(12) BẢN MÔ TẢ SÁNG CHẾ THUỘC BẰNG ĐỘC QUYỀN SÁNG CHẾ

(19) **Cộng hòa xã hội chủ nghĩa Việt Nam (VN)**
CỤC SỞ HỮU TRÍ TUỆ

(11) **1-0039292**

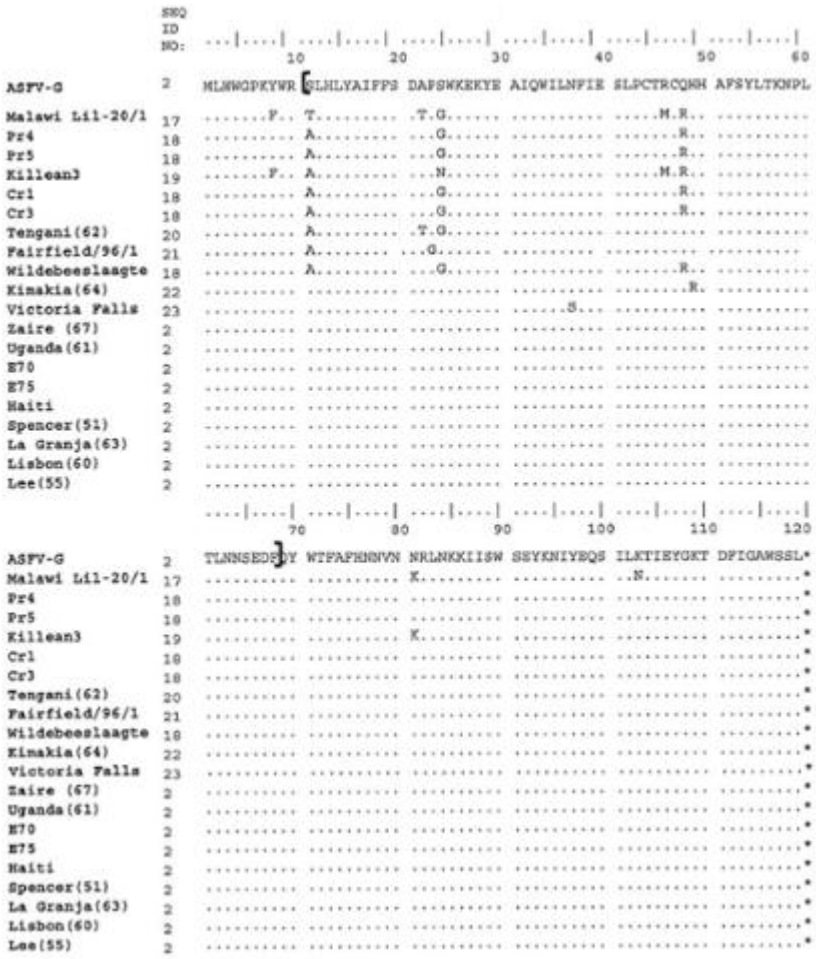
(51)[7] **C12N 15/40**; A61P 31/12; G01N 33/53; C12N 7/01; A61K 39/187

(13) **B**

(21) 1-2019-00651
(22) 26/06/2017
(86) PCT/US2017/039277 26/06/2017
(87) WO 2018/005358 04/01/2018
(30) 15/200,407 01/07/2016 US
(45) 25/04/2024 433
(43) 25/07/2019 376A
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(74) Công ty TNHH Tầm nhìn và Liên danh (VISION & ASSOCIATES CO.LTD.)

(54) VIRUT ĐỘT BIẾN ASFV-Δ9GL/ΔUK CỦA ASFV-G TÁI TỔ HỢP VÀ CHẾ PHẨM VACXIN CHỨA VIRUT NÀY

(57) Sáng chế đề cập đến virut đột biến ASFV-Δ9GL/ΔUK của ASFV-G tái tổ hợp và chế phẩm vacxin chứa virut này. Virut gây bệnh sốt ở lợn châu Phi (ASFV) do virut truyền nhiễm, thường gây chết của lợn nhà. Việc kiểm soát bệnh sốt ở lợn châu Phi (ASF) bị cản trở do không có sẵn vacxin. Vacxin thử nghiệm có nguồn gốc từ ASFV sống nhược độc có trong tự nhiên, được làm thích hợp với môi trường nuôi cấy tế bào, hoặc cải biến về mặt di truyền; tuy nhiên, các vacxin này chỉ thành công khi bảo vệ kháng lại virut đồng chủng. Virut đột biến theo sáng chế có nguồn gốc từ thể phân lập ASFV Georgia 2007 (ASFV-G) có độc lực cao bằng cách làm mất đoạn gen 9GL (B119L) và UK (DP96R) liên quan độc lực đặc hiệu.


SEQ ID NO:
10 20 30 40 50 60
ASFV-G 2 MLHWGPKYWR GLHLYAIFPS DAPSWKEKYE AIQWILNFIE SLPCTRCQHH AFSYLTKNPL
Malawi Lil-20/1 17 .....F.. T..... .T.G..... ....M.R.. .....
Pr4 18 ..... A..... ...G..... ....R.. .....
Pr5 18 ..... A..... ...G..... ....R.. .....
Killean3 19 .....F.. A..... ...N..... ....M.R.. .....
Cr1 18 ..... A..... ...G..... ....R.. .....
Cr3 18 ..... A..... ...G..... ....R.. .....
Tengani(62) 20 ..... A..... .T.G..... ....
Fairfield/96/1 21 ..... A..... ...G..... ....
Wildebeeslaagte 18 ..... A..... ...G..... ....R.. .....
Kimakia(64) 22 ....R. .....
Victoria Falls 23 ....S... ....
Zaire (67) 2 ....
Uganda(61) 2 ....
E70 2 ....
E75 2 ....
Haiti 2 ....
Spencer(51) 2 ....
La Granja(63) 2 ....
Lisbon(60) 2 ....
Lee(55) 2 ....
70 80 90 100 110 120
ASFV-G 2 TLNNSEDSY WTPAPHNNVN NRLNKKIISW SEYKNIYEQS ILKTIEYGKT DFIGAWSSL*
Malawi Lil-20/1 17 .... K..... ....N..... .....*
Pr4 18 ....*
Pr5 18 ....*
Killean3 19 .... K..... ....*
Cr1 18 ....*
Cr3 18 ....*
Tengani(62) 20 ....*
Fairfield/96/1 21 ....*
Wildebeeslaagte 18 ....*
Kimakia(64) 22 ....*
Victoria Falls 23 ....*
Zaire (67) 2 ....*
Uganda(61) 2 ....*
E70 2 ....*
E75 2 ....*
Haiti 2 ....*
Spencer(51) 2 ....*
La Granja(63) 2 ....*
Lisbon(60) 2 ....*
Lee(55) 2 ....*

Lĩnh vực kỹ thuật được đề cập

Sáng chế đề cập đến cấu trúc của vacxin chủng ứng viên sống nhược độc của virut gây bệnh sốt ở lợn châu Phi (ASFV) tái tổ hợp cho thể phân lập Georgia 2007 độc lực cao ASFV-G. Vacxin này chứa virut ASFV-G Δ9GLΔUK cải biến, ASFV-G tái tổ hợp cải biến bằng cách gây mất đoạn một phần lớn gen *9GL (B119L)* và gen *UK (DP96R)*.

Tình trạng kỹ thuật của sáng chế

Bệnh sốt ở lợn châu Phi (ASF) là bệnh do virut truyền nhiễm của lợn. Tác nhân gây bệnh, virut ASF (ASFV), là virut có vỏ bọc lớn chứa bộ gen ADN sợi kép gồm khoảng 190 cặp kilobazơ. ASFV có chung nhiều đặc điểm về cấu trúc bộ gen và chiến lược sao chép với các virut ADN sợi kép lớn khác, bao gồm *Poxviridae, Iridoviridae* và *Phycodnaviridae* (Costard *et al.* 2009. *Phil. Trans. Royal Soc. B* 364:2683-2696). Bệnh nhiễm ASFV ở lợn nhà thường gây chết và đặc trưng bởi sốt, xuất huyết, mất điều hòa và suy nhược nghiêm trọng. Tuy nhiên, tiến trình của bệnh khác nhau, trong khoảng từ gây chết cao đến cận lâm sàng, tùy thuộc vào đặc điểm của vật chủ và chủng virut cụ thể (Tulman *et al.* 2009. *Curr. Top. Microbiol. Immunol.* 328:43-87).

Hiện tại, bệnh này là bệnh địa phương ở hơn hai mươi nước châu Phi hạ Sahara. Ở châu Âu, ASF vẫn là bệnh địa phương trên đảo Sardinia (Ý) và các vụ dịch mới đã được phân bố ở vùng Kapkaz từ 2007, gây ảnh hưởng đến Georgia, Armenia, Azerbaijan và Nga. Các vụ dịch đã cách ly mới được báo cáo ở Ukraine, Belarus, Lithuania, Latvia và Ba Lan, gây ra nguy cơ phát tán thêm vào các nước lân cận. Virut gây bệnh, ASFV Georgia 2007/1, là thể phân lập độc lực cao thuộc kiểu gen II (Chapman *et al.* 2011. *Emerging Infect. Dis.* 17:599-605).

