ElectroTen-Blue
Electroporation Competent Cells

Instruction Manual

Catalog #200159

Revision C.0

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200159-12

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ElectroTen-Blue Electroporation Competent Cells

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MATERIALS PROVIDED

Materials provided	Quantity	Efficiency (cfu/μg of pUC18 DNA)
ElectroTen-Blue Electroporation Competent Cells (purple tube)	5 × 100 μl[a]	≥3 × 10^{10}
pUC18 control plasmid (0.1 ng/μl in TE buffer)	10 μl	—
StrataClean Resin	100 μl	—

[a] Each 100-μl tube is sufficient for 2 transformations.

STORAGE CONDITIONS

Electroporation competent cells: Store immediately at –80°C. Do not place the cells in liquid nitrogen.
pUC18 control plasmid: –80°C
StrataClean resin: –20°C

ADDITIONAL MATERIALS REQUIRED

Equipment

Sterile microcentrifuge tubes (1.5-ml)
Sterile cuvettes (0.1-cm gap for maximum efficiency)
14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059)

Reagents

SOC medium (see *Preparation of Media and Reagents*)

Revision C.0

INTRODUCTION

The efficient introduction of DNA into bacteria is critical to the success of many molecular biology procedures. The electroporation method of transforming *Escherichia coli* cells can produce efficiencies greater than those achieved with the best chemical methods.[1] Briefly subjecting a mixture of cells and DNA to intense electrical fields routinely results in 10^9–10^{10} transformants/µg of DNA.

We have developed a method* to produce electroporation competent cells that have greater transformation efficiencies than existing methods. The ElectroTen-Blue Electroporation Competent Cells** are a derivative of the XL1-Blue MRF´ strain that can withstand much higher levels of electrical current. This increases the survival of the cells, and thus the efficiency of transformation by ligated constructs. In addition the ElectroTen-Blue strain is deficient in all known restriction systems [Δ(*mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173*] and is endonuclease deficient (*endA1*) and recombination deficient (*recA1*). Therefore, it is an excellent strain for constructing genomic or DNA libraries using methylated cDNAs. The *lacI*^{q}*Z*Δ*M15* gene on the F´ episome allows blue-white color selection of recombinants if the cells are transformed with a plasmid capable of complementing the deletion in the β-galactosidase gene of the bacterial cell. ElectroTen-Blue cells are resistant to both tetracycline and kanamycin. These electroporation-ready cells need only be thawed, mixed with DNA, and electroporated. ElectroTen-Blue cells are not recommended for M13 single-strand rescue.

Phenol extraction and ethanol precipitation are commonly employed to clean up DNA samples prior to transformation. StrataClean Resin is provided in this kit to prepare DNA ligation reactions for electroporation. This cleanup step is an effective replacement for both phenol extraction and ethanol precipitation and takes considerably less time.

* U.S. Patent No. 6,338,965.

** U.S. Patent Nos. 6,586,249, 6,338,965, 6,040,184.

HOST STRAIN GENOTYPE

Δ(*mcrA*)*183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* Kan^r [F´ *proAB lacI*^{q}*Z*Δ*M15* Tn*10* (Tet^r)]

The genes indicated in italics signify that the bacterium carries a mutant allele. The genes on the F´ episome, however, represent wild-type bacterial alleles.

ElectroTen-Blue electroporation competent cells are kanamycin and tetracycline resistant. Plasmids that carry the Kan-resistance or Tet-resistance selection marker cannot be propagated in this strain.

PREPROTOCOL CONSIDERATIONS

The *lacI*^{q}*Z*Δ*M15* gene on the F´ episome allows blue-white color selection of recombinants. To prepare the LB agar plates for blue-white color screening, add 80 µg/ml of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), 20 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG), and the appropriate antibiotic to the LB agar. Prepare the IPTG in sterile dH_2O, and the X-gal in dimethylformamide (DMF). Alternatively, 100 µl of 10 mM IPTG and 100 µl of 2% X-gal can be spread on the LB agar plates 30 minutes prior to plating the transformations.

Some β-galactosidase fusion proteins are toxic to the host bacteria. If an insert is suspected to be toxic to the bacteria, do not plate the transformations with X-gal and IPTG. Color screening will be eliminated, but the recombinants will express lower levels of potentially toxic proteins.

PROTOCOLS

Preparation of DNA Ligation Reactions Prior to Transfection

StrataClean resin is an excellent substitute for phenol extraction and subsequent ethanol precipitation for removing modifying enzymes. Two sequential extractions are performed on each DNA sample to ensure complete removal of protein contaminants. The following protocol is adapted to clean up 10-µl ligation reactions that contain no more than 10% (v/v) enzyme and no more than 150 mM NaCl. The quantities in this protocol may be scaled up proportionally if the ligation reaction volume is greater than 10 µl.

