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权利要求书2页 说明书25页  
序列表123页(54) 发明名称  
噬菌体疗法(57) 摘要  
本发明涉及噬菌体疗法。更具体来说, 本发明涉及对金黄色葡萄球菌(Staphylococcus  
aureus)菌株具有高特异性的新的噬菌体、它们  
的制造、其组分、包含它们的组合物及其在噬菌  
体疗法中和作为伴随诊断的用途。

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1. 一种抗细菌组合物,其包含对至少一种金黄色葡萄球菌 (*Staphylococcus aureus*) 菌株具有裂解活性的至少一种噬菌体,所述至少一种噬菌体选自具有包含SEQ ID NO:2或3的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体。

2. 权利要求1的组合物,其还包含至少一种不同的噬菌体,所述噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体。

3. 权利要求1的组合物,其还包含至少两种不同的噬菌体,所述噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体。

4. 权利要求1的组合物,其还包含至少三种不同的噬菌体,所述噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体。

5. 权利要求1的组合物,其包含:

- 具有包含SEQ ID NO:1的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体;

- 具有包含SEQ ID NO:2的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体;

- 具有包含SEQ ID NO:3的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体;和

- 具有包含SEQ ID NO:4的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体。

6. 权利要求1的组合物,其包含选自以下的噬菌体混合物中的任一者:1) PN1137+PN1493;2) PN1137+PN1815;3) PN1137+PN1957;4) PN1493+PN1815;5) PN1493+PN1957;6) PN1815+PN1957;7) PN1137+PN1493+PN1815;8) PN1137+PN1493+PN1957;9) PN1137+PN1815+PN1957;10) PN1493+PN1815+PN1957;11) PN1137+PN1493+PN1815+PN1957;

其中PN1137、PN1493、PN1815和PN1957分别是指具有SEQ ID NO:1、SEQ ID NO:2、SEQ ID NO:3、SEQ ID NO:4的核苷酸序列的噬菌体。

7. 权利要求1至6任一项的组合物,其还包含可药用赋形剂或载体。

8. 权利要求1至6任一项的组合物,其是液体、半液体、固体或冻干制剂。

9. 权利要求1至6任一项的组合物,其包含 $10^1$ 至 $10^{12}$ PFU/ml之间的每种噬菌体。

10. 权利要求1至9任一项的组合物在制备用于治疗人类或动物中的细菌感染的药物中的用途。

11. 权利要求1至9任一项的组合物在制备用于通过改良人类或动物中的微生物菌群来改善所述人类或动物的状况的药物中的用途。

12. 权利要求1至9任一项的组合物用于对材料消毒的体外用途。

13. 一种用于制备权利要求1至9任一项的组合物方法,其中所述组合物包含至少两种噬菌体,所述方法包括分开地生产所述至少两种噬菌体,以及将所述噬菌体与适合的载体或赋形剂组合。

14. 一种噬菌体,所述噬菌体对金黄色葡萄球菌菌株具有裂解活性,并具有包含SEQ ID

NO:2或3的核苷酸序列或与其具有至少99%同一性的序列的基因组。

15.一种分离的核酸,所述核酸包含SEQ ID NO:2或3的核苷酸序列或与其具有至少99%同一性的序列。

16.权利要求14的噬菌体在制备用于治疗人类或动物中的细菌感染的药物中的用途。

17.权利要求14的噬菌体在制备用于通过改良哺乳动物中的微生物菌群来改善所述哺乳动物的状况的药物中的用途。

18.权利要求14的噬菌体用于对材料消毒的体外用途。

19.一种用于确定有效对抗靶金黄色葡萄球菌菌株的噬菌体混合物的方法,所述方法包括:

a) 将所述靶金黄色葡萄球菌菌株或含有所述菌株的样品分开地与下述噬菌体接触:  
(i) 具有包含SEQ ID NO:2的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体,和/或(ii) 具有包含SEQ ID NO:3的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体,和任选地(iii) 具有包含SEQ ID NO:1或4的核苷酸序列或与其具有至少99%同一性的序列的基因组的噬菌体;

b) 选择对所述菌株表现出裂解活性的噬菌体,

c) 任选地进一步选择当组合在一起时对所述菌株表现出协同活性的活性噬菌体;和/或

d) 任选地进一步选择当组合在一起时不表现出拮抗作用的活性噬菌体;和/或

e) 任选地选择属于不同属的活性噬菌体;和

f) 将所述选择的噬菌体组合。

## 噬菌体疗法

[0001] 本发明涉及新的噬菌体组合物、它们的制造及其用途。本发明特别适合于治疗人类和动物中的感染。

### 背景技术

[0002] 噬菌体是表现出侵染并杀死细菌的能力的小病毒,同时它们不影响来自于其他生物体的细胞。噬菌体最初由William Twort在一个世纪之前描述,并在其后不久被Félix d'Herelle独立地发现,迄今为止已揭示出并在形态学上描述了超过6000种不同的噬菌体,包括细菌和古菌(archeal)的病毒。这些病毒绝大多数有尾,而少部分是多面体、丝状或多形性的。它们可以按照它们的形态、遗传内含物(DNA还是RNA)、特异性宿主、生活地点(海洋病毒还是其他栖息地)和生命周期进行分类。作为细菌细胞的细胞内寄生物,噬菌体在细菌宿主内表现出不同的生命周期:裂解性、溶原性和假溶原性(Weinbauer,2004;Drulis-Kawa,2012)。作为其生命周期的正常部分,裂解性噬菌体引起宿主细菌细胞的裂解。溶原性噬菌体(也被称为温和噬菌体)可以利用裂解性生命周期进行复制并引起宿主细菌裂解,或者它们可以将其DNA并入到宿主细菌DNA中并变成非感染性前噬菌体。假溶原性可以被定义为噬菌体在宿主细胞中不增殖或复制的发育停滞阶段(M Łoś,2012)。不论噬菌体的周期是何种类型,第一步是附着于细菌细胞壁的受体,然后噬菌体的遗传材料才可能进入细菌。这个特异性过程定义了与噬菌体相互作用的细菌谱。

[0003] 噬菌体通常作为研究工具用于在实验室实验中改良细菌。

[0004] 由于它们的靶宿主细胞特异性,已考虑将噬菌体用作疗法以治疗急性和慢性感染,特别是在皮肤病学、眼科学、泌尿科学、口腔病学、儿科学、耳鼻喉科学或外科学中。然而,这种噬菌体治疗细菌感染的治疗性用途的概念,从一开始就极富争议,并且未被公众或医学界广泛接受。早期研究由于缺少适合的对照并且结果不一致而广受批评。缺乏可重复性和在各种发表的研究中获得的许多冲突的结果,使美国医学联合会药学与化学理事会(Council on Pharmacy and Chemistry of the American Medical Association)得出关于裂解滤液的治疗价值的证据在极大程度上是矛盾的、不足以令人信服的结论,并推荐进行进一步研究以确认它声称的益处。

[0005] 自从在1940年代引入抗生素以来,很少有人关注治疗学的这个领域,特别是在西方世界。但是抗生素的广泛使用已在全世界引起抗生素耐药性细菌的广泛出现,造成越来越严重的问题。因此,克服仍可用于治疗主要的多药耐药微生物的有限的剩余治疗选项,已变成主要的挑战。

[0006] 金黄色葡萄球菌(*S.aureus*)是一种革兰氏阳性球菌,其通常存在于鼻、呼吸道中和皮肤上。金黄色葡萄球菌(*S.aureus*)在金黄色菌落色素沉着和凝固酶、甘露醇发酵和脱氧核糖核酸酶试验的阳性结果的基础上与其他葡萄球菌物种区分开。金黄色葡萄球菌(*S.aureus*)是世界范围内最重要的病原体之一,并且已成为感染危重病人的重要生物体。

[0007] 金黄色葡萄球菌(*S.aureus*)可以是共生的但也是危险的病原体。大约30%的人群定殖有金黄色葡萄球菌(*S.aureus*)。金黄色葡萄球菌(*S.aureus*)感染是皮肤、软组织、呼吸

系统、骨骼、关节和血管内疾病例如皮肤脓肿、伤口感染、心内膜炎、骨髓炎、肺炎和中毒性休克综合征的主要原因。金黄色葡萄球菌 (*S.aureus*) 特别擅长于感染人类宿主中的外来物体。在这些情况下,金黄色葡萄球菌 (*S.aureus*) 通常在外来装置(例如可植入心脏装置、血管内导管、假体、支架……)的表面上形成生物膜,使得在不手术取出所述装置的情况下根除感染几乎不可能。金黄色葡萄球菌 (*S.aureus*) 可以适应于在细胞内部生活,在那里它受到保护免于宿主防御机制和大多数抗生素的攻击。

[0008] 葡萄球菌感染的数目持续增长,同时由于对多种抗生素(包括甲氧西林或万古霉素)耐药的葡萄球菌菌株的出现,这些感染的治疗变得甚至更加困难。在美国和英国,40%至60%的医院内金黄色葡萄球菌 (*S.aureus*) 菌株是多药耐药的。

[0009] 因此,对可用于毁灭或控制金黄色葡萄球菌 (*S.aureus*) 菌株,适合于在人类或动物疗法中使用以及适合于对材料消毒的新的抗菌剂或组合物,存在着极大需求。

[0010] 针对金黄色葡萄球菌 (*S.aureus*) 的实验性噬菌体疗法已在小鼠中进行测试(Capparelli等,2007),尚未开发用于人类使用。因此,鉴于金黄色葡萄球菌 (*S.aureus*) 获取耐药性的高度潜力,对可用于杀死金黄色葡萄球菌 (*S.aureus*) 菌株(包括棘手的甲氧西林耐药性菌株(MRSA))的新的抗菌剂或组合物,存在着需求。

## 发明内容

[0011] 本发明人已分离并表征了对金黄色葡萄球菌 (*S.aureus*) 菌株表现出强裂解活性的新的噬菌体。这些噬菌体单独地或者组合地提供了非常强的抗菌效果,并且可作为活性剂用于药物制剂或兽医制剂,特别是用于治疗金黄色葡萄球菌 (*S.aureus*) 细菌感染。

[0012] 本发明的一个目的是提供抗菌组合物,其包含对至少一种金黄色葡萄球菌 (*S.aureus*) 菌株具有裂解活性的至少一种噬菌体,所述至少一种噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体。

[0013] 本发明的另一个目的涉及一种噬菌体,其对金黄色葡萄球菌 (*S.aureus*) 菌株具有裂解活性,并具有包含选自SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少95%同一性的序列的基因组。

[0014] 本发明还涉及被包含在本发明的噬菌体中的分离的核酸分子,优选为包含选自SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少95%同一性的序列的分离的核酸分子,以及由所述核酸编码的分离的多肽。

[0015] 本发明的另一个目的是包含如上所定义的核酸或多肽的组合物。

[0016] 本发明的组合物通常还包含可药用或可兽医用赋形剂或载体。它们可以是液体、半液体、固体或冻干的。

[0017] 本发明的另一个目的涉及如上所定义的噬菌体、核酸、多肽或组合物,其用于治疗人类和动物中的感染,改良所述人类或动物的微生物菌群,对材料消毒,杀死或控制金黄色葡萄球菌 (*S.aureus*) 细菌,和/或损害由金黄色葡萄球菌 (*S.aureus*) 细菌产生的细菌生物膜的完整性,和/或对食品和饮料消毒。

[0018] 本发明还涉及如上所定义的噬菌体、核酸、多肽或组合物,其用于通过改良对象中的微生物菌群来改善所述对象的状况。所述微生物菌群可以通过校正、改变或恢复所述菌群中微生物的适当平衡来改良。

[0019] 本发明还涉及一种用于治疗人类或动物中的感染的方法,所述方法包括向所述人类或动物给药至少一种如上所定义的噬菌体、核酸、多肽或组合物。

[0020] 本发明还涉及一种用于对怀疑被金黄色葡萄球菌 (*S.aureus*) 细菌污染的表面或材料进行处理的方法,所述方法包括向所述表面或材料施加至少一种如上所定义的噬菌体、核酸、多肽或组合物。所述表面或材料可以是任何装置、容器、实验室材料、衣物、鞋类、军事装备、空气冷却系统、住宅等的表面。

[0021] 本发明的另一个目的涉及一种试剂盒,其包含如上所定义的组合物和用于将所述组合物施加到对象或表面的工具。

[0022] 本发明可用于任何人类或动物(优选为人类)上以及任何人类或动物(优选为人类)中,或用于处理任何材料,包括实验室材料或人类或动物内部或外部的医疗装置。

[0023] 本发明的另一个目的涉及一种用于确定有效对抗金黄色葡萄球菌 (*S.aureus*) 菌株靶的噬菌体混合物的方法,所述方法包括:

[0024] a) 将靶向的金黄色葡萄球菌 (*S.aureus*) 菌株或含有所述菌株的样品分开地与下述噬菌体接触: (i) 具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和/或(ii) 具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和/或(iii) 具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和/或(iv) 具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和(v) 这些噬菌体的一种或多种组合;

[0025] b) 选择对所述菌株表现出裂解活性的噬菌体,

[0026] c) 任选地进一步选择当组合在一起时对所述菌株表现出协同活性的活性噬菌体;和/或

[0027] d) 任选地进一步选择当组合在一起时不表现出拮抗作用的活性噬菌体;和/或

[0028] e) 任选地选择属于不同属的活性噬菌体;和

[0029] f) 将所述选择的噬菌体组合。

[0030] 发明详述

[0031] 本发明涉及新的噬菌体、其组分、包含所述噬菌体的组合物、它们的制造及其作为抗菌剂的用途,特别是用于治疗人类或动物中的感染或通过改良对象中的微生物菌群来改善所述对象的状况。

[0032] 定义

[0033] 为了便于理解本发明,下面对许多术语进行定义。

[0034] 当在本文中使用时,术语“噬菌体”是指有功能的噬菌体粒子,其包含被包装在蛋白质包膜或衣壳中的核酸基因组。该术语也指称提供基本上相同的功能活性的噬菌体的部分,包括例如头部或噬菌体组分的组装体。

[0035] 术语“表型特征”更优选地是指噬菌体的形态和/或宿主范围。用于确定噬菌体表型的方法本身在过去是公知的,并且包括例如确定细菌宿主范围和/或针对由某些细菌菌株产生的生物膜的活性。

[0036] 当在本发明中使用时,术语“裂解活性”是指噬菌体引起细菌细胞裂解的性能。可以按照本身在本领域中已知的技术在金黄色葡萄球菌 (*S.aureus*) 菌株上测试噬菌体的裂

解活性(也参见实验部分)。

[0037] 术语参比噬菌体的“变体”是指与所述参比噬菌体相比在基因组序列和/或由其编码的多肽中具有变异的噬菌体。所述变体与所述参比噬菌体相比可能具有不同的表型特征,例如不同的细菌宿主范围。在特定情况下,变体可以通过定向进化(也被称为噬菌体训练)来获得,这种技术允许所述变体获得对一种或几种细菌菌株的裂解活性。变体与所述参比噬菌体相比通常表现出相同的形态。通常,所述参比噬菌体具有包含选自SEQ ID NO:1-4任一者的序列的核酸序列。变体通常包含例如遗传物质的沉默突变、保守突变、少量缺失和/或少量复制。在优选实施方式中,本发明的变体保留取决于本发明的噬菌体的基因组的任何可观察的特征或特性,例如所述噬菌体的表型特征和/或针对金黄色葡萄球菌(*S.aureus*)菌株的裂解活性。与参比噬菌体的基因组相比,优选的变体具有少于5%、甚至更优选地少于4%、更优选地少于2%的核酸变异。可选地或组合地,与参比噬菌体的多肽相比,变体在编码的多肽序列中优选地具有少于5%的氨基酸变异。

[0038] 对于噬菌体来说,术语“特异的”或“特异性”是指所述噬菌体能够感染的宿主类型。对金黄色葡萄球菌(*S.aureus*)“特异的”噬菌体更优选地是指可以感染一种或几种金黄色葡萄球菌(*S.aureus*)菌株并且在生理条件下基本上不感染非金黄色葡萄球菌(*S.aureus*)细菌的噬菌体。

[0039] 当在本文中使用时,术语“多肽”是指任何尺寸的多肽,包括例如5至20个氨基酸的小肽、更长的多肽、蛋白质或其片段。

[0040] 在本说明书的情形中,术语“分离的噬菌体”应该被认为意指从其天然环境中取出和/或与其天然环境的组分分离的和/或由定向进化产生的噬菌体。该术语特别是指例如在体外培养、纯化和/或用任何适合的配制用产品例如稀释剂或赋形剂配制的噬菌体。对于核酸或多肽来说,术语“分离的”是指例如与其天然环境的至少一种组分例如蛋白质、脂质、糖和/或核酸分离的核酸分子或多肽。

[0041] 当在本文中使用时,术语“可药用或可兽医用”是指与在人类或动物对象中的使用相容的任何材料(例如载体、赋形剂或稀释剂)。这些材料包括对生物体无害或不引起任何显著的特异性或非特异性免疫反应或不废止活性化合物的生物活性的生理上可接受的溶液或介质。为了将组合物配制成液体制剂,可以使用盐水、无菌水、林格氏溶液(Ringer's solution)、缓冲生理盐水、白蛋白输注溶液、右旋糖溶液、麦芽糖糊精溶液、甘油、乙醇及其混合物作为可药用或可兽医用赋形剂或载体。如有必要,可以添加其他常规添加剂例如增稠剂、稀释剂、缓冲剂、防腐剂、表面活性剂、抗氧化剂和抑菌剂。此外,可以另外向组合物添加稀释剂、分散剂、表面活性剂、粘合剂和润滑剂,以制备可注射制剂例如水性溶液、悬液和乳液,口服制剂例如丸剂、胶囊、颗粒剂或片剂,或粉末制剂,以及气溶胶化制剂例如液体或粉剂。

[0042] 当在本文中使用时,“PFU”是指噬斑形成单位,正如在本领域中明确定义的。裂解性噬菌体裂解宿主细胞,在培养板上产生清空区(或噬斑)。理论上,每个噬斑由一个噬菌体形成,并且噬斑数目乘以稀释倍数等于试验制剂中噬菌体的总数。

[0043] 当在本文中使用时,“CFU”是指菌落形成单位,正如在本领域中为估算样品中活细菌的数目而明确定义的。

[0044] 术语“治疗”或“疗法”是指疾病的治愈性或预防性治疗。治愈性治疗被定义为引起



疾病治愈的治疗,或缓和、减轻、稳定或消除疾病的症状或它直接或间接地引起的痛苦的治疗,或改善对象的状况或减缓疾病进展的治疗。预防性治疗包含引起疾病预防的治疗和/或减小和/或延迟疾病的发生率或它出现的风险的治疗。

[0045] 当在本文中使用时,术语“生物膜”是指在各种不同表面上生长的非均相细菌形成物;优选为包埋在附着于固体生物或非生物表面上的胞外多糖基质中生长的细菌群落。

[0046] 当在本文中使用时,术语“损害”是指完整性的任何改变。损害细菌生物膜被理解为生物膜被噬菌体的变性和/或侵入,生物膜相关细菌的感染和/或其裂解,和/或生物膜的部分或全部清除(即通过停止定殖和/或破坏生物膜)。

[0047] 当在本文中使用时,术语“样品”是指任何样品,例如生物样品,特别是含有细胞的样品。这些样品的实例包括体液例如血液、血浆、唾液、粪便或尿液,以及活组织检查、器官、组织或细胞样品。所述样品可以被处理。

[0048] 当在本文中使用时,术语“对象”或“患者”是指动物,优选为人类,包括成年人和儿童。术语“对象”也涵盖动物,例如且不限于宠物(如狗、猫)、农场畜种如马、奶牛、山羊、猪、绵羊、家禽、非人类灵长动物和鱼类、贝类、虾等。

[0049] 当在本文中使用时,术语治疗的“功效”或对噬菌体疗法的“响应”是指与治疗前金黄色葡萄球菌(*S.aureus*)菌株的数目相比在噬菌体治疗后引起对象中金黄色葡萄球菌(*S.aureus*)菌株的数目减少的治疗。“良好响应者”对象是指当用噬菌体疗法治疗时显示出或将会显示出临床上显著的恢复的对象。

[0050] 术语噬菌体的“混合物”是指不同噬菌体的组合。混合物中的噬菌体优选被一起配制在同一容器或包装中,尽管它们也可以作为试剂盒(kits of parts)使用,在所述试剂盒中将某些噬菌体分开地配制或包装,并在使用或给药时组合。

[0051] 当在本文中使用时,术语“序列同一性”通过在比较窗口内对两个最优对齐的序列进行比较来确定,其中为了所述两个序列的最优对齐,在所述比较窗口中所述多核苷酸的片段与不含添加或缺失的参比序列相比可能包含添加或缺失(例如间隙或突出部分)。序列同一性百分率如下计算:确定在两个序列中出现一致的核酸碱基或氨基酸残基的位置的数目以获得匹配位置数目,将所述匹配位置数目除以所述比较窗口中的位置总数并将结果乘以100,以得到序列同一性的百分率。用于比较的序列的最优对齐,可以通过Smith和Waterman的局部同源性算法(Add.APL.Math.2:482(1981))、Needleman和Wunsch的同源性对齐算法(J.Mol.Biol.48:443(1970))、Pearson和Lipman的相似性搜索方法(Proc.Natl.Acad.Sci.(USA)85:2444(1988))、这些算法的计算机化实施(Wisconsin遗传学软件包中的GAP、BESTFIT、BLAST、PASTA和TFASTA,Genetics Computer Group(GCG),575Science Dr.,Madison,WI)或通过目测检查来进行。

[0052] 实施方式描述

[0053] 本发明涉及金黄色葡萄球菌(*S.aureus*)感染的新的噬菌体疗法。更具体来说,本发明涉及对金黄色葡萄球菌(*S.aureus*)菌株具有高裂解活性的新的噬菌体、它们的制造、其组分、包含它们的组合物及其在噬菌体疗法中的用途。

[0054] 噬菌体

[0055] 第一方面,本发明公开了对金黄色葡萄球菌(*S.aureus*)菌株具有裂解活性并且单独或组合地表现出卓越的裂解活性宿主范围谱的新的噬菌体的分离和表征。这些噬菌体已

被分离、测序并表征。它们单个并且组合地具有针对金黄色葡萄球菌 (*S.aureus*) 菌株的活性。它们显著有效地对抗致病性金黄色葡萄球菌 (*S.aureus*) 菌株,包括抗生素耐药性金黄色葡萄球菌 (*S.aureus*) 菌株例如甲氧西林耐药性金黄色葡萄球菌 (MRSA) 菌株。这些噬菌体可以被组合且配制成适合用作制药或兽医药剂的状态,以表现出针对受控范围的金黄色葡萄球菌 (*S.aureus*) 菌株的非常强的抗细菌效应。