Hiện tại, không có vacxin có sẵn cho ASF và các vụ dịch được kiểm soát bằng cách kiểm dịch và tiêu hủy động vật. Các nỗ lực để tiêm chủng cho động vật bằng cách

sử dụng dịch chiết tế bào bị nhiễm, dịch nổi của bạch cầu trong máu ngoại vi ở lợn bị nhiễm, virion tinh chế và bị bất hoạt, đại thực bào cố định glutaraldehyt bị nhiễm, hoặc đại thực bào phế nang bị nhiễm được xử lý bằng chất tẩy thất bại trong việc gây ra miễn dịch bảo vệ (Coggins, L. 1974. *Prog. Med. Virol.* 18:48-63; Forman *et al.* 1982. *Arch. Virol.* 74:91-100; Kihm *et al.* 1987. In: *Arfican Swine Fever*, Becker, Y. (ed), Martinus Nijhoff, Boston, pp 127-144; Mebus, C. A. 1988. *Adv. Virut Res.* 35:251–269). Tính miễn dịch bảo vệ đồng chủng phát triển ở lợn sống sót sau khi nhiễm virut. Lợn sống sót sau khi nhiễm trùng cấp tính có biến thể độc lực trung bình hoặc nhược độc của ASFV phát triển tính kháng lâu dài với việc gây nhiễm bằng virut đồng chủng, nhưng hiếm khi với virut dị chủng (Hamdy and Dardiri. 1984. *Am. J. Vet. Res.* 45:711-714; Ruiz-Gonzalvo *et al.* 1981. In: *FAO/CEC Expert Consultation in ASF Research*, Wilkinson, P. J. (ed), Rome, pp 206–216). Lợn được gây miễn dịch bằng virut ASF sống nhược độc chứa các đột biến mất đoạn được thao tác di truyền của gen liên quan đến độc lực của ASFV đặc hiệu được bảo vệ khi bị gây nhiễm bằng virut cha mẹ đồng chủng. Cụ thể là, đột biến mất đoạn đơn lẻ của gen *UK* (*DP69R*), *23-NL* (*DP71L*), *TK* (*A240L*) hoặc *9GL* (*B119L*) từ bộ gen của virut ASF gây bệnh (Malawi Lil-20/1, Pretoriuskop/96/4, E70 and Georgia 2007) làm giảm đáng kể độc lực của virut ở lợn và động vật được gây miễn dịch bằng các virut nhược độc này được bảo vệ kháng lại việc gây nhiễm bằng virut đồng chủng (Moore *et al.* 1998. *J. Virol.* 72:10310-10315; Lewis *et al.* 2000. *J. Virol.* 74:1275-1285; Zsak *et al.* 1996. *J. Virol.* 70:8865-8871; Zsak *et al.* 1998. *J. Virol.* 72:1028-1035). Các quan sát này là bằng chứng thử nghiệm mô tả việc phát triển hợp lý virut sống nhược độc có hiệu quả kháng lại ASFV.

Cụ thể là, đột biến mất đoạn của *9GL* (*B119L*) ở thể phân lập ASFV độc lực cao Malawi Lil-20/1, Pretoriuskop/96/4, và Georgia2007 (Lewis *et al.*, *supra*; Neilan *et al.* 2004. *Virol.* 319:337-342; O'Donnell *et al.* 2015. *J. Virol.* 89: 8556-8566) gây ra sự nhược độc hoàn toàn các virut này ở lợn. Việc sử dụng các đột biến Malawi Lil-20/1Δ9GL hoặc Pretoriuskop/96/4 Δ9GL hoặc E70 ΔUK cho lợn bằng cách tiêm IM ở liều virut tương đối cao đã không gây ra các dấu hiệu lâm sàng, với tất cả các động vật sống sót sau khi nhiễm. Ngoài ra, việc tiêm chủng IM cho lợn bằng các virut này đã gây ra tác dụng bảo vệ kháng lại việc gây nhiễm bằng virut cha mẹ có độc lực (Zsak *et al.* 1998, *supra*; Lewis *et al.*, *supra*; O'Donnell *et al.*, *supra*). Các quan sát này là bằng

chứng thử nghiệm duy nhất mô tả việc phát triển hợp lý virut sống nhược độc có hiệu quả kháng lại ASFV.

Vì hiện nay vẫn chưa có vacxin ASFV nên việc phát triển vacxin thử nghiệm bất kỳ mà có thể gây ra tác dụng bảo vệ thuộc kiểu bất kỳ kháng lại bệnh gây chết là mối quan tâm nhất.

Bản chất kỹ thuật của sáng chế

Các tác giả sáng chế đã phát triển virut ASFV-G Δ9GL/ΔUK đột biến tái tổ hợp mới, dạng biến đổi của ASFV-G (thể phân lập Georgia 2007 của virut gây bệnh sốt ở lợn châu Phi).

Theo phát hiện này, mục đích của sáng chế là đề xuất virut ASFV-G Δ9GL/ΔUK đột biến mới, thu được từ việc gây đột biến mất đoạn một phần lớn của cả gen *9GL (B119L)* và gen *UK (DP96R)* của ASFV-G cha mẹ. Trình tự nucleotit của ASFV-G Δ9GL/ΔUK (SEQ ID NO: 3) khác với trình tự nucleotit mã hóa ASFV-G kiểu dại (SEQ ID NO: 1). Protein được mã hóa bởi ASFV-G 9GL (kiểu dại) có 119 axit amin (SEQ ID NO: 2) và protein được mã hóa bởi ASFV-G UK (kiểu dại) có 95 axit amin (SEQ ID NO:25) khác với protein 9GL và UK đột biến được mã hóa bằng trình tự nucleotit đột biến của ASFV-G Δ9GL/ΔUK (SEQ ID NO: 3). Polypeptit 9GL đột biến có 61 axit amin (SEQ ID NO: 4) thu được từ việc gây đột biến mất đoạn axit amin # 11 đến axit amin #68 của polypeptit 9GL kiểu dại (SEQ ID NO: 2), và polypeptit UK đột biến có 10 axit amin (SEQ ID NO: 26) thu được từ việc gây đột biến mất đoạn axit amin #1 đến axit amin #85 của polypeptit UK kiểu dại (SEQ ID NO:25).

Mục đích bổ sung của sáng chế là đề xuất chế phẩm miễn dịch chứa virut ASFV-G Δ9GL/ΔUK sống.

Mục đích bổ sung của sáng chế là đề xuất vacxin ASFV-G Δ9GL/ΔUK sống nhược độc được thao tác di truyền hợp lý có hiệu quả bảo vệ động vật khỏi bệnh ASF lâm sàng khi bị gây nhiễm bởi ASFV-G gây bệnh.

Mục đích khác của sáng chế là đề xuất vacxin đánh dấu di truyền mà có thể có khả năng phân biệt giữa động vật được tiêm chủng và động vật bị nhiễm ASFV-G.

Mục đích khác của sáng chế là đề xuất phương pháp bảo vệ động vật kháng lại ASFV-G bằng cách sử dụng lượng có hiệu quả của vacxin ASFV-G Δ9GL/ΔUK sống nhược độc được thao tác di truyền hợp lý.

Mục đích bổ sung của sáng chế là đề xuất phương pháp phân biệt động vật bị nhiễm ASFV-G với động vật được tiêm chủng vacxin ASFV-G Δ9GL/ΔUK sống nhược độc được thao tác di truyền hợp lý nêu trên, bao gồm chiến lược DIVA di truyền để phân biệt động vật đã tiêm chủng với động vật bị nhiễm virut kiểu dại.

Các mục đích và ưu điểm khác của sáng chế này sẽ được hiểu rõ một cách dễ dàng từ phần mô tả.

Mô tả vắn tắt hình vẽ

Fig. 1A và Fig. 1B thể hiện sự sắp xếp thẳng hàng trình tự của polypeptit được mã hóa bởi gen ASFV-G *9GL* (*B119L*) (Fig. 1A), và polypeptit được mã hóa bởi gen ASFV-G UK (*DP96R*) (Fig. 1B). Các thể phân lập thuộc các nguồn gốc về thời gian và địa lý khác nhau, bao gồm các thể phân lập thu được từ nguồn ve và lợn, được so sánh. Đột biến mất đoạn một phần được đưa vào ASFV-G mà tạo ra virut ASFV-G Δ9GL/ΔUK được thể hiện giữa các dấu ngoặc.

Mô tả chi tiết sáng chế

Các tác giả sáng chế đã phát triển virut nhược độc mà có thể được sử dụng làm ứng viên vacxin bằng phương pháp gắn đích cả gen ASFV-G *9GL* (*B119L*) và ASFV-G *UK* (*DP96R*) cho các biến đổi di truyền. Trong bản mô tả này các tác giả sáng chế báo cáo về việc tạo ra virut Δ9GL/ΔUK tái tổ hợp của thể phân lập ASFV Georgia 2007 (ASFV-G) độc lực cao. ASFV-G Δ9GL/ΔUK được tiêm chủng trong cơ (IM) cho lợn ở liều tương đối cao (10^4 hoặc 10^6 HAD_{50}) không gây bệnh. Động vật bị nhiễm 10^4 hoặc 10^6 HAD_{50} được bảo vệ kháng lại sự biểu hiện của bệnh lâm sàng khi bị gây nhiễm ở 28 ngày sau khi nhiễm chủng cha mẹ có độc lực Georgia 2007.

