一、設計 primer

1.進入 NCBI 網站,選擇 Gene,輸入想尋找的基因名稱

S NCBI Resources 🗹 How	To 🕑				Sign in to NCBI	
SNCBI National Center for Biotechnology Information	NDRG1			Search		
Ũ	Get the lates Get th Find NCBI SARS-CoV-2 lit	COVID-19 is an emerging, rapidly evolt t public health information from CDC: <u>htt</u> t latest research from NIH: <u>https://www</u> erature, sequence, and clinical content:	ring situation. ps://www.coronavirus.gov hity.gov/coronavirus. https://www.ncbi.nlm.nih.gov/sars-cov-2/.			
NCBI Home	Welcome to NCBI			Popular Resources		
Resource List (A-Z)	Resource List (A-Z) The National Center for Richerholdow Information advances science and health by providing access to PubMed					
All Resources	All Resources biomedical and genomic information. Bookshelf					
Chemicals & Bioassays	Chemicals & Bioassays About the NCBI   Mission   Organization   NCBI News & Blog PubMed Central					
Data & Software	Data & Software					
DNA & RNA	Submit	Nucleotide				
Domains & Structures	Deposit data or manuscripts	Deposit data or manuscripts Transfer NCBI data to your Find help documents, atten into NCBI databases computer class or watch a tutorial		Genome		
Genes & Expression	into NCBI databases			SNP		
				0		

2. 往下拉,選擇物種(實驗室用的 NDRG1 是來自人類的,所以選擇 human)

Search results						
Items: 1 to 20 o	f 738 Selected: 1	<< F	First < Prev Page 1 of 37 Next >	Last >>		
See also 9 dis	scontinued or replaced items.					
Name/Gene ID	Description	Location	Aliases	MIM		
✓ <u>NDRG1</u> ID: 10397	N-myc downstream regulated 1 [ <i>Homo</i> <i>sapiens</i> (human)]	Chromosome 8, NC_000008.11 (133237175133297252, complement)	CAP43, CMT4D, DRG-1, DRG1, GC4, HMSNL, NDR1, NMSL, PROXY1, RIT42, RTP, TARG1, TDD5	605262		
D: 17988	N-myc downstream regulated gene 1 [ <i>Mus</i> <i>musculus</i> (house mouse)]	Chromosome 15, NC_000081.6 (6692931866969641, complement)	CAP43, CMT4D, DRG1, HMSNL, NMSL, Ndr1, Ndr1, PROXY1, RTP, TDD5			
D: 299923	N-myc downstream regulated 1 [ <i>Rattus</i> <i>norvegicus</i> (Norway rat)]	Chromosome 7, NC_005106.4 (107734326107775701, complement)	Ndr1			

3. 點進去後會出現許多關於 NDRG1 的資訊,往下拉,找到"mRNA and protein"

## NDRG1 N-myc downstream regulated 1 [ Homo sapiens (human) ]

Gene ID: 10397,	updated on	1-Aug-2020	
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Summary		* ?
Official Symbol	NDRG1 provided by HGNC	
Official Full Name	N-myc downstream regulated 1 provided by HGNC	
Primary source	HGNC:HGNC:7679	
See related	Ensembl:ENSG0000104419 MIM:605262	
Gene type	protein coding	
RefSeq status	REVIEWED	
Organism	Homo sapiens	
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates;	
	Haplorrhini; Catarrhini; Hominidae; Homo	
·· ·	ANY DES REAL VERY TERE ALEVA ANTIE DEA ( UNANI DETA TREAL BRANK)	