[0056] 更具体来说,已分离了下列噬菌体。它们相应的核酸序列也被注明。

[0057] 表1

| 噬菌体    | SEQ ID      |
|--------|-------------|
| PN1137 | SEQ ID NO:1 |
| PN1493 | SEQ ID NO:2 |
| PN1815 | SEQ ID NO:3 |
| PN1957 | SEQ ID NO:4 |

[0059] 已在广范围的金黄色葡萄球菌 (*S.aureus*) 菌株上确定了这些噬菌体的裂解情况。结果显示出本发明的四种噬菌体的广谱活性(特异性活性和总活性)。这四种噬菌体是有尾噬菌体目 (Caudovirales) 的成员。

[0060] 噬菌体PN1137是短尾噬菌体科 (Podoviridae) 的成员,并具有包含17,213个核苷酸的SEQ ID NO:1的核苷酸序列。它对测试的109株金黄色葡萄球菌 (*S.aureus*) 菌株中的46株表现出特异性裂解活性,占所述菌株的42.2%。此外,噬菌体PN1137对测试的109株菌株中的81株表现出总裂解活性,占所述菌株的74.31%。

[0061] 噬菌体PN1493是肌尾噬菌体科 (Myoviridae) 的成员,并具有包含134,876个核苷酸的SEQ ID NO:2的核苷酸序列。它对测试的109株金黄色葡萄球菌 (*S.aureus*) 菌株中的86株表现出特异性裂解活性,占所述菌株的78.9%。此外,噬菌体PN1493对测试的109株菌株中的108株表现出总裂解活性,占所述菌株的99.08%。

[0062] 噬菌体PN1815是肌尾噬菌体科 (Myoviridae) 的成员,并具有包含136,156个核苷酸的SEQ ID NO:3的核苷酸序列。它对测试的109株金黄色葡萄球菌 (*S.aureus*) 菌株中的59株表现出特异性裂解活性,占所述菌株的54.13%。此外,噬菌体PN1815对测试的109株菌株中的108株表现出总裂解活性,占所述菌株的99.08%。

[0063] 噬菌体PN1957是短尾噬菌体科 (Podoviridae) 的成员,并具有包含17,629个核苷酸的SEQ ID NO:4的核苷酸序列。它对测试的109株金黄色葡萄球菌 (*S.aureus*) 菌株中的71株表现出特异性裂解活性,占所述菌株的65.14%。此外,噬菌体PN1957对测试的109株菌株中的95株表现出总裂解活性,占所述菌株的87.16%。

[0064] 这些噬菌体的组合具有覆盖至少74株所述菌株 (67%) 的特异性裂解活性。具体来说,噬菌体PN1493和PN1957一起具有覆盖98株所述金黄色葡萄球菌 (*S.aureus*) 菌株 (89.9%) 的特异性裂解活性和覆盖100%的所述金黄色葡萄球菌 (*S.aureus*) 菌株的总裂解活性。

[0065] 因此,本发明的特定目的在于一种噬菌体,其对金黄色葡萄球菌 (*S.aureus*) 菌株具有裂解活性,并具有包含选自SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少95%同一性、优选地至少96%、97%、98%或99%同一性的序列的基因组。

[0066] 本发明的噬菌体可以通过标准的培养、分离和纯化方法来制备。例如,对金黄色葡

萄球菌 (*S. aureus*) 生产细菌进行培养, 用噬菌体样品感染, 然后进行处理以除去细菌细胞和碎片。可以将富集的噬菌体溶液在带有包埋的金黄色葡萄球菌 (*S. aureus*) 易感宿主菌株的培养基例如琼脂培养基中铺板, 以获得噬斑。然后, 可以挑取出单个噬斑用于随后的噬菌体纯化和扩增。本发明的噬菌体的一个或多个选择性扩增循环例如可以如下进行: 将噬菌体与感受态金黄色葡萄球菌 (*S. aureus*) 混合, 然后添加生长培养基并在所选的试验生长条件下温育。在离心后, 将澄清的扩增上清液通过过滤器过滤, 并进行另一个选择性扩增循环或测试裂解活性的存在。

[0067] 随后, 悬液中噬菌体的滴度和本发明的噬菌体的噬斑形态的可视化, 可以通过已知方法例如通过噬斑计数来评估。此外, 正如在本领域中公知的, 可以通过任何适合的方法将本发明的噬菌体加工成各种不同形式 (液体、冻干等), 以备短期、长期、冷冻或任何其他类型的储存 (参见例如 Clark, 1962)。

[0068] 本发明的噬菌体的裂解活性可以通过本领域中公知的方法例如噬斑测定法 (也称为双琼脂法), 在下述基础上进行评估: 使用潜在的宿主细菌生长所述噬菌体, 然后评估它们杀死所述宿主细菌细胞的能力。在噬斑测定法中, 在软琼脂培养基中温育一段时间后, 噬菌体引起靶金黄色葡萄球菌 (*S. aureus*) 菌株的裂解, 在平板上产生被称为噬斑的清空区。

[0069] 正如将在下文更详细公开的, 本发明的噬菌体可以被培养、扩增、分离、纯化, 并用于例如金黄色葡萄球菌 (*S. aureus*) 介导的疾病的噬菌体疗法中。此外, 可以通过本身在本领域中已知的技术来生产和/或分离这些噬菌体的保留了所述噬菌体的表型特点 (例如裂解活性) 的变体。

[0070] 核酸和多肽

[0071] 本发明涉及被包含在本发明的噬菌体中的核酸或这种核酸的任何片段。术语片段更优选地是指含有开放阅读框 (或由其构成) 的片段。所述核酸可以是 DNA 或 RNA, 可以是单链或双链的。

[0072] 所述核酸可以从保藏的噬菌体分离, 或者使用重组 DNA 技术 (例如聚合酶链反应 (PCR) 扩增、克隆)、酶促合成或化学合成或其组合, 按照本身在本领域中已知的通用技术来生产。还包括其同源序列和片段, 包括但不限于天然等位基因变体和其中核苷酸已被插入、缺失、替换和/或颠倒的修饰的核酸序列。

[0073] 在特定实施方式中, 本发明涉及包含选自 SEQ ID NO: 1-4 任一者的序列或与 SEQ ID NO: 1-4 任一者具有至少 95%、96%、97%、98%、99% 或更高序列同一性的序列的核酸。

[0074] 本发明的核酸可以是游离形式, 或者被克隆在载体例如质粒、病毒载体、表达盒、粘粒等中。

[0075] 另一方面, 本发明还涉及分离的多肽, 其由如上所定义的核酸序列, 优选由选自 SEQ ID NO: 1、SEQ ID NO: 2、SEQ ID NO: 3 或 SEQ ID NO: 4 的核酸序列编码。所述多肽可以通过本身在本领域中已知的技术例如合成、重组技术或其组合来生产。所述多肽可以被分离或纯化, 并用作抗菌剂或作为用于体外分析的试剂。

[0076] 本发明的组合物

[0077] 本发明的一个方面涉及组合物, 其包含至少一种、更优选地至少两种或更多种如上所述的噬菌体, 以及任意的可药用或可兽医用赋形剂。正如所述, 本发明的噬菌体对金黄色葡萄球菌 (*S. aureus*) 菌株具有非常强的裂解活性。可以产生这些噬菌体的组合以扩展宿

主范围并生产高度有效的抗细菌组合物。

[0078] 更具体来说,本发明涉及一种抗细菌组合物,其包含对金黄色葡萄球菌(*S.aureus*)菌株具有裂解活性的至少一种噬菌体,所述至少一种噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少90%同一性,例如与其具有至少91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的序列的基因组的噬菌体。

[0079] 一方面,与具有SEQ ID NO:1-4任一者的序列的参比噬菌体相比,具有包含与SEQ ID NO:1-4任一者具有至少90%同一性,例如与SEQ ID NO:1至4任一者具有至少91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的核苷酸序列的基因组的噬菌体可以具有相同的表型特征。通常,所述噬菌体具有相同的细菌宿主范围。这种噬菌体通常包含例如遗传物质的沉默突变、保守突变、少量缺失和/或少量复制。

[0080] 另一方面,具有包含与SEQ ID NO:1至4任一者具有至少90%同一性,例如与SEQ ID NO:1-4任一者具有至少91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的核苷酸序列的基因组的噬菌体,可能由定向进化(噬菌体训练)产生,并且可能具有不同的细菌宿主范围。通常,所述噬菌体与具有SEQ ID NO:1-4任一者的序列的参比噬菌体相比,对更多细菌菌株有活性。

[0081] 甚至更具体来说,本发明涉及一种抗细菌组合物,其包含对金黄色葡萄球菌(*S.aureus*)菌株具有裂解活性的至少两种噬菌体,所述至少两种噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少90%同一性,例如与其具有至少91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的序列的基因组的噬菌体。

[0082] 在另一个特定实施方式中,本发明的组合物包含至少三种、甚至更优选地至少四种不同的噬菌体,所述噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少90%同一性,例如与其具有至少91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的序列的基因组的噬菌体。

[0083] 本发明的组合物的具体实例包括:

[0084] -具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0085] -具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0086] -具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0087] -具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0088] -具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0089] -具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0090] -具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0091] -具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0092] -具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;或

[0093] -具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,以及具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体。

[0094] 在特定实施方式中,本发明的组合物至少包含:

[0095] -具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0096] -具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;

[0097] -具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体;和

[0098] -具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体。

[0099] 本发明的组合物可以包含如表2中呈现的噬菌体混合物:

[0100] 表2:本发明的噬菌体混合物

| 混合物 | 噬菌体                  |
|-----|----------------------|
| 1   | PN1137+PN1493        |
| 2   | PN1137+PN1815        |
| 3   | PN1137+PN1957        |
| 4   | PN1493+PN1815        |
| 5   | PN1493+PN1957        |
| 6   | PN1815+PN1957        |
| 7   | PN1137+PN1493+PN1815 |
| 8   | PN1137+PN1493+PN1957 |

|    |                             |
|----|-----------------------------|
| 9  | PN1137+PN1815+PN1957        |
| 10 | PN1493+PN1815+PN1957        |
| 11 | PN1137+PN1493+PN1815+PN1957 |

[0102] 本发明的组合物还可以包含其他抗菌剂,特别是具有不同宿主特异性的其他噬菌体。

[0103] 本发明的最优选组合物对由Centre National de Référence des Staphylocoques de Lyon确定的总共109株细菌菌株的组中超过70%的菌株具有裂解性。该保藏中心含有在欧洲和美国具有最高发病率的大量各种不同的金黄色葡萄球菌(*S.aureus*)菌株。

[0104] 本发明的组合物可以包含任何有效量的所选噬菌体。优选地,它们包含 $10^{e1}$ 至 $10^{e12}$ PFU/ml之间、优选地 $10^{e4}$ 至 $10^{e11}$ PFU/ml之间的每种所述噬菌体。本发明的组合物中每种类型噬菌体的相对量可以由专业技术人员调整。通常,当抗菌组合物包含几种( $n$ 种)不同的如上所定义的噬菌体时,组合物中每种噬菌体的总相对量% $A$ 更优选为% $A = (100/n_i) \times V$ ,其中 $n_i$ 表示不同噬菌体的数目, $V$ 是0.2至5之间的变异系数。最优选地, $V$ 在0.3至3之间,甚至更优选地在0.5至2之间,一般在0.8至1.5之间。在优选的典型实施方式中,每种类型的噬菌体以近似相等的相对量存在于本发明的组合物中。

[0105] 本发明的抗菌组合物可以是各种不同形式,例如液体、半液体、固体或冻干制剂。本发明的组合物优选地包含适合的稀释剂或载体,例如可药用或可兽医用赋形剂或载体。除了所选的噬菌体之外,本发明的组合物还可以包括任何赋形剂或载体,例如增稠剂、稀释剂、缓冲剂、防腐剂、表面活性剂等。它们包括对生物体无害或不引起任何显著的特异性或非特异性免疫反应或不废止噬菌体的生物活性的生理上可接受的溶液或介质。对于液体制剂来说,可以使用盐水、无菌水、林格氏溶液、缓冲生理盐水、白蛋白输注液、右旋糖溶液、麦芽糖糊精溶液、甘油、乙醇及其混合物作为可药用或可兽医用赋形剂或载体。如果合适的话,可以添加其他常规添加剂例如增稠剂、稀释剂、缓冲剂、防腐剂、表面活性剂、抗氧化剂和抑菌剂。此外,可以另外向组合物添加稀释剂、分散剂、表面活性剂、粘合剂和润滑剂,以制备可注射制剂例如水性溶液、悬液和乳液,口服制剂例如丸剂、胶囊、颗粒剂或片剂,具有干燥或/和挤出的粉末的粉末制剂,具有液体或干燥气溶胶的气溶胶化制剂。用于表面给药的制剂可以包括绷带、敷料、贴片、薄膜、软膏、洗剂、霜剂、凝胶、滴剂、栓剂、喷雾剂、卫生棉条、卫生巾、液体和粉剂。用于消毒或用于医学用途的制剂也可以包括气溶胶或喷雾剂。

[0106] 本发明的组合物可用于医学领域,包括人类或兽医医学领域,用于例如治疗对象感染或用于改善对象状况。所述组合物可用于减少或杀死生物体中的金黄色葡萄球菌(*S.aureus*)细菌,用于治疗感染。所述组合物也可用于通过改良对象中的微生物菌群来改善所述对象的状况。具体来说,本发明的组合物可以特异性去除对象皮肤或粘膜上的金黄色葡萄球菌(*S.aureus*)菌株,由此改良其微生物菌群并恢复适合的平衡。

[0107] 在特定实施方式中,本发明还涉及用于治疗对象中的感染的方法,所述方法包括向所述对象给药如上所定义的组合物或噬菌体或核酸或多肽。

[0108] 本发明还涉及所描述的噬菌体组合物、核酸或多肽在制造用于治疗对象中的感染或在所述对象中恢复微生物菌群的药物中的用途。

[0109] 本发明的组合物可用于治疗各种不同的金黄色葡萄球菌 (*S.aureus*) 介导的感染,特别是糖尿病或非糖尿病性足部溃疡感染,或骨骼感染例如并且不限于骨髓炎或脓毒性关节炎或关节感染或假体关节感染,或皮肤感染例如并且不限于特应性皮炎、痤疮、脓疱疮、葡萄球菌性烫伤样皮肤综合征,或软组织感染,或胸膜肺部感染,或其他临床综合征例如并且不限于脑膜炎或泌尿道感染或败血症或心内膜炎或耳炎。

[0110] 本发明的组合物可以通过任何方便的途径给药,包括静脉内、口服、透皮、皮下、粘膜、肌内、肺内、鼻内、肠胃外、直肠、阴道和表面。所述噬菌体或组合物也可以通过肺内或鼻内滴注或雾化给药。所述组合物可以直接或间接给药,例如经由支撑物或装置(例如雾化器、绷带……)给药。就此而言,所述组合物可以例如被施用或喷洒到患病区域。本发明的组合物还可以通过口服或肠胃外途径给药。适合于施用、喷洒或给药本发明的组合物的剂量,可以由本领域技术人员根据各种不同因素(包括制剂,给药方式,所治疗的对象在给药时的年龄、体重、性别、状况、饮食,给药途径和反应敏感性)来调整。具有本领域普通技术的医生或兽医可以容易地确定并开出所需的组合物的有效量。

[0111] 剂量也可以由专业技术人员调整,以便获得针对抗生素耐药性金黄色葡萄球菌 (*S.aureus*) 菌株的裂解活性。取决于给药途径,在体内获得裂解活性的有效剂量通常包括至少 $10^4$  PFU/ml、优选地约 $10^2$ 至 $10^{12}$  PFU/ml的浓度。

[0112] 在特定实施方式中,本发明的噬菌体、组合物和混合物被用于治疗糖尿病非糖尿病性足部溃疡感染、骨骼和关节感染、假体关节感染或呼吸道感染。

[0113] 在例如被甲氧西林耐药性或敏感性金黄色葡萄球菌 (*S.aureus*) (MRSA或MSSA) 感染的糖尿病性足部溃疡的情况下,患者可以接受用 $10^4$ 至 $10^{10}$  PFU/ml的噬菌体溶液浸渍的敷料,施用频率在每天至每十天之间,例如在每两天至每九天之间,优选地在每三天至每八天之间,更优选地每七天,同时使用或不使用抗生素,直至伤口闭合。所述治疗的功效可以通过细菌负荷的相对降低来度量。

[0114] 在例如髌或膝的复发性金黄色葡萄球菌 (*S.aureus*) (MRSA或MSSA) 假体关节感染的情况下,患者可以接受与使用和不使用抗生素的标准手术联合的噬菌体疗法。可以在外植结束时将含量为 $10^4$ 至 $10^{10}$  PFU/ml的噬菌体溶液散布在骨桶、关节间隙和/或肌肉组织中的手术视野中。在刚刚重新植入后和手术位点闭合之前,可以使用第二剂同样的制剂进行第二次散布。在伤口仍然开放时,例如在给伤口敷料时,可以进行额外的施用。可以使用 $10^4$ 至 $10^{10}$  PFU/ml的补充性口服噬菌体给药来支持局部治疗。

[0115] 在具有金黄色葡萄球菌 (*S.aureus*) (MRSA或MSSA) 感染的骨骼的整形手术的情况下,在骨骼刮治后可以在手术位点底部处放置吸收有 $10^4$ 至 $10^{10}$  PFU/ml噬菌体的压布,与所述被感染的刮治骨骼相接触。空腔的其余部分可以用其他无菌压布填充,并将手术位点用防水敷料遮蔽。此外可以使用真空辅助封闭 (VAC) 系统,遵从引流以从伤口或手术位点移除血液或浆液,然后进行使用或不使用抗生素的噬菌体滴注( $10^4$ 至 $10^{10}$  PFU/ml)的循环。在引流重新开始之前几小时期间,噬菌体保留。所述循环可以在手术后数天内重复几次。

[0116] 在呼吸道感染的情况下,可以使用雾化装置施用 $10^4$ 至 $10^{10}$  PFU/ml的噬菌体溶液。雾化可以使用便携式吸入器或使用连接到医疗机械通气机的附加雾化器来进行。可以将例如在1至20ml范围内的噬菌体溶液体积以各种不同的时间间隔并在治疗期间雾化。在开始第一次雾化之前并根据病情,可以使用相同的噬菌体溶液进行肺清洗。

[0117] 正如在实验部分中所示,本发明的噬菌体和组合物能够有效杀死广范围的金黄色葡萄球菌 (*S.aureus*) 细菌。所述组合物甚至在低剂量下也可以毁灭不同金黄色葡萄球菌 (*S.aureus*) 细菌的混合物。此外,本发明的组合物严格地不能影响真核细胞,因此是特异的并且在施用到人类和动物时没有副作用。

[0118] 本发明还涉及本发明的组合物、噬菌体、核酸或多肽在对材料进行消毒中的用途。由于它们的强力抗细菌效果和它们甚至损害细菌生物膜的完整性的能力,本发明的组合物可用作消毒剂,以消除材料上的细菌或至少引起细菌数量减少。这样的方法可应用于医学和非医学两种背景中各种不同的生物或非生物表面的处理,包括固体材料或装置例如隐形眼镜、待植入到身体内的装置的表面、管道、导管、实验室容器、织物、衣物、鞋类、住宅、军事装备等。

[0119] 本发明还涉及一种用于制备本发明的组合物的方法,其中所述组合物包含至少两种噬菌体,所述方法包括分开地生产所述至少两种噬菌体,以及将所述噬菌体与适合的载体或赋形剂组合。

[0120] 本发明的组合物可以与至少一种抗生素组合使用。这种共同给药允许降低使用的抗生素的量、恢复抗生素的功效或使包埋在生物膜中的细菌对抗生素敏感。

[0121] 本发明的诊断/预测试验-伴随诊断:

[0122] 本发明还涉及一种用于预测或确定噬菌体疗法在对象中的功效的方法,其中所述方法包括确定本发明的一种或多种噬菌体对来自于所述对象的样品的金黄色葡萄球菌 (*S.aureus*) 菌株的裂解活性的步骤,这种裂解活性指示高效的治疗。在优选情况下,所述方法还任选地包括用对来自于所述对象的样品的金黄色葡萄球菌 (*S.aureus*) 菌株具有裂解活性的一种或多种噬菌体治疗所述对象的步骤。

[0123] 另一方面,本发明提供了一种用于选择对象或确定对象是否易于从噬菌体疗法获益的方法,其中所述方法包括确定本发明的一种或多种噬菌体对来自于所述对象的样品的金黄色葡萄球菌 (*S.aureus*) 菌株的裂解活性的步骤,本发明的一种或多种噬菌体对至少一种金黄色葡萄球菌 (*S.aureus*) 菌株的裂解活性指示响应者对象。

[0124] 本发明的另一个目的涉及一种用于预测对象对噬菌体疗法的响应的方法,其中所述方法包括确定本发明的一种或多种噬菌体对来自于所述对象的样品的金黄色葡萄球菌 (*S.aureus*) 菌株的裂解活性的步骤,本发明的一种或多种噬菌体对至少一种金黄色葡萄球菌 (*S.aureus*) 菌株的裂解活性指示对所述疗法的良好响应。

[0125] 另一方面,本发明提供了一种用于评估金黄色葡萄球菌 (*S.aureus*) 菌株对噬菌体的敏感性的方法,所述噬菌体选自具有包含SEQ ID NO:1至4任一者的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体和/或表2的至少一种噬菌体混合物,所述方法包括:

[0126] a) 将所述金黄色葡萄球菌 (*S.aureus*) 菌株与所述至少一种噬菌体和/或所述至少一种噬菌体混合物相接触,以及

[0127] b) 确定所述噬菌体和/或混合物对所述菌株的裂解活性,由此评估所述菌株对所述噬菌体和/或混合物的敏感性。

[0128] 步骤a) 可以在体外,通过使金黄色葡萄球菌 (*S.aureus*) 菌株与所述至少一种噬菌体和/或至少一种噬菌体混合物在适合于这个步骤的任何介质(例如固体或液体介质)中发



生接触、暴露或将它们混合,来进行。

[0129] 在优选实施方式中,所述噬菌体和/或混合物的裂解活性的确定包括测量所述噬菌体在所述金黄色葡萄球菌 (*S.aureus*) 菌株中的扩增,其中所述噬菌体浓度的提高指示所述噬菌体和/或混合物对所述菌株的裂解活性。