Mặc dù việc gây đột biến mất đoạn độc lập của bốn gen khác nhau từ ASFV đã được chỉ ra là gây nhược độc virut có độc lực và mặc dù các đột biến mất đoạn độc lập của gen NL (DP71L) (Zsak *et al.* 1996, *supra*) hoặc UK (DP69R) (Zsak *et al.* 1998, *supra*) từ ASFV E75, đột biến mất đoạn của gen TK (A240L) (Moore *et al.*, *supra*) từ ASFV được làm thích hợp với tế bào Vero, Malawi Lil-20/1 và Haiti, và đột biến mất đoạn của gen 9GL (B119L) cũng từ thể phân lập Malawi Lil-20/1 (Lewis *et al.*, *supra*) và Pretoriuskop/96/4 (Neilan *et al.*, *supra*) làm cho các virut đột biến mất đoạn tái tổ hợp có độc lực giảm đáng kể ở lợn, trong tất cả các trường hợp này, động vật được chủng bằng mỗi virut đã được cải biến về mặt di truyền sống sót qua bệnh nhiễm trùng và được bảo vệ kháng lại ASFV khi bị gây nhiễm bởi virut cha mẹ có độc lực tương ứng, *tức là*, gây nhiễm đồng chủng (Lewis *et al., supra*; Moore *et al., supra*; Neilan *et al., supra*; Zsak *et al.* 1996, *supra*; Zsak *et al.* 1998, *supra*). Các phát hiện đó gợi ý rằng việc phát triển virut tái tổ hợp ASFV nhược độc bằng cách thao tác di truyền gen đích là phương pháp có hiệu quả để phát triển vacxin.

Tuy nhiên, mức độ hiệu quả của chúng ở các thể phân lập ASFV khác không thể đoán trước. Ví dụ, sản phẩm gen NL (DP71L) tồn tại ở hai dạng khác nhau, dạng dài (184 axit amin như trong 23-NL) hoặc ngắn (70 đến 72 axit amin) tùy thuộc vào thể phân lập ASFV (Zsak *et al.* 1996, *supra*). Mặc dù đột biến mất đoạn của gen này trong thể phân lập ASFV E70 (dạng ngắn) tạo ra virut nhược độc, đột biến mất đoạn của gen NL (DP71L) từ ASFV Malawi Lil-20/1 (dạng dài) hoặc Pretoriuskop/96/4 (dạng ngắn) không gây nhược độc virut này (Afonso *et al.* 1998. *J. Gen. Virol.* 79 (Pt. 10):2543-2547). Đột biến mất đoạn của gen TK (A240L), gen được bảo tồn cao trong số tất cả các thể phân lập ASFV tham gia vào tổng hợp ADN, đã được đưa vào virut Malawi Lil-20/1 và Haiti H811 được làm thích hợp với tế bào Vero gây bệnh. Virut đột biến Malawi Lil-20/1 có độc lực thấp hơn trên in vivo virut hồi biến (virut giống kiểu dại), nhưng nó không nhược độc hoàn toàn (Moore *et al.*, *supra*). Gen UK (DP69R) nằm ở vùng biến đổi bên phải của một số thể phân lập ASFV. Đột biến mất đoạn của gen này từ thể phân lập ASFV E70 làm cho virut thể hiện độc lực giảm (Zsak *et al.* 1998, *supra*). Mặc dù gen UK (DP69R) được bảo tồn, nó không có mặt trong mọi thể phân lập ASFV (*ví dụ* Malawi Lil-20/1), do đó hạn chế việc sử dụng nó làm gen đích ứng viên để sản xuất virut nhược độc.

Gen 9GL (*B119L*) được bảo tồn cao trong số ASFV được phân lập và giải trình tự cho đến nay, bao gồm các gen từ cả nguồn ve và lợn. Hiện tượng đột biến mất đoạn của gen từ Malawi Lil-20/1 (Lewis *et al.*, *supra*) và Pretoriuskop/96/4 (Neilan *et al.*, *supra*) có độc lực được giảm độc lực hữu hiệu ở lợn và gây ra tác dụng bảo vệ khiến cho 9GL (B119L) trở thành gen đích ứng viên mạnh để biến đổi và sản xuất virut nhược độc mà có thể gây ra tác dụng bảo vệ có hiệu quả kháng lại ASFV. Thật vậy, tác giả sáng chế đã phát hiện ra là đột biến mất đoạn của 9GL (B119L) từ thể phân lập ASFV-G không có tác dụng tương tự về tác dụng gây nhược độc và bảo vệ như được báo cáo đối với Malawi Lil-20/1 và Pretoriuskop/96/4. Chỉ khi ASFV-G Δ9GL được sử dụng ở liều thấp cho lợn mới có thể quan sát thấy sự giảm độc lực virut (O'Donnell *et al.*, *supra*). Các tác giả sáng chế cũng chỉ ra rằng liều không gây chết của ASFV-G Δ9GL có thể gây ra tác dụng bảo vệ có hiệu quả kháng lại sự biểu hiện bệnh lâm sàng sau khi gây nhiễm bằng virut cha mẹ đồng chủng. Các protein NL được mã hóa bởi E70 (dạng ngắn) và Malawi Lil-20/1 (dạng dài) khác nhau đáng kể và điều đó có thể giải thích sự khác nhau về kiểu hình quan sát được ở lợn được chủng bằng virut đột biến mất đoạn tương ứng. Tuy nhiên, ma trận đồng nhất protein chỉ ra rằng protein 9GL là tương đồng cao trong số các thể phân lập ASFV trong đó ASFV-G, Malawi Lil-20/1 và Pretoriuskop/96/4 có chung độ đồng nhất axit amin hơn 93%, khiến cho không chắc là việc nhược độc ASFV chỉ dựa vào sự phân kỳ protein. Vì các kiểu hình quan sát được rất có thể được tạo ra bởi tác động của nhiều gen (Lewis *et al.*, *supra*; Moore *et al.*, *supra*; Neilan *et al.*, *supra*; Zsak *et al.* 1996, *supra*; Zsak *et al.* 1998, *supra*), bằng chứng tích lũy cho đến nay làm cho khó có thể suy đoán phổ gen thực sự gây ra độc lực ở thể phân lập ASFV Georgia 2007 là gì.

Tóm lại, trong bản mô tả này các tác giả sáng chế chứng minh là đột biến mất đoạn của cả gen 9GL (*B119L*) và gen UK (*DP69R*) tạo thành virut ASFV-G Δ9GL/ΔUK tái tổ hợp nhược độc. Việc sử dụng trong cơ của ASFV-G Δ9GL/ΔUK cho lợn ở liều tương đối cao (10^4 hoặc 10^6 HAD_{50}) không gây bệnh. Động vật bị nhiễm 10^4 hoặc 10^6 HAD_{50} được bảo vệ kháng lại sự có mặt của bệnh lâm sàng khi bị gây nhiễm ở 28 ngày sau khi nhiễm chủng cha mẹ có độc lực Georgia 2007.

Vacxin được xác định trong bản mô tả này là tác nhân sinh học mà có khả năng cung cấp đáp ứng bảo vệ ở động vật đã được phân phối vacxin này và không có khả

năng gây bệnh nặng. Việc sử dụng vacxin này gây ra tính miễn dịch với bệnh; vacxin này gây ra việc sản xuất kháng thể hoặc miễn dịch tế bào kháng lại mầm bệnh gây bệnh. Tính miễn dịch được xác định trong bản mô tả này là tác dụng gây ra mức độ bảo vệ cao hơn đáng kể ở quần thể lợn kháng lại tác dụng gây chết và triệu chứng lâm sàng sau khi tiêm chủng so với nhóm không được tiêm chủng. Cụ thể là, vacxin theo sáng chế bảo vệ phần lớn động vật đã tiêm chủng kháng lại sự xuất hiện các triệu chứng lâm sàng của bệnh và tác dụng gây chết. Vacxin theo sáng chế trong bản mô tả này là vacxin virut đột biến được thao tác di truyền về mặt di truyền. Vacxin đánh dấu di truyền được xác định là vacxin mà, cùng với xét nghiệm chuẩn đoán, cho phép phân biệt về mặt di truyền động vật đã tiêm chủng với động vật bị nhiễm. Đột biến mất đoạn có thể được sử dụng để phân biệt động vật bị nhiễm với đã tiêm chủng. Đột biến được hiểu là sự thay đổi về thông tin di truyền của gen *9GL* (*B119L*) và *UK* (*DP96R*) "kiểu dại" hoặc không cải biến của chủng ASFV-G cha mẹ mà có thể biểu hiện protein 9GL và UK tự nhiên. Do đó, các polypeptit 9GL và UK được biểu hiện bởi virut đột biến ASFV-G Δ9GL/ΔUK được thay đổi: protein 9GL từ ASFV-G Δ9GL/ΔUK có ít axit amin hơn so với cả 9GL kiểu dại và UK kiểu dại, vì axit amin #11 đến #68 bị mất đoạn trong polypeptit 9GL của ASFV-G Δ9GL và axit amin #1 đến #85 bị mất đoạn ở polypeptit UK. Đột biến ASFV-G tái tổ hợp ASFV-G Δ9GL/ΔUK chứa nucleotit mã hóa đột biến ở polypeptit ASFV-G 9GL và UK. Đột biến này bao gồm đột biến mất đoạn 58 axit amin của protein 9GL và đột biến mất đoạn 85 axit amin của protein UK. ASFV-G Δ9GL/ΔUK đột biến ASFV-G tái tổ hợp là vacxin ASFV-G sống nhược độc khi được sử dụng ở liều chủng IM là 10^4 HAD_{50} đến 10^6 HAD_{50}.

