QuantStudio
qPCR System Overview

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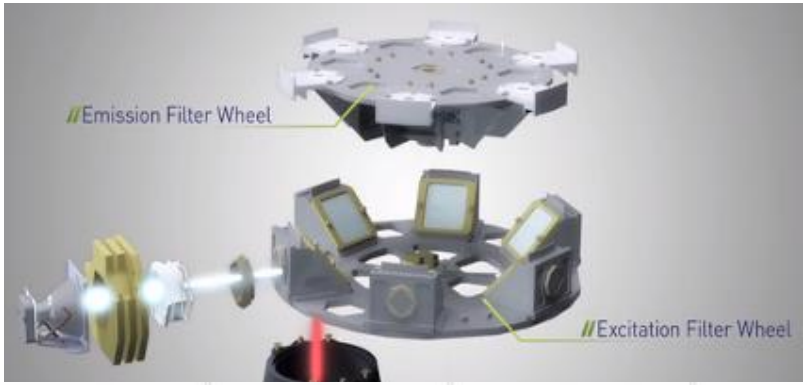
Field Applications Scientist

ThermoFisher SCIENTIFIC

The Optics: Enhanced OptiFlex™ System

- light source
- CCD Camera captures data of all filters at every cycle



	m1(520±15)	m2(558±11)	m3(586±10)	m4(623±14)	m5(682±14)	m6(711±12)
x1(470±15)	☑	☐	☐	☐	☐	☐
x2(520±10)		☐	☐	☐	☐	☐
x3(550±11)			☐	☐	☐	☐
x4(580±10)				☐	☐	☐
x5(640±10)					☐	☐
x6(662±10)						☐

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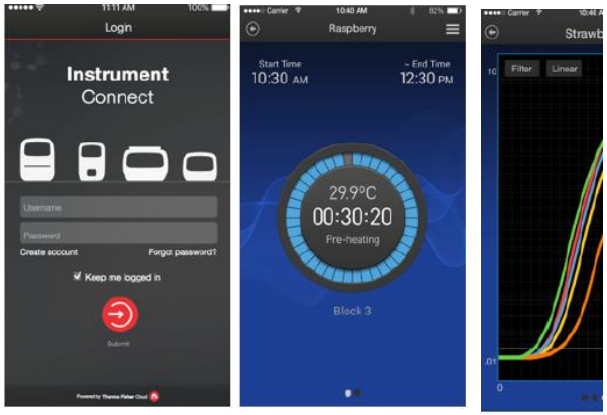
QuantStudio Connectivity

Thermo Fisher Connect™



USB

PC or Mac®

Connect with a web browser

Connect to your data anytime, anywhere

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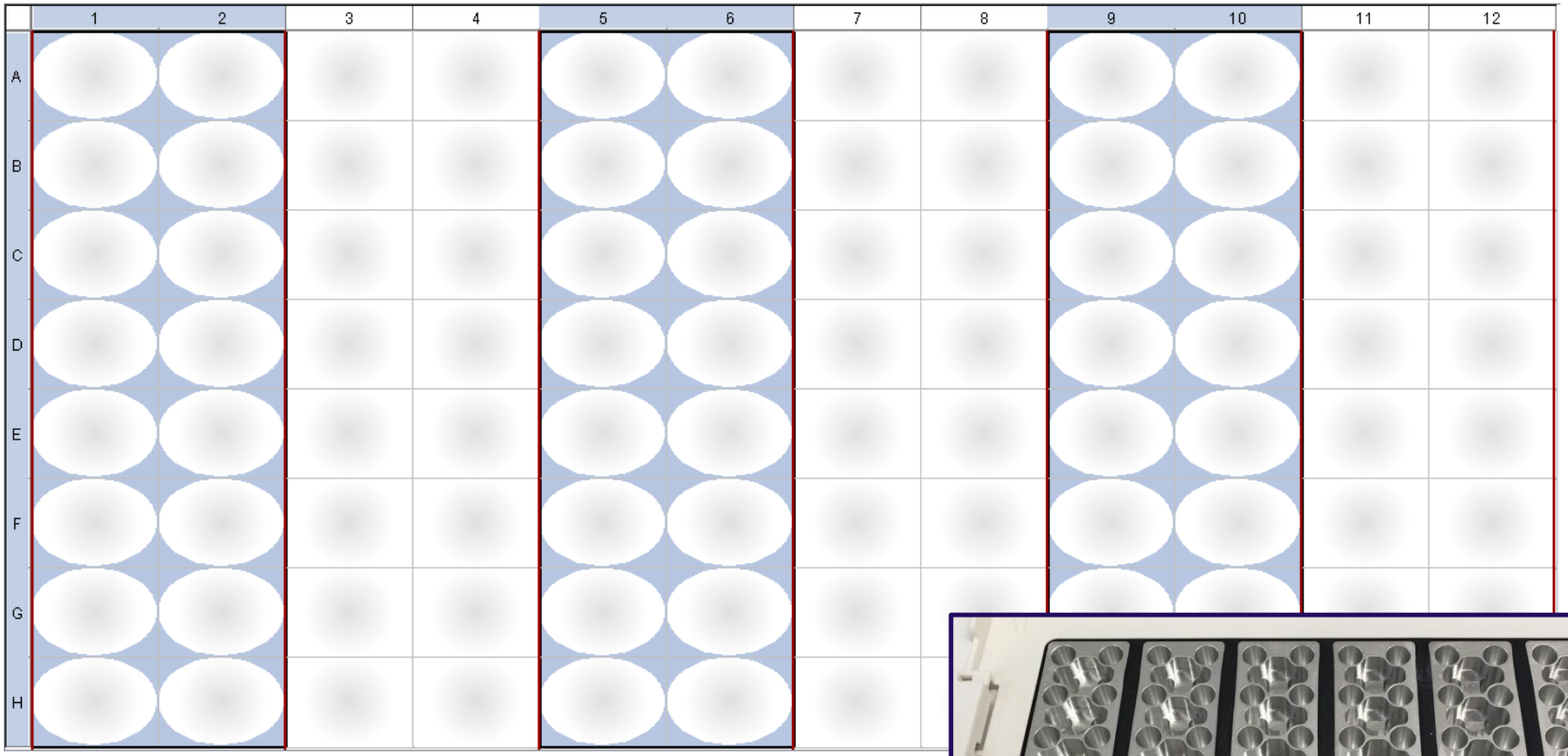
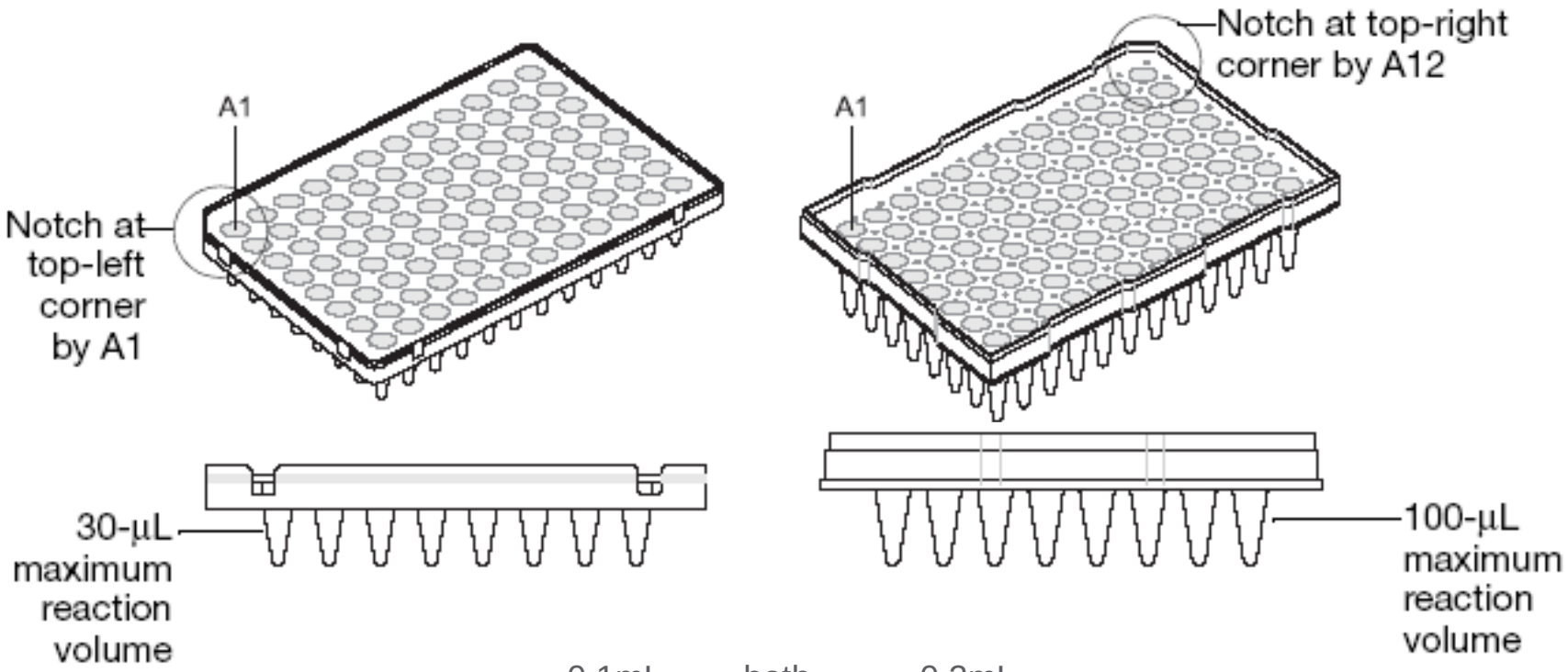
VeriFlex™ Block technology

6 independently controlled programmable zones (≤5c of adjacent zone)



96 well Fast 0.1 mL plates Standard 0.2 mL plates



	0.1mL	both	0.2mL
96 well plates	4346906		4306737
96 well plate holder		4312063	
adhesive covers		4360954	
tray	4379983		4381850
8strip tubes	4358293		4316567
8strip tube caps		4323032	
Tube Capping tool		4330015	
single tubes	4358297		N8010540
Validated RXN volume	10-30uL		10-100uL

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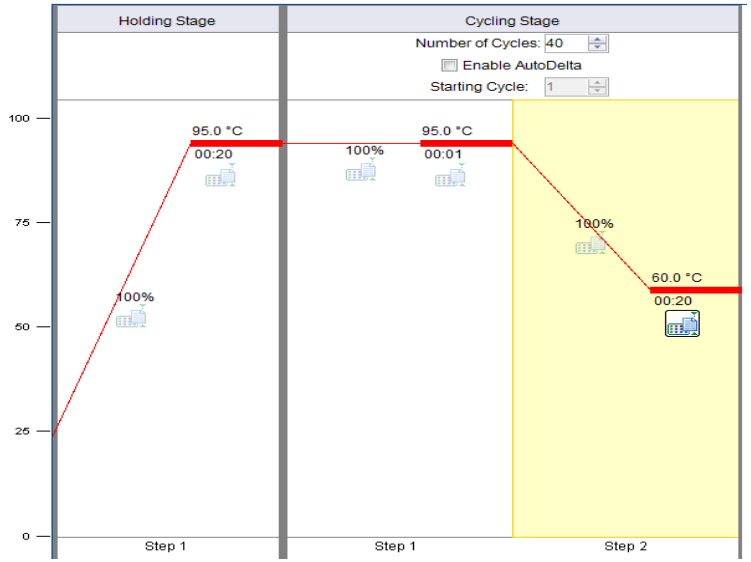
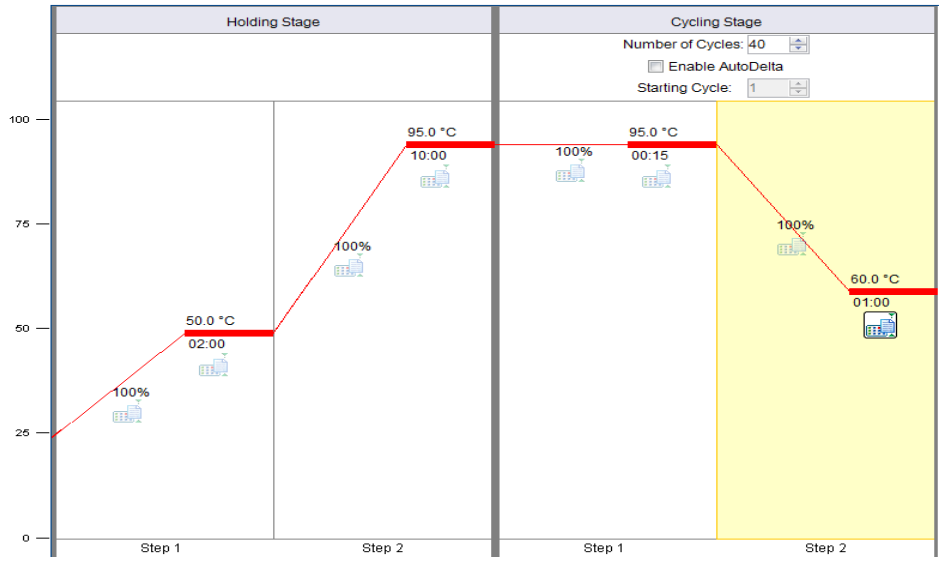
Instrument Default Cycling Options

Fast: 40 cycles in ~40min.

Standard: 40 cycles in ~1hr 45min

- faster ramping & shorter hold times





TaqMan Fast Advanced Master Mix (TaqPath ProAmp for SNP)

PowerUp SYBR Green Master Mix

There are many master mixes, read the protocol for the master mix!

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123456789

Open/close

Set up run

Sean

QuantStudio® 5 - 96-Well 0.2-mL Block

Save the last 300 runs

My Account

Load Experiment

Open Template

Run Last

Settings

February 13, 2014 10:45am

Recommended Calibration and Verification

Calibration	Recommended frequency
ROI/uniformity	**Every two years** • Always perform new Background and Dye calibrations after an ROI/Uniformity calibration.
Dye	**Every two years** • During a Dye calibration, only the dyes on the given spectral calibration plate are calibrated.
Background	**Every month** • As needed: To check for contamination • As needed: To obtain the most accurate data for the removal of background fluorescence
RNase P verification	After installing or moving the instrument. After performing instrument or block calibrations. As needed to confirm instrument performance.

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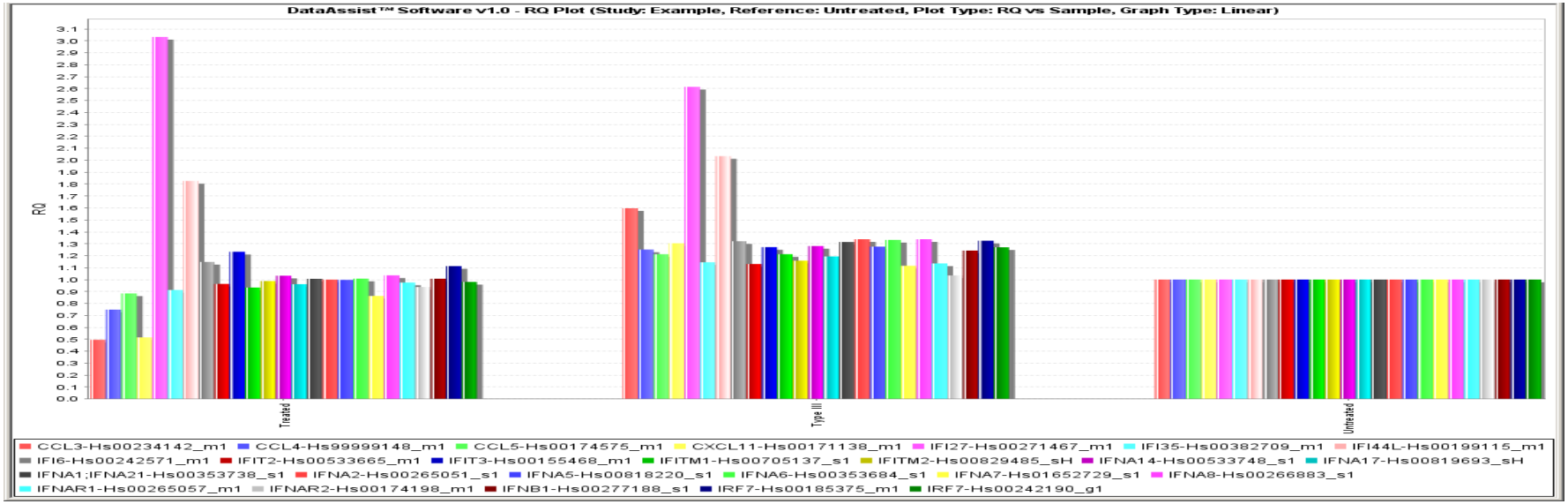
Thermo Fisher Connect

FREE web-based Gene Expression Analysis Software

How it works

- Import .eds data files from system software
- Analyze >100 plates in one study
- Graphical representation of the RQ data
- Export Analyzed Results, Data & Plots



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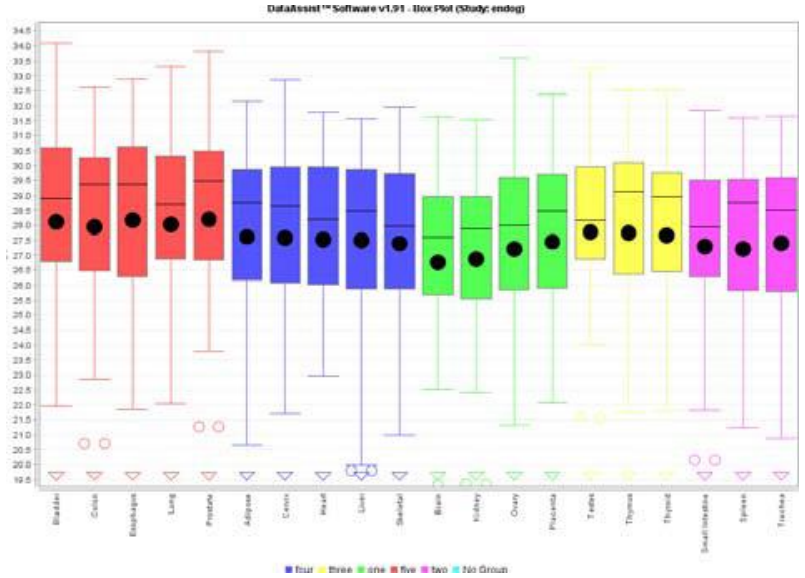
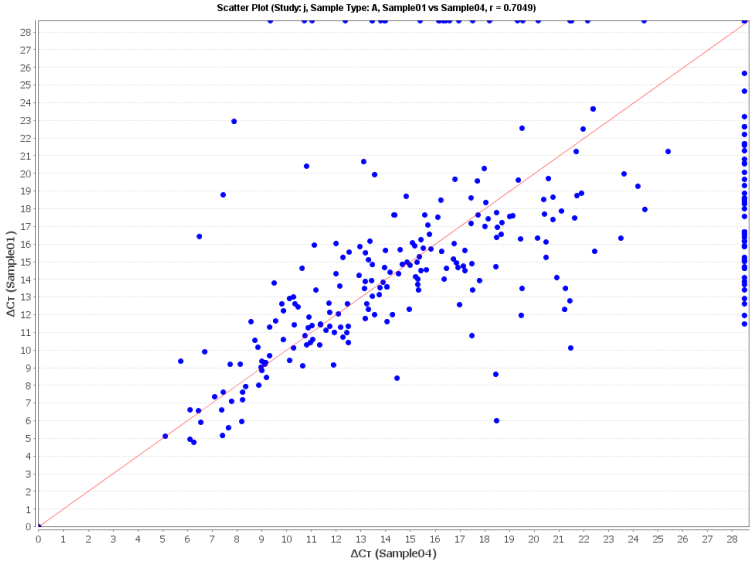
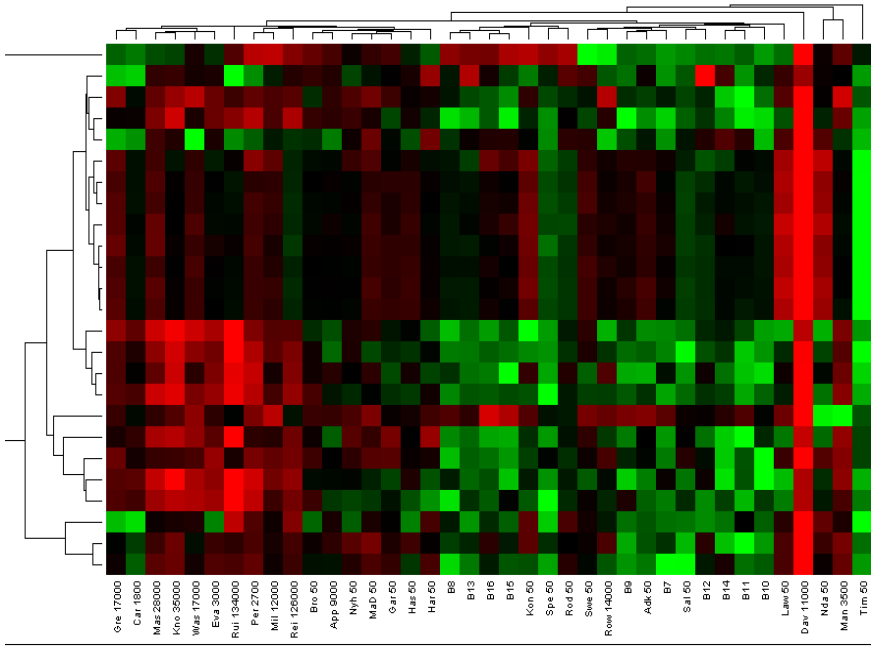
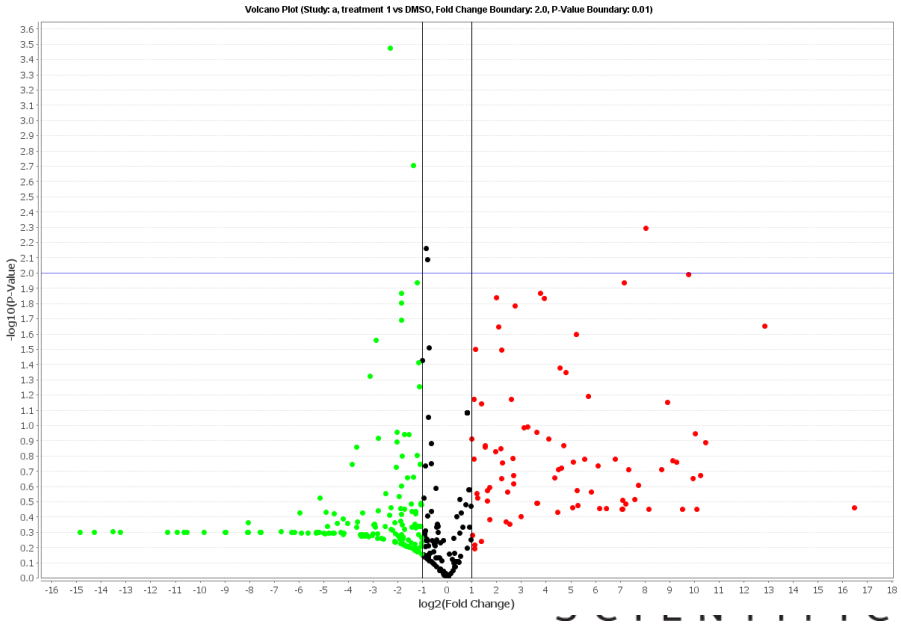
Thermo Fisher Connect









