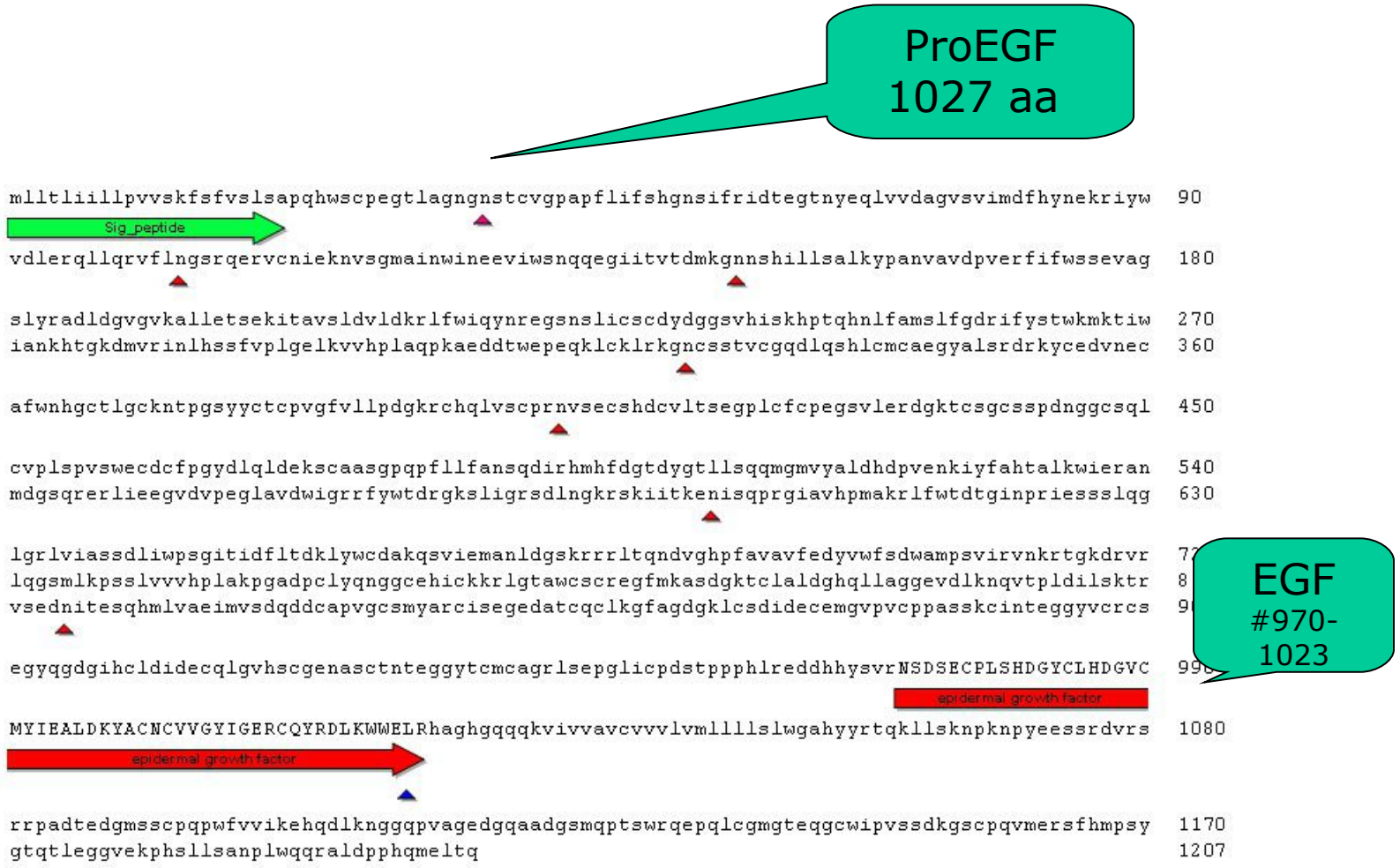
台糖重組蛋白製程開發之現況

人類表皮生長因子 (EGF)

- 分子量為 6 kD ，含 53 個氨基酸殘基，其 6 個半胱氨酸分子形成 3 對穩定的雙硫鍵。
- 最早被發現於成鼠的頷下腺；其存在於奶水、唾液、尿液、血漿及大部份的體液中。
- 被認為是一皮膚保養品中抗皺紋和老化的重要成分。

基因結構


ProEGF
1027 aa
mlltliillpvvskfsfvslsapqhwscpegtlagngnstcvgpapflifshgnsifridtegtnyeqlvvdagvsvimdfhynekriyw 90
Sig_peptide
vdlerqllqrvflngsrqervcnieknvsgmainwineeviwsnqqegiitvtdmkgnnshillsalkypanvavdpverfifwssevag 180
slyradldgvgvkalletsekitavsldvldkrlfwiqynregsnslicscdydggsvhiskhptqhnlfamslfgdrifystwkmktiw 270
iankhtgkdmvrinlhssfvplgelkvvhplaqpkaeddtwepeqklcklrkgncsstvcgqdlqshlcmcaegyalsrdrkycedvnec 360
afwnhgctlgckntpgsyyctcpvgfvllpdgkrchqlvscprnvsecshdcvltsegplcfcpegsvlerdgktcsgcsspdnggcsql 450
cvplspvswecdcfpgydlqldekscaasgpqpfllfansqdirhmhfdgtdygtllsqqmgmvyaldhdpvenkiyfahtalkwieran 540
mdgsqrerlieegvdvpeglavdwigrrfywtdrgksligrsdlngkrskiitkenisqprgiavhpmakrlfwtdtginpriessslqg 630
lgrlviassdliwpsgitidfltdklywcdakqsviemanldgskrrrltqndvghpfavavfedyvwfsdwampsvirvnkrtgkdrvr 72
lqgsmlkpsslvvvhplakpgadpclyqnggcehickkrlgtawcscregfmkasdgktclaldghqllaggevdlknqvtpldilsktr 8
vsednitesqhmlvaeimvsdqddcapvgcsmyarcisegedatcqclkgfagdgklcsdidecemgvpvcppasskcinteggyvcrcs 9
egyqgdgihcldidecqlgvhscgenasctnteggytcmcagrlsepglicpdstppphlreddhhysvrNSDSECPLSHDGYCLHDGVC 99
epidermal growth factor
MYIEALDKYACNCVVGYIGERCQYRDLKWWELRhaghgqqqkvivvavcvvvlvmllllslwgahyyrtqkllsknpknpyeessrdvrs 1080
epidermal growth factor
rrpadtedgmsscpqpwfvvikehqdlknggqpvagedgqaadgsmqptswrqepqlcgmgteqgcwipvssdkgscpqvmersfhmpsy 1170
gtqtleggvekphsllsanplwqqraldpphqmeltq 1207
EGF
#970-
1023

