*i*Porator™ 细胞转染系列产品

*i*Porator-96™

iPorator™系列产品是基于 iPoration™专利技术设计开发的新型高效细胞转染系列产品. 多项创新技术(ACE™, eInjection™ & Intelligent transfection)的运用使得该系列产品在转染贴壁细胞, 特别是转染处于生理相关状态下的不分裂和完全分化的贴壁细胞方面, 突显出其独特的技术优势和出色的转染效果.

iPoration™细胞转染系列产品非常适用于对易损原代细胞(Primary cells)和难转染的细胞线(Hard-to-transfect cells)进行多种生物分子(DNA, siRNA,和Peptide)的原位(*in situ*)转染. 并且表现出转染效率高, 超低损伤和实验结果高度一致的优秀品质.

除此之外, iPorator™系列产品的操作非常简洁, 对新的细胞品种拥有转染指令快捷优化的强大功能.

- 直观的触屏图形用户友好界面
- 简单的操作步骤
- 预置多种细胞的标准转染程序
- 内置转染指令自动优化算法(iPorator-96™)
- 用户可全面自控调整转染参数

iPorator-96™ 系统是一款高度自动化的细胞转染系统. 具有高效率, 高通量, 超低损伤, 超低干扰和应用广泛的独特技术优势. 特别适用于基因组学研究, 药物靶点验证和高通量细胞检测.

*i*Porator-LT™

秉承iPorator-96™的技术特点, **iPorator-LT™**是为适应不同客户的需求而设计开发的一款低通量, 低成本, 灵活型的细胞转染产品. 具有自动检测6孔或24孔格式细胞嵌入培养皿和转染生长在与6孔或24孔格式相兼容的单独嵌入式培养皿内的细胞等特点.特特别适用于初创公司,大专院校和医院等科研机构.

领先一步

独特技术优势

“High Five”

高效率 High Efficiency：

高达90%的DNA和Peptide转染效率, siRNA的转染效率不低于80%.

超低损伤 High viability:

近乎为“0”的细胞死亡率

超低干扰 High Fidelity:

对基因表达非特性影响极低

高通量 High Throughput:

可在一分种内转染96个样品

应用广泛 High Versatility:

可对各种贴壁细胞和细胞线进行 siRNA. miRNA, DNA和 Pepetide 的转染

PRIMAX Biosciences Inc.

江苏源脉生物科技有限公司

江苏省泰州市中国医药城A3栋7层

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Department of Pathology MSC 08 4640

April 26, 2012

Dr. Jeff X. Xi
Primax Biosciences Inc.
115 Constitution Drive, Suite 7
Menlo Park, CA 94025

RE: iPorator-LT and R&D100 award

Dear Dr. Xi:

I would like to provide an enthusiastic testimonial regarding your innovative iPoration technology.

Our morphologic and biochemical studies on human kidney and craniofacial disorders depend on being able to transfect confluent, fully polarized renal epithelia and odontoblasts on filter supports. Such conditions are well established to mimic the fully differentiated and polarized phenotype of these cells in vivo and are crucial for studying normal physiologic function. In our personal experience, lipofection offers poor transfection efficiencies (1-5%) of polarized cells. Immortalized odontoblasts are virtually untransfectable. Using the iPorator-LT we now routinely obtain transfection efficiencies as high as 60-70% for renal epithelia and up to 30-40% for odontoblasts. A >10-fold increase in efficiency saves us time and money in reagents and makes biochemical studies tractable.

We are studying primary cilia, which are only present when kidney epithelia and odontoblasts are fully differentiated. Based on our analyses 24 h post-transfection, cells retain their cilia and we are able to see the delivery of newly synthesized proteins expressed from the transfected plasmid vectors. Thus, the cells are viable and retain their differentiated status following iPoration.

We have just begun siRNA experiments and note that the transfection efficiency of both renal epithelia and odontoblasts with labeled siRNA is nearly 100% and very low siRNA concentrations yield effective knockdown.