Vacxin kháng lại ASFV-G được đề xuất chứa đột biến ASFV-G Δ9GL/ΔUK như được xác định ở trên ở dạng sống, và chất mang dược dụng hoặc chất pha loãng. Vacxin theo sáng chế chứa virut sống có thể được bào chế và được bán ở dạng huyền phù đặc hoặc ở dạng đông khô và chứa thêm chất mang dược dụng hoặc chất pha loãng thường được sử dụng cho chế phẩm này. Chất mang bao gồm chất ổn định, chất bảo quản và chất đệm. Chất ổn định thích hợp là, ví dụ SPGA (sucroza, phosphat, glutamat và albumin của người), carbohydrat (như sorbitol, mannitol, tinh bột, sucroza, dextran, glutamat hoặc glucoza), protein (như huyết thanh sữa khô, albumin hoặc casein) hoặc sản phẩm phân hủy của chúng. Chất đệm thích hợp ví dụ là

phosphat kim loại kiềm. Chất bảo quản thích hợp là thimerosal, merthiolat và gentamicin. Chất pha loãng bao gồm nước, đệm chứa nước (như nước muối đệm), rượu và polyol (như glyxerol).

Nếu muốn, vacxin sống theo sáng chế có thể chứa chất hỗ trợ. Ví dụ về hợp chất và chế phẩm thích hợp có hoạt tính hỗ trợ đã được biết rõ trong lĩnh vực kỹ thuật này. Ngoài ra, trình tự axit nucleic mã hóa polypeptit cho ứng dụng làm thuốc hoặc chẩn đoán, cụ thể là chất điều hòa miễn dịch như lymphokin, interferon hoặc xytokin, có thể được kết hợp vào vacxin.

Vacxin theo sáng chế có thể được bào chế bằng phương pháp thông thường như các phương pháp thường được sử dụng cho vacxin ASFV sống nhược độc có bán trên thị trường. Tóm lại, chất nền nhạy cảm được chủng đột biến ASFV-G Δ9GL/ΔUK và được nhân giống cho đến khi virut đã nhân rộng thành độ chuẩn mong muốn sau khi nguyên liệu chứa ASFV-G Δ9GL/ΔUK được thu hoạch. Sau đó, nguyên liệu thu hoạch được bào chế thành chế phẩm dược có đặc tính gây miễn dịch.

Mọi chất mà có thể hỗ trợ sao chép virut ASFV-G Δ9GL/ΔUK có thể được sử dụng theo sáng chế, bao gồm môi trường nuôi cấy sơ cấp của đại thực bào máu ngoại vi ở lợn.

Vacxin có thể được sử dụng bằng cách chủng hoặc tiêm trong cơ, dưới da hoặc trong mũi với lượng hữu hiệu để bảo vệ động vật kháng lại việc gây nhiễm bằng chủng ASFV-G có độc lực. Lượng này có thể thay đổi theo động vật được chủng, xem xét kích thước và khối lượng của động vật. Vacxin theo sáng chế chứa liều hữu hiệu của đột biến ASFV-G Δ9GL/ΔUK là thành phần hoạt chất, *tức là* lượng nguyên liệu ASFV-G Δ9GL/ΔUK gây miễn dịch mà sẽ gây ra miễn dịch ở động vật, lợn đã tiêm chủng kháng lại việc gây nhiễm bằng ASFV-G có độc lực. Tính miễn dịch được xác định trong bản mô tả này là sự cảm ứng tác dụng bảo vệ ở mức độ cao hơn đáng kể ở quần thể lợn kháng lại tác dụng gây chết và triệu chứng lâm sàng sau khi tiêm chủng so với nhóm không được tiêm chủng. Cụ thể là, vacxin theo sáng chế phòng chống việc xảy ra triệu chứng lâm sàng của bệnh và chết ở phần lớn động vật đã tiêm chủng. Thông thường, vacxin sống có thể được dùng ở liều 10^4 HAD_{50} đến 10^6 HAD_{50}. Lượng

hữu hiệu có thể được xác định bằng thử nghiệm khi cần bởi người có hiểu biết trung bình trong lĩnh vực kỹ thuật này theo chỉ dẫn đề xuất, ví dụ, bằng ví dụ 6.

Ngoài đột biến ASFV-G Δ9GL/ΔUK, sáng chế cũng có thể bao gồm vacxin kết hợp chứa chủng vacxin có khả năng cảm ứng tác dụng bảo vệ kháng lại mầm bệnh ở lợn khác.

Vacxin ASFV-G Δ9GL/ΔUK được mô tả ở trên, cùng với phương pháp chẩn đoán, có khả năng phân biệt giữa động vật mà đã tiêm chủng bằng vacxin này và động vật mà bị nhiễm chủng ASFV-G có trong tự nhiên hoặc được tiêm chủng bằng vacxin ASFV-G thông thường.

Sáng chế còn đề xuất công cụ cực kỳ hữu ích để theo dõi các phương pháp kiểm soát ASFV-G mà có thể dẫn đến sự tiêu diệt tận gốc ASFV-G nếu được sử dụng trong các chương trình dập tắt ở quy mô lớn. Công cụ này liên quan đến phương pháp xác định bệnh nhiễm ASFV-G ở lợn bao gồm bước kiểm tra mẫu động vật về sự có mặt của nucleotit mã hóa protein ASFV-G 9GL và UK kiểu dại so với polynucleotit mã hóa polypeptit ASFV-G Δ9GL và ΔUK ngắn hơn do các đột biến mất đoạn ở gen *9GL (B119L)* và gen *UK (DP96R)* của ASFV-G Δ9GL/ΔUK. Mẫu động vật được sử dụng ở phương pháp này có thể là mẫu bất kỳ trong đó sự khác biệt di truyền giữa ASFV-G và ASFV-G Δ9GL/ΔUK cho phép phân biệt sự nhiễm tự nhiên với việc dùng vacxin có thể được phát hiện bởi DIVA di truyền.

Ví dụ thực hiện sáng chế

Nhìn chung sáng chế sẽ được hiểu rõ hơn bằng cách tham khảo một số ví dụ cụ thể nhất định, trong bản mô tả này chỉ để minh họa thêm sáng chế và không nhằm giới hạn phạm vi của sáng chế như được xác định bởi các yêu cầu bảo hộ.

Ví dụ 1

Môi trường nuôi cấy tế bào và virut

Môi trường nuôi cấy tế bào đại thực bào ở lợn sơ cấp được tạo ra từ máu lợn được khử fibrin như được mô tả trước đó bởi Zsak *et al.* (1996, *supra*). Tóm lại, máu

lợn được xử lý heparin được ủ ở 37°C trong 1 giờ để cho phép lắng phần hồng cầu. Bạch cầu đơn nhân được phân tách bằng tuyển nổi trên gradient mật độ Ficoll-Paque (Pharmacia, Piscataway, N.J.) (trọng lượng riêng, 1,079). Phần tế bào bạch cầu đơn nhân to/đại thực bào được nuôi chủng trong bình nuôi chủng mô Primaria nhựa (Falcon; Becton Dickinson Labware, Franklin Lakes, N.J.) chứa môi trường đại thực bào, bao gồm môi trường RPMI 1640 (Life Technologies, Grand Island, NY) với 30% chất nổi bề mặt L929 và 20% huyết thanh thai bò (HI-FBS, Thermo Scientific, Waltham, MA) trong 48 giờ ở 37°C trong 5% CO_2. Tế bào bám dính được tách ra khỏi nhựa bằng cách sử dụng 10 mM EDTA trong nước muối đệm phosphat (PBS) và sau đó được gieo lại vào các đĩa Primaria T25, 6 hoặc 96 giếng với mật độ $5x10^6$ tế bào cho mỗi ml để sử dụng trong thử nghiệm 24 giờ sau.

Việc chuẩn độ virut được thực hiện trên môi trường nuôi cấy tế bào đại thực bào ở lợn sơ cấp trong tấm 96 giếng. Việc pha loãng virut và nuôi cấy được thực hiện bằng cách sử dụng môi trường đại thực bào. Sự có mặt của virut được đánh giá bằng phương pháp hấp phụ hồng cầu lên tế bào (HA) và hiệu giá virut được tính bằng phương pháp Reed and Muench (1938. *Amer. J. Hygiene* 27:493-497).

ASFV Georgia (ASFV-G) là thể phân lập ở hiện trường được cung cấp bởi Dr. Nino Vepkhvadze, từ Laboratory of the Ministry of Agriculture (LMA) in Tbilisi, Republic of Georgia.

