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[73] 专利权人 夏家辉
地址 中国医学遗传学国家重点实验室中
国湖南省长沙市湘雅路 88 号
[72] 发明人 夏家辉
审查员 柯 珂

[74] 专利代理机构 长沙正奇专利事务所有限责任
公司
代理人 卢 宏

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[54] 发明名称

人源基因引导序列、基因载体及基因表达方
法

[57] 摘要

本发明涉及人源基因引导序列、基因载体及基因表达方法。它包括如下内容：(1) 以人类 D、G 组染色体短臂上的无重要生理功能的 DNA 序列或与该 DNA 序列严格杂交的 DNA 序列作为基因的引导序列；(2) 构建含有上述引导序列的基因载体；(3) 以步骤(1)中提到的 D、G 组染色体上核仁组织区作为靶位，将目的基因导入人体细胞的 D、G 组染色体短臂的表达活跃的区域。本发明提供了一种全新的基因引导序列，由此构建的基因载体和基因表达方法。该基因表达方法可应用于人体基因治疗和制备蛋白质。

- 1、 一种载体，包括用于定点整合的基因引导序列，其特征在于所说的基因引导序列为序列表 1 中的 75590 位至 79448 位，或是与该序列严格杂交的序列。
- 2、 根据权利要求 1 所述的载体，其特征在于含有正负筛选基因。
- 3、 根据权利要求 2 所述的载体，其特征在于该载体的 DNA 序列如序列表 2，其中利用 TK 做为负筛选基因，将正筛选基因 Neo 基因插入引导序列中的 1500 位，使基因引导序列分为 1.5kb 和 2.3kb 两臂，构建成基因载体，目的基因的插入位点为 5910 位。
- 4、 外源基因表达方法，包括下列步骤：
 - (1) 构建权利要求 1 至 3 之一所述的载体；
 - (2) 将目的基因装入上述载体；
 - (3) 将目的基因导入人体细胞的 D、G 组染色体短臂的核仁组织区靶位；
 - (4) 目的基因在离体宿主细胞内表达。
- 5、 一种基因治疗药物，含有治疗基因与载体的重组体，其特征在于载体是权利要求 1 至 3 之一所述的载体。

人源基因引导序列、基因载体及基因表达方法

技术领域：

本发明涉及基因引导序列，用该基因引导序列构建的基因载体和利用该基因载体将目的基因导入人在体或离体细胞中进行基因表达的方法。

背景技术：

人类孟德尔遗传病数据库统计资料表明，到2000年6月30日止，已确定的单基因疾病有1660种，已克隆的疾病基因有989种，其中隐性遗传病大多可通过将正常基因导入患者细胞的方式进行基因治疗，随着研究的深入，显性遗传病及与体细胞突变相关的肿瘤也开始采用基因治疗的方法。虽然，美国学者E.Marshall 1995年曾在Science上提出新载体的研究和发现是研究基因治疗的核心问题，但5年后的今天，基因治疗的载体问题仍未得到解决，其主要原因是研究者未能跳出用病毒来源的元件构建载体的桎梏，而目前使用的病毒性载体普遍存在以下问题：（1）不稳定：由于基因插入效率不高，部分载体以附加体的形式存在于细胞核内，不能随细胞的分裂而稳定遗传，从而导致治疗基因不能长期稳定地表达；（2）安全性差：例如，随机插入所导致的插入突变可能影响插入位点的正常基因的功能或激活癌基因而引起其他疾病或肿瘤，另外病毒性载体还有可能产生具有复制能力的野生型重组病毒对机体造成更大的危害；近年来，在美国进行基因治疗的过程中已出现由于腺病毒载体而导致患者死亡的报道；（3）免疫原性：病毒基因产生的背景蛋白和病毒载体纯化过程中污染的蛋白都会引起机体的免疫反应，因而影响治疗基因的表达。

而80年代中期利用同源重组的原理发展起来的基因打靶载体虽能避免免疫原性和随机整合而达到定点导入的效果，但基因打靶载体用于基因治疗的定点修复或替换缺陷基因必需以基因两侧的特异性DNA片段做为引导序列，

从而受到基因特异性的局限且导入效率很低，一般常应用于胚胎干细胞（ES）或受精卵细胞的基因剔除，不宜用于成熟体细胞的定点整合（Galli-Taliadoros LA, Sedgwick JD, Wood SA, et al. J Immunol Meth 1995, 181:1-15; Hasty P, Rivera-Perez J, Chang C, et al. Mol Cell Biol 1991, 11(9):4509-4517）。因此，原美国人类基因学会主席、耶鲁大学医学院院长Leon. E. Rosenberg对基因治疗的现状做出的结论是——“在数百个基因治疗的实验中，至今还没有任何一例毫不含糊地具有临床疗效”（Rosenberg L E & Schechter A.N. Science, 2000; 287:1751）。

