96Pack® Gold Competent Cells

Catalog #200324

MATERIALS PROVIDED

Materials provided (Catalog #200324)	Quantity	Efficiency (cfu/μg of pUC18 DNA)
96Pack® Gold competent cells	Four 96-well plates (15 µl per well)	1×10^8
pUC18 DNA control plasmid (0.1 ng/µl in TE buffer)	10 µl	—
Tape	12	—

Storage: Store the cells immediately at the bottom of a –80°C freezer. Do not place the cells in liquid nitrogen. Store the control plasmid at –80°C.

ADDITIONAL MATERIALS REQUIRED

96-well thermal block
Temperature cycler, water bath, or additional 96-well thermal block

BACKGROUND

Genotype of 96Pack® Gold competent cells (XL10-Gold® strain): Tet^r Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* Hte [F´ *proAB* $lacI^qZ\Delta M15$ Tn*10* (Tet^r) Amy Cam^r].‡ (Genes listed signify mutant alleles. Genes on the F´ episome, however, are wild-type unless indicated otherwise.)

96Pack® Gold competent cells are tetracycline and chloramphenicol resistant.‡ 96Pack® Gold competent cells* are formatted for high-throughput cloning. Each plate contains 96 individual transformations for quick cloning of many constructs at once. 96Pack Gold competent cells feature the XL10-Gold®* strain to give high transformation efficiency, especially for large and ligated DNA molecules. These cells also provide large colonies that grow quickly. 96Pack Gold competent cells are ideal for constructing plasmid DNA libraries because using these cells decreases size bias and produces larger, more complex plasmid libraries. The XL10-Gold strain is deficient in all known restriction systems[Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173*]. The strain is endonuclease deficient (*endA*), greatly improving the quality of miniprep DNA, and recombination deficient (*recA*), helping to ensure insert stability. The Hte phenotype increases the transformation efficiency of ligated and large supercoiled DNA. The $lacI^qZ\Delta M15$ gene on the F´ episome allows blue-white screening for recombinant plasmids.

TRANSFORMATION PROTOCOL

Preparation

- Prepare SOC medium§ immediately before beginning the protocol (see *Preparation of Media and Reagents*).
- Prepare for the heat pulse by doing one of the following: (1) program a temperature cycler with a 96-well block to hold the temperature at 42°C and preheat the temperature cycler; (2) preheat a 96-well heating block to 42°C; or (3) preheat a water bath to 42°C. (Be careful to avoid cell contamination while heat-pulsing the transformation reaction in a water bath.)
- Place a metal 96-well thermal block on ice to chill the block.

Protocol

1. Thaw the competent cells in a 96-well plate by placing the plate in a chilled metal 96-well block. The cells should thaw within 30 seconds.
2. Carefully remove the aluminum foil seal from the plate.
3. Using a multichannel pipettor, add 1 µl of DNA (1 pg–20 ng) to each well. For uniform results, keep the volume near 1 µl. For a control, dilute the 0.1 ng/µl pUC18 DNA control plasmid 1:100 in high-quality water. Add 1 µl of the 1 pg/µl pUC18 DNA to each control well.
4. Seal the plate with tape.
5. Incubate the plate of cells and DNA in the chilled block for 20 minutes.
6. Heat-shock the cells for 20 seconds at 42°C by transferring the plate to a prewarmed temperature cycler, thermal block, or water bath. The duration of the heat pulse is ***critical*** for obtaining the highest transformation efficiency.
7. Transfer the plate back to the chilled block and allow the plate to cool for 1 minute.
8. Add 85 µl of SOC medium to each well.
9. Incubate the plate at 37°C for 1 hour. Shaking is not necessary.
10. Before plating, gently mix the cell suspensions by pipetting as cells may have settled to the bottom of the wells. Plate 10–100 µl of the suspensions on LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if color screening is desired).§ For the pUC18 control transformation, plate 20 µl of the transformation on LB–ampicillin agar plates.§

    Note *If plating <100 µl of cells, pipet the cells into a 100 µl pool of SOC medium and then spread the mixture with a sterile spreader.*
11. Incubate the plates at 37°C overnight. See *Blue-White Color Screening*, reverse side, for color screening incubation guidelines.
12. For the pUC18 control, expect 50–300 colonies (≥1×10^8 cfu/µg pUC18 DNA). For the experimental DNA, the number of colonies will vary according to the size and form of the transforming DNA, with larger and non-supercoiled DNA producing fewer colonies.

