

行政院國家科學委員會專題研究計畫 成果報告

雌激素及其受體基因心肌保護機轉探討:類胰島素生長因子, Gq 蛋白及細胞激素之角色 (3/3)

計畫類別：個別型計畫

計畫編號：NSC93-2320-B-040-007-

執行期間：93年08月01日至94年07月31日

執行單位：中山醫學大學生化暨生物科技研究所

計畫主持人：黃志揚

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中 華 民 國 94 年 10 月 30 日

行政院國家科學委員會補助專題研究計畫成果報告

(計畫名稱)

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子, Gq 蛋白及細胞激素之角色(3/3)

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計畫主持人：黃志揚

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計畫參與人員：劉忠榮, 章家倩

成果報告類型(依經費核定清單規定繳交): 精簡報告 完整報告

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國際合作研究計畫國外研究報告書一份

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執行單位：中山醫學大學生化暨生物科技研究所

中 華 民 國 94 年 10 月 30 日

(一)中文摘要。

關鍵詞：17- β -雌性素，雌性素接受體 Tet(tetracycline)-on 基因，心肌細胞
肥大·凋亡·猝死，DNA 基因晶片。

雌性素(E2)具保護心血管功能，但對心肌細胞的保護機轉仍不清楚。近來，由於心肌細胞內雌性素接受體(ER α 及 ER β)的發現,及 COS 細胞中 ER 可直接與胞膜上類胰島素接受體(IGF-IR)結合，並活化 MEK-ERK 訊息途徑，令人不禁聯想 E2-ER 與 IGF-I-IGF-IR 在調控心肌細胞功能的關聯性。且究竟 E2-ER 是透過傳統基因性(核內，genomic，slow) 作用或非基因性(膜上，non-genomic，fast)作用來調控心肌細胞，值得深究。由於病理性心肌肥大為心肌猝死主因，但病理性心肌肥大及心肌猝死的分子機轉所知不多。目前僅知，IGF-I 經 IGF-IR 活化 IP3K-AKT/PKB 為心肌細胞存活之主要途徑，同時調控心肌細胞大小，可能為生理性肥大的主要機轉。IGF-I 並可經 IRS-1 活化 Ras-Raf-MEK-ERK 調控心臟內非心肌細胞增生。因此，E2-ER 與 IGF-I-IGF-IR 的交互作用在心肌保護的角色，急待發掘。已知 G proteins coupled receptor (GPCR)之 ligands,包括血管增壓素(Ang-II)，為高血壓主因之一，因與鈣離子活化、促進心肌肥大之 Calcineurin-NFAT 途徑及影響細胞凋亡之 PKC 及 JNK 有關，所以與病理性心肌肥大及猝死關係密切。另發現血球中 TNF α 之基因表現及血液中 TNF α 蛋白含量顯著上升而 TGF β 蛋白含量減少與病人心肌衰竭極相關，在經抑制 TNF α 治療後，心肌衰竭即改善。而 IL6(interleukine-6)並與不穩定型冠狀動脈疾病患者死亡率有關，且可經活化 MEK5-ERK5 誘發擴張性心肌症，活化 P38-STAT1 引發心肌細胞凋亡。而雌性素因具有快速降低細胞鈣離子內流的非基因性功能、及抑制停經後婦女發炎細胞激素的產生，ER 更可經抑制發炎轉譯因子 NF κ B 的基因表現，而可能有心肌血管保護功能。因此雌性素與病理性心肌病的關係確實值得更進一步探討。

我們結果並發現:①腹動脈結紮大白鼠、E2-ER 可經活化 IGF-I receptor 及 IGF-I gene expression 而維持心肌細胞 survival,亦可壓制 calcineurin-NFAT3，而延緩病理性肥大，纖維化生成及心肌凋亡(附 E2-paper)。②而 IGF-IR 阻斷的心肌細胞，除引發胚胎基因 IGF-II 大量表現(圖 2)，IGF-II 並與 Ang-II 發生加成作用，導致 G 蛋白及鈣離子-calcineurin-NFAT3 過度活化，導致心肌細胞加成肥大(圖 1)、DNA 斷裂(圖 3)、細胞凋亡(圖 4) 及核破裂濃染(圖 5)。但 E2 是否直接具阻斷心肌細胞凋亡作用，透過基因性或非基因性達成，及其影響的基因種類及透過之機轉，均急待解答。

計劃將野生型(wt)及 dominant-negative (dn) ER 以 transient-transfection(圖 6)及 Tet-on 誘導系統(圖 8)建立於初級心肌細胞, H9C2 心肌 cell-line 及類心肌細胞 C2H12 之中，再以三種模式來探討 E2-ER 與這些心肌病變因子相互調控關係:目前已建立 7600 基因之 cDNA 晶片，並已歸類分析 E2-ER 在保護心肌過程中所 upregulated 及 downregulated 的基因種類。未來將進一步探討各個基因在 E2-ER 心肌保護的角色，基因性及非基因性調控差異，及相關訊息傳遞途徑及其影響基因的釐清，相信將有助於揭發心肌疾病及猝死之分子機轉，對於未來心肌疾病治療、猝死防患及強心藥物開發，將是一大突破。

(二) 英文摘要。

Key words: 17- β -estradiol, ER-Tet(tetracycline)-on gene, cardiomyocyte apoptosis, cardiac failure, cDNA-chip.

It has been proposed that estrogen has the function to protect cardiovascular, but the mechanism is not fully understood. Recently, the estrogen receptors (α & β) were observed in the cardiomyocytes, and the interaction of estrogen receptors (ERs) and membrane insulin-like growth factor receptor (IGF-IR) was also reported in CHO cells, which result in the potentiation MEK-ERK signal pathway. Consequently, if the cardioprotective effects result from the cross talking of E2 and IGF-I pathways has been causing much attentions, and the roles of E2-ER genomic and non-genomic effects should be addressed. As we know, pathological cardiomyopathy always leads to the cardiac sudden death (CSD), but the molecular mechanism of hypertrophy and how exactly it leads to CSD is unclear. According to previous studies, IGF-I, a survival factor, activates PI3K-AKT/PKB pathway via IGF-IR. This is not only resulting in cell survival but also regulating the size of cardiomyocyte during physiological hypertrophy. Furthermore, IGF-I also activates Ras-Raf-MEK-ERK mediated through IRS-1 to promote the proliferation of non-cardiomyocyte in the heart. Therefore, to reveal the cross regulation of E2-ER genomic and non-genomic effects on IGF-I pathways is urgent and important for elucidating the mechanism of E2 cardio-protective effects. However, hypertension, a risk factor for cardiac diseases, is mediated in part by G-protein coupled receptors (GPCRs) and is the response to ligand (e.g. angiotensin-II et al) binding on the surface of vessel GPCRs. Angiotensin-II and GPCRs also get involved in cardiac hypertrophy mediated by calcium influx and calcineurin-NFAT pathway activation, which will lead cell to apoptosis by activated PKC and JNK via calcineurin as well. Therefore, the GPCRs could play a key role, for cardiac hypertrophy and CSD as well. Besides, TNF α gene overexpressed and protein elevated in congestive heart failure patients, accompanied with high IL-6 and TGF1 β reduction. IL-6 related to the mortality of unstable coronary artery diseases as well. IL-6 not only activate MEK5-ERK5 to induce dilated cardiac-myopathy, but also trigger P38-STAT1 activities to cause cardiomyocytes apoptosis. However, E2 not only has non-genomic effect can rapidly reduce the calcium influx in cardiomyocytes, but also inhibit inflammation proteins in postmenopausal women, and ER even cross talk and suppress proinflammatory transcription factor NF κ B which might indeed contribute the cardioprotective effects of estrogen.

Sprague-Dawley rats were ovariectomized (OVX) one month before complete coarctation of the abdominal aorta (COX) with or without E2 treatment (100 ng/kg) and sacrificed 4 or 8 days later. Based on our findings, the up-regulation of IGF-I signaling and suppression of calcineurin/NFAT-3 pathway may be plausible mechanisms for the cardiac protective effects of E2(enclosed E2-paper1). We even applied primary cardiomyocytes of rat heart and transforming neonatal rat heart cell-line, H9C2 and skeletal C2C12 myocyte-like cells in serum-free medium, and added Ang-II with or without antisense IGF-I and IGF-IR antibodies. Results show that AngII and IGF-I resistant induced both cardiomyocytes hypertrophy and apoptosis. Which included the size enlarged (fig1), DNA fragmented (fig3), nuclei concentrated (fig5), Bad and cytochrom-c proteins elevated as well (fig4). In addition, all the Ang-II effects were fortified by IGF-I resistant. However, cardiomyocytes hypertrophy induced by IGF-I resistant was reversed after IGF-II antibodies treated. At the mean time, the upregulation of IGF-II gene expression and protein production were observed under IGF-I resistant (fig2). In summary, our results indicated that cardiomyocytes hypertrophy and apoptosis caused by Ang-II was augmented by IGF-I resistant, which probably comes from IGF-II up-regulation, and calcium-calcineurin activation.

