Made in USA

Catalog Number **200138**

Product Name **XL1-Blue MRF′ Kan Library Pack Competent Cells**

Materials Provided
XL1-Blue MRF' Kan Library Pack Competent Cells, 5 × 500 µl
pUC18 control plasmid (0.1 ng/µl in TE buffer), 10 µl
β-Mercaptoethanol (2-mercaptoethanol Library Pack), 50 µl

Certified By Todd Parsons

Quality Controlled By Tricia Molina

Shipping Conditions Shipped on dry ice.

Storage Conditions Competent 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. Competent cells are sensitive to even small variations in temperature. Transferring tubes from one freezer to another may result in a loss of efficiency.

Guaranteed Efficiency $\geq 1.0 \times 10^9$ cfu/µg pUC18 DNA

Test Conditions Transformations are performed both with and without plasmid DNA using 100-µl aliquots of cells and 100 pg of pUC18 control DNA following the protocol outlined below. Following transformation, 2.5-µl samples of the culture are plated in duplicate on LB agar plates with 100 µg/ml ampicillin. The plates are incubated at 30°C overnight and the efficiency is calculated based on the average number of colonies per plate.

Genotype and Background *Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*5* (Kan^r)]. (Genes listed signify mutant alleles. Genes on the F′ episome, however, are wild-type unless indicated otherwise.)

The XL1-Blue MRF′ Kan Library Pack competent cells have been optimized for cDNA library construction using the BacterioMatch two-hybrid system.

The XL1-Blue MRF′ Kan strain is a restriction minus (McrA$^-$, McrCB$^-$, McrF$^-$, Mrr$^-$, HsdR$^-$) and kanamycin-resistant derivative of Stratagene's XL1-Blue strain. This strain is useful for PCR cloning using vectors harboring chloramphenicol- or tetracycline-resistance genes. XL1-Blue MRF′ Kan cells are deficient in all known restriction systems [Δ(*mcrA*)*183*, Δ(*mcrCB-hsdSMR-mrr*)*173*], and are endonuclease (*endA*), and recombination (*recA*) deficient. The *hsdR* mutation prevents the cleavage of cloned DNA by the *EcoK* endonuclease system, and the *recA* mutation helps ensure insert stability. The *endA* mutation greatly improves the quality of miniprep DNA. The *lacI*^{q}*ZΔM15* gene on the F′ episome allows blue-white screening for recombinant plasmids.

Antibiotic Resistance **XL1-Blue MRF' Kan cells are resistant to kanamycin.**

Transformation Protocol

1. Pre-chill the appropriate number of 14-ml BD Falcon polypropylene round-bottom tubes on ice (one tube for the pUC18 control plus one tube for each pilot library transformation reaction). Preheat SOC medium to 42°C.
2. Thaw the XL1-Blue MRF′ Kan library pack competent cells on ice. When thawed, gently mix and aliquot 100 µl of cells into each of the pre-chilled tubes.
3. Add 2 µl of the Library Pack β-mercaptoethanol provided with this kit to each aliquot of cells.
4. Swirl the tubes gently. Incubate the cells on ice for 10 minutes.
5. Add 1 µl (100 pg) of the pUC18 control DNA to one aliquot of cells to determine the transformation efficiency. For each pilot pTRG cDNA library transformation reaction, add 1.6 µl of the pilot pTRG-cDNA library ligation reaction mixture to another aliquot of cells. Swirl the tubes gently.
6. Incubate the tubes on ice for 30 minutes.
7. Heat-pulse the tubes in a 42°C water bath for 33 seconds. The duration of the heat pulse is critical.
8. Incubate the tubes on ice for 2 minutes.
9. Add 0.9 ml of preheated (42°C) SOC medium and incubate the tubes at 30°C for 1.5 hours with shaking at 225-250 rpm.
10. For the pUC18 control transformation, plate 2.5 µl of the transformation mixture on each of two LB-ampicillin agar plates. For the pilot pTRG library transformation, plate 2 µl and 5 µl of the pilot pTRG library transformation mixture on separate LB-tetracycline agar plates. (Prior to plating the cells, place 100 µl of SOC medium on each LB-ampicillin or LB-tetracycline plate. Pipet the cells from the transformation reaction into the pool of SOC, and then spread the mixture with a sterile spreader.)
11. Incubate the plates at 30°C, inverted, for 17-24 hours.
12. For the pUC18 control, count the number of colonies on each LB-ampicillin plate and calculate the average number of colonies per plate. Expect 250 colonies ($\geq 1 \times 10^9$ cfu/µg pUC18 DNA).
13. For the pilot library transformation, count the number of colonies on each LB-tetracycline plate and calculate the transformation efficiency and yield of cfu expected from each transformation reaction. Consult the *BacterioMatch Two Hybrid System XR cDNA Library Construction Kit* manual for more information on scaling up the library ligation and transformation reactions to generate the primary BacterioMatch pTRG target library.

Critical Success Factors and Troubleshooting

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 has been optimized using 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 reduce efficiency.
Use of β-Mercaptoethanol (β-ME): β-ME has been shown to increase transformation efficiency. The Library Pack β-ME provided is diluted and ready to use. For best results, use 2 µl of this β-ME per 100 µl of cells. 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 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.
Heat Pulse Duration and Temperature: Optimal transformation efficiency is observed when 100 µl of cells are heat-pulsed at 42° C for 33 seconds. Efficiency decreases sharply when cells are heat-pulsed for <30 seconds or for >35 seconds. Do not exceed 42°C.
Plating the Transformation Mixture: When plating <100 µl of cells, pipet the cells into a 100-µl pool of SOC medium on each agar plate and then spread the mixture with a sterile spreader.

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_2O to a final volume of 1 liter and then 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)
Prepare 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_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH and then autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB-Ampicillin Agar (per Liter)
1 liter of LB agar, autoclaved and cooled to 55°C
Add 10 ml of 10 mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

LB-Tetracycline Agar (per Liter)
1 liter of LB agar, autoclaved and cooled to 45°C
Add 1.25 ml of 10 mg/ml tetracycline (prepared in 50% ethanol)
Pour into petri dishes (~25 ml/100-mm plate)
Store the plates in a dark, cool place or cover plates with foil, as tetracycline is light-sensitive

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