One Shot® TOP10F′ Competent Cells

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Overview

Contents

- 21 tubes of competent TOP10F´ *E. coli* in 50 µl aliquots (1 x 10^9 colonies/µg DNA)
- Supercoiled pUC19 plasmid (10 pg/µl in 5 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 50 µl) for testing efficiency
- SOC medium (6 ml) for plating

Genotype

F´{*lac*I^q Tn*10* (Tet^R)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

Product Qualification

Fifty microliters 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 triplicate. Transformation efficiency must be > 1.0 x 10^9 cfu/µg DNA

Untransformed cells are plated on:

- LB plates containing 100 µg/ml ampicillin, 25 µg/ml streptomycin, 50 µg/ml kanamycin, or 15 µg/ml chloramphenicol to verify the absence of antibiotic-resistant contamination
- LB plates as a lawn to verify the absence of phage contamination
- LB plates containing Tet/X-gal to verify presence of F´ episome and lacI^q

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. Transformation should be started immediately following the thawing of the cells on ice. Swirl or tap the tube gently to mix the cells and DNA. Do not pipet up and down.

Important

One Shot® TOP10F´ cells are *lac*I^q and require IPTG to induce expression from the *lac* promoter. Spread 40 µl of 100 mM IPTG onto the selection plate and let it diffuse into the agar for approximately 1 hour.

If blue/white screening is required to select for transformants, spread 40 µl of 40 mg/ml X-Gal in dimethylformamide and 40 µl of 100 mM IPTG on top of the agar. Let the X-Gal and IPTG diffuse into the agar for approximately 1 hour.

Transformation

Choosing a Transformation Procedure

Two protocols are provided below to transform One Shot® chemically competent TOP10F′ *E. coli*. Consider the following factors when choosing the protocol that best suits your needs.

If you wish to…	Then use the…
Maximize the number of transformants obtained	Regular transformation procedure (see page 5)
Use a selective agent other than ampicillin (*e.g.* kanamycin; see important note below)	
Transform cells in five minutes	Rapid transformation procedure (see page 6)

Important

The rapid transformation procedure is **only** recommended for transformations using ampicillin selection. Note that the transformation efficiency is reduced when using this protocol.

Materials Supplied by the User

- 42°C water bath (regular transformation procedure only)
- 37°C incubator
- 10 cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice

Before Starting

- Equilibrate a water bath to 42°C (regular transformation procedure only)
- Warm the vial of SOC medium to room temperature (regular transformation procedure only)
- Place an appropriate number of 10 cm diameter LB agar plates with antibiotic in a 37°C incubator to remove excess moisture (use two plates per transformation for the regular transformation procedure and one plate per transformation for the rapid transformation procedure). **Be sure to add IPTG or IPTG and X-Gal to the plates if necessary.**
- Obtain a test tube rack that will hold all transformation tubes so that they all can be put into a 42°C water bath at once (regular transformation procedure only).

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Transformation, continued

We recommend that you test the 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 50 µg/ml ampicillin
- Transform 10 pg (1 µl) into 50 µl of competent cells according to the transformation protocol below. Discard the 10 pg/µl and the 100 µg/µl solutions after use.

Calculate the transformation efficiency as transformants per 1 µg of plasmid (see the next page). The cells should have an efficiency of 10^9 transformants/µg of supercoiled plasmid.

Transformation Procedure

The instructions below are for general use. Specific instructions for particular applications such as TOPO Cloning® and Zero Background™ Cloning are provided in the respective manual. If you are using One Shot® kits in conjunction with one of these products, refer to the manual for specific instructions.

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. To transform, use one of the following:
   - 1 to 5 µl of a ligation reaction
   - 10 pg supercoiled plasmid (*i.e.* pUC19 as the transformation control)
4. Add DNA directly to the competent cells and mix by tapping gently. **Do not mix cells by pipetting.** Store the remaining ligation reactions at -20°C.
5. Incubate the cells on ice for 30 minutes.
6. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
7. Remove vial(s) from the 42°C bath and quickly place them on ice.
8. Add **250 µl** of pre-warmed SOC medium to each vial. SOC is a rich medium; practice good sterile technique to avoid contamination.
9. 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 rotary shaker-incubator.
10. Spread 10 µl to 50 µl from each transformation vial on separate, labeled LB agar plates. Be sure to plate two different volumes to ensure that at least one plate has well-spaced colonies. For plating small volumes, add 20 µl of SOC to allow even spreading. **Note**: Plate 50 µl for the transformation control.
11. Invert the plate(s) and incubate at 37°C overnight.
12. Select colonies for further analysis by plasmid isolation, PCR, or sequencing.

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Transformation, continued

Rapid Transformation Procedure

An alternative protocol is provided below for rapid transformation of One Shot® TOP10F′ *E. coli*. This protocol is **only** recommended for transformations using ampicillin selection. **Note:** It is essential that LB plates containing ampicillin (plus IPTG or IPTG and X-gal, if necessary) are prewarmed prior to spreading.

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. Transform 1 to 5 µl of a ligation reaction by adding the DNA directly to the competent cells and mix by tapping gently. **Do not mix cells by pipetting.** Store the remaining ligation reactions at -20°C.
4. Incubate the vial(s) on ice for 5 minutes.
5. Spread 50 µl from each transformation vial on a prewarmed LB agar plate containing 100 µg/ml ampicillin (plus IPTG or IPTG and X-gal, if necessary).
6. Invert the plate(s) and incubate at 37°C overnight.
7. Select colonies for further analysis by plasmid isolation, PCR, or sequencing.

Calculating Transformation Efficiency

If you have transformed cells using the regular transformation procedure, you may use the formula below to calculate the transformation efficiency using pUC19 (10 pg).

$$\frac{\text{\# of colonies}}{\text{10 pg transformed}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{\text{300 µl transformed cells}}{\text{50 µl plated}} = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

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Technical Service, continued

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