



PCR EN TIEMPO REAL: FUNDAMENTOS Y APLICACIONES EN INVESTIGACIÓN

DISEÑO DE PRIMERS

REACCIÓN EN CADENA DE LA POLIMERASA CUANTITATIVA (QPCR)

Método enzimático *in vitro* de amplificación exponencial de una región de ADN determinada que permite su cuantificación en tiempo real

Involucra ciclos de desnaturalización del molde, apareamiento de cebadores y extensión de estos iniciadores

Los iniciadores empleados (“primers”) hibridan con hebras opuestas y flanquean la región de interés



Iniciadores = primers = cebadores = oligonucleótidos



1. DISEÑO DE PRIMERS

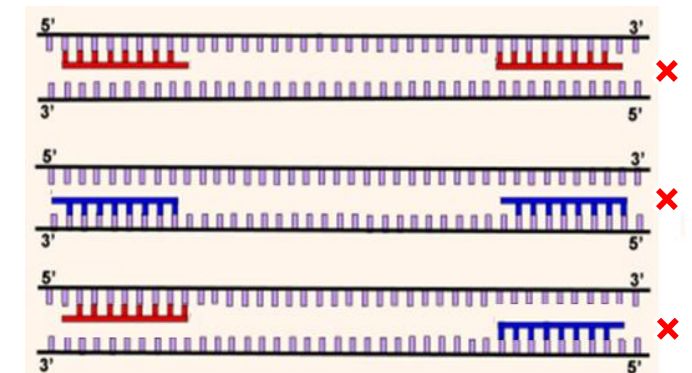
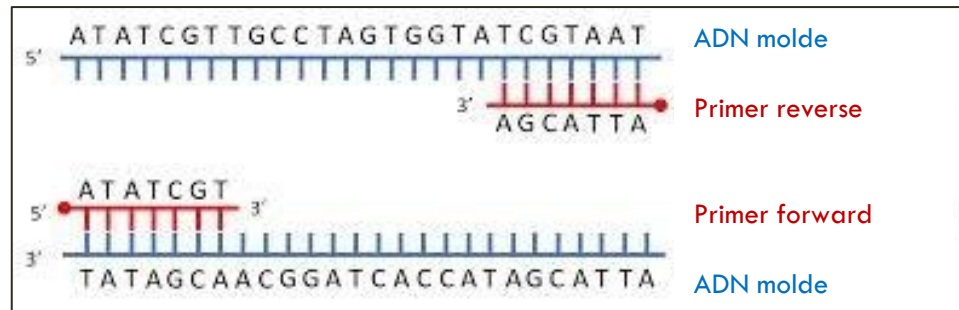
2. HERRAMIENTAS BIOINFORMÁTICAS
PARA EL DISEÑO DE PRIMERS

3. EFICIENCIA DE AMPLIFICACIÓN
DE PRIMERS



SELECCIÓN DE INICIADORES: ESPECIFICIDAD

Reconocer una SECUENCIA ÚNICA dentro del ADN molde
FLANQUEANDO la región de interés



SELECCIÓN DE PRIMERS

Forward

ATGGGAGAACTGGAGCCTTC 5'→3'

Reverse OJO!!!!

TTGGCTCCATATTCAATCGGT

AACCGAGGTATAAGTTAGCCA 3'→5' **complementaria**

ACCGATTGAATATGGAGCCAA 5'→3'

ORIGIN

```
901 gcagagtacc tgaaacagga agtatttttaa atattttgaa tcaaatgagt taatagaatc
961 tttacaaata agaataata cttctgctta ggatgataat tggaggcaag tgaatcctga
1021 gcgtgatttg ataatgacct aataatgatg ggttttattt ccagacttca cttctaataga
1081 tgattatggg agaactggag ccttcagagg gtaaaattaa gcacagtgga agaatttcat
1141 tctgttctca gttttcctgg attatgcctg gcaccattaa agaaaatatac atctttggtg
1201 tttcctatga tgaatataga tacagaagcg tcatcaaagc atgccaaacta gaagaggtaa
1261 gaaactatgt gaaaactttt tgattatgca tatgaaccct tcacactacc caaattatat
1321 atttggctcc atattcaatc ggttagtcta catatattta tgtttcctct atgggtaagc
1381 tactgtgaat ggatcaatta ataaaacaca tgacctatgc ttttaagaagc ttgcaaacac
1441 atgaaataaa tgcaatttat tttttaaata atgggttcat ttgatcacia taaatgcatt
1501 ttatgaaatg gtgagaattt tgttcactca ttagtgagac aaacgtcctc aatggttatt
1561 tatatggcat gcatataagt gatatgtggt atcttttttaa aagataccac aaaatatgca
```

EL DISEÑO DE PRIMERS PARA UNA PCR ES FUNDAMENTAL

Características a evaluar:

- Longitud
 - ✓ 18 a 25 nucleótidos (Evitar A o T en 3' si es posible)
- Contenido GC
 - ✓ Entre 40 - 60 % (Evitar repeticiones de más de 4 nt)
- Especificidad
 - ✓ Blast
- Temperatura de melting (T_m)
 - ✓ Entre 55 y 65 °C
- Complementariedad de secuencias
 - ✓ Evitar estructuras secundarias, auto-complementariedad, dímeros de primers

LONGITUD DEL PRIMER

Asegurar la unión estable entre el primer y el ADN molde

La longitud de la secuencia es fundamental para la asociación (Especificidad)

Muy cortos: falla especificidad

Muy largos: fallas en el annealing (bajo rendimiento)

TEMPERATURA DE MELTING (T_M)