In order to identify the mechanisms of E2-ER protective effects of cardiomyocytes. We set up the primary culture of adult cardiomyocytes and H9C2 cell lines with the tetracycline-on system and transient cotransfect wild type (wt), dominant negative (dn) ER, in order to reveal the roles of E2 genomic effect and its regulatory signal transduction pathways related with cardiac hypertrophy and CSD. We also set up the 7600 genes- cDNA-biochips and applied the cDNA-biochips to identify the up- and down-regulated genes of tet-on-wt,dn ER or transient transfected ER cardiomyocytes. In the near future, we are going to investigate all the genes functions. We believe all these results must supply great benefits not only on understanding the cardiac protective mechanisms of E2 and ER but also in the improvement of treatment of hypertrophy, CSD, as well as new medicine development.

(三)方法及實驗流程

目的地: 將以 cDNA 晶片 (cDNA-chip) 探討 E2-ER 在保護心肌細胞過程中所 up-regulated 及 down-regulated 的基因種類。

流程: 研究過程將包含:一萬個基因(含已知及EST)genes

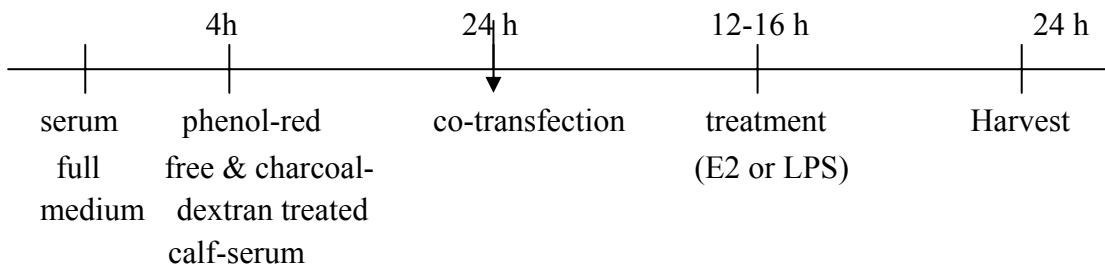
cloning→preparation of cDNA Gene Chips→extration mRNA of Cells & tissues→hybridization to Chips→up-, down-regulated genes analysis & sorting→RTPCR & Northern test→DNA sequencing。

處理:

(A) Tet-on-wtER, dnER 心肌細胞

(B) Transient-transfect (wt-ER-GFP or dn-ER-GFP)入心肌細胞

→各組分別 (treat with or without E2, genestein, raloxifene)



將 cells 分下列組別

① Control(empty vector) ② wt-ER+E2 ③ wt-ER+genestein ④

wt-ER+raloxifene

⑤ dn-ER+E2 ⑥ dn-ER+genestein ⑦ dn-ER+raloxifene

以上各組再分別以不同劑量(ER,2,4,6ug)(E2,10⁻⁶M, 10⁻⁶M, 10⁻⁶M) 來做比較

測量下列項目 :來確認其轉殖成功

1. Western-blotting 偵測 wt-,dn-ER 蛋白驗證其確實大量表現

1. Sorting 所有 up-regulated 及 down-regulated 的基因種類, 並以 RT-PCR 及 dot-blotting 或 northern-blotting 確認
3. 將來可確認各個基因功能及角色, 協助藥物之開發及心肌病機轉之探討

研究方法

H9C2 cardiac cell line 及 C2C12 類 cardiac cell line culture

H9C2, original clonal cell line derived from embryonic BDIX rat heart tissue. C2H2, a subclone derived from the mouse myoblast cell line, an excellent model to study myogen. Using culture medium; 95% DMEM with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose and 1. Growth in adherent; 5% CO₂; 37°C and split culture with trypsin-EDTA. Note: Do not allow the culture to reach confluency as this will deplete the myoblastic population in the culture. Freeze medium use with 90% culture medium + 10% DMSO.

Construction of Receptor Mutants and Expression Vectors (Which have been kindly given by Dr Benita S. Katzenellenbogen in UIUC, USA)

Constitutive plasmids:

(1) pCMV β This plasmid (Pharmacia LKB, Biotechnology Inc) was used as an internal control for transfection efficiencies during gene transfer experiments. It expresses the β -galactosidase gene (lac Z) constitutively from the SV40 early promoter.

(2) Wt-ER(pCMV-ER) & mutants(pCMV-S554fs) were made by Carol Wrenn et al (76). The 0.8-Kb fragment of hER, which includes most of the HBD was subcloned into M13 phage. The single-stranded M13-ER DNA was subjected to random chemical mutagenesis by treatment with formic acid. The formic acid concentration was selected to maximize the probability of mutating only one nucleotide per DNA molecule. The second strand was synthesized by reverse transcriptase generating double-stranded DNA carrying the mutation. These mutated regions were subcloned back into the wild-type ER cDNA, thereby replacing the homologous segment. The resulting mutants were expressed in a modified yeast- E. coli shuttle vector, YE_{ER}, and were confirmed by sequencing. The normal and mutant human ER cDNA were expressed from the multi-copy plasmid YE_{ER} in yeast, and subcloned into the eukaryotic expression vector pCTVNS.

