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LUX™

**Fluorogenic Detection System
A New Approach in qPCR**

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Genomics Product Manager**

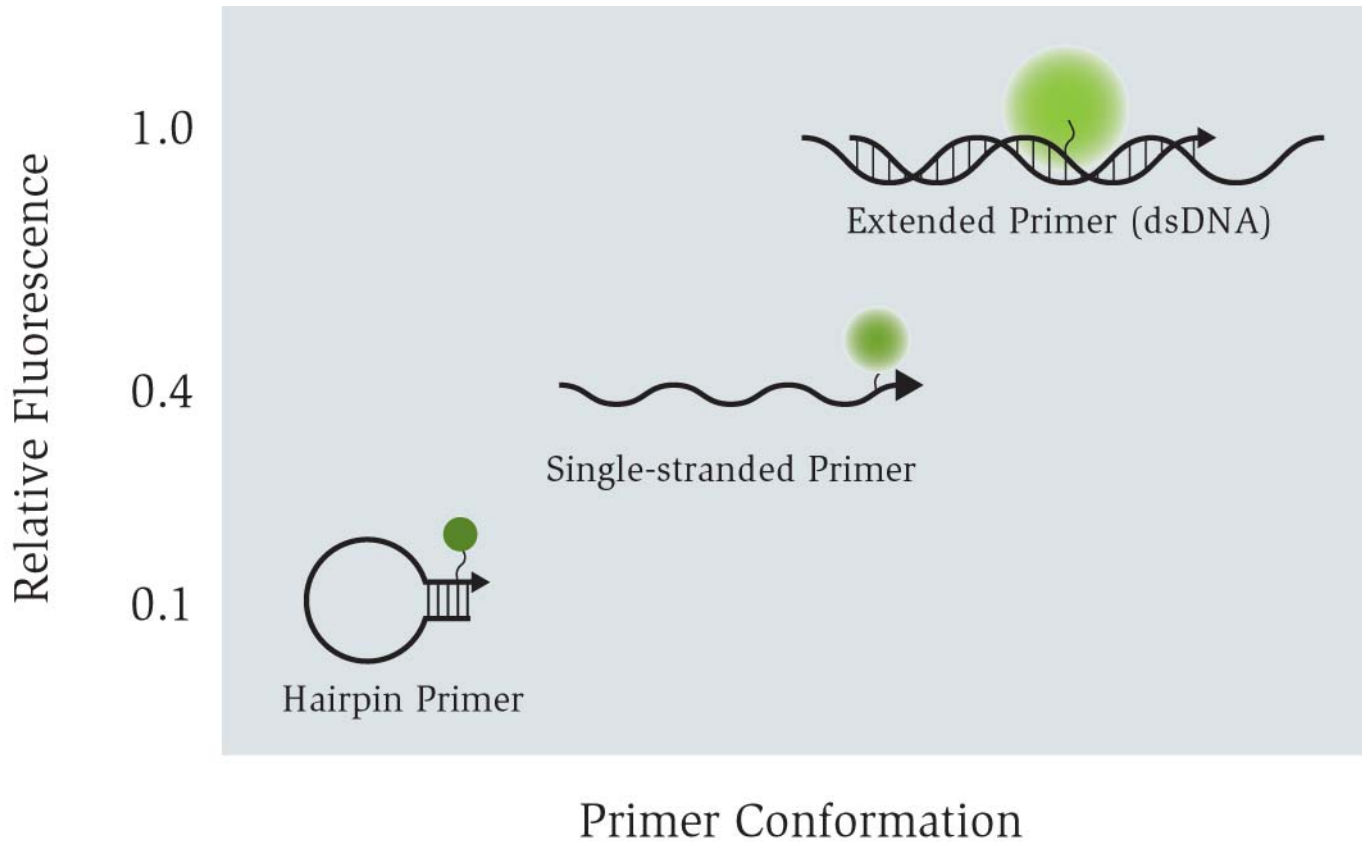
- **What is LUX™ technology?**
- **LUX™ primer platforms**
- **Comparison to other detection methods**
- **Instrument and reagent compatibility**
- **LUX™ primers applications for real-time PCR**
 - **Gene expression profiling**
 - **Pathogen detection**
- **Summary**



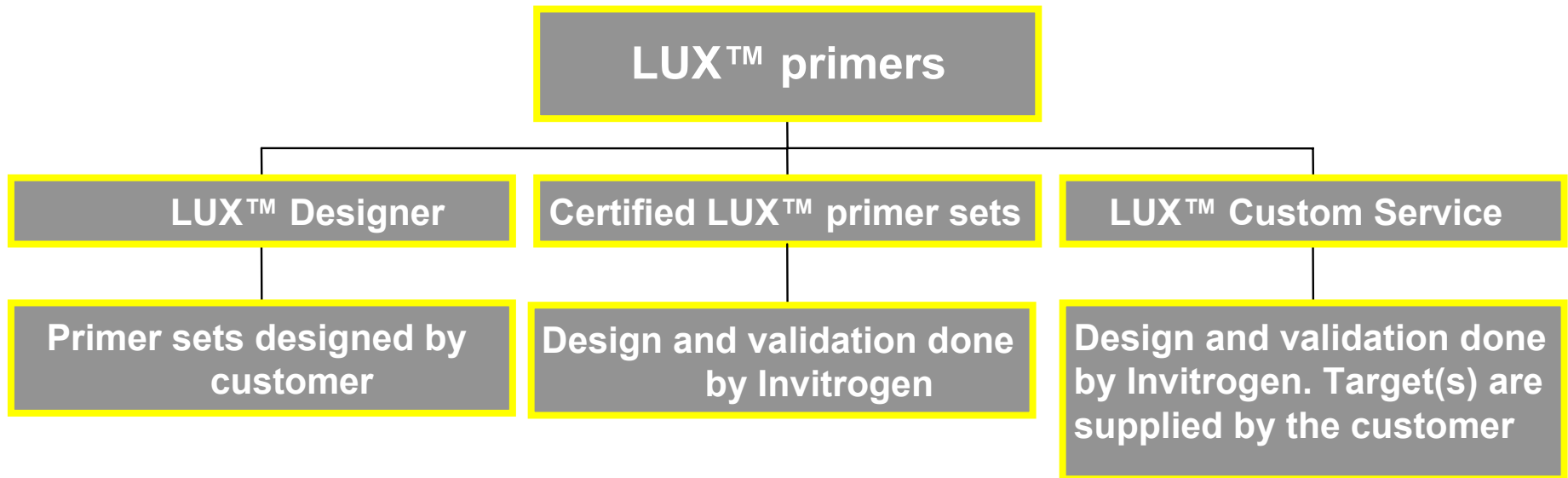
What is LUX™ technology?

- **A novel fluorescent detection system that does not require a quenching moiety for homogeneous detection.**
- **Based on oligonucleotides labeled with a single fluorophore**
 - **produce a significant increase in fluorescence intensity upon incorporation into double stranded DNA.**
 - **Light Upon eXtension**
- **Flexibility in use make this technology suitable for large-scale high throughput applications.**

Principle of the LUX™ primer Signal Generation



Invitrogen's LUX™ fluorogenic primer platforms





Comparison to other detection methods



Researchers needs for real-time quantitative PCR?

- **Dynamic range**
- **Sensitivity**
- **Precision**
- **Specificity**
- **Multiplexing**
- **Convenience**

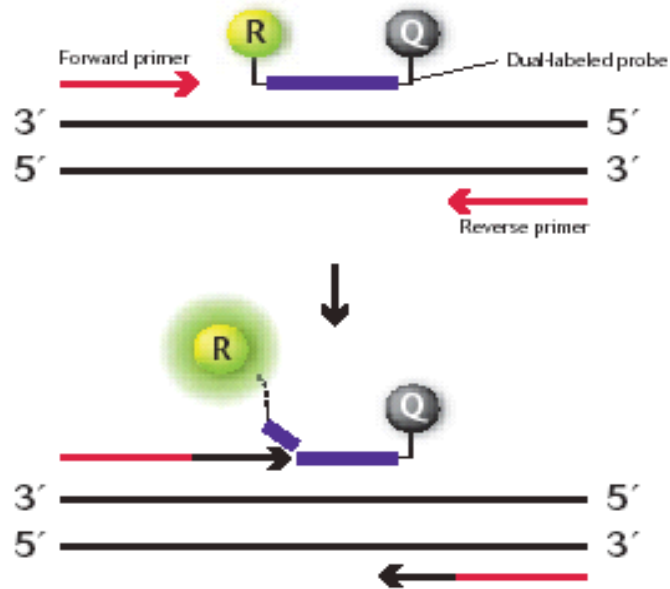


Comparison of real-time detection

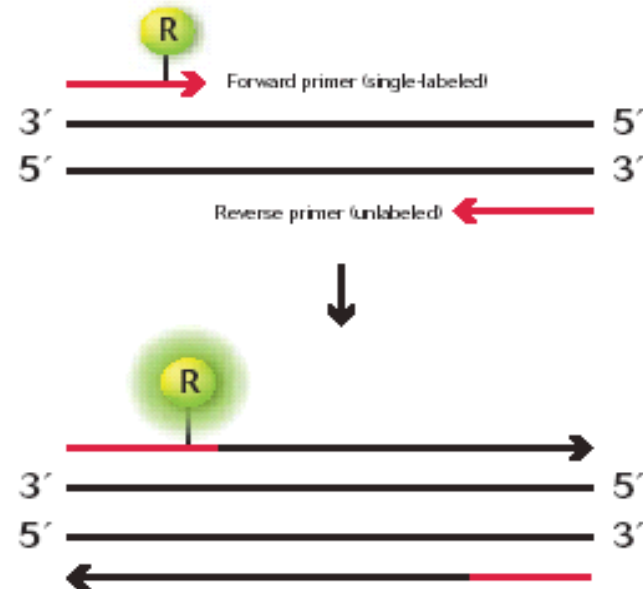
	TaqMan® Probe	Molecular Beacon	SYBR® Green	LUX™ Primer
sensitivity	+++	+++	+	+++
dynamic range	+++	+++	+	+++
specificity	+++	+++	+	++
multiplexing	++	++	N/A	+++
melting curve analysis	N/A	N/A	+++	+++
ease of primer/ probe design	+	+	+++	+++
cost effectiveness	+	+	+++	+++