Temperatura a la cual la mitad de las moléculas de primer están apareadas al ADN molde

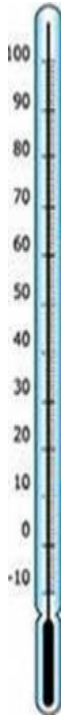
Depende de la longitud y la composición del primer. $T_m \text{ teórica} = 2(A+T) + 4(G+C)$

Los primers deben tener T_m similares entre sí (menos de 3°C de diferencia)

La temperatura de annealing depende de la temperatura de melting o fusión (T_m) de los primers:

- Temperatura de annealing por debajo de la T_m más baja del par de primers (hasta 5°C por debajo)

TEMPERATURA DE ANNEALING



Bajo rendimiento o
no amplificación

Óptima

Inespecificidad

SYBR Green

- **3-step cycling**

Cycles	Temp.	Time	Notes
1	*95 °C	*2 min	Polymerase activation
40	95 °C 60-65 °C 72 °C	5 s 10 s **5-20 s	Denaturation Annealing Extension (acquire at end of step)

- **2-step cycling**

Cycles	Temp.	Time	Notes
1	*95 °C	*2 min	Polymerase activation
40	95 °C 60-65 °C	5 s **15-30 s	Denaturation Annealing/extension (acquire at end of step)

COMPLEMENTARIEDAD DE SECUENCIAS

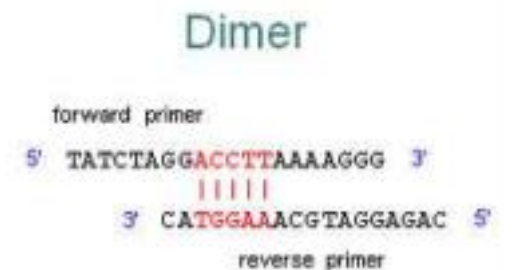
Intra-primer

El primer se pliega sobre sí mismo en una estructura doble cadena
(interfiere con el annealing al ADN molde)



Inter-primer

Formación de dímeros
Disminuye la formación de producto por competencia



1. DISEÑO DE PRIMERS



2. HERRAMIENTAS BIOINFORMÁTICAS
PARA EL DISEÑO DE PRIMERS

3. EFICIENCIA DE AMPLIFICACIÓN
DE PRIMERS



DISEÑO DE PRIMERS

Búsqueda de la secuencia de interés en bases de datos

The image shows a screenshot of the National Library of Medicine (NCBI) website. At the top, the NCBI logo and name are displayed, along with a 'Log in' button. Below the header is a search bar with the text 'mdr1 canis lupus' and a 'Search' button. A dropdown menu is open under the 'Gene' tab, listing various databases and resources. The main content area features a 'NCBI' header and a navigation menu with categories: 'Develop', 'Analyze', 'Research', 'Download', and 'Learn'. A 'Popular Resources' section lists links to PubMed, Bookshelf, PubMed Central, BLAST, Nucleotide, Genome, SNP, Gene, Protein, and PubChem. A 'NCBI News & Blog' section contains two news items: 'New ClinVar graphical display' (dated 30 Aug 2022) and 'Celebrating 1 Year of NCBI Virtual Outreach Events' (dated 26 Aug 2022).

NIH National Library of Medicine
National Center for Biotechnology Information

Log in

Gene Search

NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation

NCBI
er for Biotechnology Information advances science and health by providing access to
nomic information.
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Develop
Use NCBI APIs and code libraries to build applications

Analyze
Identify an NCBI tool for your data analysis task

Research
Explore NCBI research and collaborative projects

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Transfer NCBI data to your computer

Learn
Find help documents, attend a class or watch a tutorial

Popular Resources
PubMed
Bookshelf
PubMed Central
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem

NCBI News & Blog
New ClinVar graphical display 30 Aug 2022
Maps clinically significant variants by gene and position! ClinVar is a freely accessible, public archive of reports of
Celebrating 1 Year of NCBI Virtual Outreach Events 26 Aug 2022
We launched the NCBI Virtual Outreach Event series in the fall of 2021 to expand

DISEÑO DE PRIMERS

Búsqueda de la secuencia de interés en bases de datos



Gene

Gene

mdr1 canis lupus

Create RSS Save search Advanced

Gene sources

Genomic

Categories

Alternatively spliced
Annotated genes
Protein-coding

Sequence content

Ensembl
RefSeq

Status

✓ Current

[Clear all](#)

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Tabular 20 per page Sort by Relevance

Send to:

See [ABCB1 \(MDR1\) ATP binding cassette subfamily B member 1](#) in the Gene database

Search results

Items: 8

Showing Current items.

clear

Name/Gene ID	Description	Location	Aliases
<input type="checkbox"/> ABCB1 ID: 403879	ATP binding cassette subfamily B member 1 [<i>Canis lupus familiaris</i> (dog)]	Chromosome 14, NC_051818.1 (13410127..13507439, complement)	MDR1, p-gp
<input type="checkbox"/> ABCB4 ID: 482284	ATP binding cassette subfamily B member 4 [<i>Canis lupus familiaris</i> (dog)]	Chromosome 14, NC_051818.1 (13307604..13379530, complement)	
<input type="checkbox"/> ABCB5 ID: 482344	ATP binding cassette subfamily B member 5 [<i>Canis lupus familiaris</i> (dog)]	Chromosome 14, NC_051818.1 (34525456..34686254)	
<input type="checkbox"/> ABCB4 ID: 112674995	ATP binding cassette subfamily B member 4 [<i>Canis lupus dingo</i> (dingo)]	Chromosome 14, NC_064256.1 (13312458..13384458, complement)	
<input type="checkbox"/> ABCB11	ATP binding cassette subfamily B member 11	Chromosome 36, NC_051840.1	Abcb11e.

