



(10) 授权公告号 CN 108697785 B

(45) 授权公告日 2022.08.02

(21) 申请号 201680074939.X

(22) 申请日 2016.12.23

(65) 同一申请的已公布的文献号
申请公布号 CN 108697785 A

(43) 申请公布日 2018.10.23

(30) 优先权数据

15202585.4 2015.12.23 EP

16161068.8 2016.03.18 EP

16176025.1 2016.06.23 EP

16176049.1 2016.06.23 EP

16182845.4 2016.08.04 EP

(85) PCT国际申请进入国家阶段日
2018.06.20

(86) PCT国际申请的申请数据
PCT/EP2016/082664 2016.12.23

(87) PCT国际申请的公布数据
W02017/109225 EN 2017.06.29

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(51) Int.Cl.
A61K 39/12 (2006.01)
C07K 14/005 (2006.01)

(56) 对比文件

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W0 02074963 A1, 2002.09.26

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审查员 陈依晗

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(54) 发明名称

Zika病毒疫苗

(57) 摘要

本文描述了Zika病毒疫苗和组合物以及生产所述疫苗和将其施用于有需要的受试者的方法。

1. 一种包含灭活Zika病毒的Zika病毒疫苗,其中,所述Zika病毒疫苗能够对至少70%的施用所述Zika病毒疫苗的受试者赋予血清保护,并且其中所述Zika病毒的RNA基因组对应于由SEQ ID NO:72提供的DNA序列,并且其中所述Zika病毒疫苗含有含量低于1 μ g/mL的硫酸鱼精蛋白(PS)。

2. 根据权利要求1所述的Zika病毒疫苗,其中,所述Zika病毒能够对至少75%、80%、90%、95%、96%、97%、98%或至少99%的施用Zika病毒疫苗的接种疫苗受试者赋予血清保护。

3. 根据权利要求2所述的Zika病毒疫苗,其中,所述Zika病毒能够对至少80%的受试者赋予血清保护。

4. 根据权利要求1所述的疫苗,其中,所述Zika病毒通过化学灭活、热灭活、pH灭活或UV灭活来灭活。

5. 根据权利要求4所述的疫苗,其中,所述化学灭活包括:使Zika病毒与化学灭活剂接触的时间比通过斑块测定所测量的完全灭活Zika病毒所需的更久。

6. 根据权利要求5所述的疫苗,其中,所述化学灭活包括使Zika病毒与甲醛接触。

7. 根据权利要求6所述的疫苗,其中,所述甲醛灭活包括使Zika病毒与甲醛接触2-10天。

8. 根据权利要求4所述的疫苗,其中,所述化学灭活在约+4 $^{\circ}$ C或约+22 $^{\circ}$ C进行。

9. 根据权利要求1所述的疫苗,其进一步包含佐剂和/或一种或多种药学上可接受的赋形剂。

10. 根据权利要求9所述的疫苗,其中,所述佐剂是铝盐佐剂。

11. 根据权利要求10所述的疫苗,其中,所述铝盐佐剂是氢氧化铝或磷酸铝。

12. 根据权利要求9所述的疫苗,其中,所述疫苗包含或进一步包含:含有肽和含有脱氧肌苷的免疫刺激性寡聚脱氧核酸分子I-ODN的佐剂。

13. 根据权利要求12所述的疫苗,其中,所述肽的序列为SEQ ID NO:71,并且I-ODN为寡聚-d(IC)13,其序列为SEQ ID NO:70。

14. 根据权利要求1所述的疫苗,其中所述疫苗含有硫酸鱼精蛋白(PS),其含量低于100ng/mL。

15. 根据权利要求1所述的疫苗,其中所述硫酸鱼精蛋白(PS)能够通过质谱或其它敏感方法检测。

Zika病毒疫苗

技术领域

[0001] 本公开涉及用于纯化用于疫苗的Zika病毒的方法,具体涉及改进的蔗糖梯度处理步骤,其允许分离诸如硫酸鱼精蛋白的杂质。本公开还涉及Zika病毒疫苗和组合物以及用于生产所述疫苗和将疫苗施用于受试者以产生抗Zika病毒免疫应答的方法。

背景技术

[0002] 硫酸鱼精蛋白的不良反应已经已知多年。先前暴露于鱼精蛋白能够诱发体液免疫反应,并使易感个体易于发生随后使用该药物的不良反应。通过使用含鱼精蛋白的胰岛素或在肝素中和过程中暴露于鱼精蛋白的患者在静脉内接受大剂量鱼精蛋白后可能会发生危及生命的反应和致命的过敏反应。在没有皮下注射含鱼精蛋白的胰岛素的局部或全身性过敏反应的情况下,能够发生对静脉内鱼精蛋白的严重反应。虽然没有明确的证据表明与接种疫苗有关的硫酸鱼精蛋白过敏反应,但含有鱼精蛋白杂质的疫苗在其标签中有一项预防和禁忌警告,表明在先前剂量的含鱼精蛋白疫苗后的严重过敏反应(如IXIARO®,参见疾病预防控制中心网站<http://www.cdc.gov/japaneseencephalitis/vaccine/>)是进一步剂量的禁忌症。因此消除所述杂质是提高安全性的医学要求。另一方面,硫酸鱼精蛋白是一种极好的工具(通常比其它工具如benzonase更好)以纯化在细胞基质上生长的病毒的粗收获物。

[0003] 自从1947年在乌干达发现于猕猴以来,在2007年第一次在亚洲和非洲的流行地区以外发现了Zika病毒。从那时起,该病毒在法属波利尼西亚引起了大规模流行,到2015年在太平洋岛屿和南美和中美洲传播(世界卫生组织“Zika情况报告”,2016年2月5日)。有证据表明,除由伊蚊种类蚊子传播外,还可能存在其它载体,并且可通过输血、经胎盘传播和通过性传播来传播病毒(世卫组织Zika病毒情况说明,2016年2月)。尽管Zika病毒感染的症状包括轻度发热、皮疹和结膜炎,但感染与神经系统疾病之间可能存在相关性,包括妊娠期感染后的胎儿/新生儿中的吉兰-巴雷综合征和小头畸形。目前没有针对Zika病毒的特定治疗或疫苗,唯一预防措施是控制蚊子载体。由于伊蚊的广泛流通,多种传播途径以及潜在的严重的感染神经系统影响,Zika病毒对公共卫生构成严重威胁。

[0004] 针对Zika病毒的预防性疫苗是流行地区和传播媒介地理区域的迫切医疗需求。此外,由于Zika病毒感染对胚胎和胎儿发育具有严重后果,因此需要一种安全有效的疫苗用于生育潜能女性或怀孕妇女。怀孕期间的施用疫苗对母亲和发育中的胎儿都必须非常安全。尽管减毒活病毒疫苗非常有效,但通常认为它们施用于怀孕妇女不足够安全。在这方面,在接种疫苗的受试者中缺乏繁殖能力的灭活病毒疫苗被认为更安全。开发灭活的Zika病毒疫苗以施用于有风险的患者可以满足这种需求。

发明内容

[0005] 在病毒纯化过程中,观察到如所预期的那样,在细胞基质上产生的病毒收获物中加入硫酸鱼精蛋白不仅去除了源自宿主细胞的DNA,而且还实际上从制剂中消除了未成熟

的和非感染性的病毒颗粒。这一发现为获得高纯度的感染性病毒颗粒以用于诸如疫苗制剂的应用提供了简化、温和、可重复和广泛适用的过程。另外,令人惊讶地发现,所述硫酸鱼精蛋白能够非常有效地从病毒级分中分离,允许以高产率生产更安全的疫苗。

[0006] 本文公开了包含灭活的Zika病毒的病毒疫苗和组合物,以及生产所述疫苗和组合物的相关方法。还提供了施用Zika病毒疫苗以在受试者中预防Zika病毒感染和/或产生抗病毒免疫应答的方法,例如有风险暴露于Zika病毒的受试者。特别地,本发明涉及包含最佳灭活的Zika病毒颗粒的病毒疫苗,其中适当剂量的Zika病毒颗粒能够以至少70%的可能性,优选80%的可能性使施用病毒疫苗的受试者血清转化。本发明的另一个优点是生产所述疫苗和组合物的相关方法是非常有效的并提供很大程度上不含杂质特别是硫酸鱼精蛋白的纯组合物,从而允许大量生产疫苗。针对Zika病毒提供了上述的详细实验示例。

[0007] 本文公开的关于本发明的灭活Zika病毒疫苗的免疫原性的体内数据表明,该病毒具有惊人的有效免疫原性并且还具有高度的交叉保护性(在非洲和亚洲菌株中非常相似的免疫原性)。数据表明,免疫原性出乎意料地高于最近报道的灭活Zika病毒候选疫苗(Larocca等,2016,Nature doi:10.1038/nature18952.)。灭活病毒是最安全的疫苗之一,尤其优选用于向安全性特别关注的人群(例如孕妇、儿童和免疫受损个体)递送,这使得本文公开的灭活Zika病毒特别合适。获得高滴度的灭活病毒是该领域的挑战。本文公开的用于纯化灭活的Zika病毒的方法不仅产生高产量,而且产生非常纯的药物物质。

[0008] 本发明的每个限制能够包含本发明的各种实施方式。因此,预计涉及任何一个要素或要素组合的本发明的每个限制能够包括在本发明的每个方面中。本发明的应用不限于以下说明书中阐述的或附图中所示的组件的构造和布置的细节。本发明能够具有其它实施方式并且能够以各种方式实践或执行。

附图说明

[0009] 不意在按比例绘制附图。这些图仅是说明性的,并不是实现本公开所必需的。为了清楚起见,并非每个组件都可以在每个图中标记,使用多重比对包Jalview(Waterhouse等,2009,Bioinformatics 25(9)1189-1191)进行比对。在附图中:

[0010] 图1:平均距离树(以%同一性,nt),完整的基因组。

[0011] 图2:相邻连接树(以%同一性,nt),完整的基因组。

[0012] 图3:成对比对-Jalview(%同一性,nt),完整的基因组。

[0013] 图4:平均距离树(以%同一性,aa),E蛋白。

[0014] 图5:相邻连接树(以%同一性,aa),E蛋白。

[0015] 图6:成对比对-Jalview(%同一性,aa),E蛋白。

[0016] 图7:比对(阴影:%同一性,aa),E蛋白。

[0017] 图8:宿主细胞中病毒颗粒成熟的示例。如在黄病毒中所观察到的,颗粒的完全成熟需要通过宿主蛋白酶弗林蛋白酶对前体膜糖蛋白(prM)进行蛋白水解切割。并非所有的prM分子都被切割,导致从细胞中释放成熟的、嵌合的或未成熟样的构象。嵌合和未成熟形式通常不具感染性,只有成熟病毒颗粒具有感染性并具有血凝素(HA)/TCID50活性。(图改编自Plevka等,黄病毒的成熟起始于一个或多个二十面体独立的成核中心,EMBO reports (2011) 12,602-606)。

[0018] 图9:从粗收获物到药物物质(疫苗)制剂的示例性下游病毒纯化方法,即本发明方法(A)的优选实施方式。(B)中示出了示例性病毒灭活过程的流程图。

[0019] 图10:选择性除去Zika病毒聚集体以及Vero HCP和LMW杂质(30×浓缩Zika病毒收获第5天的SEC-HPLC)的PS处理结果。

[0020] 图11:在不同时间点的PS处理之前的单独的30×浓缩Zika收获物的SEC-HPLC。

[0021] 图12:在不同时间点的PS处理之后的单独的30×浓缩Zika收获物的SEC-HPLC。较小的图表显示随时间观察到的细胞病变效应(CPE)。

[0022] 图13:制备蔗糖梯度。

[0023] 图14:显示了来自Zika纯化的蔗糖梯度收获物的代表性SDS-PAGE。

[0024] 图15:JEV和ZikaV收获物时间表/收益的比较。

[0025] 图16:ZikaV NIV的SEC-HPLC洗脱曲线。数据在Dionex Ultimate 3000/Superose 6Increase柱上处理。两个图都来自相同的色谱图。上图是完整的洗脱图;下图是ZIKAV洗脱峰的放大图。

[0026] 图17:灭活ZikaV的SEC-MALLS分析。

[0027] 图18:Zika NIV的累积粒度分布。

[0028] 图19:用汇集的小鼠血清中和Zika病毒H/PF/2013的图示。无血清斑块的数量设定为100%。使用3-参数法计算 EC_{50} 。

[0029] 图20:用汇集的小鼠血清中和Zika病毒MR766的图示。无血清斑块的数量设定为100%。使用3-参数法计算 EC_{50} 。

[0030] 图21:通过ELISA和SEC-HPLC(Dionex Ultimate 3000,Superose 6柱)分析的中和灭活病毒(NIV)中的JEV抗原含量之间的相关性。

具体实施方式

[0031] 本文公开了包含灭活的Zika病毒的Zika病毒疫苗和组合物,以及生产所述疫苗和组合物的相关方法。还提供了施用所述病毒疫苗以在受试者中预防病毒感染和/或产生抗病毒免疫应答的方法,例如有风险暴露于病毒的受试者。具体而言,本发明涉及包含最佳灭活的病毒颗粒的病毒疫苗,其中适当剂量的病毒颗粒能够以至少70%的可能性,优选80%的可能性使施用病毒疫苗的受试者血清转化。本发明的另一个优点是生产所述疫苗和组合物的相关方法是非常有效的,并提供很大程度上不含杂质特别是硫酸鱼精蛋白的纯组合物,从而允许大量生产疫苗。针对Zika病毒提供了上述示例。

[0032] 本文公开了纯化来自粗制剂的Zika病毒颗粒的下游方法。下游方法能够应用于未适应在特定细胞底物上繁殖的病毒或应用于部分/完全细胞底物适应的Zika病毒颗粒。

[0033] 本发明的方面提供用于纯化感染性Zika病毒颗粒的方法,其包括以下步骤:(a)提供包含病毒颗粒和杂质的粗收获物(a),其中所述杂质通过在细胞基质上培养所述病毒颗粒而产生;(b)通过用包含鱼精蛋白盐,优选硫酸鱼精蛋白的试剂沉淀来减少粗收获物(a)中的杂质以获得病毒制剂(b);以及通过优化蔗糖密度梯度离心进一步纯化病毒制剂(b)以获得包含感染性病毒颗粒的病毒制剂(c)。

[0034] 在一些实施方式中,步骤(b)中的硫酸鱼精蛋白的浓度为约1至10mg/ml,更优选约1至5mg/ml,更优选约1至2mg/ml。在一个实施方式中,步骤(b)中的硫酸鱼精蛋白的浓度为

约2mg/mL。在一个实施方式中,硫酸鱼精蛋白的浓度为1.2至1.8mg/ml,更优选1.4至1.6mg/ml。在一个优选实施方式中,步骤(b)中的硫酸鱼精蛋白的浓度为约2mg/ml。

[0035] 在一些实施方式中,病毒制剂(c)的残余宿主细胞DNA小于1 μ g/mL,特别是小于900、800、700、600、500、400、300或200ng/mL,优选小于100ng/mL。在一个优选实施方式中,病毒制剂(c)的残余宿主细胞DNA小于10ng/mL。在一些实施方式中,最终病毒制剂(c)的残余宿主细胞蛋白小于10 μ g/mL,特别是小于9、8、7、6、5、4、3或2 μ g/mL,优选小于1 μ g/mL。在一个优选实施方式中,病毒制剂(c)的残余宿主细胞蛋白小于100ng/mL。在一些实施方式中,最终病毒制剂(c)的残余非感染性病毒颗粒小于10 μ g/mL,特别是小于9、8、7、6、5、4、3或2 μ g/mL,优选地小于1 μ g/mL。在一个优选实施方式中,病毒制剂(c)的残余非感染性病毒颗粒的含量小于100ng/mL。

[0036] 在一些实施方式中,残余鱼精蛋白小于1 μ g/mL,特别是小于900、800、700、600、500、400、300或200ng/mL,优选小于100ng/mL,更优选小于HPLC的检测限,特别是低于最终药物的检测限。在一些实施方式中,通过HPLC或尺寸排阻色谱法(SEC)测试PS含量。例如,HPLC在JEV蔗糖梯度池样品中作为常规释放测定进行PS测定是有效的并且非常灵敏(即,LOQ 3 μ g/mL;LOD1 μ g/mL)。在本发明中,Zika病毒DS样品中的PS含量<LOD。在一个实施方式中,PS含量的HPLC评估可以使用30%乙腈、0.1%三氟乙酸作为溶剂,以0.6ml/min的流速在25°C下并在214nm处检测,而在Superdex Peptide 10/300GL柱(GE:17-5176-01)上进行。在纯化病毒制剂中测量残余鱼精蛋白的更灵敏方法是质谱法(MS)。在一些实施方式中,Zika病毒制剂中的残余PS水平通过MS或其它此类高灵敏度的方法例如核磁共振(NMR)来测试。用这种方法,残余PS以及PS的片段和/或分解产物可以以痕量进行检测,例如水平低至例如10⁶、10⁷或10⁸个分子/典型样品负载。在一些实施方式中,在蔗糖梯度池中测试PS水平。在一些实施方式中,在药物产品中测试PS水平。在一些实施方式中,在药物物质中测试PS水平。

[0037] 在一些实施方式中,包含病毒颗粒和杂质的粗收获物(a)在步骤(b)之前进行一个或多个预纯化步骤。在一些实施方式中,一个或多个预纯化步骤包括通过酶处理在包含病毒颗粒和杂质的粗收获物(a)中消化宿主细胞基因组DNA。在一些实施方式中,一个或多个预纯化步骤包括过滤、超滤、浓缩、缓冲液交换和/或渗滤。在一些实施方式中,一个或多个预纯化步骤是使用孔径等于或小于1 μ m的过滤器进行过滤。在一些实施方式中,过滤器具有等于或小于0.2 μ m的孔径。在一个优选实施方式中,过滤器具有0.2 μ m的孔径。在一些实施方式中,通过切向流过滤(TFF)进行浓缩和/或超滤/渗滤和/或缓冲液交换。在一些实施方式中,使用截留值等于或小于300kDa的中空纤维膜对包含病毒颗粒和杂质的粗收获物(a)进行超滤/渗滤。在一个优选实施方式中,中空纤维膜具有约100kDa的截留值。

[0038] 根据本发明的方法还可以包括使用蔗糖梯度,优选优化的蔗糖梯度。蔗糖梯度优选优化用于去除硫酸鱼精蛋白,也用于去除未成熟的病毒颗粒或未感染的病毒颗粒或宿主细胞蛋白或核酸(DNA、RNA、mRNA等)或其它宿主细胞碎片。在本发明中,优化蔗糖梯度包含至少两个、至少三个、至少四个具有不同密度的蔗糖溶液层。在一个实施方式中,待纯化的病毒制剂提供于具有约8%、约9%、约10%、约11%、约12%蔗糖密度(w/w)的蔗糖溶液中,优选约10%。在一个实施方式中,一种梯度的蔗糖溶液具有约45%、约46%、约47%、约48%、约49%、约50%、约51%、约52%、约53%、约54%、约55%蔗糖密度(w/w),优选约50%。在一个实施方式中,一种梯度的蔗糖溶液具有约30%、约31%、约32%、约33%、约

34%、约35%、约36%、约37%、约38%、约39%、约40%蔗糖密度(w/w)，优选约35%。在一个实施方式中，一种梯度的蔗糖溶液具有约10%、约11%、约12%、约13%、约14%、约15%、约16%、约17%、约18%、约19%、约20%蔗糖密度(w/w)，优选约15%。在一个优选的实施方式中，蔗糖梯度包含约50%、约35%和约15% (w/w) 蔗糖的三个蔗糖溶液层，待纯化的病毒组合物包含在约10% (w/w) 蔗糖中。因为本发明提供了不仅测试宿主细胞DNA而且测试未成熟病毒颗粒的手段，本领域技术人员能够更精确地优化蔗糖梯度以进行最有效的纯化，并且包括另外的工具，例如PRNT测定以监测纯化成功。

[0039] 在一些实施方式中，病毒颗粒是活病毒、嵌合病毒、减毒活病毒、修饰的活病毒或重组活病毒。在进一步的步骤中，本发明的病毒颗粒可以任选地灭活。在一些实施方式中，病毒颗粒是病毒颗粒的减毒形式。例如，与野生型病毒相比，病毒可能具有降低的宿主感染性、毒力和/或复制。在一些实施方式中，病毒是突变或修饰的病毒，例如病毒的核酸可以含有相对于野生型病毒至少一个突变。在一些实施方式中，病毒是重组活病毒，意指重组产生的病毒，并且可含有来自不同来源的核酸。

[0040] 在一些实施方式中，Zika病毒颗粒是活病毒、减毒活病毒、修饰的活病毒或重组活病毒。在优选的实施方式中，Zika病毒是来自亚洲谱系的Zika病毒。

[0041] 在一些实施方式中，最终病毒制剂的杂质的相对减少量相对于包含病毒颗粒和杂质的液体培养基(a)在60至95%的范围内。在一些实施方式中，最终病毒制剂的残余杂质小于1%。

[0042] 在一些实施方式中，Zika病毒在选自以下的细胞系中增殖：EB66细胞系、Vero细胞系、Vero- α His细胞系、HeLa细胞系、HeLa-S3细胞系、293细胞系、PC12细胞系、CHO细胞系、3T3细胞系、PerC6细胞系、MDSK细胞系、鸡胚胎成纤维细胞系、鸭细胞系和二倍体禽细胞系。在一些实施方式中，所述细胞系是鸭细胞系。在一些实施方式中，所述细胞系是二倍体禽类细胞系。在一些实施方式中，所述细胞系是EB66细胞系。在一个优选的实施方式中，所述细胞系是Vero细胞系。

[0043] 本发明的各方面提供了本文所述的任何方法在制造抗病毒感染免疫的组合物中的用途。在一个优选实施方式中，组合物或疫苗针对Zika病毒，例如亚洲谱系的Zika病毒。

[0044] 其它方面提供了包含通过本文所述的任何方法可获得的用于治疗 and/或预防(即保护)病毒感染的Zika病毒颗粒的组合物。在一个优选实施方式中，病毒感染是由Zika病毒引起的，例如亚洲谱系的Zika病毒。

[0045] 此外，本文公开了包含灭活的Zika病毒的Zika病毒疫苗和组合物，以及生产所述疫苗和组合物的相关方法。还提供了施用Zika病毒疫苗以在受试者中预防Zika病毒感染和/或产生抗Zika病毒免疫应答的方法，例如有风险暴露于Zika病毒的受试者。

[0046] Zika病毒是一种与登革热病毒密切相关的黄病毒，并且类似地被伊蚊种类蚊子传播，尽管Zika病毒的其它节肢动物载体也是可能的。自从它于1947年首次从乌干达Zika森林的猕猴中分离出来以后，很少有人报道过人类感染的事件，特别是在非洲和亚洲流行地区之外，直到2007年法属波利尼西亚发生大规模爆发(Haddow等, PLoS Neglected Tropical Diseases (2012) 6 (2), Malone等, PLoS Neglected Tropical Diseases (2016) 10 (3))。此后，该病毒在太平洋岛屿(包括大洋洲)和南美和中美洲中传播(世界卫生组织“Zika情况报告”，2016年2月5日)。

[0047] 除了被感染的蚊子叮咬传播之外,证据还表明个体之间可能会发生传播,例如受感染个体的血液,从感染的母体到胎儿的经子宫/胎盘传播,性伴侣之间的性传播,并可能通过其它局部传输路线。孕期Zika病毒感染与胎儿/新生儿小头畸形之间可能存在关联。小头畸形是一种罕见病症,婴儿的头围显着低于基于年龄、性别和种族的平均值所预期的。这是大脑未能进行适当的胚胎发育的结果,并且90%的病例与智力低下有关(Rocha等(2016) Bull World Health Organ, 2016年2月8日)。

[0048] 已经患有Zika病毒感染的个体与吉兰-巴雷综合症的发病率之间可能存在关联,吉兰-巴雷综合征是一种神经疾病,其中个体的免疫系统破坏周围神经系统轴突周围的髓鞘(世界卫生组织“Zika情况报告”,2016年2月5日)。

[0049] 目前还没有针对Zika病毒的特定治疗或疫苗,目前唯一的防止感染的措施是通过病媒控制并避免前往发生爆发的地区。

[0050] 本文描述的是包含灭活的Zika病毒的Zika病毒疫苗和组合物,其提供了可以帮助预防Zika病毒感染的安全方法,用于产生对Zika病毒的免疫应答,包括病毒中和抗体。

[0051] 任何Zika病毒株均可用于本文所述的方法和组合物中。在一些实施方式中,Zika病毒是在Zika病毒爆发期间来自受感染受试者的分离物。在一些实施方式中,Zika病毒是从非洲或非洲病毒谱系分离的菌株。在一些实施方式中,Zika病毒是从亚洲或亚洲谱系分离的菌株(还包括来自法属波利尼西亚的菌株)。在一些实施方式中,Zika病毒是从美洲(南美洲、中美洲或北美)分离的菌株,例如苏里南Zika病毒株。在一些实施方式中,Zika病毒具有相对应于(但不限于)由GenBank登录号AY632535.2、KU321639.1、KU497555.1、KU501215.1、KU509998.1、KU527068.1、KU681081.3、KU681082.3、KU707826.1、KU744693.1或LC002520.1提供的DNA序列的RNA基因组,或本文部分或完全披露的RNA基因组(SEQ ID NO:2至69)。

[0052] 在一些实施方式中,本发明的方法导致来自包含感染性Zika病毒颗粒和非感染性病毒颗粒和其它病毒产物的粗收获物的感染性Zika病毒颗粒的富集,使得感染性病毒颗粒的富集相对于包含病毒颗粒和杂质的粗收获物(a)的总病毒颗粒含量为至少50%、至少60%、至少70%、至少80%、至少90%、至少95%、优选至少80%、尤其至少85%。

[0053] 在一些实施方式中,相对于粗收获物中的所有杂质,最终病毒制剂的残余杂质小于10%、9%、8%、7%、6%、5%、4%、3%、2%、1%,优选小于5%,如通过SEC-HPLC(尺寸排除色谱-HPLC)测定的。

[0054] 本发明的一个独特方面是认识到,可以采用用于日本脑炎疫苗(JEV)的疫苗设计和纯化方法有关的技术知识(参见Srivastava AK等人,2001,Vaccine 19,4557-4565, W099/11762)并加以改进以加速开发Zika病毒疫苗并尽快将其提供给有需要的受试者。针对IXIARO®公开的工业过程提供了针对JEV的非常有效的疫苗,其补充了本文公开的进一步显著改进以提供相比于可用的JEV疫苗更有效(更高产量)和更安全(具有过敏潜力的较少或不具有硫酸鱼精蛋白)的Zika疫苗。本文公开的疫苗的特别创新是它们通过发展改善的蔗糖梯度而促进的最终药物物质中大大降低的鱼精蛋白盐(SEQ ID NO:1)含量。所述蔗糖梯度不仅允许分离硫酸鱼精蛋白,而且还允许通过甲醛进行非常有效的灭活,并且导致Zika具有超过90%的产率(采用本文公开的改进方法),与公开的JEV方法的产率约为35%的情况,参见实验部分进行比较。

[0055] 本公开的方面涉及在Vero组织培养细胞中产生病毒的方法。Vero细胞是源自非洲绿猴肾脏的常用组织培养细胞系。本文所述方法中使用的Vero细胞是Vero (WHO) 细胞系, 其从健康保护机构总细胞集合获得, 目录号为88020401。

[0056] Vero细胞可以生长至融合单层, 例如在组织培养瓶中; 在悬浮液中(在微载体上), 例如在滚瓶中; 或用于病毒生产的任何其它细胞培养系统中。在一些实施方式中, Vero细胞在生物反应器中生长以用于病毒生产。对于斑块测定或斑块减少中和测试(PRNT), Vero细胞在组织培养瓶、培养皿或板的孔中以单层生长。为了用病毒感染Vero细胞, 用病毒接种培养基并将细胞与病毒一起温育一段时间。接种后可以洗涤细胞以去除在给定的时间内没有吸附到细胞上的任何病毒。

[0057] 本文提供的方法涉及在Vero细胞中传代病毒。如本文所用, 术语“传代”是指用病毒感染Vero细胞群体, 随后用感染第一Vero细胞群体产生的病毒接种第二Vero细胞群体。在一些实施方式中, 将来自感染的Vero细胞的培养基的一部分(含有从感染细胞释放的病毒)用于接种第二Vero细胞群体。这被称为一次传代或一轮传代。传代可以连续进行, 例如, 将来自感染的第二Vero细胞群体的培养基的一部分用于接种第三Vero细胞群体, 等等。在一些实施方式中, 将从单个斑块获得的病毒用于接种另一细胞群体。

[0058] 在一些实施方式中, 病毒在Vero细胞中传代若干次, 例如至少4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39或40次。在一些实施方式中, 病毒在Vero细胞中传代至少4次或5次。在一些实施方式中, 病毒在Vero细胞中传代至少30次。重要的是病毒群体(即病毒序列)在所述传代中尽可能保持恒定。如果发生病毒适应(即在原始病毒群中出现突变病毒), 则优选所述传代不用于制造所述病毒的情况下, 例如对于Zika, 发现直到第3代和培养到第7天都可以使用, 没有病毒种群的重大变化, 即引入具有突变的病毒种群。然而, 这种观察需要针对每种病毒株进行并且可能不同。

[0059] 在一些实施方式中, Vero细胞在接种例如通常0.01MOI(感染复数)的病毒后温育至少2天, 以允许在传代之前进行病毒生产。在一些实施方式中, 将Vero细胞在传代前的接种病毒后温育至少3、4、5、6、7、8、9、10或超过10天, 例如至少7天。Vero细胞在病毒接种后培养的天数取决于诸如用于接种细胞的感染多样性和培养基中所需的病毒滴度的因素。病毒在Vero细胞中的连续传代可能导致产生适应Vero细胞的病毒株。

[0060] 可以收获(收集)来自感染的Vero细胞的培养基以获得病毒。在一些实施方式中, 从感染的Vero细胞收获培养基并用新鲜培养基代替, 然后在另一段时间后收获。在一些实施方式中, 从感染的Vero细胞收获的培养基汇集自独立的Vero细胞培养物和/或独立的天数。例如, 感染后7或9天可以重复收获4次, 并且每单位细胞培养物产生高的病毒产量。为了最小化Zika病毒株对Vero细胞的适应性, 发现Vero细胞可以在传代前温育至少7天, 更优选5天, 随后可以在第2、3、5和7天或第2、3和5天收获上清液(另见实验部分)。收获的培养基可以保存在+4℃, 然后从培养基中纯化病毒长达2周。

[0061] 在一些实施方式中, 来自感染和裂解的Vero细胞的碎片可以从收获的培养基中去除, 称为培养基的“澄清”。收获的培养基可以通过本领域已知的常规方法澄清, 例如低速离心, 例如在1500g下10分钟, 和/或通过孔径0.45μm的过滤器过滤。收获的培养基可以在浓缩前储存在+4℃。

[0062] 为了将Zika病毒的滴度浓缩在收获的培养基中,可以通过本领域已知的任何方法对其进行浓缩。例如,可以通过包括但不限于超滤、超速离心、离心浓缩器、真空离心和冻干的方法来浓缩收获的培养基。在一些实施方式中,通过超滤浓缩收获的培养基并收集含有Zika病毒的保留物。在一些实施方式中,通过沉淀浓缩收获的培养基,其中将聚乙二醇(PEG) 8000溶解在培养基中(至多10%),并将沉淀物溶解在缓冲液例如磷酸盐缓冲盐水(PBS, pH 7.0)中。

[0063] 可以将收获的培养基沉淀以产生病毒上清液。在一些实施方式中,将收获的培养基沉淀以从收获的培养基中去除Vero细胞DNA和其它不需要的物质,例如Vero细胞碎片。在一些实施方式中,收获的培养基在沉淀之前浓缩。在一些实施方式中,通过将硫酸鱼精蛋白(例如SEQ ID NO:1)加入到收获的培养基中并且例如在+4℃或在冰上温育混合物来沉淀收获的培养基。在一些实施方式中,用benzonase处理收获的培养基以从收获的培养基中去除Vero细胞DNA和其它不需要的物质,例如Vero细胞碎片。然而,发现硫酸鱼精蛋白处理是优选的(参见实验部分)。在一些实施方式中,将沉淀的培养基离心以收集沉淀的物质,并收集称为“病毒上清液”的含有病毒的上清液。

[0064] 沉淀后可以进一步纯化病毒上清液,例如密度梯度超速离心。在一些实施方式中,通过蔗糖梯度进一步纯化病毒上清液。可以从蔗糖梯度收集级分并分析病毒的存在。分析病毒阳性级分的方法包括斑块测定、血凝测定、聚丙烯酰胺凝胶电泳和抗原测定(如Western印迹和ELISA)。根据病毒的滴度和其它杂质的含量,可以汇集含有病毒的级分。存在于病毒上清液中的杂质水平或量可以通过测试Vero细胞DNA、病毒聚集体和/或Vero细胞蛋白来估计(参见实验部分)。本发明的具体实施方式是改进的蔗糖梯度,其允许如实验部分所示的有效的鱼精蛋白分离。令人惊讶地发现,将具有10% (w/w) 蔗糖的含病毒级分添加到简单三层的蔗糖密度梯度(例如包含15% (w/w) 蔗糖溶液,35% (w/w) 蔗糖溶液和50% (w/w) 蔗糖溶液)导致有效分离硫酸鱼精蛋白,而没有很多病毒损失。因此,本发明的一个特别优选的实施方式是使用能够有效地分离硫酸鱼精蛋白的蔗糖密度梯度,其中所述蔗糖密度梯度用于纯化病毒,例如本文所述的病毒,即Zika病毒。

[0065] 为了获得用于对受试者施用的安全疫苗或组合物,病毒上清液可以是灭活的(参见Zika病毒的实验部分)。如本文所用,术语“灭活的”和“最佳灭活的”可以互换使用,指的是使病毒不能感染宿主细胞(非感染性)的过程(或其结果),但不影响或基本上不影响病毒的抗原性,例如暴露于病毒表面的免疫原性抗原能够刺激受试者的免疫应答(例如抗原特异性抗体)。所谓“不影响或基本上不影响病毒的抗原性”是指灭活的病毒保留至少50%、至少55%、至少60%、至少65%、至少70%、至少75%、至少80%、至少85%、至少90%、至少95%、至少96%、至少97%、至少98%、至少99%、或甚至基本上100%的未进行灭活的病毒的抗原性。

[0066] 本领域已知多种用于灭活病毒的方法。在一些实施方式中,通过化学灭活、热灭活、pH灭活或UV灭活使病毒灭活。

[0067] 在一些实施方式中,灭活是通过化学灭活并且包括使病毒与一种或多种化学灭活剂在使病毒灭活但抗原性表位基本上完整的条件下接触一段时间。在一些实施方式中,病毒灭活的时间长于完全灭活病毒所需的时间。在一些实施方式中,将病毒上清液灭活病毒灭活所需的天数加上至少一天的额外天数。病毒上清液的样品可以在整个灭活过程中进行

一次或多次,并通过本领域已知的任何方法,例如通过感染单层宿主细胞(即斑块试验)来评估病毒活力(感染力)。使用这样的程序,可以确定完全灭活病毒所需的时间段,并且选择较长的时间段以确保完全灭活。

[0068] 在一些实施方式中,使病毒与化学灭活剂接触1天至50天、2天至40天、2天至30天、2天至20天、2天至10天、3天至9天、4天至8天、5天至7天、2天至5天、或5天至10天。在一些实施方式中,病毒与一种或多种化学灭活剂接触至少1天、2天、3天、4天、5天、6天、7天、8天、9天、10天、11天、12天、13天、14天、15天、16天、17天、18天、19天、20天、21天、22天、23天、24天、25天、26天、27天、28天、29天、30天、31天、32天、33天、34天、35天、36天、37天、38天、39天、40天、41天、42天、43天、44天、45天、46天、47天、48天、49天或至少50天。

