



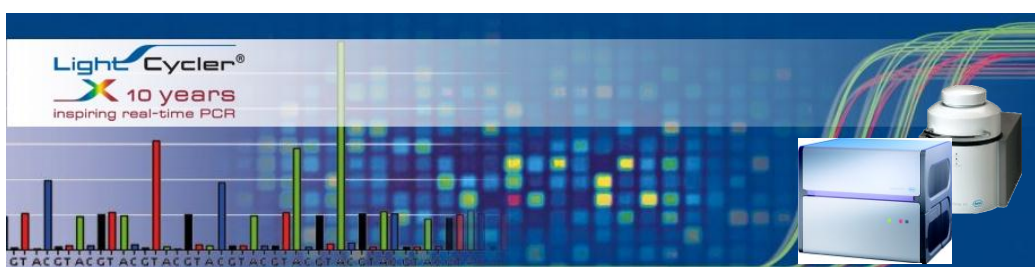
www.roche-applied-science.com

The LightCycler® 480 Real-Time PCR System

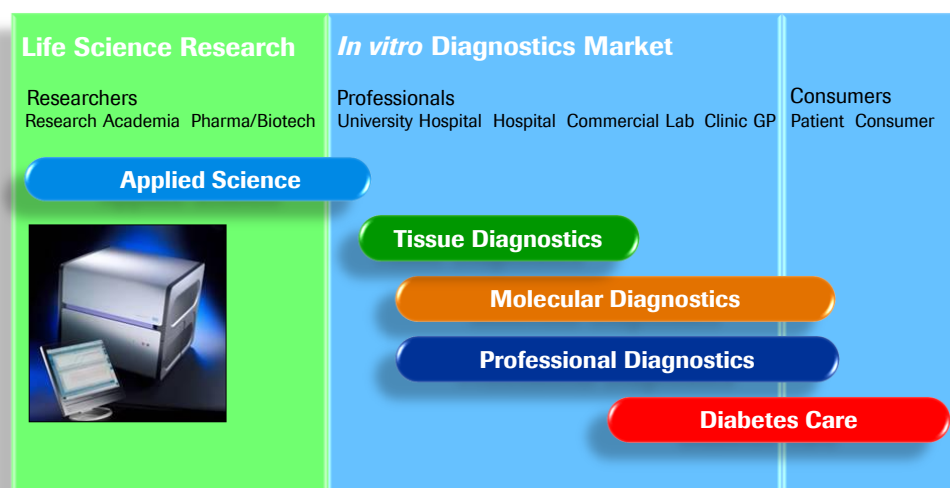
原理與應用介紹

Product Specialist 陳盈智

Angelina Chen



台灣羅氏醫療診斷設備股份有限公司



Penzberg *RAS Headquarters*



Roche Penzberg: One of the largest biotech centers in Europe with both Pharmaceuticals and Diagnostics Divisions

Established: 1972 (Boehringer Mannheim)

Employees: approx. 4,000

Area: 85 acres

Invested capital: approx. 870 million €



www.roche-applied-science.com



Integrated Solutions for Real-Time PCR *PCR Workflow System*

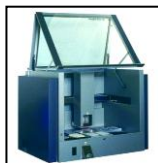


Instruments - Software - Reagents

MagNA Lyser



MagNA Pure LC



Real-time PCR



**Sample
preparation**

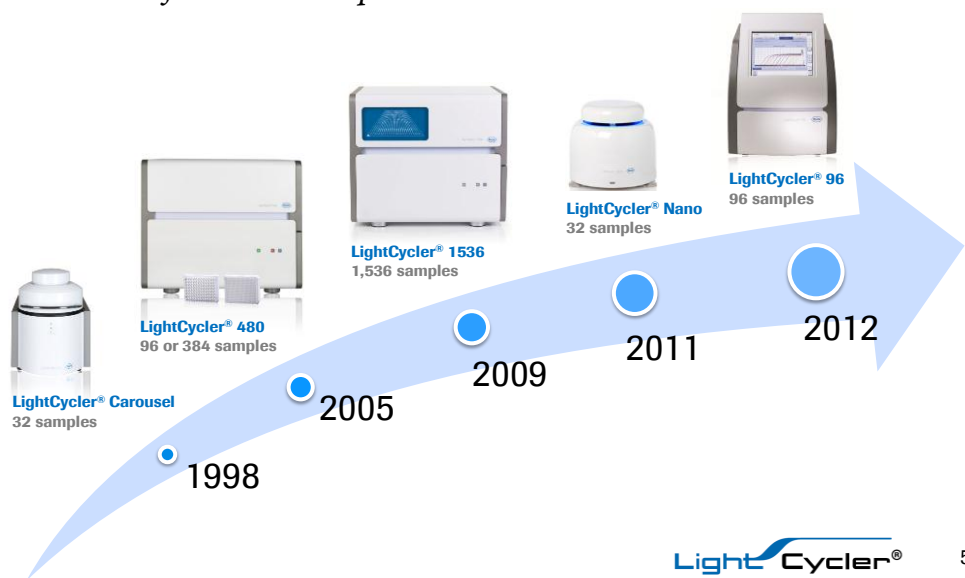
**Nucleic acid
isolation**

**Amplification &
Detection**

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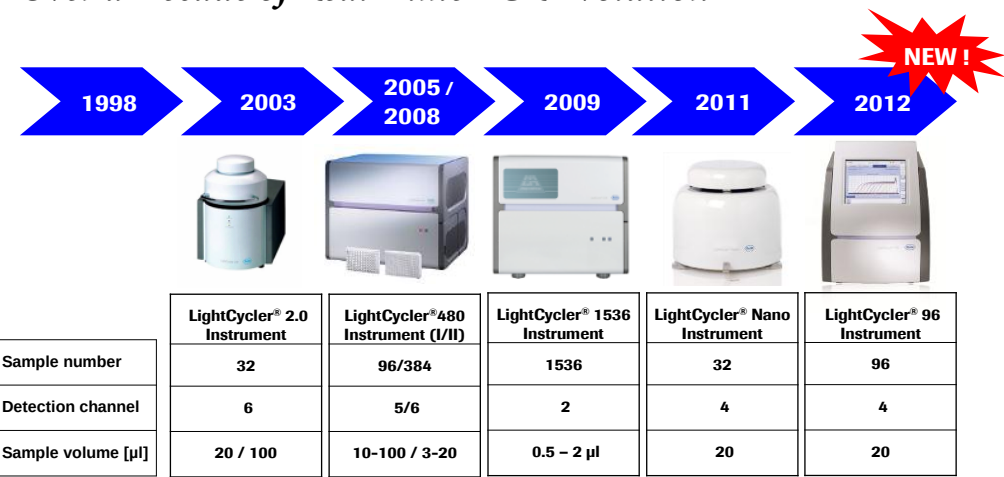
The Roche LightCycler® Story

14 Years of Continuous qPCR Innovation



LightCycler® Real-Time PCR Platform

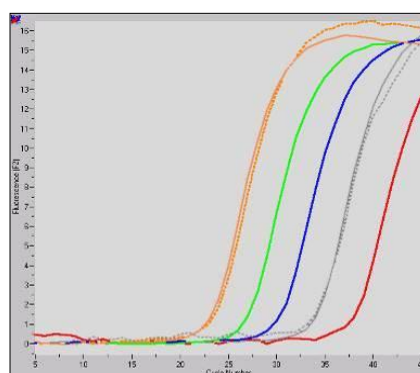
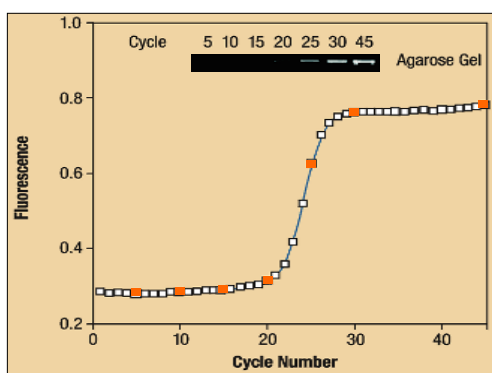
Over a Decade of Real-Time PCR Evolution



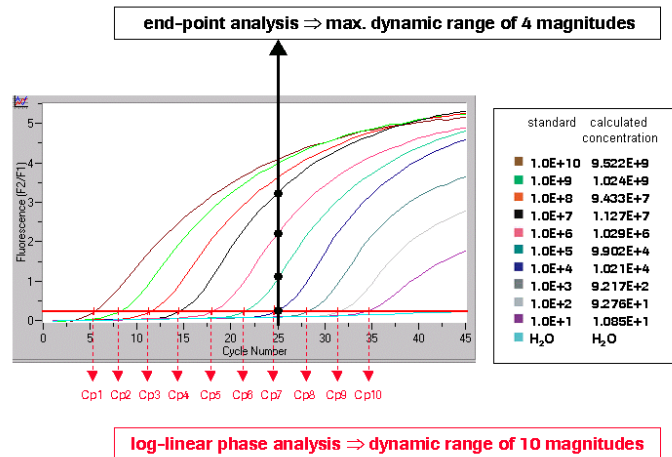
Basic Introduction

- *Detection Formats*
- *Gene Quantification Tools*
- *Instrument Features*
- *Kit Information*

Monitoring of PCR Reactions *From Block Cyclers to Real-Time PCR*



Log-linear Quantification *Dynamic Range*

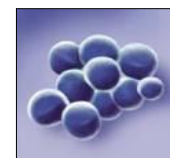


Template: Plasmid; Target: CycA; Detection Format: Hybridization Probes
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Application Fields for Real-Time PCR (1)



- **Gene detection**
 - Detection of specific DNA/RNA (e.g. oncology research)
 - Identification of a specific species (e.g. bacteria, virus)
 - Pathogen detection (e.g. legionella, anthrax)
 - Antibiotic resistance screening (e.g. MRSA, VRE)
 - Water quality monitoring
- **Gene expression**
 - Studies on minimal residual diseases (MRD)
 - Determination of mRNA expression levels (e.g. cytokines, chemokines)
 - Gene dosis quantification (e.g. chromosomal aberrations)



Staph. aureus

Application Fields for Real-Time PCR (2)



- **Genotyping**
 - Mutation in detection in cancer
 - Pharmacogenetics
 - Genetic fingerprint
 - Evolutional studies
- **Food safety analysis**
 - GMO detection
 - Pathogene in food (e.g. *Salmonella*, *E. coli*)
 - Screening for beer spoilage
- **Gene knockdown**
 - Silencing of genes with siRNA



Zea mays

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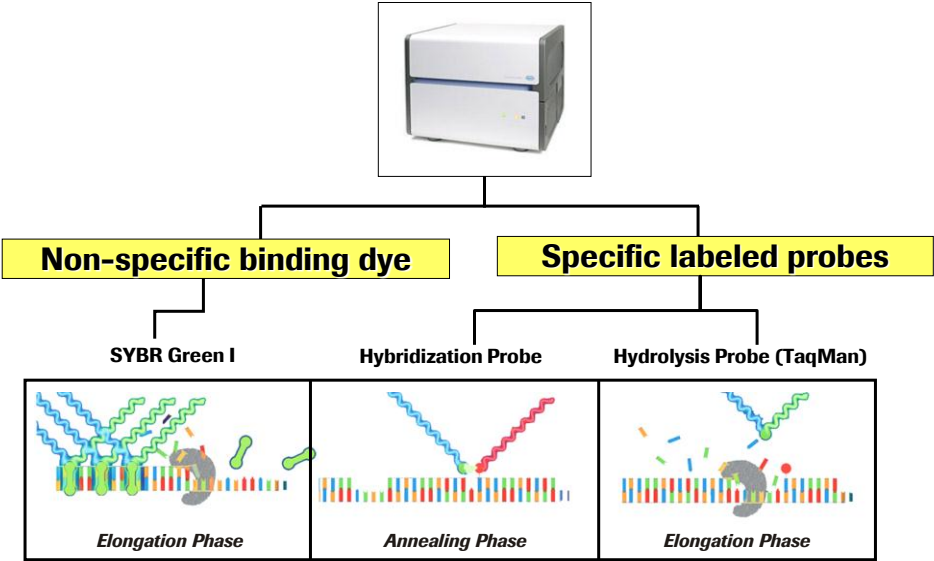


But

How can we monitor the PCR reaction?