DISEÑO DE PRIMERS

Búsqueda de la secuencia de interés en bases de datos

NCBI Reference Sequences (RefSeq) ⌵ ?

NEW Try the new [Transcript table](#)

⊕ RefSeqs maintained independently of Annotated Genomes

⊖ RefSeqs of Annotated Genomes: *Canis lupus familiaris* Annotation Release 106 [details...](#) [↗](#)

The following sections contain reference sequences that belong to a specific genome build. [Explain](#)

Reference ROS_Cfam_1.0

Genomic

1. **NC_051818.1 Reference ROS_Cfam_1.0**

Range	13410127..13507439 complement
Download	GenBank , FASTA , Sequence Viewer (Graphics)

mRNA and Protein(s)

1. [XM_038685912.1](#) → [XP_038541840.1](#) **ATP-dependent translocase ABCB1 isoform X1**

UniProtKB/TrEMBL	A0A8C0L6Y9 , A0A8COMHY8 , F1PH01
Related	ENSCAFP00845011123.1 , ENSCAFT00845014360.1
Conserved Domains (1) summary	
	PTZ00265 PTZ00265; multidrug resistance protein (mdr1); Provisional Location:48 → 1269

2. [XM_038685913.1](#) → [XP_038541841.1](#) **ATP-dependent translocase ABCB1 isoform X2**

Conserved Domains (1) summary	
	PTZ00265 PTZ00265; multidrug resistance protein (mdr1); Provisional Location:10 → 1231

DISEÑO SOBRE ADN_c SIN INTRONES

For intron spanning primers the first half of the oligo must hybridize to the 3' end of one exon and to the 5' end of the other exon. In this way only cDNA will be amplified and not gDNA (fig. 11).

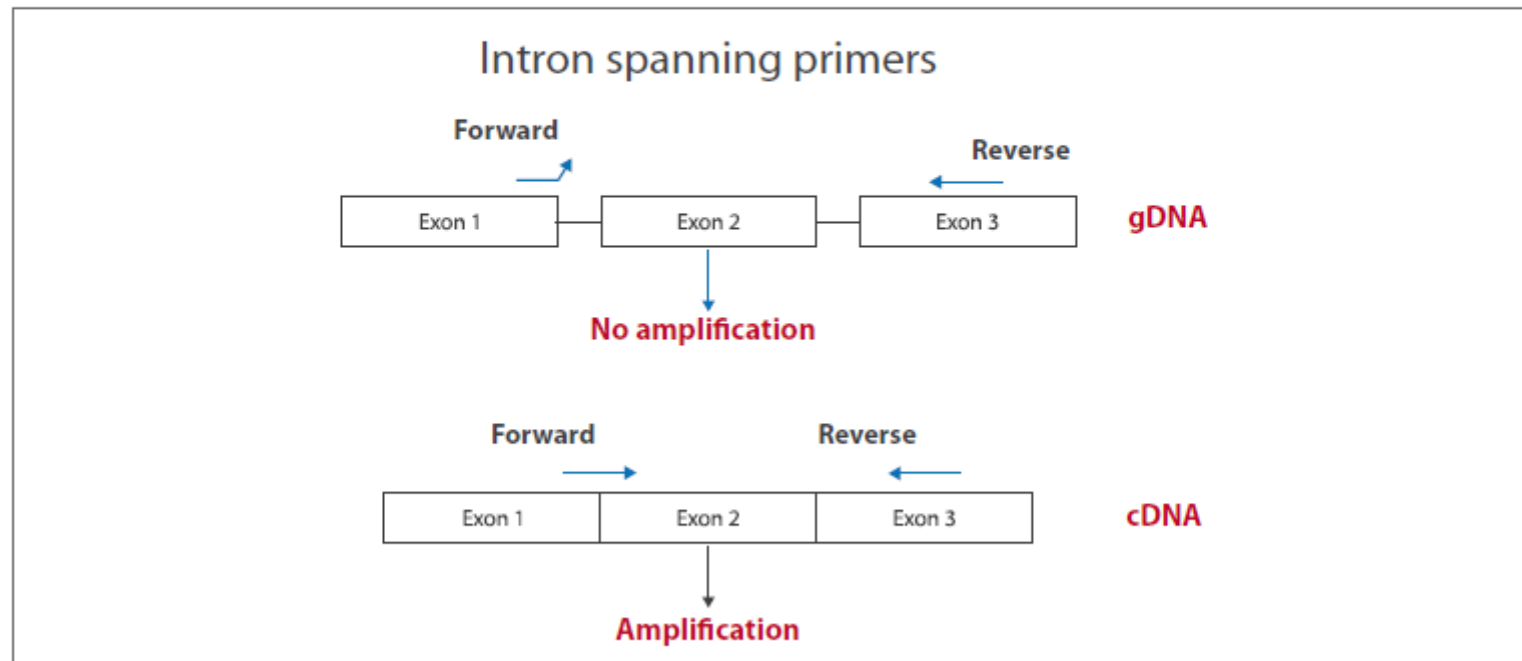



Figure 11. Intron spanning primers

DISEÑO DE PRIMERS

 **TriTrypDB** Release 59
30 Aug 2022
Kinetoplastid Informatics Resources

Site search, e.g. *Tb927.11.3120* or **reductase* or *"binding protein"*

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▶ Organisms

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▶ Genomic Sequences

▶ Genomic Segments

Overview of Resources and Tools



Take a Tour



Getting Started



Search Strategies



Genome Browser



Transcriptomic Resources



Phenotypic Data



Analyze My Data



Dov

Getting Started

VEuPathDB is packed with data, tools and visualizations that can help answer your research questions. We gather data from many sources, analyze according to standard workflows, and present the results for you to mine in a point and click interface. Here's how to get started:

News and Tweets



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 COMMUNITY CHAT

TcCLB.509153.90

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Search section names...

