AdEasy Made Easier

(www.coloncancer.org/adeasy.htm)

Use of AdEasier Cells for Generating Adenoviral Recombinants

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NOTE: **We are now distributing the AdEasier-1 cells along with other components of the AdEasy system. The AdEasier-1 cells are BJ5183 transformed with the pAdEasy-1 plasmid. They can be grown in LB-Amp/Strep medium for making competent *coli* cells. *However, you are still strongly advised to excise the Precautions (see below).***

PROTOCOL FOR PREPARING AND USING AdEasier CELLS

***Note:** This protocol is optional and can be used only if you have not received the AdEasier-1 cells, or you are not satisfied with the AdEasier-1 sent to you, or you need to use the AdEasy-2 system.*

1) Transform 50 ng of pAdEasy plasmid into BJ5183 cells and plate the transformation mix on agar plates conferring resistance to both ampicilin and streptomycin.

2) Pick 10-20 colonies and grow each in 2 ml LB-Amp/Strep medium with continuous shaking at 37^{0}C, overnight.

3) Purify the DNA from each of the cultures (see **Appendix E**: Alkaline Lysis Protocol for Plasmid Minipreps).

4) Use 20-30% of the miniprep DNA for restriction digestion (Hind III, Pst I, etc) to confirm integrity of clones. Pick one confirmed clone for subsequent use.

5) Grow the confirmed clone in LB-Amp/Strep medium.

6) Prepare electrocompetent BJAdEasy cells using the protocol described in the **Appendix**.

7) Transform the Pme I-linearized shuttle plasmid into the electrocompetent BJAdEasy cells.

8) Follow the rest of the protocol described in the **Practical Guide**.

9) Take a weeklong vocation in **Honolulu**, Hawaii or **Dundalk**, Maryland and your virus will be ready upon your return.

ADVANTAGES OF USING BJAdEasy CELLS

1) It is extremely efficient and it is almost impossible to fail.

2) It does not require preparation of high quality pAdEasy plasmids.

3) It is probably possible to generate recombinants by using conventional chemical transformation methods instead of electroporation, though we haven't explored this.

PRECAUTIONS

Although this method is extremely efficient, users are advised to note the following precaution. Because BJ5183 cells have a relatively high frequency of homologous recombination, unwanted or detrimental rearrangements and/or recombinations of the pAdEasy DNA can occur. It is thus very important to pick individual clones and characterize the clones with extensive restriction digestions, usually with Hind III and/or Pst I. The digestion pattern can be compared with the pAdEasy stock DNA made in a non-recombinant strain (like DH10B). A restriction digest characterization should optimally be carried out on the large-scale culture that is used to prepare competent cells.