(3) pVMV-wt-G α q and pCMV-dn-G α q, plasmids 由中山醫大學口研所楊肇基

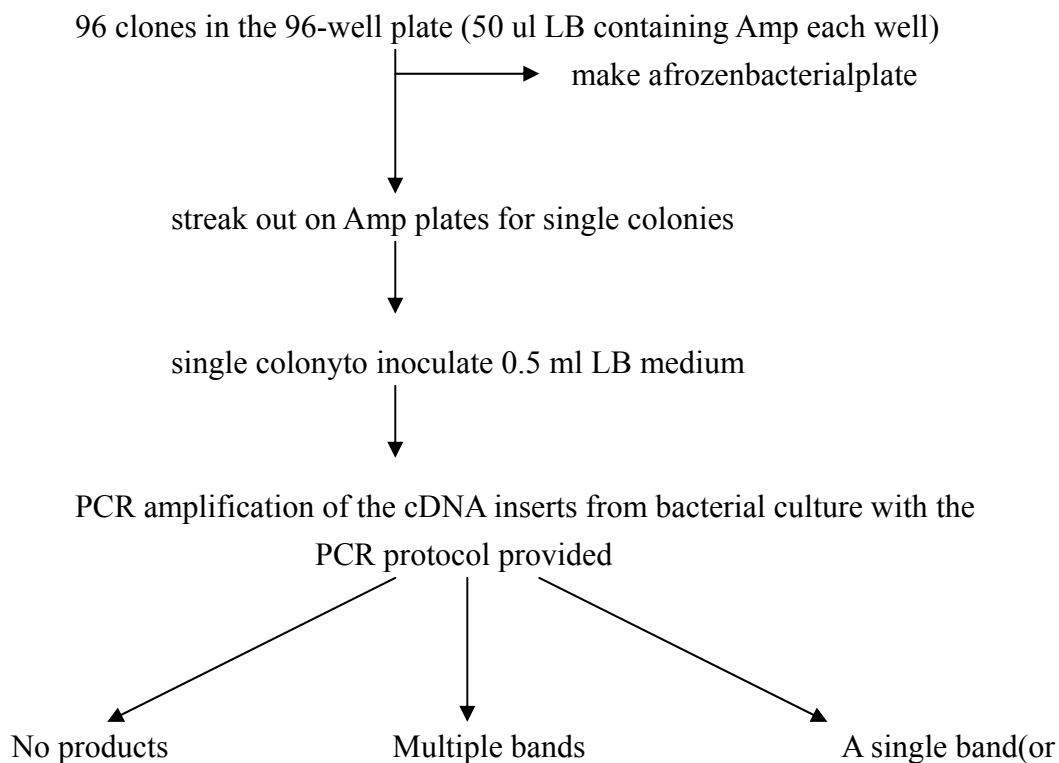
副教授提供

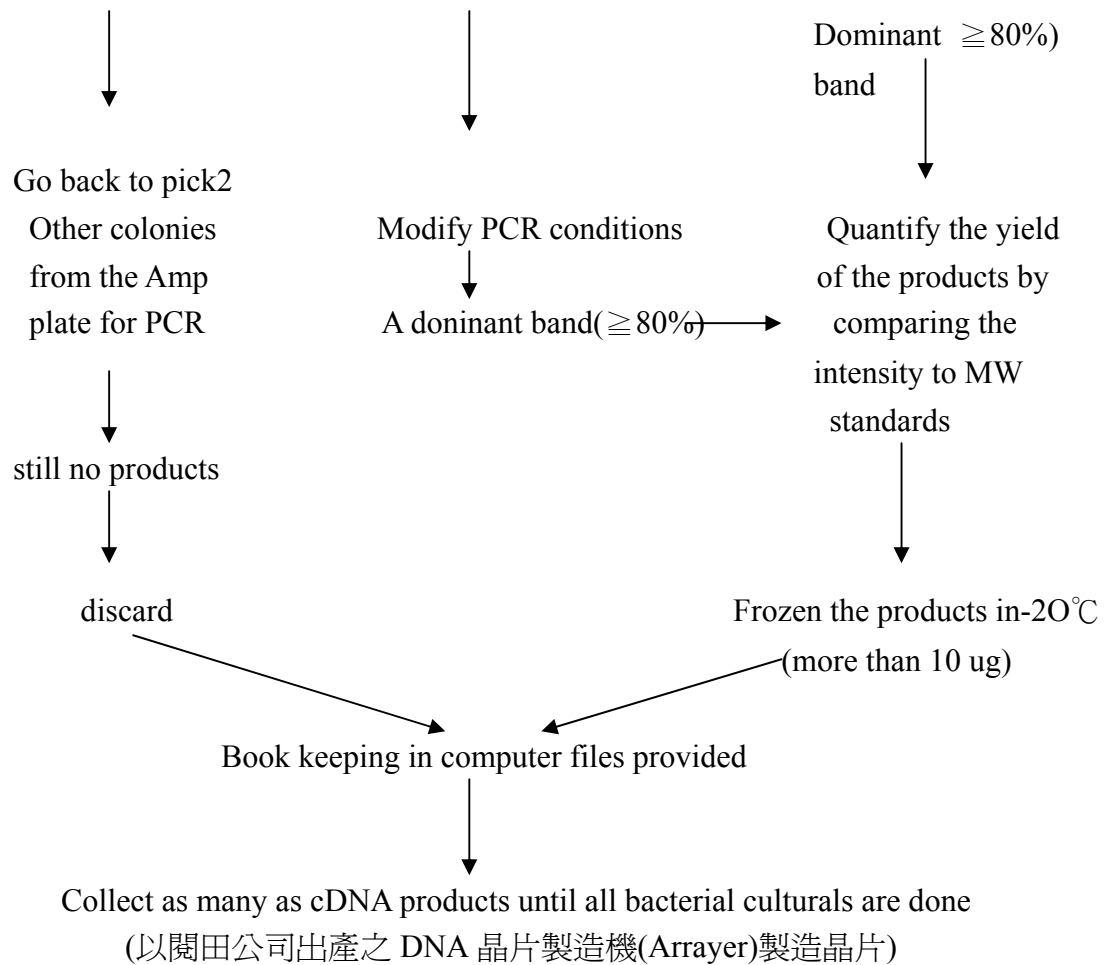
Tet-On Gene Expression System:

利用二種 plasmids 來協同調控基因的表現，首先 pTet-on 可持續表現 rtTA，當在含有 Dox 或 Tet 時，其可活化另一個 plasmid pTRE 的 promoter，當將 ER 接在 pTRE 的 promoter 之後，則可控制 ER 的表現。方法：

1. 先將 pTet-on 的 plasmid 以 transfection 的技術轉植至心臟細胞，並須經 G418 篩選二週，以獲得帶有 pTet-on 的心臟細胞，或利用 pTRE2hyg-Luc 再次 tranfect 入心臟細胞，觀察 Luciferase 的活性以判定心臟細胞在 Tet-on system 之下的表現效果，將帶有 pTet-on 的心臟細胞大量培養於含半量 G418 的濃度下以維持其 plasmid 的作用。
2. 利用 PCR 技術以獲得 ER 的 cDNA，使其 5'端帶有 BamH I 而 3'端帶有 Sal I 的 cutting sites，隨後將之接上 pTRE plasmid，以獲得 pTRE-ER。
3. 將先前經篩選正確帶有 pTet-on 的心臟細胞以 transfection 的技術將 pTRE-ER 及 pTK-Hyg 以 10:1 的 DNA 含量轉植入細胞內，利用 pTK-Hyg 所帶有抗 Hygomycin 的基因，來篩選出帶有 pTRE-ER 的心臟細胞，此時的細胞則因帶有 pTet-on 和 pTRE-ER 二種 plasmids 而可以抗 G418 和 hygomycin。
4. 實驗時則可依是否加入 Dox 或 Tet 來控制細胞是否表現 ER protein。

DNA 晶片製作(1) 7600-dot cDNA-Chip





(2) 9600 Dots PCR Protocol

➤ Prepare PCR reaction cocktail/per tube (in hood 中操作)

10X buffer	10 ul	
10 mM (2.5mM each) dNTP	2 ul	
10 ul Lafmid-F primer	2 ul	
10 ul Lafmid-R primer	2 ul	
Tag	0.12 ul	《P.S. 1》
d2H2O	80 ul	
total	96 ul	

➤ 在 hood 外加入 4 ul 菌液(直接用 glacerol stock 的菌做) , run PCR (P.S. 2)

➤ Loading 1 ul PCR product and 2.0 ul 100-bp ladder, run 1.2% gel

➤ 定量 PCR product by compare with ladder bands, check whether the total quantity is above than 10ug

P.S.1: Tag enzyme-Supertherm is from 伯昂

P.S.2: PCR program

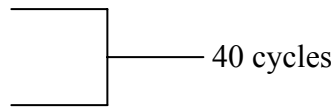
94°C 3 min

94°C 30 sec

57°C 30 sec

72°C 1min 30 sec

72°C 10 min



P.S.3 PCR: Ladder is from 波仕特 Cat.No.SAM-A1 的 100 bp ladder

每 0.6 ug 含有以下成份:

25 ng for 100,200,300,400,600,700,800 and 900 bp bands

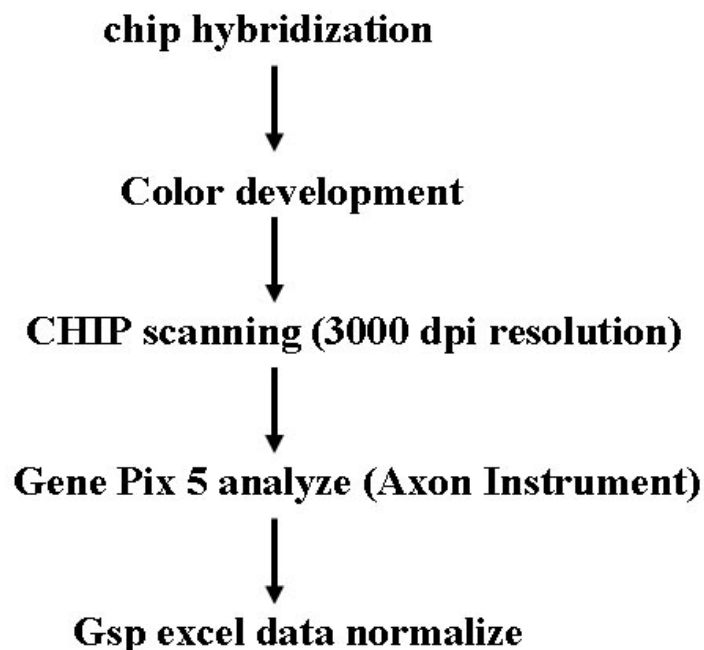
50 ng for 1000 bp band

100 ng for 1500,2000,3000 bp bands

(3) 研究過程將包含:7600 個基因(含已知及 EST)genes cloning→preparation of cDNA Gene Chips→extration mRNA of Cells & tissues→hybridization to Chips→up-, down-regulated genes analysis & sorting→RTPCR & Northern test→DNA sequencing。