- ▶ 1 Gene models
- ▶ 2 Annotation, curation and identifiers
- ▶ 3 Link outs
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- ▶ 5 Literature
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- ▶ 8 Phenotype
- ▶ 9 Genetic variation
- ▶ 10 Transcriptomics
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Predicted RNA/mRNA Sequence (Introns spliced out)

1566 bp

Copy to clipboard

```
ATGTCGCTGTTTAAAGATCCGCATGCCGGAGACGGTGGCGGAGGGGACACGTCTCGACTGCGTGCCTTTCCCTCGTTGT
AGCGGTTCGACGAGCACGGCGGCATTGGGGACGGGCGGTCGATCCCATGGAATGTGCCCGAGGACATGAAGTTTTCCGGG
ATTTGACAACAAAACAGCGGGGAAGAAGCTCAAACCATCGCCTGCGAAGCGCAACGCCGTTGTGATGGGCCGCAAGACG
TGGGACAGCATTCCGCCCAAATTCGGCCCGCTCCCGGTGCGCTGAACGTGTTTTGTCGTCCACCCTCACGACGCAACA
CCTCTTGGACGGCCTGCCAGACGAAGAAAAACGGAATTTGCATGCCGACAGCATTGTGGCGGTAATGGTGGACTCGAGC
AGGCATTGCGGTTGCTCGCGTCCCCAATTACACGCCGAGCATTGAGACGGTCTACTGCATTGGTGGCGGCTCTGTGTAT
GCGGAGGCCCTTCGCCCGCCGTGCGTTCATTTGCTGCAAGCATTACCACACCACATCCGCGCCAGCGAGTCTTCTTG
CAGCGTGTTTTTTCGGTTCGCCGAGTCGGGTACGGAGGCGGCGGGGATTGAGTGGCAGCGGGAGACAATCTCCGAGG
AGCTCACGTCCGCAACGGCAACGAGACAAAGTACTACTTTGAGAAGCTCATCCCACGCAACAGAGAGGAGGAGCAGTAT
CTAAGCCTTGTGGATCGATCATACGCGAGGGGAATGTGAAGCATGACCGCACTGGGGTGGGCACGCTCTCCATCTTTGG
CGCACAGATGCGTTTTCTCGCTCCGCAACAATCGCCTACCACCTTTTGACAACGAAGCGGGTTTTTTGGCGCGGTGTGTGCG
AGCAACTGCTGCTGTTTCTACGGGGCGCAACATATGCCAAAAGTTAAGTGACAAGGGAGTCCACATATGGGACGCAAC
```

▼ Show more

Genomic Sequence

1566 bp

Copy to clipboard

```
ATGTCGCTGTTTAAAGATCCGCATGCCGGAGACGGTGGCGGAGGGGACACGTCTCGACTGCGTGCCTTTCCCTCGTTGT
AGCGGTTCGACGAGCACGGCGGCATTGGGGACGGGCGGTCGATCCCATGGAATGTGCCCGAGGACATGAAGTTTTCCGGG
ATTTGACAACAAAACAGCGGGGAAGAAGCTCAAACCATCGCCTGCGAAGCGCAACGCCGTTGTGATGGGCCGCAAGACG
TGGGACAGCATTCCGCCCAAATTCGGCCCGCTCCCGGTGCGCTGAACGTGTTTTGTCGTCCACCCTCACGACGCAACA
CCTCTTGGACGGCCTGCCAGACGAAGAAAAACGGAATTTGCATGCCGACAGCATTGTGGCGGTAATGGTGGACTCGAGC
AGGCATTGCGGTTGCTCGCGTCCCCAATTACACGCCGAGCATTGAGACGGTCTACTGCATTGGTGGCGGCTCTGTGTAT
GCGGAGGCCCTTCGCCCGCCGTGCGTTCATTTGCTGCAAGCATTACCACACCACATCCGCGCCAGCGAGTCTTCTTG
CAGCGTGTTTTTTCGGTTCGCCGAGTCGGGTACGGAGGCGGCGGGGATTGAGTGGCAGCGGGAGACAATCTCCGAGG
AGCTCACGTCCGCAACGGCAACGAGACAAAGTACTACTTTGAGAAGCTCATCCCACGCAACAGAGAGGAGGAGCAGTAT
CTAAGCCTTGTGGATCGATCATACGCGAGGGGAATGTGAAGCATGACCGCACTGGGGTGGGCACGCTCTCCATCTTTGG
CGCACAGATGCGTTTTCTCGCTCCGCAACAATCGCCTACCACCTTTTGACAACGAAGCGGGTTTTTTGGCGCGGTGTGTGCG
```

HERRAMIENTAS DE DISEÑO DE PRIMERS

bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi <https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>

Primer3Plus

pick primers from a DNA sequence

[Primer3Manager](#)

[Help](#)

[About](#)

[Source Code](#)

Task:

- Detection
- Cloning
- Sequencing
- Primer_List
- Primer_Check

Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.

