SoloPack® Gold Supercompetent Cells

Catalog #230350

MATERIALS PROVIDED

Materials provided	Quantity	Efficiency (cfu/µg of pUC18 DNA)
SoloPack® Gold supercompetent cells	15 single-tube transformations (50 µl each)	≥1 × 10^9
XL10-Gold® β-Mercaptoethanol (β-ME) mix	50 µl	—
pUC18 control plasmid (0.1 ng/µl in TE buffer)	10 µl	—

Storage: Supercompetent cells must be placed immediately at the bottom of a –80°C freezer directly from the dry ice shipping container. Do not store the cells in liquid nitrogen.

QUALITY CONTROL TESTING

Transformations are performed both with and without plasmid DNA using 50-µl aliquots of cells and 1 µl of pUC18 control plasmid (10 pg/µl) (the pUC18 control plasmid is diluted 1:10 in sterile water) following the protocol outlined in the instruction manual. Following transformation, 5-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.

BACKGROUND

SoloPack® Gold supercompetent cells* are packaged in single-reaction aliquots, providing high performance and convenience. The single-tube transformation format eliminates the need to thaw, aliquot, and refreeze cells and allows for fewer pipetting steps because the entire transformation reaction occurs in the tube supplied.

Genotype of SoloPack Gold supercompetent cells: Tet^r Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* Hte [F´ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^r) Amy Cam^r].‡ (Genes listed signify mutant alleles. Genes on the F' episome, however, are wild-type unless indicated otherwise.)
SoloPack Gold supercompetent cells are tetracycline and chloramphenicol resistant.‡

SoloPack Gold cells are deficient in all known *E. coli* restriction systems (McrA$^-$, McrCB$^-$ McrF$^-$, Mrr$^-$, HsdR$^-$), preventing the cleavage of methylated DNA. The cells are also endonuclease deficient (*endA*), improving the quality of miniprep DNA, and are recombination deficient (*recA*), improving insert stability. The Hte phenotype increases the transformation efficiency of ligated and large supercoiled DNA and causes the production of large colonies. The *lacI*^{q}*ZΔM15* gene is present on the F´ episome, allowing blue-white screening for recombinant plasmids.

TRANSFORMATION PROTOCOL

1. Preheat NZY$^+$ broth§ or SOC broth§ to 42°C for use as the outgrowth medium in step 10.

Note *The optimized transformation protocol uses NZY$^+$ broth for maximum transformation efficiency. Use of SOC results in slightly lower efficiencies. See* Transformation Guidelines and Troubleshooting (*reverse page) for more information*.

2. Thaw two aliquots of SoloPack Gold cells on ice (one tube for each of the experimental and control transformations).
3. When the cells have thawed, swirl the tubes to gently mix the cells.
4. Add 1 µl of the β-ME mix provided with this kit to each aliquot of cells.

Note *β-ME addition may be omitted from the protocol with a slight reduction in transformation efficiency. If desired, omit the β-ME addition step and proceed to step 6. See* Transformation Guidelines and Troubleshooting (*reverse page) for more information*.

5. Swirl the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.
6. Add 0.01–50 ng of the experimental DNA to one tube of cells (see *Quantity and Volume of DNA*, on reverse page, for guidelines). Dilute the pUC18 control DNA 1:10 with sterile dH_2O, then add 1 µl of the diluted pUC18 DNA to the other tube of cells.
7. Swirl the tubes gently, then incubate the tubes on ice for 30 minutes.
8. Heat-pulse the tubes in a **54°C** water bath for 60 seconds. The temperature and duration of the heat pulse are critical for maximum efficiency. For consistent results, remove any ice trapped around the outside bottom of the tube.
9. Incubate the tubes on ice for 2 minutes.
10. Add 150 µl of preheated (42°C) NZY$^+$ broth (optimized protocol) or 250 µl of preheated SOC broth and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.
11. Plate ≤200 µl of the experimental transformation mixture on LB agar plates containing the appropriate antibiotic‡ (and containing IPTG and X-gal for color screening).§ For the pUC18 control, plate 5 µl of the transformation on LB–ampicillin agar plates.§

Note *If plating <100 µl of cells, pipet the cells into a 200 µl pool of SOC medium and then spread the mixture with a sterile spreader. If plating ≥100 µl, the cells can be spread on the plates directly. Tilt and tap the spreader to remove the last drop of cells.*

12. Incubate the plates at 37°C overnight. See *Blue-White Color Screening* (reverse page) for color screening incubation guidelines.
13. For the pUC18 control, expect 250 colonies (≥1 × 10^9 cfu/µg pUC18 DNA) when using the optimized transformation protocol.

* U.S. Patent Nos. 5,512,468 and 5,707,841 and Patents Pending.

‡ SoloPack Gold cells are chloramphenicol resistant (Cam^r) at concentrations of <40 µg/ml, but sensitive (Cam^s) to concentrations of 100 µg/ml.

§ See *Preparation of Media and Reagents*.

Transformation Guidelines and Troubleshooting

Storage Conditions: Supercompetent 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.

Use of β-Mercaptoethanol (β-ME) and NZY$^+$ broth: For the highest transformation efficiencies (1×10^9 cfu/µg pUC18 DNA), use the optimized transformation protocol that includes treatment of the cells with β-ME (see step 4) and use of NZY$^+$ as the outgrowth medium (see steps 1 and 10). When the β-ME step is omitted from the protocol and SOC is used as the outgrowth medium, expect transformation efficiencies of approximately 90% of those obtained using the optimized protocol. The β-ME mix provided is diluted and ready to use. Stratagene cannot guarantee results with β-ME from other sources.

Quantity and Volume of DNA: The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl of supercoiled DNA per aliquot of cells. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency may be lower. The volume of the DNA solution added to the reaction may be increased to up to 5 µl, but the transformation efficiency may be reduced.

Heat Pulse Duration and Temperature: Optimal transformation efficiency is observed when cells are heat-pulsed at **54°C** for 60 seconds in the tubes provided. Efficiency decreases sharply when either the temperature or the duration of the heat pulse is altered.

Blue-White Color Screening: Blue-white color screening for recombinant plasmids is available when transforming the host strain 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

LB 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
Pour into petri dishes (~25 ml/100-mm plate)

SOB Broth (per Liter)

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

NZY$^+$ Broth (per Liter)

10 g of NZ amine (casein hydrolysate)
5 g of yeast extract
5 g of NaCl
Add deionized H_2O to a final volume of 1 liter
Adjust to pH 7.5 using NaOH
Autoclave
Add the following filer-sterilized supplements prior to use:
12.5 ml of 1 M $MgCl_2$
12.5 ml of 1 M $MgSO_4$
20 ml of 20% (w/v) glucose (or 10 ml of 2 M glucose)

SOC Broth (per 100 ml)

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–Ampicillin Agar (per Liter)

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.)

Limited Product Warranty

Endnotes

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