Comparison of LUX™ to TaqMan®

TaqMan® detection



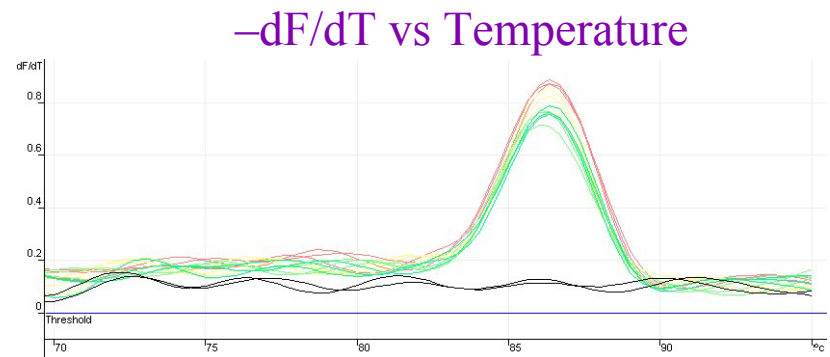
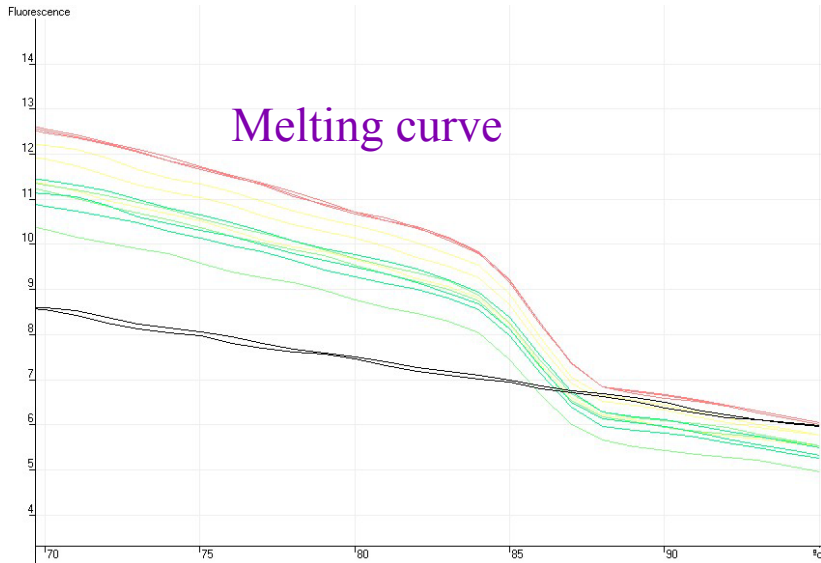
LUX™ detection





Principle of melting curve analysis

1. After amplification: 60C to 95C (slow ramp)
2. Fluorescence signal is recorded continuous during the slow temperature ramp
3. Melting curve : Fluorescence signal vs. Temperature
4. Melting curve were converted to melting peaks by plotting $-dF/dT$ vs Temperature

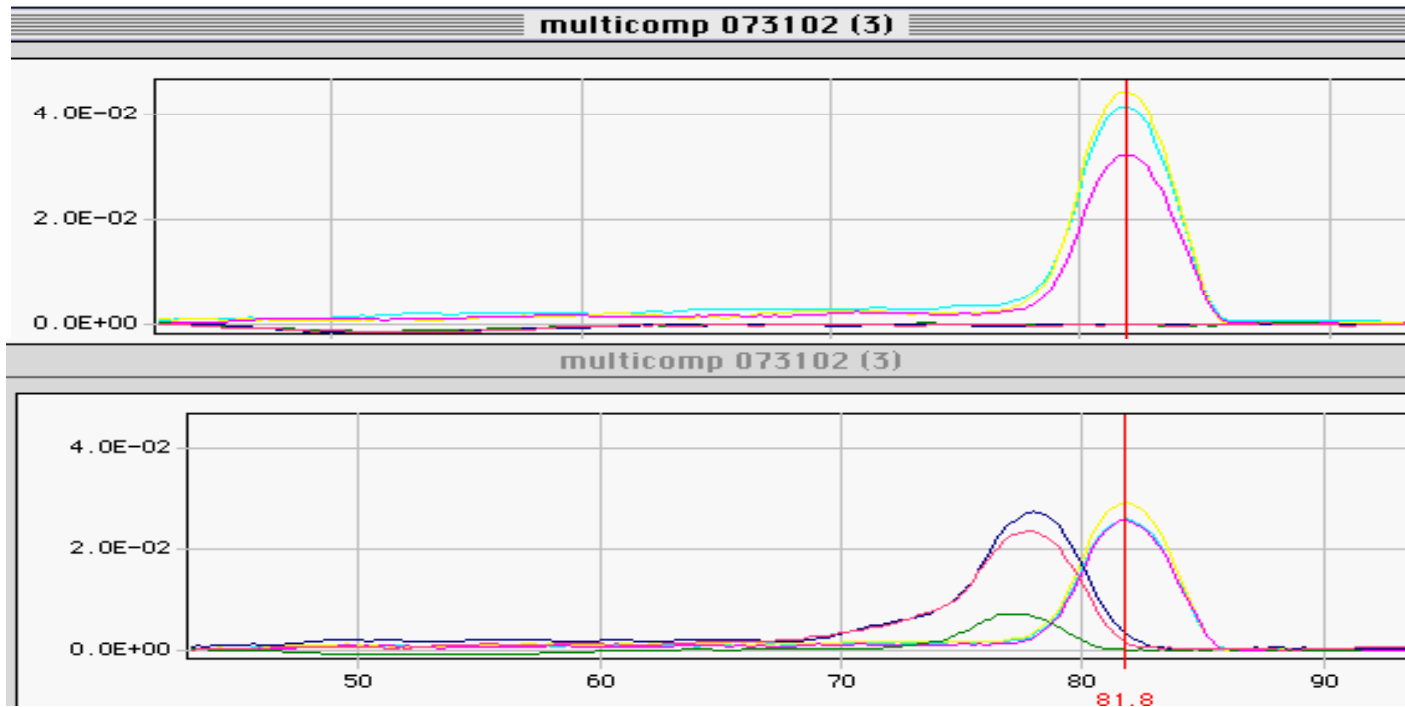


Applications:

1. Analysis of PCR product specificity: 1 peak per well if specific
2. Detection of
 - Primer dimers (low T_m in NTC),
 - contaminations (Same T_m than PCR product in NTC)



- **Detection of Primer Dimers (not detected by TaqMan® but can affect PCR efficiency)**
- **Analysis of specificity of the product or other artifacts**

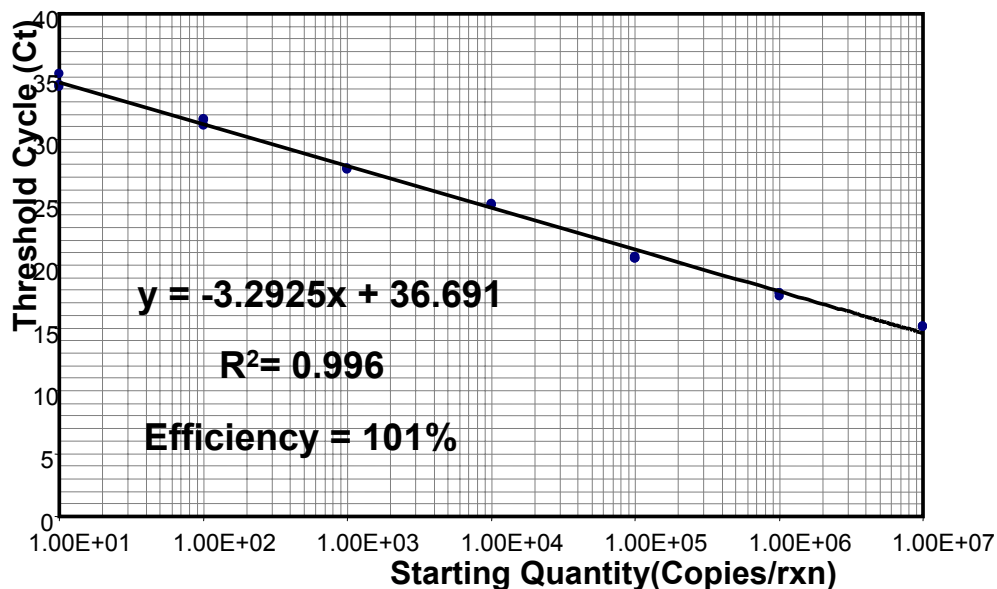
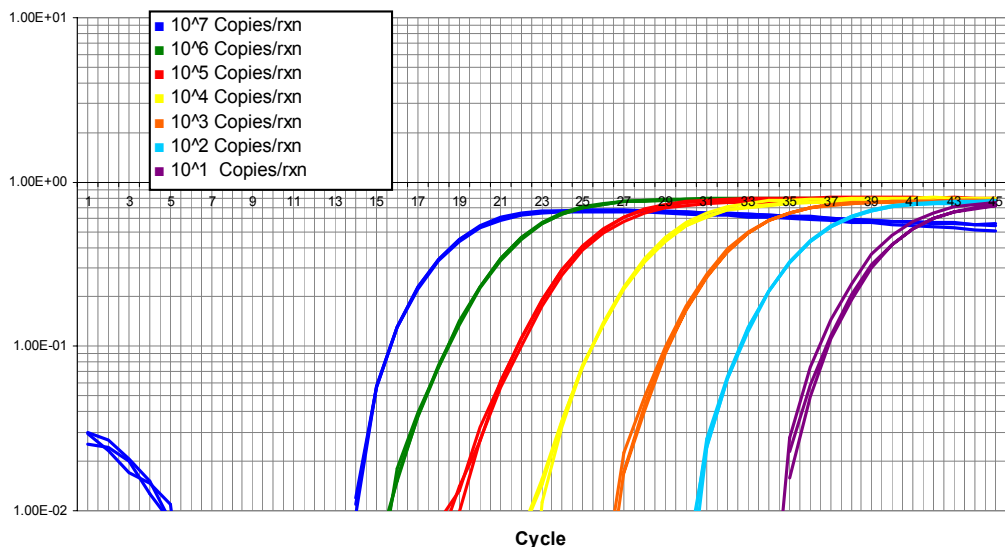