[0069] 在一些实施方式中,化学灭活在约+5℃、+10℃、+15℃、+20℃、+25℃、+30℃、+35℃、+40℃或约+45℃下进行。在一些实施方式中,化学灭活在约+4℃下进行。在一些实施方式中,化学灭活在约+22℃下进行。

[0070] 本领域已知的任何化学灭活剂可适用于在本文所述的方法中灭活病毒。本领域技术人员将会理解,诸如化学灭活剂和进行灭活的温度的因素可能影响使病毒完全灭活所需的时间长度(天数)。化学灭活剂的实例包括但不限于甲醛、酶、β-丙内酯、乙醇、三氟乙酸、乙腈、漂白剂、脲、盐酸胍、磷酸三正丁酯、亚乙基亚胺或其衍生物、以及有机溶剂如吐温、Triton、脱氧胆酸钠和磺基甜菜碱。优选的灭活是用甲醛在22℃±2℃下灭活约10天。

[0071] 在一些实施方式中,灭活剂在病毒化学灭活后被中和。在一些实施方式中,灭活剂是甲醛并且在使用硫代硫酸钠或偏亚硫酸氢钠进行化学灭活后被中和。

[0072] 在一些实施方式中,通过热灭活使病毒灭活。在一些实施方式中,热灭活包括将病毒暴露于加热(例如干燥加热或蒸气加热)一段时间。在一些实施方式中,热灭活涉及将病毒暴露于约+40℃、+45℃、+50℃、+55℃、+60℃、+65℃、+70℃、+75℃、+80℃、+85℃、+90℃、+95℃或约+100℃。在一些实施方式中,将病毒暴露于加热至少5小时、6小时、7小时、8小时、9小时、10小时、11小时、12小时、13小时、14小时、15小时、16小时、17小时、18小时、19小时、20小时、21小时、22小时、23小时、24小时、36小时、48小时、60小时、72小时、84小时、96小时或更长。优选的热灭活包括将病毒暴露于约+56℃的温度60分钟。

[0073] 在一些实施方式中,通过将病毒暴露于酸性或碱性条件一段时间以使病毒完全灭活来使病毒灭活。可以将病毒制剂的pH调节至所需的pH,例如通过向病毒制剂中加入酸、碱或具有特定pH的缓冲剂。在一些实施方式中,病毒在约2、2.5、3、3.5、4、4.5、5或约5.5的酸性pH下灭活。在其它实施方式中,病毒在约8、8.5、9、9.5、10或约10.5的碱性pH下灭活。

[0074] 在一些实施方式中,使用UV灭活使病毒灭活。UV灭活包括将病毒暴露于高能辐射(例如UV-A、UV-B或UV-C)一段时间。应该理解的是,任何两种或更多种灭活方法可以被组合并且同时或连续地执行。

[0075] 可以随后透析灭活的病毒以除去任何不需要的物质,包括灭活剂和任何中和剂,和/或用药学上可接受的缓冲液代替缓冲剂以施用于受试者。在一些实施方式中,灭活的病毒用PBS透析。另外或可选地,灭活的病毒可以通过0.22μm过滤器过滤,例如无菌过滤。

[0076] 本文所述的任何方法或用途可以用于预防受试者中的Zika病毒感染。如本文所用,术语“预防”、“防止”和“保护”包括向受试者施用病毒疫苗或组合物以减少或延迟临床或亚临床症状表现、并发症、病理的发作或者疾病或感染的生化标志,或减少或抑制Zika病

毒的扩散/传播。如本文所用,可预防性地(例如在感染之前)施用于受试者的抗原(例如灭活Zika病毒)可以被称为疫苗。

[0077] Zika疫苗

[0078] 如本文所述,Zika病毒在感染受试者后可引起多种症状中的任一种,并且通常特征在于轻度发热;面部、颈部、躯干、上臂的皮疹(皮疹);头痛;对光线敏感;非炎性关节痛;结膜炎;食欲不振;腹泻;腹痛;和/或头晕。孕期Zika病毒感染与胎儿/新生儿小头畸形相关。吉兰-巴雷综合征的发作或其症状之间也有可能存在关联。诊断暴露于Zika病毒或疑似暴露于Zika病毒的受试者的Zika病毒感染包括检测病毒特异性抗体的存在和/或分子检测,例如PCR或Zika病毒的实时PCR检测。

[0079] 本文提供用于向有需要的受试者施用治疗有效量的剂量的Zika病毒疫苗的方法。在一些实施方式中,所述受试者是哺乳动物受试者,例如人、非人灵长类动物、啮齿动物、兔子、绵羊、狗、猫、马或牛。在一些实施方式中,所述受试者是小鼠。在一些实施方式中,受试者是人受试者,例如儿童、成人或老年人。在一些实施方式中,受试者是女性受试者。在一些实施方式中,受试者是孕妇或计划成为怀孕。在一些实施方式中,受试者有风险暴露于Zika病毒。在一些实施方式中,受试者住在或前往Zika病毒存在或被认为存在的区域。在一些实施方式中,受试者先前已经感染过或接种过登革热病毒疫苗;即具有抗体依赖性疾病增强的风险。在一些实施方式中,受试者住在或前往正在经历Zika病毒感染暴发的区域。在一些实施方式中,受试者住在或前往能够传播Zika病毒载体的节肢动物载体存在或被认为存在的区域。

[0080] 本文所述的任何Zika病毒疫苗或组合物可以以治疗有效量或治疗有效量的剂量施用于受试者。如本文所用,疫苗的“治疗有效量”是导致受试者(例如本文所述的那些)的期望应答或结果的任何量,包括但不限于预防感染、对Zika病毒的免疫应答或增强的免疫应答,或预防或减少与Zika病相关的症状。

[0081] 在一些实施方式中,本文所述的Zika病毒疫苗或组合物的治疗有效量是足以产生抗原特异性抗体(例如抗Zika病毒抗体)的量。在一些实施方式中,治疗有效量足以在受试者中提供血清保护;即产生足够的抗原特异性抗体以预防/防止感染。在一些实施方式中,在接种疫苗的受试者中至少75%、80%、90%、95%、96%、97%、98%或至少99%赋予血清保护。在一些实施方式中,血清保护定义为在斑块减少中和试验(PRNT)中通过1:10以上稀释来自接种疫苗的受试者的血清而使Zika病毒斑块的量降低50%以上。在一些实施方式中,有效量的Zika疫苗足以以至少70%的可能性将受试者血清转化。在一些实施方式中,治疗有效量足以以至少75%、80%、85%、90%、95%、96%、97%、98%或至少99%的可能性将受试者血清转化。可以通过本领域已知的任何方法评估受试者是否已经血清转化,例如从受试者获得血清样品并进行测定以检测抗Zika病毒抗体。在一些实施方式中,如果来自受试者的血清样品含有超过阈值或预定基线的量的抗Zika病毒抗体,则受试者被血清转化。如果受试者血清样品中的抗Zika病毒抗体(即抗Zika E蛋白IgG抗体)与先前取自相同受试者的血清样品相比至少增加4倍,则受试者通常被认为是血清转化的。

[0082] 在一些实施方式中,通过进行斑块减少中和测试(PRNT)来评估受试者的血清转化。简而言之,与对照血清/抗体相比,PRNT用于确定将Zika病毒斑块的量减少50%所需的血清滴度(PRNT50)。PRNT50可以使用单层的Vero细胞或可以感染Zika病毒的任何其它细胞

类型/品系来进行。将受试者的血清稀释并与活的非灭活的Zika病毒一起温育。血清/病毒混合物可以应用于Vero细胞并温育一段时间。对单层Vero细胞上形成的斑块进行计数,并与不存在血清或对照抗体时Zika病毒形成的斑块数量进行比较。PRNT50中1:10稀释血清的中和抗体的阈值通常被认为是保护的证据(Hombach等,Vaccine (2005) 23:5205-5211)。

[0083] 在一些实施方式中,可以将Zika病毒配制成为组合物例如药物组合物施用。如本文所用,术语“药物组合物”是指由至少一种活性成分(例如灭活Zika病毒)与一种或多种非活性成分(其可包括一种或多种药学上可接受的赋形剂)的混合或组合而得到的产品。

[0084] 本发明的药物组合物包括疫苗,可以根据本领域熟知和常规实施的方法制备(参见例如Remington:The Science and Practice of Pharmacy,Mack Publishing Co.20nd ed.2000;和Ingredients of Vaccines-来自疾病控制和预防中心的情况说明书,例如帮助疫苗改进其作用的佐剂和增强剂、帮助疫苗保持不变的防腐剂和稳定剂(例如白蛋白、酚类、甘氨酸))。药物组合物优选在GMP条件下生产。通常在本发明的药物组合物中使用治疗有效剂量的灭活Zika病毒制剂。通过本领域技术人员已知的常规方法将灭活Zika病毒配制成药学上可接受的剂型。调整剂量方案以提供最佳的期望应答(例如预防应答)。

[0085] 本发明药物组合物中活性成分的剂量可以变化,以获得对特定受试者、组合物和施用模式有效实现期望药物应答的活性成分的量,而对受试者没有毒性。所选剂量水平取决于多种药代动力学因素,包括所用本发明特定组合物的活性、施用途径、施用时间、所用具体化合物的排泄速率、治疗持续时间、与所用特定组合物组合使用的其它药物、化合物和/或材料、所治疗的受试者的年龄、性别、体重、状况、一般健康状况和既往病史等因素。

[0086] 医师、兽医或其它受过训练的医生可以以低于实现期望治疗效果所需水平的药物组合物中使用的灭活Zika病毒疫苗的剂量开始,并逐渐增加剂量,直到达到期望效果(例如,产生抗-Zika病毒抗体)。通常,本文所述用于预防性治疗人群的本发明组合物的有效剂量取决于许多不同因素,包括施用方式、靶位点、患者的生理状态、患者是否是人或动物、所施用的其它药物以及所需的抗Zika病毒抗体的效价。需要滴定剂量以优化安全性和有效性。在一些实施方式中,给药方案需要皮下或肌肉内施用两次剂量的灭活Zika病毒,一次在第0天和一次在约第7天。在一些实施方式中,给药方案需要在皮下施用两次剂量的灭活Zika病毒,一次在第0天和一次在第14天。在一些实施方式中,给药方案需要在皮下施用两次剂量的灭活Zika病毒,一次在第0天和一次在第28天。在一些实施方式中,向受试者施用一次灭活Zika病毒。

[0087] 本文所述的任何Zika病毒疫苗或组合物可以在施用一种或多种佐剂的同时、之前或之后施用于受试者。佐剂是增强受试者中对抗原或其它分子的应答(例如免疫应答)的分子。在一些实施方式中,佐剂可稳定抗原或其它分子。确定Zika病毒疫苗或其组合物是否与佐剂一起施用取决于各种因素(例如期望响应的类型和程度),并且对于本领域技术人员而言是显而易见的。在一些实施方式中,在施用佐剂的同时、之前或之后施用本文所述的任何Zika病毒疫苗或组合物可以增强病毒中和(抗Zika病毒)抗体的产生。在一些实施方式中,在施用佐剂的同时、之前或之后施用本文所述的任何Zika病毒疫苗或组合物的受试者可能仅需要单次施用待血清转化的Zika病毒疫苗或组合物(产生抗Zika病毒抗体的水平)。佐剂的实例可以包括但不限于铝盐(氢氧化铝或磷酸铝)、磷酸氢钙、石蜡油、杀死的细菌、细菌毒素、类毒素、细菌亚基、角鲨烯、硫柳汞、去垢剂、IL-1、IL-2、IL-12、双组分佐剂(例如含有

抗菌肽和TLR9激动剂(例如**IC31®**)的双组分佐剂)、以及诸如弗氏完全佐剂和弗氏不完全佐剂的组合。在一些实施方式中,Zika病毒疫苗或组合物与氢氧化铝一起施用。在一些实施方式中,灭活Zika病毒疫苗或组合物与磷酸铝盐一起施用。优选的铝盐是Cu含量降低的氢氧化铝,例如基于Zika组合物的重量低于1.25ppb,是在W02013/083726或Schlegl等,Vaccine 33 (2015) 5989-5996中详细描述佐剂。

[0088] 在一些实施方式中,佐剂由两种组分组成。在一些实施方式中,所述双组分佐剂包含抗菌肽和TLR9激动剂。在一些实施方式中,抗菌肽由氨基酸序列KLKL5KLK (SEQ ID NO: 71) 提供。在一些实施方式中,TLR9激动剂是含脱氧肌苷的免疫刺激性寡脱氧核酸分子(I-ODN)。在一些实施方式中,I-ODN包含核酸序列(dIdC)₁₃ (SEQ ID NO: 70)。在一些实施方式中,佐剂是**IC31®**。在一些实施方式中,佐剂呈纳米颗粒形式(参见例如美国专利号8,765,148B2,其通过引用整体并入)。在一些实施方式中,佐剂是与铝盐例如氢氧化铝组合的**IC31®**,即KLKL5KLK (SEQ ID NO: 71) 和核酸序列(dIdC)₁₃ (SEQ ID NO: 70)。

[0089] 本文所述的Zika病毒疫苗或组合物可以与一种或多种疫苗同时施用于另一种传染性病原体,例如与Zika病毒存在或被认为存在于相同地理区域中的另一种传染性病原体。在一些实施方式中,另一种传染性病原体是受试者也处于接触风险中的传染性病原体。在一些实施方式中,另一种传染性病原体由与Zika病毒相同的节肢动物载体传播。在一些实施方式中,另一种传染性病原体是日本脑炎病毒、黄热病病毒、登革病毒和/或基孔肯雅病毒。

[0090] 同样在本公开内容的范围内的是用于预防性施用于受试者以例如预防或降低Zika病毒感染严重性的试剂盒。这样的试剂盒可以包括一个或多个包含含有灭活Zika病毒的组合物的容器,例如灭活Zika病毒疫苗。在一些实施方式中,试剂盒可以进一步包含一种或多种另外的含有第二组合物的附加物,例如第二疫苗。在一些实施方式中,第二疫苗是另一种虫媒病毒的疫苗。在一些实施方式中,第二疫苗是登革热病毒疫苗和/或基孔肯雅病毒疫苗。

[0091] 在一些实施方式中,试剂盒可以包含根据本文所述的任何方法使用的说明书。所包含的说明书可以包含对施用含有灭活Zika病毒的组合物的描述,以预防Zika病毒感染、延缓其发作或降低其严重性。试剂盒可进一步包括基于鉴定该受试者是否有风险暴露于Zika病毒或接触Zika病毒感染而选择适于施用的受试者的描述。在其它实施方式中,说明书包括向有风险暴露于Zika病毒或接触Zika病毒的受试者施用含有灭活Zika病毒的组合物的描述。

[0092] 涉及使用含有灭活的Zika病毒的组合物的说明书通常包括关于预期治疗的剂量、施用方案和施用途径的信息。容器可以是单位剂量、散装包装(例如多剂量包装)或亚单位剂量。在本发明的试剂盒中提供的说明书通常是标签或包装插入物(例如包括在试剂盒中的纸张)上的书面说明书,但机器可读说明书也是可接受的。

[0093] 本公开的试剂盒处于合适的包装中。合适的包装包括但不限于小瓶、瓶子、罐子和软包装等。还考虑到与特定装置(例如注射器或输注装置)组合使用的包装。该容器可以具有无菌入口,例如该容器可以是具有可被皮下注射针头刺穿的塞子的小瓶。如本文所述,组合物中的至少一种活性剂是灭活Zika病毒。

[0094] 本发明的应用不限于以下描述中阐述的或附图中所示的部件的构造和布置的细

节。本发明能够具有其它实施方式并且能够以各种方式实践或执行。而且,这里使用的措辞和术语是为了描述的目的,而不应该被认为是限制性的。本文中“包括”、“包含”或“具有”、“含有”、“涉及”及其变体的使用意在涵盖其后列出的项目及其等同物以及附加项目。

[0095] 除非在此另外定义,否则与本公开相关使用的科学和技术术语应具有本领域普通技术人员通常理解的含义。此外,除非上下文另有要求,单数形式的术语应包括复数形式,复数形式的术语应包括单数形式。本公开的方法和技术通常根据本领域众所周知的常规方法进行。通常,与本文所述的生物化学、酶学、分子和细胞生物学、微生物学、病毒学、细胞或组织培养、遗传学和蛋白质以及核酸化学有关的命名法和技术是本领域中众所周知和常用的。除非另有说明,否则本公开的方法和技术通常根据本领域众所周知的常规方法进行,并如在本说明书全文中引用和讨论的各种通常和更具体的参考文献中所述。

[0096] 通过以下实施例进一步说明本发明,这些实施例决不应被解释为进一步的限制。本申请通篇引用的所有参考文献(包括参考文献、授权专利、公开的专利申请和共同未决的专利申请)的全部内容明确地通过引用并入本文,特别是对于上文提到的教导。然而,任何参考文献的引用不打算承认该参考文献是现有技术。

[0097] 表1. 处理缓冲液和储液的概述。

缓冲液	组合物	最终 pH	最终电导率 [mS/cm]
A	0.5 M NaOH		不适用
B	0.1 M NaOH		不适用
C	25 mM Tris, 150 mM NaCl	7.4±0.2	16.5
D	1 M Tris	7.4±0.2	不适用
E	4.5 M NaCl	不适用	不适用
F	1 M NaCl	不适用	不适用
G	1 % SDS	不适用	不适用
H	50 % (w/w) 蔗糖在 25 mM Tris, 150 mM NaCl 中	7.4±0.2	不适用
I	35 % (w/w) 蔗糖在 25 mM Tris, 150 mM NaCl 中	7.4±0.2	不适用
J	15 % (w/w) 蔗糖在 25 mM Tris, 150 mM NaCl 中	7.4±0.2	不适用
K	10 x PBS	7.4±0.2	不适用
L	50 mg/mL 硫酸鱼精蛋白	7.4±0.2	不适用
M	药物制剂缓冲液(10mM Tris(羟甲基)-氨基甲烷, 5 %蔗糖, 1% (10mg/mL) rHSA)	7.4±0.2	1.3

[0099] 表2. 缩写。

[0100]

°Bx	白利糖度=水溶液的糖含量 (w/w) *
BSA	牛血清白蛋白
CC700	Capto™ Core 700
CPE	细胞病变效应
EtOH	乙醇
EU	内毒素单位
DS	药物
DP	药物产品
DSP	下游工艺
HCP	宿主细胞蛋白
hcDNA	宿主细胞 DNA
hpi	感染后数小时
HPLC	高效液相色谱
ID	内径
JEV	日本脑炎病毒
LAL	鲎变形细胞溶解物
LDS buffer	十二烷基硫酸锂上样缓冲液
LOD	检测限
LOQ	定量限

[0101]

MALLS	多角度光散射
mAU	毫吸光度单位
MS	质谱
NIV	中和的灭活病毒
PBS	磷酸盐缓冲溶液
PD	过程开发
PFU	斑块形成单位
p.i.	感染后
PS	硫酸鱼精蛋白
Rcf	相对离心力
rHSA	重组人血清白蛋白
Rms radius	均方根半径
rMSB	研究硕士种子库
RSD	相对标准偏差
SEC	尺寸排除色谱法
SGC	蔗糖梯度离心
SGP	蔗糖梯度纯化
SDS	十二烷基硫酸钠
TBS	Tris 缓冲盐水
TFF	切向流过滤
TCID50	组织培养感染剂量 50%
UF/DF	超滤/渗滤
WFI	注射用水
ZikaV	Zika 病毒

[0102] *白利糖度 (°Bx) 是水溶液的糖含量。一白利糖度是100克溶液中的1克蔗糖,代表溶液的质量百分比强度。°Bx对应于百分比 (w/w) 的蔗糖含量,例如45°Bx等于45% (w/w) 蔗糖。

[0103] 表A.Zika病毒测序的引物:小写字母表示碱基不包含在ZIKA中,但在需要时含有用于以后克隆的限制性位点(因此提供两个Tms)。

[0104]

引物对	寡聚物名称	引物序列 (5'-3') 限制位点 (小写)	T _m (基因特异性)	T _m (整个引物)	扩增子尺寸 [bp]
1	9320_Zika_PF_1F	SEQ ID NO: 74 ttaggatccGTTGTTGATCTGTGTGAAT	69.9	74.6	707
	9321_Zika_PF_1R	SEQ ID NO: 75 taactcgagCGTACACAACCCAAGTT	69.3	75.6	
2	9322_Zika_PF_2F	SEQ ID NO: 76 ttaggatccTCACTAGACGTGGGAGTG	70	73.9	704
	9323_Zika_PF_2R	SEQ ID NO: 77 taactcgagAAGCCATGTCYGATATTGAT	69.8	73.7	
3	9324_Zika_PF_3F	SEQ ID NO: 78 ttaggatccGCATACAGCATCAGGTG	72.3	74.5	712
	9325_Zika_PF_3R	SEQ ID NO: 79 taactcgagTGTGGAGTTCGGTGTCT	72	76.4	
4	9326_Zika_PF_4F	SEQ ID NO: 80 ttaggatccGAATAGAGCGAARGTTGAGATA	70.9	74	712
	9327_Zika_PF_4R	SEQ ID NO: 81 taactcgAGTGGTGGGTGATCTTCTCT	70.5	73.7	
5	9328_Zika_PF_5F	SEQ ID NO: 82 ttaggatccCAGTCACAGTGGAGGTACAGTAC	70.3	75	704
	9329_Zika_PF_5R	SEQ ID NO: 83 taactcgagCRCAGATACCATCTTCCC	71.5	77.3	

[0105]

引物对	寡聚物名称	引物序列 (5'-3') 限制位点 (小写)	Tm (基因特异性)	Tm (整个引物)	扩增子尺寸 [bp]
6	9330_Zika_PF_6F	SEQ ID NO: 84 ttaggatCCCTTATGTGCTTGGCCTTAG	70.7	72.7	698
	9331_Zika_PF_6R	SEQ ID NO: 85 taactcgagTCTTCAGCCTCCATGTG	70.4	76.9	
7	9332_Zika_PF_7F	SEQ ID NO: 86 ttaggatccAATGCCCACTCAAACATAGA	71.9	75	716
	9333_Zika_PF_7R	SEQ ID NO: 87 taactcgagTCATTCTCTTCTTCAGCCCTT	71	74	
8	9334_Zika_PF_8F	SEQ ID NO: 88 ttaggatccAAGGGTGATCGAGGAAT	70.9	75.2	703
	9335_Zika_PF_8R	SEQ ID NO: 89 taactcgagTTCCCTTCAGAGAGAGGAGC	71.9	73.4	
9	9336_Zika_PF_9F	SEQ ID NO: 90 ttaggatccTCTTTTGCAAACCTGCGATC	71.9	75	699
	9337_Zika_PF_9R	SEQ ID NO: 91 taactcgagTCCAGCTGCAAAGGGTAT	71	74.9	
10	9338_Zika_PF_10F	SEQ ID NO: 92 ttaggatccGTGTGGACATGTACATTGA	71.4	75.8	706
	9339_Zika_PF_10R	SEQ ID NO: 93 taactcgagCCCATTGCCATAAAGTC	70.4	75.8	
11	9340_Zika_PF_11F	SEQ ID NO: 94 ttaggatccTCATACTGTGTGCCATGGA	71.6	78.1	692
	9341_Zika_PF_11R	SEQ ID NO: 95 taactcgagGCCCATCTCAACCCCTTG	74	78	
12	9342_Zika_PF_12F	SEQ ID NO: 96 ttaggatccTAGAGGGCTTCCAGTGC	70.9	74	707
	9343_Zika_PF_12R	SEQ ID NO: 97 taactcgAGATACTCATCTCCAGGTTTGTG	70.2	72.2	
13	9344_Zika_PF_13F	SEQ ID NO: 98 ttaggatccGAAAACAAAACATCAAGAGTG	70.6	75.4	726
	9345_Zika_PF_13R	SEQ ID NO: 99 taactcgagGAATCTCTCTGTGTCATGTGTCCT	71.9	75.6	
14	9346_Zika_PF_14F	SEQ ID NO: 100 ttaggatccTTGATGGCAGCACCAAC	73.1	75.6	715
	9347_Zika_PF_14R	SEQ ID NO: 101 ttaggatccGTTGTTGATCTGTGTGAAT	70.8	77.9	
15	9348_Zika_PF_15F	SEQ ID NO: 102 taactcgagCAGGTCAATGTCCATTG	71.9	75.4	719
	9349_Zika_PF_15R	SEQ ID NO: 103 ttaggatccTGTGTGTTCCTATTGCTGGT	73.9	77.2	
16	9350_Zika_PF_16F	SEQ ID NO: 104 taactcgagGTGATCAGRGCCCCAGC	72.3	75.4	703
	9351_Zika_PF_16R	SEQ ID NO: 105 ttaggatccTGCTGCCAGAGAAGAGAA	72	76.3	
17	9352_Zika_PF_17F	SEQ ID NO: 106 taactcgagCACCAACAYGGGTCTCT	73.6	76	705
	9353_Zika_PF_17R	SEQ ID NO: 107 ttaggatccTCAAGGACGGTGTGGC	72	75.5	
18	9354_Zika_PF_18F	SEQ ID NO: 108 taactcgagCAATGATCTTCATGTTGGG	71.7	75.8	699
	9355_Zika_PF_18R	SEQ ID NO: 109 ttaggatccTATGGGGGAGGACTGGT	71	74.1	
19	9356_Zika_PF_19F	SEQ ID NO: 110 taactcgagGAGCCAGAACCTTGGATC	73.3	75.5	711
	9357_Zika_PF_19R	SEQ ID NO: 111 ttaggatccCAGACCCCCAAGAAGGC	71.3	76.9	
20	9358_Zika_PF_20F	SEQ ID NO: 112 taactcgagCCCCTTTGGTCTGTCT	71.7	75	706
	9359_Zika_PF_20R	SEQ ID NO: 113 ttaggatccAGGAAGGATGTATGCAGATG	71.9	73.9	
21	9360_Zika_PF_21F	SEQ ID NO: 114 taactcgagACATTGCGCATATGATTTTG	70.4	75.7	709
	9361_Zika_PF_21R	SEQ ID NO: 115 ttaggatccAGGAAGGACACACAAGAGT	71.8	75	
22	9362_Zika_PF_22F	SEQ ID NO: 116 taactcgagACAGGCTGCACAGCTTT	70	79.1	581
	9363_Zika_PF_22R	SEQ ID NO: 117 ttaggatccTCTCTCATAGGGCACAGAC	74.8	81.1	

[0106] 序列

[0107] SEQ ID NO:1

[0108] 鱼精蛋白的典型形式

[0109] PRRRRSSSRP VRRRRRPRVS RRRRRRGRRR RR

[0110] 下文提供的是可用于本文所述的方法、组合物和/或疫苗中的Zika病毒基因组的核酸序列的实例。

[0111] SEQ ID NO:2

[0112] KU321639.1 Zika 病毒株 ZikaSPH2015, 巴西, 完整的基因组

[0113] GTTGTACTGTTGCTGACTCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACA
GGTTTTATTTGGATTTGGAAACGAGAGTTTCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTCCGGATTGT
CAATATGCTAAAACGCGGAGTAGCCCGTGTGAGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGACTTCTGCTGG
GTCATGGGCCCATCAGGATGGTCTTGGCAATTCTAGCCTTTTTGAGATTACGGCAATCAAGCCATCACTGGGTCTC
ATCAATAGATGGGGTTTCACTGGGGAAAAAAGAGGCTATGGAAATAATAAAGAAGTTCAAGAAAGATCTGGCTGCCAT
GCTGAGAATAATCAATGCTAGGAAGGAGAAGAAGAGACGGGGCGCAGATACTAGTGTCCGAATTGTTGGCCTCCTGC
TGACCACAGCTATGGCAGCGGAGGTCACTAGACGTGGGAGTGCATACTATGTACTTGGACAGAAACGATGCTGGG
GAGGCCATATCTTTTCCAACCACATTGGGGATGAATAAGTGTTATATACAGATCATGGATCTTGGACACATGTGTGA
TGCCACCATGAGCTATGAATGCCCTATGCTGGATGAGGGGTGGAACCAGATGACGTCGATTGTTGGTGAACACGA
CGTCAACTTGGGTTGTGTACGGAACCTGCCATCACAAAAAAGGTGAAGCACGGAGATCTAGAAGAGCTGTGACGCTC
CCCTCCCATTCCACTAGGAAGCTGCAAACGCGGTGCGAAACCTGGTTGGAATCAAGAGAATACACAAAGCACTTGAT
TAGAGTCGAAAATTGGATATTCAGGAACCCTGGCTTCGCGTTAGCAGCAGCTGCCATCGCTTGGCTTTTGGGAAGCT
CAACGAGCCAAAAAGTCATATACTTGGTCATGATACTGCTGATTGCCCCGGCATAACAGCATCAGGTGCATAGGAGTC
AGCAATAGGGACTTTGTGGAAGGTATGTCAGGTGGGACTTGGGTTGATATTGTCTTGAACATGGAGGTTGTGTCAC
CGTAATGGCACAGGACAAACCGACTGTGACATAGAGCTGGTTACAACAACAGTCAGCAACATGGCGGAGGTAAGAT
CCTACTGCTATGAGGCATCAATATCAGACATGGCTTCGGACAGCCGCTGCCAACACAAGGTGAAGCCTACCTTGAC
AAGCAATCAGACACTCAATATGTCTGCAAAAAGAACGTTAGTGGACAGAGGCTGGGGAAATGGATGTGGACTTTTGG
CAAAGGGAGTCTGGTGACATGCGCTAAGTTTGCATGCTCCAAGAAAATGACCGGGAAGAGCATCCAGCCAGAGAATC
TGGAGTACCGGATAATGCTGTCTAGTTTCATGGCTCCAGCACAGTGGGATGATCGTTAATGACACAGGACATGAACT
GATGAGAATAGAGCGAAGGTTGAGATAACGCCCAATTCACCAAGAGCCGAAGCCACCCTGGGGGGTTTTGGAAGCCT
AGGACTTGATTGTGAACCGAGGACAGGCCCTTGACTTTTTCAGATTTGTATTACTTGACTATGAATAACAAGCACTGGT
TGGTTCACAAGGAGTGGTTCCACGACATTCCATTACCTTGGCACGCTGGGGCAGACACCGGAACTCCACACTGGAAC
AACAAAGAAGCACTGGTAGAGTTCAAGGACGCACATGCCAAAAGGCAAACCTGTCGTGGTTCTAGGGAGTCAAGAAGG
AGCAGTTCACACGGCCCTTGCTGGAGCTCTGGAGGCTGAGATGGATGGTGCAAAGGGAAGGCTGTCCTCTGGCCACT
TGAAATGTCGCCTGAAAATGGATAAACTTAGATTGAAGGGCGTGTCTACTCCTTGTGTACCGCAGCGTTCACATTC
ACCAAGATCCCGGCTGAAACACTGCACGGGACAGTCACAGTGGAGGTACAGTACGCAGGGACAGATGGACCTTGCAA
GGTTCCAGCTCAGATGGCGGTGGACATGCAAACCTTGACCCCAAGTTGGGAGGTTGATAACCGCTAACCCCGTAATCA
CTGAAAGCACTGAGAACTCTAAGATGATGCTGGAACCTTGATCCACCATTTGGGGACTCTTACATTGTCATAGGAGTC
GGGGAGAAGAAGATCACCCACCACTGGCACAGGAGTGGCAGCACCATTGGAAAAGCATTGTAAGCCACTGTGAGAGG
TGCCAAGAGAATGGCAGTCTTGGGAGACACAGCCTGGGACTTTGGATCAGTTGGAGGCGCTCTCAACTCATTGGGCA
AGGGCATCCATCAAATTTTTGGAGCAGCTTTCAAATCATTGTTTGGAGGAATGTCCTGGTTCTCACAAATTCTCATT
GGAACGTTGCTGATGTGGTTGGGTCTGAACACAAAGAATGGATCTATTTCCCTTATGTGCTTGGCCTTAGGGGGAGT
GTTGATCTTCTTATCCACAGCCGTCTCTGCTGATGTGGGGTGCTCGGTGGACTTCTCAAAGAAGGAGACGAGATGCG
GTACAGGGGTGTTCTGTCTATAACGACGTTGAAGCCTGGAGGGACAGGTACAAGTACCATCCTGACTCCCCCGTAGA
TTGGCAGCAGCAGTCAAGCAAGCCTGGGAAGATGGTATCTGCGGGATCTCCTCTGTTTCAAGAATGGAAAACATCAT
GTGGAGATCAGTAGAAGGGGAGCTCAACGCAATCCTGGAAGAGAATGGAGTTCAACTGACGGTCGTTGTGGGATCTG
TAAAAAACCCCATGTGGAGAGGTCCACAGAGATTGCCCCGTGCCTGTGAACGAGCTGCCCCACGGCTGGAAGGCTTGG

GGGAAATCGCACTTCGTCAGAGCAGCAAAGACAAATAACAGCTTTGTCTGGATGGTGACACACTGAAGGAATGCCC
ACTCAAACATAGAGCATGGAACAGCTTTCTTGTGGAGGATCATGGGTTCGGGGTATTTCACACTAGTGTCTGGCTCA
AGGTTAGAGAAGATTATTCATTAGAGTGTGATCCAGCCGTTATTGGAACAGCTGTAAAGGGAAAGGAGGCTGTACAC
AGTGATCTAGGCTACTGGATTGAGAGTGAGAAGAATGACACATGGAGGCTGAAGAGGGCCCATCTGATCGAGATGAA
AACATGTGAATGGCCAAAGTCCCACACATTGTGGACAGATGGAATAGAAGAGAGTGATCTGATCATACCCAAGTCTT
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[0114] SEQ ID NO:3

[0115] KU497555.1 Zika病毒分离株巴西-ZKV2015, 巴西, 完整的基因组

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[0117] SEQ ID NO:4

[0118] KU501215.1 Zika病毒株PRVABC59,波多黎各,完整的基因组

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[0120] SEQ ID NO:5

[0121] KU509998.1 Zika病毒株海地/1225/2014, 海地, 完整的基因组

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[0123] SEQ ID NO:6

[0124] KU527068.1 Zika病毒株Natal RGN, 巴西:Rio Grande do Norte, Natal, 完整的基因组

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[0126] KU681081.3 Zika病毒分离株Zika病毒/H.sapiens-tc/THA/2014/SV0127-14, 泰国, 完整的基因组

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[0128] KU681082.3 Zika病毒分离株Zika病毒/H.sapiens-tc/PHL/2012/CPC-0740, 菲律

宾,完整的基因组

[0129] AGTTGTTGATCTGTGTGAATCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAAC
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[0130] SEQ ID NO:9

