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(54)发明名称

膀胱癌的诊断方法

(57)摘要

本发明涉及诊断患者的膀胱癌的方法,其包括确定来自患者的DNA中的甲基化可变位置(MVP)的甲基化状态,并基于甲基化状态数据提供诊断。本发明还涉及治疗膀胱癌的方法,其包括通过本文限定的诊断方法提供膀胱癌的诊断,然后给予患者一种或多种抗癌试剂。本发明还涉及包含针对本文限定的MVP的探针的甲基化识别性阵列和包括所述阵列的试剂盒。

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1.选自由以SEQ ID NO:1至150标识并由[CG]指示的MVP构成的组的一组MVP用于制备用于在个体中诊断膀胱癌的试剂的用途,其中所述一组MVP包括至少25个以SEQ ID NO:1至150标识并由[CG]指示的MVP,其包括:

(a)提供来自所述个体的样品的DNA;

(b)确定MVP组中的每一个是否均是甲基化的;和

(c)当(b)组的至少25个MVP均是甲基化的时,在所述个体中诊断为膀胱癌,并且其中所述至少25个MVP包括以SEQ ID NO:1至3标识的MVP。

2.根据权利要求1所述的用途,其中所述MVP组包括至少40个以SEQ ID NO 1至150标识并由[CG]指示的MVP,并且其中当至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP均是甲基化的时,诊断为膀胱癌。

3.根据权利要求2所述的用途,其中所述MVP组包括至少50个以SEQ ID NO 1至150标识并由[CG]指示的MVP,或包括至少100个以SEQ ID NO 1至150标识并由[CG]指示的MVP。

4.根据权利要求3所述的用途,其中所述MVP组包括全部150个以SEQ ID NO 1至150标识并由[CG]指示的MVP。

5.根据权利要求2至4中任一项所述的用途,其中当选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的至少40个MVP均是甲基化的时,或当选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的至少50个MVP均是甲基化的时,或当选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的至少100个MVP均是甲基化的时,或当全部150个MVP均是甲基化的时,在个体中诊断为膀胱癌。

6.根据权利要求1至4中任一项所述的用途,其中所述确定为是甲基化的MVP包括以SEQ ID NO 1至5标识并由[CG]指示的MVP,或者包括以SEQ ID NO 1至10标识并由[CG]指示的MVP,或者包括以SEQ ID NO 1至40标识并由[CG]指示的MVP。

7.根据权利要求5所述的用途,其中所述确定为是甲基化的MVP包括以SEQ ID NO 1至5标识并由[CG]指示的MVP,或者包括以SEQ ID NO 1至10标识并由[CG]指示的MVP,或者包括以SEQ ID NO 1至40标识并由[CG]指示的MVP。

8.根据权利要求1所述的用途,其中所述MVP组包括全部150个以SEQ ID NO 1至150标识并由[CG]指示的MVP,其中当选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的至少40个MVP是甲基化的时,在个体中诊断为膀胱癌,且其中所述确定是甲基化的MVP包括以SEQ ID NO 1至10标识并由[CG]指示的MVP。

膀胱癌的诊断方法

技术领域

[0001] 本发明涉及诊断患者中的膀胱癌的方法，其包括确定来自患者的DNA中的甲基化可变位置(MVP)的甲基化状态，并基于甲基化状态数据提供诊断。本发明还涉及治疗膀胱癌的方法，其包括通过本文限定的诊断方法提供膀胱癌的诊断，然后给予患者一种或多种抗癌剂。本发明还涉及包含针对本文限定的MVP的探针的甲基化识别性阵列和包括所述阵列的试剂盒。

背景技术

[0002] 膀胱癌是西方世界最常见的恶性肿瘤之一，在美国排名第五位的最常见的癌症并导致所有癌症相关死亡的约3％[1,2]。出现的最主要的临床症状是血尿，在所调查的所有这样的病例的约10％中检出膀胱癌[3]。膀胱癌更可能发生于老年男性患者、现在或过去的吸烟者和接触工业致癌物的患者[4]。患有不可见血尿的年轻女性较不可能携带膀胱癌，对于这些患者而言，在误诊血尿后膀胱癌检测延误是一件常见的事[5]。膀胱镜检查是目前检测膀胱癌的黄金标准，是需要诊所或医院出诊并且具有较小但显著的感染风险的一种侵入性、不舒服的手术[6-9]。

[0003] 在英国每年大约有10300人被诊断为膀胱癌并且5000人死于膀胱癌。然而，每年有超过100000个病例被从初级保健转诊到泌尿外科血尿诊所进行膀胱镜检查和影像检查。所转诊的患者中仅10％被检出膀胱癌。

[0004] 在那些确诊病例中，三分之二是非肌层浸润性膀胱癌(NMIBC)，其中70％会复发，15％会进展为肌层浸润性膀胱癌(MIBC)。通过膀胱镜检查进行监督是检测复发所必需的，并且对于处于高复发风险的病例，实施频率为每3个月复查一次持续2年，然后每6个月复查一次，之后每年复查一次。血尿的研究及随后针对复发的监督造成估计为￡5539万的高额的健康经济费用，使得膀胱名列治疗起来的最昂贵的癌症之一[10,11]。因此，非常需要可以更好地鉴定患有疾病的患者并减少对不必要的膀胱镜检查的需求的改进的测定法。

[0005] 还没有基于尿的生物标记物被FDA批准作为膀胱癌检测的独立测试，因此，指导方针向所有患有可见血尿和持续性不可见血尿的患者推荐膀胱镜检查[10,11]。尿液细胞学常被用作膀胱镜检查的诊断辅助手段，但对高等级疾病和原位癌以外的其他癌症检测敏感性较低，不能代替膀胱镜检查[12,13]。同样，商业上可得的基于单个靶标或小的靶标组的测定法不能以足够的敏感性检测膀胱癌，并且只被批准与膀胱镜检查结合使用[14]。

[0006] 目前，一些研究已经表明，体液中的DNA甲基化生物标记物用于癌症的非侵入性检测的潜在效用，所述体液包括尿液[15-23]、血浆/血清[24-26]以及唾液[27,28]。DNA甲基化的变化在恶性转化中起关键作用，导致肿瘤抑制基因的沉默和癌基因的过表达[29]。DNA甲基化的个体发育可塑性和相对稳定性使得外遗传改变成为用于诊断的理想生物标记物。

[0007] 涉及在排泄的尿液中特定蛋白质的存在的检测测定法已被开发并商业化。在这些情况下，每次测定中检测的蛋白质的数量低，特异性和敏感性仍令人不满意[14]。所检测的蛋白质生物标记物包括人补体因子H相关蛋白、癌胚抗原(CEA)、膀胱肿瘤细胞相关的粘蛋

白和核有丝分裂器蛋白22(NMP22)。

[0008] 就评价某些蛋白质的表达的测定而言,WO2014042763描述了用于检测尿液样品中的蛋白、由IL-8、MMP-9、SDC1、CCL18、SERPINE1、CD44、VEGF-A、CA9和ANG组成的九-生物标记物组;由CA9、CCL18、MMP12、TMEM45A、MMP9、SEMA3D、ERBB2、CRH和MXRA8组成的另一个9-生物标记物组;以及由CCL18、CD44和VEGF-A组成的三-生物标记物组。

[0009] 迄今为止,用于检测膀胱癌的DNA甲基化生物标记物检测已经集中在仅对少量基因座的分析,部分是由于技术限制和具有癌症特异性的靶标的衍生[11-19]。一般而言,所报道的敏感性和特异性相对于已建立的测定来说是高的,但仍不能获得通过膀胱镜检查所达到的性能特征。先前研究的针对膀胱癌的甲基化标记物包括DAPK、BCL2、TERT、TWIST1、NID2、RARβ、E-钙粘蛋白和p16。国际专利申请公开WO2013/144362描述了一种用于膀胱癌的诊断测定法,其涉及检测ECRG4和/或ITIH5基因的启动子的甲基化。美国专利申请公开US2013224738描述了一种用于膀胱癌的诊断测定法,其涉及评估由BCL2、CDKN2A和NID2组成的基因的甲基化状态。

[0010] 寻求用于准确诊断膀胱癌的改进的测定法,这样的测定法将具有重要的临床和经济益处,特别是非侵入性的测定法。

[0011] 发明概述

[0012] 提供了具有可靠且高的敏感性和特异性的诊断方法,其可以从生物样品(特别是排泄的尿液样品)中检测膀胱癌,并且其有潜能减少在血尿患者中和进行疾病复发监测的患者中对膀胱镜检查的需求。避免膀胱镜检查将降低膀胱癌治疗的成本,并对患者的健康产生积极的影响,减少就诊次数和侵入性检查的不便。因此,本发明提供了以下内容:

[0013] 本发明提供了一种在个体中诊断膀胱癌的方法,其包括:

[0014] (a)提供来自所述个体的样品的DNA;

[0015] (b)确定选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的一组MVP中的每一个是否均是甲基化的,其中该组包括至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP;和

[0016] (c)当(b)组的至少25个MVP是甲基化的时,在所述个体中诊断为膀胱癌。

[0017] 在任一种这样的方法中,MVP组可包括至少40个以SEQ ID NO 1至150标识并由[CG]指示的MVP,其中当至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP是甲基化的时,诊断为膀胱癌。

[0018] MVP组可包括至少50个以SEQ ID NO 1至150标识并由[CG]指示的MVP,或可包括至少100个以SEQ ID NO 1至150标识并由[CG]指示的MVP。

[0019] MVP组可包括全部150个以SEQ ID NO 1至150标识并由[CG]指示的MVP。

[0020] 在上述方法中,当选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的至少40个MVP是甲基化的时,或当选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的至少50个MVP是甲基化的时,或当至少100个MVP是甲基化的时,或当全部150个MVP是甲基化的时,可以在个体中诊断为癌症。

[0021] 在上述方法中,其中确定为是甲基化的MVP可包括以SEQ ID NO 1至3标识并由[CG]指示的MVP,或者可包括以SEQ ID NO 1至5标识并由[CG]指示的MVP,或者可包括以SEQ ID NO 1至10标识并由[CG]指示的MVP,或者可包括以SEQ ID NO 1至40标识并由[CG]指示

的MVP。

[0022] 在上述方法中个,MVP组可包括全部150个以SEQ ID NO 1至150标识并由[CG]指示的MVP,其中当选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的至少40个MVP是甲基化的时,在个体中诊断为膀胱癌,且其中确定是甲基化的MVP包括以SEQ ID NO 1至10标识并由[CG]指示的MVP。

[0023] 在任一种上述方法中,其中确定每一个MVP是否均是甲基化的步骤可包括亚硫酸氢盐转化DNA。

[0024] 在任一种上述方法中,确定每一个MVP是否均是甲基化的步骤可包括:

[0025] 1)进行测序步骤以确定MVP的序列;

[0026] 2)将DNA与包含探针的阵列杂交,所述探针能够区分MVP的甲基化和非甲基化形式,将检测系统应用于所述阵列以区分MVP的甲基化和非甲基化形式;或

[0027] 3)使用甲基化特异性引物进行扩增步骤,其中通过存在或不存在扩增产物来确定MVP的状态为甲基化的或非甲基化的。

[0028] 在测序或杂交步骤之前可进行扩增步骤,其中扩增包含每个MVP的基因座。扩增可通过PCR进行。

[0029] 在测序或杂交步骤之前,可进行捕获步骤。捕获步骤可包括将包含MVP基因座的多核苷酸与对MVP基因座具有特异性的结合分子结合,以及收集包含MVP基因座和结合分子的复合物;且其中:

[0030] i.捕获步骤发生在亚硫酸氢盐转化DNA的步骤之前;

[0031] ii.捕获步骤发生在亚硫酸氢盐转化DNA的步骤之后但在扩增或杂交步骤之前;或

[0032] iii.捕获步骤发生在亚硫酸氢盐转化DNA的步骤之后且在扩增步骤之后。

[0033] 结合分子可以是对每个MVP具有特异性的寡核苷酸,优选每一个均包含与相应的MVP互补的序列的DNA或RNA分子。

[0034] 结合分子可与纯化部分偶联。

[0035] 纯化部分可包含第一纯化部分,所述收集包含MVP基因座和结合分子的复合物的步骤可包括将第一纯化部分与包含第二纯化部分的物质结合,其中第一纯化部分和第二纯化部分形成相互作用复合物。

[0036] 第一纯化部分可以是生物素,第二纯化部分可以是链亲和素;或第一纯化部分可以是链亲和素,第二纯化部分可以是生物素。

[0037] 扩增包含MVP的基因座的步骤可包括使用独立于MVP的甲基化状态的引物。

[0038] 扩增包含MVP的基因座的步骤可通过微滴PCR扩增进行。

[0039] 在任一种上述方法中,获自个体的生物样品可以为尿液、血液、血清、血浆或无细胞DNA的样品。

[0040] 在任一种上述方法中,所述方法可达到95%或更高的ROC敏感性和90%或更高的ROC特异性;优选ROC敏感性为96%,ROC特异性为97%,优选95%或更高的ROC AUC,优选98%。

[0041] 在任一种上述方法中,所述方法可达到95%或更高的阴性预测值(NPV),优选97%。

[0042] 在任一种上述方法中,在个体中诊断膀胱癌的步骤还可包括:

[0043] i.对肿瘤的等级进行分级;和/或

[0044] ii.确定肿瘤复发的风险;和/或

[0045] iii.确定非肌层浸润性疾病的进展的风险;和/或

[0046] 确定对治疗疗法的可能的反应。

[0047] 本发明还提供了一种在个体中治疗膀胱癌的方法,其包括:

[0048] (a)从来自所述个体的样品获得DNA并确定选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的一组MVP中的每一个是否均是甲基化的,其中该组包括至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP;

[0049] (b)当(a)组的至少25个MVP是甲基化的时,在所述个体中诊断为膀胱癌;和

[0050] (c)给予所述个体一种或多种膀胱癌治疗。

[0051] 本发明还提供了一种在个体中治疗膀胱癌的方法,其包括:

[0052] (a)提供来自所述个体的样品的DNA并确定选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的一组MVP中的每一个是否均是甲基化的,其中该组包括至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP;

[0053] (b)当(a)组的至少25个MVP是甲基化的时,在所述个体中诊断为膀胱癌;和

[0054] (c)给予所述个体一种或多种膀胱癌治疗。

[0055] 本发明还提供了一种在个体中治疗膀胱癌的方法,其包括:

[0056] (a)在来自所述个体的样品的DNA中确定选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的一组MVP中的每一个是否均是甲基化的,其中该组包括至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP;

[0057] (b)当(a)组的至少25个MVP是甲基化的时,在所述个体中诊断为膀胱癌;和

[0058] (c)给予所述个体一种或多种膀胱癌治疗。

[0059] 本发明还提供了一种在个体中治疗膀胱癌的方法,其包括给予所述个体一种或多种膀胱癌治疗,其中所述个体已经通过以下步骤被诊断为患有膀胱癌:

[0060] (a)提供来自所述个体的样品的DNA并确定选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的一组MVP中的每一个是否均是甲基化的,其中该组包括至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP;和

[0061] (b)当(a)组的至少25个MVP是甲基化的时,在所述个体中诊断为膀胱癌。

[0062] 本发明还提供了一种在个体中诊断膀胱癌的方法,其包括:

[0063] (a)获得识别选自以SEQ ID NO 1至150标识并由[CG]指示的MVP的一组MVP中的每一个是否均是甲基化的数据,其中该组包括至少25个以SEQ ID NO 1至150标识并由[CG]指示的MVP;和

[0064] (b)当(a)组的至少25个MVP是甲基化的时,在所述个体中诊断为膀胱癌;

[0065] 其中所述数据是通过包括以下步骤的方法获得:

[0066] i.从样品中获得DNA;和

[0067] ii.确定DNA中的MVP是否是甲基化的。

[0068] 在任一种上述方法中,癌症可以是非肌层浸润性膀胱癌(NMIBC)。癌症可以是肌层浸润性膀胱癌(MIBC)。

[0069] 本发明还提供了一种能够区分MVP的甲基化和非甲基化形式的阵列;所述阵列包

括对MVP组中的每一个MVP的甲基化形式具有特异性的寡核苷酸探针和对所述组中的每一个MVP的非甲基化形式具有特异性的寡核苷酸探针;其中所述组由至少25个选自以SEQ ID NO 1至150标识的MVP的MVP组成。

[0070] 在某些实施方案中,阵列不是Infinium HumanMethylation450BeadChip阵列。在某些实施方案中,所述阵列的MVP特异性寡核苷酸探针的数量少于482421,优选482000或更少、480000或更少、450000或更少、440000或更少、430000或更少、420000或更少、410000或更少,或400000或更少。

[0071] 在上述阵列中,所述组可由选自以SEQ ID NO 1至150标识的至少40个MVP组成;优选以SEQ ID NO 1至150标识的至少50个MVP、至少60个MVP、至少70个MVP、至少80个MVP、至少90个MVP、至少100个MVP、至少110个MVP、至少120个MVP、至少130个MVP、至少140个MVP、至少145个MVP或全部150个MVP。

[0072] 在上述阵列中,所述组可包括由SEQ ID NO 1至3限定的MVP,或由SEQ ID NO 1至5限定的MVP,或由SEQ ID NO 1至10限定的MVP,或由SEQ ID NO 1至20限定的MVP,或由SEQ ID NO 1至30限定的MVP,或由SEQ ID NO 1至40限定的MVP,或由SEQ ID NO 1至50限定的MVP,或由SEQ ID NO 1至60限定的MVP,或由SEQ ID NO 1至70限定的MVP,或由SEQ ID NO 1至80限定的MVP,或由SEQ ID NO 1至90限定的MVP,或由SEQ ID NO 1至100限定的MVP,或由SEQ ID NO 1至100限定的MVP,或由SEQ ID NO 1至120限定的MVP,或由SEQ ID NO 1至130限定的MVP,或由SEQ ID NO 1至140限定的MVP,或由SEQ ID NO 1至150限定的MVP。

[0073] 所述可阵列包括由SEQ ID NO 1至150限定的所有MVP。

[0074] 在上述阵列中,所述阵列还可包括一个或多个包含MVP的寡核苷酸,所述MVP选自SEQ ID NO 1至150中限定的任一个MVP,其中所述一种或多种寡核苷酸与所述阵列的相应的寡核苷酸探针杂交。

[0075] 所述一个或多个寡核苷酸可包括选自以SEQ ID NO 1至150标识的MVP的至少20个MVP;优选以SEQ ID NO 1至150标识的至少50个MVP、至少60个MVP、至少70个MVP、至少80个MVP、至少90个MVP、至少100个MVP、至少110个MVP、至少120个MVP、至少130个MVP、至少140个MVP、至少145个MVP、或全部150个MVP。

[0076] 所述一种或多种寡核苷酸可包含由SEQ ID NO 1至10限定的MVP,或由SEQ ID NO 1至20限定的MVP,或由SEQ ID NO 1至30限定的MVP,或由SEQ ID NO 1至40限定的MVP,或由SEQ ID NO 1至50限定的MVP,或由SEQ ID NO 1至60限定的MVP,或由SEQ ID NO 1至70限定的MVP,或由SEQ ID NO 1至80限定的MVP,或由SEQ ID NO 1至90限定的MVP,或由SEQ ID NO 1至100限定的MVP,或由SEQ ID NO 1至110限定的MVP,或由SEQ ID NO 1至120限定的MVP,或由SEQ ID NO 1至130限定的MVP,或由SEQ ID NO 1至140限定的MVP,或由SEQ ID NO 1至150限定的MVP。

[0077] 所述一种或多种寡核苷酸可包含由SEQ ID NO 1至150限定的所有MVP。

[0078] 如上所述的阵列可通过将如上所述的阵列与一组寡核苷酸杂交而获得,所述寡核苷酸每一个均包含不同的MVP,所述MVP选自SEQ ID NO 1至150中限定的任一个MVP,且其中所述组包括至少40个寡核苷酸。

[0079] 在这样的杂交阵列中,所述组可包括至少50个寡核苷酸。所述组可包括至少60、至少70、至少80、至少90、至少100、至少110、至少120、至少130、至少140、至少145或至少150个

寡核苷酸。

[0080] 在杂交阵列中,所述组可包括至少40个寡核苷酸,其包含由SEQ ID NO 1至20限定的MVP,或其中所述组可包括至少50个寡核苷酸,其包含由SEQ ID NO 1至50限定的MVP,或其中所述组可包括至少60个寡核苷酸,其包含由SEQ ID NO 1至60限定的MVP,或其中所述组可包括至少70个寡核苷酸,其包含由SEQ ID NO 1至70限定的MVP,或其中所述组可包括至少80个寡核苷酸,其包含由SEQ ID NO 1至80限定的MVP,或其中所述组可包括至少90个寡核苷酸,其包含由SEQ ID NO 1至90限定的MVP,或其中所述组可包括至少100个寡核苷酸,其包含由SEQ ID NO 1至100限定的MVP,或其中所述组可包括至少110个寡核苷酸,其包含由SEQ ID NO 1至110限定的MVP,或其中所述组可包括至少120个寡核苷酸,其包含由SEQ ID NO 1至120限定的MVP,或其中所述组可包括至少130个寡核苷酸,其包含由SEQ ID NO 1至130限定的MVP,或其中所述组可包括至少140个寡核苷酸,其包含由SEQ ID NO 1至140限定的MVP,或其中所述组可包括至少145个寡核苷酸,其包含由SEQ ID NO 1至145限定的MVP,或其中所述组可包括至少150个寡核苷酸,其包含由SEQ ID NO 1至150限定的MVP。所述组可包括至少150个寡核苷酸,其包含由SEQ ID NO 1至150限定的MVP。

[0081] 本发明还提供了一种用于制造如上所限定的杂交阵列的方法,其包括将如上所限定的阵列与一组寡核苷酸接触,所述寡核苷酸每一个均包含不同的MVP,其中所述MVP选自SEQ ID NO 1至150中限定的MVP的任一个,且其中所述组包括至少25个寡核苷酸。

[0082] 本发明还提供了一种用于制造如上所限定的杂交阵列的方法,其包括将如上限定的阵列与如上限定的一组寡核苷酸接触。

[0083] 本发明还提供了一种试剂盒,其包括任一种如上所述的阵列。

[0084] 所述试剂盒还可包括能够修饰MVP二核苷酸中的非甲基化胞嘧啶,但是不能修饰MVP二核苷酸中的甲基化胞嘧啶的DNA修饰试剂,任选地其中二核苷酸是CpG。DNA修饰试剂可以是亚硫酸氢盐试剂。

附图说明

[0085] 图1.在膀胱癌(红色)与30个正常尿路上皮(蓝色)之间的9786个MVP(1746个高甲基化MVP、8040个低甲基化MVP)的热图。

[0086] 图2.非癌症尿液(n=10)、正常尿路上皮(n=30)、膀胱癌(n=81)和血液(n=489)的150个UroMark基因座的热图。

[0087] 图3.与膀胱癌的预测状态(淡蓝色/淡红色)和实际状态(蓝/红),相比,正常尿路上皮(蓝色)和膀胱癌(红色)的UroMark测定涉及的150个基因座的热图。

[0088] 图4.基于在来自UCL和TCGA膀胱癌的膀胱癌样品中150个基因座的甲基化状态,膀胱肿瘤和正常尿路上皮的MDS图。所述MDS(多维尺度法)图代表基于150个基因座的甲基化状态的表型与该组(panel)的不同性,并清楚地表明150个标记物能准确地将肿瘤与正常膀胱分开。坐标轴表示样品之间的欧氏距离(Euclidean distance)。

[0089] 图5.用于在尿液中检测膀胱癌的UroMark模型的ROC图。

[0090] 图6.表现最佳的(A)3、(B)5和(C)10标记组的ROC图。前3个MVP列为SEQ ID NO:1至3,前5个MVP列为SEQ ID NO:1至5,前10个MVP列为SEQ ID NO:1至10,全部为等级次序(参见表1)。

[0091] 图7.由176个唯一尿液样品(98个非癌症尿液和78个癌症尿液)检测膀胱癌的UroMark模型的ROC图。

[0092] 图8.来自患者尿液的DNA品质(浓度、纯度和完整性)的比较。A)与在家收集的样品(n＝41)相比,临床中收集的样品(n＝123)的浓度和纯度,其中红色表示低产率或纯度,琥珀色表示中等产率以及中级纯度,并且绿色表示高产率和纯度。B)示出低、中等或高产率和纯度的样品百分比。C)代表性的生物分析仪电泳图谱,其表示在具有通过分光光度法定量的浓度的样品中高分子量的DNA的回收。D)与制造者的56℃进行1小时的标准方案相比,在21℃下使用延长的消化步骤,尿液DNA的增加的中值产率和改善的纯度。E)两种尿液保存方法的比较:UCL建立的标准操作程序对比Norgen尿液保存管。细菌的数量是通过rpoB基因的qPCR来定量,并表示为rpoB拷贝数/每个人类YWHAZ拷贝。数据是平均值+/-标准差。

[0093] 图9.检测来自验证同龄组2-96个唯一的尿液样品(64个非癌症尿液和32个癌症尿液)的膀胱癌的UroMark模型的ROC图。

[0094] 图10.检测来自验证同龄组3-92个唯一的尿液样品(65个非癌症尿液和27个癌症尿液)的膀胱癌的UroMark模型的ROC图。

具体实施方式

[0095] 膀胱癌

[0096] 如上所述,膀胱癌是在西方世界中最流行的癌症类型之一。移行细胞癌为最常见的类型,并占膀胱癌的约90％。移行细胞癌起源于移行上皮,所述移行上皮为衬在膀胱内表面的组织。剩余10％的膀胱癌主要由以下组成:鳞状细胞癌、腺癌、肉瘤和小细胞癌。鳞状细胞癌也起源于上皮组织,起源于鳞状细胞。这些为存在于最表面的上皮层中的薄的扁平细胞。腺癌形成于具有腺性特征和/或来源的上皮细胞。肉瘤来源于间充质来源的细胞,例如膀胱的脂肪和肌肉层的细胞。小细胞癌具有快速的倍增时间且能够更早的转移,使得它们特别具有攻击性。

[0097] 膀胱癌还可分为非肌层浸润性膀胱癌(NMIBC)和肌层浸润性膀胱癌(MIBC)。

[0098] 本文中所描述的诊断和治疗方法能够明确地所有类型的膀胱癌识别的恶性细胞。因此,本文中所描述的方法的任一种可用于诊断膀胱的移行细胞癌、膀胱的鳞状细胞癌、膀胱的腺癌、膀胱的肉瘤、膀胱的小细胞癌、转移膀胱癌、平滑肌肉瘤(在平滑肌中形成的肿瘤)、淋巴瘤(通常在淋巴结中形成的肿瘤)、恶性黑素瘤(通常在皮肤中形成的肿瘤)和大细胞神经内分泌癌。包括膀胱癌的原发形式和复发形式。如本文所述待被诊断或治疗的癌症可为尿路上皮细胞癌。因此,癌症可为输尿管癌、尿道癌或肾盂癌。

[0099] 本文中所述的诊断测定所适用的最优选的患者类型为人类。本文中所述的诊断测定还可用于识别非人类动物中的膀胱癌。例如,非人类动物可包含衍生自人类的组织,例如异种移植物。因此,诊断测定可在人类膀胱癌的动物模型中用于诊断人类膀胱癌。本文中所述的诊断测定所适用的典型的非人类动物为啮齿类动物,例如大鼠或小鼠。

