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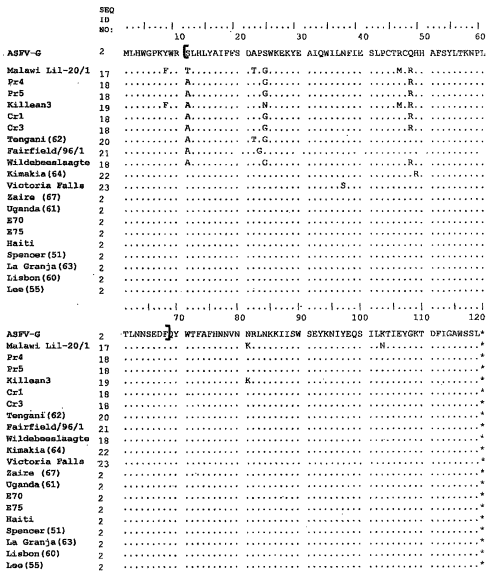
(54)发明名称

非洲猪瘟减毒病毒毒株、疫苗组合物及该病毒毒株的应用

(57)摘要

非洲猪瘟病毒(ASFV)是接触传染性病原体，通常导致家猪的致命病毒性疾病。对非洲猪瘟(ASF)的控制已受到不可获得疫苗的阻碍。已经从自然存在的、适应细胞培养的或遗传修饰的活减毒ASFV衍生了实验性疫苗；但是，这些疫苗只有在保护免于同源病毒时才是成功的。我们通过缺失特异性毒性相关的9GL(B119L)和UK(DP96R)基因，从高毒性的ASFV Georgia 2007(ASFV-G)分离株衍生了重组Δ9GL/ΔUK病毒。在体内，甚至在相当高的剂量下(10^6HAD_{50})对猪肌内施用ASFV-GΔ9GL/ΔUK也不会诱导疾病。重要的是，在感染28天后使用毒性的亲本毒株Georgia 2007攻击时，使用10^4或10^6HAD_{50}感染的动物稳定地受到保护而免于临床疾病的表现。



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1.一种重组突变体ASFV-G(非洲猪瘟病毒-Georgia 2007分离株)Δ9GL/ΔUK病毒,所述重组突变体ASFV-GΔ9GL/ΔUK病毒包含编码突变体ASFV-GΔ9GL/ΔUK多肽的cDNA,其中所述cDNA包含两个缺失,173个核苷酸的缺失导致突变体9GL蛋白比ASFV-G的未突变的野生型9GL蛋白少58个氨基酸,第11至第68氨基酸被缺失,并且255个核苷酸的第二个缺失导致突变体UK蛋白比ASFV-G的未突变的野生型UK蛋白少85个氨基酸,第1至第85氨基酸被缺失。

2.根据权利要求1的重组突变体ASFV-G(非洲猪瘟病毒-Georgia 2007分离株)Δ9GL/ΔUK病毒,其中所述cDNA是SEQ ID NO:3。

3.一种疫苗组合物,其包含根据权利要求1或权利要求2的重组突变体ASFV-GΔ9GL/ΔUK病毒。

4.一种活减毒非洲猪瘟病毒-Georgia 2007分离株(ASFV-G)的应用,用于制备Δ9GL/ΔUK疫苗以保护猪免于ASFV-G,其特征在于,所述疫苗包含根据权利要求1或权利要求2的有效量的重组突变体ASFV-GΔ9GL/ΔUK病毒,以保护所述猪免于临床ASF-G。

5.根据权利要求4的活减毒非洲猪瘟病毒-Georgia 2007分离株(ASFV-G)的应用,其中有效保护所述猪免于临床ASF-G的量是包含10^4HAD_{50}至10^6HAD_{50}的ASFV-GΔ9GL/ΔUK的疫苗。

非洲猪瘟减毒病毒毒株、疫苗组合物及该病毒毒株的应用

[0001] 发明背景

发明领域

[0002] 本发明涉及构建用于高毒性的Georgia 2007分离株ASFV-G的重组非洲猪瘟病毒(ASFV)活减毒候选毒株疫苗。所述疫苗包含ASFV-GΔ9GLΔUK修饰的病毒，一种通过缺失9GL(B119L)基因和UK(DP96R)基因的大部分来修饰的重组ASFV-G。

[0003] 相关领域描述

[0004] 非洲猪瘟(ASF)是猪的接触传染性病毒疾病。病原体ASF病毒(ASFV)是一种大型包膜病毒，其含有大约19万碱基对的双链DNA基因组。ASFV在基因组结构和复制策略方面与其它大型的双链DNA病毒共享，所述病毒包括痘病毒科(Poxviridae)、虹彩病毒科(Iridoviridae)和藻DNA病毒科(Phycodnaviridae)(Costard等人2009.Phil.Trans.Royal Soc.B 364:2683-2696)。在家猪中的ASFV感染通常是致命的，并且表征为发热、出血、共济失调和严重抑郁。但是，感染进程是不同的，从高度致死到临床症状不明显，这取决于宿主表征和具体的病毒毒株(Tulman等人2009.Curr.Top.Microbiol.Immunol.328:43-87)。

[0005] 目前，所述疾病在超过20个撒哈拉以南非洲国家流行。在欧洲，ASF仍在撒丁岛(意大利)流行，并且自2007年起已经在高加索地区宣布了新的爆发，其影响了格鲁吉亚、亚美尼亚、阿塞拜疆和俄罗斯。近期还在乌克兰、白俄罗斯、立陶宛、拉脱维亚和波兰报道了孤立的爆发，造成了进一步传染至邻国的风险。流行病毒，ASFV Georgia 2007/1，是一种属于基因型II的高毒性分离株(Chapman等人2011.Emerging Infect.Dis.17:599-605)。