Main

Settings

Advanced Settings

Internal Oligo

Penalty Weights

Sequence Quality

Sequen

[Paste source sequence below](#)

Or upload sequence file:

Ningún archi...seleccionado

Mark selected region:

[Excluded Regions:](#)

< >

[Targets:](#)

[]

[Included Region:](#)

{ }



HERRAMIENTAS DE DISEÑO DE PRIMERS

Primer3Plus pick primers from a DNA sequence	Primer3Manager	Help
	About	Source Code

Task: *Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.*

Main	General Settings	Advanced Settings	Internal Oligo	Penalty Weights	Sequence Quality
-------------	-------------------------	--------------------------	-----------------------	------------------------	-------------------------

Sequence Id:

Paste source sequence below Or upload sequence file: Ninguno ...hivo selec.

```
AGCTCACGTCGGCAAACGGCAACGAGACAAAGTACTACTTTGAGAAGCTCATCCCACGCAACAGAGAGGAGGAGCAGTAT
CTAAGCCTTGTGGATCGCATCATAACGCGAGGGGAATGTGAAGCATGACCGCACTGGGGTGGGCACGCTCTCCATCTTTGG
CGCACAGATGCGTTTTCTCGCTCCGCAACAATCGCTACCACTTTTGACAACGAAGCGGGTTTTTTGGCGCGGTGTGTGCG
AGGAACTGCTGTGGTTTTCTACGGGGCGAAACATATGCCAAAAAGTTAAGTGACAAGGGAGTCCACATATGGGACGACAAC
GGCTCGCGCGCGTTTTCTTGACAGTCGTGGCCTCACAGAGTACGAGGAGATGGACCTCGGTCTGTCTACGGCTTTGAGTG
GCGTCACTTTGGTGCCGCCTACACACACCATGATGCCAACTATGACGGACAGGGTGTGGATCAAATCAAGGCAATCGTTG
AAACGCTAAAGACAAATCCTGACGATCGCCGGATGCTATTACGGCATGGAATCCGAGCGCTTTGCCAGGATGGCCCTA
CCACCGTGCCACCTGCTCGCACAGTTTTACGTGAGCAATGGGGAATGTCTTGATGCTCTACCAGCGCTCATGTGACAT
GGGCCTTGGCGTCCCGTTCAACATTGCCTCCTACGCCCTCTTGACCATCCTCATCGCAAAGGCGACGGGGCTGCGGCCGG
GAGAGTTGGTGCACACCCTTGGTACGCTCACGTGTACAGCAATCACGTGAGCCGTGCAATGAGCAACTGAAACGTGTG
CCACGTGCCTTTCCGTATCTTGTGTTTCGCCGCGAGCGGAGTTTCTGGAGGACTACGAGGAGGGTGACATGGAGGTCAT
TGACTATGCGCCATACCCTCCCATCTCCATGAAGATGGCGGTTTAG
```

Mark selected region:

Excluded Regions: < >

Targets: []

HERRAMIENTAS DE DISEÑO DE PRIMERS

Primer3Plus pick primers from a DNA sequence	Primer3Manager	Help
	About	Source Code

Task: *Select primer pairs to detect the given template sequence. Optionally targets and included/excluded regions can be specified.*

Main	General Settings	Advanced Settings	Internal Oligo	Penalty Weights	Sequence Quality
-------------	-------------------------	--------------------------	-----------------------	------------------------	-------------------------

[Product Size Ranges](#)

Primer Size	Min: <input type="text" value="18"/>	Opt: <input type="text" value="20"/>	Max: <input type="text" value="25"/>	
Primer Tm	Min: <input type="text" value="57.0"/>	Opt: <input type="text" value="60.0"/>	Max: <input type="text" value="63.0"/>	Max Tm Difference: <input type="text" value="10.0"/>
Primer GC%	Min: <input type="text" value="40.0"/>	Opt: <input type="text"/>	Max: <input type="text" value="60.0"/>	Fix the <input type="text" value="5"/> prime end of the primer
Concentration of monovalent cations:	<input type="text" value="50.0"/>	Annealing Oligo Concentration:	<input type="text" value="50.0"/>	
Concentration of divalent cations:	<input type="text" value="0.0"/>	Concentration of dNTPs:	<input type="text" value="0.0"/>	

[Mispriming/Repeat Library:](#)

Load and Save
[Please select special settings here:](#) (use Activate Settings button to load the selected settings)
To upload or save a settings file from your local computer, choose here:
 Ningún archi...seleccionado

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pick primers from a DNA sequence

< Back

Pair 1:

Left Primer 1:

Sequence:

Start: 1010 Length: 20 bp Tm: 60.1 °C GC: 60.0 % ANY: 8.0 SELF: 2.0

Right Primer 1:

Sequence:

