分子生物學應用

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主題介紹

I. 即時定量聚合酶連鎖反應（Real-time quantitative PCR, RT-PCR/Q-PCR/RT-QPCR)

II. 報告基因分析 (Reporter gene assay)

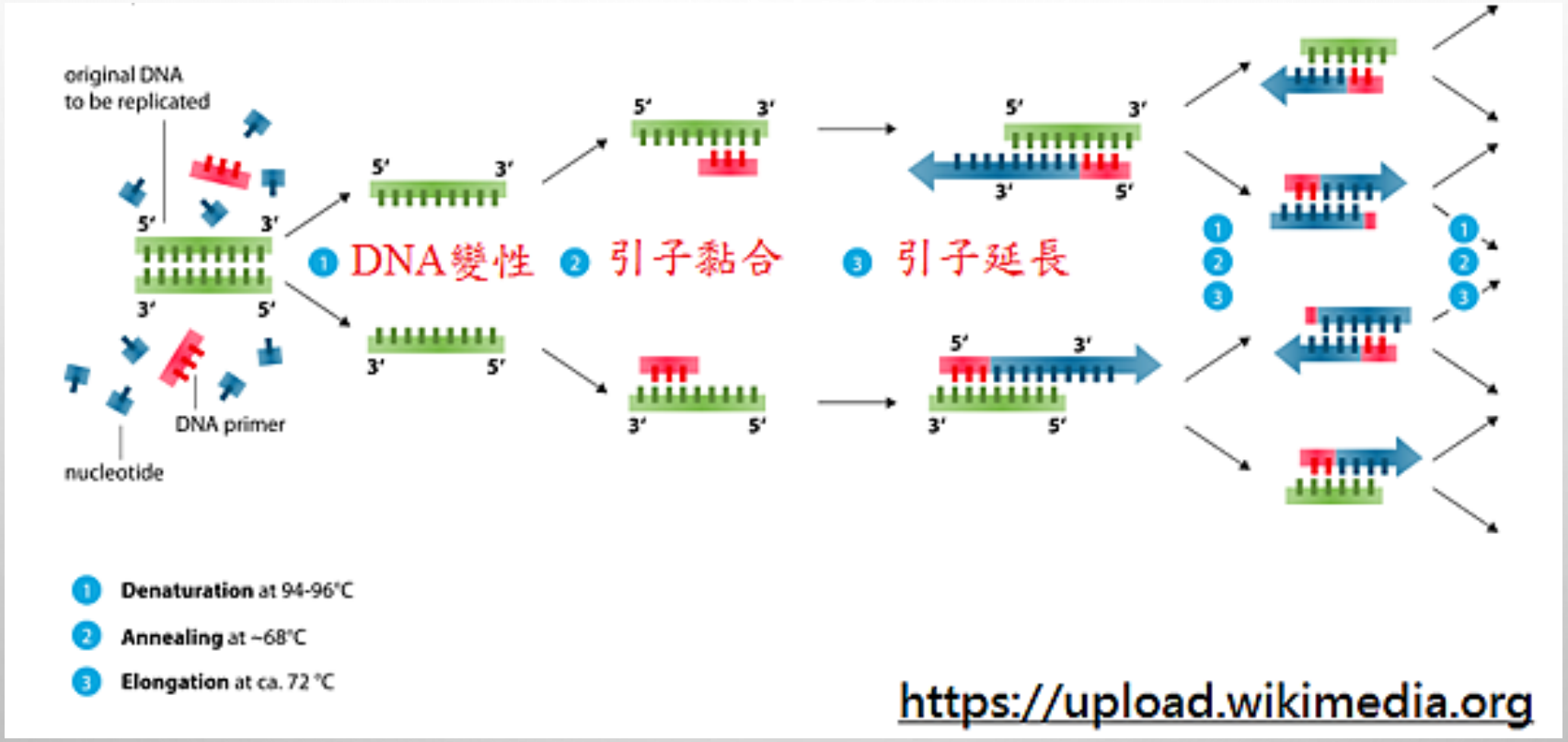
III. 核糖核酸干擾 (RNA interference, RNAi)

I. 即時定量聚合酶連鎖反應 (Q-PCR)

上課內容

- 聚合酶連鎖反應(PCR)
- Q-PCR原理
- Q-PCR步驟
- Q-PCR反應混合物
- TaqMan Probe(探針)& SYBR Green(染料)
- 使用的儀器/系統
- 如何避免Q-PCR錯誤
- Q-PCR應用

聚合酶連鎖反應
(Polymerase Chain Reaction, PCR)



The Nobel Prize in Chemistry 1993

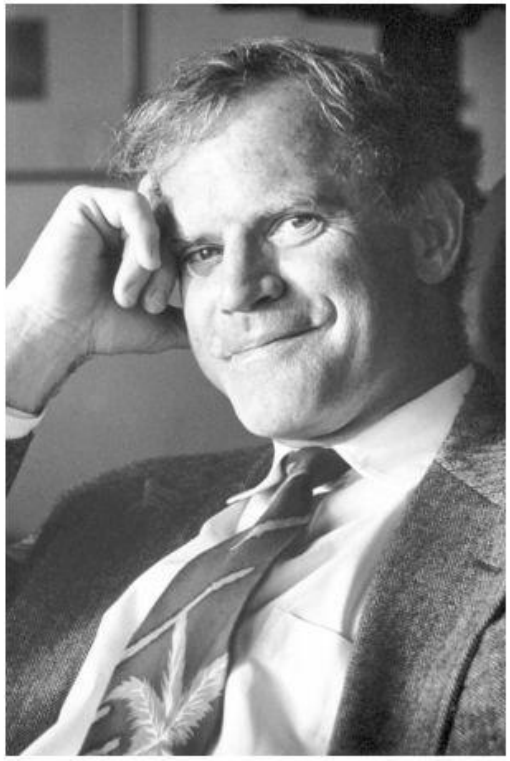


Photo from the Nobel Foundation archive.

Kary B. Mullis

Prize share: 1/2

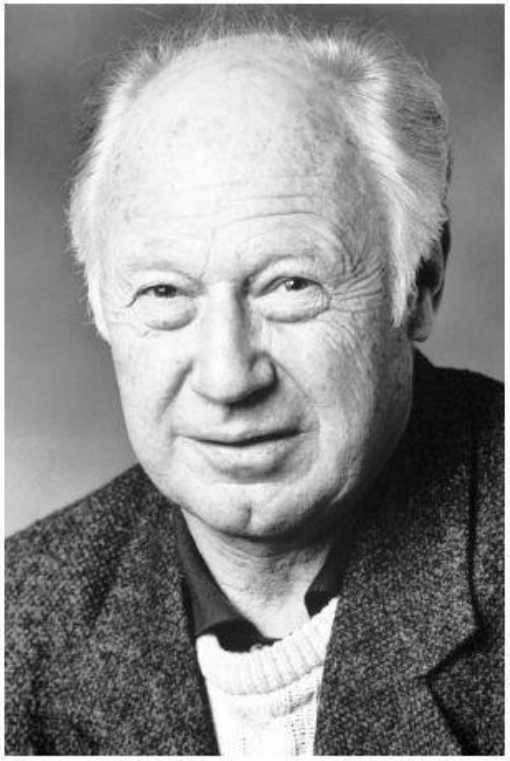


Photo from the Nobel Foundation archive.

Michael Smith

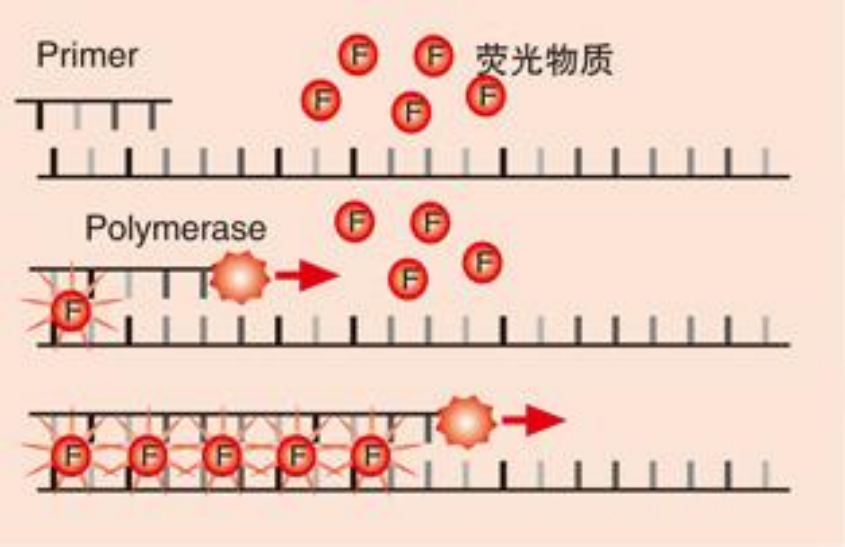
Prize share: 1/2

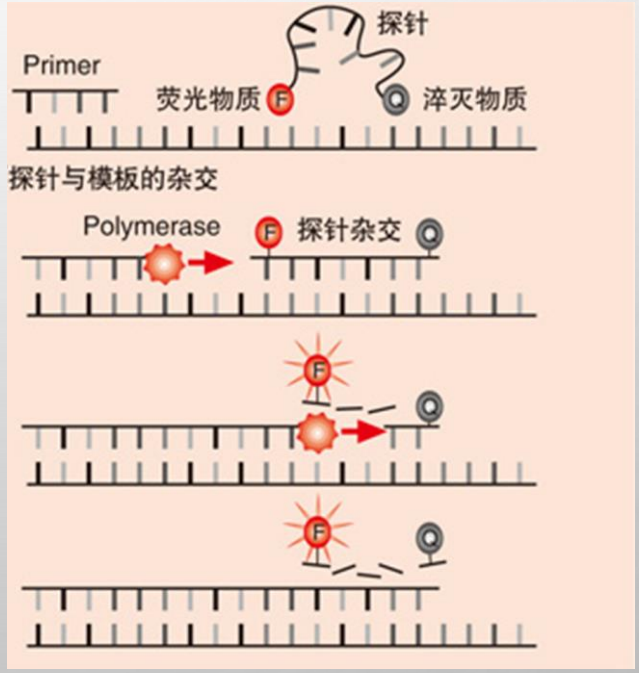
Kary B. Mullis: for his invention of the polymerase chain reaction (PCR) method.

Michael Smith: for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies.

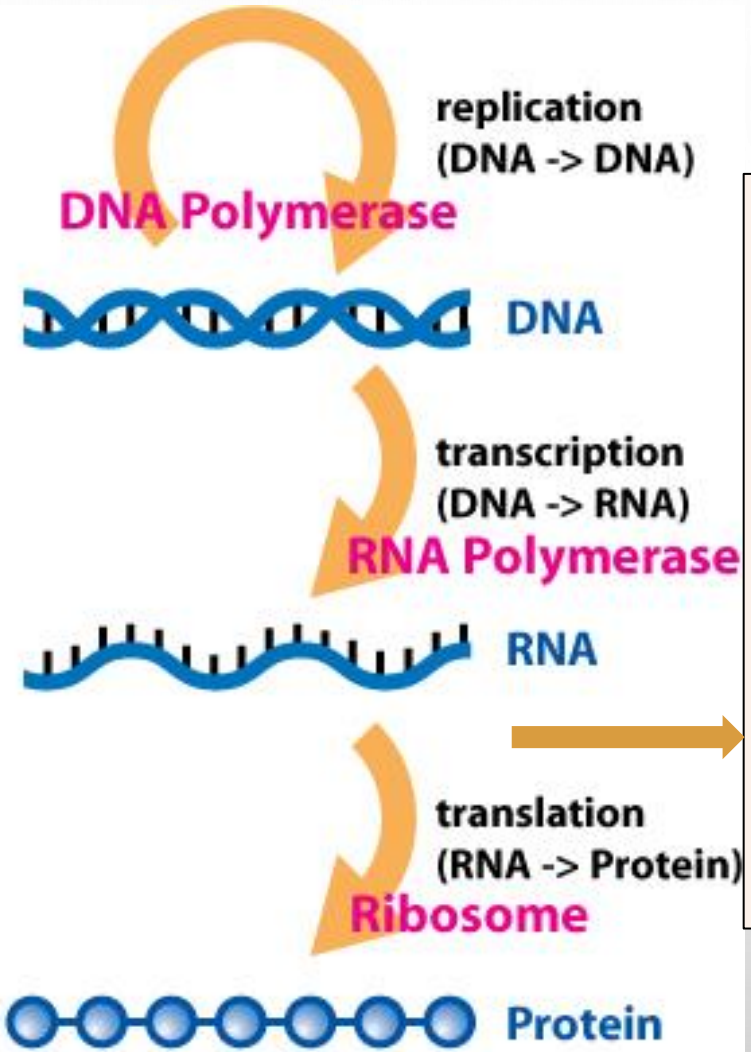
Q-PCR 原理

- 這是一種基於聚合酶鏈反應(PCR) 的技術。
- 將反應放入機器中，該機器使用相機或檢測器實時監測觀察並螢光反應檢。
- 檢測分子產生的螢光，隨著反應的進行，該分子增加。
- 可以定量相對少量的PCR產物。



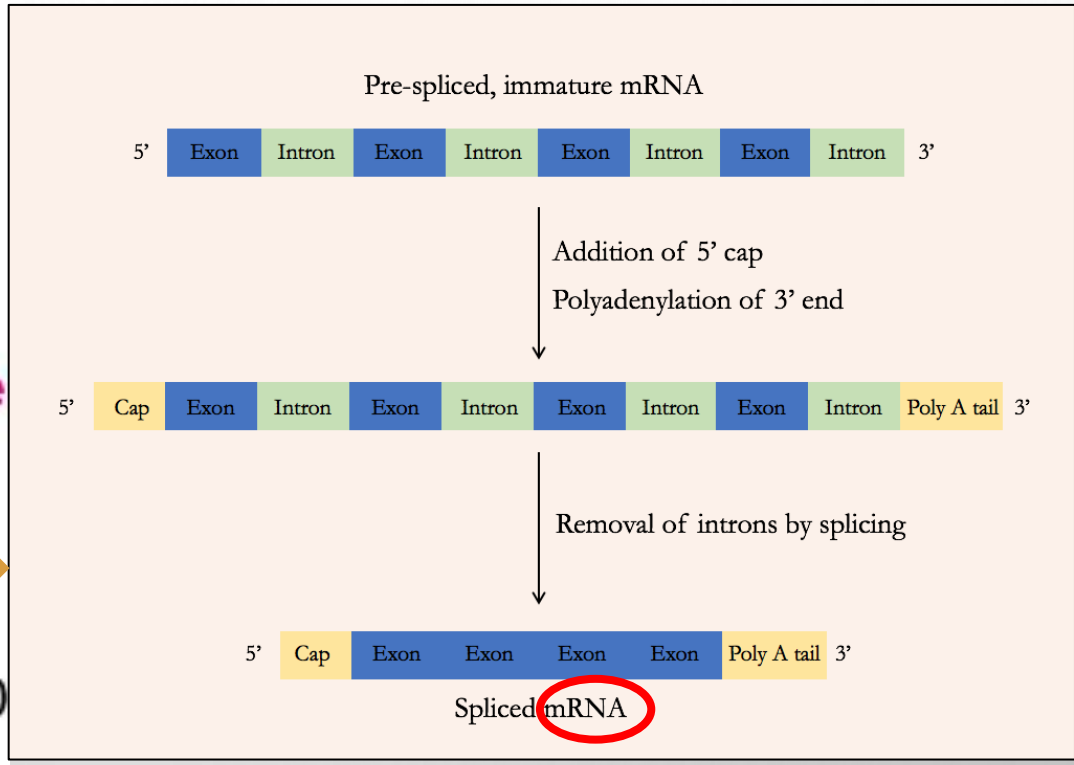


中心法則



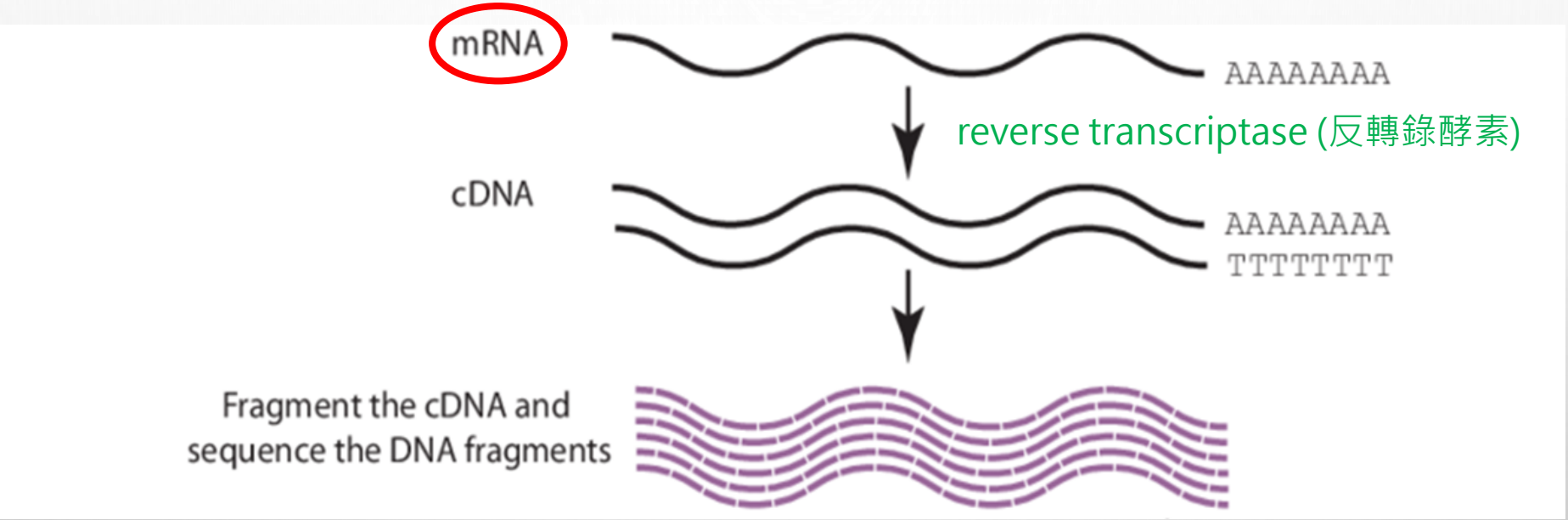
https://zh.wikipedia.org

成熟RNA修飾



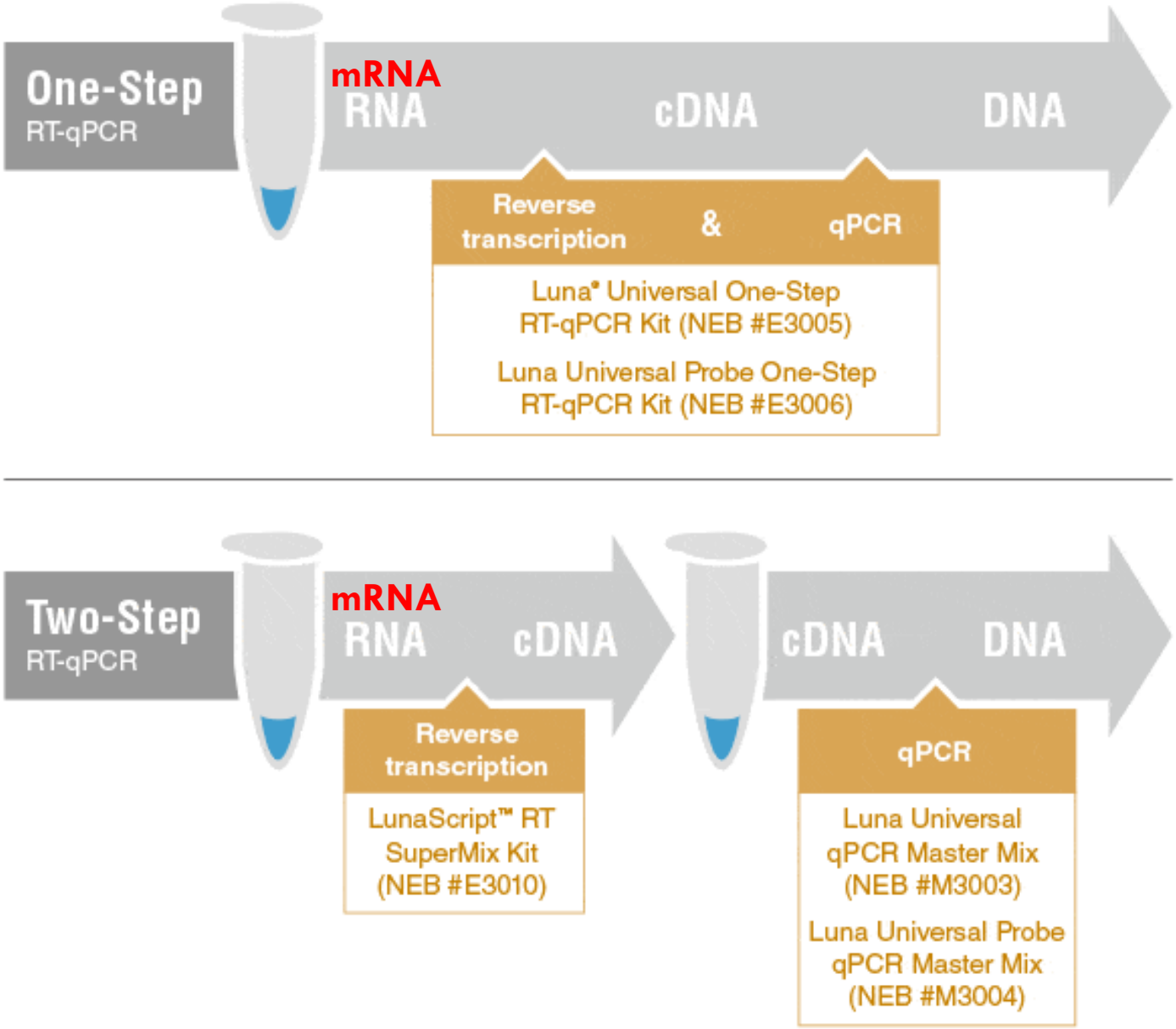
https://commons.wikimedia.org/

信使RNA (mRNA) 反轉錄成為互補 DNA (cDNA) 的 流程



Mol Cell Proteomics. 2016 Aug;15(8):2525-36.

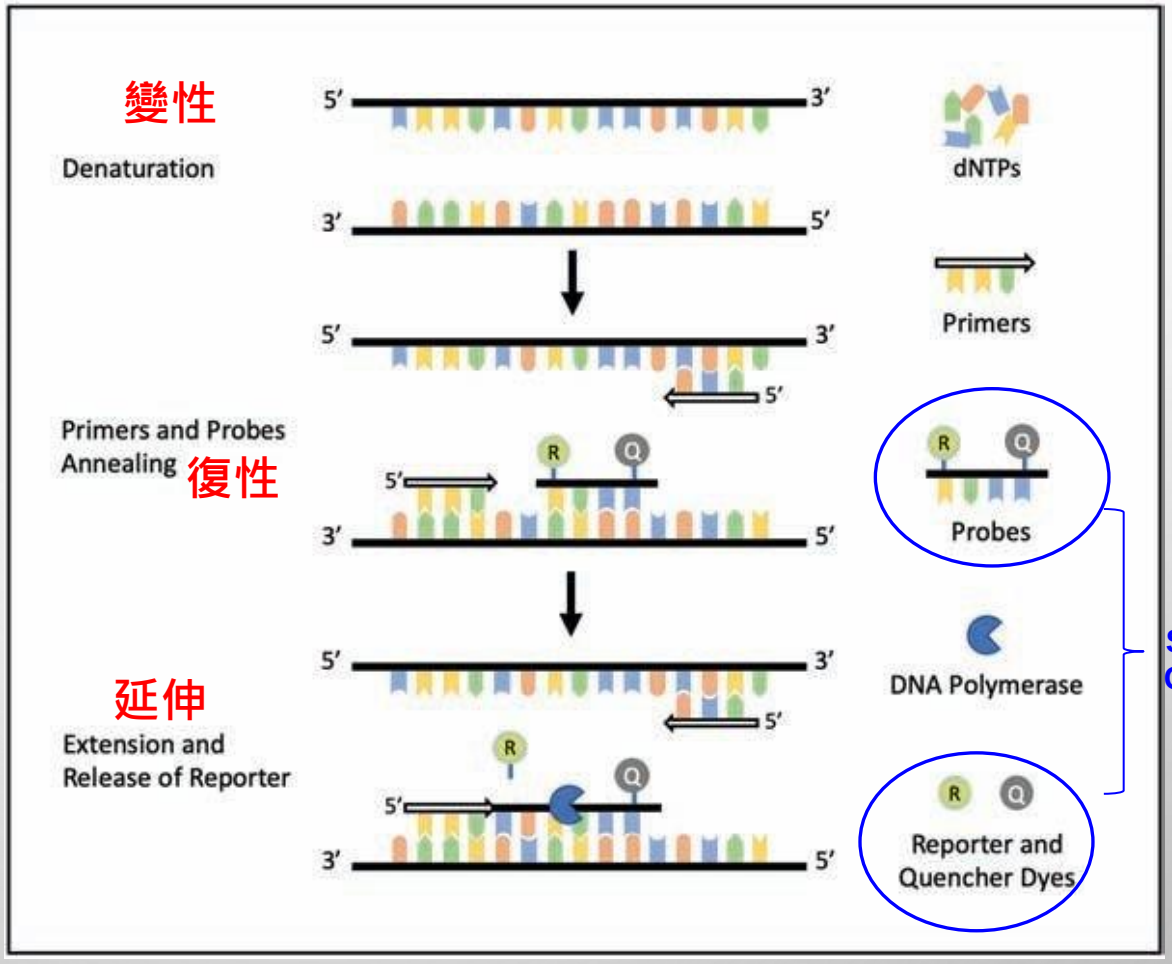
Q-PCR步驟



a. **變性**-高溫用於將雙鏈DNA「熔化」成單鏈，並鬆動單鏈DNA中的二級結構。通常使用DNA聚合酶可以承受的最高溫度（通常為95°C）。如果範本GC含量高，可以增加變性時間。

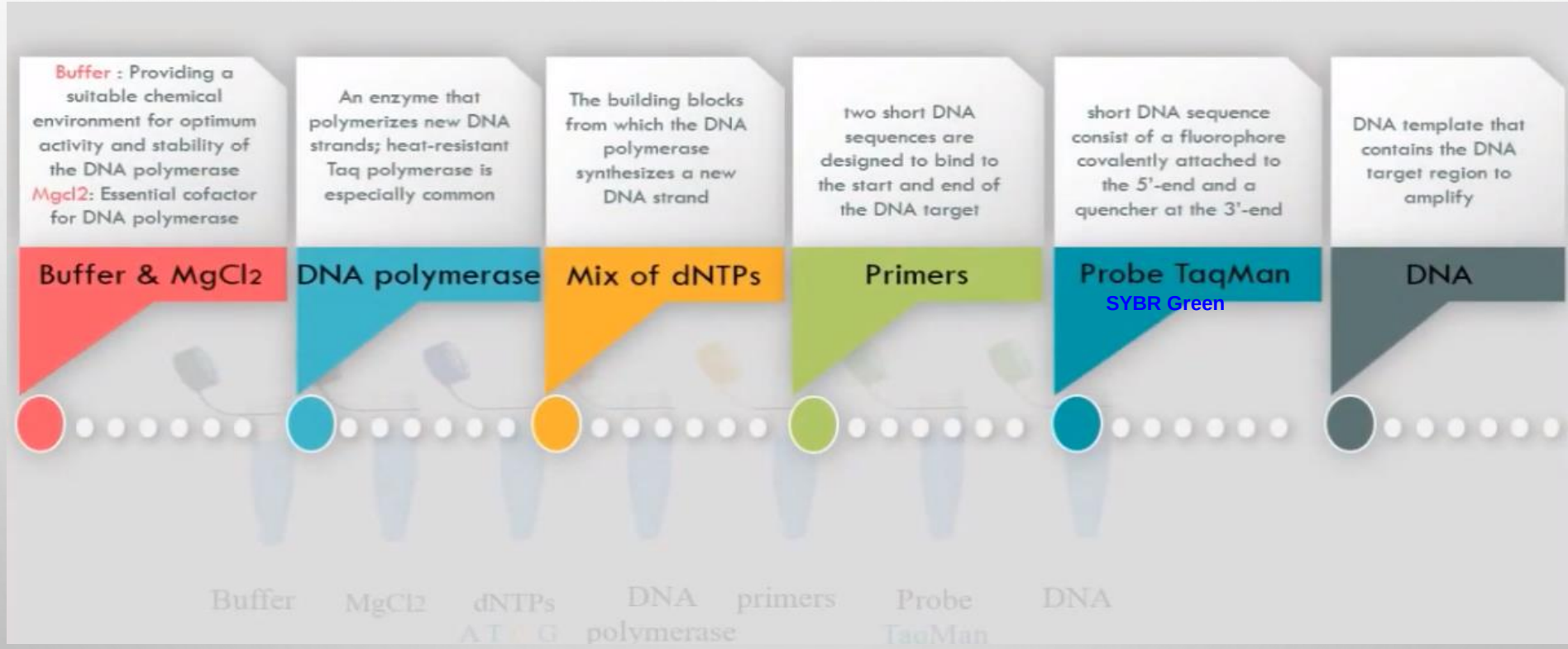
b.**復性**-在復性過程中，互補序列有機會雜交，因此使用基於引物的計算熔融溫度（Tm）的適當溫度（低於引物Tm的5°C）。

c. **延伸**-在70-72°C時，DNA聚合酶的活性是最佳的，引物延伸以高達每秒100個鹼基的速率發生。當即時螢光定量PCR中的擴增子很小時，該步驟通常與使用60°C作為溫度的復性步驟相結合。



https://www.youtube.com/watch?v=wBrNbbAIAFo

Q-PCR反應混合物



https://www.youtube.com/watch?v=wUDysO8bFbA

TaqMan Probe(探針)& SYBR Green(染料)

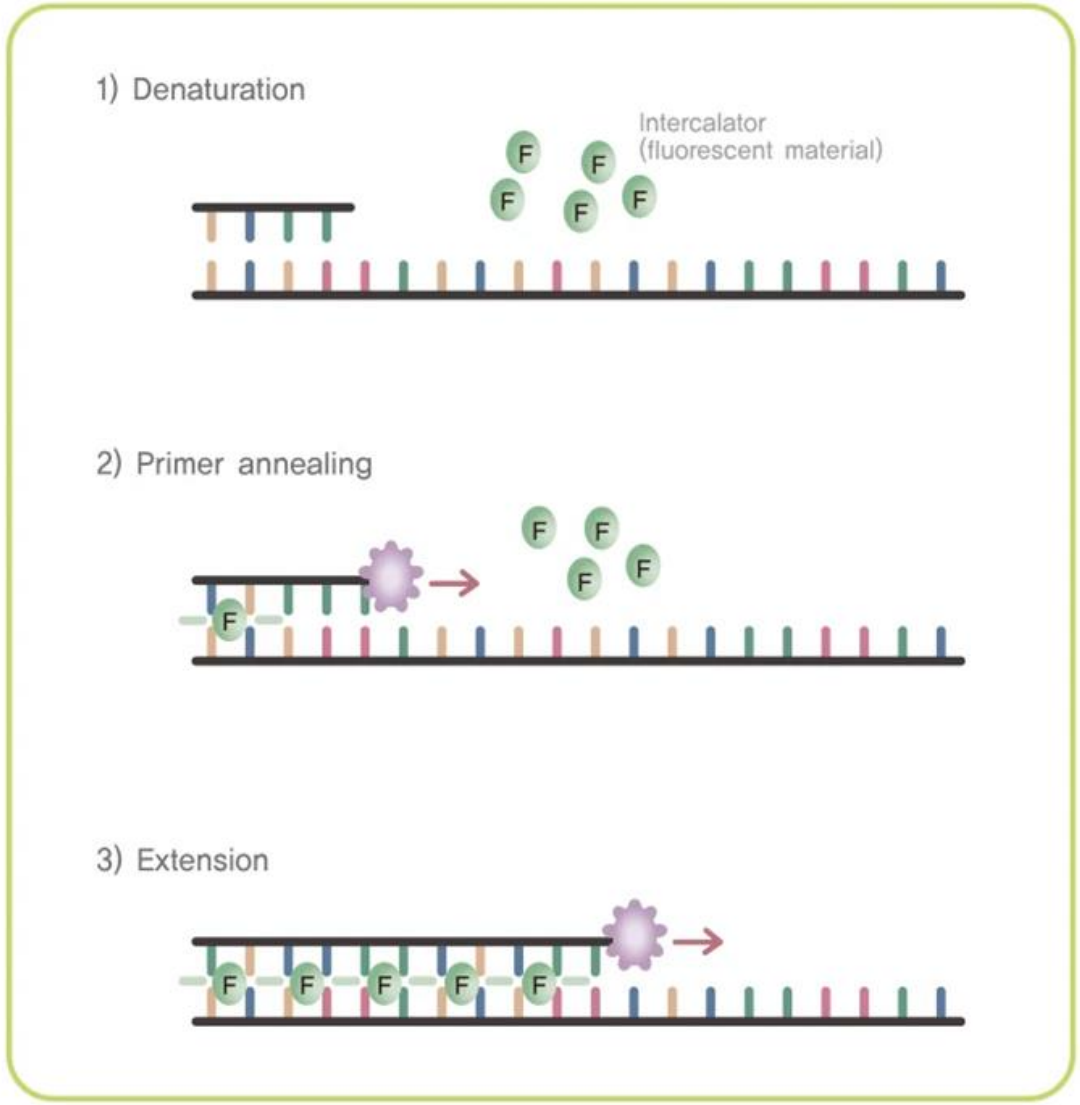
- 每個Q-PCR 都包含一個螢光報告分子（例如 TAQMAN® 探針或 SYBR® GREEN染料），用於監測 PCR 產物的積累。
- 隨著靶擴增子數量的增加，螢光團發出的螢光量也隨之增加。
- 為使用Q-PCR 進行基因表達研究而開發的兩種類型的化學物質是：

(1) SYBR Green 染料

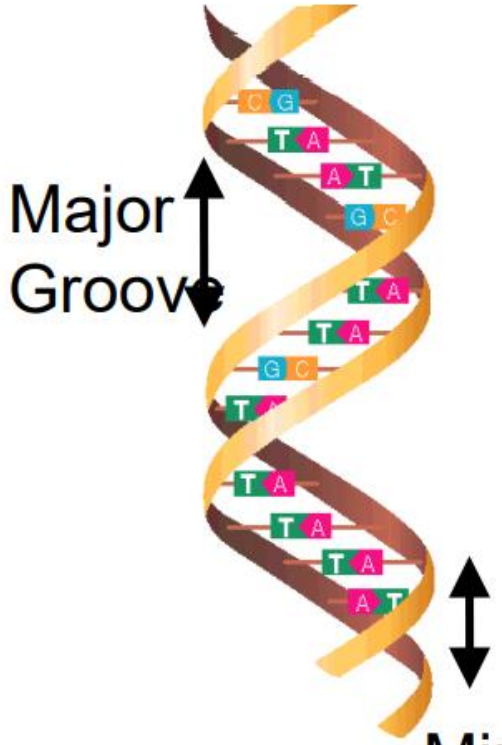
(2) TaqMan Probe 探針

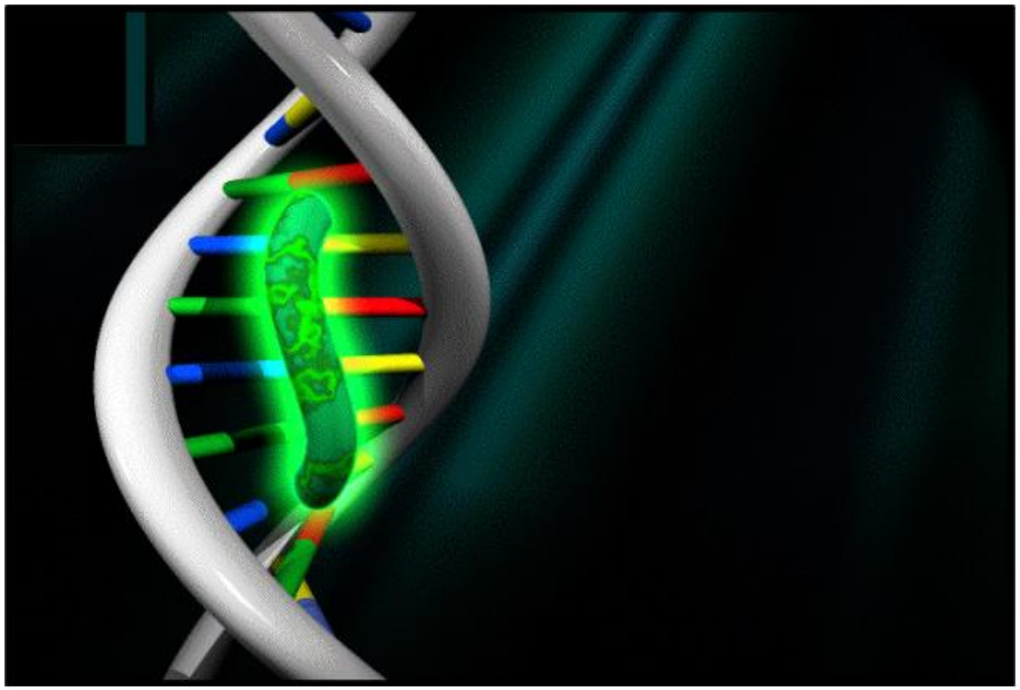
(1) SYBR GREEN 染料

- 這是一種染料，當它在DNA的微小凹槽處非特異性結合時，會發出突出的螢光信號。
- 也可以使用其他螢光染料，如溴化乙錠(EtBr)或吖啶橙(Acridine Orange)，但SYBR Green更適合用於其更高的信號強度。
- SYBR Green比TaqMan Probe更受歡迎，因為它可以提供有關每個擴增周期的資訊以及有關TaqMan Probe無法獲得的熔化溫度的資訊。
- 然而，與TaqMan Probe相比，其缺點是缺乏特異性。