Thus, in our hands the iPorator technology has proven highly effective and is hands-down the best strategy for kidney epithelial transfection that we have tested, including lipofection and magnetic nanoparticle methods. We were so impressed with our results that graduate student, Stephanie Jerman, in my lab presented the iPorator data as part of her poster at the national American Association of Dental Research meeting held in Tampa, FL, March 21-24, 2012. There was a great deal of interest in the technology generated at the meeting and therefore, I enthusiastically support the continued development of the iPoration technology through an R&D100 award.

Sincerely yours,

Angela Wandinger-Ness, PhD
Professor of Pathology & Director Fluorescence Microscopy Facility

2325 Camino de Salud, CRF 225 ♦ Albuquerque, NM 87131 ♦ 505-272-1459 ♦ FAX: 505-272-4193

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DEPARTMENT OF SURGERY
UCSF HELEN DILLAR FAMILY COMPREHENSIVE CANCER CENTER
SAN FRANCISCO, CA 94143-1351

PHONE: 415-514-0592
EMAIL: ZHIDONG.XU@UCSFMEDCTR.ORG

April 27, 2012

Dear Dr. Xi,

I am writing to evaluate the iPorator-96 apparatus used in my laboratory at UCSF. We have used the iPorator apparatus (including the iPorator-96 HT and iPorator-24 LT) in our studies since November 2011. We have used the iPorator systems for transfection of small interfering RNAs (siRNAs) into cancer cell lines as well as primary adherent cells in their more physio- and pathological relevant states.

Our data showed that the iPoraotor systems are highly suitable for in situ transfection of siRNAs into both cancer cell lines and primary cancer cells as compared to conventional transfection reagents, such as lipofection-based approaches. We found that virtually no cell damages were caused by iPorator after transfection as shown from results assessed by luciferase activity with over 90% cell survival rates as compared to negative control groups. For in situ siRNA transfection, we found that very low siRNA concentration ranging from one nanomole to picomoles could be used for effective knock down as compared with lipofection-based methods which usually require higher siRNA concentration (50-100 nanomoles) for tranfection. We have also used the iPorator-96 systems for high throughput screening of panels of multiple siRNAs per target gene (over 50 siRNAs) for discovery of lead siRNAs by setting-up of different concentrations to identify IC50 values for each siRNA within the panel.

In addition, we have found that it is advantageous to use the iPorator systems in our study in which we can seed more cells for transfection than the conventional methods. We can seed up to 70,000 cells per well in 24-well plates and up to 20,000 cells per well in 96-well plates for transfection. We can then collect enough cells after transfection for subsequent experiments, such as RNA, DNA and protein isolation, while using conventional methods, we would not obtain enough cells for further studies after transfection. We are currently testing more siRNAs using the iPorator systems. Please let me know if you need more information about our experiments using the iPorator systems.

Sincerely,

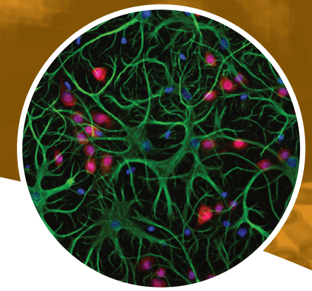
Zhidong Xu

Zhidong Xu, Ph.D.
Associate Adjunct Professor
UCSF Helen Diller Family Comprehensive Cancer Center
Department of Surgery
Thoracic Oncology Laboratory
2340 Sutter St., N261
San Francisco, CA 94115-1351

高效率的原位(*in situ*)

完全分化神经原代细胞转染

*i*Poration™



长期以来转染完全分化的神经原代细胞一直是生物学家面临的巨大挑战. 源脉生物的**iPoration™**细胞转染技术突破了传统技术的局限,实现了对 完全分化的神经原代细胞进行高效的**siRNA**和**DNA**的原位(***in situ***)转染. 由此可见, **iPoration**转染技术的成功为在神经科学研究领域中进行各种生物化学实验开创了一条新路.