Start: 1114 Length: 20 bp Tm: 60.0 °C GC: 40.0 % ANY: 4.0 SELF: 2.0

Product Size: 105 bp Pair Any: 4.0 Pair End: 1.0

1	ATGTCGCTGT	TTAAGATCCG	CATGCCGGAG	ACGGTGGCGG	AGGGGACACG
51	TCTCGCACTG	CGTGCCTTTT	CCCTCGTTGT	AGCGGTCGAC	GAGCACGGCG
101	GCATTGGGGA	CGGGCGGTCC	ATCCCATGGA	ATGTGCCCGA	GGACATGAGG
151	TTTTTCCGGG	ATTTGACAAC	AAAACCTGGG	GGGAAGAACG	TCAAACCATC
201	GCCTGCGAAG	CGCAACGCCG	TTGTGATGGG	CGCAGAGACG	TGGGACAGCA
251	TTCCGCCCAA	ATTCCGCCCG	CTTCCCGGTC	GCCTGAACGT	CGTTTTGTCC
301	TCCACCCTCA	CGACGCAACA	CCTCTTGAC	GGCCTGCCAG	ACGAAGAAAA
351	ACGGAATTTG	CATGCCGACA	GCATTTGGCC	GGTAAATGGT	GGACTCGAGC
401	AGGCAITGGG	GTTGCTCGCG	TCCCCCAATT	ACACGCCGAG	CATGAGAGCG
451	GTCTACTGCA	TTGGTGGCGG	CTCTGTGTAT	GCGGAGGCC	TTGCCCCGCC
501	GTGCGTTCAT	TTGCTGCAAG	CCATTTACCG	CACCACCATC	CGGCCAGCG
551	AGTCTTCTTG	CAGCGTGTTC	TTTTGCGTTC	CCGAGTCGGG	TACGGAGGCG
601	GCGGCGGGGA	TTGAGTGGCA	GCGGGAGACA	ATCTCCGAGG	AGTCCACGTC
651	GGCAACGGC	AACGAGACAA	AGTACTACTT	TGAGAAGCTC	ATCCCACGCA
701	ACAGAGAGGA	GGAGCAGTAT	CTAAGCCTTG	TGGATCGCAT	CATACGCGAG
751	GGGAATGTGA	AGCATGACCG	CCTGGGGTGG	GGCACGCTCT	CCATCTTTGG
801	CGCACAGATG	CGTTTCTCGC	TCCGCAACAA	TCCGCTACCA	CTTTTGACAA
851	CGAAGCGGGT	TTTTTGGCGC	GGTGTGTCCG	AGGAAGTCT	GTGGTTTCTA
901	CGGGGCGAAA	CATATGCCAA	AAAGTTAAGT	GACAAAGGAG	TCCACATATG
951	GGACGACAAC	GGCTCGCGCG	CGTTTCTTGA	CAGTCGTGGC	CTCACAGAGT
1001	ACGAGGAGAT	GGACCTCGGT	CCTGTCTACG	GCTTTCAGTG	GCGTCACTTT
1051	GGTGCCGCCT	ACACACACCA	TGATGCCAAC	TATGACGGAC	AGGGTGTGGG
1101	TCAAATCAAG	GCAATCGTTG	AAACGCTAAA	GACAAATCCT	GACGATCGCC
1151	GGATGCTATT	CACGGCATGG	AATCCGAGCG	CTTTGCCGAG	GATGGCCCTA
1201	CCACCCTGCC	ACCTGCTCGC	ACAGTTTTAC	GTGAGCAATG	GGGAACTGTC
1251	TTGCATGCTC	TACCAGCGCT	CATGTGACAT	GGGCCCTGGC	GTCCCGTTCA
1301	ACATTGCCTC	CTACGCCCTC	TTGACCATCC	TCATCGCAAA	GGCGACGGGG
1351	CTGCGGCCGG	GAGAGTTGGT	GCACACCCTT	GGTGACGCTC	ACGTGTACAG
1401	CAATCACGTC	GAGCCGTGCA	ATGAGCAACT	GAAAACGTGT	CCACGTGCCT
1451	TTCCGTATCT	TGTGTTTCGC	CGCGAGCGCG	AGTTCCCTGGA	GGACTACGAG
1501	GAGGGTGACA	TGGAGGTCAT	TGACTATGCG	CCATACCCTC	CCATCTCCAT
1551	GAAGATGGCG	GTTTAG			

Select all Primers

Herramientas de análisis de primers Oligo Analyzer (IDT)

OligoAnalyzer 3.1 <https://www.idtdna.com/pages/tools/oligoanalyzer>

[Instructions](#) | [Definitions](#) | [Feedback](#)

Sequence 0 Bases

5'- -3'

Parameter sets
SpecSheet (Default) ▼

Target type ▼

Oligo Conc μM

Na⁺ Conc mM

Mg⁺⁺ Conc mM

dNTPs Conc mM

Sequence 5' MOD ▾ INTERNAL ▾ 3' MOD ▾ MIXED BASES ▾

TGG ACC TCG GTC CTG TCT AC

Bases 20 CLEAR SEQUENCE

[Try the new batch mode here](#)

Parameter sets

SpecSheet (Default) ▾

Target type DNA ▾

Oligo Conc μM

Na⁺ Conc mM

Mg⁺⁺ Conc mM

dNTPs Conc mM

- [ANALYZE](#)
- [HAIRPIN](#)
- [SELF-DIMER](#)
- [HETERO-DIMER](#)
- [NCBI BLAST](#)
- [TM MISMATCH](#)
- [ADD TO ORDER](#)

Results






[RESUSPENSION](#)

[DILUTION](#)

SEQUENCE	5'- TGG ACC TCG GTC CTG TCT AC -3'
COMPLEMENT	5'- GTA GAC AGG ACC GAG GTC CA -3'
LENGTH	20
GC CONTENT	60 %
MELT TEMP	58 °C
MOLECULAR WEIGHT	6060 g/mole
EXTINCTION COEFFICIENT	177400 L/(mole·cm)
nmole/OD ₂₆₀ :	5.64
$\mu\text{g}/\text{OD}_{260}$:	34.16

HERRAMIENTAS DE ANÁLISIS DE PRIMERS HAIRPIN

Structures

Structure Name	Image	$\Delta G(\text{kcal.mole}^{-1})$	T_m (°C)	$\Delta H(\text{kcal.mole}^{-1})$	$\Delta S(\text{cal.K}^{-1}\text{mole}^{-1})$	Output	
1		0.16	22.9	-23.4	-79.04	Ct	Det.
2		0.42	19	-20.5	-70.17	Ct	Det.
3		0.99	4	-13.1	-47.27	Ct	Det.
4		1.01	6.2	-15	-53.69	Ct	Det.
5		1.11	4.6	-15.1	-54.37	Ct	Det.