[0006] 目前，没有可用于ASF的疫苗，并且疾病爆发通过动物检疫和屠宰进行控制。使用受感染细胞的提取物、受感染的猪外周血白细胞的上清液、纯化和灭活的病毒颗粒、受感染的戊二醛固定的巨噬细胞或洗涤剂处理的受感染的肺泡巨噬细胞对动物进行疫苗接种的尝试均没有诱导保护性的免疫力(Coggins,L.1974.Prog.Med.Virol.18:48-63;Forman等人1982.Arch.Virol.74:91-100;Kihm等人1987.In:African Swine Fever,Becker,Y.(ed),Martinus Nijhoff,Boston,pp 127-144;Mebus,C.A.1988.Adv.Virus Res.35:251-269)。发展的同源保护免疫力使得猪在病毒感染中存活。在使用ASFV的中等毒性或减毒变体的急性感染下存活的猪发展出了对同源病毒攻击的长期抵抗力，但是对异源病毒攻击则很少(Hamdy和Dardiri.1984.Am.J.Vet.Res.45:711-714;Ruiz-Gonzalvo等人1981.In:FAO/CEC Expert Consultation in ASF Research,Wilkinson,P.J.(ed),Rome,pp 206-216)。当使用同源亲本病毒攻击时，使用含有特异性ASFV毒性相关基因的工程改造缺失的活减毒ASF病毒免疫的猪受到了保护。特别地，从病原体ASF病毒(Malawi Lil-20/1、Pretoriuskop/96/4、E70和Georgia 2007)的基因组单独缺失UK(DP69R)、23-NL(DP71L)、TK(A240L)或9GL(B119L)基因显著减弱了猪中的病毒，并且使用这些减毒病毒免疫的动物受到保护而免于同源病毒的攻击(Moore等人1998.J.Virol.72:10310-10315;Lewis等人2000.J.Virol.74:1275-1285;等人1996.J.Virol.70:8865-8871;Zsak等人1998.J.Virol.72:1028-1035)。这些观察构成了描绘针对ASFV的有效的活减毒病毒的合理

开发的仅有的实验证据。

[0007] 具体地,在高毒性ASFV分离株Malawi Lil-20/1、Pretoriuskop/96/4和Georgia 2007(Lewis等人,同上;Neilan等人2004.Virol.319:337-342;0'Donnell等人2015.J.Virol.89:8556-8566)中缺失9GL(B119L)导致这些病毒在猪中完全减毒。以相当高的病毒剂量通过肌内注射将Malawi Lil-20/1Δ9GL或Pretoriuskop/96/4Δ9GL或E70ΔUK突变体施用至猪并没有诱导临床体征,而全部动物都在感染中存活。此外,使用这些病毒对猪诱肌内接种导了免于毒性亲本病毒攻击的保护(Zsak等人1998,同上;Lewis等人,同上;0'Donnell等人,同上)。这些观察构成了描绘针对ASFV的有效的活减毒病毒的合理开发的仅有的实验证据。

[0008] 因为目前没有ASFV疫苗可以获得,因此对于开发可以诱导免于疾病的致命表现的任何类型的保护的任何实验性疫苗都具有极大的兴趣。

[0009] 发明概述

[0010] 我们已经开发了新型的重组突变体ASFV-GΔ9GL/ΔUK病毒,一种ASFV-G(非洲猪瘟病毒-Georgia 2007分离株)的修饰。

[0011] 根据该发现,本发明的目的是提供新型的突变体ASFV-GΔ9GL/ΔUK病毒,其导致亲本ASFV-G的9GL(B119L)基因和UK(DP96R)基因的大部分的缺失。ASFV-GΔ Δ9GL/ΔUK(SEQ ID NO:3)的核苷酸序列不同编码野生型ASFV-G的核苷酸序列(SEQ ID NO:1)。ASFV-G(野生型)9GL编码119个氨基酸的蛋白(SEQ ID NO:2)并且ASFV-G(野生型)UK编码95个氨基酸的蛋白(SEQ ID NO:25),其不同于通过突变体核苷酸序列ASFV-GΔ9GL/ΔUK(SEQ ID NO:3)编码的突变体9GL和UK蛋白。61个氨基酸的突变体9GL多肽(SEQ ID NO:4)由野生型9GL多肽(SEQ ID NO:2)的第11氨基酸至第68氨基酸的缺失产生,并且10个氨基酸的突变体UK多肽(SEQ ID NO:26)由野生型UK多肽(SEQ ID NO:25)的第1氨基酸至第85氨基酸的缺失产生。

[0012] 本发明另外的一个目的是提供包含可存活的ASFV-GΔ9GL/ΔUK病毒的免疫原性组合物。

[0013] 本发明另外一个目的是提供当使用致病ASFV-G攻击时有效保护动物免于临床ASF疾病的合理设计的活减毒SFV-GΔ9GL/ΔUK疫苗。

[0014] 本发明进一步的目的是提供遗传标志物疫苗,其可以潜在地区分疫苗接种的动物和被ASFV-G感染的动物。

[0015] 本发明的另一个目的是提供通过施用有效量的合理设计的活减毒ASFV-GΔ9GL/ΔUK疫苗保护动物免于ASFV-G的方法。

[0016] 本发明另外的目的是提供用于将被ASFV-G感染的动物与使用所述合理设计的活减毒ASFV-GΔ9GL/ΔUK疫苗进行疫苗接种的动物区别的方法,其包括用于区分接种疫苗的动物和被野生型感染的动物的遗传DIVA策略。

[0017] 本发明的其它目的和优点将通过随后的描述变得显而易见。

[0018] 附图简述

[0019] 图1A和图1B显示了ASFV-G 9GL(B119L)基因编码的多肽(图1A)和ASFV-G UK(DP96R)基因编码的多肽(图1B)的序列比对。比较了不同时间起源和地理起源的分离株,包括从蜱虫来源和猪来源获得的那些。在括号中显示了引入ASFV-G而产生ASFV-GΔ9GL/ΔUK

病毒的部分缺失。

[0020] 发明详述

[0021] 我们通过靶向ASFV-G 9GL(B119L)和ASFV-G UK(DP96R)基因进行遗传修饰的方法,已经开发了可以作用疫苗候选的减毒病毒。在此我们报道了高毒性ASFV Georgia 2007分离株(ASFV-G)的重组Δ9GL/ΔUK病毒的构建。在相当高的剂量下(10^4或$10^6$$HAD_{50}$)对猪肌内(IM)施用ASFV-GΔ9GL/ΔUK不会诱导疾病。当在感染28天后使用毒性的亲本毒株Georgia 2007攻击时,使用10^4或$10^6$$HAD_{50}$感染的动物受到保护免于临床疾病的表现。

[0022] 尽管来自ASFV的四种不同基因的独立缺失已经显示使毒性病毒减毒,并且尽管NL(DP71L)(Zsak等人1996,同上)或UK(DP69R)(Zsak等人1998,同上)基因从ASFV E75的独立缺失、TK(A240L)基因(Moore等人,同上)从ASFV的缺失适用于Vero细胞,Malawi Lil-20/1和Haiti,和9GL(B119L)基因同样从Malawi Lil-20/1(Lewis等人,同上)和Pretoriuskop/96/4(Neilan等人,同上)分离株的缺失致使重组缺失突变体病毒在猪中具有显著减少的毒性,在所有情况下,当使用相应的亲本病毒攻击(即同源攻击)时,使用这些遗传修饰的病毒中的每种接种的动物从感染存活并且针对ASFV获得保护(Lewis等人,同上;Moore等人,同上;Neilan等人,同上;Zsak等人1996,同上;Zsak等人1998,同上)。这些发现提示通过对靶基因的遗传操作的开发减毒ASFV重组病毒是用于疫苗开发的有效方法。