[0131] KU707826.1 Zika病毒分离株SSABR1, 巴西, 完整的基因组

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[0133] SEQ ID NO:10

[0134] KU744693.1 Zika病毒分离株VE_Ganxian, 中国, 完整的基因组

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[0136] SEQ ID NO:11

[0137] LC002520.1 Zika病毒基因组RNA, 菌株:MR766-NIID, 乌干达, 完整的基因组

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[0139] SEQ ID NO:12

[0140] AY632535.2NC_012532.1Zika病毒株MR 766,乌干达,完整的基因组

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[0142] SEQ ID NO:13

[0143] KJ776791.1, Zika病毒株H/PF/2013多聚蛋白基因,完整的cds

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CACACTAGTGTCTGGCTCAAGGTTAGAGAAGATTATTCATTAGAGTGTGATCCAGCCGTTATTGGAACAGCTGTTAA
GGGAAAGGAGGCTGTACACAGTGATCTAGGCTACTGGATTGAGAGTGAGAAGAATGACACATGGAGGCTGAAGAGGG
CCCATCTGATCGAGATGAAAACATGTGAATGGCCAAAGTCCCACACATTGTGGACAGATGGAATAGAAGAGAGTGAT
CTGATCATACCCAAGTCTTTAGCTGGGCCACTCAGCCATCACAATACCAGAGAGGGCTACAGGACCCAAATGAAAGG
GCCATGGCACAGTGAAGAGCTTGAAATTCGGTTTGAGGAATGCCCAGGCACTAAGGTCCACGTGGAGGAAACATGTG
GAACAAGAGGACCATCTCTGAGATCAACCACTGCAAGCGGAAGGGTGATCGAGGAATGGTGCTGCAGGGAGTGCACA
ATGCCCCCACTGTCGTTCCGGGCTAAAGATGGCTGTTGGTATGGAATGGAGATAAGGCCCAGGAAAGAACCAGAAAG
TAACCTTAGTAAGGTCAATGGTGACTGCAGGATCAACTGATCACATGGATCACTTCTCCCTTGGAGTGCTTGTGATTC
TGCTCATGGTGCAGGAAGGGCTGAAGAAGAGAATGACCACAAAGATCATCATAAGCACATCGATGGCAGTGCTGGTA
GCTATGATCCTGGGAGGATTTTCAATGAGTGACCTGGCTAAGCTTGCAATTTTGATGGGTGCCACCTTCGCGGAAAT
GAACACTGGAGGAGATGTAGCTCATCTGGCGCTGATAGCGGCATTCAAAGTCAGACCAGCGTTGCTGGTATCTTTCA
TCTTCAGAGCTAATTGGACACCCCGTGAAGCATGCTGCTGGCCTTGGCCTCGTGTCTTTGCAAACCTGCGATCTCC
GCCTTGGAAGGCGACCTGATGGTTCTCATCAATGGTTTTGCTTTGGCCTGGTTGGCAATACGAGCGATGGTTGTTCC
ACGCACTGATAACATCACCTTGGCAATCCTGGCTGCTCTGACCACTGGCCCCGGGCACACTGCTTGTGGCGTGGA
GAGCAGGCCTTGCTACTTGCGGGGGTTTATGCTCCTCTCTCTGAAGGGAAAAGGCAGTGTGAAGAAGAAGTTACCA
TTTGTCTATGGCCCTGGGACTAACCGCTGTGAGGCTGGTGCACCCCATCAACGTGGTGGGACTGCTGTTGCTCACAAG
GAGTGGGAAGCGGAGCTGGCCCCCTAGCGAAGTACTCACAGCTGTTGGCCTGATATGCGCATTGGCTGGAGGGTTCCG
CCAAGGCAGATATAGAGATGGCTGGGCCCATGGCCGCGTTCGGTCTGCTAATTGTCAGTTACGTGGTCTCAGGAAAG
AGTGTGGACATGTACATTGAAAGAGCAGGTGACATCACATGGGAAAAAGATGCGGAAGTCACTGGAAACAGTCCCCG
GCTCGATGTGGCGCTAGATGAGAGTGGTGATTTCTCCCTGGTGGAGGATGACGGTCCCCCATGAGAGAGATCATAC
TCAAGGTGGTCCTGATGACCATCTGTGGCATGAACCAATAGCCATACCCTTTGCAGCTGGAGCGTGGTACGTATAC
GTGAAGACTGGAAAAAGGAGTGGTGCTCTATGGGATGTGCCTGCTCCCAAGGAAGTAAAAAGGGGGAGACCACAGA
TGGAGTGACAGAGTAATGACTCGTAGACTGCTAGGTTCAACACAAGTTGGAGTGGGAGTTATGCAAGAGGGGGTCT
TTCACACTATGTGGCACGTCACAAAAGGATCCGCGCTGAGAAGCGGTGAAGGGAGACTTGATCCATACTGGGGAGAT
GTCAAGCAGGATCTGGTGTCTACTGTGGTCCATGGAAGCTAGATGCCGCTGGGACGGGCACAGCGAGGTGCAGCT
CTTGGCCGTGCCCCCGGAGAGAGAGCGAGGAACATCCAGACTCTGCCCGGAATATTTAAGACAAAGGATGGGGACA
TTGGAGCGGTTGCGCTGGATTACCCAGCAGGAAGTTTCAAGGATCTCCAATCCTAGACAAGTGTGGGAGAGTGATAGGA
CTTTATGGCAATGGGGTCGTGATCAAAAATGGGAGTTATGTTAGTGCCATCACCAAGGGAGGAGGGAGGAAGAGAC
TCCTGTTGAGTGCTTCGAGCCTTCGATGCTGAAGAAGAAGCAGCTAACTGTCTTAGACTTGCATCCTGGAGCTGGGA
AAACCAGGAGAGTTCTTCTGAAATAGTCCGTGAAGCCATAAAAAACAAGACTCCGTACTGTGATCTTAGCTCCAACC

AGGGTTGTCGCTGCTGAAATGGAGGAAGCCCTTAGAGGGCTTCCAGTGCGTTATATGACAACAGCAGTCAATGTCAC
CCTACTCTGGAACAGAAATCGTCGACTTAATGTGCCATGCCACCTTCACTTCACGTCTACTACAGCCAATCAGAGTCC
CCAACCTATAATCTGTATATTATGGATGAGGCCCACTTCACAGATCCCTCAAGTATAGCAGCAAGAGGATACATTTCA
ACAAGGGTTGAGATGGGCGAGGCGGCTGCCATCTTCATGACCGCCACGCCACCAGGAACCCGTGACGCATTTCCGGA
CTCCAACCTACCAATTATGGACACCGAAGTGGAAGTCCCAGAGAGAGCCTGGAGCTCAGGCTTTGATTGGGTGACGG
ATCATTCTGGAAAAACAGTTTGGTTTGTCCAAGCGTGAGGAACGGCAATGAGATCGCAGCTTGTCTGACAAAGGCT
GGAAAACGGGTCATACAGCTCAGCAGAAAGACTTTTGAGACAGAGTTCCAGAAAACAAAACATCAAGAGTGGGACTT
TGTCGTGACAACCTGACATTTTCAAGATGGGCGCCAACTTTAAAGCTGACCGTGTCTATAGATTCCAGGAGATGCCTAA
AGCCGGTCATACTTGATGGCGAGAGAGTCATTCTGGCTGGACCCATGCCTGTCACACATGCCAGCGCTGCCAGAGG
AGGGGGCGCATAGGCAGGAATCCCAACAAACCTGGAGATGAGTATCTGTATGGAGGTGGGTGCGCAGAGACTGACGA
AGACCATGCACACTGGCTTGAAGCAAGAATGCTCCTTGACAATATTTACCTCCAAGATGGCCTCATAGCCTCGCTCT
ATCGACCTGAGGCCGACAAAGTAGCAGCCATTGAGGGAGAGTTCAAGCTTAGGACGGAGCAAAGGAAGACCTTTGTG
GAACTCATGAAAAGAGGAGATCTTCTGTTTGGCTGGCCTATCAGGTTGCATCTGCCGAATAACCTACACAGATAG
AAGATGGTGCTTTGATGGCAGCAGCAACAACACCATAATGGAAGACAGTGTGCCGGCAGAGGTGTGGACCAGACACG
GAGAGAAAAGAGTGCTCAAACCGAGGTGGATGGACGCCAGAGTTTGTTCAGATCATGCGGCCCTGAAGTCATTCAAG
GAGTTTGCCGCTGGGAAAAGAGGAGCGGCTTTTGGAGTGATGGAAGCCCTGGGAACACTGCCAGGACACATGACAGA
GAGATTCCAGGAAGCCATTGACAACCTCGCTGTGCTCATGCGGGCAGAGACTGGAAGCAGGCCTTACAAAGCCGCGG
CGGCCCAATTGCCGAGACCCTAGAGACCATTATGCTTTTGGGGTTGCTGGGAACAGTCTCGCTGGGAATCTTTTTC
GTCTTGATGAGGAACAAGGGCATAGGGAAGATGGGCTTTGGAATGGTGACTCTTGGGGCCAGCGCATGGCTCATGTG
GCTCTCGGAAATTGAGCCAGCCAGAATTGCATGTGTCCTCATTGTTGTGTTCTATTGCTGGTGGTGCTCATACCTG
AGCCAGAAAAGCAAAGATCTCCCCAGGACAACCAAATGGCAATCATCATGTTAGCAGTAGGTCTTCTGGGCTTG
ATTACCGCCAATGAACTCGGATGGTTGGAGAGAACAAAGAGTGACCTAAGCCATCTAATGGGAAGGAGAGAGGAGGG
GGCAACCATAGGATTCTCAATGGACATTGACCTGCGGCCAGCCTCAGCTTGGGCCATCTATGCTGCCTTGACAACCT
TCATTACCCAGCCGTCCAACATGCAGTGACCACTTCATACAACAATACTCCTTAATGGCGATGGCCACGCAAGCT
GGAGTGTTGTTTGGTATGGGCAAAGGGATGCCATTCTACGCATGGGACTTTGGAGTCCCGCTGCTAATGATAGGTTG
CTACTCACAATTAACACCCCTGACCCTAATAGTGGCCATCATTTTGTCTGTTGGCGCACTACATGTACTTGATCCAG
GGCTGCAGGCAGCAGCTGCGCGTGCTGCCCAGAAGAGAACGGCAGCTGGCATCATGAAGAACCCTGTTGTGGATGGA
ATAGTGGTGACTGACATTGACACAATGACAATTGACCCCCAAGTGGAGAAAAAGATGGGACAGGTGCTACTCATAGC
AGTAGCCGTCTCCAGCGCCATACTGTGCGGACCGCCTGGGGGTGGGGGGAGGCTGGGGCCCTGATCACAGCGGCAA
CTTCCACTTTGTGGGAAGGCTCTCCGAACAAGTACTGGAATCCTCTACAGCCACTTCACTGTGTAACATTTTTAGG
GGAAGTTACTTGGCTGGAGCTTCTCTAATCTACACAGTAACAAGAAACGCTGGCTTGGTCAAGAGACGTGGGGGTGG
AACAGGAGAGACCCTGGGAGAGAAATGGAAGGCCCGCTTGAACCAGATGTCGGCCCTGGAGTTCTACTCTACAAAA
AGTCAGGCATCACCGAGGTGTGCAGAGAAGAGGCCCGCCGCGCCCTCAAGGACGGTGTGGCAACGGGAGGCCATGCT
GTGTCCCAGGAAGTGCAAAGCTGAGATGGTTGGTGGAGCGGGGATACCTGCAGCCCTATGGAAAGGTCATTGATCT
TGGATGTGGCAGAGGGGGCTGGAGTTACTACGCCGCCACCATCCGCAAAGTTCAAGAAGTGAAGGATACACAAAAG
GAGGCCCTGGTCATGAAGAACCCATGTTGGTGCAAAGCTATGGGTGGAACATAGTCCGTCTTAAGAGTGGGGTGGAC
GTCTTTCATATGGCGGCTGAGCCGTGTGACACGTTGCTGTGTGACATAGGTGAGTCATCATCTAGTCTGAAGTGGA
AGAAGCACGGACGCTCAGAGTCTCTCCATGGTGGGGGATTGGCTTGAAAAAGACCAGGAGCCTTTTGTATAAAAG
TGTTGTGCCATACACCAGCACTATGATGGAAACCCTGGAGCGACTGCAGCGTAGGTATGGGGGAGGACTGGTCAGA

GTGCCACTCTCCCGCAACTCTACACATGAGATGTACTGGGTCTCTGGAGCGAAAAGCAACACCATAAAAAGTGTGTC
CACCACGAGCCAGCTCCTCTTGGGGCGCATGGACGGGCCCAGGAGGCCAGTGAATATGAGGAGGATGTGAATCTCG
GCTCTGGCACGCGGGCTGTGGTAAGCTGCGCTGAAGCTCCCAACATGAAGATCATTGGTAACCGCATTGAAAGGATC
CGCAGTGAGCACGCGGAAACGTGGTTCTTTGACGAGAACCACCCATATAGGACATGGGCTTACCATGGAAGCTATGA
GGCCCCCACACAAGGGTCAGCGTCCTCTCTAATAAACGGGGTTGTCAGGCTCCTGTCAAAACCCTGGGATGTGGTGA
CTGGAGTCACAGGAATAGCCATGACCGACACCACACCGTATGGTCAGCAAAGAGTTTTCAAGGAAAAAGTGGACACT
AGGGTGCCAGACCCCCAAGAAGGCACTCGTCAGGTTATGAGCATGGTCTCTTCCTGGTTGTGGAAAGAGCTAGGCAA
ACACAAACGGCCACGAGTCTGTACCAAAGAAGAGTTCATCAACAAGTTCTGTAGCAATGCAGCATTAGGGGCAATAT
TTGAAGAGGAAAAAGAGTGGAAGACTGCAGTGGAAGCTGTGAACGATCCAAGTTCTGGGCTCTAGTGGAACAAGGAA
AGAGAGCACCACCTGAGAGGAGAGTGCCAGAGTTGTGTGTACAACATGATGGGAAAAAGAGAAAAGAAACAAGGGGA
ATTTGGAAGGCCAAGGGCAGCCGCGCCATCTGGTATATGTGGCTAGGGGCTAGATTTCTAGAGTTCGAAGCCCTTG
GATTCTTGAACGAGGATCACTGGATGGGGAGAGAGAACTCAGGAGGTGGTGTGAAGGGCTGGGATTACAAAGACTC
GGATATGTCCTAGAAGAGATGAGTCGCATACCAGGAGGAAGGATGTATGCAGATGACACTGCTGGCTGGGACACCCG
CATCAGCAGGTTTGATCTGGAGAATGAAGCTCTAATCACCAACCAAATGGAGAAAGGGCACAGGGCCTTGGCATTGG
CCATAATCAAGTACACATACCAAAACAAAGTGGTAAAGGTCCTTAGACCAGCTGAAAAAGGGAAGACAGTTATGGAC
ATTATTTGAGACAAGACCAAAGGGGGAGCGGACAAGTTGTCACTTACGCTCTTAACACATTTACCAACCTAGTGGT
GCAACTCATTTCGAATATGGAGGCTGAGGAAGTTCTAGAGATGCAAGACTTGTGGCTGCTGCGGAGGTGAGAGAAAG
TGACCAACTGGTTGCAGAGCAACGGATGGGATAGGCTCAAACGAATGGCAGTCAGTGGAGATGATTGCGTTGTGAAG
CCAATTGATGATAGGTTTGACATGCCCTCAGGTTCTTGAATGATATGGGAAAAGTTAGGAAGGACACACAAGAGTG
GAAACCCTCAACTGGATGGGACAACCTGGGAAGAAGTTCCGTTTTGCTCCCACCACTTCAACAAGCTCCATCTCAAGG
ACGGGAGGTCCATTGTGGTTCCCTGCCGCCACCAAGATGAACTGATTGGCCGGGCGCGTCTCTCCAGGGGCGGGA
TGGAGCATCCGGGAGACTGCTTGCTAGCAAAATCATATGCGCAAATGTGGCAGCTCCTTTATTTCCACAGAAGGGA
CCTCCGACTGATGGCCAATGCCATTTGTTTCATCTGTGCCAGTTGACTGGGTTCCAACTGGGAGAACTACCTGGTCAA
TCCATGGAAAGGGAGAATGGATGACCACTGAAGACATGCTTGTGGTGTGGAACAGAGTGTGGATTGAGGAGAACGAC
CACATGGAAGACAAGACCCAGTTACGAAATGGACAGACATTCCCTATTTGGGAAAAAGGGAAGACTTGTGGTGTGG
ATCTCTCATAGGGCACAGACCGCGCACCACTGGGCTGAGAACATTAATAACACAGTCAACATGGTGGCAGGATCA
TAGGTGATGAAGAAAAGTACATGGACTACCTATCCACCCAAGTTTCGCTACTTGGGTGAAGAAGGGTCTACACCTGGA
GTGCTGTAAGCACCAATCTTAGTGTGTCAGGCCTGCTAGTCAGCCACAGCTTGGGGAAAGCTGTGCAGCCTGTGAC
CCCCCAGGAGAAGCTGGGAAACCAAGCCTATAGTCAGGCCGAGAACGCCATGGCACGGAAGAAGCCATGCTGCCTG
TGAGCCCCTCAGAGGACACTGAGTCAAAAAACCCACGCGCTTGAGGGCGCAGGATGGGAAAAGAAGGTGGCGACCT
TCCCCACCCTTCAATCTGGGGCCTGAACTGGAGATCAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAG

[0145] 在一些实施方式中,Zika病毒具有对应于由SEQ ID NO:2-13或72中的任一个核酸序列提供的DNA序列的RNA基因组。在一些实施方式中,Zika病毒具有与SEQ ID NO:2-13或72中的任一个具有至少80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%、99.5%、99.6%、99.7%、99.8%或99.9%同一性的变异基因组。

[0146] 下文提供了可用于本文所述的方法、组合物和/或疫苗中的Zika菌株的E蛋白的氨基酸序列。

[0147] SEQ ID NO:14

[0148] isol-ARB15076.AHF49784.1.Central_African_Republic/291-788黄病毒包膜糖蛋白E.

[0149] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGA
DTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSY
LCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFG
DSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGM
SWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0150] SEQ ID NO:15

[0151] isol-IbH_30656.AEN75265.1.尼日利亚/291-788黄病毒包膜糖蛋白E.

[0152] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHGSA
DTETPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSY
LCTAAFTFTKVPAETLHGTVTVEVQYAGRDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFG
DSYIVIGVGDKKITHHWHRSGSIIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGM
SWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0153] SEQ ID NO:16

[0154] ArB1362.AHL43500.1.-/291-794黄病毒包膜糖蛋白E.

[0155] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDXXXXXXXXNRAEVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0156] SEQ ID NO:17

[0157] ArD128000.AHL43502.1.-/291-794黄病毒包膜糖蛋白E.

[0158] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMXXXXXGHETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHRLVRKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWLKKGSSIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0159] SEQ ID NO:18

[0160] ArD158095.AHL43505.1.-/291-794黄病毒包膜糖蛋白E.

[0161] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA

SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0162] SEQ ID NO:19

[0163] ArD158084.AHL43504.1.-/291-794黄病毒包膜糖蛋白E.

[0164] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0165] SEQ ID NO:20

[0166] isol-ARB13565.AHF49783.1.Central_African_Republic/291-794黄病毒包膜糖蛋白E.

[0167] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0168] SEQ ID NO:21

[0169] isol-ARB7701.AHF49785.1.Central_African_Republic/291-794黄病毒包膜糖蛋白E.

[0170] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0171] SEQ ID NO:22

[0172] isol-ArD_41519.AEN75266.1.赛内加尔/291-794黄病毒包膜糖蛋白E.

[0173] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGS

QHSGMIVNDTGHETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0174] SEQ ID NO:23

[0175] MR766-NIID.BAP47441.1.乌干达/291-794黄病毒包膜糖蛋白E.

[0176] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMTVNDIGYETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0177] SEQ ID NO:24

[0178] Zika病毒基因组RNA,菌株:MR766-NIID,乌干达,黄病毒包膜糖蛋白E.

[0179] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMTVNDIGYETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0180] SEQ ID NO:25

[0181] isol-MR_766.AEN75263.1.乌干达/291-794黄病毒包膜糖蛋白E.

[0182] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGYETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0183] SEQ ID NO:26

[0184] ArD7117.AHL43501.1.-/291-794黄病毒包膜糖蛋白E.

[0185] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAVCTAAKVPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE

LDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGGVFNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0186] SEQ ID NO:27

[0187] AY632535.2/326-825NC_012532.1 Zika病毒株MR 766, 乌干达, 黄病毒包膜糖蛋白E.

[0188] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMIGYETDEDRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWA
GADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGV
YSLCTAAFTFTKVPATLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRITANPVITESTENSKMMLELDP
FGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGGVFNSLGKGIHQIFGAAFKSLFG
GMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0189] SEQ ID NO:28

[0190] MR_766.AAV34151.1. 乌干达/291-790黄病毒包膜糖蛋白E. | Q32ZE1 | Q32ZE1_9FL

[0191] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMIGYETDEDRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWA
GADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGV
YSLCTAAFTFTKVPATLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRITANPVITESTENSKMMLELDP
FGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGGVFNSLGKGIHQIFGAAFKSLFG
GMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0192] SEQ ID NO:29

[0193] MR_766.YP_009227198.1. 乌干达/1-500包膜蛋白E[Zika病毒]

[0194] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMIGYETDEDRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWA
GADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGV
YSLCTAAFTFTKVPATLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRITANPVITESTENSKMMLELDP
FGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGGVFNSLGKGIHQIFGAAFKSLFG
GMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0195] SEQ ID NO:30

[0196] KU681081.3/308-811 Zika病毒分离株Zika病毒/H.sapiens-tc/THA/2014/
SV0127-14, 泰国, 黄病毒包膜糖蛋白E.

[0197] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTYVCKRTLVDRLGWGNGCGLFGKGSVLTCAKFTCSKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHTGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRITANPVITEGTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGGVLSLGKGIHQIFGAAFK

SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0198] SEQ ID NO:31

[0199] isol-Zika_病毒%*H.sapiens*-tc%THA%2014%SV0127-_14.AMD61710.1.泰国/291-794黄病毒包膜糖蛋白E.

[0200] IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGLDCEPRTGLDFSDLYYLTMMNKHWLVHKEWFHDIPL
PWHGTADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITEGTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGVLSLKGKIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0201] SEQ ID NO:32

[0202] CK-ISL_2014.AIC06934.1.Cook_Islands/1-504黄病毒包膜糖蛋白E.(片段)OS=Zika病毒GN=E PE=4SV=1

[0203] IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGLDCEPRTGLDFSDLYYLTMMNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLKGKIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0204] SEQ ID NO:33

[0205] Natal_RGN.AMB18850.1.巴西:_Rio_Grande_do_Norte,_Natal/291-794黄病毒包膜糖蛋白E.]

[0206] IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGLDCEPRTGLDFSDLYYLTMMNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLKGKIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0207] SEQ ID NO:34

[0208] isol-Si323.AMC37200.1.哥伦比亚/1-504黄病毒包膜糖蛋白E.

[0209] IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGLDCEPRTGLDFSDLYYLTMMNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLKGKIHQIFGAAFK

SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0210] SEQ ID NO:35

[0211] KU707826.1/317-820 Zika病毒分离株SSABR1,巴西,黄病毒包膜糖蛋白E.

[0212] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKA FEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0213] SEQ ID NO:36

[0214] KU509998.1/326-829 Zika病毒株海地/1225/2014,海地,黄病毒包膜糖蛋白E.

[0215] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKA FEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0216] SEQ ID NO:37

[0217] isol-GDZ16001.AML82110.1.中国/291-794黄病毒包膜糖蛋白E.

[0218] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKA FEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0219] SEQ ID NO:38

[0220] BeH819015.AMA12085.1.巴西/291-794黄病毒包膜糖蛋白E.]

[0221] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKA FEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0222] SEQ ID NO:39

[0223] MRS_OPY_Martinique_PaRi_2015.AMC33116.1.Martinique/291-794黄病毒包膜

糖蛋白E.

[0224] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0225] SEQ ID NO:40

[0226] KU501215.1/308-811Zika病毒株PRVABC59,波多黎各,黄病毒包膜糖蛋白E.

[0227] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0228] SEQ ID NO:41

[0229] 海地%1225%2014.AMB37295.1.海地/291-794黄病毒包膜糖蛋白E.

[0230] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0231] SEQ ID NO:42

[0232] KU527068.1/308-811Zika病毒株Natal RGN,巴西:Rio Grande do Norte,Natal,
黄病毒包膜糖蛋白E.

[0233] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0234] SEQ ID NO:43

[0235] isol-Z1106027.ALX35662.1.苏里南/5-508黄病毒包膜糖蛋白E.

[0236] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA

SDSRCPTQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTG HETDENRAKVEITPNSPRAEATLGGF GSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPL
PWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAE TLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0237] SEQ ID NO:44

[0238] isol-FLR.AMM39804.1.哥伦比亚:_Barranquilla/291-794黄病毒包膜糖蛋白E.

[0239] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTG HETDENRAKVEITPNSPRAEATLGGF GSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPL
PWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAE TLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0240] SEQ ID NO:45

[0241] PLCal_ZV_isol-From_Vero_E6_cells.AHL37808.1.加拿大/254-757黄病毒包膜糖蛋白E.

[0242] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTG HETDENRAKVEITPNSPRAEATLGGF GSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPL
PWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAE TLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0243] SEQ ID NO:46

[0244] BeH818995.AMA12084.1.巴西/291-794黄病毒包膜糖蛋白E.[Zika病毒]。

[0245] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTG HETDENRAKVEITPNSPRAEATLGGF GSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPL
PWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAE TLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFGA AFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0246] SEQ ID NO:47

[0247] H/PF/2013.AHZ13508.1.法属_波利尼西亚/291-794黄病毒包膜糖蛋白E.

[0248] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTG HETDENRAKVEITPNSPRAEATLGGF GSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPL

PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0249] SEQ ID NO:48

[0250] PRVABC59.AMC13911.1.波多黎各/291-794黄病毒包膜糖蛋白E.

[0251] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0252] SEQ ID NO:49

[0253] KU321639.1/326-829Zika病毒株ZikaSPH2015,巴西,黄病毒包膜糖蛋白E.

[0254] IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0255] SEQ ID NO:50

[0256] ZikaSPH2015.ALU33341.1.巴西/291-794黄病毒包膜糖蛋白E.

[0257] IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0258] SEQ ID NO:51

[0259] 103344.AMC13912.1.瓜地马拉/291-794多蛋白[Zika病毒]。103344.AMC13912.1.
危地马拉黄病毒包膜糖蛋白E.

[0260] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEIRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE

LDPPFGDSYIVIGVEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0261] SEQ ID NO:52

[0262] isol-Brazil-ZKV2015.AMD16557.1.巴西/291-794黄病毒包膜糖蛋白E.

[0263] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0264] SEQ ID NO:53

[0265] KU497555.1/308-811Zika病毒分离株巴西-ZKV2015,黄病毒包膜糖蛋白E.

[0266] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0267] SEQ ID NO:54

[0268] isol-ZJ03.AMM39806.1.中国/291-794黄病毒包膜糖蛋白E.

[0269] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSRGSTIGKAFEATVRGARRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0270] SEQ ID NO:55

[0271] isol-FSS13025.AFD30972.1.柬埔寨/291-794黄病毒包膜糖蛋白E.

[0272] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVEKKITHHWHRSRGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0273] SEQ ID NO:56

[0274] isol-Z1106032.ALX35660.1.苏里南/291-794黄病毒包膜糖蛋白E.[Zika病毒]

[0275] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGGVLIFLSTAVSA

[0276] SEQ ID NO:57

[0277] isol-Z1106033.ALX35659.1.苏里南/291-794黄病毒包膜糖蛋白E.[Zika病毒]

[0278] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGGVLIFLSTAVSA

[0279] SEQ ID NO:58

[0280] isol-BeH828305.AMK49165.1.巴西/291-794黄病毒包膜糖蛋白E.

[0281] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHTDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDTQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0282] SEQ ID NO:59

[0283] isol-GD01.AMK79468.1.中国/291-794黄病毒包膜糖蛋白E.

[0284] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNGTGHTDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0285] SEQ ID NO:60

[0286] isol-Z1106031.ALX35661.1.苏里南/291-794黄病毒包膜糖蛋白E.

[0287] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRLGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS

QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVLAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0288] SEQ ID NO:61

[0289] ACD75819.1.密克罗尼西亚/291-794黄病毒包膜糖蛋白E.

[0290] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPAVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

[0291] SEQ ID NO:62

[0292] KU681082.3/308-811Zika病毒分离株Zika病毒/H.sapiens-tc/PHL/2012/CPC-0740,菲律宾,黄病毒包膜糖蛋白E.

[0293] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

[0294] SEQ ID NO:63

[0295] isol-Zika_病毒% H.sapiens-tc% PHL% 2012% CPC-0740.AMD61711.1.菲律宾/
291-794黄病毒包膜糖蛋白E.

[0296] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

[0297] SEQ ID NO:64

[0298] isol-BeH823339.AMK49164.2.巴西/291-794黄病毒包膜糖蛋白E.

[0299] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVSTTVSNMAEVRSYCYEATISDIA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL

PWHAGADTGTPHWNNKEALVEFKDAHAKRQTAVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

[0300] SEQ ID NO:65

[0301] isol-P6-740.AEN75264.1. 马来西亚/291-794黄病毒包膜糖蛋白E.

[0302] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMIVNDXGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWXRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFK
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

[0303] SEQ ID NO:66

[0304] KU744693.1/326-829Zika病毒分离株VE_Ganxian, 中国, 黄病毒包膜糖蛋白E.

[0305] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMLVNDTGHEHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPL
PWHAGAATGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETVDGTVTVEGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAFAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSG

[0306] SEQ ID NO:67

[0307] isol-VE_Ganxian.AMK79469.1. 中国/291-794黄病毒包膜糖蛋白E.

[0308] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
SDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGS
QHSGMLVNDTGHEHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPL
PWHAGAATGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETVDGTVTVEGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAFAFK
SLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSG

[0309] SEQ ID NO:68

[0310] ArD157995.AHL43503.1. -/291-794黄病毒包膜糖蛋白E.

[0311] ISCIGVSNRDLVEGMSGGTWVDVLEHGGCVTEMAQDKPTVDIELVTMTVSNMAEVRSYCYEASISDMA
SASRCPTQGEPSLDKQSDTQSVCKRTLGDGRWGNGCGIFGKGSVLTCSKFTCCCKMPGKSIQPENLEYRIMLPVHGS
QHSGMIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPL
PWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQSAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLE
LDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNLSLGKGIHQIFGAFAFK

SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

[0312] SEQ ID NO:69

[0313] MR_766.ABI54475.1.乌干达/291-788黄病毒包膜糖蛋白E.

[0314] IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMA
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[0315] SEQ ID NO:70

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[0318] SEQ ID NO:71

[0319] KLK肽

[0320] KLKLLLLLKLK

[0321] SEQ ID NO:72

[0322] 测序的ZIKV序列H/PF/2013

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[0324] SEQ ID NO:73

[0325] AHZ13508.1, 来自波利尼西亚爆发的Zika病毒多聚蛋白 (H/PF/2013)

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- [0327] SEQ ID NO:74
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[0355] 9329_Zika_PF_5R
[0356] taactcgagCRCAGATACCATCTTCCC
[0357] SEQ ID NO:84
[0358] 9330_Zika_PF_6F
[0359] ttaggatCCCTTATGTGCTTGGCCTTAG
[0360] SEQ ID NO:85
[0361] 9331_Zika_PF_6R
[0362] taactcgagTCTTCAGCCTCCATGTG
[0363] SEQ ID NO:86
[0364] 9332_Zika_PF_7F
[0365] ttaggatccAATGCCCACTCAAACATAGA
[0366] SEQ ID NO:87
[0367] 9333_Zika_PF_7R
[0368] taactcgagTCATTCTCTTCTTCAGCCCTT
[0369] SEQ ID NO:88
[0370] 9334_Zika_PF_8F
[0371] ttaggatccAAGGGTGATCGAGGAAT
[0372] SEQ ID NO:89
[0373] 9335_Zika_PF_8R
[0374] taactcgagTTCCTTCAGAGAGAGGAGC
[0375] SEQ ID NO:90
[0376] 9336_Zika_PF_9F
[0377] ttaggatccTCTTTTGCAAAGTGGATC
[0378] SEQ ID NO:91
[0379] 9337_Zika_PF_9R
[0380] taactcgagTCCAGCTGCAAAGGGTAT
[0381] SEQ ID NO:92
[0382] 9338_Zika_PF_10F
[0383] ttaggatccGTGTGGACATGTACATTGA
[0384] SEQ ID NO:93
[0385] 9339_Zika_PF_10R
[0386] taactcgagCCCATTGCCATAAAGTC
[0387] SEQ ID NO:94
[0388] 9340_Zika_PF_11F
[0389] ttaggatccTCATACTGTGGTCCATGGA
[0390] SEQ ID NO:95
[0391] 9341_Zika_PF_11R
[0392] taactcgagGCCATCTCAACCCTTG
[0393] SEQ ID NO:96

[0394] 9342_Zika_PF_12F
[0395] ttaggatccTAGAGGGCTTCCAGTGC
[0396] SEQ ID NO:97
[0397] 9343_Zika_PF_12R
[0398] taactcgAGATACTCATCTCCAGGTTTGTG
[0399] SEQ ID NO:98
[0400] 9344_Zika_PF_13F
[0401] ttaggatccGAAAACAAAACATCAAGAGTG
[0402] SEQ ID NO:99
[0403] 9345_Zika_PF_13R
[0404] taactcgagGAATCTCTCTGTCATGTGTCCT
[0405] SEQ ID NO:100
[0406] 9346_Zika_PF_14F
[0407] ttaggatccTTGATGGCAGACCAAC
[0408] SEQ ID NO:101
[0409] 9347_Zika_PF_14R
[0410] ttaggatccGTTGTTGATCTGTGTGAAT
[0411] SEQ ID NO:102
[0412] 9348_Zika_PF_15F
[0413] taactcgagCAGGTCAATGTCCATTG
[0414] SEQ ID NO:103
[0415] 9349_Zika_PF_15R
[0416] ttaggatccTGTTGTGTTCCCTATTGCTGGT
[0417] SEQ ID NO:104
[0418] 9350_Zika_PF_16F
[0419] taactcgaGTGATCAGRGCCCCAGC
[0420] SEQ ID NO:105
[0421] 9351_Zika_PF_16R
[0422] ttaggatccTGCTGCCAGAAGAGAA
[0423] SEQ ID NO:106
[0424] 9352_Zika_PF_17F
[0425] taactcgaGCACCAACAYGGGTTCTT
[0426] SEQ ID NO:107
[0427] 9353_Zika_PF_17R
[0428] ttaggatcCTCAAGGACGGTGTGGC
[0429] SEQ ID NO:108
[0430] 9354_Zika_PF_18F
[0431] taactcgagCAATGATCTTCATGTTGGG
[0432] SEQ ID NO:109

[0433] 9355_Zika_PF_18R
[0434] ttaggatccTATGGGGGAGGACTGGT
[0435] SEQ ID NO:110
[0436] 9356_Zika_PF_19F
[0437] taactcGAGCCCAGAACCTTGATC
[0438] SEQ ID NO:111
[0439] 9357_Zika_PF_19R
[0440] ttaggatcCAGACCCCAAGAAGGC
[0441] SEQ ID NO:112
[0442] 9358_Zika_PF_20F
[0443] taactcgagCCCCTTTGGTCTTGTCT
[0444] SEQ ID NO:113
[0445] 9359_Zika_PF_20R
[0446] ttaggatccAGGAAGGATGTATGCAGATG
[0447] SEQ ID NO:114
[0448] 9360_Zika_PF_21F
[0449] taactcgagACATTTGCGCATATGATTTTG
[0450] SEQ ID NO:115
[0451] 9361_Zika_PF_21R
[0452] ttaggatccAGGAAGGACACACAAGAGT
[0453] SEQ ID NO:116
[0454] 9362_Zika_PF_22F
[0455] taactcgagACAGGCTGCACAGCTTT
[0456] SEQ ID NO:117
[0457] 9363_Zika_PF_22R
[0458] ttaggatccTCTCTCATAGGGCACAGAC