所以，寻找和开发一种能稳定遗传，对人体无任何危害，又便于体外操作的基因载体，仍然是基因治疗亟待解决的关键问题。

发明内容：

本申请人在1981年曾发现2个携带额外双随体小染色体（BM）而表型正常的家系，该小染色体分别在2个家系的2代人和3代人之间稳定遗传，且对人体无任何危害。当时查阅国际资料发现欧、美等国总计也有7个家系报导，至今已报导这类家系17个，但没有1人提出用该小染色体为原件组装人源性基因载体的构想。本发明人1991年提出了分离该小染色体原件、组装人源基因载体的设想，并于1994年立项，1995年计划开始具体实施。在研究中申请人用FISH技术查明，该小染色体来源于人的D、G组染色体短臂即13、14、15、21、22号染色体短臂，由于D、G组染色体短臂为核仁组织区，富含核糖体DNA(rDNA)，该区域不但在人群中存在着长度不同的各种多态（即含rDNA的量有多有少），而且在细胞分裂间期该区域基因转录十分活跃，因此，本发明人推测如果能从BM克隆出具有特异性的DNA片段，并以其作为目的基因的引导序列，将目的基因定点导入人体D、G组染色体短臂核仁组织区，应该有较高的、稳定的和无害的表达。后续的实施例对此作出了有力的证明。

找到BM染色体并克隆出与D、G组染色体短臂核仁组织区同源的特异性

DNA片段，具有十分重要的意义。

本发明人首先利用显微切割的方法，构建了小染色体特异性的pUC19文库并从中筛选得到单拷贝片段，该片段经荧光原位杂交（FISH）证实来源于小染色体和D、G组染色体短臂，进一步以此单拷贝片段为探针筛选人PAC基因组DNA文库，获得了约120kb的DNA片段（BMSF），如序列表1所示，并经FISH证实来源于小染色体和D、G组染色体短臂（图1）。测序分析BMSF的序列特性，未发现与重要生理功能相关的基因，因此证明，以此为靶位点是安全的。

因此本申请人认为上述序列中基因引导序列选自人类D、G组染色体短臂上DNA序列作为目的基因的引导序列是符合发明目的的。例如基因引导序列选自序列表1表示的DNA序列。序列表1所描述的DNA序列是来源于人的。

根据本发明的实施例，申请人采用序列表1中从75590位到79448位，或与该序列严格杂交的序列为基因引导序列（GLS）构建载体。

按构建基因载体的要求可在基因载体中含有正负筛选基因。

更具体地，将序列表1中从75590位到79448位，长度为3.8kb的DNA片段为基因引导序列（GLS）装入pGEM-TK载体，利用TK做为负筛选基因，将正筛选基因Neo基因插入GLS中的1500位，使GLS分为1.5kb和2.3kb两臂，构建成基因载体，含基因载体的菌株已于2000年9月29日向中国典型培养物保藏中心提出保藏(中国.武汉武汉大学校内,邮政编码:430072)，其保藏号是：CCTCC M200030。保藏人对保藏物指定的分类命名为*Escherichia coli* JM109/JH-4/pNS2。如图2所示，其DNA序列描述见序列表2。

由于本发明提供的含有选自序列表1表示的DNA引导序列来源于D、G组染色体短臂特定的靶位，毫无疑问，由其构建的基因载体能将目的基因导入上述的特定靶位，并且由于该特定靶位的DNA片段不具有重要的生理功能相关的基因，因此目的基因的导入是安全的。

根据上述发明技术方案，本申请人进一步提出的外源基因表达方法，包括下列步骤：

- (1) 以序列表1中的75590位至79448 位，或是与该序列严格杂交的序列为目的基因的引导序列，构建载体；
- (2) 将目的基因装入上述基因载体；
- (3) 将目的基因导入宿主细胞的D、G组染色体核仁组织区靶位；
- (4) 目的基因在体外或体内的细胞内表达。

上述步骤中构建基因载体，将目的基因装入基因载体，将目的基因导入宿主细胞以及实现目的基因的表达过程都可以通过现有技术来完成。

用上述基因引导序列可构建含有正负筛选基因的载体。

本发明实施例2、3说明了上述过程：①从约120kb的BMSF选择特异性较好的3.8kb DNA片段亚克隆入pGEM-TK质粒载体，以TK为负筛选基因，同时在3.8kb DNA片段中间插入正筛选基因Neo，构建成基因载体。②选择目的基因酶切后连入Neo基因一侧的外源基因克隆位点，经PCR测序证实各部分的存在及方向。③在pGEM载体上选择一单酶切位点将基因载体线性化，并利用电穿孔或脂质体转染的方法导入靶细胞。④用G418和GCV对转化的靶细胞进行正、负筛选，获得定点整合的阳性细胞克隆。⑤对阳性细胞克隆进行目的基因的表达检测，最终达到使目的基因定点整合到D、G组染色体短臂稳定表达

的目的。

将筛选获得的表达目的基因阳性细胞通过皮下包埋或静脉注射等方式导入患者体内或将含目的基因的载体用脂质体包裹直接注入患者体内，使目的基因能在患者体内长期稳定表达，以纠正缺陷基因导致的临床症状。

本发明实施例中提供的基因载体的DNA序列如序列表2，其中引导序列为序列表1中的75590位至79448位，利用TK做为负筛选基因，将正筛选基因Neo基因插入GLS中的1500位，使GLS分为1.5kb和2.3kb两臂，构建成基因载体，目的基因的插入位点为5910位。本发明实施例中公开了目的基因治疗血栓性疾病的组织型纤溶酶原激活剂基因（TPA）在HT1080细胞内的体外表达，以及目的基因治疗血友病B的凝血因子IX基因（FIX）在HT1080细胞内的表达。实验表明：其表达是有效和稳定的。