[0100] 甲基化可变位点(MVP)

[0101] DNA的甲基化为后天修饰的可识别形式,其具有改变基因和其他元件(例如微小RNA)的表达的能力[51]。在癌症的发生和进展中,甲基化可具有例如沉默肿瘤抑制基因和/或增加癌基因的表达的作用。作为甲基化的后果,可出现其他形式的失调。DNA的甲基化出

现在其主要为由CPG基序组成的二核苷酸的分散的基因座处，但还可出现在CHH基序(其中H为A、C或T)处。在甲基化过程中，将甲基基团添加至胞嘧啶碱基的第五个碳上以形成甲基胞嘧啶。

[0102] 甲基化可在整个基因组中发生并且不限于与表达序列(如基因)相关的区域。甲基化通常但不总是在表达序列的启动子或其他调节区中发生。

[0103] 本文中所定义的甲基化可变位点(MVP)为任何二核苷酸基因座，其可在表型之间(即肿瘤与正常组织之间)显示出甲基化状态的变化。MVP优选地为CpG或CHH二核苷酸基序。本文中所定义的MPV不限于与相应的表达序列相关的基因座的位点。

[0104] 典型地，评估DNA甲基化状态包括分析DNA中甲基基团的存在或缺失，例如一种或多种胞嘧啶核苷酸的第5位上的甲基基团。优选地，评估作为CpG二核苷酸(其中C代表胞嘧啶，G代表鸟嘌呤以及p代表连接两者的磷酸基团)存在的一种或多种胞嘧啶核苷酸的甲基化状态。

[0105] 评估MVP的甲基化状态或测定MVP是否甲基化是指在后续处理之前确定在由个体获得的DNA的起始样品中MVP是否甲基化或未甲基化。

[0106] 在本文中，如果在来自患者的基因组DNA的样品中MVP的一种或多种等位基因被测定具有一个或多个甲基化的CpG二核苷酸基因座，则所述MVP被定义为甲基化的。

[0107] 在本文中所述的方法的任意一种中，测定为甲基化的MVP相对于正常的尿路上皮对照和/或全血对照为甲基化的。

[0108] 用于诊断目的的具体的MVP列于表1中，并通过SEQ ID号以及Illumina ID号(Ilmn ID)识别。用于扩增所限定的MVP的示例性引物列于表2中，并通过SEQ ID号识别。

[0109] 甲基化可变位点(MVP)状态的鉴定和评估

[0110] 多种技术可用于甲基化可变位点(MVP)的鉴定和评估，下面将简要概述。本文所述的诊断方法涵盖用于确定MVP状态的任何合适的技术。

[0111] 在常规的体外处理步骤如PCR中，甲基基团从起始DNA分子中丢失。为避免这种情况，检测甲基基团的技术通常包括在后续处理之前对DNA进行预处理，以便保存原始DNA分子的甲基化状态信息。这些预处理技术涉及三个主要类别的处理，即亚硫酸氢盐修饰、限制性酶消化和基于亲和力的分析。然后将这些技术的产品与基于测序或阵列的平台偶联，以便进行随后的鉴定或定性评估MVP甲基化状态。

[0112] 涉及DNA的亚硫酸氢盐修饰的技术已经成为最常见的检测和评估CpG二核苷酸的甲基化状态的方法。用亚硫酸氢盐(例如亚硫酸氢钠)处理DNA将胞嘧啶碱基转化为尿嘧啶碱基，但对5-甲基胞嘧啶不起作用。因此，经亚硫酸氢盐处理的DNA中胞嘧啶的存在表明存在先前在起始DNA分子中被甲基化的胞嘧啶碱基。这类胞嘧啶碱基可通过多种技术检测。例如，可产生对未甲基化的DNA与甲基化的DNA具有特异性的引物，并将所述引物用于甲基化的CpG二核苷酸的基于PCR的鉴定。可进行分离/俘获步骤，例如，使用结合分子如互补寡核苷酸序列。也可使用标准和下一代DNA测序方案。

[0113] 在其他方法中，可使用甲基化敏感性酶，其仅在甲基化的DNA的存在下进行消化或切割。对所得的片段的分析通常使用微阵列进行。

[0114] 为了富集，基于亲和力的技术利用结合相互作用力以俘获甲基化的DNA的片段。通常使用结合分子(例如抗-5-甲基胞嘧啶的抗体)，然后进行后续处理步骤(例如PCR和测

序)。

[0115] Olkhov-Mitsel和Bapat(2012)[51]提供了一篇可用于鉴定和评估基于MVP的生物标记物(包括甲基胞嘧啶)的技术的全面综述。

[0116] 为了评估本文表征和描述的基于MVP的生物标记物的甲基化状态,可使用任何合适的方法。

[0117] 优选的方法包括亚硫酸氢盐处理DNA,包括扩增所鉴定的MVP基因座以用于甲基化特异性PCR和/或测序,和/或使用甲基化鉴别微阵列对目标基因座的甲基化状态进行评估。

[0118] MVP基因座的扩增可通过多种方法实现。优选地,MVP基因座使用PCR扩增。MVP还可通过其他技术(如多重连接依赖性探针扩增(MLPA))扩增。可使用各种基于PCR的方法。例如,甲基化特异性引物可杂交至含有目的MVP序列的DNA中。这类引物可被设计为与衍生自甲基化或非甲基化MVP基因座的序列退火。退火后,进行PCR反应,随后的PCR产物的存在表明存在具有可鉴定序列的退火MVP。在这种方法中,DNA在扩增前被亚硫酸氢盐转化。这类技术通常意指甲基化特异性PCR(MSP)[53]。

[0119] 在其他技术中,PCR引物可目的MVP序列退火,而不依赖于甲基化状态,并且进一步的处理步骤可用于测定MVP的状态。测定可被设计为使得MVP位点位于引物退火位点之间。该方法方案可用于诸如亚硫酸氢盐基因组测序[54]、COBRA[55]、Ms-SNuPE[56]的技术中。在这类方法中,DNA可在扩增之前或之后被亚硫酸氢盐转化。

[0120] 优选地,使用小规模PCR方法。这类方法通常涉及样品的质量分配(例如数字PCR)。这些技术在高度微型化系统(皮升尺寸的液滴)的情况下提供了强大的准确性和敏感性,其对于随后处理可从生物样品(特别是尿液样品)中存在的潜在少量细胞材料中获得的少量DNA是理想的。各种小规模PCR技术是广泛可用的。例如,基于微滴的PCR仪器可购自多家供应商,包括RainDance Technologies,Inc.(Billerica,MA;http://raindancetech.com/)和Bio-Rad,Inc.(http://www.bio-rad.com/)。微阵列平台还可用于进行小规模PCR。这类平台可包括基于微流体网络的阵列,例如可购自Fluidigm Corp.(www.fluidigm.com)。

[0121] 在MVP基因座扩增之后,扩增的PCR产物可偶联至后续分析平台上,以便测定目的MVP的甲基化状态。例如,可直接测序PCR产物以确定在目标MVP处甲基胞嘧啶的存在或缺失,或通过基于阵列技术分析PCR产物。

[0122] 任何合适的测序技术可用于测定目标DNA的序列。在本发明的方法中,优选使用高处理量的、所谓的“第二代”、“第三代”和“下一代”技术对经亚硫酸氢盐处理的DNA测序。

[0123] 在第二代技术中,并行测序大量的DNA分子。典型地,成千上万的分子以高密度锚定到指定的位置,并且以依赖于DNA合成的方法确定序列。反应通常由以下步骤组成:连续试剂递送和洗涤步骤,例如以便能够掺入可逆的标记的终止碱基;以及扫描步骤,以确定碱基掺入的顺序。这类基于阵列的系统为市售的,例如购自Illumina,Inc.(San Diego,CA;http://www.illumina.com/)。

[0124] 第三代技术通常定义为检测步骤之间不需要停止测序方法,因此可被视为实时系统。例如,发生在掺入过程中的氢离子的碱基特异性释放可在微孔系统的环境内检测(例如参见购自Life Technologies的Ion Torrent系统;http://www.lifetechnologies.com/)。同样地,在焦磷酸测序中,焦磷酸盐(PPi)的碱基特异性释放被检测和分析。在纳米孔技术中,DNA分子通过或定位在纳米孔旁边,并且DNA分子相对于纳米孔移动后确定单个碱基的

种类。这类系统为市售的，例如购自Oxford Nanopore(https://www.nanoporetech.com/)。在另一种方法中，DNA聚合酶被限制在“零模式波导”中，并且掺入的碱基的种类是通过γ-标记的磷酸核苷酸的荧光检测来确定(参见例如Pacific Biosciences;http://www.pacificbiosciences.com/)。

[0125] 在本发明的其他方法中，可省略测序步骤。例如，基于两条互补的核酸链的退火以形成双链分子的原理，将扩增的PCR产物直接施用于杂交阵列。可以将杂交阵列设计成包括能够与MVP的扩增产物杂交并允许区分甲基化和非甲基化基因座的探针。例如，将探针设计为能够与含有胸腺嘧啶的MVP基因座选择性杂交，这表明在起始模板DNA中在未甲基化的胞嘧啶亚硫酸氢盐转化后产生尿嘧啶。相反地，将探针设计为能够与含有胞嘧啶的MVP基因座选择性杂交，这表明在亚硫酸氢盐处理后不存在尿嘧啶转化。这对应于起始模板DNA中的甲基化的MVP基因座。

[0126] 将合适的检测系统施用于所述阵列之后，可使用基于计算机的分析技术来测定MVP的甲基化状态。检测系统可包括，例如在甲基化状态特异性探针延伸反应之后加入荧光分子。这类技术使得可以进行MVP状态确定，而不用特别的需要测序MVP扩增产物。这种基于阵列的鉴别探针可被称为甲基化特异性探针。

[0127] 任何合适的甲基化鉴别微阵列可用于评估本文中所述的MVP的甲基化状态。优选的甲基化鉴别微阵列系统由Illumina,Inc.(San Diego,CA;http://www.illumina.com/)提供。特别地，Infinium HumanMethylation450BeadChip阵列系统可用于评估本文中所述的用于膀胱癌的诊断MVP的甲基化状态。在亚硫酸氢盐处理起始DNA分子之后，这类系统利用对DNA的化学修饰。简而言之，所述阵列包含与特异性针对对应于未甲基化形式的MVP的DNA序列的寡核苷酸探针偶联的小珠，以及与特异性针对对应于甲基化形式的MVP的DNA序列的寡核苷酸探针偶联的单独的小珠。将候选的DNA分子施用于所述阵列，并在适当的条件下与对应于相关的表观遗传形式的寡核苷酸探针进行选择性杂交。因此，衍生自在相应的基因组DNA中甲基化的MVP的DNA分子将选择性连接至含有甲基化特异性寡核苷酸探针的小珠上，但是不会连接至含有非甲基化特异性寡核苷酸探针的小珠上。仅杂交探针的单碱基延伸掺入标记的ddNTP，随后用荧光试剂染色并成像。MVP的甲基化状态可通过计算源自甲基化和未甲基化位点的荧光信号的比例来确定。

[0128] 因为本文中所限定的膀胱癌特异性诊断MVP生物标记物最初是使用Illumina Infinium HumanMethylation450BeadChip阵列系统来鉴别，所以相同的芯片系统可用于在本文所述的诊断测定中审查(interrogate)那些相同的MVP。然而，替代或定制的阵列可用于审查本文限定的膀胱癌特异性诊断MVP生物标记物，条件是它们包括审查用于给定方法的所有MVP的工具，如本文所限定。

[0129] 还可使用包括上述方法的组合的技术。例如，含有目的MVP序列的DNA可与微阵列杂交，然后进行DNA测序以确定上述MVP的状态。

[0130] 在上述方法中，对应于MVP基因座的序列还可进行富集过程。可通过结合与目的MVP靶向序列互补的结合分子(如寡核苷酸探针)来俘获含有目的MVP序列的DNA。可在亚硫酸氢盐转化之前或之后或者在扩增之前或之后俘获对应于MVP基因座的序列。探针可以被设计为与亚硫酸氢盐转化的DNA互补。然后可将俘获的DNA进行进一步的处理步骤以确定MVP的状态，例如DNA测序步骤。

[0131] 俘获/分离步骤可为定制设计的。或者，各种这类技术可为市售的，例如，购自Agilent Technologies(http://www.agilent.com/home)的SureSelect靶标富集系统。在该系统中，与含有目的MVP序列的DNA互补的生物素化的“诱饵”或“探针”序列(例如DNA)可与样品核酸杂交。然后，使用涂覆链亲和素的磁性小珠来俘获与诱饵序列杂交的目的序列。抛弃未结合的级分。然后(例如通过消化RNA)除去诱饵序列，从而提供从非MVP序列分离的MVP靶向序列的富集库。在本发明的一个优选的方法中，将模板DNA进行亚硫酸氢盐转化，然后通过使用独立于MVP甲基化状态的引物的小规模PCR(例如微滴PCR)扩增目标基因座。扩增之后，将样品进行俘获步骤，以便富集含有目标MVP的PCR产物，例如，如上所述，使用磁性小珠来俘获和纯化。俘获之后，进行标准PCR反应以将DNA测序条形码掺入含有MVP的扩增子中。将PCR产物再次纯化，然后进行DNA测序和分析以确定在目标基因组MVP处甲基胞嘧啶的存在或缺失[31]。

[0132] 本文中所限定的MVP生物标记基因座是例如通过**Illumina®**标识符(identifier)(IlmnID)来鉴别。这些MVP基因座标识符是指市售**Illumina®**Infinium Human Methylation450BeadChip试剂盒中使用的各个MVP位点。由每个MVP基因座标识符代表的每个MVP位点的种类可从Illumina，Inc.网站参照Infinium Human Methylation450BeadChip试剂盒中使用的MVP位点公开获得。

[0133] 关于在Illumina，Inc产品中使用的MVP基因座识别的其他信息见于2010年出版的标题为“Technical Note:Epigenetics.CpG Loci Identification.A guide toIllumina's method for unambiguous CpG loci identification and tracking for the Golden **Gate®**and**Infinium®**Assay for Methylation”的技术说明书中并见于：

[0134] http://www.illumina.com/documents/products/technotes/technote_cpg_loci_identification.pdf.

[0135] 关于Illumina Infinium Human Methylation450BeadChip系统的其他信息可见于：

[0136] http://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_humanmethylation450.pdf；

[0137] 以及见于：

[0138] http://www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/technote_hm450_data_analysis_optimization.pdf.

[0139] 为了补充不断发展的公共数据库以提供准确的MVP/CpG基因座标识符和链取向，**Illumina®**已经开发了一种方法，所述方法可根据每个单个MVP/CpG基因座的实际序列或上下文序列一致地命名MVP/CpG基因座。为了可以毫无疑义地提及任何种类的MVP/CpG基因座，**Illumina®**已经开发了一致的和确定的MVP基因座数据库，以确保在甲基化数据的记录中的统一性。**Illumina®**方法利用MVP基因座侧翼的序列来生成唯一的MVP基因座簇ID。该数字仅基于序列信息，不受基因组版本的影响。Illumina的标准化命名法也与通常用于单核苷酸多态性(SNP)命名的TOP/BOT链命名法(其表示链取向)并列。

[0140] 用于Infinium Human Methylation450BeadChip系统的**Illumina®**标识符还可来自公共存储库如Gene Expression Omnibus(GEO)(http://www.ncbi.nlm.nih.gov/geo/)。

[0141] 因此，本文中所定义的MVP是指相对于表1中列出的每个SEQ ID号和Illumina标识符(Ilmn ID)所鉴定的CG二核苷酸基序，其中可对二核苷酸的胞嘧啶碱基(在表1中列出的序列中用粗体和方括号表示)修饰或不修饰。因此，测定以指定的SEQ ID NO限定或识别的CpG的甲基化状态，或通过测定这类CpG是否甲基化，是指测定在个体的DNA的样品中的一个或多个基因座处，在表1中所示的序列中以粗体和方括号标识的CG二核苷酸基序的胞嘧啶是否被甲基化，应当认识到，由于测序错误或个体之间的差异，都可能存在任何给定的CpG的上游和下游序列中都可能存在变化。

[0142] 本发明提供在个体中诊断膀胱癌的方法，其包括：

[0143] (a)提供所述个体的样品DNA；

[0144] (b)确定选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的一组MVP中的每一个是否被甲基化，其中所述组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP；以及

[0145] (c)当至少25个(b)的组的MVP被甲基化时，在所述个体中诊断为膀胱癌。

[0146] 在任何本文中所述的这类方法中，这组MVP(即这些其甲基化状态待被确定的MVP)可包含26个或更多以SEQ ID NO 1至150标识并且由[CG]指示的MVP；或该组可包含27个或更多、28个或更多、29个或更多、30个或更多、31个或更多、32个或更多、33个或更多、34个或更多、35个或更多、36个或更多、37个或更多、38个或更多、39个或更多、40个或更多、41个或更多、42个或更多、43个或更多、44个或更多、45个或更多、46个或更多、47个或更多、48个或更多、49个或更多、50个或更多、51个或更多、52个或更多、53个或更多、54个或更多、55个或更多、56个或更多、57个或更多、58个或更多、59个或更多、60个或更多、61个或更多、62个或更多、63个或更多、64个或更多、65个或更多、66个或更多、67个或更多、68个或更多、69个或更多、70个或更多、71个或更多、72个或更多、73个或更多、74个或更多、75个或更多、76个或更多、77个或更多、78个或更多、79个或更多、80个或更多、81个或更多、82个或更多、83个或更多、84个或更多、85个或更多、86个或更多、87个或更多、88个或更多、89个或更多、90个或更多、91个或更多、92个或更多、93个或更多、94个或更多、95个或更多、96个或更多、97个或更多、98个或更多、99个或更多、100个或更多、101个或更多、102个或更多、103个或更多、104个或更多、105个或更多、106个或更多、107个或更多、108个或更多、109个或更多、110个或更多、111个或更多、112个或更多、113个或更多、114个或更多、115个或更多、116个或更多、117个或更多、118个或更多、119个或更多、120个或更多、121个或更多、122个或更多、123个或更多、124个或更多、125个或更多、126个或更多、127个或更多、128个或更多、129个或更多、130个或更多、131个或更多、132个或更多、133个或更多、134个或更多、135个或更多、136个或更多、137个或更多、138个或更多、139个或更多、140个或更多、141个或更多、142个或更多、143个或更多、144个或更多、145个或更多、146个或更多、147个或更多或148个或更多的以SEQ ID NO 1至150标识并且由[CG]指示的MVP。该组可包含149或150个以SEQ ID NO 1至150标识并且由[CG]指示的MVP。

[0147] 在任何本文中所述的方法中，当至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP被甲基化时，诊断为膀胱癌。在以下情况下可诊断为膀胱癌：当26个或更多以SEQ ID NO 1至150标识并且由[CG]指示的MVP被甲基化时；或当27个或更多、28个或更多、29个或更多、30个或更多、31个或更多、32个或更多、33个或更多、34个或更多、35个或更多、36个

或更多、37个或更多、38个或更多、39个或更多、40个或更多、41个或更多、42个或更多、43个或更多、44个或更多、45个或更多、46个或更多、47个或更多、48个或更多、49个或更多、50个或更多、51个或更多、52个或更多、53个或更多、54个或更多、55个或更多、56个或更多、57个或更多、58个或更多、59个或更多、60个或更多、61个或更多、62个或更多、63个或更多、64个或更多、65个或更多、66个或更多、67个或更多、68个或更多、69个或更多、70个或更多、71个或更多、72个或更多、73个或更多、74个或更多、75个或更多、76个或更多、77个或更多、78个或更多、79个或更多、80个或更多、81个或更多、82个或更多、83个或更多、84个或更多、85个或更多、86个或更多、87个或更多、88个或更多、89个或更多、90个或更多、91个或更多、92个或更多、93个或更多、94个或更多、95个或更多、96个或更多、97个或更多、98个或更多、99个或更多、100个或更多、101个或更多、102个或更多、103个或更多、104个或更多、105个或更多、106个或更多、107个或更多、108个或更多、109个或更多、110个或更多、111个或更多、112个或更多、113个或更多、114个或更多、115个或更多、116个或更多、117个或更多、118个或更多、119个或更多、120个或更多、121个或更多、122个或更多、123个或更多、124个或更多、125个或更多、126个或更多、127个或更多、128个或更多、129个或更多、130个或更多、131个或更多、132个或更多、133个或更多、134个或更多、135个或更多、136个或更多、137个或更多、138个或更多、139个或更多、140个或更多、141个或更多、142个或更多、143个或更多、144个或更多、145个或更多、146个或更多、147个或更多或148个或更多的以SEQ ID NO 1至150标识并且由[CG]指示的MVP被甲基化时。当149或150个以SEQ ID NO 1至150标识并且由[CG]指示的MVP被甲基化时，可诊断为膀胱癌。

[0148] 优选地，当40个或更多的以SEQ ID NO 1至150标识并且由[CG]指示的MVP被甲基化时，可诊断为膀胱癌。

[0149] 当50个或更多、60个或更多、70个或更多或80个或更多、90个或更多或100个或更多的以SEQ ID NO 1至150标识并且由[CG]指示的MVP被甲基化时，可诊断为膀胱癌。

[0150] 在任何上述方法中，确定被甲基化的MVP可包含以SEQ ID NO 1至3标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至5标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至10标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至20标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至30标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至40标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至50标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至60标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至70标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至80标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至90标识并且由[CG]指示的MVP，或可包含以SEQ ID NO 1至100标识并且由[CG]指示的MVP。

[0151] 在一个实施方案中，这组MVP(即这些其甲基化状态待被确定的MVP)可包含全部150个以SEQ ID NO 1至150标识并且由[CG]指示的MVP，并且在该方法中，当至少40个选自以SEQ ID NO 1至150标识并且由[CG]指示的MVP的MVP被甲基化时，在所述个体中可诊断为膀胱癌，并在该方法中，确定被甲基化的MVP包括以SEQ ID NO 1至10标识并且由[CG]指示的MVP。

[0152] 生物信息学工具和统计学度量

[0153] 帮助进行对亚硫酸氢盐转化的DNA序列的计算机分析和用于甲基化特异性分析目

的的引物设计的软件程序通常是可用的并且已经在前面描述过[57、58、59]。

[0154] 用于基于本文中所述的MVP甲基化状态测定的膀胱癌诊断的敏感性和特异性度量可使用标准接收器工作特性(ROC)统计分析来定义[52]。在ROC分析中,100%的敏感性对应于没有假阴性的结果,和100%的特异性对应于没有假阳性的结果。

[0155] 基于使用一组150个MVP生物标记物进行的分析,本文中所述的根据本发明的膀胱癌诊断测定可实现的ROC敏感性为90%或更高、91%或更高、92%或更高、93%或更高、94%或更高、95%或更高、96%或更高、97%或更高、98%或更高或99%。所述ROC敏感性可为100%。

[0156] 根据本发明的诊断测定可实现的ROC特异性为90%或更高、91%或更高、92%或更高、93%或更高、94%或更高、95%或更高、96%或更高、97%或更高、98%或更高或99%。所述ROC特异性可为100%。

[0157] 根据本发明的诊断测定可具有ROC敏感性和ROC特异性值的相关组合,其中所述组合为任意一个上述所列的敏感性值与任意一个上述所列的特异性值,条件是所述敏感性值等于或小于所述特异性值。

[0158] 因此,ROC特异性可为100%并且ROC敏感性可为90%或更高、91%或更高、92%或更高、93%或更高、94%或更高、95%或更高、96%或更高、97%或更高、98%或更高、99%或100%。

[0159] ROC特异性可为99%并且ROC敏感性可为90%或更高、91%或更高、92%或更高、93%或更高、94%或更高、95%或更高、96%或更高、97%或更高、98%或99%。

[0160] ROC特异性可为98%并且ROC敏感性可为90%或更高、91%或更高、92%或更高、93%或更高、94%或更高、95%或更高、96%或更高、97%或98%。

[0161] ROC特异性可为97%并且ROC敏感性可为90%或更高、91%或更高、92%或更高、93%或更高、94%或更高、95%或更高、96%或97%。

[0162] ROC特异性可为96%并且ROC敏感性可为90%或更高、91%或更高、92%或更高、93%或更高、94%或更高、95%或96%。

[0163] ROC特异性可为95%并且ROC敏感性可为90%或更高、91%或更高、92%或更高、93%或更高、94%或95%。

[0164] ROC特异性可为94%并且ROC敏感性可为90%或更高、91%或更高、92%或更高、93%或94%。

[0165] ROC特异性可为93%并且ROC敏感性可为90%或更高、91%或更高、92%或93%。

[0166] ROC特异性可为92%并且ROC敏感性可为90%或更高、91%或92%。

[0167] ROC特异性可为91%并且ROC敏感性可为90%或91%。

[0168] ROC特异性可为90%并且ROC敏感性可为90%。优选地,所述测定可实现的ROC敏感性为95%或更高并且ROC特异性为90%或更高;优选地,ROC敏感性为96%以及ROC特异性为97%。

[0169] 与代表本文中所限定的诊断方法的实施例方法相对应的ROC图示于图5和7,其表明基于MVP的测定的高度敏感性和选择性。这与用更小的生物标志物组进行的测定形成对比,这已在前面描述过。因此,比较数据证明了本文限定的试验的优良的预测能力。

[0170] 可被用于分类基于MVP的测定的准确度的另一个指标为ROCAUC。在ROC分析中,ROC

图的曲线下的面积(AUC)是二元分类的度量。在随机二元分类中,真阳性和假阳性的数量将近似相等。在这种情况下,ROC图的AUC分数将为0.5。在一个优选的二元分级中,真阳性的数量为100％以及假阳性的数量为0％。在这种情况下,ROC图的AUC分数为1。

[0171] 基于使用本文中所述的生物标记物进行的分析,本发明的膀胱癌诊断测定可实现的ROC AUC为0.90或更高、0.91或更高、0.92或更高、0.93或更高、0.94或更高、0.95或更高、0.96或更高、0.97或更高、0.98或更高、0.99或1。优选地,所述诊断测定可实现的ROC AUC为0.98或更高。

[0172] 基于本文所述的MVP甲基化状态测定的膀胱癌诊断测试也可以使用阴性预测值(NPV)度量来表征。NPV为一种其为真阴性结果的阴性结果的比例的度量。

[0173] ,基于使用一组150个MVP生物标记物进行的分析,本文中所述的根据本发明的膀胱癌诊断测试可实现的NPV为90％或更高、91％或更高、92％或更高、93％或更高、94％或更高、95％或更高、96％或更高、97％或更高、98％或更高或99％或100％。

[0174] 生物样品

[0175] 本文中所述的膀胱癌诊断测定可在获得来自患者的的任何合适的生物材料上进行。优选的生物材料为尿液。然而,可使用膀胱组织的样品,例如其通过活检或抽吸获得,或从保存的样品中获得(例如冷藏材料、组织切片等)。生物材料的样品还可包括固体组织样品、吸出物、生物液体的样品、血液、血清、血浆、腹水、淋巴、外周血、脑脊髓液、细针吸出物、唾液、痰、骨髓、皮肤、上皮样品(包括口腔上皮、宫颈上皮或阴道上皮)或其他来源于外胚层、阴道液、精液等的组织。组织刮片(scrape)可以包括来自例如口腔、食管、膀胱、阴道、尿道或宫颈刮片的生物材料。样品的细胞可包含炎性细胞,如淋巴细胞。