- A ‘minor groove’-binding molecule specific to the minor groove of double-stranded DNA
- Fluoresces at an increased intensity when bound

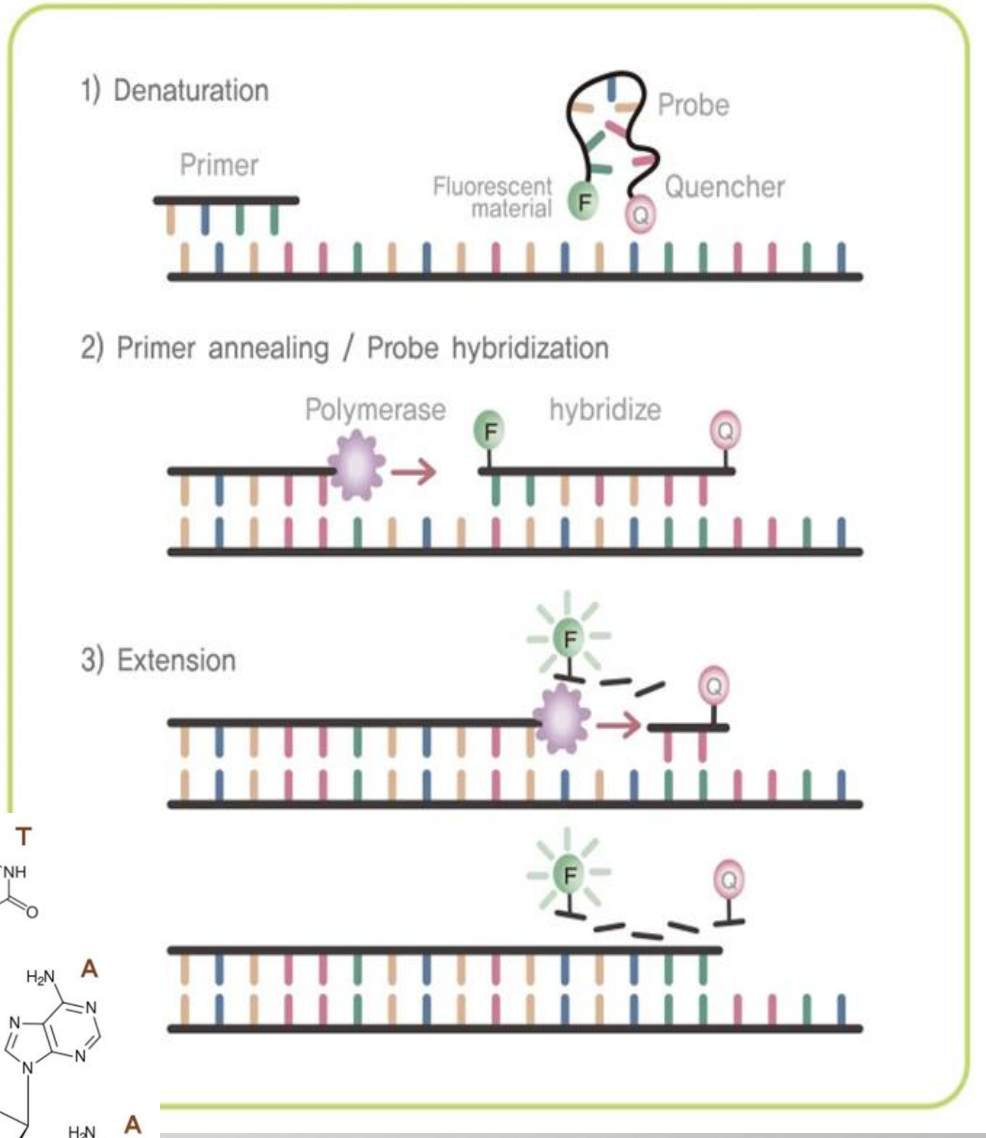


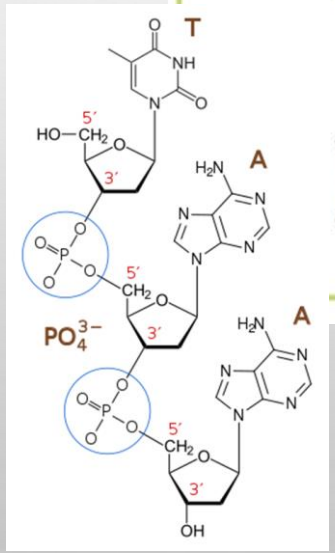


life technologies

(2) TaqMan Probe 探針

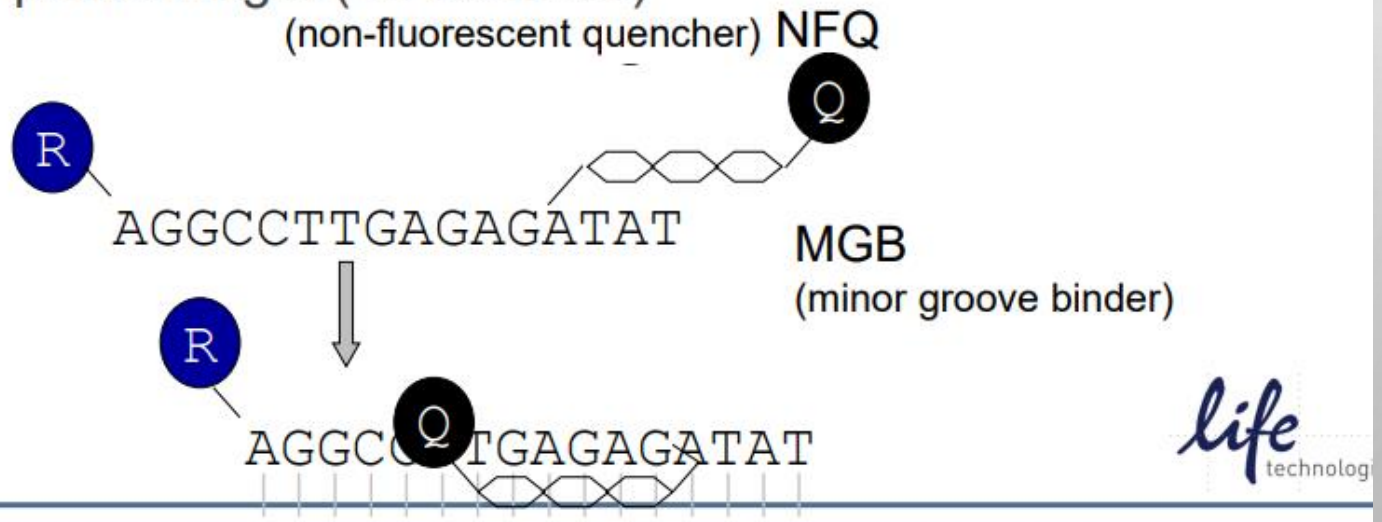
- 它是一種水解探針，帶有染料，通常在其5端有螢光素（FAM），並且在寡核苷酸的3端附著一個淬滅劑四甲基羅丹明（TAMRA）。
- 在正常情況下，探針保持盤繞在自身上，使螢光染料靠近淬滅劑，從而抑制或淬滅染料的螢光信號。
- 當聚合酶在延伸階段開始合成新的DNA鏈時，它通過5'末端核酸酶活性導致探針降解，螢光素與淬滅劑分離，從而產生螢光信號。
- 隨著該過程的繼續，在每個迴圈中信號分子的數量增加，導致螢光的增加，這與靶標的擴增呈正相關。





TaqMan® Probe: TaqMan® MGB/NFQ Probes

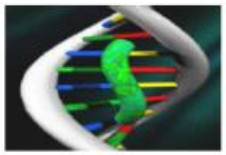
- Minor Groove Binder (MGB)
  - Small molecule that fits snugly into minor groove of duplex DNA
  - Stabilizes probe annealing
- Non-fluorescent Quencher (NFQ)
  - "Dark" quencher acts as energy transfer acceptor that doesn't emit a detectable fluorescent signal
  - MGB probe design uses a special algorithm in Primer Express® Software
- Shorter probe length (13-25-mers)



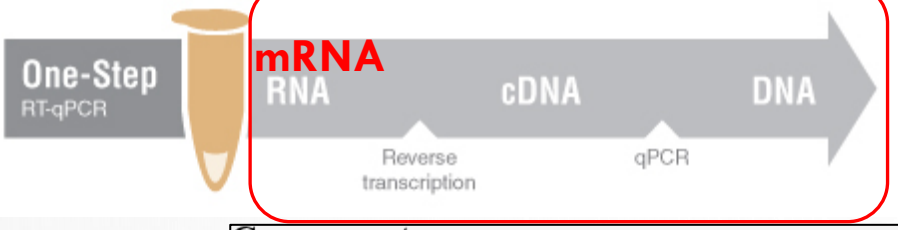
ThermoFisher SCIENTIFIC

life technologies

https://www.youtube.com/watch?v=ob3teCrpgxY

	TaqMan® Probe	SYBR® Green 1 Dye
		
Specificity	• Highly specific • Probe Hybridization	• Less specific
Sensitivity	• Very High	• Very High
Flexibility	• Multiplex PCR • SNP detection • +/- application	• No Probe is required • Screening tool
Optimization	• Ready to use 20x primer/probe mix - no need to optimize • Gold standard for MAQC • PCR efficiency 100% ±10%	• Need to optimize PCR program • Need to check primer-dimer info • Need to check PCR efficiency

一管全部反應完



Component		Volume/ reaction
Master mix		20 µl
Nuclease free water	12 µl	
5x Qiagen OneStep RT-PCR buffer	5 µl	
dNTP Mix (10 mM of each dNTP)	1 µl	
Primer Forward (100 pmol/ul)	0.5 µl	
Primer Reverse (100 pmol/ul)	0.5 µl	
Qiagen OneStep RT-PCR Enzyme Mix	1.0 µl	
Template RNA		5 µl
Total		25 µl

Reaction mixture

Master mix	Volume per one reaction
2X SYBR Green RT-PCR reaction mix	12.5 μL
Forward primer (10 μM)	1 μL
Reverse primer (10 μM)	1 μL
Nuclease-free H_2O	9 μL
RNA template (1 pg to 100 ng total RNA)	1 μL
iScript reverse transcriptase for one-step RT-PCR	0.5 μL
Final reactions volume	25 μL

Evidence-based Complementary and Alternative Medicine 2012(3):656025

分兩管反應完

1st

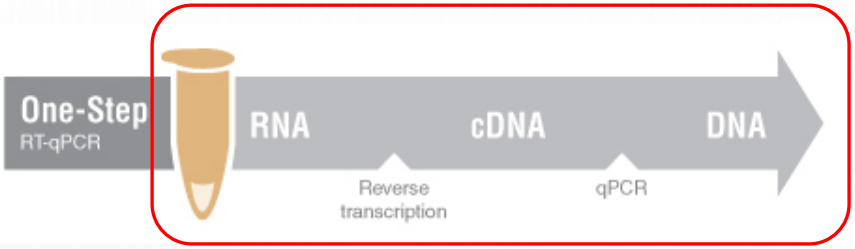
Component	Volume/Reaction	Final Concentration
Quantitect SYBR Green PCR Master Mix	25 µL	1×
Primer F	Variable	0.3 µM
Primer R	Variable	0.3 µM
RNAse Free Water	Variable	
Template cDNA	Variable	≤500 ng/reaction
Total Volume	50 µL	

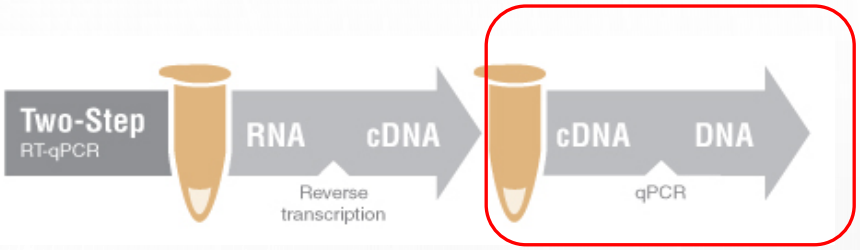
August 2018 Journal of Physics Conference Series 1073(3):032068

2nd

PCR MASTER MIX COMPONENTS

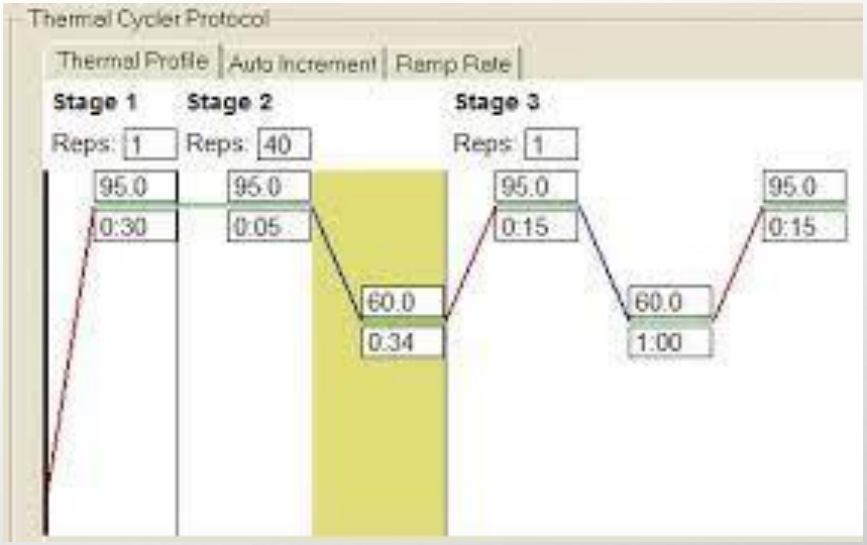
- Enzyme
- Buffer(s)
- Cofactor - Magnesium chloride ($MgCl_2$), is the most common. Sometimes $MgSO_4$ is used with particular enzymes.
- dNTP
- Primers
- Template DNA (if all samples will be uniform)
- Nuclease-free or PCR-grade water





Protocol

Process	Duration/temperature
cDNA synthesis	20 min at 50°C
iScript reverse transcriptase inactivation	4 min at 95°C
PCR cycling and detection (standard PCR cycle)	
Denaturation	10 sec at 95°C
Annealing/extension	30 sec at 61°C (data collection step)
Repeated for (30 to 45 cycles)	
	1 min at 95°C
Melt curve analysis (optional)	1 min at 55°C
	10 sec at 55°C (80 cycles, increasing each by 0.5°C each cycle)



常用哪種儀器/系統

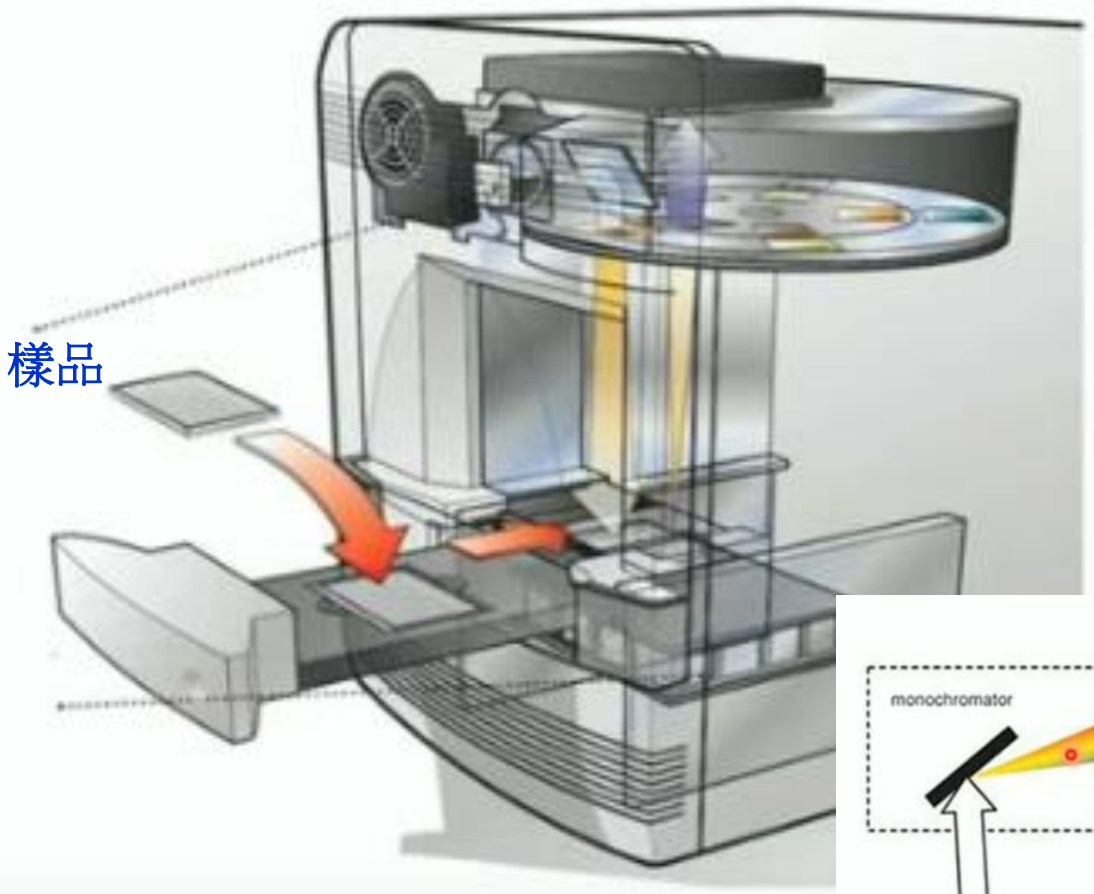
（1）ABI系統

Quantstudio systems
1,3,5,6,7

https://www.thermofisher.com/tw/zt/home/life-science/pcr/real-time-pcr/real-time-pcr-instruments/quantstudio-systems/models/quantstudio-3-5.html

https://www.thermofisher.com/tw/zt/home/life-science/pcr/real-time-pcr/real-time-pcr-instruments/quantstudio-systems/accessories.html

分光螢光計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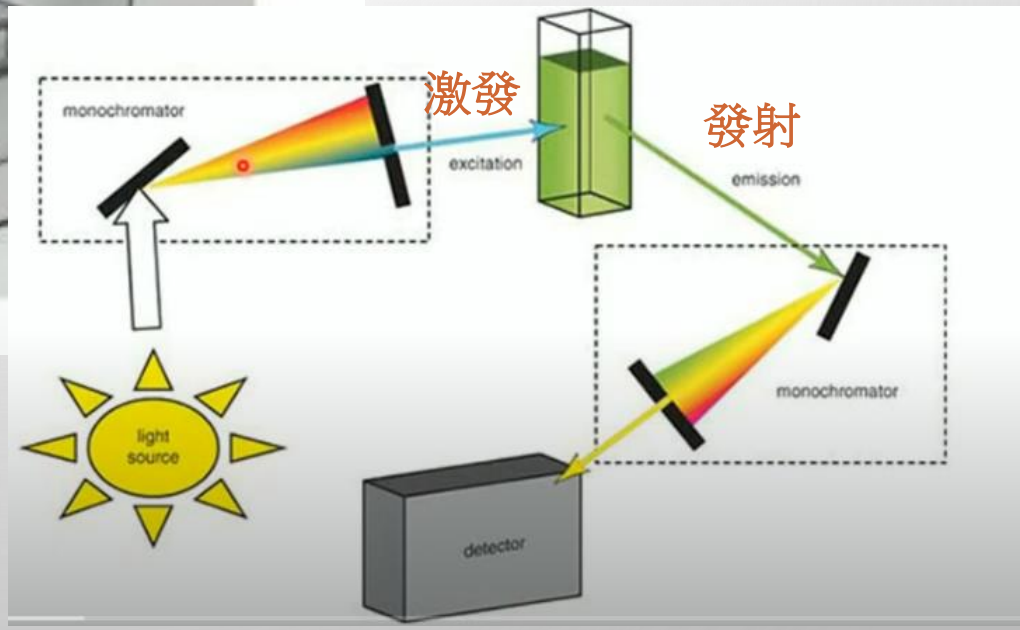
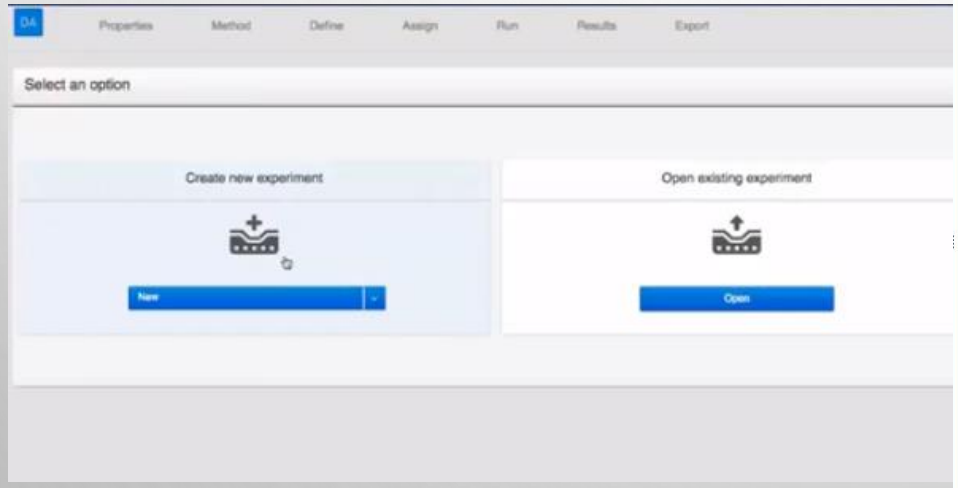

monochromator
激發
excitation
發射
emission
monochromator
light source
detector

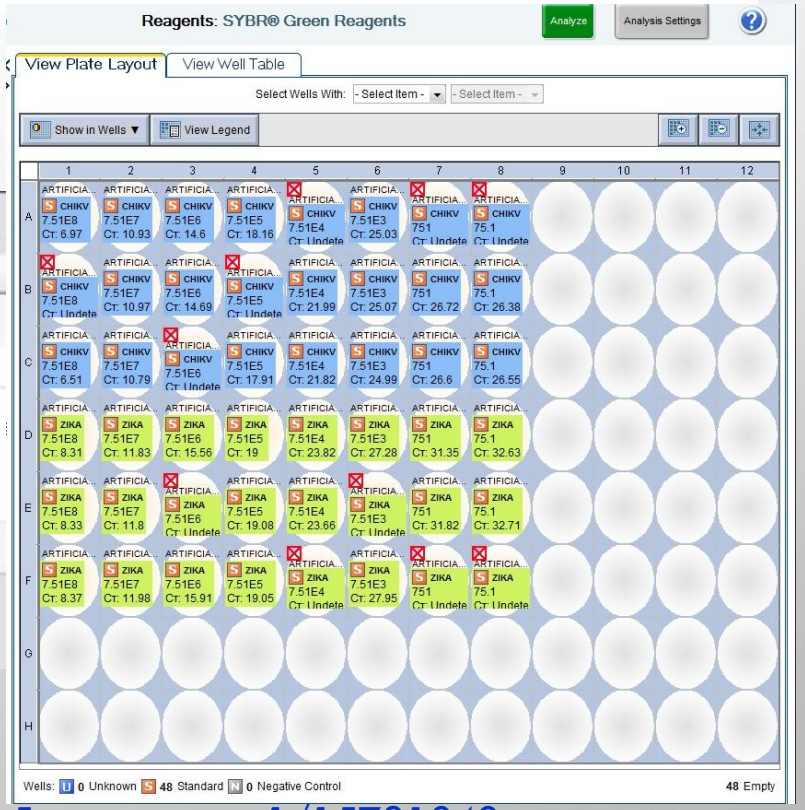
實驗材料

https://www.youtube.com/watch?v=1uh2ZxZJtgs

https://youtu.be/K5flNp46wxo

程式設置

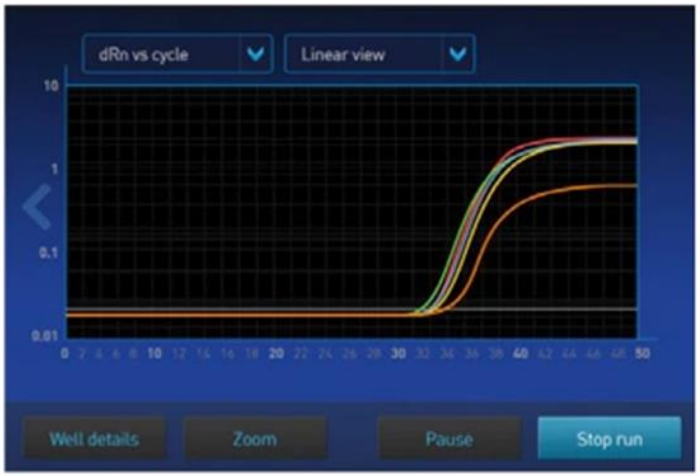


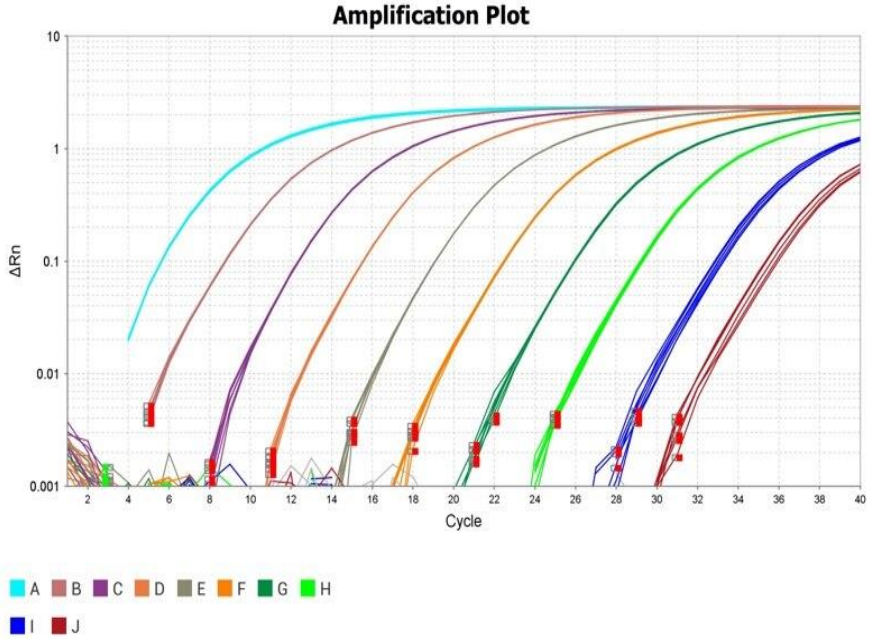


https://www.fishersci.se/shop/products/quantstudio-5-qpcr-system-1/15731248

數據分析

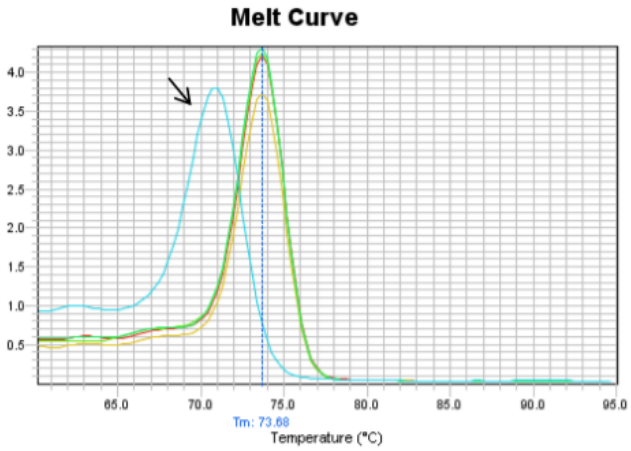
10-Log dynamic range sensitivity

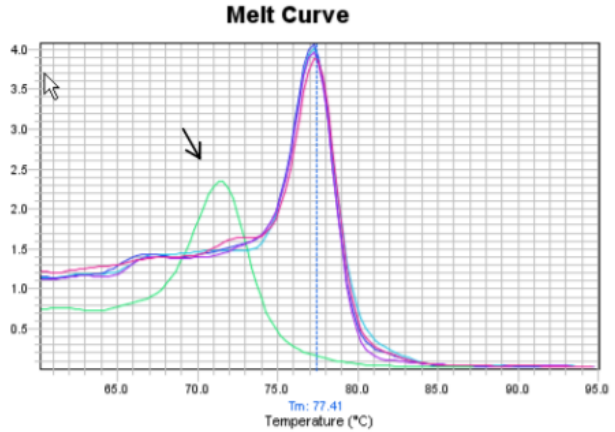


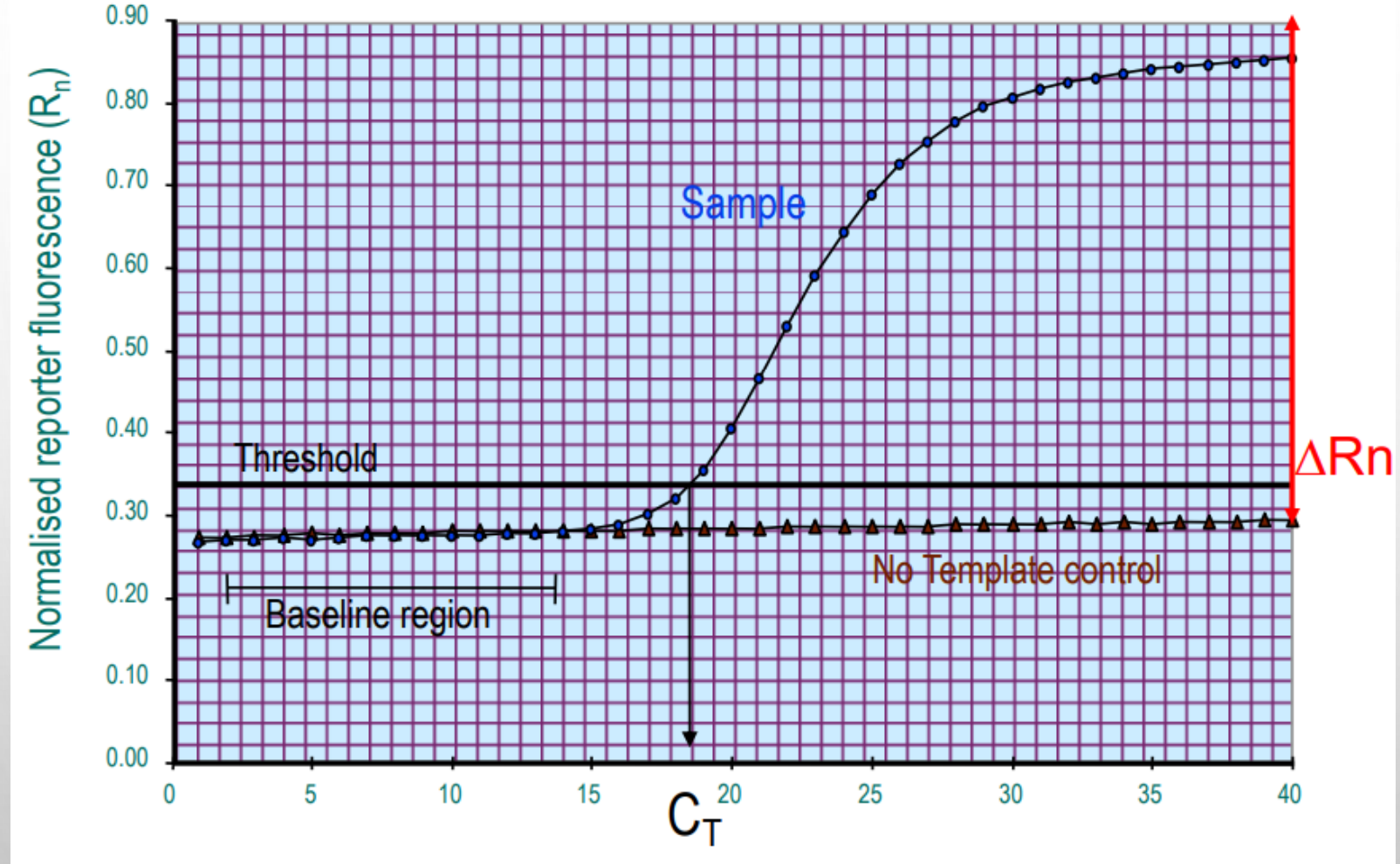


Amplification plots are displayed with the ability to drill down to a subset of sample wells.