Ví dụ 2

Cấu trúc của ASFV-G Δ9GL/ΔUK tái tổ hợp

ASFV tái tổ hợp được tạo thành bằng cách tái tổ hợp đồng chủng liên tiếp giữa bộ gen ASFV cha mẹ và vectơ chuyển tái tổ hợp ở quy trình nhiễm và chuyển nhiễm sử dụng môi trường nuôi cấy tế bào đại thực bào ở lợn (Neilan *et al.*, *supra*; Zsak *et al.* 1996, *supra*). Đầu tiên, vectơ chuyển tái tổ hợp (p72GUSΔ9GL) chứa vùng bộ gen sườn bao gồm phần 9GL ánh xạ sang trái (1,2 kbp) và phải (1,15 kbp) của gen và catxet gen báo cáo chứa gen β-glucuronidaza (GUS) với đoạn khởi đầu gen muộn ASFV p72, p72GUS được sử dụng. Cấu trúc này tạo ra đột biến mất đoạn 173 nucleotit trong 9GL ORF (gốc axit amin từ 11 đến 68) (xem Fig. 1). Vectơ chuyển tái

tổ hợp p72GUSΔ9GL thu được bằng cách tổng hợp ADN (GenScript, Piscataway, NJ, USA). Môi trường nuôi cấy tế bào đại thực bào bị nhiễm ASFV-G và được thay thế bằng p72GUSΔ9GL. Virut tái tổ hợp thể hiện vết tan sơ cấp độc lập được tinh chế để đồng nhất bởi các vòng tinh chế xét nghiệm vết tan liên tiếp. Virut tái tổ hợp thu được sau 11 lần tinh chế vết tan liên tiếp trên đơn lớp của môi trường nuôi cấy tế bào đại thực bào ở lợn sơ cấp. Virut tái tổ hợp trung gian đã tạo, ASFV-G Δ9GL, sau đó được sử dụng dưới dạng virut cha mẹ trong quy trình truyền nhiễm/chuyển nhiễm sử dụng vectơ chuyển tái tổ hợp mà tạo ra đột biến mất đoạn của gen UK từ bộ gen virut. Vectơ chuyển tái tổ hợp (p72mCherryΔUK) chứa vùng bộ gen sườn của UK ánh xạ sang trái (1,156 kbp) và phải (1,190 kbp) của gen và catxet gen báo cáo chứa gen mCherry với đoạn khởi đầu gen muộn ASFV p72, p72mCherry được sử dụng. Vectơ chuyển tái tổ hợp p72mCheryΔUK thu được bằng cách tổng hợp ADN (GenScript, Piscataway, NJ, USA). Cấu trúc này tạo ra đột biến mất đoạn 255 nucleotit trong UK ORF (gốc axit amin 1 đến 85) (xem Fig. 1). Lần tái tổ hợp thứ hai đã thay thế gen UK bằng catxet chứa gen huỳnh quang mCherry dưới đoạn khởi đầu ASFV p72. Virut tái tổ hợp được lựa chọn sau 10 vòng tinh chế pha loãng giới hạn dựa vào hoạt tính huỳnh quang. Quần thể virut thu được từ vòng tinh chế cuối cùng được khuếch đại trong môi trường nuôi cấy tế bào đại thực bào ở lợn sơ cấp để thu được nguyên liệu virut.

Ví dụ 3

Phân tích trình tự bộ gen đầy đủ: ASFV-G Δ9GL/ΔUK tương ứng với ASFV-G cha mẹ

Để đánh giá độ chính xác của việc biến đổi di truyền và tính toàn vẹn của bộ gen của virut tái tổ hợp, trình tự bộ gen đầy đủ của ASFV-G Δ9GL/ΔUK và ASFV-G cha mẹ thu được sử dụng kỹ thuật giải trình tự thế hệ mới (NGS) và được so sánh (bảng 1). Đầu tiên, sự so sánh bộ gen độ dài đầy đủ giữa ASFV-G cha mẹ và ASFV Georgia 2007/1 (Chapman *et al.*, *supra*) được thực hiện. ASFV ADN thu được từ tế bào chất của tế bào bị nhiễm sử dụng phương pháp Trizol (Life Technologies, Grand Island, NY, USA). Hàm lượng ADN được xác định sử dụng kit xét nghiệm Qubit® dsDNA HS (Life Technologies) và đọc trên Qubit® 2 Flourometer (Life Technologies). Một microgam virut ADN được phân mảnh bằng enzym để thu được

các đoạn cuối cùn trong khoảng độ dài 200-300 bp sử dụng kit chất phản ứng Ion Shear™ Plus (Life Technologies) và được ủ tại 37°C trong máy luân nhiệt ADN Peltier Engine Tetrad 2. Sau khi cắt, lưu trữ ADN phân mảnh được tải lên chip ADN (Agilent, Santa Clara, CA, USA) và phân tích sử dụng máy phân tích sinh học 2100 (Agilent) để đánh giá việc phân phối kích thước ADN và khoảng kích thước. ADN đã phân mảnh được chuyển sang thể thích nghi tương thích ion và mã vạch lưu trữ, sau đó là sửa chữa nấc để hoàn thiện mối liên kết giữa thể thích nghi và đoạn chèn ADN sử dụng kit lưu trữ mảnh thêm ion (Life Technologies). Lưu trữ buộc thể thích nghi được chọn kích thước cho độ dài tối ưu trên 2% catxet gel agaroza (Sage Science, Beverly, MA, USA) sử dụng dụng cụ Pippin Prep™ (Sage Science). Hàm lượng lưu trữ được chuẩn hóa sử dụng Ion Library Equalizer™ Kit (Life Technologies). Tiếp theo, lưu trữ ADN được khuếch đại vô tính lên Ion Sphere™ Particles (IPS) tạo ISP dương tính với mẫu sử dụng Ion PGM™ Template OneTouch™ 2 200 Kit (Life Technologies) với Ion OneTouch™ 2 Instrument (Life Technologies). Trước khi tiến hành làm giàu, đánh giá chất lượng của các ISP dương tính với mẫu không làm giàu được thực hiện sử dụng kit xét nghiệm Ion Sphere™ Quality Control (Life Technologies) và dụng cụ Qubit® 2 Flourometer. Các ISP dương tính với mẫu sau đó được làm giàu sử dụng Ion PGM™ Template OneTouch™ 2 200 Kit (Life Technologies) và dụng cụ Ion OneTouch™ ES (Life Technologies) để loại bỏ các ISP âm tính với mẫu và làm biến tính ADN trên các ISP dương tính với mẫu. Sử dụng Ion PGM™ 200 Sequencing v2 Kit (Life Technologies), ISP mẫu đã làm giàu được chuẩn bị để giải trình tự và được tải lên Ion 314™ hoặc Ion 316™ Chip v2 (Life Technologies) và chạy trên Ion PGM™ Sequencer (Life Technologies). Trình tự thu được sau đó được cắt sử dụng công cụ Galaxy (lấy từ Internet: usegalaxy.org/) NGS QC và Manipulation. Trình tự được liên kết và phân tích sử dụng phần mềm Sequencher 5.2.2 (Genecodes) và CLC Genomics Workbench (CLCBio).

Sự khác nhau sau đây quan sát được giữa hai virut này (vị trí nucleotit được đề xuất dựa vào ASFV Georgia 2007/1, số truy nhập GenBank FR682468): (i) hai đoạn chèn nucleotit, T ở vị trí 433 và A ở vị trí 441 trong phân đoạn không mã hóa của bộ gen; (ii) hai đột biến mất đoạn nucleotit, T ở vị trí 1602 và T ở vị trí 1603 trong gen MGF 360-1L ORF dẫn đến sự xê dịch khung; (iii) đột biến mất đoạn nucleotit, T ở vị

trí 1620 trong gen MGF 360-1L ORF dẫn đến sự xê dịch khung; (iv) đột biến nucleotit, A đến G ở vị trí 97391 dẫn đến đột biến câm trong ORF B438L; (v) đột biến nucleotit, C đến G ở vị trí 166192 dẫn đến phản ứng thế gốc (Ala cho Pro) ở vị trí gốc 85 trong ORF E199L; và (vi) đoạn chèn nucleotit, T ở vị trí 183303, đoạn không mã hóa của bộ gen (bảng 1). Thứ hai, sự so sánh bộ gen độ dài đầy đủ giữa ASFV Δ9GL/ΔUK và ASFV-G cha mẹ được thực hiện. Tổ hợp trình tự ADN của ASFV Δ9GL/ΔUK và ASFV-G lộ ra đột biến mất đoạn của 173 nucleotit ở gen 9GL tương ứng với biến đổi được đưa vào. Trình tự liên ứng của bộ gen ASFV Δ9GL/ΔUK thể hiện đoạn chèn của 2324 nucleotit ở gen 9GL tương ứng với trình tự catxet p72-βGUS được đưa vào để tạo thành đột biến mất đoạn 173 nucleotit trong gen đích. Ngoài ra, tổ hợp trình tự ADN của ASFV-G Δ9GL/ΔUK và ASFV-G lộ ra đột biến mất đoạn của 255 nucleotit ở gen UK tương ứng với biến đổi được đưa vào. Trình tự liên ứng của bộ gen ASFV-G Δ9GL/ΔUK thể hiện đoạn chèn của 937 nucleotit ở gen UK tương ứng với trình tự catxet p72-mCherry được đưa vào để tạo thành đột biến mất đoạn 255 nucleotit trong gen đích. Ngoài đoạn chèn của catxet, chỉ một khác biệt bổ sung quan sát được giữa bộ gen ASFV-G Δ9GL/ΔUK và ASFV-G, đột biến nucleotit G đến C ở vị trí 36,465 dẫn đến phản ứng thế gốc (Glu đến Gln) ở vị trí gốc 224 trong ORF MGF 505-4R. Tóm lại, virut ASFV-G Δ9GL/ΔUK đã không tích tụ đột biến đáng kể bất kỳ trong quy trình tái tổ hợp đồng chủng và tinh chế vết tan (bảng 1).