Seqs maintained independen	tly of Annotated Genomes
nese reference sequences exist	independently of genome builds. <u>Explain</u>
Genomic	
1. NG_007943.1 RefSeqG	ene
Range	495265085
Download	GenBank, FASTA, Sequence Viewer (Graphics), LRG_258
nRNA and Protein(s)	
mRNA and Protein(s) 1. <u>NM_001135242.2</u> → NP <u>See identical proteins a</u> Status: REVIEWED	<u>_001128714.1</u> protein NDRG1 isoform 1 and their annotated locations for NP_001128714.1
mRNA and Protein(s) 1. NM_001135242.2 → NP See identical proteins a Status: REVIEWED Source sequence(s)	2_001128714.1 protein NDRG1 isoform 1 and their annotated locations for NP_001128714.1 AF004162, AF192304, AK126924, D87953
mRNA and Protein(s) 1. NM_001135242.2 → NP See identical proteins a Status: REVIEWED Source sequence(s) Consensus CDS	<u>001128714.1</u> protein NDRG1 isoform 1 and their annotated locations for NP_001128714.1 <u>AF004162, AF192304, AK126924, D87953</u> <u>CCDS34945.1</u>
mRNA and Protein(s) 1. <u>NM_001135242.2</u> → <u>NP</u> <u>See identical proteins a</u> Status: REVIEWED Source sequence(s) Consensus CDS UniProtKB/Swiss-Prot	<u>001128714.1</u> protein NDRG1 isoform 1 and their annotated locations for NP_001128714.1 AF004162, AF192304, AK126924, D87953 CCDS34945.1 Q92597
nRNA and Protein(s) 1. NM_001135242.2 → NP See identical proteins a Status: REVIEWED Source sequence(s) Consensus CDS UniProtKB/Swiss-Prot Related	<u>_001128714.1</u> protein NDRG1 isoform 1 and their annotated locations for NP_001128714.1 AF004162, AF192304, AK126924, D87953 CCDS34945.1 Q92597 ENSP00000404854.2, ENST00000414097.6
mRNA and Protein(s) 1. NM_001135242.2 → NP See identical proteins a Status: REVIEWED Source sequence(s) Consensus CDS UniProtKB/Swiss-Prot Related Conserved Domains (1) su	<u>AF004162, AF192304, AK126924, D87953</u> CCDS34945.1 Q92597 ENSP00000404854.2, ENST00000414097.6 mmary

底下可能會有許多該基因的 isoform(異構物),選擇他們的 FASTA 格式,再將這些異構物分別丟到 MultAlin 進行比對

4.進入 MultAlin, 貼上所有 isoform 的序列進行比對

Mult,	<del>I</del> lin
Multiple sequence alignment	by Florence Corpet
"Multiple sequence alignment with hierarchical clustering" F. CORPET, 1988, Nucl. Acids Res., 16 (22), 10881-10890	
Home page LG.CAuzeville	· Made Him
Sequence data	
Cut and paste your sequences here below. 🌮	



紅字代表四組 isoform 完全相同的區域,代表該區域是 NDRG1 所有 isoform 的 重疊區域。因由重疊區域的序列來設計 primer,確保不管是哪個 isoform 皆能 以此 primer 夾到。

5. 確定重疊的片段後,回到 NCBI,複製其中一個 isoform 的序列並貼到 word, 以 word 的尋找功能找到重疊片段,將該序列全部複製。



6. 進入 NCBI "primer design tool", 將序列貼上

NIH	U.S. National Library of Medicine	NCBI National Center for	Biotechnology Information	i -	
Pri	mer-BLAST		A tool for find	ling specific primers	
		Finding pr	imers specific to your Po	CR template (using Primer3 and	BLAST).
	PCR Template Rese	t page Save search parameters	Retrieve recent results	Publication Tips for finding s	pecific primers
	Enter accession, gi, or FASTA	sequence (A refseq record is preferre	d) 😟 <u>Clear</u>	Range	
	ATCTCAGGATGGACCCAAGCTCTGCCGGACAT AATGCAGAGTAACGTGGAAGTGGTCCACACCT CTGCACCTGTTCATCAATGCTTACAACAGCGG ACACAGTCACCCTGCAGTGCCCTGCTCTGTG GGAGTGCAACTCAAAATTGGACCCAACAAAGA	GGTGGTGTCCCACCTTTTTGGGAAGGAAGA ACCGCCAGCACATTGTGAATGACATGAACCCCGGC GCGCGACCAGGAGATTGAGCGACCAATGCCGGGAA GTGGTTGGGGACAGCTCGCCTGCAGTGGATGCCGT CCACTCTCCTCAAGATGGCGGACTGTGGCGGCCTC	AAC ACCC GGT CCCG	From To	Glear
	Or, upload FASTA file	選擇檔案 未選擇任何檔案			
	Primer Parameters				
	Use my own forward primer (5'->3' on plus strand) Use my own reverse primer			<u>Clear</u>	
	(5->3 on minus strand)	Min Max			
	PCR product size	70 1000			
	# of primers to return	10			
	Primer melting temperatures (Tm)	Min         Opt           57.0         60.0	Max         Max Tm           63.0         3	difference	

修改 primer 大小, 若為 qPCR primer 通常為 100~250bp

Primer Parameters					
Use my own forward primer (5'->3' on plus strand)				0	Clear
Use my own reverse primer (5'->3' on minus strand)				Θ	Clear
	Min	Max			
PCR product size	100	250	]		
# of primers to return	10				
	Min	Opt	Max	Мах	Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3	Θ

