

Real time PCR primer and probe design

Advanced Biotechnology Lab I
Florida Atlantic University
April 2, 2008

Goals of this exercise

- Design TaqMan primers and probe for MsrA
 - We will use Integrated DNA Technologies' free software available on their website:
<http://www.idtdna.com/SciTools/SciTools.aspx?cat=DesignAnalyze>
 - You will need to set up an account and log in before starting the primer/probe design process. You will design but not actually order the primers.
- Design LUX primers for MsrA
 - We will use Invitrogen's D-LUX primer designer software available on their website:
<https://www.invitrogen.com/content.cfm?pageid=11258>

Integrated DNA Technologies - Mozilla Firefox


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Innovation & Precision In Nucleic Acid Synthesis **IDT**
INTEGRATED DNA TECHNOLOGIES

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miniGENES

Custom miniGene synthesis from IDT

50-500 bp
\$0.65/base pair!

Average turnaround
15-20 business days!

IDT SciTools

Welcome to IDT's suite of online tools. Instructions are included with each application.

Oligo Design and Analysis Tools
Advanced Oligo Design and Analysis

Blast Tools
IDT Hosted BLAST

Tutorials and Documentation
Interactive Tutorials and Documentation

- Oligo Analyzer
- TriFECTa RNAi Kits
- LNA Design
- PrimerQuest ← Click on
- WorkBench
- ddRNAi Design
- RNAi Design
- Antisense Design
- mFold

Done


start Real-time PCR Microsoft PowerPoint... 2 Firefox iTunes 11:09 PM

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Custom miniGene
synthesis
from IDT

50-500 bp
\$0.65/base pair!

Average turnaround
15-20 business days!

Basic | Standard | Advanced Display All Values | About

CALCULATE

Sequence Information

Name:

NCBI ID # **GET SEQUENCE** **Genbank or Accession (RefSeq) ID**

Sequence:

```
CATCAGTTTCAGCTTTAAATGTTAAAGCGACACATTAAATAATAACAATTTAACTAGT
GAAATCATGTCTCTGACTATTACTTCCAGTGTCACTCATCCTGAGCTCAAGGATCTGAGC
ACCGTTCGCAATGAACAAAAGGAACTGAACATTTTCGCCGGTTCACGATGTGAATGTCACC
AAAGCCACGGCCACTTTTCGGCATGGGTTGCTTTTGGGGCGCAGAATCCCTGTATGGAGCC
ACCCGTGGAGTTTTGAGAACCACCGTGGGGCTACGCCGGAGGCAGCTCGGATTTGCCAACG
TACCGTAAATGGGGCGATCATACGGAGGTGCTGGAAATCGACTATGATCCACGGTCATT
AGCTTTAAGGAGCTGCTGGATCTGTTCTGGAACAACCATGAGTATGGCCTGACCACGCC
ATCAAGAGGCGAGTACGCCTCCTTGATTCTGTACCACGATGAGGAGCAGAAGCAGGTGGCA
CACGCCTCCAAATTGGAGGAGCAAGAGCGCCGTGCACCGGAGATCATAACCACTGAGATT
GCATCCAAGGAGAACTTTTACCCAGCCGAGGCGTACCACCAGAAGTATAGGTTGCAGGGC
CACAAGGATCTCGCCTCATCGCTAAATCTCAGCCCCAACTGCTGCAGACCAGCTATGTC
```

Sequence length must be greater than the current minimum product size of 80 bases.

PrimerQuest accepts only nucleic acid bases.

Design For:

☐ PCR Primers

☒ PCR Primers with Probe

☐ Forward Sequencing Primer

☐ Reverse Sequencing Primer

☐ Probe

Use Parameter Set:

CLEAR ALL **RESET DEFAULTS**

CALCULATE

Enter a name

Enter accession

Click Get Sequence

Choose

Choose

Click

Done

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http://www.idtdna.com/SCITOOLS/Applications/PrimerQuest/Results.aspx

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for gene knockdown remain

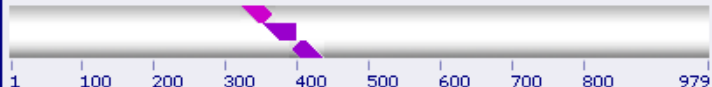
40% OFF

through the rest of 2006!

