BJ5183-AD-1 Electroporation Competent Cells

Catalog #200157

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MATERIALS PROVIDED

Materials Provided	Quantity	Transformation Efficiency (cfu/μg transformation control)[a]
BJ5183-AD-1 electroporation competent cells (green tubes)	5 × 100 μl[b]	≥ 1× 10^7
Transformation Control (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 –20°C.

ADDITIONAL MATERIALS REQUIRED

Electroporation cuvettes, 0.2 cm gap
DNase-free microcentrifuge tubes

INTRODUCTION

BJ5183-AD-1 electroporation competent cells are recombination proficient bacterial cells carrying the pAdEasy-1 plasmid that encodes the Adenovirus-5 genome (E1/E3 deleted). These cells supply the components necessary to execute a recombination event between the pAdEasy-1 vector and an AdEasy® shuttle vector containing the gene of interest, thus generating a recombinant adenovirus genome that contains the gene of interest.

GENOTYPE[1]

*end*A1 *sbc*BC *rec*BC *gal*K *met thi*-1 *bio*T *hsd*R (**Str^r**) [pAdEasy-1 (Amp^r)]
BJ5183-AD-1 cells are streptomycin and ampicillin resistant.

TRANSFORMATION GUIDELINES FOR BJ5183-AD-1 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.

TRANSFORMING THE BJ5183-AD-1 CELLS TO PRODUCE ADENOVIRUS RECOMBINANTS

Notes *In this portion of the protocol, the BJ5183-AD-1 cells are transformed with a shuttle vector containing the gene of interest (usually in linear form after cleavage with* Pme *I restriction enzyme). A recombination event that takes place in the BJ5183-AD-1 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: Stratagene recommends performing the following control transformations: 1) transformation control, 2) Pme *I-linearized pShuttle-CMV-*lacZ *recombination control.*

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-AD-1 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 μl (0.05 to 0.1 μg) of linearized shuttle vector. (Add no more than 6 μl of DNA into 40 μl of cells.)
6. Into a second tube of cells, pipet 1 μl of the provided transformation control DNA.
7. Using additional 40-μl aliquots of BJ5183-AD-1 cells, set up as many additional controls as are required.
8. Transfer the shuttle vector transformation mixture (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 linearized shuttle vector transformations, plate the entire volume of cell suspension onto LB agar plates containing the appropriate antibiotic. For Stratagene's AdEasy shuttle vectors, use LB-kanamycin agar; see *Preparation of Media and Reagents*. Spread different volumes of cells on three plates (50 μl, 100 μl, and 850 μl respectively) such that the entire volume is plated.
15. For the transformation control, plate 10 μl and 100 μl of the cells on LB-kanamycin agar. When plating less than 100 μl, first place a 100-μl pool of LB broth on the plate, pipet the cells into the broth, and then spread the mixture.
16. Incubate the plates overnight at 37°C.

Testing Colonies for Adenovirus Recombinants

1. Examine the transformation control plates to calculate the transformation efficiency (expect $\geq 1 \times 10^7$ cfu/μg).
2. Examine the linear shuttle vector transformation plates. The linear shuttle vector transformants will appear as three populations: very large colonies, intermediate-, and small-sized colonies. The small and intermediate colonies are the potential recombinants and the very large colonies represent background from the shuttle vector. The ratio of small plus intermediate colonies to very large colonies should be approximately 10:1.
3. Pick 10 of the smallest, best-isolated colonies from the recombinants plate into 5-ml cultures of LB broth containing the appropriate antibiotic (see *Preparation of Media and Reagents*).
4. Incubate the cultures at 37°C overnight with shaking at 225–250 rpm.
5. Prepare miniprep DNA from the overnight cultures using a method of choice. Resuspend the miniprep DNA in 50 μl of sterile dH_2O.

   Note *Do not store the BJ5183-AD-1 transformants after overnight growth as undesired recombinants can be generated. Prepare plasmid miniprep DNA first thing in the morning.*

6. Cut 10 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 10 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 (Stratagene recommends XL10-Gold® ultracompetent cells, Catalog #200314) with an aliquot of the miniprepped DNA and preparing maxiprep DNA from these cells. Following amplification, packaged adenovirus can be produced by transfecting a human cell line such as Stratagene's AD-293 cells (Catalog #240085) with linearized adenoviral DNA. Packaged adenovirus can then be used in gene expression studies.[2]

Note *Do not use BJ5183-AD-1 competent cells for recombinant adenovirus plasmid amplification.*

Preparation of Media and Reagents

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

References

1. Hanahan, D. (1983) *J Mol Biol* 166(4):557-80..
2. He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W. *et al.* (1998) *Proc Natl Acad Sci U S A* 95(5):2509-14.

Quality Control Testing

Following the protocol above, BJ5183-AD-1 electroporation competent cells are transformed with transformation control DNA. One microliter of the transformation control DNA is used to transform 40 μl of BJ5183-AD-1 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. 5 μl volumes are plated in duplicate on LB agar plates containing 50 μg/ml of kanamycin. The plates are incubated overnight at 37°C. The efficiency is calculated based on the average number of colonies per plate.
Following the protocol above, BJ5183-AD-1 electroporation competent cells are transformed with linearized pShuttle-CMV-*lacZ* control plasmid DNA. To assess recombination efficiency, DNA is prepared from 10 of the smallest colonies, digested with PmeI, and run on a 0.8% agarose gel. Greater than or equal to 90% of the prepared DNA samples contain the recombinant vector. The integrity of the pADEasy-1 plasmid is verified by restriction mapping following isolation from an overnight culture of BJ5183-AD-1 cells.

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Endnotes

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