NRC·CNRC

A549 EXPRESSION SYSTEM

For producing adenoviral vectors – licensing opportunity L-10624

HIGHLIGHTS

Adenoviral (AV) vectors are crucial tools for vaccine antigen delivery and immunotherapy. In these applications, the vector's E1 sequence may be deleted to prevent viral replication. However, E1-deficient AV vectors may recombine with the E1 region of the cell line, most commonly in HEK293 cells, during production. These replication-competent adenoviruses (RCA) are contaminants in the final therapeutic product and can lead to undesired replication of the vector in the patient.

To produce E1-deficient AV vectors without RCA contamination, NRC's experts developed an A549 cell line suitable for research applications as well as clinical and commercial production. They have also developed a cell line to produce protease-deficient AV that contains the E1 region.

TECHNOLOGY TRANSFER

- Non-exclusive R&D and/or commercial exploitation licence
- R&D agreement for development of custom A549 cell lines

MARKET APPLICATIONS

- Produce non-replicating, E1-deficient AV vectors for vaccines and cancer therapy
- Produce replicating, protease-deficient AV vectors for vaccines and cancer therapy
- Produce AV vectors carrying a toxic gene, using a cumate gene switch

HOW IT WORKS

E1-deficient AV: Cell line A549-BMAdE1

The NRC's bioprocess experts designed an E1 complementing cell line, **A549-BMAdE1**, where only the E1A and E1B portions of the E1 gene are present. Each portion is controlled by a separate promoter, resulting in a stable E1 construct that produces E1A and E1B proteins to complement deficient adenovirus. The NRC's experts designed the E1 expression cassette without overlapping DNA to prevent homologous recombination of an E1 element from the cell line with the deficient vector during production, eliminating the generation of undesired RCAs.

Protease-deficient AV: Cell line A549-SF-BMAd-PS

In some applications where stronger immune reactions are sought, the E1A region of the AV vector is preserved to enable AV replication, while the protease region is deleted so that the vector does not disseminate. To produce replicating, protease-deficient AV, the NRC's bioprocess experts developed cell line **A549-SF-BMAd-PS**, which contains the protease gene (PS) to complement the deletion of the PS gene in the vector. Because constitutive production of PS is toxic to the cell line, its synthesis is regulated by the coumermycin switch. Addition of coumermycin to the cell culture medium induces PS synthesis.

AV containing toxic gene: Cumate switch

Antigen DNA (genes) inserted in AV vectors for vaccine and cancer therapy applications are often toxic to the cell line required to produce the vector, and can reduce AV yield. The NRC's experts have resolved this problem by including a binding site (or sequence) for the repressor of the cumate switch in the vector's expression cassette. Activated during vector propagation, the repressor prevents synthesis of the toxic genes inserted in the AV vector, which reduces damage to the A549 cell line during production to enable higher AV yields.

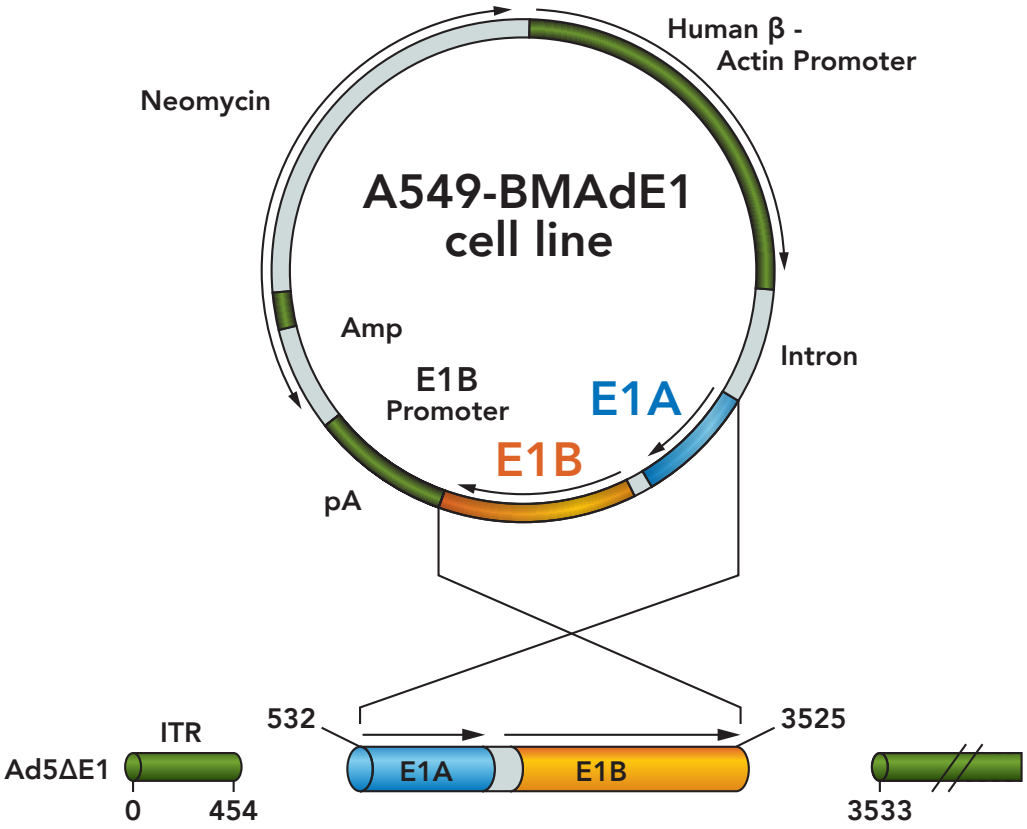

Figure A: NRC's A549-BMAdE1 cell line

Large-scale production

Large-scale production of a concentrated, pure stock can be achieved by transfection, followed by screening of plaques that are positive for AV vectors, at least two rounds of their purification, and the reinfection of the cell line using the purified AV vectors in serum-free suspension culture.

BENEFITS

- The NRC's **A549-BMAdE1** cell line expresses E1 proteins, required for production of E1-deficient AV vectors
- Unlike **HEK293** cells, the **A549-BMAdE1** cell line prevents generation of RCA, facilitating production of large-scale clinical or commercial batches
- The NRC's **A549-SF-BMAd-PS** cell line produces protease-deficient, replicating AV
- For vectors carrying a toxic gene, the cumate repressor switch enables higher yields of E1-deficient AV

PATENTS

NRC file 10624 (A549 cell lines): Patents issued in Canada and the United States

NRC File 11225/11648 (cumate switch and repressor switch): Patents granted in Canada, the United States, and Europe.

NRC file 11444 (coumermycin switch): Patents granted in Canada, the United States, Europe, Australia, New Zealand, and Japan.

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