Primer Set 1

OLIGO T_M SCALE

	< 50		60-65		FORWARD PRIMER
	50-55		65-70		INTERNAL PROBE
	55-60		70-75		REVERSE PRIMER
			> 75		



1 100 200 300 400 500 600 700 800 979

Set Options

ADD SET TO ORDER

SET DETAILS

Order only Set 1

Forward Primer

Primer Sequence:	ACGGAGGTGCTGGAAATCGACTAT	Primer Length:	24
Primer Start Position:	322	Primer Self Any:	4.0
Primer T_M :	59.6 °C	Primer Self End:	3.0
Primer GC %:	50.0 %	Primer Penalty:	0.36
Primer End Stability:	2.12		

Forward Primer Options

ADD TO ORDER

BLAST

HAIRPIN

Probe

Probe Sequence:	TAAGGAGCTGCTGGATCTGTTCTGGA	Probe Length:	26
Probe Start Position:	366	Probe Self Any:	6.0
Probe T_M :	60.8 °C	Probe Self End:	2.0
Probe GC %:	50.0 %		
Probe Penalty:	2.00		

Probe Options

ADD TO ORDER

BLAST

HAIRPIN

Reverse Primer

Primer Sequence:	TGGTCAGGCCATACTCATGGTTGT	Primer Length:	24
Primer Start Position:	415	Primer Self Any:	5.0
Primer T_M :	60.0 °C	Primer Self End:	2.0
Primer GC %:	50.0 %	Primer Penalty:	0.02
Primer End Stability:	3.32		

Reverse Primer Options

ADD TO ORDER

BLAST

HAIRPIN

Primer Pair/Product

Primer Pair Penalty:	0.38	Primer Pair Comp Any:	4.0
Primer Product Size:	94	Primer Pair Comp End:	2.0

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VIEW CART

CHECKOUT

LOG OUT

DELETE ENTIRE ORDER

WISH LIST

ACCOUNT INFO

OLIGO CARD

ORDER HISTORY

QC RESULTS

Chat with a live IDT representative

START CHAT

Results | Order [About](#)

Please Select Primer Details For Set 1

Scale: **100 nmole DNA oligo** Purification: **Standard Desalting**

Notes:

Please Select Probe Details For Set 1

Scale: **100 nmole** Purification: **Dual HPLC**

5' Mod: **5' 6-FAM** 3' Mod: **3' Black Hole Quencher 1**

Notes:

1. Paste in a sequence
2. Configure primer/probe options
3. Calculate!
4. Select the set(s) or oligos you like
5. Order your primers

Choose amount

Primer purification

Standard desalting

ok

Choose amount

Choose HPLC

Reporter dye

Quencher dye

Click

www.idtdna.com

start Integrated DNA Tech... Microsoft PowerPoint ... 10:43 AM

Integrated DNA Technologies - Windows Internet Explorer

https://www.idtdna.com/Order/Review.aspx

Logged In: David Brunell

You have 3 items in an unfinished order
Subtotal: \$199.00

CONTINUE SHOPPING
VIEW CART
CHECKOUT
LOG OUT
DELETE ENTIRE ORDER
WISH LIST
ACCOUNT INFO
OLIGO CARD
ORDER HISTORY
QC RESULTS

Chat with a live IDT representative

START CHAT

Shopping Cart

Current Order as of 4/2/2008 4:02:31 PM (CDT)

EXPAND ALL COLLAPSE ALL

#1 DroMsrA Set 1 Forward Primer Edit + Delete + Analyze + Add Complement + Move to Wish List \$12.00

Product: 100 nmole DNA oligo Length: 24 Usually Ships In: 1 business day
Purification: Standard Desalting Guaranteed Yield: 6 ODs = 24.9 nmole = 185.3 µgrams
Sequence: 5'- ACG GAG GTG CTG GAA ATC GAC TAT -3'

#2 DroMsrA Set 1 Reverse Primer Edit + Delete + Analyze + Add Complement + Move to Wish List \$12.00

Product: 100 nmole DNA oligo Length: 24 Usually Ships In: 1 business day
Purification: Standard Desalting Guaranteed Yield: 6 ODs = 26.6 nmole = 195.9 µgrams
Sequence: 5'- TGG TCA GGC CAT ACT CAT GGT TGT -3'

#3 DroMsrA Set 1 Probe Edit + Delete + Move to Wish List \$175.00

Product: 100 nm DLP 5' 6-FAM™ / 3' BHQ-1™ Length: 26 Usually Ships In: 3-5 business days
Purification: HPLC Purification Guaranteed Yield: 2.7 ODs = 10 nmole = 91.5 µgrams
Sequence: 5'- /56-FAM/TAA GGA GCT GCT GGA TCT GTT CTG GA/3BHQ_1/ -3'

☒ Ship order when complete (single shipment)
☐ Ship items as available

Subtotal	\$199.00	USD
Shipping & Handling	\$16.00	USD
Tax	\$12.94	USD
Total	\$215.00	USD

Promo Code GO

Based on your order, you may also be interested in this:
"Validated, Reliable Controls for your RT-PCR Reaction"

1 nm Human HPRT SybrTMGreen Primer Set	\$25.00	ADD
5 nm Human HPRT SybrTMGreen Primer Set	\$60.00	ADD
5 nm Mouse HPRT SybrTMGreen Primer Set	\$60.00	ADD
1 nm Mouse HPRT SybrTMGreen Primer Set	\$25.00	ADD

FINISH ORDER
SAVE ORDER
FORWARD THIS ORDER

Click here and email the order to me if you like.

