



# 創造您的人工幹細胞 ~ Induced Pluripotent Stem Cells

幹細胞是人體內可以分化為各種器官和組織的細胞，過去一直只能從胚胎中獲得，對幹細胞研究來說一直是個瓶頸，而後科學家找出臍帶血、骨髓幹細胞等等，已經擴展幹細胞研究的觸角至成體。而於2007年11月，幹細胞研究有了重大的突破，科學家可以從成體的纖維母細胞轉殖四幹細胞相關基因而得到誘導幹細胞，又名iPS細胞。目前已有實驗能將老鼠纖維母細胞誘導成 iPS 細胞，然後再使之分化成心肌細胞、造血細胞、血管平滑肌細胞，從人類纖維母細胞製成的 iPS 細胞也成功的培育出血小板等。此研究將為以往難以取得來源的幹細胞研究，帶來新旋風。

## Start iPS cells research from SBI

### Get iPS cells

- a 購買現成 human/ mouse iPS cell Line + Feeder cell
- b 建構自己的 iPS cells (iPSC source cell + iPSC reprogramming factors + Feeder cells )

### Verify pluripotency

利用 RFP 或 GFP 觀察 Pluripotent promoter 的活化: Pluripotent reporters

### Track differentiation

利用 RFP 或 GFP 觀察 Tissue specific promoter 的活化: Differentiation reporters

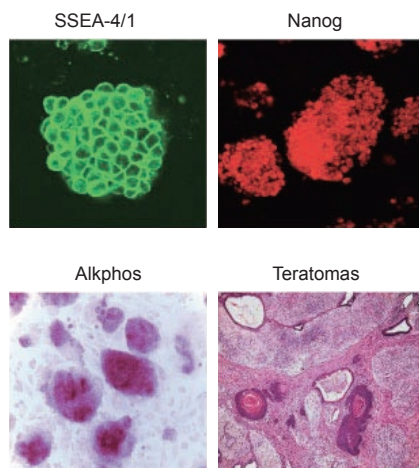
## Get iPS cells **a**

購買現成的 iPS Cells ~ 贏在起跑點 (#SC101A-1, #SC201A-1)

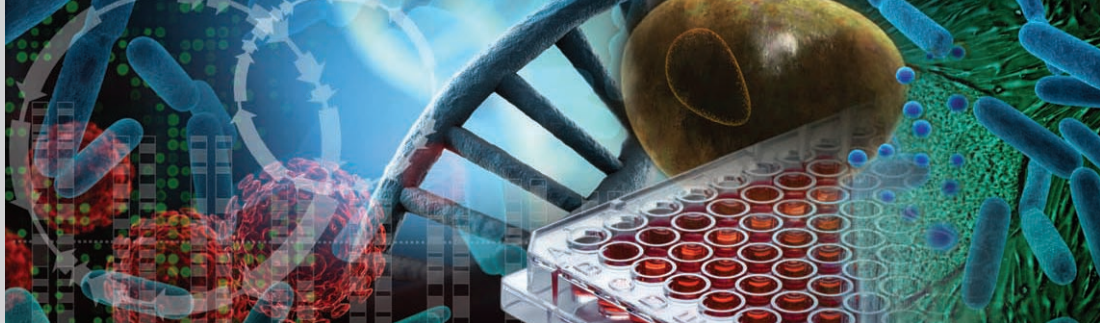
- Oct4, Sox2, c-Myc 和 KLF4 以 Lentivirus 方式成功轉殖入 Human/ Mouse fibroblast
- 嚴謹 QC 確認 pluripotency (見下圖)

廣泛應用：

- Study the induced pluripotency process
- Measure mRNA and microRNA signatures
- Identify novel epigenetic markers to study pattern formation
- Develop directed differentiation protocols