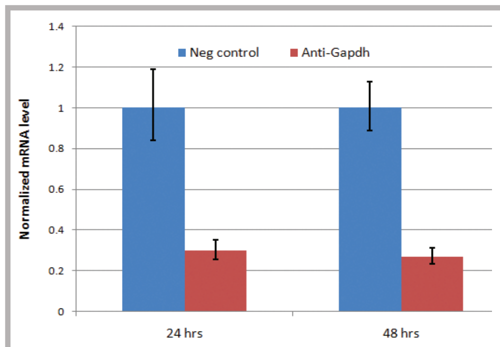
iPoration 技术的优势

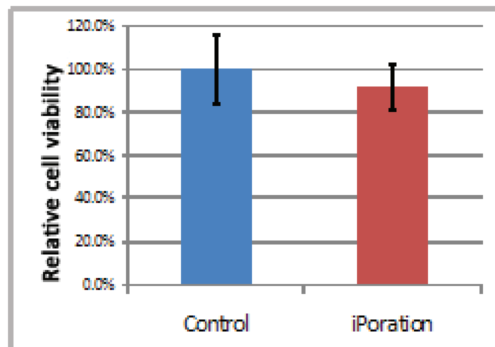
- 完全分化神经细胞的原位(*in situ*)转染,无需重新悬浮细胞
- 效率高于传统的化学试剂转染方法
- 极低的细胞死亡率
- 比传统的电穿孔方法需要更少的siRNA用量
- 无需任何特殊的化学试剂

iPoration 技术的效益

- 使应用多种生物化学方法进行下游分析成为可能
- 比传统的电穿孔方法需要更少的细胞用量
- 对细胞的生化干扰极低
- 高通量, 高效率
- 低成本

对已分化的原代海马神经元的siRNA的有效转染结果



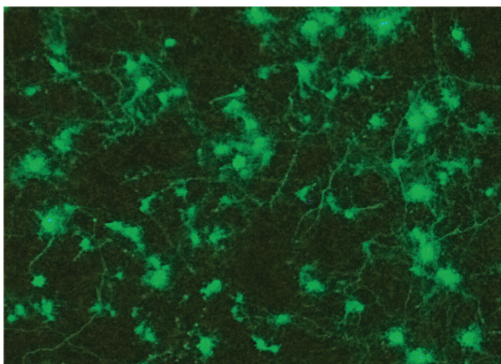


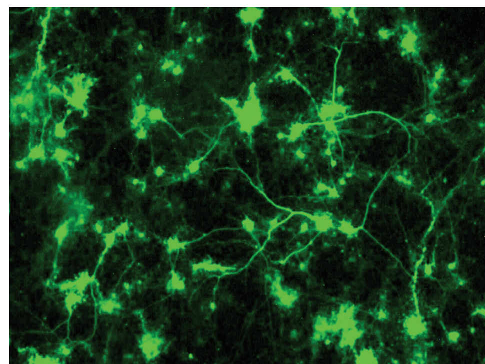
iPoration 细胞转染技术对14天大鼠海马神经元原代细胞的siRNA的原位转染结果的PCR定量和显微图片的分析

左图: 使用100nMsiRNA在转染后24和48小时的在mRNA水平上的基因沉默效果的定量分析

右图: 对转染24小时后的细胞损伤率的定量分析, 结果显示iPoration转染技术对细胞的损伤极低

对已分化的大鼠原代神经元的DNA的高效转染结果





iPoration 细胞转染技术对7天大鼠原代神经元的DNA的转染结果

左图: 大鼠原代海马神经元

右图: 大鼠原代皮层神经元

江苏源脉生物科技有限公司

PRIMAX Biosciences Inc.

