

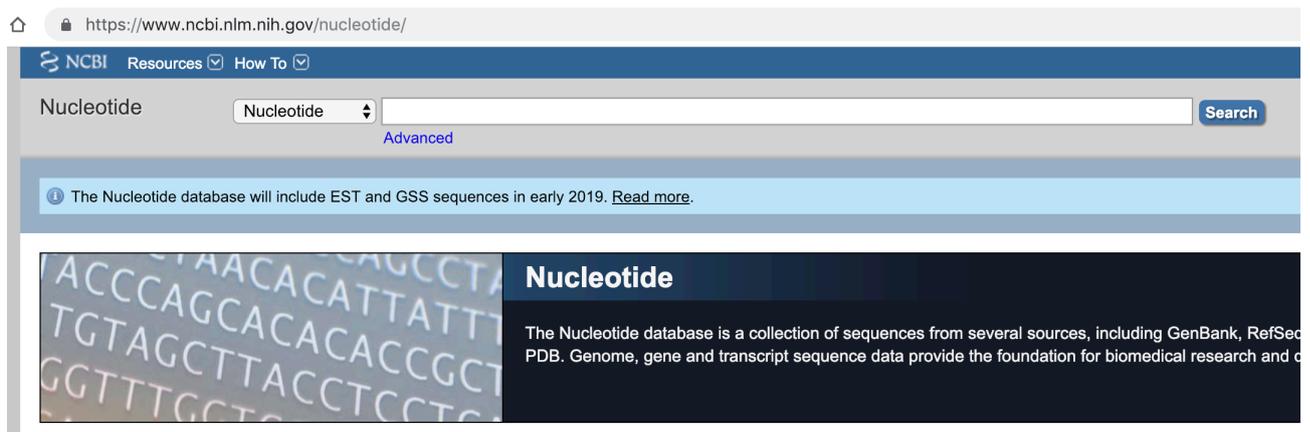
設計 primer 流程

使用時機：

1. qPCR 時使用 (片段較短, <250 bp)
2. 用於一般 PCR 驗證
3. 用於質體建構時的 target gene 製備 (需恰好使用整段完整的基因)

步驟：

1. (如果有確定好的片段則可跳過本步驟) 進入 **NCBI nucleotide** 網站頁面 <https://www.ncbi.nlm.nih.gov/nucleotide/>，並輸入感興趣的基因。



2. 選擇 “ mRNA ” 的數據，並點 “FASTA” 取得文字形式序列。

Items: 1 to 20 of 74

<< First < Prev Page 1 of 4 Next > Last >>

i Found 1737135 nucleotide sequences. Nucleotide (74) EST (4) GSS (1737057)

- [Homo sapiens full open reading frame cDNA clone RZPDo834C1011D for gene NDRG1, N-myc downstream regulated gene 1; complete cds, incl. stopcodon](#)
 - 1,185 bp linear mRNA
Accession: CR456842.1 GI: 48145800
[Protein](#) [Taxonomy](#)
[GenBank](#) [FASTA](#) [Graphics](#)
- [Cervus elaphus hippelaphus isolate Hungarian chromosome 21, whole genome shotgun sequence](#)
 2. 107,358,006 bp linear DNA
Accession: MKHE01000021.1 GI: 1207826205
[BioProject](#) [BioSample](#) [Protein](#) [Taxonomy](#)
[GenBank](#) [FASTA](#) [Graphics](#)

3. 複製序列部分，並於 **NCBI Primer-Blast** 網站頁面 <https://www.ncbi.nlm.nih.gov/tools/primer-blast/> 中貼上

Homo sapiens full open reading frame cDNA clone RZPDo834C1011D for gene NDRG1, N-myc downstream regulated gene 1; complete cds, incl. stopcodon