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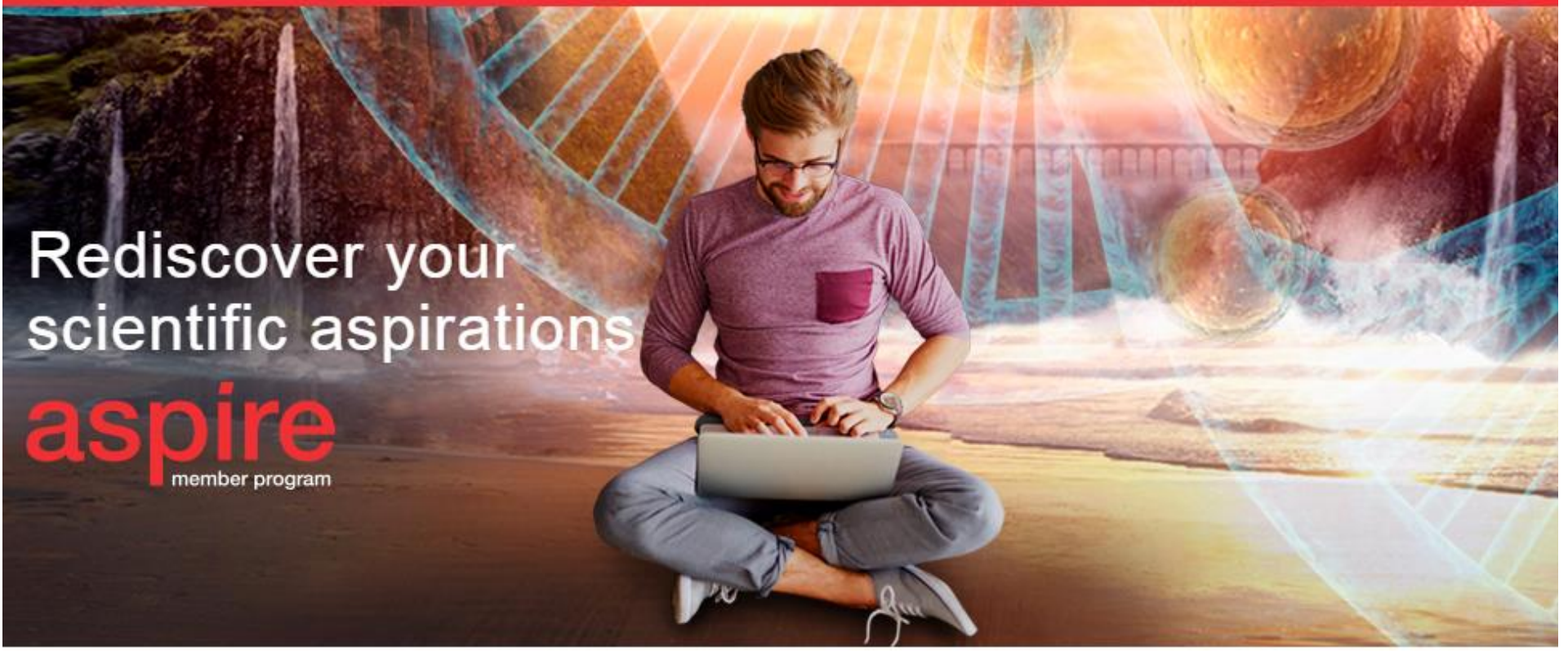

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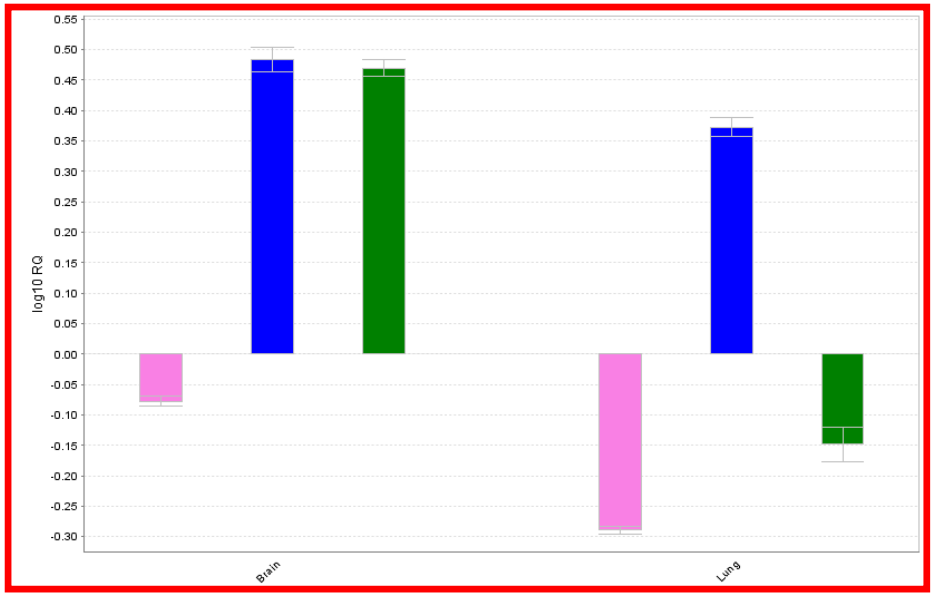
qPCR Applications & Information

Gene Expression
miRNA expression
SNP Genotyping
Copy Number Variation
CAST PCR for Mutation Analysis
Protein Thermal Shift



Digital PCR (QuantStudio 3D)
Service Plans on qPCR systems

Follow-up email with today's slides & survey

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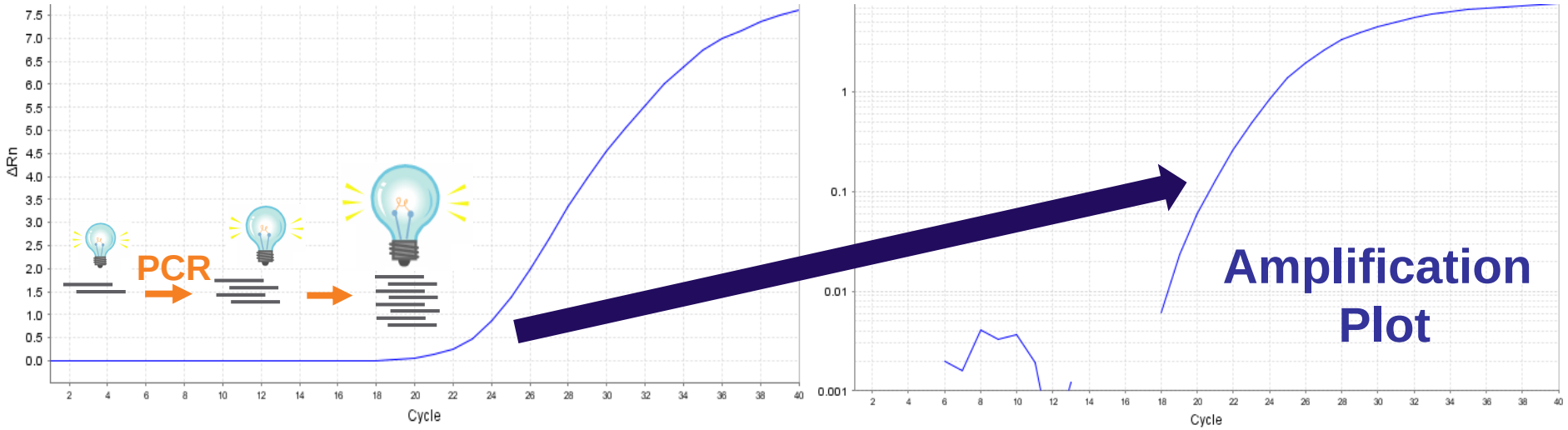
qPCR is the Technology to detect amplification of PCR products in real-time

Similar to traditional PCR…

Denature template
Anneal primers
Extension of primers → amplicons

40 cycles

A fluorescent dye is in the reaction
As Amplification proceeds, fluorescence increases with PCR product formation



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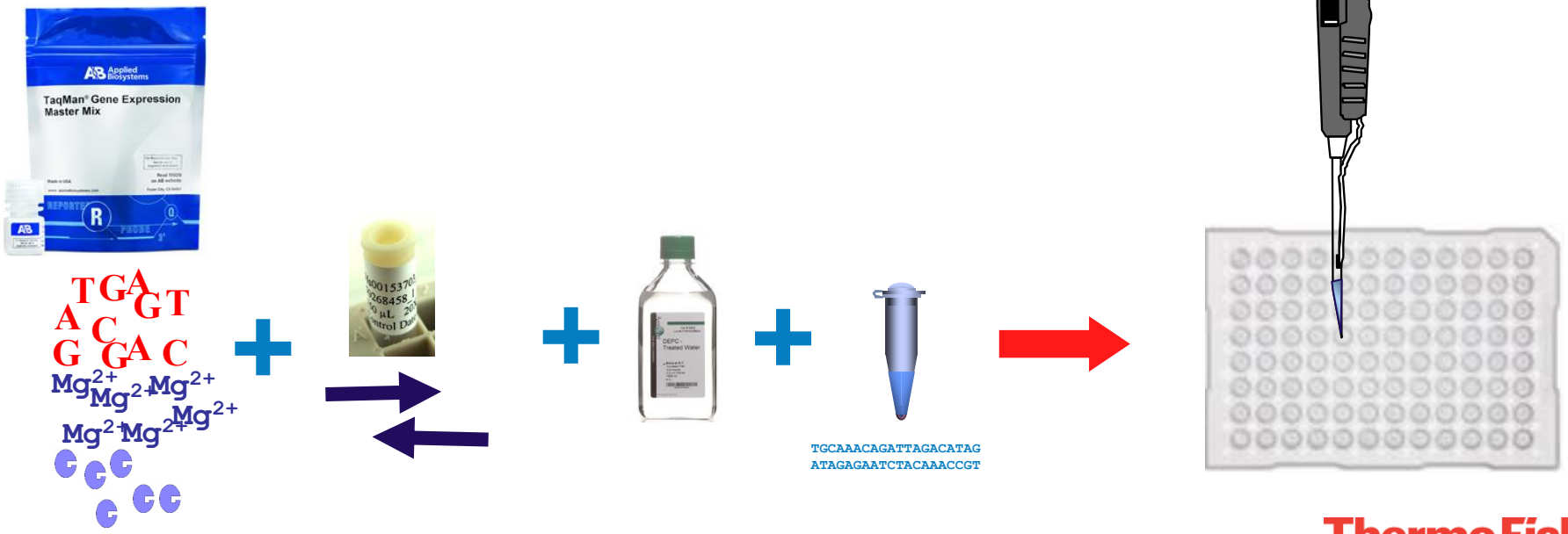
How to set up Real-Time PCR

Combine:	20uL reaction
1) TaqMan® Master Mix or SYBR® Green Master Mix	(2X) 10uL
2) TaqMan® Gene Expression Assay or SYBR® primers	(20X) 1uL
3) water (#AM9935)	7uL
4) Sample (template)	(10 ng) 2uL



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Nucleic Acid Isolation Kits

DNA & RNA Selection Guide

Displaying 7 results.

DNA | RNA

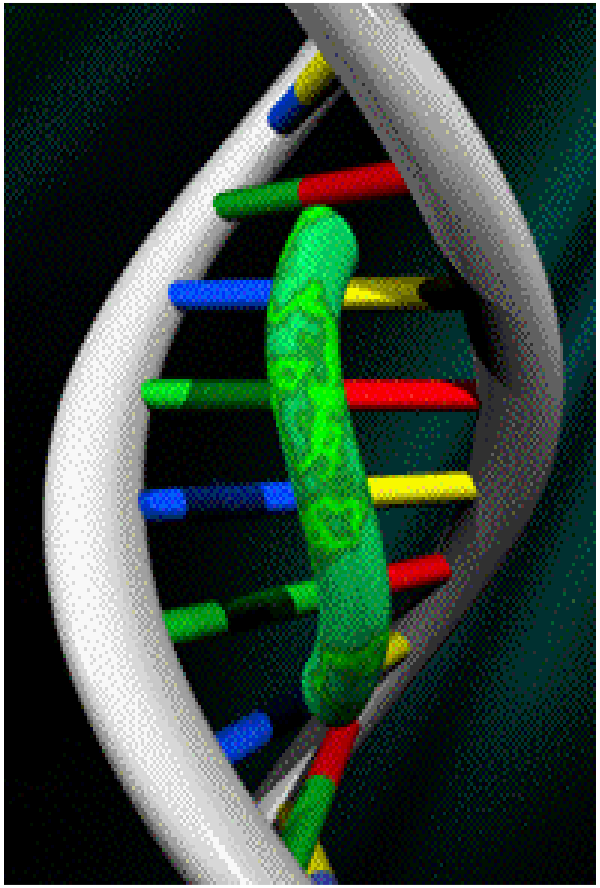
Sample Type	Starting Amount	Throughput	Application	Format
Cells	less than 1000	1-50	Real-Time	Silica matrix column
Tissue	10^3-10^4	50-100	RT-PCR	Purification-free
Blood	10^4-10^5	greater than 100	microRNA	Organic Solution
Virus	greater than 10^5		Cloning	Magnetic beads
Bacteria/Yeast			Microarrays	
Plant			Northern Blot	
FFPE			Sequencing	
LCM			messenger RNA	

KingFisher Flex

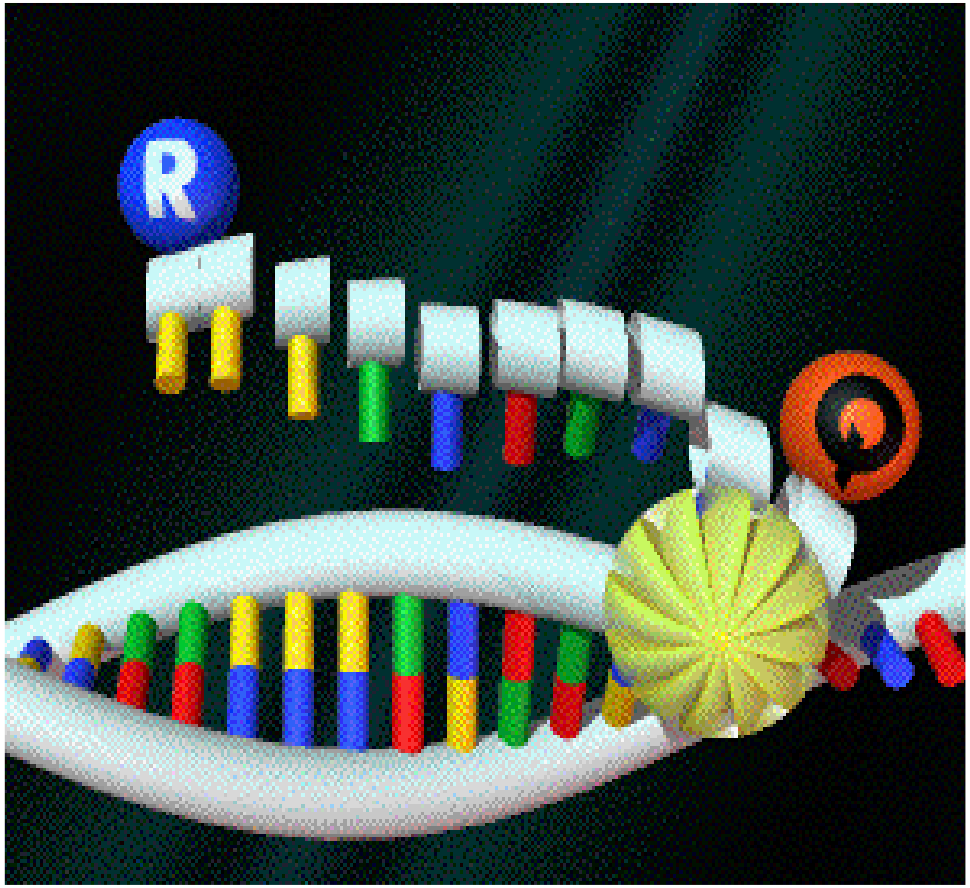
Up to 96 samples in a run

Supported Fluorescent Chemistries

SYBR® Green



TaqMan®



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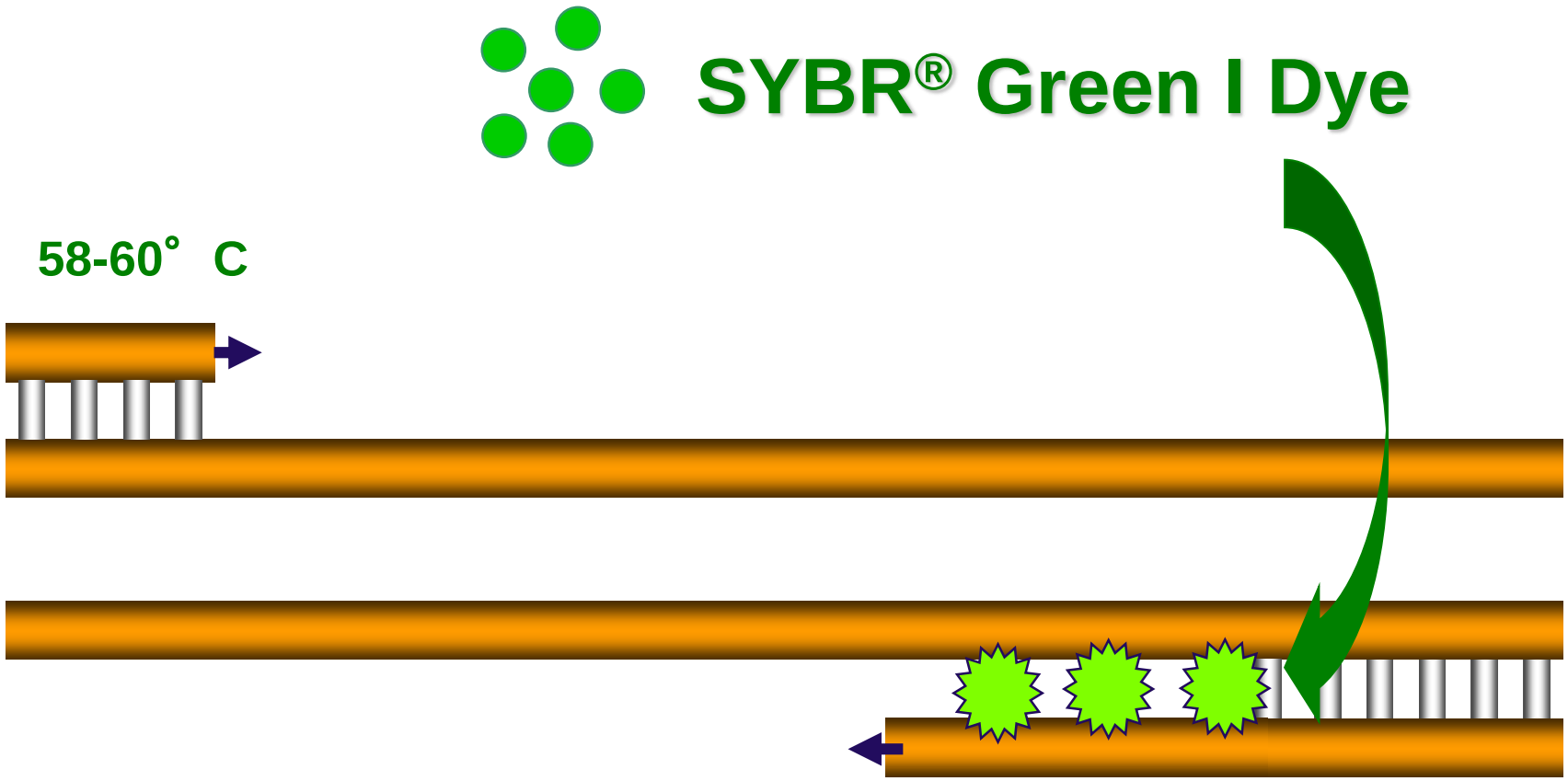
SYBR® Green Reaction



