

(12) **United States Patent**  
**Bickerton et al.**(10) **Patent No.:** **US 10,130,701 B2**  
(45) **Date of Patent:** **Nov. 20, 2018**(54) **CORONAVIRUS**(71) **Applicant:** **THE PIRBRIGHT INSTITUTE,**  
Pirbright, Woking (GB)(72) **Inventors:** **Erica Bickerton,** Woking (GB); **Sarah Keep,** Woking (GB); **Paul Britton,** Woking (GB)(73) **Assignee:** **THE PIRBRIGHT INSTITUTE,**  
Woking, Pirbright (GB)(\*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.(21) **Appl. No.:** **15/328,179**(22) **PCT Filed:** **Jul. 23, 2015**(86) **PCT No.:** **PCT/GB2015/052124**§ 371 (c)(1),  
(2) **Date:** **Jan. 23, 2017**(87) **PCT Pub. No.:** **WO2016/012793****PCT Pub. Date:** **Jan. 28, 2016**(65) **Prior Publication Data**

US 2017/0216427 A1 Aug. 3, 2017

(30) **Foreign Application Priority Data**

Jul. 23, 2014 (GB) ..... 1413020.7

(51) **Int. Cl.****A61K 39/215** (2006.01)**C12N 7/00** (2006.01)**C12N 9/12** (2006.01)**A61K 39/00** (2006.01)(52) **U.S. Cl.**CPC ..... **A61K 39/215** (2013.01); **C12N 7/00**(2013.01); **C12N 9/127** (2013.01); **C12Y****207/07048** (2013.01); **A61K 2039/5254**(2013.01); **A61K 2039/54** (2013.01); **C12N****2770/20021** (2013.01); **C12N 2770/20022**(2013.01); **C12N 2770/20034** (2013.01); **C12N****2770/20051** (2013.01); **C12N 2770/20062**

(2013.01)

(58) **Field of Classification Search**CPC ..... **A61K 39/215**

See application file for complete search history.

(56) **References Cited**

## U.S. PATENT DOCUMENTS

7,452,542 B2\* 11/2008 Denison ..... C07K 14/00  
424/221.1

## FOREIGN PATENT DOCUMENTS

## 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 responsible 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.

Armeto 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).Armeto et al., The replicase gene of avian coronavirus infectious bronchitis virus is a determinant of pathogenicity. *PLoS One*, 4(10):e7384 (2009).Armeto 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*, 6th 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 expressing 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 Application 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).Tatunova et al., BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol. Lett.*, 174(2):247-50 (1999).

(Continued)

**Primary Examiner** — Bao Q Li(74) **Attorney, Agent, or Firm** — Marshall, Gerstein & Borun LLP(57) **ABSTRACT**

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