從Melt Curve確認是因為Primer Dimer或是有汙染



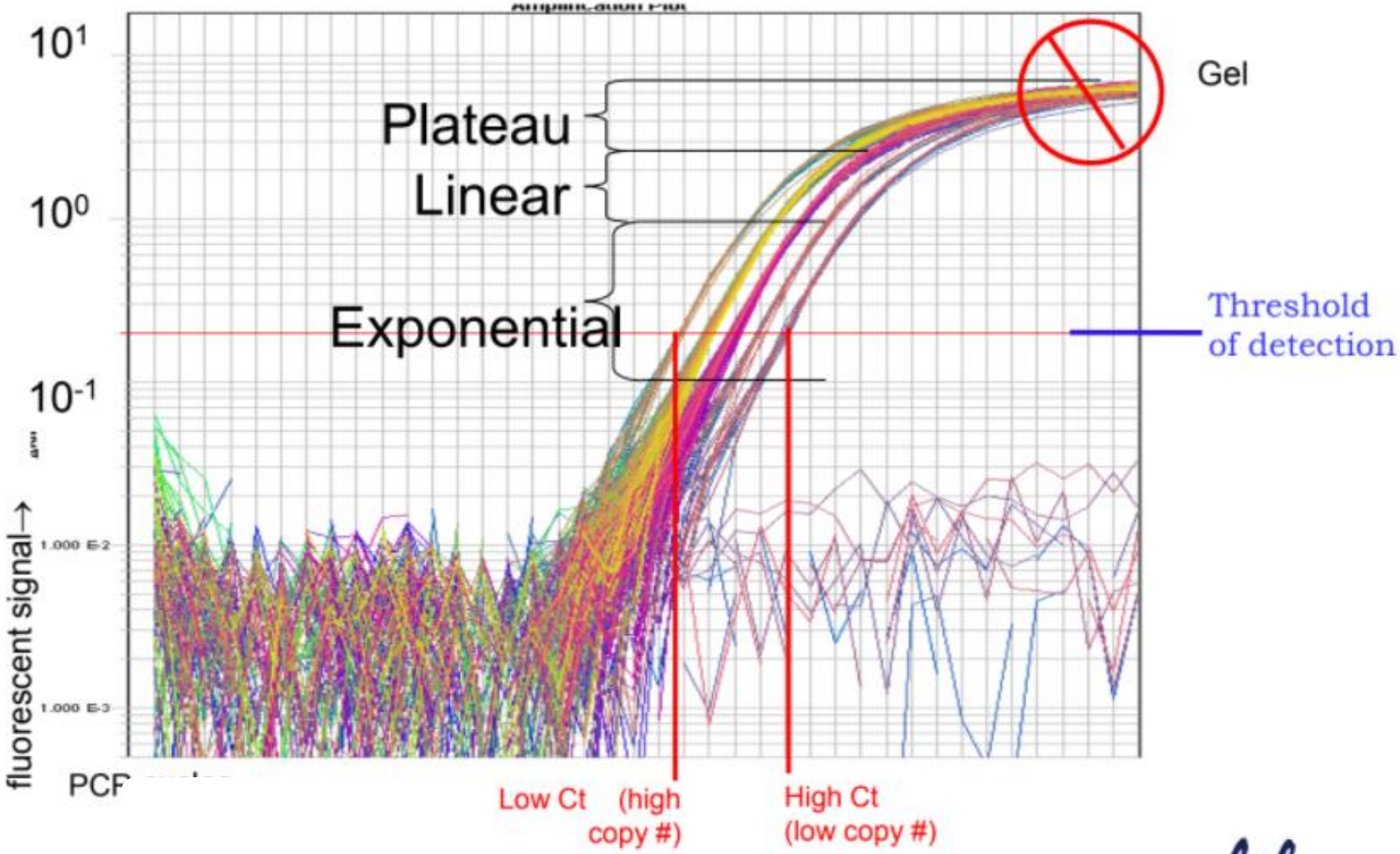




lock

C_T = threshold cycle: the calculated fractional cycle number at which the PCR product crosses a threshold of detection

ies



Y= N0 2^n，C_T 與起始濃度之對數值成反比

Samples	Raw Ct		Delta Ct	Delta Delta ct	2^delta d	A
	GAPDH	p53				
Tumor cells 1	21.00	23.00	2.00	-3.93	=2^-(F3)	
Tumor cells 2	20.50	22.00	1.50	-4.43		
Tumor cells 3	20.60	22.50	1.90	-4.03		
Normal cells 1	20.00	26.00	6.00	0.07		
Normal cells 2	20.50	26.20	5.70	-0.23		
Normal cells 3	20.30	26.40	6.10	0.17		
Avg delta Ct			5.93			

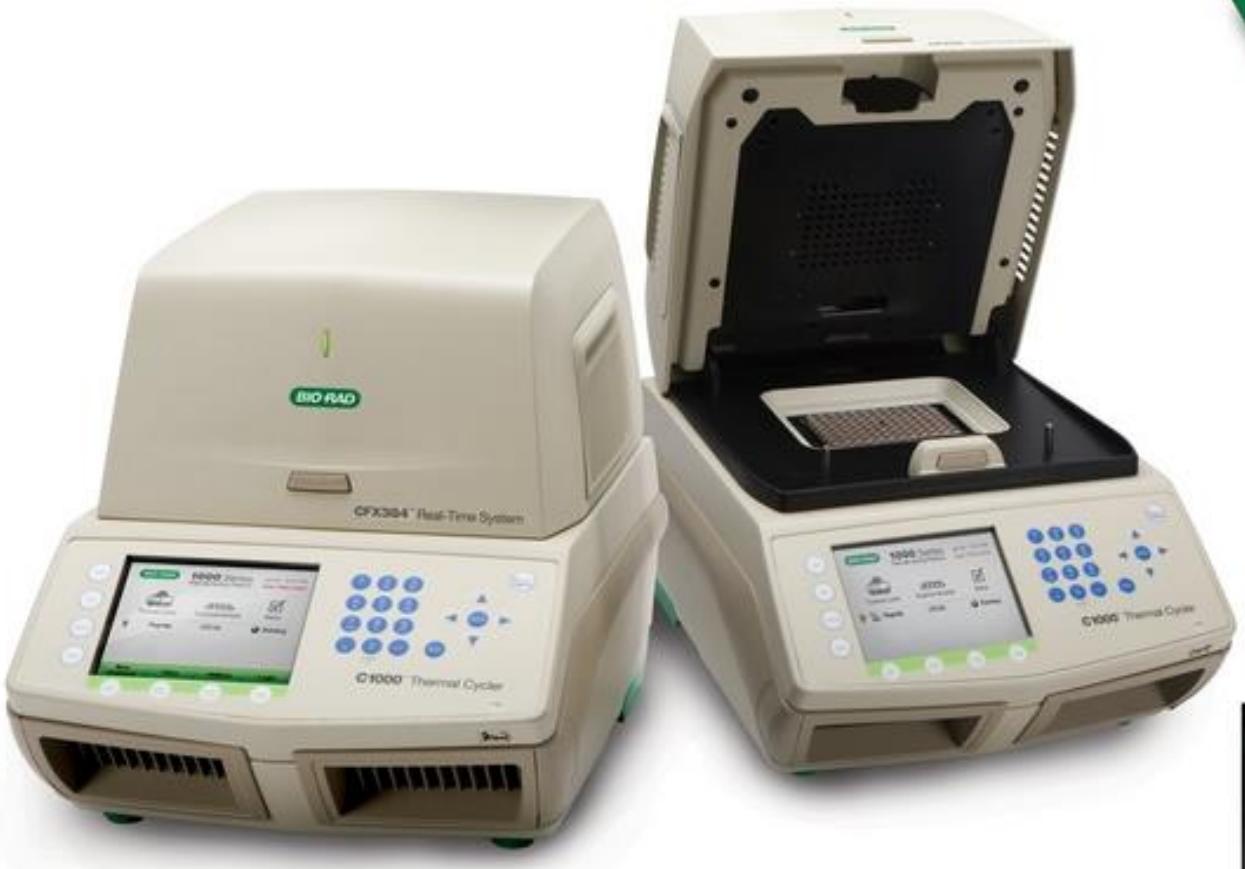
$$\Delta Ct = Ct_{target} - Ct_{18SrRNA}$$

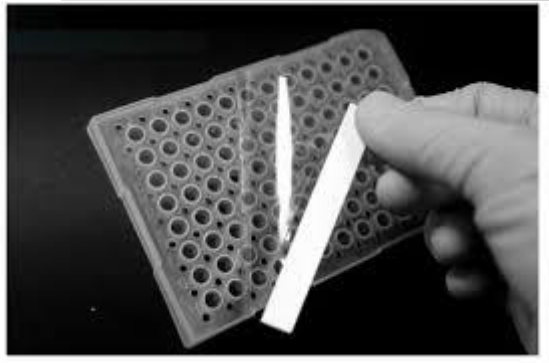
$$\Delta\Delta Ct = \Delta Ct_{(siRNA\ treated)} - \Delta Ct_{(siRNA\ nontreated)}$$

$$\text{Relative expression level} = 2^{-\Delta\Delta Ct}$$

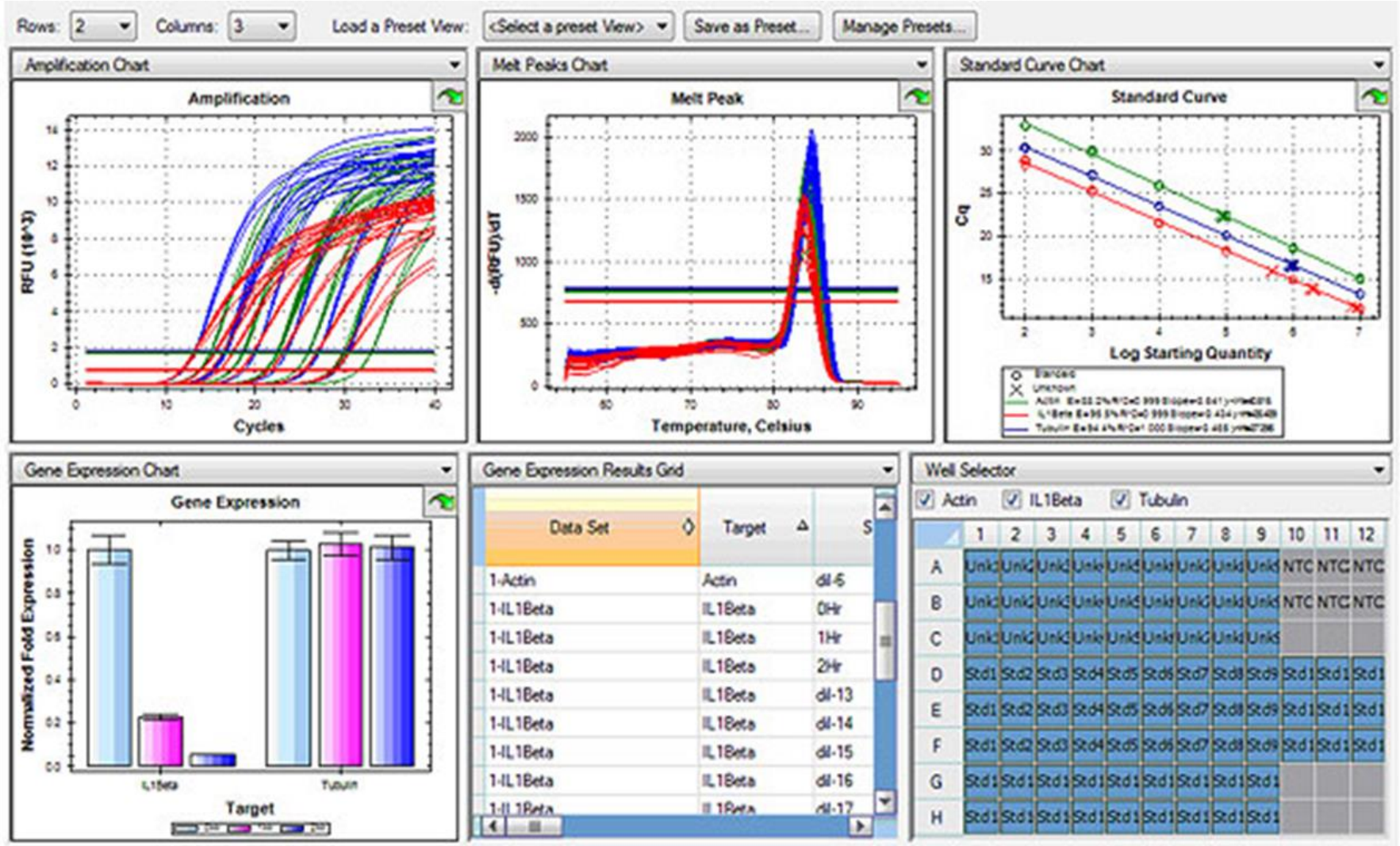
$$\%KD = 100 \times \left(1 - 2^{-\Delta\Delta Ct}\right)$$

（2）Bio-Rad系統





程式設置



https://www.youtube.com/watch?v=wUDysO8bFbA

如何避免Q-PCR錯誤

(1) 核糖核酸品質

- 是成功製備cDNA並執行高效且可重複的Q-PCR的最關鍵步驟。
- RNA對RNA酶的降解非常敏感，在核酸分離步驟中必須小心處理。降解或污染的RNA會對Q-PCR實驗的效率和產量產生負面影響。
- 您的實驗室工作台、移液器和吸頭必須不含 RNase，提取的 RNA 必須儲存在不含 RNase 的溶液中。
- 當您使用分光光度計評估RNA純度時，260和280nm（A260/280）處的吸光度比應在1.8-2.0的範圍內。如果較低，則可能表明樣品被苯酚或蛋白質污染。

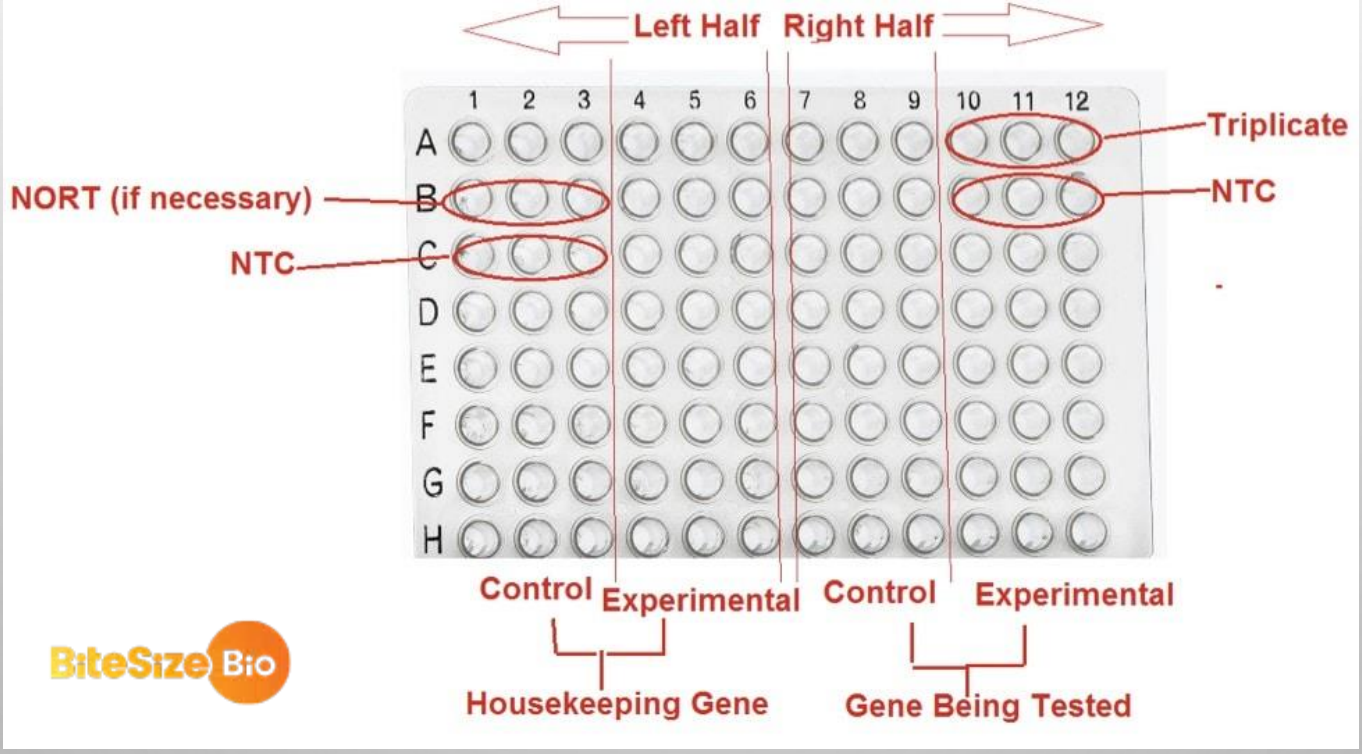
(2) 預混液與表格使用

- 使用預混液可大幅減少實驗變異性，從而減少孔間和樣品間差異來提高再現性。
- 在執行Q-PCR實驗時最好事先製作一張準備要加入每項試劑的清單與樣品相對位置表格。

Component		Volume/ reaction
Master mix		20 µl
Nuclease free water	12 µl	
5x Qiagen OneStep RT-PCR buffer	5 µl	
dNTP Mix (10 mM of each dNTP)	1 µl	
Primer Forward (100 pmol/ul)	0.5 µl	
Primer Reverse (100 pmol/ul)	0.5 µl	
Qiagen OneStep RT-PCR Enzyme Mix	1.0 µl	
Template RNA		5 µl
Total		25 µl

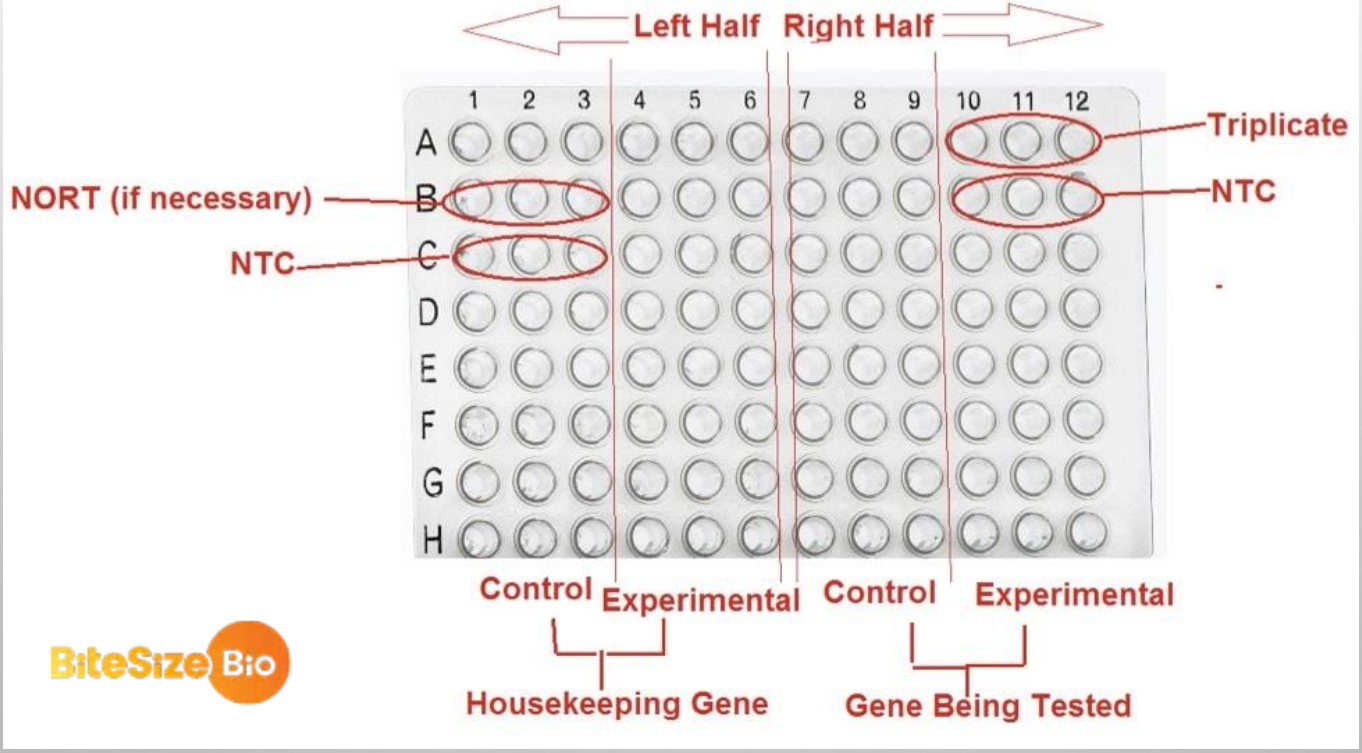
(3) 控制組

- Q-PCR測定時，適當的控制組是很重要的。
- 應該包括一個缺乏cDNA的陰性對照組，可以檢測表面或試劑的交叉污染。
- 也需要一組反轉錄的陰性對照組，其中沒有逆轉錄酶，如果觀察到產品，表明您的樣品受到DNA污染。

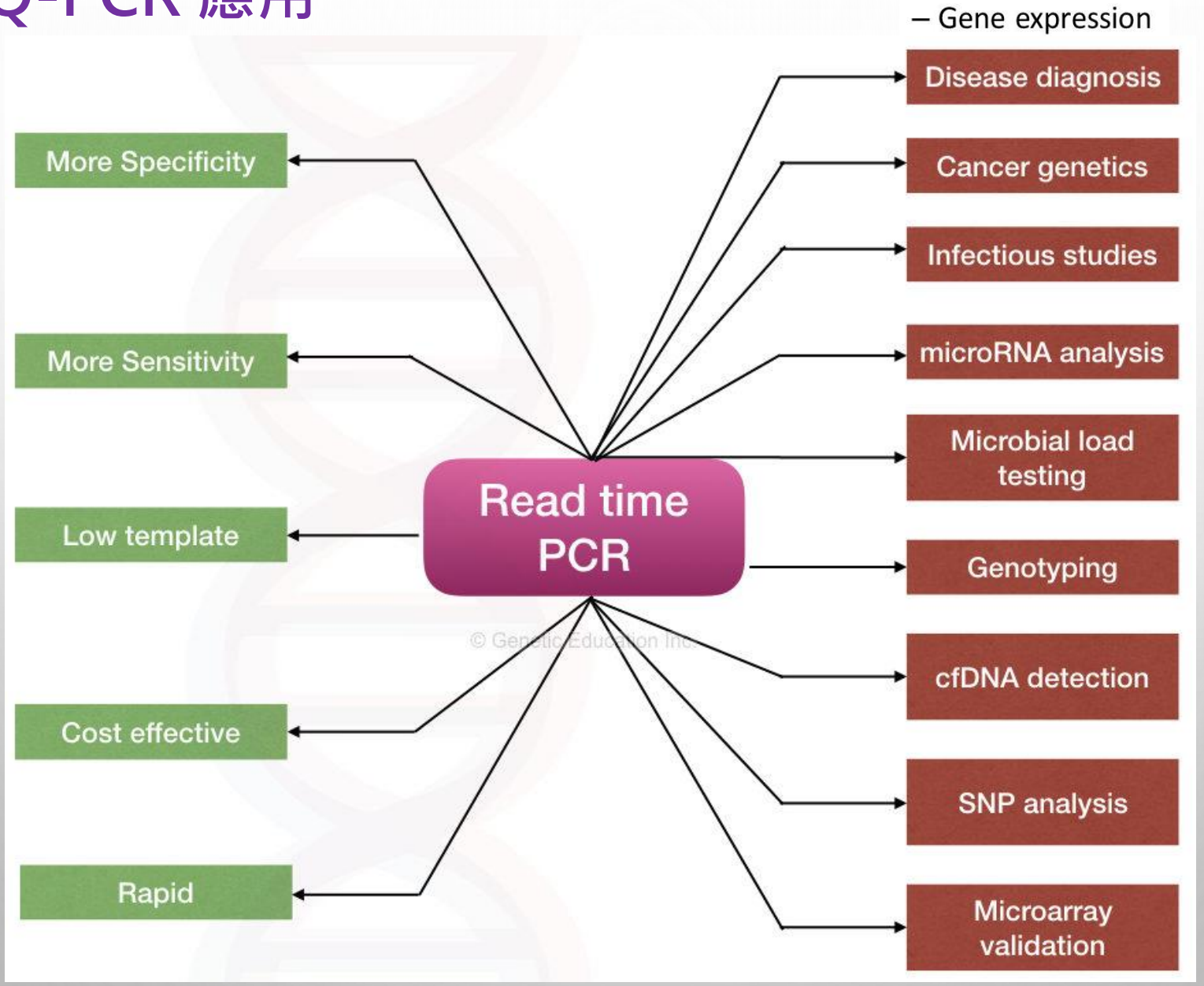


(4) 參考基因

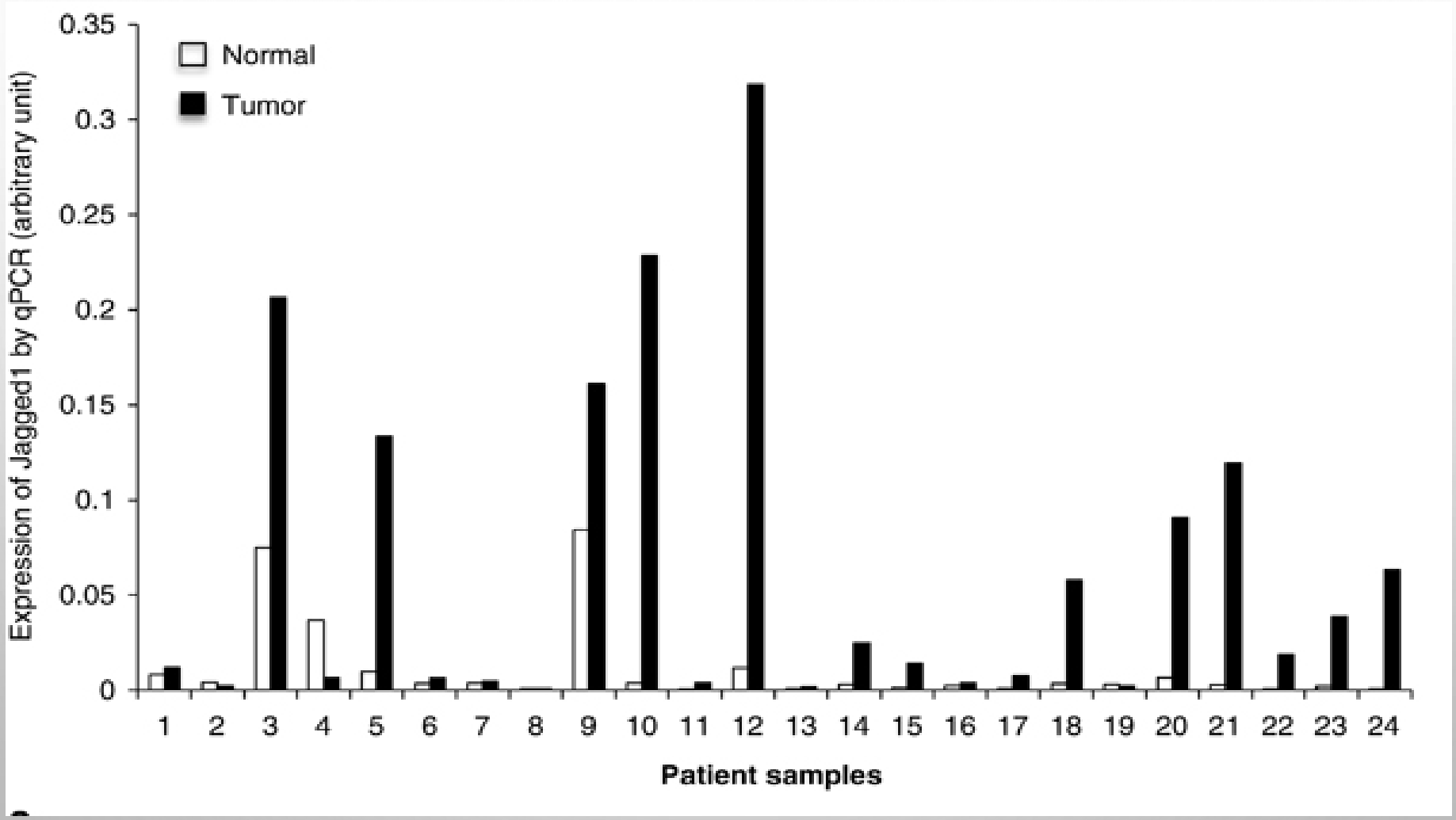
- 內源性對照基因的擴增可以解釋起始cDNA的數量或質量的差異，以及RNA製備方法或cDNA合成的差異。
- 可靠的參考基因是指其表達量不受實驗變數的影響，並且在樣品條件的相關生理狀態之間沒有差異的基因。



Q-PCR 應用

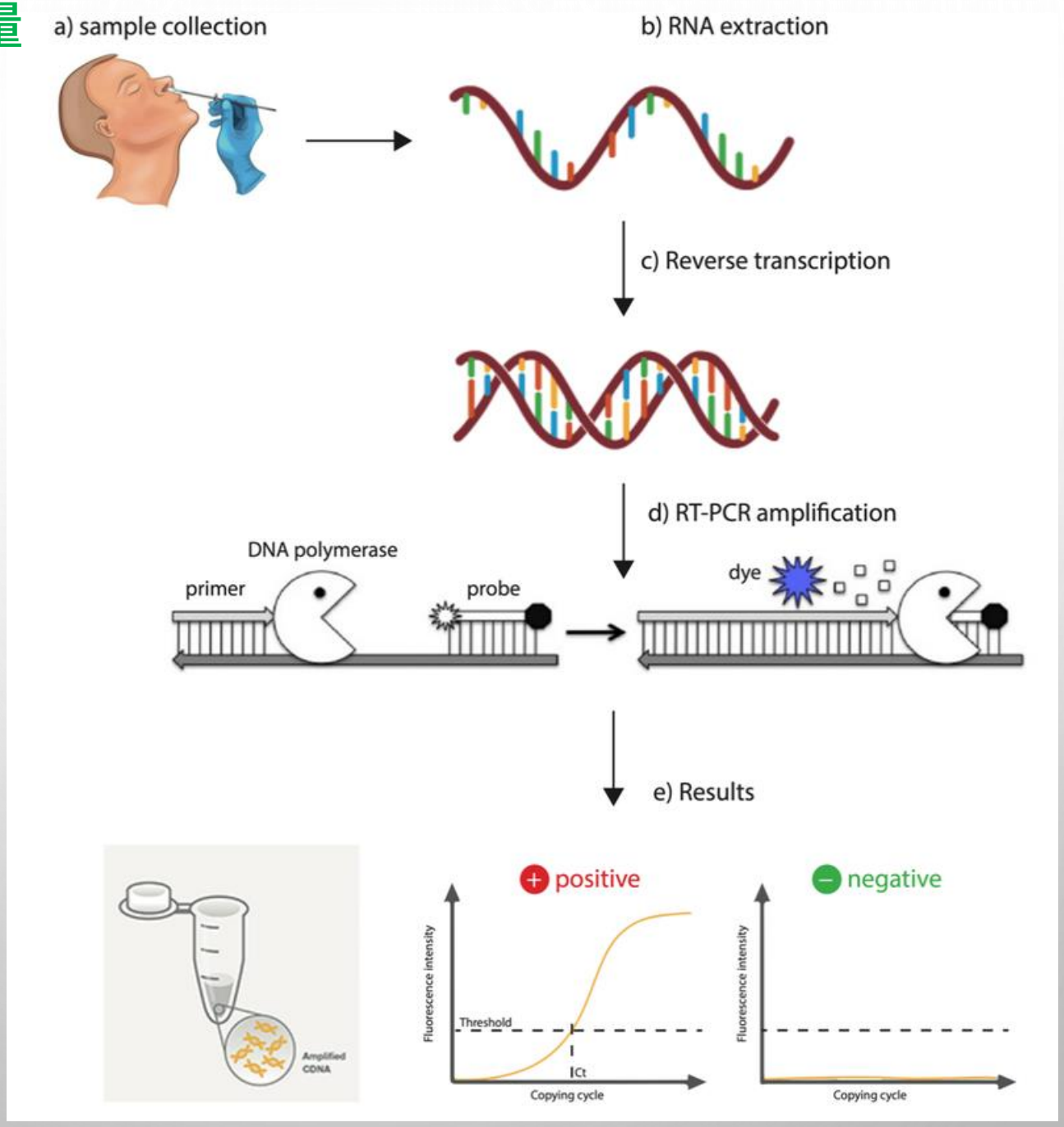

– Gene expression
Disease diagnosis
Cancer genetics
Infectious studies
microRNA analysis
Microbial load testing
Genotyping
cfDNA detection
SNP analysis
Microarray validation
More Specificity
More Sensitivity
Low template
Cost effective
Rapid
Read time PCR

（1）基因表達分析-例如病人正常細胞與癌細胞基因表現差異



World J Gastroenterol. Sep 21, 2019; 25(35): 5300-5309

（２）微生物檢測量


a) sample collection
b) RNA extraction
c) Reverse transcription
d) RT-PCR amplification
DNA polymerase
primer
probe
dye
e) Results
Amplified cDNA
positive
negative
Fluorescence intensity
Threshold
Ct
Copying cycle
Fluorescence intensity
Copying cycle

一次認識三種檢驗方式

健康醫療網

COVID19

抗原檢測（抗原快篩）

篩檢站所用

高風險地區，快速找出感染者的利器

檢測	檢體中是否含有病毒的抗原
優點	檢驗時間短 快速得到結果
缺點	準確率較PCR低，容易產生偽陽、偽陰性

PCR檢測（RT-PCR）

全球判斷染疫的標準檢測

檢測	檢體中是否含有病毒的遺傳物質
優點	準確度高，病毒量低也可檢驗出
缺點	耗時、成本高，需要專業設備及人員執行

抗體檢測（抗體快篩）

後續瞭解病毒的盛行率、研究用

檢測	血清中是否含有病毒的抗體
優點	可找出曾經感染過或打疫苗者是否有抗體
缺點	感染後期才能驗出，也可能會有偽陰性產生

資料來源：疾管署

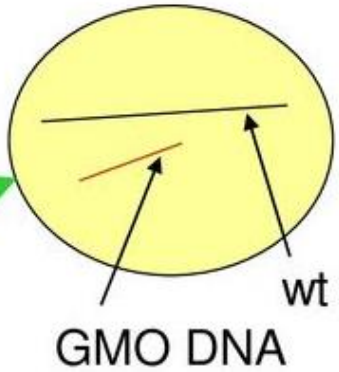
ICONS MADE BY FLATICON

(3) 基因食品檢測

Example: Determining percentage of GMO food content

Determination of percent GMO food content important for import / export regulations.

Labs use Real-Time PCR to measure amount of transgenic versus wild-type DNA.



Real-Time PCR

International shipments depend on results!

Abigayle French


Q & A


take a
BREAK

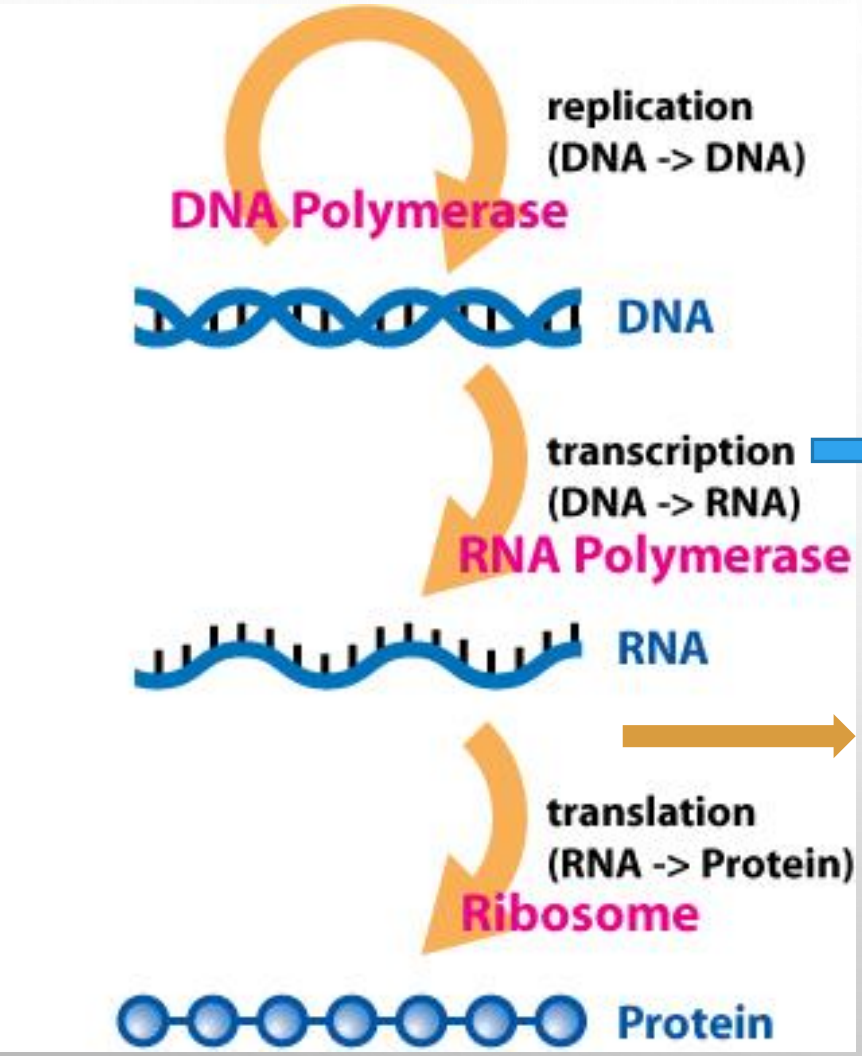
II. 報告基因分析
(Reporter gene assay)

上課內容

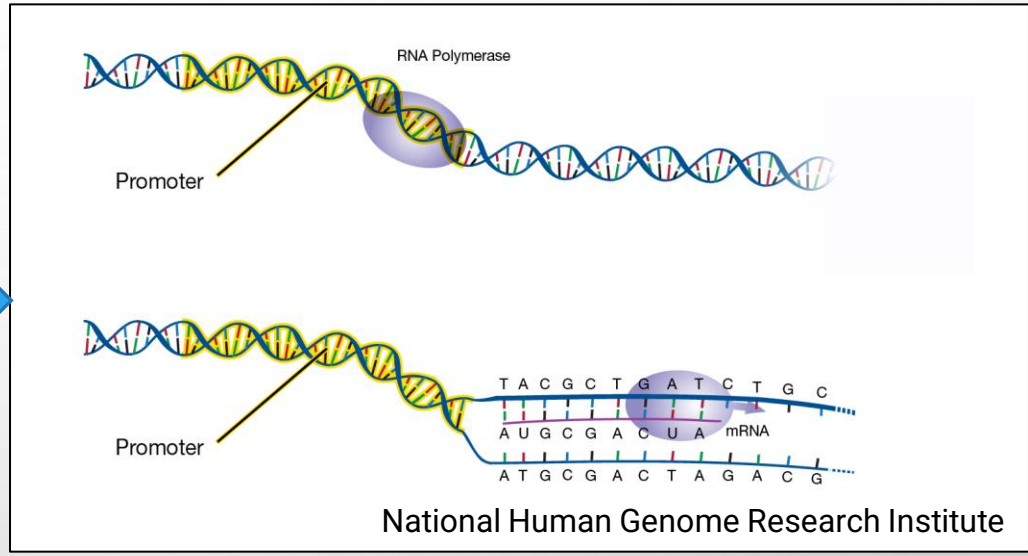
- 基因表現調節
- 什麼是報告基因分析
- 常見的報告基因
- 報告基因分析的應用