iPoration专利技术和**iPorator**产品系列受美国专利(US patent 7,687,267)及多项美国和国际专利申请的保护

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对在生理相关环境状态中的细胞进行

有效的RNAi转染

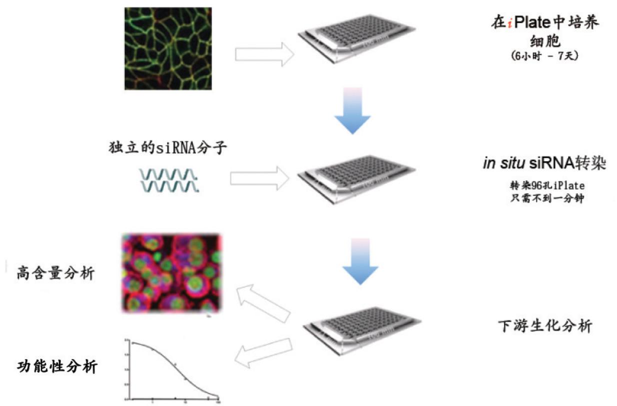
*i*Poration™

使用特定siRNA诱导的基因沉默在基因表达过程的研究和新型治疗标靶的确定中起着非常重要的作用. 由于其即可以反映出在分子层面上的生理相关性, 又能很好的表现出细胞在 *in vitro* 条件下的沉默效应和 *in vivo* 条件下的沉默效应之间的对应关系, 原代细胞和完全分化细胞被广泛地应用在siRNA的研究领域之中. 不幸的是, 目前所有已知的siRNA的转染技术 (如脂基化学转染和传统电穿孔转染技术) 对原代细胞和完全分化细胞的转染或是效率较低或是造成细胞大量死亡. 除此以外, 传统电穿孔转染技术不仅对细胞和siRNA的需要量大, 还需要耗费大量的人工和时间针对每一种特定细胞耗费做转染指令的优化, 或是使用与之配套的特殊消耗套材.

***i*Poration™**是用裸siRNA直接对原代细胞和完全分化细胞单层进行转染的最佳方法.

*i*Poration™ siRNA直接转染技术的特点:

- 简单易用
- 高效率
- 超低损伤率
- 高通量
- 超低干扰
- 广泛的适应性
- 自动指令优化功能
- 低siRNA和细胞的用量



*i*Poration™ 细胞转染1, 2, 3

用户体验:

1. 简洁明快的操作步骤

----转染一个96孔细胞培养板只需三步简单指令和不到一分钟的时间

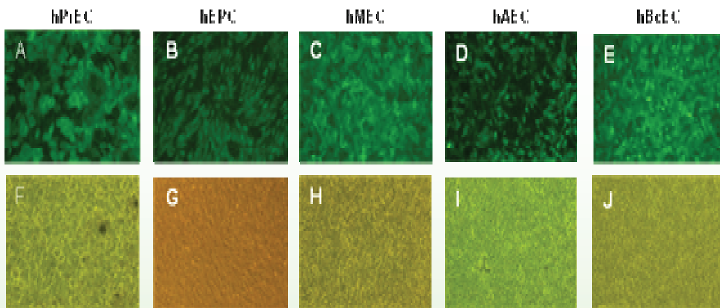
2. 优秀的基因沉默效果, 极低的siRNA用量

----可大限度的降低其他有害干扰.

3. 极低的细胞死亡率

----基本上无细胞膜损伤和外加试剂产生的毒性

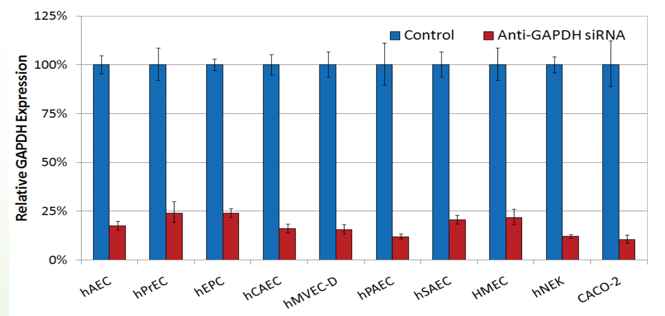
荧光标记的siRNA可以被高效率的导入多种单层人体原代细胞



使用*i*Poration™技术转染后的人体原代细胞的显微照片

PrEC: 人体前列腺上皮细胞
HEPC: 人体内皮前体细胞
HMEC: 人体乳腺上皮细胞
HAEC: 人主动脉内皮细胞
HBcEC: 人支气管/气管上皮细胞

全铺满和完全分化细胞单层的基因沉默的实验结果



图为多种人体原代细胞和Caco-2细胞线单层的基因沉默的实验结果, 数据表明*i*Poration™技术可有效用于各类贴壁源代细胞和细胞线的转染

EPC: 人体内皮前体细胞
HMVEC-d: 人体微血管上皮细胞
HCAEC: 人体冠状动脉内皮细胞
PrEC: 人体前列腺上皮细胞
HPAEC: 人体肺动脉内皮细胞
HMEC: 人体乳腺上皮细胞
HAAEC: 人体主动脉血管内皮细胞
HSAEC: 人体呼吸道上皮细胞
HUVEC: 人体脐静脉内皮细胞
Caco-2: 人体大肠癌细胞株

