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Generation of infectious genome of bovine adenovirus type 3 by homologous recombination in bacteria

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Abstract

The widely used technique of generating adenovirus vectors by homologous recombination in mammalian cells is usually not very efficient. This communication describes a simple method of generating a plasmid containing the full-length genome of an adenovirus by homologous recombination in bacteria. Following transfection of a suitable mammalian cell line with the full-length adenovirus genome, infectious virus progeny could easily be generated. Using this technique the generation of adenovirus recombinants would be efficient and straightforward. © 1999 Elsevier Science B.V. All rights reserved.

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Recombinant adenoviruses are used widely as vectors to study gene expression, to transfer genes into mammalian cells and to develop recombinant vaccines (Graham and Prevec, 1992; Rosenfeld et al., 1992; Engelhardt et al., 1994; Imler, 1995; Addison et al., 1995). Foreign gene insertion in adenoviruses is usually made at two locations: the early region (E) 1 (E1) and E3 to generate recombinant adenoviruses (Berkner and Sharp, 1984; Haj-Ahmad and Graham, 1986). The basic strategy includes construction of an E1 or E3 transfer vector containing approximately 10 kb of the left- or right-end genome with an appropriate E1 or E3 deletion, respectively replaced by a foreign gene insert. Cotransfection of a suitable cell line with the transfer plasmid and the adenovirus genome or a plasmid containing almost the entire adenovirus genome eventually results in generation of an adenovirus recombinant by homologous recombination. A number of cotransfection experiments are usually needed to obtain a recombinant virus.

The recA protein of Escherichia coli (E. coli) recognizes homologous sequences between a nascent single-stranded DNA molecule and those present in double-stranded DNA and produces their recombination (Bubeck et al., 1993; Degryse, 1995). By utilizing the highly efficient homologous recombination machinery of bacteria, a desired adenovirus recombinant could easily be constructed. This strategy has been used successfully for generating human adenovirus recombinants (Chartier et al., 1996; Crouzet et al., 1997; He et al., 1998). To determine whether the same approach could be used to construct non-human adenovirus recombinants, we exploited the homologous recombination process of bacteria to generate full-length, infectious clones of bovine adenovirus type 3 (BAV3).

In order to produce the full-length genome of BAV3 in recombination-competent bacteria, a plasmid is required containing approximately 1 kb BAV3 from both ends and the BAV3 genome. The 12 kb left-end XbaI-B fragment of BAV3 (from nucleotide 1 to 12 189; for BAV3 nucleotide numbering, see Reddy et al., 1998) was cloned into the SmaI–XbaI site of an EcoRI-deleted pUC18 to generate pMvOB02. Plasmid pMvOB02 was digested with EcoRI (present at nucleotide 1156 in the BAV3 genome) and HindIII (present in the multiple cloning site of pUC18) and a 3.8 kb fragment containing pUC18 and the left-end of BAV3 genome was gel purified. The 3.6 kb right-end EcoRI-E fragment of BAV3 genome (from nucleotide 30 833 to 34 446) was inserted into the 3.8 kb EcoRI–HindIII fragment of pMvOB02 to obtain pMvOBE1E4. To introduce a PacI site on either end of the BAV3 genome, the BAV3 sequences present in pMvOBE1E4 were amplified by polymerase chain reaction (PCR) using a single synthetic primer encoding the eight-nucleotide recognition sequence of PacI followed by the first 21 nucleotides of the BAV3 inverted terminal repeat (ITR; 195 bp long ITRs are present at both ends of the