因此，本发明提供的基因表达方法，具有非常巨大的应用前景。不但可用于制备药用蛋白质，而且为基因治疗提供了有效的途径。

本发明人利用一种新的对人体无害的染色体为独特的材料，走出了一条全新的研究路线，该发现在国际上首次找到了人类D、G组染色体短臂核仁组织区是人类基因治疗与基因表达的最佳靶位点（13、14、15、21、22号染色体短臂核仁组织区的10个靶位点），构建了一种能将目的基因定点导入这些靶位点的新载体——人源基因载体。

本发明与背景技术相比具有如下显著的优点：

1、稳定性好：按权利要求1或2所提供DNA序列作为基因引导序列来构建载体，能将目的基因定点插入到人体细胞的D组和G组染色体短臂上，并使目的基因随染色体而稳定遗传；

2、安全性好：由于在靶位点不含有与重要生理功能相关的基因，证明该靶位点安全，同时FISH证实载体能将目的基因定点插入细胞中安全的靶位点（图3，4），排除了随机插入突变，也不存在野生型病毒的危害。目的基因

在靶位点的表达是安全的。虽然本发明没有提供基因表达方法的临床证明，但申请人及国内外研究者发现的实例从另一角度证明了其安全性（实施例4）；

3、表达效率高：第一，由于本发明提供的基因载体含有来源于D、G组染色体短臂的引导序列，因此对应地在人体细胞中存在10个靶位点，插入效率比其它载体至少高5-10倍；第二，由于引导序列来自于基因转录活跃的D、G组染色体短臂，引导进入靶位点的目的基因能得到高效表达。

4、无免疫原性：由于该载体为人源性的，因此用于人体不会产生免疫原性。

图1是120kb PAC 克隆DNA的FISH定位图；

图2是本发明提供的一种基因载体的结构图（基因载体序列全长：11162bp）；其中pGEM-7（8267—11162）：载体复制元件及原核筛选系统；TK（1—2840）：真核细胞负筛选基因，该基因使用TK启动子和TK polyA信号；Neo（4342—5910）：真核细胞正筛选基因，该基因使用sv40启动子和sv40 polyA信号；GLS（2841—4341，5911—8267）：目的基因引导序列；Cloning site（5910）：目的基因插入位点；

图3是外源性tPA基因在阳性细胞克隆中的FISH定位，证明该载体能将tPA定点导入D、G组染色体短臂；

图4是外源性FIX基因在阳性细胞克隆中的FISH定位，证明该载体能将tPA定点导入D、G组染色体短臂；

图5是TPA纯化产物Western结果，1~4为TPA纯化产物，“—”示阴性对照；

图6是FIX阳性细胞Western结果，F3~F23为6个不同阳性细胞株，“—”示阴性对照。

本发明提供的实施例只是实现本发明的一种方式，不能视为对本发明的

限制。

实施例1

本发明提供的基因引导序列的制备：

1.基因引导序列PAC克隆的获得

1.1显微切割、PCR、微克隆构建BM特异性pUC19文库(Deng H-X, Yoshiura K, Dirks RW, et al. Hum Genet 1992, 89:13.)

1.2 BM特异性单拷贝DNA的获得及鉴定

(1)菌落点阵膜的制备：在两张尼龙膜上划好14×14个方格，标记为A、B，分别置于2个有固体LB的平皿中，从文库皿中随机挑取白色克隆分别点到两张膜坐标相同的方格内，共挑14×12个，第13行点100ng单拷贝DNA做阳性对照，第14行点100ng gDNA做为阴性对照，两个皿分别置37℃培养10~12小时，其中B膜置4℃保存，将A膜从皿中取出，分别在用以下溶液浸湿的滤纸上处理，10%SDS，5分钟，0.5N NaOH/1.5M NaCl，3分钟，1.5M NaCl/0.5M Tris·HCl，3分钟，2×SSC/0.2M Tris·HCl，10分钟，80℃真空干燥2小时，37℃保存待用。

(2)gDNA探针的制备

取50~75ng gDNA加无菌水至11ml，100℃煮沸变性10分钟，以下列体系做随机引物标记反应：

2mM dNTP(dATP ⁻)	3μl
primer mixture	2μl
Klenow酶	1μl
α- ³² P-dATP	3μl

点离混匀，37℃浮浴30分钟。加8μl stopmixture，过G-50柱纯化探针，取1/10测液闪值。

(3)杂交：菌落点阵膜置于2×SSC中浸泡10分钟，轻轻拭去膜表面的菌落碎片，

65℃, 5ml杂交液预杂交, 30分钟以上。根据液闪值, 按 1.2×10^6 cpm/ml杂交液的量取探针液于100℃煮沸变性10分钟, 加入5ml新鲜杂交液, 与菌落点阵膜65℃杂交12小时以上。按以下条件洗膜: $2 \times \text{SSC}/0.1\% \text{SDS}$, 室温10分钟, $2 \times \text{SSC}/0.1\% \text{SDS}$, 65℃10分钟, $0.1 \times \text{SSC}/0.1\% \text{SDS}$, 65℃10分钟, -70℃放射自显影, 杂交信号无或弱的初步认为是单拷贝。