選擇 Database 種類。一般 RNA 選用"Refseq mRNA", 如果需要 non-coding RNA 則選用"Refseq RNA"。勾選下方兩格方框,用於避免搜尋到資料庫中未 被驗證的序列。完成後送出

	Note. I alameter values that unter nom the delaut are inglinghted in yenow
Primer Pair Specificity Ch	ecking Parameters
Specificity check	Enable search for primer pairs specific to the intended PCR template
Search mode	Automatic 🗸
Database	Refseq mRNA V
Exclusion	🗹 Exclude predicted Refseq transcripts (accession with XM, XR prefix) 🗹 Exclude uncultured/environmental sample sequences 😡
Organism	Homo sapiens Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.
Entrez query (optional)	

## 7. 圖為 primer 的位置

S Query_1 •   Find:	✓	O. 📲		🔀 Tools 🗸   🏶 Tracks 🔹 🥭 🤋 🗸
(U) Primer pairs for job 1N4LsMbVy33sQ8	5GwybgdLk9-0aULuBblo	1 K 1,100 1,200	1,388 1,488 1,588 1,688	1,708 1,808 1,908  2 K  2,108  2,208 2,351
Primer 1	Primer 5	Primer 6		Primer 2
Primer 9	Frimer o		LLTINGL TO B	LLTIMEL 2 Barriel 4 Barriel 4 Barriel 4
L 100 200 300 400 500	600 700 800 90	900  1 K  1,100  1,200	1,300 1,400 1,500 1,600	1,700 1,800 1,900 2 K 2,100 2,200 2,351
Query_1: 12.4K (2,351 nt)				📈 🏟 Tracks shown: 2/4

- 選擇 primer 的原则:
- (1) 兩兩 primer 間 Tm 值差 <1
- (2) Self complementarity <5
- (3) Self 3' complementarity <2

Primer p	bair 1									
Sequence (5'->3')		equence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward prin	mer G	GAAGTGGTCCACACCTACCG	Plus	20	79	98	60.04	60.00	7.00	2.00
Reverse prin	mer G	GTCCGCCATCTTGAGGAGAG	Minus	20	327	308	59.90	60.00	4.00	1.00
Product len	gth 2	49								
Primer pair	2									
	Sequ	ence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCT	TTGCTCTTGTCGGGGT	Plus	20	1807	1826	59.96	55.00	2.00	0.00
Reverse primer	TCCA	CGGTGAGCCAAAATGA	Minus	20	2042	2023	59.89	50.00	5.00	1.00
Product length	236									
	由結果	果挑出一個最好的	,完成設計 p	rimer						
	二、p	orimer 訂購								
-	進入	「基龍米克斯」, 選	<b>差擇線上訂購</b>							

基龍米克斯
Genomics

		<u>科</u>	支服務線上訂購	聯絡我們 🌐	LANGUAGE 繁體	.GE 繁體中文 / English	
首頁	關於我們	科研服務	次世代定序	生育健康	大眾健康	委託流程	

帳號: MNT330501 密碼:107A13 (實驗室網站有)

使用者登入	
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MNT330501	
₽登入密碼	
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夏基米商城	➡訂購服務

## 選擇"引子訂購"



聯絡電話:23938235 地址:台北市中正區仁愛路一段一號10樓1009室 備註:台大院內 信箱:填自己的

【訂購者】 國立臺	彎大學醫學院一生理學研究所【賴亮全】	▼【系所/部門】	生理學研究所	【實驗室	主持人】 賴亮子	È							
聯繫人: 【1】請下拉選擇, 或	直接輸入 🗸	聯絡電話:【2】講	下拉選擇,或直接輸入		~		<b>^</b>						
通知信箱:【3】請下拉選擇,	或直接輸入 🗸 🛨	增加通知透貨地址(樓、室	(4]請下拉選指	<b>『</b> ,或直接輸入詳盡地	址 🗸								
備註:													
□ 快速型 ■ 經濟型	□ 實惠型 □ EXTRE管式 □	EXTRE盤式 新增五	列 🗗 新增十列	📝 複製資料	上資料	訂單確認	🕗 取消訂單						
AOligo													
O.D V 🚱 Quaniity V 🚱													
刪除 Name <sup>★</sup>	Sequence(5' to 3	37)*	O.D. 🕇 Quanlity	MERS GC	5' Modification 3'	Modification Internal	留作定序註記						
1 💼													
2 🛍													
3 🛍													
4 🛍													

最後分別將 F、R 填入, OD:2, Quanlity 選 OPC 之後按訂單確認及完成訂購。