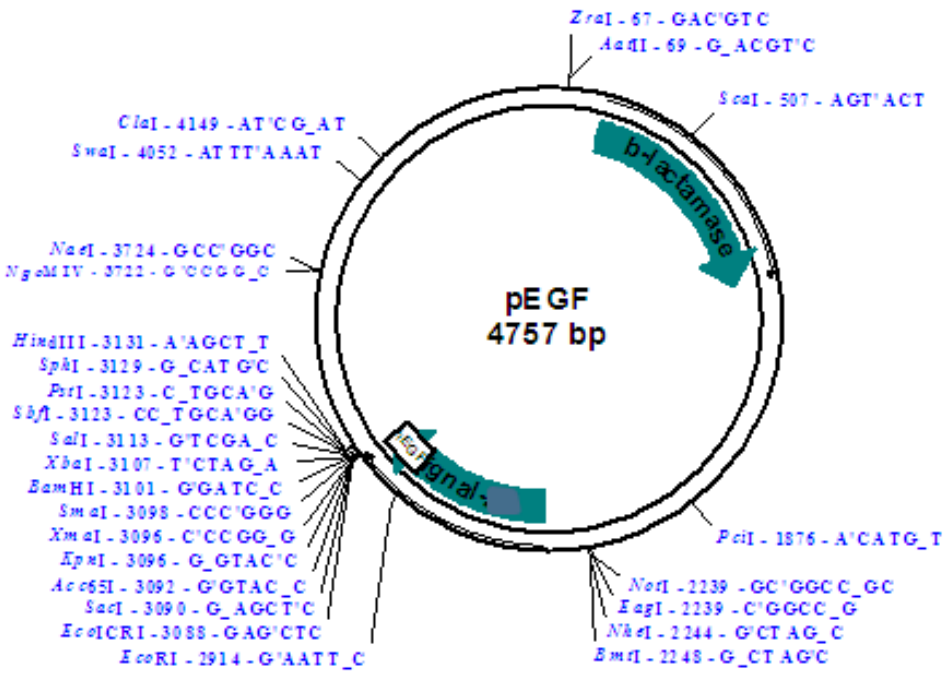
B

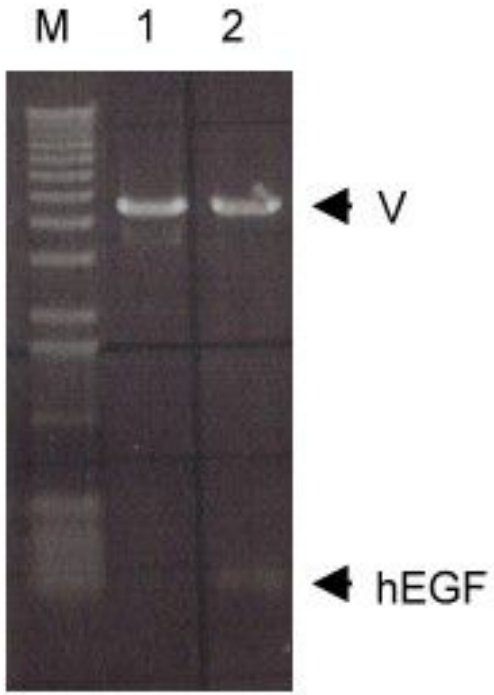
EGF 的三對雙硫鍵

NSDSE**C**PLSHDGY**C**LHDGV**C**MYIEALDK
YA**C**N**C**VVGYIGER**C**QYRDLKWWE LR

生產策略

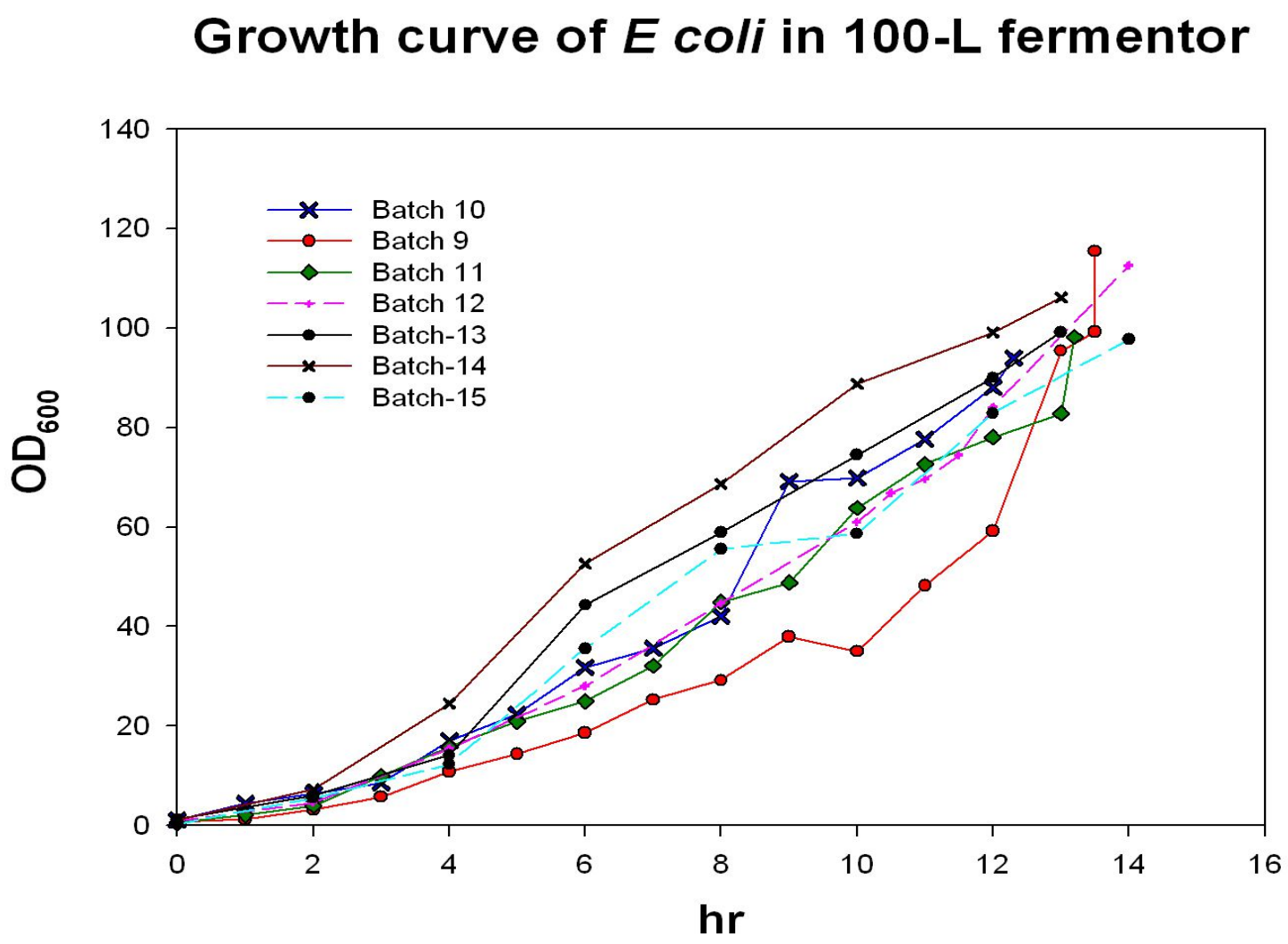
- 改變 codon usage
- 於大腸桿菌異源表現
- 產物分泌至培養基
- 降低生產成本