(4)测序, Southern杂交鉴定: 在B膜上相应位置挑取无杂交信号的克隆, 小量扩增, 抽提质粒DNA测序, 与GenBank数据库比较, 无相似性的为单拷贝。最后EcoRI酶切分离插入子DNA, 并以随机引物法, 用 α - ^{32}P -dATP标记并与EcoRI消化的gDNA转移尼龙膜杂交, 方法同上, 1条或2条杂交带的为单拷贝。

1.3 BM及D、G组染色体短臂特异性PAC克隆的获得及鉴定

(1)筛选人PAC gDNA文库获得阳性克隆编号

利用 α - ^{32}P -dATP随机引物法标记260bp的单拷贝探针P8-7→G-50柱(中等粒度)纯化→4℃待用→PAC膜7张用 $2 \times \text{SSC}$ 浸泡10分钟→55℃预杂交3小时→探针100℃变性10分钟→以 4.6×10^5 cpm/ml的量加入50ml购买的杂交液中, 与PAC膜于65℃杂交1小时→洗膜: $2 \times \text{SSC}$, 室温10分钟一次, $2 \times \text{SSC}/0.1\% \text{SDS}$ 65℃10分钟洗2次→上X光片, -70℃放射自显影12小时→冲洗X光片→按说明书方法读取阳性克隆编号。

(2)从5个不同的板上随机挑选阳性克隆编号, 购买PAC克隆。

1.4 PAC DNA与中期相细胞FISH杂交, 证实来源于D、G组染色体短臂, 如图1所示。

上述实验方法参见《分子克隆实验指南》J.萨姆布鲁克等,第2版,1989 (J. Sambrook et al. Molecular Cloning . Second Edition. Cold Spring Harbor Laboratory Press. 1989)

2. 基因引导序列DNA的获得

主要材料： β -agarose(Bio-Labs) NotI Agarose

①Not I酶切PAC169质粒;

②通过PFGE分离约120Kb大小的插入子DNA;

脉冲电泳条件：电极缓冲液： $0.5 \times$ TBE、High strength Analytical Grade Agarose:

(Bio-Rad, Low Melting point Agarose LMP) 1%、Switch Time: 2秒 $\rightarrow$ 15秒

电泳时间：18小时，电压：6V/cm，角度：120° 温度：14℃

③电泳结束后，用EB染色（0.2 μ g/ml）30分钟，根据Marker指示，用无菌小刀片切下约120kb的条带。

④切下的胶块用 β -agarase处理，乙醇沉淀。

实施例2

本发明提供的基因载体的制备：

1. 基因载体的构建及目的基因的导入

1.1载体的构建

1.1.1用NsiI和StuI（平端酶）酶切PAC DNA，普通琼脂糖凝胶回收3.8kbDNA片段，电洗脱纯化；

1.1.2用HindIII酶切pGEM-TK载体DNA，Klenow酶补平，产生一平头末端；

1.1.3 pGEM-TK/HindIII补平产物进一步用NsiI酶切；

1.1.4 3.8kb/NsiI+StuI纯化产物与pGEM-TK/HK+NsiI酶切产物于16℃连接17小时；

1.1.5连接产物转化JM109感受态细菌，氨苄皿37℃培养18小时；

1.1.6随机挑取单克隆，用NsiI及NheI双酶切鉴定阳性克隆。

1.1.7用XbaI和NheI双酶切pCDN-GPR质粒获得Neo/XbaI+NheI

1.1.8将Neo/XbaI+NheI与 pGEM-TK-3.8kb/NheI连接,构建成pNS2基因载体。

2.1 TPA/FIX的导入

2.1.1将TPA/FIX（CDS）克隆至pcDNA 3.1(-);

2.1.2设计引物TPCF和TPCR扩增TPA/FIX基因及表达元件（CMV启动子和BGH poly A信号），使其两端装上AvrII酶切位点;引物序列为：

TpcF: ATgCATCCTAggggAggTCgCTgAgTAgTg

AvrII

TpcR: TgCATgCCTAggTACCCCCTAgAgCCCAG

AvrII

2.1.3用AvrII酶切TPA/FIX基因及表达元件（CMV启动子和BGH poly A信号）并与NheI酶切的pNS2载体连接。

上述实验方法详见《分子克隆实验指南》J.萨姆布鲁克等,第2版,1989（J. Sambrook et al. Molecular Cloning . Second Edition. Cold Spring Harbor Laboratory Press. 1989）

2. 基因载体DNA的抽提

2.1 材料：

2.1.1 QIAGEN Plasmid Maxi Kit

2.1.2 培养基：液体LB

Trypton 5g

Yeast extract 2.5g

NaCl 2.5g

ddH₂O 定量至500ml

高压蒸汽灭菌

2.1.3 氨苄青霉素（Amp）：100mg/ml（1000×）

2.2 方法：

- 1) 挑取阳性单克隆至3ml LB(Amp+) 37℃，250rpm培养1小时
- 2) 取100μl上述初级培养物置100ml LB（Amp+）中，37℃，250rpm培养16小时
- 3) 于4℃，6000g离心15分钟收获细菌