Print out this page and attach it to your lab writeup

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Custom Designed qPCR Assays with D-LUX™ Designer Software

The D-LUX™ Designer is the most comprehensive tool available for designing and ordering LUX™ Primers. It offers extensive features and functions to help you design the best primers for your gene of interest, including the identification of exon-exon junctions, automatic determination of intron size, and identification and alignment of potential splice variants.

Simply enter your target sequence and the software will generate primer sets ranked in order of optimization. Primers designed through the D-LUX™ Designer are available with FAM, JOE and Alexa Fluor® 546 dyes. Primers are purified by HPLC.

For the most up-to-date BLAST capability, use the online version of our D-LUX™ Designer. If you prefer, you may download a desktop version or request a CD version be mailed to you. Click here for a [step-by-step guide to using the D-LUX™ Designer](#)

• [Design your D-LUX™ Primer Sets online](#)

• [Download Desktop version of D-LUX™ Designer Software](#)

• [Request CD version of D-LUX™ Designer Software](#)

Click

Product	Scale**
LUX™ Fluorogenic Primer Set, FAM-labeled	50 nmol
	200 nmol
LUX™ Fluorogenic Primer Set, JOE-labeled	50 nmol
	200 nmol
LUX™ Fluorogenic Primer Set, Alexa Fluor® 546-labeled *	50 nmol
	200 nmol

*When using LUX™ Fluorogenic Primers with Alexa Fluor® 546 dye labels, we recommend ordering the [qPCR Instrument Calibration Kit](#) for calibrating your real-time PCR system.

Done

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D-LUX™ Designer

- Re-order LUX™ Primers
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- LUX™ Primer Services
- qPCR Reagents
- iPath™

Step 1: Enter an accession number or provide a nucleotide sequence.

Enter Accession #

Accession number: NM_079361 Enter an Entrez Ref Seq accession number to ensure that your sequence is analyzed with our complete annotation tools (SNP location, exon/intron determination).

OR

Nucleotide sequence name(optional):

AND

Nucleotide sequence: You can only enter IUPAC letter codes: A, C, G, T, N, B, D, H, K, M, R, S, V, W, X, Y.

Check ORF

Choose species

Leave alone

Click

Step 2: Select regions for target design

☒ Open reading frame (ORF) ☐ 5' UTR ☐ 3' UTR

Specify included or excluded regions (e.g., 100,50 interpreted as region ranging from 100 to 149; multiple regions can be entered as follows: 100,50 300,50 ...).

☒ Included regions ☐ Excluded regions

Step 3: Choose database for BLAST

Fruitfly - Drosophila melanogaster

NOTE: BLAST is used to compare input sequence with sequences in the database to find unique regions against which to design LUX primers. The databases contain representative gene sequences for that species.

Step 4: Choose design parameters

The default design parameters are highly recommended for optimal results.

	Minimum	Optimum	Maximum
Amplicon size:	60	75	200
Primer temperature:	60	64	68
Primer length:	20	22	24

Design LUX primers **Reset form**

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Designed D-LUX™ Primers

? HELP

Gene: Eip71CD **Accession:** [NM_079361.2](#) **GI:** 24664626
Organism: Drosophila melanogaster **Length:** 979 **ORF Region:** 67-807
Definition: Drosophila melanogaster Ecdysone-induced protein 28/29kD CG7266-RA,transcript variant A (Eip71 CD), mRNA.

Transcript: 1 979

BLAST: 1 2150

[NM_079361.2](#)
[NM_168623.1](#)
[NM_168622.1](#)
[NM_206371.1](#)

Legend: ■ UTR ■ Coding exon ■ Unique region ■ Masked region

4 splicing variants

No.	Accession	Definition
1	NM_079361.2	Drosophila melanogaster Ecdysone-induced protein 28/29kD CG7266-RA,transcript variant A (Eip71 CD), mRNA.
2	NM_168623.1	Drosophila melanogaster Ecdysone-induced protein 28/29kD CG7266-RC,transcript variant C (Eip71 CD), mRNA.
3	NM_168622.1	Drosophila melanogaster Ecdysone-induced protein 28/29kD CG7266-RB,transcript variant B (Eip71 CD), mRNA.
4	NM_206371.1	Drosophila melanogaster Ecdysone-induced protein 28/29kD CG7266-RD,transcript variant D (Eip71 CD), mRNA.

