XL1-Red Competent Cells

Instruction Manual

Catalog #200129

Revision C.0

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XL1-Red Competent Cells

Materials Provided

Materials provided	Quantity
XL1-Red competent cells[a] (red-orange tube)	5 × 0.2-ml aliquots
XL1-Blue competent cells[b] (blue tube)	5 × 0.2-ml aliquots
pUC18 control plasmid 0.1 ng/µl in TE buffer[c]	10 µl
1.42 M β-Mercaptoethanol	50 µl

[a] Transformation efficiency is $\sim 1 \times 10^6$ cfu/µg of pUC18 DNA.
[b] Transformation efficiency is $>1 \times 10^8$ cfu/µg of pUC18 DNA.
[c] See *Preparation of Media and Reagents*.

Storage Conditions

Store the cells immediately at –80°C

Do not place the competent cells in liquid nitrogen

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INTRODUCTION

We have developed a highly efficient, rapid and reproducible method for introducing random mutations in a cloned gene of interest. This method involves propagating the cloned gene into an *Escherichia coli* strain, called XL1-Red, which is deficient in three of the primary DNA repair pathways. The *mutS* (error-prone mismatch repair),[1] *mutD* (deficient in 3´- to 5´-exonuclease of DNA polymerase III)[2] and *mutT* (unable to hydrolyze 8-oxodGTP)[3] mutations were introduced into the XL1-Red strain using basic bacterial genetics. The random mutation rate in this triple mutant strain was measured to be ~5000-fold higher than that of wild-type. This strain is particularly suitable for generating random mutations within a gene that has no selectable or screenable phenotype, and the method does not require extensive genetic or biochemical manipulations.

Because of the mutator genes engineered into the XL1-Red strain, this mutator strain possesses the following characteristics requiring special considerations:

- Although this mutator strain is recombination proficient (*recA*$^{+}$), the strain grows extremely slowly in rich media (e.g., LB media), having a doubling time of ~90–120 minutes.
- The XL1-Red strain carries the Tn*10* transposon that encodes the tetracycline-resistance gene. However, due to the rapid mutator phenotype, the cells will frequently give rise to tetracycline-sensitive (Tet^{s}) variants. Therefore, the resulting transformants may or may not be tetracycline resistant, and we do not recommend that tetracycline be added to the growth media.
- Following transformation into XL1-Red competent cells and subsequent plating on LB–ampicillin agar plates at 37°C, the transformant colonies become visible 24–30 hours later. If large-sized colonies are desired, even longer incubation times are often required.
- The high mutation rate of the XL1-Red mutator strain causes a wide range of colony sizes.
- Additionally, **the XL1-Red mutator strain should not be propagated on plates for prolonged periods of time**. The rapid mutation rate affects the chromosome, and after prolonged growth, the subsequent colonies are probably not genetically identical to the original strain.

To avoid the potential problems outlined above, the XL1-Red mutator strain is available only as competent cells, since the mutator phenotype of the XL1-Red strain cannot be guaranteed if the strain is maintained by the researcher. Each lot of XL1-Red competent cells is strictly monitored to ensure that the competent cells retain their mutator phenotype.

HOST STRAINS AND GENOTYPES

For the *E. coli* strain, the genes listed in the table below signify that the bacterium carries a mutant allele. The genes present on the F´ episome, however, represent the wild-type alleles unless indicated otherwise. Strains should be considered lambda^{-} and F^{-} unless designated otherwise.

Host strain	Reference	Genotype
XL1-Red strain	4	*endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT* Tn*10* (Tet^{r})[a]
XL1-Blue strain[b]	5	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F´ *proAB lacI^{q}ZΔM15* Tn*10* (Tet^{r})]

[a] The XL1-Red strain carries the Tn*10* transposon that encodes the tetracycline-resistance gene. However, due to the rapid mutator phenotype, the cells will frequently give rise to tetracycline-sensitive (Tet^{s}) variants. Therefore, the resulting transformants may or may not be tetracycline resistant.

[b] The XL1-Blue strain is available as higher efficiency supercompetent (Catalog #200236) and electroporation-competent cells (Catalog #200228) producing transformation efficiencies $>1 \times 10^{9}$ and $>5 \times 10^{9}$ cfu/μg of pUC18 DNA, respectively. The strain is also available as a bacterial glycerol stock (Catalog #200268).

TRANSFORMATION GUIDELINES

Important *Please read the following guidelines before proceeding with the transformation protocol!*

Storage Conditions

Competent 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. Competent cells should be placed at –80°C directly from the dry ice shipping container. Cells stored in this manner should retain their guaranteed efficiency for 6 months.

Aliquoting Cells

When aliquoting, keep the competent cells on ice at all times. It is essential that the 14-ml BD Falcon polypropylene round-bottom tubes are placed on ice before the cells are thawed and that the cells are aliquoted directly into the prechilled tubes. It is also important to use at least 100 μl of competent cells/transformation. Using a smaller volume will result in lower efficiencies.

Use of 14-ml BD Falcon Polypropylene Round-Bottom Tubes

It is important to use 14-ml BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) for the *Transformation and Mutagenesis Protocol,* since other tubes may be degraded by β-mercaptoethanol. In addition, the duration of the heat-pulse step is critical and has been optimized for the thickness and shape of these tubes.

Use of β-Mercaptoethanol

β-Mercaptoethanol (β-ME) has been shown to increase transformation efficiency two- to threefold. This kit includes prediluted β-ME, which is ready to use. Using the β-ME within 3 months is recommended. Use 1.7 µl of the β-ME provided or a fresh 1:10 dilution of a 14.2 M stock solution per 100 µl of cells.