- 4) 10ml Buffer P1重悬菌体
- 5) 加入10ml Buffer P2, 轻柔地倒转6次混匀, 室温5分钟。
- 6) 加入10ml冰上预冷Buffer P3, 轻柔倒转6次混匀, 冰上20分钟。
- 7) 4℃, 20000g离心30分钟。
- 8) 迅速将上清转移至另一40ml高速离心管, 4℃, 20000xg再次离心15分钟
- 9) 10ml Buffer QBT平衡QIGEN tip 500
- 10) 将上清转移至QIGAE tip 500过柱
- 11) 2×30ml Buffer QC洗柱
- 12) 15ml Buffer QF洗脱, 收集洗脱液
- 13) 洗脱液加(0.7倍体积) 10.7ml异丙醇, 混匀
- 14) 15000g, 4℃, 离心30分钟
- 15) 去上清, DNA沉淀加入5ml 70%乙醇洗涤, 15000g, 4℃ 离心10分钟
- 16) 去70%乙醇, 空气中干燥10分钟适量TE溶解DNA沉淀

实施例3

利用实施例2得到的带有目的基因TPA和FIX的基因载体, 分别在宿主细胞中导入目的基因, 并在体外细胞中表达。

1. 材料:

1.1细胞: HT1080

培养基: 高糖DMEM+10% FBS (HT1080) EMEM+10%FBS

1.2电穿孔仪: Bio-Rad公司

2.方法:

- 1) 细胞于75cm²培养瓶内传代后长至70%~80%满底
- 2) 收获细胞, 用HeBs缓冲液洗2次, 计数细胞
- 3) 15000rpm, 4℃离心10分钟

- 4) 适量HeBS重悬, 使细胞浓度为 $10^6 \sim 10^7/\text{ml}$
- 5) 取0.4ml电击杯, 每杯加细胞悬液0.8ml, 载体DNA $10\mu\text{g}$ 左右
- 6) 用电穿孔仪进行电击, 电击条件设定为电压260V, 电容 $550\mu\text{F}$, 电击时间一般为11~13毫秒。
- 7) 电击细胞转入 75cm^2 培养瓶中, 加14ml含双抗的培养基, 37°C , 5% CO_2 中培养, 24~48小时。
- 8) 培养基内加入终浓度 $300\mu\text{l}$ 的G418进行筛选, 2~3天换液一次, 换液时重新加G418, 同时用正常细胞做对照
- 9) 约7~10天后正常细胞全部死亡, 计数转染细胞内存活克隆数, 改用维持量G418 $150\mu\text{g}/\text{ml}$
- 10) 转染细胞继续用终浓度为 $500\text{ng}/\text{ml}$ 的GCV进行筛选
- 11) 7~10天大部分克隆死亡, 剩余存活细胞改用维持量GCV $250\mu\text{g}/\text{ml}$ 或不加经过筛的细胞长至70~80%满底后, 测定转移基因的表达活性。

3. 结果

利用该载体将TPA基因和FIX基因用电穿孔的方法分别导入HT1080细胞, 经正、负筛选得到阳性细胞株, FISH证实为定点插入(图3、图4)。TPA活性测定结果详见表1。

HT1080细胞阴性对照TPA活性为 $0\text{ U}/10^6\text{ 细胞}/24\text{ 小时}$, 导入后TPA活性为 $408\text{ U}/10^6\text{ 细胞}/24\text{ 小时}$, 转化后95天表达量为407, 表达十分稳定, 见表1。FIX活性也由阴性对照的低于 $0.5\mu\text{g}/\text{ml}$ 升高到 $2.5\mu\text{g}/\text{ml}$, 而且转化109天表达量仍为 $2.6\mu\text{g}/\text{ml}$, 见表2。二者的表达产物均已通过Western杂交证实(图5、图6)。TPA表达产物现已得到纯化。

表1 TPA转化的阳性细胞TPA活性测定

转化后	T1	转化后	T15
33天	408	54天	188

37天	396	58天	204
60天	411	95天	114
68天	430		
74天	430		
88天	441		
90天	440.9		
95天	407		

(T1、T15为两个tPA阳性细胞株)

表2. FIX活性测定: (ug/10⁶细胞/24小时)

转化后天数	克隆F23
60天	2.5
72天	2.4
100天	2.9
109天	2.6

实施例4

在人群中存在的安全性实例:

从1973年夏家辉从事人类与医学细胞遗传学研究以来,所发现和鉴定的来自全国189个实验室的470位临床细胞遗传学工作者所申报的732种世界首报核型中,有41种核型涉及D、G组染色体短臂,但不论易位到D、G染色体短臂的片段来源于1-22号哪一号染色体,或源于同一号染色体的片段长短各异,所含基因从1个到上千个,而携带者本人的表型都是正常的,说明易位到D、G染色体短臂的基因能正常表达,据此,我们将D、G组染色体短臂作为基因治疗的靶位点应该是安全的。

1、核型：46, XX, t(1;12;22;15;11;8) (1qter→1p11::8p23→8pter; 12pter→12q11::1p11→1pter; 22qter→22p11::12q11→12qter; 15pter→15q15::22p11→22pter; 11pter→11q21::15q15→15qter; 8qter→8p23::11q21→11qter).