基因表現調節

中心法則



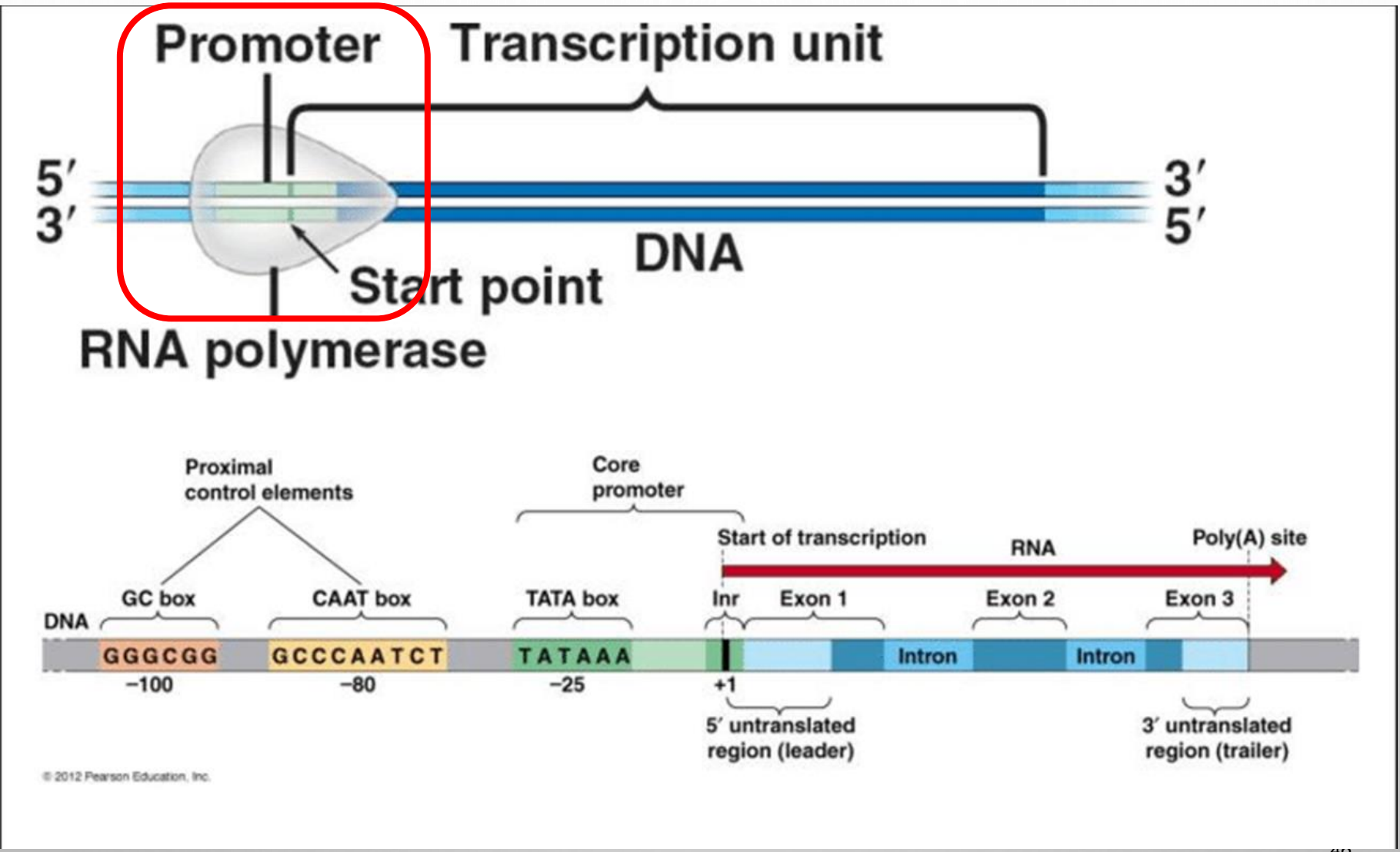
DNA→ RNA



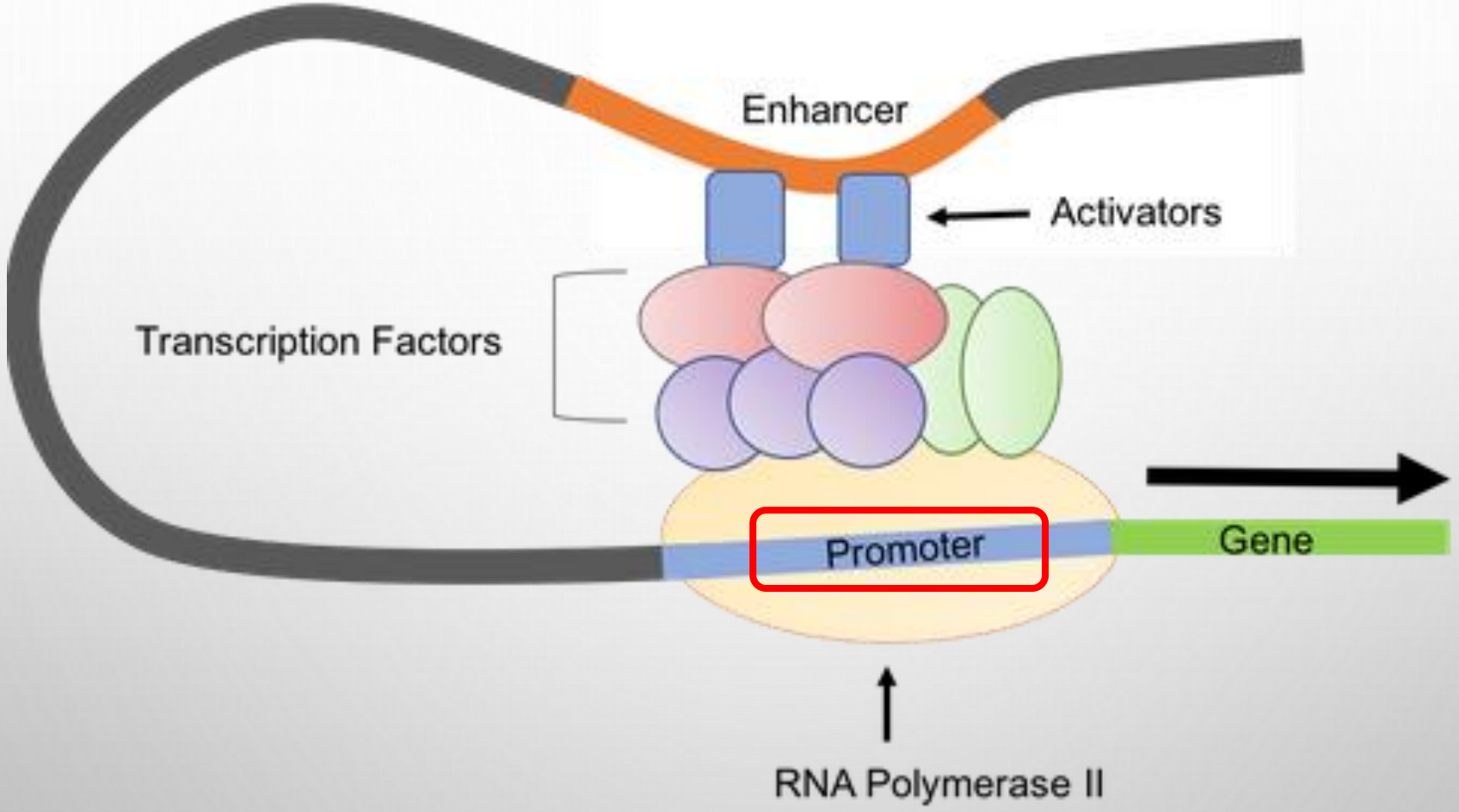
National Human Genome Research Institute

https://zh.wikipedia.org

啟動子 (Promoter)

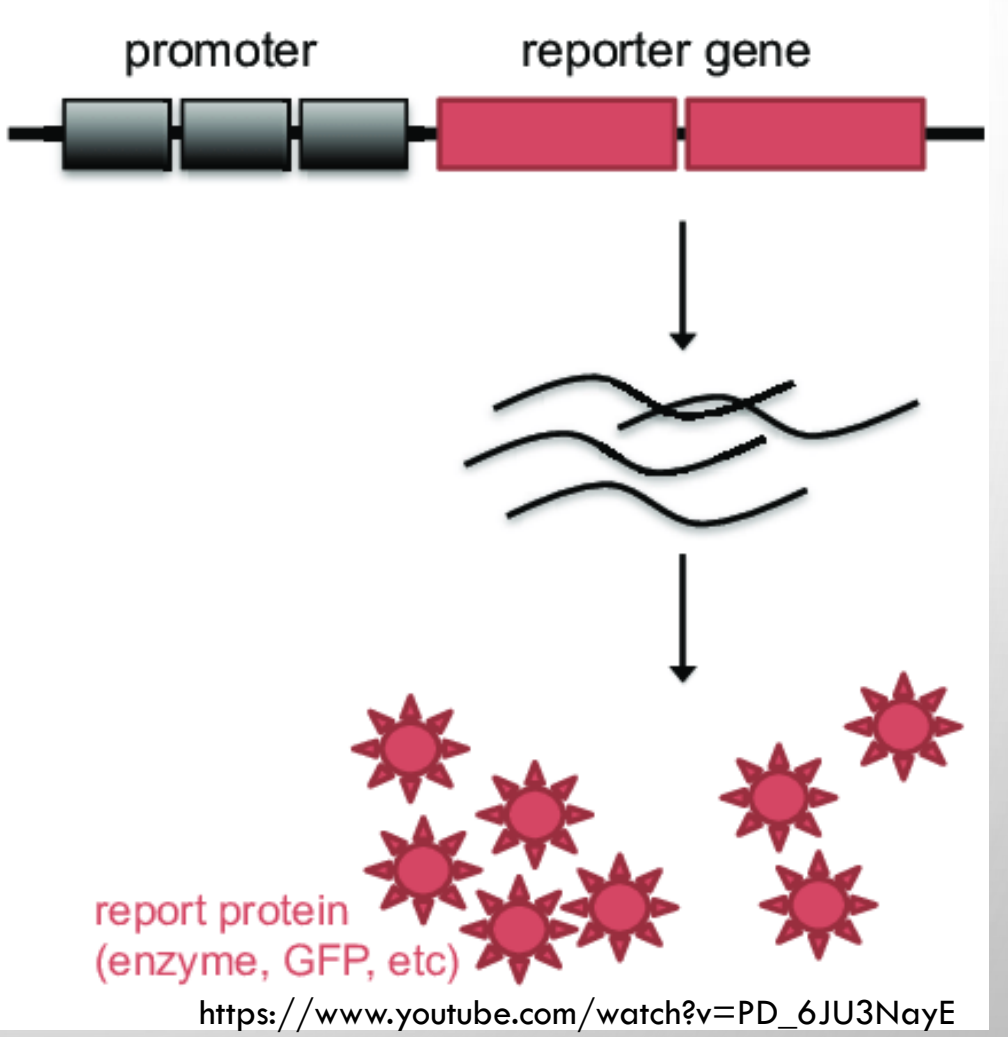


DOI:10.13140/RG.2.2.10102.34889


Enhancer
Activators
Transcription Factors
Promoter
Gene
RNA Polymerase II

什麼是報告基因分析

- 報告基因分析主要為報告基因的啟動子活性的測量。
- 報告基因是把啟動子DNA序列和報告基因DNA序列的融合。
- 報告基因可以為酵素或螢光物。



https://www.youtube.com/watch?v=PD_6JU3NayE

常見的報告基因

- β-葡萄糖醛酸酶（ β-glucuronidase , GUS ）
- β-半乳糖苷酶（ β-galactosidase, LacZ ）
- 鹼性磷酸酶 (ALP)
- 氯黴素乙醯轉移酶（CAT ）
- 綠色螢光蛋白（GFP ）
- 螢光素酶 (luciferase)

WHAT ARE THE 4 MAIN QUALITIES OF A GOOD REPORTER GENE?

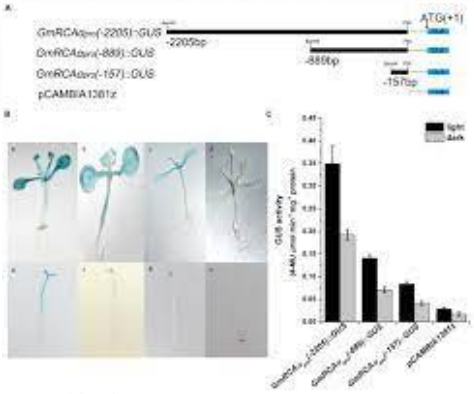
1. EASILY OBSERVABLE

2. CAN TOLERATE ADDITIONS OF AMINO ACIDS TO THE N OR C TERMINUS WITHOUT LOSING FUNCTION

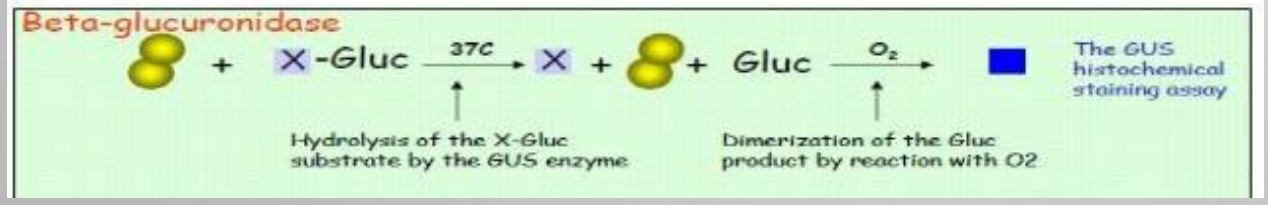
3. IT'S EASY TO ASSAY ITS FUNCTION

4. ONLY FUNCTIONAL IN A PARTICULAR REGION OF THE CELL.

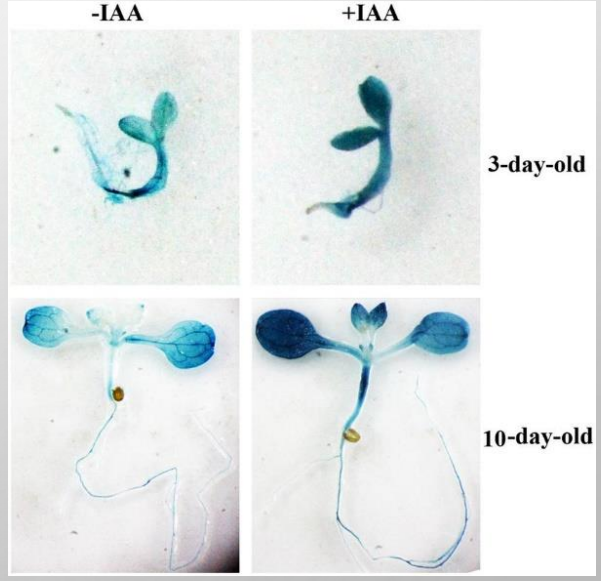
（1）β-葡萄糖醛酸酶（GUS）



Susan Jean Karcher

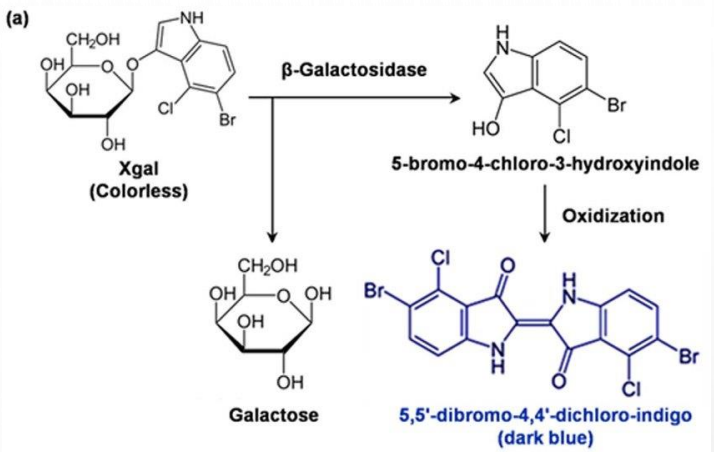


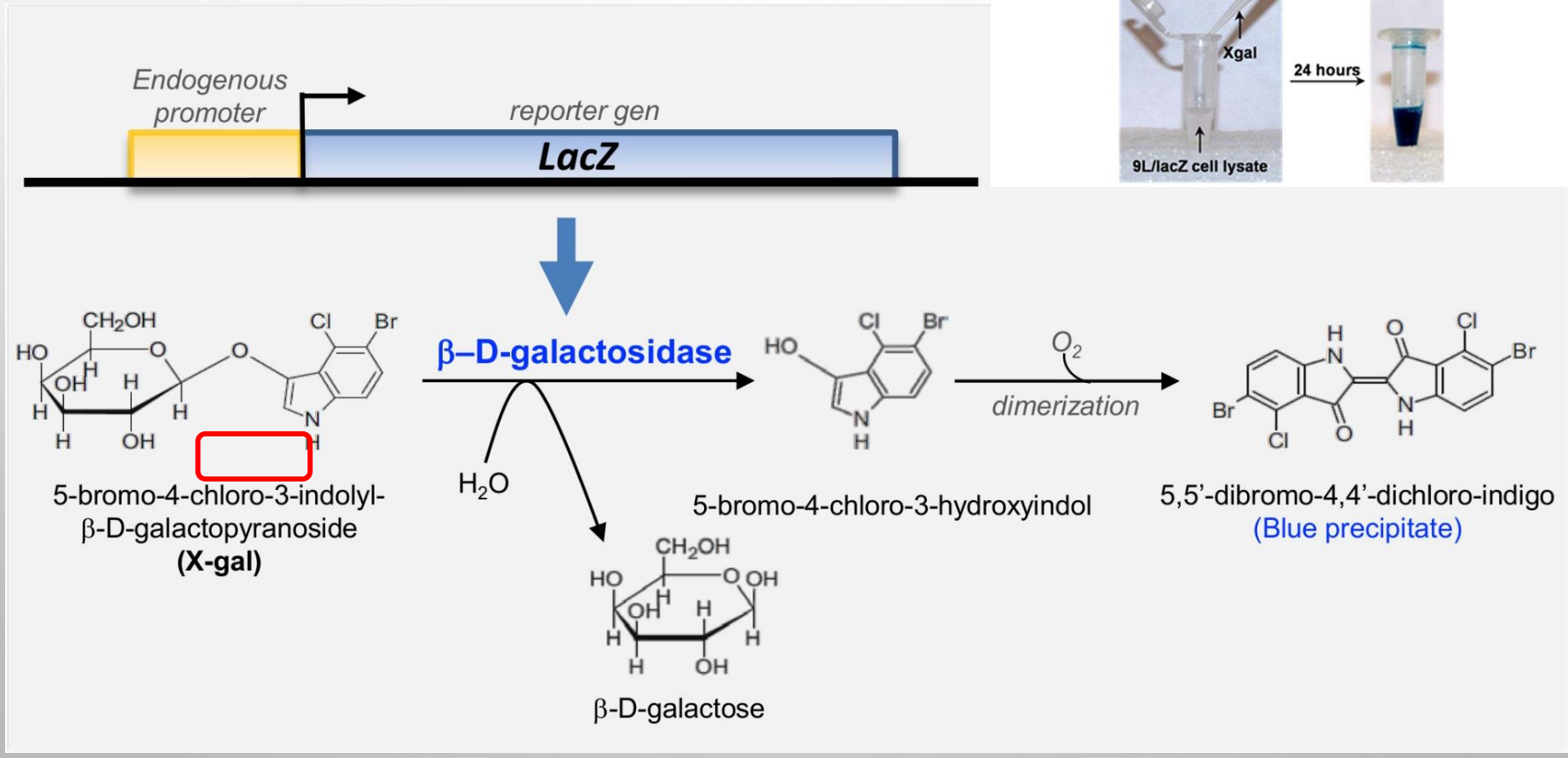
doi: https://doi.org/10.1371/journal.pone.0159875.g004



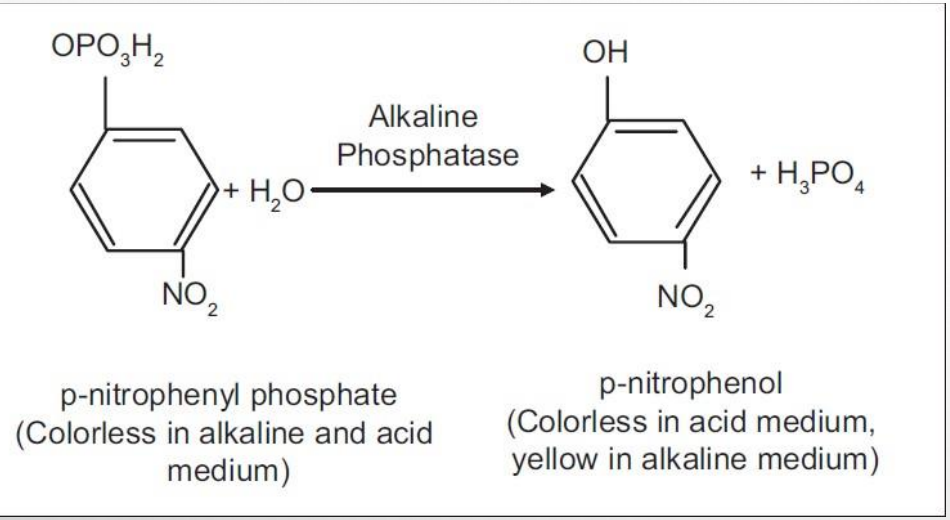
Frontiers in Plant Science 6(295) · May 2015

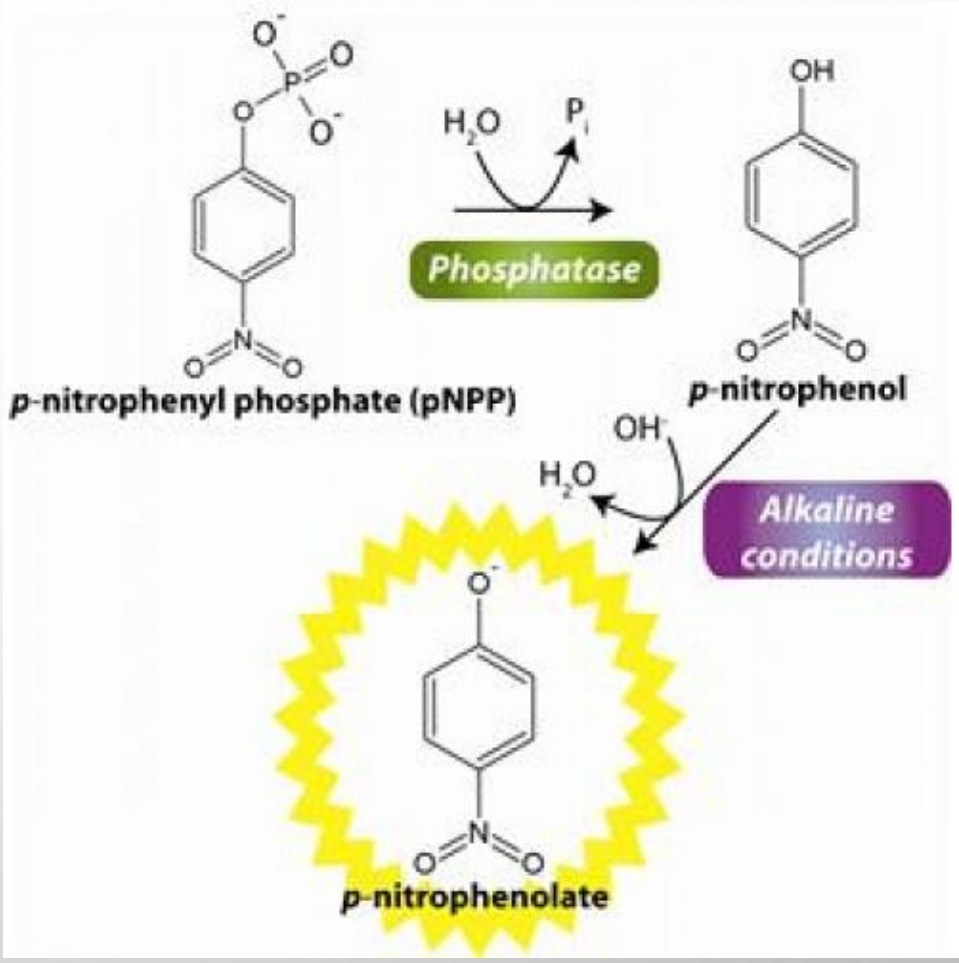
（2）β-半乳糖苷酶（LacZ）





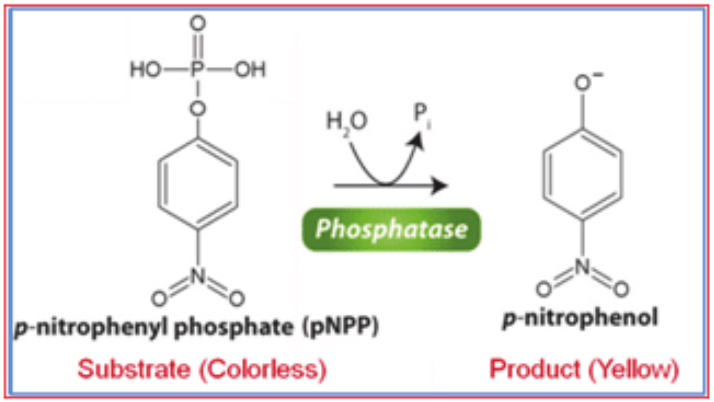
（3）鹼性磷酸酶 (ALP)





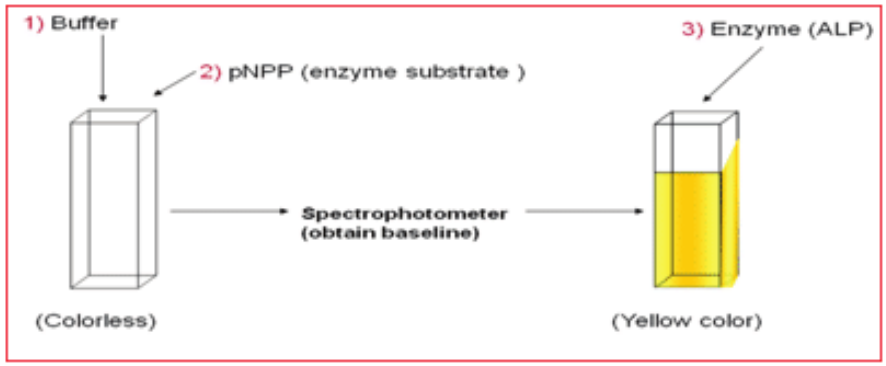
December 2010Procedia - Social and Behavioral Sciences 2(2):5507-5511

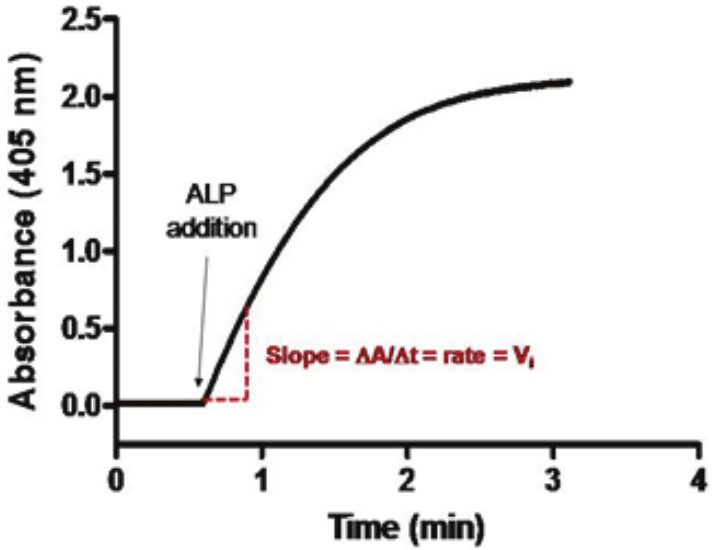
A.



B.

Alkaline Phosphatase (ALP)





December 2010Procedia - Social and Behavioral Sciences 2(2):5507-5511

(4) 氯黴素乙醯轉移酶 (CAT)

CAT:
Chloramphenicol (CAM)
acetyl transferase

CAM: chloramphenicol (CAM)
Protein synthesis inhibitor
↓ acetylation by CAT
Loss inhibitor activity

Other reporter enzymes:
β-galatosidase
luciferase

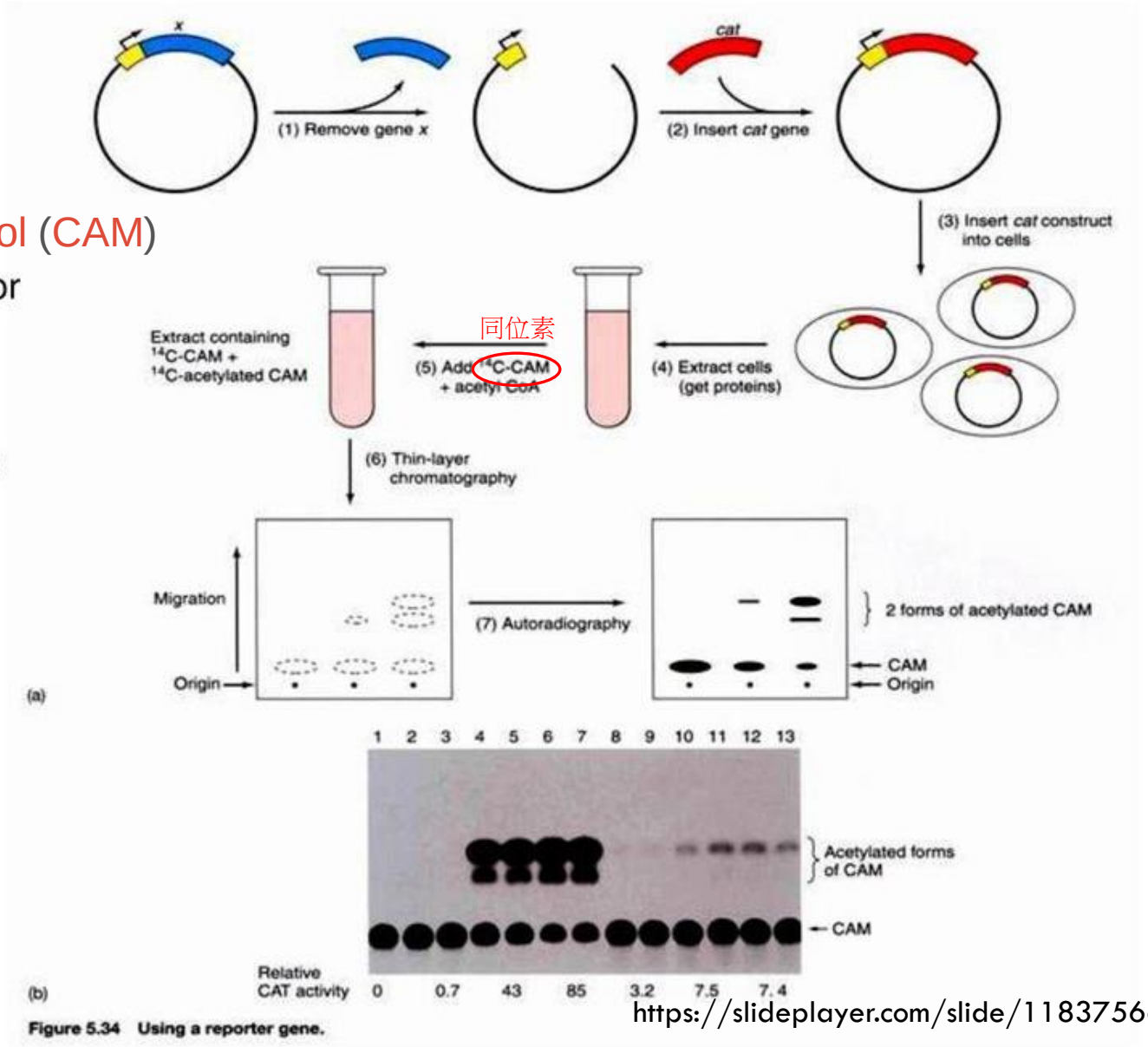
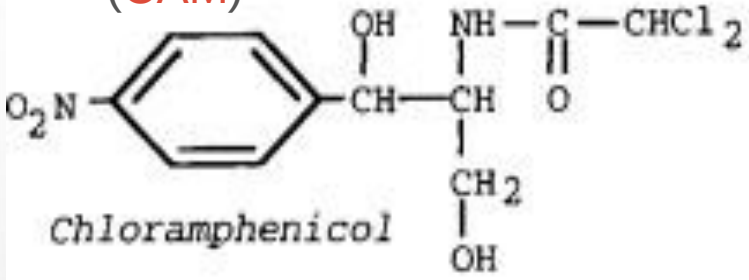


Figure 5.34 Using a reporter gene.

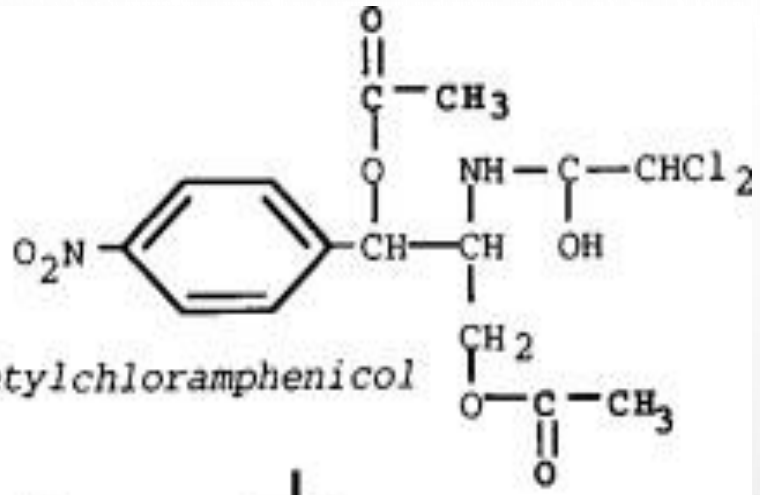
https://slideplayer.com/slide/11837566/

https://www.youtube.com/watch?v=eW3a-vXFZOI

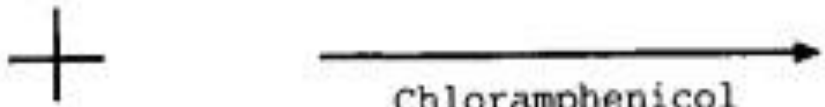
(CAM)



Chloramphenicol

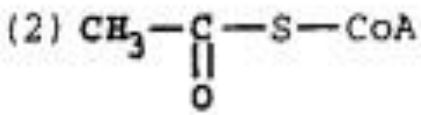


1,3-Diacetylchloramphenicol

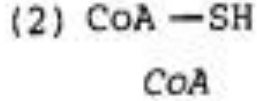


Chloramphenicol acetyltransferase

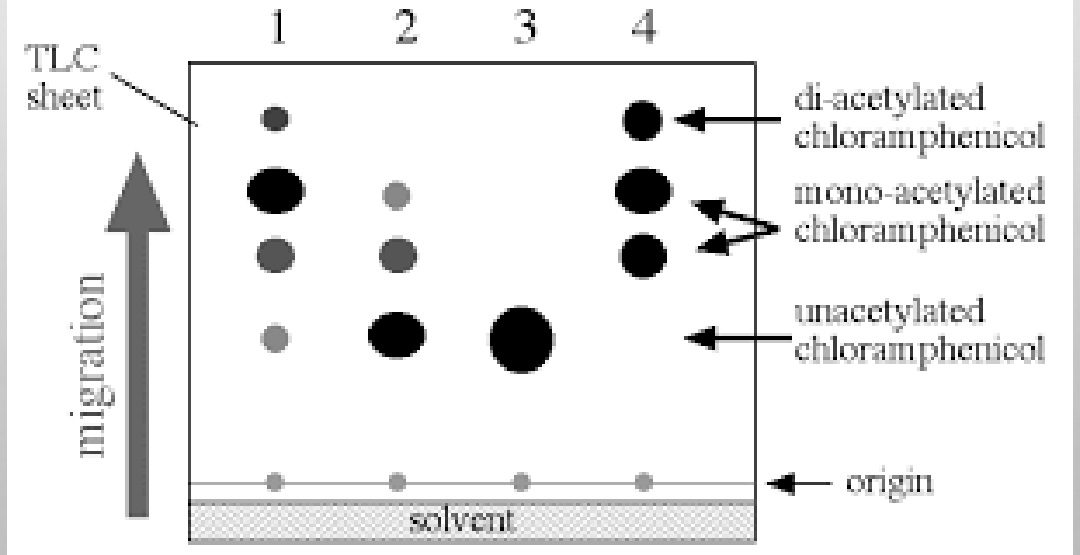
(CAT)