[0459] 在一些实施方式中,Zika病毒具有多蛋白,包括具有由SEQ ID NO:14-69或72中的任一个提供的氨基酸序列的包膜(E)蛋白。在一些实施方式中,多蛋白或E蛋白序列与SEQ ID NO:2-69或72中的任一个至少80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%、99.5%、99.6%、99.7%、99.8%或99.9%相同。

[0460] 在两个或更多个核酸或氨基酸序列的情况中,术语“相同”或百分比“同一性”是指两个或更多个相同的序列或子序列。如果两个序列具有的相同氨基酸残基或核苷酸的特定百分比(例如至少80%、85%、90%、95%、96%、97%、98%或99%同一性)在特定区域或整个序列上进行比较和比对以获得比较窗口或指定区域上的最大对应性,如使用以下序列比较算法之一或通过人工比对和目视检查所测量的。任选地,同一性存在于长度为至少约50个核苷酸(或10个氨基酸)的区域上,或更优选地在长度为100至500或1000个以上的核苷酸(或20、50、200或更多个氨基酸)的区域上。在一些实施方式中,同一性存在于蛋白质的长度上,例如E蛋白。

[0461] 对于序列比较,通常一个序列作为参考序列,将测试序列与其进行比较。用于比较的序列比对方法在本领域中是公知的。参见,例如,Smith和Waterman(1970) Adv. Appl. Math. 2:482c的局部同源性算法,通过Needleman和Wunsch J. Mol. Biol. 48:443, 1970的同源性比对算法,通过Pearson和Lipman. Proc. Natl. Acad. Sci. USA 85:2444, 1988的搜索相似性方法,通过使用这些算法的计算机化实现 (GAP, BESTFIT, FASTA, Jalview和TFASTA, 在威斯康星遗传学软件包, Genetics Computer Group. 575 Science Dr., Madison. WI), 通过使用例如CLUSTALW (Larkin等人, (2007) .Bioinformatics, 23, 2947-2948.) 或MAFFT (Katoh&Toh 2008 Briefings in Bioinformatics 9:286-298) 的多序列比对实施,或通过人工比对和目测检查(参见例如Brent Current Protocols in Molecular Biology, John Wiley&Sons, Inc. (Ringbou ed., 2003))。适用于确定序列同一性百分比和序列相似性的算法的两个示例是BLAST和BLAST 2.0算法,其分别在Altschul等, Nuc. Natl. Acad. Sci. USA, Acids Res. 25:3389-3402, 1977和Altschul等, J. Mol. Biol. 215: 403-410, 1990中描述。

[0462] 实施例

[0463] 实施例1:生产适合用作人和动物疫苗的Zika药物

[0464] 材料和方法:

[0465] 为了生产ZikaV,使用JEV加工平台 (Srivastava等, Vaccine 19 (2001) 4557-4565; US 6,309,650B1) 作为基础。某些过程步骤的微小变化适用于ZikaV特性并提高纯度。过程步骤的简要概述如下(参见图9A和B)。简而言之,在Zika病毒的纯化过程中评估了硫酸鱼精蛋白(PS)的意想不到的和新颖的纯化性质。如图10所示,非感染性病毒颗粒聚集体、HCP和其它LMW杂质通过PS沉淀除去,如通过在SEC-HPLC中去除聚集肩部所示并且通过PS处理而没有感染性病毒滴度损失。下面提供了Zika纯化方案的进一步优化。

[0466] 上游:

[0467] • 基于滚瓶的Vero细胞扩增 (25x850cm² CellBind):

[0468] • 5% CO₂, 35°C, MEM+2mM L-谷氨酰胺+10% FBS

[0469] • 在MOI 0.01下感染ZikaV研究硕士种子库 (rMSB)

[0470] • 无血清病毒生产

[0471] • 5% CO₂, 35°C, MEM+2mM L-谷氨酰胺

[0472] • 具有重新进料的多次收获 (第2、3、5和7天)

[0473] • 无菌过滤收获物并在2-8°C储存直至进一步加工

[0474] 下游:

[0475] • 通过超滤 (100kDa) 合并收获物和浓缩物

[0476] • 如果需要,可以稳定浓缩的收获物 (Tris/10% 蔗糖) 用于储存 (-80°C)

[0477] • 通过硫酸鱼精蛋白 (2mg/mL) 去除hcDNA

[0478] • 蔗糖梯度纯化 (优化三层梯度)

[0479] • 甲醛灭活 (0.02%, 22°C, 10天), 用焦亚硫酸钠中和

[0480] • 稀释至DS抗原靶标含量和具有氢氧化铝 (0.5mg Al/mL) 的制剂

[0481] 来自法属波利尼西亚的Zika病毒株H/PF/2013最初是从51岁的女性 (登录号 KJ776791.1, 本文中也是SEQ ID NO:13) 中分离出来的。样品获自欧洲病毒档案 (EVA; Ref-

SKU:001v-EVA1545)。基于此材料,在Vero细胞上制备研究主种子库(rMSB)作为细胞底物,并通过测序检查基因组序列。由于Zika病毒株H/PF/2013的5'和3'侧翼序列的基因组序列是未知的,所以在基于其它Zika病毒株的那些区域中设计了用于测序的引物,而内部引物根据公开的序列(SEQ ID NO:74至117,也参见表A)设计。通过使用这些引物由rMSB获得的序列由SEQ ID NO:72提供。该序列与公布的Zika病毒株H/PF/2013(SEQ ID NO:13)的序列有100%重叠。然而,我们测序了SEQ ID NO:72中所示的另外5'区域(另外的40bp)和3'区域(另外的160bp)。在一个优选的实施方式中,本发明的Zika病毒包含SEQ ID NO:72。基因组RNA比根据SEQ ID NO:72的序列稍长(可能是另外的200bp)。此外,适用于宿主细胞例如Vero细胞的Zika病毒预期可能含有一个或多个突变。由于这些原因,本发明的Zika病毒包含SEQ ID NO:72的序列或优选是与SEQ ID NO:72的序列具有至少95%、96%、97%、98%或至少99%序列同一性的序列。此外,因为病毒基因组可能甚至含有SEQ ID NO:72的更多侧翼区域;在一个实施方式中,本发明的Zika病毒含有SEQ ID NO:72的序列,并且任选地在5'和/或3'末端进一步包含至少10、至少20、至少30、至少40、至少50、至少60、至少70、至少80、至少90、至少100、至少110、至少120或至少130个核苷酸的延伸。在一个优选的实施方式中,Zika病毒至少包含本发明的Zika病毒株H/PF/2013的全部多蛋白质的编码序列,即SEQ ID NO:73的氨基酸序列或多蛋白质,其与SEQ ID NO:73提供的序列具有至少95%、96%、97%、98%或至少99%的序列同一性。此外,Zika病毒至少包含本发明SEQ ID NO:47的Zika病毒H/PF/2013的E-蛋白的编码序列或其与SEQ ID NO:47提供的序列具有至少95%、96%、97%、98%或至少99%的序列同一性的E蛋白。

[0482] 在Vero细胞上的病毒生长

[0483] Vero细胞在含有10%胎牛血清(FBS)的Eagle最小基本培养基(EMEM)中生长。Vero细胞单层的滚瓶培养物以每个细胞0.01个斑块形成单位(pfu)的感染复数(moi)用Zika病毒株H/PF/2013感染。病毒吸附2小时后,将培养物用PBS洗涤3次并用不含FBS的EMEM进料,并在+35℃和5%CO₂下温育。温育感染的Vero细胞培养物直至病毒滴度达到期望水平。

[0484] 在第2、3、5和7天收获培养基并从这些收获日汇集,然后在标准离心机中离心。然后过滤上清液。通过TFF超滤浓缩病毒培养物上清液以除去细胞培养基成分并减少批量。

[0485] 收获程序的评估

[0486] 当前的JEV收获过程在感染后的第3、5、7和9天具有预定收获。为了模拟JEV过程,在感染培养基(含有2%FBS的MEM+2mM L-谷氨酰胺)中以0.01的MOI用ZIKV库P4-FBS感染滚瓶2小时。除去接种物后,将细胞用PBS洗涤两次并加入200mL生产培养基(MEM+2mM L-谷氨酰胺)。

[0487] 在第2天取样后,在感染后第3天进行第一次病毒收获。此时,与在第2天除去病毒的细胞相比,可以观察到显著更高的CPE。斑块测定分析显示第2天的病毒滴度与标准收获计划相同。然而,从第3天收获开始,观察到的滴度显著较低,与标准收获计划相比观察到的CPE增加。在感染后第5天,根本没有观察到更多的活细胞,并且实验终止于最后的第5天收获。

[0488] 表3:每个斑块测定的计算滴度汇总在下表中。

[0489]

	Log 10PFU/mL
样品第2天	7.02

收获第3天	6.66
收获第5天	6.26

[0490] 这一发现导致了优化的收获计划,以更好地控制CPE并允许额外的收获第5天和第7天,参见图15。对于两个收获日,与修改的方案相比,优化的ZikaV方案产生显著更高的病毒滴度,显示出第一次收获对于产量是至关重要的。另外在第3天的第一次收获导致最多2个收获点,而在第2天的第一次收获允许4个收获点以进一步增加产量获取。

[0491] Zika病毒的下游纯化

[0492] 除非另有说明,纯化过程在室温(18-22℃)下进行。通过使用100kDa截止TFF超滤模块浓缩过滤的组合收获物而开始病毒纯化,以去除细胞培养基组分并减少批量。浓缩后,使用两种组分的储液将汇集的过滤收获物质调整至终浓度为25mM Tris pH 7.5和10%蔗糖(w/w)(参见图11,PS处理前对于不同收获物的SEC-HPLC)。如果需要,这可以在<-65℃冷冻浓缩的收获物。

[0493] 通过用硫酸鱼精蛋白(2mg/mL)沉淀,接着进行蔗糖密度离心(2-8℃)作为最终的抛光步骤,以实现宿主细胞DNA和蛋白质减少以及浓缩材料中非感染性病毒聚集体的减少(参见图20,PS处理后对于不同收获物的SEC-HPLC)。纯化过程设计为在2天内完成,SGC从第1天结束开始,随后在第2天进行分馏和SDS-PAGE分析。蔗糖梯度级分在SDS-PAGE分析期间保存在2-8℃(银染)以鉴定含有ZikaV的纯级分(参见图21)。汇集相关级分后,用福尔马林稀释并灭活该汇集物。汇集蔗糖梯度离心的相关级分后,将汇集物在PBS中以1:3稀释并通过福尔马林(0.02%v/v,200ppm)灭活。通过SDS-PAGE对级分进行分析。

[0494] PS处理对病毒恢复的影响

[0495] 通过SEC-HPLC和斑块测定进行PS处理之前(图11)和之后(图12)分析第2、3、5和7天的单个30x浓缩收获物的样品。SEC-HPLC用于测定表示为峰面积的相对总ZikaV含量(活性+非活性),而相对ZikaV峰值纯度是作为病毒单体群体相对于总病毒峰值的相对含量给出的。斑块测定表明每个样品中总活性病毒颗粒的含量。实验结果总结在表4中。通过SEC-HPLC的病毒峰回收率仅在12%至36%之间,PS处理后的峰值纯度在>90%范围内(未检测到病毒聚集体)。通过斑块测定回收的活性病毒颗粒全部>100%(130-700%,在测定变异性范围内),表明PS处理过程中没有活性病毒颗粒丢失。这些结果表明,在PS处理期间,仅去除了非感染性(未成熟和/或聚集的病毒)颗粒。

[0496] 表4:在PS处理之前和之后通过SEC-HPLC和斑块测定进行的ZikaV回收。

[0497] SEC-HPLC

	峰面积 mAU*分钟			
收获日	30x 浓缩	30x + PS	SEC 回收(%)	PS 处理后的相对病毒 单体含量(%)
[0498] 第 2 天	101.36	18.63	18	89%
第 3 天	144.51	17.48	12	90%
第 5 天	19.97	5.92	30	96%
第 7 天	68.80	24.43	36	99%

[0499] 斑块测定

	PFU/mL		
收获日	30x 浓缩	30x + PS	斑块回收(%)
[0500] 第 2 天	3E+08	5E+08	179
第 3 天	2E+08	4E+08	193
第 5 天	1E+08	9E+08	700
[0501] 第 7 天	3E+08	4E+08	132

[0502] 蔗糖梯度离心

[0503] 将PS处理的收获物分成两部分并装载在两个离心瓶上。蔗糖密度梯度离心 (SGC) 用于ZikaV材料的最终浓缩和抛光。将ZikaV PS处理的浓缩收获物装载在由三层不同密度的蔗糖组成的溶液的顶部。基于初步研究选择三个蔗糖层,如ChikV所示(图15D),其显示线性蔗糖梯度的形成和病毒颗粒与残余污染物的完全分离。蔗糖溶液的最佳体积由经验确定。表5中示出了在100mL瓶规模中离心的单个层的体积。

[0504] 表5:瓶中离心的单个层/体积。

[0505]	溶液	体积 (mL)
	在10%蔗糖中PS处理收获物 (L)	40
	15%蔗糖 (J)	15
	35%蔗糖 (I)	15
	50%蔗糖 (H)	20
	总体积	90

[0506] 通过分层单个蔗糖层来制备蔗糖梯度瓶。一根塑料管连接在蠕动泵管上。使用夹具将塑料管安装在实验室支架上,并放入离心瓶中。塑料管的喷嘴接触瓶子的底部。使用蠕动泵将ZikaV材料和蔗糖溶液泵入圆筒中。量筒用作进料容器。第一个溶液泵是ZikaV材料,因为它代表了最低密度(10%蔗糖 (w/w))的溶液。在ZikaV材料之后,以升序泵入蔗糖溶液,

以15% (w/w) 溶液J开始,随后35% 蔗糖溶液I,并以最高密度蔗糖溶液H (50% (w/w)) 结尾。所描述的设置在图14中示出。在转移所有蔗糖溶液之后,小心移除塑料管以免干扰各层。

[0507] 离心之前,将离心机预冷至4℃。将准备好的SG瓶小心地转移到预冷的转子中。(注意:为避免干扰蔗糖层,必须避免在转移到转子时突然移动瓶子)。将瓶子在4℃以~11.000RCF max离心至少20小时,不启动制动/减速。在使用具有不同转子的不同离心机系统的情况下,需要基于k系数计算必要的速度和离心时间以实现可比较的离心效率。

[0508] 采用蠕动泵手动进行蔗糖梯度的收获。使用连接到蠕动泵管的塑料管来收集蔗糖梯度。使用夹具将包含梯度的瓶子以倾斜的位置(~12°) 安装到实验室支架上。然后将塑料管放入与瓶子底部边缘接触的瓶子中,并用夹子固定在适当位置。这导致管道入口和瓶底部之间的1-2mm小间隙(参见图14)。

[0509] 使用设定为每分钟30mL流速的蠕动泵,收获梯度并手动分成2mL级分。收获每瓶32个级分的总数(约64mL),并丢弃剩余的体积。立即通过SDS-PAGE/银染检测级分以鉴定含有足够高纯度的含病毒级分。代表性SDS-PAGE显示于图14中。汇集级分10-14并进一步处理。

[0510] 通过在22℃控制温度的培养箱中在10天的时间内用0.02% 甲醛培养使纯化的病毒溶液灭活。在第十天加入焦亚硫酸钠以将甲醛中和。

[0511] 在PETG容器中,用PBS将蔗糖梯度汇集物(取样后~17mL) 进一步稀释3倍至最终体积51mL。向该汇集物中加入相当于预甲醛汇集物最终体积的1/50的1% 甲醛(10000ppm) 溶液体积,导致有效浓度为200ppm。甲醛处理的溶液在磁力搅拌器上混合10分钟。取样后,将甲醛处理的病毒溶液置于22℃±2℃的冷却培养箱内。在添加甲醛后的第5天,将经甲醛处理的病毒溶液通过0.2μm过滤器过滤,然后再次置于22℃±2℃的培养箱中。在第10天,在去除10天灭活最终样品后,将200mM焦亚硫酸钠溶液(2mM终浓度) 的1% (最终甲醛处理的病毒溶液的重量) 体积无菌转移到含有经甲醛处理的病毒溶液的PETG容器中。在磁力搅拌器上混合5分钟后,将中和的灭活病毒溶液在室温(20至25℃) 下保持至少30分钟。取样后,将中和的灭活病毒溶液储存在5℃±3℃直至进一步处理。

[0512] 通过甲醛灭活

[0513] 该步骤的关键参数是最终福尔马林浓度、温度、混合和转移到新容器中。已经在稀释汇集物预甲醛处理中设定了最大pfu/mL(通过斑块测定确定) 的初步验收标准。

[0514] 通过以下参数监测中和的灭活病毒溶液的质量:在第10天的斑块测定,SEC-HPLC, SDS-PAGE/Western印迹。

[0515] 有趣的是,在灭活期间采取的样品的SEC-HPLC分析随后用亚硫酸氢盐中和,在整个灭活期间显示或多或少恒定的峰面积。这与使用由Srivastava等人的Vaccine 19(2001) 4557-4565中公开的方法观察到病毒颗粒损失高达60%的JEV相反。在规模缩小的模型中,由于较小规模的表面/面积比和由于非特定吸附导致的高损失,病毒损失甚至更高。ZikaV灭活实验和JEV灭活的差异被注意到如下:

[0516] A) 与JEV相比,ZikaV SGP汇集物关于残余PS(<2μg/mL) 的纯度更高。因此,3倍ZikaV灭活样品含有<<1μg/mL残余PS。市售JEV SGP汇集物平均含有~120μg/mL(可能高达152μg/mL)。对灭活溶液的平均稀释度~14倍导致残余PS含量高达~11μg/mL。可能是由于与福尔马林交联/反应,较高量的残余PS会引起病毒沉淀。

[0517] B) ZikaV灭活样品含有~10% 蔗糖(含~30-35% 蔗糖的SGP汇集物的3倍稀释液)。

在用福尔马林处理期间,蔗糖可能具有病毒ZikaV颗粒的稳定作用。

[0518] 稀释到DS和氢氧化铝制剂(DP)

[0519] 为了制备在小鼠效能测定中使用的ZikaV药物,与Ixiaro相比靶向5倍的抗原含量(表示为总病毒颗粒或SEC峰面积)。确定抗原含量的基础是SEC-HPLC。简而言之,以1ml/min和25℃用PBS+250mM NaCl、pH 7.4平衡的Superose 610/300增量柱(GE Healthcare)用于检测收获样品和整个下游过程中214nm检测波长处的ZikaV。在目前的JEV过程中,NIV中的抗原含量由特定的ELISA确定。在通过ELISA和SEC-HPLC确定的抗原含量之间观察到良好的相关性。平均而言,市售NIV样品中的抗原含量在33AU/mL的范围内,对应于~5.2mAUJEV峰面积,参见图21。

[0520] ZikaV NIV第10天(Zika峰~36mAU,在Waters HPLC/Superose6Increase柱上分析)用PBS稀释至6.3(~5.7x稀释)的目标。加入氢氧化铝至最终浓度为0.5mg/mL铝(添加1/20v/v明矾2%储液)以制备ZikaV药物产品(DP)。将DP轻轻混合5分钟。取出等分试样的DP,通过离心沉淀明矾并通过SEC-HPLC分析澄清的上清液。在上清液中未检测到ZikaV峰,表明在矿物佐剂上完全吸附(估计>95%)病毒颗粒。配制的ZikaV DP在2-8℃下储存。

[0521] 灭活的Zika病毒DS的杂质谱与JEV DS的谱相当,但PS含量较低(表6)。

[0522] 表6:Zika和JEV DS样品中杂质分布的测定:

	规格(JEV DS)	JEV	Zika
	HCP (ng/mL)	<100	<LOQ
	LOQ 12 ng/mL		
	DNA (pg/mL)	<200	<40
	LOQ 40 pg/mL		
[0523]	通过 SEC-MALLS 的聚集 (%)	未标明, 表征的一部分 LOQ 5%	<LOQ
	PS (μg/mL)	仅在 SGP 汇集物中的规格说明一致的过程性能 (19-152μg/ mL), DS 中的*PS 含量基于 SGP 汇集物中的 PS 含量 (~100μg/ mL) 和 DS 的平 均稀释因子 (~28x) 计算; LOQ 2μg/mL	~4* <<LOQ

[0524] *根据Srivastava等人的Vaccine 19 (2001) 4557-4565中公开的方案生产的JEV样品中的典型PS杂质。

[0525] SEC-MALLS结果

[0526] ZikaV NIV在214nm检测波长下的代表性SEC-HPLC洗脱曲线如图16所示。注意的是,BSA (50μg/mL) 被添加到样品中,以最大程度减少HPLC玻璃瓶由于非特异性表面吸附造

成的损失。估计ZikaV单体含量约为98%，多聚体含量为~2%。

[0527] 样品的SEC-MALLS分析(图17)证实了单体ZikaV群体峰1的半径R_z为21.6nm,多聚体峰2为~49nm。累积粒度分布显示89%的所有病毒颗粒在18到25nm之间的半径范围内(图18)。

[0528] 结果证实了ZikaV NIV的纯度和均一性。

[0529] 通过斑块测定的病毒滴度

[0530] 表7:在整个过程中通过斑块测定对活性ZikaV pfus进行定量。

[0531]	样品	Pfu/mL
	收获第2天(过滤)	6.4x10 ⁷
	收获第3天(过滤)	1.0x10 ⁸
	收获第5天(过滤)	1.5x10 ⁸
	收获第7天(过滤)	1.1x10 ⁸
	PS处理的收获物300x浓缩物(=SGP上样)	9.0x10 ⁸
	SGP汇集物	8.9x10 ⁸
	灭活开始(SGP汇集物以1:3稀释)	3.4x10 ⁸
	灭活第5天	<LOD
	灭活第10天	<LOD

[0532] PS和Benzonase在工艺性能上的比较

[0533] 对浓缩的ZikaV收获汇集物的DNA去除方法进行了直接比较。一份用PS(2mg/mL,室温15min)处理,另一份用Benzonase(50U/mL,2mM MgCl₂,4小时RT,48h 2-8℃)处理。如本报告中所述,两种样品均通过蔗糖梯度进一步纯化。有趣的是,在经处理的ZikaV收获物的蔗糖梯度离心后,Benzonase处理的样品没有产生任何纯级分。在检测到特定病毒条带的那些部分中,在整个收集的级分中检测到高量的宿主细胞蛋白质。如预期的那样,PS处理的材料产生含纯ZikaV的级分。这一发现可能表明,PS不仅可以有效去除沉淀中的DNA;另外它通过破坏DNA(片段)和病毒颗粒的相互作用来提高梯度中病毒颗粒的回收率。Benzonase处理不会去除DNA,只会导致其碎裂。残余的DNA片段可能仍然与病毒颗粒和残余的HCP相互作用,导致蔗糖梯度中的交叉污染和共同纯化。汇集的SGP级分也通过SEC-HPLC分析。虽然检测到很大的峰值,但SDS-PAGE证实该样品被HCP高度污染。SEC-HPLC分析后,在UV214和280nm处可能检测到较大的峰,这是由于HCP与大型病毒颗粒之间可能发生相互作用,从而改变了紫外吸光度。

[0534] Vero生长的Zika病毒的免疫原性

[0535] 小鼠免疫

[0536] 在免疫之前,将10只6周龄雌性CD1小鼠的组通过面部静脉血管放血并制备免疫前血清。施用200μL的一次腹膜内免疫。用最终浓度为0.7%的氢氧化铝(Al(OH)₃)配制灭活Zika病毒的剂量滴定(12μg、3μg、1μg、0.33μg、0.11μg、0.037μg和0.012μg,相当于IXIARO中的蛋白量)。免疫后三周,收集血液并制备免疫血清。所有的动物实验均按照奥地利法律进行(BGB1Nr.501/1989)并经“Magistratsabteilung 58”批准。

[0537] 斑块减少中和试验(PRNT)

[0538] PRNT使用十二孔板。每孔用含有4×10⁵个Vero细胞的1mL培养基接种,并在5%CO₂

中温育35℃过夜。每个剂量组的热灭活血清汇集物一式三份进行测试。将靶病毒(H/PF/2013(SEQ ID NO:13)或MR766(SEQ ID NO:11))稀释至100pfu/165μL。等体积的靶病毒和血清稀释液在35℃和5%CO₂下温育1小时。从Vero细胞中吸出细胞培养基,向每个孔中加入330μL混合物靶病毒/血清稀释液,将平板来回摇动5次,然后在35℃和5%CO₂下温育2小时。向每个孔中加入1mL含有EMEM和营养素的2%甲基纤维素溶液,然后将平板在35℃下用5%CO₂温育5天,然后用结晶紫/5%甲醛染色细胞1小时,随后用去离子水洗涤3次。将板空气干燥并手动计数每个孔中的斑块数量。

[0539] 结果

[0540] 对于亚洲(H/PF/2013)和非洲(MR766)谱系(分别为图19和图20)的Zika病毒,在用灭活Zika病毒疫苗(H/PF/2013)免疫的小鼠的血清库中观察到中和低至37ng(剂量相当于IXIARO®中蛋白量)。在1:20血清稀释时观察到完全抑制,免疫剂量低至110ng(剂量相当于IXIARO®中蛋白量)。Zika病毒的亚洲(H/PF/2013)和非洲(MR766)谱系的中和是相同的,这表明灭活Zika病毒疫苗(H/PF/2013)的不同Zika病毒株之间的高度交叉中和。

[0541] 使用Larocca等人(2016,Nature doi:10.1038/nature18952)描述的微量中和测定进行另一种中和测定。发现本发明的灭活Zika病毒在1μg灭活的纯化病毒中具有90的MN50(微中和)滴度。

[0542] 进一步方法:使用Zika感染的小鼠模型评估灭活Zika病毒制剂的免疫原性。使用500、50或5ng灭活Zika病毒与佐剂(例如含有或不含IC31®的氢氧化铝)或无佐剂皮下(s.c.)免疫成年小鼠组。另一组小鼠接受PBS作为阴性对照。每组在t=0时施用指定的接种物,并且在一些情况下也在3至4周后(t=3/4)时施用。在最后一次免疫接种后约三周开始,每隔一定时间从每只小鼠获得血清样品。使用PRNT测试血清样品的中和抗体的存在。

[0543] 还使用Zika感染的小鼠模型即IFN-α/β受体敲除小鼠(A129)评估灭活Zika病毒制剂的体内保护效力(参见例如Dowall等,2016年3月4日,<http://dx.doi.org/10.1101/042358>),或通过向C57BL/6或BALB/c小鼠施用抗-IFN-α/β受体单克隆抗体来阻断IFN-α/β受体(参见例如Pinto等2011年12月7日,DOI:10.1371/journal.ppat.1002407)。对于保护性测定,在t=0时,将具有佐剂(氢氧化铝)或不具有佐剂的灭活Zika病毒皮下接种到10只3-8周龄的A129、C57BL/6或BALB/c小鼠组的后躯中。年龄匹配的对照用在明矾中的PBS或非特异性抗原接种。任选地在第三至第四周后第二次施用所示接种以加强小鼠。然后通过接种致命剂量的活Zika病毒在免疫后3至8周将小鼠皮下攻击。在攻击C57BL/6和BALB/c小鼠前一天,它们被动地(腹膜内)施用抗-IFN-α/β受体单克隆抗体。每天监测攻击小鼠的发病率和死亡率至多二十一天。另一种替代是攻击颅内成年接种疫苗/未接种疫苗的成年小鼠并观察保护。

[0544] 预期本发明方法产生的Zika病毒将在体外、体内和最终人体试验中提供与欧盟和美国以及其它地方目前许可的JEV疫苗IXIARO®非常相似的功能读数。剂量可能会改变,但由于非常相似的杂质谱和几乎相同的生产,将预计与目前许可的JEV疫苗(在欧盟和美国以及其它地区许可)所确定的非常相似的功效和安全性结果。

[0545] 讨论与结论

[0546] 现有的用于生产灭活JEV疫苗IXIARO®的制造平台被用作灭活ZikaV疫苗候选

者(亚洲菌株H/PF/2013)的制造可行性研究的基础。在滚瓶中培养的Vero细胞上产生病毒。通过PS处理然后用优化蔗糖梯度纯化病毒。通过福尔马林处理进行灭活(0.02%, 22°C, 10天)。对于小鼠中的探索性免疫研究,制备了与明矾一起配制的DP,其与市售JEV疫苗IXIARO®相比具有估计5倍高的病毒颗粒含量。DS的杂质分布符合IXIARO®(市售JEV疫苗)规范中定义的所有标准。Zika病毒的亚洲(H/PF/2013)和非洲(MR766)谱系的中和是相同的,这表明灭活Zika病毒疫苗(H/PF/2013)的不同Zika病毒株之间的高度交叉中和。

[0547] 关于本发明的灭活Zika病毒疫苗的免疫原性的体内数据表明,该病毒具有惊人的有效免疫原性,并且还具有高度的交叉保护性(非洲和亚洲菌株非常相似的免疫原性)。数据表明免疫原性比最近报道的灭活Zika病毒疫苗候选者更高(Larocca等,2016,同上)。灭活病毒是最安全的疫苗之一,特别优选用于递送给安全性特别关注的人群,如孕妇、儿童和免疫力低下的个体,这使得本文公开的灭活Zika病毒特别适合。获得高滴度的灭活病毒是该领域的挑战。本文公开的用于纯化灭活Zika病毒的方法不仅产生高产量,而且产生非常纯的药物物质。

[0548] 本发明的进一步更详细的方面:

[0549] A1. 包含灭活Zika病毒颗粒的Zika病毒疫苗,其中Zika病毒颗粒能够以至少70%的可能性将施用Zika病毒疫苗的受试者血清转化。

[0550] A2. A1的Zika病毒疫苗,其中Zika病毒颗粒能够以至少80%、85%、90%或95%的可能性将施用Zika病毒疫苗的受试者血清转化。

[0551] A3. A1或A2的疫苗,其中Zika病毒颗粒具有对应于由SEQ ID NO:2-13或72的核酸序列中的任一个提供的DNA序列的RNA基因组,或与SEQ ID NO:2-13或72中的任一个至少88%相同并且能够包裹毒性Zika病毒的变体核酸序列。

[0552] A4. A1-A3中任一项的疫苗,其中Zika病毒颗粒具有选自由SEQ ID NO:14-69中的任一个提供的氨基酸序列中的E蛋白,或与SEQ ID NO:14-69中的任一个至少95%相同并且能够包裹毒性Zika病毒的变体氨基酸序列。

[0553] A5. A1-A4中任一项的疫苗,其中Zika病毒通过化学灭活、热灭活、pH灭活或UV灭活来灭活。

[0554] A6. A5的疫苗,其中化学灭活包括使Zika病毒与化学灭活剂接触以如通过斑块测定所测量地、完全灭活Zika病毒。

[0555] A7. A6的疫苗,其中化学灭活包括使Zika病毒与甲醛接触。

[0556] A8. A7的疫苗,其中甲醛灭活包括使Zika病毒与甲醛接触2-10天。

[0557] A9. A5-A8中任一项的疫苗,其中化学活化在约+4°C或约+22°C进行。

[0558] A10. A1-A9中任一项的疫苗,其进一步包含佐剂。

[0559] A11. A10的疫苗,其中佐剂是铝盐佐剂。

[0560] A12. A11的疫苗,其中铝盐佐剂是氢氧化铝或磷酸铝盐。

[0561] A13. A10-A12中任一项的疫苗,其中疫苗包含或进一步包含含有肽和含有脱氧肌苷的免疫刺激性寡聚脱氧核酸分子(I-ODN)的佐剂。

[0562] A14. A13的疫苗,其中肽包含序列KLKL5KLK(SEQ ID NO:71),并且I-ODN包含寡聚-d(IC)13(SEQ ID NO:70)。

[0563] A15. A1-A14中任一项的疫苗,其还包含一种或多种药学上可接受的赋形剂。

[0564] A16.A1-A15中任一项的疫苗,其中疫苗含有硫酸鱼精蛋白或PS的片段或分解产物,其含量太低而不能被HPLC检测,即低于1 μ g/mL,尤其低于100ng/mL。

[0565] A17.A16的疫苗,其中所述硫酸鱼精蛋白或PS的片段或分解产物可以通过质谱或其它敏感方法检测。

[0566] B1.包含A1-A15中任一项的Zika病毒疫苗的试剂盒。

[0567] B2.B1的试剂盒,还包含第二疫苗。

[0568] B3.B2的试剂盒,其中第二疫苗是西尼罗河病毒疫苗、日本脑炎病毒疫苗、黄热病病毒疫苗、登革热病毒疫苗或基孔肯雅病毒疫苗。

[0569] C1.一种方法,其包括向有需要的受试者施用治疗有效量的第一剂量的A1-A15中任一项的Zika病毒疫苗。

[0570] C2.C1的方法,其进一步包括施用治疗有效量的第二剂量的Zika病毒疫苗。

[0571] C3.C1或C2的方法,其中在第一剂量的Zika病毒疫苗后约7天施用第二剂量的Zika病毒疫苗。

[0572] C4.C1或C2的方法,其中在第一剂量的Zika病毒疫苗后约14天施用第二剂量的Zika病毒疫苗。

[0573] C5.C1或C2的方法,其中在第一剂量的Zika病毒疫苗后约28天施用第二剂量的Zika病毒疫苗。

[0574] C6.C1-C5中任一项的方法,其中施用导致产生Zika病毒中和抗体。

[0575] D1.一种生产Zika病毒疫苗的方法,包括:

[0576] (i) 在Vero细胞上传代Zika病毒,由此产生包含Zika病毒的培养物上清液;

[0577] (ii) 收获(i)的培养基;

[0578] (iii) 沉淀(ii)的收获培养基,从而产生Zika病毒上清液;以及

[0579] (iv) 最佳地灭活(iii)的Zika病毒上清液中的Zika病毒,由此产生灭活Zika病毒。

[0580] D2.D1的方法,其进一步包括在步骤(iii)之前浓缩(ii)的培养基。

[0581] D3.D1或D2的方法,其中(iii)的沉淀包括使(ii)的培养基与硫酸鱼精蛋白或苯甲酸盐接触。

[0582] D4.D1-D3中任一项的方法,还包括(v)透析(iv)的灭活Zika病毒,由此产生透析Zika病毒。

[0583] D5.D4的方法,其进一步包括(vi)过滤(v)的透析Zika病毒。

[0584] D6.D1-D5中任一项的方法,其中灭活是通过化学灭活、热灭活、pH灭活或UV灭活进行。

[0585] D7.D6的方法,其中化学灭活包括使Zika病毒与化学灭活剂接触至少4天。

[0586] D8.D6或D7的方法,其中化学灭活剂包含甲醛。

[0587] D9.D6-D8中任一项的方法,其中化学活化在约+4 $^{\circ}$ C或约+22 $^{\circ}$ C下进行。

[0588] D10.D8或D9的方法,其还包括中和甲醛。

[0589] D11.D10的方法,其中用焦亚硫酸钠进行中和。

[0590] E1.A1-A15中任一项的最佳灭活Zika病毒疫苗在治疗和/或预防Zika病毒感染中的用途。

[0591] E2.E1的用途,其中灭活Zika病毒疫苗以治疗有效量的第一剂量施用于有需要的

受试者。

[0592] E3.E2的用途,其中灭活Zika病毒疫苗以治疗有效量的第二剂量施用于受试者。

[0593] E4.E3的用途,其中在第一剂量的Zika病毒疫苗后约7天施用第二剂量的灭活Zika病毒疫苗。

[0594] E5.E3的用途,其中在第一剂量的Zika病毒疫苗后约14天施用第二剂量的Zika病毒疫苗。

[0595] E6.E3的用途,其中在第一剂量的Zika病毒疫苗后约28天施用第二剂量的Zika病毒疫苗。

[0596] E7.E1-E6中任一项的用途,其中疫苗施用导致产生Zika病毒中和抗体。

[0597] F1.用于治疗或预防Zika病毒感染的药物组合物,其中药物组合物包含A1-A15中任一项的最佳灭活Zika病毒疫苗。

[0598] F2.F1的药物组合物,其中灭活Zika病毒疫苗以治疗有效量的第一剂量施用于有需要的受试者。

[0599] F3.F2的用途,其中灭活Zika病毒疫苗以治疗有效量的第二剂量施用于受试者。

[0600] F4.F3的用途,其中在第一剂量的Zika病毒疫苗后约7天施用第二剂量的灭活Zika病毒疫苗。

[0601] F5.F3的用途,其中在第一剂量的Zika病毒疫苗后约14天施用第二剂量的Zika病毒疫苗。

[0602] F6.F3的用途,其中在第一剂量的Zika病毒疫苗后约28天施用第二剂量的Zika病毒疫苗。

[0603] F7.F1-F6中任一项的用途,其中疫苗施用导致产生Zika病毒中和抗体。

[0604] G1.优化蔗糖梯度离心在从纯化的感染性Zika病毒颗粒中去除硫酸鱼精蛋白中的用途。

[0605] G2.根据G1的用途,其中所述优化蔗糖梯度离心包含含有10%+/-1% (w/w) 蔗糖溶液级分的病毒和具有不同密度的另外三层蔗糖溶液,即具有15%+/-1% (w/w) 的蔗糖的第一蔗糖溶液,具有35%+/-1% (w/w) 蔗糖的第二蔗糖溶液和具有50%+/-1% (w/w) 蔗糖的第三蔗糖溶液。