Bảng 1. Tóm tắt sự khác biệt giữa trình tự bộ gen độ dài đầy đủ của ASFV-G Δ9GL/ΔUK và ASFV-G cha mẹ so với ASFV Georgia07/1*

		Virut	
NPN*	**Loại biến đổi**	**ASFV-G**	**ASFV-G Δ9GL/ΔUK**
433	đoạn chèn T	+	+
411	đoạn chèn A	+	+
1602	đột biến mất đoạn MGF 360-1L TT FS@	+	+
1620	đoạn chèn MGF 360-1L T FS	+	+

36465	MGF 505-4R G đến C Glu224Gln	-	+
97391	B438L A đến G SM[#]	+	+
166192	E199L C đến G Ala*85*Pro	+	+
183303	đoạn chèn T in a NCR[+]	+	+

* Số vị trí nucleotit (dựa vào trình tự của thể phân lập ASFV Georgia 2007/1 được công bố bởi Chapman *et al.* 2011)

@ Biến đổi nucleotit gây ra sự xê dịch khung ở ORF tương ứng

Biến đổi nucleotit gây ra đột biến câm

+ Vùng không mã hóa

Ví dụ 4

Xác định tính độc ASFV-G Δ9GL/ΔUK ở lợn

Thử nghiệm động vật được thực hiện trong điều kiện an toàn sinh học mức độ 3 ở phương tiện động vật tại PIADC theo giao thức được cho phép bởi ủy ban chăm sóc và sử dụng động vật thí nghiệm.

ASFV-G Δ9GL/ΔUK được đánh giá cho kiểu hình độc lực của nó tương ứng với virut ASFV-G cha mẹ có độc lực sử dụng 80-90 pound lợn giống thương mại. Năm con lợn được chủng trong cơ (IM) 10^4 hoặc 10^6 HAD_{50} ASFV-G Δ9GL/ΔUK hoặc 10^4 HAD_{50} virut ASFV-G. Dấu hiệu lâm sàng (chán ăn, bệnh trầm cảm, bệnh sốt, sự đổi màu da tím, dáng đi lảo đảo, tiêu chảy và ho) và thay đổi nhiệt độ cơ thể được ghi nhận hàng ngày qua thử nghiệm. Trong thử nghiệm bảo vệ động vật được tiêm chủng IM 10^4 HAD_{50} hoặc 10^6 HAD_{50} và 28 ngày sau khi bị gây nhiễm IM 10^3 HAD_{50} chủng ASFV Georgia2007 có độc lực cha mẹ. Sự có mặt của dấu hiệu lâm sàng liên quan đến bệnh được thực hiện như được mô tả sớm hơn.

Tất cả các con lợn 80-90 pound được chủng qua IM 10^4 HAD_{50} ASFV-G biểu hiện tăng nhiệt độ cơ thể (>104°F) 3 đến 4 ngày sau khi nhiễm. Lợn có dấu hiệu lâm

sàng liên quan đến bệnh bao gồm chán ăn, bệnh trầm cảm, sự đổi màu da tím, dáng đi lảo đảo và tiêu chảy (bảng 2). Dấu hiệu của bệnh tăng dần theo thời gian và động vật chết hoặc bị tiêu hủy vào lúc chết 7 hoặc 9 ngày sau khi nhiễm. Ngược lại, động vật được chủng qua IM 10^4 hoặc 10^6 HAD_{50} ASFV-G Δ9GL/ΔUK đã không có dấu hiệu bất kỳ của bệnh lâm sàng trong toàn bộ thời gian quan sát (21 ngày). Do đó, đột biến mất đoạn của gen 9GL và UK tạo ra sự suy giảm hoàn toàn của ASFV-G có độc lực cha mẹ. Đột biến mất đoạn của gen UK mở rộng sự suy giảm của ASFV-G Δ9GL đến mức mà 10^6 HAD_{50} ASFV-G Δ9GL/ΔUK nhược độc hoàn toàn trong khi động vật được chủng 10^4 HAD_{50} ASFV-G Δ9GL có mức độ bệnh thay đổi (O'Donnell *et al.*, *supra*).

Bảng 2. Sống sót và đáp ứng bệnh sốt ở lợn theo bệnh nhiễm ASFV-G Δ9GL/ΔUK và virut ASFV-G cha mẹ.

			Bệnh sốt		
Virut	Số còn sống/ Tổng	Thời gian trung bình đến khi chết (Ngày ± SD)	Số ngày đến khi khởi phát (Ngày ± SD)	Khoảng số ngày (Ngày ± SD)	Nhiệt độ hàng ngày tối đa (°F ± SD)
ASFV-G 10^2 HAD_{50}	0/5	7,6 (0,55)	3,6 (0,55)	4 (0,5)	105,6 (0,7)
ASFV-G Δ9GL/ΔUK 10^4 HAD_{50}	5/5	-	-	-	103,2 (0,32)
ASFV-G Δ9GL/ΔUK 10^6 HAD_{50}	5/5	-	-	-	103,4 (0,59)

Ví dụ 6

Hiệu quả bảo vệ của ASFV-G Δ9GL/ΔUK kháng lại việc gây nhiễm bằng ASFV-G cha mẹ

Do lợn được chủng qua IM 10^4 HAD_{50} – 10^6 HAD_{50} ASFV-G Δ9GL/ΔUK sống sót sau khi nhiễm mà không có dấu hiệu của bệnh, nhóm động vật (n=5) bị nhiễm 10^4 hoặc 10^6 HAD_{50} ASFV-G Δ9GL/ΔUK bị gây nhiễm qua IM 10^3 HAD_{50} ASFV-G cha mẹ ở ngày 28 sau khi chủng (thử thách đồng chủng). Năm động vật chưa quen thí nghiệm mà bị gây nhiễm sử dụng cùng tuyến và liều lượng đóng vai trò nhóm đối chứng không được chủng/gây nhiễm. Năm động vật được chủng và gây nhiễm ASFV-G Δ9GL/ΔUK giữ lại không triệu chứng hoàn toàn trong tất cả thời kỳ quan sát (21 ngày) ngoại trừ hai động vật được gây miễn dịch bằng 10^6 HAD_{50} ASFV-G Δ9GL/ΔUK thể hiện sự tăng nhiệt độ cơ thể nhẹ và tạm thời (bảng 3). Tất cả các động vật ở nhóm đối chứng được chủng/bị gây nhiễm giả phát triển bệnh với khoảng lâm sàng tương tự với khoảng quan sát được ở động vật được chủng 10^4 HAD_{50} ASFV-G (xem ở trên). Do đó, ASFV-G Δ9GL/ΔUK có khả năng gây ra sự bảo vệ kháng lại bệnh lâm sàng khi bị gây nhiễm bởi virut cha mẹ độc lực cao.

Bảng 3. Sống sót và đáp ứng bệnh sốt ở lợn trong động vật bị nhiễm ASFV-G Δ9GL/ΔUK bị gây nhiễm bởi virut ASFV-G cha mẹ.

			Bệnh sốt		
Virut	Số còn sống / Tổng	Thời gian trung bình đến khi chết (Ngày ± SD)	Số ngày đến khi khởi phát (Ngày ± SD)	Khoảng số ngày (Ngày ± SD)	Nhiệt độ hàng ngày Tối đa (F° ± SD)
Giả	0/5	7,6 (0,55)	3,6 (0,55)	5 (0,50)	105,6 (0,7)
ASFV-G Δ9GL/ΔUK 10^4 HAD_{50}	5/5	-	-	-	102,7 (0,63)
ASFV-G Δ9GL/ΔUK 10^6 HAD_{50}	5/5	-	10,5 (9,45)*	4 (2,84)*	103,6 (1,21)

* Dữ liệu dựa vào 2 / 5 động vật có sự tăng nhiệt độ cơ thể tạm thời.

#Động vật IM bị nhiễm 10^4 hoặc 10^6 HAD_{50} ASFV-G Δ9GL/ΔUK bị gây nhiễm IM 28 ngày sau bằng 10^3 HAD_{50} virut ASFV-G.

Tóm lại, các tác giả sáng chế có chứng cớ rằng đột biến mất đoạn gen *9GL* và *UK* thay đổi mạnh mẽ độc lực của ASFV-G sản xuất virut hoàn toàn nhược độc được đặt tên ASFV-G Δ9GL/ΔUK. Động vật được gây miễn dịch bằng ASFV-G Δ9GL/ΔUK được bảo vệ kháng lại việc gây nhiễm bằng ASFV-G cha mẹ có độc lực.

Tất cả các công bố và bằng sáng chế được đề cập trong bản mô tả này được kết hợp vào đây bằng cách tham chiếu với cùng mức độ như thể mỗi công bố hoặc bằng sáng chế riêng được chỉ định cụ thể và riêng rẽ được kết hợp bằng cách tham chiếu.

