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(54) **CORONAVIRUS**

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See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS
7,452,542 B2 * 11/2008 Denison C07K 14/005
424/221.1

FOREIGN PATENT DOCUMENTS
WO WO-2004/092360 A2 10/2004
WO WO-2005/049814 A2 6/2005
WO WO-2007/078203 A1 7/2007
WO WO-2011/004146 A1 1/2011

OTHER PUBLICATIONS

Sperry Journal of Virology, 2005, vol. 79, No. 6, pp. 3391-3400.*
Altschul et al., Basic local alignment search tool. *J. Mol. Biol.* 215:
403-10 (1990).

Ammayappan et al., Identification of sequence changes respon-
sible for the attenuation of avian infectious bronchitis virus strain
Arkansas DPI, *Arch. Virol.*, 154(3):495-9 (2009).

Anonymous: "EM_STD:KF377577", Oct. 30, 2013.

Armesto et al., A recombinant avian infectious bronchitis virus
expressing a heterologous spike gene belonging to the 4/91 serotype,
PLoS One, 6(8):e24352 (2011).

Armesto et al., The replicase gene of avian coronavirus infectious
bronchitis virus is a determinant of pathogenicity, *PLoS One*,
4(10):e7384 (2009).

Armesto et al., Transient dominant selection for the modification
and generation of recombinant infectious bronchitis coronaviruses,
Methods Mol. Biol., 454:255-73 (2008).

Ausubel et al., *Short Protocols in Molecular Biology*, 4th edition,
Chapter 18 (1999).

Britton et al., Generation of a recombinant avian coronavirus
infectious bronchitis virus using transient dominant selection, *J.*
Virol. Methods, 123(2):203-11 (2005).

Britton et al., Modification of the avian coronavirus infectious
bronchitis virus for vaccine development, *Bioeng. Bugs.*, 3(2):114-9
(2012).

Casais et al., Recombinant avian infectious bronchitis virus express-
ing a heterologous spike gene demonstrates that the spike protein is
a determinant of cell tropism, *J. Virol.*, 77(16):9084-9 (2003).

Casais et al., Reverse genetics system for the avian coronavirus
infectious bronchitis virus, *J. Virol.*, 75(24):12359-69 (2001).

Devereux et al., A comprehensive set of sequence analysis programs
for the VAX. *Nucl. Acids Res.* 12: 387-95 (1984).

Cavanagh et al., Manipulation of the infectious bronchitis coronavirus
genome for vaccine development and analysis of the accessory
proteins, *Vaccine*, 25(30):5558-62 (2007).

International Preliminary Report on Patentability, International Appli-
cation No. PCT/GB2015/052124, dated Jan. 24, 2017.

International Search Report and Written Opinion, International
Application No. PCT/GB2015/052124, dated Oct. 9, 2015.

Larkin et al., Clustal W and Clustal X version 2.0, *Bioinformatics*,
23(21):2947-8 (2007).

Menachery et al., Attenuation and restoration of severe acute
respiratory syndrome coronavirus mutant lacking 2'-o-
methyltransferase activity, *J. Virol.*, 88(8):4251-64 (2014).

Tatusova et al., BLAST 2 Sequences, a new tool for comparing
protein and nucleotide sequences, *FEMS Microbiol. Lett.*, 174(2):247-
50 (1999).

(Continued)

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(57) **ABSTRACT**

The present invention provides a live, attenuated coronavi-
rus comprising a variant replicase gene encoding polypro-
teins comprising a mutation in one or more of non-structural
protein(s) (nsp)-10, nsp-14, nsp-15 or nsp-16. The corona-
virus may be used as a vaccine for treating and/or preventing
a disease, such as infectious bronchitis, in a subject.

25 Claims, 15 Drawing Sheets

Specification includes a Sequence Listing.