[0176] 任何本文中所述的测定和方法可包括提供来自患者的生物样品作为用于甲基化分析的患者DNA的来源。

[0177] 任何本文中所述的测定和方法可包括从预先已获自患者的生物样品中获得患者DNA。

[0178] 任何本文中所述的测定和方法可包括获得来自患者的生物样品作为用于甲基化分析的患者DNA的来源。用于获得来自患者的生物材料的方法可为非侵入性的,例如从尿液中收集细胞。或者,可使用侵入性方法例如活检。

[0179] 在本文中所述的方法中,检测水平为使得可在包含150000个细胞或更多的样品中检测到2个肿瘤细胞。在这类方法中,所述样品可包含160,000个细胞或更多、170,000个细胞或更多、180,000个细胞或更多、190,000个细胞或更多、200,000个细胞或更多、210,000个细胞或更多、220,000个细胞或更多、230,000个细胞或更多、240,000个细胞或更多、250,000个细胞或更多、260,000个细胞或更多、270,000个细胞或更多、280,000个细胞或更多、280,000个细胞或更多或者300,000个细胞或更多。

[0180] 在任何这类方法中,可被检测的肿瘤细胞的数量为10个或更多、20个或更多、30个或更多、40个或更多、50个或更多、60个或更多、70个或更多、80个或更多、90个或更多、100个或更多、200个或更多、300个或更多、400个或更多、500个或更多、600个或更多、700个或更多、800个或更多、900个或更多、1000个或更多、2000个或更多、3000个或更多、4000个或更多、5000个或更多、6000个或更多、7000个或更多、8000个或更多、9000个或更多、10000个或更多、20000个或更多、30000个或更多、40000个或更多、50000个或更多、60000个或更多、

70000个或更多、80000个或更多、90000个或更多或者100000个或更多。

[0181] 治疗方法

[0182] 本发明还包括在通过本文所述的诊断方法对膀胱癌进行阳性诊断之后进行一个或多个治疗步骤。

[0183] 因此，本发明还包括治疗个体中的膀胱癌的方法，其包括：

[0184] (a)从来自所述个体的样品获得DNA，并确定选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的一组MVP中的每一个是否被甲基化，其中所述组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP；

[0185] (b)当至少25个(a)组的MVP被甲基化时，在所述个体中诊断为膀胱癌，以及

[0186] (c)向所述个体施用一种或多种膀胱癌治疗方法。

[0187] 本发明还包括治疗个体中的膀胱癌的方法，其包括：

[0188] (a)提供来自所述个体的样品的DNA，并确定选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的一组MVP中的每一个是否被甲基化，其中所述组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP；

[0189] (b)当至少25个(a)组的MVP被甲基化时，在所述个体中诊断为膀胱癌，以及

[0190] (c)向所述个体施用一种或多种膀胱癌治疗方法。

[0191] 本发明还包括治疗个体中的膀胱癌的方法，其包括：

[0192] (a)在来自所述个体的样品的DNA中确定选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的一组MVP中的每一个是否被甲基化，其中所述组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP；

[0193] (b)当至少25个(a)组的MVP被甲基化时，在所述个体中诊断为膀胱癌，以及

[0194] (c)向所述个体施用一种或多种膀胱癌治疗方法。

[0195] 本发明还包括治疗个体中的膀胱癌的的方法，所述方法包括向所述个体施用一种或多种膀胱癌治疗方法，其中所述个体已通过以下的步骤被诊断为膀胱癌，所述步骤包括：

[0196] (a)提供来自个体的样品的DNA，并确定选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的一组MVP中的每一个是否被甲基化，其中所述组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP；

[0197] (b)当至少25个(a)组的MVP被甲基化时，在所述个体中诊断为膀胱癌。

[0198] 在任何上述治疗膀胱癌的方法中，所述选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的MVP组(即其甲基化状态待被确定的MVP的组)可包含任何数量的本文中所述和定义的MVP，条件是该组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP。在这些方法中的任一种中，当被确定为甲基化的组中MVP的数量为任何数量的本文所述和定义的MVP时，在所述个体诊断为膀胱癌，条件是至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP被确定为甲基化的。

[0199] 因此，本发明包括在阳性诊断为膀胱癌之后，施用一种或多种外科方法、一种或多种化学治疗剂、一种或多种免疫治疗剂、一种或多种放射治疗剂、一种或多种激素治疗剂，或任何上述的组合。

[0200] 外科方法包括膀胱肿瘤的经尿道切除术(TURBT)、膀胱切除术、开放式根治性膀胱切除术(ORC)、腹腔镜根治性膀胱切除术(LRC)和机器人辅助根治性膀胱切除术(RARC)。

[0201] 化学治疗剂包括以下物质。烷化剂包括氮芥、亚硝基脲、四嗪、氮丙啶、顺铂和铂基衍生物以及非经典的烷化剂。抗代谢物包括抗叶酸剂、氟嘧啶、脱氧核苷类似物和硫代嘌呤。微管破坏剂,包括长春花生物碱和紫杉烷以及多拉司他汀10及其衍生物。拓扑异构酶抑制剂,包括喜树碱(camptothecin)、伊立替康(irinotecan)和托泊替康(topotecan)。拓扑异构酶II毒素,包括依托泊苷(etoposide)、阿霉素(doxorubicin)、米托蒽醌(mitoxantrone)和替尼泊甙(teniposide)。拓扑异构酶II催化抑制剂,其包括新生霉素(novobiocin)、美巴龙(merbarone)和阿柔比星(aclarubicin)。细胞毒性抗生素,包括蒽环类抗生素(anthracyclines)、放线菌素(actinomycin)、博来霉素(bleomycin)、普卡霉素(plicamycin)和丝裂霉素(mitomycin)。

[0202] 试剂的组合包括但不限于MVAC(甲氨蝶呤(Methotrexate)、长春花碱(Vinblastine)、长春花碱和长春化碱)、Gem-Cis(GC)(吉西他滨(Gemcitabine)和顺铂)、拉帕替尼(Lapatinib)和吉西他滨。

[0203] 免疫治疗包括卡介苗(BCG)免疫疗法以及单克隆抗体和抗体-药物缀合物。抗体-药物缀合物包括与如上所述的微管破坏剂和DNA修饰试剂缀合的抗体。

[0204] 组合疗法包括膀胱内连续BCG,然后电动施用(EMDA)MMC(EMDA-MMC)以及微波诱导膀胱壁体温过高(HT)和膀胱内MMC。

[0205] 将癌症治疗剂以足以治愈的量给药至已经患有障碍或病症的受试者,减轻或部分停止所述病症或一种或多种其症状。这种治疗处理可使得降低疾病症状的严重性或提高无症状时期的频率或持续时间。将足够达到该效果的量定义为“治疗有效量”。用于给定目的的有效量将取决于疾病的严重性以及受试者的体重和常规状态。如本文中所用,术语“受试者”包括任何人。

[0206] 阵列

[0207] 本发明还包括能够鉴别本文中所定义的MVP的甲基化和非甲基化形式的阵列;所述阵列可包含对本文中所定义的MVP的甲基化形式具有特异性的寡核苷酸探针以及对本文中所定义的MVP的非甲基化形式具有特异性的寡核苷酸探针。

[0208] “特异性”意味着所述探针包含与含有MVP的寡核苷酸的序列互补的序列,使得它们可以杂交,特别是在严格条件下杂交。

[0209] 在一些实施方案中,所述阵列不是Illumina Infinium HumanMethylation450BeadChip阵列(Infinium HumanMethylation450BeadChip阵列)。

[0210] 单独地或额外地,在一些实施方案中,所述阵列的MVP-特异性寡核苷酸探针的数量为小于482421个,优选482000个或更少、480000个或更少、450000个或更少、440000个或更少、430000个或更少、420000个或更少、410000个或更少或400000个或更少、375000个或更少、350000个或更少、325000个或更少、300000个或更少、275000个或更少、250000个或更少、225000个或更少、200000个或更少、175000个或更少、150000个或更少、125000个或更少、100000个或更少、75000个或更少、50000个或更少、45000个或更少、40000个或更少、35000个或更少、30000个或更少、25000个或更少、20000个或更少、15000个或更少、10000个或更少、5000个或更少、4000个或更少、3000个或更少或2000个或更少。

[0211] 本发明还包括本文中所限定的任何阵列在任何方法中的用途,其需要在个体中确定MVP的甲基化状态以便诊断膀胱癌细胞。

[0212] 试剂盒

[0213] 本文中所限定的任何阵列可被包含在试剂盒中。

[0214] 所述试剂盒可包含本文中所限定的任何阵列。

[0215] 所述试剂盒可包含本文限定的任何阵列以及使用说明书。

[0216] 所述试剂盒可额外地包含DNA修饰试剂,例如亚硫酸氢盐试剂。

[0217] 所述试剂盒可额外地包含用于扩增DNA的试剂,例如本文中所限定的以SEQ ID NO 1至150标识的任何MVP的引物(参见表2)。

[0218] 确定样品甲基化情况的方法

[0219] 本发明还包括确定来自个体的样品的甲基化情况的方法,所述方法包括:

[0220] i.提供来自个体的样品的DNA;

[0221] ii.确定选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的一组MVP中的每一个是否被甲基化,其中所述组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP;以及

[0222] iii.基于该组MVP的甲基化状态,确定所述样品的甲基化情况。

[0223] 在任何上述确定样品的甲基化情况的方法中,所述选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的MVP组(即其甲基化状态待确定的MVP的组)可包含任何数量的本文中所述和定义的MVP,条件是该组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP。

[0224] 另外,在任何这类方法中,MVP的甲基化状态可使用本文中所述的任何阵列来确定。

[0225] 其他方法

[0226] 在本文中所述的任何诊断方法中,在个体中诊断膀胱癌的步骤可还包括:

[0227] I.对肿瘤等级进行分级;和/或

[0228] II.确定肿瘤复发的风险;和/或

[0229] III.确定非肌层侵入性疾病的进展风险;和/或

[0230] IV.确定对治疗疗法可能的反应。

[0231] 本发明还包括确定个体中发生膀胱癌的风险的方法,所述方法包括:

[0232] (a)提供来自所述个体的样品的DNA,

[0233] (b)确定选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的一组MVP中的每一个是否被甲基化,其中所述组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP;以及

[0234] (c)基于该组MVP的甲基化状态,确定所述个体中发生膀胱癌的风险。

[0235] 在任何这类确定个体中发生膀胱癌的风险的方法中,所述选自以SEQ ID NO 1至150标识且由[CG]指示的MVP的MVP组(即其甲基化状态待确定的MVP的组)可包含任何数量的本文中所述和定义的MVP,条件是该组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP。

[0236] 另外,在任何这类方法中,MVP的甲基化状态可使用本文中所述的任何阵列来确定。

[0237] 其他用途

[0238] 本发明还包括一组MVP在个体中诊断膀胱癌的用途或在个体中确定发生膀胱癌的风险的用途。

[0239] 在任何这类用途中，该组MVP选自以SEQ ID NO 1至150标识且由[CG]指示的MVP。

[0240] 在任何这类用途中，该组MVP可包含任何数量的本文中所述和定义的MVP，条件是该组包含至少25个以SEQ ID NO 1至150标识并且由[CG]指示的MVP。

[0241] 在任何上述用途中，在个体中诊断膀胱癌或在个体中确定发生膀胱癌的风险可通过本文中所述和限定的相应方法中的任何一种进行。另外，在任何这类方法中，MVP的甲基化状态可使用本文中所述的任何阵列来确定。

[0242] 本发明通过以下实施例来举例说明：

[0243] 实施例

[0244] 材料和方法

[0245] 介绍

[0246] 利用新一代DNA测序平台的新兴技术特别有希望开发出对于高敏感度的外遗传生物标记物组。例如，亚硫酸氢盐转化的DNA的基于微滴的PCR扩增，及随后的通过RainDance Technologies开发的扩增的靶基因座[30]的新一代测序，使得能够进行对高达20000个亚硫酸氢盐转化的靶基因座的灵敏的、特异的且同时的扩增。亚硫酸氢盐转化的DNA的高度并行的基于微滴的PCR扩增已经证明可用于证实一系列组织中的外遗传改变[31-33]。然而，它还没有被应用于开发用于检测膀胱癌的非侵入性诊断生物标记物。

[0247] 为了获得用于检测膀胱癌的灵敏测定法，本发明人进行了迄今为止最大的膀胱癌中全基因组甲基化的独立研究之一。由此看来，已经限定了一组膀胱特异性外遗传生物标记物，并且已经验证了使用RainDrop-BS[31]的150个基因座组对尿DNA的敏感性和特异性以用于以高度的诊断精确度检测膀胱癌。

[0248] 研究人群

[0249] 全基因组DNA甲基化分析针对来自81例膀胱癌的DNA和从UCLH(London) Department of Urology and CIEMAT(Madrid)收集的匹配30岁的正常尿路上皮样品的DNA进行。该同龄组包括35个低等级的非肌层浸润性癌症。进行代表性H&E切片的病理审查以包括肿瘤细胞性质>80％的样品。从MARMAL辅助数据库下载血液甲基化组数据(http://marmal-aid.org,[34])。

[0250] 独立的验证数据是从癌症基因组图谱计划(Cancer Genome Atlas Project)获得的(https://tcga-data.nci.nih.gov/tcga/tcgaCancerDetails.jsp?diseaseType＝BLCA&diseaseName＝Bladder%20Urothelial%20Carcinoma)，其由MIBC膀胱癌和20例正常的尿路上皮样品组成。

[0251] 对于验证研究，从就诊UCLH一站式血尿和监测膀胱镜检查诊所的患者采集连续尿液样品(n＝86例膀胱癌，n＝96例非癌症对照)。

[0252] 尿液采集

[0253] 从就诊血尿诊所或在University College Hospital为了复发的膀胱癌进行膀胱镜检查的患者获得尿液样品。为了比较诊所尿液样品与家庭尿液样品，患者被要求提供三个样品：一个诊所样品和两个家庭样品，其中一个应该是首段(first void)尿液。每个样品得到40-100ml。针对一个样品的家庭尿液试剂盒包含至多四个25ml无菌管、带有可吸收衬

垫的邮寄管和预先填好回邮地址的带衬垫信封，设计以适合通过皇家邮政信箱。

[0254] DNA提取和定量

[0255] 根据制造商的说明书使用DNeasy血液和组织试剂盒(Qiagen,UK)提取尿液DNA。通过分光光度法(Nanodrop 1000)和荧光测定法(Qubit ds DNA HS测定,Invitrogen,UK)定量DNA。

[0256] RainDance微滴PCR

[0257] 对于微滴PCR,将7.20μl亚硫酸氢盐处理的(以及任选地,全基因组扩增的)DNA加入到4.70μl 10×高保真度缓冲液(Invitrogen)、1.80μl 50mM $MgSO_4$(Invitrogen)、1.62μl 10mM dNTP溶液混合物(NEB)、3.60μl 4M甜菜碱溶液(Sigma-Aldrich)、3.60μl液滴稳定剂(RainDance Technologies)、1.80μl 100％二甲基亚砜(Sigma-Aldrich)和0.72μl 5U/μl铂Taq聚合酶高保真度(Invitrogen),至总体积为25μl。样品板使用ALPS 50V微孔板热封机(Thermo Scientific)密封。

[0258] 然后将经亚硫酸氢盐处理的基因组DNA模板混合物按照制造商的说明书施加于全自动ThunderStorm系统(RainDance Technologies)。简言之,将引物板液滴(MethylSeq Solution,RainDance Technologies)涂布到微流体芯片。DNA模板混合物在微流体芯片内转化成液滴。然后将引物对液滴和模板液滴以1:1的比率配对在一起。成对的液滴穿过电场,其诱导离散的液滴聚结成单个PCR液滴(26μl);每个样品收集约1百万个PCR液滴。

[0259] 将PCR微滴在PTC-225热循环仪(MJ Research)中进行如下处理:94℃2min;55个循环:94℃30s,54℃45s,68℃80s;接着68℃10min;4℃下直到进一步处理。缓变率设定为每秒1℃。PCR扩增后,将70μl液滴去稳定剂(RainDance Technologies)加入到每个样品中以破坏PCR微滴乳液并释放包含在液滴内的扩增子。将溶液充分混合并在室温下孵育15min。使用Agencourt AMPure XP磁性小珠(Beckman Coulter)按照制造商的方案纯化样品。对于每个样品,使用234μl小珠。样品从40μl缓冲液中的磁性小珠洗脱。纯化的扩增子DNA的完整性和浓度(片段范围:120-300bp)是使用高敏感性DNA试剂盒(Agilent Technologies)在2100生物分析仪(Agilent Technologies)上进行评估。

[0260] 甲基化阵列

[0261] 将500ng DNA进行亚硫酸氢盐转化,与Infinium 450K人甲基化阵列杂交并根据制造商的建议进行处理。DNA亚硫酸氢盐转化是使用EZDNA甲基化试剂盒(Zymo Res earch)根据制造商的说明书进行。样品在单个批次中进行处理。R统计软件(版本2.14.0[35])用于随后的数据分析。ChAMP流水线用于从iDat文件中提取并分析数据,使用BMIQ将样品标准化[36,37]。对原始β值(甲基化值)进行如下严格的质量控制分析:去除表现出降低的覆盖度的样品,并且仅保留所有样品中具有高于背景的检测水平的探针(检测P＜0.01)。DMR(差异甲基化区)是使用Lasso测定[38,39]。

[0262] 高通量DNA测序

[0263] 根据制造商的方案,将合并的测序文库(12pM)和定制的测序引物(0.5μM)施加到MiSeq循环PE消耗筒(Illumina)。定制的测序引物的DNA序列在下表2中提供。测序在MiSeq DNA测序仪(Illumina)上使用75-bp双端读段来进行。

[0264] RainDance **ThunderStorm®** 系统也用于核酸的测序(http://raindancetech.com/targeted-dna-sequencing/thunderstorm/)。

[0265] 数据和统计分析

[0266] 测序接头是使用ea-utils v1.1.2-537的fastq-mcf工具从原始测序读段中进行剪切[60]。经剪切的测序数据使用Bismark v0.7.12被映射基于计算机的亚硫酸氢盐转化的人类参考基因组(GRCh37)[40,61]。使用Bismark v0.7.12的甲基化提取器工具提取甲基化信息[61]。靶向的DNA测序分析是使用R程序包TEQC v3.2.0.25进行。

[0267] 实施例1:低等级和高等级膀胱癌的甲基化分析

[0268] 与膀胱癌相关的外遗传改变最初是通过对来自81个高等级尿路上皮和30个正常尿路上皮的DNA进行全基因组DNA甲基化分析来限定的。

[0269] 使用Wilcoxon秩和检验来指定方向性的监督分析被用于识别膀胱癌组织和正常组织之间的MVP(甲基化可变位置)。根据统计学显著性选择MVP(Wilcoxon P值＞0.001)。使用Δβ＞0.30(+/-)的额外滤器以补偿未考虑组之间甲基化的绝对差异。截断值根据经验限定为导致假发现率(FDR)＜2%,将候选基因座减少至甲基化差异最大并因此具有最大的区分效果潜力的那些基因座。总共9786个MVP符合这些要求(1746个超甲基化MVP、8040个低甲基化MVP)(图1)。

[0270] 实施例2:膀胱癌特异性尿液生物标记物

[0271] 为了限定DNA甲基化生物标记物组,识别在至少50%的癌症中确定是甲基化的(β＞50%)且在正常尿路上皮中确定是非甲基化的(β＜10%)的那些基因座。如本文中所讨论的,涉及该初始DNA甲基化生物标记物组的开发的“甲基化的(β＞50%)”,是指对于任何给定的基因座,对于该MVP而言,患者样品中>50%的细胞被确定是甲基化的。为了去除潜在的假阳性生物标记物并更好地限定膀胱癌特异性的改变,还对来自10名就诊血尿诊所并且具有确诊的非癌症诊断的患者的全血和尿液进行了分析。随后,从这些非癌症对照的尿液和血液中除去在DNA中显示任何甲基化(β＞10%)的基因座。最多识别432个基因座,其在非癌症中为非甲基化的且在大多数癌症组织中是甲基化的(β＞50%)。

[0272] 为了获得用于检测的膀胱癌DNA甲基化标记(signature),本发明人使用Random Forest框架,这得到由150个CpG基因座组成的分类标记(图2,见下表1)。使用这150个标记物的核心集合,本发明人对预测的可能值进行了分类器(classifier)的内部交叉验证,即对每个样品而言,在独立于其与样品组的关系的情况下,样品是否为癌症的可能性。这获得用于检测癌症的交叉验证的敏感性为100%、特异性为100%,表明150个外遗传基因座可以清楚地区分正常尿路上皮与膀胱癌(图3,图4)。

[0273] 为了确定使用分类算法检测膀胱癌的150CpG(MVP)标记物组的敏感性,本发明人评估了另外179个膀胱癌(144个肌层浸润性和35个非肌层浸润性)和20个正常病例的甲基化谱。该组正确分类了所有膀胱癌,其敏感性和特异性均为1。

[0274] 实施例3:检测组的验证

[0275] 为了测试用于在尿液样品中检测膀胱癌的150个基因座组(外遗传标记(epi-signature)),从包括52位膀胱癌患者(低等级＝27,中等级/高等级＝25)和34名非癌症患者对照的同龄组(n＝86)中获得来自尿沉淀细胞的DNA。在这个独立的验证同龄组中,将外遗传标记应用于该验证同龄组中,检测膀胱癌的敏感性为95%、特异性为96%、AUC为97%(图5)。

[0276] 大型标记物组也与来自训练同龄组的表现最好的单一标记物相比较,这包括来自

在包括OTX1、COD1和MEIS1的基因中在先公布作为潜在的尿液生物标记物的区域的CpG基因座。对于每个CpG，基于从训练同龄组中的非膀胱癌对照中获得的最高β值，限定了甲基化阈值。然后该值用于预测验证同龄组中可能存在的膀胱癌。将性能最好的单一标记物结合起来，并开发出一种预测分类器，以探索基于3、5或10个标记物的“寡组”的潜力。虽然敏感性比单个标记物更好(最佳单个标记物72％，最佳组合标记物70％)，但他们仍未达到替代膀胱镜检查所需的水平。

[0277] 这些数据表明，虽然单个标记物单独或组合地的性能相当好(图4-6，表1)，但使用单个基因座或寡组方法检测癌症的敏感性是有限的，低于临床实用所需的检测水平。使用大的标记物组的性能优于使用单个标记物组，表3示出了前10个最佳标记物和组合的150个基因座标记的敏感性、特异性、AUC、PPV和NPV。

[0278] 实施例4：使用高通量技术验证检测组

[0279] RainDrop BS-seq[31]允许并行地对大量区域(最高达2000个独特的扩增子)进行大规模的靶向亚硫酸氢盐测序。这项技术先前已经得到验证，并且证明与450K甲基化阵列高度相关，并且其低模板输入的应用也已得到验证[31,32]。设计亚硫酸氢盐转化的测序引物组以测量150个所选择的基因组基因座的甲基化状态(参见下表2)。设计引物用于在可能的情况下独立审查Watson和Crick链。使用RainDrop BS-seq在96个病例的第二个独立同龄组中进行尿液的外遗传标记的验证。从26名膀胱癌患者和64名非癌症患者对照中获得来自尿沉淀细胞的DNA。使用Bismark算法产生150个基因座中的每一个的甲基化评分，使用这个数据，尿液的外遗传标记预测可能存在的膀胱癌，敏感性为96％、特异性为97％且AUC为0.96。

[0280] 组合来自所有验证样品的甲基化数据可以增加所测试的样品的数量。组合样品允许评估176个独特的尿液样品：98个非癌症尿液和78个癌症尿液。外遗传标记预测膀胱癌的存在，AUC＝0.98的尿液，与分析技术无关(图7)。

[0281] 结论

[0282] 生物标记物驱动的膀胱癌早期非侵入性检测有可能从根本上改善这种疾病的治疗。高度敏感和特异性的测定法在检测到血尿诊所就诊的患者的新发疾病以及筛查现有膀胱癌患者的复发方面具有潜在效用。

[0283] 几种非侵入性检测可商购，并是基于细胞学、FISH311分析和突变检测。尽管被FDA批准，所述测试报告的敏感性为54％至86％，特异性为61％至90％[12,13,41]。性能特征不足以代替膀胱镜检查，因此尚未被纳入临床实践。因此，新型生物标记物的改进和开发具有很大的空间，生物标记物组的组合和新技术的使用可能对此目的最有帮助。

[0284] DNA甲基化模式是高度细胞特异性的，这些外遗传事件的个体发育稳定性使得DNA甲基化成为检测和诊断疾病的理想生物标记物。整体DNA甲基化模式中的变化是致瘤性转化的共同特征，并且是膀胱癌中的常见事件。以前的研究已表明，膀胱癌(非肌层浸润性膀胱癌(NMIBC)和肌层浸润性膀胱癌(MIBC))和正常尿路上皮之间的甲基化改变反映在来自膀胱癌患者的尿沉淀细胞中，并且因此可能是有用的诊断标记物。现在有几项研究已表明尿液的外遗传标记物在膀胱癌诊断中的效用。然而，尽管这些研究已经显示出良好的敏感性和特异性，但它们在临床实践中仍未被采纳，主要是因为它们仍不能拥有代替膀胱镜检查的性能特征。DNA甲基化生物标记物测定(连同其他基于DNA的标记物，例如突变)受限于

识别推定的生物标记物的主要分析的低分辨率(使用候选方法或基于低分辨率微阵列的平台),以及受限于可用于分析尿液中的候选标记物的技术中的限制。这导致在最终的生物标记物组中审查单个/小的生物标记物组[15-23]。然而,小的生物标记物组为了显示出与膀胱镜检查相匹配的敏感性和特异性,它们在很大程度上依赖于广泛疾病状态下低的肿瘤内和肿瘤间异质性[42]。

[0285] 新的技术,如下一代亚硫酸氢盐测序和大规模多重PCR,目前允许使用更大的外遗传生物标记物组[31,32]。为了确定涉及膀胱癌发生的外遗传改变以及为了限定生物标记物组,进行了迄今为止最大的无偏的膀胱癌全基因组DNA甲基化筛选之一。除了可以深入了解促进膀胱癌发生的外遗传改变外,还从这些数据中鉴别了一组外遗传生物标记物,它们对膀胱癌的检测具有高度的敏感性和特异性。

[0286] 限定了150个单个CpG基因座的生物标记物组,其可预测膀胱癌。虽然只有相对小的同龄组,但这些数据证明了与单个标记物和小的组生物标记物组相比,使用大的外遗传标记物组的效用。敏感性为96%,特异性为97%,该测试的阴性预测值(NPV)为97%。这比PSA(前列腺特异性抗原)测试(PPV=30%-43%)、乳房X线照相术(PPV=9%-19%)或大便潜血筛查(PPV=6%-11%;[43-48])所能达到的要好1.2至8.7倍,并且与膀胱镜检查相似(PPV=66.7%-98%)[49]。

[0287] 本发明人已经证明了,通过应用高度敏感的和定量的大规模高度多路复用下一代测定,能够以比先前公开的甲基化测定更高的敏感性和特异性检测尿液中膀胱癌的存在,并且具有相当于膀胱镜检查的PPV。