[0023] 然而,它们在其它ASFV分离株中的功效水平是不可预测的。例如,NL(DP71L)基因产物以两种不同形式存在,长形式(在23-NL中184个氨基酸)或短(70至72个氨基酸)形式,取决于ASFV分离株(Zsak等人1996,同上)。尽管在ASFV E70分离株(短形式)中该基因的缺失产生了减毒病毒,但是ASFV Malawi Lil-20/1(长形式)或Pretoriuskop/96/4(短形式)的NL(DP71L)基因的缺失没有造成所述病毒的减毒(Afonso等人1998.J.Gen.Virol.79(Pt.10):2543-2547)。TK(A240L)基因(一种涉及DNA合成的在全部ASFV分离株中高度保守的基因)的缺失已经被引入致病Vero细胞适应的Malawi Lil-20/1和Haiti H811病毒。Malawi Lil-20/1突变体病毒在体内比回复突变病毒(野生型样病毒)毒性更小,但是其没有完全减毒(Moore等人,同上)。UK(DP69R)基因位于某些ASFV分离株的右可变区域。该基因从ASFV E70分离株的缺失致使病毒展示出减小的毒性(Zsak等人1998,同上)。尽管UK(DP69R)基因是保守的,但是其不是在每种ASFV分离株中都存在的(例如Malawi Lil-20/1),这限制了它作为用于产生减毒病毒的候选靶基因的应用。

[0024] 9GL(B119L)基因在目前分离和测序(包括来自蜱虫或猪来源的那些)的ASFV中是高度保守的。事实表明,从毒性Malawi Lil-20/1(Lewis等人,同上)和Pretoriuskop/96/4(Neilan等人,同上)缺失所述基因有效减少了在猪中的毒性并且诱导了保护,这使得9GL(B119L)成为修饰和产生可以赋予针对ASFV的有效保护的减毒病毒的有力候选靶基因。事实上,我们发现从ASFV-G分离株缺失9GL(B119L)在减毒和保护方面没有如对于Malawi Lil-20/1和Pretoriuskop/96/4报道的相同效果。仅在当以低剂量将ASFV-GΔ9GL施用至猪时,才可能观察到病毒毒性的减少(O'Donnell等人,同上)。我们还展示了ASFV-GΔ9GL的亚致死剂量能够在使用同源亲本病毒攻击后诱导有效的保护而免于临床疾病的表现。E70(短形式)和Malawi Lil-20/1(长形式)编码的NL蛋白显著不同,并且这可以解释在使用相应缺失突变体病毒接种的猪中观察到的表型差异。然而,蛋白鉴定矩阵指示9GL蛋白在ASFV分离株中高度相似,其中ASFV-G、Malawi Lil-20/1和Pretoriuskop/96/4共享超过93%的氨基

酸同一性，这使得ASFV减毒不是仅取决于蛋白差异。因为良好观察到的表型最可能由多个基因介导(Lewis等人，同上；Moore等人，同上；Neilan等人，同上；Zsak等人1996，同上；Zsak等人1998，同上)，目前积累的证据使得推测ASFV Georgia 2007分离株中介导毒性的确切基因谱变得困难。

[0025] 综上，我们提出了缺失9GL(B119L)基因和UK(DP69R)基因导致减毒重组ASFV-GΔ9GL/ΔUK病毒的证据。在相当高的剂量下(10^4或10^6HAD_{50})对猪肌内施用ASFV-GΔ9GL/ΔUK不会诱导疾病。当在感染28天后使用毒性的亲本毒株Georgia 2007攻击时，使用10^4或10^6HAD_{50}感染的动物受到保护免于临床疾病的表现。

[0026] 在本文中疫苗被定义为能够在动物中提供保护性应答的生物制剂，其中所述疫苗已经被递送并且不能造成严重疾病。疫苗的施用导致对疾病的免疫：所述疫苗针对造成疾病的病原体刺激抗体产生或细胞免疫。在本文中免疫被定义为在疫苗接种后，相比未进行疫苗接种的组，针对死亡率和临床症状在猪群体中诱导了显著更高的保护水平。具体地，根据本发明的疫苗保护了大部分接种疫苗的动物免于出现疾病的临床症状和死亡。本发明的疫苗是遗传工程改造的突变体病毒疫苗。遗传标志物疫苗被定义为这样的疫苗，其连同诊断测试，能够遗传区分接种疫苗的动物和受感染的动物。缺失突变可以用于区分受感染的动物和接种疫苗的动物。突变被理解为在亲本ASFV-G毒株中野生型或未修饰的9GL(B119L)和UK(DP96R)基因(其能够表达天然的9GL和UK蛋白)的遗传信息中的变化。因此，由ASFV-GΔ9GL/ΔUK突变体表达的9GL和UK多肽是变化的：来自ASFV-GΔ9GL/ΔUK的9GL蛋白具有比野生型9GL和野生型UK更少的氨基酸，因为ASFV-GΔ9GL的9GL多肽中的第11至第68氨基酸被缺失，并且UK多肽中的第1至第85氨基酸被缺失。ASFV-GΔ9GL/ΔUK重组ASFV-G突变体包含编码ASFV-G 9GL和UK多肽的突变的核苷酸。所述突变包含9GL蛋白的58个氨基酸的缺失和UK蛋白的85个氨基酸的缺失。当以10^4HAD_{50}至10^6HAD_{50}的肌内接种剂量使用时，重组ASFV-G突变体ASFV-GΔ9GL/ΔUK是活减毒ASFV-G疫苗。