表型：女，28岁，为表型正常的携带者

资料提供者：吴素彬，广东省广州市，中山医科大学附属一院妇产科细胞遗传室，邮政编码：510080

2、核型：46, XY, t(1;13) (1pter→1q32::13p11→13pter; 13qter→13p11::1q32→1qter).

表型：女，24岁，为表型正常的携带者

资料提供者：肖晟，哈尔滨医科大学生物教研室，邮政编码：150086

3、核型：46, XX, t(2;15) (2pter→cen→15qter; 2qter→cen→15pter).

表型：女，26岁，为表型正常的携带者

资料提供者：郭裕萍等，江西省南昌市，江西省妇产科医院细胞遗传室，邮政编码：330006

4、核型：46, XY, t(2;21) (2pter→cen→21pter; 2qter→cen→21qter).

表型：男，32岁，为表型正常的携带者

资料提供者：康过秦等，山西省太原市，山西医学院附二院遗传研究室，邮政编码：030001

5、核型：46, XY, t(3;22) (3qter→cen→22pter; 3pter→cen→22pter).

表型：男，26岁，为表型正常的携带者

资料提供者：高永，山东省滨州市医学院毒理室，邮政编码：256603

6、核型：46, XY, t(3;22) (3pter→cen→22qter; 3qter→cen→22pter).

表型：男，29岁，为表型正常的携带者

资料提供者：石化金等，辽宁锦州市妇婴医院遗传室，邮政编码：121000

7、核型：46, XX, t(4;15) (4qter→4p13::15p13→15pter; 15qter→15p13::4p13→4pter).

表型：女，28岁，为表型正常的携带者

资料提供者：周玲等，湖北省武汉市，武汉市儿童医院遗传实验室，邮政编码：430016

8、核型：46, XY, t(4;21) (4qter→4p15::21p11→21pter; 21qter→21p11::4p15→4pter).

表型：男，25岁，为表型正常的携带者

资料提供者：徐竞芳等，上海市第六人民医院遗传室，邮政编码：200000

9、核型：46, XY, t(4;14) (4pter→4q31::14p11→14pter; 14qter→14p11::4q31→4qter).

表型：男，为表型正常的携带者

资料提供者：周明君等，河南省许昌市中心医院，邮政编码：161000

10、核型：46, XY, t(4;14) (4pter→4q35::14p11→14pter; 14qter→14p11::4q35→4qter).

表型：男，27岁，为表型正常的携带者

资料提供者：张秀泉等，广东省佛山市，佛山市妇幼保健院，邮政编码：528000

11、核型：46, XX, t(5;22) (5pter→5q13::22p11→22pter;22qter→22p11::5q13→5qter).

表型：女，32岁，为表型正常的携带者

资料提供者：曹建萍，河南安阳市妇幼保健院遗传室，邮政编码：455000

12、核型：46, XY, t(6;22) (6pter→cen6→22qter; 6qter→cen22→22pter)

表型：男，25岁，为表型正常的携带者

资料提供者：祝新霞等，山东省泰安市，山东泰安第88医院妇产科细胞遗传室，邮政编码：271000

13、核型：46, XY, t(6;22) (6qter→6p21::22p11.2→22pter; 22qter→22p11.2::6p21→6pter).

表型：男，33岁，为表型正常的携带者

资料提供者：杨兰青，山东省滨州市滨州医学院附属医院妇产科，邮政编码：256603

14、核型：45, XX, t(7;21) (7qter→7p22::21p12→21qter).

表型：女，23岁，为表型正常的携带者

资料提供者：孙吉庆等，湖北省武汉市，湖北省武汉市儿童医院医学遗传实验室，邮政编码：430016

15、核型：46, XY/46, XY, t(7;14) (7pter→7q11::14p11→14pter; 14qter→14p11::7q11→7qter)

表型：男，28岁，为表型正常的携带者

资料提供者：李麓芸、夏家辉等，湖南省长沙市，湖南医科大学医学遗传学研究室，邮政编码：410078

16、核型：46, XY, t(8;14) (8qter → 8p21::14p12 → 14pter; 14qter → 14p12::8p21 → 8pter)

表型：男，27岁，为表型正常的携带者

资料提供者：石化金等，辽宁锦州市妇婴医院遗传室，邮政编码：121000

17、核型：46, XY, t(9;14) (9pter → cen → 14pter; 9qter → cen → 14qter)

表型：男，28岁，为表型正常的携带者

资料提供者：程秋云等，衡阳医学院附一生殖医学研究室，邮政编码：421001

18、核型：46, XX, t(9;22) (9pter → 9p13::22p12 → 22pter; 22qter → 22p12::9p13 → 9pter)mat

表型：女，31岁，其母、1妹、1弟、1子具有相同核型，同为表型正常的携带者

资料提供者：李麓芸，夏家辉等，湖南医科大学医学遗传学研究室，邮政编码：410078

19、核型：46, XX, t(9;14) (9pter → 9q12::14p12 → 14pter; 14qter → 14p12::9q12 → 9qter)

表型：女，32岁，为表型正常的携带者

资料提供者：孙艳阳等，黑龙江省哈尔滨市，哈尔滨医科大学生物教研室，邮政编码：150086

20、核型：46, XX, t(9;15)(9pter→9q21::15p12→15pter; 15qter→15p12::9q21→9qter)mat.