Binds to *any* double-stranded product in PCR reaction

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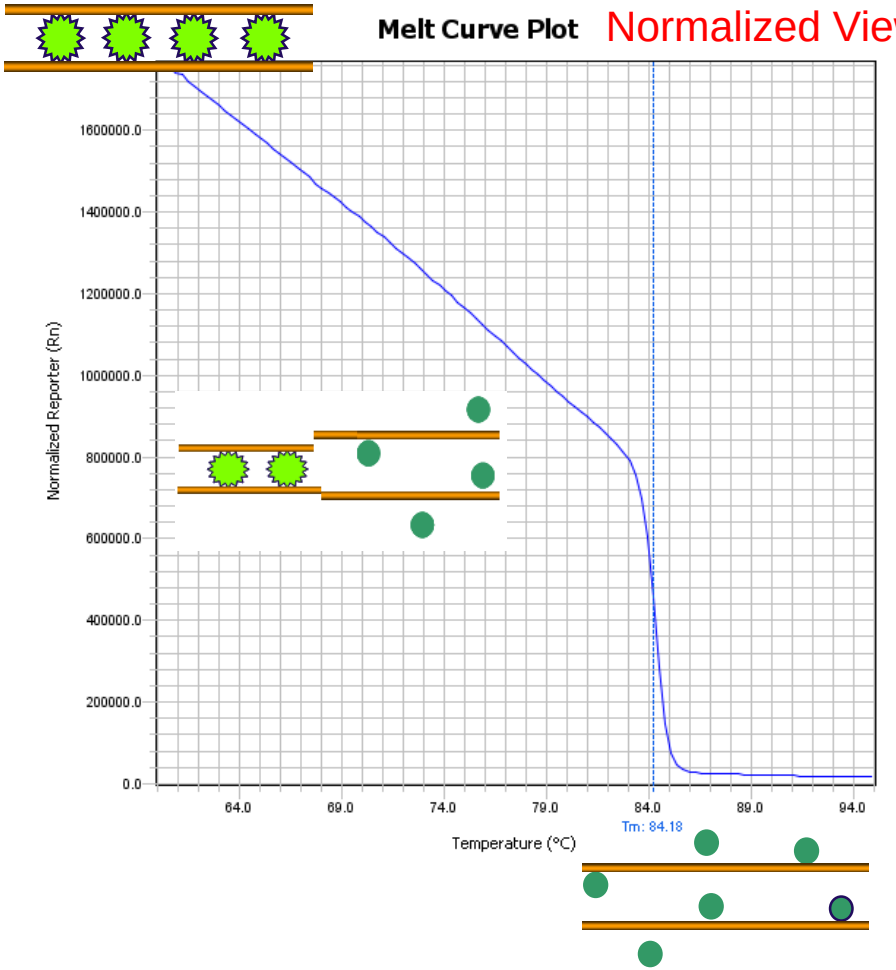
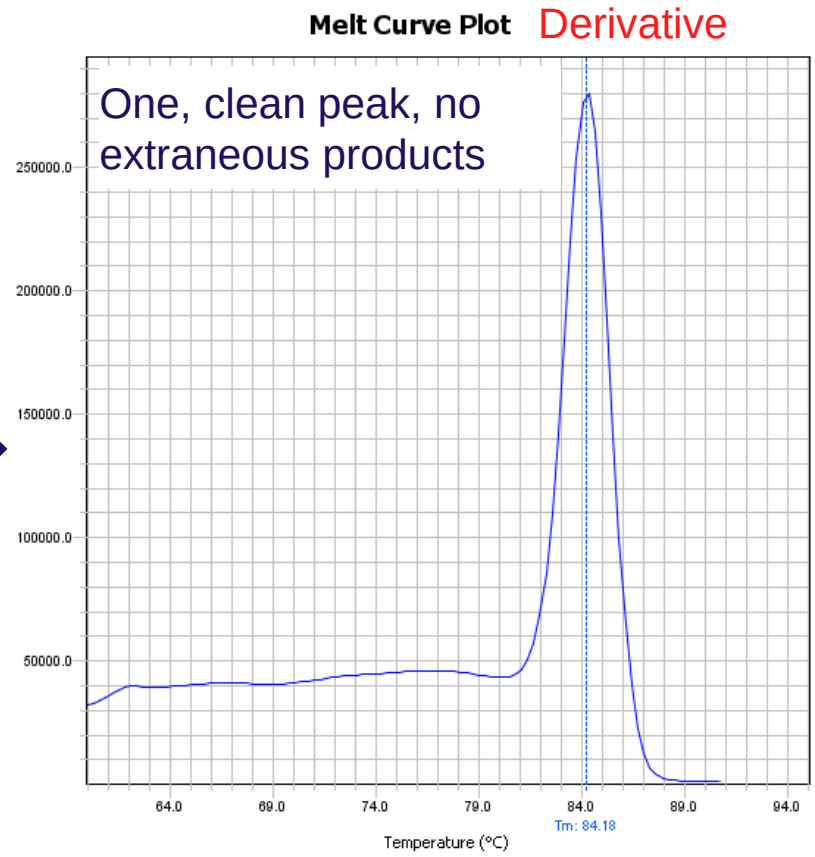
Check specificity of reactions by Melt Curve protocol





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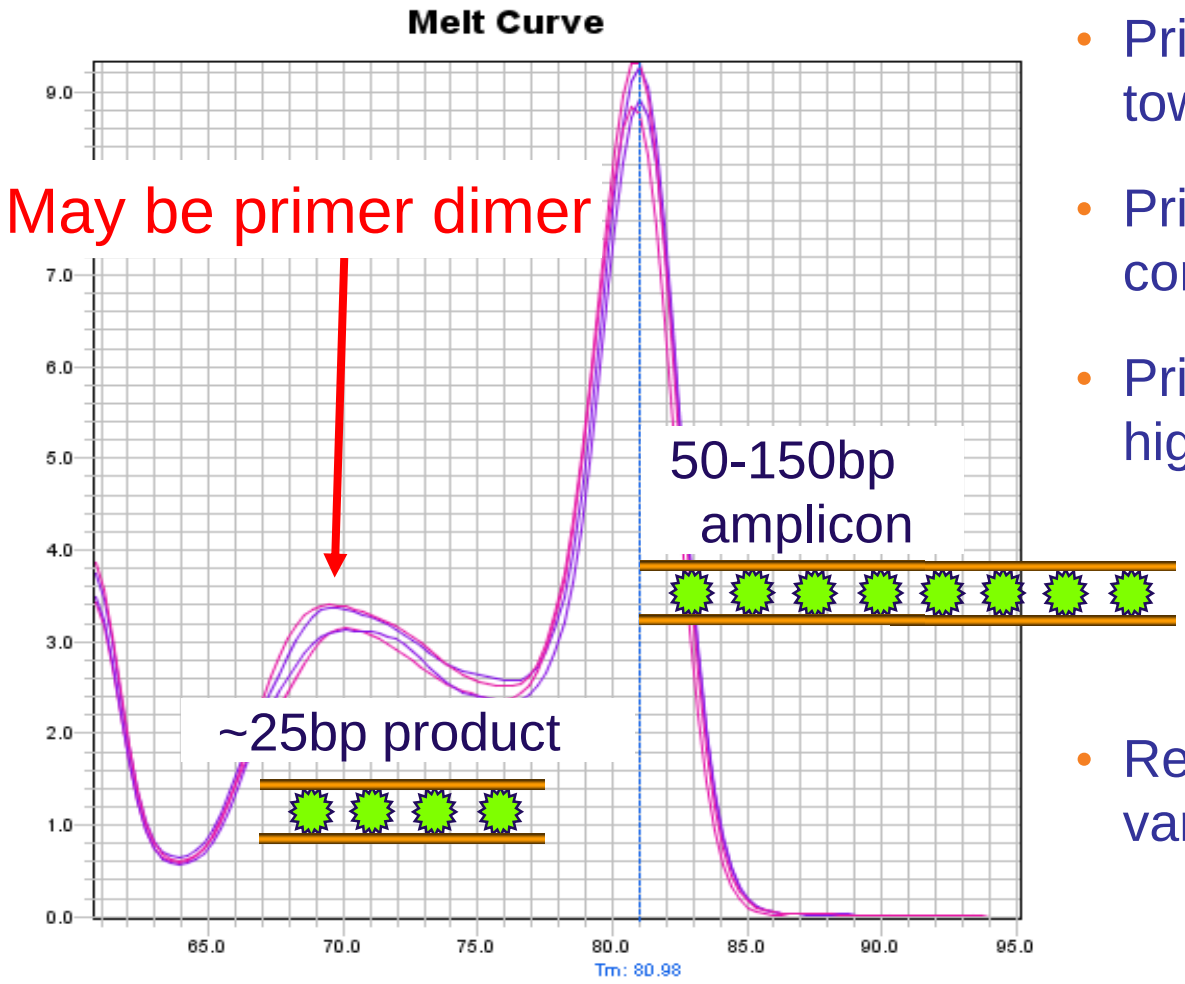
Extra peaks in melt curve



- Primer dimers can contribute towards fluorescent signal
- Primers are too complementary for each other
- Primer : template ratio is too high
- Resolved by testing out various primer concentrations

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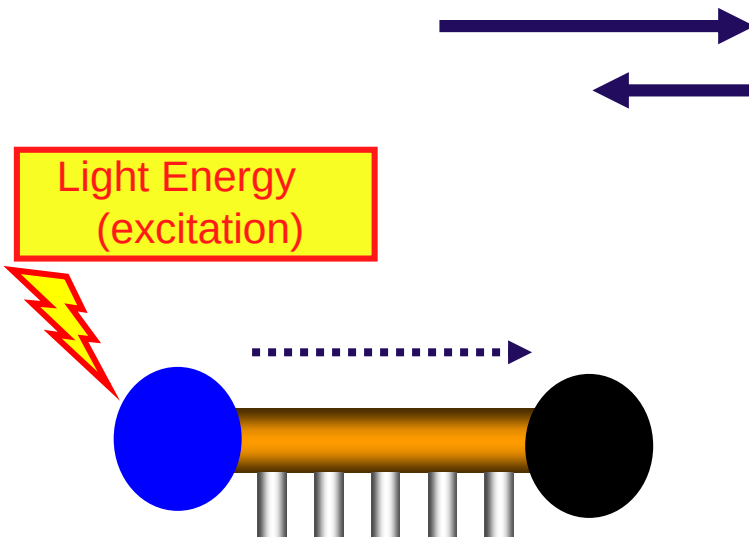
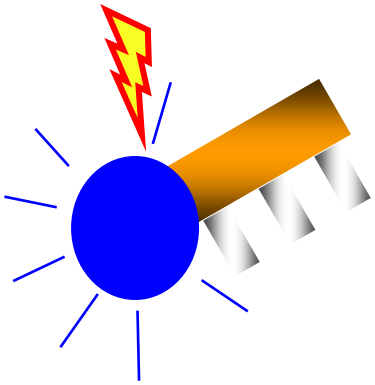
TaqMan Probe

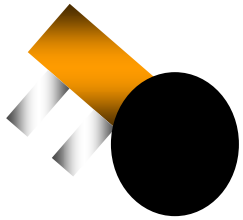
- **FRET**; phenomenon that describes an energy transfer mechanism between 2 fluorescent molecules when in close proximity.



No fluorescent signal (emission) from reporter



Signal



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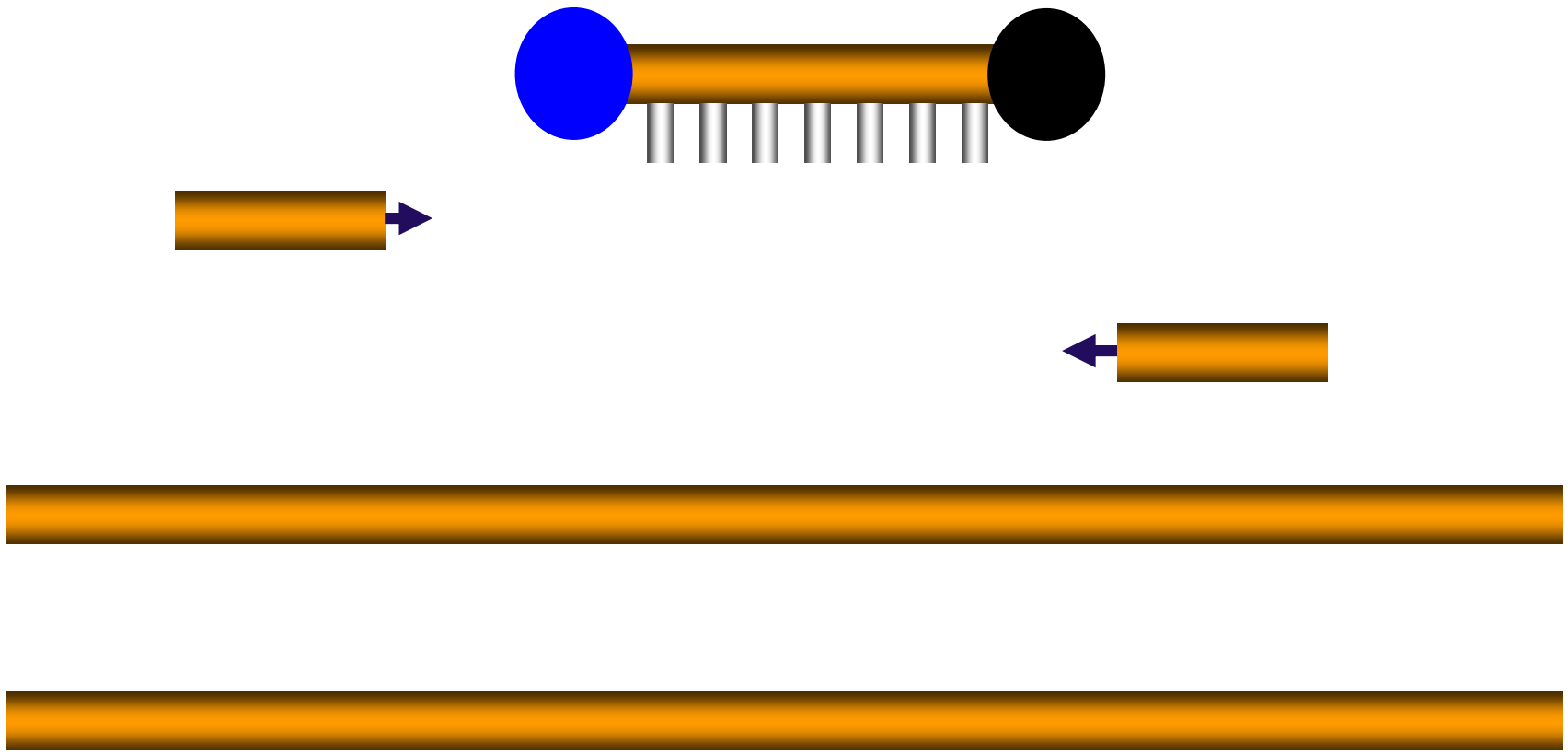
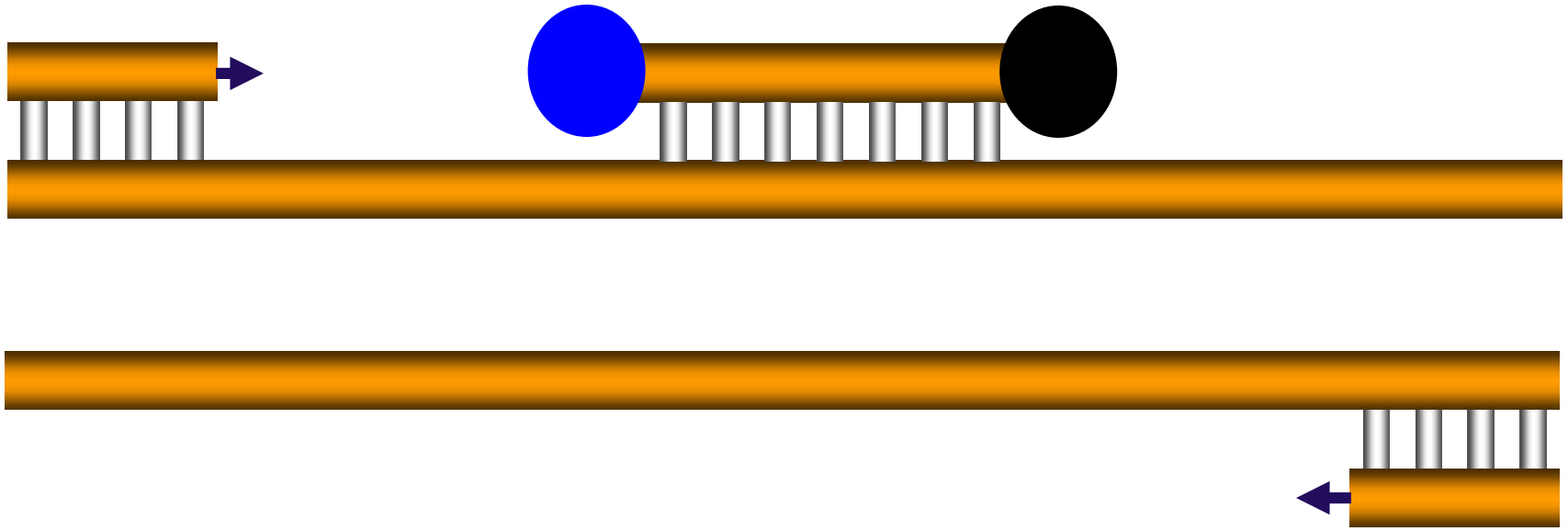
Denaturation (95º)



Annealing (60º)



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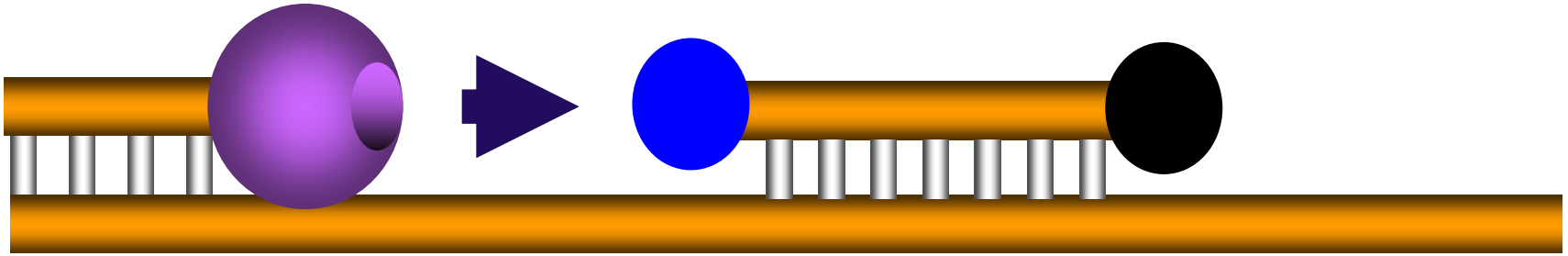
Taq polymerase binds, then extends from upstream primer