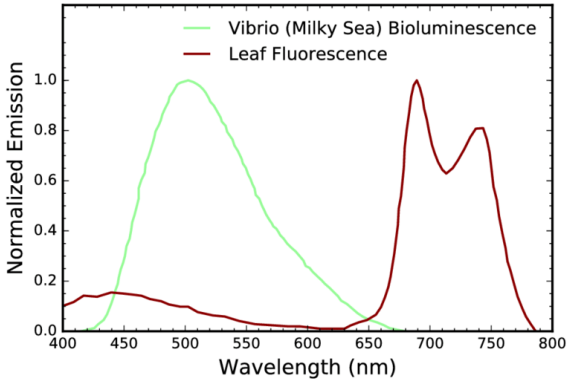
Acetyl-CoA

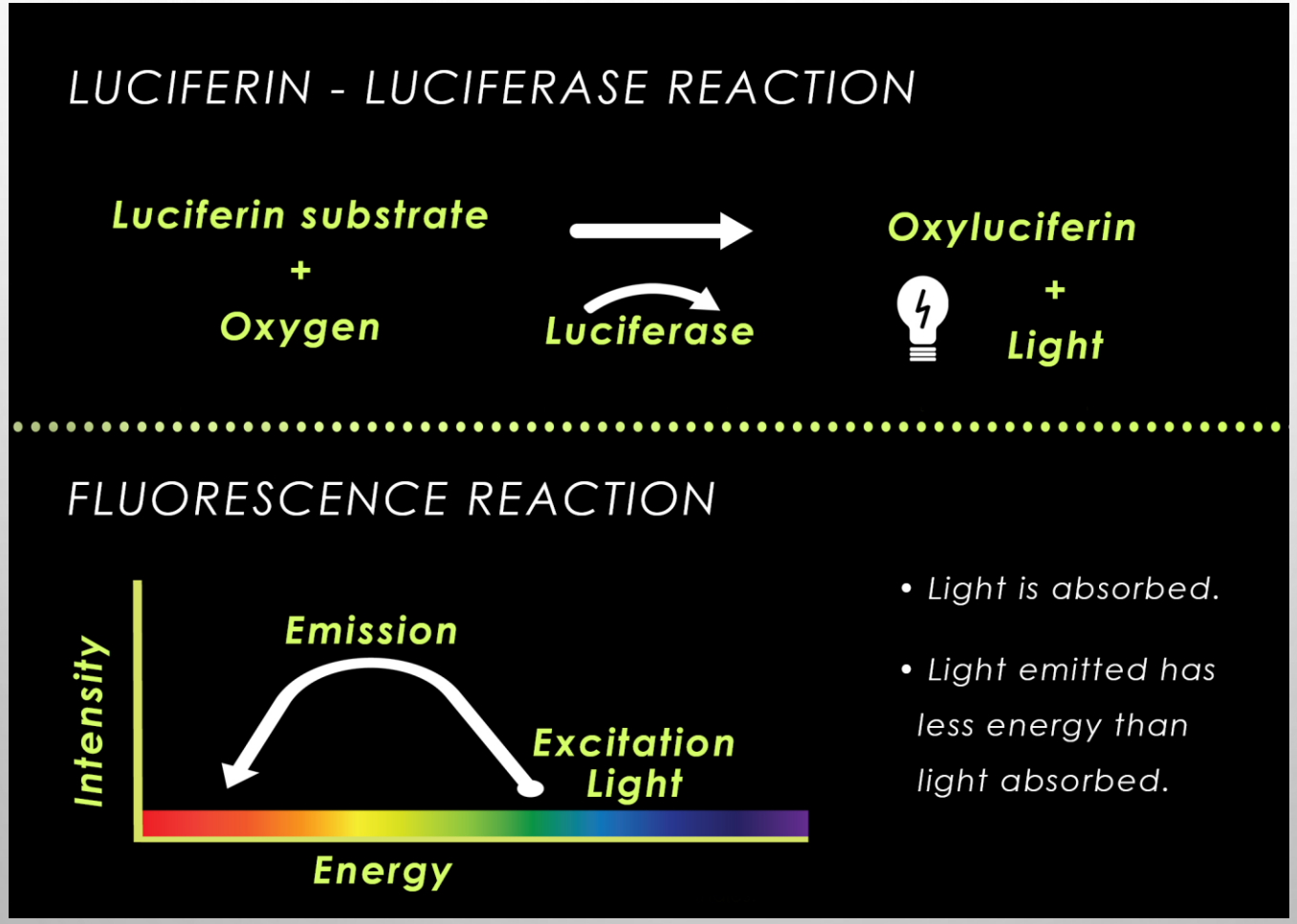


CoA



生物發光反應與螢光反應

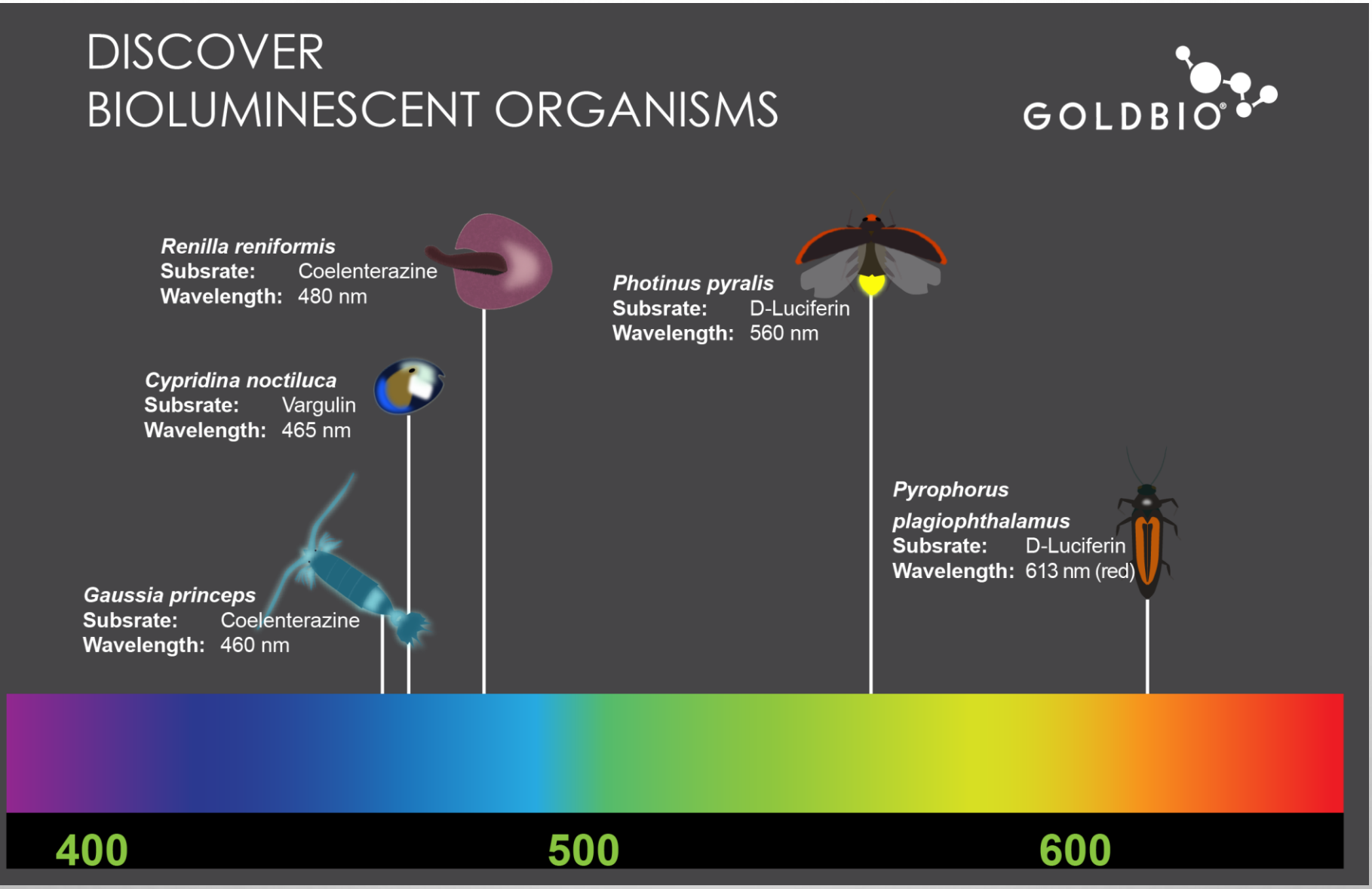




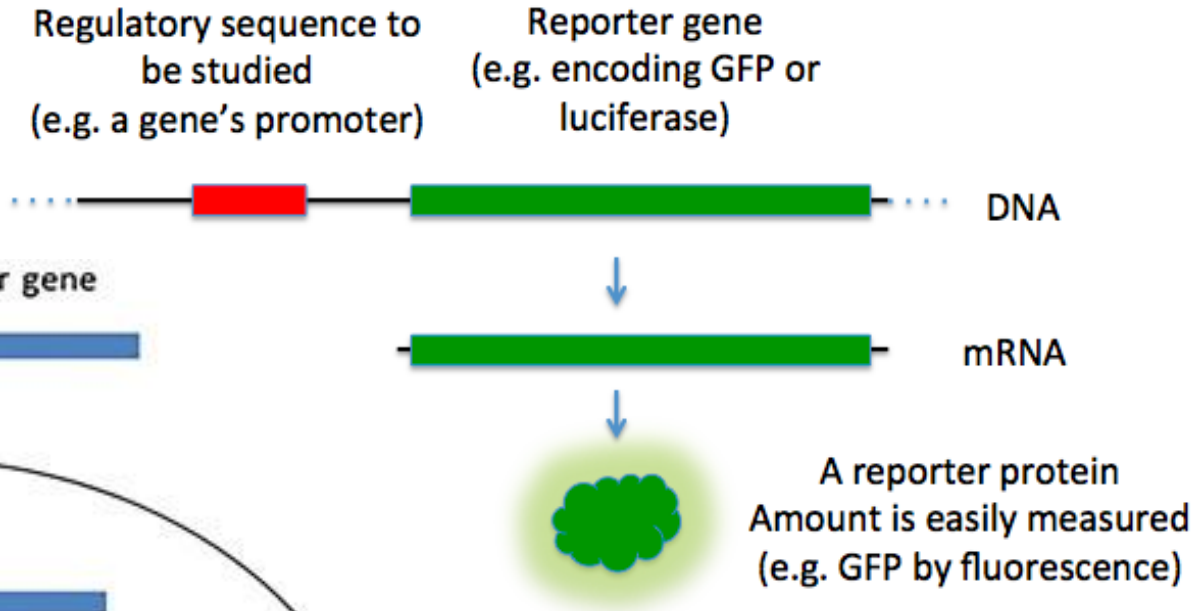
DOI:10.1007/978-3-319-30648-3_69-1

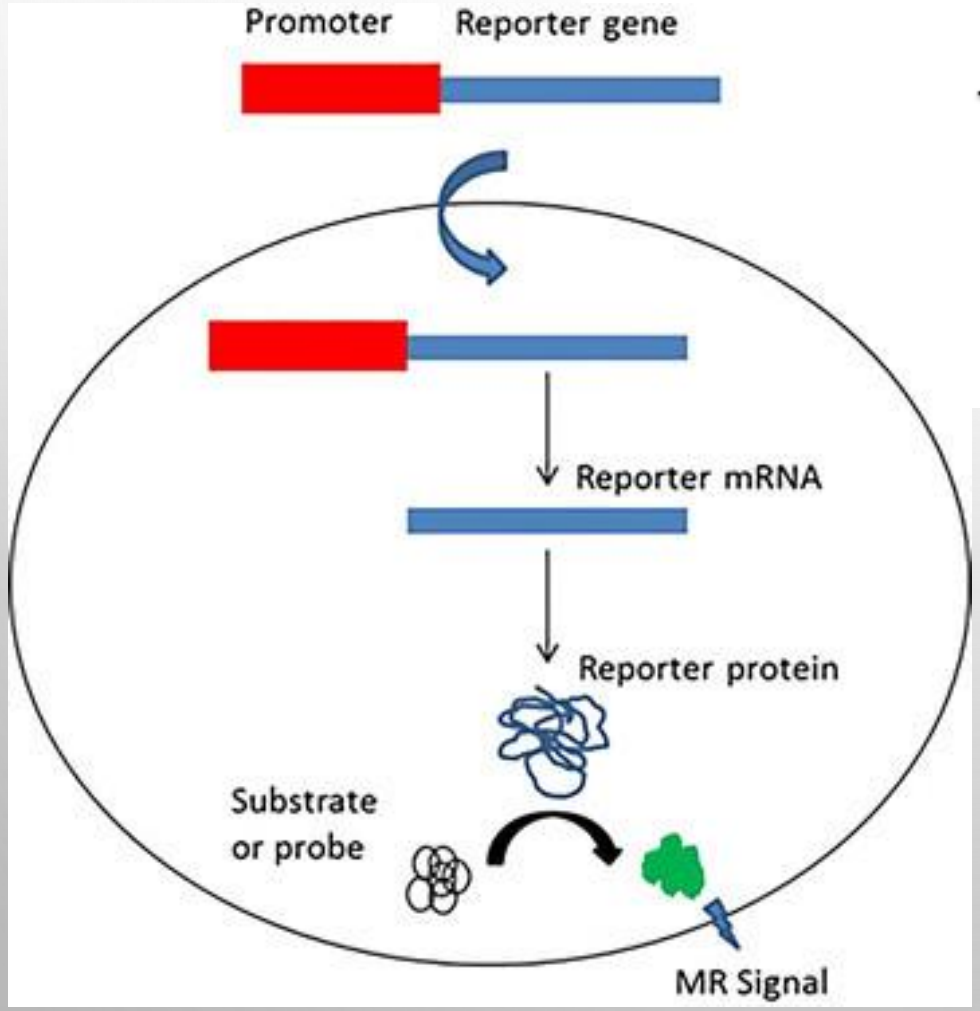
DISCOVER BIOLUMINESCENT ORGANISMS

GOLDBIO®



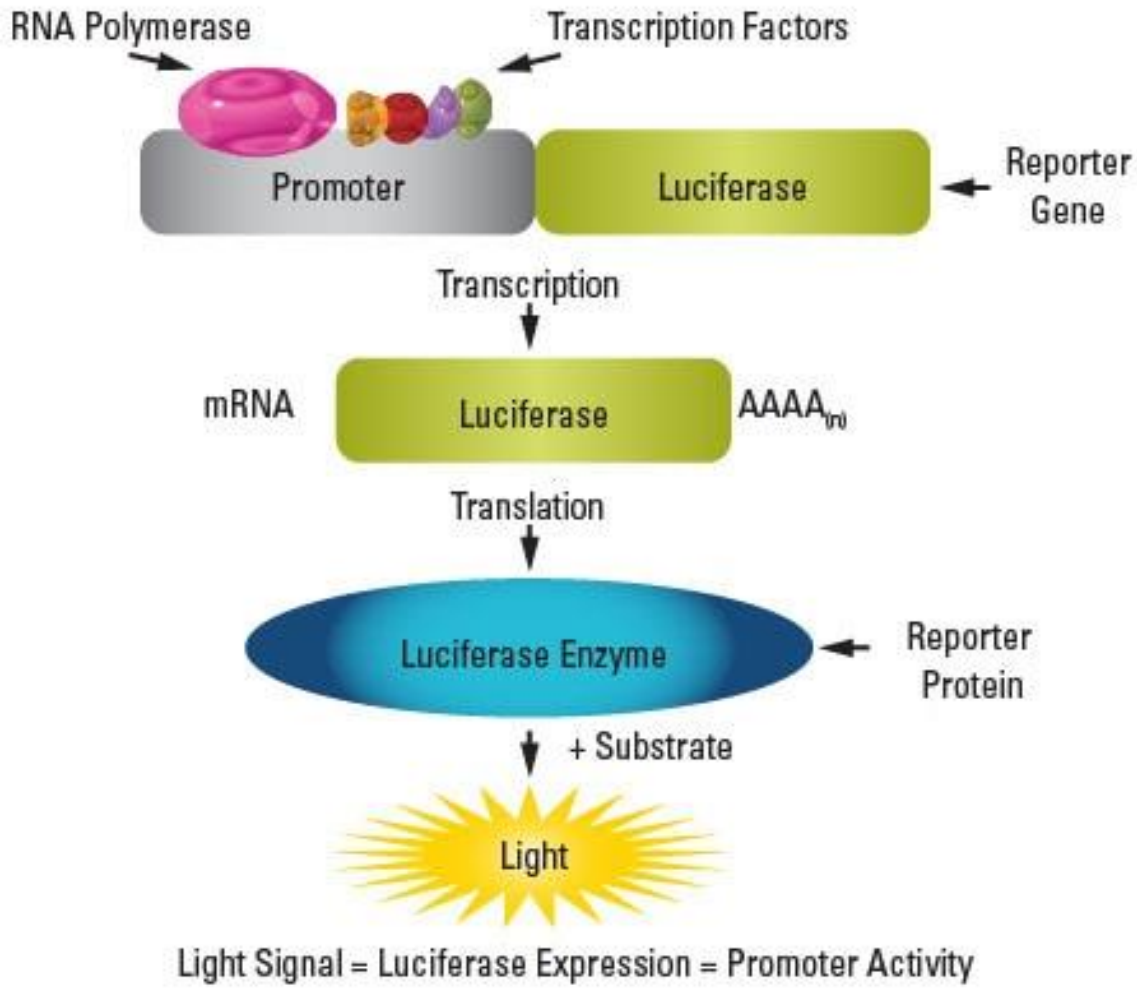
（5）綠色螢光蛋白（GFP）

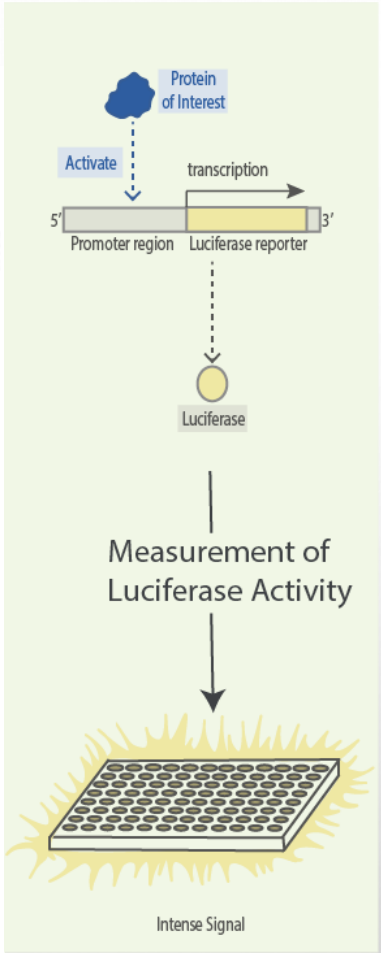


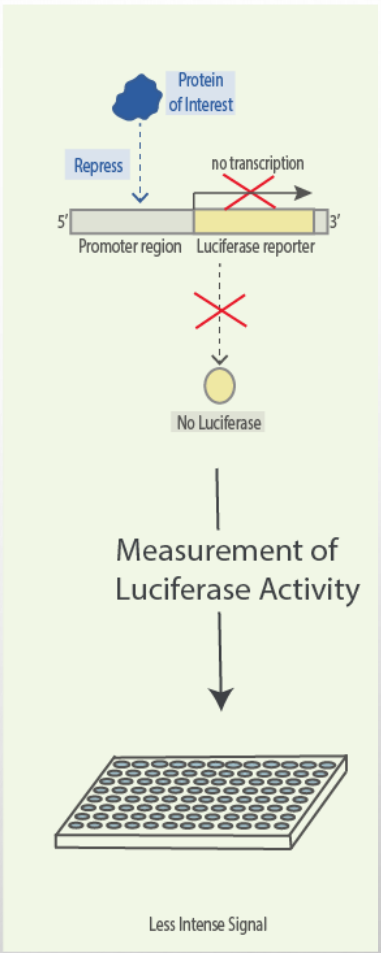


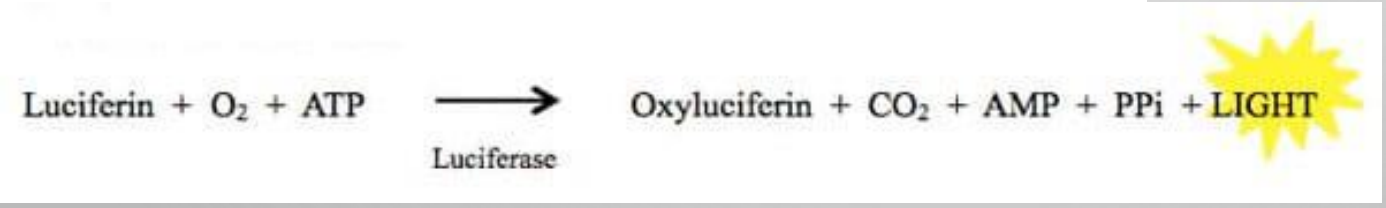
https://zh.wikipedia.org/

（6）螢光素酶 (Luciferase)









https://www.youtube.com/watch?v=PD_6JU3NayE

ThermoFisher SCIENTIFIC

The Nobel Prize in Chemistry 2008

"for the discovery and development of the green fluorescent protein, GFP"

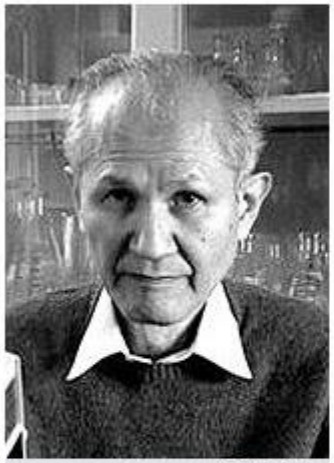


Photo: J. Henriksson/SCANPIX

Osamu Shimomura

1/3 of the prize

USA

Marine Biological Laboratory (MBL)
Woods Hole, MA, USA;
Boston University Medical School
Massachusetts, MA, USA

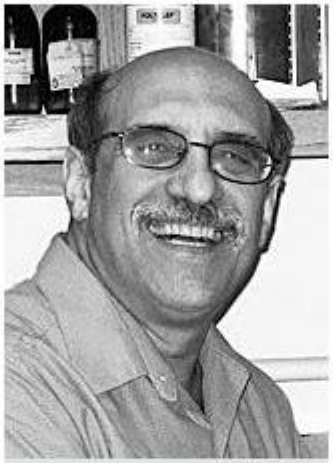


Photo: J. Henriksson/SCANPIX

Martin Chalfie

1/3 of the prize

USA

Columbia University
New York, NY, USA

Photo: UCSD

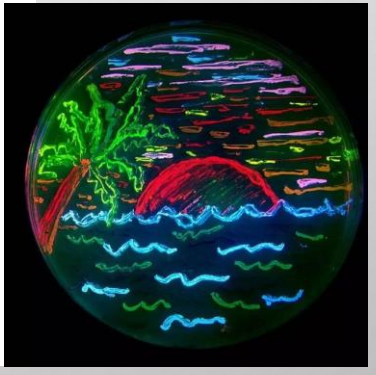
Roger Y. Tsien

1/3 of the prize

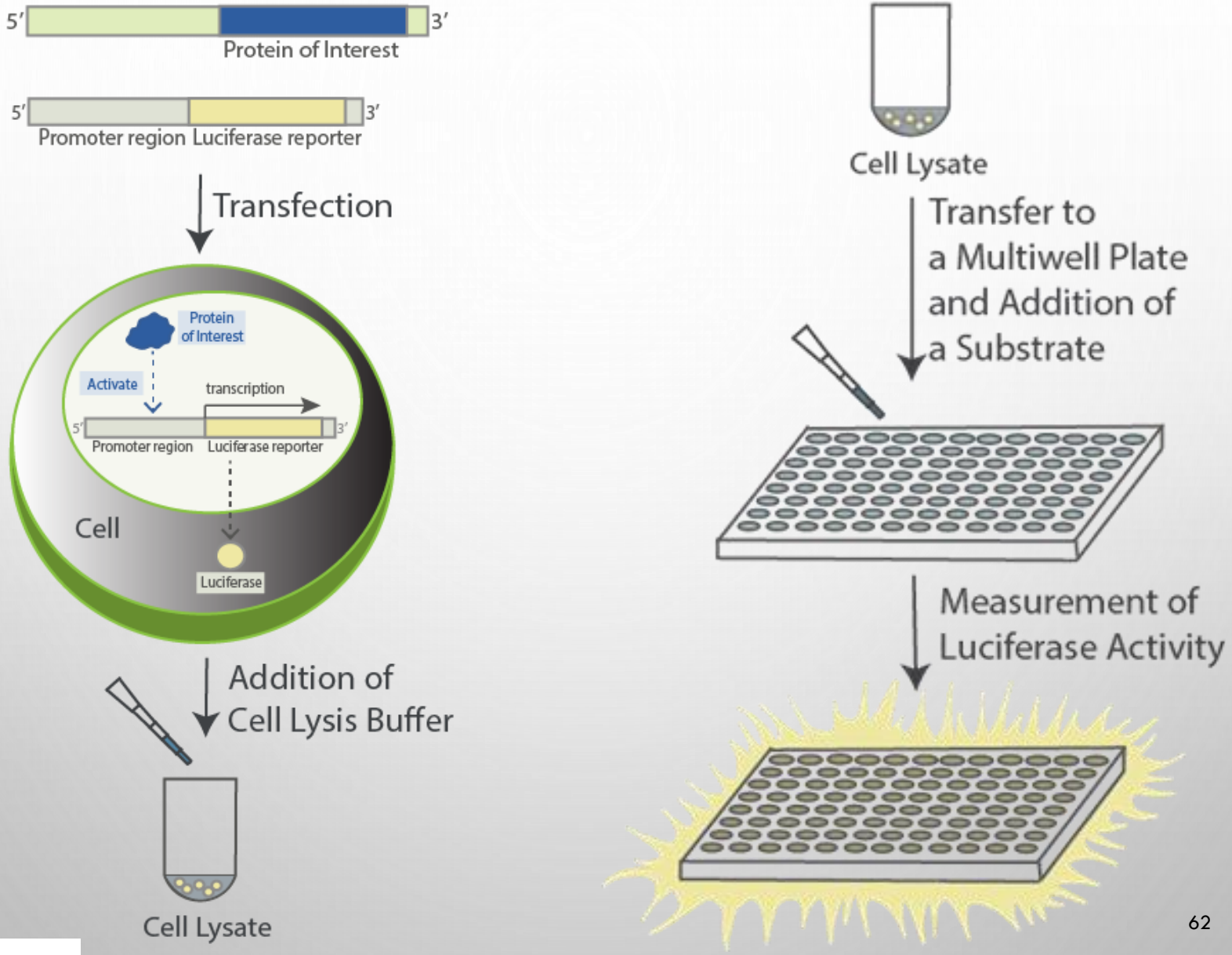
USA

University of California
San Diego, CA, USA;
Howard Hughes Medical Institute

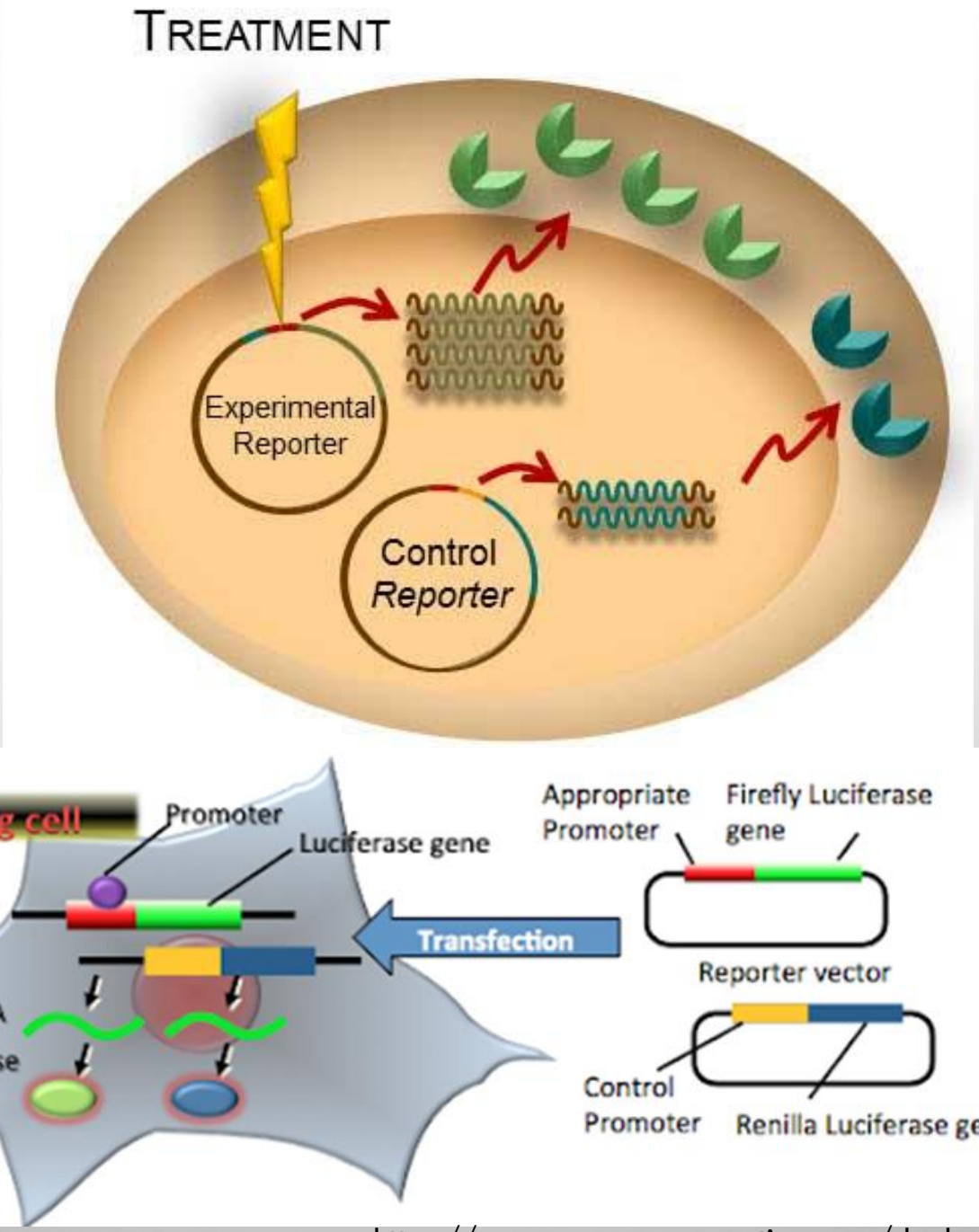
2008年10月8日，瑞典皇家科學院在瑞典首都斯德哥爾摩宣布，日本科學家下村修、美國科學家馬丁·查爾菲和美籍華裔科學家錢永健獲得2008年諾貝爾化學獎。今年得獎的三人代表在**「綠色螢光蛋白(GFP)」**研究上的三個不同時期。**下村脩是這種蛋白的發現者，查爾菲辨識出基因，錢永健則開發出能釋放更強光線、色彩更多樣化的蛋白變形，讓研究者能以不同顏色標示不同蛋白，並看到彼此交互作用。**



http://oldsue2008.blogspot.com/2008/12/2008-gfp.html

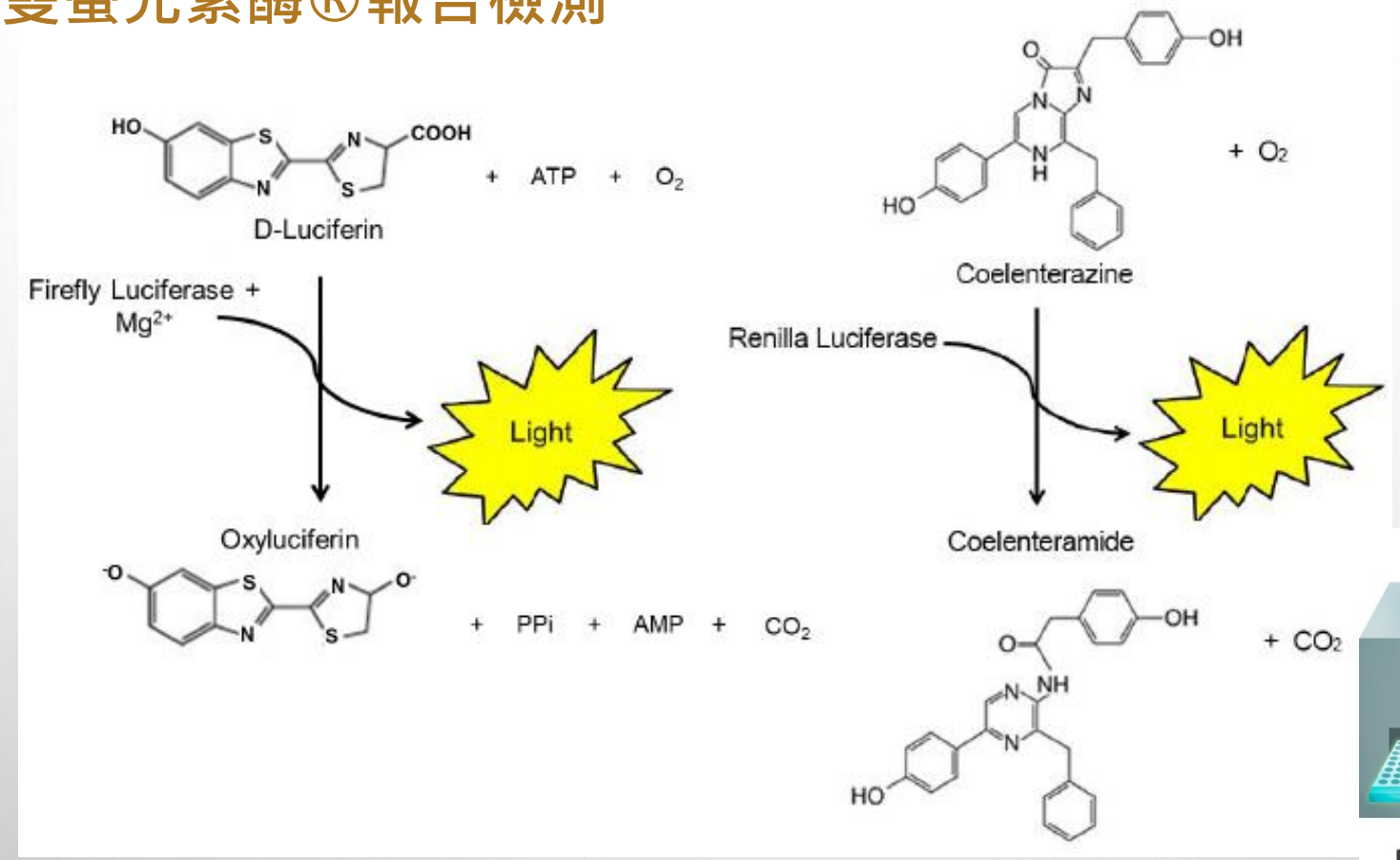


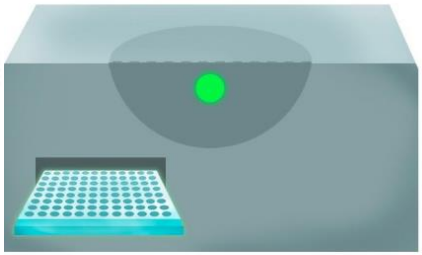
雙報告書檢測



https://www.promegaconnections.com/dual-reporter-assays/

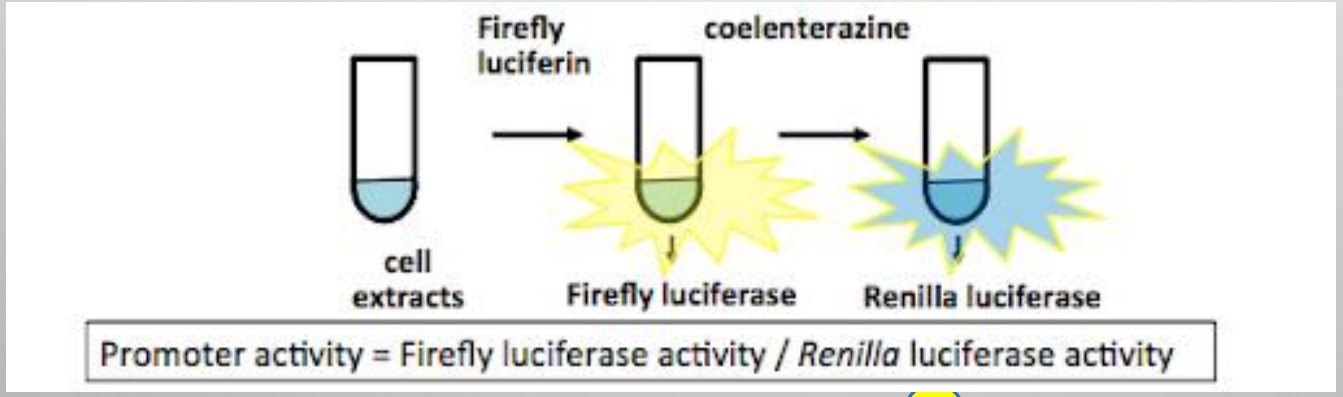
雙螢光素酶®報告檢測





Read results on any luminometer

BPS Bioscience

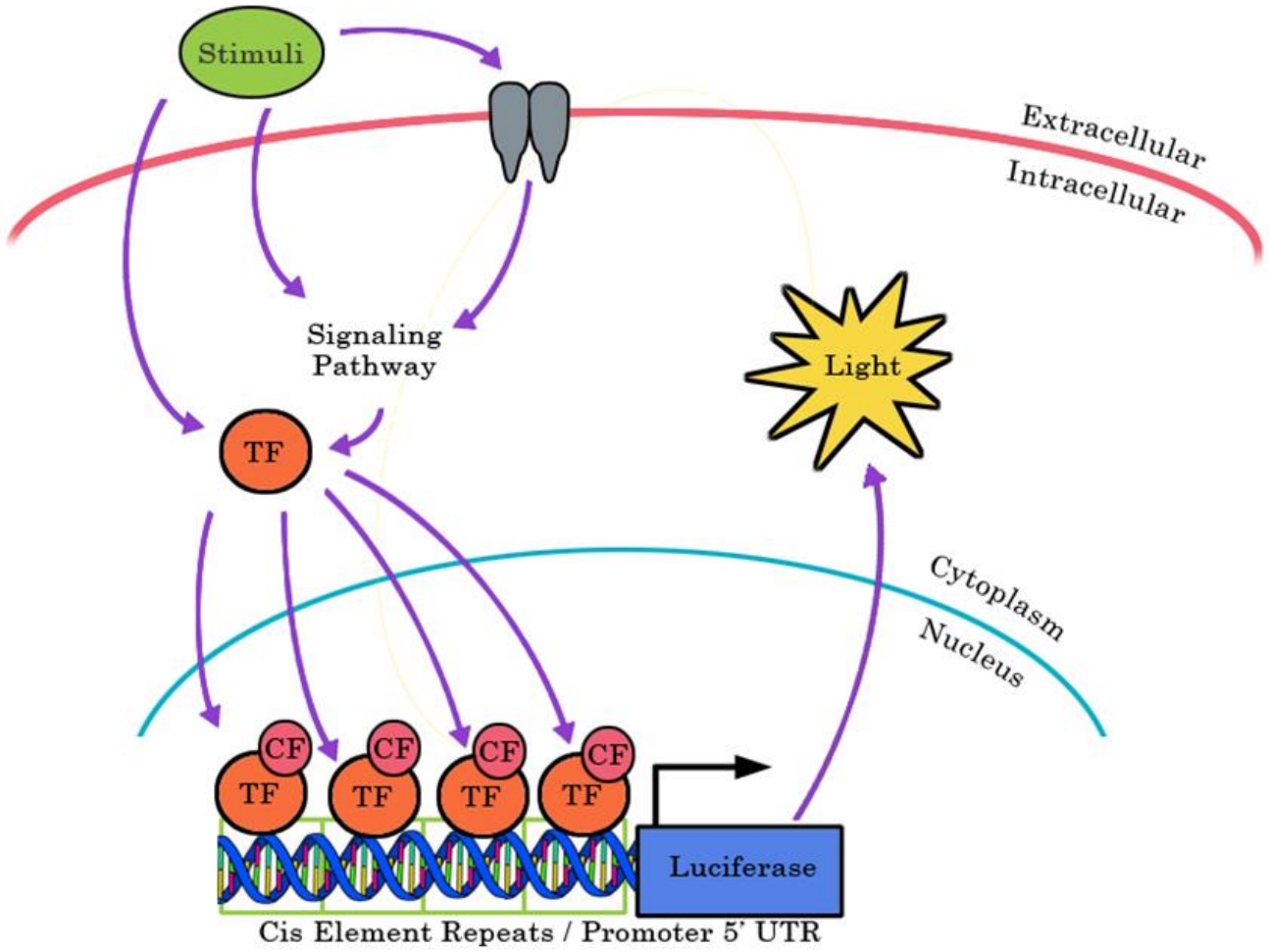


https://www.youtube.com/watch?v=yQXdpwZmwnA

報告基因分析的應用

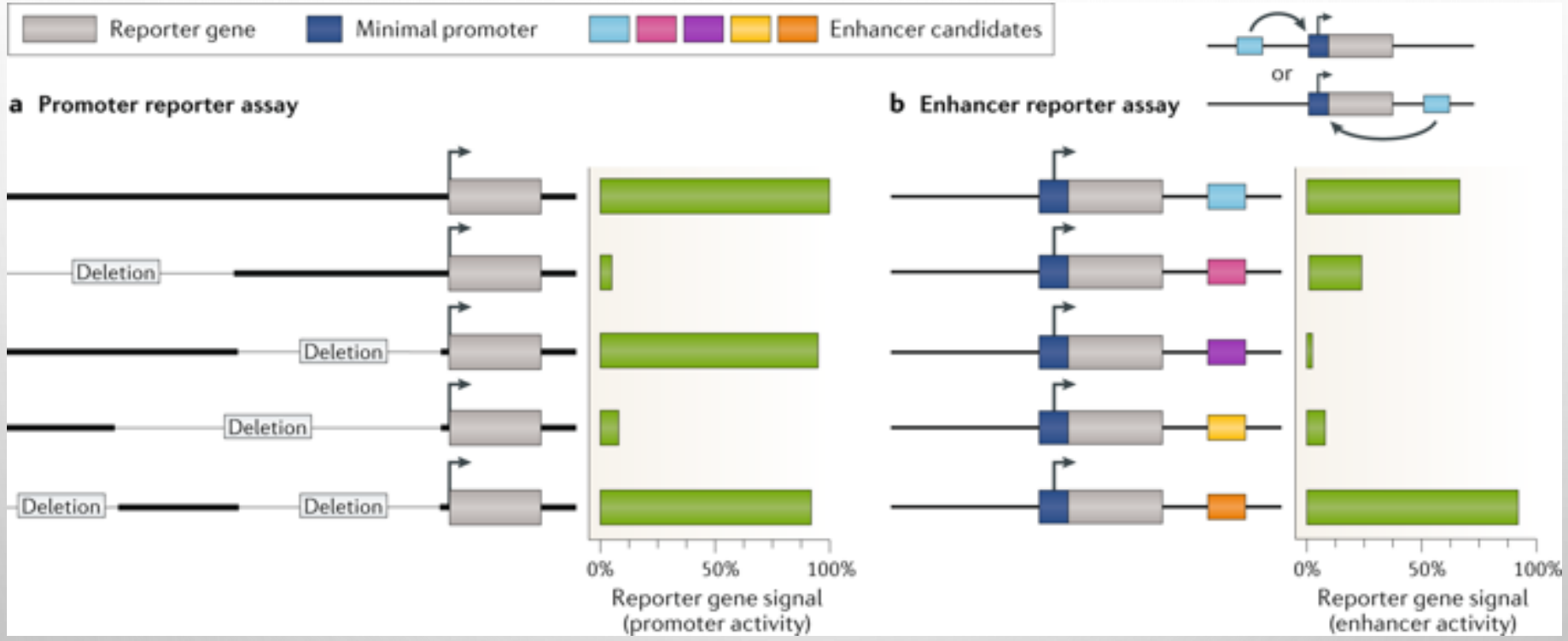
- 分析基因調控
- 分析miRNA靶標
- 分析核受體信號傳導
- 研究蛋白質相互作用
- 高通量篩選