表型：女，36岁，为表型正常的携带者

资料提供者：朱桂珍等，山东省泰安市，山东泰安第88医院妇产科细胞遗传室，邮政编码：271000

21、核型：46, XX, t(10;13)(10pter→10q24::13p11→13pter; 13qter→13p11::10q24→10qter).

表型：女，28岁，为表型正常的携带者

资料提供者：严敦清，青岛医学院附属医院妇产科研究室，邮政编码：266003

22、核型：46, XX, t(10;13)(10pter→10q24::13p12→13pter; 13qter→13p12::10q24→10qter).

表型：女，29岁，为表型正常的携带者

资料提供者：张影如等，广东省广州市，中山医科大学附一院神经科，邮政编码：510080

23、核型：46, XX, t(11;14)(11pter→cen→14pter; 11qter→cen→14qter)

表型：女，2岁，为表型正常的携带者

资料提供者：王志勇，河南省柘城县，柘城县人民医院遗传室，邮政编码：476200

24、核型：46, XX, t(11;21)(11pter→11p11::21p11→21pter; 21qter→21p11::11p11→11pter).

表型：女，26岁，为表型正常的携带者

资料提供者：郑军等，陕西省西安市，陕西省妇幼保健院医学遗传实验室，
邮政编码：710003

25、核型：46, XY, t(11;15)(11pter→11q13::15p12→15pter; 15qter→15p12::11q13→11qter)

表型：男，23岁，为表型正常的携带者

资料提供者：杨瑞芳等，山东医科大学附属医院围产医学中心，邮政编码：
250012

26、核型：46, XX, t(12;14)(12pter→cen→14pter; 12qter→cen→14qter)

表型：女，28岁，为表型正常的携带者

资料提供者：韩维田等，辽宁省计划生育科研所优生研究室，邮政编码：110031

27、核型：46, XX, t(13;16)(13qter→13p11::16p11.2→16pter; 16qter→16p11.2::13p11→13pter).

表型：女，27岁，为表型正常的携带者

资料提供者：安松兰，辽宁省大连市，大连市妇产医院遗传室，邮政编码：
110078

28、核型：46, XY/45, XY, t(13;13)(13qter→13p12::13p12→13qter).

表型：男，39岁，为表型正常的携带者

资料提供者：李麓芸、夏家辉等，湖南省长沙市，湖南医科大学医学遗传学
研究室，邮政编码：410078

29、核型：46, XY, t(14;18)(14pter→cen→18pter; 14qter→cen→18qter)

表型：男，30岁，为表型正常的携带者

资料提供者：王素桂等，北京市计划生育技术指导所，邮政编码：100006

30、核型：46, XX, t(14;15)(14pter→14q13::15p13→15pter; 15qter→15p13::14q13→14qter)

表型：女，28岁，为表型正常的携带者

资料提供者：李麓芸，夏家辉等，湖南省长沙市，湖南医科大学医学遗传学研究室，邮政编码：410078

31、核型：45, XX, t(15qter→cen→22qter).

表型：女，27岁，为表型正常的携带者

资料提供者：夏家辉等，湖南医科大学医学遗传学国家重点实验室，邮政编码：410078

32、核型：46, XY, t(15;18)(15pter→cen→18pter; 15qter→cen→18qter).

表型：男，30岁，为表型正常的携带者

资料提供者：任国庆等，北京市计划生育技术指导所，邮政编码：100006

33、核型：46, XX, t(15;20)(15pter→cen→2pter; 15qter→cen→2qter)

表型：女，26岁，为表型正常的携带者

资料提供者：王新等，湖南医科大学附属二医院妇产科遗传室，邮政编码：410011

34、核型：46, XX, t(15;22)(15pter→15q11::22p13→22pter; 22qter→22p13::15q11→15qter)。

表型：女，27岁，为表型正常的携带者

资料提供者：符生苗，海南省海口市，海南省人民医院遗传室，邮政编码：
570011

35、核型：46, XX, t(15;22)(15pter→15q22::22p11→22pter; 22qter→
22p11::15q22→qter)

表型：女，29岁，为表型正常的携带者

资料提供者：李牧尧等，新疆乌鲁木齐，新疆医学院医学遗传室，邮政编码：
830054

36、核型：46, XY, t(16;21)(16pter→16q21::21p11→21pter; 21qter→
21p11::16q12→16qter).

表型：男，29岁，为表型正常的携带者

资料提供者：张惠芳等，广东省计划生育科学技术研究所，邮政编码：510080

37、核型：46, XX, t(18;21)(18qter→cen→21qter; 18pter→cen→21pter)

表型：女，为表型正常的携带者

资料提供者：石化金等，辽宁锦州市妇婴医院遗传室，邮政编码：121000

38、核型：46, XX, t(18;21)(18pter→18q11::21p12→21pter; 21qter→
21p12::18q11→18qter)

表型：女，26岁，为表型正常的携带者

资料提供者：李修林等，辽宁省沈阳市，中国医科大学附属一院小儿科遗传室，
邮政编码：110001

39、核型：45, X, dic(Y;13) (Ypter→Yq1200::13p11→cen→13qter)

表型：男，4岁，为表型正常的携带者

资料提供者：夏家辉等，湖南医科大学医学遗传学国家重点实验室，邮政编码：41007

40、核型：46, XY, t(Y; 15) (15qter→15p12::Yq12→Ypter)pat.