[0288] 本发明证明了允许审查更大组的新技术与膀胱癌特异性外遗传生物标记物的组合可以用于检测膀胱癌,使得膀胱镜检查的次数减少,并且因此改善患者的生活质量以及减少医疗保健支出。此外,大组测定的效用允许从相同数据内评估多个临床参数的潜力。例如,肿瘤等级的分级、非肌层浸润性疾病的复发或进展、对肌肉浸润性疾病治疗的可能反应或多种疾病的差异诊断。

[0289] 实施例5:DNA样品处理和加工的优化

[0290] 进行优化研究以使来自患者尿液样品的DNA产率最大化。DNA与蛋白酶K在56℃下孵育1小时或在21℃下孵育48小时。孵育是在100mg/mL的RNA酶A的存在下进行。采用延长的孵育方案观察到DNA的量和纯度的增加(图8D)。

[0291] 将UCL家庭尿液采集试剂盒与可商购试剂盒(Norgen,Cat#18124,https://norgenbiotek.com/display-product.php?ID=424)进行比较。标准UCL尿液采集管含有70mg/ml Stabilur™尿液防腐剂。这项研究是针对来自健康志愿者的尿液进行的;每个样品的一半用UCL标准方法处理,另一半用Norgen防腐剂处理。

[0292] 观察到用这两种方法保存的尿液的DNA的完整性几乎没有差异。没有观察到在首段样品和在其他时间排泄的样品之间的DNA纯度或产率的差异(浓度对比时间,$c^1(1)=0.255$,$p=0.614$;纯度对比时间,$c^1(1)=1.046$,$p=0.306$)。

[0293] 然而,注意到在用Norgen防腐剂处理的尿液中超过8天时的DNA产率明显增加,而用UCL标准方案处理的尿液的DNA无论任何时间均具有相似的量。

[0294] 发现使用Norgen系统尿液时DNA产量的明显增加的原因是细菌随时间的增长,而使用UCL建立的方案有效抑制了细菌的生长(图8E)。

[0295] 实施例6:使用高通量技术对检测组进行额外的验证

[0296] 如上所述分析来自32个确诊的膀胱癌病例和64个非癌症病例(验证同龄组2)的96个尿液样品。亚硫酸氢盐转化后,使用RainDance ThunderStorm® 系统进行对上述150个UroMark基因座的测序。如上所述进行统计分析,得到的ROC图如图9所示(AUC=0.96,敏感性=0.97,特异性=0.97,NVP=0.98)。

[0297] 在进一步的研究中,如上所述分析来自血尿和已知癌症样品的同龄组(验证同龄组2)的92个尿液样品。该同龄组包括27例确诊的癌症病例和65例非癌症病例。同样,在亚硫酸氢盐转化之后,使用RainDance ThunderStorm® 系统进行对上述150个UroMark基因座的测序。如上所述进行统计分析,得到的ROC图如图10所示(AUC=0.955,敏感性=0.98,特异性=0.97,NPV=0.97)。

[0298]

表1

下表1提供了在本文所述方法中使用的150个MVP(CpG)的列表。为每个CpG提供的是Illumina标识符(Ilmn ID)、在人类基因组中指定CpG位置的染色体编号(CHR)和染色体位置(MAPINFO)、基因名称(如果有的话)、包括每个CpG和相应的SEQ ID编号的正向序列。经修饰的CG二核苷酸基序的胞嘧啶在每个序列中以粗体和在方括号中标识。CpG按照从1到150的排列顺序列出。排列顺序与其中给定基因座是甲基化的肿瘤的数目相关。因此,对应于SEQ ID NO:1的MVP在最大数量的肿瘤中是甲基化的,而对应于SEQ ID NO:150的MVP在最少数量的肿瘤中是甲基化的。

MVP 编号	Ilmn ID	CHR	MAPINFO	UCSC_REFGENE_名称	正向序列	SEQ ID NO.
1	cg25622366	2	63281139	OTX1	TTGCGAAGGCCGAGATCTGGGCCTGCCAGGGGCCTGCCCGAGTCCTCTATCGCGGGTCCA**[CG]**TGGCCACCAATGACCCGCGGCGCCCCCGCGTGTCCCCGCAGCCACTCCGCGGAAGCAGCG	1
2	cg20302133	1	111217194	KCNA3	CCTGCTCGCCCGCGGCCGGCAGTGAGGGCGGCAGCGGCTCGTAGCGGTCGCAGCCGCCGC**[CG]**CCACAGCCGCCTTGAGGCGGGGCCCCTCCACCATCGGCCACCTCCGGCTCCAGCAGGTGG	2
3	cg13046832	14	29254680	C14orf23	CCGGGACCCCGTCCCTCTTTCCCCTTCAGTCTTCAGGGAGGGGGAGGCGCTCCGCATTAG**[CG]**GGGCAGTTCAGCAACCCCGACCCCACCCGCGTGGCTCCAGGCCCAGGGGTCCGTTCACTT	3
4	cg17945976	2	66667433	MEIS1	GTGGAGAGCTCACTCTGCAGAATAAAATCAAGAACACCACGGTTGTAGCTGCTGTGGACT**[CG]**GCGGTCTGAGCAGCCTCCCCGAGAGCCGTAGTTGCTAGTAGAAGTAAGATTGAGCTCTGG	4
5	cg21472506	2	63283967	OTX1	CTCCAAATCAAAACCACTAAGAGTTCCTCCCGCGCAGACTGCTGCCCCTTCAGCTGCCCT**[CG]**ATTTTGCTCCACGCCTGCCGGCCAGAGCCTCCCGGCGTTTCTTCCGCCCCAGCGGAGTGC	5
6	cg26492446	20	61638574	BHLHE23	GGAGCGCTAGTACTTGCTTCTCGACTCCCCGGCCGCCGCCTCCGGCCGCCCCGGGGATTC**[CG]**CCACCAAACGCACGCGTCCCAGGTGGGCACCCGCCTCGGTCCGTCTTTGAGTCTGACCCT	6
7	cg11142705	3	42306974	CCK	GCCTCTCTTGACGCAGCTGTAAAATGCGGATGACACCATCTGGTTTTGCTCAGAGGAATC**[CG]**GTTTGGGAAAGGGATGTGTTTTCTTCCCGGGCCAAGTTACCACCACCCGCGGCGCCCACT	7
8	cg26970841	16	85932666	IRF8	TGGCTGCCGGGGGCGGGAAAGTGATTTCTCGGAAAGCAGAGCACTTCGAAGAAGGCGGGC**[CG]**CGCGAGCCAAGCTGACGCTATTGGTCGGTGTGGCCGTCGCTCTGCGCACCGCCCGTCCCC	8
9	cg23497016	2	66666684	MEIS1	GCAGTGGAGGGGACGAGGGCTTGTCGGGTGGGAAACTTAATTCAAAATGGCTGCTGGAAA**[CG]**CTTGGGTTTTATTCGTAGCAAATGTTGCCAATTTCTCCGGCCAGATACGCTAAACCGATC	9
10	cg19178853	4	174450408	NBLA00301; HAND2	GGCGGCGGCGGCAGCTGCGGCGGCGGCGGCGGCAAACGGGTAGCCCTCGTGGTGCACCAC**[CG]**GGTGGTGGGGAAAACCACCTACCAGACTCATTTCGCCCTCCGCGCCCCTCCACGCGCCCC	10
11	cg14428146	8	23563925	NKX2-6	TCTCCAAGACTGCCTCACAGGGACCCCCAGGAGGCTCCGAACCATCCAGCTTTCTGTCAC**[CG]**CCGCCGCCACCAGCGTTGTGAACCTCTGACCCTCGCGGCTCTGCGTCCATTCTCAGGTAC	11
12	cg16887264	4	147561775	POU4F2	TCCCACCGCGAGAAGCTCACCAAGCCTGAACTCTTCAATGGCGCGGAGAAGAAGCGCAAG**[CG]**CACGTCCATCGCTGCGCCAGAGAAGCGCTCGCTCGAAGCCTACTTTGCCATTCAGCCTCG	12
13	cg06392169	6	391936	IRF4	CCAGAGGTTCGACCTCCAGGGCAGCGCAGGGTACCCCGGCTTCGGAGCGGGAAGGGAGCG**[CG]**CCCCGTCCTGGAGCTCCGACTCCCACCCCATCTGCGCTGAGCCGGAGGCGCTGGTTTGGG	13
14	cg11433622	2	66667549	MEIS1	CTCTGGGCTTGTTTTCAGCTTATTTAATTCACACTGAAATGTTTCTGCGCGGGACGAACT**[CG]**GTGTCACCGGGTCCCTCCCGGAGGGTTACTTCCTGCCCCCGACAGTGTAATGAGGCAAGA	14
15	cg01791874	5	16180055	Mar-11	CGGGGGTGACGGGGCGCGGGCGCGGGGTGGGCTGGGGGCGCGGATCAGTGGGACGGAGTT**[CG]**GGGTTCGGCTCCGAGCGGGCGGGCTGGAAGTGGGGGATCCCTCAGCCGCCTCCACGGGCC	15
16	cg23180938	5	115152485	CDO1	GGAGGACGAGGCGGAGAGCCACCCAAGAAAGGTGGCGGAGGCGGGGAGACCCTGCGGGCA**[CG]**GCTCACGCGCACATCCCCGGCTTCCCCGGGCTCCGCGCCTTCCCAAGAGCCCCGTTGTCT	16
17	cg26990102	2	119606746	EN1	ACTTGTCTCCGGATTCGTTTTGGAGGACCAGTATCCGACTATGCTGAAGCCTGCGGTGGC**[CG]**AGAAAGGCGTCAGCAAACGAGGCCTTCGGGGGTGTCATCTTAGAGCTCCAGCGGACCTCC	17

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18	cg11595545	1	111217497	KCNA3;KCNA3	GGGGAAGAGGCGGCAGCGGTGAGGCCAGGTCGCTCCTCCTCGCGCTCCCCGCCCTTTCGC**[CG]**CCTCCGCCCCCGAGCCGAGCCCACCGCCTGTTGCAGCCAAAGCCGCGATGCTCTGTCTGG	18
19	cg00986824	12	75601465	KCNC2;KCNC2;KCNC2	GTGCGGTAGTAATTGAGCACATAGGCGAAGACGCCCGGGTGCCGGTCGAAGAAGAACTCG**[CG]**GCCGCCACCGGGATGGTCGCTGGCCCTGCCGCCGCGGGAACTGCAGTTGCCCGCGCCGCC	19
20	cg09462924	2	66666470	MEIS1	GGCTCTCCGAGGGCCTTGGGGTTGGGATCCCTAGGTGCAGCCCGTTGACAGTCGGCCCCA**[CG]**GCCATGGACGTCCTTTCCCCAAGTTAGCTGAGCGCCTGCCACCGAGATCCCCCGAGCCTG	20
21	cg07790615	1	237205174	RYR2	AGATTAGCCACCCCGTGCGCCCAGGTGAAAGATATCATTCTTCCGTGCGATCCGAAGTGC**[CG]**TGGAAGTTAGTGCCCTAGCCCAGTCCAGGAGGAAGGGGCGTCGTGCCGGCGGTTTTAAGC	21
22	cg06785999	14	60975964	SIX6;SIX6	GCCGAGCCCGAACCCCAAGCCGCGGAGCCAGCACCTCCTCCAGTCGGGGTCGTCCGCTCC**[CG]**GCCGTTGAGCCACCGCCGCCACCCGGTAGTGTGTCCCGCTGCCCCAATCCGCCTCATCAA	22
23	cg04550737	1	119530600	TBX15	GAGACTGCGGCTCGCGGGTCTCTCCACCCTCCCCCTGCGTCCTCCTCCGCCCTCCTCTGC**[CG]**GATCCGACCTGCGCCCCTACGCTGGCCCAGCTGCTAGGAACTAGCGCCCCGAGCGCCGCC	23
24	cg19978181	5	76923988		TTACTTGGGAGGTCCCGGGTATCTGAAGCGGATCCCGGGTCTGGGGACATGAAGGGGCGC**[CG]**TGGCCTTAGGGAAGGCCCCAAAGAGGCCTAGGCCCCGGAGGAGGCAAGAGCCGCGGCCTA	24
25	cg23065934	5	16180266	Mar-11	GGGGAGAGGCCGCGGGTCCAGAAACCGTTACTGGATGGGCCGGTGGGATGTGGCGCGGGC**[CG]**GGTGGGGCGCGACAGTCTGAGCCGAGACCCGCGTGGGCTTAAGGGTGCGCGAGGCGGGTG	25
26	cg14470895	5	115152431	CDO1	CGGGCCCCTTTTAAGCGCTTGGAGTCACTAGGAATGTACCAACGGCCCTCGGAGGGAGGA**[CG]**AGGCGGAGAGCCACCCAAGAAAGGTGGCGGAGGCGGGGAGACCCTGCGGGCACGGCTCAC	26
27	cg11036833	5	115152494	CDO1	GGCGGAGAGCCACCCAAGAAAGGTGGCGGAGGCGGGGAGACCCTGCGGGCACGGCTCACG**[CG]**CACATCCCCGGCTTCCCCGGGCTCCGCGCCTTCCCAAGAGCCCCGTTGTCTCCGGCGTCC	27
28	cg23727983	11	125774082	DDX25;PUS3	ACACGGGGCGTAAAGCGCGGCGGGGAGTCCGGGGGGCTCCCGCCTGGAGGGCTGTGTGAG**[CG]**GCGGGCCGCGGGGCGGCGCGGGGGGCGCTCTCCACTCTGCGGAAGCTGCCCCCTCTGCCC	28
29	cg16732616	1	50886782	DMRTA2	CGGCGCACACTGAACCGAAGACCTCGTAGGCGGGCCTCGGGGGGATGATGCCGTTGGCGG**[CG]**GCCAGCGCCAGCCCCTCGGCAGTGCCGTAGAGCAGCTGCAGCTCGCGCGCCTCGTTCTCC	29
30	cg16422098	10	15761881	ITGA8	CCCTGCGGGGCAAGGGGGGCTGGTGGAATCTGGCGGTCCCCAGCTGCCCGTGTCCCGGGT**[CG]**GTGCGCTCGGCGCACCCGTGGTGACAGTGCCCGGCGTCTGCTCCCACCCGCCCGCCCGCC	30
31	cg13620034	8	72755052	MSC	AAAAAGAACGTGAGATATTAGAGAGAAACGATTGTCTCAAACCGAAACAGCTCTCCTACG**[CG]**AACCCCAGATATTCCTGACTTGGAGTAGCTAAGATTTTATCAGCATTCTGGGAATTTGCA	31
32	cg09424526	2	223163809	PAX3;PAX3;PAX3;CCDC140;PAX3;PAX3;PAX3;PAX3	CTTTTACCCAAAGCTTGGTCAGGAGCCCTGAGCTGCGATTGGCCGACGGGTAGACCGTCC**[CG]**GGTGGCGGAGACACGCGCTGATTGGGCAACAGCGACCACTTTCTCTTCCCATCTCTGGTG	32
33	cg09813525	13	53420386	PCDH8;PCDH8	GGCGTGTCCCATTGCAGCACCGACCCGGACACCCCGAGCCGAGAGCCAGGCGGGCGGGAA**[CG]**CTCCGGGCTTCCTGCACTGGCAGGCGCAGCCGGCCCACGCCCGCCCCCTGCTGTTACCAC	33
34	cg18082337	3	147113726	ZIC4;ZIC4;ZIC4	GATTTTCTGATCTAGCAAAGACCTTCCCACACCCCGGGAAAGGACAAGGGAAGGGCTTCT**[CG]**CCCGTGTGCACGCGGATGTGATTTACAAGTTTGTATTTGGCTTTGAAGGGCTTTCCCTGG	34
35	cg27655158	4	1396593		GTGAAGTTCCGCAAAGTGCTTCGCAGCGGGCCGAGCCCGCGGGAGCCACCTGCCCGGCCC**[CG]**ACGCGCATGGTCATTTATAAATTTAAAACTCTTCCGTAGCAACCGGTTATGTACAGAGTC	35
36	cg26802289	7	20817859		GTGGCGCTCGCTGTCCGTGCAGCGGGCGGAGGCGGCCGCGGTGCCTTTGTGTGCGGTGGG**[CG]**CGGCGATGGGCTGCTCCGGCCCGCAGCCCGAGGGGAGCGGCGGGCCCGGGCCGGGGCCAG	36
37	cg18074954	18	44787492		CCTGTCCGCGCCGCCCGCGCGGTCTCCGGCCCCCTGCGCGAGGTCCTCCCTCCTGCCTCG**[CG]**CTCCACGGCTCCTCCGCGCTCTGCCTCCCGCGGCGCCTGCCGCCTCCCCTGCGCGGATCA	37
38	cg25947619	15	86233220	AKAP13;AKAP13;AKAP13	TTACGATTTCACTAATGGCAGCTCAAAGCTGCTGAGCTCTGCCCTGACGGCATGCGCCCC**[CG]**CTTCTGTGAGAGCACGCTTCCTGGCATGGGGGTCGAATCATGTCGATAAAATGGGCTCGG	38
39	cg15384598	14	24045549	JPH4;JPH4	TGCCTCCCTCGTCGCCCGGCAAGGGCAGGGGCGGGGGTGGCGTCGGGGGGTCGCTGTGGC**[CG]**GAATCCAGGGAGGTGCGGCGGGGCGAGCGCAGCAGCGCCGCCTGATGGTAGGGCACACTC	39
40	cg16178603	2	66667101	MEIS1	CGAGCCGGGGGTGGCGGGCGGGGACGTCTGCTCGTCAGAGTCATTCAATGAAGATATAGC**[CG]**TGTTCGCCAAACAGGTCAGCAAAATAGATGTTAAAAAGTAAAAGAAACAAAAAGAGAGCC	40
41	cg23241781	14	70653964	SLC8A3;SLC8A3;SLC8A3;SLC8A3	TTTAATTCTTCTGCCCGGGAGACCTAACCGCCGAATGCGTTCGGAGGTATGTTTTTTAAT**[CG]**CCAGGAAAGGGGGAGAGAGAGAGAAAGAGAGAGAGAGAGAGAGGAAAGGAGGAGAGAA	41
42	cg01419831	2	162283705		AGGAGGAGCACGTGCGGTCGGTGGGGCGCAGGGCCGGGGAGCCAGGGGGGTGCCGGGGCT**[CG]**AGGCCGCGGGGCCGCGGGGGCCGCGCTCTGCTCTCCGCCCAGGCTGGGCCACTGGAGCGC	42
43	cg17039236	18	77548049		AGGCGAAGGCCGCCCCGGGAGAGCGGGGTCCCGGGAGAGCGGGGTCCCGGCTGTGGGGGA**[CG]**CGGGCCGAGGCTGTCGCGAAGCCGCTGACGGCCGAGGCGCTCCCGGTTTTCGCGGCGCAC	43

[0300]

44	cg21901718	5	16180076	Mar-11	GCGGGGTGGGCTGGGGGCGCGGATCAGTGGGACGGAGTTCGGGGTTCGGCTCCGAGCGGG[CG]GGCTGGAAGTGGGGGATCCCTCAGCCGCCTCCACGGGCCGGCCCCGCGCTCACGTCGGTT	44
45	cg25191628	1	50886949	DMRTA2	CGCTGGCGCTCCGCGATGAGCGTGCACTTGGCGCACAGGCAGTCCTTCCAGCGACAGTAG[CG]TTTGTGGCCCTTGAGGGCCGACACCACGCCATGGTTGCGACAGCGCGCGCACTTGGGGGT	45
46	cg27277463	12	62585031	FAM19A2	CCGCGGTCCTGCAGTTGCCGCTCCGGTCCCCAGCGCTGGCCGGCGACCCGAGGCGCGGCT[CG]CACCTACCTGCAGCCCCGCTTCCCGGTGGCGGCAACACCTAGCGATGCTCCTGCAGCTTT	46
47	cg05311410	7	27225523	HOXA11AS; HOXA11	CTGGTGGCTTGTCCGATTTGCACGGTGACTTGATTACACTCTCTCATTCATGGTCACTTC[CG]AAGCGCTTTAGTGCCTTCCGTCCCTAAACCGCCAACAGCCAGAACGGCTTCTCCCCGCGG	47
48	cg23229261	2	63284066	OTX1	TTTCTTCCGCCCCAGCGGAGTGCGCTGGGGCGCGCCAGGGCTAGGCCCGCCGGAGGAGCG[CG]TCCCCAGCCTTCCGCGCACAGAGCCGCATCCCGCCCCGCCCTGCGCTGGACTGGTTCAAG	48
49	cg10397440	8	57359258	PENK;PENK; PENK	CCCGGGGCTTAACGGCTGCTGGAGCCACTTTATAATTAGCCCCAAACCGAAGGAGGCGCG[CG]CGCCCCAATCGCCGGCGGGCTGCAGCTGACGCAGGCCCTACGCCAGCCCCGCGCCGACGC	49
50	cg18326021	10	106401479	SORCS3	GGCGAGCGGCGGGGCCGGGGCATCCCAGCTCCTGCCAAGCTTGGCGGCGCGAGGAGGAGT[CG]CCGGGCGCAGCCCCCAATCACCCAGGAACGCGGGGACGCCTGGGCCACTGCTCCGGCCGA	50
51	cg06533244	6	27258460		CACAGGTTTCCGTGGTGTAGTGGTTATCACATTCGCCTTACACGCGAAAGGTCCTCGGGT[CG]AAACCGAGCGGAAACAACTTGCAATTTTTCGGGGTGTTTCTGTTTTCCAAGATTCCCTTA	51
52	cg03078363	12	54408664		GTAAAAACCCGTTTTATGGGGGAACGTAATTGTGAGCGGGATGCGCTCTCTTTAGAATCG[CG]TCCTCCCAAATGCTCCCGCCGTCCCATTACCGGAATGGGGACCATTCGGCTGCTGCAGAT	52
53	cg01796166	14	95239586		GGCCCCGCATCGCCTGGCGCAATTGGAATAACAAATGCGACGCACGCAAATTGTCCACCT[CG]TGTTGCTAAGCGATTGTTTGTCGGCCCCGCACGCACAGCTCAGCATGGGAGGACCGCGAG	53
54	cg00339556	5	16180048	Mar-11	GGCGCGGCGGGGGTGACGGGGCGCGGGCGCGGGGTGGGCTGGGGGCGCGGATCAGTGGGA[CG]GAGTTCGGGGTTCGGCTCCGAGCGGGCGGGCTGGAAGTGGGGGATCCCTCAGCCGCCTCC	54
55	cg03978375	16	85932668	IRF8	GCTGCCGGGGGCGGGAAAGTGATTTCTCGGAAAGCAGAGCACTTCGAAGAAGGCGGGCCG[CG]CGAGCCAAGCTGACGCTATTGGTCGGTGTGGCCGTCGCTCTGCGCACCGCCCGTCCCCCC	55
56	cg26013553	1	111217406	KCNA3	CTGCTCGCTGGGCGCTGAGGAGGGTGGGCGCGGTGGCGGGCTGAGGGCGGCGGCGGCGAG[CG]CAGAAGGCTGAGGCGCTCGTCCATGCGGCGGGGAAGAGGCGGCAGCGGTGAGGCCAGGTC	56
57	cg09734791	8	72756155	MSC	ACTGCTTGCACTCTGCGGCTGAGCCCTTGGCCGGGAGGGGCTTCTTGCCACCACCGCCCG[CG]CTACCACCTGCGCCGCCGCCCCCAGCCACACGGGGCCGCTTCCTCTTGCAGCCTTCCGCG	57
58	cg12040830	11	112833773	NCAM1;NCAM1;NCAM1	GGCGGGCCAGGGAGCACCCAGTGCGCCCCCTCCGCGGGCGGCACAAGAGCAGCGCTCGGC[CG]CCGCCTCCAGCCAACTCGGGTCCCTCCCACGGCGACCAATCAGTGCGAAGCTGGCTGGGC	58
59	cg18617005	5	140787504	PCDHGA4;PCDHGA6;PCDHGA9;PCDHGA1;PCDHGA8;PCDHGA5;PCDHGB1;PCDHGB6;PCDHGB4;PCDHGA3;PCDHGA2;PCDHGA7;PCDHGB2;PCDHGB6;PCDHGB5;PCDHGB3	ATTTCGGAGACCGAATTCAAAATGAAAAACCGGGCTGCTGTCCCGCACGGAGCCTCTGGG[CG]CCGCTGTCGGCCAGTGCAGAGCAAGCGCTGACGCCGGGGATCCGTCAGCCTCTGGCCTGG	59
60	cg19597382	4	147559423	POU4F2	ACCTCCGCTCGTATTGGGCTGGGAGTTCAGAGCCGCGCGCAGAACCCGGGTTGGCCGCAA[CG]TCTGTGTTCTCAGCGGTGGCCGGGAACCTGGGATCAGGGTCACCTGAGCTGACGGGGTGG	60
61	cg05134015	14	36973365	SFTA3	CCGGAGGTTCGATGGCCGCCGGGCCAGTGCGGGCTCAGAGGAAGACCCTGCAAAAAAGAG[CG]CTCGCCCCCACCCCTGGAGCCGACCCTGCGCAGTAGGGCCGCAGCCGGTCCCCGCGGGCA	61
62	cg02700891	3	129693586	TRH;TRH	GGCGCAGATATAAGCGGCGGCCCATCTGAAGAGGGCTCGGCAGGCGCCCGGGGTCCTCAG[CG]CTGCAGACTCCTGACCTGCCGACTGCGGATCCCGAGTCCCCGGATCCCGGACCCATCCTG	62
63	cg13476854	1	119549263		GTCGGATCTCTAAATTATCTAATCTGGCGGCTGCGTACGACTCAGGGAAAGCCCTGGCCG[CG]AGCTTTTTCACCAGGCTTGAGCTCAGCAGCCGGGCCCGCAGTGTTGCCGCCAGTGGGGAG	63
64	cg00017221	1	149719536		GTCTCTCCACGCGCTGCCGCCTAGCAAAGGCGCATCTTTAGGTCGGTAGTGAGGTGCGGC[CG]GGACGCTGCAACTCGCTCCGGGACTTGTAAACCTGGCAGGTGTTCGAAGAGGGCCACTGG	64

[0301]