Are LUX primers less specific than TaqMan® due to the absence of probes?

- **The specificity of PCR is given by the primers and reaction conditions not by the probes.**
- **The TaqMan® probe gives you the specificity just for the detection part but this can mask the presence of artifacts which can interfere with the efficiency of the PCR:**
 - **Non specific amplification**
 - **Primer dimers**



Instrument Compatibility Applications and Examples of using LUX™ Primers

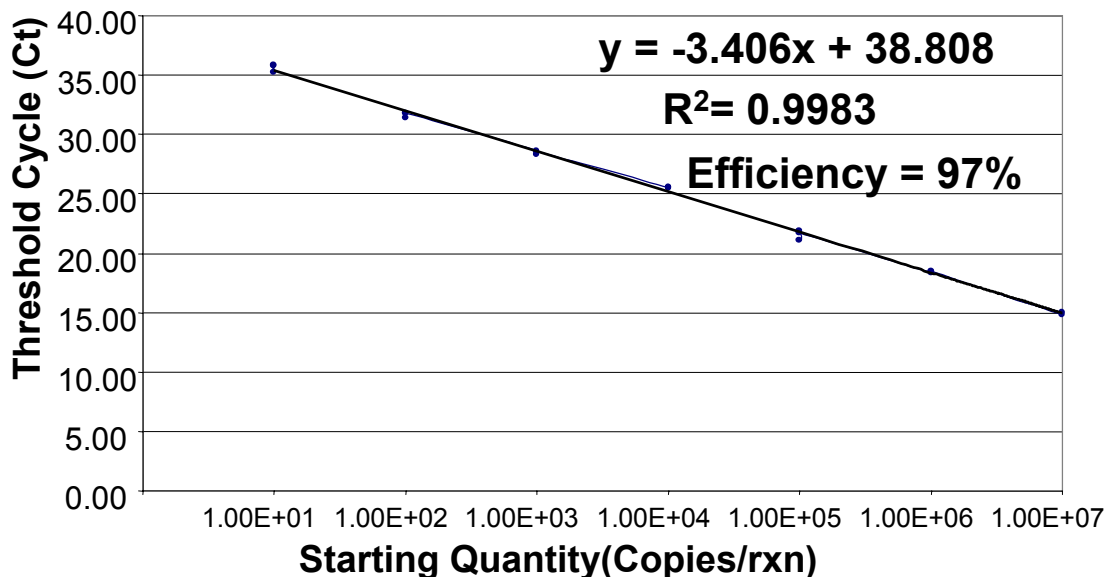
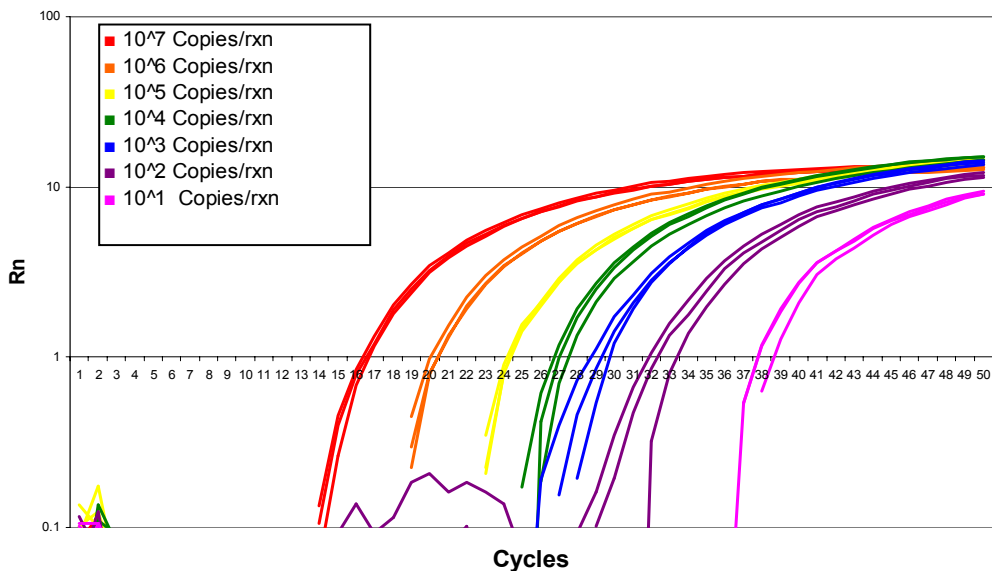


LUX™ primers against human beta actin were tested using 10 to 10⁷ copies of cloned plasmid using Platinum® SuperMix-UDG

Protocol for 96-well plate instruments (ABI 7700)

- SuperMix UDG (ROX), 200 nm each primer (FAM or JOE)
- 2 min 50°C, 2 min 95°C
- 15 sec 95°C, 30 sec 55°C, 30 sec 72°C (40-50 cycles)
- Melting curve (7700) : 40°C for 1 minute, ramp to 95°C over a period of 19 minutes followed by incubation at 25°C for 2 min

LUX™ real-time PCR on the Lightcycler



LUX™ primers against human beta actin were tested using 10 to 10⁷ copies of cloned plasmid using Platinum® SuperMix-UDG

Protocol:

500 nm FAM primer pair
Taq to 1.2 units/20 μl
BSA to 250ng/μl

PCR program:

2 min 50°C/2 min 95°C
5 sec 94°C
10sec 55°C, single acqu. F1 10 sec 72°C

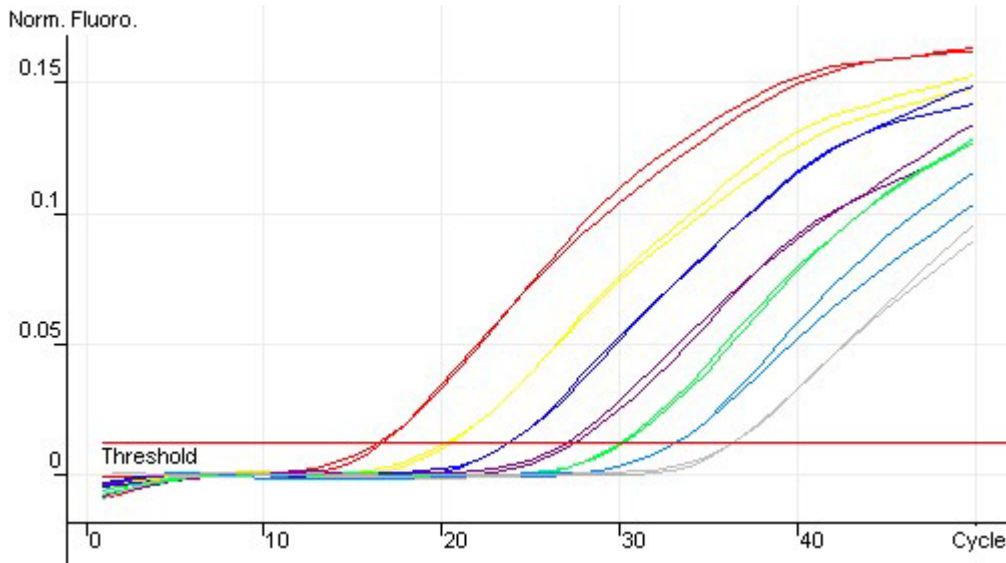
Melting curve:

0 sec 95°C/15 sec 55°C/
0 sec 95°C increase of
0.1°C/sec with cont.acqu



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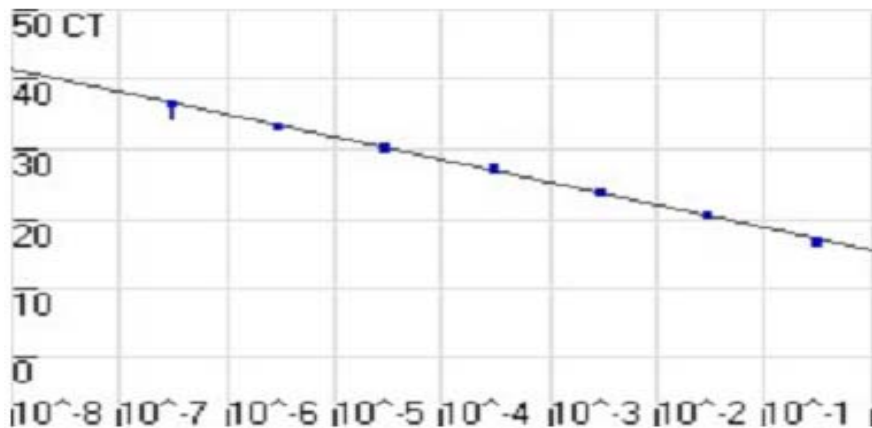
LUX™ real-time PCR on the Rotor-Gene with LUX primers