GenBank: CR456842.1

[GenBank](#) [Graphics](#)

```
>CR456842.1 Homo sapiens full open reading frame cDNA clone RZPDo834C1011D for gene
NDRG1, N-myc downstream regulated gene 1; complete cds, incl. stopcodon
ATGTCTCGGGAGATGTCAGGATGTAGACCTCGCTGAGGTGAAGCCTTTGGTGGAGAAAGGGGAGACCATCA
CCGGCCTCCTGCAAGAGTTTGATGTCCAGGAGCAGGACATCGAGACTTTACATGGCTCTGTTACAGTCAC
GCTGTGTGGGACTCCCAAGGAAACCGGCCTGTTCATCCTCACCCTACCATGACATCGGCATGAACCACAAA
ACCTGCTACAACCCCTCTTCAACTACGAGGACATGCAGGAGATCACCAGCAGCTTTGCCGCTTGCCACG
TGGACGCCCTGGCCAGCAGGACGGCGCAGCCTCCTTCCCGCAGGGTACATGTACCCCTCCATGGATCA
GCTGGCTGAAATGCTTCTCGGAGTCTTCAACAGTTTGGGCTGAAAAGCATTATTGGCATGGGAACAGGA
GCAGGCGCTACTTCCTAACTCGATTGCTCTAAACAACCCCTGAGATGGTGGAGGGCCTTGTCTTATCA
ACGTGAACCCCTTGTGCGGAAGGCTGGATGGACTGGGCCCGCTCCAAGATCTCAGGATGGACCAAGCTCT
GCCGACATGGTGGTTCACCTTTTGGGAAGGAAGAAATGCAGAGTAACGTGGAAGTGGTCCACACC
TACCCGCACACATTTGTGAATGACATGAACCCCGCAACCTGCACCTGTTTCATCAATGCCATACAACAGCC
GGCGCAGCTGGAGATTGAGCGACCAATGCCGGAACCCACACAGTCACCTGCAGTGCCTGCTCTGTT
GGTGTGGGGACAGCTCGCCTGCAGTGGATGCCGTGGTGGAGTGAACCTCAAATTTGGACCAACAAAG
ACCACTCTCTCAAGATGGCGGACTGTGGCGCCTCCCGCAGATCTCCAGCCGGCAAGCTCGCTGAGG
CCTTCAAGTACTTCGTGCAGGCATGGGATACATGCCCTCGGCTAGCATGACCCGCTGATGCGGTCCCG
CACAGCCTCTGGTTCAGCGTCACTTCTCGGATGGCACCCGAGCCGCTCCACACCAGCGAGGGCACC
CGAAGCCGCTCCACACCAGCGAGGGCACCCGAGCCGCTCGCACACCAGCGAGGGGGCCACCTGGACA
TCACCCCAACTCGGGTGTCTGGGAACAGCGCCGGGCCAAGTCCATGGAGGTCTCTGTAA
```

NIH U.S. National Library of Medicine NCBI National Center for Biotechnology Information

Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

PCR Template [Reset page](#) [Save search parameters](#) [Retrieve recent results](#) [Publication](#) [Tips for finding specific primers](#)

Enter accession, gi, or FASTA sequence (A refseq record is preferred)

ATGTCTCGGGAGATGTCAGGATGTAGACCTCGCTGAGGTGAAGCCTTTGGTGGAGAA
AGGGGAGACCATCA
CCGGCCTCCTGCAAGAGTTTGATGTCCAGGAGCAGGACATCGAGACTTTACATGGC
TCTGTTACAGTCAC
GCTGTGTGGGACTCCCAAGGAAACCGGCCTGTTCATCCTCACCCTACCATGACATCG

Or, upload FASTA file 未選擇任何檔案

Range

	From	To	
Forward primer	<input type="text"/>	<input type="text"/>	<input type="button" value="Clear"/>
Reverse primer	<input type="text"/>	<input type="text"/>	<input type="button" value="Clear"/>

Primer Parameters

Use my own forward primer (5'->3' on plus strand)

Use my own reverse primer (5'->3' on minus strand)

PCR product size
Min: 70 Max: 1000

of primers to return
10

Primer melting temperatures (Tm)
Min: 57.0 Opt: 60.0 Max: 63.0 Max Tm difference: 3

4. 修改 Primer Parameters 之 PCR product size (僅 qPCR 用途時需要)。設定值為 120 - 250。

Primer Parameters

Use my own forward primer (5'->3' on plus strand)