[0606] G3.一种纯化感染性Zika病毒颗粒的方法,其包括以下步骤:

[0607] a) 提供包含病毒颗粒和杂质的粗收获物(a),其中所述杂质通过在细胞基质上培养所述病毒颗粒而产生;

[0608] b) 通过用包含鱼精蛋白,优选鱼精蛋白盐,更优选硫酸鱼精蛋白,甚至更优选重组硫酸鱼精蛋白的试剂沉淀来减少粗品收获物(a)中的杂质,以获得病毒制剂(b);

[0609] c) 通过优化蔗糖密度梯度离心进一步纯化病毒制剂(b),其中提供优化蔗糖梯度使得鱼精蛋白可以完全或几乎完全从病毒级分分离;并且其中通过该步骤使鱼精蛋白浓度降低至最终药物中的鱼精蛋白浓度低于1 μ g/ml,优选低于0.5 μ g/mL,更优选低于0.1 μ g/mL,最优选低于0.05 μ g/mL的程度。

[0610] G4.G3的方法,其中所述优化蔗糖密度梯度离心包含包含含有10%+/-1% (w/w) 蔗糖溶液级分的病毒和具有不同密度的另外三层蔗糖溶液,即具有15%+/-1% (w/w) 的蔗糖的第一蔗糖溶液,具有35%+/-1% (w/w) 蔗糖的第二蔗糖溶液和具有50%+/-1% (w/w) 蔗糖

的第三蔗糖溶液。

[0611] G5.G3至G4中任一项的方法,其另外包含进一步纯化步骤:

[0612] (d) 填充在包含配体活化核和包含孔的无活性壳的柱中的固相基质,其中孔的分子量截断排除病毒颗粒进入配体活化核,并且其中小于孔的分子量截断的分子可以进入配体活化核并收集病毒颗粒。

[0613] G6.G3-G5任一项的方法,其中Zika病毒制剂(c)的残余宿主细胞DNA含量小于10ng/mL,最终病毒制剂(c)的残余宿主细胞蛋白含量小于100ng/mL。

[0614] G7.G3-G6中任一项的方法,其中所述包含Zika病毒颗粒和杂质的粗收获物(a)在步骤(b)之前进行一个或多个预纯化步骤。

[0615] G8.G7的方法,其中一个或多个预纯化步骤包括:

[0616] a) 使用孔径等于或小于0.2 μ m的过滤器过滤;和/或

[0617] b) 通过酶处理消化宿主细胞基因组DNA;和/或

[0618] c) 使用孔径等于或大于300kDa,优选等于或大于100kDa的中空纤维膜进行超滤/渗滤。

[0619] G9.G3-G8中任一项的方法,其中硫酸鱼精蛋白的浓度为0.5-3mg/ml,更优选为1-2mg/ml,更优选为1.2-1.8mg/ml,更优选为1.4-1.6mg/ml,最优选为1.6mg/ml或2mg/ml。

[0620] G10.G3-G9中任一项的方法,其中相对于粗收获物(a)中的总病毒产物,病毒制剂(c)或任何最终病毒制剂中的感染性Zika病毒颗粒的富集在至少50%至95%,优选至少80%的范围内。

[0621] G11.G7-G10中任一项的方法,其中G8-G11中任一项的步骤(b)之前的一个或多个预纯化步骤使用孔径等于或小于1 μ m,优选0.2 μ m的过滤器。

[0622] G12.G3-G11中任一项的方法,其中Zika病毒制剂(c)的残余杂质小于10%。

[0623] G13.G3-G12中任一项的方法,其中Zika病毒在选自以下的细胞系中增殖:EB66细胞系、Vero细胞系、Vero- α His细胞系、HeLa细胞系、HeLa-S3细胞系、293细胞系、PC12细胞系、CHO细胞系、3T3细胞系、PerC6细胞系、MDSK细胞系、鸡胚胎成纤维细胞系、鸭细胞系和二倍体禽细胞系。

[0624] G14.G13的方法,其中所述细胞系是Vero细胞系。

[0625] G15.G3-G14中任一项的方法,其中感染性Zika病毒颗粒是活病毒、活减毒病毒、嵌合病毒、修饰的活病毒或重组活病毒的感染性病毒颗粒。

[0626] G16.G3-G15中任一项的方法,其中Zika病毒优选为亚洲谱系的菌株。

[0627] G17.G3-G16中任一项的方法,其中导致最终病毒制剂(c)或(d)的所述方法之后是灭活步骤,其中病毒优选通过甲醛灭活。

[0628] G18.根据G3-G17中任一项的方法在制造用于免疫病毒感染的组合物中的用途。

[0629] G19.根据G18的用途,其中所述病毒感染是由Zika病毒引起的感染。

[0630] Q1.一种纯化感染性Zika病毒颗粒的方法,其包括以下步骤:

[0631] a) 提供包含病毒颗粒和杂质的粗收获物(a),其中所述杂质通过在细胞基质上培养所述病毒颗粒而产生;

[0632] b) 通过用包含鱼精蛋白,优选鱼精蛋白盐,更优选硫酸鱼精蛋白,甚至更优选重组硫酸鱼精蛋白的试剂沉淀来减少粗品收获物(a)中的杂质,以获得病毒制剂(b);

[0633] c) 通过优化蔗糖密度梯度离心进一步纯化病毒制剂(b), 其中提供优化蔗糖梯度使得鱼精蛋白可以完全或几乎完全从病毒级分分离; 并且其中通过该步骤使鱼精蛋白浓度降低至最终药物中的鱼精蛋白浓度低于 $1\mu\text{g}/\text{mL}$, 优选低于 $0.5\mu\text{g}/\text{mL}$, 更优选低于 $0.1\mu\text{g}/\text{mL}$, 最优选低于 $0.05\mu\text{g}/\text{mL}$ 的程度。

[0634] Q2.Q2的方法, 其中病毒颗粒来自Zika病毒。

[0635] Q3.Q1或Q2的方法, 其还包括以下步骤:

[0636] (d) 填充在包含配体活化核和包含孔的无活性壳的柱中的固相基质, 其中孔的分子量截断排除病毒颗粒进入配体活化核, 并且其中小于孔的分子量截断的分子可以进入配体活化核并收集病毒颗粒。

[0637] Q4.Q1-Q3中任一项的方法, 其中病毒制剂(c)的残余宿主细胞DNA小于 $10\text{ng}/\text{mL}$, 最终病毒制剂(c)的残余宿主细胞蛋白小于 $100\text{ng}/\text{mL}$ 。

[0638] Q5.Q1-Q4中任一项的方法, 其中包含病毒颗粒和杂质的粗收获物(a)在步骤(b)之前进行一个或多个预纯化步骤。

[0639] Q6.Q5的方法, 其中一个或多个预纯化步骤包括:

[0640] a) 使用孔径等于或小于 $0.2\mu\text{m}$ 的过滤器过滤; 和/或

[0641] b) 通过酶处理消化宿主细胞基因组DNA; 和/或

[0642] c) 使用孔径等于或大于 300kDa , 优选等于或大于 100kDa 的中空纤维膜进行超滤/渗滤。

[0643] Q7.Q1-Q6中任一项的方法, 其中硫酸鱼精蛋白的浓度为 $0.5-3\text{mg}/\text{mL}$, 更优选为 $1-2\text{mg}/\text{mL}$, 更优选为 $1.2-1.8\text{mg}/\text{mL}$, 更优选为 $1.4-1.6\text{mg}/\text{mL}$, 最优选为 $1.6\text{mg}/\text{mL}$ 或 $2\text{mg}/\text{mL}$ 。

[0644] Q8.Q1-Q7中任一项的方法, 其中相对于粗收获物(a)中的总病毒产物, 病毒制剂(c)或任何最终病毒制剂中的感染性病毒颗粒的富集在至少50%至95%, 优选至少80%的范围内。

[0645] Q9.Q5-Q8中任一项的方法, 其中Q5-Q8中任一项的步骤(b)之前的一个或多个预纯化步骤使用孔径等于或小于 $1\mu\text{m}$, 优选 $0.2\mu\text{m}$ 的过滤器。

[0646] Q10.Q1-Q9中任一项的方法, 其中病毒制剂(c)的残余杂质小于10%。

[0647] Q11.Q1-Q10中任一项的方法, 其中病毒在选自以下的细胞系中增殖: EB66细胞系、Vero细胞系、Vero- αHis 细胞系、HeLa细胞系、HeLa-S3细胞系、293细胞系、PC12细胞系、CHO细胞系、3T3细胞系、PerC6细胞系、MDSK细胞系、鸡胚胎成纤维细胞系、鸭细胞系和二倍体禽细胞系。

[0648] Q12.Q11的方法, 其中所述细胞系是Vero细胞系。

[0649] Q13.Q1-Q12中任一项的方法, 其中感染性病毒颗粒是活病毒、减毒活病毒、嵌合病毒、修饰的活病毒或重组活病毒的感染性Zika病毒颗粒。

[0650] Q14.Q1-Q13中任一项的方法, 其中Zika病毒是亚洲谱系的Zika病毒株或其免疫原性变体。

[0651] Q15.Q1-Q14中任一项的方法, 其中导致最终病毒制剂(c)或(d)的所述方法之后是灭活步骤, 其中病毒优选通过甲醛灭活。

[0652] Q16. 根据Q1-Q15中任一项的方法在制造用于免疫病毒感染的组合物中的用途。

[0653] Q17. 根据Q16的用途, 其中用于免疫病毒感染的组合物是由Zika病毒引起的感染。

[0654] Q18.包含通过Q1-Q17中任一项的方法可获得或获得的病毒颗粒的组合物,用于治疗 and/或预防感染例如Zika病毒感染。

[0655] Q19.包含在vero细胞上生长的灭活Zika病毒颗粒的Zika病毒疫苗,其中Zika病毒颗粒能够以至少70%的可能性将施用Zika病毒疫苗的受试者血清转化并且包含少量硫酸鱼精蛋白,优选低于检测限。

[0656] Q20.Q19的Zika病毒疫苗,其中Zika病毒颗粒能够以至少80%、85%、90%或95%的可能性,优选80%的可能性将施用Zika病毒疫苗的受试者血清转化。

[0657] Q21.Q19或Q20的疫苗,其中Zika病毒颗粒具有对应于由SEQ ID NO:2-13的核酸序列中的任一个提供的DNA序列的RNA基因组,或与SEQ ID NO:2-13中的任一个至少88%相同并且能够包裹毒性Zika病毒的变体核酸序列。

[0658] Q22.Q19、Q20和Q21中任一项的疫苗,其中Zika病毒颗粒具有选自由SEQ ID NO:14-69或72中的任一个提供的氨基酸序列的E蛋白,或与SEQ ID NO:14-69或72中的任一个至少95%相同并且能够包裹毒性Zika病毒的变体氨基酸序列。

[0659] Q23.Q19、Q20-Q22中任一项所述的疫苗,其中通过在Vero细胞上培养而获得的Zika病毒通过硫酸鱼精蛋白沉淀和蔗糖梯度离心进行纯化。

[0660] Q24.Q23的疫苗,其中蔗糖梯度离心是优化蔗糖梯度离心。

[0661] Q25.Q24的疫苗,其中优化蔗糖梯度离心包含含有10% (w/w) 蔗糖溶液级分的病毒和具有不同密度的另外三层蔗糖溶液,即具有15% (w/w) 的蔗糖的第一蔗糖溶液,具有35% (w/w) 蔗糖的第二蔗糖溶液和具有50% (w/w) 蔗糖的第三蔗糖溶液。

[0662] Q26.Q19、Q20-Q25中任一项的疫苗,其中Zika病毒通过化学灭活、热灭活、pH灭活或UV灭活而灭活。

[0663] Q27.Q26的疫苗,其中化学灭活包括使Zika病毒与化学灭活剂接触的时间比通过斑块测定所测量的完全灭活Zika病毒所需的更久。

[0664] Q28.Q27的疫苗,其中化学灭活包括使Zika病毒与甲醛接触。

[0665] Q29.Q28的疫苗,其中甲醛灭活包括使Zika病毒与甲醛接触2-10天。

[0666] Q30.Q27-Q29中任一项的疫苗,其中化学活化在约+4℃或约+22℃下进行。

[0667] Q31.Q19-Q30中任一项的疫苗,其进一步包含佐剂。

[0668] Q32.Q31的疫苗,其中佐剂是铝盐佐剂。

[0669] Q33.Q32的疫苗,其中铝盐佐剂是氢氧化铝或磷酸铝盐。

[0670] Q34.Q32的疫苗,其中基于包含Zika病毒、优选灭活Zika病毒的最终药物组合物,铝盐佐剂是具有小于1.25ppb铜的氢氧化铝。

[0671] Q35.Q19-Q34中任一项的疫苗,其还包含一种或多种药学上可接受的赋形剂。

[0672] R1.鱼精蛋白、优选鱼精蛋白盐在分离感染性和非感染性Zika病毒颗粒、宿主细胞蛋白和/或不确定的低分子量物质中的用途。

[0673] R2.一种纯化感染性Zika病毒颗粒的方法,其包括以下步骤:

[0674] (a) 提供包含Zika病毒颗粒和杂质的粗收获物(a),其中所述杂质通过在细胞基质上培养所述病毒颗粒而产生;

[0675] (b) 通过用包含鱼精蛋白,优选鱼精蛋白盐,更优选硫酸鱼精蛋白,甚至更优选重组鱼精蛋白硫酸盐的试剂沉淀来减少粗收获物(a)中的杂质,以获得病毒制剂(b),其中相

对于粗收获物(a)中的总病毒产物,病毒制剂(b)中的感染性病毒颗粒的富集在至少50%至95%,优选至少80%的范围内。

[0676] R3.R1的用途或R2的方法,其中病毒颗粒来自Zika病毒。

[0677] R4.一种纯化感染性Zika病毒颗粒的方法,其包括以下步骤:

[0678] (a) 提供包含Zika病毒颗粒和杂质的粗收获物(a),其中所述杂质通过在细胞基质上培养所述病毒颗粒而产生;

[0679] (b) 通过用包含鱼精蛋白,优选鱼精蛋白盐,更优选硫酸鱼精蛋白,甚至更优选重组鱼精蛋白硫酸盐的试剂沉淀来减少粗品收获物(a)中的杂质,以获得病毒制剂(b);

[0680] (c) 通过一种或多种尺寸排阻方法进一步纯化病毒制剂(b),例如(i)蔗糖密度梯度离心,(ii)填充在包含配体活化核和包含孔的无活性壳的柱中的固相基质,其中孔的分子量截断排除病毒颗粒进入配体活化核,并且其中小于孔的分子量截断的分子可以进入配体活化核并收集病毒颗粒,和/或(iii)尺寸排阻色谱以获得包含感染性病毒颗粒的病毒制剂(c),其中病毒制剂(c)的残余宿主细胞DNA低于100ng/mL,并且最终病毒制剂(c)的残余宿主细胞蛋白和感染性病毒颗粒的残余聚集体小于1 μ g/mL。

[0681] R5.R4的方法,其中病毒制剂(c)的残余宿主细胞DNA低于10ng/mL,最终病毒制剂(c)的残余宿主细胞蛋白低于100ng/mL。

[0682] R6.R2-R5中任一项的方法,其中包含病毒颗粒和杂质的粗收获物(a)在步骤(b)之前进行一个或多个预纯化步骤。

[0683] R7.R6的方法,其中一个或多个预纯化步骤包括:

[0684] (a) 使用孔径等于或小于0.2 μ m的过滤器过滤;和/或

[0685] (b) 通过酶处理消化宿主细胞基因组DNA;和/或

[0686] (c) 使用孔径等于或大于300kDa,优选等于或大于100kDa的中空纤维膜进行超滤/渗滤。

[0687] R8.R2-R7中任一项的方法,其中硫酸鱼精蛋白的浓度为0.5-3mg/ml,更优选为1-2mg/ml,更优选为1.2-1.8mg/ml,更优选为1.4-1.6mg/ml,最优选为1.6mg/ml或2mg/ml。

[0688] R9.R2-R8中任一项的方法,其中相对于粗收获物(a)中的总病毒产物,病毒制剂(c)或任何最终病毒制剂中的感染性病毒颗粒的富集在至少50%至95%,优选至少80%的范围内。

[0689] R10.R6-R9中任一项的方法,其中R6-R9中任一项的步骤(b)之前的一个或多个预纯化步骤使用孔径等于或小于1 μ m,优选0.2 μ m的过滤器。

[0690] R11.R2-R10中任一项的方法,其中病毒制剂(c)的残余杂质小于10%。

[0691] R12.R2-R11中任一项的方法,其中病毒在选自以下的细胞系中增殖:EB66细胞系、Vero细胞系、Vero- α His细胞系、HeLa细胞系、HeLa-S3细胞系、293细胞系、PC12细胞系、CHO细胞系、3T3细胞系、PerC6细胞系、MDSK细胞系、鸡胚胎成纤维细胞系、鸭细胞系和二倍体禽细胞系。

[0692] R13.R12的方法,其中所述细胞系是Vero细胞系。

[0693] R14.R2-R13中任一项的方法,其中Zika病毒是活病毒、减毒活病毒、嵌合病毒、修饰的活病毒或重组活病毒。

[0694] R15.R2-R14中任一项的方法,其中Zika病毒是亚洲谱系的Zika病毒株或其免疫原

性变体。

[0695] R16.R2-R15中任一项的方法,其中导致最终病毒制剂(c)的所述方法之后是灭活步骤,其中病毒优选通过甲醛灭活。

[0696] R17.根据R1-R16中任一项的方法在制造用于免疫病毒感染的组合物中的用途。

[0697] R18.根据R17的用途,其中用于免疫病毒感染的组合物是由Zika病毒引起的感染。

[0698] R19.包含通过R2-R16中任一项的方法可获得或获得的组合物,用于治疗和/或预防感染的病毒颗粒。

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[2412]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu		300
[2413]		305	310
[2414]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		315
[2415]		325	330
[2416]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		335
[2417]		340	345
[2418]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		350
[2419]		355	360
[2420]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		365
[2421]		370	375
[2422]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp		380
[2423]		385	390
[2424]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		395
[2425]		405	410
[2426]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		415
[2427]		420	425
[2428]	Ser Val Gly Gly Val Phe Asn Ser Leu Gly Lys Gly Ile His Gln Ile		430
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[2430]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		445
[2431]		450	455
[2432]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys		460
[2433]		465	470
[2434]	Asn Gly Ser Ile Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile		475
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 [2470] 180 185 190
 [2471] Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn
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 [2473] Lys His Arg Leu Val Arg Lys Glu Trp Phe His Asp Ile Pro Leu Pro
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 [2475] Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
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 [2477] Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val

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[2481]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Phe Ser Gly His		
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[2483]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
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[2485]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu		
[2486]	305	310	315
[2487]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[2488]	325	330	335
[2489]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[2490]	340	345	350
[2491]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
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[2493]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
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[2495]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp		
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[2497]	Leu Lys Lys Gly Ser Ser Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[2498]	405	410	415
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[2501]	Ser Val Gly Gly Val Phe Asn Ser Leu Gly Lys Gly Val His Gln Ile		
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[2505]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys		
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[2528]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
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[2530]	Leu Val Thr Cys Ala Lys Phe Thr Cys Ser Lys Lys Met Thr Gly Lys
[2531]	115 120 125
[2532]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
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[2536]	Asp Glu Asn Arg Ala Lys Val Glu Val Thr Pro Asn Ser Pro Arg Ala
[2537]	165 170 175
[2538]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro
[2539]	180 185 190
[2540]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn
[2541]	195 200 205
[2542]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro
[2543]	210 215 220
[2544]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
[2545]	225 230 235 240
[2546]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val
[2547]	245 250 255
[2548]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala
[2549]	260 265 270
[2550]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Phe Ser Gly His
[2551]	275 280 285
[2552]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser
[2553]	290 295 300
[2554]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu
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[2556]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
[2557]	325 330 335
[2558]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu
[2559]	340 345 350
[2560]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[2561]	355 360 365

[2562]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
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[2564]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp
[2565]	385 390 395 400
[2566]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
[2567]	405 410 415
[2568]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
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[2570]	Ser Val Gly Gly Val Phe Asn Ser Leu Gly Lys Gly Ile His Gln Ile
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[2572]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
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[2574]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys
[2575]	465 470 475 480
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[2587]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr
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[2589]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
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[2591]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
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[2593]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
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[2596]	85 90 95
[2597]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
[2598]	100 105 110
[2599]	Leu Val Thr Cys Ala Lys Phe Thr Cys Ser Lys Lys Met Thr Gly Lys
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[2601]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
[2602]	130 135 140
[2603]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Ile Gly His Glu Thr

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[2605]	Asp Glu Asn Arg Ala Lys Val Glu Val Thr Pro Asn Ser Pro Arg Ala			
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[2607]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro			
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[2609]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn			
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[2611]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro			
[2612]		210	215	220
[2613]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu			
[2614]	225	230	235	240
[2615]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val			
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[2617]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala			
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[2619]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Phe Ser Gly His			
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[2621]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser			
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[2623]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu			
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[2625]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp			
[2626]		325	330	335
[2627]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu			
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[2629]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser			
[2630]		355	360	365
[2631]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp			
[2632]		370	375	380
[2633]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp			
[2634]	385	390	395	400
[2635]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg			
[2636]		405	410	415
[2637]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly			
[2638]		420	425	430
[2639]	Ser Val Gly Gly Val Phe Asn Ser Leu Gly Lys Gly Ile His Gln Ile			
[2640]		435	440	445
[2641]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser			
[2642]		450	455	460
[2643]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys			
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[2645]	Asn Gly Ser Ile Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile			

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[2647]	Phe Leu Ser Thr Ala Val Ser Ala					
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[2654]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser					
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[2656]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr					
[2657]		20		25		30
[2658]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr					
[2659]		35		40		45
[2660]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser					
[2661]		50		55		60
[2662]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala					
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[2664]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu					
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[2666]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser					
[2667]		100		105		110
[2668]	Leu Val Thr Cys Ala Lys Phe Thr Cys Ser Lys Lys Met Thr Gly Lys					
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[2670]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His					
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[2672]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Ile Gly His Glu Thr					
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[2674]	Asp Glu Asn Arg Ala Lys Val Glu Val Thr Pro Asn Ser Pro Arg Ala					
[2675]		165		170		175
[2676]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro					
[2677]		180		185		190
[2678]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn					
[2679]		195		200		205
[2680]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro					
[2681]		210		215		220
[2682]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu					
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[2684]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val					
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[2686]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala					
[2687]		260		265		270

[2688]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Phe Ser Gly His
[2689]	275 280 285
[2690]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser
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[2692]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu
[2693]	305 310 315 320
[2694]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
[2695]	325 330 335
[2696]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu
[2697]	340 345 350
[2698]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[2699]	355 360 365
[2700]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
[2701]	370 375 380
[2702]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp
[2703]	385 390 395 400
[2704]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
[2705]	405 410 415
[2706]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
[2707]	420 425 430
[2708]	Ser Val Gly Gly Val Phe Asn Ser Leu Gly Lys Gly Val His Gln Ile
[2709]	435 440 445
[2710]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
[2711]	450 455 460
[2712]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys
[2713]	465 470 475 480
[2714]	Asn Gly Ser Ile Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile
[2715]	485 490 495
[2716]	Phe Leu Ser Thr Ala Val Ser Ala
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[2724]	1 5 10 15
[2725]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr
[2726]	20 25 30
[2727]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
[2728]	35 40 45
[2729]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser

[2730]	50	55	60
[2731]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
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[2733]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu		
[2734]	85	90	95
[2735]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
[2736]	100	105	110
[2737]	Leu Val Thr Cys Ala Lys Phe Thr Cys Ser Lys Lys Met Thr Gly Lys		
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[2739]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His		
[2740]	130	135	140
[2741]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Ile Gly His Glu Thr		
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[2743]	Asp Glu Asn Arg Ala Lys Val Glu Val Thr Pro Asn Ser Pro Arg Ala		
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[2745]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
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[2747]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
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[2749]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
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[2751]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		
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[2755]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
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[2759]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[2760]	290	295	300
[2761]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu		
[2762]	305	310	315
[2763]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[2764]	325	330	335
[2765]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[2766]	340	345	350
[2767]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[2768]	355	360	365
[2769]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[2770]	370	375	380
[2771]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp		

[2772]	385	390	395	400
[2773]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg			
[2774]		405	410	415
[2775]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly			
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[2777]	Ser Val Gly Gly Val Phe Asn Ser Leu Gly Lys Gly Val His Gln Ile			
[2778]		435	440	445
[2779]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser			
[2780]		450	455	460
[2781]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys			
[2782]		465	470	475
[2783]	Asn Gly Ser Ile Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile			
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[2794]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr			
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[2798]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser			
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[2800]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala			
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[2804]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser			
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[2806]	Leu Val Thr Cys Ala Lys Phe Thr Cys Ser Lys Lys Met Thr Gly Lys			
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[2808]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His			
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[2810]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr			
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[2812]	Asp Glu Asn Arg Ala Lys Val Glu Val Thr Pro Asn Ser Pro Arg Ala			
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[2814]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
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[2816]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		190
[2817]		195	200
[2818]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		205
[2819]		210	215
[2820]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		220
[2821]		225	230
[2822]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val		235
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[2824]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		255
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[2826]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Phe Ser Gly His		270
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[2828]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		285
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[2830]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu		300
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[2832]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		315
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[2834]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		335
[2835]		340	345
[2836]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		350
[2837]		355	360
[2838]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		365
[2839]		370	375
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[2841]		385	390
[2842]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		395
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[2854]	Phe Leu Ser Thr Ala Val Ser Ala		495
[2855]		500	

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[2905]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
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[2907]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
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[2909]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp		
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[2911]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
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[2919]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys		
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[2934]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr		
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[2936]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		
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[2938]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
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[2987]		450	455
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[2990]	Asn Gly Ser Ile Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile		475
[2991]		485	490
[2992]	Phe Leu Ser Thr Ala Val Ser Ala		495
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[3021]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		175
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[3031]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
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[3035]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
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[3039]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
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[3041]	Gly Pro Cys Lys Ile Pro Val Gln Met Ala Val Asp Met Gln Thr Leu		
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[3043]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
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[3078]	Tyr	Leu	Asp	Lys	Gln	Ser	Asp	Thr	Gln	Tyr	Val	Cys	Lys	Arg	Thr	Leu
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[3112]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[3113]	355 360 365
[3114]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
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[3116]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp
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[3118]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
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[3120]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
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[3122]	Ser Val Gly Gly Val Phe Asn Ser Leu Gly Lys Gly Ile His Gln Ile
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[3125]	450 455 460
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[3143]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
[3144]	50 55 60
[3145]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
[3146]	65 70 75 80
[3147]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu
[3148]	85 90 95
[3149]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser

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[3159]	Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro Arg Thr Gly Leu		
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[3161]	Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn Lys His Trp Leu		
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[3163]	Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro Trp His Ala Gly		
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[3181]	Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser Thr Glu Asn Ser		
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[3183]	Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp Ser Tyr Ile Val		
[3184]	370	375	380
[3185]	Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp His Arg Ser Gly		
[3186]	385	390	395
[3187]	Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg Gly Ala Lys Arg		
[3188]	405	410	415
[3189]	Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly Gly		
[3190]	420	425	430
[3191]	Val Phe Asn Ser Leu Gly Lys Gly Ile His Gln Ile Phe Gly Ala Ala		

[3192]	435	440	445
[3193]	Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser Gln Ile Leu Ile		
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[3195]	Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys Asn Gly Ser Ile		
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[3197]	Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile Phe Leu Ser Thr		
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[3212]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		
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[3218]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
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[3224]	Gly Ser Gln His Ser Gly Met Ile Gly Tyr Glu Thr Asp Glu Asp Arg		
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[3226]	Ala Lys Val Glu Val Thr Pro Asn Ser Pro Arg Ala Glu Ala Thr Leu		
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[3228]	Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro Arg Thr Gly Leu		
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[3232]	Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro Trp His Ala Gly		
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[3234]	Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu Ala Leu Val Glu		
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[3236]	Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val Val Leu Gly Ser		
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[3238]	Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala Leu Glu Ala Glu		
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[3242]	Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser Tyr Ser Leu Cys		
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[3244]	Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu Thr Leu His Gly		
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[3252]	Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp Ser Tyr Ile Val		
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[3254]	Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp His Arg Ser Gly		
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[3256]	Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg Gly Ala Lys Arg		
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[3258]	Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly Gly		
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[3260]	Val Phe Asn Ser Leu Gly Lys Gly Ile His Gln Ile Phe Gly Ala Ala		
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[3262]	Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser Gln Ile Leu Ile		
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[3264]	Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys Asn Gly Ser Ile		
[3265]	465	470	475 480
[3266]	Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile Phe Leu Ser Thr		
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[3283]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala			
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[3285]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu			
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[3287]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser			
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[3289]	Leu Val Thr Cys Ala Lys Phe Thr Cys Ser Lys Lys Met Thr Gly Lys			
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[3291]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His			
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[3293]	Gly Ser Gln His Ser Gly Met Ile Gly Tyr Glu Thr Asp Glu Asp Arg			
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[3295]	Ala Lys Val Glu Val Thr Pro Asn Ser Pro Arg Ala Glu Ala Thr Leu			
[3296]	165	170	175	
[3297]	Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro Arg Thr Gly Leu			
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[3299]	Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn Lys His Trp Leu			
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[3301]	Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro Trp His Ala Gly			
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[3303]	Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu Ala Leu Val Glu			
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[3305]	Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val Val Leu Gly Ser			
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[3307]	Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala Leu Glu Ala Glu			
[3308]	260	265	270	
[3309]	Met Asp Gly Ala Lys Gly Arg Leu Phe Ser Gly His Leu Lys Cys Arg			
[3310]	275	280	285	
[3311]	Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser Tyr Ser Leu Cys			
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[3313]	Thr Ala Ala Phe Thr Phe Thr Lys Val Pro Ala Glu Thr Leu His Gly			
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[3315]	Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp Gly Pro Cys Lys			
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[3317]	Ile Pro Val Gln Met Ala Val Asp Met Gln Thr Leu Thr Pro Val Gly			

[3318]	340	345	350
[3319]	Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser Thr Glu Asn Ser		
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[3321]	Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp Ser Tyr Ile Val		
[3322]	370	375	380
[3323]	Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp His Arg Ser Gly		
[3324]	385	390	395
[3325]	Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg Gly Ala Lys Arg		
[3326]	405	410	415
[3327]	Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly Gly		
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[3329]	Val Phe Asn Ser Leu Gly Lys Gly Ile His Gln Ile Phe Gly Ala Ala		
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[3331]	Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser Gln Ile Leu Ile		
[3332]	450	455	460
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[3334]	465	470	475
[3335]	Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Met Ile Phe Leu Ser Thr		
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[3348]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr		
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[3350]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		
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[3352]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
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[3354]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu		
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[3356]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
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[3358]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys		
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[3360]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His		
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[3364]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala		
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[3366]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
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[3368]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
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[3370]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
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[3372]	Trp His Thr Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		
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[3376]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
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[3378]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
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[3382]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
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[3384]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
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[3386]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
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[3388]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Gly		
[3389]	355	360	365
[3390]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[3391]	370	375	380
[3392]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[3393]	385	390	395
[3394]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[3395]	405	410	415
[3396]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[3397]	420	425	430
[3398]	Ser Val Gly Gly Val Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[3399]	435	440	445
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[3401]	450	455	460

[3402]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys
[3403]	465 470 475 480
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[3415]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr
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[3439]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro
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[3447]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
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[3449]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
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[3453]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
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[3455]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[3456]	340	345	350
[3457]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Gly		
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[3459]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[3460]	370	375	380
[3461]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[3462]	385	390	395
[3463]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
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[3471]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
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[3473]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
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[3475]	Phe Leu Ser Thr Ala Val Ser Ala		
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[3482]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser		
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[3484]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr		
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[3486]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
[3487]	35 40 45
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[3490]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
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[3494]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
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[3501]	145 150 155 160
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[3522]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
[3523]	325 330 335
[3524]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu
[3525]	340 345 350
[3526]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
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[3532]	His	Arg	Ser	Gly	Ser	Thr	Ile	Gly	Lys	Ala	Phe	Glu	Ala	Thr	Val	Arg
[3533]					405					410					415	
[3534]	Gly	Ala	Lys	Arg	Met	Ala	Val	Leu	Gly	Asp	Thr	Ala	Trp	Asp	Phe	Gly
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[3540]	Gln	Ile	Leu	Ile	Gly	Thr	Leu	Leu	Met	Trp	Leu	Gly	Leu	Asn	Thr	Lys
[3541]	465					470					475					480
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[3543]					485					490					495	
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[3561]	Tyr	Leu	Asp	Lys	Gln	Ser	Asp	Thr	Gln	Tyr	Val	Cys	Lys	Arg	Thr	Leu
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[3563]	Val	Asp	Arg	Gly	Trp	Gly	Asn	Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser
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[3573]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro			
[3574]		180	185	190
[3575]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn			
[3576]		195	200	205
[3577]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro			
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[3579]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu			
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[3581]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val			
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[3583]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala			
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[3585]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His			
[3586]		275	280	285
[3587]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser			
[3588]		290	295	300
[3589]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu			
[3590]	305	310	315	320
[3591]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp			
[3592]		325	330	335
[3593]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu			
[3594]		340	345	350
[3595]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser			
[3596]		355	360	365
[3597]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp			
[3598]		370	375	380
[3599]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp			
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[3601]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg			
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[3609]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys			
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[3611]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile			

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[3613]	Phe Leu Ser Thr Ala Val Ser Ala					
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[3630]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu					
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[3638]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr					
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[3640]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala					
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[3642]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro					
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[3644]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn					
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[3646]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro					
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[3648]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu					
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[3650]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val					
[3651]		245		250		255
[3652]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala					
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[3656]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
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[3658]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
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[3660]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
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[3662]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
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[3664]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
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[3666]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
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[3668]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
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[3670]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
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[3672]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
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[3674]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
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[3676]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
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[3678]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
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[3695]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		

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[3711]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
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[3713]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
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[3715]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
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[3719]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val		
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[3731]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
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[3733]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
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[3737]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		