18S rRNA LUX™ primers tested using 10 to 10⁷ copies of cloned plasmid using Platinum® SuperMix-UDG

Protocol:

- SuperMix UDG (20μl assays), 200 nm each primer (FAM or JOE)
- 2 min 50°C, 2 min 95°C
- 5 sec 95°C, 15 sec 72°C (40-50 cycles)
- Melting curve: 45°C to 95°C in 1° steps, 5 sec at each step
- 300 ng to 0.3 pg HeLa cDNA



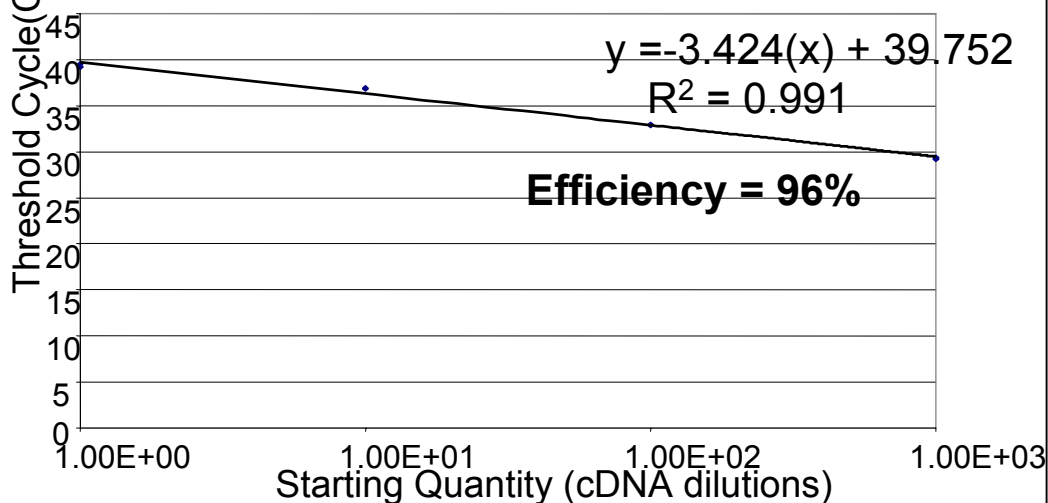
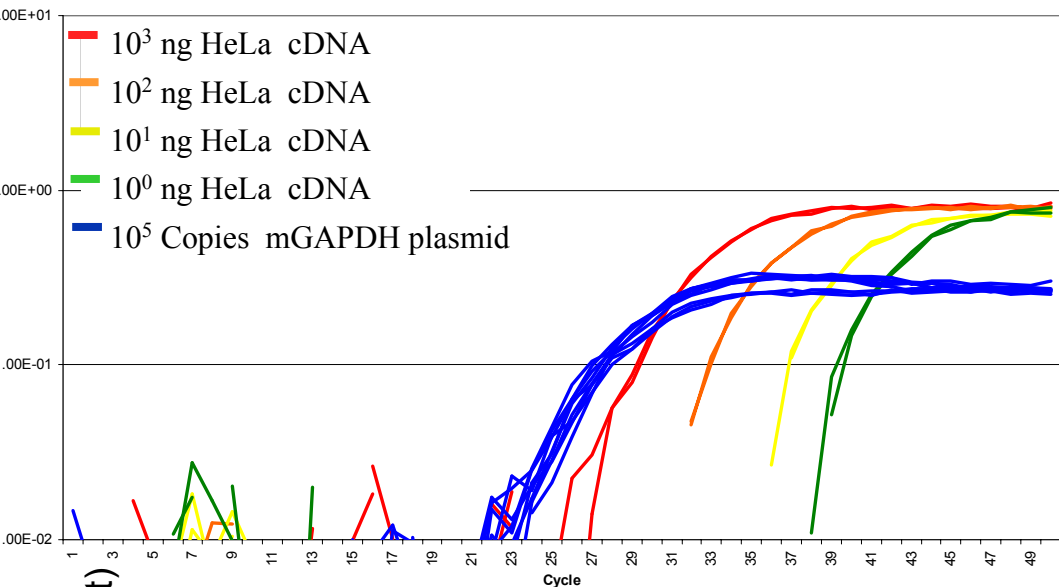
Slope = - 3.25

R² = 0.997

Efficiency = 103%



Multiplex PCR with LUX™ Primers



Human SDHA Fam LUX™ primer
Mouse GAPDH Joe LUX™ primer

- Platinum® SuperMix-UDG
- 200nm primers SDHA (LUX^{FAM}) & **100 nm primers** GAPDH (LUX^{JOE})
- 2 min 50°C, 2 min 95°C, 15 sec 95°C, 30 sec 55°C, 30 sec 72°C (40-50 cycles)

Strategies for multiplex:

Use standard protocol and determine PCR efficiency, if efficiency outside 90-110%:

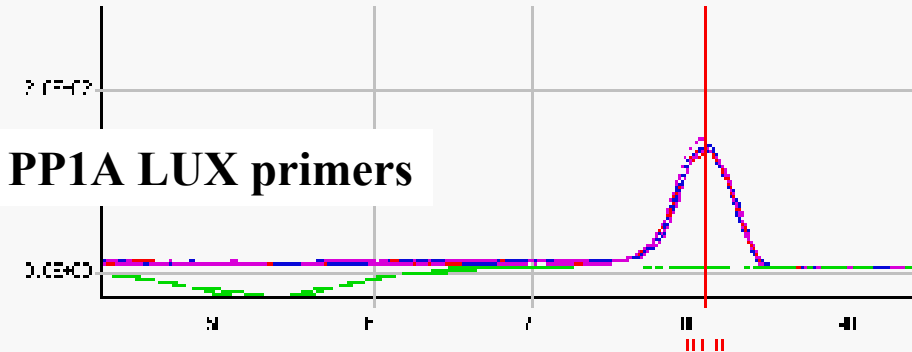
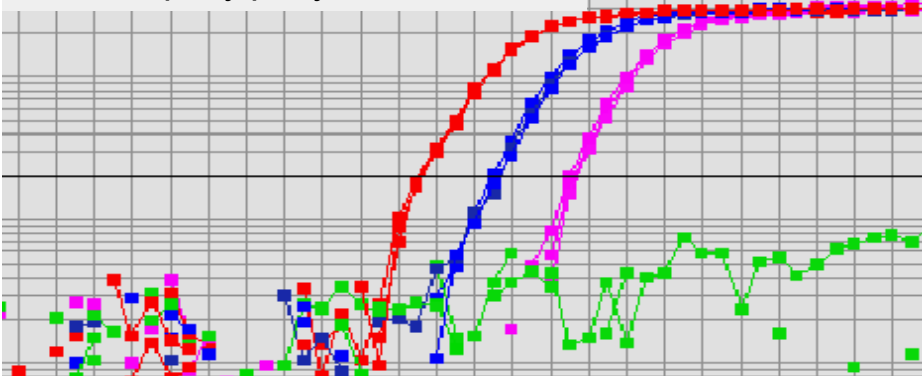
- decrease primer conc. for abundant gene
- increase Mg to **6 mM**
- Double Taq to 3u/50ul
- Double dNTP's to 400 nm



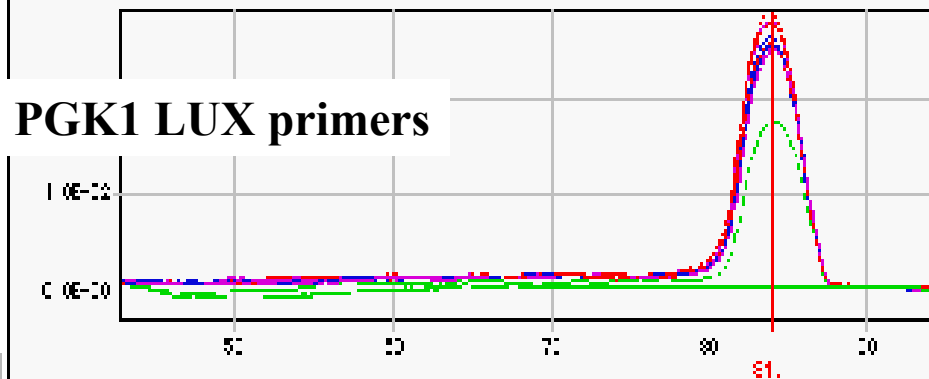
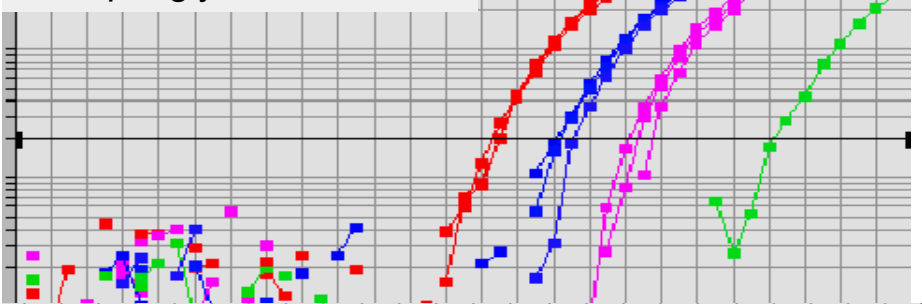
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LUX™ Primers with One-Step RT-PCR: Great performance from high to low abundance

