

TESTING COLONIES FOR ADENOVIRUS RECOMBINANTS

1. Examine the transformation control plates to calculate the transformation efficiency (expect $\geq 1 \times 10^7$ cfu/ μ g).
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 μ l of sterile dH₂O.
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 μ l 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 μ g of uncut plasmid. It is also recommended that 5 μ l 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 miniprep 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 ₂ O 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 ₂ O 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 ₂ O 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 μ l 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 μ l volumes are plated in duplicate on LB agar plates containing 50 μ g/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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