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[3747]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys			
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[3782]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		190
[3783]		195	200
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[3796]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		300
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[3798]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		315
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[3800]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		335
[3801]		340	345
[3802]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		350
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[3804]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		365
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[3806]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		380
[3807]		385	390
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[3811]		420	425
[3812]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		430
[3813]		435	440
[3814]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		445
[3815]		450	455
[3816]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		460
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[3820]	Phe Leu Ser Thr Ala Val Ser Ala		495
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[3851]	Arg	Thr	Gly	Leu	Asp	Phe	Ser	Asp	Leu	Tyr	Tyr	Leu	Thr	Met	Asn	Asn
[3852]		195				200				205						
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[3856]	225				230				235						240	
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[3869]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
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[3871]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
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[3873]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
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[3875]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
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[3877]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
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[3883]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
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[3887]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
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[3904]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
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[3970]					35					40					45	
[3971]	Thr	Val	Ser	Asn	Met	Ala	Glu	Val	Arg	Ser	Tyr	Cys	Tyr	Glu	Ala	Ser
[3972]					50					55					60	
[3973]	Ile	Ser	Asp	Met	Ala	Ser	Asp	Ser	Arg	Cys	Pro	Thr	Gln	Gly	Glu	Ala
[3974]					65					70					75	
[3975]	Tyr	Leu	Asp	Lys	Gln	Ser	Asp	Thr	Gln	Tyr	Val	Cys	Lys	Arg	Thr	Leu
[3976]					85					90					95	
[3977]	Val	Asp	Arg	Gly	Trp	Gly	Asn	Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser
[3978]					100					105					110	
[3979]	Leu	Val	Thr	Cys	Ala	Lys	Phe	Ala	Cys	Ser	Lys	Lys	Met	Thr	Gly	Lys
[3980]					115					120					125	
[3981]	Ser	Ile	Gln	Pro	Glu	Asn	Leu	Glu	Tyr	Arg	Ile	Met	Leu	Ser	Val	His
[3982]					130					135					140	
[3983]	Gly	Ser	Gln	His	Ser	Gly	Met	Ile	Val	Asn	Asp	Thr	Gly	His	Glu	Thr
[3984]					145					150					155	
[3985]	Asp	Glu	Asn	Arg	Ala	Lys	Val	Glu	Ile	Thr	Pro	Asn	Ser	Pro	Arg	Ala
[3986]					165					170					175	
[3987]	Glu	Ala	Thr	Leu	Gly	Gly	Phe	Gly	Ser	Leu	Gly	Leu	Asp	Cys	Glu	Pro
[3988]					180					185					190	
[3989]	Arg	Thr	Gly	Leu	Asp	Phe	Ser	Asp	Leu	Tyr	Tyr	Leu	Thr	Met	Asn	Asn

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[3994]	225	230	235
[3995]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val		
[3996]	245	250	255
[3997]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
[3998]	260	265	270
[3999]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[4000]	275	280	285
[4001]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[4002]	290	295	300
[4003]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[4004]	305	310	315
[4005]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[4006]	325	330	335
[4007]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[4008]	340	345	350
[4009]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
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[4011]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[4012]	370	375	380
[4013]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[4014]	385	390	395
[4015]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[4016]	405	410	415
[4017]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[4018]	420	425	430
[4019]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[4020]	435	440	445
[4021]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[4022]	450	455	460
[4023]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
[4024]	465	470	475
[4025]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
[4026]	485	490	495
[4027]	Phe Leu Ser Thr Ala Val Ser Ala		
[4028]	500		
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[4031]	<212> PRT		

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[4038]	Val	Met	Ala	Gln	Asp	Lys	Pro	Thr	Val	Asp	Ile	Glu	Leu	Val	Thr	Thr
[4039]			35					40					45			
[4040]	Thr	Val	Ser	Asn	Met	Ala	Glu	Val	Arg	Ser	Tyr	Cys	Tyr	Glu	Ala	Ser
[4041]		50						55				60				
[4042]	Ile	Ser	Asp	Met	Ala	Ser	Asp	Ser	Arg	Cys	Pro	Thr	Gln	Gly	Glu	Ala
[4043]	65					70				75					80	
[4044]	Tyr	Leu	Asp	Lys	Gln	Ser	Asp	Thr	Gln	Tyr	Val	Cys	Lys	Arg	Thr	Leu
[4045]					85				90					95		
[4046]	Val	Asp	Arg	Gly	Trp	Gly	Asn	Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser
[4047]				100				105					110			
[4048]	Leu	Val	Thr	Cys	Ala	Lys	Phe	Ala	Cys	Ser	Lys	Lys	Met	Thr	Gly	Lys
[4049]			115					120					125			
[4050]	Ser	Ile	Gln	Pro	Glu	Asn	Leu	Glu	Tyr	Arg	Ile	Met	Leu	Ser	Val	His
[4051]		130					135					140				
[4052]	Gly	Ser	Gln	His	Ser	Gly	Met	Ile	Val	Asn	Asp	Thr	Gly	His	Glu	Thr
[4053]	145					150				155					160	
[4054]	Asp	Glu	Asn	Arg	Ala	Lys	Val	Glu	Ile	Thr	Pro	Asn	Ser	Pro	Arg	Ala
[4055]					165				170				175			
[4056]	Glu	Ala	Thr	Leu	Gly	Gly	Phe	Gly	Ser	Leu	Gly	Leu	Asp	Cys	Glu	Pro
[4057]				180				185					190			
[4058]	Arg	Thr	Gly	Leu	Asp	Phe	Ser	Asp	Leu	Tyr	Tyr	Leu	Thr	Met	Asn	Asn
[4059]			195					200					205			
[4060]	Lys	His	Trp	Leu	Val	His	Lys	Glu	Trp	Phe	His	Asp	Ile	Pro	Leu	Pro
[4061]		210					215					220				
[4062]	Trp	His	Ala	Gly	Ala	Asp	Thr	Gly	Thr	Pro	His	Trp	Asn	Asn	Lys	Glu
[4063]	225					230				235					240	
[4064]	Ala	Leu	Val	Glu	Phe	Lys	Asp	Ala	His	Ala	Lys	Arg	Gln	Thr	Val	Val
[4065]					245				250					255		
[4066]	Val	Leu	Gly	Ser	Gln	Glu	Gly	Ala	Val	His	Thr	Ala	Leu	Ala	Gly	Ala
[4067]				260				265					270			
[4068]	Leu	Glu	Ala	Glu	Met	Asp	Gly	Ala	Lys	Gly	Arg	Leu	Ser	Ser	Gly	His
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[4070]	Leu	Lys	Cys	Arg	Leu	Lys	Met	Asp	Lys	Leu	Arg	Leu	Lys	Gly	Val	Ser
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[4072]	Tyr	Ser	Leu	Cys	Thr	Ala	Ala	Phe	Thr	Phe	Thr	Lys	Ile	Pro	Ala	Glu
[4073]	305					310				315					320	

[4074]	Thr	Leu	His	Gly	Thr	Val	Thr	Val	Glu	Val	Gln	Tyr	Ala	Gly	Thr	Asp
[4075]						325					330					335
[4076]	Gly	Pro	Cys	Lys	Val	Pro	Ala	Gln	Met	Ala	Val	Asp	Met	Gln	Thr	Leu
[4077]						340					345					350
[4078]	Thr	Pro	Val	Gly	Arg	Leu	Ile	Thr	Ala	Asn	Pro	Val	Ile	Thr	Glu	Ser
[4079]						355					360					365
[4080]	Thr	Glu	Asn	Ser	Lys	Met	Met	Leu	Glu	Leu	Asp	Pro	Pro	Phe	Gly	Asp
[4081]						370					375					380
[4082]	Ser	Tyr	Ile	Val	Ile	Gly	Val	Gly	Glu	Lys	Lys	Ile	Thr	His	His	Trp
[4083]						385					390					400
[4084]	His	Arg	Ser	Gly	Ser	Thr	Ile	Gly	Lys	Ala	Phe	Glu	Ala	Thr	Val	Arg
[4085]						405					410					415
[4086]	Gly	Ala	Lys	Arg	Met	Ala	Val	Leu	Gly	Asp	Thr	Ala	Trp	Asp	Phe	Gly
[4087]						420					425					430
[4088]	Ser	Val	Gly	Gly	Ala	Leu	Asn	Ser	Leu	Gly	Lys	Gly	Ile	His	Gln	Ile
[4089]						435					440					445
[4090]	Phe	Gly	Ala	Ala	Phe	Lys	Ser	Leu	Phe	Gly	Gly	Met	Ser	Trp	Phe	Ser
[4091]						450					455					460
[4092]	Gln	Ile	Leu	Ile	Gly	Thr	Leu	Leu	Met	Trp	Leu	Gly	Leu	Asn	Thr	Lys
[4093]						465					470					480
[4094]	Asn	Gly	Ser	Ile	Ser	Leu	Met	Cys	Leu	Ala	Leu	Gly	Gly	Val	Leu	Ile
[4095]						485					490					495
[4096]	Phe	Leu	Ser	Thr	Ala	Val	Ser	Ala								
[4097]						500										
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[4105]	Gly	Gly	Thr	Trp	Val	Asp	Val	Val	Leu	Glu	His	Gly	Gly	Cys	Val	Thr
[4106]						20					25					30
[4107]	Val	Met	Ala	Gln	Asp	Lys	Pro	Thr	Val	Asp	Ile	Glu	Leu	Val	Thr	Thr
[4108]						35					40					45
[4109]	Thr	Val	Ser	Asn	Met	Ala	Glu	Val	Arg	Ser	Tyr	Cys	Tyr	Glu	Ala	Ser
[4110]						50					55					60
[4111]	Ile	Ser	Asp	Met	Ala	Ser	Asp	Ser	Arg	Cys	Pro	Thr	Gln	Gly	Glu	Ala
[4112]						65					70					80
[4113]	Tyr	Leu	Asp	Lys	Gln	Ser	Asp	Thr	Gln	Tyr	Val	Cys	Lys	Arg	Thr	Leu
[4114]						85					90					95
[4115]	Val	Asp	Arg	Gly	Trp	Gly	Asn	Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser

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[4117]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys					
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[4119]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His					
[4120]		130		135		140
[4121]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr					
[4122]	145		150		155	160
[4123]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala					
[4124]		165		170		175
[4125]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro					
[4126]		180		185		190
[4127]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn					
[4128]		195		200		205
[4129]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro					
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[4131]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu					
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[4133]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val					
[4134]		245		250		255
[4135]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala					
[4136]		260		265		270
[4137]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His					
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[4139]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser					
[4140]		290		295		300
[4141]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu					
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[4143]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp					
[4144]		325		330		335
[4145]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu					
[4146]		340		345		350
[4147]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser					
[4148]		355		360		365
[4149]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp					
[4150]		370		375		380
[4151]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp					
[4152]	385		390		395	400
[4153]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg					
[4154]		405		410		415
[4155]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly					
[4156]		420		425		430
[4157]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile					

[4158]	435	440	445
[4159]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[4160]	450	455	460
[4161]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
[4162]	465	470	475
[4163]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
[4164]	485	490	495
[4165]	Phe Leu Ser Thr Ala Val Ser Ala		
[4166]	500		
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[4170]	<213> Zika病毒		
[4171]	<400> 42		
[4172]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser		
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[4174]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr		
[4175]	20	25	30
[4176]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr		
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[4178]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		
[4179]	50	55	60
[4180]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
[4181]	65	70	75
[4182]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu		
[4183]	85	90	95
[4184]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
[4185]	100	105	110
[4186]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys		
[4187]	115	120	125
[4188]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His		
[4189]	130	135	140
[4190]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr		
[4191]	145	150	155
[4192]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala		
[4193]	165	170	175
[4194]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
[4195]	180	185	190
[4196]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
[4197]	195	200	205
[4198]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
[4199]	210	215	220

[4200]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
[4201]	225 230 235 240
[4202]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val
[4203]	245 250 255
[4204]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala
[4205]	260 265 270
[4206]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His
[4207]	275 280 285
[4208]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser
[4209]	290 295 300
[4210]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu
[4211]	305 310 315 320
[4212]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
[4213]	325 330 335
[4214]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu
[4215]	340 345 350
[4216]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[4217]	355 360 365
[4218]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
[4219]	370 375 380
[4220]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp
[4221]	385 390 395 400
[4222]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
[4223]	405 410 415
[4224]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
[4225]	420 425 430
[4226]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile
[4227]	435 440 445
[4228]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
[4229]	450 455 460
[4230]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys
[4231]	465 470 475 480
[4232]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile
[4233]	485 490 495
[4234]	Phe Leu Ser Thr Ala Val Ser Ala
[4235]	500
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[4243]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr			
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[4245]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr			
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[4247]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser			
[4248]		50	55	60
[4249]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala			
[4250]	65	70	75	80
[4251]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu			
[4252]		85	90	95
[4253]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser			
[4254]		100	105	110
[4255]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys			
[4256]		115	120	125
[4257]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His			
[4258]		130	135	140
[4259]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr			
[4260]	145	150	155	160
[4261]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala			
[4262]		165	170	175
[4263]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro			
[4264]		180	185	190
[4265]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn			
[4266]		195	200	205
[4267]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro			
[4268]		210	215	220
[4269]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu			
[4270]	225	230	235	240
[4271]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val			
[4272]		245	250	255
[4273]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala			
[4274]		260	265	270
[4275]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His			
[4276]		275	280	285
[4277]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser			
[4278]		290	295	300
[4279]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu			
[4280]	305	310	315	320
[4281]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp			
[4282]		325	330	335
[4283]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu			

[4284]	340	345	350
[4285]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[4286]	355	360	365
[4287]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[4288]	370	375	380
[4289]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[4290]	385	390	395
[4291]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[4292]	405	410	415
[4293]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[4294]	420	425	430
[4295]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[4296]	435	440	445
[4297]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[4298]	450	455	460
[4299]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
[4300]	465	470	475
[4301]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
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[4303]	Phe Leu Ser Thr Ala Val Ser Ala		
[4304]	500		
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[4313]	20	25	30
[4314]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr		
[4315]	35	40	45
[4316]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		
[4317]	50	55	60
[4318]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
[4319]	65	70	75
[4320]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu		
[4321]	85	90	95
[4322]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
[4323]	100	105	110
[4324]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys		
[4325]	115	120	125

[4326]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His		
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[4328]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr		
[4329]	145	150	155
[4330]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala		
[4331]	165	170	175
[4332]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
[4333]	180	185	190
[4334]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
[4335]	195	200	205
[4336]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
[4337]	210	215	220
[4338]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		
[4339]	225	230	235
[4340]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val		
[4341]	245	250	255
[4342]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
[4343]	260	265	270
[4344]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[4345]	275	280	285
[4346]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[4347]	290	295	300
[4348]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[4349]	305	310	315
[4350]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[4351]	325	330	335
[4352]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[4353]	340	345	350
[4354]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[4355]	355	360	365
[4356]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[4357]	370	375	380
[4358]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[4359]	385	390	395
[4360]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[4361]	405	410	415
[4362]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[4363]	420	425	430
[4364]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[4365]	435	440	445
[4366]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[4367]	450	455	460

[4368]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys
[4369]	465 470 475 480
[4370]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile
[4371]	485 490 495
[4372]	Phe Leu Ser Thr Ala Val Ser Ala
[4373]	500
[4374]	<210> 45
[4375]	<211> 504
[4376]	<212> PRT
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[4378]	<400> 45
[4379]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser
[4380]	1 5 10 15
[4381]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr
[4382]	20 25 30
[4383]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
[4384]	35 40 45
[4385]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
[4386]	50 55 60
[4387]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
[4388]	65 70 75 80
[4389]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu
[4390]	85 90 95
[4391]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
[4392]	100 105 110
[4393]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys
[4394]	115 120 125
[4395]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
[4396]	130 135 140
[4397]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr
[4398]	145 150 155 160
[4399]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala
[4400]	165 170 175
[4401]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro
[4402]	180 185 190
[4403]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn
[4404]	195 200 205
[4405]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro
[4406]	210 215 220
[4407]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
[4408]	225 230 235 240
[4409]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val

[4410]	245	250	255
[4411]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
[4412]	260	265	270
[4413]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[4414]	275	280	285
[4415]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[4416]	290	295	300
[4417]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[4418]	305	310	315
[4419]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[4420]	325	330	335
[4421]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[4422]	340	345	350
[4423]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[4424]	355	360	365
[4425]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[4426]	370	375	380
[4427]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[4428]	385	390	395
[4429]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[4430]	405	410	415
[4431]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[4432]	420	425	430
[4433]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[4434]	435	440	445
[4435]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[4436]	450	455	460
[4437]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
[4438]	465	470	475
[4439]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
[4440]	485	490	495
[4441]	Phe Leu Ser Thr Ala Val Ser Ala		
[4442]	500		
[4443]	<210> 46		
[4444]	<211> 504		
[4445]	<212> PRT		
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[4447]	<400> 46		
[4448]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser		
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[4450]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr		
[4451]	20	25	30

[4452]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
[4453]	35 40 45
[4454]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
[4455]	50 55 60
[4456]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
[4457]	65 70 75 80
[4458]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu
[4459]	85 90 95
[4460]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
[4461]	100 105 110
[4462]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys
[4463]	115 120 125
[4464]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
[4465]	130 135 140
[4466]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr
[4467]	145 150 155 160
[4468]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala
[4469]	165 170 175
[4470]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro
[4471]	180 185 190
[4472]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn
[4473]	195 200 205
[4474]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro
[4475]	210 215 220
[4476]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
[4477]	225 230 235 240
[4478]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val
[4479]	245 250 255
[4480]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala
[4481]	260 265 270
[4482]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His
[4483]	275 280 285
[4484]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser
[4485]	290 295 300
[4486]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu
[4487]	305 310 315 320
[4488]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
[4489]	325 330 335
[4490]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu
[4491]	340 345 350
[4492]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[4493]	355 360 365

[4494]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
[4495]	370 375 380
[4496]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp
[4497]	385 390 395 400
[4498]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
[4499]	405 410 415
[4500]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
[4501]	420 425 430
[4502]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile
[4503]	435 440 445
[4504]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
[4505]	450 455 460
[4506]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys
[4507]	465 470 475 480
[4508]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile
[4509]	485 490 495
[4510]	Phe Leu Ser Thr Ala Val Ser Ala
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[4517]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser
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[4519]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr
[4520]	20 25 30
[4521]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
[4522]	35 40 45
[4523]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
[4524]	50 55 60
[4525]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
[4526]	65 70 75 80
[4527]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu
[4528]	85 90 95
[4529]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
[4530]	100 105 110
[4531]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys
[4532]	115 120 125
[4533]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
[4534]	130 135 140
[4535]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr

[4536]	145	150	155	160
[4537]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala			
[4538]		165	170	175
[4539]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro			
[4540]		180	185	190
[4541]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn			
[4542]		195	200	205
[4543]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro			
[4544]		210	215	220
[4545]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu			
[4546]	225	230	235	240
[4547]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val			
[4548]		245	250	255
[4549]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala			
[4550]		260	265	270
[4551]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His			
[4552]		275	280	285
[4553]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser			
[4554]		290	295	300
[4555]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu			
[4556]	305	310	315	320
[4557]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp			
[4558]		325	330	335
[4559]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu			
[4560]		340	345	350
[4561]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser			
[4562]		355	360	365
[4563]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp			
[4564]		370	375	380
[4565]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp			
[4566]	385	390	395	400
[4567]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg			
[4568]		405	410	415
[4569]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly			
[4570]		420	425	430
[4571]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile			
[4572]		435	440	445
[4573]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser			
[4574]		450	455	460
[4575]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys			
[4576]	465	470	475	480
[4577]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile			

[4578]		485		490		495
[4579]	Phe Leu Ser Thr Ala Val Ser Ala					
[4580]		500				
[4581]	<210> 48					
[4582]	<211> 504					
[4583]	<212> PRT					
[4584]	<213> Zika病毒					
[4585]	<400> 48					
[4586]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser					
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[4588]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr					
[4589]		20		25		30
[4590]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr					
[4591]		35		40		45
[4592]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser					
[4593]		50		55		60
[4594]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala					
[4595]	65	70		75		80
[4596]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu					
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[4598]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser					
[4599]		100		105		110
[4600]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys					
[4601]		115		120		125
[4602]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His					
[4603]		130		135		140
[4604]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr					
[4605]	145	150		155		160
[4606]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala					
[4607]		165		170		175
[4608]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro					
[4609]		180		185		190
[4610]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn					
[4611]		195		200		205
[4612]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro					
[4613]		210		215		220
[4614]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu					
[4615]	225	230		235		240
[4616]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val					
[4617]		245		250		255
[4618]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala					
[4619]		260		265		270

[4620]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[4621]	275	280	285
[4622]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[4623]	290	295	300
[4624]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[4625]	305	310	315 320
[4626]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[4627]	325	330	335
[4628]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[4629]	340	345	350
[4630]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[4631]	355	360	365
[4632]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[4633]	370	375	380
[4634]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[4635]	385	390	395 400
[4636]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[4637]	405	410	415
[4638]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[4639]	420	425	430
[4640]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[4641]	435	440	445
[4642]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[4643]	450	455	460
[4644]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
[4645]	465	470	475 480
[4646]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
[4647]	485	490	495
[4648]	Phe Leu Ser Thr Ala Val Ser Ala		
[4649]	500		
[4650]	<210> 49		
[4651]	<211> 504		
[4652]	<212> PRT		
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[4654]	<400> 49		
[4655]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser		
[4656]	1	5	10 15
[4657]	Gly Gly Thr Trp Val Asp Ile Val Leu Glu His Gly Gly Cys Val Thr		
[4658]	20	25	30
[4659]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr		
[4660]	35	40	45
[4661]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		

[4662]	50	55	60
[4663]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
[4664]	65	70	75
[4665]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu		
[4666]	85	90	95
[4667]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
[4668]	100	105	110
[4669]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys		
[4670]	115	120	125
[4671]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His		
[4672]	130	135	140
[4673]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr		
[4674]	145	150	155
[4675]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala		
[4676]	165	170	175
[4677]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
[4678]	180	185	190
[4679]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
[4680]	195	200	205
[4681]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
[4682]	210	215	220
[4683]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		
[4684]	225	230	235
[4685]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val		
[4686]	245	250	255
[4687]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
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[4689]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[4690]	275	280	285
[4691]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[4692]	290	295	300
[4693]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[4694]	305	310	315
[4695]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[4696]	325	330	335
[4697]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[4698]	340	345	350
[4699]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[4700]	355	360	365
[4701]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[4702]	370	375	380
[4703]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		

[4704]	385	390	395	400
[4705]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg			
[4706]		405	410	415
[4707]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly			
[4708]		420	425	430
[4709]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile			
[4710]		435	440	445
[4711]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser			
[4712]		450	455	460
[4713]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys			
[4714]	465	470	475	480
[4715]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile			
[4716]		485	490	495
[4717]	Phe Leu Ser Thr Ala Val Ser Ala			
[4718]		500		
[4719]	<210> 50			
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[4723]	<400> 50			
[4724]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser			
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[4726]	Gly Gly Thr Trp Val Asp Ile Val Leu Glu His Gly Gly Cys Val Thr			
[4727]		20	25	30
[4728]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr			
[4729]		35	40	45
[4730]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser			
[4731]		50	55	60
[4732]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala			
[4733]	65	70	75	80
[4734]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu			
[4735]		85	90	95
[4736]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser			
[4737]		100	105	110
[4738]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys			
[4739]		115	120	125
[4740]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His			
[4741]		130	135	140
[4742]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr			
[4743]	145	150	155	160
[4744]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala			
[4745]		165	170	175

[4746]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
[4747]		180	185
[4748]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		190
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[4841]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
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[5089]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala		
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[5091]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
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[5093]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
[5094]	195	200	205
[5095]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
[5096]	210	215	220
[5097]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		
[5098]	225	230	235
[5099]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val		
[5100]	245	250	255
[5101]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
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[5103]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[5104]	275	280	285
[5105]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[5106]	290	295	300
[5107]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[5108]	305	310	315
[5109]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[5110]	325	330	335
[5111]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[5112]	340	345	350
[5113]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[5114]	355	360	365
[5115]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[5116]	370	375	380
[5117]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[5118]	385	390	395
[5119]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[5120]	405	410	415
[5121]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[5122]	420	425	430
[5123]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		

[5124]	435	440	445
[5125]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
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[5127]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys		
[5128]	465	470	475
[5129]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
[5130]	485	490	495
[5131]	Phe Leu Ser Thr Ala Val Ser Ala		
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[5137]	<400> 56		
[5138]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser		
[5139]	1	5	10
[5140]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr		
[5141]	20	25	30
[5142]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr		
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[5144]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		
[5145]	50	55	60
[5146]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
[5147]	65	70	75
[5148]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu		
[5149]	85	90	95
[5150]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
[5151]	100	105	110
[5152]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys		
[5153]	115	120	125
[5154]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His		
[5155]	130	135	140
[5156]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr		
[5157]	145	150	155
[5158]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala		
[5159]	165	170	175
[5160]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
[5161]	180	185	190
[5162]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
[5163]	195	200	205
[5164]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
[5165]	210	215	220

[5166]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
[5167]	225 230 235 240
[5168]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val
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[5170]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala
[5171]	260 265 270
[5172]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His
[5173]	275 280 285
[5174]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser
[5175]	290 295 300
[5176]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu
[5177]	305 310 315 320
[5178]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
[5179]	325 330 335
[5180]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu
[5181]	340 345 350
[5182]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[5183]	355 360 365
[5184]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
[5185]	370 375 380
[5186]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp
[5187]	385 390 395 400
[5188]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
[5189]	405 410 415
[5190]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
[5191]	420 425 430
[5192]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile
[5193]	435 440 445
[5194]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
[5195]	450 455 460
[5196]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Ala Lys
[5197]	465 470 475 480
[5198]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile
[5199]	485 490 495
[5200]	Phe Leu Ser Thr Ala Val Ser Ala
[5201]	500
[5202]	<210> 57
[5203]	<211> 504
[5204]	<212> PRT
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[5206]	<400> 57
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[5211]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr			
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[5213]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser			
[5214]	50	55	60	
[5215]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala			
[5216]	65	70	75	80
[5217]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu			
[5218]	85	90	95	
[5219]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser			
[5220]	100	105	110	
[5221]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys			
[5222]	115	120	125	
[5223]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His			
[5224]	130	135	140	
[5225]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr			
[5226]	145	150	155	160
[5227]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala			
[5228]	165	170	175	
[5229]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro			
[5230]	180	185	190	
[5231]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn			
[5232]	195	200	205	
[5233]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro			
[5234]	210	215	220	
[5235]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu			
[5236]	225	230	235	240
[5237]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val			
[5238]	245	250	255	
[5239]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala			
[5240]	260	265	270	
[5241]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His			
[5242]	275	280	285	
[5243]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser			
[5244]	290	295	300	
[5245]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu			
[5246]	305	310	315	320
[5247]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp			
[5248]	325	330	335	
[5249]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu			

[5250]	340	345	350
[5251]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[5252]	355	360	365
[5253]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[5254]	370	375	380
[5255]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[5256]	385	390	395 400
[5257]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[5258]	405	410	415
[5259]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[5260]	420	425	430
[5261]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[5262]	435	440	445
[5263]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[5264]	450	455	460
[5265]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Ala Lys		
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[5267]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		
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[5269]	Phe Leu Ser Thr Ala Val Ser Ala		
[5270]	500		
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[5278]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr		
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[5282]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser		
[5283]	50 55 60		
[5284]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala		
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[5286]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu		
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[5288]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser		
[5289]	100 105 110		
[5290]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys		
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[5296]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala		
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[5300]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
[5301]	195	200	205
[5302]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
[5303]	210	215	220
[5304]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		
[5305]	225	230	235
[5306]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val		
[5307]	245	250	255
[5308]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
[5309]	260	265	270
[5310]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[5311]	275	280	285
[5312]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[5313]	290	295	300
[5314]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[5315]	305	310	315
[5316]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[5317]	325	330	335
[5318]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Thr Gln Thr Leu		
[5319]	340	345	350
[5320]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[5321]	355	360	365
[5322]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[5323]	370	375	380
[5324]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[5325]	385	390	395
[5326]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[5327]	405	410	415
[5328]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
[5329]	420	425	430
[5330]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		
[5331]	435	440	445
[5332]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
[5333]	450	455	460

[5334]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys
[5335]	465 470 475 480
[5336]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile
[5337]	485 490 495
[5338]	Phe Leu Ser Thr Ala Val Ser Ala
[5339]	500
[5340]	<210> 59
[5341]	<211> 504
[5342]	<212> PRT
[5343]	<213> Zika病毒
[5344]	<400> 59
[5345]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser
[5346]	1 5 10 15
[5347]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr
[5348]	20 25 30
[5349]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
[5350]	35 40 45
[5351]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
[5352]	50 55 60
[5353]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
[5354]	65 70 75 80
[5355]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu
[5356]	85 90 95
[5357]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
[5358]	100 105 110
[5359]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys
[5360]	115 120 125
[5361]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
[5362]	130 135 140
[5363]	Gly Ser Gln His Ser Gly Met Ile Val Asn Gly Thr Gly His Glu Thr
[5364]	145 150 155 160
[5365]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala
[5366]	165 170 175
[5367]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro
[5368]	180 185 190
[5369]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn
[5370]	195 200 205
[5371]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro
[5372]	210 215 220
[5373]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
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[5375]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val

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[5377]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
[5378]	260	265	270
[5379]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
[5380]	275	280	285
[5381]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
[5382]	290	295	300
[5383]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
[5384]	305	310	315
[5385]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
[5386]	325	330	335
[5387]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
[5388]	340	345	350
[5389]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[5390]	355	360	365
[5391]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[5392]	370	375	380
[5393]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		
[5394]	385	390	395
[5395]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[5396]	405	410	415
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[5401]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		
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[5403]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		
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[5406]	485	490	495
[5407]	Phe Leu Ser Thr Ala Val Ser Ala		
[5408]	500		
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[5414]	Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser		
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[5416]	Gly Gly Thr Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr		
[5417]	20	25	30

[5418]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
[5419]	35 40 45
[5420]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
[5421]	50 55 60
[5422]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
[5423]	65 70 75 80
[5424]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu
[5425]	85 90 95
[5426]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
[5427]	100 105 110
[5428]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys
[5429]	115 120 125
[5430]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
[5431]	130 135 140
[5432]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr
[5433]	145 150 155 160
[5434]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala
[5435]	165 170 175
[5436]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro
[5437]	180 185 190
[5438]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn
[5439]	195 200 205
[5440]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro
[5441]	210 215 220
[5442]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu
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[5444]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val
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[5447]	260 265 270
[5448]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His
[5449]	275 280 285
[5450]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser
[5451]	290 295 300
[5452]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu
[5453]	305 310 315 320
[5454]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
[5455]	325 330 335
[5456]	Gly Pro Cys Lys Val Leu Ala Gln Met Ala Val Asp Met Gln Thr Leu
[5457]	340 345 350
[5458]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[5459]	355 360 365

[5460]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
[5461]	370 375 380
[5462]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp
[5463]	385 390 395 400
[5464]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
[5465]	405 410 415
[5466]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
[5467]	420 425 430
[5468]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile
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[5470]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
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[5472]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys
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[5476]	Phe Leu Ser Thr Ala Val Ser Ala
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[5487]	Val Met Ala Gln Asp Lys Pro Ala Val Asp Ile Glu Leu Val Thr Thr
[5488]	35 40 45
[5489]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser
[5490]	50 55 60
[5491]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala
[5492]	65 70 75 80
[5493]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu
[5494]	85 90 95
[5495]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
[5496]	100 105 110
[5497]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys
[5498]	115 120 125
[5499]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
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[5501]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr

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[5505]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro			
[5506]		180	185	190
[5507]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn			
[5508]		195	200	205
[5509]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro			
[5510]		210	215	220
[5511]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu			
[5512]	225	230	235	240
[5513]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val			
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[5515]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala			
[5516]		260	265	270
[5517]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His			
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[5519]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser			
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[5523]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp			
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[5525]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu			
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[5527]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser			
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[5529]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp			
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[5531]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp			
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[5533]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg			
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[5535]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly			
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[5537]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile			
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[5539]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser			
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[5541]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys			
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[5558]	Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser					
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[5560]	Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala					
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[5562]	Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu					
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[5564]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser					
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[5566]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys					
[5567]		115		120		125
[5568]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His					
[5569]		130		135		140
[5570]	Gly Ser Gln His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr					
[5571]	145	150		155		160
[5572]	Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala					
[5573]		165		170		175
[5574]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro					
[5575]		180		185		190
[5576]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn					
[5577]		195		200		205
[5578]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro					
[5579]		210		215		220
[5580]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu					
[5581]	225	230		235		240
[5582]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val					
[5583]		245		250		255
[5584]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala					
[5585]		260		265		270

[5586]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His
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[5588]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser
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[5590]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu
[5591]	305 310 315 320
[5592]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp
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[5594]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu
[5595]	340 345 350
[5596]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser
[5597]	355 360 365
[5598]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp
[5599]	370 375 380
[5600]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp
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[5604]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
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[5606]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile
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[5608]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
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[5625]	Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr
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[5643]	Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro		
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[5645]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		
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[5647]	Lys His Trp Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro		
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[5649]	Trp His Ala Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu		
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[5653]	Val Leu Gly Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala		
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[5655]	Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His		
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[5657]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
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[5661]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
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[5663]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
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[5665]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[5666]	355	360	365
[5667]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[5668]	370	375	380
[5669]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		