[0027] 提供的针对ASFV-G的疫苗包含以存活形式的如上文限定的ASFV-GΔ9GL/ΔUK突变体和药学上可接受的载体或稀释剂。根据本发明的疫苗含有可以以悬浮液或冻干形式制备并出售的活病毒，并且另外含有习惯用于此类组合物的药学上可接受的载体或稀释剂。载体包括稳定剂、防腐剂和缓冲剂。合适的稳定剂为，例如SPGA(蔗糖、磷酸、谷氨酸和人白蛋白)、碳水化合物(诸如山梨糖醇、甘露醇、淀粉、蔗糖、葡聚糖、谷氨酸或葡萄糖)、蛋白(诸如干乳清、白蛋白或酪蛋白)或其降解产物。合适的缓冲剂为例如碱金属磷酸盐。合适的防腐剂为硫柳汞、乙汞硫代水杨酸钠和庆大霉素。稀释剂包括水、含水稀释剂(诸如缓冲盐水)、醇和多元醇(诸如甘油)。

[0028] 如果需要，根据本发明的活疫苗可以含有佐剂。具有佐剂活性的合适的化合物和组合物的实例是本领域熟知的。此外，编码用于药物或诊断应用的多肽(特别是免疫调节剂诸如淋巴因子、干扰素或细胞因子)的核酸序列可以掺入至所述疫苗。

[0029] 根据本发明的疫苗可以通过常规方法制备，诸如通常用于商业可获得的活减毒ASFV疫苗的那些。简而言之，使用ASFV-GΔ9GL/ΔUK突变体接种易感物质，并且增殖至病毒已复制到期望的滴度，在此之后收获含有ASFV-GΔ9GL/ΔUK的材料。随后，将所述收获的材料配制为具有免疫性能的药物制备物。

[0030] 能够支持ASFV-GΔ9GL/ΔUK病毒的复制的每种物质均可以用于本发明，其包括猪

外周血巨噬细胞的原代培养。

[0031] 可以以有效保护动物免于ASFV-G的毒性毒株攻击的量通过肌内、皮下或鼻内接种或注射施用疫苗。该量可以根据要接种的动物而变化，考虑所述动物的大小和体重。根据本发明的疫苗包含有效剂量的ASFV-GΔ9GL/ΔUK突变体作为活性组分，即免疫性的ASFV-GΔ9GL/ΔUK的量将(针对毒性ASFV-G的攻击)诱导接种疫苗的动物(猪)中的免疫力。在本文中免疫被定义为在疫苗接种后，相比未进行疫苗接种的组，针对死亡率和临床症状在猪群体中诱导了显著更高的保护水平。具体地，根据本发明的疫苗保护了大部分接种疫苗的动物免于疾病的临床症状的出现和死亡。通常，所述活疫苗可以以10^4HAD_{50}至10^6HAD_{50}的剂量施用。有效量可以通过根据例如实施例6提供的指导由本领域技术人员根据需要实验地测定。

[0032] 除了ASFV-GΔ9GL/ΔUK突变体，本发明还可以包括组合疫苗，其包含能够诱导针对其它猪病原体进行保护的疫苗毒株。

[0033] 上文所述的ASFV-GΔ9GL/ΔUK疫苗连同诊断方法，具有区分使用其进行疫苗接种的动物和被天然存在的ASFV-G毒株感染或使用常规ASFV-G疫苗进行疫苗接种的动物的潜力。

[0034] 本发明还提供了重要的工具以监控ASFV-G控制措施，如果实施大规模的清除项目其可以导致ASFV-G的根除。该工具涉及用于在猪中测定ASFV-G感染的方法，其包括检查所述动物的样品是否存在相对于编码更短的ASFV-GΔ9GL和ΔUK的多核苷酸(由于ASFV-GΔ9GL/ΔUK的9GL(B119L)基因和UK(DP96R)基困的缺失)的编码野生型ASFV-G 9GL和UK蛋白的核苷酸的步骤。在该方法中使用的样品可以是任何样品，其中ASFV-G相对于ASFV-GΔ9GL/ΔUK的遗传差异允许通过遗传DIVA检测将自然感染和疫苗接种进行区分。

实施例

[0035] 目前已一般性地描述了本发明，其将通过参考某些特定实例而被更好地理解，本文包括的实例仅为进一步示例说明本发明并且不意图限制由权利要求所限定的本发明的范围。

[0036] 实施例1

[0037] 细胞培养物和病毒

[0038] 如Zsak等人(1996，同上)先前所述的从脱纤维蛋白的猪血液制备原代猪巨噬细胞培养物。简而言之，将肝素处理的猪血液在37℃温育1小时以允许红细胞级分沉淀。通过在Ficoll-Paque(Pharmacia，Piscataway，N.J.)密度梯度(比重，1.079)上漂浮分离单核白细胞。在含有巨噬细胞培养基(由具有30％L929上清液和20％胎牛血清(HI-FBS，Thermo Scientific，Waltham，MA)的RPMI 1640培养基(Life Technologies，Grand Island，NY)构成)的塑料Primaria(Falcon；Becton Dickinson Labware，Franklin Lakes，N.J.)组织培养烧瓶中在37℃，5％CO_2下48小时培养单核细胞/巨噬细胞级分。使用在磷酸缓冲盐水(PBS)中的10mM EDTA将贴壁细胞从塑料分离并且随后在Primaria T25，6-或96-孔培养皿中以$5x10^6$细胞/ml的密度再接种，用于24小时后的测定。

[0039] 病毒滴度在96-孔板中培养的原代猪巨噬细胞上执行。使用巨噬细胞培养基执行病毒稀释和培养。通过血细胞吸附(HA)评估病毒的存在，并且通过Reed and Muench方法(1938.Amer.J.Hygiene 27:493-497)计算病毒滴度。

[0040] ASFV Georgia(ASFV-G)是由Nino Vepkhvadze博士友情提供的野外分离株,来自位于格鲁吉亚共和国Tbilisi的农业部实验室(LMA)。

