# FOWL ADENOVIRUS 9 (FADV-9) VECTOR SYSTEM AND ASSOCIATED METHODS

## RELATED APPLICATIONS

**[0001]** This application is a continuation of U.S. application Ser. No. 15/743,459 filed Jan. 23, 2018 (pending), which is a national phase entry of PCT/CA2016/050811 filed Jul. 11, 2016 (which designates the U.S.) which claims the benefit of priority to U.S. Provisional Patent Application No. 62/190,913 filed Jul. 10, 2015 (now abandoned), the contents of which are hereby incorporated by reference in their entirety.

## INCORPORATION OF SEQUENCE LISTING

**[0002]** A computer readable form of the Sequence Listing "6580-P48972US02_SequenceListing.txt" (110,592 bytes), submitted via EFS-WEB and created on Apr. 28, 2021, is herein incorporated by reference

## FIELD OF THE INVENTION

**[0003]** The present invention relates to the fowl adenovirus 9 (FAdV-9) and more particularly to a FAdV-9 dual delivery vector system and associated methods as well as use of the same for the prevention of disease.

## BACKGROUND OF THE INVENTION

**[0004]** Fowl adenoviruses (FAdVs) are ubiquitous poultry pathogens and are members of the family Adenoviridae.

**[0005]** Adenoviruses (AdVs) of the genus *Mastadenovirus* have been examined as anti-cancer agents (Huebner et al. (1956), Cody & Douglas (2009), Yamamoto & Curiel (2010)) and vaccine vectors (Lasaro & Ertl (2009)).

**[0006]** The problem of preexisting immunity against HAdV-5, exemplified in the STEP HIV trial that employed recombinant HAdV-5 (Buchbinder et al. (2008), McElrath et al. (2008)), has generated interest in the development of less common AdV serotypes and nonhuman AdVs as both oncolytic ((Cody & Douglas (2009), Gallo et al. (2005), Shashkova et al. (2005)) and vaccine vectors (Barouch (2008), Lasaro & Ertl, (2009), Sharma et al. (2009)). Fowl adenoviruses (FAdVs) of the genus Aviadenovirus, including species FAdV-A to FAdV-E (Adair, B. & Fitzgerald, S. (2008), Benkö et al (2005)), are being developed as vaccine vectors. The first generation of FAdV-based vaccine vectors have proven to be effective at eliciting an antibody response against a delivered transgene (Corredor & Nagy (2010b), Ojkic & Nagy (2003)), and in chickens have conferred protective immunity against infectious bursal disease virus (IBDV) (Francois et al. (2004), Sheppard et al. (1998)) and infectious bronchitis virus (Johnson et al. (2003)). Analysis of the complete genomes of FAdV-1, the chicken embryo lethal orphan (CELO) virus (Chiocca et al. (1996)), and FAdV-9 (Ojkic & Nagy (2000)) (species FAdV-A and FAdV-D, respectively), and the terminal genomic regions of FAdV-2, -4, -10, and -8 (Corredor et al. (2006), Corredor et al. (2008)) has shown that the FAdVs share a common genome organization.

**[0007]** Adenovirus-based vaccine vectors have proven to be promising tools for controlling pathogens (Bangari & Mittal (2006), Ferreira et al. (2005)). The first generation of fowl adenovirus (FAdV) based vaccine vectors have been effectively used to induce an antibody response against an inserted foreign gene (transgene) (Corredor, & Nagy (2010a), Ojkic & Nagy (2003)), and in chickens have conferred protective immunity against infectious bursal disease virus (Francois et al. (2004), Sheppard et al. (1998)) and infectious bronchitis virus (Johnson et al. (2003)).

**[0008]** Current state of the art adenoviral vectors, especially fowl adenoviruses, are hampered by the limit of the size in the foreign DNA insert size in the vector DNA. Consequently it is not possible to clone in particularly large DNA inserts representing important parts of immunogenic proteins into an independently replicating virus. Nor it is possible to clone in more than one gene. The value of having a vector capable of expressing dual or even multivalent antigens is that only one vaccine would be needed to protect against two or more diseases. This is not possible with the current state of the art adenoviral vectors due to lack of cargo space.

**[0009]** Therefore, there remains a need for novel adenoviral vectors and in particular for novel dual delivery adenoviral vectors and uses thereof.

## SUMMARY OF THE INVENTION

**[0010]** The inventors have developed novel adenoviral vectors based on recombinant fowl adenovirus 9 (FAdV-9). The vectors are particularly useful for the delivery and/or expression of exogenous sequences and as dual delivery adenoviral vectors. Inserted into the novel vector can be one or more exogenous nucleotide sequences. Optionally, the one or more exogenous nucleotide sequences code for one or more antigenic sites of a disease of concern.

**[0011]** Accordingly, in one aspect, there is provided a recombinant fowl adenovirus 9 (FAdV-9) viral vector. In one embodiment, the recombinant FAdV-9 viral vector is a dual delivery viral vector.

**[0012]** In one aspect, the FAdV-9 viral vector has a deletion at the left end of the genome. In one embodiment, the deletion at the left end of genome comprises a deletion of one or more of ORF0, ORF1 and ORF2. In one embodiment, the FAdV-9 viral vector has a deletion at the right end of the genome. In one embodiment, the deletion at the right end of the genome comprises a deletion of one or more of ORF19, TR2, ORF17 and ORF1. In one embodiment, the FAdV-9 viral vector has deletions at both the left end and right end of the genome. In one embodiment, the FAdV-9 viral vector has a deletion of ORF0, ORF1, ORF2, TR2, ORF17 and ORF11. In one embodiment, the FAdV-9 viral vector has a deletion of ORF1, ORF2, TR2, ORF17 and ORF11. In other embodiment, the FAdV-9 viral vector has a deletion ORF1, ORF2, and ORF 19. In other further embodiment, the FAdV9-viral vector has a deletion of ORF1, ORF2, and ORF19, TR2 or ORF11.

**[0013]** In one embodiment, the FAdV-9 viral vector has a deletion at the left end of the genome of about 2291 base pairs, optionally between about 1900 and 2500 base pairs. In one embodiment, the FAdV-9 viral vector has a deletion at the right end of the genome of about 3591 base pairs. In one embodiment, the FAdV-9 viral vector has a deletion at the right end of the genome of between about 3000 base pairs and 4000 base pairs.

**[0014]** In one embodiment, the viral vector comprises a nucleotide sequence with at least 80%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity to the sequence with the two deletions shown in FIG. **10**A. In one embodiment, the viral vector comprises or consists of the