Phần mô tả nêu trên và một số phương án đại diện nhất định và chi tiết của sáng chế đã thể hiện mục đích minh họa và mô tả của sáng chế. Nó không có ý định là triệt để hoặc hạn chế sáng chế với dạng chính xác được bộc lộ. Sẽ rõ ràng cho người có hiểu biết trung bình trong lĩnh vực kỹ thuật này rằng các biến đổi và biến thể có thể được thực hiện trong bản mô tả này mà không xa rời khỏi phạm vi của sáng chế.

YÊU CẦU BẢO HỘ

1. Virut đột biến ASFV-Δ9GL/ΔUK của ASFV-G tái tổ hợp (virut gây bệnh sốt ở lợn châu Phi- thể phân lập Georgia 2007) chứa cADN mã hoá polypeptit ASFV-Δ9GL/ΔUK đột biến trong đó cADN đột biến chứa hai đột biến mất đoạn, đột biến mất đoạn của 173 nucleotit tạo thành protein 9GL đột biến chứa ít hơn 58 axit amin so với protein 9GL kiểu dại, không đột biến của ASFV-G, axit amin #11 đến #68 bị mất đoạn, và đột biến mất đoạn thứ hai của 255 nucleotit tạo thành protein UK đột biến chứa ít hơn 85 axit amin so với protein UK kiểu dại, không đột biến của ASFV-G, axit amin #1 đến #85 bị mất đoạn.

2. Virut đột biến chứa cADN theo điểm 1, trong đó cADN này là SEQ ID NO: 3.

3. Chế phẩm vacxin chứa virut đột biến ASFV-Δ9GL/ΔUK của ASFV-G tái tổ hợp theo điểm 1 hoặc điểm 2.

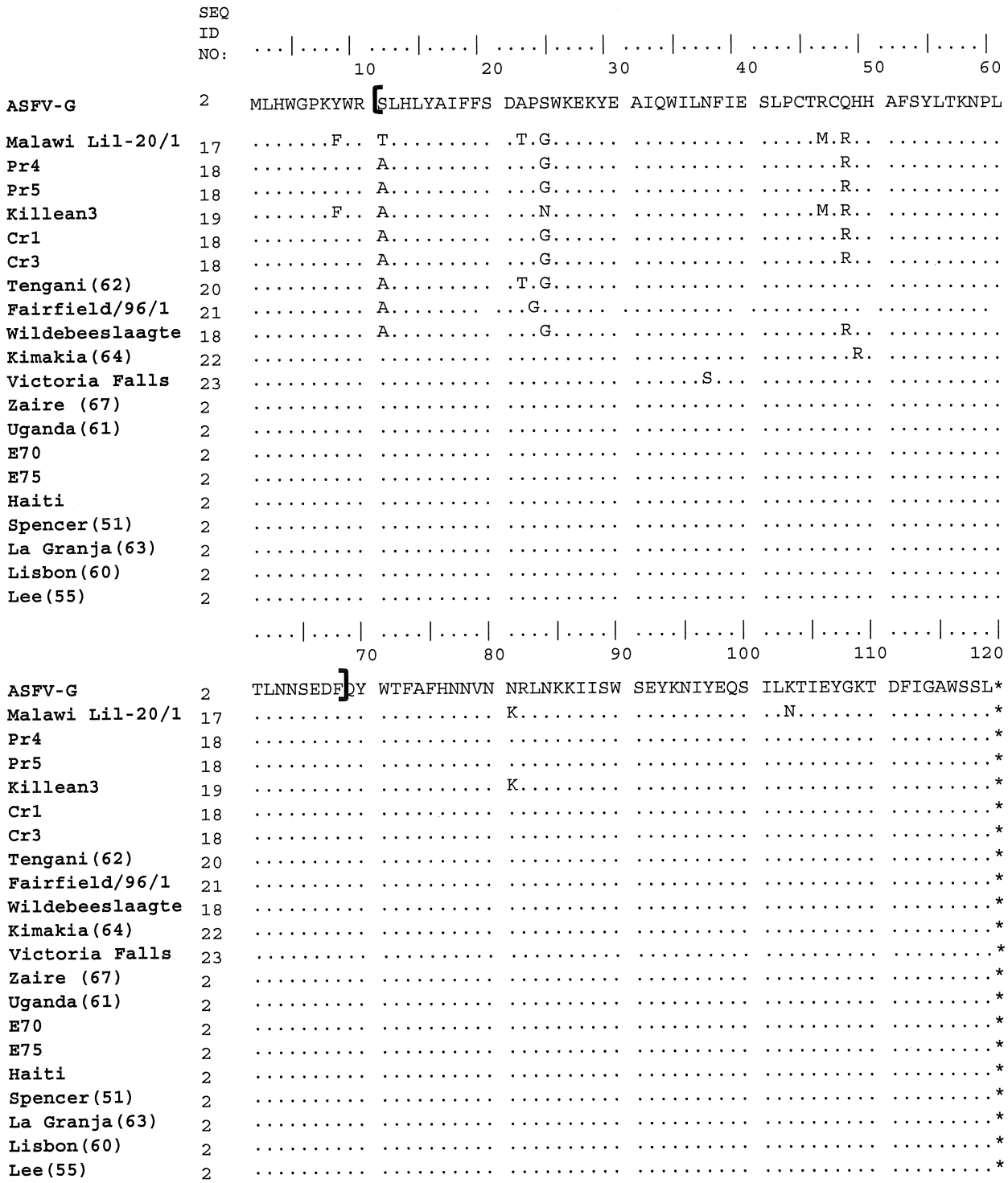

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Pr5 18 ..... A..... ...G..... ....R.. .....
Killean3 19 .....F.. A..... ...N..... ....M.R.. .....
Cr1 18 ..... A..... ...G..... ....R.. .....
Cr3 18 ..... A..... ...G..... ....R.. .....
Tengani(62) 20 ..... A..... .T.G..... ....
Fairfield/96/1 21 ..... A..... ...G..... ....
Wildebeeslaagte 18 ..... A..... ...G..... ....R.. .....
Kimakia(64) 22 ....R. .....
Victoria Falls 23 ....S... ....
Zaire (67) 2 ....
Uganda(61) 2 ....
E70 2 ....
E75 2 ....
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Spencer(51) 2 ....
La Granja(63) 2 ....
Lisbon(60) 2 ....
Lee(55) 2 ....
70 80 90 100 110 120
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Malawi Lil-20/1 17 .... K..... ....N..... .....*
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Fig. 1A

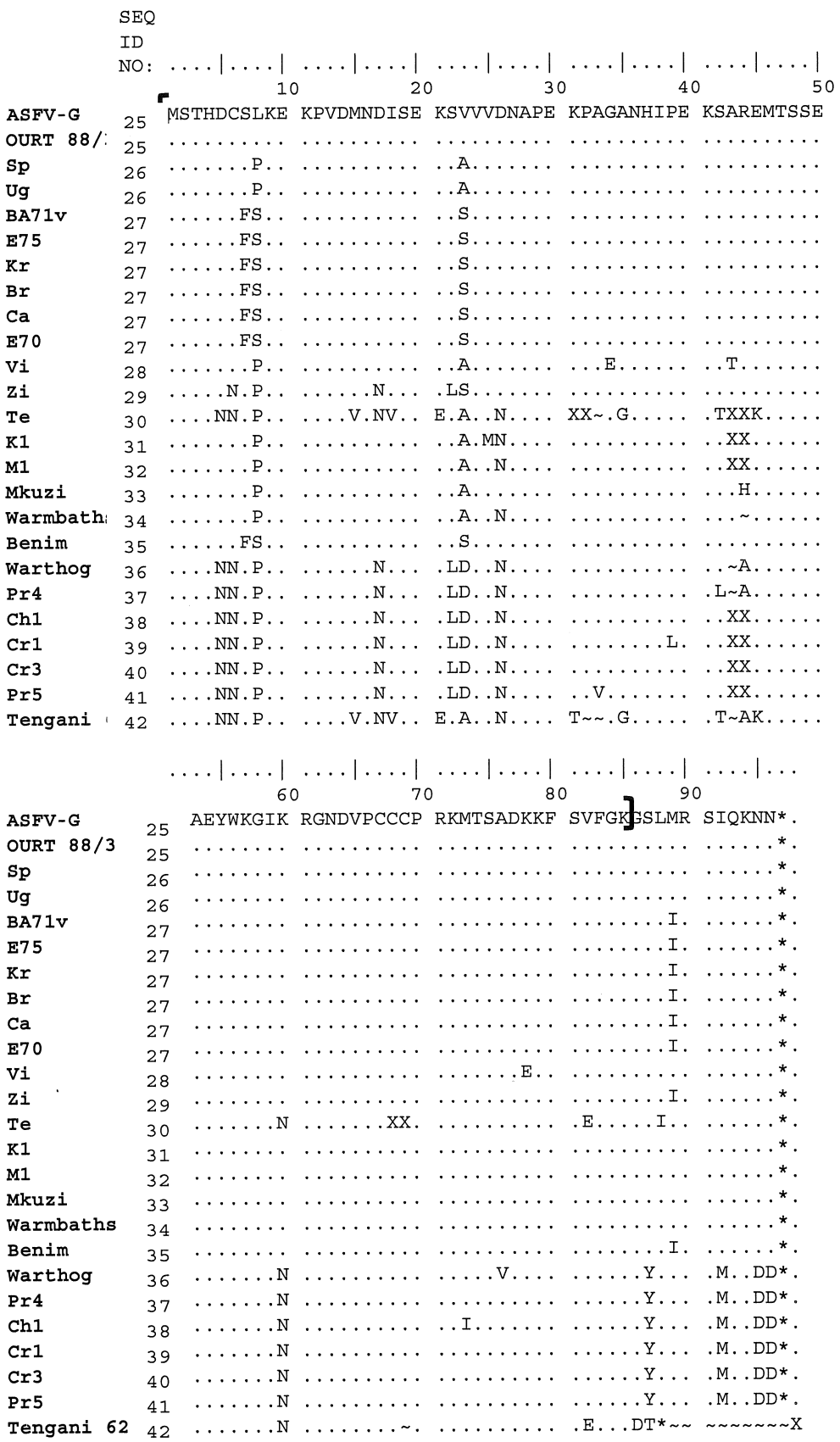

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Kr 27 .....FS.. ....S..... ....
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Fig. 1B

