

Simple and efficient method for the large-scale production of E1 deficient adenovirus

SUMMARY

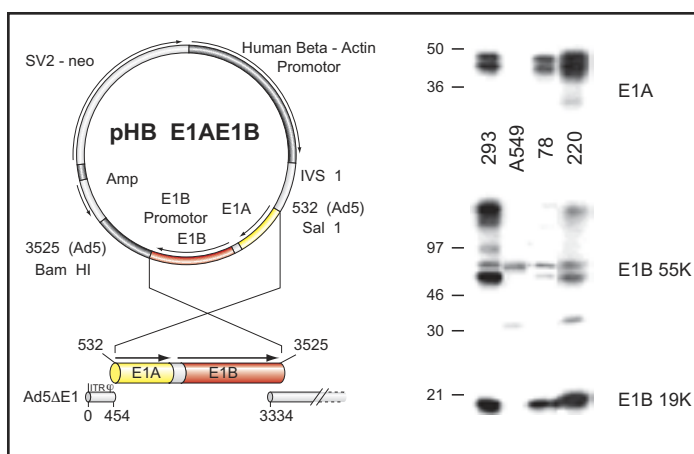
Adenoviral vector systems are one of the most powerful platforms in biotechnology for gene therapy, gene transfer and protein expression. One feature that makes this technology particularly appealing is that by simple deletion of the E1 gene sequence, the virus is incapable of replication. Under normal conditions, E1 gene products are the first to be expressed following the adenoviral infection of cells. In addition, they are essential for virus replication. Therefore, E1-deleted adenoviral vectors can only be generated in a cell line that constitutively expresses E1 proteins. This technology provides a vector and a cell line that enables easy production of replicant deficient adenovirus.

APPLICATIONS

- Large-scale production of infectious but replication-defective adenovirus viral particles that may be used for therapeutic and research applications requiring efficient gene transfer.
- Establishment of E1-complementing cells from any pharmaceutically acceptable cell line in order to significantly reduce the contamination of those cell cultures by replication-competent adenoviruses.

CONCEPT

The E1-complementing cell line has a stably integrated



complementation element comprised of only the E1A and E1B portions of the E1 gene (see Figure, left panel). Each component is placed under the separate control of different promoters. This particular construct enables the stable integration of the complementation element to produce functional proteins, E1A and E1B, which can complement, in *trans*, a defective adenoviral vector. Furthermore, the design of the E1 expression cassette is such that it will prevent any possibility of homologous recombination of an E1-element with a defective adenoviral vector, thereby preventing the generation of replication-competent adenoviruses (RCAs). The human lung A549 cell line was chosen for large-scale production of clinical grade material, instead of 293 cells known for being easily contaminated by RCAs.

FEATURES AND BENEFITS

Simple and efficient production of E1-minus adenovirus

This technology has been proven as an efficient and simple way to produce adenovirus vectors. A549E1 cells express functional E1A and E1B proteins, at a level sufficient to effectively complement the E1-defective adenoviral vector.

Scalable production of E1-deficient adenovirus

The technology supports large-scale production of substantially pure infectious E1-defective adenoviruses. In order to obtain a concentrated stock free of replication-competent viruses, the production of the infectious E1-defective particles is scaled up by the transfection of E1-defective adenoviral vector into E1-complementing cells. This is followed by the screening of plaques positive for E1-defective viruses, at least two rounds of their purification and the re-infection of E1-complementing cells with the previously purified E1-defective viruses. Large scale production can be done in suspension serum-free culture.

PROTECTION STATUS

Adenovirus E1-complementing cell lines (NRC no. 10624).

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