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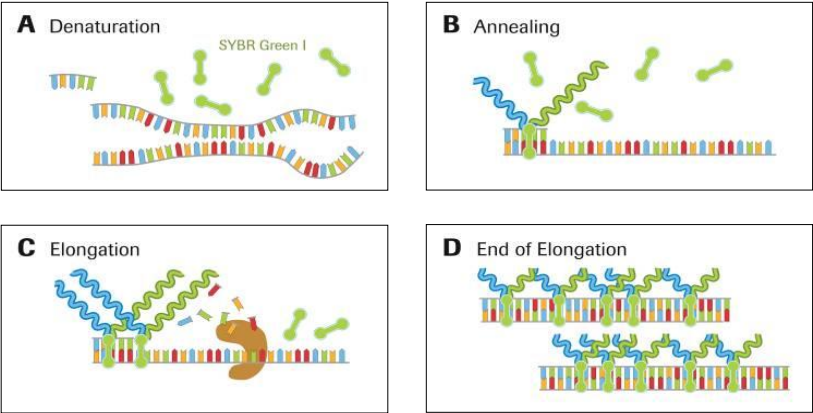
LightCycler® Detection Formats



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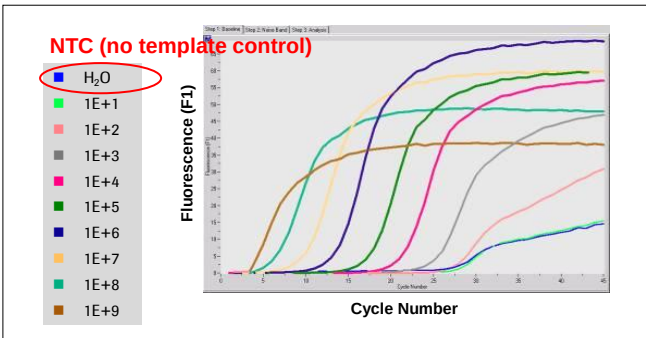
LightCycler® Assay Formats

SYBR Green I Format



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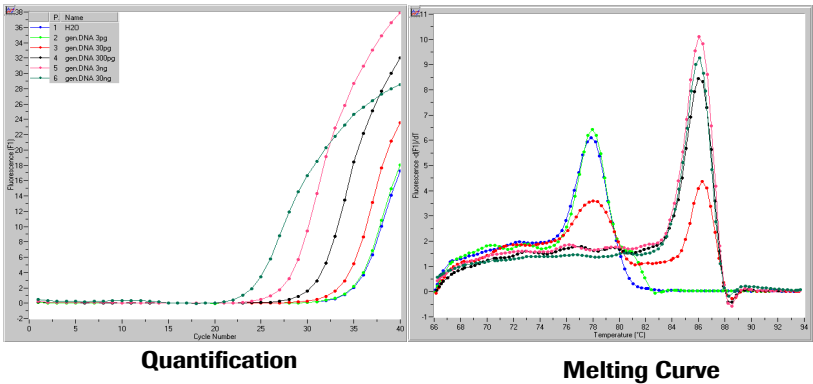
Monitoring of PCR with the LightCycler using SYBR Green I



Template: Plasmid; Target: TNF α ; Detection Format: SYBR Green I

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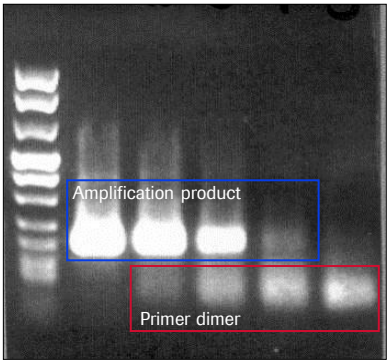
Monitoring of PCR with the LightCycler[®] Using SYBR Green I



Template: human genomic DNA; Target: β -Globin; Detection Format: SYBR Green I

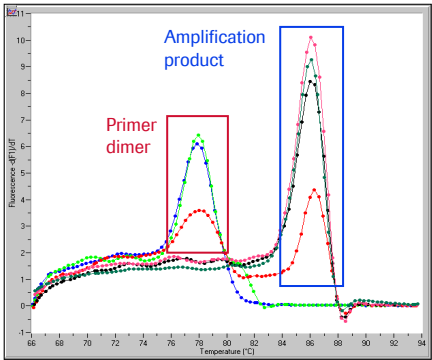
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Visualization of Primer Dimers



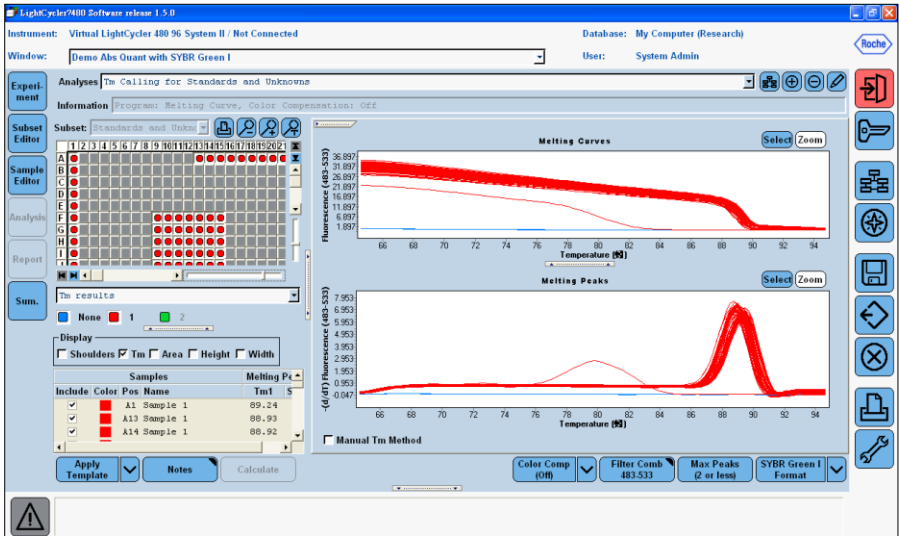
Block Cycler
gel electrophoresis

www.roche-applied-science.com



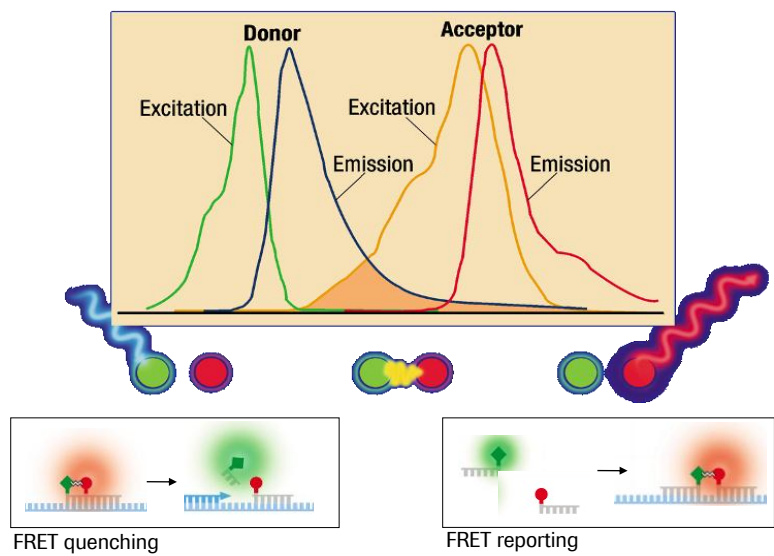
LightCycler[®]
melting curve analysis

Analysis Module Tm Calling

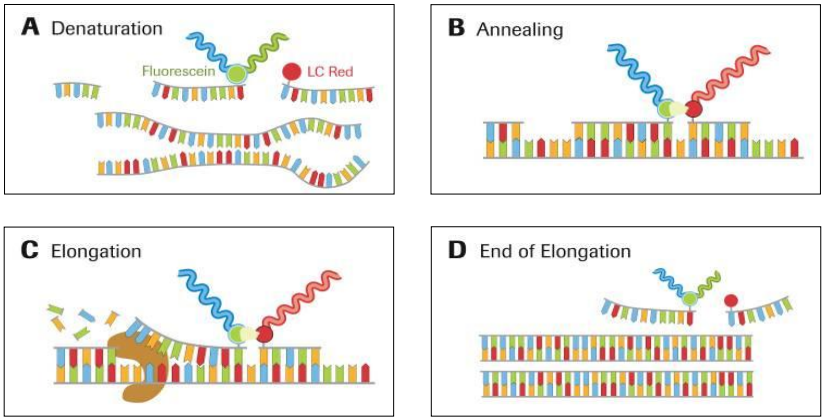


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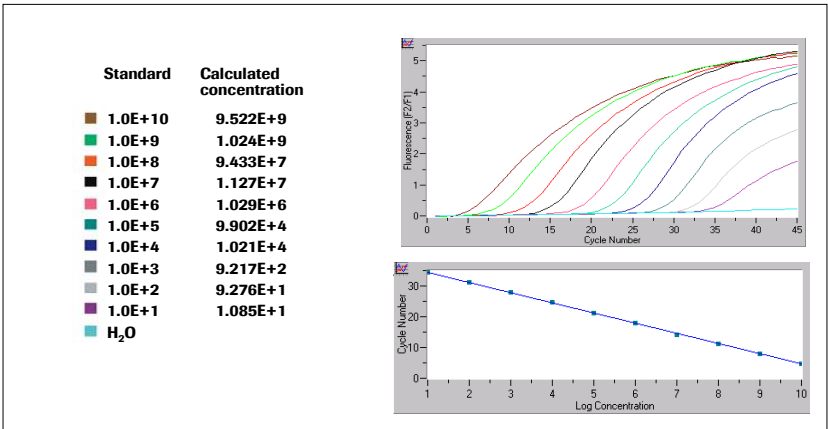
LightCycler® Assay Formats *Fluorescence Resonance Energy Transfer (FRET)*



LightCycler® Assay Formats *Hybridization Probes* HybProbe



Monitoring of PCR with the LightCycler® Using Hybridization Probes

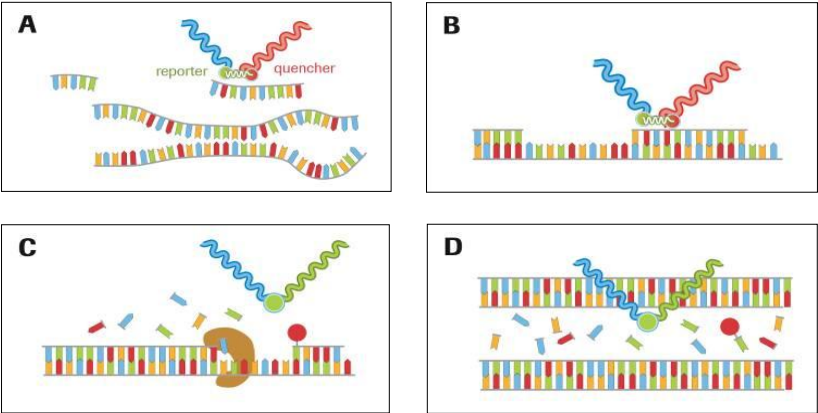


Template: Plasmid; Target: CycA; Detection Format: Hybridization Probes

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LightCycler® Assay Formats

Hydrolysis Probe-TaqMan Probe



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Universal ProbeLibrary

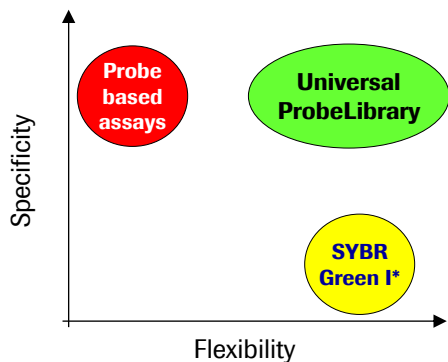
RealTime Ready Assays & Panels

23

Real-Time PCR
Specificity or Flexibility ?