[0041] 实施例2

[0042] 重组ASFV-GΔ9GL/ΔUK的构建

[0043] 在使用猪巨噬细胞培养物的感染和转染程序中通过在亲本ASFV基因组和重组转移载体之间的序列同源重组产生重组ASFV(Neilan等人,同上;Zsak等人1996,同上)。首先,重组转移载体(p72GUSΔ9GL),其含有包括映射至基因的左侧(1.2kbp)和右侧(1.15kbp)的9GL的部分的侧接基因组区域,并且使用了含有具有ASFV p72晚期基因启动子的β-葡萄糖醛酸酶(GUS)基因的报道基因盒(p72GUS)。该构建在9GL ORF(氨基酸残基11至68)(参见图1)中产生了173个核苷酸的缺失。通过DNA合成(GenScript,Piscataway,NJ,USA)获得重组转移载体p72GUSΔ9GL。使用ASFV-G感染巨噬细胞培养物并且使用p72GUSΔ9GL转染。通过连续多轮的噬斑测定纯化将代表重组病毒的单独的原初噬斑纯化以均质化。在单层原代猪巨噬细胞培养物上进行11个连续噬斑纯化事件后获得重组病毒。产生的中间产物重组病毒,ASFV-GΔ9GL随后被用作感染/转染程序的亲本病毒,所述程序使用了可以从病毒基因组产生UK基因缺失的重组转移载体。重组转移载体(p72mCherryΔUK)含有映射至基因左侧(1.156kbp)和右侧(1.190kbp)的UK的侧接基因组区域,并且使用了含有具有ASFV p72晚期基因启动子的mCherry基因的报告基因盒(p72mCherry)。通过DNA合成(GenScript,Piscataway,NJ,USA)获得重组转移载体p72mCheryΔUK。该构建产生了在UK ORF(氨基酸残基1至85)中的255个核苷酸缺失(参见图1)。第二个重组事件通过含有在ASFV p72启动子下的荧光基因mCherry的盒置换了UK基因。在10轮基于荧光活性的限制性稀释纯化后选择重组病毒。在原代猪巨噬细胞培养物中扩增从最后一轮纯化中获得的病毒群体以获得病毒贮库。

[0044] 实施例3

[0045] 全基因组序列分析:ASFV-GΔ9GL/ΔUK相对于亲本ASFV-G

[0046] 为了评估重组病毒的遗传修饰的准确性和基因组的完整性,使用下一代测序(NGS)获得ASFV-GΔ9GL/ΔUK和亲本ASFV-G的全基因组序列并且进行比较(表1)。首先,执行亲本ASFV-G和ASFV Georgia 2007/1(Chapman等人,同上)之间的全长基因组比较。使用Trizol方法(Life Technologies,Grand Island,NY,USA)从受感染的细胞的细胞质获得ASFV DNA。使用Qubit® dsDNA HS测定试剂盒(Life Technologies)测定DNA浓度并且在Qubit®2 Flourometer(Life Technologies)上读取。使用Ion Shear™ Plus试剂盒(Life Technologies)酶促片段化1微克病毒DNA以获得200-300bp长度的平端片段并在Peltier Thermal Cycler DNA Engine Tetrad 2中以37℃进行温育。在剪切后,将片段化的DNA文库加载至DNA芯片(Agilent,Santa Clara,CA,USA)并且使用2100 Bioanalyzer(Agilent)进行分析以评估DNA大小分布和大小范围。使用Ion Plus Fragment Library试剂盒(Life Technologies)将片段化的DNA连接至离子兼容的适配子和文库条形码,随后将缺口修复以完成适配子和DNA插入物之间的连接。将连接适配子的文库在2%琼脂糖凝胶盒(Sage Science,Beverly,MA,USA)上使用Pippin Prep™仪器(Sage Science)进行大小选择用于优化长度。使用Ion Library Equalizer™试剂盒(Life Technologies)将文库浓度归一化。接下来,将DNA文库在Ion Sphere™ Particles(IPS)上克隆扩增,使用Ion PGM™Template

OneTouch™ 2 200试剂盒(Life Technologies)以及Ion OneTouch™ 2仪器(Life Technologies)产生模板阳性的ISP。在进行富集前,使用Ion Sphere™ Quality Control测定试剂盒(Life Technologies)和Qubit®2 Flourometer仪器执行未富集模板阳性ISP的质量评价。随后使用Ion PGM™ Template OneTouch™ 2 200试剂盒(Life Technologies)和Ion OneTouch™ ES仪器(Life Technologies)富集模板阳性ISP,以消除模板阴性ISP并且将模板阳性ISP上的DNA变性。使用Ion PGM™200 Sequencing v2试剂盒(Life Technologies),制备富集的模板ISP用于测序并加载到Ion 314™或Ion 316™ Chip v2(Life Technologies)上,并且在Ion PGM™ Sequencer(Life Technologies)上运行。随后使用Galaxy(从因特网获取:usegalaxy.org/)NGS QC和Manipulatio工具修剪获得的序列。使用Sequencher 5.2.2(Genecodes)和CLC Genomics Workbench(CLCBio)软件比对和分析序列。

[0047] 在这两种病毒之间观察到了以下差异(核苷酸位置基于ASFV Georgia 2007/1,GenBank登录号FR682468提供):(i)两个核苷酸插入,在基因组的非编码区段的位置433的T和位置441的A;(ii)两个核苷酸缺失,在MGF 360-1L基因ORF中的位置1602的T和位置1603的T,其导致移码;(iii)核苷酸缺失,在MGF 360-1L基因ORF中的位置1620的T,其导致移码;(iv)核苷酸突变,在位置97391的A至G,其导致在ORF B438的沉默突变;(v)核苷酸突变,在位置166192的C至G,其导致在ORF E199L中残基位置85的残基取代(Ala至Pro);和(vi)核苷酸插入,在位置183303的T,基因组的非编码区段(表1)。第二,ASFVΔ9GL/ΔUK和亲本ASFV-G之间进行全长基因组比较。ASFVΔ9GL/ΔUK和ASFV-G的DNA序列组装显示在9GL基因中的173个核苷酸的缺失,其对应于引入的修饰。ASFVΔ9GL/ΔUK基因组的共有序列显示了在9GL基因中2324个核苷酸的插入,其对应于被引入以在靶基因中产生173个核苷酸缺失的p72-βGUS盒序列。此外,ASFV-GΔ9GL/ΔUK和ASFV-G的DNA序列组装显示在UK基因中的255个核苷酸的缺失,其对应于引入的修饰。ASFV-GΔ9GL/ΔUK基因组的共有序列显示了在UG基因中937个核苷酸的插入,其对应于被引入以在靶基因中产生255个核苷酸缺失的p72-mCherry盒序列。除了盒的插入,在ASFV-GΔ9GL/ΔUK和ASFV-G基因组之间观察到的仅有的另外一个差异是在位置36,465的G至C核苷酸突变,其导致在ORF MGF 505-4R中残基位置224的残基取代(Glu至Gln)。综上,ASFV-GΔ9GL/ΔUK病毒没有在同源重组和噬斑纯化过程中积累任何显著的突变(表1)。

[0048] 表1.与ASFV Georgia07/1比较的ASFV-GΔ9GL/ΔUK的全长基因组序列和亲本ASFV-G之间的差异的概述*

[0049]