晶片之操作

cDNA Microarray Procedure

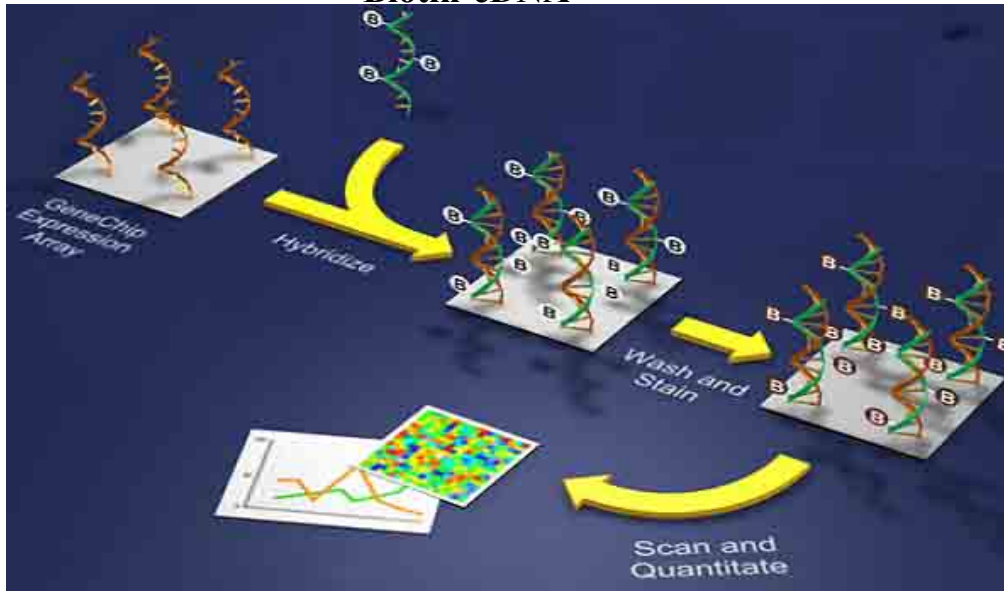


Isolation of mRNA from cardiomyocyte



Reverse Transcription

Biotin-cDNA



Housekeeping gene

1. GAPDH
2. β -actin
3. Hypoxanthine phosphoribosyl-transferase

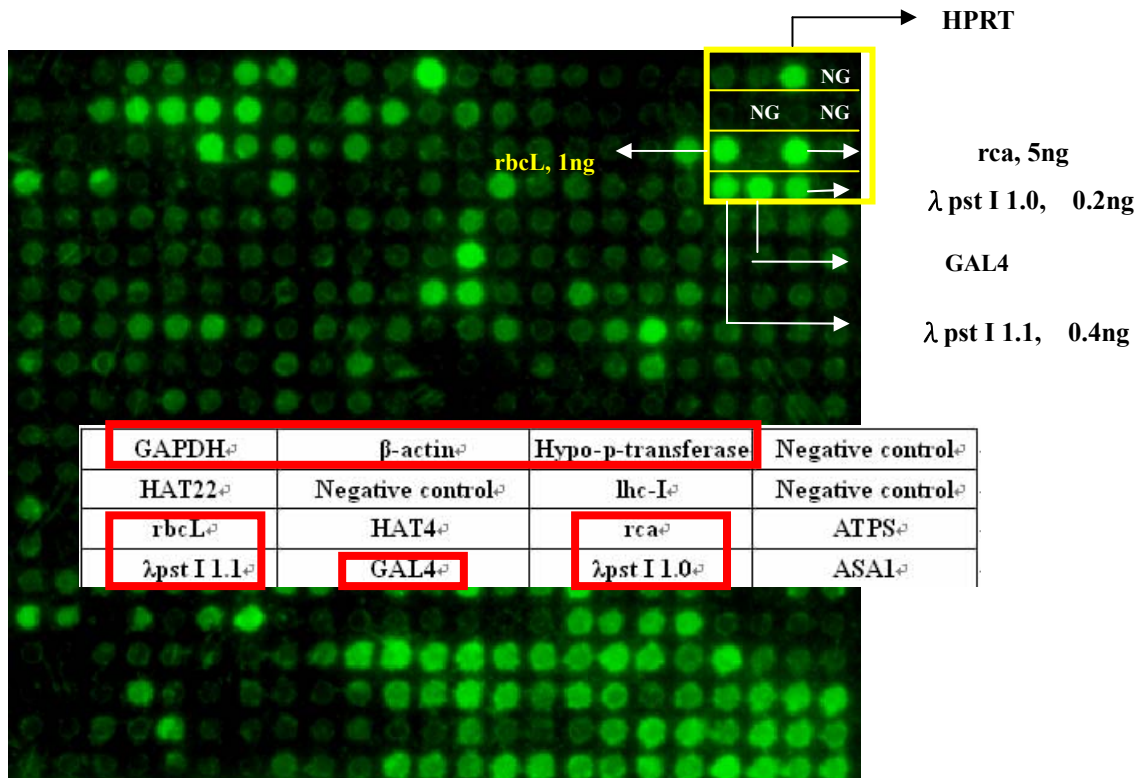
Control cDNA

1. rbcl gene (Plant).....1ng
2. λ pst I 1.1 (Plant).....0.4ng
3. λ pst I 1.0 (Plant).....0.2ng
4. rca (Plant).....5ng
5. GAL4 (yeast).....from PCR

Plant gene

1. lhcl-I
2. HAT2
3. HAT4
4. ATPS
5. ASA1

Block IV: Control region



Microarray Hybridization

I. Biotin-labeled cDNA preparation

- Add 2.5μg mRNA----- μL
 random hexamer primer (3μg/μl)----- 2.5μL
 control mRNA 2.5μL
 DEPC-H₂O----- μL.
 Total----- 25μL.
- Denaturing the RNA at 70 °C (Heat Block) for 5min, then place it on ice for 5 min
- Prepare RT mixture
 - Add (total volume of 25μl).
 10 μL 5X RT buffer,
 1.25 μL dNTP mix (final 0.5mM dATP, dCTP, dGTP and 40μM dTTP)
 2.5 μL Biotin-16-dUTP (1mM)...
 6.28 μL DTT (10mM)..... final: 1.25mM

- 1.25 μL HPR-RNase inhibitor (0.4 $\mu\text{g}/\text{ul}$),
 - 3.75 μL M-MLV reverse transcriptase
4. Transfer the mRNA mixture into the RT mixture for making total 50 μL volume
 5. Running reverse transcription process (42 °C for 90min, 94 °C for final 5min)
 6. After RT processing, place the tube on ice then add 5.5 μL NaOH (3M) at 50 °C for 30min (for denaturing and removing RNA)
 7. Then, add 5.5 μL CH₃COOH (3M) and incubating it at 50 °C again for another 30 min.(adjust the pH for neutralization)
 - 8 Add 38 μL ddH₂O,
1 μL glycogen (20mg/ml),
50 μL NH₄OAc (7.5M),
375 μL Ethanol (100%)
to precipitate the labeled cDNA at -80 °C for 30min or -20 °C overnight
 9. Centrifuge the mixture at 4 °C, 12000 rpm for 15min.
 10. The pellet will be washed twice with ice-cold 75% Ethanol
 - (1). First run:
Add 800 μl 75% ethanol, vortex then Centrifuge , 12000rpm for 5min
 - (2). Second run:
Add 500 μl 75% ethanol, vortex then Centrifuge , 12000rpm for 5min
 11. After remove the 75% ethanol, the final pellet is air-dried.(Oven)
 12. Resuspend the cDNA pellet with 10 μL ddH₂O.

II. Pre-Hybridization

1. Heat salmon sperm DNA (800 $\mu\text{g}/\text{ml}$) at Heat Block(99-100 °C) for 5min and then place it on ice
2. Prepare the pre-hybridization solution: (in 1.5mL eppendorf tube)
 - 1ml 1x Hybridization Buffer
 - 1.5 μL denatured salmon sperm DNA (800 $\mu\text{g}/\text{mL}$).
2. Put the the MillenniaChip[®] (DNA-chip) into an appropriate sized plastic bag
3. Submerge the pre-hybridization solution (1.0125mL) into the plastic

bag and seal it with a heat-sealing machine

4. Then, incubate it in shaking incubator at 60 °C at least for 1.5 hr

III. Hybridization

1. Stick one side of adhesive EasiSeal[®] square (Hybaid easiseal) to a clean glass slide .

- The inside edge of EasiSeal[®] square is needed to be trimmed to make more surface area to accommodate the size of MillenniaChip[®]
- Open the adhesive tape bottom side of Hybaid easiseal to stick it on the glass slide

2.Prepare the Hybridization solution: (in 0.5mL eppendorf tube)

10 μL Biotin- labeled cDNA

1 μL Biotin- GA4(10ng/μl)

2.5 μL oligo-dA (polyA, 3μg/μL)

and then fill up the volume with 1x Hybridization buffer to 65μL.

- use with ddH₂O for diluting the 2x Hybridization buffer to 1x

1. Heat the hybridization solution to 95 °C for 5min and chill it on ice for another 5min

4. Place pre-hybridized MillenniaChip[®] in the center of the square with the cDNA-spotted side up.

5. Loading the hybridization solution (65μL) at the center of plastic cover, then immediately completely cover MillenniaChip[®]

- Ensure there is no liquid leaking into or out of the hybridization chamber sealed onto the glass slide

3. Then, transfer the glass slide into an appropriate sized plastic bag and seal it with a heat-sealing machine

4. Submerge the plastic bag into a shaking waterbath at 63°C for overnight (or place the bag in oven)

- Hybridization of cDNA probes to DNA on MillenniaChip[®] should be taken place

for overnight with shaking.