Today you have to decide whether using a specific probe-based assay or a flexible SYBR Green I assay !



What about having both characteristics together in one assay ?

* and other intercalating dye based assays



What is a Universal ProbeLibrary?

- In total **165** specific and prevalidated hydrolysis probes
- Organism specific sets of 90 probes for: **Human, Mouse, Rat**
- UPL Extension Set of 75 probes (probes #91 – 165)
- Combination of UPL Extension Set and UPL Set, Human (probes #1-90) cover the complete UPL library
- Primers and probes for your specific intron spanning assay are designed in the web-based Assay Design Center at www.universalprobelibrary.com
- Assays for more organisms (Arabidopsis, Drosophila, *C. elegans*, Primates, Maize, Rice, Zebrafish, “Other Organism”) are also available in the Assay Design Center

www.roche-applied-science.com



Universal ProbeLibrary™

Only 165 probes for over 5 millions assays

Organism	Species Name	Assays Available	Coverage Rate
Human	Homo sapiens	> 639 500	99%
Mouse	Mus musculus	> 509 500	99%
Rat	Rattus norvegicus	> 364 000	98%
Primates	Pan troglodytes	> 519 500	96%
Drosophila	Drosophila melanogaster	> 253 500	99%
Arabidopsis	Arabidopsis thaliana	> 199 000	98%
C. Elegans	Caenorhaboditis elegans	> 134 000	95%
Maize	Zea mays	> 61 500	94%
Rice	Oryza sativa	> 898 500	98%
Zebrafish	Danio rerio	> 630 000	98%
Yeast	Saccharomces cerevisiae	> 42 000	95%
Total		> 5 000 000	

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90 Probes – One Set – One transcriptome

How can 90 probes cover one transcriptome?



- Some **8 & 9 mer** motifs are very prevalent in the transcriptome
- The same probes can be used for the same motifs in several genes
- Each probes can used to detect around 7000 different transcripts
- Each transcripts can be targeted by around 16 different probes



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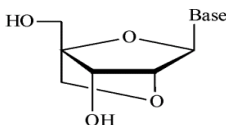
How does this work?



High melting temperature and specificity of the short real-time PCR detection probes is retained by using a proprietary nucleotide chemistry, called **Locked Nucleic Acids (LNA)**.

probe \ target	Perfect match 3'-acgaccac-5'	Single mismatch 3'-acggccac-5'	ΔT_m
DNA 8-mer 5'-tgctgggtg-3'	$T_m=35^\circ\text{C}$	$T_m=25^\circ\text{C}$	10°C
LNA 8-mer 5'-TGCTGGTG-3'	$T_m=71^\circ\text{C}$	$T_m=45^\circ\text{C}$	26°C

DNA monomers in lower case; LNA monomers in UPPER case



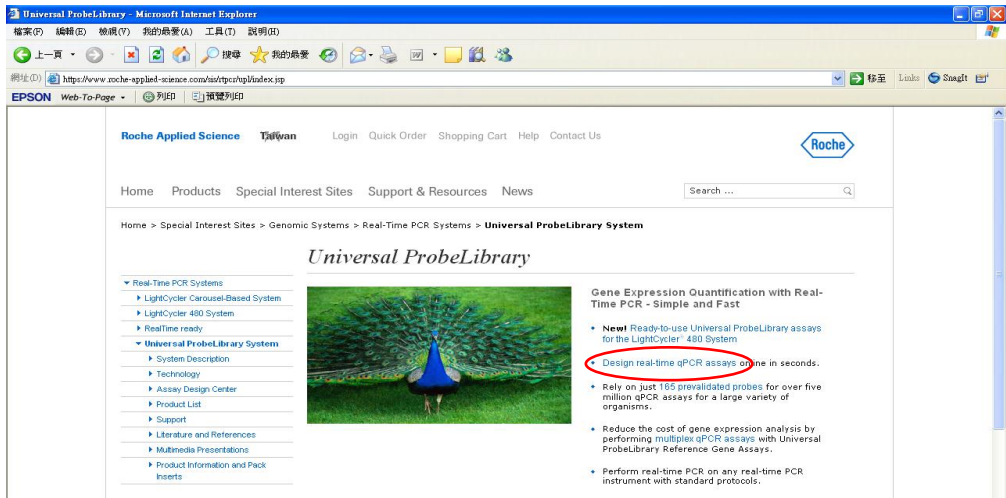
- Short LNA probes have T_m compatible with real-time PCR
- Short LNA probes show excellent single-mismatch discrimination

www.roche-applied-science.com

Assay design with ProbeFinder™



ProbeFinder™ free web tool at: <http://www.universalprobelibrary.com>



Assay design with ProbeFinder™



Step1: Choose an organism

Universal ProbeLibrary Assay Design Center

The ProbeFinder software allows you to perform a fast and easy design of real-time PCR assays for your targets of choice. It will select an optimal combination of a Universal ProbeLibrary probe and a gene-specific primer set.

To start the design process, select an organism

- enter the gene accession number, gene name, or
- paste a sequence in the appropriate field

Assays for Human, Mouse or Rat targets can now be combined with a reference gene assay.

Need more information?

① Design [batch assays](#) for up to 10 independent assays at a time

① Find [common assays](#) for all members of a gene family or

① Discriminate them as well as [splice variants](#) of a gene

The [ProbeFinder Quick reference](#) gives you a short guidance on how to

For a detailed description of all features of ProbeFinder, you can download

Select organism

Select organism

Homo sapiens (Human)

Mus musculus (Mouse)

Rattus norvegicus (Rat)

Anopheles gambiae (Mosquito)

Caenorhabditis elegans (C. elegans)

Danio rerio (Zebrafish)

Drosophila melanogaster (Fruitfly)

Pan troglodytes (Primates)

Arabidopsis thaliana

Oryza sativa (Rice)

Zea mays (Maize)

Saccharomyces cerevisiae (Baker's yeast)

Other organism (paste sequence only)

Assay design with ProbeFinder™

Step2: Enter your sequence/ gene of interest



Universal ProbeLibrary for Human

Roche Applied Science

Specify your target(s):

Advanced primer3 settings

By sequence ID, gene name or keyword

e.g. ENST00000331789, NM_001101 or X00351 or beta-actin

Option1: Enter your sequence ID

or

By sequence

e.g.
>part of X00351 Human mRNA for beta-actin
CACGGCATCTGTCACCACTGGGAGCAGATGGAGAAATCTGGACACCACTTCTACAAT
GAGCTGCTGTGGCTCCCGAGGAGCACCCTGTGCTGCTGAGACCCCTGACCCCTGACCCCT
AAGGCCAACCCGAGAGATGACCCAGATCATGTTTGGACCTTCAACACCCCAAGCCATG
TACGTTGCTATCCAGGCTGTGCTATCCCTGTACGCTCTGACCTTCAACACCTGACATGCTG
ATGGACTCCGCTGACGGGTCACCCACACTGTGCCACTACGAGGGGTATGCCCCCTCC

Option2: Paste sequences

☒ Automatically select an intron spanning assay. ☐ Design multiplex PCR with reference gene.

intron spanning assay

Design

www.roche-applied-science.com

Assay design with ProbeFinder™

Step3: Output information of optimal assay



Universal ProbeLibrary for Human

Roche Applied Science

ProbeFinder has designed the optimal real-time PCR assay for:

[NM_002751.5](#) Homo sapiens mitogen-activated protein kinase 11 (MAPK11), mRNA.

Assay details:

The optimal probe No.

Use Universal ProbeLibrary probe: #37 cat.no. 04687957001

Primer	Length	Position	Tm	%GC	Sequence
Left Primer	21	637 - 657	59	43	catgtcgaactggatgcatta
Right Primer	20	740 - 759	60	50	cgcttcagctggtaaatgta

Primer information (sequence, Tm, %GC)

Amplicon (123 nt)

catgtcgaactggatgcattacaaccaaacagtggatatctggtccgtggcctgcatcat
ggctgagctgctccaggaggaagccctcttccgggaagcgactacattgaccagctgaa
gctg

Amplicon information

Download pack insert.

PDF report

Text report

Order probes or set

www.roche-applied-science.com

Assay design with ProbeFinder™

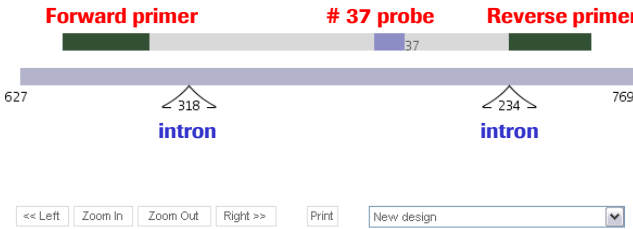
Step3: Output information of optimal assay



Transcript overview:



Detailed view:

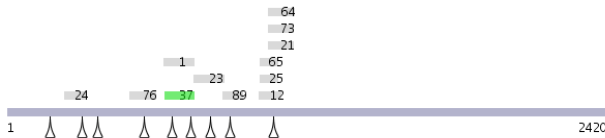


[Figure legend ?](#)

[More assays](#) or [see multiplex experiments](#)

Assay design with ProbeFinder™

Step3: Output information of optimal assay



Probe	Rank	Position	Score (mouse over for details)	
#37	1	709		All criteria met
#23	2	782		All criteria met
#1	3	706		All criteria met
#12	4	1068		All criteria met
#76	5	525		All criteria met
#25	6	1059		All criteria met
#65	7	1058		All criteria met
#89	8	918		All criteria met
#24	9	267		All criteria met
#21	10	1110		All criteria met
#73	11	1092		All criteria met
#64	12	1132		All criteria met

[Return to best assay](#) or [see all assays](#) or [see multiplex experiments](#)

UPL – Dual Color Assays



- **Reference Gene Assay** for UPL with LNA-modified hydrolysis probes and primer mix.
- Second dye **(LightCycler Yellow 555)** detectable in the VIC/HEX channel (560/568 nm).
- Reference Gene Assays are optimized against selected genes.
- **14 Reference Gene Assays are available:**
 - 10 human (PBGD, ACTB, HPRT, GUSB, PGK1, B2M, GAPD, G6PD, PPIA, TBP)
 - 2 mouse (m-ACTB, m-GAPD)
 - 2 rat (r-ACTB, r-GAPD)
- 200 x 50 µl (or 500 x 20 µl) rxns.
- ProbeFinder SW designs UPL assays and selects Reference Gene Assays with in-silico PCR test.

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Design of UPL Dual Color Assays Input Screen



By sequence ID, gene name or keyword
e.g. ENST00000331789, NM_001101 or X00351 or beta-actin

or

By sequence
e.g.
>part of X00351 Human mRNA for beta-actin
CACGGCATGTCACCACTGGAGCGACATGGAGAAATCTGGACCCACACCTTCTACAAT
GAGCTGCTGTGGCTCCCGAGGAGCACCCTGTGCTGACCGAGGCCCTTGAACCCC
AAGGCCAACCGGAGAGATGACCCAGATCATGTTTGAACCTTCAACCCCAAGCCATG
TACGTTGCTATCCAGGCTGTGCTAATCTGTACGCTCTG9CCGTACCACTGGCATGCTG
ATGGACTCCGGTGACGGGTCACCTCATCTACGAGGGGTATGCCCTCCCC

Any
ACTB
B2M
G6PD
GUSB
GUSB
HPRT
PBGD
PGK1
PPIA
TBP

☒ Automatically select an assay

☒ Design multiplex PCR with reference gene.