		病毒	
NPN*	修饰类型	ASFV-G	ASFV-G Δ9GL/ΔUK
433	T 缺失	+	+
411	A 缺失	+	+
1602	MGF 360-1L TT 缺失 FS@	+	+
1620	MGF 360-1L T 插入 FS	+	+
36465	MGF 505-4R G 至 C Glu224Gln	-	+
97391	B438L A 至 G SM#	+	+
166192	E199L C 至 G Ala85Pro	+	+
183303	T 插入至 NCR+中	+	+

[0050] *核苷酸位置编号(基于ASFV Georgia 2007/1分离株的序列,由Chapman等人公开,2011)

[0051] @导致相应ORF中移码的核苷酸修饰

[0052] #导致沉默突变的核苷酸修饰

[0053] +非编码区域

[0054] 实施例4

[0055] 猪中ASFV-GΔ9GL/ΔUK毒性的评价

[0056] 在PIADC在动物设施中在生物安全3级条件下根据由机构动物护理和使用委员会批准的方案执行动物实验。

[0057] 使用80-90磅的商业品种猪评估ASFV-GΔ9GL/ΔUK相对于毒性亲本ASFV-G病毒的毒性表型。使用10^4或10^6HAD_{50}的ASFV-GΔ9GL/ΔUK或使用10^4HAD_{50}的ASFV-G病毒对5只猪进行肌内(IM)接种。在整个实验中每日记录临床体征(厌食、抑郁、发热、紫皮肤变色、蹒跚步态、腹泻和咳嗽)和体温变化。在保护实验中使用10^4HAD_{50}或10^6HAD_{50}对动物进行肌内(IM)接种并且在28天后使用10^3HAD_{50}亲本毒性ASFV Georgia2007毒株进行攻击。与疾病相关的临床体征的出现如先前所述的进行。

[0058] 使用10^4HAD_{50}的ASFV-G肌内接种的全部80-90磅猪在感染后3-4天表现出增加的体温(>104°F)。猪出现的与疾病有关的临床体征包括厌食、抑郁、紫皮肤变色、蹒跚步态和腹泻(表2)。疾病的体征随时间进展性加重并且动物死亡或在感染7或9天后在濒临死亡时被安乐死。相反地,经由肌内使用10^4或10^6HAD_{50}的ASFV-GΔ9GL/ΔUK接种的动物在整个观察期(21天)期间没有出现任何疾病临床体征。因此,9GL和UK基因的缺失产生了亲本毒性ASFV-G的完全减毒。UK基因的缺失将ASFV-GΔ9GL的减毒扩大至10^6HAD_{50}的ASFV-GΔ9GL/ΔUK被完全减毒的程度,而使用10^4HAD_{50}的ASFV-GΔ9GL接种的动物出现了不同水平的疾病(O'Donnell等人,同上)。

[0059] 表2:使用ASFV-GΔ9GL/ΔUK和亲本ASFV-G病毒感染后的猪存活和发热反应。

[0060]

病毒	存活者的数量/总数	至死亡的平均时间（天数± SD）	发热		
			至发病天数（天数 ± SD）	持续天数（天数± SD）	最高每日温度（°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)

[0061] 实施例6

[0062] ASFV-GΔ9GL/ΔUK针对亲本ASFV-G攻击的保护功效

[0063] 因为经由肌内使用104HAD_{50}-$10^6$$HAD_{50}$的ASFV-GΔ9GL/ΔUK接种的猪从感染中存活并且没有疾病体征，在接种28天后经由肌内使用$10^3$$HAD_{50}$的亲本ASFV-G攻击（同源攻击）使用$10^4$或$10^6$$HAD_{50}$的ASFV-GΔ9GL/ΔUK感染的动物组（n＝5）。使用相同途径和剂量攻击5只原初动物作为未接种/攻击对照组。5只接种ASFV-GΔ9GL/ΔUK并被攻击的动物在全部观察时期（21天）中保持完全无症状，除了2只使用$10^6$$HAD_{50}$的ASFV-GΔ9GL/ΔUK免疫的动物显示出轻微和短暂的体温升高（表3）。在无接种/攻击对照组中的全部动物发展出具有与使用$10^4$$HAD_{50}$的ASFV-G接种的动物（参见上文）中观察到的相似的临床过程的疾病。因此，当使用高毒性的亲本病毒攻击时，ASFV-GΔ9GL/ΔUK能够诱导保护而免于临床疾病的表现。

[0064] 表3.使用亲本ASFV-G病毒攻击的受ASFV-GΔ9GL/ΔUK-感染的动物中的猪存活和发热反应

[0065]

病毒	存活者数量/总数	平均死亡时间（天数 ± SD）	发热		
			至发病天数（天数 ± SD）	持续天数（天数 ± SD）	最高每日温度（F° ± SD）
无接种	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)

[0066] *数据是基于出现体温的短暂升高的5只动物中的2只。

[0067] #使用10^4或10^6HAD_{50}的ASFV-GΔ9GL/ΔUK肌内感染的动物在28天后使用10^3HAD_{50}的ASFV-G病毒进行肌内攻击。

[0068] 综上,在此我们提供了9GL和UK基因的缺失彻底改变ASFV-G毒性的证据,其产生了命名为ASFV-GΔ9GL/ΔUK的完全减毒的病毒。使用ASFV-GΔ9GL/ΔUK免疫的动物受到保护而免于毒性亲本ASFV-G的攻击。

[0069] 在本说明书中提及的全部出版物和专利以如同各自单独的出版物或专利特别地或单独地被指示通过引用并入的相同程度通过引用并入本文。

[0070] 上述描述和某些相关实施方案和本发明的细节已经为了本发明的示例性说明和描述的目的而呈现。不意图详尽或限制本发明至公开的明确形式。对本领域实践者将显而易见的是可以进行修饰和改变而不离开本发明的范围。

序列表

<110> Borca, Manuel V.
Holinka-Patterson, Lauren G.
Gladue, Douglas
Risatti, Guillermo R.
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<120> 诱导针对同源毒性亲本病毒 Georgia 2007 分离株攻击的保护的非洲猪瘟减毒病毒毒株

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<212> DNA
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atcacctccc acaacgagga ctacaccatc gtggaacagt acgaacgcgc cgagggccgc 187380

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tttttcttga tcaaagtatg taaacgcccg cttacagcaa ggatcttaag tgagagccat 191460

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cggcgtaaca ccagttatgg tcaatttttgt ctggcccgcc gcccagccgc aaaaaaaaat 192300

caattacaac cgcaaaaaaa aatatttccg gccgcggcgt ttcaaaaaat aatctttgcg 192360

aaataattcc gcatcttgtg aaatgaacgc ctacagtaat aattttaatc tttgacacct 192420

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<210> 4
<211> 61
<212> PRT
<213> African swine fever virus (非洲猪瘟病毒)