[5670]	385	390	395	400
[5671]	His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg			
[5672]		405	410	415
[5673]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly			
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[5675]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile			
[5676]		435	440	445
[5677]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser			
[5678]		450	455	460
[5679]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys			
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[5681]	Asn Gly Ser Ile Ser Leu Thr Cys Leu Ala Leu Gly Gly Val Leu Ile			
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[5683]	Phe Leu Ser Thr Ala Val Ser Ala			
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[5714]	Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn		190
[5715]		195	200
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[5720]	Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Ala Val		235
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[5725]		275	280
[5726]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		285
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[5736]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		365
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[5738]	Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp		380
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[5742]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		415
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[5744]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile		430
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[5746]	Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser		445
[5747]		450	455
[5748]	Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu Gly Leu Asn Thr Lys		460
[5749]		465	470
[5750]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile		475
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[5752]	Phe Leu Ser Thr Ala Val Ser Ala		495
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[5799]	Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser		
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[5801]	Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu		
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[5803]	Thr Leu His Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp		
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[5805]	Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu		
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[5807]	Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser		
[5808]	355	360	365
[5809]	Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp		
[5810]	370	375	380
[5811]	Ser Tyr Ile Val Ile Gly Val Gly Asp Lys Lys Ile Thr His His Trp		
[5812]	385	390	395 400
[5813]	Xaa Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg		
[5814]	405	410	415
[5815]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly		
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[5821]	Gln Ile Leu Ile Gly Thr Leu Leu Val Trp Leu Gly Leu Asn Thr Lys		
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[5836]	Ala Met Ala Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr		
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[5871]	305					310					315				320	
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[5873]					325						330				335	
[5874]	Gly	Pro	Cys	Lys	Val	Pro	Ala	Gln	Met	Ala	Val	Asp	Met	Gln	Thr	Leu
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[5884]	Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly
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[5886]	Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile
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[5888]	Ile Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly Met Ser Trp Phe Ser
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[5892]	Asn Gly Ser Ile Ser Leu Met Cys Leu Ala Leu Gly Gly Val Leu Ile
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[5913]	Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser
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[5915]	Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys
[5916]	115 120 125
[5917]	Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His
[5918]	130 135 140
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[6395]	Glu Ser Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe					
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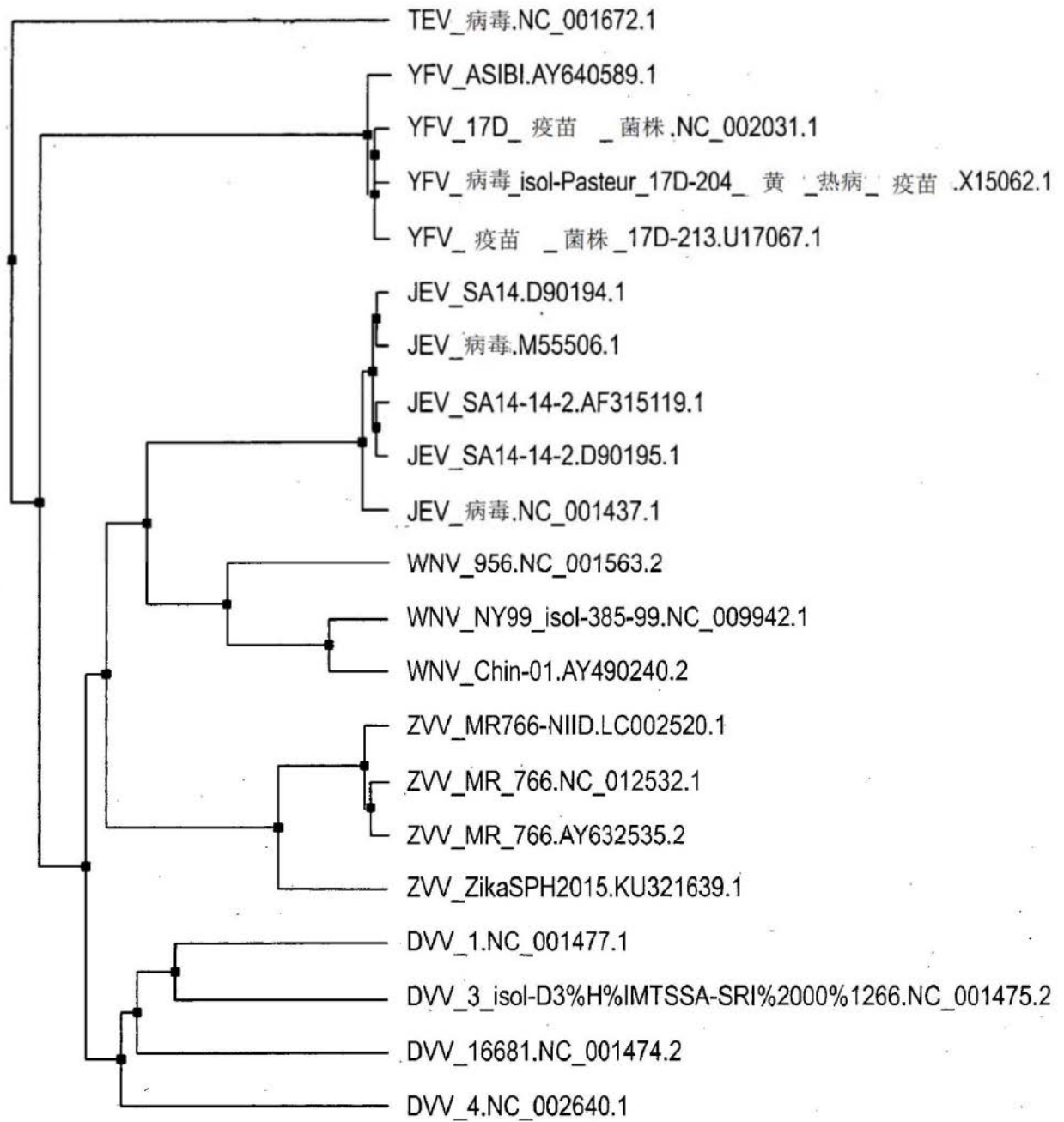


图1

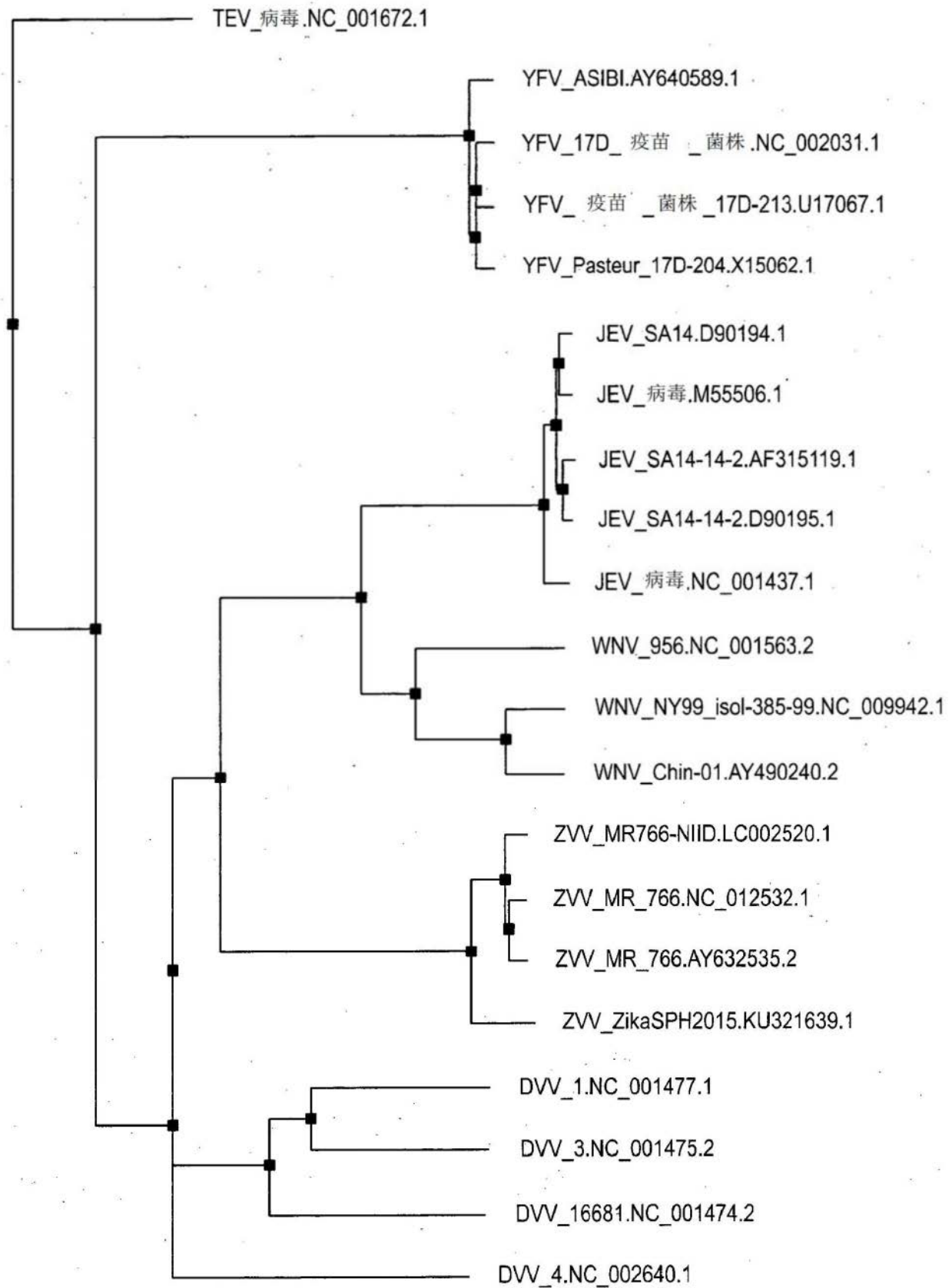


图2

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DW 1.NC 001477.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
DW 16681.NC 001474.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
DW 3.NC 001475.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
DW 4.NC 002640.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV SA14-14-2.AF315119.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV SA14-14-2.D90195.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV SA14.D90194.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV 病毒.M55506.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
JEV 病毒.NC 001437.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
TEV 病毒.NC 001672.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
WNV 956.NC 001563.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
WNV Chin-01.AY490240.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
WNV NY99 isol-385-99.NC 009942.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV 17D 疫苗 菌株.NC 002031.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV ASIB.AY640589.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV Pasteur 17D-204.X15062.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
YFV 疫苗 菌株 17D-213.U17067.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZW MR766-NIID.LC002520.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZW MR 766.AY632535.2	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZW MR 766.NC 012532.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
ZW ZikaSPH2015.KU321639.1	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0

 60% 同一性及以上
 70% 同一性及以上
 80% 同一性及以上

图3

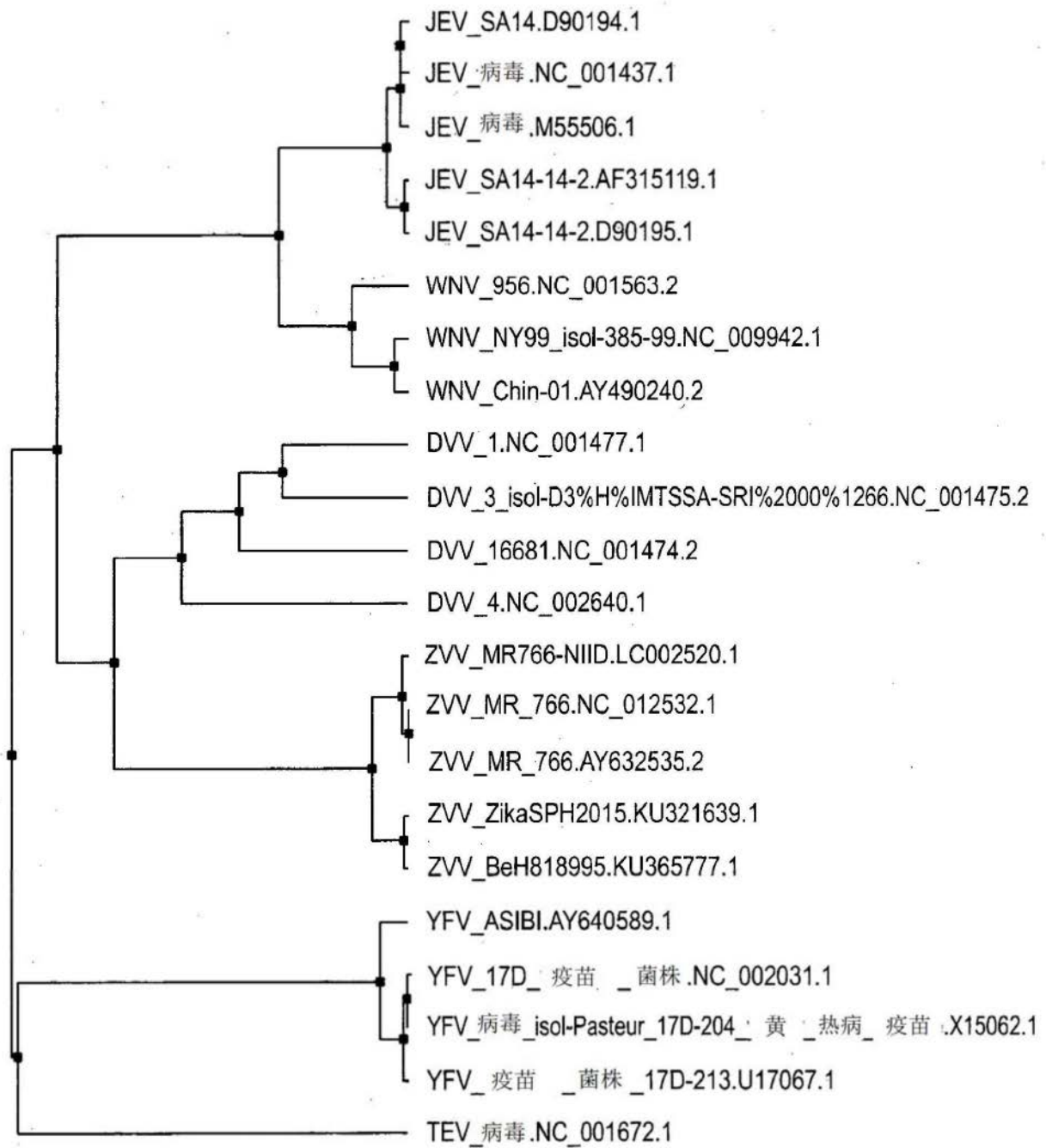


图4

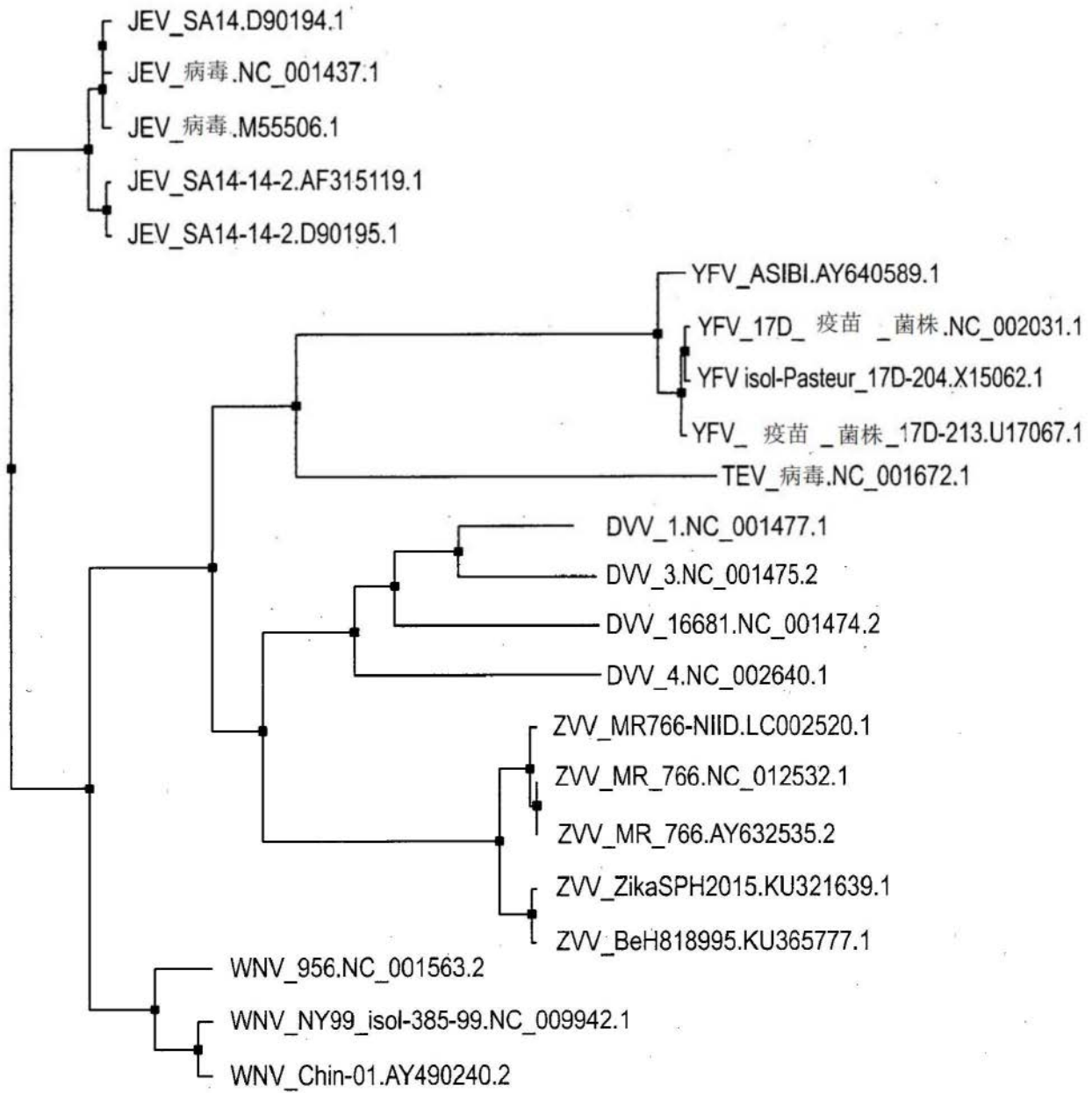


图5

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
DVV 1.NC 001477.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
DVV 16681.NC 001474.2	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
DVV 3.NC 001475.2	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
DVV 4.NC 002640.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
JEV SA14-14-2.AF315119.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
JEV SA14-14-2.D90195.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
JEV SA14.D90194.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
JEV 病毒.M55506.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
JEV 病毒.NC 001437.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
TEV 病毒.NC 001672.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
WNV 956.NC 001563.2	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
WNV Chin-01.AY490240.2	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
WNV NY99 isol-385-99.NC 009942.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
YFV 17D 疫苗 菌株.NC 002031.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
YFV ASIB.AY640589.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
YFV isol-Pasteur 17D-204.X15062.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
YFV 疫苗 菌株 17D-213.U17067.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
ZVVBetH818995.KU365777.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
ZV MR766-NIID.LC002520.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
ZV MR 766.AY632535.2	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
ZV MR 766.NC 012532.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
ZV ZikaSPH2015.KU321639.1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0

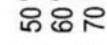
 50%同一性及以上
 60%同一性及以上
 70%同一性及以上

图6

[illegible]

图7 (1/3部分)

[illegible]

图7 (2/3部分)

YFV_AS/BI.AY640589.1/292-784	748	MG	AVL	I	W	VG	I	N	TR	N	M	T	S	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A			
YFV_17D_ 疫苗_ 菌株_NC_002031.1/292-784	748	MG	AVL	I	W	VG	I	N	TR	N	M	T	S	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A			
YFV_ 疫苗_ 菌株_17D-213.U17067.1/292-784	748	MG	AVL	I	W	VG	I	N	TR	N	M	T	S	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A			
YFV_isol-Pasteur_17D-204.X15062.1/292-784	748	MG	AVL	I	W	VG	I	N	TR	N	M	T	S	M	S	M	I	L	V	G	V	I	M	M	F	L	S	L	G	V	G	A			
IEV_SA14.D90194.1/307-806	770	MG	ALL	L	W	MG	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	G	V	L	V	F	L	A	T	N	H			
IEV_ 病毒_NC_001437.1/307-806	770	MG	ALL	L	W	MG	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	G	V	L	V	F	L	A	T	N	H			
IEV_ 病毒.M55506.1/307-806	770	MG	ALL	L	W	MG	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	G	V	L	V	F	L	A	T	N	H			
IEV_SA14-14-2.AF315119.1/307-806	770	MG	ALL	L	W	MG	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	G	V	L	V	F	L	A	T	N	H			
IEV_SA14-14-2.D90195.1/307-806	770	MG	ALL	L	W	MG	V	N	A	R	D	R	S	I	A	L	A	F	L	A	T	G	G	V	L	V	F	L	A	T	N	H			
DVV_1.NC_001477.1/294-788	752	I	G	I	L	L	T	W	L	G	L	N	S	R	S	T	S	L	S	M	T	C	I	A	V	G	M	T	L	Y	L	G	V	M	Q
DVV_16681.NC_001474.2/287-781	745	I	G	V	I	T	W	I	G	M	N	S	R	S	T	S	L	S	V	T	L	V	L	V	G	I	V	T	L	Y	L	G	V	M	Q
DVV_4.NC_002640.1/313-807	771	I	G	F	L	V	L	I	G	T	N	S	R	N	T	S	M	A	M	T	C	I	A	V	G	I	T	L	F	L	G	T	M	Q	
DVV_3.NC_001475.2/294-786	750	I	G	V	L	L	T	W	I	G	L	N	S	K	N	T	S	M	S	F	S	C	I	A	I	G	I	T	L	Y	L	G	V	Q	
TEV_ 病毒_NC_001672.1/292-787	751	L	G	V	A	L	A	L	G	L	N	M	R	N	P	T	M	S	M	S	F	L	L	A	G	G	L	V	L	A	M	T	L	G	V
WNV_956.NC_001563.2/306-802	766	L	G	A	L	L	W	M	G	I	N	A	R	D	R	S	I	A	M	T	F	L	A	V	G	G	V	L	L	F	L	S	V	N	H
WNV_NY99_isol-385-99.NC_009942.1/306-806	770	L	G	A	L	L	W	M	G	I	N	A	R	D	R	S	I	A	L	T	F	L	A	V	G	G	V	L	L	F	L	S	V	N	H
WNV_Chin-01.AY490240.2/306-806	770	L	G	A	L	L	W	M	G	I	N	A	R	D	R	S	I	A	L	T	F	L	A	V	G	G	V	L	L	F	L	S	V	N	H
ZVV_MR766-NIID.LC002520.1/326-829	793	I	G	T	L	V	L	G	L	N	T	K	N	G	S	I	S	L	T	C	L	A	L	G	G	V	M	I	F	L	S	T	A	S	
ZVV_ZikaSPH2015.KU321639.1/326-829	793	I	G	T	L	M	L	G	L	N	T	K	N	G	S	I	S	L	M	C	L	A	L	G	G	V	L	I	F	L	S	T	A	S	
ZVV_MR_766.NC_012532.1/326-825	789	I	G	T	L	V	L	G	L	N	T	K	N	G	S	I	S	L	T	C	L	A	L	G	G	V	M	I	F	L	S	T	A	S	
ZVV_MR_766.AY632535.2/326-825	789	I	G	T	L	V	L	G	L	N	T	K	N	G	S	I	S	L	T	C	L	A	L	G	G	V	M	I	F	L	S	T	A	S	
ZVV_BeH818995.KU365777.1/308-811	775	I	G	T	L	M	L	G	L	N	T	K	N	G	S	I	S	L	M	C	L	A	L	G	G	V	L	I	F	L	S	T	A	S	

图7 (3/3部分)

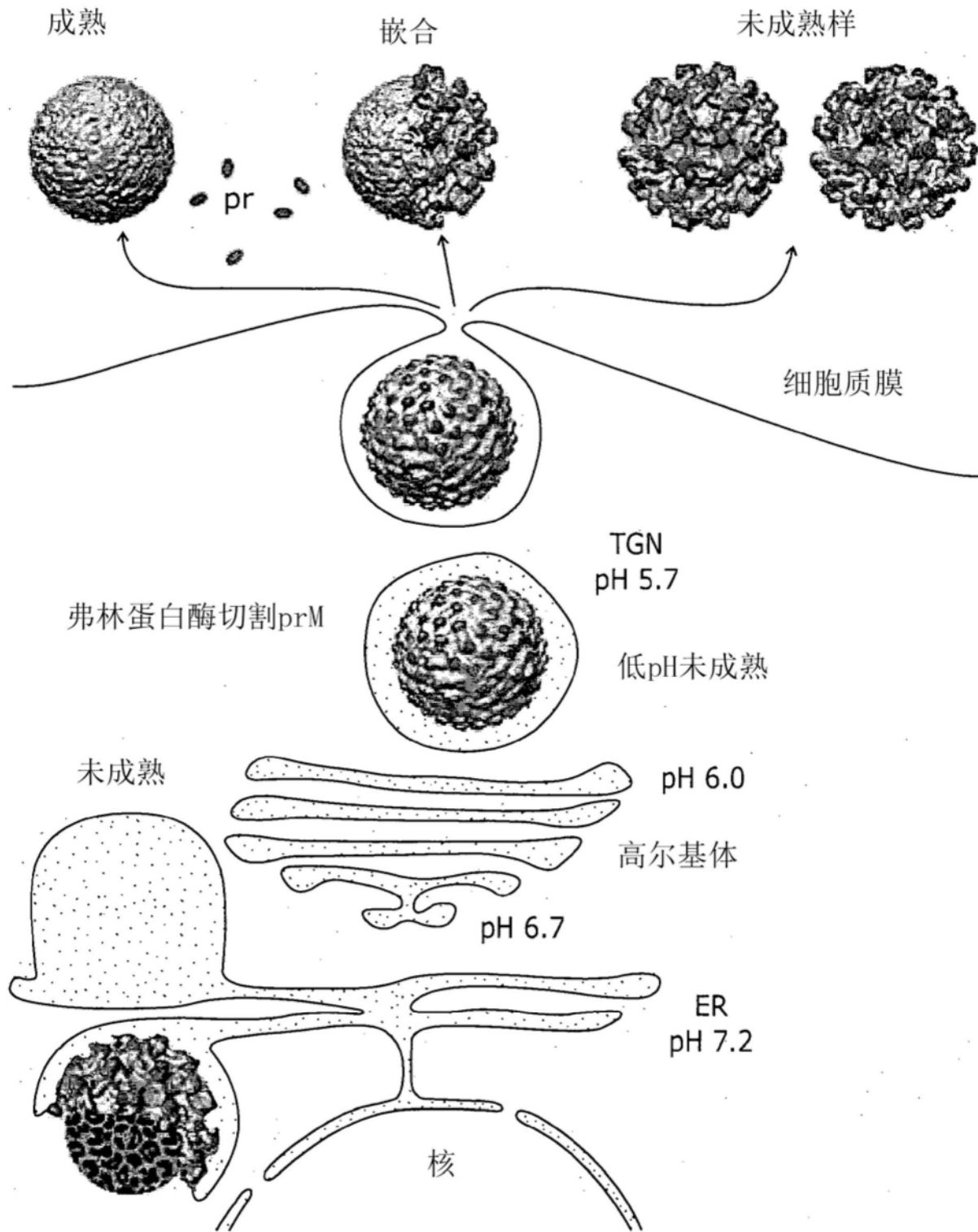


图8

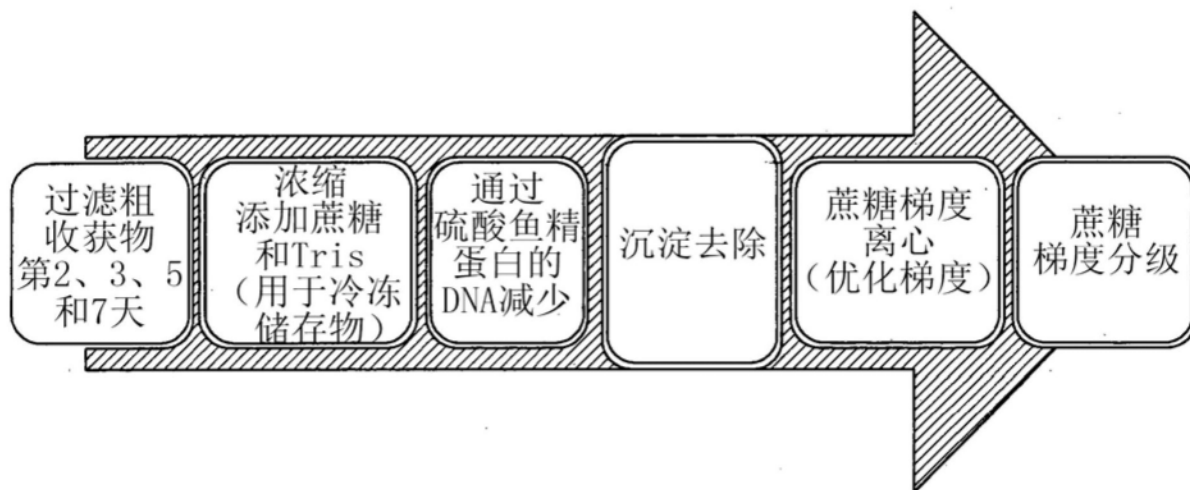


图9A

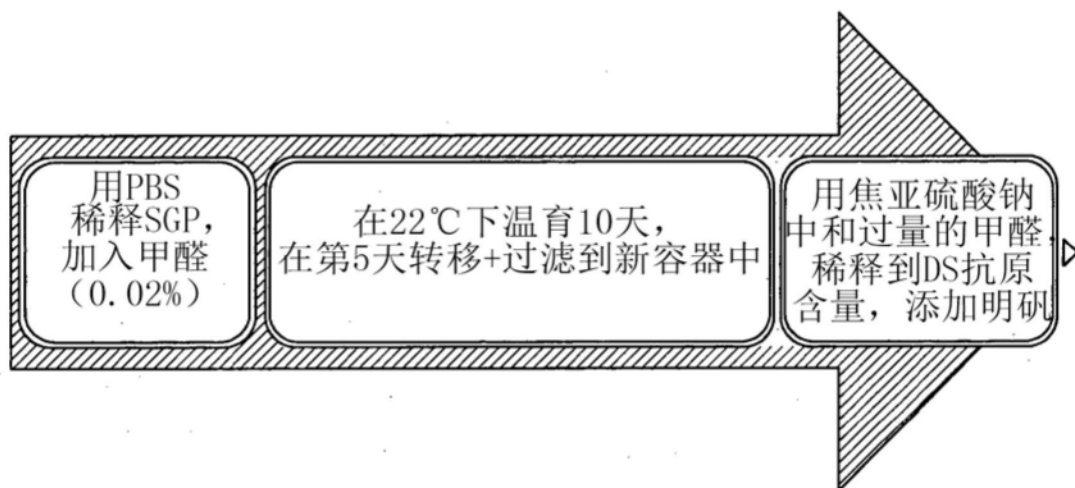


图9B

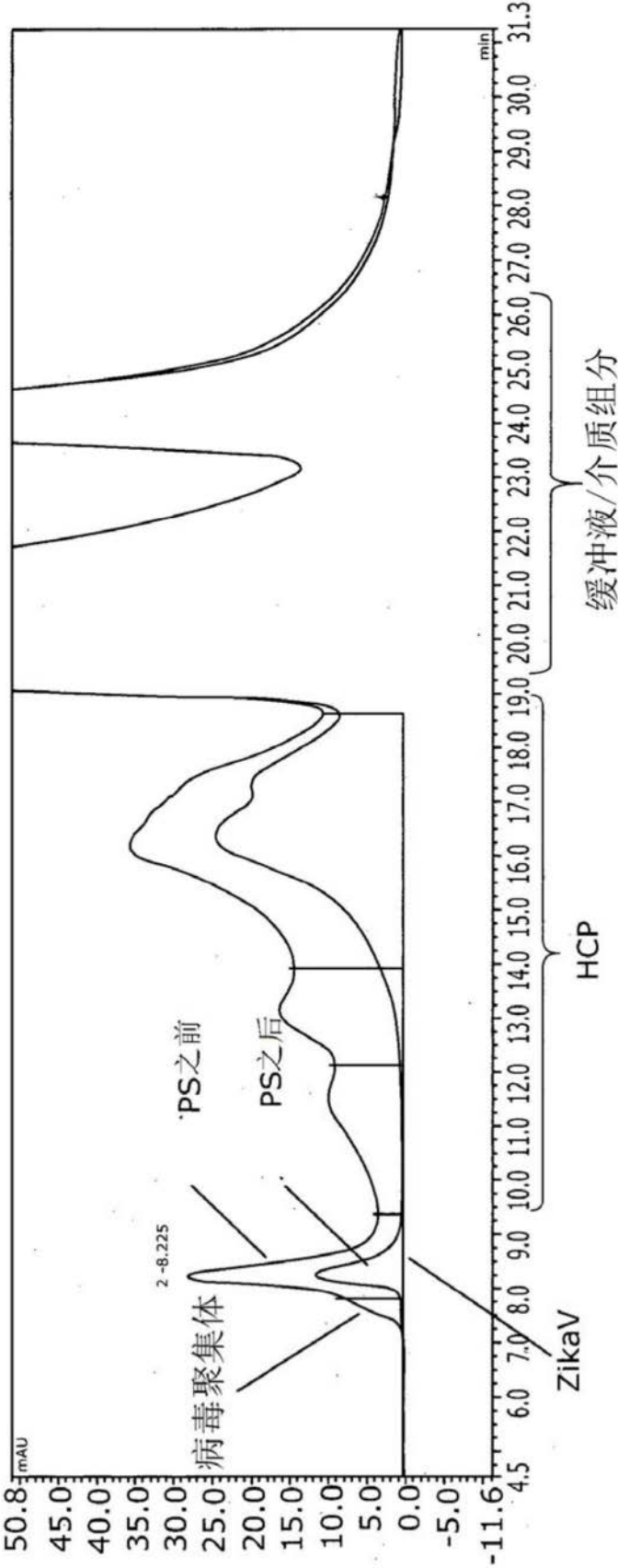
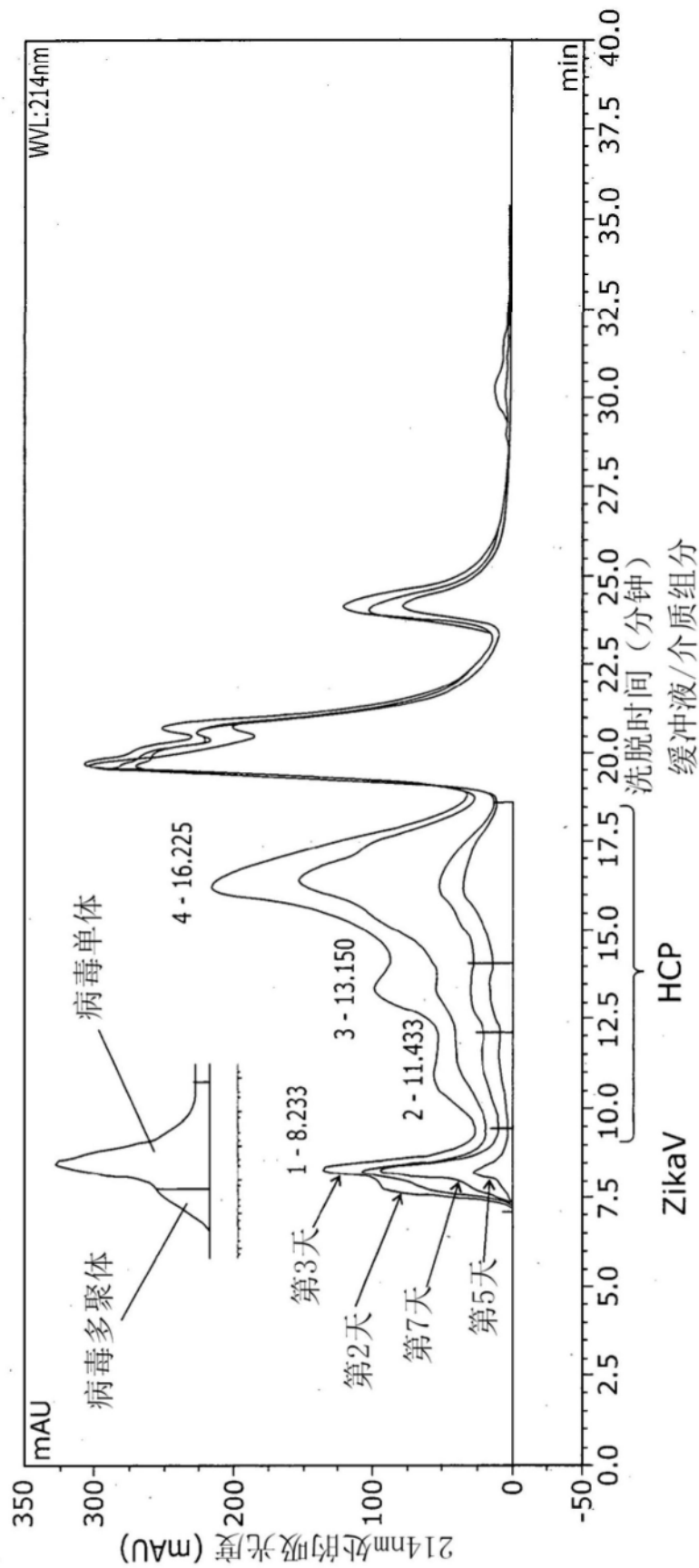