Preferred Reference Gene:

ACTB

multiplex PCR

Design

www.roche-applied-science.com

Design of UPL Dual Color Assays

Result Screen



Use Universal ProbeLibrary probe: #23, cat.no. 04686977001

Primer	Length	Position	Tm	%GC	Sequence
Left Primer	19	754 - 772	60	53	gaagcgcatcatggaagtg
Right Primer	20	858 - 877	59	55	gattgctgctcagggtccttct
Amplicon (124 nt)					
gaagcgcatcatggaagtgtgggcacacccagccctgagggtctggcaaaaatctcctc agaaacagccccgacataataccagtcctgcccccaagcccaagaggacctgagcag catc					
Download pack insert PDF report Text report Order probes or set					

Multiplex details:

Run in multiplex PCR with
HPRT Φ

www.roche-applied-science.com

50ml

TCOM

0

5

2天 (基因表現偵測速成班)

第一天

選擇有興趣的基因

利用 www.universalprobelibrary.com 免費設計 primer & probe

將免費得到的 primer 序列，向您所喜好的合成公司訂購

第二天

拿到 primer，並且從冰箱挑出適當的 probe

使用無需找條件的單一Tm值 (60°C)來完成 real-time PCR

分析 data，得到準確 gene expression 的結果



Universal ProbeLibrary (UPL) *Products*

- **Universal ProbeLibrary Set** (human, mouse, rat):
90 probe vials with 250 x 50 µl reactions (or 625 x 20 µl reactions)
- **Universal ProbeLibrary Extension Set** (Probe # 91-165)
- **Universal ProbeLibrary Reference Gene Assays**
Pre-validated assays for quantification of gene expression levels by real-time PCR
For 500 x 20 µl-reactions (or 200 x 50 µl-reactions)
- **Universal ProbeLibrary Probes**
Individual probes 1-165
500 x 50 µl reactions (or 1250 x 20 µl reactions)
- **ProbeFinder Software**
Available on RAS web site with link to e-shop

www.roche-applied-science.com

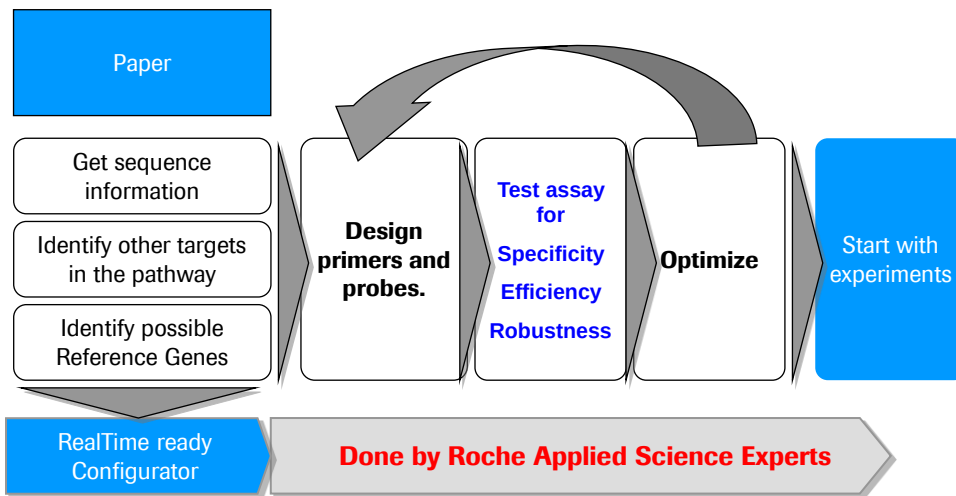


Gene Quantification Easy Tools

Universal ProbeLibrary

RealTime Ready Assays & Panels

Gene Expression Analysis in the Past ... and the Solution with RealTime ready



www.roche-applied-science.com

RealTime ready Assays *Your benefits !*



- **High performance assays** that comply with the most stringent criteria
- **Full transparency** (compliant to MIQE Guidelines)
 - Delivering assay details and Primer Sequence
- **Easy assay selection and panel configuration** with free online-tool
 - High flexibility in search functions and layouts
 - Easy ordering and online order tracking
 - Convenient download of panel content, also for use in the LightCycler® 480 Software

www.roche-applied-science.com



RealTime ready Assays

High specificity and performance guaranteed !

- **In silico Testing → high specificity**
 - Ensures detection of most transcript variances and splice variants.
 - No DNA amplification (Intron-spanning assays)
 - No cross-hybridization to other related sequences.
- **In Vitro Testing → high performance & specificity**
 - Using an universal cDNA as template, the best assay is determined by
 - qPCR analysis on the LC 480 of
 - 5 tenfold dilutions of cDNA
 - dynamic range of at least 3 logs
 - PCR efficiency of 2.0
 - linearity of the standard curve over all 5 dilutions.
 - Detection of amplification product on an agarose gel

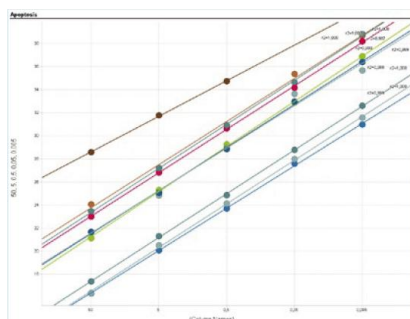
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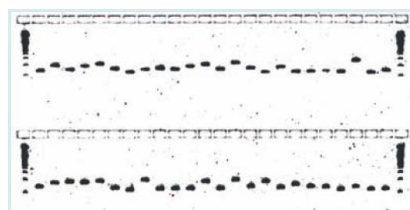
RealTime ready Assays

High specificity and performance guaranteed

Efficiency & Linearity



Specificity



No side-product in gel analysis.

www.roche-applied-science.com

RealTime ready
Products available now

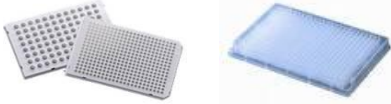


Human Focus Panels



LightCycler® 480 96 and 384 Plates

Human Custom Panels & Assays



LightCycler® 480 96, 384, and 1536 Plates

Custom Panels & Assays for mouse/rat



LightCycler® 480 96, 384, and 1536 Plates

Catalog/Designer Assay



Single Assays

RealTime ready
Focus Panels available now



Focus Panel	Content
Human Cell Cycle Regulation Panel, 96	84 targets, 5 controls, 7 reference genes
Human ABC Transporter Panel, 96	42 targets in duplicates, 5 controls, 7 reference genes
Human Nuclear Receptor Panel, 96	84 targets, 5 controls, 7 reference genes
Human GPCR Panel, 96	84 targets, 5 controls, 7 reference genes
Human Apoptosis Panel, 96	84 targets, 5 controls, 7 reference genes
Human Reference Gene Panel, 96	19 reference genes, 5 controls in quadruplicates

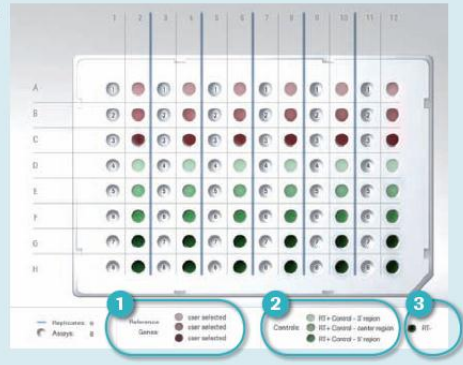
* 384-well panels are also available now!

Control Concept Supporting the MIQE Standards



Error Detection Feature

- 1 **Reference Genes** are mandatory controls on each custom panel and need to be selected during the custom panel configuration process.
- 2 **RT positive control** checks for degradation of initial RNA and the quality of the RT step. It consists of three assays, each targeting different positions of the same transcript: 3'-end, center region, and 5'-end.
- 3 **RT minus control** detects residual genomic DNA. It consists of two identical assays in two wells: the first well is for the cDNA sample and the second well is for the RNA sample.

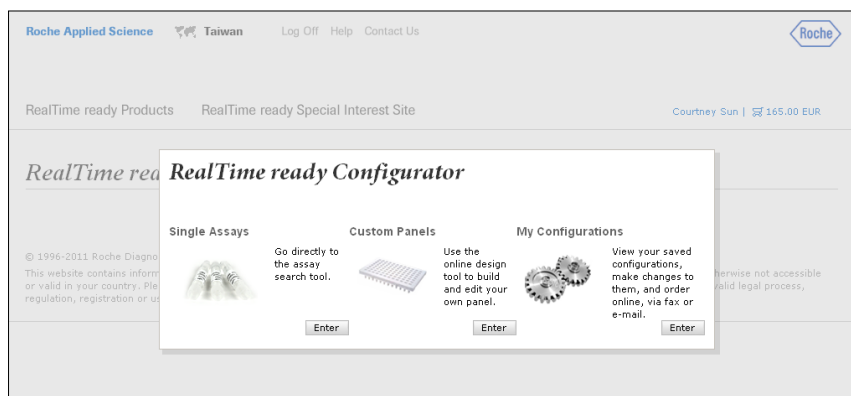


www.roche-applied-science.com

RealTime ready Configurator Product Selection



www.configurator.realtimeready.roche.com



www.roche-applied-science.com



Format Selection

Choose a plate format and name your plate

RealTime ready Custom Panels

1. Select a Format2. Configuration3. Choose Assays4. Choose Reference Genes5. Review6. OrderMy OrdersMy Configurations

You have not entered a name. Please fill out this field to continue.

Choose a plate type and describe your array

How to ...

...

How to configure your plate

Need Help?

...

For technical questions

Phone 123-45-6789

FAX 123-45-6789

To order or for price information

Phone 123-45-6789

FAX 123-45-6789

Other contact options

Name:

Description:

LightCycler960 / 96 Plate

Plate Type	Format	Targets	Residues	Control Concept	Pack Size	
☐ Lorem ipsum sic	96	5	5	yes	6	View Plate
☐ Lorem ipsum dolor	96	5	5	yes	4	View Plate
☐ Lorem ipsum	96	5	5	yes	2	View Plate

LightCycler960 / 384 Plate

LightCycler960 / 1536 Plate

Configure

RealTime ready Configurator

Options how to configure your plate

RealTime ready Custom Panels

1. Select a Format2. Configuration3. Choose Assays4. Choose Reference Genes5. Review6. OrderMy ConfigurationsMy Orders

Choose Assays

Search by Keyword

Focus Panel Overview

Batch Upload

Search by Pathway

Search by gene symbol, gene name, public accession number, biological process, or molecular function. Speed up your search even more by applying search filters for assay categories.

[Search by Keyword](#)

Your Array

Name:

Petra's Panel

Format:

96

Assigned Wells:

0

Empty Wells:

96

Assays By Design:

0

Unique Assays:

0

Reference Genes:

0

Edit Your Configuration

Use this list to see what assays and reference genes you currently have on your plate, and to delete any unwanted assays.

Edit Plate

View Your Plate

At any time during the customization process, click here to see a labeled graphic (in a new window) of your plate's current contents.

View Plate

Choose Reference Genes

Search for reference genes and other controls and add or remove them according to your specific control needs.

Choose Reference Genes

Review and Finalize

When you're done adding and removing assays and reference genes, go here to view a summary of your configuration and save your custom configuration.