La T_m debe ser menor que la temperatura de annealing propuesta para la reacción.

HERRAMIENTAS DE ANÁLISIS DE PRIMERS HETERO-DIMER

Hetero-Dimer Analysis

Primary Sequence:

5'- ATG GGA GAA CTG GAG CCT TC -3'

Secondary Sequence:

5'- -3'

CREATE COMPLEMENT

CALCULATE

HERRAMIENTAS DE ANÁLISIS DE PRIMERS HETERO-DIMER

El valor de ΔG debe estar entre 0 y -9 kcal/mol

Primary Sequence: 5'- ATGGGAGAACTGGAGCCTTC -3'

Secondary Sequence: 5'- ATGCGTATTAGC -3'

Maximum Delta G: -38.72 kcal/mole

Delta G: -3.14 kcal/mole Base Pairs2

5' ATGGGAGAACTGGAGCCTTC

: || :

3' CGATTATGCGTA

Delta G: -3.14 kcal/mole Base Pairs2

5' ATGGGAGAACTGGAGCCTTC

||

3' CGATTATGCGTA



1. DISEÑO DE PRIMERS

2. HERRAMIENTAS BIOINFORMÁTICAS
PARA EL DISEÑO DE PRIMERS

3. EFICIENCIA DE AMPLIFICACIÓN
DE PRIMERS



**UN 100% DE EFICIENCIA IMPLICA QUE EN CADA CICLO SE DUPLICA
EL NÚMERO DE AMPLICONES**

USANDO LA ECUACIÓN DE PCR

$$X_n = X_0(1 + E)^n$$

X_n = PCR product after cycle n

X_0 = initial copy number

E = amplification efficiency

n = cycle number

EFFECTO DE LA EFICIENCIA DE AMPLIFICACIÓN

$$X_n = X_0(1+E)^n$$

Caso 1: Eficiencia 90%

$$X_n = 100 (1+0.9)^{30}$$

$$X_n = 2.3 \times 10^{10}$$

Caso 2: Eficiencia 80%

$$X_n = 100 (1+0.8)^{30}$$

$$X_n = 4.6 \times 10^9$$

Resultado

Una **diferencia de 0.1 en la eficiencia** de amplificación resulta en una diferencia de **5 veces menos producto** a partir de la misma cantidad inicial luego de **30 ciclos**

EFICIENCIA DE LOS PRIMERS

A partir de un gráfico de \log_{10} concentración de molde vs C_T , la eficiencia de una reacción de PCR puede calcularse a partir de la siguiente ecuación:

$$\text{Eff} = 10^{(-1/\text{slope})} - 1$$

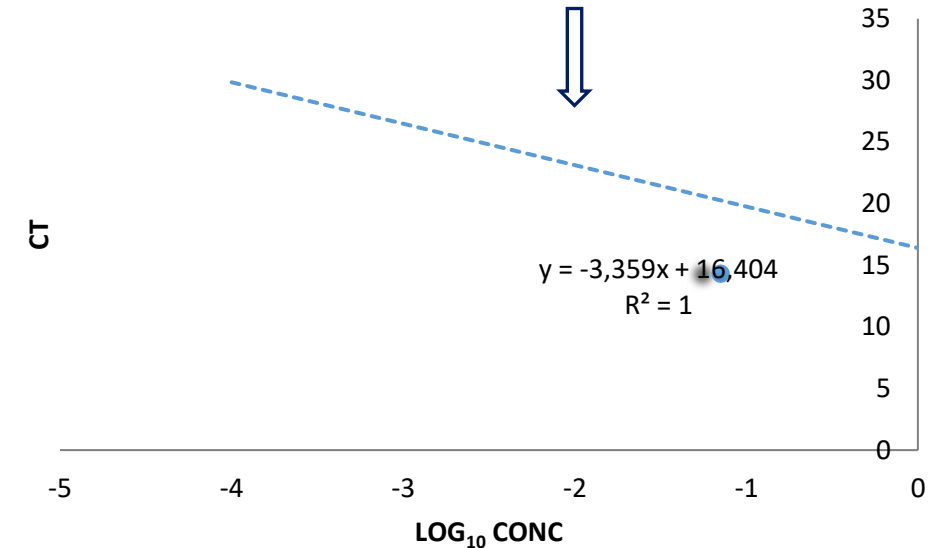
- Curva estándar con concentraciones de molde conocidas
- El molde a usar debe ser **idealmente** el mismo que se usará para los ensayos
- Duplicados o **triplicados**
- NTC