Use my own reverse primer (5'->3' on minus strand)

PCR product size
Min: 120 Max: 250

of primers to return
10

Primer melting temperatures (Tm)
Min: 57.0 Opt: 60.0 Max: 63.0 Max Tm difference: 3

- 選擇 Database 種類。一般 RNA 選用 “Refseq mRNA”，如果需要 non-coding RNA 則選用 “Refseq RNA”。
- 記得勾選下方兩格方框，主要用意在於避免搜尋到資料庫中未被驗證的序列。然後就可以送出了!

Primer Pair Specificity Checking Parameters

Specificity check Enable search for primer pairs specific to the intended PCR template

Search mode Automatic

Database Refseq mRNA

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.

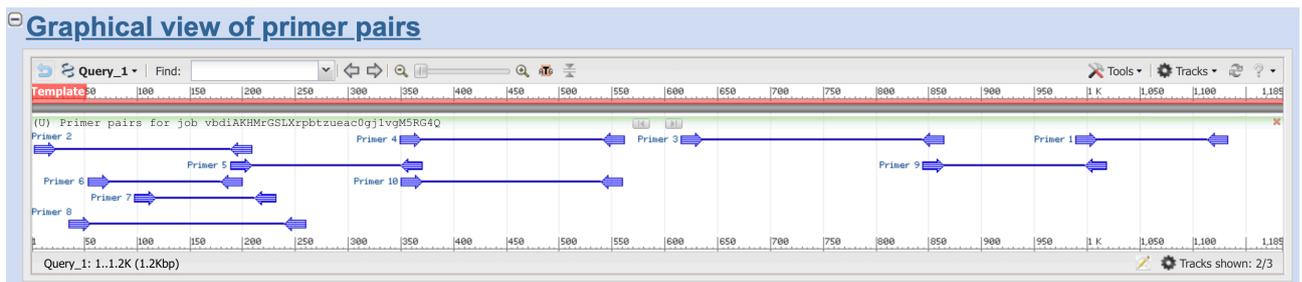
Entrez query (optional)

Primer specificity stringency Primer must have at least 2 total mismatches to unintended targets, including at least 2 mismatches within the last 5 bps at the 3' end. Ignore targets that have 6 or more mismatches to the primer.

Max target size 4000

Allow splice variants Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)

- 圖示的 primer 位置。



- 選擇 primer 的原則。
 - 兩 primer 間 Tm 值盡量 <1
 - Self complementarity <5
 - Self 3' complementarity <2

Primer pair 1

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TGGTTCCAGCGTCACTTCTC	Plus	20	990	1009	59.97	55.00	4.00	0.00
Reverse primer	GAGTTGGGGGTGATGCCAG	Minus	20	1133	1114	60.04	60.00	3.00	1.00
Product length	144								

Products on intended target

>[NM_001135242.1](#) Homo sapiens N-myc downstream regulated 1 (NDRG1), transcript variant 1, mRNA

product length = 144

```
Forward primer 1 TGGTTCCAGCGTCACTTCTC 20
Template 1576 ..... 1595
```

```
Reverse primer 1 GAGTTGGGGGTGATGCCAG 20
Template 1719 ..... 1700
```

Products on potentially unintended templates

>[NM_006096.3](#) Homo sapiens N-myc downstream regulated 1 (NDRG1), transcript variant 2, mRNA

product length = 144

```
Forward primer 1 TGGTTCCAGCGTCACTTCTC 20
Template 1179 ..... 1198
```

```
Reverse primer 1 GAGTTGGGGGTGATGCCAG 20
Template 1322 ..... 1303
```

>[NM_001258433.1](#) Homo sapiens N-myc downstream regulated 1 (NDRG1), transcript variant 4, mRNA

product length = 144

```
Forward primer 1 TGGTTCCAGCGTCACTTCTC 20
Template 1073 ..... 1092
```

```
Reverse primer 1 GAGTTGGGGGTGATGCCAG 20
Template 1216 ..... 1197
```