

TRANSFORMATION OF BJAdeasy BACTERIAL CELLS FOR GENERATING ADENOVIRAL RECOMBINANTS

by TCH 8/13/01; Updated by JK Park 5/12/04

- 1) Purify the shuttle plasmids (e.g., pAdTrack-CMV derivatives) containing your gene of interest by using the **alkaline lysis procedure**;
- 2) Digest **5 to 10ul** of miniprep DNA with **3ul** of *Pme I* (from NEB) in a 100ul reaction at 37°C for 30-60min;
- 3) Precipitate the digested DNA with ethanol followed by washing with 70% ethanol twice;
- 4) Let the pellet air-dry (or remove the residual liquid by briefly spinning the tube), and dissolve it in **10ul** ddH₂O;
- 5) Thaw an aliquot of **20ul** of electrocompetent BJAdeasy cells on ice;
- 6) Meanwhile, clean a **2-mm** cuvette by power-rinsing it with ddH₂O, then 70% ethanol and 100% ethanol. After air-drying, the cuvette should be chilled on ice;
- 7) Add the 6ul of digested DNA to the bacterial cells, and transfer the DNA/bacteria mix to a prechilled 2-mm cuvette. Bring down the mix to the bottom by gently tapping cuvette. Keep on ice;
- 8) Prepare 500ul LB (**no antibiotics!**) in 1.5 ml microfuge tubes;
- 9) Perform electroporation at **2.5KV**, followed by adding **500ul LB** to the cuvette;
- 10) Mix bacterial cells well by gently pipetting up and down, and immediately plate 100ul and 200ul onto two LB/Kan plates. [**Optional/preferred**: immediately plate 200ul onto one LB/Kan plate, and incubate the remaining cell mix (in 1.7ml microfuge tubes) at 37°C water bath for 30min, followed by plating the mix (approx. 100-200ul) onto another LB/Kan plate].

- 11) Incubate at 37⁰C overnight and pick up the **smaller clones** for miniprep confirmation.

NOTE:

- 1) More detailed information about the AdEasy system can be found at the following website: ***www.coloncancer.org/adeasy.htm***
- 2) Alkaline lysis DNA prep is the best for this procedure to make recombinants.
- 3) One must use a 2-mm cuvette because of a larger volume of DNA/bacteria mix.
- 4) A short incubation (20-30min) at 37⁰C may be desirable if only a few colonies are recovered from overnight incubation.