Quantity of DNA Added

Greatest efficiencies are observed when adding 1 µl of 0.1 ng/µl of DNA per 100 µl of cells. A greater number of colonies will be obtained when transforming up to 50 ng, although the overall efficiency may be lower. In order to ensure a population of truly random mutants, we recommend transforming the XL1-Red competent cells with at least 10 ng of supercoiled plasmid to ensure ≥200 colonies.

Length of the Heat Pulse

There is a defined "window" of highest efficiency resulting from the heat pulse in step 8 of the *Transformation and Mutagenesis Protocol*. Optimal efficiencies are observed when cells are heat-pulsed for 45–50 seconds. Heat-pulsing for at least 45 seconds is recommended to allow for slight variations in the length of incubation. Efficiencies decrease sharply when incubating for <45 seconds or for >60 seconds.

TRANSFORMATION AND MUTAGENESIS PROTOCOL

1. Pre-warm SOC medium to 42°C. (See *Preparation of Media and Reagents.*)

2. Thaw the XL1-Red competent cells on ice.

3. Gently mix the XL1-Red competent cells by hand. For each transformation reaction, aliquot 100 µl of the XL1-Red competent cells into a prechilled 14-ml BD Falcon polypropylene round-bottom tube.

4. Add 1.7 µl of the β-mercaptoethanol provided with this kit to each 100-µl of XL1-Red competent cells, giving a final concentration of 25 mM.

5. Swirl the contents of the tubes gently. Incubate the tubes on ice for 10 minutes, swirling the tubes gently every 2 minutes.

6. Add 10–50 ng of DNA to each aliquot of XL1-Red competent cells and swirl gently. As an optional transformation efficiency control, add 1 µl of the provided pUC18 control plasmid DNA to another 100-µl aliquot of cells and swirl gently.

7. Incubate the tubes on ice for 30 minutes.

8. Heat-pulse the tubes in a 42°C water bath for 45 seconds. The duration of the heat pulse is critical for obtaining the highest efficiencies.

9. Incubate the tubes on ice for 2 minutes.

10. Add 0.9 ml of SOC medium (pre-warmed to 42°C) to each tube and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.

11. Plate ≤200 µl of the transformation mixture on LB agar plates containing the appropriate antibiotic using a sterile spreader.[||] For the pUC18 control transformation, spread 100 µl of the XL1-Red transformation reaction on an LB–ampicillin agar plate.[§] (See Table 1 for the expected control transformation results.)

    Note *The cells may be concentrated by centrifuging at 1000 rpm for 10 minutes if desired. Resuspend the pellet in 200 µl of SOC medium and then spread the suspension on the plate.*

12. Incubate the plates at 37°C for 24–30 hours.

13. Using a sterile wire loop or a sterile toothpick, select ≥200 colonies at random from the transformation plates and inoculate cultures of 5–10 ml of LB broth[§] containing the appropriate antibiotic. Grow these cultures overnight at 37°C. If a higher mutation rate is desired or if a plasmid with a lower copy number than the pBluescript phagemid is being used, the cells can be diluted and grown overnight for as many cycles as desired.

14. Prepare miniprep DNA from 1.5 ml of the overnight culture in order to isolate the randomly mutated plasmid DNA. StrataPrep plasmid miniprep kits (Catalog #400761 and #400763) provide a fast, effective method for preparing high-quality plasmid miniprep DNA.

15. Transform the isolated miniprep DNA into the XL1-Blue competent cells provided with this kit, following steps 1–12 above. For plating the optional pUC18 control transformation (in step 11), spread 5 µl of the XL1-Blue transformation reaction on an LB–ampicillin agar plate.[||] (See Table 1 for the expected control transformation results.)

16. Screen for the desired mutations following the transformation into the XL1-Blue competent cells.

If toxicity of the cloned product is suspected, try transforming into an alternative strain, such as the ABLE C or ABLE K strain (Catalog #200171 and #200172, respectively), which propagate plasmids at lower copy numbers.

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

[||] When spreading bacteria onto the plate, tilt and tap the spreader to remove the last drop of cells. If plating <100 µl of the transformation mixture, plate into a 200-µl pool of SOC medium. If plating ≥100 µl, the cells can be spread directly onto the plates.

TABLE 1

Control Transformation Summary

Bacterial strain	Amount plated	Expected cfu/plate	Efficiency (cfu/μg of pUC18 DNA)
XL1-Red competent cells	100 μl	10	1×10^6
XL1-Blue competent cells	5 μl	50	1×10^8

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
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
Adjust pH to 7.0 with 5 N NaOH
Add deionized H_2O to a final volume of 1 liter
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

LB Broth (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
Adjust to pH 7.0 with 5 N NaOH
Add deionized H_2O to a final volume of 1 liter
Autoclave

References

1. Radman, M. (1980). Progress in Environmental Mutagenesis,M. Alecivic (Ed.), pp. 121–130. Elsevier/North Holland, Amsterdam, The Netherlands.
2. Scheuermann, R., Tam, S., Burgers, P. M. J., Lu, C. and Echols, H. (1983) *Proc Natl Acad Sci U S A* 80:7085–7089.
3. Cox, E. C. (1976) *Annu Rev Genet* 10:135–156.

Additional References

1. Greener, A., and Callahan, M. (1994) *Strategies* 7(2): 32–34.
2. Bullock, W. O., Fernandez, J. M., and Short, J. M. (1987) *Biotechniques* 5: 376–379.

MSDS Information

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