ZraI - 67 - GAC'GTC
AatII - 69 - G_ACGT'C
ScaI - 507 - AGT'ACT
ClaI - 4149 - AT'CG_AT
SwaI - 4052 - ATTT'AAAT
NaeI - 3724 - GCC'GGC
NgoMIV - 3722 - G'CCGG_C
HindIII - 3131 - A'AGCT_T
SphI - 3129 - G_CATG'C
PstI - 3123 - C_TGCA'G
SbfI - 3123 - CC_TGCA'GG
SalI - 3113 - G'TCGA_C
XbaI - 3107 - T'CTAG_A
BamHI - 3101 - G'GATC_C
SmaI - 3098 - CCC'GGG
XmaI - 3096 - C'CCGG_G
KpnI - 3096 - G_GTAC'C
Acc65I - 3092 - G'GTAC_C
SacI - 3090 - G_AGCT'C
EcoICRI - 3088 - GAG'CTC
EcoRI - 2914 - G'AATT_C
PciI - 1876 - A'CATG_T
NotI - 2239 - GC'GGCC_GC
EagI - 2239 - C'GGCC_G
NheI - 2244 - G'CTAG_C
BmtI - 2248 - G_CTAG'C
b-lactamase
pEGF
4757 bp
Signal


M
1
2
V
hEGF

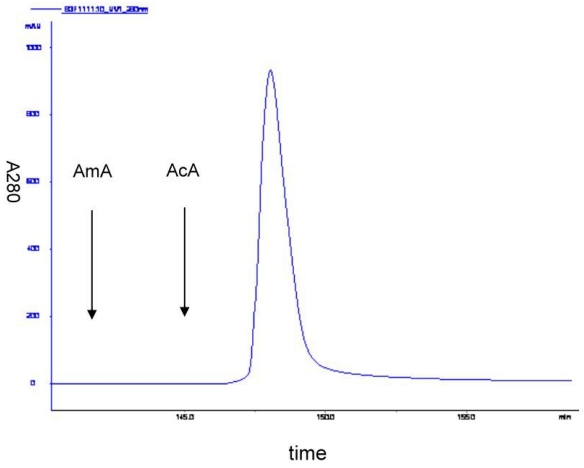
醱酵生產

100-L 醱酵槽高細胞密度培養

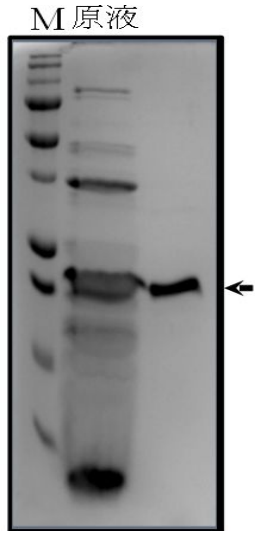


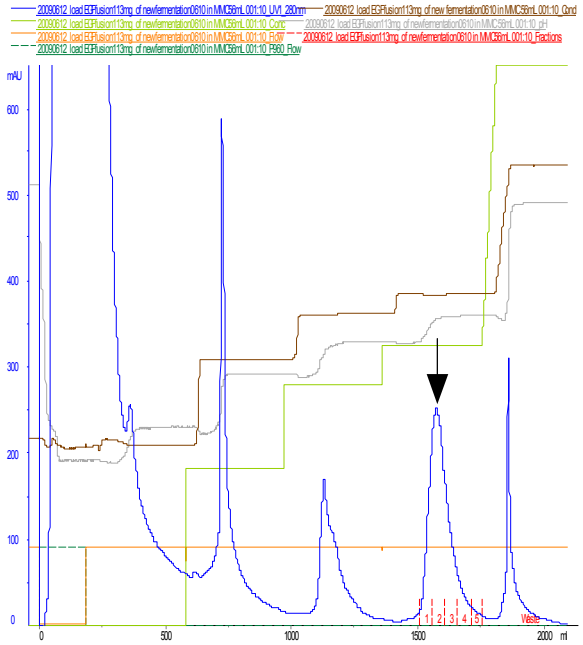
菌液分離

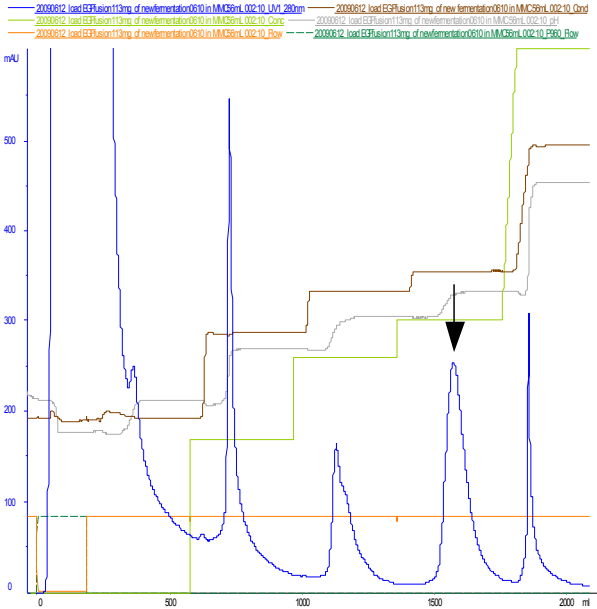
無塵室管柱層析

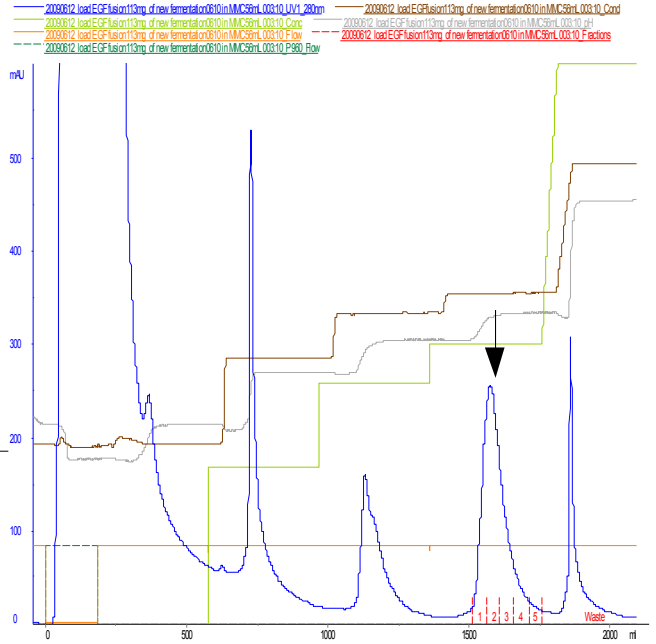


中間工場管柱層析再現性



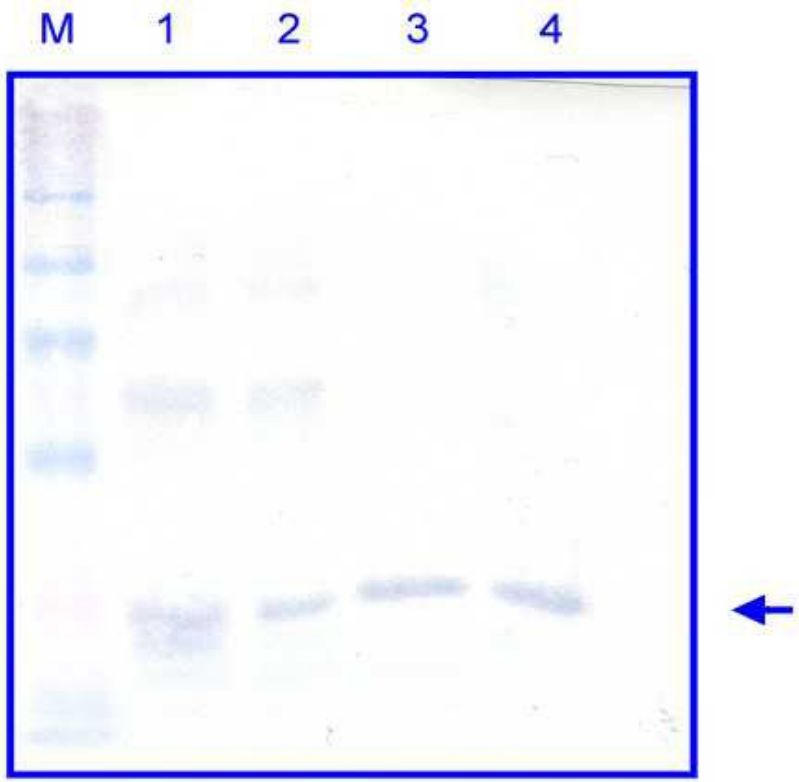






雙硫鍵結構檢測

Western blot analysis of rhEGF

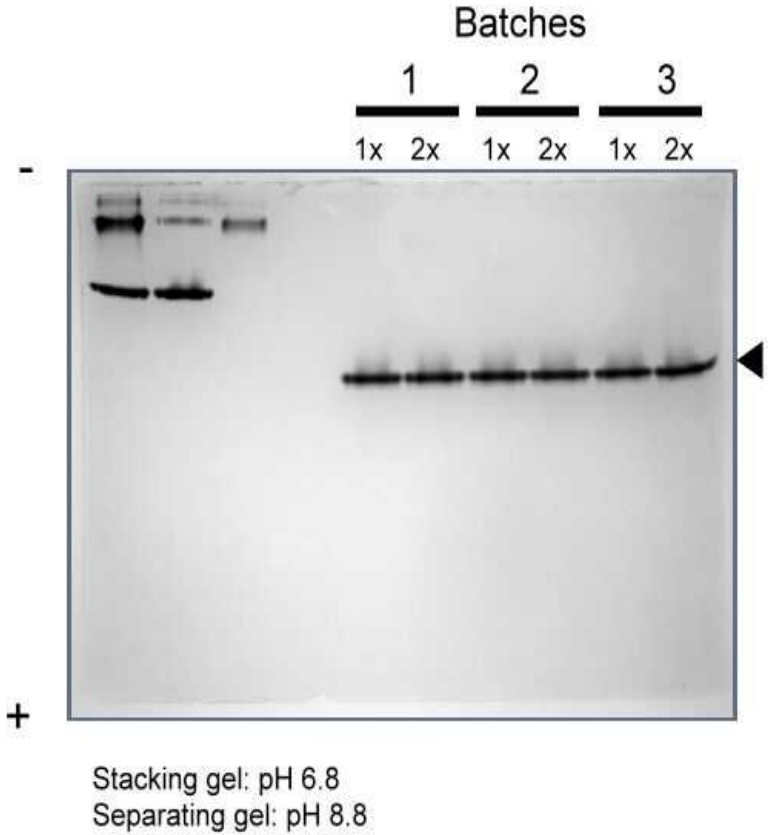


1o antibody: rabbit anti-human EGF

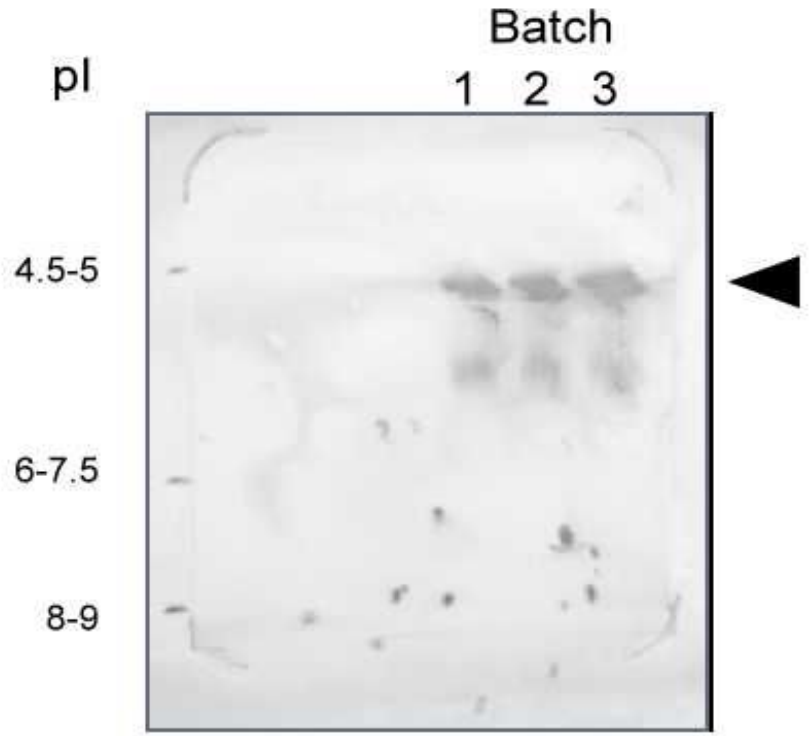
2o antibody: goat anti-rabbit HRP congugated

Lane 1,2: sample without beta-mecatoethanol
Lane 3,4: samples with beta-mecaptoethanol