Review

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Assay Search



- By keyword
- By focus panel
- By pathway
- Batch upload

RealTime ready Custom Panels

Search Keyword Search by Focus Panel Batch Upload Search by Pathway

Search for gene symbol, gene name, public accession number, biological process, or molecular function. Add up to 5 search terms, separated by "AND" or "OR" (e.g., cdk1 OR CDK5 AND cell cycle).

Search for: ALL

☐ Filtered search disabled

☐ Search all Assays

☐ Cell search:

Search Assays

Search by Keyword Search by Focus Panel Batch Upload Search by Pathway

Choose your assays from pre-generated panels of specific pathways, gene/protein families or functional group by the first letter of the name.

10 | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y

Panel (Description)	Number of Assays in Panel
Cell Cycle - Checkpoints	84
Cell Cycle - Cyclin E Lifecycle	47
Cell Cycle - G1/S Transition	74
Cell Cycle - M Phase	49
Cell Cycle - Proteins Degradation	58
Cell Cycle - S Phase	73
Cell Cycle - Selection	84
Cell Cycle - Mitosis	106
Chromatin Modification	163

[Show Assays](#)

Search by Pathway

The "Search by Pathway" function allows you to select your targets from a variety of interactive maps of cellular or signaling pathways. These maps immediately reveal possible interactions of your selected targets with other genes. Simply click on the genes of interest to add them to your assay search.

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Search Results



In the search results, all genes matching your search term are listed with the gene symbol, aliases, and description, together with the relevant part of your query. Clicking on "Details" will show a detailed description of the assay and the respective target transcript

Your Keyword Search for "cullin 1" in "All"

1-7 of 7 Results

Results per page

Assay ID	Gene Symbol	Alias	Description	Transcript	Search Term	
101454	CUL1	MGC149834, MGC149835	cullin 1	ENST00000325222	cullin 1	Details Add to Li
100309	CUL1	MGC149834, MGC149835	cullin 1	ENST00000325222	cullin 1	Details Add to Li
131024	DCUN1D1	DCUN1L1, RP42, SCCRO, SCRO, Tes3	DCN1, defective in cullin neddylation 1, domain containing 1 (S. cerevisiae)	ENST00000292782	cullin 1	Details Add to Li
tb4	DCUN1D2	C13orf17, FLJ10704, FLJ20092	DCN1, defective in cullin neddylation 1, domain containing 2 (S. cerevisiae)		cullin 1	Add to Li
44M2	DKFZ668600290		DCN1, defective in cullin neddylation 1, domain containing 2 (S. cerevisiae)		cullin 1	Add to Li

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RealTime ready Assays

Provide Full Transparency



RealTime ready Assay Details

Assay Details

Assay ID
100309

Gene Symbol
CUL1

Alias
MGC149834, MGC149835

Accession ID
ENST00000325222

1

1097

1175

3208

Close

Print

Amplicon Length: 79 bps
Intron Spanning / Position: Yes / 1140
Transcript Length: 3208 bps
Description: cullin 1
NCIC (curated): CUL1

Gene / Protein Information

Transcript Information

Array Platform IDs

Categories

GO

Gene / Protein Information

Transcript Information

Array Platform IDs

Categories

GO

Ensembl Transcript ID

Refseq DNA ID

Accession No.
ENST00000409469
ENST00000433865

Assay Location
1273
807

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RealTime ready Configurator

Search Results

Search Term: dummySearch

Add to Plate

View Plate

Edit Plate

New Search

Results per Page 10

Assay ID	Gene Symbol	Alias	Description	Transcript	RefSeq	
AS1	B667	A2B667, AB667	description1	ENST00001	Waiting for Implementation	Details Add to Plate
AS2	S114	A2S114, AS114	description2	ENST00002	Waiting for Implementation	Details Add to Plate
AS3	S114	A2S114, AS114	description3	ENST00003	Waiting for Implementation	Details Add to Plate
AS4	V3232	A2V3232, AV3232	description4	ENST00004	Waiting for Implementation	Details Add to Plate
AS5	A232	AA232, A2A232	description1	ENST00001	Waiting for Implementation	Details Add to Plate
AS6	B667	A2B667, AB667	description2	ENST00002	Waiting for Implementation	Details Add to Plate
AS7	S114	A2S114, AS114	description3	ENST00003	Waiting for Implementation	Details Add to Plate
AS8	V3232	A2V3232, AV3232	description4	ENST00004	Waiting for Implementation	Details Add to Plate

Configuration Review Page

RealTime ready Custom Panels



1. Select a Format	2. Configuration	3. Choose Assays	4. Choose Reference Genes	5. Review	6. Order	My Configurations	My Orders
--------------------	------------------	------------------	---------------------------	-----------	----------	-------------------	-----------

Configuration Review

After we process your order, you will be able to download a text file containing your plate configuration; a file download link will appear on your "My Orders" page when the file is available for download.

Format:	96	Plate Name:	Petra's Panel
Assays:	96	Description:	
Empty:	0	Selected Reference Genes:	S114, V3232, S114
Replicates:	3	Price as configured:	T000
Catalog Assays:	32		
Designer Assays:	0		
Unique Assays:	12		
Control Wells:	yes		
Reference Genes:	3 / 8		
Pack Size:	13 plates		

[View Plate](#) [Edit Plate](#) [Finalize](#) [Add to Shopping Cart](#)

Within this step in the Workflow the unique Configuration Number is assigned to the customer's Configuration

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RealTime ready Shopping List

Choose how to order your configurations



Real Time Ready Shopping Cart

1. Select a Format 2. Configuration 3. Choose Assays 4. Choose Reference Genes 5. Review 6. Order My Orders My Configurations

Shopping Cart

1. LightCycler® Assays
(Estimated Ship Time: 2 weeks)

2. LightCycler® Assays
(Estimated Ship Time: 2 weeks)

3. LightCycler® Assays
(Estimated Ship Time: 2 weeks)

Add a new Real Time Ready Product

Single Assay

1. LightCycler® Assays
(Estimated Ship Time: 2 weeks)

Custom Assay

1. LightCycler® Assays
(Estimated Ship Time: 2 weeks)

[Update Quantities](#) [Print](#) [E-Mail](#) [Continue](#)

Products	Config. No.	Pack Number	Qty	List Price	Extended Price	
1. LightCycler® Assays (Estimated Ship Time: 2 weeks)	0540383	0275000		\$9999.99	\$9999.99	Configuration Details Delete
2. LightCycler® Assays (Estimated Ship Time: 2 weeks)	9000705	0227500		\$9999.99	\$9999.99	Configuration Details Delete
3. LightCycler® Assays (Estimated Ship Time: 2 weeks)	9000705	9875207		\$9999.99	\$9999.99	Configuration Details Delete

▼ Custom Arrays

Products	Config. No.	Pack Number	Qty	List Price	Extended Price	
1. LightCycler® Low Density Array, Format 14 Your Card Number: Just a test (Estimated Ship Time: 2 weeks)	9875207	0327500		\$9999.99	\$9999.99	View Plate Delete
2. LightCycler® Low Density Array, Format 14 Your Card Number: Just a test	9000705	0327500		\$9999.99	\$9999.99	View Plate Delete

RealTime ready Shopping Cart Ordering Options



Update PricePrintE-MailE-Shop

Custom Panels

Products	Configuration No.	Catalog No.	Quantity * Pack Size	Price	Subtotal	
rfa RealTime ready Custom Panel 96 - 24+ EST: 18 days	000010250	05582610001	1 * 6 Plates	1,920,00 EUR	1,920,00 EUR	View Panel Delete
RTR Custom Panel 96 RealTime ready Custom Panel 96 - 16+ EST: 18 days	000009344	05582628001	1 * 6 Plates	1,920,00 EUR	1,920,00 EUR	View Panel Delete

Single Assays

Products	Configuration No.	Catalog No.	Quantity	Price	Subtotal	
Catalog Assay for CUL1 Assay ID: 101454 300 reactions/20µl EST: 10 days	000000505	05532957001	1	165,00 EUR	165,00 EUR	Assay Details Delete
Designer Assay for DCUN1D2 Assay ID: tbd 300 reactions/20µl EST: 15 days	000000514	05583055001	1	210,00 EUR	210,00 EUR	Delete

Related Products Chosen

Products	Catalog No.	Quantity	Price	Subtotal
----------	-------------	----------	-------	----------

Total Price: 4.215,00 EUR

Update PricePrintE-MailE-Shop

RealTime ready Shopping Cart Ordering Options



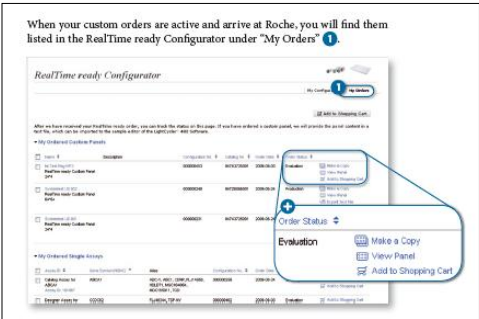
- Order by E-Mail**
Provided order form has to be completed. E-mail is sent to the respective local sales organization
- Order by Fax / Phone**
Print-out-form of the shopping cart is created. Sheet can be faxed to the local sales organization. Order by phone is also possible.

Order Tracking



After the order is placed, it is also stored under "My Orders" where:

- The present status is indicated: **Evaluation / Production / Shipping**
- When the status is Shipping, **the txt. file with the plate content** is available download into the LC 480 Sample Editor



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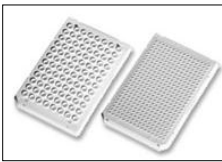
LightCycler® 480 System Components



Instrument



Block Kit



Multiwell Plates



Reagent Kits



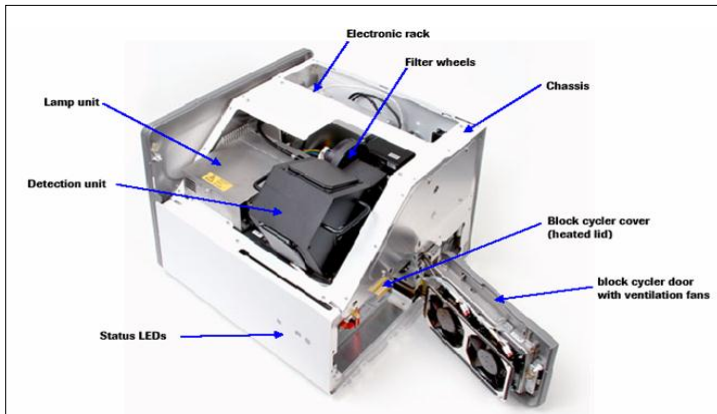
Software Modules



Prevalidated Probes

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LightCycler® 480 Instrument General Architecture



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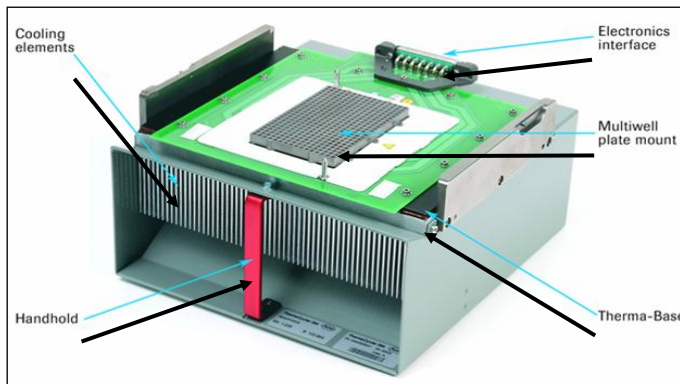
LightCycler® 480 System 96 ↔ 384 Switch



- Interchangeable thermal block cycler
- Do-it-yourself, fast
- Loading help for convenience
- No service engineer required
- **No re-calibration necessary**
- Instrument automatically detects and identifies block

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LightCycler® 480 Thermal Block Cycler *Speed and Accuracy*