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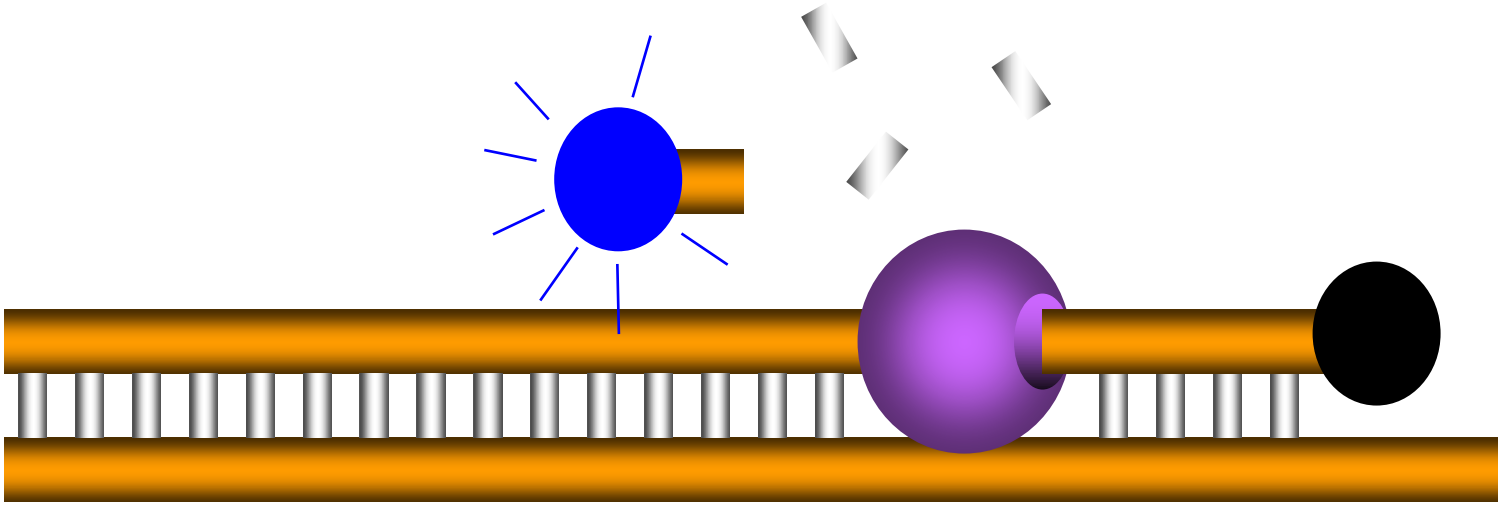
Taq cleaves probe → releases reporter dye



5’-Nuclease activity digests probe

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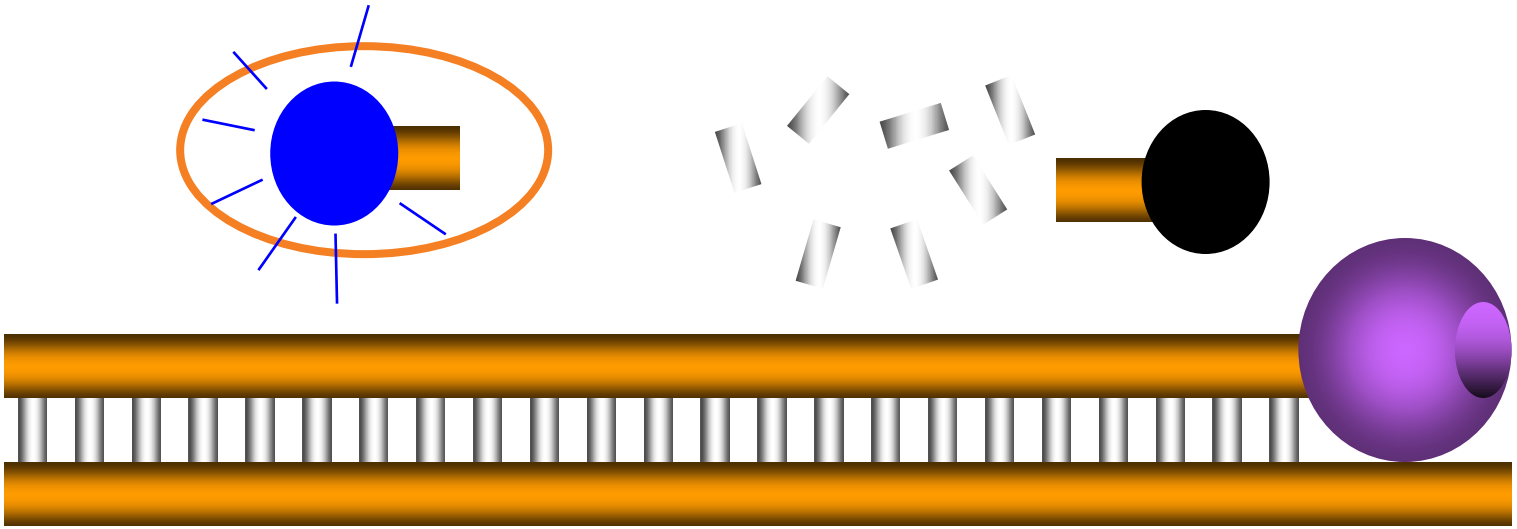
Probe digested; *Taq* completes product



Reporter signal generated in tube/well

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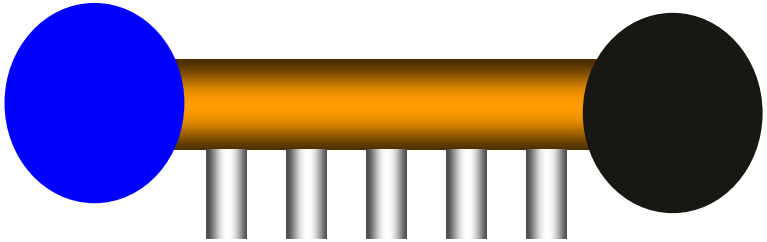
TaqMan ® probe dye Choices



Reporter

(high energy)

Quencher

(low energy)

FAM™

VIC™

ABY™

JUN™

MGB (non-fluorescent)

TAMRA™

QSY - for multiplexing >2 targets

- easily convert from BHQ

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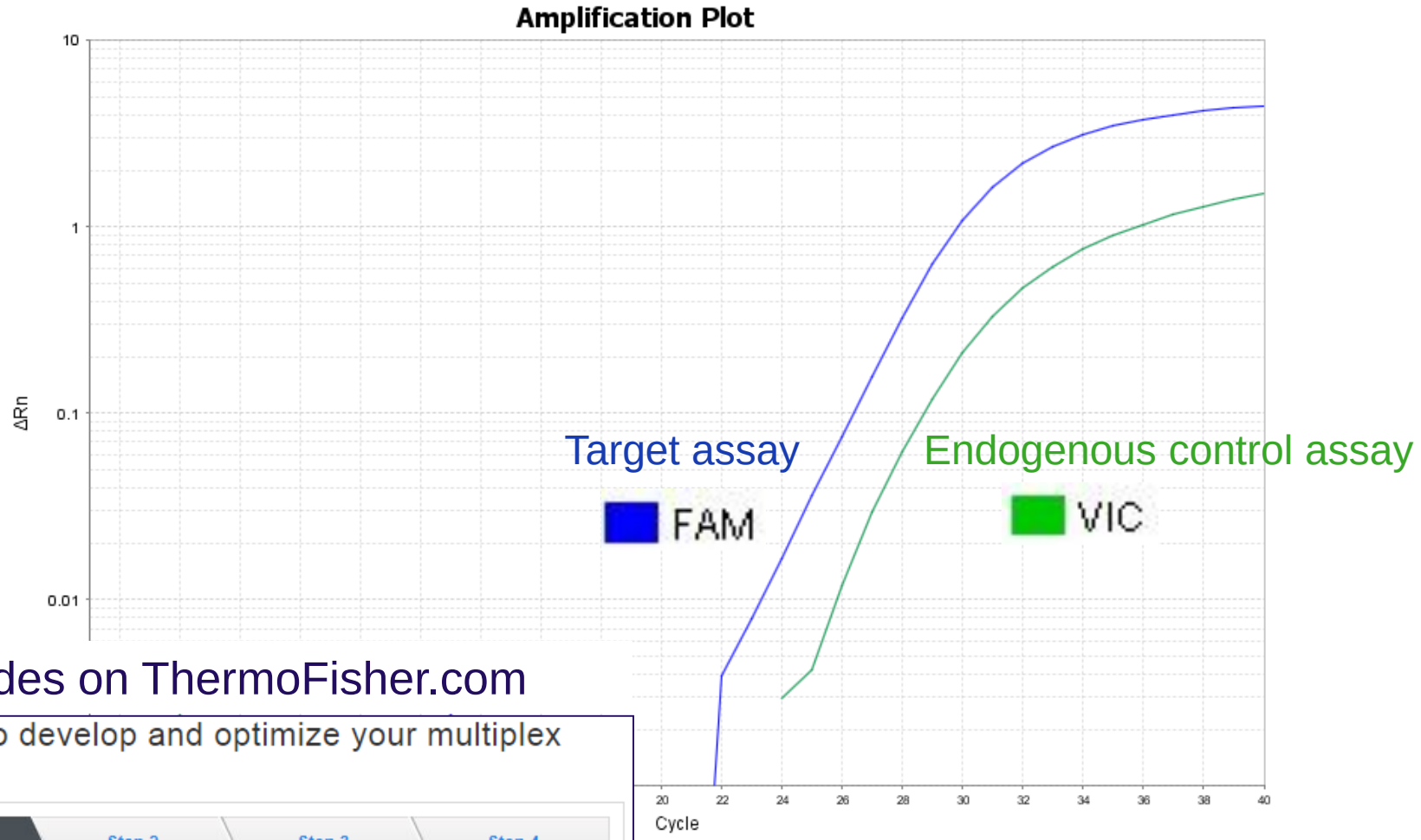
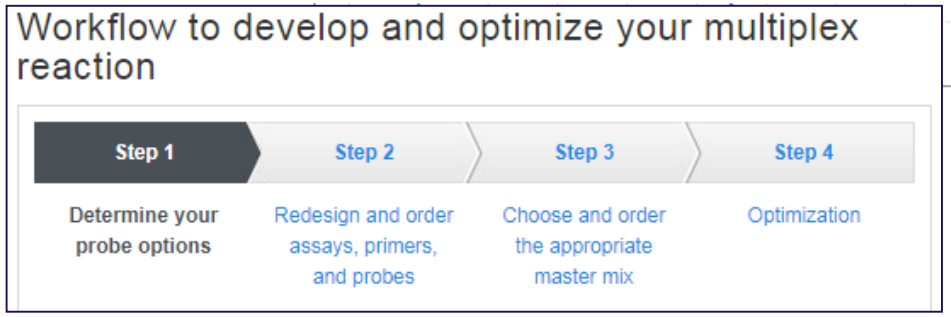
Pure Dye Spectra of System Enables Multiplexing (TaqMan chemistry)



guides on ThermoFisher.com



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Assay Set-up

SYBR

- primer optimization experiment… 300nM, 200nM, 100nM
- Use the concentration that provides the strongest amplification fluorescent signal and a single product in the melt curve

TaqMan

- Primers @ 900nM, Probe @ 250nM (final concentration in reaction).
- Probe Tm (68-70c) is ~10c higher than primers (58-60c)
- Pre-designed assays (**20X no need to worry about this!**)

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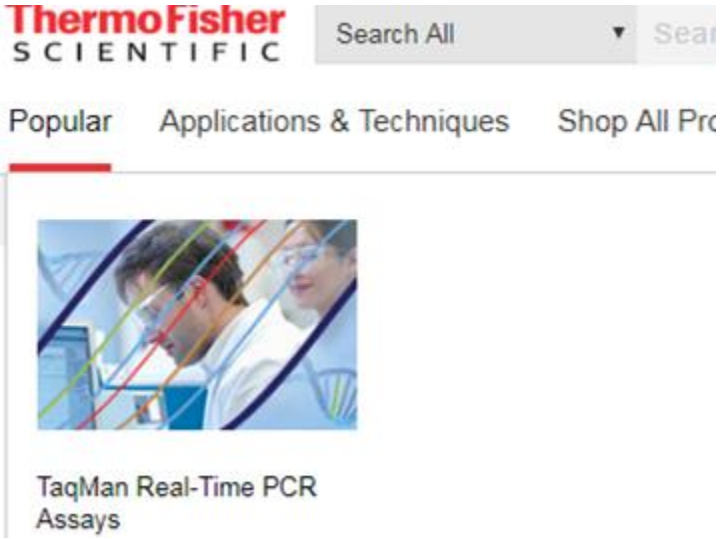
TaqMan® Gene Expression Assays



Human	Soybean	Wine grape
Mouse	Rhesus Monkey	Clawed frog
Rat	Rabbit	Chicken
Arabidopsis	Rice	Wheat
Cattlle	Pathogen	Frog
Nematode	Baker's yeast	Chinese hamster
Dog	Fission yeast	Maize
Guinea Pig	Pig	Fruit fly
Zebrafish	Bread wheat	Corn
Horse		

2,300,000
Pre-developed Assays

1 tube contains; Primers & probe (Optimized at 20x concentration)

"Probe spans Exons": no gDNA detected

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Popular Applications

Connect Your Lab

TaqMan Real-Time PCR Assays

Cell Culture Plas

Hs02786624_g1 GAPDH 4 RefSeq (NM) Both primers 157 Dye: FAM-MGB

View Details ▾ Re

99 / 100 Bioz Stars

Hs0275

View Details ▾ Re

99 / 100 Bioz Stars

Hs0442

View Details ▾ Re

99 / 100 Bioz Stars

Hs0442

Catalog number: 4331182
Target species: Human
Important Information

View Details ▾ Related Reagents ▾ Related Controls ▾ View Assay Map ▾

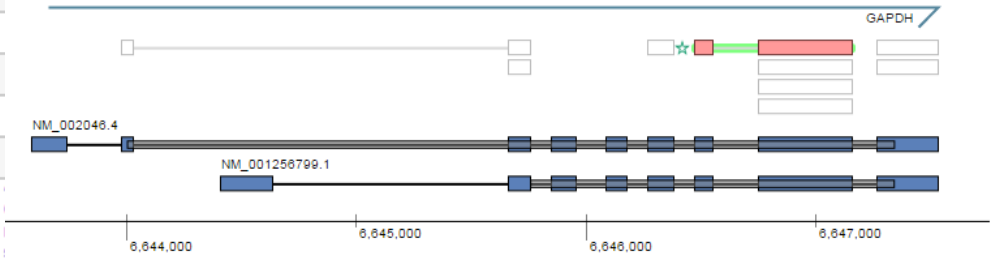
Availability: Inventoried
Price (USD): 173.00
Check your price
Add to cart

Gene Transcripts

Gene Symbol	GAPDH
Entrez Gene ID	2597
Gene Name	glyceraldehyde-3-phosphate dehydrogenase
Gene Aliases	CDABP0047, G3PD, GAPD
Location Chromosome	Chr.12: 6643657 - 6647536 on Build 37
UniGene	Hs.544577

Interrogated Sequence		Translated Protein	Exon Boundary	Assay Location	Amplicon Length
RefSeq	NM_001256799.1	NP_001243728.1	6 - 7	728	93
	NM_002046.4	NP_002037.2	7 - 8	704	93
GenBank mRNA	AB062273.1	-	7 - 8	582	93
	AF261085.1	-	7 - 8	633	93
	AK026525.1	-	7 - 8	655	93
	AK308198.1	-	7 - 8	606	93
	AY007133.1	-	7 - 8	582	93
	AY633612.1	-	6 - 7	554	93
	BC001601.1	-	7 - 8	584	93
	BC004109.2	-	7 - 8	582	93
	BC009081.1				
	BC013310.2				
	BC020308.1				
	BC023632.2				
	BC025925.1				
	BC026907.1				

Build 37.1 Human Chr.12: Hs02758991_g1 ×



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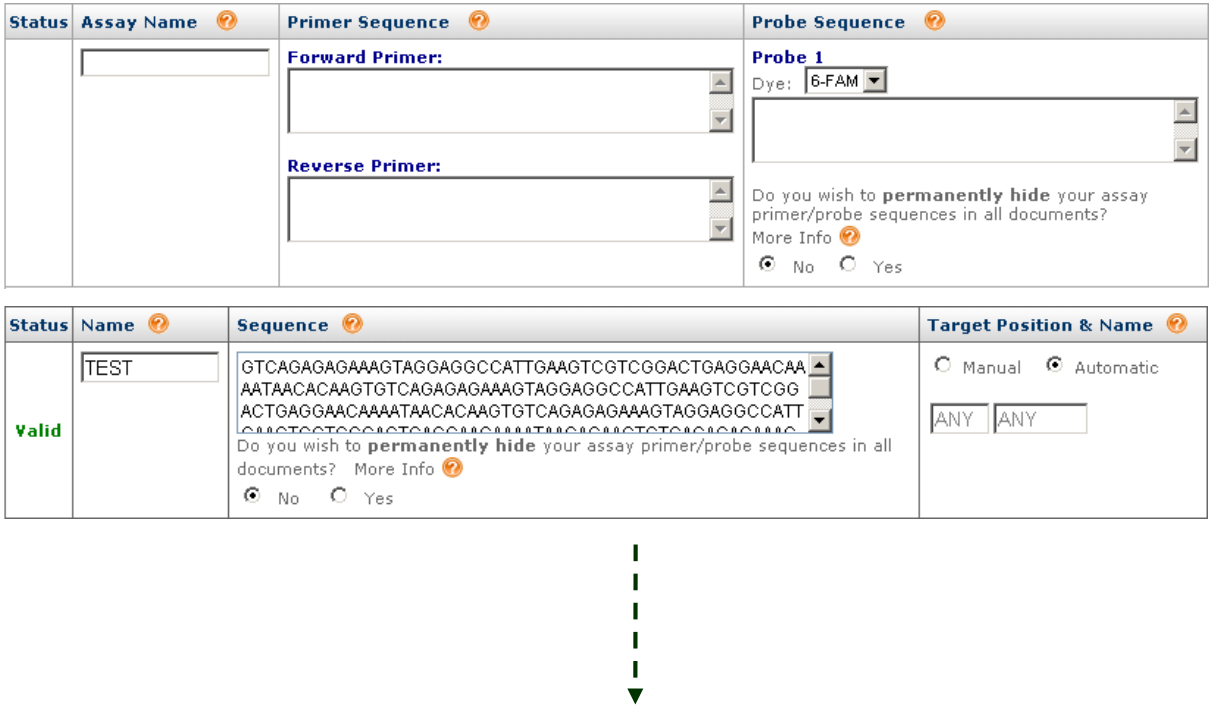
Custom TaqMan® Assay Design Tool



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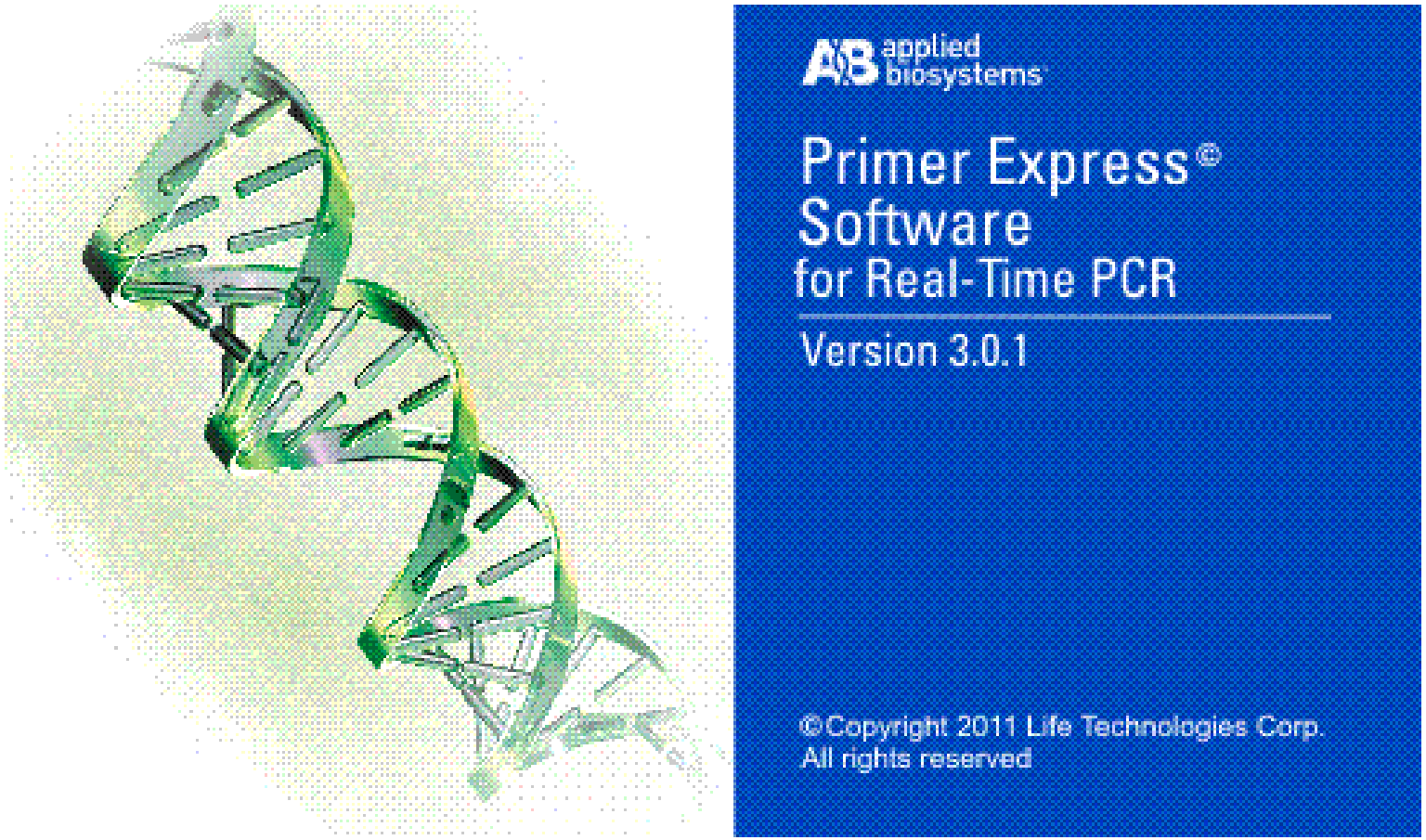
Primer Express® Software