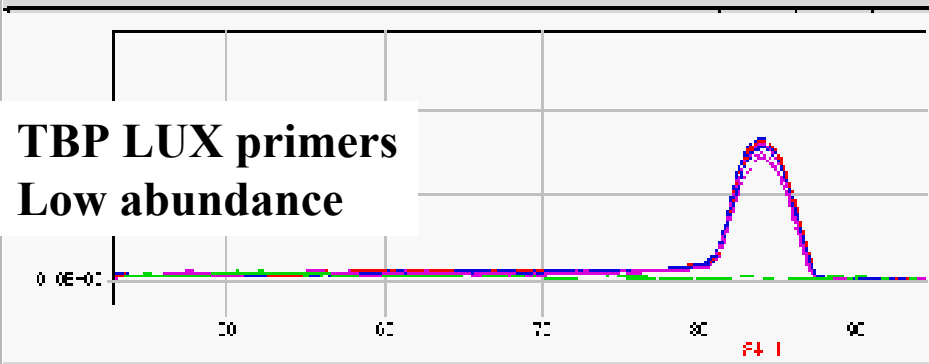
Peptidylprolyl isomerase A



Phosphoglycerate kinase I



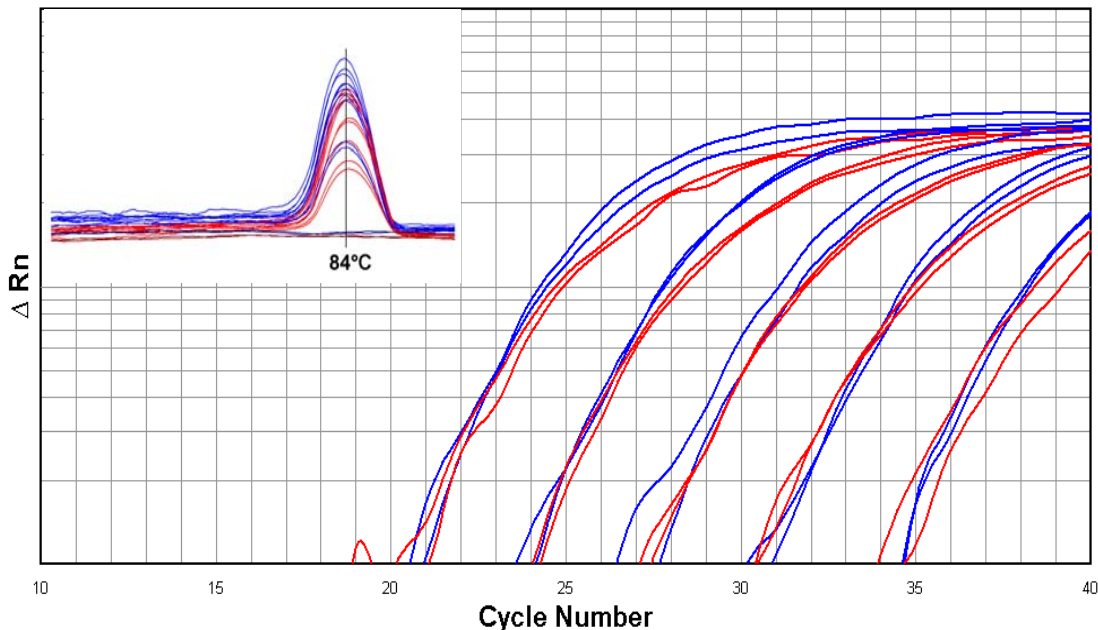
TATA box binding protein



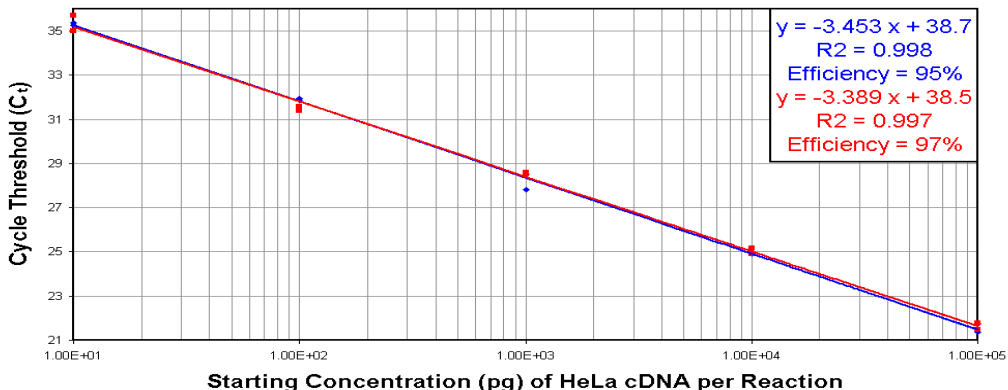


LUX™ Primers with Platinum® RTS qPCR SuperMix-UDG 96

A. Amplification Plot with Melting Temperature Plot (inserted)



B. Standard Curve



LUX™ primers against human beta actin were tested against 10-fold serial dilutions (100ng-10pg) of HeLa cDNA with:

Red: Platinum® qPCR SuperMix-UDG

Blue: Platinum® RTS qPCR SuperMix-UDG 96

Protocol:

Reactions were incubated for 2 min. at 50°C, then 2 min. at 95°C, followed by 40 cycles of 95°C for 15 sec.; 60°C for 30 sec. using the ABI PRISM® 7700.

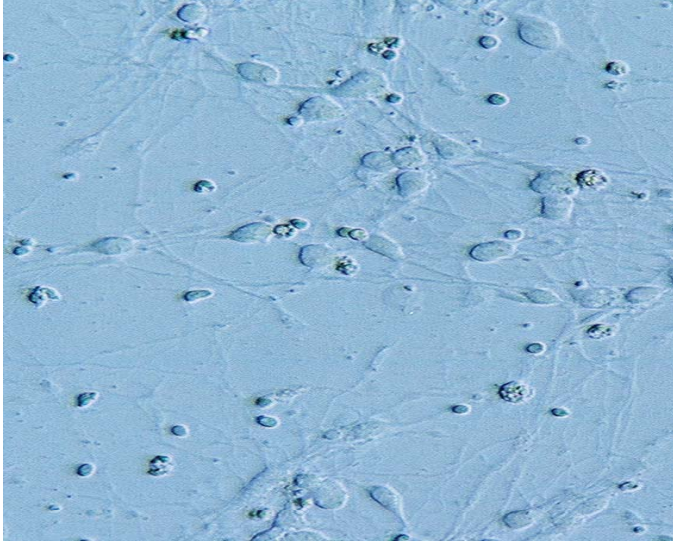


**LUX™ Primers
&
Expression Profiling**



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Use of LUX™ primers for gene expression profiling in mouse P-19 cells



Cells resembling neurons with processes were photographed (Nikon microscope, 20x objective) 7 days after they were induced to differentiate by retinoic-acid treatment.

Mouse Genes studied with LUX™ primers

GAP43 = Growth associated protein 43

NMDA1 = N-methyl-D-aspartate receptor

BMP = bone morphogenetic protein

BFGFR = basic fibroblast growth factor receptor

GLUR1 = Glutamate receptor 1

Nestin= neural progenitor marker, transiently expressed during differentiation

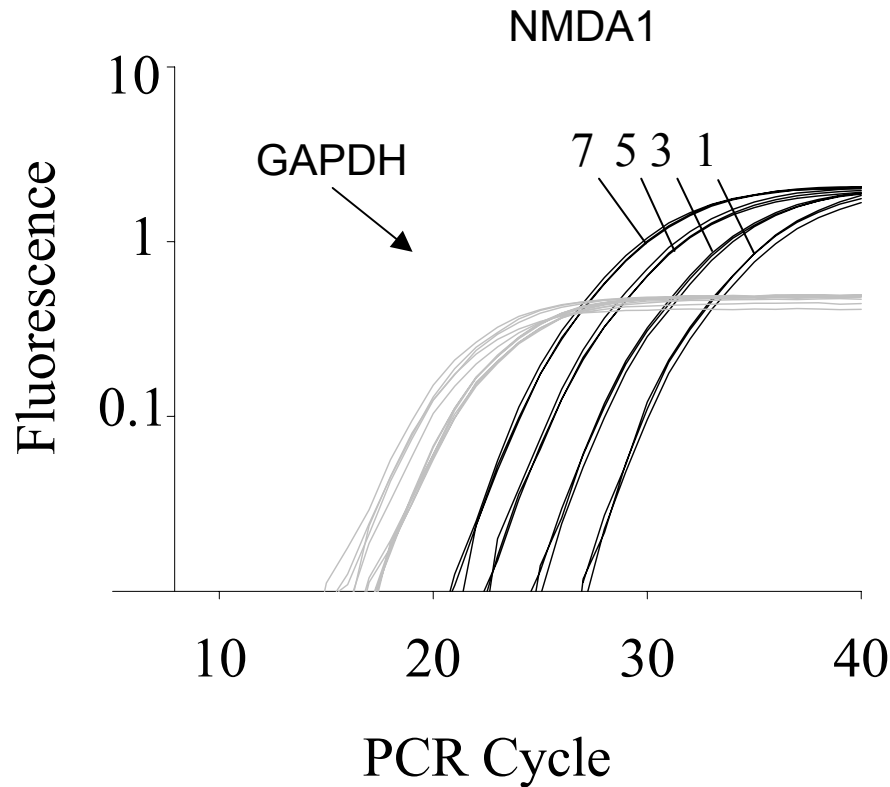
BDNFR = brain derived neurotropic factor receptor