結構再現性

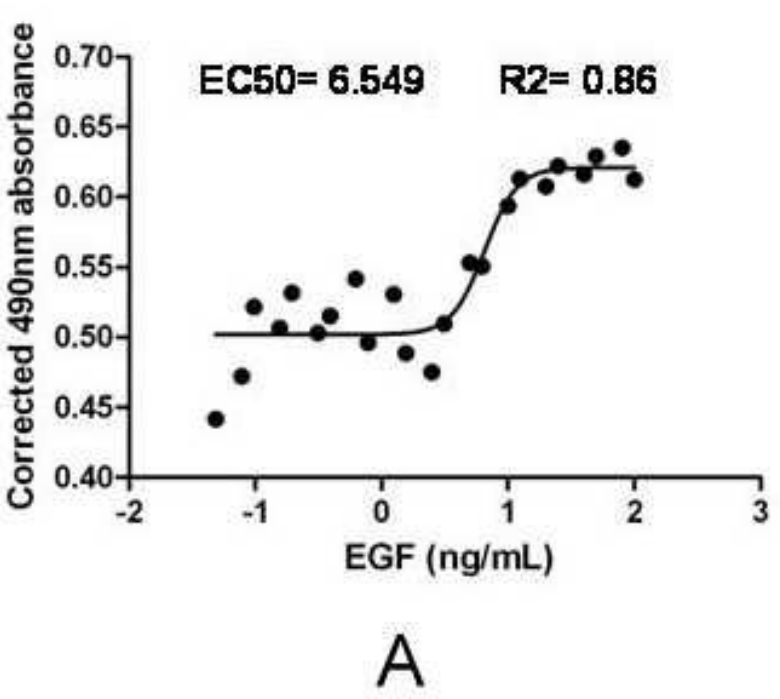


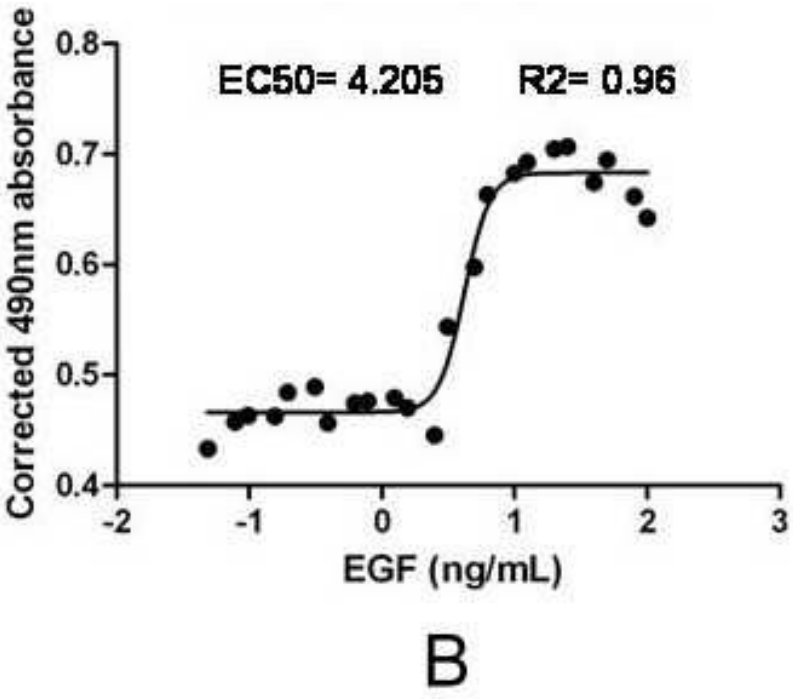
Native Gel



IEF Gel

活性比對





A: before cleavage
B: after cleavage

Balb/3T3 fibroblast

品質管制

Fusion protein stability and activity

Stability in cleavage buffer

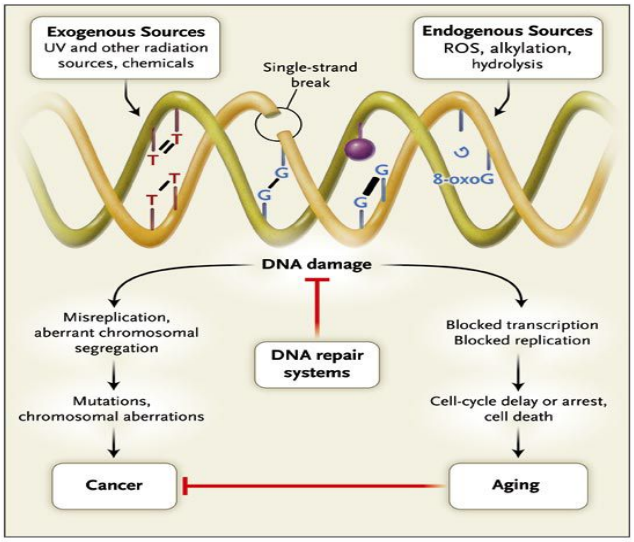
Bioburden test

Iso-electric focusing test

Native-PAGE test

Protein purity and yield: RP-HPLC

DNA 修復酵素 OGG1



圖片來源Photoageing: mechanism, prevention and therapy
M. Yaar and B.A. Gilchrest

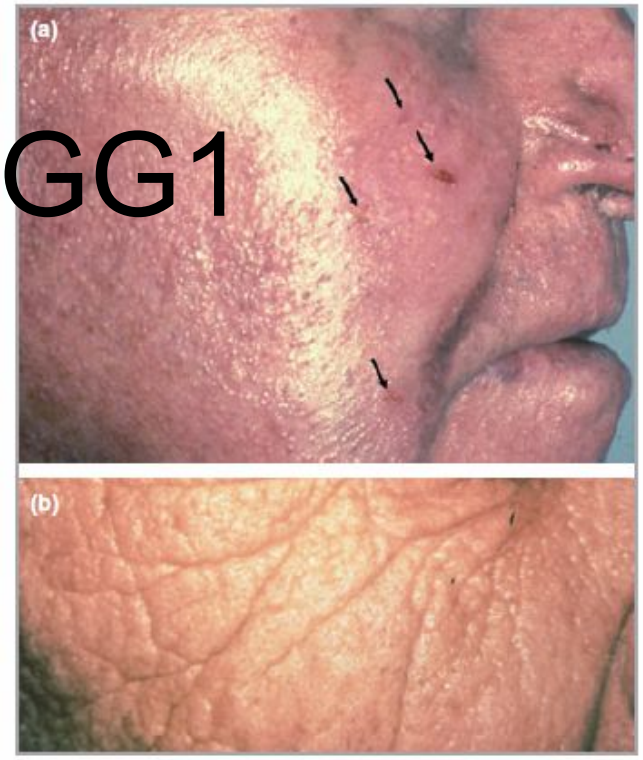


Fig 4. Photoageing. (a) An individual with skin type I displaying atrophic skin photodamage response with relatively few wrinkles but with several actinic keratoses (arrows) and a site of previous basal cell carcinoma over the lateral aspect of the nose. (b) An individual with skin type IV displaying hypertrophic skin photodamage response with deep wrinkles and leather-like coarse skin. With permission from Yaar M. The chronic effects of ultraviolet radiation on the skin: photoaging. In: Photodermatology (Lim HW, Honigsmann H, Hawk JLM, eds). New York: Informa Healthcare USA, Inc., 2007; 91–106.