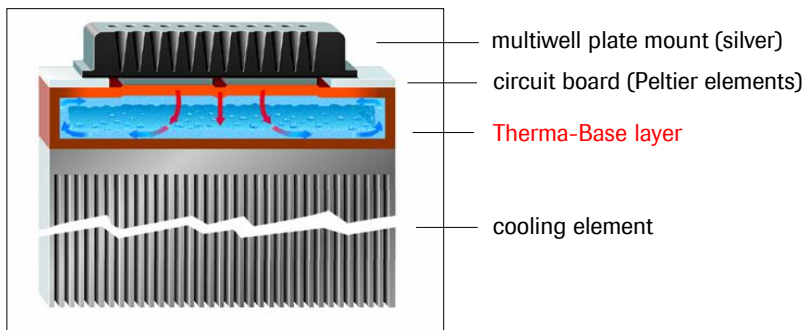
Therma-Base for optimized heat equalization

- Homogenous temperature distribution over the plate
- Fast PCR runs

96 wells in < 1 hour
384 wells in < 40 min

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LightCycler® 480 Instrument Heat Sink *Therma-Base*



- Thin sealed vacuum vessel with working fluid in a wick structure
- Rapidly transfers heat by evaporation and condensation

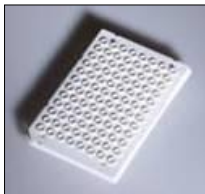
→ **Enables both rapid and accurate cycling !**

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LightCycler® 480 System *Disposables*



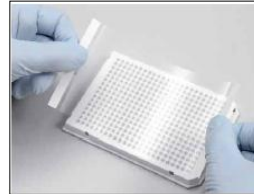
- Optimal fit for good temperature transfer, results in
 - fast cycling times (384-wells in < 40 min, 96-wells in < 1h)
 - homogenous temperature distribution in each well
- Sealing foil to prevent of evaporation and contamination



96-well plate
for 10–100 µl



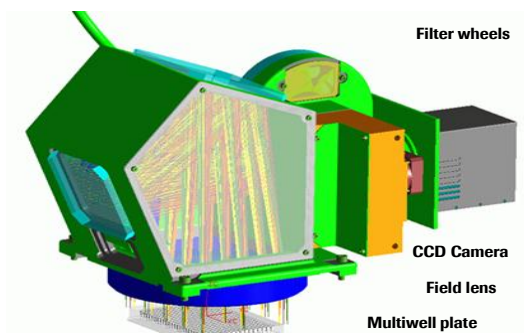
384-well plate
for 3–20 µl



Sealing foil

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LightCycler® 480 Optical Unit *Sensitivity and Homogeneity*



- Xenon lamp 氙氣弧光燈
 - high intensity
 - broad dynamic range
 - lifetime: approx. 500–1000 hrs
- CCD camera
- **Five** excitation filters
- **Six** detection filters
- Optimized arrangement of optical components
- Homogeneous excitation and fluorescence detection

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LightCycler® 480 Optical Unit Characteristics



5 excitation – and 6 emission filters for

- Easy duplexing
 - Easy and unbiased performance of dual color Hydrolysis Probes assays using universal color compensation objects for FAM/HEX (VIC) or FAM/Yellow 555 combinations
- Unmatched multiplexing
 - 4 color Hydrolysis probes
 - 4 color HybProbe probes
- The long-pass filter grants access to dyes of higher wavelength

	Instrument II Bandpass (Half Band Width)
Excitation	▪ 440 nm (35) ▪ 465 nm (25) ▪ 498 nm (40) ▪ 533 nm (25) ▪ 618 nm (35)
Detection	▪ 488 nm (20) ▪ 510 nm (20) ▪ 580 nm (20) ▪ 610 nm (20) ▪ 640 nm (20) ▪ 660 nm (95) long pass

Xenon lamp (430–630)						
Excitation filters	440	465	498	533		618
Emission filters	488	510	580	610	640	660
Dye	LightCycler® Cyan 500	SYBR Green I ResoLight	Fluorescein FAM	HEX (VIC)	LightCycler® Red 610	LightCycler® Red 640 Cy5

LightCycler® 480 System II



Allows Use of All Most Currently Used Detection Formats

Detection format	Excitation (nm)	Detection (nm)	Dye	Applications
SYBR Green I	465	510	SYBR Green I	Qualitative and quantitative PCR, Product verification
HybProbe probes	498	610 640 660	LightCycler® RED 610 LightCycler® RED 640 Cy5	Quantification, SNP/mutation analysis via melting curves
Hydrolysis probes	440 465 533 533 618	488 510 580 610 660	LightCycler® CYAN 500 FAM VIC, HEX, Joe, Yellow 555 LightCycler® RED 610 Cy5, Cy 5.5	Quantification, Endpoint genotyping
UPL	465 533	510 580	FAM Yellow 555	Quantification
High Resolution Melting	465	510	LightCycler® 480 ResoLight Dye	Gene/mutation scanning, SNP analysis

LightCycler® 480 Reagents *Optimized for Each Application*



- **LightCycler® 480 SYBR Green I Master, 2x**
 - 5 ml and 50 ml pack sizes
- **LightCycler® 480 Probes Master, 2x**
 - LightCycler® 480 Probes Master primarily developed for qPCR with [hydrolysis probes](#), incl. Endpoint Genotyping
 - 5 ml and 50 ml pack sizes
- **LightCycler® 480 Genotyping Master, 5x**
 - Genotyping Master for melting curve-based genotyping using [HybProbe](#) or [SimpleProbe](#) probes
- **LightCycler® 480 High Resolution Melting Master, 2x**
- **LightCycler® 480 Control Kit**



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Kits and Reagents For RT-PCR



Products for [Two-Step RT-PCR](#)

- **Transcriptor Reverse Transcriptase**
 - with oligo (dT) , random hexamer or specific priming
 - for transcription of long RNAs and also for GC rich templates
- **Transcriptor First Strand cDNA Synthesis Kit**
 - with oligo (dT) , random hexamer or specific priming

Products for [One-Step RT-PCR](#)

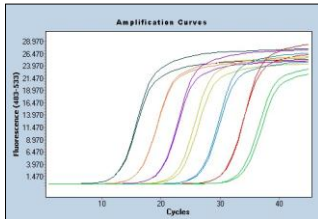
- **LightCycler® 480 RNA Master Hydrolysis Probes, 2.7x**
 - RT step done within [3 min](#)

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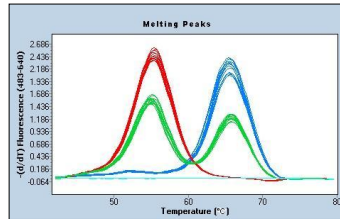
Applications - Overview



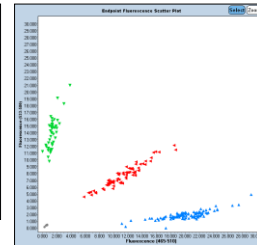
Quantification and Qualitative Detection



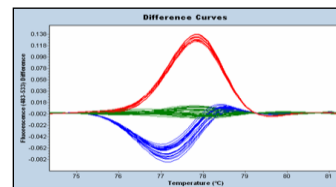
Mutation Detection (Melting Curve Genotyping)



Mutation Detection (Endpoint Genotyping)



Gene Scanning (High Resolution Melting Analysis)



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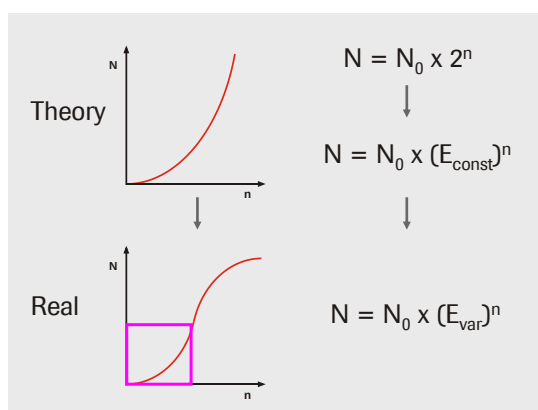
Quantification

- *Cp Calculation*
- *Absolute Quantification*
- *Relative Quantification*
 - I. Basic Method*
 - II. Advanced Method*

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PCR Quantification

Theoretical and Practical Aspects



log-phase-PCR



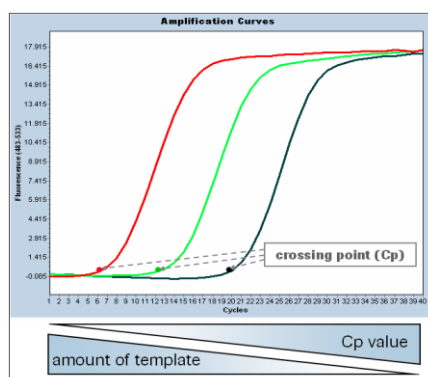
end-point-PCR

N: number of amplified molecules
 N_0 : initial number of molecules

n: number of amplification cycles
 E: amplification efficiency

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Concentrations and Crossing Points



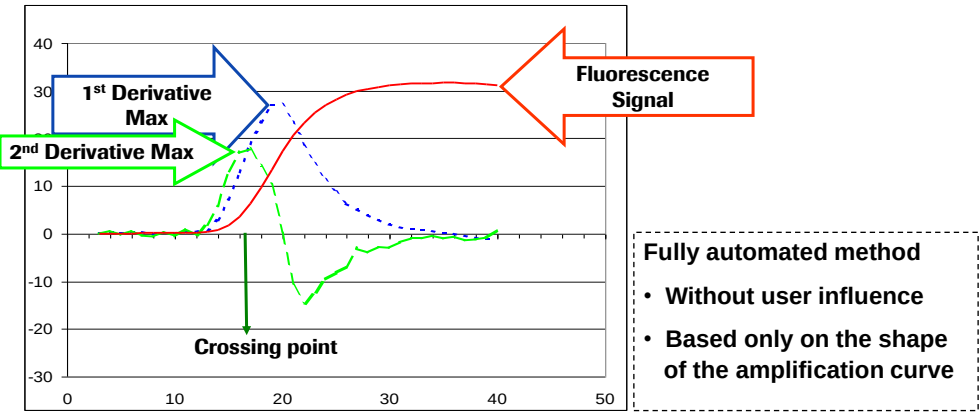
Calculation of crossing points (Cp)

- Standard method (automatic)
2nd Derivative Maximum Method
 二次微分最大値
- Optional with **Fit Point** method
 (user-influenced)