*U.S. Patent Nos. 5,512,468 and 5,707,841 and patents pending and equivalent foreign patents.

‡The strain is resistant (Cam^r) to concentrations of <40 µg/ml chloramphenicol, but sensitive (Cam^s) to 100 µg/ml chloramphenicol.

§See *Preparation of Media and Reagents*.

Transformation Guidelines and Troubleshooting

Storage Conditions: Competent cells are very sensitive to even small variations in temperature and must be stored at the bottom of a –80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency.

Quantity and Volume of DNA: Greatest efficiencies are observed when adding 1 µl of a ligation mixture. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/µg) may be lower.

Heat Pulse Duration and Temperature: There are defined windows for the temperature and duration of the heat pulse that result in the highest transformation efficiency. Optimal transformation efficiency is observed when cells are heat-pulsed at 42°C for 20 seconds. Do not exceed 42°C.

Blue-White Color Screening: Blue-white color screening for recombinant plasmids is available when transforming host strains that contain the *lacI*^{q}*Z*Δ*M15* gene on the F´ episome with a plasmid that provides α-complementation (e.g. Stratagene's pBluescript® II). When lacZ expression is induced by IPTG in the presence of the chromogenic substrate X-gal, colonies containing plasmids with inserts will remain white, while colonies containing plasmids without inserts will be blue. When performing blue-white color screening, incubate the LB agar plates containing IPTG and X-gal at 37°C for at least 17 hours to allow color development. The blue color can be enhanced by subsequent incubation of the plates for two hours at 4°C. If an insert is suspected to be toxic, plate the cells on media without X-gal and IPTG. Color screening will be eliminated, but lower levels of the potentially toxic protein will be expressed in the absence of IPTG.

Preparation of Media and Reagents

SOB Medium (per Liter)	SOC Medium (per 100 ml)
20.0 g of tryptone 5.0 g of yeast extract 0.5 g of NaCl Add deionized H_2O to a final volume of 1 liter Autoclave Add 10 ml of filter-sterilized 1 M $MgCl_2$ and 10 ml of filter-sterilized 1 M $MgSO_4$ prior to use	***Note*** *This medium should be prepared immediately before use.* 2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose SOB medium (autoclaved) to a final volume of 100 ml
LB Agar (per Liter)	**LB–Ampicillin Agar (per Liter)**
10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Add deionized H_2O to a final volume of 1 liter Adjust pH to 7.0 with 5 N NaOH Autoclave	1 liter of LB agar, autoclaved Cool to 55°C Add 10 ml of 10 mg/ml filter-sterilized ampicillin Pour into petri dishes (~25 ml/100-mm plate)

Preparation of Agar Plates for Blue-White Color Screening

To prepare plates for blue-white screening, prepare LB agar as indicated above. When adding the antibiotic, also add 5-bromo-4-chloro-3-inodlyl-β-D-galactopyranoside (X-gal) to a final concentration of 80 µg/ml [prepared in dimethylformamide (DMF)] and isopropyl-1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 20 mM (prepared in sterile dH_2O). Alternatively, 100 µl of 10 mM IPTG and 100 µl of 2% X-gal may be spread on solidified LB agar plates 30 minutes prior to plating the transformations. (For consistent color development across the plate, pipet the X-gal and the IPTG into a 100-µl pool of SOC medium and then spread the mixture across the plate. Do not mix the IPTG and the X-gal before pipetting them into the pool of SOC medium because these chemicals may precipitate.)

Quality Control Testing

Transformations are performed both with and without pUC18 plasmid DNA, following the protocol outlined above. Following transformation, 20-µl samples of the culture are plated in duplicate on LB agar plates with 100 µg/ml of ampicillin. The plates are incubated at 37°C overnight and the efficiency is calculated based on the average number of colonies per plate.

Limited Product Warranty

Endnotes

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