IV. Washing the membrane

1. Adjuste the incubator to 58 °C
2. Prepare the washing solution
 - Washing solution I: 2x SSC, 0.1% SDS (20mL)
 - Washing solution II: 0.1x SSC, 0.1% SDS (20mL)
3. Submerge the Hybridized MillenniaChip[®] into the 5ml Washing solution I in the 6ml Disc at room temperature with shaking for 5min. (for washing the unconjouted probe)
 - You will use Washing solution I for washing **twice**
4. Repeat the washing by Washing solution I
 - Transfer the chip to another container per washing
5. Transfer the Hybridized MillenniaChip[®] into the another 6mL Disc containg 5ml Washing solution II, then incubates it in the incubator at 58 °C with shaking 100rpm, 15min (for washing the nospecific material)
 - You will use Washing solution II for washing **three times**
6. Repeat twice the washing by Washing solution II

VI. Color development

1. Prepare Blocking solution (in 15mL centrifuge tube)
 - Adding 4mL Blocking dilution buffer (buffer I)
 - 0.5mL 20% Dextran sulfate (DS)
 - 0.5mL 10% blocking reagent
 - (total volume :5mL)
2. transfer the washedly Hybridized MillenniaChip[®] into the new 6mL Disc containg 5mL Blocking solution. Incubate them at room temperature with shaking for 90 min.
 - (1 chip approximately need 2.5 cc)

3. Prepare Color-Development solution (in 15mL centrifuge tube)
Adding 4.5ml 1x TBS buffer (pH7.4) with 0.3% BSA
0.5mL 10% blocking reagent
8.4μL Streptavidin β-gal (1:700)
(total volume of 5ml)
4. Then, transfer the MillenniaChip[®] into the new 6ml Disc containing Color-Development solution. Incubate them at room temperature with shaking for 60 min.
5. Use 5ml 1x TBS for washing MillenniaChip[®], Incubate them at room temperature with shaking for 10 min.
 - You will use 1x TBS buffer for washing **three times**
6. Repeat twice the washing by 1x TBS
7. Prepare X-gal substrate buffer (**Light avoid**)
Adding 50μl 0.3 M K₃Fe(CN)₆
50μl 0.3 M K₄Fe(CN)₆
5μl 1 M MgCl₂
2.5ml 2x TBS
2.395ml ddH₂O
(total volume of 5ml)
8. Prepare the Substrate solution (**Light avoid**)
Adding 4.95ml X-gal substrate buffer(make fresh every time)
50 μl 120mM X-gal
(total volume of 5ml)
 - The final Conc. of each composition: 2mM X-gal, 1mM MgCl₂, 3mM K₃Fe(CN)₆, 3mM K₄Fe(CN)₆, 1x TBS
9. Transfer the MillenniaChip[®] into the new 6ml Disc containing 5ml Substrate solution. Incubate them at 37 °C with shaking (100r.p.m.) for 1hr (**Light avoid**)
 - If the hybridization is successful, you will see the blue spots appear on MillenniaChip[®] within 1hr

10. Use with ddH₂O to wash the MillenniaChip[®], washing **twice**.

Submerge the MillenniaChip[®] into the 5ml ddH₂O in the 6ml Disc at room temperature with shaking for 5min. (Wash and stop the color development)

11. Air dried out the MillenniaChip

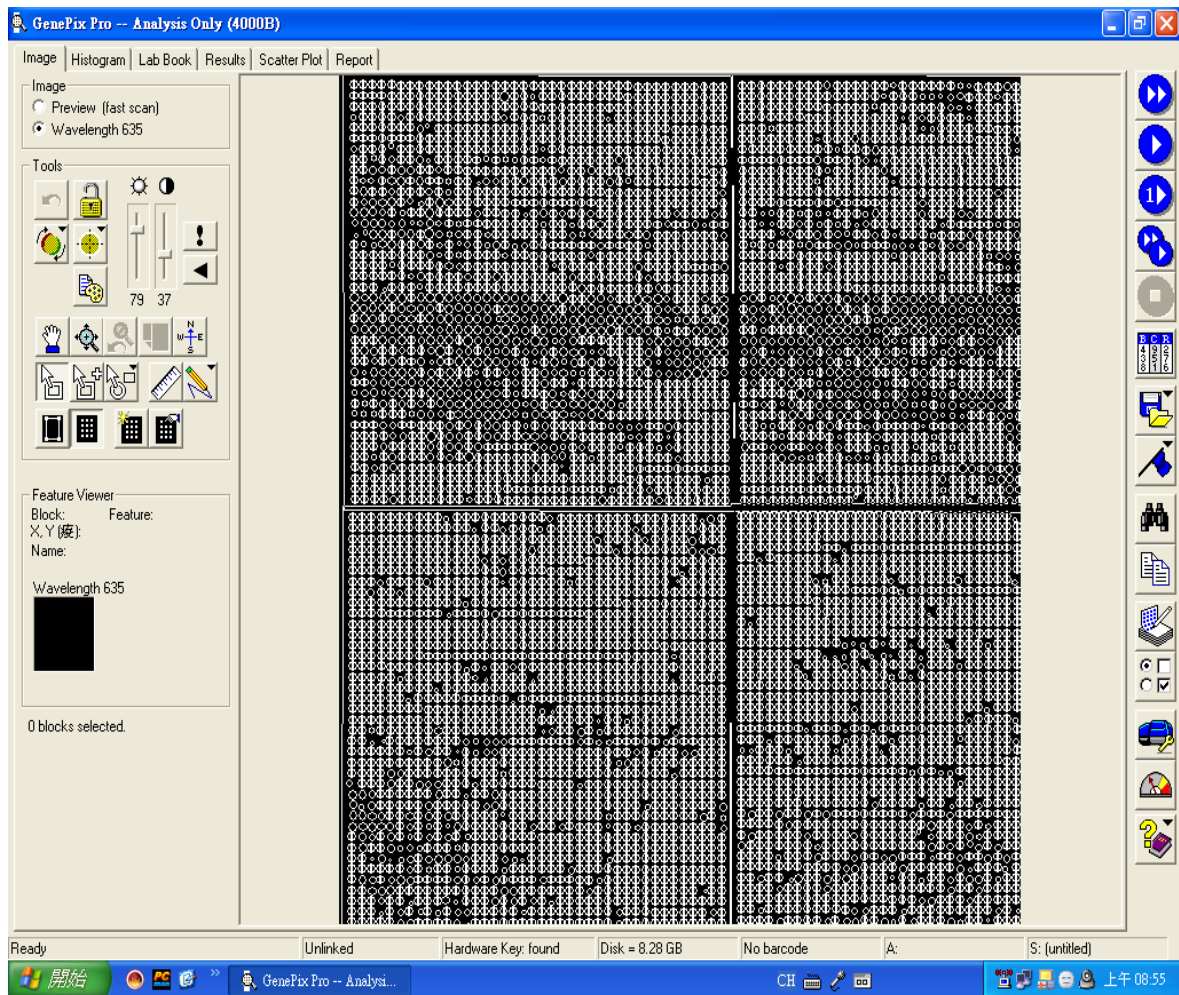
(四) 完成之工作項目及成果

(a)7600 基因之晶片建立(以完成 200 片晶片)

(b)晶片量化與分析

用 6400dpi scan

先將 chip 轉變成 16 位元、負片效果



1. 調適合的亮度與對比
2. 貼上 block 的順序：①②
③④
3. 按 select：align Features in all blocks
4. select 「result」→analyze→存檔「save result as」→得到定量結果(excel 檔)
5. 開啓「gsp excel 程式」→貼上「F520mean & B520」於「CHI & CHB」→回到主選單按分析→得到依表現強度排名的基因排序表

(C)細胞處理組別與晶片初步分析排名結果

材料: H9c2 cardiomyocyte

處理: contral, tet on ER alpha, E2+tet on ER alpha, tet on ER beta, E2+tet on ER beta

以下只把各處理與 contral 組相比後基因表現強度排序表之前一百名表示出來(因為全部貼實在太多了, 且前面部分較具意義吧!)