表型：男，新生儿，为表型正常的携带者

资料提供者：夏家辉等，湖南医科大学医学遗传学国家重点实验室，邮政编码：410078

41、核型：45, X, psu dic (Y; 22) (Ypter→Yq1209::22p12→cen→22qter)

表型：男，为表型正常的携带者

资料提供者：刘希贤等，湖北武汉同济医科大学医学遗传学研究室，邮政编码：430030

上述染色体异常携带者均未发现任何异常体征，说明在核仁组织区不但可接受外源基因，而且能让外源基因正常表达。

<110> · Xia Jiahui

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<211> ~120kb

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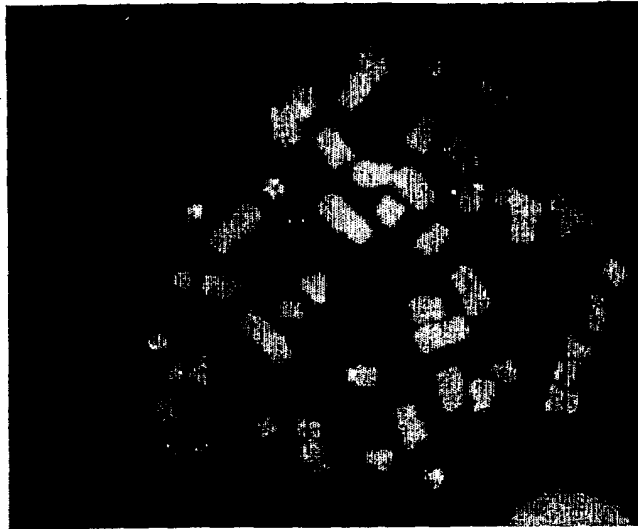


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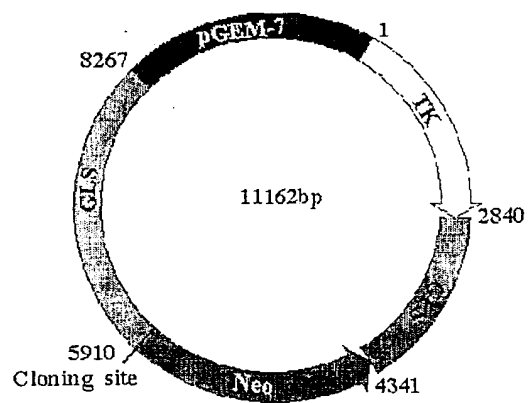


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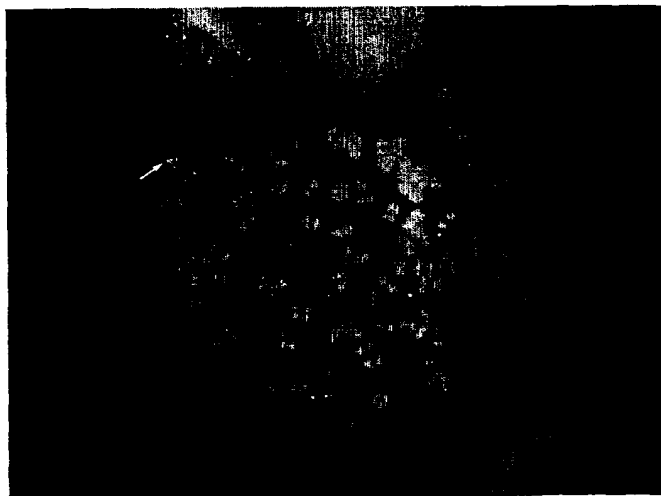


图 3



图 4

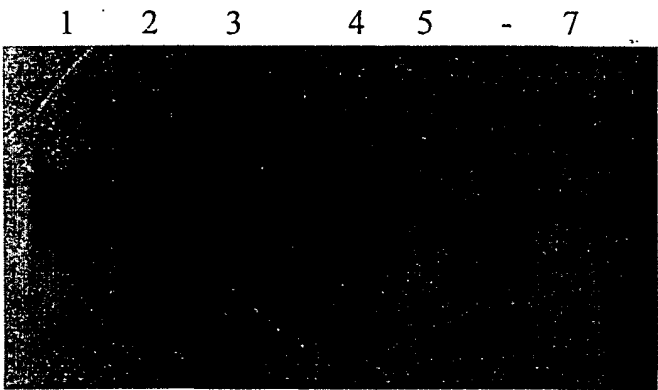


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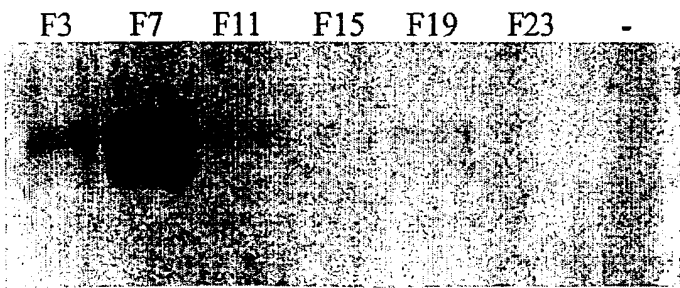


图 6