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# MAX Efficiency® Stbl2™ Competent Cells

**Cat. no.** 10268-019 **Size** 1 mL **Store at –85°C to –68°C** (Do not store in liquid nitrogen)

**Doc. Part no.** 10268019.pps **Pub. no.** MAN0000803 **Rev.** 2.0

## Description

MAX Efficiency® Stbl2™ Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). These cells are suitable for the cloning of unstable inserts such as retroviral sequences or direct repeats (2). *For optimal performance, perform expression in S.O.C. Medium and incubation on antibiotic plates at 30°C.* MAX Efficiency® Stbl2™ Competent Cells are a derivative of JM109. The *mcr*A mutation and the *mcr*BC-*hsd*RMS-*mrr* deletion allow for the cloning of genomic sequences that are methylated (3). These cells are *not* capable of blue/white selection with plasmids containing α-complementation sequences.

## Genotype

F$^-$ *mcr*A Δ(*mcr*BC-hsdRMS-*mrr*) *rec*A1 *end*A1 *lon gyr*A96 *thi sup*E44 *rel*A1 λ$^-$ Δ(*lac-pro*AB)

| Component | Amount per vial |
|---|---|
| Stbl2™ Competent Cells | 200 µL |
| pUC 19 DNA (0.01 µg/mL) | 100 µL |

## Transformation procedure

A stock pUC19 solution (0.01 µg/mL) is provided as a control to determine the transformation efficiency. To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, protein, and detergents.

1. Thaw competent cells on wet ice. Place the required number of 17 × 100 mm Falcon® 2059 tubes or similarly shaped polypropylene tubes (see the following note) on ice.

   **Note:** Falcon® 2059 tubes or other similarly shaped 17 × 100 mm polypropylene tubes are required for optimal transformation efficiency. You may use microcentrifuge tubes (1.5-mL) but the transformation efficiency will be reduced 3- to 10-fold.

2. Gently mix the cells, then aliquot 100 µL of competent cells into chilled tubes.

3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the –85°C to –68°Cfreezer. *Do not use liquid nitrogen.*

   **Note:** For optimal results, thaw each vial of cells only once. Although the cells are refreezable, subsequent freeze-thaw cycles lower the transformation frequency by approximately 2-fold.

4. To determine transformation efficiency, add 5 µL (50 pg) control DNA to one tube containing 100 µL competent cells. Move the pipette through the cells while dispensing. Gently tap the tube to mix.

5. For DNA from ligation reactions, dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Add 1 µL of the dilution to the cells (1–10 ng DNA), moving the pipette through the cells while dispensing the dilution. Gently tap the tubes to mix.

6. Incubate the cells on ice for 30 minutes.

7. Heat-shock the cells 25 seconds in a 42°C water bath; do not shake the cells.

8. Place on the cells ice for 2 minutes.
9. Add 0.9 mL of room-temperature S.O.C. Medium (Cat. no. 15544-034).

   **Note:** You may use media other than S.O.C. Medium but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold
10. Shake the tubes containing ligation reaction at 225 rpm (30°C) for 90 minutes.

    Shake the tubes containing control pUC19 DNA at 225 rpm (37°C) for 60 minutes.

### First-strand cDNA synthesis

1. Dilute the reaction containing the control plasmid DNA 1:100 with S.O.C. Medium. Spread 100 µL of this dilution on LB or YT plates with 100 µg/mL ampicillin. Incubate plates overnight at 37°C.
2. Dilute experimental reactions as necessary and spread 100–200 µL of this dilution as described in step 1. Incubate plates overnight at *30°C.*

### Grow transformants for plasmid preparations

Grow Stbl2™ Competent Cells that have been transformed with plasmids at 30°C overnight in TB (4). A 100-mL growth in a 500-mL baffled shake flask yields approximately 200 µg of pUC19 DNA.

### Calculate transformation efficiency (CFU/µg)

$$\frac{\text{CFU in control plate}}{\text{Pg pUC19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

For example, if 50 pg pUC19 yields 100 colonies when 100 µL of a 1:100 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{pg}}{\mu\text{g}} \times \frac{1 \text{ mL}}{0.1 \text{ mL plated}} \times 10^2 = 2 \times 10^9$$

## References


1. Hanahan, D. (1983) *J. Mol. Biol. 166,* 557.
2. Trinh, T., Jessee, J., Bloom, F., and Hirsch, V. (1994) *Focus 16:3,* 78.
3. Blumenthal, R. (1989) *Focus 11:3,* 41.
4. Jessee, J. (1984) *Focus 6:4,* 5.


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