图10



- + 在收获第2、3、7天病毒浓度相似；在收获第5天病毒浓度降低
- + 第2和3天的收获物比第5/7天含有更多HCP（与观察到的CPE相关）
- + 观察到病毒多聚体的最小量

图11

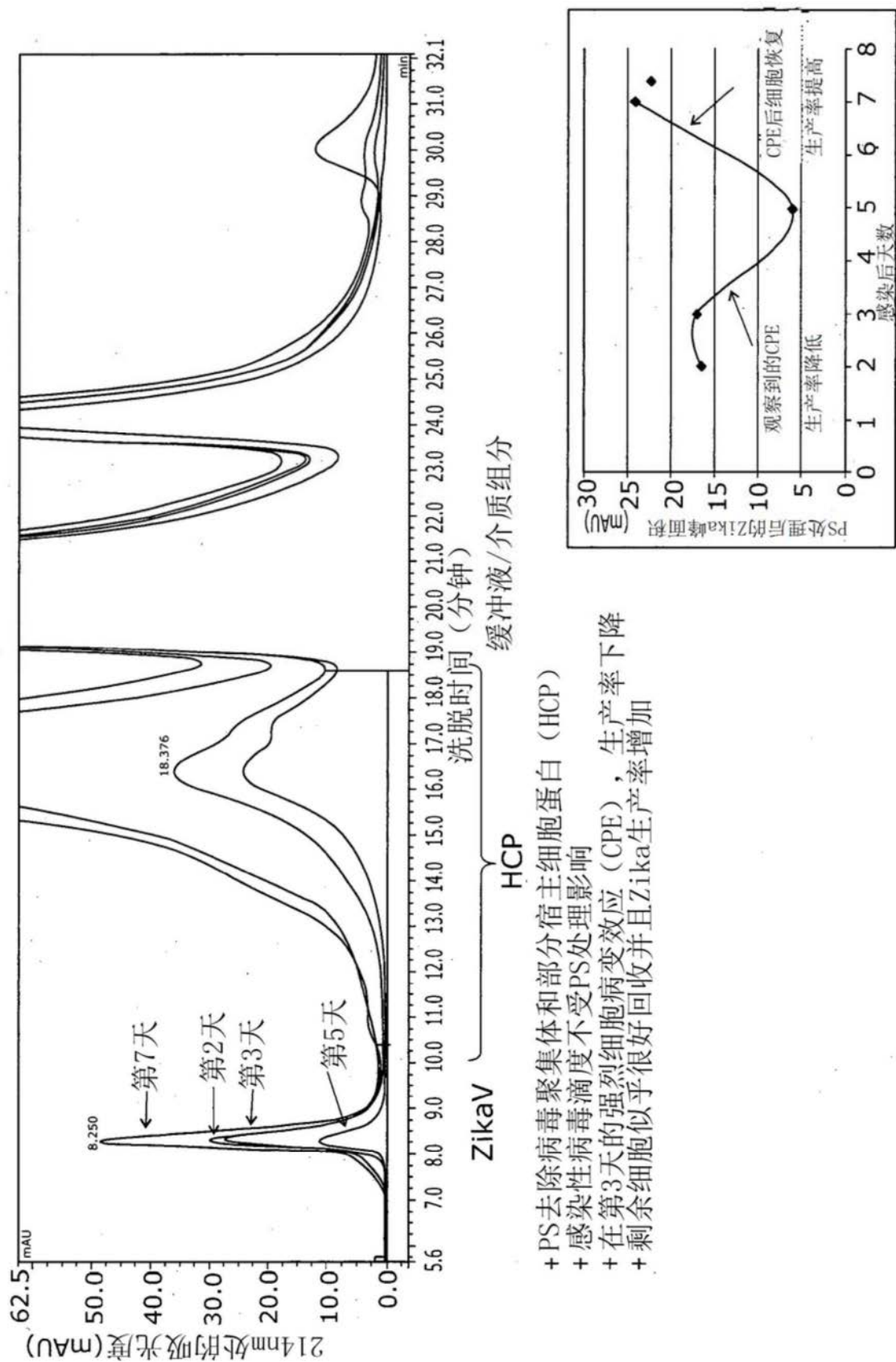


图12

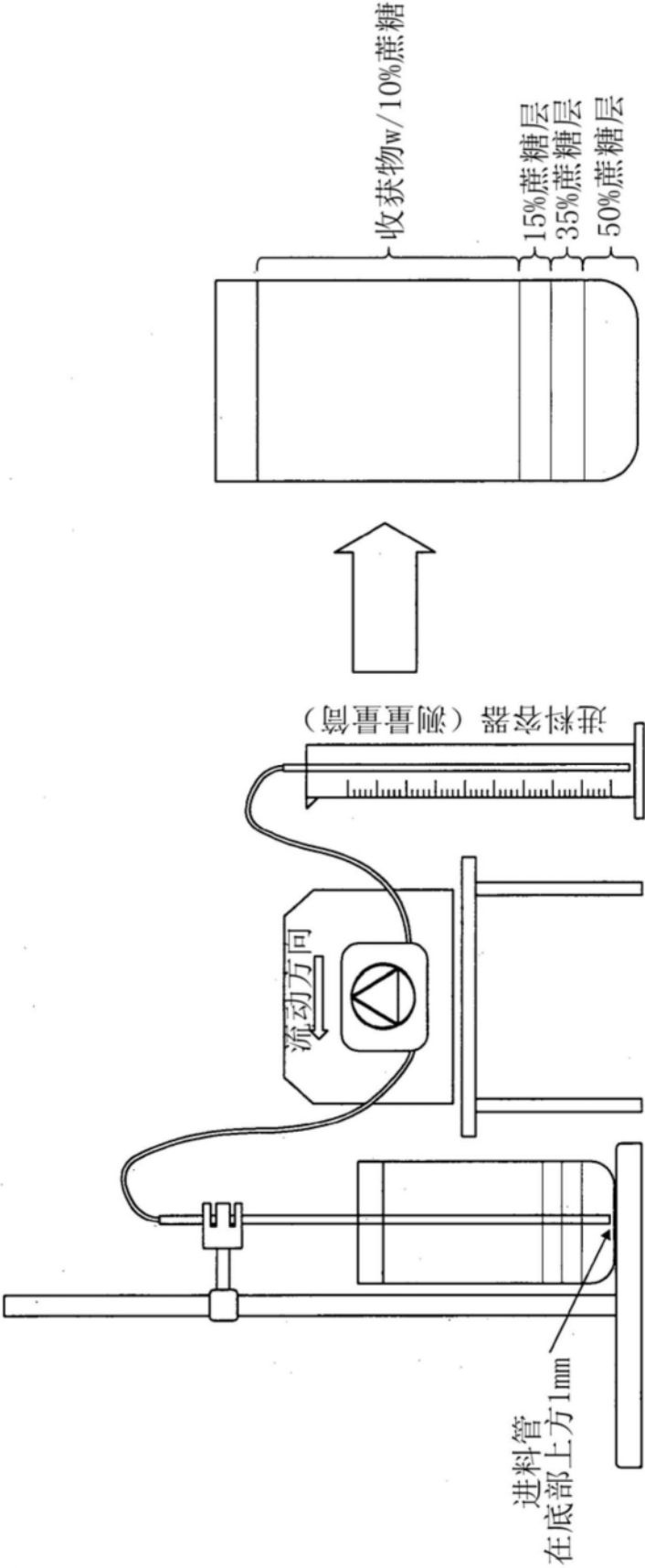


图13

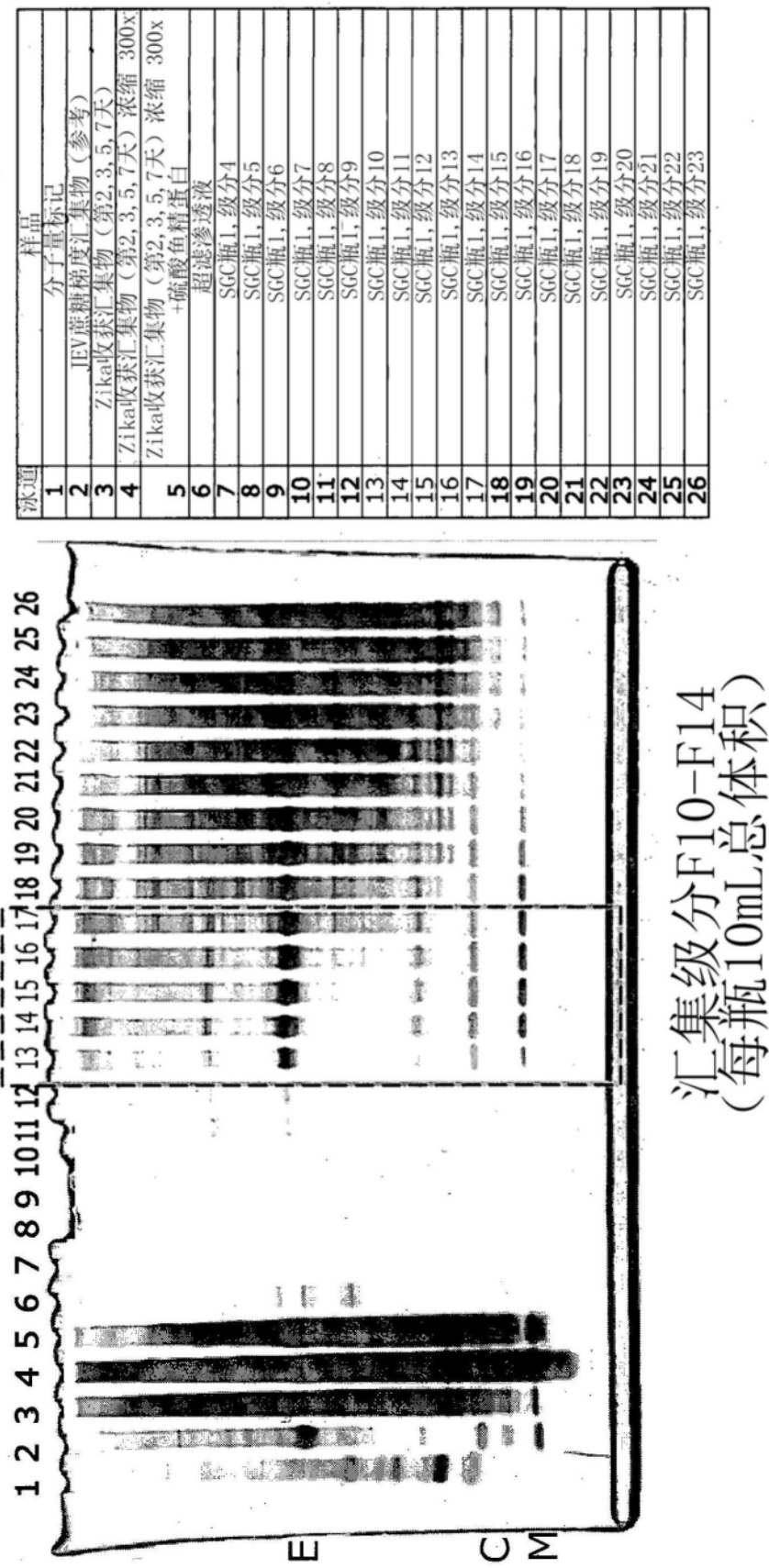


图14

获物计划的比较

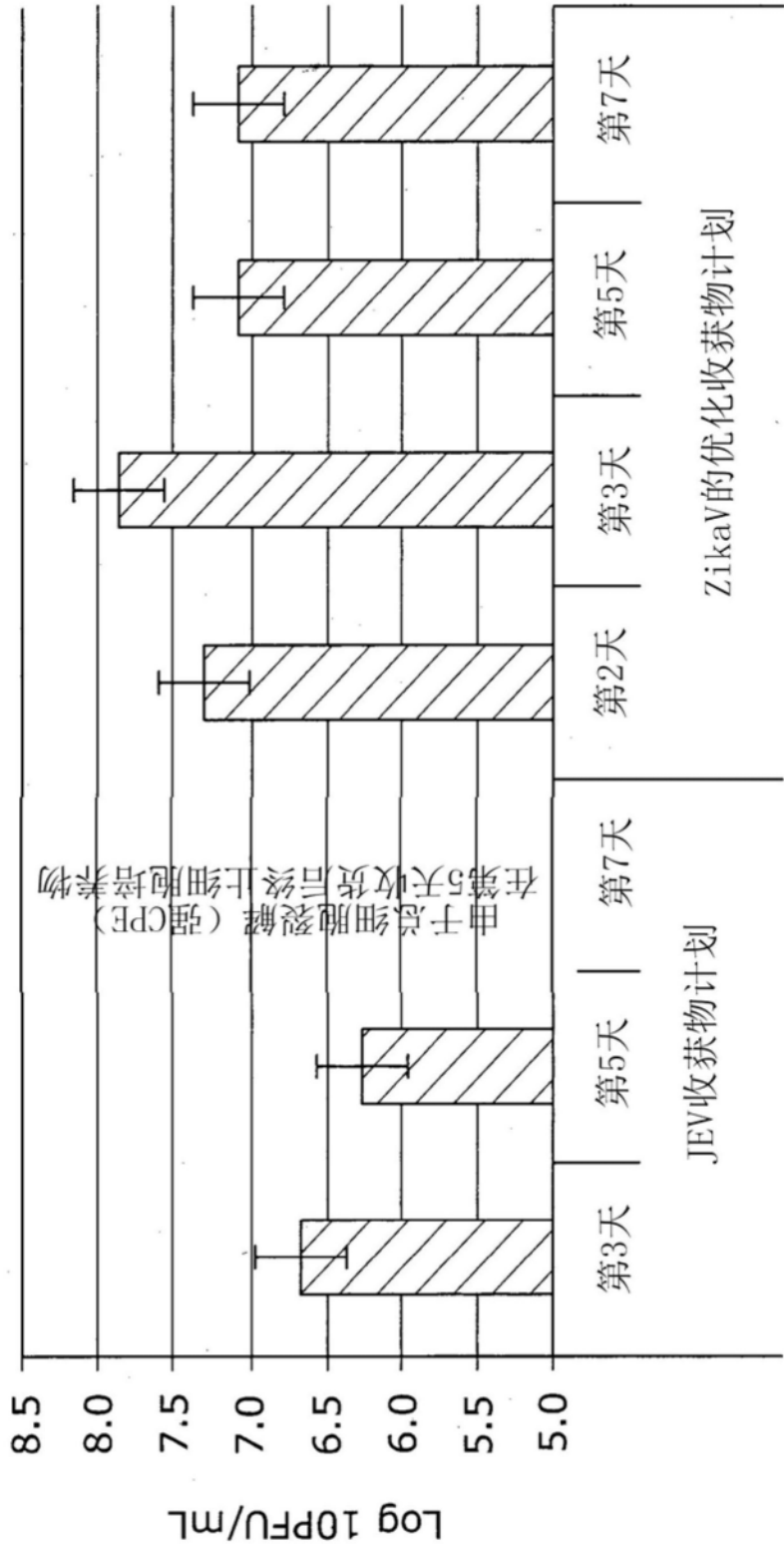


图15

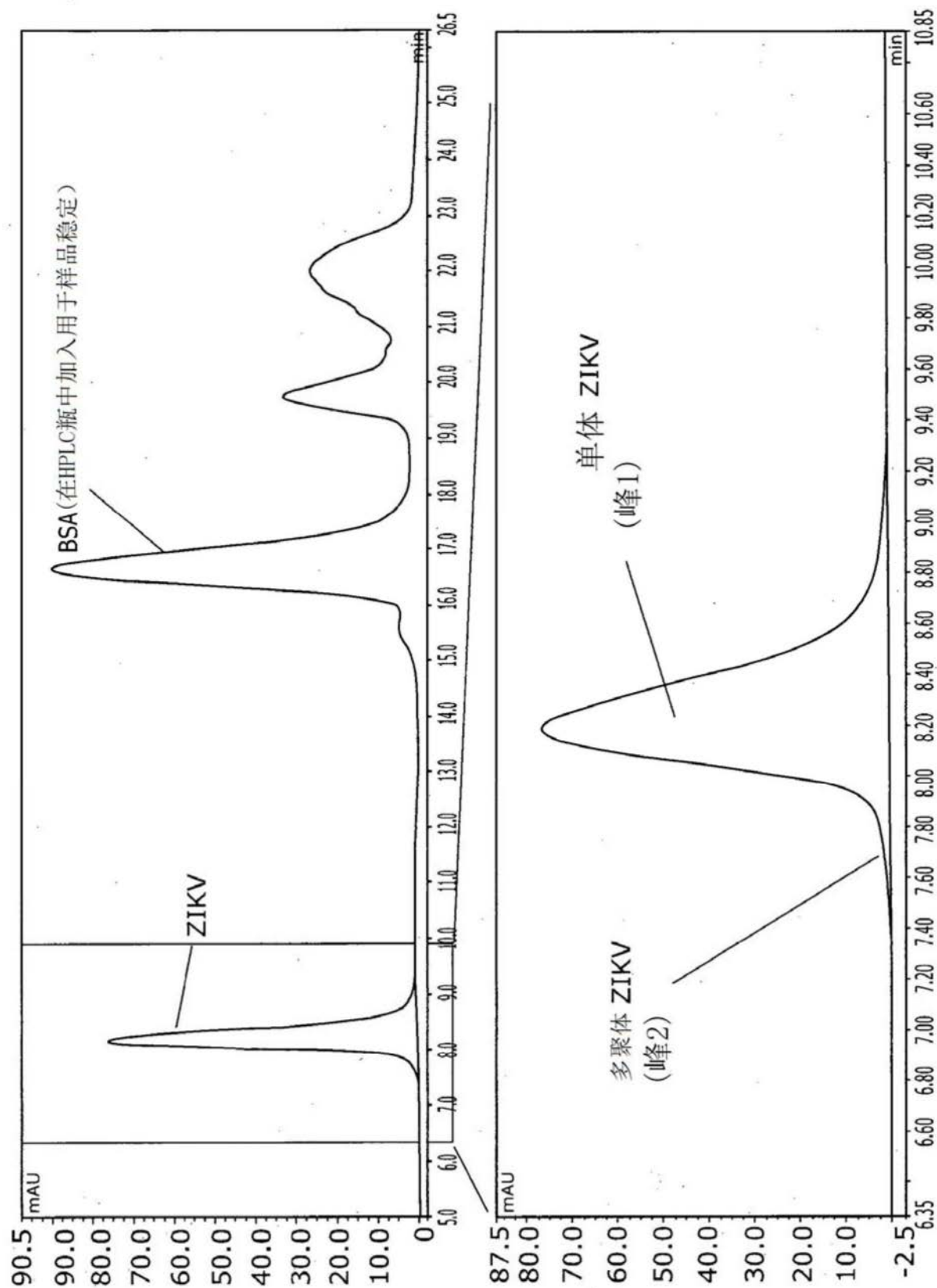


图16

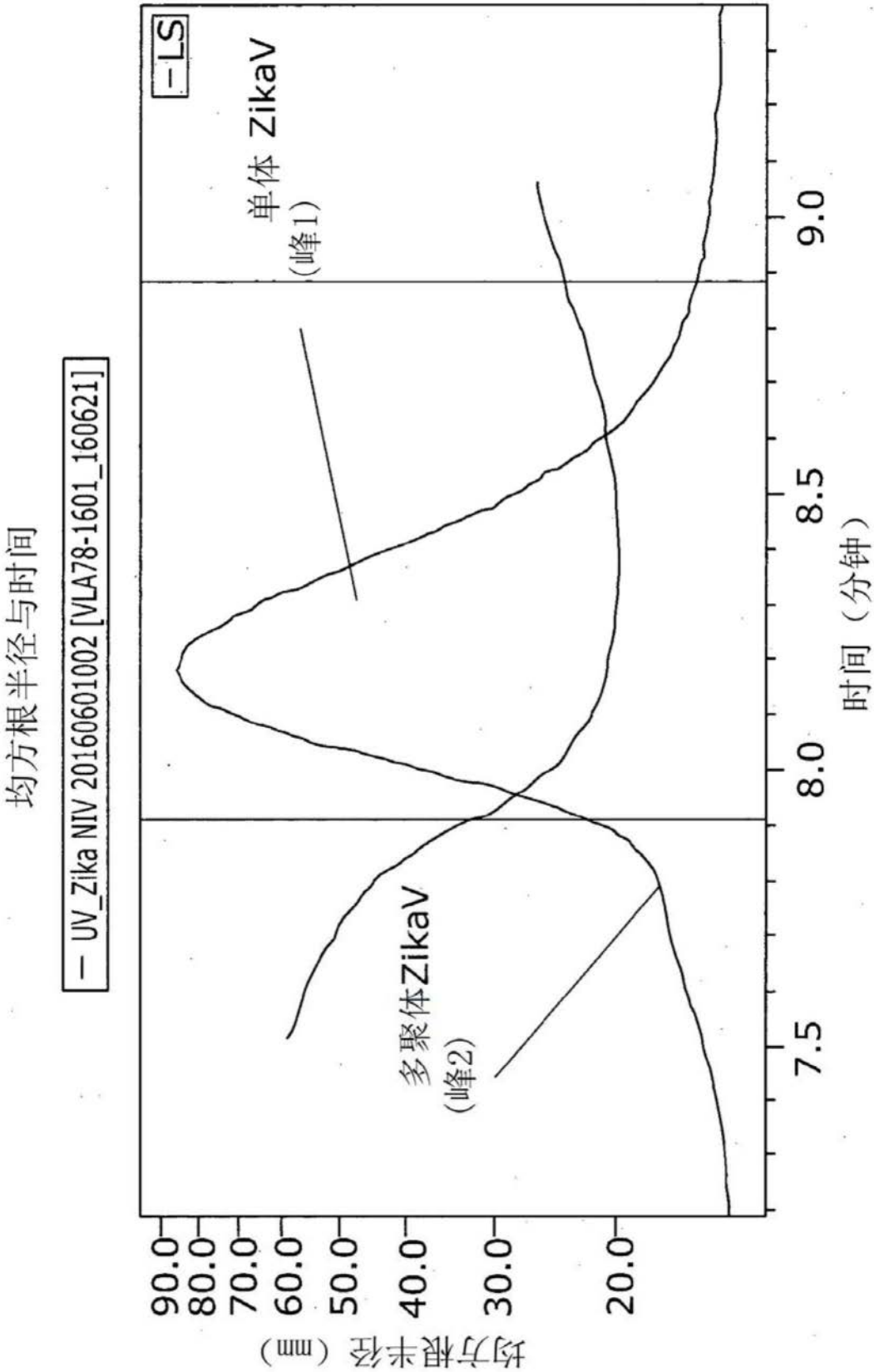


图17

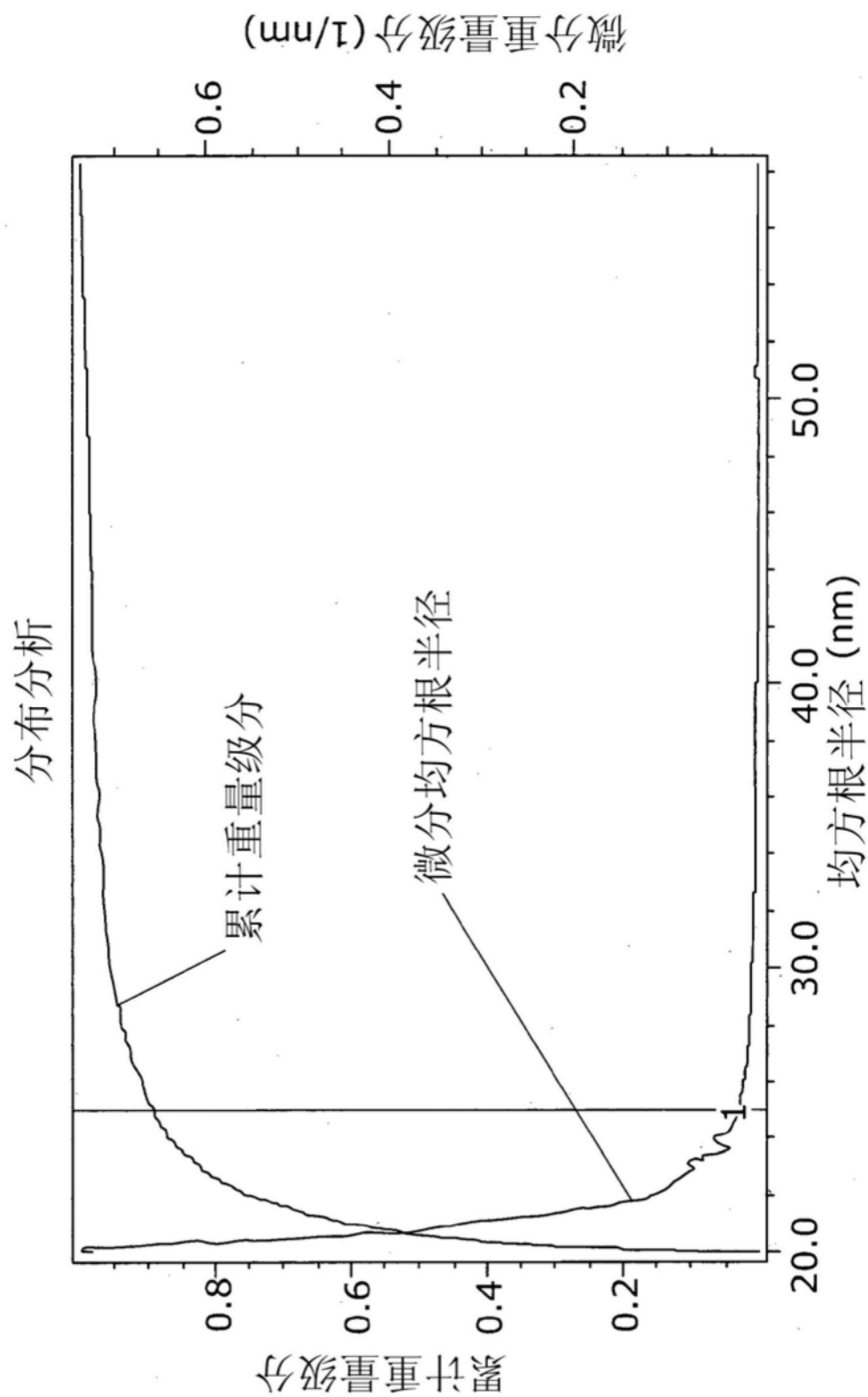


图18

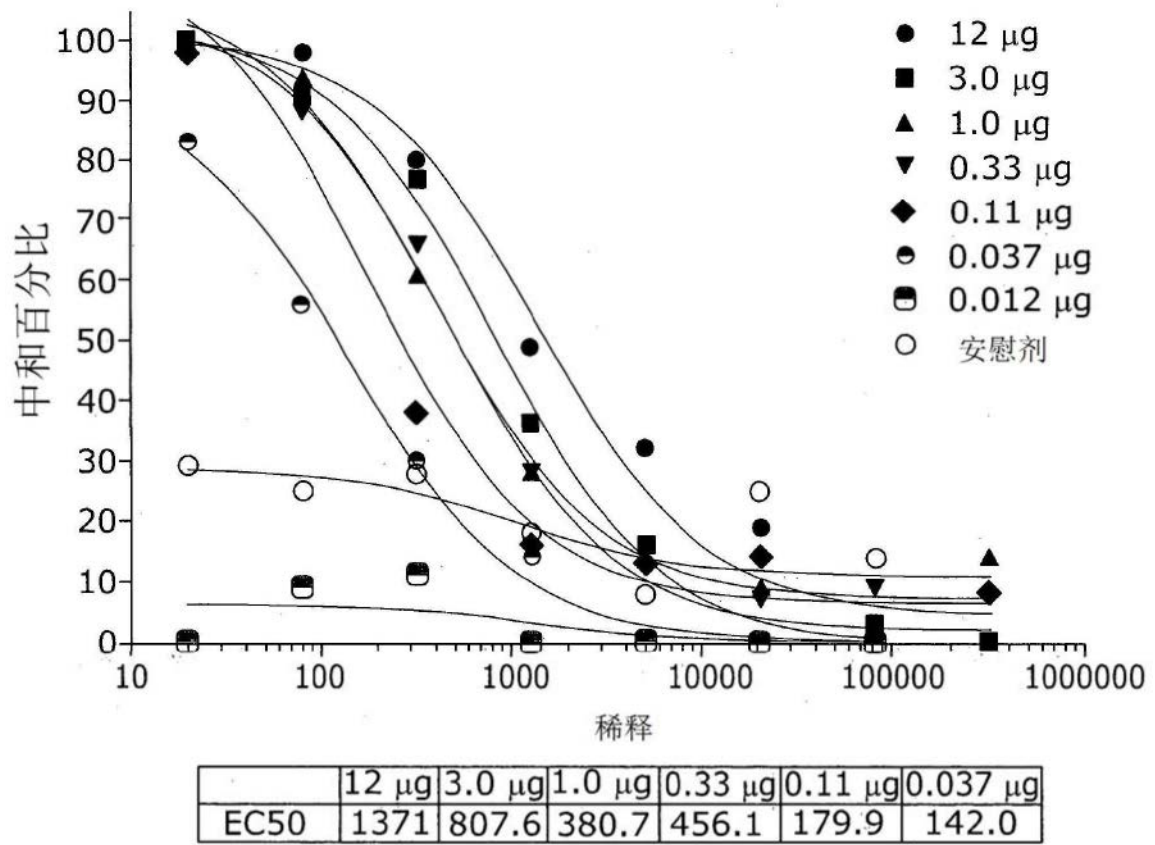


图19

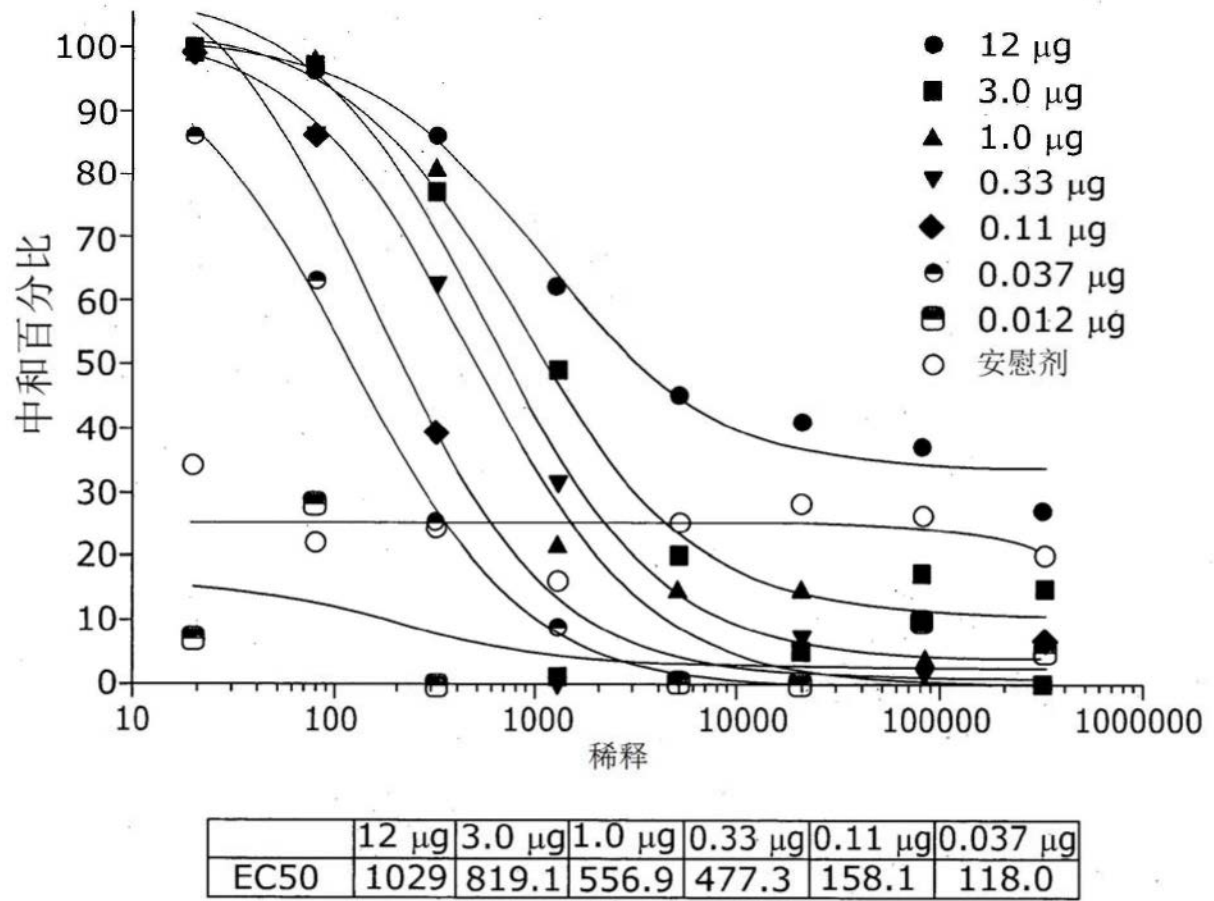


图20

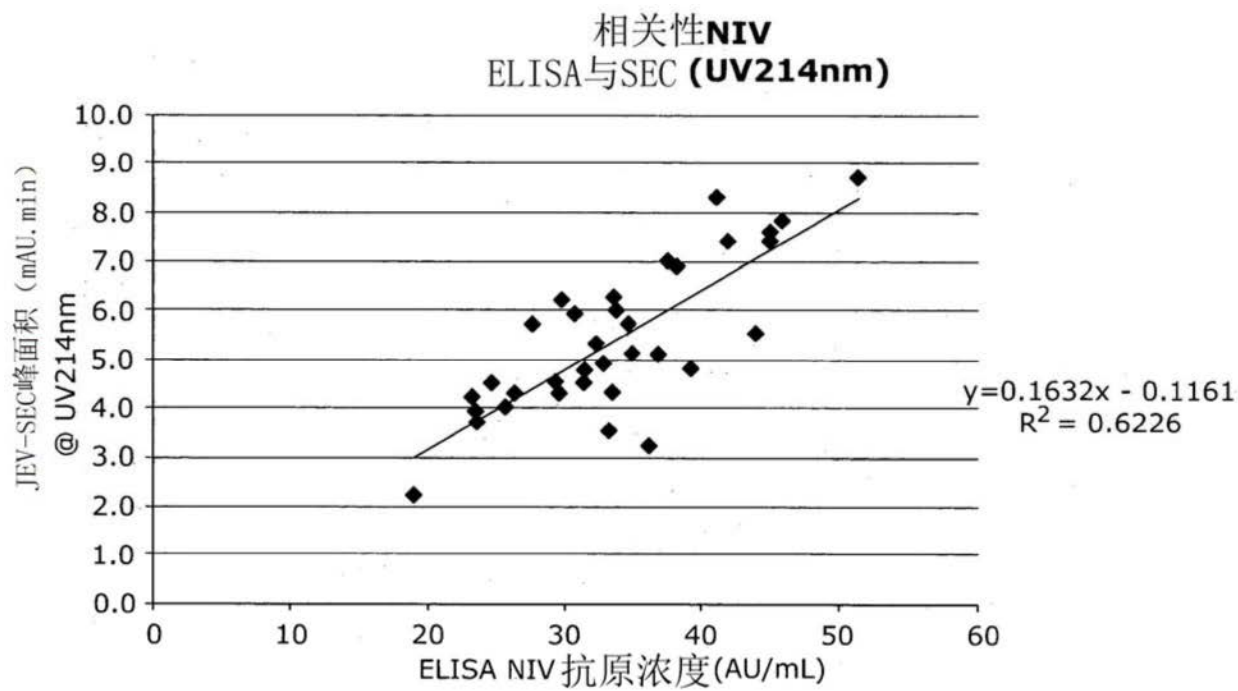


图21