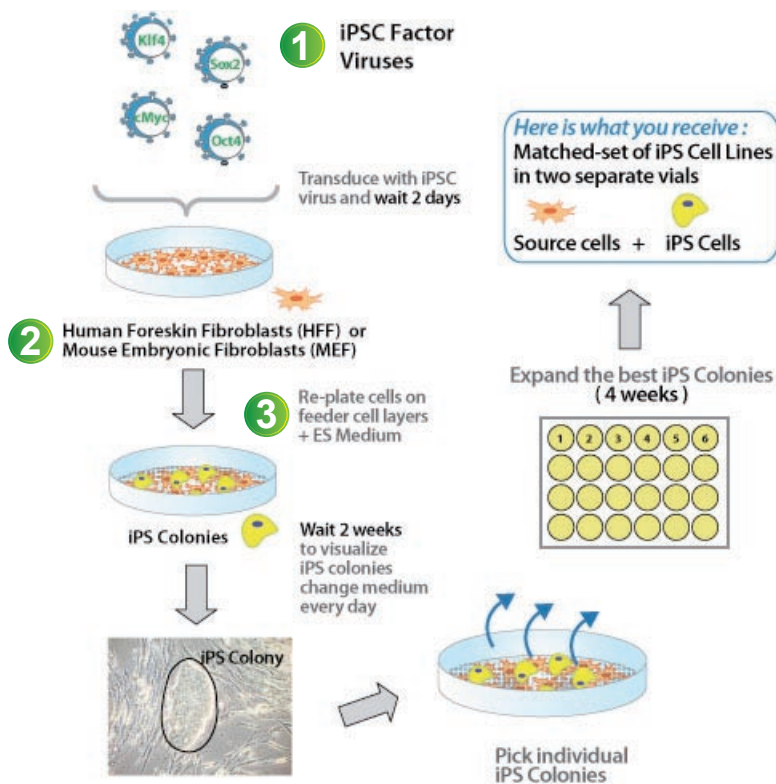


所有 iPSC 均通過 Stem Cell 特徵檢驗  
Stem cell markers for SSEA4/1 and Nanog were determined by immunocytochemistry using primary antibodies for SSEA1 (Millipore), SSEA4 (Abcam) and Nanog (Abcam) followed by fluorescent-labeled secondary Alexa594 antibodies (Invitrogen). Detection of Alkaline Phosphatase activity was performed using the AP Detection kit (Millipore). Teratoma assays were performed by subcutaneous injection of  $1 \times 10^6$  iPS cells into the dorsal flank of SCID mice. Teratomas were visualized after 4 weeks with hematoxylin and eosin staining.



## Get iPSC cells **b**

## 建構自己的 iPSC Cells ~Step by step



**1** 挑選 iPSC  
 Reprogramming factors  
 Follow Dr. Yamanaka  
 選用：  
 c-Myc + Klf4 + Sox2 + Oct4  
 Follow Dr. Thomson  
 選用：  
 Lin28 + Nanog + Sox2 + Oct4

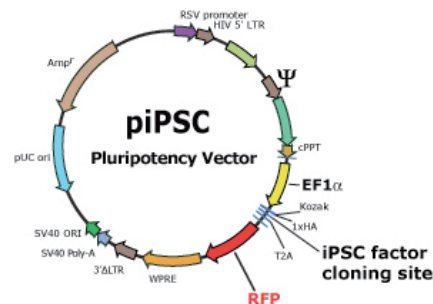
**2** Source cells for iPSC  
 (#PC501A-HFF)