<400> 4

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1 5 10 15

Phe His Asn Asn Val Asn Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp
20 25 30

Ser Glu Tyr Lys Asn Ile Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu
35 40 45

Tyr Gly Lys Thr Asp Phe Ile Gly Ala Trp Ser Ser Leu
50 55 60

<210> 5
<211> 20
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<213> 人工序列

<220>
<223> 化学合成的

<400> 5
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20

[0229]

<210> 6
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 6
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23

<210> 7
<211> 17
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 7
gacggcctgt gggcatt
17

<210> 8
<211> 17
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 8
gcgatggatt ccggcat
17

<210> 9
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 9
gtcttattgc taacgatggg aag
23

<210> 10
<211> 23
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

[0230]

<400> 10
ccaaaggtaa gcttgtttcc caa
23

<210> 11
<211> 20
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 11
gtaagatacg aaaaggcgtg
20

<210> 12
<211> 17
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 12
gacgctccta gctggaa

17

<210> 13
<211> 19
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 13
gttgttatgg aacgcgaag
19

<210> 14
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 14
gggtttctac aggacgtaac a
21

[0231]

<210> 15
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 15
ctgttgaatt acgttaagca tg
22

<210> 16
<211> 20
<212> DNA
<213> 人工序列

<220>
<223> 化学合成的

<400> 16
cattggggac ctaaatactg
20

<210> 17
<211> 119

<212> PRT
<213> African swine fever virus (非洲猪瘟病毒)

<400> 17

Met Leu His Trp Gly Pro Lys Phe Trp Arg Thr 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 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

[0232]

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

Ile Gly Ala Trp Ser Ser Leu
115

<210> 18
<211> 119
<212> PRT
<213> African swine fever virus (非洲猪瘟病毒)

<400> 18

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 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

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> 19
<211> 119
<212> PRT
<213> African swine fever virus (非洲猪瘟病毒)

<400> 19

[0233]

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> African swine fever virus (非洲猪瘟病毒)

<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

[0234] 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> African swine fever virus (非洲猪瘟病毒)

<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> African swine fever virus (非洲猪瘟病毒)

<400> 22

[0235]

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> African swine fever virus (非洲猪瘟病毒)

<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

[0236] 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> African swine fever virus (非洲猪瘟病毒)

<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> African swine fever virus (非洲猪瘟病毒)

<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

[0237]

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> African swine fever virus (非洲猪瘟病毒)

<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> African swine fever virus (非洲猪瘟病毒)

<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

[0238] 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> African swine fever virus (非洲猪瘟病毒)

<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> African swine fever virus (非洲猪瘟病毒)

<400> 29

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

[0239]

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> African swine fever virus (非洲猪瘟病毒)

<220>

<221> misc_feature
<222> (31)..(32)
<223> Xaa 可以是任何天然存在的氨基酸

<220>
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<222> (42)..(43)
<223> Xaa 可以是任何天然存在的氨基酸

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<221> misc_feature
<222> (67)..(68)
<223> Xaa 可以是任何天然存在的氨基酸

<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

[0240]

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
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<213> African swine fever virus (非洲猪瘟病毒)

<220>
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<222> (43)..(44)
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<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
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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
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<213> African swine fever virus (非洲猪瘟病毒)

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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

[0242] Ser Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
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<400> 34

Met Ser Thr His Asp Cys Ser Pro Lys Glu Lys Pro Val Asp Met Asn
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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
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Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe Ser
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Val Phe Gly Lys Gly Ser Leu Met Arg Ser Ile Gln Lys Asn Asn
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<400> 35

Met Ser Thr His Asp Cys Phe Ser Lys Glu Lys Pro Val Asp Met Asn
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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

[0243] Val Pro Cys Cys Cys Pro Arg Lys Met Thr Ser Ala Asp Lys Lys Phe
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Ser Val Phe Gly Lys Gly Ser Leu Ile Arg Ser Ile Gln Lys Asn Asn
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<213> African swine fever virus (非洲猪瘟病毒)

<400> 36

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
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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> African swine fever virus (非洲猪瘟病毒)

<400> 37

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
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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

[0244] 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

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<213> African swine fever virus (非洲猪瘟病毒)

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<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> African swine fever virus (非洲猪瘟病毒)

[0245]
<220>
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<222> (43)..(44)
<223> Xaa 可以是任何天然存在的氨基酸

<400> 39

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Met Asn
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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

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<211> 96
<212> PRT
<213> African swine fever virus (非洲猪瘟病毒)

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<223> Xaa 可以是任何天然存在的氨基酸

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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

[0246]

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> African swine fever virus (非洲猪瘟病毒)

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<223> Xaa 可以是任何天然存在的氨基酸

<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

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<212> PRT
<213> African swine fever virus (非洲猪瘟病毒)

[0247]
<220>
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<223> Xaa 可以是任何天然存在的氨基酸