Putative genes	contral	ER alpha	強度排名
EST	1	17.452025	270.66913
hypothetical protein	1	16.429257	238.06197
NA	1	16.38738	237.12256
parathyroid hormone-like hormone	1	16.241884	232.31501
KIAA0999 protein	1	15.972904	224.18784
Human mapkap kinase (3pk)	1	-15.216	202.87729
ESTs, Highly similar to CBF_HUMAN CCAAT-BINDING FACTOR [H.sapiens]	1	15.177238	200.99408
Huntington disease (HD)	1	-15.126	200.32651
Tubulin, alpha, brain-specific	1	15.091336	199.065
ubiquinol-cytochrome c reductase core protein I	1	14.625856	185.66395
toll-like receptor 4	1	14.625856	185.66395
complement component 1, s subcomponent	1	14.59418	184.80172
CDC28 protein kinase 2	1	14.479286	181.69499
H-ras-1	1	14.095412	171.48982
ESTs, Weakly similar to CATB_HUMAN CATHEPSIN B PRECURSOR [H.sapiens]	1	14.052998	170.44685
type II membrane serine protease	1	13.989646	169.09099
Homo sapiens shox gene, alternatively spliced products	1	-13.9625	168.80904
Cyclin G1	1	-13.614	159.89563
small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin)	1	13.631006	159.5423

NA	1	13.419526	155.02727
ribosomal protein L21	1	13.041504	144.99783
growth associated protein 43	1	13.029693	144.71351
peptidase (mitochondrial processing) beta	1	12.942717	142.6285
ESTs	1	12.57052	134.65957
NA	1	12.583004	134.16597
endothelial PAS domain protein 1	1	12.491733	132.05993
L13 protein	1	12.42029	131.20567
KIAA1080 protein; Golgi-associated, gamma-adaptin ear containing, ARF-binding protein 2	1	12.367074	129.99301
interferon, alpha-inducible protein 27	1	12.286642	127.6022
plastin 3 (T isoform)	1	-12.025	122.33326
glutamate receptor, ionotropic, kainate 1	1	12.027326	121.8684
v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	1	-11.676	114.75961
enhancer of zeste (Drosophila) homolog 1	1	11.634862	113.1003
KIAA0157 protein	1	11.604797	112.46172
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 (13kD, B13)	1	11.537686	111.63237
Keratin 8	1	11.53178	110.9184
inorganic pyrophosphatase	1	11.446415	109.12759
ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD, isoform 2	1	11.353927	107.98644
EST	1	11.385747	107.86374
ESTs	1	11.344621	107.79382
OX40 ligand/gp34	1	-11.334	107.57419
75 kda infertility-related sperm protein [human, testis, mRNA Partial, 2427 nt]	1	-11.3035	106.94475
UV excision repair protein RAD23	1	-11.1705	104.22171
S-adenosylmethionine decarboxylase 1	1	11.159355	103.99513

ESTs, Weakly similar to BUTY_HUMAN BUTYROPHILIN PRECURSOR [H.sapiens]	1	11.087238	102.15315
ribosomal protein L5	1	11.055562	101.11433
vimentin	1	11.037845	100.76827
hippocalcin-like 1	1	10.942816	99.575362
CGI-06 protein	1	10.955701	99.275482
AKT2-kinase	1	10.908252	98.956104
NA	1	10.876242	98.189009
ubiquinol-cytochrome c reductase hinge protein	1	10.790877	95.978807
V-erba related protein ear-1	1	10.703365	94.847342
Human N-ras mRNA and flanking regions	1	10.738799	94.844211
amylase, alpha 2B; pancreatic	1	10.716787	94.415948
zinc finger protein 217	1	10.625516	92.650563
pyruvate dehydrogenase (lipoamide) beta	1	10.587397	91.918187
Nucleobindin Precursor	1	10.489147	90.043913
pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	1	10.473041	89.738497
hypothetical protein	1	10.47143	89.707984
creatine kinase, brain	1	10.378012	88.539547
Ewing sarcoma breakpoint region 1	1	10.344725	87.32388
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	1	10.329692	87.110211
hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	1	10.300163	86.856536
ESTs	1	10.214528	85.690156
Mevalonate kinase	1	10.241106	85.398034
Cyclin H assembly factor	1	10.234126	85.363346
dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2)	1	10.101515	82.83758
EST	1	10.056954	82.114743

SNF2 (sucrose nonfermenting, yeast, homolog)-like 1	1	9.9839371	80.711127
signal sequence receptor, delta (translocon-associated protein delta)	1	9.9386068	80.681331
(clone PWHLC2-24) myosin light chain 2	1	9.9219477	80.383789
IQ motif containing GTPase activating protein 2	1	9.8561582	78.431538
pleckstrin homology, Sec7 and coiled/coil domains 3	1	9.7556539	77.444114
ESTs, Highly similar to DNA DAMAGE RESPONSE PROTEIN KINASE DUN1 [Saccharomyces cerevisiae]	1	9.7171047	75.987914
RCA	1	9.710662	75.875633
voltage-gated calcium channel beta subunit	1	-9.6215	75.112901
glycophorin C (Gerbich blood group)	1	-9.545783	73.81305
Homo sapiens clone FBA1 Cri-du-chat region mRNA	1	9.5705347	73.454065
DKFZP586I1419 protein	1	9.5640921	73.343673
phosphodiesterase I/nucleotide pyrophosphatase 2 (autotaxin)	1	9.5635552	73.334478
NA	1	-9.508394	73.175414
Human tyrosine kinase receptor (axl) mRNA, complete cds	1	9.4545673	71.479708
ESTs	1	9.4046369	71.246429
homolog of yeast SPB1	1	9.3971204	70.511632
Cyclin A1	1	-9.274	69.241715
ESTs, Highly similar to PHOSPHORYLASE B KINASE BETA REGULATORY CHAIN [Oryctolagus cuniculus]	1	9.2333701	68.419145
ESTs	1	9.2516243	68.183563
Human semaphorin (CD100)	1	-9.1995	68.014439
Proliferating cell nuclear antigen	1	9.2398128	67.894514

tropomyosin 4	1	9.1565313	67.311641
ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	1	9.1968619	67.188544
cyclin-dependent kinase inhibitor 1	1	9.1716282	66.775508
Neural cell adhesion molecule 11 precursor	1	8.9638532	63.422958
ESTs	1	8.9353983	62.970545
carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2	1	8.8744347	62.789361
NA	1	8.8458237	62.339588
Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains	1	8.8919105	62.329146
peptidylprolyl isomerase A (cyclophilin A)	1	8.8908367	62.265304

Putative genes	contral	E2ER alpha	強度排名
hypothetical protein	1	17.76226439	280.97351
CDC28 protein kinase 2	1	17.581919	274.96004
EST	1	17.09533777	259.0599
NA	1	16.35346241	235.9706
NA	1	-15.958	224.32753
ubiquinol-cytochrome c reductase core protein I	1	15.92250719	222.68122
ESTs, Highly similar to CBF_HUMAN CCAAT-BINDING FACTOR [H.sapiens]	1	15.5981197	213.1051
ESTs, Weakly similar to CATB_HUMAN CATHEPSIN B PRECURSOR [H.sapiens]	1	15.51907221	210.83496
parathyroid hormone-like hormone	1	15.34341111	205.73344
NA	1	15.31998963	205.0621
Human mapkap kinase (3pk)	1	-15.216	202.68043
Huntington disease (HD)	1	-15.126	200.12965
KIAA0157 protein	1	14.96222654	194.94377
complement component 1, s subcomponent	1	14.77368363	189.71436
KIAA0999 protein	1	14.59626592	184.85845
Homo sapiens shox gene, alternatively spliced products	1	-13.9625	168.61218
toll-like receptor 4	1	13.85087734	165.14505
Cyclin G1	1	-13.614	159.69877
growth associated protein 43	1	13.36839487	152.97719
type II membrane serine protease	1	13.18102303	148.62611
small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin)	1	12.90055082	141.62311
glutamate receptor, ionotropic, kainate 1	1	12.89118223	141.57681
peptidase (mitochondrial processing) beta	1	12.82325994	139.78948
ESTs	1	12.71017763	137.71403
L13 protein	1	12.53232064	133.58019
signal transducer and activator of transcription 1, 91kD	1	12.42977908	130.82318

endothelial PAS domain protein 1	1	12.40869975	130.15843
KIAA1080 protein; Golgi-associated, gamma-adaptin ear containing, ARF-binding protein 2	1	12.26528256	127.49236
ESTs, Weakly similar to BUTY_HUMAN BUTYROPHILIN PRECURSOR [H.sapiens]	1	12.26817088	127.25224
ribosomal protein L21	1	12.24357832	126.41805
enhancer of zeste (Drosophila) homolog 1	1	12.21898577	125.86564
Tubulin, alpha, brain-specific	1	12.10890482	123.76601
pituitary tumor-transforming 1 interacting protein	1	12.05269327	122.16203
plastin 3 (T isoform)	1	-12.025	122.1364
hippocalcin-like 1	1	11.87937432	118.89247
development and differentiation enhancing factor 2	1	11.87878878	118.34805
ubiquinol-cytochrome c reductase hinge protein	1	11.72420702	115.07509
v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	1	-11.676	114.56275
CGI-06 protein	1	11.57372401	111.90022
NA	1	11.46557051	110.11394
RCA	1	11.45720215	109.35308
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 (13kD, B13)	1	11.38283896	108.23356
OX40 ligand/gp34	1	-11.334	107.37733
75 kda infertility-related sperm protein [human, testis, mRNA Partial, 2427 nt]	1	-11.3035	106.74788
ESTs	1	11.25942877	105.84165
Keratin 8	1	11.22240182	104.4975
hypothetical protein	1	11.21420431	104.32997
ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD, isoform 2	1	11.17418019	104.09971