[0130] 噬菌体浓度可以通过本领域技术人员公知的任何技术例如噬斑形成、多态性链反应 (PCR)、生物发光等来测量。

[0131] 此外或可选地,所述噬菌体和/或混合物的裂解活性的确定包括在噬斑测定法中确定噬斑影。

[0132] 然而,当在细菌的固体培养基培养物中,在噬菌体存在下观察到细菌垫的完全或部分裂解时,所述细菌菌株也可以被认为是对所述噬菌体敏感的。

[0133] 可以使用本领域技术人员公知的其他技术来评估所述噬菌体和/或混合物的裂解活性。

[0134] 本发明的另一个目的涉及一种用于确定有效针对靶金黄色葡萄球菌 (*S.aureus*) 菌株的噬菌体混合物的方法,所述方法包括:

[0135] a) 将所述靶金黄色葡萄球菌 (*S.aureus*) 菌株或含有所述菌株的样品分开地与下述噬菌体接触: (i) 具有包含SEQ ID NO:1的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和/或 (ii) 具有包含SEQ ID NO:2的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和/或 (iii) 具有包含SEQ ID NO:3的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和/或 (iv) 具有包含SEQ ID NO:4的核苷酸序列或与其具有至少90%同一性的序列的基因组的噬菌体,和/或 (v) 这些噬菌体的一种或多种组合;

[0136] b) 选择对所述菌株表现出裂解活性的噬菌体,

[0137] c) 任选地进一步选择当组合在一起时对所述菌株表现出协同活性的活性噬菌体;和/或

[0138] d) 任选地进一步选择当组合在一起时不表现出拮抗作用的活性噬菌体;和/或

[0139] e) 任选地选择属于不同属的活性噬菌体;和

[0140] f) 将所述选择的噬菌体组合。

[0141] 这种方法可用作噬菌体测量法 (phagogramme), 以确定哪种混合物对特定金黄色葡萄球菌 (*S.aureus*) 菌株具有最佳裂解活性。

[0142] 本发明的其他方面和优点将在下面的实验部分中公开,所述实验部分仅仅是说明性的。

## 实施例

[0143] 方法

[0144] 噬菌体科的分类鉴定

[0145] 使用电子显微术研究本发明的四种噬菌体 (PN1137、PN1493、PN1815和PN1957)。结果显示所述噬菌体属于有尾噬菌体目 (Caudovirales) 并属于短尾噬菌体科 (Podovirae) 和肌尾噬菌体科 (Myoviridae), 如下所述:

[0146] -PN1137:短尾噬菌体科 (Podovirae),

[0147] -PN1483:肌尾噬菌体科 (Myoviridae),

[0148] -PN1815:肌尾噬菌体科 (Myoviridae),

[0149] -PN1957:短尾噬菌体科 (Podovirae)。

[0150] 噬菌体基因组的测序和分析

[0151] 通过用苯酚:氯仿:异戊醇 (25:24:1, V/V) 提取、乙醇沉淀和重新溶解在水中,来分离噬菌体DNA。进行全基因组测序,并使用BLAST算法来确定与国家生物技术信息中心 (National Center for Biotechnology Information) (NCBI) 数据库中所描述的基因的相似性。扫描基因组以获得潜在的开放阅读框 (ORF)。所述核酸序列被显示为SEQ ID NO:1-4。使用数据库的序列对齐产生与使用电子显微术相同的分类鉴定。

[0152] 实施例1:体外表征:宿主范围

[0153] 可能存在两种不同类型的细菌裂解。由细菌细胞壁被噬菌体的酶水解引起的细菌裂解(被称为“非特异性”裂解)和由噬菌体扩增引起的细菌裂解(被称为“特异性”裂解)。两种类型的裂解都与噬菌体的效用相关。对于每种噬菌体,使用两种方法确定了特异性和非特异性裂解两者。

[0154] 固体培养基中的噬菌体扩增

[0155] 这种方法允许评估噬菌体通过扩增裂解细菌的能力。

[0156] 所述方法包括将噬菌体悬液的多种稀释液(比率为10)沉积在含有细菌的琼脂表面处。然后将该琼脂在37℃温育18h,允许细菌垫发育。分析沉积区域。可能出现下述情况:

[0157] -情况1:在所述沉积区域处不存在所述细菌垫的裂解,

[0158] -情况2:在所述沉积区域处所述细菌垫完全裂解,

[0159] -情况3:在所述沉积区域处所述细菌垫部分裂解,和

[0160] -情况4:在所述沉积区域处观察到噬斑(噬斑形成单位(PFU))。

[0161] 只有噬斑的形成(情况4)才能证实噬菌体在所述细菌菌株中扩增并有效裂解所述细菌的能力。

[0162] 如果观察到情况1、2和/或3而不存在情况4,则在液体培养基中测试所述菌株。

[0163] 液体培养基中的噬菌体扩增

[0164] 这种方法允许评估细菌生产噬菌体的能力。

[0165] 所述方法包括将细菌菌株和噬菌体在37℃下培养18h。然后确定上清液中的噬菌体浓度。如果培养后所述噬菌体的浓度高于初始噬菌体浓度,这表明所述噬菌体已被所述细菌扩增。

[0166] 细菌生产噬菌体的能力尤其取决于感染复数(MOI),即噬菌体/细菌比率。测试了几种MOI。

[0167] 使用与斑点试验类似的方法来确定培养后的噬菌体滴度。将培养上清液的多种稀释液(比率为10)一方面沉积在含有所述噬菌体的生产菌株的琼脂表面上,另一方面沉积在含有患者菌株的琼脂表面上。然后将该琼脂在37℃温育18h。与稀释倍数相关联的沉积区域上的噬斑数目允许确定噬菌体浓度。

[0168] 特异性活性

[0169] 评估了特异性活性谱,即每种噬菌体通过扩增裂解一组菌株的能力。当出现下述情况时,认为噬菌体被细菌菌株扩增:

[0170] -在固体培养基试验中观察到情况4,和

[0171] -在液体培养基试验中,培养后的噬菌体浓度相对于初始浓度提高50倍。

[0172] 所述一组金黄色葡萄球菌 (*S.aureus*) 由Centre National de Référence des Staphylocoques de Lyon确定。它包含在欧洲和美国具有最高发病率的109株金黄色葡萄球菌 (*S.aureus*) 菌株。

[0173] 在下面的表3中,用“+”表示本发明的噬菌体能够被细菌菌株扩增,并且用“-”表示噬菌体不能被细菌菌株扩增。

[0174] 表3:PN1137、PN1493、PN1815和PN1957对一组109株金黄色葡萄球菌 (*S.aureus*) 菌株的特异性活性谱

[0175]

| #  | 金黄色葡萄球菌 ( <i>S. aureus</i> ) 菌株 | PN1137 | PN1493 | PN1815 | PN1957 |
|----|---------------------------------|--------|--------|--------|--------|
| 1  | HOCIL001                        | +      | +      | +      | +      |
| 2  | HOCIL002                        | -      | +      | +      | -      |
| 3  | HOCIL003                        | +      | +      | +      | +      |
| 4  | HOCIL004                        | +      | +      | +      | +      |
| 5  | HOCIL005                        | +      | +      | +      | +      |
| 6  | HOCIL006                        | -      | +      | +      | +      |
| 7  | HOCIL007                        | -      | +      | -      | +      |
| 8  | HOCIL008                        | +      | +      | +      | +      |
| 9  | HOCIL009                        | +      | -      | -      | +      |
| 10 | HOCIL010                        | -      | -      | -      | +      |
| 11 | HOCIL011                        | -      | +      | +      | -      |
| 12 | HOCIL012                        | +      | +      | +      | +      |
| 13 | HOCIL013                        | -      | +      | +      | +      |
| 14 | HOCIL014                        | +      | +      | +      | +      |
| 15 | HOCIL015                        | +      | +      | +      | +      |
| 16 | HOCIL016                        | +      | +      | -      | +      |
| 17 | HOCIL017                        | +      | +      | +      | +      |
| 18 | HOCIL018                        | +      | +      | +      | +      |
| 19 | HOCIL020                        | -      | +      | +      | -      |
| 20 | HOCIL022                        | +      | +      | +      | +      |
| 21 | HOCIL093                        | -      | +      | -      | +      |
| 22 | HOCIL095                        | +      | +      | +      | -      |

[0176]

|    |          |   |   |   |   |
|----|----------|---|---|---|---|
| 23 | HOCIL099 | + | + | + | + |
| 24 | HOCIL106 | - | + | - | - |
| 25 | HOCIL117 | - | - | - | + |
| 26 | HOCIL118 | - | - | - | - |
| 27 | HOCIL124 | - | - | - | + |
| 28 | HOCIL129 | - | - | - | + |
| 29 | HOCIL137 | - | - | - | - |
| 30 | HOCIL139 | - | + | + | + |
| 31 | HOCIL140 | - | - | - | + |
| 32 | HOCIL145 | + | + | + | + |
| 33 | HOCIL148 | - | + | - | - |
| 34 | HOCIL155 | + | + | - | + |
| 35 | HOCIL156 | + | + | - | + |
| 36 | HOCIL161 | - | + | + | - |
| 37 | HOCIL163 | - | - | - | - |
| 38 | HOCIL165 | + | + | + | + |
| 39 | HOCIL167 | + | - | - | + |
| 40 | HOCIL172 | + | + | - | + |
| 41 | HOCIL174 | + | + | - | + |
| 42 | HOCIL175 | - | - | + | + |
| 43 | HOCIL176 | - | - | - | - |
| 44 | HOCIL183 | + | + | - | + |
| 45 | HOCIL184 | + | + | - | + |
| 46 | HOCIL185 | + | + | + | + |
| 47 | HOCIL186 | - | + | - | + |
| 48 | HOCIL187 | - | + | - | - |
| 49 | HOCIL188 | + | + | + | + |
| 50 | HOCIL189 | - | + | - | - |
| 51 | HOCIL190 | - | + | + | + |
| 52 | HOCIL191 | + | + | + | + |

[0177]

|    |          |   |   |   |   |
|----|----------|---|---|---|---|
| 53 | HOCIL192 | - | - | - | + |
| 54 | HOCIL193 | + | + | + | + |
| 55 | HOCIL194 | + | + | + | + |
| 56 | HOCIL195 | - | - | - | - |
| 57 | HOCIL196 | - | - | - | - |
| 58 | HOCIL197 | + | + | + | + |
| 59 | HOCIL198 | + | + | + | + |
| 60 | HOCIL199 | - | + | - | + |
| 61 | HOCIL200 | - | + | + | + |
| 62 | HOCIL201 | + | + | - | - |
| 63 | HOCIL202 | - | - | - | - |
| 64 | HOCIL203 | + | + | + | + |
| 65 | HOCIL204 | + | + | + | + |
| 66 | HOCIL205 | + | + | + | + |
| 67 | HOCIL206 | + | + | + | - |
| 68 | HOCIL207 | + | + | + | + |
| 69 | HOCIL210 | - | + | - | - |
| 70 | HOCIL211 | - | + | + | + |
| 71 | HOCIL212 | + | + | + | + |
| 72 | HOCIL213 | - | + | + | + |
| 73 | HOCIL214 | - | + | - | + |
| 74 | HOCIL215 | - | - | - | + |
| 75 | HOCIL216 | - | + | + | + |
| 76 | HOCIL217 | - | - | - | - |
| 77 | HOCIL218 | - | + | - | + |
| 78 | HOCIL219 | + | + | + | + |
| 79 | HOCIL220 | - | - | - | + |
| 80 | HOCIL221 | - | + | + | - |
| 81 | HOCIL222 | - | + | + | - |
| 82 | HOCIL223 | - | + | + | - |

[0178]

|     |          |   |   |   |   |
|-----|----------|---|---|---|---|
| 83  | HOCIL224 | - | + | - | - |
| 84  | HOCIL225 | - | + | + | - |
| 85  | HOCIL226 | + | + | + | + |
| 86  | HOCIL227 | - | + | + | + |
| 87  | HOCIL228 | - | + | + | + |
| 88  | HOCIL229 | + | + | + | + |
| 89  | HOCIL230 | - | + | + | + |
| 90  | HOCIL231 | - | + | - | - |
| 91  | HOCIL232 | - | + | + | - |
| 92  | HOCIL233 | - | + | - | + |
| 93  | HOCIL234 | - | + | - | - |
| 94  | HOCIL235 | - | + | - | + |
| 95  | HOCIL236 | - | + | + | + |
| 96  | HOCIL237 | + | + | - | + |
| 97  | HOCIL238 | + | + | + | + |
| 98  | HOCIL239 | - | - | - | - |
| 99  | HOCIL240 | + | - | - | + |
| 100 | HOCIL241 | - | + | + | - |
| 101 | HOCIL242 | - | + | + | - |
| 102 | HOCIL243 | - | + | - | - |
| 103 | HOCIL244 | - | + | - | - |
| 104 | HOCIL245 | - | - | - | - |
| 105 | HOCIL246 | - | + | + | - |
| 106 | HOCIL248 | - | - | - | - |
| 107 | HOCIL249 | + | + | - | + |
| 108 | HOCIL250 | + | + | + | - |
| 109 | HOCIL251 | - | + | + | - |

[0179] PN1493和PN1957分别对所述109株金黄色葡萄球菌 (*S.aureus*) 菌株中的86和71株表现出特异性活性, 分别占78.9%和65.14%。

[0180] PN1137和PN1815也呈现出广范围的特异性活性。它们分别对46和59株所述菌株表现出特异性活性, 分别占42.2%和54.13%。

[0181] 总裂解活性

[0182] 已评估了总裂解活性谱, 即每种噬菌体在具有或不具有噬菌体生产的情况下裂解

菌株的能力。在下述情况下,认为所述噬菌体对所述金黄色葡萄球菌 (*S.aureus*) 菌株有活性:

[0183] -在固体培养基试验中观察到情况2、3或4,或

[0184] -在液体培养基试验中,培养后的噬菌体浓度与初始浓度相比提高50倍。

[0185] 在下面的表5中,用“+”表示噬菌体对细菌菌株的阳性裂解活性,并用“-”表示噬菌体对细菌菌株不存在裂解活性。

[0186] 表5:PN1137、PN1493、PN1815和PN1957对一组109株金黄色葡萄球菌 (*S.aureus*) 菌株的总活性谱

[0187]

| #  | 金黄色葡萄球菌 ( <i>S.aureus</i> ) 菌株 | PN1137 | PN1493 | PN1815 | PN1957 |
|----|--------------------------------|--------|--------|--------|--------|
| 1  | HOCIL001                       | +      | +      | +      | +      |
| 2  | HOCIL002                       | +      | +      | +      | +      |
| 3  | HOCIL003                       | +      | +      | +      | +      |
| 4  | HOCIL004                       | +      | +      | +      | +      |
| 5  | HOCIL005                       | +      | +      | +      | +      |
| 6  | HOCIL006                       | +      | +      | +      | +      |
| 7  | HOCIL007                       | +      | +      | +      | +      |
| 8  | HOCIL008                       | +      | +      | +      | +      |
| 9  | HOCIL009                       | +      | +      | +      | +      |
| 10 | HOCIL010                       | +      | +      | +      | +      |
| 11 | HOCIL011                       | +      | +      | +      | +      |

[0188]

|    |          |   |   |   |   |
|----|----------|---|---|---|---|
| 12 | HOCIL012 | + | + | + | + |
| 13 | HOCIL013 | + | + | + | + |
| 14 | HOCIL014 | + | + | + | + |
| 15 | HOCIL015 | + | + | + | + |
| 16 | HOCIL016 | + | + | + | + |
| 17 | HOCIL017 | + | + | + | + |
| 18 | HOCIL018 | + | + | + | + |
| 19 | HOCIL020 | + | + | + | - |
| 20 | HOCIL022 | + | + | + | + |
| 21 | HOCIL093 | + | + | + | + |
| 22 | HOCIL095 | + | + | + | + |
| 23 | HOCIL099 | + | + | + | + |
| 24 | HOCIL106 | + | + | + | + |
| 25 | HOCIL117 | + | + | + | + |
| 26 | HOCIL118 | + | + | + | + |
| 27 | HOCIL124 | + | + | + | + |
| 28 | HOCIL129 | + | + | + | + |
| 29 | HOCIL137 | + | + | + | + |
| 30 | HOCIL139 | + | + | + | + |
| 31 | HOCIL140 | + | + | + | + |
| 32 | HOCIL145 | + | + | + | + |
| 33 | HOCIL148 | + | + | + | + |
| 34 | HOCIL155 | + | + | + | + |
| 35 | HOCIL156 | + | + | + | + |
| 36 | HOCIL161 | + | + | + | + |
| 37 | HOCIL163 | + | + | + | + |
| 38 | HOCIL165 | + | + | + | + |
| 39 | HOCIL167 | + | + | + | + |
| 40 | HOCIL172 | + | + | + | + |
| 41 | HOCIL174 | + | + | + | + |



[0189]

|    |          |   |   |   |   |
|----|----------|---|---|---|---|
| 42 | HOCIL175 | + | + | + | + |
| 43 | HOCIL176 | + | + | + | + |
| 44 | HOCIL183 | + | + | + | + |
| 45 | HOCIL184 | + | + | + | + |
| 46 | HOCIL185 | + | + | + | + |
| 47 | HOCIL186 | + | + | + | + |
| 48 | HOCIL187 | + | + | + | + |
| 49 | HOCIL188 | + | + | + | + |
| 50 | HOCIL189 | + | + | + | + |
| 51 | HOCIL190 | + | + | + | + |
| 52 | HOCIL191 | + | + | + | + |
| 53 | HOCIL192 | - | - | - | + |
| 54 | HOCIL193 | + | + | + | + |
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[0192] 这些结果显示测试的四种噬菌体的非常广泛的活性谱,它们都对所述109株金黄色葡萄球菌 (*S.aureus*) 菌株中超过70%的菌株表现出裂解活性。具体来说,噬菌体 PN1493和PN1815显示出非常好的结果,对所述109株金黄色葡萄球菌 (*S.aureus*) 菌株中的108株具有活性,占所述组的菌株的超过99%。PN1137和PN1957也表现出广范围的裂解活性。它们分别对来自于所述组的菌株中的81和95株有效(分别为74.31%和87.16%)。来自于所述组的每一株金黄色葡萄球菌 (*S.aureus*) 菌株被至少一种所述噬菌体裂解。此外,所述菌株中的79株被所述四种噬菌体裂解。

[0193] 实施例II:混合物的生产

[0194] 混合物5:PN1493和PN1957

[0195] 这两种噬菌体具有互补的裂解活性范围。事实上,它们合在一起对测试的金黄色葡萄球菌 (*S.aureus*) 菌株中的98株表现出特异性活性,占所述菌株的89.9%。此外,它们的总裂解活性覆盖所有测试的金黄色葡萄球菌 (*S.aureus*) 菌株。优选的混合物包含比例为1:1的这两种噬菌体。

[0196] 混合物6:PN1815和PN1957

[0197] 与混合物5相同,这两种噬菌体具有互补的裂解活性范围。它们合在一起对测试的金黄色葡萄球菌 (*S.aureus*) 菌株中的87株表现出特异性活性(79.8%)。此外,它们的总裂解活性覆盖所有测试的金黄色葡萄球菌 (*S.aureus*) 菌株。优选的混合物包含比例为1:1的这两种噬菌体。

[0198] 混合物1 (PN1137和PN1493) 和混合物4 (PN1493和PN1815)

[0199] 这两种混合物也对测试的细菌菌株表现出广范围的裂解活性。混合物1和4分别对所述菌株中的89和87株具有特异性裂解活性(分别为81.6%和79.8%)。此外,它们两者都对所述菌株中的108株表现出总裂解活性,代表了对测试的金黄色葡萄球菌 (*S.aureus*) 菌株中超过99%的菌株的裂解活性。优选的混合物包含比例为1:1的所述两种噬菌体。

[0200] 混合物3、5、6、8、9、10和11

[0201] 这些混合物对100%的所述菌株表现出总裂解活性。

[0202] 实施例II:体内功效

[0203] 使用两种小鼠模型来评估本发明的噬菌体混合物的功效。

[0204] 第一种模型重现了糖尿病或非糖尿病性足部溃疡的金黄色葡萄球菌 (*S.aureus*) 感染。它基于天然小鼠 (naive mice) 或糖尿病小鼠的金黄色葡萄球菌 (*S.aureus*) 后爪感

染。

[0205] 第二种模型重现了金黄色葡萄球菌 (*S.aureus*) 骨骼和关节感染 (BJI) 以及假体关节感染 (PJI)。

[0206] 糖尿病性足部溃疡模型 (DFU)

[0207] 使用链脲佐菌素诱导糖尿病。所述小鼠的糖尿病状态是基于所述诱导后7天血糖在5g/l左右和受损的金黄色葡萄球菌 (*S.aureus*) 血液灭杀测定法。

[0208] 金黄色葡萄球菌 (*S.aureus*) 菌株

[0209] 在菌株原位留存和繁殖能力的基础上选择从假体感染分离的临床菌株。最佳接种物基于具有最高存活率的糖尿病小鼠中感染后 (p.i.) 14天的持续细菌负载来选择。

[0210] 实验设计

[0211] 通过间隔48h的两次链脲佐菌素注射来诱导糖尿病。一只后爪在第一次链脲佐菌素注射后14天进行感染。对四个组进行比较：

[0212] • 噬菌体混合物治疗组。在感染后30min以1的感染复数 (MOI 1) 局部给药 (SC注射) 所述混合物。

[0213] • 噬菌体混合物治疗组。在感染后30min以MOI 10局部给药 (SC注射) 所述混合物。

[0214] • 抗生素治疗组。在感染后30min以25mg/kg系统性给药 (IP注射) 利奈唑胺。

[0215] • 未治疗组。在感染后30min局部给药 (SC注射) PBS。

[0216] 研究了每组30只小鼠。

[0217] 在感染后的6个时间点,将小鼠安乐死,对被感染的后爪的软组织和骨骼中的菌落形成单位 (CFU) 和噬斑形成单位 (PFU) 的数目进行计数。水肿和病灶的宏观观察按照Chhibber等人中提到的进行评分。确定髓过氧化物酶 (MPO) 水平以跟踪炎症反应,并在第5天进行后爪的组织学分析以评估4个组之间的炎症反应差异。