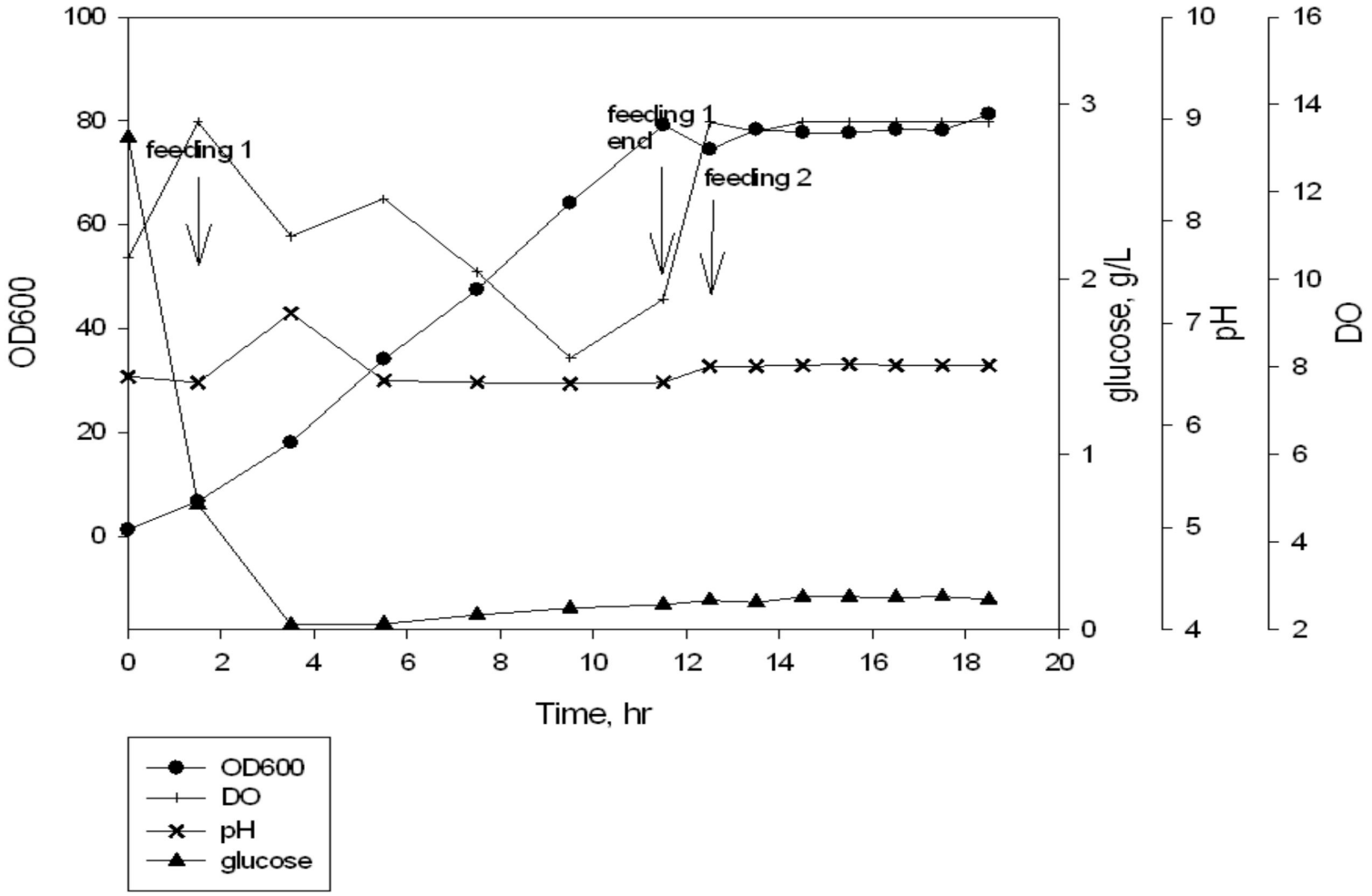
OGG1

- DNA 對於細胞有氧代謝和外源性的活性氧 (ROS) 極為敏感， ROS 可引起多種類型的 DNA 氧化損傷，形成導致基因突變作用的鹼基氧化產物。
- DNA 受氧化損傷後，產生的氧化產物 8- 羥基鳥嘌呤 (8-oxoguanine, 8-oxoG) ，如果不經修護，則會造成基因中的 G:C-->A:T 突變，此類 DNA 損傷和癌細胞發展或細胞的老化有很重要關聯。
- 8- 羥基鳥嘌呤 DNA 糖苷酶 (8-oxoG DNA glycosylase, OGG1) 能夠特異性識別受氧化的 8-oxoG ，並將其切除以協助修復，是細胞中不可缺少的酵素。