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Crossing Point Calculation

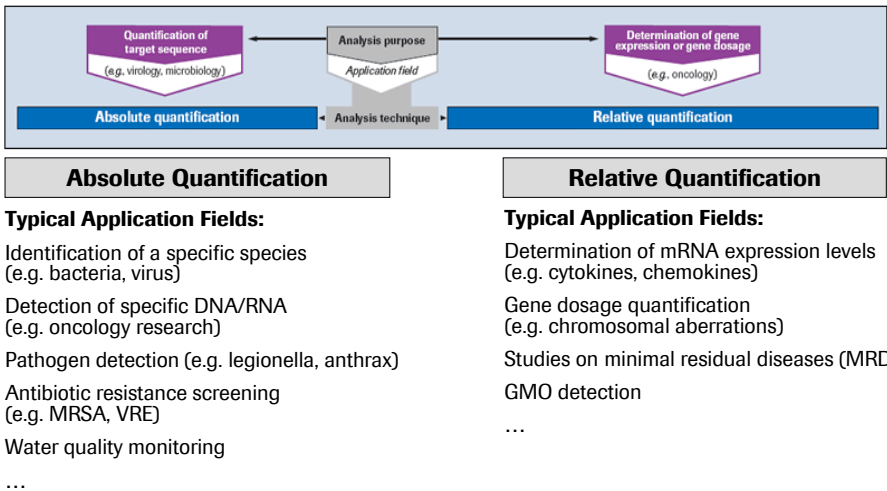
2nd Derivative Maximum Method



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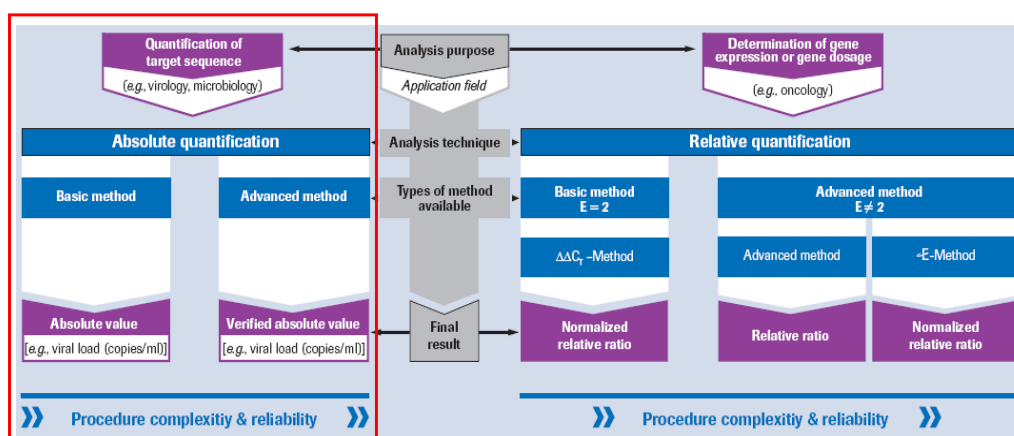
Quantitative PCR Methods

Overview



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Types of Quantification



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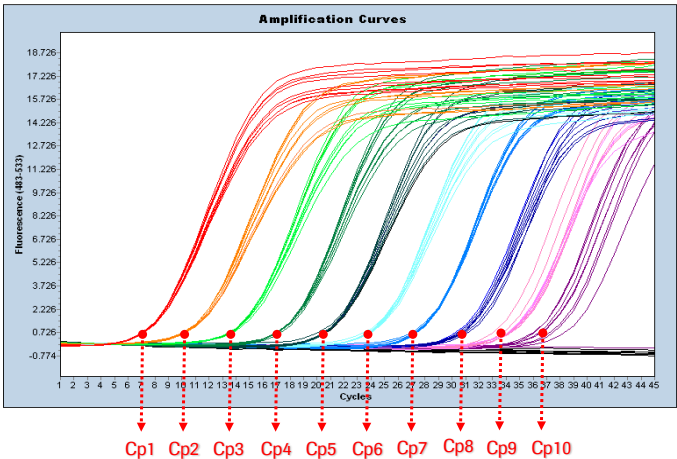
Absolute Quantification

- Is the method of choice to determine concentrations of viral or bacterial loads in cells or tissues.
- The concentration of an unknown sample is calculated by comparing its crossing point with the standard curve.

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LightCycler® 480 System

Quantification, Dynamic Range

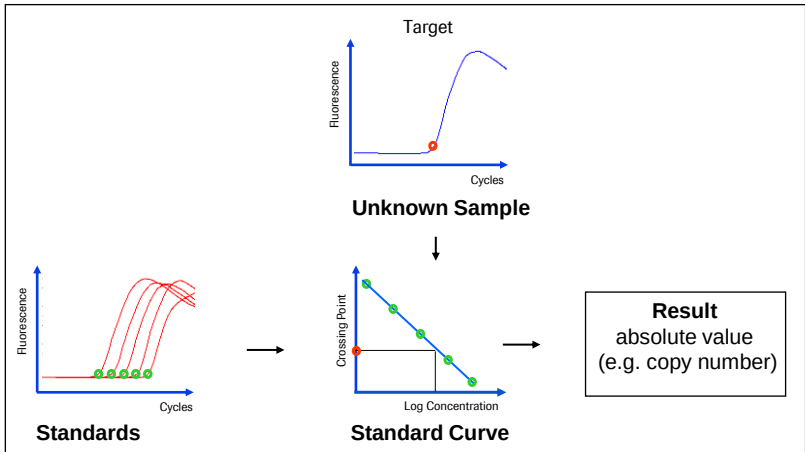


Samples	Statistics	
	Mean Cp	STD Cp
NTC		
1E0	37.69	1.08
1E1	34.94	0.49
1E2	31.65	0.27
1E3	28.21	0.05
1E4	24.85	0.01
1E5	21.56	0.02
1E6	18.17	0.01
1E7	14.81	0.01
1E8	11.46	0.02
1E9	8.18	0.02

- Serial dilution of plasmid DNA (1 copy - 10⁹ copies)
- 9 replicates each
- Detection format: UPL (FAM)
- Target: GAPDH

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Absolute Quantification with External Standards: Principle



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How to describe a PCR reaction ?



At the crossing point (C_p) the number of products (N) can be described by

$$N = N_0(E)^{C_p}$$

This can be linearised to:

$$\log N = \log N_0 + C_p \cdot \log E$$

The initial concentration of the standard (T_0) is under the control of the investigator, so should be the x value. The crossing point (C_p) is what we are measuring, so it should be the y value. Therefore we rearrange to give:

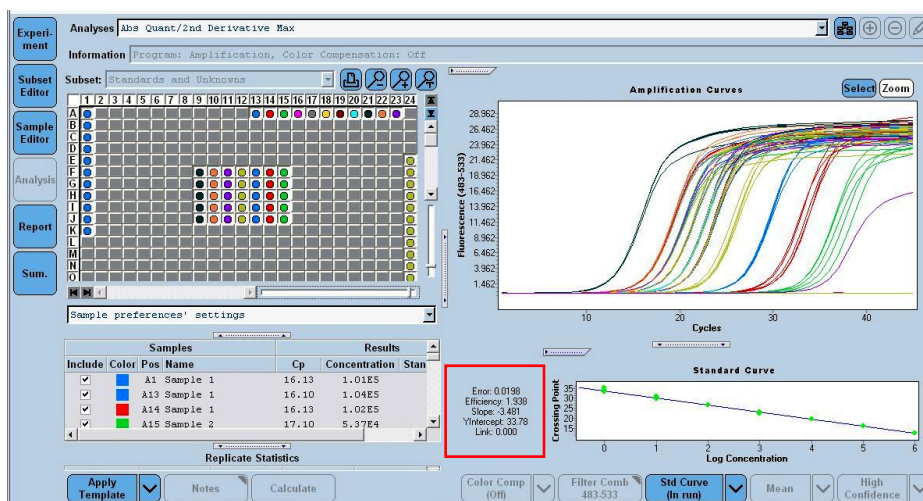
$$C_p = \frac{-(1/\log E) \cdot \log N_0 + (\log N / \log E)}$$

y slope x

$$E = 10^{-1/\text{slope}}$$

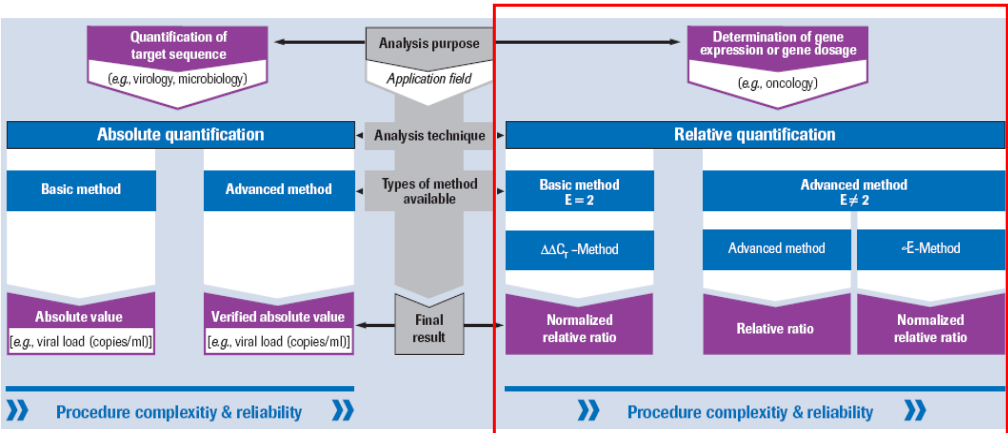
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Absolute Quantification with External Standards: Example



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Types of Quantification

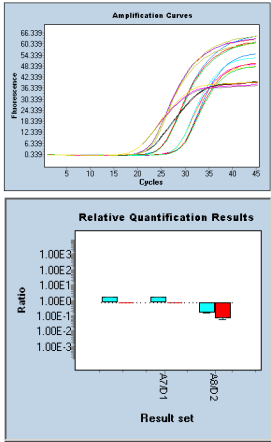
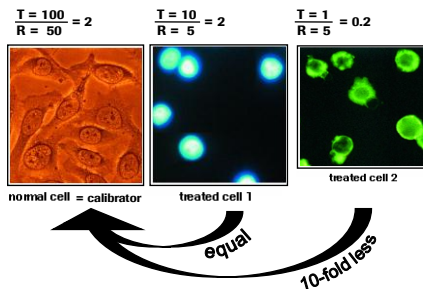


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Relative Quantification Scheme

Wanted: Target gene expression level

Required: Target gene concentration
Reference gene concentration
Calibrator sample (optional)



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Relative Quantification

Principle (I)

Step 1: Concentrations of target and reference genes from the same sample material are calculated.

Step 2: The target concentration in each sample is divided by reference gene concentration.

$$\text{Relative Ratio} = \frac{\text{concentration of target}}{\text{concentration of reference}}$$

Carefully select **reference gene(s)** for accurate normalization

- to identify reference genes unaffected by experimental conditions (non-regulated) use genes from a wide variety of cellular processes.
- one or more reference genes can be used
averaging of multiple pre-selected housekeeping genes is an additional option.

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Relative Quantification

Principle (II)

Step 3: Optional

A calibrator sample can be used in addition for further normalizing the final results

$$\text{Calibrator Normalized Ratio} = \frac{\frac{\text{concentration of target}}{\text{concentration of reference}} \text{ (sample)}}{\frac{\text{concentration of target}}{\text{concentration of reference}} \text{ (calibrator)}}$$

An assay calibrator corrects for differences in detection sensitivity between target and reference gene and between several experiments (e.g., long-term studies).

Any typical positive sample can be defined as **Calibrator**

- non treated cell line
- sample at the time point “zero“
- normal tissue
- ...