ChAT = choline acetyltransferase receptor

EGR1 = early growth response 1 gene
(transcription factor)

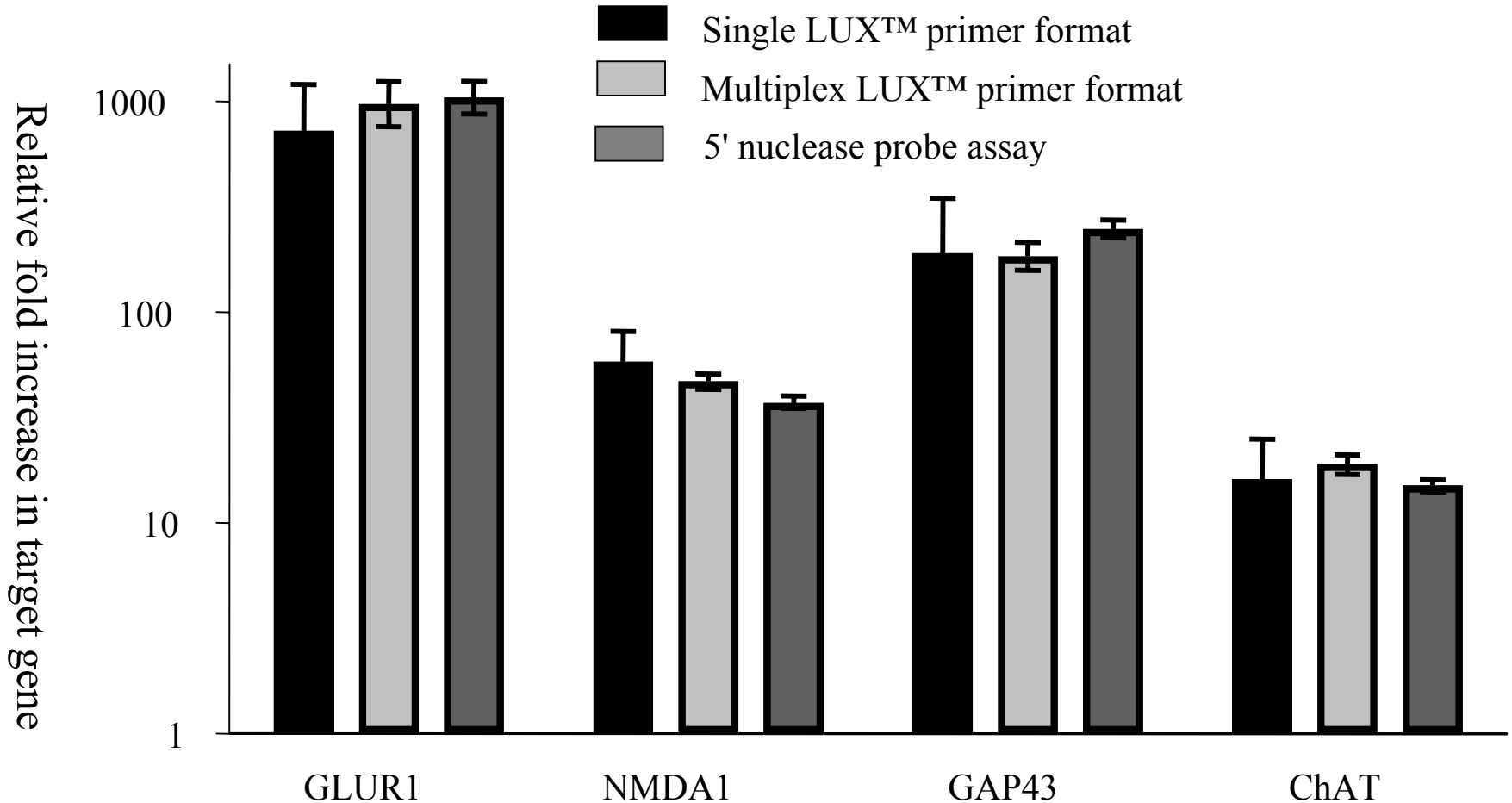
NCAM = neural cell adhesion molecule (cell-cell interaction)

Expression of the NMDA1 gene during differentiation of P-19 cells



Samples from P-19 cultures were taken at 1, 3, 5 and 7 days after induction. Real-time PCR results (3 replicates per time-point) are shown for NMDA1 (dark traces) and GAPDH (light traces) using LUX™ primers. Higher cycle numbers are correlated with lesser amount of initial template.

Comparison of different assay formats



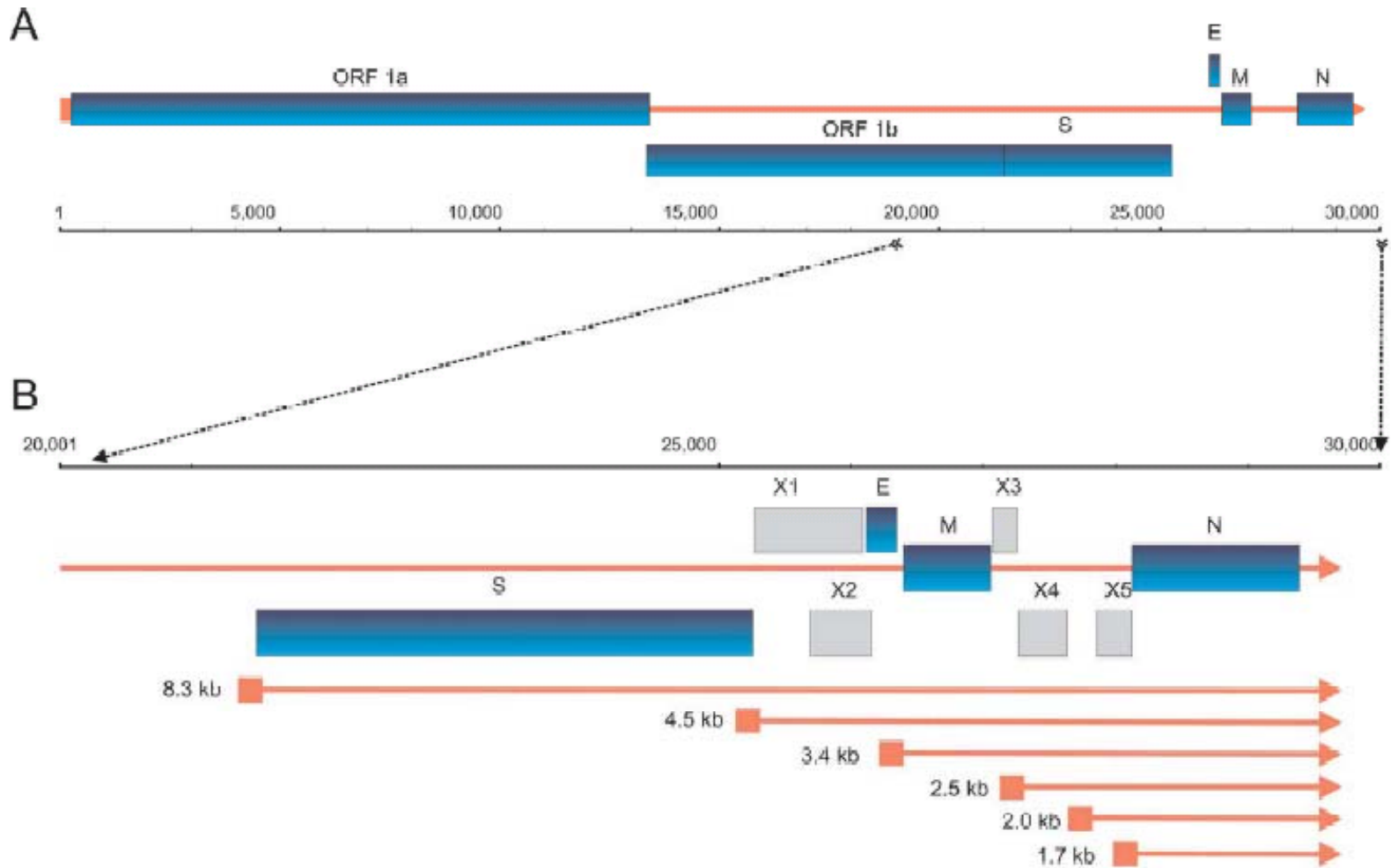
The relative fold increase between undifferentiated and differentiated (7th day of differentiation) P-19 cells was calculated using qRT-PCR data