生產策略

- 改變 codon usage
- 大腸桿菌異源表現
- 降低成本
- Auto-induction/ carbohydrate 技術誘導產物

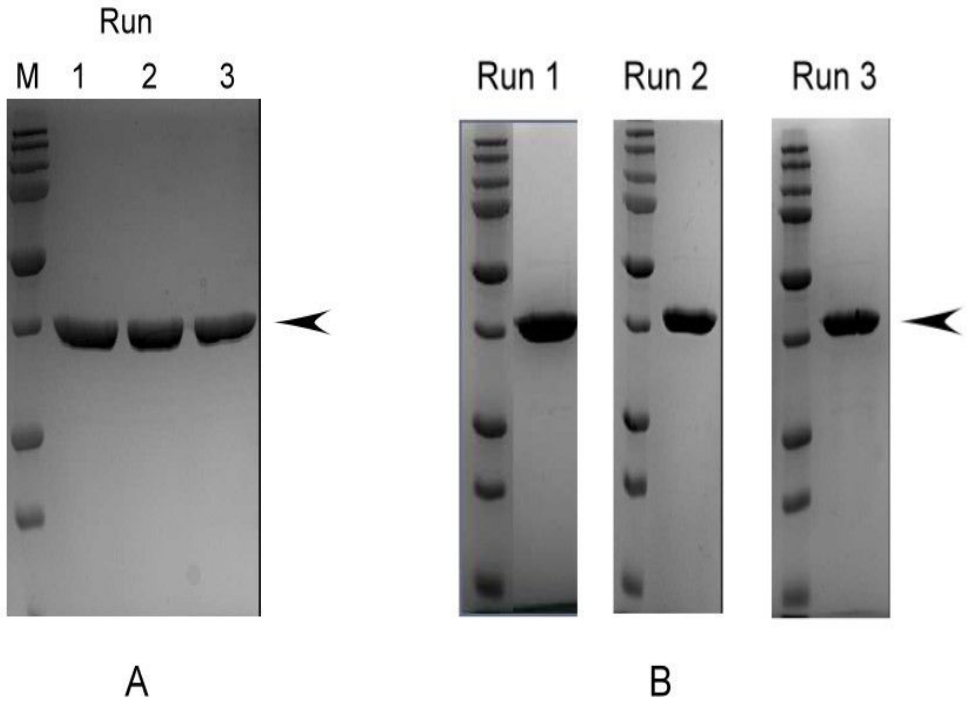
100-L 醱酵槽高密度培養



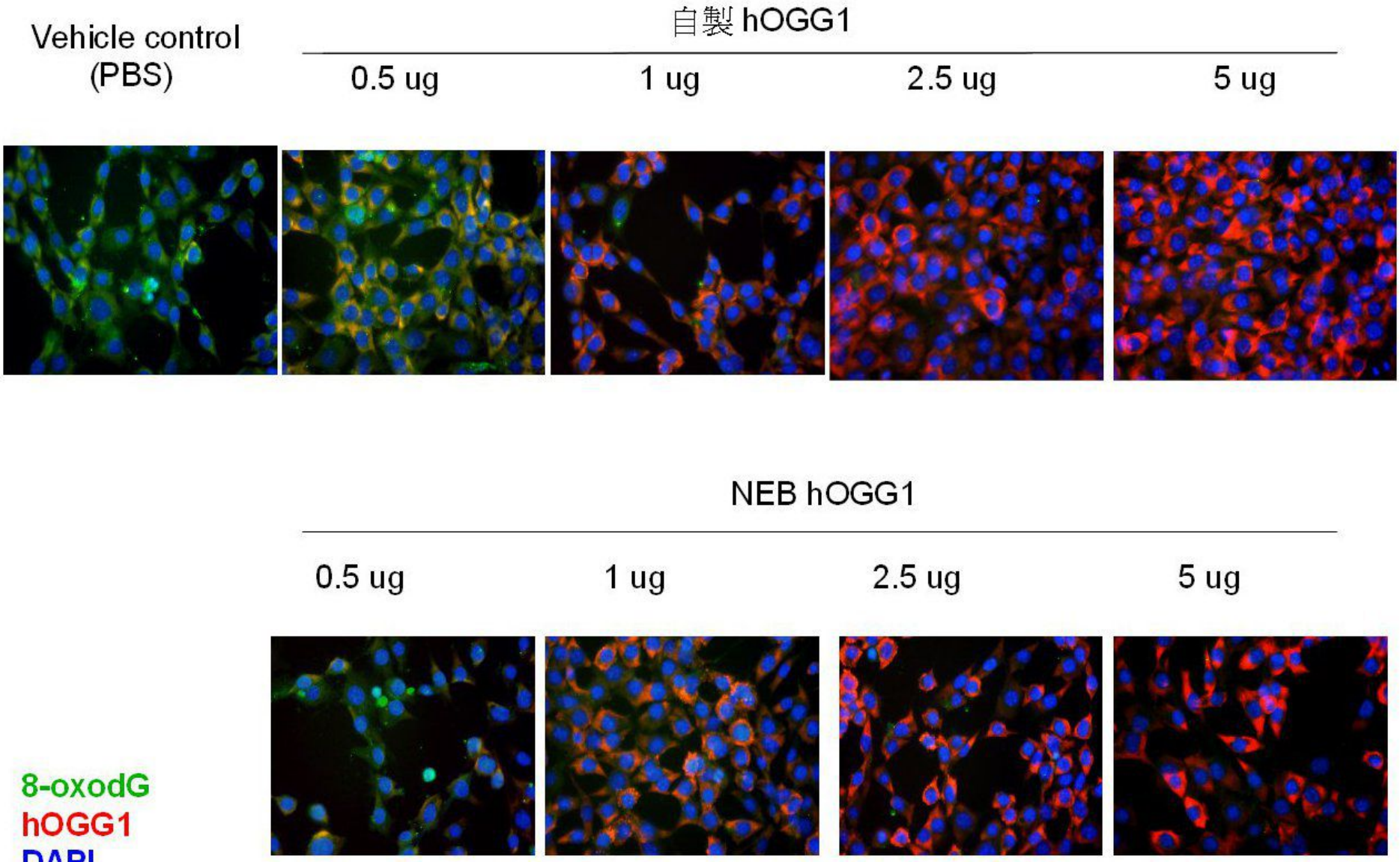
製程

- Design Space
  - pH 範圍
  - 進樣比範圍
  - 進樣濃度範圍
  - 進樣流速範圍
  - 沖洗緩衝液範圍

三重複及放大試驗結果



活性試驗 - 細胞

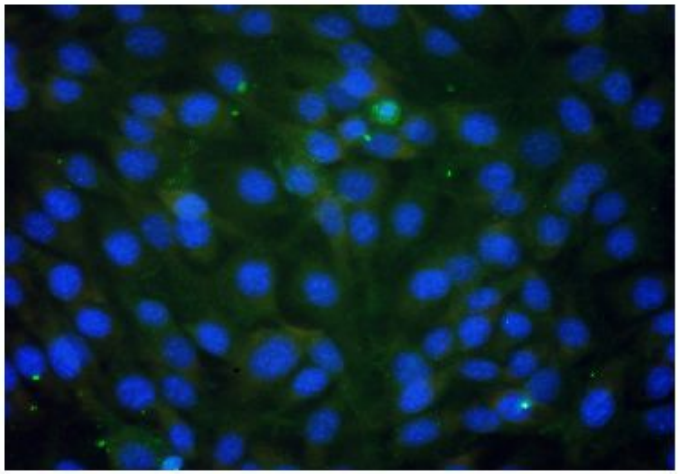

Vehicle control
(PBS)
自製 hOGG1
0.5 ug
1 ug
2.5 ug
5 ug
NEB hOGG1
0.5 ug
1 ug
2.5 ug
5 ug
8-oxodG
hOGG1
DAPI

hOGG1 保護細胞減少受到 UV 引起的 DNA 損傷 (8-oxodG)

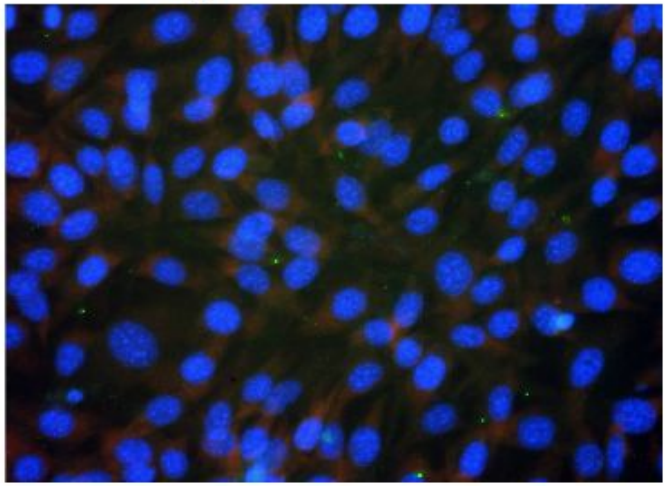
UV 28 mJ/cm^2

hOGG1

Vehicle control (0 ug)

