XL2-Blue MRF′ Ultracompetent Cells

Catalog #200151

MATERIALS PROVIDED

Materials provided	Quantity	Efficiency (cfu/μg of pUC18 DNA)
XL2-Blue MRF´ ultracompetent cells (yellow tubes)	10 × 100 μl	≥5 × 10^9
pUC18 control plasmid (0.1 ng/μl in TE buffer)	10 μl	—
β-Mercaptoethanol (β-ME) (1.22 M)	25 μl	—

Storage: Ultracompetent 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 100-μl aliquots of cells and 10 pg of pUC18 control DNA following the protocol outlined below. 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

XL2-Blue MRF´ ultracompetent cells* are high-efficiency derivatives of Stratagene's XL1-Blue MRF´ supercompetent cells. Using ultracompetent cell technology, Stratagene has achieved transformation efficiencies of greater than 5 × 10^9 transformants/μg of pUC18 DNA, making these ultracompetent cells an ideal choice for high-efficiency cloning. The XL2-Blue MRF´ (Minus Restriction) strain is deficient in all known restriction systems (McrA$^-$, McrCB$^-$, McrF$^-$, Mrr$^-$, HsdR$^-$), preventing cleavage of cloned DNA that carries cytosine and/or adenine methylation, which is often present in eukaryotic DNA and cDNA.

XL2-Blue MRF´ Genotype: Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [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.)

XL2-Blue MRF´ cells are resistant to tetracycline and chloramphenicol.‡ XL2-Blue MRF´ cells are restriction minus [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 *lacI*^{q}*ZΔM15* gene on the F´ episome allows blue-white screening for recombinant plasmids.

TRANSFORMATION PROTOCOL

1. Pre-chill two 14-ml BD Falcon polypropylene round-bottom tubes on ice. (One tube is for the experimental transformation and one tube is for the pUC18 control.) Preheat NZY$^+$ broth§ to 42°C.
2. Thaw the ultracompetent cells on ice. When thawed, gently mix and aliquot 100 μl of cells into each of the two pre-chilled tubes.
3. Add 2 μl of the β-ME provided with this kit to each aliquot of cells.
4. Swirl the tubes gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes.
5. Add 0.1–50 ng of the experimental DNA to one aliquot of cells, in a 1-μl volume for maximum efficiency (see *Quantity and Volume of DNA*). Dilute the pUC18 control DNA 1:10 with sterile dH_2O, then add 1 μl of the diluted pUC18 DNA to the other aliquot of cells.
6. Swirl the tubes gently, then incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42°C water bath for 30 seconds. The duration of the heat pulse is **critical** for maximum efficiency.
8. Incubate the tubes on ice for 2 minutes.
9. Add 0.9 ml of preheated (42°C) NZY$^+$ broth and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.
10. Plate ≤200 μl of the transformation mixture 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 5 μl of the transformation on LB–ampicillin agar plates.§

    Notes *Cells may be concentrated by centrifuging at 1000 rpm for 10 minutes. Resuspend the pellet in 200 μl of NZY$^+$ broth.*

    If plating <100 μl of cells, pipet the cells into a 200 μl pool of 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.
11. Incubate the plates at 37°C overnight. See *Blue-White Color Screening*, below, for color screening incubation guidelines.
12. For the pUC18 control, expect 250 colonies (≥5 × 10^9 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: Ultracompetent 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 100 µl of cells per transformation. Decreasing the volume will reduce efficiency.

Use of β-Mercaptoethanol (β-ME): β-ME has been shown to increase transformation efficiency. The β-ME mixture provided is diluted and ready to use. Stratagene cannot guarantee results with β-ME from other sources.

Use of NZY$^+$ broth: Transformation of the supplied ultracompetent or supercompetent cells has been optimized using NZY$^+$ as the medium for outgrowth following the heat pulse. Substitution with another outgrowth medium may result in a loss of efficiency.

Quantity and Volume of DNA: The greatest efficiency is obtained from the transformation of 1 µl of 0.01 ng/µl supercoiled pUC18 DNA per 100 µl of cells. When transforming a ligation mixture, add 2 µl of the ligation mixture per 100 µl of cells. A greater number of colonies may be obtained by transforming up to 50 ng DNA, although the resulting efficiency (cfu/µg) 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 and Temperature: Optimal transformation efficiency is observed when cells are heat-pulsed at 42°C for 30 seconds. Efficiency decreases sharply when cells are heat-pulsed for <30 seconds or for >40 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}*ZΔM15* 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

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)

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)

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 X-gal before pipetting them into the pool of medium because a precipitate may form.) Spreading the IPTG and X-gal on the plate surface produces darker blue colonies compared to the pale blue colonies produced when IPTG and X-gal are added to the medium prior to pouring.

Limited Product Warranty

Endnotes

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