UV excision repair protein RAD23	1	-11.1705	104.02484
V-erba related protein ear-1	1	11.11056426	102.73606
ESTs	1	11.10587996	102.12881
EST	1	11.07543204	101.51433
creatine kinase, brain	1	10.98760149	100.18471
EST	1	10.95715357	99.189816
ribosomal protein L5	1	10.94837052	98.970076
Human N-ras mRNA and flanking regions	1	10.9466139	98.935128
serine dehydratase	1	10.90504078	98.109833
FADD/MORT	1	10.84590154	96.941777
NA	1	10.7311363	95.172787
amylase, alpha 2B; pancreatic	1	10.68312226	93.762857
interferon, alpha-inducible protein 27	1	10.65794417	93.412724
Ewing sarcoma breakpoint region 1	1	10.65033219	93.128911
Tyrosine kinase with immunoglobulin and epidermal growth factor homology domains	1	10.61812766	92.52785
ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	1	10.57479792	91.676755
KIAA0274 gene product	1	10.5317307	91.439661
AKT2-kinase	1	10.52126666	91.24029
S-adenosylmethionine decarboxylase 1	1	10.50988944	91.023768
valosin-containing protein	1	10.43426904	89.039819
(clone PWHL2-24) myosin light chain 2	1	10.38758387	88.712502
IQ motif containing GTPase activating protein 2	1	10.40089344	88.376797
pleckstrin homology, Sec7 and coiled/coiled domains 3	1	10.20387589	85.297102
Mevalonate kinase	1	10.13681626	83.481411
ESTs, Highly similar to PHOSPHORYLASE B KINASE BETA REGULATORY CHAIN [Oryctolagus cuniculus]	1	10.06538074	82.644371

ESTs, Highly similar to DNA DAMAGE RESPONSE PROTEIN KINASE DUN1 [Saccharomyces cerevisiae]	1	9.986918789	80.764709
inorganic pyrophosphatase	1	9.972280365	80.501815
Cd44	1	9.959398551	80.270822
Corticotropin releasing hormone-binding protein	1	9.881522132	79.10036
GAPDH	1	9.879765521	78.850236
OCRL	1	9.871568003	78.704719
Nucleobindin Precursor	1	9.837021321	78.092946
Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	1	-9.69	76.101871
voltage-gated calcium channel beta subunit	1	-9.6215	74.916033
pyruvate dehydrogenase (lipoamide) beta	1	9.648478413	74.796179
vimentin	1	9.61803049	74.271355
cyclin-dependent kinase inhibitor 1	1	9.585825955	73.716407
lactotransferrin	1	9.529028868	72.744333
villin 2 (ezrin)	1	9.428902044	71.04639
SNF2 (sucrose nonfermenting, yeast, homolog)-like 1	1	9.417191304	70.849109
Homo sapiens clone FBA1 Cri-du-chat region mRNA	1	9.346926866	69.671188
Cyclin A1	1	-9.274	69.044847
ESTs	1	9.266122763	68.774181
UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1	1	9.239188061	67.916353
immunoglobulin mu binding protein 2	1	9.182132714	67.533067
homolog of yeast SPB1	1	9.205226916	67.325749
Proliferating cell nuclear antigen	1	9.045960857	64.737486
regulatory factor X, 5 (influences HLA class II expression)	1	9.027223673	64.43632
CTP synthase	1	9.001460046	64.023363
fibrinogen, B beta polypeptide	1	8.976867493	63.630415

Growth arrest and DNA-damage-inducible protein gadd45	1	8.941735274	63.071159
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Putative genes	contral	ER beta	強度排名
NA	1	15.5178	210.89591
hypothetical protein	1	15.3704	206.50945
Human mapkap kinase (3pk)	1	-15.216	202.46738
Huntington disease (HD)	1	-15.126	199.9166
parathyroid hormone-like hormone	1	15.0763	198.14233
EST	1	14.5304	183.07075
CDC28 protein kinase 2	1	14.2137	174.60264
ESTs, Weakly similar to CATB_HUMAN CATHEPSIN B PRECURSOR [H.sapiens]	1	14.1585	173.15284
Homo sapiens shox gene, alternatively spliced products	1	-13.9625	168.39913
toll-like receptor 4	1	13.7728	163.14438
Cyclin G1	1	-13.614	159.48572
Tubulin, alpha, brain-specific	1	13.5403	157.46864
KIAA0999 protein	1	13.2338	149.66643
complement component 1, s subcomponent	1	12.7816	138.80707
NA	1	12.3127	128.35013
ubiquinol-cytochrome c reductase core protein I	1	12.1961	125.35165
ESTs, Highly similar to CBF_HUMAN CCAAT-BINDING FACTOR [H.sapiens]	1	12.1773	124.93211
plastin 3 (T isoform)	1	-12.025	121.92335
type II membrane serine protease	1	11.8934	118.80001
S-adenosylmethionine decarboxylase 1	1	11.8081	117.1881
v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	1	-11.676	114.3497
endothelial PAS domain protein 1	1	11.6844	114.15648
glycophorin C (Gerbich blood group)	1	-11.3775	108.06523
OX40 ligand/gp34	1	-11.334	107.16428
small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin)	1	11.3415	106.94697

75 kda infertility-related sperm protein [human, testis, mRNA Partial, 2427 nt]	1	-11.3035	106.53484
KIAA0157 protein	1	11.1942	103.92136
UV excision repair protein RAD23	1	-11.1705	103.8118
KIAA1080 protein; Golgi-associated, gamma-adaptin ear containing, ARF-binding protein 2	1	10.9901	100.1752
ribosomal protein L21	1	10.9847	99.694236
hippocalcin-like 1	1	10.6177	92.833738
site 1 protease of sterol regulatory element binding protein mRNA	1	10.5604	91.400822
peptidase (mitochondrial processing) beta	1	10.5395	91.001737
EST	1	10.4961	90.175657
ESTs	1	10.4442	89.565741
growth associated protein 43	1	10.4623	89.535755
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9 (39kD)	1	-10.301	86.88125
calumenin	1	-10.1695	84.452459
EST	1	10.172	84.12469
glutamate receptor, ionotropic, kainate 1	1	10.0193	81.434456
Keratin 8	1	10.0091	81.163523
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 (13kD, B13)	1	9.96461	80.625043
ESTs	1	9.8733	79.108234
amylase, alpha 2B; pancreatic	1	9.82424	77.867257
L13 protein	1	9.79682	77.756711
ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD, isoform 2	1	9.77479	77.369646
voltage-gated calcium channel beta subunit	1	-9.6215	74.702991
actin related protein 2/3 complex, subunit 2 (34 kD)	1	9.64101	74.667096
ribosomal protein L5	1	9.6378	74.611553
RCA	1	9.62119	74.324905

vimentin	1	9.48939	72.06978
ESTs	1	9.36269	70.307369
eukaryotic translation initiation factor 4 gamma, 2	1	9.33027	69.414002
hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	1	9.32223	69.396304
Cyclin A1	1	-9.274	68.831805
Human semaphorin (CD100)	1	-9.1995	67.604529
NA	1	9.16901	67.027393
interferon, alpha-inducible protein 27	1	9.13472	66.236028
creatine kinase, brain	1	9.11061	66.04441
phosphodiesterase I/nucleotide pyrophosphatase 2 (autotaxin)	1	9.064	65.028068
ESTs, Weakly similar to BUTY_HUMAN BUTYROPHILIN PRECURSOR [H.sapiens]	1	8.96435	63.587249
G protein-coupled receptor 37 (endothelin receptor type B-like)	1	8.95524	63.285825
NA	1	8.95095	63.21765
NA	1	8.90832	62.91425
Human DNA-dependent protein kinase catalytic subunit (DNA-PKcs) mRNA, complete cds	1	-8.7875	61.017885
karyopherin alpha 6 (importin alpha 7)	1	8.79397	60.746045
homolog of yeast SPB1	1	8.73343	59.806001
valosin-containing protein	1	8.73022	59.762928
carcinoembryonic antigen	1	8.60592	57.850064
KIAA0274 gene product	1	8.41486	55.352882
inorganic pyrophosphatase	1	8.37394	54.37497
AKT2-kinase	1	8.31373	53.863389
development and differentiation enhancing factor 2	1	8.31608	53.524974
hypothetical protein	1	8.18214	51.583082
ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	1	8.14678	51.076408

eukaryotic translation elongation factor 1 gamma	1	8.08731	50.229916
CTP synthase	1	8.02087	49.292648
ESTs, Weakly similar to putative [C.elegans]	1	7.92711	47.984911
signal sequence receptor, delta (translocon-associated protein delta)	1	7.89271	47.882228
(clone PWHLC2-24) myosin light chain 2	1	7.88523	47.779058
heat shock 90kD protein 1, beta	1	7.85175	47.319157
APEX nuclease (multifunctional DNA repair enzyme)	1	-7.782	46.368253
KIAA0532 protein	1	7.80228	46.27104
adenylate cyclase 2 (brain)	1	-7.741	45.81381
dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2)	1	7.74013	45.429403
IQ motif containing GTPase activating protein 2	1	7.72299	45.198587
ubiquinol-cytochrome c reductase hinge protein	1	7.71924	45.169928
SNF2 (sucrose nonfermenting, yeast, homolog)-like 1	1	7.6378	44.060435
CGI-06 protein	1	7.62387	43.914043
AXL receptor tyrosine kinase	1	-7.57	43.537629
peptidylprolyl isomerase A (cyclophilin A)	1	7.52261	42.544506
LIM and cysteine-rich domains 1	1	7.52047	42.516554
Mevalonate kinase	1	7.49476	42.181848
Human N-ras mRNA and flanking regions	1	7.46904	41.848464
Prohibitin	1	-7.41	41.460829
ESTs, Weakly similar to GTR5_HUMAN GLUCOSE TRANSPORTER TYPE 5, SMALL INTESTINE [H.sapiens]	1	7.40528	41.027655
annexin A6	1	-7.375	41.013354
V-erba related protein ear-1	1	7.36564	40.841036
aldolase A, fructose-bisphosphate	1	-7.246	39.385245