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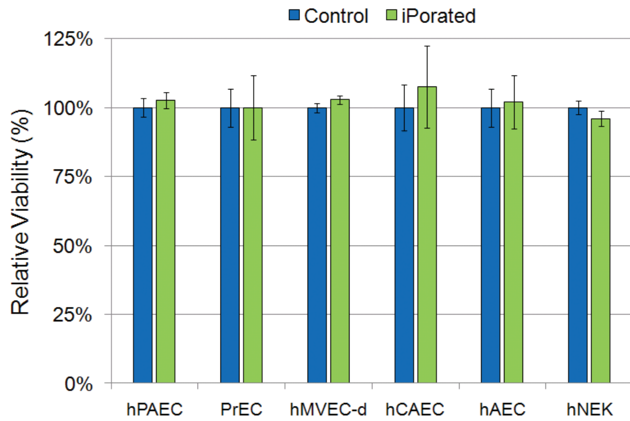
iPoration™技术对RNAi的有效转染

化学转染试剂,如脂质体和正电离子聚合物,多适用于在中低密度覆盖率下的处于分裂状态下的细胞. iPoration™细胞转染技术不仅适用于处于分裂状态下的细胞,更是对在生理相关状态下转染不分裂或完全分化贴壁细胞这一传统技术局限的重大突破.

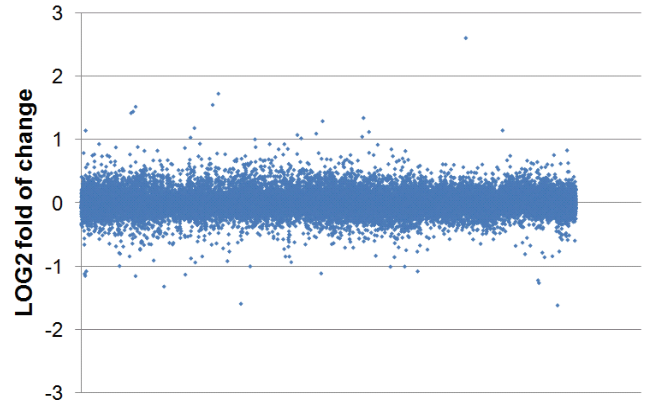
超低的细胞损伤



图为对转染前后的人体源代细胞存活率的分析报告,数据表明**iPoration**™转染技术对细胞的损伤极低

HPAEC: 人体肺动脉内皮细胞
HAEC: 人主动脉内皮细胞
PrEC: 人体前列腺上皮细胞
HMVEC-d: 人体微血管上皮细胞
HCAEC: 人体冠状动脉内皮细胞
HNEK: 人体正常表皮角质形成细胞