(1) 分析基因調控

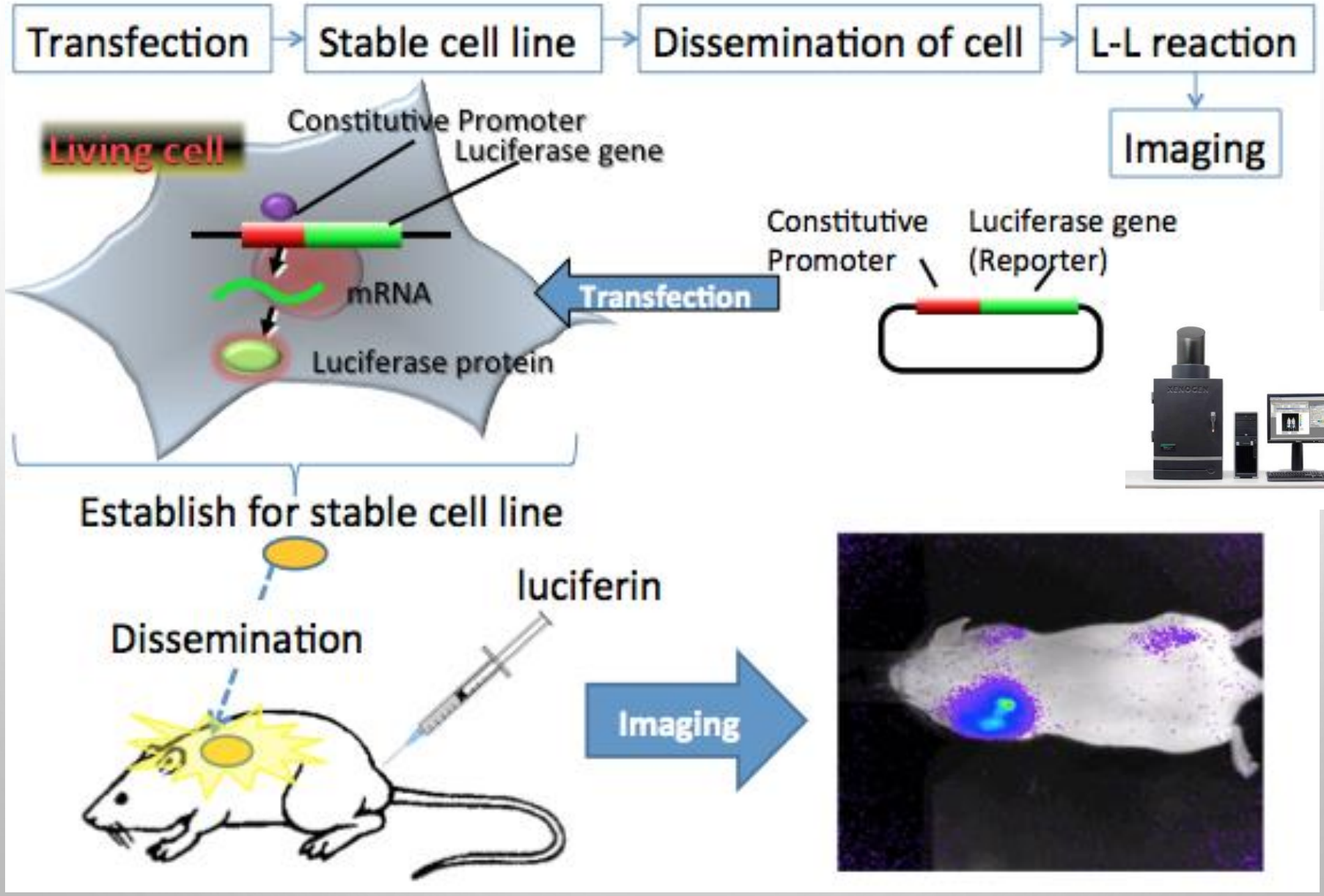


TF = Transcription Factor
CF = Co-factor

Signosis

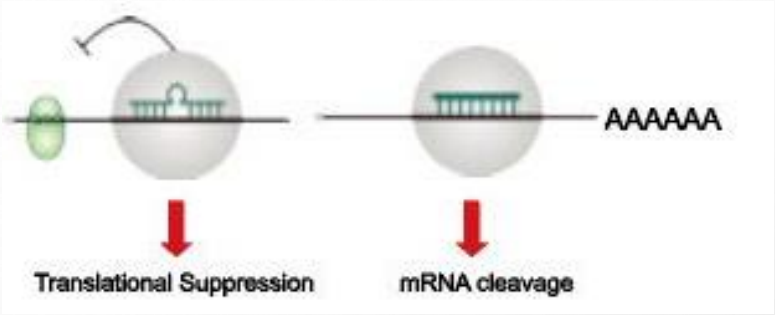


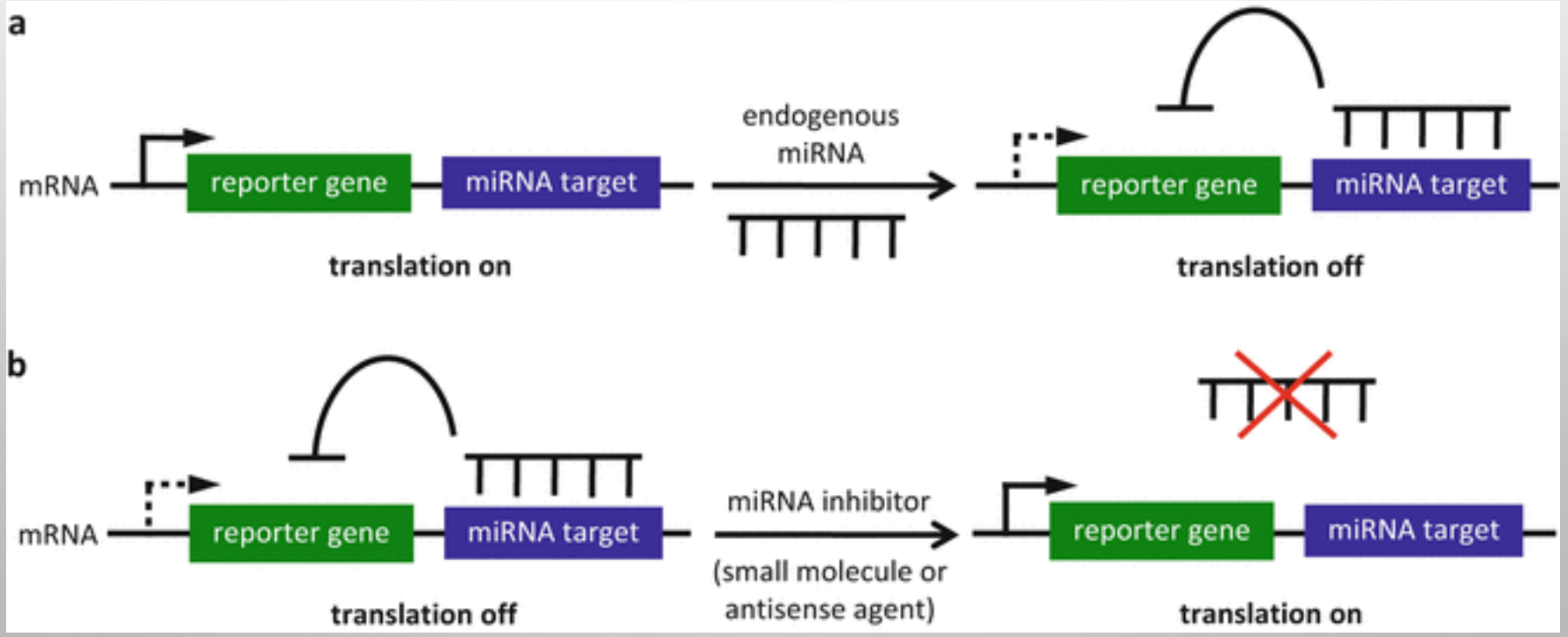
Nature Reviews Genetics (2019)


Transfection
Stable cell line
Dissemination of cell
L-L reaction
Imaging
Living cell
Constitutive Promoter
Luciferase gene
mRNA
Luciferase protein
Transfection
Constitutive Promoter
Luciferase gene (Reporter)
Establish for stable cell line
luciferin
Dissemination
Imaging

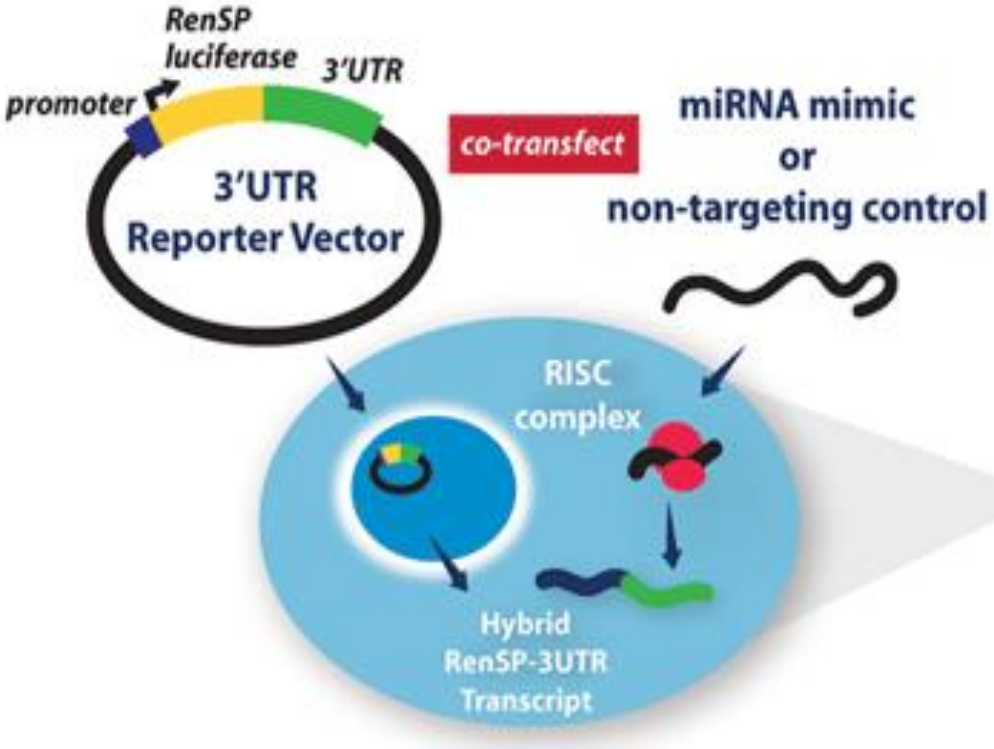
（2）分析MiRNA靶標

- MICRORNA（MiRNA）是短RNA，與轉錄本3′UTR中的靶標相互作用，導致MRNA降解或抑制翻譯。
- 觀察MiRNA介導的效應需要一個在較弱的啟動子的控制下的報告者，以便可以觀察到基因表達的細微變化。

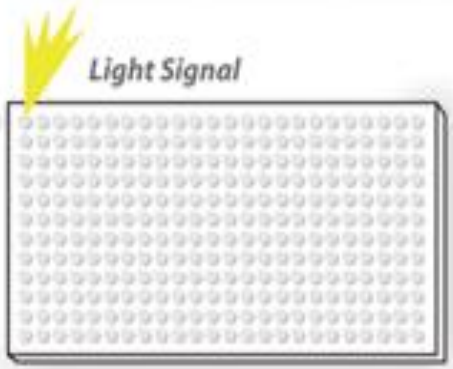




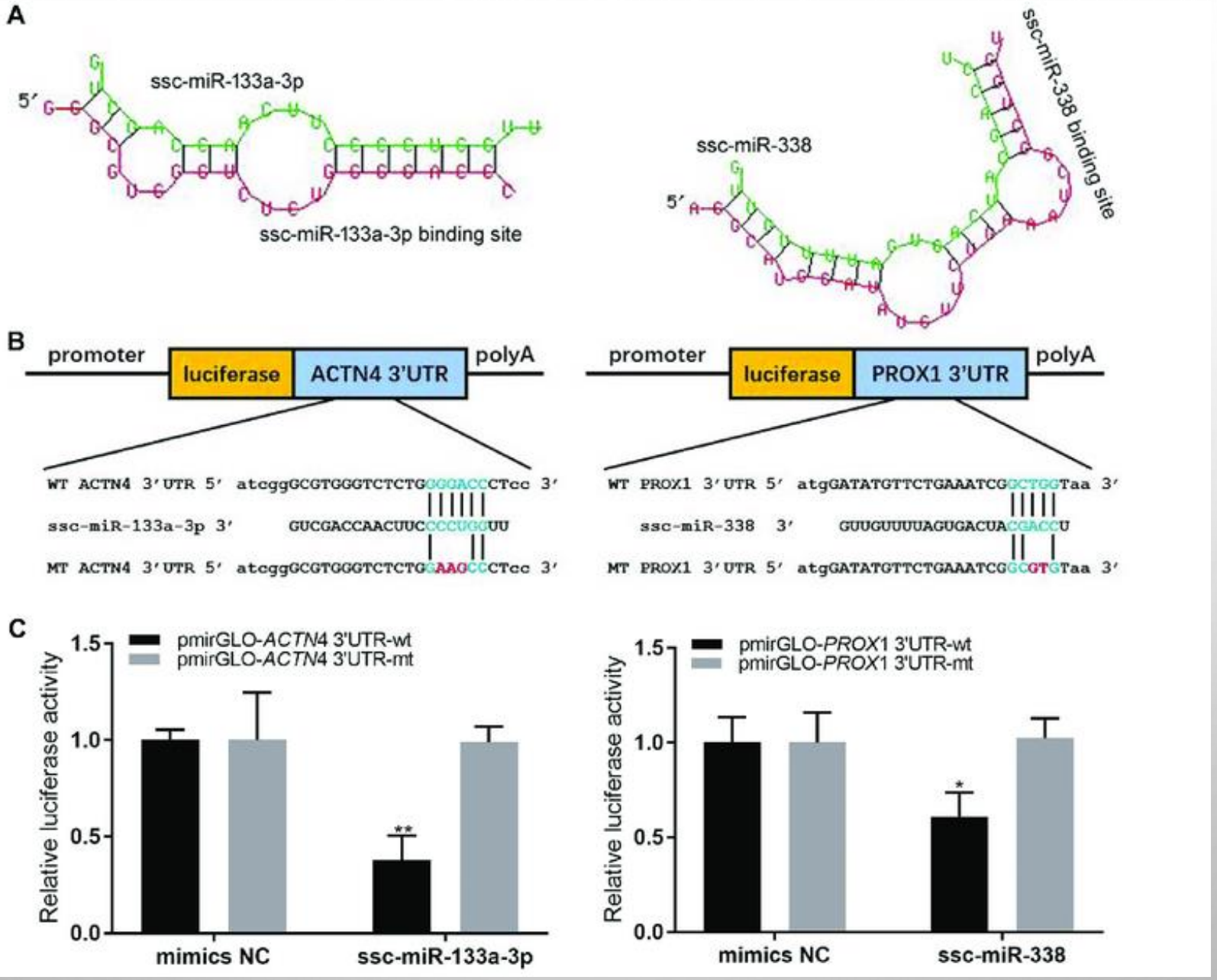
Methods Mol Biol. 2014;1095:135-46.



$$\frac{\text{Light output with miRNA mimic}}{\text{Light output with control}} = \textit{Specific activity of miRNA on target UTR}$$

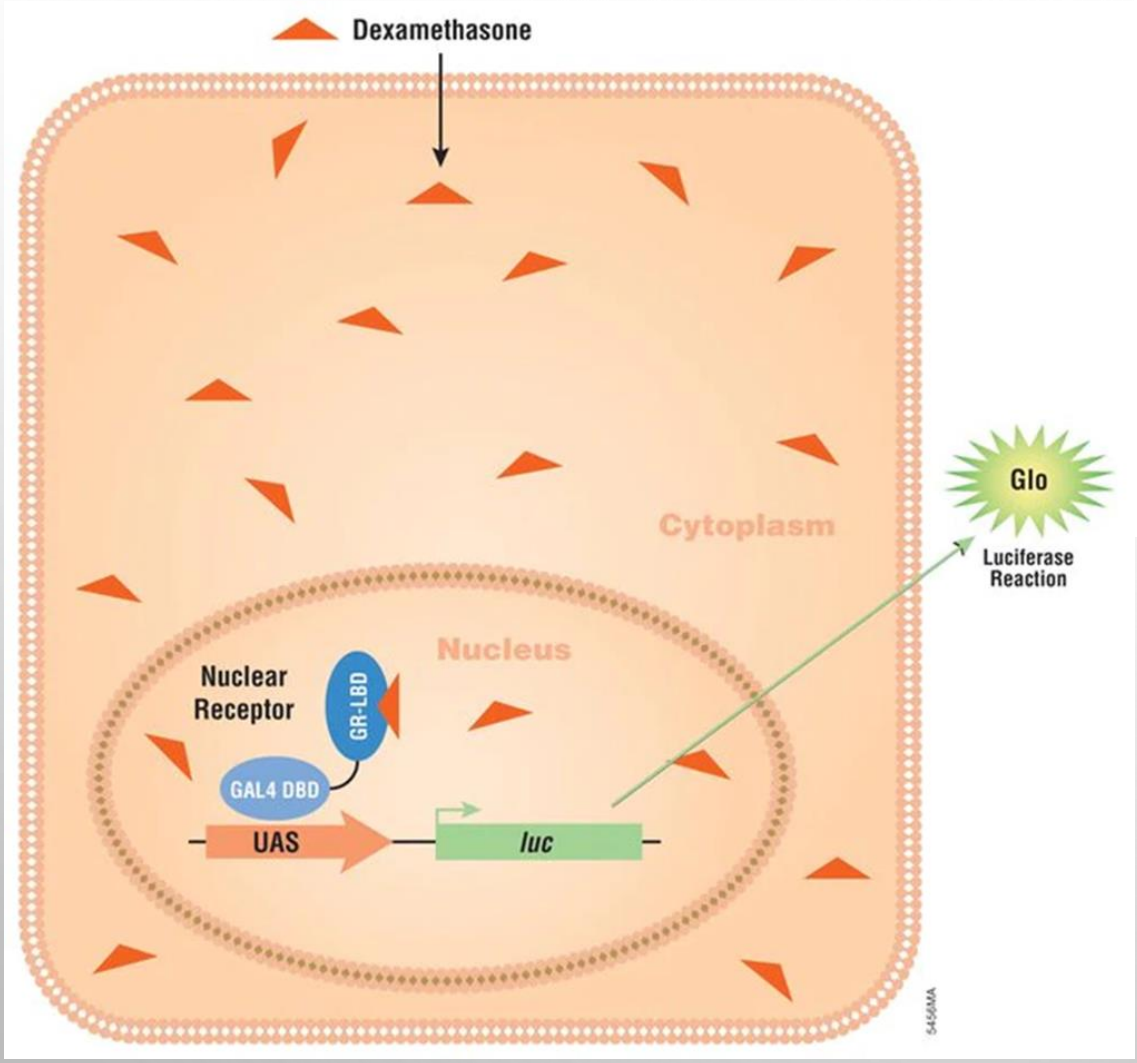


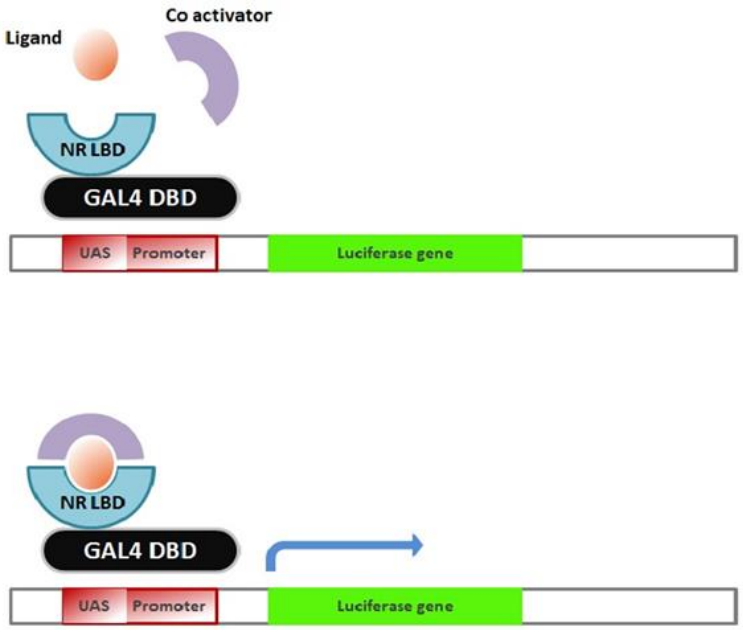
1. Transfect the GoClone 3'UTR construct into your cell line
2. A constitutive promoter drives production of a hybrid RenSP-human 3'UTR transcript
3. Total luciferase output depends on the effect of the human 3'UTR on the hybrid transcripts stability and/or translation efficiency
4. Include a co-transfected synthetic miRNA or miRNA inhibitor to measure its impact on transcript stability and/or translation efficiency
5. Use optimized LightSwitch Luciferase Reagents to obtain maximun sensitivity and dynamic range



Front Genet. 2019 Aug 28;10:756.

（3）分析核受體信號傳導

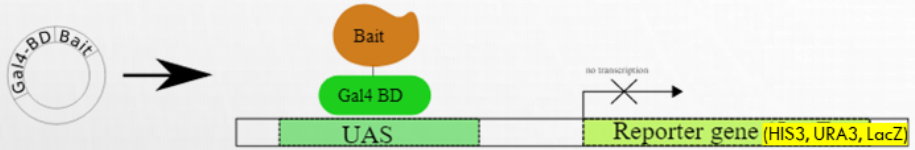




- 關鍵是一個優化的PGL4載體，其中包含9個重複的GAL4 UAS，一個最小的啟動子和LUC2P螢光素酶基因（PGL4.35）。
- 製備兩個含有雌激素受體配體結合域（PBIND-ERα）或糖皮質激素受體配體結合域（PBIND-GR）的PFN26A載體。
- 用載體、培養和檢測轉染細胞。

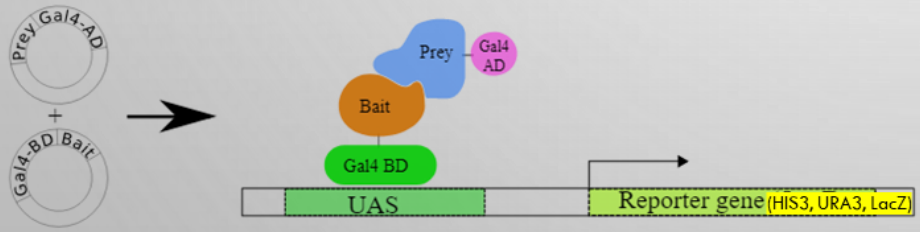
（4）研究蛋白質相互作用

A. Regular transcription of the reporter gene

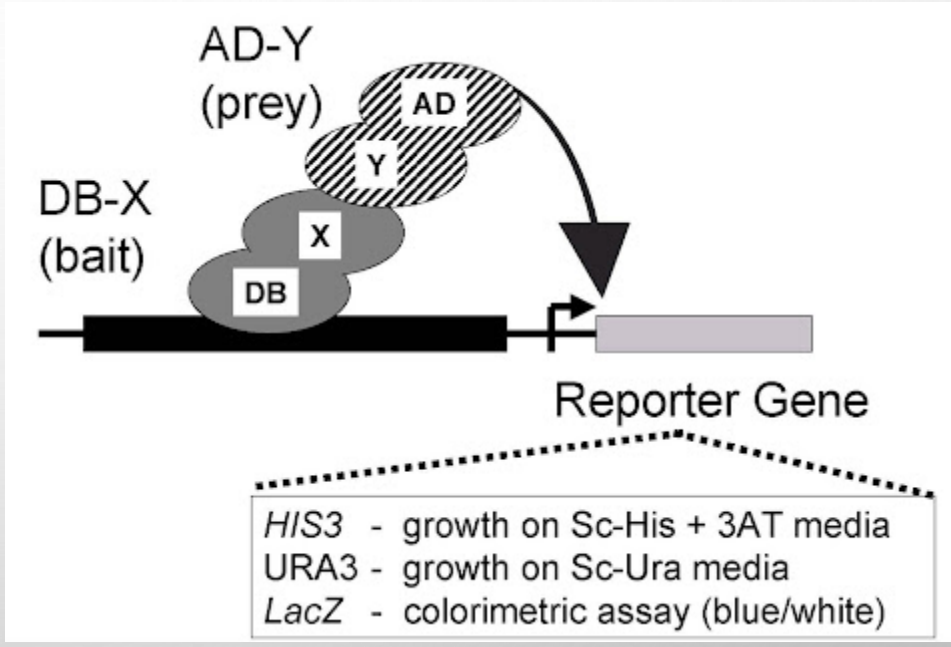


B. One fusion protein only (Gal4-BD + Bait) - no transcription

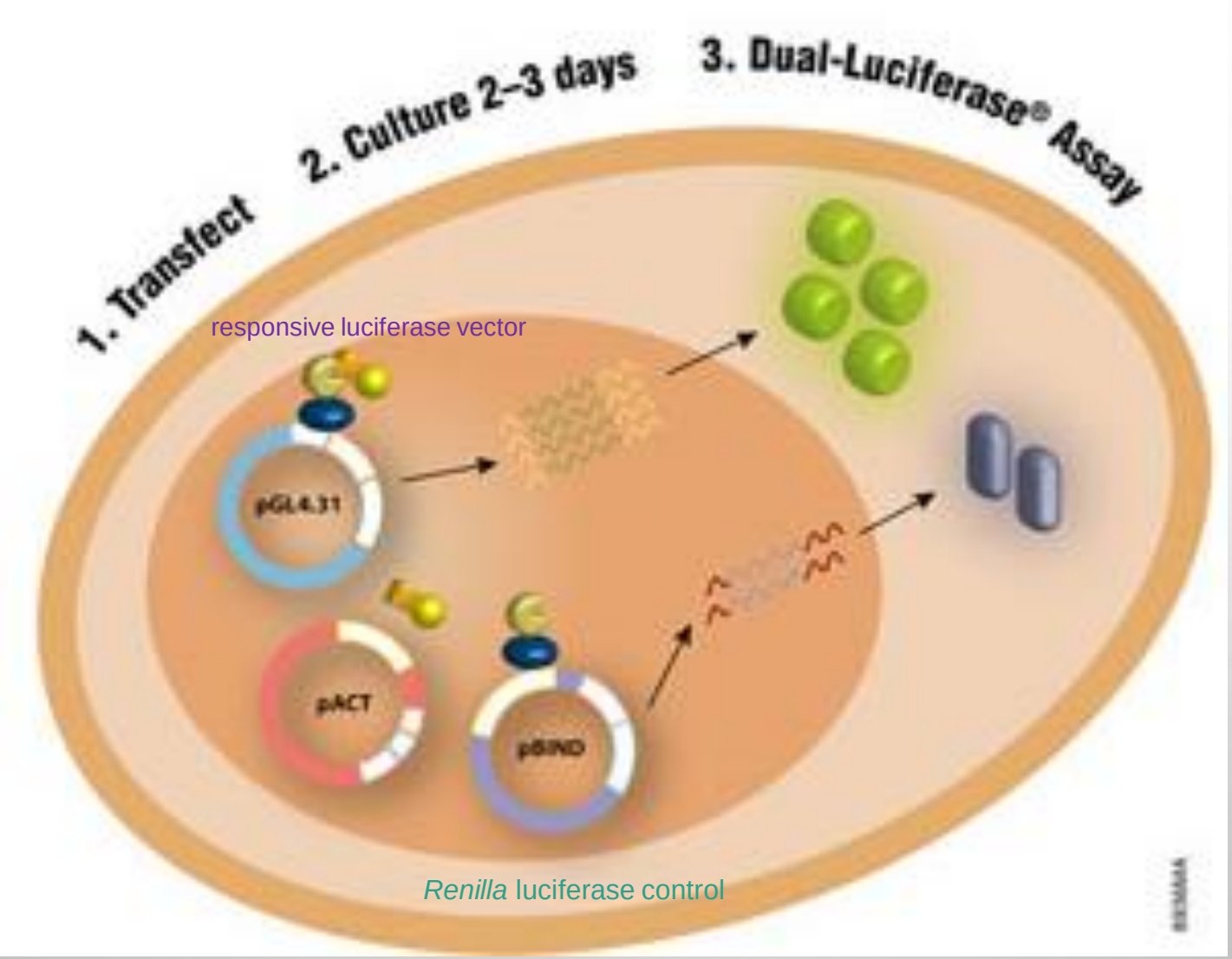
C. One fusion protein only (Gal4-AD + Prey) - no transcription



D. Two fusion proteins with interacting Bait and Prey

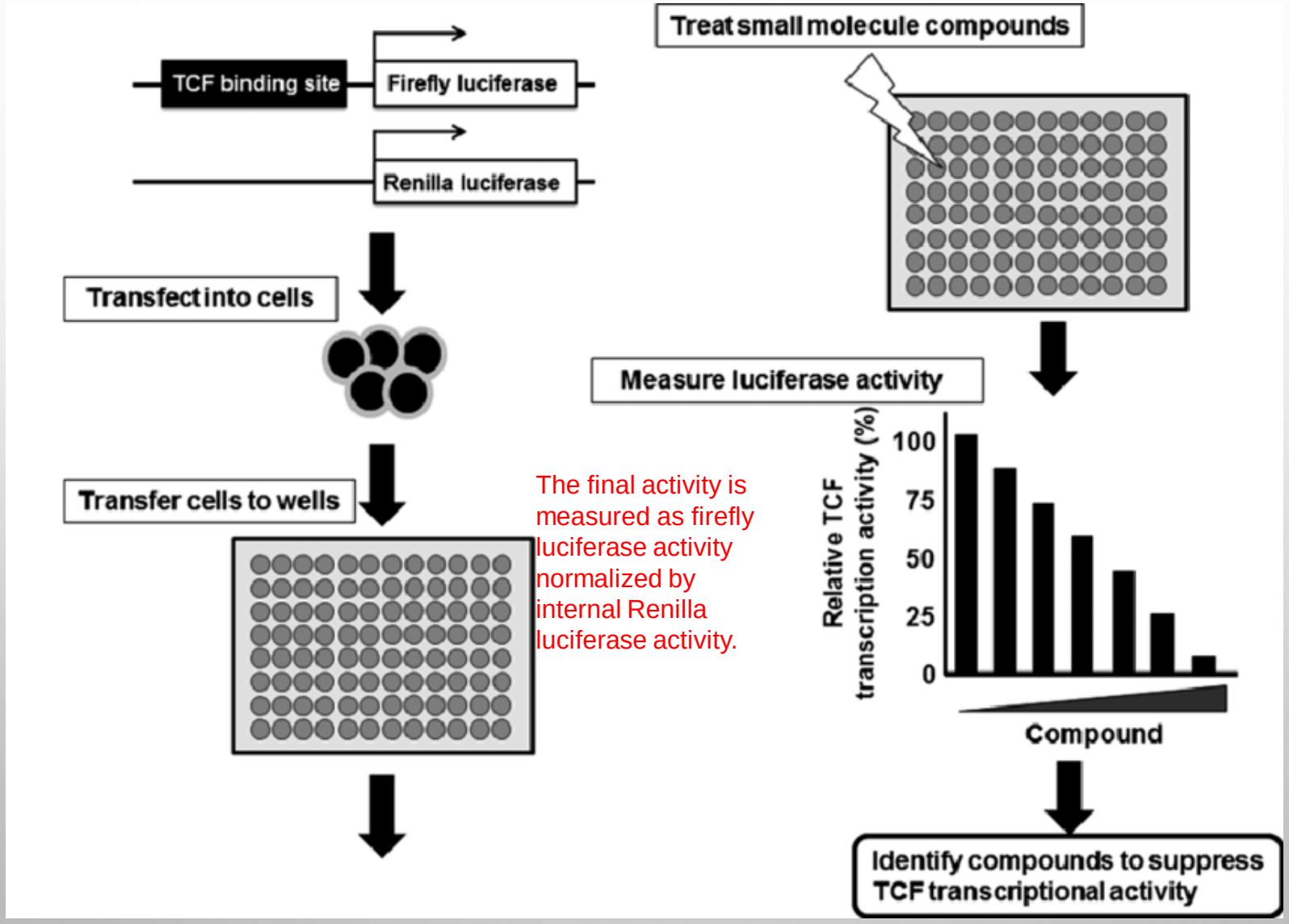


https://zh.wikipedia.org/wiki


1. Transfect
2. Culture 2–3 days
3. Dual-Luciferase® Assay
responsive luciferase vector
pGL4.31
pACT
pBIND
Renilla luciferase control

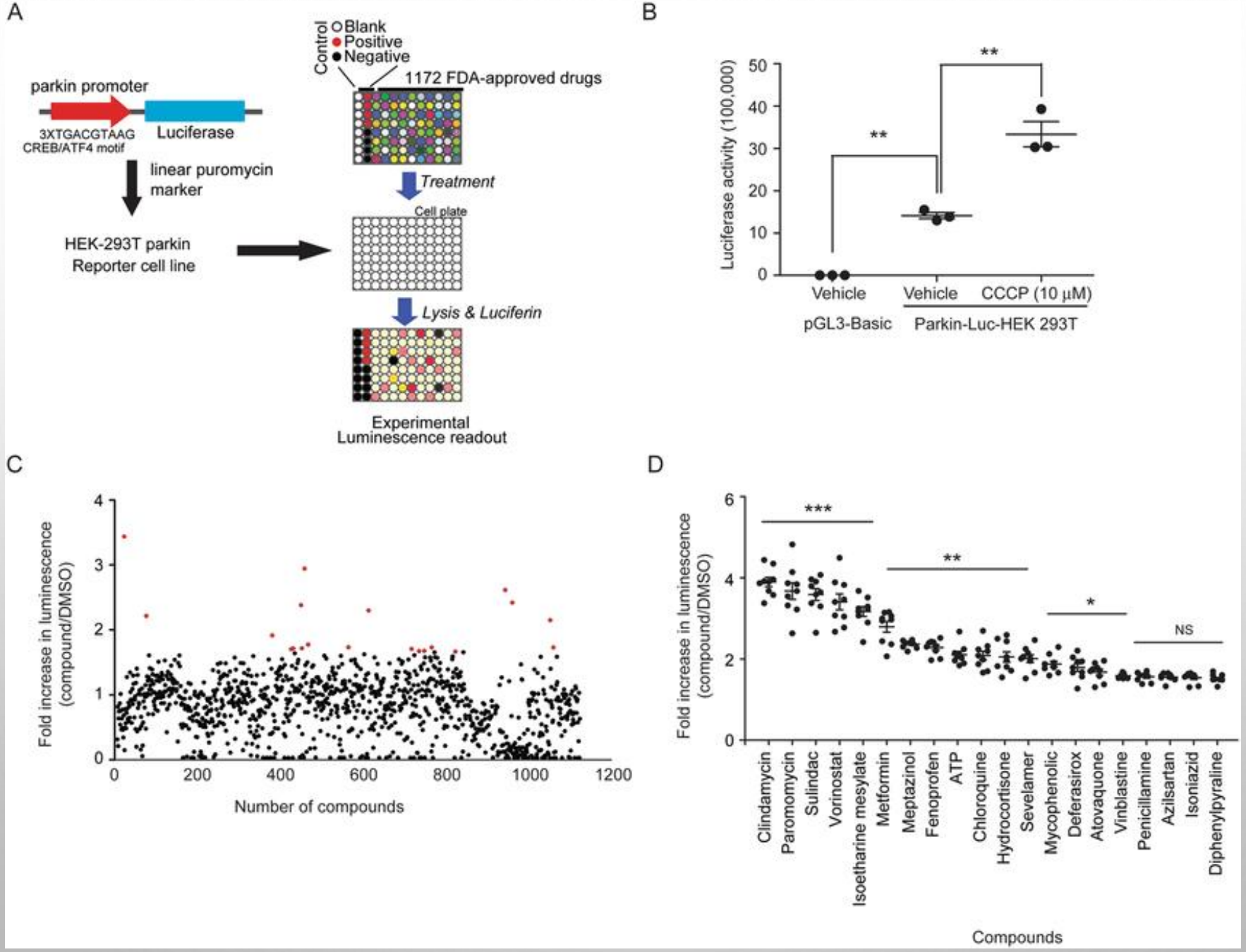
Promega

（5）高通量篩選



The final activity is measured as firefly luciferase activity normalized by internal Renilla luciferase activity.