Putative genes	contral	E2ER beta	強度排名
parathyroid hormone-like hormone	1	16.6312742	244.33673
NA	1	15.9052869	222.22133
Human mapkap kinase (3pk)	1	-15.216	202.30425
Huntington disease (HD)	1	-15.126	199.75347
Tubulin, alpha, brain-specific	1	14.8303708	191.38159
Homo sapiens shox gene, alternatively spliced products	1	-13.9625	168.236
toll-like receptor 4	1	13.94159	167.48475
Cyclin G1	1	-13.614	159.32259
complement component 1, s subcomponent	1	13.412511	154.07043
NA	1	13.2625647	150.58008
CDC28 protein kinase 2	1	12.9714123	143.31471
ubiquinol-cytochrome c reductase core protein I	1	12.8612873	140.69014
ESTs, Highly similar to CBF_HUMAN CCAAT-BINDING FACTOR [H.sapiens]	1	12.7984442	139.20329
endothelial PAS domain protein 1	1	12.5692166	133.84677
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 (13kD, B13)	1	12.3758992	129.54609
type II membrane serine protease	1	12.3232308	128.27205
hypothetical protein	1	12.2849264	127.34956
ESTs	1	12.2589262	126.97301
S-adenosylmethionine decarboxylase 1	1	12.1061102	123.55527
plastin 3 (T isoform)	1	-12.025	121.76022
ESTs	1	11.8862751	118.72058
v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	1	-11.676	114.18657
KIAA0999 protein	1	11.6151986	112.68244
L13 protein	1	11.5703131	111.94111
small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin)	1	11.5116571	110.49494
glycophorin C (Gerbich blood group)	1	-11.3775	107.9021
development and differentiation enhancing factor 2	1	11.3656218	107.44611
OX40 ligand/gp34	1	-11.334	107.00115

75 kda infertility-related sperm protein [human, testis, mRNA Partial, 2427 nt]	1	-11.3035	106.3717
UV excision repair protein RAD23	1	-11.1705	103.64866
KIAA1080 protein; Golgi-associated, gamma-adaptin ear containing, ARF-binding protein 2	1	10.9888715	99.987144
valosin-containing protein	1	10.9867677	99.735529
EST	1	10.8006325	96.052396
NA	1	10.6518649	93.368088
hippocalcin-like 1	1	10.2739475	86.189223
calumenin	1	-10.1695	84.289321
dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2)	1	10.1584359	83.876948
deoxyguanosine kinase	1	-9.9175	79.731397
NA	1	9.90945758	79.535713
NA	1	9.88970689	79.026889
microphthalmia-associated transcription factor	1	9.7514521	76.587914
Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	1	-9.69	75.725691
amylase, alpha 2B; pancreatic	1	9.69698808	75.637602
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9 (39kD)	1	-9.68289472	75.602252
voltage-gated calcium channel beta subunit	1	-9.6215	74.539853
vimentin	1	9.53180053	72.79162
integrin, alpha 7	1	9.52042893	72.597709
phosphodiesterase I/nucleotide pyrophosphatase 2 (autotaxin)	1	9.47733652	71.865234
ribosomal protein L21	1	9.40611435	70.662758
Homo sapiens cDNA FLJ10862 fis, clone NT2RP4001574, highly similar to Homo sapiens coat protein gamma-cop mRNA	1	9.39893228	70.542064
FADD/MORT	1	9.38456815	70.300983
ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD, isoform 2	1	9.36986329	70.264203

RCA	1	9.35883241	69.870079
interferon, alpha-inducible protein 27	1	9.32771011	69.367985
Cyclin A1	1	-9.274	68.668667
Human semaphorin (CD100)	1	-9.1995	67.441391
glutamate receptor, ionotropic, kainate 1	1	9.19125083	67.126453
ESTs, Weakly similar to CATB_HUMAN CATHEPSIN B PRECURSOR [H.sapiens]	1	9.155939	66.519341
ESTs	1	9.07035149	65.340164
Human DNA-dependent protein kinase catalytic subunit (DNA-PKcs) mRNA, complete cds	1	-8.7875	60.854747
Cd44	1	8.75015219	60.064859
homolog of yeast SPB1	1	8.74596265	59.999937
p300/CBP-associated factor	1	-8.68040495	59.198212
NA	1	8.64946242	58.723867
hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	1	8.59454073	57.734586
ribosomal protein L5	1	8.56641096	57.250575
KIAA0157 protein	1	8.55204683	57.033411
RAB6, member RAS oncogene family	1	-8.511	56.624712
eukaryotic translation elongation factor 1 gamma	1	8.52152304	56.573309
pleckstrin homology, Sec7 and coiled/coil domains 3	1	8.50300592	56.504689
EST	1	8.47184708	55.828499
actin related protein 2/3 complex, subunit 2 (34 kD)	1	8.44790685	55.471316
Syk	1	-8.425	55.340216
ESTs, Weakly similar to GTR5_HUMAN GLUCOSE TRANSPORTER TYPE 5, SMALL INTESTINE [H.sapiens]	1	8.43593674	55.293155
ESTs	1	8.38207123	54.495097
ESTs	1	8.33838032	53.993881
growth associated protein 43	1	8.29588642	53.229959
proteasome (prosome, macropain) 26S	1	-8.24754996	52.736572

subunit, non-ATPase, 7 (Mov34 homolog)			
NA	1	-8.1915	51.927263
peptidylprolyl isomerase A (cyclophilin A)	1	8.06007521	49.844662
heat shock 90kD protein 1, beta	1	8.03821382	49.746045
interferon (alpha, beta and omega) receptor 2	1	-8.0145	49.412801
ESTs, Weakly similar to ALU1_HUMAN ALU SUBFAMILY J SEQUENCE CONTAMINATION WARNING ENTRY [H.sapiens]	1	8.01159625	49.162482
KIAA0274 gene product	1	7.96644439	48.740939
Ewing sarcoma breakpoint region 1	1	7.96251879	48.476668
fms-related tyrosine kinase 1 (vascular endothelial growth factor/ vascular permeability factor receptor)	1	7.89668317	47.564239
carcinoembryonic antigen	1	7.86975042	47.193471
ESTs, Weakly similar to putative [C.elegans]	1	7.85059824	46.930696
zinc finger protein 217	1	7.77817906	45.943711
adenylate cyclase 2 (brain)	1	-7.741	45.650672
DKFZP586I1419 protein	1	7.72790459	45.2647
signal sequence receptor, delta (translocon-associated protein delta)	1	7.60722698	43.86504
GAPDH	1	7.61957507	43.818774
AXL receptor tyrosine kinase	1	-7.57	43.374491
IQ motif containing GTPase activating protein 2	1	7.47713073	41.953223
Prohibitin	1	-7.41	41.297691
EST, Highly similar to CPG2 protein [R.norvegicus]	1	7.38743704	41.008943
ESTs, Weakly similar to B48013 proline-rich proteoglycan 2 precursor, parotid - rat [R.norvegicus]	1	7.39752948	40.928384
annexin A6	1	-7.375	40.850216

(五).未來展望及預期貢獻:

將進一步探討各個基因在 E2-ER 心肌保護的角色，將以 antisense-oligos 及 siRNA 的方式 knock-down up-regulated 之基因及建構 down-regulated 基因之 CMV-driven 結構大量表現於心肌細胞中。來探討基因性及非基因性調控差異，及相關訊息傳遞途徑及其影響基因的釐清，相信將有助於揭釐心肌疾病及猝死之分子機轉，對於未來心肌疾病治療、猝死防患及強心藥物開發，將是一大突破。