https://www.thermofisher.com/order/catalog/product/4363991

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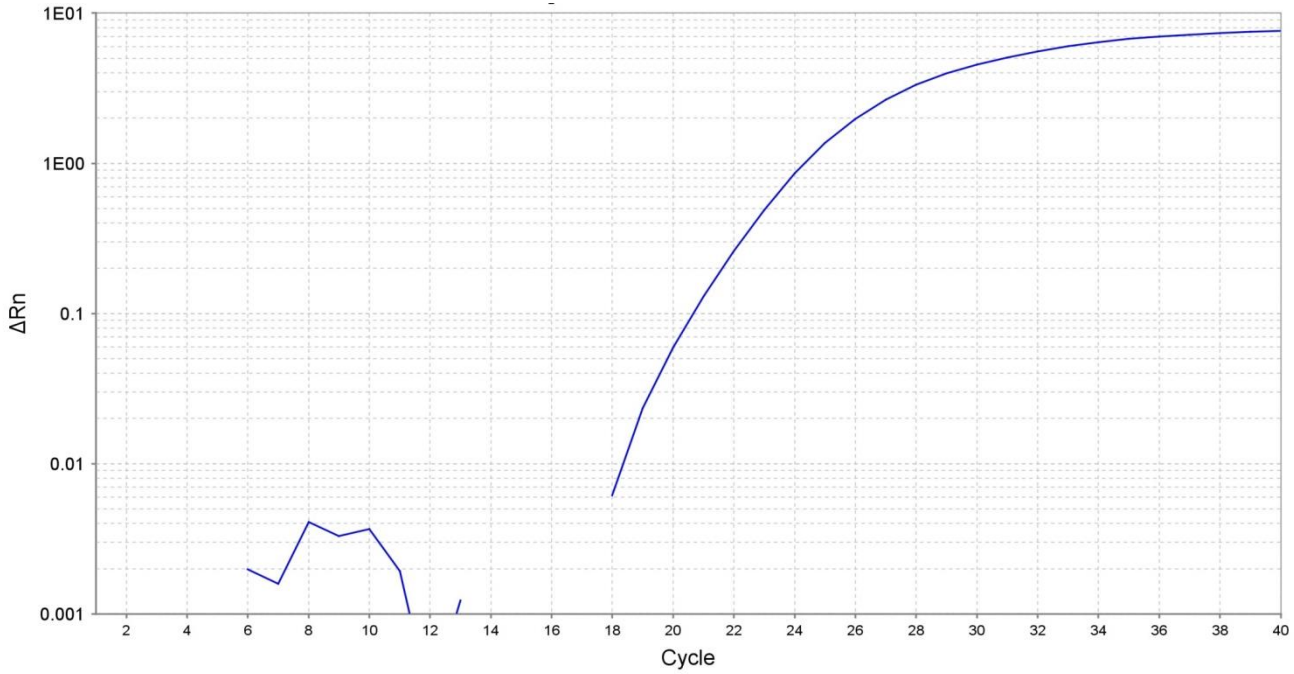
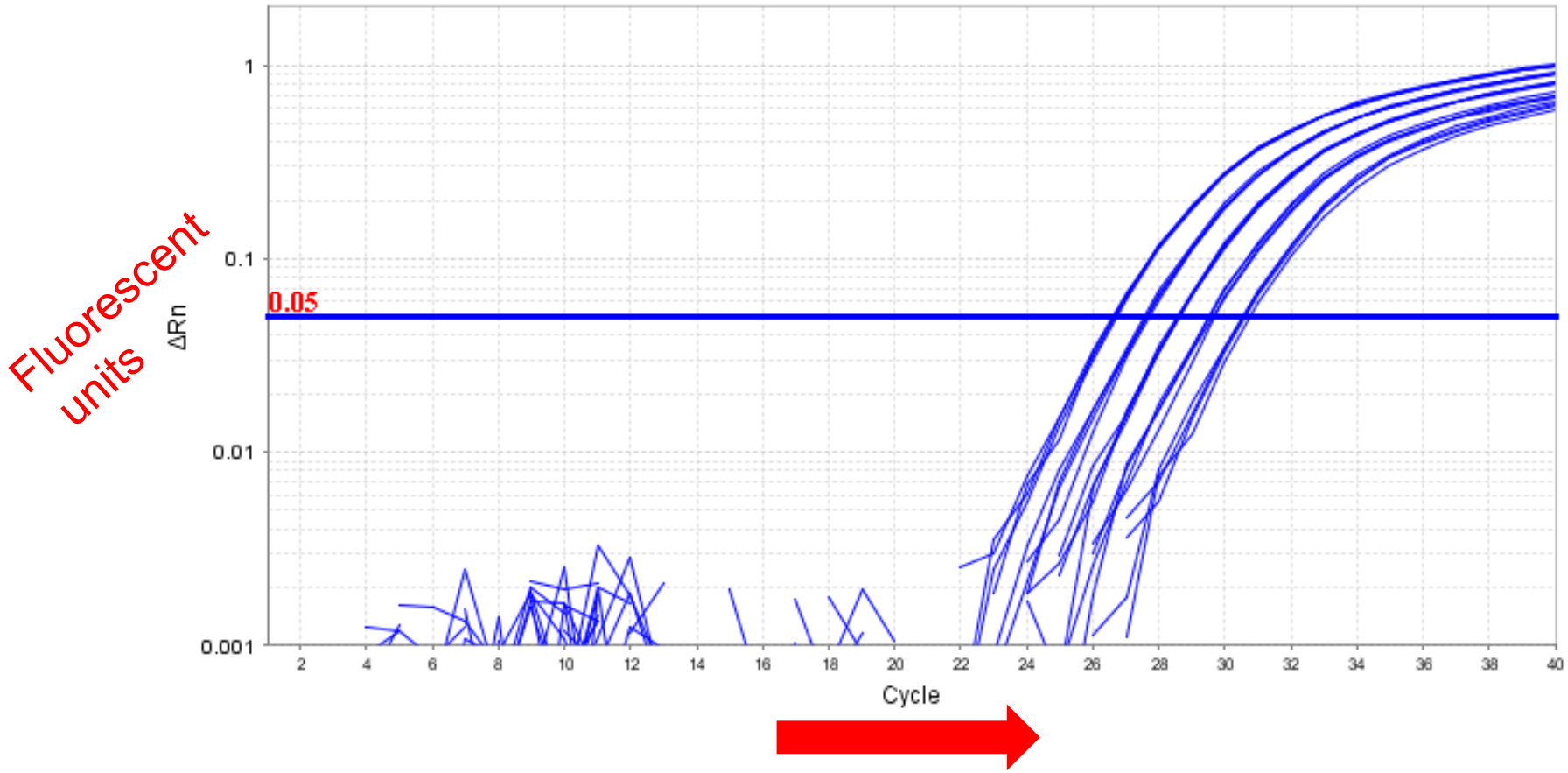
Examining qPCR amplification plots



SCIENTIFIC

Real-time PCR Terminology

Amplification plots
graph cycle vs. Δ in fluorescence



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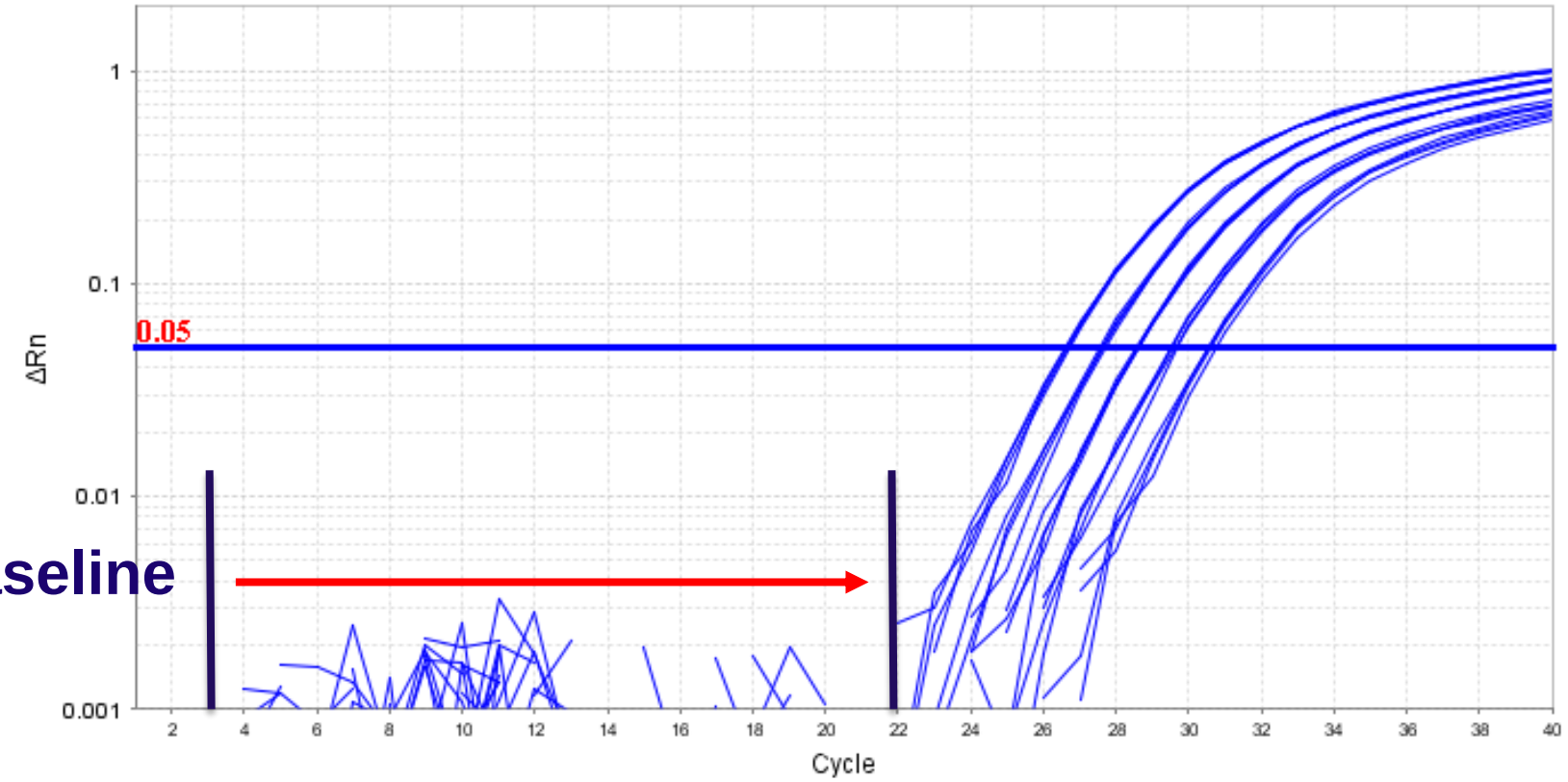
Baseline

Defines region of background noise by a cycle-to-cycle range ex. cycles 3-22



Baseline

Automatic/manual baseline

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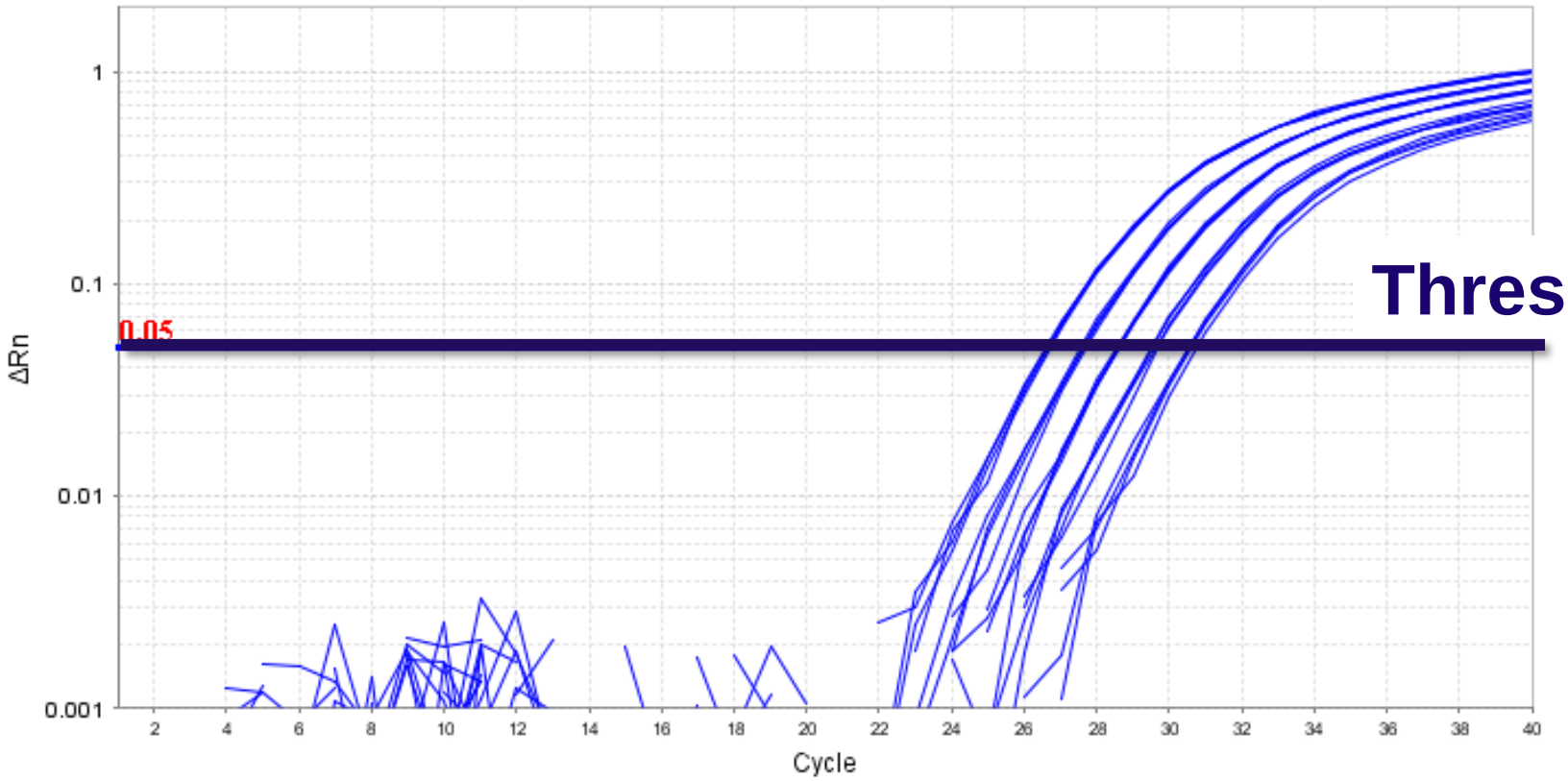
Threshold

An adjustable horizontal line in the amplification plot.
Tells the software where to capture the data.



AutoThreshold (default); Software sets the threshold for each assay.
Different assays may have different exponential phases

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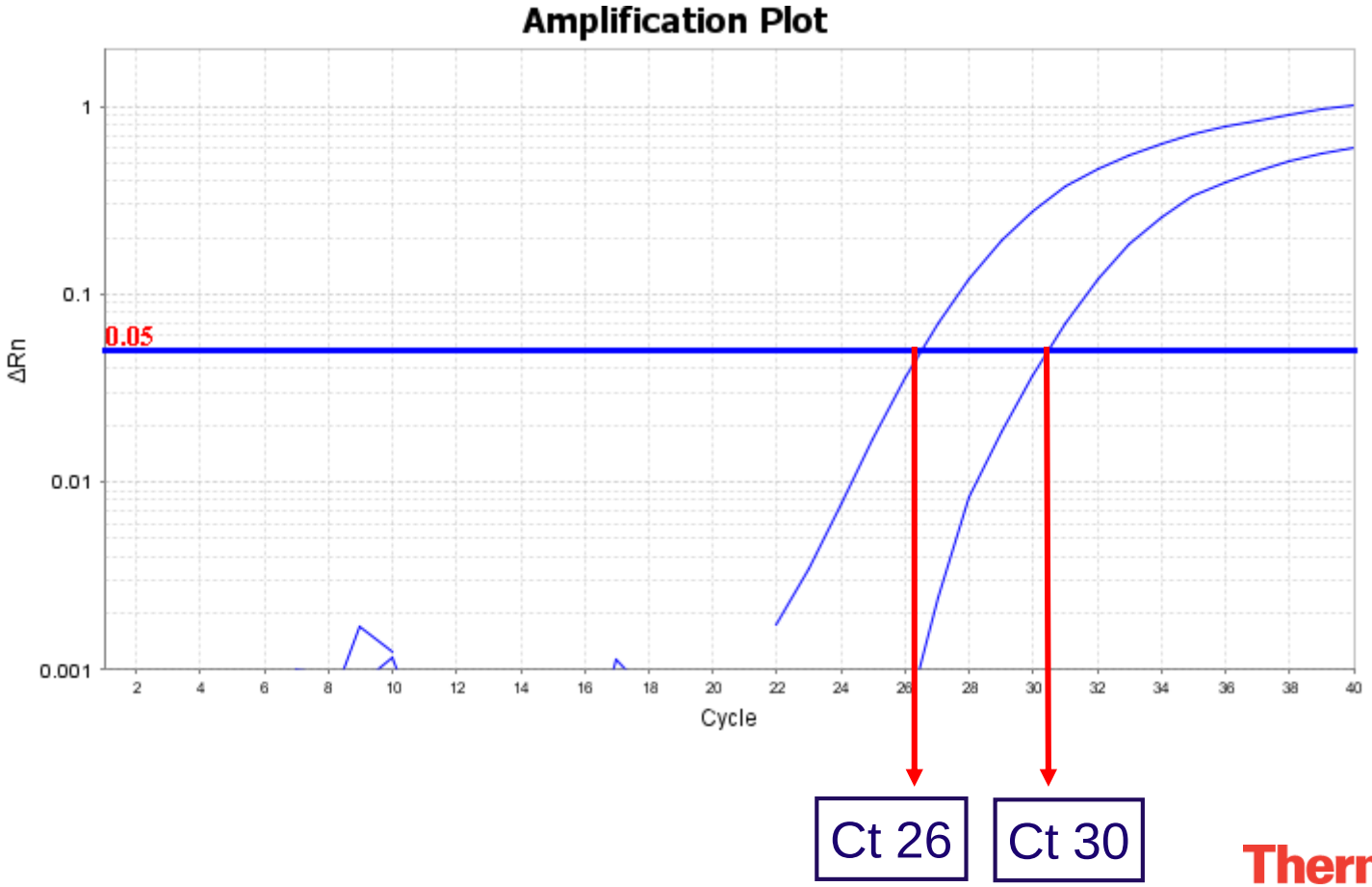
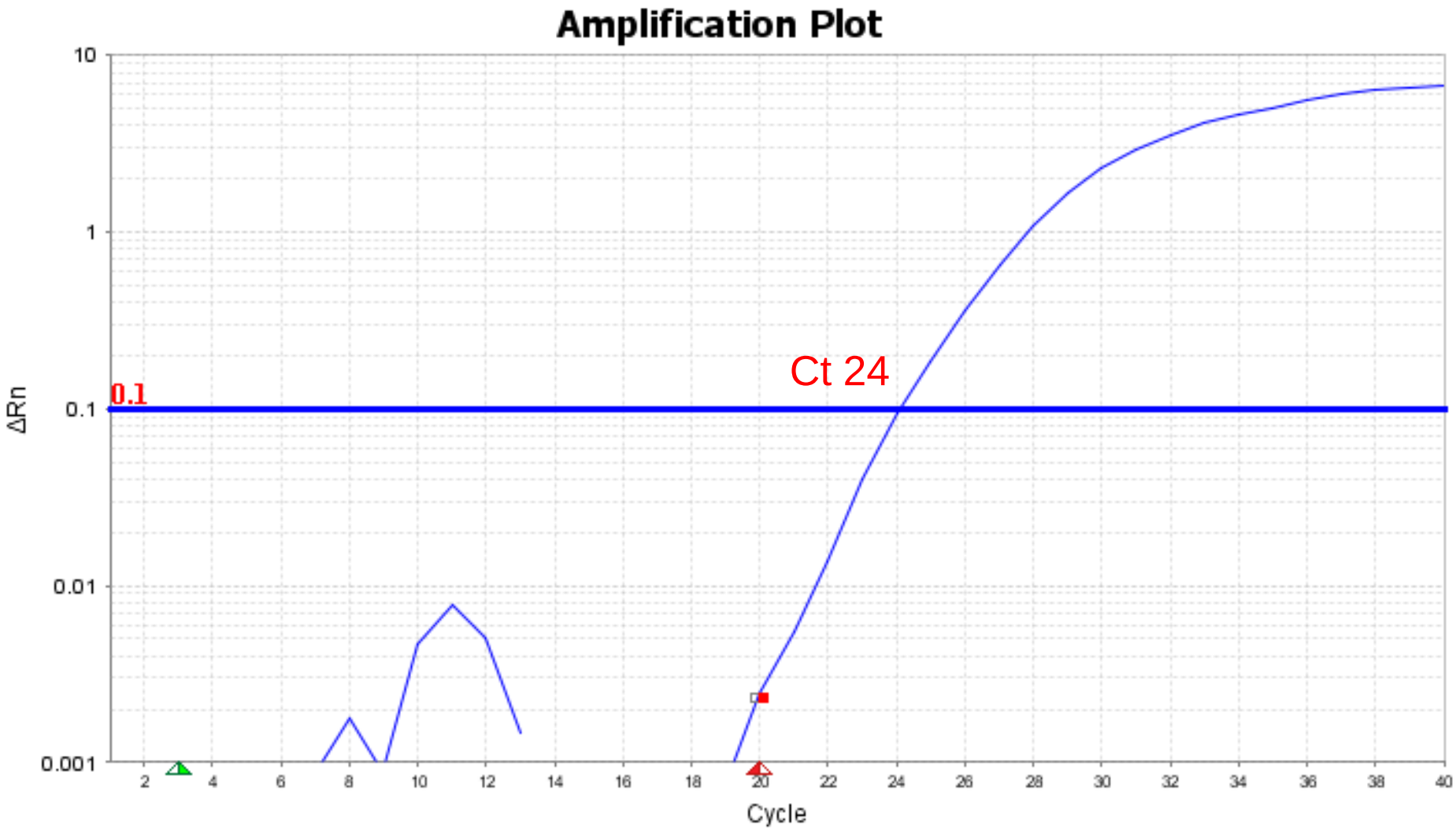
Cycle threshold (Ct)

The fractional cycle number at which each amplification curve crosses the threshold.