[0218] 这个模型允许评估本发明的噬菌体或噬菌体混合物对糖尿病性足部溃疡的金黄色葡萄球菌 (*S.aureus*) 感染的功效。

[0219] 非糖尿病性足部溃疡模型

[0220] 金黄色葡萄球菌 (*S.aureus*) 菌株

[0221] 将在糖尿病性足部溃疡模型中使用的菌株也用于非糖尿病性足部溃疡模型。使用相同的接种物。

[0222] 实验设计

[0223] 在天然小鼠中对一只后爪进行感染。在糖尿病性足部溃疡模型中比较的组也在非糖尿病性足部溃疡模型中进行研究。研究了同样的标准判据。

[0224] 这个模型允许评估本发明的噬菌体或噬菌体混合物对非糖尿病性足部溃疡的金黄色葡萄球菌 (*S.aureus*) 感染的功效。

[0225] 骨骼和关节感染模型

[0226] 金黄色葡萄球菌 (*S.aureus*) 菌株

[0227] 在菌株原位留存和繁殖能力的基础上选择从假体感染分离的临床菌株。最佳接种物基于具有最高存活率的感染后7天的持续细菌负载来选择。

[0228] 实验设计

[0229] 天然小鼠通过膝进行感染。对7个组进行比较：

- [0230] • 噬菌体混合物治疗组。将所述混合物在感染后30min以高剂量系统性给药。
- [0231] • 噬菌体混合物治疗组。将所述混合物在感染后30min以低剂量系统性给药。
- [0232] • 噬菌体混合物治疗组。将所述混合物在感染后30min以高剂量局部给药。
- [0233] • 噬菌体混合物治疗组。将所述混合物在感染后30min以低剂量局部给药。
- [0234] • 抗生素治疗组。
- [0235] • 抗生素+噬菌体治疗组。
- [0236] • 未治疗组。
- [0237] 研究了每组30只小鼠。
- [0238] 随着感染过程跟踪细菌负载和噬菌体负载、小鼠的临床状态例如体重、食物和水消耗量。
- [0239] 这个模型允许评估本发明的噬菌体或噬菌体混合物对金黄色葡萄球菌 (*S.aureus*) 骨骼和关节感染的功效。
- [0240] 假体关节感染模型
- [0241] 金黄色葡萄球菌 (*S.aureus*) 菌株
- [0242] 所述菌株以及接种物与BJI模型相同。
- [0243] 实验设计
- [0244] 将克氏针插入到天然小鼠的股骨腔中。然后将小鼠通过与克氏针相邻的膝进行感染。组比较和读出数据与骨骼和关节感染模型相似。
- [0245] 这个模型允许评估本发明的噬菌体或噬菌体混合物对金黄色葡萄球菌 (*S.aureus*) 假体关节感染的功效。
- [0246] 参考文献
- [0247] Capparelli R等, *Antimicrob Agents Chemother.*, 小鼠中针对金黄色葡萄球菌的实验性噬菌体疗法 (Experimental Phage Therapy against *Staphylococcus aureus* in Mice), 2007Aug;51 (8):2765-2773。
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- [0249] Drulis-Kawa Z, Majkowska-Skrobek G, Maciejewska B, Delattre AS, Lavigne R, 2012, 从噬菌体学到的——噬菌体和噬菌体编码的蛋白质的应用的优点和限制 (Learning from bacteriophages-advantages and limitations of phage and phage-encoded protein applications), 13 (8):699-722。
- [0250] Weinbauer MG., 原核病毒的生态学 (Ecology of prokaryotic viruses), *FEMS Microbiol Rev* 2004;28:127-81。

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| [0077] | tgcaatatca  | taaacatgaa  | cgtaaaatga  | tattgtattg  | ggatattgaa  | acattatcat  | 3840 |
| [0078] | acaataaaa   | aaacggacgt  | aataaaacca  | cgctatataa  | aaacgtaacg  | tattcagtag  | 3900 |
| [0079] | cgattgggtg  | gtttaatggt  | tacgaaattg  | atgttgaagt  | attccctagt  | tttgaagctt  | 3960 |
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| [0081] | atattatcat  | gattgcacat  | aactgtaata  | agtatgacaa  | ccatttttta  | cttaaagata  | 4080 |
| [0082] | ccatgcgtta  | ttttgataat  | atcacacgtg  | aaaatatata  | tttaaaatct  | gcagaagaaa  | 4140 |
| [0083] | acgaacacac  | actaaaaatg  | caagaggcta  | ctatttttagc | taaaaatcaa  | aatgtgattt  | 4200 |

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| [0124] | aattgctttt | ttatattttt  | ctggtaaactg | tgcatacgtc | cagttaccac | caatgacgcg | 6660 |
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| [0170] | gtgcgttttg  | ataagtgtg  | acgttcaata  | ttaacgtttg | agagttgctc  | taatacatta  | 9420  |
| [0171] | ccttggtgat  | acgtcataat | agtatcaatc  | acaaaatata | tttttaccac  | aacatcattc  | 9480  |
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| [0176] | aacggtgtgt  | tatagaaaaa | tttaaaattt  | gttaattttc | tcattttttac | ctccataaaa  | 9780  |
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| [0212] | gtgtttttaa gttatatgtg aaacttctta ttagtttcat ctttttctt aatcctgagt   | 11940 |
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| [0347] | aataaaacta aggtttataa aaaaaatta ttcttttact agttgacaag taatcttttc   | 2400 |
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| [0617] | gattaggtgg | tgaatactgt  | ttggcaatga  | atgacgatta  | taggttggtc | ttgactggtg | 18600 |
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| [0620] | aagtaaaatc | tgtacaaaac  | attatggata  | aacaaagtga  | taagtcttta | atatcaccaa | 18780 |
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| [0787] | agtaatacat acaaaactat aaccaatggt aatacacacg ataataaat tacaatggt     | 28800 |
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| [0797] | tctaacttac cagtattata taatacttcc tatctaacaa gtacagatat taacaaaggt   | 29400 |



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| [0807] | gatggtacta | aaacaggaaa | acaaaaata  | gaggaaactac | aagaacaagt | agaagcatat  | 30000 |
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| [0922] | gtagatgctg gtaaggcata tgtaaaaggt tataaagtag ataaaccagt ttctaccaga  | 36900 |
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| [0926] | tattttatcta | ataaaacagc  | ttttgaagta    | gtagagtagat   | ggacagaaac  | aagtccaggt  | 37140 |
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| [0950] | aagactgtac  | aagatgttat  | tatcaaaaca    | cgtgttacag    | taaacttagt  | agaccatta   | 38580 |
| [0951] | gcacaatcat  | tccagtatga  | tgaaagtaga    | actattagtt    | cttttaggtt  | atactttgct  | 38640 |
| [0952] | agtaaaggag  | ataagtctag  | taatgttaact   | gtacaaattc    | gtggtatggg  | agatacaggt  | 38700 |
| [0953] | taccctaaca  | agacggtata  | cgctgaaaca    | gtaatcaact    | cagatgatata | taaagtatct  | 38760 |
| [0954] | aataatgcta  | gtgctgaaac  | tagagtatac    | tttgatgacc    | ctatgatggc  | tgaagcaggt  | 38820 |
| [0955] | aaagagtatg  | ctattgtcat  | tattacagaa    | aatagtgatt    | atactatgtg  | ggtcggaaca  | 38880 |
| [0956] | agaacaaaac  | ctaagattga  | taaaccta      | at gaagttatta | gtggtaatcc  | atatgtacaa  | 38940 |
| [0957] | ggcgttctat  | ttagttcatc  | taacgctagt    | acttggaactc   | cacacaaaa   | ttcagattta  | 39000 |
| [0958] | aaatttggtg  | tatatacttc  | taagttta      | at gaaacagcta | caattgaatt  | tgaacctatt  | 39060 |
| [0959] | aagaatgtat  | cagcggatag  | attagtttta    | atgtctacgt    | acttaactcc  | tgaagaaca   | 39120 |
| [0960] | ggatgtactt  | gggaaattaa  | aatgatttta    | gatgacatgg    | ctacatctac  | tacatttgac  | 39180 |
| [0961] | caattaaaa   | tggaacctat  | tggttaactac   | caagatttag    | aggtattagg  | tttagctaaa  | 39240 |
| [0962] | caagttaaat  | taaaagctac  | atttgagtct    | aataaatata    | tttcaccttt  | attaagttct  | 39300 |
| [0963] | aatgacttaa  | cgtttactac  | attcttaact    | gagttaaaag    | gttcctatat  | cggtagagct  | 39360 |
| [0964] | attgatatga  | cagaagctcc  | atataacaca    | ataagattta    | gttatgaagc  | attcttacct  | 39420 |
| [0965] | aagggcacta  | atgttatcc   | taaatattca    | gacgatgatg    | gtaaaacttg  | gaaaacattt  | 39480 |

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| [0967] | aaagtaaacc aatctactaa gaataacaaa ttacaagtaa gattagactt atcaactgaa    | 39600 |
| [0968] | aatagtttct taagacctcg tgtacgtaga cttatggtta caacaagaga tgagtaagtt    | 39660 |
| [0969] | taaagagggt ttcataccct ctttttattt aactaaggaa gtgatattat gcctacagaa    | 39720 |
| [0970] | tatagagacc cttactcaca agctaaactc tttattccta ctgtagaaga aaaagctata    | 39780 |
| [0971] | aaagaaatgg aacaaacata taaagataaa ctacaagaag ttaatgagct tattgatgag    | 39840 |
| [0972] | cttaaaaaat tgaaagggtga ttacacacat gacgtttaac tacacacctc taacagaatc   | 39900 |
| [0973] | acaaagatta aaggatatgt accctaaagt aaatgaaatg ggtaaatact taaaagaaga    | 39960 |
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| [0975] | catacaagac tcaggtgact actttgtaac acaagccaga aacacccac aagggtgaag     | 40080 |
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| [0978] | ggttagtttt aaactagatg aaggtagact atacgattca ggaaatacaa ttaatgtaa     | 40260 |
| [0979] | agatttaaca gagtctacaa ctcaatttgc aacactcata aatcctccta aaagtaattt    | 40320 |
| [0980] | aaacacagga tggataaatt ataaggaaag taaaaatgg acctcagct tagctgagtt      | 40380 |
| [0981] | taatcctatt aattctactt caacatttaa aatgattaga aagttaccac gccagacca     | 40440 |
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| [0983] | aagaacgtat tatatagata atacatctt ttgggggtt actaataatg catctacctc      | 40560 |
| [0984] | aacagttaga tatatgggtg aaaatactat tcaattagat gatgggggg aatcatatcc     | 40620 |
| [0985] | tactgttata tctaaccgtt ttagattagg gaatgattta tatgtaggtg atacagttac    | 40680 |
| [0986] | aatgtctgta taccttaaag taaatgacac tagtttatta aatggtaact tcccttactt    | 40740 |
| [0987] | tgaaatagcg ggttatgata atgtatctca aacaaataac cttacacag gaggaaggag     | 40800 |
| [0988] | agaagttaat tactatgagt tatctacaga atggaaaaa ttctcattta cttttacatt     | 40860 |
| [0989] | gcagtattat aaccaagagg ctagcagtga cactgttaat tatatatcag cattattaag    | 40920 |
| [0990] | atttaactgt tctaactcta aaaataatgg tgctattgta tattatgcac taccaaaatt    | 40980 |
| [0991] | agaaaaatcg tactcggtaa ctccatttat tacaataact tatgataaaa gaaaatatga    | 41040 |
| [0992] | tgagatatgg agcaattgga tagagacaac aagcaaggat gaacttcaa cacatagtg      | 41100 |
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| [0998] | tcaaatgtct agtaatagtc tttgggaagg tactcttgac ttagcttcag gtaatacagt    | 41460 |
| [0999] | aaacttatcc tcttcattag ataattatga ttttattgaa gtagtatact ggactagaag    | 41520 |
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| [1001] | ttttaactta gtaaatgatg gttctaatagc tagttagtagac ttttttgaag ggtattgtga | 41640 |
| [1002] | cattacagat agagtgaatg ttactgctaa aatgtcaaaa acaatacaaa tagatgggaa    | 41700 |
| [1003] | tagaaatact atgtctgttg ctacgtttaa tcaactgggt catattgaag tattaagagt    | 41760 |
| [1004] | tgtaggacta acgtatatatt aataaggagt tttgtaaatg ataaaatatg atattagtaa   | 41820 |
| [1005] | agatgaaatt gtgttacatt tagaagatag taagtatatc atgggttaca ctattgttgg    | 41880 |
| [1006] | agggtatgat gagaacaacg gtgacgtaaa aatttctagg tcaattcttc ctgacgggtt    | 41940 |
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| [1009] | agagtattgtt | cctaaagaag  | attataaaaa | gctagaaagt  | gaagtagaag  | aattaaaaag  | 42120 |
| [1010] | aatgctacaa  | gaattgttat  | ctaagaaagg | ataaataata  | tggcactaaa  | ttttacacct  | 42180 |
| [1011] | attacagaaa  | atagtacaat  | agcagattta | acaaaacaag  | ttaataatat  | aggtggtgta  | 42240 |
| [1012] | ctaactgagg  | acagaaatgt  | ctttgaagta | acaacagact  | tacaagcaga  | tgtaagtaga  | 42300 |
| [1013] | acacagaaag  | ttaagttaac  | aacagataga | gggttagcta  | aagatattga  | ttataactaat | 42360 |
| [1014] | tacttaagag  | atattagaaa  | ggtaggtcta | tattatatag  | gagctcgtac  | attagctact  | 42420 |
| [1015] | atgtatgata  | aacctgatat  | ggaaaacatg | gatgtactac  | ttcaagtact  | aaccttagat  | 42480 |
| [1016] | actgaggata  | gagtagtaca  | gatattacat | acattatcta  | ccgctgattc  | taaagtaaaa  | 42540 |
| [1017] | gtactatata  | ggtttgttaa  | caattatcaa | acatcacaat  | ggcaagtagt  | acaaagtcta  | 42600 |
| [1018] | ccaaataata  | aatacacatc  | tcaaataggt | ggaagtcctt  | ttgatattaa  | cacacaaggt  | 42660 |
| [1019] | atatattatg  | tgtctagtat  | gtcggatatg | ccaaatgggt  | tatatgaagg  | gtttttacag  | 42720 |
| [1020] | gttattatag  | acaataatga  | aaatagaatg | ttaagattaa  | cggatattag  | cacaggaaaa  | 42780 |
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| [1022] | gtacaaaaaa  | taagtaagta  | tttattatct | aatgtagatg  | gtgatgctga  | aagtaataac  | 42900 |
| [1023] | tactctttat  | ctgtatatac  | tacagacaac | ataacatttc  | aacaggcaat  | agctaaacat  | 42960 |
| [1024] | attgaagaaa  | caagtaaaac  | tgtttttact | ttttatatac  | aaggaggtgt  | tgtaggtctt  | 43020 |
| [1025] | ccgtcctcgg  | ttagtgttag  | aggtgtattc | atatcagatt  | caactaactt  | tactaatcta  | 43080 |
| [1026] | tatggtgttt  | atactgctat  | aggtactgat | ggaagaacta  | ttaatgggtc  | tgtaaattggt | 43140 |
| [1027] | aatgtgtgga  | acacaacaaa  | aacactacca | ggattttaaag | agctgtggaa  | tggtgcacat  | 43200 |
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| [1029] | gtagaagtat  | acacaagata  | tagagctcta | caaaatacaa  | aaggtactga  | taaaacaggt  | 43320 |
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| [1032] | gatacttgga  | caattaaaga  | tagtgcagta | aataataata  | aagaccaata  | tgttacacgt  | 43500 |
| [1033] | gttgtaggta  | tctctttacc  | atagactagg | ataattccta  | gtcttttttt  | cttgactaac  | 43560 |
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| [1035] | acaacagaat  | atatatacat  | atgtagattt | tgacgaaagt  | gatgggtata  | ttaaagatat  | 43680 |
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| [1043] | ggaactctct  | catttgagac  | aatcatgagc | caagcctata  | aataggaagg  | tgcaacgact  | 44160 |
| [1044] | attatgtaca  | tccaagtgga  | tggaaacggg | gagaacctaa  | gtctcattag  | atatgggttaa | 44220 |
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| [1047] | aaaggagagc  | gtgtagcttt  | ctttacaagc | tctaccgaga  | tttttaatca  | atctgctgac  | 44400 |
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| [1049] | aaacaagtaa  | ctgtagttat  | gattcctact | gttaactcta  | acttaaaaga  | ccctacagaa  | 44520 |

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| [1052] | cctaaaacta aagtagagca gaatgtgctt gatatacttc aagatattta tcataattca   | 44700 |
| [1053] | aagacggatg ctgaagtatt aatggcatta aacaatcata atgttatttt ccaaaaagaa   | 44760 |
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| [1078] | gtagatagac ataagcattt atctgagtac tacagaaaga aaatgttaca gaatttatca   | 46260 |
| [1079] | cgagatagta tttgtaaaga cttcttttgg agtaagacat ttaatacatt ctacaacttt   | 46320 |
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| [1083] | gtaaataaaa caaatagaca aattggggaa gtagagtctt taattaagtc atctgattac   | 46560 |
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| [1088] | gtaaatgagt attcacctac tacaatacct aacaatgcta gactatctat gtttttaatg   | 46860 |
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| [1096] | atttgctaca gaatcggaag acatgggtta cttattcaca gctataaaaa gaacagcaca  | 47340 |
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| [1296] | aaaagagaaa  | gtgtftttagt | acctgagtat  | agaaatagat  | tagcagaaga  | tatgccatta  | 59340 |
| [1297] | catagagacg  | tttatttagc  | acattcttca  | tttgctaaag  | aaggcagagg  | actcacattt  | 59400 |
| [1298] | aaagaacgtc  | ttfttgtagc  | cttgcaagca  | gatggttagta | ttatttaaaaa | ataccctaaa  | 59460 |
| [1299] | tctftgaagt  | ttagatttag  | taaagaacgt  | aaaaaagata  | gactattatg  | gatatttagat | 59520 |
| [1300] | ggcttaggag  | ttgaatacac  | atataatgta  | gacaaagaca  | ataatcataa  | tattftatgft | 59580 |
| [1301] | aatatgggtg  | agaattacc   | taaagaattt  | agtaaatggg  | taaattftaca | aatgtcaca   | 59640 |

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| [1302] | caacaatggg | cttatgattt  | tattgatgag  | ttgtctcatt | gggatgctag  | tagaagaacg  | 59700 |
| [1303] | gataaatcat | ttatctacat  | gagtgtgggt  | gaacataatg | tagacattgt  | tcaagccata  | 59760 |
| [1304] | gcttctatgg | taggttataa  | atctcgtaaa  | tcagttgatt | taagggaag   | agaacctaat  | 59820 |
| [1305] | aaagccacta | tgtataaaat  | atatctatct  | gaaggtcaat | tatttgagg   | ctcaaatgtt  | 59880 |
| [1306] | actgtggaaa | cggtagaata  | taaaggtaaa  | gtatattgtg | ttgaagtacc  | tactcataaa  | 59940 |
| [1307] | cttgttgtaa | gaaggaaagg  | gcatacatta  | gtttgtggta | attctacacg  | tagaggcggt  | 60000 |
| [1308] | tttagtcctt | atttacctat  | agaacatgag  | gatattgatg | agtttttaga  | gatttggtacc | 60060 |
| [1309] | gaaghtaate | caattcaaaa  | cttaacacat  | gcagtaacag | ttactgatga  | ctggttatta  | 60120 |
| [1310] | gacatgatta | atggggataa  | agaaaaaaga  | aaaacatggg | ctaaagtatt  | aacaagaaga  | 60180 |
| [1311] | acacaaatag | gataccctta  | tatcttcttt  | catgataatg | caaatagtaa  | tacagtagat  | 60240 |
| [1312] | gtatataaag | acaaagggtt  | aactattaat  | aactcaaatt | tgtgtaaaaa  | tttgcacctt  | 60300 |
| [1313] | tagtaagtaa | ttactatcga  | aaaatccatc  | taaacggtga | atatctaagt  | taaaatatat  | 60360 |
| [1314] | gacaacaccg | tgctaaatca  | gattataaat  | ctgtaaagtc | ctaacgacta  | tctgtaacct  | 60420 |
| [1315] | attcacataa | gtgaagggat  | aagggttagag | ccaagtggta | taagtattga  | gtaataatca  | 60480 |
| [1316] | agtaaatctt | atttaaatcg  | aaatggtgga  | cttccttatt | aaggaagatg  | atatagtctg  | 60540 |
| [1317] | ctctatatgg | aaacatatag  | aagttcatat  | taacaataat | aattaagagg  | gtcgaaaatg  | 60600 |
| [1318] | gagtaccata | tttataaaat  | tacaaacaaa  | aacaataata | aaatatacat  | cgggaattaca | 60660 |
| [1319] | tcaaaaacag | ttcaacatag  | atttaaaaat  | catattagaa | aagctaagat  | aggctcaaca  | 60720 |
| [1320] | actaatttac | accaatcaat  | gatgaaacat  | ggtgaagaga | atttttatgt  | agaagaatta  | 60780 |
| [1321] | tacactttta | gtacagagga  | taaaaagttt  | gcttacatgg | tagagcaaat  | ttttatagac  | 60840 |
| [1322] | atgtacgaat | ctgtaaaaaa  | aggttataat  | atggagatag | gttttggtg   | gaatataatg  | 60900 |
| [1323] | gatagacgag | gaaaaaacia  | tccgatgtat  | ggtaaagaaa | gcgctaagtc  | taaaaaagtt  | 60960 |
| [1324] | attgttgatg | gcgtagttta  | taaaaatatt  | ggtatatgct | ctgaagagct  | aggaataaac  | 61020 |
| [1325] | agaaatacat | taaccagaag  | atgtaattct  | gaaaaattcc | ctaactataa  | atattgttaa  | 61080 |
| [1326] | ttagagaact | ggtagtgtat  | aacgaccact  | attgaacatg | taggagtga   | ataatgttac  | 61140 |
| [1327] | ctaataaaga | agatttgtca  | tttgtatgtg  | acctatctag | tatgaacctt  | gttaagtatt  | 61200 |
| [1328] | atgaatggaa | acacacggat  | gctgtagaga  | cacttggtta | cttttttagat | gctgttatgt  | 61260 |
| [1329] | ctgagtttat | tagagactta  | gaatatctaa  | gagactcaga | tgaaaaagat  | aaacaagaag  | 61320 |
| [1330] | catttaaat  | tatggagaga  | gcttataact  | tttctgttga | gaacagagcc  | ttaggaatag  | 61380 |
| [1331] | gcgttcttgg | ttaccattca  | tacctacaat  | caaaactcat | accttttgaa  | agtattaaag  | 61440 |
| [1332] | ccagtcaaat | taatgaagag  | atattttaat  | tattaaaaga | aaaaacttat  | aaagctagt   | 61500 |
| [1333] | agcagttagc | taaactttat  | ggagagcctt  | ctattttaaa | aggttatggt  | agaagaaatt  | 61560 |
| [1334] | catgtttgat | ggcggttagct | cctacgactt  | cgagctcatt | tatccttagt  | caagtttcta  | 61620 |
| [1335] | aatcaattga | accttttatg  | agtaattatt  | acgttgtaga | cactgctaaa  | gtaaagaaaa  | 61680 |
| [1336] | ctatgattaa | tccttacctt  | aaaagactat  | taaaagaaaa | aggaaaggac  | aataaaaaag  | 61740 |
| [1337] | ttattgagag | tatcagagat  | aatgatgggt  | ccgttcaaca | cctaactttc  | ttaagtgtat  | 61800 |
| [1338] | atgaaaagga | agtattttaa  | acatatggtg  | aaattaatca | atataatata  | ttagaccaag  | 61860 |
| [1339] | cttctacaag | acaaaaatat  | attgaccaag  | gacaatctat | aaacattatg  | attaacccta  | 61920 |
| [1340] | aacatgttac | agcagaggag  | ttaaatgaac  | tttatctctt | tgcatggtct  | aatggtatta  | 61980 |
| [1341] | agtcattgta | ttatcaacat  | ggtacaaatg  | catcacaaca | attcaattta  | tctaaaattt  | 62040 |
| [1342] | gtattaattg | tgaagcctaa  | agaggtgact  | aaatgaataa | aaaagaagcg  | tttggtattac | 62100 |
| [1343] | taagtagaac | tgaagatata  | ttcaaatcaa  | atgatatttt | ctcacaagta  | agtaatgtac  | 62160 |