65	cg05310764	6	27513479		CACTCAACCATTATAAGTTCACCCCAGCCGTCAGCGATGGCGTAGGTAGGTAGTCGTGGC[CG]AGTGGTTAAGGCGATGGACTTGAAATCCATTGGGGTTTCCCCGCGCAGGTTCGAATCCTG	65
66	cg04495995	7	19147157		GTGTAGAGAACAACAGTCGCTCCTTAGATATTACTCCAGGACGGAAACCTGATTGCAAAC[CG]CTGTTCCTTCGAAACTTGCAAAACCCGGAACAGAAAACTCCCGCCCAGCCAATTTTAGCT	66
67	cg01642521	11	20618250		ATTCAGACCGAATGGCTGCGCGGTGATGGATGCGGATTTACGGCCTCCTTGGCTGCGGCG[CG]CTGGGCCTGATTATCACTATAAACAGGCGTCCGCGGAGGGCGGGGGCGGAGGCCCGCGTC	67
68	cg24504927	12	85667353		TTAACCACAGAGTTGTTCTTGATTGTAAGGGACTTCGCCCACTTGGTTGAAGTGGAGAGC[CG]GTCCTCATTCCAGACGTCCCGCACGGCAGTCGCTCATGGCTCCCTCCAGGCCGGGAGCCA	68
69	cg03276408	13	112712475		GCAGGGACCCGGCTGGCCGCCTCCCCTTGCAGGAAACAGGTGTTTGAACGCGATAGCGGC[CG]CCAGTCAACTAAGGCATTAAAAGCTCGCTTTATAACATCGATTTCCTGGAGTGCGGTGGG	69
70	cg27501878	20	61638588	BHLHE23	TGCTTCTCGACTCCCCGGCCGCCGCCTCCGGCCGCCCCGGGGATTCCGCCACCAAACGCA[CG]CGTCCCAGGTGGGCACCCGCCTCGGTCCGTCTTTGAGTCTGACCCTAGCGCAAGAGTCCC	70
71	cg07495363	2	198651076	BOLL;BOLL	GGACTCCGAGCCGGGGCGTCTCAGGGGCAGAGCGCACGGCACAGCGGGGCGGGCGTGGGG[CG]TGCGGAGCGAGGGCTCGGTTCTGGGACCCCTCTCGCCTTCTCTCAGACGGCTGGGGAGGA	71
72	cg06750832	1	111217691	KCNA3	CTGCGGGGCGCGCGCCCGCCTCCGCGTCCCCTTAGGATTCCCGCCCACCGCGCGGGCGCG[CG]TCCCGCTCTCGGGGGCAGCCGCCGGGCCTGCATTTCTTGCAGCCCTCAAGGCCCCTCGGT	72
73	cg22474464	20	21492914	NKX2-2	GGAACCAGATCTTGACCTGCGTGGGCGTGAGGCGGATGAGGCTGGCCAGGTGTTCGCGCT[CG]GGCGCCGACAGGTACCGCTGCTGCCGAAAGCGCCGCTCCAGCTCGTAGGTCTGCGCCTTG	73
74	cg20146541	1	248020697	TRIM58	ACTGCGGCCACAGCTTCTGCCTCAGGTGCATCTCCGAGTTCTGCGAGAAGTCGGACGGCG[CG]CAGGGCGGCGTCTACGCCTGTCCGCAGTGCCGGGGCCCCTTCCGGCCCTCGGGCTTTCGC	74
75	cg02864844	7	149917263		GGATGCAGACGCACCAGCGGCTGCTCACACTCCCTCCACAAACCTGCCGGAGTCTCCACT[CG]CCCGCCAACTGTAGCCTCCATCTGCGCCCCACGCCCCCGCACAAGCCCCCTCCGTCGCGG	75
76	cg12323723	X	136656581		GGCGCAAAAGGGGCCGCCCCCGTGCCGGGAACAGACTTTGAAGTGGGTTTTTAGCGCGCA[CG]TGTGAGAGCCGGGCCAGGGCCGGAGCGGGGACCCGCTGGGAGGAAAGAGGAGGCTCCGGC	76
77	cg14996220	12	85673270	ALX1	CTCCCACCCATCGCCACCGCGTCCACACTCCGCCGCATTCCAGAGAAAAAGAAAAGGCCA[CG]GCCTCGTAAAGCTCCCAGCTGGCCCGGACCCCGAGCTCCTCCCGGTTAGAAGCCGAAGCG	77
78	cg23151000	2	38302892	CYP1B1	GATTTCCTTTAAAGTACCTACCACGCCACCCGCTACCTGTAATAATCCATCTGAAGAGGT[CG]CCGGGCAGCGCCTCGGCAGACAGACTGACCTGCGGGGAGGTGCGGTTTCCAGTGGCGCGG	78
79	cg00963169	1	50513927	ELAVL4	CGGCGCAGGCCCCGCACCCCCGACTCTGCCCGCCCTCTGTTACGGACACCCGCTGGGCCA[CG]TGGTCGCGACTGGCTTCTCCCAGCGGCCAGCCTGGCCACCCCGACTCCCAGGGAGGGGGA	79
80	cg13320291	10	118030970	GFRA1;GFR A1;GFRA1	TCGGCCCTCCGCCCCGGGGCATCCTGGCCTGAGCAACGACCCGGGCTCCCGGGCCACCCC[CG]GCTCCAGCCACCCGCTCCGCCCGGCTGAAACTCAGGCGCTTTCCGAGGAGAAGTGCGGCG	80
81	cg13352750	7	27225123	HOXA11AS; HOXA11	TTCTCACCGAAAGCACGTAATCGCCGGTGTAACTCATGTTGGCTGGGGGGCCTCCCGGCG[CG]CGCGGAGAGGCTGGGGTGCGCCCCCATGCAGCATGCTTGTGCTCAATTGCAGGGTCCTCG	81
82	cg26521404	7	27204981	HOXA9	GCTGAAGTCGGGGTGCTCGGCCAGCGTCGCCGCCTGCCGGGGAGGCTGGCCCAGGGTCCC[CG]GCGCATAGCGGCCAACGCTCAGCTCATCCGCGGCGTCGGCGCCCAGCAGGAACGAGTCCA	82
83	cg07573209	15	76630095	ISL2	GGGTTGGGTTGGGGCTGGAGTAGCCGAGGCCGGCCTGGGTCCGGGCAGTCAGGCCTGACG[CG]GCCCCGCGCCCTTCCCCGGCAGAGAAGCCCGGGACGGCCATGTGCGTGGGCTGCGGGAGT	83
84	cg10835584	18	55108852	ONECUT2	GCGCTTCCGGCGATCCGCCTGGGCGGCTGGGTCCGCGAAGCCAATGCGCTGAACGGTGCC[CG]AGTCTTCCTAACTATCCTGTGCTTGGCCGTTGCCACTGGGCCCTGGTGACTAAGCCCAAG	84
85	cg09474331	19	54926805	TTYH1;TTYH 1	TACCGGCCCTCAGCTTGGGTGCATCTCCTCCACCAGCTGCCCCGCGCCGACTTCCAGCTC[CG]CCCGGTGCCCAGCGTTTTCGCGCCCCAAGAGCAGGAATACCAGCAGGTGGGACCGGGCGC	85
86	cg20870512	7	1272515	UNCX	GTCCCAAATTCCCCGGGCCGCGGCTAATTATCGGGAGCTTGATGTTGATAAGTAAAGCGC[CG]GAGTGCGGGCGAAGCATGTGTGGGGCTCCGGGTCCCTGTCTCCGCCGCCGCCGCCCGCGC	86
87	cg21678445	18	22930283	ZNF521	GAGGGGCGGTCAACTTCTGGGAATGGCCAAGAGGGGTGCTCTGAGGCCCGAGCCGGGGTC[CG]GTGCCCGCGGCCGGCGGCCGGGGTCTGTTTACTCCGGCGACGTGCGGAGAGGACCCAAGC	87
88	cg05560435	5	172671526		CGCCGCCTTTCCCTCCTCGCCTCTTCCTTCCTTCCGGGTCGTGCCCTCCAACCTGCTGTG[CG]TTACCGCAGCCAAGTTTCCACCGCCCGGCGGAGCGCATTGTGAACAGCAGCTGACAAATT	88
89	cg08382226	6	108440339		CCGACTCGGGATGACAATTGACGGGGATCAAGGGATTGCCCATTCTGTGCCTGTAAGAAC[CG]ATTCGTGCCAGAGAAACTCATCAAGTGGAGGCGGAGAATAAAGACCGTTCGGGGGTAAAT	89
90	cg25510609	15	86233236	AKAP13;AKA P13;AKAP13	GGCAGCTCAAAGCTGCTGAGCTCTGCCCTGACGGCATGCGCCCCCGCTTCTGTGAGAGCA[CG]CTTCCTGGCATGGGGGTCGAATCATGTCGATAAAATGGGCTCGGTTTCATGAAGTACCCC	90
91	cg19650157	13	78493297	EDNRB;EDN RB;EDNRB	GAAAGCCGCTACTCCCTGGCTGGCTGAGCTACAGCTCCCGCAGCGCGCCCAGGAGTGCGC[CG]GAGATTCGGAAACCCGCAGAGACTTCTCAAGTCAGCAGGAACTTGGAAACCGCTGTTCCC	91
92	cg01283246	5	135266135	FBXL21;FBXL 21	CTCGCTCGGGCGTGTTCCTGCGCCGACCGGACGGCCGGACTCCAGCACCTTGGCCCGGCC[CG]CGAACGCTGAGCACGCGCGGAAACCCTTTAAAGGTAGCACATTTTTCGGGTGTCGCGGGG	92
93	cg20872937	18	74961968	GALR1	CTGGAAAAGCCGGGAGGGAGTCGGAGGCGCCAGCCCACTGGGGAGGTGGCGCTGGGCGCG[CG]GGATGCGCGGGGAGCCTTCTCTGCAGGAGCCGCACAGTGCACTGCTGCGCGCTGGGCAGT	93

[0302]

94	cg03698009	7	27204349	HOXA9	TCTCCCGTAGCCCTGCGGGCCGCTCTTCACTGCTCTCCAGACTTGGGGCCCTATCTGAGG[CG]TCCCAAACACCAACTTCTGGCTCCTGGCCCCAACTCGAGAGGCTTCCAGCGAGGACGAAG	94
95	cg01423964	1	111217575	KCNA3;KCNA3	GAGCCCACCGCCTGTTGCAGCCAAAGCCGCGATGCTCTGTCTGGGTCTGGCGCGGTCAGC[CG]GGCTCCCGCACGGGGACGCCTCCTCCCTCCTTCTCGCGCTCTCCGCCCCCTCCCCTGCGG	95
96	cg19923650	5	172659730	NKX2-5;NKX2-5;NKX2-5	CGAAGTTGTTGTTGGCGGCGGCAGTGGCCGGCTGCGCTGGGGAAGGCCCGGCGGGGTAAG[CG]GCAGTGCAGCTGTAGCCAGGGCTGCAGGCCGCGCCGCCGTAACCCGGATAGGCGGGGTAG	96
97	cg13324546	8	23564031	NKX2-6	GTCCATTCTCAGGTACTGAAAGTTTTCCGGGCTCTTCCGCACCCGCGGATGTGGCGAAGC[CG]CGGGGCAGCTCCGCTCGCGCTCCAGTCGCAGGATGTCCTTGACCGAGAAGGGGGTGGAGG	97
98	cg27357571	21	34398226	OLIG2	CGCCCCCGCCCCCGCCACCGCTGCCGCCGTCGCCGCTGCCACCGGGCTATAAAAACCGGC[CG]AGCCCCTAAAGGTGCGGATGCTTATTATAGATCGACGCGACACCAGCGCCCGGTGCCAGG	98
99	cg08448701	20	21686282	PAX1	CAGTGACGGGAACCAATGAGCTGCCAACTCGCGCGTCTCCGGCGTGACTGCCGAGATTGA[CG]TGGAGGACACGTCAAATTGATTCCCGCACGCTGCAGCCTCCCGGTCAGACGAATTTCTCC	99
100	cg16002355	4	111544387	PITX2;PITX2;PITX2	CTCCGCCTCCTCCCAGACCCTTCTCCGGGTGCGACTGACGTGGCTCCGCACCAATCAGGA[CG]CCCCGAGCCGCGGTGGAGGGACTGTCCTGCCTGCACCTATCAGCAGTGCGGGGCCGGGCT	100
101	cg05302386	14	52734525	PTGDR;PTGDR	TTAGCACCCGGGCGCCGGGGCCCTCGCCCTTCCGCAGCCTTCACTCCAGCCCTCTGCTCC[CG]CACGCCATGAAGTCGCCGTTCTACCGCTGCCAGAACACCACCTCTGTGGAAAAAGGCAAC	101
102	cg17964510	2	175199694	SP9	AGGCGCAGCCAGCGGCACTTCAAAGCGGGTGCTCCTCGCACTTAGGCTGAGTTTAGCCGG[CG]GGAGCCTGGAGTCCGCTCGGCACGAGCGCGGGGACGCGGGAGCCGCGCGGGACCCAAGCA	102
103	cg24031355	10	22634439	SPAG6;SPAG6;SPAG6;SPAG6	GGCCGCGTTCCGGTTCCGGTAGGTTGCCCGGGAGACGCGGGTACACAGAGAAGCGGCTCC[CG]TCGGAGGCCGAGTCGTCGCCACGATCGCCCCCTTGGTGGACTCGCAGGCCGAGCGGCTTC	103
104	cg24961583	3	27765409		GGGAGCGCATTTTCCGGCTGAGATGTCGGGACTCTGCTTCCCCAACCGAACGCGATCACA[CG]GGAAACTCTTCGCCCACAACAGATGAGATGGCCAAAGGATTGCTGAGTGCGCACACGCAA	104
105	cg02167020	10	102899949		GGACTCATGCAGAAGAGGACATTCCGCAGGTAGGTACAATCCCAGCGCTGGGGCCTGGGG[CG]TCCGGGGGGCGGCCTTTGAGCTTCCCGGATACCGCTCGCCTGCTCCCGGAGCTGTTCGGC	105
106	cg25026529	1	91183051	BARHL2	GGGGGAGTTAAAAAAATTTCTGAGAAAACTCGGAACTTGCGCTCCAGGAACGACTGCGCA[CG]TGGCGCGGCGGTGGCGGCGCGGAGGACCCAGGCGAAGGCGAAGGCGAAGGCGAAGGCGCA	106
107	cg05783139	2	198650985	BOLL;BOLL	GACTGCGTCGCCTCGGGTGGCAGGTGGCGGTGCGGGCGGGCGCTGCAAGCCGGAGAGGGG[CG]CGGGAGGGCGAGTTTCGGCTGTGGCCCTGGGACTCCGAGCCGGGGCGTCTCAGGGGCAGA	107
108	cg25691167	7	19184961	FERD3L	GAGGGCGTCTCGGGGAGGCCAGGGACAGGTCTGCGACGAAGTCCAGCACCGTAGTGTCCA[CG]CAGCTCTCCGGATAGGCCGCCATCGCTTCGGCTTGGCCCTGCCTCTCATCGGTTTTCCGC	108
109	cg25951981	4	46995743	GABRA4	GTATACCGCTCCACACCCTTTCGTGCCCGCGCGCTGAAGGTTCTGGGGTTCGTATCCGCG[CG]CTTGCGCTGCAAGACTCGGCAAGTTTGTTCCGACTGTAACTCCGGGGATGAGGAACGGGG	109
110	cg11601252	15	68122139	LBXCOR1	CACAGAGGAGGGGTTGGGGGCAGCGGAAAATCGGGCAGGTCGAGGCAGCCGAACCCCGGA[CG]ATGTCCCCCCACCCACCCCGAAGGTCGCAGCCTGGGCCGCGTTCTCAGCAGGAGTCGGGC	110
111	cg10698928	8	65290320	MIR124-2	CAGAAGGACCATCTGCGGACTCGTTTTCACTGCTCCAGCTCCCGAAATCGTTTCTCTGGA[CG]CGGCAGTATCCGCAGCGCATGCACCCTCCTCTGGACTTCCGCAGCCCAGACCTGCGCTTA	111
112	cg25832771	8	72756058	MSC	GGGGGCACCCAGGGCAGGCTGGTCTTGAGCCTGGAGAAGGCTTTGCTCAGCACGCGCATC[CG]GGCACGCTCACGGGCGTTGGCCGCGTTCCGCTGCGACTGCTTGCACTCTGCGGCTGAGCC	112
113	cg18507379	5	140787507	PCDHGA4;PCDHGA6;PCDHGA9;PCDHGA1;PCDHGA8;PCDHGA5;PCDHGB1;PCDHGB6;PCDHGB4;PCDHGA3;PCDHGA2;PCDHGA7;PCDHGB2;PCDHGB6;PCDHGB5;PCDHGB3	TCGGAGACCGAATTCAAAATGAAAAACCGGGCTGCTGTCCCGCACGGAGCCTCTGGGCGC[CG]CTGTCGGCCAGTGCAGAGCAAGCGCTGACGCCGGGGATCCGTCAGCCTCTGGCCTGGGAT	113
114	cg14733048	8	109095782	RSPO2;RSPO2	CTTTCTCCACGGTCACTTCACAGCTAAGATTTCTTTCTTTCCGAGCTGTAGAAGGCAGAA[CG]CTCTCGGGAGGACGAAGTGATCCGAAGGGATGTGGCAAGCGCACTTTCCGATGGAGATGC	114

[0303]

115	cg09735723	10	106402042	SORCS3	GGGAGGGATCTGTGCTCACTTTCTCCAATACTTGGCTTGGAGGGTCAGTTTTCCTGTTTG[CG]GGGTGCTTGAATTCTTGGATGAGAAAAAGGGCTGACTTGGGGCGGGAGCCGCTGAACAGA	115
116	cg05099508	10	22634432	SPAG6;SPAG6;SPAG6;SPAG6	GCAAGACGGCCGCGTTCCGGTTCCGGTAGGTTGCCCGGGAGACGCGGGTACACAGAGAAG[CG]GCTCCCGTCGGAGGCCGAGTCGTCGCCACGATCGCCCCCTTGGTGGACTCGCAGGCCGAG	116
117	cg14638883	6	166582201	T	TCTGAGAAGTGTCCTCCTCGCTCTCTTATAAAAACAGGACTTGTTGCCGAGGTCAGCGCG[CG]CATCGAGTGTGCCAGGCGTGTGCGTGGTTTCTGCTGTGTCATTGCTTTCACGGAAGGTGG	117
118	cg26673012	7	35293753	TBX20;TBX20	GGAACTACGGACAGTGAGCCCTGGCGCTCGCTGCCCTGCGCCTTAATTTGCTGGCGGCGG[CG]ATCCCGGAGGCCCGCAGCCAGTCAGCGCCGTCTCACGTCACCGCTTCCTGATTCCGCCGC	118
119	cg14763548	20	25062447	VSX1;VSX1	GCTCGGGGCCCCTGGGCGGCAGGAACGGCACGTCCGCTAGGAGCAGGCAGGGTGCTCGAG[CG]GCCGCCGGCGGCTGCGTGCCGAAGCCACAGAGGAGGCCGAGTCCCAGCGGTAGGGCCCCA	119
120	cg22884656	2	45157296		AATCACTGTAGTTAAAAATGTATGGGATTTTTGCCGTCGGAGCACCTCGTATCCGGCGCG[CG]GGCCCAGTGTGGGACTGCGGCTGGGAGCCCGGGCCCTCCGGGAGTGAAGATACCTTTGGA	120
121	cg21401879	2	45162036		GCGGGCTCCCCCTGGCCACATCCCGGGCCTCTCACACAAGAAGAATAGTTCTGTTTCCGC[CG]TAAACCCCCACACAAAGGCTGCCCGGCCACCGGGTCCCCTGTCCCCCTTCCCGGGGACTT	121
122	cg09797577	6	28778226		GTAGGCTGGACCAGGAAGGAGACCTGGTTCGTTTCGCCCAGGCTGTCACGGCTTCAAGAG[CG]CCTCTCCGCTATTTCCGTCGCTCGACAGACGGGCTGAGCTCTTTGGAGTGATGTTGGGTT	122
123	cg20567847	7	155167038		CCGGCGGGGGCTGGGCCGGGCGGGTGGGTTTCTGAGCCGCAGCGCTTGGAGCTGGGGGAG[CG]GGAGCAGGGGCGGCCCGGCGGGCGGGCCGGGACCCGGCTTTTCCGGCTACCCGTGGGCCA	123
124	cg04828133	7	155259845		GGAGGTTAATAGCGACTGACGACAAAGGGCCAAGGTGCAATTCCTAAAGCGGGGATTCGC[CG]GGTGAGGCAGAAATCAGCCTCCGGGGAGATGGGTCCCCCCTTCCCGACGCGCCCCTGCAC	124
125	cg04521510	7	27242044		CTTCGGTTTTCCGGCCAGACCCGGAAAAACGAAAACACAGCTTGGGGAGCCCCCACTAGC[CG]GCGCCTGTGCCAGCTCACCTCTGGCCATGGCGCAGCTGCCGGTGCACACGGCGGCCAAGG	125
126	cg24304093	11	14926738		TCGGCCTGGCCAGGCGGGGGGTCTTCTTCCCAGTCCGCGGAAGCCAGGTCCGGGAGACGG[CG]GGGCTCACTGTGCCTTCGGACCTACACTCTGCTCGGGGGCCGGATTTCTGCAAAGCGTCC	126
127	cg24292235	12	8171463		AGCCTAGGATTCGACTTTGAATGGTCCGTTAATGTGGTCGCAAAACGTGACTCGGTTCAT[CG]GGCGCTCCCTGTAAGCAAGACAAGCACCCACCTGCGGTCAGAGCAGGGGTCCGGCTCGCG	127
128	cg25092681	5	16180033	Mar-11	GCGGGAGGGAGGAGCGGCGCGGCGGGGGTGACGGGGCGCGGGCGCGGGGTGGGCTGGGGG[CG]CGGATCAGTGGGACGGAGTTCGGGGTTCGGCTCCGAGCGGGCGGGCTGGAAGTGGGGGAT	128
129	cg02984614	17	31618409	ACCN1;ACCN1	GGGGGCGGAACACCAGGCCTCCTGACTGCCGGGGAGTGGGGCGCAGAGGGAGCGGGTTCG[CG]CGGAGGGCAACTCACGGAGGAGAAGTTGTGCGGCCCGCAGAGCTCGCCGCGGTACTTGCA	129
130	cg07113642	20	2781262	CPXM1;CPXM1	AGCAGGAGCCCCCACATGGCGGGGATTGAGTGCCAGGGGCGCGCGGGCTACGGCGGGTGG[CG]GGTCGGTCTCTTCCTGCCGAGTGCGCCGAGCCCCCCGCCCCTTCCTGCCCCCCGCCCCTC	130
131	cg04609163	11	125774090	DDX25;PUS3	CGTAAAGCGCGGCGGGGAGTCCGGGGGGCTCCCGCCTGGAGGGCTGTGTGAGCGGCGGGC[CG]CGGGGCGGCGCGGGGGGCGCTCTCCACTCTGCGGAAGCTGCCCCCTCTGCCCTCCGGTCC	131
132	cg11193865	14	101193038	DLK1	CGGCCGCGTGCTGTACAGTGTGAGGGAACGTGTACCAAACGCTCGCGGGATACCTGTGCC[CG]TCTAGCCAAGAGTGCACCCGTGTGCGCGAGCGGGCTTCTGGGACGCCGCCGTGGTCGGGG	132
133	cg09124223	7	153749738	DPP6;DPP6	GGGGAGGGGGCGGAGGAGGCTGAGCCAGGCAGAGTCGCCAGCGGAGACTCGCGAGTGGCG[CG]CGGGAGGAGCGGCCGCCGGCGCTGGGCTTGCCTTGCTGCTGCTGCTGCTGCTGCCTCCCC	133
134	cg14768785	3	172166517	GHSR;GHSR	ACACCTCCCCTTTCCCCCACCAACTCCCCCAAAGTTTCTCCCAACACATCCTCCGGCCGG[CG]CCCACACGCATACCTGTCACCAGCCCTGCCTCGCATTTGCGTTCTCGATCCAGTTCCATC	134
135	cg12477716	17	47073436	IGF2BP1;IGF2BP1	GTATCCGGGACTCCGAAACGCGCGGCGAGCAGCCCCCTCCCCCACCGCCCAGACGGGGTG[CG]ACCGCCCACGTGTCGCCCCTTGCCCAGTCGGGTCCTTCCCTCGGGCTCCGGGAGCCGGAG	135
136	cg24826867	16	85932853	IRF8	GAACGCGGGCGGCGAGACGGCGGCAGGACGGCGGCAGGTAGGCACAGTGGGCGGGTAGGG[CG]CCCGTGTCCCGCGCGGTCCGGTCCCGCGGGGTCCCCGACGCCAGGCGGGGCGTGGGGGTG	136
137	cg24663256	4	21950307	KCNIP4;KCNIP4	ACATTCATGTCTAGGGACGCAGGGTGCAGAAGCGAGACTCGAGAGTCCACCGGCCAGGGG[CG]TCTGTCCACGGGTCTGCACGGGAGCGCACCGCCGCTCGGCCCGGGGGCGTCCGTGGCGCT	137
138	cg06653045	17	68165064	KCNJ2	TAATTCGCGAACAGTCGGGAGAACAAACAGCCAAGCGGCGCTGCAGTGGCCGCACTTGCG[CG]CGTCTCAATCCTGGGGGCTCTGCGCGCCCGCCCCAGTCCCTCGCCCCATTGACTCAGTGG	138
139	cg13592399	14	52535758	NID2;NID2	CCGGTCCCCCTCCATGCTCGCTCGGCCGTGCGCTTACCCGCTGCACAACGCGTCCCGCCC[CG]GCCTCCAGCCCACTCTCCGCGCCGCGCCAGCCTCGAACCTGGATCTCCGCGGGCGCCTGG	139
140	cg20008332	2	5833169	SOX11	CGCTGGAAAATGCTGAAGGACAGCGAGAAGATCCCGTTCATCCGGGAGGCGGAGCGGCTG[CG]GCTCAAGCACATGGCCGACTACCCCGACTACAAGTACCGGCCCCGGAAAAAGCCCAAAAT	140
141	cg02071005	2	175200844	SP9	TTCCTCTTGCCCTCCTCCTTCTTGCTCCCTCCCCCATCCCACCCACTCTAGGAAGAGCCG[CG]CTTCGGAACGACCCCGTTGGCCATGCTGGCGGCGACCTGCAACAAGATCGGCAACACGAG	141

[0304]

142	cg23302682	6	166580952	T	ATCATCTCATTGGTGAGCTCCTTGAAGCGCAGCCACAGCTCGCTCTCCTCCAGGCCCACG**[CG]**CAGTTCGCGCTCTGTGGGGTCGCCCTTCTCGCTGCCCGCCTGCAGCTCATTCTCCACGGC	142
143	cg07978472	4	122686493	TMEM155;LOC100192379	GCCAAGGAGCTGAGGAAATCCGGCGCAGACTCTCCCAGCTGGCACCAAAGCCTTCCGCTT**[CG]**CCGAGATCCTCTCAGGTGCTCTTGAGGACGCGAGCGACTTCCCTAGGAGCGAACTTCCGC	143
144	cg14732324	5	528621		CCCTCGGGGACAGCCCGGCCGGCCACGCCGCCGAACTCGCCATGGGCCTCTCTCCGCATC**[CG]**CATGTGCATCCGCAACCGCTTCCGTCCCGCTGAGCGCACGAACCCTCTCGCTCCTGTCCC	144
145	cg00228475	6	58149279		TCCTCAAGAGAGGTAGGGTCCGTTCCCCCCGGCGGGGCCGGTTAGCTCAGTTGGTTAGAG**[CG]**TGGCGCTAATAACGCCAAGGTCGCGGGTTCGATCCCCGTACGGGCCACAGGCTTTTCTAA	145
146	cg12812583	8	23567310		GAGCAGGCTCCCCAGCGTAGCGAGTCCTTGTTATGGAAAGGGTCGTTTCGGCTCAGGATG**[CG]**CGCTCCCGGCGTAGACCTGGGGATAGGGGTCCCTGTCGCGCTCGCCCCACCCCTGCAGGG	146
147	cg01163842	14	95235125	GSC	GTGGCGGCGGGACACCCCGCGCAGGCCAACAAAAGGAGGGGAGCCGCTCGCTCCCGCTTC**[CG]**CGTTTTCATTCAACTTCCTGGGCCTAAAGCGCCCTCCAGCAGCCTGCGGGCCGCCATCGG	147
148	cg17394649	6	29760164	HCG4	AAACGGCGTCTGTGGGGAGTAGCTAGGGGCCTGCCCGGCGGGGGCGCAGGAACCCGGTTG**[CG]**GTGCCGGGAGGAGGGTCGGGAGGGTCTCAGCCCCCTCCTTGCTCCCAGGCTTCCACTCCT	148
149	cg10723962	6	26240782	HIST1H4F	GCGTGACAACATACAGGGCATCACGAAGCCCGCCATCCGTCGCTTGGCCCGACGCGGCGG**[CG]**TGAAACGCATTTCGGGCCTCATTTATGAGGAGACCCGCGGTGTTCTTAAGGTGTTCCTGG	149
150	cg17466857	7	27225528	HOXA11AS;HOXA11	GGCTTGTCCGATTTGCACGGTGACTTGATTACACTCTCTCATTCATGGTCACTTCCGAAG**[CG]**CTTTAGTGCCTTCCGTCCCTAAACCGCCAACAGCCAGAACGGCTTCTCCCCGCGGTTTGT	150