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Relative Quantification Methods (1)

$\Delta\Delta C_T$ Method

This method assumes,

- that reference gene and target gene are amplified with the same efficiency and
- that the PCR efficiency of both genes is constant and identical ($E = 2$)

$$\text{normalized relative ratio} = 2^{-\Delta\Delta C_T}$$

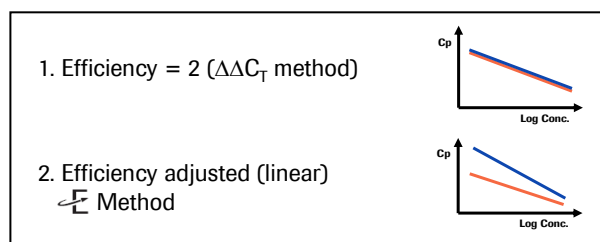
- standard curves are not required for calculation

→ Not every PCR-system is running with optimal/identical PCR efficiency

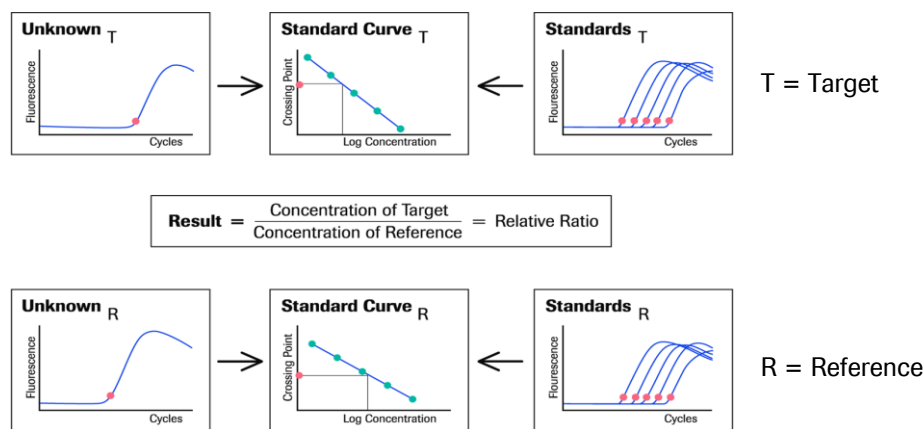
→ Not every PCR-System is following a constant PCR efficiency

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Various PCR Efficiencies between Target and Reference



Relative Quantification: - With Efficiency correction using Standard Curve

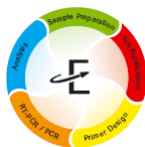


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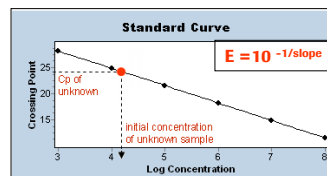
Relative Quantification Principle (III): Further Considerations



Quality of PCR – **efficiency**



Concentration is calculated via **efficiency**



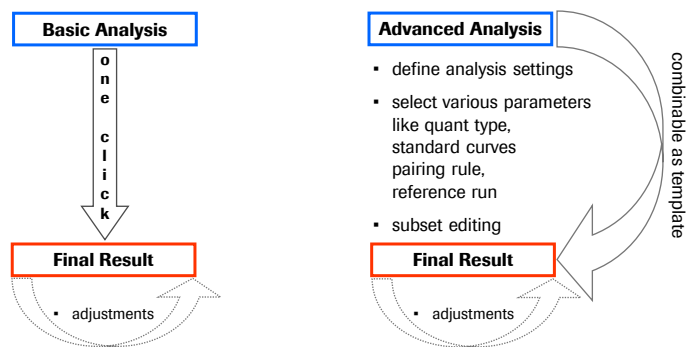
Consideration of PCR Efficiency

Without efficiency correction	E = 2 (ΔΔCT Method)	No standard curve needed
With efficiency correction	edit E ≠ 2 (linear)	Standard curve needed
	E - Method	

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LightCycler® 480 Software, Version 1.5

Relative Quantification Methods - Workflow



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Relative Quantification Analysis Modules

Basic and Advanced: Analysis Options



	Basic	Advanced
Target Gene	one / many	one / many
Reference Gene	one / many	one / many
Pairing Target - to - Reference	All-to-Mean	One-to-One All-to-Mean All-to-All Mean-to-All
Calibrator	with / without	with / without
Assay Set-up on MWP	Target & Reference on same plate Full plate analyzed	Target & Reference on same plate Target & Reference on different plates Full plate, and/or Subsets analyzed
Cp Analysis	Fit Points Method	Fit Points Method 2nd Der. Max. Method
Efficiency	$E = 2 / \neq 2$	$E = 2 / \neq 2$ standard curves: linear or non-linear fit
Multiplex	monocolor / dual color	monocolor / dual color

Basic Relative Quantification

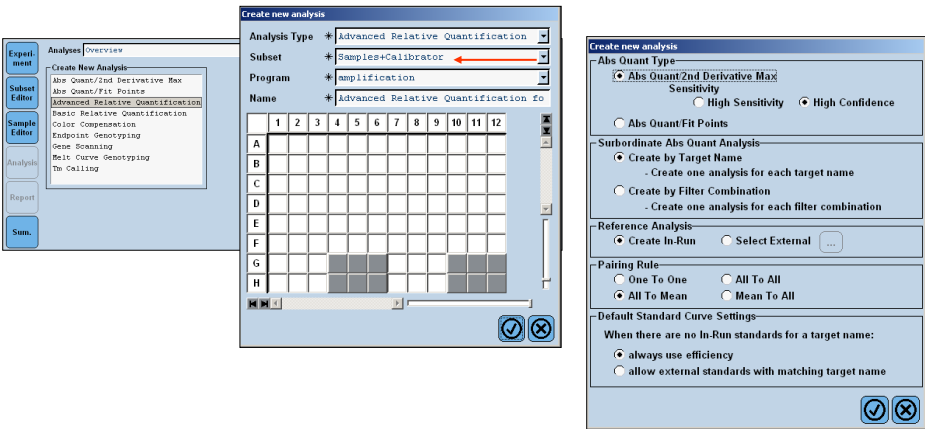
With one Click to Results !



The easiest way to export data !

Advanced Relative Quantification

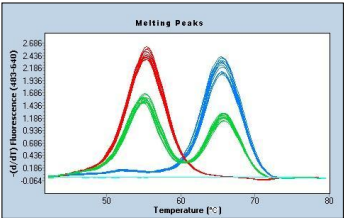
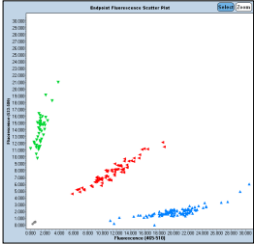
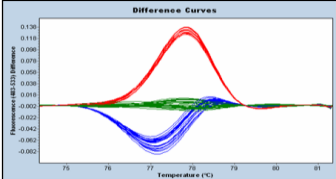
Analysis According to Your Needs !



Genetic Variation Analysis

- End Point Genotyping
- Melting Curve Genotyping
- Gene Scanning

Applications - Overview

known variants	Mutation Detection (Melting Curve Genotyping)  Mutation Detection (Endpoint Genotyping) 
any variation	Gene Scanning (High Resolution Melting Analysis) 

Genotyping by Real-Time PCR

Current Technologies and Developments

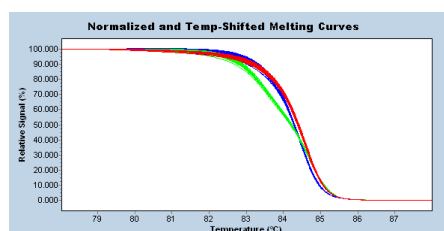
- Genotyping of **known** variants
 - Using HybProbe- or Simple Probe Probes (melting curves)
 - Using Hydrolysis Probe (endpoint genotyping)

- **Gene Scanning**
 - Finding **new** variants (e.g., **SNPs, deletions, insertions, somatic mutations, methylation patterns**) in specific regions of candidate genes
 - Using High Resolution Melting with a saturating DNA-binding dye and specific algorithms for data analysis.

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Gene Scanning

High-Resolution Melting Analysis



Example:

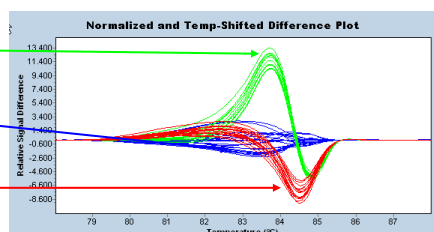
Sequence variations (SNP
G→T) in the LPLH3 gene

72 samples, 164 bp amplicon

heterozygous (homo
and heteroduplexes)

homozygous wildtype
(homoduplexes)

homozygous mutant
(homoduplexes)

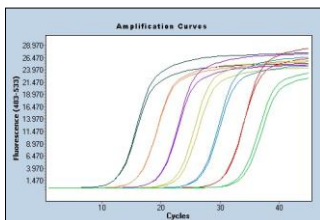


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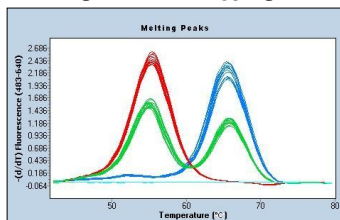
Applications - Overview



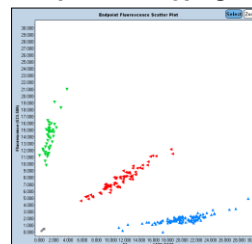
Quantification and Qualitative Detection



Mutation Detection (Melting Curve Genotyping)

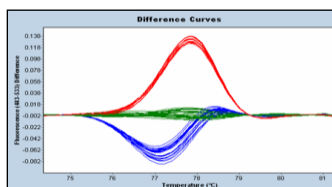


Mutation Detection (Endpoint Genotyping)



Universal ProbeLibrary
RealTime ready assays & panels

Gene Scanning (High Resolution Melting Analysis)



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LightCycler Website

<http://www.roche-applied-science.com>

The screenshot shows the LightCycler website interface. A dashed pink box highlights the following navigation links:

- Online Technical Support
- Special Interest Sites
- Product Selection Guide
- Multimedia Presentations
- Application Design Tools
- Biochemica
- E-Mail Subscription Services
- Application Notes

The main content area features the heading "Real-Time PCR Systems" and lists "Productive Real-time PCR Systems". It includes images and descriptions of three systems:

- LightCycler® Carousel-Based System**
- LightCycler® 480 System**
- LightCycler® 1536 System**

At the bottom, it states: "Function tested qPCR assays - designed to your specifications."

Package-insert download



Product Information - Microsoft Internet Explorer

檔案(F) 編輯(E) 檢視(V) 我的最愛(A) 工具(T) 說明(H)

地址(1) http://www.roche-applied-science.com/PROD_INF/index.jsp?&al=foru

EPSON Web-To-Page 列印 預覽列印

Home Products Special Interest Sites Support & Resources News

Home > Support & Resources > Product Information > **Instructions for Use**

Instructions for Use - Catalog Number Search

(Re)search the easy way

Use this Search function to obtain your **Instructions for Use** in a fast and easy searchable format.

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- ✓ Straightforward, structured, easy to use - and always on hand.
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- ✓ Display the most up-to-date information.

Catalog Number or REF
Printed on the product box
e.g., 03 003 230 001

Search

See how it works

Please enter the Catalog Number printed on the outer label of the product box and click "Search".

Service & Support
Get in touch with our sales and support teams via web, email, phone, or fax.

- Online Technical Support
- Contact
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Application Notes



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Application Notes

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NEW - xCELLigence System

- > Real-time and Dynamic Monitoring of Cell Proliferation and Viability for Adherent Cells
- > Dynamic Monitoring of Cell Adhesion and Spreading
- > Dynamic Monitoring of G-protein Coupled Receptor Activation in Living Cells
- > Dynamic Monitoring of Receptor Tyrosine Kinase Activation in Living Cells
- > Label-Free Assay for NK Cell-mediated Cytotoxicity
- > From Classical to Online Monitoring of G-Protein-Coupled Receptor Stimulation in Living Cells
- > Culture and monitoring of animal cells Basic techniques
- > Cardiac-Specific Toxicity - Real-Time Monitoring of Adverse Effects on Cardiomyocytes Derived from Embryonic Stem Cells
- > Real-time and Label-free Monitoring of Virus-mediated Cytopathogenicity

LightCycler® Real Time PCR System Notes

- > Managing LightCycler® Software 4.0/4.05 Database Files
- > Color Compensation for Hydrolysis Probe Assays
- > Developing Multicolor Applications for the LightCycler® Carousel-Based System
- > Color Compensation
- > Assay Formats for Use in Real-Time PCR
- > Sequencing of LightCycler® Amplification Products
- > Customizing LightCycler® Relative Quantification Techniques for Specific Applications
- > Selection of Housekeeping Genes
- > General Recommendations for LightCycler® Analysis of Multiple Mutations Using Hybridization Probe Color and Melting Temperature
- > Relative Quantification
- > Absolute Quantification with External Standards and an Internal Control

Online Technical Support from RAS website

<http://technical-support.roche.com>



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