



## TRANSFORMATION GUIDELINES AND TROUBLESHOOTING

**Aliquoting Cells:** Keep the cells on ice at all times during aliquoting. It is essential that the microcentrifuge tubes that the cells will be aliquoted into are placed on ice before the cells are thawed and that the cells are aliquoted directly into the pre-chilled tubes.

**Cuvette Gap Width:** Use a cuvette with a 0.1-cm gap to maximize the transformation efficiency and to minimize the possibility of arcing. A cuvette with a 0.2-cm gap is not recommended because the transformation efficiency is lower and the possibility of arcing is higher.

**Quantity and Volume of DNA:** The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl of DNA per 40 µl of cells. The volume of DNA may be increased to up to 4 µl but the transformation efficiency may be reduced and the possibility of arcing may be increased if the DNA solution contains salts. A greater number of colonies may be obtained by increasing the amount of DNA added to the cells, although the overall efficiency may be lower.

**Ionic Strength of DNA Solution:** The sample DNA to be transformed by electroporation must be in a low-ionic-strength buffer, such as TE buffer or water. DNA samples containing too much salt will cause arcing at high voltage, possibly damaging both the sample and the machine.

**Blue-White Color Screening:** Blue-white color screening for recombinant plasmids is available when transforming host strains that contain the *lacI<sup>q</sup>ZΔM15* gene on the F' episome with a plasmid that provides α-complementation (e.g. Stratagene's pBluescript® II). 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.

**Reduction of Satellite Colonies:** Due to the high concentration of bacteria in electroporation, the transformation plates should be incubated at 37°C for less than 24 hours. If the incubation is to be extended beyond 24 hours, Stratagene recommends selecting for transformants on LB plates containing tetracycline (30 µg/ml) in addition to the antibiotic used to select for the transforming plasmid. Including tetracycline helps suppress satellite colony growth and does not affect the transformation efficiency. In addition, all colonies that grow in the presence of tetracycline contain an F' episome, improving blue-white color selection. This measure for reducing growth of satellite colonies may be used for all of Stratagene's electroporation competent cells, **except** the TG1 electroporation-competent cells, which are Tet<sup>s</sup>.

## PREPARATION OF MEDIA AND REAGENTS

<p><b>LB Agar (per Liter)</b>            10 g of NaCl            10 g of tryptone            5 g of yeast extract            20 g of agar            Add deionized H<sub>2</sub>O to a final volume of 1 liter            Adjust pH to 7.0 with 5 N NaOH            Autoclave</p>	<p><b>SOB Medium (per Liter)</b>            20.0 g of tryptone            5.0 g of yeast extract            0.5 g of NaCl            Add deionized H<sub>2</sub>O to a final volume of 1 liter            Autoclave            Add 10 ml of filter-sterilized 1 M MgCl<sub>2</sub> and 10 ml of filter-sterilized 1 M MgSO<sub>4</sub> prior to use</p>
<p><b>LB-Ampicillin-Tetracycline Agar (per Liter)</b>            (Use for reduced satellite colony formation)            1 liter of LB agar, autoclaved            Cool to 55°C            Add 10 ml of 10mg/ml filter-sterilized ampicillin            Add 30 mg of filter-sterilized tetracycline            Pour into petri dishes (~25 ml/100-mm plate)</p>	<p><b>SOC Medium (per 100 ml)</b>  <b>Note</b> <i>This medium should be prepared immediately before use.</i>              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</p>
<p><b>LB-Ampicillin Agar (per Liter)</b>            1 liter of LB agar, autoclaved            Cool to 55°C            Add 10 ml of 10mg/ml filter-sterilized ampicillin            Pour into petri dishes (~25 ml/100-mm plate)</p>	<p><b>TE Buffer</b>            10 mM Tris-HCl (pH 7.5)            1 mM EDTA</p>

## 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 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<sub>2</sub>O). 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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