[0305]

表2

下表2提供了可用于扩增或测序上表1中限定的MVP的示例性引物。

Chr	有义起始	反义起始	有义序列	有义 SEQ ID NO	反义序列	反义 SEQ ID NO
2	119,606,705	119,606,804	TTGGAGGATTAGTATTYGATTATGTTGAA	152	AATCCRCTAAAACTCTAAAATAACACCC	405
19	54,926,752	54,926,852	CCTCAACTTAAATACATCTCCTCC	153	TTGTTGGTATTTTTGTTTTTGGG	406
7	70,597,038	70,597,139	GGGGTGGGGTGGATTT	154	CTCAAATCTCAATTACTCTCAAAATAAA	407
4	111,544,332	111,544,433	CCTCCTCCCAAACCCTTCT	155	TGTTGATAGGTGTAGGTAGGATAG	408
7	27,205,053	27,205,155	GTTTAGGGTTTTAGTGGTGGT	156	CCACCAAATTATTACATAAAATCTACAA	409
7	5,569,082	5,569,184	GGAAATYGGGAGGTTTTTGTGTA	157	AAAACRCCCCAAACACCAAATAAAA	410
1	237,205,102	237,205,205	ATCTCTACTTAAAAATTAACCACCC	158	TTTTGGATTGGGTTAGGGTATT	411
15	68,122,063	68,122,166	RCCTTACCAAATTCCTCACAA	159	GATTTTYGGGGTGGGTGGG	412
13	78,493,251	78,493,354	TTTGGTTGGTTGAGTTATAGTTTT	160	ACAACRATTTCCAAATTCCTACTAAC	413
7	70,597,035	70,597,140	GGYGGGGTGGGGTGGA	161	RCTCAAATCTCAATTACTCTCAAA	414
8	65,290,282	65,290,387	RTTTTCACTACTCCAACTCCC	162	TGATTGTAAGYGTAGGTTTGGGT	415
18	74,961,907	74,962,016	TTTGGAAAAGTYGGGAGGGAG	163	CAACAATACACTATACRACTCCTACAA	416
6	28,367,508	28,367,619	YGGAATTTATTAAAAGTGATTTATAAAGGT	164	ACCCCTTTCRCTCCCTTCCTA	417
2	223,163,755	223,163,866	CCCAAAACTTAATCAAAAACCCT	165	AGAGATGGGAAGAGAAAGTGG	418
6	166,582,153	166,582,264	CCTCCTCRCTCTCTTATAAAAACAAAACT	166	TTTTATTTTTYGTGAAAGTAATGATATAGTAGAA	419
8	26,723,555	26,723,669	GGGGAATTTTGGAGGATGTATT	167	ATCCTAACCCTAAAATCCCTAAAT	420
6	108,440,301	108,440,416	GGGGATTAAGGGATTGTTTATTTTG	168	CCTTCTCAATAAATACATTTACCCC	421
8	132,054,523	132,054,639	GTTTAGTGTYGGTGTTAATGATAGATG	169	AATAACCACCACCRCTCCTCC	422
5	115,152,343	115,152,460	AGGTTTAGATTTGTGGGGTTTA	170	RCCACCTTTCTTAAATAACTCT	423
10	106,401,968	106,402,085	TTAAAGATGAGTGGGGGAGG	171	CCCCAAATCAACCCTTTTTC	424
11	20,618,161	20,618,279	AAAGTAATTAAGGTYGTAGTGATTGGT	172	RCCTATTTATAATAATAATCAAACCCA	425
19	54,926,741	54,926,860	GGGTTATYGGTTTTTAGTTTGGGTG	173	RATCCCACCTACTAATATTCC	426
2	223,163,760	223,163,880	AGTTTGGTTAGGAGTTTTGAGT	174	ACCTCRACACCACCAAAAATAAA	427
22	22,862,820	22,862,940	CCTACACTCCCCCACAAAA	175	TGAGGGGAGATTTGAGGGAT	428
11	120,434,955	120,435,076	YGGGGGAAAATGTTTTTATTGA	176	AATTCCCRAACCCCCTCCC	429
8	23,563,877	23,563,998	CCTCACAAAAACCCCCAAAA	177	GGAAAATTTTTAGTATTTGAGAATGGA	430
2	198,650,902	198,651,024	TATTTGGYGGGTGGGGAGAA	178	TCRAAATCCCAAAACCACAACC	431
6	10,882,276	10,882,398	ACCTCTAACAACTACCCCCT	179	TTTTTGGGATTGTGGTGGAG	432
20	25,065,180	25,065,302	CCACCCAAAACCCAACRTCAAATTA	180	GGGTGGATYGTGGGTTAGTTTT	433

[0306]

7	27,204,952	27,205,074	GTTTGTYGGGGAGGTTGGTTT	181	AACCACCACTAAAACCCTAAA	434
5	172,671,485	172,671,608	CCTCTTCCTTCCTTCCAAATC	182	GGTGTGTGTGTGTAATATAATAATTTGT	435
3	147,113,701	147,113,826	GGGAAAGGATAAGGGAAGGG	183	ACAAACCAACCACATTTACTTCT	436
12	85,667,301	85,667,427	AGAGTTGTTTTTGATTGTAAGGGA	184	AAAACCACCTTCCTAACTCC	437
7	27,232,754	27,232,882	ACCACTCACCCRCACAAACAC	185	ATGGAGGTTTAGGTYGGTGTAAA	438
8	145,106,354	145,106,483	RTATAAAAACAAAACACATCCTATTAAC	186	TTATGGGGGYGGGTGATGGA	439
11	112,833,711	112,833,841	GGGGYGGGTTAGGGAGTATTT	187	ACTTCCCRCCCAACCAACTTC	440
7	155,259,749	155,259,880	AAATTGTAGTTTGTTAGGTTGAGT	188	ACCCATCTCCCCRAAAACTAAT	441
4	174,450,311	174,450,443	AAAAATAAAAATTCTCCTCATaactacaa	189	GAAATGAGTTTGGTAGGTGGTT	442
8	132,054,519	132,054,651	AAAAACTTAATACCRATACTAATAACAAATAAAC	190	GGTGGAGGAGGTGGTGATTA	443
10	118,030,953	118,031,086	AACTCCCRAACCACCCCC	191	YGTTGATTATGGTTGGTTTGTT	444
1	111,217,615	111,217,749	CTCTCCRCCCCCTCCCCTA	192	GAGGGGTTTTGAGGGTTGTA	445
15	86,233,181	86,233,315	CTCAAAACTACTAAACTCTACCCTA	193	TGAATTTTATTGTTATGTGGGGTATT	446
1	248,020,537	248,020,671	GGGAGGYGGGTTATGGTTTGG	194	RAAAATACACCTAAAACAAAAACTAT	447
2	66,667,006	66,667,141	TTTGAGAAATGTGAATTAGTTATTTGT	195	ACTTTTTAACATCTATTTTACTAACCTATT	448
5	16,180,236	16,180,371	TTGGATGGGTYGGTGGGATG	196	CCCTTTCCCRCTCCACTACTC	449
3	42,306,888	42,307,023	ACCACAACTAACTCTTAATATCCT	197	GGGTGGTGGTAATTTGGTT	450
7	24,323,740	24,323,875	AACTACCCRAAACCCCTCCT	198	TGGTTGGGYGGTAAGTATTATGTTG	451
10	15,761,845	15,761,980	AAAATCTAACRATCCCCAACTACC	199	AGGGTTAGAGTTTTTGGGTTAG	452
8	72,755,998	72,756,133	GGGGGTATTTAGGGTAGGTTG	200	CCCTCCCRACCAAAAACTCA	453
12	54,408,585	54,408,721	YGTTTGGAAAGAAATGGAGGTA	201	ACAACAACCRAATAATCCCCATTC	454
3	147,113,681	147,113,817	CAAAAACCTTCCCACACCC	202	TTATATTTGTTTTTGGGAGGAGTG	455
11	71,955,309	71,955,445	AAAACCCAAACACCRCCCAAAA	203	TGGTTYGGATTGGGGTAGGAT	456
6	29,760,051	29,760,188	GGGGGTTTTGGTTTTGATTTAG	204	CCTCCCRACCCTCCTCCC	457
2	66,667,480	66,667,618	AAGATTGAGTTTTGGGTTTGTT	205	TCCTAATTTCTTACCTCATTACACT	458
11	71,955,292	71,955,431	GAAGGGGYGGGGTTTAGGAG	206	ACAAAATAAAAACTCCRAAAATAAAATCCC	459
1	50,886,838	50,886,977	TTCTCCTCCTACRCCTACTACCT	207	GTGGTGTYGGTTTTTAAGGGTT	460
14	95,235,085	95,235,226	GTAGGTTAATAAAAGGAGGGGAG	208	ACCCAACCCTACTTAACTCTC	461
7	5,569,280	5,569,421	ATTATTTATGGTGAGTTGYGAGAATAGT	209	ACCCRACTTCCTTTATCCCCA	462
10	15,761,835	15,761,977	GGGGGTTGGTGGAATTTG	210	ACTAAAACCCCTAAACCAACC	463
1	119,530,560	119,530,702	TCTCCACCCTCCCCCT	211	GGTGGGTGGGAAGTAGGAT	464
4	174,450,301	174,450,443	AGTTAGTTATGGAAGTAGGGGT	212	RAAATAAATCTAATAAATAATTTTCCCC	465
7	5,569,274	5,569,417	GATATTATTATTTATGGTGAGTTGYGAGAATA	213	RACTTCCTTTATCCCCAATCTa	466
2	198,650,997	198,651,141	AGTTTYGGTTGTGGTTTTGGGA	214	TCTCTCCTCCCCAACCRTCTAAAA	467
3	42,306,941	42,307,085	GGATGATATTATTTGGTTTTGTTTAGAG	215	ATCAAATTCCCCAAAACCCT	468
7	19,184,912	19,185,056	GGGGAGGTTAGGGATAGGTT	216	TTAAAAATCCCCACCAACAAC	469

[0307]

14	52,734,499	52,734,643	CAACCTTCACTCCAACCCT	217	GGTTAGTAGGTTGTTTAGGAGG	470
13	109,147,820	109,147,965	GGGGAAYGTGGAAAGGAGGG	218	RTAAAACTAAACTCCAACTCCC	471
1	50,513,833	50,513,979	AAAAATACRCCTCAAAAACCAAATAAAAAC	219	TTGGGAGTYGGGGTGGTTAG	472
14	101,192,985	101,193,133	GTGTTGTATAGTGTGAGGGAA	220	CAATCCCTATAACCCCCTCC	473
20	21,686,229	21,686,377	RAAAACCAATAAACTACCAACTC	221	YGATTTTAGGTTTAGGGTGAATTTT	474
7	24,324,907	24,325,055	GTTTTTGTTYGTGTGTTTGGGTG	222	CCACCCACCTCTACCTAAT	475
2	66,666,426	66,666,575	TGGGGTTGGGATTTTTAGGT	223	AAAACTAATTCCTACRAATTCCTCCTA	476
5	76,923,868	76,924,017	GAGTTTTGGTAGGTGTTGGT	224	AACCTCTTTAAAACCTTCCCTAA	477
2	66,667,385	66,667,534	CTCTACAAAATAAAATCAAAAACACCA	225	AGAAATATTTTAGTGTGAATTAAATAAGTTG	478
10	106,401,990	106,402,139	TCTATACTCACTTTCTCCAATACTT	226	tgggggtaggggGAGTT	479
20	2,781,198	2,781,347	AACAAACAAAAACCCCCACA	227	GTTGTGGGYGGGTAGGAGGT	480
11	112,833,653	112,833,803	CAACTCCAAATACCRTTATACCTACCCTA	228	TGGGAGGGATTYGAGTTGGTTG	481
1	248,020,634	248,020,786	TGGATTGYGGTTATAGTTTTTGTTTTAGG	229	CCACCAAACCCRCCAACTACC	482
13	78,493,250	78,493,403	CCCTAACTAACTAAACTACAACTCC	230	TAGGTTGGGTAAAGGAAGGA	483
6	108,440,232	108,440,386	TCTAAAAATTAACTCTAACTTCCCCA	231	GGTTTTTATTTTTYGTTTTTATTTGATGAGTT	484
2	182,322,443	182,322,597	TGTTGTTGTTGTGTTTGGGG	232	TCAATCCAACTCTACTCACCAT	485
11	20,618,126	20,618,280	RAAAACAAAATCAATTACTATTTTTCATCT	233	YGTTTGTTTATAGTGATAATTAGGTTTA	486
8	145,106,394	145,106,549	GGGGAGGYGGGTTTAGTGTAG	234	CCTAACCAAAAACCRAATTTAATTTAACC	487
15	86,233,182	86,233,337	TTAAAGTTGTTGAGTTTTGTTTTGA	235	ACCTCCCCATACTTTAATCCT	488
6	29,760,062	29,760,217	TCCTAACCCAAACCTAAACAAA	236	AAGTTTGGGAGTAAGGAGGG	489
12	85,667,262	85,667,417	TCAAAATAAAAACTCCTCCACCTAT	237	TTTTGGTTTTYGGTTTGGAGGG	490
4	147,561,686	147,561,841	TGTAGGTATGGTTYGAGGAGGT	238	AAAACCRAAACTAAATAACAAAATAAACTTC	491
6	28,778,138	28,778,295	GTGAGGGAAGAGAGGTGTTT	239	CAAACCAAAACCCAACATCAC	492
7	27,225,023	27,225,180	TGTAGTTATTTTAGGGGAAGTAATAGAT	240	AACCCTACAATTAAACACAAACAT	493
14	24,045,512	24,045,669	GGTAGGGGYGGGGGTGG	241	RCAAACACCTACCAAAACCA	494
13	28,366,707	28,366,864	RTTCAACCTCCTAAACAAAAACAA	242	GTAGTATTAGYGAGTTTATTAGGAAGGAG	495
4	147,561,678	147,561,835	ACCCATCCTACAAACATAACTC	243	GAGGTTGAATGGTAAAGTAGGTT	496
10	22,518,210	22,518,368	GAAGATTTYGGGGAAGGAGTGG	244	AAATAAATTTCCAAAAACCAAACAAAA	497
7	27,225,011	27,225,169	ACTTCCTTTCTTTATAACCACCTC	245	TGAGTATAAGTATGTTGTATGGGG	498
14	95,234,999	95,235,157	RAAAAACAAAACAATTCAAATCAA	246	YGTTTTAGGTTTAGGAAGTTGAATG	499
1	50,513,832	50,513,991	GGAAGATGYGTTTTAAGAATTAGGTAGAA	247	CTCTCCCCCTCCCTAAAAAT	500
10	106,401,430	106,401,589	GGGTYGGGGTATTTTAGTTTTTGT	248	CACCTCCTCCCRAAAACCCT	501
X	136,656,476	136,656,635	GAGGTTTGGGGATTGGTTG	249	CCTCCTCTTTCCTCCCAA	502
2	66,666,980	66,667,139	CCCTCTTCCCTCTCTTAACAC	250	TTTTTAATATTTATTTTGTTGATTTGTTTGG	503
10	106,401,346	106,401,505	AAAACTACTACCCCAACAAAAC	251	TTGGGTGATTGGGGGTT	504
12	62,584,934	62,585,094	GGGGAAGGGAGAYGTGTGTA	252	CAAAAACTACAAAAACATCRCTAAATATTACC	505

[0308]

1	111,217,518	111,217,678	CCCACCRCCTATTACAACCAAAAC	253	GTGGGYGGGAATTTTAAGGGG	506
2	66,666,422	66,666,582	ACCTTAAAATTAAAATCCCTAAATACAAC	254	GTTGGGGAGGGTTGGTT	507
5	115,152,387	115,152,547	RCTTAAAATCACTAAAAATATACCAAC	255	GAGATAAYGGGGTTTTTGGGAAG	508
5	180,596,604	180,596,764	GTGTAAgttttygtagtgtagtggt	256	CCAAATAAAAATAATAATAAAATATTCCAAACT	509
22	50,216,544	50,216,704	TCCCAAAATCCCACACTACA	257	GGTTTATATTTAAGGTTAGGAAGAAGG	510
7	27,225,490	27,225,651	ACTTAATTACACTCTCTCATTCATAATC	258	AGATGAGGGGAGAGGTGG	511
11	120,434,988	120,435,149	RCAACTTCTACCTTTTATTACAAAC	259	GGAAAGGAGGGTTAYGGGTAAAG	512
1	237,205,044	237,205,207	AATTTTTATTTGATTATGAATAGAGGTAATTT	260	CCTCCTAAACTAAACTAAAACACTAAC	513
20	2,781,222	2,781,385	GGGGATTGAGTGTTAGGGG	261	ACCCTCTCCTCCTCTCC	514
7	5,569,852	5,570,016	CCACCCTACRATCCCCATTAAC	262	YGGGAGAAGTTTTTGGGTT	515
2	175,199,604	175,199,769	TCTACTCTACCTACRCCCTCATTAAA	263	TTGTTYGGAAAAAATTGTTTGGGTTT	516
18	44,787,470	44,787,635	CRAAATCCTCCCTCCTACCTC	264	GGGTGGGATAGGATAGGGT	517
11	14,926,608	14,926,773	CACAAACCCAAAAACCCCA	265	GAGTAGAGTGTAGGTTYGAAGGTAT	518
20	25,062,422	25,062,588	GTTAGGAGTAGGTAGGGTGT	266	CCAAAACAAAAATCTAACTACRAAAATCC	519
6	391,841	392,007	GTGAGGTTGATATTAGAGAGGAT	267	AACCTTAAAACCCAAACCAAC	520
7	19,184,852	19,185,019	RCAAAAACTAAATCCCCCAAAAA	268	GAAAATYGATGAGAGGTAGGGTTAAG	521
5	140,787,404	140,787,572	TTGAGGGGGATGTATATTTGTATT	269	AAAAATCCCAAACCAAAAACTAAC	522
18	74,961,845	74,962,014	RACTCTATCCACCACCAA	270	GTAGTGTATTGTGTGGTTTTTGT	523
6	27,513,424	27,513,594	AACCATTATAAATTCACCCCAAC	271	TTTGGGTAAGTTTTGAGGAAAG	524
6	26,240,675	26,240,845	GGTAAAGGTTTAGGAAAGGGAG	272	CTCCAAAAACACCTTAAAAACAC	525
6	28,778,185	28,778,355	AAACCTAATTCRTTTCACCCAAACTATC	273	GGAAGTTAGGTAATTTTTGAAGTTTTT	526
2	66,666,605	66,666,776	TCCACCRAATCCTAAATATACAATAAAAA	274	YGGAGGTTTTGAGTTATGGA	527
7	27,204,213	27,204,386	TTAAGGAGAGGGGYGGTTAGTT	275	RAATTAAAACCAAAAACCAAAAAT	528
20	25,065,065	25,065,239	ACATTTTCATAACCTCCTACAATAAA	276	TTTGGTTTTGGTTGAYGTTGAGT	529
7	20,817,774	20,817,949	CTACAAAACRAAAACAAAACACAAAATAA	277	GGTGGTGGTTTGGGGT	530
11	125,773,960	125,774,135	AACCAATTCRCTAACCTTTCTAACATC	278	gggGTAGTTTTYGTAGAGTGGAG	531
7	27,204,899	27,205,074	CCTTAAACTAAAAACTACACRAACTAAAAT	279	GGTTATTATTGGGGTTTTGGGTAA	532
5	172,671,421	172,671,597	AGTGTTGAATTGATGTTGGAAA	280	ACAACACAACAATTTATCAACTACT	533
18	55,108,733	55,108,909	TCTACACCCCCTCCCC	281	GGTTTAGTTATTAGGGTTTAGTGGT	534
14	70,653,838	70,654,015	TTTTTGATTATTTAGGAGTTTGGTTG	282	ctttcctctctctctctctct	535
6	99,841,571	99,841,748	AAACACAAATACAAAACTATACATAACT	283	GGATTAGTGGTTTTGTTTGGAAAA	536
14	24,045,441	24,045,620	CCCCRAACCCAACCAACA	284	GGGTAYGTTAGAGTGTGTTTTATTATTAG	537
2	66,666,618	66,666,799	GGATGTGTAGTGGAGGGG	285	CCTTCCTCCTAAAACCCTAAAAT	538
10	135,043,371	135,043,552	gggggtAGTATTGGGGGT	286	AATCCTCAAAAATTCTATTCTTAAACC	539
4	122,686,466	122,686,647	CCCAACTAACACCAAAACCTT	287	ATGTTGGGGGTGGAATTTT	540
20	37,352,962	37,353,143	TCCAATCCCCCACCCC	288	GGTGGTTAGTGTATTGYGGAGTTG	541

[0309]

7	27,204,196	27,204,378	ATCCTAAACCTAAACAACCAAAAA	289	GTTAGGAGTTAGAAGTTGGTGT	542
8	26,723,434	26,723,616	CCAAAACCCCAAACTCCAA	290	TTTTAGGTGATTGYGAGGTAATTTGT	543
1	50,886,642	50,886,825	RCTCTCACCTAAACCCCC	291	GTTGTAGTTGTTTTAYGGTATTGTTGA	544
6	27,513,336	27,513,519	GTTATTGATGTGTTTTGAATGAGTT	292	aaaaccccaataaatttcaaatcc	545
7	5,569,687	5,569,870	ACACCCCRAACCCCAAAAC	293	AATGGGGATYGTAGGGTGGG	546
10	22,518,180	22,518,364	CCAACACCCTAACRAAACCTAAAC	294	GGATTTTTAGGGATTAAGTAAAGAAATTAT	547
7	27,232,735	27,232,920	GTTAGTTTGTAGTTGYGGGGTTATTTA	295	CAAACCCTCCACRCTTCTACAAAAA	548
2	162,283,572	162,283,757	ACAATACATTCRTATCATCACCCACC	296	TGGTTTAGTTTGGGYGGAGAGTA	549
2	45,161,903	45,162,088	aaatctaccccaccctacc	297	GGAAGGGGGATAGGGGAT	550
6	166,582,063	166,582,250	GTTTTTATTATTTGGAAAAGGAAGGTT	298	AAAACAATAACACAACAAAAACCA	551
8	72,756,006	72,756,193	CCAAAACAAACTAATCTTAAACCTAAA	299	GGTTTYGTGTGGTTGGGGG	552
14	29,254,632	29,254,819	CCCTCTTTCCCCTTCAATCTT	300	TGAGGTTTGTAGTGAAGGGT	553
15	76,629,983	76,630,170	ATCATCTTTCCTTATAATCRCAAATCTTAAAAA	301	TGGTYGTGGATTTGATTTTTGTAGT	554
8	72,754,933	72,755,121	ACTTCTTTAATATCAAAATCCRATCTTCC	302	AGAGGATATGTAAATTTTTAGAATGTTG	555
8	23,567,163	23,567,352	atactttctaaCCCCTCTCRAAAAATA	303	GTGYGATAGGGATTTTTATTTTTAGGTTTA	556
12	62,584,867	62,585,056	ACCCRCATCTACCCTCACCT	304	GGGAAGYGGGGTTGTAGGTA	557
7	5,569,805	5,569,994	CAATAACRCCCCAACCCCCAC	305	GGAGATGGGGGATATTTTAYGTTAGTT	558
19	58,238,678	58,238,868	CTAAAACACAAAAACTACAAACACT	306	TTGATGTTTTGTAGGGATGGA	559
5	16,180,017	16,180,207	yggggtgggttgggg	307	CCTACCCCCTACTTCCCA	560
13	28,366,672	28,366,863	AGAGGTTATTGTTTTAGTTTAGGTTT	308	CAACACTAACRAATCCACCAAAAA	561
1	248,020,475	248,020,666	ACTCCTCCCCCTATACAAAC	309	TGTATTTGAGGTAGAAGTTGTGG	562
4	147,559,282	147,559,473	AAACCCTACAACCCCTCC	310	GTTTAGGTGATTTTGATTTTAGGTTTT	563
3	147,113,702	147,113,894	GGAAAGGATAAGGGAAGGGT	311	TCCAAAACTTTCAACACCATAC	564
4	147,559,273	147,559,465	AGTGAGAGAAAGTTTTGTAGTTTT	312	AACCCTAATCCCAAATTCCC	565
6	10,882,217	10,882,409	GGGYGGGGTTTTGTTTTTGGT	313	CTCCACCTCCRTTCCTAAAACTATAATAA	566
12	54,408,539	54,408,731	ACAATACCCRCTCCCCCACC	314	AGTTTTATTTGTAGTAGTYGAATGGTTTT	567
18	22,930,230	22,930,422	RATCAACTTCTAAAAATAACCAAAAA	315	YGGAGGTGGGGAGTAGTT	568
8	72,754,903	72,755,096	GTGGTTTTATATAGTTTGTTGGTTG	316	CTAATAAAATCTTAACTACTCCAAATCAA	569
2	66,667,490	66,667,683	TCTAAACTTATTTTCAACTTATTTAATTCAC	317	GTGAGAAGGAGAGGGGAG	570
20	21,492,888	21,493,082	GATGAGGTTGGTTAGGTGTT	318	TCCAAATCCCCRAAACCCTC	571
6	391,785	391,979	CCCTCAACTCCRAATCCAAAAC	319	GTTTAGYGTAGATGGGGTGGGA	572
7	24,324,845	24,325,039	CTTCTATACCTACAAATACTAAATAACAAA	320	GGTGATGAGGTTGATGTAGTGT	573
7	70,596,935	70,597,129	AAACCRATATACCCTAAAAACCCC	321	AATTATTTTTAAAGTGGGGGTAGTAT	574
5	528,491	528,685	RACAAACCCTCCCAACA	322	ggAGGGATAGGAGYGAGAGGG	575
2	66,667,301	66,667,496	TGTTTGAGTGTTTGTTTGTAGAT	323	ACCCAAAACTCAATCTTACTTCT	576
4	174,450,245	174,450,440	GGTTGTAGGATAGGGTTATGTTG	324	ATAAATCTAATAAATAATTTTCCCCACC	577

[0310]