**3** Feeder cells for iPSC  
 (#PC502B-HFF)

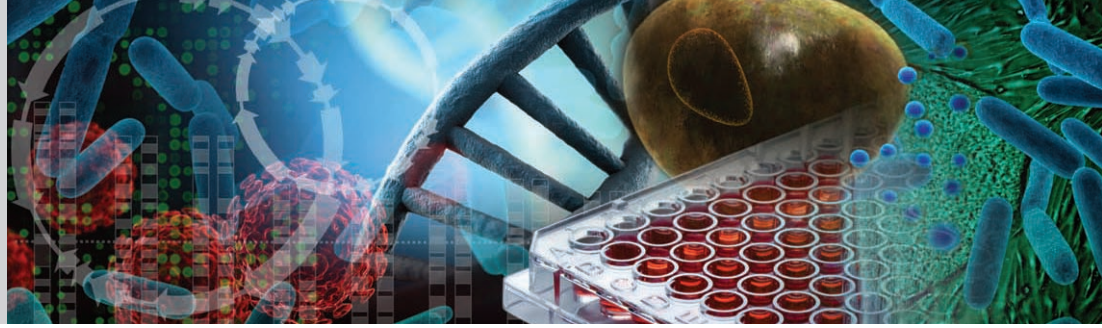
## 1 iPSC reprogramming factors

### SBI iPSC Factor construct/ virus

- 六種 Human iPSC factors : c-Myc, Sox2, Oct4, Klf4, Lin28, Nanog
- Lentiviral-based system
- 專治 hard-to-transfect cells
- 可用 RFP 檢視 transduction efficiency
- 穩定表現 iPSC Factors
- N-terminal 有 HA-tag 方便辨認



Factor	Vector	Size	Plasmid Cat #	Size	Prepacked Virus Cat#
Nonog	pPS-human Nanog-T2A-RFP Construct	10 ug	SR10060PA-1	>2X10 <sup>6</sup> IFU	SR10060VA-1
Oct-4	pPS-human Oct4-T2A-RFP Construct	10 ug	SR10061PA-1	>2X10 <sup>6</sup> IFU	SR10061VA-1
Sox2	pPS-human Sox2-T2A-RFP Construct	10 ug	SR10062PA-1	>2X10 <sup>6</sup> IFU	SR10062VA-1
cMyc	pPS-human cMyc-T2A-RFP Construct	10 ug	SR10063PA-1	>2X10 <sup>6</sup> IFU	SR10063VA-1
Lin28	pPS-human Lin28-T2A-RFP Construct	10 ug	SR10064PA-1	>2X10 <sup>6</sup> IFU	SR10064VA-1
KLF4	pPS-human KLF4-T2A-RFP Construct	10 ug	SR10065PA-1	>2X10 <sup>6</sup> IFU	SR10065VA-1
iPSC Kit	Nanog, Oct4, Sox2, cMyc, Lin28, KLF4	10 ug/each	SR10066PA-1	>2X10 <sup>6</sup> IFU	SR10066VA-1
iPSC Y Set	Oct4, Sox2, cMyc, KLF4	10 ug/each	SR10076PA-1	>2X10 <sup>6</sup> IFU	SR10076VA-1
iPSC T Set	Oct4, Sox2, Lin28, Nanog	10 ug/each	SR10077PA-1	>2X10 <sup>6</sup> IFU	SR10077VA-1