CÁLCULO DE EFICIENCIA DE PRIMERS

Tube	Sample	Tube	Ct 1	Ct 2	Promedio CT	Conc	Log ₁₀ Conc
A	Stock cDNA	A	16.37	16.4	16.385	1	0
B	1\10	B	19.78	19.79	19.785	0.1	-1
C	1\100	C	23.14	23.1	23.12	0.01	-2
D	1\1,000	D	26.45	26.54	26.495	0.001	-3
E	1\10,000	E	29.87	29.78	29.825	0.0001	-4

$$\text{Eff} = 10^{(-1/\text{slope})} - 1$$

$$\text{Eff} = 10^{(-1/-3.359)} - 1 = 0.98 = 98\%$$

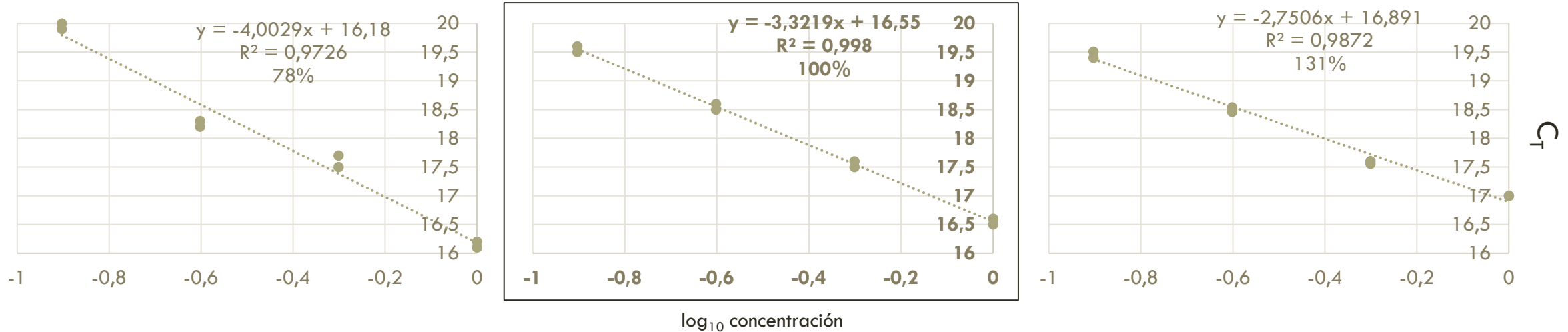


Idealmente, la eficiencia del PCR debería ser entre 90-110% (ideal slope = 3.32)

Variables que afectan la eficiencia:

- Largo del amplicon
- Estructura secundaria de los primers
- Inhibidores

Eficiencia de los primers



- Mal diseño de cebadores
- Concentraciones de reactivos no óptimas
- Condiciones de reacción no óptimas

- Inhibidores de la reacción (material remanente en la muestra o cantidades excesivas de ADN/ARN)
- Contaminantes comunes: heparina, hemoglobina, polisacáridos, clorofilas, proteinasa K, acetato de sodio. O transferidos en el paso de aislamiento: etanol, fenol y SDS

Validation Experiment

Before using the $\Delta\Delta C_T$ method for quantitation, perform a validation experiment like that in Figure 6 to demonstrate that efficiencies of target and reference are approximately equal. The absolute value of the slope of log input amount vs. ΔC_T should be < 0.1 . The slope in Figure 6 is 0.0492, which passes this test. Once this is proven, you can use the $\Delta\Delta C_T$ calculation for the relative quantitation of target without running standard curves on the same plate.

If the efficiencies of the two systems are not equal, perform quantitation using the standard curve method. Alternatively, new primers can be designed and synthesized for the less efficient system to try to boost efficiency.

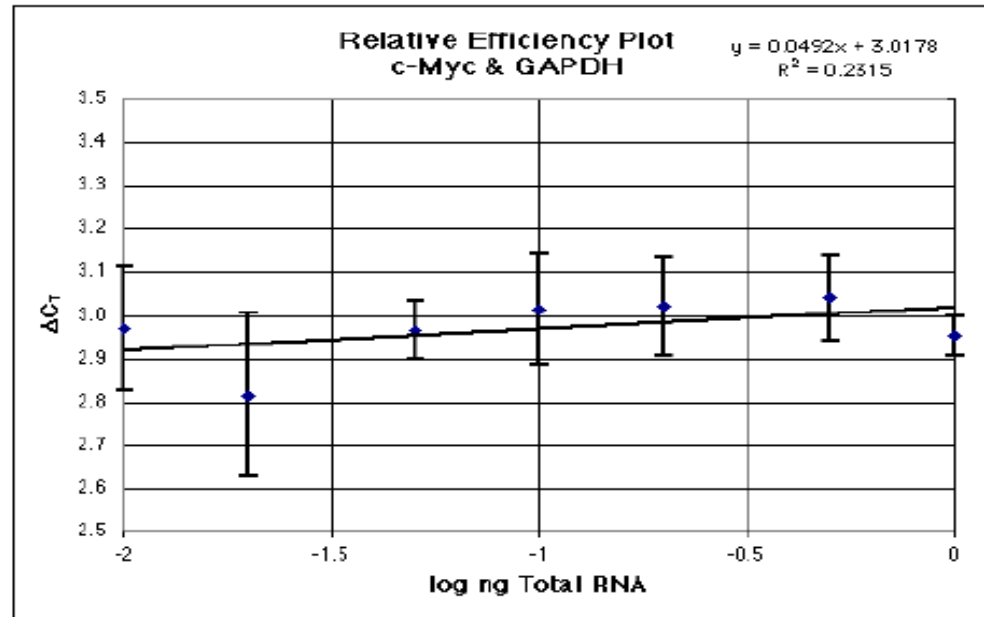


Figure 6. Plot of log input amount versus ΔC_T

Menos del 10%
de diferencia

PFAFFL METHOD

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Nucleic Acids Research, 2001, Vol. 29, No. 9 00

A new mathematical model for relative quantification in real-time RT-PCR

Michael W. Pfaffl*

Se usa para calcular la expresión génica relativa entre dos genes cuyos primers tienen **eficiencias diferentes**

$$\text{Gene expression ratio} = \frac{(E_{GOI})^{\Delta Ct_{GOI}}}{(E_{HKG})^{\Delta Ct_{HKG}}}$$



¿Preguntas?