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| [1344] | aagaccctat taagatgttt aataggggaag atgatactaa agcagatagt aaatcaagta | 62220 |
| [1345] | aatttcagct agagtttatt tataatacat atgtgtacat tcttatatat gaaggtacat  | 62280 |
| [1346] | taaagctatt taaagctgat ggtacggagc agttaacaca thtagctgat attgaaacat  | 62340 |
| [1347] | ttaaagatat tattgacaca ttagaaaagg atgatacaga aaatggaaaa agtgaatagt  | 62400 |
| [1348] | ttattagagt taaatacaac aatcagacaa aaacaggatg ttattgtcat ggttacacaa  | 62460 |
| [1349] | gatgagtgtg ctaaattgtga aattttaaaa agtgtgattc ctcttttcga agcagaagga | 62520 |
| [1350] | gatattacta aacctgttta tgttattaac ttagatgata aagatgtaga tagagaaaaa  | 62580 |
| [1351] | gcggtcaagt tatttgacat tatgagtaca cctgtactta ttggttataa agatggagaa  | 62640 |
| [1352] | cttgctaaga agttcgaaga ccaagtaaca ctaaaaaact taatggaatt agatttactt  | 62700 |
| [1353] | taatttgtaa ttacctacta cttatgctat acttatatta gtaaaaggta gtaggttttt  | 62760 |
| [1354] | tcatgaaagg atgaatagtt atcgcaaaaa ataaaacatt aactatatat aatagtgata  | 62820 |
| [1355] | gatattttta tatacatgta aaaaacaaag atgaattatc taaagctatt aaagttacaa  | 62880 |
| [1356] | ctttaaatga agatgagata gaaaaagata tggacaactt agctaataaa tcaactaggt  | 62940 |
| [1357] | atatactaag agatgataaa cattacatgc tatttaatga gaagtataac aatgataagt  | 63000 |
| [1358] | tgattgaaaa aatatgcaag cacggtggac aagtaacata ctataccgat tcagtaattc  | 63060 |
| [1359] | cttactatgt gcttaaagat ttatcagctc accctaaatc atcagttgta tatagaatgc  | 63120 |
| [1360] | gtagtaagtt tagtaacaaa gagatggata atatagcttt gagtttcatg ggaacaaaag  | 63180 |
| [1361] | tcattattga tatttcagtt gtatttcctt acgtcaatcc ttacgatatt attagaagtt  | 63240 |
| [1362] | tatacgatgt taaaacgaat gtagacgagg ttcactatc attccctcgt attagtctta   | 63300 |
| [1363] | tagacgctaa acaaggtaag tactacacgt atgataaaga agcttataaa ctaaaaccta  | 63360 |
| [1364] | ggtatacgtc tgactttgca gacaaattaa gggtttcttt gtccgtgtgg aaaatgtata  | 63420 |
| [1365] | tctatattct tgctagtaaa gaaaataaag attatgaaac aatagaagac ctacttactg  | 63480 |
| [1366] | aattaaaaag acaaagaaag attaagattt aggtgatttt gtgagtacag caaatagaag  | 63540 |
| [1367] | agatatagca agaaagatat ctgagagtac aggggtattat atccaagacg tagaagagat | 63600 |
| [1368] | actagaagca gaaacaaaag ctatttctga attactactt gaagattacg ataaggtaaa  | 63660 |
| [1369] | gaatcataag tactttcaaa tagaagtagt agagcgtaaag ggtaaaaaag catgggatgg | 63720 |
| [1370] | actaaataaa acatacttca ctttacctaa tagaaaggca ctaaagtta aacctttgtc   | 63780 |
| [1371] | tgaattagat aaagttatag atgtattaaa caaagaggaa gaaaacagtt aaatatttta  | 63840 |
| [1372] | tatagcataa gatttagtat ctactcttca tgtatttagt tggtagtata tgttttgcta  | 63900 |
| [1373] | ttatatacta tttatttaac agagattgag tcaagtttat acttgactct tttttttatg  | 63960 |
| [1374] | ttatgttata gtttaactat aaggagctga ttgaatatga aaatgaaaga tttagataac  | 64020 |
| [1375] | ttacatattg ttgtagtatg tattaagaat gatacattac ctgatgtagg gtatatggtc  | 64080 |
| [1376] | ttagaaaata taaaaagaaa gtaaaagaaa atagtacat acaatgaaat catataaaac   | 64140 |
| [1377] | agaagtaaaa ccaataaac aacaaataat tgagattaat aaaacaatta atgctttag    | 64200 |
| [1378] | aagtgtttat aataaattta ttgaagtaaa taaatttcgt tatgataacg ggttgaaatt  | 64260 |
| [1379] | tttaaacat agaaagtta gtgtttggta taataatgat tttattccta ataataaaga    | 64320 |
| [1380] | taaaaaatgg actaaggaag taaatactaa agcaactaaa caagctatgg caaatgctga  | 64380 |
| [1381] | ggatgcatat aaaagattct ttaataatat atcaggtttt cctaagtta agaagacaca   | 64440 |
| [1382] | aagtaacggt tcttactatt taataggtac tatacatgta gaacgtcata gaatacgact  | 64500 |
| [1383] | acctaattta aaatgggtta agttaaaaa aaaaggatat ataccgaaa acaatataaa    | 64560 |
| [1384] | atcagctact attattaaag aaaatgatag atattatgtg tcagtgttag tagatgaaga  | 64620 |
| [1385] | acctaagact atacttaaaa aaccacatac tgaaggattt ggtatagacc taggattaaa  | 64680 |

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| [1387] | agttaaatta | cataagtctt | taaaacgaca | acaaagaaaa  | ttatcacgaa  | aacaaaagaa  | 64800 |
| [1388] | gtccaataac | tggtctaaac | aattattaaa | agtacaaaga  | ttatatcgta  | aaataagcaa  | 64860 |
| [1389] | cattaagaag | gatattaaac | aaaagaaaat | actagaaata  | gtacaggaaa  | accacaatc   | 64920 |
| [1390] | tatcacaatt | gaaaatttaa | atattaaagg | tatgatgaaa  | aataaaagat  | tgtctaatag  | 64980 |
| [1391] | tttccaacaa | ataggattag | gctacattat | tgaatggctc  | aaatggaaat  | gttatcaata  | 65040 |
| [1392] | tgatatagaa | ctaagacaag | tagatagatt | ttacccttca  | agtcaaatat  | gcaatcaatg  | 65100 |
| [1393] | tggaataga  | caaaaaatgc | ctttaataa  | aagaatttac  | gaatgtgata  | attgtgatat  | 65160 |
| [1394] | gatagaagat | aaagatataa | acgcaagtat | taacttaaaa  | caagcaaaag  | aatatacaat  | 65220 |
| [1395] | actagtataa | aagaaaaaga | gaagttactg | agaactagta  | tcttacttaa  | agtctaaaca  | 65280 |
| [1396] | aatacggtag | gctatatcgg | aattaacgct | ctgggagagg  | tttttagacc  | tgattgttaa  | 65340 |
| [1397] | tacaatcaac | ctcgttgaat | ggagaaactt | tctaaatata  | catttaagta  | tgtttttagt  | 65400 |
| [1398] | agcaggatta | agttcctctt | ttttttattg | actactatga  | ttagatatgc  | tatactgtat  | 65460 |
| [1399] | ttaagttaaa | gaagggattg | gtaataacat | gaaagtitta  | attttatagc  | actatatcag  | 65520 |
| [1400] | agaggaacac | ttttcagtta | gtaatgatgg | tagtgtttaag | aatgtactat  | taaatacacc  | 65580 |
| [1401] | taatggtaga | gtactaaaac | aactattgtc | taatatttct  | ggtattaata  | gagataggag  | 65640 |
| [1402] | cacaaaggat | tatgacattg | attttttata | tcctaaagta  | cctacaccta  | ttaagaataa  | 65700 |
| [1403] | ctatggtaaa | acaattaagt | accaagacgt | taagttatct  | gaagttaaac  | cttactatga  | 65760 |
| [1404] | aagaatgagt | caaataatca | taaacaataa | gtacgatatt  | attattcctt  | taggtaagct  | 65820 |
| [1405] | aggggttaaa | tatttattaa | atgtatcagc | tatcggtaaa  | gttagagggtg | tacctaataa  | 65880 |
| [1406] | agtaactatt | actagtgaag | ataaaaaaca | tgatgtttgg  | gtattaccta  | cttatagcat  | 65940 |
| [1407] | agagtacaca | aacgttaata | agaatagtga | gcgtcatgta  | gtagctgacc  | ttaagttatt  | 66000 |
| [1408] | aggtaaagtt | gtagaacaag | gtgaagatgt | atttaaacct  | aaagaagtta  | agtatgaact  | 66060 |
| [1409] | tgttacaagt | attgagcgtg | taagagaaat | atttaataaa  | gaagtaaaga  | atgataatca  | 66120 |
| [1410] | tgacggtgta | gacattaccg | catgggattt | agaaactaac  | tcacttagtc  | ctgatagaga  | 66180 |
| [1411] | aggaagttaa | cccttagtat | tatcaatgag | ttggggagaat | ggtcaagggtg | ttacaatacc  | 66240 |
| [1412] | tttatataaa | tcagacttta | cttgggagaa | tgacagcaa   | gatattgatg  | aaattctttc  | 66300 |
| [1413] | cttattaaaa | gaatgggtag | ctagcaaaga | agacattaag  | gtactacata  | atgctactta  | 66360 |
| [1414] | tgatataaat | ttccttatgg | caacgcaagg | tttcacaaac  | tttgaaaata  | atcaagacac  | 66420 |
| [1415] | taaggtaggt | tggtatttag | ctgtaacaca | agaacaagca  | gagtctctaa  | gattatcaga  | 66480 |
| [1416] | ccttgcttac | gaagtaacag | atgtaggagg | atatgacaaa  | ccccttgaag  | atttttaaaga | 66540 |
| [1417] | gtggtatgta | ttgaagctac | taagattcct | atcagataaa  | ctaaaagata  | ttaaaaaaga  | 66600 |
| [1418] | aaataaaaaa | gttgctaaga | agaatataa  | cataaaagca  | aatgagtatg  | atacttggtt  | 66660 |
| [1419] | gaaaaataaa | ctcgataaca | ttgatataga | actaacagat  | gaagataagt  | attatggtat  | 66720 |
| [1420] | cacagaagag | caaaaaagat | atttagagtt | aaaactaaca  | cctgagggtta | ttacaaagaa  | 66780 |
| [1421] | tatgcttatg | gattctgaat | ttaaagaagt | agccgaatca  | tcacctgagt  | atatgagttt  | 66840 |
| [1422] | atctaacaaa | gctaaagatt | atgtattagg | tacatctatt  | aatttaatta  | acacgtataa  | 66900 |
| [1423] | agacaatact | aaagttatta | atgaggtaga | tgagggtgac  | tttaattatg  | attggattcc  | 66960 |
| [1424] | attagagctt | atgcatcctt | atgccagtgg | tgacaccgat  | gtctgtagaa  | gaatatactg  | 67020 |
| [1425] | tgatgttatt | gaaaagttaa | aagaacaaaa | tagacctaaa  | gcattggact  | taatgtcaat  | 67080 |
| [1426] | aagttaccct | agattaatta | gaacattagc | tagaatacaa  | tcaaattggtt | tacattgtga  | 67140 |
| [1427] | cctagaatac | atgcataaaa | atgatgaatt | ttatattaat  | gagatggaaa  | aaaccaccca  | 67200 |

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| [1429] | attagcctta | gcagaacatg  | agaagaaacc | atctgataga  | aataaagaaa  | tacatgagta | 67320 |
| [1430] | tagagctaaa | tttaaagatg  | aaggtttgaa | gtttaaacca  | agctcaggtg  | accataaagg | 67380 |
| [1431] | tgaagtattg | tatagcattt  | taggtataca | gttaccttat  | agtaaagaga  | cagttaagga | 67440 |
| [1432] | taaacctttc | agtaatggta  | ctaaagaaga | tgagttatca  | tggaaagatt  | acaaaacaga | 67500 |
| [1433] | tactaagtct | ataaagatgg  | cattatcttt | ggttgagaat  | gaagataata  | aaaagctact | 67560 |
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| [1435] | acctaaaaga | gtaaataaga  | atacacataa | tttacctggt  | aactataata  | gtacaggaac | 67680 |
| [1436] | tgcatgtatt | acaggtgatt  | ctttagttat | tactgataaa  | ggtataaaaa  | aaatagaaga | 67740 |
| [1437] | cttatcaaat | aatagaaaag  | aaaaagtatt | tagtagtatt  | gatgtaggta  | ttgttaatag | 67800 |
| [1438] | acagggaaat | ttagaaaaag  | cttcacactt | ttattatagt  | ggtgtaagaa  | atggattaaa | 67860 |
| [1439] | gattacttta | gaagatggta  | ctacattaac | tactacttta  | aaccaccctt  | tattacgaaa | 67920 |
| [1440] | taattattat | agtaatactg  | gtagggttct | aaataaaaaca | aaacaaacaa  | ctaagcattt | 67980 |
| [1441] | actagataat | gattggggtg  | tagctgaaga | tttaaaaata  | ggtgattata  | taaaaatgtc | 68040 |
| [1442] | ttataattct | aatctttata  | ataatagtta | tattgattta  | gatactaaag  | attttatata | 68100 |
| [1443] | tagtaaagaa | aaaagtatta  | caaacacaaa | aagctataca  | ctacctaat   | atgtatctga | 68160 |
| [1444] | agattttgct | gaatggtacg  | gtatgtatac | agctgatggg  | tctttttcta  | caaataacgg | 68220 |
| [1445] | ttcattcagt | ataagattaa  | ctaatagtaa | tcttgaagtt  | aggaatagat  | tctttaacct | 68280 |
| [1446] | aactaaagat | ttatttgata  | ttacaccata | ctacattagt  | aataaagata  | gaagtgattc | 68340 |
| [1447] | tattgaattt | tcttcaattg  | gtttaggtag | atggttagaa  | catattttta  | atatgcaaag | 68400 |
| [1448] | taaagcatta | aataaagaaa  | tcccacaaca | aatatttagat | agccctaaaa  | gtgtacaaca | 68460 |
| [1449] | agcatttctt | aagggttta   | gtttagatac | tgcaacagaa  | aagaaaaagt  | atcctagttt | 68520 |
| [1450] | gtactataat | actgtatcta  | ataaaaatgc | tttacaata   | agaacaatgc  | taatgaatat | 68580 |
| [1451] | gggtatctat | tgtaggtatt  | ctataggtaa | agtttataaa  | aataataata  | gaaaagtaca | 68640 |
| [1452] | aatgaatgc  | tattctatac  | aaataactta | tgatgcatta  | gataaatttt  | acgatgaaat | 68700 |
| [1453] | agggtttatt | gaaagtatta  | agcgtgatag | agtaaagtat  | aatcagaaa   | atctaaaggc | 68760 |
| [1454] | attaggcaga | agaaacggta  | ttcttttata | tgataatgtt  | ttattaggta  | aaataaagaa | 68820 |
| [1455] | aattgaaaaa | ataaaaagata | tggaattttt | tgattttacat | gttcctagta  | gccattcatt | 68880 |
| [1456] | tgtagctaat | aatattgtaa  | atcataatac | tagcaggett  | tctagtaaca  | accctaacct | 68940 |
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| [1458] | acgttctttt | atctctagat  | ttaaagatgg | tgtaatttta  | caagccgatt  | acagtgcctt | 69060 |
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| [1637] | atacaaaaaga aatatgcgtt agacttaatt agtgaagctg tgaatataaa gaagaaatat 79800 |

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| [1682] | atgaagataa  | acttaataaa | attaaaaatt | aatgattgac | acactcatat | gggtgtgtta | 82500 |
| [1683] | ttcttttatt  | acaacaata  | aaaggagcaa | ttgattatga | ttattacatt | aaatgaagaa | 82560 |
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| [1685] | aatacaccaa  | aagaagaata | tattgttaca | agttataata | aaggtgtacc | tgtgactgat | 82680 |
| [1686] | attgctaagg  | aagctaaagt | aagtatgggt | ttgatttata | cagtgttaaa | cttctataaa | 82740 |
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| [1688] | aagaataaag  | tacaagcctt | aattaaagat | tatcaatata | tagacttaca | aagtatttat | 82860 |
| [1689] | aaaaagtata  | acattcataa | gaatggttta | tattatattt | tagatttgta | taatattgaa | 82920 |
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| [1696] | tgagaaagcg  | catgttatgc | aaccactaat | taagatagaa | aatactgata | tagattatac | 83340 |
| [1697] | tcaggctgat  | atgattaatg | acattaaagt | aggtacaagt | attagtgatg | taaagattaa | 83400 |
| [1698] | agataaatta  | agtttaatgt | caaataatct | tattacacat | ttagaaattg | ttgataagca | 83460 |
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| [1701] | ttgtccttta  | ctgttaaaat | gtattaaagt | tatagactaa | gttattatat | tataagtagt | 83640 |
| [1702] | aaaggatggg  | gcgctatgag | taaaaaagca | ataattgctt | atgtaattat | cttagttatg | 83700 |
| [1703] | ttagccttat  | ctatatctac | ttattacgta | tcctcatact | tatatcatga | aaaaacccaa | 83760 |
| [1704] | tcacaagtta  | cagaccaatt | aacacatcac | ggtaaactaa | aaaaagataa | gaatgtagag | 83820 |
| [1705] | tacgtaggag  | attacacgct | taagaaagta | gtagacaaca | aagcatactt | tatggaaaaa | 83880 |
| [1706] | ctacctacct  | atttaccagg | aagaacaagt | gataaaagta | ttgatatgag | atattatagg | 83940 |
| [1707] | actagtaa at | ttagagaagg | tgtaaacttt | aaacttattc | gtgtgtatag | ggaggataat | 84000 |
| [1708] | gacaataatc  | cagtacataa | gtataggttt | gaagcagtag | taaataaaaa | gtaaaggtat | 84060 |
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| [1716] | taggttttat  | tttattgttt | tatgtaggtg | tatttatttg | gtacttatgg | gaagtctgga | 84540 |
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| [1719] | tattagtacc  | ttttatactt | agctatataa | tgataaaata | tttgatagga | ggttactaaa | 84720 |
| [1720] | tggaatatag  | atatgagaat | gaatgtagag | gatattatat | ttgtgtcata | gataatgaag | 84780 |
| [1721] | gtaagtatga  | agtatcatct | tttaaaaaat | cagtaaacaa | gtataccctt | gaaattacag | 84840 |