LUX™ Primers & Pathogen Detection



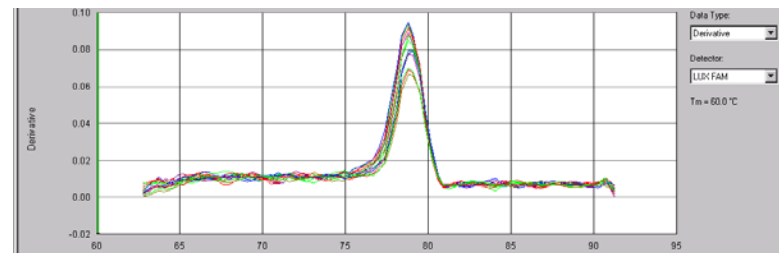
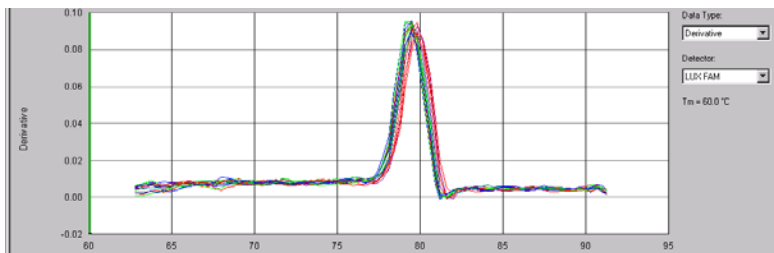
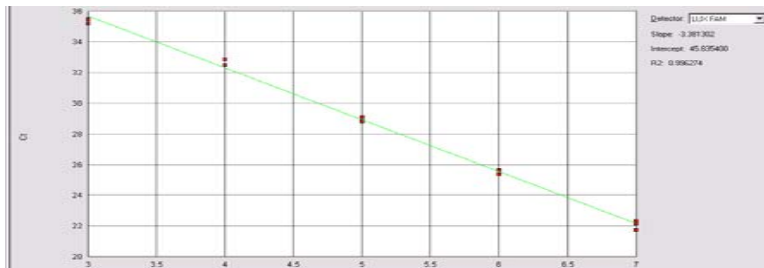
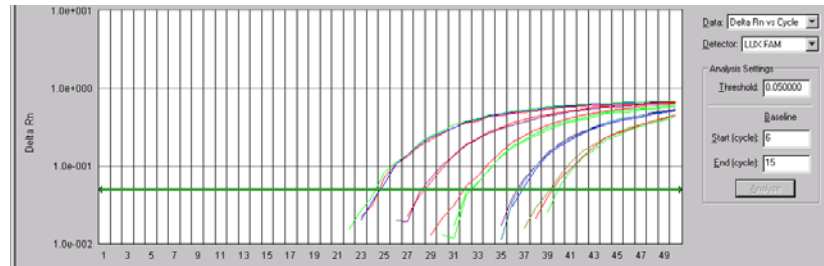
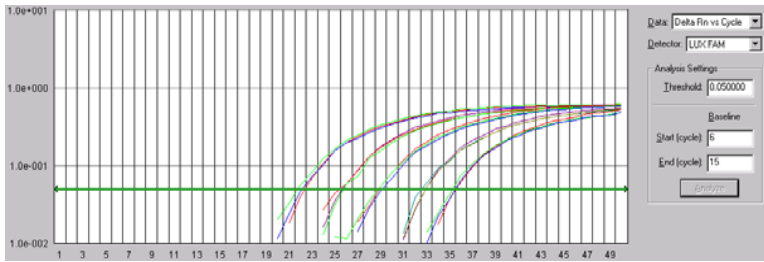
SARS representation



From Rota P.A. et al. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science express, page 1/10.1126

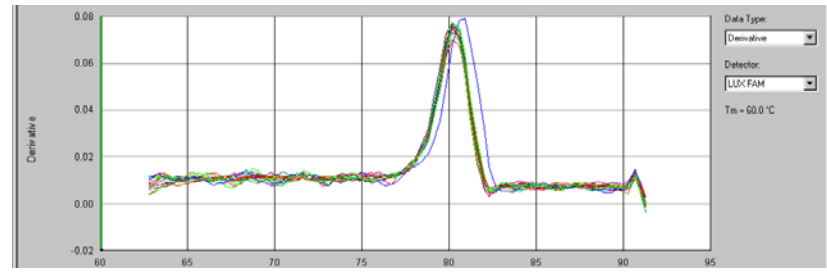
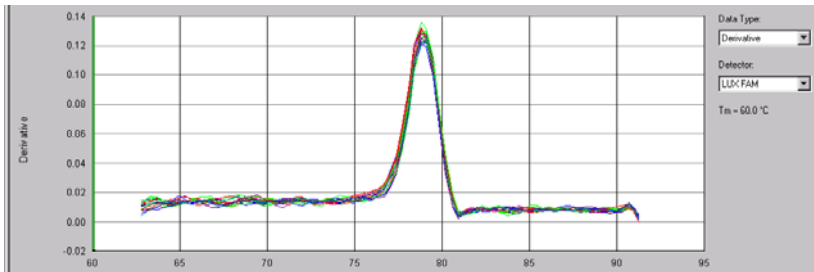
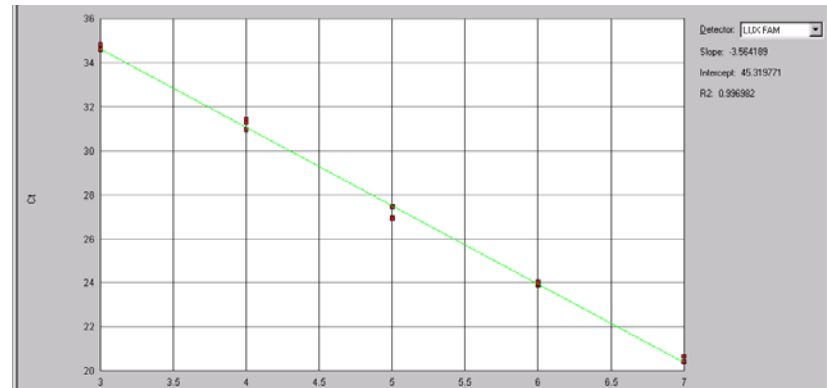
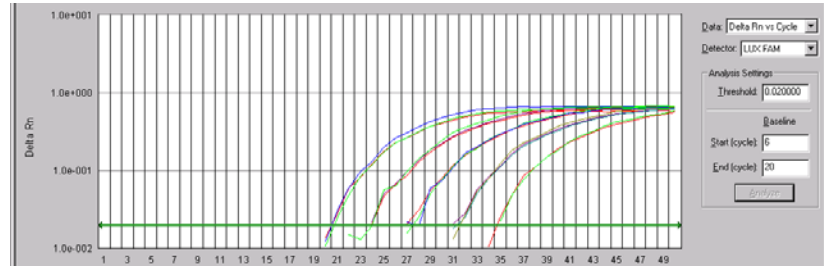
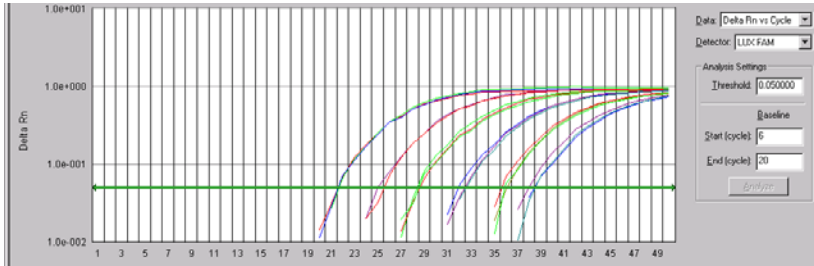
Reliable detection and quantification of SARS domains by real-time quantitative PCR

SARS Locus	RT-PCR Applications	
	Detection	Quantification
ORF 1b	+	
S Domain	+	+
M Domain	+	+
E Domain	+	+
N Domain	+	+
X1 Domain	+	+
X2 Domain	+	
X3 Domain	+	
X4 Domain	+	
X5 Domain	+	+



