

TRANSFORMATION GUIDELINES AND TROUBLESHOOTING

Storage Conditions: Competent and 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 14-ml BD Falcon polypropylene round-bottom tubes: It is important that 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) are used for the transformation protocol, since other tubes may be degraded by β -mercaptoethanol. In addition, the duration of the heat-pulse is critical and has been optimized for these tubes.

Aliquoting Cells: Keep the cells on ice at all times during aliquoting. It is essential that the polypropylene tubes are placed on ice before the cells are thawed and that the cells are aliquoted directly into pre-chilled tubes. It is also important to use the volume of cells indicated in step 2 of the *Transformation Protocol*. Decreasing the volume will result in lower efficiencies.

Use of β -Mercaptoethanol (β -ME): β -ME has been shown to increase transformation efficiency. The β -ME provided is diluted and ready to use. A fresh 1:10 dilution (from a 14.2 M stock) may be used; however, 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 0.1 ng/ μl supercoiled pUC18 DNA per reaction. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/ μg DNA) may be lower. The volume of the DNA solution added to the reaction may be increased to up to 10% of the reaction volume, but the transformation efficiency may be reduced.

Heat Pulse Duration: Optimal transformation efficiency is observed when cells are heat-pulsed at 42°C for 45–50 seconds. Efficiency decreases sharply when cells are heat-pulsed for <45 seconds or for >60 seconds.

Blue-White Color Screening: Blue-white color screening for recombinant plasmids is available when transforming host strains that contain the *lac^fZ Δ M15* gene on the F' episome with a plasmid that provides α -complementation (e.g. Stratagene's pBluescript[®] II vector). 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 .

PREPARATION OF MEDIA AND REAGENTS

SOB Medium (per Liter) 20.0 g of tryptone 5.0 g of yeast extract 0.5 g of NaCl Add deionized H ₂ O 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	SOC Medium (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 Agar (per Liter) 10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Add deionized H ₂ O to a final volume of 1 liter Adjust pH to 7.0 with 5 N NaOH Autoclave	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-indolyl- β -D-galactopyranoside (X-gal) to a final concentration of 80 $\mu\text{g/ml}$ [prepared in dimethylformamide (DMF)] and isopropyl-1-thio- β -D-galactopyranoside (IPTG) to a final concentration of 20 mM (prepared in sterile dH₂O). 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.)

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ENDNOTES

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