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| [1725] | atacagaaaa | atatagaaat | ttaatccata  | ttacatatag  | tatcttgggt  | agtattcaag  | 85080 |
| [1726] | aattagcaag | taatttatta | aataaagaag  | taaaactagt  | tacaaaagaa  | aataagtact  | 85140 |
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| [1797] | tacgtatgaa tacatctacg attctaatac aaaataccaa tacattatta ataattgaac   | 89400 |
| [1798] | atatgagtcg agtatcttat taaaacaaga tgaagtaaat aaattaaaag atatacttaa   | 89460 |
| [1799] | taaagtacct gtcacagcta agcatacatt taaaaatgag ttgcttaatc gtaggaatga   | 89520 |
| [1800] | atatgtgtta ggttatatta caatatatat ttatgatgat ttaactgcta aaattacaaa   | 89580 |
| [1801] | gaatggttat gtacaagact ttagtacact agaagatttt tgtgataagt atgaagctta   | 89640 |
| [1802] | ctgtagattt gttagaattg atttatatga ctgggtaaaa gaagtaagaa ctattaatgg   | 89700 |
| [1803] | gtataaatta aaggtagata ttaaacctta ttttaaaatt actgctacta aaacaagtaa   | 89760 |
| [1804] | atacgaattt gtagtaagtg atgatgtatc taatgttaag gtatatagag atgatatgct   | 89820 |
| [1805] | actaacaac aggtatgtgc aaaaagcaat tgatatacag gaaatagtag aggaaacatt    | 89880 |



|        |             |             |             |            |             |            |       |
|--------|-------------|-------------|-------------|------------|-------------|------------|-------|
| [1806] | gaataataat  | actgtggtat  | tttaaaaaag  | gaagaagtag | ggaaattccc  | tacttttttc | 89940 |
| [1807] | atatataagt  | gttgacatga  | ataagtgtaa  | ggtttataat | aagtatataa  | gttaaataat | 90000 |
| [1808] | tatagaggag  | acattaatca  | tgaaaaaaat  | attaagttta | gttttatccg  | tactaattgt | 90060 |
| [1809] | actaacaata  | atcaatgtgt  | tgaaaaacca  | agagcaagta | gaacaaagta  | accaagaatc | 90120 |
| [1810] | tcaaaaataat | gaagatgcag  | atgaccatac  | ttcatacgt  | cctgagaata  | atggagaaca | 90180 |
| [1811] | caaagatgtt  | agtggagaac  | acaaagatgt  | tagtgaagaa | cgtaaaagat  | tatggggtag | 90240 |
| [1812] | agagtatcaa  | gaaggtgaaa  | cacataaccc  | aaatgctaaa | gtgtttaatc  | aagatacagg | 90300 |
| [1813] | agaacagggt  | caataaagag  | taagaggagc  | acttattatg | gaatactgga  | aagaacaaga | 90360 |
| [1814] | catatattat  | tcaaaaacaa  | aacaagggtg  | tataagctat | atagacttat  | cttccttgct | 90420 |
| [1815] | ctaaacaatg  | ttcaacatta  | ccaagaagtc  | agtggataca | tgaaccacca  | gaaaacaaaa | 90480 |
| [1816] | ttatcagaca  | tagaaaagag  | agaatattgt  | aaagaaaata | gtatacaata  | tatatagaga | 90540 |
| [1817] | ttgatgctag  | aaagtctacc  | tttaaacata  | tattaagtaa | cctacaaaga  | acatttaaat | 90600 |
| [1818] | actctaatat  | agatagtaaa  | gaagtaatta  | aaagatatag | acatgtatat  | aggtaagtgt | 90660 |
| [1819] | agaagagtaa  | acaaccaaac  | agacaaacaa  | ataggtatag | agattgatgt  | aggtagaagt | 90720 |
| [1820] | tatataagta  | agtaataggt  | aaaagaaaac  | aatatatata | agtagataaa  | gaaatcagat | 90780 |
| [1821] | aagtaacaca  | taaagaaaca  | actgatatta  | taagtaccta | tatatcaagg  | aaataagtaa | 90840 |
| [1822] | tagataaaaa  | aacaataatt  | gatagcacat  | agataagcag | taggtagtat  | ataagtaata | 90900 |
| [1823] | gctaaataga  | taagtaaata  | aatactgtat  | aacagtttga | agatatgaat  | aagaagaaag | 90960 |
| [1824] | aattattaag  | taacttaagt  | attaatagat  | attctaatag | taagtaatag  | atattaagta | 91020 |
| [1825] | gtaggtaagt  | aactaaaaga  | aagtagctaa  | aagaaagtag | ctaccactat  | cacttaggtt | 91080 |
| [1826] | aggtaagaac  | agttacctag  | taatttcttt  | acatcttcta | gatagataaa  | taagtagata | 91140 |
| [1827] | ctaagtaata  | gctagtaagt  | aattaaaaga  | aaacaggtac | atggtatacc  | tatatactaa | 91200 |
| [1828] | gtagcaagta  | gcaagtagca  | agtagcaagt  | agcaagtagc | aagtagcaag  | tagcaagtaa | 91260 |
| [1829] | gaacaggtag  | atgggaatth  | ctctgcttcc  | ctccttcata | gtgtatatat  | agtttaggtg | 91320 |
| [1830] | ctaactatta  | gatatctaac  | tattttattt  | tatatagttt | taactactgt  | gaactatgtt | 91380 |
| [1831] | tatgtagtat  | taaactatgc  | tgattatatg  | ctaaactatt | ctaattgtata | gttaaactaa | 91440 |
| [1832] | gctgaagtag  | tatttactgt  | gtataaaggt  | atatttagta | ttagatattg  | tgtatatggt | 91500 |
| [1833] | atactataat  | tctgaacatt  | acttatttta  | tatatatata | gtttgtttct  | gtggtagtat | 91560 |
| [1834] | tgacttaatt  | ttccctagag  | taaaagttta  | atatatttag | taatttccga  | acaataaaga | 91620 |
| [1835] | aattttaact  | atthttacaca | tatgtgttga  | cagtatatta | cttatggttt  | atactaagta | 91680 |
| [1836] | tataaagtta  | aaggagatga  | tttattatga  | agattaaaag | agaagtttcg  | tttatagaat | 91740 |
| [1837] | taatgtatat  | gtattataaa  | ggtgatataa  | aaaaaggtcg | acactattct  | actgatagtc | 91800 |
| [1838] | agtttattgt  | gtttataact  | gttgatgata  | tatcgtttta | taatgcattg  | tatcctaata | 91860 |
| [1839] | aatctattac  | tattactata  | cctaattgaca | ctaagtttat | tgtagaagcg  | gttatagatg | 91920 |
| [1840] | cttctactga  | gttaagtagt  | atagtagtta  | cttataagca | aggtaacagt  | gtattaaact | 91980 |
| [1841] | cagtatggta  | tagaagtagt  | attaatgaag  | ttattgacta | ctatgtcgaa  | ggtaaaaata | 92040 |
| [1842] | catgggtatt  | gtctgttagt  | actgtttag   | ataatgagtt | agttactata  | tggaaagatg | 92100 |
| [1843] | gtaacgtatt  | gtgatttgg   | atataagta   | attcagtata | cttatatatg  | tacagcactt | 92160 |
| [1844] | gtattattat  | gtgtagtact  | agtatataga  | ggggttaaat | aaccgcttca  | ataaaaagg  | 92220 |
| [1845] | ggattagttt  | atgagtatat  | ttataagtat  | cgttgctata | tttttagcta  | ctattagtat | 92280 |
| [1846] | tgtactaagt  | ataaggtttg  | gtaagaagta  | ttataagcat | gtactcgata  | attatgatag | 92340 |
| [1847] | tgtttagtgat | ttagaatcag  | cttgtgtgtt  | tggtttatta | tttatgagta  | tttgtattgg | 92400 |

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| [1849] | gtatttaggt atatagagta gggcatatta agtaagggtta ttttagatg ttaatttta   | 92520 |
| [1850] | gttggtttat tatcggcata tatcttgctt gtttagttac tattacactt tttctatta   | 92580 |
| [1851] | aagatagga tattattcct gctacactag gtattgtagc tcttattata gttattattc   | 92640 |
| [1852] | ttatatctac caaagtgtta aatatgtagc ttaagtgaga ttaagtaagg aggattataa  | 92700 |
| [1853] | atgttaagtt ttattttgtt atgtgttgct ctaggtattc cagaactttt cttacttact  | 92760 |
| [1854] | attttaggtg ttatcttagt gatattctatt aaagatagga atatagtctc agctatattt | 92820 |
| [1855] | gctattgtag tttttattat aatgagtata cttatatccg cttcagtact acgtatgtta  | 92880 |
| [1856] | ggtatgtaat ttaaaaagga ttaaataagg agggtttagag atggaaaaac tacaagtatt | 92940 |
| [1857] | aaataaggta ttagaaaaca ggtaggcat aaaagcgtt acgtattctc attatagtaa    | 93000 |
| [1858] | gcataacagt aacggatata tttacagttt tcatatgaaa aaagaatctt ttacttgtca  | 93060 |
| [1859] | tattgttgta tatgatgtta gttgtgaagg tttagataca tatagagtaa aagtactaga  | 93120 |
| [1860] | caaggatgat aaggtaaatg tttcaacttt atatacttta atagaaagta tgtatgatat  | 93180 |
| [1861] | actactatat gaatatgata agtacctaaa agacaaagaa catcaaaatg aaatttactc  | 93240 |
| [1862] | tatgtttgat aaacaattag atactattta agaagaggtt tttctctagg gaaaagtagt  | 93300 |
| [1863] | caaaggtaaa aatagggtt gaacccgcat tctatggtt acatttaggt aaaattagat    | 93360 |
| [1864] | aggttttccc tagaagaaaa gtttaaaagg taaaaaagga gttggttact atgtcaaaat  | 93420 |
| [1865] | tagctaagtt ttgtaggaag tatggagaag ttactggcat aatggagaat attacagatt  | 93480 |
| [1866] | atgagatgta cttaggtagg ggttataaag taagggttag agtaggtaat agccttattt  | 93540 |
| [1867] | atgttcttgt atataaagac aaccatatca ttgctaggtt tgaggaagcg gataatatag  | 93600 |
| [1868] | tatttccttt gagtgatacg cttgagggtg ggacaattaa agacttagag gagctagaag  | 93660 |
| [1869] | agttgtataa taatatctat acattattag ggtatttata tacagaatat gaaaataaag  | 93720 |
| [1870] | ttatgtatga gtatgggagg ttagaataat ggatattacg gtaagtgagt tatttgagta  | 93780 |
| [1871] | tatattaggt cattatacct ttagtagtgg ggattggtat acttatgact taggtgaagg  | 93840 |
| [1872] | ttactgtatt aaatttaatg atgggtattg taggagcatg cttttatca aaggtagt     | 93900 |
| [1873] | agtagtaaaa gaggttctta tcgatgataa acagctctt aaagggttta gtagtattta   | 93960 |
| [1874] | tgattttatt gattactgtg aggaagcttt agatgaaaag tgcagaaaag aaaaagagcg  | 94020 |
| [1875] | cctaaaggaa attcgtacgt tcttaggtta gtagagtatt atttattatg tataaccgct  | 94080 |
| [1876] | tctatatgaa actttatacc tatagttatt atacctatag ttattatacc tatagttatt  | 94140 |
| [1877] | atacctatag ttattatacc tatagttatt ataactacag gtattttttt atatttctta  | 94200 |
| [1878] | cataaatgtg ttgattttat gtgtgttaag gtttatactt aatatataat taaaggagt   | 94260 |
| [1879] | aagttaatat ggatatgaat aaagaagtat tagaacgtt agatagtta ggtggttaagt   | 94320 |
| [1880] | tagagcaagc aggtacacat ggttatgaat ctttaattaa atacacagt actcaaggta   | 94380 |
| [1881] | ttatagattt atgttcaata agtatattat tatctataac agctatctta tgggtggctt  | 94440 |
| [1882] | tgtataagtc agataaaaag tctaatagaag gtcatagtag cttattattt gagtttgtt  | 94500 |
| [1883] | gtaagaatga aaaagagctt tctgctgcag gatgcatagt aattatattt gctgttacta  | 94560 |
| [1884] | tgtctattat ttctttttta gtgctattaa taggcttgcc tattgctatt caagagattt  | 94620 |
| [1885] | ttaatccaga aggatatttg attaaagagta cgatagataa tttaaaataa ttgtaaaaat | 94680 |
| [1886] | aaggaggaaa atataatgtt agaacaagct aaaggataa ctacagatta tattatggaa   | 94740 |
| [1887] | tttattgatg aacatgagca ttattcctat gaagacttac acaagttagc aggggagtta  | 94800 |
| [1888] | gtgttaaaaag atacacatga cacaaacct attgttatta atgatggaga tggttactca  | 94860 |
| [1889] | ggtactatta tatctaaatt taaatcaaag gtagagggtg ttccagatat cactgttagt  | 94920 |

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|--------|---------------------------------------------------------------------|-------|
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| [1891] | gaacttgaga taattagaga tttagctact atggtactac atacaattca atctatggaa   | 95040 |
| [1892] | gagcaagtag aataatgtat aagggagtgg tatagtgttt agtaagagaa aagaaattaa   | 95100 |
| [1893] | agagtataag aaacaagggtt actctgttga gtatatagga agtatttttag gtttatttgg | 95160 |
| [1894] | tattggcgat gttttatact atgtagaaac taataagaaa tacttaaagt caattagcgt   | 95220 |
| [1895] | aggtaaagat gacaaccaat atgtatatga ctttaatggg tatgaatact tatcaagaca   | 95280 |
| [1896] | taagttaa atcaaagaag aagaactaat gtctaaagct attgctcagt taagtatcaa     | 95340 |
| [1897] | aggtttaaag aatttttaag gagttaagca taaatgggat gtaggtttct tagagcaatc   | 95400 |
| [1898] | ttttaatgta catcgtaaag ctattgatat ggttaaactt aaactaagtt ttgatgaagc   | 95460 |
| [1899] | gggtaaccta tattatgaat tagaattagg ttggattaat atttataaag agtatacagg   | 95520 |
| [1900] | tgtaaaagac tttcttaata cagtagataa ctatctcaac ttcttctata aagatatagt   | 95580 |
| [1901] | cactagtggg cttgaattaa aacaagtact atataatact tttattggag gagattatga   | 95640 |
| [1902] | gtttgtagta tctccatata aggttgaaat atatagagaa gctgagttaa ttgatgtaag   | 95700 |
| [1903] | agattttaat tatagcttct gggaatatgg gtacggcact aatgtacata aacctttaca   | 95760 |
| [1904] | aacaattaag tattatctgg aataccctat gaatgaagtt gacattaatt aagtataggt   | 95820 |
| [1905] | gtatgataga tatataaaga ttaaaggaga atagataatg agtagtaaca aaaaatagca   | 95880 |
| [1906] | gaaagagtta ttgagaaatt tttagatgag actaataaag gtgtagattt tactaatcaa   | 95940 |
| [1907] | gatgaattta accgcttctt agataataca gaagacttgt atgaagtatc tgaaactcaa   | 96000 |
| [1908] | agagataaga ttattaaatg tttacaaaac attgtagtta ctgtatttat ggataaggat   | 96060 |
| [1909] | aaataataag gagaaataac aatgaaaaca ataaaaagaa aaatagtaca agaaggatatg  | 96120 |
| [1910] | aacaaagaag aaactatgag ggaagacgat tctgttgtaa tcaaaaaaat tcaaaaaata   | 96180 |
| [1911] | acaggtgggg ctattcttcc tttatccaat ggcaaggaat atgtagtagt gtttagtaaa   | 96240 |
| [1912] | gtagacccta ctaaattaat tgtatttaat gtggaaaata atcaaacta tttaaagtgc    | 96300 |
| [1913] | tctggtttcg atagtgaact gtcttatgta gacagtaaaa gcaaccctat taccttagaa   | 96360 |
| [1914] | acagttaggt taatcactaa tttattgtaa ttattatttt gaagcgggta acagtccgct   | 96420 |
| [1915] | tcttttttta aataagtagt tgacaagtat attagtatgg tttatactta gtatataaag   | 96480 |
| [1916] | attaaaggag ttggttatta tgaaaacaat tacacaagaa gaattagata aagtattaaa   | 96540 |
| [1917] | agaacataaa ttatggctga acagtgatgg cgtagaagga actagagcaa atttaagtta   | 96600 |
| [1918] | cactatgttg agacatgta atctaagagg cgctgattta agaagagcta ttctccgtaa    | 96660 |
| [1919] | agctgactta agtcatgctt acttaacagg ggctatctta agagaagttg atttgatgg    | 96720 |
| [1920] | tgctatttta agtcatattg atttaagtaa cgcggattta acacacgcca gcttaaaata   | 96780 |
| [1921] | tgttgattta agatacgctg atttaagcta tgctatttta tcagctacag atttaagtag   | 96840 |
| [1922] | tgctgatttg agattttctt atttagataa tgctatttta aagtttacta atctaagccg   | 96900 |
| [1923] | agcaagtata acaggagtta taggactgaa tatttactct atagataata ttggaacttt   | 96960 |
| [1924] | taatggtaag gtaacttata ttcctagttt agacactgta tttgcaggat gttggaatgg   | 97020 |
| [1925] | aaatttagaa gcgttttttag aaaaagggtt agaaatgaat gaaggaaaag aagcggacaa  | 97080 |
| [1926] | gattaaaaaa gcatatgaat tttttaaagt gtgtacagat taaagattat taggctttta   | 97140 |
| [1927] | aagattaagt tattttatag gataaatact actagaaaat gattaccgtt taatacagaa   | 97200 |
| [1928] | ttttaacgta cctagtttag aggtcttaaa tataaaggag ggtatttagaa tggctaaagt  | 97260 |
| [1929] | tgtaagtaga ggatatagtt ttgaagaaca agaagtaagt atgaattata accaagggca   | 97320 |
| [1930] | atggattatt tacgcaagta ggaaacctta tattaccgat attatgaaaa aatatccgga   | 97380 |
| [1931] | taaggtagaa gtactagagc aactagataa tggtagacct gtactcgta aagttatatt    | 97440 |

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|--------|-------------|-------------|------------|------------|------------|-------------|-------|
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| [1933] | aaagattgct  | aaagaaagat  | ttggtaaata | attaaatata | ctagtgaaga | agaatctgac  | 97560 |
| [1934] | attaataaac  | accaacgtga  | taccgtaaca | cttgtaggaa | ctagactaat | gttacaagat  | 97620 |
| [1935] | atctagtatg  | gttatggctc  | tattgagaat | gagtatgctt | taactaaact | agttaaagaa  | 97680 |
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| [1937] | tttatacatt  | atttacattc  | tgagtaaata | tactgtattt | aatcttggct | aataattgta  | 97800 |
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| [1943] | acagcattat  | aataatatta  | cagttatgtt | acaagttgct | aagtatgttt | tgttactaca  | 98160 |
| [1944] | atatattgaa  | tatgaaaact  | ttagaccttt | tctgcagaca | agtaattatt | acaggatatgt | 98220 |
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| [1948] | tttatacagt  | aagtctttgt  | gagggaacag | aagaagattt | actagaactt | attgagaatt  | 98460 |
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| [1951] | ggtagcgaat  | gatagaatat  | aacacacata | cagaatacga | gttagttact | cattcagtac  | 98640 |
| [1952] | ttaaagttat  | acagtatgat  | gtagataact | atactgtaat | tattgatact | aatagtata   | 98700 |
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| [1956] | atagacctat  | aagattaagg  | ctctcttact | gttataaaca | tcataagtat | aaatctatga  | 98940 |
| [1957] | ttttcctaag  | tagcaggcgc  | tgtttttact | aaaaaatgta | aatatcgtaa | gtatcaggcg  | 99000 |
| [1958] | ctgttttccc  | tttatattaa  | ccgcttcaaa | caacataaaa | aaagattagg | ttaatagcct  | 99060 |
| [1959] | aatctataac  | tcttttatat  | aattcttgta | attctttttc | taactttttt | attgttttcta | 99120 |
| [1960] | tatcattatt  | tcttttaata  | gactgacata | taaatgcaat | acctttaatc | aaattaaatg  | 99180 |
| [1961] | gttagtttta  | cactgcattg  | tatacaatta | aatcgattga | ttataataat | taatttaagc  | 99240 |
| [1962] | tgtactttct  | agtttcttct  | atataatgg  | aagaaaacat | ttattttaaa | aactttcgta  | 99300 |
| [1963] | taacctattg  | acattaaata  | caagatactg | taatataata | agcgtagtaa | acaaaccgaa  | 99360 |
| [1964] | aggaattgat  | aggatatgaa  | cgtaaataaa | attggggata | tacttgtaga | acttttagac  | 99420 |
| [1965] | atggacggtt  | acaaaagcaa  | taactactat | agaaatgact | taaaatactc | tacacattat  | 99480 |
| [1966] | caagttaa    | atttagctta  | cattgatgat | agctatgaaa | cattagaact | atttaaagaa  | 99540 |
| [1967] | acgaaagtct  | ttagcgtatc  | acattcaaat | aggttacca  | ttaacattaa | tattaaggta  | 99600 |
| [1968] | gtaaacaaac  | acttatttat  | agagctagat | aatacgttat | acctagttaa | cgagaatgca  | 99660 |
| [1969] | gaaattatta  | aagaaaaaaa  | gttaaaataa | tagttgacac | tagaattaat | atagtgtaat  | 99720 |
| [1970] | ataatagggtg | taatacaaat  | aaactaaaag | gattgattaa | ctatgaaaac | attaattact  | 99780 |
| [1971] | aatagtaagc  | cgtataagaa  | ctacggctat | gaaatggaac | aattcgagag | agaaaacaac  | 99840 |
| [1972] | gttattatca  | cacgagcata  | ccacgaacca | acaaaacaag | gctatgacct | tgtagtacat  | 99900 |
| [1973] | tatacggagg  | ttaaataata  | tgaaatacgt | agactacatt | agaggcacgt | acaacagaat  | 99960 |

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| [1976] | aataacaaca  | acgccaaaat  | gtgttaaaat  | gatacttaat  | gacggatcaa  | caaagtttgt | 100140 |
| [1977] | taaaaaaggt  | taaaataatg  | tttaacatta  | cacttaagat  | actgtaatat  | aatagattgt | 100200 |
| [1978] | gtaaaaaac   | aaatcaaaaa  | gggattgata  | cacattgata  | aatttaataa  | taacggcaag | 100260 |
| [1979] | aaataccgtt  | caacacaaaa  | gacagatgct  | attgtaacta  | ttataggtgg  | tacagcagta | 100320 |
| [1980] | atcacattga  | tagactggtt  | tatttagatta | caagataata  | ttaatatatt  | tattaaataa | 100380 |
| [1981] | gggttaacca  | cccttatttt  | tttttgtgct  | tatataaccg  | cttcacatta  | gtgtaaaact | 100440 |
| [1982] | tcccttattt  | ctttgctaaa  | atactttaca  | aatatgagct  | tatactgtaa  | tatataaagt | 100500 |
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| [1984] | acactaacat  | taatatagtg  | taatataata  | gatgtagtaa  | acaaaaacaa  | acagaaaagg | 100620 |
| [1985] | aattgattaa  | ttatgaaaaa  | tacagtaaaa  | gaaatggcaa  | acggaaacac  | taaacttgaa | 100680 |
| [1986] | agattattag  | tttttagcaat | tgaggaaagc  | gttatgacag  | agcttggtga  | atatgatgca | 100740 |
| [1987] | ttagataaat  | acaaccactt  | agaacagcag  | ttattaaaca  | atgaaacatt  | aacacaagca | 100800 |
| [1988] | gaacgtaaac  | catctaacat  | tgactatagc  | acttacgagg  | gggctatat   | agcacaagac | 100860 |
| [1989] | aaagcagaac  | actttactaa  | cgttatctta  | aaccacatga  | acaaagaaca  | atttgaacaa | 100920 |
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| [1991] | attacacatg  | aacttatctg  | agaaagaact  | tatagatata  | atggacaacg  | ttaatagctt | 101040 |
| [1992] | cactggtatt  | gatgaagcta  | ttaacgaaat  | ggaaaacaaa  | caaacaacta  | aaggcagtg  | 101100 |
| [1993] | caaagacgca  | ggagcaatta  | agaaatataa  | tagtttagat  | gagttaagac  | aagactatga | 101160 |
| [1994] | cataacaaca  | ttagcaccag  | atgtaataaa  | agaattactg  | aataatcatt  | agtgtaaaac | 101220 |
| [1995] | ttcccaaat   | aattaactaa  | aactattgca  | attaactgta  | gatttgatat  | aatgtaatta | 101280 |
| [1996] | agggaaaaca  | aataaaagaa  | aaaggattga  | taaactatga  | aattacttaa  | ccaaatgaaa | 101340 |
| [1997] | caaacagcag  | aacttgaagg  | aattgaattt  | gacttattat  | taacagctat  | ctatgaaaca | 101400 |
| [1998] | atagaggtag  | aagctgttga  | acatgattta  | ctatgtaaat  | acattcata   | tgtaaaccac | 101460 |
| [1999] | ttaaaagaga  | ataaagacat  | aacaccatta  | gaatatatac  | cttctaacct  | agactttaca | 101520 |
| [2000] | gacaatacaa  | caataagact  | agttgataat  | aaagtgtttg  | agtttagcag  | ttatataatt | 101580 |
| [2001] | gaacatatga  | gtgttaaaaga | gttttagtaa  | cacattaaag  | actatgaaca  | aattaaagca | 101640 |
| [2002] | aaagctatag  | aatgatgta   | aaacaaattg  | aaagggaata  | aactcccttt  | ctttttttta | 101700 |
| [2003] | ttattaaatta | ggattgtata  | caatagggtta | atataaatat  | attatacggg  | ggcaggggta | 101760 |
| [2004] | aaatgccact  | aaagtgggtg  | ggtcttacag  | ggctataggg  | ctacctaggg  | aacctaatga | 101820 |
| [2005] | gtgagtaata  | ttatataact  | tattattaaa  | agtcaacatt  | aaccattact  | ttattaaact | 101880 |
| [2006] | atgtcaacat  | tactaataac  | taattgtatt  | catttttaca  | taaagttagt  | aatatgtttc | 101940 |
| [2007] | cttactctct  | tacacttaat  | tagagtatta  | gtatattacg  | acataataag  | agtttagtat | 102000 |
| [2008] | ataggagcct  | ttccttatat  | atagttttct  | atggtattct  | atggtattct  | ttagtatacc | 102060 |
| [2009] | attttaataa  | ctatttatgt  | aagataccat  | cagtttagtat | gggatattac  | ataacttatt | 102120 |
| [2010] | attaaaagtc  | aacattataa  | agtacagtgt  | actaaaaagt  | caacatagta  | ttattactat | 102180 |
| [2011] | taaagtcaac  | ataataggat  | ttccattatt  | gttcttattg  | tcctacctat  | ccctattgaa | 102240 |
| [2012] | ttactagcag  | tagtggtact  | ctattttctta | caatataaaa  | aacacctata  | gtaattaata | 102300 |
| [2013] | ggtgtttaat  | gtgaggatc   | atatccatac  | tcaacaatt   | ctttatctac  | ttgttcgaaa | 102360 |
| [2014] | ctttcttttt  | tatcttttat  | aatatagtta  | ccgcgtacaa  | ctttctcaat  | acctgaaggt | 102420 |
| [2015] | gttttaataa  | acaggtctatt | gtctccttgt  | ttatctactg  | ctctaaatgt  | ttgaccttct | 102480 |

