# BJ5183 Electroporation Competent Cells

**Catalog** #200154

## MATERIALS PROVIDED

| Materials Provided | Quantity | Transformation Efficiency (cfu/μg of pUC18 DNA)[a] |
|---|---|---|
| BJ5183 electroporation competent cells (clear tubes) | 5 × 100 μl[b] | ≥ 1 × $10^8$ |
| pUC18 control plasmid DNA (0.1 ng/μl in TE buffer) | 10 μl | — |

[a] Stratagene guarantees this efficiency when the cells are used according to the protocol in this instruction manual.

[b] Each 100 μl aliquot is sufficient for two transformations.

**Storage** Store the cells immediately at the bottom of a –80°C freezer. Do not store the cells in liquid nitrogen. Store the control plasmid DNA at –80°C.

## ADDITIONAL MATERIALS REQUIRED

Electroporation cuvettes, 0.2 cm gap
DNase-free microcentrifuge tubes

## INTRODUCTION

BJ5183 electroporation competent cells are a recombination proficient bacterial strain. These cells supply the components necessary to execute the recombination event between a transfer vector containing the gene of interest and a vector containing the adenoviral genome, provided that appropriate regions of homology are shared between the two vectors.

**BJ5183 electroporation competent cellsare resistant to streptomycin.**

## GENOTYPE

*end*A1 *sbc*BC *rec*BC *gal*K *met thi*-1 *bio*T *hsd*R **(Str$^r$)** (see reference 1).

## TRANSFORMATION GUIDELINES FOR BJ5183 CELLS

### Storage Conditions

Electroporation competent cells are 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. Electroporation competent cells should be placed at –80°C directly from the dry ice shipping container.

### Aliquoting Cells

When aliquoting, keep electroporation competent cells on ice at all times. It is essential that the DNase-free microcentrifuge tubes and the electroporation cuvettes are placed on ice before the cells are thawed and that the cells are aliquoted directly into the prechilled tubes.

## COTRANSFORMING THE BJ5183 CELLS TO PRODUCE ADENOVIRUS RECOMBINANTS

**Notes** *In this portion of the protocol, the BJ5183 cells are cotransformed with the transfer vector (usually in linear form, containing the gene of interest) and the supercoiled vector representing the adenoviral genome. A recombination event that takes place in the BJ5183 cells results in the production of supercoiled recombinant adenovirus plasmid DNA. It is important that the BJ5183 host cell strain is used for this recombination step; not all bacterial strains are capable of supporting homologous recombination.*

*It is important to set up controls for the recombination event (linearized transfer vector alone, supercoiled adenoviral genome vector alone). These controls are at the user's discretion.*

1. Chill the required number of DNase-free microcentrifuge tubes and electroporation cuvettes (0.2 cm gap) on ice.
2. Referring to the instructions provided with the electroporator, set the following parameters on the instrument: 200 Ω, 2.5 kV, 25 μF.
3. Remove the BJ5183 electroporation competent cells from –80°C storage and thaw on ice.
4. Gently pipet 40 μl of the competent cells into each of the chilled microcentrifuge tubes.
5. Into one tube of cells, pipet 1 μg of linearized and dephosphorylated transfer vector and 100 ng of supercoiled adenoviral plasmid vector. Add no more than 6 μl of DNA into the 40 μl of cells.
6. Into a second tube of cells, pipet 1 μl of 10 pg/μl pUC18 control plasmid DNA (dilute stock provided 1:10 in sterile $dH_2O$).
7. Using additional 40 μl aliquots of BJ5183 cells, set up as many controls as are required.
8. Transfer the contents of the microcentrifuge tube from step 5 into a chilled electroporation cuvette and tap the cuvette gently to settle the mixture to the bottom.
9. Slide the cuvette into the chilled electroporation chamber until the cuvette connects with the electrical contacts.
10. Pulse the sample once, then quickly remove the cuvette. **Immediately** add 1 ml of sterile LB broth (see *Preparation of Media and Reagents*) and pipet up and down to resuspend the cells.
11. Transfer the cell suspension to a sterile 15-ml Falcon® 2059 polypropylene tube.
12. Repeat the electroporation for the other transformation reaction(s).
13. Incubate the cell suspensions at 37°C for 1 hour with shaking at 225–250 rpm.
14. For the adenovirus vector transformations, plate the entire volume of cell suspension onto 3–5 LB agar plates containing the appropriate antibiotic (e.g. spread three plates with 100 μl, 300 μl, and 600 μl of the cell suspension).
15. For the pUC18 transformation, first place a 100-μl pool of LB broth on an LB–ampicillin agar plate (see *Preparation of Media and Reagents*). Add 5 μl of the transformed cell suspension to the pool of LB broth. Use a sterile spreader to spread the mixture.
16. Incubate the plates overnight at 37°C.