ThermoFisher SCIENTIFIC

Summary of Ct algorithm (“baseline threshold”)



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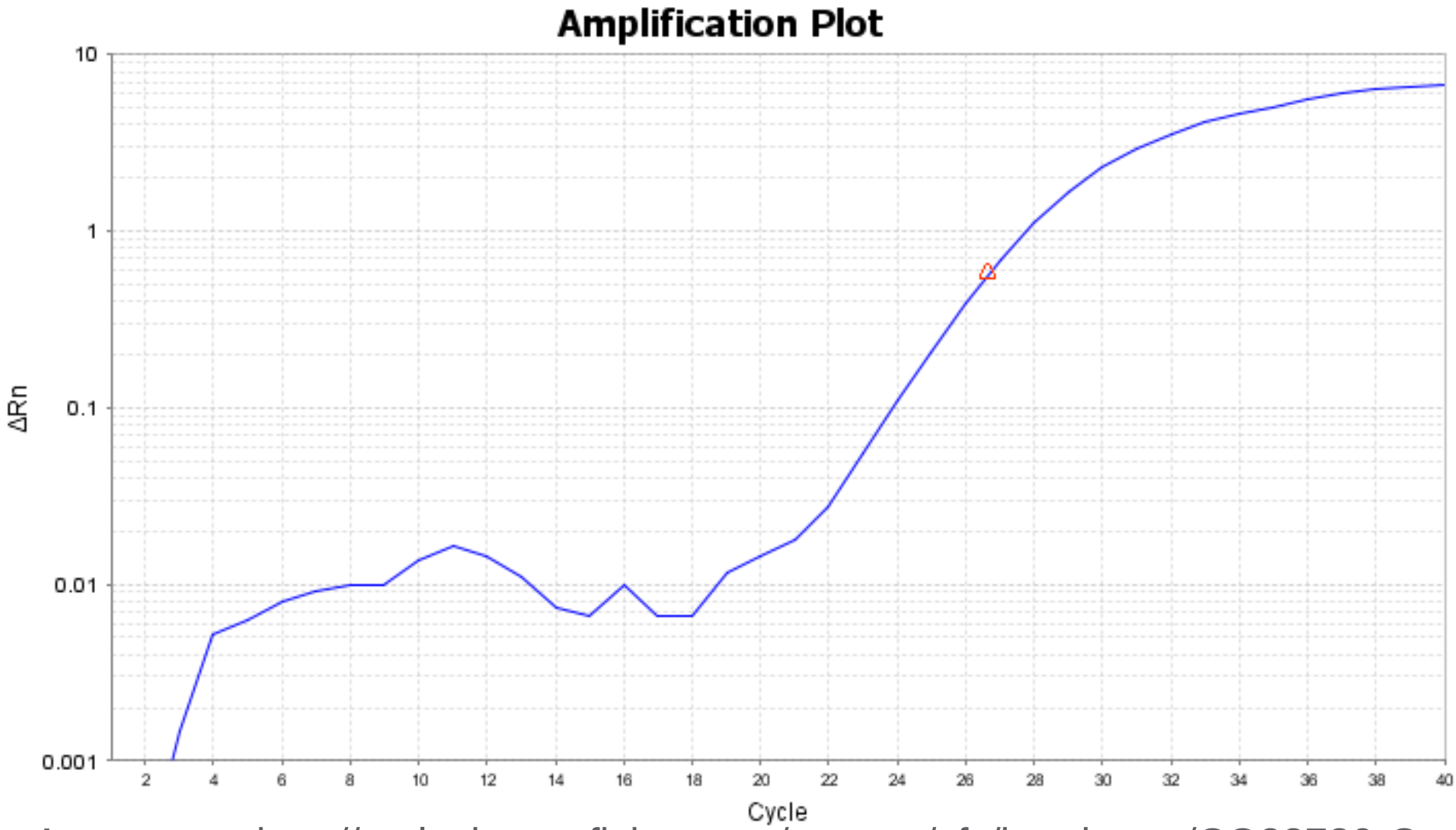
CRT algorithm (“relative threshold”)



App note: http://tools.thermofisher.com/content/sfs/brochures/CO28730-Crt-Tech-note_FLR.pdf

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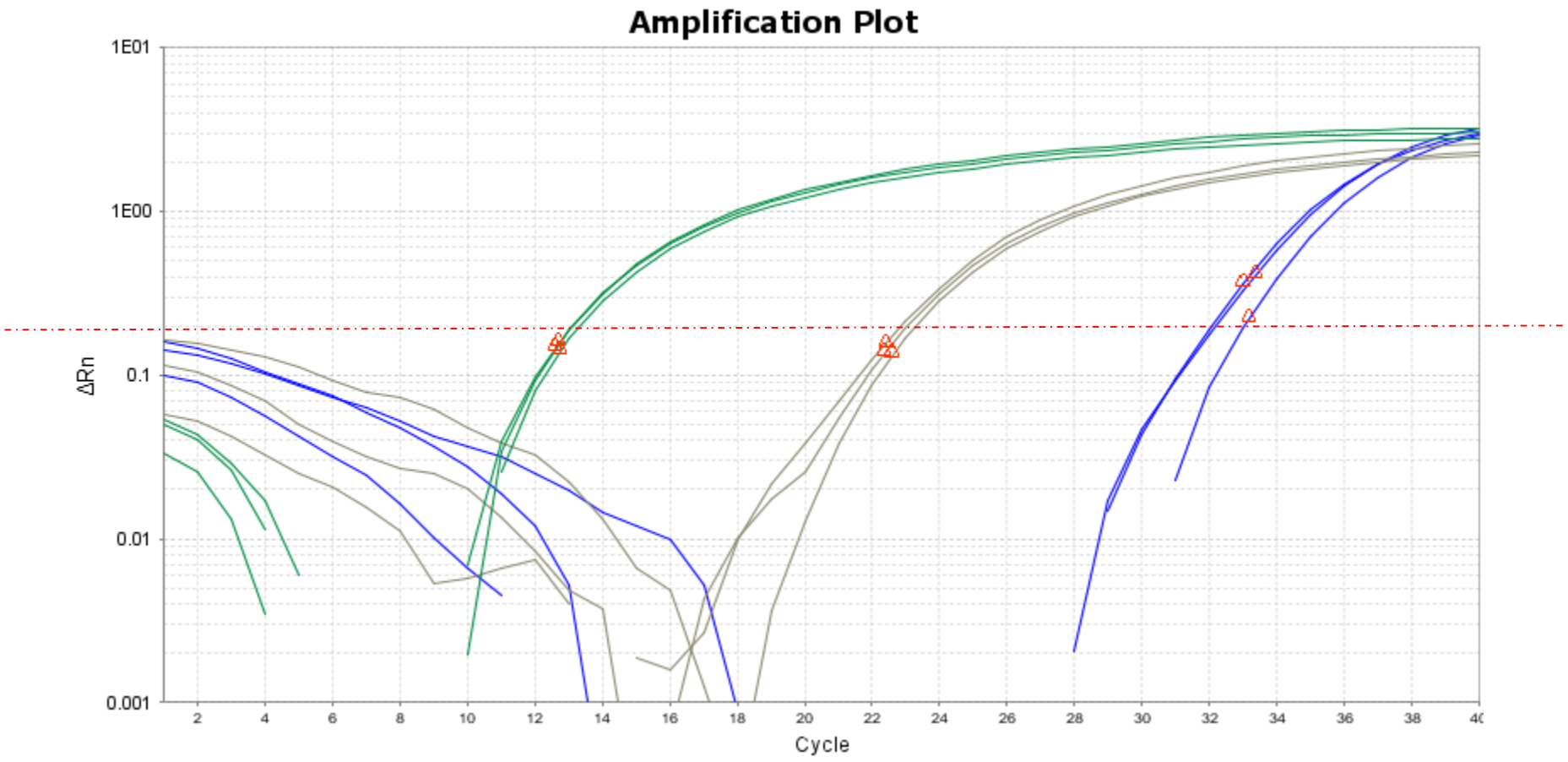
CRT may help out for samples that may need to be omitted with CT, to tighten up technical replicates.



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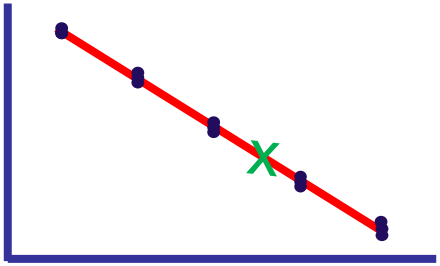
Use Ct values to provide quantitative results

Absolute Quantitation calculates a copy number by using standard curve analysis

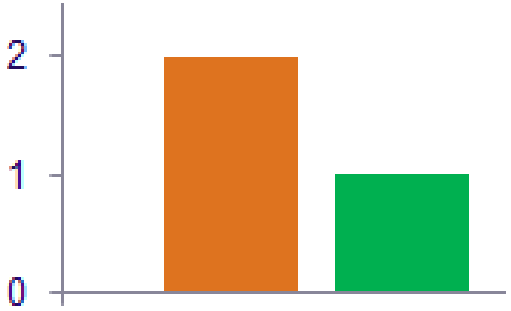
"I have 500 copies of TNFα in sample 1"



ΔΔCt method calculates relative fold change

- no standard curves, assays have similar efficiencies

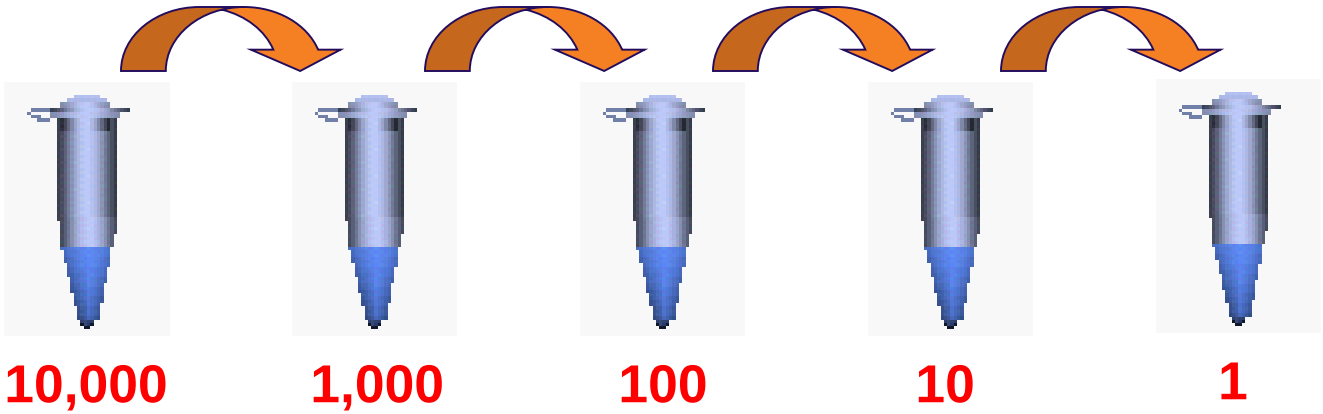
"TNFα is upregulated 2 fold in sample 1 compared to the control sample"

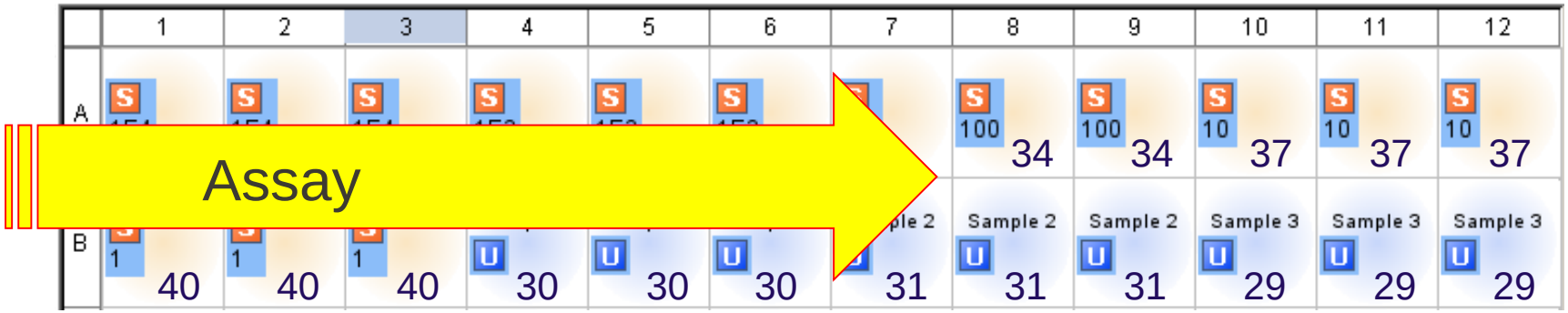


Generate an absolute standard curve

Recommend 5 or more, point standard curve of 10 fold dilutions

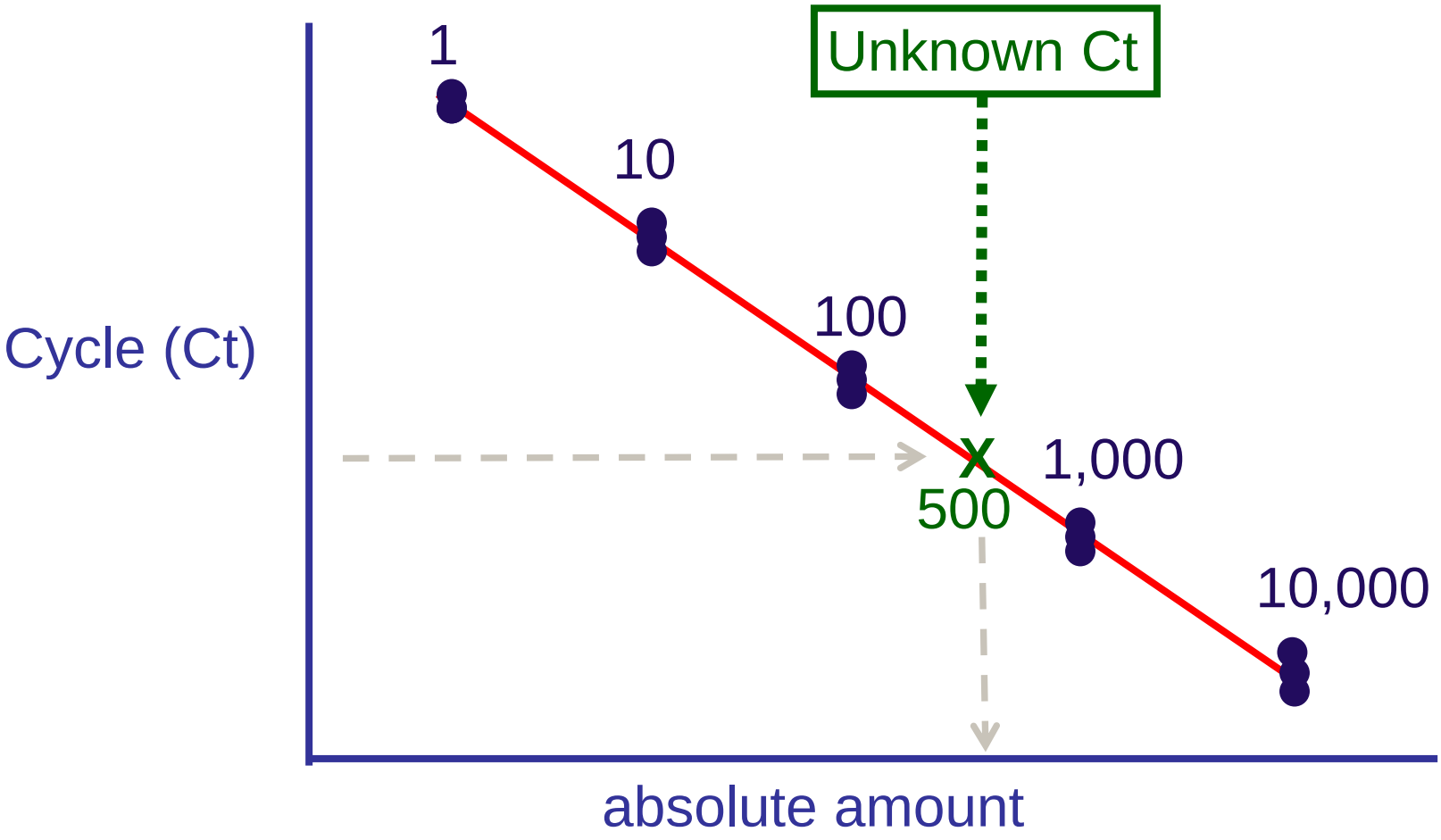
Do serial dilutions





http://www6.appliedbiosystems.com/support/tutorials/pdf/quant_pcr.pdf

After a run, plot unknown Ct on curve



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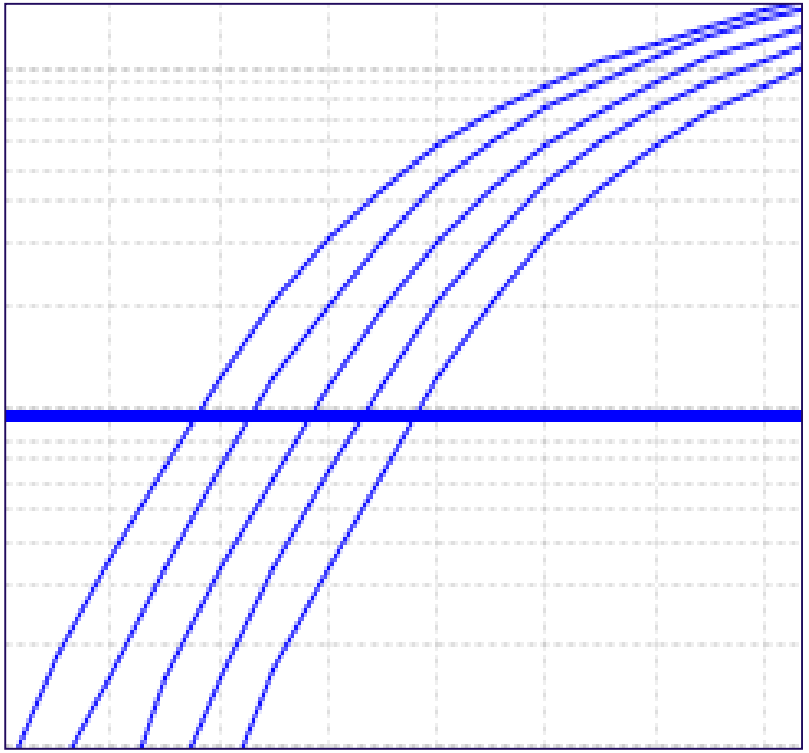
Another way to analyze expression data for relative comparisons (2^n)

ΔCt of 1 = 2^1 = 2 fold difference
ΔCt of 2 = 2^2 = 4 fold
ΔCt of 3 = 2^3 = 8 fold
ΔCt of 3.3 = $2^{3.3}$ = 10 fold
ΔCt of 4 = 2^4 = 16 fold



This assumes that the amount of product is doubling with each cycle in the exponential phase, when it does, we call this 100% amplification efficiency.

