercoiled plasmid

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Continued on next page

Technical Service, continued

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

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