## 2 & 3 Source cells and feeder cells for iPSC



由新生兒包皮分離出的 **fibroblast** 是您建構 **iPS cell** 及 **Feeder cell** 的最佳來源

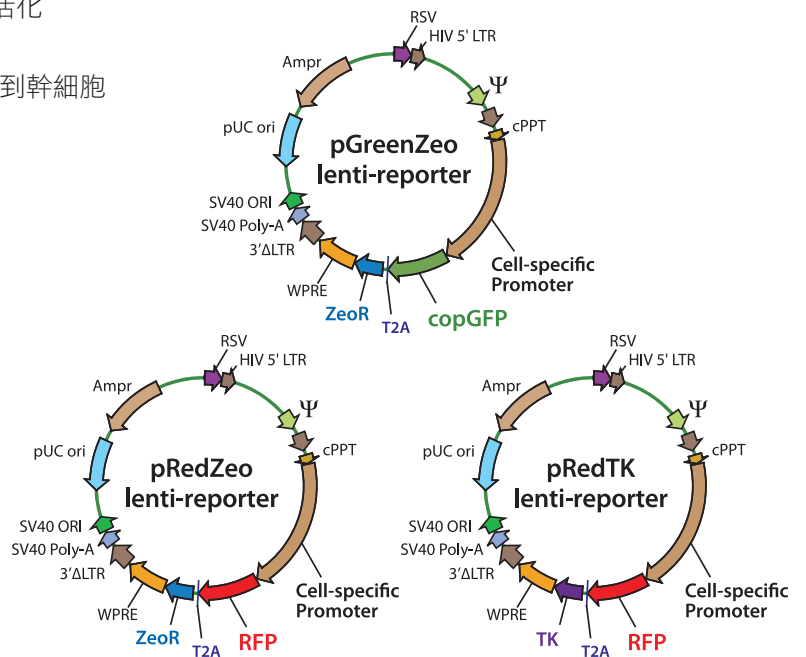
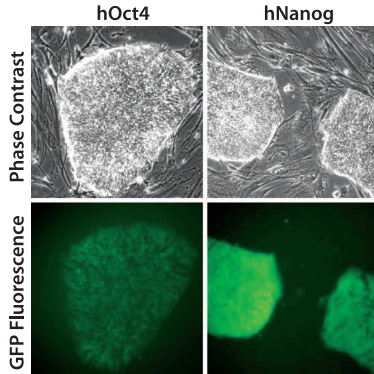
- Source cells (Cat#PC501A-HFF) 為單一個來源的 Human foreskin fibroblasts
- Feeder cells (Cat#PC502B-HFF) 為集合多個來源的 Human foreskin fibroblasts

## Verify pluripotency

利用 RFP 或 GFP 觀察 Nanog 或 Oct-4 promoter 的活化

- 利用 RFP 或 GFP 觀察 Nanog 或 Oct-4 promoter 的活化
- Human 或 mouse promoter 任您選
- 效率最高：以最高效率的 Lentivirus 幫你送 Reporter 到幹細胞
- 兩種 Reporter：紅色螢光及綠色螢光
- 兩種抗藥基因標誌：Zeocin 及 Thymidine Kinase
- Live cell imaging，無需耗損細胞染色

### Oct4 and Nanog—Human or Mouse



Promoter	Species	Reporter	Drug selection	Vector	Size	Plasmid Cat #	Prepacked virus Cat#
Oct-4	Human	GFP	Zeocin	pGreenZeo	10 ug	SR10033PA-1	SR10033VA-1
Oct-4	Human	RFP	Zeocin	pRedZeo	10 ug	SR10043PA-1	SR10043VA-1
Oct-4	Human	RFP	ganciclovir	pRedTK	10 ug	SR10053PA-1	SR10053VA-1
Oct-4	Mouse	GFP	Zeocin	pGreenZeo	10 ug	SR10029PA-1	SR10029VA-1
Oct-4	Mouse	RFP	Zeocin	pRedZeo	10 ug	SR10045PA-1	SR10045VA-1
Oct-4	Mouse	RFP	ganciclovir	pRedTK	10 ug	SR10054PA-1	SR10054VA-1
Nanog	Human	GFP	Zeocin	pGreenZeo	10 ug	SR10030PA-1	SR10030VA-1
Nanog	Human	RFP	ganciclovir	pRedTK	10 ug	SR10055PA-1	SR10055VA-1
Nanog	Mouse	GFP	Zeocin	pGreenZeo	10 ug	SR10031PA-1	SR10031VA-1
Nanog	Mouse	RFP	ganciclovir	pRedTK	10 ug	SR10056PA-1	SR10056VA-1
Nonog	Human	RFP	Zeocin	pRedZeo	10 ug	SR10042PA-1	SR10042VA-1
Nonog	Mouse	RFP	Zeocin	pRedZeo	10 ug	SR10044PA-1	SR10044VA-1