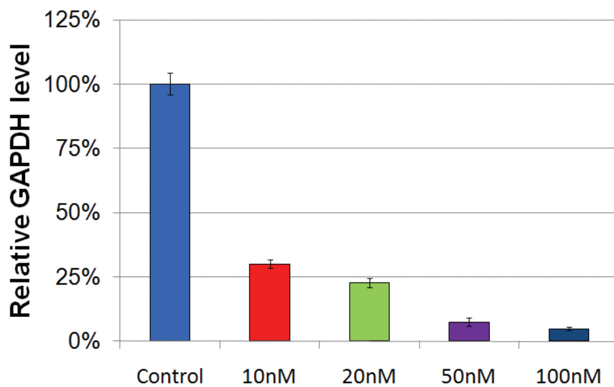
超低的基因表达干扰



图为对使用**iPoration**™技术转染后的人体心脏微血管内皮细胞基因表达的分析报告,数据表明本技术对基因表达的干扰极低

分析的基因总数: 30968
上调基因数量: 51
下调基因数量: 39
副作用百分比: 0.291%

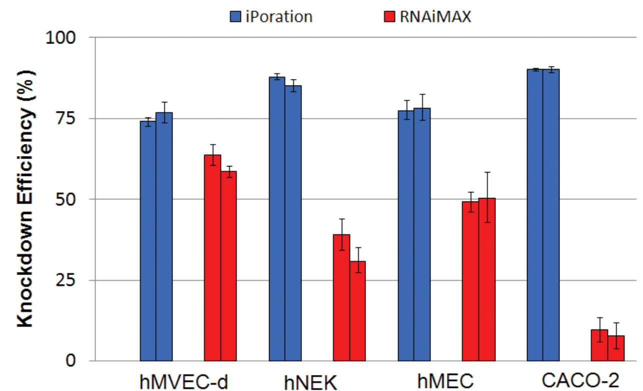
可调控的基因沉默



图为使用实时RT-PCR技术对完全分化的Caco-2细胞GAPDH基因沉默的siRNA用量的定量分析结果,数据表明**iPoration**™的siRNA需要量远低于传统电穿孔技术

CellTiter-Glo为Promega 公司的注册商标
RNAiMAX 是Invitrogen/Life Technologies的注册商标

iPoration技术与化学转染方法的 对比



图为使用实时RT-PCR技术对分别使用化学转染和**iPoration**™技术转染的完全分化细胞单层siRNA沉默效果的对比分析,结果表明**iPoration**™的siRNA的转染效率全面超过传统化学转染产品

HMVEC-d: 人体微血管上皮细胞
HMNEK: 人心脏微血管内皮细胞
HMEC: 人体乳腺上皮细胞
Caco-2: 人体大肠癌细胞线

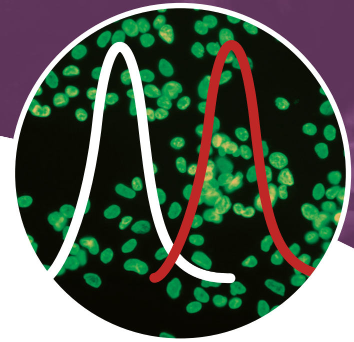
江苏源脉生物科技有限公司

iPoration专利技术和iPorator产品系列受美国专利(US patent 7,687,267)及多项美国和国际专利申请的保护

江苏省泰州市中国医药城A3栋7层

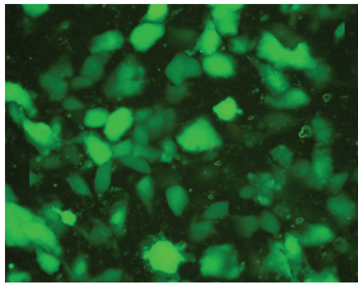
高效DNA和Peptide转染

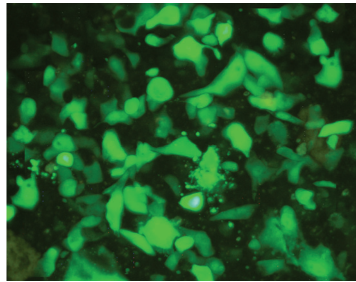
iPoration™

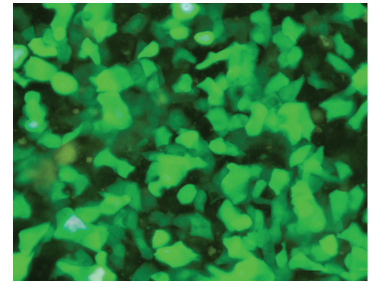


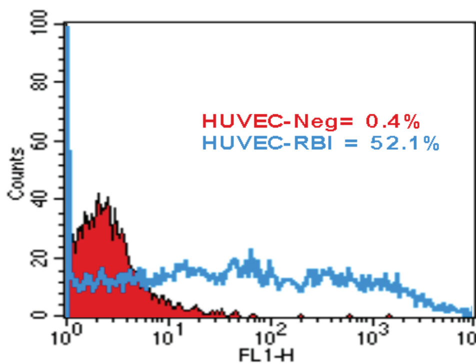
转染质粒DNA和Peptide进入原代细胞和完全分化的细胞一直是分子生物学研究中的巨大挑战. iPoration™细胞转染专利技术成功地实现了对人体原代细胞和完全分化细胞进行高效率的质粒DNA和Peptide转染.