<400> 42

Met Ser Thr His Asn Asn Ser Pro Lys Glu Lys Pro Val Asp Val Asn
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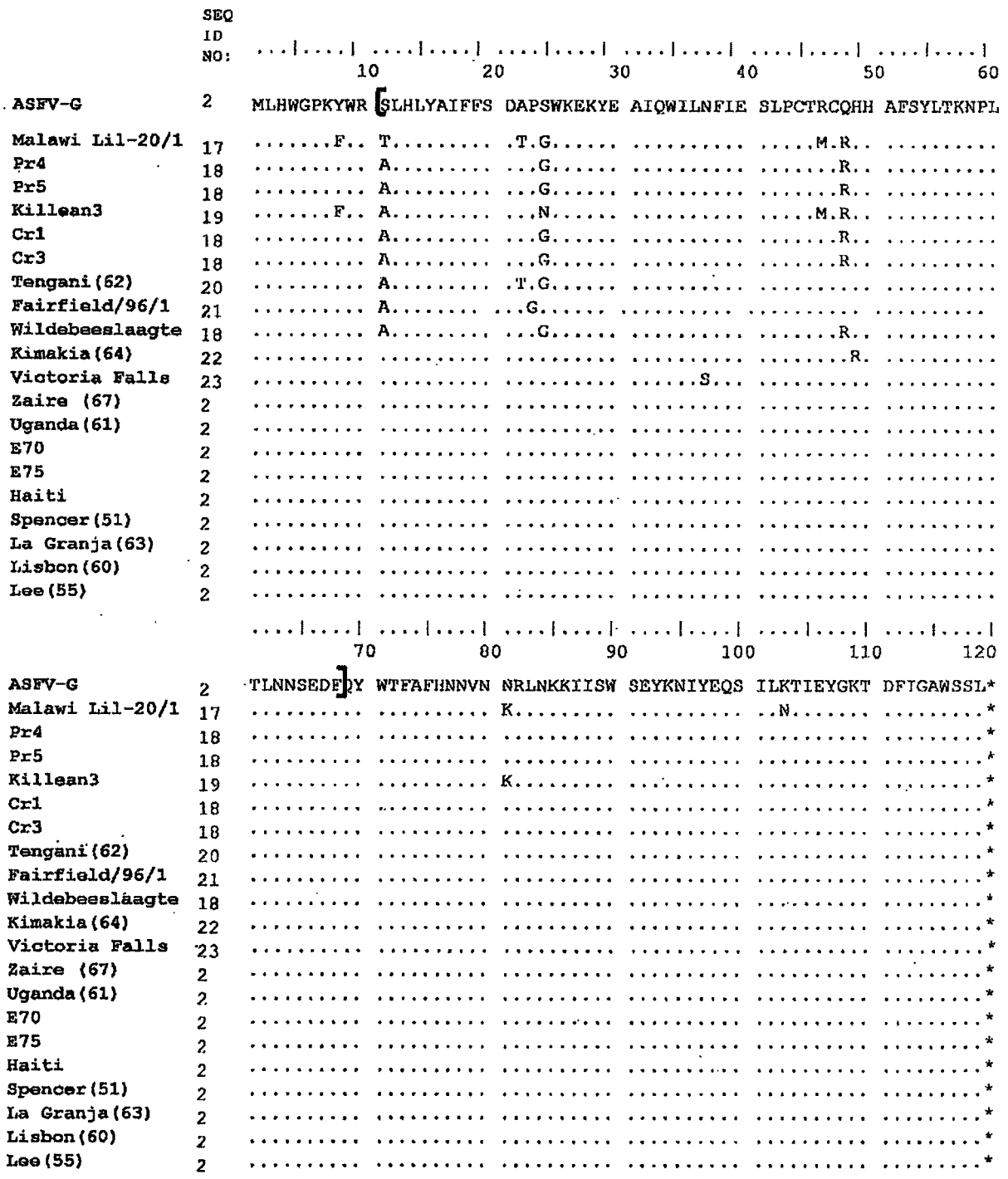
20 25 30

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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Cr1 18
Cr3 18
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Fairfield/96/1 21
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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
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Pr4 18
Pr5 18
Killean3 19
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
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图1A

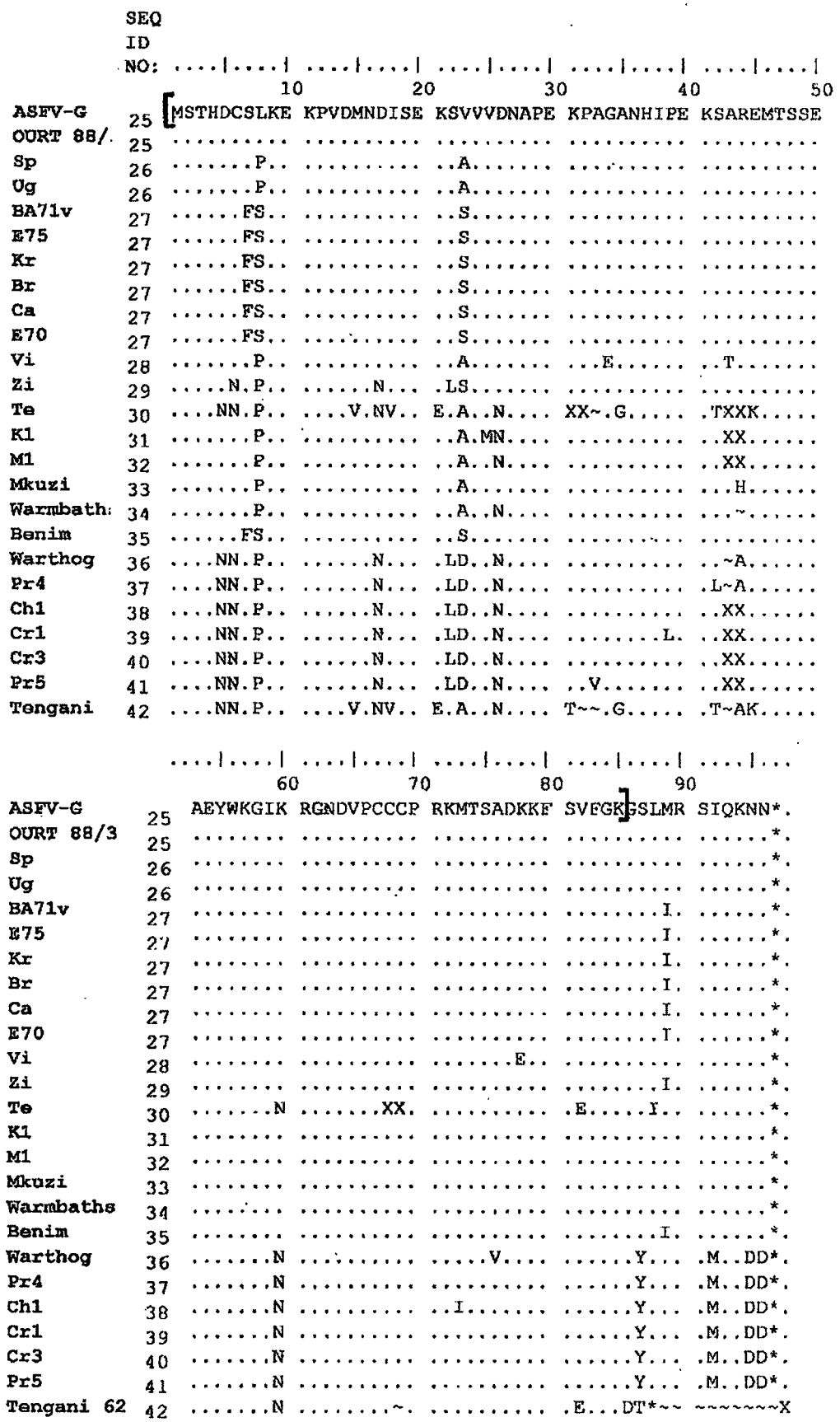
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图1B