☒ Display a selection of the best LUX™ primers ☐ Display All Designed LUX Primers

Go to order form Generate design report Sort by: Labeled primer strand/3' Loc

Done orf.invitrogen.com

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Scroll down



1. Click here to select the best primer set

[Go to order form](#)
[Generate design report](#)

Sort by:

Labeled primer strand/3' Loc

D-LUX™ Designer found 30 LUX primer sets.

Select	No.	Primer name	Strand	3' Loc	Sequence	Exon	Product size	Quality	Splicing Variants			
									1	2	3	4
<input checked="" type="checkbox"/>	1	NM_079361.2_353RL	R	353	cggtcCAGCTCCTTAAAGCTAATGACcG	3	67	★★★★★	X	X	X	X
		NM_079361.2_353RL/333FU	F	333	GCGATCATACGGAGGTGCTG	3						
<input type="checkbox"/>	2	NM_079361.2_353RL	R	353	cggtcCAGCTCCTTAAAGCTAATGACcG	3	68	★★★★★	X	X	X	X
		NM_079361.2_353RL/332FU	F	332	GGCGATCATACGGAGGTGCT	3						
<input type="checkbox"/>	3	NM_079361.2_287RL	R	287	cggatACGGTACGTTGGCAAATCcG	2	81	★★★★★	X	X		X
		NM_079361.2_287RL/251FU	F	251	GTATGGAGCCACCCGTGGAGT	2						
<input type="checkbox"/>	4	NM_079361.2_287RL	R	287	cggatACGGTACGTTGGCAAATCcG	2	80	★★★★★	X	X		X
		NM_079361.2_287RL/251FUa	F	251	TATGGAGCCACCCGTGGAGT	2						
<input type="checkbox"/>	5	NM_079361.2_287RL	R	287	cggatACGGTACGTTGGCAAATCcG	2	81	★★★★★	X	X		X
		NM_079361.2_287RL/250FU	F	250	GTATGGAGCCACCCGTGGAG	2						
<input type="checkbox"/>	6	NM_079361.2_353RL	R	353	cggtcCAGCTCCTTAAAGCTAATGACcG	3	71	★★★★☆	X			X
		NM_079361.2_353RL/329FUb	F	329	ATGGGCGATCATACGGAGGT	2, 3						
<input type="checkbox"/>	7	NM_079361.2_568FL	F	568	cggcAAGGAGAACTTTTACCCAGCcG	3	78	★★★★☆	X	X	X	X
		NM_079361.2_568FL/600RU	R	600	GATGAGGCGAGATCCTTGTTG	4						
<input type="checkbox"/>	8	NM_079361.2_568FL	F	568	cggcAAGGAGAACTTTTACCCAGCcG	3	83	★★★★☆	X	X	X	X
		NM_079361.2_568FL/604RU	R	604	TTAGCGATGAGGCGAGATCCTT	4						
<input type="checkbox"/>	9	NM_079361.2_568FL	F	568	cggcAAGGAGAACTTTTACCCAGCcG	3	84	★★★★☆	X	X	X	X
		NM_079361.2_568FL/605RU	R	605	TTTAGCGATGAGGCGAGATCCT	4						
<input type="checkbox"/>	10	NM_079361.2_696RL	R	696	cggcCCTTAAACTGCTCGATGCcG	4	109	★★★★☆	X	X	X	X
		NM_079361.2_696RL/632FU	F	632	TCGCCTCATCGCTAAATCTCAG	4						

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D-LUX™ Primer Order

HELP

Customer Number: **Contact Name/Address:**

Quote Number: **Contact Phone Number:**

Purchase Order Number: **Contact Fax Number:**

Contract Number: **Contact Email Address:**

Release Number: **Researcher Name:**

Plate or Tube:

***: Required field**

You can send your LUX primer Orders to Invitrogen by clicking "Send order" button. You can also download the order form by clicking "Download order" button and send it by email (genomicorders@invitrogen.com) later.

Buy	Primer name	Type	Sequence	Dye	Scale
<input type="checkbox"/>	NM_079361.2_287RL	Labeled	cggatACGGTACGTTGGCAAATCcG	FAM	50 nmoles
<input type="checkbox"/>	NM_079361.2_287RL/251FU	Unlabeled	GTATGGAGCCACCCGTGGAGT		50 nmoles

Note:

Click

Choose dye

Choose amount

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