Standard curve statistics example:

Slope:	-3.384	R^2:	0.995	Eff%:	97.477

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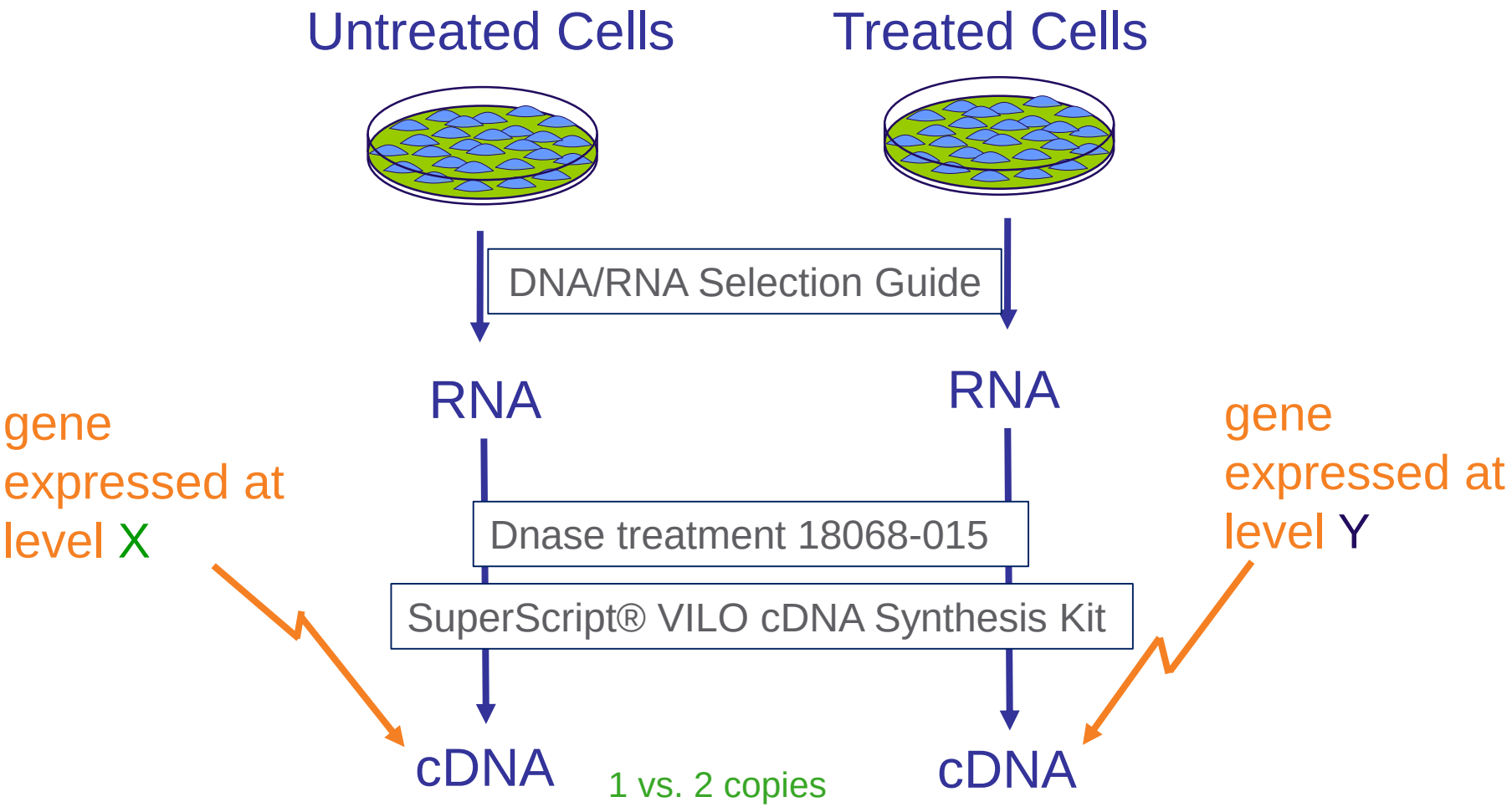
ΔΔCt

Question: What happens to the expression of a gene when I apply a treatment?



Answer: Expressed as a relative fold change

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In relative gene expression experiments, we examine two types of genes. . .

Gene of interest (target)

Normalizing gene (control)

A gene that is expressed consistently in all samples in an experiment. (a.k.a. “control gene,” “housekeeping gene,” “endogenous control”, “reference gene”)

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TaqMan® Endogenous Control Array

-Human & Rodent available
-Includes 32 commonly studied control genes
-Genes range in expression from:
high, medium, low (ex. 18s, GAPDH, HPRT1)

Gene Symbols	1	2	3	4	5	6	7	8	9	10	11	12
A	18S	GAPDH	HPRT1	GUSB	18S	GAPDH	HPRT1	GUSB	18S	GAPDH	HPRT1	GUSB
B	ACTB	B2M	HMBS	IPO8	ACTB	B2M	HMBS	IPO8	ACTB	B2M	HMBS	IPO8
C	PGK1	RPLP0	TBP	TFRC	PGK1	RPLP0	TBP	TFRC	PGK1	RPLP0	TBP	TFRC
D	UBC	YWHAZ	PPIA	POLR2A	UBC	YWHAZ	PPIA	POLR2A	UBC	YWHAZ	PPIA	POLR2A
E	CASC3	CDKN1A	CDKN1B	GADD45A	CASC3	CDKN1A	CDKN1B	GADD45A	CASC3	CDKN1A	CDKN1B	GADD45A
F	PUM1	PSMC4	EIF2B1	PES1	PUM1	PSMC4	EIF2B1	PES1	PUM1	PSMC4	EIF2B1	PES1
G	ABL1	ELF1	MT-ATP6	MRPL19	ABL1	ELF1	MT-ATP6	MRPL19	ABL1	ELF1	MT-ATP6	MRPL19
H	POP4	RPL37A	RPL30	RPS17	POP4	RPL37A	RPL30	RPS17	POP4	RPL37A	RPL30	RPS17

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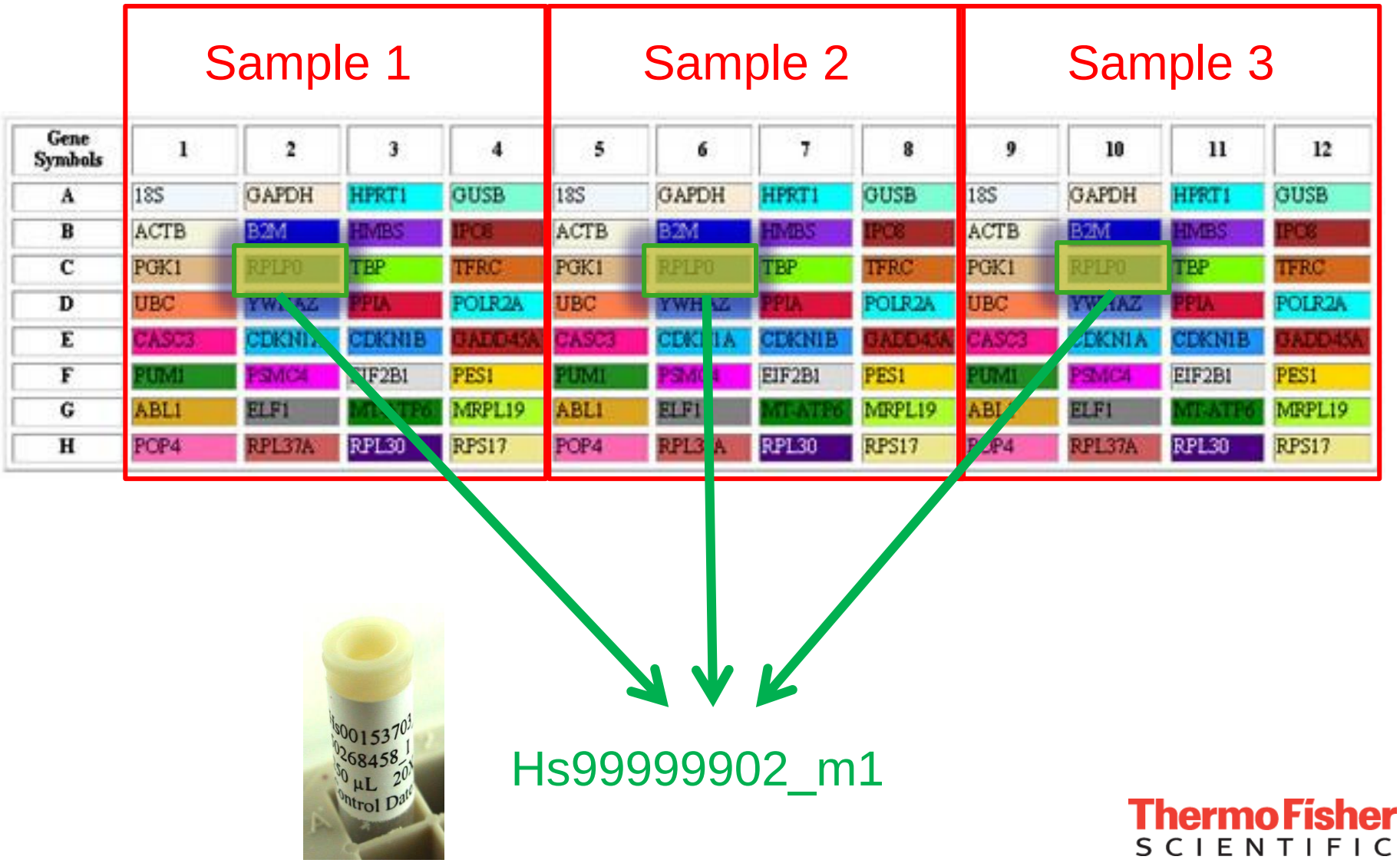
TaqMan® Endogenous Control Array
(Human & Rodent available)



Hs99999902_m1

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Example of ΔΔCt Math

Sample	X	N
Treated 1	24	14
Treated 2	20	11
Treated 3	28	12
Untreated	24	13

X = Target N = Normalizing gene

Example of ΔΔCt math

Sample	X	N		ΔCt
Treated 1	24 -	14	=	10
Treated 2	20 -	11	=	9
Treated 3	28 -	12	=	16
Untreated	24 -	13	=	11

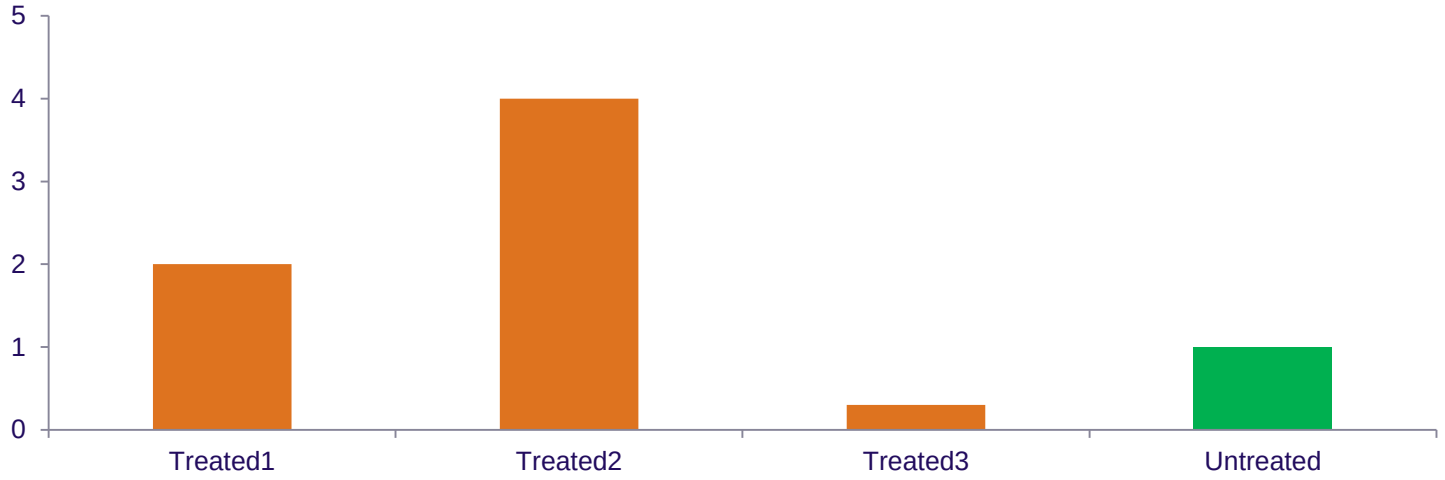
Choose a **calibrator**; all samples are relatively expressed to this sample

Sample	X	N	ΔCt	ΔΔCt
Treated 1	24	14	10 – 11 =	-1
Treated 2	20	11	9 – 11 =	-2
Treated 3	28	12	16 – 11 =	5
Untreated	24	13	11 – 11 =	0

Example of ΔΔCt math

RQ (fold change)

Sample	X	N	ΔCt	ΔΔCt	$2^{-\Delta\Delta Ct}$	
Treated 1	24	14	10	-1	2	↑
Treated 2	20	11	9	-2	4	↑
Treated 3	28	12	16	5	0.3	↓
Untreated	24	13	11	0	1	



Software can perform the calculations!

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Other Tips & Information

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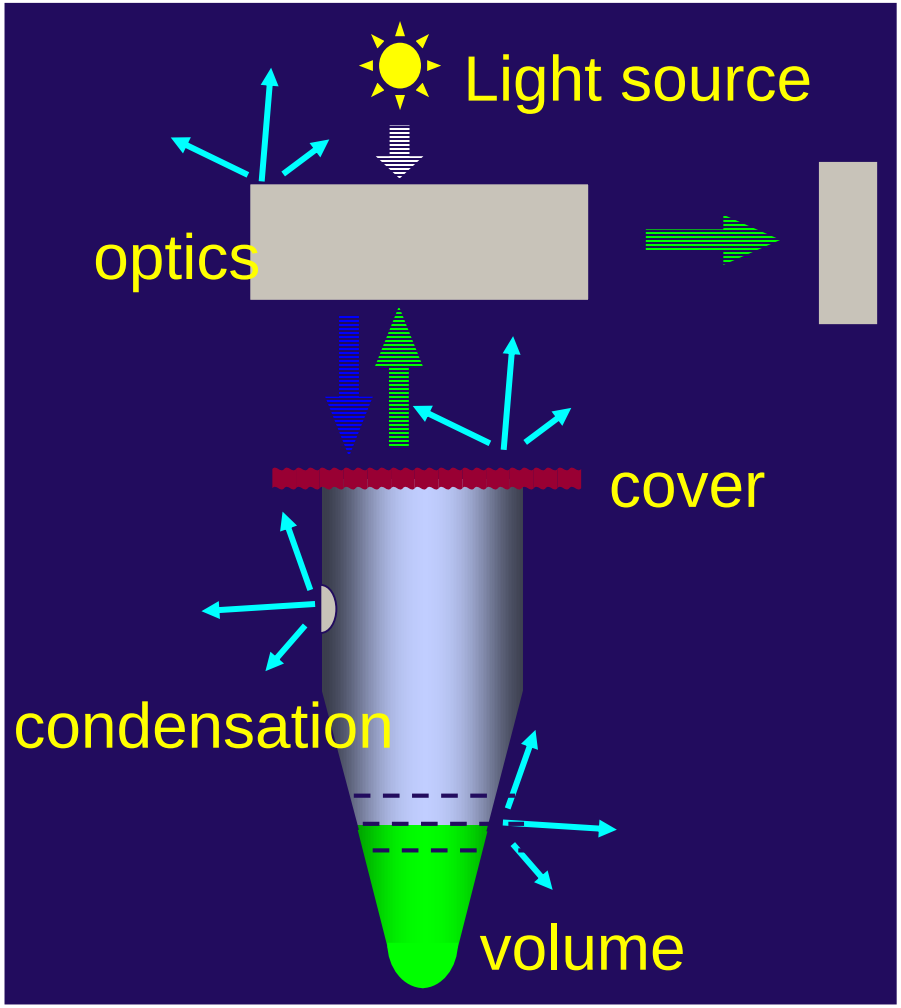

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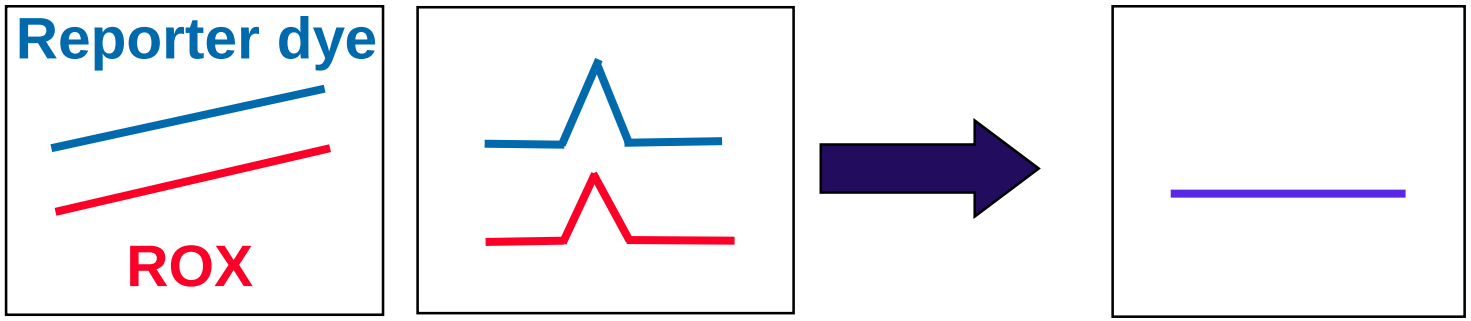
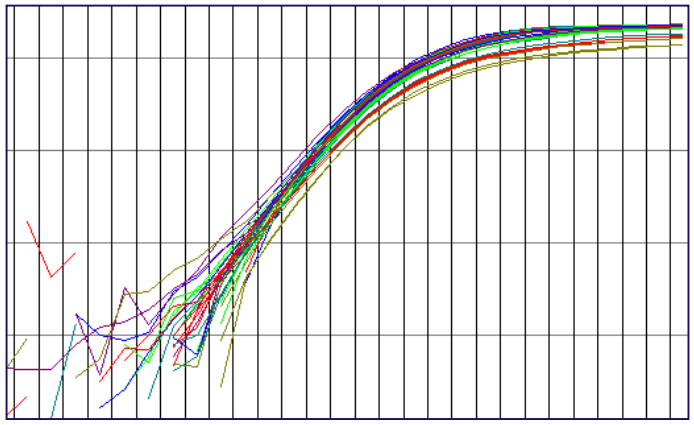
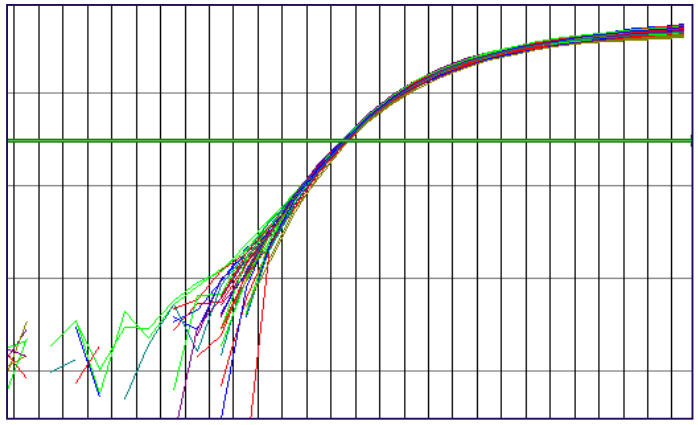
ROX™ Passive Reference Dye

Improves precision of replicates by normalizing for non-PCR related variations.







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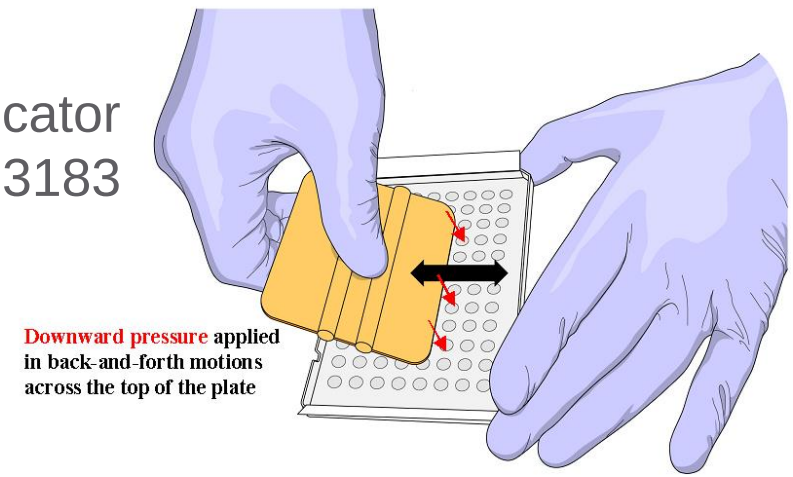
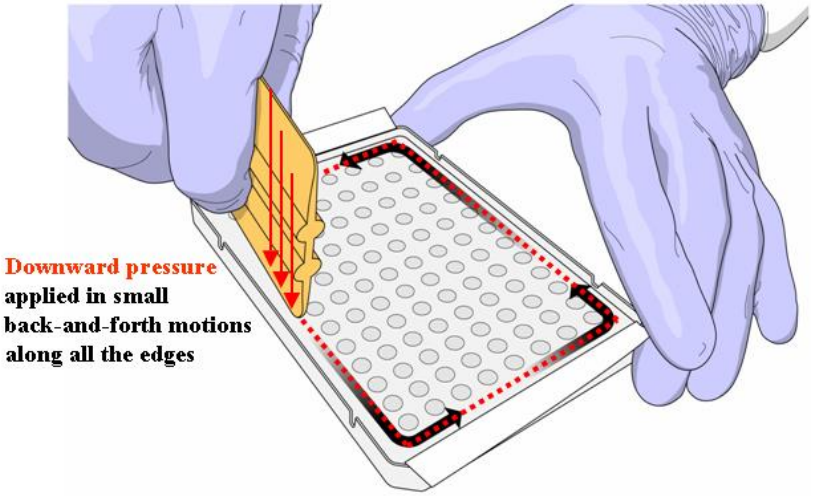
Prevent evaporation & bubbles during PCR

Use adhesive covers . . .