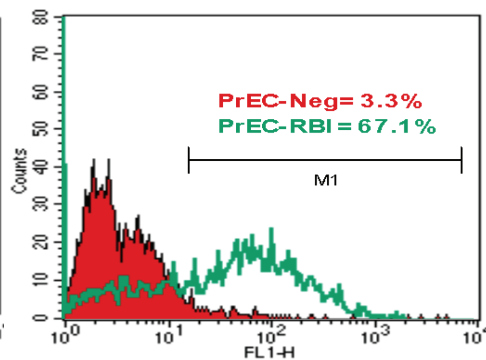
质粒DNA被成功导入多种原代细胞人体原代细胞

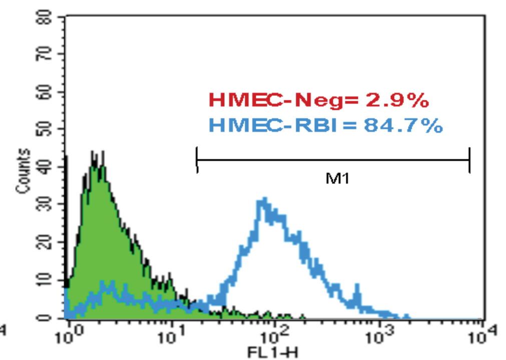








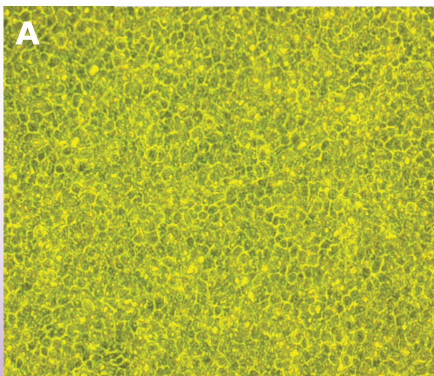


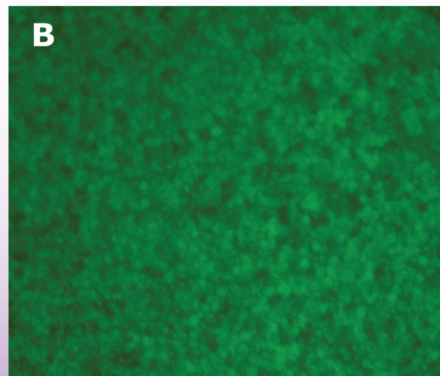


使用iPoration™ 技术对多种人体原代细胞进行质粒GFP转染效果的显微图象和FACS分析

HUVEC: 人类脐静脉内皮细胞　PrEC: 人类前列腺上皮细胞　HMEC: 人类乳腺上皮细胞

对难转染的MDCK细胞单层的高效Peptide转染





使用iPoration™技术对MDCK单层进行荧光标记Peptide的转染结果的显微图片分析

A. 普通光源下的显微图片

B: 同一细胞样本在荧光光源下的显微图片

江苏源脉生物科技有限公司

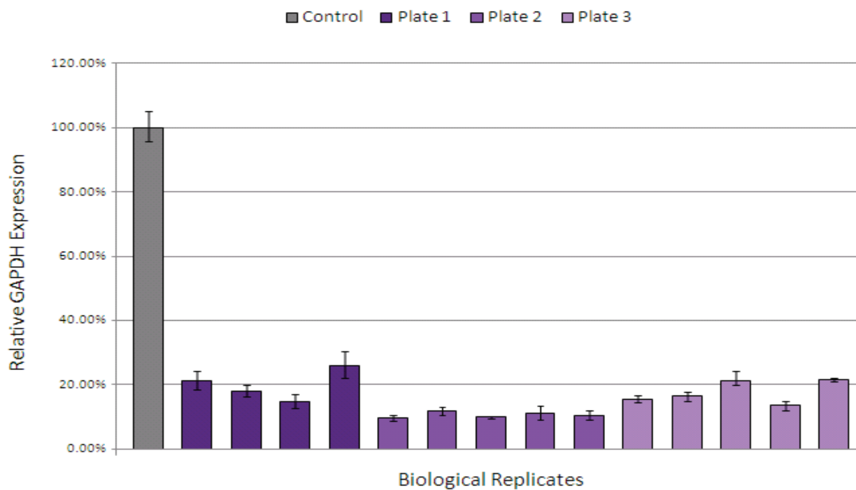
PRIMAX Biosciences Inc.

iPoration专利技术和iPorator产品系列受美国专利(US patent 7,687,267)及多项美国和国际专利申请的保护