20	21,686,179	21,686,374	GGAGGGGGTAGAGTTTTAGG	325	CCCCAAACCCAAAATAAACTTC	578
1	149,719,383	149,719,579	ATGTTGAGGGTGTTAYGGTTTTATT	326	CACCTACCAAATTTACAAATCCC	579
7	1,272,352	1,272,548	GTTTATTGAGGTGTATGTTAGGTATAAT	327	CCCRAAACCCCACACATACTT	580
2	119,607,734	119,607,930	AATTCATTTTACCTTTCATATAATAAAACC	328	TTTGGGTGGAGGTTATGGA	581
15	68,122,089	68,122,286	GGGTTGGGGGTAGYGGAAAAT	329	AACAACCRAACCCACCAAAAA	582
15	76,630,037	76,630,234	GTTGGGTTGGGGTTGGA	330	ACACTCRACACACTTAAAACAAAC	583
3	129,693,494	129,693,691	CCTCCCCACTAACCTCAC	331	GAAGGAGAGGGGTTGGG	584
5	172,659,564	172,659,761	TCCCTTCCCTACCAAACTC	332	GGTTTGTAGTTTTGGTTATAGTTGT	585
7	149,917,234	149,917,431	CCCTCCACAAACCTACCAAA	333	GAAGGTAGAGAATTTGGGTTTTT	586
14	60,975,848	60,976,045	CCAATCCRCCCACCCCAATAAC	334	TTGAGTGTGTTAGGYGTTTGTTG	587
2	162,283,566	162,283,764	AGTGGAGTAGTGTATTYGTGTTATTATTTA	335	RCTCCAATAACCCAACCTAA	588
20	21,081,785	21,081,983	GGGATGGGGAAATTATTTGATTAG	336	CCTATTTCTACCCCCACCC	589
8	23,563,886	23,564,084	GATTTTTAGGAGGTTTYGAATTATTTAGTTT	337	CCCTTCTCRATCAAAAACATCCTAC	590
16	85,932,830	85,933,028	GTAGGTATAGTGGGYGGGTAGG	338	AATCAACRACTTAAACATTCAAATTAAACT	591
13	53,420,219	53,420,417	CCCCTTTACRCACCTCCTTCTT	339	GTTGYGTTTGTTAGTGTAGGAAGTT	592
2	119,606,711	119,606,910	AACCAATATCCRACTATACTAAAACCTA	340	GGAAAATTGGAGGTAGGGG	593
7	27,205,063	27,205,262	CCAATAATAACCATCACCRTACCCAA	341	GGAGTGATTTATGYGTTATTGTTTTGT	594
22	50,216,594	50,216,793	TTTATTTTTGYGGGGGAATATAAGGAG	342	AAACTACACCCRAAAACCTCTAAAT	595
22	50,922,498	50,922,697	CAACAAAACTCAAAACATTCCC	343	GAGGTTTTTATAGGGTAGGATTAGT	596
22	22,862,796	22,862,997	GGGGATYGTGGGATTTGGTT	344	AACAACAACTAATCCRAACTAATAAAAAC	597
1	111,217,055	111,217,259	GGGAATTGGTAAAGGGTTTTTAG	345	AAAACCACCTACTAAAACCRAAAATAA	598
6	28,367,514	28,367,722	CCATCAAAAAATAACCCACAAAAAC	346	GGGTAAAGAAGAGGAGAAGATAAA	599
1	111,217,355	111,217,565	GGGYGTTGAGGAGGGTGG	347	RCCAAACCCAAACAAAACA	600
2	119,607,767	119,607,977	ATTTGTTATAGTAATGGGATGATAAATG	348	ATTCCCAATAACATATTCATTTACAAAA	601
7	149,917,184	149,917,394	AGTTATYGATTTTTGTAAGGGATGTAGA	349	CATAAATCAAACCRCAAAAATCTCCAATA	602
2	63,281,084	63,281,295	AAGGTYGAGATTTGGGTTTGTTAG	350	CCTCCTCCCRCATAAAAATATCAAAATAA	603
1	50,886,613	50,886,825	TTTGTTGTTTTTATTTGYGGTTTGGT	351	ACTACAACTACTCTACRACACTACC	604
5	135,266,111	135,266,323	RAACTCCAACACCTTAACC	352	ggAGGGGTYGTTTTAGGGGG	605
7	155,166,900	155,167,114	GGTAGGAAGAAGGGYGATGTTTTT	353	ACTAAAACCCRCACCTAACCCA	606
14	95,239,509	95,239,725	ACTAAACAAACCATCAAAACCC	354	GTTTGGGTGATAATAGGGAAAATTA	607
10	118,030,867	118,031,084	TTTTAAGGGTTTGTTTTAGAGTTTG	355	CTAACCATAACTAACCTACCCC	608
3	147,113,558	147,113,776	AGTTGGGATTTGAGGAAATTTAG	356	CCTTCAAAACCAAATACAAACTTAT	609
11	20,618,134	20,618,352	AATCAATTACTATTTTTCATCTTTAACAAAA	357	aaagaagaagaagaagaagaagaTTAG	610
10	118,030,866	118,031,086	ACTTCAAAAATTTACTCCAAAACCT	358	YGTTGATTATGGTTGGTTTGT	611
11	71,955,207	71,955,427	AATACAACRCAAATACAACCAACCC	359	GATAGGGATTTYGGGGATAGGG	612
11	14,926,603	14,926,824	GGGTGTATAGGTTTAGGGGT	360	CTACAAATAAAACTTCTTTCCAAATAAAC	613

[0311]

5	15,500,202	15,500,423	CCTCCCCRAAACTCCAACTAT	361	GAGTYGGGTAGGTTTTTGGGTT	614
2	63,281,053	63,281,282	TCTTTTATTCCCAATTCRACTTTCTTT	362	GAAGATGTTAGGGTAGYGAGTTTTG	615
14	29,254,647	29,254,879	AGTTTTTAGGGAGGGGGAG	363	CAAAACAATAATTTCTCAACTTTTCC	616
1	111,217,544	111,217,780	YGATGTTTTGTTTGGGTTTGG	364	AATACAACATAAAAACTCTTTCRCTAACAC	617
11	31,848,615	31,848,857	GTTGGGTTGGGAGAAGTTT	365	ACTCCCTCCCTTCTATTTTCA	618
4	111,544,173	111,544,425	GGGTTTTGTYGTAGTTTAGTTTTAGG	366	AATACAAACAAAACAATCCCTCCAC	619
7	27,205,054	27,205,314	TTTAGGGTTTTAGTGGTGGTT	367	ACTTTTACTATAAAAATTATAACTACAAAACATC	620
19	58,238,712	58,238,972	GGAGTYGGAGAAAGGGTGATT	368	ATCAAACTATCCCTAACCRAAATTCTA	621
5	180,596,452	180,596,712	CATCTCAACCTTCCAAATACTAAA	369	TGAAGTAATGAGATGAAAAGTATAAGAG	622
2	63,283,871	63,284,132	TGGYGTGTTTTTGTTTATTGGAGTATT	370	RAAAACTTAAACCAATCCAAC	623
15	65,116,211	65,116,473	ACAATCCRAAAACAACAACTACACT	371	TGGTTGTGGAGGAGTTGAG	624
5	140,787,349	140,787,617	ATTTAAAACACAAAACATAAAAATATCTACTA	372	TTTTGTGTGGGAGTTGGTT	625
14	95,239,425	95,239,697	GGAGGTAGGTTTYGGGAAAGG	373	ACATATTTACTACATTTCCRACCTAAAC	626
10	135,043,275	135,043,552	ACCCATCCCCTAACCTAAC	374	GGTTTTTAGGAGTTTTGTTTTTAGAT	627
19	58,238,625	58,238,903	CCCACACCCACCCAC	375	GGAGGAGATGTTGTTTTTAGTG	628
6	26,240,714	26,240,993	AAAATACTACRTAACAACATACAAAACATCAC	376	GTTGTTAAATAAAAAGTYGGGGTGAG	629
20	25,065,005	25,065,285	TGTGTTTTGGGTTATTTTGTGTT	377	ACTTTTTATCTCTTACAAACRTCTCCTAAAC	630
7	155,259,636	155,259,918	AAAATTCTACTAAAAATCATTCTATCTCC	378	GGGTAGTAGTGTGTGTAGGG	631
5	172,659,477	172,659,760	GGATTTAGGGTTATGTTGGGAG	379	ACCTACAACCCTAACTACAACTA	632
7	20,817,773	20,818,057	GTTGTAGAAYGGGAGTAGGGTATAG	380	CAACTCCTCRAAATACCCAATACA	633
14	52,734,368	52,734,652	GGAGGTTTTTGTYGTGGGGAATA	381	CAACCCCAAAACCAACAAAT	634
20	61,638,444	61,638,729	CACAAACCRAAAATAAAAACTCTAAACC	382	TTTTTAGYGGGATAGGGTGTTGG	635
18	77,547,903	77,548,190	CTAACAAAACRACCCAACCAAAAA	383	GTTAGGAGGGATTYGGGAGGT	636
11	120,434,917	120,435,205	TTAGAYGGTATTAGGTAGTTGAATTTAGT	384	AAACCCTTCCCAACCCT	637
9	37,002,424	37,002,713	GGTTAGTAAGAATGTTATAGTTTTATTTTGT	385	ACTCCATCAACRACATCCTAAACA	638
1	91,182,909	91,183,198	AACAATCTATAAATACTTTCRACACAACT	386	yggtgttaggttgtgggt	639
18	44,787,350	44,787,639	AAAACTAAAAATTCCTAAAATCCCTTTA	387	GGAGGGGTGGGATAGGA	640
1	111,217,246	111,217,537	CAACAAATAATCCCCRAACACCA	388	TGGTTGTAATAGGYGGTGGGT	641
7	1,272,400	1,272,691	RTTTCCCTACACCCAACAC	389	TGGGTATGYGGGTGTTTTAGGA	642
11	125,774,055	125,774,347	GGGTTTTYGTTTGGAGGGTTGT	390	CATCCRCTAACCAATAAACTTCCTTAAA	643
7	24,323,635	24,323,929	GGGGTGTGGGTGGTTTT	391	CTCCCACCCCTAAACAAAC	644
X	136,656,432	136,656,726	RACCCCTCCAACCTTT	392	GTGGGTGTGGGAGGTTT	645
1	91,182,902	91,183,197	GGGAAGTGGTAATTTGTGGATA	393	rataccaaactataaacaaaccc	646
7	27,225,460	27,225,755	TGTTTGGTGGTTTGTTYGATTTGTA	394	CCCCTATACCTCTATCTCTACC	647
2	182,322,235	182,322,530	CTTCCCTCTCTCCTTCCTTTA	395	GTGTTGTGGGGGTTTTGG	648
2	63,283,941	63,284,236	CAAACTACTACCCCTTCAACT	396	GGGTGTTAAGATAAGATATGTTTAGT	649

[0312]

11	20,618,153	20,618,450	TTTTTGATAAAGTAATTAAGGTYGTAGTGA	397	ACCAAACCCTAAAACAACAAAT	650
14	70,653,836	70,654,133	ATTTCTTAACCACCCAAAAACTTA	398	GGGTTAAATAGAAGAAATGTTTTTAATG	651
1	119,530,348	119,530,646	ATTAAGGTTTTAAYGGAGAAGGTATGT	399	RCTAATTCCTAACAACTAAACCAAC	652
17	47,073,268	47,073,566	CCCCTAACRCCATCCCAACC	400	GTTTTTGGGGAGGGGGGAA	653
8	23,567,042	23,567,341	GAAAAGATATTTTGTGGGgggatt	401	ACCCCTATCCCCAAATCTAC	654
17	47,073,229	47,073,528	YGAGGGGGTTTTAGGGT	402	TCCCTCRACTTTAACCAACTCA	655
2	198,650,889	198,651,188	ACCCTAAACCTACCACCTAAC	403	GTGGAGGAGAGGGGAAG	656
13	109,147,667	109,147,966	TCCTCCTACTCCCAAAATCT	404	YGTGGGGTTGAGTTTTAGTT	657

[0313] 表3

[0314] 下表3提供了包括如上表1中限定的全部150个MVP、排名前三位的MVP(SEQ ID NO：

1-3)、排名前五位的MVP(SEQ ID NO:1-5)和排名前10位的MVP(SEQ ID NO:1-10)的示例性测定法的统计信息。

[0315]

	全部150个	前3名	前5名	前10名
敏感性	0.93	0.61	0.66	0.70
特异性	0.97	0.71	0.74	0.80
PPV	0.98	0.79	0.82	0.86
NPV	0.89	0.51	0.55	0.61
AUC	0.95	0.66	0.70	0.75

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[0258] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

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[0285] cggatccgac ctgcgcccct acgctggccc agctgctagg aactagcgcc ccgagcgccg 120
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[0354] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0358] cc 122
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[0390] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

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[0402] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

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[0414] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

[0415] <400> 34

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[0417] cgcccgtgtg cacgcggatg tgatttacaa gtttgtattt ggctttgaag ggctttccct 120

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[0426] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

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[0450] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0454] ca 122
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[0462] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0474] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0486] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0489] cgtgttcgcc aaacaggtca gcaaaataga tgttaaaaag taaaagaaac aaaaagagag 120
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[0498] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0514] gc 122
[0515] <210> 43
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[0522] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0534] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"
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[0546] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

[0547] <400> 45

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[0558] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

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[0561] cgcacctacc tgcagccccg cttcccggtg gcggcaacac ctagcgatgc tcctgcagct 120

[0562] tt 122

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[0567] <220>

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[0569] <222> (61)..(61)

[0570] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

[0571] <400> 47

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[0573] cgaagcgctt tagtgccttc cgtccctaaa ccgccaacag ccagaacggc ttctccccgc 120

[0574] gg 122

[0575] <210> 48

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[0581] <222> (61)..(61)

[0582] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

[0583] <400> 48

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[0585] cgtccccagc cttccgcgca cagagccgca tcccgccccg ccctgcgctg gactggttca 120
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[0653] <222> (61)..(61)
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[0703] <400> 58

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[0706] gc 122

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[0715] <400> 59

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[0718] gg 122

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[0726] <223> /note="Nucleotide may be methylated to form 5-methylcytosine"

[0727] <400> 60

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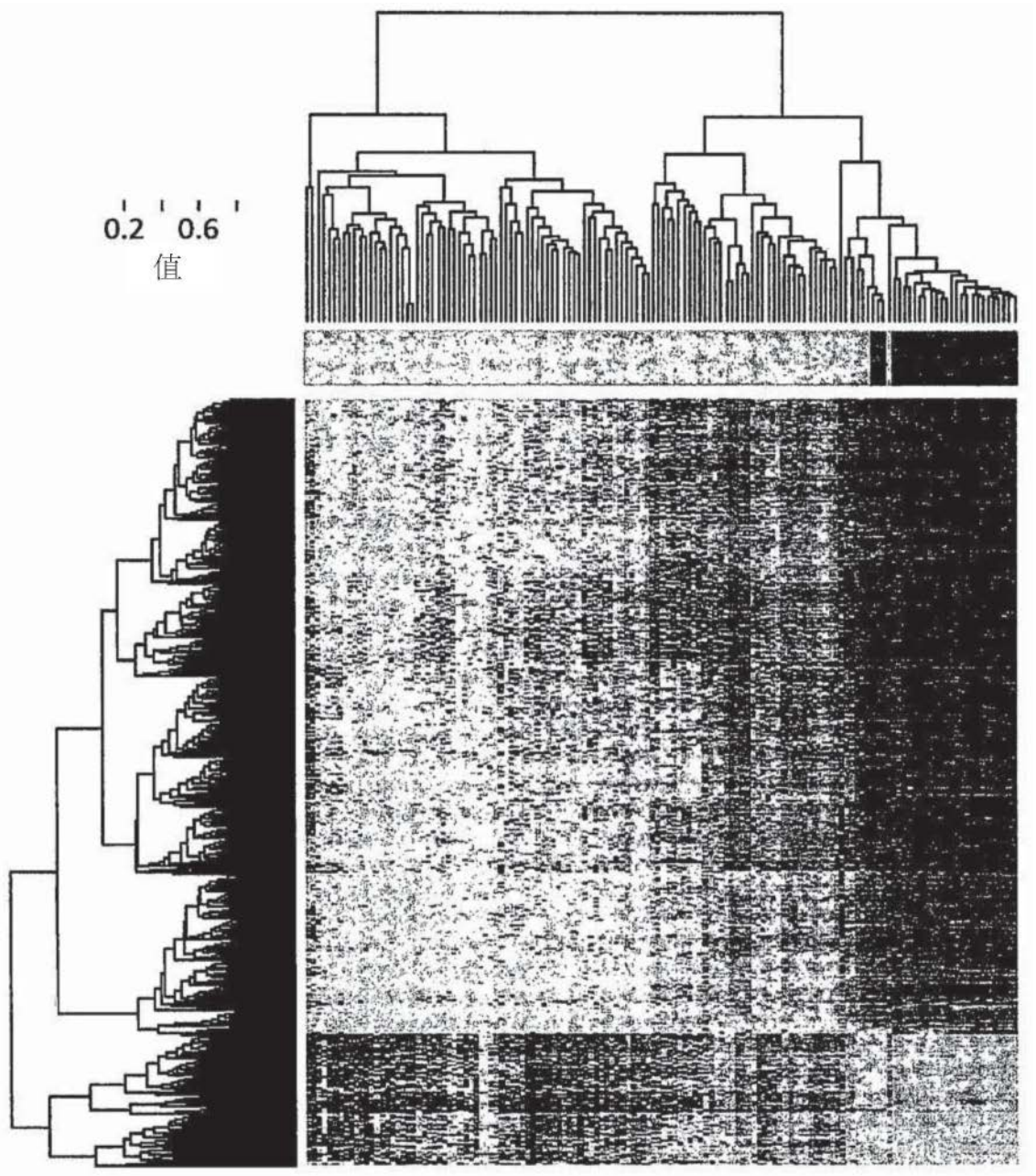

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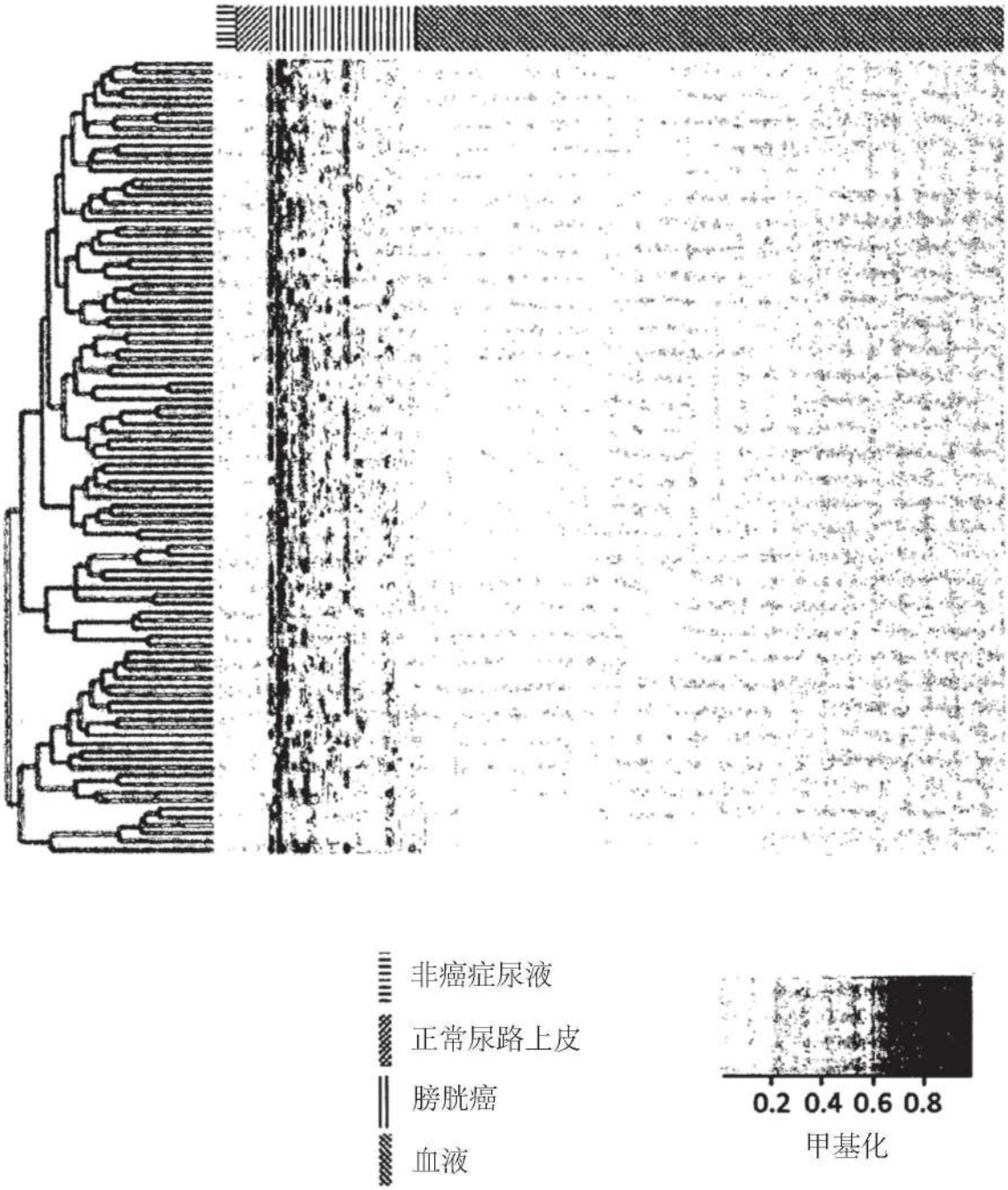

图2

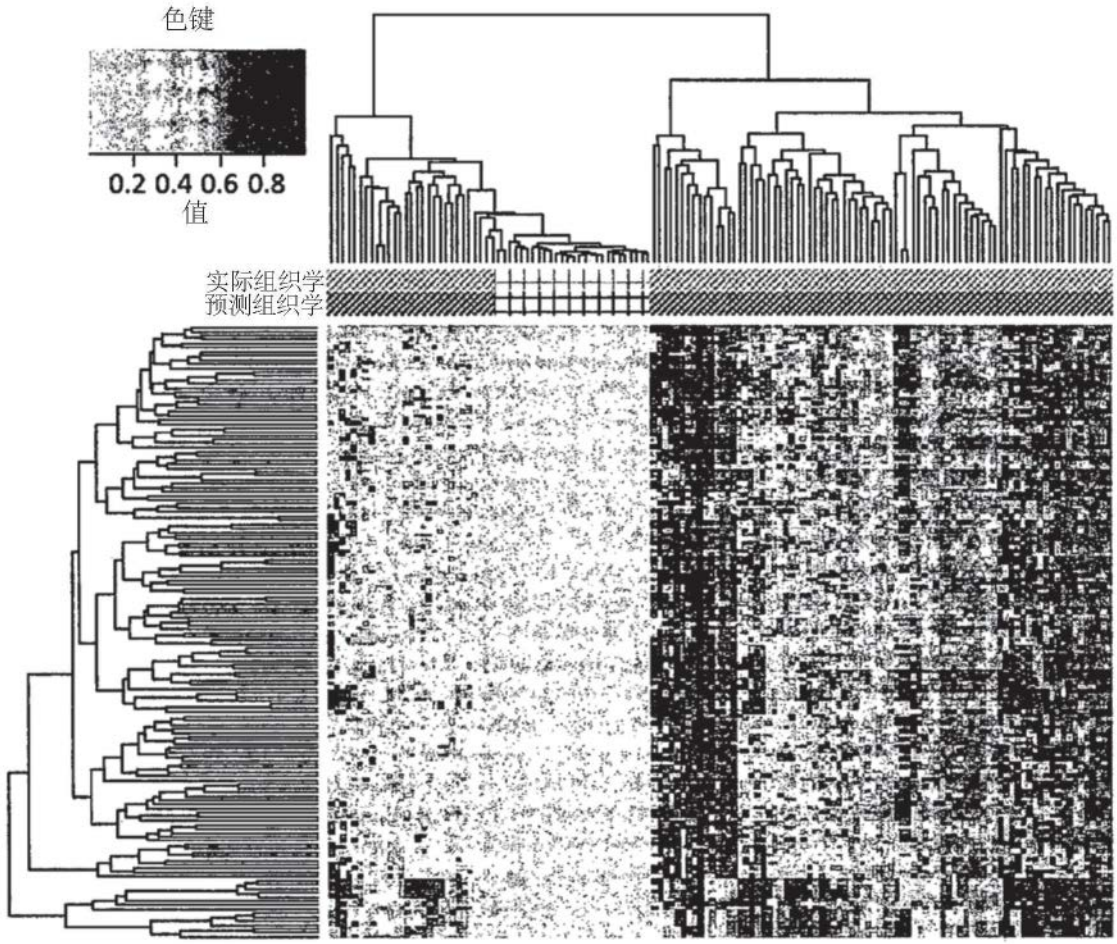

图3

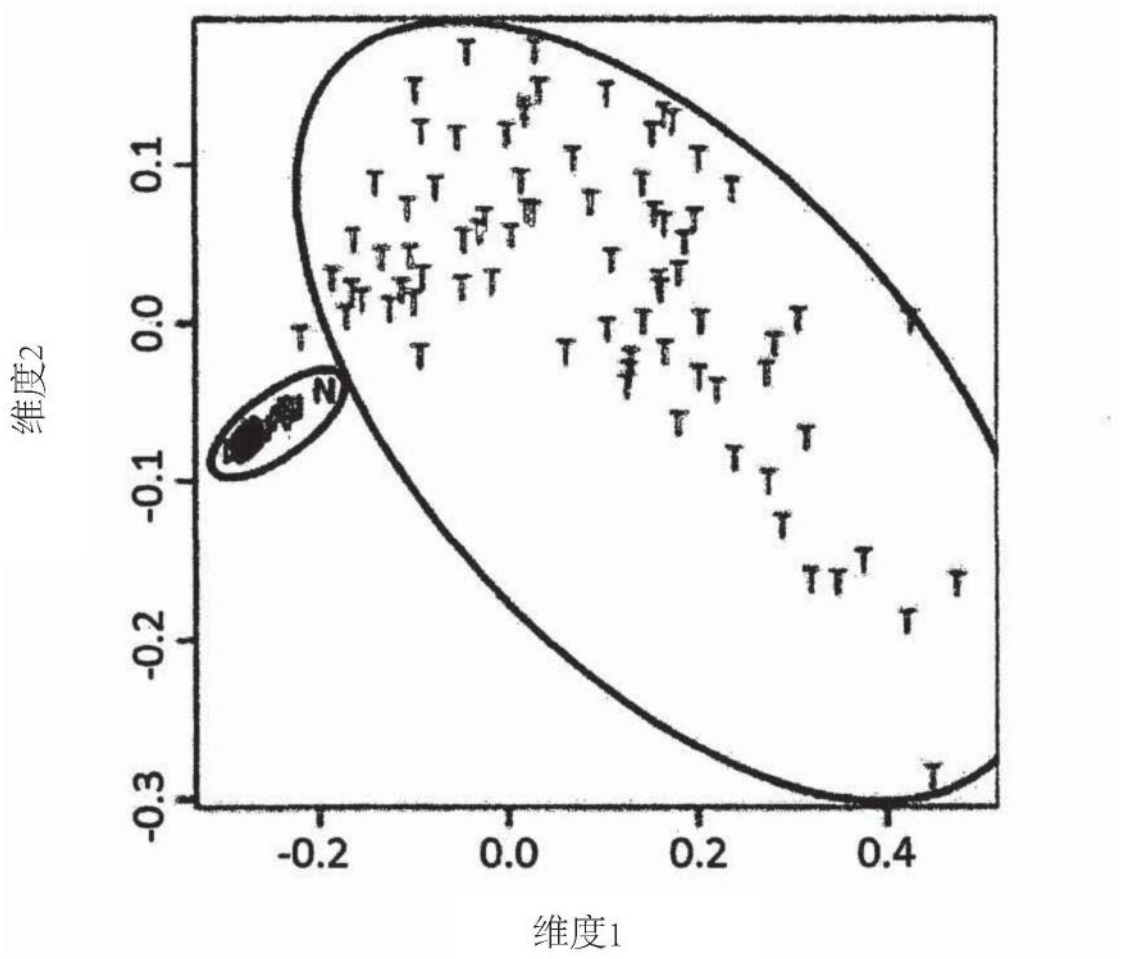

图4

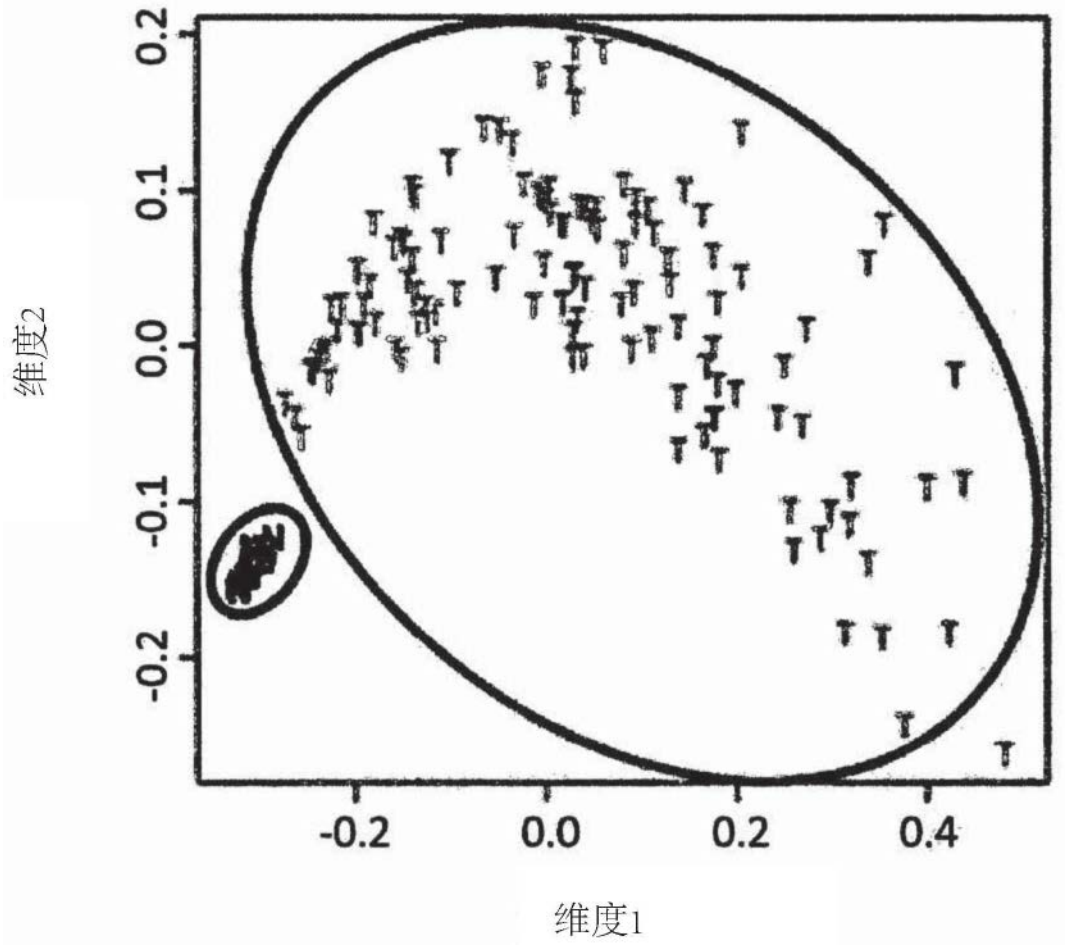

图4(续)