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| [2016] | gtccactgat taataacatg cttattatat cctgtatatt gaatatatct tactacgtct   | 102540 |
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| [2018] | gtatctaagt ctaatggttt taattttgct atattgccta tattacgttc tcttacaggt   | 102660 |
| [2019] | gtttttgaat attctacat taaatctagt aattcttcac ttgcaccta actcctaaaa     | 102720 |
| [2020] | gaatattgtt cgttgagaaa cacccaagca atagggtgtt ggttttcttt tttaataagt   | 102780 |
| [2021] | atttttctt ttctcttggt agtattttta ttgctaagg taacagtata ttctaactgt     | 102840 |
| [2022] | tttacatfff caataaatc atttgatac atagggtgta cttcctttgt ttaattatta     | 102900 |
| [2023] | tccactatgt ctatttcacg gattgtaac atgtcatctc tatactcatc tatatgctta    | 102960 |
| [2024] | aggtaactga cgccatcatg tttctagac attccttgta attctctata tccttcggct    | 103020 |
| [2025] | ttaatatctt taacaagctg ttctttgtta gtgtatactt tatcttctct atatgagtag   | 103080 |
| [2026] | ctatcctcat aaggctcaca atagtcacgt tctacttgat atagtttcat attatffffa   | 103140 |
| [2027] | ctccttttta attttatctt ctaattctgg taatacatff ttgaaatat atttatftaa    | 103200 |
| [2028] | gtcatttatt gttttaactc tataagtacg catgtttctg ttactaatat ataaattata   | 103260 |
| [2029] | taaatcctct acctacttt caatataata gaaaggatag cttatacaac tcattaatag    | 103320 |
| [2030] | agatatacta tacagtatat ttccaggtaa ttctctagta aatftftftaa ttttatftac  | 103380 |
| [2031] | ctftftftct cttcttaatt ttttatatag cttctttagg ttgtgctcat gttftftaat   | 103440 |
| [2032] | tacaagtagt tgattttggt taagtaagct atcgtcgttt ttgtttatat agtttaatat   | 103500 |
| [2033] | tcctttatat aaagtttaga attctctatc atccatatta atattatctc cttatgttct   | 103560 |
| [2034] | acatttaacc agactaaagt ataaatgact tgaggctcta tatctcattt attctatgtc   | 103620 |
| [2035] | cttctftftac tgattgggtc catgatftta gttftftcaa gaacttggag tctactftac  | 103680 |
| [2036] | cttctaattt ttggtttaga taatcatcat agaaactaaa tgttccgcct ttaaaaaagc   | 103740 |
| [2037] | aataatacgt gtctgtcgt tagttaatat taatgtactc tatatagtct ttgtttactt    | 103800 |
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| [2039] | ttaaacctgt atatctftft agcttgaac cgaacctcaa ccaaaagtct atttcattga    | 103920 |
| [2040] | ggttaaaagg ttcatftact tttftaccta aaatactatg cataaatagc atataaaca    | 103980 |
| [2041] | gttctatata ctctatacat gttgttaaaa aatgtttgtt gtcttcaaag tgatggtaag   | 104040 |
| [2042] | ccaacaaata caaaacgta aaagggaata gaacaatgga cattaaaaa tataaaatg      | 104100 |
| [2043] | atfttaatct tgtgttcatt actcagacac cttctctaca tcatatagta ttgggtatag   | 104160 |
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| [2046] | gactgttttg ttgtctaata caatatagaa cttgtcttct ttatcttgtc tttgtttata   | 104340 |
| [2047] | tttatctgta attgttctt ggtgcgtttc ttgtgttga tagctagcca ctgtatagat     | 104400 |
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| [2050] | tcttcccaa ttagtctfta cttactftga tgcataatat agaagcgtc agatfttacta    | 104580 |
| [2051] | gatttaactt catttatfta catattatga tgagtgafta tftftctgtt atctagatac   | 104640 |
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| [2055] | tagactfttg tttctgtata tgfttcttga tattcacgag gtttacaatt atcatgttct   | 104880 |
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| [2060] | tatcattacc gtttacatct ttaactcttg tgctttgcat aatttcaagg ttttctccta   | 105180 |
| [2061] | aataaccttc acaagcaaat agatgtgaat atactgtgta atctgcaaaa tcaaatctat   | 105240 |
| [2062] | caatattaaa cattctttgt gattcttcat cccatgctct aaatttcaac atcatactac   | 105300 |
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| [2064] | taatgccata ttcttagctt ctctctcatt tatactttgc acaaaataat gcttttttat   | 105420 |
| [2065] | tttgtaatca catttagata ctaagaactt gatacaaaga cttactttat aggtttgcat   | 105480 |
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| [2067] | tagaatgctt tggtagggaa agacgcgttc tctaaacgtt ctttgatact ggtatttgt    | 105600 |
| [2068] | gcagcgctga catataggct ctttctcgaa actcatatac ttcaaacaac ctatcaaact   | 105660 |
| [2069] | tagtatcttc tgtgatttcc tcttcaactt tgacttcgaa aagagtatcg atagaaataa   | 105720 |
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| [2072] | ttaactcgtc taatggcatt tcttttttag ttttgatttt catttccata tctcctaatt   | 105900 |
| [2073] | atthaatttt tctatcattt tcacaatctt ttttgattct ctaatgtcag ctttctcaca   | 105960 |
| [2074] | gttttttaat atgaaattaa ttttgttttt tacaacatta acgataatgt ccacttcttt   | 106020 |
| [2075] | ttctgttggc tcggtaggga aaagcaaccc tacaactatc tgaatttgat ttttatttag   | 106080 |
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| [2077] | tacctcatct acaattgtat ttaattcttg gataggttta aaataatgtt gttttctttg   | 106200 |
| [2078] | tatcattacc tgtgacatta gtttatatag tttctgttct ttatcaagca tttcttttgt   | 106260 |
| [2079] | aagtctttta acttccccta tctttatagt attacctcgt gcatacataat tattttta    | 106320 |
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| [2081] | gtagtaacag tcctcttcta catatatagc atatatctta ttcatactta tcaaaccttt   | 106440 |
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| [2083] | tttacagtaa acaaaaaaaaa gaaccttttt acaggttctt tactagggtta ttaatttttt | 106560 |
| [2084] | aaatcttgat ttccttttat tagcttctta tcatcaatag gatgaccatt gtataaatat   | 106620 |
| [2085] | tttacttcca tatatacatg attatccgta gccgtaaggg catctaggat atccggattt   | 106680 |
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| [2087] | acatgaaatt tttcttggtt tggtaaagag taattaaggt cttctaaatt ttgtaggaat   | 106800 |
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| [2089] | agtcttgtca tttcctcttt atttaaagtc attgtttcat aatcgttaac atcataaaaa   | 106920 |
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| [2102] | atatcaaaag  | taaactcatc  | atataaacaa  | tctatatgct | tataaataaa  | atatttttgt  | 107700 |
| [2103] | ttttccgtag  | ctttgtttaa  | ctcattgctt  | tttttacita | taaattgact  | aaagttatca  | 107760 |
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| [2105] | aatttcaccg  | taagcaaaact | ctaaacactc  | ttgtaatgac | atgtcgtggt  | gctgagctaa  | 107880 |
| [2106] | aataattaaa  | gtaactacag  | tgtccccaat  | accatcttta | agagcttcta  | ggttaccacg  | 107940 |
| [2107] | tgataatgcc  | gatgctactt  | cacctgcttc  | ttcgtagaat | ttaagagctt  | gtctatcagg  | 108000 |
| [2108] | gttggtattg  | ttcaatcctt  | tatctttact  | ccattcctct | acttgactaa  | ttaaaccctc  | 108060 |
| [2109] | tagcgtgcgg  | tcttctgttt  | caccttcatt  | aaagttaggt | ttttcatcat  | ttggtacatg  | 108120 |
| [2110] | ttcttcttgt  | tctctatcct  | ctattgtaat  | aaataaagtg | tctattttctt | tttcgtactt  | 108180 |
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| [2115] | tccatatatac | atagtttttt  | cttcaactaa  | accttctagt | atcaactcta  | catttttctgg | 108480 |
| [2116] | ttctaacacc  | gcactatcta  | agttttcaaa  | aacaatttca | atatttttaa  | tttttttagt  | 108540 |
| [2117] | cattaataat  | cattcctttt  | tttttagtata | tagtaaatac | aacacataag  | taaataagaag | 108600 |
| [2118] | tcaataaaaa  | aaaacaccta  | ctttaaaaat  | aggtagggtg | aagatatttt  | agtaggtatc  | 108660 |
| [2119] | ttgtggaaaa  | aaatcctgag  | cagggaactcg | aacccctcga | ctaggtagct  | acaactagcc  | 108720 |
| [2120] | aatgccatta  | ctcaggattg  | ctagtaacgc  | taaatagggt | cataacgtta  | ccgtagacct  | 108780 |
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| [2122] | ttactctcat  | tataagggtta | aaaaatgcta  | actgtgttgc | gcattgttaa  | gaggcggttag | 108900 |
| [2123] | ttaactacta  | taataatata  | gcataatatt  | tattgtttgt | caagttcttt  | attccattta  | 108960 |
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| [2125] | ttttctttgt  | agaacacttt  | agctaaatat  | ctaccaaag  | aatcatcttt  | gtatgtttga  | 109080 |
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| [2127] | ttttgtttct  | tttctgggtg  | gtcgacacct  | agtaatctta | ttctttgttc  | agaatatggt  | 109200 |
| [2128] | ctcattccgt  | ggtcaatagt  | tacatgtata  | gtatcccat  | ccactacttt  | gtccacgtgt  | 109260 |
| [2129] | gctttaaata  | aatataaatt  | gtcttctatc  | atcattaaat | ccccttttta  | ctaatttaaa  | 109320 |
| [2130] | attatccaat  | cagtattgct  | tgatgtacct  | ttagctacat | atacagatga  | gcctattata  | 109380 |
| [2131] | gcaatttgac  | ctttgtataa  | aggtatatta  | ctaagtcttt | cagttacacc  | tatttggtctt | 109440 |
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| [2666] | cataagtatt ttatactgtc aactattaaa ttaagaattt agcaacggat tgtcatatgc  | 6240 |
| [2667] | ttctttaagc acttagagtt tcccgtttta acaaggtttt acttgaacaa agtttatcca  | 6300 |
| [2668] | agctgtctac ttttaattac tactttattt ggggtgtgtg catttactat catttactac  | 6360 |
| [2669] | cctcggtttc ccgatattta ttaggggact agactatctc atacatgtac cttttgggtg  | 6420 |
| [2670] | acatgccctc tttctttacg ggttttatcc ctcttatact gttctaccta atcccttttg  | 6480 |
| [2671] | attagttacc agtaagttaa gtcgttaggc atttacgtaa ctaaggtata tatcctagtt  | 6540 |
| [2672] | cgatttagca attgattgcc ctctcttcta cgtaggggt ttccaatttt agaaaagggt   | 6600 |
| [2673] | tatagtcgcg atagtatgtt tacggacttg ccaaggctca tgagcttggt ctttacttct  | 6660 |
| [2674] | gtccctgttt ggaatactaa aagttatcgg gtgacctctt aacgtatgat attttagcat  | 6720 |
| [2675] | ataagtacta ggatttaaca tattgataat atggccaact tgttcttgcg taacattttg  | 6780 |
| [2676] | tgtaccgaat gtttcttggt ttatttttaa taattcttta ccgtccattc atatacacct  | 6840 |
| [2677] | ttcttttaga tgtatttgag ttctcttcgt tcataacctt ttctttatca ataatacatg  | 6900 |
| [2678] | cagtaatgtc atcagcagat agttgagata acttctctaa atctacttct ttctcacctt  | 6960 |
| [2679] | tactattctc atttgttatt tcatcaaaga gctcttggtg tggctcttgat aattgcggta | 7020 |
| [2680] | tagcaccacc accatcagaa ccatctgtaa ttaagttttg catttggttg aagataacaa  | 7080 |
| [2681] | acactttata caaatcatta gggctcttcta cttctatttc ccctctatca atctttttat | 7140 |
| [2682] | taaagtcata cataagttaa gtaatactg acataaatcc atcagctata tatttacgtt   | 7200 |
| [2683] | tatcttcgtc ttcaattttc ttcttagata acatatctct taaactttca gcgtcactc   | 7260 |
| [2684] | tatcaattca cctactttct ttaaattctt gtaacagtta ttaatatctg cgcataatgc  | 7320 |
| [2685] | aaaatataag tacttagagt actgtaaagt aaaatgatta acttttagcta tagctttacc | 7380 |
| [2686] | tttagtataa ttcatctctg aaagagactg accacataaa caacacaccc tactaggact  | 7440 |
| [2687] | tgcaatacgt ttatctcctc tttttgttaa ggaaacagcg gttgcattat cttttagctc  | 7500 |

|        |                                                                    |       |
|--------|--------------------------------------------------------------------|-------|
| [2688] | tttacgttgt tccttaatgt ccttcttcgt tttcatcgac agcatctctc tttttatctt  | 7560  |
| [2689] | ttagtactcg acgttgtgct tttgctctta ctcttacttc ctcagcagaa acattagttt  | 7620  |
| [2690] | gtgactctac aattttcttt aatacttgta attcattggt atttagctct acttctttta  | 7680  |
| [2691] | gaatataatc gggtaagggg tcttctttgt gtaataataa tttatcagtt attgttaatg  | 7740  |
| [2692] | ccacattaga aactatccca ccaaatataa agtaagtcac aactgtacta ggtcgaatag  | 7800  |
| [2693] | agtatattaa tgacagttag ttaataatag acaaaataaa caaaataaaa ataataatta  | 7860  |
| [2694] | ttagagtttt aaatataaat acagctatgt tattattcac cctattagtt cagtcctcct  | 7920  |
| [2695] | aatttaaaat ataccgtaca ctattaatgt aacattgttg atttataaac aggtttcaat  | 7980  |
| [2696] | gttatattaa agttgtatta tataagattt tattatctag ctaaaagaaa ttaggtgtaa  | 8040  |
| [2697] | ttagtggtta tactagcaag cattatagct atacctcttt ttatttttta tttttacttt  | 8100  |
| [2698] | ctgttttagct tatctaaatc actagcatag gtagtagtat taaaagagtg taaaaacact | 8160  |
| [2699] | atgaaatata taggatactt ggtgggtgtt tatatattat cttgtttttc attatggtta  | 8220  |
| [2700] | ataagtacag gtagttgttg gttgctaatt ttattaaacg taatactcat atctgctact  | 8280  |
| [2701] | acaataacta ttataacaca aaactagtgt agctcaaaat taattataag ggggtaagtt  | 8340  |
| [2702] | gatttgacta agtataaaga tatattaaaa ttagagttta aagatgcctt agctcacttt  | 8400  |
| [2703] | agacgagata gaaaattctt ccacgtctat aggattgata ggttgcttat caacggctct  | 8460  |
| [2704] | attatatatt ttgattatta ctacatacca tctgataatc ctaataccgt tattaaagaa  | 8520  |
| [2705] | atagacctcc atgagtttgg taagcttagg tttgaaataa atactaaaac gtcatatggt  | 8580  |
| [2706] | gagatagtta ctgaggatta tatgcaaatt attaatgact ttttcgaaaa ctatgacgtc  | 8640  |
| [2707] | cattcagaat ctgaaaagtt agacattaag aggtgacttt agttgaatga agaactaaaa  | 8700  |
| [2708] | aacctgtcg gacaagtaga taagataaaa gataaaatac aagatggtag ctatattgat   | 8760  |
| [2709] | aaaaatacgt ataaggaatt agaaaatgaa gttgatgaac taagaaaaat ggtagttagt  | 8820  |
| [2710] | ttagacaaag aagtagctgt taatagttag aaacaatcgt ctatctacat acagctagat  | 8880  |
| [2711] | agattagacg aaaagataaa agaactcact aacaatacag ttgttaagga cacaagaaag  | 8940  |
| [2712] | aaggatacta ccgagaaagt actactacta gtgttaggtt ctattataac tttcattttc  | 9000  |
| [2713] | aataaaattta gttaggagtt ttgatatggt aaaacatgct gaggtaacaa tgattagtgg | 9060  |
| [2714] | aaaaaaatat tatttaatct ctgatgatag tattgagtat acacaaagag aaataaatgg  | 9120  |
| [2715] | aacaattaga ggacatggac aagcatcagt tagagttaaa gtatctgagg atattaattc  | 9180  |
| [2716] | tgatacagta ttcattaatc ctttattttat agaatcattt aaattagtat ttgaaaatta | 9240  |
| [2717] | gtgactttta agtaagtcac ttttttatta ctctttgttg actttcctat atatatatta  | 9300  |
| [2718] | ttaattatta taactagtat tattatttta gtatttataa ttatttatta tatatataag  | 9360  |
| [2719] | gaatctcctt tattttccaa ataggtaaag tatctaaata ttcagaataa ttaaatataa  | 9420  |
| [2720] | ctagtaataa gcttttatca aaacaacagt atttgtattg acatcaaaat aattatatga  | 9480  |
| [2721] | tatactagct atacaaaaaa ttttaaaagg attgataatc atgaaaaaaa gacagaaaat  | 9540  |
| [2722] | gttccacagt aatttagttt gttcagaatg tggttaatact tttacagtac caagaaaaag | 9600  |
| [2723] | agctcaaaga agagaagaag gacacataaa acatttacat tgtatcaggt gtaaatgtac  | 9660  |
| [2724] | aactgtcac attgaggata atagaacaga atcagaaaaa tattgggata aaatacaaga   | 9720  |
| [2725] | agaattatca agataaaaaag aaaaaagaac ttgacaaacc tagaatatta tgttatatta | 9780  |
| [2726] | tagttaagct tgcatagaga ttaatttgtt gtcaaattga agtgttattc ctcttatttt  | 9840  |
| [2727] | ttatttgtcg aaactaggtc ttttaaaact tttttaccct agtataaaaa aactataaaa  | 9900  |
| [2728] | agttataaat ttttcttgac aaccgaataa atatatgtta tactttaaaa gtagtattat  | 9960  |
| [2729] | taaacataca tacataatgg ctgatttaaa cagggtggta cctgtaaggc gtgagattgt  | 10020 |