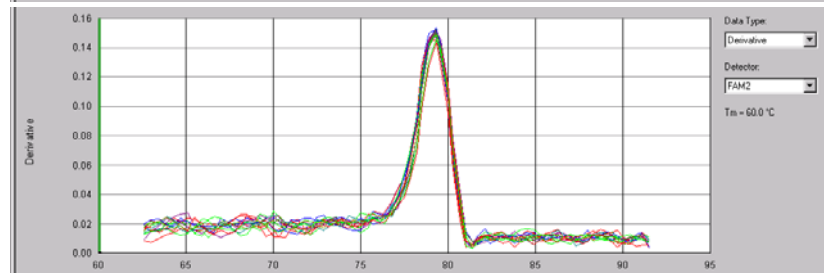
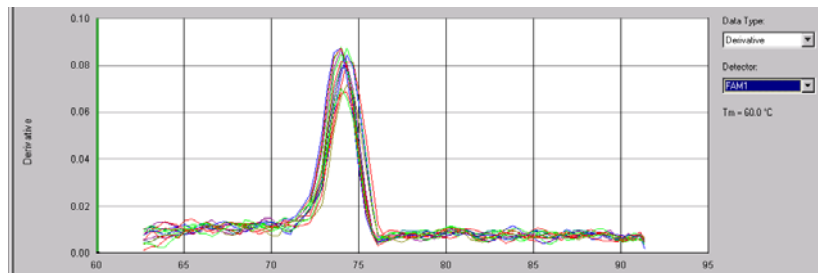
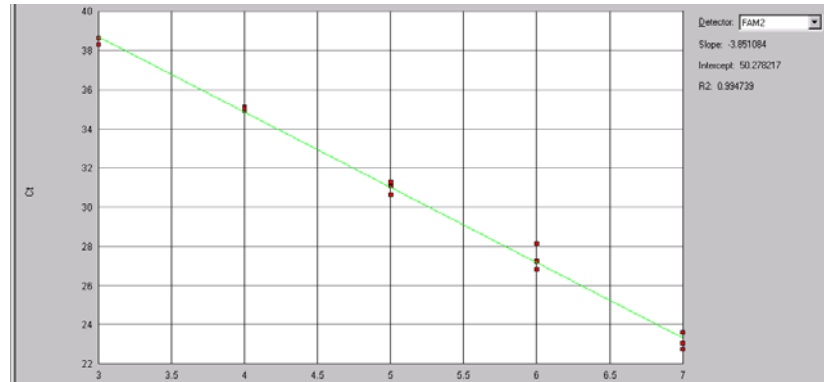
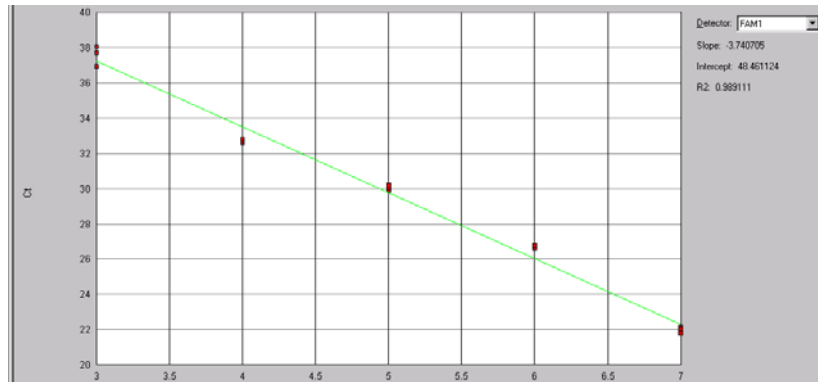
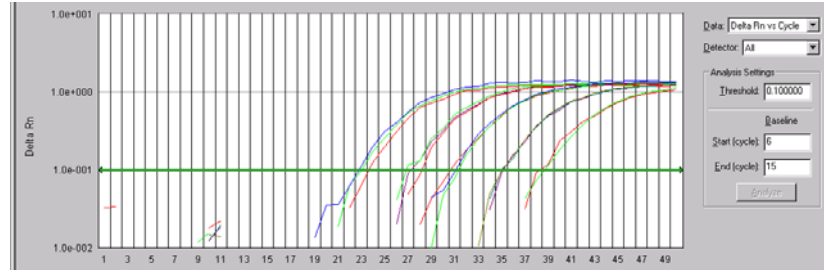
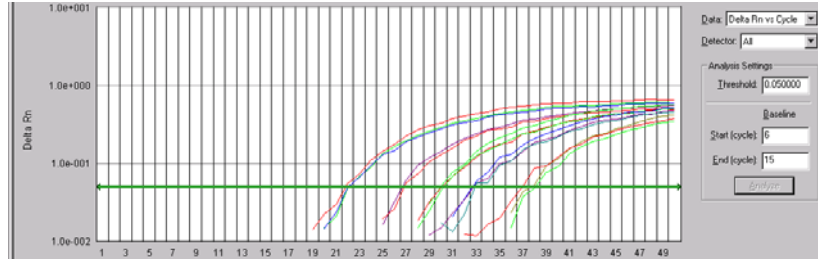
X1 151/222a
97%, R²: 0.996

X2 395/436
82%, R²: 0.995



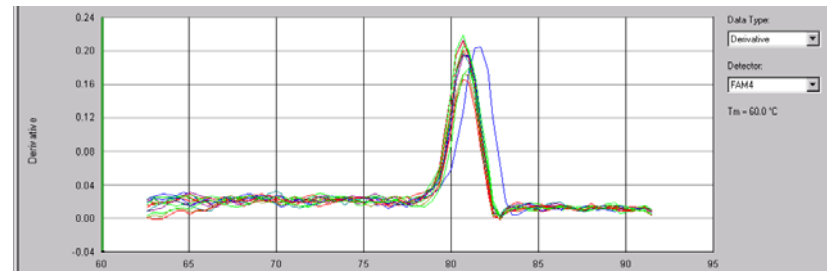
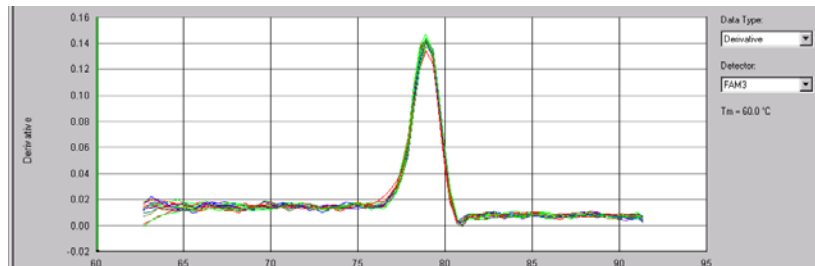
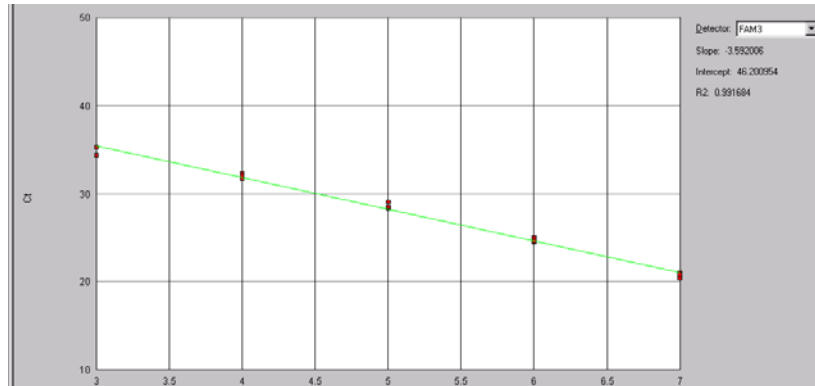
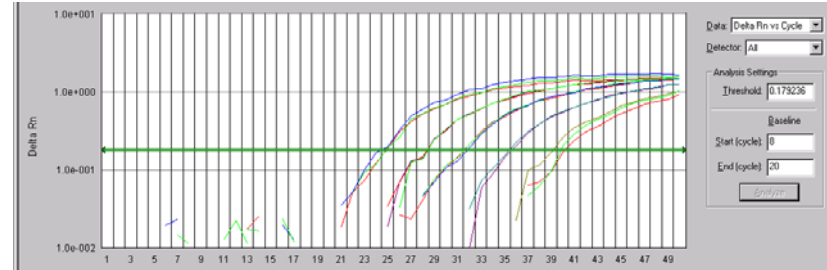
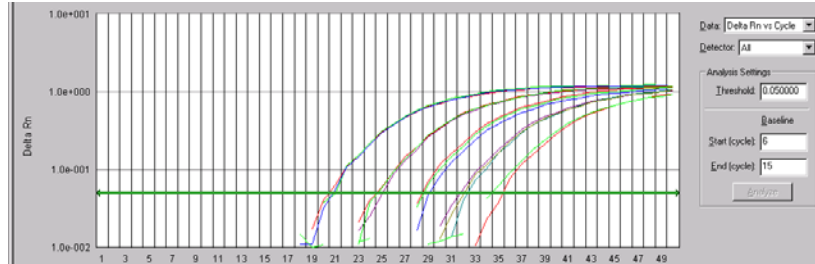
E 119/165
96%, R²: 0.996

M 314/353
91%, R²: 0.996



X3 34/71
85%, R²: 0.987

X4 151/193
82%, R²: 0.994



X5 70/141
90%, R²: 0.991

ORF1b 4654/4724
86%, R²: 0.996



Certified LUX™ Primer Sets for SARS Coronavirus domains

- **Functional validated and optimised to give accurate and reproducible results for detection and/or quantification analysis**
- **Sequence-specific, sensitive real-time detection without probes or quenchers**
- **Multiplex capability**
- **Cost-effective amplification in a ready-to-use format**
- **Melting curve analysis to confirm specific amplification**

LUX™ primers are compatible with :-

- **All real-time PCR instruments that provide detection of FAM and JOE labels**
 - **Real-time PCR**
 - **One step real-time RT-PCR**
 - **Multiplex real-time PCR**
- **Application examples:**
 - **Gene Expression Profiling**
 - **RNAi evaluation**
 - **Pathogen detection**
- **Available as LUX™ Designer, Certified LUX™ Primer Sets & LUX™ Custom Services**

- **General entry portal:** www.invitrogen.com/lux
- **Instrument protocols:**
http://www.invitrogen.com/content/world/instrument_protocols.pdf
- **LUX manual:**
http://www.invitrogen.com/Content/sfs/manuals/luxprimers_man.pdf
- **LUX Design software:**
<https://pf.invitrogen.com/primerf/pages/Default.cfm?cc=1>
- **Peer-reviewed publications**

Publications:

- Nazarenko, I., R. Pires, B. Lowe, M. Obaidy and A. Rashtchian. 2002. Effect of primary and secondary structure of oligodeoxyribonucleotides on the fluorescent properties of conjugated dyes. *Nucleic Acids Res.* 30:2089-2095.
- Nazarenko, I., B. Lowe, M. Darfler, P. Ikonomi, D. Schuster and A. Rashtchian. 2002. Multiplex quantitative PCR using self-quenched primers labeled with a single fluorophore. *Nucleic Acids Res.* 30(9):e37.
- Lowe, B., H. A. Avila, F. Bloom, M. Gleeson and W. Kusser. 2003. Quantitation of gene expression in neural precursors by RT-PCR using self-quenched, fluorogenic LUX primers. *Analytical Biochemistry* in press/published spring 2003
- Kusser, W., S. Javorschi and M. Gleeson. 2003. Quantification of gene expression by real-time PCR with LUX primers, in *PCR Primer*, 2nd editions. C.W. Diffenbach and G.S. Dveksler eds. Cold Spring Harbor Laboratory Press. In press.