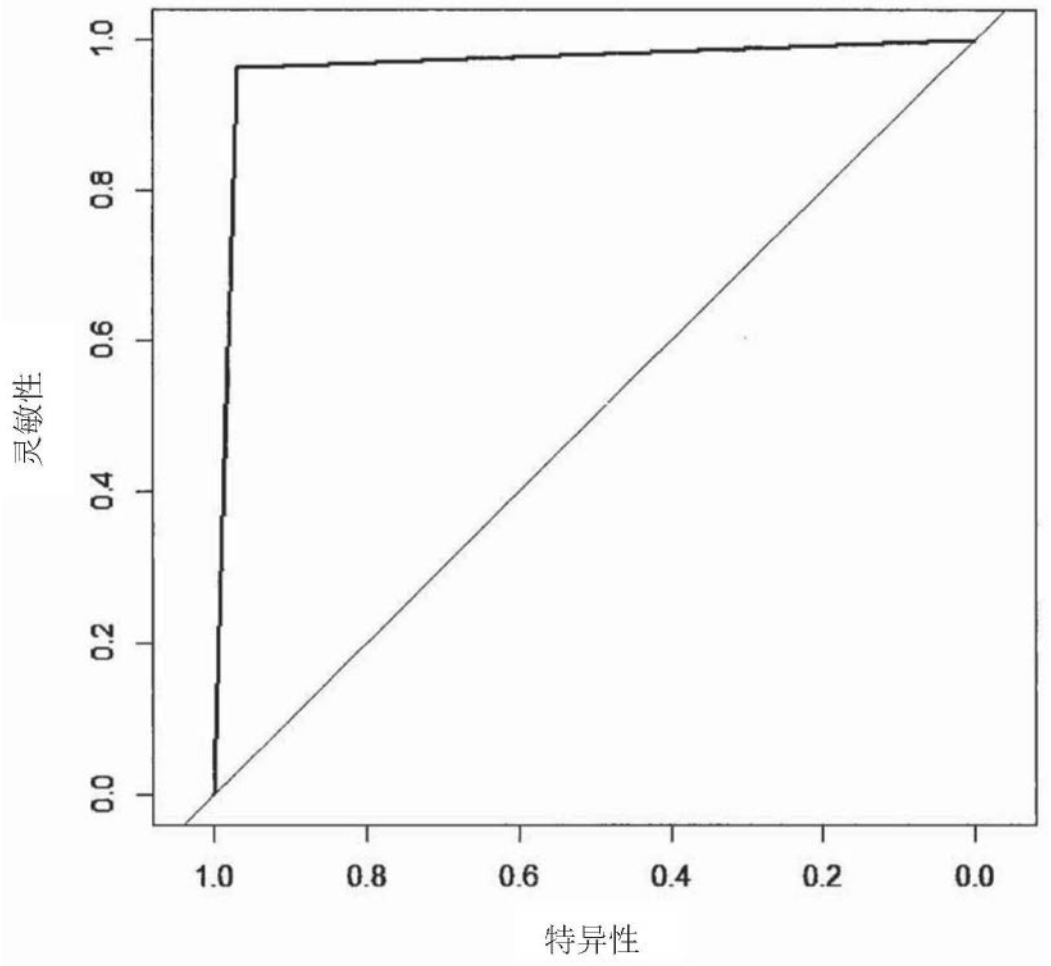

图5

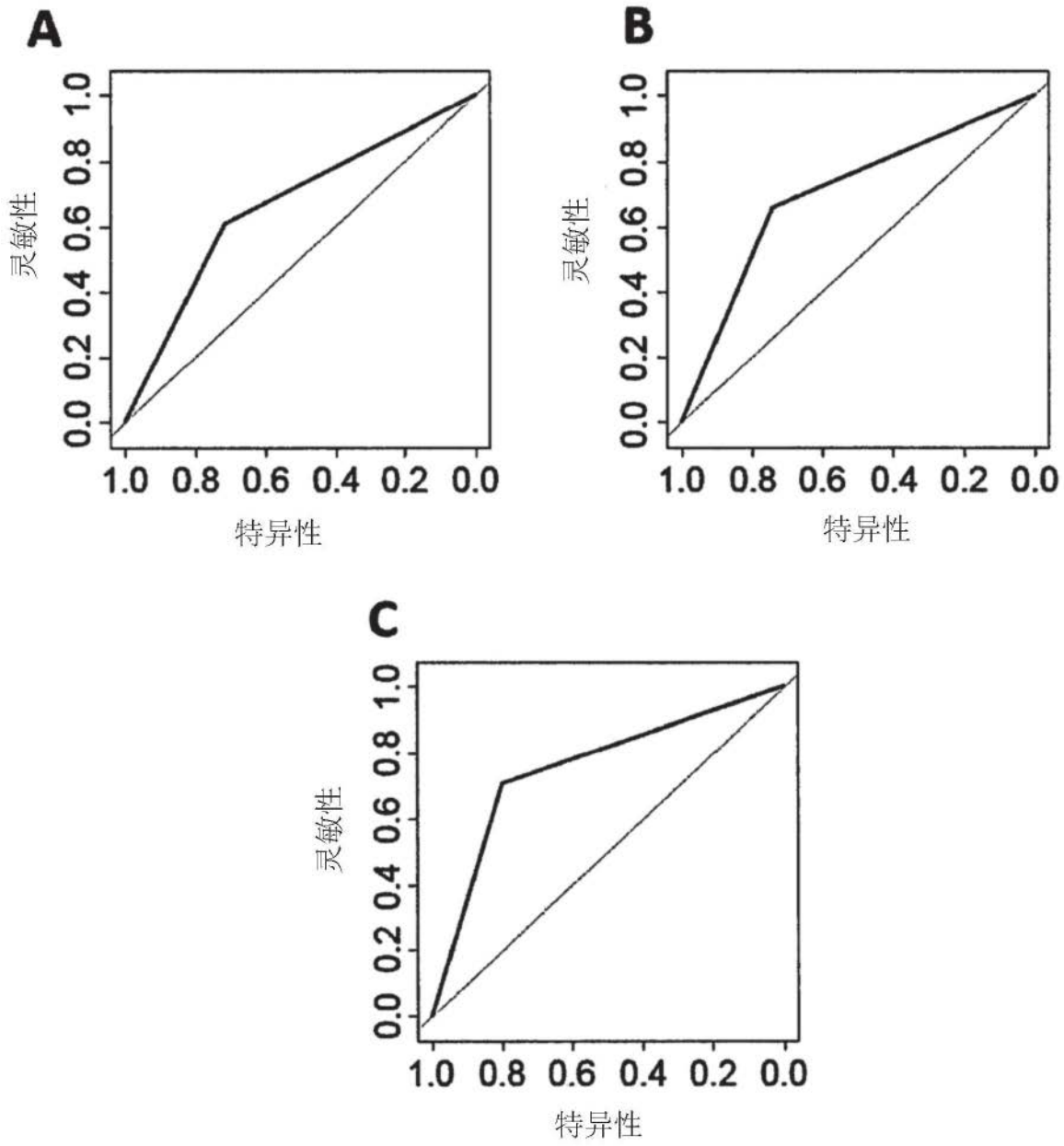
A
灵敏性
0.0 0.2 0.4 0.6 0.8 1.0
1.0 0.8 0.6 0.4 0.2 0.0
特异性
B
灵敏性
0.0 0.2 0.4 0.6 0.8 1.0
1.0 0.8 0.6 0.4 0.2 0.0
特异性
C
灵敏性
0.0 0.2 0.4 0.6 0.8 1.0
1.0 0.8 0.6 0.4 0.2 0.0
特异性

图6

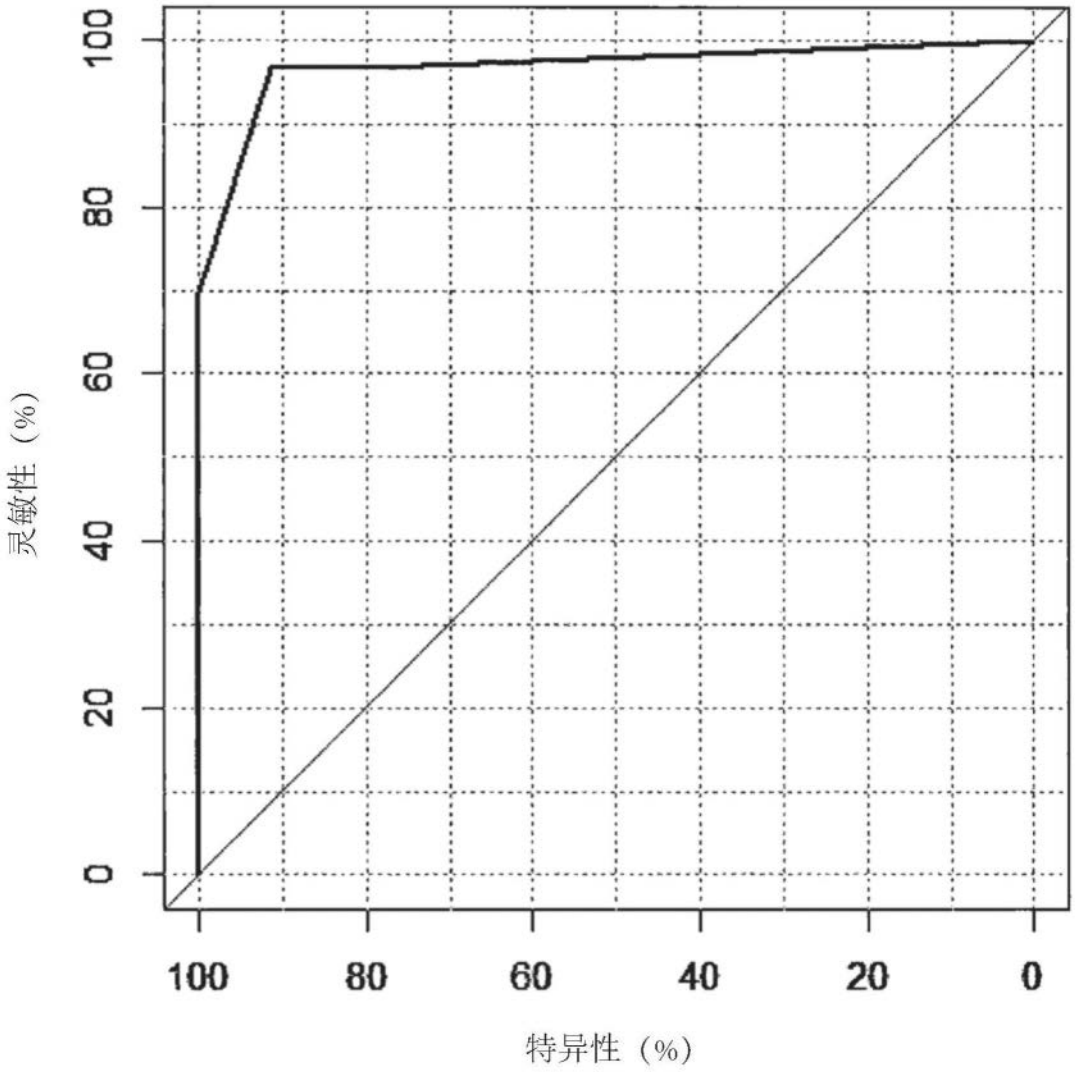

图7

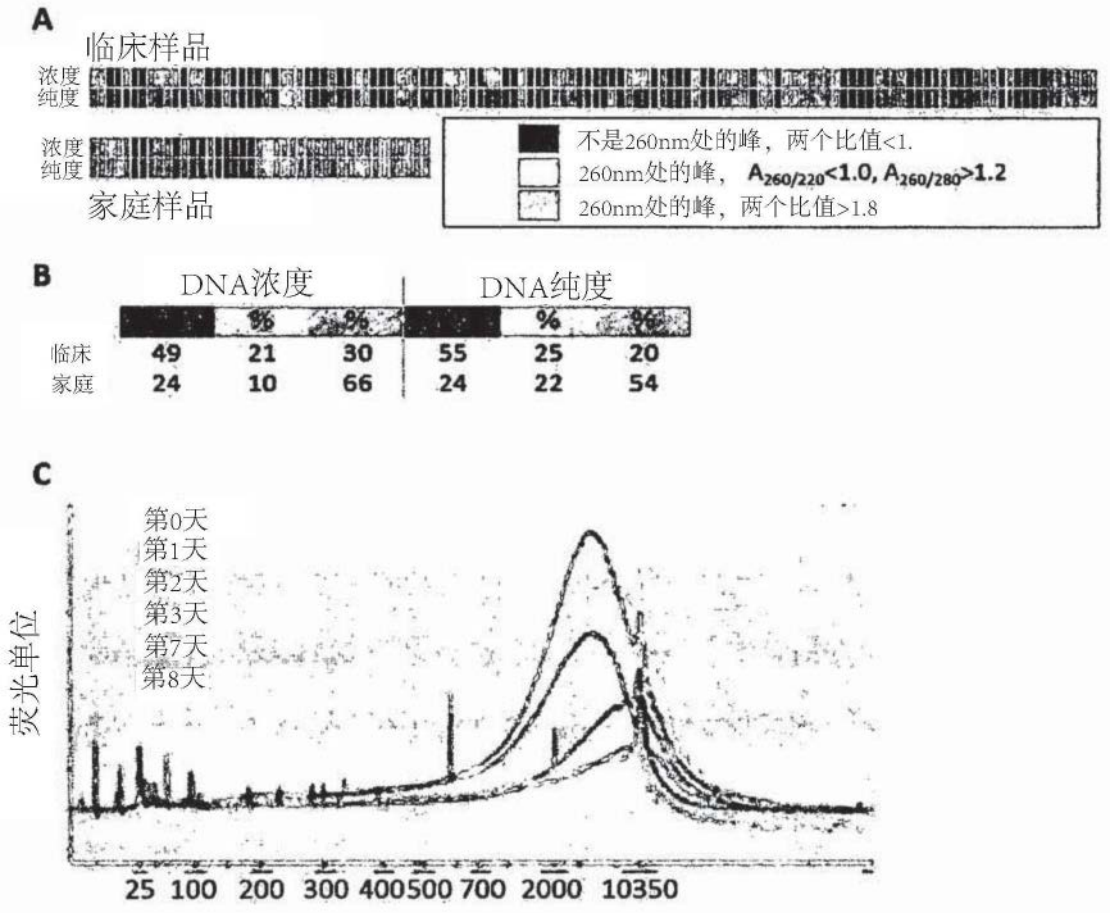
A
临床样品
浓度
纯度
浓度
纯度
家庭样品
不是260nm处的峰，两个比值<1.
260nm处的峰，$A_{260/220}$<1.0, $A_{260/280}$>1.2
260nm处的峰，两个比值>1.8
B
DNA浓度
DNA纯度
临床 49 21 30 55 25 20
家庭 24 10 66 24 22 54
C
荧光单位
第0天
第1天
第2天
第3天
第7天
第8天
25 100 200 300 400500 700 2000 10350

图8

D

消化方案	n	产率 中位数浓度 (ng/μL)	下四分位数 (ng/μL)	上四分位数 (ng/μL)	范围 (ng/μL)	纯度 $A_{260/230}$>1.79 (样品的%)	$A_{260/280}$>1.79 (样品的%)
在56℃下1 h	43	16.8	8.2	105.3	3.8-482.9	30	40
在21℃下48 h	35	29.9	14.2	55.6	4.8-264.7	43	89

E

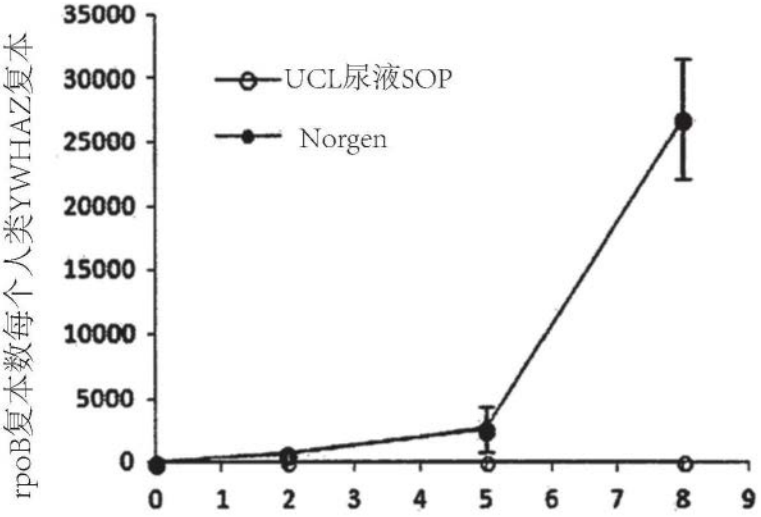

图8(续)

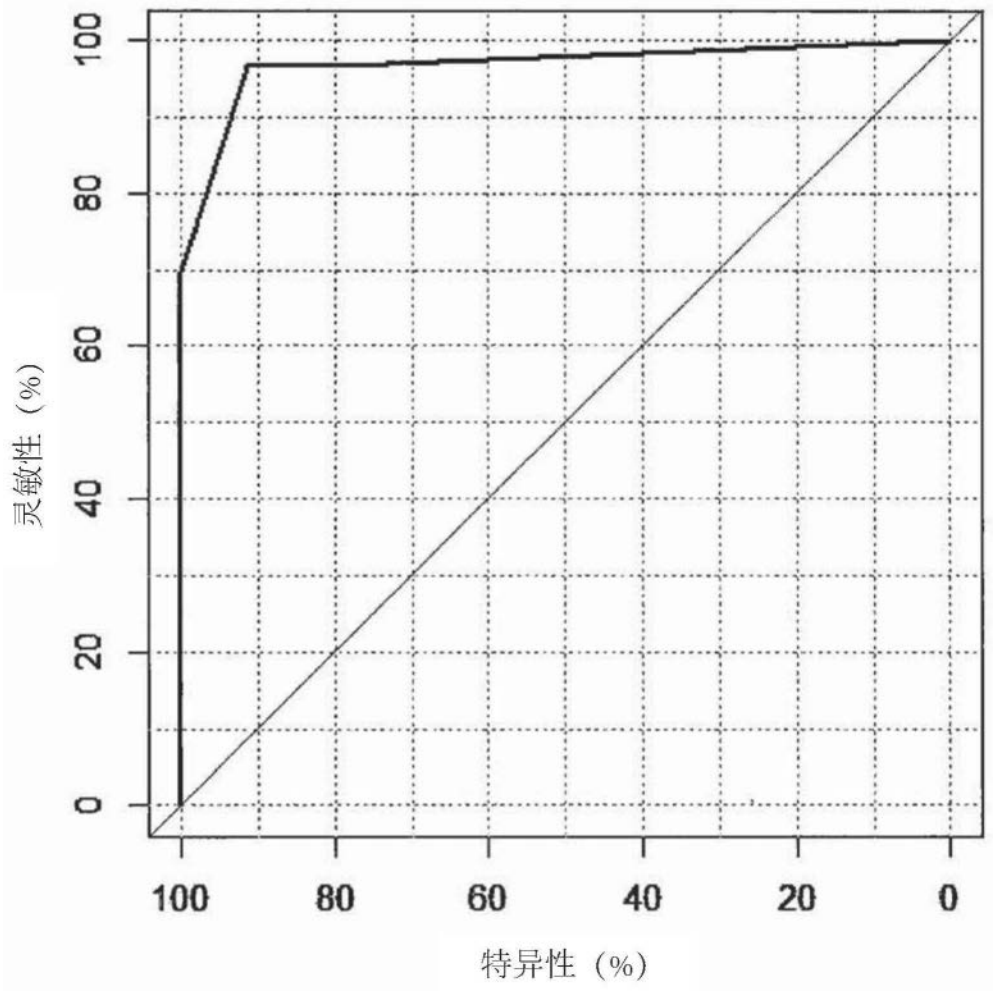

图9

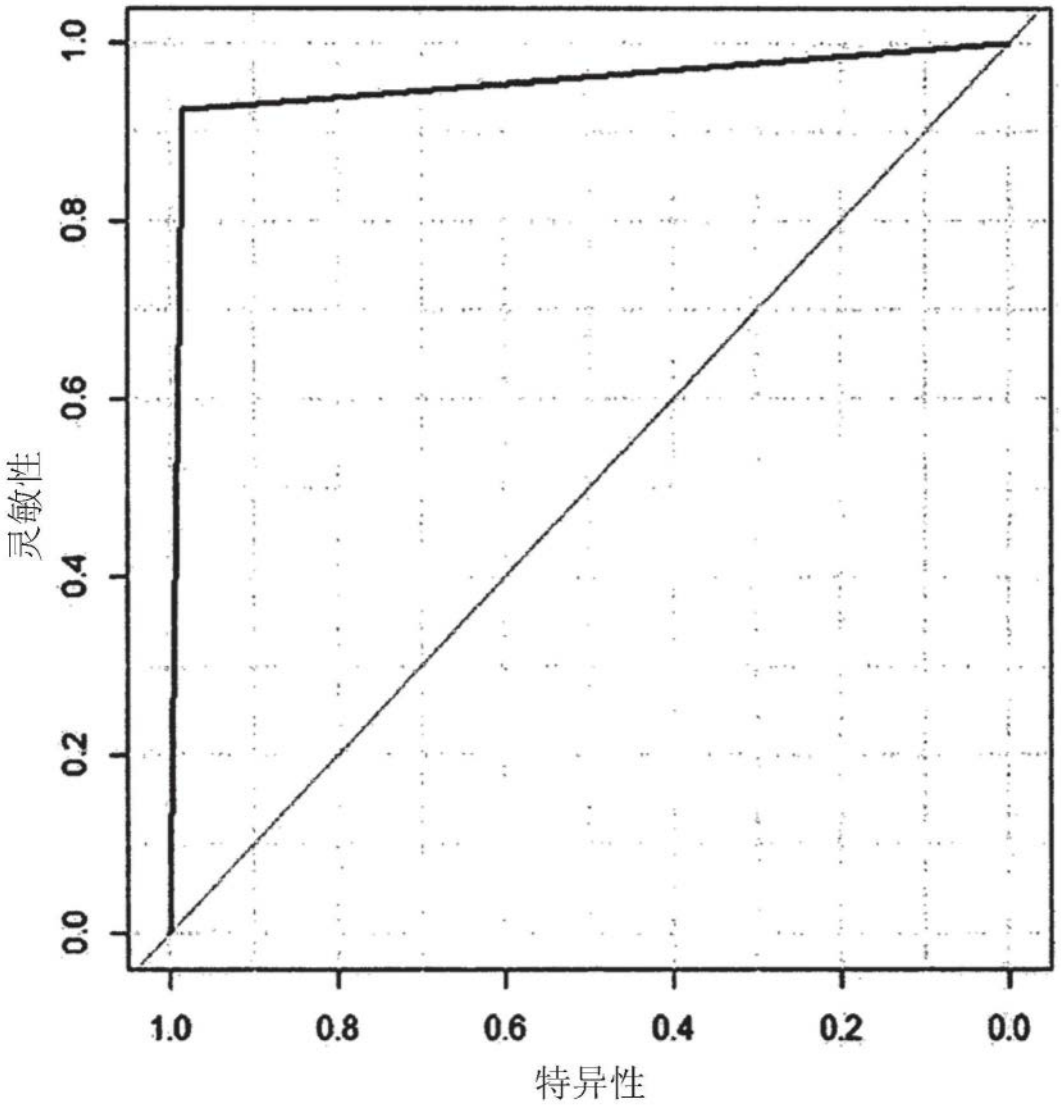

图10