iPoration™技术高度一致的转染效果

在应用高通量siRNA沉默和蛋白表达的细胞检测实验中，稳定一致的转染效果是一项非常关键的指标. 源脉生物独特的iPoration™转染技术在这一方面取得了极为出色的成绩.

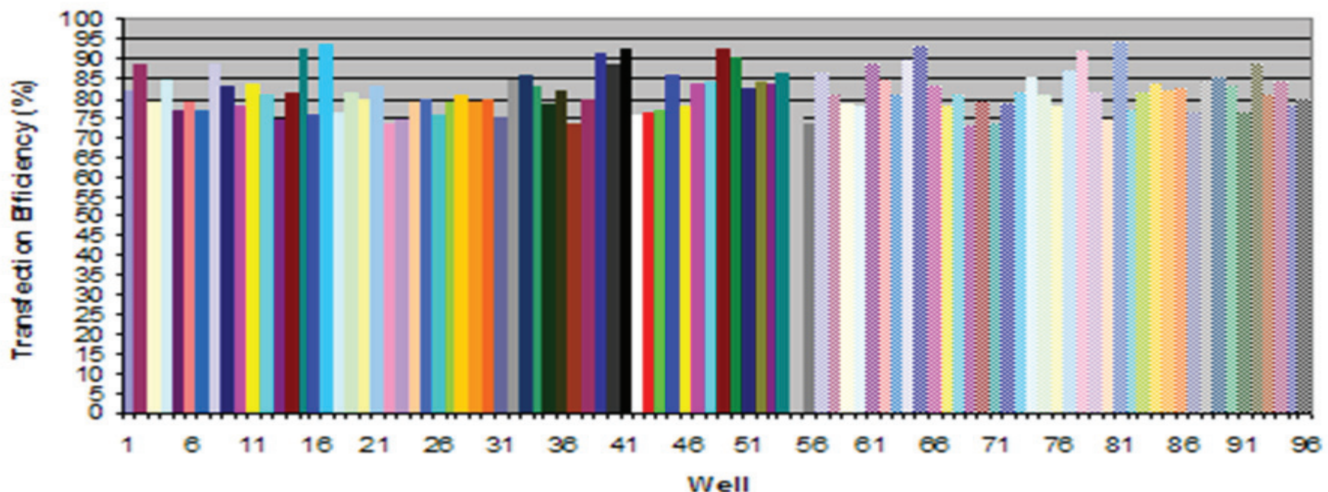
Caco-2 贴壁细胞单层siRNA转染结果的一致性



完全分化的Caco-2细胞的GAPDH基因沉默实验. 图为同一试验的多个样品间和不同实验之间的结果的对比.

ANOVA Analysis:
$F_{2,11}$= 2.095, critical $F_{2,11}$= 3.99, $p<0.05$

MDCK 单层贴壁细胞的DNA转染结果的一直性



使用iPoration™技术进行质粒GFP转染后MDCK细胞单层中GFP表达效果的荧光分析

平均转染效率：83.4% (n=288);
Std Dev (well-to-well): 5.2% (n=96) Std Dev (plate-to-plate): 3.6% (n=3)

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