## Testing Colonies for Adenovirus Recombinants

1. Examine the plates containing the pUC18 transformation to calculate the transformation efficiency (expect $\geq 1 \times 10^8$cfu/µg).
2. Compare the colonies on the linearized transfer vector alone (control) to the colonies on the recombination plates. The control plates should contain colonies of uniform size, arising from recircularized transfer vector. The transformants on the plates containing the adenoviral DNA recombinants will appear as two populations: normal-sized and tiny.
3. Pick 10 of the smallest, best-isolated colonies from the recombination plates (transfer vector plus gene of interest) into 2-ml cultures of LB broth containing the appropriate antibiotic (see *Preparation of Media and Reagents*).
4. Incubate at 37°C overnight while shaking at 225–250 rpm.
5. Prepare miniprep DNA from the overnight cultures using method of choice. The final volume of miniprep DNA should be 50 µl and the DNA should be resuspended in sterile $dH_2O$.

   **Note** *Do not store the BJ5183 cultures after overnight growth as undesired recombinants can be generated. Prepare plasmid miniprep DNA first thing in the morning.*
6. Cut 5 µl of the miniprep DNA with restriction enzymes that are diagnostic for the recombination event and run the digest on a 0.8% agarose TAE gel (see *Preparation of Media and Reagents)* next to 5 µg of uncut plasmid. It is also recommended that 5 µl of the miniprep DNA be cut with a restriction enzyme that will cleave somewhere within the gene of interest to confirm maintenance of the insert in the recombined adenovirus plasmid.

   **Note** *Reserve a small amount of each plasmid sample for amplifying by transformation in a subsequent step.*

Once the construction of the recombinant adenovirus plasmid(s) has been confirmed, amplify the plasmid stock by transforming competent bacterial cells and purifying maxiprep DNA from these. It is not necessary to use BJ5183 cells for this as no recombination event takes place during amplification and many other bacterial strains give higher yields of DNA. Following amplification, packaged adenovirus can be produced by transfecting human cell lines with the linearized adenoviral DNA. Packaged adenovirus can then be used in gene expression studies.[2]

## Preparation of Media and Reagents

| LB Broth (per Liter) | LB Agar (per Liter) | LB–Ampicillin Agar (per Liter) | 1× TAE Buffer |
|---|---|---|---|
| 10 g of NaCl | 10 g of NaCl | 1 liter of LB agar, autoclaved | 40 mM Tris-acetate |
| 10 g of tryptone | 10 g of tryptone | Cool to 55°C | 1 mM EDTA |
| 5 g of yeast extract | 5 g of yeast extract | Add 10 ml of 10 mg/ml filter-sterilized ampicillin | |
| Add deionized $H_2O$ to a final volume of 1 liter | 20 g of agar | Pour into petri dishes (~25 ml/100-mm plate) | |
| Adjust to pH 7.0 with 5 N NaOH | Adjust pH to 7.0 with 5 N NaOH | | |
| Autoclave | Add deionized $H_2O$ to a final volume of 1 liter | | |
| Cool to 55°C | Autoclave | | |
| Add antibiotic (if required) | Cool to 55°C | | |
| | Add antibiotic (if required) | | |
| | Pour into petri dishes (~25 ml/100-mm dish) | | |

## References


1. Hanahan, D. (1983) *J. Mol. Biol.* 166: 557–580.
2. He, T., Zhou, S., DaCosta, L., Yu, J., Kinzler, K., and Voglestein, B. (1998) *Proc. Natl. Acad. Sci. USA* 95:2509–2514.


## Quality Control Testing

Following the protocol above, BJ5183 electroporation competent cells are transformed with and without pUC18 control plasmid DNA. One microliter of pUC18 DNA (10 pg/µl) is used to transform 40 µl of BJ5183 cells. After electroporation, the cells are resuspended in 1 ml of LB broth and allowed to recover for 1 hour at 37°C with shaking. 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 overnight at 37°C. The efficiency is calculated based on the average number of colonies per plate. In addition, the BJ5183 electroporation competent cells are tested for adenovirus recombination proficiency using Stratagene's AdEasy® vector system.

## Limited Product Warranty



## Endnotes

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