DANH MỤC TRÌNH TỰ

<110> Borca, Manuel V.
Holinka-Patterson, Lauren G.
Gladue, Douglas
Risatti, Guillermo R.
O'Donnell, Vivian K.

<120> VIRUT ĐỘT BIẾN ASFV-Δ9GL/ΔUK của ASFV-G TÁI TỔ HỢP VÀ CHẾ PHẨM VACXIN CHỨA VIRUT NÀY

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Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
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Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
100 105 110

Ile Gly Ala Trp Ser Ser Leu
115

<210> 19
<211> 119
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 19

Met Leu His Trp Gly Pro Lys Phe Trp Arg Ala Leu His Leu Tyr Ala
1 5 10 15

Ile Phe Phe Ser Asp Ala Pro Asn Trp Lys Glu Lys Tyr Glu Ala Ile
20 25 30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Met Cys Arg
35 40 45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
65 70 75 80

Lys Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
100 105 110

Ile Gly Ala Trp Ser Ser Leu
115

<210> 20
<211> 119
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 20

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ala Leu His Leu Tyr Ala
1 5 10 15

Ile Phe Phe Ser Asp Thr Pro Gly Trp Lys Glu Lys Tyr Glu Ala Ile
20 25 30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Gln
35 40 45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
65 70 75 80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe

100 105 110

Ile Gly Ala Trp Ser Ser Leu
115

<210> 21
<211> 119
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 21

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ala Leu His Leu Tyr Ala
1 5 10 15

Ile Phe Phe Ser Asp Ala Pro Gly Trp Lys Glu Lys Tyr Glu Ala Ile
20 25 30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Gln
35 40 45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
65 70 75 80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
100 105 110

Ile Gly Ala Trp Ser Ser Leu
115

<210> 22
<211> 119
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 22

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ser Leu His Leu Tyr Ala

1 5 10 15

Ile Phe Phe Ser Asp Ala Pro Ser Trp Lys Glu Lys Tyr Glu Ala Ile
20 25 30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Gln
35 40 45

Arg His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
65 70 75 80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
100 105 110

Ile Gly Ala Trp Ser Ser Leu
115

<210> 23
<211> 119
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 23

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ser Leu His Leu Tyr Ala
1 5 10 15

Ile Phe Phe Ser Asp Ala Pro Ser Trp Lys Glu Lys Tyr Glu Ala Ile
20 25 30

Gln Trp Ile Leu Ser Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Gln
35 40 45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
65 70 75 80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
100 105 110

Ile Gly Ala Trp Ser Ser Leu
115

<210> 24
<211> 58
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 24

Ser Leu His Leu Tyr Ala Ile Phe Phe Ser Asp Ala Pro Ser Trp Lys
1 5 10 15

Glu Lys Tyr Glu Ala Ile Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu
20 25 30

Pro Cys Thr Arg Cys Gln His His Ala Phe Ser Tyr Leu Thr Lys Asn
35 40 45

Pro Leu Thr Leu Asn Asn Ser Glu Asp Phe
50 55

<210> 25
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 25

Met Ser Thr His Asp Cys Ser Leu Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Val Val Val Asp Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala Arg Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 26
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 26

Met Ser Thr His Asp Cys Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ala Val Val Asp Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala Arg Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 27
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 27

Met Ser Thr His Asp Cys Phe Ser Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ser Val Val Asp Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala Arg Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Ile Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 28
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 28

Met Ser Thr His Asp Cys Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ala Val Val Asp Asn Ala Pro Glu Lys Pro
20 25 30

Ala Glu Ala Asn His Ile Pro Glu Lys Ser Arg Arg Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Glu Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Ile Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 29
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 29

Met Ser Thr His Asp Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asn Ile Ser Glu Lys Leu Ser Val Val Asp Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala Arg Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Ile Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 30
<211> 95
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
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<222> (31)..(32)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<220>
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<222> (42)..(43)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<220>
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<222> (67)..(68)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 30

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Val Asn
1 5 10 15

Asn Val Ser Glu Glu Ser Ala Val Val Asn Asn Ala Pro Glu Xaa Xaa
20 25 30

Gly Gly Asn His Ile Pro Glu Lys Thr Xaa Xaa Lys Met Thr Ser Ser
35 40 45

Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp Val
50 55 60

Pro Cys Xaa Xaa Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe Ser
65 70 75 80

Glu Phe Gly Lys Gly Ser Ile Met Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 31
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
<221> misc_feature
<222> (43)..(44)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 31

Met Ser Thr His Asp Cys Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ala Val Met Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Xaa Xaa Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 32
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
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<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 32

Met Ser Thr His Asp Cys Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ala Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Xaa Xaa Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 33
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 33

Met Ser Thr His Asp Cys Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ala Val Val Asp Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala His Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 34
<211> 95
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 34

Met Ser Thr His Asp Cys Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ala Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala Glu Met Thr Ser Ser
35 40 45

Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp Val
50 55 60

Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe Ser
65 70 75 80

Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 35

<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 35

Met Ser Thr His Asp Cys Phe Ser Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asp Ile Ser Glu Lys Ser Ser Val Val Asp Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala Arg Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Lys Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Ser Leu Ile Arg Ser Ile Gln Lys Asn Asn
85 90 95

<210> 36
<211> 95
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 36

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asn Ile Ser Glu Lys Leu Asp Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Ser Ala Glu Met Thr Ser Ser
35 40 45

Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp Val
50 55 60

Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Val Asp Lys Lys Phe Ser

65 70 75 80

Val Phe Gly Lys Gly Tyr Leu Met Arg Ser Met Gln Lys Asp Asp
85 90 95

<210> 37
<211> 95
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<400> 37

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asn Ile Ser Glu Lys Leu Asp Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Leu Ala Glu Met Thr Ser Ser
35 40 45

Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp Val
50 55 60

Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe Ser
65 70 75 80

Val Phe Gly Lys Gly Tyr Leu Met Arg Ser Met Gln Lys Asp Asp
85 90 95

<210> 38
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
<221> misc_feature
<222> (43)..(44)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 38

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asn Ile Ser Glu Lys Leu Asp Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Leu Xaa Xaa Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Ile Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Tyr Leu Met Arg Ser Met Gln Lys Asp Asp
85 90 95

<210> 39
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
<221> misc_feature
<222> (43)..(44)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 39

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asn Ile Ser Glu Lys Leu Asp Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Leu Glu Lys Leu Xaa Xaa Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Tyr Leu Met Arg Ser Met Gln Lys Asp Asp
85 90 95

<210> 40
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
<221> misc_feature
<222> (43)..(44)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 40

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asn Ile Ser Glu Lys Leu Asp Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Ala Gly Ala Asn His Ile Pro Glu Lys Leu Xaa Xaa Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Tyr Leu Met Arg Ser Met Gln Lys Asp Asp
85 90 95

<210> 41
<211> 96
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
<221> misc_feature
<222> (43)..(44)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 41

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
1 5 10 15

Asn Ile Ser Glu Lys Leu Asp Val Val Asn Asn Ala Pro Glu Lys Pro
20 25 30

Val Gly Ala Asn His Ile Pro Glu Lys Leu Xaa Xaa Glu Met Thr Ser
35 40 45

Ser Glu Trp Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp
50 55 60

Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
65 70 75 80

Ser Val Phe Gly Lys Gly Tyr Leu Met Arg Ser Met Gln Lys Asp Asp
85 90 95

<210> 42
<211> 84
<212> PRT
<213> Virut gây bệnh sốt ở lợn châu Phi

<220>
<221> misc_feature
<222> (84)..(84)
<223> Xaa có thể là axit amin bất kỳ có trong tự nhiên

<400> 42

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Val Asn
1 5 10 15

Asn Val Ser Glu Glu Ser Ala Val Val Asn Asn Ala Pro Glu Thr Gly
20 25 30

Gly Asn His Ile Pro Glu Lys Thr Ala Lys Met Thr Ser Ser Glu Trp
35 40 45

Ile Ala Glu Tyr Trp Lys Gly Ile Asn Arg Gly Asn Asp Val Pro Cys
50 55 60

Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe Ser Glu Phe Gly

65 70 75 80

Lys Asp Thr Xaa