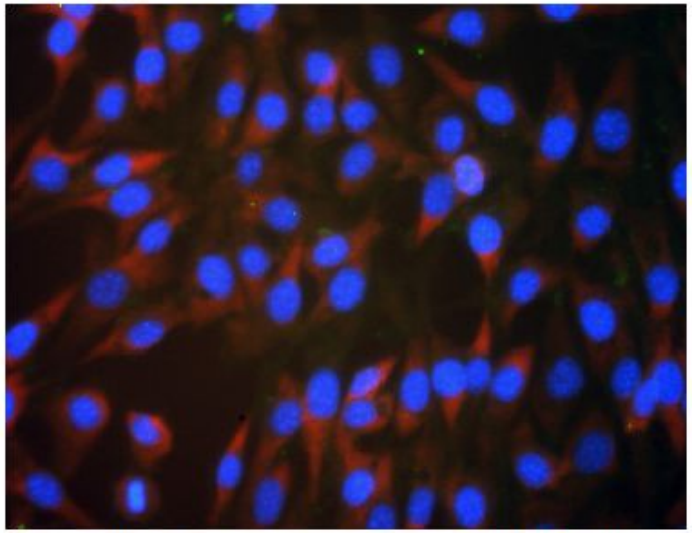


1 ug

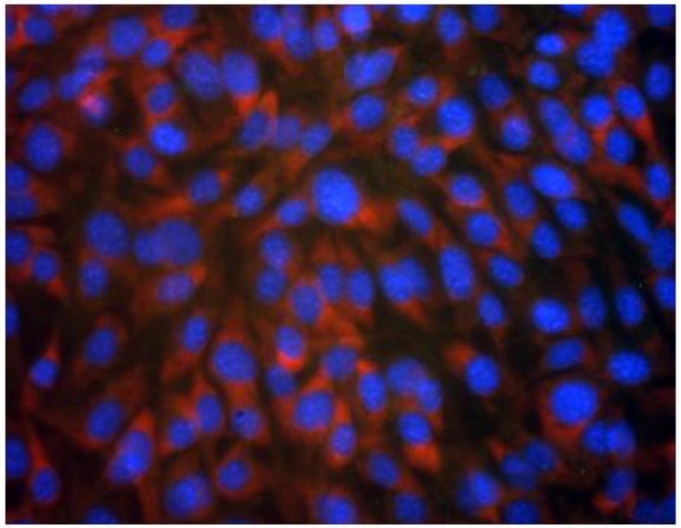


hOGG1

1.5 ug

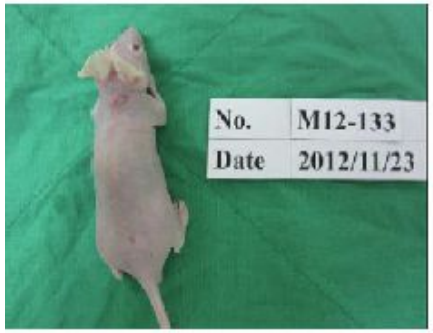


2 ug



轉染 hOGG1 2 ug 可保護 UV 引起的 DNA 損傷

hOGG1 保護 UVB 誘導皮膚腫瘤試驗



塗抹

OGG1
liposome

塗抹

blank
liposome

安全性試驗

	試驗對象	試驗樣品	試驗項目	結果
致突變性試驗	沙門氏桿菌	OGG1 liposome	沙門氏桿菌回復突變測試	不具致突變性
刺激性試驗	大白兔	OGG1 enzyme	對兔子眼睛刺激之潛在性評估	不具眼刺激性
	大白兔	OGG1 enzyme	對兔子皮膚刺激之潛在性評估	不具皮膚刺激性

OGG1 酵素溶液

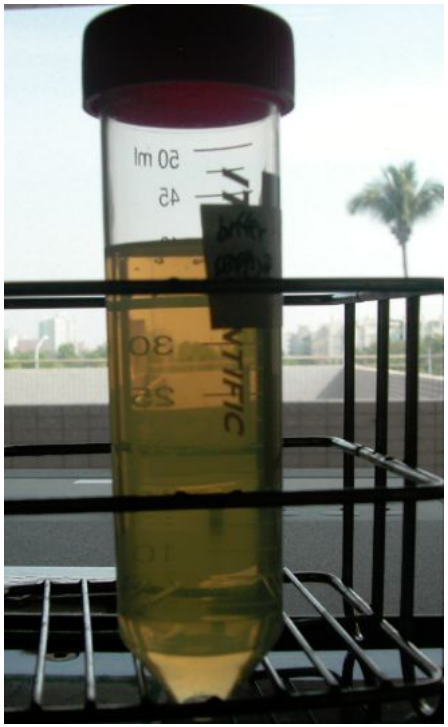
介紹：

基因工程重組蛋白	8-oxoG DNA glycosylase (OGG1)
分子量	38 kDa
等電點(PI)	8.89
組成分	OGG1
	磷酸鹽
	甘油
	甘胺酸
	水

Certificate of Analysis

項目	規格
外觀	液體
顏色	透明澄清
味道	無味道
蛋白質含量 (Bradford method)	> 110 ug/mL
總活性 (U, RFU/min/ug)	> 100
pH	6.0 ± 0.5
儲存溫度 (℃)	≤ 4

含 OGG1 酵素之微脂體



Certificate of Analysis

項目	規格	結果
外觀	液體	液體
顏色	亮黃色至霧白色	亮黃色
味道	穀物味	穀物味
蛋白質含量 (Bradford method)	> 110 ug/mL	140 ug/mL
平均粒徑	< 80 nm	55 nm
包覆率	> 60 %	75%
總活性 (U, RFU/min/ug)	> 100	128
pH	6.0 ± 0.5	6.3
儲存溫度 (℃)	4 ~ 10	4

與常用化妝品原料之相容性

項目	相容性
氯化鈉	< 1%
EDTA-2Na+	< 0.5%
乙醇	≤ 25%
1,2-丙二醇	≤ 25%
1,3-丁二醇	≤ 25%
玻尿酸	≤ 0.15%
Vit. C	≤ 2%
苯甲酸鈉	≤ 2%
pH	5~9
界面活性劑 (Triton X-100)	< 0.6%

相關設備

- 微生物醱酵槽
  - 5- 、 100- 、 700-L
- 動物細胞培養
  - Spinner flask
  - Wave disposable bag
- 回收
  - ALFA-LAVAL 連續式離心機
  - Cross-flow filtration
- 高壓連續式均質機

層析設備

- Akta Pilot, 10, 100