March 2015 Cancer Science 106(6)



April 2017 Scientific Reports 7(1):525


Q
&
A


take a
BREAK

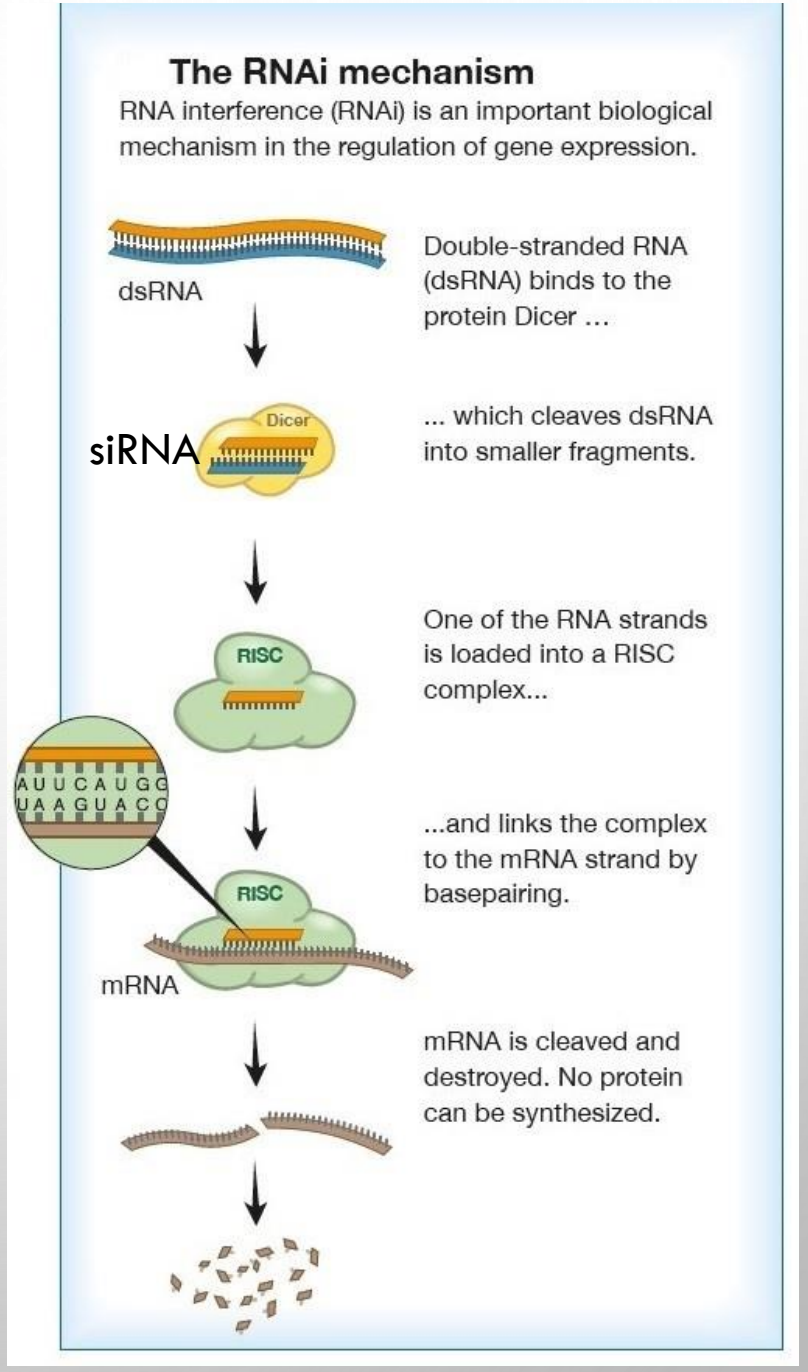
III. 核糖核酸干擾 (RNAi)

上課內容

- RNAi原理
- 誰發現了RNAi
- RNAi技術
- RNAi的應用

RNAi原理

- 雙鏈RNA（dsRNA）由RNase III家族成員Dicer處理，產生21-23nt小干擾RNA。
- siRNA由稱為RNA誘導的沉默複合物（RISC）的多組分核酸酶操縱。



誰發現了RNAi

2006 Nobel Prize in Medicine or Physiology

RNA Interference

NATURE | VOL 391 | 19 FEBRUARY 1998

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

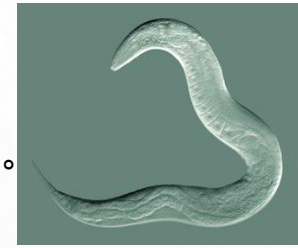
Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

A naturally occurring mechanism

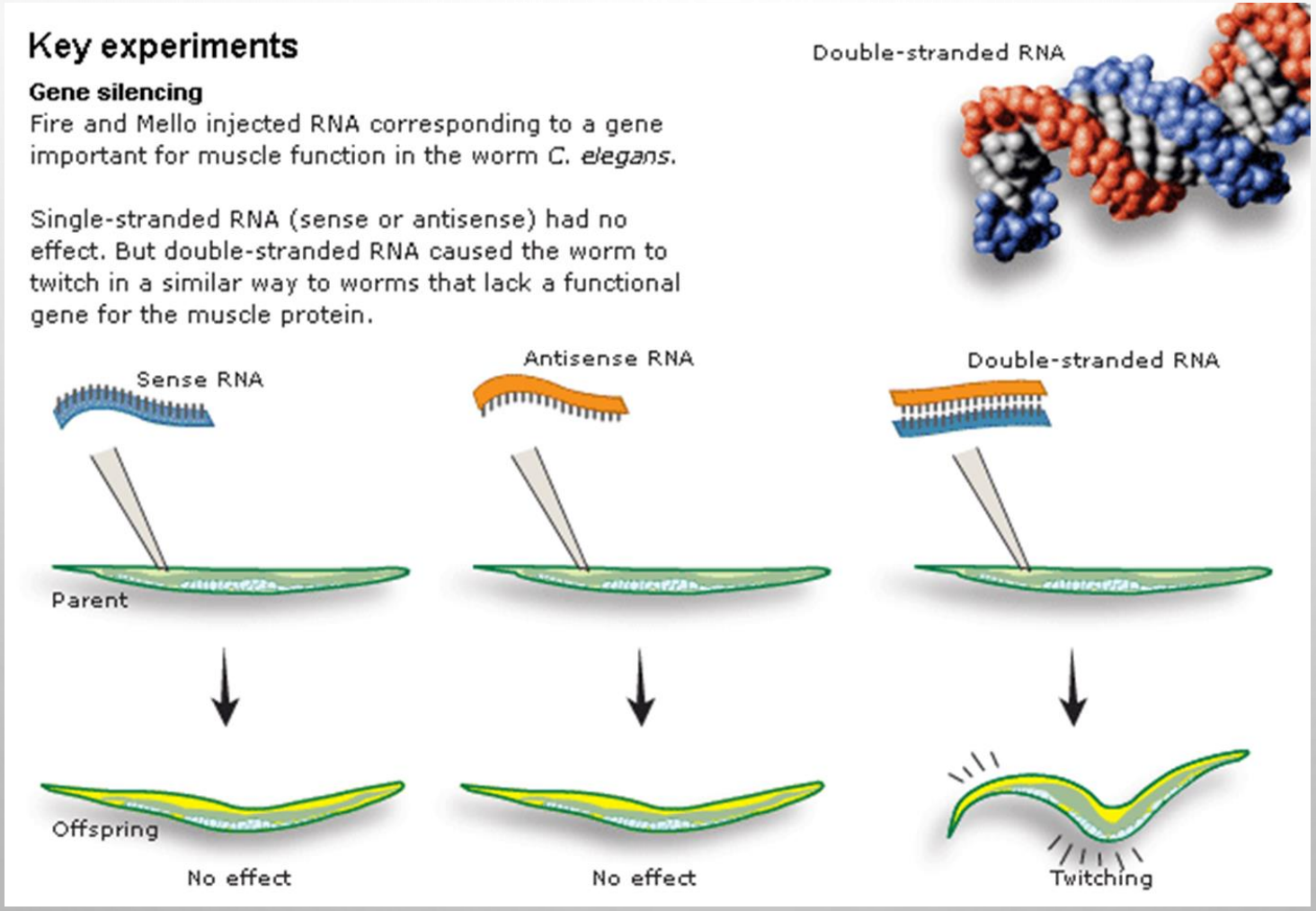
Destruction of mRNA results in the post transcriptional inhibition of gene expression and the prevention of protein synthesis.

克雷格·梅洛　　安德魯·菲爾

動物模型-線蟲

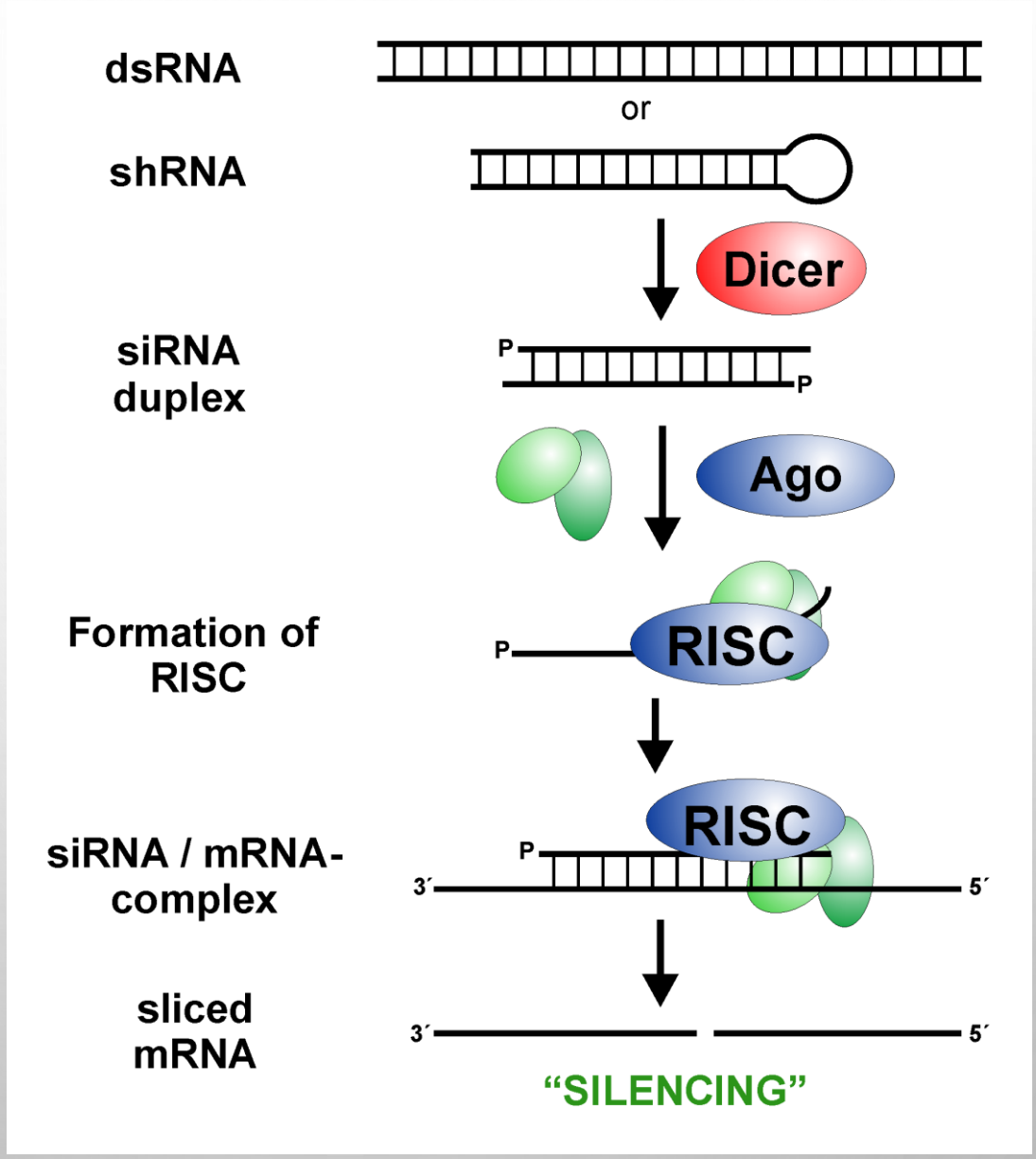


- RNAi最初被發現於秀麗隱桿線蟲 (*Caenorhabditis elegans, C. elegans*)。
- 觀察到雙鏈RNA（dsRNA）在沉默標靶基因表達方面比單獨使用反股RNA (anti-sense RNA)有效10倍。



小干擾 RNA (small interfering RNAs, siRNAs)

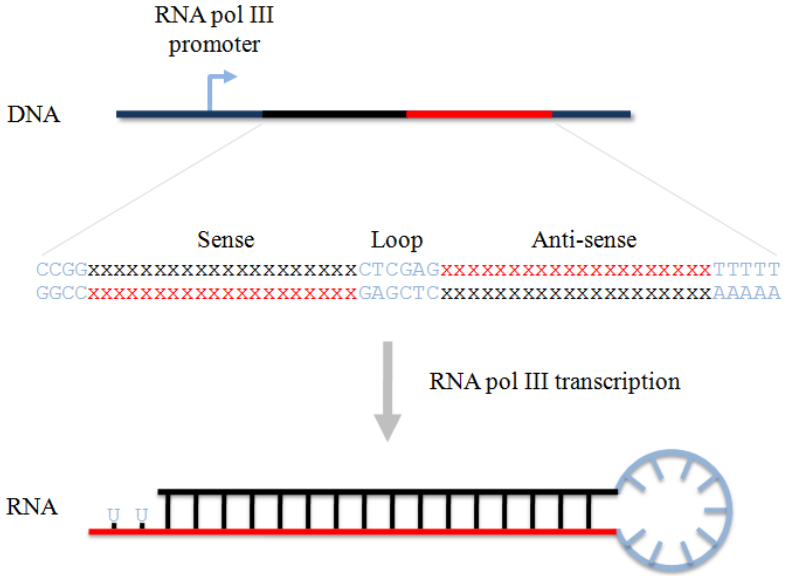
小干擾 RNA (siRNA)。具有 2 個 核甘酸， 3' 末端突出端的 dsRNA 可激活 RNAi，導致 mRNA 以依賴於靶 mRNA 的互補結合的序列特異性方式降解。

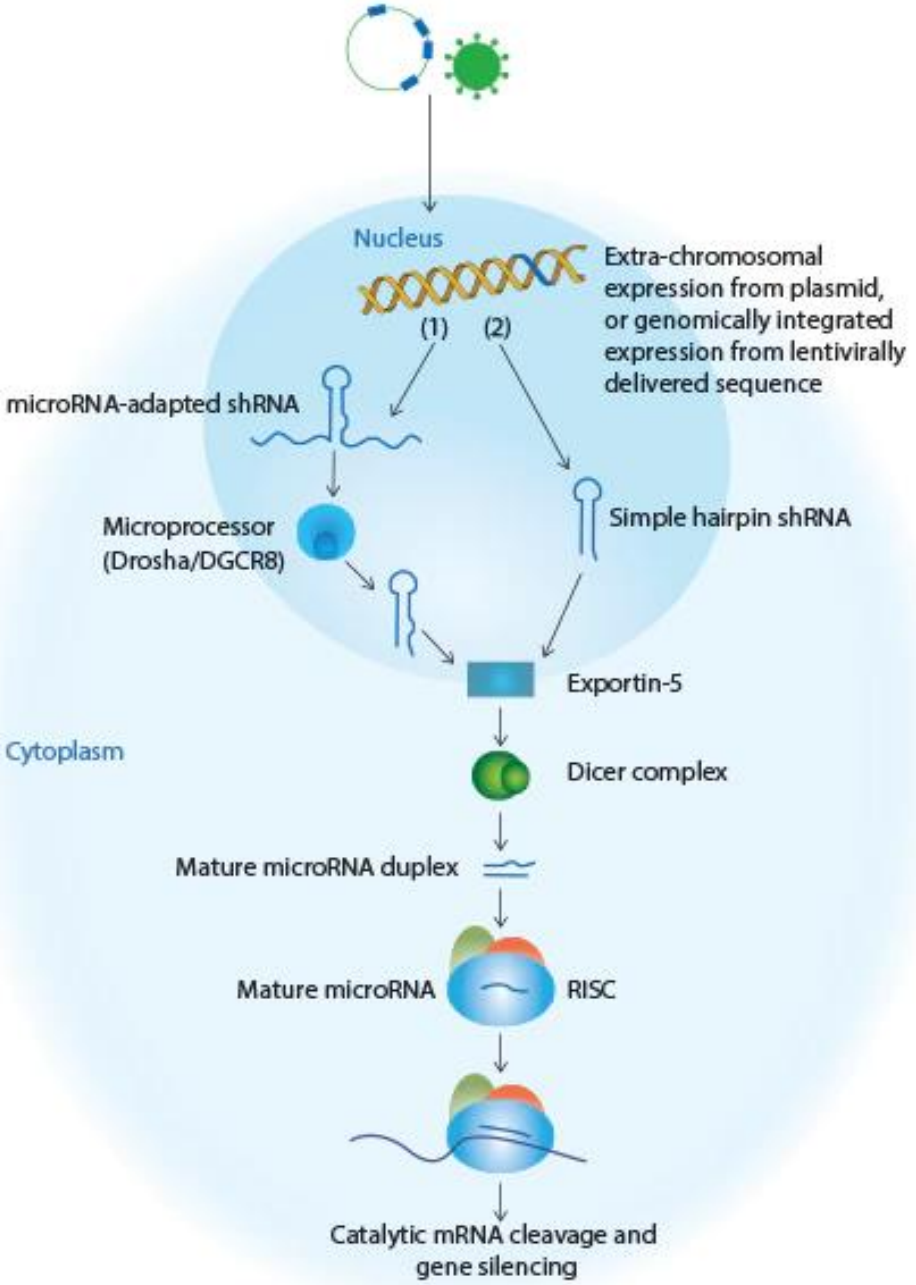


https://www.gene-quantification.de/si-rna.html

小髮夾RNA (short hairpin RNA, shRNA)

短髮夾 RNA (shRNA) 包含一個環結構，該環結構被加工成 siRNA，並且還導致 mRNA 以依賴於靶 mRNA 的互補結合的序列特異性方式降解。

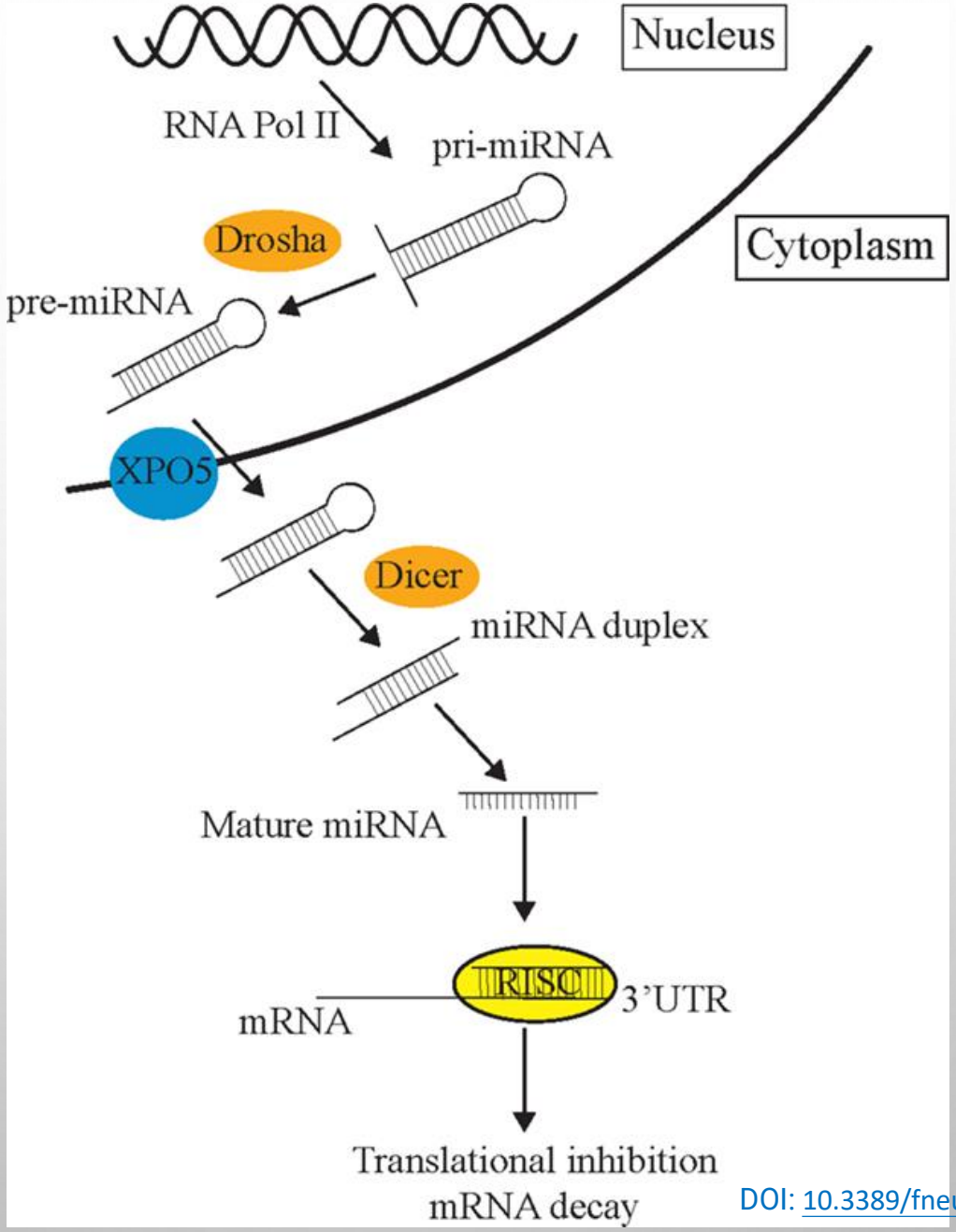




https://horizondiscovery.com

小分子核糖核酸（microRNA, miRNA)

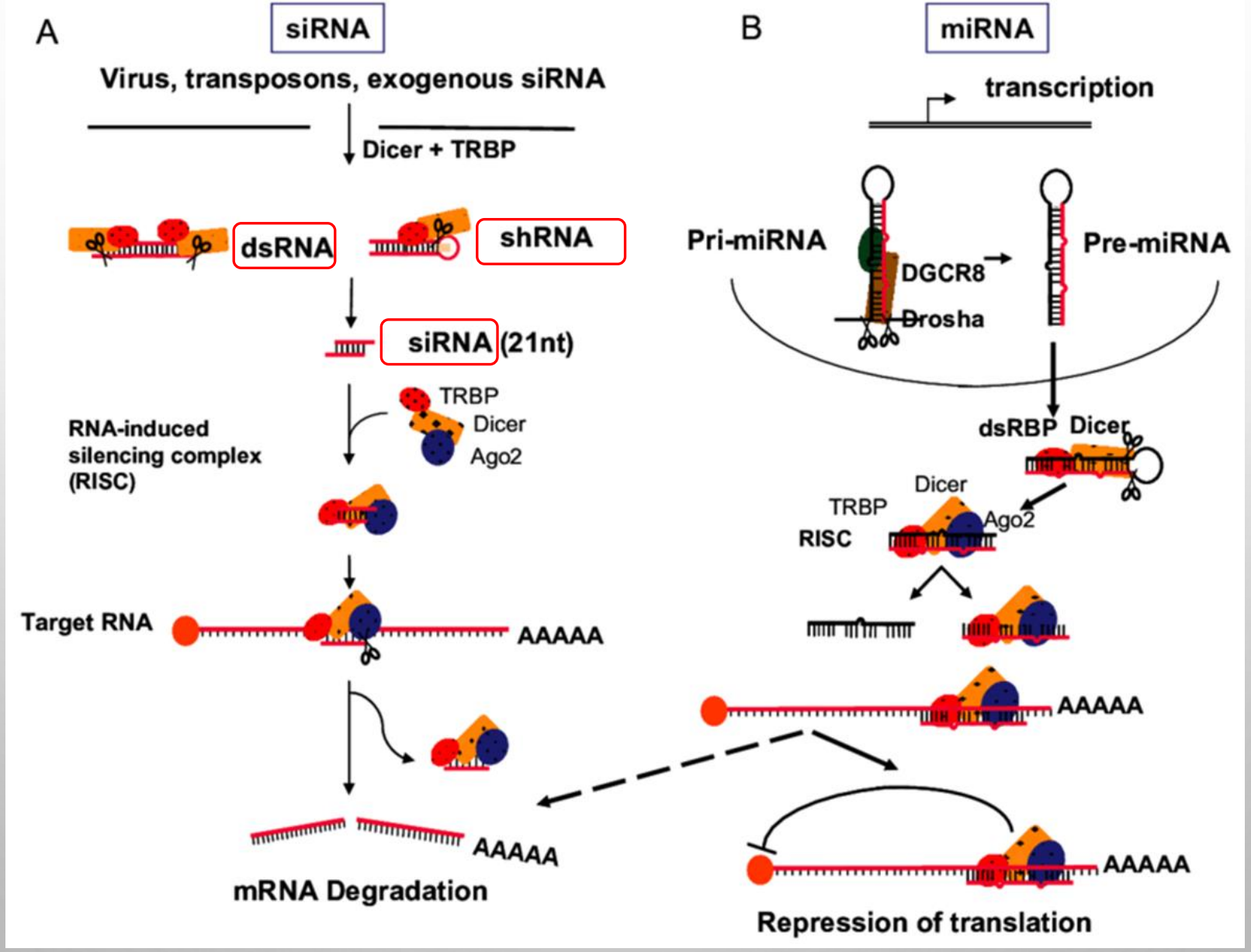
小分子核糖核酸（miRNA），是真核生物中廣泛存在的一種長約21到23個核苷酸的RNA分子，可調節其他基因的表現



DOI: 10.3389/fneur.2015.00245

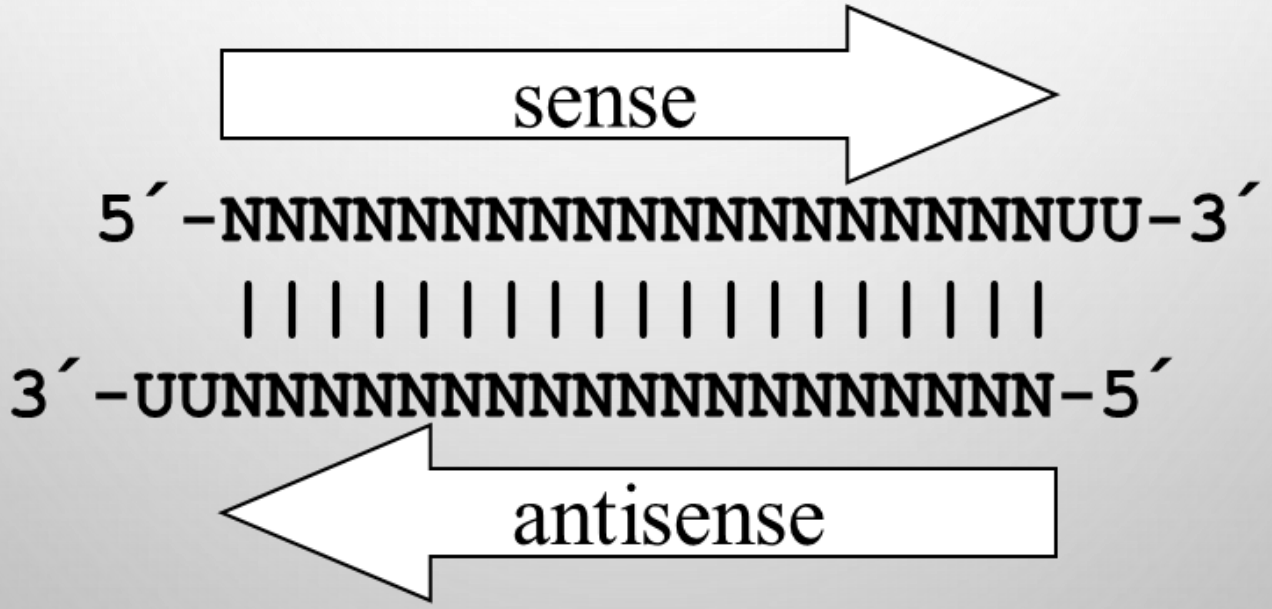
RNAi相關技術

1. siRNA化學合成

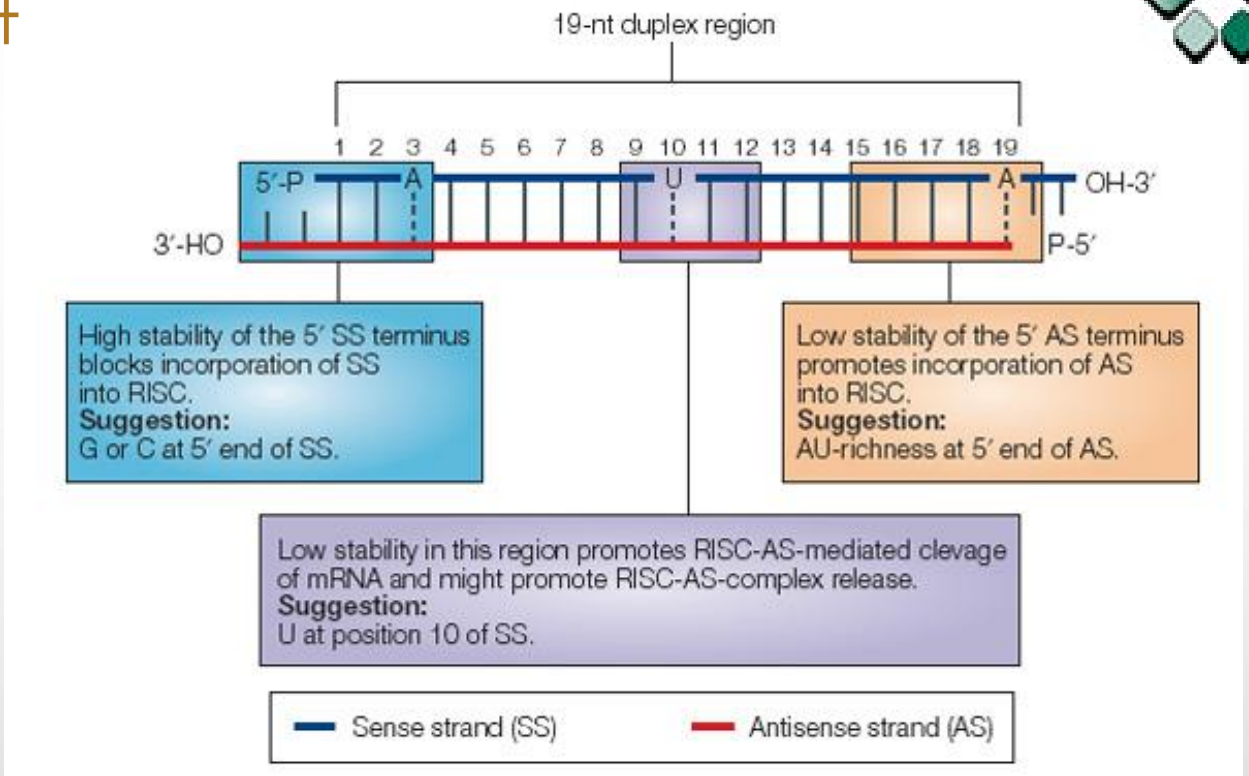


Carlos Melendez-Pena

- 靶向任何序列的合成siRNA可以通過化學合成製備
- 在哺乳動物細胞中，siRNA在敲低靶基因表達方面的有效性範圍（50-95%）
- siRNA的有效性取決於標靶序列



siRNA 設計



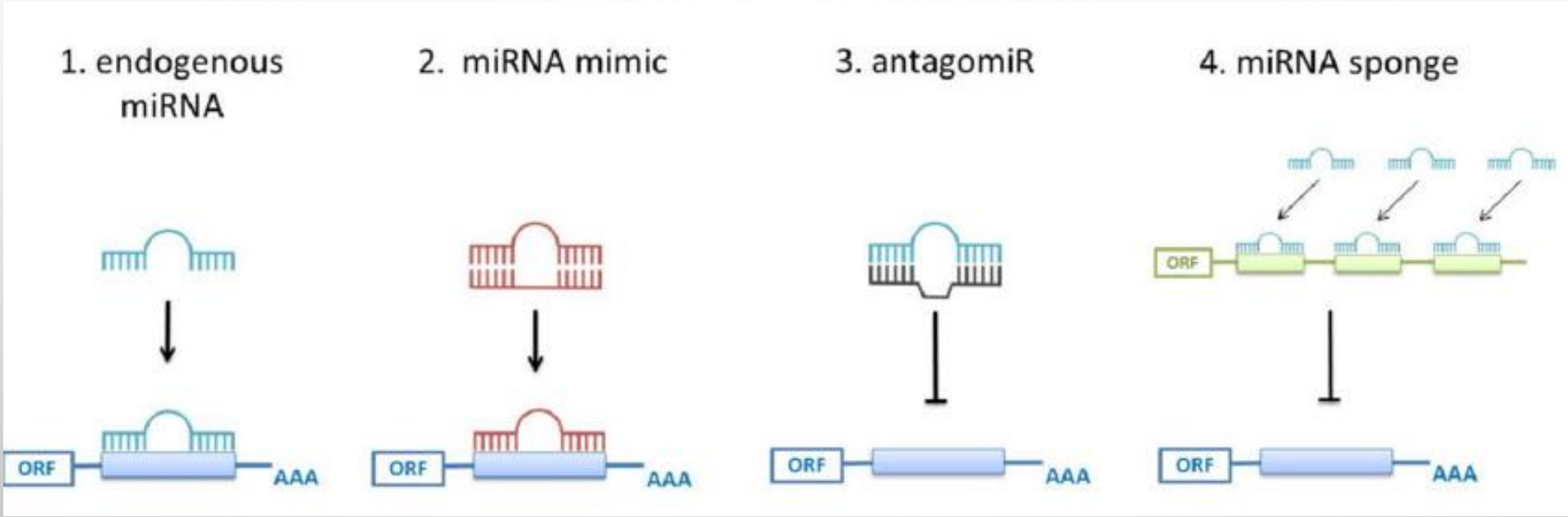
1.低至中等GC含量（30-50%）。
2. 無內部重複或回文。
3. 在感應鏈的位置 3 處存在 A。
4. 在感應鏈的位置 19 處存在 A。
5. 在感測鏈的位置 19 處沒有 G 或 C。
6. 在感應線的位置 10 處存在 U。
7. 感應鏈位置 13 處沒有 G。
8. 在感應鏈的 15-19 位置至少有 3 個 A/US。

Table 2. Names and addresses of siRNA design computational soft wares

Soft wares	Address
siDirect	http://genomics.jp/sidirect/index.php?type=fc
siDESIGN Center	dharmacon.gelifesciences.com/design-center
siRNA Design Software	http://www.genscript.com/ssl-bin/app/rnai
Block-iT RNAi Designer	https://rnaidesigner.invitrogen.com
siRNA Target Finder	http://www.ambion.com/techlib/misc/siRNA_finder.html
RNAi explorer	www.genelink.com/sirna/siRNAorder.asp

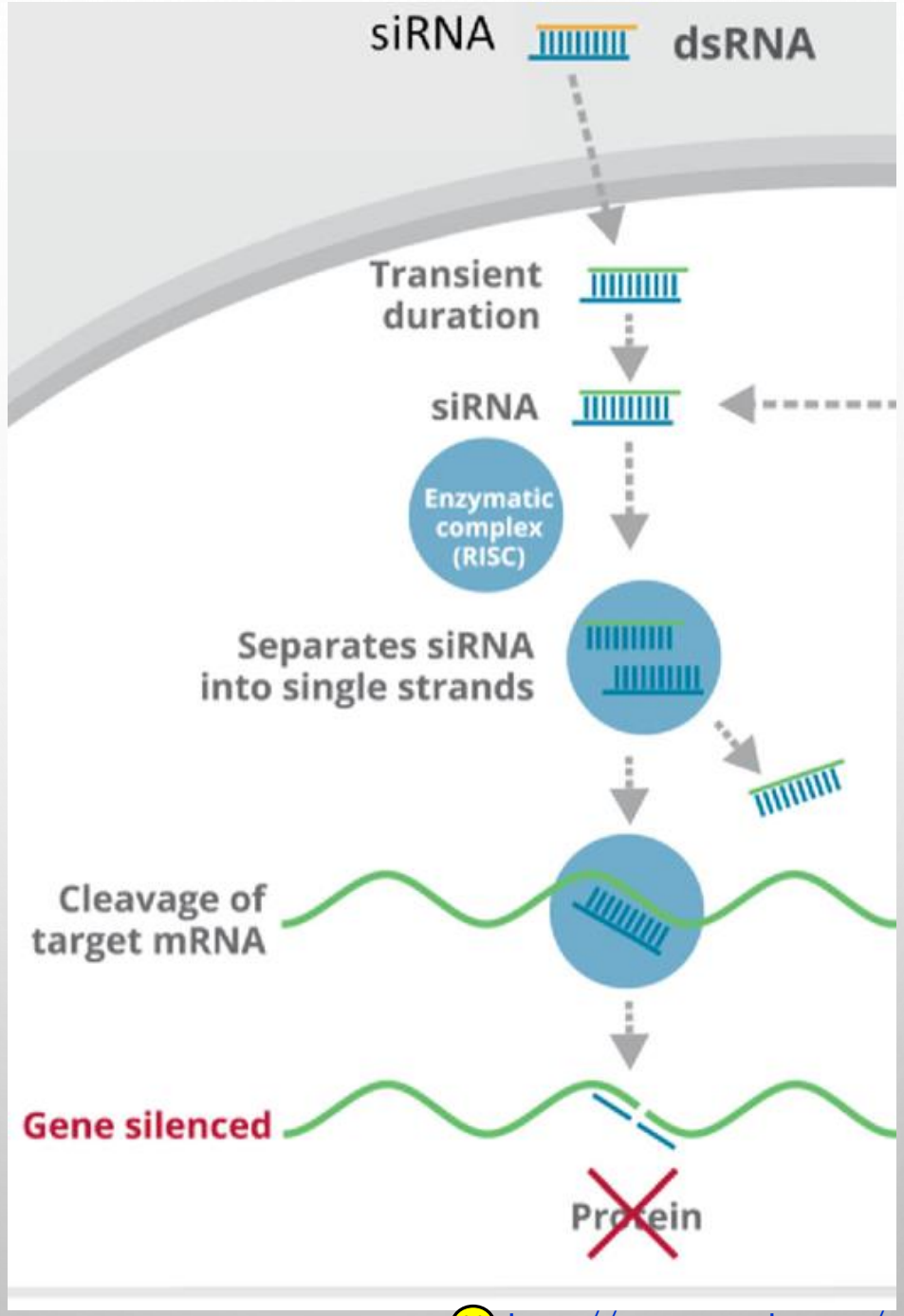
DOI:10.15171/apb.2017.072 Corpus ID: 25124047

靶向miRNA活性的不同方法



August 2017 Advances in Clinical and Experimental Medicine 26(5):868-874

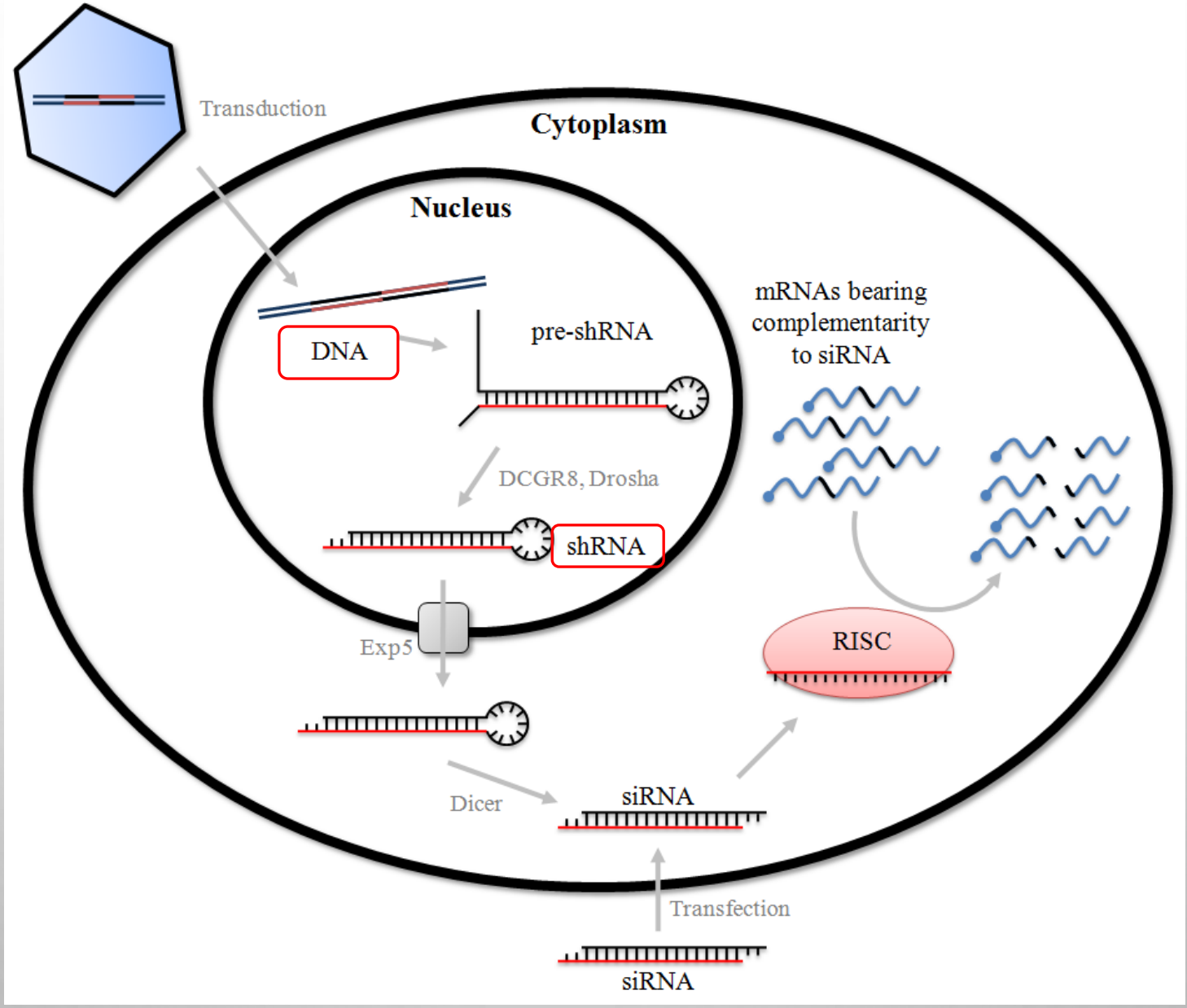
2. siRNA轉染



Transl Neurodegener. 2017; 6: 30.