Applicator
#4333183





SPIN PLATES before loading into instrument!!!

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Guides to multiplexing

Real-time multiplexing

Part 1 - What is multiplexing? How does it work?

Multiplexing is the amplification of more than one target in one tube or well on a plate. Duplexing is specifically the amplification of two targets in one tube, triplexing is the amplification of three targets, and so on. Multiplexing is possible with TaqMan® probe-based assays, where each assay has a specific probe, and that probe is labeled with a different colored dye. The instruments can detect the different dyes and measure the signal from each one separately, and use that information to quantitate the amounts of different targets.

How many different dyes can the instruments detect?

The number of different dyes that can be detected varies by instrument. The Applied Biosystems 7500 Real-Time PCR System can detect up to 5 different dyes, and the 7000, 7300, 7700 and 7900 can detect up to 4. However, AB prefers to reserve one of these dyes for a passive reference dye such as ROX™ dye. See Figure 1 and Figure 2.

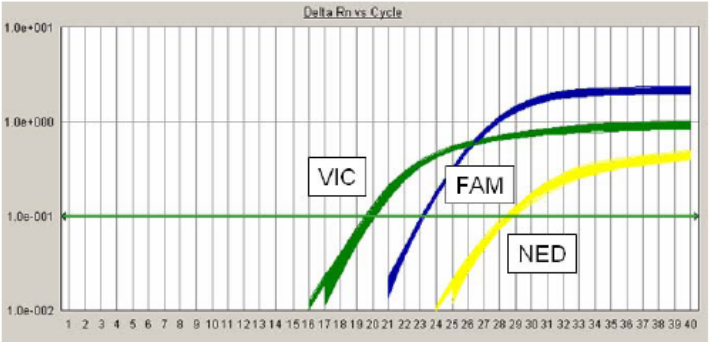

Figure 1 – Three color multiplexing on the AB 7300 instrument. Data is normalized to a fourth dye, ROX™ dye, as a passive reference.

User Bulletin #5

ABI PRISM® 7700 Sequence Detection System

August 10, 1998 (updated 01/2001)

SUBJECT: Multiplex PCR with TaqMan® VIC Probes

Overview Applied Biosystems now offers probes constructed with the new TaqMan® VIC reporter dye. The characteristics of the VIC dye make it an excellent candidate to replace existing TaqMan® JOE and HEX reporter dyes. The increased signal strength and improved spectral resolution also make VIC-labeled probes the ideal second probe for a multiplex PCR system.

This user bulletin describes the characteristics of VIC probes in relation to the existing JOE probes. It also contains guidelines for defining limiting primer concentrations in a one- or two-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) system using VIC probes.

The following topics are covered in this user bulletin:

Topic	See Page
Characteristics of TaqMan VIC Probes	2
Multiplex RT-PCR	5
Technical Support	13

IMPORTANT To use VIC probes on the ABI Prism® 7700 Sequence Detection System (SDS), you must first calibrate the instrument with the Sequence Detection Systems Spectral Calibration Kit (P/N 4305822). This kit contains the new SYBR® Green and VIC fluorescent dye standards used to update the spectra components file in the SDS software. See *User Bulletin #4: Generating New Spectra Components* (P/N 4306294).

Note All documents referred to in this user bulletin are available though the Internet at the Applied Biosystems technical support documentation library or through Fax-on-Demand (see "To Obtain Documents on Demand" on page 16 for information).

The technical support documentation library is located at:

- www.appliedbiosystems.com/techsupport

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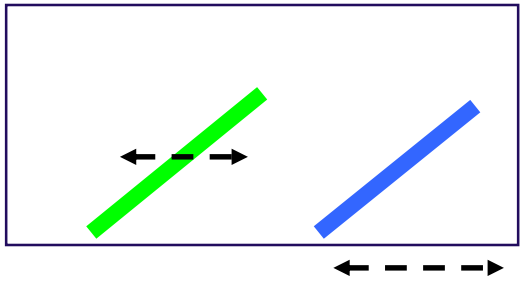
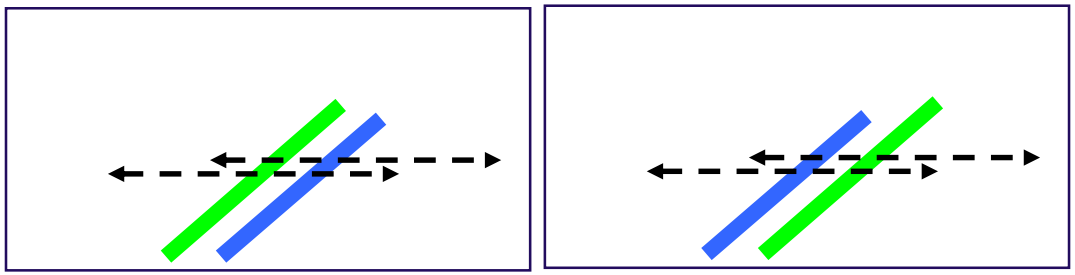
Multiplex Scenarios

#1: One gene is more abundant.

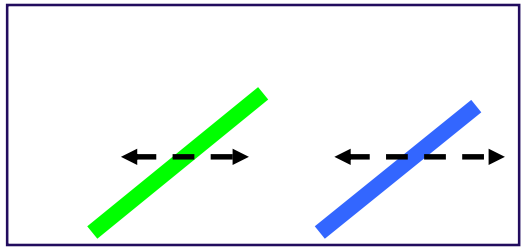


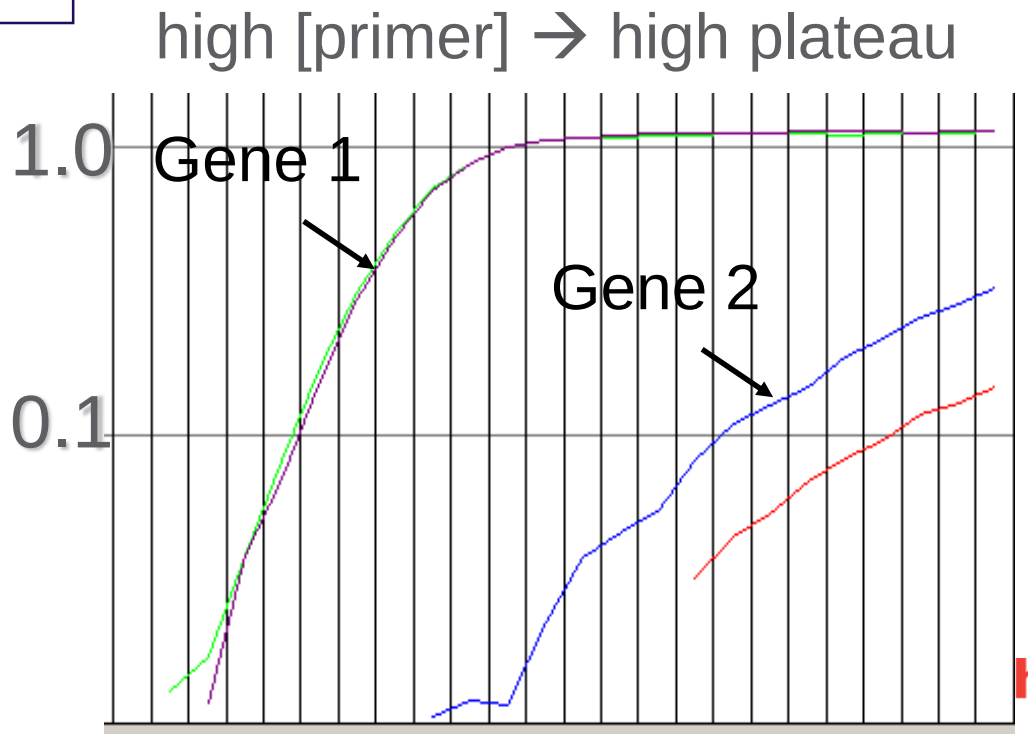
#2: Both genes are similar in expression



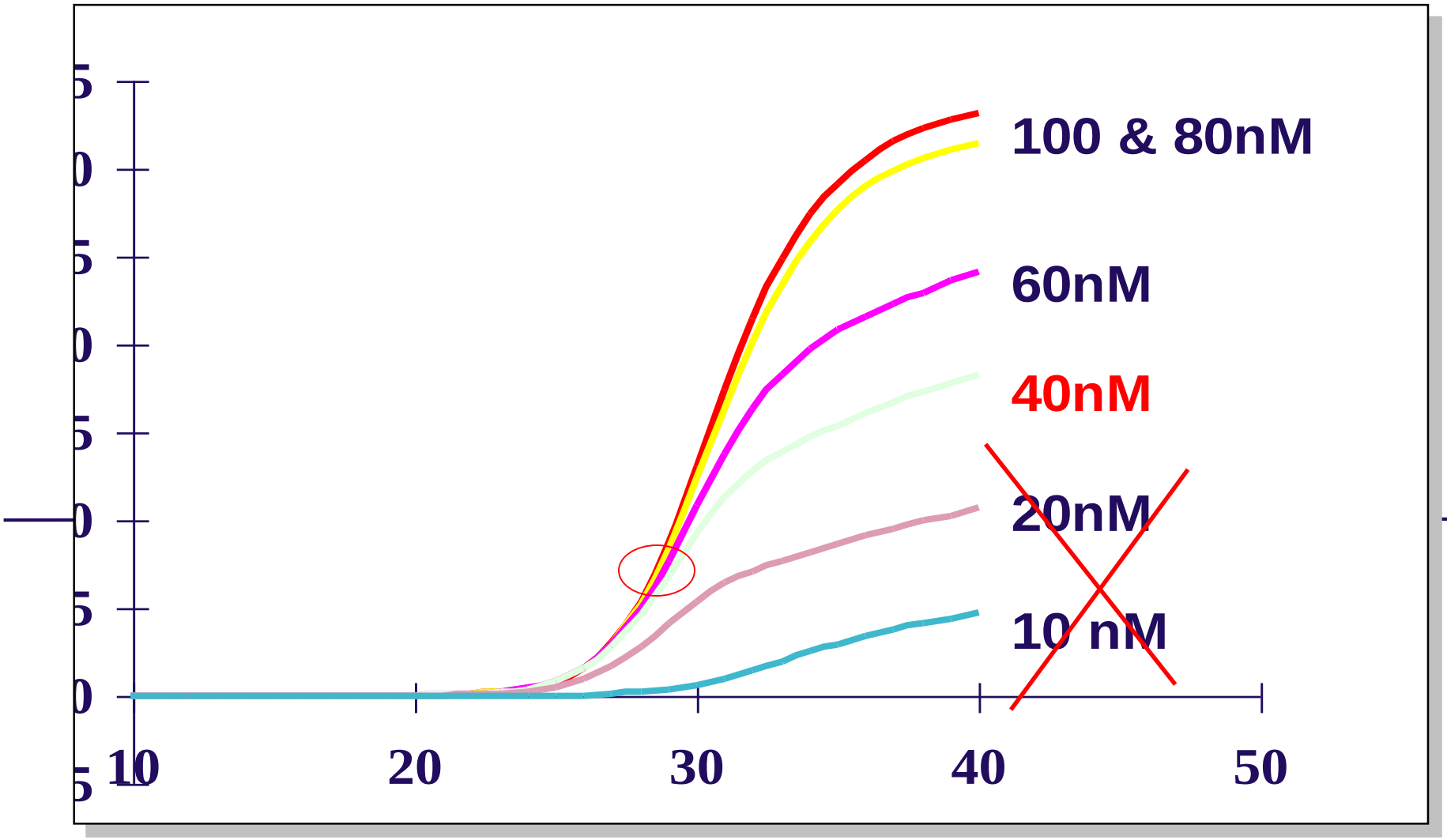
#1: One gene is more abundant

one gene uses all the reagents and there is nothing left for the other gene

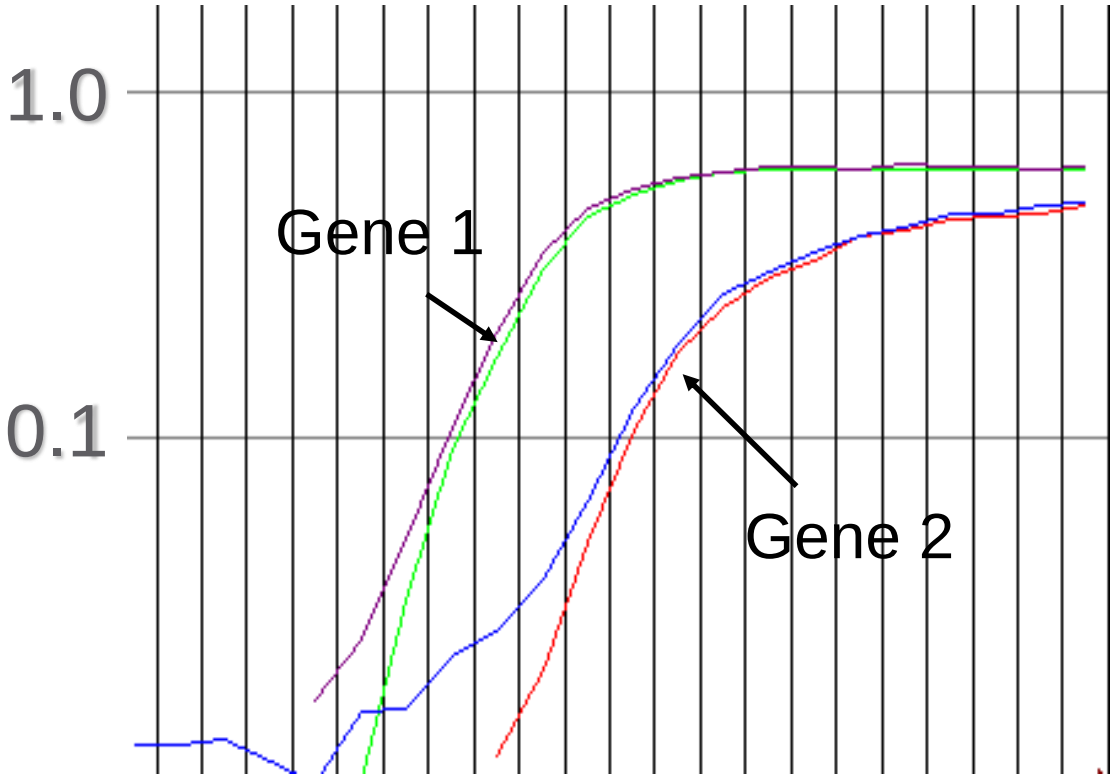




Reduce primers of more abundant gene-Optimization



Solution:
lower the [primer] of the early expressed assay → reaction plateau quickly



- Exponential phase in tact for both assays
- “primer limited” assays
- Order primers & probe separately

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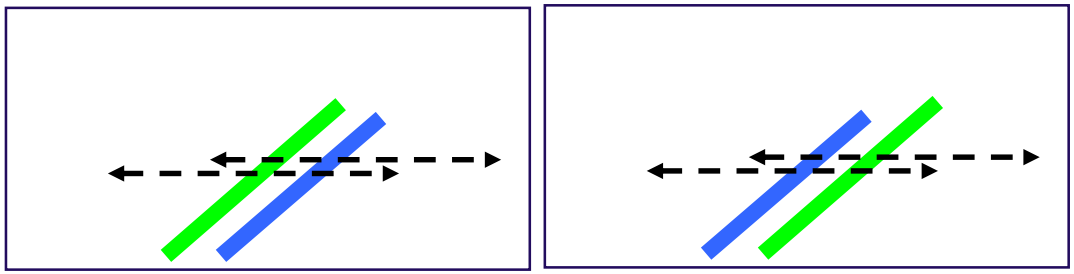
#2: Low stress multiplexing

both genes are similar in relative abundance



- Run in singleplex then duplex and ensure valid exponential phases and CTs are achieved
- If validation fails, primer limit one or both assays

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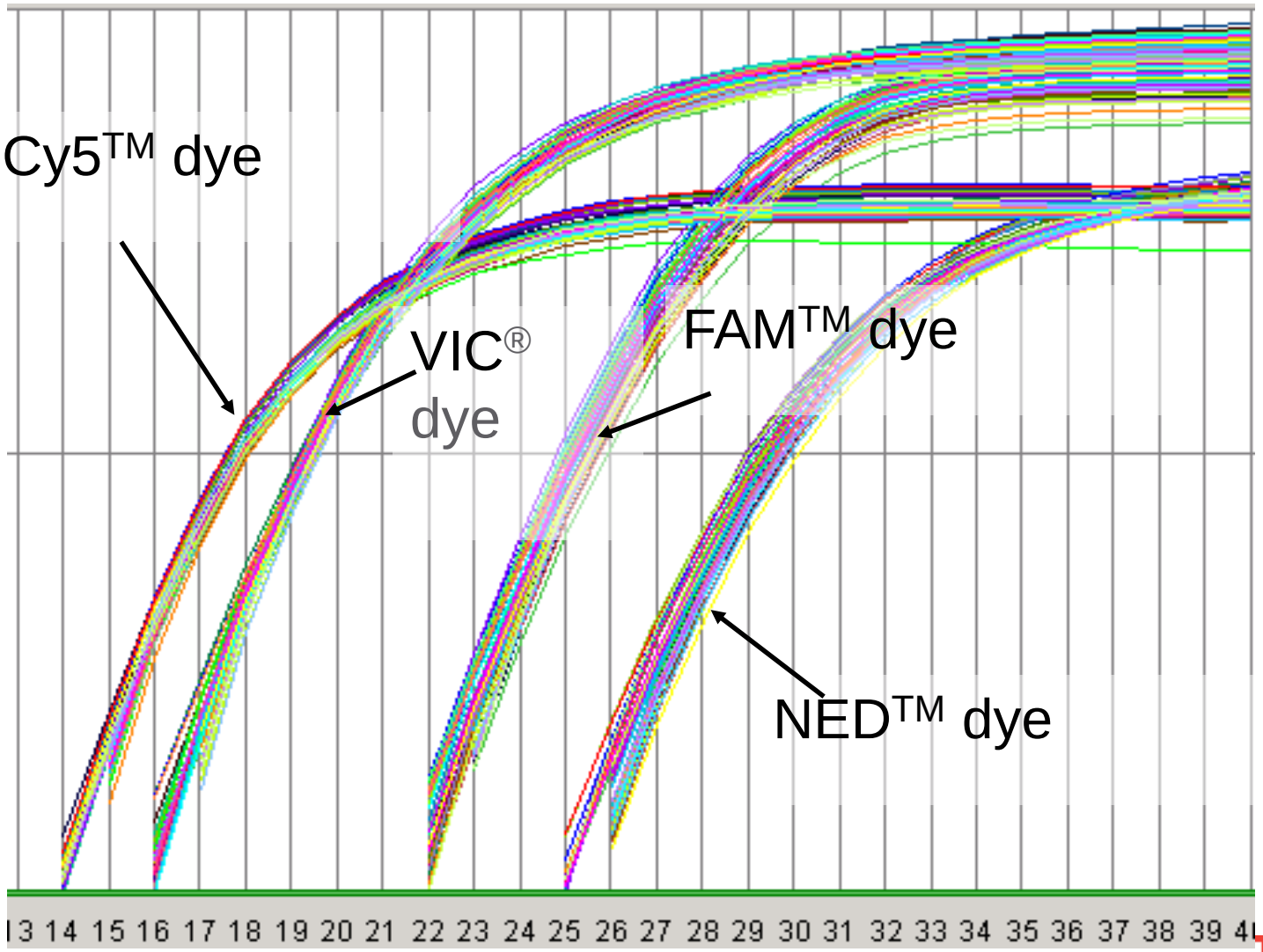
Multiplexing becomes exponentially difficult with every additional assay (triplex, quadplex…)



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Service Plans

Please contact Scott.Gardner@thermofisher.com for more details

Your qPCR system includes a 1 year manufacturers warranty. If a service plan is purchased, it will lock you into today's pricing and will start at the conclusion of the manufacturers warranty.

Discounts are available for multi-year coverage, locks you in at today's pricing.

Benefits of AB Assurance Service Plan

- Reduces downtime by providing proactive maintenance service
- Includes parts, labor, & travel at no additional cost
- Priority on-site guaranteed 2-day response time, & priority access to remote service engineer
- Scheduled on-site planned maintenance (PM) includes:
  - Calibration services (cost of the calibration kit is included)
  - Additional tests to ensure system performance
  - Computer repair and replacement

Support
(800) 955-6288
techsupport@thermofisher.com

Scott.gardner@thermofisher.com

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Instrument software

Computer login (default),

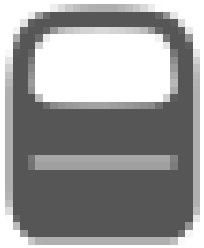
user name & password are the same

INSTR-ADMIN INSTR-ADMIN

Free Software Download (link in follow-up email)

— .edt template

— .eds single data file



Merge multiple .eds files in ThermoFisher Connect or TaqMan Genotyper (for SNP data)

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