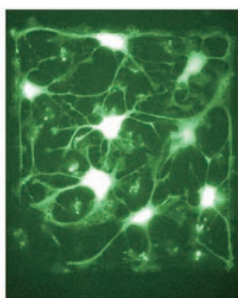


## Track differentiation

## 利用 RFP 或 GFP 觀察 tissue-specific promoter 的活化

- 最多種類：市售最多幹細胞專用的 Reporter
- 效率最高：以最高效率的 Lentivirus 幫你送 Reporter 到幹細胞
- 兩種 Reporter：紅色螢光及綠色螢光
- 兩種抗藥基因標誌：Zeocin 及 Thymidine Kinase

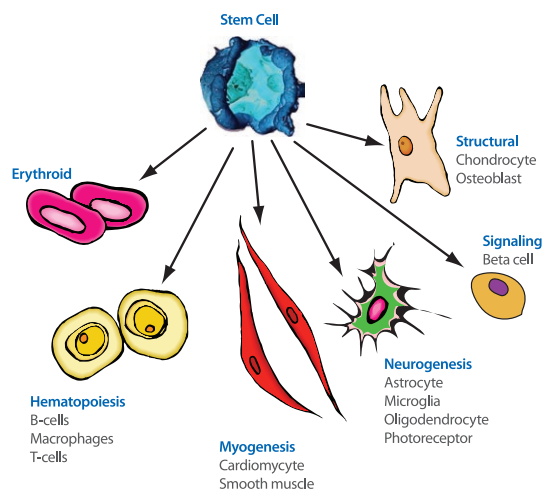
螢光顯微鏡觀察分化後的 Astrocyte



mGFAP



all cells



## SBI 提供市面最多 Differentiation reporter

### Neural

Target Cell Type	Species	Promoter Enhancer
Macrophage, microglia	Mouse	Cd68
Astrocyte	Human	GFAP
Astrocyte	Mouse	Gfap
Microglia	Human	CD11b
Microglia	Mouse	EMR1
Microglia	Mouse	Iba-1
Muller glia	Mouse	Cd44
Neuron	Human	BM88
Neuron	Mouse	Camk2 $\alpha$
Neuron	Mouse	GAD67
Neuron	Rat	NSE
Neuron	Mouse	T $\alpha$ 1 $\alpha$ -tubulin
Oligodendrocyte	Mouse	MBP
Photoreceptor	Human	Opsin
Neural Stem Cell	Rat	Nestin
Neural Stem Cell	Human	Nestin
Neuron	Human	Doublecortin
Neuron	Human	MAP2
Neuron	Human	FABP7

### Endocrine

Target Cell Type	Species	Promoter Enhancer
Beta cell	Human	Insulin
Islet	Human	PDX1
Islet	Mouse	Pdx 1

### Hematopoietic

Target Cell Type	Species	Promoter Enhancer
B-cell	Human	B29
B-cell	Mouse	B29
CD8 T-cell	Mouse	CD8
Erythroid	Human	HLA-DR $\alpha$
Macrophage, microglia	Mouse	Cd68
PanT-cell	Human	CD2
Lymphocyte	Human	LCK

### Myogenic

Target Cell Type	Species	Promoter Enhancer
Cardiomyocyte	Mouse	Actc
Cardiomyocyte	Human	MLC-2v
Cardiomyocyte	Human	TNNT2
Cardiomyocyte	Mouse	Tnnt2
Smooth muscle myocyte	Mouse	SM22 $\alpha$
Cardiomyocyte	Human	ACTC
Skeletal myocyte	Mouse	Myogenin

### Structural

Target Cell Type	Species	Promoter Enhancer
Chondrocyte	Mouse	Col2a1
Osteoblast	Human	SPP1
Osteoblast	Human	Osteocalcin
Adipocyte	Mouse	ALBP
Epithelium	Human	Keratin 14