1. Vortex the StrataClean resin to completely resuspend it.

2. Using a sterile pipet tip, pipet 5 µl of the slurry into the tube containing the ligation reaction.

3. Vortex this mixture for 15 seconds.

4. Pellet the resin by centrifugation at >2000 × *g* for one minute.

5. Using a sterile pipet tip, carefully remove the supernatant containing the DNA to a fresh microcentrifuge tube.

   Note *When removing the supernatant, remove 10 µl. DO NOT attempt to remove any more supernatant, as this may result in carry-over of resin that could interfere with subsequent enzymatic reactions.*

6. Repeat steps 1–5.

7. Use the supernatants in the following electroporation reaction. If desired, the entire 10µl supernatant sample may be added to a 40-µl aliquot of electroporation competent cells.

Performing the Electroporation

Note *The sample DNA 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. DNA prepared according to the previous protocol is suitable for use in the electroporation.*

Before beginning, ensure that the electroporation chamber, cuvettes, and 1.5-ml microcentrifuge tubes have been thoroughly chilled on ice. Preheat sterile SOC medium[§] to 37°C.

1. Thaw the electroporation competent cells on ice.

2. Aliquot 40 µl of cells into each of two chilled 1.5-ml microcentrifuge tubes.

   Note *Electroporation-competent cells are very fragile. Pipet gently when aliquotting the cells.*

3. Add experimental DNA to one tube of electroporation-competent cells. For optimal efficiency, add 1-4 µl of resin-purified plasmid DNA to 40 µl of cells. Gently mix the cells and DNA.

   Dilute the pUC18 control plasmid 1:10 with sterile, distilled water (dH_2O). Add 1 µl of the diluted pUC18 control plasmid to 40 µl of cells in a chilled 1.5-ml microcentrifuge tube.

4. Transfer each DNA-cell mixture to a **chilled** electroporation cuvette that has a 0.1-cm gap, tapping the top of the cuvette until the mixture settles evenly to the bottom.

   Note *Use of a cuvette with a 0.1-cm gap maximizes the transformation efficiency and minimizes the possibility of arcing. Use of a cuvette with a 0.2-cm gap is not recommended because the transformation efficiency is lower and the possibility of arcing is higher.*

5. Slide the cuvette into the chilled electroporation chamber until the cuvette connects with the electrical contacts.

[§]See *Preparation of Media and Reagents.*

6. Set the electroporator to the following settings:

   Note *These settings are for use with ElectroTen-Blue cells, which are able to withstand higher voltages; these settings may not be appropriate for use with other bacterial strains.*

Applied volts (0.1-cm gap)	Field strength (0.1-cm gap)	Resistance	Capacitance
2250 V	22.5 kV/cm	200 Ω	25 µF

7. Pulse the sample once, then quickly remove the cuvette. **Immediately** add 960 µl of 37°C sterile SOC medium to resuspend the cells.

8. Transfer each cell suspension to a sterile 14-ml BD Falcon polypropylene round-bottom tube. Incubate the tubes at 37°C for 90 minutes while shaking at 225–250 rpm.

9. If color screening is desired and if the agar plates were not prepared with X-gal and IPTG, spread 100 µl of 2% X-gal and 100 µl of 10 mM IPTG on the LB agar plates 30 minutes prior to plating the transformations (see *Preprotocol Considerations*).

   Note *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.*

10. Using a sterile spreader, spread 5–100 µl of the transformation on plates containing the appropriate antibiotic. To plate the cells transformed with the pUC18 control plasmid, first place a 100-µl pool of SOC medium on an LB-ampicillin agar plate (see *Preparation of Media and Reagents*). Add 1 µl of the control transformation reaction to the pool of SOC medium. Use a sterile spreader to spread the mixture.

11. Incubate the plates overnight at 37°C. If the transformation was performed with a plasmid capable of complementing the cell's truncated β-galactosidase gene, blue-white colony differentiation is possible after >17 hours. The blue color can be enhanced by incubating the plates for two hours at 4°C following the overnight incubation at 37°C. We recommend that positive colonies be selected from electroporation transformations within 24 hours of the initial plating.

Transformation Summary

Quantity of control transformation plated	Expected cfu	Efficiency (cfu/µg of pUC18 DNA)
1 µl	~300	$\geq 3 \times 10^{10}$

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)

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)

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

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
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

REFERENCE

1. Dower, W. J., Miller, J. F. and Ragsdale, C. W. (1988) *Nucleic Acids Res* 16(13):6127-45.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.

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QUICK-REFERENCE PROTOCOL

- Clean up the DNA ligation reaction using StrataClean resin
- Chill electroporation chamber, cuvettes, and microcentrifuge tubes on ice and preheat SOC medium to 37°C
- Thaw the cells and aliquot 40 µl into two 1.5-ml chilled microcentrifuge tubes
- Mix the cells with the resin-purified sample DNA (1-4 µl) or with the control DNA (10 pg)
- Electroporate with 2250 V, 22.5 kV/cm, 200 Ω, and 25 µF
- Dilute with 37°C SOC medium (960 µl)
- Incubate with shaking at 37°C for 90 minutes
- Plate 5–100 µl of the sample DNA electroporation reaction or 1 µl of the control DNA electroporation reaction in a 100-µl pool of SOC medium
- Incubate the plates overnight (or for ≥17 hours if color screening) at 37°C