|        |            |             |            |             |             |             |       |
|--------|------------|-------------|------------|-------------|-------------|-------------|-------|
| [2730] | cgaacgaatt | aaaacttgtc  | ggtttatata | ggtgagataa  | ttcgtcaata  | gtaatgctta  | 10080 |
| [2731] | ttacaatcga | aatactcagc  | agtaacatat | taggtttata  | aagtatagta  | tctgatgggc  | 10140 |
| [2732] | ttaatttttg | taaagggtct  | tggtggaatc | ctttacaaat  | acaccttaac  | aagtatatgc  | 10200 |
| [2733] | agttggagca | ttgaaaaatg  | tttgataaca | tacgatacta  | gtacaagtag  | cataaacctg  | 10260 |
| [2734] | caaaataata | attgggtcaaa | ttatagaata | ccgctaaaat  | tcttcatacc  | atgtgaaagt  | 10320 |
| [2735] | tactaattaa | tatgttagta  | tcacattaca | atattaaaca  | gaatcaaccc  | tacggttgta  | 10380 |
| [2736] | ctgctttaat | aggttacgtg  | agatggactt | aggttagcta  | cctaataagag | tcaagcgtag  | 10440 |
| [2737] | tgattaactt | aacaataggt  | ctagtatgtt | acttaccagt  | gagacggttt  | atgtatgtat  | 10500 |
| [2738] | gtttaattat | actatagaag  | ggtagatact | aatatggcat  | ctgcaaaaaca | aatttattat  | 10560 |
| [2739] | accgaaagt  | taatcggtag  | agcaattatt | aatgaaaaat  | tcacaactaa  | aaaagatatt  | 10620 |
| [2740] | tgggataaat | tagatttact  | tccacacact | aagttagaag  | acttagataa  | taaacaaatg  | 10680 |
| [2741] | tcagaagtaa | tcaaaacact  | ttctcaaatt | aacgaacaat  | aacttaaaat  | ttaataacta  | 10740 |
| [2742] | aaaccattct | ttttgttata  | ttattattag | ctttacaata  | acagaaagga  | tggtatTTTT  | 10800 |
| [2743] | ttatgtcaaa | aaaagataaa  | aaaccattat | taatagcagg  | taacaatatt  | actgcaaaag  | 10860 |
| [2744] | caaaaagtgc | tacatttttg  | acaggttttg | tacctgtttt  | agctacgttt  | attgttagtg  | 10920 |
| [2745] | tatgtagttt | actaggaatc  | aaaggtgcag | aagatattgt  | aaaccaaggc  | actgcaattg  | 10980 |
| [2746] | caggtgtagt | tattagtttc  | ttgactgggt | taggtgttct  | tgtaagtcac  | gacactaaag  | 11040 |
| [2747] | gtatagcgga | tagccctatt  | gtacaggact | ttaacaaacc  | tagagattat  | aatgaccagg  | 11100 |
| [2748] | ataaagcttt | aacatacaag  | agtgaagcaa | tgaatagtag  | ctcagagtta  | cctcctacaa  | 11160 |
| [2749] | agatgccgat | ggctcctgaa  | ggtgtcaata | atgaggatat  | agatacaagc  | catgcagaac  | 11220 |
| [2750] | cattagaaca | tggtaaagta  | gaaaaagact | tagtgtctga  | attagataaa  | gaagataacc  | 11280 |
| [2751] | caaaagaagt | aggtgaacag  | taatgataga | aatttggaaa  | ggtgtaagtg  | ttagatatga  | 11340 |
| [2752] | tttactgcct | attggaacaa  | gacgcacagg | tcaagctatg  | gcggttaaaa  | aaccggtttt  | 11400 |
| [2753] | tattgtagct | catgatacag  | ggaatattaa | tactacagct  | caaaataatg  | tagattatta  | 11460 |
| [2754] | caaaaataca | tataacattg  | attgggcttc | tactgcatcc  | gcacatatct  | ttgtagatga  | 11520 |
| [2755] | taaagaagct | atcatatgtg  | ttcctactac | agaaaaagct  | tggcatgtgt  | tgtataacgc  | 11580 |
| [2756] | tcctatggat | gacctatggt  | acggaattaa | cgctaatgat  | ggtgctattg  | gtgtagagat  | 11640 |
| [2757] | ttgttacttt | agtataaaag  | aacgctctaa | aaaatcatta  | gataatggag  | ctcgtgtcct  | 11700 |
| [2758] | agcttattta | gctgaatact  | ggggtgttga | ttacaaaaca  | aatatgccag  | gtcaccaaga  | 11760 |
| [2759] | catccaaaac | gacaaacaag  | accaggttaa | cttattagaa  | gcatgtggat  | taggtagaaa  | 11820 |
| [2760] | cactagtaac | ttagataagg  | aagtagctaa | gtattacaag  | aaagttatta  | agtcagtaga  | 11880 |
| [2761] | tgtaaagaaa | tcaggtaatg  | ctgagcctag | agtaaaaaca  | caatctacta  | aaactatgtg  | 11940 |
| [2762] | gagctggaaa | ggtacttttt  | atccagatag | agctattaaa  | gttagaaaat  | ctccaggact  | 12000 |
| [2763] | taaaggcact | atagtaaata  | gagattcatg | gctatacgat  | aaggatgatt  | gggttgagtt  | 12060 |
| [2764] | tgaccaagtt | attaaagcaa  | atggctattg | gtggattcga  | tttaaataac  | caaaaccagg  | 12120 |
| [2765] | ctctagtaaa | aattatttct  | attgtgctgt | atgtgagata  | acagataaac  | aagagcgtat  | 12180 |
| [2766] | taagaaagaa | aaatatTTTg  | gaaaaataga | ttggaaataa  | ggagtgaaaa  | cttttgaaag  | 12240 |
| [2767] | catcgatgac | tagaagtga   | tttgttcgat | ttttaaaaag  | tctagaaggc  | aaagcaatag  | 12300 |
| [2768] | actttgatgg | ttggtatggt  | cagcaatgtt | acgacttagc  | taactatgga  | tttaacaagc  | 12360 |
| [2769] | tcttcccagg | ttattcttta  | ggtggagcaa | gtgcatgtaa  | cattccatgg  | gataataaag  | 12420 |
| [2770] | ctatgttaaa | agataaagct  | agagtagtag | aaaatacact  | atcctacatt  | cctaaaccag  | 12480 |
| [2771] | gcactatggt | tatctttaat  | aattcatacg | gaggcgggtca | tggtcatgtc  | gcattgggtac | 12540 |

|        |         |          |         |        |        |        |          |         |        |        |         |        |        |       |
|--------|---------|----------|---------|--------|--------|--------|----------|---------|--------|--------|---------|--------|--------|-------|
| [2772] | taagtgc | aaaaccaa | attatag | taa    | ttgaaa | ataa   | ttggttag | gt      | ggaggt | tgga   | 12600   |        |        |       |
| [2773] | cttgggg | gaga     | tgctca  | agga   | ggcgg  | tggt   | gggaga   | aaagc   | taccgt | taga   | gcccat  | gtgt   | 12660  |       |
| [2774] | acgact  | tttcc    | tatgtg  | gttt   | ataga  | accta  | atttca   | aaaaa   | cgaggt | taca   | acaact  | ttgga  | 12720  |       |
| [2775] | attgggg | gagg     | taaatt  | tact   | gctga  | tagaa  | cgatta   | aggt    | aagacg | caca   | ccaggt  | tttaa  | 12780  |       |
| [2776] | ggggtt  | caat     | agtagg  | tgct   | gaatc  | attta  | tttatag  | tgg     | tcaat  | atgta  | aacttt  | tgacc  | 12840  |       |
| [2777] | aagttat | taa      | agcag   | acggc  | tactg  | gtgga  | ttaggt   | tttaa   | atata  | cctaca | aaccgc  | tcag   | 12900  |       |
| [2778] | ctggta  | actt     | ctatat  | ggca   | gtatg  | taaaa  | tcactg   | acaa    | gtggga | acgt   | atgcta  | aaaag  | 12960  |       |
| [2779] | agaagt  | tattg    | gggtc   | gtatt  | aactg  | gaaat  | aatgt    | actag   | ggaatt | tttcc  | ctagt   | at     | 13020  |       |
| [2780] | tttatt  | tttta    | agttg   | acaat  | gtattt | atca   | tatgct   | taca    | tacata | tata   | agtttt  | aaag   | 13080  |       |
| [2781] | gtggtt  | tataa    | taaatg  | gaaa   | aattaa | agaa   | atggtt   | taaaa   | cgta   | atgtat | taaaa   | agtga  | 13140  |       |
| [2782] | gattac  | gcag     | tacaa   | agtat  | atatta | agca   | tacacg   | ttca    | aataaa | agta   | atgttt  | tcta   | 13200  |       |
| [2783] | tgтта   | aatatg   | aaagc   | taaag  | gtaa   | agtaga | ggc      | taa     | agaa   | aaaacg | cttg    | acgttt | taaa   | 13260 |
| [2784] | taagt   | atgct    | tataa   | taaaa  | ataact | atag   | tatttt   | taaaa   | attga  | agaaa  | gatag   | gtagt  | 13320  |       |
| [2785] | ggttt   | tattat   | ggaag   | agaat  | aaagt  | attaa  | attta    | aaaaa   | agta   | agagaa | gaaaa   | aggtc  | 13380  |       |
| [2786] | atacag  | ttag     | aagtt   | tagca  | caagag | attg   | gagttc   | acta    | ctcact | tatt   | tcttatt | ggg    | 13440  |       |
| [2787] | agagt   | ggtaa    | aagaaa  | acct   | aagagt | gcta   | atctt    | atgaa   | attaga | aaaaa  | gcttt   | aaata  | 13500  |       |
| [2788] | ctaata  | gtgt     | agaatt  | at     | aaagag | gttg   | atgtt    | gatga   | ataa   | attt   | ac      | aaaatc | tatt   | 13560 |
| [2789] | attaac  | tttg     | ttgag   | cgtca  | tgag   | attgag | gat      | atgatta | atattt | tttga  | acttca  | agat   | 13620  |       |
| [2790] | gttaata | aaag     | caagtt  | gatt   | atgatt | ctaa   | aaacc    | cta     | attaga | at     | catata  | gtac   | 13680  |       |
| [2791] | tgaag   | aaaaa    | ggta    | atacaa | tgca   | aaaagt | agtag    | agcat   | ttaag  | taata  | aattag  | ataa   | 13740  |       |
| [2792] | ttgttt  | tatat    | caatc   | tacc   | ctaata | tata   | tata     | tagt    | attatt | tagaa  | gtacg   | gtga   | 13800  |       |
| [2793] | gttatt  | tagga    | gacct   | atcgt  | atatc  | gagga  | ttatt    | tact    | ggtgat | gact   | taaca   | acat   | 13860  |       |
| [2794] | tattaa  | agac     | acacat  | ctag   | aatata | taca   | caac     | attatt  | aatag  | tagtg  | aatttg  | atag   | 13920  |       |
| [2795] | tatcac  | atta     | ttag    | agcagt | ttga   | actttt | aga      | agagt   | tca    | tacag  | acttg   | aaga   | agtaaa | 13980 |
| [2796] | agaaaa  | atta     | gaag    | agctag | aacact | caga   | aaagt    | acact   | gaaatt | gatt   | taatta  | agta   | 14040  |       |
| [2797] | ccttatt | aaa      | ttata   | aaagg  | gttttt | taaa   | tgttta   | agaa    | attgtt | cgtt   | at      | tttag  | ttg    | 14100 |
| [2798] | atgg    | ttttga   | agttaa  | tggt   | attgc  | agatg  | actata   | tcga    | agcaga | agaa   | cattgt  | tata   | 14160  |       |
| [2799] | agtttt  | tttga    | tttct   | tact   | ataaa  | attac  | aagact   | taca    | agag   | attt   | at      | acaagt | gaga   | 14220 |
| [2800] | tgttcc  | cttg     | ctttg   | tagaa  | gggc   | ataaat | atgc     | ataaga  | ctgc   | atta   | at      | atac   | agat   | 14280 |
| [2801] | ggtag   | ctcat    | cttata  | ataa   | aggtc  | ttgtt  | ggag     | caggag  | ctatt  | at     | agata   | aaaat  | 14340  |       |
| [2802] | gaga    | acattg   | taaa    | agaaat | cagta  | aacct  | attg     | aaaata  | gtag   | ttta   | ta      | agtata | at     | 14400 |
| [2803] | aatgtt  | gcag     | gtgaa   | atact  | agctt  | gttgt  | tacg     | gtattg  | aagagg | ctat   | aaaact  | taggt  | 14460  |       |
| [2804] | tata    | agcagg   | ctatt   | attca  | tgtag  | attat  | atagg    | actta   | ttca   | atgg   | tga     | agg    | ttcg   | 14520 |
| [2805] | tgga    | aaacta   | aaaaca  | at     | agta   | aaaca  | tata     | ttaata  | tgcta  | aggga  | atact   | caaag  | 14580  |       |
| [2806] | tttat   | agata    | taaatt  | ttat   | aaaa   | ataaag | gctcat   | gata    | ataca  | aatg   | gaatga  | atat   | 14640  |       |
| [2807] | gtag    | atgcat   | tagc    | aaaaa  | agca   | attgga | atata    | aggag   | aaat   | attatg | atg     | aaaaa  | at     | 14700 |
| [2808] | tatc    | aaaaa    | gtttc   | aaaaa  | ggtgg  | at     | gggaa    | agtt    | at     | tttt   | at      | acag   | ttaca  | 14760 |
| [2809] | cattac  | tagt     | agtag   | ctaca  | tggttt | tttag  | tagat    | agtg    | aatgt  | ttttc  | ttaca   | acag   | 14820  |       |
| [2810] | tatta   | acatt    | ctggg   | gcttg  | gtttt  | attag  | cacct    | attgt   | agct   | ctaggt | attatt  | gctt   | 14880  |       |
| [2811] | ttag    | tatttt   | aaagt   | atcca  | agag   | taaagg | atatta   | atga    | ttact  | cggaa  | ccag    | ttagaa | 14940  |       |
| [2812] | agtact  | tata     | aggag   | actaa  | aata   | atgaca | gaatt    | aaaaag  | tta    | agatag | aaca    | ataa   | 15000  |       |
| [2813] | gtta    | aaacaa   | aagat   | gttaa  | taaa   | attag  | gagt     | acttaa  | aggata | ccta   | tgaaa   | aaacta | 15060  |       |



|        |                                                                    |       |
|--------|--------------------------------------------------------------------|-------|
| [2814] | gatatggaac aatctataga ctttcaaat agaacaccgt atttaattag tgatgatggt   | 15120 |
| [2815] | acttttattg catagtattt agaaaaagca aatgctgtag gtttttataa ggagtctatt  | 15180 |
| [2816] | atacaaggaa aagttaaaca acttggtcat gtaacacctg cagatataga acatataacg  | 15240 |
| [2817] | tggactattg aagaagatga tattgtagac ttatataacg atgaattgcc tgttgagat   | 15300 |
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| [3182] | gtattactac | tatgtagcta | taccatttac  | atagtacctg  | gacagaccga  | tataggtata  | 37200 |
| [3183] | taaaagggtg | ggtatttagt | gtattttacc  | cctgcccccg  | tataatatat  | ttatattaac  | 37260 |
| [3184] | ctattgtata | caatccta   | aaataataaa  | aaaaaagaaa  | gggagtttat  | ttcctttcaa  | 37320 |
| [3185] | tttgttttac | atcatttcta | tagcttctgc  | tttaatttgt  | tcgtagtctt  | taatccattt  | 37380 |
| [3186] | attagattct | tcaacactca | tatgttctaa  | catataatca  | gctagttaa   | acactttgtt  | 37440 |
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| [3198] | gtagtaaaag tgttctgctt tgtcttgtgc taatgtagca cctctgtaag tgctaaagtc  | 38160 |
| [3199] | aatgtttgat ggtttacgtt ctgcttgtgt taattcttcg ttgtttaata attgttgctt  | 38220 |
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| [3236] | caacgtaat actttttagg tagtatttat cctataatac ttacttgctt atataatgta   | 40440 |
| [3237] | gttaaataagg caatacaagc atataaaaaa tctagggtaa aaacaaccct agctacttaa | 40500 |
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| [5067] | tcaaaacat taacgaaacg gtcaatatct tttattaagt ctttcacttc ttctttaaag   | 13680 |
| [5068] | tcattcattt gtttcatttc tgcaacttta aataaagcaa atgcagatgt taaaccagca  | 13740 |
| [5069] | ctatattttt taaactcatc atgaataata ctgtcaatcg taccgtcgtt taaccaacct  | 13800 |
| [5070] | ctgaataagt ctttcgcttg ctctggaaat gctttcatta agtcgtccca gtttctaaaa  | 13860 |
| [5071] | cgttttttta actcaccgtc atagtcccaa atacgacgtg ctaatacttc aatgagtttt  | 13920 |
| [5072] | gataatcttg aatatataatc ataatacgat ttggaatttg tattataatc tgctctatca | 13980 |
| [5073] | tcgtaaaacg gtgtataacg ttctctcggt ttataaattt cgtctaaaaa tggacgaatg  | 14040 |
| [5074] | tcatcaaaat atttaaaatc gttttcatta tataccataa ttttcacact ttaccaaatt  | 14100 |
| [5075] | tgtaaaaaac atttttatc aaattcattt aaaattttct ttcttaaatc gtatacttta   | 14160 |
| [5076] | tcaatattat caattaaata ctgttttgaa aattgtgtac ctttcgcatt acctttttga  | 14220 |
| [5077] | ttttgattac gttttgcgtt ttgattactt tcgttacttg atttattgac ggatttaccg  | 14280 |
| [5078] | ttatcaatag tgttattgtc tgcaaatgtt aacgttggtt tatctacatc aatgttaacc  | 14340 |
| [5079] | tcgctttgtg gtaatgacgc ataagcattt ctgtttgtct tcataacctg tgaattgtct  | 14400 |
| [5080] | aatgaagtag cattttgatt tgacgtttca tcagtgttgc ttgttgatc ttcatgtgt    | 14460 |
| [5081] | tctgtaaaagc cttgtgattg tagatatit tcaacttcgc ttgatgaata aaccacattc  | 14520 |

|        |                                                                     |       |
|--------|---------------------------------------------------------------------|-------|
| [5082] | aaataatcct catgtgtgat acatacagta atcacttgca tgccaaaagc ttcaactggt   | 14580 |
| [5083] | tgtctgttga tttctctatc taaaaagtgt attgtaaatg attttttaaa aagtaagtca   | 14640 |
| [5084] | gataagtcac ctttaagttt aaaaccttta aacacttttt cattaacgat ggctaaaacg   | 14700 |
| [5085] | tctttgtcaa acttcaacat tttttgcatg aattgaaatt catcatcata aaacgttaat   | 14760 |
| [5086] | ttatcattat ttacaaattc attaaaacct tttttaatta attctgattt aataaaatca   | 14820 |
| [5087] | aataaagtca ttgtatatct agccattgta ttcactactt tcatcattag acaatgtatc   | 14880 |
| [5088] | tatcattgtg atttctgaag taacttcacg gtcgtaatac ggtttaatat ctaaaccata   | 14940 |
| [5089] | acgttttgat aagaatgtaa tcggttcacg acctttttaa taaatattac tatttgatgt   | 15000 |
| [5090] | ggtaaaacct cggttgcttt ttgcctcttc gtccgaaacg ccgctttcct tatccactgc   | 15060 |
| [5091] | tagtgaatta atacctaaat agttacttaa ttcactaatt ttattttggt attcgcggtt   | 15120 |
| [5092] | catctcagtt agtgctggaa tcacactatt actggttaaa tctataatat catcatcggc   | 15180 |
| [5093] | gttaaacata ggtgacattt taacaaatgg tgcaccgtta tatatttccg atacaagttg   | 15240 |
| [5094] | attaactgac tcatcattaa tgtctgattt aaatatattg ctaaattttg cttgcataat   | 15300 |
| [5095] | taatgaaaat cgagataaaa caacctcaga taattcatca gtataatgtt ctatgatttc   | 15360 |
| [5096] | tatatcacta ttatattgaa ttggtttatt ttgcataaca acaaagttac cactcataca   | 15420 |
| [5097] | gttatcatat attttatgaa tctgtaagca ttcatctggt attaaatagt ctgggacaat   | 15480 |
| [5098] | gaaataaata tcatcttttg ttaatcggtt ttgaaattga aagttaaagt ttgatgaaaa   | 15540 |
| [5099] | atttgggtgct tgattaaagt aagtattatt tacgtaacca agaatacataa tttgttcatt | 15600 |
| [5100] | tcttgctttg ccaaccacta cattgatggt ttgtcttaat gctgattcta attgaataaa   | 15660 |
| [5101] | atctatacca accgtatcac gattggtata gttgatgagt agtggtaaaa attccaaata   | 15720 |
| [5102] | acgattaaac ataagacgtt taaatctggt gcgatgttca acaactcttt tgttgatttc   | 15780 |
| [5103] | ttttgataat tcaacctgta cgcttttatt atgtttagtc atttatagca cctctattat   | 15840 |
| [5104] | tctgttcttg atgttacatc ttggtcagta attaaaattt tattaaagaa tgggctaatt   | 15900 |
| [5105] | gctttaaatg aatagtaatg aatccagttg gtgacttcat caaattcacc attatagaat   | 15960 |
| [5106] | ggttgtttta acataccttt tgtgtaacgt ttatatatta ttgaattaat atccaaaata   | 16020 |
| [5107] | aacgcgtata aatctgattt tggtttaatt tcttcaacac tatcttttaa ctctgatagt   | 16080 |
| [5108] | tttgacacat cataagtga tactgaacca actggaattg tatcaccttt atgagtttga    | 16140 |
| [5109] | taatcaccat aagcacgcaa gaaagctact gaatcatcac ttgaaacgac aatgtcttta   | 16200 |
| [5110] | gtaactttaa acacaccacc taaatcatca aaactaatga cgtgggtctgt gaaatcaatt  | 16260 |
| [5111] | ccagcaattt ggaaggtatt cgcaatcttt gtatctaaaa gataagattt taatgaatct   | 16320 |
| [5112] | gttgtaaaaa tcacaatata ttttaatttt gagactgttg tatattgtcc aatagcacca   | 16380 |
| [5113] | cctgaagcac gatgaacttc attatattta gcaactgttg tttgtaagtt aagaatagct   | 16440 |
| [5114] | tcaaaaactt tacttgctaa atcctctttt gatgttgctt tacgtacatt tgattcagat   | 16500 |
| [5115] | aattgattca atgagtaatc aactaacatt gcacgcattt ctttttcttc taatacgtta   | 16560 |
| [5116] | atatcagaaa ttttcttttt atagacacct aatgcataat tagttgcgtc tgctaattgtt  | 16620 |
| [5117] | tggaaattga aacgtgtatc attgttattt aatgtgaatt tttgtttctt cacaatacca   | 16680 |
| [5118] | ctaccatata acttagttgc catacgtgga taattacgtt ttaacattaa ttcttcattt   | 16740 |
| [5119] | ttagataaat ccatgttaat aggtactgta tccataatta catattcttc actgtattga   | 16800 |
| [5120] | cctataaagt cctgctcttt agctaaccac ttaaaacgat tacctaagtc aatatcgatt   | 16860 |
| [5121] | aataacgttt cattaatctt agggataaaa aatttattta caaatgtttc aaacattgta   | 16920 |
| [5122] | ttagaattat cccacttatc gccaaacgtc catgattttg aataagtatg attaaagtct   | 16980 |
| [5123] | tgtaatgcag atttagctga ctgtgcaact aatagtgtct tttcgttttt tgtactcttt   | 17040 |

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|        |                                                                    |       |
|--------|--------------------------------------------------------------------|-------|
| [5124] | tctgccatga tttattattc ctctctaca tcgccagtaa atgactgttt tgaaagtga    | 17100 |
| [5125] | tgaatttgta caccataact atcttcactt ttatttgat caattgacat attttcattt   | 17160 |
| [5126] | aattctgttc gtttatttaa tcttgaatct tcatatgatg taccatcat agaacgata    | 17220 |
| [5127] | ttgttacct catacatgtt taaattcctc ctaatctaaa tctaacttat cgactaattc   | 17280 |
| [5128] | ttcatctgaa tagtctttat cattatcatc tggttcagta acatctgggt gtgtttgttg  | 17340 |
| [5129] | tacttggtgc gattgttgca tttgtgaaga taaaaaagta gtcacttggt gttctaata   | 17400 |
| [5130] | agcaatacgt tgttctaata caacagggtc aaatttcgaa ctatcttcat ctgttgtagt  | 17460 |
| [5131] | aggttctaata ttgttttcat tttcttcttc gattgtttct actgttttat cttcagttga | 17520 |
| [5132] | ttcttcagtt gattcttcag ttgattcttc agttgattct tcagttgatt cttcagttga  | 17580 |
| [5133] | ttctttgtcg tctggtttta cgatttcac aaattctgtc atttagaca               | 17629 |