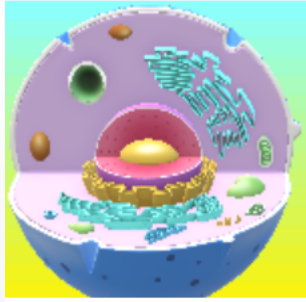
https://www.youtube.com/watch?v=UuicPthdT70

3. shRNA轉染



Springer 2010. 95-117p.

http://rnai.genmed.sinica.edu.tw/index



新服務其他客製化服務

服務項目訂購(核心服務項目&收費準則查詢)　會員登入: 帳號　密碼　Login　申請會員

C6-1 C6-2 C6-3 C6-4 C6-5 C6-6 C6-7 C6-8 C6-9 C6-10 C6-11 C6-12 C6-13 C6-14
C6-15 C6-16 C6-17 C6-18 C6-19 C6-20 C6-21

shRNA 質體細菌株分讓（點選可展開）[產品簡介] [使用須知] [技術支援]

shRNA 關鍵字查詢

INeedControlshRNA　Search

可利用 CloneId / Symbol / targetSeq 作查詢

Search Entrez Gene to find official symbols.

TRC1 vector

TRC2 vector

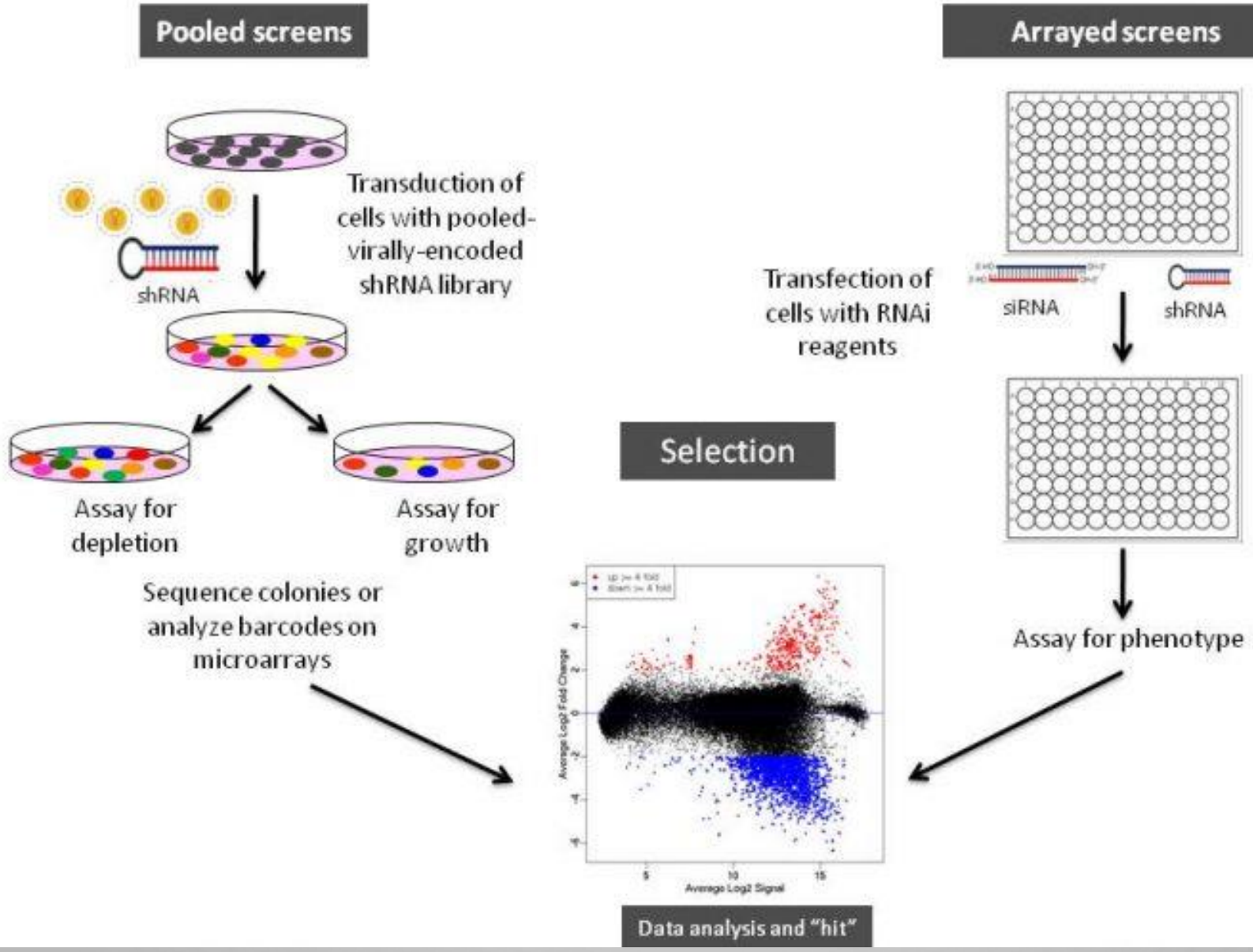
訂單取貨方式	收單(繳費完成)時間	出貨時間說明
核心取貨	※ 每週一及三早上9:00前	收單當日製作，3-4個工作天後取貨。
宅配寄送	※ 每週一及三早上9:00前	收單當日製作，每週四及隔週一出貨，預計5-8個工作天到貨。

Showing 1 to 10 of 10　<< < 1 > >>

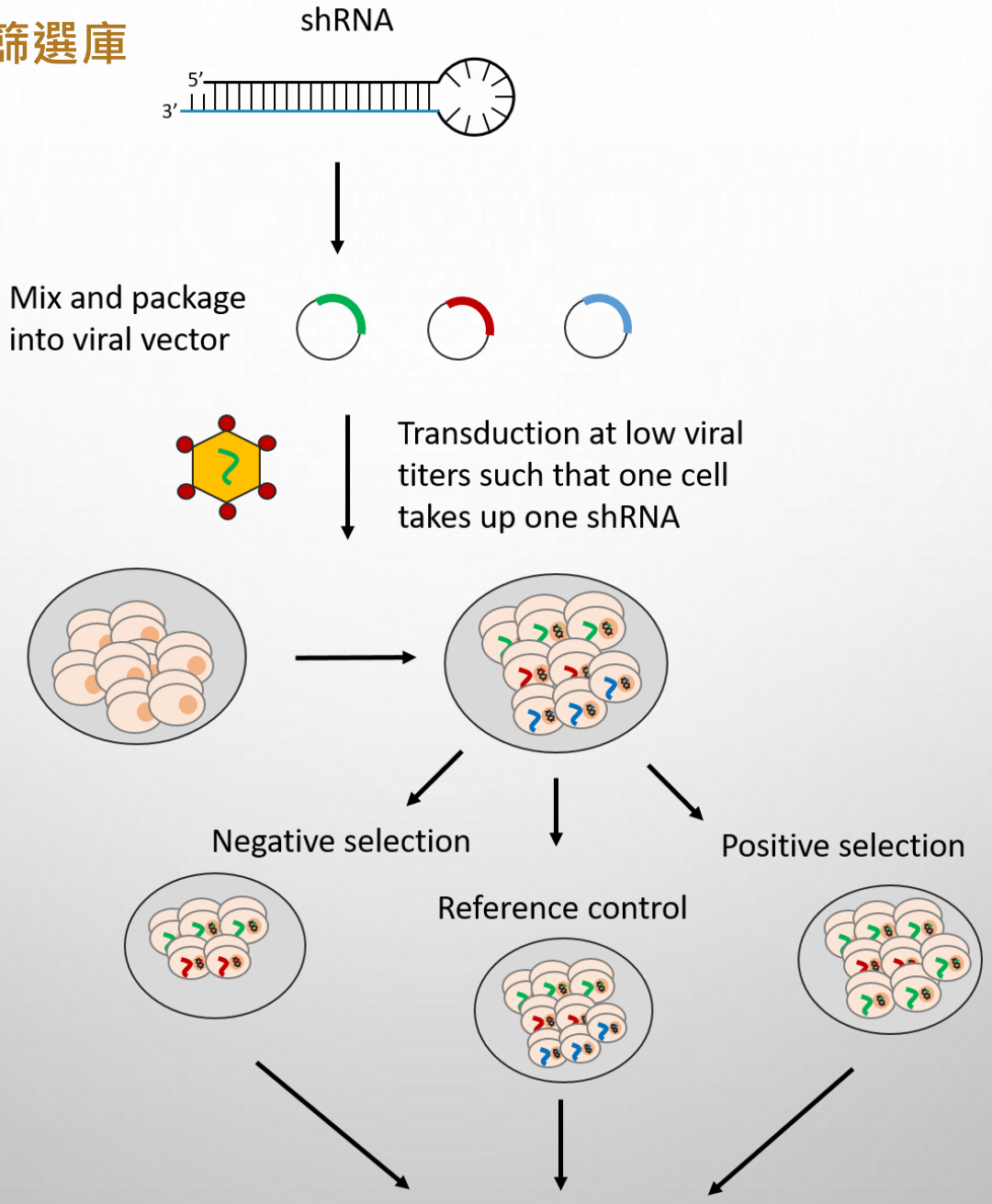
4. siRNA篩選

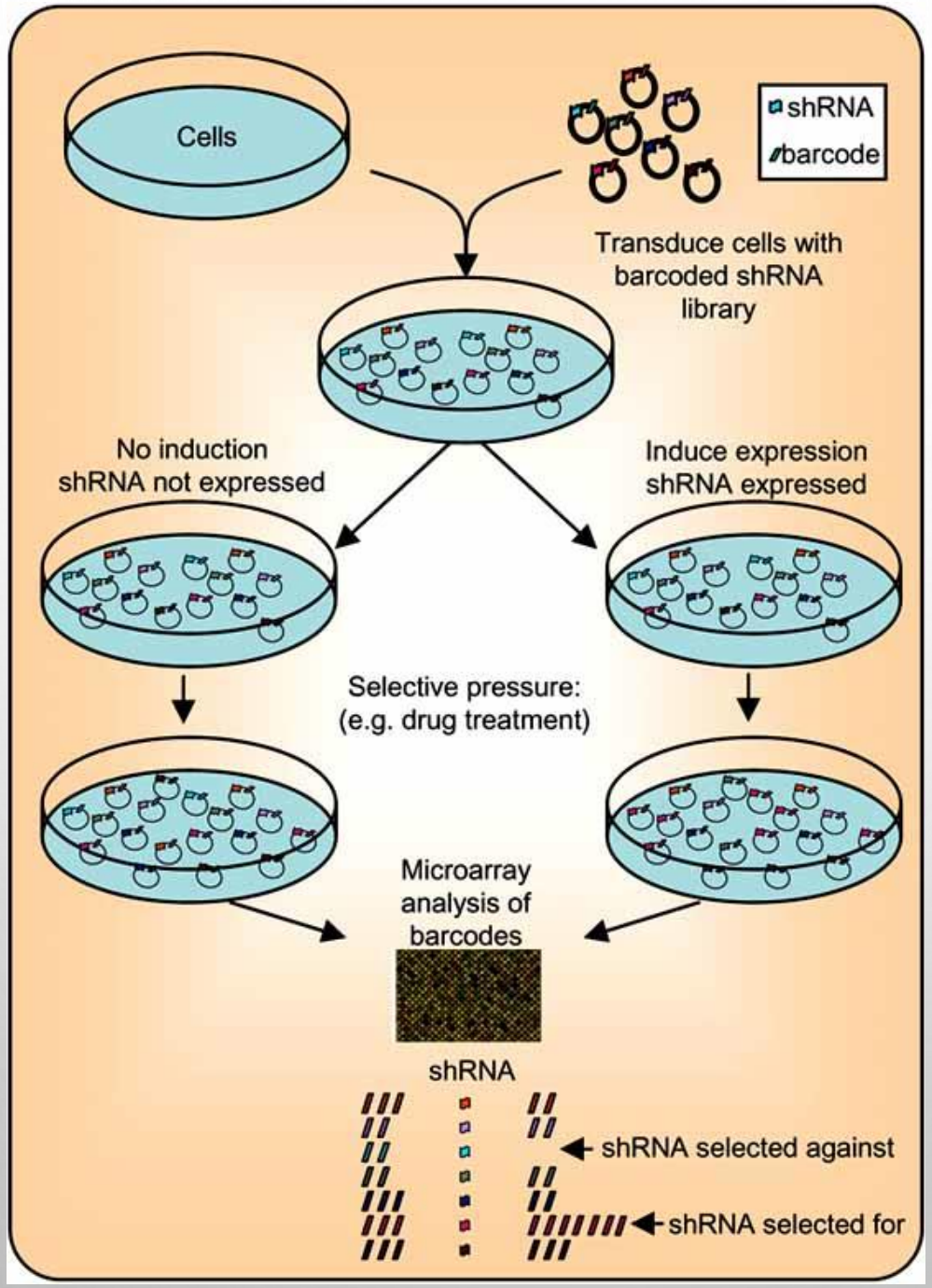
pooled RNAi screen

arrayed RNAi screen

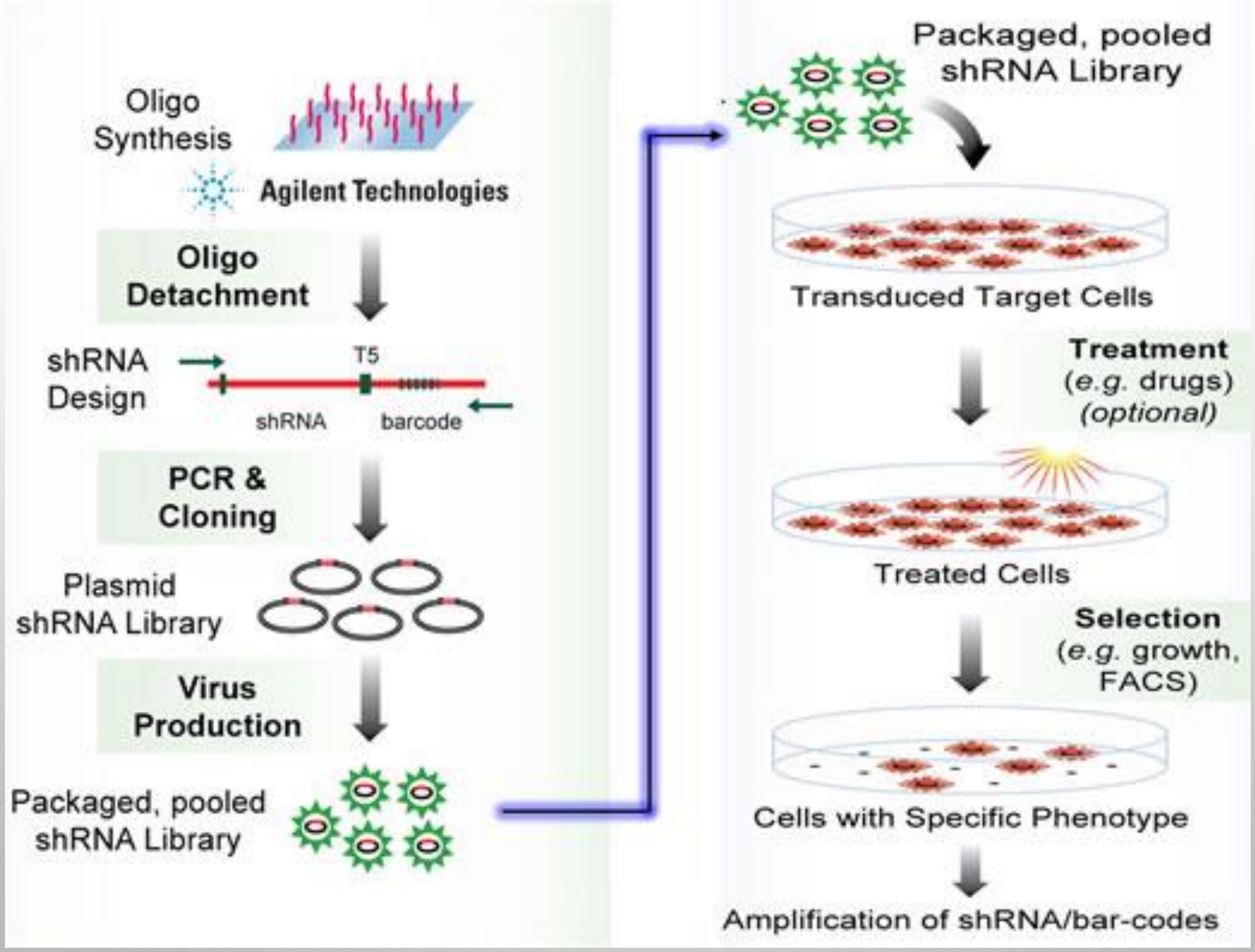


(a) Pooled RNAi 篩選庫

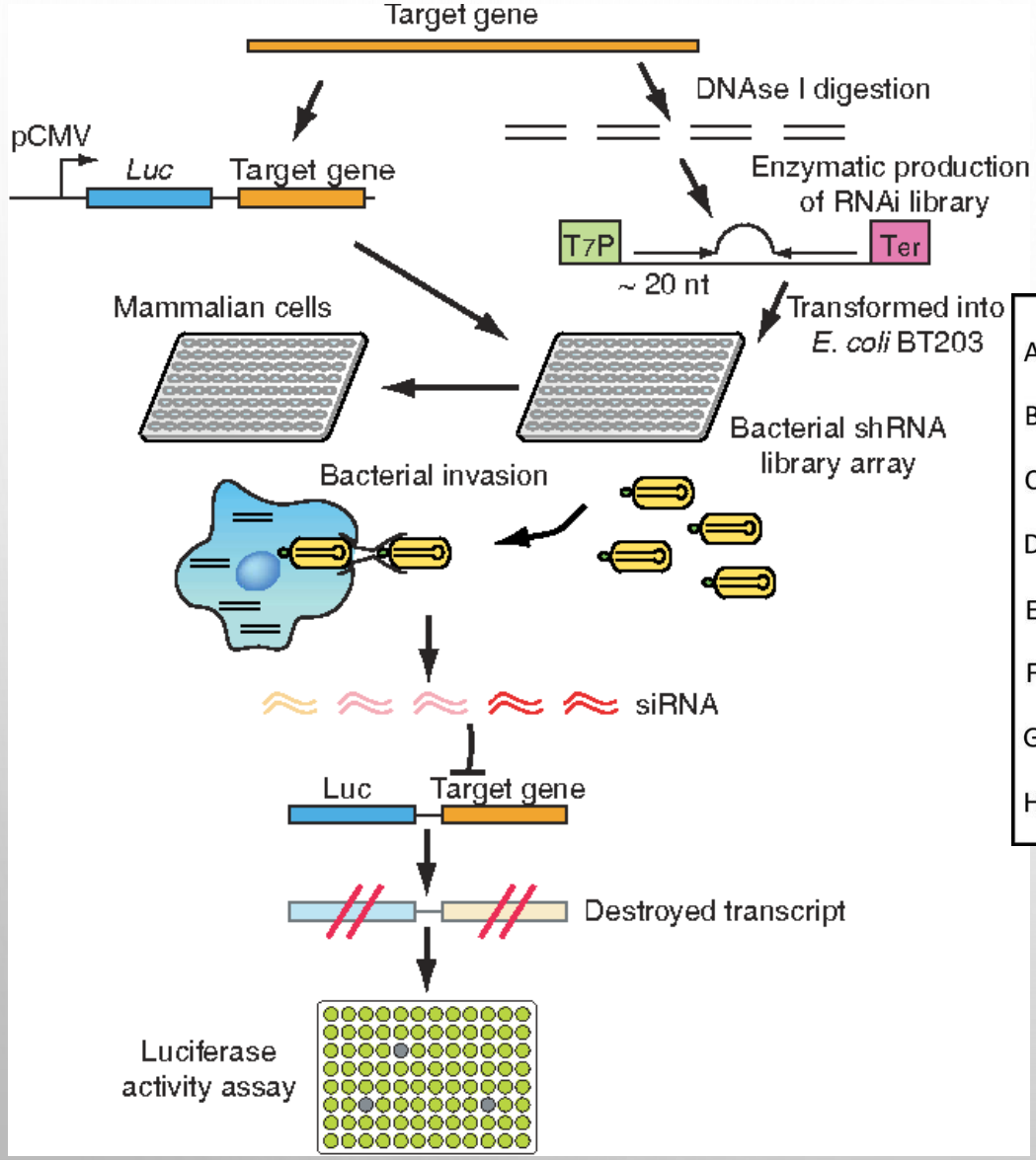




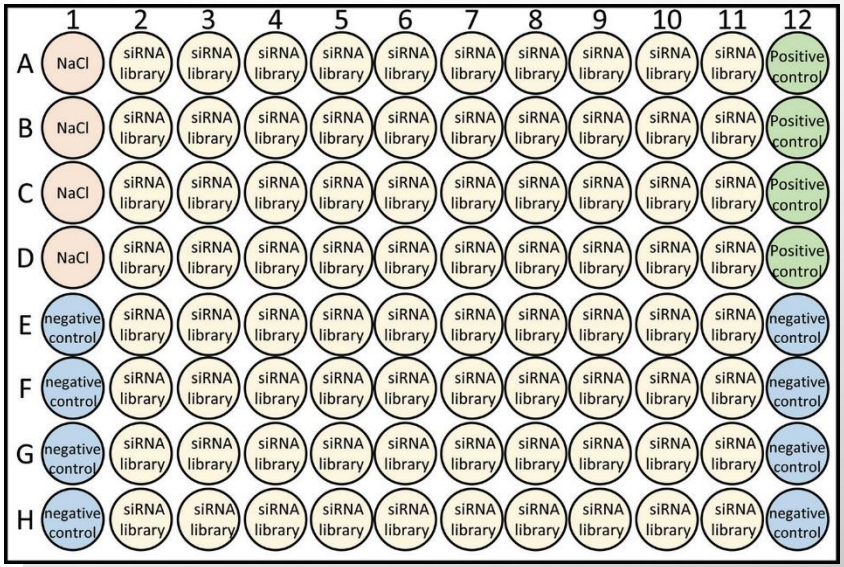
Comb Chem High Throughput Screen. 2008 Mar;11(3):175-84.


Oligo Synthesis
Agilent Technologies
Oligo Detachment
shRNA Design
T5
shRNA
barcode
PCR & Cloning
Plasmid shRNA Library
Virus Production
Packaged, pooled shRNA Library
Packaged, pooled shRNA Library
Transduced Target Cells
Treatment (e.g. drugs) (optional)
Treated Cells
Selection (e.g. growth, FACS)
Cells with Specific Phenotype
Amplification of shRNA/bar-codes

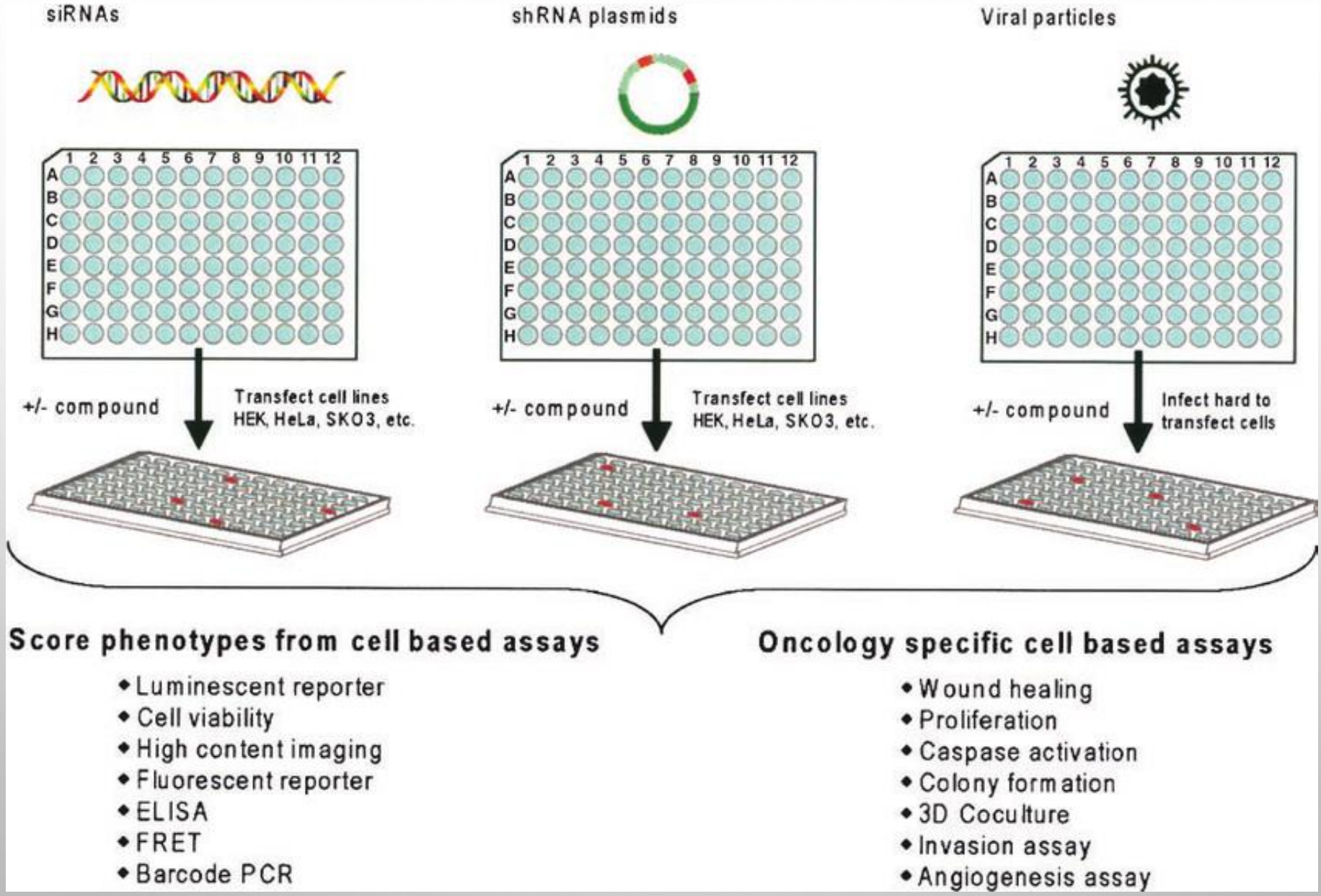
(b) arrayed RNAi 篩選庫



arrayed RNAi 篩選庫由單獨的 siRNA 或 shRNA 試劑組成，這些試劑針對不同的基因並放置在多孔板的每個孔中



Nat Methods. 2005 Dec;2(12):967-73.



Score phenotypes from cell based assays

- Luminescent reporter
- Cell viability
- High content imaging
- Fluorescent reporter
- ELISA
- FRET
- Barcode PCR

Oncology specific cell based assays

- Wound healing
- Proliferation
- Caspase activation
- Colony formation
- 3D Coculture
- Invasion assay
- Angiogenesis assay

RNAi的應用

Research Update TRENDS in Immunology Vol.23 No.12 December 2002

Research News

RNA interference of HIV replication

Miguel Angel Martínez, Bonaventura Clotet and José A. Esté

Double-stranded RNA-mediated interference (RNAi) induces sequence-specific post-transcriptional gene silencing and has emerged as a powerful tool to silence gene expression in multiple organisms. In mammalian cells, duplexes of 21 nucleotide RNAs, known as short-interfering RNAs (siRNAs), efficiently inhibit gene expression. Recent research demonstrates the general use of siRNAs to specifically inhibit HIV-1 replication by targeting viral or cellular genes. Importantly, RNAi opens a new avenue for gene-based therapeutics.

siRNA可以將HIV水準降低30-50倍!!!

Current Biology, Vol. 12, 1301–1311, August 6, 2002, ©2002 Elsevier Science Ltd. All rights reserved.

Inhibition of Retroviral Pathogenesis by RNA Interference

Short interfering RNA confers intracellular antiviral immunity in human cells

Leonid Gitlin*†, Sveta Karelsky* & Raul Andino*

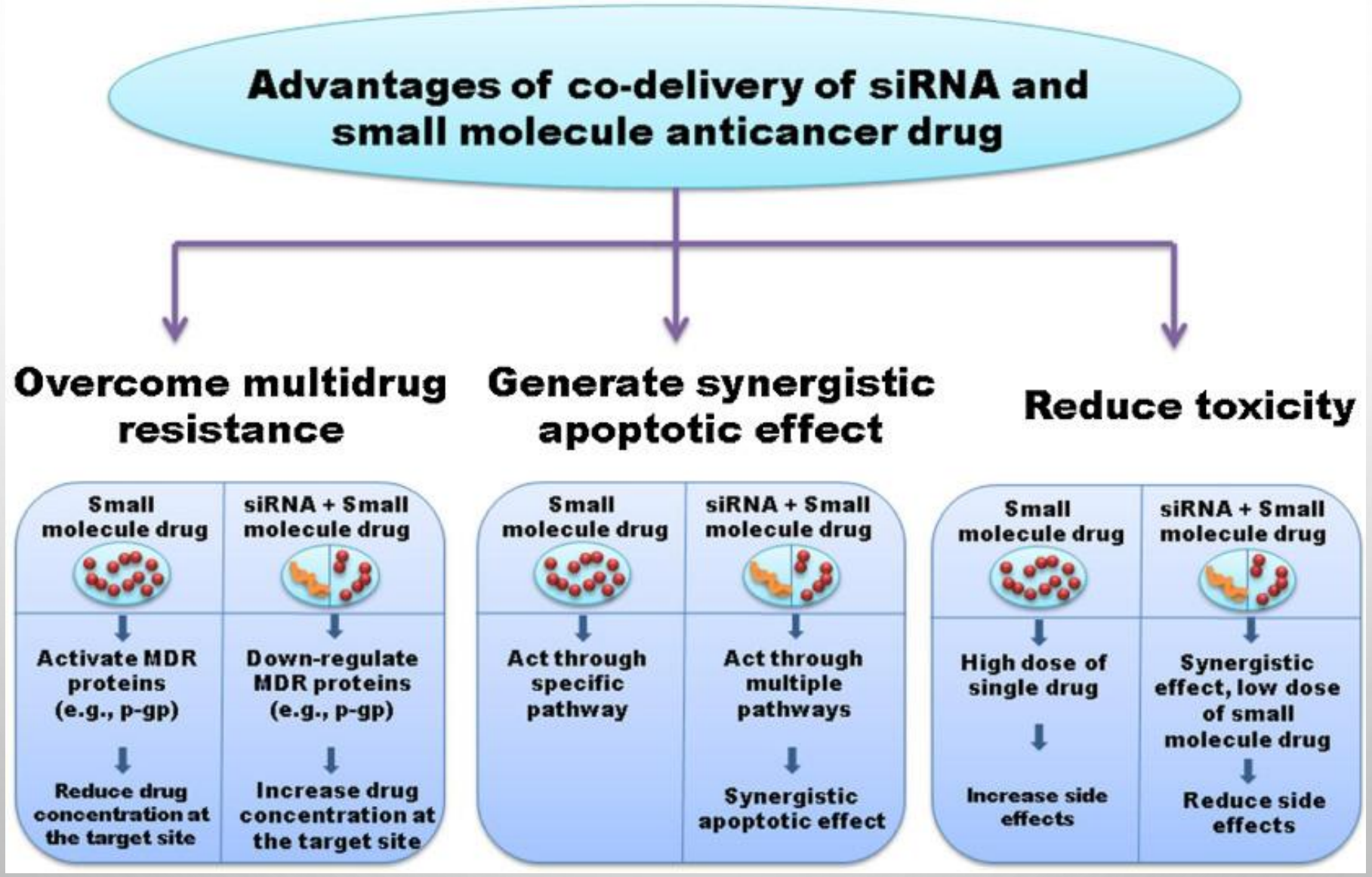
A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference

Douglas A. Rubinson[1]*, Christopher P. Dillon[1,2]*, Adam V. Kwiatkowski[1], Claudia Sievers[1,2,3], Lili Yang[4], Johnny Kopinja[5], Mingdi Zhang[5], Michael T. McManus[1,2], Frank B. Gertler[1], Martin L. Scott[5] & Luk Van Parijs[1,2]

*These authors contributed equally to this work.

Blocking oncogenes in malignant cells by RNA interference—New hope for a highly specific cancer treatment?

A little more than one year after the first demonstration that silencing of endogenous human genes is possible in cell culture, the new tool of RNA interference (RNAi) enters the field of tumor therapy.



Nanomedicine (Lond). 2008 Dec; 3(6): 761–776.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